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ASSESSMENT OF HEAVY ELEMENTS IN THE FRESH PART AND SHELL OF
THE SNAIL *Bellamyabengalensis* IN SHATT AL-ARAB RIVER, IRAQ

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ABSTRACT

Concentrations of a number of heavy elements; Aluminum, Antimony, Arsenic, Cerium, Lithium, Phosphorus, Vanadium, and Zinc were detected in the tissues and shells of the snail species *Bellamyabengalensis* collected from three sites located on Shatt al Arab River in Basrah Governorate, Southern Iraq during 2012. Another aim of the study was to determine the concentrations of such heavy elements in the sediment samples collected from the same sites. Test organisms were collected from locations at the intertidal zones of the Iraqi Estuary; Shatt al Arab River. Time of the study was during winter to autumn 2012. Samples were analyzed by using an inductively coupled plasma mass spectrometer (ICP-MS). The elements concentrations in sediment samples were also analyzed, and were found higher in magnitude, except for Zinc and Phosphorus, which were relatively lower in comparison with concentrations found in the tissues and shells samples. Comparisons of heavy metals concentrations among the tissue and shell samples showed that the tissue samples contain higher levels of all metals, except for antimony, which was lower. Arsenic concentrations were not detected in all tissue and shell samples. However, Arsenic was found detectable in the sediment samples. Statistical significance ($P < 0.05$) existed between tissue and shell loads for all tested heavy elements. The Bio-Sediment Accumulation Factor (BSAF) for heavy elements in the snail *Bellamyabengalensis* has been estimated in this study. It was observed that Zinc and Phosphorus have high (BSAF) factor in the tissue; and their factors were 12.17, and 8.65 respectively. Other elements were found within normal ranges in load for shell and tissue samples.

INTRODUCTION

Shatt al-Arab, (Arabic: “Stream of the Arabs”) is a major river in southeastern Iraq. It is formed by the meeting of the Tigris and Euphrates rivers at the town of Al-Qurnah, to the north of Basrah Governorate. It flows southeastward for (193 km) and passes the Iraqi port

of Basrah and the Iranian port of Abadan before emptying into the Arabian Gulf. Its width increases from about (37 m) at Basrah to (0.8 km) at its mouth south the Iraqi city ; Al-Fao. Along the settled banks, there are date-palm groves, which are naturally irrigated by tidal action. Three locations on the river were addressed as collection sites for this study.

For numerous reasons, the Iraqi environment is exposed to different pollutants due to action of three wars during last two decades (UNEP, 2003), and due to various anthropogenic pollution. Increase in the industrial and commercial activities, along with the growing numbers of residents, vehicles, boats, traffic activities, agricultural activities, livestock numbers, and overall population size of the Basrah Governorate. Also, the increased amounts of solid and liquid wastes, sewage discharges, and oil exploration and production, industry, and oil by-products were all caused the estuarine eco-system of the river to be loaded with various types of pollutants, such as heavy elements, and pesticides that subsequently enter the food chain causing bio-accumulation in the bodies of aquatic creatures, particularly fish and shellfish. Hence, such pollutants may be reaching to human beings and to other segments of the food web (Croteau *et al.*, 2005; Rainbow *et al.*, 2006). Factors known to influence metal concentrations and bio-accumulation in these organisms may include metal bio-availability, concentration, and season of sampling, hydrodynamics of the environment, size, sex, organ, living state, exposure period, reproductive cycle, and changes in tissue composition (Boyden and Phillips 1981; Otitoloju *et al.*, 2006; Abdullahi *et al.*, 2007). Numerous studies have estimated heavy elements concentrations in various gastropod species in diverse aquatic habitats, example are; Ezemonye *et al.*, 2006; Karadede-Akin & Unlu , 2007 ; Clinton *et al.*, 2008. Only few had compared between concentration of heavy elements in the soft tissues and shells (Ravera *et al.*, 2007; Yap and Edward 2010; Berandah *et al.*, 2010; Salahshur *et al.*, 2012; Yap and Cheng 2013). Yap *et al.* (2007) attributed metal distribution in different soft tissues could be due to different mechanisms of regulation, detoxification and/or due to physiological functions in soft tissue. On the other hand, metal distribution in the shell is found, in some parts, due to differences in the mineralogy and chemistry of the different shell layers. A study by Ravera *et al.* (2007) showed that element concentrations in the mussel tissues have a seasonal pattern of variations, contrary to the shells, due to slower earnings time of the elements in the shells than in the tissues. In Salahshur *et al.*(2012) study it was reported that higher Zinc content was observed in the soft tissue of the species *Solenbrevis*, whereas in the shell higher levels of Cadmium and lead metals were bio-accumulated due to

existence of metallothionein that binds and detoxifies toxic elements. Metallothionein has been documented to bind a wide range of metals including cadmium, zinc, mercury, copper, arsenic, silver, etc.

In Shatt al Arab River ecosystem, snails have been tested for use as bio-indicators of heavy elements bio-availability (Al-Qarooni, 2011). In Shatt al Arab River, the snail species *Bellamyabengalensis* is widely spread throughout the bank waters of the rivers on the plants, broken rocks, and on sediments.

To the best of our knowledge, there is no study conducted to measure the concentrations of heavy elements in tissue and shell's of Gastropods especially the species *B.bengalensis* from Shatt al Arab River. Thus, the main objectives of this study were; (1) to determine and compare concentrations of some heavy elements of toxicity concern, such as; Aluminum, Antimony, Arsenic, Cerium, Lithium, Phosphorus, Vanadium, and Zinc in the tissues and shells of the snails to be collected from three sites located on Shatt al Arab River in Basrah Governorate, Southern Iraq during 2012. Aim number (2) was to determine the concentrations of such heavy elements in the sediment samples to be collected from the same sites. (3) To conclude on, and to compare the findings of this study with the findings of the similar studies for the benefit of a thorough understanding of the ecosystem of Shatt al Arab River.

Materials and Methods

Gastropods samples (snails) were randomly collected from 3 sites or stations at the intertidal zone of Shatt al Arab River for the assessment of heavy elements concentrations for the period from Winter to Autumn, 2012 (Fig.1). Sediment samples were also randomly collected for analysis from the bank of the river at the same locations where the snails were picked up. Snails were collect by hands pickup, washed with distilled water, and placed in a cooler box (ice box). Samples were transported to the laboratory in the ice box few hours following the collection, and stored at -20°C for afterward analysis (Al Qarooni 2011; Al Qarooni *et al.*, 2012).

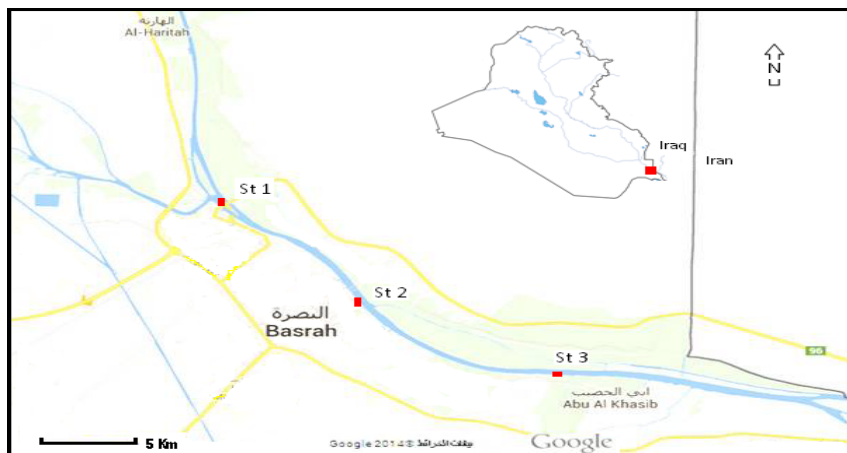


Figure 1: Shatt al Arab River Map viewing the locations of sampling sites for the study area in Basrah / Iraq.

Sample Preparation

In the laboratory, the sediment samples were let to dry, and then were further oven-dried for 24 h. at 70°C. The dried samples were grinded into fine powders using laboratory mortar and crusher pistol, then sieved by means of 2 mm mesh sieve. Sediment samples were subsequently digested according to Yap *et al.* (2002) standard procedure.

Also, in the laboratory, the snails' samples were washed with De-ionized water and dried on filter paper. The soft tissues of the organisms were carefully separated from the shells using clean plastic tweezers. The shells were washed with De-ionized water, and dried on filter papers. The samples of the soft tissue and shells were both dried for 48 h. at 80°C in an oven until reaching constant dry weight. Next, the samples were let to cool in a desiccators. Dried samples were crushed into fine powder in the same way described by Al Qarooni (2011). 1g of the dried soft tissue and shell powder was digested according to Yap *et al.* (2002, 2007). Digestion achieved by addition of 1 ml of hydrogen peroxide to further oxidize any recalcitrant (unmanageable) lipid materials in the sample (Abdullahi *et al.*, 2007). All samples solutions were filtered through filter paper (Whatman No1), transferred into 50-ml standard bottles, and then diluted with de-ionized water.

Chemical Analysis of samples

All samples were analyzed for presence of the concerned heavy elements. Analyses were carried out by using an inductively coupled plasma mass spectrometer (ICP-MS). Heavy elements concentrations in the sediments, shells and tissues were expressed in

microgram per gram ($\mu\text{g/g}$). In order to avoid any contamination, a blank digest sample was carried out in the same way after running the analyses of ten samples. The glassware was washed in diluted HNO_3 and rinsed with de-ionized water (Al Qarooni *et al.*, 2012). The metal contents of the samples were quantified against standard solutions of known concentrations, which were analyzed concurrently according to (skujins, 1998). Bio-Sediment Accumulation Factor (BSAF) for the heavy elements in the snail samples were obtained by using the equation according to Bhalchandra and Ram (2013). The equation is: $\text{BSAF} = C_{\text{organism}} / C_{\text{sediment}}$. Where;

C_{organism} : represents heavy elements concentration in the organism.

C_{sediment} : represents heavy elements concentration in the sediment.

Statistical analysis

Data represents the concentrations of heavy elements in the shell and tissue samples were compared by using independent samples T-test and correlations. Differences were considered significant at $P < 0.01$. Data analysis was carried out using the software program SPSS 16.

Results

Heavy elements in the sediment samples:

The results obtained for the sediment samples were high in concentration, except for zinc and phosphorus, which were relatively low in comparison with tissue and shell samples concentrations. The data is presented in Table (1), and results indicated the following order of magnitude for the estimated elements; $\text{Al} > \text{P} > \text{Zn} > \text{V} > \text{Ce} > \text{Li} > \text{As} > \text{Sb}$.

Heavy elements in the tissue and shell samples:

Highest concentration for Al in tissue was $13109.02 \mu\text{g/g}$ dry wet during autumn in the third station, while the lowest concentration was $1250.22 \mu\text{g/g}$ dry wet in the first station during Spring, 2012. The concentration of Al in the shell samples was between $80.42 - 113.57 \mu\text{g/g}$ dry wet as showed in Table (2). Zinc concentrations in tissue were higher than shell concentrations in about 30 times fold. The highest concentrations of zinc in the tissue and shell were 8.9970 and $262.9109 \mu\text{g/g}$ dry wet as in Table (3). Table (4) shows that V concentrations in tissue were between $1.3229 - 19.4512 \mu\text{g/g}$ dry wet in the third stations during Summer and Autumn seasons, respectively. V concentrations in shells did not pass

4.8467 µg/g dry wet in all stations. Highest concentrations of Li in tissue and shell were 6.9205 and 0.2771 µg/g dry wet during autumn in the third and second stations, respectively as presented in Table (5). Highest concentrations for Ce in tissue and shell were 3.2152 and 0.0663 µg/g dry wet as in Table (6). Antimony (Sb) concentration deviates with other elements, because metal concentrations in the shell component is more than that of the tissue component, which is about 0.1 µg/g dry wet as, presented in the Table (7). Phosphorus (P) concentrations in the tissue were higher than in shells with a magnitude of about 135 times, as shown in Table (8). Arsenic (As) concentration was not detected in all tissue and shell samples. On contrary As concentration was available in sediment.

Statistical significant difference ($P < 0.01$) was found between concentrations of metals in tissues and shells for all heavy elements independently, except for the Sb, that has a significant difference of $P = 0.024$. On the other hand, non-significant correlations were found between tissue and shell concentrations for all tested heavy elements. The correlation between seasons was significant at 0.01 levels in tissue and shell in all seasons, except for spring. This is showed in Table (8).

Bio-sediment Accumulation Factor (BSAF), which is defined as the ratio of metal concentration in the snail to that in sediments according to Bhalchandra and Ram, (2013). Results of (BSAF) calculations of heavy metals in the *Bellamyabengalensis* are presented in Table (9). From the results, it was observed that P and Zn have high (BSAF) factors in tissue. P and Zn factors were 12.1701, and 8.6516, respectively. For other elements, the factors were within normal ranges for shell and tissue loads. The factor values for Li, Al and V in the shells were lower than that found in the tissue values, as indicated in the Table (9).

Table 1: Heavy elements concentrations in sediment µg/g dry wet in the studied area.

station	Aluminium	Zinc	Vanadium	Lithium	Cerium	Antimony	Arsenic	Phosphorus
1	34890.29	30.3887	34.7860	12.3024	13.5304	0.6707	3.6796	525.7368
2	19760.61	73.4944	32.0932	8.9123	10.3134	0.7137	5.3024	482.2434
3	25931.46	41.6814	27.6837	10.6032	10.1488	0.6294	3.0297	897.9576

Table 2: Aluminium concentrations in tissue and shell of *B.bengalensis* µg/g dry wet.

Aluminium		winter	spring	summer	autumn	average
tissue	St. 1	7071.18	1250.22	4902.97	12682.47	6476.71
	St. 2	1507.96	1926.70	3006.30	1652.45	2023.35
	St. 3	1622.71	3590.26	1373.38	13109.02	4923.84
R=−0.04879						
shell	St. 1	53.99	26.2421	168.10	73.36	80.42
	St. 2	56.02	353.50	15.32	27.10	112.99
	St. 3	123.40	46.33	157.34	127.19	113.57

Table 3: Zinc concentrations in tissue and shell of *B. bengalensis* µg/g dry wet.

Zinc		winter	spring	summer	autumn	average
tissue	St. 1	287.7893	399.5345	152.92	211.3998	262.9109
	St. 2	257.7603	171.7124	186.87	62.2501	169.6482
	St. 3	312.3744	298.6185	139.11	221.5266	242.9074
R=-0.23091						
shell	St. 1	12.8516	2.6852	5.47	3.0634	6.0176
	St. 2	3.2651	7.0038	18.85	6.8692	8.9970
	St. 3	6.5472	2.9190	7.53	13.9421	7.7346

Table 4: Vanadium concentrations in tissue and shell of *B. bengalensis* µg/g dry wet.

Vanadium		winter	spring	summer	autumn	average
tissue	St. 1	11.3974	8.4324	7.8835	16.2536	10.9917
	St. 2	3.9956	8.6293	9.2616	5.3421	6.8072
	St. 3	7.0125	7.5793	1.3229	19.4512	8.8415
R=0.077807						
shell	St. 1	3.7731	4.8467	3.9724	2.6763	3.8171
	St. 2	1.7960	3.8268	4.2313	2.2423	3.0241
	St. 3	2.8931	3.1281	3.8489	3.3328	3.3007

Table 5: Lithium concentrations in tissue and shell of *B. bengalensis* µg/g dry wet.

Lithium		winter	spring	summer	autumn	average
tissue	St. 1	3.5277	3.0601	2.3200	5.7720	3.66995
	St. 2	0.7845	1.7931	2.5914	0.8370	1.50150
	St. 3	1.5063	2.3632	0.9794	6.9205	2.94235
R=0.174484						
shell	St. 1	0.3091	0.4700	0.4070	0.4594	0.41138
	St. 2	0.4298	0.7243	0.3553	0.2771	0.44663
	St. 3	0.3465	0.3984	0.4369	0.4978	0.41990

Table 6: Cerium concentrations in tissue and shell of *B. bengalensis* µg/g dry wet.

Cerium		winter	spring	summer	autumn	average
tissue	St. 1	3.5563	1.0777	2.3350	5.8919	3.2152
	St. 2	0.5669	1.5931	2.1247	0.4783	1.1908
	St. 3	1.1702	2.6900	0.5624	7.7793	3.0505
R=0.099937						
shell	St. 1	0.0006	0.0035	0.0750	0.0292	0.0271
	St. 2	0.0030	0.2348	0.0264	0.0009	0.0663
	St. 3	0.0443	0.0250	0.0848	0.0994	0.0634

Table 7: Antimony concentrations in tissue and shell of *B. bengalensis* µg/g dry wet.

Antimony		winter	spring	summer	autumn	average
tissue	St. 1	0.2613	0.4429	0.2271	0.2619	0.2983
	St. 2	0.1190	0.4062	0.3995	0.3922	0.3292
	St. 3	0.3109	0.4041	0.4481	0.3994	0.3906
R=0.401108						
shell	St. 1	0.3544	0.9121	0.3698	0.5310	0.5418
	St. 2	0.4028	0.4188	0.4345	0.3865	0.4107
	St. 3	0.3794	0.5330	0.4432	0.4432	0.4497

Table 8: Phosphorus concentrations in tissue and shell of *B. bengalensis* µg/g dry wet.

Phosphorus		winter	spring	summer	autumn	average
tissue	St. 1	6556.03	9237.23	3865.84	5934.02	6398.28
	St. 2	6070.18	5036.69	5578.93	3667.90	5088.43
	St. 3	6538.04	3991.68	6106.17	5349.79	5496.42
R=-0.06653						
shell	St. 1	41.83	28.5311	38.36	26.91	33.9078
	St. 2	29.22	84.52	18.01	49.13	45.2200
	St. 3	71.50	25.72	66.46	33.16	49.2100

Table 9: Bio-sediment accumulation factor (BSAF) of heavy elements in *B. bengalensis*

Station		Aluminum	Zinc	Vanadium	Lithium	Cerium	Antimony	Phosphorus
tissue	1	0.1856	8.6516	0.3160	0.2983	0.2376	0.4448	12.1701
	2	0.1024	2.3083	0.2121	0.1685	0.1155	0.4613	10.5516
	3	0.1899	5.8277	0.3194	0.2775	0.3006	0.6206	6.1210
shell	1	0.0023	0.1980	0.1097	0.0334	0.0020	0.8078	0.0645
	2	0.0057	0.1224	0.0942	0.0501	0.0064	0.5755	0.0938
	3	0.0044	0.1856	0.1192	0.0396	0.0062	0.7145	0.0548

Discussion

Sediment is the major depository component for metals in the aquatic ecosystem, which in some cases holds more than (99%) of the total amount of a metal presents in the aquatic system (Odiere, 1999). For instance, the data obtained by this study indicated that Phosphorus in the sediment samples for the third station was found high. This may be due to the influence of agricultural activities in this site. The sediments of Shatt al Arab river bank were found to contain high amounts of organic materials. Such organic materials represent complex compounds, with some elements formed by chemical reactions and/or exchange, as it had been indicated by (Al-Haidarey *et al.*, 2009). As a fact, Aluminum is the third most abundant metal (8.1%) on earth, forming the major component in Earth's crust. Hence, this may be is the reason for its high levels in this area, accordingly (Abdollah *et al.*, 2013). The data for Phosphorus in the sediment samples from the third station was found the highest among other concentrations. This may be due to the influence of sewage and industrial waste

discharges from downtown, beside shipwreck and ship remains, which are obvious in this site (Heba *et al.* 2004; Madkour 2005).

High Al, and P concentrations in the snail tissue are due to the presence of high concentrations of these metals in the sediment. This conclusion is in agreement with Ravera *et al.* (2007), and Berandah *et al.* (2010). These studies pointed out to the positive correlation between presence of trace elements in tissue and sediment. On the other hand, high P concentrations in the surrounding environment may lead to the increase in Al concentration in the tissue of dwellers, as stated by Walton *et al.* (2010). This is because snails exposed to P + Al had accumulated more Al than those exposed to Al alone; the loads were (291 μg – 206 μg), respectively. Also, they were found to contain higher proportion of modified Al (de-toxified) element. The average concentrations of Al in *B.bengalensis* varied within the study sites; (6476.71 – 2023.35 $\mu\text{g/g}$). This is similar to the findings of Liu and Kueh (2005) study with the species *Pernaviridis*. However, the magnitude of concentrations was lower in a comparison with the concentrations obtained here by this study. This may be due to the variations between study species and some other influencing conditions related to the environmental conditions in the study area. On the opposite of that, Arsenic (As), which was not detectable in *B.bengalensis* (this study), was found to be detectable in their study with the species *Pernaviridis*, due to Bhalchandra and Ram (2013).

Zinc (Zn) concentration average in this study was 225.1554 $\mu\text{g/g}$ dry wet in tissues was lower than the Zn concentration estimated by Bhalchandra and Ram (2013) study, which was 3498.6 $\mu\text{g/g}$ dry wet in the same species. Zn concentration was much higher in the sediments; 1565.6 $\mu\text{g/g}$ than the concentration determined in this study. Whereas, (As) and (Sb) concentrations in the sediments were generally lesser than other metals. Mori *et al.*, 1999 study showed that (As) and (Sb) contamination reached (9450, 1108 $\mu\text{g/g}$ dry wet), hence may exert a strong impact on the benthic invertebrate community. However, this may not lead to an important decrease in the abundance of the benthic communities in the ecosystem.

All sites samples for both, tissues and shells have revealed a lower (Sb) concentration. This is due to the fact that natural concentrations of the (Sb) element were low, and its occurrence in the Earth's crust is between 0.2 - 0.3 $\mu\text{g/g}$ dry wet, as stated by Foler and Goering (1997). This result is in agreement with (Agah *et al.*, 2009). On the other hand, the present study revealed that the soft tissue of the snail had high levels of all heavy elements except for (Sb). This result disagrees with the findings by (Aboho *et al.*, 2009). Aboho *et al.*(2009) explained

that by the ability of the shell on adsorption of the metals is limited. Also, involves that the trace metals in these organisms are gradually leached out from the viscera (soft tissue) to the shell with time.

The other observed differences among heavy elements accumulation in tissues, that it was the higher concentration for (P) and lower concentrations for (Ce). Bhalchandra and Ram (2013) indicated that the magnitude of heavy metals accumulation in the snail's tissues may depend on the type of heavy metal and the species of the snail tested. Studies on bio-accumulation of (Ce) in invertebrate are rare, and that's why this study may be considered as an important database for this element in Shatt al Arab River.

Statistical significant differences ($P < 0.01$) were found obvious between tissue and shell concentrations for all heavy elements. This result is mainly in agreement with the similar investigations of Ravera *et al.*, 2007; Yap and Edward (2010), Berandah *et al.*, 2010; Salahshur *et al.*, 2012; Yap and Cheng, 2013.

A comparison of heavy metals concentrations among the tissues and shells showed that tissue contains higher level of all metals at Shatt al Arab River. The explanation for this finding can be attributed to the fact that tissues are more capable of binding various types of metals and/or pollutants, due to several mechanisms they possess; such as the active transport mechanisms they have, and due to binding enzymes they possess. This may be making them good bio-accumulation sites. This fact is obvious, and is in agreement with Yap and Cheng, 2013, and with Berandah *et al.*, 2010, too.

Heavy metals concentrations in the sediments were higher than the concentrations estimated in the soft tissue tested, except for (P) and (Zn). Accordingly, the (BSAF) factors were high for (P) and (Zn). This result is in a total agreement with the findings of Bhalchandra and Ram (2013), which had recorded a higher (BSAF) value for zinc; (2.24) in *B.bengalensis*. Thus, this investigation indicates that the snail *B.bengalensis* is able to bio-accumulate higher concentrations of the heavy metal (Zn).

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