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Original Article

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Abstract

Giza governorate has unique characteristics which induces mosquito proliferation and thus magnified the risk of diseases transmission. In the present study, entomological field surveys integrated with lab analysis were utilized to characterize water quality associated with mosquito breeding habitats in the Nile Valley zone of Giza governorate. Field survey was conducted for a total number of 20 accessible breeding sites during the period of 6th –9th December 2019. From each visited site, mosquito larvae and water were sampled to identify larval species and analyze physico-chemical characteristics of water. Fourteen different water quality parameters were measured including pH, Total Dissolved Solids (TDS), Oxidation-Reduction Potential (ORP), Conductivity (EC), Turbidity, Chlorophyll, HDO, Crude Oil (CO), Salinity, OM, Phosphate ion (PO_4^{3-}), Nitrate ion (NO^{3-}), Cadmium (Cd), Lead (Pb). Spearman correlation coefficient was used for data analysis. A total of 8,435 mosquito larvae belonging to 4 species representing one genus were collected from fixed larval breeding localities. *Culex pipiens* was the dominant species except in two sites where *Cx. pusillus* and *Cx. theileri* were predominant. There was a significant positive correlation between the density of *Cx. pusillus* and *Cx. theileri* with salinity, OM, nitrate, phosphate, and heavy metal. It could be concluded that the water quality characteristics are considered a good indicator to predict the existence of certain mosquito species.

1. Introduction

Mosquito-borne infectious diseases account for the highest number of reported cases, mortality, and disability-adjusted life years. Nearly 700 million people contract a mosquito-borne illness every year resulting in greater than one million deaths. Mosquitoes act as vectors of several important pathogens such as viruses (West Nile virus, Dengue virus, Chikungunya virus, etc.), parasites (*Dirofilaria spp*.) or even bacteria (*Francisella spp*.) (Caraballo, 2014).

Physicochemical characteristics of breeding habitats are important for mosquitoes' oviposition and development. Different characteristics of the oviposition sites such as: vegetation, temperature, turbidity, pH, concentration of ammonia, nitrite and nitrate, sulphate, phosphate, chloride, calcium, and water hardness affect mosquito larval abundance. Changing these factors in larval habitats may create conditions preferable or unpreferable for mosquito breeding (Nikookar *et al.* 2017).

Previous studies evaluate natural and artificial habitats as well as elucidate relationships between occurrences of species and larval habitat characteristics, identify some environmental and climatic variables that serve as drivers of vector larval abundance, examine the physical characteristics of the mosquito breeding habitats, identify areas with suitable habitats and evaluate the risk for disease transmission (Kenawy *et al*., 1996; Fischer *et al.*, 2002; Estallo *et al.*, 2018).

Information on the ecological factors affecting mosquito larval abundance such as the physicochemical properties of the water of the breeding sites and interspecific associations are important in survival, spatio-temporal distribution, biodiversity, affinity, and association indices of disease vectors. The information may serve as the basis for designing and implementation of adequate vector control programs (El-Zeiny and Sowilem, 2016).

Despite the huge literature on the distribution of mosquito larvae and physicochemical factors, the data set seems to be inadequate in leading to a prediction of the presence of larvae in different habitats. Therefore, more studies and systematic reviews with proper generalization are required. On the other hand, Giza has a unique environment fit for spreading the breeding of various species of mosquito. Differences in environmental and geographical characteristics of the governorate have caused a fertile environment for the development of mosquitoes. The area had a history of diseases transmitted by vectors, the most important of which has been Filaria (Amusan *et al*., 2005). To date, there is no information on physicochemical characteristics of mosquito larval habitats and coefficient of interspecific association in the governorate and this is the first such study in the governorate.

Consequently, the present study aims to identify and assess mosquito breeding habitats, water quality parameters of these habitats, and document the relationship between water quality and larval densities in the Nile-Valley of Giza governorate.

2. Materials and Methods

Study Area

Giza governorate, which is the third largest city in Egypt, is located at the Middle of the Egypt on the west bank of the Nile River at about 20 km southwest of central Cairo. It extends between latitudes 27º 40´ N and 30º 20´ N, longitude 27º 20´ E and 31º 80´ E with a total area of 1579.75 km^2 (609.94 mi^2).

The meteorological data indicates that the average annual temperature is 21.2 °C. The quantity of rainfall in Giza does not vary significantly over the course of the year, staying within 3 mm. Relative humidity records indicate extreme monthly variations between 16 and 63%, and the average annual humidity is 39.5%. As shown in Figure (1), the targeted area is the Nile-Valley zone of Giza, with exception of eastern and western desert as these areas are non-suitable for mosquito reproduction. It extends between latitudes 29º 15´ N and 30º 20´ N, longitude 30º 50´ E and 31º 20´ E and occupies an area of 1331.69 km2.

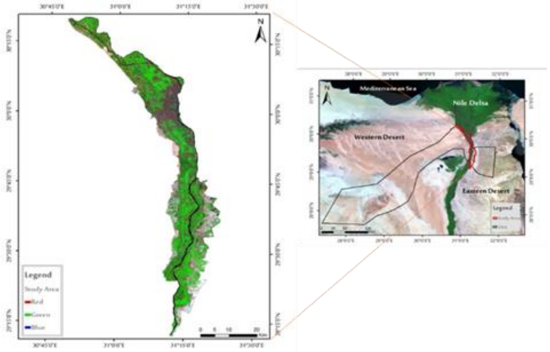

Figure (1): Location map of the study area

Mosquito larval collection and water sampling

A field trip was conducted to Giza governorate during the period of 6th –9th December 2019. Various breeding sites were visited as illustrated in Figure (2,3). A total number of 20 various breeding sites were investigated. From each visited site, mosquito larvae and water were collected and prepared to be transferred into laboratory for further analyses.

In regard with the sampled immature stages of mosquitoes, a standard dipper was utilized for sampling from stagnant aquatic habitats. A number of 2-10 dips were taken around according to size of site and larval densities. Collected larvae were counted to calculate their densities then preserved in 70% Ethyl Alcohol in labeled glass vials. Procedures and precautions, regarding larval collection and transportation, were carried out according to WHO (1975) guidelines. Natural and physical characteristics of each habitat site were fully described and geo-referenced using GPS.

Lab work

Collected mosquito's larvae were identified and classified using Keys of Harbach et al. (1988) and Glick (1992). On other hand, sampled water was analyzed for some physico-chemical characteristics. Fourteen different water quality parameters were measured including; pH, TDS, Oxidation-Reduction Potential (ORP), EC, Turbidity, Chlorophyll, HDO, Crude Oil (CO), Salinity, OM, Phosphate ion (PO43-), Nitrate ion (NO3-), Cadmium (Cd), Lead (Pb). Manta-2 instrument was used for determination of physico-chemical characteristics of water except Turbidity, OM, PO43-, NO3-, Cd, Pb which was measured at lab, following standard method of water analyses American Public Health Association (APHA, 1992).

Statistical analysis

For statistical analyst purposes, mosquito larval density in each breeding habitat was calculated using the following formula: $D = l / L \cdot 100\%$, where; D: Density, l: Number of specimens of each mosquito species, L: Number of all specimens. The means and standard deviations of physicochemical parameters of each breeding site was calculated. The Spearman correlation coefficient was used to examine the relation of the mosquito larval abundance to the physicochemical factors. Figure (4) summarizes the methodology adopted to achieve the goal of the current study.

3. Results and Discussion

Mosquito breeding habitat assessment

Despite the short duration of field survey and its implementation at a period of the year that was not optimal for summer-breeding mosquitoes, four mosquito species have been observed; Culex pipiens, Cx. perexigues, Cx. pusillus, and Cx. theileri with a total number of 8,435 larvae. Tabulated results indicated that Cx. pipiens was the most dominant species in the whole study area representing 95.04% (n=8017 larvae) of the total collection, while the least abundant species was Cx. theileri recording 0.10% (n= 10 larvae) of the total collection.

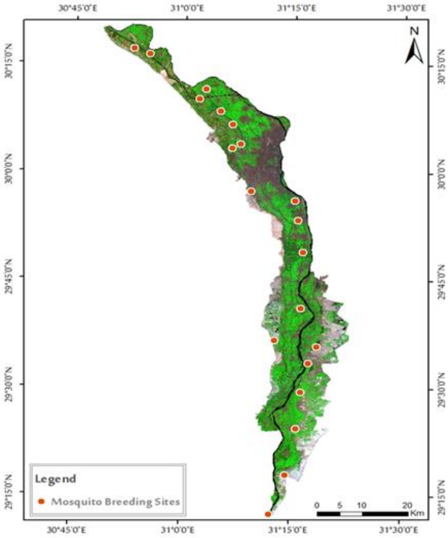

Figure (2): Mosquito breeding sites at Nile Valley of Giza Governorate.

Figure (3): Different mosquito breeding habitats in Nile Valley of Giza Governorate as captured from the field.

These findings are similar to previous studies achieved by El-Said and Kenawy (1983), Bahgat et al. (2004), Sowilem et al. (2017) in different localities in Egypt and other studies by Mohamed et al., (1981), Abdel-Shafi (2016) in Giza governorate (Table 1).

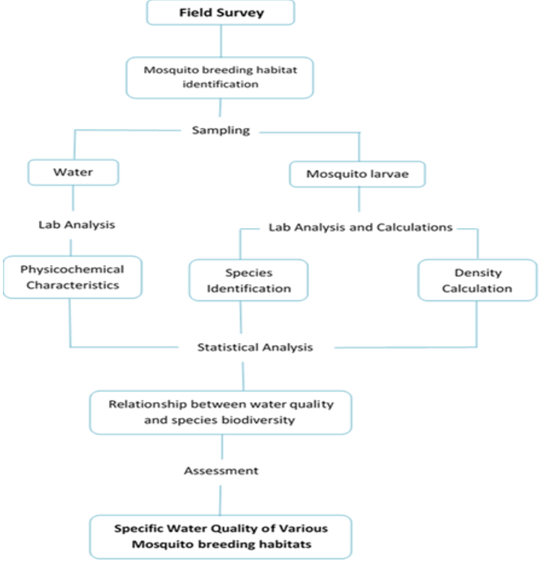

Figure (4): Flow chart showing methodology adopted in characterization of mosquitoes breeding habitat.

Inspected larval breeding habitats were categorized into four habitat types; sewage, seepage, drainage, and irrigation canals. According to larval density, the habitats can be ordered as follows; irrigation canals > sewage > drainage canals > seepage. Habitats identification helps to assess the frequent larval species and the associated characteristics of each habitat separately. As indicated in Table (2), *Cx.*

Table (1): Statistics of mosquito larval species in all breeding habitats within the study area.

Species	Number	Percentage (%)	Min.	Max.	Mean
Cx. pipiens	8017	95.04	0	4500	400.85
Cx. pusillus	230	2.73	0	150	11.5
Cx. perexigues	178	2.13	0	100	8.9
Cx. theileri	10	0.10	0	10	0.5
Total Number	8435	100			
Density			4	4500	421.75

Table (2): Relative abundance and breeding preference (%) of each mosquito species in relation to all breeding habitats at Nile Valley Zone – Giza governorate.

Habitat type	*Cx. pipiens* No (%)	*Cx. perexigues* No (%)	*Cx. busillus* No (%)	*Cx. theileri* No (%)
Drainage canals	330 (4.12)	125 (70.22)	0 (0)	0 (0)
Irrigation canals	5287 (65.94)	53 (29.75)	0 (0)	0 (0)
Sewage	2400 (29.94)	0 (0)	0 (0)	0 (0)
Seepage	0 (0)	0 (0)	230 (100)	10 (100)
Total	8017	178	230	10

Water quality assessment and mosquito breeding habitats preference

Mosquito can breed in a wide range of habitats with different physico-chemical characteristics. A total number of 14 physico-chemical characteristics were investigated in the different surveyed breeding sites as shown in Table (4). The measured parameters include pH, Turbidity, Salinity, TDS, Specific Conductance, Chlorophyll, HDO, ORP, CO, OM, anions (PO43-, NO3-), and heavy metals (Cd, Pb). Most of the investigated mosquito breeding habitats are neutral tendency. However, acidic habitats (< 7) are restricted to agriculture drains where Cx. pipiens and Cx. perexiguus breed. These findings are in line with Hamdy's records (1987) in Ismailia (Figure 5).

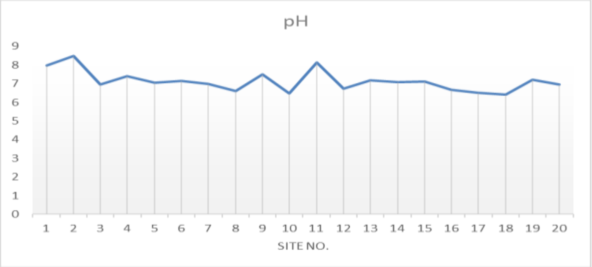

Figure (5). Levels of pH levels of the investigated breeding habitats.

As shown in Table (3), Turbidity showed high variations among investigated habitats. However, high mean levels of turbidity were observed in sewage (107.75 NTU) and drainage canals (73.30 NTU). Presence of culicine mosquito larvae in various habitats with turbid and slightly turbid stagnant water demonstrates that no significant correlation between turbidity and larval density, which agreed with Abd El-Meguid's study (1987). Whereas, Kenawy et al. (1996) observed that water turbidity significantly affects breeding of Cx. pipiens and Cx. Perexiguus (Figure 6). On other hand, Salinity, TDS and EC levels were very high in seepage water compared to other habitats. It was found that Cx. pusillus and Cx. theileri can breed in water with high salinity since the higher densities were recorded (Figure 7).

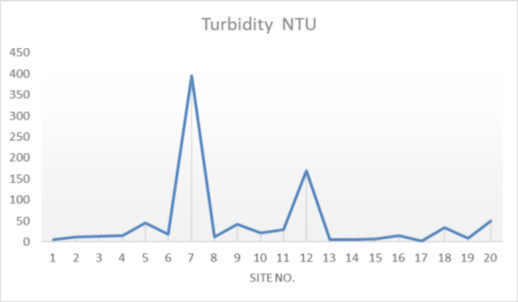

Figure (6). Turbidity levels of the investigated breeding habitats.

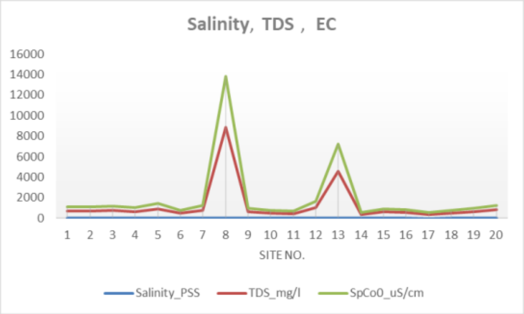

Figure (7). Salinity, TDS and EC levels of the investigated breeding habitats.

Chlorophyll levels remarked a great fluctuation, within the studied breeding sites. Generally, high chlorophyll mean level (5.56 µg/l) was detected in breeding sites characterized by sewage wastewater. On the other hand, low chlorophyll mean (4.48 µg/l) was observed in association with irrigation canals (Figure 8).

Levels of **HDO** were extensively related to levels of organic pollutants where bacteria consume DO for degradation of OM (El-Zeiny and Sowilem, 2016). The maximum level of HDO was reported at seepage (75.58 %Sat) and sewage (72.50 %Sat), while low level detected in drainage canals (68.43 %Sat) and irrigation canals (69.45 % Sat) as shown in figure (9).

ORP is a measurement that indicates the degree to which a substance is capable of oxidizing or reducing another substance. Majority of the investigated breeding habitats are oxidizing agent as their ORP positive. It has been noticed that seepage water has the highest records (830.85 mV). However, lower oxidation-reduction potential values were represented by habitats inhibited by *Cx. pipiens*. This finding is compatible with Golding *et al.* (2015), who reported that *Cx. pipiens* favored habitats with low levels of ORP (Figure 10).

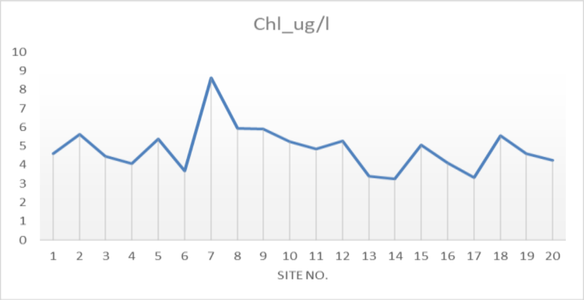

Figure (8). Chlorophyll levels of the investigated breeding habitats.

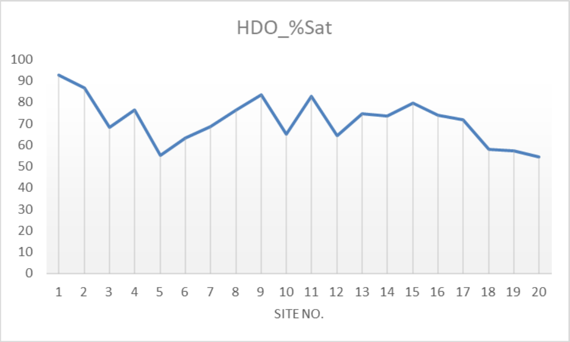

Figure (9). HDO levels of the investigated breeding habitats.

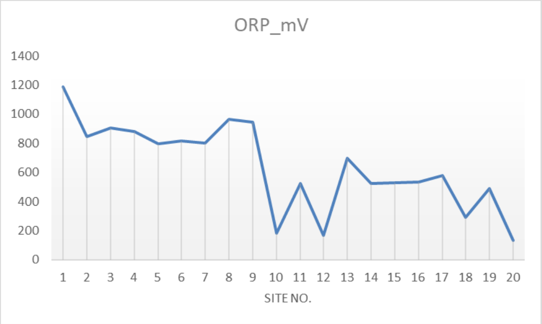

Figure (10). ORP levels of the investigated breeding habitats.

CO could be defined as a mixture of naturally occurring hydrocarbons that is refined into diesel, gasoline, heating oil, jet fuel, kerosene, and literally thousands of other products called petrochemicals. Heavier crudes yield more heat upon burning in comparison to light (or sweet) crudes. In the current study, CO showed high levels fluctuating from 451.95 rfu to 582844 rfu. Mean high level of CO (293550.82 rfu.) was reported at irrigation canals. On other hand, **OM** at mosquito breeding habitats, remarkably fluctuated from 0.005 to 0.109 mg/l with a mean of 0.032 mg/l. The high levels of OM were clearly observed in seepage water (Figure 11).

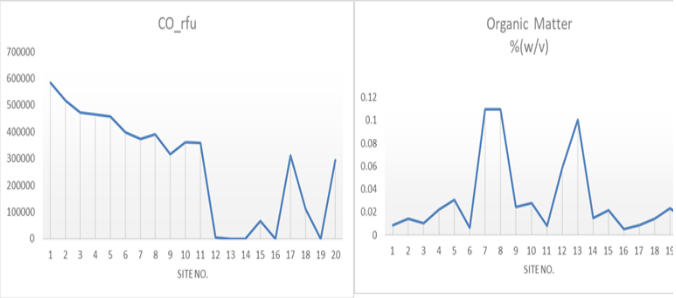

Figure (11). CO (left) and OM (Right) levels of the investigated breeding habitats

Figure (12) shows that the chemical analyses of anions (PO43- & NO3-) in sampled water showed great fluctuation among different breeding habitats. Phosphate and nitrite tend to be higher within habitats related to seepage and drains associated with agricultural activities (i.e; because of using fertilizers). Current study showed that culicine mosquito could tolerate different degrees of these elements. Previous findings also showed significant general increase of culicine mosquito larval populations with the use of fertilizers. These fertilizers enhance the growth of microbes that are a major source of nutrition for larvae (Mutero et al., 2004).

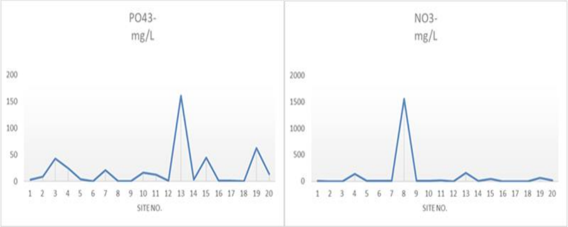

Figure (12). PO43- (left) and NO3- (Right) levels of the investigated breeding habitats.

The presence of culicine larvae in habitats with different concentrations of **Pb** and **Cd** suggests relatively high tolerance of this species to these metals. Anthropogenic factors, poor planning and drainage contribute to the accumulation of heavy metals. Mireji *et al.* (2008) demonstrated greater tolerance of culicines to elevated levels of heavy metals in different habitats, which is compatible with the current findings. However, seepage water recorded higher levels of Cd (24.50 µg/L) and Pb (390.25 µg/L) where *Cx. pusillus* and *Cx. theileri* can breed (Figure 13).

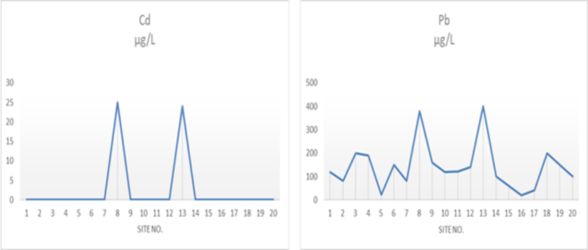

Figure (13). Cd (left) and Pb (Right) levels of the investigated breeding habitats.

Correlation coefficient:

In order to study the statistical relationship between physicochemical characteristics and mosquito species, a correlation matrix was calculated between water quality parameters and the reported mosquito species as shown in Table (5). In line with the findings of Zang et.al (2011) and Hyslope et.al (2015), significant correlation (0.63) was observed between pH and HDO.

Significant positive correlations were observed between turbidity with chlorophyll (0.75), and OM (0.54), which indicates to the presence of algae and organic components in water in the suspended state. In addition, strong correlation exists between salinity and OM (0.73), NO3- (0.92), Cd (0.95), and Pb (0.78). The significant correlation between OM with chlorophyll (0.50), NO3- (0.75), Cd (0.73), and Pb (0.59) declared that the source of pollutants and decaying matter were as a result of agriculture and human activities. Significant correlation was noticed between ORP with HDO (0.58) and CO (0.60). This may be explained that ORP depends on the amount of dissolved oxygen that may be influenced by the amount of CO in water.

On other hand, significant positive correlations were observed between salinity, TDS, EC with Cx pusillus (0.79) and Cx theileri (0.89). Several studies in Egypt and other countries support the present findings. El-Zeiny and Sowilem (2016) demonstrated that these species preferred breeding in salty water (like Seepage water). Moreover, mild to strong positive correlation was found between these species with OM, nitrate, phosphate, and heavy metal, except that the correlation between Cx theileri and phosphate is negligible. This may be interpreted as these two species can tolerate high levels of nitrate, phosphate, and heavy metal because of nutrients availability.

Conclusion

In the present study, field surveys and laboratory analysis of mosquito breeding habitats were integrated in order to get more precise characterization and assessment of the proliferation sites. Results showed that mosquito can survive and breed in a wide range of environmental and climatic conditions. Mosquitoes can proliferate in clean and contaminated sites, fresh and saline water, neutral and alkaline water, oxidation and reduction media, high and low algae content, high and low crude oil content, high and low OM content, and in sites with high and low anions like phosphate and nitrates. It could be concluded that physicochemical factors of breeding sites may determine the distribution and abundance of culicine mosquito. High interspecific association between pair of species *Cx pusillus* and *Cx theileri* show that these species have common needs and adaptability for sympatry. These findings could be useful in understanding the ecology of mosquito larvae that may be beneficial in designing and implementing larval control programs.

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Table (3): Physico-chemical (Mean) characteristics of mosquito breeding habitats

Parameter	**Drainage canals**	**Irrigation canals**	**Sewage**	**Seepage**
pH	7.15	7.15	6.93	6.90
Turbidity (NTU)	73.30	21.98	107.75	8.58
Salinity (PSS)	0.59	0.39	0.52	6.11
TDS (mg/l)	767	512.85	671.93	6730.60
EC (uS/cm)	1198.70	801.25	1049.70	10512.93
Chl (ug/l)	5.26	4.48	5.56	4.67
HDO (%Sat)	68.43	69.45	72.50	75.58
ORP (mV)	533	486.08	691.60	830.85
CO (Rfu)	107195.96	293550.82	227975.26	195862.96
OM (%(w/v))	0.04	0.01	0.04	0.10
PO_4^{3-} (mg/L)	21.11	9.19	27.35	161
NO^{3-} (mg/L)	24.60	6.64	13.99	859
Cd (µg/L)	0.09	0.08	0.08	24.50
Pb (µg/L)	150.47	114.26	90.68	390.25

Table (4): Physico-chemical characteristics of mosquito breeding habitats at Nile Valley Zone – Giza governorate (6th-9th December 2019).

No	pH	Turbidity NTU	Salinity PSS	TDS mg/l	EC uS/cm	Chl ug/l	HDO %Sat	ORP mV	CO Rfu	OM %(w/v)	PO_4^{3-} mg/L	NO^{3-} mg/L	Cd µg/L	Pb µg/L
1	7.96	6.10	0.55	718.80	1123	4.61	92.80	1187.50	582844	0.009	3.20	3.80	0.09	120
2	8.49	12.70	0.55	710.50	1110	5.63	86.80	844.50	516082	0.014	8.66	2.40	0.09	80.70
3	6.96	13.30	0.59	757.60	1183	4.44	68.20	902.50	471386	0.010	42.50	0.35	0.08	200.20
4	7.41	15.20	0.51	659	1029	4.07	76.30	882.10	464147	0.022	24.90	138.0	0.08	190.40
5	7.05	44.60	0.72	916.40	1432	5.38	55.20	795.80	458537	0.031	3.61	6.90	0.10	22.50
6	7.14	18.60	0.36	477.60	746.20	3.68	63.30	814.60	397811	0.006	0	9.90	0.08	150.30
7	6.99	395	0.61	784.70	1226	8.64	68.60	802.60	373673	0.109	20.80	9.50	0.09	80.90
8	6.62	11.60	8.02	8855	13830	5.96	76.50	964.80	391082	0.109	0	1557	25.0	380.20
9	7.50	42.50	0.48	630.70	985.50	5.92	83.50	943.10	317708	0.025	0.14	10.60	0.11	160.40
10	6.48	21.30	0.37	482.60	754.10	5.24	65.20	181.70	360397	0.027	15.90	3.60	0.10	120.50
11	8.13	30.1	0.34	443.9	693.6	4.83	82.7	523.2	358630	0.008	12.7	12.7	0.08	120.70
12	6.74	169	0.83	1051	1643	5.27	64.4	165.3	3427.92	0.059	0.8	0.8	0.08	140.60
13	7.17	5.56	4.19	4606.2	7195.85	3.38	74.65	696.9	643.925	0.100	161	161	24	400.30
14	7.07	5.90	0.28	375.30	586.40	3.27	73.4	523.40	657.57	0.015	2.90	2.90	0.06	100.50
15	7.10	7.80	0.45	594.20	928.50	5.05	79.40	526.80	66261	0.021	45.10	45.10	0.08	60.90
16	6.67	14.90	0.42	551.20	861.30	4.12	73.80	534.50	581.02	0.005	1	1	0.08	20.70
17	6.50	2.40	0.25	336.10	525.20	3.32	71.90	580.50	310485	0.008	1.40	1.40	0.07	40.50
18	6.40	34.30	0.36	476.50	744.50	5.57	57.90	289.30	110643	0.014	0	0	0.09	200.30
19	7.22	8.40	0.47	619.30	967.60	4.59	57.40	490.60	451.95	0.023	62.40	62.40	0.08	150.40
20	6.97	50.50	0.62	800.30	1250	4.26	54.40	131.45	293701	0.013	13.60	13.60	0.07	100.60

Min.	6.40	2.40	0.25	336.10	525.20	3.27	54.40	131.45	451.95	0.005	0	0	0.06	20.70
Max.	8.50	395	8.02	8855	13830	8.64	92.80	1187.50	582844	0.100	161	1557	25	400.30
Mean	7.10	45.50	1.05	1242.35	1940.73	4.86	71.31	639.10	273957.50	0.030	24.70	107.5	2.50	142.08

Table (5). Correlation matrix between water analysis and mosquito species.

	pH	**Turbidity NTU**	**Salinity PSS**	**TDS mg/l**	**EC uS/cm**	**Chl ug/l**	**HDO %Sat**	**ORP mV**	**CO rfu**	**OM %(w/v)**	**PO_4^{3-} mg/L**	**NO^{3-} mg/L**	**Cd µg/L**	**Pb µg/L**	***Cx* *pipiens***	***Cx* *perexigues***	***Cx* *pusillus***	***Cx* *theileri***
pH	1.00																	
Turbidity NTU	-0.12	1.00																
Salinity PSS	-0.18	-0.09	1.00															
TDS mg/l	-0.18	-0.09	1.00	1.00														
SpCond uS/cm	-0.18	-0.09	1.00	1.00	1.00													
Chl ug/l	0.03	0.75	0.10	0.10	0.10	1.00												
HDO %Sat	0.63	-0.18	0.12	0.12	0.12	0.01	1.00											
ORP mV	0.47	-0.06	0.26	0.27	0.27	0.13	0.58	1.00										
CO rfu	0.41	0.02	-0.01	0.00	0.00	0.25	0.29	0.60	1.00									
OM %(w/v)	-0.23	0.54	0.72	0.73	0.73	0.50	-0.02	0.14	-0.11	1.00								
PO_4^{3-} mg/L	0.05	-0.10	0.28	0.27	0.27	-0.24	-0.01	0.02	-0.35	0.39	1.00							
NO^{3-} mg/L	-0.20	-0.11	0.92	0.93	0.93	0.17	0.12	0.28	0.11	0.57	-0.03	1.00						
Cd µg/L	-0.15	-0.14	0.95	0.94	0.94	-0.05	0.14	0.23	-0.13	0.73	0.53	0.76	1.00					
Pb µg/L	-0.10	-0.16	0.78	0.78	0.78	-0.07	0.08	0.21	-0.07	0.59	0.54	0.63	0.85	1.00				
Culex pipiens	-0.10	0.02	-0.12	-0.11	-0.11	-0.10	-0.30	-0.43	-0.11	-0.17	-0.06	-0.11	-0.14	-0.22	1.00			
Culex perexigues	-0.21	0.26	-0.09	-0.08	-0.08	0.09	-0.15	-0.36	-0.28	0.08	-0.21	-0.11	-0.13	0.03	-0.14	1.00		
Culex pusillus	-0.09	-0.14	0.79	0.79	0.79	-0.15	0.12	0.17	-0.22	0.68	0.73	0.51	0.95	0.82	-0.13	-0.12	1.00	
Culex theileri	-0.22	-0.09	0.89	0.89	0.89	0.21	0.11	0.26	0.14	0.53	-0.13	0.99	0.70	0.56	-0.09	-0.09	0.43	1.00