

Effects of Sewage Sludge on the Growth of Potted Salt Marsh Plants Exposed to Natural Tidal Inundation

**Richard R. Vance^{1*}, Richard F. Ambrose², Sean S. Anderson¹, Spencer
MacNeil², Timothy McPherson², Irene Beers², and Thomas W. Keeney³**

¹Department of Organismic Biology, Ecology, and Evolution

University of California

Los Angeles, California 90095

telephone: (310) 825-2685; fax: (310) 206-3987; email: rvance@biology.ucla.edu

*corresponding author

²Environmental Science and Engineering Program and

Department of Environmental Health Sciences

University of California

Los Angeles, California 90095

³Environmental Division

Naval Air Station Point Mugu

Point Mugu, California 93042

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Abstract

A growth experiment with native plants in pots exposed to natural environmental conditions evaluates the use of sewage sludge as a soil amendment in restoration of a southern California salt marsh. Sludge containing desirable organic matter but also undesirable heavy metal contaminants was mixed with a readily available matrix soil to reduce metal concentrations to levels below legal limits for land applications of sludge. Soil nutrient analysis revealed expected increases in total nitrogen and total phosphorus content with increasing sludge concentration. Soil metals analysis, however, revealed decreases in metal content with increased sludge concentration, a trend evidently caused by higher than expected metal content in the matrix soil.

Five artificial soil mixtures ranging from 0% to 70% sludge were accompanied by natural wetland soil controls. Pots containing these soils were placed into a natural salt marsh. The pots were then planted with two native salt marsh plant species, *Salicornia virginica* and *Frankenia grandifolia*. Above ground biomass was harvested after 12 months. Plant growth displayed no obvious change with increasing sludge concentration. Over the concentration ranges employed, increased nutrient content did not stimulate plant growth, and increased metal content did not inhibit plant growth. Plants grew better in natural wetland soil than in artificial soil mixtures, a trend probably caused by the substantially finer texture and higher organic content of natural soil. All sludge treatments differed more from the natural soil than from each other, implying that within the ranges examined, soil texture and organic content exerted more influence on plant growth than did metal or nutrient concentration. These results suggest that incorporating this sewage

sludge in the soil of the restored salt marsh will neither benefit nor harm the plants that will live there and that greatest plant growth will be achieved by mixing the sludge with a fine-grained matrix soil.

keywords: fertilizer, *Frankenia grandifolia*, heavy metals, nutrients, *Salicornia virginica*, salt marsh, sewage sludge, soil amendment

Introduction

The success of any salt marsh restoration project depends heavily on three main elements of environmental engineering: establishing the correct elevation of the restoration site, producing natural tidal inundation, and providing soil that can support natural growth of salt marsh plant species (Zedler 1996). Although appreciation of the importance of soil quality has grown in recent years, salt marsh restoration projects rarely include a serious attempt to provide suitable soil. Large-scale soil replacement or soil enrichment is usually judged either impractical or prohibitively expensive. Lacking restoration projects that present practical opportunities for soil treatment, salt marsh restorers have not invested much effort in field experiments that systematically examine effects of various soil properties upon salt marsh plants.

The few experimental salt marsh soil studies that have been performed (Langis et al. 1991; Zedler & Langis 1991; Gibson et al. 1994; Zedler 1996; Boyer & Zedler 1998; Padgett & Brown 1999; Huckle et al. 2000) reveal that soil quality can exert powerful effects on plant growth and also that these effects seem to be caused by several different soil properties acting together. Numerous previous experiments in salt marshes around

the world have shown repeatedly that artificial nutrient addition can greatly enhance plant growth, at least for short periods (Valiela et al. 1973, 1975; Valiela & Teal 1974; Valiela et al. 1982; Zedler & Nordby 1986; Sardá et al. 1996; Levine et al. 1998; Lin et al. 1999). Unfortunately, however, treatment of otherwise poor soil with a one-time nutrient enrichment performed during marsh construction typically does not ensure sustained plant vigor. Not surprisingly, greatest long-term success arises in soils most similar to the soils of natural marshes. Producing high quality marsh soil artificially from readily available materials is usually no simple task. Commonly used artificial soils, like dredge spoils for example, contain too little organic matter and too little fine-grained material. Healthy plant growth requires soil organic matter whose gradual decomposition provides a steady supply of essential nutrients. Healthy plant growth also requires abundant fine-grained soil particles that reduce nutrient loss through soil leaching either by binding the nutrients chemically (Foth & Turk 1972) or by decreasing the flow rate of ground water (Aber & Melillo 1991) or both.

Fortuitous circumstances in the southern California salt marsh at Mugu Lagoon (36° 6' N. Lat., 119° 6' W. Long.), whose general geology and ecology have been well described by Warne (1971), Zedler (1982), and Onuf (1987), have encouraged pre-restoration study of soil effects on plant growth. A 15 ha plot of upper tidal salt marsh was converted to sewage oxidation ponds in the 1960s. Environmental concerns led to pond closure in the mid-1990s and motivated a plan to restore the area back to its natural state. The project necessarily began with consideration of what to do with the 40-100 cm thick layer of sewage sludge that accumulated in the ponds. Vigorous growth of terrestrial weedy plants that invaded the area shortly after pond draining suggests that the

sludge can support healthy growth of salt marsh vegetation. The high cost (\$5-50 million) of transporting and landfilling this roughly 100,000 m³ of sludge makes the alternative of using the sludge in place particularly attractive. Similar economic considerations have led Sawhill & Furguson (1998) to consider this issue in a somewhat different setting.

Unfortunately, using this sewage sludge as a soil amendment creates an environmental risk. Like sludge from most municipal sewage treatment plants, this sludge is contaminated with heavy metals. Previous chemical analysis (CH2M Hill 1994, CDM 1996) has revealed that concentrations of cadmium, copper, lead, and zinc, though highly variable spatially throughout the ponds, in the worst places exceed or lie near the monthly average allowable concentrations for land application of sludge (Table 1). Employing this sludge in salt marsh restoration could potentially compromise vegetation health (Valiela et al. 1985), pass heavy metals into the food chain via herbivores and detritivores that consume plant material laced with heavy metals (Banus et al. 1974; Valiela et al. 1974; Giblin et al. 1980; Teal 1986; Otte et al. 1991; Otte et al. 1993), and possibly cause marine pollution directly by the leaching of heavy metals from sludge-containing soil (Banus et al. 1975; Valiela et al. 1976; Bourq et al. 1979; Teal et al. 1982; Giblin 1985).

This potential environmental benefit and potential environmental cost motivated pre-restoration study of possible consequences of using sewage sludge in salt marsh restoration at Mugu Lagoon. Our ongoing investigation is proceeding in steps. The first step exploits the convenience of salt marsh mesocosms discussed by Leendertse et al. (1996) and Callaway et al. (1997) and used creatively by Kelley & Mendelssohn (1995) and DesRoches (1998) to study the feasibility of using cuttings produced by undersea gas and

oil drilling to restore coastal marshes in Louisiana. Following Gallagher & Wolf (1980) and Gallagher & Kibby (1980), we examined growth of salt marsh plants in pots containing carefully prepared sludge-soil mixtures. We first placed the pots into holes dug into the natural marsh. We then filled them with artificial soils formed by mixing sludge, in a range of concentrations, with a conveniently available “matrix” soil. Finally, we transplanted a single plant into each pot and measured its growth during the following 12 months. This experiment seeks answers to four questions: (1) Does sludge concentration in artificial soil mixtures affect salt marsh plant growth? (2) Do heavy metal concentrations in artificial soil mixtures affect salt marsh plant growth? (3) How does plant growth in the artificial soils compare to plant growth in natural salt marsh soil? (4) If plants in artificial and natural soils do perform differently, which soil properties are most likely responsible?

Methods

To achieve the greatest practical physical and chemical homogeneity in the artificial soil mixtures, we collected all sludge from a single pond area far from any sewage influent pipe from which variable inflow current might have created local spatial variation in sludge properties. Minimizing differences between replicate pots containing each soil mixture required making these mixtures homogeneous on as small a spatial scale as possible. This requirement forced thoroughly drying the sludge ahead of time so that the clods could be broken apart prior to mixing. We accomplished this drying simply by exposing freshly mined sludge to direct sunlight and the dry air of southern California over a period of several weeks, by the end of which the sludge had lost not all of its moisture

but enough to allow effective mixing. This procedure may have altered sludge bulk density somewhat, and it almost certainly caused some decomposition of the organic matter present. These changes, however, probably do not greatly exceed sunlight- and dry air-induced organic decomposition that takes place at least at the soil surface in any mid- to high-elevation salt marsh locally. Such habitat in southern California routinely experiences long periods of desiccation between spring tide periods, truly qualifying as a “xeric wetland” ecosystem.

The material selected for use as the matrix soil was obtained from the top of the berm that surrounds the sewage ponds. This choice was motivated by restoration cost considerations; as restoring the entire pond area requires berm removal anyway, the berm provides the most economical matrix soil available. Berm soil was also collected from a single area and air-dried for several weeks. We passed both soils through a 12 mm (.5 in) mesh to remove rocks and large, firm clods. Measured volumes of the sieved material were then placed in a portable cement mixer and thoroughly mixed to form the finished artificial soils.

Sludge concentrations in the mixtures were selected on the basis of previous chemical analysis (CH2M Hill 1994, CDM 1996) of heavy metal concentrations in the sludge. These measurements yielded crude guesses of average metal concentrations throughout the sewage ponds area. The sludge concentration in our richest mixture was chosen so that, after dilution of “average” sludge with a metal-free matrix soil, the concentration of each metal in the mixture would not exceed the monthly average allowable concentration for land application of sludge. The five sludge mixtures we examined contained 70%, 50%, 30%, 10%, and 0% sludge. To these first five treatments using artificial soil mix-

tures, we added two control treatments (described below) that employed natural salt marsh soil. This soil was obtained from the holes in the marsh dug to accommodate the pots. This marsh soil was air-dried, sieved, and thoroughly homogenized in the cement mixer, just as sludge and berm soil were, prior to replacement in the marsh.

The experimental containers were 3.8 L (1 gal) cylindrical plastic pots obtained from a commercial plant nursery supply company. Prior to pot use, about seventy ~5 mm (13/64 in) holes, spread roughly evenly over each pot's sides and bottom, were drilled to allow subsurface water flow into and out of the pot. Also, four small rectangular areas, evenly spaced around each pot's rim, were cut out to allow surface water flow into and out of the pot. We considered these perforated pots as providing a suitable compromise between complete soil and root containment and completely unimpeded subsurface and surface water flow. However, because these perforated pots created a somewhat artificial environment for plant roots, the experiment included two additional control treatments. The first employed natural (but dried and mixed) marsh soil in a pot identical to those used in the sludge mixture treatments. This control provides a direct comparison between effects of natural and artificial soils on plant growth in pots. The second control employed no pot at all. Rather, processed natural soil was simply placed into an unlined pot-size hole in the natural marsh. Comparing the two controls allows examining the effect of the presence of a pot on plant growth in order to attempt to evaluate the similarity of the entire experiment to conditions that might prevail in a continuous area of restored salt marsh enriched with sludge mixture.

Physical conditions in natural salt marshes vary through space, of course, and this environmental variation causes different marsh regions to support different plant species.

To examine effects of at least some of this variation, we deployed this experiment in two sites and using two common plant species with greatly contrasting physiology. Although the two sites lie at almost exactly the same elevation, one is far better watered than the other by virtue of a nearby major tidal creek that surrounds the site on three sides. We call this the “wet site” (elevation +1.62 m MSL with respect to the North American Vertical Datum of 1988) and the other, which lies farther from any regular water supply, the “dry site” (elevation +1.65 m MSL). One study species is *Salicornia virginica* (family Chaenopodiaceae) whose foliage consists entirely of relatively large-diameter (~3 mm) succulent stems, and the other is *Frankenia grandifolia* (family Frankeniaceae) whose smaller diameter (~1 mm) nonsucculent stems bear deciduous leaves that provide most of the plant’s photosynthetic area. Both species are perennials, and they are the two most common plant species in the Mugu Lagoon upper tidal salt marsh.

The experiment was initiated by transplanting small plants cultivated from cuttings by Tree of Life Nursery of San Juan Capistrano, California. At the time of planting, all plants of each species appeared healthy and roughly the same size (mean above ground biomass \pm standard deviation was 1.21 ± 0.39 g for *Salicornia* and 1.06 ± 0.25 g for *Frankenia*). We established 10 replicates of each soil treatment for each species at each site. Thus, with 7 soil treatments in the experiment, each site received 70 plants of each species, for a total of 280 plants in the entire experiment. The 140 experimental plants at each site were arranged into a regular 7×20 array located within a $6 \text{ m} \times 12 \text{ m}$ area from which all above ground vegetation had been previously cleared. Centers of neighboring plants were separated by approximately 50 cm. The locations of all replicates were assigned randomly. Planting took place on 18 June 1996. Throughout the 12-month grow-

ing period that followed, the experimental sites were weeded so that test plants experienced no competition with non-experimental plants.

Harvesting took place on 2-3 June 1997 when some experimental plants were just beginning to experience shading by their largest neighboring experimental plants. At harvesting time, the plants were clipped at ground level, transported to the laboratory, and dried for several days at 60° C, and then the above ground dry weight of each plant was measured. We did not have sufficient resources to perform corresponding measurement of plant roots. Also at harvesting time, two cores were collected for soil analysis. Two replicate pots subjected to each treatment applied to each species at each site were selected at random, for a total sample of $2 \times 7 \times 2 \times 2 = 56$ pots. Two cores extending from the top to the bottom of each selected pot were collected using a metal-free protocol. These cores were transported to the laboratory and stored frozen until analysis.

In the laboratory, one member of each pair of cores was split in half along its length. One half-core was subjected to particle size analysis by the method of Bouyoucos (1962) which yields the fraction of the sample's mass consisting of sand (particles > 62 μm diameter), silt (particles between 2 μm and 62 μm), and clay (particles < 2 μm). The other half-core was oven-dried at 60° C for several days, weighed, placed in a muffle furnace at 400° C for 18 hours, and weighed again to determine the ash-free dry weight, a simple measure of soil organic content. The second soil core from each sampled pot was analyzed chemically for Cd, Cr, Cu, Pb, Mo, and Zn by Columbia Analytical Services of Canoga Park, California, using EPA methods 3050 and 6010, and for total Kjeldahl nitrogen and total phosphorus by DANR Analytical Laboratory at the University of California in

Davis, California. Cd, Cu, Pb, and Zn were analyzed because their maximum concentrations in the sludge exceed or lie near the monthly average allowable concentrations for land application of sludge. Cr and Mo were analyzed because they contaminate several nearby areas and consequently are metals of potential concern to the landowner.

Because of budgetary and logistic constraints, we were unable to perform metals analysis of our sludge, berm material, or natural marsh soil at the beginning of the experiment.

Statistical analysis employed two standard methods for different groups of samples. Analysis that focused on effects of sludge concentration employed linear regression with sludge concentration as the independent variable. These analyses examined only the five artificial soil mixtures formed from sludge and berm soil. The regression of final plant dry weight on sludge concentration included the square of sludge concentration as a second independent variable to allow for the possibility that plants grow best at some intermediate sludge concentration. Regression proceeded in a forward stepwise manner and terminated when the partial correlation of each independent variable not in the regression equation lacked statistical significance with the critical value of P set at .05.

Analyses that contrasted effects of the sludge mixtures with those of natural salt marsh soils employed one-way analysis of variance. This ANOVA examined all seven treatments for each species at each site separately, followed by Tukey's honest significant difference method of contrasting individual treatments. This conservative method evaluates all contrasts within an experiment using a single experiment-wide type I error, chosen as $P = .05$ here. Analyses involving only plant mass examined the full within-group

sample of 10 replicates, and hence all 280 plants in the experiment, except for the 7 individuals that died prior to harvest. Because soil analyses were performed on only 2 replicates within each group, however, all analyses involving measured soil properties employed just these 56 cases.

Results

Chemical and physical analyses revealed several trends in the artificial soil mixtures. Organic and nutrient content (Fig. 1) display different patterns. Contrary to expectation, increasing sludge concentration did not give rise to a discernible increase in soil organic content ($P = .25$, $R^2 = .03$). However, increasing sludge concentration did produce significant ($P < .001$) increases in both total Kjeldahl nitrogen and total phosphorus. Although accompanied by substantial unexplained variation ($R^2 = .34$), the nitrogen regression suggests that total Kjeldahl nitrogen increased from about 38 parts per thousand (ppt) in the treatment containing no sludge to 50 ppt in the 70% sludge treatment, a 32% increase. The phosphorus regression explains more of the variation ($R^2 = .86$) in these samples, and phosphorus concentration more than doubles from 11 parts per million (ppm) in the sludge-free mixture to 25 ppm in the 70% sludge mixture.

The concentrations of all six metals studied (Fig. 2) displayed the same unexpected pattern: metal content significantly *decreased* with increasing sludge concentration ($P < .001$ for Cr, Cu, Pb, Mo, and Zn; $P < .01$ for Cd). On a relative basis, the magnitude of this decrease differed for the different metals, ranging from a high of 48% decrease in Cr over the range of sludge concentrations to a low of 24% decrease in Zn. The fraction of total variation explained by the regression lines ranges from $R^2 = .23$ for Cd to $R^2 = .74$

for Cr. These decreasing trends establish that the sewage sludge actually contained less heavy metal contamination than did the berm soil, at least in the specific areas from which we obtained sludge and berm material. Measured concentrations of both Cd and Mo, however, lay close to the detection limits of the chemical analysis procedure. Consequently, despite their statistical significance, the trends involving these metals are somewhat suspect.

Grain size analysis (Fig. 3) revealed that with increasing sludge content, the artificial mixtures contained less sand, more silt, and less clay. All regression lines are significant, but the clay line is barely so ($P = .049$) and explains only 10% of the total variation in clay content. The sand and silt regressions are both highly significant ($P < .001$) and explain about half of the total variation. If one regards sand particles as “coarse” and both silt and clay particles as “fine”, then the sand regression implies both that coarse-grained material decreased and also that fine-grained material increased as sludge formed a larger fraction of the artificial soil mixture.

Of the 280 plants in the experiment, 273 survived the 12 months between planting and harvest. The 7 deaths that occurred involved plants of both species, plants at both sites, plants growing in natural as well as artificial soil, and plants that experienced low as well as high soil metal content. This small amount of widely scattered mortality, displayed incidentally as sample size information in Fig. 5, suggests that metals at the concentrations used here did not directly cause plant mortality.

Plant growth in this experiment (Fig. 4) displayed no clear relation to soil sludge content. In *Frankenia grandifolia* at both sites, sludge concentration entered the regression

equation first, and its square failed to improve the regression ($P > .05$). Growth showed a significant ($P < .05$) decrease with sludge enrichment at the wet site, due largely to exceptionally high growth by one plant in the 0% sludge mixture, but a significant ($P < .05$) increase with sludge enrichment at the dry site. Neither regression, however, accounts for much of the observed variation ($R^2 = .087$ and $.125$, respectively). *Salicornia virginica* growth showed no significant relation to sludge concentration or its square at either site ($P > .05$), and at neither site did the best fitting straight line explain much of the overall variation ($R^2 = .062$ and $.001$ at the wet and dry sites, respectively).

A possible cause of this absence of clear growth patterns is that all artificial mixtures differed greatly from natural marsh soil such that plants could hardly detect, much less respond to, the comparatively small differences between mixtures. These plant species do tend to grow faster in natural soils (Figure 6). Mean final *Frankenia* mass in wetland soil exceeded mean final mass in the mixtures, significantly so ($P < .05$) for four of the five mixtures at the wet site and three of the five at the dry site. For *Salicornia*, wetland soil produced greater mean growth at the wet site, significantly ($P < .05$) for three mixtures. *Salicornia* displayed no such difference at the dry site where growth conditions appeared uniformly poor for this species. For both plant species, no significant differences ($P < .05$) occurred between any pair of artificial mixtures at either site. Thus, growth differences between plants in natural and artificial soils exceeded differences between plants in different artificial mixtures except where growth conditions were uniformly poor.

Comparisons between the two control treatments test for the existence of a pot effect. The fact that no significant difference ($P < .05$) occurred in either species at either site

(Fig. 5) suggests that the presence of a pot does not influence plant growth under the conditions of this experiment. Thus, trends in the potted plants of this experiment may resemble trends in plants not constrained by pots.

Our search for possible causes of plant growth differences between natural and artificial soils began with linear regression analysis of the 56 pots for which all soil data as well as plant data exist. This analysis (Table 2) reveals no obvious general trends. For *Frankenia* at the dry site, plant growth correlated positively ($P < .05$) with phosphorus and silt content and negatively ($P < .05$) with clay content. In *Salicornia* at the wet site, plant growth correlated positively ($P < .05$) with ammonium content. In no case did the regression explain as much as 60% of the variation, however. Furthermore, comparing all soil characteristics with $P < .20$ reveals that no characteristic appears more than once in the list of all such factors for both species at both sites (Table 2). Thus, no individual soil characteristic exerted a universal strong effect on plant growth under the conditions of this experiment, even though many pronounced and significant differences between natural and artificial soil characteristics did exist (Table 3). Fig. 6 contrasts artificial and natural soils with respect to organic content and particle size composition. It reveals that natural soils contained much more organic matter than did all sludge mixtures. Natural soils also contained more silt and more clay but less sand than did the mixtures. In natural soils, none of these characteristics differed statistically ($P > .05$, Tukey's honest significant difference test) between wet and dry sites, but all characteristics differed significantly ($P < .05$) between each natural soil and all artificial soils. Some significant differences in sand and silt content did occur between some of the mixtures; but for all four soil properties, natural soil differed more from artificial soils than artificial soils did from

each other. Thus, in this experiment with potted plants, the same features found in earlier studies (Zedler & Langis 1991; Gibson et al. 1994; Zedler 1996; Padgett & Brown 1999; Huckle et al. 2000) distinguish good soil from poor soil: good soil is rich in organic matter and silt and clay, while poor soil contains little organic matter and much sand.

Discussion

This experiment yielded two main categories of information concerning use of sewage sludge as fertilizer in salt marsh restoration. These are effects on plant performance of heavy metal contamination and effects on plant performance of soil texture and organic and nutrient content. We will discuss these two effects in turn.

This experiment yielded no clear evidence that heavy metal contamination harmed *Salicornia virginica* or *Frankenia grandifolia*, the two most common plant species in the Mugu Lagoon salt marsh. There was virtually no plant mortality in this experiment. Only 7 of the 280 experimental plants of both species combined died during the experiment, and 5 of these deaths occurred at the dry site, suggesting that desiccation may have been the main cause of death. In addition, metal contamination, at the range of concentrations examined, probably did not affect plant growth, because regression analysis reveals no significant relationship between the growth of either plant species and sludge content of the soil mixture. Analysis of variance of the same data corroborates this conclusion.

Prior to this study, heavy metal effects on salt marsh plant growth have received very little experimental study, and the few published studies describe contradictory results. From observations in salt marshes experimentally fertilized for many years with con-

taminated sewage sludge, Valiela et al. (1985) assert that heavy metals in low to moderate concentrations cause no direct physiological harm to salt marsh plants. In contrast, Gallagher & Wolf (1980) found from a potted plant experiment like ours that *Salicornia virginica* died when grown in metal-contaminated soil. However, their metal concentrations ranged from 10 to 100 times greater than ours, and in any case the authors point out that the observed plant deaths could have been caused by factors other than metal poisoning.

Several authors have discovered that metal content of wetland plants usually correlates poorly or not at all with metal content of the surrounding environment. Albers & Camardese (1993a) found that experimental acidification that releases metals from soil into the water causes the freshwater wetland plant *Sparganium* to take up more Zn but not more Cd, Cu, or Pb. Taylor & Crowder (1983) observed a positive but weak correlation between concentrations in plants and in soil of Cu but not Zn. Albers & Camardese (1993b) observed a negative correlation between *Potamogeton* tissue and soil concentration in Zn but no correlation in Pb. Franzin & McFarlane (1980) found that submerged aquatic plants with high leaf surface area contained more trace metals than did fleshier plants. They observed a positive correlation between plant Zn concentration and the Zn concentration of water but not of the soil, and no correlations in Cd, Cu, and Pb. Similarly, Sprenger & McIntosh (1989) found that submerged aquatic plants contained more Cd, Pb, and Zn than did plants with floating leaves. None of these authors mentioned adverse effects of trace metals on plant size or health. Gillespie et al. (2000) observed that freshwater plants exposed to Zn-contaminated water do contain more Zn as a result, but these authors too did not mention observing any obvious harm to these more Zn-

contaminated plants. Mays & Edwards (2001) found that even when wetlands constructed for sewage treatment effectively cleansed metals from contaminated water, there were no clear correlations between metals in plant tissue and metals in either the soil or the water to which they were exposed. Mungur et al. (1997) observed elevated Cu, Pb, and Zn in the roots of plants in wetlands constructed for sewage treatment, but they too noted no ill effects on the plants. In contrast, in a somewhat polluted but natural marsh, Pourang (1995) actually found lower concentrations of these same metals in plants than in the soil beneath them. Wall et al. (2001) could discern no ill effects on *Spartina alterniflora* of elevated PCB and Hg concentrations that motivated classifying their Georgia habitat as an Environmental Protection Agency superfund site. Finally, Ansedè et al. (1999) have shown that *Spartina alterniflora* responds to Se contamination by converting highly toxic Se-containing compounds into different compounds that are far more environmentally benign. The main message that emerges from all of these findings is that most aquatic plants growing in environments moderately polluted with heavy metals do not experience obvious harm and may not even contain elevated concentrations of metals in their tissues.

Together with these previously published findings, this potted plant experiment reduces concern about the use of local sewage sludge in salt marsh restoration at Mugu Lagoon. The presence of this heavy metal-contaminated sludge in the soil is likely to cause little or no harm to at least the two most common plant species that will occupy the restored marsh.

The experiment yielded a second category of information. It concerns the effects of soil texture and soil organic and nutrient content on plant performance. Although plant

growth in the various sludge concentrations did not differ from each other, plants in the artificial soil mixtures grew less well than plants in natural salt marsh soil. This difference probably arose from the coarser texture (~ 60% sand) and reduced organic content (~ 1.5%) of the artificial mixtures compared to natural marsh soils (~ 20% sand and ~ 3% organic matter locally). Sandy soil retains both water and nutrients less effectively than do fine-grained natural soils to which most salt marsh plant species are evolutionarily adapted, and soil with low organic content deprives plants of a continuing supply of nutrients from ongoing organic decomposition. Langis et al. (1991) and Gibson et al. (1994) obtained similar results in southern California experiments with *Spartina foliosa*, as did Huckle et al. (2000) in experiments with *Spartina anglica*. Lin et al. (1999), in an experiment concerning oil pollution, found incidentally that *Spartina alterniflora* growing in sandy soil responded most strongly to nutrient addition, probably because those growing in finer grained soils already experienced a better natural nutrient supply. The results are consistent with Padgett & Brown's (1999) finding that *Spartina alterniflora* in sandy soil grows better if the soil is enriched with peat.

The low organic content of our soil mixtures resulted from three causes. First, most of the sludge we used actually entered the Mugu Lagoon sewage oxidation ponds some 20-30 years previously, and most organic matter originally present had long since decomposed. Second, our procedure of drying the sludge in direct sunlight under hot and dry conditions probably decomposed much of the organic matter that remained. Third, the matrix soil with which we mixed the sludge came from the surface of a region of sewage pond berm that had been exposed to direct sunlight and severe desiccation over a

period of decades, and this soil probably contained very little organic matter even at the time of pond construction.

The coarse texture of our soil mixtures resulted from two causes related to those just described. First, the sludge was sandy because most of the finer grained particles originally present were largely organic, and consequently organic decomposition through the years left behind a digested sludge dominated by sand size particles. Second, the berm material consisted mostly of sand.

The methods and results of this potted plant experiment inspire four guidelines for performing salt marsh restoration at the Mugu Lagoon sewage ponds site. First, metal concentrations in sewage sludge, in candidate matrix soils, and in natural marsh soil in the general vicinity of the sewage ponds should be measured ahead of time. Not much ecosystem damage can arise from using metal-contaminated sludge if local natural marsh soil contains higher metal concentrations than the sludge and candidate matrix soils do.

The second guideline is that a non-sludge matrix soil should be used in the restoration to improve plant growth even if doing so does not reduce metal contamination. This matrix soil should be as fine grained and organically rich as possible. Berm soil is not suitable. Two candidate soils are available, the natural salt marsh soil adjacent to the sewage ponds that presently supports degraded marsh, and the fine grained material that underlies the sludge and constitutes the sewage ponds' "clay" liner. Determining which is better will require a further experiment.

The third restoration guideline is that sludge and matrix soil should be mixed without removing sludge from the ground, thereby minimizing decomposition of the small

amount of organic matter present. On-the-ground mixing will necessarily involve heavy earthmoving and farming equipment and cannot possibly duplicate the fine scale homogeneity of the soil mixture that this experiment employed. This experiment's finding that the mixture's sludge content exerts little or no influence on plant growth establishes that finer scale homogeneity is actually not necessary. On-the-ground soil mixing has the further advantage of being far less expensive than would mixing soil in a pug mill, the only practical alternative.

The fourth restoration guideline arises from the observation that *Salicornia virginica* survived slightly better and grew much better at this experiment's wet site than at the dry site. The two sites were similar in elevation and in degree of desiccation at the soil surface between spring tide periods. They differed in subsurface moisture content due to the proximity of a tidal creek on three sides of the wet site and no such water supply at the dry site. The guideline is simply that restoration construction should attempt to establish hydrological conditions that keep the subsurface soil moist most of the time. This guideline is already followed in most well-planned salt marsh restoration projects; this experiment merely provides one more demonstration of its importance.

A general observation accompanies these four guidelines. The use of sewage sludge as a soil component in salt marsh restoration actually has two potential benefits, better plant growth on what might otherwise be poor soil for salt marsh plants, and easy sludge disposal in metropolitan areas with a shortage of landfill space. Because the sludge at Mugu Lagoon is so old, sludge use in salt marsh restoration there will reap only the second benefit. However, restoration projects that use sludge fresh from a sewage treatment plant have the potential to capture both of these benefits. The ideal sludge for this pur-

pose has low heavy metal (and other toxic substance) content, high organic content, and fine physical texture. Because problems that accompany use of fresh sludge in salt marsh restoration will differ from the problems described here, we encourage pre-restoration experiments to discern sludge effects and to guide restoration methodology.

Conclusions

This experiment yielded four main conclusions. These answer, or partially answer, the four questions stated in the introduction.

1. Sludge concentration in the soil mixtures employed in this experiment did not significantly affect above ground growth of either *Salicornia virginica* or *Frankenia grandifolia*.
2. Heavy metal concentrations employed in this experiment (see Fig. 3) exerted no statistically detectable effect on above ground growth or survival of either plant species.
3. Both plant species experienced significantly greater above ground growth in natural salt marsh soil than in this experiment's artificial soil mixtures.
4. Properties most likely to have caused reduced plant growth in the soil mixtures of this experiment are coarse soil texture and low soil organic content.

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Table 1. Concentrations of metals in sludge, berm and wetland soils at Mugu Lagoon. CFR refers to limits for monthly average allowable concentrations for land application of sludge, as specified in section 40:503 of the Code of Federal Regulations. Sludge values are the maximum concentrations reported by CDM (1996). Berm and wetland values are the maximum of three and two samples, respectively, collected before the initiation of the experiment. All values are ppm dry mass. NL = no limit given in CFR. * = dry mass values not given in CDM (1996).

metal	CFR	sludge	berm	wetland
cadmium	39	79	4	1
chromium	NL	*	100	23
copper	1500	1016	50	17
lead	300	425	7	6
molybdenum	18	*	4	3
zinc	2800	3058	200	54

Table 2. Linear regressions of plant growth on individual soil characteristics. Features of all single-variable regressions with $P < .2$ are shown.

species	site	soil characteristic	P	sign of R	R ²
<i>Frankenia</i>	wet	chromium	.17	+	.23
<i>Frankenia</i>	dry	clay	.01	-	.57
		silt	.01	+	.56
		phosphorus	.03	+	.45
		sand	.09	-	.33
		nitrate	.13	-	.26
<i>Salicornia</i>	wet	ammonium	.01	+	.59
		cadmium	.14	-	.25
<i>Salicornia</i>	dry	total nitrogen	.15	+	.31
		copper	.15	+	.31

Table 3. Comparison of metal, nutrient, organic material, and grain size class concentrations in natural soils and sludge mixtures. Shown for each site separately is how many of the five sludge mixtures differ significantly from natural soil in the concentration of each constituent measured. A + sign in the second column for each site indicates higher concentration in natural soil than in sludge mixtures, and a – sign indicates the opposite. Statistical significance of these differences was evaluated using Tukey’s honest significant difference test following one-way analysis of variance applied to each soil property individually.

variable	concentration difference: natural soil – mixture			
	dry site soil		wet site soil	
	no. of mixtures with P < .05	sign of difference	no. of mixtures with P < .05	sign of difference
cadmium	5	–	5	–
chromium	5	–	5	–
copper	5	–	4	–
lead	0		5	+
molybdenum	3	–	2	–
zinc	2	–	2	+
total nitrogen	3	+	5	+
ammonium	5	+	5	+
nitrate	0		5	+
total phosphorus	5	+	4	+
organic material	2	+	5	+
sand	5	–	5	–
silt	5	+	5	+
clay	0		5	+

Figure Captions

Fig. 1. Organic and nutrient content of the sludge mixtures; ppt = parts per thousand, ppm = parts per million. Eight replicates were examined at each sludge concentration, 40 cases in all, but some points in these graphs exactly coincide.

Fig. 2. Content of six selected heavy metals in the sludge mixtures; ppm = parts per million. At each sludge concentration, each graph contains 8 points, some of which coincide.

Fig. 3. Particle size composition of the sludge mixtures. At each sludge concentration, each graph contains 8 points, some of which coincide.

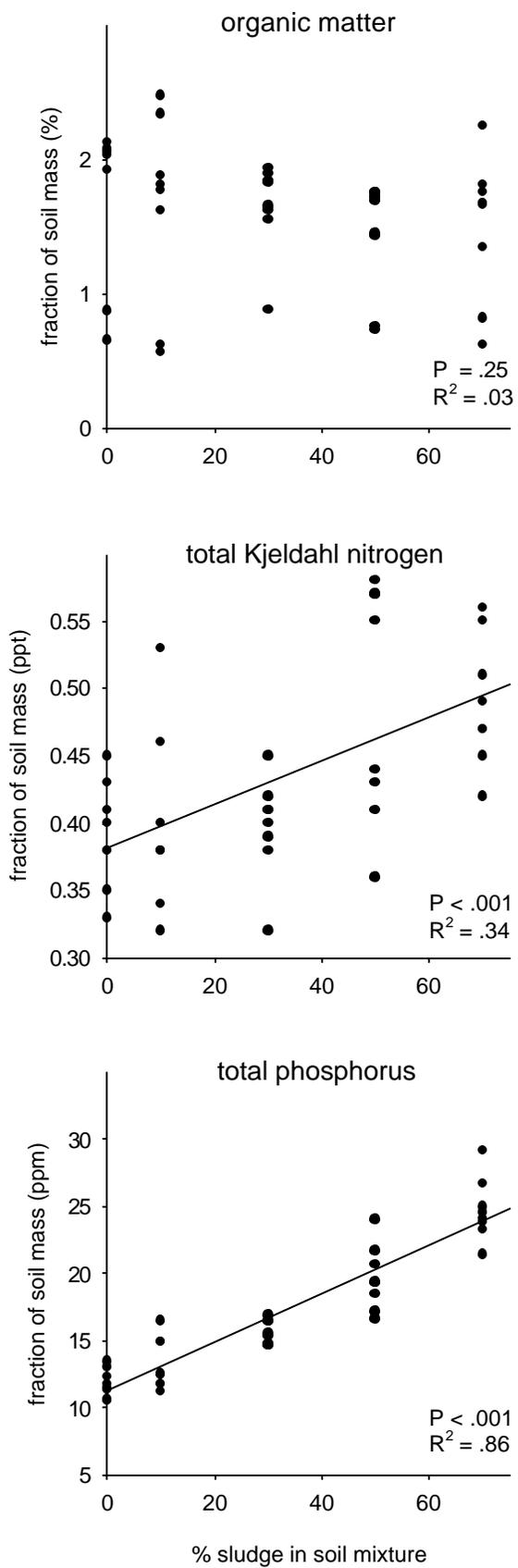
Fig. 4. Plant growth in the sludge mixtures. In each graph, the number of replicates at each soil concentration is shown in Figure 6. Some points in these graphs exactly coincide. Note the different scales on the graphs' vertical axes.

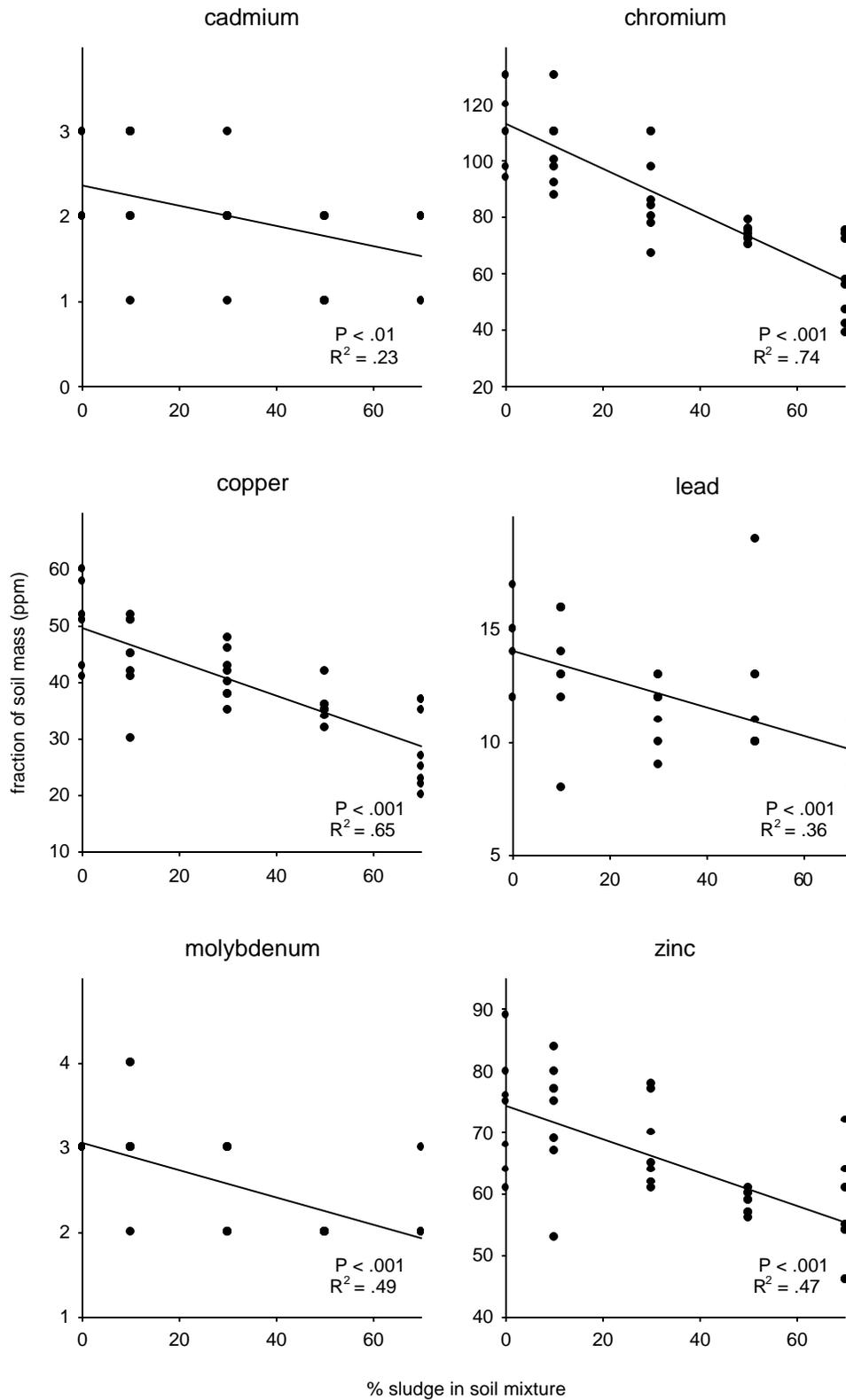
Fig 5. Mean plant growth in the sludge mixtures and in the controls that employed natural soil obtained locally from each planting site. In the controls labeled "wetland soil" and "wetland soil np", this soil was placed into holes lined or not lined, respectively, with pots identical to those containing artificial soil mixtures. The solid bars, error bars, and numbers within solid bars represent sample means, standard errors, and numbers of replicates, respectively. This last number is the number of plants, of the 10 replicates in

each treatment originally planted, that remained alive at the time of harvest. In each graph, mean concentrations that share the same letter above the bar do not differ statistically ($P > .05$), and those that do not share the same letter do differ statistically ($P < .05$). Note the different scales on the graphs' vertical axes.

Fig. 6. Mean organic content and particle size composition of the sludge mixtures and natural soils at the two experimental sites. The solid bars and error bars represent sample means and standard errors, respectively, of the 8 replicates in each sample. In each graph, mean concentrations that share the same letter above the bar do not differ statistically ($P > .05$), and those that do not share the same letter do differ statistically ($P < .05$).

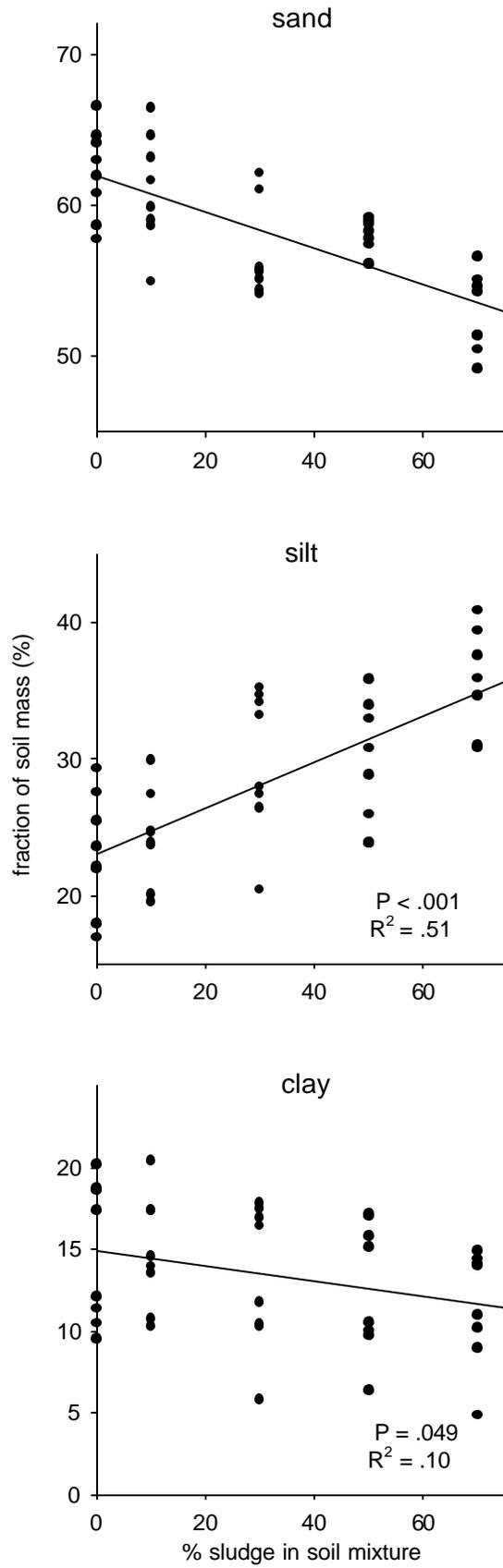
[Figure 1]

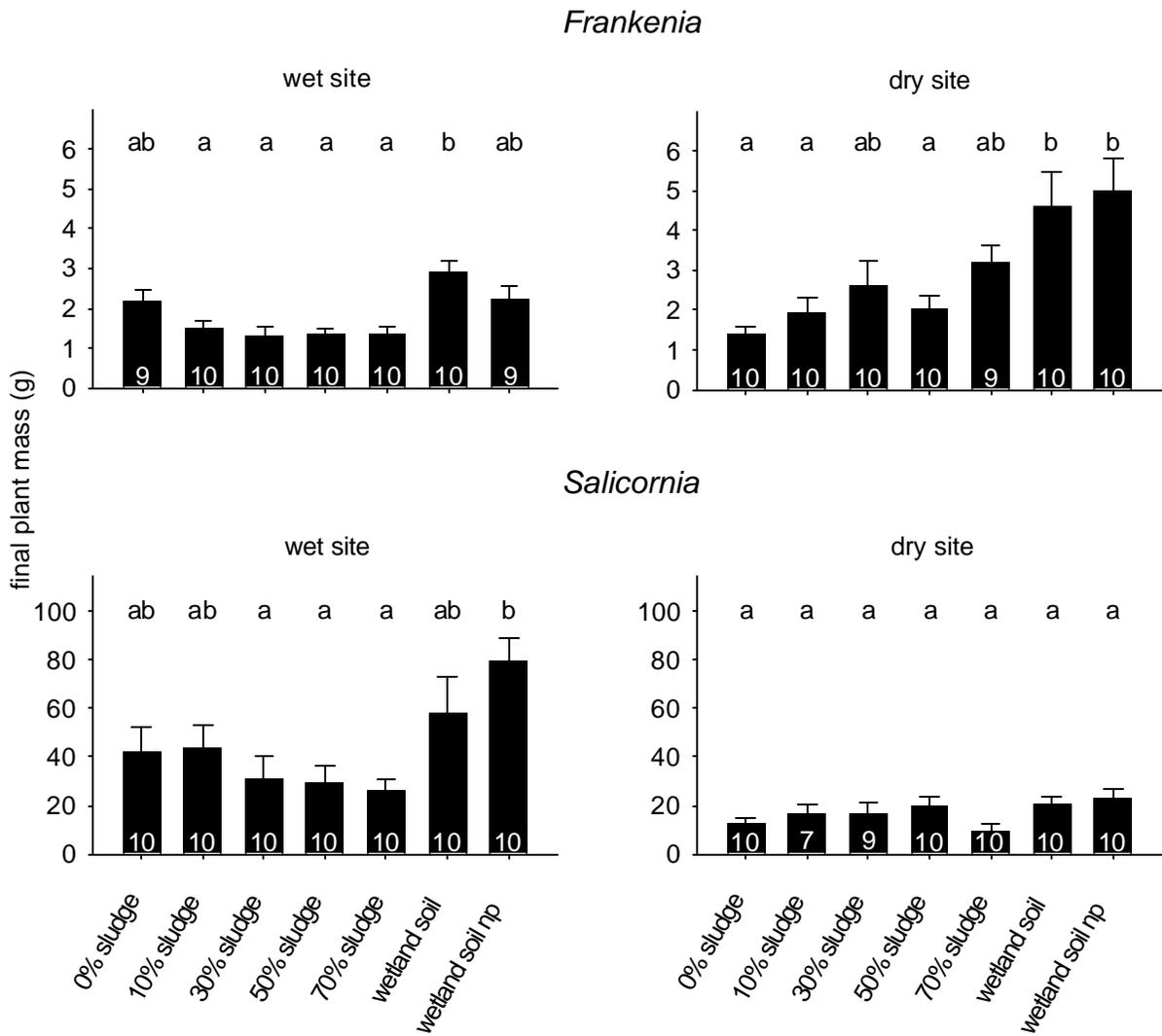




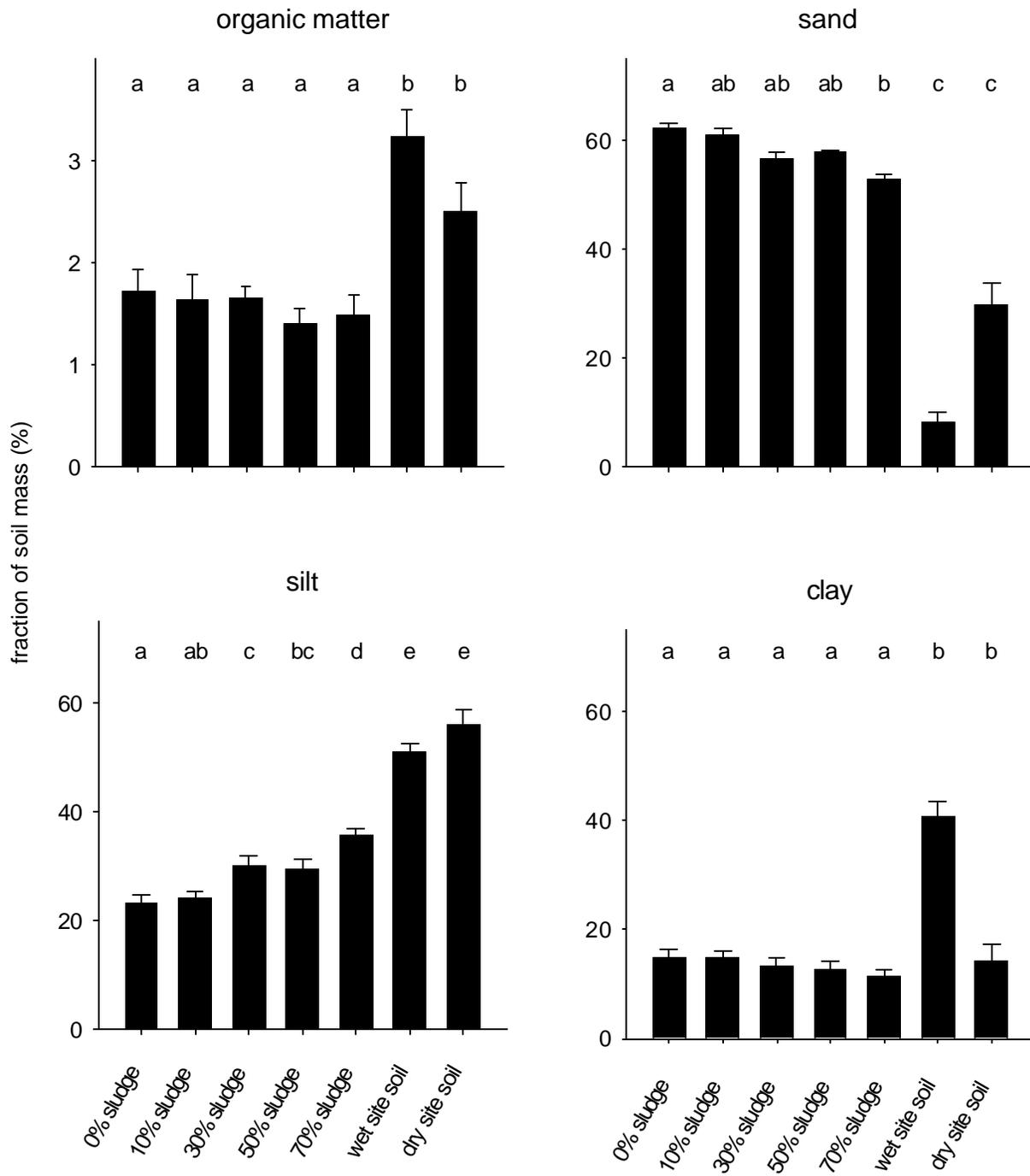
[Figure 2]

[Figure 3]





[Figure 5]



[Figure 6]