Hindawi Publishing Corporation
Physiology Journal
Volume 2014, Article ID 545967, 15 pages
http://dx.doi.org/10.1155/2014/545967

Research Article

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

G. Singh,[1] Madhulika Bhati,[1] T. R. Rathod,[1] and U. K. Tomar[2]

[1] *Division of Forest Ecology, Arid Forest Research Institute, New Pali Road, Jodhpur 342005, India*
[2] *Division of Forest Genetics and Tree Breeding, Arid Forest Research Institute, New Pali Road, Jodhpur 342005, India*

Correspondence should be addressed to G. Singh; gsingh@icfre.org

Received 5 February 2014; Revised 25 June 2014; Accepted 2 July 2014; Published 24 July 2014

Academic Editor: Deyue Yu

Leaf water potential (Ψ_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 Ψ_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 m^3 (i.e., size of 2 m × 2 m × 2 m) at the experimental field of Arid Forest Research Institute, Jodhpur (26°45′N latitude and 72°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 Camborthides, 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-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 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 × 2 × 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 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 CO_2 Gas

Analyzer, 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 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 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°C, ground in a palvnizer, and digested with triacid mixture ($HNO_3 : H_2SO_4 : HClO_4$ 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 H_2SO_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 μmol m^{-2} s^{-1} in December, 1999, to 2140 μmol m^{-2} s^{-1} in May, 2000 (Figure 1).

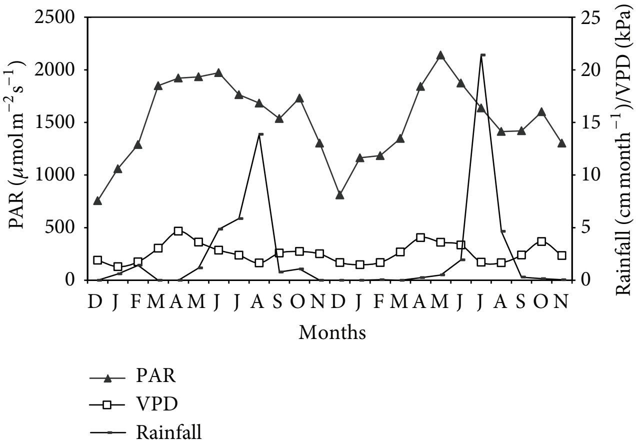


Figure 1: Monthly changes in environmental variables. (a) Rainfall, (b) vapour pressure deficit (VPD), and (c) photosynthetically active radiations (PAR).

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 ± SE of three replications in parentheses.

S	T	N	P	K	Ca	Mg	Na	Cu	Fe	Mn	Zn
		$\mathrm{g\,kg^{-1}\,DM}$						$\mathrm{mg\,kg^{-1}\,DM}$			
Ec	T_1	20.67^{fg}	0.96^{fg}	11.64^{f}	11.09^{g}	3.66^{h}	1.39^{g}	26.80^{b}	661.69^{f}	169.00^{b}	30.66^{e}
		(0.36)	(0.05)	(0.64)	(0.78)	(0.05)	(0.02)	(1.29)	(18.50)	(12.15)	(1.32)
	T_2	24.71^{de}	1.23^{ef}	12.63^{ef}	16.31^{def}	4.03^{h}	1.62^{def}	28.40^{b}	825.30^{e}	404.00^{a}	32.53^{e}
		(0.79)	(0.09)	(0.39)	(0.51)	(0.08)	(0.06)	(0.42)	(17.03)	(25.75)	(1.13)
	T_3	31.72^{b}	1.56^{cd}	15.06^{de}	19.95^{bc}	5.34^{c}	2.06^{b}	28.60^{b}	959.30^{cd}	458.00^{a}	43.50^{d}
		(0.63)	(0.06)	(0.65)	(0.64)	(0.43)	(0.05)	(2.60)	(27.22)	(19.20)	(2.06)
	T_4	18.92^{g}	0.45^{h}	13.02^{ef}	17.73^{cde}	4.16^{gh}	1.93^{bc}	23.40^{ab}	570.20^{g}	134.70^{b}	26.87^{e}
		(1.05)	(0.03)	(0.65)	(0.63)	(0.16)	(0.04)	(2.01)	(18.57)	(10.89)	(0.72)
An	T_1	22.67^{ef}	0.49^{h}	18.41^{c}	17.56^{bc}	4.87^{de}	1.42^{fg}	42.02^{b}	699.00^{f}	64.01^{cd}	45.02^{d}
		(0.56)	(0.07)	(0.43)	(2.10)	(0.12)	(0.04)	(2.58)	(20.05)	(6.87)	(3.20)
	T_2	25.20^{cd}	0.78^{gh}	21.22^{bc}	19.87^{bc}	5.97^{b}	1.78^{cd}	53.01^{ab}	898.01^{de}	78.02^{cd}	60.33^{b}
		(1.23)	(0.02)	(0.55)	(0.35)	(0.17)	(0.08)	(2.28)	(26.86)	(3.80)	(1.60)
	T_3	36.72^{a}	1.01^{fg}	23.41^{ab}	22.00^{ab}	8.87^{a}	2.90^{a}	76.80^{a}	1477.01^{a}	117.02^{bc}	78.40^{a}
		(1.27)	(0.03)	(2.11)	(0.47)	(0.32)	(0.08)	(2.22)	(21.21)	(5.79)	(2.18)
	T_4	20.78^{fg}	0.45^{h}	12.34^{ef}	14.67^{ef}	4.67^{ef}	1.68^{de}	38.40^{b}	684.02^{f}	52.01^{d}	39.62^{d}
		(1.13)	(0.03)	(1.52)	(0.37)	(0.07)	(0.13)	(1.30)	(17.34)	(2.26)	(0.52)
Ds	T_1	27.87^{c}	3.70^{a}	12.71^{ef}	13.78^{fg}	2.29^{i}	1.33^{g}	34.80^{b}	575.92^{g}	66.00^{cd}	39.45^{d}
		(0.45)	(0.04)	(1.04)	(0.59)	(0.04)	(0.05)	(1.18)	(15.81)	(3.66)	(2.38)
	T_2	31.67^{b}	4.10^{a}	13.78^{ef}	15.78^{def}	4.70^{h}	1.53^{efg}	43.70^{b}	705.34^{f}	77.80^{cd}	51.30^{c}
		(0.25)	(0.11)	(0.36)	(0.56)	(0.07)	(0.07)	(2.63)	(25.31)	(2.45)	(1.04)
	T_3	37.68^{a}	5.70^{a}	20.14^{c}	18.78^{bcd}	5.32^{c}	2.01^{b}	51.60^{ab}	1023.50^{b}	138.59^{b}	63.45^{b}
		(0.66)	(0.40)	(0.59)	(0.37)	(0.07)	(0.05)	(2.25)	(62.86)	(2.05)	(2.39)
	T_4	19.23^{g}	2.80^{b}	13.78^{ef}	10.71^{g}	1.79^{i}	1.38^{g}	24.50^{b}	515.40^{g}	60.30^{cd}	29.70^{e}
		(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

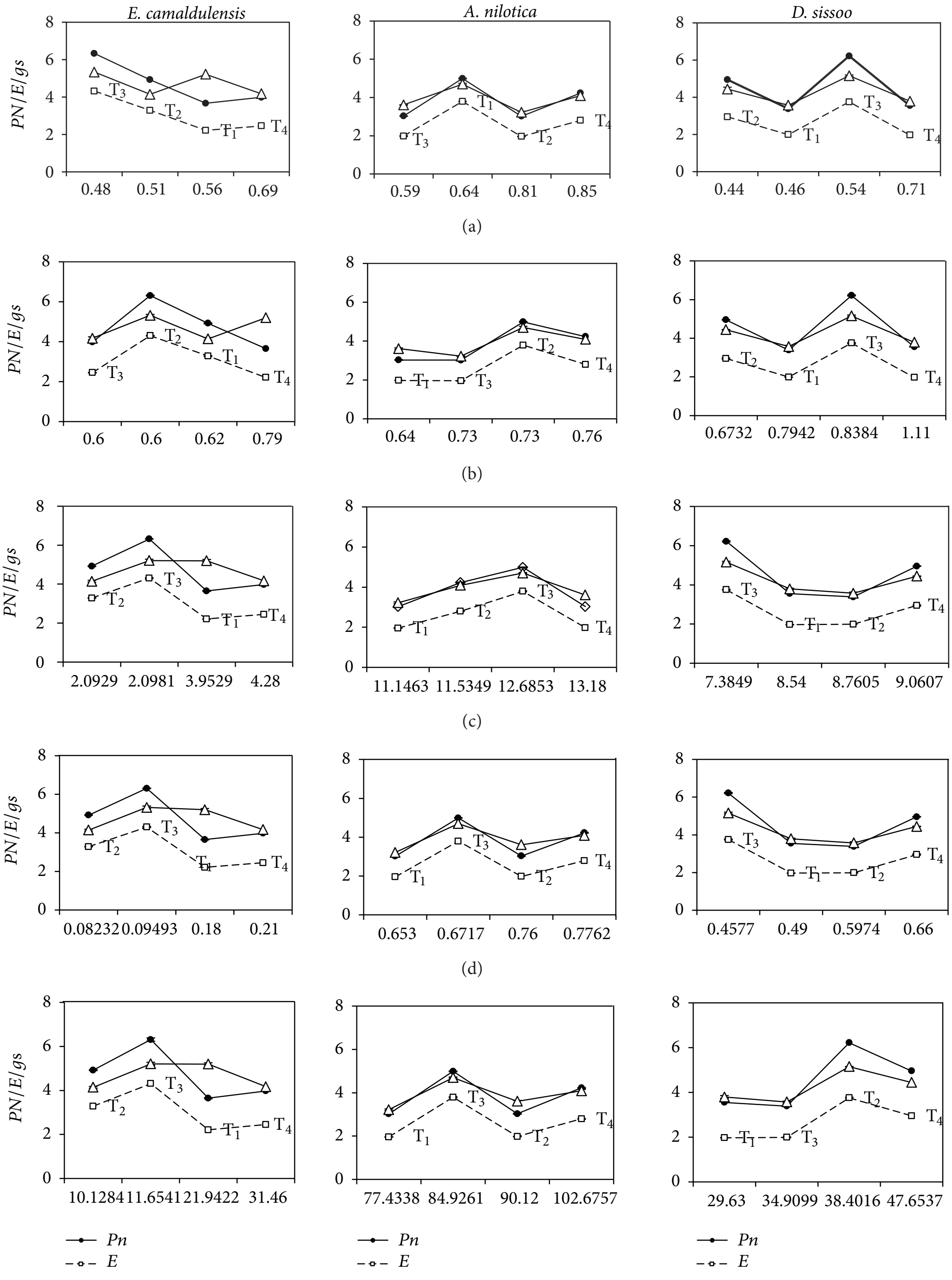
*ANOVA results are significant at $P < 0.01$ except for Cu, which was significant at $P < 0.05$ for species but not significant (NS) at $P > 0.05$ for treatment and species (S) × treatment (T) interaction.

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_1 to T_4) 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 × 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_4, and Mg : Na, Zn : Mn and Mg : Mn were in T_2 treatments (Figure 2). The lowest ratios of K : N, Fe: Mn, and Zn: Mn were in the seedlings of T_3, Mg : Na in T_4, K : Ca, and Mg in T_2, and Zn : Mn in T_3 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 (Ψ_l) was the highest ($P < 0.01$) in the seedlings of T_3 across species (Table 2). The highest Ψ_l was in *E. camaldulensis*, but it did not differ with *D. sissoo* for Ψ_l ($P > 0.05$, DMRT) across the treatments. The lowest Ψ_l was in *A. nilotica*. *Dalbergia sissoo* indicated the highest Ψ_l in T_2, whereas *E. camaldulensis* showed greater Ψ_l in T_3 and T_4 treatments (DMRT). The Ψ_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 Ψ_l was recorded for *A. nilotica* seedlings in most of the months.



(a)

(b)

(c)

(d)

(e)

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 ±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	gs	WUE
Average values across municipal effluent treatments for species					
E. camaldulensis	-2.01^{a}	4.72^{a}	3.06^{a}	42.96^{a}	1.56^{b}
A. nilotica	-2.20^{b}	3.82^{c}	2.63^{c}	39.04^{b}	1.44^{c}
D. sissoo	-1.99^{a}	4.53^{b}	2.67^{b}	42.41^{a}	1.66^{a}
Average values across tree species for municipal effluent treatment					
T_1	-2.28^{d}	3.35^{d}	2.05^{d}	34.45^{d}	1.57^{b}
T_2	-2.01^{b}	4.70^{b}	3.01^{b}	42.25^{b}	1.55^{b}
T_3	-1.81^{a}	5.84^{a}	3.95^{a}	50.63^{a}	1.48^{c}
T_4	-2.15^{c}	3.52^{c}	2.13^{c}	38.56^{c}	1.62^{a}
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: (μmol CO_2 m^{-2} s^{-1}); E: rate of transpiration (mmol m^{-2} s^{-1}); gs: stomatal conductance ($\times 10^{-3}$ mol m^{-2} s^{-1}), and WUE: instantaneous water use efficiency (P_N/E). 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. **Significant at $P < 0.01$. The same letter in the same column means no significant difference ($P > 0.05$) between treatments/species.

The Ψ_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 ($66.59 \pm 2.44 \times 10^{-3}$ mol m^{-2} 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_1, 1.7-fold in T_2 and T_3 and 1.6-fold in the seedlings of T_4 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_1. Average E was 4% lesser in the seedlings of T_1, but it increased by 46% and 85% in the seedlings of T_2 and T_3 treatment, respectively, as compared to E value in T_4 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 ± 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. E ranged from 0.87 to 3.96 mmol m^{-2} s^{-1} in T_1, 1.40 to 5.60 mmol m^{-2} s^{-1} in T_2, 1.96 to 7.8 mmol m^{-2} s^{-1} in T_3, and 1.0 to 3.99 mmol m^{-2} s^{-1} in T_4 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_3 treatments indicated the highest P_N in all species. The lowest P_N was in the seedlings of T_1 in most of the months and in T_4 in April, May, August, and September. When compared with the seedlings of T_4, P_N increased by 34% and 66% in the seedlings of T_2 and T_3, respectively, whereas it was 5% less in T_1 treatment. Across the treatments, the highest and lowest values of P_N were in *E. camaludensisis* 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 ± 0.34 μmol CO_2 m^{-2} s^{-1} in August 2000, mean ± 1SE), in *D. sissoo* in April, May, June, and July (6.35 ± 0.36 μmol CO_2 m^{-2} s^{-1}) and in *A. nilotica* in October 2000 (5.2 ± 0.05 μmol CO_2 m^{-2} s^{-1} in T_3). 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_1, 4.0- to 5.2-fold in T_2, 3.9- to 4.5-fold in T_3, and 4.0- to 5.7-fold in the seedlings of T_4 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 (μmol CO_2 $mmol^{-1}$ H_2O)

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
Ec	T_1	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)
	T_2	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)
	T_3	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)	(0.05)	(0.08)	(0.04)	(0.05)	(0.04)	(0.06)	(0.02)
	T_4	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)	(0.04)	(0.07)	(0.06)	(0.07)	(0.04)	(0.06)	(0.03)
An	T_1	1.95	1.92	2.24	2.75	2.80	3.19	2.87	2.47	2.10	2.14	2.01	2.01
		(0.06)	(0.03)	(0.04)	(0.14)	(0.04)	(0.06)	(0.06)	(0.05)	(0.06)	(0.06)	(0.06)	(0.07)
	T_2	1.85	1.81	1.98	2.23	2.37	2.82	2.51	2.01	1.93	1.98	1.90	1.87
		(0.02)	(0.02)	(0.03)	(0.04)	(0.05)	(0.10)	(0.03)	(0.05)	(0.04)	(0.06)	(0.04)	(0.04)
	T_3	1.80	1.73	1.89	2.03	2.15	2.63	2.30	1.93	1.82	1.92	1.89	1.82
		(0.03)	(0.02)	(0.05)	(0.09)	(0.02)	(0.08)	(0.04)	(0.07)	(0.03)	(0.04)	(0.04)	(0.04)
	T_4	1.93	1.91	2.13	2.55	2.61	2.93	2.83	2.32	2.02	2.10	1.99	1.96
		(0.02)	(0.02)	(0.04)	(0.05)	(0.03)	(90.1)	(0.02)	(0.02)	(0.07)	(0.05)	(0.03)	(0.05)
Ds	T_1	1.96	1.87	1.95	2.13	2.23	2.39	2.31	2.09	2.01	2.29	2.28	2.09
		(0.02)	(0.05)	(0.04)	(0.03)	(0.04)	(0.04)	(0.02)	(0.03)	(0.03)	(0.02)	(0.02)	(0.03)
	T_2	1.79	1.57	1.73	1.89	2.07	2.27	2.18	1.92	1.90	2.20	2.07	1.87
		(0.03)	(0.05)	(0.03)	(0.06)	(0.05)	(0.03)	(0.01)	(0.02)	(0.02)	(0.02)	(0.03)	(0.02)
	T_3	1.63	1.40	1.62	1.77	1.89	2.22	2.01	1.85	1.73	1.89	1.82	1.71
		(0.05)	(0.04)	(0.02)	(0.06)	(0.04)	(0.03)	(0.06)	(0.08)	(0.02)	(0.02)	(0.04)	(0.04)
	T_4	1.91	1.82	1.84	2.07	2.21	2.26	2.22	1.97	1.98	2.19	2.17	2.01
		(0.04)	(0.04)	(0.04)	(0.06)	(0.03)	(0.03)	(0.03)	(0.01)	(0.02)	(0.03)	(0.04)	(0.02)

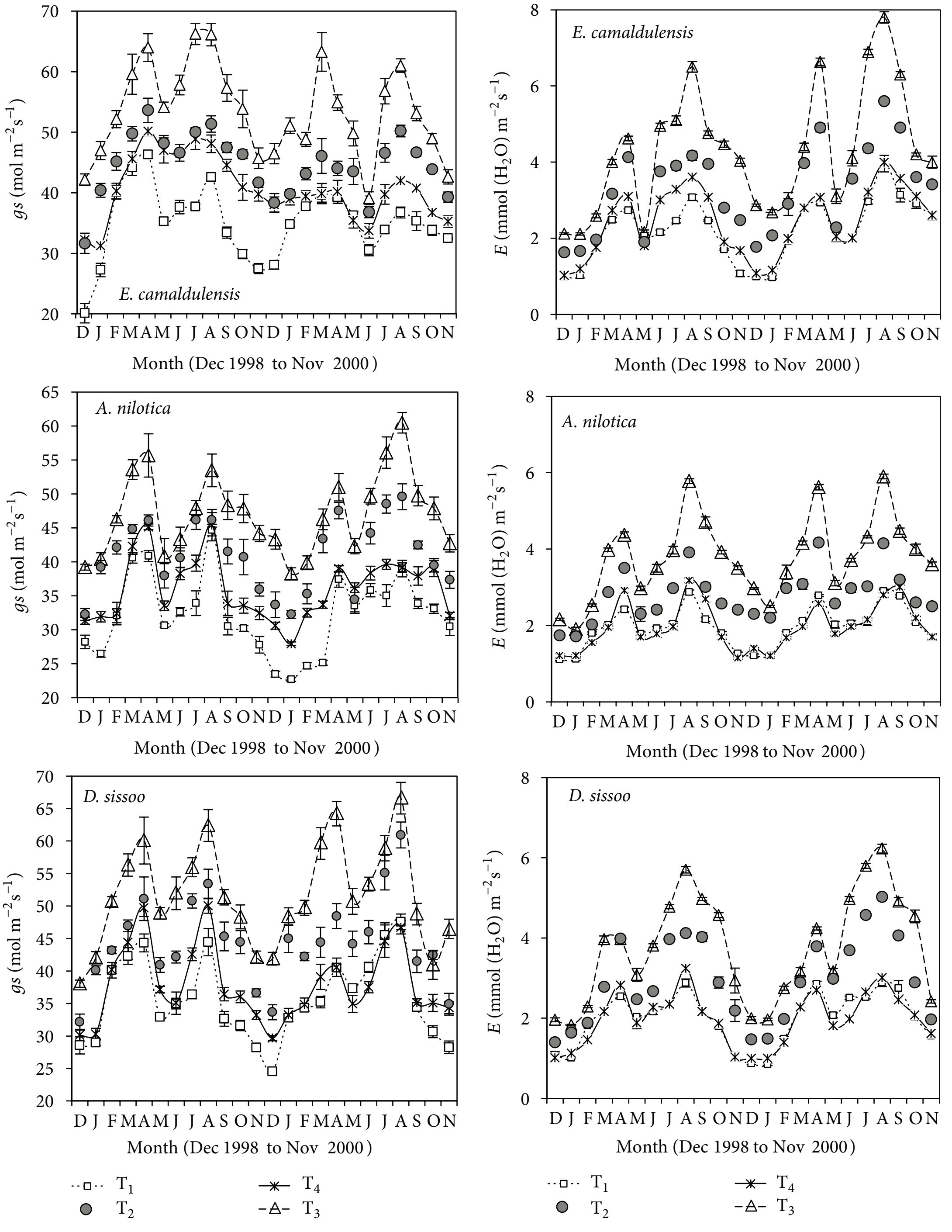
Repeated Measure ANOVA*

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 × 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.

between the species, treatments, and months. WUE was the highest ($P < 0.01$) in T_4 and the lowest in T_3 seedlings across the species. However, WUE did not differ significantly between the seedlings of T_1 and T_2 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_1, whereas the increase was 1.9- to 2.4-fold in T_2, 1.7- to 2.6-fold in T_3, and 1.9- to 2.2-fold in T_4 treatments. Relative increase in WUE was highest in *D. sissoo* (Figure 4(a)).

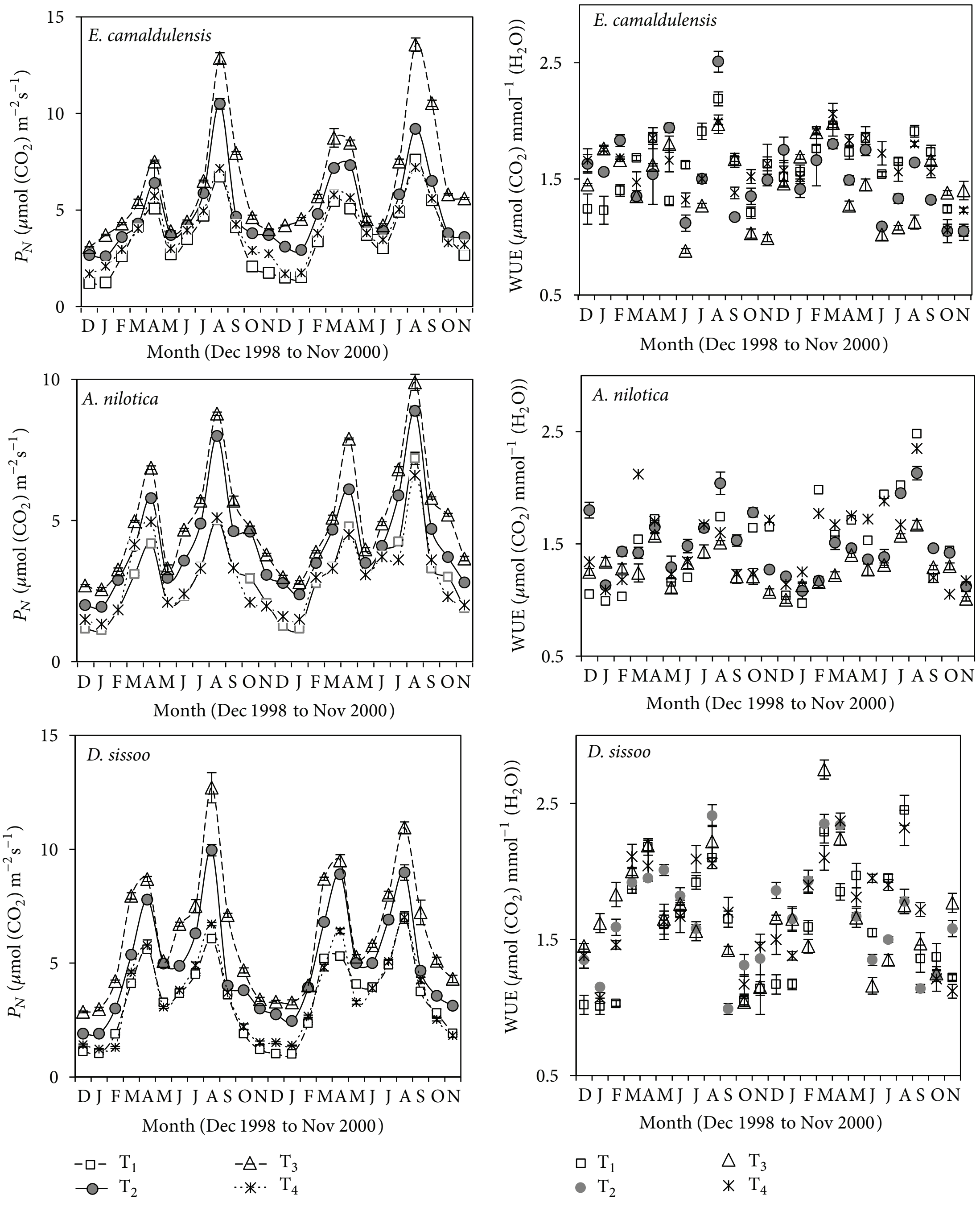
3.8. Correlations and Regressions. Nutrient concentrations in foliage of the seedlings were positively correlated ($r = 0.453$ to 0.841, $P < 0.05$, $n = 27$) to total applied municipal effluent (except for P and Mn). Fe, P, Na, Mn and N showed positive ($r = 0.426$ to 0.716, $P < 0.05$) and K : N ratio showed negative ($r = -0.496$, $P < 0.05$) relationship with Ψ_l. Total quantity of applied effluent showed positive relationship with average Ψ_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.



(a) (b)

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.

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



(a)

(b)

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 ±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.

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 (gs)									
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

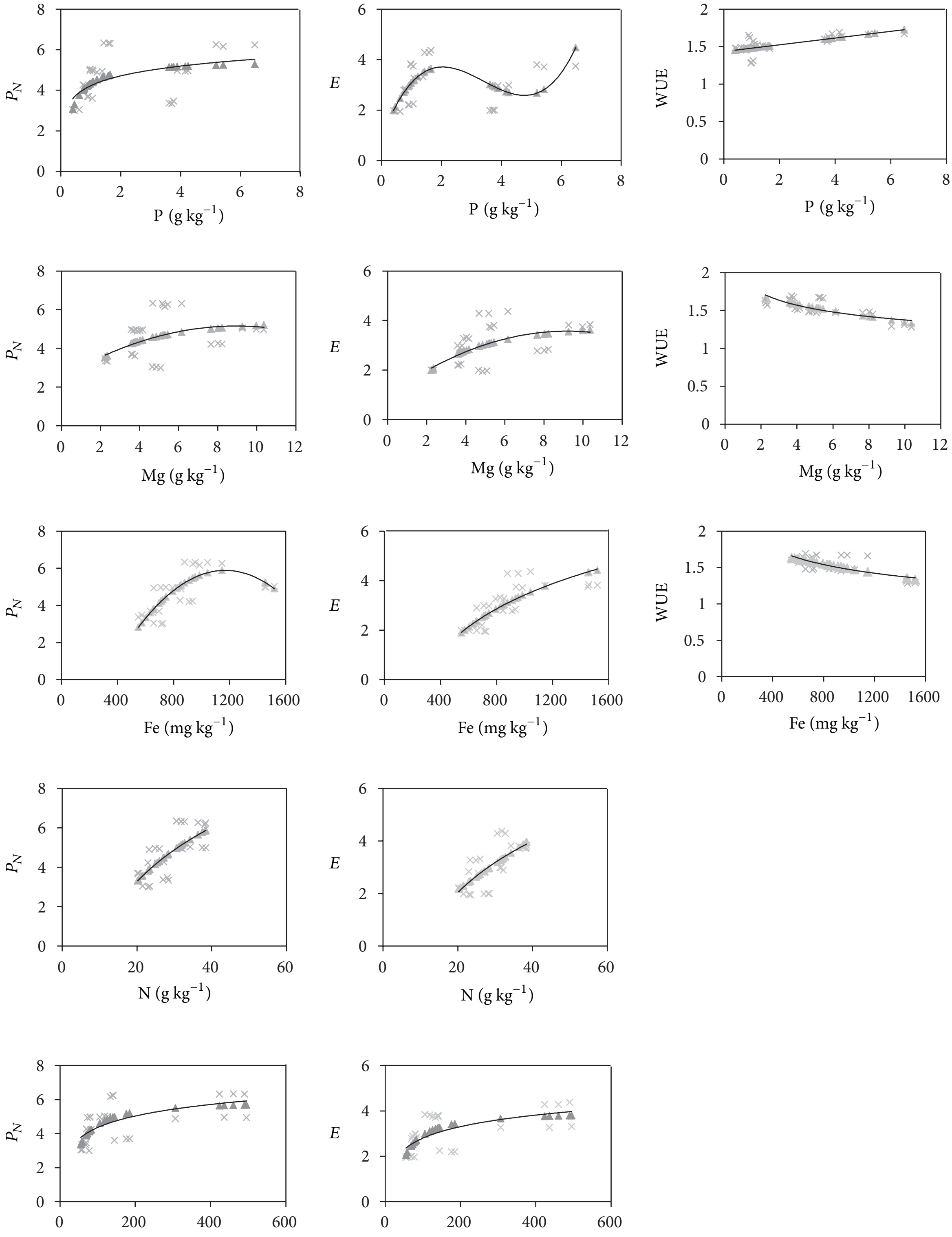


FIGURE 5: Relationship of mineral concentrations with average rate of net photosynthesis (P_N, μmol CO$_2$ m^{-2} s^{-1}), transpiration (E, mmol m^{-2} s^{-1}), and instantaneous water use efficiency in tree seedlings irrigated with varying levels of municipal effluent. The observed and estimated trend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

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 Ψ_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 Ψ_l and physiological functions by increasing solute concentration. Lesser concentrations of these nutrients in *E. camaldulnsis* 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 Ψ_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 ($P < 0.05$) Ψ_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 Ψ_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 Ψ_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 Ψ_l from January to May in all the three species indicated negative relations between Ψ_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 Ψ_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 Ψ_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 gs (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. camaldulesis* were the effects of lower Ψ_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, Ψ_l, 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.

Acknowledgment

The authors are thankful to the Director of Arid Forest Research Institute, Jodhpur, for providing necessary facilities.

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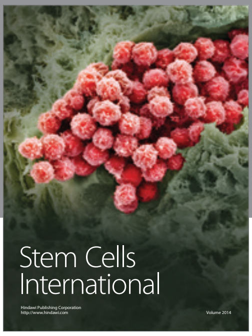
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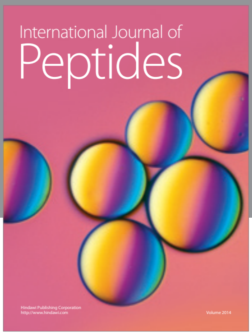
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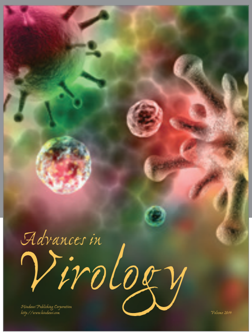
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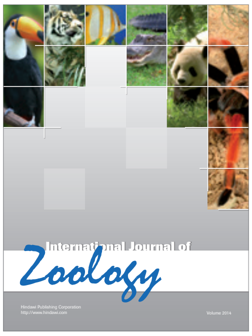
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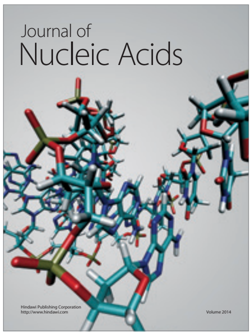
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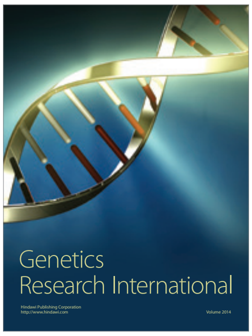
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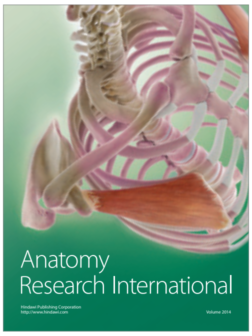
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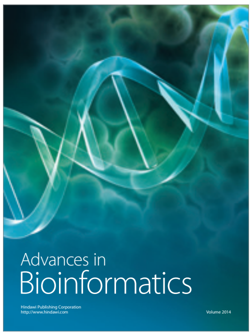
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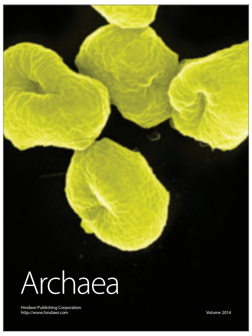
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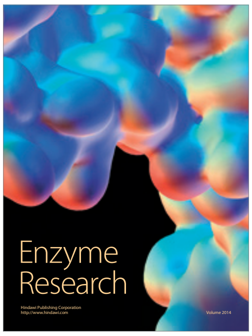
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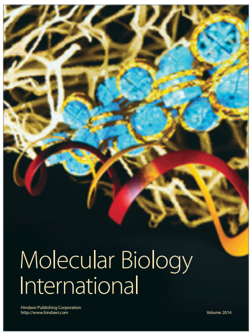
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