

# Soil and plant mineral composition and productivity of *Acacia nilotica* (L.) under irrigation with municipal effluent in an arid environment

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## SUMMARY

Municipal effluent is becoming an increasing environmental threat and needs appropriate disposal measures to safeguard soil and environmental quality. As an important source of water and nutrients, municipal effluent could be used to increase productivity in nutrient deficient dry areas. *Acacia nilotica* L. (Babool) seedlings were grown using municipal effluent. Five treatments comprised: irrigation of soil (without seedlings) with municipal effluent at potential evapotranspiration (PET) (treatment T<sub>1</sub>), irrigation of seedlings at 0.5 PET (T<sub>2</sub>), PET (T<sub>3</sub>), and 2 PET (T<sub>4</sub>), and canal water irrigation of seedlings at PET (T<sub>5</sub>). Seedlings in the T<sub>3</sub> and T<sub>4</sub> treatments attained greater height and collar diameter, and produced 22% and 54% more biomass than those in T<sub>5</sub>. After 24 months, biomass production was 7.43–12.96 t ha<sup>-1</sup> as compared to 5.73 t ha<sup>-1</sup> in T<sub>5</sub>. Nutrient concentrations in the seedlings were positively correlated with effluent quantity ( $r = 0.82$ ,  $p < 0.01$ ), being greater in foliage than in stems and roots. Uptake of nitrogen (N) was 2.70%, and of iron (Fe) 0.11%, of seedling biomass. Relative increase in metal concentration was greater than that in nutrients. The availability of potassium (K), copper (Cu), Fe, manganese (Mn) and zinc (Zn) in the soil increased twofold and that of NH<sub>4</sub>-N and PO<sub>4</sub>-P by 4.5- and 7.9-fold, respectively, in T<sub>4</sub> relative to T<sub>5</sub>. Available NH<sub>4</sub>-N, PO<sub>4</sub>-P, Mg and K were highest in the 0–15 cm depth soil, whereas NO<sub>3</sub>-N, Na, Cu, Fe, Mn and Zn availability were highest in deeper soil layers. Relatively low soil nutrients in T<sub>3</sub> compared to T<sub>1</sub> indicated withdrawal and accumulation of these nutrients in the seedlings. In several respects municipal effluent benefited *A. nilotica* seedlings and soil properties, and produced 5.59–12.96 t ha<sup>-1</sup> dry biomass. Municipal effluent could help to meet the fuel need of suburban areas, although long-term application of effluent would lead to metal accumulation in soil and plants.

*Keywords:* arid region, biomass, effluent irrigation, India, plant nutrients, soil nutrients, tree seedlings

## INTRODUCTION

Low soil water availability is often one of the main factors limiting plant productivity (Honeysett *et al.* 1992). Lack of soil nutrient elements, particularly nitrogen (N) and phosphorus (P), is another constraint affecting plant productivity in many dry areas (Gutierrez-Boem & Thomas 1999). Increasing amounts of wastewater from many desert cities produce major problems for safe disposal, resulting in degradation of land and environment. It may be viable to use municipal effluent to grow trees and increase biomass productivity in dry areas. For example, about 132 million litres of municipal effluent are generated daily by Jodhpur city in the Indian desert (Public Health Engineering Department, personal communication 2004), and some of this effluent is already used to grow vegetables and agricultural crops, whereas more than 80% of the effluent is disposed of into adjacent water courses. When discharged into water bodies, sewage and other effluents with a high nutrient load cause major ecological and public health problems (Johnson *et al.* 2004). Indeed, eutrophication and accumulation of nutrients in biological systems are serious pollution issues (Driscoll *et al.* 2004).

Trees can remove nutrients from land-applied wastewater for their growth without any harmful effect on the environment (Stewart *et al.* 1990). Using municipal effluent to grow trees in suburban areas may help solve the dual problems of wastewater disposal and lack of fuel wood supply (Paliwal *et al.* 1998). *Acacia nilotica* (L.) (Babool) is an important species for fuel wood and timber production, and its growth has been found to increase when sufficient soil water and nutrients are available (Singh & Singh 2000; Bhutta & Chaudhary 2000). However, productivity of *A. nilotica* in dry areas is limited because of low soil water and nutrient availability. Productivity may be increased by municipal effluent application to meet the fuel wood and timber needs of desert cities.

However, this effluent contains metal ions in addition to essential macro- and micronutrients. The essential nutrients improve soil conditions and can therefore benefit irrigated agricultural and horticulture crops, forests and recreational areas (Stewart & Salmon 1986). Loading rates or concentrations of metals in effluent may become toxic to the plants because of mineral accumulation in the soil (Nriagu 1991). The level of effluent and possible changes in soil properties and plant mineral status need to be studied prior to their use to reduce pollution by subsurface drainage of excess nutrients (Johnson *et al.* 2004). Optimum levels of sewage application could benefit growth and productivity of plants in addition to opening new options for safe disposal.

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Little information is available on the use of municipal effluent in growing *Acacia nilotica* in desert regions because most studies are limited to agricultural crops, grasses or eucalypt trees (Myers *et al.* 1996; Johnson *et al.* 2004). In this study changes in soil and plant mineral composition were monitored following irrigation with different levels of municipal effluent to assess plant nutrient withdrawal from the soil and its effect on growth and biomass production of *A. nilotica* seedlings. The specific objectives were to: (1) investigate the effect of municipal effluent on fuel wood productivity, and (2) determine the point at which mineral toxicity would develop in the plants and soil system.

## MATERIALS AND METHODS

### Site description

An experiment was conducted in a non-weighing infilled type of lysimeter measuring 2 m × 2 m × 2 m in the experimental field of the Arid Forest Research Institute, Jodhpur (26° 45'N, 72° 03'E), India. The lysimeters were filled with soil up to a depth of 185 cm, leaving 15 cm for irrigation. The soil was a loamy sand (coarse loamy, mixed, hyperthermic family of Typic Camborthides, according to USA soil taxonomy) with 82% sand, 12% silt and 5% clay. The soil had a bulk density of 1.5 t m<sup>-3</sup> and low contents of organic matter (0.13%), and available PO<sub>4</sub>-P (5.00 mg kg<sup>-1</sup>), NO<sub>3</sub>-N (6.00 mg kg<sup>-1</sup>) and NH<sub>4</sub>-N (4.50 mg kg<sup>-1</sup>). The pH and electrical conductivity (EC) of the soil were 7.61 and 0.71 dSm<sup>-1</sup>, respectively.

The climate of the site is tropical and with hot and dry summers, hot rainy seasons, warm autumns and cool winters. Summer is dominant in terms of plant production and has strong winds. Mid-July to September is the monsoon season with an average annual rainfall of 420 mm. The mean annual pan evaporation is 2025 mm, indicating a high water deficit at the site.

### Experimental design, planting and irrigation

One-year-old seedlings of *Acacia nilotica* of single provenance were planted in the lysimeters in July 1998 (Singh 2001). There was one seedling in each lysimeter to a density of 2500 seedlings ha<sup>-1</sup>. The plantation was in a completely randomized design with three replicates for treatment. Approximately 53 million litres of municipal effluent passed daily through the experimental area via a sewerage drain and drained into a river. Municipal effluent treatments were initiated in the first week of September 1998 after proper establishment of the seedlings. The seedlings were irrigated daily for maximum use of effluent. Irrigation levels were based on the potential evapotranspiration (PET), which varies seasonally and has an annual value of 1843 mm (Rao *et al.* 1971). The five treatments comprised: irrigation of soil (without seedlings), with municipal effluent at PET (treatment T<sub>1</sub>), irrigation of seedlings at 0.5 PET (T<sub>2</sub>), PET (T<sub>3</sub>), and twice PET (T<sub>4</sub>), and canal water irrigation of

seedlings at PET (T<sub>5</sub>). Rainfall was an additional input. At the start of the experiment average seedling height and collar diameter were 37 cm and 0.5 cm, respectively.

### Effluent, plant and soil analysis

Municipal effluent was sampled daily for seven days during the second weeks of the months of July and August 1998 (monsoon), December and January 1998–99 (winter) and May and June 1999 (summer) at two-hour intervals from 0600–1800 hours to make a composite daily sample; 14 samples of municipal effluent were collected in each season. Samples of canal water were collected in triplicate in each season at the time of irrigation. Thus, there were 51 samples, which included 42 samples of effluent and nine samples of canal water in the year. These samples were filtered through Whatman 42 filter paper, and stored at 4°C (OMA [Official Methods of Analysis] 1990). Each sample was analysed for pH, electrical conductivity (EC), chemical oxygen demand (COD), biochemical oxygen demand (BOD) and concentrations of macro- and micronutrients using standard procedures (OMA 1990). Total dissolved solids (TDS), total solids (TS) and total suspended solids (TSS) were determined gravimetrically, while chemical oxygen demand (COD) was determined by the reflux method, and total alkalinity was determined by the titration (OMA 1990). Nitrogen and phosphorus were analysed colorimetrically (Jackson 1973). Copper (Cu), iron (Fe), manganese (Mn) and zinc (Zn) were estimated by Jackson's (1973) aqua-regia method followed by measurement of concentrations using an atomic absorption spectrophotometer (model-3110, Perkin-Elmer, Boesch, Huenenberg, Switzerland).

Foliage, stem and root were sampled both from the harvested and the standing seedlings at the time of seedling harvest, washed with tap water and rinsed with distilled water. These samples were oven dried at 80°C, ground and used for mineral analysis. Measurements of N and P content were performed after a wet digestion using UV-VIS spectrophotometry (Systronix model 117, Ahmedabad, India) at 490 and 420 nm, respectively. Soil pH and electrical conductivity (EC) were determined in 1:2 soil water suspensions, whereas soil organic carbon (SOC) was determined using a standard procedure (Jackson 1973). Soil available nitrogen was determined after 2M KCl extraction, whereas available phosphorus was determined by Olson's extraction method (Jackson 1973). For determination of macro and micronutrients, soil samples were extracted with ammonium acetate and the sodium salt of diethylene tri-amine penta-acetic acid (DTPA) solution, respectively, whereas plant samples were wet digested (Jackson 1973). Calcium (Ca), magnesium (Mg), Cu, Zn, Mn, Fe, potassium (K) and sodium (Na) were estimated using double beam atomic absorption spectrophotometry.

### Observations on plants

Seedling height, collar diameter and number of branches were measured at monthly intervals. One seedling in each treatment

**Table 1** Main characteristics of effluent and canal water. Values are means ( $\pm$  SE) of 14 replicates (seven days  $\times$  two months in each season) for municipal effluent and nine replicates (three replicates  $\times$  three seasons) of canal water. EC = electrical conductivity; TS = total solids; TDS = total dissolved solids; TSS = total suspended solids; PA = phenolphthalein alkalinity; TA = total alkalinity; COD = chemical oxygen demand; and BOD = biochemical oxygen demand. Within rows similar letters indicate non-significant ( $p > 0.05$ ) differences.

Quality parameters	Monsoon 1998	Winter 1999	Summer 1999	Canal water
pH	7.60 $\pm$ 0.01 <sup>a</sup>	7.66 $\pm$ 0.03 <sup>a</sup>	8.02 $\pm$ 0.04 <sup>b</sup>	7.40 $\pm$ 0.01 <sup>a</sup>
EC (dS m <sup>-1</sup> )	0.91 $\pm$ 0.01 <sup>a</sup>	1.83 $\pm$ 0.02 <sup>b</sup>	2.14 $\pm$ 0.02 <sup>c</sup>	0.56 $\pm$ 0.01 <sup>d</sup>
TS (g L <sup>-1</sup> )	1.00 $\pm$ 0.02 <sup>a</sup>	1.43 $\pm$ 0.03 <sup>b</sup>	3.02 $\pm$ 0.08 <sup>c</sup>	0.13 $\pm$ 0.004 <sup>d</sup>
TDS (g L <sup>-1</sup> )	0.62 $\pm$ 0.02 <sup>a</sup>	1.09 $\pm$ 0.02 <sup>b</sup>	1.83 $\pm$ 0.11 <sup>c</sup>	0.10 $\pm$ 0.01 <sup>d</sup>
TSS (g L <sup>-1</sup> )	0.37 $\pm$ 0.01 <sup>a</sup>	0.34 $\pm$ 0.01 <sup>b</sup>	1.20 $\pm$ 0.11 <sup>c</sup>	0.03 $\pm$ 0.002 <sup>d</sup>
PA (CaCO <sub>3</sub> mg L <sup>-1</sup> )	12.5 $\pm$ 0.12 <sup>a</sup>	14.0 $\pm$ 0.35 <sup>b</sup>	2.0 $\pm$ 0.12 <sup>c</sup>	–
TA (CaCO <sub>3</sub> mg L <sup>-1</sup> )	92.0 $\pm$ 0.76 <sup>a</sup>	126.4 $\pm$ 1.84 <sup>b</sup>	145.4 $\pm$ 2.71 <sup>c</sup>	–
CO <sub>3</sub> <sup>-</sup> (mg L <sup>-1</sup> )	–	–	4.23 $\pm$ 0.05	–
HCO <sub>3</sub> <sup>-</sup> (mg L <sup>-1</sup> )	8.78 $\pm$ 0.02 <sup>a</sup>	9.99 $\pm$ 0.04 <sup>a</sup>	13.76 $\pm$ 0.10 <sup>b</sup>	–
Cl <sup>-</sup> (mg L <sup>-1</sup> )	97.0 $\pm$ 2.60 <sup>a</sup>	99.0 $\pm$ 2.44 <sup>a</sup>	126.0 $\pm$ 2.61 <sup>b</sup>	7.6 $\pm$ 0.12 <sup>c</sup>
COD (mg L <sup>-1</sup> )	220.0 $\pm$ 1.9 <sup>a</sup>	190.0 $\pm$ 3.1 <sup>b</sup>	270.0 $\pm$ 3.0 <sup>c</sup>	6.0 $\pm$ 0.5 <sup>d</sup>
BOD (mg L <sup>-1</sup> )	36.0 $\pm$ 0.9 <sup>a</sup>	39.0 $\pm$ 1.3 <sup>a</sup>	56.0 $\pm$ 1.3 <sup>b</sup>	3.0 $\pm$ 0.4 <sup>c</sup>
Na (mg L <sup>-1</sup> )	13.30 $\pm$ 0.21 <sup>a</sup>	9.30 $\pm$ 0.11 <sup>b</sup>	17.30 $\pm$ 0.18 <sup>c</sup>	4.20 $\pm$ 0.21 <sup>d</sup>
K (mg L <sup>-1</sup> )	20.40 $\pm$ 0.42 <sup>a</sup>	30.48 $\pm$ 1.612 <sup>b</sup>	41.01 $\pm$ 1.24 <sup>c</sup>	20.48 $\pm$ 0.62 <sup>b</sup>
Ca (g L <sup>-1</sup> )	0.30 $\pm$ 0.003 <sup>a</sup>	0.23 $\pm$ 0.002 <sup>b</sup>	0.24 $\pm$ 0.001 <sup>b</sup>	0.16 $\pm$ 0.001 <sup>c</sup>
Mg (g L <sup>-1</sup> )	0.14 $\pm$ 0.01 <sup>a</sup>	0.16 $\pm$ 0.01 <sup>b</sup>	0.16 $\pm$ 0.01 <sup>c</sup>	0.05 $\pm$ 0.002 <sup>a</sup>
NH <sub>4</sub> -N (mg L <sup>-1</sup> )	6.00 $\pm$ 0.007 <sup>a</sup>	8.50 $\pm$ 0.61 <sup>b</sup>	11.80 $\pm$ 0.32 <sup>b</sup>	2.10 $\pm$ 0.02 <sup>c</sup>
NO <sub>3</sub> -N (mg L <sup>-1</sup> )	2.00 $\pm$ 0.11 <sup>a</sup>	0.40 $\pm$ 0.01 <sup>b</sup>	0.60 $\pm$ 0.02 <sup>c</sup>	0.02 $\pm$ 0.001 <sup>d</sup>
PO <sub>4</sub> -P (mg L <sup>-1</sup> )	12.00 $\pm$ 0.56 <sup>a</sup>	12.80 $\pm$ 0.86 <sup>b</sup>	13.60 $\pm$ 0.77 <sup>c</sup>	0.02 $\pm$ 0.001 <sup>d</sup>
Cu (mg L <sup>-1</sup> )	0.25 $\pm$ 0.01 <sup>a</sup>	0.26 $\pm$ 0.009 <sup>a</sup>	0.54 $\pm$ 0.02 <sup>b</sup>	.008 $\pm$ 0.002 <sup>c</sup>
Fe (mg L <sup>-1</sup> )	2.66 $\pm$ 0.05 <sup>a</sup>	3.64 $\pm$ 0.03 <sup>b</sup>	6.24 $\pm$ 0.05 <sup>c</sup>	0.08 $\pm$ 0.002 <sup>d</sup>
Mn (mg L <sup>-1</sup> )	0.26 $\pm$ 0.02 <sup>a</sup>	0.32 $\pm$ 0.009 <sup>b</sup>	0.80 $\pm$ 0.02 <sup>c</sup>	0.02 $\pm$ 0.001 <sup>d</sup>
Zn (mg L <sup>-1</sup> )	0.40 $\pm$ 0.02 <sup>a</sup>	0.48 $\pm$ 0.02 <sup>b</sup>	0.80 $\pm$ 0.02 <sup>c</sup>	0.02 $\pm$ 0.001 <sup>d</sup>

was harvested at 24 months of age. Foliage (leaves + small twigs) was separated from stem and wet weight determined. The stem was removed from the collar region and length and collar diameter measured. Roots were excavated manually and soil particles adhering to the root surface removed. The single longest root was measured for data on primary root length and diameter. Secondary roots were counted. Wet mass of roots was recorded immediately after measurement. Dry mass values of foliage, stem and root were recorded after oven drying the samples for 72 hours at 80°C. Root volume was measured by water displacement (Singh 2001).

### Statistical analysis

Data were statistically analysed using the SPSS statistical package for social and environmental sciences (Lindaman 1992). Since the experiment was laid out in complete randomized design, treatments were compared using a one-way ANOVA model with average growth variables and nutrients in seedling parts as the dependent variables, and treatments considered the main factor. Soil nutrient data were analysed using a two-way ANOVA considering treatments and soil layers as the main effects. To monitor the changes in soil properties after 24 months of effluent application compared to the nutritional status in July 1998, soil variables were tested using Student *t*-tests. Since one seedling was harvested for root study and biomass recoding in each treatment, root growth variables and biomass of seedling parts

were analysed using single sample *t*-tests. Least significant differences were separated at a probability (*p*) level < 0.05.

### RESULTS

Raw municipal effluent had pH of 7.60–8.02, with electrical conductivity of 0.91–2.14 dSm<sup>-1</sup> and being highest in summer (Table 1). Mean concentrations of total solid (TS), total dissolved solid (TDS) and total suspended solid (TSS) were high in summer and lowest in monsoon months (Table 1). Biochemical oxygen demand (BOD) and chemical oxygen demand (COD) were at a minimum in the monsoon and winter seasons, respectively, and at a maximum in summer (Table 1). The BOD and COD values ranged between 36–56 mg L<sup>-1</sup> and 190–270 mg L<sup>-1</sup>, respectively. Mean chloride concentration was 97–126 mg L<sup>-1</sup>. Availability of NH<sub>4</sub>-N, NO<sub>3</sub>-N, PO<sub>4</sub>-P, K, Fe, Cu, Mn and Zn was higher in municipal effluent than in the canal water. In most cases, concentrations of these nutrients were low during the monsoon and increased during summer, except for NO<sub>3</sub>-N, which was higher in monsoon (Table 1), probably because of additional wastewater from the suburban area and run-off water from fertilized fields.

### Seedling growth

Mean initial height and collar diameter of the seedlings were 37 cm and 0.5 cm, respectively, and did not differ among treatments. After two years of effluent irrigation, height and

**Table 2** Effect of municipal effluent application on the height and collar diameter of *A. nilotica* seedlings. Values are means of three replicates ( $\pm$  SE). T<sub>2</sub> = municipal effluent irrigation at 0.5 PET, T<sub>3</sub> = municipal effluent irrigation at PET, T<sub>4</sub> = municipal effluent irrigation at 2 PET, and T<sub>5</sub> = canal water irrigation at PET.

Treatment	Height (cm)		Collar diameter (cm)	
	Initial	24 months	Initial	24 months
T <sub>2</sub>	37 $\pm$ 6.0	242 $\pm$ 6.0	0.4 $\pm$ 0.00	3.9 $\pm$ 0.15
T <sub>3</sub>	36 $\pm$ 5.9	270 $\pm$ 13.2	0.5 $\pm$ 0.03	5.1 $\pm$ 0.12
T <sub>4</sub>	36 $\pm$ 4.7	285 $\pm$ 15.2	0.4 $\pm$ 0.06	5.8 $\pm$ 0.36
T <sub>5</sub>	39 $\pm$ 3.4	273 $\pm$ 8.6	0.6 $\pm$ 0.03	4.3 $\pm$ 0.15

collar diameter were 242–285 cm and 3.9–5.8 cm, respectively, and differed significantly among treatments (height  $F = 4.36$ ,  $p < 0.023$ ; diameter  $F = 6.61$ ,  $p < 0.007$ ; Table 2). Height and collar diameter were greatest in treatment T<sub>4</sub> seedlings and smallest for T<sub>2</sub> seedlings. Number of branches did not vary significantly between the treatments. Seedling growth indicated a seasonal pattern, per cent increment in height and collar diameter being greater in July–August than February–March.

### Root growth

*Acacia nilotica* seedlings had a deep primary root supported by fibrous secondary and tertiary roots. The concentration of roots was greater in 0–30 cm depth layer than the soil layer > 30 cm depth. Primary root length, root diameter and root volume were greater for the seedlings in treatment T<sub>4</sub> than those in the other treatments (Table 3). Treatment T<sub>3</sub> seedlings had longer roots than T<sub>5</sub> seedlings. Root diameter in T<sub>2</sub> seedlings was 15% less than T<sub>5</sub> seedlings. Mean root length and root diameter could be ranked in the following order: T<sub>4</sub> > T<sub>3</sub> > T<sub>2</sub> > T<sub>5</sub>. Number of secondary roots and root volume were also greater in T<sub>3</sub> and T<sub>4</sub> treated seedlings than T<sub>5</sub> seedlings.

**Table 3** Root growth and dry biomass of 24-month old *A. nilotica* seedlings under different levels of municipal effluent application. RL = primary root length; RD = primary root diameter; SR = number of secondary roots; RV = root volume; R:S = root:shoot dry weight ratio. For T<sub>2</sub> to T<sub>5</sub> see caption for Table 2.

Parameters	Treatments				One sample t-test	
	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>	T <sub>5</sub>	t	p
<b>Root variables</b>						
RL (cm)	160	160	165	150	50.46	<0.01
RD (cm)	3.9	5.1	5.7	4.1	11.08	<0.01
SR	25	29	31	25	18.33	<0.01
RV (cm <sup>3</sup> )	815	1280	1505	825	6.45	<0.01
<b>Dry biomass</b>						
Foliage (g seedling <sup>-1</sup> )	1210	1490	2925	1275	4.27	<0.05
Stem (g seedling <sup>-1</sup> )	1065	1480	2260	1015	4.94	<0.05
Root (g seedling <sup>-1</sup> )	1487	1681	2500	1520	7.55	<0.01
Total (g seedling <sup>-1</sup> )	3782	4650	5851	3810	5.34	<0.05
R:S	0.65	0.53	0.46	0.66	13.50	<0.01
Total (t ha <sup>-1</sup> )	5.59	7.43	12.96	5.73	4.58	<0.05

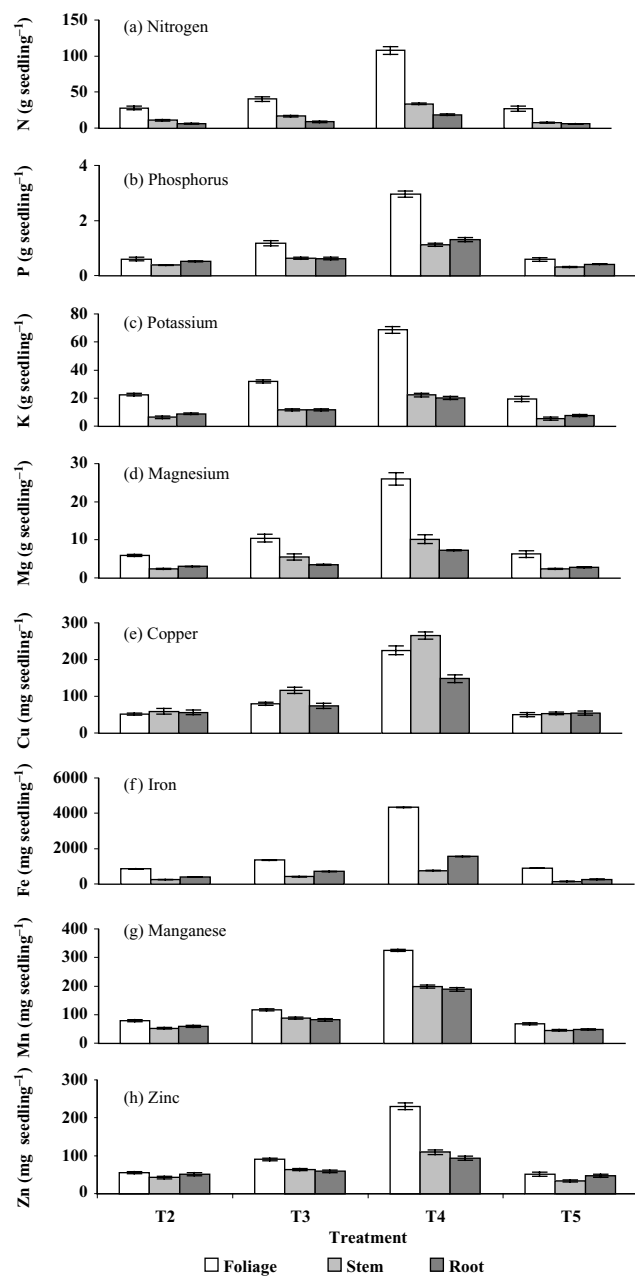
### Seedling biomass

T<sub>3</sub> and T<sub>4</sub> seedlings produced 22% and 54% more dry biomass than T<sub>5</sub> seedlings. T<sub>2</sub> and T<sub>5</sub> seedlings produced similar mean biomass to each other. Considering biomass partitioning in the seedling parts, foliage accounted for 37% of biomass in the T<sub>4</sub> treatment, in which it was highest (Table 3). Foliage biomass in the seedlings was similar between the T<sub>2</sub> and T<sub>5</sub> treatments. Stem contributed 32% of the total biomass in the T<sub>3</sub> treatment and 27% in the T<sub>5</sub> treatment (Table 3). The stem biomass was 46% higher in T<sub>3</sub> seedlings than in T<sub>5</sub> seedlings. Seedling root biomass was similar between the T<sub>2</sub> and T<sub>5</sub> treatments. Root biomass increased by 11% in the T<sub>3</sub> seedlings and by 64% in the T<sub>4</sub> seedlings compared to T<sub>5</sub> seedlings. Mean contribution of root to total biomass was 33% in T<sub>4</sub> seedlings, resulting in a low root/shoot dry mass ratio (R/S). Root contributed 40% to total biomass in T<sub>2</sub> and T<sub>5</sub> seedlings, which had a high root/shoot dry weight ratio.

### Seedling nutrient concentrations

At 24 months, N, P, K, Ca, Mg, Na, Cu, Fe, Mn and Zn concentrations were higher in effluent-irrigated seedlings (i.e. increased from treatment T<sub>2</sub> to T<sub>4</sub>) than in T<sub>5</sub> seedlings (Fig. 1). Concentrations of these nutrients were highest in foliage and lowest in roots, except for Cu, which was highest in stems, and Fe, which was lowest in stems. The increase in concentration over 24 months was about 45.5% for N, P, Ca, Mg and K, and 42.8% for Cu, Fe, Mn and Zn, compared to the T<sub>5</sub> seedlings. Nutrient concentration was highest (> 2.0-fold for Cu, Fe, Mn and Zn, and < 2.0-fold for N, P, Ca, Mg, K and Na) in the foliage of T<sub>4</sub> seedlings. T<sub>2</sub> seedlings had a mineral concentration equal to or higher than T<sub>5</sub> seedlings (i.e., 20.78 g kg<sup>-1</sup>, 0.45 g kg<sup>-1</sup>, 15.01 g kg<sup>-1</sup>, 14.67 g kg<sup>-1</sup>, 4.67 g kg<sup>-1</sup>, 38.40 mg kg<sup>-1</sup>, 684.00 mg kg<sup>-1</sup>, 52.00 mg kg<sup>-1</sup> and 39.60 mg kg<sup>-1</sup> seedling dry mass for N, P, K, Ca, Mg, Cu, Fe, Mn and Zn, respectively). Concentration of Na increased by 6% and 73% in the foliage of T<sub>3</sub> and T<sub>4</sub> seedlings, respectively, as compared to 1.68 g kg<sup>-1</sup> dry mass





**Figure 1** Nutrient and metal accumulation (mg per g kg<sup>-1</sup> dry biomass) in 24-month-old seedlings irrigated with different levels of municipal effluent. (a) Nitrogen, (b) phosphorus, (c) potassium, (d) magnesium, (e) copper, (f) iron, (g) manganese and (h) zinc. Error bars are ± SE.

in T<sub>1</sub> seedlings. In the stems, concentrations of N, P, K, Ca, Cu, Mn and Zn increased by about 40% in T<sub>3</sub> seedlings, and Fe concentrations increased more than twofold compared to concentrations of 6.97 g kg<sup>-1</sup>, 0.29 g kg<sup>-1</sup>, 5.08 g kg<sup>-1</sup>, 6.37 g kg<sup>-1</sup>, 52.00 mg kg<sup>-1</sup>, 43.00 mg kg<sup>-1</sup>, 32.70 mg kg<sup>-1</sup> and 127.50 mg kg<sup>-1</sup> in T<sub>5</sub> seedlings. Concentrations of these nutrients were highest in the stems of T<sub>4</sub> seedlings. Differences in mineral concentrations in roots were very similar to those observed in the stems. However, the concentration

of Zn in roots did not differ among the treatments. K:Na, Na:Mg and K:Ca + Mg ratios were highest (12.96, 4.66 and 0.82, respectively) in the foliage of T<sub>2</sub> seedlings, whereas the Mg:Mn ratio was highest in the T<sub>5</sub> treatment. K:Na and K:Ca + Mg ratios were lowest in T<sub>4</sub> seedlings.

**Nutrient accumulation in seedlings**

Total accumulation (g or mg seedling<sup>-1</sup>) of N, P, K, Ca, Mg, Na, Fe, Mn and Zn was greatest in the foliage, whereas accumulation of Cu was greatest in stems of the seedlings irrigated with both effluent and canal water. Accumulation of P, Ca, Na and Fe was higher in root than in stem. The accumulation of K, Mg, Zn and Mn was higher in roots than the stems only for the T<sub>2</sub> and T<sub>5</sub> seedlings (Fig. 1). T<sub>4</sub> seedlings accumulated the highest amounts of nutrients, the concentrations positively correlated (mean *r* value) with quantity of municipal effluent (*r* = 0.82, *p* < 0.01). The T<sub>4</sub> irrigated seedlings accumulated 2.7% N, 0.11% Fe and 0.22% Na on a dry mass basis (mean of all seedling parts).

**Soil chemical changes**

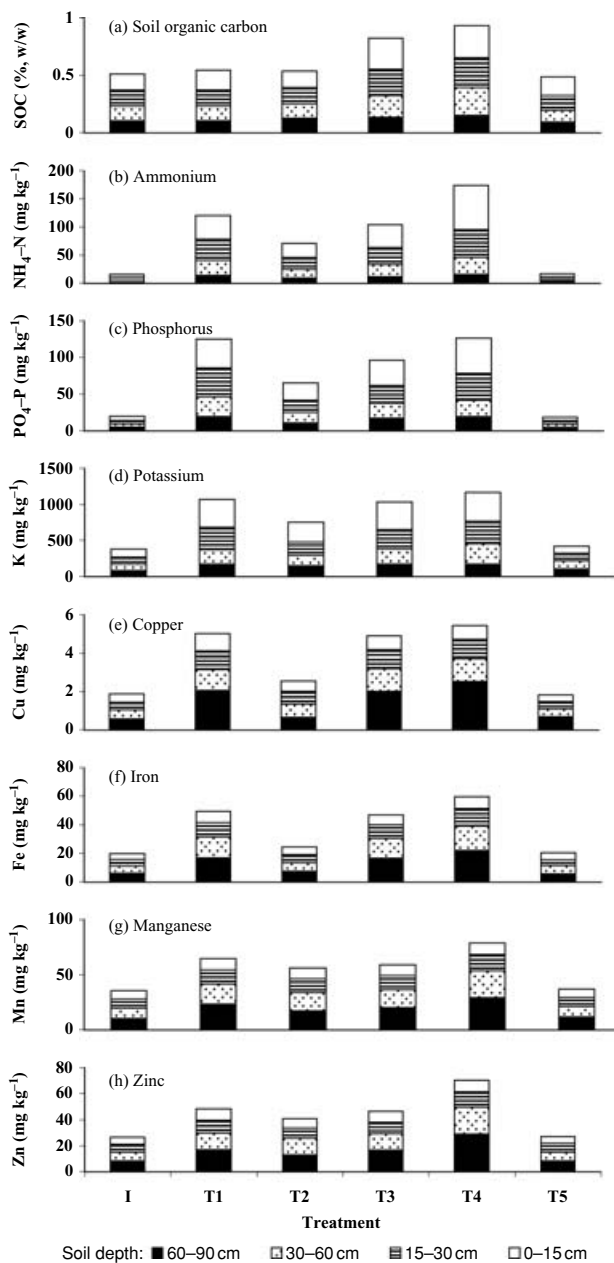
Compared to the initial values of July 1998, application of municipal effluent resulted in 2- to 2.5-fold increases in mean Mn, Zn, Fe, Cu and K concentrations in the 0–90 cm soil layer of the T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub> treatments (Fig. 2). Na, soil organic carbon (SOC), and available NH<sub>4</sub>-N and PO<sub>4</sub>-P concentrations increased 1.3-fold, 1.4-fold, 4.5-fold and 7.9-fold, respectively. Available NO<sub>3</sub>-N decreased, whereas pH, EC, Ca and Mg concentrations did not show significant changes over the period. There was a decrease in pH, EC, SOC, PO<sub>4</sub>-P and Cu values in the soil of T<sub>5</sub> compared to their respective values in July 1998.

The above-mentioned soil variables increased in the soil of the T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub> treatments, and reached their highest values in the soil of T<sub>4</sub> (0–90 cm, mean of soil layers). For T<sub>3</sub> and T<sub>4</sub> treatments, the respective increases were 66% and 77% for SOC, 6.6- and 11.7-fold for NH<sub>4</sub>-N, and 5.2- and 6.4-fold for PO<sub>4</sub>-P, and 1.2–2.7-fold and 1.3–3.0-fold in Na, K, Cu, Fe, Mn and Zn concentrations compared to T<sub>5</sub>. Concentrations of these nutrients were low in T<sub>2</sub> soil, and greater in T<sub>1</sub> soil compared to that of T<sub>3</sub> (Fig. 2). The values of pH, EC, SOC, NH<sub>4</sub>-N, PO<sub>4</sub>-P, Mg and K were highest in the 0–15 cm soil layer and decreased with increasing soil depth, whereas the concentrations of NO<sub>3</sub>-N, Ca, Na, Cu, Fe, Mn and Zn indicated a reverse trend. A 30% and 45% decrease in soil EC and SOC was observed in the 60–90 cm soil layer, respectively. The treatment by soil layer interaction was significant (*p* < 0.01).

**DISCUSSION**

**Seedling growth and biomass**

Increase in water and nutrient availability through effluent application influenced the growth of *A. nilotica* seedlings. An



**Figure 2** Soil mineral concentration under *A. nilotica* after irrigation with varying levels of municipal effluent. (a) Soil organic carbon, (b) available phosphorus, (c) available ammonium, (d) potassium, (e) iron, (f) copper, (g) manganese, and (h) zinc. I = initial state, T<sub>1</sub> = municipal effluent irrigation at PET (soil only), T<sub>2</sub> = municipal effluent irrigation at 0.5 PET, T<sub>3</sub> = municipal effluent irrigation at PET, T<sub>4</sub> = municipal effluent irrigation at 2 PET and T<sub>5</sub> = canal water irrigation at PET.

increase in foliage is expected to have captured more solar energy for metabolic use, fixed more CO<sub>2</sub>, and produced more photosynthates and increased growth and biomass (Ceulemans *et al.* 1993; Myers *et al.* 1996). Higher growth and biomass in T<sub>3</sub> and T<sub>4</sub> seedlings were attributable to effects of available nutrients in the effluent on leaf initiation and growth-enhancing CO<sub>2</sub> fixation and photosynthate levels (Boyer 1988). Carswell *et al.* (2000) suggested that increased nutrient

supply results in an increase of photosynthetic activity and carbohydrates. In our study a positive correlation of height ( $r=0.79$ ,  $p < 0.01$ ) and collar diameter ( $r=0.89$ ,  $p < 0.01$ ) with quantity of effluent added supports this inference. However, smaller seedling height and collar diameter values in T<sub>2</sub> compared to those in T<sub>3</sub> and T<sub>4</sub> treatments were a result of low water and nutrient supply. Reductions in growth and photosynthesis have been previously recorded in *Eucalyptus marginata* seedlings under low water supply (Stoneman *et al.* 1994). The higher growth and biomass of T<sub>3</sub> seedlings compared to T<sub>5</sub> seedlings suggested nutrient-induced growth and productivity consistent with the observations of Paliwal *et al.* (1998) on *Hardwickia binata* and Sheriff *et al.* (1986) on *Pinus radiata*. Facilitative effects of municipal effluent on *A. nilotica* seedlings were suggested by the similar biomass produced in T<sub>5</sub> and T<sub>2</sub> seedlings, despite half the quantity of water being applied in T<sub>2</sub> than that in T<sub>5</sub>. Beneficial effects of municipal effluent have also been reported for *Eucalyptus grandis*, which accumulated as much biomass in four years as it did in eight years in a rainfed site (Stewart *et al.* 1990). The high loading rate would have resulted in nutrient accumulation in the perennial parts and enhanced capacity of the seedlings to form new leaves, sequester more carbon and produce more biomass (Peterson *et al.* 1993). However, long-term application of effluent at a high loading rate may cause excessive accumulation of metals resulting in soil toxicity. The increase of 11–64% in root biomass and 55–82% in root volume of T<sub>3</sub> and T<sub>4</sub> seedlings was a result of a higher number of fine secondary roots. The availability of water and nutrients probably had positive effects on root biomass, particularly the fine secondary roots, for better use of soil resources (Souch & Stephens 1998). Cavelier *et al.* (1999) also recorded high root biomass in irrigated (biomass of 1.8 t ha<sup>-1</sup>) than in non-irrigated (biomass of 1.25 t ha<sup>-1</sup>) plant communities. The relatively greater biomass in roots than in foliage of the T<sub>2</sub> seedlings as compared to other treatments suggests more photosynthate allocation to the roots under low water supply conditions. However, higher availability of water and nutrients (particularly N) favoured biomass partitioning towards foliage in T<sub>4</sub>. Li *et al.* (1991) observed that high N conditions resulted in higher growth of leafy shoots through reinvestment of assimilates towards photosynthetic parts, whereas low N favoured dry matter allocation to the root system because of its ability to acquire more of the limited resource.

### Seedling nutrient concentration and uptake

Soil nutrient availability, and nutrient absorption from the soil and its further transport in seedling parts influenced nutrient concentration in the seedlings (Al-Harbi 1999). The higher concentrations of nutrients in foliage compared to stems and roots are similar to the observation of Hopmanns *et al.* (1993). The increasing quantity of municipal effluent from treatment T<sub>2</sub> to T<sub>4</sub> resulted in increasingly greater nutrient concentrations in the seedlings. In the absence of any negative effect on the seedlings, the effluent loading

rates were thought to be beneficial. Basiouny (1984) observed substantially higher foliar N, P and Fe concentrations in peach trees irrigated with municipal wastewater than in controls. Availability of water and nutrients and higher root mass through increased numbers of secondary roots facilitated absorption and transport of the nutrients from the soil to the seedling parts in treatments T<sub>3</sub> and T<sub>4</sub>. Theodorou and Bowen (1983) observed a significant correlation of foliage N and P concentration with number of first and second order lateral roots. Nutrient concentrations in the seedlings of T<sub>2</sub>, which received the lowest level of effluent, indicated a positive relationship between water and nutrient availability and seedling nutrient concentration. Low concentration of nutrients in the seedlings of T<sub>2</sub> (Fig. 1) was probably the effect of restricted transport under low soil water supply (Singh & Singh 2000), however differences induced by competition between ions could not be ruled out. The decreased K:Na ratio suggested a relative increase in concentration of Na rather than K in foliage with increasing effluent quantity. Gadullah (1994) also observed an increase in Na, Cl and Zn concentrations in plants irrigated with sewage and other effluents. There was a greater relative increase in Cu, Fe, Mn and Zn concentrations compared to N, P, Ca, Mg, K and Na concentrations in the T<sub>4</sub> seedlings compared to T<sub>2</sub> and T<sub>3</sub> seedlings, probably because the high rate of effluent application increased the solubility and mobility of Cu, Fe, Mn and Zn. Siebe (1995) also recorded an increase in heavy metal content in both soil extracts and alfalfa (*Medicago sativa*) plants irrigated with wastewater. The increase in micronutrient concentration is evinced by the decreased Mg:Mn ratio in the effluent-irrigated seedlings. The comparatively higher concentration of Cu in stems than in the foliage and roots might be because of the strong binding and poor translocation of this ion.

Total nutrient uptake in the seedlings depended upon the dry biomass and mineral concentration of the seedlings. The significantly higher accumulation of nutrients in the T<sub>4</sub> seedlings was as a result of the higher levels of nutrients per unit mass, as well as the dry biomass of the seedlings (Fig. 1). Other studies also suggest greater accumulation of nutrients under effluent irrigation (Gadullah 1994; Hopmanns *et al.* 1993). However, greater accumulation of K, Mg, Zn and Mn in roots than in the stems or foliage of T<sub>2</sub> seedlings was because of decreased soil water availability and restricted transport in the plants. However, accumulation of N, K, Ca, Na and Fe to 2.71%, 1.89%, 1.96%, 0.23% and 0.11% of biomass (mean of foliage, stem and roots), respectively, indicated significant nutrient removal by the *A. nilotica* seedlings, suggesting that the seedlings had an ameliorative impact on soil properties.

### Soil properties

Effluent application influenced soil properties and depended upon the physico-chemical properties of the municipal effluent. Average decrease in pH and EC was because of the withdrawal of salts and nutrients by the *A. nilotica* seedlings. However, an increase in pH and EC in the 0–15 cm soil layer of the T<sub>1</sub>, T<sub>3</sub> and T<sub>4</sub> treatments was due to the

alkaline nature of the effluent (Mitra & Gupta 1999). Increase in SOC content was because of addition of organic matter by municipal effluent irrigation and was similar to that observed by Kumar *et al.* (1998). However, increase in SOC was also caused by litter addition. The low SOC in T<sub>1</sub> compared to T<sub>3</sub> was probably the result of litter mineralization by high solar radiation and soil temperature in the absence of planted seedlings (Davidson 1995) and the role of tree seedlings in SOC improvement in T<sub>3</sub>. Significantly greater concentrations of NH<sub>4</sub>-N, PO<sub>4</sub>-P, Mn, Zn, Fe, Cu and K in soil indicated a higher nutrient-loading rate relative to nutrient removal by the seedlings (Fig. 2). However, increase in seedling growth without any nutritive or morphological deformities suggested the beneficial effect of these nutrients. Low availability of the soil nutrients in T<sub>3</sub> compared to those in T<sub>1</sub> (received equal quantity of municipal effluent) reflected their absorption and accumulation by seedlings, as suggested by the decreased PO<sub>4</sub>-P availability compared to the initial July 1998 value in T<sub>5</sub>. Higher concentrations of NH<sub>4</sub>-N, PO<sub>4</sub>-P, Mg and K in the 0–30 cm layer as opposed to the deeper soil layer was probably because of surface application of effluent and nutrient retention in soil micelles. However the significantly higher concentration of NH<sub>4</sub>-N and PO<sub>4</sub>-P in the topsoil layer may have increased vulnerability to pathogens and insect attack and needs to be reduced before application or the loading rate decreased (Arora *et al.* 1985). Increased concentration of Na, metal ions and EC in the deeper soil layer was probably because of high infiltration rates and low SOC resulting in salt accumulation at that depth. However, absorption of these nutrients in the upper soil layer by the planted seedlings may be another reason for low concentrations at that depth.

### CONCLUSIONS

Irrigating with municipal effluent provided supplementary organic matter and essential nutrients and water to the soil. Application of municipal effluent enhanced the growth and usable biomass production of *A. nilotica* after 24 months (5.59–12.96 t ha<sup>-1</sup>). The comparatively lower soil nutrient levels in soils with seedlings treated by municipal irrigation at PET (T<sub>3</sub>) compared to soils without seedlings treated at the same irrigation rate (T<sub>1</sub>) and the higher mineral accumulation in seedling parts suggests that *A. nilotica* seedlings ameliorated soil properties. Using municipal effluent to raise *A. nilotica* seedlings is an appropriate means to increase the productivity of dry areas and meet local fuel needs. Use of municipal effluent in plantations improves the environmental quality of suburban areas without degrading soil health if long-term application at high loading rates is avoided.

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