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# Heavy Metals Levels in the Blood of *Oreochromis niloticus niloticus* and *Clarias gariepinus* as Biomarkers of Metal Pollution in the River Nile

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**Abstract:** A combination of biological monitoring (Biomonitoring) and measurements of water and sediment quality can provide a good indication of conditions and potential risks to any water body, which is an essential step in the development of efficient decision support tools for environmental managers. This study was carried out to investigate the possibility of using blood metal concentrations of two fish species *Oreochromis niloticus niloticus* and *Clarias gariepinus* as biomarkers of metal pollution, for the first time, to evaluate the health of the River Nile environment. Water, sediment and fish samples were collected seasonally from eighteen different sampling points, representing six different sites (three points from each site) along the whole course of the River Nile in Egypt. The present result concluded higher mean concentrations of nearly all the detected heavy metals in water and sediment samples collected from sampling sites downstream River Nile (polluted sites) compared to those collected from upstream river. The mean concentrations of all the detected metals were significantly ($P<0.05$) higher in the blood of fish collected from the polluted sites. Pb and Cd in blood serum collected from *O. niloticus niloticus* were significantly correlated ($P<0.05$) with corresponding levels in water and sediment samples collected from same sites. Likewise, Pb in blood serum collected from *Clarias gariepinus* was significantly correlated ($P<0.05$) with corresponding Pb in water and sediment samples collected from same sites, while Cr and Zn were significantly correlated only with sediment collected from same study sites. The results revealed species specific different sensitivities, suggesting that Nile tilapia may serve as a more sensitive test species compared to the African catfish. These results indicate that the blood metal concentrations of the selected species are adequate biomarkers of metal pollution and could be included in monitoring programmes to indicate the response of such animals to metal pollution.

**Keywords:** Biomonitoring, Blood Metal, Sediment, water

## 1. Introduction

The contamination of freshwater with a wide range of pollutants has become a matter of great concern over the last few decades; not only because of the threats to public water supplies, but also due to the damage they cause to the aquatic life [1].

Metals are among the most common environmental pollutants; their accumulation and distribution in soil and water are increasing to an alarming rate, causing deposition and sedimentation in water reservoirs and affecting the aquatic organisms as well [2, 3]. Metals like chromium, lead, cadmium and tin exhibit extreme toxicity even at trace levels. Rivers are a dominant pathway for metals transport [4], and metals become significant pollutants of many riverine systems [5]. The behaviour of metals in natural waters is a function of the substrate sediment composition, the suspended sediment composition, and the water chemistry. During their transport, the metals undergo numerous changes in their speciation due to dissolution, precipitation, sorption and complexation phenomena [6] which affect their behaviour and bioavailability [7]. Hence, metals are sensitive indicators for monitoring changes in the water environment. However, to assess the environmental impact of contaminated sediments, information on the total concentrations is not sufficient, and particular interest is the fraction of the total metal content that may take part in further biological processes [8].

The River Nile is the principal fresh water resource for Egypt, meeting nearly all demands for drinking water, irrigation, and industry [9, 10]. Fishing, aquaculture, and navigation are in stream uses of the River Nile water. Approximately 99 percent of the population of Egypt lives within the Nile Valley and Delta, which constitutes less than 4 percent of Egypt's total area. Any disruption or impairment to these from natural or anthropogenic threats cannot be without far reaching economic and social implications. For these reasons, continuous monitoring for quality parameters is necessary. Even with the presence of relevant legislation, the Nile River receives numerous non-point and point source discharges during its transit through Egypt [11].

Biomonitoring is the use of biological response to assess changes in the environment [12]. Biomonitoring is a valuable assessment tool that is receiving increased use in water quality monitoring programs of all types [13]. Biomonitoring measures pre-selected endpoints (biomarkers) that reflect ecosystem health, of which the results can be interpreted to answer specific questions [14]. A combination of biological monitoring (biomonitoring) and measurements of water and sediment quality can provide a good indication of conditions and potential risks to the water body.

Fish provide suitable models for monitoring aquatic toxicity and wastewater quality because of their ability to metabolize xenobiotics and bio-accumulate pollutants [15, 16]. Fish are often used as bio-indicators because they play a number of roles in the trophic web, bio-accumulate toxic substances, and respond to low concentrations of contaminants [15-17]. Fish are widely used to evaluate the health of aquatic ecosystems, and their physiological changes serve as biomarkers of the environmental pollution [18].

A commonly used fish species in environmental studies are the Nile tilapia and African catfish. The Nile tilapia, *Oreochromis niloticus niloticus*, is one of the most common freshwater fish used in toxicological studies [19]. It is one of the aquatic organisms affected by heavy metals; so in many cases, *Oreochromis niloticus niloticus* was used as a metal biological marker in toxicological studies in which it was substantiated with the highest sensitivity to toxic effect [20]. *Oreochromis niloticus niloticus* presents a number of characteristics that may make it an appropriate model that can be used as an indicator species in biomonitoring programmers [21]. The Nile tilapia is a native fish species of Egypt that has become a popular species worldwide; mainly as a valuable fish, easy to breed and grow in a variety of aquaculture systems [22]. The African catfish (*Clarias gariepinus*) is distributed throughout Africa [23]. It is a ubiquitous benthic catfish distributed throughout the great River Nile [9]. It is one of the most important tropical cultured fish due to its high growth rate, high stocking-density capacities, high consumer acceptability and high resistance to poor water quality and oxygen depletion [24]. Moreover, it has been used in fundamental research and has been considered as an excellent model for toxicological studies [25] since it has a well-documented biology [26].

The aim of this study is to investigate the possibility of using blood metal concentrations of two fish species, *Oreochromis niloticus niloticus* and *Clarias gariepinus,* as biomarkers of metal pollution as a new approach to evaluate the health of the River Nile environment.

# 2. Materials and Methods

### *2.1. Study Area*

The Nile is the longest river of the world, stretching north for approximately 4,000 miles from East Africa to the Mediterranean Sea. While the River Nile is often associated with Egypt, it actually flows through Ethiopia, Zaire, Kenya, Uganda, Tanzania, Rwanda, Burundi and Sudan, as well as Egypt. It flows 1,300 kilometres in Egypt before it empties into the Mediterranean Sea near Alexandria. At a distance of 25 km north of Cairo, the River Nile is divided into two branches (Rosetta and Damietta), forming a delta resting with its base on the Mediterranean Sea shore.

Eighteen different sampling points, representing six sites (three points from each site), were selected along the whole course of the River Nile in Egypt from Aswan, in the south, to its estuaries at Rosetta and Damietta branches, in the north (Fig. 1).

Water, sediment and fish samples were collected seasonally (autumn, winter, spring and summer) from November 2009 to October 2010 from the selected six sites (Aswan, Qena, Assuit, Bany-Suief and Damietta and Rosetta branches).

### *2.2. Water Sampling and Analysis*

Water samples were collected by polyvinyl chloride Van Dorn bottle at two meter depth from the selected sites along the main course of the River Nile. Water samples were kept into a one-litre polyethylene bottle in an ice box till analysed in the laboratory.

A mixture of nitric acid and the material to be analysed 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 sample was filtered, allowed to settle and prepared for analysis according to

APHA (2005). The total cadmium (Cd), chromium (Cr), iron (Fe), manganese (Mn), lead (Pb) and zinc (Zn) were measured after digestion using Graphite Furnace Atomic Absorption, (Model: AA-6800), at water quality laboratory (an ISO 17025 certificated lab), Egyptian Environmental Affairs Agency. The correlation coefficients between the quality parameter pairs of the river water samples were calculated in order to indicate the nature and sources of the polluting substances.

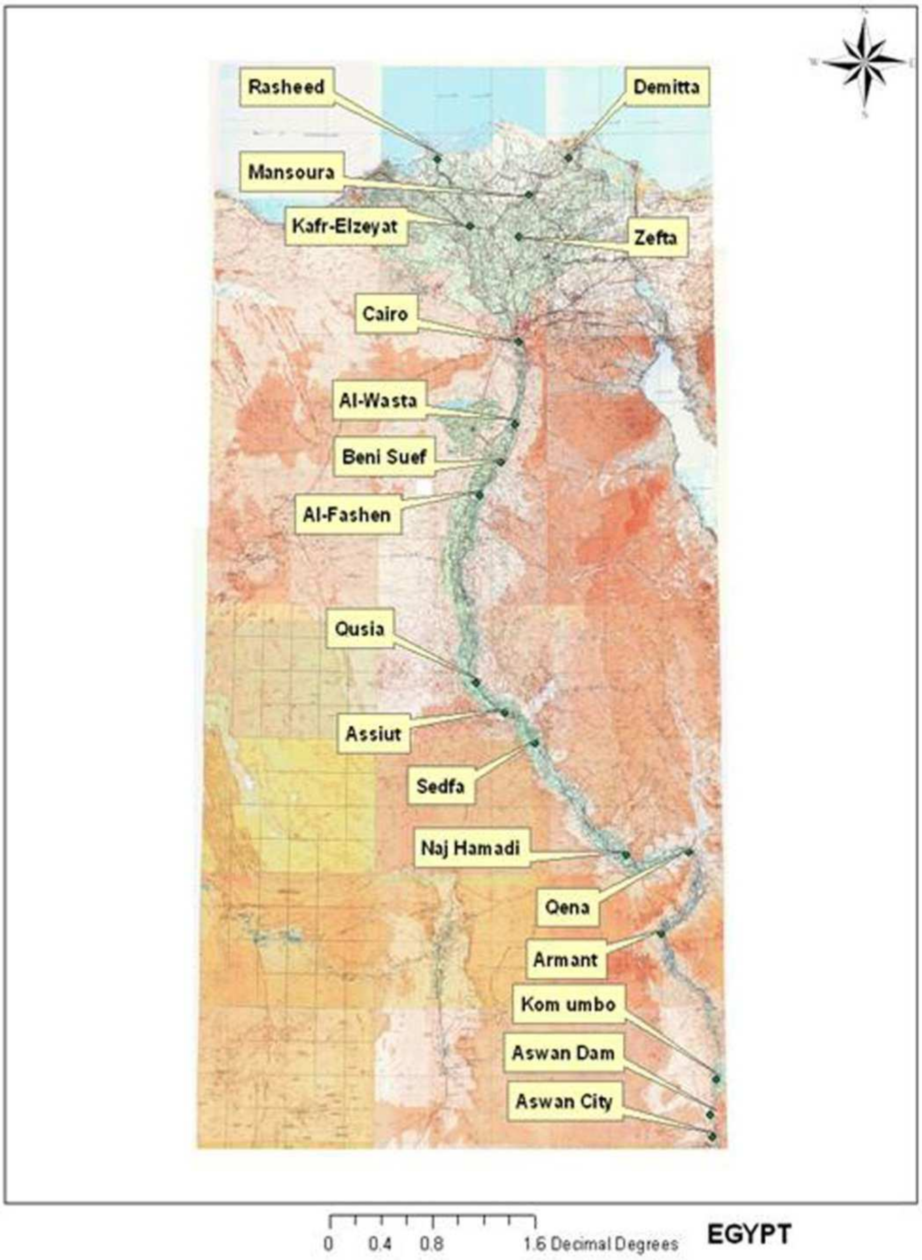


*Fig. 1. Map showing the sampling sites along the whole course of the Egyptian River Nile from its start at Aswan to its estuaries at Damietta and Rosetta sites..*

### *2.3. Sediment Sampling and Analysis*

Sediment samples were collected from the selected sites along the main course of the River Nile by using Ekman dredge and kept frozen until analysed [27]. For total metals, sediment samples were allowed to defrost, then air-dried in a circulating oven at 30°C and sieved mechanically using a 2 mm sieve. For the digestion of samples, one gram from sieved sediment was digested with repeated addition of nitric acid and hydrogen peroxide according to Jackwerth and Würfels [28] method to complete dissolve all elements present in the sediments. The resultant digestate was reduced in volume and then diluted to a final volume of 100 mL. The elements of concern (Cd, Cr, Fe, Mn, Pb and Zn) were determined by Atomic absorption spectrophotometer (AAS), (Model: AA-6800), at water quality laboratory, Egyptian Environmental Affairs Agency.

### *2.4. Fish Specimens Collection*

420 fish samples (35 samples per site per species) were

collected using long line or net. The *Oreochromis niloticus niloticus* total length ranged between 15 and 22 cm (18.5±2.5), while that of *Clarias gariepinus* ranged between 35 and 50 cm (43.5±3.2).

### *2.5. Blood Sampling and Analysis*

Blood samples were withdrawn from caudal puncture [29]. No anesthetic was applied to fish, as it may affect blood parameters and hemolysis tissues [30]. Blood serum is obtained by centrifuging the whole blood for 15 min and is stored in plastic tubes at -20°Cfor metals analysis.

The Cd, Cr, Fe, Mn, Pb, and Zn in serum were measured after digestion using Graphite Furnace AA (GFAA) spectroscopy [31].

### *2.6. Statistical Analysis*

All results were presented as means ± the standard deviation of the mean. Data obtained were analysed by a one-way analysis of variance (ANOVA) using statistica8© (data analysis software system) and figured by Microsoft Excel©. Means were tested using the Fisher Least Significant Difference (LSD) test. The correlation between metals in blood serum, water and sediment samples were analysed by a correlation matrix test using statistica8©.

## 3. Results

### *3.1. Metals Concentrations in Water*

The total trace metals exhibited a number of behaviour along the Nile course. Pb values fluctuated within a narrow range (0.016 to 0.068 ppm). The highest value was recorded at Rosetta branch (0.068 ppm) which is higher than the permissible limit (0.05 ppm) according to the Egyptian law No 48 of 1982 (Fig. 2). Cd exhibited a wide range of variation between 0.0029 and 0.016 ppm. Downstream sites (Rosetta and Damietta branches) had a very high Cd concentration (higher than the permissible limit (0.01)) compared to the upstream sites (Aswan and Qena). Zinc concentrations fluctuated between 0.084 and 0.79 ppm, where the maximum values were recorded at Rosetta branch (Fig. 2). Zn level was considerably elevated at Damietta and Rosetta branches. The concentrations of Cr, Mn and Fe were notably higher in the water sampled from Rosetta branch sites compared to the upstream ones. The lowest Cr concentration was recorded at Aswan (0.0078 ppm) while the highest was recorded at Rosetta (0.061 ppm) where it was higher than the permissible limit (0.05) (Fig. 2). Mn concentrations fluctuated between 0.029 ppm and 0.14 ppm. They showed a slight increase from upstream to downstream sites. The maximum value was recorded at the Rosetta branch (0.14 ppm). Fe fluctuated between rise and fall; the highest value was at Rosetta branch (0.91 ppm) while the lowest was at Aswan (0.31 ppm). The recorded level of Fe, Zn and Mn were lower than the permissible limits according to the Egyptian Law No 48 of 1982 in all the sites (Fig 2).

### *3.2. Metals Concentrations in Sediment*

The concentrations of Pb exhibited a wide range of variation ranging from 3.57 to 20.4 mg/kg. Pb was very low in the sediment of Assuit and Aswan when compared to its concentrations in the sediment of Damietta and Rosetta (Fig.3). Cd exhibited a narrow range of variation ranging between 0.45 and 0.85 mg/kg. The lowest concentration was recorded at Aswan while the highest one was recorded at Damietta. In general, the concentrations of Cd fluctuated between sites, but they still showed a slightly increase from Aswan toward Damietta and Rosetta. The level of Cd was higher than the permissible limit nearly in all sites, except in Aswan and Qena (Fig. 3). Cr exhibited a wide range of variation ranging from 5.6 to 15.1 mg/kg. The highest concentrations were recorded at Rosetta and Damietta branches while the lowest were at Qena. The Cr level was lower than the permissible in the sediment of all sites (Fig. 3). Mn and Zn concentrations represented the second and third highest metals in the sediment after iron, respectively. Such concentrations ranged between 225.6 and 379 mg/kg for Mn and from 114.2 to 323.9 mg/kg for Zn. The level of Zn was higher than the permissible limit in the sediment of Bany-Suief, Damietta and Rosetta branches (Fig. 3). Fe showed higher values at all sites compared to other metals, ranging from 407.4 to 702.1 mg/kg (Fig. 3), indicating that this metal is naturally high in the sediments.

### *3.3. Metals Concentrations in Blood Serum*

The mean values of the Pb, Cd, Cr, Zn, Fe and Mn in blood serum of *Oreochromis niloticus niloticus* and *Clarias gariepinus* are shown in (Fig. 4). The mean concentrations of all the detected metals were significantly ($P<0.05$) higher in the blood of fish collected from down river Nile (Rosetta and Damietta) compared to those sampled from upstream areas. Nearly for all the detected metals, the mean concentrations in the blood of the African catfish was higher than that in the blood of Nile tilapia sampled from the same site and at the same sampling time (Fig. 4).

The correlations between metals concentrations in blood serum collected from *Oreochromis niloticus niloticus* and metals concentrations in water and sediment are shown in (Figure 5). Pb and Cd concentrations in blood serum were significantly correlated ($P<0.05$) to the corresponding Pb and Cd concentrations in water and sediment samples collected from same sites (Fig. 5).

The correlations between metals concentrations in blood serum collected from *Clarias gariepinus* and metals concentrations in water and sediment are shown in (figure 6). Pb in blood serum was significantly correlated ($P<0.05$) to the corresponding Pb in water and sediment samples collected from same sites, while Cr and Zn concentrations were significantly correlated only with that of sediment collected from same sites (Fig. 6).

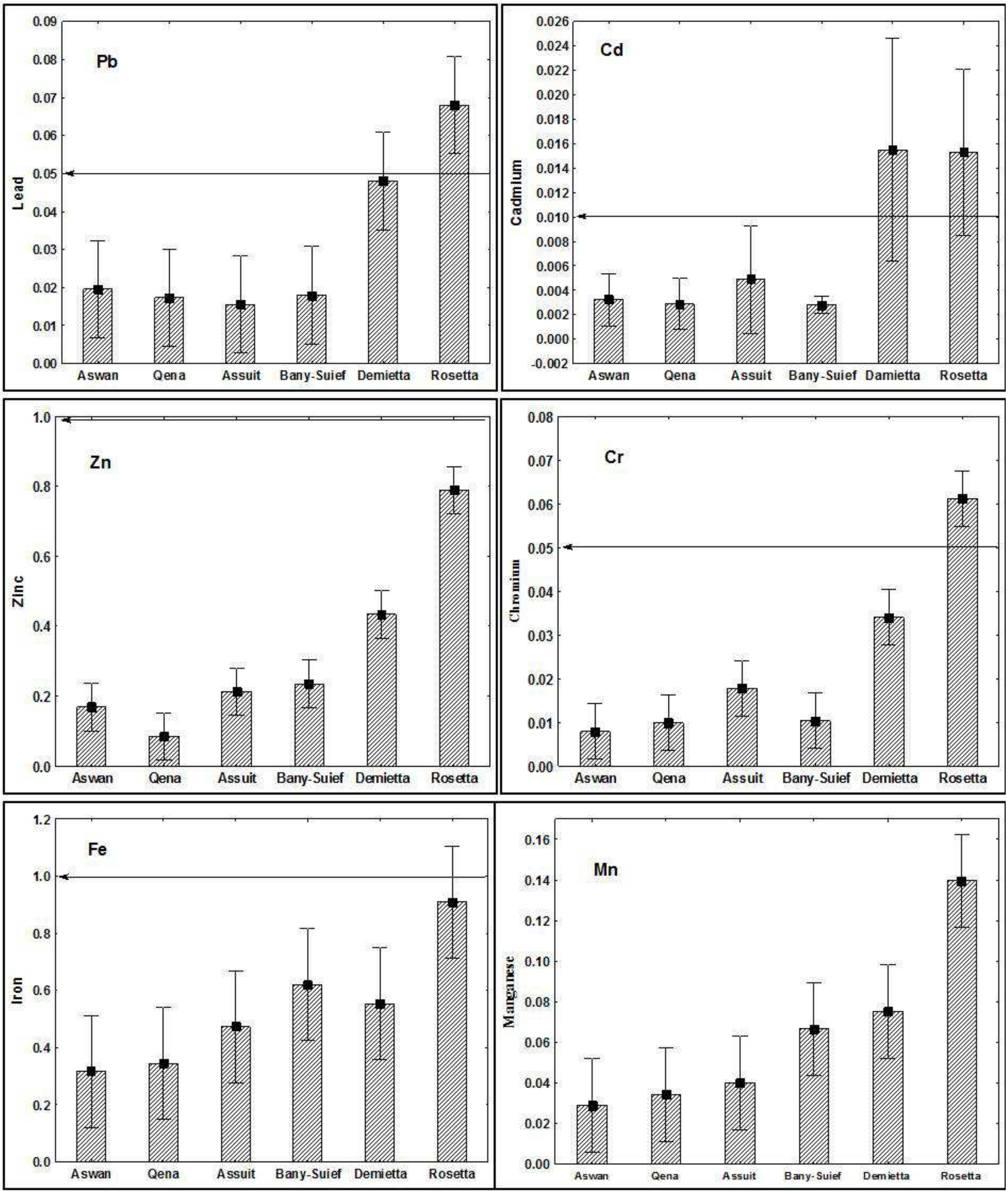


***Fig. 2.*** *Metals concentrations (ppm) (mean ± SD) of the water collected from different sites along the river Nile, Egypt, (____) Permissible limit according to the Egyptian Law No. 48 of 1982 on the Protection of the River Nile and waterways from pollution.*

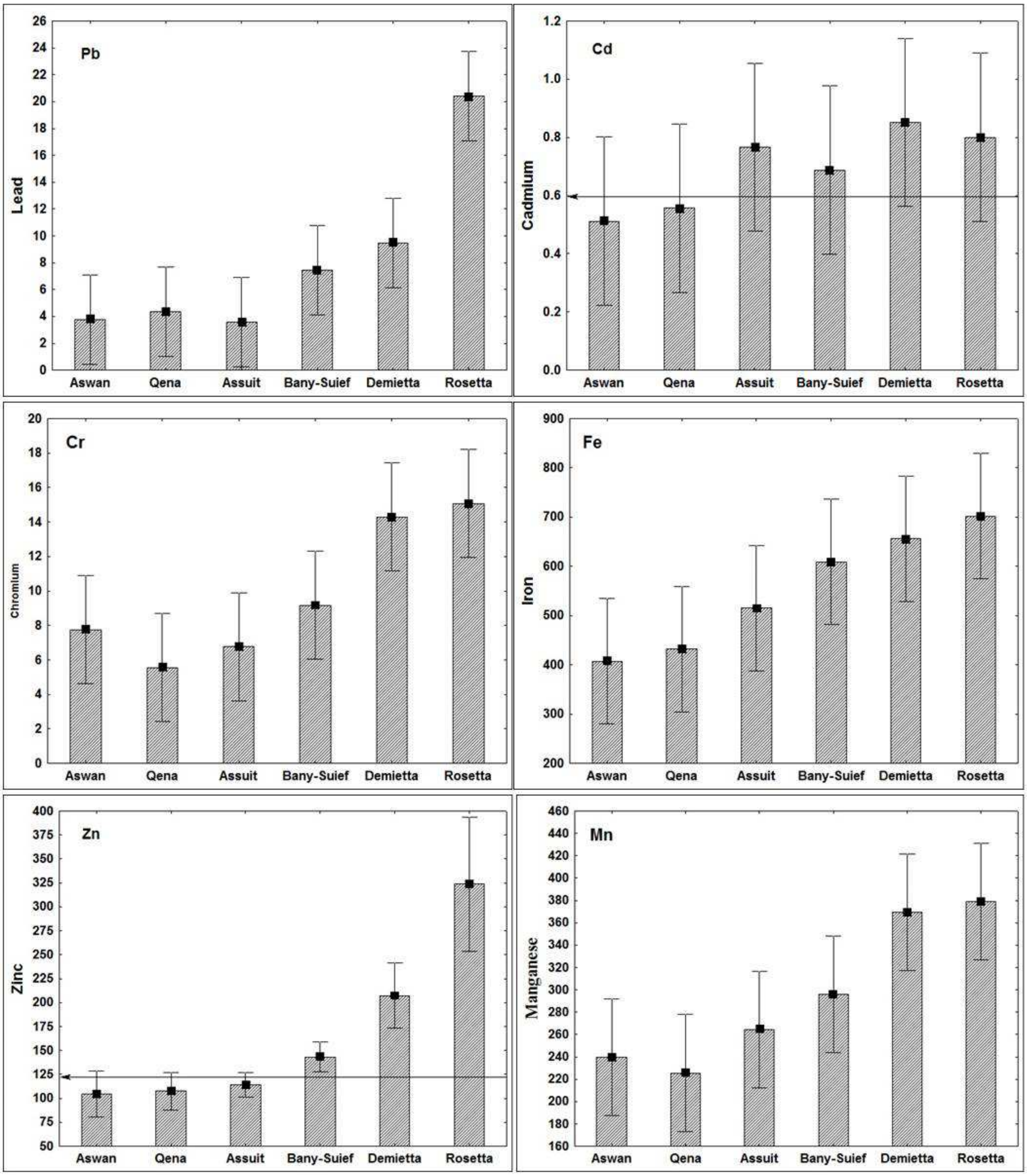


***Fig. 3.*** *Metals concentrations (mg/Kg) (mean ± SD) of the sediment collected from different sites along the River Nile, Egypt, (__) Permissible limit according to Canadian standard.*

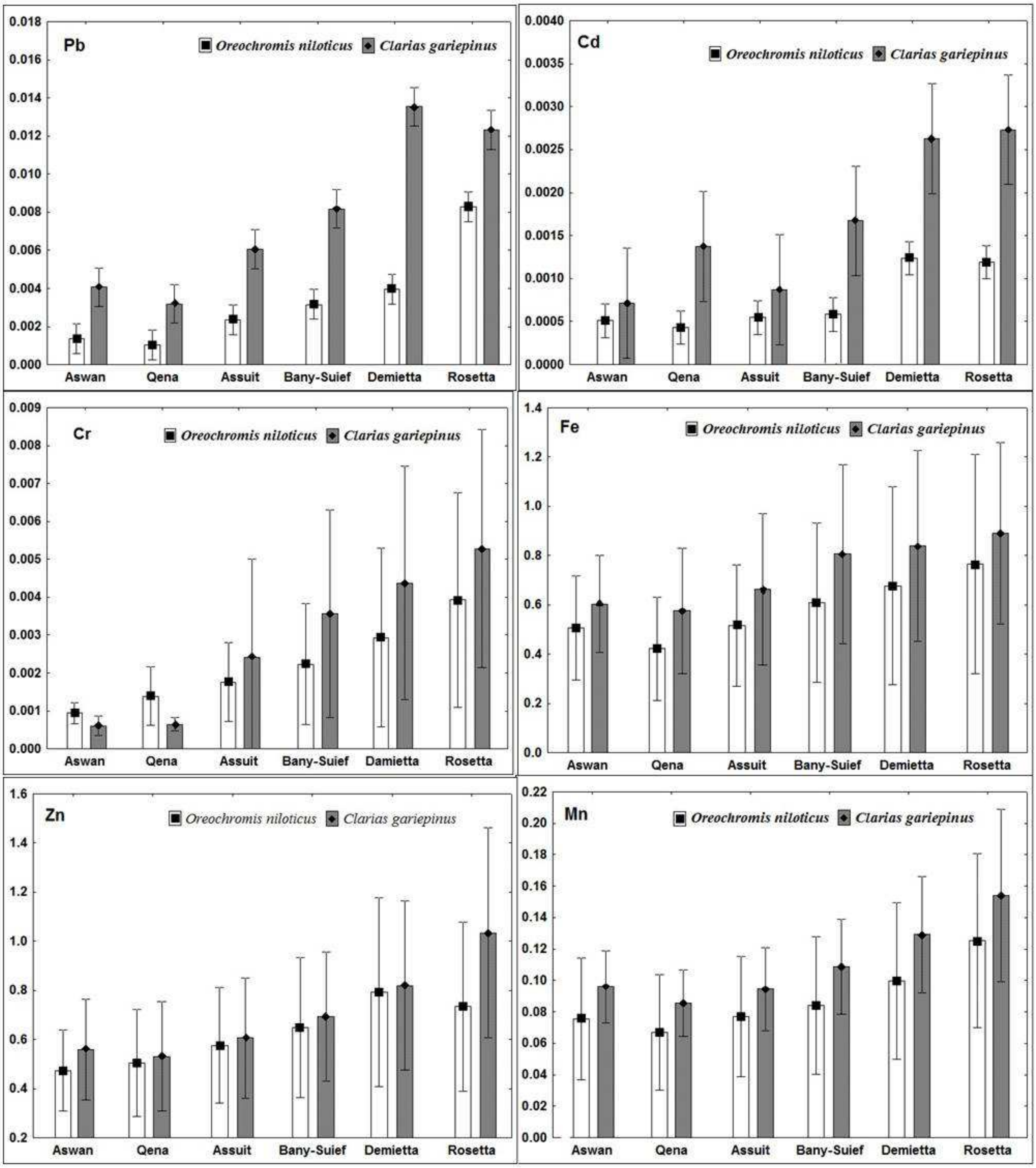


***Fig. 4.*** *Blood metals concentrations (ppm) (mean ± SD) of the Oreochromis niloticus niloticus and Clarias gariepinus collected from different sites along the River Nile, Egypt.*

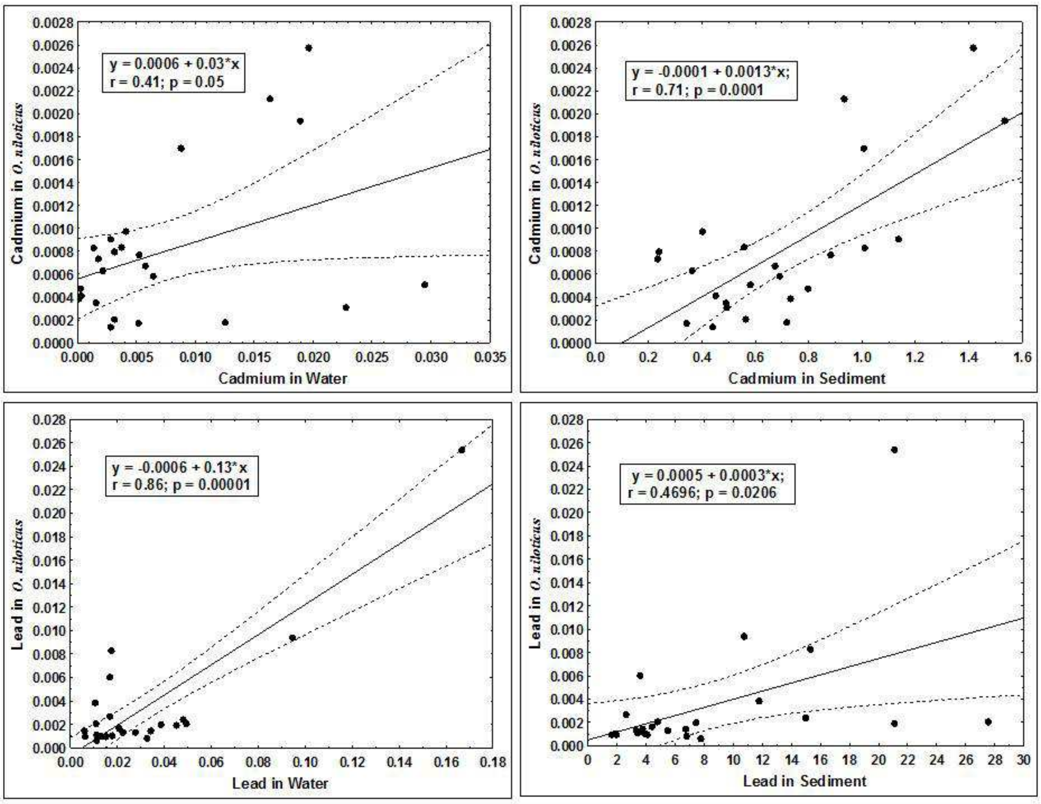


***Fig. 5.*** *Correlations between Cd and Pb in blood serum collected from Oreochromis niloticus niloticus and that in water and sediment collected from different sites along of the River Nile, Egypt.*

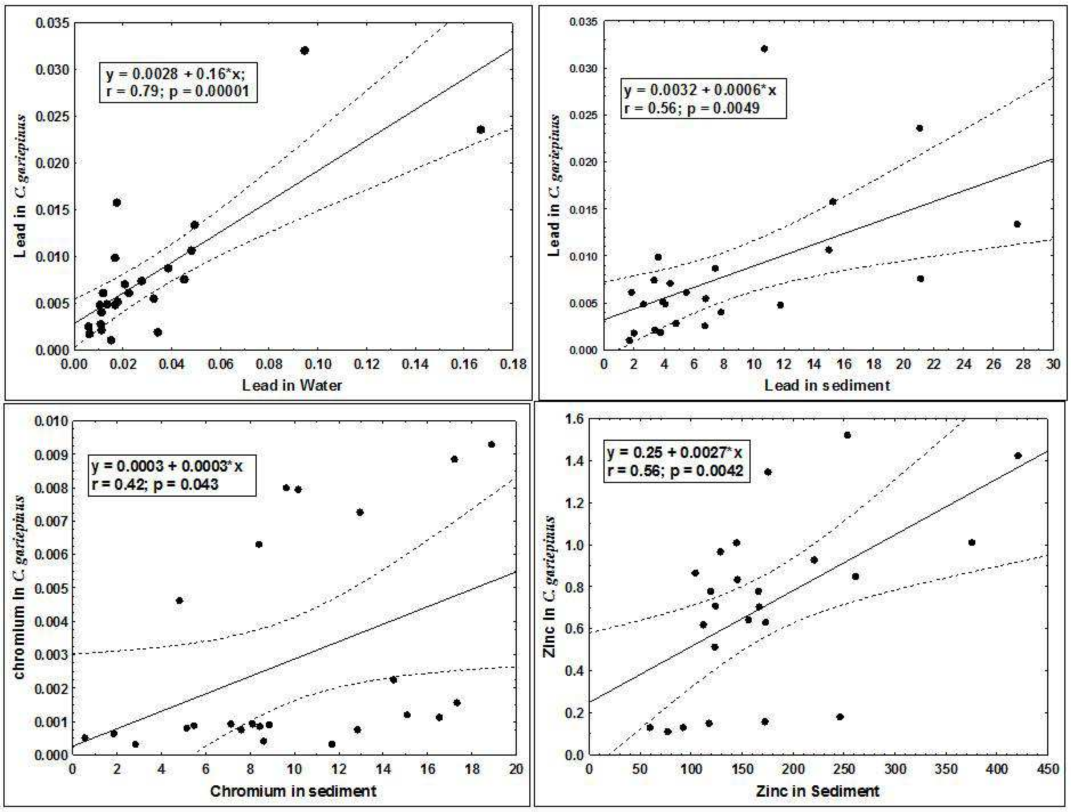


***Fig. 6.*** *correlations between Pb, Cr and Zn in serum blood collected from Clarias gariepinus and, Pb in water and Pb, Cr and Zn in sediment collected from different sites along of the River Nile, Egypt.*

# 4. Discussion

According to this study results, the water quality variables of the River Nile at upstream areas were better than those at downstream River Nile. The detailed investigation [32] of water quality assessment along the whole course of the River Nile concluded higher mean values of nearly all the detected physicochemical parameters in water collected from sampling sites downstream River Nile compared to those collected from upstream River Nile. The same results were also obtained by EEAA [33-36], Elewa and Goher [37] for the same sites. The better water quality at upstream River Nile was due to the limitation in the pollution sources. The sources of pollution along the Nile in Upper Egypt areas are mainly agro-industrial. Sugar cane industries significantly influence the Nile water quality at Upper Egypt-South zone, while hydrogenated oil and onion drying factories influence the Nile water quality at Upper Egypt-North zone [14].

Lower water quality at downstream River Nile was due to the discharge of a number of agricultural drains, which are heavily polluted with industrial and domestic sewage. These sewage and domestic wastes discharge directly without treatment into Rosetta branch. The drains receive large parts of the wastewater of Cairo. The Rosetta branch is impacted by several industrial activities which discharge directly at the east bank of the branch. It was estimated that the aquatic environment of Rosetta branch receives more than 3 million cubic meters daily of untreated or partially treated domestic and industrial wastes in addition to agricultural drainage water. Along Damietta branch, there are Talkha fertilizer plant, Kafer Saad Electric Power Station, Delta Milk, Edfina Factories, besides the sewage and domestic wastes discharging from the neighboring villages (El- Serw and Ras El-Bar cities) without any treatment into the Damietta branch. The Fertilizer Company is considered as the major point source of industrial pollution at Damietta branch. The Damietta Branch also receives polluted water of a number of agricultural drains [14].

Metals are usually divided into two subclasses: Cu, Fe, Mn, and Zn, which are essential for the correct functioning of biochemical processes; while Cd, Cr, and Pb have no established biological function and represent the most important contaminants in the aquatic environment [38]. The primary source of metals in irrigation and drainage canals is the discharge of domestic wastewaters, which contain high concentrations of metals such as copper, iron, lead, and zinc, which are derived from household products such as cleaning materials, toothpaste, cosmetics, and human faeces [38].

The result of the present work concluded higher mean concentrations of nearly all the detected heavy metals in water and sediment samples collected from sampling sites downstream River Nile compared to those collected from upstream river Nile. The lead and chromium concentration were higher than the permissible limit in the water of Rosetta Branch. Cadmium and mercury concentrations were higher than the permissible limit in the water of Rosetta and Damietta Branches. The increase in Pb concentrations in the Nile water may be due to the direct inputs from different sources (industrial wastes and atmospheric inflow of dust containing car exhaust) [39]. Concentration of Cd in the River Nile depends on the quality of sewage discharge, agricultural discharges, domestic wastes, and industrial discharges inflow to the river [40]. The high concentrations of the studied elements may be attributed to the effect of intrusion of water borne Fe, Mg, Zn, Pb and Cd coming from agricultural, domestic and industrial effluents, and the small grain size of the sediment facilitates the adsorption of these metals to bottom sediments.

Sediment contamination poses one of the worst environmental problems in ecosystems, acting as sinks and sources of contaminants in aquatic systems. Fine grain particles in sediment usually act as effective collectors and carriers of dissolved metals from the water column to the sediments, and thus elevate the concentration of heavy metals in sediment. Their analysis plays an important role in assessing the pollution status of the environment [41] and in the interpretation of water quality [42]. They reflect the current quality of the water system and can be used to detect the presence of contaminant that does not remain soluble after discharge into water. The sediments are also used for determining the presence of heavy metals when the concentrations in water are undetectable with present analyzing methods [43]. The concentrations of the selected heavy metals, in the present work, in sediments samples were very high in all the sites compared to the concentration of the same heavy metals in water samples collected from the same sites and at the same sampling time. Higher levels of nearly all the selected heavy metals were recorded in downstream River Nile compared to upstream areas. Such increase proves the presence of large quantities of organic and inorganic pollutants in Rosetta and Damietta water. This finding was expected due to the fact that the level of contamination is greater downstream River Nile, compared to upstream sampling sites, where more domestic and industrial effluents have been released into the Nile without adequate treatment. Domestic and industrial effluents are the major sources of the observed higher level of Pb. Zn, Co, [44]. The lead concentration was higher than the permissible limit in the sediment collected from Rosetta Branch. The level of Cadmium was higher than the permissible limit in all the sites, except in the sediment of Aswan and Qena. The concentration of Zinc was higher than the permissible limits in Rosetta and Damietta branches and in Bany-Suef. Generally, the element mobilization in the sediment environment is dependent on physicochemical changes in the water at the sediment–water interface. The precipitation of heavy metal elements in the form of insoluble hydroxides, oxides and carbonates might be the result of alkaline pH. The given elements, such as Cr, Cu and Co, have interacted with the organic matter in the aqueous phase and settled resulting in a high concentration of these elements in the sediment [45].

Heavy metals play important roles in many biochemical reactions and in the life processes of living organisms. Due to the ability of heavy metals to form complex compounds [46], some metals (e.g. Ca, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni and Zn) are essential; they serve as micronutrients and are used for redox processes to stabilize molecules through electrostatic interactions and regulate the osmotic pressure [47]. Many other metals have no biological role, (e.g. Ag, Al, Cd, Pb, and Hg), and are nonessential [47] and potentially toxic to living organisms. The toxicity of nonessential metals occurs through the displacement of essential metals from their native binding sites, or through ligand interactions alterations in the conformational structure of nucleic acids and proteins, or the interference with oxidative phosphorylation and osmotic balance [47]. In the present study, the concentrations of the selected heavy metals (Pb, Cd, Cr, Zn, Mn, and Fe) increased significantly in the blood serum of *Oreochromis niloticus niloticus* and *Clarias gariepinus* from upstream to downstream River Nile. Metals concentrations in blood serum and that in water and sediment were significantly correlated ($P<0.05$) in case of Pb and Cd in *Oreochromis niloticus* and Pb in *Clarias gariepinus*, while Cr and Zn concentrations in blood serum of *Clarias gariepinus* were significantly correlated to that of the sediment. Higher levels of these metals in the serum samples might indicate that fish have been recently exposed to these metals and that an acute response occurred. Effectively, after the absorption, metals in fish are then transported through bloodstream to the organs and tissues where they are accumulated [48].

Higher levels of both, essential and nonessential metals, can damage cell membranes, alter enzyme specificity, disrupt cellular functions, bind with greater affinity to thiol-containing groups and oxygen sites than the essential metals do, and damage the structure of DNA [49]. Previous studies have shown that certain metals can cause either increased or decreasing levels of serum protein, cortisol, glucose, and cholesterol, as well as changes in serum enzymes activities [50]. In toxicological studies of acute exposure, changes in activities of some enzymes may reflect cell damage in specific organs [51]. The activity of transaminases in fishes may be significantly changed under the influence of different toxic agents. Some metals, such as zinc, copper and cadmium significantly increase the activity of serum transaminases in some fresh water fishes [52]. Oxidative stress caused by different metals may damage certain tissues and liberate various transaminases into the plasma [53]. Thus, it was argued that several biochemical parameters in fish blood could be used as an indicator of heavy metal toxicity [54].

The selection of fish species is a critical issue in biomonitoring studies. Nile tilapia avoid long-term exposure to metal pollution at a particular site by actively swimming, while African catfish have a more stationary behaviour and thus may be exposed to metal pollution for a longer time. This could explain the recorded lower levels of all the detected heavy metals in the blood of Nile tilapia than that in the blood of African catfish collected from the same sites and at the same sampling time, proving that Nile tilapia is more sensitive bio-indicator for genotoxicity. The difference in sensitivity of Nile tilapia and African catfish to metals bio-accumulation might be attributed to their feeding peculiarities [9]. These findings could explain that the African catfish are more abundant in many polluted sites along the river Nile compared with the Nile tilapia.

In conclusion, the present work reckoned higher mean concentrations of nearly all considered metals in water and sediment samples collected from sampling sites downstream River Nile compared to those collected from upstream, where more domestic and industrial effluents have been released into the Nile without adequate treatment. The mean concentrations of all the detected metals were significantly ($P<0.05$) higher in the blood of fish collected from downstream River Nile (Rosetta and Damietta) compared to those sam

led from upstream areas. These results indicate that blood metal concentrations of selected species are adequate biomarkers of metal pollution and could be included in monitoring programmes to indicate the response of such animals to metal pollution.

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