



The effects of acid mist rain on the growth of *Albizia lebbbeck* (L.) Bth.

¹Muhammad Zafar Iqbal and ^{*1}Muhammad Shafiq

¹Department of Botany, University of Karachi, Karachi, 75270, Pakistan.

^{*1}Corresponding author e-mail shafiqeco@yahoo.com

mziqbalbotuokpk@yahoo.com

Abstract: The wet and dry deposition of acidic substance in environment results due to human, automobile and industrial activities. Acid deposition is worldwide environmental problems. As a results these acidic materials are increasingly in the environment both in developed and developing countries, including Pakistan. In addition, acid mist found responsible for producing negative effect on loss in crop yield and vegetation growth. *Albizia lebbbeck* (L.) Benth. (Fabaceae: Mimosoideae) has significant importance to human beings for its multipurpose use. This study aim to record the seedling growth performances of *A. lebbbeck* raised in different media of pH in pot trials. To test for the effects that precipitation pH may have upon the growth and development of plants, *A. lebbbeck* seedlings were exposed, for 3 weeks, to artificial acid mists ranging in pH from 2.82, 3.45, 4.46 and 5.55 marked four level as T1, T2, T3, T4, respectively. It has been examined that the foliar spray of acid mist in a small amount pH 2.82-5.55 affected the seedling growth of *A. lebbbeck* as compared to other treatment. Decline was notice in the shoot and root growth at 5.55 pH as compared to other treatment. An increase was recorded in root, shoot, and plant length and total plant dry weight of *A. lebbbeck* at 3.45 pH as compared to other treatments.

Short title: Effects of acid rain on *A. lebbbeck*.

[Muhammad Zafar Iqbal and Muhammad Shafiq. **The effects of acid mist rain on the growth of *Albizia lebbbeck* (L.) Bth.** *N Y Sci J* 2023;16(7):27-33]. ISSN 1554-0200 (print); ISSN2375-723X (online). <http://www.sciencepub.net/newyork>. 05.doi:10.7537/marsnys160723.05.

Key words: biomass, damage, root, shoot, tolerance, toxicity

Introduction

The rapid growth of population, industrial and automobile activities has given rise to many common problems, such as air, water, soil pollution and environmental degradation. Sources of pollution can vary according to specific industrial, geographical, geological, urban environment, contamination, climatic and sociological conditions important ones and it influences all parts of the environments (Sanka et al., 1995). The release of sulfur dioxide and nitrogen oxide into the atmosphere and reaction with water forms acid rain. This problem is especially serious in acidic soils with low buffering capacity.

During the past two decades increased amounts of strong inorganic acids have appeared in the precipitation over much of northern Europe and the Northern Eastern United States, causing the pH of the rain fall to drop to levels of 4.0 and less. European scientist have found that rain in northwestern Europe shows a trend toward increased acidity. There are tenfold differences between each unit. Thus, pH 6 is ten times more acid than pH 7, pH 5 is 100 times more acidic than pH 7 and so on (Granat 1972; Likens et al., 1972). A significant ($p < 0.01$) decrease in seedling growth of yellow birch (*Betula alleghaniensis* Britt.) were

observed at pH 2.3 and foliar tissue damage was observed at pH 3.0 and below (Wood and Bormann 1974). This trend appears to be linked to mounting levels of gaseous pollutants such as sulfur and nitrogen oxides which can be converted chemically in the atmosphere to strong acids (H_2SO_4 and HNO_3) deposited and transported hundreds of kilometers from the original source of emission (Reiquam 1970). The potential effects of acidic precipitation is considered best in terms of the long term critical load of pollutants to the soil (Cape, 1993). The reduction in forest productivity, water quality, the availability of nutrients due to acid stress (Dahl and Skre, 1971; Sheppard et al., 1993; Neal et al., 2010).

Acid rain and increase in precipitation acidity has become a dominant feature of changes in the chemical climate of earth, on trees, leaf growth, enzymatic and non-enzymatic antioxidant activities, ecosystem may also decrease in the pH and an increase in foliar leaching losses (Maklenburg et al. 1966; Cowling, 1983; Debnath et al., 2018; Du et al., 2020). The significant degradation of natural ecosystem, photosynthetic performance, pigment composition, soil physiochemical and microbial properties due to pollutant stress reported (Yao et al., 2016; Wei et al.,

2021; Zhang et al., 2021). In a study about the comparison of forest susceptibility to acid stress estimated a relative growth reduction in forest productivity in Sweden and north eastern United States (Jonsson and Sundberg, 1972a; Jonsson and Sundberg, 1972b). The simulated acid rain is a global issue and induced high effects of on soil pH, soil microbial community, litter decomposition, leaf injury, root, sapling and woody tree growth (Zhang et al., 1996; Pietri and Brookes, 2008; Ouyang et al., 2008; Wang et al., 2010; Wang et al., 2014; Liu et al., 2018a; Liu et al., 2018 b).

Seeds and seedlings of five hardwood species were subjected to a simulated acid rain adjusted to pH values of 2.0, 3.5, 5.0, 6.0, and to distilled water (the control). Seed germination, foliar damage, decline in chlorophyll contents and retardation was remarkably inhibited by pH 2.0 treatment for three hardwood species while seedling growth was stimulated at pH levels between 3.5 and 5.0. The pH 2.0 treatment seemed to be a threshold level for inhibition of seed germination and seedling growth for all the treated hardwood species (Fan and Wang, 2000). Munzuroglu et al. (2002) reported that pH levels below 3.1 destroyed the pollen tubes and that pollen development stopped near pH 3.0 and concluded that both the pH value and quantity of the rain was an important factor in the development of apples.

The species selected, *Albizia lebbek* (L.) Bth. belongs to family Mimaceae found in Swat, eastwards ascending to 4000' and commonly planted in Sind, Baluchistan and the Punjab plain (Pakistan). It is introduced as perennial tree species and is growing as multipurpose tree in the arid regions. *A. lebbek* is well adapted in wide range of acidic, alkaline and saline soil condition (Prinsen, 1986). *A. lebbek* is vitally important tree for firewood, fodder, heavy shade tree, industrial raw material and timber and relevant to the arid zone (Khoshoo and Subrahmanyam 1985; Habib et al., 2016; Mariod et al., 2017).

However, short-term studies have shown that acid mist has affected on the growth of plants. Karachi is situated on the coast and therefore sea breezes aid pollutant dispersion during summer. Despite this, maximum sulfur dioxide concentrations, exceed WHO guideline in some areas of Pakistan (UNEP 1992). Air pollution studies are a matter of utmost concern. Great concern has been expressed, in developed countries, about the role of acid rain. The occurrence of incased precipitation acidity over wide areas of the city raises serious question, as it can effects on growth and vigor of trees. Although the effects of acidified rain on trees has not intensively studies. *A. lebbek* is growing at main busy roads of the Karachi, city and is under pressure of environmental pollution. The information on impact of

acid mist on *A. lebbek* is scarce. The aim of the present experiment was designed with purpose to determine the effect of simulate acid mist on the growth of multipurpose tree species, *A. lebbek*.

2. Materials and Methods

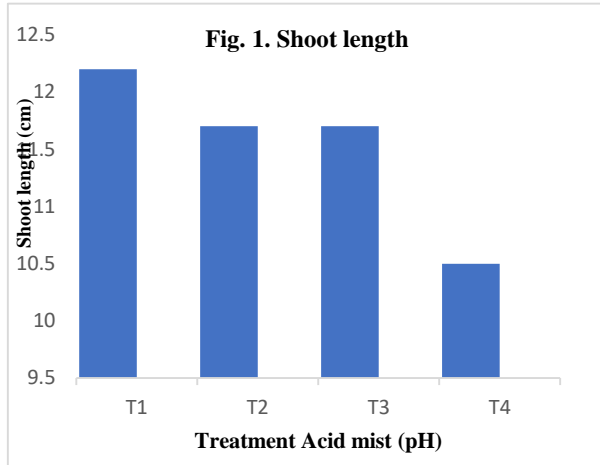
The effect of acid mist on seedlings of *A. lebbek* was investigated by a series of pot trials using a garden loam soil substrate with varying amounts of added pH. This experiment was designed to examine effects of aqueous acid mist at various pH levels on the subsequent growth and development of three week old *A. lebbek* seedlings. Experiment was conducted in the Experimental field located at the Department of Botany, University of Karachi. The acid mist was adjusted to different pH levels 2.82, 3.45, 4.46 and 5.55 through additions of technical grade sulfuric acid. The pots were prepared by mixing soil and fertilizer. The pots selected were of uniform size. The healthy seeds of *A. lebbek* were randomly collected from the Karachi University Campus and the top end were slightly cut with a clean scissor to remove any possible seed coat dormancy. The surface of seeds of *A. lebbek* were treated with 1% dilute solution of Sodium-hypochlorite to prevent any fungal contamination and prepared for seedlings. Three weeks old seedlings were irrigated with different concentrations of acid mist after every four days. Each irrigation episode represented 2 ml of rain fall. The irrigations solution was sprayed with a sprayer in order to moisten the whole plant equally. The seedlings were allowed to grow under natural conditions. After 20 days, the seedlings were harvested. Root, shoot and total plant length were recorded. The seedling dry weight was determined by drying the plant materials in an oven at 80 °C for 24 hours and dry biomass was measured with electrical balance. Each treatment had five replicates.

Statistical analysis

Statistical analysis was conducted by using COSTAT software version 3. The data variance was analyzed by using one-way analysis of variance (ANOVA). Comparison of the means (mean values \pm standard error) was analyzed using a Duncan Multiple Range Test with a probability level of 5 % error. $P < 0.05$ was used to define statistical significance.

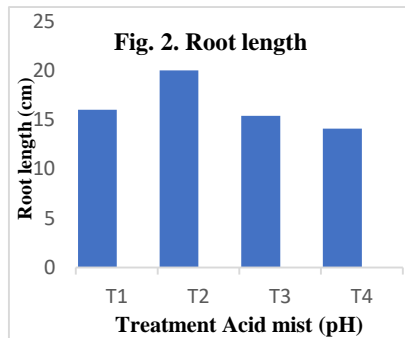
Results and Discussion

Acid rain is an environmental problem. An important factor governing germination is the pH (Hora and Baker, 1972). Acid mist toxicity is deleterious to plant growth. Normally, rainfall is slightly acid, but its pH value must be lower than 5.6 to be considered acid rain. It is well known that acidic precipitations are harmful for plants, in fact, they can damage the photosynthetic machinery, plant physiology, reduce the chlorophylls content and increase the production of reactive oxygen species, while at agroecosystem levels they are responsible for the crop yield losses, above and



below ground plant parts (Shu et al., 2019; Shi et al., 2021; Shu et al., 2023). Inhibition to germination and retardation of plant growth are commonly reported effects due to acidity. It was observed that all measured growth variable of *A. lebbeck* were responded differently to acid treatment (Fig. 1-4; Table 1).

Table 1. Effects of different concentrations of acid mist pH (T1-2.82, T2-3.45, T3-4.46, T4-5.55) on root, shoot, seedling height and seedling dry weight of *Albizia lebbeck*.



Treatem ents	Root length (cm)	Shoot length (cm)	Plant height (cm)	Seedlin g dry weight (g)
T1	16.00	12.20	28.10	2.856
T2	20.00	11.70	31.70	2.992
T3	15.40	11.70	27.10	2.878
T4	14.10	10.50	24.60	2.308
L.S.D. P<0.05	9.32	2.28	10.64	1.421

The pH value of a substance determines its acidity. The pH value is the concentration of hydrogen ions (H¹⁺) in a substance and is measured on a scale of 0.0 to 14.0. The pH values less than 7.0 are acidic, and those more than 7.0 are basic and pure water (H₂O) has a pH of 7.0, making it neutral (CBEF, 2013). Acid rain availability produces harmful impact on herbs, shrub and trees. In present study, the variance between pH levels 5.55 to 2.82 indicates that seedling growth means are not significantly different for shoot, root, and total plant height and total plant dry weight of *A. lebbeck* (Table 1).

Fig. 1. Effects of different concentrations of acid mist pH (T1-2.82, T2-3.45, T3-4.46, T4-5.55) on shoot length of *A. lebbeck*.

It was examined that the foliar spray of acid in a small amount 2.82-5.55 affected the shoot growth of *A. lebbeck* (Fig. 1). The shoot growth of *A. lebbeck* at pH 4.46 was found promotory. A sharp decline in shoot growth of *A. lebbeck* was noticed in pH 5.55 and 3.45 followed by pH 2.82 and 4.46 treatment, respectively. The inhibition of plant growth due to acid rain not only affects the aerial parts of plant that are directly exposed to acid rain but also degrade the fertility of soil and increases the vulnerability of plants to toxic metals (Du et al. 2017). The maximum reduction in shoot growth of *A. lebbeck* at 5.5 pH was recorded. The results of the present study support the findings of Singh and Agrawal (2004) reported that the shoot lengths of both types of wheat cultivar declined significantly at or below pH 4.0.

Fig. 2. Effects of different concentrations of acid mist pH (T1-2.82, T2-3.45, T3-4.46, T4-5.55) on root length of *A. lebbeck*.

The deleterious effects of simulated acid rain on chlorophyll contents, chlorophyll fluorescence, chlorosis, nutrient loss, enzyme activity changes in foliage of plant (Ren et al., 2018; Ma et al., 2021). Root systems provide mechanical support and helps in nutrient uptakes. The research work of Ju et al., (2017) and Huang et al., (2019) shows that acid rain increases the accumulation of reactive oxygen species and inhibits roots growth. The results indicated that acid mist also differently inhibited root growth of *A. lebbeck* in all the treatments, especially in T4 and T1 followed by T3 and T2 (Fig. 2). The decrease in root growth of *A. lebbeck* at 5.5 pH was recorded. It has been examined that the foliar spray of acid mist in a small amount pH 2.82-5.55 affected the root growth of *A. lebbeck* as compared to other treatment. Decline was notice in the root length at 2.85 pH as compared to other treatment. This research revealed that all highly acidic (pH = 2.5) significantly

inhibited the total root length of *A. lebeck*. The present experimental results are confirm and in agreement with the previous studies published on soybean and rice root systems also confirmed the inhibitory effects of highly acidic acid rain on root (Sun et al. 2013).

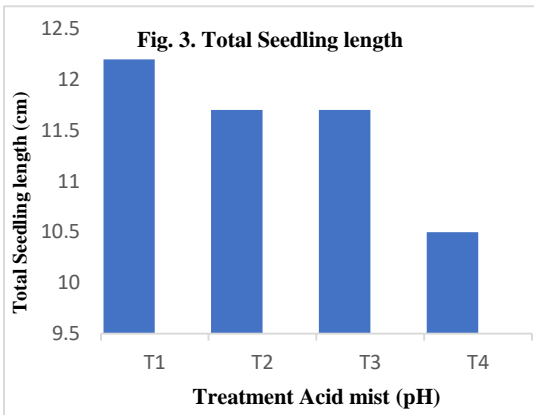


Fig. 3. Effects of different concentrations of acid mist pH (T1-2.82, T2-3.45, T3-4.46, T4-5.55) on total seedling length of *A. lebeck*.

The artificial precipitation did not significantly affect the growth of *A. lebeck* seedling while the pH of the misting solution reached values less than 2.82 (Fig. 3). Similarly, Pignattelli *et al.*, (2021) found reduction in physiology and growth of *Lepidium sativum* due to acid rain stress. It was noticed that T1 treatment produced promotory effects on total plant height as compared to other treatments. It has been examined that the foliar spray of acid mist in a small amount pH 5.55 affected the total seedling length of *A. lebeck* as compared to other treatment. Decline was notice in the seedling length at 3.45 pH as compared to other treatment. In a short term study the simulated acid rain (pH range 5.6 to 2.3) affected seedling tissue chemistry, seedling productivity growth and nutrient relations of Eastern White Pine seedlings (*Pinus strobus*, L.) grown in a sandy loam soil (Wood and Bormann, 1977).

The effects of simulated acid rain, at varying pH levels of 2.82, 3.45, 4.46, and 5.55 on yields of *A. lebeck* was recorded (Fig. 4). The results of total seedling dry weight changes after treated at different level of pH. The reduction seedling dry weight in *A. lebeck* was evidently due to poor growth of shoot and root due to acid mist. These data suggests, as dose Gordon's work (1972) with pines, that developing tissue may be the most susceptible to injury from acids. The reductions in this study was most probably a result of foliar tissue damage caused by the mist acidities and agrees with the finding of Wood and Bormann (1977), who had found decrease growth reduction in yellow

birch seedling. Many countries have adopted energy conservation measures, mainly on economic grounds, and have effectively limited energy consumption and demand and also improved generation and distribution efficiency (UNEP 1992). An obvious examples is the use of low temperature hydrometallurgical techniques which reduce the SO₂ emissions associated with traditional metal smelting methods. More plantation are required to overcome the pollution problems.

The effects of simulated acid rain, at varying pH levels of 5.7, 4.0, 3.1, and 2.7 on yields of radish, garden beet, kidney bean, and alfalfa recorded. The results showed no significant difference in the yields of radish, kidney bean, and alfalfa when treated with simulated acid rain when compared to the yields of garden beet treated with pH 5.7 simulated rain (Evans et al., 1982).

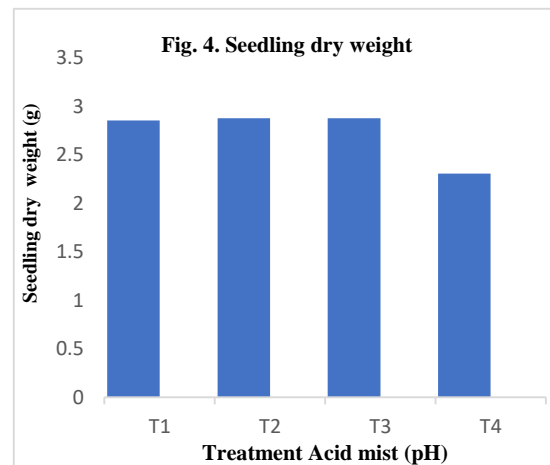


Fig. 4. Effects of different concentrations of acid mist pH (T1-2.82, T2-3.45, T3-4.46, and T4-5.55) on seedling dry weight of *A. lebeck*.

Conclusion

It is concluded that the sulfuric acid mist acidity produce toxic effect upon shoot, root, and total plant height at pH 2.28. This suggest that reduced growth may ultimately occur with longer period of acid misting. *A. lebeck* tree is a one component of natural ecosystem, and in under natural conditions acidity can produced synergistic effects on seedling growth. Further research into the screening for better acid mist tolerant species are also recommended. There is a need of coordination in multidisciplinary research and development programme leading to utilization of *A. lebeck* for transplantation at the industrial, urban centers and acid mist deposit areas.

References

- [1]. Cape JN. Direct damage to vegetation caused by acid rain and polluted cloud: definition of critical levels for forest trees. *Environ Pollut.* 1993;82(2):167-80. doi: 10.1016/0269-7491(93)90114-4. PMID: 15091786.
- [2]. CBEF 2013. CBEF (Chesapeake Bay Ecological Foundation, Inc. Acid Raid Overview. Retrieved from <http://www.chesbay.org/acidRain/>
- [3]. Cowling EB. 1983. Acid Precipitation: Scientific Progress and Public Awareness. *The American Biology Teacher* (1983) 45 (4):194–202. <https://doi.org/10.2307/4447678>
- [4]. Dahl E, Skre O. 1971. En undersøkelse av virkningen av sur nedbør på produktiviteten i landbruket. *Nordforsk, Miljøvernssekretariatet*, 1:27-40.
- [5]. Debnath B, Irshad M, Mitra S, Li M, Rizwan HM, Liu S, Pan T, Qiu D. 2018. Acid rain deposition modulates photosynthesis, enzymatic and non-enzymatic antioxidant activities in tomato. *Int. J. Environ. Res.*, 12:203-214.
- [6]. Du E, Dan Dong, Xuotong Zeng, Zhengzhong Sun, Xiaofei Jiang, Wim de Vries. Direct effect of acid rain on leaf chlorophyll content of terrestrial plants in China. *Science of The Total Environment*, 605–606,2017, 764-769, <https://doi.org/10.1016/j.scitotenv.2017.06.044>.
- [7]. Du J, Qv M, Zhang Y, Cui M, Zhang H (2020) Simulated sulfuric and nitric acid rain inhibits leaf breakdown in streams: a microcosm study with artificial reconstituted fresh water. *Ecotoxicol Environ Saf* 196:110535
- [8]. Evans, L. S., Lewin, K. F., Cunningham, E. A., Patt, M. J. 1982. Effects of simulated acidic rain on yields of field grown crops. *New Phytol*, 91:429-441.
- [9]. Fan HB, Wang YH. 2000. Effects of simulated acid rain on germination, foliar damage, chlorophyll contents and seedling growth of five hardwood species growing in China. *Forest Ecology and Management*, 126(3):321-329. [https://doi.org/10.1016/S0378-1127\(99\)00103-6](https://doi.org/10.1016/S0378-1127(99)00103-6).
- [10]. Granat L. 1972. On the relation between pH and the chemical composition in atmospheric precipitation. *Tellus*, XXIV(6):550-560. <https://doi.org/10.1111/j.2153-3490.1972.tb01581.x>
- [11]. Habib G, Khan NA, Sultan A, Ali M. 2016. Nutritive value of common tree leaves for livestock in the semi-arid and arid rangelands of Northern Pakistan. *Livestock Science*, 184:64-70. <https://doi.org/10.1016/j.livsci.2015.12.009>.
- [12]. Huang J, Wang H, Zhong Y, Huang J, Fu X, Wang L et al (2019) Growth and physiological response of an endangered tree, *Horsfieldia hainanensis* Merr., to simulated sulfuric and nitric acid rain in southern China. *Plant Physiol Biochem* 144:118–126
- [13]. Jonsson B, Sundberg R, 1972a. Supporting Studies to Air Pollution Across National Boundaries. The Impact on the Environment of Sulfur in Air and Precipitation. In *Sweden's case study for the United Nations Conference on the Human Environment*. Stockholm: Royal Ministry of Foreign Affairs, Royal Ministry of Agriculture.
- [14]. Jonsson B & Sundberg R. 1972b. Has the acidification by atmospheric pollution caused a growth reduction in Swedish forests? A comparison of growth between regions with different soil properties. Research Note No. 20, Department of Forest Yield Research, The Royal College of Forestry, S-104 05 Stockholm 50, Sweden.
- [15]. Ju S, Yin N, Wang L, Zhang C, Wang Y (2017). Effects of silicon on *Oryza sativa* L. seedling roots under simulated acid rain stress. *PLoS ONE* 12:e0173378
- [16]. Khoshoo TN, Subrahmanyam GV. 1985. Ecodevelopment of arid lands in India with non-agricultural economic plants — a holistic approach. In: Wickens, G.E., Goodin, J.R., Field, D.V. (eds) *Plants for Arid Lands*. Springer, Dordrecht. https://doi.org/10.1007/978-94-011-6830-4_18
- [17]. Likens GE, Bormann FH, Johnson NM. 1972. Acid Rain. *Environment*, 14(2): 33-40. <https://doi.org/10.1080/00139157.1972.9933001>
- [18]. Liu X, Zhao WR, Meng MJ, Fu ZY, Xu LH, Zha Y, Yue JM, Zhang SF, Zhang J. 2018a. Comparative effects of simulated acid rain of different ratios of SO₄²⁻ to NO₃⁻ on fine root in subtropical plantation of China. *Sci. Total Environ.*, 618:336-346.
- [19]. Liu X, Fu Z, Zhang B, Zhai L, Meng M, Lin J, Zhuang J, Wang JG, Zhang J. 2018b. Effects of sulfuric, nitric, and mixed acid rain on Chinese fir sapling growth in Southern China. *Ecotoxicol. Environ. Saf.*, 160:154-161.
- [20]. Ma S, Liu X, Jia Z, Meng M, Li C, Ren Q, Zhai L, Zhang B, Zhang Y, Zhang J. 2021. Response of *Quercus acutissima* foliage to different types of simulated acid rain. *Atmospheric Pollution Research*, 12(7): 2021, 101112, <https://doi.org/10.1016/j.apr.2021.101112>.
- [21]. Mariod AA, Mohamed Elwathig Saeed Mirghani, Ismail Hussein. 2017. Chapter 41 - *Albizia lebbek* (L. Benth.) Lebbek Tree Seed, Editor(s): Abdalbasit Adam Mariod, Mohamed Elwathig Saeed Mirghani, Ismail Hussein, Unconventional Oilseeds and Oil Sources, Academic Press, 2017, Pages 277-281,

- <https://doi.org/10.1016/B978-0-12-809435-8.00041-X>.
- [22]. Mecklenburg RA, Tukey HB Jr., Morgan JV. A mechanism for the leaching of calcium from foliage. *Plant Physiology*, 41(4):610–613, <https://doi.org/10.1104/pp.41.4.610>
- [23]. Munzuroglu, O., Obek, E., Geckil, H. (2003). Effects of simulated acid rain on the pollen germination and pollen tube growth of Apple (*Malus sylvestris* Miller CV. Golden). *Acta Biologica Hungarica*, 54(1):95-103.
- [24]. Neal C, Robinson M, Reynolds B, Neal M, Rowland P, Grant S, Norris D, Williams B, Sleep D, Lawlor A. Hydrology and water quality of the headwaters of the River Severn: Stream acidity recovery and interactions with plantation forestry under an improving pollution climate. *Sci Total Environ*. 2010, 408(21):5035-51. doi: 10.1016/j.scitotenv.2010.07.047. Epub 2010 Aug 13. PMID: 20708776.
- [25]. Ouyang X, Guo-Yi ZHOU, Zhong-Liang HUANG, Ju-Xiu LIU, De-Qiang ZHANG, Jiong LI. 2008. Effect of simulated acid rain on potential carbon and nitrogen mineralization in Forest Soils 1 Project supported by the Knowledge Innovation Program of the Chinese Academy of Sciences (No. KSCX2-SW-120) and the National Natural Science Foundation of China (No. 30470306). *Pedosphere*, 18(4): 503-514, [https://doi.org/10.1016/S1002-0160\(08\)60041-7](https://doi.org/10.1016/S1002-0160(08)60041-7).
- [26]. Pietri JCA, P.C. Brookes. 2008. Relationships between soil pH and microbial properties in a UK arable soil. *Soil Biol. Biochem.*, 40:1856-1861.
- [27]. Pignattelli S, Andrea Broccoli, Manuela Piccardo, Antonio Terlizzi, Monia Renzi. 2021. Effects of polyethylene terephthalate (PET) microplastics and acid rain on physiology and growth of *Lepidium sativum*. *Environmental Pollution*, 282, 2021, 116997, <https://doi.org/10.1016/j.envpol.2021.116997>.
- [28]. Prinsen, J.H. 1986. Potential of *Albizia lebbek* (Mimosaceae) as a tropical fodder tree – a review of literature. *Trop. Grasslands* 20(2):78-83.
- [29]. Reiquam H. Sulfur: simulated long-range transport in the atmosphere. *Science*. 1970, 170(3955):318-20. doi: 10.1126/science.170.3955.318. PMID: 5460034.
- [30]. Ren X, Zhu J, Liu H, Xu X, Liang C (2018) Response of antioxidative system in rice (*Oryza sativa*) leaves to simulated acid rain stress. *Ecotoxicol Environ Saf* 148:851–856
- [31]. Sanka M, Strnad M, Vondra J 1995. Sources of soil and plant contamination in an urban environment and possible assessment methods. *International Journal of Environmental Analytical Chemistry*, 59(2/4):327-343.
- [32]. Sant'Anna-Santos BF, da Silva LC, Azevedo AA, de Araujo JM, Alves EF, da Silva EAM, Aguiar R (2006) Effects of simulated acid rain on the foliar micromorphology and anatomy of tree tropical species. *Environ Exp Bot*, 58(1–3):158–168
- [33]. Sheppard LJ, Cape JN, Leith ID. Influence of acidic mist on frost hardiness and nutrient concentrations in red spruce seedlings: 2. Effects of misting frequency and rainfall exclusion. *New Phytol*. 1993,124(4):607-615. doi: 10.1111/j.1469-8137.1993.tb03850.x. PMID: 338 74441
- [34]. Shi Z, Jiaen Zhang, Zeheng Xiao, Tiantian Lu, Xiaoqiao Ren, Hui Wei. 2021. Effects of acid rain on plant growth: A meta-analysis. *Journal of Environmental Management*, 297, 2021, 113213, <https://doi.org/10.1016/j.jenvman.2021.113213>.
- [35]. Shu X, KeRong Zhang, QuanFa Zhang, WeiBo Wang. 2019. Ecophysiological responses of *Jatropha curcas* L. seedlings to simulated acid rain under different soil types. *Ecotoxicology and Environmental Safety*, 185, 2019, 109705, <https://doi.org/10.1016/j.ecoenv.2019.109705>.
- [36]. Shu X, Zhang K, Zhang Q, Wang W. 2023. Changes in the composition of rhizosphere bacterial communities in response to soil types and acid rain. *Journal of Environmental Management*, 325, Part A, 2023, 116493, <https://doi.org/10.1016/j.jenvman.2022.116493>.
- [37]. Singh, B., Agrawal, M. (2004). Impact of simulated acid rain on growth and yield of two cultivars of wheat. *Water, Air, and Soil Pollution*, 152:71-80.
- [38]. Sun Z, Wang L, Zhou Q, Huang X (2013) Effects and mechanisms of the combined pollution of lanthanum and acid rain on the root phenotype of soybean seedlings. *Chemosphere* 93:344–352.
- [39]. UNEP 1992. United Nation Environmental Programme (UNEP). Urban Air Pollution in Mega cities of the world. Blackwell Publisher Oxford.
- [40]. Wang CY, P. Guo, G.M. Han, X.G. Feng, P. Zhang, X.J. Tian. 2010. Effect of simulated acid rain on the litter decomposition of *Quercus acutissima* and *Pinus massoniana* in forest soil microcosms and the relationship with soil enzyme activities. *Sci. Total Environ.*, 408:2706-2713.
- [41]. Wang L, Z. Chen, H. Shang, J. Wang, P. Zhang. 2014. Impact of simulated acid rain on soil microbial community function in Masson pine seedlings. *Electron. J. Biotechnol.*, 17:199-203.
- [42]. Wei H, Yalan Liu, Jiaen Zhang, Saifei Li, Xiaolan Zhong, Huimin Xiang. 2021. Leaching of

- simulated acid rain deteriorates soil physiochemical and mechanical properties in three agricultural soils. *CATENA*, 206, 2021, 105485, <https://doi.org/10.1016/j.catena.2021.105485>.
- [43]. Wood T, Bormann FH. 1974. The effects of an artificial acid mist upon the growth of *Betula alleghaniensis* Britt. *Environmental Pollution*, 7(4):259-268. [https://doi.org/10.1016/0013-9327\(74\)90035-4](https://doi.org/10.1016/0013-9327(74)90035-4).
- [44]. Wood, T., Bormann, F.H. Short-term effects of a simulated acid rain upon the growth and nutrient relations of *Pinus strobus*, L. *Water Air Soil Pollut* 7, 479–488 (1977). <https://doi.org/10.1007/BF00285546>
- [45]. Yao FF, Ding HM, Feng LL, Chen JJ, Yang SY, Wang XH (2016) Photosynthetic and growth responses of *Schima superba* seedlings to sulfuric and nitric acid depositions. *Environ Sci Pollut Res* 23:8644–8658
- [46]. Zhang Y, Tian C, Yu T, Dayananda B, Fu B, Senaratne SL, Wu C, Li J. 2021. Differential effects of acid rain on photosynthetic performance and pigment composition of the critically endangered *Acer amplum* subsp. *catalpifolium*. *Global Ecology and Conservation*, 30, 2021, e01773, <https://doi.org/10.1016/j.gecco.2021.e01773>.
- [47]. Zhang Y, Wu L, Wang X (1996) Effects of acid rain on leaf injury and physiological characteristics of crops. *AgroEnviron Prot.*, 15:197-208.

Dear sir

Kindly check below reference to avoid any duplication error.

Iqbal, M.Z. and S.A. Rehman 2005. Effects of acid mist on the growth of *Albizia Lebbeck* (L.) Benth. and *Leucaena Leucocephala* (LAM.) De Wit. *International Chemical, Pharmaceutical & Medical Journal* 2: 263-266.

June 20, 2023