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Relationship between Some Heavy Metals and *Schistosoma mansoni* Infection Rates in *Biomphalaria alexandrina* Snails Collected from Different Egyptian Localities

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Abstract: The present study aimed to investigate the impact of water pollution with chemicals on the susceptibility of *Biomphalaria alexandrina* snails to infection with *Schistosoma mansoni* parasite. *Biomphalaria alexandrina* snails were collected from different Egyptian governorates and reared in the laboratory. Water samples were also collected from the same snail collecting sites for water analysis. The analysis of water samples using atomic absorption spectrophotometer indicated the presence of several heavy metals such as Cu, Cd, Pb and Hg with various concentrations in the snails collecting sites. The snails offspring showed clear differences in their infection rates with *Schistosoma mansoni*, the snails were classified depending on their infection rates into highly susceptible as in Schistosome Biological Supply Center group (50.3%), moderately susceptible (Giza group 43.3%) and low susceptible as *Biomphalaria alexandrina* snails of Fayoum, Ismailia and Kafr El-Sheikh Governorates. The infection rate of *B. alexandrina* snails was found to be positively correlated with the concentration of Cd ($r= 0.924$, $p<0.001$). However, negative correlations were observed between infection rate and the concentrations of each of the following metals Pb ($r=-0.233$, $p<0.05$), Cu ($r=-0.09$, $p<0.05$) and Hg ($r=-0.620$, $p<0.01$).

Key words: *Biomphalaria alexandrina* · *Schistosoma mansoni* · Heavy metals · Pollution · Susceptibility

INTRODUCTION

Water pollution is considered to be one of the most dangerous hazards affecting Egyptian human beings. Pollution in the River Nile system (main stem Nile, drains and canals) has increased in the last few decades due to increasing human activities along the River Nile [1]. Moreover, Marshall [2] concluded that many of the aquatic habitats in which schistosome-transmitting snails occur are now incredibly fouled by water pollution. The most important heavy metals from the point of view of water pollution are Zn, Cu, Pb, Cd, Hg, Ni and Cr. Some of these metals (e.g. Cu, Ni, Cr and Zn) are essential trace metals to living organisms, but become toxic at higher concentrations. Other, heavy metals such as Pb and Cd have no known biological function but are toxic element [3]. Abd Allah *et al.* [4] demonstrated by spectrophotometric analyses that *B. alexandrina* snails exposed to Pb, Cd or Hg has significantly elevated levels of these materials in their bodies within four weeks at 17°C or 28°C, indicating that these species and/or its close relatives may prove valuable as biomonitors of heavy-metal pollution. Ansaldo *et al.* [5] found that the acute exposure (96 hr) to cadmium, lead and arsenic altered the reproduction of *B. glabrata*, modifying the total number of laid eggs, hatching time and embryonic survival. The ions commonly present in nature water are Ca^{+2}, Mg^{+2}, Na^{+}, HCO_3^{-}, CO_3^{-2}, Cl-and SO^{-2}_4. Upatham [6] observed that the increasing NaCl concentration from 1200ppm to 4200ppm resulted in more reduction in the infection rate of *B. glabrata* with *S. mansoni* from 41.6% to 2.1%.

The present study aimed to evaluate the impact of water chemistry contents on the susceptibility of offspring of *Biomphalaria alexandrina* snails from different Egyptian localities to *Schistosoma mansoni* infection.

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MATERIALS AND METHODS

Collection, Rearing and Infection of *B. alexandrina* Snails: Populations of *B. alexandrina* snails were collected from five Egyptian Governorates (Giza, Fayoum, Kafr El-Sheikh, Ismailia and Damietta) and a 6th group was obtained from Schistosome Biological Supply Center-Theodor Bilharz Research Institute (SBSC-TBRI) as a reference control. The snails from each group were reared in the laboratory and their first generations (F_1) were used throughout the infection experiments. Three replicates, each of 30 lab-bred *B. alexandrina* snails (4-6 mm in diameter), from each Governorate offspring were exposed individually to 10 newly hatched *S. mansoni* miracidia (SBSC-TBRI).

Starting from the day 21 post miracidial exposure, the snails were examined individually and repeatedly for cercarial shedding in multi dishes containing 2 ml of dechlorinated tape water/snail under artificial light for two hours (stimulant period). After initial shedding was observed, snails were screened individually once weekly till the death of snails. All snails that died during the prepatent period were crushed between two slides and inspected under a microscope for immature parasite stages [7]. The classification of snail susceptibility to infection was dependent on the infection rate of the snails according to the method of Saoud [8] in which snails were considered refractory below 10%, low susceptible 10-25%, moderate susceptible 25-50% and high susceptible at infection rate over 50%.

Chemical Analysis of Water: The water samples, collected from the same snail sampling sites, were preserved and prepared for the determination of minerals and heavy metals concentrations according to APHA [9] after that the clear solution was used for heavy metals measurements by using the atomic absorption spectrophotometer (GBC AVANTA 3000, Australia).

The following elements were measured against standard crude ones (Sigma company-Egypt).

- Na, Ca and K were measured using the flame system
- Cd, Pb and Cu were measured using graphite furnace system
- Hg was measured using hydride system

The results were obtained automatically from the device.

Statistical Analysis: The analyses included the calculation of the mean value, standard deviation and a "*t*" value at level is $p \leq 0.05$ (according to Zar [10]. These determinations were calculated for both control (SBSC-TBRI snails) and other snail groups. Correlation, homogeneity chi-square (continuity adjusted), analysis of variance and linear regression analyses were conducted using SPSS version 11.5.0 (SPSS, Inc., Chicago, IL).

RESULTS AND DISCUSSION

Infection Rate: Table (1) shows that offspring of snails collected from Fayoum, Kafr El-Sheikh and Ismailia were low susceptible with infection rates ranged from 15-20%. The total number of cercariae produced from all infected snails belonging to the previous three governorates ranged from 5,070-14,971 cercariae, the mean number of cercariae per snail ranged from 1175.3 to 2495.2 and the mean number of cercariae per snail per week was from 163.55 to 598.8. Moderate susceptibility exhibited by Giza and Damietta snails characterized by infection rates of 33.3% and 43.3%, total number of cercarial production of 17448 and 62378 with a mean number of cercariae per snail being 1744.8 and 5198.17 and the mean number of cercariae/ snail/ week 335.5 and 533.15, respectively for the two Governorate's snail groups.

SBSC-TBRI snails showed high susceptibility (50.3 %) and the total number of cercarial production was 33,486, mean number of cercariae per snail 2575.85 cercariae and the mean number of cercariae per snail per week was 446.48. No refractory snails were found among *B. alexandrina* snail groups investigated.

Water Chemistry

A-Heavy Metals: Cadmium, lead, mercury and copper concentrations in water courses belonging to five governorates are shown in Table (2), for cadmium, the highest concentration was obtained from Giza Governorate being 4.247 ± 1.8 ppb. While the lowest one (0.41 ± 0.12 ppb) was reported from Ismailia. The concentrations of lead ranged from 5.34 ± 1.2 ppb (Ismailia Governorate) to 81.28 ± 8.3 (Fayoum Governorate).

In the case of Cu, higher concentrations were detected in Giza, Fayoum and Kafr-El-Sheikh being 144.03 ± 25.1, 74.74 ± 12.4 and 313.72 ± 28.2 ppb, respectively, while the concentration of the metal in Ismailia and Damietta Governorates were much lower being 29.40 ± 5.6 ppb, 5.08 ± 3.1 ppb, respectively. The concentration of Hg was highest in Fayoum Governorate (0.346 ± 0.04 ppb) while it was not detected in Kafr-El-Sheikh Governorate.

Table 1: Classification of snail's susceptibility according to their infection rates

	Low susceptible	Moderately susceptible	Highly susceptible	Refractory
Snails origin	Fayoum Kafr El-Sheikh Ismailia	Giza Damietta	SBSC	-
Infection rate %	15-20	43.3-33.3	50.3	-
Total number of cercarial production	5,070-14,971	17,448-62,378	33,486	
Mean number of cercariae/ Snail	1175.3-2495.2	1744.8-5198.17	2575.85	-
Mean number of cercariae/ snail/ week	163.55-598.8	335.5-533.15	446.48	-

Table 2: The concentrations of the elements measured in the water samples obtained from the field

Elements	Ca	Na	K	Cd	Pb	Cu	Hg
Concentrations	ppm			ppb			
Ismailia (canal)	8.58±1.4	76.26±13.2	3.735±0.82	0.41±0.12	5.34±1.2	29.40±5.6	0.24±0.06
Fayoum (canal)	11.53±2.6	141.3±18.3	5.02±1.2	1.01±0.2	81.28±8.3	74.74±12.4	0.346±0.04
Mean Canals	10.055±2	108.8±15.75	4.38±1.01	0.71±0.16	43.31±4.75	52.07± 9.8	0.293±0.05
Kafr-El-Sheikh (drain)	16.12±4.3	315.32±31.2	20.582±3.4	1.31±0.4	14.11±2.2	313.72±28.2	0.0±0.0
Damietta (drain)	15.73±2.6	705.0±45.6	13.442±2.3	2.07±0.6	35.94±3.4	5.08±3.1	0.11±0.08
Giza (drain)	29.5±3.8	425.17±25.2	40.26±4.8	4.247±1.8	27.65±3.2	144.03±25.1	0.024±0.02
Mean Drains	22.615±3.2*	565.1±35.4*	26.8±3.55*	3.16±1.2*	31.8±3.3*	74.5±14.1*	0.067±0.05*

Data was expressed as mean ± SD *: Significance p< 0.05

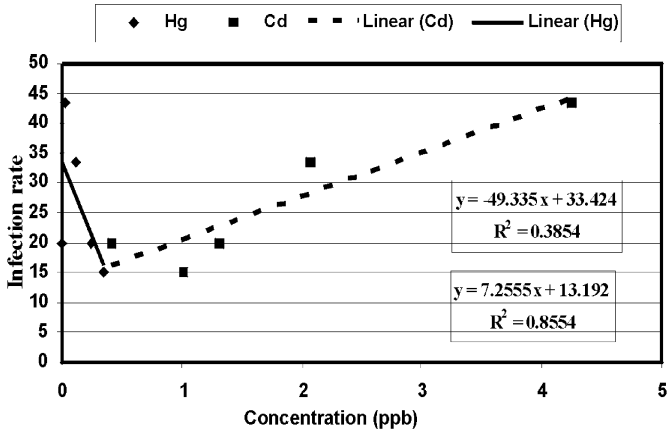

Fig. (1a): Correlations between infection rates of *Biomphalaria alexandrina* snails and concentrations of cadmium and mercury

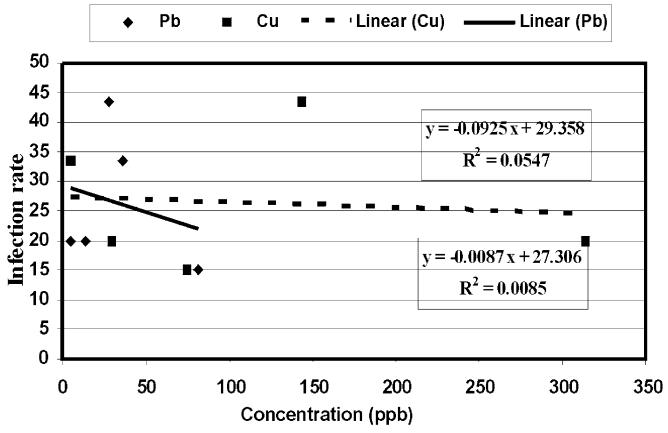

Fig. (1b): Correlations between infection rates of *Biomphalaria alexandrina* snails and concentrations of lead and copper

B-Minerals (Sodium, Calcium and Potassium): The minerals concentrations in water collected from snail's sampling sites ranged from 76.26 ± 13.2 to 705.0 ± 45.6, 8.58 ± 1.4 to 29.5 ± 3.8 and 3.735± 0.82 to 40.26 ± 4.8 ppm for Na, Ca and K, respectively. Giza Governorate has significantly ($p<0.001$) higher mean concentrations of Ca and K than the other Governorate's samples. Na in Damietta sample showed the highest concentration ($p< 0.001$). Ca, Na, K, Cd and Cu concentrations were significantly higher in drains than in canals. However, the concentrations of Pb and Hg were significantly higher in canals than in drains (Table 2).

Correlation Between Heavy Metals Concentrations and Infection Rates of Different *B. alexandrina* Snails: The infection rate of offspring of *B. alexandrina* snails was found to be positively correlated with the concentration of Cd ($r= 0.924$, $p<0.001$). However, negative correlations were observed between infection rate and the concentrations of each of the following metals Pb ($r=-0.233$, $p<0.05$), Cu ($r=-0.09$, $p<0.05$) and Hg ($r=-0.620$, $p<0.01$) (Fig. 1a&b).

In the present study, several heavy metals were found in the habitats of *B. alexandrina* snails such as Cd, Cu, Pb and Hg but with various concentrations. In the same respect, Tolba and Awad [11] studied the quantitative analysis of irrigation water and indicated the presence of significantly higher levels of lead, copper and cadmium in natural habitats of snails in Egypt. In the present work, it was noted that the concentration of these heavy metals in the canals were found to be lower than that in drains. Similar results were obtained by Rizk *et al.* [12].

The level of the metals in the sampling sites were found to be higher than safe levels mentioned by National Academy of Science [13] which should not exceed 0.9 to 9 ppb for Cu and 0.59 to 5.9 ppb for Pb. In the case of cupper, higher concentrations were found in Giza, Fayoum, Kafr-El-Sheikh and Ismailia Governorates being 144.03 ± 25.1, 74.74 ± 12.4, 313.72 ± 28.2 and 29.40 ± 5.6 ppb, respectively, while the concentration of the metal in Damietta Governorate was in its normal range (5.08 ± 3.1 ppb). The concentrations of lead ranged from normal value in Ismailia Governorate (5.34 ± 1.2 ppb) to extremely high value (81.28 ± 8.3 ppb) in Fayoum Governorate. For cadmium, the highest concentration was obtained from Giza Governorate (4.247 ± 1.8 ppb), while the lowest concentration was (0.41 ± 0.12 ppb) reported from Ismailia. The concentration of mercury was the highest in Fayoum Governorates being 0.346 ± 0.04 ppb, while the metal was not detected in Kafr-El-Sheikh Governorate.

The variations noticed in the susceptibility rates (ranged from 15% "Fayoum" to 43.3% "Damietta") of different snail groups from different localities may be, partially, attributed to variations seen in the levels of heavy metals in the canals and drains from which the snails were collected as it was found that the infection rate of *B. alexandrina* snails positively correlated with the concentration of Cd ($r= 0.924$, $p<0.001$). Meanwhile, negative correlations were observed between infection rate and the concentrations of each of the following metals Pb ($r=-0.233$, $p<0.05$), Cu ($r=-0.09$, $p<0.05$) and Hg ($r=-0.620$, $p<0.01$). The effects of heavy metal toxicity on host-parasite interactions in freshwater snails have been the subject of many investigations. Metal-induced changes in host survival [14], cercarial emergence [15,16] and the physiochemical properties of host hemolymph [17]. Ibrahim [18] studied the effects of both parasitism and environmental stress on the growth, reproduction and survival of *B. alexandrina* snails and suggested that resource allocation strategies may be influenced by both biotic and a biotic factors. Using the planorbid snail *B. alexandrina* and *S. mansoni*, this hypothesis was examined by raising snails fed the same diet under two stressors (infection and Cd exposure). Infection status and Cd exposure had significant effects on snail growth and reproduction. The infected and Cd-exposed infected snails exhibiting reduced survival relative to snails of other treatments. It was found that parasite development, also, influenced by Cd exposure. Results of this study suggest that energy allocation patterns are context-dependent in *B. alexandrina* snails, influenced by infection and Cd exposure.

B. alexandrina snails can tolerate such high concentrations of these metals. The lethal effects of copper, cadmium and lead on *B. alexandrina* (LC_{50}-96 hrs) were 0.169, 1.09 and 143.83 ppm, respectively according to Rizk *et al.* [12]. Also, Abd Allah *et al.* [15] found that Pb at concentrations from 0.25-100 μM, Cd from 0.075-0.25 μM and Hg from 0.25-1 μM significantly reduced growth and survival of normal snails. With each metal the effects increased with increasing concentration. Snails exposed to heavy metals continued to be reproductively active, but the egg production was highly variable and no significant effect of heavy metal exposure was demonstrated. Snails can accumulate Cd, Cu and Pb in their tissues [19]. Many investigators studied the effect of the accumulation of these elements on total protein content of exposed snails [20] and their egg laying capacity [18]. The toxicity arises owing to copper adverse effects on the structure and function of macromolecules such as DNA and proteins [21].

In the present study, the minerals concentrations in water collected from sampling sites ranged from 76.26 ± 13.2 to 705.0 ± 45.6, 8.58 ± 1.4 to 29.5 ± 3.8 and 3.735 ± 0.82 to 40.26 ± 4.8 ppm for Na, Ca and K, respectively. Giza Governorate has significantly ($p<0.001$) higher mean concentrations of Ca and K than the other Governorate's samples. Na in Damietta sample showed the highest concentration ($p< 0.001$). Snails can live in a wide range of mineral content in water till they approached by certain limiting values [22]. Moreover, El-Hawary [23] showed that habitat preferred by *B. alexandrina* snails contains higher concentration of various common ions (Na, K and Ca).The importance of calcium in shell formation is well known [24], this finding supports the present results where high concentrations of Calcium were reported in the sites of snails. The same results were obtained by El-Khayat *et al.* [25] who found higher concentration of Ca in snail sites. Also, Horsak and Hajek [26] found a linear correlation between mollusca species richness and water calcium concentration. Sodium occurs in the water in the form of chloride, sulfate and carbonate. An estimation of the sodium content of the water is important in an evaluation of the quality of the water for snail vectors. Water with a high concentration of Na compared to other cations, especially to calcium, is not favorable to snails. The ratio of cations present in the water is based on the general principle of Base Exchange if sodium ions predominate in the water, they will tend to displace the beneficial calcium and less calcium will be absorbed [27]. Also, Caquet [28] stated that in the habitat of snails, water with a high concentration of Na is not favorable to snails.

In conclusion, this work has revealed that some snail habitats were contaminated with heavy metals such as Cd, Hg, Pb and Cu which could have an impact on the susceptibility of offspring of these snails to infection with *S. mansoni*.

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