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Identification of Factors from Agricultural Runoff Water on the Viability of Embryos of the Earthworm *Dendrobaena veneta*

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ABSTRACT

Agricultural runoff water (inlet and outlet points of water storage ponds) was evaluated for its effect on earthworm using *Dendrobaena veneta*. Chemical analysis revealed a significant difference in pH, phosphate and inorganic nitrogen from inlet and outlet samples as well as differences in embryo viability. Among the factors tested, pH was the most important factor for viability, while phosphate and nitrate appeared to stimulate the development of the embryos. These results demonstrate the utility of *Dendrobaena veneta*, a water-tolerant earthworm species useful for environmental water testing.

Keywords: environmental water testing, nitrate, phosphates, soil, annelid, toxicity

INTRODUCTION

Assessment of the impact of agricultural run-off water upon various ecosystems is important for balancing environmental concerns with the economic demands of agriculture (Stringfellow *et al*. 2007; Stringfellow 2008; Stringfellow *et al*. 2008), while innovation in sustainable agriculture has reduced the amount of pesticides and fertilizers, their use is unavoidable for a wide variety of agricultural areas. This is especially true in northern California, which includes orchard and row-crop farming. In both of these agricultural settings, practices that enhance the activity of many soil dwelling organisms may have long-term economic and environmental benefits. Among these soil organisms are a wide variety of earthworm species (Edwards 1983). Earthworms play an important role in organic matter processing, inorganic processing, soil aeration and water drainage. When possible, their activity is generally encouraged throughout most agricultural regions in the US and worldwide. For example, in Kansas grasslands, approximately 10% of all organic matter appears to be processed by earthworms. This includes approximately 10 and 50% of mineral nitrogen and phosphorus, respectively (James 1991). The earthworm contributes to macroporosity of the soil facilitating water drainage and aeration (Lee and Foster 1991; Edwards 2004). Evaluation of soil samples for earthworm population estimates and the addition of a wide variety of nutrients and pesticides to soils to examine adult viability and reproduction have been crucial. The expression of alkaline phosphatases in embryonic and immature earthworms may play a role in the metabolism of organophosphates, and their metabolites as well as inorganic phosphorus, with implications for embryonic development (Park *et al*. 1996). Until recently, the limited commercial availability of earthworm embryos (in casings or "cocoons") has limited the studies of factors which specifically affect embryonic development. With the large scale commercial development of earthworm embryos for soil enrichment and related applications, the study of environmental factors that influence the development of these embryos becomes crucial. The current study examines selected factors for the development of *Dendrobaena veneta* (the European Nightcrawler), a water-tolerant species in commercial use (W. Kreitzer, Advanced Prairie, Inc., pers. comm.). This species is suitable for temperate zone agriculture and vermicomposting, with a similar habitat to common North American species *Lumbricus rubellus* and *Lumbricus terrestris* (Kurek and Plytycz 2003). The purpose of this study was to determine the chemical factors that influence the viability of *Dendrobaena veneta* embryos with input and output water samples from agricultural water storage ponds. The differences in chemical composition of these samples and the associated viability differences would be useful indicators for subsequent testing of these factors in artificial media.

MATERIALS AND METHODS

Dendrobaena veneta embryos were obtained from Advanced Prairie, Inc. (Elliot, IL). Sodium bicarbonate, sodium nitrate and dibasic sodium phosphate were of reagent grade and obtained from Sigma-Aldrich (St. Louis, MO). Petri dishes (10 cm diameter) were obtained form Corning with Whatman No. 1 filter paper inserts (9 cm diameter). Earthworm embryos (in natural embryo casings or "cocoons") were harvested commercially and stored at 4°C until used (7-10 days). For evaluation, embryo casings were placed into Petri dishes lined with Whatman No. 1 filter paper (100 embryo casings/dish). To each dish, 15 ml of water sample was added (for all studies). These cultures were incubated at 20.4°C. The cultures were examined periodically for the emergence of immatures from the embryo casings. Upon emergence, the immatures were counted and removed from the cultures. Distilled water was added to each culture (1–3 ml) to maintain a water vapor saturated environment that is suitable for *D. veneta* embryos. Distilled water (pH 6.5) cultures were used as a universal control. Each water sample source was evaluated in triplicate.

Culture counts were terminated in 60 days or until no embryos were observed developing in the embryo casings within any of the Petri dishes. In the first study, input and output samples were evaluated from each of three different surface water sources (at two different seasonal sampling times) in the San Joaquin Valley of California. These water sources were evaluated as part of a larger water quality evaluation. All sample collection, data evaluation, and analysis in the project was collected in accordance with rigorous, SWAMP compatible, QA/QC procedures (Puckett 2002;

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Research Note

Stringfellow 2005; Borglin *et al*. 2006; California Department of Fish and Game 2007). Water samples were collected in glass 1000 ml bottles (Wheaton Science Products, Millville, NJ). All bottles were rinsed with sample water prior to collection of a depth-integrated sample. Samples were immediately stored at 4°C after sampling and were received by the laboratory the same day they were sampled, logged in and inspected for damage, and stored at 4°C until filtering and analysis. Filtration and preservation of samples were completed within 24 h.

Samples were collected, preserved, stored, and analyzed by methods outlined in *Standard Methods for the Analysis of Water and Wastewater* (American Public Health Association 2005) unless otherwise indicated. Total ammonia nitrogen (TAN), dissolved nitrate (NO_3-N), and total nitrogen (TN) were quantified using the TL-2800 ammonia analyzer made by Timberline Instruments (Boulder, CO) (Carlson *et al*. 1990). Total phosphorus (TP) was determined on 5.0 ml of unfiltered sample by persulfate digestion (Yu *et al*. 1994). Prior to each analysis the instrument was checked for proper calibration and re-calibrated as needed. Accuracy was insured by duplicate samples, laboratory check standards, travel blanks, and matrix spikes at a rate of one each per 20 samples. In a second study, data from the chemical analysis of the input and output samples were used to test for specific factors that may be responsible for enhanced embryonic development found in the first study. These included sodium bicarbonate (0.44 gm/l pH 8.0) and phosphate buffered nitrate (sodium nitrate 21.3 mg/l and dibasic sodium phosphate 25.8 mg/l at pH 8.0). This second study was limited to inorganic ions, due to the complexity of organic materials and microbial growth present in the surface water samples.

RESULTS AND DISCUSSION

Dendrobaena veneta has been used for vermicomposting applications and is noted for its high moisture tolerance; with an optimal range from 67.4 to 84.3% relative humidity at 15°C when reared in cattle manure media (Muyima *et al*. 1994). When used for water testing in the present study, analysis of input and output surface water samples revealed a statistically higher emergence rate for specific input samples (**Table 1**). In cases were emergence data were statistically significant, there were also differences in inorganic phosphate and nitrogen. In addition, the pH difference between the surface water samples and distilled water (8.0 and 6.5, respectively) was an important factor for consideration. For the second study, three test conditions were evaluated (**Table 2**). It was noted that incubations containing phosphate-buffered nitrate has an initially higher emergence rate than bicarbonate at the same pH (40 days), although the total emergence numbers did not differ at the end of 51 days. Previous studies evaluating emergence rates in cattle manure media noted that the mean incubation period for emergence was temperature dependent; 42 days at 25°C and 71 days at 15°C (Vilgoen *et al*. 1992). It must be noted that the incubation temperature with commercially obtained embryos in the present study was within this range (20.4°C) with the exception of 4°C during transport and storage 7-10 days prior to water testing. In addition, the emergence rates in the present study are reasonably within the range reported (Vilgeon *et al*. 1992).

From these studies, we conclude that a slightly basic pH is optimal for *D. veneta* embryonic development and that trace phosphate and inorganic nitrogen may be an important environmental stimulus. The mechanisms for this stimulus may not be fully understood, although hypothetically, increasing soil temperatures and microbial enzyme activity upon organics and inorganics released into water permeating the soil (and in contact with embryo casings) could be a signal for earthworm embryos that an optimal environment for development is present. While the analysis in this study was limited to inorganics, the comparative analysis of surface water content and embryo viability may be adaptable for toxicological evaluation of pesticides and other xenobiotics in surface water.

Table 1 Effect of surface water samples upon emergence of immature *Dendrobaena veneta*

Samples used		% Immatures emerging[a]	pH	Nitrate[b]	Phosphate[b]
Distilled water		67 ± 9	6.5	0	0
Surface water samples					
Region 1	Inlet	89 ± 6*	7.9	3.299	0.1205
July	Outlet	76 ± 4	8.3	2.021	0.0064
Region 1	Inlet	90 ± 4	8.4	3.549	0.5789
May	Outlet	82 ± 1	8.2	1.718	0.1387
Region 2	Inlet	87 ± 10	7.7	1.273	0.1594
June	Outlet	89 ± 6	8.1	2.738	0.1313

[a]Mean ± S.D. of triplicate determinations of immatures emerging after 49 days
[b]mg/l
* Statistically significant means from corresponding outlet sample $P < 0.05$

Table 2 Effect of inorganic culture media upon emergence of immature *Dendrobaena veneta*.

Incubation conditions	% Immatures emerging[a]	
	After 40 days	After 51 days
Distilled water	65 ± 9	67 ± 8*
Bicarbonate	75 ± 6	97 ± 5
Phosphate buffered nitrate	86 ± 3*	93 ± 3

[a]Mean ± S.D. of triplicate determinations for immatures emerging after specified days of incubation
*Statistically significant (ANOVA) from other incubation conditions at the specified days of incubation; $P < 0.05$

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