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Anatomical Response of Cenchrus Grass *Cenchrus Celiaris* to the Paperboard Effluent Irrigation

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Abstract: Land treatment of wastewater using grasses as range lands has potential to be a sustainable method for disposal of industrial effluent. Plants that grow in effluent irrigation show specific structural modification to survive with the altered physiological and biochemical mechanism, while maintaining their life cycle. However, the knowledge on morphological and anatomical response to excess ions in the effluent is still incomplete. The objective of this experiment was to document the effect of increasing concentration of the ions in the effluent led out from the paperboard mill effluent on the root anatomy of the *Cenchrus celiaris* treated grass in comparison with their control. The rooted cuttings were grown in soils irrigated with water, treated effluent and raw effluent. Here the grass irrigated with the water is taken as control. Root anatomy gets strongly influenced by the nature of ions in the effluent. The anatomical changes of root tissues with effluent irrigation were observed with those in the water irrigation, including greater number of cortical layer and thick endodermis. Among the effluent irrigated grass the raw effluent had damaged the phloem and the total vascular region seems to be reduced. The anatomical alterations documented in the experiment are produced to survive in the stress condition imposed by the ions in the effluent.

Key words: Anatomical changes · paperboard mill effluent · *Cenchrus celiaris* · root anatomy

INTRODUCTION

Phytoremediation is the use of vegetation for *in situ* treatment of contaminated soils, sediments and water. It is applicable at sites containing organic, nutrient, or metal pollutants that can be accessed by the roots of plants and sequestered, degraded, immobilized, or metabolized in plant. Instead of groundwater recharge with pollutant, discharge using grasses maintained as rangelands has been the subject of interest for some years.

Pollution control acts and regulations of Asian country like India have listed the pulp and paper mills as one among the 20 polluting industries with water consumption at the rate of 75-100 m^3 t^{-1}[1] is significantly higher than the global average of 10-25 m^3 t^{-1} (Green Rating of Pulp and Paper Sector, CSE). Pulp and paper industry ranks as the third in the annual wastewater discharge (695 million cubic meters) [1].

The demand for paper and paper products has increased from 1.2 mt in 1980-81 to 3.6 mt in 2000. The per capita consumption of paper in India is one of the lowest in the world at 5 kg. The present level of paper consumption in the country is 4.2 mt and is expected to grow with a rise in literacy. Going by the current trend, the demand for paper and allied products is expected to cross 10 mt by 2015. At present there are an estimated 525 pulp and paper mills with a total installed capacity of around 6.25 million TPA with a capacity utilization of about 67 per cent. The aggregate installed capacity by 2010 for paper and paperboard is expected to reach 8.3 mt and 1.5 mt for newsprint. It is important that the industry has to reduce pollution and eventually moves to zero effluent mills.

The main problem with the paperboard mill effluent is sodium and chloride ion concentration. The Na that enters the xylem stream may enter through many putative uptake pathways. Some of those pathways have been identified through molecular [2] and the electrophysiological level [3], but the identity of the primary Na^+ uptake pathway is still not clear. These pathways include several different K^+ carriers [2] as well as non-selective cation channels [4]. In certain plant species such as rice, pathways that bypass membrane transport processes may be involved in the translocation of Na^+ into the transpiration stream and translocation to the shoot [5].

In comparison with Na^+ uptake, less is known about how Cl^- is taken up even though it is an essential micronutrient and must be transported to the shoot where it is involved in biochemical and physiological

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processes including photosynthesis [6]. At the mechanistic level, Cl^- is believed to be transported into the cell by a H^+/Cl^- symport, but at the molecular level the identity of that symport is not known. At high salinities (e.g., >60 m M Cl^-) Cl^- might enter passively through anion channels although at low salinities (25 mM) this is unlikely. Chloride uptake and transport by roots has been the subject of a number of recent reviews [4, 7, 8].

Modification in structural arrangement of the root tissue and cells of *Cenchrus celiaris* has to undergone. A better understanding of this will give a clear idea of the mechanisms involved in the inhibition of plant growth by salinity, which may accelerate the introduction of environmental and genetic manipulation aimed at increasing crop salinity resistance.

For the experiment purpose under controlled condition we arranged for the raw effluent along with the treated effluent, which is otherwise, not let out without the treatment. This study was carried out with the objective to document the histological changes in the grass root due to the influence of the paperboard effluent irrigation.

MATERIALS AND METHODS

Characteristics of effluent: The colour of the treated effluent let out for irrigation from paper factory was colorless whereas the raw effluent was light brownish. The pH of the raw effluent was acidic (6.3) and neutral (7.5) after treatment. The EC of the raw and treated effluent was 1.53 and 1.31 dS m^{-1}, respectively. The total dissolved solids and suspended solids of the treated effluent were around 811 and 30 mg L^{-1} respectively.

The organic carbon of the raw and treated effluent was around 0.71 and 0.51 per cent, respectively. The values of dissolved oxygen, BOD and COD were 3.3, 12.5 and 87 mg L^{-1}, respectively in the treated effluent. Nutrient content of the treated effluent were 158, 1.23 and 17.1 mg L^{-1} of NH_4–N, P and K, respectively. The cations like calcium sand magnesium were also present in the treated effluent at the levels of 11.00 and 6.00 cmol L^{-1}, respectively. The sodium content of the treated effluent was 11.50 cmol L^{-1}. The anions like chloride (421 mg L^{-1}) and sulphate (297 mg L^{-1}) were also present in the treated effluent.

Anatomical procedure: The root sample of grasses were washed thoroughly with water and cut into a size of 2-5 mm. The root materials were killed and fixed in FAA (formalin, alcohol and acetic acid) solution containing 40 percent formalin, 80 percent alcohol and acetic acid in the ratio 90:5:5. Following which dehydration was done using Tertiary Butyl Alcohol (TBA) as dehydrating agent. The dehydrated root section was infiltered by placing it in vials containing solidified wax with 1:1 TBA-liquid paraffin (maintained at 50-55°C). After that embedding of the root sections was done by pouring with liquid paraffin wax into paper trays or boats and solidified. The wax is then cut into thin blocks along with the root material and sectioning was done in the form of thin ribbons using a rotary microtome of transverse serial sections 10-15 μm thick [9]. The root sections were mounted on the glass slides, before that a thin smear of egg albumen mixed with streptomycin (to avoid bacterial growth) was applied over the slides and the slides were rapidly passed over the flame for proper mounting. Finally the slides were stained, by treating with hexane, saffranin [10] and ethanol for 15 minutes each. It was observed through microscope this is another evidence for the plant resistance to pollutant. Photographs of different magnifications were taken using Nikon lab photo 2- microscopic unit.

Transpiration rate and leaf stomatal conductance: Transpiration rate and leaf stomatal conductance were measured at 210 DAP using a steady-state porometer (EGM4-PMR5) and were corrected for leaf surface area. Both the transpiration rates and stomatal conductance are expressed as mM m^{-2} s^{-1}.

RESULTS

Grasses grown in raw effluent showed growth inhibition from the beginning of the treatment. This was intensified as the salt concentration was built in the soil showing visual phototoxic symptom in the surviving plants as chlorosis and necrosis. For this reason the anatomical structure in the cross-section of the root and the possible modification of root tissue were corresponded with the changes due to the effluent irrigation.

The transverse section of *Cenchrus celiaris* root grown under three irrigation sources of water, treated effluent and raw effluent is shown in Fig. 1-3.

Anatomical structure of *Cenchrus celiaris* root

Anatomical character of the control plant: There is a typical anatomical difference in the samples with different irrigation water sources. The cortex consisted of three to four layers of thin walled parenchyma cells, farming large arenchyma sacks. The pericycle adjacent to the endodermis consisted of single layer of thin walled parenchyma cells. The vascular tissues were

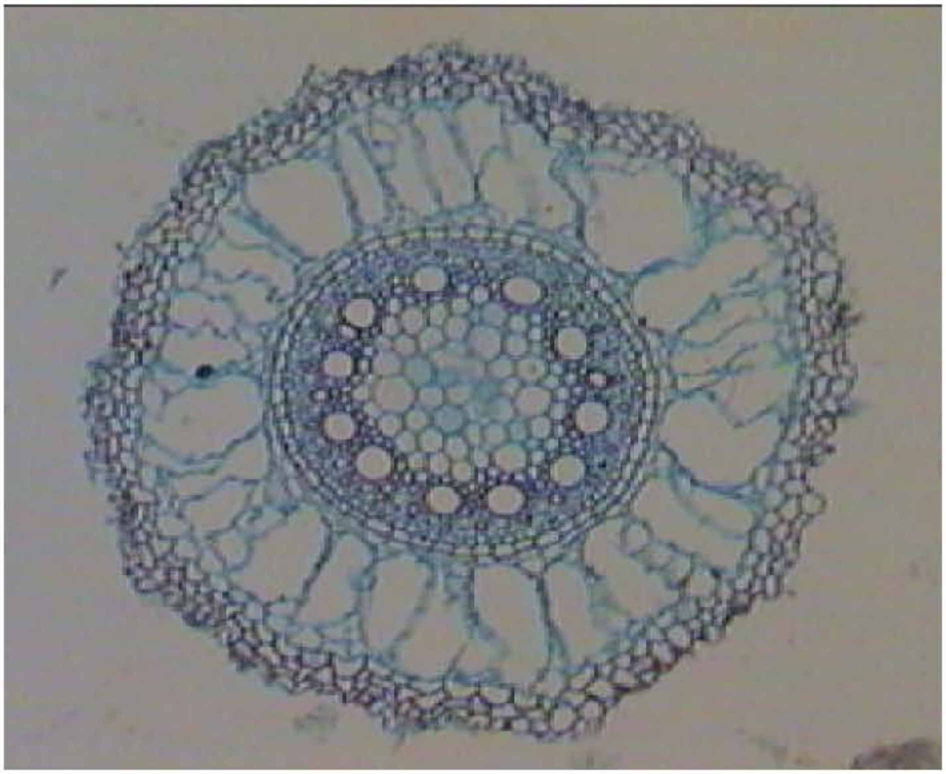

Fig. 1: Transverse section of *Cenchrus celiaris* root grown with water irrigation

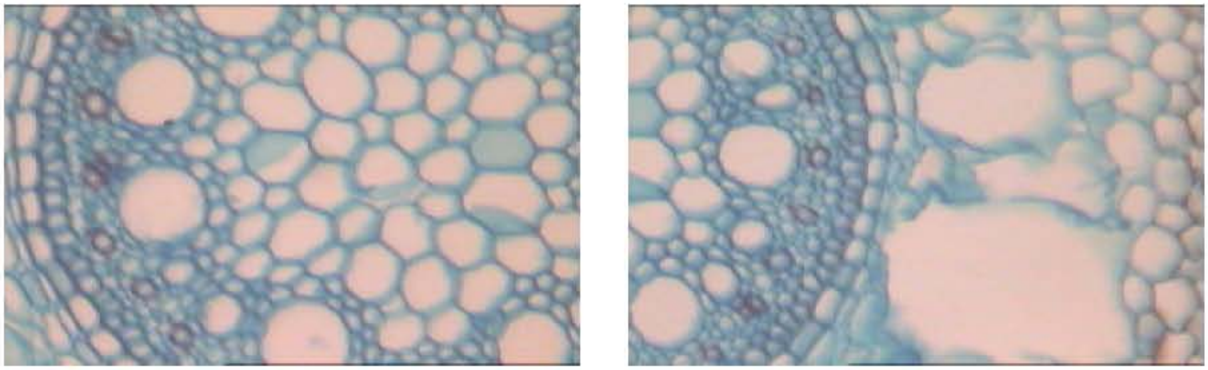

Fig. 1a: Transverse section of *Cenchrus celiaris* root grown with water irrigation under the high resolution fluorescent microscope

represented by eleven phloem cells at the periphery alternating with central group of xylem cells extended as strands. The anatomy is still clear in the high resolution fluorescent microscope.

Anatomical characters of the treated plant: The roots collected from the treated effluent irrigated grass had (Fig. 2 and 2a) 2 to 3 more parenchyma cell layer in their cortex. Nevertheless they had similar diameter to the water irrigated root because their parenchyma cell had no arenchyma chamber. The endodermis was thickened covered with 2 layers of pericycle of thin walled parenchyma cells in different size makes difficult to recognize (Fig. 2a). The structure of these root varied from 8 to 10 complete phloem cells.

The root form the grass irrigated with raw effluent had shown damage in the cortical layer and vascular tissue (Fig. 3). The cells of the endodermis were larger than in the control roots covered with 2 layers of pericycle of thin walled parenchyma cells in different size makes difficult to recognize as in the case of grass irrigated with treated effluent. The vascular region was

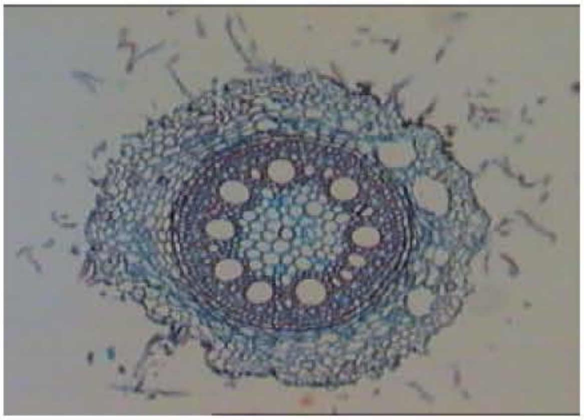

Fig. 2: Transverse section of *Cenchrus celiaris* root grown with treated effluent irrigation

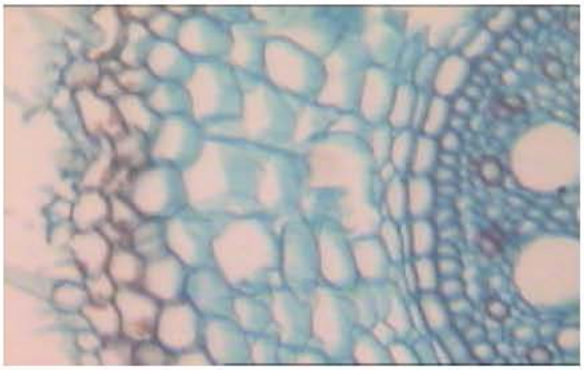

Fig. 2a: Transverse section of *Cenchrus celiaris* root grown with treated effluent irrigation under high resolution fluorescent microscope

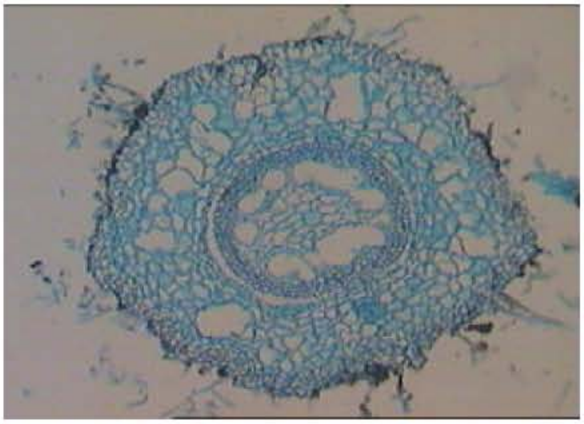

Fig. 3: Transverse section of *Cenchrus celiaris* root grown with raw effluent irrigation

less developed and the phloem cells were not complete. The central xylem vessel was poorly developed.

Table 1: Physical and chemical analysis of raw and treated effluent

Parameters	Unit	Raw effluent	Treated effluent
pH	-	6.30	7.10
EC	dS m^{-1}	1.53	1.31
TDS	mg L^{-1}	1230.00	811.00
TSS	mg L^{-1}	480.00	30.00
Organic carbon	per cent	0.71	0.51
Dissolved oxygen	mg L^{-1}	3.00	3.30
BOD	mg L^{-1}	170.00	12.50
COD	mg L^{-1}	2105.00	87.00
HCO_3	mg L^{-1}	215.00	115.00
Ammonical-N	mg L^{-1}	23.70	158.00
Phosphorus	mg L^{-1}	1.30	1.23
Potassium	mg L^{-1}	19.00	17.10
Calcium	cmol L^{-1}	18.00	11.00
Magnesium	cmol L^{-1}	10.00	6.00
Sodium	mg L^{-1}	22.00	11.50
Chloride	mg L^{-1}	617.00	421.00
Sulphate	mg L^{-1}	322.00	297.00

Transpiration rate and stomatal conductance of *Cenchrus celiaris* (mmole m^{-2} sec^{-1}): Transpiration rate and stomatal conductance of *Cenchrus celiaris* is presented in the Table 2. The treatments were effective in influencing grass transpiration rate and stomatal conductance. The grass transpiration rate ranged from 1.08 to 1.27 mmole m^{-2} sec^{-1}. Among the treatments, water irrigated grass recorded the highest value (1.27 mmole m^{-2} sec^{-1}) Raw effluent irrigated grass recorded the lowest value (1.08 mmole m^{-2} sec^{-1}). Grass with the raw effluent irrigation recorded lowest value (39.90 mmole m^{-2} sec^{-1}), whereas the highest value (65.10 mmole m^{-2} sec^{-1}) was observed in water irrigated grass.

DISCUSSION

One of the developmental features that characterize halophytic species is a marked reduction in the cortical region [11]. This feature was observed in *P. strombu flera* [12] and in *P. tamarugo* [13] grown in high NaC1 salinity. In the present work also this feature is observed in *C. celiaris* grown in effluent irrigation. Although the cortical layer was reduced a greater number of cortical layers with small vacuolated parenchyma cells were observed. This is in agreement with Strogonov [14] who proposed that SO_4^{2-}influences cell division more than expansion, which constitutes an adaptive mechanism to regulate ion concentration. Reinoso *et al.* [12], also documented the greater number of cortical layers with arenchyma reduction in *P. strombu flera* treated with Na_2SO_4.

Table 2: Transpiration rate and stomatal conductance of *Cenchrus celiaris* (mmole m^{-2} sec^{-1})

	Irrigation		
	Water	Treated effluent	Raw efflunet
Transpiration rate	1.27	1.19	1.08
Stomatal conductance (mmole m^{-2} sec^{-1})	65.10	54.60	39.90

The reduction in the arenchyma cells may be the adoption to keep the excess ions from entering the xylem vessel. In grapevine and in soybean, Cl^- transport to the shoots has been shown to be controlled by the roots [15-17], which suggests that this trait may be controlled by a single physiological mechanism.

Transpiration is a phenomenon related to the soil salt content and the turgor pressure created by the water. Since, in soils containing high levels of salts, the problem can be made worse by evaporative water loss directly from soils or evapotranspiration processes in a plant, which leaves salts behind and effectively concentrates them at the soil surface. Hence, the plant tends to increase the stomatal resistance to avoid the loss of water in stress condition. Low plant transpiration rate and high stomatal conductance is a survival mechanism developed by the grass to avoid death due to the accumulation of salt.

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