Research Article

Physiological Responses to Nutrient Accumulation in Trees Seedlings Irrigated with Municipal Effluent in Indian Desert

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Leaf water potential (Ψ), net photosynthesis rate (PN), transpiration rate (E), stomatal conductance (gS), and water use efficiency (WUE) are greatly influenced by the nutrient composition of water which is used for irrigating trees. The above-mentioned physiological variables and foliage mineral concentrations were observed for Eucalyptus camaldulensis, Acacia nilotica, and Dalbergia sissoo plants irrigated with municipal effluent (ME) at 1/2 PET (potential evapotranspiration; T1), 1 PET (T2), and 2 PET (T3) rates and the control plants irrigated with canal water at 1PET (T4). Increased mineral concentrations in order T1 < T2 < T3 enhanced Ψ, PN, E, and gS. Relatively greater increase in E than PN reduced WUE. Available nutrient in ME enhanced physiological function in T2, whereas reduced quantity of water lowered it in T1 than in T4 plants. Differential minerals uptake increased concentrations of N and P in D. sissoo, Mn in E. camaldulensis, and the rest in A. nilotica. PN was more sensitive to environment than E. Enhanced mineral concentration through ME was beneficial but its differential uptake and accumulation influenced physiological functions and WUE. E. camaldulensis is better for high and continuous loading of effluent and A. nilotica is best for high nutrient uptake. D. sissoo is efficient water user.

1. Introduction

Land degradation and contamination of environment from a variety of anthropogenic sources such as smelters, power station industry, the application of metal-containing pesticides, fertilizers and sewage sludge are widespread [1]. Metals/minerals released into environment do not only become irreversibly immobilized in soil components but are also toxic to animals, plants, and microorganisms [2]. Zinc, nickel, and copper are important constituents of pigments and enzymes. Cadmium, lead, mercury, and copper are toxic at high concentrations because of their disrupt enzyme functions, replace essential metals in pigments, or produce reactive oxygen species [3]. Although some plants have tremendous potential to hyperaccumulate minerals [4, 5], their excess accumulation could have adverse effect on the physiological functions thereby affecting growth and biomass production of various tree species when exposed to wastewater disposal. The problem is further aggravated due to the prevalence of crosstalk across different elements. Incidents of interaction between phosphorus and other macro-and microelements have been reported in crop species [6], whereas nutrient interactions in A. thaliana corroborated the prevalence of crosstalk across P and Fe [7]. In addition, Zn deficiency induced accumulation of P in barley, whereas P deficiency in A. thaliana resulted in the suppression of high-affinity Zn transporter ZIP9 [6, 8]. However, the studies pertaining to nutrient interaction have been confined largely to crop species or model plant system.

Unrelenting disposal of effluent of varying chemical constituents is responsible for contamination of land and water bodies, though increased water and nutrients availability by effluent disposal improve photosynthetic capacity of plants [9]. Municipal effluent is a precious resource available in dry regions and is rich in nutrients required for the plants. Rate of photosynthesis, carbon assimilation, and biomass production can be increased in tree by making available this water and nutrients to the nutrient poor soil of the desert region [10]. Though increased photosynthetic efficiency is the most important way of increasing productivity, simultaneous
increase in mineral concentrations may affect the efficiency of the species towards efficient utilization of this resource [11]. Protective mechanisms of plants by absorption and uptake of minerals from the soil reduces soil toxicity and safeguards environment [12]. But long term disposal may lead to excess accumulation of mineral in biological system and affect physiology and productivity [13]. The extent of influences both on the plant and soil needs to be assessed to avoid mineral toxicity during long term effluent application. The influences may be assessed by measuring foliage mineral concentration in and the physiological functions of tree seedlings used in plantation for efficient utilization of the effluent along with environmental and aesthetic benefits.

Present investigation was undertaken to monitor the effect of varying levels of municipal effluent on minerals accumulation in *Eucalyptus camaldulensis* Dehn., *Acacia nilotica* (L.) Willd. ex Delile and *Dalbergia sissoo* Roxb. ex DC. seedlings, and the physiological responses in these seedlings in relation to the accumulated minerals. Objectives of this study were to monitor changes in physiological functions of tree seedlings influenced by the mineral accumulation due to municipal effluent irrigation/disposal.

2. Materials and Method

2.1. Site Description. Experiment was conducted in non-weighing in-filled type of lysimeters of capacity 8 m$^3$ (i.e., size of 2 m $\times$ 2 m $\times$ 2 m) at the experimental field of Arid Forest Research Institute, Jodhpur (26$^\circ$45'N latitude and 72$^\circ$03'E longitude), in Rajasthan, India. The climate of the site is characterized by hot and dry summer, hot rainy season, warm autumn, and cool winter. The mean annual rainfall of 1998, 1999, and 2000 was 420 mm and the mean annual pan evaporation was 2025 mm. Averages of minimum and maximum air temperatures of a month were 14.5°C and 25.0°C in January, which increased gradually to 34.4°C and 40.7°C, respectively, in May. The soil was loamy sand (coarse loamy, mixed, hyperthermic family of Typic Camborthids, according to US soil taxonomy) with 82% sand, 12% silt, and 6.0% clay. Soil organic matter was 0.13% and available PO$_4$-P, NO$_3$--N, and NH$_4$-N were 5.00, 6.00, and 4.50 mg kg$^{-1}$, respectively. Soil pH and electrical conductivity (EC) were 7.61 and 0.71 dSm$^{-1}$, respectively [14].

2.2. Sampling. Preservation and Analysis of the Effluent. Samples of municipal effluent were collected and analyzed as described earlier [14, 15]. Samples were analyzed for pH, electrical conductivity, chemical oxygen demand, biochemical oxygen demand, macro- and micronutrients, total dissolved salts, total solids, and total suspended solids [16]. Nitrogen (N) and phosphorus (P) were analyzed following standard procedure [17]. Calcium (Ca), magnesium (Mg), potassium (K), sodium (Na), copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn) were estimated by the aqua-regia method of Jackson [17] followed by a measurement of concentrations using an atomic absorption spectrophotometer (model-3100, Perkin-Elmer, Boesch, Huenenberg, Switzerland). Municipal effluent was alkaline (pH 7.60 to 8.02); whereas electrical conductivity ranged from 0.91 to 2.14 dSm$^{-1}$ as described earlier [14]. Biochemical and chemical oxygen demand ranged between 36 and 56 mg L$^{-1}$ and 190 and 270 mg L$^{-1}$, respectively. Availability of NH$_4$-N, NO$_3$--N, PO$_4$-P, K, Fe, Cu, Mn, and Zn was always higher in municipal effluent than in the canal water. Calcium and iron were highest in concentrations among the basic cations and micronutrients, respectively. The ratios of K: N, K: Ca and Mg, Mg: Na, Mg: Mn, Fe: Mn, and Zn: Mn in municipal effluent were 0.04, 0.21, 0.31, 0.91, 9.09, and 1.22, respectively (see Supplementary Table 1 in Supplementary Material available online at http://dx.doi.org/10.1155/2014/545967). These effluent parameters increased during summer (due to high temperature and concentration) and decreased during rainy season (because of addition of runoff water), but highest concentration of NO$_3$--N during monsoon was due to its addition from the suburban area and the fertilized field through runoff water [14].

2.3. Plantation and Experimental Design. Nursery raised one-year-old seedlings of *Acacia nilotica*, *Dalbergia sissoo*, and *Eucalyptus camaldulensis* from a single provenance were planted in July 1998 in the lysimeters of 2 $\times$ 2 $\times$ 2 m$^3$ capacity, which were filled with soil up to 185 cm leaving 15 cm space for irrigation. There was one seedling in each lysimeter. The plantation was done in completely randomized design with three replications. Irrigation with municipal effluent was initiated in the first week of September 1998 after seedling establishment. Irrigation was based on the potential evapotranspiration (PET) calculated by multiplication of pan evaporation (Class A evaporation pan fixed at the site) rate and pan coefficient (i.e., 0.70) considering the crop coefficient value of 1.2 to 1.5 for *Eucalyptus*/*Alfaalfa* [18–21]. Water uses by tree plantation was considered not less than 1.5 times that of agriculture crop or about 1.25 times of Class A pan [22]. Four treatments comprised T$_1$: irrigation of seedlings with municipal effluent at 1/2 PET; T$_2$: irrigation of seedlings with municipal effluent at 1 PET; T$_3$: irrigation of seedlings with municipal effluent at 2 PET, and T$_4$: irrigation of seedlings with canal water (potable water with low mineral concentration) at 1 PET as control. At the time of treatment application, average seedling heights and collar diameters (12 plants) were 37.3 ± 0.5 (mean ± SE) cm and 0.5 ± 0.0 cm in *E. camaldulensis*, 37.8 ± 2.1 cm and 0.5 ± 0.1 cm in *A. nilotica* and 49.8 ± 0.3 cm, and 0.5 ± 0.0 cm in *D. sissoo*, respectively.

2.4. Observation Recording. Leaf water potential (LWP) was measured monthly on leaf discs in a leaf chamber (L-52; Wescor, Logan, Utah, USA) connected to a dew point microvoltmeter (Wescor HR-33T) between 0500 and 0700 hr from December 1998 to November 1999 before the reirrigation of the seedlings in each treatment. Leaf disc of 0.5 cm diameter was punched out from the attached leaves (without leaf abrision) and was transferred into a leaf chamber and after 15 minutes of equilibration the water potential was determined [23]. The discs were collected at the time of observation recording for each measurement. Net photosynthetic rate ($P_N$), transpiration rate ($E$), and stomatal resistance ($R$) were recorded with open system of portable CO$_2$ Gas
Analyser, Model CI-301 (CT-301 PS0), CID Inc., Vancouver, USA. Stomatal conductance ($g_S$) was calculated as $1/stomatal resistance$. These physiological variables were recorded between 10:00 and 11:00 hours and at one-month interval from December 1998 to November 2000 (24 months). All these observations were recorded on leaves of middle canopy of the seedlings in three replicates. Self-shading within the cuvette was minimised by ensuring that the leaves did not overlap. Instantaneous water use efficiency (WUE) was calculated as $P_{ET}/E$. Atmospheric CO$_2$ concentration during the experiment period was 380 ppm.

2.5. Mineral Nutrient Analysis. Irrigation quality criteria of municipal effluent and canal water were assessed as described earlier [14, 15]. Leaf samples from the 24-month-old planted seedlings were collected in June 2000, washed with tap water, and then rinsed with distilled water. The leaf samples were then oven-dried at 80°C, ground in a pestle and mortar, and digested with a triacid mixture ($\text{HNO}_3: \text{H}_2\text{SO}_4: \text{HClO}_4$ in 10 : 4 : 1 ratio). Concentration of K, Ca, Mg, Na, Cu, Fe, Mn, and Zn was determined using atomic absorption spectrophotometer [17]. Measurement of N and P content was performed after wet digestion with 12 mL $\text{H}_2\text{SO}_4$ and two Kjeltab (Cu/3.5) catalyst tablets at 350°C for half an hour and estimated using UV-VIS spectrophotometer model 117 at 490 and 420 nm wavelengths, respectively [17].

2.6. Statistical Analysis. Data were statistically analyzed using SPSS statistical package. There were three species and four treatments; hence, the foliage nutrient data were analysed using a two way ANOVA. Tree species and treatments were the factors. Since the physiological data were recorded repeatedly at one-month interval, these data were analysed using repeated measure ANOVA. Physiological parameters per month were the response variables. Month was the within subject factor and tree species and treatments were the between subject factors. Before analysis, average data of these variables were log or reciprocal of square root transformed for normality [24] and homocedasticity [25] in order to make valid statistical inferences about population relationships. Duncan Multiple Range Tests (DMRT) were also performed on each set of data for homogeneous subsetting for treatments and species. Pearson's correlation was performed to monitor the relations of foliage nutrients concentrations with the physiological variables and total effluent applied. Regressions were performed to observed relations between 24 months average physiological parameters and foliage mineral concentrations.

3. Results

3.1. Environmental Factors. Rainfall was 588.5 mm and total pan evaporation was 5420 mm during December 1998 to November 2000 showing high water deficit. Air temperature, photosynthetically active radiation (PAR), and vapour pressure deficit (VPD) varied between the months (Figure 1). Averages of minimum and maximum air temperatures of a month increased from the lowest at 08:00 hr to the highest at 13:00 hr and decreased in the evening (17:00 hr). Vapour pressure deficit (VPD) increased from 1 290 Pa in January, 1999, to 4660 Pa in April, 1999. PAR was highest at midday (13:00) and oscillated between 811 $\mu$mol m$^{-2}$ s$^{-1}$ in December, 1999, to 2140 $\mu$mol m$^{-2}$ s$^{-1}$ in May, 2000 (Figure 1).

3.2. Foliage Nutrient Concentrations. Seedlings irrigated with municipal effluent at $T_3$ and $T_4$ levels had higher ($P < 0.05$) concentration of N, P, K, Ca, Mg, Cu, Fe, Mn, and Zn than in the canal water ($T_4$) irrigated seedlings across the species (Table 1). Uptake and accumulation of the abovementioned nutrients increased ($P < 0.01$) with irrigation quantity from $T_1$ to $T_3$. When $T_1$ and $T_4$ treatments were compared, concentration of Na in all the species, Ca and Mg in $E. camaldulensis$ and K in $D. sissoo$ seedlings were the lowest in $T_1$, whereas other nutrients were the lowest in the seedlings of $T_4$ treatment. Concentrations of K, Ca, Mg, Cu, and Mn did not differ ($P > 0.05$) between the seedlings of $T_1$ and $T_4$ treatments (DMRT) despite of twofold water applied in latter than in the former treatment (Supplementary Table 2). There was 2% (Mg in $T_1$) to 2.9-fold (Mn in $T_3$) increase in nutrient concentration in ME irrigated seedlings than the respective concentrations in the seedlings of $T_4$ treatment (Supplementary Table 2). Across the treatments, the concentrations of K, Ca, Mg, Na, Fe, Cu, and Zn were the highest ($P < 0.05$) in $A. nilotica$, N and P were the highest in $D. sissoo$ and Mn was the highest in $E. camaldulensis$ seedlings. We observed nonsignificant differences in Ca and Cu concentrations between $E. camaldulensis$ and $D. sissoo$ and in Mn concentration between $A. nilotica$ and $D. sissoo$ (DMRT). The concentrations of these nutrients were adequate to high (N, Ca, Mg, Cu, and Fe in $E. camaldulensis$, Mg, K, Fe, Zn, and Cu in $A. nilotica$, and P, Ca, Cu, Fe, and Zn in $D. sissoo$) except in treatment $T_4$ (i.e., N and Cu in marginal concentrations) when compared with the reported literatures [27, 29, 32]. Compared to the nutrient concentrations in the seedlings of $T_4$, the increase in N and P concentrations in the seedlings of $T_3$ was 1.7 and 3.5-fold in $E. camaldulensis$, 1.8-
Table 1: Foliage nutrient concentration in 24-month-old planted seedlings of *E. camaldulensis* (*Ec*), *A. nilotica* (*An*), and *Dalbergia sissoo* (*Ds*) irrigated with varying levels of municipal effluent. Mean ± SE of three replications in parentheses.

| S | T   | N  | P  | K  | Ca | Mg | Na | Cu  | Fe  | Mn  | Zn  |
|---|-----|----|----|----|----|----|----|-----|-----|-----|-----|
|   |     | g kg⁻¹DM |     |     |    |    |    |     |     |     |     |
| T₁ |     | 20.67⁶ | 0.96⁶ | 11.6⁴ | 11.09⁶ | 3.66⁸ | 1.39⁸ | 26.80⁸ | 661.69⁸ | 169.00⁶ | 30.66⁷ |
|    | (0.36) | (0.05) | (0.64) | (0.78) | (0.05) | (0.02) | (1.29) | (18.50) | (12.15) | (1.32) |
| T₂ |     | 24.71¹ | 1.23 | 12.63 | 16.3⁴ | 4.03 | 1.62⁴ | 28.40 | 825.30 | 404.0⁴ | 32.5¹ |
|    | (0.79) | (0.09) | (0.39) | (0.51) | (0.08) | (0.06) | (0.42) | (17.03) | (25.75) | (1.13) |
| T₃ |     | 31.7⁴ | 1.56 | 15.0⁶ | 19.95 | 5.3⁴ | 2.0⁶ | 28.60 | 959.3⁴ | 458.0⁴ | 43.5⁴ |
|    | (0.63) | (0.06) | (0.65) | (0.64) | (0.43) | (0.05) | (2.60) | (27.22) | (19.20) | (2.06) |
| T₄ |     | 18.9² | 0.4³ | 13.0² | 17.7³ | 4.1⁶ | 1.9³ | 23.40 | 570.2⁰ | 134.7⁰ | 26.8⁷ |
|    | (1.05) | (0.03) | (0.65) | (0.63) | (0.16) | (0.04) | (2.01) | (18.57) | (10.89) | (0.72) |

**Table 2:** Leaf water relations (Ψₑ) in January (December in *Ds*) for *A. nilotica* and *E. camaldulensis*. The highest (Ψₑ = 3.7) in January for *E. camaldulensis* was higher than that of *A. nilotica* (Ψₑ = 2.7), but it did not differ with *Ds* (Ψₑ = 2.4), showing a significant difference between species (Table 2). The lowest Ψₑ was in *Ds* (Ψₑ = 0.5), whereas *E. camaldulensis* showed higher Ψₑ in *T₂* and *T₃* treatments (DMRT). The Ψₑ was the highest (Ψₑ = 3.7) in January for *E. camaldulensis* that decreased gradually to the lowest value in May and then rose in July-August (Table 3). The lowest Ψₑ was recorded for *A. nilotica* seedlings in most of the months.

Among the species, ratios of K : N, K : Ca and Mg, Mg : Na, Fe : Mn, Mn : Mn, and Zn : Mn ranged from 0.4 to 0.9, 0.6 to 1.1, 1.3 to 4.5, 2.1 to 13.2, 10.1 to 102.7, and 0.1 to 0.8 (Figure 2), respectively.

3.3. Leaf Water Relations. Leaf water potential (Ψₑ) was the highest (P < 0.01) in the seedlings of *T₃* across species (Table 2). The highest Ψₑ was in *E. camaldulensis*, but it did not differ with *Ds* for Ψₑ (P > 0.05, DMRT) across the treatments. The lowest Ψₑ was in *A. nilotica*. Dalbergia sissoo indicated the highest Ψₑ in *T₂*, whereas *E. camaldulensis* showed greater Ψₑ in *T₁* and *T₃* treatments (DMRT). The Ψₑ was the highest (P < 0.01) in January for *E. camaldulensis* that decreased gradually to the lowest value in May and then rose in July-August (Table 3). The lowest Ψₑ was recorded for *A. nilotica* seedlings in most of the months.

and 2.2-fold in *A. nilotica* and 1.9- and 2.0-fold in *Ds*. Concentrations of K, Ca, Mg, and Na were 1.1- to 1.3-fold in *E. camaldulensis*, 1.5- to 1.9-fold in *A. nilotica* and 1.5- to 3.0-fold in *Ds*. The increase in Cu, Fe, Mn and Zn was 1.2- to 6.6-fold across species (Table 3). The highest Ψₑ (3.7) in January for *E. camaldulensis* was higher than that of *A. nilotica* (Ψₑ = 2.7), but it did not differ with *Ds* (Ψₑ = 2.4), showing a significant difference between species (Table 2). The lowest Ψₑ was in *Ds* (Ψₑ = 0.5), whereas *E. camaldulensis* showed higher Ψₑ in *T₂* and *T₃* treatments (DMRT). The Ψₑ was the highest (Ψₑ = 3.7) in January for *E. camaldulensis* that decreased gradually to the lowest value in May and then rose in July-August (Table 3). The lowest Ψₑ was recorded for *A. nilotica* seedlings in most of the months.
Figure 2: Ratios of mineral elements concentrations and their relationship with Pn, E, and gs (×10^{-2}) influenced by tree species and level of municipal effluent application. Error bars are ±SE of three replicates. T1, T2, and T3 are municipal effluent irrigation levels of 1/2 PET, 1 PET, and 2 PET, respectively.
Table 2: Average values of physiological variables. Values are mean of 12 data (across treatments) for species and 9 data (across species) for treatments.

| Species/treatment | LWP | \( P_N \) | E | gs | WUE |
|-------------------|-----|--------|---|----|-----|
| Average values across municipal effluent treatments for species | | | | | |
| E. camaldulensis | −2.01<sup>a</sup> | 4.72<sup>a</sup> | 3.06<sup>a</sup> | 42.96<sup>a</sup> | 1.56<sup>b</sup> |
| A. nilotica | −2.20<sup>b</sup> | 3.82<sup>c</sup> | 2.63<sup>d</sup> | 39.04<sup>b</sup> | 1.44<sup>c</sup> |
| D. sissoo | −1.99<sup>c</sup> | 4.53<sup>d</sup> | 2.67<sup>b</sup> | 42.41<sup>a</sup> | 1.66<sup>c</sup> |

Average values across tree species for municipal effluent treatment

| Treatment | Species | gs | E | WUE |
|-----------|---------|----|---|-----|
| T<sub>1</sub> | 4.00 | 3.35<sup>d</sup> | 2.05<sup>d</sup> | 34.45<sup>d</sup> | 1.57<sup>b</sup> |
| T<sub>2</sub> | 4.00 | 4.70<sup>b</sup> | 3.01<sup>b</sup> | 42.25<sup>b</sup> | 1.55<sup>b</sup> |
| T<sub>3</sub> | 4.00 | 5.84<sup>b</sup> | 3.95<sup>a</sup> | 50.63<sup>c</sup> | 1.48<sup>c</sup> |
| T<sub>4</sub> | 4.00 | 3.52<sup>c</sup> | 2.13<sup>c</sup> | 38.56<sup>c</sup> | 1.62<sup>c</sup> |

LWP: leaf water potential (MPa, Mega Pascal); \( P_N \): rate of photosynthesis (\( \mu \)mol CO\(_2\) m\(^{-2}\) s\(^{-1}\)); E: rate of transpiration (mmol m\(^{-2}\) s\(^{-1}\)); gs: stomatal conductance (×10\(^{-3}\) mol m\(^{-2}\) s\(^{-1}\)); and WUE: instantaneous water use efficiency (\( P_N/E \)). \( \Psi_s \) was highest for D. sissoo in the seedlings of T<sub>1</sub> in August 2000, whereas A. nilotica showed the highest seasonal variations in E. camaldulensis seedlings. Species \( \times \) treatment interaction was also significant (\( P < 0.05 \)).

3.4. Stomatal Conductance. Stomatal conductance (gs) across the species increased (\( P < 0.01 \)) in order T<sub>1</sub> < T<sub>2</sub> < T<sub>3</sub> < T<sub>4</sub> (Table 2). Considering species, gs was highest (\( P < 0.01 \)) in E. camaldulensis and lowest in A. nilotica. However, DMRT showed non-significant difference in gs between E. camaldulensis and D. sissoo. D. sissoo indicated highest (\( P < 0.05 \)) gs during April to August (66.59 ± 2.44 × 10\(^{-3}\) mol m\(^{-2}\) s\(^{-1}\)) in E. camaldulensis in most of the months and in A. nilotica in all treatments.

3.5. Transpiration Rate. Rate of transpiration (E) varied (\( P < 0.01 \)) within months, species, and treatments. Across the species, E was the lowest in the seedlings of T<sub>1</sub>. Average E was 4% lesser in the seedlings of T<sub>1</sub>, but it increased by 46% and 85% in the seedlings of T<sub>2</sub> and T<sub>3</sub> treatment, respectively, as compared to E value in T<sub>4</sub> treatment (Table 2). Across treatments, E was the highest (\( P < 0.01 \)) in E. camaldulensis and the lowest in A. nilotica seedlings. E. camaldulensis indicated the highest values (maximum of 7.80 ± 0.26 mmol m\(^{-2}\) s\(^{-1}\) in August 2000) of E during January to April and July to September. A. nilotica indicated the highest (2.97 ± 0.04 mmol m\(^{-2}\) s\(^{-1}\) in December 1999) E during December to February, whereas D. sissoo indicated the highest (4.97 ± 0.10 mmol m\(^{-2}\) s\(^{-1}\) in June 2000) E in May and June.

Rate of transpiration was the lowest in December/January (Figure 3(b)). It increased in March and April and decreased again in May before approaching the highest value in August.

3.6. Net Photosynthesis Rate. Repeated measure ANOVA indicated variations (\( P < 0.01 \)) in net photosynthesis rate (\( P_N \)) due to species, treatments, and months. Average \( P_N \) increased with quantity of applied effluent and seedlings of T<sub>3</sub> treatments indicated the highest \( P_N \) in all species. The lowest \( P_N \) was in the seedlings of T<sub>1</sub> in most of the months and in T<sub>4</sub> in April, May, August, and September. When compared with the seedlings of T<sub>4</sub>, \( P_N \) increased by 34% and 66% in the seedlings of T<sub>2</sub> and T<sub>3</sub>, respectively, whereas it was 5% less in T<sub>1</sub> treatment. Across the treatments, the highest and lowest values of \( P_N \) were in E. camaldulensis and A. nilotica, respectively (Table 2). However, temporal variation indicated the highest \( P_N \) in E. camaldulensis in most of the observations (maximum of 13.56 ± 0.34 mmol CO\(_2\) m\(^{-2}\) s\(^{-1}\) in August 2000, mean ± 1SE), in D. sissoo in April, May, June, and July (6.35 ± 0.36 mmol CO\(_2\) m\(^{-2}\) s\(^{-1}\)) and in A. nilotica in October 2000 (5.2 ± 0.05 mmol CO\(_2\) m\(^{-2}\) s\(^{-1}\)). There was a significant (\( P < 0.01 \)) seasonal pattern in \( P_N \) with two maxima, that is, August and again in March/April (Figure 4(a)). \( P_N \) value in August was 6.2- to 7.1-fold in T<sub>1</sub>, 4.0- to 5.2-fold in T<sub>2</sub>, 3.9- to 4.5-fold in T<sub>3</sub>, and 4.0- to 5.7-fold in the seedlings of T<sub>4</sub> as compared to the respective \( P_N \) value in December/January. Seedlings of D. sissoo showed the highest seasonal variations in \( P_N \) among the species.

3.7. Instantaneous Water Use Efficiency. Repeated measure ANOVA showed significant (\( P < 0.01 \)) variation in water use efficiency (WUE), that is, \( P_N/E \) (\( \mu \)mol CO\(_2\) mmol\(^{-1}\) H\(_2\)O)
Table 3: Leaf water potential (MPa) of trees seedlings of *E. camaldulensis* (Ec), *A. nilotica* (An), and *Dalbergia sissoo* (Ds) irrigated with varying levels of municipal effluent. Mean ± SE of three replications in parentheses.

| S | T    | December | January | February | March | April | May | June | July | August | September | October | November |
|---|------|----------|---------|----------|-------|-------|-----|------|------|--------|-----------|---------|----------|
|   |      |          |         |          |       |       |     |      |      |        |           |         |          |
| 1 | T1   | 1.65     | 1.71    | 1.92     | 2.09  | 2.65  | 3.00| 2.76 | 2.76 | 2.38   | 2.48      | 2.65    | 2.10     |
|   |      | (0.04)   | (0.03)  | (0.04)   | (0.05)| (0.05)| (0.02)| (0.04)| (0.05)| (0.09) | (0.06)    | (0.06)  | (0.04)   |
| 2 | T2   | 1.38     | 1.43    | 1.60     | 1.93  | 2.15  | 2.52 | 2.11 | 1.97 | 2.29   | 2.35      | 2.35    | 1.48     |
|   |      | (0.06)   | (0.02)  | (0.05)   | (0.03)| (0.04)| (0.04)| (0.04)| (0.05)| (0.05) | (0.04)    | (0.03)  | (0.03)   |
| 3 | T3   | 1.00     | 1.14    | 1.43     | 1.72  | 1.81  | 2.20 | 2.05 | 1.65 | 1.50   | 1.84      | 2.07    | 1.33     |
|   |      | (0.04)   | (0.05)  | (0.02)   | (0.02)| (0.04)| (0.08)| (0.04)| (0.05)| (0.05) | (0.04)    | (0.06)  | (0.02)   |
| 4 | T4   | 1.57     | 1.63    | 1.83     | 2.01  | 2.04  | 2.81 | 2.19 | 2.19 | 2.32   | 2.42      | 2.47    | 1.62     |
|   |      | (0.05)   | (0.03)  | (0.04)   | (0.04)| (0.05)| (0.07)| (0.06)| (0.07)| (0.04) | (0.06)    | (0.06)  | (0.03)   |

Repeate Measure ANOVA*

| Tests of within subjects effects | Tests of between subjects effects |
|---------------------------------|----------------------------------|
|                                 | df  | MSE  | F value | P value | df  | MSE  | F value | P value |
| M                               | 11  | 2.507| 435.84  | <0.001  | S   | 2    | 1.948  | 147.99  | <0.001 |
| M × S                           | 22  | 0.372| 64.69   | <0.001  | T   | 3    | 4.417  | 335.54  | <0.001 |
| M × T                           | 33  | 0.031| 5.32    | <0.001  | S × T| 6    | 0.261  | 19.81   | <0.001 |
| M × S × T                       | 66  | 0.041| 7.06    | <0.001  |      |      |        |         |        |

* ANOVA results are significant at *P* < 0.01 except in February and March when it was significant at *P* < 0.05 for species × treatment interaction. M, month; S, species and T, treatments.

3.8. Correlations and Regressions. Nutrient concentrations in foliage of the seedlings were positively correlated (*r* = 0.453 to 0.841, *P* < 0.05, *n* = 27) to total applied municipal effluent (except for P and Mn). Fe, P, Na, Mn and N showed positive (*r* = 0.426 to 0.716, *P* < 0.05) and K: N ratio showed negative (*r* = -0.496, *P* < 0.05) relationship with Ψₛ. Total quantity of applied effluent showed positive relationship with average Ψₛ, *Pₛ*, *E*, and *gₛ* (*r* = 0.429 to 0.939, *P* < 0.05). Concentrations of foliage N, P, Fe, Mn, and Zn (*r* = 0.385 to 0.728, *P* < 0.05) were positively correlated with average *Pₛ*. Average *E* showed positive correlations (*r* = 0.451 to 0.715, *P* < 0.05) with N, Ca, Mg, Fe, Mn, and Zn concentrations, but we did not find relations (*P* > 0.05) of these nutrients with stomatal conductance. Ratios of mineral elements did not show significant relationship with physiological variables except for negative correlation of *Pₛ* (*r* = -0.412, *P* < 0.05) with K:N; and WUE with K:N (*r* = -0.465, *P* < 0.05), Mg:Na (*r* = -0.554, *P* < 0.01), and Mg:Mn (*r* = -0.488, *P* < 0.01). WUE showed negative correlations (*r* = -0.435 to -0.684, *P* < 0.05) with foliage nutrients concentration in tree seedlings.
Regressions equations (irrespective of species and treatments) between physiological functions and foliage nutrients concentration showed nonlinear relationships ($P < 0.05$). Nitrogen and P concentrations showed linear relations to $P_N$ and WUE, respectively (Table 4). Net photosynthesis and transpiration rates showed linear relations with slope value of 1.266, 1.067, and 1.605 for $E.\ camaldulensis$, $A.\ nilotica$, and $D.\ sissoo$ (Supplementary Figure 1) Both $P_N$ and $E$ increased ($P < 0.05$) with increase in nutrient concentrations except Cu, which did not indicate any relation with $E$. WUE.

Figure 3: Monthly changes in stomatal conductance ($g_s \times 10^{-3}$, (a)) and transpiration rate ($E$, (b)) of tree seedlings irrigated with canal water and varying levels of municipal effluent during 1998-99 and 1999-00. Error bars are ±SE. $T_1$, $T_2$, $T_3$, and $T_4$ are irrigation of seedlings with municipal effluent at $1/2$ PET, $1$ PET, $2$ PET, and canal water at $1$ PET, respectively.
was influenced by foliage biochemistry resulting in variations in $P_N/E$ ratio, which decreased with increase in nutrient concentration, but P concentration was positively related (Figure 5). WUE increased with increase in Mg: Na, Fe: Mn, Zn: Mn and Mg: Mn ratios but decreased when their ratio increased above 1.9, 6.67, 0.2, and 34.1, respectively. Increase in Ca, Na, and Fe concentrations influenced ($P < 0.05$) $P_N$ positively but their respective concentration of greater than 22.26 g kg$^{-1}$, 2.76 g kg$^{-1}$, and 1146 mg kg$^{-1}$ reduced $P_N$ (Table 4, Figure 5). Likewise greater than 1.65 g kg$^{-1}$, 21.09 g kg$^{-1}$, and 2.76 g kg$^{-1}$ concentrations of P, Ca, and Na respectively, reduced $E$. 

**Figure 4:** Monthly changes in net photosynthetic rate ($P_N$, (a)) and instantaneous water use efficiency (WUE, (b)) irrigated with canal water and varying levels of municipal effluents during 1998-99 and 1999-00. Error bars are ±SE. T$_1$, T$_2$, T$_3$, and T$_4$ are irrigation of seedlings with municipal effluent at 1/2 PET, 1 PET, 2 PET, and canal water at 1 PET, respectively.
Table 4: Regression equations* between mineral element concentrations and net photosynthesis rate, transpiration rate, stomatal conductance, and instantaneous water use efficiency as the dependent variables and mineral concentrations as independent variables.

| Variable   | Equation | $a_0$   | $b_1$   | $b_2$   | $b_3$     | $R^2$ | SE     | F value | P value |
|------------|----------|---------|---------|---------|-----------|-------|--------|---------|---------|
| Rate of photosynthesis ($P_{net}$) | N Logarithm | -8.62930 | 3.97209 | —       | —         | 0.5319 | 0.7837 | 28.40   | 0.000   |
|           | P Sigmoid | 1.69905 | -0.23188 | —       | —         | 0.3166 | 0.2094 | 11.58   | 0.002   |
|           | Ca Cubic  | 12.29345 | -2.00749 | 0.14362 | -0.0031 | 0.3759 | 0.9434 | 4.62    | 0.011   |
|           | Mg Sigmoid | 1.75381 | -1.10272 | —       | —         | 0.1790 | 0.2295 | 5.45    | 0.027   |
|           | Na Quadratic | -8.92904 | 12.89990 | -2.77332 | —         | 0.7060 | 0.6338 | 28.82   | 0.000   |
|           | Fe Quadratic | -5.02941 | 0.01869 | -0.00001 | —         | 0.5924 | 0.7464 | 17.43   | 0.000   |
|           | Mn Inverse | 5.97239 | -1.44654 | —       | —         | 0.4325 | 0.8629 | 19.05   | 0.000   |
|           | Zn Power   | 6.71215 | 0.50522 | —       | —         | 0.1710 | 0.2306 | 5.16    | 0.032   |
|           | K/N Compound | 6.71215 | 0.50522 | —       | —         | 0.1710 | 0.2306 | 5.16    | 0.032   |
|           | Fe/Mn Inverse | 4.07304 | 2.71389 | —       | —         | 0.1638 | 1.0474 | 4.90    | 0.036   |
| Rate of transpiration ($E$) | N Linear | 0.16705 | 0.09866 | —       | —         | 0.5111 | 0.5815 | 26.13   | 0.000   |
|           | P Cubic | 0.85675 | 3.25457 | -1.13713 | 0.1113 | 0.3623 | 0.6823 | 4.36    | 0.014   |
|           | K Sigmoid | 1.45824 | -6.13854 | —       | —         | 0.1242 | 0.2677 | 3.55    | 0.071   |
|           | Ca Cubic  | 12.73548 | -2.24278 | 0.14900 | 0.002986 | 0.5185 | 0.6016 | 8.25    | 0.000   |
|           | Mg Sigmoid | 1.45201 | -2.17157 | —       | —         | 0.3397 | 0.2325 | 12.86   | 0.001   |
|           | Na Quadratic | 5.60495 | 7.83469 | -1.57456 | —         | 0.7883 | 0.3901 | 44.80   | 0.000   |
|           | Cu Cubic | 1.96642 | -729.363 | —       | —         | 0.6725 | 0.1637 | 51.33   | 0.000   |
|           | Mn Inverse | 4.01221 | -108.593 | —       | —         | 0.4637 | 0.6090 | 21.61   | 0.000   |
|           | Zn Compound | 1.86315 | 1.00904 | —       | —         | 0.2454 | 0.2485 | 8.13    | 0.008   |
|           | Fe/Mn Inverse | 2.58987 | 2.01328 | —       | —         | 0.1711 | 0.7571 | 5.16    | 0.032   |
|           | Zn/Mn Inverse | 2.68570 | 0.07460 | —       | —         | 0.1564 | 0.7638 | 4.63    | 0.041   |
| Stomatal conductance ($g_s$) | N Compound | 28.39680 | 1.01473 | —       | —         | 0.1622 | 0.2003 | 4.83    | 0.037   |
|           | Na Sigmoid | 4.24489 | -0.79956 | —       | —         | 0.2381 | 0.1910 | 7.81    | 0.009   |
|           | Fe Sigmoid | 4.08435 | 256.357 | —       | —         | 0.1420 | 0.2027 | 4.14    | 0.052   |
|           | Mn Sigmoid | 4.07692 | -22.8573 | —       | —         | 0.2967 | 0.1835 | 10.55   | 0.003   |
| Instantaneous water use efficiency (WUE) | P Linear | 1.43590 | 0.04460 | —       | —         | 0.4673 | 0.0869 | 21.93   | 0.000   |
|           | K Compound | 1.81403 | 0.98970 | —       | —         | 0.3293 | 0.0655 | 12.27   | 0.001   |
|           | Ca Compound | 1.8993 | 0.98773 | —       | —         | 0.3669 | 0.0636 | 14.49   | 0.000   |
|           | Mg Compound | 1.76437 | 0.97289 | —       | —         | 0.6349 | 0.0483 | 43.48   | 0.000   |
|           | Na Compound | 1.86695 | 0.89376 | —       | —         | 0.4765 | 0.0579 | 22.75   | 0.000   |
|           | Cu Compound | 1.71655 | 0.99728 | —       | —         | 0.3036 | 0.0667 | 10.90   | 0.002   |
|           | Zn Compound | 1.71518 | 0.99764 | —       | —         | 0.2151 | 0.0708 | 6.85    | 0.014   |
|           | K/N Inverse | 1.28251 | 0.13853 | —       | —         | 0.2370 | 0.1039 | 7.76    | 0.010   |
|           | Mg/Na Cubic | 0.41929 | 1.46011 | -0.54740 | 0.0606 | 0.4673 | 0.0905 | 6.72    | 0.002   |
|           | Fe/Mn Quadratic | 1.34984 | 0.09269 | -0.00715 | —         | 0.7007 | 0.0665 | 28.10   | 0.000   |
|           | Zn/Mn Cubic | 1.30100 | 2.78703 | -0.07910 | 3.5243 | 0.2924 | 0.1044 | 3.17    | 0.043   |
|           | Mg/Mn Cubic | 1.30661 | 0.02323 | -0.00047 | 0.000003 | 0.6924 | 0.0688 | 17.26   | 0.000   |

* Nonsignificant ($P > 0.05$) relations have not been shown. $a_0$, $b_1$, $b_2$, and $b_3$ are regression constants. $R^2$: coefficient of determination and SE: standard error.

4. Discussion

4.1. Foliage Nutrients and Water Relations. The results of this study showed beneficial effects of municipal effluent on the physiological functions of *E. camaldulensis*, *A. nilotica*, and *D. sissoo* seedlings. Because of essential in nature, these nutrients were transported to and accumulated ($P < 0.01$) in foliar parts with increased effluent quantity from T1 to T3. Greater concentrations of the most of the nutrients in the seedlings in T2 and N, P, Fe, and Zn in T1 as compared to the seedlings in T3.
Figure 5: Relationship of mineral concentrations with average rate of net photosynthesis ($P_N$, $\mu$mol CO$_2$ m$^{-2}$ s$^{-1}$), transpiration ($E$, mmol m$^{-2}$ s$^{-1}$), and instantaneous water use efficiency in tree seedlings irrigated with varying levels of municipal effluent. The observed and estimated trend in values are shown by cross (×) and solid line, respectively.
T₄ treatment (despite same quantity of water in T₂ and half quantity of water in T₁ treatment) were due to the nutrients applied through municipal effluent. Relatively greater accumulation of Mn, Fe, Cu, and Zn (10% to 2.9-fold in effluent irrigated than in T₁) as compared to N, K, Ca, and Mg (2% to 93%) showed an increase in absorption and mobility of the former elements under increased level of effluent irrigation [26]. However, absence of any toxic effect on the tree seedlings showed that the nutrient concentrations were adequate [27–29] or lesser than the critical concentrations observed in other studies [30, 31]. The differential accumulation of nutrients varied with species characteristics influencing Ψₛ and ratios of the nutrient concentrations in plant system. The highest concentration of Ca, Mg, K, Na, Cu, Fe, and Zn in A. nilotica seedlings particularly basic cations (Table 1) was related to reduced Ψₛ and physiological functions by increasing solute concentration. Lesser concentrations of these nutrients in E. camaldulensis and D. sissoo were due to dilution effects because these species had much broader leaf blades (and increased growth and biomass) than A. nilotica [32]. Relatively greater accumulation of Fe as compared to Mn (high Fe : Mn ratio) in A. nilotica indicated impairing effect of Ca and K reducing Mn concentration, an important constituent (together with Cu, Zn and Fe) of many enzymes influencing physiological function [33]. Higher plants have also evolved sophisticated antioxidant defense system and glyoxalase system to scavenge the oxidative effects of metals [34]. However, the lowest ratio of Fe : Mn in E. camaldulensis was an adaptation/defense mechanism through antioxidative systems (superoxide dismutase) for success of this species under high water availability or waterlogged conditions as observed for Populus angustifolia [35, 36].

Increased Ψₛ in the seedlings from T₁ to T₃ was positively influenced by increased level of effluent application-soil water availability [37]. However, higher (P < 0.05) Ψₛ in the seedlings of T₂ as compared to the seedlings of T₁ treatment was due to two-fold higher water applied to T₂. A difference of 1.14 to 1.35 MPa between the lowest and highest Ψₛ in the seedlings of E. camaldulensis as compared to those of 0.83 to 1.24 MPa in A. nilotica and 0.35 to 0.59 MPa in D. sissoo was due to higher E in former than in the latter two species (Table 3). High Ψₛ during December and January indicated low water loss or reduced E as a function of low PAR, VPD, low air temperature, and rainfall in January and February, 1999 (Figure 1). However, gradual increase in PAR, VPD, and air temperature with concomitant decrease in Ψₛ from January to May in all the three species indicated negative relations between Ψₛ and these environmental factors.

4.2. Foliage Nutrients and Gas Exchange. Application of municipal effluent had no toxic effect on Pₙ, E, and gₛ together with Ψₛ in the seedlings of T₄ indicated negative impact of low water supply [38]. Relatively greater increase in these variables during August (monsoon period, Figures 3 and 4) further suggests that the seedlings of this treatment suffered of water stress [39]. A 15% decrease in Pₙ has been reported in a two-year-old Picea ruben seedlings at water potential averaging –2.45 MPa [40]. Greater values (P < 0.05) of Pₙ, E, and gₛ in T₂ than in T₁ seedlings (except in February, 1999, March, May, August, and October, 2000, for Pₙ, February and May 1999, and February to June, 2000, for E, and May 2000 for gₛ) were due to two-fold water applied [41]. Despite of similar level of irrigation (IPET) increased values of the physiological variables in T₂ as compared to the seedlings of T₄ were due to nutritional effects of municipal effluent (Figures 3 and 4). Carswell et al. [42] observed an enhanced rate of electron transport and velocity of carboxylation in Cedrela odorata seedlings at 5% rate of macro- and micronutrient supply compared to that at 1% rate. Highest level of irrigation and corresponding increase in water and nutrient supply induced absorption and transport of the nutrients to the seedling resulted in the highest Pₙ and E in the seedlings of T₂. This increase in Pₙ, E and gₛ was positively related to Ψₛ and foliage N and other nutrients as observed in Pseudotsuga menziesii (Mirb.) Franco. [43]. However, the higher value of gₛ was not parallelly by increased Pₙ or E, which may reflect a partial limitation in foliage biochemistry or leaf structure and varying effects on these physiological variables [44].

Linear/nonlinear increase in Pₙ with nutrient concentrations suggests a close link of photosynthetic capacity with nutrient supply, but simultaneous increases in E and gₛ (Figure 5) are indicative of rapid growth and biomass production [45]. A decline/saturation, after an initial increase in Pₙ and E with increase in concentrations of Ca, Na, and Fe, was as a result of the effects of accumulated minerals and limitations due to other nutrients and their ratios [45, 46]. A reduction in net photosynthetic rate and stomatal conductance due to a toxic effect of Na⁺ has also been reported in Citrus limonina Osbeck and Olea europaea L. [47]. Though increases in N, K, Fe, and Mn concentrations were beneficial, but relatively greater increase in N and Mn than K and Fe, respectively, from T₁ to T₄ seemed to facilitate Pₙ to a greater extent than E evidenced by increased WUE as observed in D. sissoo discussed later (Supplementary Figure 1). A 4.0- to 7.1-fold variation in Pₙ compared to 2.2- to 4.0-fold variation in E among the months further indicated greater sensitivity of Pₙ to foliage chemistry as well as environmental factors. Increase in Pₙ, E, and gₛ during monsoon and spring due to reduced VPD, PAR, and air temperature though rainfall suggests the effects of environmental factors in influencing physiological variables. Despite of lower nutrient concentrations except Mn (highest) higher Pₙ, E, and gₛ in E. camaldulensis were the effects of lower Ψₛ and tolerance to Mn because of scavenging system composed of antioxidants as reported for Mn-tolerant maize (Zea mays L.) [48]. Decrease in the values of these physiological variables during winter (due to plant senescence and reduced VPD and transpiration losses) and summer (due to increase in VPD, PAR, air temperature, desiccating wind velocity, and probably mineral concentrations)
was similar to that in *Pseudotsuga menziesii* [49]. Drops in $P_N$ and $E$ as a function of high irradiance/temperature through stomatal control have also been reported by Van Assche and Clijsters [50] and Castillo et al. [51].

4.3. Foliage Nutrients and Water Use Efficiency. Nutrient concentration influenced $P_N$ and $E$ and thus instantaneous water use efficiency (WUE). A negative relation of nutrients concentration with WUE suggested impaired effects of K, Ca, Na, and Zn on $E$ than on $P_N$. Increase in $P_N$ was associated with increase in $E$ and $g_s$ from $T_1$ to $T_3$ treatments, but greater increase in $E$ as compared to $P_N$ due to increased water and nutrient supply from $T_1$ to $T_3$ impaired WUE. Ewers et al. [52] observed an increase in transpiration rate in irrigated trees, relative to unirrigated trees by the effect of irrigation combined with fertilization. Low WUE in municipal effluent irrigated seedlings as compared to control ($T_4$ treatment) was due increased water availability which enhanced $E$ to a greater extent (increased by 46% in $T_2$ and 85% in $T_3$) than $P_N$ (increased by 34% in $T_2$ and 66% in $T_3$). Higher ($P \lt 0.01$) WUE in *D. sisoos* than the other species in most of the months (Figure 4(b)) was due to enhanced foliage $N$ and $P$ concentrations with greater positive influence on $P_N$ than on $E$. Thus *D. sisoos* was able to maintain high rates of $P_N$ with relatively low $g_s$ and $E$ is considered to be tolerant to low moisture availability and has high WUE [50]. An inverse relation between K : N ratio and WUE (Table 3; Figure 5) also suggests foliar chemistry regulated variations in $E$ and $P_N$. Lowest WUE in *A. nilotica* was due to relatively greater concentrations (than in other species) of basic cations together with Fe and Zn and lesser concentrations of P and Mn influencing $P_N/E$ ratio. This type of species-specific response in WUE had also been observed in *Vismia japurensis*, *Bellucia grossularioides*, and *Laetia procera* when treated with $P$, Ca, and gypsum [53]. After initial increase, a decrease in WUE with increase in ratios of Mg : Mn, Fe : Mn, and Zn : Mn suggested an adverse effect of Mg, Fe, and Zn on WUE at enhanced concentrations. It seemed that Mn played a part in stabilizing mineral ratio to maintain up right $P_N : E$ ratio (WUE).

5. Conclusions and Recommendation

Irrigating tree seedlings with municipal effluent showed positive influence on nutrient accumulation and physiological functions, that is, $\Psi_f$, $P_N$, $E$, and $g_s$. Enhanced $P_N$ together with $E$ and $g_s$ with increased water and nutrient from $T_1$ to $T_3$ indicated a fast growth in the tree seedlings. Increase in physiological functions in $T_3$ as compared to $T_1$ was the nutrient effects, whereas their increase in $T_3$ than in $T_1$ was the effect of water. Relatively higher and lower concentrations of basic cations and Fe influenced gas exchange negatively in *A. nilotica* and positively in *E. camaldulensis*, respectively, affecting WUE. A positive effect of N and P on net photosynthesis and that of K on transpiration rate influenced WUE in these seedlings. *D. sisoos* was efficient water user by maintaining up right ratio between $P_N$ and $E$ by accumulating higher $N$ and $P$, and lower Mg, Na, and Fe concentrations than other mineral nutrients. Adequate concentration of Mg, Na, Fe and Zn enhanced physiological functions, but their higher concentrations adversely affected gas exchange and WUE. Conclusively, higher nutrient accumulation and low WUE in *A. nilotica* seedling were adaptations towards higher nutrient load and this species can safely be categorized as best soil ameliorator [32]. *D. sisoos* maintained relatively greater $P_N$ and lesser $E$ (a characteristic of efficient water user). *E. camaldulensis* maintained higher gas exchange by reducing concentration of basic cations and stabilizing Fe : Mn and Mg : Mn ratios and can be better species for long term disposal of municipal effluent.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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References

[1] B. Robinson, C. Russell, M. Hedley, and B. Clothier, "Cadmium adsorption by rhizobia: implications for New Zealand pastureland," *Agriculture, Ecosystems and Environment*, vol. 87, no. 3, pp. 315–321, 2001.

[2] F. I. Khan, T. Husain, and R. Hejazi, "An overview and analysis of site remediation technologies," *Journal of Environmental Management*, vol. 71, no. 2, pp. 95–122, 2004.

[3] S. Cenkci, I. H. Ci˘gerci, M. Yıldız, C. Özay, A. Bozda˘g, and H. Terzi, "Lead contamination reduces chlorophyll biosynthesis and genomic template stability in *Brassica rapa L.*," *Environmental and Experimental Botany*, vol. 67, no. 3, pp. 467–473, 2010.

[4] R. R. Brooks, *Plants that Hyperaccumulate Heavy Metals*, CAB International, Wallingford, UK, 1998.

[5] H. Sarma, "Metal hyperaccumulation in plants: a review focusing on phytoremediation technology," *Journal of Environmental Science and Technology*, vol. 4, no. 2, pp. 118–138, 2011.

[6] C. Huang, S. J. Barker, P. Langridge, F. W. Smith, and R. D. Graham, "Zinc deficiency up-regulates expression of high-affinity phosphate transporter genes in both phosphate-sufficient and -deficient barley roots," *Plant Physiology*, vol. 124, no. 1, pp. 415–422, 2000.

[7] J. T. Ward, B. Lahner, E. Yakubova, D. E. Salt, and K. G. Raghothama, "The effect of iron on the primary root elongation of Arabidopsis during phosphate deficiency," *Plant Physiology*, vol. 147, no. 3, pp. 1181–1191, 2008.

[8] A. Jain, M. D. Poling, A. P. Smith et al., "Variations in the composition of gelling agents affect morphophysiological and molecular responses to deficiencies of phosphate and other nutrients," *Plant Physiology*, vol. 150, no. 2, pp. 1033–1049, 2009.

[9] A. S. Walcroft, D. Whitehead, W. B. Silvester, and F. M. Kellih, "The response of photosynthetic model parameters to temperature and nitrogen concentration in *Pinus radiata* D. Don, " *Plant, Cell and Environment*, vol. 20, no. 11, pp. 1338–1348, 1997.
[10] J. S. Pereira, M. M. Chaves, F. Fonseca et al., "Photosynthetic capacity of leaves of Eucalyptus globulus (Labill.) growing in the field with different nutrient and water supplies," Tree Physiology, vol. 11, pp. 381–389, 1991.

[11] V. R. Nenova, "Growth and photosynthesis of pea plants under different iron supply," Acta Physiologiae Plantarum, vol. 31, no. 2, pp. 385–391, 2009.

[12] Z.-G. Shen, X.-D. Li, C.-C. Wang, H.-M. Chen, and H. Chua, "Lead phytoextraction from contaminated soil with high-biomass plant species," Journal of Environmental Quality, vol. 31, no. 6, pp. 1893–1900, 2002.

[13] R. Mata-González, R. E. Sosebee, and C. Wan, "Physiological impacts of biosolids application in desert gardens," Environmental and Experimental Botany, vol. 48, no. 2, pp. 139–148, 2002.

[14] G. Singh and M. Bhati, "Growth, biomass production and nutrient composition of Eucalyptus camaldulensis seedlings irrigated with municipal effluent in loamy sand soil of Indian desert," Journal of Plant Nutrition, vol. 26, no. 12, pp. 2469–2488, 2003.

[15] G. Singh and M. Bhati, "Mineral accumulation, growth, and physiological functions in Dalbergia sissoo seedlings irrigated with different effluents," Journal of Environmental Science and Health A, vol. 38, no. 11, pp. 2679–2695, 2003.

[16] OMA, Official Methods of Analysis, Association of Official Analytical Chemists, Arlington, Va, USA, 15th edition, 1990.

[17] M. L. Jackson, Soil Chemical Analysis, Prentice Hall of India, New Delhi, India, 1973.

[18] K. N. Rao, C. J. George, K. S. Ramasastri et al., "Climatic classification of India," Scientific Report 158, India Meteorological Department, New Delhi, India, 1971.

[19] E. Stibbe, "Soil moisture depletion in summer by an eucalyptus grove in a desert area," Agro-Ecosystems, vol. 2, no. 2, pp. 117–126, 1975.

[20] M. L. Sharma, "Evapotranspiration from a eucalyptus community," Agricultural Water Management, vol. 8, no. 1–3, pp. 41–56, 1984.

[21] R. G. Allen, M. E. Jensen, J. L. Wright et al., "Operational estimates of reference evapotranspiration," Agronomy Journal, vol. 81, pp. 650–662, 1989.

[22] A. E. Heuperman, A. S. Kapoor, H. W. Denecke et al., "Biodrainage: principle, experiences and application," in Proceedings of the International Programme for Technology and Research in Irrigation and Drainage (IPTRID ’02), p. 15, FAO, Rome, 2002.

[23] E. C. Campbell, G. S. Campbell, and W. K. Barlow, "A dewpoint hygrometer for water potential measurement," Agricultural Meteorology, vol. 12, pp. 113–121, 1973.

[24] S. S. Shapiro and M. B. Wilk, "An analysis of variance test for normality: Complete samples," Biometrika, vol. 52, pp. 591–611, 1965.

[25] H. Levene, "Robust tests for equality of variances," in Contributions to Probability and Statistics, pp. 278–292, Stanford University, Palo Alto, Calif, USA, 1960.

[26] D. R. R. Mallanthi, M. Moritsugu, and K. Yokoyama, "Effects of low pH and AI on absorption and translocation of some essential nutrients in excised barley roots," Soil Science & Plant Nutrition, vol. 41, no. 2, pp. 253–262, 1995.

[27] P. Drechsel and W. Zech, "Foliage nutrient levels of broad-leaved tropical trees: a tabular review," Plant and Soil, vol. 131, no. 1, pp. 29–46, 1991.

[28] N. E. Marcar, "Fodder values of salt tolerant Australian Acacias," in Proceedings of the International Workshop on Nitrogen Fixing Trees for Fodder (IWNTF ’95), pp. 20–25, Pune, India, 1995.

[29] B. Bargali K and S. Bargali, "Acacia nilotica: a multipurpose leguminous plant," Nature and Science, vol. 7, pp. 11–19, 2009.

[30] S. M. Reichman, C. J. Asher, D. R. Mulligan, and N. W. Menzies, "Seeding responses of three Australian tree species to toxic concentrations of zinc in solution culture," Plant and Soil, vol. 235, no. 2, pp. 151–158, 2001.

[31] S. M. Reichman, N. W. Menzies, C. J. Asher, and D. R. Mulligan, "Seeding responses of four Australian tree species to toxic concentrations of manganese in solution culture," Plant and Soil, vol. 258, no. 1–2, pp. 341–350, 2004.

[32] M. U. Shirazi, M. A. Khan, M. Ali et al., "Growth performance and nutrient contents of some salt tolerant multipurpose tree species growing under saline environment," Pakistan Journal of Botany, vol. 38, no. 5, pp. 1381–1388, 2006.

[33] E. J. Hewitt, "The role of mineral elements in the activity of plant enzyme systems," Encyclopedia of Plant Physiology, vol. 4, pp. 427–481, 1958.

[34] M. A. Hossain, P. Piyatidha, J. A. T. da Silva, and M. Fujita, "Molecular mechanism of heavy metal toxicity and tolerance in plants: central role of glutathione in detoxification of reactive oxygen species and methylglyoxal and in heavy metal chelation," Journal of Botany, vol. 2012, Article ID 872873, 37 pages, 2012.

[35] S. Alam, F. Akiha, S. Kamei, S. M. I. Huq, and S. Kawai, "Mechanism of potassium alleviation of manganese phytotoxicity in barley," Journal of Plant Nutrition, vol. 28, no. 5, pp. 889–901, 2005.

[36] K. A. Barrick and M. G. Noble, "The iron and manganese status of seven upper montane tree species in Colorado, USA, following long-term waterlogging," Journal of Ecology, vol. 81, no. 3, pp. 523–531, 1993.

[37] R. Tognetti, A. Longobucco, F. Miglietta, and A. Raschi, "Water relations, stomatal response and transpiration of Quercus pubescents trees during summer in a Mediterranean carbon dioxide spring," Tree Physiology, vol. 19, no. 4-5, pp. 261–270, 1999.

[38] H. Schnabl and H. Ziegler, "The mechanism of stomatal movement in Allium cepa L.," Planta, vol. 136, no. 1, pp. 37–43, 1977.

[39] N. Mohanty and I. Vass, "Impairment of PSII activity at the level of secondary quinone electron acceptors in chloroplast treated with Co2+, Zn2+, Ni2+ and ions," Physiology Plant, vol. 76, pp. 386–390, 1989.

[40] H. Seiler and B. H. Cazel, "Influence of water stress on the physiology and growth of red spruce seedlings," Tree Physiology, vol. 6, no. 1, pp. 69–77, 1990.

[41] B. Singh and G. Singh, "Biomass partitioning and gas exchange in Dalbergia sissoo seedlings under water stress," Photosynthesis, vol. 41, no. 3, pp. 407–414, 2003.

[42] C. Carswell, J. Grace, M. E. Lucas, and P. G. Jarvis, "Interaction of nutrient limitation and elevated CO2 concentration on carbon assimilation of a tropical tree seedling (Cedrela odorata)," Tree Physiology, vol. 20, no. 14, pp. 977–986, 2000.

[43] A. K. Mitchell and T. M. Hinkle, "Effects of foliar nitrogen concentration on photosynthesis and water use efficiency in Douglas-fir," Tree Physiology, vol. 12, pp. 403–410, 1993.

[44] R. Tognetti, L. Sebastiani, and A. Minnocc, "Gas exchange and foliage characteristics of two poplar clones grown in soil amended with industrial waste," Tree Physiology, vol. 24, no. 1, pp. 75–82, 2004.
[45] G. Singh and M. Bhati, “Changing effluent chemistry affect survival, growth and physiological function of *Acacia nilotica* seedlings in northwestern region of India,” *Environmentalist*, vol. 28, no. 3, pp. 175–184, 2008.

[46] G. Rubio, J. Zhu, and J. P. Lynch, “A critical test of the two prevailing theories of plant response to nutrient availability,” *The American Journal of Botany*, vol. 90, no. 1, pp. 143–152, 2003.

[47] J. C. Melgar, J. P. Syvertsen, V. Martínez, and F. García-Sánchez, “Leaf gas exchange, water relations, nutrient content and growth in citrus and olive seedlings under salinity,” *Biologia Plantarum*, vol. 52, no. 2, pp. 385–390, 2008.

[48] S. Doncheva, C. Poschenrieder, Z. Stoyanova, K. Georgieva, M. Velichkova, and J. Barceló, “Silicon amelioration of manganese toxicity in Mn-sensitive and Mn-tolerant maize varieties,” *Environmental and Experimental Botany*, vol. 65, no. 2-3, pp. 189–197, 2009.

[49] F. C. Meinzer, “The effect of light on stomatal control of gas exchange in Douglas fir (*Pseudotsuga menziesii*) saplings,” *Oecologia*, vol. 54, no. 2, pp. 270–274, 1982.

[50] F. van Assche and H. Clijsters, “Inhibition of photosynthesis in *Phaseolus vulgaris* by treatment with toxic concentrations of zinc: effects on electron transport and photophosphorylation,” *Physiologia Plantarum*, vol. 66, no. 4, pp. 717–721, 1986.

[51] J. M. Castillo, A. E. Rubio Casal, C. J. Luque, T. Luque, and M. E. Figueroa, “Comparative field summer stress of three tree species co-occurring in Mediterranean coastal dunes,” *Photosynthetica*, vol. 40, no. 1, pp. 49–56, 2002.

[52] B. E. Ewers, R. Oren, T. J. Albaugh, and P. M. Dougherty, “Carry-over effects of water and nutrient supply on water use of Pinus taeda,” *Ecological Applications*, vol. 9, no. 2, pp. 513–525, 1999.

[53] C. C. Ma, Y. B. Gao, H. Y. Guo, and J. L. Wang, “Photosynthesis, transpiration, and water use efficiency of *Caragana microphylla*, *C. intermedia*, and *C. korshinskii*,” *Photosynthetica*, vol. 42, no. 1, pp. 65–70, 2004.
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