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Terrestrial snails as bioindicators of environmental degradation

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Abstract

The snails are very important part of the food chain and excellent source of calcium in the ecosystem for the birds during their breeding season. They are intermediate hosts of many parasites. This study was conducted to estimate the species relative abundance, the effects of abiotic factors on different snail species, which led us to the finding of the indicator species in the agro ecosystem of Faisalabad,. Total of 19,290 snails were collected from crop lands of 24 villages. The species were identified with the help of recent identification keys and RAPD markers. Three snail species i.e. *Physa fontinalis, Zooctecus insularis* and *Ceciliodes acicula* were found completely absent from most of the villages except a few, indicating that the possible cause may be presence of arsenic. Other abiotic factors i.e. Cd, Pb, pH and Electrical conductivity are within the permissible limits. These species can be considered extremely important for biomonitoring. This study can be used as a predictive model to estimate environmental degradation.

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Introduction

Recently there is an increasing trend of using land snails as ecological models to estimate anthropogenic stress (Dedov and Penev, 2004) and the environmental degradation (Gotmark *et al*., 2008; Kappes *et al*., 2009). The snails can be used as good ecological indicators due to their small body size, restricted mobility and position in the food chain (Baur and Baur 1993; Kerney and Cameron, 1979). The inventories of various areas regarding snails are available yet the impact of anthropogenic stress on these little creatures to study them for predictive models and biodiversity conservation, is still missing (Triantis *et al.* 2009). In the urban areas such studies on the snails have not been carried out (Horsa'k *et al*., 2009). In Pakistan even the baseline information about this tiny creature is fragmentary and missing in most of the regions. The information generated previously by Ali (2005) and Rehman (2010) is an attempt which has augmented the information of the malacofaunna in Faisalabad. The contribution of Auffenberg (2009) in studying the distribution of land snails in South Asia, including Pakistan, is recent and quite important.

Lately researches have been conducted on the biodiversity of soil macroinverteberate in the low and high input fields of Wheat and Sugarcane in District Faisalabad as reported by Rana (2012) with the major focus on impact of chemical on the diversity of different macroinverteberates as molluscs are very important for monitoring the toxins in aquatic ecosystem as previously stated by (Salanki *et al*., 2003). These studies can help us to enter into a new avenue to understand the level of biomagnifications of different pollutants in snails leading to serious concerns.

Studies on the status of the snails and their role as bioindicators have not been undertaken in this part of the world due to the fact that their role is being undermined. Most species of the snails play a dominant role in the fresh waters by providing food for other organisms like fish and improve water quality by consuming large quantities of detritus and algae (Johnson, 2003). In the forest ecosystem a decline in the reproduction in the passerine birds due to decrease in the snail abundance on acidic soils (Graveland *et al*., 1994).

Their status in this part of the world faced ignorance. The objective of this study is to assess the effects of heavy metals on different species in various parts of Faisalabad

Materials and method

Physical and Chemical Parameters of Soils

The physical and chemical parameters like pH, electrical conductivity and heavy metals (Pb and Cd) of the soil samples were determined. Brief description of the protocols has been given as under.

Soil Saturated Paste

The soil saturated paste was made following USSLS, (1954). For preparation of soil paste known weight (200g) of soil was soaked with distilled water and allowed to stand overnight. The saturated paste was made glistened, did not accumulate water if depression is made in the surface and fell freely from spatula.

pH of Saturated Soil Paste (pH)

With the help of pH meter Jenway Model -671 P, pH was recorded after standardizing it with buffer solution of pH 4.02 and 9.20 (Method 21a).

Electrical Conductivity of Saturation Extract (EC)

Electrical Conductivity of a saturation extract was determined by following Method 4b as prescribed by USSLS, (1954) with the help of conductivity meter Jenway Model 4070, EC was measured after standardizing it with 0.01N KCl solution.

Heavy Metal Analysis

Soil sampling after extracting reducible fraction were extracted with 2 mL of 0.02 M HNO_3 and 5 mL of 30% H_2O_2. The mixture was heated to 85°C for 2 h with occasional agitation. Another 3 mL of 30% H_2O_2 (buffered to pH 2 using concentrated HNO_3) was again heated to 85°C for 3 h with intermittent agitation. After cooling 5 mL of 3.2 M CH_3COONH_4 in 20% HNO_3 (v/v) was added and the samples were diluted to 20 mL and then agitated it continuously for 30 minutes. Metals in the filtrate were measured by using Hitachi Polarized Zeeman 8200 Atomic Absoption.

Results

The accession number of the snails has been given in (Table 1). The maximum limit for Cadmium and Lead, according to National Environmental Quality Standards are 0.1 mg L^{-1} and 0.5 mgL^{-1} respectively. All the villages of Faisalabad under study have much less concentration of Cadmium and Lead than the allowed limit (Table 2, Fig. 1, 2). The maximum limit for pH is 10 (GOP, 1997). The villages under study have slightly alkaline to neutral pH, reaching maximum up to 8.41 in 121 J.B. 222 R.B. with least value of 7.79 in 223 R.B. The pH of the soil in Faisalabad villages is from neutral to slightly alkaline (Fig. 3). The Electrical conductivity of the soil ranges from 0.75-5.22 dSm^{-1} with least EC in 202 R.B. and maximum in 73 G.B. (Fig. 4).

Table 1. Specimen number of different species.

Specimen Number	Species
1	*Ariophanta bistrialis ceylanica*
2	*Ariophanta bistrialis cyix*
3	*Ariophanta bistrialis taprobanensis*
4	*Ariophanta bistrialis*
5	*Ariophanta solata*
6	*Ariophanta belangeri bombayana*
7	*Oxychilus draparnaudi*
8	*Monacha catiana*
9	*Cernuella virgata*
10	*Pupoides fontinalis*
11	*Physa fontinalis*
12	*Zooctecus insularis*
13	*Juvenile Zooctecus insularis*
14	*Cecilioides acicula*
15	*Oxyloma elegans*

Table 2. Comparison of soil parameters with national environmental quality standards (Pakistan,1997).

Villages	Soil Samples			
	Cd mg/L	Pb mg/L	pH	EC dSm−1
204 R.B	0.0045	0.0400	8.1700	2.3200
07 J.B	0.0070	0.0430	7.9000	1.2100
119 J.B	0.0090	0.0460	8.1700	5.2200
214 G.B	0.0032	0.0510	8.2000	1.9200
121 J.B	0.0120	0.0340	8.4100	2.7100
123 J.B	0.0110	0.0370	7.9100	1.6100
124 J.B	0.0100	0.0480	8.1700	1.2300
217 J.B	0.0100	0.0370	8.1500	1.0800
202 R.B	0.0050	0.0450	8.0500	0.7500
208 R.B	0.0052	0.0420	8.0700	3.8500
103 J.B	0.0120	0.0330	8.1200	4.1000
214 R.B	0.0056	0.0320	8.3100	4.3200
221 R.B	0.0044	0.0430	8.1700	1.4700
219 R.B	0.0040	0.0410	8.2500	0.9300
222 R.B	0.0045	0.0330	8.4100	3.2100
223 R.B	0.0042	0.0300	7.7900	0.8200
225 R.B	0.0052	0.0440	8.2100	1.2000
235 R.B	0.0060	0.0350	7.9000	1.7600
295 R.B	0.0050	0.0420	8.1000	1.3300
71 J.B	0.0033	0.0430	7.9100	2.1000
105G.B	0.0031	0.0510	8.0000	3.1000
65 G.B	0.0051	0.0021	7.8000	2.2500
56 G.B	0.0045	0.0030	7.7100	5.1300
73 G.B	0.0032	0.0051	8.2000	3.9000
Pak. Environmental Legislation	0.1000	0.5000	10.000	3.9000

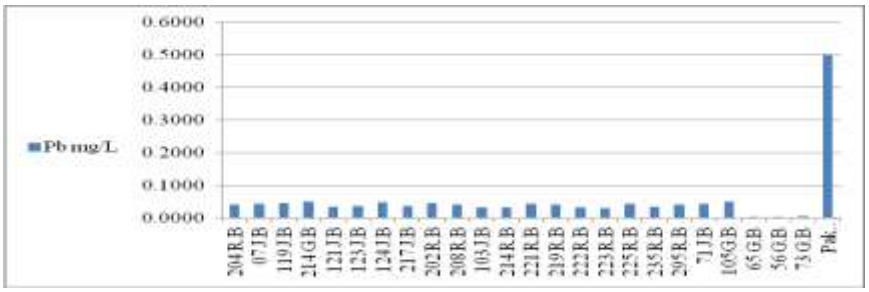

Fig. 1. Comparison of soil lead concentration with national environmental quality standards (Pakistan).

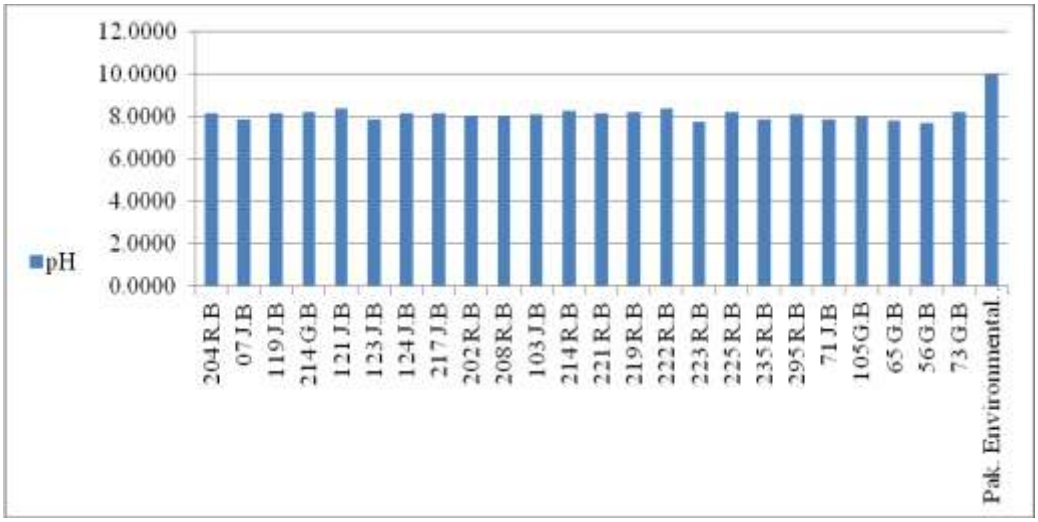

Fig. 2. Comparison of soil ph with national environmental quality standards (Pakistan).

The number of snails is a dependent variable (Y) and the Cadmium concentration, Lead concentration, pH, Electrical conductivity of the soil is independent variable. There is found no significant effect of the Cadmium concentration, Lead concentration, pH and electrical conductivity of the soil on the number of snails (Table 3 Fig. 5, 6, 7, 8). Juvenile *Zooctecus insularis* and *Cecilioides acicula* were absent from all of the villages linked to Jhang Branch and very less in villages.

Linked to Ghogera branch. The 235 R.B., village linked to Rakh branch, there is least diversity and *Zooctecus insularis,* Juvenile *Zooctecus insularis* and *Cecilioides acicula* are absent. So these species can be considered as bioindicators. The *Physa fontinalis* have been found absent in most of the villages linked to Ghogera branch and Jhang branch leading to a conclusion that these areas must be monitored for their contamination of soil and water (Table 4).

Table 3. Regression analysis of soil parameters and number of snails.

(Y)	(X)	N	R	r^2	Slope	Y intercept	t- value	df	p-value
No. of Snails	Cd. Conc.	24	-0.22	0.05	20300.5	928.09	-1.06	22	0.15NS
	Pb. Conc.	24	-0.13	0.02	-2515.95	894.55	-0.63	22	1.27NS
	pH	24	0.085	0.01	-120.42	1777.51	-0.40	22	0.35NS
	EC	24	0.205	0.04	-39.29	897.82	-0.98	22	0.17NS

Table 4. The Presence_ Absence of Snail Species in Different Villages of Faisalabad.

Species/Villages	204 R.B	07 J.B	119 J.B	214 G.B	121 J.B	123 J.B	124 J.B	217 J.B	202 R.B	208 R.B	103 J.B	214 R.B	221 R.B	219 R.B	222 R.B	223 R.B	225 R.B	235 R.B	295 R.B	71 J.B	105 G.B	65 G.B	56 G.B	73 G.B
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
2	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1
3	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
4	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1
5	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
6	1	1	1	1	1	0	0	1	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1
7	1	1	0	1	0	1	1	0	1	1	1	1	0	0	1	1	1	0	0	1	1	1	0	1
8	1	1	1	1	1	1	0	0	1	1	1	0	1	1	1	1	1	1	0	1	0	1	1	0
9	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	0	1	1	1	0	0
10	1	1	1	1	0	1	0	0	1	1	1	1	1	0	1	1	1	0	1	1	0	1	0	0
11	1	0	1	1	0	0	0	1	1	0	1	0	0	1	1	0	0	0	0	1	1	1	1	0
12	1	1	0	1	0	0	0	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
13	1	1	0	0	0	0	0	0	1	1	1	1	0	1	1	1	0	0	0	0	1	1	0	0
14	1	0	0	0	0	0	0	0	1	1	1	0	0	1	0	1	1	0	0	0	1	0	1	1
15	1	0	1	1	0	0	0	1	1	1	1	0	0	1	1	1	1	1	0	1	1	1	1	0

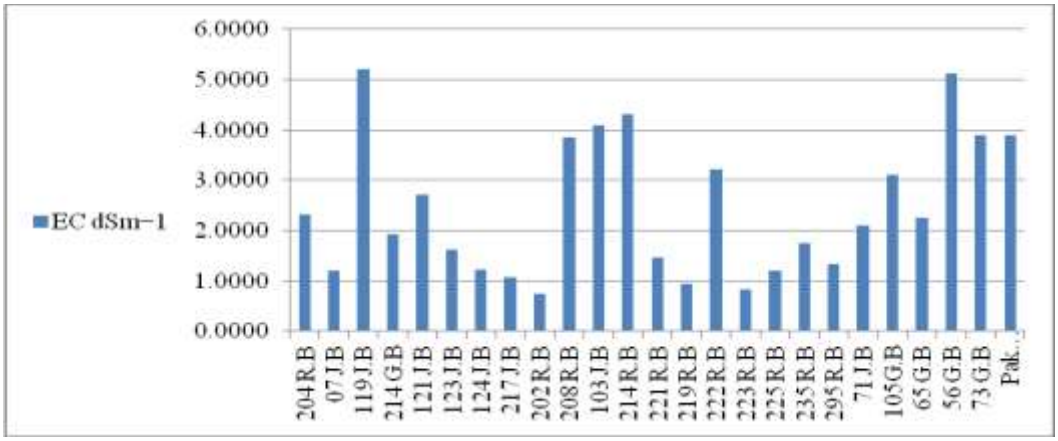

Fig. 4. Comparison of soil electrical conductivity with national environmental quality standards (Pakistan).

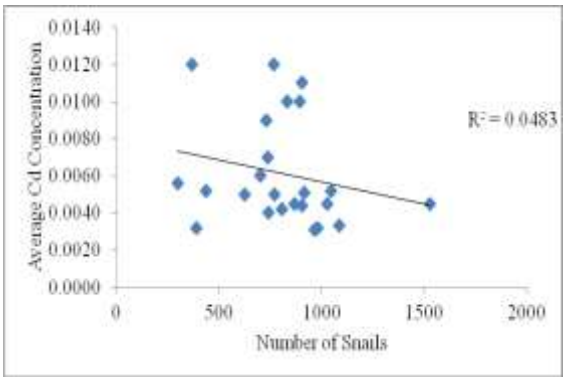

Fig. 5. Effect of Average Cadmium Concentration on the Number of Snails.

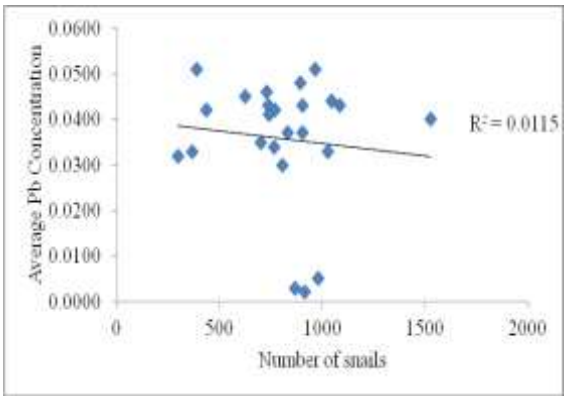

Fig. 6. Effect of Average Lead Concentration on the Number of Snails.

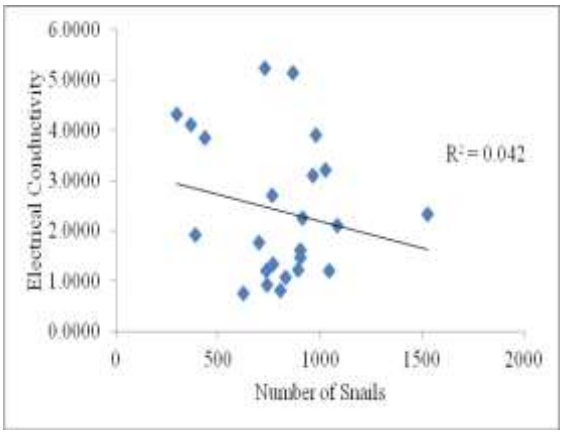

Fig. 7. Effect of Average Electrical Conductivity on the Number of Snails.

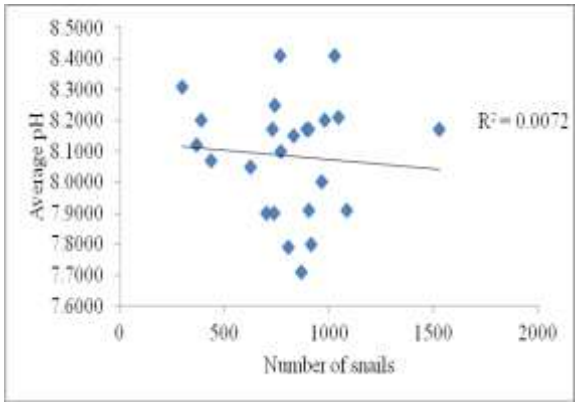

Fig. 8. Effect of Average pH on the Number of Snails.

DISCUSSION

The Physa fontinalis has been found absent in eleven out of twenty four villages and was found in very less numbers in the remaining villages. This species belongs to order Bassomatophora and is generally found in the aquatic habitat which as confirmed by Watson and Dallwitz (2005).

The main source of food of these snails is algae or fish food and they can survive in all conditions due to which they are called as the cockroaches of malacology (Sturm *et al*, 2005), however, they have been found absent in most of the villages linked to Ghogera branch and Jhang branch leading to a

conclusion that these areas must be monitored for their pesticidal contamination of soil and water. Chromium is found to be more toxic for crustaceans as compared to arsenic while on the other hand arsenic is more toxic for the Physa fontinalis and insect larvae (Canivet, *et al*., 2001). There are certain natural sources of arsenic in the environment but anthropogenic stress factors are playing an additive role through fuel combustion, mining activity, chemical input and addition of arsenic in the livestock feed especially poultry as reported by Smedley and Kinniburgh (2002). Snails are extremely sensitive to certain chemicals such as petroleum, certain metals and agricultural pesticides and fertilizers, in very small amounts; many species are excellent water quality indicators as reported by Johnson (2003). Recently, it has been reported by (Gilani *et al*., 2013) that the drinking water in the industrial area of Sheikhupura, Faisalabad, Pakistan is being contaminated by industrial discharge and was found with high arsenic levels when sampled at four sites. The most important heavy metals causing water pollution are Cu, Zn, Pb, Hg, Cd, Ni, and Cr. Some of these metals i.e. Cu, Cr, Ni, and Zn are essential trace metals to living organisms, while some cause toxicity at higher concentrations. Fresh water snails are also an important source of food for many fish, turtles and other species of wild life including pathogens, ground beetles, toads, snakes, turtles, and birds as previously stated by (Johnson, 2003). These heavy metals not only bioaccumulate in the snails but also disturb the food chain. These high concentrations of arsenic have clear cut effect on the natural fish fauna and these metals bioaccumulations have critical impact on the human health (Jankong, 2007). Investigations are still required as snails have an important position in the food chain of fish. The diversity index was found highly significant in sugarcane, wheat, fodder, and vegetables fields however the results were non-significant in case of ditches (Altaf *et al*., 2016).

Our results show that the soil pH of the different villages of Faisalabad is ranging from 7.71 to 8.41 showing that the soil pH of Faisalabad is much less than given National Environmental Quality Standards (Pakistan) 1997.

However, out of 24 villages *Cecilioides aciculae* were absent in 14 villages or previously have not been recorded. which may be a reason of the absence of this species or may be it has not been recorded in Faisalabad previously.

Cecilioides acicula were found in terrestrial regions which is in accordance with the findings of Watson and Dallwitz, 2005 showing the snail species *Cecilioides acicula* were found in the soil near the roots of the plants . It is a subterranean species associated with deep calcareous soils and limestone pavement, the latter a threatened habitat (Byrne *et al*., 2009). It is found to be an open country species and these are synanthropes which may have been imported via humans as stated by (Siarzewska, 2013).

These snails are found in the loose ground below the stones and near roots and can reach to 1m as recorded by (Anderson, 2008). These species inhabit underground, some distance below the surface as previously stated by (Kerney and Cameron, 1979). Snails generally have high calcium requirement for their shells and reproduction (Graveland *et al*., 1994). The abundance of the snails is positively and strongly correlated with calcium carbonate and in turn with pH. There is a strong correlation of their abundance with high pH and Calcium carbonate concentration as previously stated by (Waldén, 1995; Schilthuizen *et al*., 2003, Schiltuizen 2005a; Burch, 1955; Hotopp, 2002; Aravind, 2005).

The concentration of lead and cadmium in the soils of Faisalabad is much less which may be a reason of reduced pH as it is concluded that calcium carbonate adsorb the heavy metals ion from aqueous solution. The calcium carbonate may reduce the level of heavy metals more than 90%. Furthermore, the adsorption ability of Pb^{2+} ion and Cd^{2+} ions was significantly high as stated by (Aziz *et al*. 2008). Still we cannot give a definite reason for these values as studies are needed to be linked in the natural system. The soil lacks calcium ions and is very porous, which allows for quicker leaching of nutrients and harmful metals through the soil.

The accumulation of metals in soils and devastation to trees and shrubs decreases the health of an ecosystem. Liming lowers the acidity and increases the concentrations of calcium and magnesium in soil. The liming technique also makes toxic metals change to a solid form thereby reducing their bioavailabilty hence increasing the bioavailabilty of calcium.

Our study has revealed that there is no significant effect of the cadmium and lead concentration which is supported by Everard and Denny, (1984). The macro inverteberates, that are extremely disturbed due to the changes in the soil chemistry, are strong bioindicators of the envoirnment as stated by Paoletti (1999). These kinds of explorations of envoirnment use organisms like nematodes, earthworms, Collembola as well as molluscs (Otitoloju *et al*., 2009) and ants. However, the last two organisms are quite resistant to this type of contamination (Grzés, 2009) but still this type of contamination can lead to reduction in the diversity of species as changes in the composition of the community eliminates the most sensitive species (Del Val *et al*., 1999 and Beyrem *et al*., 2007), while on the other hand the tolerance of the opportunistic species (Syrek *et al*., 2006) or invasive species (Piola and Johnston, 2008) is promoted. Not only these tolerant species are found in extremely contaminated sites, there is however a positive correlation between the abundance of some groups of arthropods such as larvae of beetles of the subfamily Hoplinae and family Staphylinidae as reported by Nahmani and Lavelle, (2002) and with an increase in the heavy metal pollution, there is an increase in the abundance of Protura, Diplura and Collembola a reported by (Migliorini *et al*. 2004). The same is in the case of the diversity of ant species which increases with the increase in heavy metal pollution as stated by Grzés, (2009).

The increase in the either abundance or richness and uniformity have not been commonly studied however, some studies reported these types of changes occur with the increase of pollution by heavy metals as stated by Russell and Alberti, (1998).

There is a record of physicochemical environment in the isotopic and elemental form in the shells and the tissues of the gastropods as stated by (Mills, *et al*., 2007). The snails are good biomonitors for heavy metals. There is a strong relation between sediments and soft tissues as significant correlations were found between sediments and the soft tissues for Cu ($P<0.05$) and Pb ($P<0.01$).

The operculum and the shells have significant correlation, for Cd and Pb, with the sediments as recorded by Cheng, (2008). This increase in the concentration of heavy metals is in consequence of the population growth and technological advancement contaminating the environment and due to this the situation becomes more adverse as they cannot be mineralized to their total innocuous forms (Bonaventura and Johnson, 1997). Due to this non-biodegradeability, these heavy metals tend to accumulate in the plants and animal tissues a stated by (Otitoloju *et al*., 2009). The pollution has an indirect effect on the diversity and abundance at the community level as well as at the individual level of the land snails by reducing the size of the shell belonging to the same species. Not only this shells pose reproductive problems on the birds that consume these shell as a calcium supplement during their breeding season (Eeva *et al*., 2010) leading to biomagnifications.

The juvenile *Zooctecus insularis* is absent from 13 villages out of 24 villages this may be due to a reason that this species has not been previously recorded from Faisalabad but according to the findings of Gude, (1914) they are mainly reported in the arid and barren regions of Pakistan while these snails species have also been found in Thar Desert of Pakistan (Auffenberg, 1997). The rivers have played a very important role in the dispersal of snails throughout Punjab, through their tributaries (Arshad *et al*., 2011).

Conclusions

Three snail species i.e. Physa fontinalis, is a strong candidate of being a bioindicator species and future studies may lead us to the exploration of some bioactive agents produced in their bodies against pollutants.

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Conflict of interest

The authors hereby confirm that there are no known conflicts of interest associated with this publication.

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