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Germination response of five tree species on hospital wastewater

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Abstract

World is facing water scarcity and wastewater may be important source of water and nutrients for irrigation. Estimates reveal that about 10% of agri-land is irrigated usingwastewater with both positive and negative effects on ecosystem. Since wastewater reuse is currently necessary (due to water shortage). The best feasible management practice is to use wastewater for forest irrigation in and around cities. Species with higher tolerance index can be used in urban forestry. In the present study, germination studies of five tree species were investigated, using hospital wastewater (HWW). Germination experiment was conducted in Petri dishes in completely randomized design, each containing four seeds on filter paper moistened with different concentrations of HWW. Petri dishes in triplicate were placed under 16 hrs photoperiod. Statistical analysis revealed that germination %, seedling length andweight increased with increase in HWW concentration but concentration beyond 50% imparted negative effects. Study showed that only *Millettia peguensis* Ali, *Pongamia pinnata* L. Pierre, *Albizia lebbeck* L. Benth, *Bauhinia purpurea* L. and *Dalbergia sissoo* L. can withstand toxicity of HWW and thus can be the potential candidate for urban forestry. These species showed significantly high vigor index and tolerance index. Results support the use of HWW with 50% dilution for forest irrigation. This study suggests the possible use of HWW for forest irrigation and highlights the multiple benefits of HWW use in irrigation such as to improve forest cover and to reduce; pollutant migration in environment and water scarcity.

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Introduction

Water scarcity is the growing problem at world level. In developing countries the use of wastewater is increasing due to two main factors. Firstly due to unavailability of fresh water, secondly, wastewater has fertilizing properties (Miscellaneous, 2003). Developing countries do not have segregated system for hospital and sewage wastewater as a result of which the hospital wastewater getsmixed with sewage wastewater. Small farmers in peri-urban areas are fulfilling their irrigation water requirements with wastewater to grow fresh vegetables and fodder (Ensink *et al*. 2004b). Irrigation with wastewater has both advantages and disadvantages. Some of the common advantages are; high yield, whole year availability, whole year production, availability in arid and semi arid zones, reduction in fertilizer amount, cost effective, low cost water disposal, fresh water conservation, ground water recharge (Khaleel *et al.*, 2013), whereas, limitations or disadvantages of wastewater include; presence of pathogens, presence of toxic components, contamination of ground water, require sustainable planning (Pena *et al.*, 2014).

According to United Nations estimates, about 20 million hectares of land in 50 countries is under wastewater irrigation (United Nations, 2003); however no concrete information is available (Figure 1). Another estimate advocates that about one tenth of world's population is using products (mostly vegetables) growing on wastewater (Smit and Nasr, 1992). As in Hanoi (Vietnam) about 80% vegetables are irrigated with wastewater (Ensink *et al.*, 2004a).

Due to growing concern over the extensive use of wastewater for irrigation. Number of studies has recommended the use of wastewater for tree irrigation, as trees are non-edible. Another benefit is that the metals and toxic pollutants will remain bonded in tree wood and will not spread in food chain/food web. Researchers are planning to plant forests within cities (as urban forests) using wastewater irrigation. It will offer multiple benefits like; wastewater use, immobilization of toxic metals, provide urban green belts, carbon sinks and heat sinks (Mongkhonsin *et al.*, 2011).

Wastewater may be applied to forest in many ways like, without treatment (raw wastewater), with dilution and with treatment (reclaimed water) (Han *et al.*, 2014). One of the pioneer experiments for the use of wastewater in forest irrigation was conducted at CSSRI (Central Soil Salinity Research Institute), Karnal Haryana India. This experiment estimated the practicability of using raw wastewater (untreated) to irrigate trees like, *Leucaena leucocephala, Populus deltoids* and *Eucalyptus tereticornis*. The experiment lasted for 5 year and the selected tree species did not showed any negative growth sign (Das and Kaul, 1992). The present study is designed to investigate germination potential of five tree species under hospital wastewater irrigation for their potential use in urban forestry.

Materials and methods

Plant and seed selection

Based on the literature survey and in collaboration with Punjab Forest Department, following tree species of Fabaceae were selected for research; *Dalbergia sissoo* L., *Pongamia pinnata* (L.) Pierre, *Millettia peguensis* Ali, *Bauhinia purpurea* L., *Albizia lebbeck* (L.) Benth,. Seeds of all species were collected from Punjab Forest Department, Lahore. Physical screening was conducted and unhealthy and damaged seeds were discarded.

Waste water collection and analysis

Hospital wastewater was collected as per standard protocols from outlets of Combined Military Hospital, Services Hospital and Mayo Hospital, Lahore. Physiochemical analysis was conducted in Botany Department Government College University, Lahore. Different dilutions were prepared, as follow;

T0 = 100% tap water
T1 = 25% Hospital Wastewater
T2 = 50% Hospital Wastewater
T3 = 75% Hospital Wastewater
T4 = 100% Hospital Wastewater

Germination experiment

Seed germination experiment was conducted in Petridishes. In Petri dishes, filter paper was placed as base and 4 seeds were arranged in each petridish.

Petri dishes were placed in triplicates over lab table in complete randomized block design. Initially seed were irrigated with 3 ml HWW (respective dilution) and later 2ml HWW was used daily, under aseptic conditions. Emergence of radical was considered as the sign of germination. Germination parameters noted were; mean time for germination, germination %, roots and shoots length, seedling length, fresh weight, tolerance index and vigor index. Different equations used are as follow;

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

$$\text{Seedling vigor index} = \text{Germination } \% \times \text{Seedling length}$$

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

Where: n = number of germinated seeds

d = incubation time

N = total number of germinated seeds

Statistical analysis

Germination data was statistically analyzed using Co-stat. Tests applied was ANOVA and Duncan's Multiple Range.

Results and discussion

Wastewater analysis

Hospital wastewater analysis revealed that all the parameters like COD, BOD, EC, pH, TS, TSS, TDS, etc. (Table 1) were above the National Environmental Quality Standards (NEQS). Nutrients like potassium, sodium and nitrogen were close to acceptable limit. The TDS and TSS were quite low compared to NEQS, probably due to dilution factor or due to settlement of suspended particles. Hospital wastewater is the combination of lot of drugs, metabolites, chemicals and sewage waste.

Table 1. Physio-chemical characteristics of hospital wastewater.

Parameters	Values
pH	7.9
EC (µs)	94
TDS (mgL^{-1})	0.0043
TSS (mgL^{-1})	0.0021
COD (mgL^{-1})	654
BOD(mgL^{-1})	335
N (%)	2.16
K (ppm)	732
P (ppm)	487
Cl^{-1} (mgL^{-1})	367
Mg^{+2}(mgL^{-1})	98
Ca^{+2} (mgL^{-1})	201

All the values are the mean of 3 replicates.

This complex nature of HWW makes it both a 'resource' and 'problem'. Presence of sewage waste in HWW makes it a resource, as it consists of biodegradable organic waste, whereas, presence of toxic chemicals and metabolites makes it a problem. Sewage sludge degrades into non-toxic fulvic and humic acid. Generally, biodegradation of organic compounds is more facilitated in soil compared to water bodies (Han *et al.*, 2014).

With controlled irrigation the BOD or TOC drops rapidly within a few meter wastewater percolations(Mansell *et al.*, 2004; Khaleel *et al.*, 2013). Nitrogen removal from WW during irrigation depends on 2 main factors. Firstly the nitrogen content and secondly the WW quantity. Studies recommend the rotation of flooding and drying periods to enhance nitrogen removal.

Table 2. Relationship between soaking time and seed germination.

	Time (hrs)			
	3	4	5	6
D. sissoo	+	-	-	-
A. labbeck	+	+	-	-
B. purpurea	+	-	-	-
P. pinnata	+	+	+	-
M. peguensis	+	+	+	+

Table 3. Relationship between seed germination and moisture requirement.

	Moisture requirement (ml)			
	5	10	15	20
D. sissoo	+	++	+++	+
A. labbeck	+	++	+++	+
B. purpurea	+	++	+++	+
P. pinnata	+	++	+++	+++
M. peguensis	+	++	++	+++

This rotation facilitates nitrification/denitrification process and may lead to 75% (up to) nitrogen removal (Ali *et al.*, 2011; Jelusic *et al.*, 2013). One drawback in SW irrigation is that the potassium ions binds with iron, aluminum or calcium and is converted into insoluble form. With the passage of time these immobilized phosphate may become soluble (Barbera *et al.*, 2013).

Soaking time

Research studies have revealed that the seed germination is dependent on optimum soaking time (Mosse *et al.*, 2010; Khaleel*et al.*, 2013). Its optimum value is critical, too short or too long soaking time is detrimental for germination. Soaking basically serves three important functions, firstly it provides aqueous environment which is essential for activating enzymes.

Table 4. Relationship between seed germination % and HWW.

	Concentration of HWW			
	25%	50%	75%	100%
D. sissoo	95.8 ±	90.0 ±	82.0 ±	70.2 ±
	1.64 [a]	1.56 [a]	2.35 [a]	1.37 [a]
A. labbeck	92.4 ±	84.9 ±	73.1 ±	63.1 ±
	2.54 [b]	2.43 [b]	1.43 [b]	2.02 [b]
B. purpurea	88.5 ±	76.8 ±	61.6 ±	51.3 ±
	2.04 [c]	2.11 [c]	1.55 [c]	2.07 [c]
P. pinnata	83.2 ±	67.8 ±	48.8 ±	37.6 ±
	2.50 [d]	1.45 [d]	1.32 [d]	1.54 [d]
M. peguensis	76.5 ±	61.6 ±	43.7 ±	33.6 ±
	2.54 [e]	2.23 [e]	2.08 [e]	1.54 [e]

Treatment means followed by different letters in each column are significantly different at p=0.05 according to Duncan's Multiple Range Test.

Secondly, it dissolves monosaccharides and oligosaccharides and also helps in the hydrolysis of polysaccharides. Thirdly, water softens the seed coat and makes it possible for radical and plumule emergence(Pena *et al.*, 2014). Statistical analysis revealed that the maximum socking time of 5.8 hrs is required by the *M. peguensis* and the least socking time of 3 hrs is of *D. sissoo* (Table 2).

Duncan Multiple range test revealed that the *D. sissoo and B. Purpurea* does not differ significantly between them. Similarly, *P. pinnata* and *A. lebbeck* show the same socking time of 4.5 hrs without any statistical difference (Kanwal *et al.*, 2015). Generally species with less socking time requirement have more practical applications (Barbera *et al.*, 2013).

Table 5. Impact of HWW on seedling fresh weight (g).

	Concentration of HWW				
	0%	25%	50%	75%	100%
D. sissoo	0.278 ± 0.0083[a]	0.308 ± 0.0092[a]	0.332 ± 0.0099[a]	0.347 ± 0.0104[a]	0.325 ± 0.0097[a]
A. labbeck	0.232 ± 0.0070[b]	0.241 ± 0.0072[b]	0.274 ± 0.0082[b]	0.291 ± 0.0087[b]	0.273 ± 0.0082[b]
B. purpurea	0.135 ± 0.0040[c]	0.197 ± 0.0059[c]	0.225 ± 0.0067[c]	0.224 ± 0.0067[c]	0.196 ± 0.0059[c]
P. pinnata	0.114 ± 0.0034[d]	0.151 ± 0.0045[d]	0.181 ± 0.0054[d]	0.179 ± 0.0054[d]	0.166 ± 0.0050[d]
M. peguensis	0.097 ± 0.0029[e]	0.120 ± 0.0036[e]	0.120 ± 0.0036[e]	0.116 ± 0.0035[e]	0.112 ± 0.0034[e]

Treatment means followed by different letters in each column are significantly different at p=0.05 according to Duncan's Multiple Range Test.

Moisture requirement

Moisture requirement is one of the prerequisites for seed germination.All species show hyperbolic trend, which means that seeds need optimum water quantity. Increase or decrease from optimum value will decrease the germination percentage. All 5 species respond differently at different water concentration. Optimum water requirement for *B. Purpurea, A. Lebbeck, D. Sissoo* and *M. Peguensis* is 12 to15 ml, whereas *P. Pinnata* showed optimum germination at 18 to 20 ml (Table 3). Statistical analysis showed significant difference among 5 species (Kanwal *et al.*, 2015). At low moisture, germination is effected due to the lack of moisture and mostly metabolic processes are dependent on water (Ali *et al.*, 2011), whereas, at high moisture content germination was reduced due to the poor aeration, reduced oxygen, leaching of essential molecules (enzymes, soluble food reserves, etc.) from seed by ex-osmosis (Khaleel *et al.,* 2013).

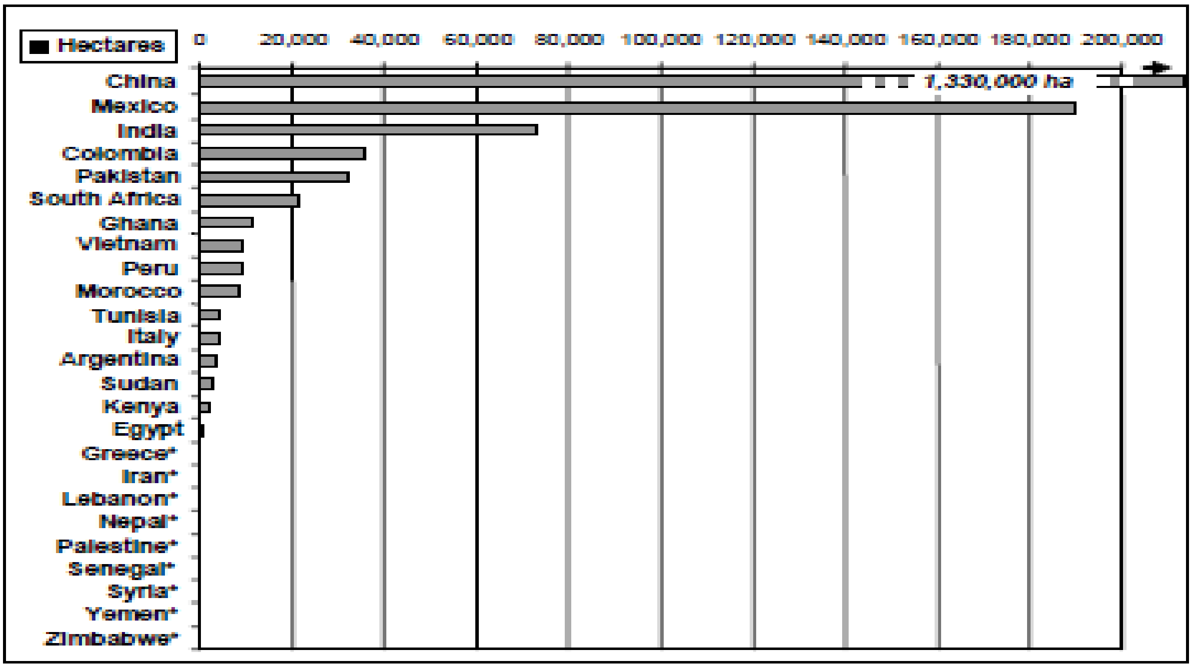

Fig. 1. Area under wastewater irrigation at international level.

Percentage germination

Hospital wastewater is rich in nutrientsalongwith toxic materials like pharmaceutics, detergents and surfactants etc. Theoretically, it is expected that due to more nutrients wastewater irrigation will enhance germination. The same trend is visible in the Table 4. At low HWW concentration, germination is increased as compared to the control and as the concentration of waste water wasincreased the germination percentage was decreased (Prabhakar *et al.*, 2004), however, the 5 experimental species differed significantly in their germination response. Better response was shown by *D. Sissoo* and the lesser response was of *B. Purpurea*. Statistically, *M. Pegaensis* and *P. Pinnata* do not differ significantly at 25% hospital wastewater. Similarly, *D. Sissoo* and *A. lebbeck* responded in same manner at highest concentration.

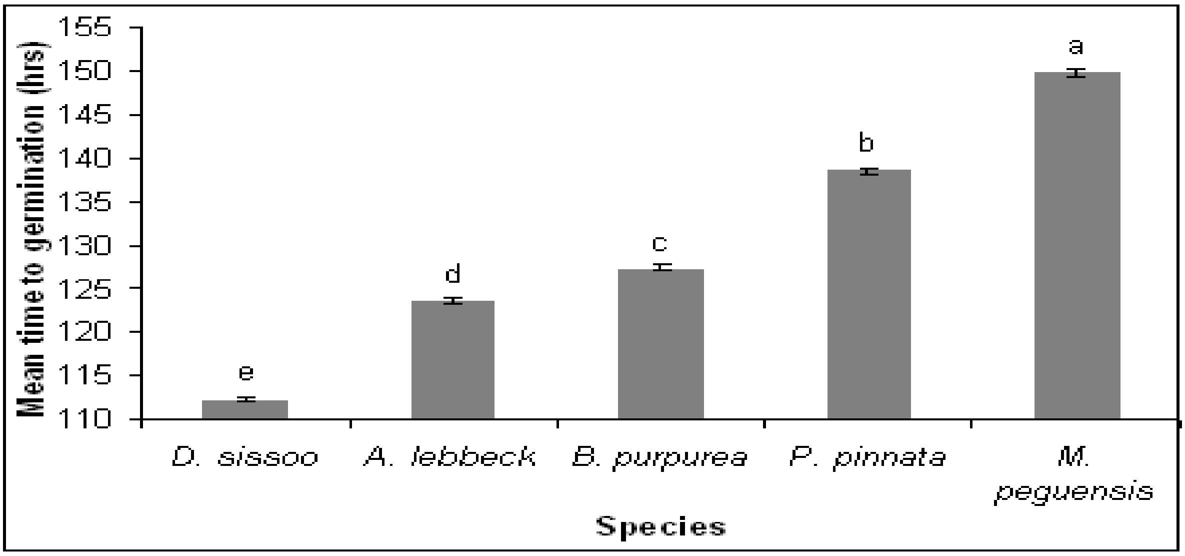

Fig. 2. Impact of HWW on germination time.

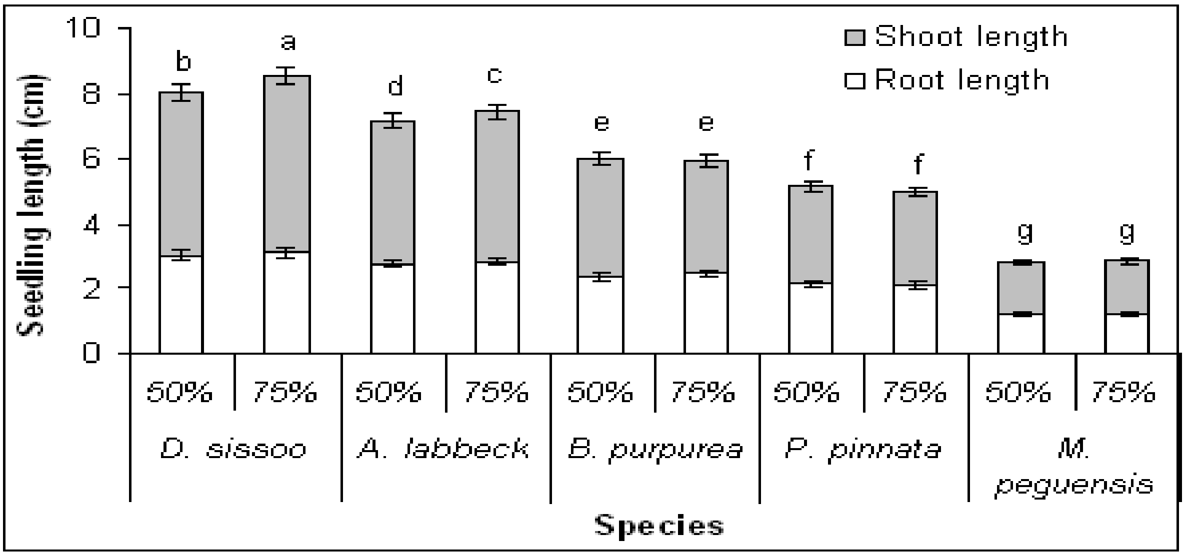

Fig. 3. Influence of HWW on root and shoot length.

This difference in germination response may be due to the variable needs of difference species, some need more nutrients and others need lesser nutrients (Hussain *et al.*, 2010). At high concentration, the toxicity of wastewater was more apparent than its effectiveness. This parameter was useful in optimizing the wastewater quantity in urban forestry (Manu *et al.*, 2012).

Mean time for germination

Hospital wastewater significantly reduced mean time for germination (Adam and Duncan, 2002). All the five species differed significantly in mean time for germination (MTG). The minimum time was exhibited by *D. sissoo* and maximum was reported by *M. peguensis* (Fig. 2).

The MTG values are 112, 124, 127, 137 and 150 (hrs) for *D. sissoo, A. lebbeck, B. purpurea, P. pinnata* and *M. peguensis,* respectively. 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 influence of salts and phenolics in delaying MTG (Mosse *et al.*, 2010).

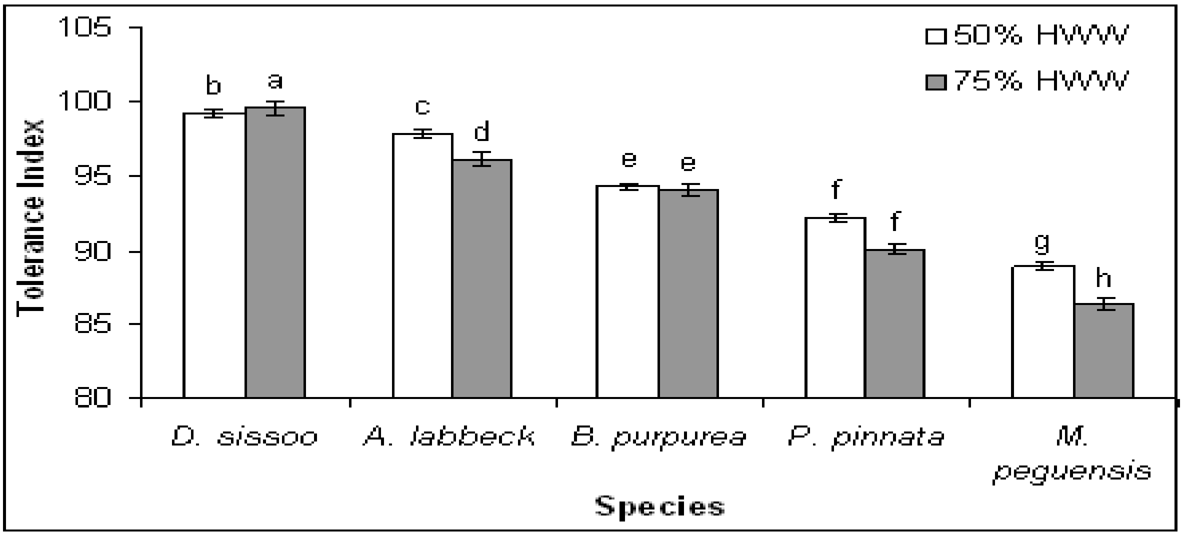

Fig. 4. Tolerance response in HWW.

Seedling length

Seeding length was affected by wastewater but was dependent on the nature and concentration of wastewater. Data for root and shoot length showed (Fig. 3) that *D. sissoo* represented the longestshoots and roots at 75% of wastewater. Other species also exhibited considerably better length at 75% of HWW, which may be due to availability of the more nutrients (Barbera *et al.*,2013). Statistical analysis revealed the seedling length in the following order:

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

Seeding fresh weight

Seedling fresh weight was measured in order to optimize the treatment for irrigation. Again the trend was somewhat similar as was in germination percentage. With the increase in HWW concentration the fresh weight increased up to 75% but further increase in concentration decrease the fresh weight due to the toxicity (Table 5).

B. purpurea did not differ significantly at 50-75% of HWW. *Milletia peguensis* showed least difference from 25-75%. It is not clear that which component of HWW exhibited phyto-toxicity at higher concentration, however, some evidences suggest the involvement of phosphorous, sodium, ethanol and polyphenols (Stutte *et al.*, 2006). The variation in species may be due to the tolerance and differential nutrient requirements (Mosse *et al.*, 2010).

Tolerance index

Tolerance index is another important parameter to get specie response. From the results it is clear that the *D. sissoo* is the most tolerant out of the five species (Fig. 4). Statistical analysis (one way ANOVA and Duncan's Multiple Range test) also revealed that the tolerance index decreased with the increase in wastewater concentration. The maximum tolerance was shown by *D. sissoo* at 75% of HWW (TI-99) and the least tolerance was exhibited by *M. peguensis* (TI-86). All the rest species differedsignificantly from one another.

At high concentration of WW the salt accumulation increased which interfered with translocation and photosynthesis (Jelusic *et al.*, 2013). Salt accumulation also disturbs the osmotic balance between soil and roots which creates problems in water absorption. Those species which are more tolerant are useful in agro-forestry (Pena *et al.*, 2014).

Vigor index

When different dilutions of wastewater were compared for vigor index (VI), very promising results were exhibited. Statistical analysis showed that the concentrations of HWW and the species differ from one another. The maximum VI was shown by *D. sissoo* in 25-75% HWW.

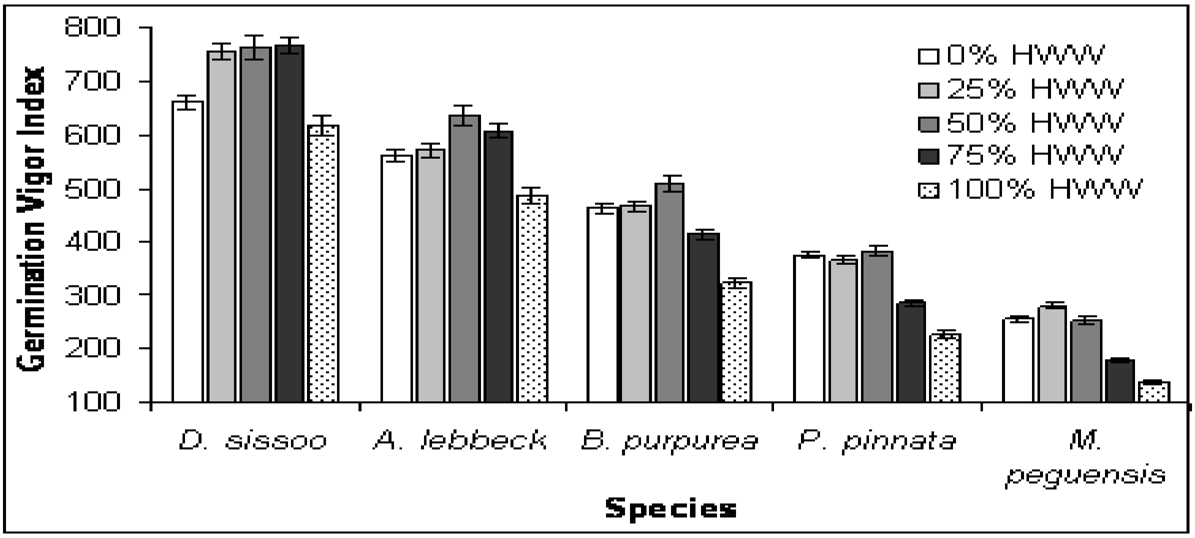

Fig. 5. Effect of HWW on vigor index.

The VI increased with the increase in HWW concentration but after certain concentration it was decreased (Fig. 5), probably due to the fact that in dilute solution the toxic molecules are less and as the concentration increases the amount of toxic molecules also increases (Ali *et al.*, 2011).

Conclusion

This study suggests solution to reduce pollution of HWW with indigenous species. Instead of artificial and man-made water purification systems we may opt nature-based system with some modifications, as they are more reliable, eco-friendly and sustainable. It may provide multiple benefits like reduction in air pollution; increase in forest cover, urban green spots and also to serve as heat sink.

References

Adam G, and Duncan H. 2002. Influence of diesel fuel on seed germination. Environmental Pollution **120,** 363-370.
https://www.ncbi.nlm.nih.gov/pubmed/12395850

Ali HM, EL-Mahrouk EM, Hassan FA, EL-Tarawy MA. 2011. Usage of sewage effluent in irrigation of some woody tree seedlings. Part 3: *Swietenia mahagoni* (L.) Jacq. Saudi Journal of Biological Sciences **18,** 201-207.
http://dx.doi.org/10.1016/j.sjbs.2010.08.001

Barbera AC, Maucieri C, Ioppolo A, Milani M, Cavallaro V. 2013. Effects of olive mill wastewater physico-chemical treatments on polyphenol abatement and Italian ryegrass (*Lolium multiflorum* L) germinability. Water research **52(1),** 275-281.
http://dx.doi.org/10.1016/j.watres.2013.11.004

Das DC, Kaul RN. 1992. Sewage water: utilization through forestry. Karnal, India, Central Soil Salinity Research Institute. Greening wastelands through waste water. New Delhi, National Wastelands Development Board.
www.fao.org/3/a-w0312e/w0312e09.htm

Ensink J, Mahmood T, VanderHoek W, Raschid-Sally L, Amerasinghe F. 2004. A nationwide assessment of wastewater use in Pakistan: An obscure activity or a vitally important one? Water Policy **6,** 197-206. http://wp.iwaponline.com/content/6/3/197

Han P, Kumar P, Bee-Lian O. 2014. Remediation of nutrient-rich waters using the terrestrial plant, *Pandanus amaryllifolius* Roxb. Journal of Environmental Science **26(2),**404-414. https://www.ncbi.nlm.nih.gov/pubmed/25076532

Hussain F, Malik SA, Athar M, Bashir N, Younis U, Hassan MU, Mahmood S. 2010. Effect of tannery effluents on seed germination and growth of two sunflower cultivars. African Journal of Biotechnology **9(32),** 5113-5120. www.ajol.info/index.php/ajb/article/viewFile/92138/81572

Miscellaneous, IWMI (International Water Management Institute). 2003. Confronting the realities of wastewater use in agriculture. Water policy briefing 9. Colombo: IWMI.http://www.iwmi.cgiar.org/publications/briefs/water-policy-briefs

Jelusic M, Grcman H, Vodnik D, Suhadolc M, Lestan D. 2013. Functioning of metal contaminated garden soil after remediation. Environmental Pollution **174,** 63-70. https://www.ncbi.nlm.nih.gov/pubmed/23246748

Khaleel RI, Ismail N, Ibrahim MH. 2013. The Impact of Waste Water Treatments on Seed Germination and Biochemical Parameter of *Abelmoschus Esculentus* L. Procedia - Social and Behavioral Science **91,** 453-460. http://www.sciencedirect.com/science/article/pii/S1877042813025743

Mansell J, Drewes J, Rauch T. 2004. Removal mechanisms of endocrine disrupting compounds (steroids) during soil aquifer treatment. Water Science and Technology **50(2),** 229–237. http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.483.3265&rep=rep1&type=pdf.

Manu KJ, Kumar M, Mohana VS. 2012. Effect of Dairy Effluent (treated and untreated) on Seed Germination, Seedling Growth and Biochemical Parameters of Maize (*Zea mays* L.). International Journal of Research, Chemistry and Environment **2(1),** 62-69. http://journals.indexcopernicus.com/abstract.php?icid=1060467

Mekki A, Dhouib A, Sayadi S. 2007. Polyphenols dynamics and phytotoxicity in a soil amended by olive mill wastewaters.Journal of Environmental Management **84,** 134-140. http://dx.doi.org/10.1016/j.jenvman.2006.05.015

Mongkhonsin B, Nakbanpote W, Nakai I, Hokura A, Jearanaikoon N. 2011. Distribution and speciation of chromium accumulated in *Gynura pseudochina* (L.) DC. Environmental and Experimental Botany**74,** 56-64. http://dx.doi.org/10.1016/j.envexpbot.2011.04.018

Mosse KPM, Patti AF, Christen EW, Cavagnaro TR. 2010. Winery wastewater inhibits seed germination and vegetative growth of common crop species. Journal of Hazardous Material **180,** 63-70. http://www.sciencedirect.com/science/article/pii/S0304389410002839

Pena A, Mingorance MD, Guzmán I, Sánchez L, Espinosa AJ, Valdés B, Rossini-Oliva S. 2014. Protecting effect of recycled urban wastes (sewage sludge and wastewater) on ryegrass against the toxicity of pesticides at high. Journal of Environmental Managment**142,**23-29. https://www.ncbi.nlm.nih.gov/pubmed/24797639

Prabhakar PS, Mall M, Singh J. 2004. Impact of fertilizer factory effluent on Seed Germination, Seedling growth and Chlorophyll content of Gram (*Ciceraeritenum*). Journal of Environmental Biology **27(1),** 153-156. https://www.ncbi.nlm.nih.gov/pubmed/16850894

Smit J, Nasr J. 1992. Urban agriculture for sustainable cities: Using wastes and idle land and water bodies as resources. Environment and Urbanization **4(2),** 141-152. http://www.klamathsustainablecommunities.org/articles/urbanAg/urbanAgForSustCities.pdf.

Stutte GW, Eraso I, Anderson S, Hickey RD. 2006. Bioactivity of volatile alcohols on the germination and growth of radish seedlings. Hortriculture Science **41,** 108-112.

United Nations. 2003. Water for people, water for life. The United Nations world water development report. Barcelona: UNESCO.