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Ecological risk assessment and probability of human health risk of two phenolic compounds in different watercourses and *Oreochromis niloticus*

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ABSTRACT

This study aimed to detect the actual levels of phenol and nonylphenol ethoxylate 9 (NPEO 9) in some freshwater courses and muscles of the Nile tilapia, *Oreochromis niloticus*, in three different Egyptian Governorates (Al-Giza, El-Dakahlya and Port-Said). Moreover, the ecological and probability of public health risks were evaluated for 6 sites in the three governorates. Results revealed that all the detected levels of phenol and those of total investigated compounds in water were above the reported permissible limits. Phenol had medium ecological risk in water at all sampling sites during the study period except in El-Mataria City at El-Dakahlya (Lake Manzala), where a high ecological risk was recorded. Meanwhile, high levels of ecological risk of NPEO 9 were observed in all water samples collected from the six investigated sites during the four seasons of the study period. In addition, the calculated hazard index (HI) for investigated phenolic compounds indicated high non-cancer risks for generalized habitual consumers at all sites during different seasons.

INTRODUCTION

Contaminants of emerging concern (CEC) are characterized as any synthetic or naturally occurring chemical that is not generally controlled in the environment, but has the ability to get in the soil and aquatic ecosystems, causing harmful impacts to humans, wildlife and environment **(Focazio *et al.*, 2008; Anderson *et al.*, 2012)**. CECs incorporate industrial, agricultural, and household chemicals and nanomaterials, as well as pharmaceuticals and personal care products **(Thomaidis *et al.*, 2012)**.

The most stressful outcome of the wide use and environmental dispersion of contaminants of emerging concern (CECs) is the addition in the possible exposure pathways for humans **(Weber *et al.*, 2005; Molnar *et al.*, 2013)**.

Previous investigations have shown that phenolic compounds can be detected in the environment, and can be delivered to aquatic organisms through the food chain, and into the human body principally through pathways of dermal contact and ingestion. **(Mielke *et al.*, 2001; Michalowicz and Duda, 2007; Zhou *et al.*, 2017)**. For their non-biodegradable and persistent characteristics, the accumulation of phenolic compounds in water and sediment may present risks to the aquatic ecosystem and human health **(Marianna, 2004)**.

The Egyptian National Standards have a relatively zero tolerance for phenol and phenolic compounds (0.005 ppm) as defined in Subject 66/Egyptian National Decree 8/1983. In water, the World Health Organization **(WHO, 1993, 2002, 2003)** recommended 1.0 µg/L as the permissible concentration of phenol. **FAO (1983)** announced that the permissible level of synthetic phenolic resins compounds in fish is 0.01 µg/g. **Soliman *et al.* (2020)** reported that phenol levels were 20 µg/L in water collected from the El-Tebin (Helwan) and 1.4 µg/g in the liver of *O. niloticus*; whereas, **Moustafa *et al.* (2007)** assessed that phenol level in water samples from Helwan was 420 µg/L and in *O. niloticus* muscles was 0.009 µg/g. They added that phenol in Bahr El Baquar was 100 µg/L and 0.003 µg/g in collected water samples and fish muscles, respectively. Furthermore, phenol levels ranging from 0.370 to 3.92 mg/L have been recorded in the rivers of Brazil **(Moraes *et al.*, 2016)**. On the other hand, **Shao *et al.* (2005)** demonstrated that NPEOs levels in water samples of Changjiang River and Jialingjiang River in China during the periods from April to December ranged from 1.0 to 97.6 µg/L. In addition, they recorded fluctuating levels from 0.5 to 1.3 µg/g in the muscles of different fish species

Therefore, the aims of this study were to know the actual presence and seasonal variations in the levels of phenol and Nonylphenol ethoxylate 9 (NPEO 9) in different watercourses from three governorates (Al-Giza, El-Dakahlya and Port-Said) and assess the impacts of the pollution with these phenolic compounds as a mean of ecological assessment of water quality. Another critical goal of the current study was to use the Nile tilapia, *Oreochromis niloticus*, as a bio-indicator species for investigating the probability of public health risks.

MATERIALS AND METHODS

The survey was done from autumn 2019 to summer 2020 during the four seasons in three Egyptian governorates: Al-Giza, El-Dakahlia, and Port-Said. Six selected sites with different water sources were chosen from different areas in the governorates. The selection depended on the presence of *O. niloticus* fish, and at least one of the pollution resources. The watercourses including the River Nile, the Manzala Lake, and agriculture drains were as follows:

I. Al-Giza governorate (the River Nile)

The River Nile is the main water source for Egypt. It forms 98% of the fresh water resources. The River Nile is receiving huge amounts of pollutants including the agriculture and domestic sewage as well as the industrial wastes **(El-Amier *et al.*, 2015)**, which find their way into the river and its branches. The study areas in the River Nile were **Al-Hawamdeya city (I)** near Hawamdeya sugar factory and **Saqayl village (II)** near West Cairo power station.

II. El-Dakahlia governorate (the Manzala Lake)

The Manzala Lake lies within the borders of five Egyptian Governorates (Dakahlia, Damietta, Port Said, Ismailia and Sharkiya). The lake receives polluted water from various drains; namely, Bahr El-Baqar, Ramsis, El-Mataria, Hadous, Faraskur, El-Serw, and Lissa El-Gamalia **(Zahran *et al*., 2015)**.

The selected sites were: **El-Mataria City (III)**, where the nearest effluents are Ramsis and Hadous drains and **Lissa El-Gamalia Village (IV)**, where the nearest effluents are Lissa El-Gamalia and El-Serw drains.

III. Port-Said Governorate

Two sites were chosen: **El- Qabuty region and Shader Azzam Village**.

El- Qabuty region (v): located near El-Qabuty canal that links the Manzala Lake to the Suez Canal and receives effluents of TCI Sanmar Chemicals Factory.

Shader Azzam Village (VI): It uses sewage directly from Bahr El-Baqar drain. Bahr El-Baqar drain is considered as one of the most polluted drains in Egypt **(Elkiki, 2018)**.

Collection and preparation of water samples

Water samples were manually collected from each study site at a depth of 30 cm under the water surface and kept in clean glass containers. Water samples were filtered immediately after collection through Millipore glass-fiber filters (diameter 47mm, nominal pore size 0.45mm). Water sample with an amount of 250 mL was prepared by adjusting the pH to 2.0 with a phosphoric acid solution (0.1 mol/L) **(Zhai, 2012)**. Water samples were transferred in an ice box to the laboratory and kept at 4°C till being analyzed.

The Nile tilapia *O. niloticus* collection

Fish samples were caught by fishermen using “gobiah” nets. Fish were transferred alive to the laboratory, and the muscles of the fish were carefully excised. Muscles of *O. niloticus* were studied to determine the residue content as they are a source of phenolic residues bioaccumulation in higher food chain organisms, especially humans.

Preparation of fish muscles

Phenolic compounds determination was erformed according to the procedure of **Goupy *et al.* (1999)** as follows: 5 g of each tissue sample was mixed with methanol and centrifuged at 10000 rpm for 10min, and the supernatant was filtered through a 0.2 µm Millipore membrane filter. Then, 1-3 mL were collected in vials for injection into HPLC.

HPLC analysis of phenolic content

Separation and determination of phenolic compounds were performed by HPLC Agillant 1260 equipped with Perkin Elmer Brownlee Validated Aqueous C18, 5 µm, 4.6 x 250-mm, auto sampling injector, solvent degasser, and ultra violet (U.V) detector were set at 280 nm, and the column temperature was maintained at 35°C. The solvent systems used were gradients of A (8% CH_3COOH/H_2O), and B (acetonitrile). The separation was done with the following gradient at 0.0 - 20min 5% B, 95% A, at 20 - 50min,10% B, 90% A, at 50 - 55min, 30% B, 70% A, at 55 - 100min, 50% B, 50% A, at 100 - 120min, 100% B the solvent flow rate was 1 mL/min, the separation was at 35°C, and the injection volume was 10 µL of the standards and extracts. Phenolic compounds were assayed by external standard calibration at 280nm. Laboratory reagents were of analytical and HPLC grade and were purchased from Sigma.

Ecological Risk Assessment:

A risk quotient (RQ) was utilized to calculate the potential ecological risks of each target pollutant by comparing the levels of phenolic compounds in water against their corresponding quality values, which is described as follows (USEPA, 1986):

$$RQ = MEC / PNEC$$

Where MEC is the detected environmental concentration and PNEC is the predicted no effect concentration. The PNEC value used for phenol is 7.7 µg/L according to the European Union risk assessment report (EU RAR) (2006) and the Environment Agency UK (2008), and for NPEO 9 is 0.3 µg/L according to the European Commission (EC) (2005 and 2013). It is considered that $RQ > 1$ indicates a high risk; $0.1 < RQ < 1$ indicates a medium risk; while $RQ < 0.1$ indicates a low risk (USEPA, 1986).

Human Health Risk Assessment:

Health risk is characterized by non-carcinogenic risk which is considered by the hazard quotient (HQ) as follows (USEPA, 2001): $HQ = CDI / RfD$,

$$CDI = (C \times DR \times EF \times ED) / (BW \times AT)$$

Where CDI means chronic daily intake (mg/kg/d), RfD is the chronic reference dose (mg/kg/d). The USEPA's Integrated Risk Information System (IRIS) has established the chronic oral Reference Dose (RfD) for phenol as 0.3 mg/kg/day (USEPA, 2002). Meanwhile the RfD for nonylphenol ethoxylate is 0.1 mg/kg/day (Bakke, 2003). C is the concentration of the contaminant (mg/kg/d), DR is the daily consumption rate (0.0312 and 0.1424 kg/day for generalized normal and habitual fish consumers, respectively as reported by USEPA (2000) and 0.0435 kg/day proposed by FAO (2010) specifically for adult Egyptians.

EF is exposure frequency (d/a), ED is exposure duration (a), BW is body weight (kg) and AT is averaging time (d). When exposure includes more than one chemical, the sum of the individual hazard quotients for each chemical is used as a measure of the potential for harm. This sum is called the hazard index (HI):

$$HI = \text{Sum of hazard quotients}$$

It is considered that HQ > 1 indicates a high non-cancer risk, while HQ < 1 indicates a low non-cancer risk.

RESULTS AND DISCUSSION

Occurrence and seasonal variation of phenol and NPEO 9 in water:

Fig. (1) illustrats the fluctuated seasonal variations in the concentrations of phenol and NPEO 9 in water. Between the two phenolic compounds, the successive order of concentration in water was phenol > NPEO 9. The highest recorded mean values were 26.5 and 21.0 µg/L for phenol and NPEO 9, respectively, at site III during winter. While, the lowest detected level of NPEO 9 was 0.55 µg/L in site III during spring. Zhou *et al.* (2017) detailed that phenol record the highest concentration with 137.35 ng/L of five phenolic compounds analyzed in water samples of Shitou Koumen Reservoir of Yinma River in China during August. Furthermore, they indicate the concentrations of phenol in the water of the Yinma River Basin showed seasonal variations with the highest value observed in the wet season in China (August) then followed by normal season (May) and finally dry season (November), which might be because of the variations in hydrological conditions and environmental factors like temperature and sunlight intensity.

Meanwhile, the study carried out by Khairy (2013) to determine the levels of phenolic compounds in Lake Maryut, Egypt during July revealed that the concentration of phenolic compounds ranged from < LOD to 106 µg/L with an average concentration of 35.9 µg/L in effluents discharged from Qalaa Drain, which discharges mainly treated municipal wastewater and agricultural effluents into the south eastern part of Lake Maryut. Also, results of the risk assessment of his study revealed that phenol was one of the contaminant of concern and that adverse ecological impacts could possibly occur to benthic species from the exposure to these pollutants in Lake Maryut. Therefore, phenols should be listed in monitoring and pollution prevention programs in the Egyptian aquatic environment influenced by anthropogenic activities.

On the other hand, as this is the first study to detect NPEO 9 in Egyptian watercourses and the previous reported studies were concerned about the concentrations of nonylphenol (NP) which is the main product of NPEOs breakdown, so data about NP concentration will be discussed. Abdel-Wareth and Sayed (2019) found that freshwater concentrations of NP were in the range of 400 to 1600 µg/L in six studied sites at Al-Giza during summer. While, in two major rivers in Lagos, Nigeria, 4-nonylphenol concentrations varied from 43.9 to 79.4 ng/L (Oketola and Fagbemigun, 2013). Fu *et al.* (2007) and Xu *et al.* (2008) revealed a seasonal trend of dissolved nonylphenol (NP) concentrations with higher values in summer than in winter and the finding was attributed firstly to high temperatures and related microbial activity, prompting enhanced degradation of NPEOs in sediments and subsequently, increased NP concentrations in the water column during summer.

This clearly demonstrates the impact of the seasonality of natural or anthropogenic sources on pollutant abundance in aquatic environment and also the great effect of point and nonpoint sources of pollution along the study sites. It was noted that all detected levels of phenol in water and also for total investigated compounds in water were above the permissible limit reported by WHO (2003) and Egyptian National Standards detailed in Subject 66/Egyptian National Decree 8/1983.

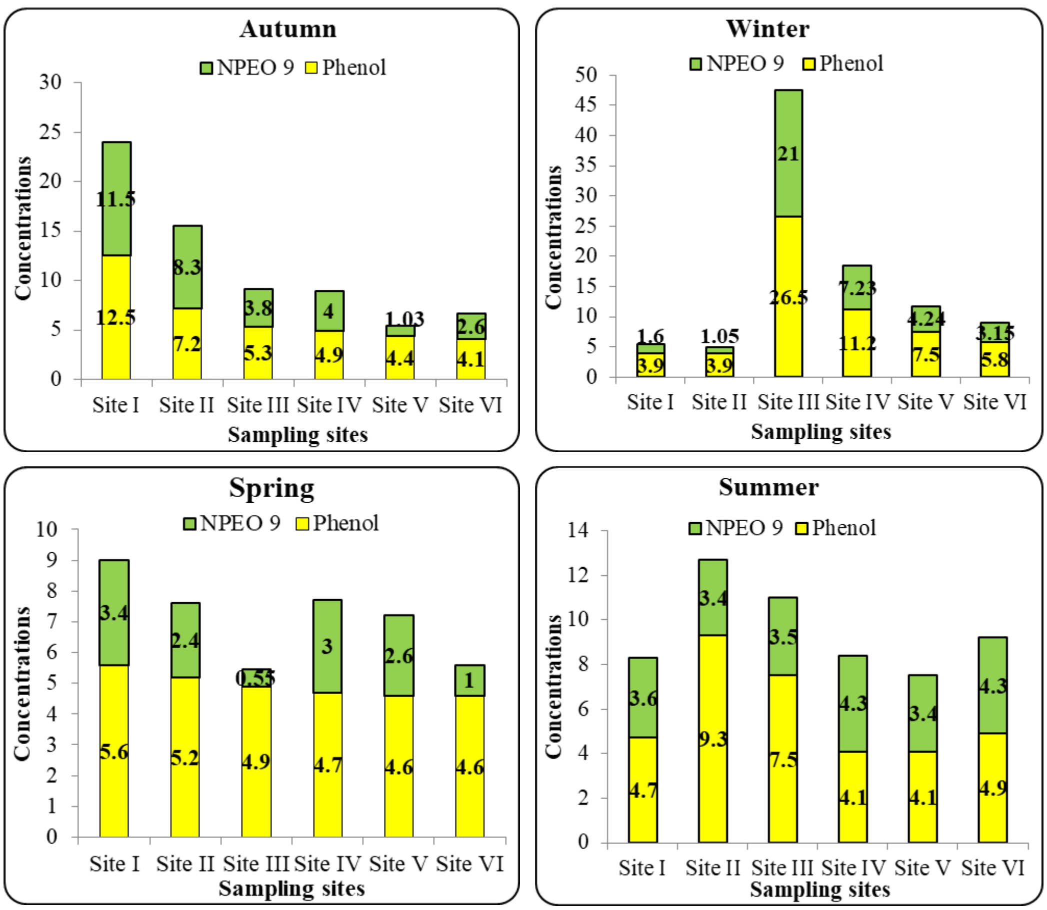

Fig. (1): Seasonal distributions of the concentrations of phenol and NPEO 9 (µg/L) in water from different sampling sites during the study period.

- **Site I**: Nile River, **Site II**: Nile River, **Site III**: Manzala Lake, **Site IV**: Manzala Lake, **Site V**: Manzala Lake and **Site VI**: Bahr El Baqar drain.

Occurrence and seasonal variation of phenol and NPEO 9 concentrations accumulated in *O. niloticus* fish:

Considering the high residual concentration of phenol and NPEO 9 in the water of different sampling sites, it was necessary to further investigate the residual levels in fish since they are one of the most important indicators in aquatic environment that used for the assessment of pollution and risk potential of human consumption (Authman *et al.*, 2013). **Fig. (2)** reveals phenol and NPEO 9 varied seasonal concentrations in muscle of *O. niloticus* (µg/g wet wt.) in the six studied sites. Also, there were high exceeded levels of phenol and NPEO 9 in fish muscles in all investigated sites during four seasons. Moustafa *et al.* (2007) mentioned that the phenol level in *O. niloticus* muscle from Helwan was 0.009 µg/g, whereas phenol in *O. niloticus* muscle from Bahr-El-Baquar was 0.003 µg/g. Kannan *et al.* (2003) found the NP concentrations ranged from 3.3 to 29.1 ng/g in fish collected from the Kalamazoo River, Michigan, USA. Shao *et al.* (2005) reported that NPEOs levels in muscles of fish samples of Changjiang River and Jialingjiang River in China during periods from April to December were 0.4 µg/g in *Rhinogobio ventralis*, 0.5 µg/g in *Rhinogobio typusfrom* and *Coreius heterodon*, 0.8 µg/g in *Coreius Guichenoti* and 1.3 µg/g in *Leptobotia elongata.* They indicated that NPEOs are more easily bioconcentrated in fish than 4-NP. These great differences in detected levels may be due to the fact concluded by Zhou *et al.* (2007) that bioconcentration from water via the gills, skin, and food is a possible pathway for pollutants to accumulate in tissue, but the route relies mainly on the feeding preference, general behavior and trophic level.

Ecological risk assessment of phenol and NPEO 9 in water:

Table (1) shows the risk quotient (RQ) for the two phenolic compounds in water from all sampling sites in the four studied seasons. Seasonal variations in the RQ values for the two phenolic compounds in water were observed. The total RQ values of the two compounds were more than 1.00 in all investigated sites during each season which reflects the high ecological risk of these compounds in water. Meanwhile, Zhou *et al.* (2007) revealed middle risks in several sampling sites in the Yinma River Basin in China of total concentrations for five phenolic compounds. Also, high ecological risks of phenol were noted at the site I during autumn, site II during summer, site III during winter, and site IV during winter. The highest risk of phenol pollution was in site III during the winter with RQ value of up to 3.44. Other RQ values in water were more than 0.1 but less than 1.0, which means that there were medium levels of risk in the water at sampling sites during the study period. Moreover, there were high levels of risk in the water of NPEO 9 in the six investigated sites during the four seasons of the study period. The highest RQ value of NPEO 9 in water was recorded at site III during winter.

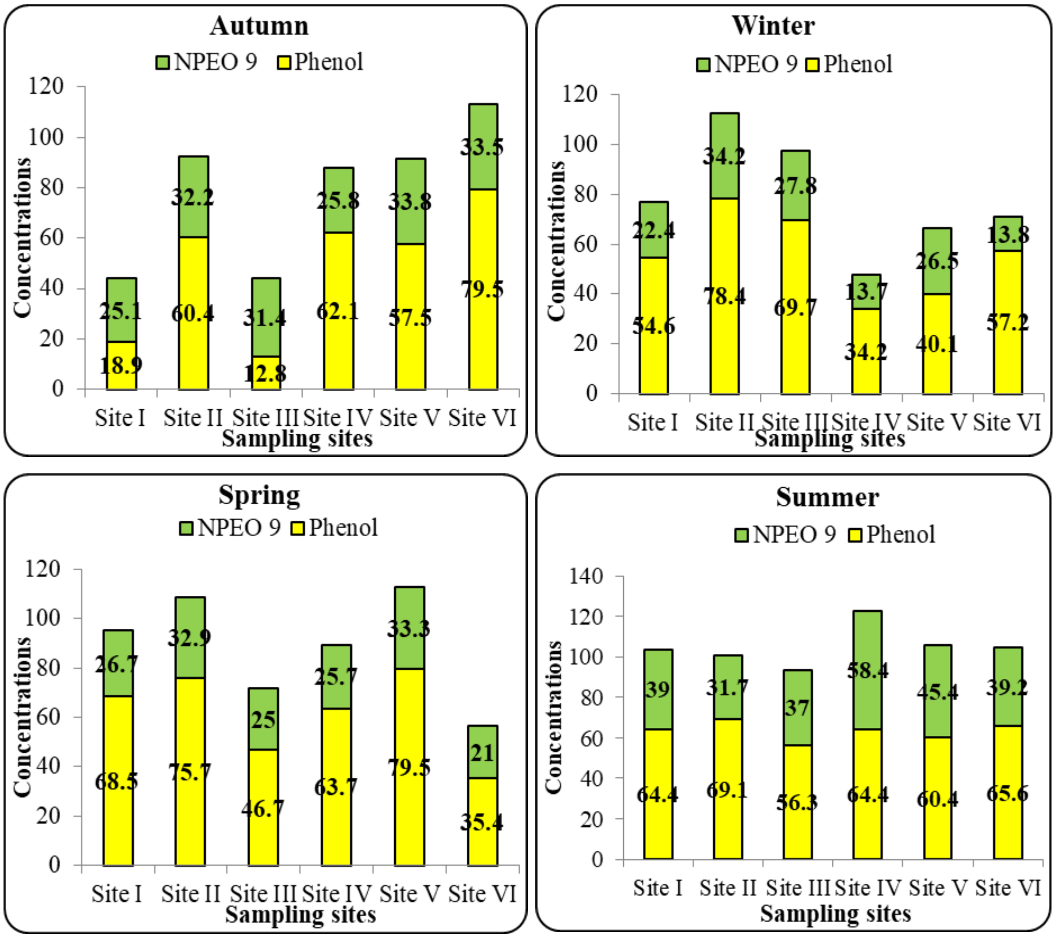

Fig. (2): Seasonal distributions of the concentrations of phenol and NPEO 9 in muscles of *O. niloticus* (µg/g wet wt.) from sampling sites during the study period.

- **Site I**: Nile River, **Site II**: Nile River, **Site III**: Manzala Lake, **Site IV**: Manzala Lake, **Site V**: Manzala Lake and **Site VI**: Bahr El Baqar drain.

Probability of human health risk assessment

Fig. (3) reveals the calculated HQ values of phenol. It was clearly obvious that all values of HQ for phenol did not pose unacceptable risks at the three proposed ingestion rates (for generalized normal and habitual fish consumers and also Egyptian consumers). Also, **Fig. (4)** indicates that all values of HQ for NPEO 9 did not represent risks at the three proposed ingestion rates (for generalized normal and habitual fish consumers and also Egyptian consumers) except for habitual consumers in site IV during summer. While, the calculated total hazard quotients (HI) of both compounds in the edible muscle of *O. niloticus* fish, as indicated in the **Table (2)** shows a high risk for habitual fish consumers during spring and summer in the site I, the four seasons in site II, winter and summer in site III, summer in site IV, autumn, spring and summer in site V, and autumn as well as summer in site VI. Generally, habitual consumers have HI value of more than 1.00 in all sites during summer. This obviously shows the importance of merging the consumption rates in pollutants' risk assessments. In accordance with this, Omar *et al.* (2015) inferred that use of the proposed human risk assessment (dose and consumption dependent variables) is more reliable in predicting the risks presented on human consumers rather than the application of regular known permissible levels or the upper level of intake in food for human consumption which are not consumption dependent variables.

Table (1): Risk quotient (RQ) values of phenol and NPEO 9 in water of investigated sites during the study period.

Risk quotient (RQ)					
Sites	**Compounds**	**Autumn**	**Winter**	**Spring**	**Summer**
Site I	**Phenol**	1.62	0.50	0.72	0.61
	NPEO 9	38.33	5.33	11.33	12.0
	Total	39.95	5.83	12.05	12.61
Site II	**Phenol**	0.93	0.50	0.67	1.20
	NPEO 9	27.66	3.50	8.00	11.33
	Total	28.59	4.00	8.67	12.53
Site III	**Phenol**	0.68	3.44	0.63	0.97
	NPEO 9	12.66	70.0	1.83	11.66
	Total	13.34	73.44	2.46	12.63
Site IV	**Phenol**	0.63	1.45	0.61	0.53
	NPEO 9	13.33	24.1	10.0	14.33
	Total	13.96	25.55	10.61	14.86
Site V	**Phenol**	0.57	0.97	0.59	0.53
	NPEO 9	3.43	14.13	8.66	11.33
	Total	4.00	15.10	9.25	11.86
Site VI	**Phenol**	0.53	0.75	0.59	0.63
	NPEO 9	8.66	10.50	3.33	14.33
	Total	9.19	11.25	3.92	14.96

- **Site I**: Nile River, **Site II**: Nile River, **Site III**: Manzala Lake, **Site IV**: Manzala Lake, **Site V**: Manzala Lake and **Site VI**: Bahr El Baqar drain.

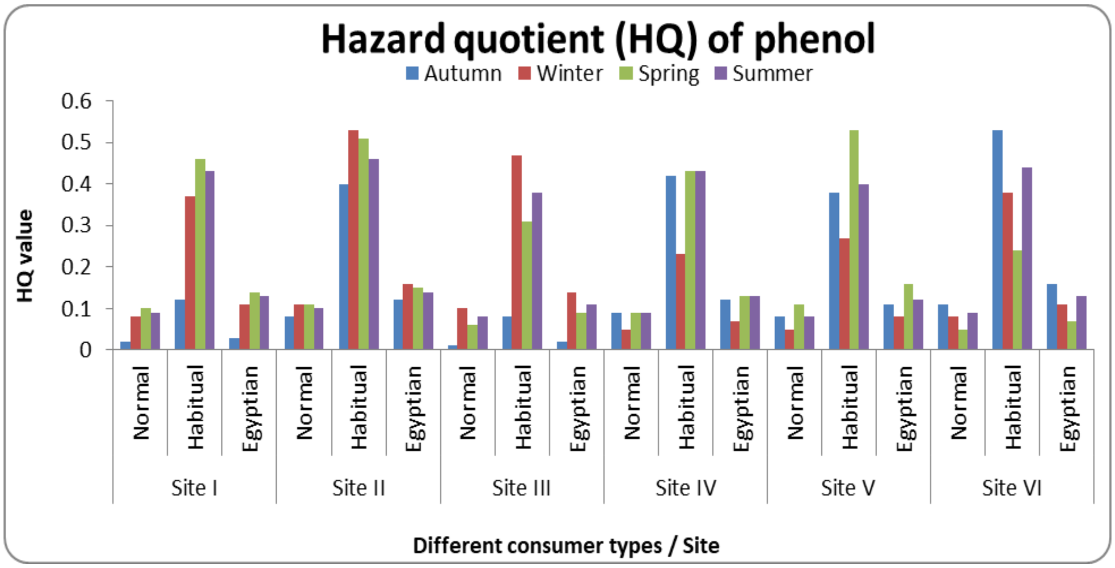

Fig. (3): Hazard quotient (HQ) of phenol for consumers in investigated sites during four studied seasons.

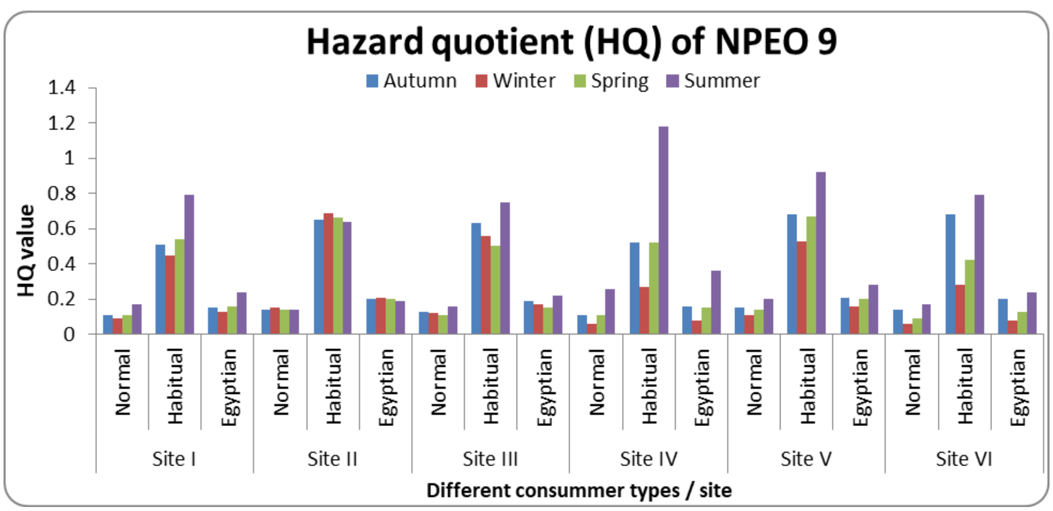

Fig. (4): Hazard quotient (HQ) of NPEO 9 for consumers during study period in investigated sites.

- **Site I**: Nile River, **Site II**: Nile River, **Site III**: Manzala Lake, **Site IV**: Manzala Lake, **Site V**: Manzala Lake and **Site VI**: Bahr El Baqar drain.

Table (2): Total hazard quotients (HI) of phenol and NPEO 9 in fish edible muscles of investigated sites during the study period.

Sites	Consumers	Autumn	Winter	Spring	Summer
Site I	Normal	0.13	0.17	0.21	0.26
	Habitual	0.63	0.82	**1.0**	**1.22**
	Egyptian	0.18	0.24	0.3	0.37
Site II	Normal	0.22	0.26	0.25	0.24
	Habitual	**1.05**	**1.22**	**1.17**	**1.1**
	Egyptian	0.32	0.37	0.35	0.33
Site III	Normal	0.14	0.22	0.17	0.24
	Habitual	0.71	**1.03**	0.81	**1.13**
	Egyptian	0.21	0.31	0.24	0.33
Site IV	Normal	0.2	0.11	0.2	0.35
	Habitual	0.94	0.5	0.95	**1.61**
	Egyptian	0.28	0.15	0.28	0.49
Site V	Normal	0.23	0.16	0.25	0.28
	Habitual	**1.06**	0.8	**1.2**	**1.32**
	Egyptian	0.32	0.24	0.36	0.4
Site VI	Normal	0.25	0.14	0.14	0.26
	Habitual	**1.21**	0.66	0.66	**1.23**
	Egyptian	0.36	0.19	0.2	0.37

- **Site I**: Nile River, **Site II**: Nile River, **Site III**: Manzala Lake, **Site IV**: Manzala Lake, **Site V**: Manzala Lake and **Site VI**: Bahr El Baqar drain.

CONCLUSION

This work mainly investigated seasonal variation in concentrations of phenol and NPEO 9 and seasonality in the risks of the two phenolic compounds in the freshwater and muscles of *O. niloticus*. The results indicated that the concentrations of two phenolic compounds in water were all above permissible limit reported by WHO (2003) and Egyptian National Standards that detailed in Subject 66/Egyptian National Decree 8/1983. Also, there were high exceeded levels of phenol and NPEO 9 in fish muscles in all investigated sites during four seasons. The results of the RQ indicated that there were high levels of ecological risk in water of NPEO 9 in the six investigated sites during the four seasons of the study period. Moreover, there were high ecological risks of the total phenolic compounds in all investigated sites during each season. Based on the results of HI, the habitual consumers were under a high non-cancer risk in all sites of investigation during summer.

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ARABIC SUMMARY

تقييم المخاطر المحتملة على البيئة وصحة الانسان لإثنين من المركبات الفينولية في المجاري المائية المختلفة وأسماك البلطى النيلى "*Oreochromis niloticus*"

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تهدف هذه الدراسة إلى تقدير المستويات الفعلية من الفينول والنونيل فينول إيثوكسيلات ٩ (NPEO 9) فى بعض مجارى المياه العذبة وايضا في عضلات أسماك البلطى النيلى فى ٣ محافظات مصرية (الجيزة - الدقهلية - بورسعيد). وقد تم دراسة المخاطر البيئية والصحية العامة المحتملة من هذه المركبات فى ٦ مواقع من المحافظات الثلاثة حيث أوضحت النتائج أن جميع التركيزات التى تم تقديرها في الماء من الفينول وكذلك مجموع المركبين معا كانت أعلى من الحد المسموح به. كما وجد أن مستويات الفينول تمثل مخاطر بيئية متوسطة في الماء فى كل المواقع خلال فترة الدراسة فيماعدا مدينة المطرية بالدقهلية (بحيرة المنزلة) حيث أظهرت النتائج وجود مخاطر بيئية عالية. بينما كان هناك مستويات عالية من المخاطر البيئية من النونيل فينول إيثوكسيلات ٩ في جميع عينات المياه التي تم جمعها من جميع مواقع الدراسة خلال الفصول الأربعة. وفي الوقت نفسه لوحظ أن مؤشر الخطر المحسوب (HI) للمركبات الفينولية محل الدراسة يشكل مخاطر غير مقبولة للمستهلكين المعتادين بشكل عام في جميع المواقع خلال المواسم المختلفة.