

**PLANT TOXICITY TESTING WITH
SEDIMENT AND MARSH SOILS**

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EXECUTIVE SUMMARY

Contaminated sediments are a major problem in many ponds, lakes, and marshes of the United States. Besides creating problems *in situ*, they are significant non-point sources for contamination downstream.

The National Park Service is aware of potential dangers of contaminated sediments to water resources and supports efforts to detect and mitigate those dangers. This report, which describes principles and methods for laboratory detection of potential effects of contaminated sediments on aquatic plants, may be used by park personnel wherever sediment contamination is suspected. The report was prepared as supplementary reading for a course entitled "Soil and Plant Toxicity Assessment" given at the 12th Annual Meeting of the Society of Environmental Toxicology and Chemistry, Seattle, WA, on November 3, 1991. It should be of value to those who are interested in environmental toxicology.

ABSTRACT

A short account of the principles and practices of toxicity testing with aquatic plants and sediments is given. Aquatic (wetland, marsh) plants have been shown to be sensitive to toxicants in natural and synthetic sediments, and advantages and disadvantages of each type of sediment in toxicity testing are described. Toxicological studies with *Echinochloa crusgalli*, *Sesbania macrocarpa*, and *Spartina alterniflora* are described, but other experimental species need to be adapted for use in impact analysis and risk assessment. It is concluded, after comparison of results from seed germination, hydroponic, and sediment tests, that the latter best simulate the unique field conditions under which plants are exposed to pollutants.

INTRODUCTION

Submerged and emergent vascular plants are dominant features of wetlands and, in association with sediments, determine the structure and function of these important ecosystems. In fact, wetlands have been defined in relation to their sediments as "areas that are inundated or saturated by surface- or ground-water, at such a frequency and duration that under natural conditions they support organisms adapted to poorly aerated and/or saturated soil" (Lugo, 1990). Wetland (aquatic) plants are adapted to the conditions of such sediments, and risk analyses of probable effects of toxicants on them must be conducted under the unique aquatic plant/sediment system.

Sediments are major sinks or reservoirs for pollutants. Pesticides, other toxic substances, and nutrients adsorb to organic and inorganic particles in water and are deposited with them in sedimentary areas such as lakes, ponds, and freshwater and saltwater marshes. Because sediments act as reservoirs, toxic effects may be felt long after the original pollution event is past. When in place in aquatic ecosystems, toxic sediments serve as major non-point sources of pollution to the benthos, water column, or downstream areas by redeposition and erosion. Toxic sediments are now recognized as a major pollution problem, and new methods are rapidly being developed for their analysis with algae, plants, and animals. The American Society for Testing and Materials (ASTM) supports a large subcommittee for development of ecotoxicological studies on sediments (Subcommittee E47.03, "Sediment Toxicology").

This short account of toxicity testing with marsh plants and sediment is designed as an introduction to the subject. Its basic assumptions are that structure and functions of wetlands can be affected by toxicants in sediments and that laboratory tests can detect possible or probable injury to aquatic plants under specific circumstances. The methods given here may be modified or used directly for routine toxicity testing or for experimental studies in which environmental variables are manipulated.

PRINCIPLES OF SEDIMENT TOXICITY TESTING WITH VASCULAR PLANTS

Aquatic vascular plants are potentially of great value for estimation of soil and sediment toxicity. Their roots are in close contact with the particles of these substrata, and they absorb and translocate substances, including toxics, from interstitial water to other parts of the plants. Because they are in such close association, rooted aquatic vascular plants modify their substrata and are affected by them. They are also sensitive to pollutants and are thus good test subjects for identification of toxic sediments and marsh soils.

Although terrestrial vascular plants are used commonly for toxicity testing of soil, aquatic vascular plants have not been used extensively for such studies of sediment. A major text on aquatic toxicity testing (Rand and Petrocelli, 1985) mentions only microalgae (unicellular algae, phytoplankton) for use in phytotoxicity tests, and an otherwise outstanding review of aquatic toxicity testing (Munawar *et al.*, 1989) describes studies on only one species of aquatic vascular plant, *Lemna minor* (duckweed), a floating plant that does not have contact with a solid substratum. In the same publication, Ahlf *et al.*, (1989) used germination and initial growth of rye grass (*Lolium multiflorum*) and cress (*Lepidium sativum*) in phytotoxicity tests on sediments. In a review of sediment toxicity and bioaccumulation testing, Ingersoll (1991) did not mention use of vascular plants.

The fact that rooted aquatic plants are sensitive to toxicants in sediment has been demonstrated by Walsh *et al.*, (1990, 1991a, b, *in press* a,b). Copies of these reports are appended. As with other toxicity tests, the conditions under which plants are exposed to bioactive substances strongly determine response. Many environmental variables affect survival and growth of aquatic plants (Table 1). All of these can interact with pollutants and must be considered when interpreting toxicity data. Thus, structure, composition, and physical and chemical conditions of soil or sediment, inherited plant sensitivity or resistance, age and size of the plant, length of time of exposure, temperature, light intensity and quality, polarity of the toxic molecule and its degradative properties all affect response.

A brief introduction to the properties of sediments is given below, followed by a discussion on toxicity testing with plants. The term "sediment" will be used to designate the substratum upon which and within which aquatic plants grow. Aquatic plants are "those species which normally start development in water and must grow for at least a part of their life cycle in water, either completely submersed or emersed" (Muenscher, 1972).

PROPERTIES OF SEDIMENTS

Sediment is particulate matter that has been transported by wind, water, or ice, or that has been precipitated from water. Its chemical composition and physical properties are determined by its origin and how it was changed by physical, chemical, and biological processes before and after deposition in aquatic ecosystems.

Inorganic Sediment - This sediment is composed of particles classified by size (Table 2) and formed predominantly from igneous granite (mainly SiO) or sedimentary limestone (mainly CaCO₃). Structures of three secondary minerals derived mainly from granite are illustrated in Fig. 1. Sediment textural classes are defined by the relative amounts of sand, silt, and clay (Fig. 2).

Table 1. Aspects of environmental variables that affect survival and growth of aquatic plants. Modified from Scott (1974).

<u>Climatic</u>	
Solar radiation	Spectral composition, intensity, direction, periodicity
Terrestrial back radiation	Intensity, integration
Temperature, air	Degree, periodicity, integration, lateral and vertical variation
Temperature, sediment	Degree, periodicity, integration, lateral and vertical variation, freeze-thaw phenomena
Water, vapor	Evaporation, transpiration
Water, precipitation	Cloud, fog, dew, salt spray, rain, snow, pH
Water, soil	Content, tension, supply rate, aeration
Gasses, atmospheric	Oxygen and carbon dioxide contents, ozone, pollutant gasses
Weather phenomena	Wind, frequency, force, direction, evaporation, transpiration, abrasive agents
<u>Edaphic</u>	
Parent material	Minerals present, weathering
Physical properties	Texture, mechanical analysis, moisture, stability
Chemical properties	Clay mineralogy, organic compounds, cation exchange capacity, pH, redox, macro- and micro-nutrients, toxic substances
Biotic properties	Soil flora and fauna

Table 2. Particle size categories of geological materials. The phi value is the logarithmic transformation of particle diameter in mm based on the negative log to the base 2. (Lincoln *et al.*, 1982)

<u>Diameter mm</u>	<u>Phi value</u>		
		Boulder	
256	-8		
		Cobble	
64	-6		Gravel
		Pebble	
4	-2		
		Granule	
2	-1		
		Very Coarse	
1	0		
		Coarse	
0.5	1		Sand
		Medium	
0.25	2		
		Fine	
0.125	3		
		Very Fine	
0.0625	4		
		Silt	
0.0039	8		Mud
		Clay	
0.00024	12		
		Colloid	

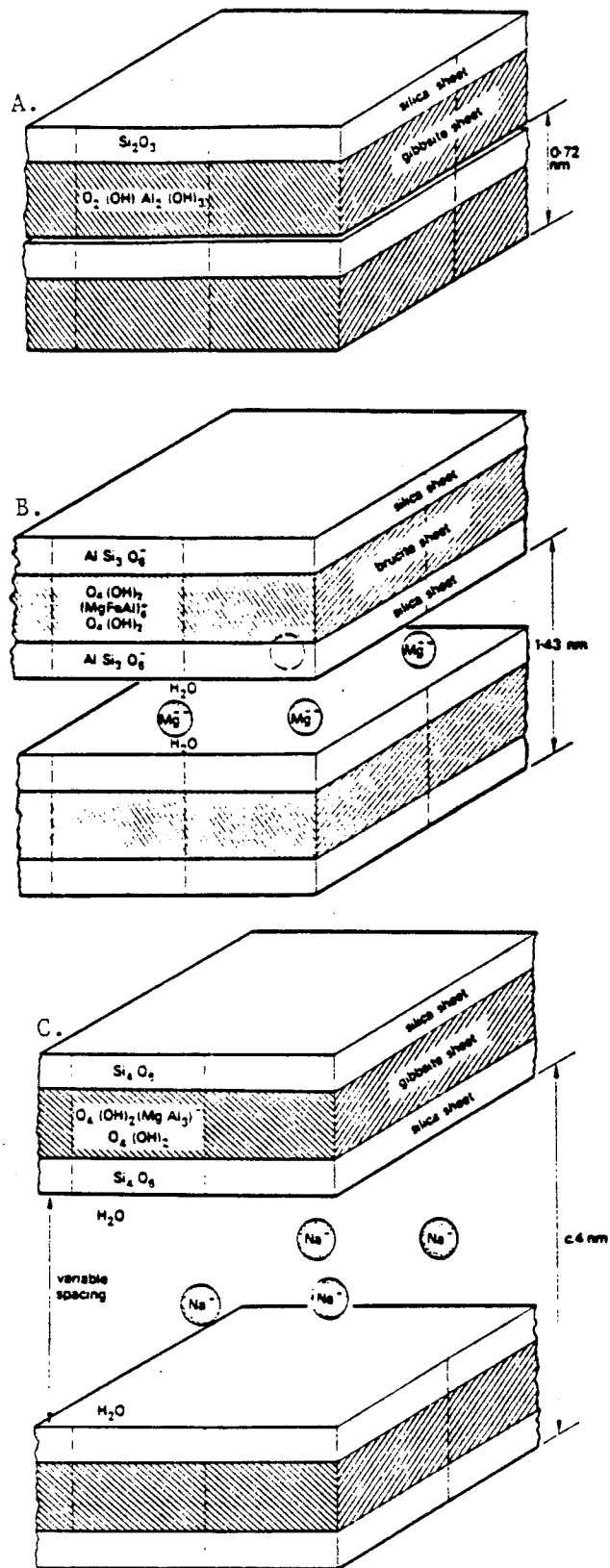


Figure 1. Structure of A. kaolinite, B. vermiculite, and C. montmorillonite. From White (1987)

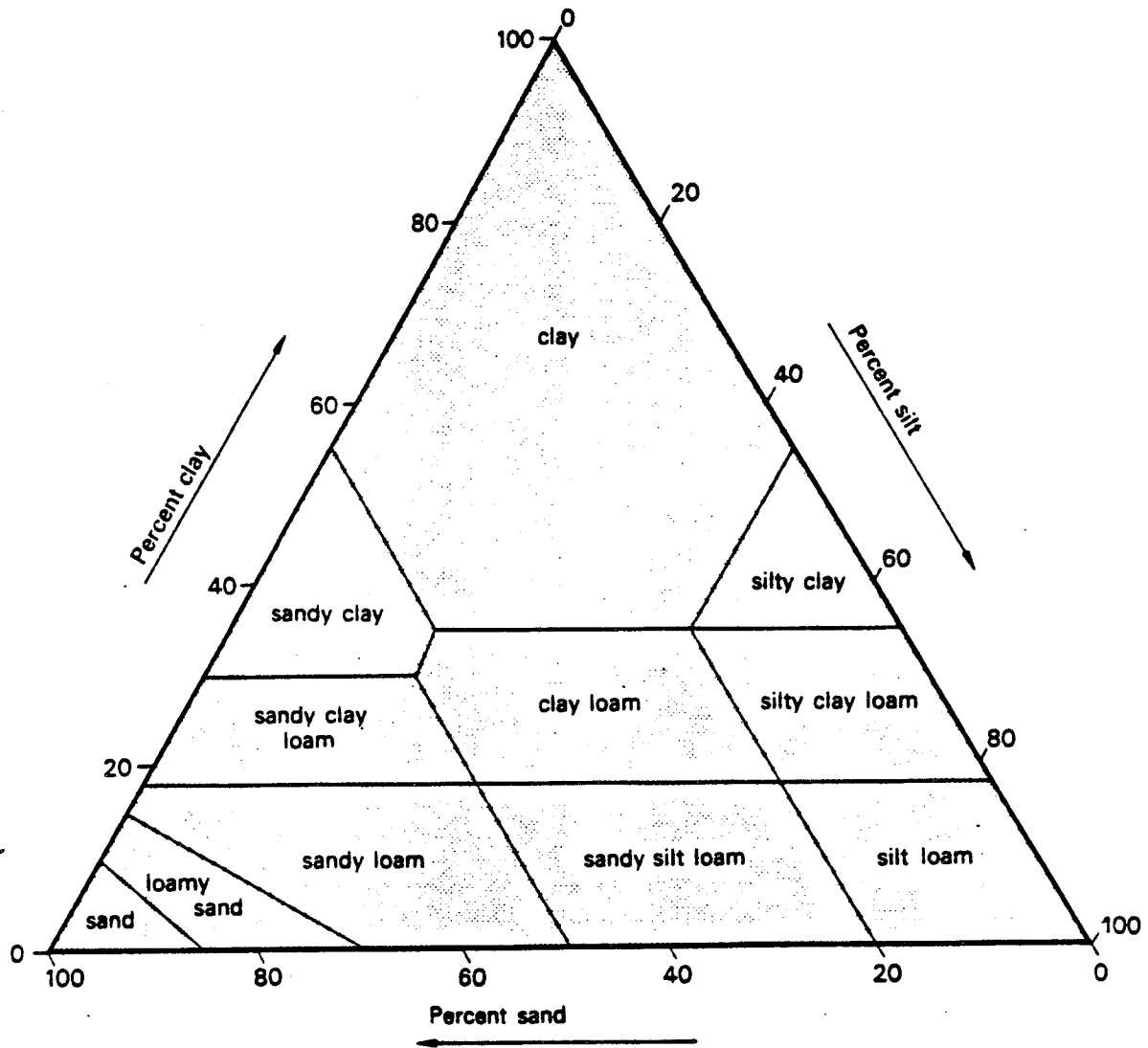


Figure 2. Soil textural classes. From White (1987) after Hodgson (1974).

Table 3. Cation exchange capacity and size of sediment particles. Black, (1968).

	Diameter of particles, mm	Surface area cm ² /g	CEC Cmols (+)/kg
Silt	0.02-0.005	1,800	3
	0.005-0.002	6,200	7
Coarse clay	0.002-0.001	16,000	22
	0.001-0.0005	30,000	35
	0.0005-0.0001	74,000	52
Fine clay	0.0001-0.00005	320,000	56
	<0.00005	920,000	63

Texture - Texture is defined as the size distribution of the particles that form the sediment. The term "structure" is often used synonymously with "texture," although technically they are different. Texture is a very important property of sediments because it controls the drainage properties, amount of pore space between particles (and thus the ease with which roots and rootlets may penetrate), volume of pore (interstitial) water, and soil temperature. Texture controls the amount of water available to plants (the amount is greater in sediments of moderately fine texture than in sediments of coarse texture), and availability of sediment nitrogen, a limiting nutrient, usually increases as texture becomes finer. This combination of availability of water and essential nutrients can strongly affect plant growth and results of toxicity tests.

Relative amounts of sand, silt, and clay may also affect the dissolved constituents of pore water. Texture is important here because dissolved substances are the only ones available for uptake by plants. Clays adsorb more dissolved organic matter than do sands or silts, so that plant response to contaminated soil of high clay content may be less than that in a similarly contaminated sandy soil.

The property of sediment most important in regulation of concentrations of dissolved constituents of pore water is the cation exchange capacity (CEC). This is defined as the total amount of exchangeable cations that soil or sediment holds, as determined by leaching with a neutral salt such as 1N KCl or 0.1N BaCl₂ (Russell, 1973). The CEC of the mineral fractions of soils is derived from the dissociation of cations from particle surfaces, and therefore increases with the surface area of the particles. Surface area and CEC per unit weight increase rapidly with decrease in particle size (Black, 1968; Table 3). Thus, the clay fraction is extremely important in the binding of ionic and polar substances to sediments, including the amount bound and the strength by which it is bound.

Organic Matter - The organic matter of sediments is composed of living organisms such as bacteria, fungi, algae, plant roots, protozoa, etc., the remains of dead organisms in various states of

decomposition, and exudates of living organisms (bacteria, fungi, root exudates, etc.). White (1987) suggested that non-living soil organic matter may be broadly classified as (a) macro-organic matter: plant and animal debris, (b) light fraction: partly humidified and very fine plant and animal remains, and (c) humified fraction: organic matter that has been reduced to humus. Aiken *et al.*, (1985) defined humic substances as "A general category of naturally occurring biogenic, heterogeneous organic substances that can generally be categorized as being yellow to black in color, of high molecular weight, and refractory." They described three fractions of humic substances in relation to their solubilities: humin, humic acid, and fulvic acid. These substances have been the subjects of numerous studies (Schnitzer and Khan, 1978; Tate, 1987; Kumada, 1987; Drever, 1988; Saiz-Jimenez *et al.*, 1989), and they affect toxicity of organic and inorganic substances in sediment (Suffet and MacCarthy, 1989).

Organic matter in sediment may be in the dissolved, colloidal, or particulate phases. The dissolved and colloidal portions may be free in the pore water or they may be adsorbed to clay, forming a clay-humus complex. The main products of humification are organic colloids with a high surface area, a high CEC, and the ability to chelate metal ions. These properties make organic matter extremely important in sediment toxicity, especially in relation to clay concentration. Toxicity tends to be inversely related to concentrations of clay and organic matter.

Particulate organic matter, because it can hold a weight of water greater than its own weight, can influence the water holding capacity of a sandy soil (Thompson and Troek, 1973), and thus affect the growth rates of plants by adsorption of toxicants and provision of water.

Aeration - The proper sediment atmosphere is vital to plant survival and normal growth. Sediment pores that are not filled with water contain lacunae that contain the sediment atmosphere. This atmosphere contains higher amounts of carbon dioxide and lower amounts of oxygen than the atmosphere above the sediment because of uptake of oxygen and release of carbon dioxide from metabolism by roots and soil organisms and by decomposition of organic matter. In aquatic systems, gaseous exchange between sediment and the overlying water is facilitated by bioturbation and water motion. In marshes, it is also facilitated by burrowing organisms.

Most plants require sediment atmospheric oxygen for root function, although some, such as mangroves, have anatomical devices for conduction of oxygen from the atmosphere above the sediment to the roots. It is important that the sediments of toxicity tests with plants never become anaerobic to ensure that effects on growth are due only to the toxicant.

pH - For the purpose of pH measurement, sediment is considered to be a suspension of particles in water. The pH of such a system is determined by the ionic atmosphere around the particles, i.e., the relative amounts of acidic (H^+ and Al^{+++}) and base (Ca^{++} and Mg^{++}) cations on its cation exchange sites. The pH of a sediment depends on the salt concentration in the soil solution and the carbon dioxide concentration in the sediment atmosphere (Russell, 1973).

By itself, pH has no effect on plant survival and growth. However, it does control edaphic factors that can affect plants. pH influences the solubility of plant nutrients, the amounts of nutrients stored on cation exchange sites, and the rate of weathering (Thompson and Troek, 1973). Cation exchange capacity is directly related to pH: a rise in pH produces an increase in CEC (Black, 1968).

Control of pH is extremely important in toxicity testing of sediments. Many pollutants are affected directly by pH, being more or less active, susceptible to degradation, or adsorbed to particles at various pHs. For example, at pH 4, aluminum (Al^{+++}) becomes soluble and can be strongly toxic to aquatic biota, whereas at higher pH, Al is complexed and not detrimental

Redox potential - Redox (oxidation - reduction, Eh) reactions are those in which a molecule or ion is reduced from a more oxidized state to a less oxidized state, or *vice versa*, through the transfer of electrons (White, 1987). The redox potential is a measurement, expressed in volts or millivolts (mV), of the tendency for a redox reaction to occur. High redox potential is associated with an oxidizing atmosphere, low redox potential with a reducing atmosphere. For a clear and succinct discussion of redox potential in waterlogged soil, see Russell (1973).

Since low redox potential is associated with reducing conditions (low pH and dissolved oxygen concentration, presence of hydrogen sulfide), higher potentials must be maintained to ensure healthy plants. Toxicity tests are best conducted at redox potentials of 200 mV or greater at pH between 6 and 7. This can usually be achieved easily by avoidance of anaerobic sediment conditions.

PLANT REQUIREMENTS

Texture - Individual plant species have optimal requirements for sediment texture. Texture affects the rate of growth, plant form, and function of roots, which in turn affect the well-being of the plant. It affects root penetration and branching, production of root hairs, root cellular morphology, water and nutrient uptake, the amount of photosynthate required to form and sustain roots, oxygen utilization by roots, and activities of symbiotic root bacteria and fungi (Glinski and Lipiec, 1990).

Pore space, which is related to mechanical impedance to root growth, is probably the most important attribute of sediment with regard to growth. Pore space, or porosity, is expressed as the ratio:

$$\text{Pore Space Ratio} = \frac{\text{volume of pores}}{\text{total soil volume}}$$

Porosity does not indicate the size or shape of the pores, which are dependent upon the size and shape of the sediment particles, nor does it indicate the relative amounts of space occupied by water and air.

A general rule is that a pore space ratio of $0.5 \text{ m}^3/\text{m}^3$, or 50% pore space is desirable for most plants. Between 10 and 25% clay and approximately equal amounts of silt and sand and several percent of organic matter makes a very good soil for most uses (Thompson and Troek, 1973). However, it is best to determine the optimal texture for growth of test plants before initiation of toxicity studies.

Nutrients - Plants require an array of nutrients in specific concentrations and relative amounts for survival and growth. Insufficient quantities of nutrients limit growth, whereas quantities above those needed for optimal growth may be toxic. Carbon dioxide from the air and water from the sediment

are the sole sources of carbon and hydrogen ions for plants. Mineral nutrients include salts of nitrogen, phosphorus, potassium, sulfur, magnesium, calcium, iron, zinc, copper, manganese, boron, and molybdenum. Cobalt, silicon, and aluminum may also be necessary. Fertilizers that contain correct amounts of these nutrients are available commercially, but it is usually better to prepare liquid fertilizers from laboratory chemicals for improved quality assurance. A good fertilizer was described by Hoagland and Arnon (1950).

Numerous excellent books on plant nutrition have been published, and one should be consulted before tests with plants are begun. The fertilization regime is dependent upon the needs of the test species and whether or not the test pots are drained or closed.

Light - Light is required by plants for photosynthesis and normal growth rate and form. Too little light will result in low productivity and elongation and thinning (etiolation) of the plant axis. Too much light causes reduced productivity and damage to plant structures.

Intensity and quality of light should be carefully controlled in toxicity tests with plants. Most plants will grow normally under 300-600 $\mu\text{E}/\text{m}^2/\text{sec}$ of photosynthetically active radiation (PAR, 400-720 nm wave length) with a diel light: dark cycle determined by experimental needs. A 16h light: 8h darkness cycle is generally acceptable.

Water - Water is not a problem in toxicity tests with aquatic plants because the sediment is kept waterlogged in short-term tests. A waterlogged (flooded) sediment is one whose pore space is filled to capacity. In long-term tests, the sediment is kept moist constantly.

Temperature - Temperature of the air and sediment affect plant growth. Each species has an optimal temperature that must be maintained, within limits, in toxicity tests. Some species require diel variation in temperature for optimal growth. It is necessary, therefore, to expose plants to toxic sediments in plant growth chambers or greenhouses where temperature variation is minimal or under controlled temperature regimes.

Humidity - Transpiration rate is related indirectly to relative humidity: as humidity falls, transpiration rate increases. If the transpiration rate is too high, the plant may wilt. Toxicity tests should be conducted at a relative humidity that does not place stress upon the plant.

CHOICE OF TEST SPECIES

Only a few marsh plant species have been used for sediment toxicity testing in the laboratory, and there is a need for development of tests with new species. At present, toxicity testing is limited to species for which seeds are available. Seeds may be collected in the field and planted in uncontaminated natural or synthetic sediment in the laboratory. The plants that develop from them may be grown to maturity and their seeds harvested and replanted to build a seed source whose background is known. Otherwise, seeds may be purchased from commercial dealers in bulk and used directly in toxicity tests and as starters of laboratory cultures for seeds. Seeds and grown individuals ready for transplanting may be purchased from Wildlife Nurseries, Ashkosh, WI (freshwater) and Environmental Concern, St. Simons, MD (saltwater).

Although several species of freshwater and saltwater marshplants have been used in hydroponic uptake and toxicity studies (Lee *et al.*, 1981), I am familiar only with sediment methods for *Echinochloa crusgalli* (Linneaus) Palisot de Beauvois var. *crusgalli* and var. *zelayensis* (Poaceae) and *Sesbania macrocarpa* Muhl. ex Raf. (Leguminosae) (freshwater) and *Spartina alterniflora* Loisel (Poaceae) (saltwater). Publications that describe methods and studies with these species are described in Appendices A, B, and C.

METHODS OF TOXICITY TESTING WITH SEDIMENT/PLANT SYSTEMS

Toxicity testing with vascular marsh plants is currently in a developmental stage, and I know of no "standard" methods for such tests. Sediment/plant tests are potentially of great value for estimation of possible effects of field sediment samples and of specific toxic substances.

The only published methods for sediment/plant toxicity tests that I am aware of are given in detail in Appendices A, B, and C. Comparisons of natural and synthetic sediments are presented in Appendices D, E, and F. The methods address preparation of seeds and sediments, transplantation of seedlings to toxic sediments, length of exposure time, endpoints of toxicity tests, and statistical analyses. They are simple and adaptable for most requirements.

Synopsis of Method

1. Germinate seeds in uncontaminated sand under environmental conditions to be used in the toxicity exposure.
2. Transplant seedlings to control and contaminated soils or sediments.
3. Grow the seedlings for a predetermined period of time under carefully controlled conditions of light, temperature, and watering regime.
4. Harvest seedlings, measure height, and weigh while fresh.
5. Divide seedlings into roots, stems, and leaves; dry and weigh.
6. Apply statistical analyses to determine effects on weights of plant parts.

DISCUSSION

It is clear that the sediment/plant system is, like natural systems, very complex, and that toxicity tests must be carefully controlled. All of the sediment and plant considerations presented here can affect results.

The major concern of laboratory toxicity tests is how results aid in prediction of possible effects in natural ecosystems. Results from sediment/plant, seed germination, and hydroponic tests (Appendices A, B, and C) often differ, and it is concluded that germination and hydroponic tests are valuable for estimation of effects and uptake of dissolved substances. However, most vascular plants are exposed to toxic substances in the rooting sediment. As shown above, the complex features of sediments, and plant interactions with sediment, light, and atmospheric conditions,

greatly modify the toxic response, suggesting that germination and hydroponic tests give little, if any, information on possible field effects. Sediment tests consider the roles of sediment in plant growth and pollutant adsorption/desorption kinetics. Also, other factors that affect plant growth (water, nutrients, light, etc.) are supplied in a more field-related manner in sediment tests.

Sediment/plant tests are potentially of great value for estimation of possible effects of field samples and specific toxic substances. Papers in the appendices demonstrate that plant growth was affected by heavy metals, organic substances, and effluents in sediment, that synthetic sediments are valuable in toxicity tests, and that degree of plant response to toxicants is related to sediment composition. Also, some plant species are more sensitive to toxicants than others. Since plants are susceptible to toxicants in soils and sediments, information from most batteries of tests, e.g. the Sediment Quality Triad (Chapman, 1990), could be broadened by addition of plant tests.

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APPENDIX 1

**TOXICITY TESTS OF EFFLUENTS WITH MARSH
PLANTS IN WATER AND SEDIMENT**

TOXICITY TESTS OF EFFLUENTS WITH MARSH PLANTS IN WATER AND SEDIMENT

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Abstract—Methods are described for toxicity testing of water and sediment with two varieties of the freshwater marsh plant *Echinochloa crusgalli* (Linneaus) Palisot de Beauvois (Poaceae), and complex effluents. Two tests are described: a seed germination and early seedling growth test in water, and a survival and seedling growth test in natural and synthetic sediments. Effects of effluents from a sewage treatment plant, tannery, textile mill, pulp and paper mill, coking plant and sewage treatment plant included inhibition of germination, chlorophyll synthesis and growth. The tests with rooted marsh plants were sensitive to pollutants and detected toxicity of a range of pollutants in water and sediment. Synthetic sediments similar to natural sediments allowed toxicity tests to be done under carefully controlled conditions of particle size distribution, organic content, pH, electrode potential (Eh) and cation exchange capacity (CEC).

Keywords—*Echinochloa* Effluents Germination Survival Growth

INTRODUCTION

Freshwater and salt-marsh sediments may serve as sinks for industrial, municipal and agricultural pollutants. The pollutants, usually carried to marshes by rivers, tides and longshore currents, are partitioned between sediment particles and pore water [1]. In sediment, relative amounts of sand, silt, clay and organic matter (particulate and dissolved) and salinity of pore water affect partition coefficients of pollutants between pore water and sediment particles. Because distribution of the sediment components differs greatly among marshes and even within a single marsh, it is often difficult or impossible to predict effects of pollutants on marshes with accuracy. Also, as most marshes are contaminated to some extent, it is difficult to obtain sediment that permits study of possible effects of single substances or effluents without interference from other toxic substances.

This report describes methods for examination of effects of single toxicants, mixtures of toxicants and complex effluents in freshwater whole sediment and in pore water. It describes formulation of

synthetic sediments that simulate natural sediments with regard to relative amounts and particle sizes of sand, silt, clay and particulate organic matter. It also describes a method for analysis of small amounts of pore water. The experimental methods utilize rooted marsh plants and the processes of seed germination and early seedling growth in water under conditions of light and darkness, and of seedling survival and growth in contaminated sediments. Although used here with vascular marsh plants, the artificial sediments may also be used in studies with other sediment-associated species.

MATERIALS AND METHODS

Test species

Two varieties of the common freshwater marsh grass *Echinochloa crusgalli* (Linneaus) Palisot de Beauvois (Poaceae) were used: var. *crusgalli* and var. *zelayensis*. Seeds were obtained from Wildlife Nurseries, Oshkosh, Wisconsin, and stored dry at 4°C. They were identified in our laboratory by growing to seed with confirmation of the varietal name according to the descriptions of Correll and Correll [2].

Effluents

Liquid effluents (Table 1) were collected in glass or polyethylene containers by personnel of EPA Region IV or of state agencies. The samples were

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Use of trade names in this report is for identification purposes only and does not constitute endorsement by the U.S. Environmental Protection Agency.

Table 1. Chemical and physical characteristics of effluents used in plant tests

Effluent	Color	Odor	Suspended solids	pH	Salinity (‰)	Ammonia N (µg/L)	Nitrite N (µg/L)	Nitrate N (µg/L)	Phosphate P (µg/L)
Sewage	pink	none	yes	7.6	0	1,945	2.0	1.4	46.0
Textile	none	none	yes	8.2	0	25.0	2.4	20.5	21.2
Pulp and paper	brown	none	yes	7.8	0	10.6	50.9	10.5	35.4
Plating	none	slight	no	9.2	0	132	2.3	10.8	0.7
Coke	brown	strong	yes	7.9	0	157	0	0	5.7
Tannery	brown	none	yes	8.1	8	460	2.2	10.5	1.1

packed in ice, shipped to the Gulf Breeze laboratory in insulated containers and stored at 4°C immediately upon receipt. They were received within 24 h of shipment, and tests were begun the day after receipt. Visual examination was made for color and suspended particles, and presence or absence of odor was recorded; salinity was determined with an American Optical Co. temperature-compensated refractometer, and pH with a Beckman Co. Phi 12 pH/ISE meter. Nitrogen in ammonia, nitrite and nitrate, and phosphorus in phosphate, were determined by the methods of Parsons et al. [3].

Seed germination and early growth

Seed germination and early growth tests were performed in 47-mm clear polystyrene petri dishes with tightly capped lids (Millipore Corp., Bedford, MA). Seeds were surface-sterilized by immersion in 1% sodium hypochlorite for 20 min and rinsed in deionized water. Twelve seeds of *E. crusgalli* were placed in 10 ml of deionized water (freshwater control), undiluted effluent or effluent diluted with deionized water in the petri dishes. Each control and exposure concentration of effluent was prepared in triplicate. Thus, 36 seeds of *E. crusgalli crusgalli* and *E. crusgalli zelayensis* were used in controls and each treatment. In tests with tannery and pulp and paper mill effluents, seed germination and early growth tests were conducted only in light (approximately 35 µE/m²/s on a cycle of 8:16 h dark:light). Also, because tannery effluent was of 8‰ salinity, solutions of the same salinities as the effluent and its dilutions prepared with NaCl were tested. Duplicate tests were conducted with textile mill, sewage plant, coking plant and metals plating plant effluents under the same light regime and in total darkness. Germination tests were conducted for 7 d, and germinated seedlings were counted at selected intervals between day 2 of exposure and the end of the test. At the end of the exposure period, the remains of the bracts were removed from

each seedling. The seedlings from each petri dish were combined and dried for 24 h at 103°C and weighed to the nearest 0.1 mg on a Mettler Model AE 163 balance. Weights of exposed seedlings were compared to weights of control seedlings.

Survival and growth

Sediments. Natural freshwater sediment was collected from a wetland near Milton, Florida. Leaves, twigs and other large particles were removed, and the sediment was dried in air. Particle size distribution of the natural sediment was determined by sieving and by settling rate in water [4]. Organic content was determined by ashing at 550°C for 24 h.

Synthetic sediments were formulated from washed sand, silt, clay and organic matter. Sand (Tables 2 and 3) was obtained from New England Silica, Inc., South Windsor, Connecticut. Three types of sand were used: Mystic White® No. 85 (fine), No. 45 (medium) and No. 18 (coarse). Each type was sieved to obtain the proper grain size for use in synthetic sediments. Silts and clays (Tables 2 and 3), manufactured by Englehard Corp., Edison, New Jersey, were obtained from Gulf Coast

Table 2. Composition^a of sand, silt and clay used in formulation of artificial sediments

Sand (as oxides)	%	Clay and silt	%
SiO ₂	97.70	SiO ₂	65.9
Al ₂ O ₃	1.50	Al ₂ O ₃	12.2
K ₂ O	0.29	MgO ₂	11.5
CaO ₂	0.08	CaCO ₃	4.3
Fe ₂ O ₃	0.07	Fe ₂ O ₃	3.6
MgO ₂	0.06	P ₂ O ₅	1.1
TiO	0.04	K ₂ O	0.8
Na ₂ O	0.01	TiO	0.5
Loss on ignition	0.25	Trace elements	0.1

^aExpressed as percentage by weight.

Table 3. Particle size distribution of natural^a and synthetic sediments used in toxicity tests with rooted freshwater plants

Class	Particle size (μm)	Weight %	
		Natural	Synthetic
Coarse sand	500-1,500	0.6	0.6
Medium sand	250-499	9.5	8.7
Fine sand	63-249	67.4	69.2
Silt	4-62	10.3	10.2
Clay	<4	6.7	6.4
Organic matter	-	4.9	4.9

^a0.6% of weight of natural sediments was lost during handling.

Chemical Corp., Tampa, Florida. Particulate organic matter was composed of commercial peat humus milled to pass the 840 μm (20-mesh) screen on a Wiley mill.

After particle size analysis of the natural sediment, the sand, silts, clays and organic matter were used to formulate a sediment with similar particle size ratios and organic contents (Table 3). Natural and synthetic sediments were hydrated by mixing sediment into deionized water or effluent at the ratio of 42 ml of water or effluent per 135 g of sediment. Survival and growth of seedlings in sediments hydrated with saline solution, as described above, were also determined when tannery effluent was tested. Mixing was by spatula in a glass beaker, and the mixture was stirred until smooth and homogeneous.

After hydration, approximately 100 ml of sediment was apportioned to each of three Styrofoam[®] cups, 5.5 cm high \times 7.4 cm diameter. This yielded a system in which the sediment was overlain by approximately 5 mm of water or effluent. In tests with effluents from a textile mill, plating works and coking plant, three cups received an additional 20 ml of effluent at the surface of the sediment at 5-d intervals between planting of seedlings and harvest. Three other cups received 20 ml of deionized water. Sediment pH was measured [5] immediately before planting the seedlings. Redox potential (Eh) was measured with a Radiometer/Copenhagen PHM pH meter fitted with a platinum electrode [5], and cation exchange capacity (CEC) was measured by the ion exchange procedure [6] in natural and synthetic sediments prepared with deionized water. The Eh and CEC of natural and synthetic sediments were similar (Table 4). The pH of natural sediment (5.8) was lower than that of synthetic

Table 4. Redox potential (Eh), cation exchange capacity (CEC) and pH of natural and synthetic sediments used in toxicity tests with rooted freshwater plants

	Natural	Synthetic
Eh, mV	380	315
CEC, meq/100 g	16.6	19.0
pH	5.8	7.5

sediment (7.5) (Table 4), but, as discussed below, there were no statistically significant differences in responses to effluents in natural or synthetic sediments.

Exposure of seedlings. Seeds were soaked in 1% sodium hypochlorite for 20 min, rinsed with deionized water and set to germinate 4 d before tests were started. *E. crusgalli* was germinated in deionized water as in the seed germination and early growth tests.

Twelve seedlings were planted in each cup, and each control and treatment was done in triplicate. Seedlings were placed into holes in the sediment with their coleoptiles above the surface of the sediment. Roots remained intact during planting. Twenty milliliters of Hoagland nutrient solution [7] was added to the surface of each sediment.

Echinochloa crusgalli was grown for two weeks under the temperature and lighting conditions given above. At the end of the growth period, surviving seedlings were enumerated and collected carefully by peeling the Styrofoam cup from the sediment and washing the sediment from the roots. Remains of bracts were removed, and the seedlings of each cup were combined, dried and weighed as described above.

Statistical analyses

Data were evaluated statistically by a general linear model for analysis of variance (ANOVA). When *F* values of the ANOVA were significant ($P = 0.05$), means of control and treated groups were compared by Tukey's Studentized Range Test [8], which allowed for calculation of the lowest observed effect concentration (LOEC) ($\alpha = 0.05$). In the test with tannery effluent, effects of the effluent and saline water on growth were compared by analysis of covariance [8] to separate the effects of salinity from those of the effluent. This was possible due to the linear growth response to salt concentrations.

RESULTS

Sewage treatment plant

Germination and early growth. Undiluted sewage treatment plant waste inhibited germination and early growth of *E. crusgalli* var. *crusgalli* and var. *zelayensis* (Table 5). There was total inhibition in light through 4 d of exposure, but by day 6, germination percentages were similar in control and treated groups. Average weights of treated seedlings were significantly lower than those of controls because they germinated later. Germination was inhibited in darkness throughout the test, suggesting that the toxicant(s) was photolabile.

Survival and growth. One application of sewage treatment plant effluent to natural and synthetic sediments had no effect on survival or growth of either variety.

Textile mill

Seed germination and early growth. Textile mill effluent had no effect on germination of *E. crusgalli* var. *crusgalli* or var. *zelayensis*. Percentage germination was not significantly different in controls and effluent in light and darkness between 2 and 7 d of exposure.

The effluent inhibited seedling growth by both varieties in the light, but not in darkness (Fig. 1). The LOEC for var. *crusgalli* was 50% effluent; for var. *zelayensis* it was 75%.

Survival and growth. Textile mill effluent had no effect on survival and growth of either variety in natural or synthetic sediment.

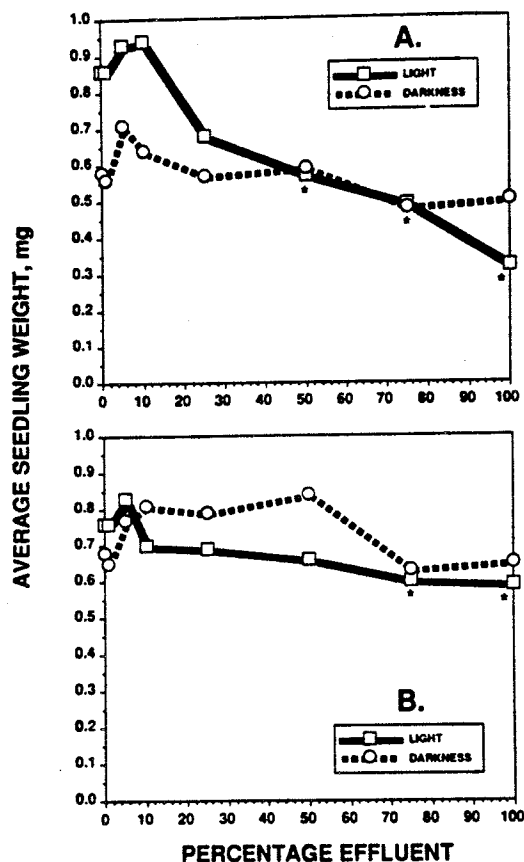


Fig. 1. Average weights of seedlings of *Echinochloa crusgalli* var. *crusgalli* (A) and var. *zelayensis* (B) exposed for 7 d to dilutions of textile mill effluent in early growth tests. ★ = significantly different from control in light; $P = 0.05$.

Table 5. Effects of undiluted sewage treatment plant effluent on germination and seedling weight of *Echinochloa crusgalli* var. *crusgalli* and var. *zelayensis* in light and darkness

Variety	Treatment	Percent seed germination (days of exposure)				Seedling wt. ^a (mg)
		2 d	4 d	6 d	7 d	
<i>crusgalli</i>	control, light	86.1	86.1	86.1	86.1	0.8
	effluent, light	0 ^b	0 ^b	75.0	83.3	0.2 ^b
	control, darkness	80.6	94.4	94.4	94.4	0.5
	effluent, darkness	0 ^b	0 ^b	0 ^b	8.3 ^b	<0.1 ^b
<i>zelayensis</i>	control, light	94.4	94.4	94.4	94.4	0.9
	effluent, light	0 ^b	0 ^b	86.1	91.7	0.3 ^b
	control, darkness	83.3	94.4	97.2	97.2	0.6
	effluent, darkness	0 ^b	0 ^b	8.3 ^b	11.1 ^b	<0.1 ^b

Thirty-six seedlings were used in each test.

^aAt Day 7.

^bStatistically different from control ($P = 0.05$) of same treatment.

Pulp and paper mill

Seed germination and early growth. Effluent from a pulp and paper mill had no effect on germination of *E. crusgalli* var. *crusgalli* and var. *zelayensis* in light. The test was not done in darkness. The effluent did inhibit early growth of seedlings (Fig. 2), and the LOECs were 75% effluent for both varieties.

Survival and growth. One treatment of sediments with undiluted effluent did not affect survival and growth of either species.

Metals plating works

Germination and early growth. Metals plating works effluent did not affect the germination rate of *E. crusgalli* var. *crusgalli*, but it did inhibit germination of var. *zelayensis* (Table 6). The LOEC increased between 2 and 7 d of exposure. In light, the LOEC on days 2 and 3 was 50% effluent, on days 4 and 5 it was 100% and there was no effect on days 6 and 7. Toxicity to germination was slightly greater in darkness: The LOEC rose from 25% on day 2 to 75% on day 7.

The effluent also caused significant reduction in weight of seedlings of both varieties in light and darkness (Fig. 3). The LOEC for each was 50% effluent, except for var. *zelayensis* in darkness, during which it was 75%.

Survival and growth. Metals plating works effluent had no effect on survival of seedlings in natural and synthetic sediments. Seedling weight was

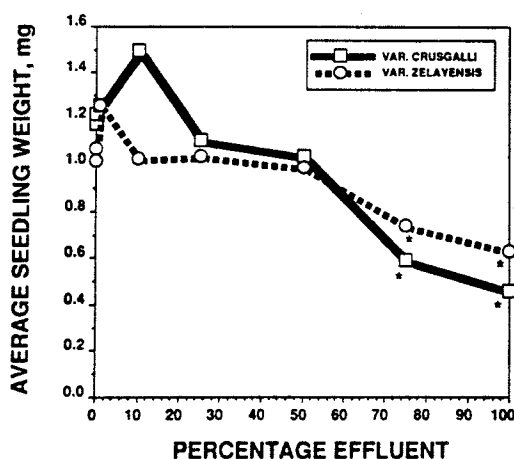


Fig. 2. Average weights of seedlings of *Echinochloa crusgalli* var. *crusgalli* and var. *zelayensis* exposed to pulp and paper mill effluent in light in early growth tests. ★ = significantly different from control, $P = 0.05$.

Table 6. Percentage of *Echinochloa crusgalli* var. *zelayensis* seedlings germinated in metals plating works effluent in light and darkness

Dilution	Percentage germination (days of exposure)					
	2 d	3 d	4 d	5 d	6 d	7 d
In light						
Control	44.4	80.6	86.1	86.1	86.1	86.1
1	66.7	91.7	97.2	97.2	97.2	97.2
5	69.4	80.6	86.1	86.1	86.1	88.9
10	61.1	83.3	86.1	88.9	91.7	94.4
25	38.9	69.4	75.0	77.8	77.8	77.8
50	13.9 ^a	50.0 ^a	69.4	91.7	91.7	91.7
75	0 ^a	33.3 ^a	63.9	83.3	88.9	94.4
100	0 ^a	19.4 ^a	25.0 ^a	55.6 ^a	80.6	83.3
In darkness						
Control	16.7	41.7	52.8	52.8	55.6	55.6
1	47.2	63.9	69.4	75.0	75.0	75.0
5	22.2	41.7	52.8	55.6	61.1	61.1
10	38.9	50.0	61.1	66.7	66.7	66.7
25	11.1 ^a	19.4 ^a	25.0 ^a	41.7	44.4	44.4
50	0 ^a	25.0 ^a	25.0 ^a	27.8 ^a	30.6	30.6
75	0 ^a	8.3 ^a	11.1 ^a	16.6 ^a	19.4 ^a	22.2 ^a
100	0 ^a	0.1 ^a	13.9 ^a	16.6 ^a	22.2 ^a	25.0 ^a

Thirty-six seeds were exposed in each control and treatment.

^aSignificantly less than control, $P = 0.05$.

reduced significantly by the first and third treatments with undiluted effluent (Table 7).

Coking plant

Germination and early growth. Coking plant effluent inhibited germination of both varieties of *E. crusgalli*, and its effect was greater in var. *zelayensis* (Table 8). The effluent LOECs after 2 d of exposure for var. *crusgalli* were 75% effluent (light) and 50% (darkness). For var. *zelayensis* they were 5% (light) and 10% (darkness). The LOECs became greater with time of exposure; by day 7 they were 100% effluent in light and darkness for var. *crusgalli* and 10% (light) and 50% (darkness) for var. *zelayensis*.

Early growth of seedlings in light and darkness also was inhibited by coking plant effluent, and growth of both varieties was inhibited more strongly in light than it was in darkness (Fig. 4). The LOEC concentrations were considerably lower for weight than they were for germination after 7 d of exposure, at which time they were 5% effluent (light) and 5% (darkness) for var. *crusgalli* and 1% (light) and 5% (darkness) for var. *zelayensis*. Growth of var. *zelayensis* was inhibited completely by 75% effluent.

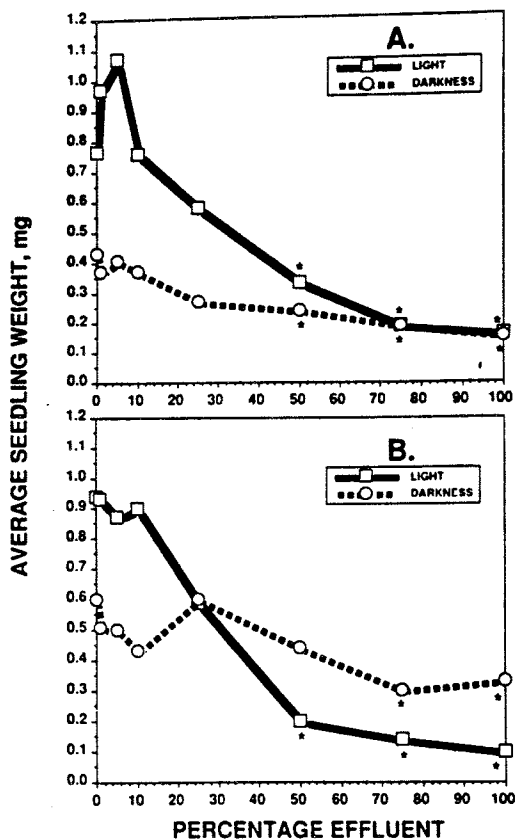


Fig. 3. Average weights of seedlings of *Echinochloa crusgalli* var. *crusgalli* (A) and var. *zelayensis* (B) exposed for 7 d to dilutions of plating works effluent in early growth tests. ★ = significantly different from control, $P = 0.05$.

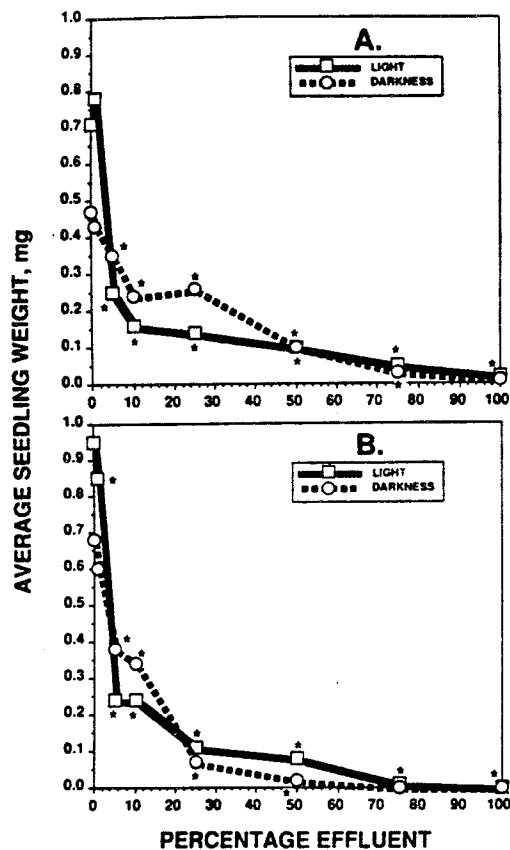


Fig. 4. Average weights of seedlings of *Echinochloa crusgalli* var. *crusgalli* (A) and var. *zelayensis* (B) exposed for 7 d to dilutions of coking plant effluent in early growth tests. ★ = significantly different from control, $P = 0.05$.

Effects of the effluent were greater in light than they were in darkness, suggesting the presence of a nonlabile inhibitor of photosynthesis. It is likely that inhibition of photosynthesis was caused by inhibition of chlorophyll synthesis. Plants in all of the exposure concentrations were white and without any visual trace of green pigmentation.

Survival and growth. The first and third treatments of natural and synthetic sediments, respectively, with undiluted effluent had no effect on survival of either variety. They did, however, inhibit growth significantly (Table 7).

Tannery

Germination and early growth. There were no effects of undiluted tannery effluent and effluent diluted with 8‰ salinity water or water of 8‰ salinity on germination. There was an inverse relationship between concentration of effluent or salt

and average seedling weight (Fig. 5). The LOECs were 75% effluent (equal to 6‰ salinity), diluted with deionized water, and 4‰ salinity water. Because 8‰ salinity was toxic, all seedlings exposed to effluent diluted with 8‰ salinity water were significantly lower in weight than those in the deionized water control.

Survival and growth. Tannery effluent and saline water did not inhibit survival in synthetic sediment, but growth of var. *crusgalli* was inhibited (Table 7, Fig. 6). Variety *zelayensis* was not tested. When effluent was diluted with water of 8‰ salinity, seedling weights were depressed strongly at all waste concentrations (Fig. 6). The LOEC for effluent diluted with deionized water was 50% (equal to 4‰ salinity); the LOEC for saline water was 4‰. Those data indicate that salt was the major toxic factor for inhibition of growth by tannery effluent. However, analysis of covariance of effluent and

Table 7. Average weights and percentage inhibition of growth of *Echinochloa crusgalli* var. *crusgalli* and var. *zelayensis* exposed to one and three treatments with industrial effluents in natural and synthetic sediments

Effluent	Variety	Sediment	Treatments	Avg. wt. (mg)	Inhibition (%)
Plating	<i>crusgalli</i>	natural	0 (control)	9.0	—
			1	5.9 ^a	34.4 ^a
			3	6.0 ^a	33.3 ^a
		synthetic	0 (control)	16.1	—
			1	11.3 ^a	29.8 ^a
			3	6.1 ^a	62.1 ^a
	<i>zelayensis</i>	natural	0 (control)	8.6	—
			1	5.6 ^a	34.9 ^a
			3	7.7	11.1
		synthetic	0 (control)	9.9	—
			1	7.0 ^a	29.3 ^a
			3	7.0 ^a	29.3 ^a
Coke	<i>crusgalli</i>	natural	0 (control)	10.2	—
			1	3.8 ^a	62.7 ^a
			3	3.6 ^a	64.7 ^a
		synthetic	0 (control)	15.8	—
			1	7.7 ^a	51.3 ^a
			3	6.0 ^a	62.0 ^a
	<i>zelayensis</i>	natural	0 (control)	9.1	—
			1	4.4 ^a	51.5 ^a
			3	4.2 ^a	53.8 ^a
		synthetic	0 (control)	12.3	—
			1	10.0 ^a	18.7 ^a
			3	7.8 ^a	36.6 ^a
Tannery	<i>crusgalli</i>	synthetic	0 (control)	19.0	—
			1	3.0 ^a	84.2 ^a

^aStatistically significant inhibition compared to control ($P = 0.05$) of same sediment.

salt concentration demonstrated that depression of average seedling weight was greater in diluted effluent than it was in saline water of comparable salinity at effluent concentrations of 50% (4‰ salinity) and above. This indicates that another, unidentified toxic factor was present in the effluent. The factor was not pH, which did not affect response to effluent or salinity (Fig. 6).

DISCUSSION

Rooted marsh plants are shown here to be useful experimental organisms for detection of toxicity of effluents and herbicides in water and sediments. They are responsive in a variety of ways and can be tested in natural sediments and artificial sediments of any desired formulation. Toxic end points, such as germination, rate of early growth in light and darkness, survival and later growth, are easily measured [9]. Germination and early growth studies can be conducted in a small volume of water and can thus be used to test toxicity of sediment pore water. Germination and

growth are separate physiological events; tests in light and darkness allow identification of effects on imbibition of water and cell elongation (germination) and mobilization of nutrients and photosynthesis (growth). In addition, photolabile toxicants can be identified by such tests.

The tests described below demonstrate responses of marsh plants to toxicants in water and sediments:

Sewage treatment plant effluent inhibited germination of seeds, but the toxic factor was photolabile and the effect was lost after 4 d of exposure in light.

Textile and pulp and paper mill effluents had no effect on germination. They inhibited early seedling growth in light but not in darkness.

Metals plating works effluent inhibited germination of one plant variety and early growth of both varieties in light and darkness. It also reduced growth rates of both varieties in natural and synthetic sediments.

Coking plant effluent inhibited both germination and early growth, with greater effects in light

Table 8. Number of *Echinochloa crusgalli* var. *crusgalli* and var. *zelayensis* seeds germinated in coking plant effluent in light and darkness

% Effluent	No. germinated in light (days of exposure)						No. germinated in darkness (days of exposure)					
	2	3	4	5	6	7	2	3	4	5	6	7
<i>crusgalli</i>												
Control	32	32	32	32	32	32	36	36	36	36	36	36
1	35	35	35	35	35	35	32	33	33	33	33	33
5	29	29	29	29	29	32	32	33	33	33	33	33
10	29	31	31	31	32	32	29	30	31	32	32	32
25	24	30	30	30	31	31	25	26	27	27	27	27
50	30	32	32	32	32	33	14 ^a	18 ^a	23	23	24	25
75	11 ^a	26	27	28	28	28	4 ^a	10 ^a	15 ^a	20	20	20
100	3 ^a	10 ^a	15 ^a	19 ^a	19 ^a	19 ^a	0 ^a	2 ^a	7 ^a	12 ^a	12 ^a	13 ^a
<i>zelayensis</i>												
Control	33	36	36	36	36	36	9	11	12	17	17	17
1	30	32	34	35	35	35	10	13	15	15	15	15
5	14 ^a	25	31	33	33	33	6	14	19	19	20	21
10	9 ^a	18 ^a	21 ^a	25 ^a	25 ^a	25 ^a	0 ^a	3 ^a	3 ^a	7 ^a	8	9
25	5 ^a	10 ^a	18 ^a	31	34	35	0 ^a	2 ^a	2 ^a	3 ^a	5 ^a	8
50	0 ^a	2 ^a	7 ^a	18 ^a	18 ^a	22 ^a	0 ^a	0 ^a	1 ^a	1 ^a	1 ^a	3 ^a
75	0 ^a	1 ^a	2 ^a	7 ^a	7 ^a	8 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a
100	0 ^a	0 ^a	0 ^a	2 ^a	2 ^a	2 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a

Thirty-six seeds were used in each control and effluent concentration.

^aSignificantly less than control, $P = 0.05$.

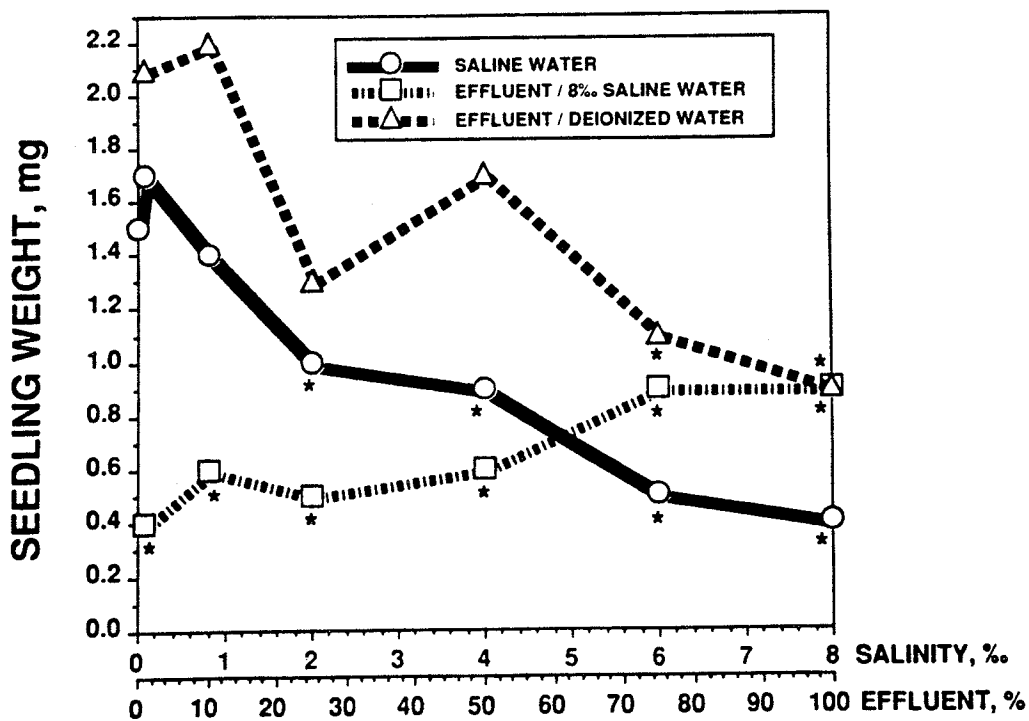


Fig. 5. Average weights of *Echinochloa crusgalli* var. *crusgalli* seedlings grown in saline water, tannery effluent diluted with 8‰ saline water and tannery effluent diluted with deionized water. ★ = significantly different from control, $P = 0.05$.

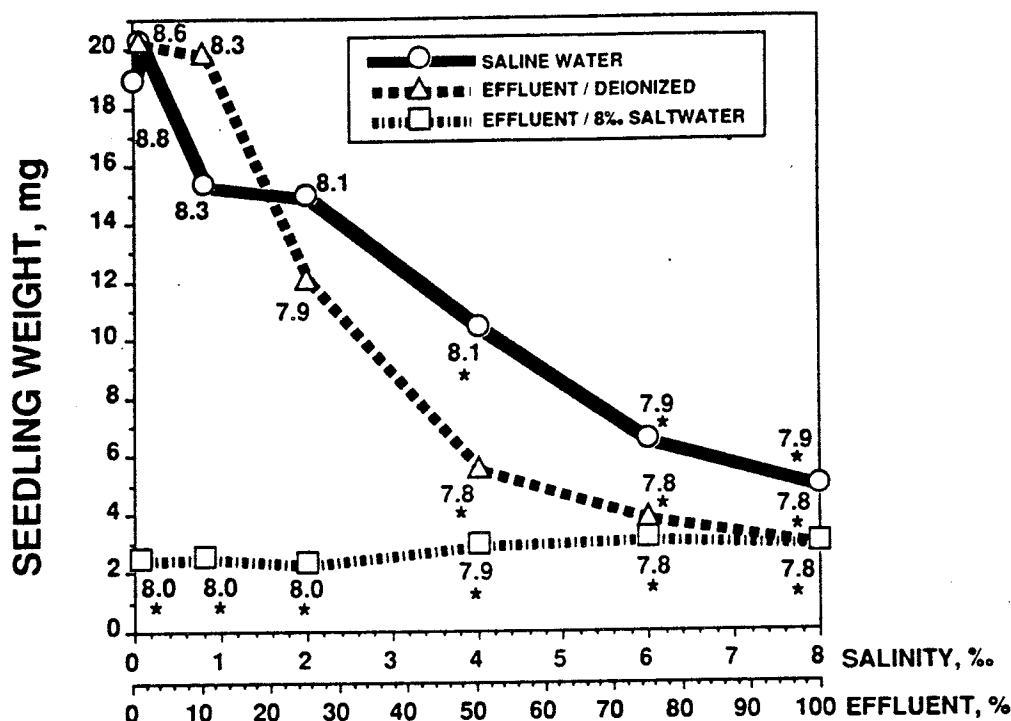


Fig. 6. Average weights of *Echinochloa crusgalli* var. *crusgalli* seedlings exposed to saline water, tannery effluent diluted with 8‰ saline water and tannery effluent diluted with deionized water in synthetic sediment. Number at each point = pH of sediment. ★ = significantly different from control, $P = 0.05$.

because it inhibited chlorophyll synthesis. It also inhibited growth in natural and synthetic sediments.

Tannery effluent inhibited early and later seedling growth.

CONCLUSIONS

Germination, survival and growth of freshwater marsh plants were inhibited by effluents in standardized tests with water and sediment. The tests described here provide reliable toxicity data for estimation of potential effects of effluents in marshes and can be used for regulation of effluent discharges to natural systems.

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APPENDIX 2

USE OF MARSH PLANTS FOR TOXICITY

TESTING OF WATER AND SEDIMENT

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Use of Marsh Plants for Toxicity Testing of Water and Sediment²

ABSTRACT: The freshwater wetland plants, *Echinochloa crusgalli crusgalli* and *Echinochloa crusgalli zelayensis*, and the saltmarsh plant, *Spartina alterniflora*, were exposed to the herbicides, metolachlor and norflurazon, in two types of toxicity tests: seed germination and early seedling growth in water, and seedling survival and growth in natural and artificial sediments. The artificial sediments were formulated to simulate the natural sediments with regard to particle size distribution and organic content. The herbicides did not affect rate of germination, but significantly inhibited rate of early growth, and survival and rate of growth of older seedling in sediments. *Echinochloa* was more sensitive than *Spartina* to both herbicides. Inhibition of the growth rates of the two varieties of *E. crusgalli* was similar in natural and simulated sediments, but inhibition of growth of *S. alterniflora* was greater in simulated than in natural sediment. It is concluded that the

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species tested may be used for estimation of potential effects of toxicants on wetland plants and that simulated sediments of known composition may be used in sediment toxicity tests.

KEYWORDS: wetland plants, Echinochloa crusgalli, Spartina alterniflora, metolachlor, norflurazon, germination, survival, growth, natural sediment, simulated sediment

Freshwater and estuarine wetlands serve as sinks for waterborne pollutants [1]. These pollutants may be in the dissolved state, adsorbed to suspended particles [2], or bound to dissolved organic matter [3], but the ultimate locations of many pollutants are in interstitial water or on particles of wetland sediments [4]. Whether dissolved or adsorbed to particulates, toxic substances in sediments can be taken up by the roots of wetland plants and translocated to aerial organs, where they may inhibit growth, injure foliage or kill the plants [5]. Also, wetland plants generally produce numerous seeds which germinate at the sediment-water interface, where they may be exposed to toxic substances in water.

Rooted plants are the dominant life forms that control the physical, chemical, and biological characteristics of wetland ecosystems. They are the major primary producers and sources of detritus, their roots and rhizomes stabilize sediment, and they provide food and habitat for animals. Chemical hazard to rooted wetland plants also constitutes a threat to their ecosystems. Such hazards could occur through effects of toxic substances on seed germination, seedling growth, and survival.

Few studies describe effects of toxic substances in sediments on wetland plants [6], and we are not familiar with methods devoted specifically to development of artificial sediments for toxicity testing with such plants. The research reported here was designed to (1) develop methods for exposure of freshwater and estuarine marsh plants to toxicants in water and sediment, (2) identify marsh plant species that can be used in toxicity tests, and (3) conduct toxicity tests in natural and artificial sediments with substances known to be toxic to plants.

Use of artificial sediments was deemed critical to evaluation of effects of toxicants in sediments. In early studies, we found natural sediments to be unacceptable for toxicity tests because while wet and without amendment, pH decreased with time and weed seeds germinated. When dried in air, pHs of reconstituted natural sediments were as low as 2, and weed seeds continued to germinate. Moreover, structure of natural sediments could not be varied experimentally, they contained unknown quantities of nutrients and, perhaps, toxicants. We required sediments whose properties could be controlled to simulate the variety of sediments found in nature. Artificial sediments that we formulated varied in grain size distribution and organic content. Their characteristics and formulation methods are reported here.

The methods for toxicity testing of plants and sediments consisted of (1) a germination and early growth test in water, and (2) a seedling survival and growth test in natural and artificial sediments. Although the natural sediments were altered by drying, they were used as a standard to which tests with artificial sediments were compared. Two varieties of a freshwater marsh species and one species of estuarine plant were tested with two herbicides.

Effects of the herbicides in water, natural, and artificial sediments are reported and the value of the tests is discussed.

Materials and Methods

Plant Species

Freshwater - The common freshwater wetland plant, *Echinochloa crusgalli* (Linnaeus) Palisot de Beavois, (Gramineae) was used. Two varieties, *crusgalli* and *zelayensis*, were obtained as seed from Wildlife Nurseries, Oshkosh, Wis., and stored dry at approximately 4°C. The varietal names were confirmed by growing plants to seed, with identification according to Correll and Correll [7].

Estuarine - *Spartina alterniflora* Loisel (Gramineae) seeds were obtained from Environmental Concern, St. Michaels, Md. Upon receipt, the dry seeds were placed in natural seawater diluted with deionized water to 4 parts per thousand (ppt) salinity and stored at 4°C. Identity was confirmed from the description given by Hotchkiss [8].

Toxicity Tests

Germination and Early Growth - Seed germination and early growth tests were performed in 47-mm clear polystyrene Petri dishes with tightly capped lids (Millipore Corp., Bedford, MA). Seeds were surface-sterilized by immersion in 1% sodium hypochlorite for 20 min and rinsed in deionized water. Twelve seeds of *Echinochloa* were placed in 10 ml of deionized water (freshwater control) and up to 7 concentrations of herbicide in Petri dishes. Each control and

exposure concentration was prepared in triplicate. Thus, 36 seeds were exposed in the control and each treatment. Tests with *Echinochloa* were conducted for 7 days under cool white fluorescent lights at approximately $35 \mu\text{E}/\text{m}^2/\text{sec}$ with a diel cycle of 16 h light: 8 h darkness. With *Spartina*, eight surface-sterilized seeds were placed in 10 ml of 4 ppt diluted seawater (seawater control) and up to 7 dilutions of herbicide. Controls and exposure concentrations were prepared in triplicate, so that 24 seeds of *Spartina* were used in each. The tests were conducted for 10 d under cool white fluorescent lights at approximately $35 \mu\text{E}/\text{m}^2/\text{sec}$ and a temperature regime of 16 h at $18 \pm 1^\circ\text{C}$ and 8 h at $35 \pm 1^\circ\text{C}$ [9]. Germinated seedlings were enumerated each day. At the end of the exposure, the roots and stems were cut from the caryopses and the plant material from each Petri dish was combined and dried for 24 h at 103°C and weighed to the nearest 0.1 mg on a Mettler Model AE 103 balance.

Seedling Survival and Growth - Survival and growth tests were conducted in natural and artificial sediments. Natural freshwater sediment was collected from a marsh near Milton, Fla., and saltmarsh sediment was collected from a marsh near Pensacola, Fla. Leaves, twigs, and other large particles were removed and the sediments dried in air at room temperature. Particle size distributions were determined by dry sieving and by settling rate in water [10]. Organic content was determined by ashing at 550°C for 24 h. Artificial sediments were formulated to simulate the physical properties of the natural sediments (Table 1).

Simulated sediments were formulated from washed quartz sand, silt, clay, and organic matter. Fine, medium, and coarse sands (New England Silica, Inc., South Windsor, Conn.) were sieved to obtain the proper grain size for each

simulated sediment. Silts (average particle sizes 4.8 and 1.8 μm) and clays (average particle sizes 0.1 and 2.0 μm) were produced by Englehard Corp., Edison, N.J. Particulate organic matter was air-dried commercial peat humus (Greenleaf Products, Inc., Haines, Fla.) milled to an average particle size of 840 μm on a Wiley Mill.

The dry components of simulated sediments, mixed in the desired proportions, and air dried natural sediments were reconstituted for survival and growth studies by mixing with either deionized water or 4 ppt diluted seawater at the ratio of 42 ml water: 135 g sediment. Treated sediments were prepared with water that contained dissolved herbicide. Sediments were mixed with a spatula in a glass beaker until smooth and homogeneous. Approximately 100 ml of wet sediment were added to each of three styrofoam cups, 5.5 cm high x 7.5 cm diam. Sediment pH was determined by addition of 100 g sediment to 100 ml deionized water in a glass beaker. The mixture was stirred for one min and allowed to settle for one h, at which time pH was determined with a Beckman Phi 12 pH/ISE meter. Cation exchange capacity (CEC) was determined by the ion-exchange analysis procedure [12].

Young seedlings were used in the survival and growth tests. Seeds were surface-sterilized in 1% sodium hypochlorite and set to germinate 4 d (*Echinochloa*) or 10 d (*Spartina*) before tests were to begin. *Echinochloa* seeds were germinated in deionized water at $24 \pm 1^\circ\text{C}$ under cool white fluorescent lights at approximately $35 \mu\text{E}/\text{m}^2/\text{sec}$ and a 16 h light: 8 h darkness cycle. *Spartina* was germinated in 4 ppt diluted seawater in a temperature regime of 16 h at $18 \pm 1^\circ\text{C}$ and 8 h at $35 \pm 1^\circ\text{C}$ with 16 h light and 8 darkness.

Twelve seedlings were planted in each cup and triplicate cups were prepared for each control and herbicide concentration was done in triplicate. Seedlings were planted in holes in sediment without damage to roots and with coleoptiles above the sediment. *Echinochloa* was grown for two weeks and *Spartina* for four weeks at $24 \pm 1^\circ\text{C}$ under cool white fluorescent lights at approximately $35 \mu\text{E}/\text{m}^2/\text{sec}$ on a 16 h light: 8 h darkness cycle. Twenty ml of Hoagland solution [11] were added to *Echinochloa* immediately after planting and on the 4th and 12th d, and to *Spartina* immediately after planting and on the 4th, 12th, and 20th d. At the end of the growth period, surviving seeds were enumerated and collected carefully by peeling the styrofoam cup from the sediment and washing sediment from the roots with deionized water. Shoots and roots were cut from the caryopses and the plant material of each cup was dried at 103°C for 24 h and weighed to the nearest 0.1 mg.

Preparation of Herbicide Solutions

The herbicides, metolachlor (2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl) acetamide, 98% pure, from Ciba-Geigy Corp., Greensboro, N.C.) and norflurazon (4-chloro-5-methylamino-2-(3-trifluoromethylphenyl)-pyridazin-3 (2H) one, > 98% pure, Sandoz, Inc., Homestead, Fla.) were used without carrier. In seed germination and early growth tests, the highest concentration to be used was dissolved in deionized water or 4 ppt diluted seawater and diluted as needed. In seedling survival and growth tests, saturated solutions were prepared and diluted as needed for the desired concentration in sediment.

Purity of the herbicides and all concentrations in water were confirmed by gas chromatography. Concentrations in sediments were not confirmed because percentage recovery was very low. Samples of freshwater or diluted seawater containing herbicides were extracted with solvent or mixtures of solvents and analyzed by gas chromatographs equipped with packed columns and either electron-capture or nitrogen phosphorus detectors. Average recovery of these compounds spiked into freshwater and seawater to validate analyses of test water was greater than 85% for all compounds. Depending on concentrations and sensitivity of the detector, sizes of samples extracted with solvent ranged from 2 ml to 20 ml. The types and amounts of solvents were different for each compound and were as follows: metolachlor (hexane, 2-10 ml); norflurazon (40% ethyl acetate/60% hexane [v/v] 2-20 ml).

Statistical Analyses

Two statistical procedures were used for analysis of germination, survival, and weight data. The mean number of germinated seeds or surviving seedlings, and the average weights of seedlings per dish or cup were calculated. Comparisons of the means in each test, the lowest observed effect concentration (LOEC) and differences between each treatment within each test were computed with Tukey's Studentized Range Test [13]. Comparisons of responses in natural vs simulated sediments were made by two-way analysis of variance (ANOVA) [13], $\alpha = 0.05$.

Results and Discussion

ph and Cation Exchange Capacity

The ph of dried natural sediments was low (Table 2). Simulated sediments were slightly basic and had no added sulfur. It is probable that oxidation of sulfide in the wetland sediments contributed to the low pHs. Herbicidal activities at the pHs of the natural sediments are discussed below.

Cation exchange capacities of natural and simulated sediments were similar (Table 2).

Metolachlor

Seed Germination and Early Growth - Metolachlor did not affect the germination rate of either species. It did, however, suppress growth of both varieties of *E. crusgalli* and of *S. alterniflora* (Fig. 1, Table 3). The LOEC for metolachlor and seedling weights with *E. crusgalli crusgalli* and *E. crusgalli zelayensis* was 0.25 mg/L; for *S. alterniflora*, it was 0.5 mg/L. In each case, concentrations at and greater than the LOEC were not statistically significantly different from each other. The highest concentration in tests with *Echinochloa* was 20 times greater than the LOEC; with *Spartina*, the highest concentration was four times greater than the LOEC. This phenomenon, in which increasing concentrations of herbicide did not reduce final seedling weight, occurred in all tests with metolachlor and norflurazon. Metolachlor is a chloroacetamide preemergence herbicide that inhibits protein [14] and lipid [15] synthesis, but does not inhibit seed germination. It is suggested that, in these tests, the seeds germinated and the seedlings grew by utilization of stored nutrient reserves, imbibition of water, and cell elongation, none of which was affected by metolachlor. However, when

photoautrophic growth began, protein synthesis was inhibited and seedling weight gain was arrested at that point. Thus, average seedling weights were the same in all concentrations at and above the LOEC.

Seedling Survival and Growth - Metolachlor inhibited survival of *E. crusgalli crusgalli* and *S. alterniflora* in natural and simulated sediments but did not affect survival of *E. crusgalli zelayensis* (Fig. 2). The LOEC for survival in metolachlor was 0.5 mg/kg in natural and simulated sediments with *E. crusgalli crusgalli*; it was 2.5 mg/kg in simulated sediment and 7.5 mg/kg in natural sediment with *S. alterniflora*.

Metolachlor in sediment significantly inhibited growth of seedlings (Fig. 3, Table 3). However, effect of the herbicide with *S. alterniflora* was significantly greater in simulated than in natural sediment. Metolachlor is stable even at pH 1 [15], but is degraded rapidly in aerobic natural soil [15]. It is possible that the microbial flora of the natural saltmarsh sediment contributed to degradation of metolachlor over the 28-d exposure.

Metolachlor is applied to crops as a preemergence herbicide for control of broadleaf and grassy weeds. It is stable in loamy soil for over 64 d [15] and has been detected in surface and groundwaters in the United States [15]. The data suggest that metolachlor could affect germination, survival, and growth of marsh plants when present in water or sediment.

Norflurazon

Seed Germination and Early Growth - Norflurazon did not affect the rate of germination of the species tested. It did reduce the rate of early growth of the two freshwater species (Fig. 4), but the highest concentration, 1 mg/L,

did not affect early growth of *Spartina* (Table 3). The LOEC for norflurazon and *Echinochloa* was 0.05 mg/L. As with metolachlor, average weights of seedlings exposed to norflurazon concentration at and about the LOEC were similar.

Norflurazon is a phenylpyridozone herbicide that inhibits carotenoid synthesis [16], and because carotenoids protect chlorophyll from degradation by light, norflurazon treatment results in bleached seedlings (Fig. 5). Autotrophic growth of treated seedlings was arrested after initial growth by stored nutrient mobilization, imbibition of water and cell elongation at the LOEC concentration and above, resulting in similar weights.

Seedling Survival and Growth - Norflurazon reduced survival of *E. crusgalli crusgalli* in natural and simulated sediments and of *E. crusgalli zelayensis* in natural sediments (Fig. 6). It did not affect survival of *E. crusgalli zelayensis* in simulated sediment or *S. alterniflora* in either sediment.

The LOEC for growth for norflurazon and *Echinochloa* in both sediments and for *Spartina* in simulated sediment was 0.25 mg/kg (Fig. 7, Table 3). As for metolachlor, effect of norflurazon on average seedling weight of *Spartina* was significantly greater in simulated sediment. Norflurazon is stable under acid conditions [17] but susceptible to degradation by bacteria [18]. As for metolachlor, it is possible that the bacterial flora in the natural saltmarsh soil caused degradation of norflurazon in these tests.

Significance of the Research

There are, at present, no tests that address the problem of effects of contaminated water or sediment on wetland plants. Current toxicity tests with

plants utilize commercial species [19, 20], germination on filter paper [21], or growth substrata that do not simulate natural soils [22,23]. The approach reported here demonstrates that acute exposure of seeds to toxicants in water may inhibit germination and early growth of wetland plants and that chronic exposure of seedlings to toxicants in artificial sediments that are similar to natural sediments may cause death or inhibit growth.

Choice of Test Species

The U.S. Environmental Protection Agency [24] described desirable attributes of organisms for use in toxicity tests with benthic species. The attributes include ecological relevance, variety of endpoints (acute and chronic), all potential routes of exposure should be possible, there should be an adequate amount of tissue for analysis, and ease of organism culture and handling. Also, a plant test species should grow normally in sediments of disparate composition because natural sediments vary widely in composition. The three plants described here satisfy all of these requirements.

Echinochloa is a widely distributed wetland genus found in North and South America, Europe, Africa, Asia, Australia and the Pacific islands [25]; *Spartina* is often the dominant genus in many marshes of the Atlantic and Gulf Coasts of the United States, and one species, *pectinata*, is found in freshwater of the Northern United States [26]. Acute endpoints are used in the short-term germination and early growth tests and chronic endpoints are used in the survival and seedling growth test. Both tests provide ample tissue for analysis of uptake from water and sediment. Seeds, readily available from suppliers, can be stored in a refrigerator and have a germination rate of approximately 90% [27] and both species grow well in

sediments of diverse composition [27]. There is also a large literature on the biology of both species. *Echinochloa crusgalli crusgalli* and *E. crusgalli zelayensis* were shown to be sensitive to industrial and municipal effluents [28]. The effluents inhibited germination, survival, and growth, and when germination and early growth tests were conducted in light and total darkness, effects of toxicants on imbibition of water, cell elongation, utilization of stored nutrients, and photosynthesis were identified.

Choice of Sediments

Composition and structure of sediments are probably the most important factors in substratum toxicity [29], and laboratory use of substances that are not similar to those in which the plant naturally grows may not provide data applicable to field conditions [30, 31]. Grain size [31] and organic content [32, 33, 34] strongly influence the process of equilibrium partitioning of toxicants between sediment particles and pore water. Although natural sediments may be amended in some cases [35], they are often unsuitable for use in toxicity tests because they cannot be duplicated and data from toxicity tests with them must be normalized [30].

Standard sediments are needed for toxicity studies with plants. The standard sediment should be representative of a variety of natural sediments with regard to particle and pore sizes, chemical composition (e.g. quartz vs calcareous), organic content, and nutrient content. Bradshaw [36] gave the qualities of soil required for good plant growth: productive growth, response to fertilizers, good drainage, good water retention, and free of weeds. Others have described the principles of managing man-made soils [37] and procedures for assessment of substances suitable for growing plants [38].

Artificial sediments can be formulated to satisfy the above requirements for plant growth [27]. This report demonstrates that wetland plants responded to herbicides in artificial sediments. In all cases, average seedling weights in simulated sediments were equal to or greater than those in natural sediments. This indicates that the artificial sediments were good growth media and do not contain factors that may inhibit plant growth or confound interpretation of toxicity data.

Conclusions

The wetland plants, *E. crusgalli crusgalli*, *E. crusgalli zelayensis*, and *A. alterniflora*, may be used for evaluation of toxicity of herbicides in water and sediment. Acute (7-d) tests detect effects on early seedling growth; chronic (14- or 28-d) tests detect effects on survival and growth of older seedlings in sediment. Artificial sediments that simulate natural sediments are of value in plant toxicity tests because they support productive growth and allow for assessment of toxic responses. Furthermore, their compositions may be held constant from test to test or may be varied in relation to experimental requirements.

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TABLE 1 - Composition of natural and simulated freshwater and saltmarsh sediments.

Class	Particle Size μm	% Composition, by weight	
		Freshwater	Saltmarsh
Coarse sand	500 - 1500	0.6	33.6
Medium sand	250 - 499	9.5	58.8
Fine sand	63 - 249	67.4	4.9
Silt	4 - 62	10.3	0.6
Clay	< 4	6.7	0.7
Organic	-	4.9	0.8
Lost during analysis	-	0.6	0.6