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PHYTOMORPHOLOGY

Effects of various physicochemical treatments on seed germination of selected tree species in Delhi, India

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Abstract

Seed germination is a critical factor for the establishment of a green microclimate in urban areas, which could be hindered by seed dormancy. The period of seed germination is very sensitive to ambient environmental conditions so, can be used as indicator of Pb (lead) pollution. For the present study, eight commonly growing tree species in Delhi were selected to identify the species with maximum germination under five pre-sowing treatments i.e., control, cold water, hot water, acid treatment and mechanical scarification to select the best treatment. Among selected species, *Terminalia arjuna*, *Cassia fistula* and *Millettia pinnata* showed highest seed germination and survival and were further used to study the impact of Pb contamination. For *T. arjuna*, seed germination and survival ranged from 46 to 88% and 75.86 to 95.45%, respectively, with maximum germination and survival in hot water and minimum in control. For *C. fistula*, both germination and survival ranged from 30% (control) to 88% (mechanically scarified) and from 80% (acid treated) to 96.15% (hot water), respectively. *M. pinnata* showed the highest germination and seedling survival in mechanical scarification treatment i.e., 88 and 85.37%, respectively, and lowest in control i.e., 42 and 75.76%, respectively. Further, both parameters decreased with increase in Pb concentrations in soil. Germination varied from 42 to 78% in *T. arjuna*, 40 to 82% for *C. fistula*, and 42 to 86 % for *M. pinnata.* Similarly, seedling survival varied from 57.14 to 84.62% for *T. arjuna*, 45 to 90.24% for *C. fistula*, and from 47.62 to 93.02% for *M. pinnata.*

Keywords: Germination, Pre-sowing treatments, Pb-toxicity, Seed dormancy, Seedling survival

Introduction

Seedling is a very important stage of the plant life cycle that depends on processes like seed germination, seedling growth, and survival percentages (Gunaga & Vasudeva, 2011; Bhadouria et al., 2017). Seed dormancy is of several types, including mechanical dormancy which limits the maturity of the embryo and can be lowered either by routinely weakening the enclosing seed coat or pericarp (Bachelard, 1967; Amen, 1968). A fruit enclosure or seed coat that is waterresistant prevents imbibition, and in some cases gaseous exchange, resulting in physical dormancy (Rolston, 1978). Physical dormancy can be broken using various pre-sowing treatments such as hot water treatment, cold water treatment, acid treatment, and physical scarification of the seed coat using tools such as a knife, needle, or abrasion paper (Azad et al., 2006, 2011; Salim et al., 2010). Therefore, understanding the germination, survival rate, and seedling growth of many widely distributed tree species is necessary. The future plant community organization is controlled by processes that have an impact on the seedling stage (Bhadouria et al., 2017). Seed germination and seedling growth and survival phases are considered to be the critical factors for the establishment of new individuals and the founding of a population to maintain biodiversity (Rees, 1996; Azad et al., 2011; Missanjo et al., 2014). It will also help to establish and develop a green microclimate in urban areas such as green belts, urban parks, urban forests, roadside patches, etc. However, seed dormancy interrupts the process of the establishment of tree populations in particular areas (Schmidt, 2000).

The period of seed germination is very sensitive to ambient environmental conditions. Thus, it can be used as an indicator of environmental pollution (Ma et al., 2013; Yang et al., 2016). Among various pollutants, heavy metals are major concern as many of them are dangerous even at extremely low amounts. Lead (Pb) is the most prevalent heavy metal contaminant in soil and has drawn a lot of attention due to its widespread distribution and possible damage to the environment and ecosystem (Yang et al., 2016). Numerous studies have examined the impacts of Pb toxicity on seed germination and seedling survival in various plant species (Ma et al., 2013, Yang et al., 2016). Pb is a significant ecological threat because of its long-term persistence in the ecosystem and its effects on human health via the food chain (Shu et al., 2012). High levels of Pb in the soil prevent seeds from germinating, reduce plant growth, and result in a variety of metabolic changes in plants (Kumar et al., 2012; Srivastava et al., 2014). Previous studies have found that phytoremediation is the most efficient and sustainable way to remove Pb from soil (Shu et al., 2012; Yang et al., 2016). In order to remediate the polluted soil, it is crucial to choose suitable plant species that can endure Pb toxicity even at the seedling stage and can accumulate Pb (Srivastava et al., 2014).

Therefore, the present study was conducted to identify the best commonly growing tree species (selected on the basis of their abundance and seed availability) with high seed germination and seedling survival under various pre-sowing treatments. For assessment of seed germination, five treatments were used viz., control (no treatment), cold water, hot water, acid treatment, and mechanical scarification. Consequently, the best performing species under the best-suited treatments were selected with the highest germination and seedling survival. Further, these shortlisted species were used to study the impact of Pb contamination in soil on their seedling stage by estimating the variation in seed germination and seedling survival with the variations in Pb concentrations in soil.

Material and methods

Seed collection

The present study was carried out in the NCT (National Capital Territory) of Delhi

(28°24'17" N and 28°53'00" N latitudes and 76°50'24" E and 77°24'17" E longitudes) with an area of 1483 km^2. Delhi features a dry-winter humid subtropical climate with a hot-summer semi-arid climate. Seeds were collected from the campus of the University of Delhi (DU) which is a commercial and moderately polluted site because of more anthropogenic activities. The experiment was carried out in open-air conditions under a rain-protected net house located in the garden of the Department of Botany, University of Delhi. Seeds were collected from commonly grown plant species that were high in abundance and seed availability. These plant species include Arjuna (*Terminalia arjuna*), Indian beech (*Millettia pinnata*), Cemetery tree (*Polyalthia longifolia*), Indian rosewood (*Dalbergia sissoo*), White siris (*Albizia lebbeck*), White leadtree (*Leucaena leucocephala*), Golden shower tree (*Cassia fistula*) and Captain cook tree (*Cascabela thevetia*). The general description of selected plant species is given in Table 1.

Seed viability test

Seed viability was tested using TTC test with the help of 2,3,5-tetrazolium chloride (0.1%, pH- 7) which was prepared in phosphate buffer solution. This method involves 12 hr soaking of seeds in water at room temperature for proper imbibition so that de-coating of seeds would be done easily. Soaking was followed by de-coating of seeds and then the seeds were kept in 0.1% TTC solution in darkness at room temperature for a staining period. Seeds that were stained red were classified as viable, while improperly stained or unstained seeds were considered unviable (Copeland, 2001; Takos & Efthimiou, 2003; Srivastava et al., 2014; Nakar & Jadeja, 2014).

Experimental set-up

First set-up: For the germination experiment setup, the garden soil used was dried and air-passed through a 5 mm sieve to remove the large stone and grass debris. Before Pb treatment, the soil was first characterized (sandy loam with pH- 7.6 ± 0.41, EC- 388 µS, Pb- 23.33 mg/kg dry soil) and mixed with compost (in 7:3 ratio). Large crates with dimensions of 60 × 40 × 20 cm were filled with 25 kg of compost mixed soil and in replicates for each of pre-sowing treatment. All seeds were sterilized with 70% ethanol for 30 sec followed by a 5 min treatment with 2.5% sodium hypochlorite and washed three times with distilled water. Sterilization followed by pre-germination treatments (Kumar et al., 2007; Azad et al., 2011; Humtsoe et al., 2018):

Treatment 1: Control seeds were directly sown in the soil for germination experiments.

Treatment 2: Cold water treatment which includes soaking of seeds in cold water (4°C) and afterward immersed in it for 24 hr.

Treatment 3: Hot water treatment for which water was boiled at 85°C and seeds were immersed in the hot water then remain immersed for 24 hr.

Treatment 4: Acid treatment where seeds were treated with conc. H_2SO_4 for 30 minutes followed by washing with distilled water and soaked for 24 hr.

Treatment 5: Mechanical scarification of seeds done by damaging the seed coat using sandpaper, and blade, followed by soaking in water for 24 hr. similar to other treatments.

Seeds after each treatment were transferred to the already prepared plastic crates and each crate was labeled and kept in open-air conditions under a rain-protected net house located in the garden of the Department of Botany, University of Delhi. Crates were regularly watered to maintain sufficient moisture and monitored for germination for the next three months, the emergence of radical was considered germination.

Second set-up: Among the eight plant species tested for pre-sowing treatment, only three species (viz., *T. arjuna*, *C. fistula* and *M. pinnata*) which had the highest germination and seedling survival rates were selected. Among the five treatments, seeds treated with mechanical scarification and hot water were found to have maximum germination and seedling survival rates. Therefore, all of the seeds of the three selected tree species were mechanically scarified then the seeds were thoroughly washed and sterilized using mercuric chloride. Followed by hot water treatment and soaking at room temperature for 24 hr. The soaked seeds were transferred to crates filled with 25 kg of compost-mixed garden soil supplemented with lead. Following the method of Chen et al. (2019), Pb was supplemented in the form of lead nitrate ($PbNO_3$) by spiking and thoroughly mixing. Four treatments viz., 0, 320, 720, and 960 mg of $PbNO_3$ kg^{-1} dry soil corresponding to 0 (control), 200, 400, and 600 mg kg^{-1} of Pb treatments in dry soil, which were selected from a consideration of safe limits of Pb levels in the soil given by Indian standards, WHO and European Union standards (Awashthi et al., 2000; Joint FAO/WHO, 2007; Kinuthia et al., 2021). Similar to the above-mentioned experiment, crates were regularly watered to maintain sufficient moisture and monitored for germination for the next three months.

Data evaluation

Germination was recorded daily for the experimental time period and the daily germination was summed up to calculate the cumulative germination for each treatment for each species. Germination percentage (GP) is calculated by dividing the total number of germinated seeds in each treatment by the total number of seeds sown initially and multiplied by 100 (Feizi & Mousavi, 2016; Zazai et al., 2018).

Further, seedling survival percentage was also calculated using recorded readings at end of three months after seed sowing. Survival percentage (SP) is calculated by dividing the number of seedlings survived by the total number of seeds germinated and multiplied by 100 (Zazai et al., 2018).

Statistical analysis

For each parameter data is represented as mean value ± standard error. Statistical analysis was done using SPSS (version 21). To observe the effect of plant species and various treatments on the GP and SP, two-way ANOVA (analysis of variance) with Tukey's post-hoc test in a completely randomized design (CRD) was performed. Further, one-way ANOVA was done to observe the significance of the effects of various treatments on each species, separately. The level of statistical significance was recorded at $P \leq 0.05$.

Results

Viability of seeds using TTC test indicate that among selected eight species highest viability (%) was for *L. leucocephala* (95%), followed by *M. pinnata* (92.4%), *C. fistula* (92.3%), *P. longifolia* (90%), and *T. arjuna* (86.5%). *D. sissoo* had lowest viability (67.3%)(Table 1).

The percentage of both germination and seedling survival was found to be highest for *T. arjuna*, *C. fistula* and *M. pinnata*.

Table 1. General description of selected plant species

Scientific name	Common name	Family	Origin	Type of tree	Viability (%)
Terminalia arjuna (Roxb. Ex DC.) Wight & Arn	Arjuna	Combretaceae	India	Deciduous	86.5
Millettia pinnata (L.) Panigrahi	Indian beech	Fabaceae	India	Deciduous	92.4
Polyalthia longifolia (Sonn.) Thwaites	Cemetery tree	Annonaceae	India	Evergreen	90.33
Dalbergia sissoo Roxb. Ex DC.	Indian rosewood	Fabaceae	India	Deciduous	67.32
Albizia lebbeck (L.) Benth.	White siris	Fabaceae	Indomalayan	Deciduous	77.34
Leucaena leucocephala (Lam.) de Wit	White leadtree	Fabaceae	Mexico	Evergreen	95.43
Cassia fistula L.	Golden shower	Fabaceae	India	Deciduous	92.32
Cascabela thevatia (L.) Lippold	Captain cook tree	Apocynaceae	America	Evergreen	72.83

Table 2. Two-way ANOVA for germination and seedling survival percentage for eight selected plant species under various pre-sowing treatments

	df	Germination (%)	Survival (%)
Plant	7	1770.50**	236.23**
Treatment	4	455.53**	69.97**
Plant × Treatment	28	51.54**	15.91**

**Significant at the 0.01 level (p<0.01)

Two-way ANOVA indicated that both plant species and treatments significantly affect both germination and seedling survival (Table 2).

Further, among pre-germination treatments, hot water treatment and mechanical scarification were found to be best as these treatments showed the highest germination and seedling survival. For *T. arjuna*, the germination and seedling survival ranged from 46 to 88% and from 75.86 to 95.45%, respectively, with the highest value in hot water treatment followed by mechanical scarification, cold water, acid treatment and control. Similarly, for *C. fistula*, germination ranged from 30% in control to 88% in mechanically scarified and survival from 80% in acid treated to 96.15% in hot water. *M. pinnata* showed the highest germination and seedling survival in mechanical scarification i.e., 88 and 85.37%, respectively, and the lowest in control i.e., 42 and 75.76%, respectively. *Dalbergia sissoo* had the highest germination in hot water treatment (10%), and lowest in control (6%). The seedling survival was also least in *D. sissoo*, highest in mechanically scarified seeds (50%) and lowest in cold water (25%) (Fig. 1, 2).

The three species with the highest germination and seedling survival were

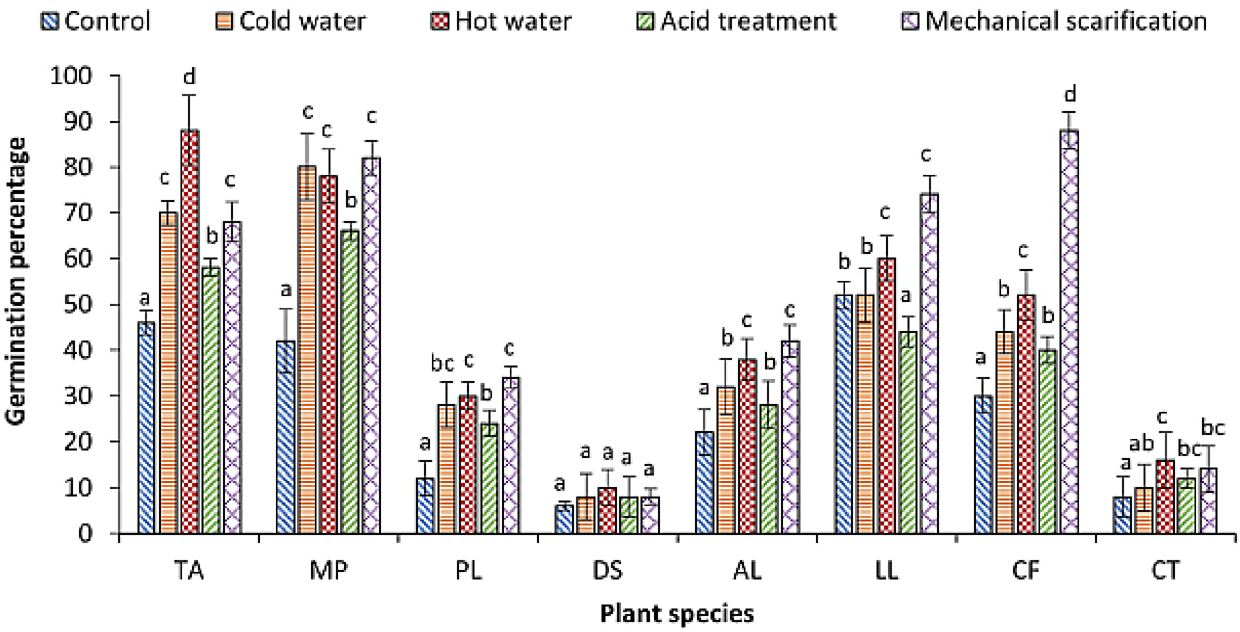

Fig. 1. Germination percentage (%) of seeds of various tree species (i.e, TA: *T. arjuna*, MP: *M. pinnata,* PL: *P. longifolia,* DS: *D. sissoo*, AL: *A. lebbeck,* LL: *L. leucocephala*, CF: *C. fistula,* and CT: *C. thevetia*) after various pre-germination treatments viz., control, cold water, hot water, acid treatment, and mechanical scarification

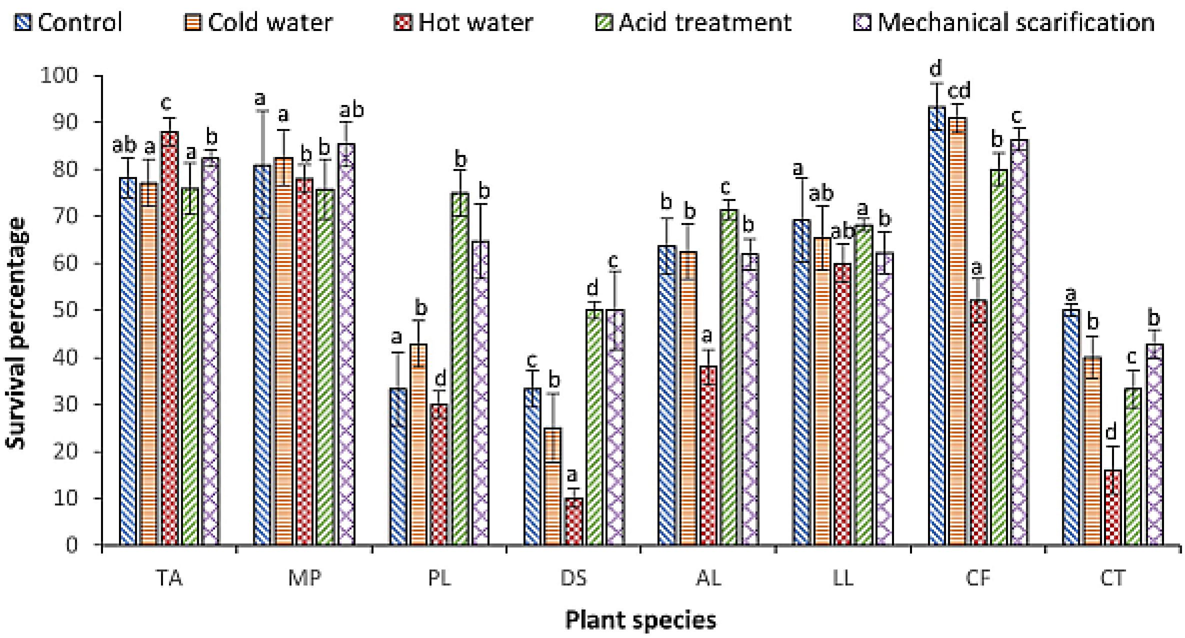

Fig. 2. Seedling survival percentage (%) of seeds of various tree species (i.e, TA: *T. arjuna*, MP: *M. pinnata*, PL: *P. longifolia*, DS: *D. sissoo*, AL: *A. lebbeck*, LL: *L. leucocephala*, CF: *C. fistula*, and CT: *C. thevetia*) after various pre-germination treatments viz., control, cold water, hot water, acid treatment, and mechanical scarification

Table 3. Two-way ANOVA for germination and seedling survival percentage for eight selected plant species under Pb treatments (viz., 0, 200, 450, and 600 mg/kg of Pb in dry soil)

	df	Germination (%)	Survival (%)
Plant	2	20.25**	26.77**
Treatment	3	810**	587.27**
Plant × Treatment	6	27.25**	30.99**

**Significant at the 0.01 level ($p<0.01$)

further sown to observe the impact of Pb treatments by using different concentrations of Pb in soil. The two-way ANOVA showed both the parameters were significantly affected by tree species and Pb treatments (Table 3).

Seed germination and seedling survival were highest for *M. pinnata*, followed by *C. fistula*, and *T. arjuna*. Further, both these parameters decreased with the increase in Pb concentration in soil. Germination was highest in control (0 mg/kg of Pb in soil) and lowest at 600 mg/kg. For *T. arjuna,* germination varied from 42 to 78%, for *C. fistula* from 40 to 82%, and from 42 to 86% for *M. pinnata* (Fig. 3). Similarly, the seedling survival for *T. arjuna* varied from 57.14 to 84.62%, for *C. fistula* from 45 to 90.24%, and from 47.62 to 93.02% for *M. pinnata* (Fig. 4).

Discussion

Enhancement in the germination of seeds after various pre-sowing treatments such as hot water, cold water, mechanical scarification, acid, and other treatments was reported by many studies (Salim et al., 2010, Azad et al., 2011; Feizi & Mousavi, 2016). These treatments help seeds to break dormancy and accelerate their germination process. Further, it is also reported that seed dormancy is species-specific and therefore, shows a variation from species to species, it also depends on the stage of maturity of the seed and physiological conditions of the seeds (Azad et al., 2006; Salim et al., 2010). Thus, the pre-sowing treatments must be modified according to the seed type, dormancy, and status of the seeds (Alamgir and Hossain, 2005). It is well known that physical seed dormancy can be overcome by breaking and dissolving the seed coats which could be achieved using various physical treatments. Among the pre-germination treatments used in the present study, hot water treatment and mechanical scarification were found to be best as seeds with these treatments showed the highest germination and seedling survival. The order of success of various pre-sowing treatments is hot water treatment followed by mechanical scarification, cold water acid treatment, and control. Similar findings were reported by Azad et al. (2011) for seeds of *Melia azedarach*. Both hot water and machinal scarification treatments were reported to show the highest germination in *Albizzia saman* (Alamgir & Hossain, 2005). Feizi and Mouvasi (2016) also observed substantial enhancement in seed germination due to the mechanical scarification of seeds of *C. fistula.* Further, Kumar et al. (2007) reported escalated

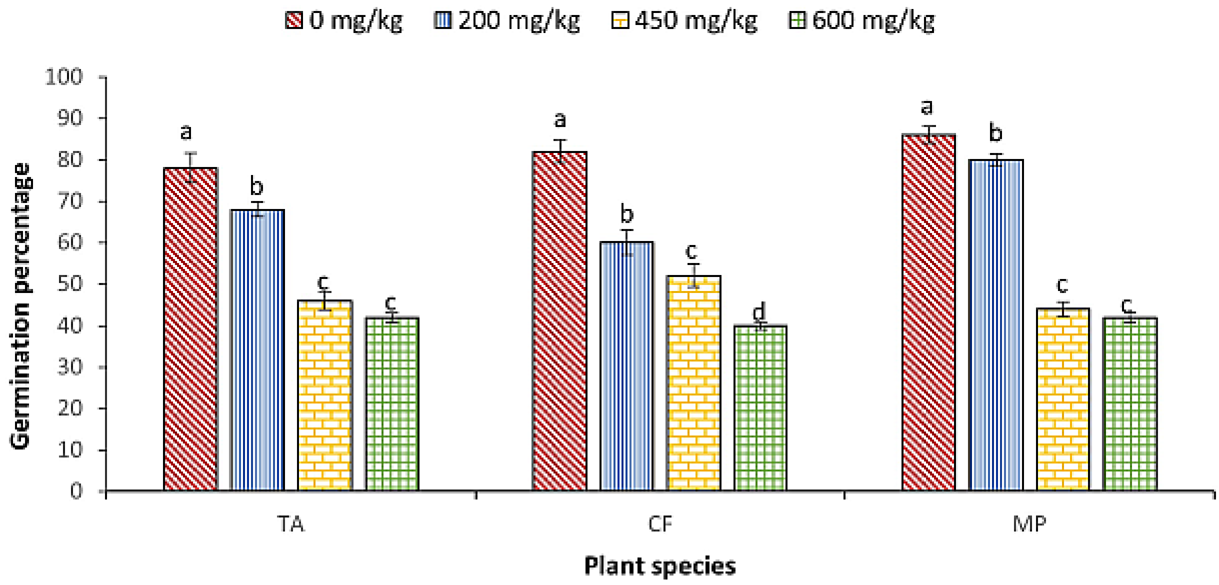

Fig. 3. Germination percentage (%) of three tree species viz., *T. arjuna*, *C. fistula*, and *M. pinnata* under various Pb treatments viz., 0, 200, 450, 600 mg/kg dry soil

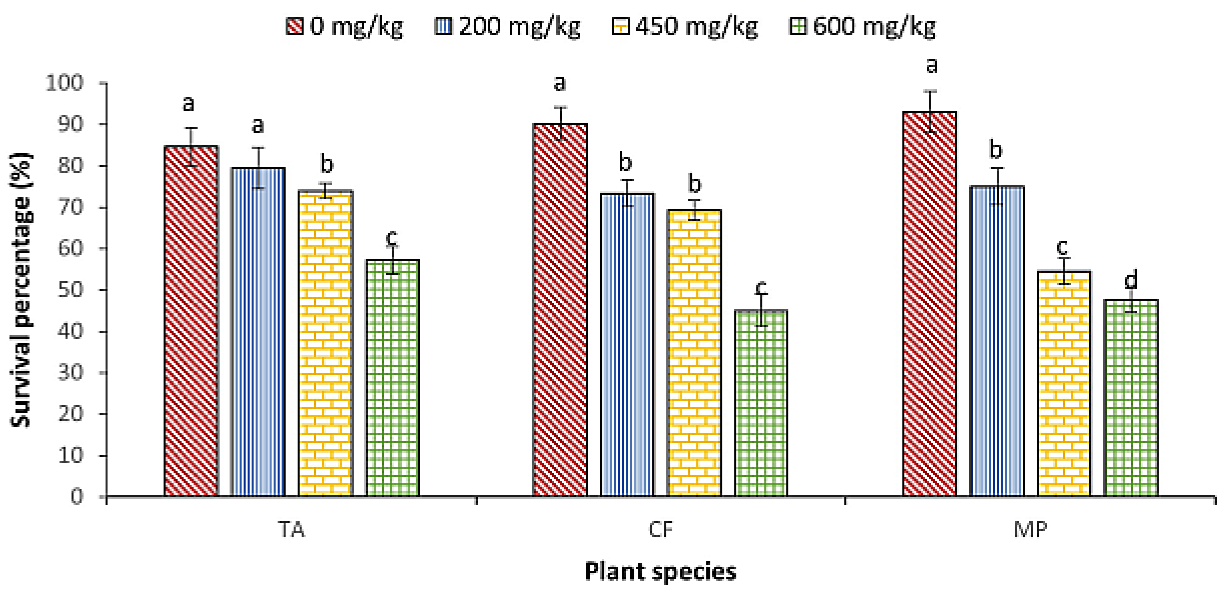

Fig. 4. Survival percentage (%) of three tree species viz., *T. arjuna, C. fistula*, and *M. pinnata* under various Pb treatments viz., 0, 200, 450, 600 mg/kg dry soil

germination in hot water and mechanically scarified treated seeds in *M. pinnata*. From the present study, it was also found that among the eight commonly growing tree species, *T. arjuna*, *C. fistula*, and *M. pinnata* showed the highest germination and seedling survival percentage, and the lowest percentages were recorded in *D. sissoo*. The reason for the difference in germination rates in various treatments for different plant species might be due to variations in seed coats (Azad et al., 2006, 2011). Bhadouria et al. (2017) also reported good seedling germination and survival under forest's environmental conditions for *T. arjuna*. Sainkhediya et al. (2021) also reported speedy and good germination rates for *T. arjuna*, *C. fistula*, and *M. pinnata* among 29 commonly growing tree species that were selected for germination experiments. They also mentioned the role of seed vigour, germination, environmental plasticity, fast seedling growth, and their survival to be decisive factors in the selection of suitable tree species to maintain the local biodiversity.

In the present study increase in Pb concentration in soil significantly affected the seed germination and seedling survival. Similar decline was also reported in different tree species with increasing Pb contamination in soil (Kabir et al., 2008; Shafiq et al., 2008; Farooqi et al., 2009). The decline in seed germination could be due to Pb toxicity which affects the amylase activity in seeds and induced deformation of the radicle and plumule growth (Yang et al., 2016). While the decline in seedling survival could be due to reduced meristematic cell activity and also because of accumulation of Pb in cells of radicle which affects various enzyme activities involved in food breakage and their transfer (Shafiq et al., 2008). Further, Yang et al. (2016) explained that when the seed germinates after breaking the seed coat, the radicle is immediately exposed to Pb-contaminated soil. This exposure causes entry of Pb^{2+} ions in the meristematic cells and reduced the activity of RNA and DNA and ultimately disrupts mitosis, eventually influencing cell division, growth, and therefore, the seedling survival. Additionally, the variations in response among tree species to the same treatments of Pb-contamination in the soil are because of the difference in tolerance of particular species to Pb-toxicity (de Souza et al., 2012).

Conclusion

The present study revealed that pre-sowing treatments of seeds help to break the physical dormancy due to their hard seed coats in various tree species and therefore, enhance the germination rate. It was found that various pre-sowing treatments such as hot water, cold water, mechanical scarification, and acid treatments showed an improvement of germination in comparison to control treatment. Among these hot water treatment and mechanical scarification were found to be best followed by cold water, acid treatment. Among the eight selected commonly growing tree species *T. arjuna*, *C. fistula*, and *M. pinnata* showed the highest germination and seedling survival, while *D. sissoo* had the lowest. Thus, for further studies *T. arjuna*, *C. fistula*, and *M. pinnata* with pre-sowing treatments hot water treatment and mechanical scarification can be considered as most suitable. Further, the impacts of Pb toxicity on the seed germination and survival were lowest for *M. pinnata*, followed by *C. fistula*, and *T. arjuna*.

Increase in Pb concentration in soil significantly affected the seed germination and seedling survival. In the Pb-contaminated regions, further research is needed to determine the effects of Pb contamination on seed germination and seedling growth, and the survival of a large number of tree species. Also, it is recommended to extend the study to the next level to investigate the different levels of Pb in the environment and various parts of the selected tree species to determine their Pb-phytoremediation potential.

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