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TOLERANCE TEST OF *EISENIA FETIDA* FOR SODIUM CHLORIDE

MICHA KERR AND ARTHUR J. STEWART

ABSTRACT

Saltwater spills that make soil excessively saline often occur at petroleum exploration and production (E&P) sites and are ecologically damaging. Brine scars appear when produced water from an E&P site is spilled onto surrounding soil, causing loss of vegetation and subsequent soil erosion. Revegetating lands damaged by brine water can be difficult. The research reported here considers earthworms as a bioremedial treatment for increasing the salt mobility in this soil and encouraging plant growth and a healthy balance of soil nutrients. To determine the practical application of earthworms to remediate brine-contaminated soil, a 17-d test was conducted to establish salt tolerance levels for the common compost earthworm (*Eisenia fetida*) and relate those levels to soil salinity at brine-spill sites. Soil samples were amended with sodium chloride in concentrations ranging from 1 to 15 g/kg, which represent contamination levels at some spill sites. The survival rate of the earthworms was near 90% in all tested concentrations. Also, reproduction was noted in a number of the lower-concentration test replicates but absent above the 3-g/kg concentrations. Information gathered in this investigation can be used as reference in further studies of the tolerance of earthworms to salty soils, as results suggest that *E. fetida* is a good candidate to enhance remediation at brine-damaged sites.

INTRODUCTION

There are many damaging environmental problems related to the extraction of petroleum from the earth, not the least of which are brine spills. When oil deposits are tapped, petroleum isn't the only thing brought to the surface. For every barrel of crude oil produced, there are approximately 10 barrels of brine water produced (USGS 1997). Normally, this produced water is re-injected after it has been separated from the oil. However, when the water is spilled onto the surrounding land, a brine scar may appear after vegetation dies and soil is eroded, leaving a near-barren area. More than 2 billion gallons of brine water are re-injected each day into injection wells in the United States alone (USEPA 2002), and an indeterminate amount of this briny water is spilled annually. When the spills do occur, they can be very damaging to soil, plants, and soil biota; the damage can last for decades. Data for the Tallgrass Prairie Preserve (northeast Oklahoma) may provide insight into the significance of brine spills, however. The Tallgrass Prairie Preserve (TPP) is 15,720 hectares (ha) in area, and contains some 337 oil wells. In association with oil extraction activities on the TPP, 126 brine spills have been reported. The total area of the brine spills is ~ 17 ha (Tom Ashwood, Environmental Sciences Division, ORNL, personal communication).

This brine water is more than just salt and water. Chemical composition of brine varies greatly from well to well and from oil-field to oil-field. Additionally, the salinity and constituents of co-produced water from a single site depends upon the depth from which the oil and water is being extracted. Of more than 57,000 samples of this water that the United States Geological Survey (USGS) has information on, the mean amount of total dissolved solids is 89.27 g/L, and the mean concentration of sodium ions is 28.36 g/L

(Breit 2002). Other ions such as bicarbonate, sulfate, potassium, magnesium, chloride, and calcium can be present at high concentrations as well (Breit 2002). In most cases, sodium chloride (NaCl) dominates ionic composition of briny co-produced water (Keiffer and Ungar 2001). For this reason, pure NaCl (rather than a mixture of salts) was used as the representative brine in the experiments reported here. The salinity levels of brine water vary greatly, from 1.0 g/L up to 400 g/L (USGS 1997). To put these levels into context, ocean water salinity is 35 g/L, and the NaCl limit for drinking water is 0.5 g/L (USGS 1997).

Part of the charge of oil producers and ecologists alike is to bring land scarred by brine water back into use or to its near-undisturbed state. Current techniques for achieving this goal focus on bioremediation, which generally refers to degradation of contaminants by living organisms. Bioremediation techniques are usually associated with microbes, but the practice can and does utilize other living things. The extent to which brine-contaminated soil can be remediated is yet to be fully seen, however, bioremediation has already been successfully addressing environmentally problematic incidents associated with the petroleum industry. In November, 2002, a proposal led by Dr. Kerry Sublette, University of Tulsa, was submitted to the Integrated Petroleum Environmental Consortium (IPEC) to address the question of how best to accelerate the restoration of oil- and brine-impacted sites using a combination of soil organic amendments, plants, and soil invertebrates. Studies and field trials have indicated that halophytes can remediate brine spill sites— at the very least, revegetate them (Keiffer and Ungar 2001, 2002).

This research examines the feasibility of using the earthworm, *Eisenia fetida*, as a potential bioremedial agent in the treatment of salty soils. Earthworms may facilitate increased salt mobility in soil and encourage plant growth and a healthy balance of soil nutrients. Earthworms also are good candidates because of their hardiness, as illustrated in this work.

Revegetating lands damaged by brine water can be difficult. Research on the tolerance of earthworms to salt is needed to assess the real potential of *E. fetida* to help restore brine-damaged soils. This study tests that potential.

MATERIALS AND METHODS

Endpoints of this bioassay were earthworm survival and reproduction. The species of earthworm chosen for this test was *Eisenia fetida*, or the common red worm (Edwards and Coulson 1992, Linz and Nakles 1997). The individuals were harvested from a culture kept on-site at Oak Ridge National Laboratory (ORNL) in Oak Ridge, Tennessee. The *Eisenia* cultures at ORNL were started in 1995 with juveniles obtained from East Tennessee State University (ETSU). The ETSU cultures, in turn, were started with individuals obtained from EPA (Region IV) in Atlanta, Georgia. A common, commercially available brand of topsoil ("Garden Basics"; Southern Importers, Greensboro, North Carolina) was used for this test. Soil samples were weighed and dried at 60°C for 48 h, then reweighed to determine the water content of the soil.

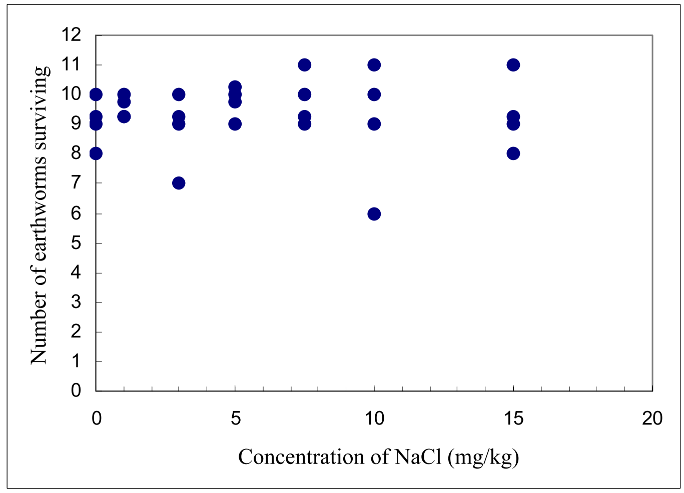

Figure 1. Survival of *Eisenia fetida* adults (17-d test) in soil containing various concentrations of NaCl. Eleven *E. fetida* were found in three replicates (one replicate each in the 7.5-, 10-, and 15-g/kg concentrations) at the end of the test; other replicates received 10 worms each at the start of the test. Data points are offsite slightly to allow the number surviving in each replicate to be determined.

Soil was treated with salt by application of solutions. The salt solutions were prepared using NaCl and deionized water. The tested concentrations of salt in soil (g/kg) were 0 (control), 1, 3, 5, 7.5, 10, and 15. Four replicate containers were used for each concentration. Each container received 50 mL of its assigned salt solution.

A 400-g quantity of soil (dry weight) was added to each replicate container that had been designed to allow aeration but prevent escape by the earthworms. The soil and test solutions were placed in these bags. Containers were then sealed and shaken before adding earthworms to facilitate the dispersion of the salt throughout the soil.

The earthworms were weighed in groups of 10 and added to the containers of test soil. All worms were incubated in beakers overnight to empty their guts before being weighed and placed into the test environments. After the earthworms were added, food (~2 g) was added to each container. The food consisted of Premium Rabbit Diet pellets (Pet Products; Hauppauge, New York), slurried into water (Gibbs et al. 1996).

Enough additional distilled water (25-75 mL) was added to each replicate to ensure moist but not saturated soil conditions. All replicates were allowed to incubate in a climate-controlled chamber at 25°C in darkness for 17 d. Replicates were periodically watered to maintain moisture levels friendly (moist to touch) to *E. fetida*.

At the end of 17 d, the replicates were disassembled and the worms counted and removed from the soil by hand. The recovered *E. fetida* again stayed overnight in beakers to

	Y= Yes	N=No		
Salt Consentration (g/kg soil)	Reproduction in Replicate Groups			
	A	B	C	D
0	Y	Y	Y	Y
1	Y	Y	Y	Y
3	Y	Y	N	N
5	N	N	N	N
7.5	N	N	N	N
10	N	N	N	N
15	N	N	N	N

Table 1. Evidence of *E. fetida* reproduction (Yes or No) in each replicate, for each concentration of salt tested. Evidence was "Yes" if juvenile worms or cocoons were observed, and "No" otherwise.

empty their guts before being weighed. The next day the earthworms were put into pre-weighed jars for drying. Next, the jars with the worms in them were weighed. They were then dried at 60°C for 6 d. After drying, the containers were weighed. (A weight subtraction adjustment of 0.09337 g was made to each empty vial weight in the calculation of earthworm dry weight to compensate for water lost from the vial's cap. This number represents the mean difference between weights of wet and dry caps.)

The test soils were returned to their containers and again placed in the climate chamber. Three weeks later, the soil containers were emptied and hand-sorted to check for evidence of reproduction: the presence of juvenile *E. fetida*.

Subtest A

On day five of the test, a 0.5-g sample of soil was taken from each replicate, combined with the 0.5-g samples from the other replicates in its concentration class, and pH was measured. The 2-g soil samples from each test concentration were added to 50 mL of deionized water before the measurement was made.

Subtest B

On day seven of the test, a 50-g sample was taken from each replicate and added to 100 mL of deionized water. The sample was stirred, allowed to settle, and the conductivity was measured. Conductivity measurements were also made on NaCl solutions to establish a reference for comparison with the conductivities of the soil samples. Class concentrations of 0, 0.5, 1, 1.5, 2.5, 3, 3.75, 5, 7.5, 10, 15 g/L were tested using 100 mL samples of deionized water each.

Subtest C

In order to assess the influence of the earthworm food on the salt concentration in test soils, a conductivity measurement was made on control soils with and without food added. Neither sodium chloride nor earthworms were added to these test samples, and three replicates each were tested. Approximately 100 g of wet soil was used in each container; 1.6 g of food was added to three of the containers. All containers then received 50 mL of deionized water to disperse the food (where present). The samples were then covered and placed in a dark climate chamber at 25°C to ferment for 6 d to simulate feeding conditions of earthworm-containing soil replicates. On day six, 100 mL of deionized water was added to each replicate and conductivities were measured.

RESULTS

Survival

Earthworm survival (Fig. 1) was unaffected by the salt concentrations used in this experiment. The survival rate in replicates for the control soil ranged from 80%-100%. In replicates with a salt concentration of 1 g/kg soil, survival rates ranged from 90%-100%. Replicates with a salt concentration of 3 g/kg soil had a survival rate ranging from 70%-100%. At a salt concentration of 5 g/kg soil, survival rates ranged from 90%-100%. At 7.5 g of salt per kilogram of soil, survival rates ranged from 90%-100%. In replicates with a salt concentration of 10 g/kg soil, survival ranged from 60%-100%. In the replicates with the highest salt concentration (15 g/kg soil), survival ranged from 80%-100%.

Conductivity for soil extracts, per g of added NaCl, after correcting for background (105 µS) (µS)	NaCl concentration in soil (g/kg)	Conductivity[a] of water extract of NaCl-amended soil	NaCl concentration in pure water (g/L)	Conductivity of NaCl in pure water (µS)
0	0	105 (90-120)	0	2
413	1	518 (405-684)	0.5	999
404.3	3	1318 (1084-1738)	1	1894
435.6	5	2283 (1960-2480)	1.5	2910
433.1	7.5	3353 (2630-3850)	2.5	3880
373.3	10	3838 (3300-4550)	3	5530
400.2	15	6108 (4120-7670)	3.75	6070
			5	9310
			7.5	13950
			10	16980

Table 2. Conductivity of various concentrations of NaCl in water, and of water extracts of NaCl-amended topsoil. To prepare water extracts of NaCl-amended soil, for each replicate, a 100 mL volume of deionized distilled water was added to a 50-g amount of soil.

Reproduction

Cocoons were observed in many of the replicates upon breakdown. Successful reproduction (Table 1) occurred in all of the control and the lowest-concentration replicates and in half of the replicates containing NaCl at a concentration of 3 g/kg. No successful reproduction occurred in replicates with a salt/soil concentration above 3 g/kg. During the experiment, it was observed that the worms in replicates of higher salinities were much more sluggish throughout the experiment than the worms in lower concentrations of salt and the control. Some of the worms in a few higher-concentration replicates also exhibited escape behavior at the start of the test; they crawled out of the soil to the top seam of the container-bag and would have escaped if it had not been sealed.

pH

The pH measurements on the soil samples were found to be slightly acidic, ranging from 5.06-5.60. A marked drop in pH was noted between the 1 g/kg and 3 g/kg concentrations; it decreased from 5.5 to 5.15.

Conductivity

A comparison of conductivities of soil replicates and salt solutions is presented in Table 2. The differences found in leachable salts from soil treated with and without food were very small. Untreated soil samples' conductivities ranged from 103-122 µS and food-treated soils' conductivities ranged from 130.4-150.4 µS.

DISCUSSION

The survival of *E. fetida* in this project speaks to their hardiness. Data obtained in this experiment indicate that this species can tolerate soils nearly half as salty as seawater. This level of tolerance falls in line with the findings of Piearce and Piearce (1979, 1982), who noted that immersion in a solution half as salty as the sea was quickly fatal to several species of earthworms, while immersion in a solution one quarter as salty as seawater was tolerated. When viewed with results presented here, saltwater may be toxic to *E. fetida* at NaCl concentrations of 15-17 g/L.

There is, however, a difference between submerging earthworms in a saltwater solution for an acute test and confining them for longer periods to salty soils. Would the findings of Piearce and Piearce (1979) that *Lumbricus terrestris* and *Aporrectodea longa* were most tolerant to saltwater solutions accurately predict that those earthworm species would be most likely to thrive at brine spill sites? Findings here support that though they may not be the most saltwater-tolerant of earthworm species (Piearce and Piearce 1979), *E. fetida* have sufficient tolerance of salty soils to merit use at some produced-water spill sites.

Other studies have been conducted on the salt tolerance of invertebrates; the aquatic nematode *Caenorhabditis elegans* was shown to be quite tolerant in an acute test to salt solutions in concentrations of 15.46-20.5 g/L (Khanna et al. 1997). Hota and Rao (1985) found that the survival of three species of tropical earthworms (*Perionyz millardi, Octocheaetona surensis, Drawida calebi*) was 50% (LC_{50}) in saline solutions of 9.5, 8.5, and 7 g NaCl per L, respectively. The study presented here suggests that North American *E. fetida* is more salt tolerant than the tested species of tropical earthworms.

Behavioral observations indicate that *E. fetida* may require a period of captive acclimation if they are to be used at higher-salinity field sites. As this 17-d tolerance test progressed, less escape behavior was observed. This leads the author to suggest that earthworms in captive or field conditions may acclimate. If allowed free range of mobility and travel, the earthworms may leave a test or field site upon introduction. This does not rule them out as candidates as remediators because when contained, they seemed to adjust to the adverse conditions.

Evidence presented here also suggests that *E. fetida* cannot only tolerate lower-salinity soils, but also continue their natural life cycles. They can successfully reproduce in soils that may be representative of some soils at brine-damaged sites (Table 1).

Further investigation of the tolerance of *E. fetida* to salt and the effects of different soil types on this tolerance will be needed. A threshold concentration of NaCl for mortality and reproduction for this species will be useful if they are to be considered for field studies or as treatments for the bioremediation of site-specific brine-contaminated soils. A behavioral study could gauge the extent/intensity of their instinct to escape inhospitable soils. Investigation into the earthworms' ability to acclimate would provide information that could be directly applied to a field test or effort of bioremediating a brine spill site, indicating how long the creatures may need to be contained to an area or whether they would require the addition of uncontaminated soil to dilute sites of higher salinity.

Aside from the high concentrations of NaCl in produced water, the question remains, would *E. fetida* tolerate the concentrations of the myriad of other ions and compounds in the brine waters extracted at petroleum exploration and production sites. Information from the USGS's samples of produced water suggests that no two wells are chemical copies of each other. Are there elements in some of these waters that are acutely toxic to *E. fetida* in the soil environment? Further tolerance testing and field studies are called for to answer these questions.

CONCLUSIONS

With such a high tolerance to NaCl as seen in this investigation, *E. fetida* holds promise as a resource in the remediation of lands damaged by brine water at oil exploration and production sites. Differences in effects due to varying water and soil composition will require further investigation. Earthworms are an important segment of a healthy soil ecosystem; with their help, the application of bioremediation can keep us from taking the loss of surface earth with merely a grain of salt.

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