



## Effects of Heavy Metals with Different Concentrations on Some Biological Properties of *Hyphantria cunea* Drury (Lepidoptera: Arctiidae) Larvae

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**Abstract:** The aim of this study was to determine the effects of zinc (Zn), iron (Fe), nickel (Ni), copper (Cu), and cobalt (Co) in different amounts on the total consumption amount, pupal weight, pupal protein and lipid content, the development time of the last instar *Hyphantria cunea*. For this purpose, ten artificial diets were prepared using 0.788 mg/ml and 2.364 mg/ml of each metal. A total of 11 diets were made, including one control diet. Chloroform was used to determine the pupal lipid amounts. Protein analysis was performed by semi-micro Kjeldahl method with Kjelttec Auto 1030 analyzer. ANOVA-Dunnet test was used for statistical analysis. With increasing iron and copper amounts in the diet, the larval consumption amounts, pupal weights, pupal protein and pupal lipid amounts of the insect decreased, while the development time was prolonged. Consumption amounts, pupal weights, pupal protein and pupal lipid amounts of the groups containing nickel and cobalt were found to be higher than the control group. It was determined that with increasing zinc amount, consumption amounts, pupal weights, and pupal protein amounts of the larvae increased, but pupal lipid amounts decreased. In this study, it has been shown that heavy metals affect the physiological processes of *H. cunea* larvae.

**Keywords:** Development time, *Hyphantria cunea*, lipid, protein, pupal weight.

## Farklı Konsantrasyonlardaki Ağır Metallerin *Hyphantria cunea* Drury (Lepidoptera: Arctiidae) Larvalarının Bazı Biyolojik Özelliklerine Etkileri

**Öz:** Bu çalışmanın amacı, farklı miktarlarda çinko (Zn), demir (Fe), nikel (Ni), bakır (Cu) ve kobaltın (Co) son evre *Hyphantria cunea*'nın toplam diyet tüketim miktarı, pupa ağırlığı, pupa proteini ve lipit miktarı ve gelişme zamanı üzerindeki etkilerini araştırmaktır. Bu amaçla, her bir metalden 0.788 mg/ml and 2.364 mg/ml kullanılarak 10 diyet hazırlandı. 1'i kontrol diyeti olmak üzere toplam 11 diyet yapıldı. Pupal lipit miktarlarının belirlenmesinde kloroform kullanıldı. Protein analizi semi-mikro Kjeldahl metodu ile Kjelttec Auto 1030 analizörü ile yapıldı. İstatistik analizlerde ANOVA-Dunnet testi kullanıldı. Diyetteki artan demir ve bakır miktarları ile larvaların tüketim miktarları, pupa ağırlıkları, pupa protein ve pupa lipit miktarları azalırken, gelişme süreleri uzadı. Nikel ve kobalt içeren grupların tüketim miktarlarının, pupa ağırlıklarının, pupa protein ve pupa lipit miktarlarının kontrol grubundan fazla olduğu bulundu. Artan çinko miktarı ile larvaların tüketim miktarlarının, pupa ağırlıklarının, pupa protein miktarlarının arttığı fakat pupa lipit miktarlarının azaldığı belirlendi. Bu çalışmada, ağır metallerin *H. cunea* larvalarının fizyolojik süreçlerini etkilediği gösterildi.

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**Anahtar Kelimeler:** Gelişme süresi, *Hyphantria cunea*, lipit, protein, pupa ağırlığı.

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## INTRODUCTION

Heavy metal pollution is one of the most important causes of environmental pollution. Heavy metals enter the environment from natural (mineral decomposition, erosion, and volcanic activities) and anthropogenic sources (mining, smelting, electroplating, pesticides and fertilizers, as well as the use of biosolids in agriculture, sludge discharge, industrial discharge, atmospheric deposition, etc.) (Ali et al., 2013). Various applications, including chemical fertilizers, cause heavy metals to accumulate in the soil, and this is not only a serious threat to plants but also a potential danger to many organisms, including humans (Hejazizadeh et al., 2016). Because heavy metals cannot be broken down, they accumulate in the body tissues of living organisms (bioaccumulation) and their amounts increase as they move from low trophic levels to higher trophic levels (a phenomenon known as biomagnification) (Ali et al., 2013). Considering the food chain, this situation can reach dangerous levels for living things.

Regarding their role in biological systems, heavy metals are classified as essential and non-essential. Essential heavy metals such as Fe, Mn, Cu, Zn, and Ni are required by living organisms in very small amounts for vital physiological and biochemical functions (Cempel & Nikel, 2006; Göhre & Paszkowski, 2006). Non-essential metals such as Cd, Pb, As, Hg, and Cr are metals that are not needed by living organisms for any physiological and biochemical functions (Dabonne et al., 2010; Sánchez-Chardi et al., 2009). Many heavy metals are toxic even at very low amounts and can cause serious problems (Arora et al., 2008; Kara, 2005). The harmful effects of heavy metals have been demonstrated in studies with various animals (Brasso & Cristol, 2008; Pedersen & Saether, 1999; Witeska et al., 2014).

The effects of metals on insects have been shown in various studies. For example, the longevity and fecundity of adult *Chromatomyia milii* Kaltenbach (Diptera: Agromyzidae) and the survival of the offspring decreased with increased exposure to cadmium (Scheier et al., 2006). It was determined that crickets and locusts that consumed the leaves of *Stanleya pinnata* and *Brassica juncea* with high selenium died (Freeman et al., 2007). Görür (2010) found that cabbage and radish plants contaminated with zinc or cadmium had detrimental effects on the growth of aphids. It was determined that the survival of *Heliothis virescens* Fabricius (Lepidoptera: Noctuidae) decreased with an increase in zinc and cadmium amounts (Kazemi-Dinan et al., 2014).

The fall webworm, *Hyphantria cunea* Drury (Lepidoptera: Arctiidae), is a devastating invasive insect

commonly found in North America, its region of origin (Gomi, 2007). It causes unprecedented damage and economic losses due to its excessive fecundity and wide host range, including forest and shade trees and even crops (Zhang et al., 2016). These insects can cause serious crop losses each year in Turkey.

It is essential to know the biology of the species in the fight against pest species. There are studies on the effects of various factors on the protein, lipid, and carbohydrate content of insects (Büyükgüzel & Kalender, 2008; Sharma et al., 2011). In our study, we aimed to determine how five heavy metals (Fe, Zn, Cu, Ni, and Co) in different amounts affect the consumption amounts, pupal weights, pupal protein and pupal lipid amounts, and development time of *H. cunea* larvae.

## MATERIAL and METHODS

*Hyphantria cunea* larvae were collected in Turkey's Samsun Province Çarşamba District on the field studies. The artificial diet developed by Yamamoto (1969) was used as a control diet to feed the larvae. The content of Yamamoto's artificial diet was wheat germ, casein as the protein, saccharose as the carbohydrate, torula yeast, vitamin mixture, salt mixture, cholesterol, sorbic acid, methyl paraben, linseed oil, agar, and water. Different diets were prepared by adding different amounts of Fe, Zn, Cu, Ni, and Co into this diet. For this, heavy metals prepared as 0.788 mg/ml and 2.364 mg/ml were added to the diets. In total, 11 diets were prepared, one of which was the control diet (Table 1).

**Table 1.** Diet types and diet contents.

Diet types	Diet contents
A	Control Diet (CD)
B	0.788 mg/ml Fe
C	2.364 mg/ml Fe
D	0.788 mg/ml Zn
E	2.364 mg/ml Zn
F	0.788 mg/ml Cu
G	2.364 mg/ml Cu
H	0.788 mg/ml Ni
J	2.364 mg/ml Ni
K	0.788 mg/ml Co
L	2.364 mg/ml Co

Thirty last instar larvae were put into plastic containers containing artificial diets, and the feeding experiment was started every other day. Every other day, the new diet was weighed on a 0.001 sensitive scale and given to the larvae, and the remaining nutrients were weighed after drying in the incubator. This process was continued until the larvae pupated.

Pupae were placed in an incubator set at 45°C to be dried and kept in the incubator until they reached a constant weight. Dried pupae were taken from the

incubator and placed in tubes to determine the lipid amount. It was kept in pure chloroform for 24 hours on a rotating shaker, and this process was repeated 3 times (Simpson & Raubenheimer, 2001). Thus, lipid content was removed from pupae. Then, the pupae were taken into the incubator again and dried until they reached a constant weight. The dried pupae were weighed, and their lipid-free weight was noted. Nitrogen determination of lipid-free *H. cunea* pupae was made by semi-micro Kjeldahl method with Kjeltac Auto 1030 analyzer (Tecator, Sweden). The % nitrogen amounts found as a result of this process were multiplied by the constant of 6.25, and the % protein amounts were found (Ooninx et al., 2015).

In the study, whether the total diet consumption amounts, pupal weights, pupal lipid and pupal protein amounts, and development times of *H. cunea* larvae were statistically different from each other was determined by ANOVA test, and Dunnet test was used. SPSS 21.0 software was used for statistical analysis.

## RESULTS and DISCUSSION

Among all the groups, the lowest consumption was in the larvae fed with the diet containing the highest amount of copper (G diet; Mean=277.0±0.4; F=6801.7;

P<0.001). Consumption amounts decreased with the increase in iron and copper amounts added to the diet, and it was found that the consumption amounts of these groups were lower than the control group. These results we found in our study coincide with Vesik and Reichman's (2009) study that metals can deter invertebrate insects. Deterrence causes a decrease in the consumption amount of the insect, and this situation may negatively affect the development stages of the insects. It was found that the consumption amounts of all groups containing nickel and cobalt were higher than the control group. Gonçalves et al., (2007), in a study with *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae), found that as the Ni amount increased, the larvae were significantly less fed and rejected the diet discs with the highest Ni amount, which is the opposite of the result we found in our study (Table 2).

In our study, it was noted that pupal weights decreased with the increase of iron and copper amounts in the diet. Consumption amounts were also low in these groups. In this case, it can be concluded that the low consumption amount negatively affects pupal weight. Similar results were found in other studies examining the effects of metals on pupal weights.

**Table 2.** Total consumption amount, pupal weight, pupal protein and lipid amount, and development time of *Hyphantria cunea* larvae according to their diet types.

	Diet types	Total consumption amount (mg)	Pupal weight (mg)	Pupal protein amount (mg)	Pupal lipid amount (mg)	Development time (day)	
Mean ± standard error	A	365.6±0.4	58.0±1.0	27.1±0.4	17.3±0.2	3.0±0.1	
	B	358.6±0.5	39.9±0.2	19.6±0.1	10.0±0.1	3.6±0.1	
	C	306.6±0.4	28.7±0.2	13.7±0.1	7.2±0.2	4.5±0.1	
	D	343.7±0.5	45.7±0.6	21.0±0.3	18.5±1.1	2.9±0.1	
	E	385.0±0.5	54.1±0.9	25.9±0.5	9.0±1.1	2.7±0.1	
	F	283.7±0.4	30.3±0.2	19.9±0.1	8.2±0.2	2.9±0.1	
	G	277.0±0.4	26.2±0.2	12.7±0.1	6.5±0.2	3.2±0.1	
	H	380.8±0.6	66.4±1.5	29.0±0.5	19.8±0.3	3.1±0.1	
	J	386.0±0.6	69.6±1.3	26.7±0.4	22.1±0.3	3.1±0.1	
	K	374.0±0.6	64.1±1.8	27.0±0.4	18.0±0.3	3.1±0.1	
	L	379.0±0.6	67.3±1.7	29.8±0.5	20.3±0.3	3.5±0.1	
	ANOVA	df	164	164	164	164	164
F		6801.7	437.6	284.7	615.8	23.3	
P		< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	
Dunnet test			B<0.001	B<0.001	B<0.001	B<0.001	B<0.05
			C<0.001	C<0.001	C<0.001	C<0.001	C<0.001
			D<0.001	D<0.001	D<0.001	D<0.05	E<0.05
			E<0.001	E<0.05	F<0.001	E<0.001	L<0.05
			F<0.001	F<0.001	G<0.001	F<0.001	
			G<0.001	G<0.001	H<0.05	G<0.001	
			H<0.001	J<0.001	J<0.001	H<0.001	
			J<0.001	H<0.001	L<0.001	J<0.001	
			K<0.001	K<0.001		L<0.001	
		L<0.001	L<0.001				

Wu et al., (2014) found that in susceptible strains of *Boettcherisca peregrina* Robineau-Desvoidy (Diptera: Sarcophagidae), body weight decreased with increasing copper amount. In another study in which *Panolis flammea*

Denis & Schiffermüller (Lepidoptera: Noctuidae) and *Bupalus piniarius* Linnaeus (Lepidoptera: Geometridae) were used (Heliövaara et al., 1989), it was found that pupae whose larval nutrients were closer to the emission source

were smaller. The decrease in the pupal size of insects affects fecundity (Barah & Sengupta, 1991). If the fecundity is negatively affected, it means that less number of offspring occurs and; therefore, the population is negatively affected by this situation. We found that all groups containing nickel and cobalt had greater pupal weights than the control group. Also, pupal weight increased with increasing zinc amount in the diet.

Determining the changes in the total protein amount in the whole body or a specific tissue of an insect is essential in determining whether the ingredients in the diet are used effectively by the insect and whether it is effective on the growth of the insect (Büyükgüzel, 2002; Büyükgüzel & İcen, 2004). While the pupal protein amount decreased with increasing amounts of iron and copper added to the diet, an increase in the amount of protein was observed with increasing amounts of zinc and cobalt. Heavy metals affect protein homeostasis; they can also bind to proteins and inhibit their biological activities (Tamás et al., 2014). In our study, it was determined that the pupal protein amount in the groups containing iron, zinc, and copper was less than control. It indicates that the pupal protein amount is negatively affected by the presence of these metals.

Insects rely on lipid reserves to survive in periods when they are not physiologically fed or to meet the energy requirements of flying, starvation and developing eggs (Patel et al., 2005). Lipid metabolism is necessary for growth and reproduction and provides the energy needed in long-term non-feeding periods (Arrese & Soulages, 2010). We found that the pupal lipid amount in the groups containing iron and copper was less than the control group. Also, as the amounts of iron, zinc, and copper added to the diet increased, the pupal lipid amounts decreased. In this case, it can be said that these metals may adversely affect vital functions by reducing the pupal lipid amounts. Besides, in our study, it was determined that the lipid amount increased with the increase in nickel and cobalt amounts, and the pupal lipid amounts of these groups were high than control.

Some heavy metals such as Cu, Zn, Fe, Ni, and Co that we used in our study are essential micronutrients for the development of animals (Yaldız & Şekeroğlu, 2013). While necessary in trace amounts, they can cause adverse effects at high amounts. For example, we found that with the increase of iron, copper, and cobalt amounts in the diet, the development time of the larvae was prolonged, with the longest development time in the diet group containing the highest amount of iron (C diet; Mean=4.5±0.1; F=23.3; P<0.001). Prolonged development time may increase the risk of parasitization of larvae (Kaitaniemi & Ruohomäki, 1999). In this case, it can be said that copper and cobalt, in

addition to iron, negatively affect the development time. Also, the development time shortened with increasing zinc amount, the shortest development time in the diet group with the highest zinc amount (E diet; Mean=2.7±0.1; F=23.3; P<0.05).

## CONCLUSIONS

With the development of industrialization and urbanization, heavy metals have become highly toxic environmental pollutants, which seriously impair normal metabolism and the physiological and genetic development of organisms. In our study, it was determined that *H. cunea* was physiologically and developmentally affected by heavy metals in different amounts. The results of this study will shed light on other studies in determining the effects of heavy metal contamination on different insect species.

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