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HOW DO YOU DETERMINE THE OPTIMUM FORMULATED SEDIMENTS FOR YOUR STUDY?

APPLICATION NOTE

Comparison Of The Use Of Natural Versus Formulated Sediment In Sediment Toxicity Testing

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ABSTRACT

Formulated sediments are mixtures of materials that mimic the physical components of natural sediments. However, some organisms perform better in natural sediments which contain more micronutrients. Formulated sediments have been used to standardize tests and to serve as a reference for labs to measure performance. The use of formulated sediment eliminates interferences caused by the presence of indigenous organisms, eliminates variation in sediment physico-chemical characteristics and provides a consistent method for evaluating the fate of chemicals in sediment. An acceptable formulated sediment should (1) support the survival, growth, or reproduction of a variety of benthic invertebrates, (2) provide consistent performance, and (3) be consistent from batch to batch. Natural sediments should (1) come from a clean source, (2) be fully characterized, and (3) free of organisms that might compete with or consume the test organisms. Two formulated and two natural sediments that have been extensively used at EAG Laboratories' Easton facility were compared for differences in sediment characteristics, classification, pesticide contaminants, and composition of metals, many of which are micronutrients. Similar results between sediments were observed for acute tests while some of the chronic tests performed dramatically better with natural sediments. Differences in micronutrients may help explain why some tests only do well in natural sediment.

SEDIMENT TYPES

There are 4 major sediment types we use in testing and have undergone extensive analyses for characterization and screening for pesticides, and metals.

1. Formulated Sediment with alpha cellulose (FSA) as the source of organic carbon following OECD 218 and 219 Guideline recommendations.

2. Formulated Sediment with peat (FSP) as the source of organic carbon following OECD 218 and 219 Guideline recommendations.

3. West Bearskin Lake Sediment (WBS) which is a natural sediment from the Boundary Waters of Minnesota that has been used as a reference sediment by EPA, USGS, and USFWS in development of guidelines for *Hyalella* and chironomid species.

4. Wye River Sediment (WRS) which is a natural sediment from the Wye River of the Eastern Shore of Maryland used in developing saltwater guidelines for Leptocheirus.

SEDIMENT CHARACTERIZATION

Samples of sediments are also routinely sent for soil characterization at Agvise Laboratories. Mixed batches of formulated sediment are sent out for characterization. Summaries of these analyses are presented in Table 1.

	FSA	FSP	WBS	WRS
	Mean	Mean	Mean	Mean
% Sand	86.2	78.1	67.0	91.3
% Silt	4.7	6.1	32.0	5.5
% Clay	9.1	15.8	1.0	3.3
USDA Textural Class	Loamy sand	Sandy loam	Loamy sand	Sand
Bulk Density	1.2	1.2	0.9	1.2
Cation Exchange	3.0	6.9	7.6	10.8
% Moisture at 1/3 bar	10.0	13.4	23.3	6.1
% Organic Carbon (Walkley Black)	2.2	1.6	3.8	0.3
% Organic Matter (Walkley Black)	3.7	2.8	6.6	0.5
pH in 1:1 soil-to-water ratio	6.8	7.6	5.4	7.5

Base Saturation | Cation (ppm)				
Calcium	375	739	631	237
Magnesium	63	276	120	285
Sodium	56	18	25	1,483
Potassium	14	16	33	122
Hydrogen	4	8	32	5

Table 1. Sediment Characterization Analysis

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PESTICIDES AND METALS ANALYSES OF SEDIMENT

As part of GLP requirements our sediment is periodically screened for pesticides and metals to insure our source of sediment is acceptable for testing. The analyses are performed by Lancaster Laboratories using EPA approved methods. An example a report with list of analytes is presented below.

Table 2. Pesticides and Organics Concentration

Component	µg/kg	Component	µg/kg
Aldrin	< 0.82	Dursban (Chlorpyrifos)	< 66
Gamma BHC – Lindane	< 0.82	o,p-DDE	< 1.7
Alpha BHC	< 0.82	Endosulfan I	< 0.82
Gamma Chlordane	< 0.82	o,p-DDT	< 1.7
Alpha Chlordane	< 0.82	Endosulfan II	< 1.7
Guthion (Azinphos-methyl)	< 66	p,p-DDD	< 1.7
Beta BHC	< 0.99	Endosulfan Sulfate	< 1.7
HCB	< 0.82	p,p-DDE	< 1.7
Bolstar	< 66	Endrin	< 1.7
Heptachlor	< 0.82	p,p-DDT	< 1.7
Chlordane	< 17	Endrin Aldehyde	< 1.7
Heptachlor Epoxide	< 0.82	Phorate	< 66
Coumaphos	< 66	Endrin Ketone	< 1.8
Kepone	< 6.9	Ronnel	< 66
Delta BHC	< 0.89	EPN	< 66
Malathion	< 66	Stirofos	< 66
Demeton-O	< 66	Ethion	< 66
Merphos	< 66	Sulfotepp	< 66
Demeton-S	< 66	Ethoprop	< 66
Methoxychlor	< 6.6	Telodrin	< 1.2
Diazinon	< 66	Ethyl Parathion	< 66
Methyl Parathion	< 66	Thionazin	< 66
Dichlorvos	< 66	Famphur	< 66
Mevinphos	< 66	Tokuthion	< 66
Dieldrin	< 1.7	Fensulfothion	< 200
Mirex	< 1.7	Toxaphene	< 33
Dimethoate	< 66	Fenthion	< 66
Naled	< 66	Trichloronate	< 66
Disulfoton	< 66	Trithion	< 66
o,p-DDD	< 1.7		

[1] Analyses performed by Lancaster Laboratories on samples collected on December 7, 2016.

Table 3. Metals Concentration

Component	mg/kg	Component	mg/kg
Aluminum	5,400	Antimony	< 1.57
Magnesium	1,230	Manganese	2.29

Table 3. Metals Concentration (Continued)

Component	mg/kg	Component	mg/kg
Arsenic	< 1.57	Silver	< 0.394
Mercury	< 19.0	Chromium	3.30
Barium	34.7	Sodium	< 78.7
Nickel	0.904	Cobalt	< 0.394
Beryllium	< 0.394	Sulfate by IC (solid)	94.5
Nitrate Nitrogen by IC (solid)	< 1.5	Copper	1.01
Bromide by IC (solid)	< 5.1	Thallium	< 2.36
Nitrite Nitrogen by IC (solid)	< 1.0	Fluoride by IC (solid)	< 1.0
Cadmium	< 0.394	Vanadium	1.85
Potassium	74.6	Iron	278
Calcium	2,370	Zinc	2.69
Selenium	< 1.57	Lead	6.60
Chloride by IC (solid)	13.4		

[1] Analyses performed by Lancaster Laboratories on samples collected on December 7, 2016.

RESULTS

Pesticide screens came back at below the limit of detection in all four sediment types. However there were marked differences in metals, especially between natural and artificial sediments. These metals are key micronutrients for aquatic organisms. The figure below provides a comparison of key micronutrients identified by Wetzel (2001) among the four sediment types.

Figure 1. A comparison of key micronutrients identified by Wetzel (2001) among the four sediment types.

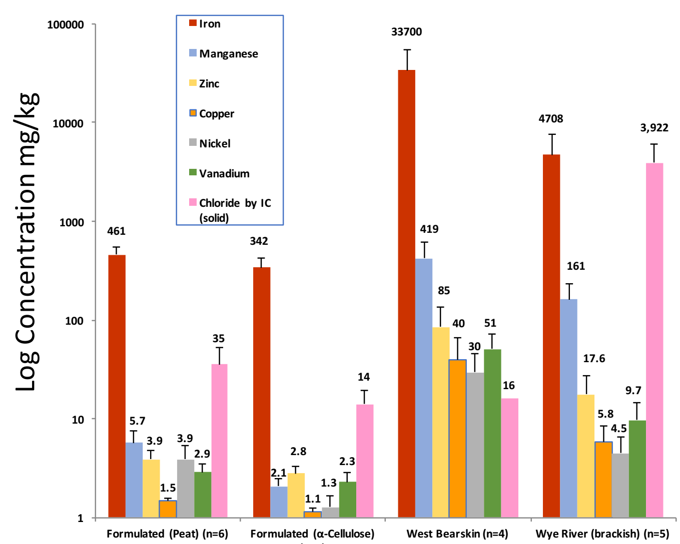

Table 4. Primary Physiological Functions of Micronutrients Generally Accepted as Essential or Potentially Essential for Aquatic Organisms (from Wetzel 2001)

Micronutrient	Primary Physiological Function
Iron	Electron transport in redox systems of respiration and photosynthesis; enzyme activation, oxygen carrier in N2 fixation

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Table 4. Primary Physiological Functions of Micronutrients Generally Accepted as Essential or Potentially Essential for Aquatic Organisms (from Wetzel 2001) (Continued)

Manganese	Enzyme activation; electron transport reactions particularly in photosynthesis, detoxification of superoxide radicals, synthesis if secondary metabolites. Ribosome structure
Zinc	Membrane integrity, enzyme activation, particularly carbonic anhydrase, gene structure, expression and regulation, carbohydrate metabolism, anaerobic respiration, protein synthesis, ribosome structure, detoxification of superoxide radicals; phytohormone activity
Copper	Redox reactions of respiration and photosynthetic electron transport; detoxification of superoxide radicals; lignification; haemocyanin in aquatic invertebrates
Nickel	Iron absorption; nitrogen fixation, several constitutive enzymes, particularly urease; reproductive growth in plants
Boron	Cell wall formation and stabilization; membrane integrity; carbohydrate utilization, pentose phosphate metabolism, lignification; xylem differentiation, stomatal regulation, heterocyst structure and nitrogen fixation of cyanobacteria
Molybdenum	Electron transfer reactions, nitrate reduction and nitrogen fixation, sulfate oxidation; protein synthesis
Chloride	Osmoregulation; cation uptake, photosynthesis; reactivity of enzymes
Selenium	Essential growth regulator among algae; enhancement of phosphorous metabolism; amino acid and protein synthesis; mitosis, cuticle integrity
Cobalt	Essential for growth among many microbiota, particularly algae; essential component of vitamin B12 ; potential substitution for zinc
Vanadium	Unclear functions and absolute requirements; enhancement of nitrogenase and nitrate reductase activities; substitution for molybdenum in some algae; phosphorylation
Cadmium	Unclear if required nutrient in algae; implicated in functions in carbonic anhydrase activity; possible substitution for zinc

SEDIMENT ASSOCIATION WITH BIOLOGICAL RESULTS

The differences in performance of typical tests among different sediment types are summarized in the next Table. The differences observed in micronutrients between natural and artificial sediments correlate with the ability of our lab to meet control performance criteria with certain sediment organisms. The only way we could successfully culture and successfully run the 28-day for the chronic test with *Leptocheirus pulmulosous* was by using the natural sediment from the Wye River. While we can culture in *Chironomous dilutus* sand, we had trouble meeting control acceptance criteria in this test for emergence and reproduction in formulated sediments. We found for this type of testing that control performance in *Chironomous dilutus* chronic tests were consistently much better in West Bearskin Lake sediment.

CONCLUSIONS

Sediment type does not matter as much with acute tests, since these are 10-day tests, with much shorter exposures than chronic tests. The increased concentrations of micronutrients in natural sediments seem to be more limiting in longer term chronic tests. It is uncertain as to the exact amounts of specific micronutrients that are limiting, but a greater understanding might help develop a better formulated sediment.

REFERENCES

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Guideline Studies	Species	Duration	Formulated Sediment α-Cellulose	Formulated Sediment Peat	Natural Sediment West Bearskin Lake	Natural Sediment Wye River
OECD 218	*Chironomid*	28-day	Yes	Yes	Yes	—
OECD 219	*Chironomid*	28-Day	Yes	Yes	Yes	—
OECD 233	*Chironomid*	44-Day	—	Yes	—	—
OECD 225	*Oligochaete*	28-Day	—	Yes	—	—
OCSPP 850.1735	*Chironomid*	10-Day	Yes	Yes	Yes	—
OCSPP 850.1735	*Hyalella*	10-Day	Yes	Yes	Yes	—
OCSPP 850.1740	*Leptocheirus*	10-Day	Yes	Yes	—	Yes
EPA under revision	*Chironomid*	60-Day	Marginal	Marginal	Best	—
EPA under revision	*Hyalella*	42-Day	Yes	Yes	Yes	—
EPA under revision	*Leptocheirus*	28-Day	No	No	—	Yes