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Regular research paper

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EFFECTS OF HOST PLANT CONTAMINATED WITH HEAVY METALS ON THE LIFE HISTORY TRAITS OF APHIDS (*BREVICORYNE BRASSICAE* L.)

ABSTRACT: Heavy metal contaminations can be considered as one of the serious environmental pollution and may have adverse effects on different organisms. This study was carried out to search how rearing the cabbage aphid (*Brevicoryne brassicae* L.) on heavy metal contaminated host plants affects aphid life history traits. The results revealed that both radish and cabbage host plants (*Raphanus sativus* L., *Brassica oleracea* L) contaminated separately with copper (Cu) and lead (Pb) have an important effects on life history traits of aphids. The life history traits such as fecundity (number of offspring produced per day) and fitness (intrinsic rate of population increase) were reduced about 30% when aphids were reared on contaminated host plants. However, no concise effects were found in case of development time (from birth to beginning of first reproduction). Reproduction potential of aphid population significantly decreased and their mortality tended to be higher about 20 % on contaminated host plants. Cu and Pb had different impacts on life history traits of aphids living on the same host plant, and the same heavy metal had different effects on aphid life history traits across host plants. The uptake of Cu and Pb by cabbage aphids feeding on contaminated host plants affected life history traits due to direct toxicity or reduced food quality of host plants. Results are discussed in the context of the possible effects of heavy metal pollution on herbivore-host plant relationships. This study indicates that aphid-host plant system has

several advantages to study the heavy metal pollution effects on food chain in nature.

KEY WORDS: *Brevicoryne brassicae*, heavy metal contamination, copper, lead, life history traits

1. INTRODUCTION

Plants can be seen as the most important route for the transfer of heavy metals from soil to herbivorous insects. Accumulation of heavy metals in the body of these herbivores by feeding on their heavy metal contaminated host plants heightens the potential for metal introduction into food chains and subsequent usage by consumers (Smith 1996). Herbivore insects-host plant relationships can be considered as a model system to study possible heavy metal effects on population and individual levels in a short time. Aphids are mostly host specific and their development time is relatively shorter than other groups. The importance of accumulation of heavy metals in phytophagous insects, especially phloem feeders such as aphids has been shown in a number of laboratory, greenhouse and field studies (Crawford *et al.* 1990, 1996, Merrington *et al.* 1997). Merrington *et al.* (1997) showed a significant

increase in the uptake of cadmium (Cd) and zinc (Zn) in *Rhopalosiphum padi* (L.) feeding on wheat. It makes that the aphid-host plant system is particularly suitable for the study of the contamination effect.

Comparative studies on the uptake of metals by aphids and its subsequent effects are limited. Crawford *et al.* (1995) demonstrated that although black bean aphid, *Aphis fabae* Scopoli, could accumulate higher amount of Cd when feeding on highly contaminated host plants, high metal concentration appeared to have few adverse effects on aphid growth, development and reproduction. Hanson *et al.* (2004) showed that selenium (Se) protects plants from green peach aphid, *Myzus persicae* Sulzer, due to both deterrence and toxicity. Aphids even did not choose the plant leaves contaminated with Se and if they forced to feed on, they died.

Feeding behaviour of aphids may increase in an accumulation of metals as they obtain their food from sieve tubes in plant phloem tissue. Sap within the tubes is sugar-rich but poor in amino acids. Therefore aphids ingest large amount of sap to have sufficient amino acids (Dixon 1973). Crawford *et al.* (1995) showed that while *A. fabae* accumulated Cd in significant amount, it did not accumulate Cu. Merrington *et al.* (1997) showed clear accumulation of Cd and Zn within the aphid tissue compared with their host plant. Some heavy metals ingested from the contaminated plants are accumulated within the soft part of the aphid body and they are easily excreted (Hughes *et al.* 1980). Merrington *et al.* (2001) observed higher amount of Cd and Zn accumulated in aphids but there were differences in the level of accumulation. The subsequent impact of heavy metal increase in soil on herbivorous insect populations can be either positive or negative (Bagatto and Short-house 1996). Viskari *et al.* (2000) demonstrated that life history traits of *Cinara pilicornis* (Hartig) were differently affected by traffic pollution.

There are not enough detailed research to reach general conclusions about heavy metal effects on the host plant-herbivorous insects relationships. But there is a strong evidence that the changes in nitrogen metabolism in

host plants have an important effect on herbivorous insect performance. Aphids, in particular, are going to be affected dramatically due to their feeding style from heavy metal accumulation in their host plant. As they are consumed by a wide range of predators, the other species involved in the food chain could be also contaminated.

The goals of this study are to find out: 1) Are there differences in life history traits of the cabbage aphid individuals reared on heavy metal contaminated (lead and copper) host plant?, 2) Are there any differences between individuals reared on two host plants? Is there any host specific effect?

2. MATERIAL AND METHODS

2.1. Experimental design

Cabbage aphid, *B. brassicae*, individuals were continuously reared on heavy metal contaminated and uncontaminated (as a control) cabbage and radish plants under laboratory conditions. Both host plants were exposed to copper as $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (3.14 mg l^{-1}) and lead as PbNO_3 (1.39 mg l^{-1}) separately beginning from planting. An amount of 50 ml metal solution was given to each contamination pot daily and 50 ml water was given to control plant. For the initial establishment, one adult apterous was placed in a clip-cage, allowed to give a birth and then removed along with all but single offspring was left. Left offspring was numbered and confined singly in the clip-cage on upper surfaces of leaves. Aphids were reared on potted plants in transparent acrylic boxes ($40 \times 40 \times 55 \text{ cm}$) in a controlled environment at 20°C and relative humidity 75% at 16 h day light. Observations were made on 20 days old radish and 30 days old cabbage plants and host plants were changed every two weeks regularly.

2.2. Assessments

Individuals in each clip-cages were followed at 24 h intervals on both metal contaminated and uncontaminated (control) host plants. Development time (d , time from birth to beginning of first reproduction) and relative fecundity (Rf , average number of

3. RESULTS

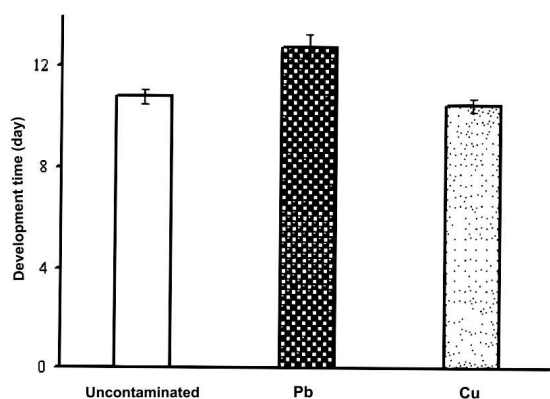


Fig. 1. The development time (d – period from birth to first reproduction, in days) of *B. brassicae* individuals reared on uncontaminated, copper and lead contaminated cabbage plants (each bar represents the mean \pm SE).

offspring produced per day) were recorded. The intrinsic rate of natural increase (r_m) was calculated to determine the fitness of *B. brassicae* populations.

r_m was calculated by the formula of Wyatt and White (1977):

$$r_m = (0.738 \times \ln(Md))/d \quad (1)$$

Where:

d – is the development period from birth to beginning of first reproduction, in days

Md – is the number of nymphs born in the period from d to $2d$ days from birth.

Significance of the metal effects was detected by performing one-way ANOVA. The post-hoc analyses were performed to determine which means differed significantly according to ANOVA results. In addition to these parameters, offspring mortality and adult mortality were also recorded. The offspring mortality was assessed as the per cent of offspring that have not reached the adulthood and the adult mortality was assessed as the per cent of adults that did not reach the age equals two times of development (d) period. Mortality results were compared using arcsine transformation and significance was tested by applying the two-sample t test (Zar 1996). The statistical program SPSS 10.01 was used for all analyses.

The development time of aphids was the longest on Pb contaminated cabbage. Calculated mean values of the development time (d) on both uncontaminated (as a control) and Cu contaminated cabbage plants were almost the same and both were shorter than on Pb contaminated host plants (Fig. 1). An overall analyses indicated significant differences among treatments in development time ($F_{[2,99]} = 17.22, P < 0.0001$) among treatments and post-hoc analyses indicate that this is due to Pb treatment (Tukey HSD $_{[33,8]} = 2.01, P < 0.0001$).

There were significant differences in development time between aphids reared on uncontaminated and Pb contaminated host plants ($F_{[1,66]} = 16, P < 0.0001$) and between individuals reared on Cu and Pb contaminated cabbage plants ($F_{[1,69]} = 25, P < 0.0001$), respectively.

Heavy metal contamination affects also the relative fecundity (Rf) of the aphids. There were differences in the relative fecundity between individuals reared on Cu and Pb contaminated cabbage plants (Fig. 2). There was an overall significant effect of metal treatment on relative fecundity ($F_{[2,99]} = 7, P = 0.002$), and post-hoc analyses revealed that it is due to lead contamination (Tukey HSD $_{[33,8]} = 0.64, P < 0.001$), the copper treatment shows no significant deviation from the uncontaminated control.

There were differences in relative fecundity (Rf) between individuals reared on uncontaminated and on Pb contaminated cabbage plants ($F_{[1,66]} = 11, P = 0.001$). In contrast, there were no significant differences between individuals reared on uncontaminated and on Cu contaminated cabbage plant ($F_{[1,63]} = 4, P = 0.063$) and populations reared on Pb contaminated and on Cu contaminated cabbage plant ($F_{[1,66]} = 4, P = 0.065$) respectively. It seems that heavy metal accumulation affected survival ability and reproductive success of the aphids. In addition to development time and relative fecundity, there were also differences in intrinsic rate of increase (r_m) between aphid populations (Fig. 3). Although r_m of aphid population was higher on uncontaminated host plant, there were not statistically significant differences between

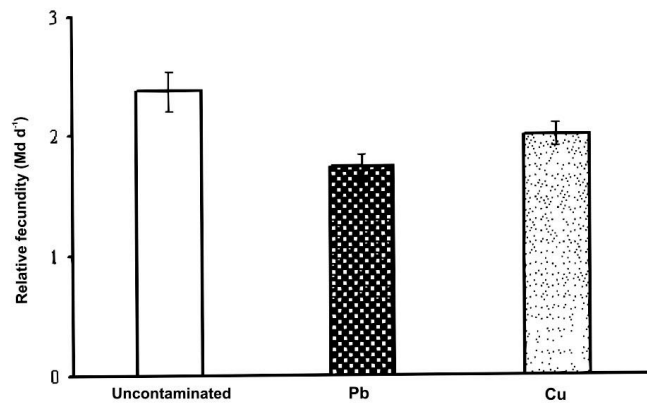


Fig. 2. Relative fecundity (R_f) of *B. brassicae* individuals reared on uncontaminated, copper and lead contaminated cabbage plants (each bar represents the mean \pm SE). (Md – the number of nymphs born in the period from d to $2d$ days from birth, d – the development period from birth to beginning of first reproduction, in days).

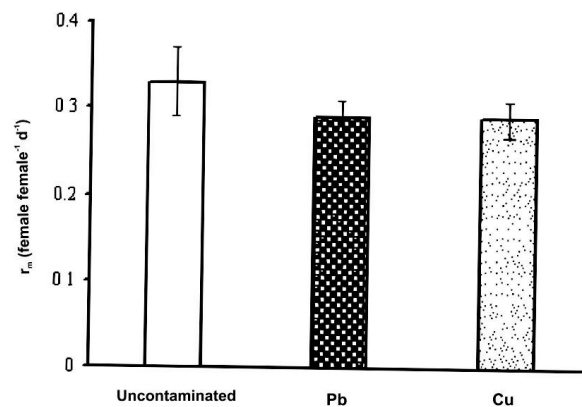


Fig. 3. Fitness (r_m) of *B. brassicae* populations reared on uncontaminated, copper and lead contaminated cabbage plants (each bar represents the mean \pm SE) (r_m – intrinsic rate of increase, see equation (1)).

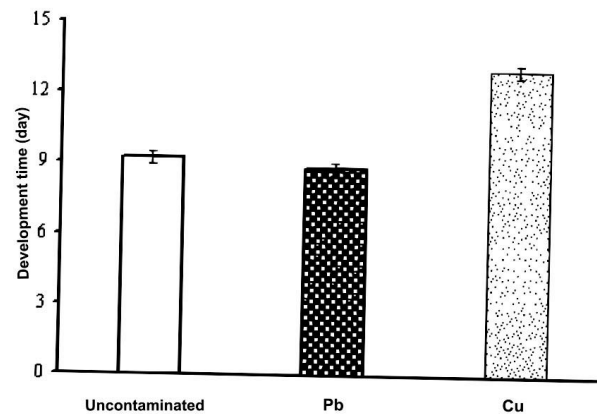


Fig. 4. The development time (d) of *B. brassicae* individuals (in days) reared on uncontaminated, copper and lead contaminated radish plants (each bar represents the mean \pm SE).

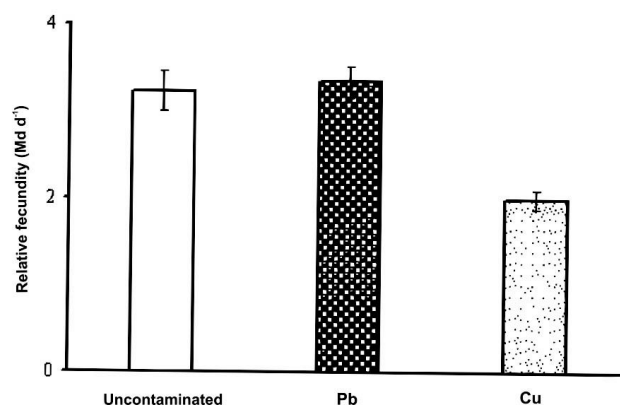


Fig. 5. Relative fecundity (Rf) of *B. brassicae* individuals reared on uncontaminated, copper and lead contaminated radish plants (each bar represents the mean \pm SE). (Md – the number of nymphs born in the period from d to $2d$ days from birth, d – the development period from birth to beginning of first reproduction, in days).

populations reared on contaminated and uncontaminated cabbage plants ($F_{[2,99]} = 2$, $P = 0.135$).

There were also differences in mortality between populations. Offspring mortality and adults mortality on uncontaminated cabbage was found to be 30 and 17%, respectively. The mortality rate on metal contaminated host plant was higher. On Cu contaminated plant, offspring mortality and adult mortality were 54 and 24% respectively and on Pb contaminated host plant – 47 and 64% respectively. Differences in offspring mortality between uncontaminated and Cu contaminated plants were not statistically significant after arcsine transformation ($Z = 0.73$, $P > 0.05$). However, there were significant differences in adult mortality between populations reared on Pb contaminated and uncontaminated cabbage plants ($Z = 4.54$, $P < 0.001$).

There were also important effects of heavy metal contamination on the development time, relative fecundity and intrinsic rate of increase of cabbage aphids when reared on contaminated radish plants. The development time (d) of the population reared on Cu treated radish was longer than both on uncontaminated and Pb contaminated radish plants, respectively (Fig. 4). There was an overall significant effect of metal treatment ($F_{[2,99]} = 79.54$, $P < 0.0001$), and post-hoc analyses indicate that it is due

to Cu treatment (Tukey HSD $_{[33,14]} = 3.79$, $P < 0.0001$) whilst Pb treatment shows no significant deviation from the control.

Aphids reared on Cu contaminated radish have significantly longer development time than the individuals reared on Pb contaminated radish ($F_{[1,68]} = 125$, $P < 0.0001$) and those reared on uncontaminated radish ($F_{[1,62]} = 99$, $P < 0.0001$).

Aphid individuals reached the lowest relative fecundity (Rf) on Cu contaminated radish plants and there was almost no difference between individuals reared on uncontaminated and Pb contaminated radish plants (Fig. 5). An overall effect of metal treatment is significant ($F_{[2,99]} = 17.22$, $P < 0.0001$), and post-hoc analysis shows that it is due to copper treatment (Tukey HSD $_{[31,12]} = 1.24$, $P < 0.0001$) whilst lead contamination shows no important deviation from the control. The relative fecundity of the individuals reared on Cu contaminated radish was significantly different from both populations reared on the uncontaminated ($F_{[1,58]} = 27$, $P < 0.0001$) and Pb contaminated radish plants ($F_{[1,64]} = 39$, $P < 0.0001$), respectively.

There were also differences between intrinsic rates of increase (r_m) of populations. Aphid population reached the highest r_m on Pb contaminated host plants, which was slightly higher than on uncontaminated plants (Fig. 6). Metal accumulation in host plants has a significant effects ($F_{[2,93]} = 13.66$,

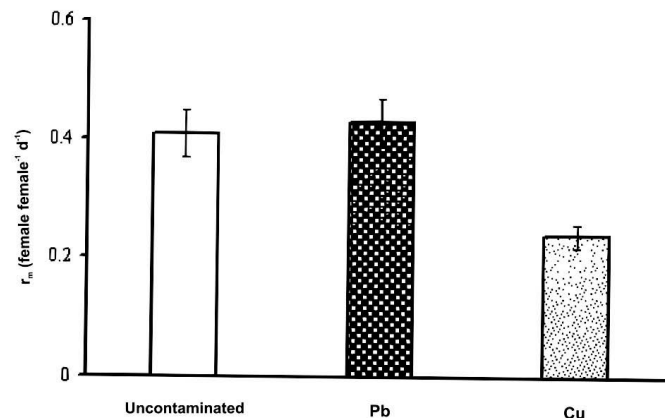


Fig. 6. Fitness (r_m) of *B. brassicae* populations reared on uncontaminated, copper and lead contaminated radish plants (each bar represents the mean \pm SE). (r_m – intrinsic rate of increase, (equation 1)

$P < 0.0001$), and post-hoc analysis indicates that it is due to copper treatment (Tukey HSD $_{[31,12]} = 0.138$, $P = 0.001$) while the lead treatment expressed no considerable deviation from the control. The values of the intrinsic rate of increase of the population reared on Cu contaminated radish were significantly different from both populations reared on uncontaminated ($F_{[1,60]} = 19$, $P = 0.0001$) and Pb contaminated radish ($F_{[1,64]} = 23$, $P = 0.0001$). However, metal contamination did not produce a consistent change in mortality of individuals. For example,

there was a 45% offspring mortality on Cu contaminated radish plants compared to 41% mortality on Pb contaminated and 29% mortality on uncontaminated plants, but none of these differences was statistically significant ($Z = 1.40$, $P > 0.05$).

Heavy metal accumulation affects differently aphid life history traits across host plants. The same metal in two host plants also differently influenced on measured traits. Two-way ANOVA analyses indicated that it is due to the significant host-metal interaction (Table 1).

Table 1. Analyses of variance of life history traits of the cabbage aphid (*B. brassicae*) populations reared on uncontaminated, copper and lead contaminated cabbage and radish plants (Two-way ANOVA, host as fixed factors, metals as random factors). (r_m , equation (1) – intrinsic rate of increase, Rf – average number of offspring produced per day, d – time from birth to beginning of first reproduction).

| Traits | Source | Df | F-ratio | Significance (P) |
|--------|---------------------|-----|---------|----------------------|
| r_m | Host | 1 | 0.750 | =0.478 |
| | Metal | 2 | 2.050 | =0.328 |
| | Host \times Metal | 2 | 4.749 | = 0.010 |
| | Error | 166 | | |
| Rf | Host | 1 | 2.870 | =0.232 |
| | Metal | 2 | 0.961 | =0.510 |
| | Host \times Metal | 2 | 15.134 | < 0.0001 |
| | Error | 190 | | |
| d | Host | 1 | 0.221 | =0.685 |
| | Metal | 2 | 0.239 | =0.807 |
| | Host \times Metal | 2 | 68.164 | < 0.0001 |
| | Error | 196 | | |

4. DISCUSSION

It was shown in numerous studies that aphids accumulate heavy metal in their bodies more than host plant tissues (Crawford *et al.* 1995, Merrington *et al.* 2001) and this might be due to feeding mode. The potential for the uptake of heavy metals by aphids and other herbivorous insects has been shown in a various laboratory and field studies but in most of the studies their effects upon herbivorous insects life history traits such as development time, fecundity, fitness and mortality rate were ignored. Culliney and Pimentel (1986) demonstrated that survival and fecundity of the green peach aphid, *Myzus persicae*, were considerably reduced when they were reared on collard plants grown in pots of soil treated with chemically contaminated sewage sludge. Görür (2006) demonstrated the negative effects of the heavy metal accumulation in host plants on aphid morphological development process. Aphid populations reared on heavy metal accumulated host plants showed greater variation in development process i.e. developmental instability.

The results of the presented study indicated clear adverse effects of heavy metal contamination on measured life history traits of the cabbage aphid, *B. brassicae*. On both host plants (cabbage and radish), all measured life history traits were greatly reduced by heavy metal contamination. Aphid population also showed higher mortality rate on contaminated host plants. Thus, it can be considered that higher amount of heavy metal uptake by aphids through feeding on contaminated hosts resulted in reduction of life history traits. *B. brassicae* individuals showed the higher mortality rate and the lower fecundity on contaminated radish and cabbage plants. Aphid population also demonstrated the lowest fitness on heavy metal contaminated host plants. These results indicated that heavy metal accumulation created physiological stress and lead to reduction in main life history traits.

Regarding the impact on all life history traits, there were also interesting differences between two heavy metal effects on both host plants. Two-way ANOVA analyses in-

dicated significant host-metal interactions. Pb had stronger effects on life history traits of aphid population reared on cabbage, while Cu has been showing the strongest negative effect on population reared on radish. These differences can be explained by a different uptake strategy or by different physiological pathway involved in the metabolism of these two metals. Crawford *et al.* (1995) demonstrated the different level of accumulation for the various heavy metals and possibility to regulate it by aphids through digestion system.

The experimental concentrations of Cu and Pb used in this study are higher than those occurring in arable plant occurred within normally polluted areas. However, they prove that the effects of elevated heavy metal pollution can be strong. The study results clearly indicate that the possible effects of heavy metal pollution to aphid life history traits could be adverse. In an ecological context, the important point is to assess their possible ecological consequences. Further studies are required to clarify how heavy metal pollution influences the aphid-host plant relationships in field. Especially in what way the changes in life history traits of aphids influence their natural enemies-parasites and predators.

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