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Sampling of Resident Earthworms Using Mustard Expellant to Evaluate Ecological Risk at a Mixed Hazardous and Radioactive Waste Site

by

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DISCLAIMER

ABSTRACT

As residents of contaminated soils and as prey for many species of wildlife, earthworms can serve as integrative biomonitors of soil contamination, which is biologically available to the terrestrial food chain. The assessment of contaminants within earthworm tissue provides a more realistic measurement of the potential biological hazards and ecological risks than physical and chemical measurements of soil. A unique sampling procedure using a mixture of ground mustard powder and water was implemented for cost-effectively collecting earthworms without digging; the procedure minimized occupational exposure to soil contaminants and reduced the quantity of investigation-derived wastes. Earthworms were maintained in the laboratory for four days to allow passage of the contents of the digestive tract. Earthworm body burdens, castings, and soil were analyzed for gamma-emitting radioisotopes (potassium-40, cobalt-60, cesium-137), strontium-90, trace metals (arsenic, cadmium, chromium, mercury, lead, and selenium), and polychlorinated biphenyls (PCBs). Ecological effects of soil contamination on the earthworms were also assessed through analysis of weight, abundance, and reproductive success.

INTRODUCTION

Ecological risk is a function of toxicological hazard and environmental exposure. Hazards data often consist of laboratory-derived levels of contaminants in the diet of biota; these contaminants can cause a measurable adverse effect on the biota, which is often measured by a reduction in numbers or a change in the kind or age of the organisms. As residents of contaminated soils and as prey for many species of wildlife, earthworms can serve as integrative biomonitors of soil contamination, which is biologically available to the terrestrial food chain. The assessment of contaminants within earthworm tissue provides a more realistic measurement of the potential biological hazards and ecological risks of soil contamination than physical and chemical measurements of soil.

A unique sampling procedure using a mixture of ground mustard powder and water was implemented for cost-effectively collecting earthworms without digging; the procedure minimized occupational exposure to soil contaminants and reduced the quantity of investigation-derived wastes.

Knowing the levels at which organisms are adversely affected is useless without having an estimate or measurement of the contact or exposure of the organisms to the contaminants. For air and water, the toxicity of the contaminants is directly related to the levels in the environmental media. Exposure to sediments is estimated by the extent that the chemical becomes bioavailable in the sediment interstitial pore water. However, exposure pathways to soil contaminants are not simple predictions.[1] Few animal species are directly exposed to soil contaminants except through incidental ingestion during cleaning of fur (preening) or breathing of dust. However, they can be linked to soil contaminants by using earthworms as prey and can be indirectly linked by eating prey that eats earthworms.

Earthworms are the natural bridge between soil contaminants and wildlife.[2] Earthworms are an ideal biological monitor because they are abundant, are easily collected, do not range long distances for food or cover, occupy a variety of habitats, and ingest soil particles.

Sampling of earthworms residing in soil (not artificially introduced) was proposed by Ashwood[3] to monitor the uptake, bioaccumulation, and potential transfer of soil contaminants to higher trophic layers in the terrestrial food chain. The rate of contaminant uptake into earthworm tissue is estimated by determining the bioaccumulation factor, which is the ratio of the levels in tissue divided by the levels in soil. Factors greater than 1 indicate that contaminants are concentrating in earthworm tissue at levels greater than those in soil.

The objective of this study was to determine whether earthworms could be

- collected in sufficient quantities with minimal occupational exposure to soil contaminants,
- cost-effectively maintained in the laboratory, and
- analyzed to allow quantitative estimates of the uptake of soil contaminants in earthworm tissue and, subsequently, the terrestrial food chain.

The study site is located at a closed burial ground for low-level radioactive waste and transuranic waste that lies within the Valley and Ridge Physiographic Province of East Tennessee. The site is on a moderately sloping hillside consisting of southwest-northeast

oriented ridges of sandstone, shale, and cherty dolomite. The soils are members of the Ultisol Group, which is highly weathered, low-base, forest soils. The soil texture is predominantly silty with considerable clay subsoil.

Mixed radioactive and hazardous wastes were disposed of in shallow trenches (10 to 14 ft deep) and auger holes and covered with weathered shale previously removed from the excavation. The movement of contaminants from the trenches is aided by the abundant rainfall (54 to 70 in./year) and seasonally shallow depth to groundwater (1 to 15 ft below surface) but is retarded by the high sorptive and ion-exchange capacity of clays in the subsoil (kaolinite and illite). The primary contaminants present are tritium (^{3}H), cobalt (^{60}Co), stron um (^{90}Sr), cesium (^{137}Cs), uranium (^{233}U,^{235}U), polonium (^{238}Pu, ^{239}Pu), americium (^{241}A n), and cerium (^{244}Cm). Metals (including mercury, lead, and selenium) and some orga ics (such as PCBs) are also present.

MATERIALS AND METHODS

Field Sampling

Three contaminated and three uncontaminated areas were sampled by using a ground mustard and water expellant. The contaminated areas were selected based on prior radiological survey data. The sampling locations were originally selected at random and then, for improved efficiency, by using systematic grids. The locations were then preferentially chosen to contain high organic matter and constant moisture inputs. The habitats sampled were two contaminated pasture areas (05.SB049 and 05.SB046), one contaminated forest area (05.SB008), and three uncontaminated wooded areas (05.SB0057, 05.SB062, and 05.SB063). A 10 by 10 sampling grid was superimposed on each sampling area. Each sampling location was 0.5 by 0.5 m for a total area size of 5 by 5 m.

A stainless steel frame (50 × 50 × 10 cm) was inserted into the ground to demarcate the sampling area and to contain the expelled earthworms. Adjacent sampling locations were not sampled to prevent effects of one site on another. Ground mustard (Colman's English Mustard) and deionized water were mixed at a concentration of one teaspoon per liter, respectively. After the percentage of ground cover was estimated, the vegetation and litter were removed immediately before the expellant was applied. Usually 3 L of mustard-water expellant was slowly applied with a watering can at a rate approximately equal to the infiltration rate (to prevent puddles). The mustard-water irritant infiltrated the ground and forced the earthworms to the surface, where they were collected with stainless steel entomological forceps. The sampling duration was 20 minutes, and the number of individuals expelled in each 1-minute interval was recorded.

After each earthworm was collected, it was rinsed with deionized water in the field to remove soil and expellant, placed in a clean plastic petri dish, and put in a cooler with ice. For identification, the petri dishes were marked with the sampling location (05.SB0XX), the grid number (1-100), the collection date, and a unique accession number. Information was recorded in controlled field and laboratory logbooks.

Onsite Laboratory Maintenance During Depuration

The earthworms were transported under a custody seal to the onsite laboratory, where they were weighed, moistened, and placed in an incubator (20°C ± 2°C) for a four-day depuration period to purge their digestive tracts. The worm castings (feces) were gathered on the first and fourth days after the worms were collected. The earthworms that expired before three full days of depuration were not used in tissue samples. However, the earthworms that lived for three days of depuration but died on the fourth day were included in the composite tissue sample. On the fourth day, the earthworms were weighed, composited in designated sample containers, and frozen. Before they were shipped to an offsite laboratory for radiological and chemical analysis, all tissue samples were fully homogenized in a clean, acid-washed, stainless steel blender and frozen until analysis.

Soil Sampling

Soil sampling followed earthworm sampling and consisted of collecting composite samples with 2-in. hand augers to a depth of 8 in. at the lower left corner of and adjacent to each earthworm sampling location. Certain physical and chemical properties of the soil that influence the distribution and abundance of earthworms were measured. These properties include cation exchange capacity, total organic carbon, soil temperature, texture, soil moisture (tension), and soil pH. The isotopic and chemical analyses of the soil were identical to the analyses of the earthworm tissue and castings.

Quality Control

Quality control procedures required that duplicate samples and equipment rinsate be analyzed. Duplicate earthworm samples collected at one uncontaminated location, 05SB057, were used as a relative measure of the precision of the collection technique. Identical procedures, equipment, and containers were used in the collection of the duplicates. All equipment was rinsed with organic-free water, and the rinsate was collected as a sample. The rinsate was analyzed to evaluate the decontamination of nondedicated equipment. The organic-free water was frozen and added to the stainless steel blender to simulate the homogenization of frozen worm tissue.

Laboratory Analysis

Composite samples of tissue, castings, and soil were analyzed for gamma-emitting radioisotopes (potassium-40, cobalt-60, cesium-137), strontium-90, trace metals (arsenic, cadmium, chromium, mercury, lead, selenium), and PCBs. The minimum sample sizes for inorganic, organic, and radiological analysis were 10, 35, and 30 g (wet weight), respectively, for tissue and castings. The high moisture content of the tissue samples greatly increased the detection limits for organics. Radiological analyses were not performed on the castings because of insufficient quantities.

Organic Analyses

Soil, worm tissue, and worm casting samples were analyzed for Target Compound List PCBs in accordance with the Environmental Protection Agency (EPA) Contract Laboratory Program Statement of Work for Organic Analysis, Revision OLMO1.8. Only analysis for PCBs was requested, but the reports received from the laboratory contained the results for the pesticides associated with the statement of work.

All samples were extracted and analyzed within the required holding time of 14 days. The worm tissue and casting samples (processed as soil samples) were extracted at a reduced volume, ranging from 10.1 g to 15.6 g in weight. Each of these samples was concentrated to a final volume of 5 ml, instead of 10 ml, to achieve the best detection limits possible by compensating for the reduced sample aliquot. The soil samples were extracted using approximately 30 g of soil and then concentrated to a final volume of 10 ml. The soil, tissue, and casting samples were spiked with the same amounts of the surrogate solutions, in lieu of varied final volumes. The sample extracts were not brought through gel permeation chromatography cleanup or Florisil cleanup. The sample extracts were subjected to sulfuric acid cleanup because the only compounds requested were PCBs (aroclors). Reported detection limits for tissue and casting samples were elevated because of the high level of moisture in the samples. All of the worm tissue and casting results were reported in units of μg/kg consistent with a soil matrix.

Inorganic Analyses

Soil, worm tissue, and worm casting samples for metals analysis were prepared and analyzed in accordance with the EPA Contract Laboratory Program Statement of Work for Inorganic Analyses, Revision ILMO2.1. The analyses requested for these samples included arsenic, cobalt, cadmium, chromium, mercury, and lead.

Selected soil samples were also analyzed for total organic carbon in accordance with the EPA Test Methods for Evaluating Solid Waste (SW-846, 3rd edition) method 9060.

All samples for metals analysis were prepared and analyzed within the required holding time of six months. Each of the worm tissue, casting, and soil samples were digested using a 1-g aliquot. The samples were prepared in accordance with the procedure outlined in Exhibit D of the statement of work, which resulted in a final sample volume of 200 ml. Samples were analyzed for arsenic and lead using a Thermo-Jarrell Ash 61E Trace Analyzer. Cobalt, cadmium, and chromium were analyzed using a Thermo-Jarrell Ash 61. Samples were prepared and analyzed for mercury using the cold vapor technique. Sample results were corrected for moisture content and were reported in units of mg/kg consistent with a soil matrix.

Selected soil samples were analyzed for total organic carbon using a carbonaceous analyzer (Dohrman Model DC80). The reported detection limit for this method is 1 mg/kg.

Radiological Analyses

Soil, worm tissue, and worm casting samples were submitted for the radiological analyses gamma spectroscopy, gross alpha, gross beta, and total strontium. Gamma spectroscopy was performed by method ITAS-RD-3219, which is based on EPA method 9310. Gross alpha and gross beta were analyzed by method ITAS-RD-3222, which is based on EPA method 901.1. Total strontium was analyzed by method ITAS-RD-3204, which is based on EPA method 600.

Samples analyzed by gamma spectroscopy did not require sample preparation. Sample aliquots of 31.1 to 68.0 g were placed in a 25-ml styrene jar geometry and counted on a germanium detector. Preparation of samples for gross alpha and gross beta involved using 17.0 to 209.7 mg of the samples to make a slurry. The slurry was vortexed and flamed onto a planchet. The samples were diluted to achieve less than 50 mg of total dissolved solids for gross alpha and less than 250 mg of total dissolved solids for gross beta. Gross alpha was counted by alpha scintillation and gross beta by gas flow proportional counting.

Samples analyzed for total strontium were prepared by wet ashing 0.0102 to 25.7 g of the samples. Samples were digested with concentrated nitric acid and peroxide and were counted by gas flow proportional counting.

RESULTS

Depuration Production

Daily casting collection was discontinued after three days because of the high collection cost. However, the decline in production (see Figure 1) demonstrates that after four days, the worms produced little additional casting, and the digestive tract was mostly clear of ingested material.

Sampling and Expellant Efficacy

At six different locations, the average rate of worms that surfaced after expellant was added did not appear to differ between contaminated (05SB008, -49, -46) and uncontaminated (05SB057, -62, -63) areas or between pasture (05SB049, -46) and forest habitats. Concentrated solutions (2 teaspoons per liter) of the expellant were often applied at locations that were nearly saturated. However, additional or concentrated applications of expellant did not increase the sampling efficiency. Most earthworms were collected during the first five minutes, and few were collected during the last ten minutes (see Figure 2).

Population Effects

The average number of earthworms in each sampling area was significantly different between contaminated and uncontaminated areas, but the average weight per worm was

consistent (see Table 1). Uncontaminated areas contained more individuals than contaminated areas.

Body Burdens

On a dry weight basis, bioaccumulation factors (the ratio of contamination levels in earthworm tissue to those in soil) were used as an estimate of uptake. The only measured radioisotope to exhibit levels greater in tissue than in soil was cobalt-60. Apparently, radioactive contaminants do not accumulate more in earthworm tissue than in soil (see Table 2). Results for strontium-90, a beta emitter, show no accumulation in earthworm tissue at levels greater than in the soil. Trace metals that bioaccumulated include selenium, cadmium, and mercury. The bioaccumulation factors increased as the concentrations in soil decreased for cadmium and mercury but not for selenium. Total PCBs also accumulated in earthworm tissue at levels exceeding those in soil. The highest bioaccumulation factor for PCBs appeared in the most highly contaminated soil. Preliminary results show that other gamma-emitting radioisotopes (such as potassium-40, cesium-137, and strontium-90) and lead, cobalt, chromium, and arsenic did not accumulate in earthworm tissue at levels exceeding those in soil.

Discussion

An innovative and effective sampling procedure was implemented for collecting earthworms without disturbing contaminated soil. Using the procedure lowered the requirements for personal protective equipment from those required for hand-sorting (modified level D [face shield, Tyvek, two pairs of gloves, and rubber boots]) to simply level D (safety glasses, gloves, and boots) for use with the expellant.

Traditionally, watering the ground with a mixture of formaldehyde and water has been used to expel worms for population assessment.[4] However, use of formaldehyde could be considered an unintended or improper use of the chemical, and the formaldehyde could be designated as a discarded commercial chemical product (according to 40 CFR 261.33) and, therefore, a hazardous waste listed as U by the Resource Conservation and Recovery Act. Also, formaldehyde is classified as a hazardous substance by the Comprehensive Environmental Response, Compensation, and Liability Act (in 40 CFR 302.4), and a release of it would induce liability regardless of whether it exceeds the reportable quantity. Therefore, using this traditional expellant would have been unacceptable.

A vinegar-mustard suspension ("ready mixed emulsion") has been used as an expellant;[5] when mixed with water in a concentrated solution, it expelled earthworms from the soil. However, the mustard emulsion did not mix well with water. A "glutinous mass" was reported to result from very high concentrations of mustard emulsion in water. Therefore, a finely ground mustard powder and water solution was used as an alternative mustard expellant.

Limitations of Expellant Collection Method

A sampling window and a prejudice against deep-dwelling worms may exist. Mustard-water expellant sampling was effective only under cool, moist, early spring conditions. One of the pasture areas (05SB049) was sampled in early April before mowing, and only 2 of the 25 locations had no worms. However, in early May after the first cutting, 3 of the 6 locations had no worms.

Depuration Period

A starvation or depuration period to allow contents to pass through the digestive tract is necessary to accurately assess the bioavailability of soil contaminants and uptake by earthworm tissue. If a contaminant is present in high concentrations in soil but is not assimilated by earthworms, the measurement of whole worms (including the soil remaining in their guts) will overestimate the tissue uptake and bioaccumulation factors. Food chain studies may not require separation of castings and tissue because vermivorous predators ingest tissue and gut contents. However, contaminants in the tissue may be more digestible and available for uptake to predators of earthworms than undigested soil remaining in their guts. A study[6] showed that dissecting earthworms and washing soil from their guts is preferable to the more laborious and time-consuming practice of starvation, but the study used mature, larger specimens. The diminutive size of the mustard-expelled worms precluded any attempts at dissection.

Population Effects

Representatives of all functional groups of earthworms were collected including unpigmented, subsurface-dwelling, geophagous species (endogeic) and pigmented, surface-dwelling, litter-feeding earthworms (epigeic and anecique). Unpigmented specimens belonged mostly to the genus *Octolasion,* but some *Diplocardia* and *Apporectodea* were also identified. Most of the pigmented individuals belonged to the genus *Lumbricus,* mostly *L. rubellus* and some *L. terrestris;* a few individuals from the genus *Amynthas* were collected, too. Besides *Diplocardia,* which were collected by hand-digging, almost all of the 5,000 individual worms were collected by using the mustard-water expellant. Digging produced many injured and severed specimens unsuitable for the depuration period.

The survey of 6 areas plus 1 duplicate took 21 days of sampling to complete. During each sampling day, 3 persons—2 in the field and 1 in the laboratory—collected and weighed 165 to 562 worms per day at 4 to 13 locations per day. See Table 1 for location-specific population details.

Although the abundance of earthworms differed among the areas, earthworm growth—as measured by the average worm weight—did not differ between contaminated and uncontaminated areas. A study of the measurable effects of 21-day laboratory toxicity tests using *Eisenia andreia* found that worm weight, as a measure of earthworm growth, was less sensitive than measurements of earthworm reproduction. The most sensitive measurement endpoint of earthworm reproduction is the total number of juveniles produced per worm.[7]

Subsequent analysis will assess the ratio of mature to immature worms as a measurement of reproductive success and possible ecological effect of the soil contamination.

Uptake

Bioaccumulation is defined as the uptake and retention of substances by an organism from its surrounding media and from food. The ratio of the contaminant level in the organism to the level in soil is defined as the bioaccumulation factor. This factor describes the degree to which an organism or tissue can acquire a higher concentration of a chemical in its body than is available in its environment. Different rates of uptake for different species and for different life stages have been observed,[8] and the comparability of uptake among contaminated sites is tenuous.

Observed levels of strontium-90 are somewhat higher than those reported in the literature. A surface-dwelling species was found to uptake strontium-90, but not at concentrations greater than in the soil. The bioaccumulation factors range from 0.111 to 0.191 for *Allobophora caliginosa* after a three-day depuration period.[9] The decrease in the bioaccumulation factor with increasing cadmium concentrations in soil is substantiated in other reports.[8] Relatively low tissue accumulation of trace metals such as lead in tissue may suggest strong binding of the metals to soil or organic separates. Measurements of these trace metals in earthworm tissue may reflect a more relevant bioavailable fraction of metal within the soil.[10] Most metal concentrations in castings are reported to be similar to the concentrations in the soils in which the worms were living.[11] However, because of the difference in the composition of silt, sand, and clay particles in soil and castings, this comparison may not be equitable.

Summary

Collection of earthworms for use in an ecological risk assessment can be performed in a cost-effective and timely manner with little disturbance of contaminated soils. Ground mustard and water expellant is an effective twist of an old technique for collecting large numbers of earthworms for tissue analysis, as long as the limitations of the technique are understood. The measurement of contaminants in earthworm tissue estimates the ecologically significant phase of contaminants in soil to wildlife predators of earthworms. Because of soil, species, and life stage differences, site-specific uptake may not be comparable to other contaminated sites.

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Table 1
Summary of Earthworm Population Effects[a]

Location[a]	No. of Grids Sampled	Avg. No. of Worms/Site at Collection	Avg. Weight/Worm (g) at Collection	Mature to Immature Ratio	Immature to Mature Ratio
05.SB008	26	24.9 a	0.3085 a	0.302	0.691
05.SB049	31	13.7 b	0.5858 a		
05.SB046	27	27.8 a	0.3649 a	0.170	0.828
05.SB063	22	41.7 c	0.3285 a	0.322	0.684
05.SB057	24	61.0 d	0.2283 a	0.104	0.897
05.SB062	13	59.1 d	0.2368 a	0.262	0.736

[a]Locations with different letters are significantly different (alpha = 0.5).

Table 2
Bioaccumulation Factors as Indicators of Soil Uptake by Earthworms

Sampling Location	Analyte	Bioaccumulation Factor[a]
	Organic	
05.SB063	Aroclor 1260	2.6
05.SB046	Aroclor 1260	22.5
05.SB049	Aroclor 1260	2.1
05.SB057	Aroclor 1260	2.2
05.SB008	Aroclor 1260	6.1
	Inorganic	
05.SB008	Selenium	1.10
05.SB063	Selenium	13.73
05.SB062	Selenium	18.55
05.SB057	Selenium	15.58
05.SB046	Selenium	10.00
05.SB049	Selenium	7.06
05.SB046	Mercury	0.28
05.SB063	Mercury	2.10
05.SB057	Mercury	2.91
05.SB008	Mercury	4.44
05.SB062	Mercury	16.25
05.SB049	Mercury	5.17
05.SB063	Lead	0.12
05.SB062	Lead	0.10
05.SB046	Lead	0.12
05.SB057	Lead	0.14
05.SB008	Lead	0.11
05.SB049	Lead	0.15
05.SB063	Chromium	0.16
05.SB049	Chromium	0.46
05.SB057	Chromium	0.19
05.SB062	Chromium	0.15
05.SB046	Chromium	0.29
05.SB008	Chromium	0.35
05.SB008	Cadmium	2.08
05.SB046	Cadmium	6.41

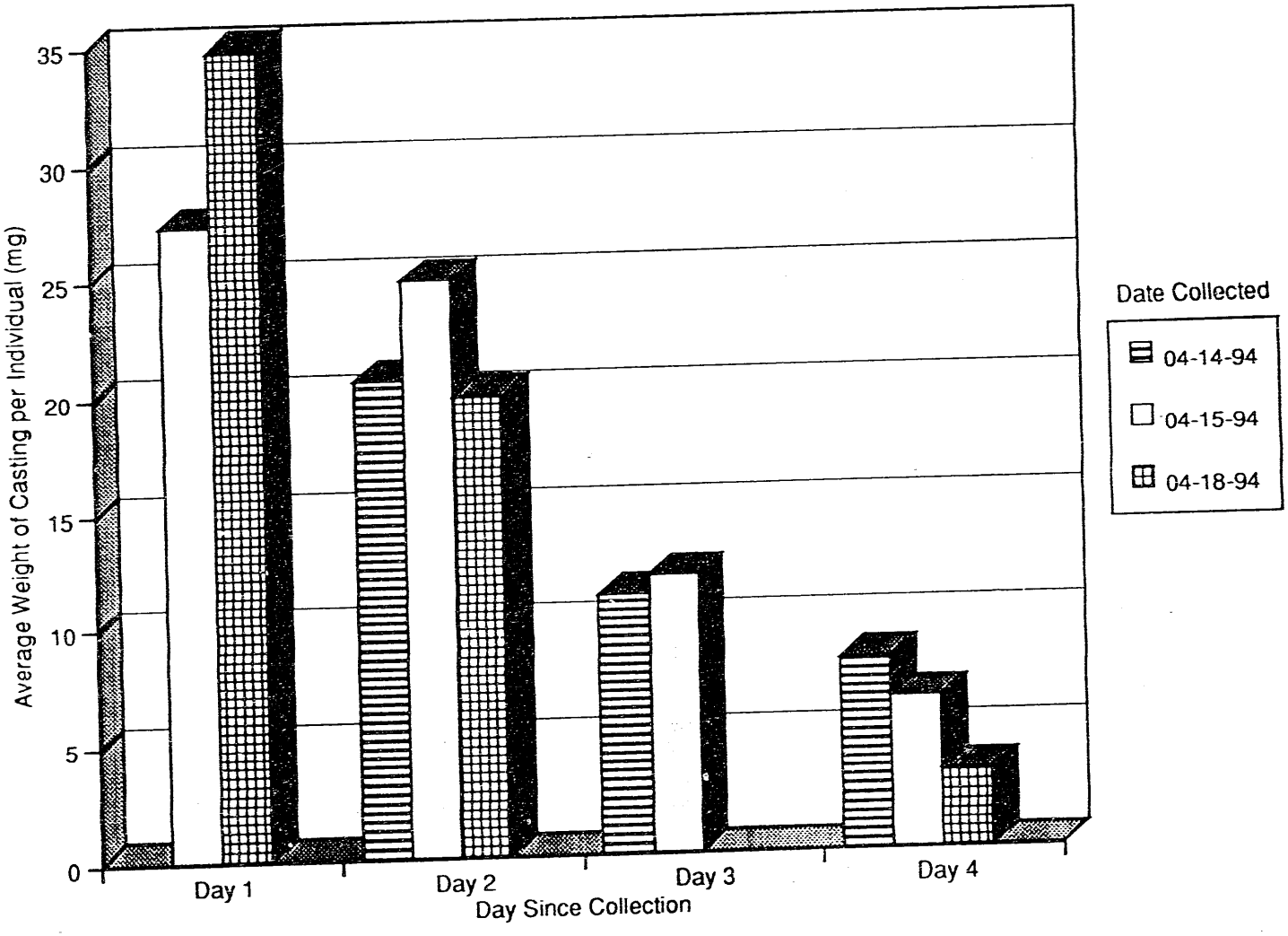
Table 2 (continued)

Sampling Location	Analyte	Bioaccumulation Factor[a]
	Inorganic (continued)	
05.SB057	Cadmium	5.93
05.SB049	Cadmium	4.83
05.SB063	Cadmium	22.69
05.SB062	Cadmium	11.54
05.SB046	Arsenic	0.43
05.SB049	Arsenic	0.44
05.SB057	Arsenic	0.45
05.SB008	Arsenic	0.81
05.SB063	Arsenic	0.91
05.SB062	Arsenic	0.56
	Radiological	
05.SB008	Total radioactive strontium	0.25
05.SB046	Total radioactive strontium	0.03
05.SB049	Total radioactive strontium	0.03
05.SB063	Total radioactive strontium	0.17
05.SB057	Total radioactive strontium	0.38
05.SB062	Total radioactive strontium	0.00
05.SB063	Potassium-40	0.26
05.SB057	Potassium-40	0.07
05.SB049	Potassium-40	0.11
05.SB062	Potassium-40	0.07
05.SB008	Potassium-40	0.06
05.SB046	Potassium-40	0.27
05.SB046	Cesium-137	0.00
05.SB049	Cesium-137	0.00

Table 2 (continued)

Sampling Location	Analyte	Bioaccumulation Factor[a]
	Radiological (continued)	
05.SB063	Cesium-137	0.02
05.SB057	Cesium-137	-0.06
05.SB008	Cesium-137	-0.02
05.SB062	Cesium-137	0.01
05.SB046	Cobalt-60	0.01
05.SB063	Cobalt-60	0.17
05.SB008	Cobalt-60	1.99
05.SB062	Cobalt-60	2.37
05.SB049	Cobalt-60	-6.34
05.SB057	Cobalt-60	-3.18
05.SB046	Beta particle	0.01
05.SB049	Beta particle	0.00
05.SB008	Beta particle	0.07
05.SB063	Beta particle	0.10
05.SB057	Beta particle	0.05
05.SB062	Beta particle	0.24
05.SB046	Alpha particle	0.01
05.SB049	Alpha particle	0.02
05.SB057	Alpha particle	0.05
05.SB008	Alpha particle	0.01
05.SB062	Alpha particle	-0.02
05.SB063	Alpha particle	0.02

[a]Bioaccumulation factor ratios are listed in the order of decreasing analyte concentrations in soil.



Stopped daily harvest because of time and money constraints.

Figure 1. Earthworm Casting Production During 4-Day Depuration/Starvation Period.

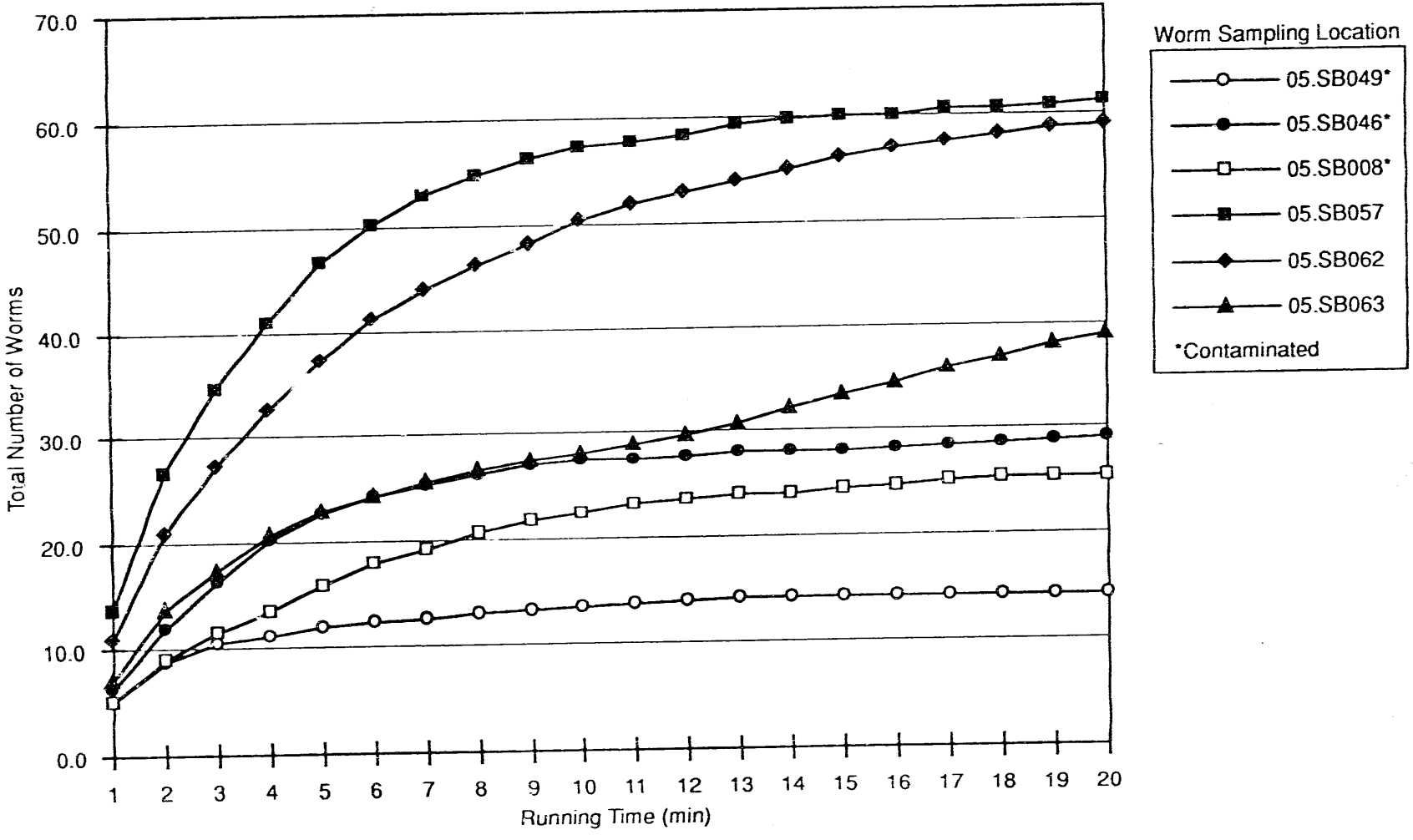
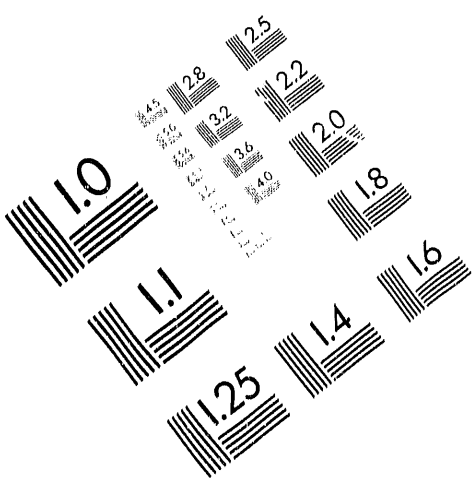
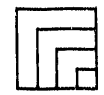


Figure 2. Cumulative Total of Mustard-Water Expelled Earthworms.

5.81 5057.2





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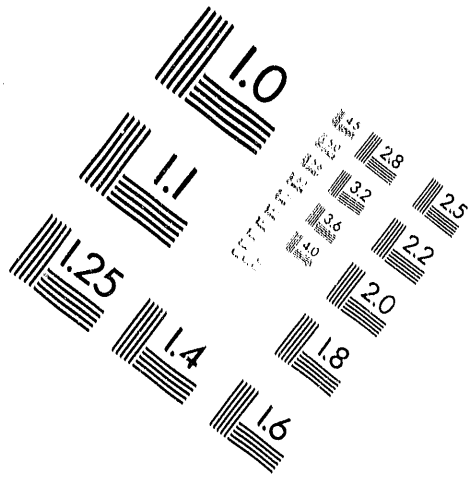

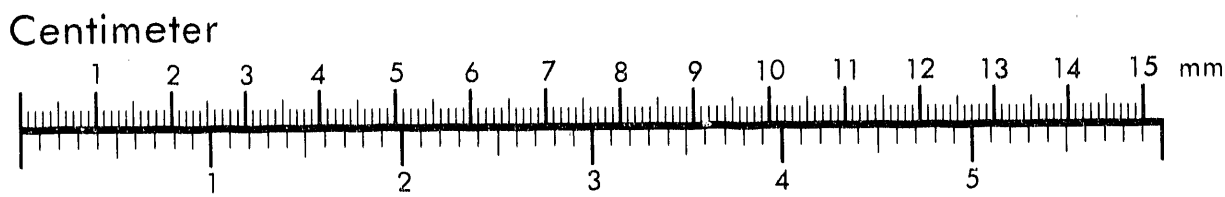

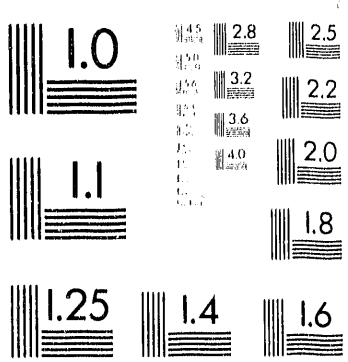

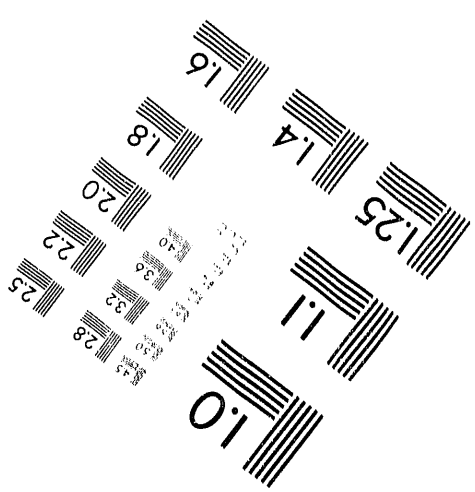

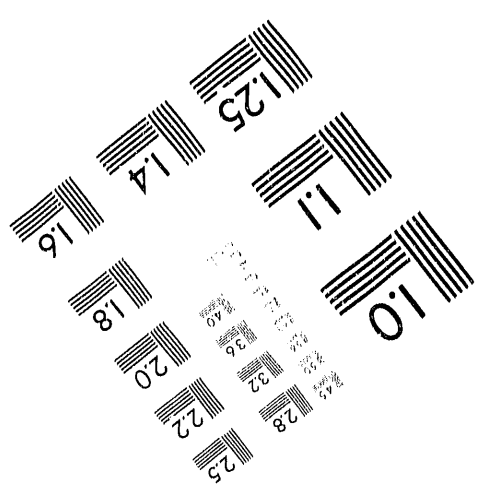

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