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Effects of trace elements on morphological and biochemical characteristics of poplar hybrid (*Populus ciliata* x *Populus maximowiczii*)

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Abstract
The present investigations were carried out to study the effect of heavy metals *viz*. As and Pb on morphological and biochemical parameters and their uptake in Poplar hybrid (*Populus ciliata* x *Populus maximowiczii*) seedlings. Pot experiment was conducted on one-year-old cuttings. Experiment was laid out with 16 treatments consisting of various combinations of different levels of as and Pb at 0, 5, 10 and 20 ppm. Arsenic had significant influence on all the morphological parameters, whereas, lead significantly influenced seedling height, stem diameter, root dry weight and total dry biomass and had no significant impact on number of leaves, collar diameter and leaf area of Poplar hybrid. Biochemical parameters like chlorophyll content and total soluble sugar in leaves decreased with increasing level of as and Pb. The species was capable of tolerating the applied concentrations of as and Pb and could be a good candidate for planting in the heavy metal contaminated soils. The present investigations widen the scope for studying the effect of higher concentration of heavy metals beyond 20 ppm in future to confirm the phytoremediation ability of this Poplar hybrid species.

Keywords: heavy metals, Poplar hybrid, morphological and biochemical characteristics

Introduction

Environment pollution is one of the severe problems the world is facing today. Heavy metals are major environmental pollutants which are discharged into the atmosphere from the burning of fossil fuels, release of industrial wastes and use of agrochemicals. Anthropogenic activities such as mining, fossil fuel combustion, smelting and agricultural processes have locally increased the levels of heavy metals such as Cd, Cu, Cr, Pb, As, Ni in the soil up to levels that are dangerous for plants, animals and human beings (Flora and Busselberg, 2006) [1].

Heavy metals are present in natural environment and many of them such as copper, zinc and nickel are essential for plants at certain low concentrations, but at higher levels they are toxic (Liu *et al*., 2007) [2]. In soil, the presence of heavy metals decreases microbial activity and soil fertility, leading to inhibition of plant growth by indirect and direct effects. When present in the soil they are translocated to different parts of the plant thereby affecting its various morphological and biochemical parameters (Pandey and Tripathi, 2011) [3].

Arsenic is toxic to a wide range of organisms, including plants. As is a metalloid having properties of both metals and non-metals, and can undergo different ranges of chemical interactions in plants. Arsenic enters the soil either from natural or anthropogenic sources (Gonzaga *et al*., 2006) [4] Natural sources include weathering reactions, volcanic eruptions and biological activities (Niazi *et al*., 2012) [5] and anthropogenic activities such as use of pesticides in agriculture, smelting, paints, dyes, cosmetics also contribute in As contamination of soils (Abid *et al*., 2016) [6].

Prolonged exposure to as results in restricted shoot and root growth of plants (Niazi*et al*., 2017) [7]. At higher concentrations, As causes many physiological and biochemical dysfunctions in plants such as limited uptake of water and nutrients, disruption of ATP, enzymes and chloroplast structure, reduced photosynthetic capacity, chlorosis and necrosis (Khalid *et al*, 2016) [8]. When plants were exposed to excess arsenic either in soil or in solution culture, they exhibited toxicity symptoms such as: inhibition of seed germination, decrease in plant height, depress in tillering, reduction in root growth, decrease in shoot growth, lower fruit and grain yield and sometimes, lead to death (Johan *et al*., 2003) [9].

Lead (Pb) is known to be one of the most abundant toxic metals in the biosphere. It is one of the potentially toxic heavy metal pollutants with no known biological function and its concentrations are rapidly increased in agricultural soil (Hamis *et al*., 2010) [10]. Elevated Pb in soils may compromise soil productivity and even a very low concentration can inhibit some vital plant processes such as photosynthesis, mitosis and water absorption with toxic symptoms of dark leaves, wilting of older leaves, stunted foliage and brown short roots (Patra *et al*., 2004) [11].

The effect of lead depends on the concentration, type of salts, soil properties and plant species involved. In general, effects are more pronounced at higher concentrations and durations. In some cases, lower concentrations stimulate metabolic processes and enzymes involved. The major processes affected are seed germination, seedling growth (shoot and root growth), photosynthesis, plant water status, mineral nutrition, and enzymatic activities. Higher concentrations of lead significantly affect plant water status causing water deficit. Transpiration intensity, osmotic pressure of cell sap, water potential of xylem, and relative water content were significantly reduced (Parys *et al*., 1998) [12]. Lead also reduces the size of stomata but increases their number and diffusion resistance. Lead is reported to reduce the uptake and transport of nutrients in plants, such as Ca, Fe, Mg, Mn, P and Zn by blocking the entry or binding of the ions to ion-carriers making them unavailable for uptake and transport from roots to leaves (Xiong, 1997) [13].

Forest trees have a great economic and ecological value, as well as unique biological properties of basic scientific interest. The genus *Populus* is regarded as model system for forest trees since it offers several advantages including rapid growth, high biomass production and prolific sexual reproduction combined with a relatively small genome. In addition, it is amenable to coppicing and short-rotation harvest, as well as in- vitro propagation and genetic transformation. Poplar is also 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. Plantation of trees not only helps in reclamation of polluted soils but also provides many other environmental benefits. Tolerance to metal/metalloid is a basic requirement for plants species to be used for phytoremediation (Tong *et al*., 2004) [14].

Fast growth, deep root system, high biomass production as well as broad species and clonal variability predetermine the use of poplar species (*Populus* sp.) and their hybrids for phytoremediation techniques (Marmiroli *et al*., 2011) [15]. Compared to herbaceous species, poplar trees have several advantageous characteristics such as a deeper root system, a higher productivity and transpiration activity. The genus *Populus* is geographically widespread in various climatic areas and its presence can be observed in the severe soil conditions (pioneer species) that characterize heavily contaminated areas (Pulford and Watson, 2003) [16]. Various *Populus* species are recognized as perennial plants, easy in propagation with high metal tolerance and soil stabilization potential (Wan *et al*., 2008) [17]. Poplars are also well suited for phytoremediation because they can remove contaminants in several ways, including degrading them, confining them, or by acting as filters or traps (Isebrands and Karnosky, 2001) [18]. Several studies have focused the potentiality of willows and poplars for phytoextraction (Kuzovkina and Quigley, 2005) [19].

Therefore, keeping in view the potential of Poplar hybrid for biomass production and accumulation of heavy metals a study was carried out to evaluate the effect of different doses of As and Pb on morphological and biochemical characteristics of Poplar hybrid and to study the uptake of As and Pb in Poplar hybrid.

Methodology

Location and climate

The research farm of Department of Environmental science 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 ranged from 17.3°C to 32.6°C and 2.4°C to 18.6°C, respectively, whereas the annual rainfall was between 1000-1300 mm (average 1150 mm). About 70 per cent of rainfall was 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 per cent.

Plant Material and Treatments

Young cuttings of uniform size from one year old cut back stems and branches of Poplar hybrid (*Populus ciliata* x *Populus maximowiczii*) were planted in pots of 15 kg capacity. 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. The selected treatment combinations were applied after complete establishment of seedlings in the pots. To study the effect of Arsenic and Lead toxicity on seedlings of Poplar hybrid, field experiment was conducted by applying four levels of heavy metals *viz*. 0, 5, 10 and 20 ppm each of Us and Pb. Arsenic was applied through As_2O_3 (M.W. 197.84 g/mol) and Pb through $PbCl_2$ (M.W. 278.10 g/mol). In the field experiment, Poplar hybrid seedlings were exposed to total 16 treatment combinations of as and Pb as per the detail given below:

Table 1

Treatment combinations	
T_1 -As_0Pb_0	T_9 - $As_{10}Pb_0$
T_2 -As_0Pb_5	T_{10} -$As_{10}Pb_5$
T_3 - As_0Pb_{10}	T_{11} - $As_{10}Pb_{10}$
T_4 -As_0Pb_{20}	T_{12} -$As_{10}Pb_{20}$
T_5 -As_5Pb_0	T_{13} - $As_{20}Pb_0$
T_6 - As_5Pb_5	T_{14} - $As_{20}Pb_5$
T_7 - As_5Pb_{10}	T_{15} - $As_{20}Pb_{10}$
T_8 - As_5Pb_{20}	T_{16} - $As_{20}Pb_{20}$

The treatment combinations were replicated four times and the selected doses were applied once in a week in solution form. The seedlings were exposed to the selected treatment combinations.

Plant Sampling and Analysis

At the end of the experiment, after recording the morphological parameters, plant sampling was done. Freshly matured leaf samples were collected and taken to laboratory in paper bags for analysis of biochemical parameters. At the termination of experiment, whole plant was uprooted. The leaves, stems and roots of each seedling were separated, taken to laboratory, weighed separately for their fresh and dry weights, dried at 60 ± 5° C, powdered and kept in polythene bags for further chemical analysis.

Observations recorded

Observations on various morphological and biochemical parameters of *P. ciliata* x *P. maximowiczii* seedlings were recorded and are presented as under:

Morphological Parameters

Randomly five recently matured leaves were collected from each plant and leaf area was measured with Leaf area meter using Model-LI-COR-3100 in cm^2. The total numbers of leaves were counted manually at the end of the experiment. 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 in meter. The stems of seedlings in each pot were marked with red paint at the base.

Diameter of stem (cm) was measured at three points with the help of vernier calliper at the base, middle and tip of shoot to minimize the effect of taper. Average diameter was calculated for each seedling. After the completion of experiment, the seedlings were uprooted and divided into various plant parts *viz*. leaves, stems, etc. and then collected and weighed. Samples were kept in separate paper bags and were dried in oven at 60±5ºC till constant weight was reached. Total dry biomass was measured after adding dry weight of all parts and expressed as gram per plant. Roots were separated after uprooting the seedlings and dried at 60±5ºC in hot oven till they attained a constant weight. Root dry weight was expressed in gram per plant.

Biochemical Parameters

Chlorophyll content

The leaf chlorophyll content was estimated by the method of Hiscox and Israelstam, (1997) [20]. The fresh leaves were chopped to fine pieces under subdued light. Sample of 100 mg of chopped leaves was placed in vials containing 7 ml of Di-methyl sulphoxide. The vials were incubated at 65°C for half an hour, extract was then transferred to graduate test tube and the final volume was made to 10 ml with Di-methyl sulphoxide. The optical density (O.D) values of the above extract were recorded on Spectrophotometer (Model-Spectronic-20D) at 645 and 663 nm wavelength against Di-methyl sulphoxide blank. The total chlorophyll content was calculated by using formula:

$$\text{Total chlorophyll (mg g}^{-1}) = \frac{20.2\ A_{645} + 8.02\ A_{663}}{a \times 1000 \times w} \times V$$

Where;

V = Volume of extract made

a = Length of light path in cell (usually 1cm)

w = Weight of sample

A_{645} = Absorbance at 645nm

A_{663} = Absorbance at 663nm

Phenol content

The phenol contents in plant samples were estimated by Bray HC and Thorpe WV (1954) [21] method. The dried leaf samples (0.5g) were centrifuged at 10,000 rpm for 20 minutes in 80 per cent ethanol and then supernatant was evaporated to dryness. The residue of the sample was dissolved in 5 ml of distilled water. Out of this 0.2 ml was taken in test tubes to which 0.5 ml Folin Ciocalteu Reagent was added and final volume was made 3 ml by adding water. After 3 minutes, 2 ml of 20 per cent Na_2CO_3 solution was added in the test tube. The optical density (O.D) values of the above extract were recorded on Spectrophotometer (Model-Spectronic-20D) at 650 nm wavelength against standard catechol blank.

Total soluble sugar content in leaves

Total sugar contents in leaves of Poplar hybrid were determined based on the method given by Dubois *et al*. (1956) [22]. Fresh leaves (0.1g) were added with 5 ml of 8% ethanol to test tubes, placed in water bath, and heated for 1 hour at 80 ºC. Then, 1 ml of the sample extract was taken in another set of test tubes and mixed with 1 ml each of 18% phenol and distilled water, and then allowed to stand at room temperature for an hour.

Finally, 5 ml of sulphuric acid was added and the whole mixture was vortexed. The absorbance was read at 490 nm wavelength on the UV spectrophotometer. Ethanol 80% was used as blank sample. The results were expressed in mg g^{-1}.

$$\text{Total Soluble Sugar Content} = \frac{\text{Absorbance of sample} \times \text{K Value} \times \text{Dilution Factor}}{\text{Weight of Sample} \times 100}$$

Heavy metal analysis in plants

The dried leaf, stem and root samples were digested in di-acid (HNO_3 + $HClO_4$) mixture by taking 1 gm of sample as per standard procedure given by Singh *et al*. (2005) [23]. Heavy metals *viz*; as and Pb were estimated by using Inductively Coupled Plasma Emission Spectrometer (Model-ICAP 6300 Duo) and concentration was expressed as mg/kg.

Soil Samping and Analysis

At the termination of experiment, representative soil samples from each of the experimental pots growing Poplar hybrid seedlings were collected for each replication. The samples were air dried in shade, powdered, sieved in 2 mm sieve and stored in cloth bags for further laboratory analysis.

Soil microbial biomass (Soil Fumigation-Extraction Method)

Soil microbial biomass carbon was determined by soil fumigation-extraction method as detailed by Vance *et al*. (1987) [24]. Sample of 10 g of soil was fumigated with 20 ml of $CHCl_3$ in vacuum desiccators for 24 hours in dark and other 10 g of same soil sample was refrigerated, Both the samples (fumigated and unfumigated) were extracted with 0.5 M K_2SO_4 for half an hour and then treated with potassium dichromate, H_2SO_4 and ortho-phosphoric acid heated on hot plate at 120 ^{0}C for 30 minutes. Thereafter, they were diluted to 250 ml with distilled water and 2-3 drops of diphenylamine were added and titrated against 0.05 N FAS (Ferrous Ammonium Sulphate) and microbial biomass carbon was calculated by using the following formula:

$$\text{MB-C } (\mu\text{g/g soil}) = \frac{OC_F - OC_{UF}}{K}$$

OC_F = Total amount of extractable C in fumigated soil

OC_{UF} = Total amount of extractable C in unfumigated soil

K = Factor (0.45) which represents the efficiency of extraction of microbial biomass carbon.

The soil pH was determined in 1: 2.5 soil and water suspension with the help of pH meter (Model 510 of EIA make). The EC was estimated in 1:2 soil-water suspension (Jackson, 1973) [26] by using microprocessor based meter (Model- 1601 EIA make) and expressed as dS/m. To estimate the heavy metals in each collected soil sample, 0.5 g of soil sample was digested in concentrated HNO_3 and H_2O_2 mixture by using US-EPA 3050 B method of APHA, 2005. Heavy metals *viz*; As and Pb were estimated by injecting filtered samples in solution form into Inductively Coupled Plasma Emission Spectrometer (Model-ICAP 6300 Duo) and concentration was expressed as mg/kg.

Results and Discussion

Morphological Parameters

The impact of graded doses of As and Pb on various morphological parameters *viz*., seedling height, number of leaves, leaf area, stem diameter, root dry weight and total dry biomass of Poplar hybrid grown in metal contaminated soils revealed that arsenic (As) had significant influence on all the morphological parameters, whereas, lead (Pb) significantly influenced seedling height, stem diameter, root dry weight and total dry biomass and had no significant impact on number of leaves and leaf area of Poplar hybrid. The interaction effect of as and Pb was non-significant for all the morphological parameters, though the characteristics had decreasing trend with increasing concentration of different doses in soil. The different doses of as decreased the leaf area with the range lying between 652.18-749.37 cm^2. Leaf area at 20 ppm as concentration was about 13% less than the control. Number of leaves per plant followed a decreasing trend of 20 ppm (13.16%) > 10 ppm (6.4%) > 5 ppm (5.2%) with increasing doses of As (Table 1).

Table 2: Effect of different doses of as and Pb on leaf area (cm^2) and leaves number of Poplar hybrid

Pb levels (ppm) / As levels (ppm)	0	5	10	20	Mean
0	780.00	742.50	755.00	720.00	749.37
5	762.50	722.50	725.00	757.50	741.87
10	697.50	725.00	697.50	660.00	695.00
20	667.50	660.00	645.00	636.25	652.18
Mean	726.87	712.50	705.62	703.43	
$CD_{0.05}$ As: 38.66 Pb: NS As × Pb: NS					
Pb levels (ppm) / As levels (ppm)	**0**	**5**	**10**	**20**	**Mean**
0	27.15	26.00	25.75	24.75	25.91
5	25.25	25.00	24.50	23.50	24.56
10	24.50	23.50	24.50	24.50	24.25
20	23.25	23.00	21.50	22.25	22.50
Mean	25.03	24.37	24.06	23.75	
$CD_{0.05}$ As: 1.45 Pb: NS As × Pb: NS			s		

The highest reduction of 13.16% in leaf number of Poplar hybrid was with 20 ppm concentration of as which was followed by 6.4% and 5.2% at 10 and 5 ppm, respectively (Table 1).

As and Pb at 20 ppm levels resulted highest decrease in seedling height as compared to control followed by 10 ppm and 5 ppm levels (Table 2). The results are in agreement with the findings of Hussain *et al*., (2017) [27] who reported decrease in plant height of *P. deltoides* with increase in soil as levels (Control, 5, 10 and 15 mg/kg). Similar results were reported by Ansari *et al*. (2013) [43] and Gomes *et al*. (2013) [28] according to them there was a reduction in growth and biomass of plants treated with increasing as concentrations in the growth medium. Higher As concentration can inhibit growth because of interference with plant metabolic processes that often leads to death. Several reports described the morphological changes under as-rich growing medium (Mokgalaka *et al*., 2008) [29].

Table 3: Effect of different doses of As and Pb on seedling height (m) and stem diameter of Poplar hybrid

Pb levels (ppm) / As levels (ppm)	0	5	10	20	Mean
0	2.45	2.17	2.15	2.13	2.22
5	2.33	1.97	1.94	1.91	2.04
10	2.17	1.93	1.89	1.86	1.96
20	2.01	2.04	1.88	1.90	1.95
Mean	2.24	2.03	1.95	1.94	
$CD_{0.05}$ As: 0.07 Pb: 0.07 As × Pb: NS					
Pb levels (ppm) / As levels (ppm)	**0**	**5**	**10**	**20**	**Mean**

0	1.60	1.58	1.56	1.52	1.56
5	1.52	1.65	1.50	1.47	1.53
10	1.52	1.42	1.50	1.36	1.45
20	1.40	1.35	1.25	1.39	1.34
Mean	1.51	1.50	1.45	1.43	
$CD_{0.05}$ As: 0.11 Pb: 0.11 As × Pb: NS					

The range of stem diameter varied between 1.25-1.65 cm which decreased with increase in the levels of as and Pb (Table 2). The highest reduction of 14% was recorded at 20 ppm of as followed by 7% and 2% at 10 ppm and 5 ppm, respectively. Stem diameter of plant followed a decreasing trend of 20 ppm (5.2%) > 10 ppm (4%) > 5 ppm (0.6%) with increasing doses of Pb. The results are in close agreement with the findings of Salehi *et al*., (2014) [30] who reported significant reduction in stem diameter and stem length of *Populus nigra* and *Populus alba* clones after two months of Pb treatment. This stress may be due to the presence of Pb beyond permissible limit.

Table 4: Effect of different doses of as and Pb on total dry biomass (g) and root dry weight (g) of Poplar hybrid

Pb levels (ppm) / As levels (ppm)	0	5	10	20	Mean
0	129.87	129.25	128.25	128.38	128.94
5	127.50	124.91	126.50	125.00	125.97
10	127.97	126.97	122.50	118.00	123.86
20	128.65	124.00	119.25	113.50	121.35
Mean	128.50	126.28	124.12	121.22	
$CD_{0.05}$ As: 3.05 Pb: 3.05 As × Pb: NS					
Pb levels (ppm) / As levels (ppm)	**0**	**5**	**10**	**20**	**Mean**
0	47.62	44.25	45.25	42.30	44.85
5	45.00	43.80	37.50	44.87	43.04
10	38.25	41.37	38.37	35.75	38.43
20	36.75	36.00	30.75	28.25	32.93
Mean	41.90	41.25	37.96	37.79	
$CD_{0.05}$ As: 2.91 Pb: 2.91 As × Pb: NS					

The total dry biomass of Poplar hybrid grown in as and Pb contaminated soils ranged from 113.50-129.87g (Table 3). The application of different doses of as and Pb in soils of Poplar hybrid significantly influenced the root dry weight which ranged from 32.93-44.85 g and 37.79-41.90 g, respectively. Pandey and Tripathi (2011) [3] also reported reduction in biomass of *Albizia procera* with increase in concentration of As from 1-10 ppm. Maximum reduction of 75 per cent in biomass as compared to control was caused by 10 ppm of As. Tsakou *et al*. (2003) [31] also reported that as the level of concentration of heavy metal increased there was significant decrease in dry biomass of *Populus* x *euramericana* by metal treatment under field conditions.

Biochemical Parameters

The Poplar hybrid seedlings varied in chlorophyll content within the range of 1.44-1.92 mg/g. Higher doses of As and Pb at 20 ppm influenced chlorophyll content by reduction upto 21.21% and 10.55%, respectively over control followed by 10 ppm and 5 ppm (Table 4). These results are in conformity with those of Pandey *et al*. (2011) [32] who observed similar trend in *Albizzia procera*. Wang *et al*. (2011) [33] also reported that chlorophyll content of *Populus deltoides* decreased with increase in concentration of As.

Table 5: Effect of different doses of as and Pb on chlorophyll content (mg g^{-1}) in leaves of Poplar hybrid

Pb levels (ppm) / As levels (ppm)	0	5	10	20	Mean
0	1.92	1.60	1.67	1.42	1.65

5	1.52	1.66	1.45	1.65	1.57
10	1.57	1.46	1.51	1.43	1.52
20	1.43	1.27	1.25	1.24	1.30
Mean	1.61	1.50	1.46	1.44	
$CD_{0.05}$ As: 0.12 Pb: 0.12 As × Pb: 0.24					

Different doses of as also increased phenol content with the range lying between 1.01-4.27 mg/g. The highest (4.27 mg/g) phenol content was observed at 20 ppm of as followed by (3.18 mg/g) at 10 ppm of as and lowest (1.01 mg/g) phenol content over control (Table 5). According to Pandey and Tripathi (2011) [3] 10 ppm concentrations of As exhibited the maximum (66.6%) increase in polyphenol content of *Albizzia procera* as compared to control.

Table 6: Effect of different doses of as and Pb on phenol content (mg g^{-1}) in leaves of Poplar hybrid

Pb levels (ppm) / As levels (ppm)	0	5	10	20	Mean
0	0.76	0.90	0.99	1.40	1.01
5	1.60	1.52	1.83	1.62	1.64
10	2.66	3.49	3.03	3.56	3.18
20	3.02	4.12	4.30	4.39	4.27
Mean	2.41	2.45	2.39	2.47	
$CD_{0.05}$ As: 0.11 Pb: NS As × Pb: 0.21					

Total soluble sugar content values lied within the range of 0.38-0.57 mg g-1was recorded in the control (Table 6). As and Pb at 20 ppm levels resulted minimum total sugar content in leaves with respect to control. The present results are in congruence with those of Wang *et al*., (2011) [33]; Pandey and Tripathi, (2011) [3]. The decrease in the total sugar content with increasing concentration of heavy metals has also been reported in agricultural crops by Hemalatha *et al*. (1997) [34].

Table 7: Effect of different doses of as and Pb on total soluble sugar content (mg g^{-1}) in leaves of Poplar hybrid

Pb levels (ppm) / As levels (ppm)	0	5	10	20	Mean
0	0.57	0.57	0.55	0.52	0.54
5	0.55	0.52	0.47	0.55	0.52
10	0.48	0.51	0.49	0.45	0.48
20	0.46	0.46	0.40	0.38	0.43
Mean	0.52	0.51	0.48	0.47	
$CD_{0.05}$ As: 0.03 Pb: 0.03 As × Pb: NS					

Effects of Heavy Metals on Soil Properties

The application of different doses of as and Pb to the soils of Poplar hybrid had non-significant effect on pH and EC of the soil (Table 7). The range of soil microbial biomass was 63.50 mg/kg to 86 mg/kg at various levels of heavy metals. Studies of Wang *et al*., (2011) [33] also revealed that soil microbial carbon was negatively affected by elevated heavy metal levels in soil and correlated to heavy metal stress. Many reports have shown that short-term or long-term exposure to toxic metals results in the reduction of microbial diversity and activities in soil (Lasat, 2002) [35].

Table 8: Effect of different doses of as and Pb on soil microbial biomass (mg kg^{-1})

Pb levels (ppm) / As levels (ppm)	0	5	10	20	Mean
0	86.00	74.36	75.50	72.75	77.15
5	67.75	72.75	74.50	79.25	73.56
10	71.50	72.25	80.00	65.00	72.18
20	79.50	80.75	64.50	63.50	72.06
Mean	76.18	75.02	73.62	70.12	
$CD_{0.05}$ As: 2.52 Pb: 2.52 As × Pb: 5.05					

The soil biomass followed a decreasing trend with increased concentration of as and Pb levels. As reduction of soil microbial biomass followed the order 20 ppm (16%) > 10 ppm (11%) > 5 ppm (1.8%) and the highest reduction in soil microbial biomass (8%) was recorded at 20 ppm Pb level followed by 10 ppm Pb level (3.4%) and 5 ppm Pb level (1.5%). Concentration of as and Pb in soil was found to be in the range of 4.07- 22.65 mg/kg and 7.49-51.75 mg/kg respectively. The application of 20 ppm as induced maximum built up of As concentration in soil (22.65 mg kg^{-1}) and the lowest As concentration in soil (1.87 mg kg^{-1}) was recorded at 0 ppm As level. Similarly, Pb concentration in soil was found to increase with increase in levels of Pb applied. Highest built up of 51.75 mg/kg was under 20 ppm dose. The heavy metal concentration in leaves ranged from 0.017-0.142

mg/kg and 0.011-0.140 mg/kg for as and Pb, respectively. The highest concentration of as in leaves (0.142 mg/kg) was recorded at 20 ppm As^{+} 10 ppm Pb level. Highest Pb concentration in leaves (0.140 mg/kg) was recorded at 20 ppm. These results are in line with the findings of Ciurli *et al*., (2014) [36] who also found that as concentration in plant tissues increased with increasing as concentration in the growing medium. Borghi *et al*. (2008) [37] also reported that poplar clone grown has higher copper concentration in leaves than in control plants and highest copper content was in root parts followed by stem and leaves of *P. alba* and *P. canadensis.* Increase in as and Pb uptake in plants was recorded with increase in heavy As^{+} 10 ppm Pb level. Metal concentration in soil with values ranging between 0.02-0.54 mg per plant and 0.07-0.56 mg per plant for as and Pb, respectively. The present results are in conformity with the findings of Vamerali *et al*., (2009) [38] and Wang *et al*., (2011) [33] who also have observed similar trends while studying uptake of as and other heavy metals in different species of poplar.

Conclusions

There was decrease in all the morphological parameters, chlorophyll content and total soluble sugar content with increase in the different doses (0, 5, 10 and 20 ppm) of As and Pb, except for phenol content which increased with increase in As and Pb concentration. The soil microbial biomass followed a decreasing trend with increased concentration of as and Pb levels. The accumulation and uptake of as and Pb in leaves from soil increased with increase in concentration. The observed ability of poplar hybrid to continue growth at different doses of as and Pb and the ability to accumulate metals in its tissues demonstrated its resistance to moderate to high levels of metals. 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 these species. The data, thus generated through this study will be very helpful in detecting the lethal levels of heavy metals for particular plant species, its tolerance and remediation capacity.

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Conflict of interest

There is no conflict of interest in respect to this paper.

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