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Effect of Water Pollution by Organophosphate Pesticides on Seasonal Distribution of Freshwater Macrophytes and Snails in Egypt

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ABSTRACT

Water pollution caused by pesticides is one of the urgent issues, especially in the developing countries. Organophosphate pesticides residues reach water canals through the discharge from agricultural fields. The aim of the present study was to assess the effect of water pollution caused by organophosphate pesticide residues on the aquatic life using macophytes and snails as indicators. The study was carried out over the course of a year in Qalubeya Governorate, Egypt, where the samples were seasonally collected. The results indicated that fenamiphos, fenitrothion, diazinon, malathion and pirimiphos-methyl showed the highest concentrations in both water and sediment samples. *Eichhornia crassipes* and *Lemna gibba* were the highly represented macrophytes in the investigated canals, and the most widespread snail species was *Physa acuta*. The correlation analysis between different parameters indicated that diazinon and fenamiphos negatively affected the population of the *Lymnaea natalensis* and *P. acuta* snails, whereas malathion and phorate pesticides had negative effects on *Succinea cleopatra*. On the other hand, fenitrothion, prothiophos and pirimiphos-methyl showed positive correlations with each of the *L. natalensis* and *P. acuta* snails. For the relationships between the detected pesticides and macrophytes, it was found that diazinon and fenamiphos deleteriously affect the distribution of *Azolla pinnata*, *Phragmites australis* and *Atropa belladonna*. While fenitrothion, prothiophos and pirimiphos-methyl had negative effects on the distribution of *E. crassipes* and *Echinochloa* sp. Meanwhile, these two macrophyte species can tolerate high concentrations of diazinon and fenamiphos. In conclusion, water pollution with high concentrations of organophosphate pesticides can affect the population and distribution of macrophytes and snails, which subsequently affect the functioning of the aquatic ecosystem.

INTRODUCTION

Organic pollution of water sources is one the biggest problems, especially in developing countries. The growing population leads to an increased food demand, this led to the expansion of agricultural areas. To increase the production, millions of tons of pesticides are used in agriculture fields **(FAO, 2016)**. This has contributed to higher pollution levels in the water **(Schwarzenbach *et al*., 2010; Mateo-Sagasta *et al*., 2017)**.

These pesticides may contain carcinogenic and other poisonous substances that can deleteriously affect the aquatic life. On the other hand, they may be bioaccumulated in the aquatic organisms and through the food chain, they reach humans causing harmful effects **(Mateo-Sagasta *et al*., 2017)**. Acute pesticide poisoning causes significant human morbidity and mortality worldwide, especially in low income countries, where poor farmers often use highly hazardous pesticides **(Mateo-Sagasta *et al*., 2017)**.

Organophosphate pesticides are used as alternatives to organochlorine ones, and their residues reach water canals **(Aktar *et al*., 2009)**. These residues not only affect the water quality but also affect the aquatic fauna and macrophytes **(Tallarico *et al*., 2014)**. Macrophytes are one of the biological elements for assessing the ecological status of watercourses **(Tarkowska-Kukuryk & Grzywna, 2022)**. As major primary producers, they play a crucial role in the trophic structure of the aquatic ecosystems, interacting with higher trophic levels by providing food and refuge for the macroinvertebrates and fish **(Elosegi *et al*., 2018)**. In addition, macrophytes affect the water quality through their involvement in nutrient cycling and sediment resuspension **(Kleeberg *et al*., 2010)**. Macrophytes that are common in freshwater systems include the *Lemna* spp., *Azolla pinnata*, and *Eichhornia crassipes* (**Marie *et al*., 2015; El-Deeb *et al*., 2017)**.

As photosynthesis is an important ecosystem process that influences the water quality, the inhibition of photosynthesis by an extensive application of pesticides results in a lower concentration of dissolved oxygen and lower water pH during daytime. This deterioration of the water quality can lead to a decrease in the herbivore populations due to food limitation and habitat loss. Hence, this represents indirect detrimental impacts on the sensitive invertebrate species **(Schäfer *et al*., 2011)**. The molluscs, including snails, represent 80% of the aquatic invertebrate species **(Haszprunar & Wanninger, 2012)**. Snails are the most abundant molluscan group in the aquatic systems, and they have been used as excellent indicators of the ecosystem's health **(Sheir *et al*., 2020)**. Moreover, snails are amongst the most vulnerable species since they are part of the slow-moving benthic fauna, which is easily influenced by pesticides discharged into the environment.

Therefore, the aim of the present study was to assess the effect of water pollution caused by organophosphate pesticides residues on the aquatic life using macophytes and snails as indicators.

MATERIALS AND METHODS

Study area

This study was carried out in the Qalubeya Governorate, Egypt. Four water canals were chosen from 4 sites; two sites from the Kanater Center and two sites from Tokh Center. The study extended for one year, during which the samples were collected seasonally.

Collection of water and sediment samples

In one liter glass bottles, water samples were collected seasonally from the studied water canals **(Dahshan *et al*., 2016)**, transferred to the laboratory, filtered and prepared for analysis. Whereas, sediment samples were seasonally collected in plastic bags from the shallowest depth of water canals using a net with small pore sizes. These samples were transported to the laboratory, air dried, ground and prepared for analysis.

Analysis of organophosphate pesticides in water samples

A volume of 2.5mL of acetonitrile (1% acetic acid) was added to 10mL of each water sample in a 15mL centrifuge tube. The tubes were capped and kept in the freezer at -18°C for 15min, and then 4g $MgSO_4$ and one gram NaCl were added. The tubes were shaken for 1min and centrifuged under cooling conditions for 5min at 4400rpm. From the upper layer, one milliliter was transferred to a 2mL vial containing 500mg $MgSO_4$. The vials were shaken and centrifuged under cooling conditions for 2min at 4400rpm. The extract was transferred to a PTFE capped vial (obtained from Shimadzu™, Japan) for GC-MS analysis using chromatograph A Shimadzu GC/MS-Q2010 Ultra (Shimadzu Corporation™, Japan) to determine pesticides residues **(Abdel Ghani & Hanafi, 2016)**.

Analysis of organophosphate pesticides in sediment samples

In a 50mL tube, three grams of air-dried sediment sample were placed, and 7mL H_2O was added. The mixture was stirred for 30min, followed by the addition of 10mL acetonitrile, and shaking for 5min to extract pesticides. Then, citrate buffered salts were added to each centrifuge tube, and samples were shaken for 2min and centrifuged for 5min at ≥3000 rcf. A volume of one mL aliquot of the supernatant was transferred to a 2mL CUMPSC18CTdSPE tube ($MgSO_4$, PSA & C18), followed by vortexing for 1min, centrifugation for 2min at a high rcf (e.g. ≥ 5000) to cleanup samples. Then, the purified supernatant was filtered through a 0.2µm syringe filter into a vial and analyzed by LC-MS/MS **(Sa *et al*., 2012)**.

Collection, identification and frequency of macrophytes

The occurrence of different macrophytes was observed *in situ* and recorded in the field sheets. A sample from each plant was put in a plastic bag, labeled, and transferred to the lab, where their identification was assessed by a botanist in Cairo University, Egypt. The frequency percentage of each plant was determined by dividing the number of sites where it heavily appeared by the total number of sites **(Nunes *et al*., 2019)**.

Collection, identification, and enumeration of snails

Using a dip net (30×40cm), samples of snails were collected from the investigated water canals (three spots for each sample), put in plastic aquaria, and transferred to the laboratory. In the lab, they were sorted out, counted, and identified according to **Ibrahim *et al*. (1999)**.

Statistical analysis

Principal component analysis was used to determine the correlation between different parameters using Graph Pad Prism (Ver.9).

RESULTS AND DISCUSSION

Fig. (1) shows the mean concentrations of the investigated organophosphate pesticides in the watercourses of the Qalubeya Governorate. The results indicated that fenamiphos recorded the highest concentration in spring, summer, and autumn, with mean concentrations of 20.23, 54 and 43µg/ L, respectively. In winter, the highest concentration observed was that of fenitrothion (9µg/ L), followed by that of fenamiphos (8.78µg/ L). On the other hand, pirimiphos-methyl showed its highest concentration in autumn (31.51µg/ L).

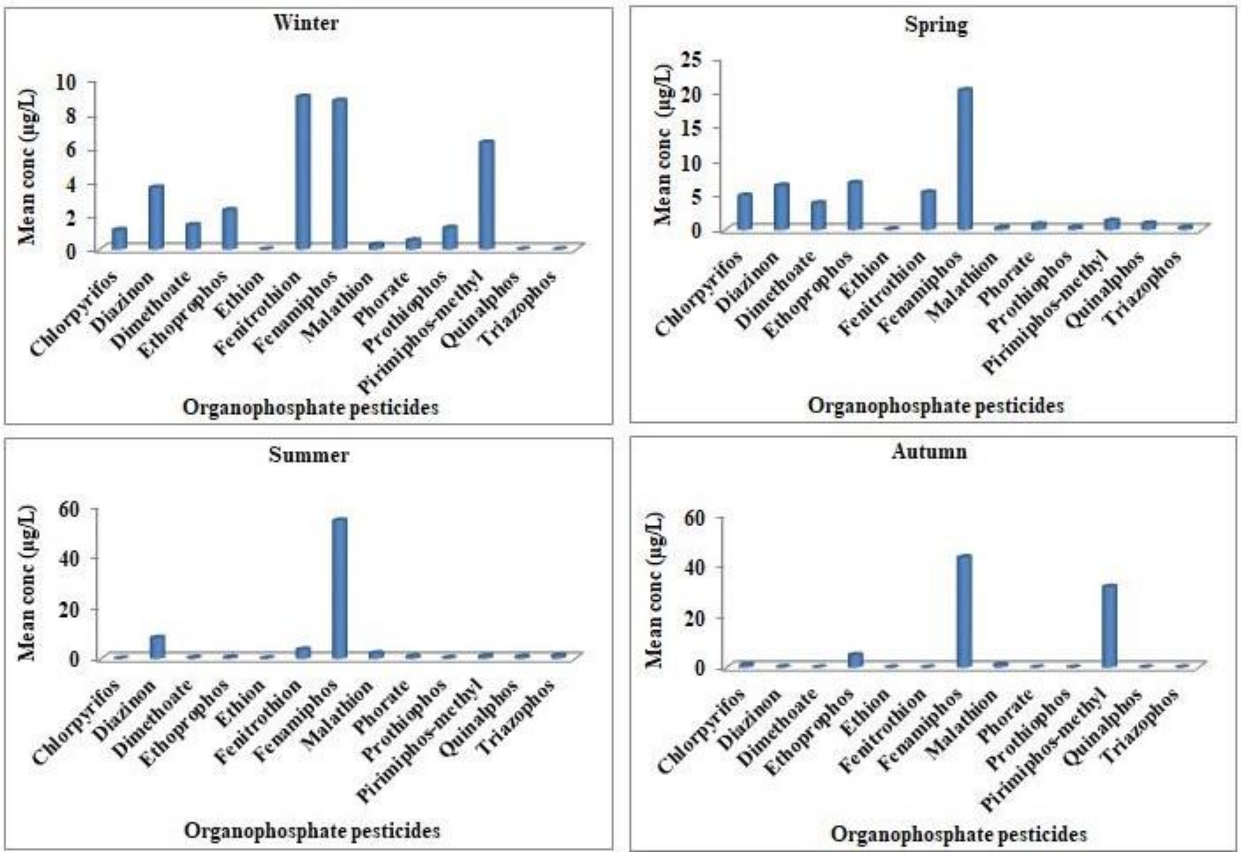


Fig. 1. Seasonal mean concentrations of different organophosphate pesticides in the water samples from canals of the Qalubeya Governorate, Egypt

Fig. (2) displays the mean concentrations of the organophosphate pesticides in the sediment samples of the investigated waterbodies of the Qalubeya Governorate. Generally, all the detected concentrations in sediment samples were higher than those observed in water ones. It was found that fenitrothion showed the highest mean concentration in summer (744.93µg/ g), winter (467.15µg/ g), and spring (203.04µg/ g). While in autumn, the highest concentration was that of pirimiphos-methyl (157.75µg/ g). Diazinon and malathion also recrded high concentrations in summer, with concentrations of 215.28 and 222.37µg/ g, respectively.

Organophosphate pesticides have countless adverse effects on the aquatic ecosystem; upon reaching humans, they might be carcinogenic, endocrine disrupting or neurotoxic compounds. Therefore, it is important to monitor their concentrations regularly, and to investigate their effects on the aquatic life. The present study indicated that fenitrothion and fenamiphos showed the highest mean concentrations in the water samples, while in sediment samples, the highest concentrations were those of fenitrothion, pirimiphos-methyl, diazinon, and malathion. This might be due to the affordable price of these pesticides for the Egyptian farmers **(Shalaby *et al*., 2018)**, and the growing use of the organophosphate pesticides instead of the organochlorine ones. Since the latter were banned due to their confirmed hazards **(Abbassy, 2000)**. The observed higher concentration of the organophosphate pesticides in the sediment samples of this work can be attributed to the low solubility of these hydrophobic compounds in the water, and hence their tendency to accumulate on a particulate matter **(Musa *et al*., 2011)**. Similarly, **Gotz *et al*. (1998)** and **Abbassy *et al*. (1999)** recorded significant concentrations of organophosphate pesticides in the River Elbe near Hamburg, Germany, and in the Nile River at the estuaries of the Rosetta and Damitta branches, Egypt.

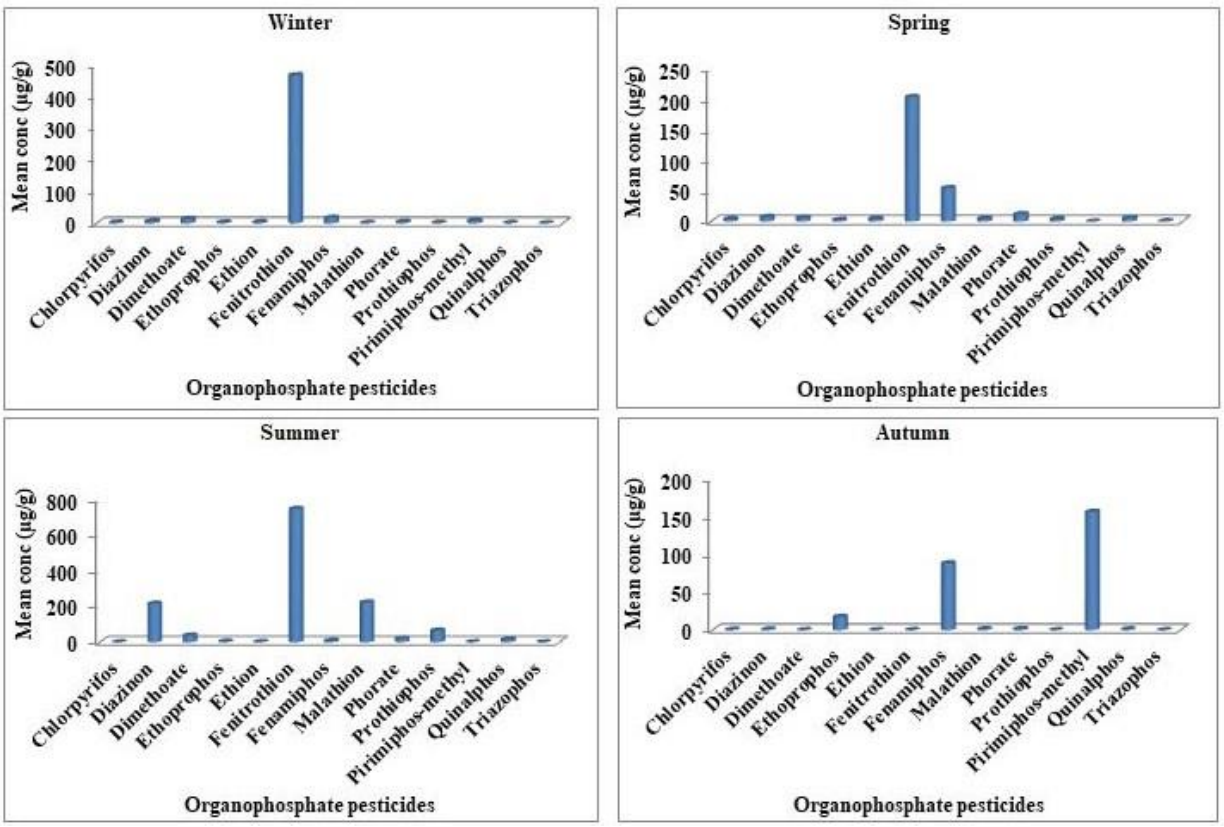
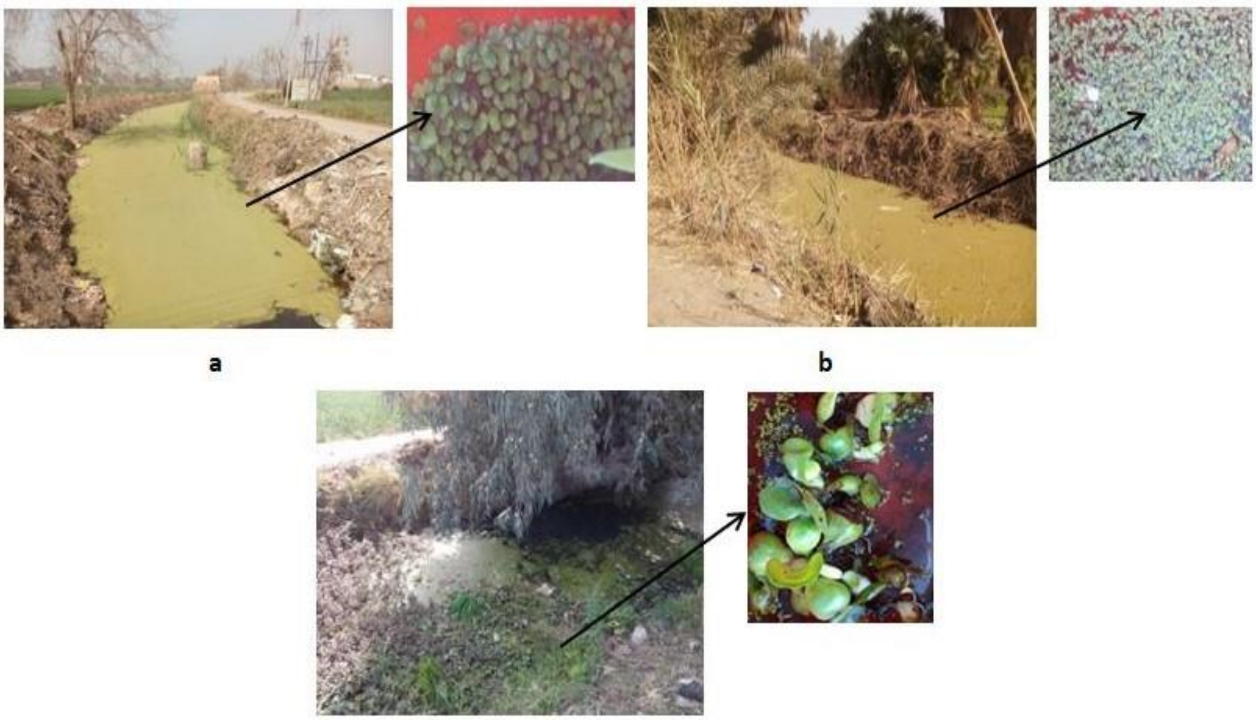


Fig. 2. Seasonal mean concentrations of different organophosphate pesticides in sediment samples from water canals of the Qalubeya Governorate, Egypt

With respect to the macrophytes, generally, some water canals were totally covered by the vegetation, as shown in Fig. (3). The highest frequency in the present study was that of *Lemna gibba*; it was densely represented in the water bodies of the four investigated sites during both spring and autumn (Fig. 4). Identically, **El-Deeb *et al*. (2017)** found that *L. gibba* was the most abundant macrophyte in different water bodies of Giza and Kafr El-Sheikh governorates of Egypt. The high density of the *Lemna* sp. as a free floating plant was attributed to numerous abiotic factors, such as the water flow, shading periods, and high nutrients concentrations **(Netten *et al*., 2011)**. *Eichhornia* *crassipes*, on the other hand, appeared in a high density at three sites in spring, summer, and autumn (Fig. 4). In the same vein, **Marie *et al*. (2015)** reported on the dominance of *E. crassipes* and *L. gibba* in all the investigated water canals of Ismailia and Menofeya governorates of Egypt. The dominance of *E. crassipes* over other indigenous flora, and its ability to invade the aquatic ecosystems on almost every continent can be attributed to its fast reproduction, easy dispersion, and ecological adaptability **(Ogwang & Molo, 2004; Sharma *et al*., 2016)**.



a b c

Fig. 3. Photos of some water canals in the Qalubeya Governorate, Egypt, showing dissemination of **a.** *Lemna gibba*; **b.** *Azolla pinnata*, and **c.** *Eichhornia crassipes*

In the present study, only three snail species were found in the investigated water bodies of the Qalubeya Governorate. These snails were *Lymnaea natalensis*, *Succinea cleopatra* and *Physa acuta*. *S. cleopatra* only appeared in spring, whereas *L. natalensis* and *P. acuta* were detected during all seasons. Generally, *P. acuta* showed the highest abundance, their total number was 370, 238, 167 and 106 snails in winter, spring, autumn, and summer, respectively. *L. natalensis*, on the other hand, showed their highest number (69) in winter (Fig. 5). These results are in line with the findings of **Abdel-Kader *et al*. (2016)** who found that, *P. acuta* snails had the highest percentages among other snails throughout all the year seasons in Giza Governorate, and exhibited its maximum percentage during spring. Additionally, **Abdel-Wareth and Sayed (2023)** postulated that *Physa acuta* was the most abundant snail in both Giza and Gharbeya governorates, showing its highest percentage in Giza during spring followed by summer , and in winter followed by spring for the Gharbeya Governorate. Moreover, *P. acuta* was reported as a widespread freshwater snail in northern Morocco **(Taybi *et al*., 2024)**.

The authors described it as a highly *euryceous* organism that can thrive in various habitats, including the degraded ones; this was attributed to its ability to adapt easily and to have low requirements in terms of habitat quality **(Taybi *et al*., 2024)**.

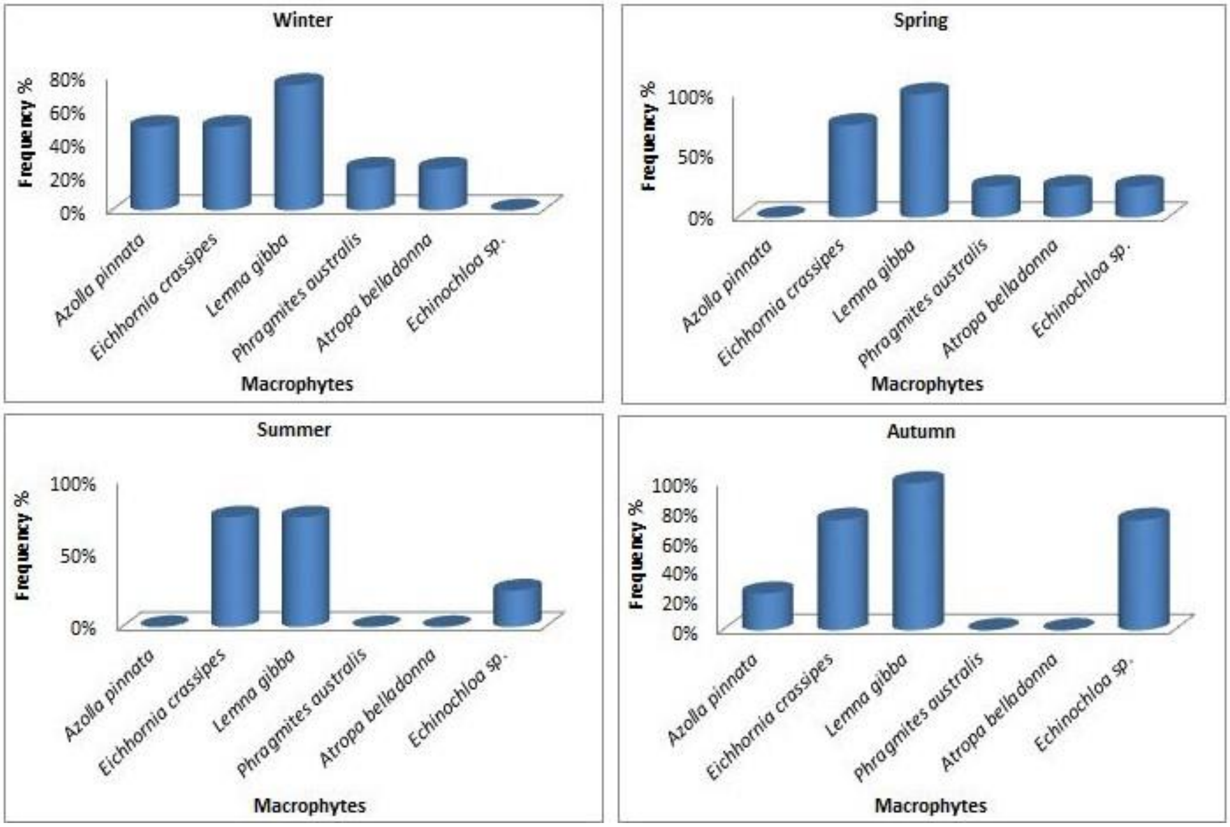


Fig. 4. Seasonal frequency percentage of different macrophytes in the investigated water canals of Qalubeya Governorate, Egypt

The principal component (PC) analysis was applied in the current study to determine the correlation between the organophosphate pesticides that showed the highest concentrations and both the snails and macrophytes. PC1 showed that diazinon (-0.897) and fenamiphos (-0.982) had strong negative correlations with each of *L. natalensis* (0.961), *P. acuta* (0.966), *Azolla pinnata* (0.907), *Phragmites australis* (0.817) and *Atropa belladonna* (0.817). On the other hand, diazinon and fenamiphos had strong positive correlation with each of *Eichhornia crassipes* (-0.907) and *Echinochloa* sp. (-0.907). While for fenitrothion (0.999), prothiophos (0.999), and pirimiphos-methyl (0.921), they showed positive correlations with each of *L. natalensis* (0.961), *P. acuta* (0.966), *Azolla pinnata* (0.907), *Phragmites australis* (0.817) and *Atropa belladonna* (0.817). On the other hand, fenitrothion, prothiophos and pirimiphos-methyl had strong negative correlation with each of the *Eichhornia crassipes* (-0.907) and *Echinochloa* sp. (-0.907). PC2 showed that each of malathion (0.721) and phorate (0.777) were negatively correlated with *S. cleopatra* (-0.995) and *Lemna gibba* (-0.995) (Table 1).

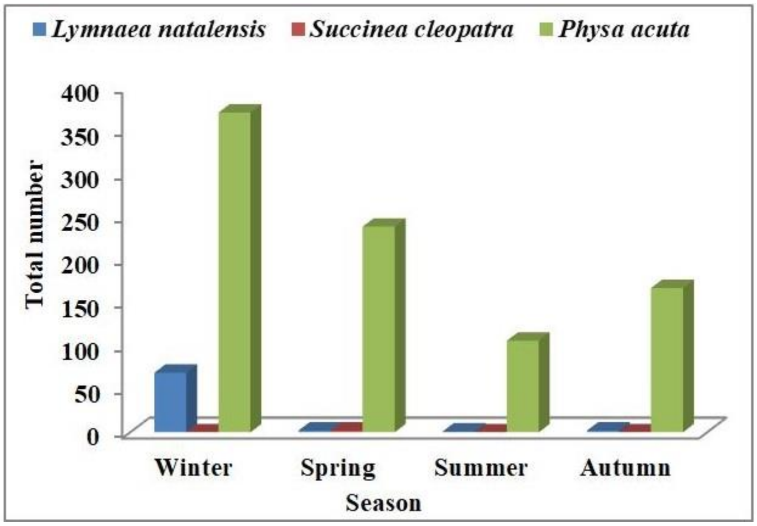


Fig. 5. Seasonal total number of different snail species in the investigated water canals of the Qalubeya Governorate, Egypt

The present correlation results revealed that diazinon and fenamiphos negatively affected the population of *L. natalensis* and *P. acuta* snails, whereas malathion and phorate pesticides had negative effects on *S. Cleopatra*. This means that these snail species are sensitive to water pollution caused by such organophosphate pesticides. On the other hand, fenitrothion, prothiophos, and pirimiphos-methyl showed positive correlations with each of *L. natalensis* and *P. acuta* snails. This indicated the ability of these snail species to tolerate high concentrations of the mentioned pesticides, and their tendency to accumulate their residues. Many studies highlighted the adaptability of *P. acuta* snails, as they can survive and reproduce in unstable environments and colonize highly polluted habitats inaccessible to other snail species due to their high resistance to pollution **(Tomkins & Scott 1986; Strzelec, 1999)**. For the *Lymnaea* spp., they have been widely used in ecotoxicological studies and water pollution biomonitoring programs **(Pirger *et al*., 2018; Amorim *et al*., 2019)**. They were found to be tolerant to some organic pollutants **(Erben & Pisl, 1993)**.

The relationships between the detected pesticides and macrophytes recorded in the present study showed that diazinon and fenamiphos deleteriously affect the distribution of *Azolla pinnata*, *Phragmites australis* and *Atropa belladonna*. While, the pollution of water with high concentrations of fenitrothion, prothiophos, and pirimiphos-methyl had a negative effect on the distribution of *Eichhornia crassipes* and the *Echinochloa* sp. Meanwhile, these two macrophyte species can tolerate high concentrations of diazinon and fenamiphos. The positive correlation observed in the current work between fenitrothion, prothiophos and pirimiphos-methyl and each of *Azolla pinnata*, *Phragmites australis* and *Atropa belladonna* might be due to the ability of these aquatic plants to accumulate high concentrations of the mentioned pesticides and that highlighted their tolerance. Moreover, the results demonstrated that *L. gibba* was sensitive to water polluted with malathion and phorate. The free floating macrophytes such as *E. crassipes* and *A. pinnata* have a better chance to survive than rooted macrophytes as the concentrations of the investigated pesticides in the current study were generally higher in sediment samples than in water samples. Moreover, *E. crassipes* was reported as a successful bioaccumulator of numerous organic pollutants **(Laet *et al*., 2019)**.

Table 1. Principal component analysis of different variables in water canals of Qalubeya Governorate, Egypt (carried out using Graph Pad Prism version 9)

Variable	**PC1**	**PC2**
Diazinon	**-0.897**	-0.442
Fenitrothion	**0.999**	0.013
Fenamiphos	**-0.982**	0.186
Malathion	-0.692	**0.721**
Phorate	-0.629	**0.777**
Prothiophos	**0.999**	0.003
Pirimiphos-methyl	**0.921**	0.390
Lymnaea natalensis	**0.961**	0.276
Succinea cleopatra	-0.091	**-0.995**
Physa acuta	**0.966**	-0.255
Azolla pinnata	**0.907**	0.419
Eichhornia crassipes	**-0.907**	-0.419
Lemna gibba	-0.091	**-0.995**
Phragmites australis	**0.817**	-0.576
Atropa belladonna	**0.817**	-0.576
Echinochloa sp.	**-0.907**	-0.419
Eigenvalue	**11.18**	**4.82**
Proportion of variance	**69.84**	**30.16**

CONCLUSION

Some aquatic flora and fauna were proved to be excellent indicators of water pollution caused by organophosphate pesticides since their population and distribution were deleteriously affected by the high concentrations of pesticides residues. In addition, the investigated canals in the Qalubeya Governorate, Egypt were highly polluted by organophosphate pesticides. Therefore, a continuous monitoring of surface water sources is mandatory to maintain the aquatic ecosystem's functioning and human health.

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