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Short Research Article

Studies on Effect of Cd and Hg on Morphological Characteristics of *Populus deltoides* (W. Bartram ex. Marshall)

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Abstract

The present investigation entitled "Studies on Effect of Cd and Hg on Morphological Characteristics of *Populus deltoides* (W. Bartram ex. Marshall)." was carried out at the Research Farm of Department of Environmental Science, College of Forestry, Dr .Y. S. Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh during 2014- 2015. Seedlings of *P. deltoides* were grown with different graded doses of cadmium (Cd) and mercury (Hg) viz, 0, 5, 10 and 20 ppm in experimental pots in four replications in CRD. Cadmium had significant influence on all the morphological parameters, whereas, mercury significantly influenced seedling height, root dry weight and total dry biomass and had no significant impact on number of leaves, collar diameter and leaf area of *P. deltoides*. The interaction effect of the two metals was non-significant for all the morphological parameters, though the characteristics had decreasing trend with increasing concentration of graded doses in soil. Highest level of Cd and Hg (20 ppm) had lowest seedling height of 2.02 and 2.25 m and lowest number of leaves i.e. 57.19 and 53.25, respectively. Collar diameter decreased from 1.71-1.45 cm and 1.63-1.50 cm for Cd and Hg, respectively. Root dry weight and total dry biomass were significantly influenced by Cd and Hg which varied from 35.97-45.66 gm (Cd) and 39.03-45.69 gm (Hg) and 100.74-130.50 gm (Cd) and 106.50-123.98 gm (Hg), respectively.

1. Introduction

Environment pollution is one of the severe problems the world is facing today. Our planet is becoming increasingly polluted with organic and inorganic compounds, primarily as a result of human activities. There is growing concern on presence of heavy metals in the environment. Toxic heavy metals in air, soil, and water are global threats to the environment. Anthropogenic activities viz., mining, fossil fuel combustion, smelting and agricultural processes have locally increased the concentration of heavy metals such as cadmium, copper, chromium, lead, arsenic, nickel, mercury etc. in the soil up to levels that are dangerous for plants, animals and human beings.

Among the various heavy metals Cd (Cadmium) and Hg (Mercury) are considered potentially important environmental pollutants. Cadmium is associated with outbreak of itai-itai disease in Japan and recently it has become of much concern because of its high toxic effect to organisms on long term exposure to low concentrations. In organisms it inhibits a large number of metabolic enzyme system, forms complexes with amino acids, peptides and proteins. At low concentrations, Cd is not toxic to plants but retards root growth and cell division, whereas, at higher concentration it inhibits chlorophyll biosynthesis and decreases total chlorophyll contentand chlorophyll a/b ratios. It is frequently accumulated by agriculturally important crops and enters the food chain with asignificant potential to impair animal and human health (Di Toppi and Gabrielli, 1999). Mercury (Hg) enters into the environment from industrial emissions, use of mercuric chloride, fungicides such as mancozeb (16% Mn and 2% Hg), Zineb and Ziram (1−18% Hg) in agriculture is another source of mercury in the environment. High concentration of $HgCl_2$ causes significant decline in chlorophyll content which in turn lead to depletion in primary and total production. If Hg enters into aquatic systems it undergoes chemical conversion from one form to the other in a cyclic manner. Mercury is a unique pollutant and oscillates between all the phases of the environment (Brosset, 1981) with high tendency for biomagnification in trophic levels. It affects photosynthesis and oxidative metabolism of plants by interfering with electron transport in chloroplasts and mitochondria. Toxic level of Hg^{2+} can induce visible injuries and physiological disorders in plants.

Plants have evolved different strategies to cope up with heavy metal stresses including detoxification and exclusion. Multipurpose fast growing tree plantations play important role in reclamation of polluted soils and are good candidates for stabilizing metal contaminated soils (Sebastiani et al., 2004). Poplar is regarded as a suitable candidate for use in phytoremediation of polluted soils due to relevant features such as the ability to withstand environmental stresses, the extensive root system and the high water uptake. It is geographically wide spread in various climatic areas, is adapted to contaminated or polluted soils and has the capacity to accumulate heavy metals.Therefore, the potential of *P. deltoides* for accumulation of heavy metals and biomass production has been evaluated in the present study.

2. Materials and Methods

The present investigation was carried out at the Research Farm of Department of Environmental Science of College of Forestry of Dr. Y. S. Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh during the year 2014–2015. It is located at an altitude of 1273 m amsl and at latitude of 35.5°N, longitude of 77.8°E which falls in the mid hill zone of Himachal Pradesh having sub–temperate and semi-humid type of climate. The annual maximum and minimum temperature ranges from 17.3 °C to 32.6 °C and 2.4 °C to 18.6 °C, respectively, whereas the annual rainfall is between 1000-1300 mm (average 1150 mm). About 70 percent of rainfall is received in the monsoon season i.e. during June to September. Mean temperature during the crop season varied from 11.77–25.22 °C, while the relative humidity was in the range of 41–60%. Young cuttings of uniform size from one year old cut back stems and branches of *P. deltoides* were planted in pots. The growing media was prepared by using sand, soil and FYM in the ratio 1:1:1. The cuttings sprouted completely after 30–40 days of planting and the selected treatment combinations were applied after complete establishment of the seedlings. In order to study the effect of cadmium and mercury toxicity on seedlings of *P. deltoides*, field experiment was conducted by applying four levels of heavy metals viz. 0, 5, 10 and 20 ppm each of Cd and Hg. Cd was applied through $CdCl_2.H_2O$(M.W. 201.324 g mol^{-1}) and Hg through $HgCl_2$ (M.W. 271.524 g mol^{-1}). In the field experiment, *P. deltoides* seedlings were exposed to total of 16 treatment combinations of Cd and Hg as per the detail given below:

Treatment combinations were as follows: $T_1(Cd_0Hg_0)$, $T_2(Cd_0Hg_5)$, $T_3(Cd_0Hg_{10})$, $T_4(Cd_0Hg_{20})$, $T_5(Cd_5Hg_0)$, $T_6(Cd_5Hg_5)$, $T_7(Cd_5Hg_{10})$, $T_8(Cd_5Hg_{20})$, $T_9(Cd_{10}Hg_0)$, $T_{10}(Cd_{10}Hg_5)$, $T_{11}(Cd_{10}Hg_{10})$, $T_{12}(Cd_{10}Hg_{20})$, $T_{13}(Cd_{20}Hg_0)$, $T_{14}(Cd_{20}Hg_5)$, $T_{15}(Cd_{20}Hg_{10})$ and $T_{16}(Cd_{20}Hg_{20})$.

2.1. Seedling height

Stem of each seedling was tagged on similar peripheral location in each pot. The data was recorded along the length of stem i.e. from tagging location till the tip of stem. Plant height was expressed in metre.

2.2. Number of leaves

The total number of leaves were counted manually at the end of the experiment.

2.3. Collar diameter

The collar of seedlings in each pot were marked with red paint at base. Diameter of collar was measured at three points with the help of vernier calliper at base, middle and tip of shoot to minimize the effect of taper. Average diameter was calculated for each seedling and expressed in cm.

2.4. Leaf area

Randomly five recently matured leaves were collected from each plant and leaf area was measured with Leaf area meter (Model-LI-COR-3100). The average leaf area was expressed as cm^2.

2.5. Root dry weight

Roots were separated after uprooting the seedlings and dried at 60±5 °C in hot oven till they attained a constant weight. Root dry weight was expressed in g plant^{-1}.

2.6. Total dry biomass

After the completion of experiment, the seedlings were uprooted and divided into various plant parts *viz.* leaves, stems, etc. collected and weighed. Samples were kept in separate paper bags and then dried in oven at 60±5^{o}C till constant weight was reached. Total dry biomass was measured after adding dry weight of all parts and expressed as g plant^{-1}.

3. Results and Discussion

3.1. Effect of graded doses of Cd and Hg on seedling height of P. deltoides

The application of various levels of Cd to soils of *P. deltoides* seedlings had a significant influence on seedling height which varied from 2.02-2.26 m (Table1). Maximum height of seedling i.e. 2.26 m was recorded at 0 ppm dose while lowest i.e. 2.02 m at 20 ppm. With increase in Cd concentration there was decrease in the plant height. The results are in conformity with the findings of Durand et al. (2011) who reported significant reduction (26%) in stem height of (*Populustremulax P. alba*) after 61 days of Cd treatment. Yanbao et al. (2007) also observed the maximum plant height (105.80 cm) of *Populus cathayana* where no Mn was applied which decreased to 66.20 cm with increase in concentration to 1 mM. Mercury (Hg) also influenced the seedling height significantly, which ranged from 2.03-2.25 m under different levels of Hg. The minimum height of 2.03 m was recorded with 20 ppm level, whereas, the highest height of 2.25 m was noticed where no mercury was applied. Methyl mercury compound of Hg is reported of much greater toxicity than inorganic mercury salts to a variety of life forms. Mercury is known to affect photosynthesis and oxidative metabolism by interfering with electron transport in chloroplasts and mitochondria in plants (Bernier et al., 1993).

3.2. Effect of graded doses of Cd and Hg on number of leaves of P. deltoides

Under all the combinations of Cd and H_g the number of leaves per plant ranged from 53.25-72.75. Highest number of leaves (65.69) was recorded where no Cd treatment was applied (Table 1). The *P. deltoides* seedlings exposed to 20 ppm level of Cd registered significantly lowest number of leaves (57.19). The increasing level of Cd was found to decrease the number of leaves per seedling. The results are in agreement with the findings of Elobeid et al. (2012) who reported decrease in number of leaves of Populus×canescens with increase in Cd concentration to 50 µm in hydroponic solution with respect to control. Yanbao et al. (2007) also reported that high concentration of Mn cause significant decrease in leaf number of *P. cathayana*.

Table 1: Effect of graded doses of Cd and Hg on seedling height (m) and number of leaves of *P. deltoides*

Hg levels / Cd levels	Seedling height: Hg (0 ppm)	Hg (5 ppm)	Hg (10 ppm)	Hg (20 ppm)	Mean	No. of leaves: Hg (0 ppm)	Hg (5 ppm)	Hg (10 ppm)	Hg (20 ppm)	Mean
Cd (0 ppm)	2.50	2.33	2.15	2.06	2.26	72.75	67.50	64.00	58.50	65.69
Cd (5 ppm)	2.27	2.34	2.18	2.21	2.25	61.75	61.50	60.25	61.75	61.31
Cd (10 ppm)	2.13	2.12	2.01	1.98	2.06	59.50	59.25	58.25	54.50	57.88
Cd (20 ppm)	2.10	2.05	2.05	1.87	2.02	58.75	58.50	58.25	53.25	57.19
Mean	2.25	2.21	2.10	2.03		63.19	61.69	60.19	57.00	
CD(p=0.05)	0.151					CD(p=0.05)	6.03			
Cd	0.151					Cd	NS			
Hg	NS					Hg	NS			
Cd×Hg						Cd×Hg				

3.3. Effect of graded doses of Cd and Hg on collar diameter of P. deltoides

The collar diameter of *P. deltoides* seedlings recorded among the interaction effect ranged from 1.41 to 1.86 cm and all the interaction values were at par with each other (Table 2). The collar diameter decreased with increasing levels of Cd. Highest diameter (1.71 cm) was observed in seedlings grown at 0 ppm, whereas, significantly, lowest collar diameter of 1.45 cm was noticed with 20 ppm level of Cd. The results are in concordance with the findings of Durand et al. (2011) who observed reduced stem diameter of 822 µm for *Populustremula×P. alba* clone after 61 days of exposure to soil containing 50 mg Cd Kg^{-1} as compared to zinc (1100 µm) and control (1126 µm). In an other study Tanvir and Siddique (2010) reported 22% decrease in collar diameter of *P. deltoides* under municipal wastewater irrigation than domestic waste water. Irrespective of Cd, different doses of Hg resulted in non significant influence on collar diameter of *P. deltoides* though it ranged from 1.50−1.63 cm.

3.4. Effect of graded doses of Cd and Hg on leaf area (cm²) of P. deltoides

The interaction of heavy metals Cd and Hg did not influence leaf area in *P. deltoides* significantly, though the values ranged from 640.30 to 850.55 cm^2 (Table 2). Different doses of Hg resulted in non significant influence on leaf area of *P. deltoides*. However, different levels of Cd had significant effect on the leaf area which ranged from 675.11 cm^2 (Cd 20 ppm) to 827.92 cm^2 (Cd 0 ppm).The leaf area of *P. deltoides* plants followed a decreasing trend with increasing levels of Cadmium. The results are in confirmation with the findings of Durand et al.(2011) who reported 28 per cent

Table 2: Effect of graded doses of Cd and Hg on collar diameter and leaf area of *P. deltoides*

Hg levels / Cd levels	Collar diameter					Leaf area				
	Hg (0 ppm)	Hg (5 ppm)	Hg (10 ppm)	Hg (20 ppm)	Mean	Hg (0 ppm)	Hg (5 ppm)	Hg (10 ppm)	Hg (20 ppm)	Mean
Cd (0 ppm)	1.86	1.85	1.61	1.50	1.71	850.55	836.10	814.10	810.93	827.92
Cd (5 ppm)	1.62	1.65	1.54	1.61	1.60	814.48	843.43	809.55	810.40	819.46
Cd (10 ppm)	1.52	1.49	1.48	1.48	1.49	780.38	774.73	734.08	708.95	749.53
Cd (20 ppm)	1.51	1.46	1.44	1.41	1.45	689.58	686.05	684.50	640.30	675.11
Mean	1.63	1.61	1.59	1.50		783.74	785.08	760.56	742.64	
CD(p=0.05)	0.132					CD(p=0.05)	60.68			
Cd	NS					Cd	NS			
Hg	NS					Hg	NS			
Cd×Hg						Cd×Hg				

inhibition in the total leaf area of *P. tremula×P. alba* after 61 days of exposure to Cd treatments as compared to control, whereas, no significant changes in leaf area occurred under Zn exposure. Milan et al. (2012) in another study, obtained similar results of decrease in the leaf area with increased Cd concentration in the soil as compared to control in *Salix alba*, which could be due to change in the photosynthetic activity in effected seedlings.

3.5. Effectof graded doses of Cd and Hg on root dry weight (g) of P. deltoides

The root dry weight at various treatments varied from 31.50-55.25 g (Table 3). Whereas, a significant relationship between root dry weight and Cd levels applied to the *P. deltoides* seedling was observed. The highest root dry weight (45.66 g) was recorded at 0 ppm level of Cd, whereas, minimum (35.97 g) was recorded at Cd 20 ppm. Similarly, different graded doses of Hg also resulted in significant influence on root dry weight of *P. deltoides*. Hg at 0 ppm level recorded maximum root dry weight of (45.69 g), whereas, minimum root dry weight was observed in Hg 20 ppm level (39.03 g). High level of Hg^{2+} interfere with the mitochondrial activity and induces oxidative stress, leading to the disruption of biomembrane lipids and cellular metabolism in plants Cargnelutti et al. (2006).

The results are in confirmity with the findings of Borghi et al. (2007) who reported highest root biomass 0.42 g of *P. deltoides×P. nigra* at 500 µm copper concentrations which decreased to 0.15 g at 1000 µM after 15 days of treatment. Durandet al. (2011) also reported reduction in root dry weight of *P. tremula×P. alba* after 61 days of exposure to a soil containing 50 mg Cd kg^{-1} where a root dry weight of 2.76 g was recorded as compared to 4.07 g in control. Cd can be accumulated to high concentrations in roots and it causes inhibition of photosynthesis resulting in biomass loss which may eventually cause plant death (Di Toppi and Gabrielli, 1999).

3.6. Effect of graded doses of Cd and Hg on total dry biomass (g) of P. deltoides

Table 3: Effect of graded doses of Cd and Hg on root dry weight (g) and total dry biomass (g) of *P. deltoides*

Hg levels / Cd levels	Root dry weight					Total dry biomass				
	Hg (0 ppm)	Hg (5 ppm)	Hg (10 ppm)	Hg (20 ppm)	Mean	Hg (0 ppm)	Hg (5 ppm)	Hg (10 ppm)	Hg (20 ppm)	Mean
Cd (0 ppm)	55.25	45.13	39.75	42.50	45.66	151.50	133.38	122.63	114.50	130.50
Cd (5 ppm)	45.00	43.25	46.63	43.13	44.50	124.00	128.75	125.53	116.29	123.64
Cd (10 ppm)	43.75	43.63	39.38	39.00	41.44	113.53	108.08	110.01	103.58	108.80
Cd (20 ppm)	38.75	37.88	35.75	31.50	35.97	106.88	106.88	97.57	91.64	100.74
Mean	45.69	42.47	40.38	39.03		123.98	119.27	113.93	106.50	
CD(p=0.05)	4.63					CD(p=0.05)	10.90			
Cd	4.63					Cd	10.90			
Hg	NS					Hg	NS			
Cd×Hg						Cd×Hg				

Total dry biomass of *P. deltoides* at various graded doses of Cd and Hg varied significantly with increase in Cd and Hg levels (Table 3). The total dry biomass among the interaction effect ranged from 91.64-151.50 g and all the interaction values were at par with each other. Cd had significant effect on the total dry biomass of *P. deltoides* which ranged from 100.74-130.50 g. Highest biomass was recorded at Cd 0 ppm (130.50 g) and the lowest was recorded at Cd 20 ppm (100.74 g). The present results find support from the findings of Pandey and Tripathi (2011) who reported reduction in biomass of *Albizia procera* with increase in concentration of Cd from 1-10 ppm. Maximum reduction of 0.12% in biomass as compared to control was caused by 10 ppm of Cd. Hg concentrations had significant effect on *P. deltoides*, highest biomass was recorded at Hg 0 ppm (123.98 g) and lowest biomass was observed at Hg 20 ppm (106.50 g). The results however are contradictory to the findings of Wang et al. (2005) who reported that root and shoot biomass increased in willow (*Salix viminalis*×*S. schwerinii*) when grown in Hg contaminated soil. The decrease in biomass with high concentration of heavy metals may be due to sensitivity of enzymes of the photosynthetic carbon reduction cycle to Cd as reported by De Filippis and Zeigler (1993) which in turn effectthe plant biomass.

4. Conclusion

The impact of graded doses of Cd and Hg on various morphological parametersof *P. deltoides* grown in metal contaminatedsoils revealed that cadmium had significant influence on all themorphological parameters, whereas, mercury significantly influenced seedling height, root dry weight and total dry biomass and had no significant impact on number of leaves, collar diameter and leaf area. The observed ability of *P. deltoides* to continue growth even at higher doses of Cd and Hg and the ability to accumulate metals in its tissues demonstrated its resistance to moderate to high levels of metals.

5. Further Research

The present study widens the scope for studying the effect of higher concentration of heavy metals beyond 20 ppm in future to confirm the phytoremediation ability of this species.

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