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Heavy Metals Accumulation in the Barnacle *Amphibalanus amphitrite* from Alexandria, Mediterranean Sea

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ABSTRACT

Concentrations of eight heavy metals (Cd, Pb, Cu, Cr, Zn, Mn, Fe and Ni) were determined in *Amphibalanus amphitrite* Darwin collected from two coastal areas (El-Montaza Bay and El-Dekhela Harbour) along the Egyptian seaside of the Mediterranean Sea of Alexandria. Both sites have different levels of pollutions. The sampled animals were collected during the dry season of 2009 (April, May and June) and the wet season of 2010 (January and February). Significant spatial and temporal differences in the concentrations of the studied metals were found. *Amphibalanus amphitrite* accumulated the heavy metals at relatively high concentrations in their tissues. These accumulations may relate to their very efficient storage detoxification systems and/or due to the biological needs of animal for these metals. Zn, Pb, Fe and Cu presented the highest level of concentration. Among them Zn accumulation was the peak level with the mean value 94.46 $\mu g\ g^{-1}$ dry weight followed by Pb. In addition, the study investigated the spatial and temporal variance of the heavy metals in soft tissues of *Amphibalanus amphitrite*.

Key words: Heavy metals, barnacle, *Amphibalanus amphitrite*, Alexandria, Egypt

INTRODUCTION

During recent decades, the high population in Alexandria, Egypt has soared to a steady rural exodus to this urban centre beside considered as the principal seaside summer resort on the Mediterranean (Mostafa *et al*., 2000). With the rapid increase in the industries and population, changes in water quality would have potential consequences for the large rapidly growing population of the Alexandria region (Frihy *et al*., 1996). In addition, there have been many environmental problems in the coastal areas, including beach erosion, environmental degradation and destruction of natural habitats. Hence, continuous monitoring studies of trace elements concentration in the Alexandria are needed.

Marine water quality has become a matter of serious concern because of its effects on human health and aquatic ecosystems, including a rich array of marine life. Heavy metals are persistent, toxic, tend to bioaccumulate and they pose a risk to marine species diversity, humans and ecosystem. They have the potential to cause ecotoxicological effects in coastal habitats if present in sufficiently high availabilities, usually as a result of anthropogenic activities (Philips and Rainbow, 1994; Szefer, 2002; Rainbow, 2006). Metals, such as iron, copper, zinc and manganese, are essential since they play important roles in biological systems, where as lead and cadmium are toxic, even in trace amounts. Lead

and cadmium have been included in the regulations of the European Union for hazardous metals (EC, 2001), while USFDA (1993a, b, c) has included chromium and nickel in the list.

Marine organisms, in general, accumulate contaminants from the environment and therefore have been extensively used in marine pollution monitoring programs (Uthe *et al.*, 1991; UNEP, 1993).

Organisms suitable for use as biomonitors provide integrated measures of the supply of trace metals available to them in an environment, accumulating the metal taken up from all sources such as from water and from food (Phillips and Rainbow, 1993; Rainbow, 1995a, b). Biomonitors therefore offer a direct measure of metal pollution as it will affect the local ecosystem (Rainbow *et al.*, 2000). The criteria by which organisms are accepted as biological indicator for the assessment of contamination were proposed more than twenty five years ago and remain unchanged (Phillips, 1976; Fowler and Oregioni, 1976).

Barnacles are present in different types of locations, with different degrees of pollution, so this group of organisms may be considered ideal for contaminants biomonitoring programs (Ruelas-Inzunza and Paez-Osuna, 1998). Indeed, barnacles normally satisfy all the main characteristics for biomonitoring species proposed by Barbaro *et al.* (1978).

The barnacle bioindicator *Amphibalanus amphitrite* is one of these organisms, which has been successfully used in monitoring programs for heavy metals especially in the Indo-Pacific region (Rainbow, 1987, 1995a; Phillips and Rainbow, 1988, 1993; Rainbow and Phillips, 1993; Blackmore *et al.*, 1998; Blackmore and Rainbow, 2001; Reis *et al.*, 2011).

This study has set out to investigate the spatial variation of the bioavailabilities of the heavy metals, zinc, lead, copper, manganese, iron, cadmium, nickel and chromium in *Amphibalanus amphitrite*. In addition, to study the effect of seasonal variation in the accumulations of these heavy metals.

MATERIAL AND METHODS

Sampling locations: The sampling sites are located along the marine coastal water of Alexandria. They were selected on the basis of potentially different pollution input levels (Fig. 1).

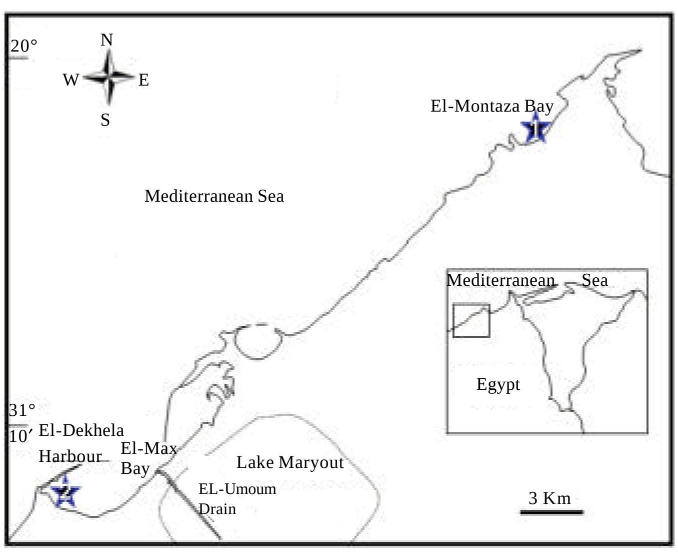

Fig. 1: Map showing the coastal line of Alexandria and sampling sites

Location No. 1 (El-Montaza Bay): It is a semi enclosed bay, the estimated area of the bay that calculated from admiralty chart is about 0.075 km^2 (El-Sammak, 1999). It is a famous summer beach and it receives about 1000 m^3 of sewage per day (El-Rayis *et al.*, 1997a).

Location No. 2 (El-Dekhela Harbour): El-Dekhela Harbour is a semi-enclosed basin constructed after 1986 on the western side of El-Mex Bay west of Alexandria (Fahmy *et al.*, 1997). The harbour's water is subjected to several sources of wastewater. A huge volume of brackish water is discharged into El-Mex Bay through the El-Umoum drain. At its downstream part and before reaching the sea its water mixes with water effluent (surplus water) from a neighboring sewage polluted lake called Lake Maryout, rate 262.8×106 m^3 $year^{-1}$ (El-Rayis and Abdallah, 2005). This bay also receives industrial wastes from several sources as Chloro alkali plant and petroleum refinery.

Animal sampling: The studied animal, barnacle *Amphibalanus amphitrite* Darwin (Crustacea, Cirripedia) were removed from the substratum by a stainless steel scraper from the studied sites along the coast of Alexandria. After washing by the sea water they placed into plastic bags and transferred to the laboratory at which they frozen at -20°C until dissection. The shell heights ranged 5-8 mm. The animals were collected during the dry season of 2009 (April, May and June) and during the wet season of 2010 (January and February).

Chemical analysis: The procedure used for measuring concentrations of trace elements has been described formerly (Anan *et al.*, 2001). Tissue samples were dried for 12 h at 80°C and weighed and digested in a microwave oven using nitric acid in a Teflon PTFE tube. Concentrations of studied heavy metals (Cd, Pb, Cu, Cr, Mn, Fe, Ni and Zn) were measured using an Atomic Absorption Spectrophotometer (AAS, Varian Techtron-Model 1250). Before each metal determination all reagents used were of analytical grade (Analar). Results were expressed as ($\mu g\ g^{-1}$) dry weight.

The analytical blanks were run in the same way as the samples and concentrations were determined using standard solutions prepared in the same acid matrix.

The accuracy and precision of our results were checked by analyzing standard references materials (materials SRM Dorm-2 National Research Council Canada).

Data analyses: Data collected were subjected to statistical tests of significance using the Student t-test and Analysis of Variance (one-way ANOVA) at $p<0.05$ to access whether heavy metal Concentrations varied significantly between sites and seasons. Probabilities less than 0.05 ($p<0.05$) were considered statistically significant. All statistical calculations were performed with SPSS 9.0 for Windows.

RESULTS AND DISCUSSION

Figure 2 and 3 illustrate the heavy metals concentrations in the whole soft tissues of the studied animal, *Amphibalanus amphitrite*, obtained from the two studied locations. The peak values of all metals were observed in location No. 2 (El Dekhela Harbour) in wet season. Zinc was the highest accumulated metal and lead was the second. Concentrations of all metals in the studied locations are differ significantly ($p<0.005$) in both, the dry season of 2009 and the wet season of 2010.

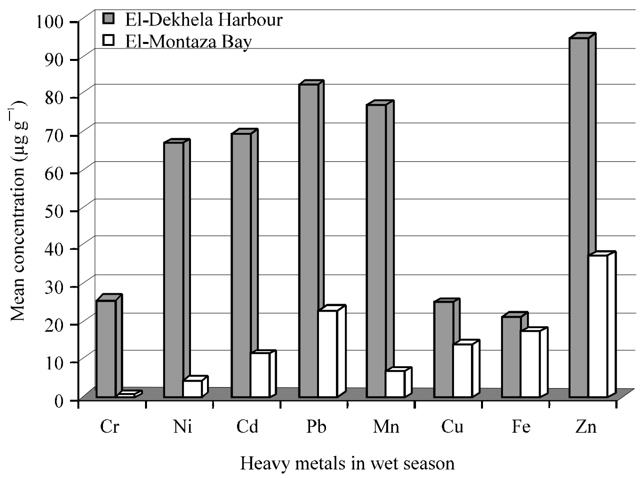

Fig. 2: Mean concentrations (µg g^{-1}) of the studied elements in wet season of both locations

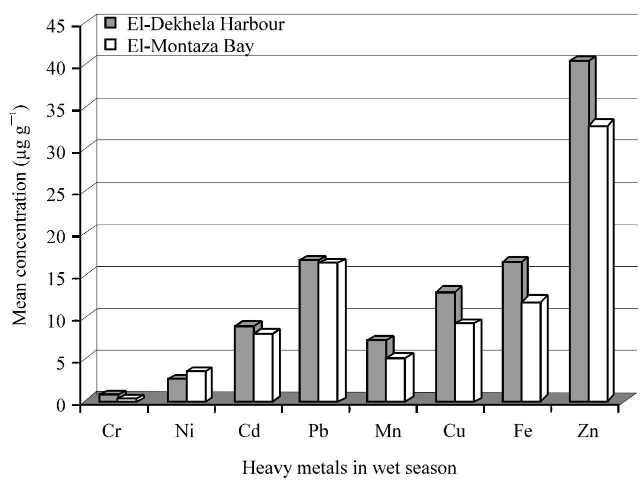

Fig. 3: Mean concentrations (µg g^{-1}) of the studied elements in dry season of both locations

Metal accumulations in the soft tissues: The zinc content, in the soft tissues of *Amphibalanus amphitrite*, represents the highest mean concentration when compared to the other metals (Fig. 2, 3) in both locations during the dry and the wet seasons. According to Rainbow (1988) the high range of Zn concentrations is normally reported for marine species caught from relatively polluted areas of the world.

Walker *et al.* (1975a) used *Semibalanus balanoides*, to study Zn contamination in coastal waters of North Wales (UK). This work revealed that barnacles accumulated Zn in their soft tissues, mainly in granules of the mid-gut, in levels which directly reflected the concentrations of the coastal environment.

Earlier pioneering studies using X-ray microanalysis show numerous granules containing Zn phosphates beneath the gut epithelium, which serve as an important detoxification mechanism for the deposition of Zn in barnacles (Walker *et al.*, 1975a, b; Rainbow, 1987). In confirmation, Rainbow and White (1989) and Rainbow (1998) reported that all zinc taken up from solution by

barnacles is accumulated without excretion and the accumulated body concentrations reach very high levels (e.g., 50,000 μg Zn g^{-1} or more) and most of this accumulated zinc inevitably is in detoxified form.

Copper (Cu), an essential nutrient for all species (Uriu-Adams and Keen, 2005), can become toxic at high cellular concentrations (Irwin, 1997), interfering with several metabolic pathways and thereby inducing different responses at cellular and/or higher levels. Figure 2 and 3 showed that the mean Cu concentrations ranged 9.3-24.9 μg g^{-1} dry wt. in the samples collected from El-Montaza Bay and El-Dekhela Harbour. Clark (2001) declared that anti-fouling paint for ships' hulls is one of the major sources of Cu pollution in an aquatic environment. On the other hand, Moore and Ramamoorthy (1984) conclude that the major discharged of copper are anthropogenic sources such as waste incineration and domestic water discharges. The anti-fouling paint for ships' hulls is one of the major sources of Cu pollution in an aquatic environment (Clark, 2001). In the present study, the high levels of copper concentrations observed are probably caused by the proximity to shipyards and harbours where copper-based, antifouling paints are used in both locations.

In copper-contaminated sites, barnacles have the potential to increase their body concentrations of copper well above those of amphipods and carideans (Phillips and Rainbow, 1988). For example, the concentrations of Cu in *Balanus amphitrite* sampled from the contaminated sites accumulated was 3472 μg g^{-1} and those from the non contaminated sites was 59.3 μg g^{-1} (Phillips and Rainbow, 1988). Making many assumptions such as the equal contribution of each enzyme to the total enzyme load, they estimated that metabolizing tissue needs approximately 26.3 μg Cu g^{-1} and 34.5 μg Zn g^{-1} to fulfill enzyme requirements. Anil and Wagh (1988) collected *Amphibalanus amphitrite* from the Zuari Estuary in west coast of India and showed that it could accumulate 865 μg Cu g^{-1} and 1937 μg Zn g^{-1} in its soft tissues, when the coastal waters only presented 1-11 μg Cu L^{-1} and 13-46 μg Zn L^{-1}.

Iron is the most abundant transition element and probably the most well known metal in biologic systems (Forstner and Wittmann, 1983). In the present study iron mean concentrations in locations No. 1 and 2 ranged from 11.9 to 21.2 μg g^{-1} dry wt. (Fig. 2, 3). These high accumulated values may due to the importance of iron as essential element for the physiological and biological activities of the studied animals.

Lead is a non-essential metal, accumulated rather-than regulated by most aquatic taxa and exerts toxic effects at lower concentrations than many other metallic contaminants (MacFarlane *et al.*, 2000). It ranks as the metal of largest diffusion through the atmosphere (SEMA, 1998).

It has been shown that Pb is the second accumulated metal in the soft tissues of *Amphibalanus amphitrite*. The highest values detected were in animals collected from El-Dekhela Harbour (Fig. 2, 3). This result is not surprising as this location is subject to much shipping activities. Beside the release to the atmospheric fumes evolved from a cement factory, steel and Iron factory in addition to the agriculture drainage from El-Umoum Drain (Abdel-Moati and El-Nady, 1991; El-Rayis and Abdallah, 2005).

Cadmium has no known biological function; it is considerably toxic for both vertebrates and invertebrates alike because of its long biological lifetime (Elinder, 1982). The barnacle *B. amphitrite* is a useful bioindicator, especially for Cd, because it exhibits a low depuration rate and a direct relation between the metal in the dissolved phase and in its tissue (Wang *et al.*, 1999a, b; Rainbow *et al.*, 2003).

No crustacean regulates the body concentration of the non-essential metal cadmium (Rainbow, 1998). In barnacles (*Elminius modestus*), all cadmium taken up from solution is accumulated without excretion over at least a 28 day period (Rainbow and White, 1989).

Phillips and Rainbow (1988) studied the accumulation of Cd in *B. amphitrite* collected from both contaminated and non contaminated sites and the values were ranged between 7.3 and 5.5 μg g^{-1}, respectively. Blackmore (1996) mentioned that the concentrations of Cd (4 to 12 μg g^{-1} dry wt.) in *B. amphitrite* are several times higher than its typical concentrations in other marine invertebrates. Results of the present study showed high levels of Cd in the soft tissues (Fig. 2, 3) and this may refers to the high bioavailability of Cd in the sampling sites. Cd concentration in El-Mex Bay is highly widespread and its concentration in sediments was 8.09 μg g^{-1} (Abdallah, 2007; Abdallah and Abdallah, 2007).

The highest mean content of manganese was found in the soft tissues of the animals sampled from El-Dekhela Harbour (Fig. 2, 3). This may indicate to the high concentrations of Mn in the water of this site and/or the available of planktons that rich in this metal. Rainbow (1987) showed that *Amphibalanus improvisus* from Thames estuary (United Kingdom) bioaccumulated high concentrations of Mn in its body tissues. It was possible to assume that areas where barnacles presented high metal concentrations had high metal bioavailabilities (Rainbow *et al.*, 2000, 2002).

Concerning the nickel level, results show remarkable variations in mean concentrations of the collected samples. Ni mean values ranged between 3.8 to 66.9 μg g^{-1}dry wt. (Fig. 2, 3). These wide differences in concentrations may indicate the difference in bioavailability of this metal between sites and seasons.

Chromium can accumulate in barnacles' soft tissues 543 times the levels found in sea water (Van Weerelt *et al.*, 1984). In the present study, the highest mean value recorded in animals collected from El-Dekhela Harbour (Fig. 2, 3). Although chromium is an essential metal, it is also an element that at high levels may produce toxic, mutagenic and carcinogenic effects in biological systems. Its presence in the environment is mainly linked to anthropogenic sources being used in industrial sectors as metal plating and refining, leather industry, processing of steel (Anderson, 1997; Storelli and Marcotrigiano, 2005). In El-Mex Bay there are several of those types of industries (Abdallah, 2007). This may be the explanation for the high Cr concentrations that accumulated in *A. amphitrite* collected from El-Dekhela Harbour.

In general, the present study recorded high accumulations of metals as Zn, Pb and Cu in the soft tissues of the barnacle, *A. amphitrite*. This result may due to the high bioavailability of these metals in both sites. Meanwhile, the results obtained from the analysis carried out on water samples of the Alexandria coastal region confirmed the presence of high concentrations of heavy metals (Pb, Zn and Cu) as well as progressive enrichment in their levels (Abd El-Hakim, 1990; Fahmy *et al.*, 1997).

In this study, the highest accumulations of heavy metals in the barnacle, *A. amphitrite*, were recorded in El-Dekhela Harbour that located in El-Mex Bay. Many studies have recorded an elevation of heavy metal concentrations in this site. Levels of trace elements in water of huge drain (6×106 m^3 day^{-1}) called El-Umoum Drain have increased over the past 15 years (Dahab *et al.*, 1990; El-Rayis *et al.*, 1997a, b ; El-Rayis and Abdallah, 2005). Coastal water of El-Mex Bay receives huge amounts of untreated industrial wastes (Fe, Mn, Cu, Zn, Cd, Pb and Ni) as revealed sediment analysis (Shriadah and Emara, 1996) and water analysis (Fahmy *et al.*, 1997). Abdallah (2008) reported the increase in contamination of El-Mex Bay by trace elements, especially toxic elements (Cd, Cr and Pb), with a risk to human population through consumption of fish.

In wet season, the accumulation of heavy metals increased in animals collected from location No. 1 and 2. The peak mean values detected in El-Dekhela Harbour (location No. 2) that may due to that the wet season is flushing more metals into the system and that enough extra metal is present to promote uptake (Rainbow, personal communication).

Moreover, this location received many heavy metals from the agriculture wastes from El-Umoum Drain. Otchere *et al.* (2003) found that metals present in fertilizers include Cd, Cu, Cr, Ni, Mn, Mo and Zn, eventually, many of them may accumulate in soils and become exposed to run-offs during the rainy season.

Spatial and temporal trend: Multiple factors including season, physical and chemical properties of water can play a significant role in metal accumulation in different animal tissues.

In the present study, the accumulated metal concentrations varied over time for each metal at each site. It appears that over the time period of the dry season (April, May and June, 2009) and the wet season (January and February, 2010) the mean concentrations values of the studied metals are increased in the *A. amphitrite* that collected from location No. 1 and 2 in the wet season.

The statistical test of significance using the Student t-test and analysis of variance (one-way ANOVA), showed significant differences ($p<0.05$) between the levels of heavy metals in both dry and wet seasons and between both studied sites.

So, in this study, the barnacle, *A. amphitrite*, highlighted their ability to detect temporal and/or spatial changes in metal availabilities. These differences may well be attributable to changes in anthropogenic input of metals over even that limited period or to changes in physicochemical factors such as salinity which affect the uptake of many trace metals.

In a similar study on barnacles (*Balanus improvisus*) collected from 5 station between February 2000 to September 2001, zinc and manganese concentrations in barnacles have risen and cadmium concentrations have fallen (Rainbow *et al.*, 2004).

Blackmore (1996) and Blackmore *et al.* (1998) measured metal concentrations in soft tissues of *Amphibalanus amphitrite* and *T. squamosa* from harbour coastal areas, which showed high spatial and temporal variations, reflecting different environmental and anthropogenic sources of metals in each region.

Ireland (1974) measured Cu, Mn, Pb and Zn in soft tissues of *Semibalanus balanoides* collected from the Cardigan Bay (Wales, UK) and found seasonal and spatial variation in metal concentrations, possibly as a result of environmental changes in the river flow rates and in the phytoplankton productivity.

Ruelas-Inzunza and Páez-Osuna (1998, 2000) and Páez-Osuna *et al.* (1999) showed that different barnacle species were suitable biomonitors for metals in Mexican coastal waters and sensitive to the temporal and spatial variations of metal bioavailability.

CONCLUSION

Concentrations of essential metals (Zn, Cu, Fe, Ni, Cr and Mn) and some non-essential metals (Pb and Cd) were determined in soft tissues of *Amphibalanus amphitrite* from El-Montaza Bay and El-Dekhela Harbour. Samples were collected in both wet and dry seasons. Metals were accumulated in soft tissues of the studied species in different concentration values. These differences may well be attributable to changes in anthropogenic input of metals over even that limited period or to changes in physicochemical factors such as salinity and temperatures and/or the availabilities of foods which affect the uptake of many trace metals.

Amphibalanus amphitrite accumulated the heavy metals at relatively high concentrations in their tissues. Of the eight metals studied Zn, Pb, Fe and Cu concentrations were the highest. These accumulations may relate to their very efficient storage detoxification systems and/or due to the biological needs of animal for these metals.

A. amphitrite accumulated the studied heavy metals in the studied sites, revealing significant spatial variations. In addition, these metal concentrations in the barnacle also presented significant temporal variations, suggesting different environmental and anthropogenic sources of metals in each region and/or changes in food sources and in their relative abundance.

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