Sediment-Water *Hyalella* Reproduction Test: Challenges Using Plant Material as Food Source.

Markus Simon[1]

[1]Fraunhofer IME, Applied Ecology, Auf dem Aberg 1, 57392 Schmallenberg, Germany (contact: markus.simon@ime.fraunhofer.de)

Introduction

For Environmental Risk Assessment in the frame of several legislations, toxicity tests with sediment dwellers have to be conducted. Internationally accepted OECD guidelines exist for *Lumbriculus* (OECD 225[1]) and *Chironomus* (OECD 218[2]/219[3]) and Hyalella (ASTM E1706[4]). All test designs represent sediment-water compartments. However, according to the OECD and ASTM guidelines, there are two serious differences regarding the feeding regimes for strongly adsorbing substances.

According to the OECD guidelines, ingestion of contaminated food may be a significant exposure route and therefore, the use of food added to the sediment before application of the test substance may be considered. In such a case, finely ground plant material added to the formulated sediment before test substance application must be used. According to the ASTM E1706 guideline, such a feeding regime is not considered. Independent from the test substance properties, always food suspensions have to be applied frequently via the overlying water.

OECD currently develops an own sediment-water *Hyalella* guideline considering different feeding regimes. However, one problem regarding a change in food source and quality could be a reduced survival rate, growth and maybe a total lack of reproduction within the test duration of six weeks. So far, no guideline, ring test or research project published regarding *Hyalella* development and reproduction used plant material as the only food source.

Materials & Methods

Modified 42-day Sediment-Water *Hyallea azteca* control tests following ASTM E1706[4] in a flow-through test system were applied. Finely ground plant material was used as the only food source in the 4 week sediment-water phase. In the subsequent 2 week lasting water-only phase, the standard food suspensions (diatoms/fish food) were applied.

300 mL glass beakers with an outlet tube, equipped with a metal gauze to provide from loss of test specimens were applied as test vessels. For the sediment-water phase, 130 and 90 g dry mass artificial OECD sediment were submerged by 255 and 270 mL water, respectively. Ten 7-8 days old juveniles were applied per vessel, four replicates per treatment. Water exchange rate was 2 volumes per day, conducted continuously by a peristaltic pump. Overlying water in the test vessels was aerated. For the water-only phase, 7 g clean quartz sand were submerged by 300 mL water. The test was run at 23 °C with a L:D ratio of 16:8 h.

Different kind of food sources and sediment amounts were tested.

- A) Green plant meal (lucerne) incorporated into 130 g dry sediment before test start
- B) Green plant meal (lucerne) incorporated into 90 g dry sediment before test start
- C) Green plant meal (lupine) incorporated into 90 g sediment before test start

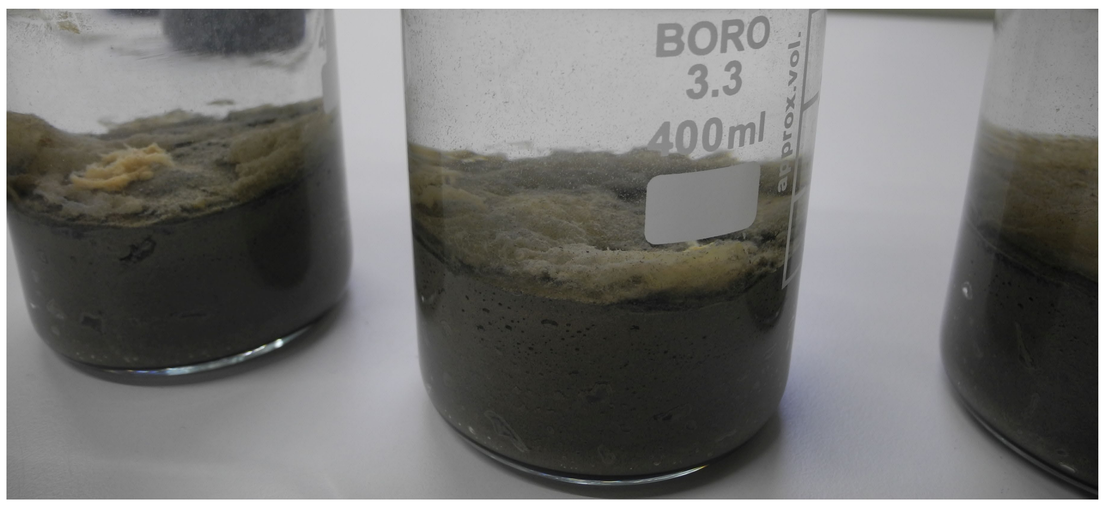

Figure 1: Fungal growth on the sediment surface

Fungal growth after 28 days in 130 g dry sediment assays with incorporated lucerne green meal (A). Photo © Fh-IME

	Survival rate (%)	Mean dry mass (mg)	Juveniles per female
(A) 130 g sediment lucerne	60	0.8	not examined
	70	0.8	
	40	1.0	
	90	0.8	
(B) 90 g sediment lucerne	90	0.8	6.8
	90	0.8	7.5
	80	1.0	8.5
	100	0.8	6.3
(C) 90 g sediment lupine	30	0.8	5.0
	90	0.8	7.3
	100	1.0	4.0
	60	0.8	5.7

Table 1: Relevant endpoints after 42 days

Relevant biological endpoints in an ASTM E1706 42-day Hyalella Test: Survial rate and mean dry mass of adults and juveniles per female.

Results & Discussion

In all test vessels, early after test start fungal growth on the sediment surface occured. This was also mentioned by the OECD expert group coordinating the OECD *Hyalella* guideline development. Comparison with OECD chironomid and *Lumbriculus* assays give hints that mainly the higher temperature in the ASTM *Hyalella* assay may be the reason. Visual comparison between assays showed more fungal growth in the 130 g sediment assays, but no dependency from the food source (lucerne/lupine).

With 40 – 90% survival rate after 42 days, 130 g sediment assays with lucerne food (A) showed high variance. The same is true for 90 g sediment assays with lupine food (C; 30 – 100%). With 80 – 100%, 90 g sediment assays with lucerne food (B) showed best survival rates.

There was no obvious difference in growth between the assays. Mean dry mass per specimen was 0.8 – 1.0 mg (A), 0.9 – 1.1 mg (B) and 1.0 – 1.3 mg (C).

Food quality affected clearly reproduction rate. Lucerne (B) resulted in at mean 7.3 juveniles per survived female, Lupine (C) in at mean 5.5. Assay (A) could not been examined due to the huge fungal mass.

Conclusions

- Incorporated plant green meal can be used for valid 42-day Sediment-Water *Hyallea azteca* tests in the 4 week sediment-water phase. However, food source/quality is important.
- The less sediment mass used in a test, the better the results.
- In this project, 90 g sediment and lucerne food was the only test design surpassing all acceptability criteria of the ASTM guideline for day 42: 80% survival rate, 0.5 mg dry mass per adult specimen and 6.0 juveniles/female.

1 OECD 225 (16.10.07): OECD guideline for testing of chemicals – Sediment-Water Lumbriculus Toxicity Test using Spiked Sediment.
2 OECD 218 (13.04.04): OECD guideline for testing of chemicals – Sediment-Water Chironomid Toxicity Test using Spiked Sediment.
3 OECD 219 (13.04.04): OECD guideline for testing of chemicals – Sediment-Water Chironomid Toxicity Test using Spiked Water.
4 ASTM E1706-20 (2020): Standard test method for measuring the toxicity of sediment-associated contaminants with freshwater invertebrates