Egyptian Journal of Animal Health

P-ISSN: 2735-4938 On Line-ISSN: 2735-4946
Journal homepage: https://ejah.journals.ekb.eg/

Bioaccumulation and oxidative stress induction of heavy metals in ecosystem of an urban region: A case study to assess ecotoxicological risk on freshwater mollusk, *Lanistis carinatus* in Kafr El-Zayat region, Egypt

Khaled Y. Abdel-Halim[*1], Fayz A. Shoukr[2], Safaa R. Osman[1], Mohamed H. Mona[2]
[1]Mammalian & Aquatic Toxicology Department, Central Agricultural Pesticides Laboratory (CAPL), Agricultural Research Center (ARC), 12618-Dokki, Giza, Egypt
[2]Department of Zoology, Faculty of Science, Tanta University, Tanta, Egypt

Article History
Received in 6/11/2020
Received in revised from 23/11/2020
Accepted in 24/12/2020

Keywords
Heavy metals
oxidative stress
ecosystem
Bioconcentration factor
urban region, Egypt.

ABSTRACT

Bioaccumulation pattern of heavy metals (HMs) emitted from urban region of Kafr El-Zayat city was studied in attributing to oxidative stress induction in freshwater mollusk, *Lanistis carinatus*. Regional distribution of HMs in water, sediments, mollusk's tissues in collected samples from sites near industrial units showed significantly differences, respect to reference site (rural). Measured metals: cadmium (Cd), lead (Pb), manganese (Mn), copper (Cu), zinc (Zn), and iron (Fe) in the tissues were greater than those found in water and sediments, representing bioconcentration factors (BCFs) in range (0.83-49.13) during summer and (0.00-24.85) during winter for ratio of tissue/water. However, its ranged (0.09-29.25) during summer and (0.00-8.05) during winter for ratio of tissue/sediments. On the other hand, levels of malondialdehyde (MDA) in digestive glands of mollusk from contaminated sites were greater than those of reference site. Moreover, significantly alterations were recorded for catalase (CAT), glutathione peroxidase (GPx), and carbonyl protein (CP) in a positively correlation with measured HMs. From all findings, it was obtained that use of cumulative biomarkers with BCF may provide diagnostic tool to assess ecotoxicological risk in ecosystem of industrial and urban regions.

INTRODUCTION

Environmental pollution with heavy metals (HMs) is known to be one of the most important problems in urban regions. HMs, dependent on their oxidation state, can be highly reactive and as a consequence toxic to most organisms (**Pinto et al. 2003**). Aquatic ecosystems can be considered as final sinks for environmental contaminants. Practically, watercourses of urban regions are affected by high

Corresponding Author: Khaled Y. Abdel-Halim, Mammalian & Aquatic Toxicology Department, Central Agricultural Pesticides Laboratory (CAPL), Agricultural Research Center (ARC), 12618-Dokki, Giza, Egypt
E-mail address: khaled_yassen68@yahoo.com
DOI: 10.21608/EJAH.2021.134824

inputs of mixtures of organic and inorganic compounds from various sources, such as domestic and industrial sewage waters, abrasion from streets and vehicles (**Papiri et al. 2003, Mahler et al. 2005, van Metre and Mahler, 2005**).

Biomarkers studies are applied in the field (biomonitoring), because they provide an integrated view on how organisms are affected by the bioavailable fraction of the pollution present in the around media. So, HMs can bioconcentrate and bioaccumulate in food chain and contribute the chronic toxicity to aquatic organisms (**Ahmad et al. 2010**). Potential toxic metals such as cadmium (Cd), nickel (Ni), lead (Pb) and chromium (Cr) mainly have oxidative potential effects than other metals such as iron (Fe), zinc (Zn), copper (Cu), selenium (Se) and manganese (Mn) which are essential for metabolism. However, they become toxic when their concentrations are excessive (**Chang et al. 1996**).

The cytotoxicity of metals has been extensively linked to oxidative damage Numerous metals are already capable to induce oxidative stress as well as in the presence of transition metals, like Fe and Cu, $O_2^{\cdot}$ and H_2O_2 can generate $^{\cdot}OH$ through Fenton reaction (**Valko et al. 2005**). Other non-transition metal ions can also be implicated in reactive oxygen and nitrogen species (ROS/RNS) generation in mitochondria. Thus, potential role of ROS to damage tissues and cellular components is called oxidative stress. Oxidative stress occurs in living organisms when the rate of generation of oxygen radicals exceeds the rate of their decomposition (**Sies 1986**). The enzymatic defenses degrade ROS directly such as superoxide dismutase (SOD) which catalyzes the dismutation of two superoxide radicals to hydrogen peroxide. Catalase (CAT) and selenium-dependent glutathione peroxidase (GPx) degrade H_2O_2 (**Halliwell and Gutteridge 1999**). On the other hand, production of ROS can initiate lipid peroxidation (LPO) causing an intracellular excess of malondialdehyde (MDA). Also, exposure to ROS is known to cause a range of reversible and irreversible covalent modification of amino acid side-chains of proteins (**Chezzi and Bonetto, 2003**). They alter proteins structure or function. It has been suggested that induction of protein carbonylation may be a powerful addition to the battery of biochemical endpoints in study of oxidative stress in the environment (**McDonagh et al. 2005**).

Mollusks are used as a good biomonitoring of water pollution with HMs by being sedentary, hardly tolerant to high concentration of pollutants, easy to identify and having enough tissues for analysis. They reflect the high degree of environmental contamination and are the most useful biomarker tools. The use of oxidative stress biomarkers is considered a potential interest for pollutants impact assessment or seasonal variation in animals under field conditions (**Regoli and Principato 1995**). Moreover, the interaction between xenobiotic and the components of the antioxidants defense system plays an important role in the ecotoxicological response of an organism to its environment (**Regoli et al. 2006**). Therefore, this study was conducted to use freshwater mollusk, *Lanistis carinatus* to assess bioaccumulation and oxidative stress effect of water pollution with HMs in Kafr El-Zayat region as an urban model.

2. MATERIAL AND METHODS

2.1. Studied area

This study was conducted in the Kafr El-Zayat district of Egypt, which lies on the Nile River (Rosetta branch). This area is one of the main industrial regions and a number of large factories, especially chemical industries: C1 (pesticides), C2 (fertilizers), C3 (oils & detergents), C4 (brick making factories), C5 (salts & acids) and C6 (textile) are located there. Surface waters in this area are thought to be adversely affected by discharges emitted from industrial and urban sources. Five locations were chosen near factories and urbanized areas from which invertebrate were collected: S1 (Belshay village), S2 (El-Demer area) and S3 (Afify area) are near urbanized discharges, salts and pesticide factories, respectively. S4 (Benufer village) and S5 (Rosetta branch) are near factories making pesticides, fertilizers, textile and bricks, respectively (Figure 1). Another site, situated in a rural region was selected as a likely less contaminated reference area (S6), to compare risk factors.

2.2. Heavy metals analysis

2.2.1. Water

Water samples from different sites as marked in the geographic map were collected periodically every season from 2018 to 2019. Heavy metals (HMs) in water samples were determined according to the method (**APHA 1998**). About 250 ml of collected water were filtered through Whattman filter paper (No.1) and 5 ml of concentrated nitric acid were added. The samples were boiled to constant volume (5 ml). After cooling, the remained mixture was dissolved in 3 ml of the acid, filtered and completed to 25 ml with deionized water.

2.2.2. Sediments

The samples were taken by using simplified equipment (auger) at a depth of 5 cm of sediment. The water was removed from the sediments before packed in a labeled polyethylene bag and transferred to the laboratory in an ice-box. The samples were air dried in the laboratory for 72 hr, before analysis. Five g of sieved sediment were taken into 150 ml conical flasks. So, 50 ml of 0.1 M HCl was added and the flask was agitated on an orbital shaker for 30 min. The content was filtered into 50 ml standard flask and made up to mark with 0.1M HCl for the determination of desirable metals (**Olowu et al. 2010**).

2.2.3. Tissues

Gastropod, *L. carinatus* were collected and transferred to laboratory in glass jars. Digestive gland was dissected and frozen at -20 °C until used. The samples were dried in an oven at 100 °C until constant weight and then mineralised (**Salvo et al. 1998**). Ten millilitres of a nitric acid/ sulphuric acid (4:1 v/v) mixture were added to 1 g of sample and heated about 1 hr on a hot plate provided with a magnetic agitator and reflux condenser. After cooling, 10 ml of concentrated HCl were added and the volume of the samples was reduced by heating mantles. Finally, the samples were reduced to a quantity of around 5 ml and diluted to 30 ml with deionized water for analysis by Inductive Coupled Plasma Optical Emission Spectroscopy (ICP-OES).

2.2.4. ICP-OES

All measurements were performed on Agilent microwave plasma model 4200 MP-AES. An auto-sampler was used to deliver samples to instrumental cyclonic spray chamber with mass flow-controlled nebulizer gas flow as 0.65 L/min. The instrument was operated in a fast sequential mode at rinse time 30 sec with stabilization time 15 sec and featured to cooled CCD detection. Background and spectral interferences could be easily corrected and accurately using Agilent's MP Expert Software.

The limits of detection (LODs) of measured metals were calculated as double the standard deviation of a series of measurements of a solution against the blank absorbance (**ISO/IEC 1990**). Working standards were used and quality assurance procedures and precautions were carried out to ensure reliability of the results. The samples were carefully handled and deionized water was used to avoid contamination. A recovery experiment was carried out by spiked the blank with 50 and 100 ppm of multi-standards of desired metals and the procedures were done as described above. Table 1 highlights the validation and accuracy among studied conditions.

Bioconcentration factor (BCF) is defined as the ratio between the chemical concentration in organism ($C_{org.}$) to the respective concentration in the surrounding media (C_{media}) (**EC 2011**).

$$BCF = Corg./Cmedia$$

2.3. Biochemical quantifications

Half g was homogenized in 10 volumes (w/v) of ice-cold saline solution using a polytron homogenizer for 15 sec. The homogenate was centrifuged at 5000 rpm for 20 min at 4 °C. The homogenate was used as a source for LPO, while supernatant was used for CAT and GPx and total protein content.

2.3.1. LPO

The barbituric acid reactive substances (TBARS) were used as an index of lipid perox-

idation according to **Rice-Evans et al. (1991)**. TBARS was determined by spectrophotometric quantification of MDA content in tissues. Briefly, 250 µl of tissue homogenate was mixed with 1ml of 15% (w/v) trichloroacetic acid (TCA) in 25 mM HCl and 2 ml of thiobarbituric acid (TBA) (0.37%). The samples were boiled for 10 min, quickly cooled, and immediately centrifuged at 5000 rpm for 5 min. Spectrophotometric determination was carried out at 535 nm. MDA was quantified using an extinction coefficient of 156 Mm^{-1} and its concentration was expressed as mM/g tissue.

2.3.2. CAT

The enzyme activity was measured following the decrease of absorbance at 240 nm due to hydrogen peroxide (H_2O_2) consumption **Beers and Sizer (1952**). The reaction mixture consisted of 1 ml of 12.5 mM H_2O_2 (substrate), 2 ml of 66.7 mM phosphate buffer, pH 7.0 and an aliquot of enzyme source. The activity was expressed as U/g wet mass. The unit of CAT is the amount of enzyme which liberates half the peroxide oxygen from hydrogen peroxide solution of any concentration in 100 at 25 °C.

2.3.3. Glutathione peroxidase (GPx)

The enzyme activity was measured according to **Flohe and Gunzler (1984**) by mixing phosphate buffer solution (100 mM), EDTA (50 mM), sodium azide (250 mM), H_2O_2 (10 mM) and enzyme in a cuvette. The change in absorbance at 340 nm was recorded every 3 sec for 40 sec. The activity was expressed as mU GPx/mg protein. One unit of GPx is defined as the amount of enzyme necessary to oxidize 1µM of NADPH per min.

2.3.4. Carbonyl protein (CP)

Oxidative damage to protein was quantified as a carbonyl protein (CP) (**Stedman and Levine 2000**). Frozen samples of tissues were weighed, homogenized (1:20 w/v) in ice-cold 5% (w/v) sulfosalicylic acid and then centrifuged at 13000 rpm for 15 min at 4 °C. The supernatant was removed and 0.5 ml of 2, 4-dinitrophenylhydrazine (10 mM/L in 2 mM of HCl) solution was added to the pellet. The samples were kept at room temperature for 1 hr with vigorous vortex every 15 min, then 0.5 ml of 20% w/v TCA was added and the tubes were centrifuged as described above. The supernatant was again discarded and the excess of 2, 4-dinitrophenylhydrazine was removed by washing the pellet three times with 1 ml of ethanol: ethyl acetate (1:1 v/v), followed by vigorous vortex and re-centrifuging as described before. Finally, the pellet was dissolved in 6 M of guanidine chloride and incubated for 15 min at 37 °C. The maximum absorbance in the range of 360-370 nm was recorded and the find carbonyl protein values were expressed by using the extinction coefficient of 22 mM^{-1}. The blank was prepared by replacing 2,4-dinitrophenyl hydrazine with 2 mM HCl.

2.3.5. Protein content

Total protein was determined according to the method of **Lowry et al. (1951)**. Bovine serum albumin (BSA) was used as a standard.

2.4. Statistical analysis

Analysis of variance (ANOVA) was used to compare means among treatments. The least square means were compared for significant differences between treatments using Student-Newman-Keels test. The regression coefficient (RC) between biochemical parameters and HMs concentrations was estimated, where the analysis was performed on Costat program (**Cohort software Inc. 1985**).

RESULTS

Determination of heavy metals in ecosystem.

Water, sediment and mollusk's samples from the study area were screened for the levels of Zn, Cu, Mn, Cd, Pb and Fe, respectively.

Levels of HMs in water samples are presented in Table (1). Distribution of metals in summer was higher than those of winter of a regional mean value 120.23 µg/L. During summer, levels of Mn and Fe were higher than other metals in all studied zones ranged from 668.04 to 64.31 µg/L for Mn and from 583.08 to 276.84 µg/L for Fe. However, mean levels of metals were distributed in the following order: Fe> Mn> Zn > Cu> Pb with mean values: 453.82, 241.57, 20.91, 4.90 and 0.20 µg/L, respectively. During winter, Mn values were higher than other metals which distributed

moderately, and mean values of metals were in the order as follows: Mn> Cu> Zn> Pb> Fe> Cd accounting for 168.26, 27.36, 25.83, 14.36, 9.02 and 4.16 µg/L, respectively.

Table (2) represents HMs content in sediment samples from the studied zones. The data showed that, Fe exhibited the greatest value (325.72 µg/g) in S3, while Mn exhibited the greatest one (320.52 µg/g) in S4 during summer. The metals were distributed in the following order: Mn> Fe> Pb> Zn > Cu with mean values: 108.63, 85.95, 40.03, 13.30 and 8.23 µg/g, respectively. During winter, Fe and Mn exhibited the greatest values: 629.8 and 411.15 µg/g in S5, while other metals displayed no significant differences in all selected sites. The distribution pattern of HMs was as follows: Fe> Mn> Cu> Zn > Pb> Cd with mean values: 105.68, 73.54, 3.26, 3.09, 0.81 and 0.26 µg/g, respectively.

In case of mollusk's tissues, levels of HMs during summer were greater than those during winter. In fact, Fe exhibited the high contaminated status during both seasons with mean values: 1891.42 and 224.15 µg/g during summer and winter, respectively. During summer, HMs levels were as follows: Fe> Cu> Mn> Zn> Pb> Cd with mean values: 1891.42, 240.75, 199.38, 19.28, 3.85 and 1.07 µg/g, respectively. However, the distribution pattern during winter was as follows: Fe> Cu> Mn> Zn with mean values: 224.15, 114.25, 26.25 and 14.43 µg/g, respectively. Moreover, Cd and Pb were below detection limits (Table 3).

The measured HMs exhibited BCFs in animal's tissue respect to their levels in the surrounding media (Table 4). The measured HMs displayed BCF pattern from tissue/sediment greater than those from tissue/water. Between water and mollusk's tissues, positively HM levels showed BCF ranged from to 0.83 to 49.13 during summer and from 0.00 to 24.85 during winter, arising mean values: 0.75, 25.05, 0.76, 0.54, 9.63 and 14.52 for Zn, Cu, Mn, Cd, Pb, and Fe, respectively. In case of BCF obtained from ratio of tissue/sediment, examined HMs exhibited the range (0.09-29.25) during summer and the range (0.00-8.05) during winter with mean values: 3.06, 18.65, 1.71, 0.54, 0.05 and 12.07 for metals as described above.

Biochemical responses.

MDA

Levels of MDA in digestive gland of freshwater mollusk, *L. carinatus* seasonally collected from different sites of studied area are illustrated in Figure (2). The obtained data showed that, MDA levels during summer were greater than those during other seasons as follows: summer> winter> spring> autumn. During summer, MDA levels exhibited the order: S3> S2> S6> S4> S1>S5 with mean values: 5.27, 3.21, 2.67, 2.65, 1.81 and 1.59 mM/g tissue, respectively. The lowest levels of MDA were found during autumn with mean values: 0.17, 0.69, 2.37, 0.79, 1.28 and 0.84 mM/g tissue for sites as described above. However, level in mollusk's samples from S3 was greater than other sites during all seasons.

Catalase (CAT).

Activity of CAT in haemolymph and tissue homogenate of collected mollusk seasonally are illustrated in Figures (3). The presented data displayed that, mean values during autumn were greater than others. During this season, the activity exhibited the following order: S1> S3> S2> S4> S5> S6 with mean values: 6.59, 5.49, 4.79, 3.48, 3.07 and 2.35 nM/mg tissue, respectively. In case of heamolymph, CAT activity during autumn was greater than others. In this season, the activity was in the following order: S5> S2> S3> S4> S1> S6 with mean values: 70.37, 58.87, 45.11, 40.52, 31.28 and 19.88 nM/g tissue, respectively. In fact, S6 (rural site) displayed the lowest values during all seasons.

Glutathione peroxidase (GPx).

The activities of GPx in tissue's homogenate of seasonally collected mollusk are illustrated in Figure (). The highest mean values of GPx activity were recorded during winter season as follows: S4> S5> S3> S6> S1> S2 with mean values: 2.06, 1.56, 0.09, 0.08, 0.04 and 0.03 nM/mg tissue, respectively. However, the lowest values were recorded during autumn, 0.02, 0.03, 0.05 and 0.08 nM/mg for sites: S3, S6, S2, S4, S1 and S5, respectively. Among seasonally impact of pollutants on enzyme activity, winter exhibited the highest activity, fol-

lowed by spring and summer, respectively.

Carbonyl protein (CP).

Levels of CP in tissue's homogenate of seasonally collected mollusk are illustrated in Figure (3)**.** Levels of CP during winter was greater than those during other seasons as follows: winter> autumn> spring> summer. Site (S1) exhibited the greatest value of CP levels during autumn and winter (0.237and 0.289 mM/L), respectively, while S5 exhibited the lowest values of CP levels: 0.027, 0.017, 0.006 and 0.026 mM/L during spring, summer, autumn and winter, respectively.

Relationship between HMs and biomarker responses.

Among antioxidant enzymes or carbonyl protein (CP), it revealed negative correlation with most metals, while Mn and Pb were the only observed positive correlation with LPO and carbonyl protein. In case of CAT, Zn, Cu and Fe revealed positive correlation, while Mn, Cd and Pb observed the negative one (Table 5).

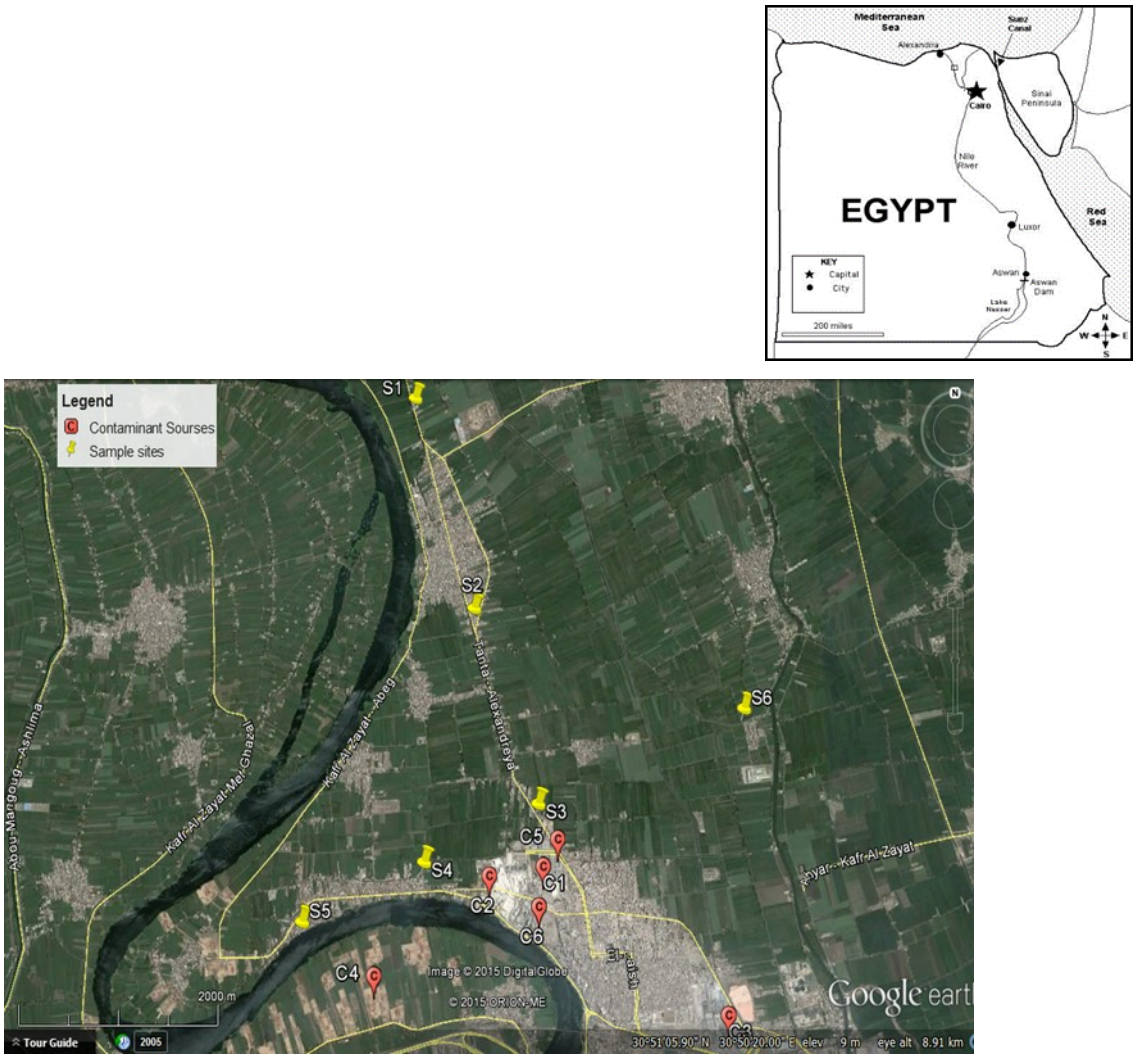

Figure 1: Sampling sites and contaminants sources of Kafr El-Zayat region (http://earth.google.com). Exposure sites are in order: S1 (Balshay v.); S2 (El-Demer zone.); S3 (Afify zone); S4 (Benufer v); S5 (Rosetta branch) and S6 (Abdallah Basha v.)(reference zone), respectively.

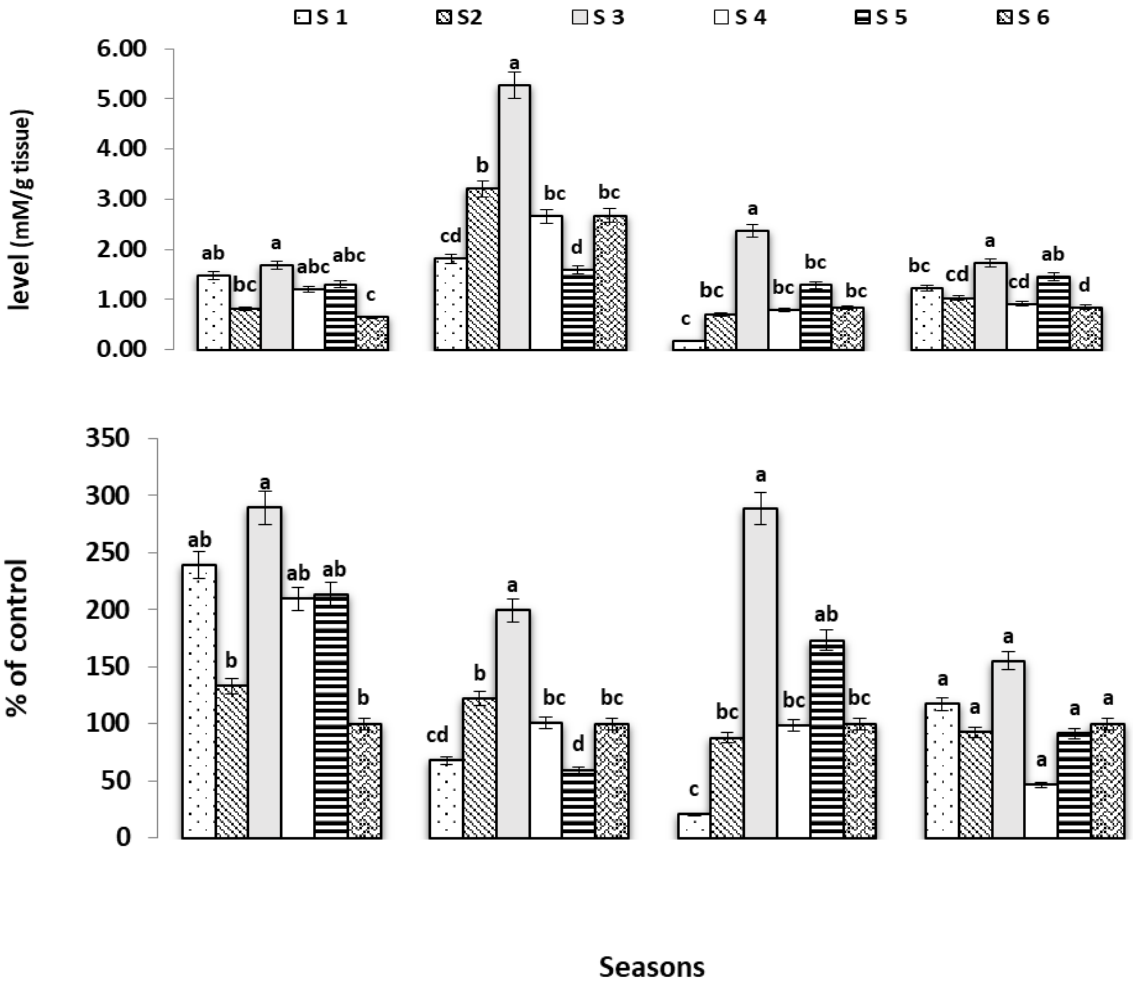

Figure 2: (A) Levels of MDA In tis sue homogenate of *L. carinatus* (mM/g tissue) and (B) % of control. Each value is the mean of 3 replicates ±SE. The same letters indicate no significant differences at 0.05 levels.

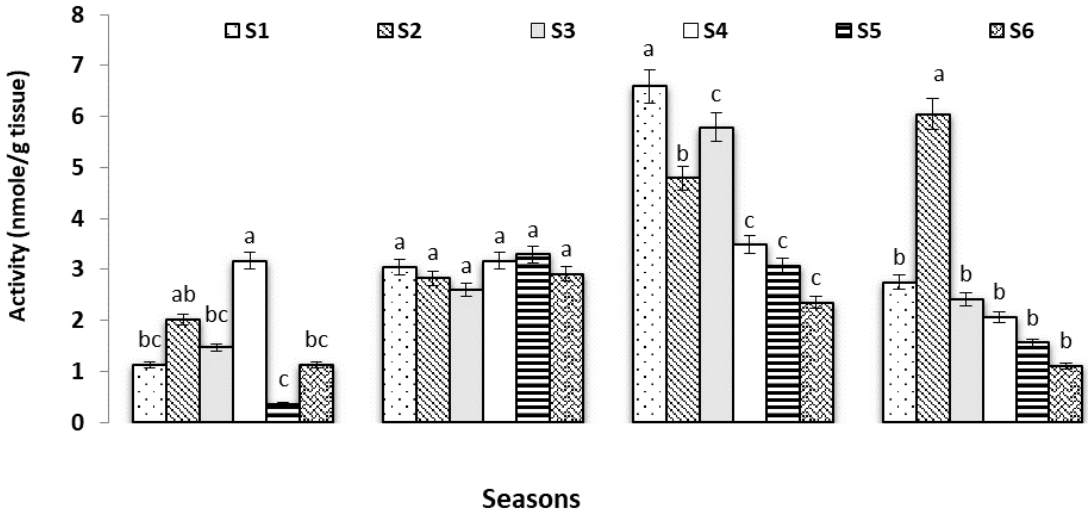

Figure 3: Activity of CAT (nM/g tissue) in whole body tissue of freshwater mollusca, *L. carinatus* collected from Kafr El-Zayat region.

Table (1) Heavy metals levels (ppb) in water samples collected from different zones of Kafr El-Zayat district during 2018-2019.

Sites	Concentrations (ppb)						Regional Mean
	Zn	Cu	Mn	Cd	Pb	Fe	
Summer							
1	38.64 ±0.002	7.44 ± 0.00	668.04 ± 0.001	BDL	BDL	583.08 ± 0.03	
2	19.80 ± 0.003	9.12 ± 0.007	299.42 ± 0.003	BDL	1.200	653.1 ± 0.03	
3	18.10 ± 0.003	BDL	130.13 ± 0.006	BDL	BDL	539.64 ± 0.03	
4	12.96 ± 0.005	BDL	64.31 ± 0.12	BDL	BDL	276.84 ± 0.04	120.23
5	20.28 ± 0.003	BDL	68.78 ± 0.01	BDL	BDL	325.08 ± 0.06	
6	15.72 ±0.004	12.86 ± 0.00	218.75 ± 0.004	BDL	BDL	345.10 ± 0.05	
Mean	20.91 ± 0.003	4.90 ± 0.013	241.57 ± 0.003	BDL	0.200	453.82 ± 0.04	
LSD 0.05	0.08	0.12	1.43	-	0.07	30.05	
Winter							
1	17.4 ± 0.003	36.48 ± 0.001	193.82 ± 0.00	3.00 ± 0.13	16.44 ± 0.03	4.2 ± 0.01	
2	18.6 ± 003	25.32 ± 0.002	68.76 ± 0.001	2.52 ± 0.15	16.32 ± 0.03	4.32 ± 0.01	
3	62.76 ± 0.001	29.52 ± 0.002	371.28 ± 0.00	3.24 ± 0.12	21.0 ± 0.02	4.08 ± 0.01	
4	33.48 ± 0.002	27.84 ± 0.002	271.17 ± 0.00	16.20±0.02	20.4 ± 0.02	4.68 ± 0.01	41.49
5	0.6 ± 0.09	29.04 ± 0.002	3.48 ± 0.02	BDL	12.0 ± 0.04	31.09 ± 0.00	
6	22.08 ± 0.002	15.96 ± 0.003	101.04 ± 0.001	BDL	BDL	5.89 ± 0.01	
Mean	25.83 ± 0.002	27.36 ± 0.002	168.26 ± 0.00	4.16 ± 0.09	14.36 ± 0.03	9.02 ± 0.005	
LSD 0.05	0.10	0.085	0.109	0.704	0.872	0.063	
Seasonal Mean	23.36	16.1	204.89	4.16	7.28	231.41	

Table (2) Heavy metals levels (µg/g) in sediment samples collected from different zones of Kafr El-Zayat district.

Sites	Concentrations (µg/g)						Regional Mean
	Zn	Cu	Mn	Cd	Pb	Fe	
Summer							
1	4.14 ± 0.1	1.50 ± 0.00	4.92 ± 0.08	BDL	0.05 ± 0.12	105.84 ± 0.00	
2	1.55 ± 0.2	BDL	13.35 ± 0.03	BDL	1.38 ± 0.00	83.72 ± 0.00	
3	3.71 ± 0.1	BDL	45.22 ± 0.01	BDL	7.18 ± 0.00	325.72 ± 0.00	
4	37.88 ± 0.01	26.40 ± 0.00	320.52 ± 0.00	BDL	121.18 ± 0.00	BDL	
5	0.16 ± 0.3	BDL	1.76 ± 0.23	BDL	BDL	0.44 ± 0.01	42.69
6	32.36 ± 0.01	21.47 ± 0.00	266.0 ± 0.00	BDL	110.36 ± 0.00	BDL	
Mean	13.30 ± 0.03	8.23 ± 0.01	108.63 ± 0.00	BDL	40.03 ± 0.00	85.95 ± 0.00	
LSD (0.05)	0.67	0.09	0.74	-	0.01	0.01	
Winter							
1	0.89 ± 0.006	0.54 ± 0.013	3.28 ± 0.01	0.38 ± 0.02	1.2 ± 0.04	1.52 ± 0.02	
2	0.49 ± 0.012	0.36 ± 0.02	0.06 ± 0.6	0.45 ± 0.01	1.18 ± 0.04	0.26 ± 0.02	
3	0.71 ± 0.008	0.97 ± 0.007	0.32 ± 0.1	0.38 ± 0.02	1.2 ± 0.04	0.28 ± 0.14	
4	0.92 ± 0.006	1.21 ± 0.006	13.2 ± 0.003	0.36 ± 0.02	1.33 ± 0.04	0.55 ± 0.07	
5	14.41 ± 0.00	15.92 ± 0.00	411.15 ± 0.00	BDL	BDL	629.8 ± 0.00	31.1
6	1.15 ± 0.005	0.6 ± 0.012	13.26 ± 0.003	BDL	BDL	0.34 ± 0.1	
Mean	3.09 ± 0.012	3.26 ± 0.002	73.54 ± 0.00	0.26 ± 0.00	0.81 ± 0.00	105.68 ± 0.00	
LSD 0.05)	0.01	0.012	0.672	0.011	0.09	0.072	
Seasonal Mean	8.195	5.745	91.085	0.13	20.42	95.815	

* Each value is the mean of three samples ± SE.
* The concentrations of heavy metals are expressed as (µg/g). *BDL = below detection limit

Table (3) Heavy metals levels (µg/g) in Mollusca's tissue samples collected from different zones of Kafr El-Zayat district.

Sites	Concentrations (µg/g)						Regional Mean
	Zn	Cu	Mn	Cd	Pb	Fe	
Summer (2014):							
1	2.43 ± 0.02	109.42 ± 0.00	39.26 ± 0.01	BDL	3.19 ± 0.00	547.22 ± 0.00	**378.43**
2	18.20 ± 0.002	17.26 ± 0.03	87.15 ± 0.01	BDL	2.85 ± 0.00	1912.40 ± 0.00	
3	18.38 ± 0.002	1235.70 ±0.00	529.65 ± 0.02	1.01 ±0.01	4.03 ± 0.00	5183.80 ± 0.00	
4	35.67 ± 0.001	82.14 ± 0.01	403.0 ± 0.02	2.97 ±0.00	5.13 ± 0.00	2780.80 ± 0.00	
5	30.18 ± 0.001	BDL	37.77 ± 0.01	BDL	BDL	704.83 ± 0.00	
6	10.81 ± 0.003	BDL	99.36 ± 0.001	2.42 ±0.00	7.92 ± 0.00	219.45 ± 0.00	
Mean	19.28 ± 0.00	240.75 ± 0.00	199.38 ± 0.00	1.07 ±0.01	3.85 ± 0.00	1891.42 ± 0.00	
LSD (0.05)	0.07	0.89	0.09	0.012	0.007	0.085	
Winter (2015):							
1	2.46 ± 0.02	BDL	66.11 ± 0.001	BDL	BDL	5.07 ± 0.16	**63.18**
2	18.92 ± 0.03	62.03 ± 0.00	44.6 ± 0.001	BDL	BDL	281.88 ± 0.003	
3	18.38 ± 0.03	77.26 ± 0.00	26.97 ± 0.001	BDL	BDL	213.68 ± 0.004	
4	35.67 ± 0.01	0.84 ±0.006	43.27 ± 0.001	BDL	BDL	410.72 ± 0.002	
5	0.36 ± 1.04	17.35 ± 0.00	37.93 ± 0.001	BDL	BDL	348.62 ± 0.002	
6	10.81 ± 0.05	BDL	466.62 ± 0.00	BDL	BDL	84.95 ±0.01	
Mean	14.43 ± 0.03	26.25 ± 0.00	114.25 ± 0.00	BDL	BDL	224.15 ± 0.004	
LSD (0.05)	0.92	0.009	0.066	-	-	1.48	
General Mean	16.855	133.5	156.815	0.54	1.925	1057.785	

* Each value is the mean of three samples ± SE. *The concentrations of heavy metals are expressed as (µg/g).
*BDL = below detection limit

Table (4) Bioconcentration factors (BCFs) of heavy metals in mollusca's tissue in association with surrounding media of Kafr El-Zayat region, Egypt.

Element	BCF Water/tissue			BCF Sediment/tissue		
	Summer	Winter	Mean	Summer	Winter	Mean
Zn	0.93	0.56	0.75	1.45	4.67	3.06
Cu	49.13	0.96	25.05	29.25	8.05	18.65
Mn	0.83	0.68	0.76	1.84	1.55	1.71
Cd	1.07	0.00	0.54	1.07	0.00	0.54
Pb	19.25	0.00	9.63	0.09	0.00	0.05
Fe	4.18	24.85	14.52	22.01	2.12	12.07

Table (6) Relationship between heavy metals levels and biomarker response.

Biomarker response (Y)	Metals (x)	r	r2	df	F	Intercept (a)	egression coefficient (b)
LPO	Zn	-0.325	0.105	1	0.15	1.395	0.010
LPO	Cu	-0.300	0.090	1	46.84**	1.248	0.002
LPO	Mn	0.053	0.003	1	0.45	1.693	-0.001
LPO	Cd	-0.087	0.008	1	0.00	1.580	-0.021
LPO	Pb	0.252	0.064	1	0.01	1.601	-0.017
LPO	Fe	-0.372	0.138	1	14.46**	0.994	0.001
GP_x	Zn	0.645	0.416	1	2.85	0.004	0.015
GP_x	Cu	-0.116	0.014	1	0.05	0.278	-0.000
GP_x	Mn	-0.453	0.205	1	1.03	0.339	-0.000
GP_x	Cd	0.236	0.056	1	0.24	0.212	0.092
GP_x	Pb	-0.405	0.164	1	0.78	0.416	-0.080
GP_x	Fe	0.217	0.047	1	0.20	0.200	0.000
CAT	Zn	0.092	0.008	1	0.03	2.769	0.006
CAT	Cu	0.216	0.047	1	0.20	2.785	0.001
CAT	Mn	-0.610	0.372	1	2.37	3.193	-0.003
CAT	Cd	-0.381	0.145	1	0.68	3.117	-0.446
CAT	Pb	-0.215	0.046	1	0.19	3.125	-0.128
CAT	Fe	0.356	0.127	1	0.58	2.576	0.000
CP	Zn	-0.325	0.105	1	0.47	0.109	-0.001
CP	Cu	-0.300	0.090	1	0.39	0.093	-0.000
CP	Mn	0.053	0.003	1	0.01	0.082	0.000
CP	Cd	-0.087	0.008	1	0.03	0.088	-0.007
CP	Pb	0.252	0.064	1	0.27	0.065	0.009
CP	Fe	-0.372	0.138	1	0.64	0.105	-0.000

Regression follow the mode y = a + b x obtained by simple linear regression; r^2 = coefficient of determination, df = degree of freedom, value 1 = a simple linear regression of equation and F = variation
(* = mean the significant at 0.05; ** means the significant at 0.01 and *** = the significant at 0.001)

DISCUSSION

The present study focused on the distribution of HMs into aquatic environment surrounding industrial plants of Kafr El-Zayat district. Additionally, it may state the impact of these metals on some of freshwater mollusks which is commonly distributed in this region (*L. carinatus*). Overall data indicated the major ecotoxicological effects of HMs in the ecosystem.

As mentioned above in POP_s residue levels, HMs elevated the same concept, where their concentrations during summer were greater than those during winter. Moreover, Fe and Mn recovered the greatest levels in most sampling sites, followed by other essential metals: Cu and Zn. This concept may due to emission of fertilizers companies into water course or air, followed by that emitted from oil extraction and brick making factories in Kafr El-Zayat. In previous studies, **Abdel-Halim *et al.* (2013)** investigated that land snail, *Helix aspersa* showed hyperaccumulation capacity for HMs emitted in atmosphere of Kafr El-Zayat, where tissues of gastropod showed regional means of Cd, Mn, Ni, Pb, Zn and Cu at values: 0.71, 7.09, 0.71, 2.68, 41.44 and 18.01 mg/kg b.w, respectively. On the other hand, several studies demonstrated that, freshwater mollusks are considered good bioindicators for water pollution with HMs (**Lau *et al.* 1998, Zhou *et al.* 2008;, Kanakaraju *et al.* 2008, Moloukhia and Saleem 2011, Bhalchandra and Ram 2013, Kesavan *et al.* 2013, Yap and Cheng**

2013). In the present data Pb and Cd exhibited low concentrations in water, sediments and mollusk's tissues compared with other metals, except in some sites. These findings explain the limitation of Pb and Cd source in Kafr El-Zayat region, except those emitted from brick making factories near these sites. These data are in agreement with that obtained by **Abdel-Halim (2014)** where Pb and Ni were the mostly detected in freshwater alga collected from sites near brick making factories of Kafr El-Zayat region considering the use of fuel in these factories is the major source of potential toxic metals. Moreover, sewage water is considered another source of metals in aquatic media of this region as stated previously by **Chen et al. (2005)** and **Wannaz et al. (2006**).

Biochemical responses.

The obtained results demonstrate the assessment of the biological impact and risk of HMs from industrial and urban discharge in the aquatic media of Kafr El-Zayat.

The use of freshwater mollusk, *L. carinatus* may represent a relative easy process to improve the actual monitoring techniques even in the absence of native organisms. Additionally, the biological significant of the results presented here is important both in terms of the accumulated chemicals and appear once of toxicological responses. The digestive gland was chosen as a target organ for biochemical and toxicants assessment according to its ability to uptake and concentrate the contaminants by 5-10 folds higher than other organs (**Gomot de-Vaufleury and Pihan 2000, Beeby and Richmond 2003**). On the other hand, heamolymph of collected animals was used a source to assess the activities of both CAT and MDA in correlation to induction in genotoxic effects of contaminants were proven to be related to the production of ROS in chosen organism which affect various cellular processes, mostly the function of membrane system (**Pinto et al. 2003** and **Valko et al. 2005**). The potential of ROS to damage tissues and cellular components is called oxidative stress. In this study, CAT, LPO, GPx and CP were used as good parameters in oxidative stress, where ROS cause intracellular excess of MDA and LDH. Moreover, they are capable to interact with DNA to form DNA adduct in different forms.

The obtained data revealed the ability of measured chemical which emitted from this region into aquatic media to induce oxidative stress in contaminated sites compared with reference site (S6). Most studies in aquatic animals centered on oxyradicals such as $O^{\bullet -}$, H_2O_2 and $OH^{\bullet}$, respectively, are main form of ROS. Rates of amount of ROS production can be increased by the presence of a wide range of natural and man–made xenobiotic. Possible anthropogenic–related sources of enhanced ROS and other pro–oxidant free radical production includes organic contaminants such as nitro aromatics, PAHs, PCB_s, pesticides and heavy metals (**Lemaire and Livingstone 1993, Di Giulio et al. 1995, Halliwell and Gutteridge 1999**). In the present study, ROS cause intracellular excess of MDA in all sites more than reference site. The formation of lipid peroxidation in membranes disrupts the normal cellular metabolism, triggering adaptive response causing cell death (**Girotti 1998**). Previous studies demonstrated the levels of lipid peroxidation (as malondialdehyde, MDA) in mussel species submitted to different stresses (**Almeida et al. 2003, 2004, 2005**).

On the other hand, catalase and glutathione peroxidase have complementary roles in hydrogen peroxide detoxication. In the present study, CAT activity in animals collected from all sites observed increases in either tissues or heamolymph compared with reference site. These increases are due to regulation by ROS as stated previously by **Hermes-Lima (2004)**. GPx activity in the collected animals from the polluted sites of this region decreased during most seasons in comparison with other animals in the reference site. Among effect of pollutants discharge into water, S4 (near fertilizers factories) demonstrated the highest responsibility for oxidative stress than other sites, followed by S5 and S3, respectively. Previous studies demonstrated the oxidative stress in different species of mollusks impacted different pollutant in coastal areas, marine and freshwater. As example, in mussels, *Mytilus galloprovincialis* (**Vlahogianni et al. 2007**), *Perna perna* (**Almeida et al. 2007**), and freshwater mussel (**Falfushynska et al. 2014**).

The levels of LPO and CP in selected gastropod were significantly higher in polluted

sites than other animals of reference site. This fact indicated that, some oxidative injury may be related to POP_s toxicity and accumulation. Previous studies in other polluted regions marked an increase of LPO and CAT levels in the digestive gland than those of the reference organs of mussels *M. yuyanensis* (**Torres et al. 2002**) and snails; *Theba pisma* (**El-Gendy et al. 2009** and **Radwan et al. 2010**), *Helix aspersa* (**Abdel-Halim et al. 2013b**).

Formation of CP was checked in this study as an index of protein oxidation of exposed mollusk to industrial effluents in this region. The obtained data revealed the induction of CP in most collected animals from desired sites. Sites 1, 2 and 4 displayed the most response among CP levels in their exposed mollusk. This induction in digestive gland samples disclosed the relationship between the distance of the sampling sites and contamination source, where the level of detected CP increased in a direct proportional manner with the distance to emission source (S1> S2> S4). Since digestive gland is the principal feeding organ of mollusk and the first organ to come into contact with xenobiotic, it is plausible that metals and/or other xenobiotic entering the animal *via* this organ may cause transient oxidative stress resulting in protein glutathionylation. Overall, our data suggest that long term chronic exposure to pollutants can result in higher levels irreversible modification such as carbonylation of protein (**McDonagh *et al.* 2005**). Moreover, winter season was the mostly polluted period with chemicals resulting in CP induction more than other season compared with reference zone (S6). Other literatures stated that, tissue-specific formation of protein carbonylation by environmental by pollutants such as POP_s and heavy metals had been demonstrated by proteomic approach in few mollusk's species (**McDonagh et al. 2005, 2006, Dowling et al. 2006** and **Chora et al. 2008**). Other findings previously established inducting levels of CP in response to oil exposure (**Prevodnik et al. 2007**) or PAHs (**Almorth et al. 2005** and **Kaloyanni et al. 2009**). On the other hand, exposed gastropods to OP_s and PAH_s observed significantly CP content in their digestive gland tissue (**Itziou et al. 2011**). In Kafr El-Zayat region, **Abdel-Halim et al. (2013b)** stated that, land snail *Helix aspersa* affected with breathing contaminated air of industrial emission resulting in significantly CP induction in digestive glands, especially in groups near brick making and pesticides factories. In agreement with the previous data, **Gupta *et al.* (2007)** also supported the correlation between CP content formation and ROS generation with the up-regulation of the latter in environmental toxicants exposed organism, indicating the involvement of ROS in induction protein modification.

From these findings, we would to focus on the use of cumulative biomarkers as approaches of oxidative stress, immune functions, DNA adducts, and protein profile of selected mollusks may state imposed risk from industrial effluents and urbanized activity. These outcomes investigate good information among ecosystem status of Kafr El-Zayat region and arise predicted risk of chemicals emitted from industrial units on aquatic organisms. Moreover, governmental and management decisions and control options must be done for environmental remediation resulting in good quality of ecosystem arising human health protection outcome.

REFERENCES

Abdel-Halim KY. 2014. Biomarkers of freshwater algae, *Lemna minor* as a model for urban pollution with pesticides and heavy metals. Alex. Sci. Exch. J. 5(2): 96-106.

Abdel-Halim KY, Abo El-Saad AM, Talha MM, Hussein AA .2013b. Protein profile in land snail (*Helix aspasra*) exposed to environmental stress in an urban District. Proc. of (ICES), 21-22: 1-10

Abdel-Halim KhY, Abo El-Saad AY, Talha MM, Hussein AA, Bakry NM. 2013a. Oxidative stress on land snail *Helix aspersa* as a sentinel organism for ecotoxicological effects of urban pollution with heavy metals. Chemosphere 93, 1131-1138.

Ahmad MK, Islam S, Rahman S, Haque MR, Islam MM. 2010. Int. J. Environ. Res. 4(2): 321.

Almeida EA, Bainy ACD, Dafre AL, Gomes OF, Medeiros MHG, Di Mascio P. 2005. oxidative stress of digestive gland and gill of the brown mussel (*Perna perna*) exposed to air and re-submersed. J. Exp. Mar. Biol.

Ecol. 318: 21-30.
Almeida EA, Marques SA, Klitzke CF, Bainy, ACD, Medeiros MHG, Di Mascio P. 2003. DNA damage in digestive gland and mantle tissue of the mussel *Perna perna.* Comp. Biochem Physiol. C 135: 295-303.
Almeida EA, Miyamoto S, Bainy ACD, Medeiros MHG, Di Mascio P. 2004. Protective effect of phospholipid hydroperoxide glutathione peroxidase (PHGPx) against lipid peroxidation in mussels *Perna perna* exposed to different metals. Mar. Pollut. Bull. 49: 386-392.
Almeida EA, Bainy ACD, Loureiro APM, Martinez GR, Miamoto S., Onuki J. 2007. Oxidative stress in *Perna perna* and other bivalve indicators of invironmental stress in the Brazilian marine environment: Antioxidants, lipid peroxidation and DNA damage. Comp. Biochem. Physiol. 146(A): 588-600.
Almorth CB, Sturve J, Berglund A, Forlin L. 2005. Oxidative damage in eelpout (*Zoarces viviparous*) measured as protein carbonyls and TBARS, as biomarkers. Aquat. Toxicol. 73: 171-180.
APHA 1998. Standard methods for the examination of water and waste water. 20th Ed. American Public Health Association. (APHA), Washington, D.C.
Beeby A, Richmond L. 2003. Do the soft tissues of *Helix aspersa* serve as quantitative sentinel of predicted free lead concentrations in soil. Appl. Soil. Ecol. 22: 159-165.
Beers JRA, Sizer RF. 1952. Spectrophotometric method for measuring the breakdown of hydrogen peroxide by Catalase. J Biol Chem. 195: 133-140.
Bhalchandra W, Ram P. (2013). Bioaccumulation of heavy metals in freshwater snails *Bellamya bengalensis*, and *Lymnea accuminata* from Malangon wetland of Dhule district (Maharashtra) Indea. Nature to Survive 8(3): 1043-1047.
Chang LW, Magos L, Suzuki T. 1996. Toxicology of Metals. CRC Lewis, Boca Raton, FL.
Chen TB, Zheng YM, Lei M, Huang ZC, Wu, HT, Chen H, Fan KK, Yu K, Wu X, Tian QZ. 2005. Assessment of heavy metals pollution in surface soils of urban parks in Beijing, China. Chem. J. 60: 542-551.
Chezzi P, Bonetto V. 2003. Redox proteomics: identification of oxidatively modified proteins. Proteomics 3: 1145-1153.
Chora S, Mc Donagh B, Sheehan D, Atarita-Geribalidi M, Romes M, Bebianno J. 2008. Ubiquination and carbonylation as marker of oxidative stress in *Ruditapes decussates*. Mar. Environ. Res. 66, 95-97.
Cohort Software Inc (1985). Costat User Manual, Version 3 cohort Tucson, Arizona, USA.
Di Giuilo RT, Benson WH, Sanders BM, Van Veld PA. 1995. Biochemical mechanisms: metabolisms, adaptation, and toxicity. In Fundamental of Aquat. Toxicol. Effects of environmental fate, and risk assessment, ed. G. Rand. Taylor and Francis, London.
Dowling V, Hoarau PC, Roméo M, O'Halloran J, van Pelt F, O'Brien N. 2006. Protein carbonylation and heat shock response in Ruditapes decussates following *P, P'*-dichloro-diphenyldichloroethylene (DDE) exposure: a proteomic approach reveals that DDE causes oxidative stress. Aquat. Toxicol. 77: 111-118.
EC 2011. Common implementation strategy for the water framework directive (2000/60/ec): Guidance document No. 27. Technical guidance for driving environmental quality standards. European communities.
El-Gendy KS, Rawan MA, Gad AF. 2009. In vivo evaluation of oxidative stress biomarkers in the land snail, *Thepa pisana* exposed to copper –based pesticides. Chemosphere 77: 339-344.
Falfushynska HI, Gnatyshyna LL, Osadchuk, OYU, Farkas A, Vehovszky A, Carpenter, A. 2014. Diversity of molecular responses to separate wastewater effluents in freshwater effluents in freshwater mussels. Comp. Biochem. Physiol. 164: 51-58.
Flohe L, Gunzler WA. 1984. Assays of glutathione peroxidase. In Methods of Enzymology. Academic Press, New York, USA.
Girotti AW. 1998. Lipid hydroperoxide generation, tumover, and effector action in bio-

logical system. J. Lipid Res. 39: 1529-1542.
Gomot de-Vaufleury A, Pihan F. 2000. growing snails as sentinels to evaluate terrestrial environment contamination by trace elements. Chemosphere. 40: 275-284.
Gupta SC, Siddique HR, Mathur N, Vishwakarma AL, Mishra RK, Saxena DK.. 2007. induction of hsp70, alterationsin oxidative stress markers and apoptosis against dichlorovos exposure in transgentic Drosophila melanogaster: modulation by reactive oxygen species. Biochim Biophys Acta. 1770: 1382-1394.
Halliwell B, Gutteridge JMC. 1999. Free Radical in Biology and medicine. Oxford University Press, Oxford.
Hermes-lima M. 2004. Oxygen in biology and biochemistry: role of free radicals. In: Storey, K.B. Functional Metabolism: Regulation and Adaptation. John Wiley & Sons, New York, pp. 319-368.
ISO/IEC 1990. EN 45001 guide 25-general requirements for competence of calibration and testing laboratories. Geneva.
Itzoiu A, Kaloyianni M, Dimitriadis VK (2011). Effects of organic contaminants in reactive oxygen species, protein carbonylation and DNA damage on digestive gland and heamolymph of land snails. Chemosphere 85: 1101-1107.
Kaloyianni M, Dailianis S, Chrisikopoulou E, Zannou A, Koutsogiannaki S, Alamdari L. 2009. Oxidative effects of inorganic and organic contaminants in haemolymph of mussels. Comp. Biochem. Physol. C 149: 631-639.
Kanakaraju D, Jios C, Long Sh.M. 2008. Heavy metals concentrations in the Razor clams *(Solen spp)* from Muara Tebaz Sarawak. Malaysian J Anal Sci. 12 (1): 53-58.
Kesavan K, Murugan A, Vekatesan V, Vijkumar BS. 2013. Heavy metals accumulation in molluscs and sediment from Vppanar Esruary, southeast coast of India. Int J Mar Sci. 29(2): 15-21.
Lau S, Mohamed M, Yen ATC, Suiut S. 1998. Accumulation of heavy metals in freshwater molluscs. Sci. Total Environ. 214, 113-121.
Lemaire P, Livingstone DR. 1993. Prooxidant/antioxidant processes and organic xenobiotic interactions in marine organisms, in particular the flounder *Platichthys fleuses* and mussel *Mytilus edulis*. Trends in Comp. Biochem. & Physiol. 1: 1119-1150.
Lowry OH, Rasebrough NJ, Farr AL, Randall RJ. 1951. Protein measurement with the folin phenol reagent. J Biol Chem. 193, 265-275.
Mahler BJ, van Metre PC, Bashara TJ, Wilson JT, Jehns DA. 2005. Parking lot sealcoat: an unrecognized source of urban polycyclic aromatic hydrocarbons. Environ Sci Technol J. 39: 5560-5566.
McDonagh, B., Tyther, R., Sheehan, D. 2005. Carbonylation and glutathionylation of proteins in the blue mussel *Myrilus edulis* detected by proteomic analysis and Western blotting: actin as a target for oxidative stress. Aquat Toxzcol 73: 315-326.
Moloukhia H, Sleem S. 2011. Bioaccumulation, Fate and Toxicity of Two Heavy Metals Common in Industrial Wastes in Two Aquatic Molluscs. J. Amer. Sci. 7(8): 459-469.
Olowu RA, Ayejuyo OO, Adewuyi GO, Adejoro IA, Denloye AAB, Babatunde A.O. 2010. Determination of heavy metals in fish tissues, water and sediment from Epe and Badagry Lagoons, Lagos, Nigeria. http: / www.e-journals.net. 7(1): 215-221.
Papiri S, Ciappi C, Capodaglia A, Collivignarelli C, Bertanza G, Swartling F. 2003. Field monitoring and evaluating of innovative solutions for cleaning storm water runoff. Water Sci Technol J. 47: 327-334.
Pinto E, Segaud-Kutner TCS, Leitae MAS, Okamoto OK, Morse D, Colepicolo P. 2003. Heavy metals-induced oxidative stress in algae. Physiol. 39, 1008-1088.
Prevodnik A, Gardesttromb J, Lilja K, Elfwing T, McDonaghc B, Petrovic N. 2007. Oxidative stress in response to xenobiotics in the blue mussel *Mytilus edulis* L.: evidence for variation along a natural salinity gradient of the Baltic sea. Aquat. Toxicol. 82: 63-71.
Radwan MA, El-Gendy KS, Gad AF. 2010. Biomarker of oxidative stress in the land snail *Theba pisana* for assessing ecotoxicological effects of urban metal pollution. Chemosphere 79: 40-46.
Regoli F, Gorbi S, Fattorini D, Tedesco S, Notti A, Machella N, Bocchetti R, Benedetti

M, Piva F. 2006. Use of the land snail Helix aspersa as sentinel organism for monitoring ecotoxicological effects of urban pollution: an integrated approach. Environ. Health Prespect. 114: 63–69.

Regoli F, Principato G. 1995. Glutathione, glutathione dependent and antioxidant enzymes in mussel, *Mytilus galloprovincialis*, exposed to metals under field and laboratory conditions. Toxicol. 31: 143-164.

Rice-Evans CA, Diplock AT, Symons MCR. 1991. Technique in Free Radical Research. Elsevier, Amsterdam.

Salvo F, Ziino M, Signorino D, Lezzi U, Dugo G, Chioalo B. 1998. Chemical composition (*Thunnus thunnus* L). from the strait of on Bluefin tuna Messina waters. La riv Di Sci. dell Aliment. 1: 27.

Sies H. 1986. Biochemistry of oxidative stress. Angew Chem Int. 25, 1058-1071.

Sokel RR, Rohlf FJ. 1969. Biometry W.H., Freeman, San Francisco, USA.

Stedman EP, Levine RL. 2000. Protein oxidation. Ann NY Acad Sci. 899: 191-203.

Torres MA, Testa CP, Gaspari C, Masutti MB, Pagitz CMN. 2002. Oxidative stress in the mussel *Mytella guyanensis* from polluted mangroves on Santa Cattarina Island, Brazil. Mar Pollut Bull. 44: 923-932.

Valko M, Morris H, Cronin MTD. 2005. Metals toxicity and oxidative stress. Curr Med Chem. 12: 1161-1208.

Van Metre PC, Mahler BJ. 2005. Trends in hydrophobic organic contaminants in urban and reference lake sediments across the United States, 1970-2001. Environ Sci Technol J. 39: 5567-6674.

Wannaz ED, Carreras HA, Perez CA, Pignata ML. 2006. Assessment of heavy metals accumulation in two species of (*Till andsia*) in relation to atmospheric emission sources in Argentina. Sci. Total Environ. 361: 267-278.

Yap CK, Cheng WH. 2013. Distributions of Heavy Metal Concentrations in Different Tissues of the Mangrove Snail *Nerita lineata* Sains Malaysiana 42(5)**:** 597-603.

Zhou Q, Zhan J, Fu J, Shi J. Jiang G. 2008. Biomonitoring: An Appealing Tool for Assessment of Metal Pollution in the Aquatic Ecosystem. Anal Chim Acta. 606 (2): 135-150.