Assessment of physiological and antioxidant responses in African catfish, *Clarias gareipinus* as biomarkers of metal pollution in River Nile

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Abstract

The present study was planned to assess the harmful effects of heavy metals on the physiological/antioxidant statuses and histopathological alterations of African catfish, *Clarias gareipinus* collected from two polluted sites in River Nile, Egypt; El-Rayah El-Tawfeky (Industrial polluted site) and Moshtahr (Agricultural polluted site) as well as, El-Qanater El-Khayriyah (Reference site) during spring and summer 2015. Results revealed marked ($P < 0.05$) variations in cadmium (Cd), copper (Cu), iron (Fe), lead (Pb), and zinc (Zn) in water samples among sites. The overall pollution was ordered as agricultural polluted site, industrial polluted site, and reference site. Serum level of glucose, creatinine, urea, uric acid, as well as aspartate transferase (AST), alanine transferase (ALT), and alkaline phosphatase (ALP) were significantly ($P<0.05$) higher in fish collected from the two polluted sites especially in Moshtahr site during summer season compared to the reference site. On the other hand, serum levels of total protein, albumin, globulin, and A/G ratio were significantly lower during both seasons in fish collected from the two polluted sites compared to those of the reference site. Highest reduction in total proteins was more evident during summer in fish collected from Moshtahr site. However, fish tissues of both polluted sites exhibited a significant rise in lipid peroxidase (LPO) and reduced glutathione (GSH) values as well as superoxide dismutase (SOD), catalase (CAT), and glutathione S-transferase (GST), which were more obvious in Moshtahr site during summer than spring season. Histopathological examination of liver tissues of the collected fish from polluted sites showed vacuolar degeneration of the hepatocytes, haemorrhage, haemosidirin, and focal areas of necrosis compared to the fish from EL- Qanater El- Khayreya. The study indicates that the alterations in the biochemistry profile and antioxidant defense system of

African catfish, *C. gariepinus* can be used as biomarkers of metal pollution for monitoring aquatic life.

Keywords: Heavy metals; Biochemistry; Antioxidant biomarkers; *Clarias gariepinus*; Histopathology.

1. Introduction

In the last decades, heavy metals discharges into aquatic ecosystems are increased due to the increase in many industrial and agricultural activities. Heavy metal pollution have devastating effects on the ecological balance of aquatic ecosystems and aquatic organisms (Ashraj, 2005; Vosyliene and Jankaite, 2006; Farombi et al, 2007). Fish have been pointed on the top of the aquatic food chain and may concentrate large amounts of some metals from the water. In addition, fish represents an important indicative factor in freshwater systems, to estimate metal pollution and the potential risks of the human consumption (Aktar et al, 2011). Therefore, Elia et al (2007) stated that fish can be considered as an early indicator of environmental risk due to their sensitive responses to environmental fluctuations.

Heavy metals are known to alert the integrity of physiological and biochemical mechanisms in fish metabolism (Basha and Rani, 2003; Gioda et al, 2007) as well as alteration in enzyme activities (Lionetto et al, 2000). Moreover, heavy metals promoted an oxidative damage by raising the cellular concentration of reactive oxygen species (ROS) in fish, consequently, a response of antioxidative defenses (Pinto et al, 2003; Ruas et al, 2008; Monteiro et al, 2010). Overproduction of ROS in restraint to heavy metals pollution causing an increscent in lipid peroxidation (LPO) and affecting the cell viability by damage the cell membrane (Nordberg and Arne´r , 2001). The produced ROS can detoxify by enzymatic and non-enzymatic cell defense systems, such as CAT, SOD, and GSH and GST as their induction or inhibition aimed to protect cells and tissues from oxidative damages as a response to pollutants (Reme´o et al, 2000; Jee and Kang, 2005; Asagba et al, 2008).

African catfish, *Clarias gariepinus* is an important commercial fish due to its high growth rate, high consumer acceptability, and tolerance to poor water quality, and oxygen depletion (Adewolu et al, 2008; Karami et al, 2010). It is widely distributed through different aquatic ecosystems in Africa (Nguyen and Janssen, 2002). Moreover, African catfish inhabits different ecosystems, which have different water and pollution characteristics for different seasons and be exposed to different water temperatures. Consequently, this study was aimed to investigate effects of heavy metals, throughout spring and summer seasons, on the biochemical parameters and oxidative defenses responses in African catfish, *C. gariepinus* sampled from different aquatic ecosystems. Histological alterations in the hepatic tissues were also investigated.

2. Materials and Methods

2.1. Study Area

Water, and fish samples were collected from three selected sites (El-Qanatir El-Khayriyah) (a reference site), El-Rayah El-Tawfeky (an industrial polluted site) and canal in Moshtahr village (an agricultural polluted site), (from March 2015 to August 2015) during spring and summer seasons 2015 (Figure 1).

2.2. Determination of water quality parameters

30 water samples (5 samples from each site) were collected by one-liter polyvinyl chloride Van Dorn bottle at two meter depth from the selected sites during spring and summer seasons. Temperature and Dissolved oxygen (DO) were measured on site with using a YSI oxygen meter (model 58, Yellow Spring Instrument Co., Yellow Springs, Ohio, USA). Whereas pH degree was measured using a pH-meter (Fisher Scientific, Denver, USA). After that, water samples were kept into a one-liter polyethylene bottle in an ice box till analyzed in the laboratory, where, Ammonia was measured using HACH kits (HACH Co., Loveland, Colorado, USA) Alkalinity and total hardness were measured by titration according to Boyd (1984).

2.3. Fish samples

A total number of 150 African catfish, *Clarias gariepinus* with average weight of 200±50g were collected from three different sites during Spring and Summer seasons for surveying biochemical and oxidative defense parameters and histopathology of catfish.

2.4. Diagnostic Kits

Serum creatinine, urea, uric acid, AST, ALT, ALP, Glucose, Total protein , and Albumin. In addition to LPO, GSH, SOD, CAT, and GST were determined using Commercial Diagnostic Kits purchased from (Biodiagnostic Com., Giza, Egypt) using spectrophotometer.

2.5. Determination of water heavy metals concentrations

A mixture of nitric acid and water sample is refluxed in a covered Griffin beaker. After the digestive, has been brought to a low volume, it was cooled and brought up in dilute nitric acid (3% v/v). The water sample was filtered, allowed to settle and prepared for analysis according to APHA (2005). The total cadmium (Cd), copper (Cu), iron (Fe), lead (Pb) and zinc (Zn) were measured after digestion using Atomic Absorption spectrophotometer (Pye-Unicam Ltd, Cambridge, England) at Soils, Water and Environment Unit, Soils, Water and Environment Institute, Agricultural Research Centre, Giza, Egypt.

2.6. Blood Sampling and biochemical analysis

2.6.1. Blood sampling

Blood samples have been collected from the caudal vessels by using plastic syringes in dry sterilized vials (Andrew,1990) and were left without anticoagulant in centrifuge tube for serum separation. Serum was separated by centrifugation at 3000 x g for 20 minutes, and then clean serum supernatant was separated carefully and kept frozen at -20°C till time of analysis of the serum constituents.

2.6.2. Biochemical analysis

Serum glucose was evaluated according to the method of Trinder (1959). Total protein was determined by Biuret test according to Gornal et al (1949). Serum albumin and globulin were assessed according to the method described by Dumas and Biggs (1972), and Coles (1974), respectively. Albumin / Globulin ratio was calculated from the albumin present in the serum in relation to the amount of globulin. Serum creatinine, urea, and uric acid were measured by colorimetric method described by Fabiny and Eringhausen (1971), Henry et al (1974), and Barham and Trinder (1972), respectively. Serum AST and ALT were assessed as described by Reitman and Frankel (1957). ALP was measured by the colorimetric method of Belfield and Goldberg (1971).

2.6.3. Oxidative stress biomarkers

Samples of liver tissue were homogenized in a Potter–Elvejhem glass/Teflon homogenizer and centrifuged then the supernatant was collected for estimation the following parameters: lipid peroxidase (LPO) was measured according to the method described by Ohkawa et al (1979). Reduced glutathione (GSH) was determined by Beutler et al (1963). Superoxide dismutase (SOD) was assessed calorimetrically according to the method described by Nishikimi et al (1972). Catalase (CAT) enzyme was estimated according to Aebi (1984). Glutathione-S-transferase (GST) level was determined according to the method described by Habig et al (1974).

2.6.4. Histopathological studies

Moribund fish were anesthetized using (0.02%) benzocaine solution. Tissue specimens from the liver as the site of detoxification were taken, fixed in 10% neutral buffered formalin, dehydrated in alcohol, cleared in xylol and embedded in paraffin, 4µm thick sections were prepared and stained with H & E (Bancroft et al, 1996; Roberts, 2001).

2.6.5. Statistical analysis

The data obtained in the present investigation have been statistically analyzed by using the analysis of variance, two way "ANOVA" followed by "Tuckey" multiple tests as a post hoc test, SPSS.

3. Results

3.1. Water quality

In the present study, water temperature was significantly lower during spring in the water samples collected from all sites and during summer season (Table 1). Water alkalinity, salinity, total hardness, pH, and ammonia were increased significantly in water samples collected from both sites during spring and summer. On the other hand, water dissolved oxygen (DO) was decreased significantly during spring and summer in water samples collected from both the polluted sites compared to the reference site (Table 1).

3.2. Water heavy metals concentrations

In the current work, water Cd, Cu, Fe, Pb, and Zn concentrations were significantly higher in water samples collected from sites of El- Rayah El- Taweky and Moshtahr compared with those from the reference site which were more obvious in Moshtahr's samples during spring and summer seasons and exceeded the permissible limits compared with the reference site (Table 2).

3.3. Serum biochemical analysis

Serum glucose, creatinine, urea, uric acid, AST, ALT, and ALP levels were significantly ($P<0.05$) higher during spring and summer seasons. Their highest values were recorded in Moshtahr during summer season (Table 3). Serum total protein, albumin, globulin, and A/G ratio were significantly ($P<0.05$) lower in fish collected from Moshtahr site during spring and summer compared to the collected fish from EL- Qanatir El- Khayriyah site (Table 3).

3.4. Oxidative stress biomarkers

The current investigation showed that the hepatic LPO, GSH, SOD, CAT, and GST levels were significantly ($P< 0.05$) higher in fish collected from both, El- Rayah El- Tawfeky and Moshtahr sites during summer than those collected during spring season (Table 4). On the other hand, hepatic SOD concentration was markedly higher in Moshtahr site during spring and summer seasons than that in El- Rayah El- Tawfeky site (Table 4).

3.5. Histopathological investigation

The histological examination of liver specimens in African catfish collected from El-Qanater El-Khirya site in both seasons showed normal structure of hepatocytes (Figure 2 a). Meanwhile, the liver samples of fish collected from El- Rayah El- Tawfeky (an industrial polluted site) and Moshtahr canal (an agricultural polluted site) during spring and summer seasons showed degeneration of the hepatocytes,congested dilated central vein, infiltration of chronic inflammatory cells between hepatocytes, haemorrhage, dilatation of blood Vessel and the appearance of iron pigments (haemosiderin), respectively (Figure 2 b, c, and d). Fibrous tissue invaded with inflammatory cells observed in livers of cat fish collected from El- Rayah El- Tawfeky and vacuolar necrosis with pyknotic nuclei in hepatic samples collected from Moshtahr site during summer season, respectively (Figure 3 e, and h). Infiltration of fibrous connective tissue in between hepatocytes (Figure 4 j) as well as, necrotic changes and scattering of haemosiderin were noticed (Figure 4 l).

4. Discussion

4.1. Water quality

Water quality analysis is generally used to examine if water is suitable for various uses or processes (Chapman and Chapman, 1996). The water quality described as the concentration and state of the organic and inorganic materials found in the water, together with the physical characteristics of the water (Mapfumo et al, 2002). Water temperature is a critical parameter, since it directly influences DO availability to aquatic organisms (APHA, 2005). Water temperature was significantly ($P<0.05$) lower during spring in water samples collected from and El- Rayah El- Tawfeky and Moshtahr sites and during summer in the water samples collected from Moshtahr site compared to the control area. This reduction was more pronounced in El- Rayah El- Tawfeky site (25.78±0.40). On the other hand, water temperature was significantly ($P<0.05$) increased during summer in water samples collected from El- Rayah El- Tawfeky site compared to the control area (Table 1). These results are verified with Robert (1994) ; Stephen (1995) ; Carvalho et al (2012). The variation in water temperature of the investigated areas depends on several factors such as the climatic condition, sampling times, and the number of sunshine hours and the specific characteristics of water environment like turbidity, wind velocity, plants density and humidity (Mahmoud, 2002 ; Tayel et al, 2008). Alkalinity of water is defined as the acid-neutralizing capacity. It is also considered as the sum of all titratable bases (APHA, 2005). It represents an indication of the concentration of carbonate, bicarbonate and hydroxide content in water (Osman and Kloas, 2010). Water alkalinity was significantly ($P<0.05$) increased during spring and summer in water samples collected from El- Rayah El- Tawfeky and Moshtahr sites compared to the control area (Table 1). This elevation was more obvious in Moshtahr site during spring and summer seasons. The results are agreed with Osman et al (2010). Salinity is the total amount of salts (in grams) dissolved in one liter of water. Water salinity was significantly ($P<0.05$) higher during spring and summer seasons in water samples collected from El- Rayah El- Tawfeky and Moshtahr sites compared to the control area (Table 1). This elevation was more evident in El- Rayah El- Tawfeky (3.72±0.14) during summer. Water hardness was significantly ($P<0.05$) increased during spring and summer in the water samples collected from El- Rayah El- Tawfeky and Moshtahr sites compared to the reference site (Table 1). This elevation was more pronounced in El- Rayah El- Tawfeky (106.81±0.80) during summer seasons. The results are supported with Osman and Kloas (2010). pH value is the most important and frequently used tests in water chemistry (APHA, 1995), as it has effect on the solubility and availability of nutrients, and how they can be utilized by aquatic organisms (Osman and Kloas, 2010). Water pH increased significantly ($P<0.05$) during spring and summer in the water samples collected from El- Rayah El- Tawfeky and Moshtahr sites compared to the control area (Table 1). In this study, all pH values were in alkaline side (7.75 to 7.97). These results are confirmed with Carvalho et al (2012) and Oss et al (2013). This elevation may be attributed to the thermal pollution which leads to high density of vegetation and phytoplankton, which were accompanied by increment in photosynthetic activity and consumption of CO_2 (Sabae, 2004). Dissolved oxygen (DO) is a vital factor to the aquatic organisms, because it affects their biological processes, respiration and oxidation of the organic matter in water and sediments (Tayel et al, 2008). In the present study, water DO was significantly ($P<0.05$) decreased during spring and summer in water samples collected from El- Rayah El- Tawfeky and Moshtahr sites compared to the reference site (Table 1). The decrease in DO values were more evident in Moshtahr site during spring and summer seasons, which may be attributed to the elevation of water temperature and increase in oxidative processes of organic matter. Ammonia is the most common components of nitrogen excretion in aquatic ecosystems (APHA, 2005). Our results showed that water ammonia increased significantly ($P<0.05$) during spring and summer seasons in the water samples collected from El- Rayah El- Tawfeky and Moshtahr sites compared to the reference site (Table 1). The increase in ammonia level was more obvious in Moshtahr site (6.18±0.37) during summer. The results are verified with Carvalho et al (2012) who explained this by the aggregation of large amounts of organic matter sources and their decomposition of organic matter exhausting dissolved oxygen and production of high level of ammonia (Saad et al, 2011).

4.2. Water heavy metals concentrations

In aquatic environments, heavy metal pollution results from many process naturally such as direct atmospheric deposition, geologic weathering and through anthropogenic activity like the discharge of agricultural, municipal, residential or industrial waste products, also via wastewater treatment plants (WWTPs) (Maier et al, 2014; Dhanakumar et al, 2015; Garcia et al, 2015). In this investigation, water Cd, Cu , Fe, Pb, and Zn was significantly ($P<0.05$) increased during spring and summer seasons in water samples collected from El- Rayah El- Tawfeky and Moshtahr sites compared to the control area (Table 2). This elevation was more evident in Moshtahr site during spring and summer seasons. The results are agreed with Authman et al (2012) ; Carvalho et al (2012). Therefore, the high levels of heavy metals found in water during summer season could be attributed to the seasonal variations causing fluctuations of the amount of agricultural drainage water, sewage effluents, and industrial wastes discharged into water (Zyadah, 1995).

4.3. Serum biochemical analysis

Biochemical parameters helped to identify the target organs of toxicity and the general health status of animals. It also provided an early warning signal in stressed organism (David et al, 2010). Glucose level was significantly ($P<0.05$) increased during spring and summer seasons in El- Rayah El- Tawfeky and Moshtahr compared to the reference site (Table 3). The elevation was evident during summer in El- Rayah El- Tawfeky and Moshtahr sites. These results are supported with Bakhshwan et al (2009) ; Hamed (2015 a); Hamed (2015 b) ; Kim and Kang (2015) ; Canli and Canli (2015). Elevation in glucose level may be resulted from the increase in glucogenesis and glycogenolysis as well as inhibition of glucogenolysis and glycogenesis to produce the energy used in combating the stress induced on the fish by the environmental pollution (Yekeen and Fawole, 2011; Hamed, 2015b). Total proteins are useful in diagnosis of fish disease. The majority of proteins which are synthesized in the liver are used as an indicator of liver dysfunction (Yang and Chen, 2003). In the current investigation, serum total proteins, albumin, globulin, and A/G ratio were significantly (P<0.05) lower in catfish collected from El- Rayah El- Tawfeky and Moshtahr sites during spring and summer seasons (Table 3). The reduction in total proteins was more pronounced in summer season in El- Rayah El- Tawfeky and Moshtahr sites. The results are consistent with Marzouk et al (2012) ; Harabawy and Ibrahim (2014) ; El gamal et al (2015). The reduction of total proteins may be due the disturbances in liver protein metabolism (Dange and Masurekar, 1984; Abdel-Tawwab et al, 2007a ; Abdel-Tawwab et al, 2007b) and the inhibition of the hepatic synthesis of blood protein (Fontana et al, 1998; Aly et al, 2013).

Kidneys are playing an essential role in the water and electrolyte balance and in the maintenance of a stable internal body environment (Palaniappan et al, 2009). Serum renal products, including creatinine, urea, and uric acid were significantly (P<0.05) higher during spring and summer seasons in El- Rayah El- Tawfeky and Moshtahr sites compared to the control area (Table 3). This elevation was more pronounced in Moshtahr site during summer season in serum creatinine (0.88±0.02 mg/dl), urea (30.24±0.38 mg/dl), and uric acid (2.26±0.06 mg/dl). The results are confirmed with Sabae and Mohamed (2015) ; Hamed (2016) ; Hamed and Osman (2017) ; Sayed and Hamed (2017). The rising levels of creatinine and urea in the blood is an indicative of impaired kidney function (Patel et al, 2006), which attributed to the high production of ROS and kidney injury (Upasani and Balaraman, 2003). The kidney dysfunction may be as a result of heavy metal -induced nephrotoxicity, and glomerular insufficiency (Yu et al, 2004).

Transaminases are important enzymes which are playing a key role in mobilizing L-amino acids for gluconeogenesis and they are function as links between carbohydrate and protein metabolism under altered physiological and pathological conditions (Manjunatha et al, 2015). In the current study, serum AST, ALT, and ALP were significantly (P<0.05) elevated during spring and summer seasons in El- Rayah El- Tawfeky and Moshtahr sites compared to the reference site (Table 3). These elevations were more obvious in Moshtahr during spring and summer seasons. These results agreed with Canli and Canli (2015) ; Sabae and Mohamed (2015). Elevations in activities of serum AST, ALT and ALP reflect hepatic impairment, leading to extensive liberation of these enzymes into the blood circulation. The elevation of the hepatic enzymes may be due to liver dysfunction as a result of the hepatocellular damage or cellular degradation (Mohamed and Gad, 2009).

4.4. Oxidative stress biomarkers

Oxidative stress is another mechanism for toxicity which leading to cell death and disturbance of the physiological processes in fish (Banaee, 2013). Oxidative stress results from the imbalance between the antioxidant enzyme activities and ROS production, also when the antioxidant system becomes unable to eliminate or neutralize the excess of ROS (Nishida, 2011; Hamed**,** 2015b). Lipid peroxidation emerges from the reaction of free radicals with lipids and this is an important feature of the cellular injury caused by free radical attack (Hoek and Pastorino, 2002). In the present work, the activity of hepatic LPO was significantly higher in African catfish sampled from both El-Rayah El- Tawfeky and Moshtahr sites (Table 4). The increment in LPO activity was more evident during summer season. Previous investigations have reported the induction of LPO in different fish species by pesticides such as atrazine (Kadry et al, 2012), bisphenol-A (Hamed and Abdel-Tawwab, 2017) and heavy metals (Souid et al, 2013; Kumari et al, 2014). The SOD-CAT system, the first line of defense system against oxidants varied according to the response of fish antioxidant system to counteract with the toxicity of metal exposure (Garcia et al, 2008; Atli and Canli, 2010). The obtained results showed that SOD and CAT activities were markedly higher ($P<0.05$) in liver of African catfish exposed to heavy metals during summer and spring seasons in El-Rayah El-Tawfeky and Moshtahr sites compared to the reference site (Table 4). The increase in SOD and CAT enzymes may be due to the elimination of ROS from the cell induced by exposure to pollutants which convert superoxide anions (O_2^-) into H_2O_2 and then into H_2O and O_2 (Jin et al, 2010 ; Hamed, 2016). Concurrently, hepatic GSH and GST levels in African catfish collected from El-Rayah El-Tawfeky and Moshtahr sites were significantly raised during summer and spring seasons compared to El- Qanatir El- Khayriyah (Table 4). GST plays an important role in homeostasis, detoxification and clearance of many xenobiotic compounds. Where, GST can catalyze the synthetic conjugation reactions of xenobiotics or other pollutants to GSH, and facilitates the excretion of chemicals by the addition of more polar groups (Luo et al, 2006; Hamed**,** 2015b). The increase of GSH level in fish tissues could be due to defense system to protect the fish from the oxidative stress or could be appear as an antioxidant adaptation to metal exposure. The results are in accordance with the previous investigators (Souid et al, 2013; Saglam et al, 2014).

4.5. Histopathological investigations

Histopathological investigation is crucial to determine cellular changes that may occur in target organs, such as the liver (Sabae and Mohamed, 2015). The liver is a detoxification organ and vital for both the metabolism and excretion of toxic substances (Abdel-Tawwab et al, 2007b). In the present study, several histopathological alterations were observed in the liver tissue of fish collected from El-Qanatir El- Khayriyah (a reference site), El- Rayah El- Tawfeky (an industrial polluted site) and Moshtahr canal (an agricultural polluted site) during spring and summer seasons (Figure 2 & 3). The histopathological alterations may be due to direct toxic effects of pollutants on hepatic cells because the liver is considered as the site of detoxification of all types of toxins and chemicals, hence, the cellular degeneration observed in the liver specimens of the studied fish is most probably due to vascular dilation and intravascular haemolysis as well as, it may be due to thrombosis formation in the blood vessels with subsequent stasis of blood (Sabae and Mohamed, 2015). The necrotic changes detected in hepatic cells were most probably a consequence of the cellular degenerations. The observed degeneration in the liver may be also due to disruption in the lysosomal membrane, which is very sensitive to toxicants and thus their enzymes released and caused degeneration and vacuolation of cytoplasm of hepatocytes (Mohamed and Gad, 2009). The vacuolization of hepatocytes indicated to the disproportion between the rate of synthesis of substances in the parenchymal cells and the rate of their liberation into the circulation system (Gingerich, 1982). Oxygen deficiency as a result of gill degeneration being the most common cause of the cellular degeneration in the liver (Eder and

Gedigk, 1986). According to Walter and Israel (1974), the presence of haemorrhagic areas and aggregations of inflammatory cells in the liver of the studied fish could be explained that the necrotic cells lead to release of different chemostatic factors which attract different inflammatory cells. However, Ram and Singh (1988) reported that the rupturing of blood vessels causing invasive infiltration of inflammatory cells and detrimental focal necrosis. The results are supported with (El-Naggar et al, 2009; Hamed and Osman, 2017).

5. Conclusion

This study presented an investigation of the hazard effects of heavy metals pollution on African catfish which collected from El- Qanatir El Khayriyah (a reference site), a canal in Moshtahr village (an agricultural polluted site), and El- Rayah El- Tawfeky (an Industrial polluted site) during spring and summer seasons. It showed that heavy metals induced a harmful effect on the biochemical profile of African catfish, *C. gariepinus* and damages the hepatic tissues which manifested by the histopatholoical examination and oxidative damage in fish livers of collected samples. Additionally, the results showed that Moshtahr is more polluted than El- Rayah El- Tawfeky which is more obvious during summer season. On the other hand, increase the temperature during summer season has a marked affection on the collected fish samples. Moreover, it caused antioxidant effects which clarified as increment of the oxidative stress indicators (LPO, GSH, CAT, SOD and GST). Hence, the biochemical analysis and oxidative stress are considered as important biomarkers of water pollution by heavy metals.

Conflict of interest

Authors declare that they have no conflict of interest.

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Figure 1: Map showing the study sites of El- Qanatir El Khayriyah (A; Reference site), Moshtahr canal (B; Agricultural polluted site), and El-Rayah El-Tawfeky (C; Industrial polluted site).

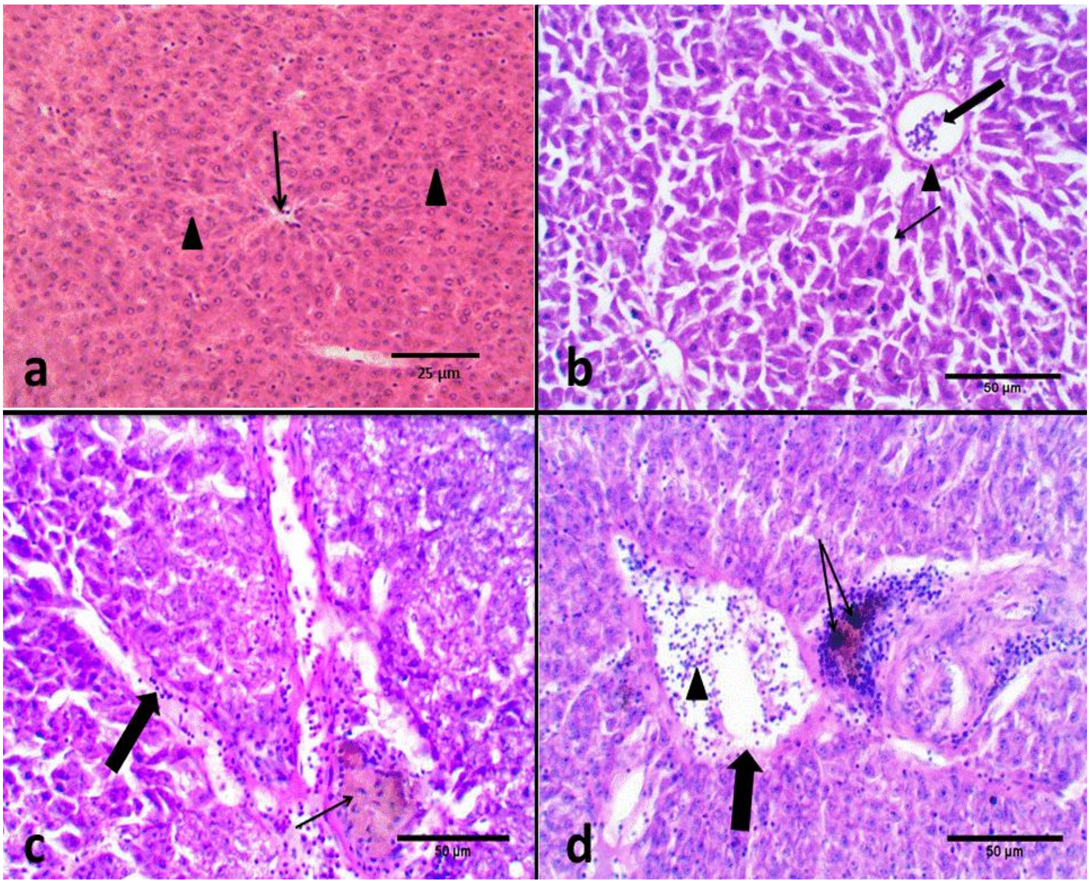

Figure 2 a) Section in liver of *Clarias gariepinus* collected from El-Khanater site (Reference site) showing the normal histological structure of hepatocytes (head) and central vein (thin arrow) (H & E, × 200). b) Section in liver of *C. gariepinus* collected from Moshtahr site during spring showing degenerative changes (thin arrow) and congested dilated central vein (head) infiltrated with chronic inflammatory cells (thick arrow) (H & E, × 400). c) Section in liver of *C. gariepinus* collected from Moshtahr site during spring showing infiltration of chronic inflammatory cells between hepatocytes (thick arrow) and haemorrhage is quite clear (thin arrow) (H & E, × 400). d) Section in liver of *C. gariepinus* collected from Moshtahr site during spring showing dilatation of blood Vessel (thick arrow) filled with inflammatory cells (head) beside the appearance of iron pigments (haemosiderin) (thin arrow) (H & E, × 400).

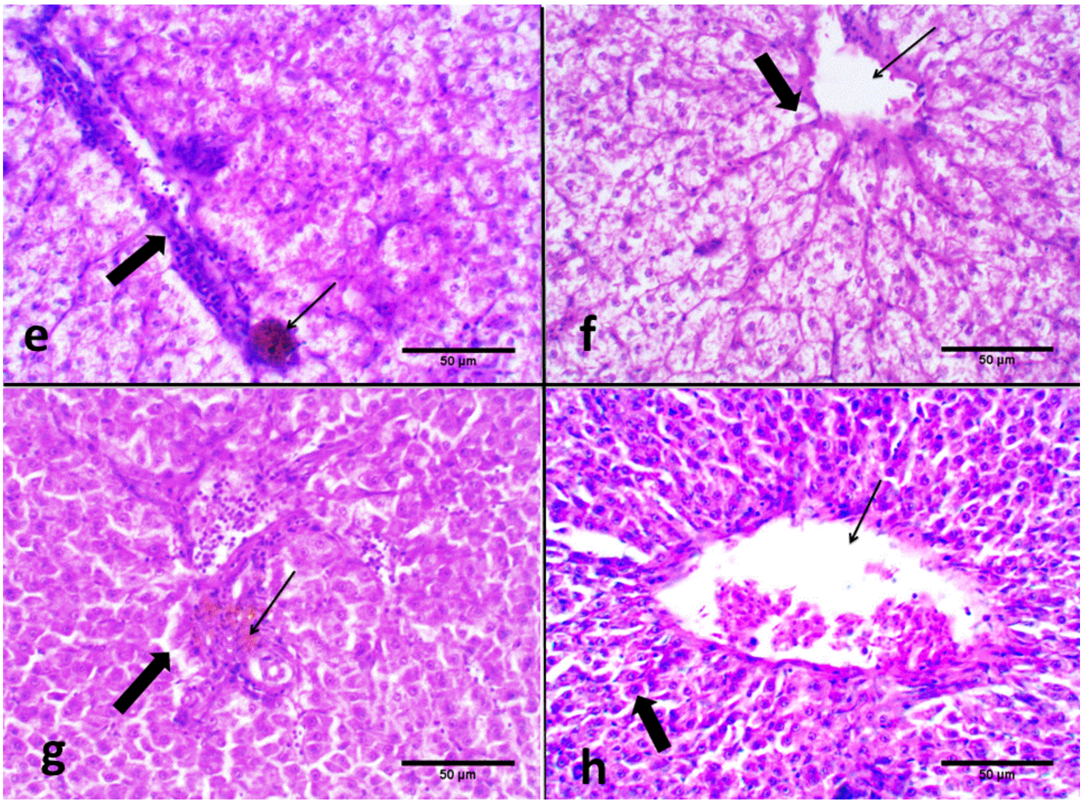

Figure 3 e) Section in liver of *C. gariepinus* collected from El- Rayah- El- Tawfeky site during spring showing fibrous tissue invaded with inflammatory cells (thick arrow), haemorrhage is clear through hepatic cells (thin arrow) (H & E, × 400). f) Section in liver of *C. gariepinus* collected from El- Rayah- El- Tawfeky site during spring showing severe vacuolar degeneration (thick arrow) and congested dilated hepatic central vein (thin arrow) (H & E, × 400). g) Section in liver of *C. gariepinus* collected from Moshtahr site during summer showing degeneration of hepatocytes (thick arrow) and haemorrhageic signs (thin arrow) (H & E, × 400). h) Section in liver of *C. gariepinus* collected from Moshtahr site during summer showing dilated blood vessel infiltrated with chronic inflammatory cells (thin arrow) and vacuolar necrosis with pyknotic nuclei (thick arrow) (H & E, × 400).

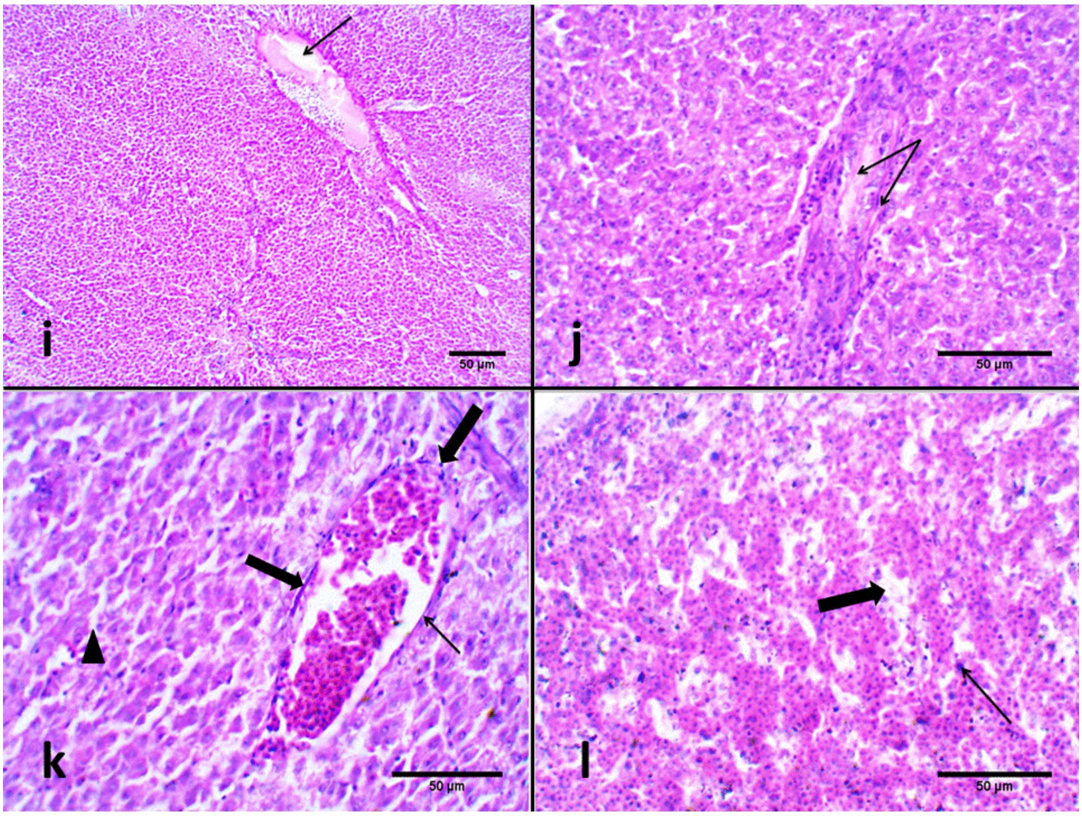

Figure 4 i) Section in liver of *C. gariepinus* collected from Moshtahr site during summer showing thickened dilated blood vessel (thin arrow) (H & E, × 200). j) Section in liver of *C. gariepinus* collected from El- Rayah- El- Tawfeky site during summer showing infiltration of fibrous connective tissue in between hepatocytes (thin arrow) (H & E, × 400). k) Section in liver of *C. gariepinus* collected from El- Rayah- El- Tawfeky site during summer showing degeneration of hepatic cells (head), dilated blood vessel (thin arrow) and Haemosedrin is clear (thick arrow) (H & E, × 400). l) Section in liver of *C. gariepinus* collected from El- Rayah- El- Tawfeky site during summer showing necrotic changes (thick arrow) and scattering of iron pigments (haemosiderin) (thin arrow) (H & E, × 400).

Table 1: Physicochemical characteristics of water samples collected from El- Qanatir El Khayriyah (Reference site), El-Rayah El-Tawfeky (Industrial polluted site), and Moshtahr canal (Agricultural polluted site) during spring and summer seasons.

Seasons	Spring			Summer		
parameters / sites	Reference site	El- Rayah El-Tawfeky site	Moshtahr site	Reference site	El- Rayah El-Tawfeky site	Moshtahr site
Temperature (°C)	27.42±0.19[a]	25.78±0.40[b]	27.33±0.64[ab]	31.19±0.97[a]	31.54±0.32[a]	30.45±0.43[a]
Alkalinity (ppm)	23.33±1.67[c]	29.24±0.53[b]	34.00±0.61[a]	24.23±0.88[c]	30.50±0.13[b]	36.34±0.33[a]
Salinity (‰)	1.38±0.04[c]	3.59±0.35[a]	2.57±0.15[b]	1.72±0.17[c]	3.72±0.14[a]	2.56±0.16[b]
Total hardness (ppm)	75.29±0.90[c]	104.88±2.47[a]	85.08±1.05[b]	76.97±1.46[c]	106.81±0.80[a]	81.49±0.94[b]
pH	7.42±0.01[a]	7.66±0.24[a]	7.75±0.02[a]	7.41±0.01[a]	7.97±0.24[a]	7.754±0.16[a]
Dissolved O_2 (mg/l)	8.03±0.26[a]	7.44±0.12[a]	6.37±0.19[b]	8.00±0.17[a]	7.40±0.12[a]	6.40±0.10[c]
Ammonia (mg/l)	2.60±0.10[b]	3.10±0.32[ab]	3.76±0.14[a]	2.83±0.12[c]	4.90±0.21[b]	6.18±0.37[a]

Means with different superscript letter in the same row for each parameter are significantly different ($P < 0.05$).

Table 2: Concentrations of heavy metals in water samples collected from El- Qanater El- Khayriyah) (Reference Site), El- Rayah El- Tawfeky (Industrial polluted site), and Moshtahr canal (Agricultural polluted site) during spring and summer seasons.

Seasons	Spring			Summer		
Heavy metals / sites	Reference site	El- Rayah El- Tawfeky site	Moshtahr site	Reference site	El- Rayah El- Tawfeky site	Moshtahr site
Cd (µg/l)	0.005±0.001[b]	0.022±0.004[b]	0.540±0.050[a]	0.006±0.001[c]	0.036±0.010[b]	0.507±0.009[a]
Cu (µg/l)	0.015±0.003[b]	0.037±0.007[b]	0.483±0.019[a]	0.020±0.006[b]	0.032±0.002[b]	0.467±0.019[a]
Fe (µg/l)	0.001±0.000[c]	0.144±0.003[b]	0.169±0.010[a]	0.001±0.000[c]	0.129±0.001[b]	0.167±0.008[a]
Pb (µg/l)	0.015±0.008[c]	0.697±0.027[b]	1.216±0.047[a]	0.012±0.004[c]	0.687±0.024[b]	1.32±0.036[a]
Zn (µg/l)	0.009±0.001[c]	0.047±0.007[b]	0.163±0.012[a]	0.007±0.001[c]	0.040±0.006[b]	0.163±0.005[a]

Means with different superscript letter in the same row for each parameter are significantly different ($P < 0.05$).

Table 3: Biochemical profile of African catfish, *C. gariepinus* collected from El- Qanatir El Khayriyah (Reference site), El- Rayah El- Tawfeky (Industrial polluted site), and Moshtahr canal (Agricultural polluted site) during spring and summer seasons.

Seasons	Spring			Summer		
parameters / sites	Reference site	El- Rayah El- Tawfeky site	Moshtahr site	Reference site	El- Rayah El- Tawfeky site	Moshtahr site
Glucose (mg/dl)	43.00±1.53^{c}	84.33±3.33^{b}	102.00±1.03^{a}	50.00±0.58^{c}	111.33±3.18 ^{b}	129.00±1.73^{a}
Total protein (g/dl)	4.26±1.53^{c}	3.61±0.11^{b}	3.15±0.13^{c}	4.26±0.13^{a}	3.07±0.05^{b}	2.52±0.14^{c}
Albumin (g/dl)	2.30±0.21^{a}	1.76±0.03^{b}	1.45±0.06^{b}	1.85±0.12^{b}	1.60±0.08^{b}	1.26±0.03^{c}
Globulin (g/dl)	1.95±0.26^{a}	1.85±0.12^{b}	1.70±0.18^{b}	1.97±0.16^{a}	1.47±0.03^{b}	1.26±0.15^{b}
A/G ratio	1.24±0.23^{a}	0.96±0.07^{b}	0.88±0.12^{b}	1.17±0.10^{a}	1.09±0.07^{b}	1.03±0.13^{b}
Creatinine(mg/dl)	0.37±0.01^{c}	0.53±0.03^{b}	0.76±0.01^{a}	0.36±0.01^{c}	0.66±0.02 ^{b}	0.88±0.02^{a}
Urea (mg/dl)	8.65±0.51^{c}	12.37±1.02^{b}	16.43±0.68^{a}	9.77±0.16^{c}	23.79±1.63^{b}	30.24±0.38^{a}
Uric acid (mg/dl)	0.71±0.06^{c}	1.22±0.13^{b}	1.67±0.07^{a}	0.91±0.06^{c}	1.68±0.17^{b}	2.26±0.06^{a}
AST(U/l)	106.33±5.24^{b}	124.00±2.31^{a}	133.67±2.33^{a}	109.33±5.21^{b}	132.00±2.52^{a}	142..67±2.58^{a}
ALT (U/l)	220.67±1.45^{c}	237.00±1.00^{b}	246.67±2.60^{a}	222.00±0.58^{c}	240.67±0.33^{b}	253.67±2.03^{a}
ALP (U/l)	35.84±2.46^{c}	43.96±1.00^{b}	53.20±2.62^{a}	34.24±2.96^{c}	50.49±1.04^{b}	66.72±1.28^{a}

Means with different superscript letter in the same row for each parameter are significantly different ($P < 0.05$).

Table 4: Oxidative stress biomarkers in the liver tissue of African catfish, *C. gariepinus* collected from El- Qanatir El Khayriyah (Reference site), El- Rayah El- Tawfeky (Industrial polluted site), and Moshtahr canal (Agricultural polluted site) during spring and summer seasons

Seasons	Spring			Summer		
parameters / sites	Reference site	El- Rayah El- Tawfeky site	Moshtahr site	Reference site	El- Rayah El- Tawfeky site	Moshtahr site
LPO (nmol/g tissue)	$50.33±0.67^{b}$	$80.33±7.97^{a}$	$91.00±5.29^{a}$	$51.00±2.08^{c}$	$96.00±1.53^{b}$	$108.33±4.91^{a}$
GSH (nmol/g tissue)	$16.30±0.30^{c}$	$21.23±1.00^{b}$	$25.22±1.10^{a}$	$18.39±0.82^{b}$	$27.40±1.70^{a}$	$30.85±1.15^{a}$
CAT (µg/mg tissue)	$1.79±0.05^{b}$	$1.84±0.02a^{b}$	$1.92±0.01^{a}$	$1.79±0.01^{c}$	$1.98±0.01^{b}$	$2.52±0.02^{a}$
SOD (µg/mg tissue)	$515.00±47.43^{c}$	$544.33±18.67^{ab}$	$599.67±9.70^{a}$	$516.67±22.85^{b}$	$546.00±14.36^{ab}$	$640.67±39.44^{a}$
GST (µg/mg tissue)	$0.37±0.04^{c}$	$0.44±0.03^{ab}$	$0.54±0.01^{a}$	$0.42±0.01^{b}$	$0.73±0.03^{a}$	$0.83±0.05^{a}$

Means with different superscript letter in the same row for each parameter are significantly different ($P < 0.05$).