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Trends in genetic divergence among *Biomphalaria alexandrina* snails and *Oreochromis niloticus* fish in response to water quality of Lake Burullus, Egypt.

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ABSTRACT

Water quality of Lake Burullus was investigated through evaluating their impact on health and genetic alterations of native Lake Burullus *Biomphalaria alexandrina* snails and *Oreochromis niloticus* fish samples and non-native ones exposed in simulating experiments for six weeks to lake-water/sediment from three lake sites; El-Hamoul, Mottobus and Sidi-Salem. Water in El-Hamoul and Sidi-Salem lake sites and their simulating experiments showed higher levels of total dissolved solids and conductivity while higher levels of Zn and Cd were recorded in their simulating experiments. Also, most of highest bioaccumulation levels in native and non-native samples were recorded in samples of either El-Hamoul or Sidi-Salem while Mottobus samples showed the least levels in most of water quality creiteria and bioaccumulation. Higher Cu bioaccumulation was observed in the native snails and in kidney of native Sidi-Salem fish samples while most non-native fish showed higher bioaccumulation in all organs. All snail hemolymph samples showed increase in granulocytes, decrease in hyalinocytes and total hemocytes while Hb decreased in native and slightly increased in non-native Sidi-Salem and El-Hamoul samples and vice versa in Mottobus. Fish blood showed highly significant increase in WBCs of native El-Hamoul and Sidi-Salem samples while samples of native and non-native Mottubus showed the highest Hb level. Genetic divergences were investigated by ISSR-PCR technique in snail and fish samples: native lake, non-native non-exposed (control), and non-natives of El-Hamoul, Sidi-Salem & Mottobus experiments. Results of PCR demonstrated 33 and 26 amplified bands of which 30 % and 35% were polymorphic among snail and fish samples, respectively. Cluster analysis using dendrograms based on genetic similarity matrices showed cluster between control, lake, and non-native Mottobus groups; the non-native El-Hamoul sample was the most divergent followed by the non-native Sidi-Salem sample; indicating that water quality in Lake Burullus exerted divergence among snail and fish genomic DNA graded from El-Hamoul > Sidi-Salem > Mottobus samples.

INTRODUCTION

The northern Delta Lakes in Egypt comprise Edku, Burullus, Manzala, and Mariut make as reservoirs for the irrigation drain water before flowing into the

Mediterranean Sea. These lakes are an important natural resource for fish production in Egypt.

However, the lakes were subjected to a gradual shrinkage during the last few years due to land reclamation and transformation of the lakes to fish farms along their southern regions. Also, large parts of the lakes are overgrown by aquatic vegetation, besides, the lakes proper contains high numbers of islands that reduce their open water (Saeed, and Shaker, 2008). The Egyptian Lakes have been the main source of fish for a long time and have always contributed more than 40% of the country's total fish production, but at present this has decreased to less than 12.22% (GAFRD, 2006). Lake Burullus lies on the eastern side of Rosetta branch of the River Nile, occupying a central position along the Mediterranean Nile Delta coast of Egypt. It extends between longitude 30º 30' and 31º 10' E and latitudes 31º 21' and 31º 35' N. It is the second largest lake of the Nile Delta coastal lakes and is about 65 km long, and its width varies between 6 and 16 km with an average of about 11 km the depth of lake ranging from 0.5 to 2.5m (Frihy and Dewidar, 1993). Lake Burullus is connected to the sea at its northeastern edge through El-Burullus inlet (El-Boughaz), which is about 250 m wide and 5m deep and connected with the River Nile by Prembal canal. The present area of the lake is about 410 Km² (100,000 Fadden) of which 370 km^2 open water, it receives approximately 4 billion m^3 of drainage water per year from the Nile Delta agricultural lands (El- Shinnawy, 2002), which accounts for 97% of the water inflow (Shaltout and Khalil, 2005;Eid, 2012). The eastern and southern parts of the lake receive agriculture, sewage, drainage water through 8 drains (Nafea and Zyada, 2015). The lake includes a noteworthy number of environments, with swamps and sand plains prevailing, and constitutes an ideal habitat for 135 land and water plant species as well as an important stop-over point for migrating birds. The area is densely populated, with approximately one million people living around the lake (MedWet Culture Network, 2016).

Fish are often at the top of the food chain and have the tendency to concentrate heavy metals from water. Therefore, bioaccumulation of metals in fish can be considered as an index of metal pollution in the aquatic bodies. Nwajei *et al.* (2012) and El-Naggar *et al.* (2009) mentioned that, tilipia fish is a good bio-accumulator of heavy metals and it can be used as indicator for environmental pollution monitoring. Moreover, freshwater molluscs play an important role in aquatic ecosystems, providing food for many fish species and vertebrates (Maltchik *et al.*, 2010). They are abundant in many terrestrial and aquatic ecosystems, being easily available for collection. They are highly tolerant to many pollutants and exhibit high accumulations of them, particularly heavy metals. The Northern Delta lakes heavy metal levels were studied by many authors by estimating their level in water (Farag, 2002) and bioaccumulation in lake cichlid fish species (El-Ghobashy *et al.*, 2001). Mason (2002) elucidated that metals can be accumulated by aquatic organisms through a variety of pathways including respiration, adsorption and ingestion. Turkmen *et al.*, (2008) revealed that the coastal water of the Mediterranean Sea in Turkey was faced by metal pollution; Cd, Cr and Pb and these metals concentrations in muscles of fish were higher than the international permissible safety levels for human uses. Metal bioaccumulation in animal tissues depends on a number of factors which may be physiological like metal detoxifying proteins in the animal body and may be environmental such as temperature, the presence of other ions in the environment and distance of the organism from the contamination source, specific food habits, species, age, size of animal and exposure time, (Chouvelon *et al.*, 2011

and 2012). Also, Karakoc (1999) confirmed that there are many factors, physico-chemical and biological, affecting the accumulation and toxicity of metals in aquatic organisms. For instance, aqueous Cd, Pb, and Zn levels have been related to pH, dissolved oxygen, carbonate, and nutrients (Prahalad and Seenayya, 1989). Also, Mason (2002) interpreted that metal ions can be incorporated into food chains and concentrated in aquatic organisms to a level that affects their physiological state. The effective pollutants are the heavy metals which have drastic environmental impact on all organisms. Siegel *et al* . (1994) explained that high concentrations of heavy metals in lake sediments contaminated fish, especially bottom feeders, the case that realized in most of the Northern lakes that receive sewage discharges of major cities (Hamza, 2006). In the last years the problems of the drainage canals in Egypt have extremely increased. These problems include the presence of high concentrations of different metals and pesticides in both water and aquatic animals (Authman *et al*., 2008 a&b).

The use of biomarkers as indicators for water quality has been developed to detect its impacts on the inhabting aquatic funa. Prominent among these biomarkers are physiological variables, such as plasma levels of metabolites (DiGiulio *etal*., 1995) and hematological data (Lohner *et al*., 2001 and Cazenave *et al*., 2005). Interesting reports concerning the mechanisms of metal uptake, accumulation, transport, and elimination of metals in molluscs are usually focused on chemical, biochemical, molecular, and physiological aspects (Langston *et al.,* 1998). Also, inter simple sequence repeat (ISSR) is a PCR based molecular marker technique that has been proven to be highly useful for studying genetic diversity and population genetic structure of various animal species (Pazza *et al,*. 2007 and Moysés *et al.,* 2010).Thus, the ISSR-PCR strategy is especially attractive because it avoids the need to carry out costly cloning and sequencing inherent in the original microsatellite based approach (Nagaraju *et al.,*2002).This PCR-based method uses primers annealing to microsatellite repeats to amplify the regions between adjacent SSRs provided. They are close enough to allow exponential multiplication (Kramer *et al.,* 2007). In respect with, variable ISSR patterns have potentials as dominant markers for studying genetic diversity of many fishes (Tong *et al.,* 2005). Also, the ISSR technique has been reported as a good marker to differentiate between geographically different *Lymnaea natalensis* populations and a laboratory isolate (El-Khayat *et al.* 2015).

The present work aimed to investigate the effect of water quality in Lake Burullus expressed by certain physicochemical parameters and heavy metals on bioaccumulation, hematological variables, and genetic alterations using ISSR-PCR technique in native snail (*B. alexandrina*) and tilapia species (*O. niloticus*) samples collected from different lake sites and non-native *B. alexandrina* and *O. niloticus* samples exposed in simulating experiments to water and sediment from the examined lake sites.

MATERIALS AND METHODS

This study was carried out during the years 2015-2016 in Lake Burullus in 3 different centers, Kafr El-Sheikh governorate; El-Hamoul (El-Khashaa site), Sidi-Salem (Shakhloba site) and Mottobus (Prembal site), in North Delta, Egypt (Fig.1).

Field investigation and sampling:

Physico-chemical water quality criteria were recorded (e.g. temperature and conductivity were measured in the selected lake sites by temperature conductivity meter (HANNA instrument, HI 9635). Also, hydrogen ion concentration (pH) was

measured by pH meter (HI 8543) and dissolved oxygen was measured by dissolved oxygen meter (HANNA HI 9146). From each site samples of *B. alexandrina* were collected using scope net and placed in separate plastic aquaria then transferred to the laboratory to be tested for their natural trematode infections looking for negative snails that would be prepared and used for heavy metal bioaccumulation, hematological and molecular analysis. *O. niloticus* fish samples were collected with the help of professional local fishermen. After fish anesthesia (by ethyl alcohol), blood samples were collected by a cardiac puncture using a sterile syringe into tubes containing EDTA for hematological and genetic analysis. The blood tubes were preserved cool till transferring to laboratory. In addition, water samples were collected 30 cm below the water level in 1 liter polyethylene bottles from sites under study then were acidified and transferred to laboratory where they were filtered with filter papers then syringe filter 0.45 um and kept at 4°C till determination of the heavy metals: copper (Cu), cadmium (Cd) and zinc (Zn) levels; using atomic absorption spectrophotometer (AAS) (GBC AVANTA 3000, Australia) in the Environmental Research Laboratory, Theodor Bilharz Research Institute (TBRI), Egypt.

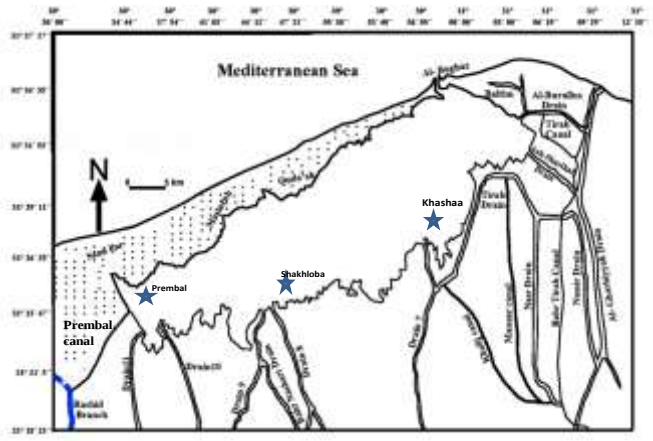

Fig. 1: Map of Lake Burullus (Egypt) indicating the locations of the three sampling sites (*).

Samples preparation and assessment:

To study the accumulation of heavy metals in the native lake samples, snails from each site were dissected free form their shells within 12 hours of collection while the fish were dissected to get muscle, liver, and kidney within one hour. The dissected tissues were dried at about 80ºC in an electric oven. After complete drying, the tissues of each sample were weighted and transferred separately to a labeled clean screw-capped glass bottle and digested with 10 ml of HNO3 solution (FAO, 1983). Digestion was conducted by heating at 40–45°C for 1 h in water bath and then raised to 70°C till the end of digestion. After cooling at room temperature, the digested sample was diluted to 25 ml with deionized water and filtered out into a volumetric flask and kept at -20ºC till determining the concentrations of Zn, Cd, and Cu using AAS.

Hemolymph was collected from snails represented each site according to Michelson (1966) via small hole made in the shell situated directly above the heart then a capillary tube was introduced into the heart to collect hemolymph. Also, snails' head-foot tissues were dissected free, fixed in 70% ethanol and maintained at 4◦C till used for DNA extraction. Hematological parameters: The total hemocytes of the hemolymph samples were determined by Burker-Turk haemocytometer. For differential count, monolayer of hemocytes were stained with Giemsa stain for 20

minutes, according to the methods of Abdul Salam & Michelson (1983) and counted by light microscopy. Fish blood samples were taken from 10 fish/ each lake site (~1.0 ml/fish) in EDTA-containing tubes for determining hematological and molecular analysis. Hematological analysis included hemoglobin content (Hb), red blood cell count (RBC), packed cell volume (PCV), mean cell volume (MCV), mean cell hemoglobin concentration (MCHC), and white blood cell (WBC) according to Rauthan *et al. (*2006).

Laboratory exposure:

Non-native *B. alexandrina* snails were collected from Giza watercourses then maintained in plastic aquaria (16 x 23 x 9 cm) containing de-chlorinated tap water and kept for four weeks under observation for their natural trematode infections and acclimatization. Fresh lettuce leaves were used for feeding twice a week and water in the aquaria was changed weekly. Dead snails were removed and healthy un-infected ones were used in the experimental exposure. In parallel, non-native apparently healthy *O. niloticus* fish (30 to 50 g in weight) were purchased from Abbassa fish aquaculture, Abou-Hammad, Sharkia governorate. Then transported in a sterile water tanks to the laboratory and acclimatized for two weeks in glass aquaria of 70 × 60 × 50 cm dimensions (15 fish / aquariaum).Water and sediment samples were obtained from the three examined sites of Lake Burullus (El-Hamoul, Sidi–Salem and Mottobus) for the experimental study. About 100 L of lake water and 10 Kg of sediment were collected from each site before sunrise then immediately transported to the laboratory.

Experimental design:

The tested snails and fish were divided into 4 groups each with two replicates in eight glass aquaria each containing 35 liter water and 4Kg sediment; one control group exposed to de-chlorinated water and autoclaved sediment from TBRI garden and three treated groups each exposed to water and sediment collected from one of the three examined sites of Lake Burullus; El-Hamoul, Sidi–Salem and Mottobus. Each aquarium contains 15 fish and 40 snails. The snails were maintained in two cylindrical plastic net (20 snails/ net) that hanged to the aquaria and immerged into the water with stone (about 100 g). The experiment continued for six weeks where the snails were fed twice a week with fresh lettuce leaves and fish were fed daily with artificial food meal. During the whole experimental period, the physico-chemical parameters (Temp, EC, TDS, pH and DO) were recorded weekly. Also, water samples from each aquarium were collected weekly, acidified with concentrated nitric acid, filterated with filter papers then syringe filter 0.45 um and kept at 4 ^{o}C till analysis as the field ones to determine Cu, Cd and Zn levels using AAS. At the end of the experiment, snail and fish samples were collected and prepared also in the same way as field samples for determination of hematological parameters, heavy metal bioaccumulation and DNA extraction.

Molecular study:

DNA was extracted from snails and fish groups; one native lake group (pooled from equal samples represented the examined sites) and three non-native groups each exposed to lake-water/sediment from one of El-Hamoul, Mottobus and Sidi-Salem sites and one control group, non-native non-exposed.

DNA extraction: Each group snail or fish was pooled from at least of five specimens. The genomic DNA was extracted from snail-feet and fish blood or liver using the methods of (Junghans *et al.,* 1990; Hillis *et al.,* 1996; Turtinen and Juran, 1998; Qamar *et al.,* 2017). From each sample, 0.1g to 0.5 g of snails'head-foot tissues were ground in a mortor and pestle in liquid nitrogen until a fine powder was

obtained. After grinding, thawing of ground tissues was prevented and transferred to 1.5 ml Eppendorf tube. Then 700 µl of extraction buffer (10 ml 150 mM Tris-HCl, 10 ml mM Nacl, 10 ml 50 mM EDTA, 10 ml 0.5% sodium dodecyl sulfate (SDS), 100ul/100 ml Mercato ethanol, complete wih ultra pure water to 100 ml) was added and mixed well. The tubes were incubated at 4ºC for ten min then centrifuged at 12000 r.p.m for ten min. The supernatant was transferred to a new sterile Eppendorf tube and 500 µl of phenol: chloroform: isoamyl at a ratio of 25:24:1were added to the supernatant and mixed. The tubes were centrifuged at 12000 r.p.m for five min then the aqueous phase was transferred to a new sterile tube and 750 µ of cold isopropanol was added and mixed then incubated at 4ºC for 20 min. Then the tubes were centrifuged for five min. to aggregate the DNA pellets which were washed in 70% ethanol and left to dry for about 30 min.

Collected fish blood samples were processed for DNA extraction within 72 h of collection or preserved in 95% ethanol at a proportion of 1:3. Genomic DNA from the whole blood was extracted in the following steps: In a 15 ml centrifuge tube 1-1.5 ml blood was taken and 4 -6 ml RBC lysis solution was added (150 mM NH4 Cl, 10 mM NaHCO3 and 0.1 mM disodium EDTA). Tubes were placed in tube rotator for 5 min then were centrifuged for 10 min at 300 r.p.m, supernatant was discarded and the white cell pellet was re-suspended in 500 µl phosphate buffered saline (PBS) (137 mM NaCl, 2.7 mM KCl, 10 mM Na2 HPO4 and 1.8 mM KH2 PO4) and pH adjusted to 7.4. This RBC lysis and removal step was repeated for three times and at the end, a RBC-free clean white pellet was obtained and re-suspended in 500 µl PBS. 1.5 ml WBC lysis buffer was added (0.1 M Tris-HCl, 0.1 M EDTA, 0.01 M NaCl, 1% SDS, pH 8.0). The lysates were treated with 50 µl freshly made proteinase K (150 mg/mL) incubated at 55 ºC for 2 h or at 37°C overnight. After incubation the samples were removed from the water bath and cooled down at room temperature. 500 µl of 7.5 M ammonium acetate was added in each sample and gently vortex-mixed vigorously for 15 seconds until the solution was homogenous, chilled on ice for 5–10 min, followed by centrifugation at 1200 g for 30 min to precipitate partially hydrolyzed polypeptides. The DNA pellet was then washed in 70% ethanol and moderately dried.

The dried DNA pellets extracted from both snail and fish groups were re-suspended in 500 mL of TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) overnight and stored at 4°C. The pellets were re-dissolved in 100 µ TE buffer then RNAase (5 units/µl) for each sample 10 µl was added to remove RNA from samples and was incubated at 37ºC for two hours to have pure DNA which was kept in refrigerator at -80ºC till use. DNA concentrations were measured by UV-Spectro-Photometer at a wave length of 260-280nm. DNA integrity and concentrations were estimated by comparison with molecular weight standard on 0.7% agarose gel electrophoresis.

PCR: Genomic DNA of different groups was subjected to polymerase chain reaction (PCR) using primers. ISSR-PCR reactions were conducted using randomly selected five ISSR primers, for each the genotypes of snails and fish (Table 1). Amplification was carried out in start gene PCR 96 which was programmed as follows: Denaturation (one cycle) 94ºC for 2min; followed by 30 cycles: as follows 94ºC for 30 second, 44ºC for 45 second, 72ºC for 1 min; and finally one cycle extension at 72ºC for 20 min; and 4ºC (infinitive).

Gel Electrophoresis: 15 µl of PCR-product were resolved in 1.5% GTG agarose gel electrophoresis with 1x TAE running buffer. The run was performed at 80 V for 180 min. and the gel was stained with ethidium bromide. A marker of 1 Kb plus DNA

ladder 1µg/µl (Invitrogen) that contains a total of twenty bands ranging from 12000 to 100 bp was used. Bands were detected on UV- Tran illuminator and photographed by gel documentation system UVP2000.

Statistical analysis:

One-way ANOVA and T-test were used to evaluate the significant difference in the studied parameters with respect to the different sites and experiments. A probability at level of 0.05 or less was considered significant.

Table 1: ISSR primers names and their sequences:

Samples	Primer name	Sequence	
Snail	ISSR1	5' TAT (CA)7 C 3'	TATCACACACACACACAC
	ISSR2	5' CAC (TCC)5 3'	CACTCCTCCTCCTCCTCC
	ISSR3	5' TTT (TCC)5 3'	TTTTCCTCCTCCTCCTCC
	ISSR4	5' CAT (CA)7 T 3'	CATCACACACACACACAT
	ISSR5	5' ACA G (TG)7 3'	ACAGTGTGTGTGTGTGTG
Fish	ISSR6	5' (GA)8 CG 3'	GAGAGAGAGAGAGAGACG
	ISSR7	5' ATT A (CA)7 3'	ATTACACACACACACACA
	ISSR8	5' (AG)8 CT 3'	AGAGAGAGAGAGAGAGCT
	ISSR9	5' AAC (TG)7 T 3'	AACTGTGTGTGTGTGTGT
	ISSR10	5' (TCC)5 AC 3'	TCCTCCTCCTCCTCCAC

RESULTS

Water quality criteria

Observations of water temperature, pH, DO, TDS and EC were recorded in each examined lake site during samples collection and weekly in each experimental aquaria. The observations presented in Table (2), showed that water temperature range was 18.2– 27.5 °C in the lake and experimental aquaria, pH range was 7.02-8.2, DO range was 4.70 – 7.6 mg/l , TDS range was 924 - 3033 mg/l, and EC range was 1310 - 4515 µmohs/cm. The mean value of the observed temperature and pH in water samples from the examined lake sites and aquaria of the three experiments were in the normal range according to permissible limit approved by (USEPA, 2009), while TDS and EC values were exceeded the permissible limit in all observations except in aquaria representing Mottobus site (924±112 and 1310±141, respectively), DO showed slightly lower values than the permissible limit in El-Hamoul and Sidi-Salem lake sites (4.7±0.14 and 4.85±0.07, respectively). As well, results of Cu, Zn and Cd determination in water samples collected from the examined lake sites and experimental aquaria are presented in Table (2) in comparison with the level of concern determined by USEPA (2009). Cu, Zn and Cd ranged between 0.02 – 0.11 mg/l, 0.03 – 0.5 mg/l, and 0.21- 3.9 µg/l, respectively. Cu showed normal levels in all examined lake sites, Zn showed normal levels in El-Hamoul and Mottobus sites while experiments of El-Hamoul and Sidi-Salem showed Cu and Zn levels that exceeded the levels of concern. Cd level exceeded the concern level in Mottobus site and in all the three experimental simulating aquaria.The mean concentrations of the heavy metals which determined in lake and experiments were in the following order: Zn >Cu > Cd.

Results of Cu, Zn and Cd bioaccumulation in snails' tissues are presented in Fig. (2). It was observed that Cd showed higher bioaccumation in all non-native snail tissues than native samples while Cu levels showed the higher bioaccumulation in the lake native samples graded as follows: El- Hamoul > Sidi-Salem > Mottobus. Zn was

slightly more bioaccmulated in native El-Hamoul and Mottobus samples while its bioaccumulation in Sidi-Salem non-native sample was higher than native one. Bioaccumulation of Zn and Cd in non-native control exceeded that of the exposed in Sidi-Salem experiment and together represented the most bioaccumulation of these metals while the most bioaccumulation of Cu was observed in El-Hamoul native sample

Table 2: Physico-chemical parameters measured in water samples collected from the three examined sites in Lake Burullus and from the aquria of the three simulated experiments for the three examined lake sites; El-Hamoul, Sidi Salem, and Mottobus (using lake-water/sediment from these sites, Mean ± SD).

parameters	Lake sites			Experimental aquaria											P.L (USEPA, 2009)
	El-Hamoul	Sidi-Salem	Mottobus	El-Hamoul		Sidi-Salem		Mottobus		Mean					
				Control	Experiment	Control	Exp	Control	Experiment	Control	Expe	% of change	Lake	% of change	
Temp (°C)	27.5 ±0	27.5 ±0	23.0 ±0	18.23 ± 0.95	18.18 ±0.90	18.17 ±0.21	19.01 ±0.8	18.68± 2.08	18.46 ±1.84	18.36 ±0.3	18.6 ±0.4	1.3	26.0	-28	21-25
pH	7.90 ±0	7.02 ±0.02* **	7.48 ±0.06	7.75 ±0.31	7.80 ±0.2	7.97 ±0.10	8.2 ±0.1***	7.80 ±0.2	7.7 ±0.2	7.89 ±0.12	8.0 ±0.24	1.4	7.5	6.6	7-8
DO (mg/l)	4.70 ±0.14***	4.85 ±0.07* **	5.0 ±0.08***	8.34 ±0.73	7.6 ±0.5***	6.33 ±0.91	6.6 ±0.5***	6.05 ±0.43	6.3 ±0.5***	6.90 ±1.2	6.8 ±0.7	-1.4	4.9	39	>5
TDS (mg/L)	3006 ±0	1845 ±21.2	1441 ±31.8	824.0 ±255.0	3033 ±363xxx	892.8 ± 77.6	1400 ±86xxx	746.0 ±222.0	924 ±118	821 ±73	1786 ±1106	118	2097	-14	400-1200
EC (µmohs/cm)	4350 ±70.7	2670 ±28.3	2065 ±49.5*	1177.0 ± 325	4515 ±922 xxx,a	1224.0 ±395.0	2035 ±109xx	1063.0 ±302.0	1310 ±141*, a ,x	1154 ±83	2620 ±1681	127	3028	-13.4	400-1400
Cu (ppm)	0.06 ±0.06	0.035 ±0.02	0.02 ±0.007	0.1 ±0.05	0.13 ± 0.06	0.02 ±0.01	0.042 ± 0.02	0.086 ±0.056	0.11 ±0.13	0.06 ±0.04	0.09 ±0.04	0.5	0.04	125	0.09
Zn (ppm)	0.036 ± 0.005	0.38 ± 0.53	0.03 ± 0.004	0.27 ±0.16	0.46 ± 0.27xxx	0.28 ±0.16	0.3 ± 0.1	0.07 ±0.08	0.05 ±0.07	0.21 ±0.11	0.3 ±0.2	43	0.15	100	0.12
Cd (ppb)	0.26 ±0.03 *	0.21 ±0.2	0.31 ±0.12	3.82 ± 1.25	4.2 ± 1.88*	1.09 ±0.27	1.91 ± 0.74	0.29 ±0.18	0.55 ±0.46	1.73 ±1.8	2.2 ±1.8	27	0.26	746	0.25

*,** and *** P< 0.05, 0.01 and 0.001 respectively in comparison between native and non-native.

a, aa and aaa P< 0.05, 0.01 and 0.001 respectively in comparison between different site in the same group.

x, xx and xxx P< 0.05, 0.01 and 0.001 respectively in comparison between control and all groups.

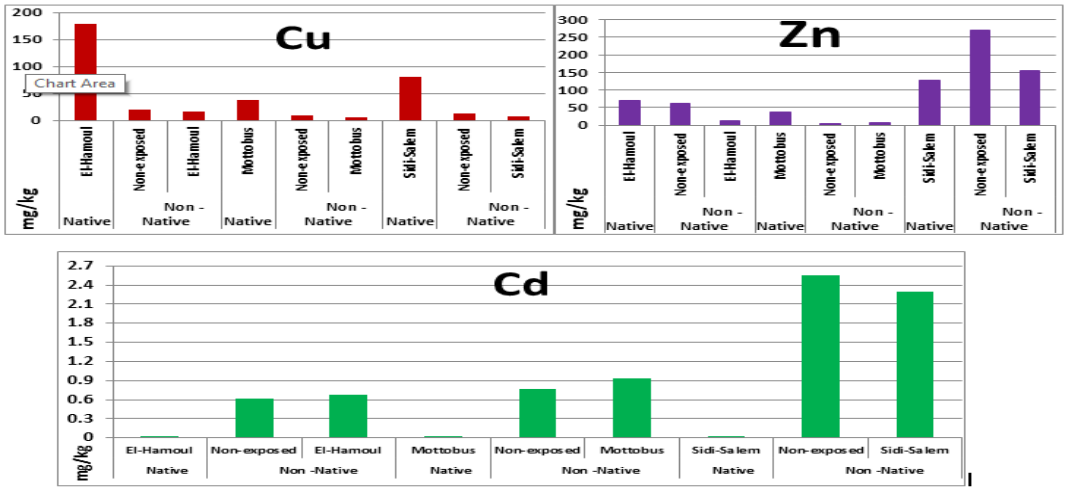

Fig. 2: Bioaccumulation of the heavy metals Cu, Zn and Cd in *Biomphalaria alexandrina*snails'tissue samples, native samples collected from three examined lake sites, control samples (non-native & non-exposed), and three non-native snail samples exposed to water and sediment from El-Hamoul, Sidi Salem and Mottobus sites.

Results presented in Figure (3) showed comparison of bioaccumulation of Cu, Zn and Cd values in the fish organs; muscles, liver and kidney in native and non-native samples (control and exposed). The highest bioaccumulation level of Cu was recorded in liver samples of control groups in El-Hamoul and Mottobus experiments

and in kidney of Sidi-Salem native samples. Zn and Cd were most bioaccumulated in kidneys of the exposed samples in Sidi-Salem experiment. In comparison to control, most of the exposed fish samples showed higher levels of bioaccumulation as follows: Cu, Zn & Cd bioaccumulation in fish kidney samples of all experiments (except for Zn in El-Hamoul experiment), Cu bioaccumulation in fish liver and muscle of Sidi-Salem experiment, Zn bioaccumulation in fish liver of Mottobus experiment and muscle in Sidi-Salem experiments, and Cd bioaccumulation in fish liver and muscle of Mottobus experiment.

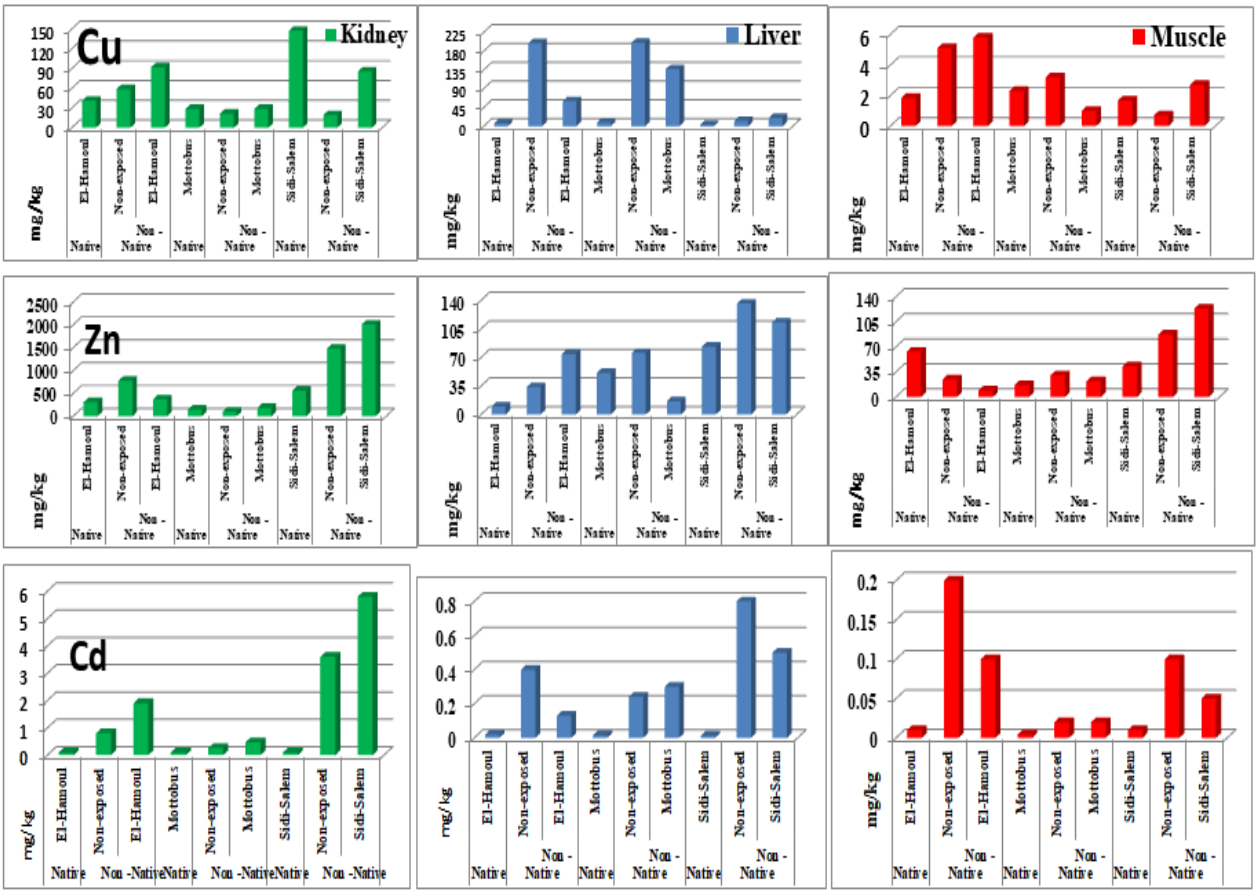

Fig. 3: Bioaccumulation of heavy metals Cu, Zn and Cd in muscles, liver and kidney of the examined *Oreochromis niloticus* fish samples; native samples collected from three examined lake sites, control samples (non-native & non-exposed), and three non-native snail samples exposed to water and sediment from El–Hamoul, Sidi Salem and Mottobus sites.

Hematological parameters:

Snail hemolymph showed hemoglobin (Hb) fluctuation around the normal level of control (-37% to 25). All snail samples showed non-significant decrease in total hemocytes count as compared to control and the highest percent of reduction (-71%) was recorded in non-native *Biomphalaria* exposed to lake-water/sediment from Mottobus site. The differential count in native and non-native snail hemolymph showed significant increase in granulocytes (large and/or small), the most increase of large granulocytes 93% was recorded in non-native Mottobus samples and the most increase in small granulocytes 107% was recorded in native Mottobus snail samples. Also, all samples showed significant decrease hyalinocytes, the most reduction -59% was recorded in native El-Hamoul snail samples.

Table 3: Hematological parameters in different *Biomphalaria alexandrina* snail samples; native collected from Lake Burullus sites and non-native from Giza watercourses, control (non-exposed) and experimentally exposed to lake water/sediment from the examined sites El-Hamoul, Sidi-Salem and Mottobus (Mean ± SD).

parameters	Control	Native from lake sites						Non –Native exposed to lake water/sediment					
		El-Hamoul	% of Change	Sidi-Salem	% of Change	Mottobus	% of Change	El-Hamoul	% of Change	Sidi-Salem	% of Change	Mottobus	% of Change
HB g/dl	1.6 ±0.2	1.3 ±0.1	- 18	1.01 ±0.1	- 37	1.7 ±0.2	6	1.63 ±0.5	1.8	2.0 ±0.4	25	1.15 ±0.3	-28
Total hemocytes	2.95 ±0.6	2 ±0.2	-32	1.14 ±0.16	- 61	2.3 ±0.1	-22	2.07 ±0.43	-30	1.85 ±0.4	-37	0.69 ±0.01	-76
Large granulocytes %	14.5 ±0.5	25*** ±3	72	25*** ±2	72	16 ±2	10	20.5 ±1.5 ***, aa	41	22.0 ±1.0 ***, aa	52	28.0 ±1.0 ***, aa	93
Small granulocytes %	27 ±2	51 ±9***	88	39.5 ±3.5	46	56 ±4***	107	32.0 ±5.0	19	25.0 ±4.0	-7	30.0 ±3.0	11
Hyalinocytes %	58.5 ±1.5	24 ±6***	-59	35.5 ±5.5***	- 39	35 ±2***	-40	47.5 ±6.5 ***,aaa	-19	53.0 ±5.0 aaa	-9	42.0 ±2.0 ***, aaa	-28

*, ** and *** P< 0.05, 0.01 and 0.001 respectively in comparison between native, non-native and control.
a, aa and aaa P< 0.05, 0.01 and 0.001 respectively in comparison between different site in the same group.

The hematological parameters in the examined fish samples showed slight non-significant increase in Hb in native Mottobus samples (18%) and non-significant decrease in other samples (native and non-native) (-18 to -50%). In spite of non-native Mottobos showed reduction in Hb than control (-27%) it showed the highest level among other non-native samples. RBCs showed reduction in all blood samples with % of change ranged between -17% and -54% in the non-native Sidi-Salem and El-Hamoul samples, respectively except for El-Hamoul native sample, was in normal range. Also, PCV% showed reduction in El-Hamoul native sample and in all non-native ones.WBCs showed significant increase in native El-Hamoul and Sidi-Salem samples (250 and 213%, respectively), non-significant increase in native and non-native Mottobus (change of 50% & 27%, respectively) and non-native Sidi-salem samples (22%) while non-significant reduction in non-native El-Hamoul sample (-41%). MCV showed increase in all samples (3% in native Sidi-Salem to 203.5% in native Mottobus and non-native El-Hamoul). MCH showed reduction in native El-Hamoul samples (significant) and non- native Sidi-Salem samples while showed significant increase in all other samples (11% in native Sidi-Salem to 50% in native Mottobus). All samples showed non-significant slight reduction in MCHC (-11% to -20%), (Table 4).

Table 4: Hematological parameters in different *Oreochromis niloticus* fish samples;native collected from Lake Burullus sites and non-native from Abbasa fish aquaculture, control (non-exposed)and experimentally exposed to Lake water/sediment from the examined sites El-Hamoul, Sidi-Salem and Mottobu (Mean ± SD).

parameters	Control	Native from lake sites						Non –Native exposed to lake water/sediment					
		El-Hamoul		*Sidi-Salem*	% of Change	*Mottobus*	% of Change	*El-Hamoul*	% of Change	*Sidi-Salem*	% of Change	*Mottobus*	% of Change
HB g/dl	10.9 ±0.5	6.3 ±0.6	-33	8.9 ±0.35	-18	12.6 ±0.8	18	6.9 ±0.5	-37	6.9 ±0.95	-37	7.6 ±1.2	-27
WBCs 10^3/cmm	7.8 ±3.6	27.5 ±1.3**	250	24.4 ±10*	213	11.7 ±3.5	50	4.6 ±0.66	-41	9.5 ±2.7	22	9.92 ±4.7	27
RBCs ×10^4/cmm	2.3 ±0.15	2.3 ±0.14	0	1.87 ±0.7	-19	1.82 ±0.11	-21	1.05 ±0.2	-54	1.9 ±0.45	-17	1.5 ±0.6	-34
PCV%	28.5 ±4.5	20.4 ±0.8	-28	27.8 ±2.3	0	37 ±1.4	30	20.7 ±1.56	-27	21.0 ±3.0	-26	24.0 ±3.0	-16
MCV (ft)	120 ±11.5	88.9 ±9.1**, aaa	-26	160.7 ±70***, aaa	3	203.5 ±21***, aaa	70	203.5 ±43.5***, aaa	70	124.6 ±46.4a aa	4	173.0 ±45.0***, aaa	44
MCH (Pg)	46.4 ±0.8	27.4 ±4.2**, aa, aaa	-41	51.4 ±20.2 aa, a	11	69.7 ±8.5***, aaa, a	50	67.8 ±14.5 ***, a	46	40.7 ±15.1 a	-12	54.3 ±12.4	17
MCHC (g/dl)	38.7 ±4.2	30.1 ±0.5	-20	32.3 ±1.34	-17	34.2 ±0.8	-11	32.5 ±0.9	-16	32.6 ±0.1	-16	31.5 ±1.1	-18

*, ** and *** P< 0.05, 0.01 and 0.001 respectively in comparison between native, non-native and control.
a, aa and aaa P< 0.05, 0.01 and 0.001 respectively in comparison between different site in the same group.

ISSR markers in assessing genetic variation

Among the different types of molecular markers ISSR can be considered to be essential tool for assessment of genetic variability and to study the phylogenetic relationships within, and among different examined groups. Genetic diversity was assessed by similarity coefficient (Jaccard) and cluster analysis among *B. alexandrina* snails (S) and *O. niloticus* (F) fish samples. Five samples; native Lake Burullus "B", experimentally non-exposed non-native control "C" and the three non-native each exposed to water and sediment collected from one of the three lake sites, Mottobus "M", El-Hamoul "E", and Sidi-Salem "S" were investigated by ISSR-PCR technique using ten primers. All of the tested primers successfully amplified products from genomic DNA. The number of amplified, monomorphic and polymorphic bands generated by each primer is shown in Tables (5, 6,7 & 8) and Fig.(4). A total of amplification fragments in snails and fish were 33 & 26 ranging from 205 to 1120 bp and 205-1205 bp, respectively (Table 7). The highest total number of bands (10) in snails was obtained using primer ISSR 4, and (8) in fish by the primers ISSR 9 and 10, (Table 5).

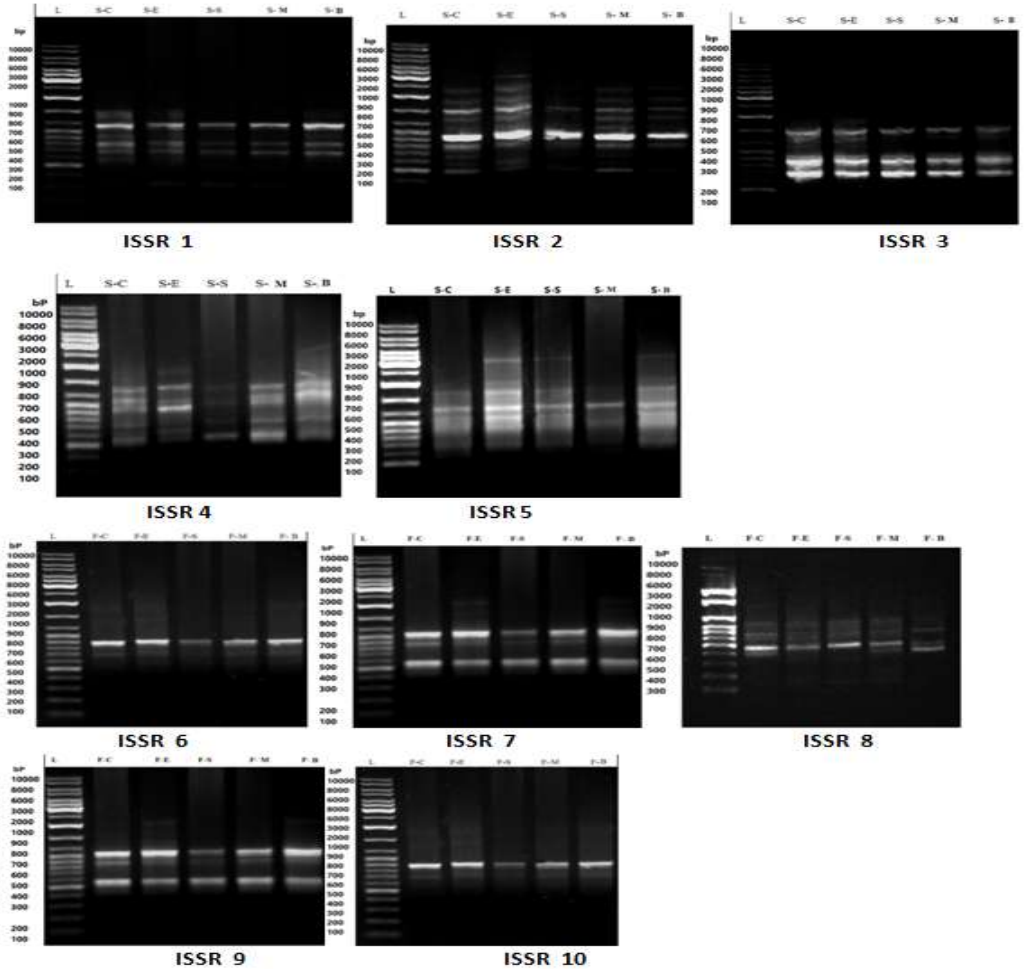

Fig. 4: PCR products of genomic DNA from *Biomphalaria alexandrina* snails and *Oreochromis niloticus* fish; samples, included control field snail from Giza control fish from Abbasa aquaculture reared in de-chlorinated tap water and autoclaved garden sediment (S-C) 1st lane, Giza snails & aquaculture fish (non-native) exposed to water and sediment from El–Hamoul (S-E & F-E) 2nd lane, from Sidi Salem (S-S & F-S) 3rd lane, and from Mottobus (S-M& F-M) 4th lane, and Lake Burullus snail sample pooled from snails and fish collected from Sidi Salem, El-Hamoul and Mottobus sites (S-B& F-B) 5th lane,The first lane showed DNA Leader (L).

Table 5: Survey of ISSR markers using five primers in *B. alexandrina* snail and *O. niloticus*fish samples included control field snails from Giza and control fish from Abbasa aquaculture reared in de-chlorinated tap water and autoclaved garden sediment (S-C), Lake Burullus snail sample pooled from snails and fish collected from Sidi Salem, El-Hamoul and Mottobus sites (S-B & F-B), Giza snails & aquaculture fish (non-native) exposed to water and sediment from Sidi Salem (S-S & F-S), from El–Hamoul (S-E & F-E), and from Mottobus (S-M & F-M). Band present was indicated by 1while band absence was indicated by 0.

ISSR 01					
samples	1	2	3	4	5
MW	S-C	S-E	S-S	S-M	S-B
1120	0	1	1	0	1
805	0	1	1	0	1
750	1	1	1	1	1
635	1	1	1	1	1
560	1	1	1	1	1
450	1	1	1	1	1
Total	4	6	6	4	6
ISSR 02					
MW	S-C	S-E	S-S	S-M	S-B
920	0	1	0	0	0
775	1	1	1	1	1
605	1	1	0	1	1
425	1	1	1	1	1
360	0	1	0	0	0
250	1	1	1	1	1
Total	4	6	3	4	4
ISSR 03					
MW	S-C	S-E	S-S	S-M	S-B
610	1	1	1	1	1
530	1	1	1	1	1
410	1	1	1	1	1
340	1	1	1	1	1
245	1	1	1	1	1
Total	5	5	5	5	5
ISSR 04					
MW	S-C	S-E	S-S	S-M	S-B
1080	0	1	0	0	0
840	1	1	0	1	1
760	1	1	1	1	1
640	1	1	1	1	1
595	1	1	1	1	1
515	1	1	1	1	1
455	1	1	1	1	1
360	1	1	1	1	1
255	1	1	1	1	1
205	1	1	1	1	1
Total	9	10	8	9	9
ISSR 05					
MW	S-C	S-E	S-S	S-M	S-B
760	1	1	0	0	1
620	1	1	1	1	1
525	1	1	0	0	0
490	1	1	1	1	1
410	1	1	1	1	1
375	1	0	0	0	0
Total	6	5	3	3	4

ISSR 06					
samples	1	2	3	4	5
MW	F-C	F-E	F-S	F-M	F-B
865	1	1	1	1	1
670	1	1	1	1	1
510	1	0	0	1	1
Total	3	2	2	3	3
ISSR 07					
MW	F-C	F-E	F-S	F-M	F-B
865	1	1	1	1	1
670	1	1	1	1	1
510	1	0	0	1	1
Total	3	2	2	3	3
ISSR 08					
MW	F-C	F-E	F-S	F-M	F-B
850	1	1	1	1	0
710	1	1	1	1	1
640	0	1	0	0	0
475	1	1	1	1	1
360	1	0	0	1	1
Total	4	4	3	4	3
ISSR 09					
MW	F-C	F-E	F-S	F-M	F-B
1205	1	1	0	0	1
1130	0	1	0	0	0
865	1	1	1	1	1
710	1	1	1	1	1
660	1	1	1	1	1
610	0	1	0	0	0
475	1	1	1	1	1
325	1	1	1	1	1
Total	6	8	5	5	6
ISSR 10					
MW	F-C	F-E	F-S	F-M	F-B
985	0	1	0	0	0
620	1	1	1	1	1
560	1	1	1	1	1
465	1	1	1	1	1
410	1	1	1	1	1
365	1	1	1	1	1
220	1	1	1	1	1
205	0	1	0	1	0
Total	6	8	6	7	6

Table 6 Number of amplified bands in 5 samples of each of *Biomphlaria alexandrina* snails and *Oreochromis niloticus* fish*;* groups from Lake Burullus and experimental study by using five ISSR primers:

Samples	**Snails**					**Fish**					
Primers	**1**	**2**	**3**	**4**	**5**	**Primers**	**1**	**2**	**3**	**4**	**5**
	S-C	**S –E**	**S-S**	**S-M**	**S –B**		**F-C**	**F-E**	**F-S**	**F-M**	**F-B**
ISSR01	**4**	**6**	**6**	**4**	**6**	**ISSR06**	**3**	**2**	**2**	**3**	**3**
ISSR02	**4**	**6**	**3**	**4**	**4**	**ISSR07**	**3**	**3**	**3**	**3**	**3**
ISSR03	**5**	**5**	**5**	**5**	**5**	**ISSR08**	**4**	**4**	**3**	**4**	**3**
ISSR04	**9**	**10**	**8**	**9**	**9**	**ISSR09**	**6**	**8**	**5**	**5**	**6**
ISSR05	**6**	**5**	**3**	**3**	**4**	**ISSR10**	**6**	**8**	**6**	**7**	**6**
Total	**28**	**32**	**25**	**25**	**28**	**Total**	**22**	**25**	**19**	**22**	**21**

By using all primers and according to the total number of amplified bands in each of snails and fish samples, non-native Giza snail and aquaculture fish exposed to water and sediment from El-Hamoul (S-E & F-E) showed the highest number of bands (32 and 25, respectively), while the snail and fish exposed to water and sediment from Sidi-Salem showed least number of bands (25 and 19, respectively) (Table 6). Snail genetic analysis revealed that El-Hamoul snail sample was the most unique by the presence of 3 polymorphic bands by using all primers. It was characterized by two bands of 920 and 360 bp using the primer ISSR 02, and one band of 1080 bp using the primer ISSR 4, followed by Sidi-Salem that characterized

by the absence of 2 bands of 605 bp using the primer ISSR02 and 840 bp using the primer ISSR 04, and control that characterized by presence of one band of 375 bp using primer ISSR05. Fish genetic analysis revealed that El-Hamoul fish group was the most unique by the presence of 4 polymorphic bands by using all primers, It was characterized by one band of 640 pb using the primer ISSR 08, and two bands of 1130 bp and 610 bp using the primer ISSR 09 and one band of 985 bp using the primer ISSR 10, followed by Lake Burullus samples that characterized by the absence of one band of 850 bp using the primer ISSR08, (Table 5). The similarity coefficients estimated among the snail samples; native lake, non-exposed non-native control, and the three non-native exposed snails groups; was ranged from 0.686 to 0.973. The highest value (0.973) was recorded between the native Lake Burullus (S-B) and non-native exposed to water and sediment from Mottobus (S-M), while the lowest (0.868) was detected between non-native Sidi-Salem (S-S) and control snail group (S-C) (Table 8).

Table 7: Total number of amplified and the level of polymorphism as revealed by five ISSRs markers among five samples of each of *Biomphalaria alexandrina* snails and *Oreochromis niloticus* fish.

Samples	Snails				Fish				
Primers	Total number of amplified bands	Polymorphic amplified bands	No. of unique bands	Polymor-phism %	Primers	Total number of amplified bands	Polymorphic amplified bands	No. of unique bands	Polymor-phism %
ISSR 1	6	2	0	33 %	ISSR 06	3	1	0	33 %
ISSR 2	6	0	3	50 %	ISSR 07	3	0	0	0 %
ISSR 3	5	0	0	0 %	ISSR 08	5	2	2	80 %
ISSR 4	10	0	2	20 %	ISSR 09	8	1	2	38 %
ISSR 5	6	2	1	50 %	ISSR 10	8	1	1	25 %
Total	33	4	6	30%	Total	26	5	5	40%

Table 8: Proximity and similarity matrices based on ISSR fragment analysis among five samples of each of *B. alexandrina*snails and *O. niloticus* fish included control field snail from Giza control fish from Abbasa aquaculture reared in de-chlorinated tap water and autoclaved garden sediment (S-C & F-C), Lake Burullus snail sample pooled from snails and fish collected from Sidi Salem, El-Hamoul and Mottobus sites (S-B & F-B), Giza snails & aquaculture fish (non-native) exposed to water and sediment from El–Hamoul (S-E & F-E), from Sidi Salem (S-S & F-S), and from Mottobus (S-M & F-M).

Groups	Snails						Fish				
	S- C	S-E	S-S	S-M	S-B		F-C	F-E	F-S	F-M	F-B
S-C	--	0.900	0.868	0.943	0.929	F-C	--	0.851	0.927	0.955	0.977
S-E		--	0.877	0.877	0.933	F-E		--	0.864	0.851	0.826
S-S			--	0.920	0.943	F-S			--	0.927	0.900
S-M				--	0.973	F-M				--	0.930
S-B					--	F-B					--

UPGMA dendrogram (Fig. 5) based on genetic similarity matrices between all snail samples showed the first cluster was between non-native Motobus (S-M) and native lake samples (S-B), that in turn cluster with control sample while the non-native El-Hamoul sample (S-E) was the most divergent followed by non-native Sisi-Salem sample (S-S). Meanwhile in fish the similarity coefficients estimated among the fish groups was ranged from (0.826 to 0.977) the highest value was recorded between native Lake Burullus sample (F-B) and non-native non-exposed control sample (F-C) while the least value (0.826) was recorded between samples (F-B) and non- native El- Hamoul sample (F-E), (Table 8). Also, dendrogram based on genetic similarity matrices between all Fish samples showed that the first cluster was between control sample (F-C) and lake group (F-B) that in turn clustered with non-native fish

sample from Mottobus (F-M), non-native sample from El-Hamoul (F-E) was the most divergent followed by non-native Sidi-Salem sample (F-S), (Fig.6).

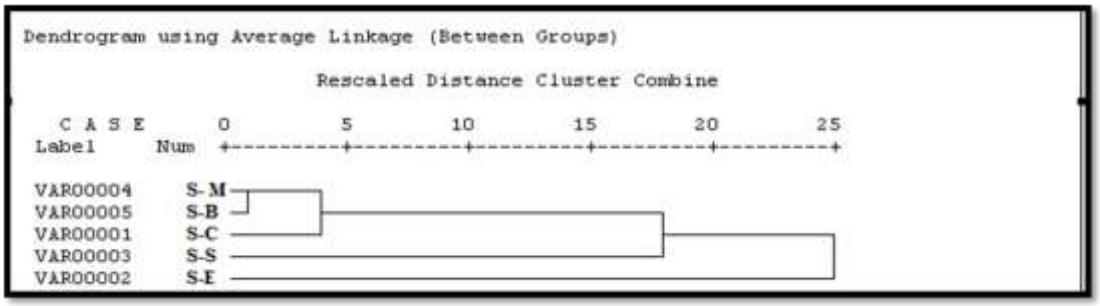

Fig. 5: Dendrogram demonstrating the diversity and relationships based on ISSRs among five *B. alexandrina* snail samples; included control field snail from Giza reared in de-chlorinated tap water and autoclaved garden sediment (S-C), Lake Burullus snail sample pooled from snails collected from Sidi Salem, El-Hamoul and Mottobus sites (S-B), Giza snails (non-native) exposed to water and sediment from El–Hamoul (S-E), from Sidi Salem (S-S), and from Mottobus (S-M).

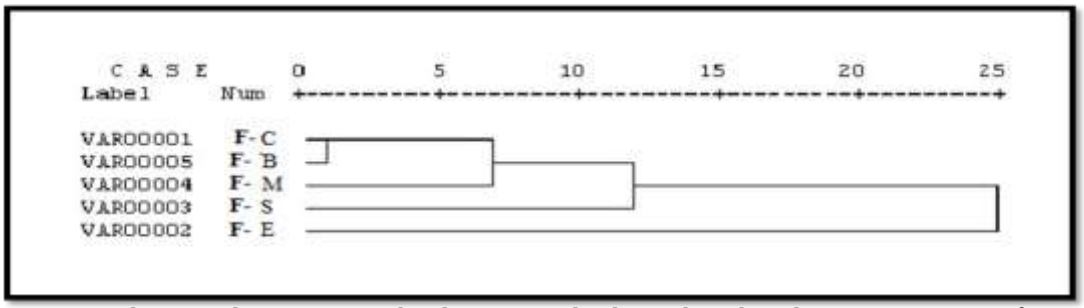

Fig. 6: Dendrogram demonstrating the diversity and relationships based on ISSRs among five *O. niloticus* fish samples; included control field fish from Abbasa aquaculture reared in de-chlorinated tap water and autoclaved garden sediment (F-C), Lake Burullus fish sample pooled from fish collected from Sidi Salem, El-Hamoul and Mottobus sites (F-B), aquaculture fish (non-native) exposed to water and sediment from El–Hamoul (F-E), from Sidi Salem (F-S), and from Mottobus (F-M).

So, it could be concluded from snail and fish cluster analysis that the closest relationship was between the samples native Lake Burullus , control group , and the exposed non-native Mottobus while the exposed non-native El-Hamoul and Sidi-Salem were the most divert.

DISCUSSION

Lake Burullus is a brackish water coastal lake on the Mediterranean Sea, Egypt. Before the construction of the Aswan High Dam, the lake received the Nile floods in late summer and autumn. Lake ecosystem has been deteriorated since Aswan High Dam construction, 1965. Lake salinity has been suffered from significant decrease due to excess unregulated drainage water effluent into the lake, which affected the lake flora and fauna. In the present work, the quality of surface water in Lake Burullus was assed and their impact was investigated on health and genetic alterations of native *B. alexandrina* snails and *O. niloticus* fish samples collected from the lake and non-native ones exposed in simulating experiments for six weeks to lake-water/sediment from the examined lake sites; El-Hamoul, Mottobus and Sidi-Salem. The examined water quality criteria in lake sites and their corresponding

simulating aquaria showed normal temperature of 18.1-27.5 ºC range, slightly alkaline side pH values (8.2 - 7.02), and normal DO values (6-8 mg/l) in Mottobus site and all simulating experiments while DO values were slightly lower than normal in El-Hamoul and Sidi-Salem lake sites that may be attributed to these sites are near to the discharge points of drains (El-Gharbia and drains No. 8&9 respectively). The findings of DO lower level are in consequence with that of Zaghloul, *et al.*,(2007) who recorded lower value of DO content at eastern than that observed in western sectors, the much suffered from the industrial and agricultural discharge causing high levels of organic compounds, inorganic salts and heavy metals that lead to fish hypoxia. TDS and EC showed high levels in El-Hamoul and Sidi-Salem lake sites that exceeded the permissible limits that determined by United States Environmental Protection Agency (USEPA, 2009). Heavy metals may enter an aquatic ecosystem from different natural and anthropogenic sources, including industrial or domestic sewage, shipping and harbor activities and atmospheric deposits (Rajeshkumar and Munuswamy, 2011). Heavy metals analysis in the aquatic system can provide important information on the degree of environmental pollution (Kowalezyk and Czepiel, 2013). However, high concentration of heavy metals affected fish and snail populations, reducing their growth, reproduction, physiological activity and maturity and/or survival and may even kill fishes (Calabrese *et al.*, 1977; Zaghloul *et al.*, 2000 &2005). The present study recorded high level that exceeded the permissible limit of Cu in El- Hamoul and Mottobus experiments, Zn in Sidi-Salem lake site and El Hamoul and Sidi-Salem experiments, and high Cd in all lake sites and experiments. These findings was agreed with findings of Gad (2005) and Emara *et al.*(2016) who referred to bad conditions of the aquatic ecosystems expressed by high heavy metal levels that may be attributed to gasoline motors, industrial, agricultural effluents and municipal sewage contamination which are rich with fertilizers and chemicals that feed the lake. The mean levels of physicochemical parameters showed alteration in simulating experiments than their corresponding lake sites, a slight alteration with percentage of change equal to -28% in temperature , -14%, in TDS, 13.4% in EC, and 39% in DO while a higher alteration was observed in the mean level of metals equal to 83, 100 & 746% in the levels of Cu, Zn and Cd, respectively indicating the role of the natural lake recovery in decreasing metal concentration.

In aquatic ecosystem, heavy metals are considered the most important pollutants, since they are present throughout the ecosystem and are detectable in critical amounts. Molluscs have long been regarded as promising bio-indicator and bio-monitoring subjects. In the present study bioaccumulation of heavy metals in snails' tissues showed higher Cu bioaccumulation in all the native snail samples while non-native showed higher levels of Zn & Cd bioaccumulation. All non-native exposed fish in the three simulating experiments showed higher bioaccumulation in all organs than native fish from Lake Burullus except for Cu bioaccumulation in Kidney of native Sidi-Salem samples and Zn bioaccumulation in liver of native El-Hamoul samples. Heavy metals were accumulated in the tissues of *B. alexandrina* in the order of Zn > Cu >Cd mg/kg respectively, this is may be due to the soft tissues of the snail could be the target organs for Zn &Cu. This result was confirmed by Rainbow *et al.* (1993) and Rainbow (2002) who stated that accumulation strategies of invertebrates vary intra-specifically between metals and inter-specifically for the same metal in closely related organisms. Also, Moolman *et al.* (2007) showed that *Melanoides tuberculata* had a much higher uptake of Zn in a mixed Cd/Zn exposure. Cd in the present work showed lower level of bioaccumulation in all organs of native fish samples from all lake sites than non-native (control & exposed). This

could be attributed to higher mean of Cd level in simulating experiments than lake sites up to 746%. The higher level of bioaccumulation in the non-native exposed samples than non- native control observed in 21/36 cases is reflecting the impact of exposure to lake water and sediment in increasing bioaccumulation rates while in the other cases 15/36 non-native control showed higher bioaccumulation, this could be related to either water quality where non-native samples were reared (water canals and aquaculture or de-chlorinated tap water & garden sediment) and/or reflecting the recovery effect of lake water and sediment. Present data showed that heavy metals were accumulated in the different organs of *O. niloticus* as follows: Zn and Cd - kidney> liver > muscles while Cu- kidney> liver > muscles in native samples, and in non-native exposed samples as follows: Cu- liver >kidney > muscles which agree with the findings of Authman *et al.* (2013) who mentioned that fish muscle tissue is generally lower in trace metal content. Trace elements in fish tissues were always higher than water, the finding that was confirmed and elucidated by Riani (2015) that its mechanism was through the regular diffusion, biomagnification, and bioconcentration.

No doubt that the hemolymph of mollusks is of critical importance as the hemocytes react against foreign biotic and abiotic bodies, digest and transport nutrients, accumulate various substances such as heavy metals, pesticides and molluscicides (Malker and Chong, 1974). The present work demonstrated three types of hemocytes in the hemolymph of *B. alexandrina* snails; large and small granulocytes and hyalinocytes, the findings that confirmrd by Sharaf El- Din (2003) and Kamel *et al.* (2006). Most native snail Lake Burullus samples and non-native exposed showed non-significant decrease in total hemolymph cell count, highly significant decrease in hyalinocytes, and increase in small and large granuolocytes. Also, Hb showed non significant decrease in El-Hamoul and Sidi-Salem native snails, while showed non-significant increase in native Mottobus samples. The decrease in hemcytes may be considered as a haemolysis response to the multiple pollution in Lake Burullus. This was mentioned by (Helal *et al*., 2003) that hemocytosis represents a response to external stress or certain stimuli and may originate from a variety of biotic or abiotic sources (Wolmarans & Yssel 1988). These results were in agreement with (El-Kayat *et al.*, 2015a*)* who found that the snails collected from El-Manzala Lake showed decrease in hemolcytes. Hematological components have been developed for evaluation of fish health conditions. As a matter of fact, blood serves as the most convenient indicator of the general conditions of the animal body. Subsequently, hematological studies are promising tools for investigating physiological changes caused by environmental pollutants (Zaghloul *et al.*, 2005). Meanwhile blood of native fish samples collected from El-Hamoul and Sidi-Salem sites showed highly significant increase of WBCs count, this increase could be due to their function as they are defensive in nature also, leukocytosis is directly proportional to the severity of damage and stress which as a consequence result in the stimulation of immunological defense, (Javed and Usmani 2012). The increase in small and large granulocytes of snail hemolymph and in WBCs of fish was suggested to indicate alteration in defense mechanism against the action of heavy metals toxicity bio-accumulated in snail and fish tissues as previously reported by Zaghloul (2001), (Helal et al., 2003) and Zaghloul et al. (2005). Mottobus native fish samples showed the highest Hb level (% of change was 18 in comparison to control) and in spite of non-native Mottobos showed reduction in Hb than control (-27%) it represented also, the highest level among other non-native exposed samples, this is side by side with the present results showing that Mottobus site had the least levels

in most of water quality creiteria and its samples showed the least bioaccumulation confiming the more healthy lake ecosystem in Mottobus. In the same consequence, the low Hb recorded in native and non-native El-Hamoul and Sidi-Salem samples side by side with the present results showing that water in these lake sites and their simulating experiments showed higher levels of TDS, EC, Zn and Cd, and higher tissue bioaccumulation in their samples, confirming their impaired ecosystem. As well, Adakole (2012) reported that decreased Hb level might be as result influx of water from the farms, industrial, sewage that contained heavy metals such as Cd and Pb, which alter the properties of hemoglobin by decreasing their affinity towards oxygen binding capacity. Gafaar *et al.* (2010) reported that prolonged reduction in hemoglobin content is deleterious to oxygen transport and degeneration of the erythrocytes could be due to pathological condition in fish exposed to toxicants.

ISSR-PCR technique was used in the present study to asess genetic variations between *B. alexandrina and O.niloticus* native collected from Lake Burullus and non-native exposed to lake water and sediment. El-Hamoul snail and fish experimental samples were the most unique indicating that they were the most genetically altered followed by Sidi-Salem then each of Lake Burullus and control. On the other hand Mottobus snail and fish samples did not show any unique bands indicating that they were the least genetically alterated. As well, the dendrogram based on ISSR fragment analysis confirmed that El-Hamoul site was the most divergent detected in samples exposed to lake-water and sediment followed by Sidi-Salem site. The same as with fish dendrogram showed cluster between non-native non-exposed and native lake group that in turn clustered with non-native Mottobus fish sample. Non-native El-Hamoul sample was the most divergent followed by non-native Sidi-Salem sample. So, the present results reflect higher genetic and hematological alterations and metal bioaccumulation in El-Hamoul and Sidi-Salem snail and fish samples under the effect of water quality that suffered from higher levels of total dissolved solids TDS, EC, Zn &Cd. As well, El-khayat *et al.* (2015) used ISSR-PCR technique for studying genetic variations of *Lymnaea natalensis* collected from four Egyptian Governorates and compared it with laboratory snails. In addition, El-Khayat *et al.* (2017) succeeded to differentiate among five snail groups; three were under the selective pressure for Cd tolerance through successive three generation; G1, G2 &G3; and two non-selected snail groups, parent field and lab *B. alexandrina*. Their results revealed that G3 snail group was the most unique by total 25 polymorphic bands produced by ten primers, 16 absence and 9 excess.These results were in agreement with Ebied *et al.*(2014) who identifying that petroleum oil components pollution have distinct effect on genetic structure of fishes and with Saravanan *et al*., (2011) who reported that fish are used as excellent indicator of aquatic pollution due to their high sensitivity to environmental contamination which may change certain physiological and biochemical processes when contact with the organs of fishes. The efficiency of ISSR markers in this study was very satisfactory, supporting previous studies of genetic diversity evaluation in fish species, such as *Cichla* (Almeida-Ferreira *et al.*, 2011), *Astyanax fasciatus* (Pazza *et al.*, 2007).

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ARABIC SUMMARY

تأثير نوعية المياه فى بحيرة البرلس على الاختلاف الجيني لقواقع *Biomphalaria alexandrina* وأسماك البلطي النيلي*Oreochromis niloticus*.فى مصر

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تم دراسة تأثير جودة المياه في بحيرة البرلس على التغيرات الحيوية والوراثية فى عينات قواقع *Biomphalaria alexandrina* واسماك البلطي النيلي التى تم تجميعها من البحيرة والمعرضة فى تجارب معملية لمياه البحيرة / الرواسب من ثلاثة مواقع؛ الحامول ، مطوبس وسيدى سالم لمدة سته أسابيع. أظهرت مواقع الحامول وسيدى سالم فى البحيرة مستويات عالية من المواد الصلبة الذائبة الكلية (TDS) ، التوصيل الكهربائي (EC)، فى حين سجلت مستويات عالية من عناصر الزنك والكادميوم فى التجارب المعملية. كما سجلت معظم مستويات التراكم المعادن فى عينات الحامول وسيدى سالم المجمعه من البحيرة والمعرضة معمليا فى حين أظهرت عينات مطوبس ادنى المستويات فى معظم معايير جودة المياة والتراكم الاحيائى. وأظهرت أنسجة القواقع التى تم جمعها من البحيرة (سيدى سالم) إرتفاعا فى مستوى تراكم النحاس وكذلك الكلى فى الاسماك بينما أظهرت معظم الاسماك المعملية فى التجارب الثلاثة إرتفاعا فى تراكم المعادن فى جميع الاعضاء. وقد أظهر الهيموليمف فى القواقع زياده معنوية فى الخلايا المحببه granulocytes وانخفاض فى خلايا hyalinocytes , وخلايا الدم الكلية ،فى حين انخفض الهيموجلوبين فى العينات المجمعه من البحيرة وزاد بشكل طفيف فى عينات سيدى سالم والحامول المعرضة وبالعكس فى مطوبس . أما الاسماك التى تم تجميعها من الحامول وسيدى سالم فقد اظهرت زياده معنوية فى خلايا كريات الدم البيضاء بينما اظهرت العينات المعرضة الى مياة وراسب مطوبس اعلى مستوى فى الهيموجلوبين . تم إجراء فحص التغيرات الجينية بواسطة تقنية التحليل التتابعى التكرارى البسيط (ISSR-PCR) حيث تبين فى عينات القواقع والاسماك المجمعه والمعرضه الى وجود درجات متفاوته من التقارب والاختلاف فى هذه الاماكن. فمثلا درجة التقارب كانت عالية بين العينات المجمعه من البرلس والمعملية المعرضه للمياه والراسب من منطقة مطوبس والمجموعه الضابطة الغير معرضة لمياه البحيرة بينما كانت أكثر تباعدا فى عينات الحامول، يليها عينات سيدى سالم. ولهذا فقد أشارت نتائج تراكم المعادن الثقيله فى الانسجة والتغيرات الجينية و الدموية فى كلا من القواقع والاسماك الى أن نوعية المياه فى بحيرة البرلس لها تأثيرات سلبية تتدرج تنازليا كما يلى: الحامول > سيدى سالم > مطوبس.