Lead Accumulation And Elimination During Metamorphosis Of *Bercaea Cruentata* (Meigen) (Diptera: Sarcophagidae)

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Abstract: First-instar larvae of the fleshfly *Bercaea cruentata* were reared on ground beef media containing various concentration of lead 0, 25, 50, 100 and 200 µg/g, all based on dry weight. The content of lead was analyzed in the bodies of feeding larvae, wandering larvae, pupae, pupal shells and adults. There was a positive correlation between lead concentration in larval food and content of lead in all stages. Concentration of lead remained significantly lower in feeding larvae than in their food, as well as the content of lead decreased rapidely during metamorphosis. Pupae discharged about 34.4-43.7% of the total administered lead in pupal shells. Concentration of lead in adults bodies at emergence, were significantly higher in males than in females. The highest lead content was found in the abdomen. Adults eliminated about 75.5% of their lead contents within their first three days. There was indication of excretion of accumulated lead with faeces, in sloughed off larvae and pupa exuviae.

Key words: fleshfly, Bercaea cruentata, metamorphosis, lead, accumulation, elimination.

Introduction

The fleshfly, *Bercaea cruentata* (Meigen) has a world-wide distribution from Nepal, India and Pakistan through Africa and Europe to North and South Americas (Amoudi, 1993). It is of medical importance as it is commonly allied with facultative intestinal and traumatic myiasis in man and animals (Lapage, 1968, James and Harwood, 1969). Aspects of biology and behaviour of *B. cruentata* were described by Knipling (1936), Zumpt (1965), James and Harwood (1969), Madubunyi (1986), Al-Misned *et al.* (1999) and Al-Misned and Abou-Fannah (2000).

High levels of heavy metals including lead have been found in various organisms commonly infested by fleshfly larvae such as snails (Moller, 1978, Williamson, 1980; Paval and Povolny, 1993, 1994; Bartosova and Povolny, 1997), earthworms (Beyer *et al.*, 1987, Paval and Povolny, 1993, 1994, Bartosova and Povolny, 1997), fungi (Stijve and Besson, 1976, Lodenius, 1981) and turtles (Davenport and Wrench, 1990). Moreover, fleshfly larvae were found to accumulate heavy metals from their food sources into their tissues (Paval and Povolny, 1993, 1994, Bartosova and Povolny, 1997).

Little studies have been conducted on the effects of heavy metals on biology of sarcosaprophagous flies (Al-Misned 2000, 2001, 2003b, 2003c).

The accumulation of metals from food in sarcosaprophagous flies has been observed in several cases (Nuorteva and Nuorteva, 1982;

Aoki and Suzuki 1984; Al-Misned, 2003a). However, detailed bioaccumulation studies of heavy metals are lacking for the fleshfly stage.

The objectives of this study is to determine the lead content in various larval stages of fleshflies' *B. cruentata*, larvae reared on lead-contaminated media. In additional studies, the lead content in various body parts of adult flies and the excretion of lead from adults was studied.

Materials and Methods

Bercaea cruentata larvae were collected from Riyadh city in December 1997 using plastic jar (20 cm deep x 15 cm diameter) containing 500 g decaying ground beef. These larvae were used to establish a laboratory colony that was maintained on ground beef in an environmentally controlled room at 25°C, 60 -65% RH, and a 15:9 (L:D) h photoperiod as described by Amoudi *et al.* (1992). Larvae of *B. cruentata* were reared in contaminated ground beef by lead as described by Al-Misned (2003b).

Egg batches were removed from the colony within one hour of egg deposition. Within 30 minute of hatching, the 100 first-instar larvae were placed in rearing jars (14 cm diameter) containing the 100 g of ground beef (24.3 g dry weight) mixed homogeneously with lead acetate solution (25 ml) at lead concentrations of 0 (control), 25, 50, 100 and 200 µg Pb/g dry ground beef. The jars were covered by cotton cloth, held by rubber bands to permit ventilation. Five replicate jars were used for each lead concentration. After 4 days, the media were covered with 4 cm deep sawdust moistened with distilled water.

The larvae were checked during wandering in the sawdust at 12-h interval until pupation. Fresh pupae were daily collected and placed into clean jars, which were provided with moistened sawdust. The jars were covered with a cotton cloth held by rubber band until adult emergence. Upon adult emergence, the flies were held in rearing cages, given water and sugar until termination by subjecting to freezing temperature for 3 h.

Lead analysis

Approximately 15 feeding larvae (0.410-0.480 g dry weight) at the 4th day of exposure, 15 wandering larvae (0.420-0.500 g dry weight) at 6th day, 15 fresh pupae (0.415-0.470 g dry weight) and 40 pupae shells (0.215-0.290 g dry weight) were collected from each lead concentration, transferred into clean beakers, killed by hot distilled water, washed with distilled water to remove dust particles, and then re-washed with fresh 95% ethanol and again with distilled water to remove surface lead from the specimens (Middleton *et al.*, 1972). Approximately 20 insect specimens (0.31-0.42 g dry weight) of adult males or females were randomely pooled from each concentration. The adults developing from larvae reared on media containing the highest lead concentration (200 ug Pb/g dry media) were used for determining of the Pb contents in adult stages at different time after emergence (0-27.5 d after emergence) and in different parts of the adult body (0-6h after emergence). The dissection of flies was performed promptly after killed them by freezing temperature and each sample consisted of organs from 50 males and 50 females (0.158-0.504 g dry weight). Then specimens were placed in petri-dishes and oven dried for 48 h at 85°C. They were weighed directly from the oven. Samples were transferred to 10 ml

Table 1. Mean lead concentration (ppm dry wt) in the different developmental stages of *B. cruentata* larvae reared on media containing various concentrations of lead.

Stage	Lead concentration µg/g dry media				
	Control	25	50	100	200
Feeding larvae (4 days age)	0	9.73	16.18	31.18	56.76
Wandering larvae (6 days age)	0	1.28	2.19	3.75	6.56
Pupae	0	1.23	2.13	3.67	6.11
Pupa shells	0	1.86	3.14	6.43	11.43
Adults ♂ + ♀	0	1.06	1.90	3.02	5.32
♂	0	1.24	1.95	3.62	7.14
♀	0	0.88	1.85	2.41	3.49

Volumetric-flasks and treated with 2 ml of concentrated nitric acid (Analar) and allowed to stand for 24 h. The samples were digested at 85°C, heated to near dryness, and then diluted to 10 ml After cooling, the samples were brought to 10 ml of distilled water (Donald, 1979). Finally the solutions were filtered and stored in vials until the analysis. All samples collected for this study were analyzed for lead content using a flame atomic absorption spectrometry AAS (Perkin-Elmer 380). Standard solutions with appropriate concentrations were prepared from 1000 µg/ml standard stocks. The lead content (µg) per larva, pupa, pupa shell, different parts of the adult body and adult stage were calculated by multiplying the mean concentration of lead (ppm) by the mean dry weight (g).

Statistical methods

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

Results and Discussion

The results obtained in the present study showed that lead is rapidly accumulated in the bodies of feeding larvae (4 days of age), wandering larvae (6 days of age) and in pupae and pupa shells depended on the concentration of Pb in the larval food (Table 1). The concentrations of Pb in the feeding larvae, wandering larvae, pupae and pupa shells increased with increasing Pb concentration in larval food (Table 1). There was a significant positive correlation between lead concentration in larval food and content of lead in immature stages (Fig. 1). Obtained results are differ from those of Andrzejewska *et al.* (1990) who found that the increased concentration of lead in larval

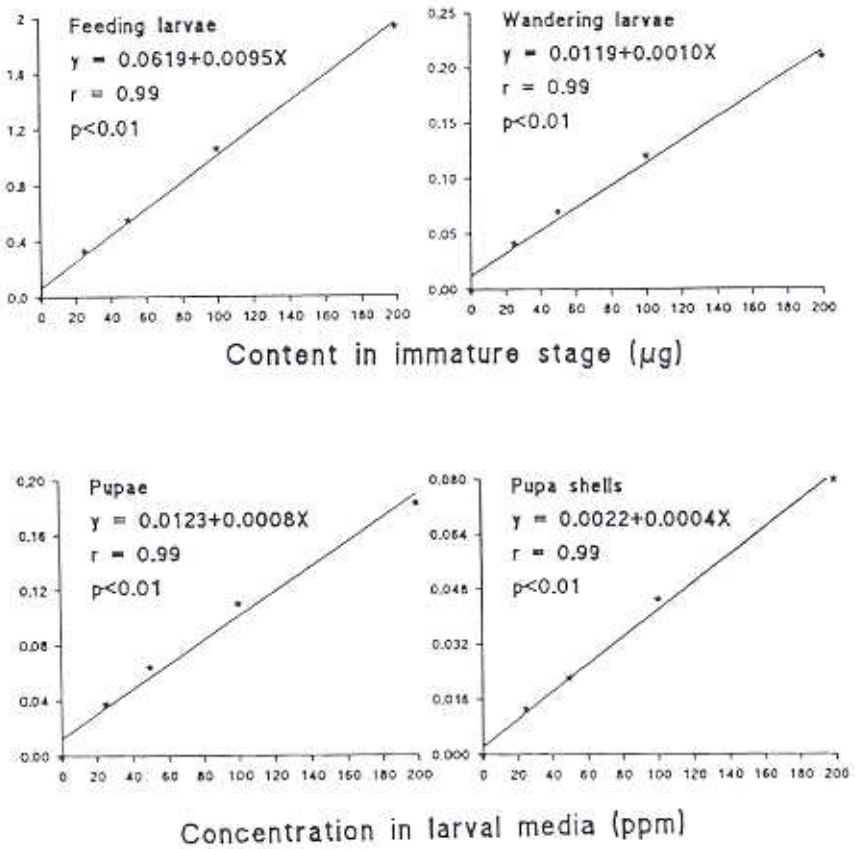

Fig 1. The correlation between the lead concentrations in larval media and the lead content in immature stages of *B. cruentata*.

food (Dandelion leaves of *Taraxacum officinale* Wiggers) did not always correspond to proportionally greater than content in caterpillars' bodies of the moth *Spodoptera littoralis L.*

Concentration of Pb was found lower in the feeding larvae than in the devoured food. It was 9.73, 16.18, 31.18 and 56.76 ppm at 25, 50, 100 and 200 ug Pb/g dry food, respectively, and it was about 38.9, 32.4, 31.2, and 28.4% of Pb concentration in larval food, respectively. Concentrations of lead were lower in feeding larvae than in their food, which agreed with the findings of Lindqvist (1994), who found that the concentration of lead in larvae of a butterfly (*Aglais urticae* L.) were lower than in their food of leaves, *Urtica dioica* L. throughout the rearing period. On the other hand, these results are differ from those of Beyer and More (1980) who found that the lead concentrations in tent caterpillars *Malacosoma americanum)* averaged 76% as high as those in host leaves *(Prunus serotina)*. Also Andrzejewska *et al.* (1990) noted that the concentration of the examined metals in the insects' tissues was usually higher than in the devoured food.

The concentrations of lead in wandering larvae (at 6 days of age) were lower than in feeding larvae (at 4 days of age). The amounts of lead in the feeding larvae increased markedly during rearing, however, the amount of lead in

Table 2. Lead contents (µg/part) found in different parts of the body adult stage at emergence of *B. cruentata*, resulted from larvae reared on media containing 200 µg Pb/g dry media.

Parts	Mean dry weight g	Concentration ppm	Content	
			µg	%
Head	0.00158	10.13	0.016	15.24
Thorax	0.00730	2.88	0.021	20.00
Wings & legs	0.00330	6.97	0.023	21.90
Abdomen	0.00502	8.96	0.045	42.86
Total body	0.01720	6.08	0.105	100.00

Table 3. Lead concentration (ppm dry wt) and content (µg/fly) in the adult stages and percent eliminating at different time after emergence of *B. cruentata*, larvae reared on media containing 200 µg Pb/g dry media.

Time (days)	Pb concentration ppm	Pb content µg / fly	Elimination %
0	5.68	0.098	0.0
1	4.88	0.088	10.2
2	2.62	0.044	55.1
3	1.45	0.024	75.5
27.5	0.41	0.006	93.9

wandering larvae is less than those in feeding larvae. Between 10.9 and 12.8% at 200 and 50 µg Pb/g dry food, respectively of the amounts of lead ingested in food during rearing was retained in the wandering larvae, the remainder being (87.2-89.1%) may be excreted in faeces or discharged in sloughed off larvae exuviae in the sawdust. These results are similar to the results found by Lindqvist (1994), who found that the lead concentration at the end of rearing larvae is less than those at the start of rearing.

Concentrations of Pb which were usually lower in the pupae than in the wandering larvae. About 8.3-12.9% of lead in wandering larvae may be excreted with faeces. Amount of lead were discharged outside organisms of pupating larvae throughout the pupa shells. The shells contained 35.1, 34.4, 40.9 and 43.7% of the analyzed lead found in pupae at 25, 50, 100 and 200 µg Pb/g dry food, respectively. This finding is in agreement with Andrzejewska *et al* (1990), who found that the shells contained from 70-80% of the analyzed heavy metals (Pb, Cu, Mn and Fe), which were found also in pupae.

The amount of the content of lead accumulated in the bodies of adults were found to be depending on the concentration of Pb in the larval food (Table 1). The concentrations of Pb in adults, males, females and combined sexes increased with increasing Pb concentration in larval media (Table 1). There was a

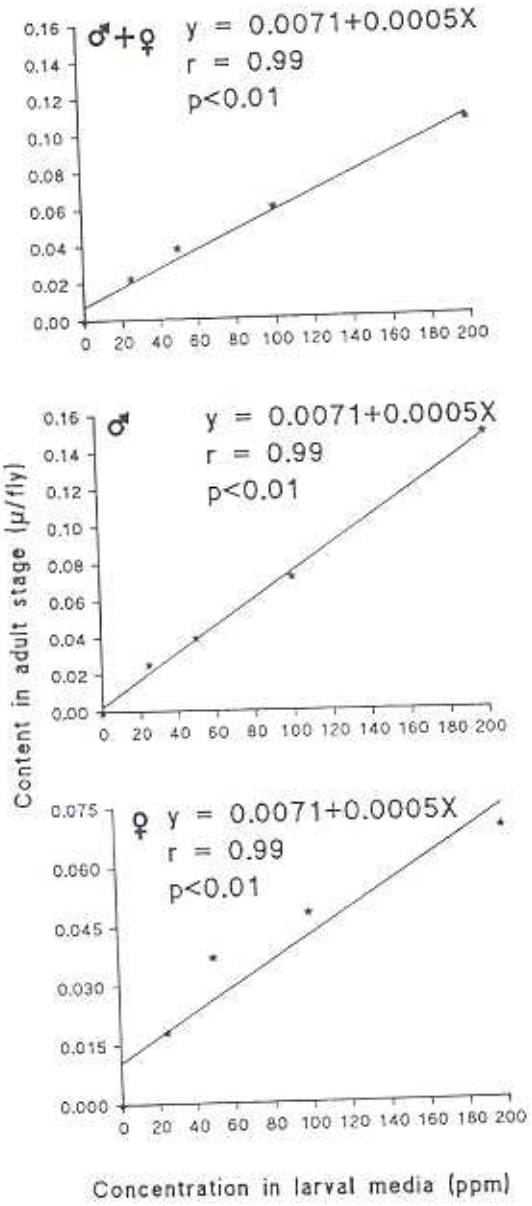

Fig 2. The correlation between the lead concentrations in larval media and the lead content in adult stages of *B. cruentata*.

positive correlation between lead concentration in larval food and content of lead in the adult stage (Fig. 2). Similar observations were noted by Paval and Povolny (1993), who studied heavy metal contents in fleshflies and their hosts (snails, earthworms) in selected habitats of Central Europe. In the case of lead, a significant positive relationship was found between lead levels in fleshflies and their hosts (earthworms). The concentration of lead in adults bodies were usually lower than in larval food. In adults combined sexes at emergence, lead concentrations were 1.06, 1.90, 3.02 and 5.32 ppm at 25, 50, 100 and 200 µg Pb/g dry food, respectively, and it were about 4.2, 3.8, 3.0 and 2.7% of lead concentration in larval food, respectively. The concentration of lead in the adult bodies of fleshflies was usually smaller than in tissues of instars larvae. These results are similar to those found by Andrzejewska *et al.* (1990) in their experiments on caterpillars.

Concentrations of lead for all groups were higher in males than in females (Table 1). Concentrations of lead in males were 1.4, 1.1, 1.5 and 2.0-fold than in females for 25, 50, 100, 200 µg Pb/g, respectively. The differences in lead concentrations between male and female emergent fleshflies might have resulted from differences in body size and body composition. 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 cadmium and mercury concentrations between sexes in mayflies may have resulted from different factors, including differences in body size and body composition. Al-Misned (2003a) described that the differences in Cd concentrations between sexes in blowflies may have resulted from differences in developmental time and body composition.

The amounts of lead found in the various body parts are presented in Table 2. When we analyzed the lead contents in different body parts of the flies, we observed that the highest

lead contents occurred in the abdomen and the wings & legs. They were 42.86 and 21.90% of the total body content, respectively. In agreement with the present study, Schmidt and Ibrahim (1994) analyzed the heavy metals (Hg, Cd and Pb) content in different parts of adult grasshoppers (*Aiolopus thalassinus*) resulted from eggs laid in treated soil. They found that the highest heavy metals concentrations were found in abdomen (testes, gut and ovaries) compared to other body parts. Nuorteva and Nuorteva (1982) found similar results in their study of the accumulation of Hg in different parts of the body of blowflies (*Calliphora vicina*).

The results of the present study indicated that adult fleshfly have a surprising ability to decrease their lead content. There was a significant negative correlation (r=-0.72) between adult age per day and lead concentration in adults. Within their first three days as adults, they eliminated about 75.5% of their lead load (Table 3). Lead accumulated in the bodies of adult insects, may be excreted with faeces. These results are similar to the results found by Al-Misned (2003a) in his experiments on the adult blowflies. Nuorteva and Nuorteva (1982) have found that the rapidity of mercury excretion in adult blowflies is, however, an artifact, because 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). Also metal concentrations in adult insects are influenced by activities such as mating (Engebretson and Mason, 1981) and egg laying (Galtney *et al.*, 1981). It is clear from the obtained results in the present study that due to the ability of *B. cruentata* to dispose of lead, it may not be used for monitoring purposes.

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تراكم الرصاص والتخلص منه خلال تطور ذبابة اللحم برشيا كروينتاتا *Bercaea cruentata* (Meigen) التابعة لعائلة ذباب اللحم من رتبة ثنائية الأجنحة

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ملخص البحث: تم في هذا البحث تغذية يرقات ذبابة اللحم *Bercaea cruentata* ابتداءا من الطور اليرقي الأول على لحم بقري مفروم كغذاء يرقي معامل بأربعة تركيزات مختلفة من الرصاص (٢٥، ٥٠، ١٠٠، ٢٠٠ ميكروجرام رصاص/ جرام من الغذاء اليرقي الجاف) بالإضافة للمجموعة الضابطة وذلك لدراسة انتقال عنصر الرصاص من الغذاء اليرقي إلى الأطوار المختلفة لدوره الحياة وكذلك تراكم وطريقة التخلص من هذا العنصر في كل طور من الأطوار المختلفة. وبتحليل الرصاص في كل من الطور اليرقي المتغذي والطور اليرقي المتجول والطور العذري وأغلفه العذارى والطور اليافع أظهرت الدراسة وجود علاقة طردية بين تركيزات الرصاص في الغذاء اليرقي وكمية الرصاص المتراكم في كل الأطوار. كما أثبتت الدراسة أن تركيز الرصاص في الطور اليرقي المتغذي أقل من تركيزه في الغذاء اليرقي وكانت كمية الرصاص تقل أثناء تطور كل طور إلى الطور الذي يليه. كما وجد أن العذارى قد تخلصت من ٣٤,٤ - ٤٣,٧% من إجمالي محتواها من الرصاص من أغلفتها. وبتحليل الذباب اليافع بعد بزوغه مباشرة من أغلفة العذارى وجد أن تركيز الرصاص في الذكور أعلى منه في الإناث. وبتحليل مناطق الجسم المختلفة للذباب اليافع وجد أن منطقة البطن تحتوي على أعلى كمية من الرصاص. كذلك وجد أن الذباب اليافع قد تخلص من حوالي ٧٥,٥% من كمية الرصاص خلال الثلاثة أيام الأولى من البزوغ. وقد أشارت جميع النتائج إلى أن الحشرات خلال الأطوار المختلفة كانت تتخلص من الرصاص المتراكم في أجسامها عن طريق البراز وكذلك من خلال أغلفة كل من اليرقات والعذارى.