Anadolu Tarım Bilimleri Dergisi,
Anadolu Journal of Agricultural Sciences

e-ISSN: 1308-8769, ANAJAS Ekim 2024, 39 (3): 637-648

Effects of Mercury on the Growth and Development of *Musca Domestica* (Diptera: Muscidae)

Civanın *Musca Domestica*'nın Büyüme ve Gelişimi Üzerindeki Etkileri

Nazlı SERT[1], Meltem KÖKDENER[2]

[1]Department of Forensic Science, Ondokuz Mayıs University, Institute of Science, Samsun, Türkiye
· **nzlisertt@gmail.com** · ORCİD > 0009-0005-3796-3251

[2]Faculty of Health Sciences, Ondokuz Mayıs University, Samsun, Türkiye
· **kokdener@omu.edu.tr** · ORCİD > 0000-0002-0107-3274

Makale Bilgisi/Article Information

Makale Türü/Article Types: Araştırma Makalesi/Research Article
Geliş Tarihi/Received: 29 Ocak/January 2024
Kabul Tarihi/Accepted: 18 Temmuz/July 2024
Yıl/Year: 2024 | **Cilt-Volume:** 39 | **Sayı-Issue:** 3 | **Sayfa/Pages:** 637-648

Atıf/Cite as: Sert, N., Kökdener, M. "Effects of Mercury on the Growth and Development of *Musca Domestica* (Diptera: Muscidae)" Anadolu Journal of Agricultural Sciences, 39(3), Ekim 2024: 637-648.

Sorumlu Yazar/Corresponding Author: Nazlı SERT

https://doi.org/10.7161/omuanajas.1427175

EFFECTS OF MERCURY ON THE GROWTH AND DEVELOPMENT OF *MUSCA DOMESTICA* (DIPTERA: MUSCIDAE)

ABSTRACT

Mercury is a highly toxic heavy metal and a serious source of environmental pollutants. The purpose of the present study was to determine the effects of mercury on some life history parameters of *Musca domestica* Linnaeus, 1758 (Diptera: Muscidae). Forty larvae of *M. domestica* were placed on rearing media with three different concentrations of mercury (1.5 µg/g, 2 µg/g, 2.5 µg/g), and some life history parameters recorded (larval and pupal periods, larval, pupal and adult weights, larval and pupal survival rate). The development of *M. domestica* was studied at 30°C, 50% RH, and a photoperiod of 12:12 (L:D) h.

In the present study, larval and pupal survival decreased as mercury concentrations increased and mercury decreased the pupal weight compared to the control. It has been demonstrated that the life-history parameters of *M. domestica* are sensitive to mercury residue and mercury changes in the environment. This study provides basic knowledge about the biology of this species, suggesting that the effect of the presence of mercury on larval development in corpses found in industrialized areas with high heavy metal pollution should be kept in mind in criminal investigations.

Keywords: Entomotoxicology, Heavy Metal, Housefly, Life History Parameters.

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CİVANIN MUSCA DOMESTİCA'NIN BÜYÜME VE GELİŞİMİ ÜZERİNDEKİ ETKİLERİ

ÖZ

Civa oldukça toksik bir ağır metaldir ve ciddi bir çevre kirletici kaynağıdır. Bu çalışmanın amacı, *Musca domestica* Linnaeus, 1758 (Diptera: Muscidae) türünün yaşam parametreleri üzerinde civanın etkilerini belirlemektir. Kırk adet *M. domestica* larvası, üç farklı civa konsantrasyonu (1.5 µg/g, 2 µg/g, 2.5 µg/g) içeren besleme ortamlarına yerleştirilmiştir ve bazı yaşam öyküsü parametreleri kaydedilmiştir (larva ve pupa dönemleri, larva, pupa ve ergin ağırlıkları, larva ve pupa hayatta kalma oranları). *M. domestica*'nın gelişimi, 30°C sıcaklık, %50 bağıl nem ve 12:12 (A:K) fotoperiyot koşullarında incelenmiştir.

Mevcut çalışmada civa konsantrasyonları arttıkça larva ve pupa sağkalımı azalmış ve civa, pupa ağırlığını kontrolle karşılaştırıldığında azaltmıştır. *M. do-*

*mestica'*nın yaşam öyküsü parametrelerinin civa kalıntısına ve çevredeki civa değişikliklerine duyarlı olduğu ortaya konulmuştur. Bu çalışma, bu türün biyolojisi hakkında temel bilgi sağlamaktadır ve ağır metal kirliliğinin yüksek olduğu sanayileşmiş bölgelerde bulunan cesetlerde civa varlığının larva gelişimi üzerindeki etkisinin kriminal soruşturmalarda dikkate alınması gerektiğini önermektedir.

Anahtar Sözcükler: Ağır Metal, Entomotoksikoloji, Ev Sineği, Yaşamsal Parametreler.

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1. INTRODUCTION

Heavy metals are elements characterized by their high densities and atomic weights (Fu and Zi, 2019). Heavy metal pollution is one of the most important environmental problems in the world (Kökdener et al., 2022). Heavy metals are naturally found in the earth's crust; and dispersed to the environment through natural processes such as volcanic eruption, spring waters, erosion, and bacterial activity (Ali et al., 2019). Also heavy metals generally arising from industrialization, transport, metal mining, increased vehicle use, and modern farming practices conduce to environmental pollution that are impacting diverse terrestrial and aquatic organisms (Tchounwou et al., 2012).

Although heavy metals, such as chromium, copper, iron, cobalt, and nickel, are necessary for various physiological and biological processes in the human body (Fu and Zi, 2019), high doses of heavy metals may be toxic to humans and affect many processes. People are exposed to heavy metals directly and indirectly. Long term exposure to heavy metals may result in various disorders such as Parkinson's, muscular dystrophy, Alzheimer's, and cancer (Fu and Xi, 2019).

Heavy metal, is present in the soil, water, and air. Insects are exposed to various heavy metals in their ecosystems (Azam et al., 2015) and these compounds in insects have negative effect on the growth, behavior, physiology, and development of insects (Mogren and Trumble, 2010). Heavy metals can enter the arthropod body through skin penetration ingestion, and respiration (Jiang et al., 2021).

Mercury (*Hg*) exists in elemental, inorganic, and organic forms and is a unique element which is liquid at room temperature. Mercury metal is extensively utilized in various industries such as battery and thermostat production, dentistry, caustic soda production, pharmaceutical preservatives, and nuclear reactors (Tchounwou, 2012). Human may be exposed to mercury under different circumstances, including eating contaminated fish and inhalation of mercury vapor (Kamensky et al., 2019). Mercury poisoning leads to severe dysfunctions, such as bloody diarrhea,

intestinal necrosis, colitis, kidney failure (Mahurpawar, 2015), stomach irritation, nausea, diarrhea, circulatory and respiratory failure (Bai et al., 2020). These compounds can cause organotoxicity and carcinogenic effects (Risher et al., 1999).

M. domestica Linnaeus, 1758, also known as the house fly, is a cosmopolitan species and important sanitary pest of humans and animals (Malik et al., 2007; Iqbal et al., 2014; Davies et al., 2016; Khamesipour et al., 2018). It is found in close association with human activities and a carrier of a large number of vectors of many human and animal diseases. Houseflies are medically and forensically important flies (Chin et al., 2008). Many diseases transmitted by *M. domestica* include hepatitis, cholera, dysentery, and tuberculosis (Erdoğan, 2017; El-Hamid et al., 2018). It may be used as an alternative protein source for poultry and fish (Fitches et al., 2018). Houseflies can be employed in environmental studies and forensic investigation as biological indicators.

Although there have been several studies showing the adverse impacts of heavy metals on *M. domestica* development (Niu C. Y. et al. 2002; Borowska and Pyza, 2011; Haq et al., 2012; Wang et al. 2021; Kökdener, 2022), there has been little research about the effects of mercury on the development of *M. domestica* (Raina et al., 2001; Mishra and Tewari, 2011). The presence of toxic substances in corpses can directly impact fly development and lead to the calculation of an inaccurate postmortem interval.

Therefore, given its economic, forensic, and veterinary importance, it is clear that further research is needed on the biology and life history of the house fly. The purpose of the current study was to determine the effects of mercury on the life cycle of *M. domestica*. Hence, the present comparative study focuses on the effects of mercury on the developmental stages and different vital parameters of this species.

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2. MATERIALS AND METHODS

2.1. M. Domestica Colony

This study was carried out in the the animal physiology laboratory at the Faculty of Science of Ondokuz Mayıs University, Samsun (41° 15′ N, 36° 19′ S), Turkey between May and September 2023 to examine the effects of some life history parameters of *M. domestica* fed on diets with different mercury concentrations. Some of the eggs collected were used to maintain the colony and some of the eggs were used for experiments. *M. domestica* adults were collected from Ondokuz Mayıs University campus in 2021 and added periodically with wild-caught adults.

Adult flies were cultured in fly cages (50 cm X 50 cm X 50 cm) at 30 °C and 50 % humidity with a photoperiod of 12:12 (L:D). Water and sugar were supplied ad libitum. Wheat bran-milk mixture was used as oviposition substrates and larval rearing diet. Five days after adult emergence, a Petri dish (9 cm) containing wheat bran diet was provided as an oviposition substrate for 24 hr. Newly hatched first instar larvae were placed on a wheat bran diet in a 500 ml glass jar (Paşabahçe, Turkey) and reared under the same conditions described earlier.

2.2. Experimental Design

The compound mercuric chloride ($HgCl_2$) at three concentrations (1.5 µg/g, 2 µg/g, and 2.5 µg/g) was used in this study. The metal salts were dissolved in distilled water to prepare a stock solution of 2,000 ppm. which was diluted with distilled water to prepare the series of various concentrations. All metal concentrations were left for 3 hours in laboratory conditions until used. The wheat bran diet was prepared by mixing different concentrations of mercury and wheat bran that was mixed with milk served as the control treatment without heavy metals (untreated).

Forty first-instar larvae were transferred into a 500 ml jar containing a diet, and a total of 1080 larvae were utilized. The labeled plastic containers were transferred to the growth room.

Two larvae were collected and measured daily until pupation. Larval length was measured using a ruler, and larval weight was measured with microbalance (AUW 220D, Shimadzu Corporation, Kyoto, Japan). Pupae were monitored every 12 hours until adult emergence. The total number of pupae and adults, larval length and weight, the duration of larval and pupal development stages, and larval and pupal mortality were recorded. The resulting adults were checked every 8 hours and not provided food and water. After adults died, weight and sex were recorded.

2.3. Statistical Analysis

The data analysis was performed on SPSS 21.0 software (SPSS, Inc., an IBM Company, Chicago, IL). Shapiro-Wilk and Levene's tests were used to check the data for homogenous variances and normality. Larval and pupal survival, and pupal, larval and adult weight, and larval length were compared using One Way ANOVA, followed by the Tukey HSD test for means comparison. Chi-square analysis was used to test the dose-dependent relationship between the number of pupae, adults, and the number of females and males. In all tests, significance levels were determined at the level of at **α =0.05).**

3. RESULTS

3.1. Larval and Pupal Development Durations

The duration of larval and pupal development are shown in Table 1. Our results showed that the larval and pupal development periods were impacted by mercury. Larvae exposed to different concentrations of mercury developed slower compared to the control group. Mean larval development duration was similar to the control when reared on a diet with mercury at concentration 3 (2.5 µg/g). The pupal development period decreased with increasing mercury concentration compared to the control group. The shortest pupal development period (3d) was recorded at a concentration 3 (2.5 µg/g).

Table 1. Larval and pupal development durations under varying mercury concentrations

Concentration	Larval Period (day) (Mean ±SE)	Pupal Period (day) (Mean±SE)
1	4.27±0.04b	5.25±0.06b
2	4.17±0.06b	4.20±0.02b
3	3.15±0.06a	3.12±0.04a
Control	3.07±0.04a	6.15±0.06c
	F=118.738	F=531.037
	P<0.001	P<0.001

* Concentration 1 (1.5 µg/g) concentration 2 (2 µg/g), and concentration 3 (2.5 µg/g), the differences between the means indicated by different letters in the same column are statistically significant (p<0.05)

3.2. Larval Length and Weight

The mean larval length and weight are presented in Table 2 and Table 3, respectively. The findings of the current study suggested that mercury exposure affects the larval weights negatively. The maximum larval weight was recorded (0.0215 g) in the control group. The results of this study also demonstrate that mercury exposure negatively affects the larval length. The maximum larval length was observed at the control (14 mm). The total larval length significantly differed among concentrations (F =15.495; *P< 0.001).* In the present study, the larval weight and length of *M. domestica* decreased with increasing mercury concentrations.

Table 2. Larval length (mm) of *M. domestica* at different mercury concentrations

Concentration	1st Instar Larvae	2nd Instar Larvae	3rd Instar Larvae
1	2.08±0.20b	9.06±0.60b	10.33±0.44b
2	2.00±0.18a	8.00±0.51b	8.56±0.60ab
3	1.50±0.18a	5.08±0.37a	7.00±0.57a
Control	2.76±0.08b	9.95±0.47b	13.33±1.05c
	F=9.462	F=15.172	F=15.495
	P<0.001	P<0.001	P<0.001

* Concentration 1 (1.5 µg/g) concentration 2 (2 µg/g), and concentration 3 (2.5 µg/g), the differences between the means indicated by different letters in the same column are statistically significant (p<0.05)

Table 3. Larval weight (mg) of *M. domestica* at different mercury concentrations

Concentration	1st Instar Larvae	2nd Instar Larvae	3rd Instar Larvae
1	0.0024±0.001b	0.0104±0.011b	0.0129±0.010b
2	0.0013±0.001ab	0.0092±0.004b	0.0123±0.009b
3	0.0007±0.000a	0.0015±0.005a	0.0024±0.008a
Control	0.0029±0.002b	0.0117±0.008b	0.0154±0.020b
	F=39.537	F=45.170	F=21.593
	P<0.001	P<0.001	P<0.001

* Concentration 1 (1.5 µg/g) concentration 2 (2 µg/g), and concentration 3 (2.5 µg/g), the differences between the means indicated by different letters in the same column are statistically significant (p<0.05)

3.3. Pupal and Adult Weights

The mean pupal and adult sampled from different concentrations of mercury are presented in Table 4. Pupal weight was significantly different among concentrations (F=5.465; *P<0.001*). Adult weights decreased with increasing mercury concentrations. Male weight was significantly different among concentrations (F=3.070; *P=0.033*) while female weight was not significantly different among concentrations (F=0.500; *P<0.683*). The highest pupal, female, and male weights were observed at control, while the lowest pupal, female, and male weights were observed at concentration 3 (2.5 µg/g). The lowest pupal weight (0.0053 g) was observed in a concentration of 3 (2.5 µg/g).

Table 4. Pupal and adult weight (mg) values at different concentrations of mercury

Concentration	Pupa Weight (Mean±SE)	Female Weight (Mean±SE)	Male Weight (Mean±SE)
1	0.0137±0.005b	0.0022±0.001	0.0020±0.001ab
2	0.0132±0.006b	0.0021±0.001	0.0018±0.001ab
3	0.0079±0.006a	0.0020±0.001	0.0017±0.002a
Control	0.0201±0.006c	0.0023±0.003	0.0024±0.001b
	F=5.465	F=0.500	F=3.070
	P<0.001	P<0.683	P=0.033

* Concentration 1 (1.5 µg/g) concentration 2 (2 µg/g), and concentration 3 (2.5 µg/g), the differences between the means indicated by different letters in the same column are statistically significant (p<0.05)

3.4. Numbers of Pupae and Adults of M. Domestica

Larval and pupal survival are summarized in Table 5. Larval survival was significantly different between concentrations (F=29.588; *P<0.001*). The results from this study showed that larval and pupal survival impacted as mercury concentrations. The lowest number of pupae (12) was recorded at concentration 3, while the highest number of pupa (25) was observed in the control group. Pupal survival was significantly different among concentrations (F=34.272; *P<0.001*). Male and female survival was significantly different among concentrations (for male: F=30.983; *P<0.001*; for female: F=7.132, *P<0.005*). Chi-square analysis was used to investigate whether there was a relationship between dose and the number of pupae, adults, females, and males. According to the results of the chi-square analysis, there was a statistically significant relationship between dose and number of pupae and adult, with chi-square values of 22.868 and *P<0.001* significance level. Similarly, a statistically significant relationship was observed between the dose and number of females and males, with a chi-square value of 46.667 and a *P<0.001* significance level.

Table 5. Numbers of pupae, adult, female and male of *M. domestica* at different mercury concentrations

Concentration	Number of Pupae	Number of Adult	Number of Female	Number of Male
1	16.00±0.64a	11.00±0.85a	5.00±0.62a	6.00±1.15a
2	15.00±.0.64a	11.00±0.86a	7.00±0.70a	4.00±0.28a
3	12.00±1.08a	9.00±1.64a	6.00±0.47a	3.00±0.25a
Control	25.00±1.54b	21.00±1.47b	9.00±1.00b	12.00±0.95b
	F=29.588	F=34.272	F=7.132	F=30.983
	P<0.001	P<0.001	P<0.005	P<0.001

* Concentration 1 (1.5 µg/g) concentration 2 (2 µg/g), and concentration 3 (2.5 µg/g), the differences between the means indicated by different letters in the same column are statistically significant (p<0.05)

4. DISCUSSION

Heavy metals are remain persistent in the environment (Butt et al. 2018). Heavy metals can enter many organisms through different pathways and lead to harmful effects on enzymatic processes and metabolism. In addition, they have destructive impacts on the ecological balance and can damage DNA structures to living organisms. Thus, arthropods are sensitive to ecological and environmental changes and are used as environmental pollution bioindicators. Heavy metals adversely impact population dynamics, density, survival, development, reproduction, and biodiversity of insects (El-Sheikh et al., 2010). The extent of heavy metal accumulation in insects depends on several factors, including specific heavy metals, type of substrate, insect species, and environmental conditions (Meyer et al., 2021).

Heavy metals have adverse impacts on the process of metamorphosis, development, and growth (Beamish et al., 2021; Rebolloso Hernández et al., 2023). Our results suggested that mercury has a substantial adverse effect on some life-history parameters of *M. domestica*. Previous studies showed that different heavy metals impacts on the development and survival of *M. domestica* (Borowska and Pyza, 2011; Mishra and Tewari, 2011; Haq et al., 2012; Wang et al., 2021; Kökdener 2022).

In the current study, mercury exposure is associated with affected development time of house. Larvae exposed to different concentrations of mercury developed slower compared to the control group and pupal development duration decreased with increasing mercury concentrations. Similarly, Borowska and Pyza (2011) showed that larval development and metamorphosis of *M. domestica* were prolonged by heavy metals exposure such as lead (*Pb*), zinc (*Zn*), copper (*Cu*), and cadmium (*Cd*). Wang et al. (2021) reported that larvae took more hours to reach the pupal stage as mercury concentrations increased.

Raina et al. (2001) showed that the total development duration of *M. domestica* larvae treated with mercuric chloride was longer than the development duration in the control group. Similarly, Kökdener and Yılmaz (2021) showed that the total development duration of *Lucilia sericata* (Meigen, 1826) (Diptera: Calliphoridae) decreased with increasing antimony (*Sb*), barium (*Ba*), and *Pb* concentrations. Contrary to our study, Frat et al. (2021) reported that the larval development duration increased with increasing *Hg* concentrations in diets. In contrast, Kaur (2016) showed that a high concentration of mercury caused delayed pupal duration of *Chrysomya megacephala* (Fabricius, 1784) (Diptera: Calliphoridae). Abnoos et al. (2013) showed that larval and pupal periods of *Drosophila melanogaster* (Meigen, 1830) (Diptera: Drosophilidae) prolonged with increasing mercury concentrations. Also, Beamish et al. (2021) showed that larval survival of *D. melanogaster* was affected, and the larval development period was prolonged by mercury exposure. Kökdener (2022) showed that increasing lead concentrations prolonged the develop-

ment period of *M. domestica*. Heavy metals could adversely affect the metabolism of carbohydrates, lipids, and proteins and alter development durations of arthropods.

Our study showed that the weights of larvae and pupae decreased with increasing of mercury concentration in the diets is a pattern similar to that observed by Kaur (2016) in larvae of *L. sericata* and *C. megacephala*. This is supported by observations made by Niu et al. (2002) who showed that high Cd^{2+}concentrations enriched diets significantly reduced larval weight of *M. domestica*. The lowest pupal weight (0.0053 g) was observed in larvae reared the diet with concentration 3 (2.5 µg/g). Heavy metals can effect decreased diet or food absorption (Frat et al., 2021) and its toxic effect caused smaller pupae, adults, and larvae (Bayley et al. 1995, Servıa et al. 2006, Safaee et al. 2014).

In the present study, the female weight is greater than the male in a pattern similar to that observed by Zheng et al. (2010) in larvae of *Cryptotympana atrata* (Fabricius, 1775) (Hemiptera; Cicadidae), Kaur (2016) in larvae of *C. megacephala*. Adult weight was negatively associated with the concentration of mercury in the present study. Al-Misned, 2001 and Simkiss et al., 1993 found that a diet with cadmium significantly reduced the adult weight of blowflies. Similarly, Kökdener (2022) demonstrated that the pupal and adult weight of *M. domestica* was adversely affected by increasing concentrations of lead. Kökdener and Yılmaz (2021) showed that a diet with *Pb*, *Sb*, and *Ba* significantly reduced the pupal and adult weight of *L. sericata.*

The results of the present study also indicate that mercury exposure negatively affects the larval length. The results are similar to Kaur (2016) who indicated the larval length of the *C. megacephala* decreased with the increase of mercuric chloride concentrations. Abnoos et al., (2013) found that a diet with mercury significantly reduced the length and width of *D. melanogaster.* Several studies have also shown that heavy metal exposure adversely impacts the larval length (Kökdener and Yılmaz., 2021; Kökdener et al., 2022).

The results from this study also showed that larval and pupal mortality of *M. domestica* increased with increasing heavy metals. The possible reason for decreasing larval and pupal survival is exposure to heavy metals that may impact the essential enzyme function, which is responsible for arthropod organogenesis and pupal morphogenesis (Al-Momani and Mossadeh 2005). Similarly, Raina et al. (2001) and Mishra and Tewari (2011) reported that larval mortality of *M. domestica* increased with increasing mercury concentrations.

Several studies showed that the mortality rate increased with increasing lead concentration of *M. domestica* (Haq et al., 2012; Wang et al., 2021). Also, Niu et al. (2002) reported that the emergence of *M. domestica* decreased with the increasing cadmium concentration. Similarly, Kökdener (2022) reported that the pupal

and larval mortality of *M. domestica* increased with increasing lead concentrations. Kökdener and Yılmaz (2021) reported that the larval mortality of *L. sericata* increased with increasing heavy metals concentrations. Kaur, 2016 and Abnoos et al., 2013 indicated that the percentage of pupa and egg hatching decreased with increasing mercury concentrations. Heavy metals have an adversely impact on metabolic genes, their expression (Al-Momani and Massadeh 2005; Safaee et al. 2014; Heer and Singh 2019) and result in reduced body mass and lipid storage (Ilahi et al. 2020). Mercury toxicity induced changes in reactive oxygen species (ROS), which damages proteins and nucleic acids. This toxicity may impact the activities of enzymes responsible for pupal morphogenesis causing increased mortality (Zaman et al., 1994).

5. CONCLUSION

Larval and pupal development durations were affected in the presence of mercury, and larval and pupal mortality increased with increasing concentrations of mercury in the diets. *Hg* exposure significantly reduced adult and pupal weight and adversely affected their life history parameters.

The pupal development duration was shortened compared to the control group. The total development period was approximately 3 days shorter than the control group. The results of our study showed that *M. domestica* is sensitive to environmental stress caused by mercury exposure and is a suitable model organism to examine the effect of heavy metals on the developmental stages of the organism. It would be useful to conduct further laboratory and field studies to determine the effects of heavy metals on insects in a corpse found in industrial areas with high heavy metal pollution.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethics

This study does not require ethics committee approval.

Author Contribution Rates

Design of Study: NS (%50), MK (%50)

Data Acquisition: NS (%50), MK (%50)

Data Analysis: NS (%50), MK (%50)

Writing up: NS (%50), MK (%50)

Submission and Revision: NS (%50), MK (%50)

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