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Km Swaileh
Birzeit University

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Heavy metal concentrations in common freshwater snails of Azraq Oasis, Jordan

Khaled M. Swaileh, Mohamed N. Mesmar & Naim S. Ismail*
Department of Biology, Yarmouk University, Irbid, Jordan (author for correspondence)*

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Abstract

Heavy metal concentrations in three freshwater snails, an aquatic plant, sediment, and water from Azraq Oasis pools in the Jordanian desert were measured by atomic absorption spectrophotometry. There were significant differences in metal accumulation among snail species. These snails are ranked according to their metal accumulation capacity in the order: *Melanoides tuberculata* > *Melanopsis praemorsa* > *Theodoxus jordani*. Although there were no significant differences among the size classes of these snails in metal concentrations, larger snails contained generally higher levels of metals than the smaller ones. The concentration factors for the selected snails exceeded 10^3, while it ranged from $0.1–10.2 \times 10^3$ for *Typha angustata* plants. Among sampling sites, metal concentrations in *M. praemorsa*, *T. angustata*, sediment, and water were not significantly different, except for Fe in *M. praemorsa* which was highest at station 3, and Cd and Cr which were highest in water and sediment of station 4. The seasonal variation in metal content was found to be species and metal-dependent.

Introduction

Metal pollution is becoming important with the rapid development in industry and technology. The use of living organisms as bioindicators of metal pollution has several advantages: they integrate pollution over a period of time; indicate the bioavailability and biomobility of the pollutant; may concentrate the pollutant to levels that exceed those in the abiotic environment (Martin & Coughtrey, 1982; Moriarty, 1983). Thus, for a species to act as an effective indicator of metal pollution it must have the ability to concentrate metals, and be abundant and easily collected in the study area.

Azraq Oasis lies in the Jordanian desert at about 100 km east of Amman, the capital city of Jordan. It provides drinking water for Amman and some other cities in Jordan, and has a special importance in being a national park for migrating birds between Africa and Eurasia (Nelson, 1973). However, there have been no studies on the heavy metals in Azraq Oasis inspite the potential pollution from the increasing traffic on the highways passing through Azraq to Iraq and Saudi Arabia.

The freshwater bodies of Azraq are heavily populated with three prosobranch snails: *Melanoides tuberculata* (Muller, 1774) (Thiaridae), *Melanopsis praemorsa* (Linnaeus, 1758) (Thiaridae), and *Theodoxus jordani* (Sowerby,

1836) (Neritidae). The cat tail, *Typha angustata*, is the most common aquatic plant in Azraq (Nelson, 1973).

The purpose of the present study is to determine the concentration of Cu, Zn, Fe, Pb, Cd, Co, Mn, and Cr metals in water, sediment, common snails, and the aquatic plants in Azraq, in order to select the best bioindicators for future monitoring programs.

Materials and methods

Study area

Azraq has a true desert climate, with hot dry summers and cool wet winters. The average annual rainfall is below 100 mm and precipitation occurs mainly during December–January. The extreme annual air temperature range is 10–45 °C with an average of 29 °C (Nelson, 1973).

There are two major springs in Azraq which provide water for its pools. Three pools in South Azraq and one in North Azraq were selected for this study (Fig. 1). Two pools in South Azraq (Stations 1 and 2) and the pool at North Azraq (Station 4) are closed to people since they are located in the vicinity of the water pumping stations. However, station 3 at South Azraq is not closed and is used for activities such as fishing and swimming.

Sampling

Selected snail species were randomly collected by hand picking from edges of the pools and transferred into clean plastic bags. *Melanoides tuberculata* was commonly found in the thin layer of detritus covering the bottom of the pools. *Melanopsis praemorsa* and *Theodoxus jordani* were usually found attached to leaves of the common aquatic plant *Typha angustata*. The collected snails were kept in an ice-box and transferred to the laboratory where they were washed with distilled water, identified according to Brown (1980), measured to the nearest millimeter of length using a calliper, divided into size classes, dried at room temperature over a filter paper, and deep frozen for further analysis.

Submerged pieces of 3 to 5 *T. angustata* plants were collected from each pool, washed with distilled water, and deep frozen for later analysis. Similarly, 3 to 5 sediment samples were taken from each pool and deep frozen for later analysis. Three, one liter, water samples were collected from each pool and stored at 4 °C until analyzed.

The sampling protocol was repeated for four successive seasons: Summer (July 1989), Autumn (October, 1989), Winter (January 1990), and Spring (April 1990).

Analytical procedures

A total of 30–225 snails of each size class from each pool were crushed, and their tissues were dried at 85 °C to constant weights. Weighed dry samples (0.1 gm) were digested in a hot 4:1 mixture of concentrated 'Analar' nitric and perchloric acids until a clear solution was obtained (Andrews, 1989). The clear solutions were then diluted to 100 ml using distilled water. Similarly the collected specimens of *T. angustata* from each pool were dried and digested as described above. The sediment samples were oven dried and two grams from each sample were treated with 30% H_2O_2 in a boiling water bath to destroy the organic matter (Malo, 1977). Samples were diluted to 200 ml with distilled water and 10 ml of 6M HCl were added and the suspension was heated for 30 minutes. The hot mixture was filtered through a Whatman No. 42 filter paper. The residues on the filter paper were washed three times with hot dilute HCl (1:19, v:v) and the filtrate was cooled and brought up to 250 ml with the same dilute HCl. The water samples were filtered through 0.45 μm millipore filter paper to separate particulates (Wahbeh *et al.*, 1985). The concentrations of 8 heavy metals (Zn, Co, Cu, Cr, Cd, Fe, Mn, and Pb) in the above solutions and in the filtered water samples were measured using a flame atomic absorption spectrophotometer (Pye Unicam).

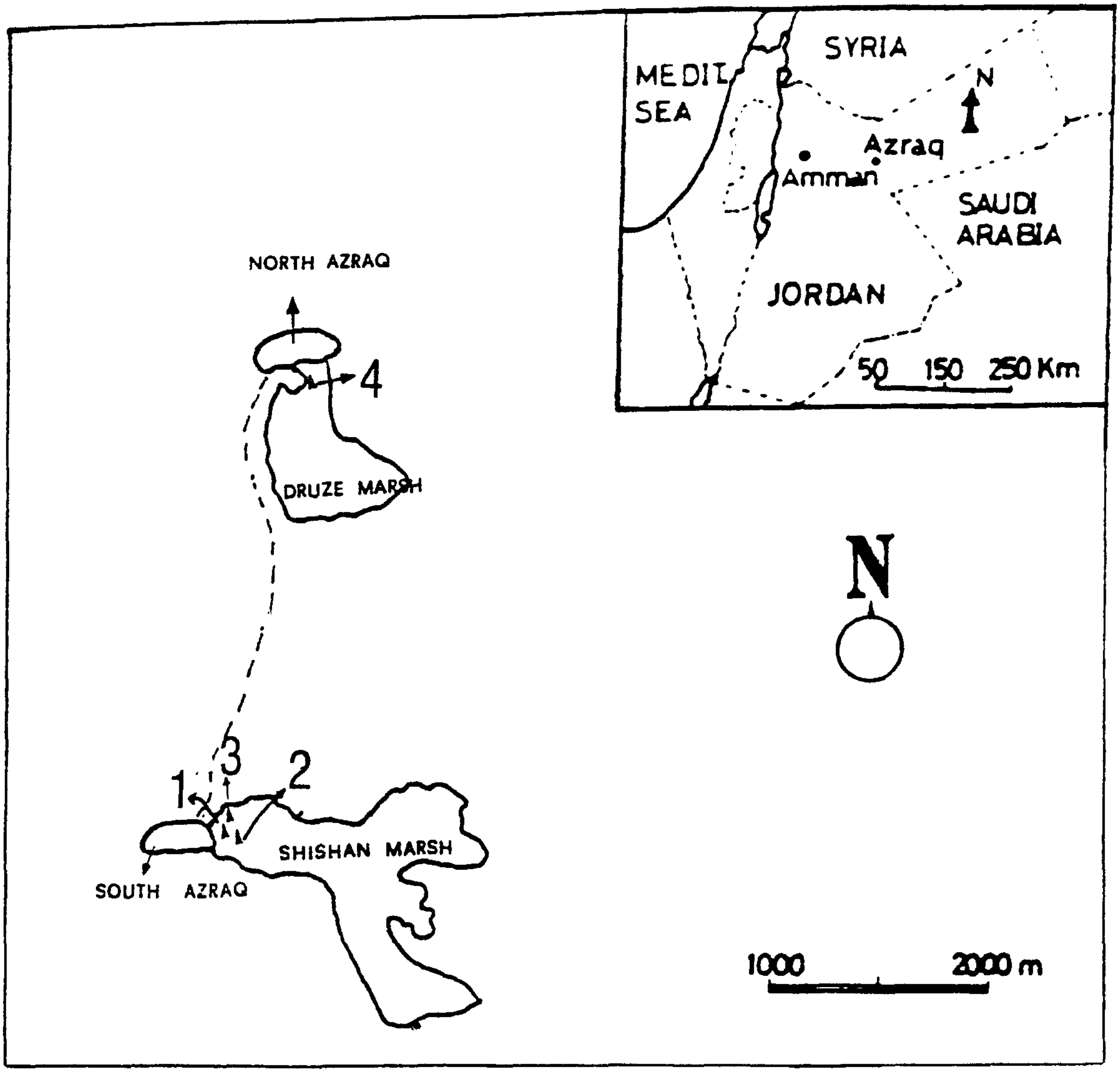

Fig. 1. Location map of Azraq Oasis showing the sampling stations (1, 2, 3, and 4).

Statistical analysis

Data were expressed as mean concentrations (ppm) ± S.D. The main effects of snail size, sampling sites, and the seasonal variation of the metal concentration were tested using the one way analysis of variance. $p = 0.05$ was taken as the level of significance. Duncan's multiple range test was further employed to identify the highest concentrations that are significantly ($p < 0.05$) different from the lowest concentrations. The concentration factor, which reflects the ratio of a metal concentration in a selected snail to that in water, was calculated for each snail species and the aquatic plant. The biotransference factor, which is the ratio of a metal concentration in a selected snail to that in the aquatic plant, was measured for the three snail species.

Results

There were significant differences in the mean concentrations of the heavy metals Cu, Zn, Fe, Pb, Cd, Co, Mn, and Cr among the three snail species (Table 1). In general, *M. tuberculata* contained significantly higher levels of metals other than *M. praemorsa* and *T. jordani*, except for Fe and Mn which were highest in *M. praemorsa*. Significantly lower means of the heavy metals were found in *T. jordani*. No significant difference in the mean concentrations of metals were found among the size classes of the selected snail species. However, larger (> 10 mm) *M. tuberculata* snails contained higher levels of the heavy metals than the smaller ones (< 10 mm). Large snails (> 15 mm) of *M. praemorsa* contained also higher concentrations of Cu, Zn, Fe, Cd, Mn, and Cr than the medium (11–15 mm) and small (< 5 mm) snails. Large snails (> 5 mm) of *T. jordani* contained higher concentrations of Cu, Cd, and Cr than the smaller (< 5 mm) snails.

Table 2 shows a comparison of the heavy metals in *M. praemorsa*, the plant *T. angustata*, sediment, and water from various sampling sites in Azraq. There were no significant differences in the mean concentrations of metals in *M. praemorsa* collected from different sites, except that Fe was significantly higher in station 3. Similarly, the levels of metals in *T. angustata*, sediment, and water were not significantly different among sampling stations, except Cd and Cr which were significantly higher in water and sediment, respectively, at station 4.

The seasonal variation in the metal concentration of the snails, the aquatic plant, sediment, and water is shown in Fig. 2. There were significant seasonal variations in the mean concentrations of Cu, Fe, Pb, Cd, and Cr in *M. praemorsa*. The level of Cu and Fe were highest during autumn and winter, while Cd and Cr levels were highest during winter and spring. The concentration of Pb was highest during autumn. Moreover, Duncan's test indicated significantly higher concentrations of Zn and Co during spring and winter, respectively, than those during autumn and winter, respectively. There were significant seasonal variations in the Cu, Mn, and Cr concentrations of *M. tuberculata*. The highest concentrations of Cu and Cr were during winter. The level of Mn was highest during summer. Similarly, Duncan's test indicated significantly high concentration of Fe during summer and Zn and Cd during winter. In *T. jordani* and *T. angustata* plants, there were no significant seasonal variations in the metal concentration, except for Zn in the former and Cd in the latter. The level of Zn was highest during winter and Cd during winter and spring. In sediment and water there were no significant seasonal variations in the metal concentration, except Cu and Fe in the former. Copper concentration was highest during winter and Fe during autumn.

The concentration factors for the metals were highest in *M. tuberculata*, except for Fe and Mn which were concentrated more in *M. praemorsa* (Table 3). Moreover, the latter snail was more

Table 1. Mean concentrations ± S.D. (ppm) of heavy metals in the various size classes of the selected species from Azraq Oasis. M.p., *Melanopsis praemorsa*; M.t., *Melanoides tuberculata*; T.j., *Theodoxus jordani*.

Species	Length	Cu	Zn	Fe	Pb	Cd	Co	Mn	Cr
M.p.	< 10	74.0 ± 9.6	140 ± 12.4	1573 ± 551	26.9 ± 12.7	3.4 ± 0.5	7.4 ± 4.1	160 ± 16.7	18.3 ± 5.3
	11–15	79.3 ± 7.6	162 ± 23.2	1550 ± 300	27.6 ± 7.0	3.1 ± 0.8	6.1 ± 0.4	156 ± 8.8	16.7 ± 2.9
	> 15	88.6 ± 10.2	186 ± 19.1	1744 ± 387	23.2 ± 4.8	3.6 ± 0.6	6.0 ± 0.6	179 ± 27.6	18.9 ± 9.2
Total		80.6 ± 9.2	163 ± 18.8	1622 ± 426	25.9 ± 8.8	3.4 ± 0.7	6.5 ± 2.4	165 ± 19.3	18.0 ± 6.3
M.t.	< 10	101.4 ± 28.8	231 ± 44.0	1384 ± 596	43.9 ± 20.9	4.6 ± 1.9	11.6 ± 1.4	72 ± 34.0	23.8 ± 9.0
	> 10	103.8 ± 45.5	248 ± 75.0	1562 ± 223	58.5 ± 21.1	5.3 ± 4.0	12.3 ± 4.9	79 ± 21.0	25.8 ± 8.6
Total		102.6 ± 38.1	240 ± 61.5	1473 ± 450	51.1 ± 21.0	5.0 ± 3.1	12.0 ± 3.6	76 ± 28.3	24.8 ± 8.8
T.j.	< 5	13.9 ± 7.1	39.4 ± 9.8	773 ± 122	42.2 ± 19.3	4.2 ± 1.2	10.9 ± 3.6	40 ± 8.1	10.8 ± 5.6
	> 5	20.2 ± 22.2	37.0 ± 6.3	632 ± 286	31.5 ± 9.6	4.7 ± 0.7	10.6 ± 3.7	31 ± 11.8	11.9 ± 10.5
Total		17.0 ± 16.5	38.2 ± 8.2	703 ± 220	36.9 ± 15.2	4.5 ± 1.0	10.8 ± 3.7	35 ± 10.1	11.4 ± 8.4

Table 2. Comparison of mean concentrations + S.D. (ppm) of the heavy metals in *Melanopsis praemorsa* (M.p.) snails, *Typha angustata* (T.a.) plants, sediment, and water from various sampling stations in Azraq Oasis.

Species	Site	Cu	Zn	Fe	Pb	Cd	Co	Mn	Cr
M.p.	1	76.2 ± 16.5	181.9 ± 21.3	1254 ± 231	27.5 ± 5.5	3.6 ± 1.0	6.7 ± 4.0	162 ± 24.3	15.9 ± 3.8
	2	85.0 ± 23.9	165.6 ± 26.4	1353 ± 477	25.1 ± 7.5	3.3 ± 0.7	6.5 ± 2.2	145 ± 36.0	16.7 ± 7.9
	3	81.9 ± 8.9	145.8 ± 34.9	2718 ± 616	35.1 ± 12.3	3.7 ± 0.6	5.0 ± 1.0	169 ± 41.0	20.7 ± 6.6
	4	72.4 ± 9.6	157.3 ± 27.7	1162 ± 174	25.9 ± 12.1	2.8 ± 1.1	7.9 ± 1.3	186 ± 38.4	18.5 ± 6.9
T.a.	1	5.1 ± 5.8	14.9 ± 10.1	75 ± 69	22.4 ± 29.6	1.1 ± 0.6	1.0 ± 0.8	34 ± 11.4	1.8 ± 3.6
	2	2.7 ± 2.5	13.3 ± 3.2	52 ± 27	11.2 ± 5.7	0.8 ± 0.9	1.0 ± 0.9	22 ± 5.6	1.3 ± 2.2
	3	3.9 ± 2.4	20.3 ± 9.8	85 ± 69	6.4 ± 3.4	0.8 ± 0.6	1.8 ± 1.5	30 ± 15.1	3.4 ± 3.5
	4	4.1 ± 3.6	35.8 ± 20.3	205 ± 166	10.6 ± 4.6	0.7 ± 0.9	1.1 ± 1.4	82 ± 70.6	12.4 ± 21.1
Sediment	1	12.3 ± 5.9	39.6 ± 16.6	2792 ± 641	34.5 ± 20.7	2.3 ± 0.6	6.1 ± 1.9	88 ± 45.6	37.5 ± 16.2
	2	14.2 ± 3.2	87.5 ± 79.9	3042 ± 1140	21.5 ± 4.1	2.2 ± 0.8	6.9 ± 2.0	60 ± 22.8	56.0 ± 40.6
	3	11.4 ± 3.0	35.7 ± 9.6	3284 ± 878	22.9 ± 5.6	2.1 ± 1.3	8.2 ± 2.6	151 ± 75.4	33.5 ± 12.2
	4	9.5 ± 4.4	36.9 ± 11.8	3877 ± 745	18.9 ± 11.3	1.0 ± 0.4	5.7 ± 1.3	84 ± 28.8	126.3 ± 71.1
Water	1	0	0.003 ± 0.005	0.013 ± 0.10	0.025 ± 0.013	0	0.010 ± 0.008	0.003 ± 0.005	0.005 ± 0.010
	2	0	0	0.020 ± 0.022	0.013 ± 0.013	0.003 ± 0.005	0.005 ± 0.006	0.003 ± 0.005	0
	3	0	0	0.005 ± 0.006	0.015 ± 0.013	0	0.008 ± 0.005	0.005 ± 0.010	0.015 ± 0.013
	4	0.006 ± 0.013	0.006 ± 0.006	0.036 ± 0.025	0.046 ± 0.028	0.008 ± 0.005	0.018 ± 0.011	0.006 ± 0.006	0.002 ± 0.005

efficient in concentrating Cu, Zn, Fe, Mn, and Cr than *T. jordani*. In general, lower concentration factors were calculated for *T. angustata* plants. The biotransference factors, in relation to *T. angustata*, ranged from 20.9 for Cu to 2.1 for Pb in *M. praemorsa*. Higher ratios were found for *M. tuberculata*, where it ranged from 1.8 for Mn to 26.3 for Cu. In *T. jordani* the factors ranged from 8.9 for Co to 0.8 for Mn.

Discussion

The results indicate that different snail species have different abilities to concentrate a certain metal, and each species shows different ability to concentrate different metals. For example, *M. tuberculata* concentrated metals in the order: Zn, Fe, Cu, Mn, Cr, Pb, Cd, and Co, while *M. praemorsa* accumulated metals in the order:

Table 3. Mean concentration factors (C.F.)* and biotransference factors (B.T.F.)** for *Melanopsis praemorsa*, *Melanoides tuberculata* and *Theodoxus jordani* snails and the aquatic plant *typha angustata*.

	M. praemorsa		*M. tuberculata*		*T. jordani*		*T. angustata*
	C.F. 10^3	B.T.F.	C.F. 10^3	B.T.F.	C.F. 10^3	B.T.F.	C.F. 10^3
Cu	45.2	20.9	57.0	26.3	9.5	4.4	2.2
Zn	67.1	7.6	99.8	11.4	15.9	1.8	8.8
Fe	85.0	15.9	75.9	14.2	36.2	6.8	5.4
Pb	1.0	2.1	2.0	4.0	1.4	2.9	0.5
Cd	1.1	3.7	1.7	5.4	1.6	5.0	0.3
Co	0.6	5.3	1.1	9.9	1.0	8.9	0.1
Mn	40.5	4.0	18.5	1.8	8.6	0.8	10.2
Cr	1.7	3.8	2.3	5.3	1.1	2.4	0.4

* Measured in relation to concentration of metals in water.

** Measured in relation to concentration of metals in *T. angustata*.

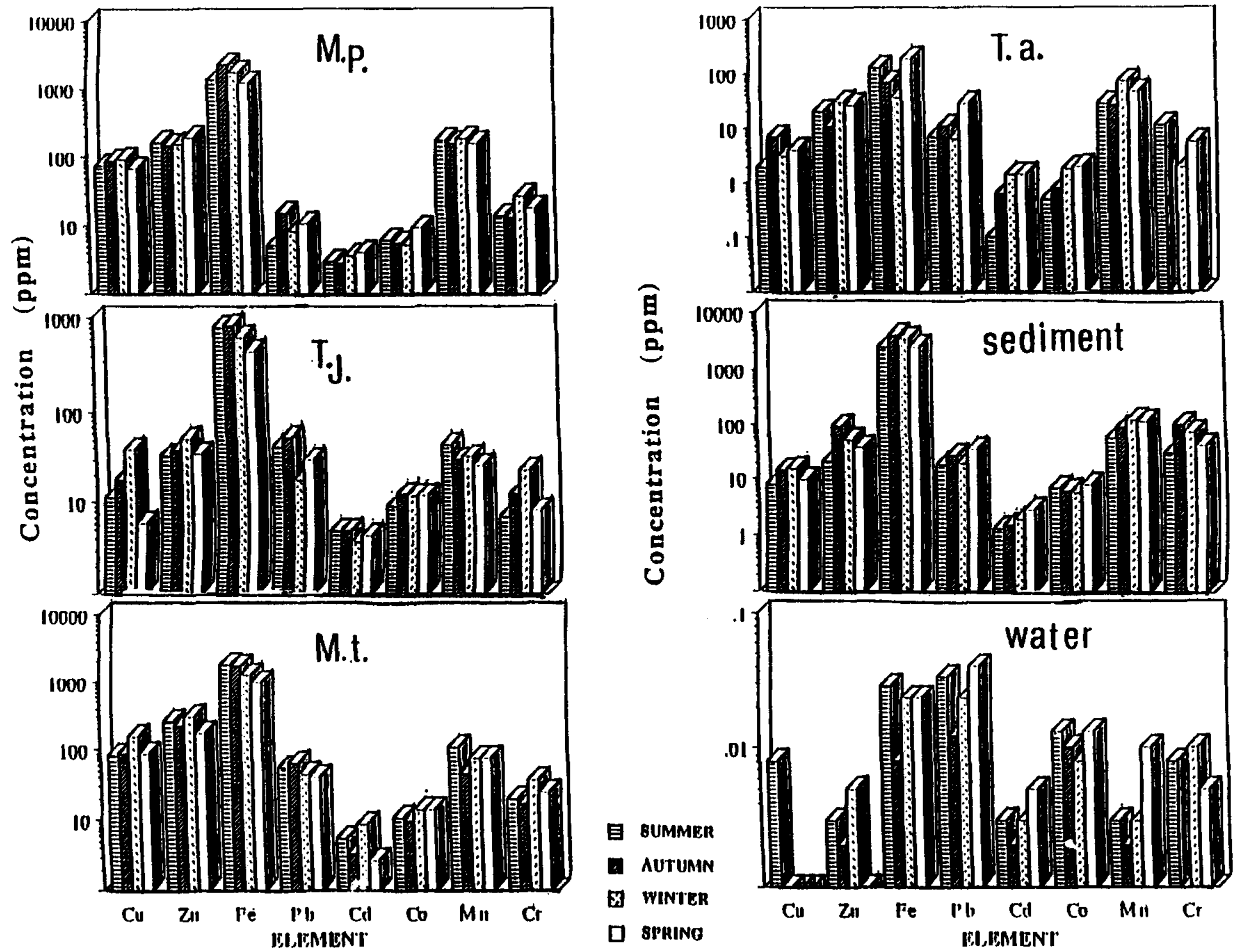

Fig. 2. The seasonal variation in the mean concentrations of heavy metals in *Melanopsis praemorsa* (M.p.), *Melanoides tuberculata* (M.t.), and *Theodoxus jordani* (T.j.) snails, the aquatic plant *Typha angustata*, sediment, and water collected from the pools of Azraq Oasis.

Fe, Zn, Cu, Mn, Cr, Cd, Pb, and Co, and *T. jordani* accumulated metals in the order: Fe, Zn, Cu, Mn, Cd, Pb, Cr, and Co. In addition, the ability of snails to concentrate the investigated metals are ranked (as described by Greville & Morgan, 1989) in the series: *M. tuberculata* > *M. praemorsa* > *T. jordani* (Table 4). The species differences in the metal concentrations can probably be partially attributed to the snails' feeding habits. *Melanoides tuberculata* is a detritus feeder, so it takes sediment-bound metals in addition to the organic matter-adsorbed metals (Adriano, 1986). *Melanopsis praemorsa* is usually found attached to the leaves and stems of the aquatic plant *T. angustata*, but it can also be found crawling on the bottom of the pools. Thus, it depends mainly on the aquatic plant as source of food, although it can feed on sediment detritus. *Theodoxus jordani* feeds on the aquatic plant and the microalgae growing on large stones. Table 2 shows that the mean concentrations of the metals in sediments were higher than those in *T. angustata*. Thus, it is expected that *M. tuberculata* is exposed to higher levels of metals than *M. praemorsa* and *T. jordani*. Greville and Morgan (1989) concluded that by measuring the accumulated metal concentration within slug tissues, it is possible to establish whether differ-

Table 4. Total rank scores of the selected snail species according to their capacity to concentrate the investigated metals.

Metal	Ranking scores for snail species		
	Melanopsis praemorsa	*Melanoides tuberculata*	*Theodoxus jordani*
Cu	2	3	1
Zn	2	3	1
Fe	3	2	1
Pb	1	3	2
Cd	1	3	2
Co	1	3	2
Mn	3	2	1
Cr	2	3	1
Total rank score	15	22	11

ent species do share available sources. However, *M. praemorsa* accumulated more Fe and Mn than *M. tuberculata*, and *T. jordani* accumulated more Cd and Co than *M. praemorsa* (Table 1). It is possible that physiological factors are responsible for these differences. Hopkin *et al.*, (1985) suggested that the recorded differences in the metal concentration in three species of isopods could perhaps be due to differences in alimentary physiology; for example, relatively small differences in gut pH would result in large differences in the solubility and potential availability of the heavy metal.

The seasonal variation in the concentration of Fe and Cu in *M. praemorsa* and *M. tuberculata* is probably due to seasonal changes of these metals in the sediment of the pools. Fe and Cu concentrations were highest during autumn and winter in both the snails and sediments. Although the other metals did not vary seasonally in the sediment, some were seasonally variable in the snails. Phillips (1980) reported that indicator organisms, such as mollusks, undergo dramatic physiological changes with season which are related to maturation of gametes and spawning. These changes involve fluctuations of all biochemical components as well as animal weight, water content and condition.

There were no significant differences in the mean concentrations of metals among the size classes of the selected species. In mollusks, the relationship between metal content and body size is species and metal dependent (Boyden, 1974). Strong *et al.*, (1981) found positively sloped, negatively sloped, and insignificant relationships between metal content and size. It is noteworthy, however, that larger snails from Azraq contained higher concentrations, particularly of Cu, Fe, Zn and Mn. The increase in Cu level is possibly due to the fact that these gastropod snails have the Cu containing respiratory pigment known as hemocyanin (Barns, 1980).

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