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Effects of copper and benzo(a)pyrene concentration on life history characteristics of *Aphelenchus* and *Acrobeloides*

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Introduction

Nematodes have promise as bioindicators because of their ubiquity, known response to chemical and physical perturbations to soil and water, and current consideration in regional and national monitoring programs (Freckman 1988, Gupta et al. 1997, Heinz 2002, van Straalen and van Gestel 1993). Although biology and genetics of *Caenorhabditis elegans* are well known, the use of this species for ecotoxicological testing is rather artificial (Kammenga et al. 1996b). Apart from *C. elegans*, toxicity tests have been conducted using the aquatic nematode species, *Panagrellus redivivus*. The nematode has been used to determine toxic effects of about 400 single chemicals (Samoiloff, 1987). For terrestrial ecosystems, a community-based maturity index (MI) is a promising index of soil biological health (Heinz, 2002). MI is based on the allocation of nematode taxa into five groups (from 1 to 5) representing different life strategies and ecological requirements (Bongers 1990). It is a measure of the ecological successional status of a soil community. Because succession can be interrupted at various stages by common agricultural practices, such as cultivation and applications of fertilizers and pesticides (Wasilewska 1979), the succession status of a soil community may reflect the history of disturbance. Basically, MI is a weighted mean of individual colonizer-persister (CP) values ranging from 1 to 5. Smaller weights are assigned to taxa with relative tolerance to disturbance and large weights to taxa more sensitive to disturbance (Bongers 1990). Nematode taxa have specific life history traits that affect their sensitivity to stress.

Current assignments of many nematodes have been inferred based on morphology rather than confirmed by convincing criteria, thus, a difference between the CP value of some species and their real status are inevitable (Korthals et al. 1998, Fiscus and Neher 2002). A weight of 2 infers tolerance, but in fact, it may demonstrate sensitivity. However, nematode species with similar morphology and, thus, index values sometimes respond oppositely to chemical pollutants (Korthals et al. 1998, Fiscus and Neher 2002).

In this study, we compare the effects of two classes of genotoxicants, heavy metals and polycyclic aromatic hydrocarbons (PAHs) on life history characteristics of two nematode genera, *Acrobeloides* and *Aphelenchus*, that have been assigned identical CP values for the MI. Our goal was two-fold, that is to test whether these two genera 1) respond to both classes of chemical pollutants in the same manner, and 2) have similar levels of tolerance to chemical pollution.

Methods

Two species of nematodes, *Acrobeloides* (Rhabditidae) and *Aphelenchus* (Aphelechidae), were chosen that had identical index values of 2, but different diets. An index value of two suggests both genera are tolerant to environmental disturbance. Nematode species were chosen for their presence in local sites contaminated with either heavy metals or polycyclic aromatic hydrocarbons and respective control sites paired for common soil type. These two genera have been reported to have responses to environmental stress that contrast the index value, assigned on the basis of morphology (Korthals et al. 1998, Fiscus and Neher 2002). We can successfully culture *Acrobeloides* on *E. coli* bacteria and *Aphelenchus* on *Rhizoctonia solani* fungi.

There are two sites in this study; one site is located at the Fostoria Wastewater Treatment Plant near Fostoria, Ohio. Soil samples analyzed in 1998 and 1999 indicate that soil are neutral to basic and contain high concentrations of copper, cadmium, manganese, lead and zinc with median values of 4830, 4476, 263, and 1160 mg/kg, respectively. The other site is located at an abandoned lumber treatment site in Toledo, historically polluted by creosote. The most abundant compounds at the site were fluoranthene, pyrene, phenanthrene and benzo (α) pyrene (BaP). Their respective maximum concentrations were 605.68, 775.03, 240.46, and 2077.55 mg/kg, respectively (Blakely et al. 2002).

In these experiments, characteristics of survival, development and reproduction are measured for each of two nematode species exposed to each of two contaminants, copper and benzo(a)pyrene. Measurements in these experiments include survival as lethal concentration (LC_{50}), development (size and rate), and reproduction (egg number, size, hatch rate).

Acute survival experiments were designed as a completely randomized design. Each genus was tested in at least four concentrations of copper and BaP. For each of three replicates of each concentration, 10 adults (J4, just entering adult stage, no eggs laid) were placed on each of three culture dishes and incubated at 20 C for 5 days after which percent mortality was determined. Death was determined to occur if an individual did not respond when touched by a probe. Mortality in treatments is corrected for mortality in control treatments. Linear regression is performed with proportion mortality as the dependent variable and concentration as the independent variable. A median lethal concentration (LC_{50}) was computed for each genus.

Growth and development for both genera were quantified on a range of concentrations of copper and BaP. For *Acrobeloides*, 0, 5, 10, and 5 and 20 mg/kg copper and 0, 0.125, 0.25, 0.5 and 1 mg/kg BaP were tested. For *Aphelenchus*, 0, 10, 20 and 50 mg/kg copper and 0, 0.5, 1 and 2 mg/kg BaP were used. These concentrations were chosen based on preliminary tests demonstrating that greater concentrations inhibited survival and/or growth of each respective genus. Size measurements were made using video image analysis at 4, 8, 12, 16, and 20 days after inoculation. Inoculation comprised of placing gravid females on the plate, and then removed after laying eggs for three days. Each treatment was comprised of 15 culture dishes (3 replicates x 5 measurements through time). Average generation time (egg to egg) was approximately 14 days for A*crobeloides* and 20 days for *Aphelenchus*. Biomass (ng C) was computed based on length and width measurements according to Andrássy (1956).

Reproductive output was measured as number and size of eggs in two successive generations. Hatching rate was quantified as the number of juveniles that emerged from eggs and survived. Reproduction was expressed both as total and daily numbers of eggs per culture dish. Each culture dish was seeded initially with 10 gravid females. In the first generation, the adults were removed after 3 days of laying eggs. In the second generation, egg production for single females was monitored. Every five days, the female was transferred to fresh media, and allowed to continue to lay eggs until she died (sensu Kammenga 1996a). Size measurements of at least five eggs per experiment unit were measured by video imaging.

To meet assumptions of normality, proportion of eggs hatched was transformed as arcsine of the square root, number of eggs was transformed as the natural log $(x + 1)$ where x is the number of eggs, and worm weight was transformed as $(x + 0.1)$. All statistical analyses were performed using GLM and MIXED procedures of the Statistical Analysis System 6.08 (SAS Institute Inc., 1989).

Results

Survival. Notably, neither species of nematode could survive or reproduce on copper or BaP concentrations observed in the field. However, we know nematodes are prevalent in soils with these concentrations (Blakely et al. 2002). Bioavailablity of contaminants depends on their solubility in water and exchange capacity of soil, determined by clay and organic matter content. For example, organic contents greater than 2% decrease bioavailability of PAHs (Alexander 2000). Bioavailability of copper is 63 times less than the total amount added to soil (Kammenga et al. 1996b).

Obviously, individual nematodes gained weight through time as they matured. Generally, BaP affected survival of *Aphelenchus* and *Acrobeloides* more than copper (Table 1). These results suggest that responses are pollutant-specific. Quantitative associations between development stage relative to body length aid in interpretation of biomass estimates (Table 2).

Table 1. Survival of two nematode genera to acute exposure of a heavy metal and polycyclic aromatic hydrocarbon. Expressed are Lethal Concentration doses (mg/kg) for 50% of the population (LC_{50}).

Nematodes	copper	Benzo(a)pyrene
Aphelenchus	74.5	> 20[a]
Acrobeloides	27.8	3.93

[a] 6.7 % mortality at this concentration

Table 2. Body length (μm)[a] associated with specific life stages of *Aphelenchus*.

Development Stage	Body length ($\bar{x}$ ± std dev)
J1	278.4 ± 26.7
J2	370.4 ± 23.6
J3	478.4 ± 45.2
J4	727.4 ± 63.7

[a]: Biomass = ((D2*L)/1600000 where D and L are the width and length of nematode (Andrássy, 1956)

Development. Aphelenchus were generally smaller in size than *Acrobeloides* under control conditions (Figure 1). The two species responded differently toward copper and BaP (Figure 1). Development of *Acrobeloides* was impeded more by BaP ($F = 3.30$, $P = 0.0225$) than copper ($P = 0.9894$). Not only was *Aphelenchus* tolerant of greater concentrations of both chemicals, but the opposite pattern was observed. Development of *Aphelenchus* was impeded by copper ($F = 15.51$, $P < 0.0001$) but not BaP ($P = 0.6423$).

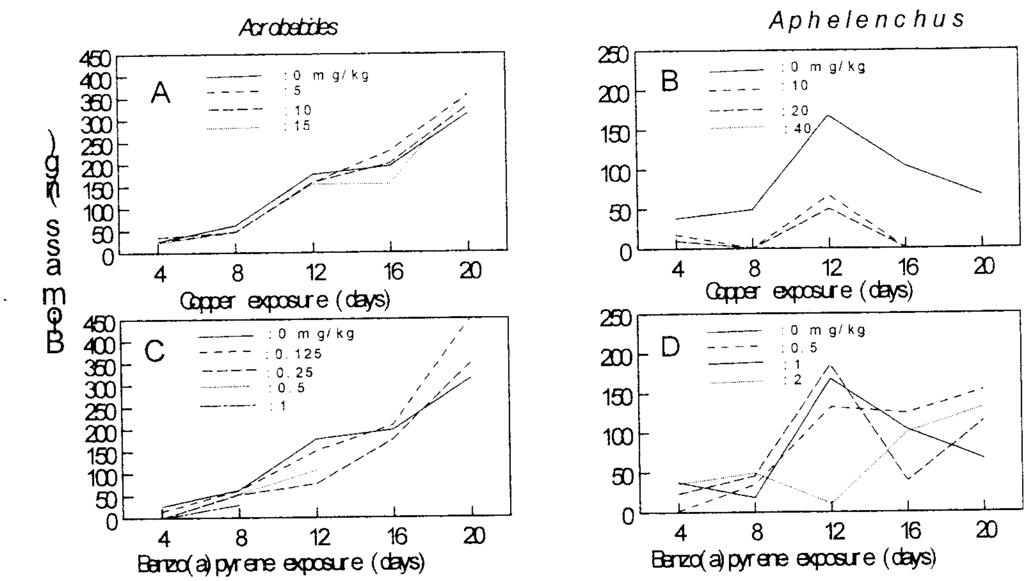

Figure 1. Effect of contrasting concentrations (mg/kg) of copper (A, B) and benzo(a)pyrene (C,D) on growth and development of *Acrobeloides* (A,C) and *Aphelenchus* (B,D).

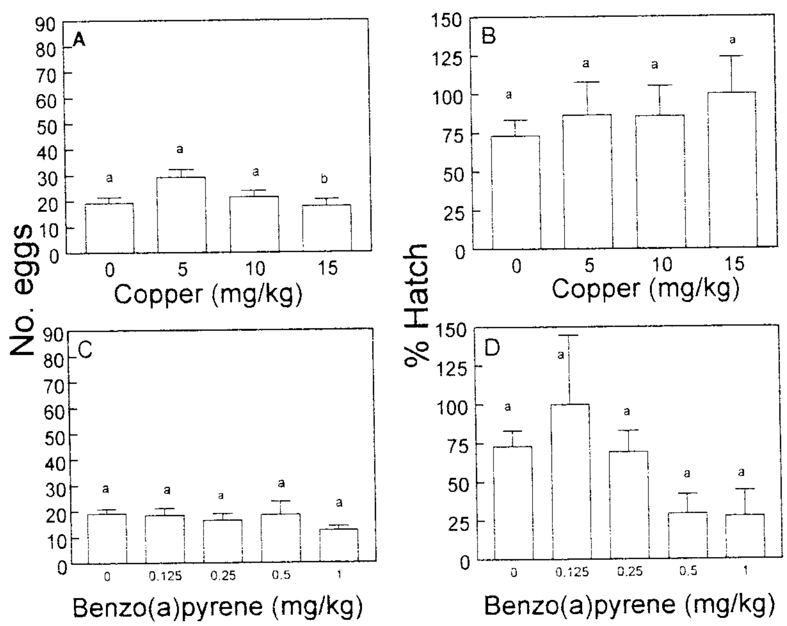

Figure 2. Effect of copper (A,C) and benzo(a)pyrene (B,D) on reproduction of *Acrobeloides* under acute exposure.

Reproduction. Numbers of offspring were reduced by copper in both *Acrobeloides* (F = 3.91, P = 0.0165) and *Aphelenchus* (F = 10.19, $P < 0.0001$). Eggs of *Aphelenchus* were able to tolerate up to 40 mg/kg of copper, whereas *Acrobeloides* eggs perished at 20 mg/kg (Figures 2, 3). Although numbers of offspring were reduced, size was not affected ($P > 0.2$).

Under acute exposure, numbers of eggs of neither *Acrobeloides* (P = 0.7511) nor *Aphelenchus* (P = 0.2406) were affected by BaP concentrations. However, in contrast to copper, BaP reduces egg size in *Acrobeloides* (F = 5.52, P = 0.0445) but not *Aphelenchus* (P = 0.24).

Neither copper (P = 0.9360) nor BaP (P = 0.1425) affected the percentage of eggs that hatch for *Acrobleoides* (Figure 2). However, both copper (F = 6.96, P = 0.0011) and BaP (F = 3.86, P = 0.0153) reduced hatching rate of *Aphelenchus* eggs (Figure 3).

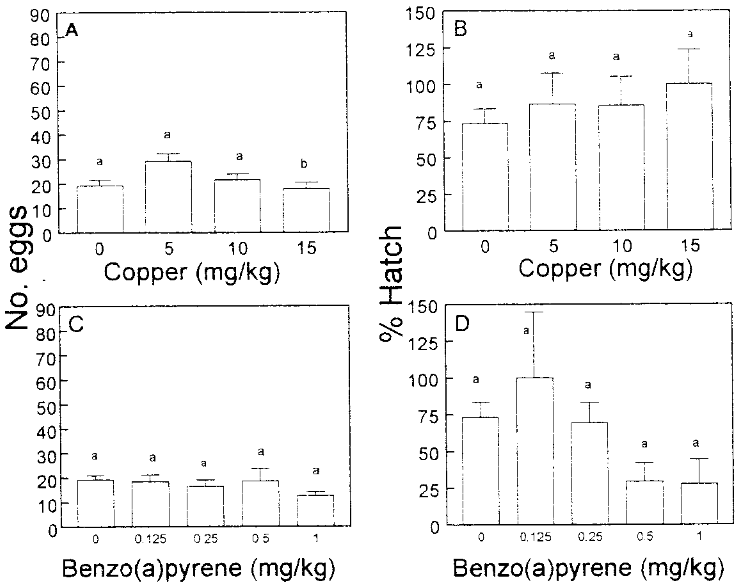

Figure 3. Effect of copper (A,C) and benzo(a)pyrene (B,D) on reproduction of *Aphelenchus* under acute exposure.

In generation 2, neither copper or BaP affected generation time (20.2 ± 0.2 days, mean ± standard error), number (19.8 ± 1.3), size (49.5 ± 3.7 ng) or hatching rate of eggs (83.4 ± 3.6) or juvenile size (196.8 ± 19.4 ng) in *Acrobleoides* ($P > 0.5$). Experiments of *Aphelenchus* are in progress.

Discussion

The survivorship and fecundity of *Acrobeloides* and *Aphelenchus* during acute exposure to BaP was examined. Although they have the same CP values, they respond to these genotoxicants differently. *Acrobeloides* reacts to PAHs and copper differently. PAHs are more toxic than copper on *Acrobeloides* reproduction, affecting hatching rate and egg size, but not egg number. Exposure to > 20 mg/kg of copper did not inhibit the maturity of eggs, but PAH did reduce egg number and size, i.e., both quantity and quality of eggs. The impact of copper and BaP has a different mechanism for *Aphelenchus*. It appears that reproductive rates are more sensitive than survival. Early stages of life, i.e., egg and early juvenile are the most sensitive, and later stages are more tolerant. Once they make it through the juvenile stage, toxicity has little impact on maturation.

Large differences in heavy metal uptake can be observed in closely related species. The observation that the subphylum Secernentia was less susceptible to pentachlorophenol than subphylum Penetrania might be the result of differences of cuticle characteristics (Kammenga et al. 1994). Unlike *Acrobeloides*, it is well established that *Aphelenchus* has a cuticular structure and metabolism to avoid stress caused by extreme drought and temperatures.

Genotoxicants may damage organisms directly by increasing their mortality rates, or interfering with the process of resource acquisition and uptake, and so reducing reproduction rates. These effects on individuals result in slower population growth. In our experiments, by examining the effect of Cu on nematodes and their eggs, the slower individual growth was observed when *Acrobeloides* and *Aphelenchus* were exposed to 10 and 20 ppm Cu, respectively. For *Aphelenchus*, it seems that Cu stress reduced fecundity, but not size of eggs. Perhaps this is a trade-off between reproduction and survival. Kammenga et al. (1996a) suggest that survival and period of juveniles (somatic growth and survival) may be more important to population growth rate than fecundity.

Life history theory provides tools to gain insight into relationship between toxicant-induced changes in life-cycle variables and fitness, which is defined as the intrinsic rate of population increase. Generally, vulnerability of species to chemical stress is determined by relationships between life-cycle traits and fitness. Life-history strategy reflects the ability to adapt to heterogeneous or unfavorable environments by either genetic alterations or phenotypic plasticity. Life history analysis provides the key to identifying important life stages, which can be used as suitable, and ecologically relevant effect parameters in toxicity tests. Unfortunately, most present ecotoxicological tests are not based on considerations from life-history perspective (Kammenga et al. 1996b). Many ecotoxicology studies focus on single sensitive traits as a suitable end point for comparing sublethal toxicity among species. The problem with this approach is that sensitivity of traits per se does not adequately provide ecologically relevant information concerning species performance.

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Acknowledgments

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