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Morpho-Ecological Study of Freshwater Mollusks of Margalla Foothills, Pakistan

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ABSTRACT

For a long time, freshwater snails have attracted the attention of biologists. The ecological attributes like diversity, species richness and equitability of freshwater gastropods are important due to various reasons like intermediate hosts for many trematodes and bio-indicator. Snails are cosmopolitan in distribution and diversity of habitat to perform ecological performance is abosolute. The freshwater malacological information is sparse within Pakistan specifically in the foothills of Margalla hills. Therefore, the present study was designed to evaluate the habitat preference of freshwater snails to environmental factors. However, only 3 species of snail were found from the 13 sites of Islamabad during the period of study. The presence of the snails like *Melanoides tuberculata, Indoplanorbis exustus* and *Gyraulus convexiusculus* belonging to Family Planorbidae and Family Thiaridae. Freshwater snail samples were collected from Islamabad and Rawalpindi in screw capped sterilized bottles from various localities during the months of July 2017 to December 2017. Physical parameters affect the presence of snails e.g. alkalinity of the soil indicates the survival of more number of species. A significant correlation was found between diversity and equitability (Pearson correlation value=0.961, *p*-value=0.00). Higher the electrical conductivity of soil lower is the diversity of snail. A significant correlation was found between diversity and equitability (Pearson correlation value=0.961, *p*-value=0.00). Electrical conductivity and pH showed a significant correlation (Pearson correlation value=0.665; *p*=0.015). The saturation in soil was significantly correlated to calcium in the soil (Pearson correlation value=0.640; *p*=0.025). The study indicated that the freshwater snails of twin cities need such type of investigations, so urban benthic macrofauna may be well understood in-terms of ecology, phylogenetic analysis, spatio-temporal variation and diversity.

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Authors' Contribution
HA conceived and supervised the project. QW, ST and MAJ collected the samples and did initial laboratory investigations. MAG helped in the taxonomy of mollusks and preparation of manuscript. WH helped in statistical analysis. AK and QR helped in preparation of the final manuscript.

Key words
Freshwater snails, Mollusks, Ecology, Distribution, Abundance, Islamabad

INTRODUCTION

Biodiversity is globally admired as key component to understand the genetic abundance, ecological role and the elasticity of the ecosystem (Schultze and Mooney, 1993; Haywood *et al.,* 1995; Macintosh *et al.,* 2002). The macrobenthic fauna plays several important roles due to many reasons. There are many proven relationships between benthic and pelagic populations comprise various communities (Herman *et al.,* 1999). The structure of community in these ecosystems is quick in response to varying climate, that impact at both higher and lower trophic levels. The diversity and the distribution patterns of benthic fauna are affected by variation in both abiotic and biotic factors. Snails are important members of macro-benthos that has a prominent effect on determining the properties of sediments generally as well as specifically. Snails are cosmopolitan in distribution, so they are distributed among diverse habitats.

Ecological investigations of freshwater and terrestrial snails have shown that the population dynamics and ecology of animals depends on various factors such as the physical structure of a given region, topography, type of bottom soil sediment, hydrography, climate change; physicochemical parameters such as temperature, nitrate level, pH, dissolved gases, alkalinity, calcium ions and biological factors such macrophytes (Ghani *et al.,* 2017). The freshwater snails are one of the important component of the aquatic ecosystem. Therefore, representing a large group of consumers of primary production and secondary producers (Brönmark, 1989; Yeung and Dudgeon, 2014). Many consumers like invertebrates and vertebrates may include snails in their diet and some are specialized molluscivores (Brönmark, 1992; Dillon, 2000; Michelson, 1957; Onyishi *et al*., 2018). The snails constitute a vital part of the food chain so an excellent source of calcium in the ecosystem for the birds species during their breeding

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season. Altaf *et al*. (2017) conducted a study to determine the relative abundance of species, affected by abiotic factors to determine the indicator species in agriculture based ecosystems of Pakistan. Dillon (2000) reported the distribution of freshwater gastropods and their abilities to colonize a habitat and survive there.

Habitats of snails vary from deserts to all types of cooler climates e.g. mountain areas and marshes. They are also found in high altitude, mountainous regions, hot and cold places. Mostly terrestrial snails live in gardens, agricultural areas, river sides or streams, suburbs, swamps, cities, jungles, and forests. House gardens are home to many snails too. Snail culture is easy while, some people keep them as farm animals to use them as food. Many snail species prefer to stay close to the rocks, ditches, and plants to have a place to protect themselves from their natural predators. Some freshwater snails serve as intermediate host to many trematode parasites (Appleton, 1996). Many workers reported taxonomic description of freshwater snails from Pakistan (Kahtoon and Ali, 1978; Nazneen and Begum, 1990). The summer rainy season favours the abundance of snails (Brown, 1980). Various factors *e.g.* type of vegetation, type of snail predators and amount of soil moisture encourage the presence of snails (Afshan *et al*., 2012). The effects of benthic macrofauna especially snails on sediment characterization has been well understood by several reasons like recycling of minerals, transfer of energy to other trophic levels, aeration of soil and promotion of decomposition (Tenore *et al.,* 1984; Schaffner *et al.,* 1987; Hutchings and Saenger, 1987). In addition, they are an important energy source for many trophic level members *e.g.* epibenthic crustaceans, fishes and birds (Aller and Aller, 1998). They maintain a connection between the unavailable nutrients in sediments and useful biotic materials like proteins in fish and shellfish. Many benthic organisms depend upon detritus that reside on the bottom of the water and serve as food source for a wide range of aquatic fauna (Imevbore and Bakare, 1970; Adebisi, 1989; Ajao and Fadage, 1990; Oke, 1990; Idowu and Ugwumba, 2005).

Taxonomic account of the freshwater mollusks of Pakistan was reported by many workers from Pakistan about occurrence of mollusks (Pelecypod) (Khan and Dastagir, 1972; Tirmizi, 1973). Although many investigators (Ziaullah *et al*., 2018; Afsar *et al*., 2012; Ahmed and Hameed, 1999; Ahmed and Ayub, 1996; Ahmed *et al*., 1982) worked on several aspects of marine gastropods, still knowledge is lacking on freshwater snails of Pakistan. Therefore, present study was aimed to describe some ecological attributes of freshwater gastropods of freshwater bodies in and around Islamabad.

MATERIALS AND METHODS

Study sites

Monthly samples of freshwater snails were collected from different localities *e.g.* Rawal lake, Rose and Jasmine garden, Shakarparian, E9 green belt Islamabad, Falcon complex Rawalpindi, Korang town Islamabad and COMSATS University, Islamabad. Rawalpindi and Islamabad are situated in a climatic region known as sub-tropical and sub-humid (latitude 30°N - 34° N and longitude 70°E - 74°E). The annual rainfall of the area is 737 mm with minimum temperature does not exceed beyond 01°C in winter and average maximum temperature remains 38.5°C in summer.

Sample analysis

Freshwater snail samples were collected in screw capped sterilized bottles from different localities of Islamabad and Rawalpindi (July2017 - December 2017) by applying hand scoop and hand picking methods. The snails were taken to the laboratory, clean water was used to remove the sediments, and details of fresh specimen were noted. They were classified according to the shell morphology as described by El-Gindy (1960). The specimens were put in 90% alcohol for 24 h before removing the soft parts. The shells were dipped in dilute solution of Oxalic acid and rinsed with tap water to prevent the etching by the acid. Scrubbing with soft brush was used to remove the deeply impacted minerals and alga from shells; this helped to note the features of sculpture of shell (Mandahl-Barth, 1962; Thompson, 2004). During transfer from wild area to laboratory, natural vegetation was used as food for snails, while spinach leaves were also provided as food and keeping at room temperature (25.0±2.0°C). The shells were dried at room temperature and preserved for future studies.

Statistical analysis of snails

Shanon-Weaver index, and Margalef index were used to calculate the diversity and species richness. Pearson correlation was calculated for ecological parameters using MiniTab ver. 18.0.

Shannon-Weaver index was expressed as (Shannon-Weaver, 1963):

$$Hs = \sum Ni/N \text{ Log2 } Ni/N$$

Where, Hs is Shannon-Weaver index, N is total number of individual in the sample, and Ni is the number of individuals of species in the sample.

Margalef Index of richness:

$$d=S-1/\ln N$$

RESULTS

Distribution and abundance

A total of 3 (n=537) species of snail were found from various sites of Islamabad (*e.g.* Rawal Lake, Rose and Jasmine Garden, Shakarparian, E-9 green belt Islamabad, Falcon Complex Rawalpindi, Korang Town Islamabad and CIIT, Islamabad) during the period of study. These species were belonging to Family Planorbidae and Family Thiaridae. Total number of *Gyraulus convexiusculus*, *Indoplanorbis exustus* and *Melanoides tuberculata* were 320, 117 and 100, respectively.

Table I.- Diversity, species richness, equitability and number of individual members of various species snails collected from Islamabad.

Sites	Species	No. of species	Diversity	Equitability	Sp. Richness	No. of individual
1	*Gyraulus convexiusculus*	22	0.834	0.834	0.219	83
	Indoplanorbis exustus	0				
	Melanoides tuberculata	61				
2	*Gyraulus convexiusculus*	15	1.465	0.924	0.309	94
	Indoplanorbis exustus	44				
	Melanoides tuberculata	35				
3	*Gyraulus convexiusculus*	79	0.278	0.278	0.219	83
	Indoplanorbis exustus	4				
	Melanoides tuberculata	0				
4	*Gyraulus convexiusculus*	8	0	0	0.35	8
	Indoplanorbis exustus	0				
	Melanoides tuberculata	0				
5	*Gyraulus convexiusculus*	18	0.954	0.954	0.289	48
	Indoplanorbis exustus	0				
	Melanoides tuberculata	30				
6	*Gyraulus convexiusculus*	16	0	0	0.25	16
	Indoplanorbis exustus	0				
	Melanoides tuberculata	0				
7	*Gyraulus convexiusculus*	18	0.954	0.469	0.447	20
	Indoplanorbis exustus	0				
	Melanoides tuberculata	2				
8	*Gyraulus convexiusculus*	13	0.958	0	0.436	16
	Indoplanorbis exustus	0				
	Melanoides tuberculata	8				
9	*Gyraulus convexiusculus*	29	0.210	0.210	0.365	30
	Indoplanorbis exustus	0				
	Melanoides tuberculata	1				
10	*Gyraulus convexiusculus*	22	0	0	0.213	22
	Indoplanorbis exustus	0				
	Melanoides tuberculata	0				
11	*Gyraulus convexiusculus*	6	0	0	0.409	6
	Indoplanorbis exustus	0				
	Melanoides tuberculata	0				
12	*Gyraulus convexiusculus*	15		0	0.258	15
	Indoplanorbis exustus	0	0			
	Melanoides tuberculata	0				
13	*Gyraulus convexiusculus*	6	0	0	0.409	6
	Indoplanorbis exustus	0				
	Melanoides tuberculata	0				

Sites: 1, 7, Shakarparian; 2, 8, Rawal lake; 3, 9, Rose and Jasmine Garden; 4, 10, E-9 Green Belt; 5, 11, Falcon Complex; 6, 12, 13, COMSATS University.

Table II.- Pearson correlation among the various ecological and physical attributes with the Specie richness of snails.

	Diversity	Equitability	Spp. richness	EC	pH	Organic matter	Phosphorous	Calcium
Equitability	0.961 0.000							
Spp. Richness	0.033 0.915	0.054 0.860						
EC	-0.298 0.322	-0.302 0.316	0.157 0.608					
pH	-0.039 0.899	-0.094 0.761	0.087 0.779	-0.655 0.015				
Organic matter	0.500 0.082	0.488 0.091	-0.062 0.840	-0.525 0.065	0.496 0.085			
Phosphorous	-0.325 0.303	-0.244 0.445	0.221 0.489	-0.045 0.889	0.344 0.273	0.363 0.246		
Calcium	-0.314 0.321	-0.232 0.468	0.221 0.490	-0.156 0.629	0.570 0.053	0.310 0.326	0.526 0.079	
Saturation	-0.464 0.128	-0.417 0.177	0.203 0.527	0.185 0.564	0.422 0.171	0.094 0.772	0.396 0.203	0.640 0.025

Gyraulus convexiusculus *(Family: Planorbidae)*

Shell shape cape, number of whorls 3 to 6, umbilicus open, shell pale brown or pale horn, whorls convex, last whorl expanded, periphery rounded to angulated only in last whorl, but not influencing the aperture, aperture is ovate-lunate, ribs transverse, aperture opening right, umbilicus broad, surface of the shell closely striated.

Indoplanorbis exustus *(Family: Planorbidae)*

Shell shape discoidal or cape, number of whorls 4 to 7, umbilicus perforate or open, shell greenish or greyish brown in color, concavely flattened on both sides, body whorl large, aperture broad, umbilicus open, ribs transverse, sculpture with spiral striations (Fig. 1).

Melanoides tuberculata *(Family: Thiaridae)*

Shell conical, 7whorls, shell light or white dusky in color, elongate and tapering, whorl rounded, convex and increasing in size downward, aperture small and ovate, ribs transverse, aperture opening right, sculpture with finer spiral striations.

Diversity indices

The highest diversity was found in at site 2 (1.465), whereas the lowest diversity was found in site 9 (0.210) (Table I). Similarly the equitability was highest at site 5 as compared to lowest equitability (0.210) estimated at site 9. The maximum species richness was found at site 7 (0.477) than lowest species richness was estimated at site 10 (0.213) (Table I).

Correlation

A significant correlation was found between diversity and equitability (Pearson correlation value=0.961, *p*-value=0.00) as shown in Table II. Electrical conductivity and pH showed a significant correlation (*p*=0.015). The saturation in soil was significantly found correlated to calcium in the soil (*p*=0.025). However no significant correlation was found among other parameters like species richness, phosphorus and organic matter as Table II indicates.

Morphometric analysis

The average values and ranges for shell height, width and length as well as aperture of *Gyraulus convexiusculus, Indoplanorbis exustus* and *Melanoides tuberculata* measured are shown in Table III. These indicate great heterogeneity in shell height, length and weight of these populations. Data for these characteristics were also compared in a box plot (Figure 7). These box plots represent summaries of measurements of the height, length, width of the shell as well as aperture in the principal component analysis.

Gyraulus convexiusculus

Gyraulus convexiusculus showed minimum 3.0 mm length and maximum 20mm whereas mean value is 85.38mm. These values are also shown in Figure 1A. Shell weight and shell width are shown in Figure 1B and C. The minimum value of sell weight was 0.040g and maximum value was 7.430g whereas mean value was 0.724g. The minimum value of shell width was 6.0 mm and maximum value was 30.0mm and means value was 15.71mm. Comparison between different variables (length/weight,

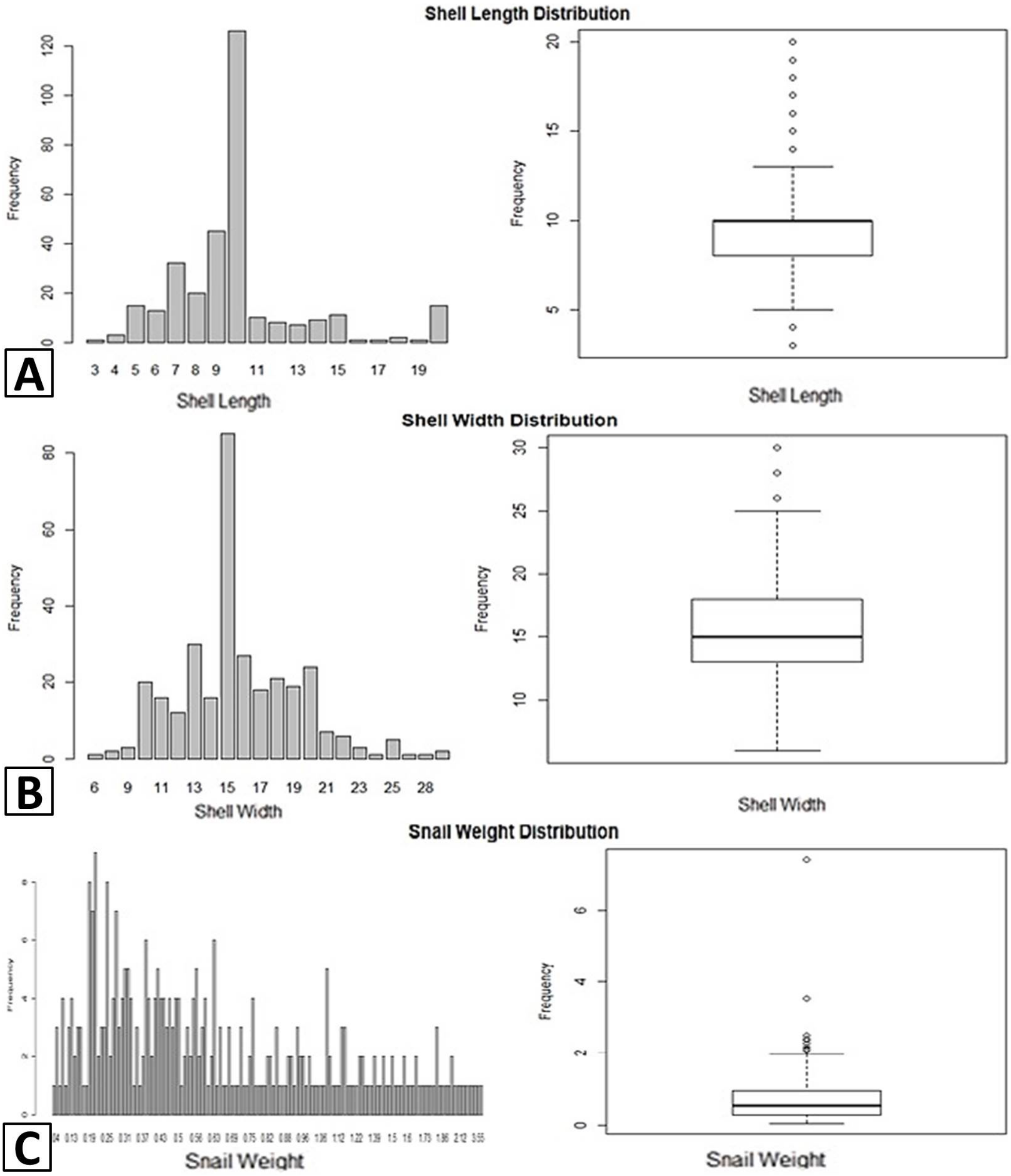

Fig. 1. Graphical and box plot presentation of shell length (A), shell width (B), and shell weight (C) distribution of *Gyraulus convexiusculus*.

Weight/width, length/width of *Gyraulus convexiusculus* is shown in Figure 2.

Melanoides tuberculate

Melanoides tuberculate shows minimum 1.0 mm length and maximum 19 mm whereas mean value was 10.41mm. These values are also shown in Figure 3A. Shell weight and shell width are shown in Figure 3B and C. The minimum value of shell weight was 0.09g and maximum value was 0.94g whereas mean value was 0.49g. The minimum value of shell width was 1.0 mm and maximum value was 10.0 mm and means value was 6.01 mm.

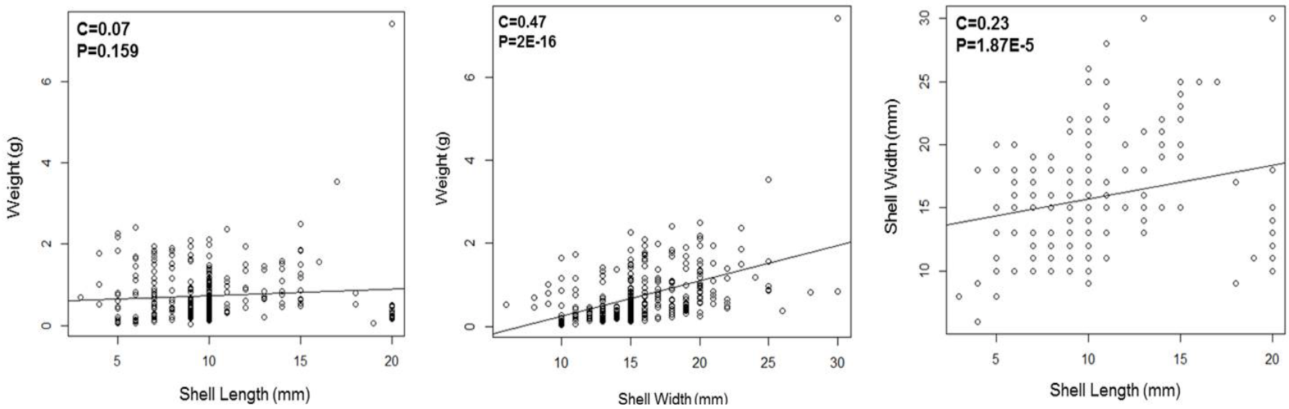

Fig. 2. Comparison between different variables (length/weight, weight/width, length/width of *Gyraulus convexiusculus.*

Table III.- Average values and ranges for shell height, width and length as well as aperture of *Gyraulus convexiusculus, Indoplanorbis exustus* and *Melanoides tuberculata.*

S. No.	Species	Shell width(mm) mean (range)	Shell height (mm) mean (range)	Shell weight(g) mean (range)	Aperture(mm) mean (range)
1	*Gyraulus convexiusculus*	15.71 (6.00-30.00)	9.97 (3.00-20)	0.72 (0.0400-7.43)	9.66 (3.00-20.00)
2	*Melanoides tuberculate*	6.01 (1.00-10.00)	18.71 (12.00-23.00)	0.49 (0.09-0.94)	5.44 (1.00-9.00)
3	*Indoplanorbis exustus*	16.06 (5.00-23.00)	10.41 (1.00-19)	0.94 (0.10-2.68)	10.06 (1.00-15.00)

Comparison between different variables (Length/weight, Weight/width, length/width of *Melanoides tuberculate* is shown in Figure 4).

Indoplanorbis exustus

Indoplanorbis exustus shows minimum 1.0 mm length and maximum 19mm whereas mean value is 10.41mm. These values are also shown in Figure 5A. Shell weight and shell width are shown in Figure 5B and C. The minimum value of shell weight was 0.10g and maximum value was 2.68g whereas mean value was 0.94g. The minimum value of shell width was 5.0 mm and maximum value was 23.0 mm and means value was 16.09mm. The comparison between different variables e.g. length/weight, weight/width and length/width has been shown in Figure 6.

DISCUSSION

The freshwater molluscan fauna of Pakistan is not very well known globally. The literature showed that few studies generated little information on the identification of freshwater snail fauna. They form an important component of the aquatic biodiversity and very important consumers of primary production and secondary producers (Brönmark, 1989; Yeung and Dudgeon, 2014). Many animals of wide range of taxa may include snails in their diet and some species maintain molluscs as primary food (Dillon, 2000; Michelson, 1957). Three types of gastropods *viz*., *Gyraulus convexiusculus, Indoplanorbis exustus* and *Melanoides tuberculata* were identified from various localities of Rawalpindi and Islamabad. The snails were characterized on various morphological characteristics e.g. shell sculpture, color pattern, shapes and slightly on soft body parts (Preston, 1915; Begum and Nazneen, 1991, 1992a, b). The diversity and abundance of these three species were also unknown to date and local investigators did not take interest to enhance the knowledge about the local urban fauna of twin cities of Rawalpindi and Islamabad.

Present study revealed that the freshwater snail fauna was more diverse in soils containing more moisture as compared to soil with low moisture. The first three sites (*e.g.* Ataturk Garden, Islamabad; Green belt near Motorawy Chowk, Islamabad; Freshwater stream in Shakarparian Hills) counted more number (n=83, n=94, n=83, respectively) of individuals from all three species collected. A very interesting correlation was found between diversity and equitability. The correlation was found very strong and

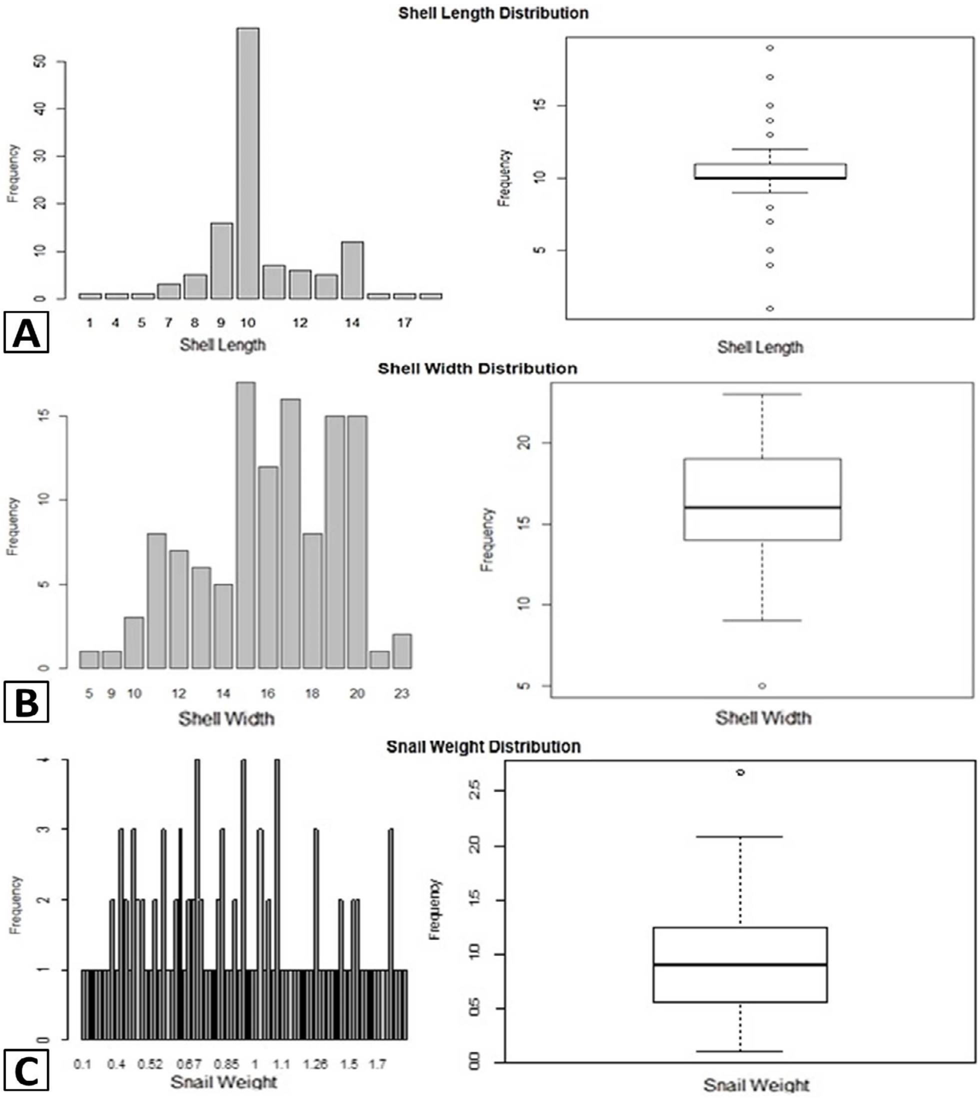

Fig. 3. Graphical and box plot presentation of shell length (A), shell width (B), and shell weight (C) distribution of *Melanoides tuberculate*.

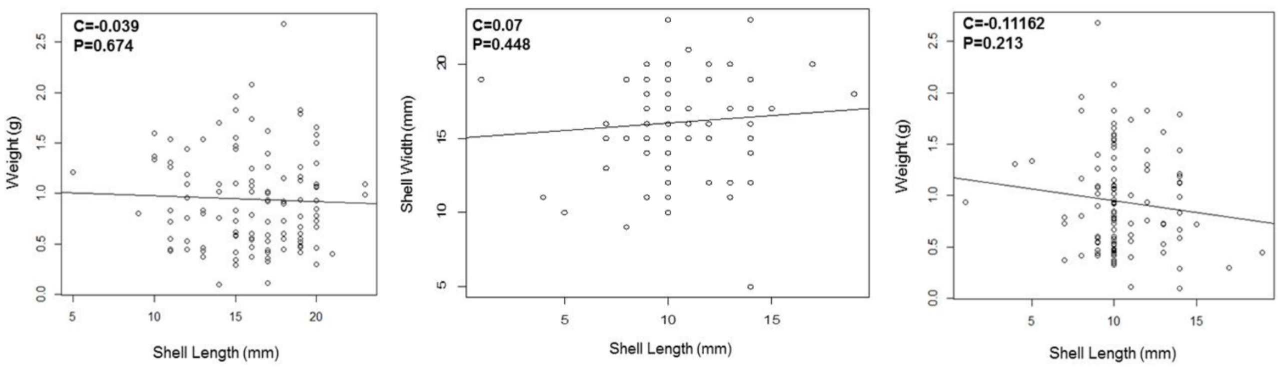

Fig. 4. Comparison between different variables (length/weight, weight/width, length/width of *Melanoides tuberculate*.

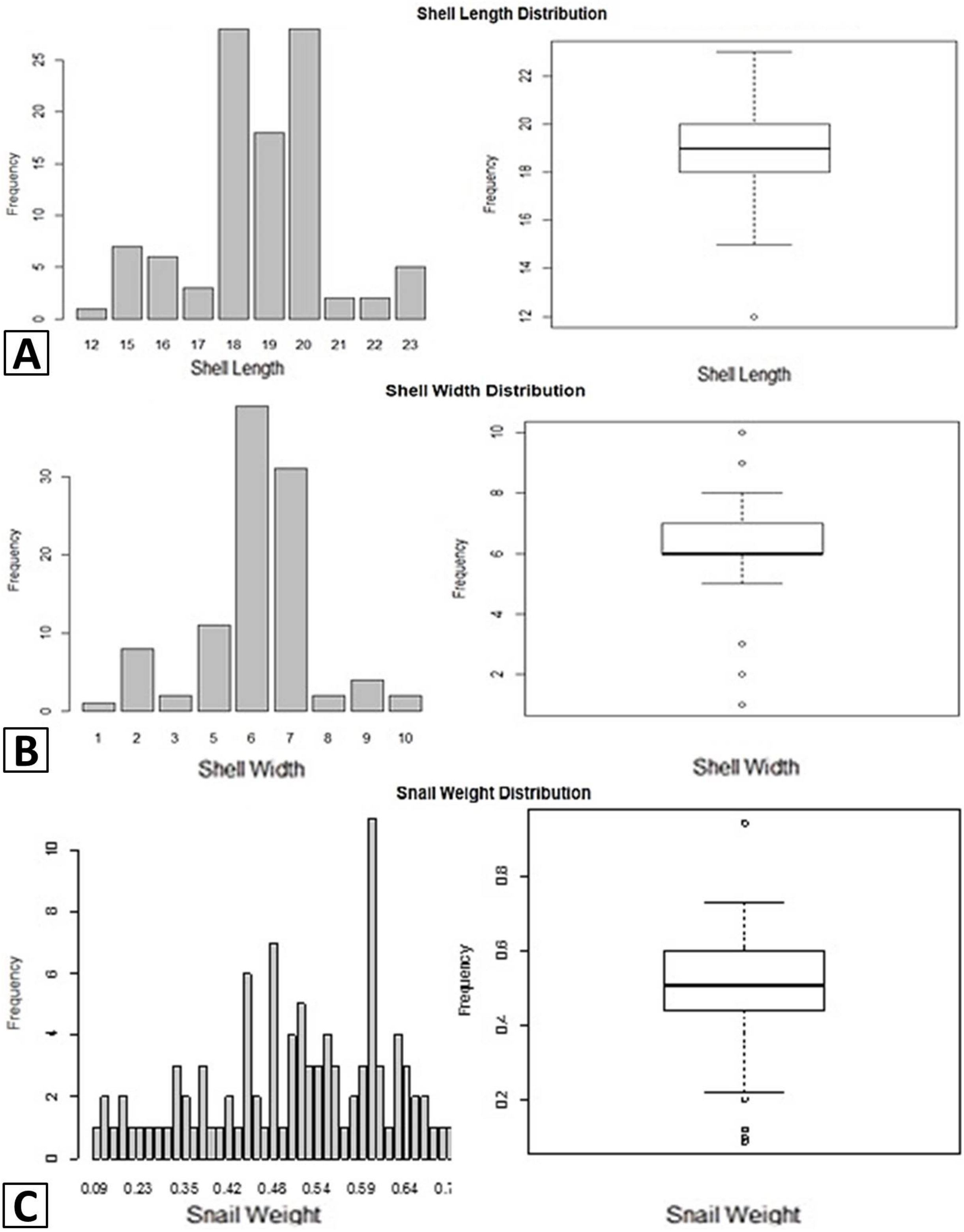

Fig. 5. Graphical and box plot presentation of shell length (A), shell width (B), and shell weight (C) distribution of *Indoplanorbis exustus*.

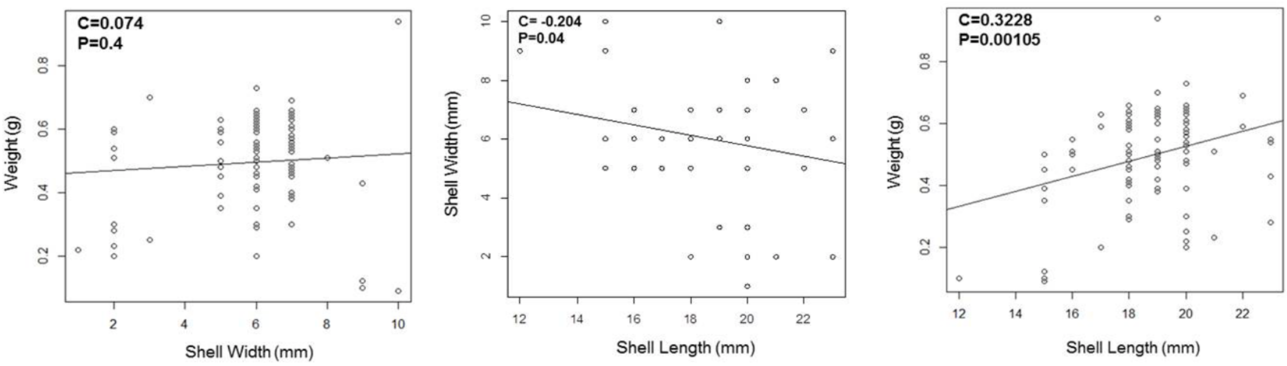

Fig. 6. Comparison between different variables (length/weight, weight/width, length/width) of *Indoplanorbis exustus.*

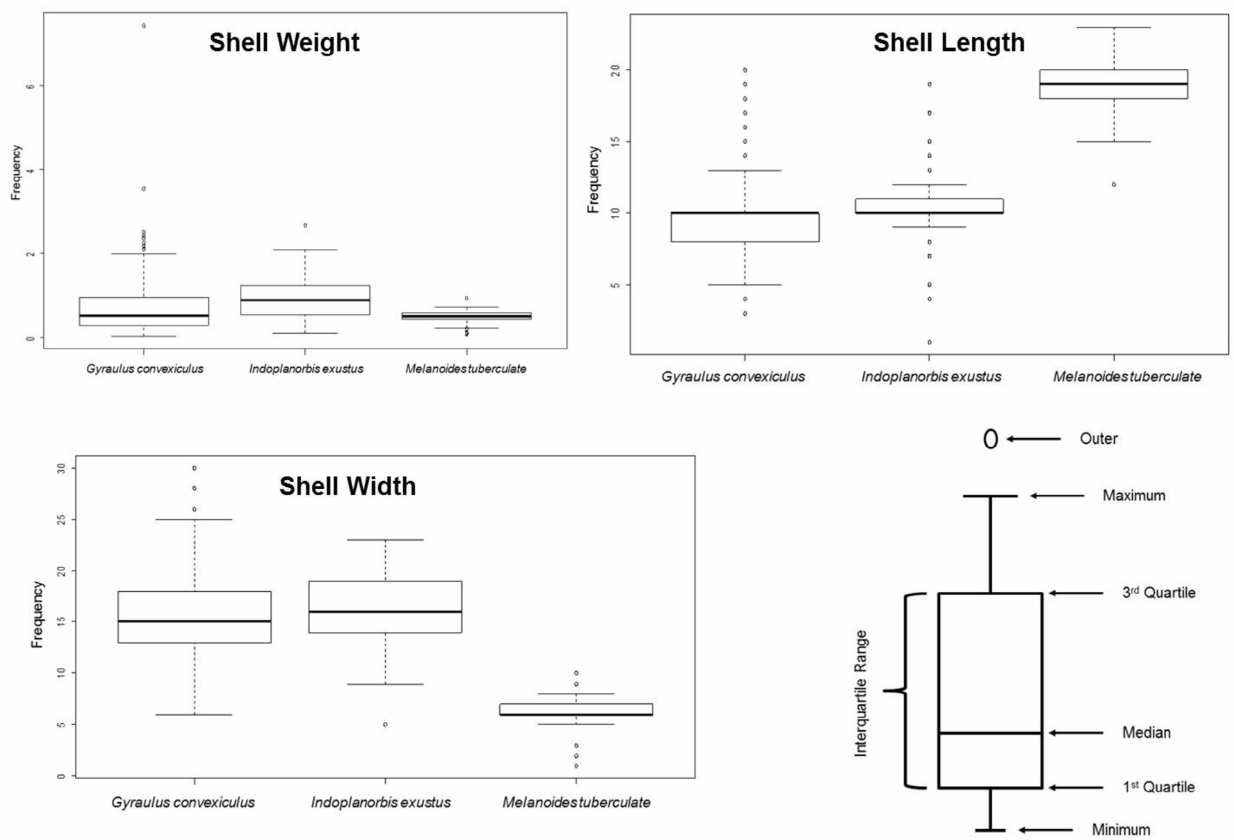

Fig. 7. Box plots of morphometric comparison of characteristics for *Gyraulus convexiusculus, Melanoides tuberculate, Indoplanorbis exustus:* A, shell weight; B, shell length; C, shell width.

significant as results showed. Although there were only three species found in thirteen different sites of Islamabad, the individuals of each specie showed even distribution for likeliness of physical factors such as pH and electrical conductivity of the soils. The two factors complimented to each other and made favorable conditions along with calcium that supported for growth of gastropods in the soils of Islamabad. Gleason *et al*. (1975) found that *P. paludosa* egg cluster counts were positively correlated with specific conductivity, calcium concentration, and pH in wetlands of south Florida; Hurdle (1974) observed a similar association along a gradient of water chemistry conditions in a spring-fed creek in central Florida. Perera *et al*. (1989) reported, however, that *P. paludosa* (in Cuba) can persist in water with pH<6. The pH remained favorable as basic to neutral level, since the condition supporting for life in soil. Lower the pH value the more acidic conditions become so this refers to polluted environment restricting the selected growth of living organisms. Dalesman (2011) inferred about freshwater snail *Lymnaea stagnalis* (L.) as a calciphile that showed reduced growth and survival in environments containing less than 20 mg/l environmental calcium with no apparent effect on survival at 20 mg/l, since reducing environmental calcium increases metabolic demand, and as such this level of calcium acted as a stressor on the snail. However another study by Martin *et al*. (2001) described waters inhabited by *Pomacea canaliculata* as having an average of 29.8 mg Ca/l compared with those not inhabited by snails as having 14.8 mg/l [pH (8 in all sites)]. Ollerenshaw (1958), Yilma (1985) and Dillon (2000) described that factors like temperature, hardness, pH, altitude, size of water bodies and vegetation and pollution were among the significant aspects affecting the distribution and abundance of mollusks. Herbst *et al*. (2008) investigated that many slow moving waters in the range of 25–200 µS cm^{-1} cannot support productive New Zealand mud snails and that nuisance invasions may be most prevalent in waters above 200 µS cm^{-1} where sufficient dissolved mineral content is present for growth. Whereas, a significant correlation was found between saturation and calcium. Similar observations were revealed by Burdi *et al*. (2008) that number of individuals in a population was large during June and a strong positive relationship (R^2= 0.777) with elevated temperature while a negative correlation with the hardness was obvious. The same was found in our study too, where phosphorus and calcium in soil was not significantly correlated with any attributes like diversity, species richness and equitability. Kotzian *et al*. (2013) studied the diversity and distribution of molluscan fauna along the Contas River, Northeastern Brazil, and concluded that the longer the hydroperiod (especially the local precipitation) more survival of molluscs either bivalves or gastropods happened. This study favours the former comments as we found more clusters and distribution of gastropods in moist shady places along the water channels and small water ponds in Islamabad. The precipitation rate (1000mm) in monsoon (15 June-15 August) was higher as compared to other regions of Pakistan (PMD, 2018).

The freshwater snail's biodiversity in the localities has linkage with vegetation, presence and absence of snail predators, sediment structure and its chemical composition. Localities *e.g.* parks, gardens and green belts in Islamabad favour the presence and distribution of snails. Trussell (2000) suggested that geographic variation in morphology and its plasticity in the intertidal snail (*Littorina obtusata*) were associated with differences in the abundance of principal crab predators (*Carcinus maenas*). Consequently, quality of freshwater and environmental parameters is referred as good biological indicators to support the freshwater snail biodiversity richness in a given habitat. Thus, seasonal population density of gastropods of each species is associated with varying moisture content in the substratum.

CONCLUSIONS

The study infers that biodiversity of urban area like Islamabad and Rawalpindi needs much attention to investigate. The components of urban biota especially benthic fauna like snails play vital role in reforming the characteristics of soil and relevant phenomenon. A continuous study to describe the variations in physical and biological factors influencing the diversity and distribution of terrestrial snails is required. Although the results are promising and indication of habitat preference for land snails in wild areas of Islamabad, refer to characterization and qualitative interpretations of localized ecosystems comprising of seasonal water streams, small water ponds develop after summer monsoon, and low hilly jungles with suitable habitat for snails' growth and population. Therefore it is suggested to investigate the role of each species of snail is important and may be studied separately in detail.

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Statement of conflict of interest

The authors declare no conflict of interest.

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