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The Evaluation of Alkali Grass (*Puccinellia ciliata* Bor) Populations in Aydin Province of Turkey

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A R T I C L E I N F O

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A B S T R A C T

Alkali grass grows in waterlogged, saline and alaline soils. The main problem in these soils is minerals at toxic level. The toxic ions are chloride, sodium and boron. A number of techniques have been investigated for removing toxic metals from the soil. Today, the cost-effective and environmentally technique is phytoremediation, using hyperaccumulator plants. Alkali grass (*Puccinellia ciliata* Bor) is suggested as a hyperaccumulator plant by the combination of more favourable characteristics with salt and waterlogging tolerance, high biomass value and convincing nutritive value for adverse environmental conditions. For this reason, we collected alkali grass and soil samples from five different locations in Aydın-Muğla highway, Turanlar and Sınırteke villages in Germencik-Aydın. In the soil analysis, we observed that K accumulation varies between root, shoot and panicle at least whereas Na and B shows more variation on whole plant portions among locations. Intense aerenchyma development on the root tips of *Puccinellia* plant was observed and it is determined as radial lysogenic aerenchyma formation. Average plant height and dry matter values were between 47.2-74.4 cm and 15.61-80.85 g/plant according to locations. The highest plant height value was obtained from the first location whereas the highest dry matter yield was detected in the fifth location. In conclusion, plants from fifth location can be regarded as fodder plants in these areas. Our results indicated that alkali grass can be effective for phytoextraction of sodium and boron from contaminated sites.

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Introduction

The presence of toxic metals in soil and water has a negative impact on human, environment and agriculture. Instead of using pricy traditional remediation techniques for improvement of heavy metals-contaminated areas, researchers focused on cost-effective, sustainable and eco-friendly technique called phytoremediation. This thriving method is using specially selected and engineered metal accumulating plants in the name of environmental clean up (Salt et al., 1995).

When particular ions accumulated in the leaves of plant during water transpiration at a certain level via soil water uptake, the plants suffer from toxicity. The degree of the damage depends on growing stage, ion concentration, plant sensitivity and water consumption. The damage can be severe enough to cause loss of production. Phytoextraction is the most promising approach in removal of toxic heavy metals from the soil, by using hyperaccumulator plants. They accumulate and detoxify higher quantities of metal ions in their aboveground parts and can survive under metal (Kachout et al., 2009; Marques et al., 2009; Nazir et al., 2011; Paz-Ferreiro et al., 2014). In order to perform the objectives of phytoremediation technique, the selected hyperaccumulator plants must have some abilities as well as accumulating higher amounts of toxic metal ions in the target area.

According to numerous researchers, there are some important criteria in selecting a hyperaccumulator plant for phytoremediation; being adaptive to adverse environmental conditions (e.g., waterlogging, high pH, salinity, etc.), having tolerance to the heavy metal toxicity, the level of heavy metal ion accumulation, translocation and uptake potential, having high biomass value, high growth rate, depth of root zone, root characteristics, being available in various habitats; terrestrial, aquatic, semi aquatic etc. (LeDuc et al., 2005; Pilon-Smits, 2005; Sarma, 2011).

Especially in Aegean region of Turkey agricultural areas irrigated with surface water is contaminated with boron significantly. The high salt and boron levels reduce yields of many of the selected crops. One of the most important factors affecting the amount of boron in the soil is the amount of $CaCO_3$. There is a negative relationship between the boron intake of plants and amount of water soluble Ca in the soil. Precautions against boron toxicity are considered temporary and inadequate. For this reason, the best action to tolerate boron toxicity is the usage of boron tolerant plants.

Alkali grass is a native plant to Turkey. It is a member of the Poacea family. “Menemen” is the only cultivar collected fom the Kaklıç village near to Menemen (where the cultivars name came from Menemen province-İzmir). It was stated that alkali grass is a perennial bunch grass with 40 cm tall, tolerant to salinity, moderate to high with an EC range 5 to 40 dS/m (Barret-Lennard, 2003). Plant has a great waterlogging tolerance and also tolerates submergence conditions. It can live in soil conditions where soil pH values range from 5.5 to 8.0. It has also high drought and frost tolerance. Alkali grass has a convincing nutrititive value during winter and early spring and has good dry matter digestibility (DMD) (60-78 %) and protein (10-18 %) values in winter and early spring. The plant is usually sown as a pioneer plant for soil erosion control or soil restoring of saltlands in South Australia and it is marketed in South Australia as "restora sweet grass”. As a salt tolerant plant, Alkali grass has low salt content, makes it ideal for supplementary feed for stock.

The aim of this study was to reveal the cultivation of the hypercumulator plant such as *Puccinellia distans* which is tolerance to boron and its ability to accumulate it in its tissues and economical value of uncultivated areas is a good solution. Therefore *Puccinellia ciliata* Bor was considered a suitable plant for this work

Material and Methods

Sample Collection Procedure

Alkali grass (*Puccinellia ciliata* Bor) and the soil samples were collected prior to surveys from five different locations in this study. The coordinates of locations were 37°48’N-27° 50 E for location I, in Aydın-Mugla railway 6. km, 37°81’N-27° 63’E for location II, 37°82’N-27° 64’E for location III, 37°83’N-27° 65’E for location IV and 37°83’N-27° 67’E for location V in Turanlar/ Sınırteke (Figure 1). At each location, four randomly samples were collected for a total of 20 soil samples (4 samples × 5 locations). Also, the *Puccinellia* plant samples were collected at the stage of panicle during April and May.

Soil Analyses

Soil characterizations and heavy metal concentrations in different locations were analyzed according to Wolf (1971) and observed in Table 1.

Plant Characteristics

Plant height was measured from the plant base to the tip of the panicle and mean values were expressed in centimeter. Green herbage yields were determined by weighing the harvested plants from each location. 0.5 g of wet samples were taken from each location for dry hay yield. And the samples were stored at 70°C for 48 hours in a drying cabinet. Dry hay yield values were obtained from multpliying green hay yield and dry hay ratio.

Table 1 Soil characterizations in different locations where *Puccinellia ciliata* (Bor) plant shows wide distribution in Aydın.

Properties	Location I		Location II		Location III		Location IV		Location V	
	Explanation		Explanation		Explanation		Explanation		Explanation	
Structure	L	Loam	C	Clay	L	Loam	L	Loam	SICL	SCL
Sand (%)	48.57		24.42		48.16		32.96		10.61	
Silt (%)	36.43		29.26		37.09		43.77		58.99	
Clay (%)	15.00		46.32		14.75		23.27		30.40	
Satur. (%)	42.1		122.8		39.5		58.5		47.4	
Org.Matter.	1.01	Low	0.95	Very Low	0.59	Very Low	0.30	Very Low	0.30	Very Low
pH	9.19	K. Alkaline	8.91	K. Alkaline	8.31	Alkaline	10.39	K. Alkaline	10.83	K. Alkaline
Lime	7.87	High	13.93	High	7.57	High	8.48	High	20.89	Excessive
Salt	0.0897	Salt-free	0.1418	Salt-free	0.0887	Salt-free	0.1198	Salt-free	0.1447	Salt-free
P (ppm)	18	Medium	13	Medium	5.40	Low	12	Medium	20	High
K (ppm)	446	Very high	457	Very high	342	Very high	224	Medium	352	Very high
Ca (ppm)	2256	Medium	2845	Medium	2453	Medium	2354	Medium	1962	Medium
Mg (ppm)	242	High	1328	Very high	1347	Very high	462	Very high	131	Medium
Na (ppm)	1301	Very high	3234	Very high	1237	Very high	3435	Very high	5479	Very high
Fe (ppm)	25.52	High	53.34	High	19.82	High	40.54	High	38.02	High
Zn (ppm)	0.90	Critical	0.68	Critical	0.86	Critical	0.60	Critical	0.56	Critical
Mn (ppm)	13.72	Adequate	10.44	Adequate	11.32	Adequate	9.08	Adequate	6.74	Adequate
Cu (ppm)	1.70	Adequate	2.28	Adequate	3.30	Adequate	2.00	Adequate	1.46	Adequate
B (ppm)	8.62	Toxic	5.65	Adequate	6.00	Toxic	14.47	Toxic	13.74	Toxic

SCL: Silty Clay Loam

Calculation of Hyperaccumulation Criteria

The definition of metal hyperaccumulation requires the consideration of metal concentrations in the aboveground biomass and the soil. Both the enrichment factor (EF) and the translocation factor (TF) have to be considered when determining whether a particular plant is a metal hyperaccumulator. The enrichment factor is calculated as the ratio of the plant shoot concentration to the soil concentration ($[Metal]_{shoot}$ / $[Metal]_{soil}$), while the translocation factor is the ratio of the metal concentration in the shoot to that in the root ($[Metal]_{shoot}$ / $[Metal]_{root}$). A hyperaccumulator plant should possess an EF or TF > 1 (Kutty and Al-Mahaqeri, 2016).

Statistical Analysis

The basic parameters such as mean, standart error and cofficient of variation values were analyzed using the TARİST statistical program (Açıkgöz et al., 1994)

Results

Soil Characterization

Table 1 given that soil characteristics of different locations where Alkali grass shows wide distribution in Aydın. According to the analysis, soils showed different structures; in I. III. and IV. locations soils were loam, in the II. location it was clay and in the V. location it was silty clay loam. Saturation values (%) of these locations ranged from 39.5-122.8 and generally found to be very low in organic matter. pH values were changed from 8.31 to 10.83. Soil samples from each location varied in P levels and differ between low and high values. While K values were generally very high, Ca values were moderate and Mg was generally defined as high-very high. Despite the presence of low levels of salt, very high level of Na examined and this could be interpreted as an indication of sodic soils of these areas. As Fe was found in high levels, Zn was found to be critical in all areas. The amount of Mn and Cu were adequate. The B values ranged from 5.65-14.47 and is often found to be on toxic levels.

When the soil characteristics were evaluated it could said that *Puccinellia ciliata* Bor plant shows propagation on the areas which are usually low in organic matter, having excess alkali values, high sodium problem, and the toxic levels of B. (Table 1).

Plant Nutrition Contents

Plants collected from different locations randomly. Plant nutrition contents (Na, K, Ca, Mg and B) per root, stem and cluster are given in the Table 2. Plant Na, K, Ca, Mg analyzes were determined according to Kacar (2009) and B analyze was stated according to Wolf (1971) with Azomethin-H method. The amounts of Na and B accumulated in root tissue were 35.25% and 31.22%, respectively. Na values of parameters were changed from 0.09% to 0.26%, B values ranged from 449 to 1078 ppm. K and Mg values which had the coefficient of variation below 10% had shown mean values of 0.18 and 0.70 respectively. Similar changes occured in Ca values which were ranged from 0.66 to 1.13. Location IV showed much more accumulation except Na in the name of accumulation. When the accumulation in stems examined, we observed that the accumulation of all elements except K showed variation. The accumulation of K was 0.93% on average. Na accumulation was 0.09 to 0.43%; Ca accumulation was 0.18 to 0.30%, Mg accumulation was 0.14 to 12.27%, and B values ranged from 64 to 816 ppm, respectively. Location IV showed much more accumulation except Mg in terms of accumulation. Cluster showed a variation between locations in terms of all elements. Na accumulation was 0.03 to 0.14%, K was 0.25 to 0.35%, Ca accumulation was 0.09 to 0.18%, Mg accumulation was 0.18 to 0.34%, and B values ranged from 169 to 794 ppm, respectively (Table 2).

Figure 1 *Puccinellia ciliata* distribution in Aegian Reagion of Turkey

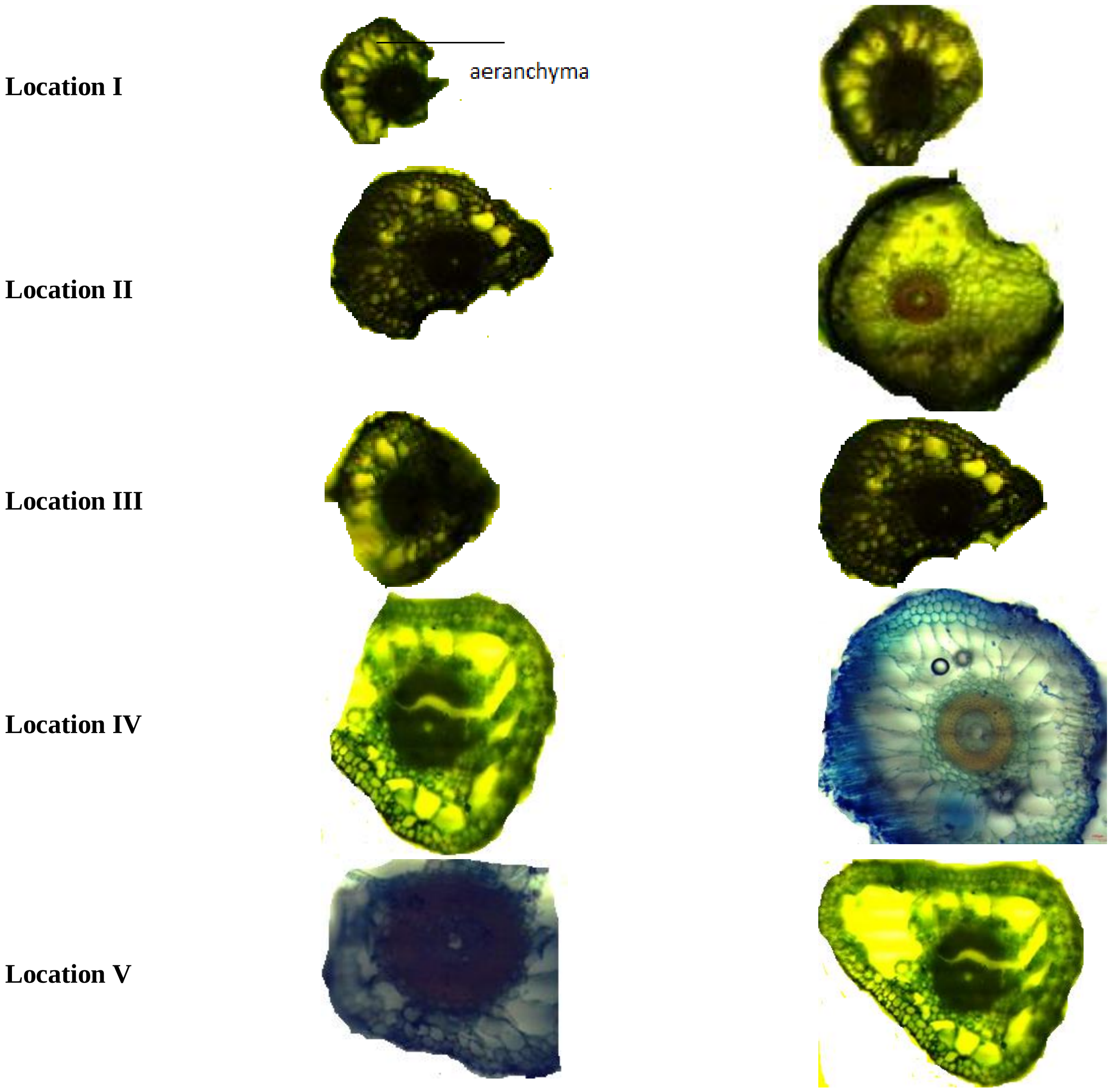

Figure 2 Aeranchyma formation of *Puccinellia ciliata* plants from different locations

Plant Height, Green Herbage Yield and Hay Yield Values

According to Table 3 Average plant height and hay yield values were; 47.2 to 74.4 cm. and 15.61 to 80.85 g/plant respectively. Highest plant hight obtained from Location I, however highest hay yield gained from Location V. We concluded that the plants on Location V can be considered as forage crops (Table 3).

Adventitious Root Anatomy

Adventitious root anatomy of *Puccinellia ciliata* Bor was evaluated and the pictures of roots from each location captured by a Novex B series 86041 Model Trinocular Microscope. Intensive aeranchyma formation on root tips can easily be recognised from the pictures on Figure 2.

We assessed aeranchyma formation type as radial lysigeny as seen on rice (*Oryza sativa*) relying on Jung et al. 2008. Lysigeny type of aeranchyma forms by programmed cell death under submergence conditions or whenever the soil is waterlogged, Puccinellia ciliata Bor roots can be able to adequetly respire because of its roots adaptaion to insufficient oxygen environment by this aeranchyma formation mechanism.

Discussion

Alkali grass is usually found on alkaline and strong alkaline soils (8.31-10.83) in Australia (Brown, 2004). These results parallel our findings. pH values of 10.39 and 10.83 in IV and V locations indicated that alkali grass can grow in strong alkaline areas. Brown (2004) determined that the EC values in the areas of alkali grass ranged from 9.7 to 41.0 dS/m. It has been reported that the Menemen variety developed from alkali grass is grown in Australia with salinity of 10.0-40.0 dS/m (Barett-Lennard et al., 1999). Soil analysis results showed that all locations were in the unsalted class whereas Na values were in very high class. It was observed that the problem in these regions was especially sodium rather than salinity. Therefore alkali grass has been grown successfully in these areas. It was observed that the soil of the alkali grass is over-calcified and has moderate calcium content. alkali grass grows in areas with waterlogging and drainage problems. Setter et al. (2009) was indicated that waterlogging causes many changes in soil chemistry and particularly the amount of Mn and Fe exceeding the critical toxic level. It was observed that the alkali grass grows in very unfavorable conditions in our region, unlike the other regions. It was shown that the amount of Fe, Mn and B were high, sufficient and toxic, respectively in our region. Therefore using alkali grass, itself for phytoremediation and/or phytoextraction will remove excess boron from contaminated soil. Therefore this will be useful for soil depollution and for simple boron purification (Öztürk et al., 2017).

Table 2 Na, K, Ca, Mg and B contents per root, stem and cluster of plants from different locations

L	Root					Stem					Cluster				
	Na (%)	K (%)	Ca (%)	Mg (%)	B (ppm)	Na (%)	K (%)	Ca (%)	Mg (%)	B (ppm)	Na (%)	K (%)	Ca (%)	Mg (%)	B (ppm)
I	0.24	0.18	0.74	0.74	449	0.09	0.89	0.18	0.26	64	0.03	0.35	0.14	0.34	169
II	0.09	0.19	0.78	0.63	654	0.37	1.04	0.19	0.14	546	0.07	0.25	0.09	0.18	542
III	0.26	0.16	0.66	0.72	802	0.16	0.96	0.20	0.27	425	0.07	0.29	0.12	0.25	502
IV	0.20	0.19	1.13	0.70	1078	0.40	0.96	0.30	0.15	734	0.14	0.34	0.16	0.22	647
V	0.15	0.16	0.85	0.70	938	0.26	0.81	0.26	0.16	816	0.07	0.30	0.18	0.26	794
M	0.19	0.18	0.83	0.70	784	0.26	0.93	0.22	0.20	517	0.08	0.31	0.14	0.25	531
$St._E$	0.031	0.007	0.081	0.019	109.45	0.059	0.039	0.023	0.028	132.5	0.018	0.018	0.016	0.026	103.59
CV	36.74	8.62	21.66	5.94	31.21	51.87	9.27	22.91	32.39	57.30	52.30	13.19	25.31	23.66	43.64

L: Locations

Table 3 Plant Height, Green Herbage Yield and Hay Yield values of five locations.

R	Location I			Location II			Location III			Location IV			Location V		
	PH	GHY	HY	PH	GHY	HY	PH	GHY	HY	PH	GHY	HY	PH	GHY	HY
1	62	18,5	11,9	65	48,2	23,3	64	68,7	31,6	40	88,4	43,9	76	367,5	185,4
2	67	15,1	7,6	65	33,1	17,4	70	42,9	20,0	58	53,9	38,8	61	89,8	49,2
3	73	52,6	27,9	50	13,4	7,8	62	11,5	5,8	50	72,8	36,7	57	166,4	83,8
4	70	10,2	6,8	40	3,5	3,0	86	108,3	54,9	59	47,3	29,1	50	279,5	148,9
5	80	56,2	27,8	45	49,9	17,8	82	28,9	18,2	34	278,9	131,7	57	104,3	48,7
6	87	62,4	32,6	45	11,3	7,6	69	68,7	31,6	39	75,8	28,3	70	50,4	27,6
7	74	16,2	8,0	35	0,8	0,4	45	41,0	22,5	43	56,8	23,5	66	217,6	103,4
8	77	81,3	36,9	33	2,6	0,8	69	92,6	43,6	48	201,3	96,6	41	28,8	15,5
9	80	54,6	27,1	35	5,3	3,2	64	26,3	12,5	57	54,7	39,8	61	136,5	68,7
10	74	22,2	12,9	59	143,9	74,8	73	75,4	42,8	51	71,9	28,9	68	150,9	77,0
M	74.4	38.9	19.9	47.2	31.2	15.6	68.4	56.4	28.4	47.9	100.2	49.8	60.7	159.2	80.8
$St._E$	2.26	5.91	3.66	3.86	13.84	7.04	3.57	9.87	4.87	2.75	24.34	11.23	3.22	33.10	16.79
CV	9.59	48.00	58.02	25.87	140.27	142.60	16.50	55.30	54.32	18.16	76.84	71.39	16.77	65.75	65.71

R: Replication, M: Mean, PH: Plant Height; GHY: Green Herbage Yield; HY: Hay Yield

It was determined that the K accumulation in plants is the element showing the least variance between the locations in terms of root, stem and bunch. Similarly, Jenkins (2007) reported that the alkali grass increased the salinity and Na content of the growing media, but the K accumulation remained constant. The most variable elements in our study were Na and B in terms of all plant parts. *P. distans* has an efficient root B-exclusion capability and B tolerance in shoots (Hamurcu et al., 2016). *Puccinellia distans* was evaluated as a boron hyperaccumulator against high boron contents (Hamurcu et al., 2009; Öztürk et al., 2017; Stiles et al., 2010). When the "Translocation Factor" (TF) evaluated as shoot / root content and values above 1.0 are considered as hyperaccumulators; the TF value in plants was found to be less than 1.0 in soil containing high lime, medium calcium and very high magnesium. The dry matter average production of alkali grass is 3-7.5 ton/ha^{-1} (Changgui and Shuning, 1992). Dry hay yields of halophyte species in saline grasslands were significantly different among species. The differences could be due to the the genetic structures, genotype or species because of the physiological and biological properties, root structure and habitat (Temel et al., 2015). The aerenchyma tissue we observed was similar to the radial lysigeny (RL) aerenchyma type which schizogenous separations are accompanied or followed by collapse and death of cells along radial sectors of the mid cortex found by Jung et al. (2008) (Figure 2).

In Aydın, alkali grass plants are usually found in toxic areas of boron, low organic matter, extremely alkaline and sodium, waterlogged soils. Overall, considering the results of this study, it could be concluded that alkali grass can be suggested as a hyperaccumulator plant by the combination of more favourable characteristics are salt tolerance, waterlogging tolerance, high EC range, suitable for adverse environmental conditions, high biomass value and convincing nutritive value. These species can play also a significant role as an alternative forage resource in recovered of the grazing lands losing the production potential due to unconscious utilization and salinity. For this purpose, the plants in the fifth localition can be used as feed in these areas.

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