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Research Article

Freshwater Snail Distribution Related to Physicochemical Parameters and Aquatic Macrophytes in Giza and Kafr El-Shiekh Governorates, Egypt

Abstract

A field work was conducted to study the density of the freshwater snail in relation to the vegetation cover as well as the physical and chemical properties in different watercourses. Two sites were selected in Giza governorate while, three sites were selected at Kafr El-Shiekh governorate. Water temperature, conductivity, total hardness, and pH were measured in the selected sites as well as bisphenol A (BPA) levels. Snail sampling was carried out and all types of macrophytes found in each site were collected, identified and coverage. Nine snails species namely *Biomphalaria alexandrina*, *Physa acuta*, *Planorbis planorbis*, *Lymnaea natalensis*, *Bulinus truncates*, *Bellamya unicolor*, *Melanoides tuberculata*, *Helisoma duryi* and *Lanistes carinatus* were identified. *B. alexandrina* was the most enumeration of snail species. The percentage of total snail species (75.47 %) was recorded at 28 °C as compared to 5.86 % recorded at 34 °C. Five species of aquatic vegetation were identified, two of them correlated positively and significantly with various snail species. Sites in which snails associated with macrophytes were characterized with higher ranges of chemicals, dissolved oxygen, and conductivity. In conclusion, the most important of the associating vegetation was *L. gibba* which correlated with *B. alexandrina* and served as an indicator plant.

Introduction

The freshwater ecosystem is under increasing threat due to rapidly expanding population and the subsequent modernization process resulted in inconspicuous exploitation of nature leading to the pollution crisis. Rivers are vulnerable since waste effluents from industries, domestic and farms open directly into them. Industrial and domestic effluents which account for the pollution that endangers the aquatic life contain various toxic substances [1].

Bisphenol A [4,4'-(propane-2,2-diyl) diphenol; BPA] is one of the environmental contaminants widely used in the manufacture of polycarbonate plastic and epoxy resins [2]. Bisphenol A (BPA) is a pseudo-persistent chemical, which despite its short half-life is ubiquitous in the environment because of continuous release [3]. Surface-water concentrations of BPA vary considerably depending on the location, sampling period, and how the results are reported. It has noted that although BPA dissolved in surface water has a short half-life because of photo and microbial degradation, while, metabolites may persist [4]. The most values reported for BPA in surface water are below 1.0 mg/L [4]; BPA concentrations can vary with depth [5].

Submersed macrophytes like *Ceratophyllum demersum* and *Lemna gibba* have major effects on productivity and biogeochemical cycles in freshwater because they occupy key interfaces in stream and lake ecosystems. Moreover, some aquatic plants are known to accumulate industrial radionuclides and heavy metals [6]. Freshwater pulmonate snails are commonly found in association with macrophytic vegetation and their epiphyton [7]. These macrophytes provide sites for snail oviposition, access to the air-water interface and shelter, and provide a surface for epiphyton development, which constitutes a major source of the food of freshwater snail [8]. It has been proposed that the close association of plants and snails in freshwater habitats since the cretaceous may have led to the development of mutually beneficial interactions [8]. This hypothesis is supported by laboratory experiments which show that the presence of freshwater snails can increase macrophytes growth and leaf longevity [9]. This effect appears to be due to both nutrient exchange and removal of epiphyton by feeding snails [10].

The mechanisms of competition between *Melanoides tuberculatus* and *Biomphalaria sp.* are not yet understood, but the competition for food probably occurs because these snails have a similar diet, including fine detritus and epiphytic algae

[11, 12]. *M. tuberculatus* is capable of reaching high densities; hence, competition for space is also possible [13]. However, the outcome of the interactions between *M. tuberculatus* and *Biomphalaria* spp. seems to be related to the habitat type where both species occur. In a former study in Kenya [14], no evidence was found of negative effects due to the presence of *M. tuberculatus* on *Biomphalaria* spp. Populations [15].

Continuous field studies are needed to detect changes in the distribution and abundance of the snails that are due to global climate and ecological changes [16]. The Egyptian freshwater habitat has been deteriorating primarily due to the discharge of municipal wastewater, industrial and agricultural into various water bodies across the country.

A field work was conducted to study the possible relation between the distribution of the freshwater snail with BPA concentrations. The correlation of snails with the density as well as the types of aquatic plants was determined in different watercourses in Giza and Kafr El-Sheikh governorates.

Materials and Methods

Study area: This study was conducted in two Egyptian governorates; Giza and Kafr El Shiekh. Some selected sites were chosen from different centers in each governorate. At Giza governorate, two sites were chosen namely El-Salmawy and Kafr Hakim (Figure 1). At Kafr El-Shiekh governorate, three sites were selected namely Qulin, Shinu and Kafr EL-Shiekh (Figure 2). The watercourses included irrigation canals and agricultural drains.

Physical and chemical parameters of water: Water temperature, conductivity, dissolved oxygen (DO) and total dissolved solid (TDS) were measured directly in the selected watercourses to the nearest °C, $\mu\text{s}/\text{m}$, ppm, and mg/L, respectively using temperature conductivity meter (HANNA instrument, HI 9635) and a portable D.O. meter (HI 8543). Moreover, hydrogen ion concentration (pH) was measured by pH meter electrode (HI 9124 and HI 9125). All the physical parameters were measured between 11: 00 am to 3: 00 pm and were recorded in the field survey sheets.

Snail survey: Snail sampling was carried out during spring 2013 and sampling was performed using a standard dip net (33 x 33 Cm) [17]. At each sampling site, three adjacent dip nets were taken, covering a length of about one meter. The collected snails were sorted and recorded in field survey sheet [18].

Aquatic plants: All types of macrophytes found in each site were collected, properly labeled and identified to species level. Coverage was determined with some modification by a simple estimation of the proportion of site covered by each species and scored 1 for $\leq +$ (very low coverage), 2 for ++ (low coverage), 3 for +++ (moderate coverage), 4 for ++++ (high coverage) and 5 for $\geq +++++$ (very high coverage) according to [19].

Determination of BPA in water sample: The extraction and analysis of BPA from collected water sample were done using HPLC system equipped with Smart line pump 1000, UV detector model basic 2500 set as 230 nm and model YL9100 Berlin manual injector with a 20 μL sample loop. The system used for this work was equipped with two detectors in series, ultraviolet (230 nm) and fluorescence (Ex 225 nm, Em 310 nm). Sample processing using solid phase extraction (SPE) was selected to demonstrate the ability of this technique to perform both extraction and concentration tasks. An Ascentis Express C18 column (5 cm \times 4.5 mm, particle size: 5 μm) from Berlin (Germany) was utilized to obtain a fast HPLC analysis at 35 °C. The mobile phase was water/acetonitrile (60:40, v/v) and it was delivered with a flow rate of 1 mL/min at room temperature and pressure was 3268 psi (225 bars) [20].

The system was calibrated with several standards and a response factor for BPA was generated for each detector. The pH values of aqueous solutions were measured with a Metrohm pH-meter (model: 827) supplied with a glass electrode. This allowed recovery data of the spiked sample to be calculated as $\mu\text{g}/\text{L}$.

Statistical analysis: The data was statistically analyzed for the significance difference was demonstrated at $p \leq 0.05$ by using T-test and values were expressed as means \pm S.E. Correlations of the changes in snails distribution with the other parameters were determined by Pearson's correlation and stepwise multiple regression analysis. The significance level was set at $P < 0.05$.

Results

Results of physicochemical parameters were recorded at each site (Table 1). It was noticed that Giza sites have almost the same temperature, conductivity, DO, and pH. There were significant differences among Kafr El-Shiekh sites in conductivity and TDS, where, Qulin had the highest water conductivity (1302 $\mu\text{s}/\text{m}$) and TDS (901 mg/L).

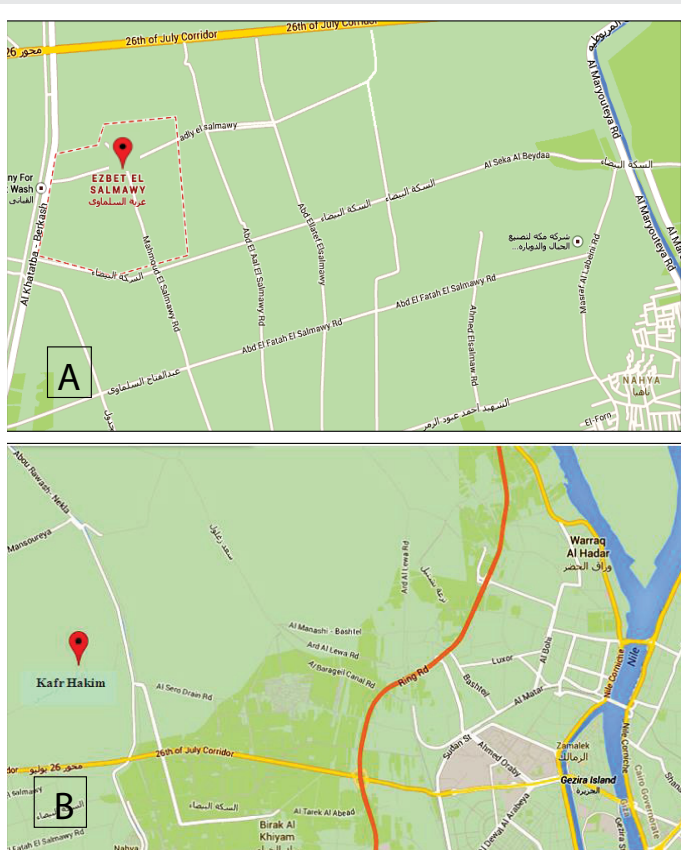


Figure 1: Sites of the collection at Giza governorate. (A) El-Salmawy. (B) Kafr Hakim.

A total of nine snail species were collected from the five sites of investigation during the study period (Table 2). The number of total specimens is 2711, 189, 323, 189, 4, 6, 18, 9 and 33 for *B. alexandrina*, *Physa acuta*, *Planorbis planorbis*, *Lymnaea natalensis*, *Bulinus truncates*, *Bellamya unicolor*, *M. tuberculata*, *Helisoma duryi* and *Lanistes carinatus*, respectively.

B. alexandrina snails were the most distributed species with 94.3% and 88.5% at Qulin and Kafr hakim, respectively. The results revealed that *B. alexandrina* represented 45.96% and 83.29% of all abundant snails in Giza and Kafr El Sheikh collected-sites, respectively.

The association pattern between *B. alexandrina* and other snail's species is presented in (Table 2). The highest association was found between *B. alexandrina* and *P. acuta* followed by *L. natalensis*. However, *B. alexandrina* snails showed the lowest association with *M. tuberculata*, *P. planorbis*, and *B. truncatus*.

The frequency and relative coverage by the site of macrophytes are represented in (Table 3). During the survey study, five aquatic plant species; *L. gibba*, *C. demersum*, *Eichhornia crassipes*, *Jussiaea repen* and *Pistia stratiotes* (Water lettuce) were observed. Results showed that *L. gibba* was the most represented aquatic plant with relative coverage 15, 13, 11, 0 and 0 in El Salmawy, Kafr Hakim, Qulin, Shinu and Kafr El-Sheikh, respectively. However, *P. stratiotes* confined presence in one site with total coverage 5.

In descending pattern, the most density of aquatic plants / site was recorded as 29, 22 and 15 at El-Salmawy, Kafr hakim,

Table 1: The physicochemical properties of the selected sites in Giza and Kafr El-Sheikh.

Governorates	Giza		Kafr El-Sheikh		
Watercourse properties	El-Salmawy	Kafr Hakim	Qulin	Shinu	Kafr El-Sheikh
Width (cm)	60 cm	1 m	2 m	2 m	6-7 m
Color	Pale green	turbid	Pale brown	Pale green	Turbid
Odor	Bad	No	Bad	No	No
Current	No	No	No	No	Slightly
Temperature (°C)	31.0 ± 0.5	32.0 ± 1.0	28.0 ± 0.6	36.0 ± 0.5 ^b	34.0 ± 0.5 ^b
Conductivity (µS/m)	190.5 ± 2.3	190.3 ± 1.2	1302 ± 2.5	367 ± 1.4 ^b	337 ± 1.3 ^b
TDS (mg/L)	130.7 ± 0.8	120.1 ± 1.2 ^a	901 ± 3.2	254 ± 0.9	235 ± 1.6
DO (ppm)	2.65 ± 0.2	2.68 ± 0.3	2.71 ± 0.5	2.86 ± 0.1	3.22 ± 0.2 ^b
pH	7.22	7.33	7.26	7.71	7.55

The data presented as mean ± S.E (n= 3).

^a Significant different as compared to El-Salmawy site and ^b Significant different as compared to Qulin site.

Table 2: *B. alexandrina* and other non-target snail's species collected from the selected sites.

Governorates	Giza		Kafr El-Sheikh		
Snails species	El Salmawy	Kafr Hakim	Qulin	Shinu	Kafr El-Sheikh
<i>B. alexandrina</i>	224 (88.5%)	9 (3.5%)	2478 (94.3%)	-	-
<i>L. natalensis</i>	-	10 (3.9%)	120 (4.6%)	43 (30.1%)	16 (7.8%)
<i>B. truncatus</i>	-	-	4 (0.2%)	-	-
<i>P. acuta</i>	11 (4.3%)	12 (4.7%)	26 (0.9%)	-	140 (68.6%)
<i>P. planorbis</i>	-	223 (87.8%)	-	100 (69.9%)	-
<i>M. tuberculata</i>	18 (7.1%)	-	-	-	-
<i>H. duryi</i>	-	-	-	-	9 (4.4%)
<i>B. unicolor</i>	-	-	-	-	6 (100%)
<i>L. carinatus</i>	-	-	-	-	33 (100%)
Total number	253	254	2628	143	204
All snails at site (%)	7.3	7.3	75.3	4.11	5.9
<i>B. alexandrina</i> (%)	45.96		83.29		

The data presented between brackets as % of *B. alexandrina* as compared to the total collected snails/site. - Indicated that there were no snails.

and Kafr El-Sheikh, respectively. Data revealed that *L. gibba* was the most predominant plant with *B. alexandrina* snails.

There is a positive correlation between the abundance of snails and the presence of aquatic plants. In Giza, the number of snails increased in the presence of *L. gibba*, *C. demersum*, and *Pistia stratiotes*. In Kafr El-Sheikh, the number of snails increased in the presence of *L. gibba* and *J. repen*.

Determination of BPA was performed with a micellar mobile phase. An example of a chromatogram obtained under these conditions from a standard mixture of BPA (0.19 mg / mL). Chromatogram obtained from a standard solution of BPA showed the retention time (4.2 min). The recoveries of BPA were in the range of 98.3%–100%. The concentrations of BPA

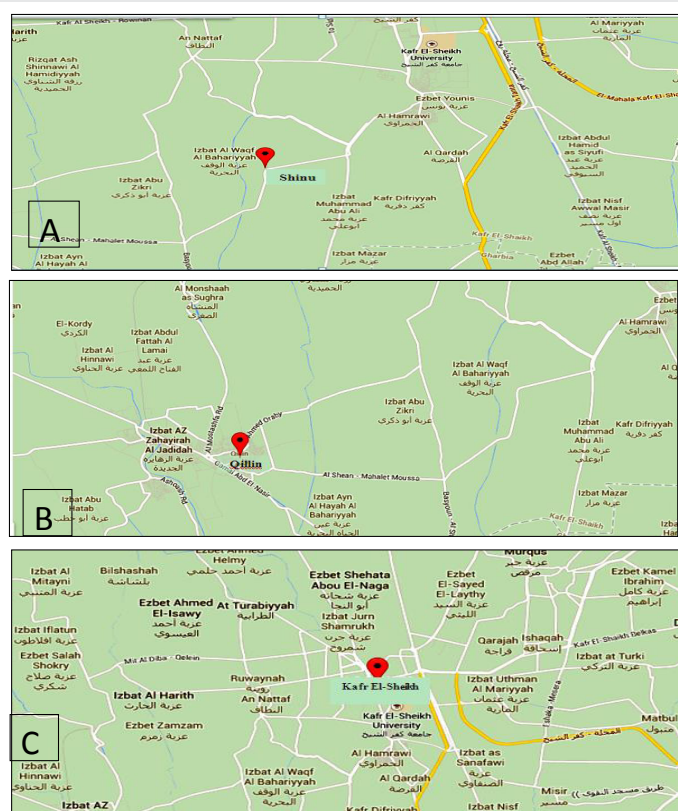


Figure 2: Sites of the collection at Kafr El-Sheikh governorate. (A) Shinu. (B) Quillin. (C) Kafr El-Sheikh.

at Kafr Hakim, Qulin, and Shinu sites were 7.0, 0.36, and 1.17 mg/L, respectively (Figure 3).

Discussions

It is apparent that conditions in polluted field sites present a more complex picture than laboratory studies. This is not unexpected when one considers the numerous interactions occurring between biotic and abiotic factors. Nevertheless, a few generalizations are apparent [21].

The prevalence and diversity of snails could be increased or decreased depending on the class of pollutants. Under natural condition, snails are exposed to several environmental factors which produce a collective effect on the snails. In the present study, it was found that the highest percentage of total snail species (75.47 %) was collected at 28°C in Qulin, but when the temperature increased more than 34°C, the percentage was decreased to 5.86 % and 4.11% in Kafr El Sheikh and Shinu sites, respectively. The highest occurrence of *B. alexandrina* snails was recorded to be 94.3% and 88.5% at 28°C and 31°C,

respectively. This is in coincidence with El-Khayat et al. [22], who revealed that snails can tolerate a wide range of temperature 19 – 34°C. However, it has been showed that snails can tolerate low temperature rather than a high temperature which can lethally affect them [23]. This indicates that snail species is highly sensitive to an elevation in temperature that may cause thermal stress on snail and also reduces the dissolved oxygen content of water body [24]. However, significant link was not found between snail abundance and water temperature [25].

The present study found that the highest percentage of *B. alexandrina* snail was collected under conductivity 1302 µS/m. This finding is in agreement with the previous study of Berrie [26], who promulgated that snails are not found in waters with low conductivity. This may be attributed to the ability of snails to tolerate a wide range of water hardness and these results were supported by [27], where the water with low hardness showed a reduction in the individual number and snail's shells become relatively thin.

B. alexandrina was collected at TDS ranges of 120.1-901 mg/L. These results give an indication that the snails could survive under high concentrations of TDS. Our results could be confirmed by the observation of Hairson et al. [28], who reported that snails are not found in waters with low concentrations of TDS. Moreover, it has been postulated that certain snails found in habitat with higher salinity more suitable [29]. The snails can live in a wide range of mineral content in water till certain limiting [30]. On the contrary, it was found that the highest numbers of *B. truncatus* snails were found under low dissolved salts (100-300 ppm) while *B. alexandrina* snails were obtained from a range of 601-1000 ppm [31].

From the current work, it was found that the *B. alexandrina* species were collected at DO range of 2.65 – 2.71 mg/L. This result was almost within the range mentioned by [32], who found that the desired concentration of DO for snails ranged between 2.2 – 8.5 mg/L.

In the present work, *B. alexandrina* species were observed in almost the same range of water pH (7.22-7.33). it was stated that pulmonata snails *Lymnaea sp.*, *Bulinus sp.* and *B. alexandrina* were collected from large as well as small canals in Behera Governorate and from narrow ditches, where pH ranged from 7.2 to 7.6 [33]. The present pH range found to be nearly similar to the observation by [34], who found that pH range was 7.2 – 10.9 for all the sites that harbored snails.

The present study has identified a total of nine snail species namely *B. alexandrina*; *B. truncatus*; *L. natalensis*; *P. acuta*; *H. duryi*; *L. carinatus*; *B. unicolor*; *P. planorbis* and *M. tuberculata* in five selected sites from two Egyptian governorates (Giza and Kafr El-Sheikh) during a survey period. This gives an indication of stable coexistence found only in habitats, which are capable of supporting mutually exclusive and conductive niches for different species population.

The present field survey detected that the *B. alexandrina* was the most enumeration of snail species. This may be attributed to that *B. alexandrina* was more tolerant than the other snail

Table 3: The frequency and relative coverage (represented as scores) of aquatic plants at the selected sites.

Governorates	Plants sp.	Giza		Kafr El-Sheikh			Total
		El Salmawy	Kafr Hakim	Qulin	Shinu	Kafr El-Shiekh	
L. gibba	N.(Co)	5,5,5	5,4,4	4,4,3	-	-	39
	Sum of (Co.)	15	13	11	0	0	
C. demersum	N.(Co)	4,3,2	-	-	-	3,3	15
	Sum of (Co.)	9	0	0	0	6	
E. crassipes	N.(Co)	-	2,2,1	-	3,4,3	2,1,1	19
	Sum of (Co.)	0	5	0	10	4	
J. repen	N.(Co)	-	1,2,1	2,2,1	-	1,1	9
	Sum of (Co.)	0	4	5	0	2	
P. stratiotes	N.(Co)	2,3	-	-	-	-	5
	Sum of (Co.)	5	0	0	0	0	
Total density		29	22	16	10	12	89

N=frequency of occurrence, Co= Coverage (represented as scores):- (absent), 1 for ≤ + (very low coverage), 2=++ (low coverage), 3= +++ (moderate coverage), 4= ++++ (high coverage), 5 for ≥ +++++ (very high coverage).

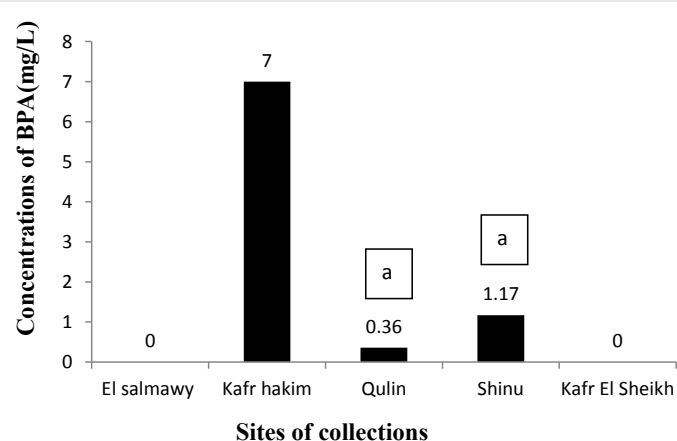


Figure 3: Concentrations of BPA at five sites of collections. a Significant different as compared to Kafr hakim.

species to some of the examined parameters. This was in agreement with results of [23,35,36]. It was noted that *B. alexandrina* snails invested in smaller canals and drains. This notice is in accordance with Dazo et al. [37], who reported that *B. truncatus* was most abundant in large canals while *B. alexandrina* was most abundant in drains.

The highest percentage of total snail species (75.47 %) was collected at 28 °C, but when the temperature increased more than 34°C this percentage was decreased to 5.86 % and 4.11% in Kafr El-Sheikh and Shinu sites, respectively. However, *B. alexandrina* snails were recorded with the highest occurrence (94.3% and 88.5%) at 28 °C and 31 °C, respectively. *B. alexandrina* snail was found at TDS ranges from (120.1–901 mg/L) and dissolved oxygen range (2.65 – 2.71 mg/L).

The distribution of *B. truncatus* and *B. alexandrina* in two villages, El-Garda, and Salamoniya, in Menoufia Governorate was studied and differed greatly in the degree of chemical and fecal pollution of the watercourses [38]. This was probably due to the existence of the sewage disposal system in El-Garda village and its absence in Salamoniya. In spite of the high pollution of watercourses in Salamoniya, both *Bulinus* and *Biomphalaria* snails were found and were often infected. On the other hand, in El-Garda, in spite of the lower pollution of its watercourses, which would have been expected to be associated with higher snail counts, particularly in Kafr Tambidy canal which was less chemically polluted, *B. truncatus* was the only snail found and with very low counts.

The survey study observed five aquatic plant species; *L. gibba*, *C. demersum*, *E. crassipes*, *J. repen* and *P. stratiotes* where *L. gibba* and *E. crassipes* were the mostly infested aquatic plants. It has been showed that sites in which snails associated with macrophytes (64%) were characterized with higher ranges of chemicals, DO, and conductivity than that observed in sites with snails only indicating the helpful role of macrophytes for increasing snail tolerance to unfavorable conditions [22]. Moreover, a significant association between vegetation density and snail occurrence was found [39]. In addition, it has been reported that in general, adverse effects of water pollution on snail biology were modified by biotic factors including food supplies, aquatic plants, behavioral and physiological adaptation [40].

In the current study, there is a positive correlation between the abundance of snails and the presence of aquatic plants. We observed an association between moderate density of the recorded plants especially *L. gibba* and *B. alexandrina* in most sites. This is in agreement with findings of De souza and De melo [41,42], that considered the aquatic vegetation (such as *Eichhornia* sp. and *Lemna* sp.) consequently, providing shelter and food resource for the snails. Moreover, it has been found that *E. crassipes* and *L. gibba* were positively correlated with *B. truncatus* and *B. alexandrina*, respectively [19]. The reasons could be because the snails depend directly or indirect on the aquatic plant, where they cannot live or reproduce without aquatic vegetation. They prefer to deposit their egg masses on the plant materials as well as on hard and broad leaves [43].

Environmental degradation information is factored into

estimates of exposure. The environmental degradation of polycarbonate grade BPA, used in the manufacture of plastics, was measured using waters samples from the five sites. The concentrations of BPA in Kafr Hakim, Qulin, and Shinu sites were 7.0, 0.36, and 1.17 mg/L, respectively. This variation in BPA concentration could be illustrated as surface-water concentrations of BPA vary considerably depending on the location, sampling period, and how the results are reported [44]. It was found that BPA concentrations from monthly samples on multiple rivers ranged 0.07–4.0 µg /L [45]. However, studies the levels of BPA in landfill leachate in Japan reported that the concentrations range and 1.3–17,200 µg/L [46].

In conclusion, the most important of the associating vegetation was *L. gibba* which correlated with *B. alexandrina* and could be served as an indicator plant for the snail in selected sites.

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