Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 27(1): 179 – 201 (2023)
www.ejabf.journals.ekb.eg

Association Pattern Between Freshwater Snails and Fungi in Relation to Water Quality Parameters in Two Egyptian Governorates

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ARTICLE INFO

Article History:
Received: Nov. 26, 2022
Accepted: Jan., 2022
Online: Feb 7, 2023

Keywords:
Aspergillus,
Biodiversity,
Biomphalaria alexandrina,
pH,
Physa acuta ,
Temperature

ABSTRACT

Water quality is one of the most important issues, especially due to the water scarcity that the world is currently facing. Surface water in Egypt is receiving agricultural, industrial, and municipal effluents. The aim of the present study was to investigate the quality of some water canals in Giza and Gharbeya governorates using abiotic and biotic factors. Physicochemical parameters, snail distribution, and frequency of fungal genera were determined from March 2020 to February 2021. The highest water temperatures were recorded during summer and spring in Giza and Gharbeya governorates, respectively, and of electrical conductivity was observed during winter and summer, respectively. *Physa acuta* snails were the most abundant in all seasons. Also, *Aspergillus* sp. was the most frequent amongst all identified fungi during all seasons, except winter in Gharbeya Governorate, where *Trichoderma* sp. was the most frequent. According to the Shannon-Wiener index, autumn showed the highest diversity of snails and fungi in Giza Governorate. For Gharbeya Governorate, snails' diversity showed the highest equal value in summer and autumn, while that of fungi was in spring. In Giza Governorate, Dissolved oxygen showed a strong positive association with *Physa acuta* and *Aspergillus* sp. In Gharbeya Governorate, temperature, electrical conductivity, and total dissolved solids exhibited a strong positive correlation with *Aspergillus* sp., while they showed negative correlations with *Lymnaea natalensis* and *Bulinus truncatus* snails. The relationships between fungal genera and snail species varied according to the governorate. In conclusion, low water quality and global warming might result in a decrease of schistosomiasis and fascioliasis dissemination but also can lead to the incidence of more fungal diseases.

INTRODUCTION

Clean, safe and adequate freshwater is of utmost importance to human existence and the survival of all living components in the ecosystem. Water quality issues are complex and diverse, deserving urgent global attention and action (Breabăn *et al*., 2012). Water quality is generally defined as "the chemical, physical and biological characteristics of water usually in respect to its suitability for a designated use" (Johnson *et al*., 1997). The past decade has seen a remarkable impact of man on the environment due to the unprecedented increase in population and rapid rate of urbanization, as well as the intensification and expansion of agricultural practices. This has led to progressive and continual degradation of resources especially surface water. The impaired surface water quality always results in an unhealthy socio-economic environment (Bullard, 1972), as polluted water represents an important vehicle for the spread of diseases.

Aquatic snails are key organisms in freshwater ecosystems; they play major roles in the public and veterinary health (Supian and Ikhwanuddin, 2002), and some of them transmit serious diseases to human beings and their livestock such as schistosomiasis, fasciolosis, amphistomiasis, etc. They are used as indicator organisms for biological monitoring and in risk assessment strategies (Borcherding and Volpers, 1994). The composition and spatial relationships of aquatic communities are related to the habitat structure and variation of environmental factors (Pérez-Quintero, 2011). One of the goals of freshwater ecology is to understand how communities of freshwater species are structured and how environmental factors affect their distribution. According to Pemola *et al*. (2015), snail communities are influenced by the prevailing physicochemical parameters, which determine their abundance, occurrence and seasonal variations. These parameters include dissolved oxygen, pH, water temperature, physical nature of the substratum, depth, current velocity and nutritive content of the water body (Duft *et al*., 2007; Rai and Jauhari, 2016). In addition, as aquatic ecosystems are subjected to anthropogenic disturbance due to sewage drainage, agricultural and industrial effluents, this disturbance being a menace for the integrity of the aquatic ecosystem has to be considered. Therefore, it is very important to examine environmental factors influencing the distribution of snails in the freshwater ecosystem (Igbinosa *et al*. 2015). Hence, effective control of vector snails can be achieved as long as the characteristics of their habitats are well understood (Owojori *et al*. 2006).

Aquatic fungi are widespread biotic components of aquatic ecosystems (Voronin, 2008). The biomass and abundance of these fungi depend on hydrochemical conditions, and are significantly correlated with parameters such as pH, electrical conductivity, and certain nutrients (Sole *et al*., 2008; Pietryczuk *et al*., 2013a, 2013b, 2013c). Processes involving aquatic fungi are very important to the functioning of aquatic ecosystems but have received little attention in the literature. Fungi fulfill very substantial functions in such environments, as they are important elements of the microbial loop. Together with bacteria, they initiate the decomposition of organic matter, especially that of plant origin, preparing the substratum to be inhabited by other organisms, particularly invertebrates such as snails (Edet *et al*., 2011). Moreover, fungi inhabiting water ecosystems actively participate in the production of autochthonic humic substances (Raj and Shaji, 2017). These microorganisms also actively participate in the circulation of nutrients such as nitrogen and phosphorus (Ameen *et al*., 2018). Fungi can be suspended in the water depths, settled on the bottom or periphyton, be directly supplied to waters with surface runoff, or be of anthropogenic origin. Certain fungal species supplied to the water depths cause diseases in fish and other aquatic animals, as well as in humans (Abtahi *et al*., 2015). Some fungal species also produce secondary metabolites such as mycotoxins that can cause much harm to humans, and can lead to deterioration in the organoleptic properties of water, leaving it unfit for use (Oliveira *et al*., 2016; Russell *et al*., 2005). Moreover, fungal infections are becoming of increasing concern due to the increasing numbers of immunocompromised patients and those with other risk factors. On the other hand, fungi have been evidenced to actively participate in the biotransformation of xenobiotics (Krauss *et al*., 2005) and heavy metals (Sridhar *et al*., 2001) that reach the aquatic environment, potentially contributing to the alleviation of the effects of anthropogenic stress, and improving water quality. Due to this, a lot of authors have proposed to include these organisms in the group of bioindicators of anthropogenic alterations in the monitoring of the ecological state of aquatic ecosystems (Biedunkiewicz, 2011; Pascoal *et al*., 2005; Solé *et al*., 2008), and the sanitary state of waters (Biedunkiewicz *et al*. 2007; Cudowski *et al*. 2015). Research regarding the abundance (Jorgensen and Stepanauskas, 2009; Pietryczuk *et al*., 2013a) and taxonomic identification of aquatic fungi in various types of waters (Biedunkiewicz *et al*., 2013; Jobard *et al*., 2010; Pietryczuk *et al*., 2014), has frequently been increased in the recent years.

The aim of the present study is to evaluate the quality of watercourses representing Giza and Gharbeya governorates in Egypt via measuring physicochemical parameters, determining seasonal distribution of snails and fungi, and finding the relations between these abiotic and biotic factors in an attempt to figure out the consequences of these correlations.

MATERIALS AND METHODS

Study area

The study was carried out seasonally from March 2020 to February 2021 in water courses representing two Egyptian governorates; Giza and Gharbeya. Each governorate was represented by four waterbodies. Giza Governorate was represented by El-Zumareya Canal, Tabeq Canal, El-Mansoureya Canal, and El-Prinsessa Canal, while Gharbeya Governorate was represented by Ezbet El-Sabeel Canal, Ekhnaway Canal, Balkeem Canal, and Kafr Abou Dawood Canal.

Determination of physicochemical parameters

A portable pH meter [Hanna Instruments (HI) 9024] was used to measure water temperature and hydrogen ion concentration (pH). A portable conductivity meter (HI 9635) was used to determine electrical conductivity (EC) and total dissolved solids (TDS) values. Dissolved oxygen was measured using a portable DO meter [Hanna Instruments (HI) 98193]. All the parameters were measured in situ at midday 20 cm below the water surface (Musa *et al*., 2018; Jannat *et al*., 2019; Liu *et al*., 2019).

Collection of snails

Snail samples were seasonally collected (three times/season) during the study period from the investigated waterbodies (three spots for each sample) using a sieve (300 mm standard dip-net) (Barbour *et al*., 1999). Snails were transported to laboratory, sorted out, identified according to Ibrahim *et al*. (1999) and counted; they were also examined for trematode natural infection (Boothroyd and Stark, 2000).

Isolation and identification of fungi

For isolation of fungi, water samples were collected in sterilized plastic containers, 20 cm below the water surface (each waterbody was represented by three samples) (Kaufmann *et al*., 1988). The container was completely filled with water, and then the cap was replaced immediately. They were labeled, kept in an ice box, and transported to the laboratory for analysis within 12 h. One ml of each water sample was spread, in triplicate, onto Petri dishes containing Czapek agar, malt extract agar, and Sabouraud dextrose agar to which 500 mg/l of chloramphenicol was added, and then the cultured plates were incubated at 28°C for 7 days (Anon, 1996). Isolated fungi were identified by mycologists in Regional Centre for Mycology and Biotechnology, Al-Azhar University, Egypt. Firstly, the morphology of the fungal colonies was studied by naked eyes (Ajello *et al*., 1963), then identification of fungal species was carried out according to the morphology and diameters of their structures under microscope using an image analysis system, soft imaging system GmbH software (analySIS® pro ver. 3.0) (Raper and Fennell, 1965; De Hoog *et al*., 2000). The identified species were maintained by continuous subculturing on Sabouraud dextrose agar at constant intervals and the slants were kept in refrigerator.

Statistical analysis

Diversity Index (H') was used according to Shannon-Wiener (1949) formula:

$H' = -\sum [ni/N] \ln [ni/N]$…………………………….. ………………………….(1)

Where ni is the number of individuals in each species, N equals the total number of individuals in the sample, ln is the natural logarithm and ∑ is the total number of species in the sample. Results that are >4 indicate high status, 3- 4 indicate good status, while 1-2 mean poor status.

Evenness Index (J) was used according to Pielou (1966) formula:

$J = H'/ In\ S\ H'$…………………………………………………………………… (2)

Where H' is Shannon-Wiener diversity index, ln means natural logarithm, and S is the total number of species in the sample. The values are between 0-1. When the value is getting closer to 1, it means that the individuals are equally distributed.

Data of the physicochemical parameters were statistically analyzed for the significance of differences between different seasons by one-way ANOVA test at $P < 0.05$, using the statistical program SPSS version 23 (SPSS, Inc., Chicago, IL) for windows. The Principal component analysis (PCA) was determined according to Kaiser's rule (Hayton *et al*., 2004) to extract a small number of factors for analyzing the relationships among the observed variables.

RESULTS

Physicochemical parameters

In Giza Governorate, although water temperature values in autumn and winter were not significantly different, that of summer showed significant difference ($p < 0.05$). The highest mean temperature was 28.3˚C in summer, while the lowest was 21.5˚C in autumn. Meanwhile, pH values were almost neutral during all seasons. The highest values of electrical conductivity (EC) and total dissolved solids (TDS) were 1326 µ mhos/cm and 1719.3 mg/l in winter, respectively. Also, the significantly highest dissolved oxygen (DO) value was 7.8 mg/l in winter, while the lowest was 0.8 mg/l in autumn (**Figure 1)**.

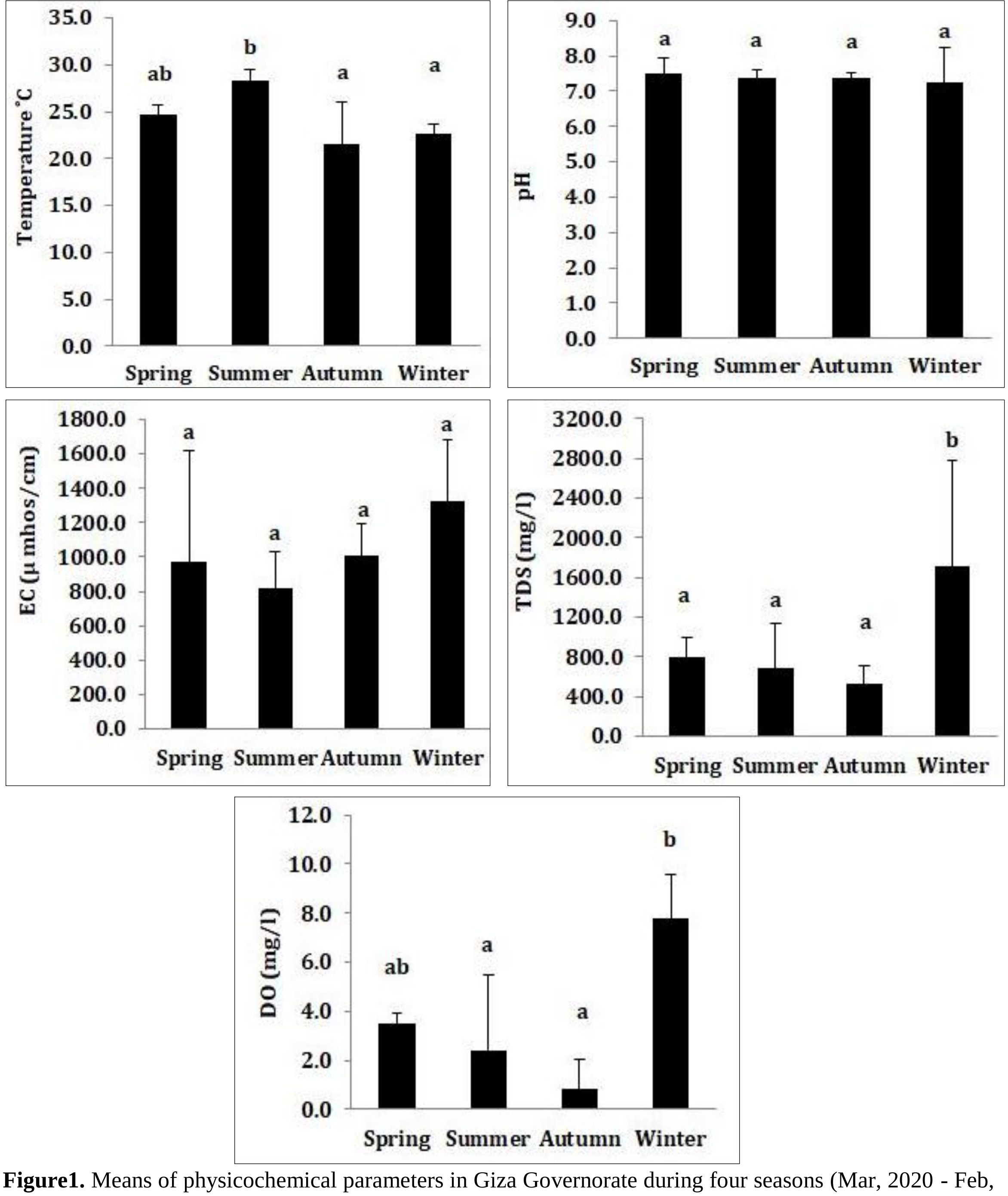

Figure1. Means of physicochemical parameters in Giza Governorate during four seasons (Mar, 2020 - Feb, 2021). EC: Electrical conductivity, TDS: Total dissolved solids, DO: Dissolved oxygen. Different letters indicate significant difference amongst values in each season at $P < 0.05$, while the same letters indicate insignificant difference at $P > 0.05$

In Gharbeya Governorate, the maximum temperature was observed in spring (32.2˚C) followed by summer (27.3˚C), whereas the minimum value was recorded in winter (17.9˚C). pH values showed no significant differences among different seasons.

The maximum EC and TDS values were observed in summer and spring, respectively, and winter witnessed the lowest values of both parameters. The maximum DO value (6.4 mg/l) was recorded in autumn, while the lowest (2.3 mg/l) was noticed in summer (**Figure 2**).

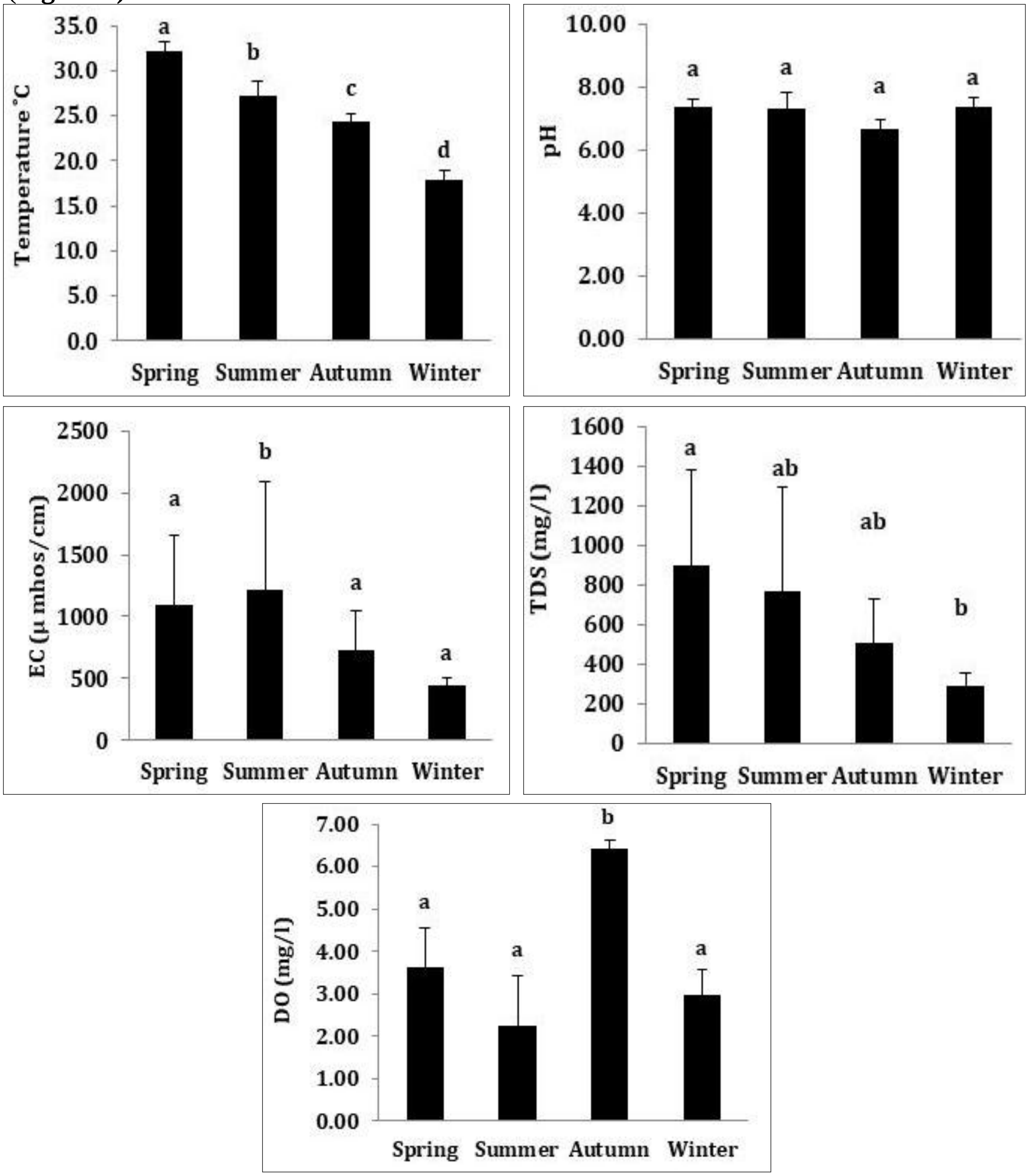

Figure 2. Means of physicochemical parameters in Gharbeya Governorate during four seasons (Mar, 2020 - Feb, 2021). EC: Electrical conductivity, TDS: Total dissolved solids, DO: Dissolved oxygen. Different letters indicate significant difference amongst values in each season at $P < 0.05$, while the same letters indicate insignificant difference at $P > 0.05$

Distribution of snails

Figure (3) shows the distribution of collected snails species from Giza Governorate during different seasons. *Physa acuta* was the most abundant snail, showing its highest percentage in spring (83%), followed by summer (69%). *Helisoma duryi* was also recorded in all seasons where its percentage was the highest in autumn (21%). Although *Planorbis planorbis* was not observed in summer, it was highly abundant in winter (32%). Other snail species showed high frequency rates in certain seasons; such as *Cleopatra bulimoides* (20%) and *Succinea cleopatra* (12%) in autumn, and *Gabbiella sinariensis* (17%) in summer.

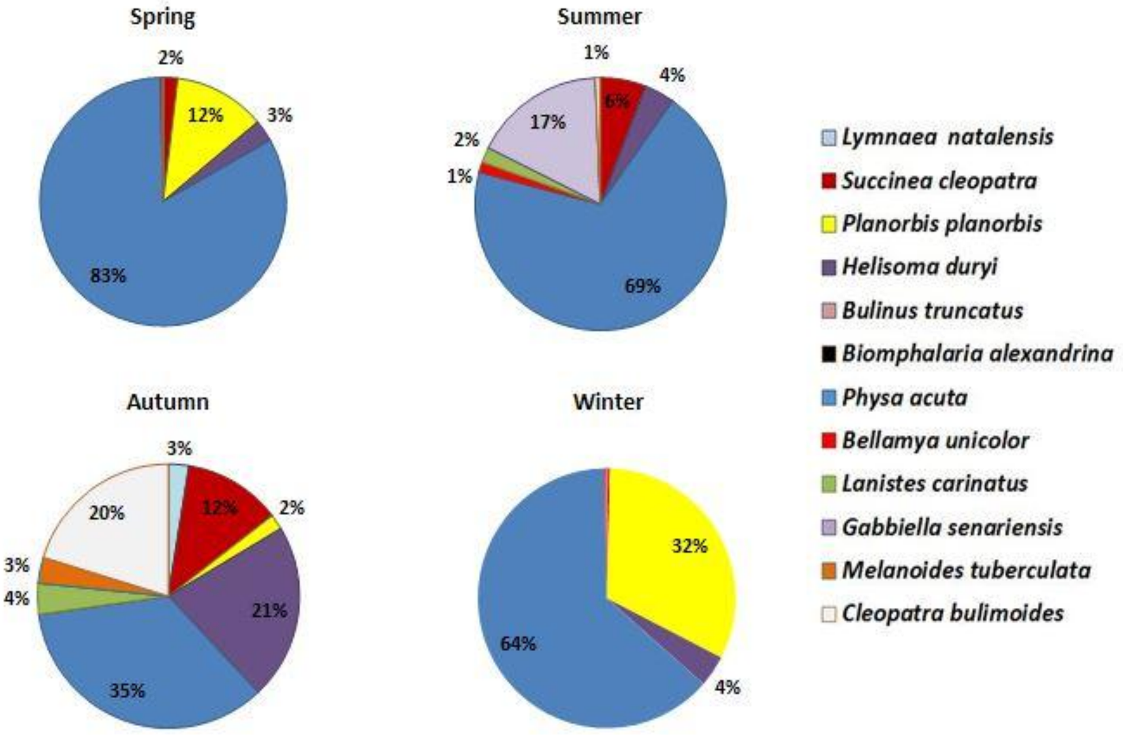

Figure 3. Seasonal distribution of snails species collected from Giza Governorate (Mar, 2020 - Feb, 2021)

In Gharbeya Governorate, *Physa acuta* showed the highest frequency amongst all collected snails, as its percentage reached 90% in winter, followed by 64% in spring. *Biomphalaria alexandrina* was recorded in all seasons, where its highest abundance was noticed in summer (33%), followed by autumn (28%). *Planorbis planorbis* was noticeably abundant in spring with 24% frequency. In addition, *Lymnaea natalensis* was found in all seasons, with the highest abundance percentage (11%) in autumn **(Figure 4)**.

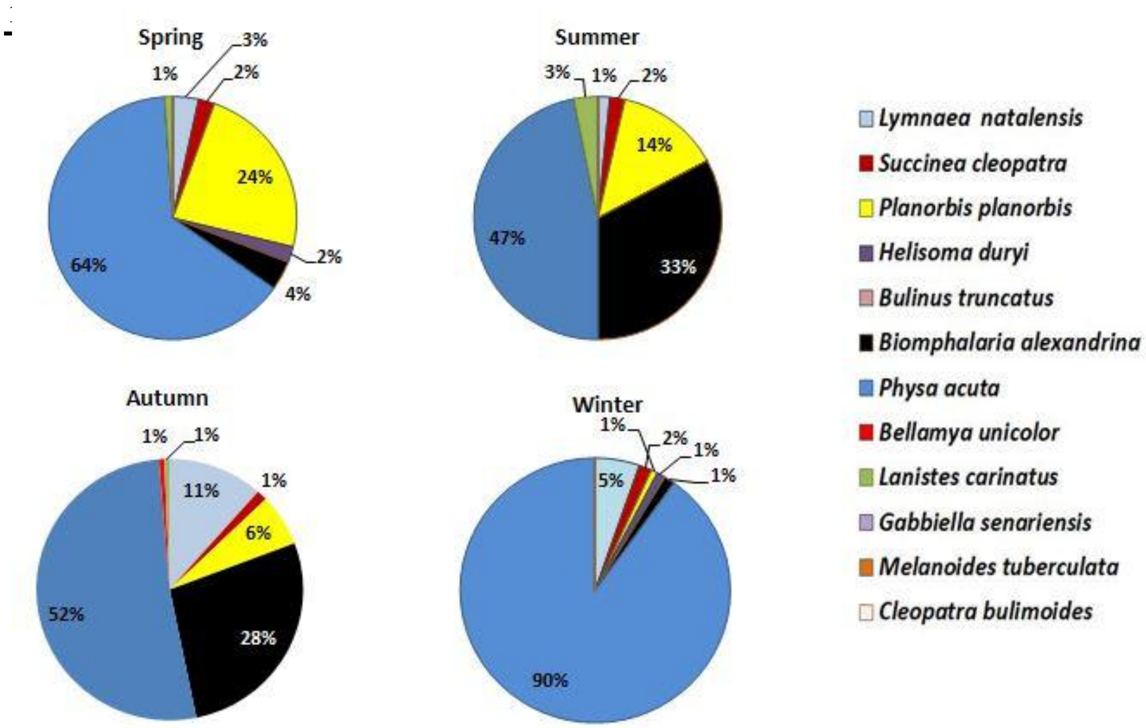

Figure 4. Seasonal distribution of snails species collected from Gharbeya Governorate (Mar, 2020 - Feb, 2021)

In Giza Governorate, the Shannon-Weiner diversity index values were 1.68 and 1.02 during autumn and summer, respectively **(Figure 5a)**, indicating higher diversity of snail species than other seasons. Also, Evenness index showed the same pattern. On the other hand, the Shannon-Weiner diversity index values were >1 during spring, summer, and autumn in Gharbeya Governorate. Also, Evenness index values were compatible with Shannon-Weiner diversity index results (**Figure 5b)**.

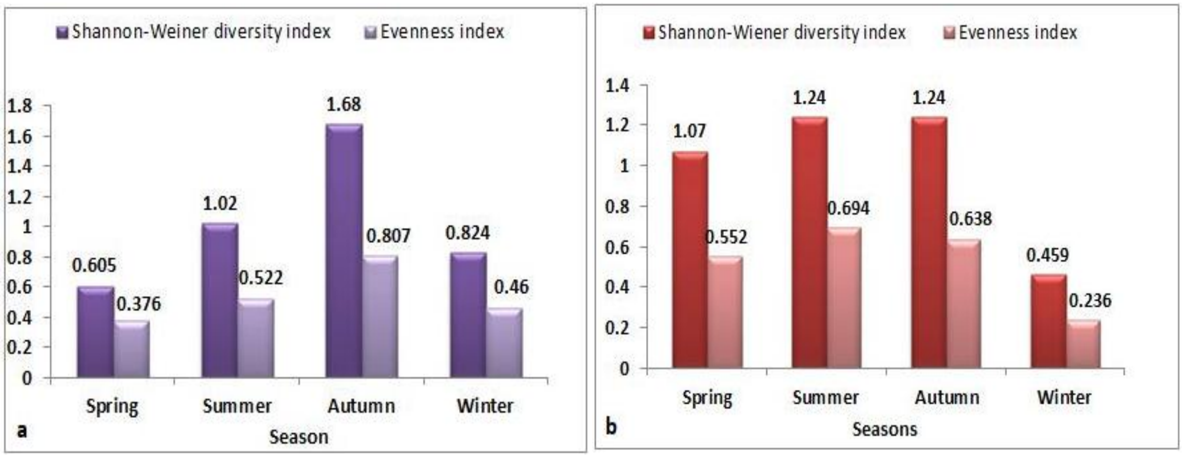

Figure 5. The values of Shannon-Weiner diversity index and Evenness index for collected snail species during different seasons (Mar, 2020 – Feb, 2021): (a): Giza Governorate and (b): Gharbeya Governorate

Frequency of fungi

In Giza Governorate, the most frequent fungal species was *Aspergillus*, as its percentage reached 78 % in spring, followed by 66% in winter. *Rhizopus* sp. was the second to be recorded in pronounced percentages in all seasons, with the highest frequency (18 %) in winter. *Trichoderma* sp., *Fusarium* sp. and *Rhizopus* sp. showed noticeable frequency rates in summer (13% and 10% and 10 %, respectively), *Penicillium* sp. recorded 12 % frequency in autumn, while *Microascus* sp. and *Drechslera* sp. showed the same frequency in autumn (9 %) (**Figure 6)**.

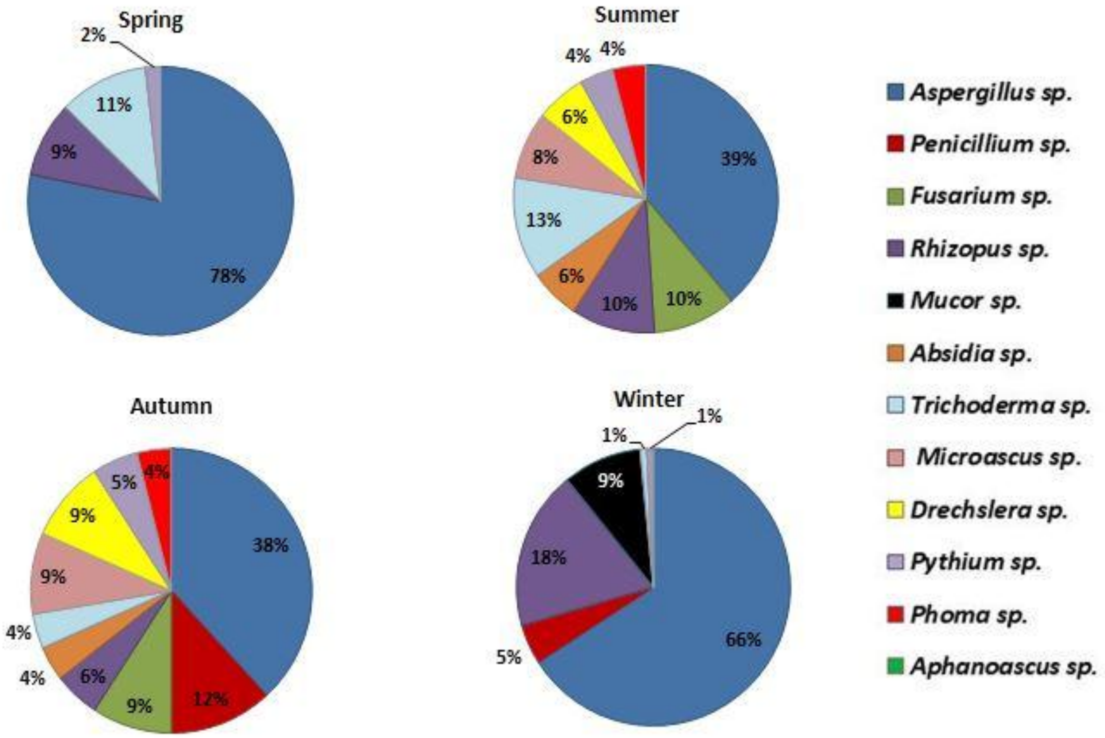

Figure 6. Seasonal frequency of fungi in Giza Governorate (Mar, 2020 - Feb, 2021)

In Gharbeya Governorate, *Aspergillus* sp. showed the highest frequency amongst all isolated fungi, with a maximum abundance in autumn (52 %). In the same season, *Trichoderma* sp. and *Drechslera* sp. showed their highest frequencies (20 and 21%). The frequency rate of *Rhizopus* sp. was the highest (20%) in winter. *Fusarium* sp. was recorded with slightly high frequency (17 %) in winter, while *Mucor* sp. showed a pronounced frequency rate (14 %) in summer (**Figure 7**).

Shannon-Weiner diversity index showed that the highest season in fungal biodiversity in Giza Governorate was autumn (1.97), followed by summer (1.85), and is consistent with the results of the Evenness index during the same seasons **(Figure 8a)**. In Gharbeya Governorate, the diversity index values were >1 in all seasons, with spring showing the highest value, while Evenness index was of the highest value in winter **(Figure 8b).**

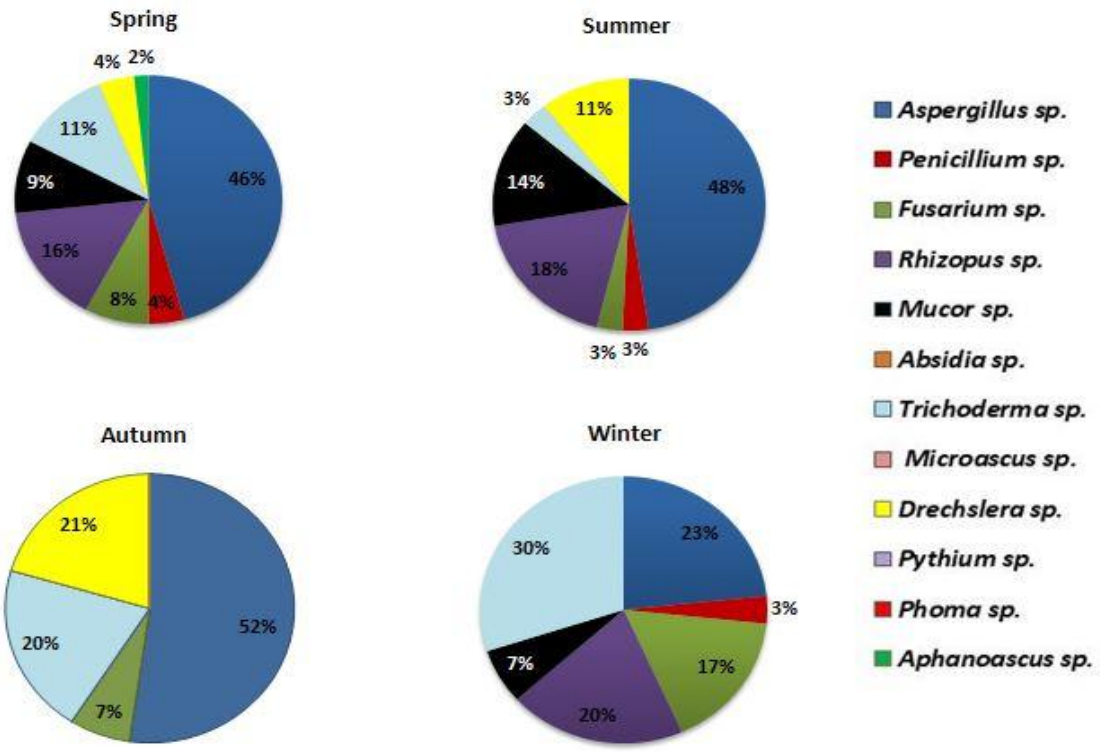

Figure 7. Seasonal frequency of fungi in Gharbeya Governorate (Mar, 2020 - Feb, 2021)

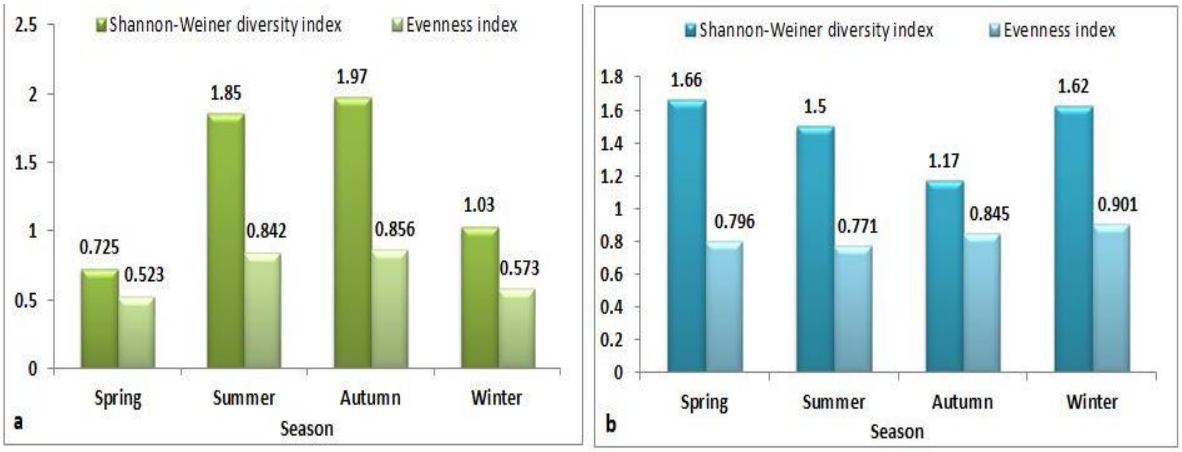

Figure 8. The values of Shannon-Weiner diversity index and Evenness index for fungi during different seasons (Mar, 2020 - Feb, 2021): (a): Giza Governorate and (b): Gharbeya Governorate

Correlation among snails, fungi and physicochemical parameters

The principal components (PC) were extracted from the variables in Giza by Kaiser's rule. The first three principal components have Eigen values > 1. The first two components represented 89.9 % of the variation in the data **(Table 1)**. The 1st principal component (PC1) contained DO (-0.963), which showed strong positive associations with *Planorbis planorbis* (-0.893) and *Physa acuta* (-0.990) snails, and *Aspergillus* sp. (-0.848), *Mucor* sp. (-0.741) and *Rhizopus* sp. (-0.792). Therefore, this component mainly measured the favorable range of DO that may affect the distribution and abundance of snail species with the mentioned fungal genera **(Figure 9)**.

In contrast, the 2nd PC contained temperature (-0.943), which showed a strong negative association with *Lymnaea natalensis* (0.971), *Helisoma duryi* (0.976) snails, and *Penicillium* sp. (0.983), while it showed a strong positive correlation with *Trichoderma* sp. (-0.820). Therefore, this component mainly measured the impact of temperature on the distribution of snail species with the mentioned fungal genera.

Regarding the association between fungi and snails, the analysis showed an excellent positive correlation between *Succinea cleopatra*, *Lanistes carinatus*, *Cleopatra bulimoides* and *Melanoides tuberculata* snails, and *Absidia* sp., *Fusarium* sp., *Microascus* sp., *Drechslera* sp., *Pythium* sp., and *Phoma* sp. **(Figure 9)**.

Table1. The number of principal components extracted from the variables in Giza Governorate

Variable	PC1	PC2	PC3
T	-0.135	**-0.943**	0.304
pH	-0.349	-0.418	-0.839
EC	-0.679	0.732	0.055
TDS	0.480	**0.834**	-0.273
DO	**-0.963**	-0.042	0.266
Ln	0.223	**0.971**	0.090
Sc	**0.971**	0.103	-0.217
Pp	**-0.893**	0.431	-0.125
Hd	-0.185	**0.976**	0.116
Pa	**-0.990**	0.064	0.124
Bu	**-0.723**	-0.335	0.604
Lc	**0.971**	0.138	0.197
Gs	0.357	-0.713	0.604
Mt	0.732	0.643	-0.227
Cb	**0.842**	0.527	-0.113
Asp	**-0.848**	0.526	-0.065
Pen	0.095	**0.983**	0.154
Fus	**0.962**	0.023	0.274
Rhiz	-0.792	0.589	0.157
Muc	-0.741	0.488	0.462
Abs	**0.943**	-0.061	0.326
Trich	0.429	**-0.820**	-0.378
Micr	**0.969**	0.085	0.233
Drech	**0.970**	0.168	0.176
Pyth	**0.940**	0.339	0.047
Pho	**0.967**	0.069	0.243
Eigen value	**15.003**	**8.383**	**2.613**
Variance %	57.7 %	32.2 %	10.1 %
Cumulative	57.7 %	89.9 %	100.0 %

T: Temperature, EC: Electrical conductivity, TDS: Total dissolved solids, DO: Dissolved oxygen, Sc: *Succinea cleopatra*, Pp: *Planorbis planorbis*, Pa: *Physa acuta*, Hd: *Helisoma duryi*, Gs: *Gabbiella senariensis,* Mt: *Melanoides tuberculata,* Ln: *Lymnaea natalensis*, Bu: *Bellamya unicolor*, Lc: *Lanistes carinatus,* Cb: *Cleopatra bulimoides,* Asp: *Aspergillus* sp., Pen: *Penicillium* sp., Fus: *Fusarium* sp., Rhiz: *Rhizopus* sp., Muc: *Mucor* sp., Abs: *Absidia* sp., Trich: *Trichoderma* sp., Micr: *Microascus* sp., Drech: *Drechslera* sp., Pyth: *Pythium* sp. and Pho: *Phoma* sp.

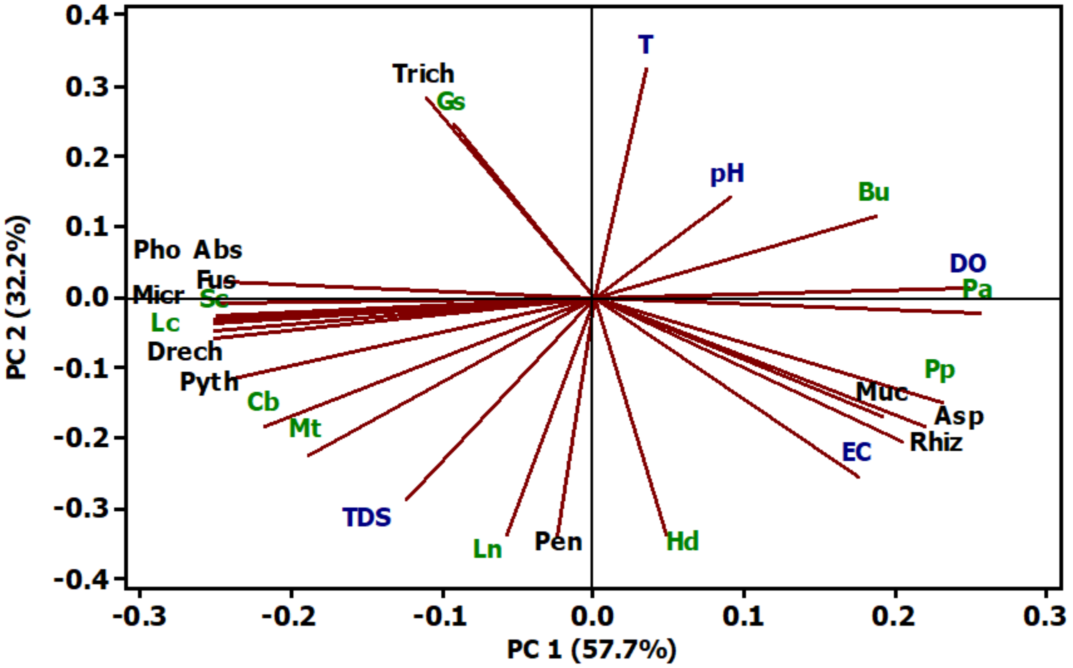

Figure 9. Principal component (PC) analysis showing the association patterns among the original variables in Giza Governorate (Mar, 2020 - Feb, 2021). T: Temperature, EC: Electrical conductivity, TDS: Total dissolved solids, DO: Dissolved oxygen, Sc: *Succinea cleopatra*, Pp: *Planorbis planorbis*, Pa: *Physa acuta*, Hd: *Helisoma duryi*, Gs: *Gabbiella senariensis*, Mt: *Melanoides tuberculata*, Ln: *Lymnaea natalensis*, Bu: *Bellamya unicolor*, Lc: *Lanistes carinatus*, Cb: *Cleopatra bulimoides*, Asp: *Aspergillus* sp., Pen: *Penicillium* sp., Fus: *Fusarium* sp., Rhiz: *Rhizopus* sp., Muc: *Mucor* sp., Abs: *Absidia* sp., Trich: *Trichoderma* sp., Micr: *Microascus* sp., Drech: *Drechslera* sp., Pyth: *Pythium* sp. and Pho: *Phoma* sp.

The principal components (PC) extracted from the variables in Gharbeya Governorate showed that the first three principal components have Eigen values > 1. The first two components represented 81.6 % of the variation (**Table 2**). The 1st principal component (PC1) contained temperature (0.923), EC (0.995), and TDS (0.919), which showed strong positive correlations with *Planorbis planorbis* (0.856), *Lanistes carinatus* (0.985) snails and *Aspergillus* sp. (0.908), while it showed negative correlations with *Lymnaea natalensis* (-0.936) and *Bulinus truncatus* (-0.864) snails. So, this component mainly determined some physicochemical parameters which might affect the distribution of different snail species **(Figure 10)**.

On the other hand, the 2nd principal component (PC2) contained DO (- 0.680), which showed negative correlations with *Rhizopus* sp. (0.851), *Penicillium* sp. (0.837), and *Mucor* sp. (0.717), and *Succinea cleopatra* (0.903) and *Helisoma duryi* (0.901) snails. Therefore, this component mainly measured the impact of DO levels on the distribution of some fungal genera and snail species.

Regarding the association between snails and fungi, the analysis showed a good positive correlation between *Aspergillus* sp., *Lanistes carinatus* and *Planorbis planorbis* snails, while exhibited a negative association with *Lymnaea natalensis* and *Bulinus truncatus* snails **(Table 2)**.

Table 2. The number of principal components extracted from the variables in Gharbeya Governorate.

Variable	PC1	PC2	PC3
T	**0.923**	0.073	0.378
pH	-0.629	0.175	0.757
EC	**0.995**	0.099	-0.017
TDS	**0.919**	0.263	0.295
DO	-0.217	**-0.680**	0.701
Ln	**-0.936**	0.179	0.303
Sc	-0.307	**0.903**	-0.3
Pp	**0.856**	0.457	0.243
Hd	-0.336	**0.901**	0.273
Bt	**-0.864**	0.354	-0.359
Ba	0.642	-0.687	-0.341
Pa	-0.619	0.758	-0.204
Bu	-0.169	**-0.852**	0.496
Lc	**0.985**	0.058	-0.162
Asp	**0.908**	0.061	0.415
Pen	0.548	**0.837**	0.02
Fus	-0.153	**0.781**	0.606
Rhiz	0.423	**0.851**	-0.309
Muc	0.647	**0.717**	-0.258
Trich	-0.454	0.326	0.829
Drech	0.781	-0.532	0.327
Aph	0.404	0.672	0.621
Eigen value	**10.21**	**7.74**	**4.05**
Variance %	46.4 %	35.2 %	18.4 %
Cumulative	46.4 %	81.6 %	100.0 %

T: Temperature, EC: Electrical conductivity, TDS: Total dissolved solids, DO: Dissolved oxygen, Sc: *Succinea cleopatra*, Pp: *Planorbis planorbis*, Pa: *Physa acuta*, Hd: *Helisoma duryi*, Ba: *Biomphalaria alexandrina*, Ln: *Lymnaea natalensis,* Bu: *Bellamya unicolor,* Lc: *Lanistes carinatus,* Bt: *Bulinus truncatus*, Asp: *Aspergillus* sp., Pen: *Penicillium* sp., Fus: *Fusarium* sp., Rhiz: *Rhizopus* sp., Muc: *Mucor* sp., Trich: *Trichoderma* sp., Drech: *Drechslera* sp. and Aph: *Aphanoascus* sp.

DISCUSSION

In the present study, the highest mean temperature in Giza Governorate was recorded in summer. Similarly, Saad *et al*. (2014) found that the highest mean temperature (27.3 °C) in Giza Governorate watercourses was observed in summer. Also, Okasha *et al*. (2021) reported on the highest temperature (25.45-27.59°C) in three sites representing Giza Governorate during summer. Meanwhile, pH values in the current work were almost neutral during all seasons. In the same vein, El-Deeb *et al*. (2017) observed mean pH values of 7.22 and 7.33 in two canals representing Giza Governorate, and Okasha *et al*. (2021) observed more or less similar neutral pH values in two sites of the same governorate. The highest recorded values of EC and TDS in this study were in winter. This matches the findings of Saad *et al*. (2014), as the highest TDS and EC values were recorded in Giza canals in autumn followed by winter. In addition, Okasha *et al*. (2021) observed the highest EC value in winter in one of the investigated Giza canals. Moreover, the significantly highest DO value in the present study was observed in winter, while the lowest was detected in autumn, in contrast, Saad *et al*. (2014)

demonstrated that autumn witnessed the highest DO value, while winter showed the lowest value.

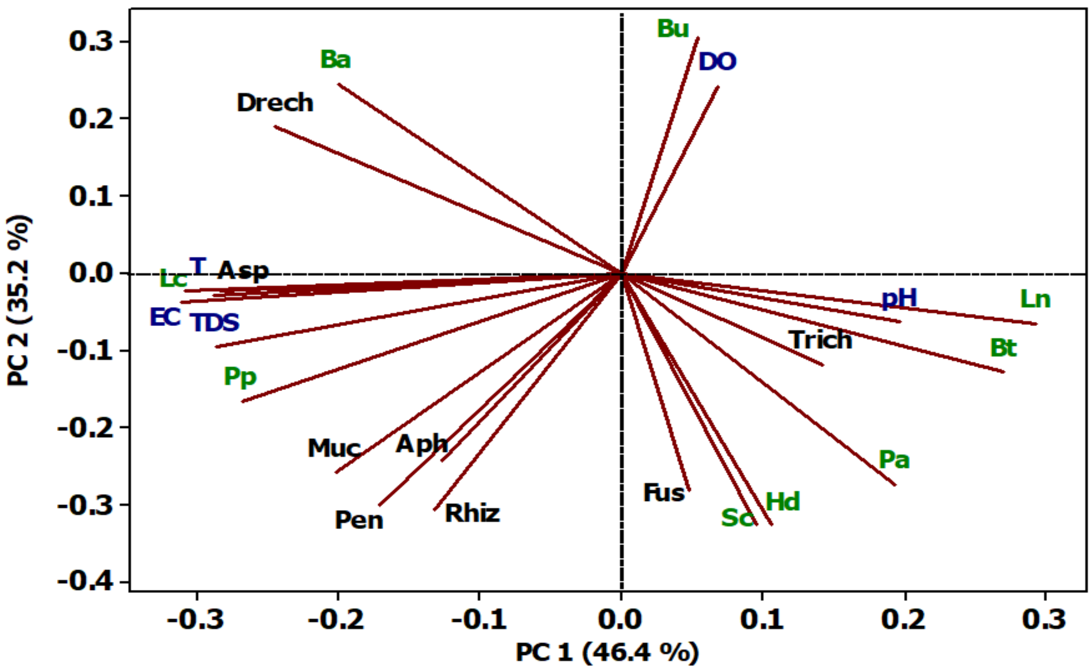

Figure 10. Principal component (PC) analysis showing the association patterns among the original variables in Gharbeya Governorate (Mar, 2020 - Feb, 2021). T: Temperature, EC: Electrical conductivity, TDS: Total dissolved solids, DO: Dissolved oxygen, Sc: *Succinea cleopatra*, Pp: *Planorbis planorbis*, Pa: *Physa acuta*, Hd: *Helisoma duryi*, Ba: *Biomphalaria alexandrina*, Ln: *Lymnaea natalensis*, Bu: *Bellamya unicolor*, Lc: *Lanistes carinatus*, Bt: *Bulinus truncatus*, Asp: *Aspergillus* sp., Pen: *Penicillium* sp., Fus: *Fusarium* sp., Rhiz: *Rhizopus* sp., Muc: *Mucor* sp., Trich: *Trichoderma* sp., Drech: *Drechslera* sp. and Aph: *Aphanoascus* sp.

In Gharbeya Governorate, the maximum temperature was detected in spring followed by summer. On contrary, Saad *et al*. (2014) and Abdel-Raheem *et al*. (2018) recorded the highest temperature in summer followed by spring in the same governorate. pH values in the current work showed no significant differences among the four seasons, this coincides with the results of Saad *et al*. (2014) and Abdel-Raheem *et al*. (2018), where they recorded similar pH values that ranged from 6.2 to 7.6. The maximum EC and TDS values in this study were observed in summer and spring, respectively, and winter witnessed the lowest values of both parameters. On the reverse, the highest TDS value recorded by Saad *et al*. (2014) was in winter, while autumn witnessed the highest EC value. Whereas, Abdel-Raheem *et al*. (2018) reported on the highest EC and TDS values in Gharbeya Governorate during autumn. It was declared that the high values of EC might indicate that these watercourses receive excessive discharge from land runoff or industrial effluents (Ezzat *et al*. 2012; Shammi *et al*. 2016). The maximum DO value in Gharbeya Governorate in the present study was recorded in autumn, while the lowest was noticed in summer, on the contrary, Saad *et al*. (2014) observed the highest DO value in Gharbeya Governorate in summer.

The differences of our results, especially in Gharbeya Governorate, from those of other researchers in 2014 and 2018 might be attributed to the increased urbanization and anthropogenic activities in the investigated sites, as the anthropogenic discharges (municipal and industrial waste water effluents) are considered a constant polluting and deteriorating source of the watercourses (Najafpour *et al*., 2008). Also, climate change participates in altering not only the values of the detected physicochemical parameters, but also the seasons that witnessed the maximum and minimum values, as it was reported that surface runoff is a seasonal phenomenon largely affected by climatic conditions (Najafpour *et al*., 2008).

Snail distribution results indicated that *Physa acuta* was the most abundant snail in both Giza and Gharbeya governorates, showing its highest percentage in spring followed by summer in Giza, and in winter followed by spring in Gharbeya. Similarly, Marie *et al*. (2015) reported on the highest peak of *Physa acuta* in spring. additionally, Abdel Kader *et al*. (2016) found that *P. acuta* snails had the highest percentages among all collected snails in Giza Governorate, and exhibited its maximum percentages during spring. In this study, *Biomphalaria alexandrina* was recorded in Gharbeya Governorate in all seasons, where its highest abundance was noticed in summer, followed by autumn. Whereas, Saad *et al*. (2014) and Marie *et al*. (2015) showed that the highest frequency of *B. alexandrina* in the same governorate was recorded in spring.

To determine the degree of snail species diversity in both governorates, Shannon-Weiner diversity index was applied. It showed its highest value during autumn in Giza Governorate. Whereas, in Gharbeya Governorate, the diversity index values reached their maxima equally during summer and autumn. These results indicated higher diversity of snail species in these two seasons. Also, Evenness index values were compatible with Shannon-Weiner diversity index results reflecting a better balance in the abundance of different snail species in autumn and summer. The variation of snail species which was clearly observed in the present study during autumn and summer can be attributed to the favorable conditions in these seasons which allow snails to survive, feed and reproduce (Yousif *et al*. 1993). Also it might be due to keeping the stagnancy of such water bodies during the dry seasons (summer and autumn) as there is no rainfall adversely affect snail habitat (Kloos *et al*., 2004).

The frequency of fungi in both Giza and Gharbeya governorates was investigated, where the most frequent fungal species was *Aspergillus*, as its percentages were the highest in spring and autumn, respectively. This matches the findings of Saad *et al*. (2014), as they reported that *Aspergillus* sp. were the most frequent in watercourses of four Egyptian governorates including Giza and Gharbeya. In addition, Badran (1986), Khallil (1990) and Hashem (2010) declared that *Aspergillus* sp. was reported as the most prevalent genus in water samples collected from River Nile and El-Gharbeya drainage. Furthermore, Abdel Hameed *et al*. (2008) investigated the prevalence and distribution of waterborne fungi along the main stream of River Nile (eight sites), as they identified 27 genera with *Aspergillus* sp. on the top of the predominant fungal types list. By the same token, Saad *et al*. (2016) isolated 129 fungal species belonged to *Aspergillus* out of 196 fungal isolates from El-Giza, El-Ismailia, El-Menoufiya and El-Gharbeya governorates. The predominance of *Aspergillus* sp. can be explained in the light of Barron (1968) statement that *Aspergillus* sp. is biologically one of the most successful of all fungi and is expected to occur in all sorts of habitats. It was found that *Aspergillus* species possess the ability to grow in oxygen-rich environments, carbon- rich substrates and even nutrient-depleted environments (Domsh *et al*., 1980). It was mentioned that the more obvious types of origin of these most commonly isolated terrestrial fungi from fresh water would be animals or plants (either living or dead) and soil litter having been in contact with soil (Park, 1972; Bandh *et al*., 2012).

Shannon-Weiner diversity index was used to highlight fungal diversity. It showed that the highest season in fungal biodiversity in Giza Governorate was autumn, followed by summer, and this was consistent with the results of the Evenness index during the same seasons, indicating better balance in the abundance of different fungal species in these seasons. This is in agreement with the findings of Saad *et al*. (2014), as they recorded the maximum variation of fungal groups during autumn in El-Giza Governorate. On the other hand, in Gharbeya Governorate, Evenness index was of the highest value in winter, indicating equal distribution of fungal species, while the diversity index values were more than one in all seasons, with spring showing the highest value, this coincides with the results of Saad *et al*. (2014), as they observed the highest diversity of fungal species during spring in El-Gharbeya Governorate. The results of the present study matches those declared by Nassar *et al*. (2002), where they denoted that the richest water and mud samples in fungal populations were those collected during the moderate temperature months. This could be explained according to Okpokwasili and Odokuma (1994), who declared that the effects of higher hydrocarbon contents (which commonly occur in the dry seasons) on microorganisms could be stimulatory.

The correlation between the biotic (snails and fungi) and the abiotic (physicochemical parameters) factors in the canals of the investigated governorates was determined. The correlation results in Giza Governorate showed that DO had strong positive associations with each of *Planorbis planorbis* and *Physa acuta* snails, and *Aspergillus* sp., *Mucor* sp. and *Rhizopus* sp. As DO is negatively affected by pollution, our results indicated that the numbers of these snail species and fungal genera might decrease as the pollution of the investigated watercourses increased, as a consequence, the biodiversity would decrease as well. Temperature, on the other hand, showed a strong negative association with *Lymnaea natalensis*, *Helisoma duryi* snails, and *Penicillium* sp. Similarly, Marie *et al*. (2015) and Abdel-Kader *et al*. (2016) found that temperature was negatively correlated with *L. natalensis*, and *Helisoma duryi* in Giza Governorate. These findings indicated that these snail species are highly sensitive to high temperature that causes thermal stress to such snails, and reduces dissolved oxygen content of the water body (Hofkins *et al*., 1991). On the other hand, temperature showed a strong positive correlation with *Trichoderma* sp. This positive relation might lead to an increase in the numbers and strains of *Trichoderma* sp. in the near future, as global warming resulted in the increase of water temperature, hence the diseases that *Trichoderma* sp. is known as their etiologic agent might increase, and deleteriously affect both plants and humans (Groll and Walsh, 2001).

Regarding the association between fungi and snails in Giza Governorate, the analysis showed a strong correlation between *Succinea cleopatra*, *Lanistes carinatus*, *Cleopatra bulimoides* and *Melanoides tuberculata* snails, and *Absidia* sp., *Fusarium* sp., *Microascus* sp., *Drechslera* sp., *Pythium* sp., and *Phoma* sp. This positive association can be explained in the light of the ability of freshwater-derived fungi to degrade dead plant material. This results in the production of fungal biomass, reproductive spores, and litter transformation products such as dissolved organic matter (DOM) and fine particulate organic matter (FPOM), so this process could increase the food quality and availability for aquatic invertebrates including snails (Gessner *et al*. 2007).

In Gharbeya Governorate, temperature, EC and TDS showed strong positive correlations with *Planorbis planorbis*, *Lanistes carinatus* snails and *Aspergillus* sp., while they showed negative correlations with *Lymnaea natalensis* and *Bulinus truncatus* snails. These findings can be interpreted as follows, as long as pollution of water canals increased (represented in the high values of EC and TDS), the intermediate hosts of fascioliasis and schistosomiasis decreased. Also, these vector snails might decrease in number as the global warming increased. On the other hand, as EC, TDS and global warming increased, the frequency of *Aspergillus* sp. might increase. This can lead to the incidence of more plant, fish and human diseases that are caused by the enormous strains of *Aspergillus* (Javey *et al*., 2009; Bobadilla-Carrillo *et al*., 2020). So, it is hard to determine whether it is better for human beings and their domestic animals to encounter such water conditions or not. Our results coincide with the findings of Saad *et al*. (2014) who recorded a similar negative correlation between *Bulinus truncatus* and each of EC and TDS. Also, they declared that temperature and *Aspergillus* sp. are strongly positively correlated in Gharbeya Governorate. Additionally, Marie *et al*. (2015) reported on a negative association between *Bulinus truncatus* and EC.

Regarding the association between snails and fungi in Gharbeya Governorate, the analysis showed a good positive correlation between *Aspergillus* sp., *Lanistes carinatus* and *Planorbis planorbis* snails, while exhibited a negative association with *Lymnaea natalensis* and *Bulinus truncatus* snails. In the same vein, Saad *et al*. (2014) found a negative correlation between *Aspergillus* sp. and *Bulinus truncatus*. This negative correlation might be due to the metabolites that *Aspergillus* spp. produce (Hernández-Carlos and Gamboa-Angulo 2011). These metabolites might negatively affect different invertebrates, especially sensitive snails such as *Lymnaea natalensis* and *Bulinus truncatus*, so the number of snails decreased in the sites where *Aspergillus* spp. showed high frequency.

CONCLUSION

The low quality of water in the investigated watercourses due to pollution (as reflected in the high values of electrical conductivity and total dissolved solids) might decrease the abundance and biodiversity of certain medically important snails and fungi. Also, both pollution and global warming might result in an increase in the frequency of certain pathogenic fungi that can cause various plant, fish and human diseases.

Funding

This work was supported by Theodor Bilharz Research Institute, Egypt, Grant 102 C.

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