Treatments of Severely Boron-Contaminated Soils for Phytorestoration

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ABSTRACT

As a result of the boron refining process, the Rio Tinto Borax site in California has experienced boron contamination, leading to desertification and the loss of local vegetation. Increasing industrial demand for the energy reduction ability of boron promotes continued mining. As such, the development of an effective and low-cost method for long-term reclamation is important. To establish this method, I analyzed the effects of amendments on the growth of a boron tolerant plant. I tested the effectiveness of water and organic matter on the growth of weeping alkaligrass (*Puccinellia distans*) naturalized in the USA in severely boron-contaminated soil at the Rio Tinto Borax site. I conducted a multi-factorial experiment with 4 levels of dilution of contaminated soil with sands, 3 levels of organic matter (OM), and 3 concentrations of polyethylene glycol (PEG) with 3 replicates. This study aimed to determine if: (1) there is a significant difference in plant growth and water content in the soils and plants between the levels of each factor: (2) there are interaction effects between each combination of two factors: and (3) there is an interaction effect for all three factors. Sand and OM significantly affected the plant growth and water content. PEG significantly affected water in the soil, but did not affect the plant growth. The combination of sand and OM significantly affected the plant growth and water content. This study shows the importance of amendments for reclaiming boron-contaminated soils.

INTRODUCTION

Boron contamination is a serious environmental problem affecting both ecosystems and human activities. There are many causes of boron accumulation in the surface soil and/or deep ground including irrigation, a dry local climate, fertilizer, industrial wastewater, and mining (Camacho-Cristobal *et al.* 2008, Col & Col 2003, Nable *et al*. 1997, Shani & Hanks 1993, Sommer & Sorokin 1928). Both irrigation and dry local environment cause the accumulation of salts in the ground which, because of insufficient water, can increase boron to a toxic level. Boron contamination caused by these factors degrades the vegetative quality and decreases the amount of crops supplied to people (Shani & Hanks 1993). In western Central California, the arid and semiarid environment and weathering of boron-containing parent rocks from California's coastal ranges has led to boron accumulation in the subsurface and groundwater and has interrupted sustainable agriculture because excess boron degrades the physiological functions of plants (Banuelos *et al*. 1993). Other studies in Turkey have indicated that the wastewater containing excess boron (25-30 mg/L) discharged into a river from the power plants can decrease crop production, particularly for citrus trees (Akar 2007).

Although many studies have reported the negative effects of boron accumulation, mining cannot be stopped because of increasing industrial demand for boron for its energy saving ability (Lyday 2000). For example, adding boron to certain chemical reactions can reduce the time and gas required to heat a reaction. Thus, because reduced energy requirements directly impact company sales in terms of efficiency of cost, time, and materials, the energy reduction ability of boron is significant (Lyday 2000). The United States and Turkey, the world's largest boron producers, have the most significant boron contamination problems (Lyday 2000). In California, the Rio Tinto Borax Mining Company that refines borate (BO_3^{3-}) is now facing boron contamination problems as a result of boron refining process.

The Rio Tinto Borax site has experienced desertification as local vegetation has died out and soils have lost a considerable amount of nutrients and water. As a result of substantial water loss in soils, boron concentration in the water system in the ground increases, which makes boron more available for plants to uptake. Through the process of desertification, this excess boron dissolved in water degrades several physiological functions of plant by retarding metabolism, reducing root cell division, decreasing leaf chlorophyll contents and photosynthetic rates, decreasing lignin and suberin levels, and reducing growth of shoots and roots (Camacho-Cristobal *et al.* 2008, Nable *et al*. 1997). Another study also showed that boron toxicity could cause leaf burn that make plants unable to photosynthesize (Eaton 1944). Because most plants die from these kinds of boron toxicity, less organic matter from leaf litter falls to the ground and the soil loses its ability to provide nutrients and retain water (Parton *et al*. 1987). In addition, by the multiplier effect, a lack of vegetation and degraded soil quality promotes conditions in which plants cannot grow.

Given these conditions, the most effective and low-cost method for long-term reclamation would be the improvement of soil quality by boron-tolerant plants (Nable *et al*. 1997). Boron-tolerant plants, which can survive exposure to boron without significant physiological damage, extract boron from the soil and eventually reduce the boron content in the ground. Although the mechanism of their tolerance to boron has not been determined in detail, there are some potential boron-tolerant species already reported: saltbush (Watson *et al*. 1994), wheat (Paull *et al*. 1988), and tall fescue (*Festuca arundinacea*) (Banuelos *et al*. 1995). These boron-tolerant plants provide organic matter that helps the soil retain moisture and acts as an excellent food source to support soil microbes as an initial vegetative cover when they die and decay (Reiley & Shry 2000). At the same time, boron tolerant plants can remediate other conditions caused by lack of vegetation and drought, such as erosion, increased concentration of heavy metals and salts, and reduced microbial population in soils (Skujins & Allen 1986). Therefore, by introducing boron-tolerant plants the soil would recover its natural conditions that can accommodate native vegetation again.

Even for boron-tolerant plants, enhancing soil conditions by adding amendments is a necessary step of reclamation. According to a Chambers' Group report (unpublished data), the soil at Rio Tinto Borax has high levels of boron, insufficient organic matter, high levels of salinity and alkalinity, possible potassium and phosphorus deficiencies, and insufficient soil moisture. These soil problems quickly kill plants even though they are tolerant to boron. To reduce these effects of those soil characteristics, amendments are usually utilized for restoration projects (Prather 1977). For example, organic matter enhances plant growth by increasing the capability of soil to hold moisture and providing nutrients (Reiley & Shry 2000). Adding sand to contaminated soil decrease the concentrations of toxic substances.

Among the potential boron-tolerant plants, weeping alkaligrass (*Puccinellia distans*) naturalized in the USA is expected to be highly effective for reclamation of boron. It is already known that *Puccinellia distans* native to Turkey (PDT), the same species as PDU, has high boron tolerance (unpublished data from Prof. Terry lab). Also, there are more PDU seeds available of lower cost in the United States. Once the feasibility of PDU as a boron-tolerant plant is determined, the possibility of low-cost reclamation by boron-tolerant plants in U.S.A is expected. However, there is a doubt that PDU and PDT share the same or similar characteristics in boron tolerance because their appearance is different.

In this study, I analyzed the effects of adding sand, organic matter (OM), and polyethylene glycol (PEG) on the growth of weeping alkaligrass in severely boron-contaminated soil at the Rio Tinto Borax site by using a multi-factorial approach. I assessed all combinations of all three factors by measuring fresh and dry weight of shoots and by calculating shoot moisture content (SMC) and soil gravimetric water content (SGWC). Dilution of contaminated soil by adding sand should increase the rate of growth by lowering the concentration of boron contained in the water system. Organic matter should improve the condition of soils by increasing the capacity of water retention and providing nutrients. Because PEG reduces the amount of water taken up by plants by lowering the rate of transpiration (Michel and Kaufmann 1973), it should lower the amount of boron extracted by plants. Also, PEG is safe for environments. Working *et al.* confirmed the relative safety in PEG administration by reviewing the literatures about PEG (1997)._The individual effects of these three factors are already known, but it has not been examined that each amendment actually helps plant growth in the severely boron-contaminated soil. Also, their combined effects are still unknown. This study will determine if: (1) there is a significant difference in plant growth and water content in the soils and plants between the levels of each factor: (2) there are interaction effects between each combination of two factors: and (3) there is an interaction effect for all three factors. Because PEG reduces both the amount of water and boron taken up by plants, it should have a negative effect on plant growth when boron is not abundant. As such, healthier plants should have higher biomass, less browning, less wilting, and be more greenish in color.

METHODS

Study Organism and Germination The plant species used in this study is weeping alkaligrass (*Puccinellia distans*) naturalized to the USA. This species was introduced from Eurasia to North America and naturalized in the USA where the Rio Tinto Borax site is located. Weeping alkalgrass is highly expected for restoration because it is adapted to sites with high salt concentrations as a facultative halophyte (Tarasoff 206). Also, PDU is adapted to high pH and low available water, which are soil characteristics at Rio Tinro Borax site.

The soil used for the germination of the seeds was a normal potting soil for gardening, Scott Potting Medium (Scotts-Sierra Horticultural Products Co, Marysville, OH, USA) provided from a greenhouse at University of California, Berkeley. I sowed the seeds of weeping alkaligrass and watered them with tap water regularly. Approximately 5 weeks from sowing PDU seeds, they established a suitable size (about 15 cm) to be used in experiments.

Experimental Design for Multi-factorial Analysis To determine how each factor affects the plant growth and water in the soils and the plants in severely boron-contaminated soils, I conducted a multi-factorial analysis by using sand, peat moss as organic matter, and PEG as soil amendments. In this study, there were 36 treatments with 3 replicates: all combinations of 4 levels of dilution by adding sands (0 %, 30 %, 60 %, and 90 % by weight), 3 levels of peat moss (0 %, 25 %, and 50 % by volume in a pot), and 3 concentrations of PEG (0, 45, and 90 g/L). In total, there were 108 pots.

Severely boron-contaminated soils were collected at the Rio Tinto Borax site (870 mg of boron per litter). I put all pots into a clear bag that holds leaching from pots. Then, I placed an additional larger pot under the pot with the bag and rotated smaller pots 45 degrees, which makes enough space to hold leaching between smaller and larger pots and prevent smaller pots to soak into leaching (Fig. 1). If there is no space under smaller pots, then the pots are submerged into water for a long time and the roots of plants may die because of too much water.

Figure 1. Bag System. A bag holds leached water from a smaller pot. There is a space created between two pots by rotating the smaller one 45 degrees.

To prepare the soil, I first added dry gypsum powder at the concentration of 1.2 g per 100g of contaminated soil to reduce the effects of boron. Then, I diluted the contaminated soils with the proper amounts of sands to 0, 30, 60, and 90 % by weight. Each pot held 300 g of soil and/or sands or 40 g of peat moss. I mixed 0, 25, and 50 % of peat moss by volume in each pot with the proper amount of mixed soil to make them 100 % in total. I poured these soils into each pot and gave them 200 ml of half strength Hoagland solution containing 0, 45, or 90 g of PEG per litter. After about 3 days, these solutions spread throughout the pots and made a homogeneous environment. I transferred seedlings to pots without removing potting soil, so that potting soils acts as a buffer and prevent transplant shock. Twice a week, I watered each plant with 50 ml of distilled water. I first poured distilled water into a clear bag catching the leached water and returned it to the pots after shaking it, so that constant environments were created by pouring back all leached water containing boron, nutrients, and PEG to pots. Throughout the study, I monitored plant health in terms of browning, leaf color, and wilting.

Fresh and Dry Weight Measurements To determine the effects of addition of sand, organic matter, and PEG and their interaction effects on plant growth, I measured the fresh and dry weight of plant shoots harvested 3 weeks after transplanting. I cut the above ground part of shoots and measured their weight. For dry weight measurements, shoots were placed in an incubator oven at 70°C for 72 hours, and subsequently weighted (unpublished data from Prof. Norman's lab).

Calculations To determine the effects of sand addition, OM, and PEG on the water in the soils and the plants in severely boron-contaminated soils, I calculated the shoot moisture content (SMC) and Soil Gravimetric Water Content (SGWC). SMC is the ratio of weight of water in shoots to shoot fresh weight and expressed on a percent basis. SGWC is the ratio of weight of water to soil dry weight and expressed on a percent basis.

Data and Statistical Analysis To determine if: (1) there is a significant difference of plant growth and water content in soil and plant between the levels of each factor: (2) there are interaction effects between two factors: and (3) there is an interaction effect of all three factors, I analyzed the data by using factorial ANOVA (R 2.10.1 for Mac OS X, Rcmdr XQuartz 2.3.4).

RESULTS

All plants of weeping alkaligrass showed some response to boron-contaminated soils although the extent of response varied with different treatments of sand, organic matter, and PEG. Most plants lost their green color and turned browner as the experiment progressed. Although many plants wilted as the experiment went further, some plants didn't show much wilting. There were two types in those plants that did not wilt: plants with green color and little browning and ones with less green color and much browning. In addition, plants that showed a greater response to boron (less green and more browning and/or more wilting) didn't grow tall, compared to plants, which showed little response (more green, less browning, and less wilting).

Table 1. P-value of each thee factor and combinations of two or more in fresh and dry weight, shoot moisture content, and soil gravimetric content (factorial ANOVA). P-value that is less than 0.05 indicates significance and is expressed as the symbol of *.

	Fresh Weight p value	Dry Weight p value	SMC p value	Soil GWC p value
Sand	**< 0.0001***	**< 0.0001***	**< 0.0001***	**< 0.0001***
OM	**< 0.0001***	**0.028***	**0.001***	**< 0.0001***
PEG	0.853	0.736	0.228	**< 0.0001***
Sand*OM	**< 0.0001***	**< 0.0001***	**< 0.0001***	**< 0.0001***
Sand*PEG	0.286	0.291	0.122	**0.012***
OM*PEG	0.152	0.084	0.316	**0.001***
Sand*OM*PEG	0.762	0.404	0.682	0.39

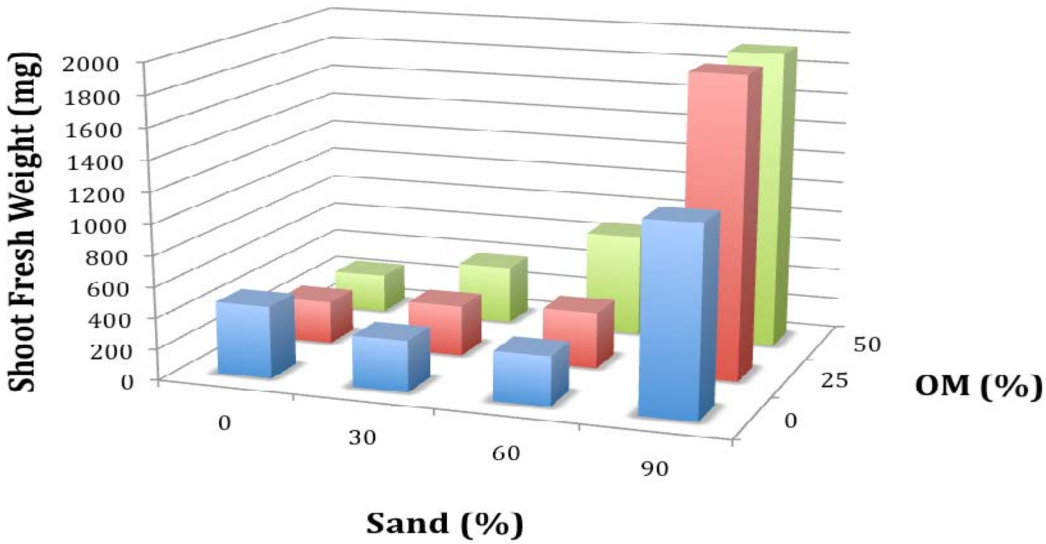

Figure 2. Shoot fresh weight of weeping alkaligrass in milligram in different levels of sand (% by weight) and organic matter addition (% by volume). I took the averages of different levels of PEG addition.

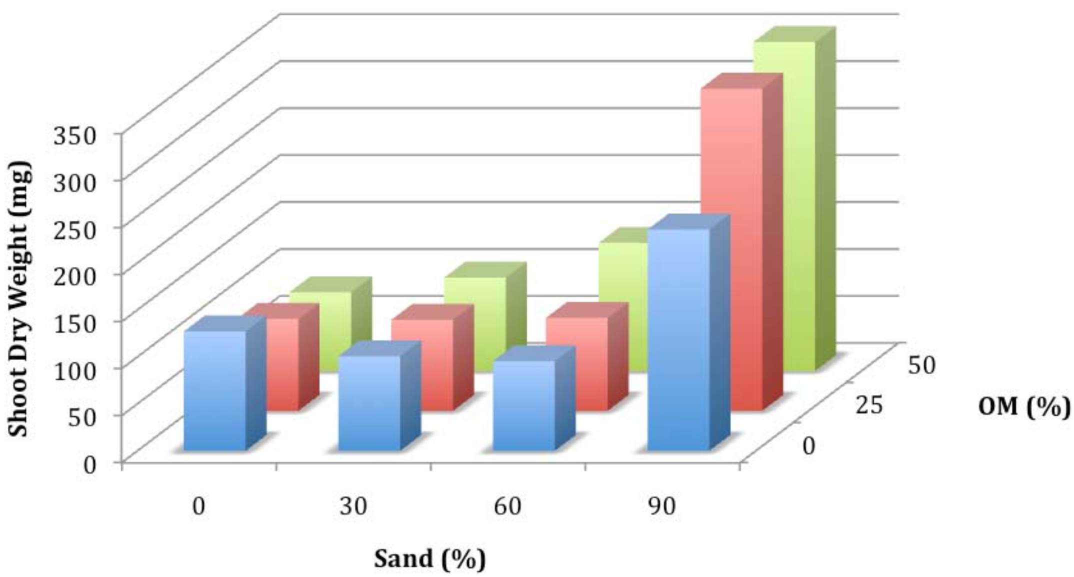

Figure 3. Shoot dry weight of weeping alkaligrass in milligram in different levels of sand (% by weight) and organic matter addition (% by volume). I took the averages of different levels of PEG addition.

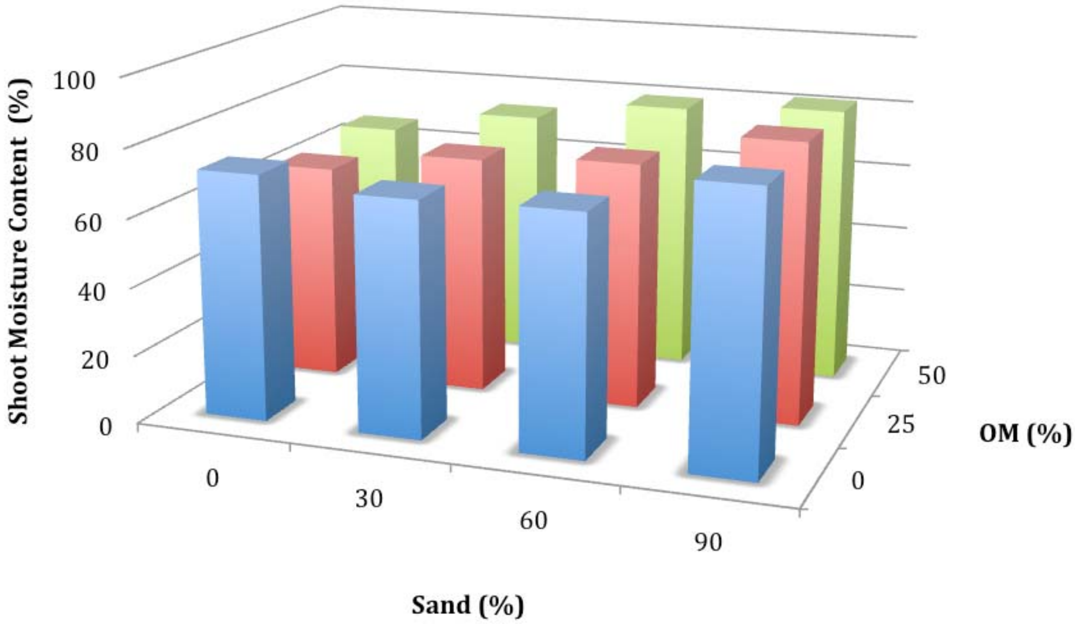

Figure 4. Shoot moisture content of weeping alkaligrass on a percentage basis in different levels of sand (% by weight) and organic matter addition (% by volume). I took the averages of different levels of PEG addition.

Effects of Single Factor There was a significant difference in the shoot fresh weight and dry weight between the different levels of sand addition (Table 1, $p<0.0001$ for both the fresh and dry weight). Sand addition largely increased the shoot fresh and dry weight (Fig. 2 and 3). Sand addition significantly affected the shoot moisture content and the soil gravimetric water content (Table 1, $p<0.0001$ for both SMC and SGWC). Although there were some exceptions at the 0 % organic matter, sand addition slightly increased the shoot moisture content (Fig. 4). Also, sand decreased SGWC at any levels of organic matter and PEG, except for the 0 % organic matter (Fig. 5 and 6).

Different levels of organic matter showed a significant effect on the fresh and dry weight (Table 1, p=less than 0.0001 and 0.028 respectively). Organic matter increased the fresh and dry weight at the higher levels of sand addition (Fig. 2 and 3). This trend was more prominent in 90 % sand addition. There was a significant difference in the SMC and SGWC between different levels of organic matter (Table 1, $p=0.001$ and less than 0.0001 respectively). Organic matter slightly increased SMC, but not at the 0 % sand. Organic matter increased SGWC, but not at 90 % sand addition (Fig. 5).

Unlike sand and organic matter, different concentrations of PEG did not significantly affect the fresh and dry weight and shoot moisture content (Table 1, p=0.853, 0.736, and 0.228 respectively). However, PEG significantly affected SGWC (Table 1, p= less than 0.0001). PEG increased the soil gravimetric water content (Fig. 6 and 7) although the extent of the effects varied.

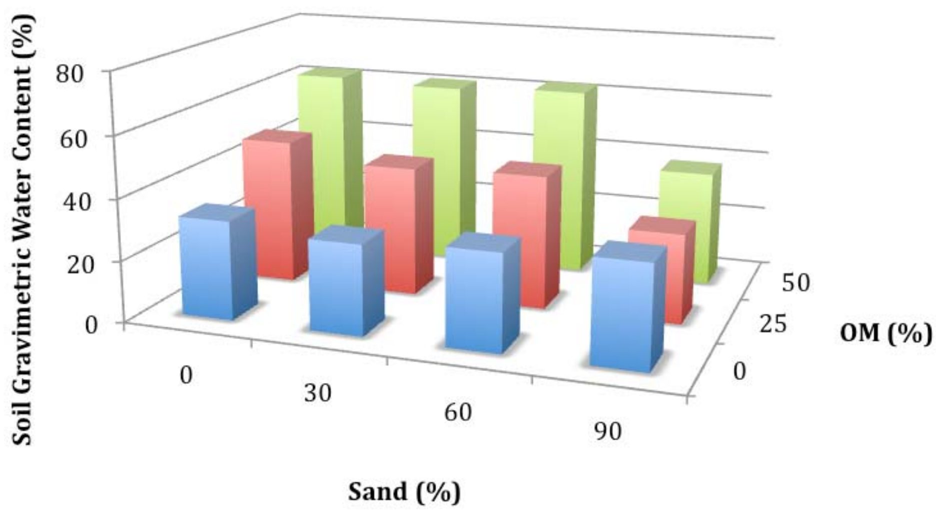

Figure 5. Soil gravimetric water contetnt on a percentage basis in different levels of sand (% by weight) and organic matter addition (% by volume). I took the averages of different levels of PEG addition.

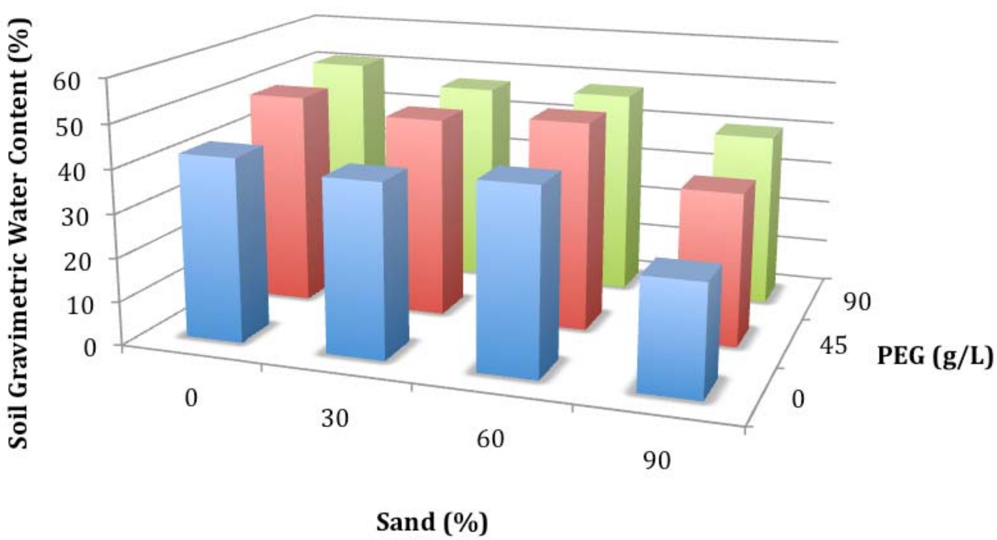

Figure 6. Soil gravimetric water contetnt on a percentage basis in different levels of sand (% by weight) and PEG (g/L). I took the averages of different levels of OM addition.

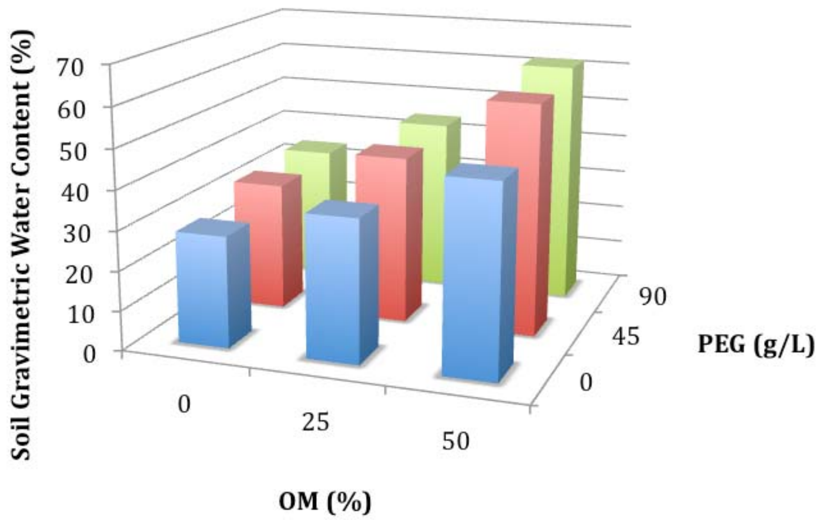

Figure 7. Soil gravimetric water contetnt on a percentage basis in different levels of sand (% by weight) and PEG (g/L). I took the averages of different levels of OM addition.

Interaction Effects of Two or More Factors The interactions effects between sand and organic matter were significant in the fresh and dry weight, SMC, and SGWC (Table 1, $p<0.0001$ for all). In the fresh and dry weight, sand progressively increased the plant growth in the presence of organic matter (Fig. 2 and 3). However, the increase of the fresh and dry weight by sand addition was less prominent without organic matter. Diluting the soils actually decreased the shoot fresh and dry weight at 0 % organic matter. Also, organic matter increased the shoot fresh and dry weight more when the soil-sand mixture contained the larger amount of sand. But, organic matter decreased the fresh and dry weight without sand. Sand increased the SMC more progressively with the higher levels of organic matter (Fig. 4). Organic matter increased SGWC progressively, but not at the 90 % sand.

The interaction effects between sand and PEG and OM and PEG were significant only in the soil gravimetric water content (Table 1, $p=0.012$ and 0.001 respectively). PEG increased the soil gravimetric water content especially at the 90 % sand (Figure 6). The increase of SGWC by adding PEG was more prominent with higher levels of organic matter (Fig. 7). The interaction effect of the three factors was not significant in any measurements and calculations (Table 1).

DISCUSSION

Many cases of boron contamination that leads to soil degradation and vegetation loss have been reported, but an effective method to reclaim boron is still unknown. Finding a way to reclaim boron is really important for humans and environments. I tested 4 levels of sand addition, 3 levels of organic matter, and 3 levels of PEG as soil amendments in a multi-factorial analysis. The results showed a significant effect of each sand and organic matter in the shoot fresh and dry weight, SMC, and SGWC and a significant effect of PEG on soil gravimetric water content. The interactions effects between sand and organic matter were significant in the fresh and dry weight, SMC, and SGWC. The combinations of sand and PEG and organic matter and PEG significantly affected SGWC. This study investigated how each factor and their combinations of two or more factors affect the plant the growth of weeping alkaligrass and water content in the soils and the plants in severely boron-contaminated soils.

All plants showed symptoms of boron toxicity (browning, bleach of green color, and wilt) at least to some extent. The symptoms plants showed in this study matched with the results in the study by Blevins and Lukaszewski (1998). According to Camacho-Cristobal *et al.* (2008), these symptoms are due to the degradation of chlorophylls and the cell walls.

Effects of Single Factor There was a significant difference in the fresh and dry weight between the different levels of sand addition, which indicates that diluting the boron-contaminated soils by adding sand affect the plant growth. According to Keren *et al.* (1985), the decrease of plant dry weight is correlated to the increase of boron content in plants. Fig. 2 and 3 showed that sand addition has increased the shoot fresh and dry weight, which means that sand addition increases plant growth by decreasing boron content in plants (Reeve and Doering 1966). Sand addition also significantly affected the shoot moisture content and the soil gravimetric water content, indicating sand affects the capacity of water retention in soil and eventually the amount of water taken up by plants. Because the size of sand is larger than the soil size, sand holds less water than soil does. This explains that sand actually decreased SGWC (Fig. 5 and 6). However, at the same time, sand increases the amount of water available to plant roots (Keren *et al.* 1985). Even if soil contains a lot of water in it, all water is not always available to plant roots because soil holds water and does not easily take it away. Because of that, sand addition slightly increased the shoot moisture content.

Different levels of organic matter significantly affected the fresh and dry weight, indicating organic matter is important for the plant growth. Also, organic matter increased the shoot fresh and dry weight at higher levels of dilution (Fig. 2 and 3), which indicates that organic matter provides nutrients more effectively in low levels of boron in soil. There was a significant difference in the SMC and SGWC between different levels of organic matter. This indicates that organic matter affects water content in the soil and plants. Organic matter increased SGWC because organic matter retains more water (Parton *et al*. 1987). Because organic matter also increases the amount of water available to plant roots, it slightly increased the SMC.

PEG did not significantly affect the fresh and dry weight and the shoot moisture content, indicating PEG alone does not affect the plant growth and water in the plants. However, PEG significantly affected the soil gravimetric water content, indicating PEG actually controls the amount of water taken up by plants by lowering the rate of transpiration (Michel and Kaufmann 1973).

Interaction Effects of Two or More Factor The interactions effects between sand and organic matter were significant in the fresh and dry weight, SMC, and SGWC, indicating sand and organic matter together has the different effects on the plant growth and water content in plants and soil from those when they are a single factor. According to Keren *et al.* (1985), plant growth is controlled by boron in soil solution, not by total boron content in soils. Also, plants uptake more boron and dry weight decreases when there is more boron in soil solution (Keren *et al.* 1985). Moreover, sand increases boron level in soil solution although organic matter decreases boron content in solution (Keren *et al.* 1985). Based on these facts, the decrease of the plant growth by adding sand at 0 % organic matter and the increase of the plant growth by sand addition at 50 % organic matter can be explained. Because sand increases available boron in solution, plants take up more boron and the dry weight decreases. At 50 % organic matter, I assume more nutrients provided by organic matter increased the plant growth as the positive effects of nutrients on plant growth offset the negative effects of boron. Also, organic matter decreases the fresh and dry weight without sand. This is because there is more boron available in soil solution by organic matter addition. This result matched with the study conducted by Yermiyahu *et al*. (2001); excess organic matter encourages the boron uptake by plants. Organic matter decreases the soil pH (McCauley et al. 2003), but the soil decreases the ability to absorb boron in low pH. As a result of that, more boron becomes available in soil solution. The same thing would have happened when sand increased the SMC more progressively with the higher levels of organic matter. Organic matter increased SGWC progressively, but not at the 90 % sand. This is because too much sand does not hold water and water drain out from the pots even if there is some organic matter.

The interaction effects between sand and PEG and OM and PEG were significant only in the soil gravimetric water content. Also, PEG increased the soil gravimetric water content especially at the 90 % sand. These results indicate that PEG improves soil water retention only when the soil contains a lot of sand. The increase of SGWC by adding PEG was more prominent with higher levels of organic matter, indicating PEG works better in more nutrient rich condition. The interaction effect of the three factors was not significant in any measurements and calculations, indicating the three factors together do not affect the plant growth and water in soil and plants.

Conclusion To reclaim severely boron-contaminated soils, it is essential that: soil boron is diluted: organic matter is added to improve the condition of the soil: and PEG is added especially when plants are exposed to drought. As a result, PEG increases soil water retention only when soil contains a lot of sand, which indicates PEG help plants to retain water when they are exposed to drought. This is because a lot of sand encourages drainage. The important thing is that there are some interaction effects between factors. Diluting boron by adding sand is actually beneficial for plant growth, but it is not in certain conditions. Further study is required for interaction effects between many factors.

High levels of sand and organic matter addition are the most effective on the plant growth, but it is hard to conduct this method in field scale experiments. Finding an alternative way to dilute contaminated soils or to limit boron uptake by plants is important. Also, the use of mycorrhizae is another approach to reduce boron effects. Polanco *et al*. (2008) inoculated jack pine (*Pinus banksiana*) with several species of mycorrhizae and determined that all inoculated seedlings had higher dry weight and chlorophyll concentrations in solutions with high boron concentration. In addition, highly boron tolerant bacteria (*Bacillus boroniphilus*) have been found (Ahmed *et al*. 2007). If the genes and/or mechanism to tolerate boron are determined, it would also help remediation projects.

Although I tried to simulate conditions in the field, there are still many limitations to this method. Because the field has many variables, the conditions easily change. Based on the results of this study, there are interaction effects between factors and the concentrations in combination are important to support and enhance growth of plants. Therefore, in the field, it is hard to maintain the best combination in the real field for a long time. Moreover, I focused more on creating the conditions that plants can grow in boron-contaminated soils. In this method, boron goes back into the soils after the first introduction of plants to the field because the dead leaves that is decomposed and become nutrients later still contains boron in their tissues. However, the method in this study at least provides organic matter, which supplements essential nutrients for plants and improves the soil condition. Analysis of boron content in plant tissues will be conducted. If the plants that grew well in this study did not extract much boron, I suggest cultivating a method to effectively remove boron from soils, which would be the use of another boron tolerant plant species.

Implications of Study The effects of each factor and their interaction effects on the plant growth in the boron-contaminated soils at the Rio Tinto borax site help to plan reclamation methods in many organizational group. This study determined how each factor act and how the three factors interact in the presence of boron. In planning a reclamation method, the results from this study would be useful. Improving a boron reclamation method is important for not only people working on reclamation projects and people in mining companies but also people in the agricultural field and people living normally. Because there are many factors that cause boron contamination, many people are suffering from the effects of boron accumulation. The agriculture in dry local climate where boron accumulation is usually found would be improved once an effective boron reclamation method is cultivated. Also, the economy would be encouraged because the agriculture in many fields is improved once boron is reclaimed. Therefore, determining the effects of many factors and the interaction effects on plant growth in soils containing high levels of boron leads to the establishment of more effective reclamation method, healthier vegetation (agriculture), and the active economics in the world.

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