Biomarkers of Freshwater Algae *Lemna minor* as a Model for Urban Pollution with Pesticides and Heavy Metals

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ABSTRACT

Biochemical changes in collected algal cells of *Lemna minor* were examined as biomarkers for pesticides and heavy metals pollution for different sites of an urban district. Four contaminated sites were chosen for the biomonitoring programs, while another site (rural zone) was considered as a reference to compare the risk factors. Aspartate aminotransferase (AST) reached the lowest values in winter season to account for 3.22, 6.75, 5.43 and 2.35 U. L^{-1} in Potato International Center (PIC)(S1), El-Nasaria (S2), Kafr Hashaad (S3) and Bounfer (S4), respectively. Multiresidue of pesticides and potential toxic metals were examined in linear regression analysis with some biochemical components in algal cells. During the same season, the activity of alanine aminotransferase (ALT) showed decrease only in S3 and S4, but recorded the highest value (10.00 U. L^{-1}) in S2.

Carbohydrates and total protein levels were significantly decreased in all sites compared with reference. The algal pigments reached the lowest values in S1 to account for 4.76, 1.83, and 1.97 mg. L^{-1} for chlorophyll a, b and carotenoides, respectively. Therefore, this study showed the importance of freshwater algae in biomonitoring programs especially for the urban regions.

INTRODUCTION

Biomarker 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 (Contardo-Jara and Wiegand, 2008). Biomonitoring can be conducted by sampling organisms living in the investigated areas (passive biomonitoring) or by exposure of organisms from either reference site or laboratorial culture to the investigated area (active biomonitoring) (Franzle, 2006).

Aquatic ecosystems can be considered as final sinks for many environmental contaminants. Practically, watercourses of urban areas are affected by high 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). Generally, industrial influents cause significant distribution especially in aquatic media and their biota (Longston, 1990; Claisse and Alzieu, 1993). In addition, certain kinds of contaminants such as pesticides and heavy metals are considered the main industrial effluents introduced into water around urban communities (Akcali and Kucuksezgin, 2011).

Consequently, only the species showing some tolerance against pollution can establish sustainable populations. Biomarker research has already focused to find sensitive organisms and specific physiological reactions to detect low pollution levels in the aquatic system (Hallare et al., 2005). The use of freshwater organisms as bioindicators for pesticides and heavy metals pollution is currently very common. Algae and mollusks are considered suitable sentinel animals for this purpose (Rainbow, 1995). In addition, microalgae have ecological significance as a result of their position at the base of the aquatic food webs. Those attribute the microalgae to be used as sentinel organisms in environmental studies in order to evaluate the toxicity of various chemicals or pollution discharges, and particularly inputs of metals (Wei et al., 2006; Labra et al., 2007; Liebig et al., 2008).This group (algae, *Lemna minor*) is smallest flowering plants in the world which grow in foliage cover on freshwater bonds or streams. Sometimes, it is called lesser duckweed. Ducks and some fish species consume it as well as muskrat, beaver, birds and frogs. Environmental scientists use duckweeds to remove unwanted substances from water or assessing their toxicity. For this study, it was used as a bioindicator for aquatic pollution with pesticides and heavy metals in Kafr El-Zayat district resulting from industrial emission and discharge.

MATERIALS AND METHODS

1. Description of the study area

Kafr El-Zayat region, El-Gharbia governorate, Egypt was selected for this study as an urban model. It is considered as one of the main region due to the fact that, a number of large factories are established in it, especially chemical industries. Ecosystem around this region has an extensive exposure to chemical influents (Abdel-Halim et al., 2013).

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Algae, *L. minor* (family; Lemnaceae) was used as a bioindicator for urban pollution of freshwater bonds. Algae were seasonally collected during 2012-2013 from four locations as illustrated in figure 1. The samples were transferred to the laboratory, cleaned up and stored at -20 $^{\circ}$C until used. The chosen sites were as follows: S1, Potato International Center (PIC), S2 (Kafr El- Nasriyyah) and S3 (Kafr Hashaad) are near the brick factories. Site 4 (Binufar village) is near the pesticide and fertilizer factories. In addition, S5 (rural zone) was selected as a reference to compare the risk factors.

2. Chemical analysis

2.1. Pesticide residues.

Five g of algal tissues were homogenized using a polytron homogenizer (Janke&Kunkel, Gmb Hu Co KG) for 1 min. The suspension was mixed with 10 ml of acetonitrile and shaken for 1 min. One g of NaCl and 4g of $MgSO_4$ were added, vortexed immediately for 1 min and centrifuged at 4000 rpm for 10 min. An aliquot (1 ml) was placed into a micro centrifuge tube (2 ml) containing 50 mg primary-secondary amine (PSA) and 150 mg $MgSO_4$ vortexed for 1 min and re-centrifuged at 6000xg for 10 min. The supernatant was checked on gas chromatography (Jiang et al., 2009). The analysis was carried out using gas chromatograph (Agilent 7890) equipped with electron capture detector (ECD) and HP-5MS capillary column (30m × 0.25mm i.d. with 0.25µm film thickness). The oven temperature was programmed from an initial temperature of 80 $^{\circ}$C (2min hold) to 260 $^{\circ}$C at a rate of 5 $^{\circ}$C. min^{-1}, while injector and detector temperature were maintained at 300 and 320 $^{\circ}$C, respectively. Nitrogen was used as a carrier gas at flow rate of 3ml. min^{-1}. DB-17 capillary column was applied as a confirmatory procedure.

Methods and instruments were fully validated as part of a laboratory quality assurances system (ISO/IEC, 1990). The Codex Committee's Criteria for quality assurance were followed to determine the performance of the multi-residue method. Recovery, accuracy, and limit of detection (LOD) were determined for every compound.

2.2. Heavy metals quantification.

Half g of dry algae was digested with perchloric: nitric acid (1:4 v/v) to constant volume. After cooling, the samples were filtered and made up to the final volume of 25 ml using deionized water (Akhbar Jan et al., 2010). Potential toxic metals Cr, Pb, Cd and Ni were examined on wavelengths 357.9, 217, 228.8, and 232nm, respectively, in duplicates by using atomic absorption spectrometer (Spectra AA 220; Variance) at the department of water and soil (Alexandria University, Egypt). The concentration of each metal was calculated as mg. kg^{-1} dry weight and LOD was calculated as double the standard deviation of a series of measurements. All measurements were closed to the blank absorbance measurement (USEPA, 1983).

Appropriate quality assurance procedures and precautions were carried out to ensure reliability of the results. The samples were carefully handled to avoid contamination. Deionized water was used throughout the study.

2.3. Biochemical quantification.

2.3.1. Plant pigments.

Chlorophyll a, b and carotenoids were determined in algal suspension according to the method of Moran and Porath, (1980) by using N, N-dimethylformamide as an extraction solution. The absorbance was measured at 662, 644 and 440 nm for chlorophyll a, b and carotenoids, respectively, by using spectrophotometer (JENWAY 6305 UV/VIS). The pigment's concentrations were estimated as follows: chlorophyll a = 9.784 × E.662-0.99 × E.644; chlorophyll b= 21.426 × E.644-4.65×E.662 and carotenoides = 4.695×E.440-0.268×(chl. a +chl. b), respectively. The concentration was expressed as mg. L^{-1}.

2.3.2. Total carbohydrate.

After pigments extraction, the algal cells were extracted with 1N NaOH in a boiling water bath for 2hr. The final extract was used for protein and carbohydrate quantification (Payne and Stewart, 1955). Total carbohydrate was determined as glucose by the method of Dubois et al. (1956)**.**

2.3.3. Total soluble protein.

The protein content was determined according to the method of Lowry et al., (1951).

2.3.4. Aspartate aminotransferase (AST) & Alanine aminotransferase (ALT)

The algal cells were washed several times and suspended in 60 ml of 100mM of Tris HCl buffer, PH 7.6 containing 1mM EDTA and 6mM mercaptoethanol. The all were sonnicated for a total of 10 min (1 min each) and centrifuged at 20000xg for 20 min. The supernatant was subjected to ammonium sulphate precipitation (between 35 and 65%). The protein was dissolved in 20mM of the pervious buffer. All procedures were carried out at 4 $^{\circ}$C (Ohmori et al., 1988). The activities were measured according to the method of Gello et al., (1985) by using pyridoxal phosphate reagent against blank at 340nm. The activity was expressed as U. L^{-1}.

2.4. Statistical analysis.

All the data were presented as a mean value ±SE and subjected to analysis of variance (ANOVA). In addition, the means were compared for significance by Lethal Significant Difference (LSD) at 0.05. The regression coefficient (RC) between biochemical activities and pollutants concentration was estimated. The statistical analysis was performed using the Costat program (Cohort Software Inc, 1985).

RESULTS AND DISCUSSION

1. Chemicals quantification.

The residue levels of both pesticides and heavy metals in algal cells were quantified as presented in Table 1. The maximum residue levels were 1893.33, 1811.03, 663.31, 501.42 and 176.98 µg. Kg^{-1} wet mass for permethrin, deltamethrin, γ-BHC, α-BHC and HCB, respectively. On the other hand, *P,P′*-DDE, heptachlor, *P,P′*-DDD showed the lowest seasonal means to be 0.34, 0.41 and 0.63 µg. Kg^{-1} wet mass, respectively. Among heavy metals levels, Cr and Ni recorded the maximum values 67.66 and 54.12 mg. Kg^{-1} dry weight, respectively. However, the lowest mean was recorded for Cd (1.71 mg. Kg^{-1} dry weight). The limits of detection and percentages of recovery were showed in Table 2.

2. Biochemical quantification

2.1.1. Plant pigments.

The pigments in algal cells e.g. chlorophyll a, b, and carotenoids were measured and illustrated in figure 2. The locational mean of chlorophyll a content reached the lowest value (4.76 mg. L^{-1}) in S1 compared with S5 which not exceeded than 6.78 mg. L^{-1}. However, chlorophyll b content reached this value (1.68 mg. L^{-1}) in S2 followed by S1 which accounted for (1.83 mg. L^{-1}). Among carotenoids, the ascending order was as follows: S5>S4>S2>S3>S1, respectively.

2.1.2. Total carbohydrates.

Total carbohydrates were determined colorimetrically in algal cells as illustrated in figure 3a. The carbohydrates levels reached the highest value during autumn season in S1 (0.51 mg. L^{-1}). However, the locational mean showed the lowest value (0.23 mg. L^{-1}) in S4 compared with S5 which not exceeded than 1.11 mg. L^{-1}. Among seasonal mean, the lowest value was 0.24 mg. L^{-1} during summer season.

2.1.3. Total protein.

The protein levels were determined in algal cells as illustrated in figure 3b. The protein levels decreased in most sites, where S2 observed the lowest values (2.02 mg. L^{-1}) during autumn season. In addition, locational mean reached the highest value (1.46 mg. L^{-1}) in S3 followed by S4 which accounted for 1.33 mg. L^{-1}. No significant difference obtained in seasonal mean in all sites compared with reference which not exceeded than 1.14 mg. L^{-1}.

2.1.4. AST & ALT.

The enzymes activity was seasonally assayed in algal cells as illustrated in figure 4. The activity of AST recorded the highest values in all sites during summer season. Moreover, S4 recorded the highest value (17.62 U. L^{-1}) in S2, while the lowest value observed in S1 to account for 7.63 U. L^{-1}. However, ALT activity was lower than those of AST in most sites. Thus, the activity reached the highest value 20.08 U. L^{-1} in S1. Generally, the locational mean reached the highest value in S1 followed by S2 to account for 11.73 and 10.52 U. L^{-1}, respectively.

3. Relationship between pollutants and algal biomarkers.

To investigate this relation, a simple linear regression analysis of biomarker responses on metals concentrations was conducted in algal cells for seven different sites of Kafr El-Zayat district as illustrated in Table 3. The data showed that, potential toxic metals were in a positively relation with AST, while in positively relation with ALT. Among algal pigments, they were in negatively relation with all pigments. In case of pesticides, multi-linear regression analysis was conducted as illustrated in Table 4.

DISCUSSION

The present study investigated the impact of aquatic pollution along urban discharge of Kafr El-Zayat district on some biochemical components of freshwater algae *L. minor*. In this region, industrial discharge and urban wastes disposal into water are the major source of pollution to selected sites near emission sources. Practically, watercourse of urban areas are affected by high inputs of mixtures of organic and inorganic compounds from various sources such as domestic and industrial sewage water, abrasion from streets and vehicles (Papiri et al., 2003; Mahler et al., 2005; van Metre and Mahler, 2005). According to algal distribution in freshwater, uptake of unwanted substances, and easy to sample, the *Lemna minor* was selected as a bioindicator for aquatic pollution with pesticides and toxic heavy metals in Kafr El-Zayat district resulting from industrial effluents and urban discharge.

The obtained results showed that, seasonal residue levels of pesticides reached the highest values for ΣBHC, aldrin, methoxychlor and heptachlor epoxide, respectively, and other group e.g. permethrin and deltamethrin.

Table 1. Seasonal residue levels of pesticides and heavy metals in algal cells of *Lemna minor*.

compound	min	max	mean	n	variance	Stdev
			pesticides (µg. kg^{-1} wet mass)			
α-BHC	BDL	501.42	59.21	20	19017.21	274.01
HCB	BDL	176.98	28.34	20	2227.51	95.06
γ-BHC	BDL	663.31	93.57	20	30972.92	359.01
β-BHC	BDL	63.73	18.32	20	401.38	32.81
heptachlor	BDL	5.75	0.41	20	3.21	1.90
aldrin	BDL	156.63	14.03	20	1261.04	86.66
hept. epoxide	BDL	100.61	11.78	20	510.18	55.00
γ-chlordane	BDL	6.31	1.11	20	3.99	3.37
dieldrin	BDL	59.02	5.53	20	191.90	32.59
P,P'-DDE	BDL	5.36	0.34	20	3.00	1.54
endrin	BDL	36.18	6.83	20	108.87	19.22
P,P'-DDD	BDL	6.33	0.63	20	3.54	3.49
P,P'-DDT	BDL	66.22	9.19	20	410.49	35.88
methoxychlor	BDL	115.43	13.53	20	920.76	30.34
cyhalothrin	BDL	74.65	3.73	20	278.63	16.69
permethrin	BDL	1893.33	94.67	20	17923.9	423.36
deltamethrin	BDL	1811.03	629.14	20	6939987.0	2634.39
			heavy metals (mg. kg^{-1} dry weight)			
Cd	BDL	16.23	1.71	20	15.06	8.91
Pb	BDL	37.50	12.56	20	90.47	19.09
Cr	BDL	67.66	7.29	20	317.85	37.26
Ni	BDL	54.12	5.86	20	156.78	29.69

BDL= below detection limit; n= number of samples.

Table 2. Recovery percentage and the minimum detection limits for various compounds

compound	% recovery	SD	LOD (ng)
α-BHC	97	4	0.02
HCB	82	12	0.05
γ-BHC	93	9	0.01
β-BHC	95	5	0.04
heptachlor	98	10	0.03
Aldrin	92	8	0.01
hept. Epoxide	100	8	0.03
γ-chloredane	91	6	0.02
Dieldrin	102	6	0.02
P,P'-DDE	98	5	0.05
Endrin	96	3	0.01
P,P'-DDD	95	7	0.05
P,P-DDT	99	10	0.05
methoxychlor	91	10	0.02
cyhalothrin	80	10	0.06
permethrin	87	11	0.06
Fenvelerate	89	8	0.06
deltamethrin	94	9	0.06
Pb	100.0	30	0.1
Cd	77.0	3	0.02
Cr	73.9	15	0.06
Ni	81.3	20	0.1

SD= standard deviation and LOD=limits of detection.

Table 3. Simple linear regression analysis of biomarker responses (Y) on metals concentrations (X) in algae from contaminated sites of Kafr El-Zayat region

Biomarker response (y)	Metal (x)	r	r^2	*df*	*F*	Intercept (a)	Regression coefficient (b)
AST	Cr	-0.375	0.141	1	0.33	12.959	-0.239
AST	Cd	-0.381	0.145	1	0.34	12.927	-1.017
AST	Ni	-0.284	0.081	1	0.18	12.814	-0.226
AST	Pb	0.678	0.460	1	1.70	3.43	0.678
ALT	Cr	0.04	0.002	1	0.001	9.011	0.0059
ALT	Cd	0.049	0.002	1	0.002	9.05	0.030
ALT	Ni	0.026	0.001	1	0.001	9.018	0.0047
ALT	Pb	-0.127	0.016	1	0.030	9.397	-0.0292
Total protein	Cr	-0.885	0.783	1	4.260	1.108	-0.0029
Total protein	Cd	-0.832	0.692	1	4.510	1.108	-0.0123
Total protein	Ni	-0.747	0.558	1	2.520	1.109	-0.0033
Total protein	Pb	0.581	0.338	1	1.020	1.053	0.0032
Carbohydrates	Cr	0.779	0.607	1	3.080	0.398	0.0148
Carbohydrates	Cd	0.786	0.618	1	3.220	0.397	0.0626
Carbohydrates	Ni	0.669	0.448	1	1.910	0.390	0.0166
Carbohydrates	Pb	-0.592	0.350	1	1.080	0.690	-0.0176
Chloro. a	Cr	-0.175	0.031	1	0.060	6.478	-0.0466
Chloro. a	Cd	-0.175	0.031	1	0.050	6.444	-0.176
Chloro. a	Ni	-0.283	0.080	1	0.170	6.708	-0.0944
Chloro. a	Pb	-0.85	0.723	1	5.190	10.537	-0.356
Chloro. b	Cr	-0.324	0.105	1	0.230	4.525	-0.065
Chloro. b	Cd	-0.307	0.094	1	0.210	4.493	-0.26
Chloro. b	Ni	-0.427	0.182	1	0.450	4.720	-0.1077
Chloro. b	Pb	-0.812	0.659	1	3.88	7.287	-0.257
Carotenoids	Cr	-0.186	0.035	1	0.07	2.996	-0.0375
Carotenoids	Cd	-0.169	0.029	1	0.06	2.971	-0.1428
Carotenoids	Ni	-0.282	0.080	1	0.17	3.156	-0.0709
Carotenoids	Pb	-0.759	0.576	1	2.71	5.693	-0.2396

Regression follow the mod y = a+bx obtained by simple linear regression; r^2 =coefficient of determination; df =degrees of freedom value=1means a simple linear regression of equation) and F= variation.

Table 4. Multilinear regression analysis of biomarker responses (y) on pesticides concentration (x) in freshwater algae from seven different sites of Kafr El-Zayat district.

factors	Biomarker responses (y)						
	AST	ALT	TP	Carbohy.	Chloro. a	Chloro. b	Carotene.
df	18	18	18	18	18	18	18
f	1.961*	0.0897	0.473	1.421*	7.912**	4.527*	2.318*
a	6.483	7.998	1.089	0.521	7.133	4.998	3.238
Pesticides concentration (x)							
α-BHC	-0.072	0.038	-0.0008	0.0038	0.097	0.067	0.072
HCB	-0.052	-0.142	0.0008	-0.0024	-0.296	-0.217	-0.232
γ-BHC	0.122	0.036	0.0002	-0.0018	0.023	0.017	0.025
β-BHC	1.144	0.167	0.004	-0.025	-0.093	-0.058	-0.013
heptachlor	3.989	3.280	0.0056	-0.074	6.783	5.204	5.382
aldrin	-0.722	-0.008	-0.004	0.022	0.198	0.124	0.121
heptachlor Epoxide	0.319	-0.030	0.0011	-0.006	-0.257	-0.196	-0.178
γ-chlordane	6.098	1.852	0.0019	-0.135	2.14	1.711	1.938
dieldrin	-0.466	-0.002	0.0046	0.024	-0.0397	-0.073	-0.028
P,P'-DDE	-12.028	-7.639	-0.015	0.195	-13.746	-10.472	-11.154
endrin	0.868	0.539	-0.0018	-0.00001	0.699	0.492	0.597
P,P'-DDD	2.122	-1.163	0.018	-0.085	-3.489	-2.542	-2.589
P,P'-DDT	-1.931	-0.745	-0.0018	0.023	-0.748	-0.541	-0.688
methoxychlor	0.606	0.103	0.0016	-0.011	-0.05	-0.037	-0.005
cyhalothrin	1.100	0.514	-0.0023	0.002	-0.386	0.234	0.378
permthrin	-0.005	0.0002	-0.0001	0.0006	-0.006	-0.006	-0.004
deltamethrin	0.004	-0.0001	0.0001	-0.0001	-0.002	-0.001	-0.001

Regression follow the mod y = a+bx obtained by simple linear regression; r^2 =coefficient of determination; df =degrees of freedom value=1means a simple linear regression of equation) and F= variation (*= means the significant at 0.05; **=means the significant at 0.01 and *** = means the significant at 0.0001).

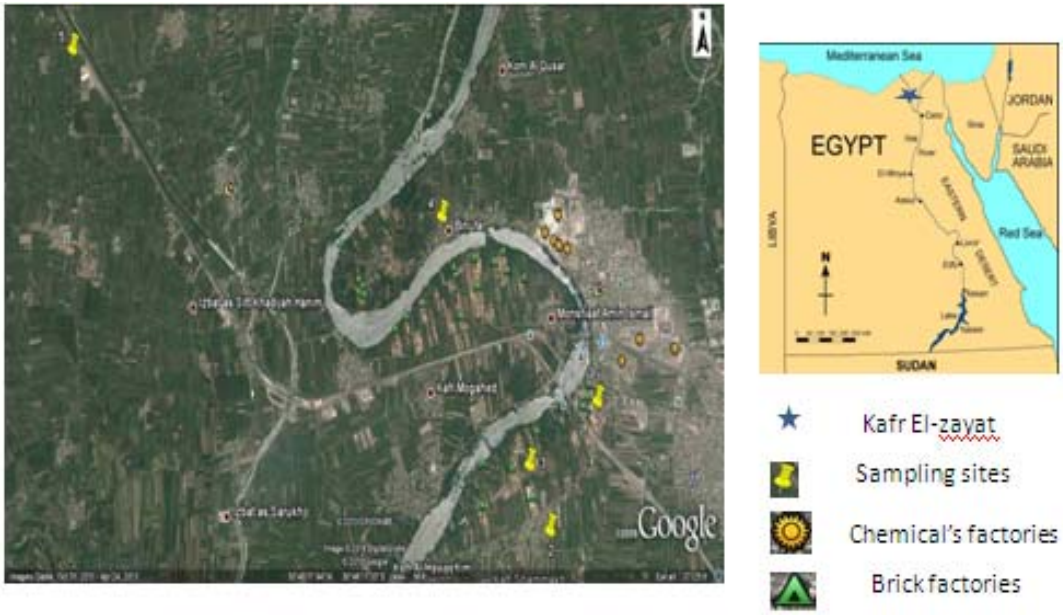

Figure1: Sampling sites and contaminants sources of Kafr El-Zayat city (http://earth.google.com).

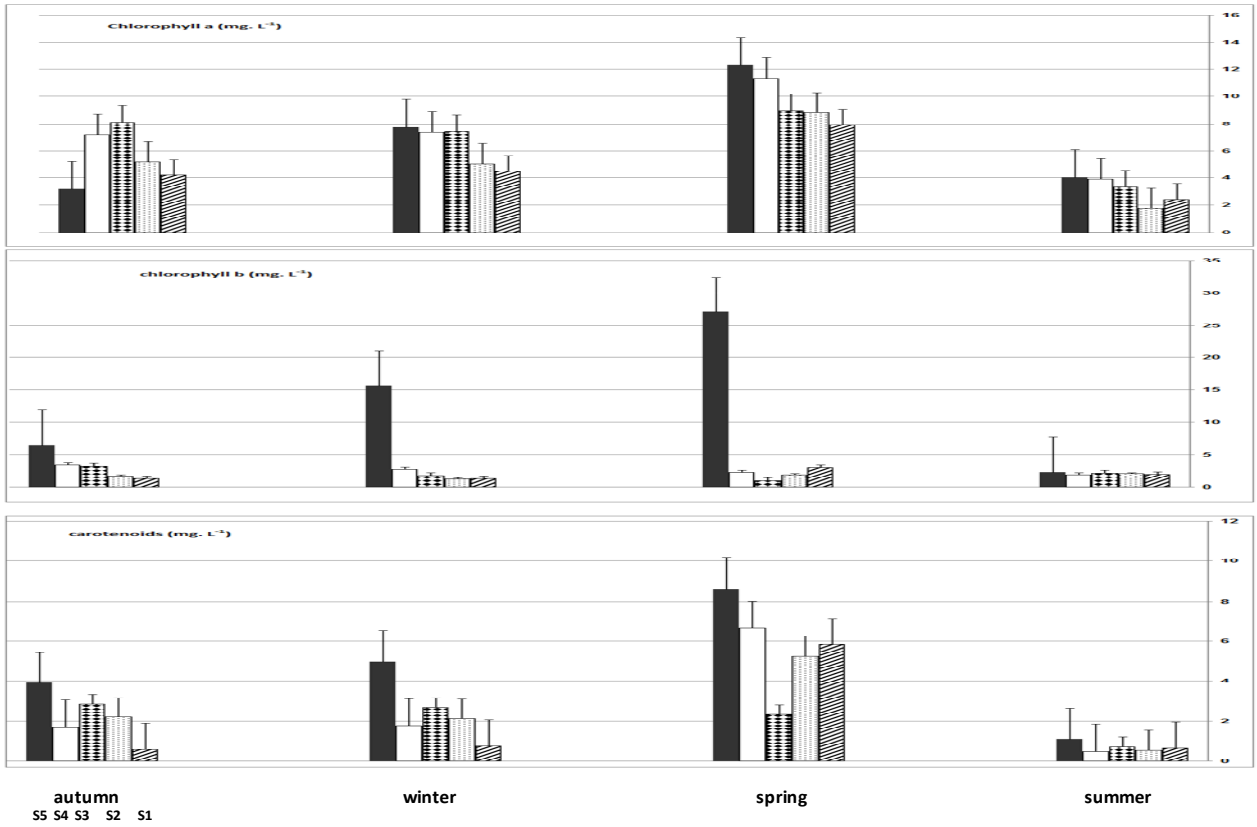

Figure 2. Alterations in algal pigments (2a) chlorophyll a; (2b) chlorophyll b; and (2c) carotenoids .Vertical bars indicate standard errors.

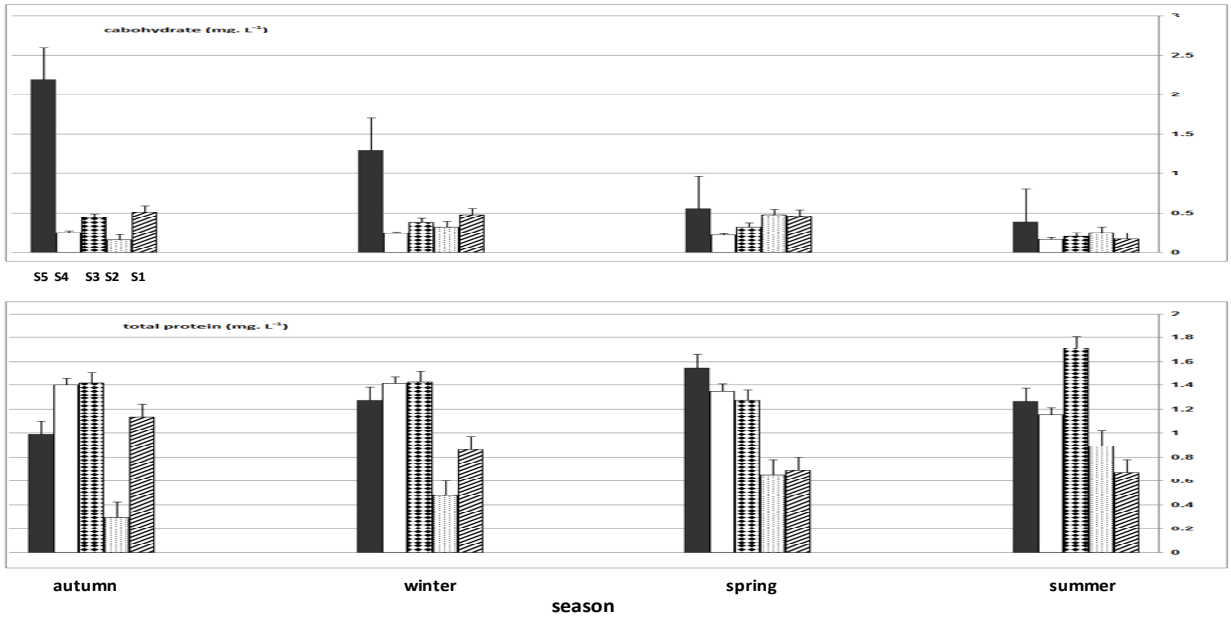

Figure 3. Alterations in biochemical components (3a) carbohydrate and (3b) total protein. Vertical bars indicate standard errors

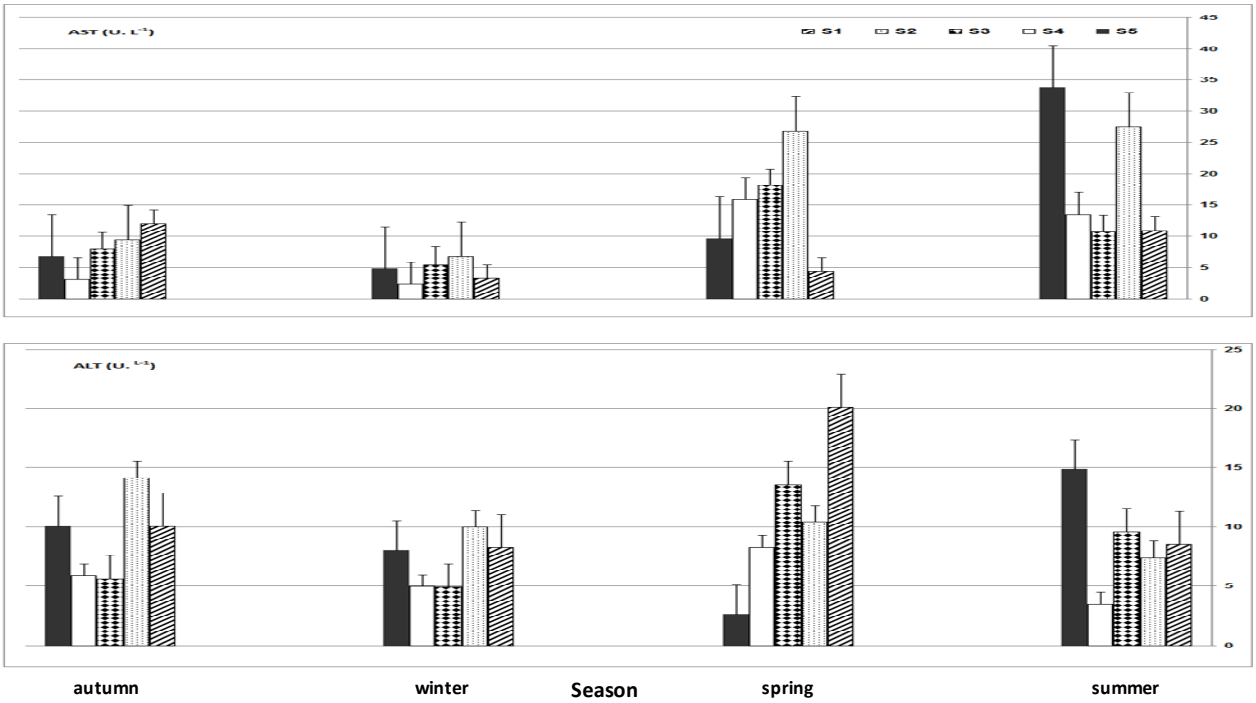

Figure 4. Alterations in enzymes (4a) AST and (4b) ALT. Vertical bars indicate standard errors.

Additionally, overuse or industrial emission of pesticides increases the probability of negative impacts on non-target organisms such as aquatic biota, terrestrial, plants, mammals and soil microorganisms (Tremolada et al., 2004).

On the other hand, Pb was detected in all seasons followed by Ni especially in S3 and S4 which are near brick or pesticides factories. The use of fuels in brick making factories is a major source of Pb and Cd, while Ni is mostly emitted from fertilizers and chemical industries near the sites as presented in figure 1. In addition, different industries such as plant oils extraction, textile, fertilizers, acids, salts, pesticides and detergents are major source of POP_s and heavy metals which are emitted in air. Another source is urban sewage water, where metals pollution has been associated with sewage outlets (Chen et al., 2005; Wannaz et al., 2006). The emission of these factories mostly precipitates on land and aquatic media such as streams and canals around them.

In worldwide, several studies investigated that, major source of air contamination is the non-ferrous metals industry which emits Cd, Pb, Ni, As, Cu, Se and Zn (Liu et al., 2003; Lewtas, 2007; Blake et al., 2007). Fuels in factories is the major source of Hg, As, Cr, and Se (Zhuang et al., 2004; Keegan et al., 2006; Guijian et al., 2007), while combustion of oil is the most important source of Ni and Vanadium (V)(US EPA, 2002c; Dundar, 2006).

Therefore, algae play an important role in the disposal, chemical transformation and bioaccumulation of many toxic compounds (Wang et al., 1998; Okay et al., 2000; Todd et al., 2002; Lei et al., 2002, 2007; Bopp and Lettieri, 2007). In addition, macro and microalgae also play an important role in the removal of polychlorinated biphenyls from the euphotic zone by direct sinking of the cells (Wang et al., 1998; Gerofke et al., 2005).

On the other hand, the movement of most POP_s in the environment is a complex task due to the distribution and exchange dynamics of these compounds in different physical phases. In addition, the cycle of volatilization and deposition may be repeated many times with the result that POP_s accumulate in an area far removed from where they initially used or emitted (Jones and Voogt, 1999). These concerns may link to the changes which done in the residue levels of POP_s in biota of contaminated sites of this region.

The changes and interactions of pollutants with algal biochemical components have important consideration as biomarkers of urban pollution. As mentioned previously, the study of physiological and biochemical alterations as well as the identification and quantification of pollutants in organisms are essential diagnostic tools (Van Gestel and Van Brummelen,

1996; Handy and Depledge, 1999; Handy et al., 2003). Additionally, photosynethyic organisms such as algae are early and timely indicators of potential hazard in aquatic systems and should be seriously considered in any environmental assessment program (Kowalewska, 1999; Okay et al., 2000). Many studies depending on organisms had been reported as regionally important tools in environmental programs e.g. fish in Australia, Asia and America (Edwards et al., 2001; Ueno et al., 2005; Carrasco-Letelier et al., 2006), land snails (Reogli et al., 2006; Radwan et al., 2010; Abdel-Halim et al., 2013), macroalgae (Fytianos et al., 1999; Sanchez-Rodrignez et al., 2001; Conti and Cecchetti, 2003) and microalgae (Siripornadulsil et al., 2002; Nishikawa et al., 2003; Pinto et al., 2003; Tripathi et al., 2006). Moreover, biochemical responses of the organisms exposed to POP_s, PAH_s and pesticides have been reported for the last two decades and documented by the international organizations such as Economic Corporation and Development (OECD) and the United States Environmental Protection Agency (USEPA) which recommended to use algae because of their wide distribution and their sensitivity with respect to the environmental evaluation of freshwater ecosystems (Bauer et al., 2012).

CONCLUSION

The obtained results of this study may provide information about the role of freshwater algae in biomonitoring of urban pollution with chemical contaminants. Algae; *L. minor* is considered a useful tool for diagnosis of aquatic system pollution, especially in urban regions. Biochemical components and enzymes of algae may be useful biomarkers to assess biomonitoring programs. Risk management programs must be done in this region for prevention ecosystem disruption and non-target organism's outcomes.

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الملخص العربي

الدلائل البيولوجية لطحاب المياة العذبة *Lemna minor* كنموذج لتلوث المناطق الحضرية بالمبيدات والعناصر الثقيلة

خالد ياسين عبد الحليم

تم تقييم الدلائل الحيوية في خلايا الطحاب المجمعة موسميا من مناطق مختلفة في منطقة كفر الزيات-محافظة الغربية-مصر. أربعة مناطق ملوثة تم اختيارها مقارنتا بموقع ٥ (منطقة ريفية). أنزيم اسبارت امينوترانسفيراز وصل لأعلى القيم في موسم الشتاء بواقع ٣.٢٢ – ٦.٧٥ – ٥.٤٣ – ٢.٣٥ وحدة/ لتر في موقع ١ (المركز الدولي للبطاطس) وموقع ٢ (كفرالنصارية) وموقع ٣ (كفر حشاد) وموقع ٤ (قرية بونفر) على التوالي. في نفس الموسم نشاط إنزيم الانين امينوترانسفيراز أوضح انخفاض فقط في موقعي ٣ و٤ بينما وصل لأعلى قيمة ١٠.٠٠ وحدة/لتر في موقع ٢.

مستوى الكربوهيدرات والبروتين سجل انخفاضا ملحوظا في كل المواقع مقارنة بالكنترول. على الجانب الآخر الصبغات النباتية وصلت لأعلى مستوى في موقع ١ لتمثل ٤.٧٦ – ١.٨٣ – ١.٩٧ مجم/لتر لكل من كلوروفيل ا وكلوروفيل ب والكاروتين على التوالي. من هذه الدراسة تتضح أهمية استخدام الطحالب في الرصد البيولوجي خاصة في المناطق المعرضة للتلوث الصناعي.