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Natural plant based solution for industrial wastewater

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Abstract

In developing countries like Pakistan, industries are increasing rapidly and are reluctant to manage industrial wastes and wastewaters. This industrial wastewater (IWW) containing number of toxic chemicals, which join natural water streams to disturb the whole ecosystem. Among physical and chemical methods to treat IWW, nature based systems are gaining popularity being more eco-friendly, less laboriously and economical. One way is to irrigate forests with IWW where toxic chemicals/metals can be taken up by plants and is stored for longer time. We conducted seed germination studies on 5 tree species of family fabaceae. Healthy seeds of each species were surface sterilized and placed as 10 per Petri plate on 1 g cotton bed moistened with 15 ml of IWW diluted to 4 concentrations along with control, the Petri plates were placed in growth room for 15 days. Only 4 species responded well in the following sequence; *Dalbergia sissoo* L. > *Albizia lebbeck* (L.) Benth > *Bauhinia purpurea* L. > *Pongamia pinnata* (L.) Pierre. Seed germination percentage, germination time, seedling length and seedling fresh weight showed positive correlation with concentration of IWW. Heavy metal concentrations found in IWW were 0.006(Cu), 0.0097(Mn), 0.0014(Cr) and 0.0017(Pb) mgL^{-1}. At higher concentration of IWW the germination response was reduced to nil, may be due to the increased toxicity level. *Dalbergia sissoo* showed 65% germination in 100% IWW. The maximum mean time to germination (115 hrs) was observed in *Millettia peguensis* and the maximum tolerance index (122) was exhibited by *Dalbergia sissoo*. Based on germination index, mean time to germination, tolerance index and vigor index these species can be potential candidates to be used in forestry with diluted IWW irrigation. This study highlighted the use of IWW for forest irrigation benefits like, IWW management, IWW treatment, irrigation water scarcity and low forest cover.

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Introduction

Heavy metal values above threshold level affect long-term fertility of soils and ecosystem health (Baebera *et al*., 2013). Use of industrial effluents for irrigation (Prabhakar *et al*., 2004) and sludge used as manure are the major sources of heavy metal contamination (Hussain *et al*., 2010). In many developing countries due to non-avability of irrigation water, farmers are irrigating their crops with industrial effluents having high level of toxic metals like Cu, Pb, Cr, Ni, Mn, Fe, Zn and Cd (Ensink *et al*., 2004).

These heavy metals through food chain affect all the components of ecosystem. Bio-accumulation of these metals lead to chronic diseases in humans (Mansell *et al*., 2004). Removal of toxic metals from environment is essentially needed to render the animals including human beings lead healthy life.

Phytoremediation is the plant use for the purpose of heavy metal elimination/lessening from soil (Mongkhonsin *et al*., 2011; Naser *et al*., 2012). Some tree species like popular, pine and spruce have the capability to respond against toxic levels of heavy metals by root exudation of organic acids in soil that avoid metal uptake (Khaleel *et al*., 2013). Hyper-accumulation of heavy metal ions is a striking phenomenon exhibited by approximately < 0.2% of angiosperms (Rascio and Navari-Izzo, 2011). Table 1 show some species which have been investigated as metal hyper-accumulators. The best known angiosperm hyper-accumulator of metals is *Thlaspi* (now: Noccaea) *caerulescens* (pennycress), which can accumulate large amounts of Zn i.e. 39,600 mg/kg (Zhang *et al*., 2002).

Fabaceae or leguminoseae, is a large and economically important flowering plant family, members of which are found to grow in many different climates and environments around the world (Stevens, 2001). The present study was designed to investigate germination response of five tree species (family Fabaceae) to evaluate their use in natural plant based IWW treatment and urban forestry.

Materials and methods

Seed collection

Seeds of 5 tree species (*Dalbergia sissoo* L., *Albizia lebbeck* L. Benth, *Bauhinia purpurea* L., *Pongamia pinnata* L. and *Millettia peguensis*) of family Fabaceae were collected from Punjab Forest Department, Cooper Road, Lahore. Healthier seeds were selected stored in paper bags and used for germination experiments.

Surface sterilization of seeds

Healthy seeds of each species were surface sterilized by washing in distilled water, soaking in 10% bleach for 2 min, 0.5% $HgCl_2$ for 5 minutes, each solution added with 2 drops of Tween-20 and thorough rinsing with sterilized distilled water after each treatment. Seeds were then rinsed three times with distilled water.

Wastewater collection and analysis

Industrial wastewater (IWW) was collected from Quaid-e-Azam industrial state, Kot Lakpat, Lahore and analyzed for heavy metals according to standard protocols. Five treatments of industrial wastewater were prepared by making dilutions including 0% dilution as control (Table 2).

Seed Germination

Bed of one gram cotton was used in Petri plates soaked with 15 ml of IWW (respective treatment). These Petri plates were sterilized in autoclave under 121°C, 15 lb/inch^2 pressure for 15 minutes. Ten surface sterilized seeds were place in each Petri plate and were covered properly. Petri plates containing seeds were placed in growth room under darkness. Each treatment had 3 replicates and all were placed in completely randomized block design for 15 days.

Germination parameters

Following parameters were recorded from germination setup, percentage germination, mean time to germination (MTG), seedling length, seedling fresh weight and tolerance index.

$$\text{Mean time to germination (MTG)} = \frac{\sum n \times d}{N}$$

Where; n = number of germinated seeds

d = time period to incubated

N = total number of seeds

$$\text{Tolerance Index} = \frac{\text{Wastewater treated seedling FW (g)}}{\text{Control seedling FW (g)}} \times 100$$

Seedling vigor index = Seedling length x germination %

Statistical analysis

ANOVA and Duncan's multiple range tests was conducted using SPSS (version 18).

Results and discussion

Industrial wastewater analysis

Industrial wastewater drained into natural streams carries heavy metals (Table 3) which cause serious threats to ecosystem. These metal ions disturb soil fertility and are carcinogenic for animals (Ensink *et al.*, 2004). Trend of using wastewater for irrigation is increasing day by day due to number of factors including irrigation water scarcity (Jelusic *et al.*, 2013).

Table 1. Important plant species which have been reported as metal hyper-accumulators.

Metal	Number of hyper-accumulator species reported	Plant species that accumulate particular metal specifically
Ni	320	*Berkheya coddii*
		Phidiasia lindavii
Cu	34	*Commelina communis*
		Crassula helmsii
Co	34	*Crotalaria cobalticola*
		Haumaniastrum robertii
Zn	18	*Thlaspi caerulescens*
		Arabis gemmifera
Pb	14	*Sesbania drummondii*
		Hemidesmus indicus
Cd	4	*Thlaspi caerulescens*
		Arabidopsis halleri
Cr (VI)	Not available	*Salsola kali Leersia hexandra Gynura*

(Naser *et al.,* 2012).

Table 2. Different treatments of IWW.

Treatments	To	T1	T2	T3	T4
Concentration of industrial wastewater	0% control	25%	50%	75%	100%

Percentage germination

Germination is an important parameter in urban forestry or agro-forestry study, species which show better germination are considered potential candidates for phyto-remediation. The results of the present study reveled that all the species differed significantly from one another in all treatments (Fig.1). It was noted that the increase in IWW lead to the decrease in germination percentage. The most drastic effect was observed with undiluted IWW. Among the 5 species the *D. sissoo* exhibited maximum germination percentage, i.e. 90% at 25% IWW, the minimum germination was shown by *M. peguensis* which was 68% with 25% of IWW.

Table 3. Heavy metal concentration in IWW.

Metal	Values (mg/L)
Cu	0.001–0.006
Mn	0.0013–0.0097
Cr	0.0003–0.0014
Pb	0.0004–0.0017

It was observed that at lower IWW concentration the difference in germination among 5 species was minor, may be at high concentration germination difference was well pronounced. This difference in germination response may be due to the variable needs of different species, some species requiring more nutrients than the other (Hussain *et al.*, 2010). At high concentration, the toxicity of wastewater was more apparent than effectiveness (Naser *et al.*, 2012). This parameter was useful in optimizing the wastewater quantity for urban forestry (Manu *et al.*, 2012).

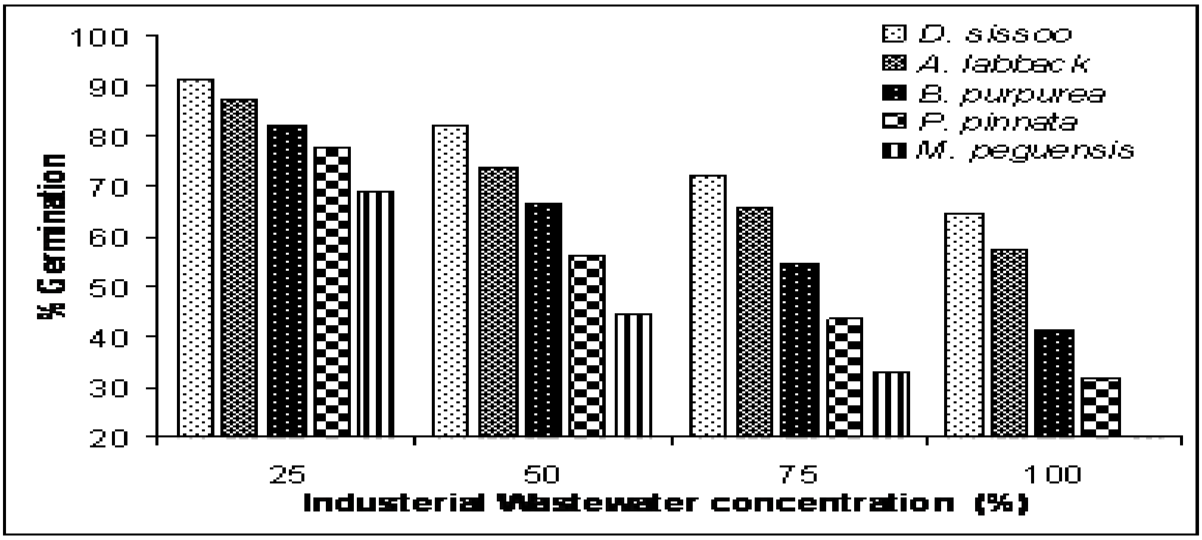

Fig. 1. Effect of IWW on germination.

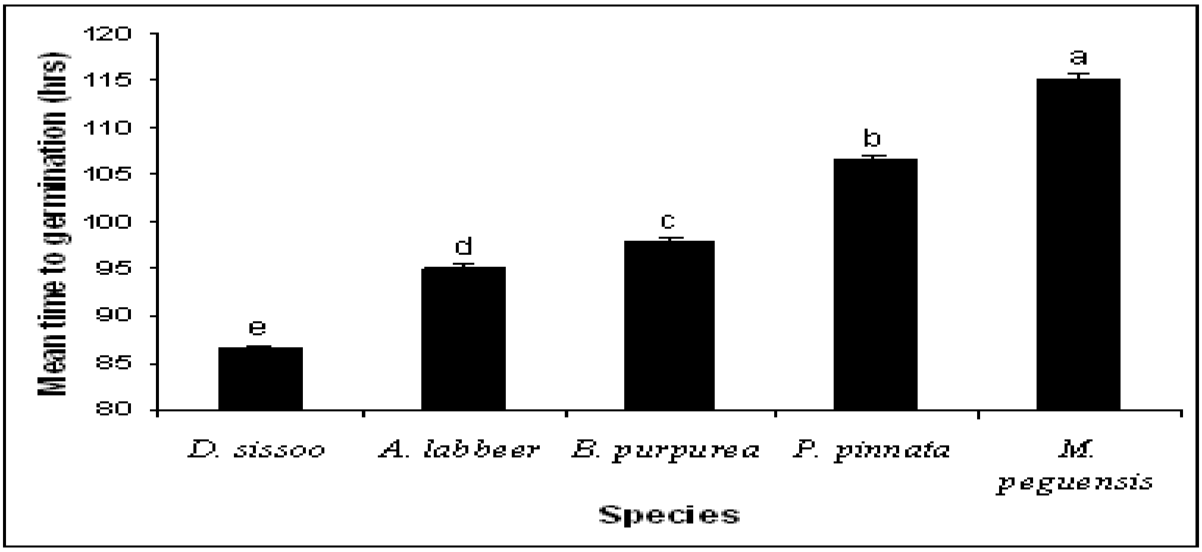

Fig. 2. Effect of IWW on mean time to germination.

Mean time to germination

Statistically all the five species differed significantly in mean time to germination (MTG). The MTG values were 87, 94, 97, 107 and 115 hr for *D. sissoo*, *A. lebbeck*, *B. purpurea*, *P. pinnata* and *M. peguensis*, respectively (Fig. 2).

Generally the species having less MTG are preferred over those who have high MTG. *Dalbergia sissoo* and *M. peguensis* showed sharp difference among the species, however, *A. lebbeck and B. purpurea* exhibited less sharp difference. The delay in germination differed between species and might be due to variation in seed size, seed coat permeability, differential uptake of nutrients, indigenous toxins and metabolites (Jelusic *et al.*, 2013).

Difference in MTG depends on seed size, seed coat thickness/permeability, degree of difference in toxin/nutrient uptake (Mekki *et al.*, 2007). Studies have reported strong negative impact of salts and phenolics on MTG (Mosse *et al.*, 2010).

Delay in germination has been reported in studies of olive mill wastewater (Mekki *et al.*, 2007) and diesel oil (Adam and Duncan, 2002).

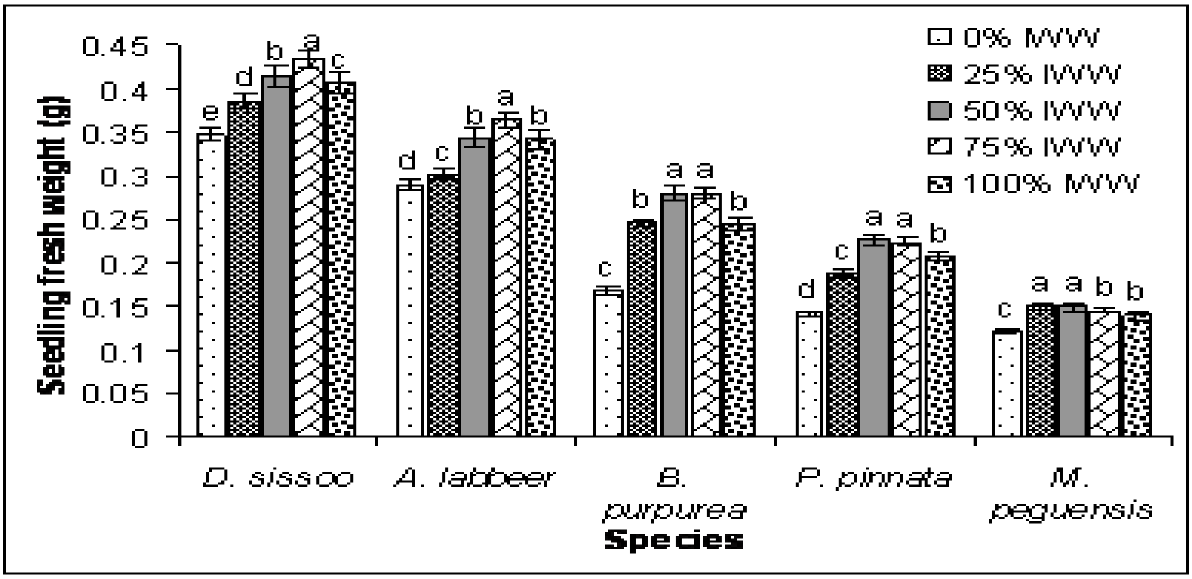

Fig. 3. Effect of IWW on seedling fresh weight.

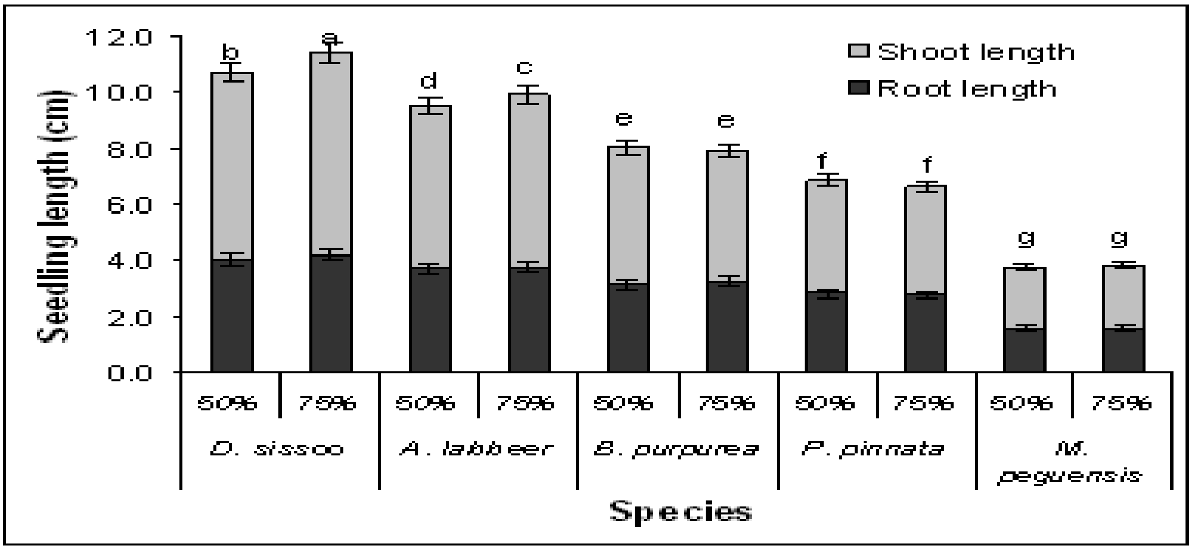

Fig. 4. Effect of IWW on seedling length.

Seedling fresh weight

Seedling fresh weight represents the health and tolerance of species, in this study five species differed significantly from one another, whereas same specie also differ significantly at different concentrations of IWW with reference to seedling fresh weight. Among species, *D. sissoo* exhibited maximum seedling fresh weight and *M. peguensis* showed lowest fresh weight (Fig. 3).

The order of fresh weight was:

D. sissoo > *A. lebbeck* > *B. purpurea* > *P. pinnata* > *M. peguensis*

Concentrations of IWW between 25-75% usually contribute better results. In an applied sense, it is important to know the effects of wastewater application on growth at different growth stages, to optimize IWW application. In seeds moistened with IWW, the time of emergence was likely to be affected, but the percentage of emergence was unlikely to be impacted (Mongkhonsin *et al.*, 2011).

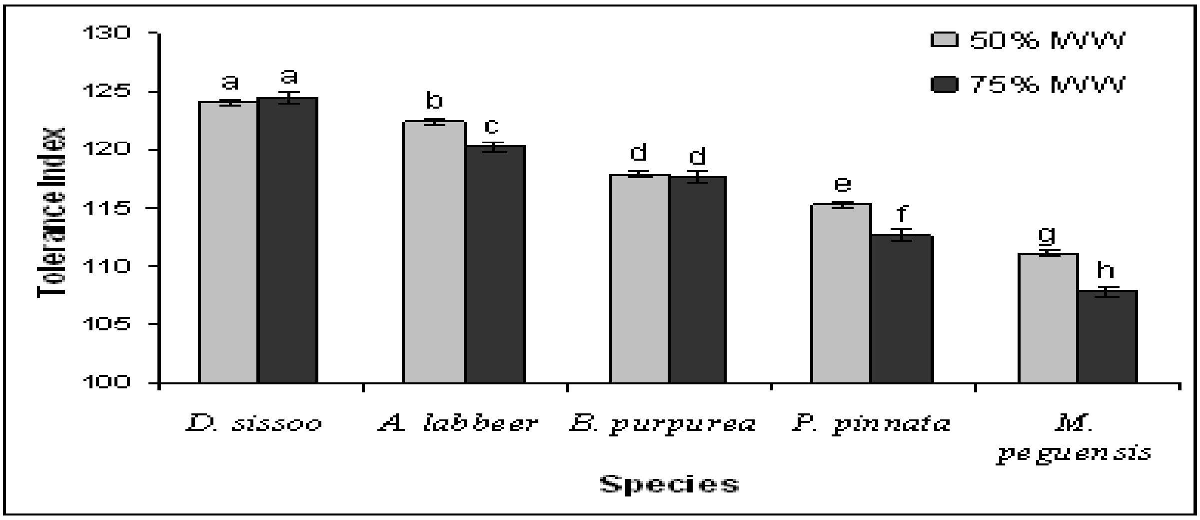

Fig. 5. Effect of IWW on tolerance index.

Industrial wastewater is complex in chemical properties (Manu *et al.*, 2012). At low concentrations the availability of nutrients are limited which decrease the seedling fresh weight, where as at high concentration, the abundance of nutrient make them toxic which hinder the physiochemical processes, leading to the decrease in seedling weight (Pena *et al., 2014*).

Seedling length

Seedling length was influenced softly by IWW irrigation. In *D. sissoo* and *A. lebbeck* the 75% concentration of IWW showed better results as compared to the 50%, however, *B. purpurea, P. pinnata* and *M. peguensis* did not show significant difference at 50% and 75% IWW. Among the five species root length showed less variation at different concentrations, whereas shoot length exhibited considerable variation (Fig. 4).

Uptake of metal ions by roots is influenced by many factors like, bioavailability in rhizosphere, metal concentration and binding affinity with other soil particles (Ali *et al.*, 2011).

During uptake, first hindrance is created by cell wall, but has low selectivity (Stutte *et al.*, 2006). Plasma membrane is more selective in metal ion uptake and it has secondary transporters, H^+ coupled carrier and channel proteins (Barbera *et al.*, 2013). Translocation through shoot is primarily controlled by root pressure and transpiration pull (Jelusic *et al.*, 2013).

Tolerance index

Tolerance index is effective tool to identify the tolerance level of species against different metal concentrations along with determination of the extent of IWW use for irrigation. In present study, *D. sissoo* showed high tolerance at 75% IWW, the other four species showed better tolerance at 50% IWW (Fig. 5).

This indicates that *D. sissoo* is more tolerant to higher metal concentration which makes it suitable candidate for phytoextraction and IWW irrigation. Statistical analysis reveled that all the species differed significantly from one another and for IWW concentration. Generally it is observed that high concentration is more toxic for plants. Plant has several mechanisms to minimize the effect of metal toxicity. One is to store metal ions in vacuoles so that they may not hinder cyto-chemical processes (Mosse *et al.*, 2010). Second is the binding of metal ions with ligands/chelatins like proteins, peptides and organic acids (Prabrakar *et al.*, 2004), however detailed evidences and mechanisms of ligands/chelatins are still not very clear (Manu *et al.*, 2012). Metal ions are converted into less toxic oxidation state to further reduce toxicity, this typically include reduction of Cr^{6+} to Cr^{2+} (Han *et al.*, 2014).

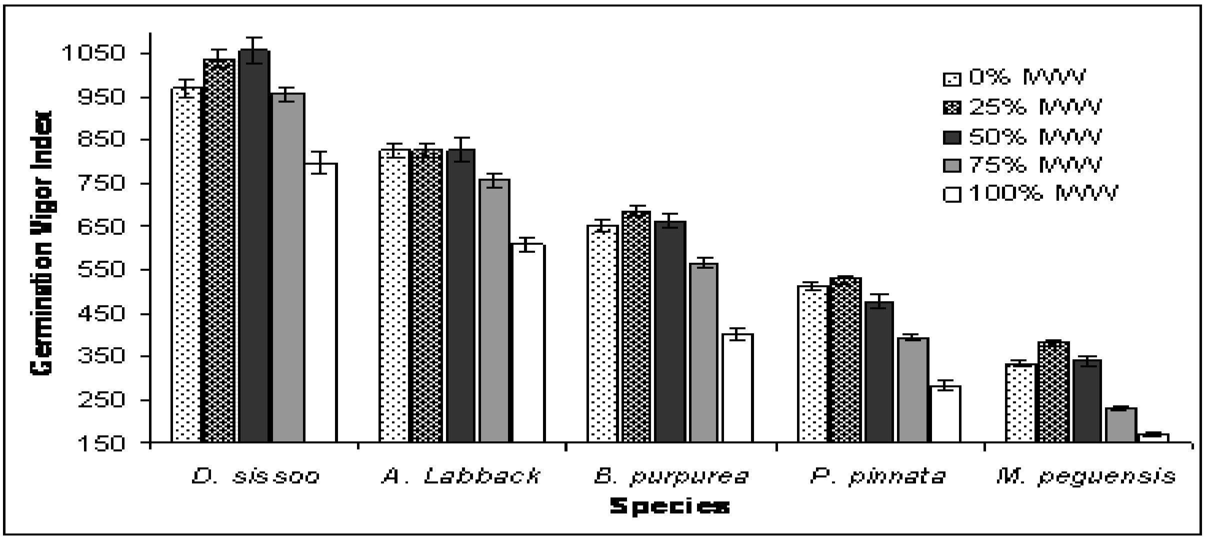

Fig. 6. Vigor index of selected species in IWW.

Vigor index

Promising results were obtained when different dilutions of IWW were compared for vigor index (VI).

The order of VI was: *D. sissoo* > *A. lebbeck* > *B. purpurea* > *P. pinnata* > *M. peguensis* (fig 6). *Dalbergia sissoo* stood more vigorous in terms of health and it showed positive signs of growth up to 75% of IWW, whereas *A. lebbeck* and *B. purpurea* seemed to show negative trend with 50% dilution of IWW and above. *Millettia peguensis* had least VI for only up to 25% of IWW and further increase in IWW concentration caused decrease in VI. Statistical analysis reveled that all the 5 species were significantly different from each other for VI. Other studies also reported somewhat similar results (Ali *et al.*, 2011; Naser *et al.*, 2012).

Conclusion

This study indicates that, *Dalbergia sissoo* L., *Albizia lebbeck* (L.) Benth, *Bauhinia purpurea* L., *Pongamia pinnata* (L.) Pierre and *Millettia peguensis* Ali are considerably tolerant in IWW and can be successfully used for phytoextraction processes.

The tolerance index was as follows: *Dalbergia sissoo* > *Albizia lebbeck* Benth > *Bauhinia purpurea* > *Pongamia pinnata* > *Millettia peguensis*. This idea to plant urban forests with above mentioned species and irrigating them with IWW (in diluted form), can reduce multiple and serious problems like, wastewater toxicity, lack of urban green areas, air pollution and serve as heat sinks. This study provides eco-friendly and sustainable solution for multiple problems.

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