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B. DEVKOTA, G.H. SCHMIDT

# Life span and fecundity of *Aiolopus thalassinus* exposed to dietary heavy metals (Hg, Cd, Pb)

**Abstract** - *Aiolopus thalassinus* (Fabr. 1781) (Orthopteroidea, Caelifera Acrididae) from Kenya was reared and fed with wheat seedlings grown in water contaminated by different heavy metal salts. Additionally, the egg-laying substrate was contaminated.

The effects of dietary heavy metals (Hg, Cd, Pb) on the life span and fecundity of *A. thalassinus* were studied. The life span of *A. thalassinus* kept on Hg, Cd and Pb contaminated food was shorter than that of control grasshoppers, where Hg showed the highest effect, followed by Pb and Cd. Females exposed to Hg and Pb had significantly shorter life spans than those exposed to Cd. Differences in the life span between the female and male within the same exposure was significant only during exposure to cadmium. The accumulation to Hg was propotional to the life span in both sexes, as it increased steadily with the age. The concentration factors were in the order Hg > Cd > Pb. Cadmium accumulation in both sexes increased during the maturing phase (first 8-10 days of adult stage), but it decreased steadily when the individuals became older. Decontamination of Cd was faster and greater in males than in females. Bioaccumulation of Pb in the grasshoppers was significantly lower than in food.

Dietary Hg reduced the fecundity, whereas females exposed to Cd laid more eggs and egg pods than in control. The effect of dietary Pb was not as negative as that of Hg. The females during Hg exposure required 4.21 days for one oviposition, on the average, whereas those exposed to Cd and Pb contaminated food oviposited in shorter intervals, 3.17 and 3.26 days. respectively. A higher number of egg pods was deposited in substrates containing higher concentrations of metal salts. Higher number of eggs per female per day and more frequent oviposition were accompanied by decreasing Cd concentration in the female. The life span of such individuals were longer than those exposed to Hg and Pb.

**Zusammenfassung** - *Lebenszeit und Fruchtbarkeit von* Aiolopus thalassinus *nach Aufnahme von Schwermetallen (Hg, Cd, Pb) in der Nahrung.*

Eine Population von *Aiolopus thalassinus* (Fabr., 1781) aus Kenya wurde auf Weizenkeimlingen gezogen, die in Wasser mit verschiedenen Schwermetallsalzen kultiviert waren. Zur Eiablage stand unterschiedlich kontaminiertes Sandsubstrat zur Verfügung.

Nach Aufnahme von mit Schwermetallen (Hg, Cd, Pb) kontaminierter Nahrung war die Lebenszeit von *A. thalassinus* verkürzt, wobei mit Hg behandelte Weizenkeimlinge eine stärkere Wirkung hatten als solche, die in Wasser mit Pb- oder Cd-Salzen kultiviert worden waren. Weibchen, die Hg- oder Pb-haltiger Diät ausgesetzt waren, lebten signifikant kürzer als solche, die Cd-haltige Keimlinge fraßen. Unterschiede in der Lebenszeit zwischen Weibchen und Männchen waren bei gleicher Expositionszeit nur bei Cd-Aufnahme signifikant. Eine Anreicherung von Hg im Körper erfolgte bei beiden Geschlechtern der Lebenszeit proportional und stieg dem Alter entsprechend an. Der Konzentrierungsfaktor war für Hg > Cd > Pb. Die Cd-Akkumulation stieg in beiden Geschlechtern während der Reifungsphase (die ersten 8-10 Tage nach der Adulthäutung) an und sank dann stetig mit dem Alter ab. Die Dekontamination von Cd war schneller und stärker bei Männchen als bei Weibchen. Die Bioakkumulation von Pb in den Insekten war signifikant niedriger als in der Nahrung.
Quecksilber in der Nahrung reduzierte die Fruchtbarkeit. Aber Weibchen auf Cd-haltiger Nahrung produzierten mehr Eier und Ootheken als auf Kontrolldiät. Die Wirkung von Pb in der Nahrung war nicht so negativ wie die von Hg. Bei Hg-haltiger Nahrung benötigten Weibchen im Mittel 4,21 Tage für eine Eiablage, während jene, die Cd- oder Pb-haltige Nahrung aufnahmen, in kürzeren Intervallen Ootheken absetzten (nach 3,17 bzw. 3,26 Tagen). Ootheken wurden vermehrt in Substraten mit höherer Metallsalzkonzentration abgelegt. Eine höhere Anzahl von Eiern pro Weibchen pro Tag und eine häufigere Oviposition waren von einem Abfall der Cd-Konzentration im Insektenkörper begleitet. Die Lebenszeit solcher Weibchen war länger als von denen, die Hg oder Pb-haltige Nahrung aufgenommen hatten.

**Riassunto** - *Longevità e fecondità di* Aiolopus thalassinus *allevato con diete contaminate da metalli pesanti (Hg, Cd, Pb).*

*Aiolopus thalassinus* (Fabr. 1781) originario del Kenia è stato allevato con piantine di frumento sviluppatesi con acqua contenente diversi metalli pesanti. Anche il substrato per l'ovideposizione è stato contaminato in modo analogo. E' stato studiato l'effetto di Hg, Cd, e Pb sul ciclo vitale e sulla fecondità dell'insetto. La durata della vita è risultata più breve che nel testimone; Hg evidenzia effetti più spiccati, seguito da Pb e Cd. Le femmine esposte a Hg e Pb hanno una durata di vita significativamente più breve di quelle esposte a Cd. Una differenza nella vitalità tra maschi e femmine sottoposti al medesimo trattamento è risultata significativa solo nel caso di esposizione al cadmio. L'accumulo di Hg è proporzionale alla durata della vita in ambedue i sessi. I fattori di concentrazione sono nell'ordine Hg > Cd > Pb. L'accumulo di cadmio in ambedue i sessi aumenta durante la fase di maturità (primi 8-10 gg dello stadio di adulto), ma decresce quando gli individui progressivamente invecchiano. La decontaminazione del Cd è più rapida e forte nei maschi che non nelle femmine. Il bioaccumulo di Pb nelle cavallette è significativamente inferiore che nel cibo. Hg nella dieta riduce la fecondità, mentre le femmine esposte a Cd depongono più uova ed ooteche che quelle del controllo. L'effetto di una dieta con Ph non è tanto negativo quanto quello con Hg. Durante il trattamento con Hg le femmine richiedono in media 4,21 gg per una ovideposizione, mentre quelle a cui è somministrata una dieta contaminata con Cd e Pb ovidepongono a intervalli più brevi, rispettivamente 3,17 e 3,26 giorni. Un numero maggiore di ooteche è deposto nei substrati contenenti più elevate quantità di metalli pesanti. Una superiore quantità di uova per femmina, per giorno e una più frequente ovideposizione sono correlate con una decrescente concentrazione di Cd nelle femmine. La vita di questi individui è più lunga di quella degli insetti esposti a Hg e Ph.

**Key words**: *Aiolopus thalassinus*, life span, fecundity, dietary heavy metals, Hg, Cd, Pb, oviposition.

## INTRODUCTION

Increasing emission of toxic metals in the environment, their flow within the food chain and increasing toxicity as well as the fate of organisms forced to adapt in such environments are of ecological concern (Wieser, 1979). Anthropogenic contributions to the atmosphere now exceed the natural sources by a factor of around twentyfold in the case of Cu, Ni and Pb, and up to tenfold for Cd and Zn (Nriagu, 1979). The major part of the total ecosystem burden of heavy metals is found in the soil and organic litter components (Martin & Coughtrey, 1987).

When the toxicant enters an ecosystem it will selectively remove susceptible species, ultimately leading to a reduction in species diversity and a change in the community structure (Connell, 1987). Not only the biological effects of toxicants such as changes in the individual, population and ecosystem are important, but also their effects on survival and reproduction are of relevance to ecotoxicology (Beijer & Jernelöv, 1986, Van der Hoeven, 1991).

Because pollutants are not emitted singly, it may not be possible to pin the blame for harmful effects on a specific contaminant (Hopkin, 1989). Still, the toxicants do not act singly; their synergistic effect (Biesinger *et al.*, 1986) might be greater than their individual effects together. Organisms take up chemicals from two principal sources: either directly from the ambient environment or from food, though the toxicant in the food is also derived from the ambient environment (Connell, 1987). Pollutants affect both the abundance and distribution of aquatic insects (Hare, 1992), and such changes occur also in the terrestrial ecosystems. Organisms may exhibit a variety of negligible to sublethal reactions such as reduced growth or reproduction, behavioural effects or ultimately death, so a clear understanding of the nature and effects of potentially hazardous chemicals is needed (Connell, 1987). The grasshoppers in a contaminated environment not only forage on contaminated grasses, but also lay their eggs in contaminated soils. They are thus under continuous exposure to many toxic substances through several paths.

In summer short-horned grasshoppers can constitute about 20-30% of the arthropod biomass in grassland ecosystems (Schmidt, 1986). Heavy metals and persistent chemicals accumulate in the soil and along with the food chain can exterminate whole populations of distinct species (Wieser, 1979, Schmidt, 1986). Low level ecological disturbance due to pollution cannot be distinguished from natural fluctuations in ecosystem composition and structure (Depledge, 1990). An investigation in simulated laboratory conditions would allow to identify the effects of individual toxicant in question on the organisms and an extrapolation of the laboratory results in the field conditions would help to elucidate the question how and in which extent the organisms in the field get adversely affected. So, the effect of dietary heavy metals on the life span and fecundity of *A. thalassinus* was studied to see how the population of such grasshopper species can be affected by the toxic metals.

## MATERIAL AND METHODS

*Aiolopus thalassinus* (Fabr.1781), collected in Kenya, were reared under laboratory conditions (25 ± 5°C and 12/12 hours light-dark rhythm) for many generations in the Department of Zoology-Entomology, University of Hannover, Germany. This species of grasshopper could propagate well under laboratory conditions.

The freshly emerged grasshoppers from the stock rearing were marked on wings and/or the thoracic shield and were placed in cages (15 females and 15 males in each) for this study. Each cage had an upper 25 cm high wooden frame wrapped with nylon gauze, leaving one side for a plexi glass door, and this was mounted on a plexi glass base (18 x 25 x 21 cm). Each cage contained food for the grasshoppers (wheat seedlings grown in a hydroculture pot and also a mixture of dry oats and finely ground dog food - Frolic, as dry food), moistened sand in a shallow petri dish (for extra water /moisture for the grasshoppers) and six plastic pots, each 3 x 3 x 3 cm filled with washed and oven dried sand as substrate for egg laying. The sand (<0.43 mm grains) was washed several times to remove as much organic matter as possible and dried at 250°C for several hours to sterilise it.

### *Exposure to contaminated food*

Wheat seedlings grown in plastic pots (20 x 10 x 5 cm) containing 500 ml of water, separately contaminated by adding different heavy metals (1 µg/ml $Hg^{2+}$, 5 µg/ml $Cd^{2+}$ or 25 µg/ml $Pb^{2+}$ at the time of sowing), were placed in the cages. Additionally, dry food, also separately contaminated by adding 0.15 µg/g $Hg^{2+}$, 1.5 µg/g $Cd^{2+}$ or 3.75 µg/g $Pb^{2+}$, was offered to the grasshoppers. Grasshoppers in the control cages were given not contaminated food and all cages were placed parallel under similar laboratory conditions. Chlorides of the heavy metals ($HgCl_2$, $CdCl_2$ and $PbCl_2$ manufactured by Merck, Germany) were used to contaminate the food as well as the substrates for egg laying.

### *Contamination of egg laying substrates*

About 8 days after exposure to the contaminated food but before the females started to oviposit 5 females and 5 males were taken out from each cage to determine the metal concentrations in sexually mature grasshoppers. The other 10 females and 10 males were reared further. A set of six egg laying pots, each filled with 75 g of washed and oven dried (sterilised) sand, as substrate for egg laying, was placed at the centre of each cage. In adaptation to Schmidt *et al.* (1991, 1992) each set of the egg laying substrates was contaminated by adding different concentrations of each heavy metal salt: 0, 0.121, 0.605, 1.21, 6.05 and 12.1 µg/g $Hg^{2+}$ or 0, 2, 10, 20, 50 and 100 µg/g $Cd^{2+}$ or 0, 25, 50, 100, 250 and 500 µg/g $Pb^{2+}$. Different concentrations of these metal salt solutions were prepared in such a way that with the addition of 9 ml of salt solution 12% humidity was achieved in the substrates. Any loss of moisture from the substrate was compensated by adding deionized water during daily checking. The contaminated substrates were placed in the cages in such a way that the grasshoppers were exposed to the same heavy metal through the diet and the substrate. A similar set of such pots containing not contaminated substrate was placed in the control cage. This experiment was conducted in triplicate.

### *Life span of adults and fecundity*

The grasshoppers which were found dead in the cages were collected during daily checking and individually registered. With the help of the special markings on the wings and prothoracic shield the life span (in days) of each adult grasshopper could be exactly calculated. The life span (mean ± S.D.) of females and males was calculated for different exposures. Heavy metal accumulation in grasshoppers having shorter (less than average: mean - S.D.), medium (average: mean life span) and longer (more than average: mean + S.D.) life spans was determined to see the relation between the life span and the metal accumulation in both sexes.

Every seven days the sets of egg laying pots were exchanged with new ones as long as the females were surviving. The removed pots with egg pods were kept in an incubator for hatching of the nymphs (at 30 ± 1°C). The total number of eggs and egg pods laid by the grasshoppers in differently contaminated substrates were recorded. For each exposure the total life span of the females as well as the total number of eggs and egg pods laid by the females were calculated to see the role of individual heavy metals on the egg laying capacity (eggs per female and day). Number of eggs per pod and the interval between the two consecutive ovipositions were calculated for different exposures.

The egg laying pots in the cages were rotated clockwise twice daily to avoid any possible preference to a particular pot due to its position in the cage, and in the meantime to see whether the degree of contamination had any influence on the females in choosing the substrates for oviposition. The percentage of egg pods laid in differently contaminated substrates was calculated.

### *Metal determination*

Grasshoppers attaining maturity (8-10 days after emergence) and those having different life spans (shorter, medium and longer) were washed with deionised water, freeze dried and then ground (two grasshoppers per sample) in a vibrating mill (manufacturer- Retsch, Germany) to fine and homogenous powder. Five replicates each of both females and males were prepared and the concentrations of Hg, Cd and Pb were determined in atom absorption spectrophotometers attached with different accessories. Metal concentrations in freshly contaminated dry oats as well as in wheat seedlings, collected after 9 days of sowing, were also determined. The concentrations were calculated on the dry weight basis of the samples and the presented concentration values are the means of five determinations with their standard deviations.

For Hg determination homogenous powder (200 mg) of the samples was wet digested in 3 ml aqua regia, a 2:1 v/v mixture of sulphuric acid 95-97% (analytical grade) and nitric acid 65% (supra pure), manufactured by Merck (Germany), at 140°-150°C for 3 hours (Devkota & Schmidt, 1992; Welz, 1985). The sample aliquot was diluted to 20 ml. Mercury was determined by using cold vapour technique in Perkin Elmer AAS 1100 MHS 10. The diluted sample solution (10 ml per determination) was reduced by 3% $NaHBO_4$ and 1% NaOH in the presence of $KMnO_4$ and atomic absorption of elemental mercury, carried by extra pure nitrogen to the quartz tube, was measured by using hollow cathode mercury lamp at 253.6 nm wave length and 0.7 nm slit width. Fixanal Mercury standard (manufactured by Riedel-de Häen, Germany) was used for the calibration and Hg in a parallel empty sample was determined to avoid any unwanted contamination due to reagents and glass wares.

Cadmium and Pb were analysed in a Zeeman AAS SM30 (manufactured by Gruen Optiker Wetzlar, Germany), equipped with a graphite tube furnace and suitable for the determination of elements in solid samples (Steubing *et al.*, 1980; Grobecker & Kurfuerst, 1990; Devkota & Schmidt, 1992). The samples (40-60 µg), weighed in a graphite boat, was introduced into the graphite tube furnace. After stepwise pyrolysis and ashing, the sample was atomised in an argon atmosphere at 2100°C during Cd and at 2300°C during Pb determinations. The concentration of the element was determined by measuring the atomic absorption at 228.3 nm for Cd and at 283.3 nm for Pb using respective hollow cathode lamps. Certified reference materials (CRM 061: pig kidney and CRM 185: bovine liver for grasshopper samples and CRM 060 & CRM 061: aquatic plants for wheat seedlings and dry oat samples) from Bureau of Community Reference (BCR), Brussels, were taken for the calibration. Along with the samples the reference materials were also determined in their Cd and Pb concentrations. The obtained data were within the certified values.

*Analysis of data*

The differences in the life span between the control and heavy metal exposures as well as the differences in the concentrations of heavy metals between the food and grasshoppers were analysed by using Student's T-test (Wardlaw, 1985). Significance of difference in the life spans as well as in the metal concentrations between the females and the males within the same exposure period was statistically analysed by comparing their variances (F-ratio). Significant differences are indicated by: *** $p<0.001$,

** $p<0.01$, *$p<0.05$ and ns – not significant different. The correlation coefficient (R) between the life span (x) of grasshoppers exposed to contaminated food and metal concentration (y) in their bodies as well as the linear trend ($y = mx + b$) were calculated.

## RESULTS

### *Metal concentration in food*

The average concentrations of heavy metals in the wheat seedlings, grown in contaminated water, after 9 days of sowing were 1.01 ± 0.16 µg/g for Hg, 10.5 ± 0.16 µg/g for Cd and 40.5 ± 16.6 µg/g for Pb (Fig. 1a-c). The lower part of the growing seedlings always had higher concentrations than upper parts with concentration factors of 1.4, 2.8 and 2.3 for Hg, Cd and Pb, respectively. In control wheat seedlings, 0.46 ± 0.2 µg/g for Hg and 10.50 ± 7.5 µg/g for Pb could be determined, whereas the concentration of Cd was very low (0.16 ± 0.1 µg Cd/g). Determined concentrations of the heavy metals (0.58 ± 0.19 µg/g for Hg, 2.20 ± 0.8 mg for Cd and 4.20 ± 1.2 µg/g for Pb) in dry oats were higher than the added concentrations (0.15 µg Hg/g, 1.50 µg Cd/g and 3.75 µg Pb/g). This showed that the dry food already had considerable amounts of these heavy metals.

### *Life span*

The life span of individuals kept on Hg, Cd and Pb contaminated food was shorter than of control grasshoppers (Table 1). The highest effect was evident during Hg exposure, where the average life spans of females and males were the shortest of all exposures. Some of the grasshoppers were found to live for less than 12 days, whereas others had a prolonged life span (more than 50 days in females exposed to Cd contaminated food). During Cd exposure, the females were rarely affected, whereas the males had significantly shorter life span than their control neighbours. The males always had a shorter life span than the females from the same exposure, but the difference was significant only in grasshoppers exposed to Cd. The difference in the life span between the males from different exposures was not significant, whereas the

*Table 1 - Life span (days ± S.D.) of female and male* A. thalassinus *exposed to mercury (HgF), cadmium (CdF) and lead (PbF) contaminated food. The differences in the life span between the control and heavy metal treated grasshoppers are indicated as *** $P<0.001$, **$P<0.01$, *$P<0.05$ and ns – not significant differences.*

| | Life span (days ± s.d.) of grasshoppers exposed to | | | |
|---|---|---|---|---|
| | Control | HgF | CdF | PbF |
| Female | 41.5 ± 12.42 | 30.1 ± 14.16** | 40.45 ± 12.08$^{ns}$ | 31.3 ± 12.96** |
| Male | 40.0 ± 15.92 | 23.9 ± 11.01*** | 30.2 ± 15.85** | 28.75 ± 11.89** |
| | Difference in life span between the female and male within the same exposure was significant (*$P<0.05$) only in grasshoppers exposed to CdF. | | | |

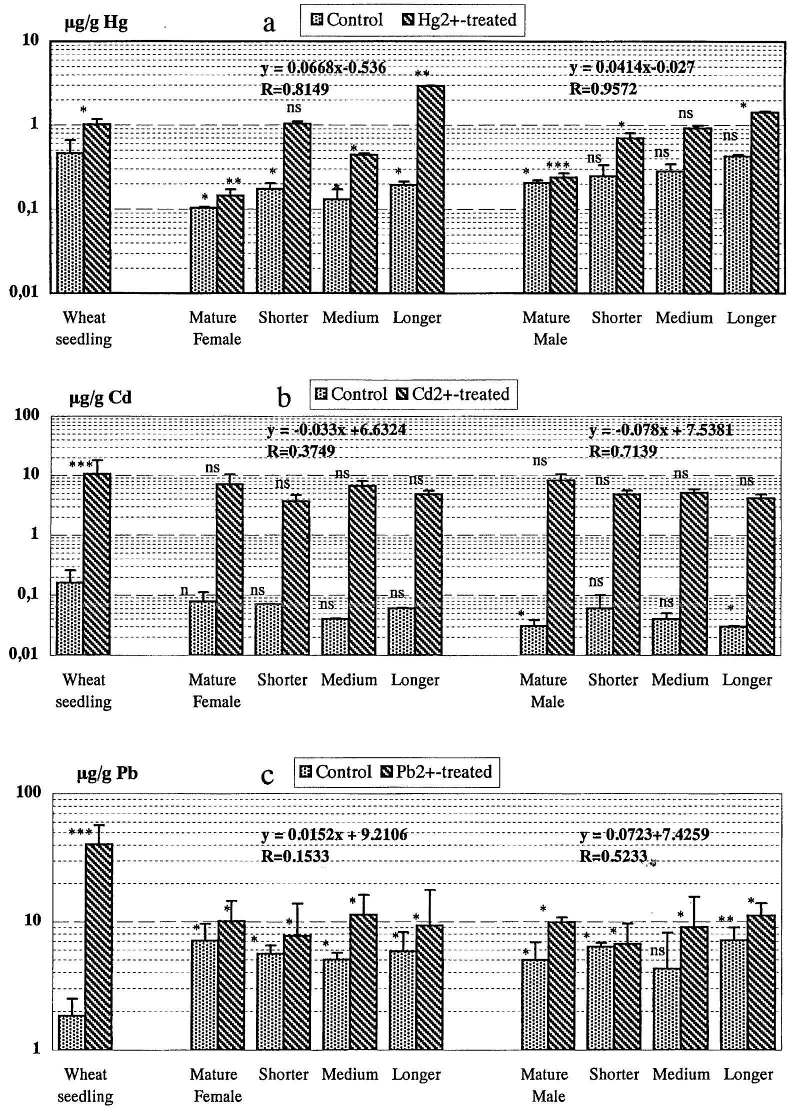


Fig. 1(a-c): Logarithmic presentation of heavy metal concentrations (a) µg Hg/g, (b) µg Cd/g and (c) µg Pb/g in female and male *A. thalassinus* kept on Hg, Cd and Pb contaminated food. Each bar represents the mean of 5 measurements with S.D. Differences in metal concentrations (mean ±S.D., N=5) between the wheat seedlings and body concentrations of *A. thalassinus* (Mature – sexually mature and those having different life spans - Shorter, Medium and Longer) are indicated by ***P<0.001, **P<0.01, *P<0.05 and ns – not significant differences. Correlation coefficients (R) between the life spans (x) of grasshoppers exposed to contaminated foods and metal concentrations (y) in their bodies as well as the linear trends (y = mx + b) are presented.

females from Hg and Pb exposures had significantly shorter life spans than such exposed to Cd contaminated food.

Comparison of variances between the life span of females and males of *A. thalassinus* feeding on the same food showed no significant difference between the standard deviations of the life spans and the effect of a heavy metal on the longevity of both sexes of grasshoppers was similar.

*Life span and heavy metal accumulation*

The accumulation of dietary Hg (Fig. 1a) ranged between low (accumulation factor <1) in sexually mature individuals and high (accumulation factor > 1) in those having longer life spans. A higher accumulation of Hg could be found in females, which also had a longer life span than the males. The concentration of mercury increased steadily with the age and the Hg accumulation and life span were in the same order in both sexes. The correlation between the Hg concentration (y) and the life span (x) was positive with the coefficients 0.8149 and 0.9572 in females and males, respectively. An increase of one unit of life span (x) caused 0.0668 and 0.0414 increase in Hg concentration in females and males, respectively. The greater slope in females (0.0668) than in males (0.0414) showed a faster rate of Hg accumulation in females than in males.

Concentration of Cd in grasshoppers was always lower than in contaminated food (accumulation factor <1: 0.67 in mature female and 0.78 in mature male) and the difference between the concentrations in food and in grasshoppers was also not significant (Fig. 1b). Cd accumulations in females reached to the maximum during the ripening stage (first 8-10 days after emergence), but it decreased steadily in the later phase of adult life. With one unit of life span (x) Cd concentrations (y) in females and males decreased by 0.033 and 0.078, respectively, showing an inverse relation between Cd accumulation and advance in the age of adult grasshoppers. The greater slope in males, which had a shorter life span than the females, showed that the decontamination is faster and greater in males than in females.

The rate of Pb bioaccumulation in *A. thalassinus* was even lower (highest accumulation factors: 0.28 in females with medium life span and 0.27 in males with longer life span) than Hg and Cd accumulations and the Pb concentrations in both females and males were always lower than in contaminated food (Fig.1c). There was an increase in Pb concentration in the grasshoppers exposed to Pb contaminated food, and it was slightly greater in males than in females (slope 0.0723 in male and 0.0152 in female per unit life span).

Comparison of variances between the standard deviations (Table 2) of Cd concentration in females and males of *A. thalassinus* showed that the variances of Cd concentration between different sexes did not vary significantly. Such difference in Hg concentration was slightly significant ($p<0.05$) only between females and males having medium life span, where the males had a significantly higher concentration of Hg than the females. Intraspecific comparison of the variance in Pb concentration between

*Table 2 - Comparison of variances (F-ratio) between metal concentrations in females and males of* A. thalassinus *having similar life spans (sexually mature and those which had shorter, medium and longer life spans) and belonging to the same exposure group (HgF, CdF and PbF) with ratios at 1% (**) and 5% (*) significant levels and ns – not significant variances.*

| Exposed to ⇓ | Mature | Shorter life span | Medium life span | Longer life span |
|---|---|---|---|---|
| HgF | 1.13 (ns) | 2.04 (ns) | 12.25 (*) | 1.00 (ns) |
| CdF | 2.29 (ns) | 1.86 (ns) | 2.54 (ns) | 1.32 (ns) |
| PbF | 23.64 (**) | 4.25 (ns) | 1.81 (ns) | 8.96 (*) |

females and males was significant in sexually mature ($p<0.01$) and in those having longer life spans only at $p<0.05$. Here, the concentrations varied with greater deviations in females than in males, though the mean values did not differ greatly.

*Influence of metal concentrations on fecundity and oviposition*

The fecundity of grasshoppers was found differently affected by three different heavy metals (Table 3). Steady increase of Hg concentration in the females seemed to negatively affect the fecundity (3.71 eggs per female per day) and ovipositions (7.15 egg pods per female), whereas Cd induced it with increased egg laying (6.00 eggs per

*Table 3 - Number of eggs and egg pods laid by* A. thalassinus *exposed to differently contaminated food (Hg contaminated food - HgF, Cd contaminated food - CdF and Pb contaminated food - PbF) and percent ovipositions in substrates (1-6) contaminated by adding different concentrations (µg/g) of $Hg^{2+}$, $Cd^{2+}$ and $Pb^{2+}$. The substrates numbered 1-6 represent the similar positions in all the cages and the figures in the parentheses are the added concentrations of three heavy metal salts in the substrates.*

| Number of ⇓ | Control | | HgF | | CdF | | PbF | |
|---|---|---|---|---|---|---|---|---|
| Eggs/female/day | 5.31 | | 3.71 | | 6.00 | | 5.88 | |
| Egg pods/female | 11.0 | | 7.15 | | 12.75 | | 9.60 | |
| Eggs/egg pod | 20.01 | | 15.62 | | 19.02 | | 19.16 | |
| Days / ovipositon | 3.77 | | 4.31 | | 3.17 | | 3.26 | |
| Ovipositions ⇓ | Substrates Control | Egg pods (%) | Substrates (µg $Hg^{2+}$/g | Egg pods (%) | Substrates (µg $Cd^{2+}$/g) | Egg pods (%) | Substrates (µg $Pb^{2+}$/g) | Egg pods (%) |
| Egg pods laid in different substrates (1-6) | 1 | 15.91 | 1(0) | 16.08 | 1(0) | 14.12 | 1(0) | 13.54 |
| | 2 | 19.55 | 2(0.121) | 13.99 | 2(2) | 15.29 | 2(25) | 15.10 |
| | 3 | 15.00 | 3(0.605) | 16.78 | 3(10) | 15.29 | 3(50) | 15.63 |
| | 4 | 15.91 | 4(1.21) | 13.99 | 4(20) | 13.273 | 4(100) | 15.10 |
| | 5 | 18.18 | 5(6.05) | 13.99 | 5(50) | 18.82 | 5(250) | 19.79 |
| | 6 | 15.45 | 6(12.1) | 25.17 | 6(100) | 22.75 | 6(500) | 20.83 |

female per day) and increased ovipositions (12.75 egg pods per female). The effect of dietary Pb was lower than that of Cd, but not as negative as that of Hg. The females in Hg exposure required 4.21 days for one oviposition, whereas those exposed to Cd and Pb contaminated food laid in shorter intervals, 3.17 and 3.26 days, respectively. In control, there was an interval of 3.77 days between two consecutive ovipositions, on the average.

*Influence of substrate contamination on oviposition*

The behaviour of females in choosing the substrates for egg laying was noteworthy (Table 3). The percent distribution of egg pods in differently contaminated substrates showed that *A. thalassinus* preferred the substrates having higher concentrations of heavy metal salts (12.1 µg $Hg^{2+}$/g; 50 and 100 µg $Cd^{2+}$/g; 250 and 500 µg $Pb^{2+}$/g) to deposit the eggs. The females exposed to mercury laid about 25% of all the egg pods in the most contaminated substrate, whereas between 13.9 and 16.78 % in substrates containing lower concentrations of $Hg^{2+}$. In a parallel control, the percent oviposition was more uniform with a difference of about 4.0% between the substrates. Thus, a preference to more contaminated substrates for egg laying did not seem to be a mere coincidence.

## DISCUSSION

In plants Hg and Pb normally occur in concentrations less than 0.4 µg/g and 10 µg/g, respectively. (Keller *et al.*, 1986). Slightly higher values of Hg found in control wheat seedlings grown in the laboratory could be due to foliar uptake of ambient mercury.

*Life span and metal accumulation*

The life span of *A. thalassinus* adults was reduced by dietary heavy metals, where Hg and Pb were more effective than Cd, but in *Eyprepocnemis plorans* (Charp.) (Acrididae), which had an average life span longer *A. thalassinus*, was still prolonged during Hg exposure (Devkota & Schmidt, 1999). In *A. thalassinus, the* females had a longer life span than the males, but in *E. plorans* the opposite was the case. The accumulation of dietary mercury increased steadily with the age and the concentration was proportional to the life span in both grasshopper species. Sudden increase in Hg concentration in *A. thalassinus* females having shorter life span might indicate a faster uptake and efficient accumulation. The higher accumulation of Hg in females than in males of *A. thalassinus*, but higher in males than in females of *E. plorans* (Devkota & Schmidt, 1999) showed that the length of life span was deciding for higher accumulation of Hg. During early stage of adult life assimilation of Cd and Pb in the isopod *Procellio scaber* Latr. exceeded the growth leading to rapidly increasing concentrations, but during the later stage of life the concentrations of Cd and Pb remained on a stabilised level, where the rate of accumulation was proportional to the rate of growth (Witzel, 1998).

Schmidt *et al.* (1991) found a shorter life span of F1 and F2 adults of *A. thalassinus*, whose P-adults laid the eggs in $Hg^{2+}$, $Cd^{2+}$ and $Pb^{2+}$ contaminated substrates and the offsprings of successive generations were not exposed to toxic substances. An exposure to toxic substances in one generation showed the long term toxicity. Hopkin & Martin (1984) found a shorter life span in the centipede *Lithobius variegatus* Leach from an uncontaminated area and fed with Cd-contaminated wood lice tissues than in those individuals originating from contaminated environment and also fed with Cd-contaminated wood lice tissues. The shorter life span of the centipede individuals could be due to intoxication of midgut tissues by Cd. The adult isopod *P. scaber.* from a highly contaminated medium (12 800 µg Pb/g substrate) had a shorter life span (Beyer & Anderson, 1985), but Schütt & Nuorteva (1983) found no influence of methyl mercuric chloride on the life span of the flour beetle *Tenebrio molitor* L., although a reduced activity of the adult beetles was observed after 11 weeks, when the concentration of Hg exceeded 200 µg/g body weight. Thus, different heavy metals had different levels of toxicity in different insects. Hackstein *et al.* (1985) found a higher accumulation of dietary Cd in the aquatic environment in *Gammarus tigrinus* Sexton (Crustracea; Amphipoda) to be responsible for postponed sexual maturity and prolonged life span, but these could not be observed in *A. thalassinus* exposed to different heavy metals.

In *A. thalassinus,* the concentration factors of three heavy metals Hg>Cd>Pb showed different affinities to get accumulated, where the concentrations of Hg and Cd between females and males did not vary significantly. Dukerschein *et al.* (1992), on the other hand, found significant differences in concentrations of Hg and Cd between females and males of the may fly *Hexagenia bilineata*, where males had higher body burden of both metals and Cd concentration varied more than that of Hg. This might be due to different food uptake.

Hunter *et al.* (1987) found the highest concentration of Cd in older *Chorthippus brunneus* (Thunbg.) (Acrididae), but in *A. thalassinus* it decreased steadily during the later phase of adult life. Thus, different species of grasshoppers seemed to differently accumulate the heavy metals and show different effects.

No significant effect on the life span of *A. thalassinus* exposed to dietary Cd could be due to their better ability to excrete Cd (Devkota & Schmidt, 1992) or due to higher tolerance to this metal, i.e. the toxicity limit of Cd on *A. thalassinus* might be still higher than the highest determined concentrations (7.06 ± 3.06 and 8.20 ± 2.22 µg Cd/g in sexually mature females and males, respectively) and the concentration of Cd in food (10.5 ± 0.16 µg/g) might not be of toxicity level. The shorter life spans of adults kept on Hg and Pb contaminated foods than those exposed to dietary Cd might indicate that the dietary concentrations of Hg (1.01 ± 0.16 µg/g) and Pb (40.5 ± 16.6 µg/g) were of the toxicity level. Kay & Haller (1986) found a body burden of 36.67 µg Cd/g and 44.45 µg Pb/g in the weevil *Neochetina eichhorniae* (Warne), kept on leaves of the waterhyacinth *Eichhornia crassipes* (17.2 µg Cd/g and 9.84 µg Pb/g), but there was no visible toxic effects. On the other hand, with total body concentrations of at least 20 mg and 50 mg Cd/g the isopod *P. scaber* showed a reduced metabolism during a laboratory experiment (Coenen-Stass, 1998). Compared to the Pb concentration in food the concentration in *A. thalassinus* was low, but the dietary concentration of Pb seemed to have a negative influence of the life span of adult grasshoppers. Wong & Cheung (1986) found lower concentrations of Pb in *Pieris canidia* caterpillars (Lepidopera) than in food reared on waste grown vegetables and Roth-Holzapfel (1990) also found lower Pb accumulation in different arthropods (bark beetles and diplopods) of a forest ecosystem than in their foods.

*Substrate concentration and oviposition*

Deposition of higher number of egg pods by *A. thalassinus* in substrates containing higher concentrations of heavy metal salts could be due to salt effects. More frequent ovipositions in substrates containing higher concentrations of heavy metal salts might indicate that the females perceive the salt concentrations and prefer the substrates with higher ionic concentration for oviposition, but this has to be proved. On the other hand, the isopod *Procellio laevis* Latr. was able to avoid Pb contaminated leaves which would cause accumulation of leaf litter, and thus a reduction of the decomposition rate (Odendaal & Reinecke, 1998).

*Heavy metal exposure and fecundity*

Both the germinal and somatic cells of the reproductive system of *Locusta migratoria* L. were adversely affected by Hg (Martoja *et al.*, 1983) and such toxic effect might be responsible for the reduced fecundity of *A. thalassinus*, ultimately affecting the population. Haney & Lipsey (1973) observed a lower fecundity in the plant louse *Macrosiphum gei* Ashmed kept on tomato plants grown in methyl mercury hydroxide contaminated substrate. In the testes and eggs of *A. thalassinus* Hg and Cd were enriched significantly when the grasshoppers were given highly contaminated food throughout the nymphal development (Schmidt, 1992; Schmidt & Ibrahim, 1994). Devkota & Schmidt (1992) found higher accumulation of Hg in malpighian tubules and midgut epithelium, but not in oocytes and ovarioles of *A. thalassinus*, when the individuals were contaminated as adults.

Rabitsch (1997) found the highest concentrations of heavy metals (Pb, Cd, Cu, Zn) in the midgut followed by the malpighian tubules and the hindgut in the workers of three ant species *Formica pratensis* (Retz.), *F. polyctena* (Foerster) and *Camponotus ligniperda* (Latr.). Higher accumulation of Cd in midgut epithelium, fat bodies, malpighian tubules, muscles and ovarioles of *A. thalassinus* (Devkota & Schmidt 1992) showed the uptake and accumulation as well as decontamination of Cd. In *A. thalassinus* decontamination seemed to be due to excretion (through malpighian tubules) and internal decontamination (deposition in muscles and fat bodies) and storage excretion (in oocytes). A higher number of eggs per female per day and more frequent ovipositions by Cd treated females than by Hg and Pb treated ones might indicate a better decontamination of Cd, which could be supported by the decreasing Cd concentration and longer life span of Cd treated females. Thus, increased oviposition might be one mechanism to get rid of Cd. Quimby *et al.* (1979), on the other hand, found no influence of Cd (18 μg/g food) on the fecundity of *Cyperus rotundus* L. (Lepidoptera), but observed a decreased oviposition (only 4 eggs laid) in the beetle *Agasicles hygrophila* Selman & Vogt feeding on the leaves of alligator weed *Alternanthera philoxeroides* (Mart.) Griseb. containing 8.7 μg Cd/g, compared to 53 eggs in control beetles. Gintenreiter *et al.* (1993) observed that higher doses of dietary metals (50 μg Cd/g, 200 μg Cu/g and 500 μg Zn/g body weight) reduced the survival and inhibited the reproduction in *Lymantria dispar* L. (Lepidoptera), but Pb in a concentration of 500 μg/g body weight had no effect. Dietary administration of Pb (40.5±16.6 mg/g in diet) in *A. thalassinus* (11.34 mg/g in female and 10.94 mg/g in male) also showed no negative effect, which may indicate that the concentrations of Pb were below the toxicity levels. In third instar larvae of the mosquito *Aedes aegypti* L (Diptera) exposed to heavy metals for 24 h the mortality rate was metal and dose dependent; the $LC_{50}$ endpoints were 3.1, 16.5 and 33 ppm for Hg, Cd and Cu, respectively (Rayms-Keller *et al.*, 1998).

The concentrations of individual pollutants might lie well below the toxicity level or within the `safety levels, but their synergistic effects can be harmful to the organisms. Different concentrations of Cd, Hg, and Zn in water (0.09, 9.19 and 0.34 μg Cd/L, 0.9, 1.9 and 3.5 μg Hg/L and 35, 70 and 140 μg Zn/L) had no effect on the reproduction in *Daphnia magna*, but it was significantly reduced by the mixtures of these metal salts (Hg-Cd, Cd-Zn, Hg-Zn) (Biesinger *et al.*, 1986).

Dietary Hg by shortening the life span and by reducing the fecundity had adverse effects on the population of *A. thalassinus*, whereas the concentration of Cd in food during the present study did not seem to be of toxicity level and to adversely affect the population of this species. Though Cd is a more toxic metal than Pb, its dietary concentration in the present study did not seem to be as toxic as dietary Pb.

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DR. BHUPENDRA DEVKOTA - Department of Ecology, Faculty-5, University of Osnabrück, Barbarastr.11, D-49076 Osnabrück, Germany.

PROF. DR. GERHARD H. SCHMIDT - Department of Zoology-Entomology, University of Hannover, Herrenhäuserstraße. 2, D-30419 Hannover, Germany

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