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**TRANSPORT OF CADMIUM FROM TREATED LARVAL MEDIA TO
THE BLOWFLY *CHRYSOMYA ALBICEPS* (WIEDEMANN) LIFE
STAGES (DIPTERA: CALLIPHORIDAE)**

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Key words: *Chrysomya albiceps*; Cadmium; Content; Accumulation.

ABSTRACT

Larvae of the blowfly *Chrysomya albiceps* were reared on media containing 0, 100 and 200 µg cadmium/g dry media. The concentration of cadmium in the feeding and wandering larvae, pupae, pupal shells and adults were increased with the increase of cadmium concentration in prepared food. Cadmium concentration was lower in the feeding larvae than in the devoured food. Body concentration of cadmium was usually lower in every stage than in the preceding one. Pupae discharged about 33-37% of cadmium in their pupal shells. Concentration of cadmium in adult bodies at emergence, were higher in females than in males. Adults eliminated about 67% of their cadmium load within their first three days (72 hours). Excretion of accumulated cadmium in faeces, in sloughed off larvae and pupa exuviae was indicated.

INTRODUCTION

In the last two decades, it is quiet clear that cadmium concentration has been increased to toxic levels in respect to several organisms (Quimby et al., 1979; Martoja et al., 1983; Schmidt et al., 1991, 1992 and Schmidt and Ibrahim, 1994). At the same time, it is well known that cadmium is easily transferred and biomagnified in the food chain (Lindqvist, 1988), and could therefore lead to serious problems, such as high mortality rate, deformations and toxic effects in higher trophic levels.

The life cycle of the blowfly *Chrysomya* from egg to adult stage takes from 10 to 28 days. The eggs are laid in masses on carcasses; they hatch within 24 hours, and the larvae mature in a period of 4 to 12 days. The pupal stage lasts from 5 to 15 days (Zumpt, 1965; Prins, 1982 and Al-Misned et al., 2002). *Chrysomya albiceps* occurs in different regions of the world: Africa, Southern Europe, South West Asia and East and North West India (Zumpt, 1965), South America (Baumgartner and Greenberg, 1985). It is ubiquitous in Saudi Arabia, commonly in different places such as

hospitals, homes, slaughterhouses, markets, garbage and gardens (Buttiker et al., 1979). *C. albiceps* is essentially scavenger and could be considered as beneficial biological control agent because of its ability in reducing populations of carrion flies, which are of medical and veterinary importance (Omar, 1995). This species is of veterinary importance as it is a carrion-breeder, and also causes secondary myiasis in sheep (Zumt, 1965; Oldroyd and Smith, 1973; Erzinclioglu and Whitcombe, 1983, and Leite et al., 1983). The third instar larvae have been found to be predacious upon dipterous larvae (Zumt, 1965), and larvae of *C. marginalis* (Coe, 1978), meanwhile the third instar of this species behave as a predator on the pupae of their own species (Tantawi et al., 1996).

Various studies dealing with transport of cadmium in life stages of insects have been notated (Lindqvist, 1988; Andrzejewska et al., 1990; Postma et al., 1994; Postma and Davids, 1995 and Rayms- Keller et al., 1998). Cheng (1980) fed larvae of *Drosophila melanogaster* on media mixed with cadmium > 1000 ppm and noticed that the adult flies died early without laying eggs. Studies of the grasshopper *Aiolopus thalassinus* reported that high concentrations of cadmium affect significantly the development throughout the whole life stages (Schmidt et al., 1991; 1992; and Schmidt and Ibrahim, 1994).

Biological effects of larval media treated with 100, 200, 400, 800 and 1600 µg Cd/g dry media on the developmental time, mortality and

weights of pupal and adult of *C. albiceps* were previously studied by Al-Misned (2001). Total developmental time was extended and the percentage of larval and pupal mortality were increased with increasing cadmium concentration in larval media. This was accompanied by loss in pupal and adult weight. Under similar conditions, the influence of larval media treated with 100 and 200 µg Cd/g dry media on adult longevity and fecundity of the same species were investigated (Al-Misned, 2002). Moreover, the life span was shortened and the total number of eggs per female was decreased with increasing cadmium concentration in the larval media. On the other hand, to the best of my knowledge no studies have been carried out on the transport of cadmium throughout the life stages of the blowfly *C. albiceps* yet.

Therefore the present work was carried out and based primarily on bioaccumulation studies of blowflies' *C. albiceps* stages, larvae reared on media contaminated by varying amount of cadmium. In addition to the excretion of cadmium from pupae during the whole period of their activity:

MATERIALS AND METHODS

Rearing of the Insect:

Egg-batches of 6th generation of the blowflies *C. albiceps* were obtained from the adult fly colony from Entomology Laboratory, Department of Zoology, King Saud University, Riyadh City, Saudi Arabia within 0-6 h. of egg

deposition. The colony was maintained in the laboratory in an environmental chamber at 25 °C, 60 - 65% RH, and a 15: 9 (L: D) h photoperiod as described by Amoudi *et al.* (1992). Larvae of *C. albiceps* were reared in cadmium contaminated ground beef as described by Al-Misned (2001).

One hundred first - instar blowfly larvae were removed from the colony within (0 - 6) h of egg hatching and placed into rearing beakers (11 cm diameter) containing contaminated ground beef. The ground beef was artificially contaminated by mixing of fresh ground media with solutions containing different concentrations of cadmium to obtain the following series: 0 (control), 100 and 200 µg Cd /g dry ground beef. The toxicants were mixed homogeneously with the fresh ground beef. After 4 days covered 3 - 4 cm deep moistened sawdust with distilled water were added to the rest media to allow pupation. The beakers were covered by cotton clothes held by rubber band to permit ventilation. Five replicates sampling beakers were used at each cadmium concentration.

Larvae were checked during wandering phase through the sawdust strata at 12-h intervals until pupation takes place. The fresh pupae were daily collected for each group, isolated and kept in clean beakers, which were provided with moistened sawdust. Beakers were covered with a cloth held by rubber bands until the emergence of adults. Upon adults

One hundred feeding larvae were collected at the 4th day, and at the 6th day, other one hundred wandering larvae were also collected from each concentration then transferred into clean beakers, killed by hot distilled water and washed.

After pupation, one hundred fresh pupae were collected from each concentration, transferred into clean beakers, washed by distilled water, then by fresh 95% ethanol, and lastly by distilled water again. Washed pupae were dried for 48 h at 85 °C.

Cadmium analysis

All materials used in preparation of specimens and analyses were cleaned in a three step procedure. Starting with an acid detergent treatment, materials were soaked in 0.1 N HNO₃ for 24 h, and finally rinsed at least three times with distilled water.

Insect specimens of larvae, pupae and exuviae were washed with distilled water in glass containers to remove dust particles, then washed with fresh 95% ethanol and again with distilled water to remove surface cadmium from the specimens (Middleton *et al.*, 1972). Then specimens were placed in petri-dishes and oven dried for 48 h at 85 °C. The adult insects were dried directly without washing. They were weighed together directly from the oven. Samples were ground, divided into convenient portions and reweighed, before digestion. It was impossible to count insects included in each sample.

concentrated nitric acid (Analar) and allowed to stand for 24 h. The samples were digested at 85 °C, heated to near dryness and after cooling, brought to 10 ml with distilled water (Donald, 1979). Finally the solutions were filtered and stored in plastic vials until the analysis. All samples collected for this study were analyzed for cadmium content using a flame atomic absorption spectrometer (module Perkin-Elmer 380). Standard solutions with appropriate concentrations were prepared from 1000 µg AAS stock standards. The Cd content were determined on the basis of dry weight.

Statistical methods

Statistical analyses were conducted using the MINITAB Computer Program. Relationships between the cadmium concentrations in larval media and the cadmium content in immature and adult stages were tested using correlation coefficients. For univariate models, significance of the correlation coefficient, r , was tested with analysis of variance (Edwards, 1985).

RESULTS AND DISCUSSION

The results obtained in the present study showed that Cd accumulated in the bodies of feeding (4 days age) and wandering (6 days age) larvae. Regarding pupae and pupa shells this was dependent on the concentration of Cd in the larval food (Table 1). The concentrations of Cd in the feeding and wandering larvae,

0.93, $p > 0.05$), ($r = 0.97, p > 0.05$) and ($r = 0.94, p > 0.05$), respectively.

Concentration of Cd was usually lower in the feeding larvae than in the devoured foods. It was 82.9 and 137.5 ppm at 100 and 200 µg Cd/g dry food, respectively, compared to 82.9% and 68.8% of Cd concentration in larval food, respectively. Seidman *et al.* (1986) found the same results in his study dealing with accumulation of Cd in *Chironomus thummi* (Europ. lab. strain). He exposed the larvae to 10 and 250 µg radiolabeled Cd/L (ppb) for up to four days. After four days, average cadmium accumulation was 6.6 and 177 ng Cd/g dry weight (ppb), respectively

The concentration of cadmium in wandering larvae (at 6 days age) was lower than that in feeding larvae (at 4 days age). Wandering larvae may excrete cadmium accumulated during wandering in the sawdust with faeces or discharged it in sloughed off larvae exuviae. Amounts of cadmium were discharged to outside throughout the pupa shells of pupating larvae. These shells contained 37.0 and 32.9% of the analyzed cadmium found in pupae at 100 and 200 µg Cd/g dry food respectively. These results are similar to that found by Andrzejewska *et al.* (1990) in their experiments on caterpillars.

The amount of cadmium content accumulated in the bodies of adults were dependent on the concentration of Cd in the larval food

Cd concentration in larval media ($r=1.00, p<0.01$), ($r=0.97, p>0.05$) and ($r=0.98, p>0.05$), respectively.

Vogel (1986) determined heavy metals concentration (Cd, Pb, Mn, Zn) in bark and wood of *Picea abies* Karst. from different locations and compared them with that of the bark-beetles feeding on it. In case of cadmium and zinc, a positive correlation of metal concentration between beetles and food were observed. This was in agreement with the present study. Cadmium concentration in adult bodies combined sexes at emergence was 50.3 ppm and 75.5 ppm at 100 and 200 $\mu\text{g Cd/g}$ dry food, respectively which represent 50.3% and 37.8% of cadmium concentration in larval food respectively. On the other hand Lindqvist (1988) analyzed the cadmium content in 14 herb species and 6 adult insects species and one in larval stage, from two Central Swedish sites with equal cadmium burdens. The Cd concentration of herbivorous insects were about twice as high as those recorded from herbs. Differences between insect species in metals content could be due to a number of factors such as differences in food types, the period of feeding, size, age, developmental stage and sex, bioavailable form (Hare, 1992).

Concentrations of accumulated cadmium were higher in females than in males (Table 1) for both groups of contaminated rearing media (100 and 200 $\mu\text{g/g}$). Concentration of cadmium in females were 3.7- fold that in males for group 100 $\mu\text{g Cd/g}$ and 2.5- fold for group 200 $\mu\text{g Cd/g}$. The differences in cadmium concentration

between male and female emergent blowflies may have resulted from differences in their developmental time and body composition. Al-Misned (2001) described the effects of cadmium on developmental time of this species, and he found that the larval developmental time of females was longer than that of males at all cadmium concentrations applied in his study. Phillips (1980) and Smock (1983) found that the differences in the growth rates, rates of feeding, metal uptake and partitioning within the body could partly account for differences in metal content between sexes. Dukerschein *et al.* (1992) found that the differences in Cd concentrations between sexes in mayflies may have resulted from different factors including body composition.

At cadmium concentration 200 $\mu\text{g/g}$ dry media, the mean concentration of cadmium in pupae, pupal shells and adults (combined sexes) were 61.4, 45.9 and 75.5 $\mu\text{g/g}$ dry weight respectively. The mean dry weight of pupal, pupal shells and adults (combined sexes) were 10.1, 3.9 and 6.1 mg, respectively. The mean of cadmium content of the whole body was calculated to be 0.616, 0.179 and 0.463 μg for pupal, pupal shells and adult, respectively (calculated by multiplying the mean concentration of cadmium by the mean dry weight of life stages). These results indicated that the pupal stage eliminated about 29.1% of their cadmium content in their pupal shells.

The results of this study indicated that adult blowflies have a surprising ability to decrease their

cadmium content. There was significant negative correlation ($r = -0.95$, $P < 0.05$) between adult age per hour and cadmium concentration in adults. Within their first three days (72 hrs), as adults, they eliminated about 67.9% of their cadmium load (Fig. 1). Cadmium accumulated in the bodies of adult insects, may be excreted with faeces. Nuorteva and Nuorteva (1982) have stated that the rapidity of mercury excretion in adult blowflies is, however, an artifact, because of the true excretory functions occurred during the pupal stage, and the role of the adult fly is restricted to emptying the excrement reservoirs (meconium from the intestine). It is clear from the results obtained in the present study that *C. albiceps* may not be used for monitoring purposes due to their ability to dispose cadmium.

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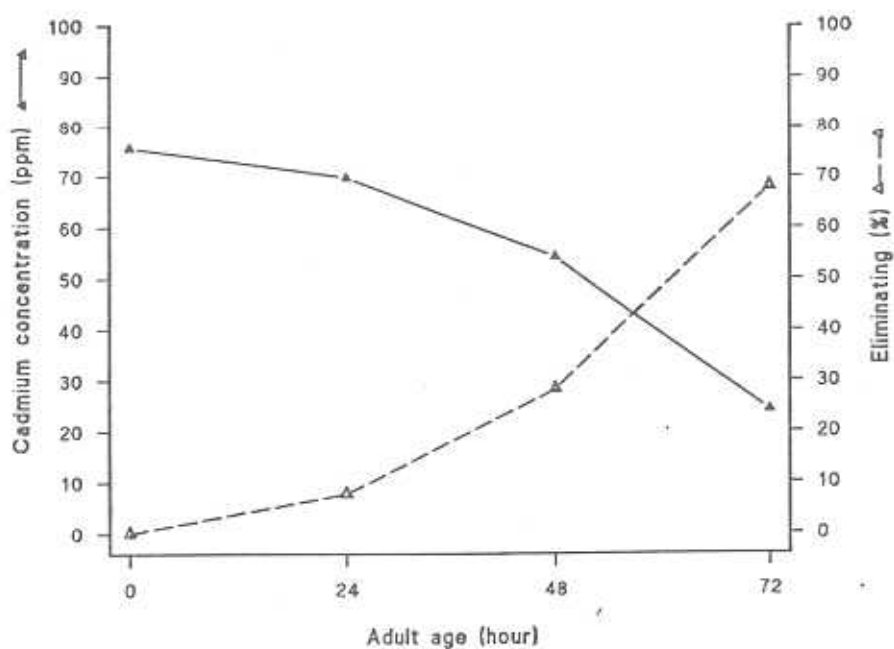
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Table 1. Mean cadmium concentration (ppm dry weight) in the different developmental stages of *C. albiceps*, larvae reared on media containing various concentrations of cadmium.

Stage	Cadmium concentration $\mu\text{g/g}$ dry media		
	Control	100	200
Feeding larvae (4 days age)	0	82.9	137.5
Wandering larvae (6 days age)	0	70.1	82.3
Pupae	0	44.7	61.4
Pupa shells	0	37.4	45.9
Adults $\text{♂} + \text{♀}$	0	50.3	75.5
♂	0	22.5	45.2
♀	0	82.9	114.8



**انتقال الكادميوم من الغذاء البرقي المعامل الى الاطوار المختلفة لذبابة كريبزومايا
البيسييس *Chrysomya albiceps* (Wiedemann) التابعة لعائلة الذباب الملون من
رتبة ثنائيات الأجنحة**

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ملخص البحث: تم في هذا البحث تغذية يرقات ذبابة *Chrysomya albiceps* على غذاء معامل بالكادميوم بتركيزين مختلفين (١٠٠، ٢٠٠ ميكروجرام كادميوم/جرام من الوزن الجاف بالإضافة للمجموعة الضابطة وذلك لدراسة انتقال عنصر الكادميوم من الغذاء البرقي الى الأطوار المختلفة لدورة الحياة. وقد اثبتت الدراسة ان تركيزات الكادميوم في كل من الطور اليرقي المتغذي والطور اليرقي المتجول والطور العذري واغلفة العذارى والطور الياقع تزداد بزيادة تركيزه في الغذاء البرقي. كما بينت الدراسة ان تركيز الكادميوم في الطور اليرقي المتغذي (عمر ٤ أيام) اقل من تركيزه في الغذاء البرقي وان التركيز كان يقل في كل طور عن الطور الذي يسبقه. كما وجد ان العذارى قد تخلصت من ٢٣-٣٧% من الكادميوم في اغلفتها. وتحليل الذباب الياقع بعد بزوغه مباشرة من اغلفة العذارى وجد ان تركيز الكادميوم في الإناث اعلى منه في الذكور. كذلك وجد أن الذباب الياقع قد تخلص من حوالي ٦٧% من كمية الكادميوم خلال الثلاثة ايام الأولى من البزوغ. وقد اشارت جميع النتائج الى أن الاطوار المختلفة للحشرة كانت تتخلص من الكادميوم المتراكم في البراز وكذلك من خلال اغلفة كل من اليرقات والعذارى.