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Toxicological Effects of Some Heavy Metal Ions on *Culex pipiens* L. (Diptera: Culicidae)

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ABSTRACT

Different concentrations of selected heavy metals in the form of cadmium chloride ($CdCl_2$), copper sulphate ($CuSO_4$), lead nitrate (Pb $(NO_3)_2$) and mercuric nitrate (Hg $(NO_3)_2$) were tested against immature and mature stage of *C. pipiens* to assess the toxicity, LC_{50}, total carbohydrate and lipid content. The survival potential of 2nd instar larvae was highly affected by the contamination with the tested heavy metals. On the basis of LC_{50}, Cd was the most toxic metal against the larval stage followed by Hg, Cu and Pb. The late toxicity of heavy metals tested on the adult females resulted from larvae treated with the LC_{50} of each heavy metal decreased significantly the number of eggs laid by female. The fecundity recorded 81.7±5.03, 90.3±2.52, 92.7±3.5 and 78.6±1.52 eggs/♀ for females resulted from larvae treated with the LC_{50} of $CdCl_2$, $CuSO_4$, Pb $(NO_3)_2$ and Hg $(NO_3)_2$; respectively, compared to 186±4 eggs/♀ for control females. The hatchability percent of eggs laid with the LC_{50} of $CdCl_2$, $CuSO_4$, $Pb(NO_3)_2$ and Hg $(NO_3)_2$ was significantly decreased to 37, 73, 80 and 39 %; respectively, compared to 97 % for eggs laid by untreated females. A significant decrease in total carbohydrate content in the whole body of males and females, *C. pipiens* resulted from larvae treated with the LC_{50} of $CdCl_2$ or the LC_{50} of Hg $(NO_3)_2$ was observed. Also, the present study showed a significant decrease in total lipid content of females by the LC_{50} $CuSO_4$ and the LC_{50} of Hg $(NO_3)_2$, while in males a significant increased was caused by the LC_{50} $CuSO_4$ and the LC_{50} of $Pb(NO_3)_2$. It is clear from the results obtained in this study that the presence of such elements in the environmental system of the mosquito *C. pipiens* water as possible to contribute to the reduction of mosquito breeding.

Key words: Heavy metals, Toxicity, Fecundity, Hatchability, *C. pipiens*.

INTRODUCTION

Heavy metals such as cadmium, chromium, copper, iron, lead, manganese and zinc are environmentally dangerous substances, and this necessitates their surveillance in aquatic environments. Low concentrations of heavy metals occur in natural aquatic ecosystems, but recent expansions in human population growth, industry, and peri-urban agricultural activities in African cities have led to an increase in heavy metal occurrence in excess of natural loads (Biney *et al*., 1994).

Heavy metal pollution can have a devastating effect on the ecological balance of aquatic environments, limiting the diversity of aquatic organisms and plants. For instance, there are indications that the level of pollution in water bodies directly influences the diversity and abundance of larval stage mosquito species (Chinery, 1984; Coluzzi, 1993 and Coene, 1993).

Anopheles mosquito populations are typically lower in urban environments as compared to rural environments because of high levels of human pollution and perturbation (Trape and Zoulani, 1987). The level of heavy metal contamination may play a limiting role on *Anopheles* mosquito populations in urban environments (Mireji *et al*., 2008).

The biological impact of heavy metals on aquatic insects has been extensively studied in nature and in the laboratory (e. g. Cain *et al*., 1992; Clements and kiffiney, 1994; Dallinger, 1994 and Rayms-Keller *et al*., 1998). Aquatic insects accumulate heavy metals and have long been exploited as indicator species of environmental pollution and for bioassays of pollutants (Hare, 1992). In addition to mortality, exposure of aquatic insects to heavy metals can result in changes in fecundity and fertility. However, only scattered information on effects of heavy metal stress on metabolism, structure and function of the reproductive organs in mosquitoes is available.

Mosquitoes serve as vectors of many vertebrate blood pathogens; *Culex pipiens* is a very common mosquito species in Egypt and is the predominant vector of *Wuchereria bancrofti* that causes filariasis or elephantiasis in humans (Khalil *et al*., 1930 and Gad *et al*., 1996), Rift Valley fever virus (Meagan *et al*., 1980; Darwish and Hoogstraal, 1981) and West Nile virus (Pelah *et al.,* 2002).

The present investigation was carried out to study the larvicidal effects of exposure to selected heavy metal ions namely; cadmium (Cd), copper (Cu), lead (Pb) and mercury (Hg) on *C. pipiens*. Moreover, to study certain biological effects such as total carbohydrate, lipid, female fecundity and hatchability.

MATERIALS AND METHODS

1- Origin and laboratory maintenance of the mosquito colony:

Mosquitoes used in this study were *Culex pipiens* L., they were collected from Abu Rawash, Giza governorate, then were reared for several generations, in the insectary of Medical Entomology at the Department of Zoology, Faculty of Science, Al-Azhar University, under controlled conditions at temperature of 27±2 °C, relative humidity 70±10% and 12-12 light-dark regime. Adult mosquitoes were kept in (30 x 30 x 30 cm) wooden cages and daily provided with sponge pieces soaked in 10% sucrose solution for a period of 3-4 days after emergence. After this period the females were allowed to take a blood meal from a pigeon host, which is necessary for laying eggs (anautogeny). Plastic cup oviposition (15x15cm) containing dechlorinated tap water was placed in the cage.

The resulting egg rafts picked up from the plastic dish and transferred into plastic pans (25 x 30 x 15 cm) containing 3 liters of tap water left for 24 h. The hatching larvae were provided daily with fish food as a diet. This diet was found to be the most preferable food for the larval development and a well female fecundity, (Kasap and Demirhan, 1992).

2- Heavy metals tested:

The salts of heavy metals used in this work were; cadmium chloride ($CdCl_2$), copper sulphate ($CuSO_4$), lead nitrate ($Pb(NO_3)_2$) and mercuric nitrate ($Hg(NO_3)_2$), each of these salts was dissolved in distilled water (dist.) to make a stock solution of 1000 ppm. The stock solution was then diluted to make a series of different concentrations. The concentrations were: A) $CdCl_2$: 0.05, 0.1, 0.15, 0.2 and 0.3 ppm. B) $CuSO_4$: 1, 2, 4, 8 and 10 ppm. C) $Pb(NO_3)_2$: 8, 16, 32, 50 and 75 ppm. D) $Hg(NO_3)_2$: 0.1, 0.2, 0.5, 0.7 and 1 ppm.

3- Experimental bioassay:

In order to study the toxicity of these heavy metals, different range of concentrations of each heavy metal salt was used. The 2nd instar larvae were collected from the established colony and placed in plastic cup its diameter was 12 cm and its hight was 7 cm containing 250 ml of the metal salt solution as recommended by (WHO). Control larvae were placed in cups contained 250 ml dechlorinatedtap water (25 of 2nd instar larvae/cup). At least three replicates were used in each experiment. All plastic cups were incubated under controlled conditions at temperature of 27±2 °C, relative humidity 70±10% and 12-12 light-dark regime. The following biological aspects were used to evaluate the effect of the four heavy metals on *C. pipiens*.

3-1- Larvicidal activity:

Mortality was recorded daily and dead larvae removed until adult emergence. Mortality of the larvae was indicated by a failure to respond to mechanical stimulation (Williams *et al.* 1986). Larval mortality percent was estimated by using the following equation: Larval mortality % = A – B / A × 100 (Briggs, 1960): Where: A = number of tested larvae. B = number of tested pupae.

3-2- Female fecundity:

The adult females that succeeded to emerge from the 2nd instar larvae treated with each concentration were collected and transferred with normal adult males obtained from the colony to the wooden cages (20×20×20 cm) by using an electric aspirator recommended by (WHO), and fed with 10% sugar solution for three days, then, the adult males and females leaved one day without sugar solution. At five day, the starved females were allowed to take a blood meal from a pigeon and allowed to lay egg rafts on clean water. The number of eggs/raft was counted by using binocular microscope and the mean value was taken.

3-3- Egg hatchability:

The eggs of females resulted from the 2nd instar larvae treated with $CdCl_2$, $CuSO_4$, Pb $(NO_3)_2$ and Hg $(NO_3)_2$ were counted by using a binocular microscope. The eggs were sorted into two categories: hatched and non-hatched eggs according to the method used by Hassan *et al.* (1996).

The Egg-hatchability was calculated by using the following equation: Egg-hatchability % = A / B × 100, **Where:** A = total No. of hatched eggs. B = total No. of eggs laid.

4- Biochemical studies:

The resulting adult males and females from treated *C. pipiens* larvae with the LC_{50} of each of $CdCl_2$, $CuSO_4$, Pb $(NO_3)_2$ and Hg $(NO_3)_2$ were collected daily, weighed and kept under freezing condition at 4°C until the biochemical determinations. For the determination of the total protein, lipid and carbohydrate, adults were homogenized in saline solution (40 adults/ 1 ml saline solution) using a fine electric homogenizer, tissue grinder for 2 minutes (min.). Homogenates were centrifuged at 4000 r.p.m. (rotate per min.) for 15 min. at 2°C in a refrigerated centrifuge. The supernatant was used directly or stored at 4°C until biochemical determination.

4-1-Determination of the total carbohydrate content:

The total carbohydrate content of the whole adult body was determined.

4-2- Determination of the total lipid content:

The total lipid content was determined by colourimetric method of (Frings *et al.*, 1972). A sample of whole body extract was heated with conc. sulphuric acid and the mixture was then reacted with phosphoric acid-vanilline reagent to give red to purple colour. The intensity of colour was measured by photoelectric colourimeter (Carlzziss).

Statistical analysis:

The statistical analysis of the obtained data was done according to Armitage (1974) and Lentner *et al.* (1982). The analysis was revised and graphics were drown by Excel for windows program version 2 Microsoft office 2010. The obtained data were assessed by calculation of the mean (M), standard deviation (SD) and student t-test. LC_{50} was calculated using multiple linear regressions (Finney, 1971).

RESULTS

1- Biological activity of heavy metals against *Culex pipiens*:

1-1- Toxicity:

A- Cadmium:

The mortality percentages of *C. pipiens* larvae as influenced by different concentrations of cadmium chloride ($CdCl_2$) are given in table (1). The obtained data indicated that there was a positive correlation between the concentration of the $CdCl_2$ and the mortality percent i.e. the increase of $CdCl_2$ concentration led to the increase of larval mortality percent.

The larval mortality percent increased gradually from 30.6% at the concentration of 0.05 ppm to 92.0% at the concentration of 0.3 ppm. The larval mortality percent among the control group was 10.6%. The calculated LC_{50} from the different mortality percentages recorded 0.11 ppm.

Table 1: Effect of different concentrations of $CdCl_2$ on larval mortality of *C. pipiens*.

Conc. (ppm)	No. of larvae tested	No. of larvae died	Mortality percent	LC_{50} (ppm)
0.05	75	23	30.6	0.11
0.1	75	39	52.00	
0.15	75	47	62.6	
0.2	75	56	74.7	
0.3	75	69	92.00	
Control	75	8	10.6	

B- Copper:

The data given in table (2) Showed the effect of different concentrations of copper sulphate ($CuSO_4$) on the larval mortality percentages. There was a positive correlation between the concentration of $CuSO_4$ and the mortality percent. The larval mortality percent increased from 22.6% at the concentration of 1.0 ppm to 76.0% at the concentration of 10 ppm. The larval mortality percent among the control group was 13.3%. The LC_{50} as calculated from the different mortality percentages recorded 5.09 ppm.

Table 2: Effect of different concentrations of $CuSO_4$ on larval mortality of *C. pipiens*.

Conc. ppm	No. of Larvae tested	No. of larvae died	mortality Percent	LC_{50} (ppm)
1	75	17	22.6	5.09
2	75	24	32.00	
4	75	38	50.6	
8	75	53	70.6	
10	75	57	76.00	
Control	75	10	13.3	

C- Lead:

Data given in table (3) Showed the larval mortality percentages among the larvae treated with different concentrations of lead nitrate ($Pb(NO_3)_2$) as well as the untreated ones (control). The results indicated that the larval mortality percent increased as the concentration of $Pb(NO_3)_2$ increased. The larval mortality percent increased from 4.0% at the concentration of 8.0 ppm to 93.3% at the concentration of 75 ppm. The larval mortality percent was 4.0% among the control group. The calculated LC_{50} from the different mortality percentages recorded 45.36 ppm.

Table 3: Effect of different concentrations of Pb $(NO_3)_2$ on larval mortality of *C. pipiens*.

Conc. ppm	No. of Larvae tested	No. of larvae died	mortality Percent	LC_{50} (ppm)
8	75	3	4.00	45.36
16	75	9	12.00	
32	75	12	29.3	
50	75	22	48.00	
75	75	36	93.3	
Control	75	3	4.00	

D- Mercury:

The results presented in table (4) indicated that the mortality percent among the larvae treated by different concentrations of mercuric nitrate (Hg $(NO_3)_2$) increased as the concentration increased.

The larval mortality percent increased from 28% at the concentration of 0.1 ppm to 93.3% at the concentration of 1.0 ppm. The larval mortality percent was 14.6% among the untreated control group. The calculated LC_{50} recorded 0.44 ppm.

Table 4: Effect of different concentrations of Hg $(NO_3)_2$ on larval mortality of *C. pipiens*.

Conc. ppm	No. of larvae tested	No. of larvae died	Mortality percent	LC_{50} (ppm)
0.1	75	21	28	0.44
0.2	75	28	37.3	
0.5	75	37	49.3	
0.7	75	55	73.3	
1.0	75	70	93.3	
control	75	11	14.6	

From the aforementioned results it is obvious that the toxicity values of the tested heavy metals based on LC_{50} (Table 5) may be arranged in descending order as follows: $CdCl_2$ > Hg $(NO_3)_2$ > $CuSO_4$ > Pb $(NO_3)_2$.

Table 5: Toxicity of different heavy metal salts against larvae of *C. pipiens*.

Heavy metal salts	LC_{50} (ppm)	Slope (b)	Correlation Coefficient (r)
Cadmium chloride	0.11	268.46	0.9796
Copper sulphate	5.١	6.2996	0.9830
Lead nitrate	45.٤	1.1997	0.9815
Mercuric nitrate	0.44	75.194	0.9903

1- 2- Fecundity:

The number of eggs laid per female (fecundity) for *C. pipiens* females resulted from treated larvae with the LC_{50} of $CdCl_2$, $CuSO_4$, Pb $(NO_3)_2$ and Hg $(NO_3)_2$-salts and others (untreated) is given in table (6). As shown from the results there was a significant decrease ($p>0.05$) of eggs laid by females resulted from larvae treated with the LC_{50} of $CdCl_2$, $CuSO_4$, Pb $(NO_3)_2$ and Hg $(NO_3)_2$, where the fecundity was 81.7±5.03, 90.3±2.52, 92.7±3.5 and 78.6±1.52 eggs/♀; respectively, compared to 186±4 eggs/♀ for the untreated females (control).

Table 6: Fecundity and egg hatchability of *C. pipiens* as affected by treatment of 2nd larval instars with the LC_{50} of different heavy metal salts.

Heavy metal salts Conc.(ppm)	LC_{50} (ppm)	No. of females tested	No. of egg laid/female		No. of egg hatched	
			Total	Mean ± SD	Total	%
Cadmium Chloride	0.11	25	2041	81.7±5.03***	755	37.0
Copper Sulphate	5.1	25	2258	90.3±2.52***	1648	73.0
Lead nitrate	45.4	25	2316	92.7±3.5***	1853	80.0
Mercuric nitrate	0.44	25	1966	78.6±1.52***	767	39.0
Control	--	25	4650	186.4±4	4510	97.0

*** = Very highly significant

1-3- Egg hatchability:

The hatchability percent of eggs laid by *C. pipiens* females resulted from treated larvae with the LC_{50} of $CdCl_2$, $CuSO_4$, $Pb(NO_3)_2$ and Hg $(NO_3)_2$, and the other resulted from untreated larvae is also given in table (6). The results indicated that heavy metal salts used decreased significantly the hatchability percent of eggs laid by females resulted from treated larvae as compared with the control. The hatchability percent recorded was 37, 73, 80 and 39% for eggs laid by females treated with the LC_{50} of $CdCl_2$, $CuSO_4$, Pb $(NO_3)_2$ and Hg $(NO_3)_2$; respectively, compared to 97% for eggs laid by females resulted from untreated larvae.

2- Effect of heavy metals on some biochemical parameters in *C. pipiens*:

2-1- Total carbohydrates content:

Data given in table (7) show the changes in the total carbohydrate content in the homogenate of the whole body of *C. pipiens* adults resulted from larvae treated with the LC_{50} of $CdCl_2$, $CuSO_4$, Pb $(NO_3)_2$ and Hg $(NO_3)_2$.

The results indicated a very highly significant ($p>0.001$) and a highly significant ($p>0.01$) decrease in total carbohydrate content in the whole body of males and females resulted from larvae treated with the LC_{50} of $CdCl_2$; respectively. The total carbohydrate recorded 0.09 and 0.08 mg/ml compared to 0.13 and 0.13 mg/ml for untreated males and females; respectively. Also a very highly significant ($p>0.001$) decrease in the total carbohydrate content in both males and females resulted from larvae treated with the LC_{50} of Hg $(NO_3)_2$ was found, where it recorded 0.07 and 0.08 mg/ml compared to 0.13 and 0.13 mg/ml for untreated males and females; respectively.

On the other hand, the change in total carbohydrate content in males and females resulted from larvae treated with the LC_{50} of $CuSO_4$ was non significant, while in males and females resulted from larvae treated with the LC_{50} of Pb $(NO_3)_2$ was significant ($p>0.05$). It recorded 0.12 and 0.12 mg/ml in males and females resulted from larvae treated with the LC_{50}

of $CuSO_4$ and 0.11 and 0.11 mg/ml in males and females resulted from larvae treated with the LC_{50} of Pb $(NO_3)_2$; respectively. By comparing the percentages of reduction in the total carbohydrate content of males and females resulted from larvae treated with $CdCl_2$ and Hg $(NO_3)_2$ it seemed that mercury caused the highest reduction percent 46.15% in the total carbohydrate content of males, while cadmium caused reduction percentages of 30.76 and 38.46 in total carbohydrate of males and females; respectively.

Table 7: Changes in the total Carbohydrate Contents of *C. pipiens* resulted from larvae treated with the LC_{50} of different heavy metal salts.

Treatments	LC_{50} ppm	Carbohydrates (mg/ml) Mean±SD	Change percent
Control (male)	--	0.13±0.01	--
Control (female)	--	0.13±0.01	--
$CdCl_2$ (male)	0.11	0.09**±0.002	-30.76
$CdCl_2$ (female)	0.11	0.08**±0.009	-38.46
$CuSO_4$ (male)	5.1	0.12±0.001	-7.69
$CuSO_4$ (female)	5.1	0.12±0.001	-7.69
$Pb(NO_3)_2$ (male)	45.4	0.11*±0.002	-15.38
$Pb(NO_3)_2$ (female)	45.4	0.11*±0.001	-15.38
$Hg(NO_3)_2$ (male)	0.44	0.07***±0.008	-46.15
$Hg(NO_3)_2$ (female)	0.44	0.08**±0.005	-38.46

2-2-Total lipids content:

As shown from the results given in table (8) a very highly significant (p>0.001) and highly significant (p> 0.01) increase in total lipids content in males resulted from larvae treated with LC_{50} of $CdCl_2$, $CuSO_4$ and Pb $(NO_3)_2$ was occurred, where it recorded 0.88+0.0013, 0.16±0.01 and 0.14±0.004 mg/ml; respectively, compared to 0.09±0.015 mg/ml for untreated control males. The percentage of increase in total lipids was 87.8%, 77.78% and 55.56% for $CdCl_2$, $CuSO_4$ and Pb $(NO_3)_2$-treated males; respectively.

Table 8: Changes in the total Lipid Contents of *C. pipiens* resulted from larvae treated with the LC_{50} of different heavy metal salts.

Treatments	**LC_{50} ppm**	**Lipids (mg/ml) Mean±SD**	**Change percent**
Control (male)	--	0.09±0.015	--
Control (female)	--	0.96±0.005	--
$CdCl_2$ (male)	0.11	0.88***±0.0013	+87.8
$CdCl_2$ (female)	0.11	0.09***±0.003	- 90.62
$CuSO_4$ (male)	5.1	0.16**±0.01	+ 77.78
$CuSO_4$ (female)	5.1	0.14***±0.012	- 85.42
$Pb(NO_3)_2$ (male)	45.4	0.14**±0.004	+55.56
$Pb(NO_3)_2$ (female)	45.4	0.99±0.05	+ 3.13
$Hg(NO_3)_2$ (male)	0.44	0.09±0.0002	0
$Hg(NO_3)_2$ (female)	0.44	0.81*±0.09	- 15.62

On the other hand, $CdCl_2$ and $CuSO_4$ caused a very highly significant (p>0.001) decrease in the total lipids content of females, where it recorded 0.09+0.003 and 0.14±0.012 mg/ml; respectively, compared to 0.96±0.005 mg/ml for untreated control females. The percentages of reduction were 90.62%

and 85.42% as induced by cadmium or by copper; respectively. Also, there was a significant decrease in the total lipids content of females resulted from larvae treated with the LC_{50} of Hg $(NO_3)_2$, where it recorded 0.81+0.09 with percentage of 15.62%. However, the other changes in the total lipids as induced by other heavy metals (Table 8) were insignificant ($p>0.05$).

DISCUSSION

1- Effect of heavy metals on some biological parameters in *Culex pipiens*.

Metal pollution often reduces the fitness of organisms to such extent that species diversity in polluted environments is strongly reduced (Brown, 1977; Clements *et al*., 1988 and Gunn, 1995). Aquatic insects, which are often the most abundant and diverse group of benthic animals in fresh water ecosystems accumulate heavy metals and have long been exploited as indicator species of environmental pollution and for bioassays of pollutant (Hare, 1992). Moreover, aquatic insects are sensitive bioreporters of heavy metals contamination because exposure occurs during critical stage of insect development such as embryogenesis, larval development and pupation.

The biological impact of heavy metals on aquatic insects has been extensively studied in nature and in the laboratory (e.g. Clements *et al*., 1988; Cain *et al*., 1992; Timmermans *et al*., 1992 and Clements & Kiffney, 1994).In addition to mortality, exposure of aquatic insects to heavy metals can result in changes in fecundity and fertility. However, some mosquitoes can survive in polluted waste water (Kitvatanachai *et al*., 2005). The toxicity of some heavy metals against these mosquitoes is not yet known.

In this study, the results obtained revealed that the heavy metals namely; Cd, Cu, Pb and Hg were found to exert biological effects on the larvae of *C. pipiens*. The survival potential of the 3rd instar larvae was highly affected by the contamination with the heavy metals tested. A concentration dependent mortality percent was obtained i.e. the larval mortality percent increased as the concentrations of heavy metals increased. However, the present data revealed that the toxicity of heavy metals tested against the larval stage varied from one metal to another. On the basis of LC_{50}, Cd was the most toxic metal against the larval stage followed by Hg, Cu and Pb. These results are in agreement with those obtained by Migula (1989), where he reported high toxicity of cadmium followed by lead against *Acheta domesticus.*

Heavy metal effects on larval mortality were not unexpected and similar results have been observed in other dipterans (Hare, 1992). In general, deleterious effects were directly proportional to metal concentration. The present results are in a harmony with that of Hafez *et al.* (1999) who found that survivorship of *C. pipiens* larvae significantly decreased as the concentration of cadmium increased. Also, Salama (2002) proved that the larval mortality percent of *C. pipiens* increased as the concentration of contaminants namely; Cd, Hg and Pb increased.

The present data have shown also that the LC_{50} was 0.11, 5.09, 45.36 and 0.44ppm for Cd, Cu, Pb and Hg; respectively, against *C. pipiens* larvae. Meanwhile, Jiang *et al*. (1988) reported that, the LC_{50} was 10.5 ppm on the larvae of *C. pipiens* pallens. The LC_{50} of the heavy metals studied may be comparable with that obtained by Rayms-Keller *et al*. (1998) against *Aedes aegypti* larvae and Salama (2002) against *C. pipiens* larvae.

The toxicity action of heavy metals has been reported also against several species of insects. Pascoe *et al*. (1989) found that, relatively high larval mortality occurred during the first instar larvae of *Chironomus riparius* (Meigen) treated with cadmium and this action increased as the concentration of cadmium increased. The present data were in harmony with these observations. Contrary, Timmermans *et al*., (1992) showed that long term exposure experiments with low cadmium concentrations resulted in high mortality in first instar stages of *C. riparius* (Meigen).

Concerning the effect of heavy metals on reproduction, reports on the acute and chronic toxic effects of heavy metals on insect reproduction are frequent in literature. Several studies have demonstrated pleiotrophic chronic effects of Cd on insect physiology, affecting processes such as growth, development, reproduction and/or hatchability (Van-Straalen *et al*., 1989; Mathova, 1990; Schmidt *et al*., 1992; Gintenreiter *et al*., 1993; Rayms-Keller *et al*., 1998 and Sildanchandra & Crane, 2000). However, the interruption of insect reproduction is an important and potent effect for heavy metals.

The present study has shown that the delayed toxicity of heavy metals on the adult females resulted from larvae treated with the LC_{50} of the heavy metals tested decreased significantly the number of eggs. The fecundity was 81.7±5.03, 90.3±2.52, 92.7±3.5 and 78.6±1.52 eggs/♀ for females resulted from larvae treated with the LC_{50} of $CdCl_2$, $CuSO_4$, Pb $(NO_3)_2$ and Hg $(NO_3)_2$; respectively, compared to 186±4 eggs/♀ for untreated females (control). These results may be comparable with those obtained by Salama (2002) using different concentrations against the 3rd larval instar of *C. pipiens*. Also, the reduction in fecundity of females resulted from larvae treated with the LC_{50} as indicated in the present results was in agreement with that of William *et al.* (1987) who demonstrated that female, *C. riparius* laid fewer eggs in high cadmium concentrations (300 and 100 mg/l) than in lower concentrations or clean water. Moreover, the present study has shown that lead significantly reduced the fecundity of *C. pipiens* females which was in accordance with observations of Kitvatanachai *et al*., (2005) on *Culex quinquefasciatus*.

The observed inhibition of hatching of eggs laid by females resulted from larvae treated with the LC_{50} of the heavy metals tested as indicated in the present study was in agreement with Rayms-Keller *et al*., (1998) using Cd, Cu, Pb and Hg against *A. aegypti,* Salama (2002) using Cd, Cu, Pb, Hg and Zn against *C. pipiens* and Kitvatanachai *et al*., (2005) using Pb against *C. quinquefasciatus*. However, Romi *et al*. (2000) found that there were no effects on egg hatchability of *Aedes albopictus* treated with $CuSO_4$.

2- Effect of heavy metals on some biochemical parameters in *C. pipiens*.

With regard to environmental contamination, research on heavy metals effects on mosquito biochemistry has started only recently. Only scattered information on effects of heavy metal stress on metabolism is available. Conclusion on how heavy metals interfere with general metabolic pathways can probably be drawn from the determination of total proteins, carbohydrates and lipids in the haemolymph or the target organ or even the whole body.

The present study has shown a significant decrease in total carbohydrates content in the whole body of males and females, *C. pipiens* resulted from larvae treated with the LC_{50} $CdCl_2$ or the LC_{50} of Hg $(NO_3)_2$. Meanwhile, the change in total carbohydrates content in males and females, *C. pipiens* resulted from larvae treated with the LC_{50} $CuSO_4$ or Pb $(NO_3)_2$ was insignificant. The present study has shown also a significant decrease in total lipids content of females by the LC_{50} of $CuSO_4$ and the LC_{50} of Hg $(NO_3)_2$, while a significant increase was caused in males by the LC_{50} of $CuSO_4$ and LC_{50} of $Pb (NO_3)_2$.

The effect of heavy metals tested on total carbohydrates and lipids in adults, *C. pipiens* is in accordance with observations of other authors. Cadmium toxicity on metabolic processes has already been demonstrated for *A. domesticus*, where Cd-contaminated food caused a strong inhibition of the respiratory metabolism (Migula, 1989), reduced the assimilation efficiency and increased the energetic maintenance costs during development (Migula *et al*., 1989). Radhakrishnaiah and Busappa (1986) demonstrated shifts in the carbohydrate metabolism in the fresh water field crab, *Oziotelphusa senex senex* due to exposure to sublethal concentration of cadmium. Bischof (1995) reported a drastical decrease of glycogen in the body tissue of *Lymantria dispar* larvae after cadmium and zinc contamination. In addition, lipid concentration declined in the haemolymph and total body tissue due to the two heavy metals. Also, the present results agree with Ortel (1996), where he demonstrated that whole body lipid concentration of day-3 (4^{th} instar) larvae of *L. dispar* was significantly reduced due to cadmium concentration.

There are also some reports dealing with effects of heavy metals on total carbohydrates and lipids in insects which support the present results. For example, Islam and Roy (1983) reported a significant decrease in levels of lipids and carbohydrates in haemolymph, fat body and ovaries of bug, *Chrysocharis stolli* after injection with 5 µg Cd per individual. Also, a significant decrease in total lipid content in Cd-contaminated larvae and pupae of the greater wax moth, *Galleria mellonella* was observed by Byung-Silk *et al*. (2001).

Generally, the present results have shown that the two heavy metals, cadmium and mercury were found to be the most effective ones in inducing the decline of the main metabolites which agree the previous finding of the aforementioned authors. Meanwhile, copper and lead were insignificant in this respect. These results are in harmony with that of Cass and Hill (1980), whom found that copper and zinc are metals of great importance in biological processes and metabolism, where they are enzymatic factors. Also copper is a constituent of several insect enzymes including phenol oxidase and tyrosinae (Nilsson, 1970; Hackman, 1974; McFarlane, 1974 and Bagatto & Shorthouse, 1996) and this may explain that copper is an essential ion in insect metabolism.

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ARABIC SUMMARY

التأثيرات السمية لبعض ايونات العناصر الثقيلة على بعوضة كيولكس بيبنز (ثنائية الأجنحة: كيولسيدى)

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تم في هذه الدراسة معاملة الدور اليرقى الثاني لبعوضة *كيولكس بيبنز* بتركيزات معينة من العناصر الثقيلة: كلوريد الكادميوم، كبريتات النحاس، نترات الرصاص و نترات الزئبق وذلك لتقدير السمية والتركيز النصف المميت لكل منها و كذلك محتوى الكربوهيدرات و الدهون للطور اليافع.

أظهرت نتائج الدراسة أن للعناصر الثقيلة: الكادميوم، النحاس، الرصاص والزئبق تأثيرات حيوية على بعوضة *كيولكس بيبنز*. وأن إمكانية معيشة الدور اليرقى الثالث تتأثر بتلوثه بالعناصر الثقيلة المختبرة وقد كانت نسبة الوفيات في صورة علاقة طردية تزداد بزيادة التركيز.

وعلى أساس التركيز النصف المميت لها، إتضح أن الكادميوم هو العنصر الأكثر سمية على الدور اليرقى الثالث يليه الزئبق ثم النحاس ثم الرصاص.

كما إتضح أن السمية المتأخرة للعناصر الثقيلة المختبرة على الإناث اليافعة الناتجة من اليرقات المعاملة بالتركيز النصف المميت من كل عنصر سببا في إنقاص إنتاجية البيض من الأنثى بشكل معنوي ملحوظ. حيث كان عدد البيض الموضوع ٨١.٧ ±٥.٠٣ ، ٩٠.٣ ±٢.٥٢ ،٩٢.٧ ±٣.٥ ، ٧٨.٦ ±١.٥٢ للإناث الناتجة من اليرقات المعاملة بالتركيز النصف المميت من عناصر: كلوريد الكادميوم، كبريتات النحاس، نترات الرصاص و نترات الزئبق على الترتيب مقارنة ب ١٨٦ ±٤ للمجموعة الضابطة. وأوضحت النتائج أن نسبة فقس البيض الموضوع بواسطة الإناث الناتجة من اليرقات المعاملة بالتركيز النصف مميت من عناصر: الكادميوم، النحاس، الرصاص و الزئبق قلت بشكل ملحوظ الى ٣٧، ٧٣، ٨٠ و ٣٩ % على الترتيب مقارنة ب ٩٧% للبيض الموضوع بواسطة الإناث الضابطة.

وكذلك أظهرت الدراسة الحالية نقص معنوي ملحوظ في المحتوى الكلى للكربوهيدرات في الجسم الكامل لذكور وإناث بعوضة *كيولكس بيبنز* الناتجة من اليرقات المعاملة بالتركيز النصف المميت من عنصري: الكادميوم والزئبق. بينما كانت التغيرات في المحتوى الكلى للكربوهيدرات في الإناث والذكور الناتجة من اليرقات المعاملة بالتركيز النصف المميت لعنصري: النحاس والزئبق غير معنوية. أيضا أظهرت الدراسة نقص معنوي ملحوظ في المحتوى الكلى للدهون بالنسبة للإناث المعاملة بالتركيز النصف المميت من عنصري: النحاس والزئبق. بينما كانت هناك زيادة معنوية ملحوظة في الذكور المعاملة بالتركيز النصف المميت لعنصري النحاس والرصاص.

ويتضح من النتائج المتحصل عليها في هذه الدراسة أن وجود مثل هذه العناصر في النظام البيئي المائي لبعوض *الكيولكس* ممكن أن تساهم في الحد من تكاثر البعوض.