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Simple Whole-Soil Bioassay Based on Microarthropods

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Development of bioassays for whole soils remains a challenge. It is complicated by the heterogeneity of the solid, liquid, and gas phases of the soil matrix and the complexity of the many interactions of organisms with soils. Many bioassays for soils avoid some of these difficulties by using soil extracts. We sought to develop bioassays for whole soils, with emphasis on simplicity coupled with reliability (Sheppard and Evenden 1992; Sheppard et al. 1993).

Soil invertebrates are good subjects for whole-soil bioassays (Koehler 1992; Paoletti et al. 1991; Tomlin 1975). They are exposed to soil contaminants by contact, by direct ingestion of soil solids and soil water, and through food-chain transfers. They have short life-cycles and are sufficiently mobile that they can be extracted with simple, heat gradient devices (Crossley and Blair 1991). Their use in bioassays is somewhat limited by our lack of full understanding of their ecological relationships (van Straalen and Denneman 1989). Earthworms are perhaps the simplest to study, and earthworm bioassays typically involve survival counts and may include some physiological measurements. Relatively large numbers of earthworms are needed to obtain acceptable precision in survival counts (Sheppard and Evenden 1992). This is a statistical issue; to get meaningful counts of surviving earthworms, a large number of animals must be tested. Smaller invertebrates, such as microarthropods, occur in very large numbers in soil, and for this reason we directed our interests towards them.

Microarthropods have been used for many years in studies of the side effects of pesticides in soils (Koehler 1992; Tomlin 1975), and several studies have used them for other contaminants. Pirhonen and Huhta (1984) found microarthropods to be more sensitive to two types of oil in soil than either Enchytraeidae or Nematoda. The data of Neuhauser et al. (1989) seem to indicate that microarthropods were less variable indicators than earthworms for the impacts of oily waste in field plots. Bengtsson et al. (1985) and Posthuma et al. (1992) report on detailed investigations of the responses of microarthropods to metal-polluted soil. Denneman and van Straalen (1991) specifically developed a

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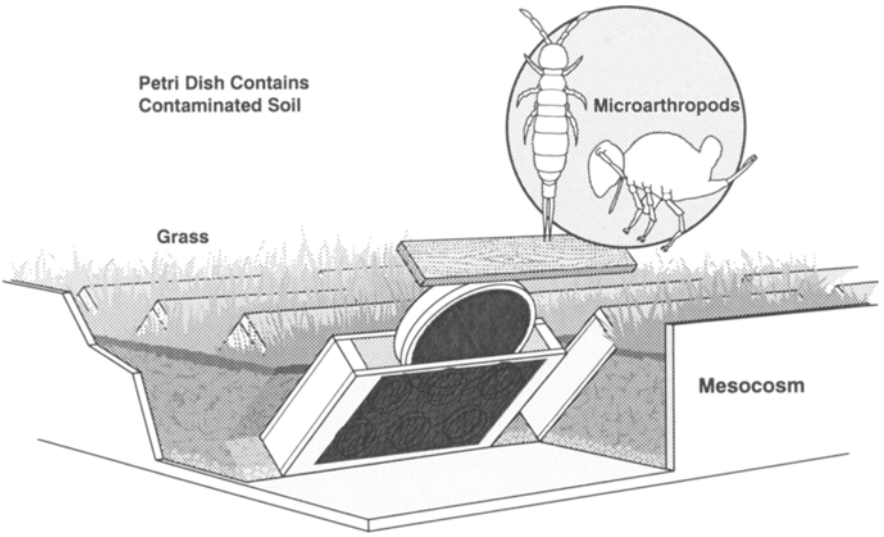

Figure 1. Schematic of the apparatus, showing the sleeves buried in the litter-and-soil-filled mesocosm, the modified petri dishes that contain the contaminated soil baited with ground alfalfa, and illustrations of two common springtail microarthropods that invade the contaminated soil.

bioassay for metals using soil-dwelling mites, but their protocol did not include soil.

The objective was to develop a simple bioassay based on counts of microarthropods. Following the multispecies approach of Sugiura (1992) and the general experimental concept of Huhta et al. (1991), we developed a microcosm method and report here the protocol and the initial indications of sensitivity using mercury (Hg) and iodine (I).

MATERIALS AND METHODS

Two pieces of apparatus, constructed from simple laboratory supplies, are required (Figure 1). The first, which we refer to as a mesocosm, is a plastic-lined wooden box with inside dimensions of 0.6 x 1.2 m and 20-cm deep. The bottom 2 cm is filled with gravel, nominally 2 to 10 mm diameter, to provide drainage. The next 12 cm is a mixture of fresh forest litter and soil. We have used a mixture of 46% (by volume) commercial sterilized potting soil,

46% litter horizon from a mixed boreal aspen and birch stand, and 8% of a sandy calcareous agricultural soil. The litter provides the initial arthropod population and substrate. The top 2 cm of the mesocosm is a layer of potting soil in which are planted seeds of a shade-tolerant lawn grass blend.

During assembly, 24 empty plastic sleeves are placed vertically in the soil. These sleeves are 19-cm-long, open-ended boxes of Plexiglas with cross-section inner dimensions of 3 x 15 cm. The upper end of the sleeves are positioned just above the soil surface and are covered with an opaque, removable lid. The lower ends are open to the soil. The sleeves are inclined about 30° from vertical, and the downward facing side of the sleeves are perforated with six, 3-cm-diameter holes. These holes are covered with 2-mm-mesh nylon screens.

We positioned the mesocosms on a laboratory bench to receive indirect sunlight and continuous supplemental light from fluorescent tubes. Tap water was applied to the surface when needed. Water accumulation in the gravel could be viewed through a 5-cm acrylic vertical access tube in one corner of the mesocosm, and excess water was avoided. The mesocosm was established, and the grass was cultivated for at least one month prior to the first bioassay so that the microarthropod populations could stabilize. We have done bioassays in individual mesocosms for up to a year with consistent results.

The second piece of apparatus is a plastic petri dish, 15-cm diameter and 2.5-cm deep, to hold the contaminated soil. The lid is modified by cutting out the centre to leave only a 1-cm-wide rim, and 2-mm-mesh nylon screen is glued onto the rim. With cleaning, the lids may be reused.

The soil was prepared by partial drying and grinding to pass a 4-mm mesh. Few viable microarthropods survive this treatment, but the soil can be heated to 30°C for 8 hr to eliminate indigenous meso fauna. The soil was amended with ground alfalfa at 10% by weight. We used ground, unmedicated laboratory rodent feed. The soil was then placed in the petri dish and moistened to 80% of its predetermined water-holding capacity. The petri dishes were fit, with the mesh lid downward, into the sleeves in the mesocosm.

Within 2-3 d, a considerable amount of fungal growth was visible in the petri dishes. After 11 d, the petri dishes were removed from the mesocosm and immediately placed, mesh lid downwards, in large funnels. These were supported in a plant growth chamber where temperature was controlled at 22 ± 1°C and where the lights could be lowered to 2 cm above the clear plastic bottom of the petri dishes. Under the spout of each funnel was a 50-mL beaker containing 10 mL of denatured ethanol. Parafilm was used to partially cover the beaker, but there was some ventilation so that ethanol fumes did not kill the microarthropods before they exited the soil. This apparatus operated as a

Tullgren extractor and was a derivative of that described by Crossley and Blair (1991). The capture of escaping microarthropods in the ethanol was essentially complete within 24 h, and after 96 h the ethanol traps are collected and soils discarded. With care in handling the petri dishes, the traps were virtually free of detritus.

The numbers of microarthropods were counted with up to 40-fold magnification, and counts of springtails (Collembola), mites (Acari), and remaining meso fauna were recorded separately. More detailed taxonomic differentiation would be possible, especially with computer-aided keys (Moldenke et al. 1991), but the costs would not likely be justified by improved bioassay sensitivity. The traps generally contained many more arthropods than could be economically counted. We evaporated some of the ethanol from the traps and adjusted the volume to 10 mL, then stirred and removed an aliquot by pipette for counting. We selected the volume of the aliquot so that actual counts from control soils were 20 to 100 microarthropods. Although this procedure may have under-sampled large meso fauna, it was accurate for the 0.1 to 2 mm organisms important to this protocol. The ethanol traps may be stored, with additions of glycerol, for archival purposes.

In order to compare the sensitivity of the proposed bioassay with other methods, the soils contaminated with Hg from the experiment described by Sheppard et al. (1993) were used. The concentrations of Hg were initially 0, 10, 22, 46, 100, 220, 460, and 1000 mg Hg kg^{-1} soil, applied as $HgCl_2$. An acidic sand, a humus-rich garden soil and a clay were used. Aliquots of the same three soils were treated with I at concentrations of 0, 10, 22, 46, 100, 220, 460, and 1000 mg I kg^{-1} soil, applied as KI. All contaminant treatments and controls were done in triplicate within the same mesocosm. The soils were used in the bioassay shortly after they were treated, and the Hg and I concentrations were confirmed at that time by analyses. There was some loss of Hg presumably by volatilization. There was no detectable methyl mercury in the Hg-treated soils. Sheppard et al. (1993) indicated the relative sensitivity of other bioassays to the Hg treatments. They used earthworm survival, lettuce seed emergence and timing of bloom initiation in *Brassica rapa* as whole-soil bioassays. We present here results of these bioassays for the I-treated soils using the same methods.

RESULTS AND DISCUSSION

The counts of microarthropods in this system are a holistic measure of the ecology of the treated soil, encompassing effects on fungal proliferation on the alfalfa substrate, invasion of fungivore and other microarthropods from the mesocosm, and then survival of those microarthropods in the contaminated soil.

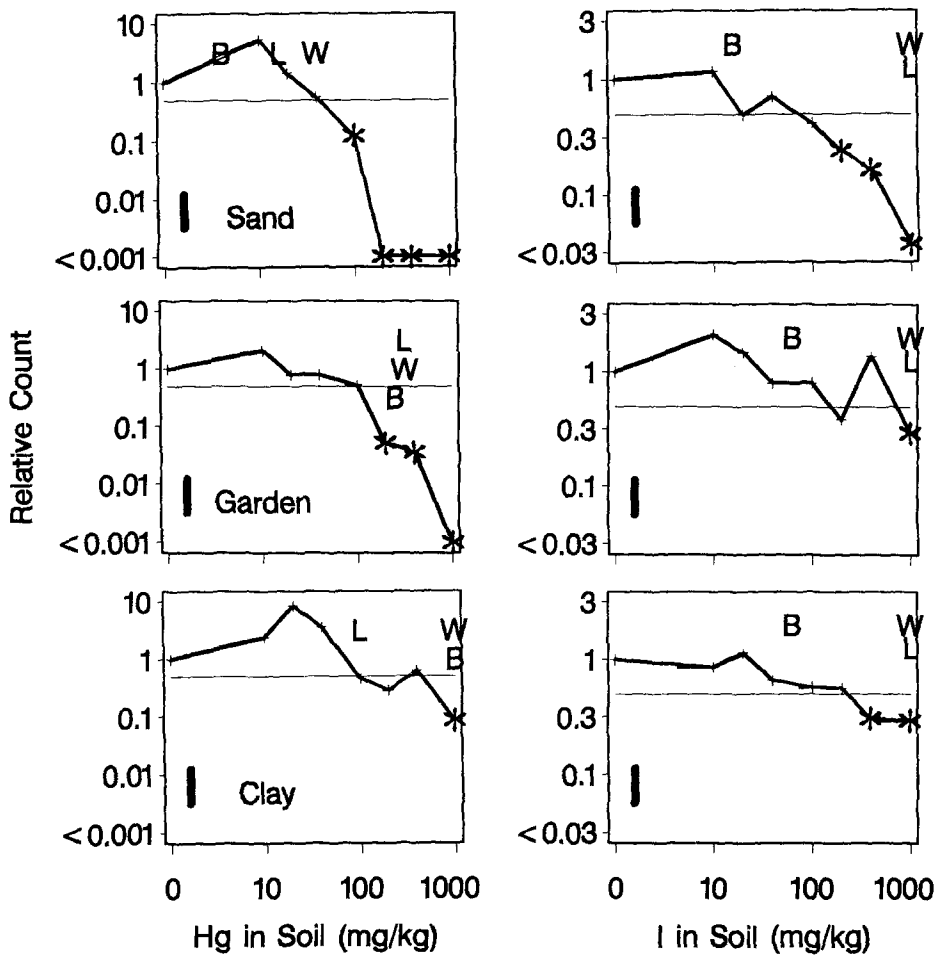

Figure 2. Counts of microarthropods, relative to controls, in the acidic sand (a), the garden (b) and the clay (c) soils contaminated with Hg and I. The horizontal line is 50% of the control (EC_{50} for microarthropods) and the short bold vertical line is the pooled (geometric) standard deviation. Points indicated by * were significantly lower than the control by one-tailed test ($P < 0.05$). The EC_{50} for earthworm survival, lettuce emergence, and the most sensitive of either bloom or emergence counts in *B. rapa* are indicated by the position of **W**, **L** and **B**, respectively, along the concentration axes.

There may be effects at several trophic levels. Longer exposures would be needed to ensure inclusion of effects on reproductive success.

The microarthropod counts spanned several orders of magnitude (Figure 2), and consequently were logtransformed for interpretation. Although variation was considerable, the trends were approximately monotonic, and treatments that had counts less than 50% of the controls were statistically different from the controls ($P < 0.05$ by single-degree-of-freedom contrasts, using the pooled estimate of error from analyses of variance). Whole-soil bioassays are somewhat more variable than bioassays on aqueous samples or aqueous soil extracts, and these results are considered useful. For Hg concentrations above 220 mg kg^{-1} in the sand soil, there were no microarthropods found in the samples.

The EC_{50} (the soil contaminant concentration that reduces test performance to 50% of the control) is indicated on the graphs and compared to EC_{50} for the other bioassays (Figure 2). The relative sensitivity of the four bioassays varied among the three soils and two contaminants, but clearly the microarthropod counts were as sensitive, and in some cases more sensitive, than the other bioassays. The costs of operation of the microarthropod bioassay is about one fifth that of the *B. rapa* bioassay, and about equal to that of the earthworm survival and lettuce seed emergence bioassays. We conclude that the simplicity and sensitivity of microarthropod bioassay is good and that this method deserves further development.

Further work is needed to examine the effectiveness of mesocosms supplied with litter and colonizing species from other habitats. The usable life time of the mesocosm is yet to be defined, as well as the importance of residual effects from one bioassay to the next in the same mesocosm. Smaller soil containers may prove to be effective, and the relationship between the size of individual soil samples and the number of replicate soil samples needs to be defined. Greater replication may markedly reduce the variation and improve precision. The method has not been tested for organic contaminants, and for this application the plastic components may have to be replaced.

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