Analysis of Earthworm Depuration Conditions and Kinetics

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Introduction

- Bioaccumulation Tests assess the bioaccumulation factor [1] (BAF) of contaminants using animal models (e.g. earthworms)
- The depuration conditions during these tests are not specific and lead to inconsistencies across the literature
- Incomplete gut voidance before tissue analysis results in an overestimation of said BAF [2]

Objective

- The goal of this project is to determine the effects of **coprophagy** and **light conditions** on gut voidance during bioaccumulation test protocols

Methodology

- Subjects were identified as *Eisenia fetida* using a taxonomic guide of North American Lumbricidae species [3]
- Earthworms and petri dishes were prepared according to ASTM-E1676 and OECD Test No. 317 except for the variable condition (light condition and coprophagy prevention) [4] [5]
- The Coprophagy Prevention study contained triplicate petri dishes of 3 worms each (Fig. 1)
- The Alternative Light Conditions study contained triplicate petri dishes of 2 worms each (Fig. 2)

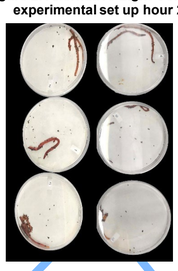
Coprophagy Prevention

Figure 1. Coprophagy Prevention experimental set up hour 24

Filter Paper Changed Every 12 Hours | Filter Paper Changed Every 24 Hours | No Filter Paper Change (Control)

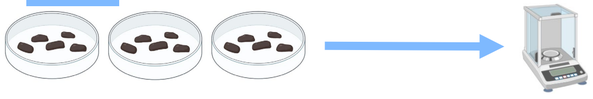
Alternative Light Conditions

Figure 2. Alternative Light Condition experimental set up hour 24



Maintained Under < 0.001 Lux | Maintained Under 700 Lux (Control)

Dry @ 40° C for 24 hours



- Dry weight of egesta was recorded after filter paper collection at various intervals
- After 48 hours, it was noted whether material was visible along the gastrointestinal tract of each worm

Current Results

Coprophagy Prevention

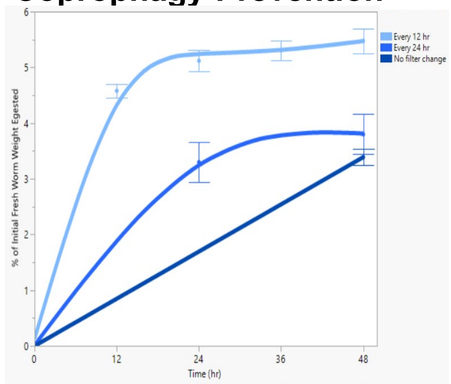


Figure 3. % of initial fresh earthworm weight egested over the course of 48 hours

$$\%\ initial\ fresh\ weight = \frac{dry\ weight\ of\ egesta}{(\sum_{i=1}^{3} initial\ fresh\ weight_i)/3}$$

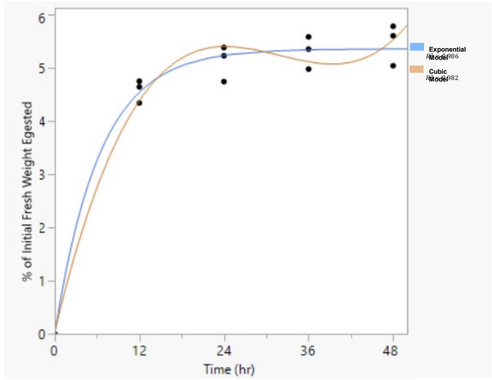


Figure 4. Egestion Kinetics for the Filter Change Frequency: Every 12 Hours

Exponential Model $y = 20.87058 + -20.8599e^{(-0.15764x)}$

Cubic Model $y = 0.1887 + 2.169x - 0.07233x^2 + 0.000758x^3$

- For the all groups that had egesta collected before the endpoint, **~90% of the total egesta for each petri dish was produced during the first 24 hours** (Fig. 3)
- The exponential model best fit the egestion kinetics data for filter change frequency: Every 12 Hours (Fig. 4)

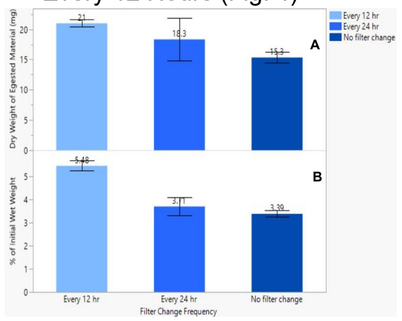


Figure 5. Egested material after 48 hours of gut voidance with different filter change frequencies

Table 1. Initial Characterization of Earthworm Fresh Weight for the Coprophagy Prevention Study

Variable Group	Average Fresh Weight Per Worm	Standard Deviation
Filter Change Every 12 Hours	391 ± 36	0.111
Filter Change Every 24 Hours	493 ± 89	0.270
No Filter Change (Control)	443 ± 24	0.078

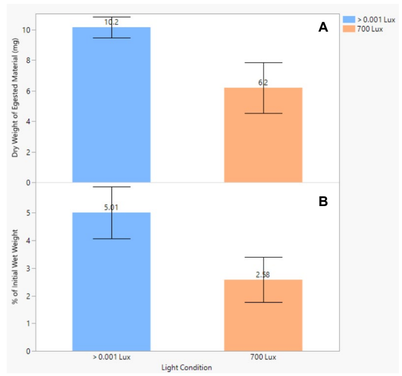


Figure 6. Egested material after 48 hours of gut voidance in light (700 lux) and dark (< 0.001 lux) conditions

- **A filter paper change every 12 hours resulted in 62% more** egested material per mg of earthworm weight compared to the control (Fig. 5B)
- A filter paper change every 24 hours resulted in 10% more egested material per mg of earthworm weight compared to the control (Fig. 5B)
- The normalized data (Fig. 5B) is a more accurate representation of the egesta since the standard deviation and fresh weight averages vary across the variable groups (Table 1)
- Visual inspection of the subjects' GI tract showed complete gut voidance of all worms experiencing a filter paper change every 12 hours

Alternative Light Conditions

- **Dark conditions resulted in 94% more** egested material per mg of earthworm weight compared to the control (Fig. 6B)
- The egesta pattern of the petri dishes in the dark was more erratic, while the egesta produced in the brighter light were deposited mainly along the boundary of the petri dishes
- Earthworms in the dark were observed to be more active

Discussion

- Light-induced hemiparesis could explain the lower percentage of egesta produced in the 700 lux conditions
- Paralysis and mortality under prolonged light exposure have been documented for the worm phylum Nematoda [6], but the phylum used in this study (Annelida) has not had light-related paralysis documented in the literature in the same way
- Coprophagy prevention methods, i.e. changing of filter papers during gut voidance, are used commonly in the literature yet standards do not mention coprophagy and justification is rarely given.
- While more frequent filter paper changes resulted in a higher percentage of worm fresh weight egested, visual evidence of material in the anterior portion of the GI tract was not observed.

Future Work

- The results of this study can be applied to future bioaccumulation tests and be used to build upon the current bioaccumulation test procedures
- Egestion kinetics can help researchers increase bioaccumulation test throughput by providing justification for a depuration time period that fits the sensitivity of the specific study
- The effect of light conditions and coprophagy prevention methods on BAF will be investigated
- This study will be repeated using species besides *Eisenia fetida*
- Coprophagy will be further investigated using visual analysis

References

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