

## Research Article

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

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Leaf water potential ( $\Psi_l$ ), net photosynthesis rate ( $P_N$ ), transpiration rate ( $E$ ), stomatal conductance ( $g_s$ ), 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;  $T_1$ ), 1 PET ( $T_2$ ), and 2 PET ( $T_3$ ) rates and the control plants irrigated with canal water at 1PET ( $T_4$ ). Increased mineral concentrations in order  $T_1 < T_2 < T_3$  enhanced  $\Psi_l$ ,  $P_N$ ,  $E$ , and  $g_s$ . Relatively greater increase in  $E$  than  $P_N$  reduced WUE. Available nutrient in ME enhanced physiological function in  $T_2$ , whereas reduced quantity of water lowered it in  $T_1$  than in  $T_4$  plants. Differential minerals uptake increased concentrations of N and P in *D. sissoo*, Mn in *E. camaldulensis*, and the rest in *A. nilotica*.  $P_N$  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 wide spread [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 they 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 Pi 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 mechanism 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* Dehnh., *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  $8\text{ m}^3$  (i.e., size of  $2\text{ m} \times 2\text{ m} \times 2\text{ m}$ ) at the experimental field of Arid Forest Research Institute, Jodhpur ( $26^\circ 45'\text{N}$  latitude and  $72^\circ 03'\text{E}$  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  $14.5^\circ\text{C}$  and  $25.0^\circ\text{C}$  in January, which increased gradually to  $34.4^\circ\text{C}$  and  $40.7^\circ\text{C}$ , respectively, in May. The soil was loamy sand (coarse loamy, mixed, hyperthermic family of Typic Camborthides, according to US soil taxonomy) with 82% sand, 12% silt, and 6.0% clay. Soil organic matter was 0.13% and available  $\text{PO}_4\text{-P}$ ,  $\text{NO}_3\text{-N}$ , and  $\text{NH}_4\text{-N}$  were 5.00, 6.00, and  $4.50\text{ mg kg}^{-1}$ , respectively. Soil pH and electrical conductivity (EC) were 7.61 and  $0.71\text{ dSm}^{-1}$ , 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-3110, Perkin-Elmer, Boesch, Huenenberg, Switzerland). Municipal effluent was alkaline (pH 7.60 to 8.02); whereas electrical

conductivity ranged from 0.91 to  $2.14\text{ dSm}^{-1}$  as described earlier [14]. Biochemical and chemical oxygen demand ranged between 36 and  $56\text{ mg L}^{-1}$  and 190 and  $270\text{ mg L}^{-1}$ , respectively. Availability of  $\text{NH}_4\text{-N}$ ,  $\text{NO}_3\text{-N}$ ,  $\text{PO}_4\text{-P}$ , 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  $\text{NO}_3\text{-N}$  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  $2 \times 2 \times 2\text{ m}^3$  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  $37.3 \pm 0.5$  (mean  $\pm$  SE) cm and  $0.5 \pm 0.0$  cm in *E. camaldulensis*,  $37.8 \pm 2.1$  cm and  $0.5 \pm 0.1$  cm in *A. nilotica* and  $49.8 \pm 0.3$  cm, and  $0.5 \pm 0.0$  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 abrasion) 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  $\text{CO}_2$  Gas

Analyzer, Model CI-301 (CT-301 PS0), CID Inc., Vancouver, USA. Stomatal conductance ( $g_s$ ) was calculated as  $1/\text{stomatal resistance}$ . These physiological variables were recorded between 10:00 and 11:00 hrs 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  $P_N/E$ . Atmospheric  $\text{CO}_2$  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 seedling were collected in June 2000, washed with tap water, and then rinsed with distilled water. The leaf samples were then oven-dried at  $80^\circ\text{C}$ , ground in a palvniizer, and digested with 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  $350^\circ\text{C}$  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

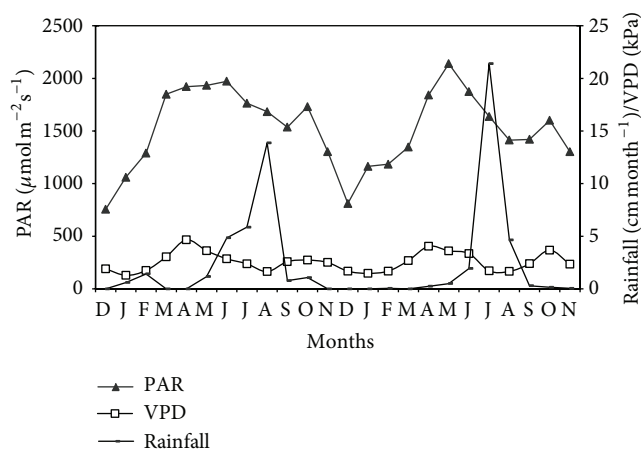


FIGURE 1: Monthly changes in environmental variables. (a) Rainfall, (b) vapour pressure deficit (VPD), and (c) photosynthetically active radiations (PAR).

at 13:00 hr and decreased in the evening (17:00 hr). Vapour pressure deficit (VPD) increased from 1290 Pa in January, 1999, to 4660 Pa in April, 1999. PAR was highest at midday (13:00) and oscillated between  $811 \mu\text{mol m}^{-2} \text{s}^{-1}$  in December, 1999, to  $2140 \mu\text{mol m}^{-2} \text{s}^{-1}$  in May, 2000 (Figure 1).

**3.2. Foliage Nutrient Concentrations.** Seedlings irrigated with municipal effluent at  $T_2$  and  $T_3$  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 above-mentioned 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  $\pm$  SE of three replications in parentheses.

S	T	N	P	K	Ca	Mg	Na	Cu	Fe	Mn	Zn
				g kg <sup>-1</sup> DM				mg kg <sup>-1</sup> DM			
Ec	T <sub>1</sub>	20.67 <sup>fg</sup>	0.96 <sup>fg</sup>	11.64 <sup>f</sup>	11.09 <sup>g</sup>	3.66 <sup>h</sup>	1.39 <sup>g</sup>	26.80 <sup>b</sup>	661.69 <sup>f</sup>	169.00 <sup>b</sup>	30.66 <sup>e</sup>
		(0.36)	(0.05)	(0.64)	(0.78)	(0.05)	(0.02)	(1.29)	(18.50)	(12.15)	(1.32)
	T <sub>2</sub>	24.71 <sup>de</sup>	1.23 <sup>ef</sup>	12.63 <sup>ef</sup>	16.31 <sup>def</sup>	4.03 <sup>h</sup>	1.62 <sup>def</sup>	28.40 <sup>b</sup>	825.30 <sup>c</sup>	404.00 <sup>a</sup>	32.53 <sup>c</sup>
		(0.79)	(0.09)	(0.39)	(0.51)	(0.08)	(0.06)	(0.42)	(17.03)	(25.75)	(1.13)
	T <sub>3</sub>	31.72 <sup>b</sup>	1.56 <sup>cd</sup>	15.06 <sup>de</sup>	19.95 <sup>bc</sup>	5.34 <sup>c</sup>	2.06 <sup>b</sup>	28.60 <sup>b</sup>	959.30 <sup>cd</sup>	458.00 <sup>a</sup>	43.50 <sup>d</sup>
		(0.63)	(0.06)	(0.65)	(0.64)	(0.43)	(0.05)	(2.60)	(27.22)	(19.20)	(2.06)
	T <sub>4</sub>	18.92 <sup>g</sup>	0.45 <sup>h</sup>	13.02 <sup>ef</sup>	17.73 <sup>cde</sup>	4.16 <sup>gh</sup>	1.93 <sup>bc</sup>	23.40 <sup>ab</sup>	570.20 <sup>g</sup>	134.70 <sup>b</sup>	26.87 <sup>e</sup>
		(1.05)	(0.03)	(0.65)	(0.63)	(0.16)	(0.04)	(2.01)	(18.57)	(10.89)	(0.72)
An	T <sub>1</sub>	22.67 <sup>ef</sup>	0.49 <sup>h</sup>	18.41 <sup>c</sup>	17.56 <sup>bc</sup>	4.87 <sup>de</sup>	1.42 <sup>fg</sup>	42.02 <sup>b</sup>	699.00 <sup>f</sup>	64.01 <sup>cd</sup>	45.02 <sup>d</sup>
		(0.56)	(0.07)	(0.43)	(2.10)	(0.12)	(0.04)	(2.58)	(20.05)	(6.87)	(3.20)
	T <sub>2</sub>	25.20 <sup>cd</sup>	0.78 <sup>gh</sup>	21.22 <sup>bc</sup>	19.87 <sup>bc</sup>	5.97 <sup>b</sup>	1.78 <sup>cd</sup>	53.01 <sup>ab</sup>	898.01 <sup>de</sup>	78.02 <sup>cd</sup>	60.33 <sup>b</sup>
		(1.23)	(0.02)	(0.55)	(0.35)	(0.17)	(0.08)	(2.28)	(26.86)	(3.80)	(1.60)
	T <sub>3</sub>	36.72 <sup>a</sup>	1.01 <sup>fg</sup>	23.41 <sup>ab</sup>	22.00 <sup>ab</sup>	8.87 <sup>a</sup>	2.90 <sup>a</sup>	76.80 <sup>a</sup>	1477.01 <sup>a</sup>	117.02 <sup>bc</sup>	78.40 <sup>a</sup>
		(1.27)	(0.03)	(2.11)	(0.47)	(0.32)	(0.08)	(2.22)	(21.21)	(5.79)	(2.18)
	T <sub>4</sub>	20.78 <sup>fg</sup>	0.45 <sup>h</sup>	12.34 <sup>ef</sup>	14.67 <sup>ef</sup>	4.67 <sup>ef</sup>	1.68 <sup>de</sup>	38.40 <sup>b</sup>	684.02 <sup>f</sup>	52.01 <sup>d</sup>	39.62 <sup>d</sup>
		(1.13)	(0.03)	(1.52)	(0.37)	(0.07)	(0.13)	(1.30)	(17.34)	(2.26)	(0.52)
Ds	T <sub>1</sub>	27.87 <sup>c</sup>	3.70 <sup>a</sup>	12.71 <sup>ef</sup>	13.78 <sup>fg</sup>	2.29 <sup>i</sup>	1.33 <sup>g</sup>	34.80 <sup>b</sup>	575.92 <sup>g</sup>	66.00 <sup>cd</sup>	39.45 <sup>d</sup>
		(0.45)	(0.04)	(1.04)	(0.59)	(0.04)	(0.05)	(1.18)	(15.81)	(3.66)	(2.38)
	T <sub>2</sub>	31.67 <sup>b</sup>	4.10 <sup>a</sup>	13.78 <sup>ef</sup>	15.78 <sup>def</sup>	4.70 <sup>h</sup>	1.53 <sup>efg</sup>	43.70 <sup>b</sup>	705.34 <sup>f</sup>	77.80 <sup>cd</sup>	51.30 <sup>c</sup>
		(0.25)	(0.11)	(0.36)	(0.56)	(0.07)	(0.07)	(2.63)	(25.31)	(2.45)	(1.04)
	T <sub>3</sub>	37.68 <sup>a</sup>	5.70 <sup>a</sup>	20.14 <sup>c</sup>	18.78 <sup>bcd</sup>	5.32 <sup>c</sup>	2.01 <sup>b</sup>	51.60 <sup>ab</sup>	1023.50 <sup>b</sup>	138.59 <sup>b</sup>	63.45 <sup>b</sup>
		(0.66)	(0.40)	(0.59)	(0.37)	(0.07)	(0.05)	(2.25)	(62.86)	(2.05)	(2.39)
	T <sub>4</sub>	19.23 <sup>g</sup>	2.80 <sup>b</sup>	13.78 <sup>ef</sup>	10.71 <sup>g</sup>	1.79 <sup>i</sup>	1.38 <sup>g</sup>	24.50 <sup>b</sup>	515.40 <sup>g</sup>	60.30 <sup>cd</sup>	29.70 <sup>e</sup>
		(0.47)	(0.05)	(0.29)	(0.52)	(0.11)	(0.08)	(0.61)	(15.67)	(1.48)	(1.63)
Tow way ANOVA*											
F	S	39.64	966.5	34.99	20.57	484.6	25.73	9.39	67.37	345.3	119.96
	T	203.3	136.1	26.44	19.13	255.5	90.31	2.93 NS	221.8	112.1	123.68
	S × T	7.10	9.09	7.54	7.40	32.72	8.80	1.82 NS	6.56	3.50	5.19

\* ANOVA results are significant at  $P < 0.01$  except for Cu, which was significant at  $P < 0.05$  for species but not significant (NS) at  $P > 0.05$  for treatment and species (S)  $\times$  treatment (T) interaction.

The same letter in the same column means no significant difference ( $P > 0.05$ ) between treatments (all three species).

and 2.2-fold in *A. nilotica* and 1.9- and 2.0-fold in *D. sissoo*. 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 *D. sissoo*. The increase in Cu, Fe, Mn and Zn was 1.2- to 3.4-fold, 2.0- to 2.3-fold and 2.0- to 2.3-fold in the respective species.

Differences in relative uptake of nutrients affected the treatments order (T<sub>1</sub> to T<sub>4</sub>) with increasing nutrients concentration ratios (Figure 2). The ratios of K : N, Mg : Na, Zn : Mn, and Mg : Mn differed ( $P < 0.01$ ) due to both tree species and treatments. But significant variation ( $P < 0.05$ ) in Fe : Mn ratio was observed only between the species. Species  $\times$  treatment interactions were also significant ( $P < 0.01$ ). Among the treatments, the highest K : N, K : Mg and Ca, and Fe : Mn ratios were in T<sub>4</sub>, and Mg : Na, Zn : Mn and Mg : Mn were in T<sub>2</sub> treatments (Figure 2). The lowest ratios of K : N, Fe : Mn, and Zn : Mn were in the seedlings of T<sub>3</sub>, Mg : Na in T<sub>4</sub>, K : Ca, and Mg in T<sub>2</sub>, and Zn : Mn in T<sub>3</sub> treatments.

Among the species, ratios of K : N, K : Ca and Mg, Mg : Na, Fe : Mn, Mg : 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 ( $\Psi_l$ ) was the highest ( $P < 0.01$ ) in the seedlings of T<sub>3</sub> across species (Table 2). The highest  $\Psi_l$  was in *E. camaldulensis*, but it did not differ with *D. sissoo* for  $\Psi_l$  ( $P > 0.05$ , DMRT) across the treatments. The lowest  $\Psi_l$  was in *A. nilotica*. *Dalbergia sissoo* indicated the highest  $\Psi_l$  in T<sub>2</sub>, whereas *E. camaldulensis* showed greater  $\Psi_l$  in T<sub>3</sub> and T<sub>4</sub> treatments (DMRT). The  $\Psi_l$  was the highest ( $P < 0.01$ ) in January (December in *E. camaldulensis*) that decreased gradually to the lowest value in May and then rose in July-August (Table 3). The lowest  $\Psi_l$  was recorded for *A. nilotica* seedlings in most of the months.

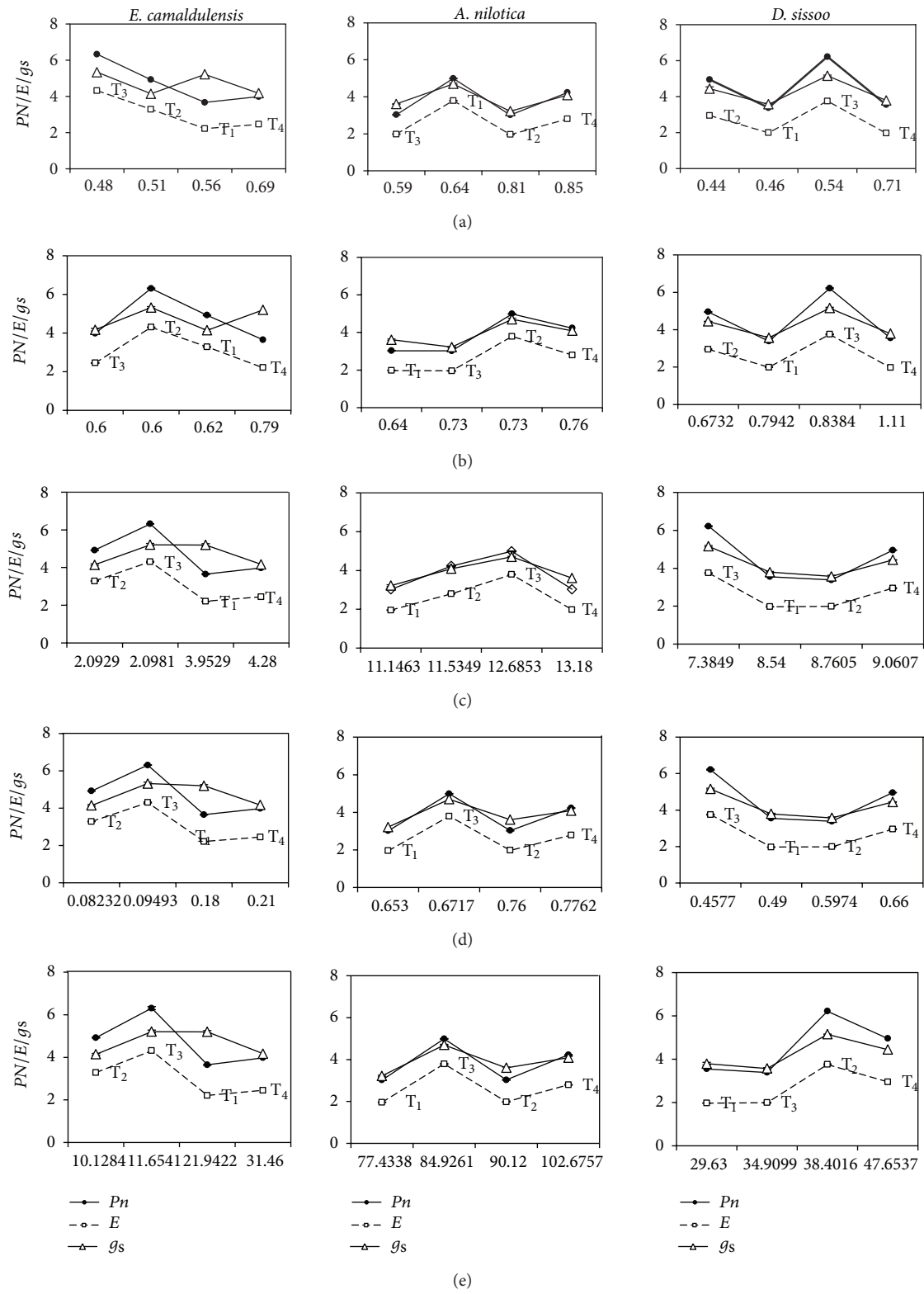


FIGURE 2: Ratios of mineral elements concentrations and their relationship with  $P_N$ ,  $E$ , and  $g_s$  ( $\times 10^{-2}$ ) influenced by tree species and level of municipal effluent application. Error bars are  $\pm$ SE of three replicates.  $T_1$ ,  $T_2$ , and  $T_3$  are municipal effluent irrigation 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	Physiological variables				
	LWP	$P_N$	$E$	$g_s$	WUE
Average values across municipal effluent treatments for species					
<i>E. camaldulensis</i>	-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>
<i>A. nilotica</i>	-2.20 <sup>b</sup>	3.82 <sup>c</sup>	2.63 <sup>c</sup>	39.04 <sup>b</sup>	1.44 <sup>c</sup>
<i>D. sissoo</i>	-1.99 <sup>a</sup>	4.53 <sup>b</sup>	2.67 <sup>b</sup>	42.41 <sup>a</sup>	1.66 <sup>a</sup>
Average values across tree species for municipal effluent treatment					
T <sub>1</sub>	-2.28 <sup>d</sup>	3.35 <sup>d</sup>	2.05 <sup>d</sup>	34.45 <sup>d</sup>	1.57 <sup>b</sup>
T <sub>2</sub>	-2.01 <sup>b</sup>	4.70 <sup>b</sup>	3.01 <sup>b</sup>	42.25 <sup>b</sup>	1.55 <sup>b</sup>
T <sub>3</sub>	-1.81 <sup>a</sup>	5.84 <sup>a</sup>	3.95 <sup>a</sup>	50.63 <sup>a</sup>	1.48 <sup>c</sup>
T <sub>4</sub>	-2.15 <sup>c</sup>	3.52 <sup>c</sup>	2.13 <sup>c</sup>	38.56 <sup>c</sup>	1.62 <sup>a</sup>
F with significant level of two-way ANOVA					
Species	147.99**	1683.38**	709.21**	61.38**	336.17**
Treatment	335.54**	7552.44**	7335.46**	483.82**	61.14**
Species × treatment	19.81**	86.79**	22.29**	9.16**	25.38**

LWP: leaf water potential (MPa, Mega Pascal);  $P_N$ : rate of photosynthesis  $P_N$ : ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ );  $E$ : rate of transpiration ( $\text{mmol m}^{-2} \text{ s}^{-1}$ );  $g_s$ : stomatal conductance ( $\times 10^{-3} \text{ mol m}^{-2} \text{ s}^{-1}$ ), and WUE: instantaneous water use efficiency ( $P_N/E$ ). T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub>, and T<sub>4</sub> are irrigation of seedlings with municipal effluent at 1/2 PET, 1 PET, 2 PET, and canal water at 1 PET, respectively. \*\*Significant at  $P < 0.01$ . The same letter in the same column means no significant difference ( $P > 0.05$ ) between treatments/species.

The  $\Psi_l$  was the highest for *D. sissoo* from April to August and that of *A. nilotica* from September to October.

**3.4. Stomatal Conductance.** Stomatal conductance ( $g_s$ ) across the species increased ( $P < 0.01$ ) in order  $T_1 < T_4 < T_2 < T_3$  (Table 2). Considering species,  $g_s$  was highest ( $P < 0.01$ ) in *E. camaldulensis* and lowest in *A. nilotica*. However, DMRT showed non-significant difference in  $g_s$  between *E. camaldulensis* and *D. sissoo*. *D. sissoo* indicated highest ( $P < 0.05$ )  $g_s$  during April to August ( $66.59 \pm 2.44 \times 10^{-3} \text{ mol m}^{-2} \text{ s}^{-1}$ ) 2000 (August 2000 in all treatments) and in *E. amaldulensis* in rest of the observations. From the lowest value in December/January,  $g_s$  increased by 1.8-fold in T<sub>1</sub>, 1.7-fold in T<sub>2</sub> and T<sub>3</sub> and 1.6-fold in the seedlings of T<sub>4</sub> with wide temporal variation (Figure 3, left panels).

**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). Average 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  $7.80 \pm 0.26 \text{ mmol m}^{-2} \text{ s}^{-1}$  in August 2000) of  $E$  during January to April and July to September. *A. nilotica* indicated the highest ( $2.97 \pm 0.04 \text{ mmol m}^{-2} \text{ s}^{-1}$  in December 1999)  $E$  during December to February, whereas *D. sissoo* indicated the highest ( $4.97 \pm 0.10 \text{ mmol m}^{-2} \text{ s}^{-1}$  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.

$E$  ranged from 0.87 to  $3.96 \text{ mmol m}^{-2} \text{ s}^{-1}$  in T<sub>1</sub>, 1.40 to  $5.60 \text{ mmol m}^{-2} \text{ s}^{-1}$  in T<sub>2</sub>, 1.96 to  $7.8 \text{ mmol m}^{-2} \text{ s}^{-1}$  in T<sub>3</sub>, and 1.0 to  $3.99 \text{ mmol m}^{-2} \text{ s}^{-1}$  in T<sub>4</sub> treatment, where the highest values were in *E. camaldulensis* seedlings. Species × treatment interaction was also significant ( $P < 0.05$ ).

**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. camaludensis* and *A. nilotica*, respectively (Table 2). However, tempral variation indicated the highest  $P_N$  in *E. camaldulensis* in most of the observations (maximum of  $13.56 \pm 0.34 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$  in August 2000, mean  $\pm$  1SE), in *D. sissoo* in April, May, June, and July ( $6.35 \pm 0.36 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) and in *A. nilotica* in October 2000 ( $5.2 \pm 0.05 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$  in T<sub>3</sub>). 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,  $P_N/E$  ( $\mu\text{mol CO}_2 \text{ mmol}^{-1} \text{ H}_2\text{O}$ )

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  $\pm$  SE of three replications in parentheses.

S	T	December	January	February	March	April	May	June	July	August	September	October	November
<i>Ec</i>	T <sub>1</sub>	1.65 (0.04)	1.71 (0.03)	1.92 (0.04)	2.09 (0.05)	2.65 (0.05)	3.00 (0.02)	2.76 (0.04)	2.76 (0.05)	2.38 (0.09)	2.48 (0.06)	2.65 (0.06)	2.10 (0.04)
	T <sub>2</sub>	1.38 (0.06)	1.43 (0.02)	1.60 (0.05)	1.93 (0.03)	2.15 (0.04)	2.52 (0.04)	2.11 (0.04)	1.97 (0.05)	2.29 (0.05)	2.35 (0.04)	2.35 (0.03)	1.48 (0.03)
	T <sub>3</sub>	1.00 (0.04)	1.14 (0.05)	1.43 (0.02)	1.72 (0.02)	1.81 (0.04)	2.20 (0.05)	2.05 (0.08)	1.65 (0.04)	1.50 (0.05)	1.84 (0.04)	2.07 (0.06)	1.33 (0.02)
	T <sub>4</sub>	1.57 (0.05)	1.63 (0.03)	1.83 (0.04)	2.01 (0.04)	2.04 (0.05)	2.81 (0.04)	2.19 (0.07)	2.19 (0.06)	2.32 (0.07)	2.42 (0.04)	2.47 (0.06)	1.62 (0.03)
	T <sub>1</sub>	1.95 (0.06)	1.92 (0.03)	2.24 (0.04)	2.75 (0.14)	2.80 (0.04)	3.19 (0.06)	2.87 (0.06)	2.47 (0.05)	2.10 (0.06)	2.14 (0.06)	2.01 (0.06)	2.01 (0.07)
	T <sub>2</sub>	1.85 (0.02)	1.81 (0.02)	1.98 (0.03)	2.23 (0.04)	2.37 (0.05)	2.82 (0.10)	2.51 (0.03)	2.01 (0.05)	1.93 (0.04)	1.98 (0.06)	1.90 (0.04)	1.87 (0.04)
	T <sub>3</sub>	1.80 (0.03)	1.73 (0.02)	1.89 (0.05)	2.03 (0.09)	2.15 (0.02)	2.63 (0.08)	2.30 (0.04)	1.93 (0.07)	1.82 (0.03)	1.92 (0.04)	1.89 (0.04)	1.82 (0.04)
	T <sub>4</sub>	1.93 (0.02)	1.91 (0.02)	2.13 (0.04)	2.55 (0.05)	2.61 (0.03)	2.93 (90.1)	2.83 (0.02)	2.32 (0.02)	2.02 (0.07)	2.10 (0.05)	1.99 (0.03)	1.96 (0.05)
<i>Ds</i>	T <sub>1</sub>	1.96 (0.02)	1.87 (0.05)	1.95 (0.04)	2.13 (0.03)	2.23 (0.04)	2.39 (0.04)	2.31 (0.02)	2.09 (0.03)	2.01 (0.03)	2.29 (0.02)	2.28 (0.02)	2.09 (0.03)
	T <sub>2</sub>	1.79 (0.03)	1.57 (0.05)	1.73 (0.03)	1.89 (0.06)	2.07 (0.05)	2.27 (0.03)	2.18 (0.01)	1.92 (0.02)	1.90 (0.02)	2.20 (0.02)	2.07 (0.03)	1.87 (0.02)
	T <sub>3</sub>	1.63 (0.05)	1.40 (0.04)	1.62 (0.02)	1.77 (0.06)	1.89 (0.04)	2.22 (0.03)	2.01 (0.06)	1.85 (0.08)	1.73 (0.02)	1.89 (0.02)	1.82 (0.04)	1.71 (0.04)
	T <sub>4</sub>	1.91 (0.04)	1.82 (0.04)	1.84 (0.04)	2.07 (0.06)	2.21 (0.03)	2.26 (0.03)	2.22 (0.03)	1.97 (0.01)	1.98 (0.02)	2.19 (0.03)	2.17 (0.04)	2.01 (0.02)

## Repeated 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 $\times$ S	22	0.372	64.69	<0.001	T	3	4.417	335.54	<0.001
M $\times$ T	33	0.031	5.32	<0.001	S $\times$ T	6	0.261	19.81	<0.001
M $\times$ S $\times$ 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  $\times$  treatment interaction. M, month; S, species and T, treatments.

between the species, treatments, and months. WUE was the highest ( $P < 0.01$ ) in T<sub>4</sub> and the lowest in T<sub>3</sub> seedlings across the species. However, WUE did not differ significantly between the seedlings of T<sub>1</sub> and T<sub>2</sub> treatments (DMRT). Among the tree species, the highest ( $P < 0.05$ ) WUE was in the seedlings of *D. sissoo* and the lowest in *A. nilotica* (Figure 4(b)). When compared with the lowest WUE (observed either in winter or in summer), a 2.1- to 2.6-fold greater WUE was observed in the seedlings of T<sub>1</sub>, whereas the increase was 1.9- to 2.4-fold in T<sub>2</sub>, 1.7- to 2.6-fold in T<sub>3</sub>, and 1.9- to 2.2-fold in T<sub>4</sub> treatments. Relative increase in WUE was highest in *D. sissoo* (Figure 4(a)).

**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  $\Psi_l$ . Total quantity of applied effluent showed positive relationship with average  $\Psi_l$ ,  $P_N$ ,  $E$ , and  $g_s$  ( $r = 0.429$  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  $P_N$ . 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  $P_N$  ( $r = -0.412$ ,  $P < 0.05$ ) 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.

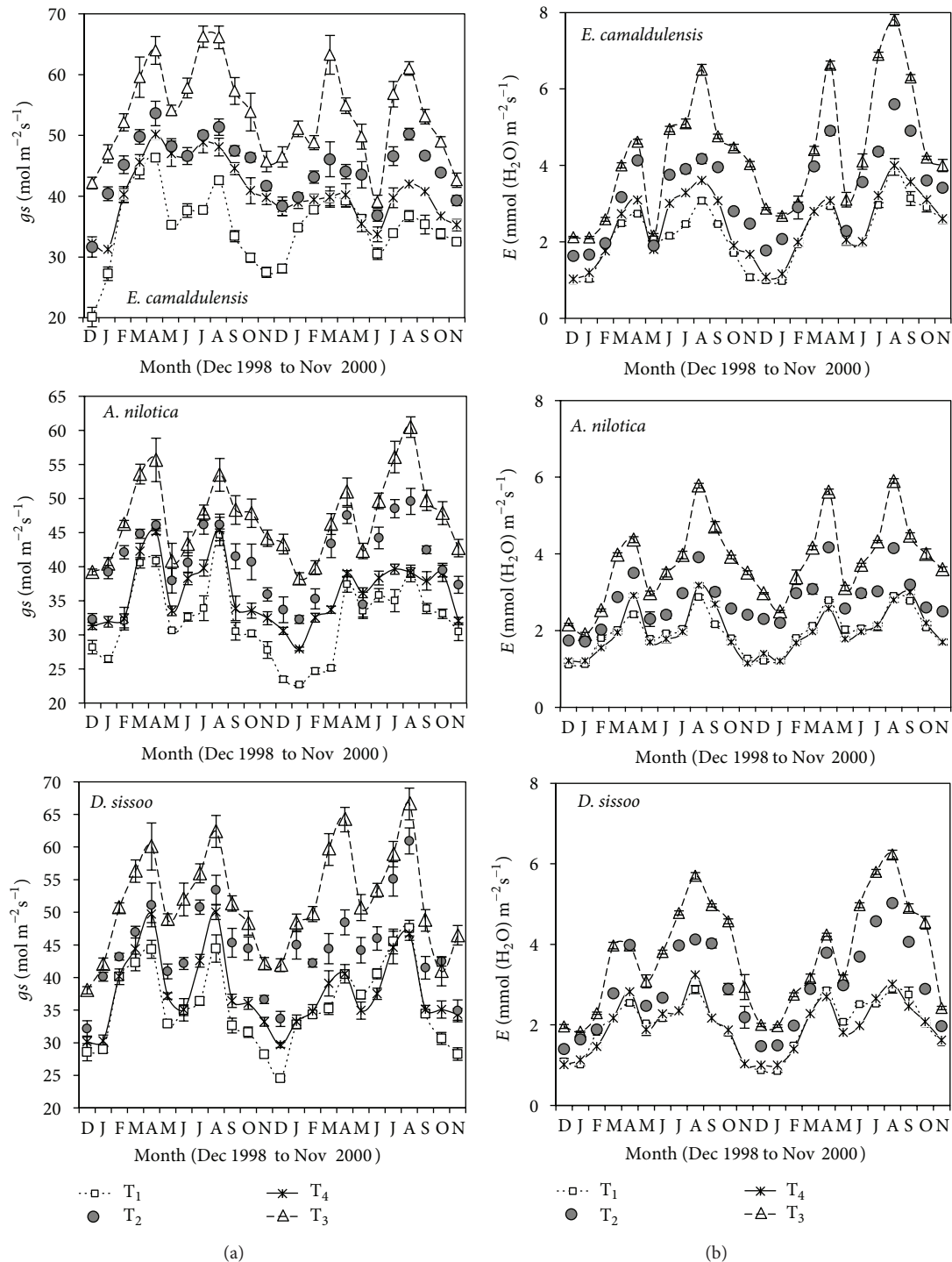


FIGURE 3: Monthly changes in stomatal conductance ( $g_s \times 10^{-3}$ , (a)) and transpiration rate ( $E$ , (b)) of tree seedlings irrigated with canal water and varying levels of municipal effluent during 1998-99 and 1999-00. Error bars are  $\pm$ SE.  $T_1$ ,  $T_2$ ,  $T_3$ , and  $T_4$  are irrigation of seedlings with municipal effluent at 1/2 PET, 1 PET, 2 PET, and canal water at 1 PET, respectively.

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



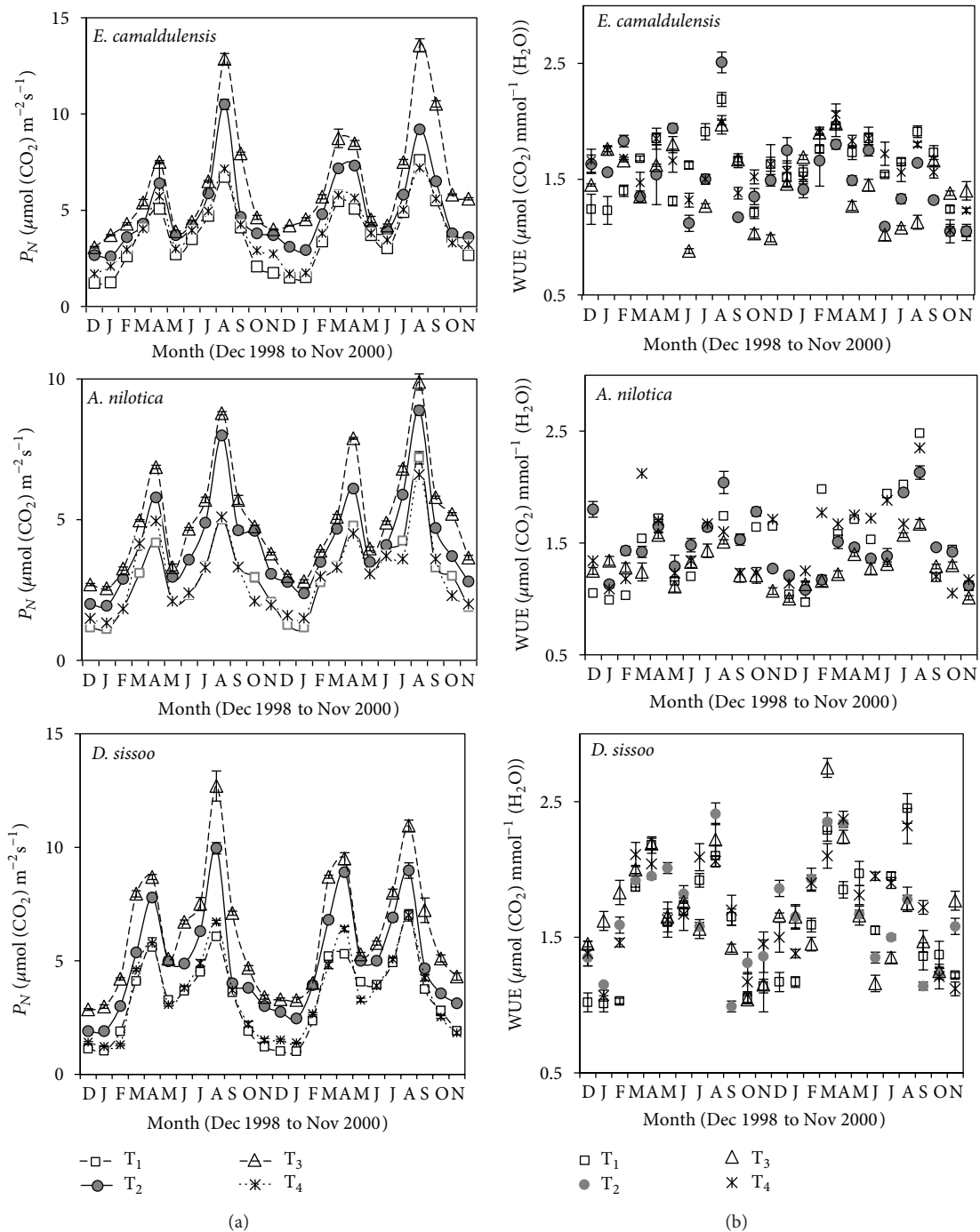


FIGURE 4: Monthly changes in net photosynthetic rate ( $P_N$ , (a)) and instantaneous water use efficiency (WUE, (b)) irrigated with canalwater and varying levels of municipal effluents during 1998-99 and 1999-00. Error bars are  $\pm$ SE.  $T_1$ ,  $T_2$ ,  $T_3$ , and  $T_4$  are irrigation of seedlings 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  $22.26 \text{ g kg}^{-1}$ ,  $2.76 \text{ g kg}^{-1}$ , and  $1146 \text{ mg kg}^{-1}$  reduced  $P_N$  (Table 4, Figure 5). Likewise greater than  $1.65 \text{ g kg}^{-1}$ ,  $21.09 \text{ g kg}^{-1}$ , and  $2.76 \text{ g kg}^{-1}$  concentrations of P, Ca, and Na respectively, reduced  $E$ .

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 ( $P_N$ )									
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	1.25817	0.33138	—	—	0.1712	0.2306	5.16	0.031
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	-1.17157	—	—	0.3397	0.2325	12.86	0.001
Na	Quadratic	-5.60495	7.83469	-1.57456	—	0.7883	0.3901	44.80	0.000
Fe	Sigmoid	1.96642	-729.363	—	—	0.6725	0.1637	51.33	0.000
Cu	Cubic	13.22883	-0.68615	0.01381	-0.000084	0.2865	0.7323	3.08	0.047
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	3.9789	-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
Fe	Compound	1.83373	0.99979	—	—	0.5011	0.0565	25.10	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	0.41192	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	1.30110	2.78703	-6.07910	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  $T_1$  to  $T_3$ . Greater concentrations of the most of the nutrients in the seedlings in  $T_2$  and N, P, Fe, and Zn in  $T_1$  as compared to the seedlings in

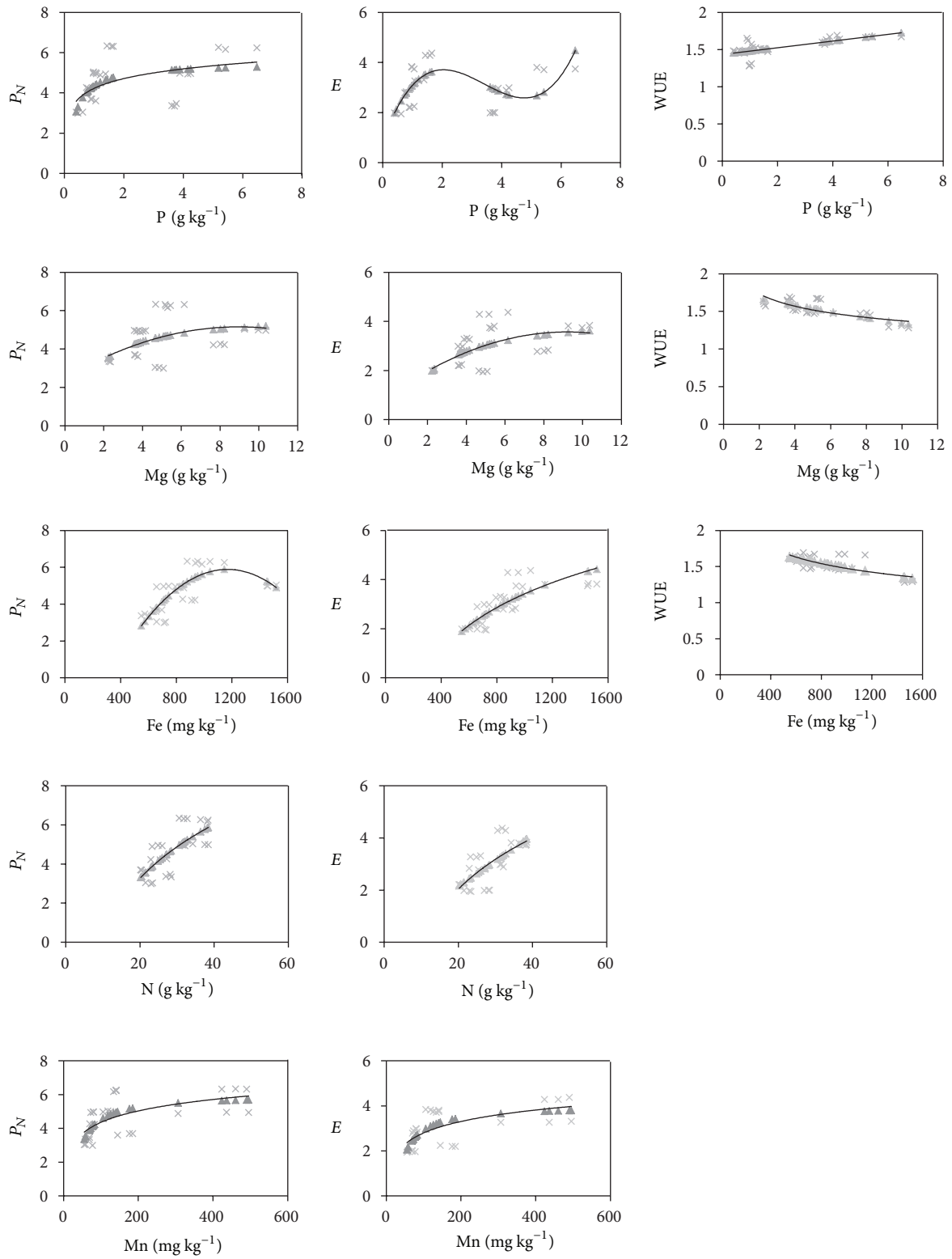


FIGURE 5: Relationship of mineral concentrations with average rate of net photosynthesis ( $P_N$ ,  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ), transpiration ( $E$ ,  $\text{mmol m}^{-2} \text{ s}^{-1}$ ), and instantaneous water use efficiency in tree seedlings irrigated with varying levels of municipal effluent. The observed and estimated trend in values are shown by cross ( $\times$ ) and solid line, respectively.

$T_4$  treatment (despite same quantity of water in  $T_2$  and half quantity of water in  $T_1$  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  $T_4$ ) 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  $\Psi_l$  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  $\Psi_l$  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  $\Psi_l$  in the seedlings from  $T_1$  to  $T_3$  was positively influenced by increased level of effluent application-soil water availability [37]. However, higher ( $P < 0.05$ )  $\Psi_l$  in the seedlings of  $T_4$  as compared to the seedlings of  $T_1$  treatment was due to two-fold higher water applied to  $T_4$ . A difference of 1.14 to 1.35 MPa between the lowest and highest  $\Psi_l$  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  $\Psi_l$  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  $\Psi_l$  from January to May in all the three species indicated negative relations between  $\Psi_l$  and these environmental factors.

**4.2. Foliage Nutrients and Gas Exchange.** Application of municipal effluent had no toxic effect on  $P_N$ ,  $E$ , and  $g_s$  and seedlings grown with municipal effluent irrigation were capable of maintaining efficient photosynthetic activity throughout the growing season. Higher values of these physiological variables in the seedlings grown in  $T_2$  and  $T_3$  treatments than in the control ( $T_4$ ) further suggested that leaf function was unimpaired by the municipal effluent irrigation. Reduced  $P_N$ ,

$E$ , and  $g_s$  together with  $\Psi_l$  in the seedlings of  $T_1$  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  $P_N$  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  $P_N$ ,  $E$ , and  $g_s$  in  $T_4$  than in  $T_1$  seedlings (except in February, 1999, March, May, August, and October, 2000, for  $P_N$ , February and May 1999, and February to June, 2000, for  $E$ , and May 2000 for  $g_s$ ) were due to two-fold water applied [41]. Despite of similar level of irrigation (1PET) increased values of the physiological variables in  $T_2$  as compared to the seedlings of  $T_4$  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  $P_N$  and  $E$  in the seedlings of  $T_3$ . This increase in  $P_N$ ,  $E$  and  $g_s$  was positively related to  $\Psi_l$  and foliage N and other nutrients as observed in *Pseudotsuga menziesii* (Mirb.) Franco. [43]. However, the higher value of  $g_s$  was not paralleled by increased  $P_N$  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  $P_N$  with nutrient concentrations suggests a close link of photosynthetic capacity with nutrient supply, but simultaneous increases in  $E$  and  $g_s$  (Figure 5) are indicative of rapid growth and biomass production [45]. A decline/saturation, after an initial increase in  $P_N$  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 limonia* 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  $T_1$  to  $T_3$  seemed to facilitate  $P_N$  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  $P_N$  compared to 2.2- to 4.0-fold variation in  $E$  among the months further indicated greater sensitivity of  $P_N$  to foliage chemistry as well as environmental factors. Increase in  $P_N$ ,  $E$ , and  $g_s$  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  $P_N$ ,  $E$ , and  $g_s$  in *E. camaldulensis* were the effects of lower  $\Psi_l$  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 ( $P < 0.01$ ) WUE in *D. sissoo* 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. sissoo* 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,  $\Psi_t$ ,  $P_N$ ,  $E$ , 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  $T_2$  as compared to  $T_4$  was the nutrient effects, whereas their increase in  $T_4$  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. sissoo* 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. sissoo* 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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