INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
1560–8530/2006/08–4–504–507
http://www. fspublishers.org

Growth, Nutrient Homeostatis and Heavy Metal Accumulation in *Azadirachta indica* and *Dalbrgia sissoo* Seedlings Raised from Waste Water

H. FAROOQ[1], M.T. SIDDIQUI, M. FAROOQ†, E. QADIR AND Z. HUSSAIN
Department of Forestry and †Crop Physiology, University of Agriculture, Faisalabad–38040, Pakistan
[1]Corresponding author's e-mail: farooqcp@gmail.com

ABSTRACT

In a pot study, the growth, nutrient homeostatis and heavy metal accumulation in *Azadirachta indica* and *Dalbrgia sissoo* raised from different combinations of waste and canal water was investigated. The irrigation treatments included 100% canal water (100C), 100% effluent water (100E), 25% canal water + 75% effluent water (25C + 75E), 50% canal water + 50% effluent water (50C + 50E) and 75% canal water + 25% effluent water (75C + 25E). Under all the irrigation treatments, growth of *Azadirachta indica* was more vigorous than *Dalbrgia sissoo*. Maximum growth and minimum metal accumulation was observed in the seedlings raised from 100C followed by 75C + 25E. However, minimum growth and maximum metal accumulation was observed in the seedlings raised from 100E followed by 25C + 75E. The present study suggests that effluent water may be mixed with canal water (75C: 25E) for successful growth of both forest species.

Key Words: Waste water; Growth; Heavy metals; *Azadirachta indica; Dalbergia sissoo*

INTRODUCTION

Increased urbanization and industrialization has resulted in huge quantity of waste water. Moreover, underground water is un-fortunately increasingly becoming contaminated with municipal and industrial waste (Saleem, 2001). This fact can be appreciated better by considering that municipal waste water from just three municipalities of Pakistan (Lahore, Faisalabad & Multan) accounts to over 7000 cubic feet per second, which can be translated to 4.5 million acre feet irrigation water per year (Saleem, 2001).

Although the sewage effluents are considered to be a source of organic matter and plant nutrients (Ghaffor *et al.*, 1994) but municipal waste water effluents may contain a number of toxic elements including heavy metals (trace elements) like Fe, Mn, Co, Zn, Pb and Ni. Under practical conditions, wastes from many small and informal industrial sites are discharged into the common sewer system (Ghafoor, 1994). Some of them may be removed during the treatment process but others will persist and could present phytotoxic problems (Ghafoor *et al.*, 1994). Revegetation of plant species using industrial and municipal waste water may not only help us to minimize the use of fresh water as irrigation source but may also help for saving precious foreign exchange now being spent on the import of wood and wood products.

In Pakistan, *Azadirachta indica* is one of the most useful multipurpose tree specie for rural and urban communities. Almost all parts of the tree have multiple uses (Kumar & Sadhna, 1999). *Dalbergia sissoo* is also an important woody specie in Pakistan. The cultivation of these species may be extended to industrial and urban areas where the only source of irrigation water could be the industrial and municipal waste water.

The present study was designed to study the growth behavior of *Azadirachta indica and Dalbergia sissoo* to industrial and municipal waste water, to determine the heavy metals uptake by these species and to determine the fresh water percentage that could be saved by utilizing the industrial and municipal waste water as irrigation source.

MATERIALS AND METHODS

Experimental material. Seedlings of *Azadirachta indica* and *Dalbergia sissoo* were used as experimental material. Two-month-old uniform sized seedlings of both tree species were obtained from the Nursery, Department of Forestry, University of Agriculture, Faisalabad, Pakistan

Effluent was collected from two different sites of Faisalabad (viz. drain carrying industrial waste water at Dhoodi Wala & drain carrying municipal effluent at Aakhri Stop in Ghulam Muhammad Abad) and was mixed. Chemical composition of the mixed effluent is given in Table I.

Experimental detail. The irrigation treatments included 100% canal water (100C), 100% effluent water (100E), 25% canal water + 75% effluent water (25C + 75E), 50% canal water + 50% effluent water (50C + 50E) and 75% canal water + 25% effluent water (75C + 25E). After transplanting the seedlings of both the species into the earthen pots (45 cm x 30 cm) containing 6 kg soil. The soil was sandy clay loam having pH 7.4. Water was applied twice a day during the 1st week, once a day for the next two weeks and on each alternate day till the end of

Table I. Chemical composition of effluent water

Characteristic	*Value*
pH	3 ds m^{-1}
Cd	0.05 ppm
Cr	2.35 ppm
Cu	6.24 ppm
Fe	13.11 ppm
Pb	5.94 ppm
Mn	4.63 ppm
Ni	1.88 ppm
Zn	5.84 ppm

the experimental period. The experimental units were arranged in completely randomized design with four replications. Seventy five days after transplanting shoot length, root length, shoot and root fresh and dry weight, their dry weight and collar diameter of the seedlings was recorded.

The plant samples were oven dried at 85°C and ground to pass 2 mm sieve. The ground plant samples were digested in concentrated Nitric acid. The material was then diluted 10 times with distilled water and filtered through Whatman's filter paper No. 1. Nitrogen was determined by "Gunning and Hibbards" method of sulphuric acid digestion and distillation was made with micro Kjeldhal's apparatus (Jackson, 1962). Phosphorous was determined calorimetrically on the perchloric acid digest. Color was developed with ammonium molybdate and ammonium vandate solutions. Jenway PEP- 7 flame photometer was used to determine K in diluted extracts of plant material by using potassium filter. Cd, Cr, Mn and Fe were determined by atomic absorption spectrophotometer (Model No Varian AA 1475) by following conditions given in operation manual of Varian.

RESULTS

In *Azadirachta indica*, all the treatments resulted in lower shoot and root length, shoot fresh and dry weight, root fresh and dry weight and collar diameter compared with control (100C) except 75C + 25E, which behaved similar to that of control in case of root length and shoot fresh and dry weight and 50C + 50E in case of root fresh weight (Table II). Minimum shoot and root length, shoot fresh and dry weight, root fresh and dry weight and collar diameter was observed when 100E was applied that was similar to that of 25C + 75E and 50C + 50E in case of shoot dry weight and collar diameter and 25C + 75E in case of root fresh weight (Table II). Similar to *Azadirachta indica*, in *Dalbergia sissoo*, all the treatments resulted in lower shoot and root length, shoot fresh and dry weight, root fresh and dry weight and collar diameter compared with control (100C) except 75C + 25E, which behaved similar to that of control in case of shoot dry weight and collar diameter (Table II). Minimum shoot and root length, shoot fresh and dry weight, root fresh and dry weight and collar diameter was observed when 100E was applied that was similar to that of 25C + 75E in case of shoot length and collar diameter, and 25C + 75E and 50C + 50E in case of root length, shoot dry weight and root fresh and dry weight (Table II).

In *Azadirachta indica*, maximum nitrogen, potash and phosphorus percentage was recorded in seedlings irrigated with 100C that was similar to 100E irrigated seedlings in case of nitrogen and, 100E, 25C + 75E, and 50C + 50E in case of K and P (Table III). However, in *Dalbergia sissoo* maximum N and P were recorded in the seedlings irrigated with 100E, while maximum K was noted in seedlings raised from 100C (Table II). Minimum N was recorded in the seedlings raised from 100C that was similar to 75C + 25E and 50C + 50E (Table III). While minimum P and K were recorded in the seedlings irrigated with 75C + 25E and were similar to the seedlings treated with 100C in case of P (Table III).

In *Azadirachta indica*, maximum Cd contents were measured in 100E followed by 25C + 75E, while zero Cd contents were recorded in the seedlings raised from 100C and 75C + 25E (Fig. 1a). While, in *Dalbergia sissoo*, maximum Cd contents were measured in the seedlings irrigated by 25C + 75E and followed by 100E. The lowest cadmium content was recorded in the 75C + 25E, which was similar to 100C (Fig. 1b).

In both *Azadirachta indica* and *Dalbergia sissoo*, maximum Cr contents were recorded in 100E followed by 25C + 75E, while minimum Cr contents in *Azadirachta indica* were measured in seedlings raised from 75C + 25E

Table II. Influence of waste water on the seedling growth of *Azadirachta indica* and *Dalbrgia sissoo*

Treatments		*Shoot length (cm)*	*Root length (cm)*	*Shoot fresh weight (mg)*	*Shoot dry weight (mg)*	*Root fresh weight (mg)*	*Root dry weight (mg)*	*Collar diameter (cm)*
Azadirachta indica	100C	93.25 a	62.50 a	14.35 a	3.697 a	8.760 a	2.758 a	1.500 a
	100E	62.88 d	40.13 d	9.047 c	2.783 c	5.145 b	0.832 d	0.663 c
	25C+75E	74.75 c	47.50 c	10.69 b	2.515 c	5.117 b	1.205 c	0.875 bc
	50C+50E	81.38 b	50.88 b	11.93 b	2.875 bc	7.485 a	1.752 b	0.975 bc
	75C+25E	86.88 b	61.00 ab	13.54 a	3.488 ab	8.485 a	2.080 b	1.100 b
	LSD at 0.05	5.667	5.751	1.375	0.625	1.518	0.337	0.312
Dalbrgia sissoo	100C	55.88 a	49.63 a	7.775 a	2.235 a	6.885 a	2.252 a	1.300 a
	100E	36.00 d	25.85 c	3.610 d	0.995 b	2.660 c	0.617 c	0.850 b
	25C+75E	38.63 d	28.27 c	4.627 c	1.230 b	2.880 c	0.780 c	0.922 b
	50C+50E	42.75 c	26.88 c	4.930 c	1.220 b	3.000 c	0.947 c	1.188 a
	75C+25E	48.38 b	34.30 b	6.250 b	2.092 a	4.253 b	1.795 b	1.325 a
	LSD at 0.05	3.799	3.930	0.706	0.289	0.513	0.452	0.228

Table III. Influence of waste water on the nutrient homeostatis in *Azadirachta indica* and *Dalbrgia sissoo* seedling

Treatments		*Nitrogen (%)*	*Phosphorous (%)*	*Potassium (%)*
Azadirachta indica	100C	2.345 a	1.905 a	2.880 a
	100E	2.290 ab	1.015 a	2.805 a
	25C+75E	2.015 c	0.810 ab	2.475 ab
	50C+50E	2.115 bc	0.805 ab	2.610 a
	75C+25E	1.790 d	0.710 b	2.154 b
	LSD at 0.05	0.215	0.215	0.422
Dalbrgia sissoo	100C	2.005 c	1.300 cd	2.995 a
	100E	2.740 a	1.900 a	2.765 b
	25C+75E	2.345 b	1.620 b	2.700 b
	50C+50E	2.130 bc	1.410 c	2.615 c
	75C+25E	2.040 c	1.245 d	2.500 d
	LSD at 0.05	0.269	0.162	0.812

followed by that of 100C (Fig. 2a); while in *Dalbergia sissoo*, maximum minimum Cr contents were measured in 100C followed by 75C + 25E (Fig. 2b).

In *Azadirachta indica*, maximum Mn contents was noted in the seedlings irrigated with 100E followed by that of 100C, however minimum Mn contents were recorded in the seedlings raised from 75C + 25E (Fig. 3a). While, in *Dalbergia sissoo*, maximum Mn contents were recorded in 100E that was similar to 25C + 75E, while minimum Mn contents were measured in seedlings raised from 100C (Fig. 3b).

In both *Azadirachta indica* and *Dalbergia sissoo*, maximum Fe contents were noted in the seedlings raised from the 100E followed by 25C + 75E. While minimum Fe contents were recorded in the seedlings raised from 75C + 25 in *Azadirachta indica* (Fig. 4a) and 100C in *Dalbergia sissoo* (Fig. 4b).

DISCUSSION

The present study suggests that waste water can be used to irrigate the forest trees if mixed with canal water. Waste water alone suppressed the plants growth that seems the result of higher salt contents in particular the elements, which are required in trace amount and are toxic at higher concentrations (Table I). Kamini and Shrabani (1999) observed the effect of effluent water on *Allium cepa* L. and *Allium sativum* L. and reported similar inhibited growth of the plants. In another study, Kumar (1999) applied the sewage effluent to *Hordeum vulagre* and observed the reduction in mass and volume due to heavy metals toxicity. The comparison of both tree species showed that *Azadirachta indica* is hardier and grow more vigorously than *Dalbergia sissoo* that might be due to hardy bark and rapid growth rate of the former (Tables II, III).

Woody plants have to strive for survival despite of un-favorable growth conditions. Salts and metals may deposit in the plants in vacuole and may disturb ion balance and whole water relations of the plants (Gupta & Sharma, 1997).

Fig. 1. Influence of waste water on the Cd accumulation in (a) *Azadirachta indica* and (b) *Dalbrgia sissoo* seedling ± s.e

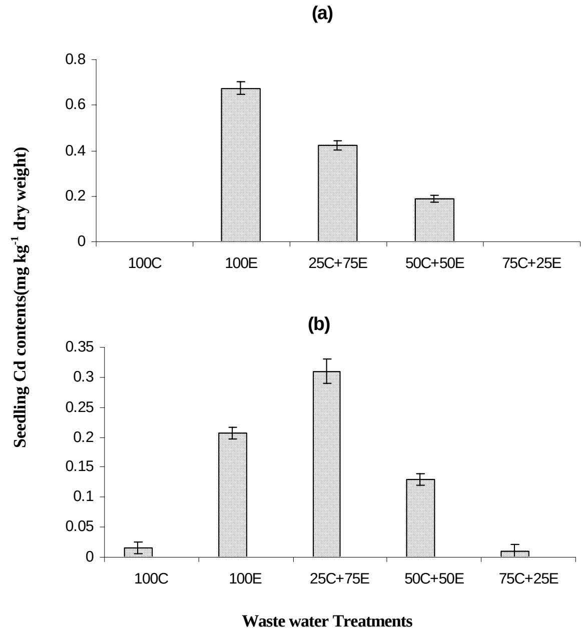
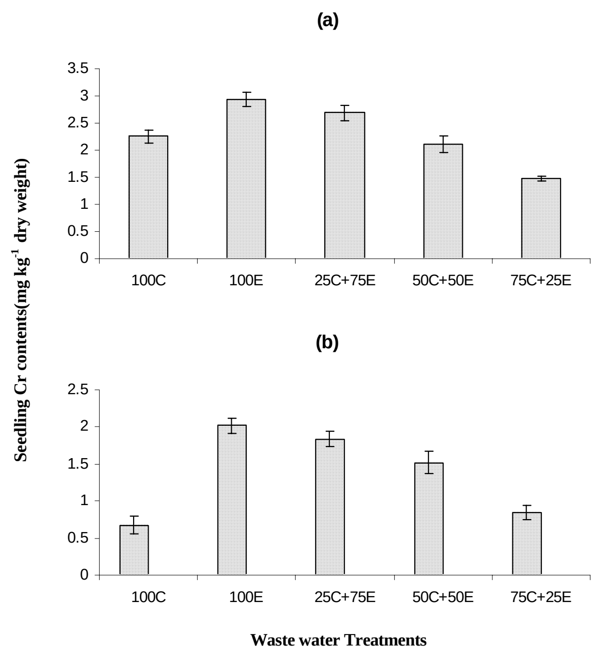


Fig. 2. Influence of waste water on the Cr accumulation in (a) *Azadirachta indica* and (b) *Dalbrgia sissoo* seedling± s.e



Heavy metals not only affect the nutrient homeostatis but also inhibit the photosynthetic apparatus (Jaswant, 1999; Pandit & Kumar, 1999). However, when canal water is mixed with the waste water, proportion of both determines the effectiveness of the mixture. The present study suggests that lower effluent water with higher canal water proportion

Fig. 3. Influence of waste water on the Mn accumulation in (a) *Azadirachta indica* and (b) *Dalbrgia sissoo* seedling± s.e

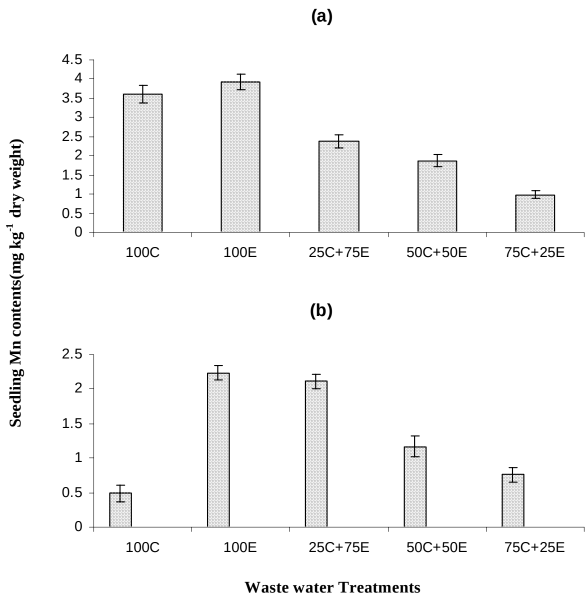
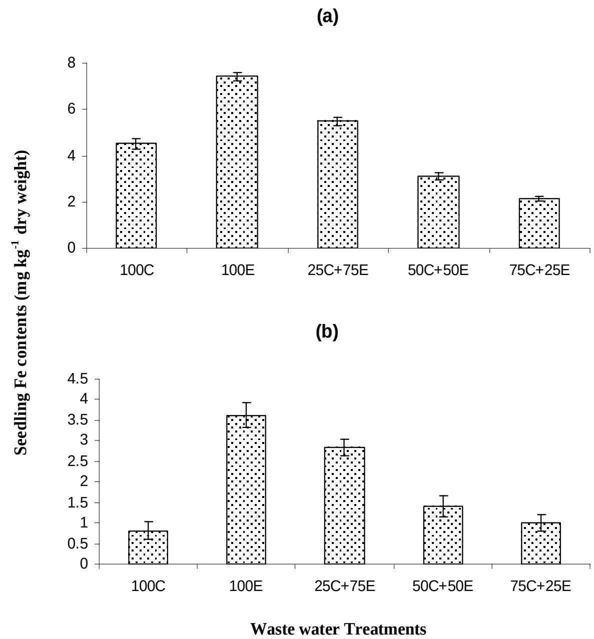


Fig. 4. Influence of waste water on the Fe accumulation in (a) *Azadirachta indica* and (b) *Dalbrgia sissoo* seedling± s.e



might be used effectively without an economic loss (Tables II, III).

These results also suggest that *Azadirachta indica* is hardy and grow more vigorously than *Dalbergia sissoo*. Growth and nutrient homeostatis were disturbed by higher levels of waste water; however, it can be used if mixed with canal water (25E + 75C).

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(Received 09 March 2006; Accepted 22 May 2006)