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The Effect of Seasonal Metals Pollution in Two Hotspots (El-Mex, Abu-Qir) Bays on ATPases in Gills of *Siganus rivulatus*

Hegazi M. M.[1, *], Mostafa A. H.[1], Assem E. H.[2], Mourad H. M.[2], Hasanein S. S.[2]

[1]Zoology Department, Faculty of Science, Tanta University, Tanta, Egypt
[2]Fish Physiology, National Institute of Oceanography and Fisheries, Alexandria, Egypt

Email address:

m_a_m_hegazi@hotmail.com (M. M. Hegazi), hala.mostafa@science.tanta.edu.eg (A. H. Mostafa), assem31745@yahoo.com (E. H. Assem), mourad_mohamed54@yahoo.com (H. M. Mourad), Soharafat1@yahoo.com (S. S. Hasanein)

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Abstract: The present study aimed to study the effect of pollution on rabbitfish Marbled spinefoot (*Siganus rivulatus*) seasonally caught from the two polluted area El-Mex Bay and Abu-Qir Bay and non-polluted area as controls. The rocky coast between Kayet Bey Fork and El-Boughaz opening in the Eastern Harbor toward the open sea was taken as control area. The activity of ATPases in gills and heavy metals accumulation in white muscle, gills, and liver of rabbitfish was assessed. These could be helpful for the understanding of physiological regulation mechanism for seasonal adaptation. The activities of Na^+/K^+-ATPase, Ca^{2+} Mg^{2+}-ATPase reached maximum activity in summer in fish caught from control area but the activity decreased in the two polluted areas. The physicochemical characteristics of water in these areas were assessed. The accumulation of zinc (Zn), iron (Fe), cupper (Cu), lead (Pb) and cadmium (Cd) were assessed white muscle, gills, and liver. The accumulation of heavy metals increased in the season's summer, spring than autumn and winter. The order of accumulation was Fe>Zn>Cu>Pb>Cd. The combined effects of increased heavy metals accumulation and low salinity of El-Mex Bay influence on osmoregulation and decreased the activity of ATPases.

Keywords: *Siganus rivulatus,* Seasonal Heavy Metals Bioaccumulation, El-Mex Bay, Abu-Qir Bay, Gill ATPases

1. Introduction

The fast progress of industrial accomplishments has resulted in heavy metals pollution, which is a momentous health threat to human beings over food chain [1]. Metals occur less than 1% of the earth's crust, with trace quantity generally found in the environment and when these exceed a postulated limit, they may toxic to adjacent environment [2]. The studied areas (El-Mex, Abu Qir Bays) were loaded by continuous flow of diverse form of pollutants [3]. El-Mex Bay receives about 2.547×10^9 m^3 y^{-1} of agricultural wastes mixed with water effluents (surplus water) from an adjacent sewage-polluted lake (Lake Mariut) with a rate of 262.8×10^6 m^3 y^{-1} via the Omoum Drain. In addition, the Bay receives 13×10^6 m^3 y^{-1} of industrial discharge, as well as water from the Western Harbor amounting to 1.13×10^6 m^3 y^{-1} [4]. Abu Qir Bay else receives diverse pollutants contributing to various waste cleared through three main beginnings namely, 1) El-Tabia pumping station, outlet of Lake Edku (Boughaz El-Maddya), 2) Rosetta mouth of the Nile River. Besides, 15,000 m^3 day^{-1} of manufacturing wastes is cleared directly into the bay [5, 6].

Rabbitfish Marbled spinefoot (*Siganus rivulatus*) inhabits subtropical environments and nourishes mainly on green algae. Rabbitfish is an economically valued species in the Eastern Mediterranean and Indo-West Pacific regions, it is a marine fish of the herbivorous family Siganidae and can reach sizes of 31.9 cm and 318.2 g in length and body weight, respectively [7]. Rabbitfish is identified as a potential candidate for marine warm water aquaculture because of its good taste, herbivorous feeding habits, tolerance to high stocking densities [8] and tolerance to wide ranges of environmental factors such as salinity [9] and temperature [8].

Salinity affects the speciation and bioavailability of trace metals, influencing their uptake by aquatic entities [10]. Thus,

it directly controls the amount of incoming metal that potentially altering the ATPase enzymes in osmoregulatory system [11, 12]. Osmoregulation is the ability to actively maintain osmotic concentrations in extracellular fluids, in spite of the osmolarity (salinity) of the surrounding environment. It is a fundamental physiological adaptation of animals living in estuarine environments [12]. Salinity has a profound effect on a fish's osmoregulatory and ionoregulatory physiology and interferes with the metal uptake by changing their availability [10, 13, 14 and 15]. ATPases are the enzymes concerned with the intermediate release of energy useful for the maintenance for physiological functions. ATPases are involved in the intracellular ionic regulation and also help in the osmoregulation of the whole animal. ATPases, membrane-bound enzymes which regulate the cellular volume, osmotic pressure, and membrane permeability due to the transport of ions through biological membranes [16, 17]. ATPases enzymes are very sensitive to trace metal toxicity. The enzyme Na^+/k^+-ATPase (EC 3.6.3.9) is strictly concerned with the active transport of Na^+/K^+ of the cells. It is present in most all the cell membranes that act some type of active transport, aid in maintaining the intracellular ionic gradient and transport of organic molecules of low molecular weight [18, 19]. The enzyme Ca^{2+}-ATPase (EC 3.6.3.8) is also a momentous ATPase, acts to remove the Ca^{2+} ions from cytoplasm to maintain the low Ca^{2+} levels [10, 20]. The enzyme Mg^{2+}ATPase (EC 3.6.3.2) plays avital role in oxidative phosphorylation and ionic transport and acts for transepithelial regulation of Mg^{2+} ions [21]. The assessment of ATPase activity may therefore be used as an early caution signal of metal-induced injury to the osmoregulatory and acid-based regulatory system in osmoregulatory organs such as gills, kidney, and intestine [22, 23, 24 and 25].

Gill ATPases activity and of heavy metals accumulation in tissue of rabbitfish seasonally collected from the two polluted hot spot (El-Mex, Abu-Qir) Bay (polluted) and the rocky coast between KayetBey Fork and El-Boughaz opening in the Eastern Harbor toward the open sea, as control area (non-polluted) were the goal of this study. These have gained importance for metal toxicity monitoring program biomarkers because it can be beneficial in evaluating the physiological condition of aquatic animals before toxic effects occur.

2. Materials and Methods

Fish samples were seasonally collected from the coastal areas of El-Mex Bay and Abu-Qir Bay (polluted) and the rocky coast between KayetBey Fork and El-Boughaz opening in the Eastern Harbor toward the open sea, as control area (control) during the year of (2014 -2015). Samples were brought to the laboratory on ice boxes (at 4ºC). In the same day of fish capture the fish samples were dissected for analysis. Composite samples of white muscle, gills, and liver were taken, using stainless steel instruments on a clean glass working surfaces. Liver, white muscle and gill samples were excised and weighted and used for heavy metal determination, and assessment ATPases enzymes in tissues.

2.1. Determination of Heavy Metals

The total concentrations of heavy metals were determined according to [26], A sample of tissue sample was digested using a mixture of nitric, perchloric and hydrofluoric acids in a previously cleaned and dried Teflon beaker, then evaporated to near dryness at 80°C. After complete digestion, the residue was transferred to 25 ml volumetric flask with 0.1 M HC1. The concentration of trace metals were measured using atomic absorption spectrophotometer (AAS), Shimadzu model (6800), metals were determined and measured in $\mu g\ g^{-1}$ wet mass.

2.2. Assays of Gill Na^+/K^+-, Ca^{2+}-, and Mg^{2+}-ATPase Activities

Gill samples was weighed and homogenized by Omni TissueMaster-125 variable speed homogenizer at 35,000 rpm in 11:10 w:v 65 mM L^{-1} in imidazole buffer (pH 7.4). The activity of ATPases was assayed in the crude homogenate by determining the inorganic phosphate (Pi) liberated from the hydrolysis of the substrate ATP at 25ºC [27]. Incubation media was prepared as described by [28]. Final assay concentrations of chemicals used for incubation media were (in mML^{-1}):Tris–HCl (pH 7.4) 30, NaCl 100, KCl 5, $MgCl_2$ 5, ATP (Vanadium free) 3 and EDTA 0.1 for Na^+, K^+-ATPase, Tris–HCl (pH 7.4) 135, $MgCl_2$ 6, ATP 3 and EDTA 0.1 for Mg^{2+}-ATPase, Tris–HCl (pH 7.4) 135.85, $MgCl_2$ 6, $CaCl_2$ 0.5, ATP 3 and EDTA 0.1 for Ca^{2+}-ATPase.

After pre-incubation of the medium for 5 min. at 25°C, the reaction was initiated by adding the samples and ATP, appropriately. The reaction was continued for 30 min. It was stopped after putting the samples on ice for 10 min and adding of an ascorbic acid-molybdic acid (1:6, v/v) mixture. Samples were kept at room temperature for 10 min. Inorganic phosphate was determined at 390 nm using 1 mL aliquots of the incubated mixtures. The (Ca^{2+},Mg^{2+})-ATPase activity was calculated from the slope of the plot, with the activity in the presence of EDTA subtracted from that in its absence. All assays were carried out in triplicate against the reaction blank. The ATPase activity was expressed as μM Pi/min/g wet tissue. The change in absorbance was monitored at 390 nm using UV/VIS Spectrophotometer (JENWAY 6505) UK.

2.3. Statistical Analysisand Chemicals

Each reading represents Mean±SD of 6 fish. Data were analyzed by one-way analysis of variance (ANOVA) using Dunnett's test using a software program (GraphPad 6 InStat Software, Inc.). The level of $P \leq 0.05$ was regarded statistically significant. Analytical grade chemicals were used (Sigma-Aldrich Chemical Co., St. Louis, MO, USA).

3. Results

3.1. Physicochemical Characteristics of Water

The temperature, pH, dissolved oxygen, conductivity, and

total dissolved solids at different areas during the four seasons in comparison with the control non-polluted area were tabulated (table 1). The pH values of the samples ranged from 7.2 to 8.2, dissolved oxygen ranged from 1.1 to 10.2 (mgL^{-1}), and total dissolved solids ranged from 15 to 128 (gL^{-1}) at different localities during the four seasons. The physicochemical parameters of El- Mex Bay water were significantly different in comparison with water of the other areas.

Table (1). *Seasonal physicochemical characteristics of water collected from El- Mex Bay, Abu–Qir Bay (polluted) and control (non-polluted).*

Season	Site	Temp (°C)	pH	DO (mg L^{-1})	EC (µs cm^{-1})	TDS (g L^{-1})	Salinity (‰)
Spring	Control	20.0	8.2 ± 0.05	9.5±1.3	201 ±15	128.6±12	35 ±0.1
	Abu- Qir	20.15	8.1 ± 0.03	8.1±2.0	198 ±21	87.±12*	35 ±0.5
	El-Mex	20.3	7.7 ± 0.06*	1.1±0.4*	36 ±18*	27 ±11*	2.3±0.1*
Summer	Control	27.8	8.2 ± 0.04	8.1±1.4	169 ±13	111 ±23	35 ±0.3
	Abu-Qir	28.4	8.0 ± 0.02	8.1±3.2	170 ±22	86 ±14*	35 ±0.5
	El-Mex	26.2	7.5 ± 0.07*	2.5±1.1*	13.0 ±2.*	20 ±1.1*	4.2±0.2*
Autumn	Control	25.3	8.2 ± 0.05	10.2±3.1	146.0±31	93 ±32	35 ±0.1
	Abu-Qir	26.8	8.0 ± 0.04	9.1±2.0	113 ±12*	88.6±2*	35 ±0.3
	El-Mex	25.2	7.2 ± 0.05*	3.8±2.2*	25.3 ±4*	16 ±2.3*	3.8±0.2*
Winter	Control	18.5	8.2 ± 0.03	9.1±3.1	171.2±13	123 ±13	35 ±0.1
	Abu-Qir	18.3	8.1 ± 0.06	8.7±4.1	176 ±17	104±11*	30 ±0.6
	El-Mex	18.1	7.5 ± 0.06*	4.1±1.5*	20.4 ±3*	15 ±3.3*	2.4±0.2*

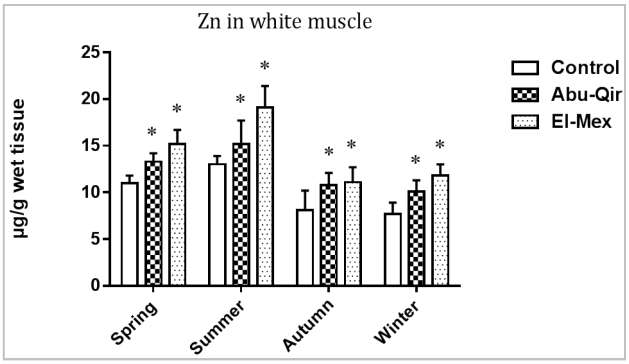
- Each reading represents Mean± SD of 6 samples
- The significant of difference was checked by Dunnett's test (compare all vs. control using a computer program (Graph pad In State Software, Inc.).
- * The difference was significant at $P \leq 0.05$ in comparison with control.
- Temp (temperature), DO: Dissolved oxygen, EC: Conductivity of Water, TDS: Total dissolved solids.

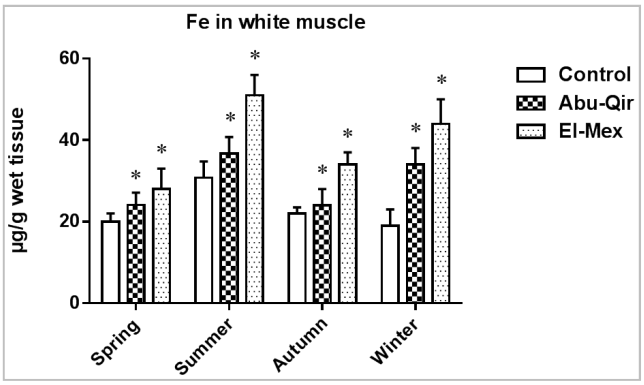
3.2. Accumulation of Heavy Metals (µg g^{-1} Wet Weight Tissue)

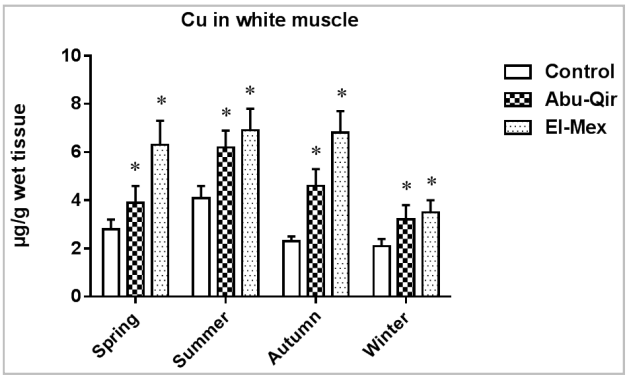
There was significant increase in the metals Zn, Cu, pb and Cd accumulated in white muscle of rabbitfish caught seasonally from Abu-Qir Bay and El-Mex Bay in comparison with control. The metal Fe was significantly increased in white muscle of rabbitfish caught only seasonally from El-Mex Bay in comparison with control (figure 1).

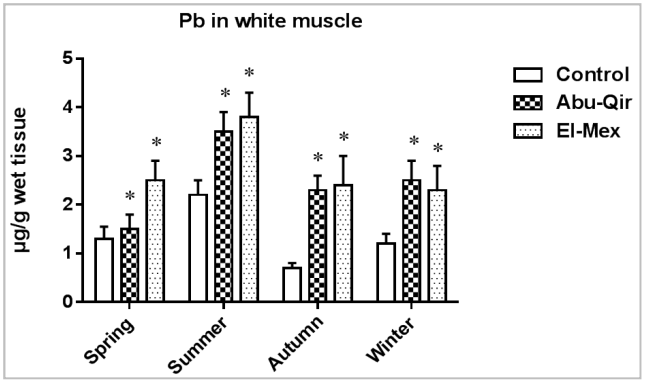
There was significant increase in Zn accumulated in gills of rabbitfish only caught in summer from Abu-Qir Bay and caught seasonally from El-Mex Bay in comparison with control. There was significant increase in Fe and Cu accumulated in gills of rabbitfish caught seasonally from Abu-Qir Bay and El-Mex Bay in comparison with control. There was significant increase in pb accumulated in gills of rabbitfish only caught in summer, spring and winter from Abu-Qir Bay and El-Mex in comparison with control. There was significant increase in Cd accumulated in gills of rabbitfish only in spring, summer, and autumn from Abu-Qir Bay (figure 2) in comparison with control.

There was significant increase in Zn, pb accumulated in liver of rabbitfish caught seasonally from Abu-Qir Bay and El-Mex Bay in comparison with control. There was significant increase in the of Cu accumulated only in spring, summer and autumn accumulated in liver of rabbitfish caught seasonally from Abu-Qir Bay and El-Mex Bay in comparison with control. There was significant increase in the Cd accumulated only in liver of rabbitfish caught seasonally from El-Mex Bay in comparison with control (Figure 3).









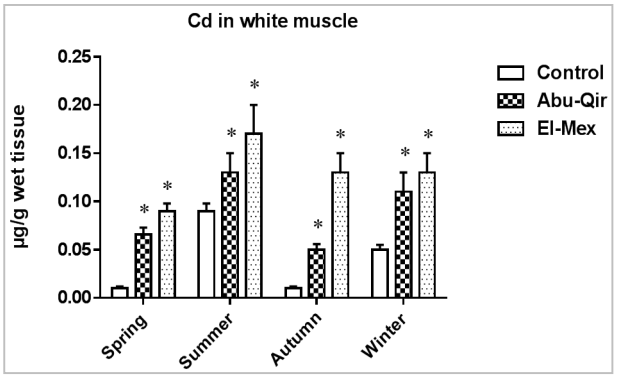
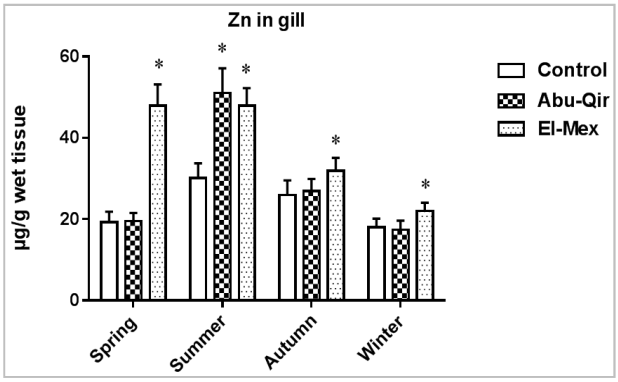
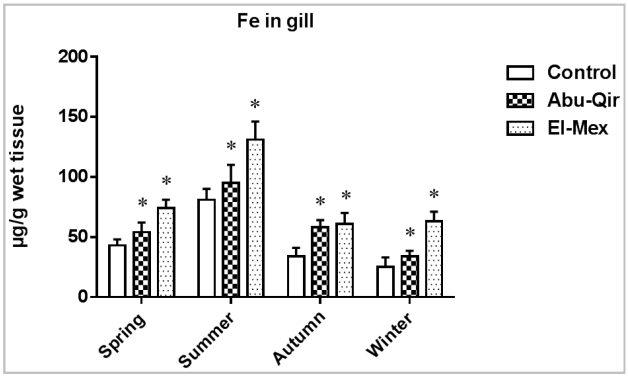
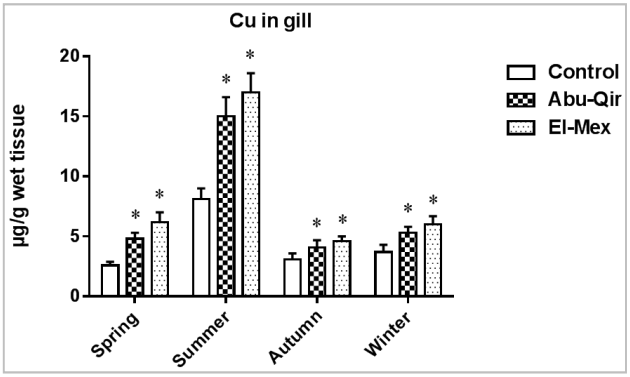
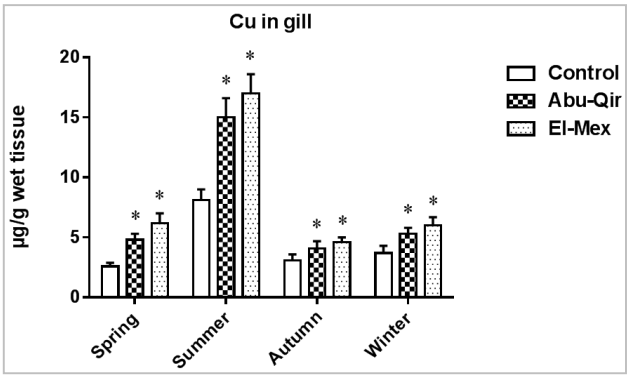


Figure (1). *Heavy metals in white muscle of rabbitfish seasonally caught from El-Mex Bay, Abu-Qir Bay in comparison with control. Each reading represents Mean±SD of 6 fish. Data were analyzed statistically by one-way ANOVA and Dunnett's test at P ≤ 0.05 (*)in comparison with control.*









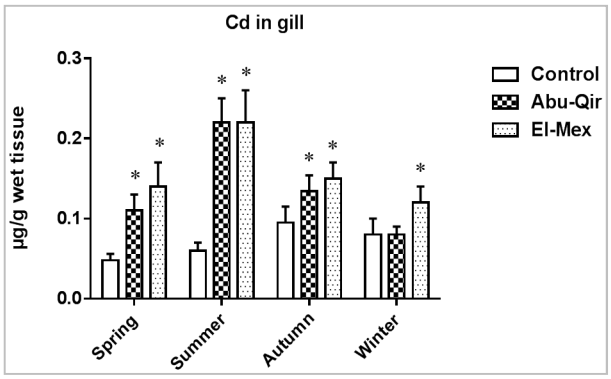
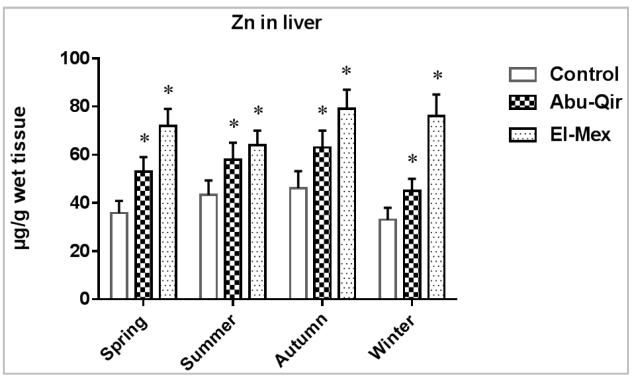
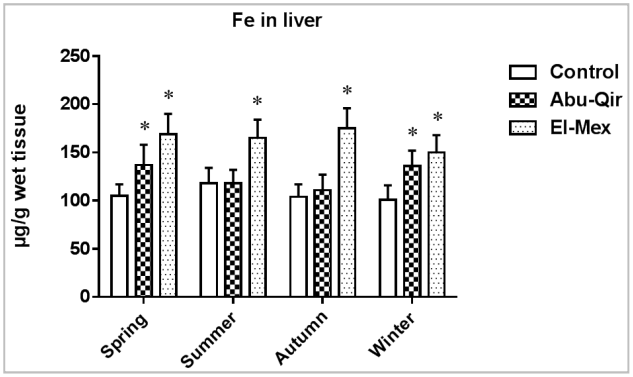
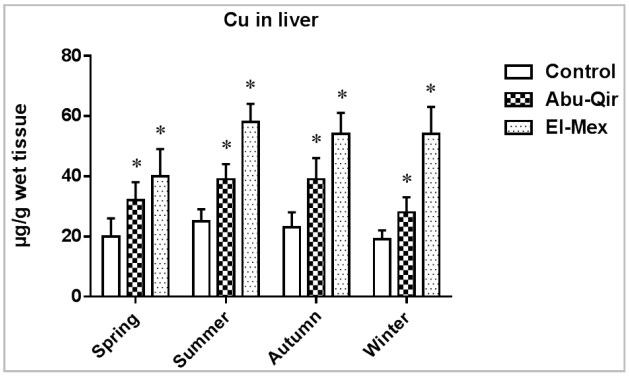
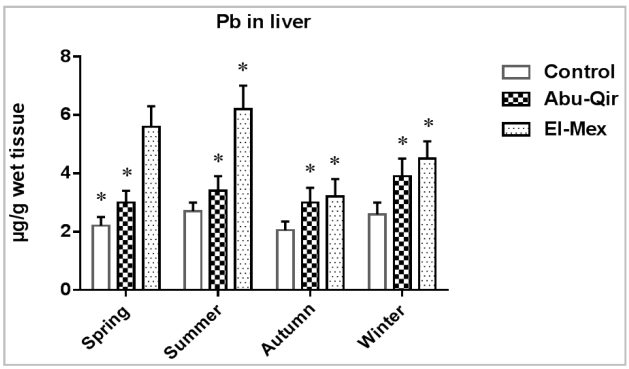


Figure (2). *Heavy metals in gills of rabbitfish seasonally caught from El-Mex Bay, Abu-Qir Bay in comparison with control. Each reading represents Mean±SD of 6 fish. Data were analyzed statistically by one-way ANOVA and Dunnett's test at P ≤ 0.05 (*) in comparison with control.*









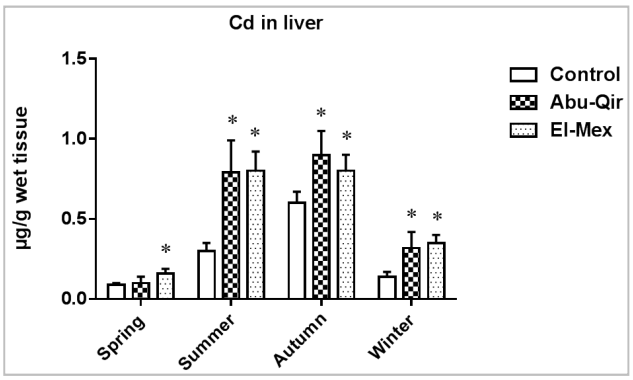
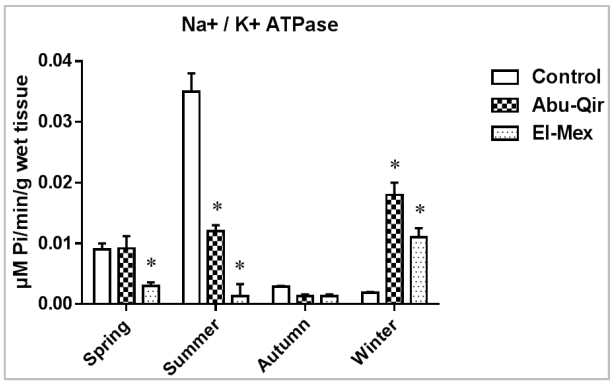
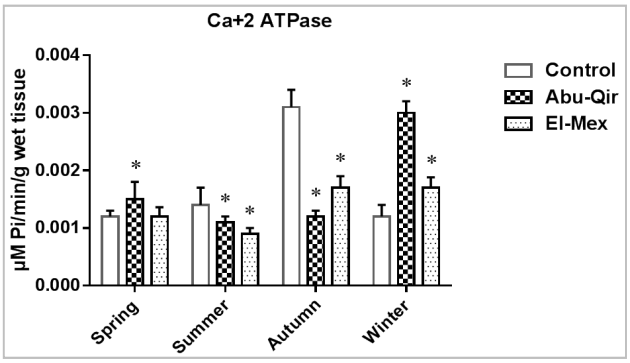


Figure (3). *Heavy metals in liver of rabbitfish seasonally caught from El-Mex Bay, Abu-Qir Bay in comparison with control. Each reading represents Mean±SD of 6 fish.Data were analyzed statistically by one-way ANOVA and Dunnett's test at P ≤ 0.05 (*) in comparison with control.*

3.3. Gill ATPases Activities

The activities of Na^+K^+/Ca^{2+} and Mg^{2+}-ATPase in gills (µM Pi/min/g wet weight tissue) of rabbitfish caught seasonally from El-Mex Bay, Abu–Qir Bay in comparison with control were illustrated in Fig. (4).





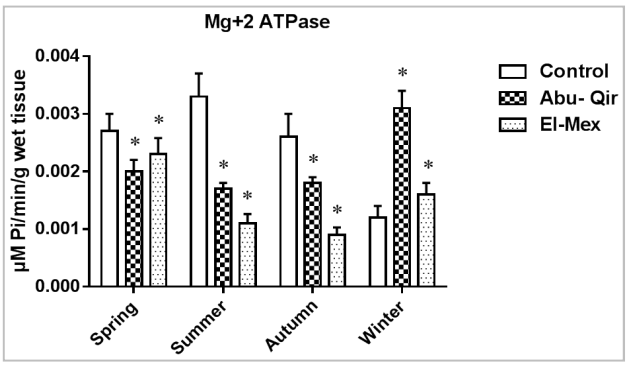


Figure (4). *Na^+K^+/Ca^{2+} and Mg^{2+}-ATPase activities (µM Pi/min/g wet weight tissue) in gills of rabbitfish caught seasonally from El-Mex Bay, Abu–Qir Bay and control. Each reading represents Mean±SD of 6 fish. Data were analyzed statistically by one-way ANOVA and Dunnett's test at P ≤ 0.05 (*) in comparison with control.*

In gills of rabbitfish caught from Abu-Qir Bay, there was significant decrease in the activity of Na^+/K^{+-}ATPase in only in summer and significant increase in winter in comparison with control. However, there was significant decrease in the activity of Na^+/K^{+-}ATPase in gills in spring, summer, and significant increase in winter of rabbitfish caught seasonally from El-Mex Bay in comparison with control.

There was significant decrease in the activity of Ca^{2+}-ATPase in gills of rabbitfish caught from Abu-Qir Bay in autumn and significant increase in winter. However, there was significant decrease in spring, summer and autumn but significant increase in winter in rabbitfish caught from El-Mex in comparison with control.

There was significant decrease in the activity of Mg^{2+}-ATPase in gills of rabbitfish caught from Abu-Qir Bay in spring, summer and autumn, but significant increase in winter in comparison with control. In El-Mex there was significant decrease in spring, summer, autumn, in comparison with control.

4. Discussion

It was of our interest to study the physicochemical characteristics of water which, demonstrated that the conductivity of water samples of El-Mex Bay decreased significantly which may influence on uptake and toxicity of metals in fish in this area. Conductivity of water can affect bioavailability, uptake, and toxicity of metals. When the conductivity of water increased, metal bioavailability, and consequent metal toxicity decreases as a result of chelation processes. Thus, one can say that metals are more toxic to fish when they are exposed in soft water compared to hard water [12, 23, 29, 30 and 31]. Salinity affects the speciation and bioavailability of trace metals, influencing uptake of heavy metals by aquatic organisms [10]. Thus, it directly controls the amount of incoming metal that potentially altering the ATPase enzymes in osmoregulatory system [11, 12]. Salinity decreased significantly from El-Mex Bay vs. Abu-Qir and control and may influence on the activity of ATPases this agrees with previous study of [32] who studied single and combined effects of Cu^{2+}after salinity challenge in *Anguilla anguilla* and showed decrease in ATPase activities at low salinity. The activity of Na^{+}/ K^{+}, $Ca^{2+}$and Mg^{2+}ATPase enzymes reach their maximum activities in high temperature season (summer) in control this agree with [33], while the activity of these enzymes in polluted area (El-Mex Bay and Abu-Qir Bay) decrease in summer this may be due to high accumulation of heavy metals especially lead in this season. There was significant increase in accumulation of Zn, Cu, Fe, pb, and Cd in three tissues of rabbitfish from El- Mex Bay, Abu Qir Bay vs. control seasonally. The accumulation of metals in white muscle, gills, and liver increased in summer, spring rather than other seasons this may be related to increase human activities in this seasons and increase rate of metabolism due to increase of temperature this confirmed by previous studies such as [34]. Metals accumulated in order Fe>Zn>Cu>pb>Cd. The accumulation of heavy metals increase in liver>gill> white muscle in Zn, Fe, Cu and Cd. Lead is a non-essential metal with no biological function and can be toxic to aquatic animals when given in access amounts. Previous studies have shown that pb^{2+} causes disruption of Na^{+}, Cl and Ca^{2+} regulation and disruption in hemoglobin synthesis [35, 36 and 37]. pb^{2+} interacts with other elements synergistically or antagonistically. There are evidences on the antagonism between Pb^{2+} and Ca^{2+}, by which this metal directly competes with Ca^{2+} for uptake at calcium binding sites and can enter the cells through similar transport pathways [31, 38, 39, 40, 41, 42 and 43]. Similarly, waterborne Ca^{2+} has a marked protective effect against waterborne Cd^{2+} toxicity to brook trout [44], tilapia [45, 46], and rainbow trout [47]. Our previous study showed that there were exposure dependent decreases in the branchial Na^{+} /K^{+}-ATPase activity in the tissues of *Tilapia zillii* exposed to Cu^{2+} and pb^{2+} [48]. Similarly,[49] showed that effect of Pb^{2+} with competitive inhibition of apical Ca^{2+} channel in fish gills disrupt Ca^{2+} homeostasis. In another study, demonstrated that activities of ATPases in tissues of Nile tilapia altered significantly after exposure to metals (Cd^{2+}, Cu^{2+}, Zn^{2+} and pb^{2+}) for 14 days with an inhibition trend of Na^{+} /K^{+}-ATPase activity in the gills and Ca^{2+}-ATPase activity in white muscle [25]. ATPase activities in the gills, kidney, and white muscle of *Nile tilapia* exposed to Cd^{2+}, Cr^{6+}, and Ag^{+} for 96 h decreased, in general [50]. They concluded that decreased ATPase activities were due to the direct effects of metals, these agree with [31, 42, and 43]. When particularly Ca^{2+}-ATPase and Mg^{2+}-ATPase decreased, excessive Ca^{2+}would be accumulated in the cytosol and resulted in metabolic malfunction and cellular abnormalities [51]. Lead could potentially have an impact at any one of steps of Ca^{2+}entry and subsequently lead disturbance of Ca^{2+} homeostasis by and caused competitive inhibition at apical of Ca^{2+} channels in the gills of fish [19,53]. Lead causes inhibition in all ATPase enzymes. The inhibition of Na^{+}/K^{+} occurred because the enzyme molecule is very susceptible to the metals from sulfhydryl groups. Lead also degraded phosphorylation activity by bonding with the carboxyl group in the active region of the enzyme as a result of the damage to the membrane or irregularities occurring in the ion hemostasis [18, 19]. We can conclude that many environmental factories influence on the activity of three ATPases in rabbitfish such as seasonal variation in temperature, Salinity and accumulation of heavy metals especially lead in these hot spots areas. Data from the present study indicated that physicochemical characteristics of water and the metabolic activity of a specific organism are very important factor to measure the effects of metals in water, especially on ATPases and emphasized water ion levels should be measured in the evaluation data from the field.

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