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Bioremediation of Tributyltin Contaminated Sediment using Spartina alterniflora in a Created Tidal Wetland

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BIOREMEDIATION OF TRIBUTYL TIN CONTAMINATED SEDIMENT USING
SPARTINA ALTERNIFLORA IN A CREATED TIDAL WETLAND

A Thesis

Presented to

The Faculty of the School of Marine Science
The College of William and Mary in Virginia

In Partial Fulfillment

Of the Requirements for the Degree of
Master of Science

by

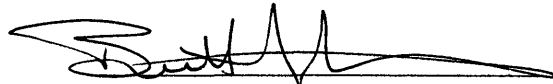
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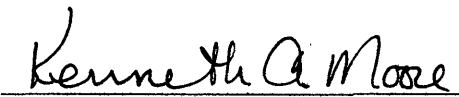
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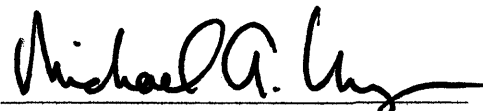
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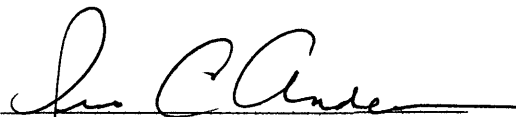
Master of Science


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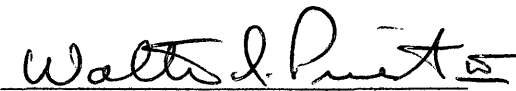

Walter I. Priest, III

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ABSTRACT

Tributyltin (TBT), the active ingredient in many marine antifouling paints, has been strictly regulated over the last decade because of its toxicity to many non target species. Regulatory actions have reduced TBT concentrations in the water column, however TBT has accumulated in the sediment where degradation is very slow with a half life on the order of years. Dredging has the potential to re-expose sediment historically contaminated with high concentrations of TBT. These dredge spoils then require some form of remediative treatment. The goal of this study was to determine the feasibility of bioremediation of TBT contaminated sediment using created wetlands. This was accomplished by measuring the loss of TBT from amended sediments, its uptake by the marsh macrophyte *Spartina alterniflora*, and its potential release via plant decomposition. TBT levels up to 250 ng/g did not inhibit *S. alterniflora* growth over a sixteen month period. TBT disappearance in vegetated versus unvegetated treatments were similar with half lives of 300 and 330 days respectively. Measured DBT levels suggest that the breakdown of TBT is occurring in the wetland. Measured levels of TBT in aboveground biomass ranged from 1- 2.5 ng/g. Belowground biomass TBT levels were higher at 43 ng/g of TBT suggesting little translocation within the plant. During a fifty-three day plant degradation study, plant tissue underwent physical changes but much of the TBT was retained in the detrital material. Based on the results of this study in a relatively sandy created marsh, only 40% of the sediment TBT would be expected to remain after 16 months. Of the initial amount of TBT only 0.4% would be expected to accumulate in *S. alterniflora* biomass. While *S. alterniflora* did not expedite the degradation of TBT, using dredge spoils to create wetlands is plausible. However this should only be undertaken after further investigation into the potential for the plant biomass to become a biovector for TBT to different trophic levels.

BIOREMEDIATION OF TRIBUTYL TIN CONTAMINATED SEDIMENTS USING
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INTRODUCTION

Tributyltin

Uses and Effects

Since organotins were first described by Lowig in 1852, these compounds have been put to many uses. Organotins were first used in 1925 as the active ingredient in moth proofing agents. In the mid 1930s, organotins were employed as stabilizers first in chlorinated benzenes and biphenyls used in transformers and capacitors as heat stabilizers and then in polyvinyl chloride (PVC) copolymers for heat and light. This use has continued today in industrial and food packaging with PVCs. The benefit to the food packaging industry is that organotin stabilizers result in PVCs which are clear, allowing the consumer to examine the food product (Bennett, 1996; Champ & Seligman, 1996). In 1954 organotins were even introduced in the medical world as treatment for staphylococcal infections. This was quickly discontinued when it resulted in the deaths of 102 people. It was not until 1961 that organotins were first recognized as efficient, effective active ingredients for antifouling paints. Throughout the 1970s and 80s their use in this manner became widespread (Champ & Seligman, 1996). Other uses of organotins include wood preservation, fungicides in the textile industry, biocides in the agricultural industry, insecticides, molluscides, stone preservatives, and as disinfectants (Bennett, 1996). As illustrated by their prolific and diverse usage, organotins are broad spectrum toxins, and their use and production has escalated to more than 50,000 tons annually worldwide (Bennett, 1996).

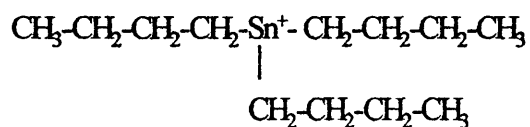
Of most concern to the study presented here is the use of the tributyltin (TBT) cation

in marine antifouling paints. Preventing the fouling of vessel hulls has been a goal of the shipping industry since its beginning. Fouling by seaweeds, algae, barnacles, worms, bryozoans, and other colonizing marine animals on a ship's hull causes increased drag and as a result, increased fuel consumption and decreased maneuverability. Copper based antifouling paints had been utilized before organotin based paints were introduced to the market. It became apparent that TBT containing paints were more effective at preventing fouling. In one US Navy study, copper based antifouling systems worked for less than a year while TBT paints were effective for 5-7 years (Champ & Pugh, 1987; Champ & Seligman, 1996). Both by extending the periods between drydocking and repainting and by decreasing fuel consumption, the use of TBT was estimated to save the world fleet \$US3 billion annually (de Mora, 1996; Rouhi, 1998). An indirect benefit of using TBT based paints for the environment is reduction in the emissions of greenhouse gases due to reduced fuel usage. The paints also prevent the spread of non-native, invasive species because they keep the hulls of ships free of fouling organisms (Evans & Nicholson, 1999; Evans, 1992).

There are two types of TBT based antifouling paints, free association paints and self-polishing copolymers (SPCs). The free association paints include those manufactured with both soluble and insoluble matrices. Paints that employ a soluble matrix release TBT through diffusion and periodically the top layer has to be sloughed off to expose a layer still rich in TBT. The normal life of these paints is approximately 2 years. Paints utilizing an insoluble matrix initially release high levels of TBT. These release rates decrease exponentially with time and become more controlled as TBT moves through small channels in the paint matrix. Eventually these channels become plugged rendering the paint

ineffective. At that time the paint must be removed. High levels of TBT remain in the paint which is removed and then disposal of the contaminated waste becomes an issue. The latest paints are the self polishing copolymers (SPCs) which use TBT incorporated into a methylmet-*n*-acrylate matrix (TBTM). A gradual, constant release is attained through the slow alkaline hydrolysis of the matrix surface. This results in the eroding of the surface layer and the exposure of fresh TBTM. These paints contain lower overall concentrations of TBT and have an extended life time of up to and beyond 5 years (de Mora, 1996). The problem with TBT as an antifouling agent is its toxicity to non target species once it enters the water column.

Tributyltin (TBT) is an extremely biologically reactive organometallic compound. The TBT moiety is made up of three butyl groups bound covalently to a tin atom in the +4



oxidation state (Muller et al., 1989). The carbon-tin bonds are relatively weak and more polar than the bonds formed within the organic component of the molecule. In the aquatic environment tributyltin speciation is controlled by pH, salinity, and the dissolved carbon dioxide concentration in the water. At a pH of 8, roughly that of seawater, the most common species are tributyltin hydroxide and tributyltin carbonate but it can also exist as a hydrated tin cation or tributyltin chloride (de Mora, 1996).

As an environmental contaminant, TBT has been shown to impact multiple species

and trophic levels at concentrations as low as ng/L (ppt). Responses to TBT exposure have included imposex in snails (the superimposition of male genitalia on the female reproductive system), disruption of normal hormonal activity in multiple species, reduced growth rates in mussels and oysters, sterility, and premature death (Lee, 1991). Concentrations of TBT in contaminated water normally range from >100 ng/L to <300 ng/L (Batley, 1996) but ranges of 20-1800 ng/L have been observed (Hall, 1986). Sediment concentrations can be even higher because of the propensity of TBT to be associated with particles which quickly settle out. Normally the water surface micro-layer is also enriched due to the increased concentration of organic and inorganic anthropogenic compounds as well as hydrophobic materials to which TBT adsorbs (Hardy, 1982; Cleary & Stebbing, 1985). During peak boating season, levels of TBT in the surface micro-layer can exceed 1000 ng/L (Mathias et al., 1988). Studies have shown that these levels of TBT are toxic to many species. Evans and Smith (1999), reported that the no observed effects concentration (NOEC) for plankton growth and development was 0.4 ng/L. Lindblad et al. (1989) exposed *Fucus vesiculosus* communities including epifauna and algal components to 1.6-5.0ng/L TBT. Before exposure, net community productivity was between 10 and 30mgO₂ g⁻¹ d⁻¹, following exposure that had fallen to 0-5mgO₂ g⁻¹ d⁻¹. Oysters including *Ostrea edulis* (European oyster), *Crassostrea gigas* (Pacific oyster), and *Crassostrea virginica* (Eastern oyster) exhibited a range of 96 hour LC₅₀ values from 210-560µg/L. The larvae and embryos of these species exhibited lethal effects at much lower concentrations of TBT (1 and 2µg/L). Serious physiological effects on larvae resulted after exposure to 0.05µg/L TBT (Hall, 1996). Langston and Burt (1991) observed that many sediment dwelling organisms such as the

infaunal deposit feeding bivalves *Scrobicularia plana* develop body burdens of 25-30 μ g/g within a few days of exposure to sediment with a TBT concentration of 10 μ g/g. Within two weeks of exposure, all the clams were dead. Fish, fish eating birds, and marine mammals have been found with TBT in body tissues (Kannan & Falanaysz, 1997; Guruge et al., 1996; Iwate et al., 1994; & Kannan et al., 1996). Microorganisms, fish, and algae have also shown toxicities to TBT (Muller et al., 1989).

Regulations of Use

As an antifouling agent TBT is extremely effective, but it became apparent in the early 1980s that there were problems with some non-target, economically important species. The first restrictions placed on the use of TBT occurred in France in 1982. They were based on circumstantial evidence summarized in Alzieu (1996) which attributed damage to the oyster population on the French coast to exposure to TBT. Oyster production had fallen to 30-50% of that in the late 1970s and early 1980s (Evans & Smith, 1991). A temporary ban was put in place that would remain until more scientific evidence could be collected. This ban excluded the use of paints which contained more than 3% organotin on all non-aluminum vessels less than 25m (82ft) in length in ports along the French Atlantic coast. This was later extended to include all organotin containing paints (de Mora, 1996; Huggett et al., 1992; Clark et al., 1988). In 1985 the United Kingdom followed suit with a set of regulations which banned the sales of SPC paints containing greater than 7.5% total tin and free association paints containing greater than 2.5 % total tin. It also set a water quality target for TBT of 20ng/L. In 1987 the water quality goal was lowered to 2ng/L. A ban,

similar to that in France, was also placed on boats less than 25m in length. Beginning in 1987, the United States followed the French model in the passage of the Organotin Antifouling Paint Control Act (PL 100-333). This act prohibited the use of paints on non-aluminum craft less than 25m in length and set the maximum daily leach rate at $4\mu\text{g TBT}/\text{cm}^3/\text{day}$ on vessels greater than 25m in length. In 1990, the US EPA also began requiring that all paints be registered and applicators of the paint be certified. In the early 1990s, Canada, South Africa, and the majority of the European Community followed suit, with similar guidelines (Bosselman, 1996). In 1996, Austria, Switzerland, and New Zealand were the only countries that had total bans in place. Not surprisingly, none of these nations relied heavily on the shipping industry for their economic stability. Brazil, China, Hong Kong, Korea, Singapore, Malaysia, Russia, the Ukraine, and the United Arab Emirates are among the nations which have very few restrictions. This is significant because these countries have large drydocking facilities in conjunction with their shipping industries (Bosselman, 1996; de Mora, 1996; Hall, 1988).

The United Nations became involved in the TBT debate in 1990 when at the 3rd Annual International Organotin Symposium it was recognized that the International Maritime Organization, which was established by the UN in 1948, was the body that should regulate the use of organotin paints and develop regulatory strategies. In November 1990, the Marine Environmental Protection Committee (MEPC) of the IMO passed resolution MEPC 46(30). In this resolution the following general guidelines were put in place to aid countries in forming regulations regarding use of TBT based paints.

1. Eliminate use on non aluminum vessels less than 25m in length.

2. Eliminate use of paints with daily release rates greater than $4\mu\text{g organotin}/\text{cm}^3/\text{day}$.
3. Develop sound management practices related to ship maintenance and construction activities which eliminate discharge of TBT.
4. Encourage development of alternative, safer antifouling products.
5. Engage in monitoring to evaluate effectiveness of regulatory actions.
6. Consider appropriate movement towards a possible total ban of TBT.

These guidelines are echoed very strongly in the regulations discussed previously. Recent actions of the MEPC of the IMO are continuing to move towards the total ban of TBT use as a marine antifouling agent by 2003 and the goal of all hulls being TBT free by 2008 (Evans, 1999).

Fate & Degradation

The fate of tributyltin in the environment is controlled by the characteristics of the compound. TBT has a $\log K_{ow}$ (water-octanol partitioning coefficient) of 3.7 at a salinity of 25 psu (Laughlin et al., 1986). The K_{ow} is a relative indicator of the propensity of a compound to partition into organic material. To a large extent TBT in the water column is associated with particles of various sizes which then settle out. This is one of the most important mechanisms of loss from the water column. The typical measured $\log K_d$ (partitioning coefficient) values in sediment range from 2 to 5 but these are very site specific (Langston & Pope, 1995). It is important to realize that this does not mean that TBT is then no longer available to organisms. TBT partitioning to sediment is a reversible process (Unger et al., 1988) and furthermore, the organisms that live in the sediment are exposed to

higher levels than organisms in the water column. Other losses from the water column are due to assimilation by organisms, aerobic metabolism, photolysis, hydrolysis, and volatilization (de Mora, 1996). Under some conditions, such as when the compound is in the water column, degradation to less toxic daughter products, dibutyltin and monobutyltin, can be relatively quick, on the order of 1-3 weeks (Muller et al., 1989). However, TBT adsorbed to particles and buried in sediment, or in anoxic conditions may have a half-life on the order of years (de Mora et al., 1995; Dowson et al., 1996). TBT buried in the sediments of harbors and marinas is not lost to the environment. Dredging and other physical processes may re-expose organisms such as clams and mussels, certain species of which can bioaccumulate the compound to levels three orders of magnitude above ambient levels (Clark et al., 1988).

TBT degradation can occur in several ways. In the upper water column the most important of these is photolytic degradation. Direct photolysis of TBT occurs when the molecule is exposed to near UV light in the wavelength range of 300-350nm. Because of the low transmittance of UV light through water, this process only occurs in the very top layer of the water column (Clark et al., 1988). Indirect photolysis has also been shown to occur with the assistance of fulvic acids (Muller et al., 1989). Muller et al. (1989) demonstrated that degradation rates with light exposure increased by a factor of 2 to 3 when fulvic acids were present. Chemical cleavage, thermal cleavage, and volatilization are also potential pathways of degradation. Both nucleophilic and electrophilic reagents can act on the Sn-C bonds of the TBT molecule resulting in chemical cleavage. Most studies involving these reactions were done with very high starting concentrations and in non-aqueous solutions.

Half lives ranged from one minute to 115 days and results are not directly applicable to the environment (Clark et al., 1989). Thermal cleavage is rare in the environment due to the high level of heat needed to break the bonds which are stable up to 200°C (Clark et al., 1989). Volatilization does not appear to be a major mechanism of TBT removal. The vapor pressure of TBT is relatively low, in the range of 6.4×10^{-7} to 1.2×10^{-4} mm of Hg at 20°C (de Mora, 1996). One freshwater study showed negligible amounts of TBT lost due to volatilization over a time period of months. In the saltwater experimental study, significant loss due to volatilization was reported, but the possibility that photolysis was also occurring in the surface micro-layer could not be ruled out (de Mora, 1996). Over all, loss of TBT from the environment due to volatilization is also negligible.

Biotic degradation is an important process affecting TBT concentrations in the environment. In fact much of the photolysis that has been observed was biologically mediated. Both microbial and fungal populations have been shown to degrade TBT. Loss is accelerated in unsterilized versus sterilized cultures (de Mora, 1996). In sediment the process appears to be a debutylation although in water samples both debutylation and methylation can occur depending on the dominant microbial population. The dominant end products of these reactions are dibutyl and monobutyl tin. TBT can also be metabolized by a number of organisms, some with more efficiency than others (de Mora, 1996). Green algae, diatoms, eelgrass, crabs, oysters, and fish are a few examples of organisms that are capable of degrading the contaminant. In the higher organisms such as fish, the hydroxylated products indicate that a cytochrome P-450 dependant mono-oxygenase detoxification system was likely responsible for this metabolism (Lee, 1991).

Created Wetlands and Remediation

Goals

Wetland creation and restoration is becoming a prominent aspect of coastal wetland management as the functional roles of these created habitats become better understood. Although wetlands are created each year in order to compensate for acreage destroyed by expansion of urban areas and industry, increasingly, creation projects are undertaken for the purpose of targeting specific wetland functions. Targeted functions range from flood control, wastewater treatment, storm water or nonpoint source pollution control, and water quality improvement to coastal restoration, and wildlife and fisheries habitat enhancement (Mitsch & Gosselink, 1993). Erwin (1990) further expands this list to encompass groundwater recharge, flood storage, sediment trapping, and nutrient retention. More recently the importance of wetlands in bioremediation projects has come to the forefront of wetland science. The use of wetlands for the treatment of contaminated sediment and water is perhaps the least understood function of created wetland habitats. Standards for creation and criteria for success by which wetlands should be evaluated are being established in order to better define and subsequently refine these projects (Kusler & Kentula, 1990).

Contaminant Remediation with Wetlands

The use of natural wetlands and created wetlands for remediation of contaminated water and sediment has been utilized in many areas including freshwater and tidal/saltwater environments. A broad range of contaminants have been studied including metals, radio-nuclides, and multiple organic compounds consisting of chlorinated solvents, PCBs, PAHs,

pesticides, insecticides, explosives, nutrients, and surfactants (Miller, 1996). Initially these attempts at remediation focused on microbial communities which inhabit the wetlands, but as studies continued it became obvious that the plants populating these sites were contributing more to the degradation of chemicals than simply supplying a favorable medium in which microbes would flourish. In the case of some toxicants, there is evidence to suggest that certain vascular plants may not only sequester the toxicants, but promote their degradation to less toxic daughter products (Cunningham & Ow, 1996; Schnoor et al., 1995; Rai et al., 1995). Plants can aid in the removal and breakdown of contaminants by multiple avenues. Direct mechanisms include uptake, metabolism, and release of exudates and enzymes that stimulate microbial and biochemical degradation, enhancing these processes in the rhizosphere. Indirectly, plants may increase both particulate and dissolved organic material in the soil, thereby enhancing microbial activity. Microbial activity is also enhanced by the oxygen which diffuses into the sediment surrounding the roots and rhizomes of plants. In addition, they stabilize soil preventing the spread of contaminants, and through transpiration, plants can decrease the downward migration of chemicals via percolation (Schnoor et al., 1995).

The use of wetlands to aid in the transformation and removal of nutrients (nitrate, nitrite, phosphate, and ammonium), organic matter, fecal coliforms, total suspended solids (TSS) and biochemical oxygen demand (BOD) in wastewater has been the focus of many mesocosm and full scale field studies. These studies have incorporated a broad range of fresh and saltwater macrophytes and macroalgae. Removal efficiencies reported include 85% reduction in TSS using *Scirpus validus* (Gearheart et al., 1990), 74% reduction in fecal

coliform and 66% removal of total phosphate by *Eleocharis dulcis* (Maddox and Kingsley, 1989), 70% decrease in organic matter with *Scirpus lacustris* and *Typha augustifolia* (Ansola et al., 1995), and reduced total nitrogen using *S. validus*, *T. latifolia*, and *Phragmites communis* (Gersberg et al., 1986). Removal rates depended on a number of factors including combination of plants, substrate, and retention times, but in all cases the use of wetlands in remediation was successful and more cost effective than traditional means.

Reclamation of industrial wastes is a second area in which wetlands have been successfully utilized to reduce contamination of soil and water. The Tennessee Valley Authority (TVA) created a wetland in the vicinity of an impoundment area near an old mine using *T. latifolia* and *Scirpus cyperinus*. The successful reduction in levels of iron, manganese, TSS loads and lowering of the pH of acid mine drainage (AMD) allowed the TVA to maintain suitable effluent levels in the adjacent soil and streams, a goal that had eluded them for years (Brodie et al., 1990). For example, the number of macroinvertebrate taxa in the adjacent stream increased from 2 to 19 in six months. In the actual AMD impoundment, the number rose to thirty-two taxa. In thirteen months the two species of plants introduced had increased to twenty. In other projects, multiple species of floating and rooted emergent plants have been successfully used in the reduction of chromium levels in tannery effluent (Valpayee et al., 1995). Wetlands have also been effective in remediating ash pond seepage, pulp mill effluents, and refinery effluents (Brodie et al., 1989; Thut, 1989; Litchfield & Schatz, 1989).

The success of wetland plants for remediation of water and sediments with high levels of metals has also been shown. Rai et al. (1995) assessed the potential of free floating,

submersed, and emergent plants to remove heavy metals. Chromium, iron, manganese, cadmium, and lead accumulated in at least two of the plant species and concentrations of many of the metals were reduced to levels below the maximum permissible limit in the wastewater tested. It has been demonstrated that *Spartina alterniflora* will take up metals found in salt marsh sediments including Al, Cu, Fe, Hg, Mn, and Zn (Alberts et al., 1990). In addition, Kornuc (Pers. Com., 1998) has found that in the created wetland used in this study, after one growing season the concentrations of lead, chromium, and arsenic had increased in the tissues of the *S. alterniflora*, while concurrent decreases in the concentration of these metals in the surface sediments were observed.

TBT Remediation

Studies on TBT contamination in the past have focused on sediment and water column processes, microbial degradation, and the effects of TBT on organisms. Levine et al. (1990) and Kelly et al. (1990) examined the influence of *Thalassia testudinum* seagrass beds on TBT behavior and degradation. Using microcosm experiments they concluded that degradation was faster in vegetated compared to unvegetated cores. Furthermore, uptake of TBT was rapid. Half lives of ten to twenty hours were observed, but the assimilation was temporary. When cores were sacrificed, only 20-30% of the initial TBT concentration remained and half of this was as degradation products. Even with rapid assimilation by grasses and degradation, fauna in the microcosms still had accumulated TBT (Levine et al., 1990). In addition, studies involving *Zostera marina* and TBT accumulation have demonstrated the propensity of eelgrass to take up TBT (Francois et al., 1989). While the

rate of degradation of TBT was slower in the plants than in the water, the rate of dibutyltin decomposition was faster. Further investigation revealed that monobutyltin, the least toxic of the butyltin species, was then slowly released into the water. Upon decay in clean water, relatively large amounts of MBT were released (300-500ng/L), a small quantity of DBT was released and the water contained <30ng/L of TBT. This was a much lower concentration than the original concentrations to which the water was spiked (540-1940ng/L).

One problem with the use of wetland plants for remediation of substances such as TBT is the potential of contaminated plants to become vectors for transferring the toxins to higher trophic levels. Francois et al. (1989) found that TBT in *Zostera marina* was reduced to MBT, which was subsequently released into the water column. Potential pathways of TBT transfer within the food chain were not determined. Another question left unanswered was whether decaying plants release contaminants. In some studies plants have been harvested and incinerated, thereby removing the contaminant from the natural environment and reducing the mass of the contaminated material (Cunningham & Ow, 1996). Furthermore, there is the problem of what to do with plant material if it merely stores the toxin and does not degrade it.

The previously described seagrass - TBT studies indicated that vascular plants may play a potentially important role in TBT degradation. However, relationships between marsh environments, and TBT accumulation and decomposition have not yet been elucidated through research. The presence of *S. alterniflora* in contaminated treatments could have multiple effects on the concentration of TBT in sediment. The plants could act directly by taking up TBT and degrading it, releasing less toxic daughter products or indirectly by

providing an environment that encourages microbial growth. It would then be the microbes that act directly on the TBT. The mode of uptake of TBT by *S. alterniflora* is likely through the roots and rhizomes of the plants. Due to its bulky nature, we expect most of the TBT to remain in the belowground biomass of the plant.

Objectives and Hypotheses

For both created and natural salt marshes to be considered effective in the remediation of TBT contaminated soils, a more detailed understanding of the rates of degradation and fate of the daughter products must be determined. The first objective of this study was to investigate, in the field, the potential of *S. alterniflora* to aid in the degradation of tributyltin in enriched marsh sediments by measuring TBT, DBT, and MBT in both plants and sediments from vegetated and unvegetated plots throughout a growing season. This experiment was accomplished in a created tidal wetland in order to subject the treatments to natural conditions which could effect the degradation of the contaminant. The second objective was to investigate the fate of TBT once it had been taken up by the *S. alterniflora*. In a laboratory experiment, the release of TBT and its daughter products by decaying plants was examined.

Hypothesis #1

TBT will be taken up by *Spartina alterniflora* from contaminated sediments in detectable amounts. Tissue concentrations of TBT in plants growing in contaminated sediments will be significantly greater than in plants growing in uncontaminated

sediments.

Hypothesis #2

TBT concentrations typical of those found in contaminated dredge spoils will not be detrimental to *Spartina alterniflora* growth.

Hypothesis #3

Presence of *Spartina alterniflora* will increase the disappearance rate of TBT.

Hypothesis #4

The rate of TBT disappearance will increase as plant biomass increases throughout the growing season.

Hypothesis #5

TBT concentration will be higher in plant roots and rhizomes than in shoots.

Hypothesis #6

Upon plant senescence and decay, TBT and its daughter products will be released into the water column. Relative concentrations of TBT species released will be in the following order MBT>DBT>TBT.

METHODS

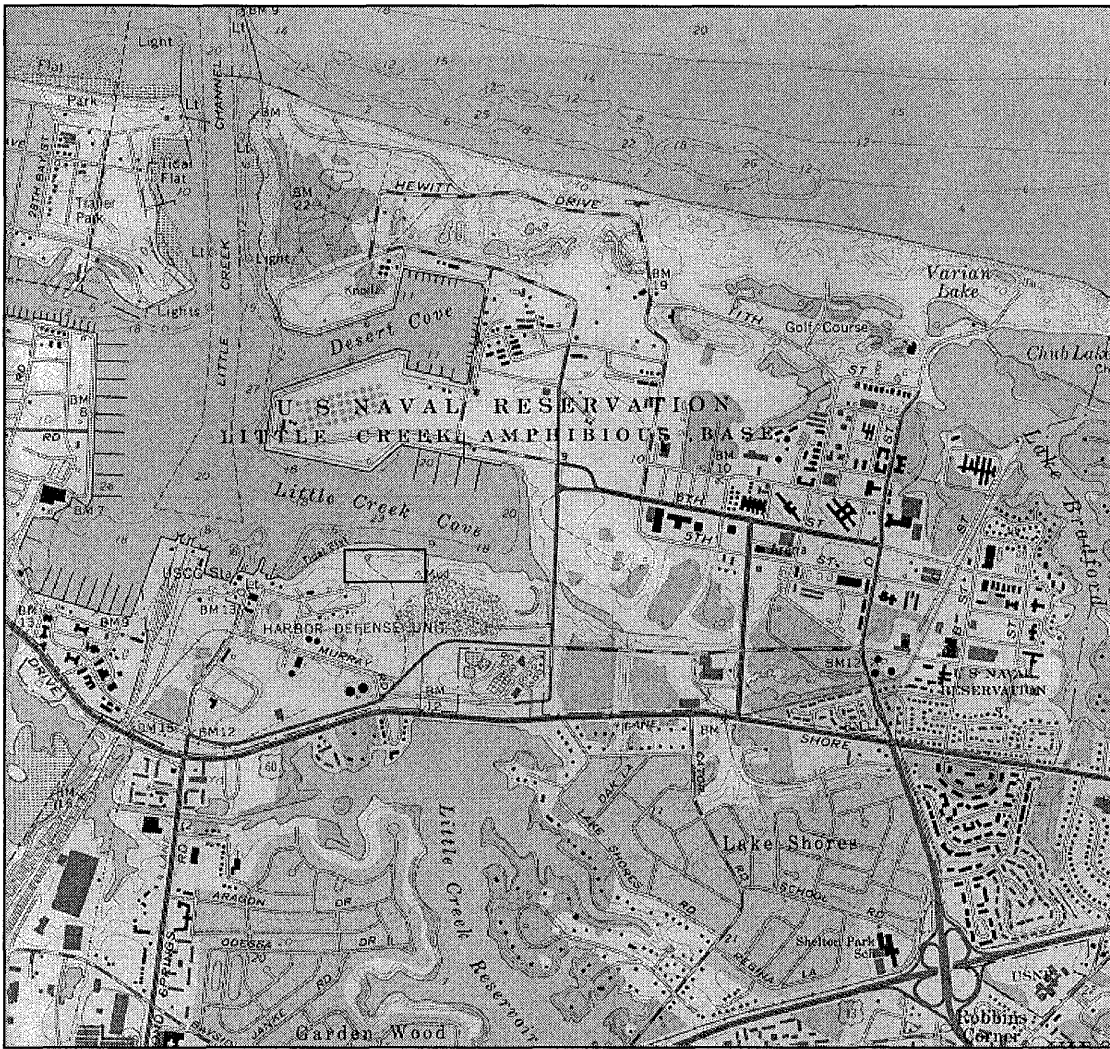
Study Site

The wetland chosen as the study site for this work was created in the summer of 1996 by the Naval Facilities Engineering Service Center (NFESC) of Port Hueneme, California at Little Creek Naval Amphibious Base in Norfolk, Virginia (36°55'N, 76°10'W) (Figure 1). This tidally-influenced salt marsh was created on a previous dredge spoils site to investigate the treatment of non-point source pollution, in this case, storm water runoff. The wetland consists of two cells each measuring 60 x 650 feet, graded to mean sea level. The cells are fed by a tidally influenced drainage ditch via 24-inch diameter pipes equipped with one-way check valves (Figure 2). This forces a one-way flow of tidal water from the storm water channel through the wetland cells and into an adjacent cove. The wetland was planted with 20,000 greenhouse-raised marsh plants in the summer of 1996. *S. alterniflora* was planted up to the mean high tide line and *S. patens* was planted on the slopes down to the *S. alterniflora*. *Panicum amarum* and *Iva frutescens* were planted on the upper side of the slopes and on top of the berm. This study was initiated after the wetland had been growing for two years. The wetland presented an environment in which the experimental treatments would be subjected to tidal flow, animal usage, and plant growth that would simulate a natural marsh system.

Field Experimental Design

The capacity of *S. alterniflora* to aid in the degradation of TBT was assessed through a field study at the created wetland site using a randomized complete block design

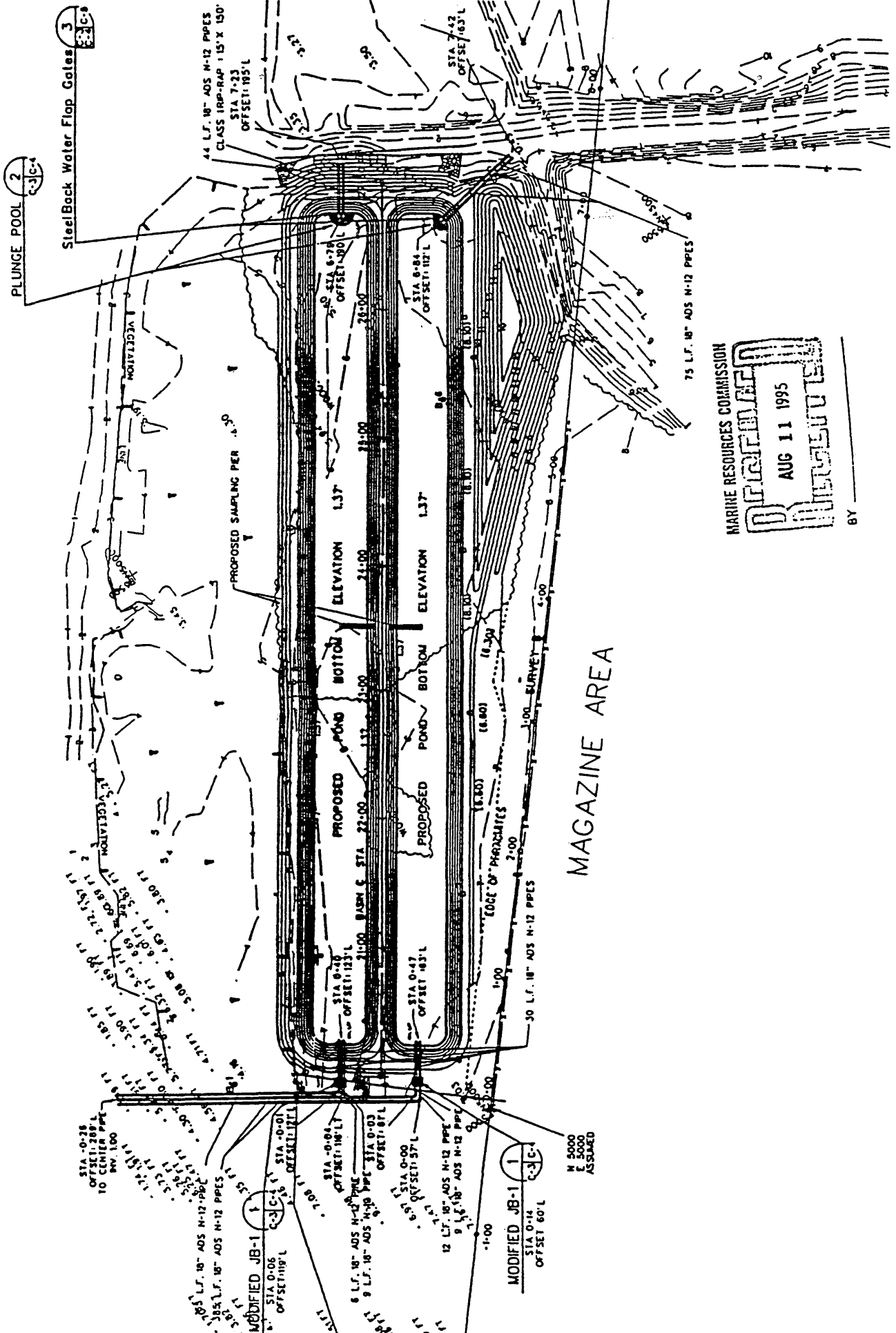
Figure 1. Map of Little Creek Naval Amphibious Base, Norfolk, Virginia and the location of the created wetland used in this study



Location of the Created Wetland

Figure 2. Site, Grading, and Drainage Plan for the constructed wetland at the Little Creek Naval Amphibious Base, Norfolk, Virginia.

LITTLE CREEK COVE



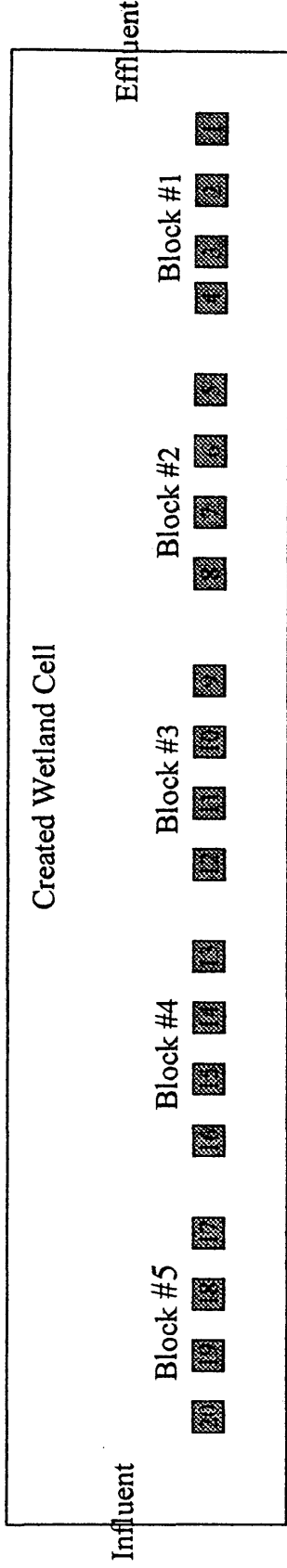
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(Figure 3). A total of twenty 18-inch by 12-inch by 6-inch fiberglass bins were placed in the *S. alterniflora* zone of the wetland (Figure 4). There were five replicates of four treatments including: 1) vegetated, TBT contaminated, 2) vegetated, uncontaminated, 3) unvegetated, TBT contaminated and 4) unvegetated, uncontaminated. The wetland cell furthest from the cove was used for the experiment because of its hydrological conditions. Through observation, this cell appeared to be draining more completely, and *S. alterniflora* coverage was greater than the other cell. The first fifty feet at the influent and effluent ends of the cell were not used and the remaining 550 feet were divided into five equal lengths. For the purposes of the experiment, each section was considered a block to assess potential effects caused by location in the marsh. Within each block replicates of each treatment were randomly placed at equal intervals. The individual experimental units were contained in 18-inch by 12-inch by 6-inch steel reinforced, fiberglass bins (C&H Distributors). The fiberglass bins were prepared in advance by drilling holes in the sides and bottom and covering the holes with squares of fiberglass mesh window screen attached with 100% silicone sealant (Dow Corning) to prevent loss of sediment, but to allow the rise and fall of the water level with the tide. The use of bins enabled the environmental processes to occur on a realistic, measurable, controllable scale while reducing the potential contamination of the surrounding areas with butyltins.

The sediment for experimental treatments was removed from the wetland in late August 1998. It was transported to the Virginia Institute of Marine Science in four large containers and stored with a layer of water over the sediment as well as a shade cloth in order to prevent drying. Within 48 hours the homogenizing and dosing took place. This was

Figure 3. Diagram of the wetland cell used in the study including approximate placement of experimental bins in the cell and location of the blocks and treatments

Experimental Randomized Block Design



Block #1

- 1 Unvegetated, No TBT
- 2 Vegetated, No TBT
- 3 Vegetated, TBT
- 4 Unvegetated, TBT

Block #3

- 9 Vegetated, TBT
- 10 Vegetated, No TBT
- 11 Unvegetated, NoTBT
- 12 Unvegetated, TBT

Block #5

- 17 Vegetated, NoTBT
- 18 Unvegetated, No TBT
- 19 Unvegetated, TBT
- 20 Vegetated, TBT

Block #2

- 5 Vegetated, No TBT
- 6 Unvegetated, No TBT
- 7 Unvegetated, TBT
- 8 Vegetated, TBT

Block #4

- 13 Unvegetated, TBT
- 14 Vegetated, TBT
- 15 Unvegetated, NoTBT
- 16 Vegetated, NoTBT

Figure 4. Prepared bins before placement into the created wetland at Little Creek, September 1998 and an experimental bin located in the *S. alterniflora* zone of the wetland at the beginning of the growing season 1999



accomplished by first manually removing large pieces of plant and detrital matter and homogenizing sediment by hand. In order to amend the sediment, 10ml of ethanol was used as a carrier for a dose of 15.7mg TBT-Cl that would amend the sediment to approximately 200mg/kg TBT. This concentration was determined to be representative of a relatively high, but reasonable value found in sediments from harbor areas of the Chesapeake Bay, VA, San Diego Bay, CA, and Pearl Harbor, HI (Espourteille et al., 1993; Govhough et al., 1996). Ten-ml of ethanol minus the TBT-Cl spike was added to uncontaminated sediment in order to rule out any carrier effects from the ethanol. Sediment for the uncontaminated bins was handled first. Sediment sufficient to fill two bins was placed into a pre-rinsed cement mixer. The 10ml of ethanol was added to a quart Mason jar with 200ml of York River water, shaken, then added to the sediment followed by two rinses of 100ml each. The sediment was mixed for 15 minutes at which time it was placed back into two bins. This was repeated 5 times until all 10 uncontaminated bins were filled. The same procedure was then followed for the contaminated bins with the exception that the ethanol contained a dose of 15.7mg TBT-Cl. After all 20 bins were prepared, a subsample of known volume was removed from each of the bins for determination of percent moisture. After the sediment was dosed, each vegetated bin was planted with twelve plugs of two year old *S. alterniflora* grown in two inch by two inch peat pots. The *S. alterniflora* was purchased from Environmental Concern of St. Michaels, Maryland, where they had been grown in freshwater and then adapted to 20psu. The day following transplanting, the bins were transported to the wetland location and placed into the marsh. Initial sediment samples for TBT analysis were taken at this time to establish background levels (uncontaminated bins) and attained dose levels (contaminated bins).

Several plants from the stock that were not used for planting were rinsed free of sediment and frozen in order to determine background levels of TBT, DBT, and MBT.

In the Spring of 1999, a second set of four bins were contaminated using the same method but with twice the amount of TBT-Cl. This brought the target spike to 400mg/kg TBT. These bins were also planted with *S. alterniflora* from Environmental Concern. The *S. alterniflora* were of the same cohort as the original plants used in September 1998. These plants were to be harvested and utilized in the plant degradation laboratory study.

TBT Sampling - Sediment

In order to determine concentrations of TBT, DBT, and MBT in the sediment and plants, samples were taken at regular intervals, frozen, and processed back in the lab. Each month, four 8.5cm sediment core samples were collected from each bin using a modified 20cc syringe. The cores were homogenized in a 500ml plastic beaker and approximately 50g of sediment was placed into a Whirl-Pak[®] bag. The samples were then placed on ice and transported back to the lab where they were subsequently frozen until they could be processed.

TBT Sampling - Plant Material

Plant tissue was collected at intervals throughout the experiment to determine levels of TBT, DBT, and MBT in the aboveground and belowground portions of the plants. Sampling occurred at the beginning of the experiment (September 1998), the middle of the 1999 growing season (June 1999), and at the end of the experiment (December 1999). Entire

plants, including shoots, roots, and rhizomes were cut from the bins, placed in Zip-Loc[®] bags, put on ice, and transported back to the lab. Once in the lab, the plants were gently rinsed using deionized water in order to remove any sediment. Above and belowground tissue were separated and frozen until analysis.

Spartina alterniflora Biomass

Each bin was delineated into six overlapping zones, each zone consisted of four of the original twelve planted culms and had an area of approximately one third of the entire bin (approximately 464 cm²). Within each bin one zone was randomly selected at the beginning of the experiment. The biomass in the same zone was then estimated each month throughout the duration of the field study. During the first growing season of the experiment, September 1998 through December 1998, biomass was measured monthly by recording the height and basal width of the stem and length and width of each leaf of new growth around the original culm. This was considered a period of establishment for the new plants. The original culm that was placed into the bin was not measured and all dead standing material was trimmed to an equal height during the first winter (February 1999). Beginning in March 1999, with the onset of the growing season, new shoots within the designated zones of each bin were marked with # 12, numbered, monel, bird-bands (National Band & Tag Co.). In each bin, working in a clockwise orientation around the designated zone, the first 10 shoots were measured for shoot height and basal width and the width and length of each leaf on the shoot. All remaining shoots after the tenth were measured for shoot basal width and height. Basal width was defined as the width of the shoot at the level of the sediment. Leaf width

was taken at the point where the leaf joined the shoot and leaf length from that same point to the tip of the leaf.

In September of 1999, 65 shoots were sacrificed from the constructed wetland surrounding the bins. The plants selected were within the size range of the plants growing in the bins. The shoots were taken back to the lab and measurements of basal width and height and leaf length and width, were made. The individual plants were then dried and weighed and a relationship between plant size and weight was developed (c.f. Dai, 1996).

Pulse-Amplitude Modulated Fluorometry

A pulse-amplitude modulated (PAM) fluorometer was utilized to measure stress in the *S. alterniflora*. PAM fluorometry provides information about the health of the photosystem II (PSII) photochemical processes (Schreiber et al., 1994). The two parameters which were of greatest interest in this study were light adapted, effective quantum yield ($\Delta F/F_m'$) and dark adapted, maximum quantum yield, or photochemical efficiency (F_v/F_m).

These ratios includes measurements, ΔF or F_v and F_m' or F_m . ΔF and F_v are the differences between two parameters, F_0' or F_0 which are the minimal fluorescence yield under light and dark adapted conditions and F_m' or F_m which are the maximum fluorescence yields under these same conditions. Minimal fluorescence yield is the amount of fluorescence when all of the PSII reaction centers are open. Maximal fluorescence yield is the amount of fluorescence when all of the PSII reaction centers are closed. Typical photochemical efficiency values range from 0.75 to 0.85 (Bolhar-Nordenkampf & Oquist, 1993). These parameters can be used as stress indicators because it has been shown that PSII function is

particularly sensitive to many stress factors including; high temperature, chilling, freezing, drought, and excessive radiation (Bolhar-Nordenkampf & Oquist, 1993). Other factors can also indirectly affect PSII health. PAM fluorometry has been used in the past to examine stress in the seagrass, *Halophila ovalis*, caused by herbicides, petrochemicals and heavy metals (Ralph, 1999; Ralph & Burchett, 1998a; Ralph & Burchett, 1998b).

PAM measurements were taken in the field in August 1999 using a Walz DIVING-PAM (Germany). Ten shoots were selected from each bin and the second newest leaf on the shoot was used to collect measurements. Dark-adaption clips (DLC-8) were placed on the leaves 2cm above the stock clasp on the adaxial side of the leaf. Effective quantum yield was measured and then the leaf was dark adapted for 10 minutes at which time the maximum quantum yield was measured. The same measurements were taken on shoots growing in the high dose experimental treatments.

Redox Potential

Redox potential was measured monthly in each of the contaminated bins beginning in March 1999 with a Fisher Scientific AP10 E_p/pH Meter. Readings were taken using platinum electrode probes at three locations within the bins at four different depths: 1cm, 2cm, 5cm, and 10cm. These electrodes were tested for accuracy each month using a quinhydrone standard solution (pH 4) before the field measurements were taken. A reference electrode was placed on the surface of the wet sediment and the platinum electrode probe at the first depth. After a period of equilibration, a reading in millivolts, was taken and the probe was then advanced to the second depth until all readings were taken and recorded.

A sediment temperature reading was also obtained in order to correct the reading. The mean of the three readings measured at each depth was used to characterize the redox potential with depth in the contaminated bins. Five control measurements were also collected within the marsh surrounding the bins, one in each of the five blocks.

Nutrients

Sediment nutrients were sampled in each bin seasonally in order to detect possible variations in the conditions between bins and to help explain any potential variations in plant growth. Sediment cores were taken to the full depth of the bin and divided into three segments; 0-2cm, 2-5cm, and 5-15cm. These segments were then divided in half. One half was used to determine bulk density of the sediment. The other half was placed into Whirl-Pak[®] bags containing 2N KCl in a 2:1 ratio with the sediment. The bag with KCl and sediment was placed on a shaker table and allowed to extract for 1 hour. The liquid was then poured into a centrifuge tube and spun using a Centra MP4 (International Equipment Co.) at 2300 rpms for 10 minutes. The supernatant was then decanted into a 30cc syringe and filtered (Gelman Supor, 0.45 μ m) into a Whirl-Pak[®] bag. The samples were then frozen until analysis for dissolved ammonium, nitrate, and nitrite. An Alpkem autoanalyzer was used to determine the concentration of nitrate/nitrite in the samples. In this method, nitrate was reduced to nitrite by cadmium metal. With the addition of a color reagent, which consisted of sulfanilamide and N-1-naphthylethylenediamine dihydrochloride in a phosphoric acid solution, the total nitrite was determined spectrophotometrically at 540nm. In a replicate sample, which was not reduced, the amount of nitrite originally there was determined.

Nitrate concentration was then derived by calculating the difference in nitrite between the two samples (Perstorp, 1992). To determine the amount of ammonium in the samples, a set volume of 5ml was pipetted into a test tube. A volume of 0.143ml of each of the following reagents was added in the order listed; citrate, phenol, and hypochlorite. Between each addition the sample was vortexed. The samples were then stored in the dark for 24 hours. At that time, a color change indicated the presence of ammonium in the samples. This was detected spectrophotometrically at 630nm with a Shimadzu UV 1601 spectrophotometer. (Parsons et al., 1984; Solorzano, 1969). In the case of both methods a standard curve was developed using standard stock solutions of ammonium sulfate or potassium nitrite/potassium nitrate dissolved in dionized water. This solution was then further diluted into a series of concentrations ranging from zero to a value at the high end of the estimated sample concentration.

Statistical Analyses

The concentration of TBT over time in the bins was analyzed using a two way ANOVA with main effects of vegetation, treatment, and time. Regression analysis was used to test the relationships between TBT sediment concentration over time and *S. alterniflora* biomass. A one-way ANOVA was used on redox data to determine if there was any difference in the redox potential at each depth interval over time. The same was done for the nutrient data.

Analytical Methods

Sediment

TBT sediment analysis was performed according to the protocols in *A Manual for the Analysis of Butyltins in Environmental Samples* (Unger, 1996). The analytical methodology flowchart in Figure 5 gives a general overview of the process. Samples were thawed and homogenized in the Whirl-Pak® bags and then a 10g aliquot was placed into a 500ml Nalgene Teflon bottle (VWR). At the same time a subsample of the sediment was placed into a pre-weighed aluminum weigh pan for water content determination. These were weighed, dried overnight in a 100°C degree oven, and weighed again. A value of the total percent solids was calculated for each sample. A blank consisting of 10g of pre-extracted sand was also placed into a Teflon bottle. The samples were then spiked with the surrogate standard, triphenyltin chloride in hexane, at a concentration similar to the expected TBT concentration of the samples. Twenty-ml of deionized water with the pH adjusted to 2 was added to each sample. A 0.2% tropolone hexane/tropolone mixture made with 98% tropolone (Aldrich) and high purity Burdick & Jackson Hexane UV (VWR) was added to the samples in two 100ml aliquots. The samples were shaken for one hour on a Wrist Action Shaker after each of these additions and the hexane layer was decanted into a 500ml round bottom flask. Using a rotary evaporator, the volume was reduced to approximately 2ml and the samples were then transferred to a 50ml centrifuge tube. The volume was reduced to 2ml under a stream of nitrogen.

The next step in the process was the removal of the sulfur from the sediment extract. This was accomplished by eluting the samples through columns packed with fine granular

Figure 5. Flowchart of tributyltin analytical method

Tributyltin Analytical Methodology

Sample spiked with triphenyltin chloride and
extracted hexane solvent



Extract volume reduced
Butyltins derivatized with Grignard reagent
(n-hexyl magnesium bromide)



Derivatized butyltins separated from other
contaminants by open column chromatography
on Florisil



Extract volume reduced and spiked
with tetrabutyltin



Sample analyzed by GC with modified flame
photometric detection

(-40 mesh) activated 99.5% copper (Aldrich). This was followed by derivatization of the butyltins with 0.5ml of Grignard reagent, hexylmagnesium bromide, 20%, in tetrahydrofuran (THF). Excess reagent was neutralized with 2 ml of concentrated HCl. The samples were shaken, vented, and then allowed to settle for 30 min. to allow separation of the phases. The bottom aqueous layer was then removed and discarded. The samples were purified by elution through 20g of activated (110°C) florisil (Fisher) and 2g of granular anhydrous sodium sulfate (JT Baker) which had been rinsed with 75ml of hexane. The samples were collected in 500ml round bottom flasks. After sample volume was reduced by rotary evaporator, samples were transferred into centrifuge tubes and spiked with an internal standard, tetrabutyltin in hexane, to a concentration similar to the surrogate standard. TBT concentrations were calculated relative to the surrogate internal standard. Total recovery for the sample was calculated relative to the tetrabutyltin internal standard added just prior to injection on the GC. Samples were then reduced further to a volume of 0.1ml and analyzed on a Varian 3300 GC with a modified flame photometric detector. GC settings and a sample chromatogram are included in Appendix A. Detection limits for this method were approximately 1ng/g for sediment and tissue samples and 1ng/L for water samples.

Plant Tissue Analysis

Frozen plant material was thawed and cut into 0.5cm segments using clean hexane rinsed utility shears. A 10g subsample was placed into a pint Mason jar and spiked with triphenyltin in hexane. A desiccant consisting of a 1:9 ratio of precipitated silica (QUSO®) and anhydrous granulated sodium sulfate was then added in a 3:1 ratio to the plant tissue.

The sample was homogenized further in a blender using a metal blade and frozen for 24 hours.

The frozen samples were placed in a Soxhlet apparatus with a coarse frit (VWR) and extracted with hexane for 24 hours. A blank was prepared with each batch of samples using the desiccant materials. The surrogate standard was added to the blank after extraction, the sample volume was reduced to approximately 2ml using a rotary evaporator, and the samples were transferred to a centrifuge tube. The extracts were then put through the derivatization and cleaning steps described above and were analyzed by gas chromatography with modified flame photometric detection.

Water Analysis

Water samples were warmed to room temperature and 500ml was poured into a separatory funnel. An equal amount of acidified (pH 2), deionized water was extracted as a blank with each batch of samples. All samples were spiked with the surrogate standard (tripentyltin chloride in ethanol) to a concentration near to the expected TBT concentration. Samples were swirled to mix and then extracted for 3 minutes with three 40ml aliquots of 0.2% tropolone in hexane. The extract was reduced to approximately 2ml, transferred to centrifuge tube, and the aqueous layer (bottom) was removed from the tube. Derivatization and sample clean-up followed procedures which were described in detail above.

Recovery Experiments - Standard Curves

Two standard curves, one for plant material and one for sediment were created to

establish TBT recovery in the analytical process. For the sediment, a series of butyltin spikes were added to 10g aliquots of sediment taken from the constructed wetland. By using this sediment, the same which was used for the actual experiment, any matrix effect would be taken into consideration in the recovery of the butyltins. The series of spikes included 0.0ng/g, 5.6ng/g, 9.8ng/g, 56ng/g, 98ng/g, and 270ng/g. This range was inclusive of concentrations that we would expect to see within the samples obtained from the experiment. The results, shown in Figure 6, give a percent recovery for TBT of 105%, DBT 73%, and MBT 19%. Background sediment levels of TBT were below the detection limit which for sediment samples is approximately 1ng/g..

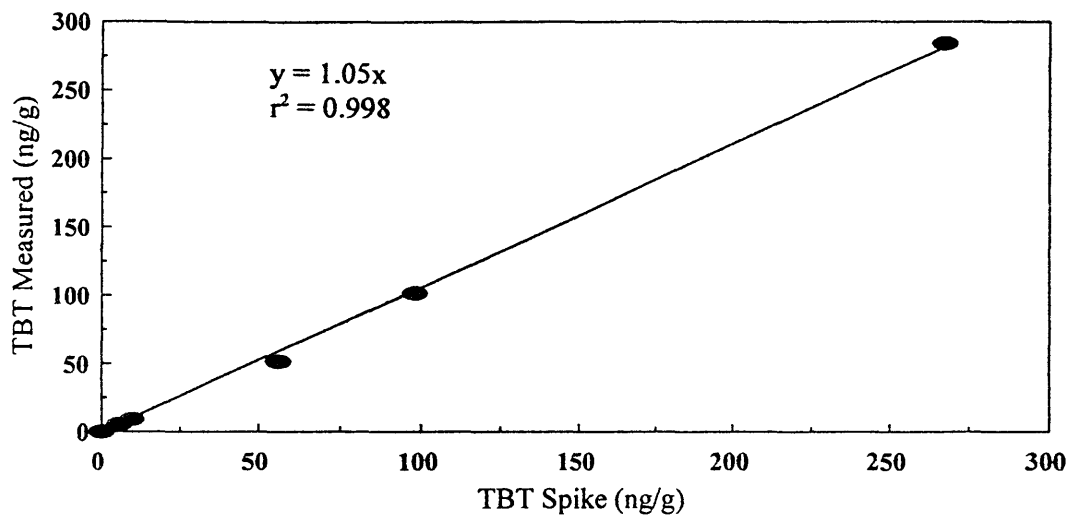
To test the recovery from *Spartina alterniflora*, a series of TBT spikes were added to plant material which was collected from the wetland. The series of spikes included 0.0ng/g, 1.1ng/g, 11ng/g, and 23ng/g. There was a 0.42ng/g background level of TBT which was subtracted out of the series before calculating recoveries. Again, this series included a range of TBT concentrations which spanned all expected plant sample values. Percent recovery was 88% while the r-squared value for the regression was 0.9969 (Figure 7).

Laboratory Experimental Design

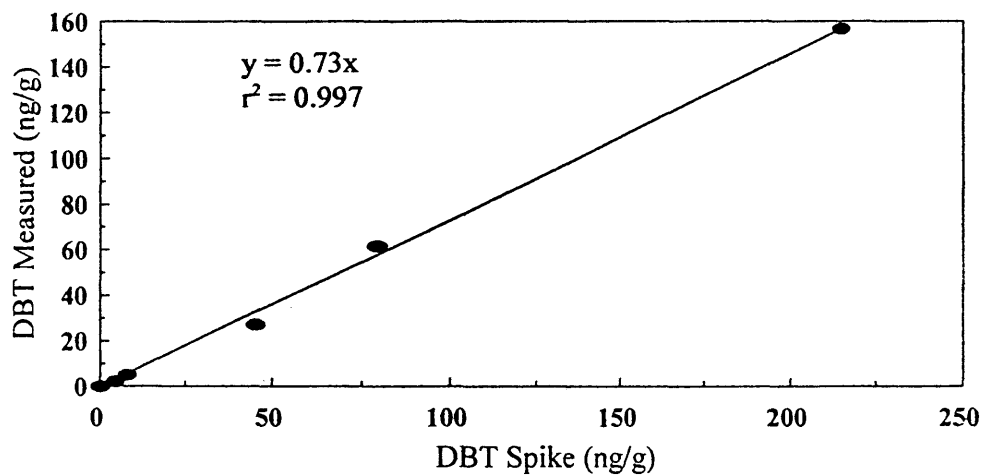
A study of the potential re-release of TBT, DBT, and MBT from decomposing plants into the water column was conducted in the lab using TBT contaminated *S. alterniflora* gathered from the contaminated bins at the end of the experiment in December 1999. The average measured TBT in the aboveground plant material was 1.2ng/g with a range of

Figure 6. Standard curves for sediment TBT, DBT, and MBT recovery experiments

TBT Sediment Recovery



DBT Sediment Recovery



MBT Sediment Recovery

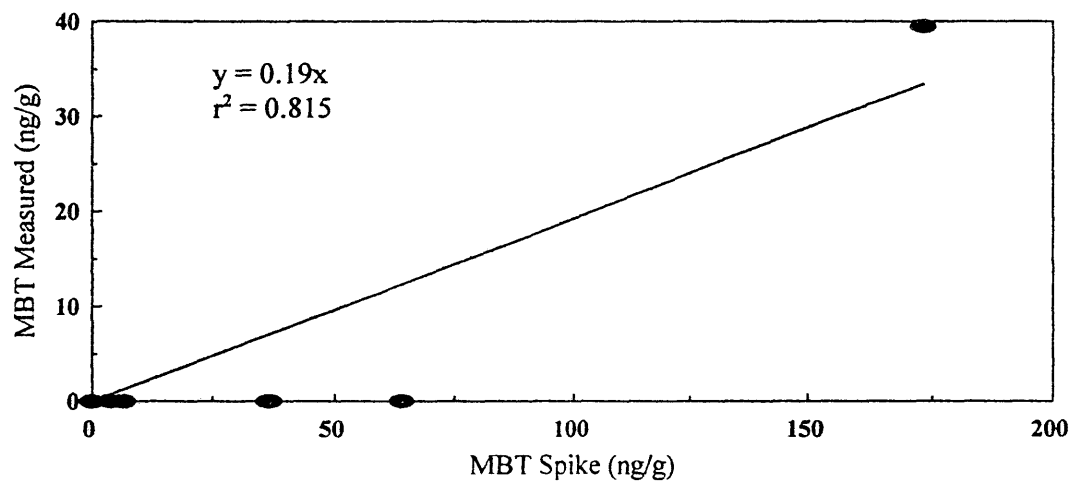
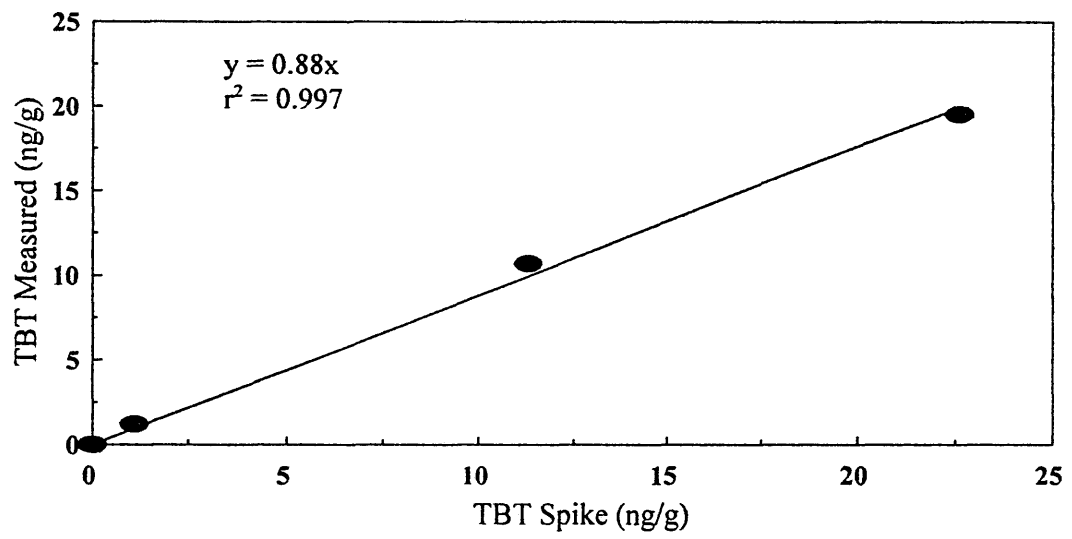


Figure 7. Standard curves for plant TBT recovery experiments

TBT Plant Recovery

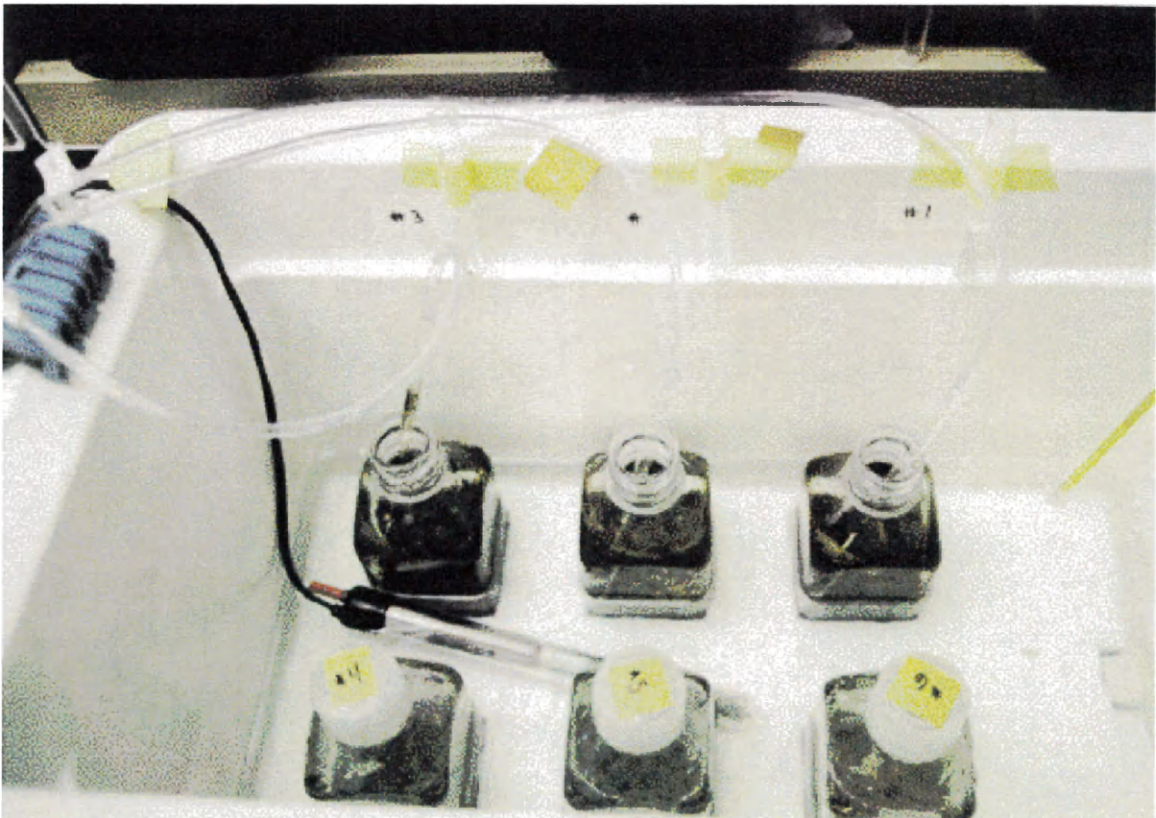


1.0ng/g to 1.4ng/g. The plant material was thawed, cleaned gently with deionized water to remove sediment, cut into approximately 0.5cm lengths, and well mixed.

At the onset of the experiment, 50g subsamples of the prepared plant material were placed into 500ml square polycarbonate Nalgene bottles (Fisher). Two treatments (aerobic and anaerobic) were replicated in three bottles. Aerobic treatments remained open to the air throughout the experiment. Anaerobic treatments were bubbled with nitrogen and then capped. After plant material was added, a stock solution consisting of 25psu Hawaiian Marine Mix, 30 μ m ammonium, and 4 μ m phosphate was poured into the aerobic bottles up to the 500ml mark. An aliquot of the stock solution was bubbled with nitrogen gas for 30 minutes and then poured into each of the three anaerobic treatment bottles. These were then each bubbled with nitrogen for 5-7 minutes and capped. All of the experimental bottles were then placed in a cooler which had been prepared by removing the lid and mounting a styrofoam holder for the bottles in the bottom. Water was added in the cooler, surrounding the bottles and a second constructed styrofoam piece was placed over the bottle necks to hold them in place and prevent water from splashing into the aerobic, open treatments (Figure 8). A fish tank heater was placed in the water bath and temperature was maintained throughout the experiment between 25° and 27° Celsius. Glass Pasteur pipets were connected with aquarium tubing to a Whisper® air filter and air was bubbled through the aerobic treatment bottles and the blank for the duration of the experiment. The cooler was set on a shaker table and agitated continuously at 700rpms. The experiment was run for 53 days.

Due to the typical degradation rate of TBT in the upper water column of 7-14 days (Muller et al., 1989), a water sample was collected every four days. This was accomplished

Figure 8. Diagram of the laboratory plant degradation experiment- Cooler was placed on shaker table with two treatments replicated three times in the cooler held in place with styrofoam. Blank was set outside the cooler. Aerobic treatments and blanks were aerated constantly and temperature was kept constant using an aquarium heater.



using a modified Nalgene Teflon lid which had the center drilled out and a brass screen (150 micron) installed. The water could then be poured off while filtering out large pieces of detrital plant matter. At each sampling, approximately 400ml of water was poured into a 2L Nalgene polycarbonate bottle and replaced with stock solution. The sample was acidified to a pH of 2 using hydrochloric acid to preserve the TBT and daughter products and refrigerated at 4°C until analysis. Water samples from four consecutive sampling dates were combined in each 2L bottle resulting in a total of three composite samples from each of the replicates for the duration of the experiment. Plant material was also collected during the course of the experiment. This occurred on the 12th, 28th, and 44th days. After the water was drained, forceps were used to withdraw approximately 10g of plant material. This was then frozen until analysis. The concentrations from each water sample, examined individually, were used to provide a measure of any change in the rates of release over the month. Total net release over the 53 days was estimated by adding total butyltin mass obtained from each sample. The butyltin concentrations measured in the plant samples combined with butyltins measured in water samples over time were used to model the fate of TBT in the plant/water system. One of the aerobic water samples was also divided in half and one half was extracted whole while the other half was filtered using a type A-E glass fiber filter to remove suspended material prior to extraction. Results from this analysis were used to estimate the bioavailable fraction of TBT released to the water column.

RESULTS

Field Experiment

Butyltin Background Levels

Background concentrations of TBT, DBT, and MBT in incoming water in December 1997 in the non-experimental wetland cell were determined to be 1-3ng/L TBT, 6-7ng/L DBT and 1-4ng/L MBT. In the experimental wetland cell in January 1998, the levels were 1-2ng/L TBT, 2ng/L DBT, and MBT was below the detection limit (<1ng/L). Highest butyltin levels in the water would be expected during this period when water from the cove, where ship traffic occurred, was coming into the wetland. It was determined that these low background levels would not effect the field experiment due to the high initial TBT spike that would be used to amend the sediment.

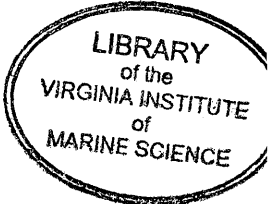
When the experimental bins were placed in the wetland in September 1998, sediment samples from the uncontaminated bins were analyzed to insure that butyltin concentrations were low. One uncontaminated bin was then selected and samples from that bin were analyzed every other month of the experiment for butyltins. Levels remained below the detection limit throughout the study period. This demonstrated that there was no measurable input of TBT to the sediment either from outside sources or from the experimental bins themselves over the course of the experiment. In September 1998, some of the *S. alterniflora* that was not used in the experimental bins was frozen to determine background levels of butyltins. All butyltin species were found to be below the detection limit.

Butyltins in the Sediment

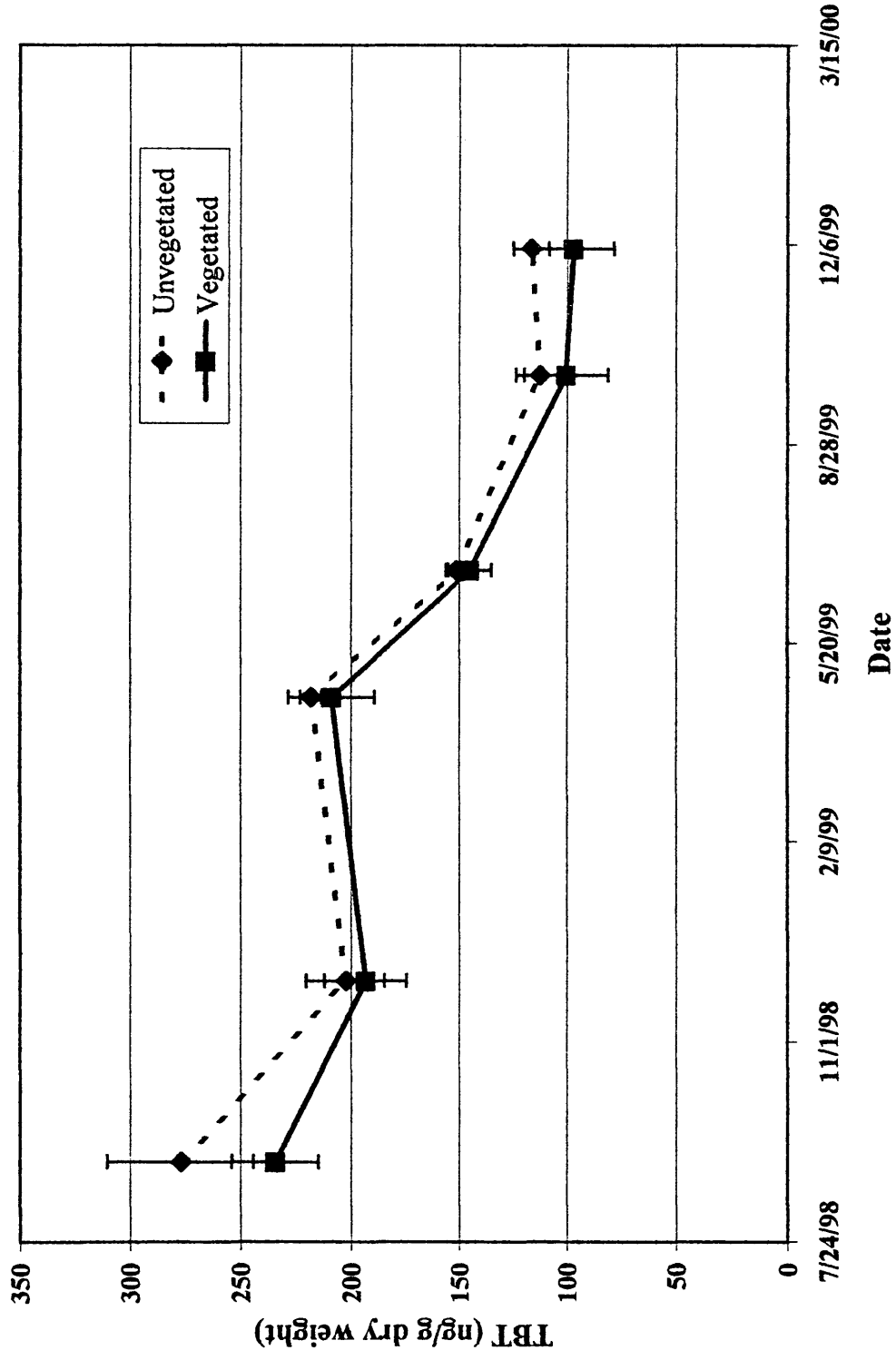
TBT levels in the vegetated bins ranged from 230-300ng/g dry weight, with an average of 250 ng/g at the beginning of the experiment. Unvegetated bin levels ranged from 240-310ng/g dry weight with an average of 280ng/g. These levels attained by the amendment of the sediment show that the spike resulted in concentrations near the target dose and the small range demonstrate that the sediment was well mixed. Though sediment samples were taken every month for 16 months, samples from only 6 dates were processed due to the time and cost of the analysis since a trend of disappearance could be established from these samples.

TBT disappearance was not linear throughout the experimental period. The greatest decrease occurred during the spring and summer of 1999. Over the first four months of the experiment (Sept. - Dec. 1998) TBT concentrations in both treatments fell to approximately 200ng/g, with the vegetated bins ranging from 170-220ng/g and the unvegetated bins ranging from 190-230ng/g (Figure 9). During the winter months, there was little change in the levels of TBT. With the onset of spring and the 1999 growing season, TBT sediment concentrations decreased once again. During the interval from April to June the TBT in the bins fell to an average of 150ng/g, ranging from 130-160ng/g and 140-160ng/g in vegetated and unvegetated bins respectively. A further decrease in TBT occurred over the second part of the growing season when TBT ranged from 83-130ng/g in the vegetated bins and 94-120ng/g in the unvegetated bins. From October 1999, until the experiment ended in December, little change was noted in the levels of TBT. There was no significant difference ($p>0.05$) between vegetated and unvegetated treatments in sediment TBT concentrations at

Figure 9. TBT concentrations in vegetated and unvegetated experimental bins in the created wetland over the course of the experiment



TBT in Experimental Bins



each of the sampling dates suggesting that the *S. alterniflora* was not contributing to the disappearance of TBT. The calculated half lives of TBT for the vegetated and unvegetated bins were not significantly different ($p>0.05$) at 300 days and 330 days respectively.

DBT demonstrated a different pattern of change over time compared to TBT, reflecting both its generation due to TBT degradation and the degradation of the DBT itself. DBT was present in the bins at the beginning of the experiment. In the vegetated bins there was an average of 30ng/g DBT (19-37ng/g) and in the unvegetated bins 50ng/g (41-75ng/g). Like TBT, the concentration of DBT decreased between September and December 1998 and remained at a stable level during the winter with averages at 24ng/g in the vegetated bins and 25ng/g in the unvegetated bins (Figure 10). An increase in DBT was then seen in both the vegetated and unvegetated bins with averages of 41ng/g and 34ng/g respectively. There was then a decrease of DBT in the bins through October to an average of 17ng/g in vegetated bins and 20ng/g in unvegetated bins. There was no change in these levels through December, the end of the experiment.

Butyltins in the Plants

Initially, in September 1999, butyltins in the plant tissues were below the detection limit but over time TBT was at measurable concentrations in the plants. By June 1999 the levels of TBT had risen to an average of 1.2ng/g (Figure 11) in the aboveground material and to 26ng/g in the belowground material (Table 1). These levels remained constant through the remainder of the study period. DBT and MBT in the aboveground plant tissue remained below the detection limit throughout the experiment.

Figure 10. DBT concentrations in vegetated and unvegetated experimental bins in the created wetland over the course of the experiment

DBT in Experimental Bins

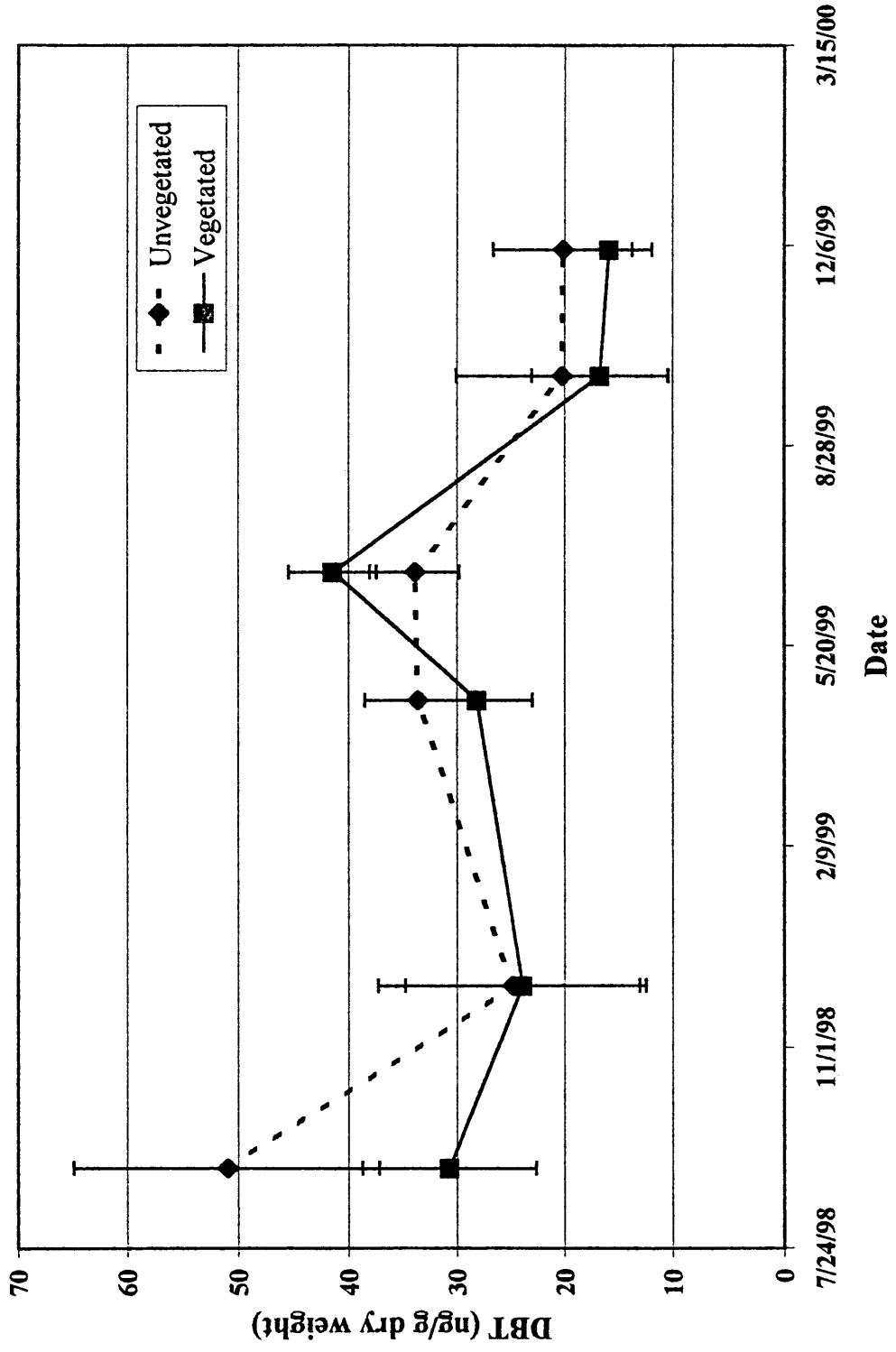


Figure 11. Butyltin concentrations in *S. alterniflora* aboveground biomass from the experimental bins

TBT in Plant Tissue from Experimental Bins

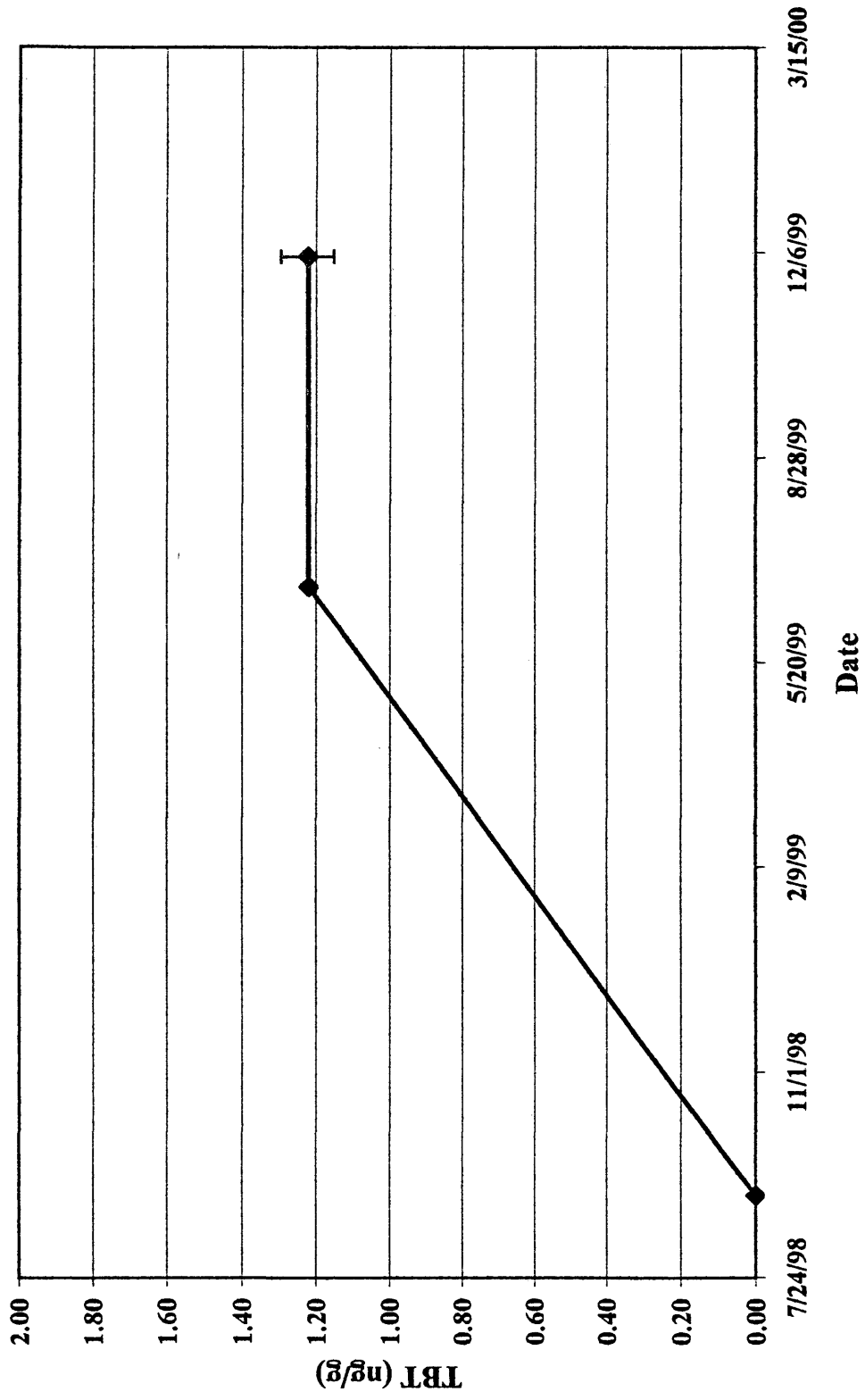


Table 1. Butyltin concentrations in *S. alterniflora* belowground biomass from the experimental bins

Butyltin Concentrations in *Spartina alterniflora*
Belowground Biomass
(concentrations in ng/g wet weight)

| Sample | Date | TBT | DBT |
|---------------|-------------|------------|------------|
| Cumulative | 6/26/99 | 26 | ND |
| A3 VEG-T | 12/4/99 | 27 | 2.3 |
| B4 VEG-T | 12/4/99 | 34 | 2.8 |
| C1 VEG-T | 12/4/99 | 52 | 3.1 |
| D2 VEG-T | 12/4/99 | 48 | 2.2 |
| E4 VEG-T | 12/4/99 | 54 | 2.6 |

Sediment Nutrients Data

Nutrient concentrations (Table 2) varied with season but overall the vegetated bins and the surrounding marsh were similar ($p > 0.05$) while the concentrations of nutrients were higher in the unvegetated bins ($p < 0.05$) compared to the vegetated bins and the surrounding marsh. Concentrations of nitrite in the sediment was determined to be minimal ($0.0\text{--}0.08\mu\text{mole/m}^2$). Mean NO_x concentrations did not vary significantly (Table 2; $p > 0.05$) with respect to the treatment but did vary significantly ($p < 0.05$) over time at the 0-2cm ($p < 0.05$) and the 5-10cm ($p < 0.05$) depth intervals. Mean NH_4 concentrations did vary significantly ($p < 0.05$) among both treatment and date at each of the three depth intervals (except for the 0-2cm depth interval with respect to date). There were significant differences between the vegetated and unvegetated treatments, but no effect of TBT. The unvegetated treatments had much higher concentrations of NH_4 compared to vegetated treatments and were similar to sediments in the surrounding created marsh ($p > 0.05$).

Sediment nutrient data were also analyzed for each of the quarterly sampling dates in order to minimize the temporal variability in the data, as well as highlight differences due to the treatments which each bin represented. A summary of each quarterly nutrient concentration is presented in Table 3. In February 1999, the NO_x concentrations did not vary significantly between vegetated and unvegetated or TBT contaminated and uncontaminated treatments but NH_4 showed a significant effect of treatment over the 2-5cm and 5-10cm depth intervals with the higher concentration in the unvegetated versus vegetated treatments ($p > 0.05$). There was no TBT effect ($p > 0.05$). The same results were found for the May 1999 nutrient data. In August 1999 NO_x data demonstrated no significant difference

Table 2. Summary of annual means and ANOVA results for sediment nutrient data from the experimental bins and the surrounding created wetland

Sediment Nutrient Data - 1999 (All Dates)

(nutrient concentrations in mmole/m³)

Annual Mean Nitrate/Nitrite Concentration

| Depth | Treatment | | | | |
|--------|-----------|--------|-------|--------|-------|
| | UNV-T | UNV-NT | VEG-T | VEG-NT | MARSH |
| 0-2 cm | 39.93 | 76.97 | 44.72 | 77.17 | 39.06 |
| 2-5 cm | 36.17 | 50.12 | 68.77 | 64.53 | 29.49 |
| 5-10cm | 124.90 | 39.56 | 41.53 | 51.06 | 60.83 |

Nitrate/Nitrite ANOVA

| Depth | Date Effect | | | Treatment Effect | | |
|--------|-------------|-------|--------|------------------|--------|--------|
| | df | F | P | df | F | P |
| 0-2 cm | 80 | 5.215 | 0.0024 | 80 | 1.537 | 0.1993 |
| 2-5 cm | 80 | 1.798 | 0.1543 | 80 | 0.4088 | 0.8018 |
| 5-10cm | 80 | 7.298 | 0.0002 | 80 | 1.1019 | 0.3615 |

Annual Mean Ammonium Concentration

| Depth | Treatment | | | | |
|--------|-----------|---------|--------|--------|--------|
| | UNV-T | UNV-NT | VEG-T | VEG-NT | MARSH |
| 0-2 cm | 1639.49 | 1773.55 | 808.12 | 744.25 | 678.12 |
| 2-5 cm | 4531.07 | 4454.30 | 660.07 | 790.84 | 556.30 |
| 5-10cm | 8645.07 | 8509.19 | 764.90 | 921.03 | 644.29 |

Ammonium ANOVA

| Depth | Date Effect | | | Treatment Effect | | |
|--------|-------------|--------|--------|------------------|----------|--------|
| | df | F | P | df | F | P |
| 0-2 cm | 80 | 1.0378 | 0.3805 | 80 | 6.8061 | 0.0001 |
| 2-5 cm | 80 | 3.1193 | 0.0306 | 80 | 40.75661 | >.0001 |
| 5-10cm | 80 | 3.8801 | 0.0121 | 80 | 30.5706 | >.0001 |

Table 3. Summary of quarterly means and ANOVA results for sediment nutrient data from the experimental bins and the surrounding created wetland

Quarterly Sediment Nutrient Data - Feb. 1999

(nutrient concentrations in mmole/m³)

February 1999 Mean Nitrate/Nitrite Concentration (S.E.)

| Depth | Treatment | | | | |
|--------|------------|------------|--------------|--------------|------------|
| | UNV-T | UNV-NT | VEG-T | VEG-NT | MARSH |
| 0-2 cm | 17.85 (15) | 27.88 (14) | 20.37 (15) | 92.00 (68) | 35.49 (12) |
| 2-5 cm | 7.88 (4) | 25.37 (22) | 172.57 (170) | 149.25 (144) | 14.13 (10) |
| 5-10cm | 34.58 (21) | 2.26 (2) | 8.57 (5) | 5.37 (5) | 67.19 (57) |

February 1999 Nitrate/Nitrite ANOVA

| Depth | Treatment Effect | | |
|--------|------------------|--------|--------|
| | df | F | P |
| 0-2 cm | 20 | 0.8660 | 0.5013 |
| 2-5 cm | 20 | 0.6555 | 0.6299 |
| 5-10cm | 20 | 1.0155 | 0.4231 |

February 1999 Mean Ammonium Concentration (S.E.)

| Depth | Treatment | | | | |
|--------|---------------|---------------|--------------|---------------|--------------|
| | UNV-T | UNV-NT | VEG-T | VEG-NT | MARSH |
| 0-2 cm | 1890.0 (1310) | 1136.4(79) | 695.2 (1352) | 859.3 (2457) | 738.0 (2629) |
| 2-5 cm | 4502.0 (3361) | 4379.6 (495) | 1044.0 (47) | 1181.2 (2695) | 606.9 (573) |
| 5-10cm | 9497.0 (158) | 12991.4 (867) | 857.2 (304) | 1780.0 (86) | 721.1 (3283) |

February 1999 Ammonium ANOVA

| Depth | Treatment Effect | | |
|--------|------------------|---------|--------|
| | df | F | P |
| 0-2 cm | 20 | 1.3268 | 0.2943 |
| 2-5 cm | 20 | 11.9610 | >.0001 |
| 5-10cm | 20 | 11.0227 | >.0001 |

Quarterly Sediment Nutrient Data - May 1999

(nutrient concentrations in mmole/m²)

May 1999 Mean Nitrate/Nitrite Concentration (S.E.)

| Depth | Treatment | | | | |
|--------|------------|------------|------------|------------|------------|
| | UNV-T | UNV-NT | VEG-T | VEG-NT | MARSH |
| 0-2 cm | 27.41 (12) | 63.26 (40) | 43.59 (25) | 67.86 (37) | 18.61 (10) |
| 2-5 cm | 38.75 (31) | 50.46 (31) | 22.25 (13) | 22.9 (6) | 3.77 (2) |
| 5-10cm | 16.77 (11) | 51.53 (47) | 3.59 (2) | 40.66 (34) | 22.22 (17) |

May 1999 Nitrate/Nitrite ANOVA

| Depth | Treatment Effect | | |
|--------|------------------|--------|--------|
| | df | F | P |
| 0-2 cm | 20 | 0.6180 | 0.6548 |
| 2-5 cm | 20 | 0.7417 | 0.5748 |
| 5-10cm | 20 | 0.4836 | 0.7475 |

May 1999 Mean Ammonium Concentration (S.E.)

| Depth | Treatment | | | | |
|--------|---------------|---------------|--------------|--------------|--------------|
| | UNV-T | UNV-NT | VEG-T | VEG-NT | MARSH |
| 0-2 cm | 994.2 (1574) | 1073.8 (178) | 956.3 (1588) | 801.0 (943) | 658.2 (1610) |
| 2-5 cm | 4516.6 (3598) | 4143.5 (1604) | 625.8 (61) | 674.0 (1940) | 617.5 (137) |
| 5-10cm | 9850.3 (243) | 6697.9 (1893) | 968.2 (81) | 814.7 (69) | 850.7 (1061) |

May 1999 Ammonium ANOVA

| Depth | Treatment Effect | | |
|--------|------------------|---------|--------|
| | df | F | P |
| 0-2 cm | 20 | 2.0912 | 0.1199 |
| 2-5 cm | 20 | 18.6698 | >.0001 |
| 5-10cm | 20 | 7.6488 | 0.0006 |

Quarterly Sediment Nutrient Data - Aug.1999

(nutrient concentrations in mmole/m²)

August 1999 Mean Nitrate/Nitrite Concentration (S.E.)

| Depth | Treatment | | | | |
|--------|-----------|------------|-----------|------------|------------|
| | UNV-T | UNV-NT | VEG-T | VEG-NT | MARSH |
| 0-2 cm | 26.94 (8) | 59.33 (28) | 35.29 (8) | 30.17 (10) | 25.02 (9) |
| 2-5 cm | 25.04 (6) | 11.83 (9) | 15.88 (4) | 18.3 (5) | 14.16 (10) |
| 5-10cm | 9.82 (5) | 13.16 (12) | 4.03 (4) | 34.25 (26) | 30.69 (9) |

August 1999 Nitrate/Nitrite ANOVA

| Depth | Treatment Effect | | |
|--------|------------------|--------|--------|
| | df | F | P |
| 0-2 cm | 20 | 0.8901 | 0.4879 |
| 2-5 cm | 20 | 0.4917 | 0.7418 |
| 5-10cm | 20 | 0.9405 | 0.4609 |

August 1999 Mean Ammonium Concentration (S.E.)

| Depth | Treatment | | | | |
|--------|---------------|---------------|--------------|--------------|--------------|
| | UNV-T | UNV-NT | VEG-T | VEG-NT | MARSH |
| 0-2 cm | 1893.6 (1509) | 2010.9 (373) | 1005.9 (169) | 702.6 (1190) | 898.4 (1268) |
| 2-5 cm | 5934.6 (4512) | 6047.5 (2369) | 597.2 (92) | 797.4 (2413) | 580.2 (45) |
| 5-10cm | 11087.9 (157) | 9771.9 (1644) | 684.7 (108) | 683.7 (166) | 560.7 (2249) |

August 1999 Ammonium ANOVA

| Depth | Treatment Effect | | |
|--------|------------------|--------|--------|
| | df | F | P |
| 0-2 cm | 20 | 4.9088 | 0.0064 |
| 2-5 cm | 20 | 9.4711 | 0.0002 |
| 5-10cm | 20 | 7.6296 | 0.0007 |

Quarterly Sediment Nutrient Data - Nov. 1999

(nutrient concentrations in mmole/m²)

November 1999 Mean Nitrate/Nitrite Concentration (S.E.)

| Depth | Treatment | | | | |
|--------|--------------|-------------|-------------|-------------|-------------|
| | UNV-T | UNV-NT | VEG-T | VEG-NT | MARSH |
| 0-2 cm | 87.52 (19) | 157.41 (75) | 79.61 (11) | 118.66 (61) | 77.11 (17) |
| 2-5 cm | 73.02 (12) | 112.82 (65) | 64.35 (31) | 67.67 (27) | 85.9 (11) |
| 5-10cm | 438.43 (285) | 91.28 (16) | 149.91 (19) | 123.96 (18) | 123.35 (11) |

November 1999 Nitrate/Nitrite ANOVA

| Depth | Treatment Effect | | |
|--------|------------------|--------|--------|
| | df | F | P |
| 0-2 cm | 20 | 0.5799 | 0.6806 |
| 2-5 cm | 20 | 0.3190 | 0.8619 |
| 5-10cm | 20 | 1.2454 | 0.3239 |

November 1999 Mean Ammonium Concentration (S.E.)

| Depth | Treatment | | | | |
|--------|--------------|---------------|------------|-------------|-------------|
| | UNV-T | UNV-NT | VEG-T | VEG-NT | MARSH |
| 0-2 cm | 1780.2 (651) | 2873.2 (1240) | 575.1 (69) | 614.2 (71) | 417.62 (86) |
| 2-5 cm | 3170.9 (540) | 3246.6 (1073) | 373.3 (82) | 510.8 (124) | 420.6 (78) |
| 5-10cm | 4145.2 (700) | 4578.5 (1440) | 549.6 (76) | 405.7 (99) | 444.7 (75) |

November 1999 Ammonium ANOVA

| Depth | Treatment Effect | | |
|--------|------------------|--------|--------|
| | df | F | P |
| 0-2 cm | 20 | 2.8384 | 0.0515 |
| 2-5 cm | 20 | 7.8923 | 0.0005 |
| 5-10cm | 20 | 8.9083 | 0.0003 |

between treatments but NH_4 concentrations were significantly different between vegetated and unvegetated treatments at all depth increments. There was no effect of TBT seen on the NH_4 concentrations. The same was true for the November 1999 data. Vegetated and marsh samples were not significantly different in nutrient concentration at all sampling dates showing that as far as the sediment nutrient environment is concerned, the experimental bins and surrounding created marsh were similar.

Redox Potential

Trends in redox show a higher, less negative redox potential in the vegetated treatments and in the marsh outside of the bins compared to the unvegetated treatments. Redox potential decreased with depth in all treatments. Redox measurements from April, August, and October 1999 (Figure 12) are representative of conditions throughout the growing season. Because of the spatial variation and the range of values of the redox data (Table 4), there appears to be few differences between the treatments as determined by ANOVA (Table 5). In April 1999, there were significant differences in redox at the 1cm, 2cm, and 5cm depths between the vegetated and unvegetated plots. At the 10cm depth there were significantly lower redox potentials in the unvegetated treatments and in the surrounding marsh ($p < 0.05$). In July, at the 10cm depth sediment redox potentials in the unvegetated treatments and the surrounding marsh were significantly different ($p < 0.05$).

Figure 12. Redox potential profiles for the vegetated and unvegetated experimental bins and the surrounding created wetland April, August, and October 1999.

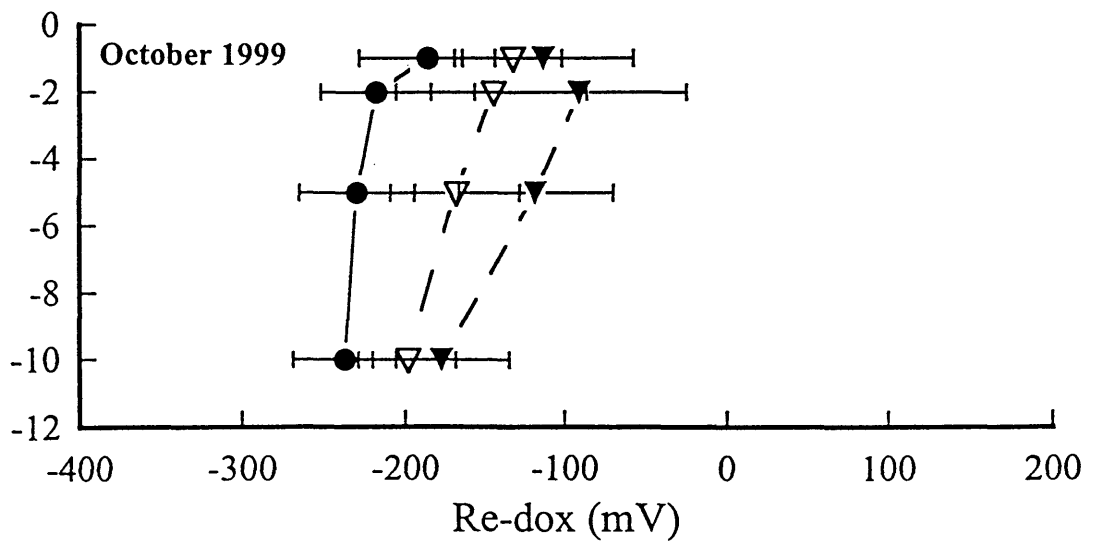
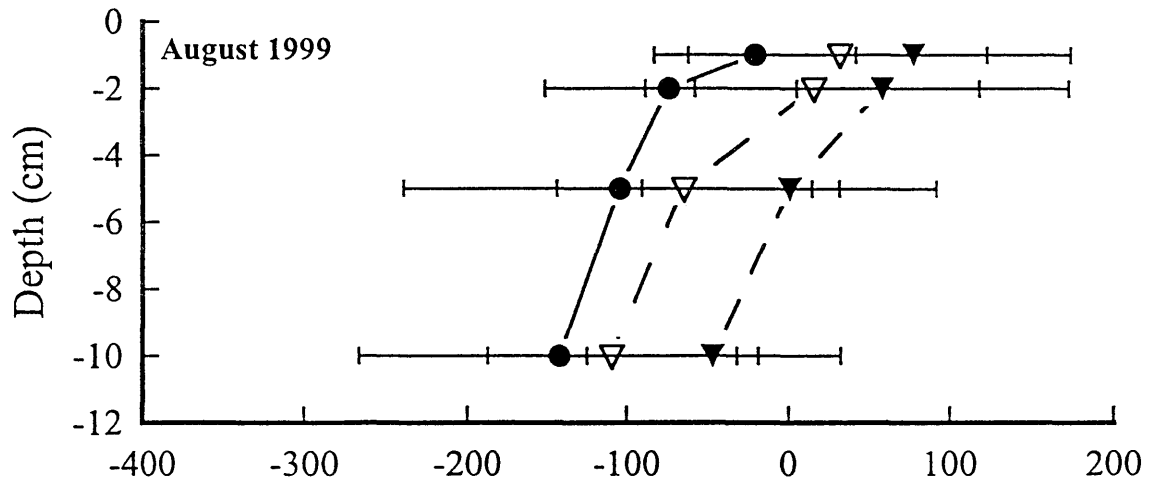
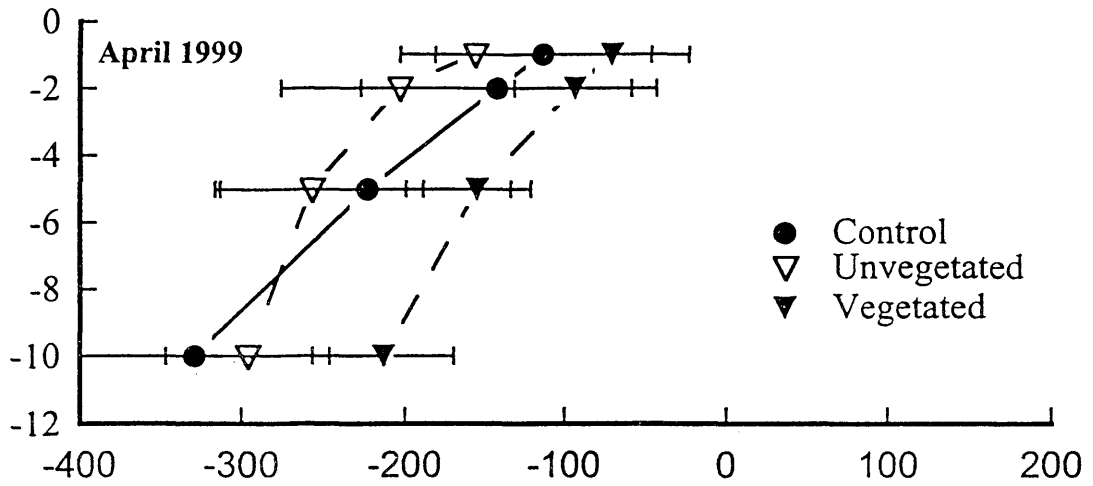


Table 4. Monthly redox mean values at four depth intervals in the vegetated and unvegetated experimental bins and the surrounding created wetland

Monthly Re-Dox Mean Values

April 1999 Mean Re-Dox Values

| Depth | Treatment | | | | | |
|-------|-----------|------------|---------|------------|---------|------------|
| | UNV-T | UNV-T S.E. | VEG-T | VEG-T S.E. | MARSH | MARSH S.E. |
| 1 cm | -156.60 | 20.68 | -70.08 | 21.13 | -113.60 | 30.17 |
| 2 cm | -203.67 | 32.24 | -93.00 | 22.47 | -142.67 | 37.81 |
| 5 cm | -257.60 | 26.17 | -155.00 | 15.08 | -223.33 | 39.98 |
| 10 cm | -296.33 | 22.35 | -213.17 | 19.52 | -328.73 | 52.12 |

July 1999 Mean Re-Dox Values

| Depth | Treatment | | | | | |
|-------|-----------|------------|--------|------------|---------|------------|
| | UNV-T | UNV-T S.E. | VEG-T | VEG-T S.E. | MARSH | MARSH S.E. |
| 1 cm | 112.83 | 25.57 | 133.70 | 33.56 | 107.63 | 24.5 |
| 2 cm | 80.23 | 30.76 | 83.77 | 61.2 | 40.37 | 40.93 |
| 5 cm | 33.77 | 36.25 | 6.90 | 50.84 | -45.10 | 53.82 |
| 10 cm | -5.37 | 30.69 | -44.3 | 43.97 | -156.17 | 98.62 |

June 1999 Mean Re-Dox Values

| Depth | Treatment | | | | | |
|-------|-----------|------------|--------|------------|--------|------------|
| | UNV-T | UNV-T S.E. | VEG-T | VEG-T S.E. | MARSH | MARSH S.E. |
| 1 cm | 46.13 | 41.21 | 69.07 | 35.31 | 41.67 | 37.16 |
| 2 cm | 25.73 | 40.8 | 46.87 | 34.28 | 8.40 | 37.15 |
| 5 cm | -27.53 | 33.51 | -27.47 | 48.05 | -49.60 | 38.41 |
| 10 cm | -72.4 | 24.77 | -59.2 | 37.2 | -108.4 | 38.78 |

August 1999 Mean Re-Dox Values

| Depth | Treatment | | | | | |
|-------|-----------|------------|--------|------------|---------|------------|
| | UNV-T | UNV-T S.E. | VEG-T | VEG-T S.E. | MARSH | MARSH S.E. |
| 1 cm | 30.73 | 41.23 | 77.27 | 43.13 | -20.60 | 27.73 |
| 2 cm | 14.80 | 46.14 | 57.53 | 51.42 | -73.60 | 35.05 |
| 5 cm | -64.87 | 35.47 | 0.67 | 40.61 | -104.20 | 60.5 |
| 10 cm | -109.87 | 34.82 | -46.53 | 35.18 | -142.67 | 55.41 |

October 1999 Mean Re-Dox Values

| Depth | Treatment | | | | | |
|-------|-----------|------------|---------|------------|---------|------------|
| | UNV-T | UNV-T S.E. | VEG-T | VEG-T S.E. | MARSH | MARSH S.E. |
| 1 cm | -132.70 | 14.06 | -112.80 | 25.09 | -185.70 | 18.84 |
| 2 cm | -145.30 | 26.7 | -90.33 | 29.59 | -217.50 | 15.16 |
| 5 cm | -168.37 | 18.11 | -117.73 | 21.69 | -229.43 | 15.85 |
| 10 cm | -198.57 | 13.56 | -177.27 | 19.07 | -237.1 | 14.17 |

Table 5. Monthly redox ANOVA results at four depth intervals in the vegetated and unvegetated experimental bins and the surrounding created wetland

Monthly Re-Dox ANOVA Results

April 1999 Re-Dox ANOVA

| Depth | Treatment Effect | | |
|-------|------------------|--------|--------|
| | df | F | P |
| 1 cm | 11 | 7.2177 | 0.0099 |
| 2 cm | 11 | 5.3950 | 0.0233 |
| 5 cm | 11 | 6.8327 | 0.0118 |
| 10 cm | 11 | 6.7955 | 0.012 |

July 1999 Re-Dox ANOVA

| Depth | Treatment Effect | | |
|-------|------------------|--------|--------|
| | df | F | P |
| 1 cm | 12 | 0.4041 | 0.6764 |
| 2 cm | 12 | 0.3848 | 0.6887 |
| 5 cm | 12 | 1.1079 | 0.3618 |
| 10 cm | 12 | 6.2577 | 0.0138 |

June 1999 Re-Dox ANOVA

| Depth | Treatment Effect | | |
|-------|------------------|--------|--------|
| | df | F | P |
| 1 cm | 12 | 0.2671 | 0.7700 |
| 2 cm | 12 | 0.3420 | 0.7170 |
| 5 cm | 12 | 0.1462 | 0.8655 |
| 10 cm | 12 | 0.6873 | 0.5217 |

August 1999 Re-Dox ANOVA

| Depth | Treatment Effect | | |
|-------|------------------|--------|--------|
| | df | F | P |
| 1 cm | 12 | 4.2245 | 0.0408 |
| 2 cm | 12 | 5.7031 | 0.0182 |
| 5 cm | 12 | 1.4808 | 0.2662 |
| 10 cm | 12 | 1.6778 | 0.2278 |

October 1999 Re-Dox ANOVA

| Depth | Treatment Effect | | |
|-------|------------------|--------|--------|
| | df | F | P |
| 1 cm | 12 | 1.1444 | 0.3508 |
| 2 cm | 12 | 3.0138 | 0.0869 |
| 5 cm | 12 | 2.5742 | 0.1174 |
| 10 cm | 12 | 2.0076 | 0.177 |

Biomass Data

Regression to Estimate Biomass

Non-destructive shoot morphometric measurements were compared to measured shoot biomass to develop a predictive model of plant biomass. Total stem length was found to be the best predictor of biomass ($r^2=0.804$; Figure 13). This relationship ($y=0.0019x^{1.7974}$) was therefore used to predict shoot biomass and growth from the measurements of shoot height.

Monthly Field Measurements

Results indicated a gradual decrease in mean *S. alterniflora* shoot density and shoot height over time during the fall of 1998 (Table 6). There were no significant differences ($p>0.05$) between plants growing in TBT and non-TBT amended sediments. During the 1999 growing season the mean number of shoots per meter squared decreased over the growing season as the mean shoot height increased (Figure 14). Shoot biomass increased from April to June, then decreased during July and increased again through September. Final winter dieback of shoot material was evident in November and December (Figure 15). Growth parameters paralleled this biomass trend. There were no significant differences ($p>0.05$) observed in the monthly measured shoot morphometric and biomass parameters between the TBT treatments (Table 7). Belowground biomass was measured at the end of the experiment only. Belowground biomass ranged from 620-3100g dry wt/m². Mean belowground biomass was 1700g dry wt/m² and 1900g dry wt/m² for the TBT and non TBT amended treatments were not significantly different ($p>0.05$).

Figure 13. Regression relating *S. alterniflora* stem length to shoot biomass

Stem Length vs Shoot Biomass

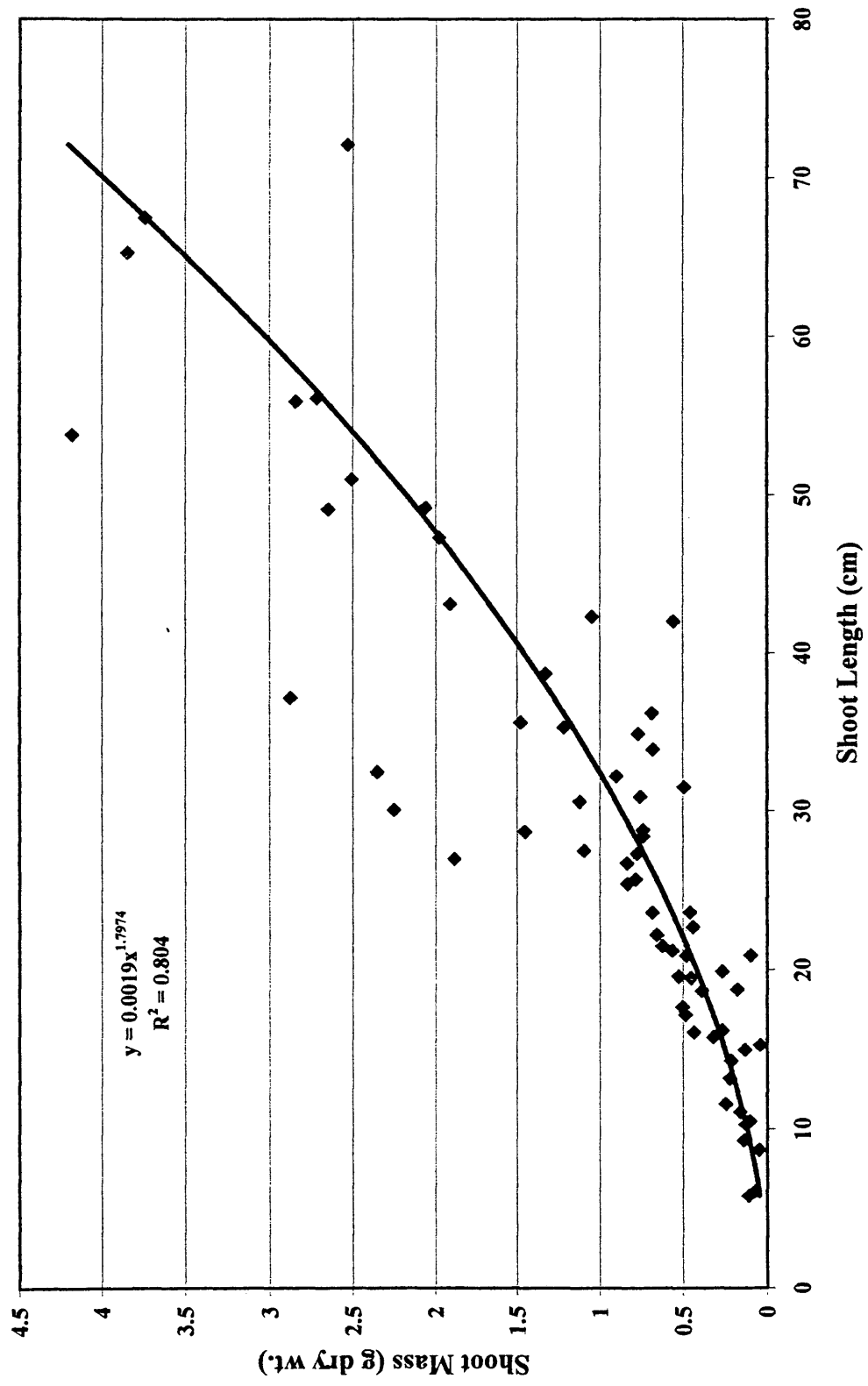


Table 6. *S. alterniflora* average stem length and shoot density for September to December 1998

Spartina alterniflora Data - 1998

Parameters for new growth shoots only

Monthly Treatment Means for Biomass Parameters

| Parameters | 10/8/98 | | 11/4/98 | | 12/2/98 | |
|-----------------------|---------|--------|---------|--------|---------|--------|
| | TBT | No TBT | TBT | No TBT | TBT | No TBT |
| Shoots/m ² | 172.17 | 129.21 | 116.21 | 116.21 | 94.69 | 81.78 |
| Mean Shoot Ht. (cm) | 7.40 | 5.80 | 6.50 | 6.40 | 3.90 | 4.1 |

Figure 14. *S. alterniflora* density and canopy height during the 1999 growing season

Spartina alterniflora Density and Canopy Height

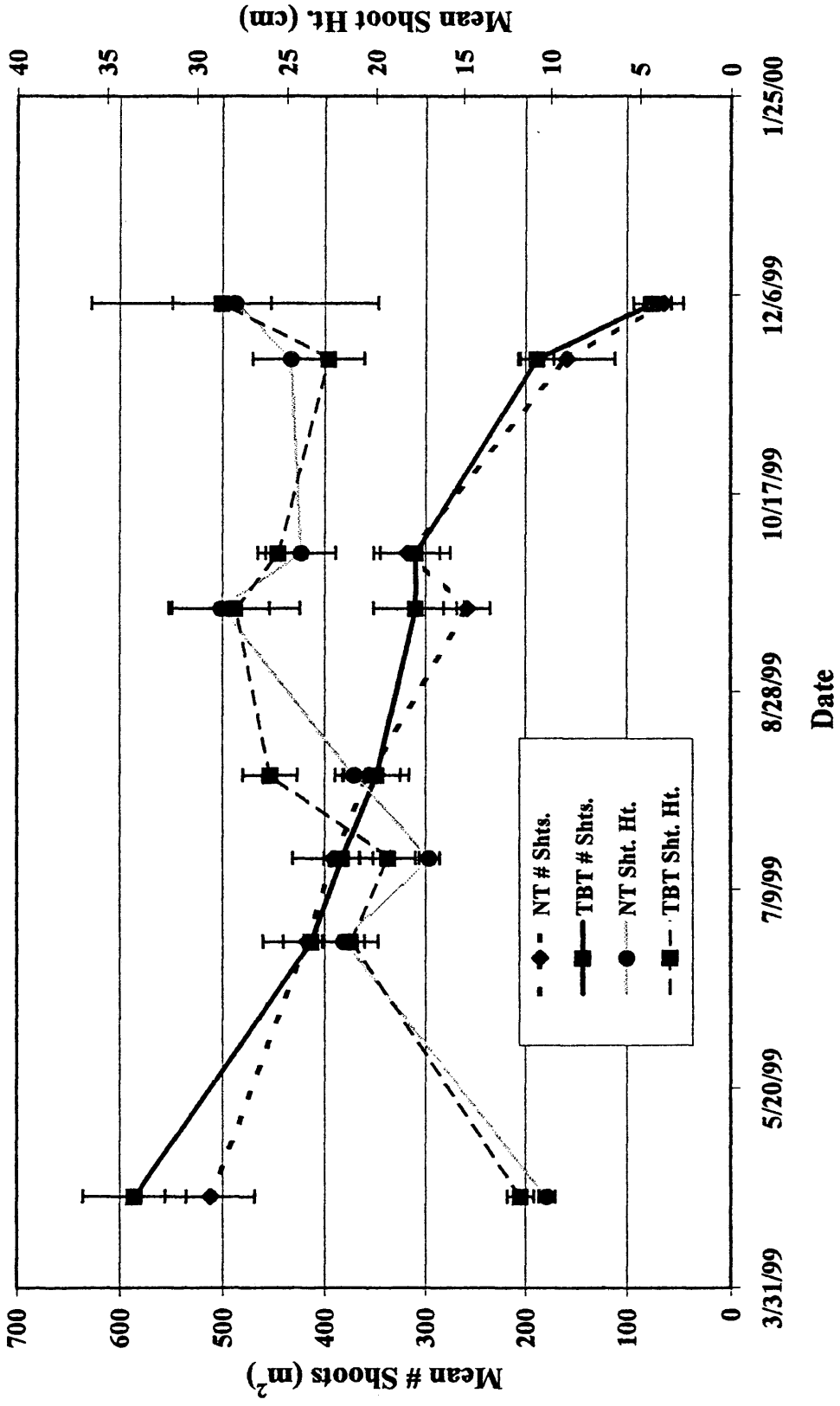


Figure 15. *S. alterniflora* biomass during the 1999 growing season

Spartina alterniflora Biomass

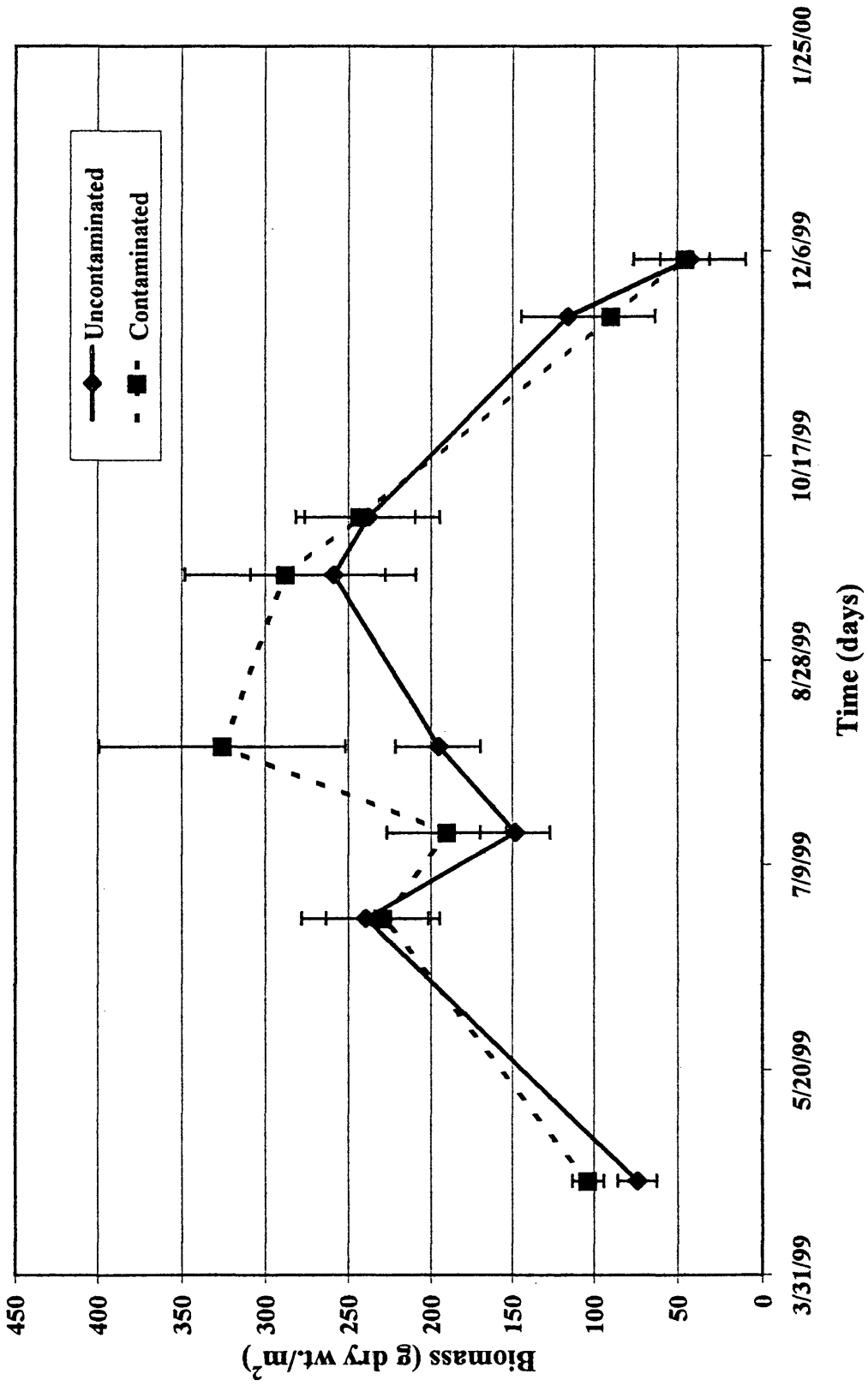


Table 7. *S. alterniflora* biomass parameters - monthly means and ANOVA results

***Spartina alterniflora* Data - 1999**
Means in experimental bins by date and treatment

Monthly Treatment Means for Biomass Parameters

| Parameters | 4/23/99 | | 6/26/99 | | 7/17/99 | | 8/7/99 | |
|----------------------------|---------|--------|---------|--------|---------|--------|--------|--------|
| | TBT | No TBT | TBT | No TBT | TBT | No TBT | TBT | No TBT |
| Shoots/m ² | 585.55 | 512.36 | 413.33 | 417.64 | 383.19 | 391.8 | 348.75 | 357.36 |
| Mean Shoot Ht. (cm) | 11.70 | 10.21 | 21.41 | 21.78 | 19.29 | 16.9 | 25.93 | 21.2 |
| Biomass/m ² | 103.68 | 74.06 | 229.05 | 239.82 | 190.13 | 148.46 | 325.37 | 195.26 |
| Growth/m ² /day | - | - | - | - | 3.86 | 3.15 | 8.71 | 6.37 |

| Parameters | 9/18/99 | | 10/2/99 | | 11/20/99 | | 12/4/99 | |
|----------------------------|---------|--------|---------|--------|----------|--------|---------|--------|
| | TBT | No TBT | TBT | No TBT | TBT | No TBT | TBT | No TBT |
| Shoots/m ² | 310.00 | 258.33 | 310.00 | 318.61 | 188.37 | 159.3 | 75.35 | 64.58 |
| Mean Shoot Ht. (cm) | 27.98 | 28.66 | 25.46 | 24.20 | 22.63 | 24.75 | 28.62 | 27.85 |
| Biomass/m ² | 287.83 | 259.06 | 243.00 | 238.01 | 89.68 | 116.12 | 45.25 | 42.96 |
| Growth/m ² /day | 2.5 | 3.15 | 7.13 | 7.29 | 0.69 | 0.95 | 3.02 | 1.87 |

Monthly Treatment ANOVA Results for Biomass Parameters

| Parameters | 4/23/99 | | | 6/26/99 | | | 7/17/99 | | |
|----------------------------|---------|--------|--------|---------|--------|--------|---------|--------|--------|
| | df | F | P | df | F | P | df | F | P |
| Shoots/m ² | 9 | 1.023 | 0.4927 | 9 | 0.8897 | 0.571 | 9 | 0.8932 | 0.5688 |
| Mean Shoot Ht. (cm) | 9 | 1.1972 | 0.4052 | 9 | 0.8928 | 0.5691 | 9 | 1.1089 | 0.4474 |
| Biomass/m ² | 9 | 1.3201 | 0.353 | 9 | 0.8937 | 0.5685 | 9 | 0.9959 | 0.5078 |
| Growth/m ² /day | - | - | - | - | - | - | 9 | 1.0933 | 0.4553 |

| Parameters | 8/7/99 | | | 9/18/99 | | | 10/2/99 | | |
|----------------------------|--------|--------|--------|---------|--------|--------|---------|--------|--------|
| | df | F | P | df | F | P | df | F | P |
| Shoots/m ² | 9 | 0.8928 | 0.5691 | 9 | 1.0179 | 0.4954 | 9 | 0.8924 | 0.5693 |
| Mean Shoot Ht. (cm) | 9 | 1.8139 | 0.2069 | 9 | 0.8921 | 0.5695 | 9 | 0.9233 | 0.5503 |
| Biomass/m ² | 9 | 1.1962 | 0.4056 | 9 | 0.9039 | 0.5622 | 9 | 0.8898 | 0.5709 |
| Growth/m ² /day | 9 | 1.0115 | 0.499 | 9 | 0.9727 | 0.521 | 9 | 0.8907 | 0.5704 |

| Parameters | 11/20/99 | | | 12/4/99 | | |
|----------------------------|----------|--------|--------|---------|--------|--------|
| | df | F | P | df | F | P |
| Shoots/m ² | 9 | 0.891 | 0.5702 | 9 | 0.945 | 0.5373 |
| Mean Shoot Ht. (cm) | 9 | 1.0672 | 0.4689 | 9 | 0.9302 | 0.5462 |
| Biomass/m ² | 9 | 0.9455 | 0.537 | 9 | 0.8896 | 0.5711 |
| Growth/m ² /day | 9 | 1.0584 | 0.4735 | 9 | 0.9546 | 0.5316 |

PAM Results

In situ plant stress measurements of photosystem effective quantum yield and maximum quantum yield were obtained during the perceived peak of biomass in August 1999 with the Pulse Amplitude Modulated (PAM) fluorometer. The measurements revealed no significant effect of TBT on the plants, even in the high dose bins (TBT > 400ng/g). Effective quantum yields ranged from 0.663 to 0.747 and maximum quantum yields from 0.707 to 0.768. Typically maximum quantum yields of 0.75-0.85 are considered representative of non-stressed conditions. The data from the PAM is summarized in Table 8.

Plant Degradation Laboratory Experiment

Butyltins in the Water

During the course of the laboratory experiment, TBT and DBT were observed to be released into the water in the aerobic treatments (Figure 16). Only data from the aerobic treatments is available due to an analytical problem with TBT extraction in the anaerobic water samples. An average of 13ng of TBT was released into the water during the first twelve days of the experiment with a range of 14-17ng. After 25 days a the total released averaged of 14ng. This level remained constant through the end of the experiment. This represents a release of approximately 20% of the initial level of TBT into the water (Figure 17). This did not, however, account for the TBT lost to degradation which was approximately 10% of the initial TBT. DBT followed the same trend with an average of 7ng released over the first 12 days. This amount increased to 7.5ng after 24 days and to 9.0ng

Table 8. Summary of pulse modulated amplitude fluorometry

Summary of PAM Data - August 1999

| Bin & Treatment | Effective Quantum Yield | Effective QY Std. Error | Maximum Quantum Yield | Maximum QY Std. Error |
|-----------------|----------------------------|----------------------------|--------------------------|--------------------------|
| A2 VEG-NT | 0.747 | 0.003 | 0.768 | 0.007 |
| A3 VEG-T | 0.740 | 0.005 | 0.765 | 0.016 |
| B1 VEG-NT | 0.737 | 0.006 | 0.764 | 0.006 |
| B4 VEG-T | 0.746 | 0.009 | 0.777 | 0.007 |
| C1 VEG-T | 0.735 | 0.008 | 0.763 | 0.006 |
| C2 VEG-NT | 0.730 | 0.010 | 0.765 | 0.008 |
| D2 VEG-T | 0.741 | 0.009 | 0.768 | 0.006 |
| D4 VEG-NT | 0.731 | 0.012 | 0.767 | 0.009 |
| E1 VEG-NT | 0.743 | 0.005 | 0.718 | 0.011 |
| E4 VEG-T | 0.663 | 0.018 | 0.707 | 0.009 |
| HIGH DOSE #1 | 0.672 | 0.017 | 0.713 | 0.017 |
| HIGH DOSE #2 | 0.716 | 0.012 | 0.735 | 0.013 |

Figure 16. Cumulative TBT released into the water during the plant degradation experiment

Butyltins in Aerobic Water

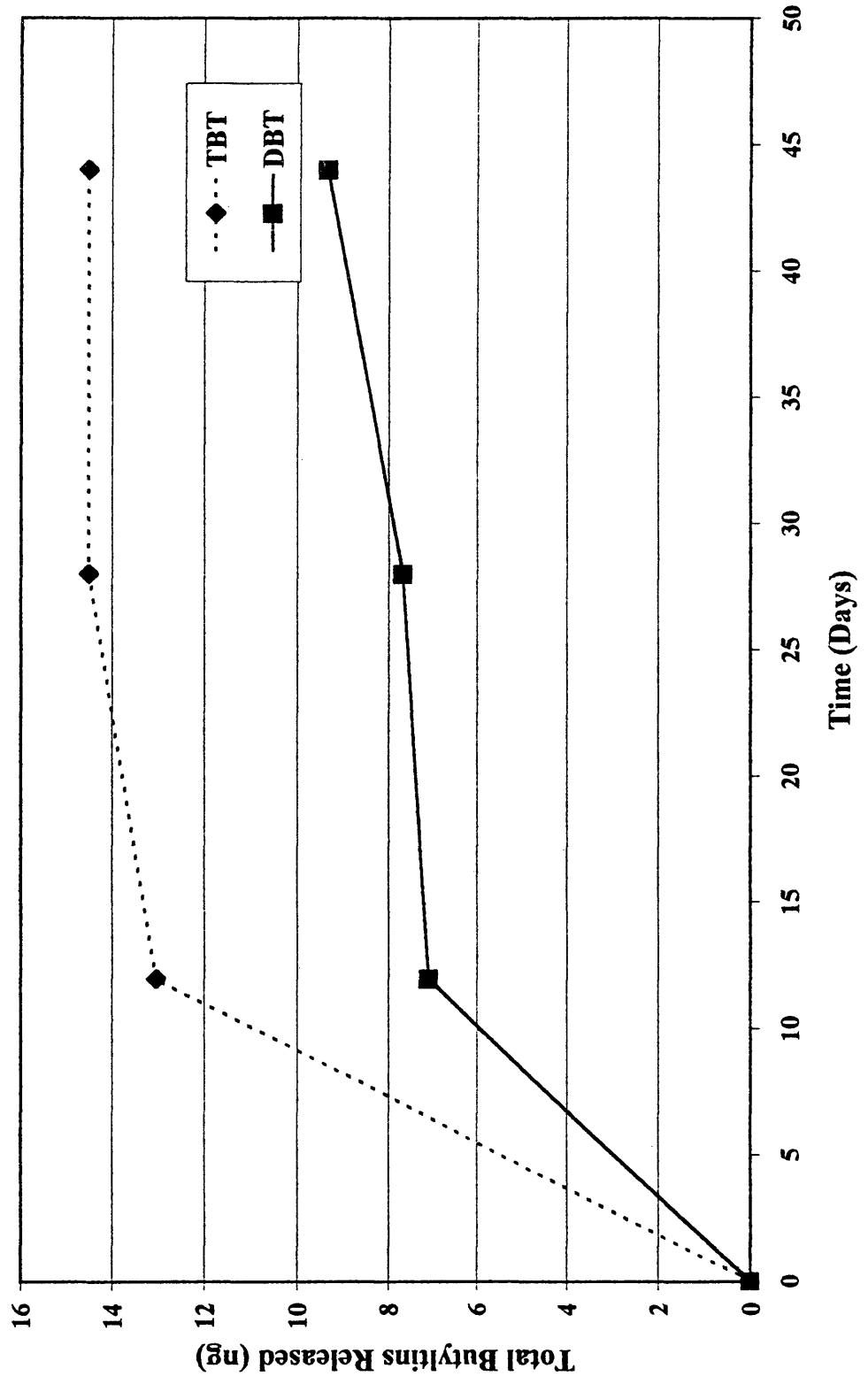
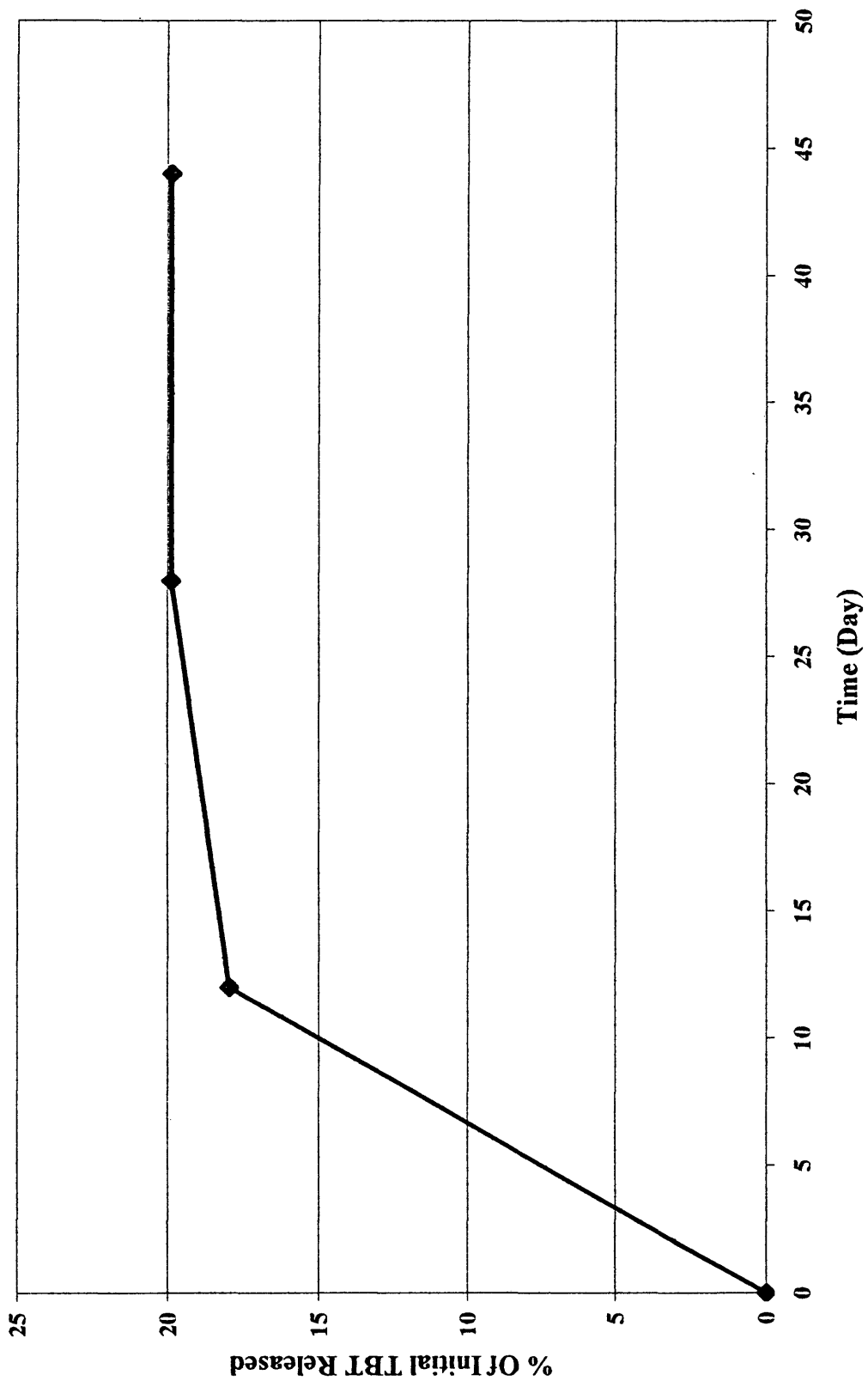


Figure 17. Percent of initial TBT released into the water

Percent of Initial TBT Released



after 44 days. MBT release was negligible at 1.5ng after 44 days.

One of the aerobic water samples was filtered and run to determine the percent of TBT associated with the particulate phase. In the unfiltered sample, there was 28ng/l TBT and in the filtered sample 17ng/l TBT remained. Therefore in the water associated with the plant decomposition study, roughly 61% of the TBT was associated with the dissolved phase and 39% with the particulate phase.

Butyrtins in the S. alterniflora

Normally tissue concentrations are presented as a wet weight but due to the changing quality of the plant material throughout the experiment, the concentrations were normalized to dry weight to better compare concentrations over time. When converted to dry weight, there was 3.8ng/g TBT in the plant material which translates to a total beginning mass of TBT in the experiment of 73ng. Due to the abrupt change in the quality of the plant material over the first twelve days, it appears as if the TBT concentration actually went up (Figure 18). In the aerobic treatments, the concentration rose to 14ng/g then fell to approximately 11ng/g by the end of the experiment. The anaerobic also rose to approximately 13ng/g but then stayed at that level. DBT levels also appeared to rise initially to 6ng/g then fall to 3ng/g (Figure 19). The aerobic treatment increased to 11ng/g while the anaerobic treatment level remained unchanged. MBT levels in the anaerobic plant material increased to approximately 5ng/g then fall at the end to 3.5ng/g (Figure 20). There was never any detectable MBT detected in the aerobic plant material. This apparent rise in the concentration of TBT in the plant material corresponded with the initial release of TBT into the water. The percent total

Figure 18. TBT concentration in the aerobic and anaerobic plant material over the course of the plant degradation experiment

TBT in Experimental Plant Material

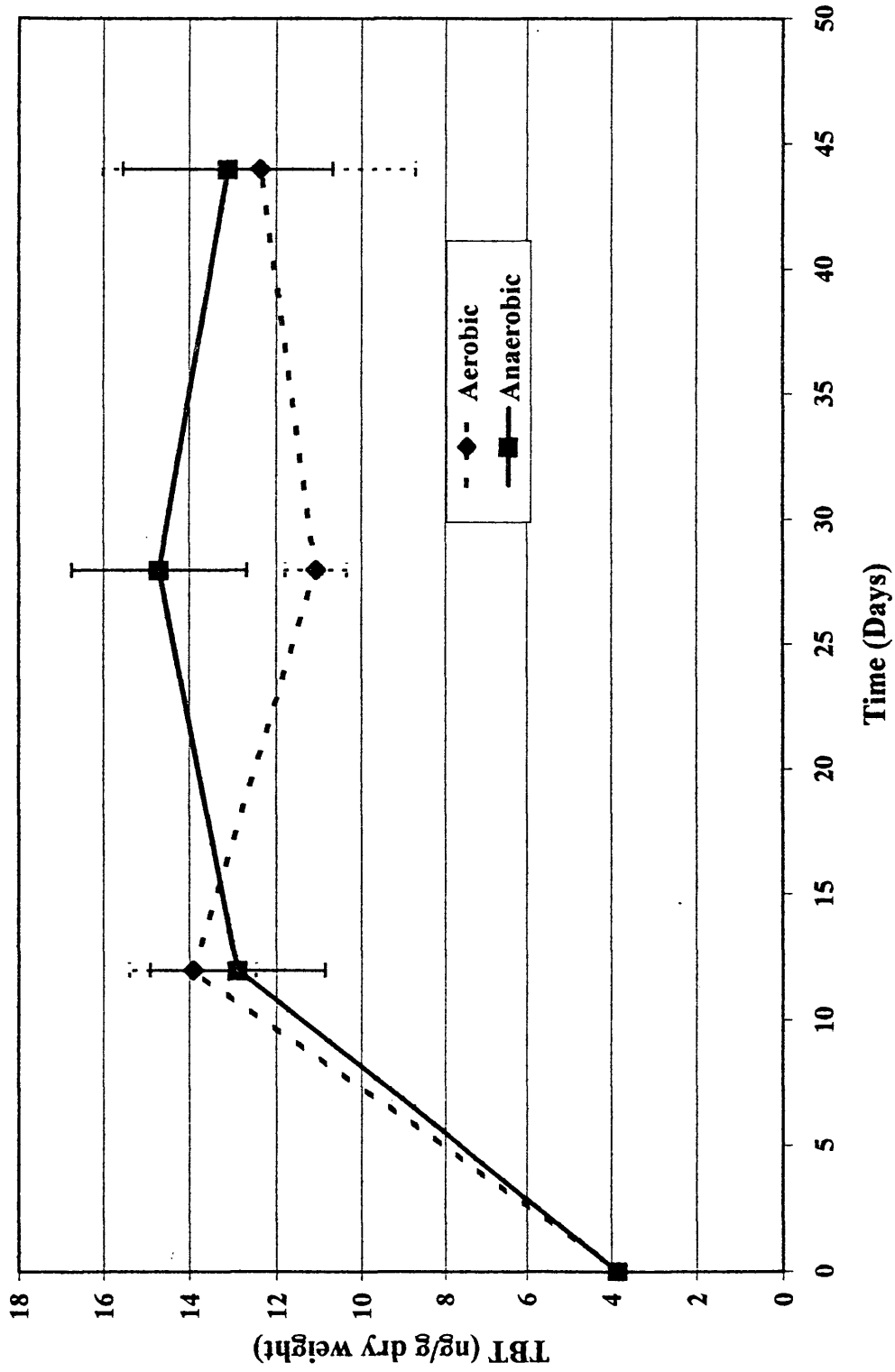


Figure 19. DBT concentration in the aerobic and anaerobic plant material over the course of the plant degradation experiment

DBT in Experimental Plant Material

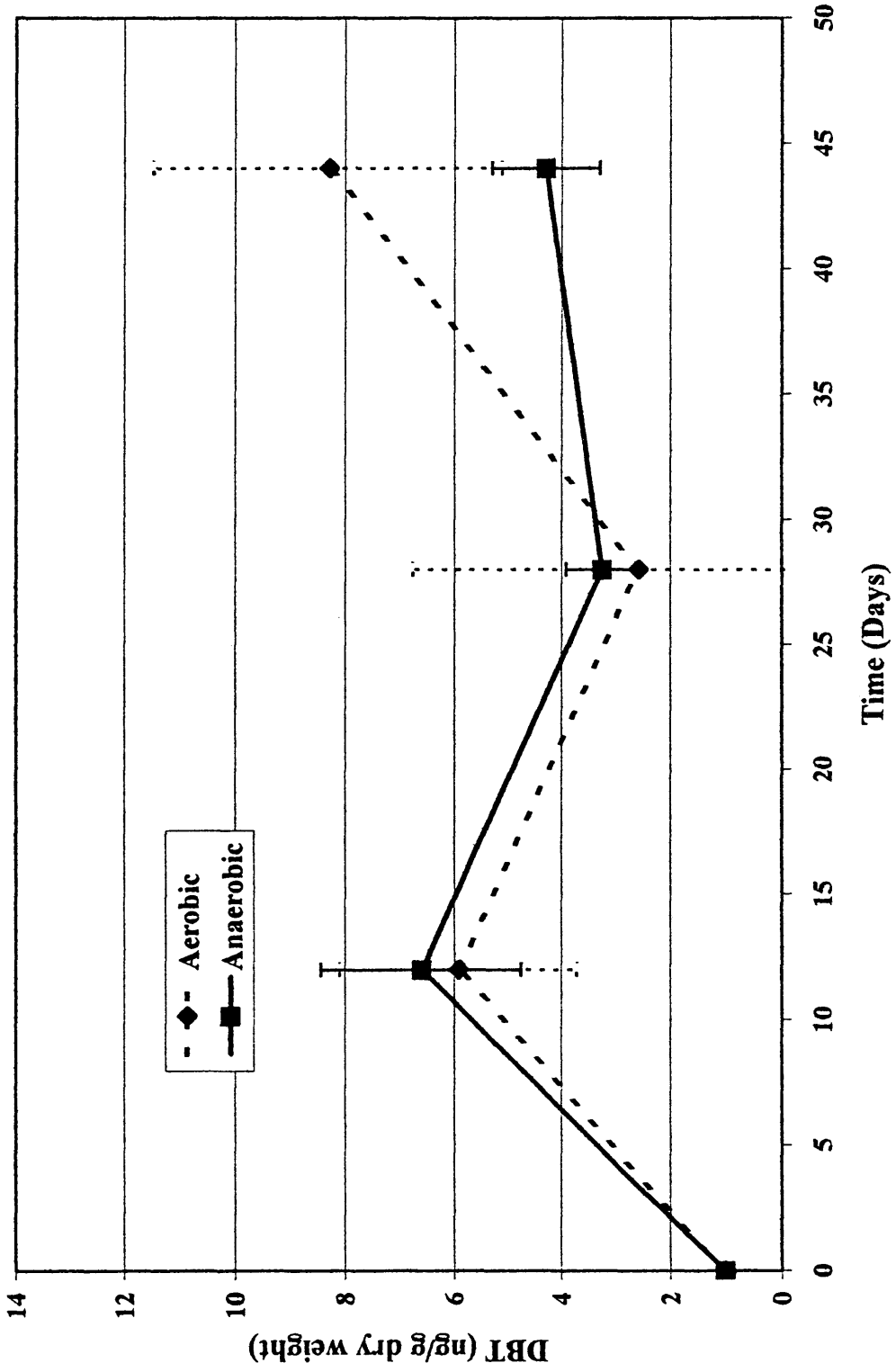
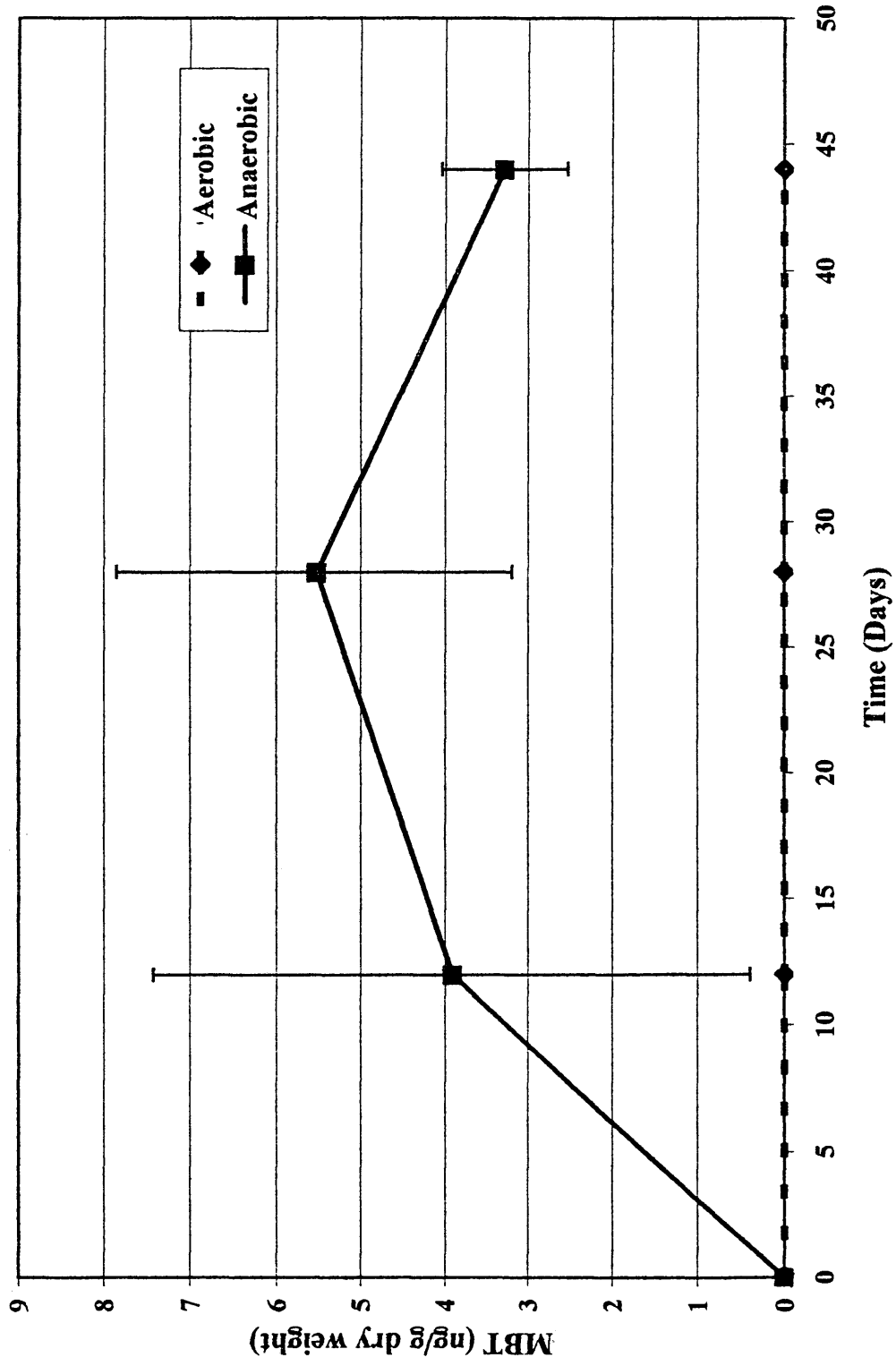


Figure 20. MBT concentration in the aerobic and anaerobic plant material over the course of the plant degradation experiment.

MBT in Experimental Plant Material



solid of the plant material decreased from 32% initially to an average of 12.7% after 12 days. It then decreased further to 11% and remains constant through the end of the experiment. The percent organic carbon did not change throughout the 53 days, staying at approximately 75%.

DISCUSSION

Field Experiment

Natural Versus Created Wetland Conditions

The biomass and growth of the *S. alterniflora* in this study were found to be consistent with that of other of created tidal wetlands, and therefore the results are likely good models of bioremediation for these wetland habitats. Biomass production in *S. alterniflora* dominated low marsh systems typically varies with latitude and has been found to range from 1,300 to 3,700g dry wt/m²/ yr in North Carolina and Georgia (Mitsch & Gosselink, 1993). The average annual production for the vegetated treatments at the Little Creek study site was 410g dry wt./m²/yr. However, four year old created marshes have been found to typically have 1.3 - 5.5 times less above ground biomass and 2.1-12.4 times less below ground biomass than similar natural marshes (D'Avanzo, 1990; Hardisky, 1978).

Nutrient concentrations in the created wetland were found to be similar to those observed in a study by Anderson et al (1997) for a natural *S. alterniflora* saltmarsh on the Eastern Shore of Virginia. D'Avanzo (1990) found that newly created wetlands are normally lower in nutrient concentrations than their established counterparts, but become similar within 2-5 years of development. When the study at Little Creek began, the created marsh was 3 years old so these concentrations were indicative of a created marsh at a 3-4 year stage of development. The sediment nutrients in the vegetated treatment bins were also similar to the surrounding marsh and therefore the experimental treatments within the bins were representative of the adjacent created wetland. During periods of increasing biomass, nutrient concentrations in the vegetated treatments were found to be significantly lower than

those of the unvegetated treatments. Again, patterns of decrease were similar between both vegetated bins and the adjacent vegetated marsh suggesting little impact of the bins on sediment geochemistry.

*Effect of *Spartina alterniflora* on disappearance of TBT*

The TBT concentration in the contaminated sediment decreased from an average of 260ng/g to 110ng/g over the 16 month duration of the experiment (Figure 9). The greatest decrease occurred during the fall of 1998 and the spring/summer of 1999 with little change evident during the winter. It was hypothesized that if the plants did not directly accumulate or degrade TBT, they would enhance the surrounding environment making it more favorable to microbial growth. This could occur by oxygenating the sediment and by providing microbial nutrients through exudates in the root and rhizome zone. However in this study, *S. alterniflora* growth did not enhance the disappearance of TBT, as both vegetated and unvegetated treatments followed the same trend with half lives of 300 and 330 days. There were also no significant affects of the vegetation on the levels of DBT in the sediment. Previous studies have shown that microbially mediated degradation is a primary means of disappearance of TBT from sediment. In one study, when poison was used to inhibit microbial activity, degradation slowed significantly (Clark et al., 1988). It is likely that the microbial communities in the saltmarsh sediment were the major factor affecting TBT degradation rate and they were inhibited by the colder temperatures. The MBT was below the detection limit for all of the sediment samples analyzed but, initial recovery experiments showed a low recovery of MBT at 19%. This is due in part to the method used to analyze the

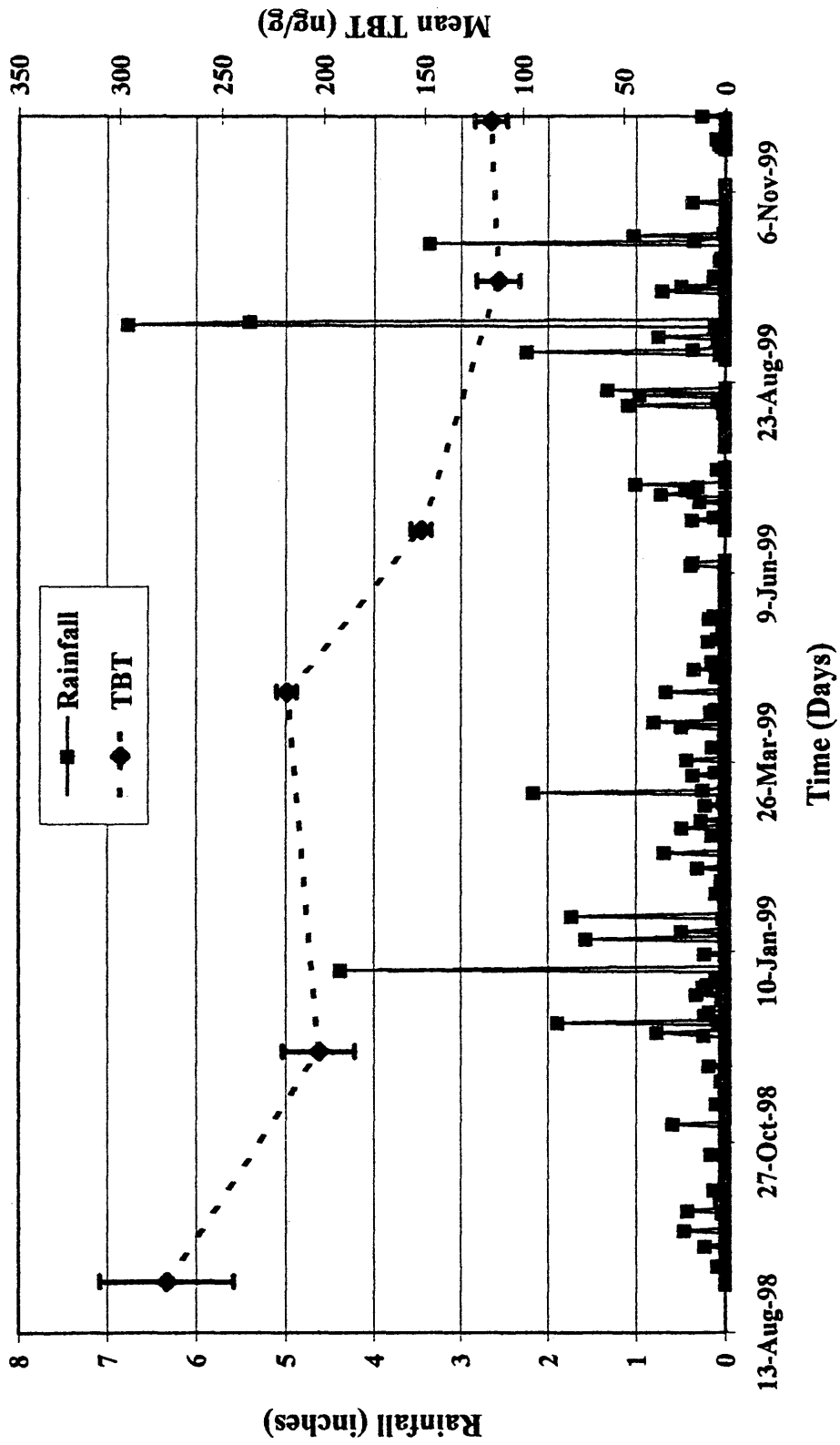
sediment which is optimized for the recovery of TBT. Francois et al. (1989) found that in seagrass tissue (*Zostera marina*), MBT was the major degradation product. They hypothesized that while decomposition was a step wise debutylation reaction, the seagrass tissue actually increased the rate of DBT degradation. In our study DBT is the major degradation product in the sediment and water. De Mora (1996) demonstrated two paths of degradation, methylation and debutylation depending on the dominant microbial activity. The lack of apparent effects of the plants on TBT degradation, even at the levels of plant biomass and growth found here, suggest that degradation processes other than those affected by the plants were likely regulating TBT degradation.

Other Processes Affecting TBT Degradation

The most likely explanation for the decrease in the concentration of TBT within the constructed wetland was biologically mediated degradation. Other possible mechanisms to consider include removal by tidal flushing and/or rainfall, volatilization, and photolysis. Processes such as photolysis and volatilization are unlikely, even in the top layer of sediment due to low transmittance of UV light beyond the top of the sediment and the low vapor pressure of TBT. The effect of tides and rain seem unlikely because of the hydrophobic character of TBT. Water-octanol partitioning coefficients (K_{ow}) as well as partitioning coefficient (K_d) values suggest a much greater affinity of TBT for sediment particles than water. In order to investigate the effect of rain events on the flushing of TBT from the bins, daily rainfall was compared to TBT concentration in the sediment for the duration of the experiment (Figure 21). However, no correlation between TBT disappearance and rainfall

Figure 21. TBT concentration and rainfall over the duration of the field study

TBT Concentration Versus Rainfall



could be found suggesting effects were small.

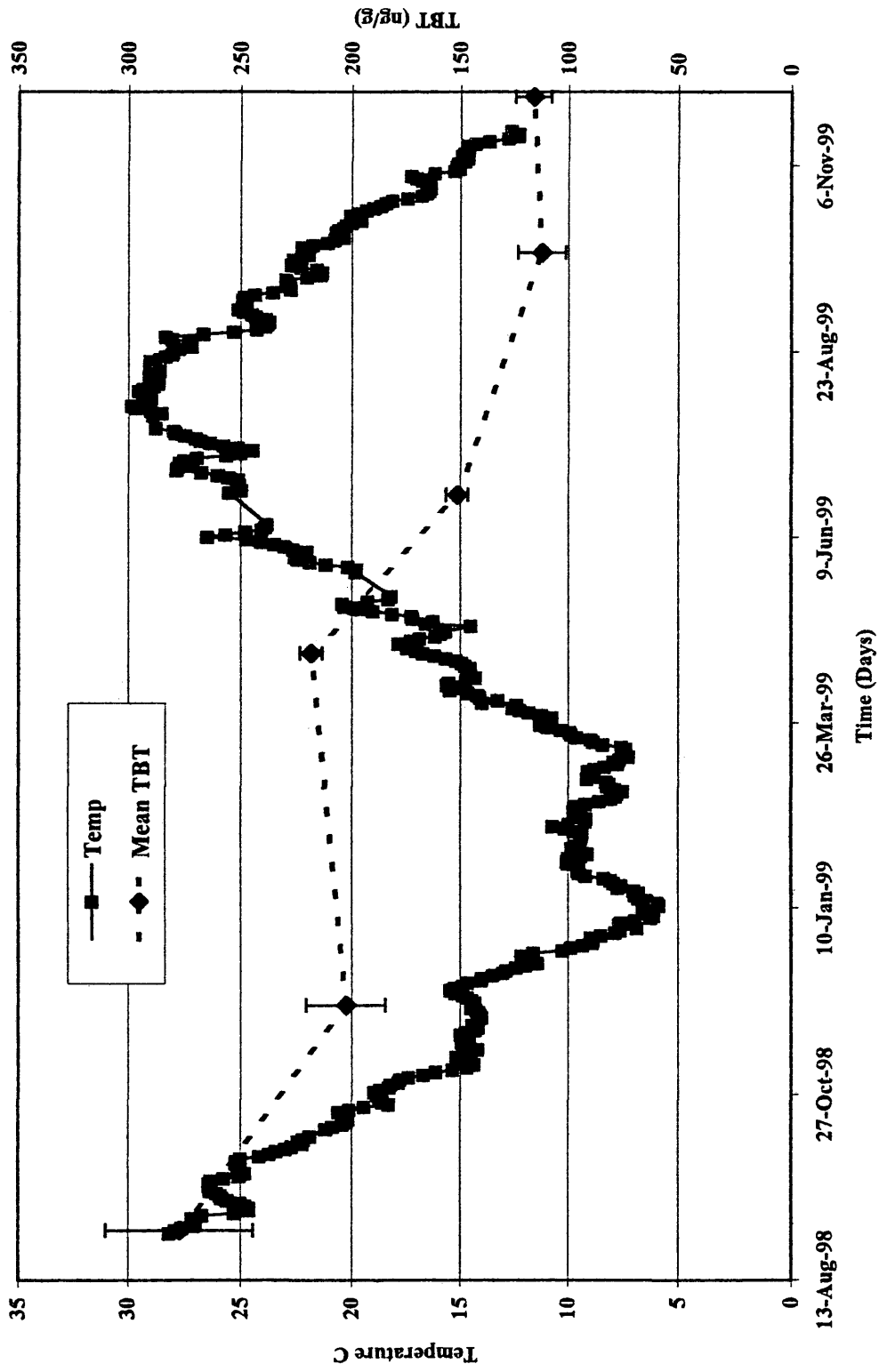
The disappearance of TBT seemed to be best explained by microbial degradation. Sediment microbial activity typically decreases with temperature, with the lowest amount of activity during the winter months. Slower rates of disappearance in winter months (Figure 22) suggests that there was a potential relationship between temperature and TBT disappearance with a temperature threshold of approximately 15°C. Below this temperature the rate of TBT disappearance was negligible. When the temperature increased above this threshold in the spring, the degradation rates increased.

Redox

Overall there were no consistent significant differences in the redox potentials between the vegetated and unvegetated treatments throughout the study. This may explain the lack of difference between the vegetated and unvegetated plots with regard to the disappearance rates of TBT. Dowson et al. (1996) found that TBT degradation rates were related to the aerobic condition of the sediment. The redox conditions measured in the created wetland and experimental treatments suggest relatively aerobic sediments even without vegetation. Therefore the potential additional effects of vegetation on sediment oxygenation may have been minimized in this study. The redox measurements which were taken at low tide during the day likely represent, temporally, the most oxygenated periods at the site. Under waterlogged conditions of high tide the diffusion of oxygen down into the sediment would be reduced and redox would be expected to be lower. Given the relatively sandy nature of the sediments in this created wetland (1-4% organic carbon) and the lack of

Figure 22. TBT concentration and water temperature over the duration of the field study

TBT Concentration Versus Water Temp



waterlogging at low tide, it is likely that the disappearance rates for TBT observed here represent relatively high rates for salt marsh areas. Rates for marshes with poorer drainage or more organic-rich sediments might be less than those reported in this study.

Effects of TBT on Spartina alterniflora

Prior to this study there has been no reported information on the effects of TBT on saltmarsh macrophytes. Results here suggest that TBT effects on *S. alterniflora* should be minimal up to sediment levels of 270ng/g. Growth rates of the plants in contaminated and uncontaminated sediments were similar and measurements of photo-system stress indicated relatively unstressed conditions. Therefore, the use of TBT contaminated sediments at these representative levels of contamination (Espoueyille et al., 1993; Govhoug et al., 1996) should not inhibit macrophyte growth and development.

In the Spring of 1999 additional bins were amended to 400ng/g TBT and planted with *S. alterniflora* of the same cohort as that which was used to vegetate the original bins. Unlike the experimental bins, the *S. alterniflora* in these bins did not grow well. A composite sample of plant material from these bins was analyzed and the TBT levels in the aboveground portion of the plant were found to be higher than in the regular experimental bins (16ng/g versus 1.2ng/g). While this was a single sample that was not replicated, it does suggest that for higher sediment concentrations the TBT concentrations in the plants would also increase. There was no evidence of photo-system stress in the surviving shoots. Those shoots that did grow were healthy. There could have been other factors that may have affected the health of the plants during the transplanting process such as temperature, time

out of soil before they were transplanted, and uncompacted soils which may have affected shoot viability. However, future work investigating the effect of TBT at levels higher than those used here may be warranted.

TBT Concentration in Plants and Trophic Transfer

TBT concentrations in *Spartina alterniflora* growing in the contaminated treatments remained relatively low throughout the experiment, achieving a maximum of 1.2ng/g in aboveground tissue and in 43ng/g in belowground tissue. TBT concentrations as low as 0.03µg/L in the water column have been shown to cause adverse effects to the copepod *Acartia tonsa*, an important estuarine organism in the Chesapeake Bay (Bushong et al., 1990). The levels measured in the *S. alterniflora* are very low compared to those measured in eelgrass tissue (*Zostera marina*) by Francois et al (1989) (61-819ng/g after two weeks) but the TBT water spike used in that study was much higher than levels typically found in natural waters. Additionally the mode of uptake in eelgrass tissue differs from a marsh macrophyte in that uptake occurs through the leaf tissue from the surrounding water column as well as in the roots and rhizomes. An earlier survey of eelgrass tissue from the Great Bay Estuary, NH by Francois and Weber (1988) revealed tissue levels to be below detection limits (<1ng/g) even in areas of heavy boating traffic.

There has been little published information concerning the toxicity of TBT contaminated food, therefore it is difficult to predict the effect of the TBT contaminated *S. alterniflora* tissues on higher trophic levels. While this study demonstrates that most of the non-degraded TBT remains associated with the sediment, there is some potential for

exposure to organisms of the lower trophic levels as well as secondary consumers. Many studies have investigated the importance of salt marshes for wintering waterfowl, insects, detrital feeders, and small vertebrates. Uptake by bacteria could result exposure to copepods, larval crustaceans, and small fish. Waterfowl including Snow Geese, Geyleg Geese, Atlantic Brant, Canada Geese, and Mallards feed on both the leaves and the roots and rhizomes through grubbing of many salt marsh plants including *S. alterniflora* (Mason, 1995; Esselink et al., 1997; Buchsman & Valiela, 1987; Priest pers. com., 2000; Havens pers. com., 2000). Insects such as grasshoppers, and leafhoppers feed on *S. alterniflora* as well as certain vertebrates including nutria, muskrats, and grazers such as deer and livestock (Priest, Havens, & Perry pers. com., 2000). Some smaller birds will also ingest the seeds of marsh grasses. There are therefore many possible vectors for transfer of TBT, not only to higher trophic levels in the aquatic system but also to the terrestrial environment. While this study only dealt with *S. alterniflora*, it is essential to carefully consider the animals utilizing the flora in a remediative system and to evaluate the consequences of their grazing on the potentially contaminated tissue. Considering the diverse group of consumers that can feed on both the aboveground and belowground tissue of *S. alterniflora*, trophic transfer is a concern in these systems and this issue warrants further investigation.

Laboratory Experiment

Plant Degradation

The plant degradation experiment was undertaken in order to assess the possibility of re-release of TBT into the environment from contaminated plants upon their senescence.

Plant decomposition takes place in multiple stages as described in Hicks et al. (1991). The first stage involves mostly physical changes with the leaching of the soluble components of the tissue. During the second stage, microbial activity on the tissue is the predominant method by which the plant continues to degrade. In the third stage, which is of the longest duration, refractory components like cellulose and lignins make up a large percentage of what remains and microbial activity associated with the litter is diminished. The plant degradation experiment conducted here simulated the early stages of senescence.

Over the initial 12 days of the experiment, the quality of the plant material changed. This was associated with a relative increase in TBT concentration within the plant material (3.8 to 14ng/g). Possibly, when the tissue was beginning to decompose and the labile portions of the plant were released there was little TBT associated with this material therefore the relative concentration in the remaining tissue increased. This is similar to what Drifmeyer & Redd (1981) found in an experiment involving *S. alterniflora* and heavy metals. Higher concentrations were detected in the dead versus live *S. alterniflora* biomass and they hypothesized this was due to the association of the metals with the more refractory component of the plant tissue. Of the initial 73ng of TBT in the plant material only 15ng were released into the water over the course of the experiment. The TBT released by the decomposing plant material was therefore only a small percentage (20%) of the total taken up by the plants. Most of that which was released occurred within the first 2 weeks after that little loss occurs. Therefore, in a marsh system, much of the TBT associated with the plant material may eventually be incorporated into the marsh sediment through burial and degradation. This would prevent its exportation as a dissolved component from the marsh.

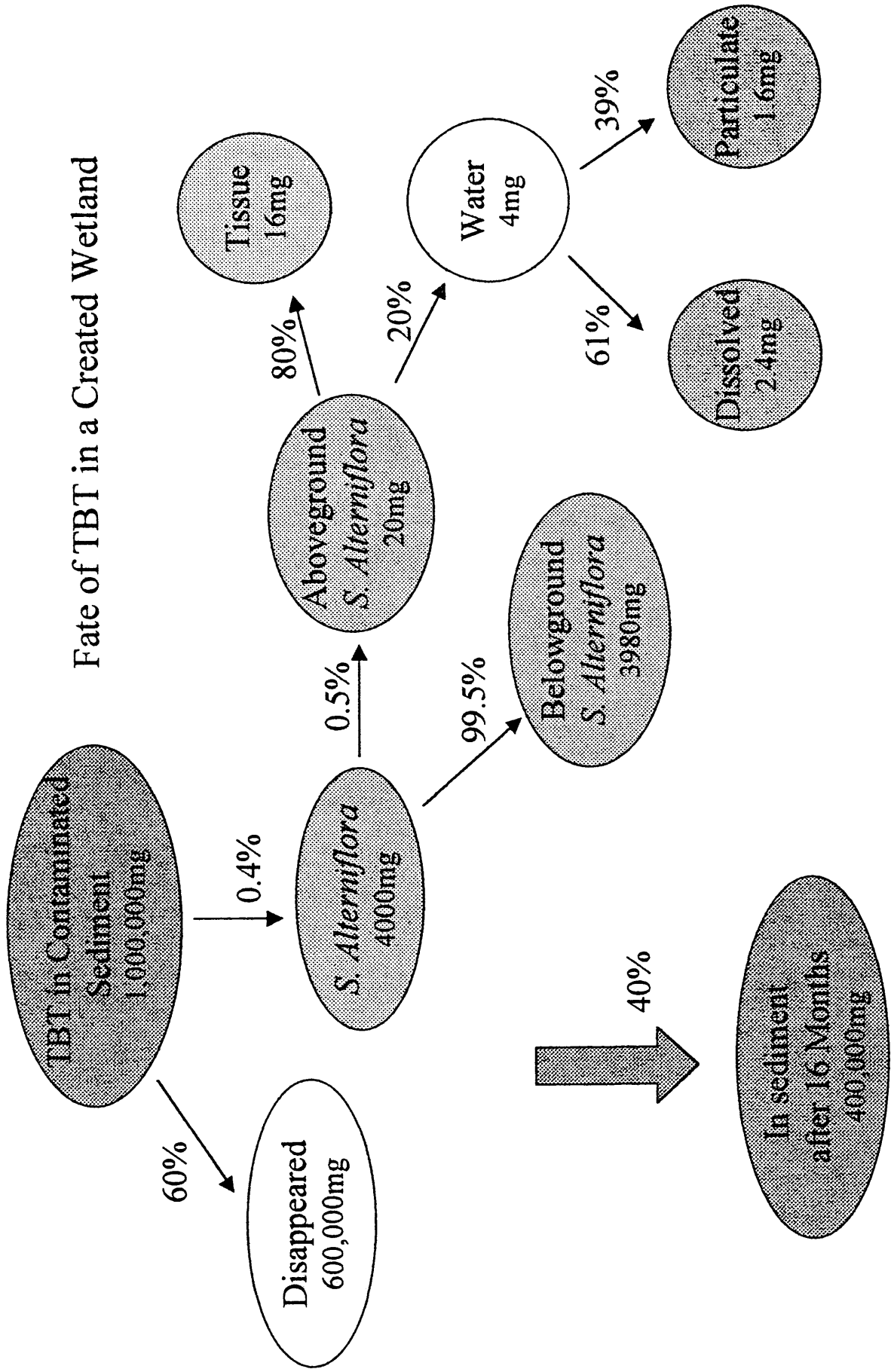
Unlike the aerobic treatments, the TBT concentrations in the anaerobic plant material here did not decrease over time after an initial relative increase. This is in agreement with what has been reported in the literature for the fate of TBT in marsh sediments. When TBT is buried in anaerobic sediments there has been normally very little degradation of the compound and a half life of years has been reported (de Mora et al., 1995; Dowson et al., 1996).

Overall Observations of the Fate of TBT in a Created Wetland

Based on the results of this study an overall budget of sediment TBT in a created wetland was developed and a theoretical amount of TBT (1kg) was used to illustrate the amount that would be in each compartment (Figure 23). After 16 months 40% of introduced TBT would still be present in the marsh sediment. Only 0.4% of the original TBT would have accumulated in *S. alterniflora* tissue. Of this amount, 0.5% would be in the aboveground shoot tissue and 99.5% in the belowground portions of the plant. Not taking into account the small portion which was degraded, approximately 20% of the shoot TBT, or 0.0004% of the original amount of sediment TBT, would be expected to be released into the water after 53 days of senescence. The remainder of the TBT accumulated by the plants may be reincorporated into the sediment via burial where decomposition would be reduced. Of the TBT released into the water column, 61% would likely be associated with the dissolved fraction and 39% with the particulate fraction. This budget suggests that most of the TBT associated with sediments would either disappear or remain associated with the sediment in a created, *S. alterniflora* dominated salt marsh. While *S. alterniflora* does not

Figure 23. Fate of TBT in a created marsh system

Fate of TBT in a Created Wetland



seem to increase the rate of TBT disappearance in relatively well oxygenated sediments, TBT in contaminated sediment will degrade. Creating wetlands on contaminated dredge spoils for the sole purpose of remediation does not seem viable, but using dredge spoils to create wetlands would be plausible.

CONCLUSIONS

The first objective of this study was to determine if *S. alterniflora* contributed to the degradation of TBT in created marsh sediments. The presence of *S. alterniflora* did not expedite TBT degradation and disappearance rates did not differ significantly between vegetated and unvegetated treatments. While this potential mechanism remediating TBT contaminated sediment was not found to work here, TBT was accumulated by the marsh macrophyte. Greater amounts of TBT were found in the belowground portion of the *S. alterniflora* which averaged 43ng/g than in the aboveground tissue which averaged 1.2ng/g. These concentrations could potentially affect the consumers of *S. alterniflora*. The toxicity of TBT to animals which utilize *S. alterniflora* dominated salt marshes deserves further study if this common marsh macrophyte is to be planted on TBT contaminated dredge spoils.

The second objective of the study was to investigate the fate of the TBT if it was accumulated by the plants. It was hypothesized that upon plant senescence, butyltins would be released into the water with concentrations of MBT and DBT being higher than TBT. Here it was found that TBT was released in the greatest quantity followed by DBT. MBT remained below the detection limit. However, most of the TBT remained in the plant material over the 53 day experiment.

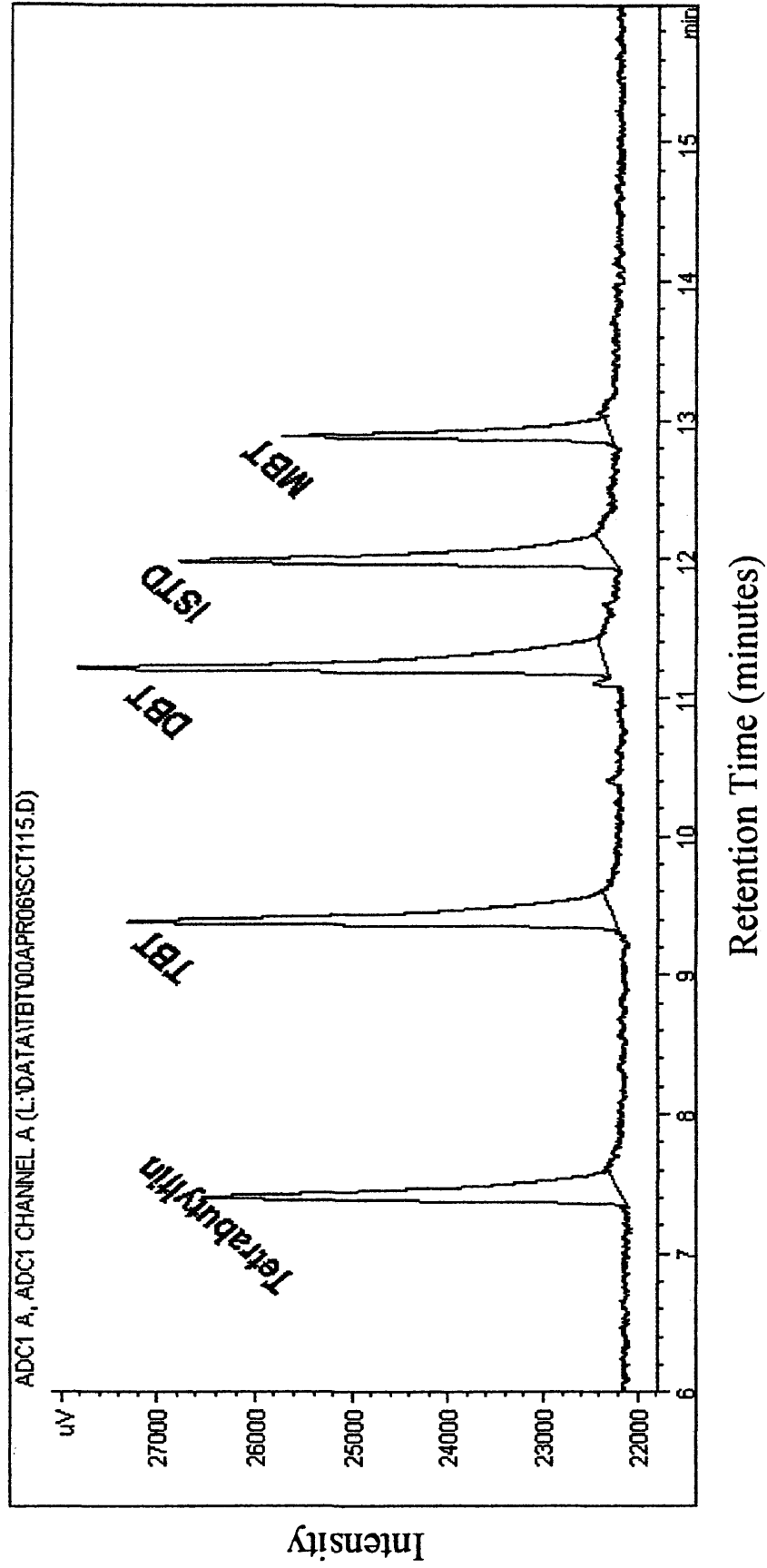
The use of *S. alterniflora* to speed the remediation of TBT contaminated dredge spoils was not supported in this marsh with sandy, well drained sediment. During this study, degradation rates did not differ significantly between vegetated and unvegetated treatments. Half lives of the TBT in these treatments were 300 and 330 days respectively, and presence

of DBT in sediments confirmed that the degradation of TBT to less toxic daughter products was occurring. In a created wetland using TBT contaminated sediment approximately 60% of the contaminant would be lost after 16 months. Approximately 0.5% would be accumulated by plants in the marsh and 0.3% of this would be in the aboveground biomass. While the TBT does degrade, the concern of the marsh macrophytes becoming vectors for TBT movement up the trophic level and potentially into the terrestrial ecosystem must be considered.

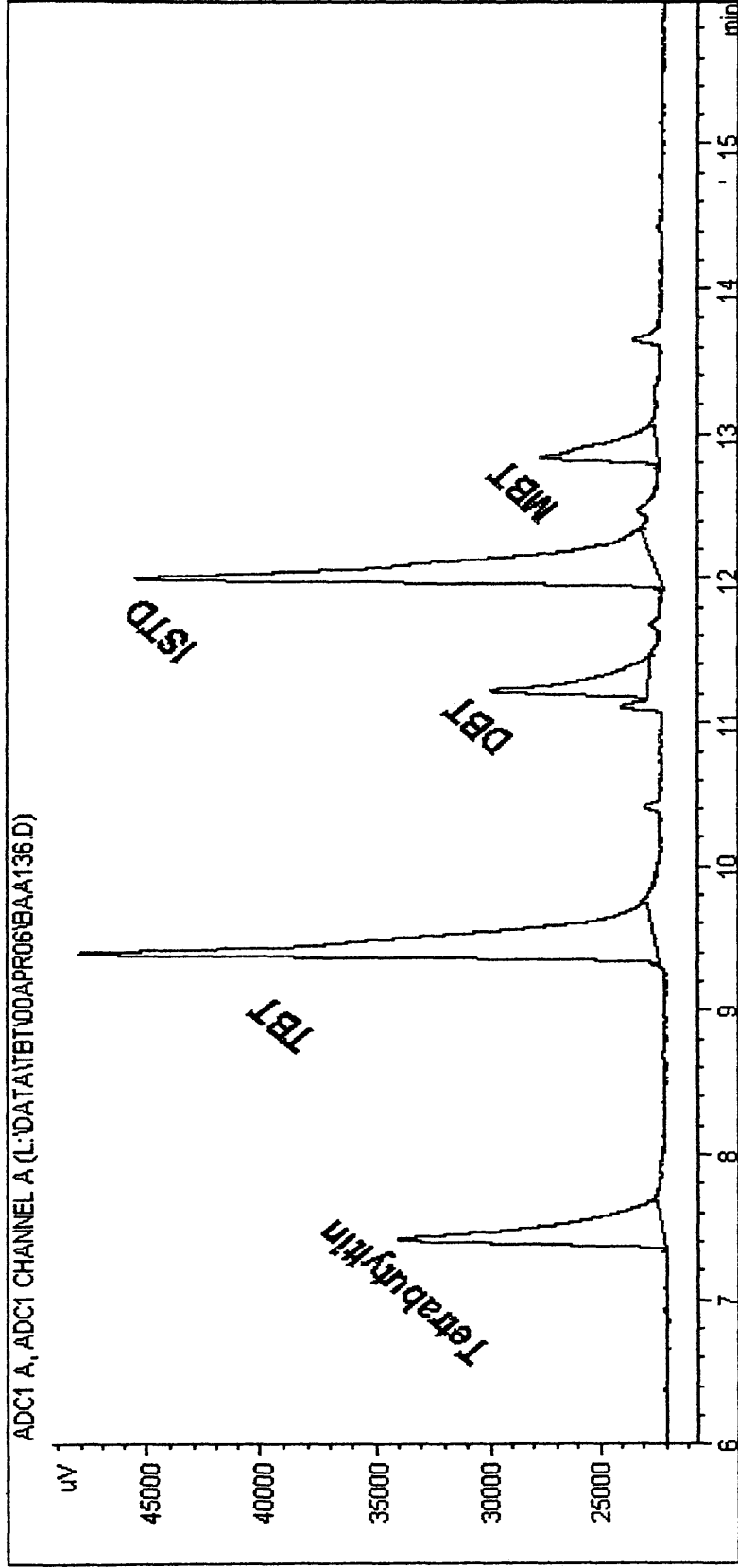
Appendix A. GC Settings and sample chromatogram of TBT standard and sediment standard
from early spring 1999

Gas Chromatography Settings for Butyltin Analyses

GC: Varian 3300 GC
Detector: Modified Flame Photometric
Carrier Gas: Helium at 4 ml/min.
Detector: Hydrogen 152 ml/min.
Air1 97 ml/min.
Air2 188 ml/min.
Filter: 600nm band pass
Column: DB-5; 30m x 0.32id. x 1.00 μ m
Injector Temperature: 280°C
Detector Temperature: 280°C
Initial Column Oven Temp.: 135°C
Final Column Oven Temp.: 300°C
Temperature Program Rate: 10°C/min.
Final Oven Temp. Hold Time: 5min.



Chromatogram: TBT GC Standard



Retention Time (minutes)

Chromatogram: Sediment Sample, Spring 1999

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