Feeding of the willow leaf beetle *Lochmaea capreae* L. (*Coleoptera*, *Chrysomelidae*) on leaves of birch (*Betula pendula* Roth) contaminated by heavy metals

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ABSTRACT: The effect of contamination of birch (*Betula pendula* Roth) leaves with heavy metals on the feeding of imagoes of the willow leaf beetle (*Lochmaea capreae* L.) was studied under laboratory conditions. The imagoes preferred feeding on leaves less contaminated by Cd, Mn and Zn. The Pb content was tolerated on all the studied levels. The repellent effect of Zn 8,000 μg/ml in the Pb + Zn regime was compensated by Pb 500 μg/ml; the effect of Cd 250 μg/ml in relation to Mn 10,000 μg/ml was similar.

Keywords: *Lochmaea capreae*; cadmium; manganese; lead; zinc; feed preference; laboratory conditions

The willow leaf beetle (*Lochmaea capreae* L., 1758, *Chrysomelidae*) occurs in Europe, Lapland, the British Isles, the Balkans and Lake Baikal (Pfeffer 1954). Imagoes winter in tufts of grass or in plant litter; in early May, when average daily temperatures rise above 12°C, they begin maturation feeding (Brovdij 1973). The imagoes make holes (windows) in the leaves of the genera *Salix* and *Betula* that damage them, and they naturally prefer leaves higher up in the crown. They also attack the buds and bark of annual shoots (Urban 1981). After repeated copulation (VI) the female lays 85–190 eggs in the soil at the foot of the tree in clusters of 10–25 eggs. Embryonic development takes 1–2 weeks. Negative phototropic larvae skeletonise the leaves from the bottom side (VII to VIII). After 3–4 weeks they pupate in the surface soil layers and 10 days later the imagoes of the new generation leave the pupal chamber and begin maturation feeding (VIII–½ IX). In the Czech Republic *L. capreae* rears one generation (Urban 1981). In 1985–1987 it gradated in young birch stands established in the air-polluted area of the Krušné hory Mts. and the Děčín Sandstone Uplands (Kula 1988).

In the terrestrial food chain the phytophagous insects are an important link in the distribution of heavy metals (Kowalczyk, Watala 1989). Among the defence mechanisms of the body eliminating heavy metals is, in addition to secretion in the excrements and exuviae and deposition in the skin or body organs, a capacity to search for and consume less contaminated feed (e.g. Heliövaara, Väisänen 1990; Lindqvist 1992; Weismann, Řeháková 1993; Hrdlička et al. 1999). If the content of heavy metals in feed increases, on oral contact with the contaminated part the insects stop feeding, they do not consume the feed, or they choose the less contaminated or not contaminated parts (Weismann et al. 1983). The interruption of feeding, lower feed consumption, metabolic disorders and the use of the feed may indirectly intensify the impact of heavy metals on the physiology (development, fertility, enzyme activity, energy metabolism, deformation, behaviour, growth, respiration, vitality, mortality), ecology (abundance, diversity, density, competition) and insect evolution (industrial melanisms, resistance) (Starý, Kubizňáková 1987; Heliövaara, Väisänen 1993; Habuštová, Weismann 2001).

Imagoes of the willow leaf beetle (*L. capreae*) feeding on birch (*B. pendula*) were used in laboratory conditions to specify the degree of preference to untreated and heavy-metal-contaminated feed under different levels of application.

Supported by the Grant research projects MSM 434100005 and NAZV 1144 funded by Ministry of Agriculture and by the following firms and companies: Netex; Alcan extruded products; Municipal Offices all in Děčín, Setuza in Ústí nad Labem, CEZ Ltd, Lafarge Cement Works in Čížkovice, North-Bohemian Mines in Chomutov, Dieter Bussmann in Ústí nad Labem.

Table 1. Spatial arrangement of the sequence

Regressive			Progressive			Parallel		
metal	leaf[a]	(µg/ml)	metal	leaf[a]	(µg/ml)	level	leaf[a]	metal (µg/ml)
Cd	1	2	Cd	1	250	low	1	Pb 4
	2	10		2	50		2	Mn 100
	3	50		3	10		3	Cd 2
	4	250		4	2		4	control 0
Mn	1	100	Mn	1	10,000	increased	1	Pb 20
	2	500		2	5,000		2	Mn 500
	3	5,000		3	500		3	Cd 10
	4	10,000		4	100		4	control 0
Pb	1	4	Pb	1	500	high	1	Pb 100
	2	20		2	100		2	Mn 5,000
	3	100		3	20		3	Cd 50
	4	500		4	4		4	control 0
Control	1	0	control	1	0	very high	1	Pb 500
	2	0		2	0		2	Mn 10,000
	3	0		3	0		3	Cd 250
	4	0		4	0		4	Control 0

[a] vertical position of the leaf from the upper part of the twig (1, 2 – apical; 3, 4 – basal leaves)

Table 2. Area arrangement of the sequence

Individual		Parallel		Combined	
metal	(µg/ml)	level	metal (µg/ml)	metals	(µg/ml)
Cd	0	low	control 0	Pb + Zn	0
	2		Cd 2		4 + 80
	10		Mn 100		500 + 8,000
	50		Pb 4		4 + 8,000
	250		Zn 80		500 + 80
Mn	0	increased	control 0	Mn + Cd	0
	100		Cd 10		100 + 2
	500		Mn 500		10,000 + 250
	5,000		Pb 20		100 + 250
	10,000		Zn 400		10,000 + 2
Pb	0	high	control 0	Cd + Zn	0
	4		Cd 50		2 + 80
	20		Mn 5,000		250 + 8,000
	100		Pb 100		2 + 8,000
	500		Zn 4,000		250 + 80
Zn	0	very	control 0		
	80	high	Cd 250		
	400		Mn 10,000		
	4,000		Pb 500		
	8,000		Zn 8,000		

Table 3. Scale of qualified estimate of feeding

Degree	Feeding	(%)
0	without feeding	0
1	poor feeding	< 10
2	medium feeding	10–25
3	heavy feeding	25–50
4	very heavy feeding	50–75
5	defoliation	> 75

MATERIAL AND METHODS

Imagoes of the willow leaf beetle (*L. capreae*) collected in the field in early spring (Sněžník, altitude 600 m) were reared in a laboratory (temperature 20°C, relative humidity 60%, normal light regime). They were fed leaves of birch (*B. pendula*) contaminated by steeping in solutions containing heavy metals (Cd 2–250, Mn 100–10,000, Pb 4–500, Zn 80–8,000 µg/ml). Agrovital (ANONYM 1992) was used as a soaking agent to ensure better adhesion of the solution. The control leaves were steeped in Agrovital-containing distilled water. After drying up the treated leaves were placed into breeding vessels and changed in 24-hour intervals. Each variant had three replications. Dead specimens were continuously replaced by specimens from maintenance breeding.

The imagoes were removed into 4 l jars in a 20 ♀♀:6 ♂♂ ratio and they were repeatedly given a twig with 4 contaminated leaves (spatial arrangement). With a regressive sequence of the heavy metal on the leaves the concentration decreased from the lowest position to the top of the twig. With a progressive sequence the arrangement was opposite. Twigs with uncontaminated leaves in two replications were used as controls. A set of 4 leaves of the twig made up a parallel sequence to the control below; Cd, Mn and Pb-treated leaves were towards the top of the twig. The respective replications differed in the level of feed contamination (low, increased, high and very high) (Table 1).

In order to eliminate the apical phenomenon, we chose the area arrangement of leaves in Petri dishes (ø 16.5 cm) with filter paper on the bottom, and the willow leaf beetle in a 15 ♀♀:5 ♂♂ ratio. Leaves were contaminated in an individual sequence of the heavy metal, in a set of control leaf and leaves treated with parallelly increased concentration, with a combination of two metals (Pb + Zn, Mn + Cd, Cd + Zn) respectively (Table 2).

The average degree of feeding on the leaf based on qualified daily estimates during the studies of ethology according to the scale in Table 3 was decisive for evaluations of the selection of feed by the willow leaf beetle. The AAS method was used to analyse the natural content of heavy metals in the birch leaves and the content of heavy metals in the control and contaminated leaves (Table 4).

Table 4. Average content of heavy metals in birch leaves (µg/g)

Metal	Feed contamination (µg/ml)	Cd	Mn	Pb	Zn
Distilled water[a]	0	0.26	124.8	2.11	148
Control[b]	0	0.26	147.2	2.33	203
Cd	2	56.5	153	5.87	
	10	120	115	1.65	
	50	213	175	1.39	
	250	347.5	377	1.24	
Mn	100	0.38	348	1.85	
	500	0.51	1,072	7.10	
	5,000	0.31	9,823	1.83	
	10,000	0.71	11,740	3.56	
Pb	4	0.65	90.9	91.7	
	20	0.81	74.9	351	
	100	1.21	77.2	731	
	500	9.30	145	1,035.5	
Zn	80	0.38	93.7	3.68	340
	400	0.23	89.6	5.98	967
	4,000	0.36	98.4	3.03	5,894
	8,000	0.39	92.4	4.35	13,066
Pb + Zn	4 + 80	1.45	132	28.7	271
	500 + 8,000	0.89	112	2,795	5,949
	4 + 8,000	5.41	104	23.9	6,751
	500 + 80	1.09	102	1,515	529
Mn + Cd	100 + 2	9.89	201	1.89	164
	10,000 + 250	474	10,143	5.73	175
	100 + 250	616	262	2.96	187
	10,000 + 2	5.66	9,695	5.27	157
Cd + Zn	2 + 80	10.3	108	1.56	355
	250 + 8,000	528	120	2.3	5,982
	2 + 8,000	10	88.3	2.52	6,721
	250 + 80	618	110	2.28	299

[a] natural leaf composition, [b] distilled water + Agrovital

RESULTS

In control breeding the imagoes confirmed their preference to leaves situated higher up on the twigs. Apical leaves were severely damaged by feeding, while the damage to leaves of the basal part was medium (Fig. 1). The regression equation $y = 3.5000 - 0.3750 \times x$ ($R = -0.4116$; $R^2 = 16.94\%$) showed that the degree of feeding changed by 10.7% based on the position of the leaf.

Within the framework of a regressive sequence of leaf contamination, the degree of feeding decreased

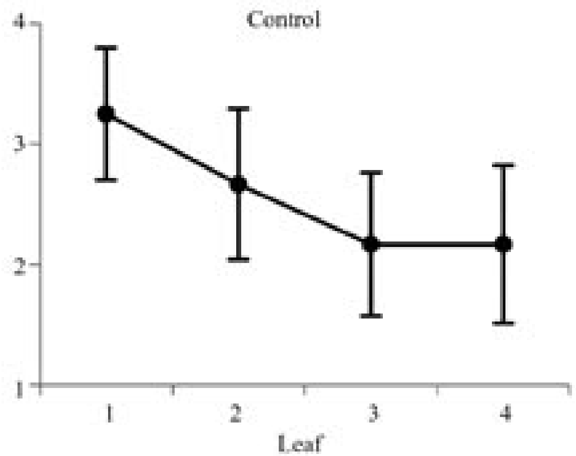


Fig. 1. Average degree of feeding on uncontaminated leaves (spatial arrangement)

with increasing amounts of Cd. The regression analysis (y = 2.8047 – 0.0064 × x; R = –0.6493; R^2 = 42.16%) showed that with increased Cd contamination of the leaf by 10 µg/ml we can assume that the degree of feeding will decrease by 2.3%. The statistically different degree of feeding on the leaves was observed with an application of Mn 100 µg/ml and 500 µg/ml. If the feed was contaminated by Mn 5,000 µg/ml, the area of feeding further decreased and contamination by Mn 10,000 µg/ml minimised leaf damage. The regression analysis (y = 3.4717 – 0.0003 × x; R = –0.7468; R^2 = 55.77%) showed that the degree of feeding might decrease by 8.6% if the Mn contamination of feed increased by 1,000 µg/ml. Applications of different doses of Pb did not affect feeding as much as Cd or Mn. Heavy to very heavy feeding seriously damaged leaves treated with Pb 4 µg/ml, and with Pb 20–500 µg/ml the degree of feeding was classified as medium to heavy (Fig. 2a).

If the level of Cd increased towards the apical part of the twig, regression analysis defined the decreased extent of feeding on the leaves as not statistically significant. The effect of Mn on the intensity of feeding of the willow leaf beetle was also negative if the sequence of the metal was progressive. When feeding on leaves contaminated by Mn 100 or 500 µg/ml, the imagoes showed heavy to very heavy feeding; contamination by 5,000 µg/ml mostly resulted in poor feeding and the imagoes consumed leaves treated with 10,000 µg/ml only occasionally. Regression analysis characterised this correlation as y = 3.3326 – 0.0003 × x (R = –0.7110; R^2 = 50.55%). A 9% change in the degree of feeding after feed contamination by Mn 1,000 µg/ml was very marked with extreme exposure. Pb-treated leaves were damaged by medium to heavy feeding regardless of the concentration (Fig. 2b).

Poor, occasionally medium, feeding was discovered on leaves with a low concentration of Cd and Pb (2 and 4 µg/ml, respectively), while leaves with natural composition or treated with Mn 100 µg/ml resulted in poor feeding of the willow leaf beetle imagoes. The beetle fed on leaves with an increased level of contamination by heavy metals (Cd 10, Mn 500 and Pb 20 µg/ml) much more than on the control leaves. The degree of imago feeding on Pb-treated leaves (medium to heavy feeding) was different from feeding on control leaves. The damage to Mn and Cd intoxicated leaves was classified as medium. When the imagoes took up feed with a high level of contamination, they preferred uncontaminated feed (poor to medium feeding) or feed treated with Pb 100 µg/ml (medium feeding predominated). Feeding was poor when the feed was contaminated by Cd 50 µg/ml and particularly by Mn 5,000 µg/ml. If the feed was contaminated by very high levels of heavy metals (Cd 250, Mn 10,000 and Pb 500 µg/ml), preference was given to leaves containing Pb (medium feeding) or uncontaminated leaves (poor to medium feeding). Feeding on Mn-treated leaves differed considerably from the feeding on Pb-treated ones. The imagoes fed on leaves containing 10,000 µg/ml only sporadically. Feeding on Cd-treated leaves was classified as poor (Fig. 2c).

The apical phenomenon was eliminated by random placement of leaves with different levels of heavy metal contamination onto Petri dishes. Imagoes of the willow leaf beetle generally consumed leaves treated with Cd 10 µg/ml. By regression analysis the correlation between the exposure of feed to Cd and the degree of feeding was y = 3.1553 – 0.0021 × x (R = –0.2280; R^2 = 5.20%). The decrease in the degree of feeding was 6.7% as a response to 100 µg/ml of feed contamination by Cd. In the Mn regime the preference to feed uncontaminated by a heavy metal and intoxicated with Mn 100–500 µg/ml to leaves contaminated by Mn 5,000–10,000 µg/ml was statistically significant. Based on regression analysis (y = 3.4853 – 0.0002 × x; R = –0.6765; R^2 = 45.76%) it was found that with the increasing Mn contamination of feed by 1,000 µg/ml the degree of leaf feeding decreased by 5.7%. Leaves treated with various doses of Pb were damaged by heavy feeding. The attack on control leaves and leaves intoxicated with Zn 80–400 µg/ml was classified as heavy and was significantly different from feeding on leaves contaminated by Zn 4,000–8,000 µg/ml (Fig. 3a). Regression analysis determined the equation y = 3.3898 – 0.0002 × x (R = –0.6961; R^2 = 48.46%) and the change with the increasing contamination of feed by Zn by 5.9% to 1,000 µg/ml.

Heavy feeding predominated on control leaves and on leaves with low (Cd 2, Mn 100, Pb 4 and Zn 80 µg/ml) and increased (Cd 10, Mn 500, Pb 20 and Zn 400 µg/ml) doses of heavy metals. The feeding of the willow leaf beetle on leaves with a high level of contamination showed a statistically significant preference to feed treated with Pb 100, Cd 50 µg/ml and to uncontaminated leaves against the leaves intoxicated with 5,000 and Zn 4,000 µg/ml. Preference was given to control leaves and leaves treated with Pb 500 µg/ml against the feed with a very high level of contamination (Cd 250, Mn 10,000 and Zn 8,000 µg/ml) (Fig. 3b).

Intensive feeding occurred on control leaves and on leaves where Pb 4 + Zn 80 µg/ml and Pb 500 + Zn

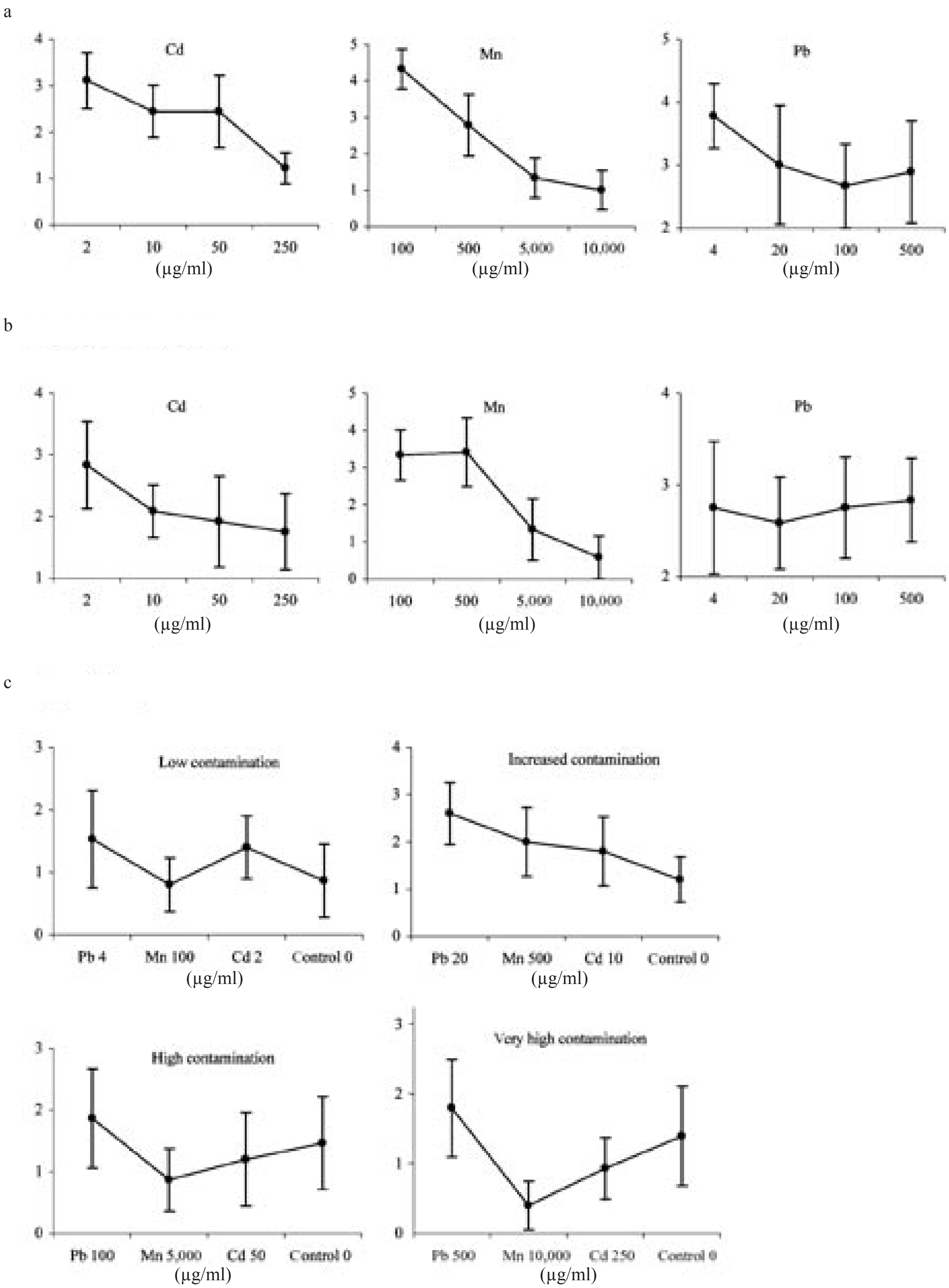


Fig. 2. Average degree of feeding on leaves contaminated by heavy metals (spatial arrangement)
a) regressive sequence
b) progressive sequence
c) parallel sequence

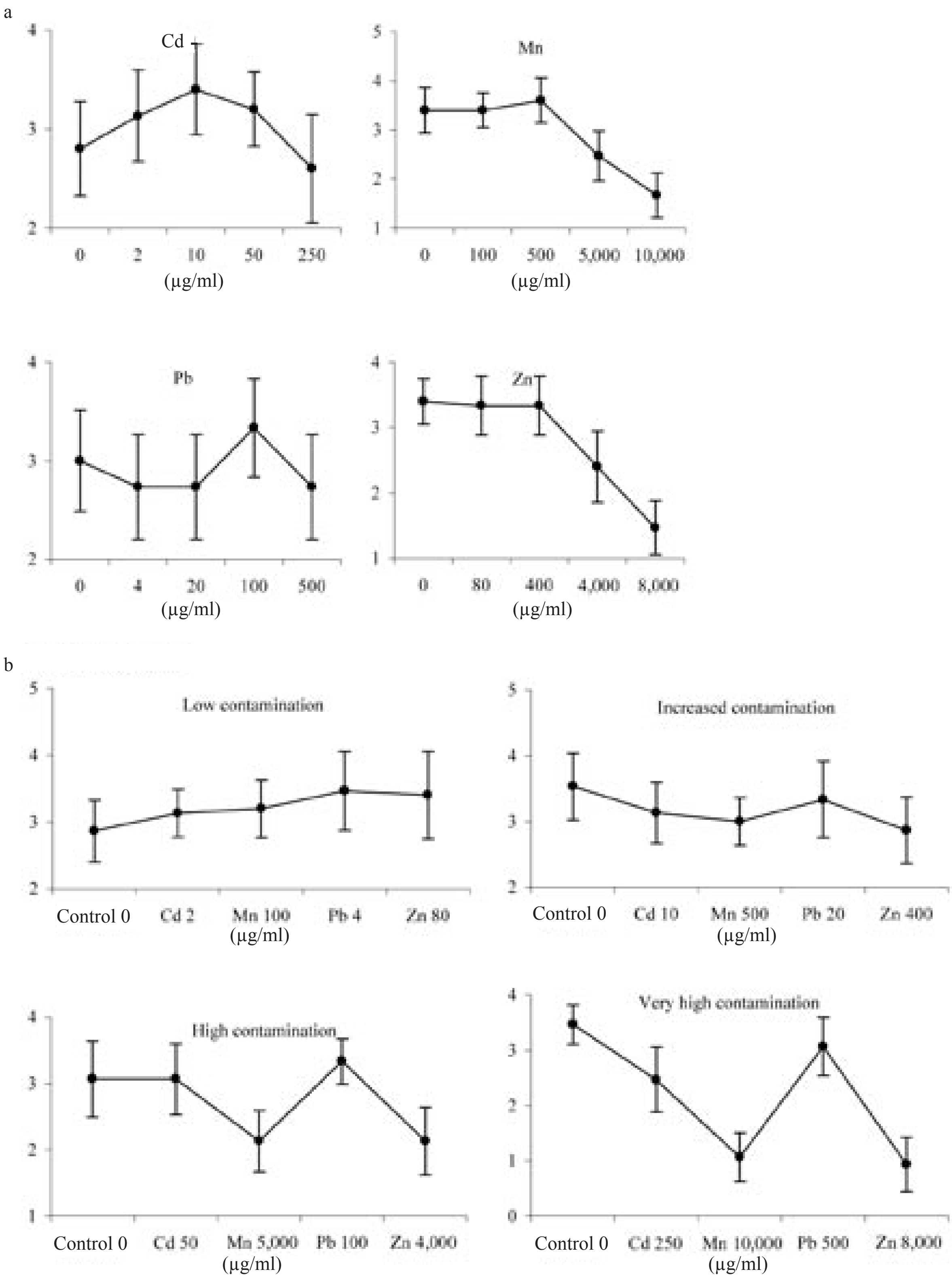
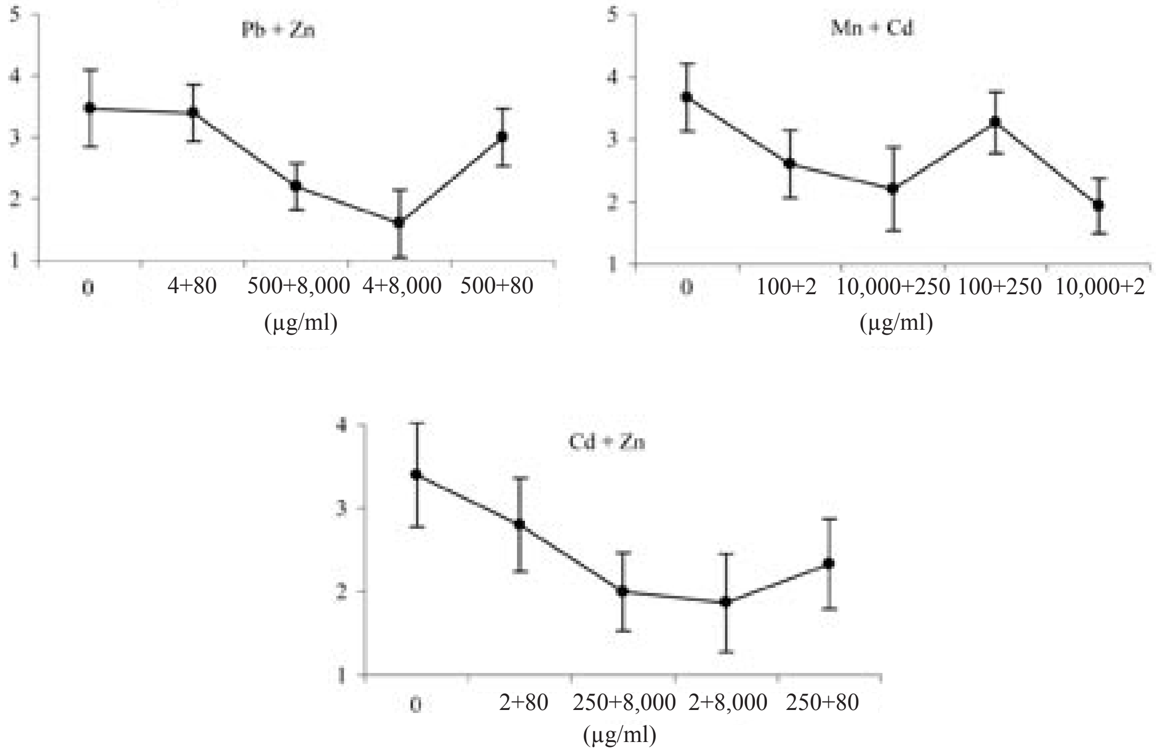


Fig. 3. Average degree of feeding on leaves contaminated by heavy metals (area arrangement)
a) individual sequence
b) parallel sequence
c) combined sequence (see next page)

80 µg/ml was applied. A dose of Zn 8,000 µg/ml (regardless of the Pb concentration in the feed) reduced the feeding intensity, even though Pb 500 µg/ml partly eliminated the effect of a very high concentration of Zn. The resulting feeding was very similar on control leaves and on leaves contaminated by Mn 100 µg/ml in combination with Cd 250 µg/ml. The feeding of imagoes was medium to heavy under a low level of contamination by Mn 100

c



+ Cd 2 µg/ml. Predominantly medium feeding occurred on leaves where Mn 10,000 µg/ml was applied (regardless of the Cd concentration in the feed), whereas the negative effect of a very high level of Mn was partly eliminated by a concentration of Cd 250 µg/ml in contrast to Cd 2 µg/ml. Intoxication with Cd 2 + Zn 80 µg/ml and Cd 250 + Zn 80 µg/ml insignificantly reduced feeding compared to heavy feeding of controls. An important change came by when the leaves were treated with Zn 8,000 µg/ml, whereas with an application of this dose of Zn with Cd 250 µg/ml feeding was discovered to be only insignificantly higher than in interaction with Cd 2 µg/ml (Fig. 3c).

DISCUSSION

Heavy metals were used for the contamination of birch (*B. pendula*) leaves in laboratory breeding of the willow leaf beetle (*L. capreae*); the impact was evaluated in the larvae of *Lymantria dispar* L. (GINTENREITER et al. 1993), *Agrotis segetum* Den. et Schiff., *Mamestra brassicae* L. and the leaf beetle *Leptinotarsa decemlineata* Say (HABUŠTOVÁ, WEISMANN 2001). In the Krušné hory Mts., where the willow leaf beetle is an important pest (KULA 1988), an abnormal content of Cd (0.2–0.3 µg/g), background content of Pb (1.1–1.6 µg/g) and a high level of Mn (10,000 µg/g) were discovered in the dry matter of leaves of birch (*B. pendula)*, which is characterised by its high degree of tolerance (HRDLIČKA, KULA 1998, 2002, 2003). In the air-polluted areas the contamination of feed is reflected in the pollutant load of the soil and crown fauna of the forest ecosystems. The ground beetles (C*arabidae*) contained as much as Cd 51.28 µg/g, Mn 1,316.73 µg/g, Pb 117.17 µg/g and Zn 464.98 µg/g of dry matter (KULA et al. 2002). No essential function of Cd and Pb was proved. The presence of a certain amount of the minor elements Mn and Zn as enzyme co-factors is necessary. For normal growth the larvae of the meal-beetle (*Tenebrio molitor* L.) require at least 6 µg/g of Zn of fresh feed (HOPKIN 1989) although a higher level is toxic (FRIBERG et al. 1979).

The nutritional composition of the feed and its repellent and/or toxic effects might affect the ethology of feeding. In breeds where it was possible to select untreated feed and feed contaminated by heavy metals, preference to leaves with a low and increased contamination against leaves with a high and very high load was confirmed. The specimens avoided such leaves and concentrated on untreated leaves. Feeding leaves containing Pb was not dependent on its concentration. Parallel application of two heavy metals reduced feeding with a gradient of concentration. Due to stress the plants undergo physiological changes that alter their attractiveness for phytophagous insects (WHITE 1984; HELIÖVAARA, VÄISÄNEN 1993). The mobilisation of nitrogen and free amino acids is a condition of a higher nutritional value and thus also attractiveness of the plants, while the production of terpenoids in secondary metabolism protects the plants against the attack of the phytophages. LARSSON (1989) sees the causes of different reactions of insects to the host plant stress in feeding behaviour (species with superficial feeding, with biting or piercing suction mouthparts, miners, cambiophagous insects). It was found during laboratory rearing of the willow leaf beetle imagoes where feed selection was not possible that with the gradient of heavy metal concentrations the amount of feed taken up during maturation feeding decreased, whereas the consumption of the controls was lower than under stress, indicating that with the intoxication of the organism with heavy metals the feed utilisation was probably less effective; to be capable of reproduction the imagoes had to compensate this condition during maturation feeding by taking up a larger amount of feed (not published). In air-polluted areas relatively less contaminated feed need not always be available in addition to the potentially toxic feed (for instance, younger leaves or leaves inside the tree crowns). The organism either starves or by feeding risks toxic effects. Some insect species have defensive mechanisms to eliminate the ingested heavy metals (they secrete them in excrements and pupae, they deposit them in the skin, in the walls of the alimentary tract, fat body, Malpighian tubes, ovaries, hepatopancreas, haemolymph, integuments, exoskeleton, mandibles, and they get rid of them in the exuviae and meconium during moulting) (HOPKIN 1989; HELIÖVAARA, VÄISÄNEN 1990; POLEK 1991; LINDQVIST 1992; HRDLIČKA et al. 1999; HABUŠTOVÁ, WEISMANN 2001).

CONCLUSION

Feed contamination (leaves of *B. pendula*) by heavy metals (Cd, Mn, Zn, Pb) affected the feeding ethology of the willow leaf beetle (*L. capreae*), which was seen as:

- giving preference to leaves with a lower level of intoxication,
- reduction of feed consumption with the Cd, Mn and Zn gradient accompanied by toxicity of the metals,
- tolerance to Pb 4–500 µg/ml in the feed,
- inhibition of feeding when two heavy metals were applied in parallel with a gradient of concentration,
- antagonistic Pb 500 + Zn 8,000 µg/ml and/or Cd 250 + Mn 10,000 µg/ml correlation.

Acknowledgements

We thank the State Phytosanitary Administration in Brno for helping to conduct the experiment.

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Received for publication October 7, 2003
Accepted after corrections December 12, 2003

Žír bázlivce vrbového *Lochmaea capreae* L. (*Coleoptera*, *Chrysomelidae*) na listech břízy (*Betula pendula* Roth) kontaminovaných těžkými kovy

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ABSTRAKT: V laboratorních podmínkách byl hodnocen vliv kontaminace listů břízy bílé (*Betula pendula* Roth) těžkými kovy na žír imag bázlivce vrbového (*Lochmaea capreae* L.). Imaga preferovala listy se sníženou zátěží Cd, Mn a Zn. Obsah Pb byl tolerován ve všech sledovaných úrovních. V režimu Pb + Zn byl repelentní účinek Zn 8 000 µg/ml kompenzován Pb 500 µg/ml, podobně působilo Cd 250 µg/ml ve vztahu s Mn 10 000 µg/ml.

Klíčová slova: *Lochmaea capreae*; kadmium; mangan; olovo; zinek; potravní preference; laboratorní podmínky

Imaga bázlivce vrbového (*Lochmaea capreae* L.) žeroucí na bříze bílé (*Betula pendula* Roth) byla užita v laboratorních podmínkách ke stanovení míry preference potravy neošetřené a kontaminované těžkými kovy v odlišných úrovních aplikace.

Listy byly kontaminovány namočením do roztoků těžkých kovů (Cd 2–250; Mn 100–10 000; Pb 4–500; Zn 80–8 000 µg/ml) se smáčedlem Agrovital. Souběžně se uskutečnil kontrolní chov na listech namočených v destilované vodě s Agrovitalem. Ve 24hodinovém intervalu byla potrava předkládána do chovných nádob. Pokus s prostorovým, resp. plošným uspořádáním listů byl realizován ve třech opakováních každé varianty. Rozhodující pro hodnocení výběru potravy bázlivcem vrbovým byl průměrný stupeň žíru na listu posuzovaný denně kvalifikovaným odhadem. Metodou AAS byla provedena analýza obsahu těžkých kovů v listech břízy bílé.

V kontrolním chovu byla prokázána preference imag výše položených listů na větvičce. Zvýšený žír na listech s nižší kontaminací Cd byl částečně ovlivněn vrcholovým fenoménem. Imaga preferovala listy se sníženou zátěží Mn a Zn. Obsah Pb byl tolerován ve všech sledovaných hladinách.

Spotřeba potravy při nízkém a zvýšeném stupni kontaminace Cd, Mn a Zn byla vyšší než u kontrolních listů. Žír listů s obsahem Pb nezávisel na jeho dávce a rozsahem byl srovnatelný s kontrolou.

Souběžná aplikace dvou těžkých kovů s gradientem koncentrace žír redukovala. V režimu Pb + Zn byl repelentní účinek Zn 8 000 µg/ml kompenzován Pb 500 µg/ml, podobně působilo Cd 250 µg/ml ve vztahu s Mn 10 000 µg/ml.

Bázlivec vrbový je v podmínkách stresu Cd, Mn a Zn schopen vybrat si méně toxickou potravu. Vysoké hladiny Pb žír neovlivňují. Kvalita a množství potravy přijaté během zralostního žíru imag má zásadní význam pro reprodukci i vývoj juvenilních stadií hmyzu.

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