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# Water pollution evaluation in Aswan, Egypt, utilizing biochemical and molecular indicators in aquatic Chironomidae larvae in natural and laboratory settings

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**ABSTRACT**

Heavy metal pollution has a harmful impact on the health of all living beings. The goal of this study was to compare the impacts of zinc (Zn), lead (Pb), iron (Fe), and manganese (Mn) on two Egyptian streams in Aswan Governorate. In addition, aquatic Chironomidae larvae were exposed to a variety of heavy metal concentrations for two days. The mortality rate was determined, and the median lethal concentrations (LC50) were estimated. The most harmful metal for Chironomidae larvae was Pb, followed by Zn, Fe, and Mn. Metal bioconcentration in Chironomidae larvae increases with increasing concentrations of metals. The enzymatic response to heavy metals uptake in Chironomidae larvae; namely, lipid peroxidation, superoxide dismutase, catalase, glutathione-S-transferase, and glutathione peroxidase were determined. Although the Pb-treated group recorded the highest lipid peroxidation, it showed the lowest glutathione peroxidase activity. The glutathione-S-transferase enzyme was less active in the Zn-treated group, as was the superoxide dismutase enzyme, and the catalase enzyme was the least active in the Zn-treated group. Changes in randomly amplified polymorphic DNA (RAPD) profiles were detected following heavy metal treatments at various doses when compared to controls. This study gives insight into how cruise ship activities and industrial pollution in rivers endanger aquatic life and hence, human life.

## INTRODUCTION

Water is the primary component of all species' chemistry. Freshwater environments are critical; since industrial waste contains a significant amount of heavy metals, freshwater conservation is a critical environmental concern **(Ho *et al.*, 2012)**. Though industrial water pollution accounts for around one-fourth of all water pollution sources, it is certainly the most hazardous **(Desai SmtVanitaben, 2014)**.

Water contamination has a direct and indirect effect on the health of all species. For example, pollution modifies the structure and function of biological systems at every organizational level, from the molecule to the community **(Wokoma, 2019; Dalzochio *et al.*, 2022)**. These modifications include behavioral, respiratory, and reproductive changes, as well as histological, biochemical, and haematological changes, in addition to

inhibition, mortality, euthrophication and turbidity **(Abdel-Gawad & Mola, 2014)**. Macroinvertebrate communities are critical for a range of biological processes in lakes and rivers due to their role in material cycling and secondary production, besides their vulnerability to environmental contaminants **(McCall & Soster, 1990; Shuhaimi-Othman *et al.*, 2012)**. The benthos degrades organic waste stored in sedimentary reservoirs into dissolved nutrients. Such nutrients can be absorbed into the surface waters **(Abdel-Gawad & Mola, 2014)**. As a result of their sediment mixing, benthic invertebrates can have a direct or indirect effect on microbial production and release of greenhouse gases, carbon dioxide and methane, poisonous gases Hydrogen sulphide, ammonium, and nitrogen **(Covich *et al.*, 1999)**.

Certain species of aquatic insects such as the Chironomidae serve as indicators of low water quality and are resistant to contamination **(Fishar & Abdel Gawad, 2009; Ahmad *et al.*, 2015)**. Aquatic insects are the most frequently used creatures in freshwater biomonitoring for human influence because they are sensitive to environmental changes **(Bonada *et al.*, 2006; Corbi *et al.*, 2013; Strayer, 2013; Ceneniva-Bastos *et al.*, 2017)**. For many years, biomarkers have been used as surrogate measures of biological effects in laboratory and field investigations since their usage is a key strategy to evaluate ecosystem health **(McCarty *et al.*, 2002; Dalzochio *et al.*, 2016)**. Bioavailable heavy metals constitute a fraction of the overall ambient metal load that is directly ecotoxicologically significant due to their ability to influence biochemical and physiological processes in organisms **(Gall, 2010; Mohammad *et al.*, 2022)**.

Many pollutants produce oxidative stress. Pro-oxidants (including external stressors like pollution) and antioxidants can be utilized to assess environmental stress. The sensitive indicators of oxidative stress are antioxidant enzymes, especially in aquatic organisms **(Valavanidis *et al.*, 2006)**. Oxidative stress may rise in cells because of heavy metal toxicity at a biochemical level. This can lead to the inactivation of lipid peroxidation enzymes and DNA damage **(Gichner 2003; Sun *et al.*, 2007)**.

The change in the RAPD (random amplified polymorphic DNA) profile following genotoxic exposure is readily identifiable by the variation in the strength of the bands as well as the gain or loss of bands. These changes in the RAPD reaction patterns of the bands may be attributable to DNA damage caused by genotoxins, such as structural alterations or rearrangements **(Atienzar *et al.*, 2002)**. The RAPD method is very useful for ecotoxicological studies because it is simple, sensitive, cheap, and effective at detecting DNA damage **(De Wolf *et al.*, 2004; Pal 2016)**.

In Egypt, the Nile River is used for many purposes, including agriculture, industry, hydropower, fishing and recreation **(Bream *et al.*, 2017)**. Heavy metal poisoning of the soil and water, particularly of lead and cadmium, has become a severe problem in Egypt and all over the world and is currently the subject of lively discussion **(El-Komi *et al.*, 2002)**.

The aim of this study was to determine the extent of water pollution in selected Egyptian streams in Aswan Governorate, based on the accumulation of heavy metals in the water and sediment, as well as the accumulation of heavy metals in aquatic Chironomidae larvae, and estimate their biological responses. The study used both natural and laboratory settings to assess the species' ability to uptake and tolerate heavy metals by integrating biochemical and molecular indicators.

## MATERIALS AND METHODS

### Collection sites

Samples were collected from two locations along the Nile in Aswan (Fig. 1). One of these locations was designated as reference site 1 (next to the Cataract hotel), While the second site is on the west side of Aswan City, next to various cruise ship ports and opposite to the Egyptian Chemical Industries Company's (Kima), where the industrial drainage outlet is found in the Nile.

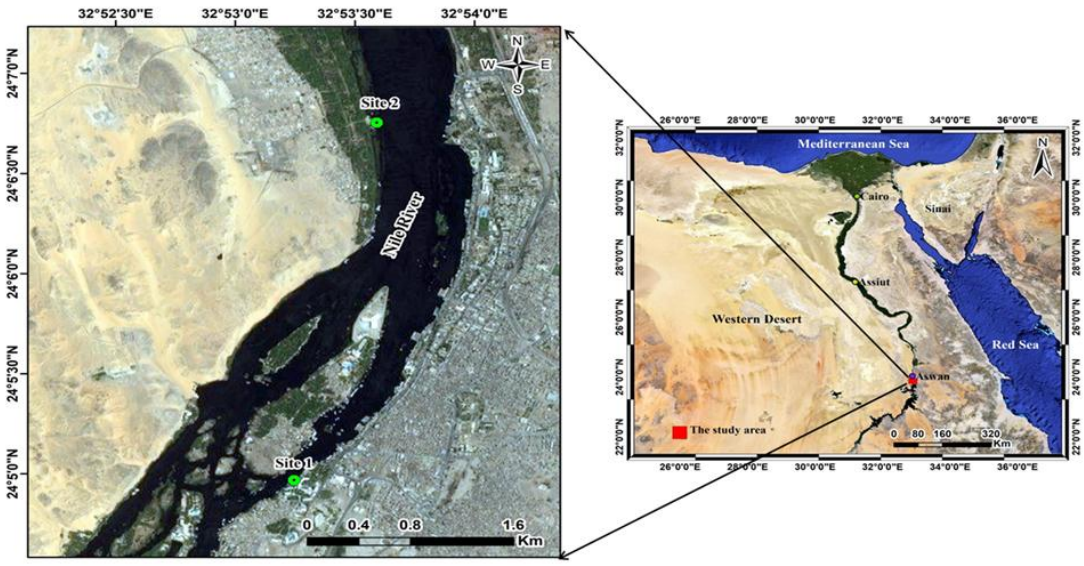


**Fig. 1. A map showing the sampling sites; site 1 (next to the Cataract hotel) and site 2 (west Aswan)**

Sampling from the sediment was manually carried out. Both water and sediment samples were carefully transferred in appropriate containers (plastic bottles). The Chironomidae larvae were collected using hairbrushes and forceps. In the laboratory, samples were tagged and then frozen at -80$^{o}$C. The RAPD-PCR samples were stored in 70% alcohol at -20$^{o}$C.

### Analysis of heavy metals

Water samples were digested with 37% hydrochloric acid and 65% nitric acid on a thermostatic hot plate (Merck). Then, the residual solution was transferred to a 100ml volumetric flask, diluted to the specified concentration, and thoroughly mixed. An atomic absorption spectrophotometer (model iTE 3000 SERIES) was then used to determine the metals according to the method of **A O A C (1995)**. Further, aqua-regia was used to decompose dry sediment (1:3 $HNO_3$: HCl). After heating the acidified mixture to the boiling point, it was cooled to room temperature. The acidified mixture was then filtered, and the filtrate was added to distilled water in a volumetric flask up to the 50ml. The heavy metal concentration of digestion solutions was then determined using atomic absorption spectrophotometry **(Ilie *et al.*, 2014)**. In addition, the measurement of heavy

metals in soft tissue was carried out in compliance with the method of **Federici *et al.* (2007)**. The toxicological analyses were carried out at the Environmental Studies and Development Unit in Aswan University, which is accredited by the EgAC (Egyptian Accreditation Council) and has an ISO/IEC 17025 accreditation.

**Acute toxicity test**

The analytical grade metallic salts of ZnSO4•7H2O, Pb (NO3)2, FeCl3, and MnSO4•H2O were used to make the standard stock solution (100 mg $L^{-1}$) of Zn, Pb, Fe, and Mn (Merck, Darmstadt, Germany). Followingthe procedures of **Shuhaimi-Othman *et al.* (2011)**, toxicity testing was carried out for 48 hours.

**Determination of antioxidant enzymes activities**

Tissue samples were washed with deionized water and then crushed in an ice-cold mortar and pestle with 0.01 M cooled phosphate buffer at pH 7, followed by 25 minutes of centrifugation at 4°C and 14,000 rpm **(Bakshi *et al.*, 2018; Banaee *et al.*, 2019)**. The lipid peroxidation (LPO) determination method was adapted from the study of **Ohkawa *et al.* (1979)**. Glutathione peroxidase (GPx) activity was determined in tissue homogenates using the method of **Paglia and Valentine (1967)**. Glutathione-s-transferase (GST) activity was determined using the spectrophotometric method described in the work of **Habig *et al.* (1974)** for tissue homogenate. The activity of superoxide dismutase (SOD) was determined based on its inhibitory effect on the oxidation of epinephrine at 480nm **(Misra & Fridovich, 1972)**. Catalase (CAT) activity was determined following the steps of **Beers and Sizer (1952)**. The assay was performed in accordance with the reagent kit's manufacturer's instructions (Biodiagnostic, Egypt).

**DNA analyses**

Using a standard procedure, genomic DNA (gDNA) was isolated from frozen ethanol-preserved tissues. Then, genetic variation was determined by RAPD-PCR in accordance with the method of **Yousif *et al.* (2009)** and **Abdel-Halim *et al.* (2019)**, with minor adjustments using six 10-mer oligonucleotide primers (OPB 4- OPB 5- OPB 6- OPB 7-OPB 17- OPB 18). RAPD was performed as described in the wok of **Williams *et al.* (1990)** with minor modifications. The PCR reaction contained 30–60 ng of gDNA, 5 Pico mole random primer, 0.2 mM dNTPs, 2.5µl PCR buffer (10X), 2.0 mM $MgCl_2$, and 0.5U Taq DNA polymerase in a total volume of 25µl. Amplifications were carried out using the following conditions: 3min at 95°C followed by 40 cycles of 2min at 95°C (Denaturation), 1min at 35°C (Annealing), and 2min at 72°C (Extension), with a final extension of 10min at 72°C. The amplification products were analyzed on 1% (w/v) agarose gels stained with 0.2 µg/ml ethidium bromide, viewed under UV illumination, and photographed **(Jing *et al.*, 2005)**.

**Statistical analysis**

Analysis of variance on the SPSS software package (version 23) (SYSTAT statistical program) was used to test the present data. In the case of a significant difference, the multiple range comparison (LSD) was selected from the postHoc window of the same statistical package to detect the distinct variance between the means **(Sparks, 2000)**. Probability value ($\leq 0.05$) was defined as significant through the study; however, the value >0.05 was defined as non-significant, whereas those less than 0.01 were defined as highly significant. Analysis of probit line regression on MedCalc application software (version 19.5) was used to test LC50.

## RESULTS

### Concentration of the elements in water, sediment and Chironomidae larvae tissue at the two sites

The highest concentrations of Zn, Pb, and Fe in water were detected at site 2, whereas the highest concentrations of Mn in water were found at site 1. On the other hand, the highest concentrations of Zn, Pb, Fe, and Mn in both sediment and the soft body of samples were found at site 2, as illustrated in Fig. (2).

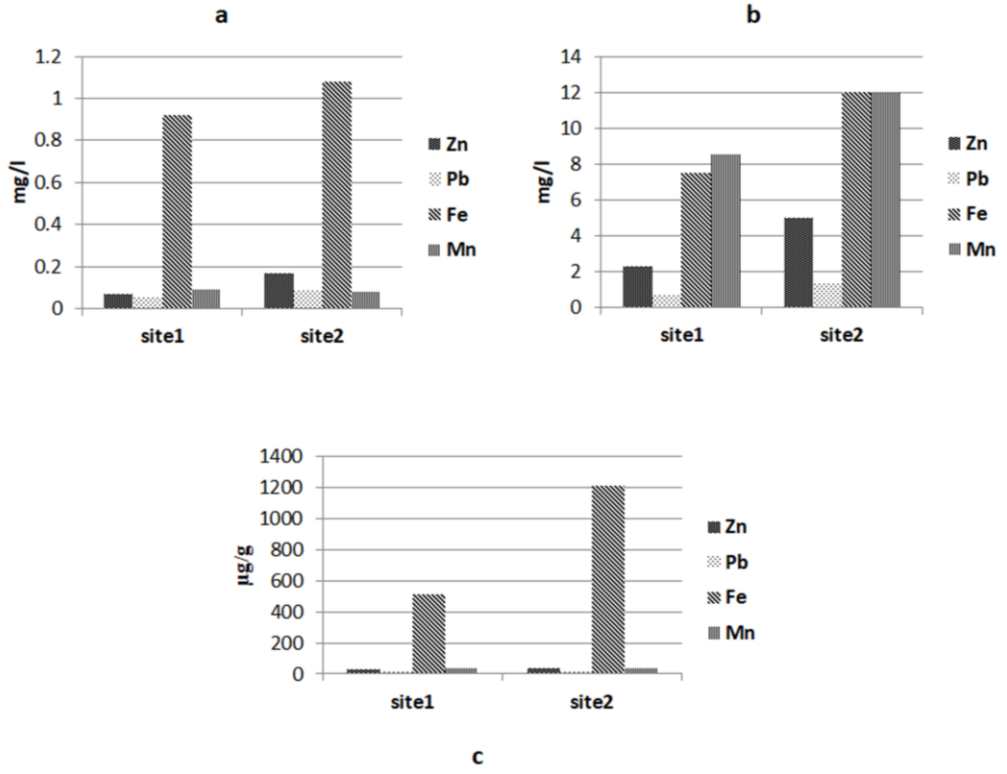


**Fig. 2.** Concentration of the studied elements (Zn, Pb, and Fe) in water, sediment and Chironomidae larvae tissue at site1 and site2. **(a)** Concentration of the studied elements in water; **(b)** Concentration of the studied elements in sediment, and **(c)** Concentration of the studied elements in Chironomidae larvae tissue.

### Bio-accumulation factor (BAF)

To evaluate the ability of Chironomidae larvae to extract and accumulate heavy metals in their tissues, the bioaccumulation factor (BAF) was calculated. According to bio-water accumulation factor (BWAF) data, the uptake of heavy metals by Chironomidae larvae samples followed the succeeding order, where Fe > Zn > Mn > Pb at site 1 and Fe > Mn > Pb > Zn at site 2. According to bio-sediment accumulation factor

(BSAF) data, the uptake of heavy metals by Chironomidae larvae samples followed the order of Fe > Pb > Zn > Mn at site 1 and Fe > Pb > Zn > Mn at site 2, as shown in Fig. (3).

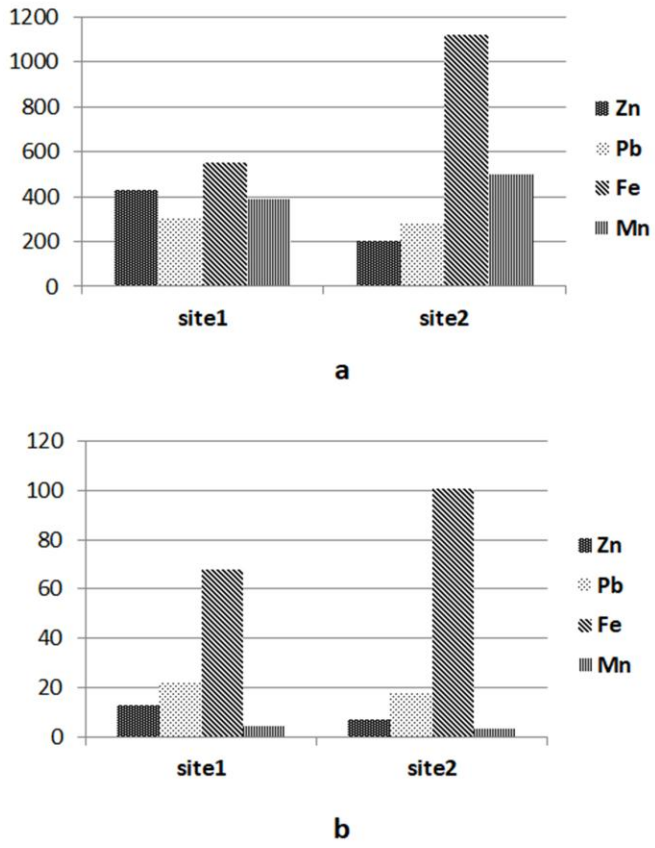


**Fig. 3** Bio-accumulation factor at sites 1 and 2. **(a)** Bio-water accumulation factor at site1 and site2, and **(b)** Bio-sediment accumulation factor at site1 and site2.

## Acute toxicity

### *Probit line regression graph of mortality after 48h*

As shown in Fig. (4a, b, c, d), the median lethal concentrations (LC50) in the 48hr exposure for Pb, Zn, Fe and Mn in Chironomidae larvae were 5.6 mg/l, 7.5 mg/l, 11.9 mg/l and 115.6 mg/l, respectively.

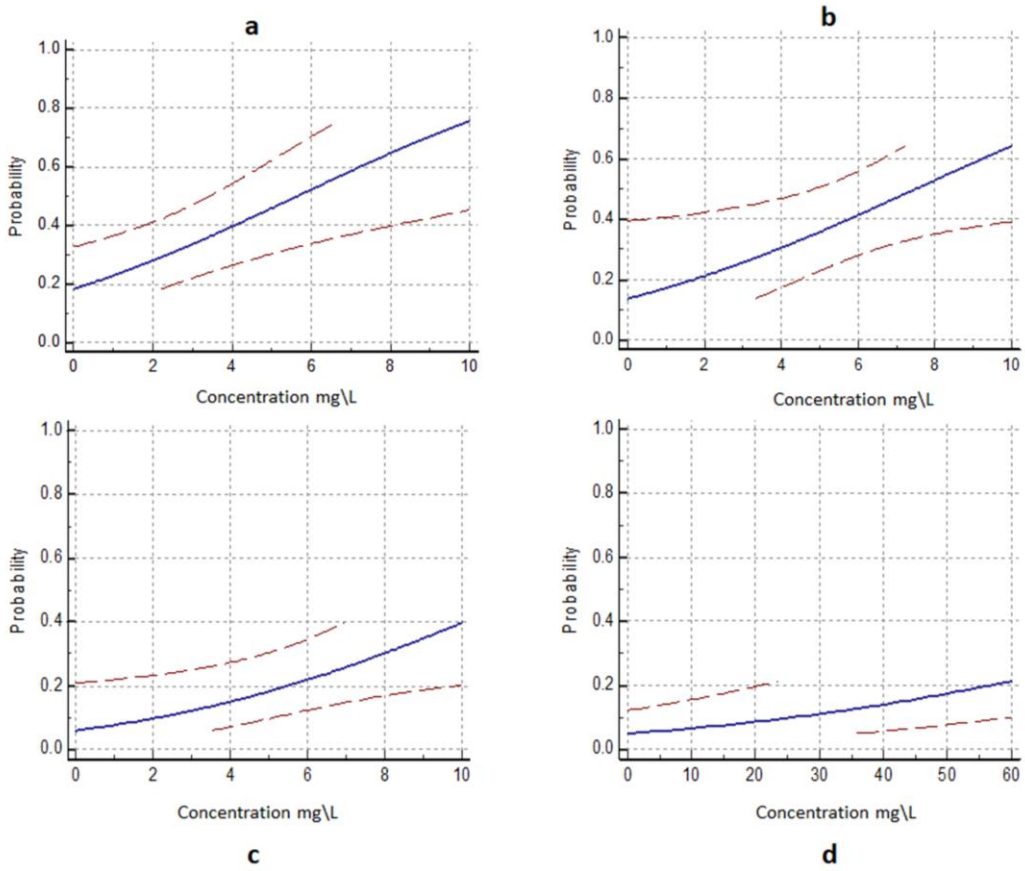


**Fig.4** Probit line regression graph of mortality after 48h in toxicity test for Chironomidae larvae. **(a)** Pb toxicity test; **(b)**Zn toxicity test; **(c)** Fe toxicity test and **(d)** Mn toxicity test.

## Bioconcentration of Zn, Pb, Fe and Mn in surviving Chironomidae larvae after 48h exposure

After 48 hours of exposure, the bioconcentrations of Zn, Pb, Fe, and Mn in Chironomidae larva soft tissue at the five different concentrations of each element in toxic solution were increased with increasing the concentration during exposure, recording ranges between (170 µg/g - 500 µg/g) for Zn, (155 µg/g -1200 µg/g) for Pb, (830 µg/g - 4500 µg/g) for Fe, and (35 µg/g -520 µg/g) for Mn, respectively (Fig. 5).

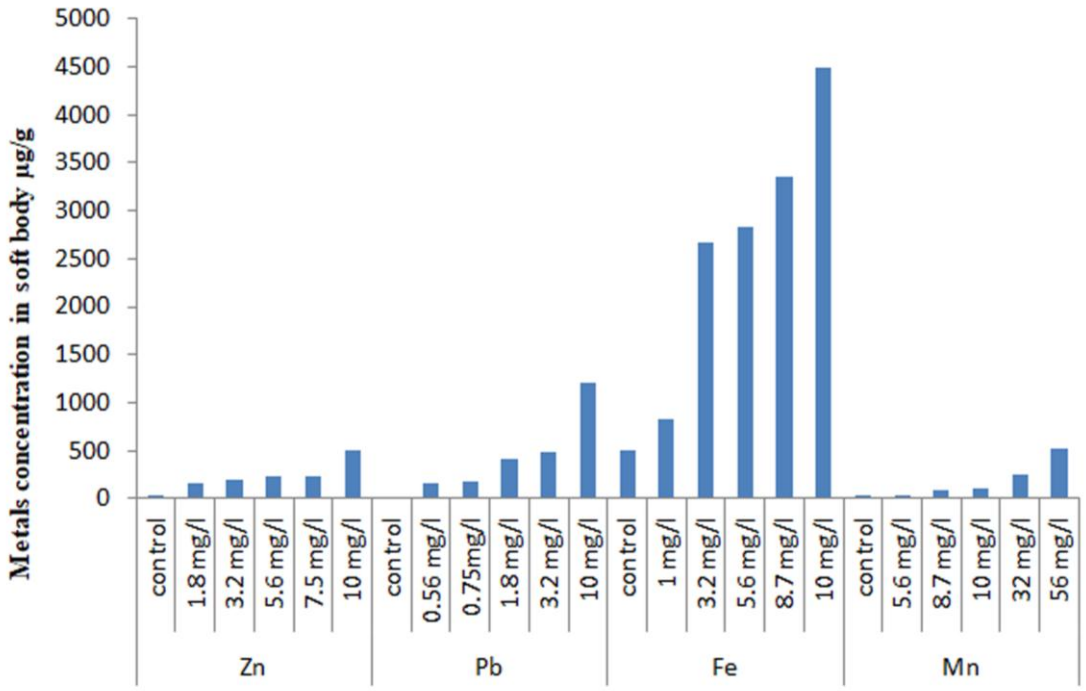


**Fig. 5** Bioconcentration of Zn, Pb, Fe and Mn (mean) in Chironomidae larvae soft tissues (µg/ g) after a two-day exposure to different concentrations of Zn, Pb, Fe and Mn.

**Biochemical analyses in Chironomidae larvae**

According to LSD multiple comparisons between SOD, LPO, GST, GPx, CAT, and (sites and treated groups), there were multiple differences as shown in Table (1).

By comparing LPO activity, it was shown that the maximum activity of the enzyme was found in the Pb-treated group, with values ranging from ranging from 32 to 25.7 nmol/mg tissue, with highly significant differences between Pb-treated group and sites1,2 ($p \leq 0.01$). Site 2 had a higher level of LPO activity than Site 1. Further, by comparing GPx activity, it revealed that the less active enzyme was in the treated group of Pb, with values ranging between 32.4 µM/min/mg tissue and 49.1 µM/min/mg tissue, with significant differences between Pb-treated group and sites 1,2 ($p \leq 0.05$). Site 2 had less GPx activity than site 1. By comparing GST activity, it was discovered that the enzyme had less activity in the Zn-treated group, with values ranging from 6.1 to 11.1 µM/min/mg of tissue, with highly significant differences between the Zn-treated group and sites 1,2 ($p \leq 0.01$). Site 2 had less GST activity than site 1. While comparing SOD activity, it revealed that the lowest activity of the enzyme was in the treated group of Zn, with values ranging from 9.3 and 18.5U/min/mg tissue, with highly significant differences between the Zn-treated group and sites 1,2 ($p \leq 0.01$). Site 2 had less SOD activity than site 1. Although by comparing CAT activity it revealed that the less active enzyme was in the treated group of Zn, with values ranging from 3.9U/ and 10.4 U/min/mg tissue, there was no significant difference between the Zn-treated group and sites 1,2 ($p > 0.05$). Site 2 had less CAT activity than site 1 (Fig. 6).

**Table (1):** The Multiple Range Comparison (LSD) for Chironomidae larvae between superoxide dismutase (SOD), Lipid peroxidation (LPO), glutathione-S-transferase (GST), glutathione peroxidase (GPx) and catalase (CAT) and (sites and treated groups).

| Sites & treated groups | SOD | LPO | GST | GPX | CAT |
|---|---|---|---|---|---|
| Site 1&Site 2 | NS | NS | NS | NS | NS |
| Site 1& ZN | ** | * | ** | NS | NS |
| Site 1 & Pb | ** | ** | ** | * | NS |
| Site 1& Fe | ** | NS | NS | NS | * |
| Site 1& Mn | ** | NS | NS | NS | * |
| Site 2& Zn | ** | * | ** | NS | NS |
| Site 2&Pb | ** | ** | ** | * | NS |
| Site 2& Mn | * | NS | NS | NS | * |
| Site 2& Fe | ** | NS | NS | NS | * |
| Pb & Zn | NS | ** | NS | NS | NS |
| Fe&Mn | ** | NS | NS | NS | NS |
| Fe&Zn | ** | * | * | NS | ** |
| Fe&Pb | ** | ** | NS | NS | * |
| Mn&Zn | ** | NS | * | NS | * |
| Mn &Pb | * | ** | * | NS | * |

LSD multiple comparisons for Chironomidae larvae, (*) the mean difference is significant at the 0.05 level., (**) the mean difference is significant at the 0.01 level., and (NS) the mean difference is not significant.

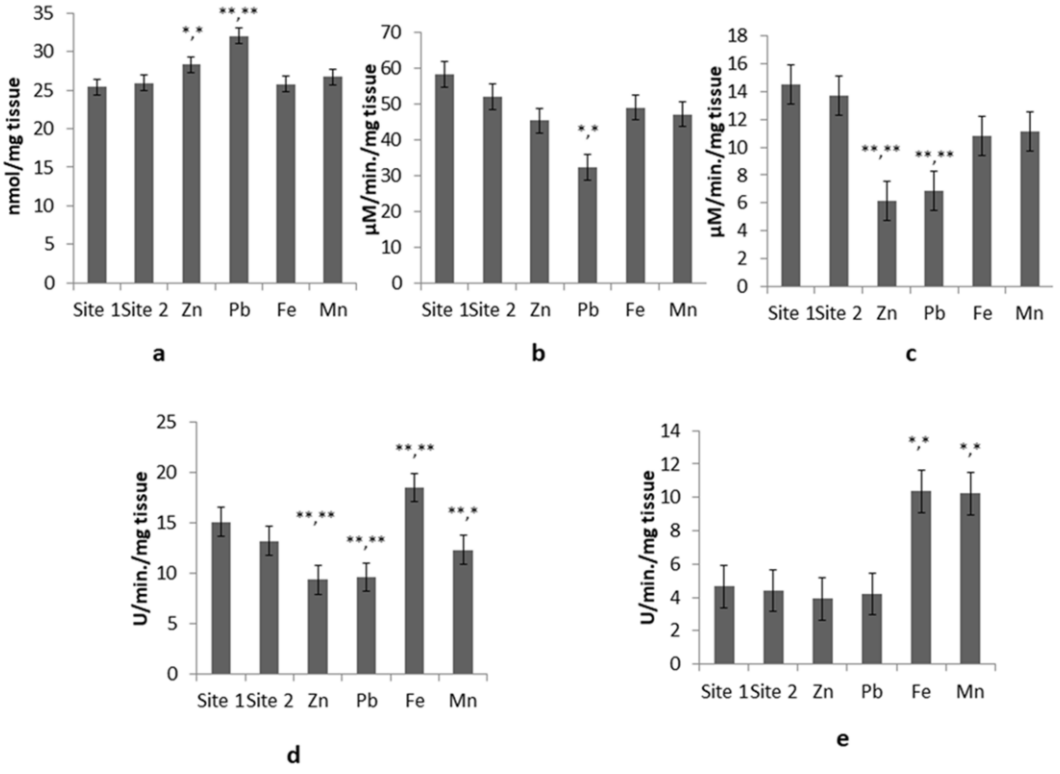


**Fig. 6** Enzymatic activities activity measured in Chironomidae larvae samples from the two sites of Aswan city and in treated samples that survived in toxicity test. (a) LPO activity, (b) GPx activity, (c) GST activity, (d) SOD activity and (e) CAT activity

## DNA analyses

The RAPD-PCR test for acute toxicity (2-day exposure) of Zn, Pb, Fe, and Mn was used. For the six 10-mer oligonucleotide primers used, the primers OPB 5 and OPB 17 produced the most distinguishable banding profiles. Figures 7, 8 and Table 2 illustrate representative RAPD profiles, where in the OPB 5 primer the most distinguishable banding profiles (four strong bands) were produced by Fe = 3.2mg/l, Zn = 3.2mg/l and Pb = 0.75mg/l groups, and there is one distinguishing band in the PCR profile with Primer OPB 17 in the Mn = 8.7mg/l, Mn = 56mg/l, Fe = 10mg/l, Zn = 3.2mg/l, and Pb = 0.75mg/l groups.

The RAPD pattern in samples exposed to Zn, Pb, Fe, and Mn showed variations in the appearance and disappearance of bands, as well as changes in band intensity in both control and treated groups. The number of polymorphic bands and varied bands were 4, 11 bands in Mn = 8.7mg/l, 8, 16 bands in Mn = 56mg/l, 5, 9 bands in Fe = 3.2mg/l, 7, 12 bands in Fe = 10mg/l, 7, 18 bands in Zn = 3.2mg/l, 13, 19 bands in Zn = 10mg/l, 9, 20 bands in Pb = 0.75mg/l, and 4, 7 bands in Pb = 10mg/l. The number of new bands was low (2 in Mn = 8.7mg/l group, 3 in Fe = 3.2mg/l group, 5 in Zn = 3.2mg/l group and 2 in Pb = 10mg/l group). For each treated group, genomic template stability (GTS) was shown in Table 2.

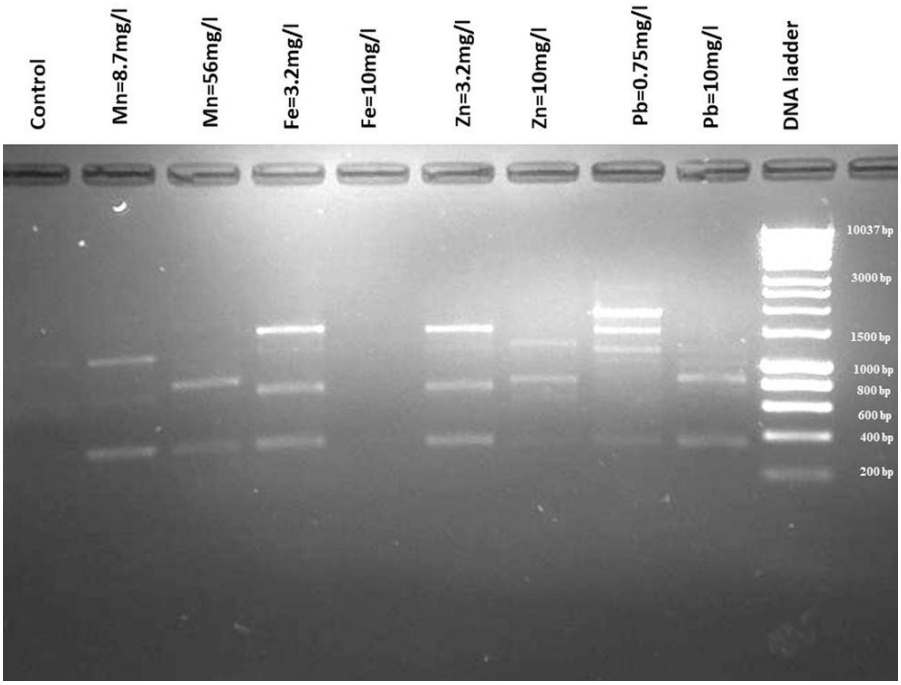


**Fig. 7**. RAPD profiles of different groups of genomic DNA represents PCR products with primer (OPB 5).

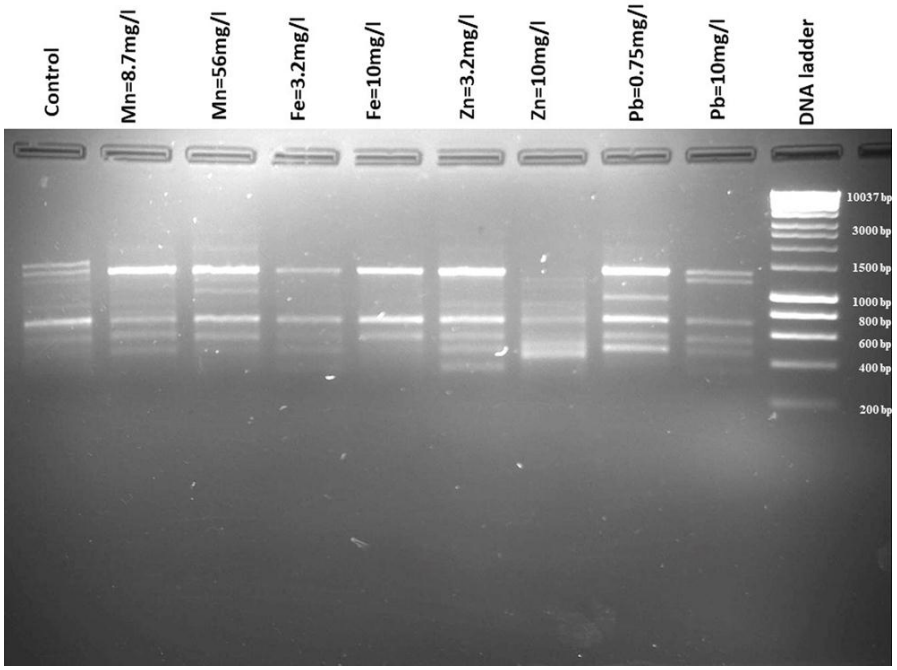


**Fig. 8.** RAPD profiles of different groups of genomic DNA represents PCR products with primer (OPB 17).

**Table (2):** Changes in total bands in control, and of polymorphic bands and varied bands in treated group. a: appearance of new bands, b: disappearance of normal bands, c: decrease in band intensity, and d: increase in band intensity. a+b denotes polymorphic bands, and a+b+c+ d denotes varied bands.

| Primers | Control | Mn=8.7mg/l | Mn=56mg/l | Fe=3.2mg/l | Fe=10mg/l | Zn=3.2mg/l | Zn=10mg/l | Pb=0.75mg/l | Pb=10mg/l |
|---|---|---|---|---|---|---|---|---|---|
| | | a-b-c-d | a-b-c-d | a-b-c-d | a-b-c-d | a-b-c-d | a-b-c-d | a-b-c-d | a-b-c-d |
| OPB 4 | 1 | 0-0-0-1 | 1-0-0-1 | 2-0-0-1 | 3-0-0-1 | 3-0-1-0 | 3-0-1-0 | 0-0-0-1 | 0-0-0-1 |
| OPB 5 | 3 | 0-0-0-2 | 1-1-0-2 | 1-1-0-3 | 0-0-0-0 | 1-1-0-3 | 2-1-0-2 | 4-1-0-4 | 1-0-0-2 |
| OPB 6 | 1 | 0-0-0-0 | 0-0-0-0 | 0-0-0-0 | 0-0-0-0 | 0-0-0-1 | 2-0-0-2 | 0-0-0-0 | 0-0-0-0 |
| OPB 7 | 8 | 1-1-0-3 | 1-1-0-3 | 0-1-0-0 | 0-1-0-3 | 2-1-0-5 | 1-2-0-0 | 2-1-0-5 | 1-2-0-0 |
| OPB 17 | 1 | 1-1-0-1 | 2-0-0-2 | 0-0-0-0 | 1-1-0-1 | 0-0-0-1 | 1-1-0-1 | 1-0-0-1 | 0-0-0-0 |
| OPB 18 | 1 | 0-0-0-0 | 1-0-0-0 | 0-0-0-0 | 1-0-0-0 | 1-0-0-0 | 0-0-0-0 | 0-0-0-0 | 0-0-0-0 |
| Total bands | 15 | 2-2-0-7 | 6-2-0-8 | 3-2-0-4 | 5-2-0-5 | 5-2-1-10 | 9-4-1-5 | 7-2-0-11 | 2-2-0-3 |
| a+b | | 4 | 8 | 5 | 7 | 7 | 13 | 9 | 4 |
| a+b+c+d | | 11 | 16 | 9 | 12 | 18 | 19 | 20 | 7 |
| Genomic Template Stability (GST%) | | 73.3 | 47 | 67 | 53 | 53 | 13 | 40 | 53 |

## DISCUSSION

The present investigation demonstrates that while the amounts of Zn and Mn in water were below the permitted limits in both study sites, the quantities of Pb and Fe were much higher, particularly at site 2. The high concentrations of Pb and Fe in the water can be linked to floating ships and their maintenance activities, as well as industrial company discharges. The concentrations of iron and lead exceeded the standards for aquatic life **(CCME 2007)**. According to Egyptian Law 48 (1982), the concentration of zinc in water must be less than 1 mg/l, the concentration of lead must be less than 0.001 mg/l, the concentration of iron must be less than 0.5 mg/l, and the concentration of manganese must be less than 0.2 mg/l.

The present findings revealed that Zn, Pb, and Fe concentrations in sediment were increasing during sampling at site 2, which might be attributed to an increase in element concentrations in the water at this site. Heavy metals can settle in sediment. A trace amount of these fixed heavy metals will re-enter the adjacent water body and be absorbed by aquatic biota. The elevated Fe content in the sediments may be a result of natural deposits and industry **(El Bouraie *et al.*, 2010)**. It is critical to assess the status of heavy metals in Nile sediments because they act as a reservoir for all contaminants and decomposing organic matter that fall from the above-ground environment **(Hamed 1998; Nguyena *et al.*, 2005)**.

The present study revealed that an increase in the heavy metals in the soft body by an increase in water and sediment is consistent with the findings of **Abdel Gawad (2018)**. Metal concentrations in living organisms may serve as a barometer of their environmental conditions. They are consumed by some birds and fish, which are then eaten by humans via the food chain. Soft tissues accumulated more Fe, Mn, Zn, and Pb than hard tissues **(Gundacker, 2000; DeWolf *et al.*, 2001; Yap *et al.*, 2003; Cravo *et al.*, 2004)**. The most toxic metal to larvae was determined to be Pb, followed by Zn, Fe, and Mn. Pb was found to be more toxic to *Chironomus tendipes* than Zn by **Rao and Saxena (1981)**. However, **Béchard *et al.* (2008)** observed that the order of toxicity for different metals in first instar larvae of *C. riparius* was Pb > Cu > Cd > Ni > Zn and in

third instar larvae of *C. tentans* was Cu > Cd > Zn > Pb > Ni (Khangarot and Ray 1989). This is most likely due to age sensitivity, as *Chironomus* larvae in the first instar are more sensitive than larvae in the third and fourth stages (William et al. 1986). The present study revealed that Zn, Pb, Fe, and Mn bioconcentration increased with increasing concentration exposure in surviving Chironomidae larvae, which is consistent with **Shuhaimi-Othman *et al.* (2011)**. Metals accumulated in animals can either be deposited without excretion, resulting in elevated body concentrations (accumulators), or they can be maintained at a low constant body concentration (regulators) by balancing intake and excretion rates **(Gillis and Wood, 2008)**. Chironomidae larvae are capable of regulating or managing the accumulation of copper, nickel, and zinc, but not of lead or cadmium **(Gillis and Wood, 2008)**.

The present study showed that the LC50s for 48 hours of Pb, Zn, Fe, and Mn to Chironomidae larvae were 5.6 mg/l, 7.5 mg/l, 11.9 mg/l, and 115.6 mg/l, respectively. As explained by **Vedamanikan and Shazilli (2008)**, the higher sensitivity of Chironomidae larvae may correspond to their low body mass. Toxicity tests are used to quantify the cumulative effect of polluted medium on organisms. These effects are a result of the medium's properties (for example, hardness and pH in the case of water), interactions between pollutants, and interactions between contaminants and the media. As a result, observed toxicity test findings frequently differ from those predicted just by chemical data **(ASTM 1992)**. A few studies reported on the acute toxicity of metals to Chironomidae larvae showed that the 48 h-LC50s of Pb were 220 mg/l **(Qureshi *et al.*, 1980)**, 50 mg/l **(Rao and Saxena, 1981)** and 6.53 mg/l **(Shuhaimi-Othman *et al.*, 2011)**. Additionally, the 48 h-LC50s of Zn were 2.62 mg/l (Watts and Pascoe 2000), 62.5 mg/l (Rao and Saxena 1981) and 8.71 mg/l **(Shuhaimi-Othman et al., 2011)**. Further, the 48 h-LC50s of Fe and Mn were 1.65 mg/l and 8.82 mg/l, respectively **(Shuhaimi-Othman et al., 2011)**.

LPO, antioxidant enzymes; SOD, CAT, GST and GPx were studied as enzymatic responses to heavy metal uptake in Chironomidae larvae. The present study showed that the Pb-treated group had the highest LPO, but it also had the lowest GPx activity. LPO is a highly sensitive indicator of oxidative stress **(Guéraud *et al.*, 2010; Negre-Salvayre *et al.*, 2010)**. This result coincides with previous results on macro-invertebrates that demonstrated a decrease in antioxidant enzyme activity and the peroxidase pool resulted in an increase in LPO **(Doyotte *et al.*, 1997; Choi *et al.*, 2001)**. The decrease of GPx activity in the Pb-treated group is in agreement with the findings of **Farid *et al.* (2009)**. The decrease in the activity of GPx might be due to overproduction of ROS. The GST enzyme was less active in the Zn-treated group, as was the SOD enzyme, and the CAT enzyme was the least active in the Zn-treated group. This could be related to the alterations in antioxidant enzyme activities and other biomarkers of oxidative stress that may cause biochemical dysfunction, which agrees with **Farombi *et al.* (2007) and Joseph and Kafilat (2012)**. The current results of biochemical markers (LPO, GPx, GST, SOD, and CAT) in the soft tissue of Chironomidae larvae were decreased by metal toxication as metal toxicity led to free radicals and oxidative damage in the tissue. This is in accordance with **Talas et al. (2008)** and **Cleoni Dos *et al.* (2012)**. These biomarkers may detect the frequency of pollution exposure **(Berra *et al.*, 2002)**. The differences in biomarker levels between the two sites and treatment groups during the toxicity test may be related to the severity and duration of the stress exposure. Statistical analysis (LSD) of

enzyme activity in Chironomidae larvae illustrated that enzyme activity varied significantly between treatment groups and collection sites. This indicates the influence of stress intensity and duration on the activities of LPO, GPx, GST, SOD, and CAT.

The current work used the RAPD-PCR technique to assess the potential for acute (2-day) genotoxicity of Zn, Pb, Fe, and Mn in Chironomidae larvae. From the DNA of Chironomidae larvae, unique finger patterns were obtained. Primers used in Chironomidae larval DNA exposed to Zn, Pb, Fe, and Mn resulted in RAPD patterns distinct from those observed in control samples. The primary modifications in the RAPD profiles shown in this investigation were the inclusion or deletion of several bands, as well as differences in their intensity. These effects may be a result of structural alterations in DNA produced by a variety of different types of DNA damage. As previously stated, the emergence of new bands or disappearance of bands can be attributed to a variety of DNA structural alterations, including breaks, transpositions, recombinations and deletions **(Arillo *et al.*, 1981; Cenkci *et al.*, 2010; Maurizio *et al.*, 2012)**.

The current results of genomic template stability were consistent with **Salarizadeh and Kavousi's (2015)** findings that genomic template stability decreased as heavy metal concentrations increased, with the exception of genomic template stability increasing as Pb concentrations increased, which is consistent with **Asseri *et al.* (2017), Ciğerci *et al.* (2016) and Cenkci *et al.* (2010)** findings. The degree of DNA damage, as well as the effectiveness of DNA repair and replication, all have an effect on the stability of the genomic template. As a result, a high level of DNA damage does not always imply decreased genomic template stability (as compared to a low level of DNA changes), because a high level of DNA damage inhibits DNA repair and replication **(Atienzar *et al.*, 2000)**. Overall, alterations in gDNA in response to heavy metal exposure imply that abiotic environmental influences, including heavy metals, may have an effect on the emergence of new individual features in animals, which is consistent with **Ozyigit *et al.*, (2016)**. RAPD determines the stability of the genomic template, which is related to the degree of DNA damage. It facilitates the detection of a variety of environmental pollutants that have the potential to be cytotoxic **(Pal, 2016)**. The RAPD test has been successfully used to monitor DNA modifications caused by heavy metals such as lead, cadmium, and copper **(Körpe and Aras, 2011)**. DNA fragmentation occurs as a result of such metal ions complexing with DNA. Between nucleosomes, DNA breaks down, which can cause a ladder-like pattern of fragmentation, which is a sign of apoptosis **(Yakovlev *et al.*, 2000)**.

## CONCLUSION

This study focused on the contamination and toxicity of Zn, Pb, Fe, and Mn in Chironomidae larvae, as well as in water and sediment at two sites along the Nile River in Aswan city, Egypt. The study showed that the west of Aswan city, adjacent to multiple cruise ship ports, and directly across the Nile from the industrial drainage outlet of the Egyptian Chemical Industries Company (Kima) was a highly polluted area. Laboratory investigations employing biochemical indicators confirmed the field findings (SOD, CAT, LPO, GST, and GPx). Due to heavy metal exposure and bioaccumulation, there was a genotoxic consequence. The current study shade light on the sources of pollution in aquatic life, that could be affected Egyptian citizens' lives. Current research supports

increasing regulations governing cruise ships that consume irrigation and drinking water, as well as exploring ways to reuse treated industrial waste rather than dumping it into rivers.

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## ARABIC ABSTRACT

التلوث بالمعادن الثقيلة له تأثير ضار على صحة جميع الكائنات الحية. لذا كان الهدف من هذه الدراسة هو مقارنة تأثيرات الزنك (Zn) والرصاص (Pb) والحديد (Fe) والمنغنيز (Mn) على موقعين في مجرى النيل في مدينة أسوان أحدهما يتعلق بملوثات السفن والنشاط الصناعي والآخر لا يوجد به مصار للتلوث . بالإضافة إلى ذلك ، تعرضت يرقات الحشرات المائية من النوع كيرونوميدي لمجموعة متنوعة من تركيزات المعادن الثقيلة على مدار يومين في المعمل. ومنها تم تحديد معدل النفوق ، وتم تقدير التركيزات المميتة لنصف العينات (LC50) . وقد اظهرت النتائج أن المعدن الأكثر ضررًا لليرقات هو الرصاص ، يليه الزنك و الحديد ثم المنجنيز . هذا بالإضافة إلي وجود زيادة في التركيز الحيوي للمعادن داخل أجسام اليرقات مع زيادة تركيزات المعادن. كما تمت دراسة بيروكسيد الدهون (LPO) ، والإنزيمات المضادة للأكسدة مثل ديسموتاز الفائق (SOD) ، والكتالاز (CAT) ، والجلوتاثيون S- ترانسفيراز (GST) ، والجلوتاثيون بيروكسيديز (GPx) كاستجابات إنزيمية لامتصاص المعادن الثقيلة في اليرقات. وعلى الرغم من أن المجموعة المعالجة بـالرصاص كانت لديها أعلى نشاط لإنزيم بيروكسيد الدهون ، إلا أنها كانت تمتلك أيضًا أقل نشاط لانزيم الجلوتاثيون بيروكسيديز. كما كان إنزيم الجلوتاثيون S- ترانسفيراز أقل نشاطًا في المجموعة المعالجة بالزنك ، مثل إنزيم ديسموتاز الفائق ، وكان إنزيم الكتالاز هو الأقل نشاطًا في المجموعة المعالجة بالزنك. وتم الكشف عن التغييرات الجينية واحتمالية حدوث تلف للحمض النووي DNA نتيجة التعرض للمعادن الثقيلة عن طريق تقنية RAPD (ظهور واختفاء البندات ). في هذه الدراسة تم رصد التلوث الناتج عن أنشطة السفن السياحية والتلوث الصناعي في نهر النيل بمدينة أسوان والذي يهدد الحياة المائية وبالتالي قد يوثر صحة علي الإنسان.