Effect of Salinity on the Growth and Cadmium Accumulation of *Vetiveria nemoralis*

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ABSTRACT

One of the major targets for phytoremediation is the removal of toxic heavy metals from the contaminated sites. This study investigated the effect of the salt stress (EC 3.5 dS/m) on the growth and removal of Cadmium (333 mg/kg) from spike contaminated soils by growing vetiver grass (*Vetiveria nemoralis*) in a pot experiment for forty five days. There were four treatments, including uncontaminated soil, uncontaminated saline soil, contaminated soil, and contaminated saline soil. *V. nemoralis* survived and grew well in the saline soil with or without contaminants. The presence of salt did not affect the total amount of chlorophyll in the plant tissues. No toxicity symptoms were evident from plant morphology after forty five days of exposure. Cd had accumulated mainly in the roots of *V. nemoralis*, while plants grown in saline soil showed the highest Cd accumulation in the roots (191.5 mg/kg) and uptake (2.8 g/plant) . The results suggested that Cd accumulation in plants was not affected by either salinity or contaminant, while slight reduction in shoot height was caused by contaminants.

1. INTRODUCTION

Heavy metal contamination in soil with salinity is a widespread problem that limits crop yields throughout the world. Cadmium (Cd) is one of the most toxic heavy metals. It has a negative impact on people, animals and plants (Kabata-Pendias, 2010). There are many sources of Cd pollution in the environment, mainly found in fuel combustion, industrial sludge, phosphate fertilizer and mine tailings. The regulatory limit for Cd and compounds in agricultural soil in Thailand is 37 mg/kg (Pollution Control Department, 2004). However in Thailand, the largest reported Cd deposit is situated in the Mae Sot district in Tak province. The results of the International Water Management Institute (IWMI) survey of Cd levels in agricultural soils were presented in 2000, revealing that the soil Cd concentration ranges from 3.4 to 284 mg/kg (Unhalekhaka and Kositanont, 2008). Mining has the potential for a long-term and serious environmental impact on soil and plants, whilst soil and irrigation water of arid regions around the mining areas are characterized by high salinity levels, which may aggravate heavy metal pollution problems. The chemical characteristics of soil are adversely affected by salinity because it results in increased heavy metal ion concentrations, and Cd availability to plants is affected by both biotic and non-biotic factors. Recently, Cd contamination of soil with high salinity levels could pose a big problem in terms of the remediation of many mined areas in arid regions.

On the other hand, phytoremediation is an emerging technology for contaminated sites and is attractive due to its low cost, high public acceptance and environmental friendliness (Eapen et al., 2007). In addition, salinity and heavy metals, such as Cd, accumulation can be detrimental for plant growth and development (DalCorso et al., 2010; Chen et al., 2015). Therefore, for the remediation of Cd contaminated saline soils, a selection of crop plant species that can tolerate pollutants with a large biomass under salt stress is one of the most important criteria for phytoremediation. There are few studies, however, of the phytoremediation of Cd contaminated soil under salt stress. Vetiver grass was selected based on its high tolerance to heavy metal pollution because of its dense root system and wide climatic tolerance to extreme environmental conditions (Roongtanakiat and Chairoj, 2001). Moreover, there are no reports that both the negative and positive effects of salinity stress with either higher or lower on Cd uptake and accumulation by vetiver grass. In addition, several halophytes species had rather limited uses. This is because halophytes each show low biomass productions and a shallow root system (Saifullah et al., 2009). The present investigation aimed to assess the phytoremediation potential of *Vetiveria nemoralis* A Camus (Ratchaburi ecotype) for Cd removal from contaminated saline soil at Cd concentrations of 333.10 mg/kg. Moreover, the slight influence of saline (3.5 dS/m) in Cd accumulation and the response of plant growth, chlorophyll content to Cd and a saline environment were also investigated.

2. METHODOLOGY

2.1 Plant material and soil

Vetiveria nemoralis A Camus (Ratchaburi ecotype) were obtained from the Khao Cha-Ngoom Royal Soil Remediation Study Center, Photharam District, Ratchaburi Province, Thailand. The vetiver grass used was about one year old. They were allowed to grow for four weeks before the experiments. Clean soil was obtained from Chatuchak Market, Bangkok, Thailand (Table 1). Soil pH was determined using a pH meter in a 1:1 soil to water ratio extraction. EC was measured in the saturation extract of soils using an EC meter (Peech, 1965). Organic matter was determined by the wet oxidation method of Walkley and Black (1934), total N by the Kjeldahl method (Black, 1965), and CEC using the method described by Chapman (1965). Available P was determined by the Bray II method (Bray and Kurtz, 1945), available K by flame photometry, and the texture was measured using the hydrometer method (Allen et al., 1974). The artificial and freshly spiked, contaminated soil was prepared by mixing with 33.25 g of $Cd(NO_3)_2{\cdot}4H_2O$) per kg soil. The initial concentration of Cd in the spiked soil was 333.10 mg/kg. Salinity was induced by watering the soil with distilled water containing sodium chloride (NaCl). Soil salinity with an EC value of 3.5 dS/m (slightly saline) was prepared by adding 0.5 g of NaCl to 1 L distilled water per 1 kg soil. The soil was air dried and stored at room temperature.

2.2 Pot experiment

Plant leaves and stems were cut to fifteen centimeter lengths from the root to the top and five centimeters of the root system needed to be cut. The initial plant biomass was 16.19±0.3650 g/pot (mean, ±SE). Uniform *V. nemoralis* (1-year-old) were planted into plastic pots (nineteen centimeters in diameter and twenty two centimeters in height) containing five kilograms of soil. The pot experiment was arranged in four treatments: uncontaminated soil (T1), uncontaminated saline soil (T2), contaminated soil (T3), and contaminated saline soil (T4). Each treatment was prepared in triplicate (one plant per pot represented one replicate). A complete randomized experimental design was used. Plants were grown in the greenhouse under prevailing natural environmental conditions for forty five days. Two hundred milliliters of tap water were supplied to each plant every other day (Basumatary et al., 2012).

2.3 Growth measurement

The plants from each pot were harvested thirty and forty five days after planting and analyzed for growth and Cd accumulation. Plant samples were thoroughly washed with tap water and deionized water, separated into shoots and roots, and oven-dried (65°C for 72 h) to determine the dry weights of shoots and roots. The stem height was measured from the aboveground portion to the top of the stem. The root length was measured from root tips to the base of the stem. Any symptoms of salt or Cd induced toxicity exhibited by plants were visually noted throughout the experimental period.

2.4 Chlorophyll content measurement

Chlorophyll a and chlorophyll b content were determined by taking fresh leaf samples from each pot in darkness and washed with deionized water and 0.5 g of fresh leaf was cut into small pieces and homogenized with 5 mL of acetone (80% v/v). There were three replicates performed for each treatment. The absorbance was measured using a UV/visible spectrophotometer at 663.6 and 646.6, which are the major absorptions peaks of chlorophyll a and chlorophyll b, respectively. The chlorophyll content was calculated according to the equations of Porra et al. (1989) and Holm (1954) on the basis of mg chl/g fresh weight (Korkmaz et al., 2010).

$$\text{Chl a (mg/g FW)} = 12.25 \times A_{663.6} - 2.55 \times A_{646.6}$$
$$\text{Chl b (mg/g FW)} = 20.31 \times A_{646.6} - 4.91 \times A_{663.6}$$
$$\text{Chl a+b (mg/g FW)} = 17.76 \text{ x } A_{646.6} + 7.34 \text{ x } A_{663.6}$$

Where; Chl a = chlorophyll a, Chl b = chlorophyll b

$A_{663.6}$ = Absorbance at a wavelength of 663.6 nm
$A_{646.6}$ = Absorbance at a wavelength of 646.6 nm

2.5 Determination of cadmium

The dried plants were ground into powder and sieved through a two millimeter mesh sieve. A subsample (0.5 g) of each group plant sample was digested in 2:1 HNO_3:$HClO_4$ (v/v) used the open tube digestion method (Simmons et al., 2005). After digestion, the total concentrations of Cd in plants were analyzed by FAAS. All analyses were conducted in triplicate per sample. Changes in Cd concentrations in soil were measured after thirty and forty five days of exposure (data not shown).

2.6 Data analysis

Bioconcentration factor (BCF): The bioconcentration factor was calculated as a ratio between Cd concentration in the plant tissue and Cd concentration in the soil (Garg and Chandra, 1994). The calculation of BCF was expressed as shown below:

$$\text{BCF} = \frac{\text{Cd concentration in plant or part of plant (mg/kg dry weight)}}{\text{Initial Cd concentration in soil (mg/kg dry weight)}}$$

BCF > 1 indicates that the plant is a metal accumulator.

Translocation factor (TF): The Cd translocation in these plants from root to shoot was measured using TF (Cui et al., 2007), which is given below:

$$\text{TF} = \frac{\text{Concentration of Cd in shoot (mg/kg dry weight)}}{\text{Concentration of Cd in root (mg/kg dry weight)}}$$

TF>1 indicates that the plant translocated Cd effectively from the roots to the shoots (Baker and Brooks, 1989).

Cd uptake: Total uptake of Cd in plants was calculated as follows (Zhang et al., 2012).

$$\text{Cd uptake} = \frac{\text{Total Cd concentration in plant (mg/kg)} \times \text{plant dry weight (g/plant)}}{1{,}000}$$

Relative growth rate (RGR) was calculated according to Hunt’s equation (1978).

$$\text{RGR} = \frac{\ln W_2 - \ln W_1}{T_2 - T_1}$$

RGR is the relative growth rate (g/g/d) and W_1, T_1 and W_2, T_2 are the initial and final dry weights and times for each treatment, respectively.

2.7 Statistical analysis

The mean and standard deviations of three replicates were calculated and the statistical significance was evaluated using SPSS-17.0 statistical software package (SPSS, Inc.) with ANOVA. A significant level of 0.05 was used in all treatment comparisons and applied using the least significant difference (LSD).

3. RESULTS AND DISCUSSION

3.1 The physico-chemical properties of experimental soils

The physico-chemical properties of the experimental soil are shown in Table 1. The results showed that the soil had a neutral pH (7.3). The soil texture was clay with high organic matter levels (12.95 g/kg) and a CEC value was 76.80 cmol/kg. In terms of the soil, the amounts of essential nutrients were relatively high, especially the available phosphorus (35 mg/kg) and potassium (177 mg/kg), and a moderate level of total nitrogen (0.11%).

Table 1. Physico-chemical properties of uncontaminated soil

Parameter	Value
Soil pH	7.3
Organic matter (OM)	12.95 g/kg
Soil texture	Clay
Cation exchange capacity (CEC)	76.80 cmol/kg
Total nitrogen (N)	0.11%
Available phosphorus (P)	35 mg/kg
Available potassium (K)	177 mg/kg

3.2 The impact of cadmium and salinity on plant growth

All plants grown in both non-saline and saline soils appeared healthy and there were no abnormalities or discoloration of plant organs. They maintained that one hundred percent survival rate for the duration of the experiment, a period of 45 days. Dry plant biomass in each treatment significantly increased with exposure time (Table 2) ($p < 0.05$). At the end of the experiment, the highest biomass production was found in the control (T1; 15.38 g/plant). The lowest dry weights were observed in plants grown in contaminated non-saline soil (T3; 11.12 g/plant). The dry weight yield of plants in each treatment showed no significant mean difference ($p > 0.05$). The lowest RGR values were also found in plants grown in non-saline soil with contamination at 45 days after planting. The growth of a plant was not influenced by salinity or Cd and root length increased in all treatments ($p > 0.05$) with increasing exposure time (Figure 1(a)). However, after forty five days of growth, the stem height of plant was found to be influenced by contaminants (Figure 1(b)). Plants grown in contaminated soils T3 and T4, respectively showed 86.0 and 87.0% reduction in stem height relative to the control (T1, 100%). No significant differences between treatments were found in T3 and T4, while there were significant differences between the stem heights of plants grown in non-saline and saline soil without contamination (T1, T2) at the end of the experiment ($p < 0.05$). Our study demonstrated the effects of salinity slightly and Cd in combination had no overall effects on the growth of *V. nemoralis*.

Table 2. Effects of Cd and soil salinity on dry biomass production (DW, g/plant) and relative growth rate (RGR, g/g/d) of *V. nemoralis* after 30 and 45 days of treatment, values are mean±SD (n=3). Those followed by the same letter did not differ; small letters showed differences between treatments; capital letters showed differences between exposure times at $p < 0.05$, according to the LSD test.

Treatments	Exposure time (days)			
	30		45	
	DW	RGR	DW	RGR
T1: uncontaminated soil	8.59±0.430^{a,A} (100%)	0.0277^{a,A} ±0.0017	15.38±0.565^{a,B} (100%)	0.0314^{a,B} ±0.0008
T2: uncontaminated saline soil	8.81±0.309^{a,A} (100%)	0.0281^{a,A} ±0.0011	13.29±0.420^{a,B} (86.41%)	0.0285^{a,B} ±0.0007
T3: contaminated soil	7.70±0.827^{a,A} (89.64%)	0.0136^{a,A} ±0.0070	11.12±0.571^{a,B} (72.30%)	0.0242^{a,B} ±0.0011
T4: contaminated saline soil	9.13±0.566^{a,A} (100%)	0.0251^{a,A} ±0.0338	14.01±0.574^{a,B} (91.09%)	0.0293^{a,B} ±0.0009

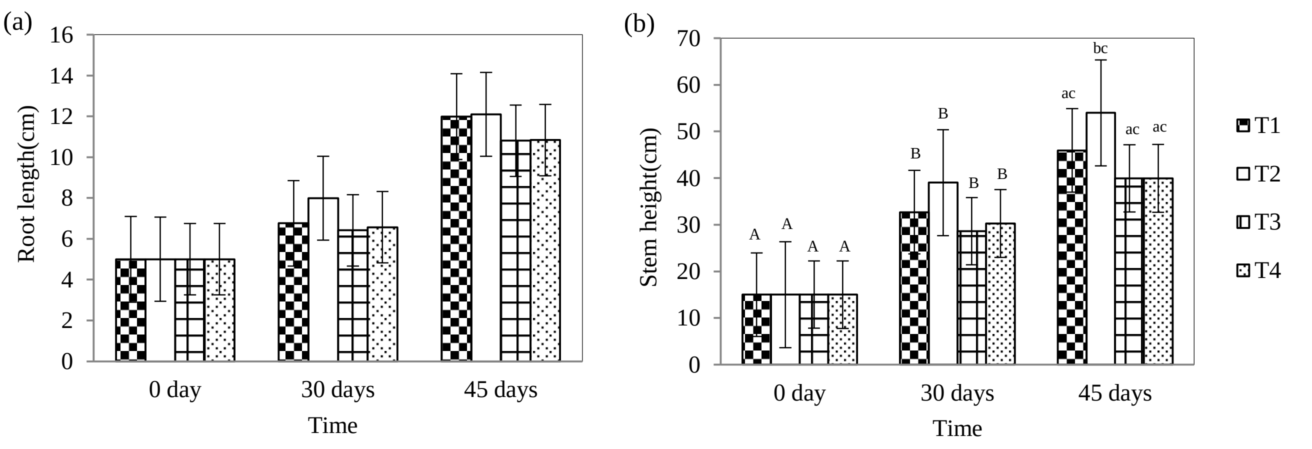

Figure 1. Effects of Cd and salinity on root length (a) and stem height (b) of *V. nemoralis* grown in non-saline and saline soils with and without contamination for 0, 30 and 45 days, values are mean±SD (n=3). Those followed by the same letter did not differ; small letters showed differences between treatments; capital letters showed differences between exposure times at $p < 0.05$, according to the LSD test.

The usefulness of plants for phytoremediation is assessed based not only on their tolerance to contamination, but also the possibility of obtaining a suitable amount of biomass. Exposure to slight salinity and/or Cd had no effect on the biomass yield of *V. nemoralis*. This is strong evidence that vetiver grass grows well in various soil types and different conditions, including salt stress. Our results were similar to other studies which reported that vetiver grass could have a tolerance to the toxicity of heavy metals and could grow in soil contaminated with Cd, As and Pb (Roongtanakiat and Chairoj, 2001; Jampasri et al., 2010). Although Cd is generally considered a toxic element that inhibits plant growth and development (Nazar et al., 2012), some authors found that excess amounts of this metal in soil increased the biomass of grasses instead of decreasing it. In contrast to other studies, our results indicated that the shoots were more sensitive to Cd than roots. However, *V. nemoralis* was not affected by the presence of salt. It might be concluded that salinity in the soil, even at the level below critical value to plant growth, had no negative effects on the growth of *V. nemoralis*. According to Shu et al. (2001), most plant species can survive in an EC range of 0-2 dS/m and sensitive species are affected by EC of 4-8 dS/m.

3.3 The effect of cadmium and salinity on the contents of chlorophyll

It is clear from Figure 2 that the chlorophyll a content predominated over chlorophyll b. The lowest total chlorophyll content was found in plants grown in saline soil without contamination (T2), which showed 64.76% reduction relative to controls (T1, 100%) after forty five days of exposure. The observed differences in total chlorophyll content among treatments and between treatments and control were not significantly different ($p > 0.05$) after forty five days of treatment, while a slight but non-significant reduction in the chlorophyll content was observed in plants grown in saline soils T2 and T4 treatments. The results suggested that the chlorophyll content of plants was unaffected by salt and contaminant stress.

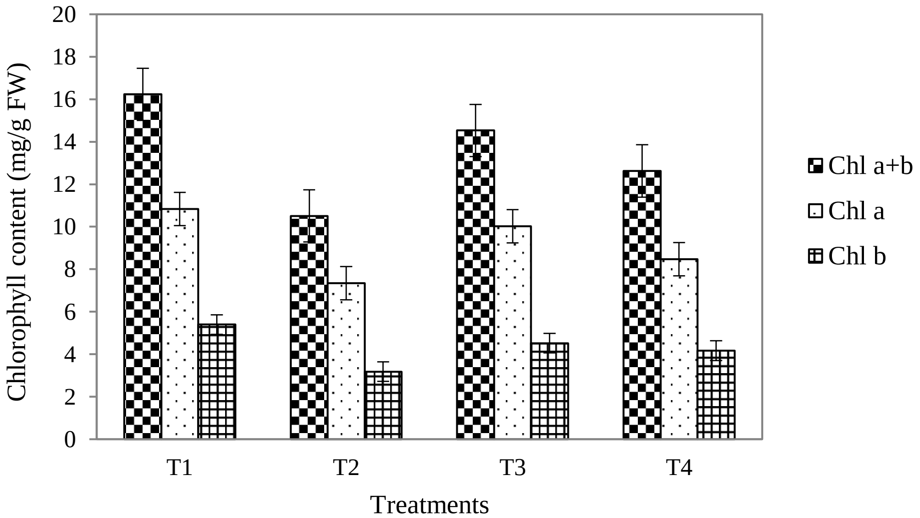

Figure 2. Effects of Cd and salinity on chlorophyll content (mg/g fresh weight of leaf) of *V. nemoralis* after 45 days of treatment, values are mean±SD (n=3).

Our results clearly display the effect of both salinity and Cd on the chlorophyll contents of *V. nemoralis*. The total chlorophyll content exhibited a slight decrease in all treatments (T2, T3 and T4) as compared with control (T1). Heavy metals and salinity often have deleterious effects on photosynthesis in plants. Several previous studies have reported reductions in chlorophyll content in plants exposed to heavy metals and high salinity level (Doganlar et al., 2010; Rahdari et al., 2012). However, the total chlorophyll content of *V. nemoralis* was not significantly affected by the presence of either Cd, or by salinity. It might be that the initial salt contents of the spiked soil were less than 4 dS/m. Our results agreed with those of Lutts et al. (2004) and Manousaki et al. (2008) in the salt-tolerant plants, *Tamarix smyrnensis* and *Atriplex halimus*. Increased salinity caused a reduction in plant growth but no reduction in chlorophyll content in *T. smyrnensis* (Manousaki et al., 2008). Lutts et al. (2004) observed that *A. halimus* growth and chlorophyll content were unaffected by heavy metals (Cd, Pb) or salinity.

3.4 The concentration of cadmium in roots and shoots

Higher Cd content was found in roots rather than shoots, and in addition, exhibited a TF and BCF values <1.0 in all treatments (Table 3). Plants grown in saline soil had a higher capacity to accumulate Cd in their roots than plants grown in non-saline soil after forty five days of treatment. There was a significant difference in the accumulation of Cd in planted non-saline and saline soil ($p > 0.05$). On the other hand, the translocation of Cd from roots to shoots was negatively affected by salt stress. By the end of the trial, the results revealed that the amount of Cd accumulation in plant was unaffected by salt stress.

According to Brooks (1998), the ability to accumulate more than 100 mg/kg of Cd in shoots is one of the conditions that the plant can be considered hyperaccumulators. The findings of our present study show that the concentration of Cd in roots ranged from 127.63 to 191.50 mg/kg, while the shoots were from 6.38 to 35.75 mg/kg. The high accumulation of Cd in roots of *V. nemoralis* makes them useful for phytoremediation. Although vetiver is not a hyper-accumulator, several previous studies have reported the accumulation of much larger quantities of Cd in the roots than that in the shoots of plants, including grasses growing in the contaminated soil (Deram et al., 2006; Guo et al., 2014; Zhang et al., 2014; Quezada-Hinojosa et al., 2015; Stanislawska-Glubiak et al., 2015). A much higher Cd accumulation in the roots than that in the shoots was also reported in the hydroponic experiment for *Vetiveria zizanioides* (Aibibu et al., 2010).

Table 3. Accumulation of Cd in different plant parts, Cd uptake, TF and BCF values of *V. nemoralis* grown in non-saline and saline soil for 45 days, values are mean±SD (n=3). Those followed by the same letter did not differ; small letters showed differences between treatments at $p < 0.05$, according to the LSD test.

Treatments	Dry biomass (g/plant)	Cd accumulation (mg/kg)		Cd uptake (g/plant)	TF	BCF
		Shoot	Root			
T3: non-saline soil	11.12^a±0.571	35.75^b±0.687	127.63^a±0.260	1.82	0.28	0.49
T4: saline soil	14.01^a±0.574	6.38^a±0.317	191.50^b±.363	2.77	0.03	0.59

Evidently, previous studies have reported the positive effects of salinity on metal accumulation and uptake, especially for Cd. Increasing salinity increased Cd uptake and accumulation in halophyte species *T. smyrnensis* and *A. halimus*, sunflower and sudangrass (Lutts et al., 2004; Manousaki et al., 2008; Kadkhodaie et al., 2012). A 2013 study reported that the increase of salinity promoted metal mobility, following the order: Cd > Mn > Cu > Pb (Zhao et al., 2013). The present study also observed higher Cd uptake and accumulation in *V. nemoralis* grown in saline soils rather than non-saline soils. In saline soil treatment (T4), for many reasons, including the formation complexes of Cd with chloride, the solubility of Cd and its absorption are increased by plant. Also, the results obtained by some researchers showed that salinity with Cl has an effective role in increasing the solubility of Cd and its absorption by plants. Moreover, the increase of Cd absorption in saline soils has been reported in many crops (Norvell et al., 2000; Zhao et al., 2013).

Our result clearly showed a TF and BCF values in both treatments (T3, T4) was lower than one. It indicates a weak Cd translocation from the roots to shoots of *V. nemoralis*. This may be due to its larger biomass, apart from the strongest Cd uptake ability. A similar TF values was obtained by Zhang et al. (2014) for *V. zizanioides*. Furthermore, salinity is shown to be a key factor in the translocation of Cd from roots to shoots as also observed in this study. This might suggest that the reduction of metal translocation from the roots to the shoots can be considered to be one of the defence mechanisms that protect the aboveground parts of plants from the detrimental effects of heavy metals and salt stress (Aibibu et al., 2010). According to Mendez and Maier (2008), TF is one of the most important factors to consider when evaluating the phytoremediation potential of plants. It can be assumed that plants with a low TF (<1) are suitable for the phytostabilisation of heavy metals in the soil (Malik et al., 2010; Cheraghi et al., 2011; Korzeniowska and Stanisławska-Glubiak, 2015).

4. CONCLUSIONS

It was clearly demonstrated that the growth and performance of *V. nemoralis* were not affected by exposure to Cd and salt stress. The total chlorophyll content was not affected by either Cd or salt, while only a slight reduction of the shoot height was caused by Cd. The roots presented higher Cd contents in their tissues than the shoots. Combined exposure to Cd and salt positively affected Cd removal from contaminated soil by *V. nemoralis*, but salt-exposure may negatively affect the Cd translocation from roots to shoots. Thus, our findings demonstrated that the tolerance of *V. nemoralis* (Ratchaburi ecotype) to concentrations of Cd (333.10 mg/kg) made them good candidates for use in the phytoremediation of contaminated soil, including slightly saline soil with an EC value of 3.5 dS/m.

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