Populus nigra as a Phytoremediator for Cd, Cu, and Pb in Contaminated Soil

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The contamination of agricultural soil with heavy metals is a complex phenomenon that causes negative consequences for various organisms. Poplars may have considerable phytoremediation potential, and this plant species can tolerate Cd, Cu, and Pb up to 15.6, 63.6, and 173.3 mg kg^{-1} soil, respectively, with 100% survival. The analyzed data revealed significant reduction in vegetative growth traits and leaf N, P, K, and carbohydrate (%) and leaf green color degree. However, a simultaneously significant increase in enzymatic activities and electrolyte leakage were recorded in comparison to control plants. A bioconcentration factor of plant organs was < 1, and the translocation factors (TF) of Cd and Cu were < 1 (<100%) under various concentrations of each heavy metal, while TF of Pb was > 1(>100%), except for the first level. More Cd, Cu, and Pb contents were localized in roots compared to leaves or stems. Thus, the risk of contamination through leaf can be minimized. *Populus nigra* has defense mechanisms against Cd, Cu, and Pb up to 7.8, 29.8, and 91.1 mg/kg soil, respectively because the tolerance index (TI) of either biomass or root was >0.8. Finally, it is a good candidate for research of phytoremediation and phytoextraction.

Keywords: Phytoremediation; Contamination; Heavy metals; Cadmium, copper; Lead; Populus nigra

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INTRODUCTION

Pollution of agricultural soils by heavy metals is a complicated and dangerous phenomenon (Puschenreiter *et al.* 2005). Heavy metals are the main components of inorganic contaminants, which pose a different problem than organic contaminants (Wu *et al.* 2018). Heavy metals are ubiquitous, highly persistent, and non-biodegradable (Gardea-Torresdey *et al.* 2005). Rocks in the soil are subjected to natural weathering, which is a source of metals ions. Also, the disposal of waste and use of fertilizers, pesticides, insecticides, and industrial effluent can contaminate the soil by increasing their concentrations of heavy metals (Abdullahi *et al.* 2009). Among the most common heavy metals are Cd and Pb, and Cu at a higher concentrations. Of these, Cd is one of the big HMs poisons and is not known for any essential biological process (Campbell 2007). Cd can cause harm to various organless entities including chloroplasts, nucleus, vacuole, mitochondria, and it inhibits activities of many enzymes, such as those rich in sulfurhydryl groups (Clemens 2006). Pb is one of the toxic HMs (Yang *et al.* 2005). Pb leads to inhibition of photosynthesis, oxidative stress, and geno toxicity including DNA damage and defects in mytosis (Küpper 2017). Cu becomes a toxic component when its concentration in the tissue of plants raises above optimal levels (Lombardi and Sebastiani 2005). Cu ions can catalyze the productin of highly toxic hydroxyl radicals, particularly through Fenton chemistry, causing the adverse impact to macromolecules and disturbance of metabolic pathways (Hänsch and Mendel 2009).

Many mechanically or physiochemically based remediation technologies have been conducted to treat contaminated soil (Marques *et al.* 2009). These technologies are expensive and disturb the soil, sometimes rendering the land useless for plant growth. Hence, phytoremediation is an alternative using plants to clean heavy metals from contaminated soils, sediments, and water. Phytoremediation is environmentally friendly and low cost, and it is effective in reducing ions in contaminated soils to very low levels (Rakhshaee *et al.* 2009). It is applicable for a wide range of toxic metals and radionuclides (Liu *et al.* 2000). Zárubová *et al.* (2015) mentioned that the bioavailability of metals in soil and different physiological processes in plants affect HM toxicity, adsorption, and distribution in the plant. These variables are dependent on plant growth stage, time of exposure and heavy metal concentrations in soil, soil pH, organic matter, added fertilizers, and temperature.

Heavy metals induce oxidative stress through the production of reactive oxygen species (ROS) that promote membrane lipid peroxidation, protein oxidation, enzyme inhibition, and damage to nucleic acid, resulting in cell death. To improve oxidative stress, plants employ the antioxidative machinery by alternations in antioxidative enzyme activities (*e.g.*, superoxide dismutase, catalase, ascorbate peroxidase, glutathione reductase, and glutathione s-transferase) and the levels of low molecular weight antioxidants (ascorbic acid, reduces glutathione, carotenoids, phenolics (Hossain *et al.* 2012). Redovniković *et al.* (2017) supported this fact on *P. nigra* ' Italica' cv. The balance between ROS and the antioxidative system is a key factor for plant adaptation and survival under stressful conditions such as heavy metals (Sharma *et al.* 2012)

Poplars are able to remove soil contaminants by phytoextraction, phyostabilization, phytovolatilization, and rhizofiltration. Advantageous characteristics of poplars for phytoremediation include quick stablishment, fast growth, large biomass accumulation, extensive and deep root systems, high transpiration rates, easy asexual propagation, exceptional growth on marginal lands, not being part of the food chain, long life (25 to 30 years), and poplars ability to be harvested and then regrown (Sebastiani *et al.* 2004; Zalesny *et al*. 2008). The plants can decrease the HMs concentration in the soil by phytoextraction in which the plants uptake heavy metals from soil by concentrating the metal in areal plant organs (Sebastiani *et al*. 2004). Phytostabilization is the use of plants to stabilize the soil surface *via* retaining the metals in the roots (Marques *et al*. 2009), and rhizolphiltration is where the plant roots absorb or adsorb metals from water and aqueous waste streams (Erakhrumen and Agbontalor 2007).

It is important to estimate whether a tree under any phytoremediation strategy will contaminate the wider environment through leaf fall. The heavy metals content in the green and fallen leaves is dependent on plant species and metal concentration and metal type in the soil. Rafati *et al*. (2011) who reported that the fallen leaves of *Populus alba* had more Cd content than the green leaves, while *Morus alba* had more Cd content in the green leaves than the fallen leaves. The same authors added that Cr was high in the fallen leaves and Ni was high in the green leaves.

Populus nigra L. (Salicaceae) is native to Europe, southwest and central Asia, and northwest Africa. It is widely distributed in Egypt and used for many purposes. *Populus nigra* produces economically valuable non-food biomas that is exploitable for energy production. Most previous studies have been conducted in acidic soil or a hydrobonic system for a short period (less than one year). Data of the effect Cd, Cu, and Pb on leaf chemical and biochemical traits and electrolyte leakage in *P. nigra* are limited in the previous studies. Moreover, there are no available data about the using of *P. nigra* as phytoremediator for Cd, Cu, and Pb in Egypt. Furthermore, large areas in Egypt have a high amount heavy metals, particularly Cd, Cu, and Pb owing to the irrigation with sewage effluent, industrial effluent or drainage water, and also usage of sewage sludge as organic fertilizer for fertilization the soil. This study investigated the effect of heavy metals such as Cd, Cu, and Pb on vegetative growth traits, chemical compositions, enzymatic activity, electrolyte leakage, and efficiency of metal phytoextraction of *P. nigra*. Its use as a phytoremediator for heavy metal-contaminated slightly alkaline soils was examined.

EXPERIMENTAL

Plant Material

Black poplar is a medium to large deciduous tree, reaching 20 to 30 m and rarely 40 m tall, and 1.5 m in diameter. Shoot cuttings (one-year-old wood) that were 15 cm in length and 0.5 cm in diameter were taken from a 10 year-old mother tree of *P. nigra* L. grown in the Experimental Farm of the Fac. Agric. Kafrelsheikh Univ. Egypt, on the first of February. Cuttings were cultured in the plastic bags of 10 cm diameter (one cutting per bag) filled with clay soil, which was used for experiments. The cultured bags with cuttings were placed in an air-condition in plastic greenhouse adjusted 25 ± 2 °C, 40 to 50% relative humanity, and a photoperiod 16 h light/8 h dark, with a light intensity of 300 µmol $m^{-2}s^{-1}$. The cuttings were watered manually every 10 days using 10-L watering cans; the same amount of water was applied to each bag.

Heavy Metals Treatments

The heavy metals were applied as follows: cadmium chloride [$CdCl_2.H_2O$] at rates of 20, 40, 60, and 80 mg/kg soil, equal to 3.9, 7.8, 11.9, and 15.6 mg Cd/kg soil, respectively; copper chloride [$CuCl_2.2H_2O$] at rates of 50, 100, 150, and 200 mg/kg soil, equal to 14.4, 29.8, 47.7, and 63.6 mg Cu/kg soil, consecutively; and lead acetate trihydrate [$(CH_3COO)_2Pb.3H_2O$] at rates of 250, 450, 650 and 850 mg/kg soil, equal to 50, 91.1, 132.1, and 173.3 mg Pb /kg soil, each in turn. The soil was placed in the plastic pots of 40 cm in diameter with 9 kg air-dried weight soil per pot, spiked with solutions of the aforementioned concentrations of heavy metals, and equilibrated for 60 days outdoor under a waterproof tarpaulin. Each metal was added separately to prevent interaction effects. World soil average of Cd, Cu, and Pb are 0.41, 27, and 39.9 mg/kg soil and maximum allowable concentrations of such metals are 1 to 5, 60 to 150, and 20 to 300 mg/kg soil, respectively (Kabata-Pendias 2011).

Planting Date

Identical growth three month-old transplants (average 35 cm height and 0.9 cm stem diameter at the soil surface) were transplanted into the pots (one transplant/ pot) on May 1, 2015. The transplants were placed in the replicated plots after the planting. The plants were irrigated with tap water to reach the field capacity when needed.

Design of the Experiment

The experiment was designed using a complete randomized design (Snedecor and Cochran 1980). The experiment replicated 3 times, each replicate consisted of 13 treatments (3 heavy metals × 4 concentrations, and the control). Each replicate was represented by three plants; thus, each treatment was represented by nine transplants.

Recorded Measurements

At the end of the experiment on August 1, 2017, six plants (30 months old) for each treatment (two plant of each replicate) were chosen randomly to determine the following parameters described below.

Vegetative growth traits

Plant height (from the soil surface to shoot apex, cm), the number of branches per plant, the stem diameter (measured 5 cm from the soil surface, cm), the area per leaf (cm^2) using a C1-202 laser area meter (Cid Bio-Science, Camas, WA, USA), and length of the longest root (cm) were determined. To determine the dry weight, harvested plants were separated into roots, stems, and leaves, which were washed twice (first with tap water to remove soil, then with deionized water) and then oven-dried at 80 °C for 24 h (Rautio *et al.* 2010). The degree of greenness was measured for the fifth leaf from the apex meristem using a portable leaf chlorophyll meter (SPAD-501, Minolta, Osaka, Japan), as described by Markwell *et al.* (1995).

Leaf chemical composition and Cd, Cu, and Pb contents and uptake in the plant parts (leaves, stems, and roots)

Dry samples were ground to obtain a homogenous powder in a metal-free mill (IKa-Werke, M 20, Staufen, Germany). Dried samples of 0.2 g were wet digestion to produce a clear solution according to Evenhuis and de Waard (1980) as follows: concentrated sulfuric acid (95%, 5 mL) was added to the sample (0.2 g), and the mixture was heated for 10 min. on a sand hot plate.Then, 0.5 perchloric acid was added, and heating was continued until a clear solution was obtained, The solution was left to cool bfore it was filtered and diluted to 50 mL with distilled water.

The digested samples were prepared for measuring nitrogen (N%) using a modified micro Kjeldahl methods as described by Chemists and Horwitz (1990). Phosphorus (P%) was extracted according to Murphy and Riley (1962) and determined calorimetrically using spectrophotometer (Beijing Purkinje instrument, T 80^+, London, UK). Potassium (K%) was extracted as described by Cotteine *et al.* (1982) and determined using an atomic adsorption spectrophotometer (GPC Avanta E, Victoria, Australia). The total carbohydrate percentage in leaves was determined according to Herbert *et al.* (1971). The cadmium, copper, and lead concentrations (mg/kg D.W.) in different plant parts were determined according to Page *et al.* (1982) using an atomic absorption spectrophotometer (GPC Avanta E, Victoria, Australia). The uptake of heavy metal (mg/organ d.w.) in different plant parts and total uptake /plant(mg/plant) were calculated as follows,

$$MU = \frac{MC\ x\ ODW}{1000} \tag{1}$$

where MU is metal uptake, MC is metal concentration (mg/kg d.w), and ODW is organ dry weight (g).

$$TPU = LU + SU + RU \tag{2}$$

In Eq. 2, TPU is total plant uptake (mg/plant), LU is leaves uptake (mg), SU is steam uptake (mg) and RU is root uptake (mg).

Biochemical assays of antioxidant enzymes and electrolyte leakage in the leaves

To determine antioxidant enzyme activities, 0.5 g of fully expanded young leaves were homogenized in liquid nitrogen with 3 mL of extraction buffer [50 mM TRIS buffer (pH 7.8) containing 1 mM EDTA-Na2 and 7.5% polyvinylpyrrolidone)] using a prechilled mortar and pestle. The homogenate was filtered through four layers of cheesecloth and centrifuged at 12,000 rpm for 20 min at 4 °C. The supernatant, which was re-centrifuged at 12,000 rpm for 20 min at 4 °C, was used for the total soluble enzyme activity assay using an ultraviolet-160A spectrophotometer (Shimadzu, Japan).

Catalase assay. Catalase (CAT; EC 1.11.1.6) activity was measured by following the consumption of H_2O_2 at 240 nm (Aebi 1984). A total of 1 mL of the reaction mixture contained 20 mg total protein, 50 mM sodium phosphate buffer (pH 7.0), and 10 mM H_2O_2. The reaction was initiated by adding the protein extract. For each measurement, the blank corresponded to the absorbance of the mixture at time zero, and the actual reading corresponded to the absorbance after 1 min. One unit of CAT activity was defined as a 0.01 decrease in absorbance at 240 nm/mg of protein/min.

Polyphenol oxidase assay. Polyphenol oxidase (PPO; EC 1.10.3.1) activity was determined according to the method described by Malik and Singh (1980). The reaction mixture contained 3.0 mL of buffered catechol solution (0.01 M) freshly prepared in 0.1 M phosphate buffer (pH 6.0). The reaction was initiated by adding 100 mL of the crude enzyme extract. Changes in the absorbance at 495 nm were recorded at 30 s for 3 min. Enzyme activity was expressed as an increase in the absorbance min–1·g–1 fresh weight.

Peroxidase assay. Peroxidase (POD; EC 1.11.1.7) activity was determined according to the procedure proposed by Hammerschmidt *et al*. (1982). The reaction mixture consisted of 2.9 mL of a 100-mM sodium phosphate buffer [pH 6.0 containing 0.25% (v/v) guaiacol (2-methoxy phenol) and 100 mM H_2O_2]. The reaction was started by adding 100 mL of crude enzyme extract. Changes in absorbance at 470 nm were recorded at 30^{-s} intervals for 3 min. Enzyme activity was expressed as an increase in the absorbance $min^{-1} \cdot g^{-1}$ fresh weight.

Electrolyte leakage. Electrolyte leakage (EL) measurements were performed as described by Szalai *et al*. (1996), with some modifications. Twenty leaf discs (1 cm^2) were placed individually into flasks containing 25 mL of deionized water (Milli-Q 50, Millipore, Bedford, MA). Flasks were shaken for 20 h at an ambient temperature to facilitate electrolyte leakage from injured tissues. Initial EC measurements were recorded for each vial using an Acromet AR20 EC meter (Fisher Scientific, Chicago, IL). Flasks were then immersed in a hot water bath (Fisher Isotemp, Indiana, PA) at 80 °C (176 °F) for 1 h to induce cell rupture. The vials were again placed on the Innova 2100 platform shaker for 20 h at 21 °C (70 °F). Final conductivity was measured for each flask. The percentage of electrolyte leakage for each bud was calculated as the initial conductivity/final conductivity %100.

Evaluation of Cd, Cu, and Pb phytoextraction potential of Populus nigra

The phytoextarction potential of heavy metals was determined by calcatating bioconcentration factor (BCF), translocation factor percentage (TF%), tolerance index biomas (TI_b), and root tolerance index (TI_r) as follows,

$$BCF = \frac{CO}{CS} \tag{3}$$

where CO is the metal concentration in the plant organ (mg/kg D.W.), and CS is the metal concentration in the soil (mg/kg soil D.W.)

Depending on BCF values, accumulation efficiency was estimated using one of four groups: BCF >1 [intensive], 1 to 0.1 [medium], 0.1 to 0.01 [weak], and 0.01 to 0.001 [no accumulation] (Kabata-Pendias and Pendias 1999),

$$TF\% = \frac{CH}{CR} \times 100 \tag{4}$$

where CH is metal content in the shoots (mg/kg D.W.), and CR is the metal content in the roots (mg/kg D.W.)

TF % was calculated to estimate the metal efficiency ion transport from roots to aerial plant organs (Maiti and Jaiswal 2008) whereas, shoots=leaves and stems.

$$TI_b = \frac{MP}{CP} \tag{5}$$

In Eq. 5, MP is D.W. of metal- treated plant (g/plant), CP is D.W. of control plant (g/plant). TI_b was calculated according to Wilking (1978) to estimate the heavy metal resistance through there values as follow $TI_b < 1$ (a net decrease in biomass and a stressed condition of plants), $TI_b = 1$ (no difference relative to control treatment) and $TI_b >$ (a net increase in biomass and correct plant development).

TI_r was calculated according to Wilkins (1978),

$$TI_r = \frac{RM}{RC} \tag{6}$$

where RM is root length of the metal- treated plant (cm), RC is root length of control plant (cm).

Statistical Analysis

Data were subjected to analysis using the SAS program (Version 6.12; SAS Institute, Cary, NC), and the mean separation was performed using Duncan's Multiple Range Test method. The significance was determined at $p \leq 0.05$.

Soil Analysis

The used soil (virgin soil without contamination) was analyzed prior to and after plantation (one sample of each replicate were mixed carefully as one sample, then it was analyzed)**.** The particle size distribution was analysed by the hydrometer method (Gee and Bauder 1986) before planting only (Table 1). Samples of three replicates (one sample of each replicate) of each treatment were taken from the soil around roots at the end of the experiment and mixed carefully in one sample for the analysis according to the procedure of Cools and De Vos (2011). The soil samples were prepared to determine the following parameters. The EC was measured in a 1:5 ratio (soil: deionized water) using an EC-Meter (MI 170, Szeged, Hungary). The pH was measured in a 1:1 ratio (soil: deionized water suspension) using a calibrated pH-meter (JENEWAY3510, Staffordeshire, UK). Soluble ions in saturated extracted samples were measured according to Jackson (1973). Total carbonate was determined by volumetric calcimeter (Nelson and Sommers 1996). Organic matter content was determined by the dichromate oxidation method (Nelson and Sommers 1996). The available N (%) was determined using the micro Kjeldahl method (Bremner and Mulvaney 1982), and available P (%) was determined according to Olsen and Sommers (1982). Both Ca and Mg contents were measured according to Jackson (1973). The concentrations of Cd, Cu, and Pb were quantified through atomic absorption spectrophotometer (AAS; Page *et al.* 1982). The Na and K contents were examined according the methods described by Black *et al.* (1965) and determined using a flame photometer PSP7 (JENEWY, Staffordeshire, UK). Chloride content was determined by titration with a standard solution of silver nitrate (Jackson 1973).

RESULTS AND DISCUSSION

Soil Analysis

Table 1 shows the soil characteristics before and after contamination. The texture of soil was clayey, with a pH of 7.84, EC of 3.30 dsm^{-1}, and organic matter (OM) of 1.31%. The cations, anions, and EC increased after plantation compared with before, except for the control treatment and Na under 250 and 450 mg Pb acetate kg^{-1} soil. The available N, P, and K values and, Cd, Cu, and Pb concentrations decreased at the end of the experiment relative to their concentrations prior to the planting. Where Cd after plantation in soil was 3.47, 6.47, 7.04, and 9.03 mg/kg soil against the added concentration of 3.9, 7.8, 11.9, and 15.6 mg/kg soil , repectively. The Cu in soil was 1.65, 5.38, 10.19, 16.88, and 29.51 mg/kg soil against the added concentration of 3.6, 14.4, 29.8, 47.7, and 63.6 mg/kg soil, respectively. The Pb in soil was 13.14, 16.20, 19.95, and 23.61mg/kg after plantation against 50.00, 19.11, 132.10, and 173.30 mg/kg before planting, respectively.

At the end of experimentation, there were negligible changes in pH values. Vyslouzilová *et al.* (2006) reported that soil pH did not differ among rhizobax compartments Cd, Pb, and Zn polluted soil after plantation of two willow clones (*Salix* × *rubens*). Soil pH, OM, and cations exchange capacity (CEC) positively correlated with Cu retention (King 1988) and Cd and Pb retention (Jopony and Young 1994). A rise in EC values might have taken place due to the addition of the tested metals. The presence of a large amount of ionic substances and soluble salts have resulted in raising the value of EC in industrial effluents treated soil sample (Sharma and Raju 2013). The reduction in available NPK and Cd, Cu, and Pb concentrations after plantation of poplar might be attributed to uptake by plant roots and the loss fractionally with water irrigation.

Vegetative Growth and Leaf Chemical Composition

In general, the tested levels of Cd, Cu, and Pb significantly decreased the values of the growth traits (Table 2) in comparison to the control ($p \leq 0.05$). The highest significant values of plant height, stem diameter, branches number per plant, area per leaf, root length, dry weights of leaves, stems and roots, and leaf green color degree were the outcomes of control plants. The negative impact was higher with increasing heavy metal concentrations (*e.g.*, more than 50% reduction of leaves dry weight, 23.40, 21.55, and 22.44% in stem dry weight and 34.62, 32.96, and 33.17 % in root dry weight at the high level of each heavy metal, respectively). Meanwhile, the reduction in root length was 27.62, 30.73 and 31.03% at the higher level of each heavy metal.

Table 1. Soil Analysis Before and After Plantation

Parameter	Soil before plantation	Soil after plantation												
		Treatments (mg/kg) soil D.W.												
		Cont.	Cd-20	Cd-40	Cd-60	Cd-80	Cu-50	Cu-100	Cu-150	Cu-200	Pb-250	Pb-450	Pb-650	Pb-850
pH	7.84	7.72	7.80	7.76	7.75	7.73	7.82	7.81	7.81	7.79	7.81	7.81	7.780	7.78
EC(ds/m)	3.30	2.48	4.32	4.45	4.48	4.78	3.6	3.78	4.31	4.72	3.51	3.78	4.35	4.71
$CaCo_3$%	3.36	3.27	3.12	3.12	3.11	3.27	3.16	3.16	3.16	3.14	3.11	3.14	3.16	3.17
O.M%	1.31	1.22	1.23	1.24	1.26	1.27	1.24	1.24	1.27	1.28	1.23	1.24	1.24	1.26
Soluble cations (meq/L)														
Ca^{++}	7.71	4.87	10.71	11..22	10.97	13.31	8.72	8.91	13.37	14.36	9.71	10.41	12.02	12.91
Mg^{++}	4.82	2.38	8.91	9.18	9.22	8.31	5.82	6.87	5.98	7.53	5.91	6.12	7.42	7.71
Na^+	20.12	17.43	22.02	22.87	22.89	24.22	20.12	21.44	22.61	22.90	18.31	19.37	22.61	24.87
K^+	0.35	0.12	1.56	1.23	1.72	1.96	1.44	2.41	1.14	3.41	1.17	1.90	1.45	1.61
Soluble anions (meq/L)														
Cl^-	19.73	12.51	24.21	24.81	24.92	26.21	22.31	22.51	24.22	24.91	22.11	22.45	24.23	25.98
CO_3^{--}	----	----	----	----	----	----	----	----	----	----	----	----	----	----
HCO_3^-	2.50	1.75	4.56	4.61	4.67	5.17	4.12	4.32	4.61	4.92	3.87	4.12	4.58	5.37
SO_4^{--}	10.77	10.54	14.43	15.08	15.42	16.42	9.67	10.97	14.27	17.37	9.12	11.23	14.69	15.75
Available N (ppm)	2.75	1.78	2.16	2.41	2.36	2.43	2.21	2.46	2.46	2.48	2.31	2.31	2.33	2.38
Available P (ppm)	3.51	1.79	2.24	2.34	2.51	2.61	2.16	2.24	2.24	2.33	2.31	2.41	2.46	2.52
Available K (ppm)	216	148	167	167	178	181	171	172	182	186	156	168	174	178
HMs(mg/kg)														
Cd	00	--	3.47	6.47	7.04	9.03	-----	-----	-----	----	--	--	--	--
Cu	3.6	1.62	--	--	--	--	5.38	10.19	16.88	29.51	--	--	--	--
Pb	00	--	--	--	--	--	--	--	--	--	13.14	16.2	19.95	23.61
Practical size distribution														
Sand (%)	24.03													
Silt (%)	22.92													
Clay (%)	50.05													
Soil texture	Clayey													

Note: Each value represents the average of three replicates after plantation.

The obtained results are in agreement with those of Borghi *et al.* (2008), who found a general reduction of *P.× canadensis* (Adda clone) biomass and growth variable at Cu equals or higher than 100 µM. Too total leaf area in Yillow clones was affected by 38.5 mg/L Cd sulphate (Zacchini *et al.* 2009) and by 0.19 mg/L Cu in *Salix viminalis* (Gąsecka *et al.* 2012). Also, Houda *et al.* (2016) reported that there were inhibition effects on the growth prameters on *Populus nigra* and *P. alba* irrigated by treated wastewater that contained nickel, zinc, cadmium, and lead at rates of 0.5, 0.4, 0.2, and 0.2 mgL^{-1}, respectively. Additionally, Redovniković *et al.* (2017) concluded that there was a reduction in dry root biomass of *P. nigra* 'Italica' treated with 50 mg Cd and 1200 mg Pb kg^{-1} soil. In spite of the fact that the heavy metals tested exerted adverse effects on the vegetative growth, the survival was 100%. This agrees with the study of Polle *et al.* (2013), who found that *P. eurphratica* and *P. canescens* could grow in treated soil with Cd. Similer results were obtained by Kubátová *et al.* (2016), who reported that *P. maximowiczi × P. nigra* could grow in 7.3 mg Cd, 1368 mg Pb, and 218 mg Zn /kg soil in spite of the inhibition effects on the growth parameters that correlated with higher levels of Cd, Pb, and Cu in the soil.

At the same time, the impact of HMs used on the percentages of N, P, K (Fig. 1, 2, and 3), and total carbohydrates (Fig. 4) showed a similar downward tendency in the presence of increasing HMs concentations. Moreover, the highest and least significant percentages of such chemical contents were the results from the control plants and the highest levels of heavy metals, respectively. Meanwhile, non-contaminated plants (control) had 2.35, 0.20, 2.14, and 14.98% for N, P, K, and total carbohydrates, consecutively. On the other side, the contaminated plants with 80 mg $CdCl_2$/kg soil resulted in lower significant N and P% of 0.83 and 0.03% each it turn. Meanwhile, the plants received 850 mg Pb acetate produced 1.28 and 9.10 % for K and total carbohydrates, respectively. Furthermore the treatments of 80 mg $CdCl_2$ and 850 mg Pb acetate gave non-significant values of P, K, and total carbohydrates % .The adverse effects of Cd, Cu, and Pb on N, P, and K% may be owing to the competition between them and essential nutrients. HMs exert toxicities in plants through: (i) similarities with the nutrient cations, which result into a competition for absorption at root surface; for example, As and Cd compete with P and Zn, respectively, for there absorption, (ii) displacement of essential cations from specific binding sites that lead to a collapse of function (Sharma and Dietz 2009; Dalcorso *et al*. 2013). Further, Cd affects N metabolism by inhibiting nitrate uptake and transportation and nitrate reductase (Lea and Miflin 2003). Cu can also substitute for Mg in the chlorophyll present in both antenna and reaction centres (Küpper *et al*. 1996). This adversely affects the structure and function of chlorophyll, leading to a reduction in carbohydrate accumulation. In addition, Pb accumulated in plant tissue reacts with the phosphate group of ADP or ATP and replaces essential ions, thus impaired essential elements uptake such as Mg and Fe and inducing deficiency of CO_2 resulting from stomatal closure (Pourrut *et al*. 2011).

Likewise, Pieterini *et al*. (2010) found that $CdSO_4$ at 50 mM interferes with the uptake, transport and use of different elements (*e.g*., Fe, Zn, and Mg) in poplar clones. In addition, Gąsecka *et al*. (2012) pointed out that the disturbance in starch hydrolysis and inhibition of soluble carbohydrate transport to different organs of *S. viminalis* under Cu stress (at 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 mM) probably leads to a decrease in leaf length, root biomass, and root length. This is because carbohydrates formed during photosynthesis are the source of carbon and energy necessary for growth, lignification, and biomass formation. Overall, foliage elements differ according to their concentrations in soil or soil chemistry and due to the plant species or clones of Salix (Mosseler and Major 2017). According to El-Mahrouk *et al*. (2019), the decrement in N, P, and K % in leaves of *Salix mucronata* was increased with raising the concentrations of $Cd\ Cl_2$ (20 to 80 mg/kg soil), $CuCl_2$ (50 to 200 mg/kg soil), and Pb acetate (250 to 850 mg/kg soil) in the soil.

Table 2. Effect of Different Levels of Cd, Cu, and Pb on the Vegetative Traits of *Populus nigra* L.

Treatments (mg/kg soil)	**Plant height (cm)**	**Stem diameter (cm)**	**Number of branches / plant**	**Area per leaf (cm^2)**	**Leaves dry weight/ Plant (g)**	**Stem dry weight/plant (g)**	**Root dry weigh/plant (g)**	**Length of the longest root (cm)**	**Degree of leaf green color (SPAD) units**
Cont.00	224.1±1.13 a	3.14±0.02 a	13.0 ±1.00 a	12.06±0.51a	11.07±0.19 a	54.21±1.04 a	41.76±0.49a	78.82±0.07 a	56.42±0.83a
Cd--20	218.8±0.81 b	2.78±0.01 b	10.0 ±0.00 c	11.63±0.64a	9.47±0.07 c	50.15±0.16 d	36.14±0.06c	72.57±0.81 c	54.07±0.55 c
Cd--40	188.1±0.35 e	2.26±0.02 c	7.3 3 ±0.58 e	10.31±0.19b	7.60±0.14 e	44.71±0.03 g	33.58±0.01e	67.03±2.27 d	49.57±0.12 d
Cd--60	180.3±0.69 f	1.97±0.01de	5.33±0.58 g	9.47 ±0.12 c	6.28±0.10 g	43.20±0.33 h	30.55±0.06 g	62.07±1.29 e	46.03±0.49 f
Cd--80	166.0±1.39 i	1.35±0.01 g	4.0±0.00 h	8.40 ±0.33d	4.92±0.09 i	41.53±0.94 j	27.68±0.56 h	57.05±0.79 fg	42.82±0.24gh
Cu -50	222.5±0.53 a	2.88±0.06 b	11.67±0.58 b	11.97±0.12a	9.88±0.03 b	51.38±0.42 c	36.84 ±0.02 b	75.84±1.36 b	55.07±0.45 b
Cu -100	218.7±0.35 b	2.25±0.05 c	7.67±0.58de	10.59±0.05b	7.78±0.10de	47.02±1.20 e	34.14±0.56 de	66.15±0.60 d	50.14±0.49 d
Cu -150	190.4±0.95 d	2.08±0.09 d	5.67±0.58 fg	9.71 ±0.17 c	6.64±0.07 f	45.41±1.07 f	30.66±0.60 fg	58.67±0.72 f	48.00±0.53 e
Cu - 200	176.5±2.61 g	1.70±0.17 f	3.67±0.58 h	8.67±0.14d	5.00±0.15 i	42.77±0.08 h	28.11±0.20h	54.60±0.29gh	43.72±25.2g
Pb -250	218.3±0.38 b	2.87±1.66 b	10.33±0.58 c	11.53±0.50a	9.90±0.13 b	53.23±0.72 b	36.44±0.47 bc	75.93±0.17 b	53.94±0.29 c
Pb -450	213.2±0.58 c	2.37±0.10 c	8.33 ±0.58 d	10.56±0.10b	7.80±0.02 d	51.22±1.03 c	34.41±0.05 d	65.85±0.74 d	49.74±0.78 d
Pb -650	191.4±0.96 d	1.91±0.01 e	6.33±0.58 f	9.62 ±0.07 c	6.44±0.10 g	42.57±1.56hi	31.27±0.56 f	61.37±0.19 e	46.70±0.10f
Pb -850	168.2±0.64 h	1.38±0.11 g	4.33±0.58 h	8.27 ±0.22d	5.20±0.05 h	42.05±0.58ij	27.91±0.51 h	54.37±0.63 h	42.82±0.24gh

Means followed by a similar letter within a column are not significantly different at 0.05 level probability by Duncan's Multiple Range Test. P≤0.05.

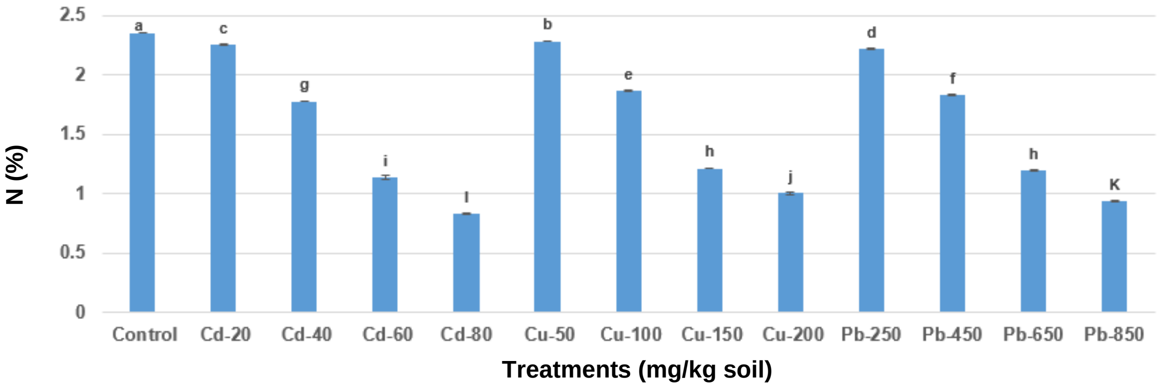

Fig. 1. Effect of different levels of Cd, Cu and Pb in soil on the leaf N% of *Populus nigra* L. Means followed by a similar letter within a figure are not significantly different at 0.05 level probability by Duncan's Multiple Range Test (P ≤ 0.05)

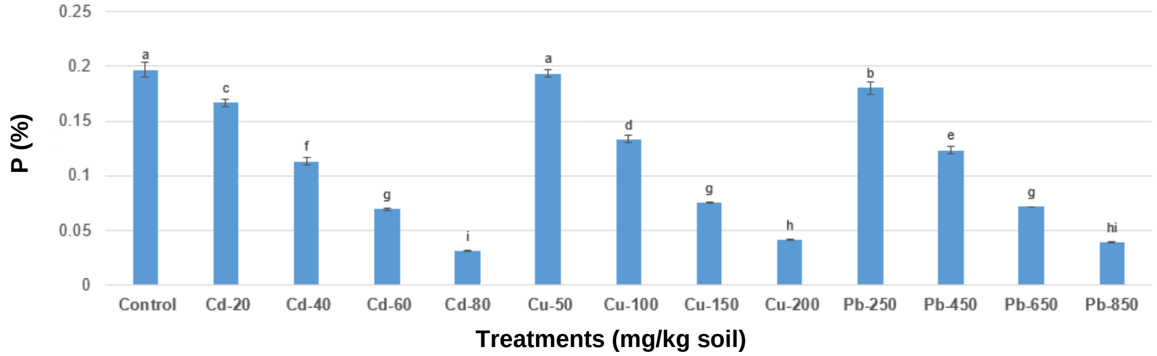

Fig. 2. Effect of different levels of Cd, Cu and Pb in soil on the leaf P %of *Populus nigra* L. Means followed by a similar letter within a figure are not significantly different at 0.05 level probability by Duncan's Multiple Range Test (P ≤ 0.05).

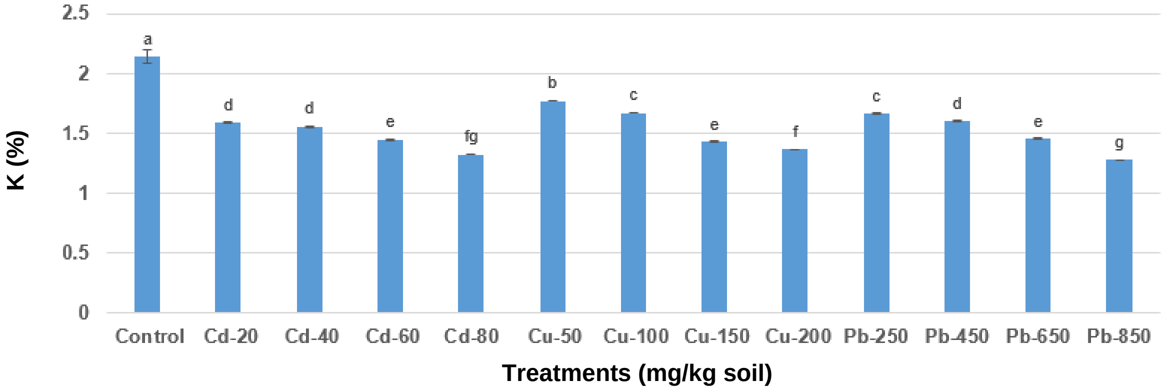

Fig. 3. Effect of different levels of Cd, Cu and Pb in soil on the leaf K %of *Populus nigra* L. Means followed by a similar letter within a figure are not significantly different at 0.05 level probability by Duncan's Multiple Range Test (P ≤ 0.05).

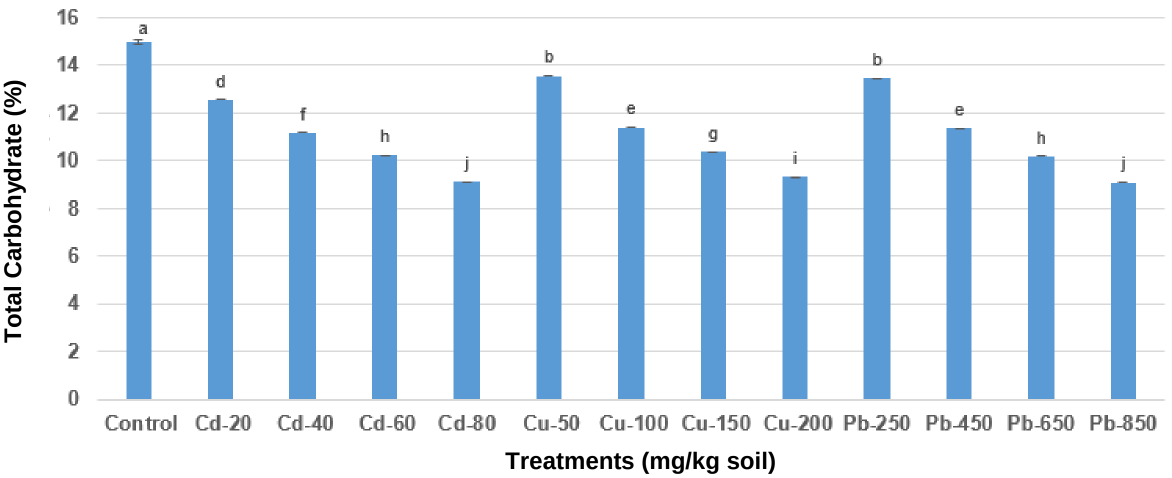

Fig. 4. Effect of different levels of Cd, Cu and Pb in soil on total carbohydrate% of *Populus nigra* L.
Means followed by a similar letter within a figure are not significantly different at 0.05 level probability by Duncan's Multiple Range Test ($P \leq 0.05$).

Cd, Cu, and Pb Contents and Uptake in the Plant Parts and Plant Total Uptake

The obtained results showed that all tested treatments of Cd, Cu, and Pb caused a significant ($p \leq 0.05$) effect on their contents and uptakes in the different plant parts, as well as plant total uptake (Table 3). Differences among treatments of each heavy metal reached a significant level. In the same time period, Cd content reached 3.53, 2.57, and 6.65 mg/kg D.W., and its uptake reached 0.017, 0.107, and 0.180 mg, Cu content reached 30.67, 20.38, and 61.78 mg/kg D.W., and its uptake reached 0.15, 0.87, and 1.74 mg, and Pb content reached 47.63, 35.15, and 76.20 mg/kg D.W., and its uptake reached 0.25, 1.48, and 2.13 mg in leaves, stems, and roots, respectively, when 80 mg $CdCl_2$, 200 mg $CuCl_2$, and 850 mg Pb acetate/kg soil were applied, respectively. Meanwhile, total plant uptake of Cd, Cu, and Pb were 0.31, 2.76, and 3.85 mg/plant at 80 mg $CdCl_2$, 200mg $CuCl_2$, and 850 mg $Pb(CH_3COO)_2$ / kg soil, respectively. The content of Cd, Cu, and Pb in the plant parts depended on their concentration in the soil, and their contents were found to be in the order of roots > leaves > stem, except for $CdCl_2$ at 20 and 40 mg/kg soil, where the content of Cd in the stem was more than leaves. At the same time, the uptake of the used HMs in the different plant parts was in order of roots >stems>leaves.

The present results showed that the roots had Cd, Cu, and Pb content and uptake more than aerial parts. This finding was supported by Zhivotovsky *et al.* (2011) in various tree species, where Cd and Pb mainly accumulated in the roots rather than leaves and stem. The results matched those of Samuilov *et al.* (2016) on *Populus tremula* × *P. alba*, where the concentration of Pb in the different plant parts depends on concentrations in soil. The plant roots can accumulate Pb more than the above-ground parts. Redovniković *et al.* (2017) exposed *P. nigra* 'Italica' cv. to Cd (10, 25, and 50 mg kg^{-1} soil) and Pb (400, 800, and 1200 mg kg^{-1} soil) and found that in the majority of cases Cd and Pb accumulated.

Thus the roots of *Populus nigra* had the highest accumulation of Cd, Cu, and Pb, when compared to the stems and leaves; in this case, the contamination risk of the wider environment through leaf fall can be considered minimal. This was supported by Baker (1981), who reported that many deciduous plant species are considered to translocate accumulated heavy metals to their above-ground tissues before senescence. Also, Vervaeke *et al*. (2003) mentioned that willow colonies, which do not accumulate metals in their leaves, can be used to minimize the contamination risk of the wider environmental through leaf fall. Additionally, Rafati *et al*. (2011) reported that the green leaves of *P. alba* had Ni less than the fallen leaves under 120 mg Ni nitrate/kg soil. Therefore, these results suggest that *P. nigra* is a suitable alternative to deciduous hyperaccumulators. To keep the soil at low heavy metals levels, short rotation forestry systems can be used to remove heavy metals contamination from the soil through repeated harvest of the biomass for energy purposes (Punshon and Dickinson 1997).

Table 3. Effect of Different Levels of Cd, Cu, and Pb on Their Content and Uptake in the Different Plant Parts as well as Plant Total Uptake

Treatments (mg/Kg soil)	**Leaves**		**Stems**		**Roots**		**Total uptake (mg/plant)**
	Content (mg/kg d.w)	**Uptake(mg)**	**Content (mg/kg d.w)**	**Uptake(mg)**	**Content (mg/kg d.w)**	**Uptake(mg)**	
Cd Cl_2							
Cont.	0.00±0.00e	0.000±0.0000e	0.00 ±0.00e	0.000±0.000e	0.00±0.00e	0.00±0.000e	0.00±0.000e
20	0.07±0.04d	0.001±0.0004d	0.42 ±0.07d	0.021±0.004d	2.07±0.07d	0.07±0.003d	0.10±0.001d
40	0.95±0.03c	0.007±0.0003c	0.97±0.04c	0.043±0.002c	3.35±0.03c	0.11±0.001c	0.16±0.002c
60	1.70±0.03b	0.011±0.0003b	1.55 ±0.06b	0.067±0.003b	4.38±0.02b	0.13±0.001b	0.21±0.003b
80	3.53±0.04a	0.017±0.0001a	2.57 ±0.06a	0.107±0.002a	6.65±0.19a	0.18±0.005a	0.31±0.007a
Cu Cl_2							
Cont.	0.68±0.06e	0.01±0.0010e	0.24 ±0.04e	0.01±0.000e	1.07±0.07e	0.05±0.003e	0.07±0.004e
50	8.58±0.07d	0.09±0.0010d	6.43 ±0.92d	0.33±0.002d	10.65±0.03d	0.39±0.003d	0.81±0.004d
100	10.15±0.31c	0.08±0.0030c	10.46±0.53c	0.49±0.004c	30.97±0.70c	1.06±0.026c	1.63±0.020c
150	18.33±0.30b	0.12±0.0020b	15.00±0.45b	0.68±0.014b	41.83±0.15b	1.28±0.009b	2.09±0.022b
200	30.67±0.03a	0.15±0.0020a	20.38±0.04a	0.87±0.012a	61.78±0.02a	1.74±0.004a	2.76±0.009a
$(CH_3COO)_2Pb$							
Cont.	0.00±0.02e	0.00±0.0000d	0.00±0.00e	0.00±0.00e	0.00±0.13e	0.00±0.00e	0.00±0.000e
250	16.22±1.16d	0.16±0.0010c	5.72 ±0.04d	0.30±0.003d	25.70±0.38d	0.94±0.005d	1.40±0.008d
450	28.42±0.80c	0.22±0.0090b	17.47±1.92c	0.90±0.048c	44.68±0.59c	1.54±0.011c	2.65±0.050c
650	36.95±1.58b	0.24±0.0050a	24.15±0.53b	1.03±0.023b	59.50±2.14b	1.86±0.012b	3.13±0.033b
850	47.63±0.02a	0.25±0.0090a	35.15±0.45a	1.48±0.029a	76.20±0.13a	2.13±0.069a	3.85±0.090a

Means followed by a similar letter within a column are not significantly different at 0.05 level probability by Duncan's Multiple Range Test. P≤0.05.

Enzymatic Activities and Electrolyte Leakage (EL) of the Leaves

There were significant increases in PPO, POD, and CAT activities under the tested independent variables levels in comparison to the control treatment (Fig. 5, 6, and 7). The activities of PPO, POD, and CAT enzymes were increased up to the second concentration then decreased gradually from the third concentration of each tested heavy metal, but the enzymatic activities were higher in control plant leaves. The differences among all tested treatments reached the significance level for most treatments. The significantly maximum activities of PPO, POD, and CAT were observed at 100 ppm Cu Cl_2/kg soil in comparison to the other tested heavy metal levels.

The visual toxicity symptoms of leaves as yellowish, chlorosis, and necrotic spots were noticed on the adult leaves, and browning of roots of poplar under study may take place due to inducing ROS under heavy metals stress (Wang *et al.* 2008). The same authors added that elevated Cd increased the enzymatic activities rapidly in metal-accumulator plants, especially SOD and CAT. ROS decreases with increased exposure time to heavy metals stress (Jakovljević *et al.* 2014). This finding was supported by Redovniković *et al.* (2017), who found that *P. nigra* 'Italica' cv., subjected to Cd (50 mg/kg) and Pb (1200 mg/kg) heavy metals, induced increasing oxidative stress during short-term exposure (one month), compared to long-term HM exposure (4 months). Increasing enzymatic activities with increasing heavy metal concentrations then decreased at high levels of such metal; this was explained by Nagajyoti *et al.* (2010), who reported that the activity of antioxidative enzymes under heavy metal stress (*e.g.*, Cd, Cu, Pb) is concentration-dependent and can be retarded or stimulated. This was also reported by Stobrawa and Lorenc-Plucińska (2007) in the fine roots of *P. nigra* grown in Cu (849.7 and 1174.75 mg/kg) and Pb (255.30 and 411.13 mg kg^{-1}) polluted soil. Tauqeer *et al.* (2016) on *Alternanthera bettzickiana* concluded that under lower levels of both Cd and Pb (0.5 and 1.0 mM), both POD and CAT activities increased, while under higher Cd and Pb levels (2.0 mM) the activeties of such enzymes were decreased. Also, Zou *et al.* (2017) mentioned that the enzymatic acitvity in *Salix matsudana* was increased under stress of Cd (10, 50, and 100 μM). Emamverdian *et al.* (2018) reported increasing enzymatic activities in *Indocalamus latifolius* due to Cu, Pb, and Zn at rates of 0, 500, 1000, and 2000 mg kg^{-1} soil. Bankaji *et al.* (2019) studied *Atriplex halimus*, finding that raising the Pb level from 0 to 600 µM in the soil increased CAT activity.

Concerning electrolyte leakage (EL), Fig. 8 shows that with increasing heavy metal levels in the soil, the EL gradually increased. The maximum significant electrolyte leakage value reached 95.74 $\mu S/cm^2$ owing to the application of 80 mg $CdCl_2$/kg soil, followed by Pb acetate and $CuCl_2$ at 850 and 200 mg/kg soil. Each in turn resulted in 88.95 and 87.81 $\mu S/cm^2$, respectively but without significant difference between themselves against 37.17 $\mu S/cm^2$ for the negative heavy metals treated plants. EL is ubiquitous among various species, tissues, and cell types and can be triggered by all stress factors including heavy metals (Demidchik *et al.* 2003). The membrane damage that resulted from Cu can be assessed by EL determintion (Liu *et al.*2004). Elevated Cd levels (200 $mgkg^{-1}$) and exposure time increase the EL in *Brasica juncea* (Ahmad *et al.* 2016). Furthermore, EL in *Alternanthera bettzckiana* increases with increasing Cd and Pb from 0.5 to 1.0 mM (Tauqeer *et al.*2016). Thus, reduced vegetative growth traits, leaf chemical composition, and TI may be due to the increase in EL with the increasing heavy metal levels. Thus, PPO, POD, and CAT activities and EL can be used as environmental biomarkers of heavy metal concentrations.

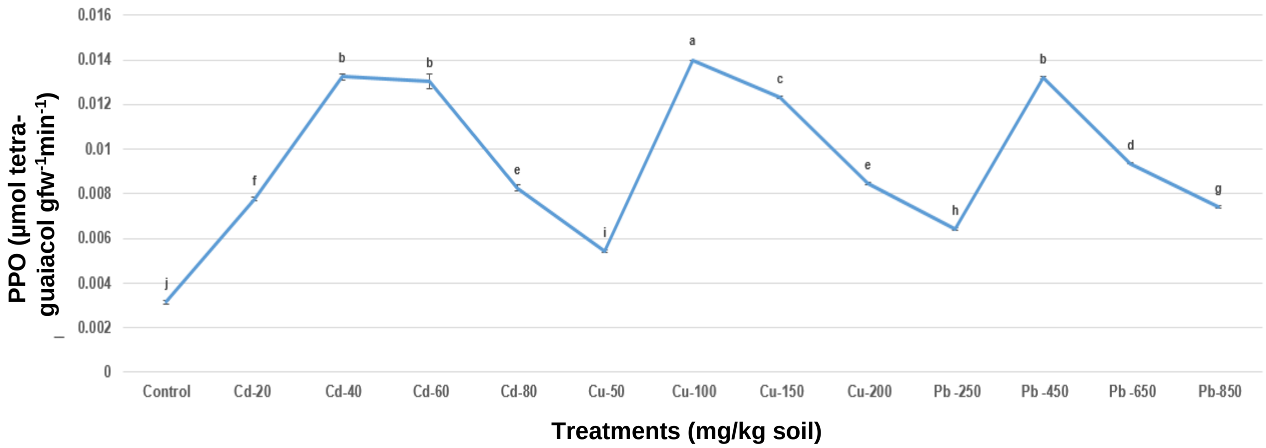

Fig. 5. Effect of different levels of Cd, Cu, and Pb in soil on peroxidase (PPO) activities in the leaves of *Populus nigra* L. Means followed by a similar letter within a figure are not significantly different at 0.05 level probability according to Duncan's Multiple Range Test (P≤0.05).

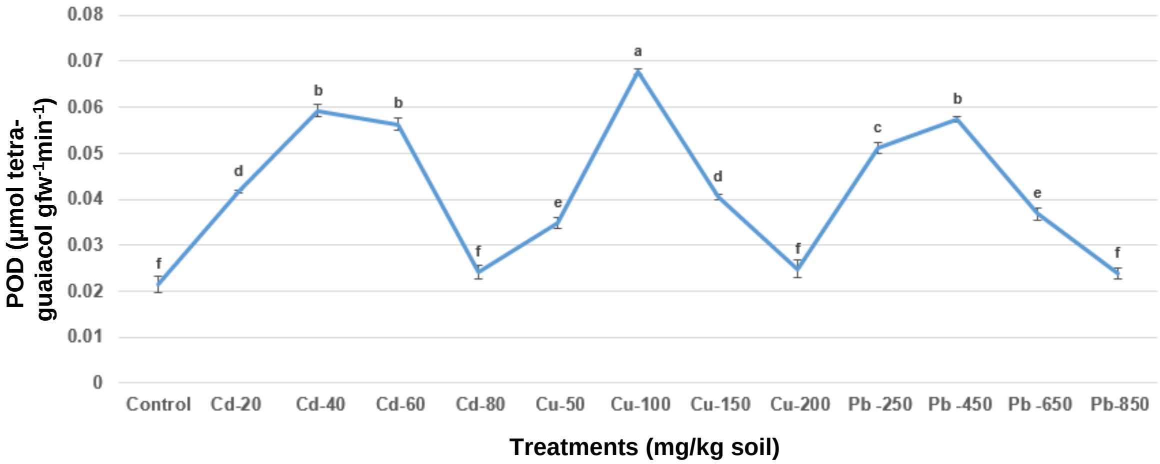

Fig. 6. Effect of different levels of Cd, Cu, and Pb in soil on peroxidase (POD) activities in the leaves of *Populus nigra* L. Means followed by a similar letter within a figure are not significantly different at 0.05 level probability according to Duncan's Multiple Range Test (P≤0.05).

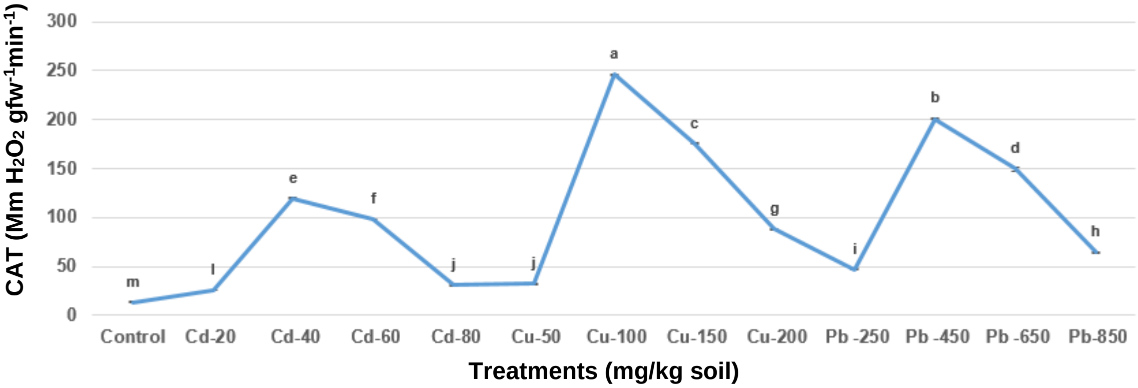

Fig. 7. Effect of different levels of Cd, Cu, and Pb in soil on catalase (CAT) activities in the leaves of *Populus nigra* L. Means followed by a similar letter within a figure are not significantly different at 0.05 level probability according to Duncan's Multiple Range Test (P≤0.05).

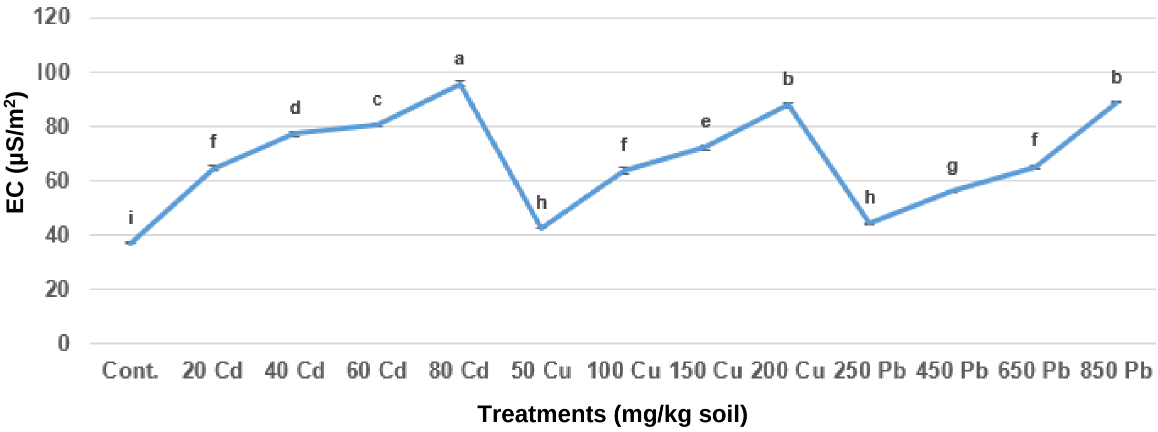

Fig. 8. Effect of different levels of Cd, Cu, and Pb in soil on electrolyte leakage activitiy in the leaves of *Populus nigra* L. Means followed by a similar letter within a figure are not significantly different at 0.05 level probability according to Duncan's Multiple Range Test (P≤0.05).

Bioconcentration Factor (BCF), Translocation Factor (TF, %), and Tolerance Index (TI)

To evaluate the Cd, Cu, and Pb phytoextraction ability in poplar heavy metals accumulation, BCF and TF were calculated (Table 4). The BCF was calculated to estimate ion transport from soil to the plant organs. Higher significant BCF Cd of leaves, stem, and root were found at 80, 80, and 20 mg Cd Cl_2 kg^{-1} soil, respectively. Also, higher significant BCF Cu of leaves and root were recorded for the treatments of 50 and 200 mg $CuCl_2$/kg soil, without a significant difference between themselves. Higher significant BCF Cu of stem was found at 50 mg Cu Cl_2/kg soil. On the other side, higher significant BCF Pb of leaves and root were achieved on 250 and 450 mg Pb acetate/kg soil, and the difference between such treatments did not reach the significant level ($P \leq 0.05$). Meanwhile, higher significant BCF Pb of stem resulted from 450 and 850 mg Pb acetate/kg soil, without a significant difference between such treatments. Soil properties such as pH, CEC, and Ca concentration have a significant ($p \leq 0.05$) effect on the bioavailability of Pb in soil (Zhang *et al.* 2019). BCF and TF are key values that evaluate a plant's potential for phytoextraction or phytoestabilization (Rafati *et al.* 2011). In this study, the BCF of plant organs at concentrations of each metal was estimated as medium (BCF = 1 to 0.1), which is in accordance to Kabata-Pendias and Pendias (1999). Also, shoots BCF of Cd, Cu, and Pb at the various concentrations of each metal was less than the unity (<1). This is incompatible with the previous study of Redovniković *et al.* (2017) on poplar 'Italica'cv., where they mentioned that shoots BCF of Cd or Pb < 1 under Cd at 10, 25, and 50 mg/kg soil and Pb at 400, 800, and 1200 mg/kg soil. The results indicated that root BCF at the levels of Cd, Cu, or Pb was < 1. Rafati *et al.* (2011) concluded that root BCF of *P. alba* was <1 under 40, 80, and 160 mg Cd/kg soil. Additionally, at levels of each heavy metal, roots BCF Cd, Cu, and Pb was higher than of leaves or stems. Thus, *P. nigra* roots were more effective in storing these metals than other organs.

Concerning TF, there was a significant ($p \leq 0.05$) increase in TF of Cd and Pb values with increasing their concentrations in soil. While TF of Cu did not show a clear trend. It is obvious from data of tested heavy metal levels that TF Cd or TF Cu < 1 (less than 100%), with an exception of 50 mg $CuCl_2$, while TF Pb is > 1 (over than 100%), with an exception of 250 mg Pb acetate. Higher TF of Cd, Cu, and Pb is 91.82, 141.01 and 108.79% for 80 mg $CdCl_2$, 50 mg $CuCl_2$ and 850 mg Pb acetate/kg soil, respectively.

The movement of heavy metals from roots to shoots probably occurs within the xylem. The free Cd, Cu or Pb levels can be influenced by cellular sequestration of these metals, and therefore, it can affect their movement through the plant (Niu *et al.* 2007; Fulekar *et al.* 2009; Pourrut *et al.* 2011). The TF Cd values at various levels were not higher because it is often restricted due to its ability to create Cd-phytoextraction complex by sequestration in the vacuole (Lux *et al.* 2010). Also, the present results indicated that TF Cd or Cu is <1 (< 100%) at levels of each, with an exception of TF Cu, which was 50 mg $CuCl_2$ kg^{-1} >1 (> 100%). This is in agreement with the result of Redovniković *et al.* (2017) on *P.nigra* 'Italica'cv, who found that TF Cd is <1 under 10 and 50 mg Cd kg^{-1} soil. Meanwhile, TF Pb was >1(> 100%) under its concentrations, with the exception of TF Pb at 250 mg Pb acetate kg^{-1} <1 (< 100%). This was contrary to TF Pb of *P.nigra* 'Italica'cv, which was <1 under 400, 800, and 1200 mg Pb kg^{-1} soil (Redovniković *et al.* 2017). In contrast, TF Cd >1 of *P. alba* grown in 40 to 160 mg Cd kg^{-1} soil (Rafati *et al.* 2011). Whereas, Saraswat and Rai (2009) and Zacchini *et al.* (2009) concluded that plants recording a shoot BCF>1 are suitable for phytoextraction and those with a root BCF>1 and TF<1 are suitable for phytostabilization. From the results either shoot BCF or TF Cd or Cu <1, *P. nigra* has a potential for phytostabilization of these ions. At the same time BCF Pb of *Populus nigra* root was <1 and TF Pb was >1 (>100%) under the used levels of Pb in this study, with the exception of TF Pb at 250 mg Pb acetae /kg soil, which was <1 (<100%). This means that *Populus nigra* can be used as a phytoextractor of Pb contaminated soil at 450 to 850 Pb acetate /kg soil. The results of Rafati *et al.* (2011) indicated that BCF Cd of *P. alba* and *Morus alba* roots were <1 and TF Cd of such spesies were >1 under Cd at levels of 40, 80, and 160 mg/kg soil, and they added that the two species were suitable for phytoextraction of Cd contaminated soil at such levels.

To estimate the black poplar resistance for Cd, Cu, and Pb phytoextraction and to determine the plant ability to grow in the presence of tested heavy metals, TI_b and TI_r were calculated, as shown in Table 4. A significant reduction in TI_b and TI_r values was recorded with increasing heavy metal concentrations in the soil. Likewise, the values of either TI_b or TI_r of Cd, Cu, and Pb were less than the unity at different heavy metal levels. The TI_b reached 69, 71, and 70% and TI_r reached 72, 69, and 69% relative to control treatment (100%) at higher levels of Cd, Cu, and Pb, respectively. The result indicated that the values of TI_b under all tested levels of Cd, Cu, or Pb were lower than 1; the plants were under stress and the biomass production was limited or a net decrease in biomass and stressed condition of plants (Wilkins 1978). Upon $TI_r < 1$, there was a limited length of the root. The results in this study showed that TI_b of *Populus nigra* was 0.80, 0.83, and 0.87 and TI_r of *Populus nigra* was 0.85, 0.83, and 0.84 at 40 mg $CdCl_2$, 100 mg Cu Cl_2, and 450 mg Pb acetate /kg soil. This means that *P. nigra* has better tolerance against 40 mg $CdCl_2$, 100 mg $CuCl_2$, and 450 mg Pb acetate. Metal tolerance varied greatly among 20 different clones of willow and poplar spesies treated with Cd and Zn (Dos Santos Utmazian *et al.* 2007). The present results were supported by the result of Wu *et al.* (2010), who revealed that TI of *P. deltoides* x *P. nigra* was significantly decreased at 4.35g Cd/kg purble soil. Melczek *et al.* (2013) showed that tolerance index of biomas or root of *S. viminalis* was <1 under 3 mM Cu (NO3)$_2$ and at ratios of Ca/Mg (20:1 and 1:10). According to El-Mahrouk *et al.* (2019), the decrement in TI_b or TI_r of *Salix macrunata* was increased with increasing the levels of Cd, Cu, and Pb. In general, BCF, TF, and TI_b or TI_r factors could be used to define Cd, Cu, and Pb hyperaccumulators. The suitable plant for phytoremediation should have much more metal tolerances and high accumulation capacity in its tissues (especially in harvestable parts) (Shi *et al.* 2009). Thus, the results imply that *P. nigra* is a good candidate plant for phytoremediation applications in Cd, Cu, and Pb contaminated soil.

Table 4. BCF, TF%, TI_b and TI_r of *Populus nigra* as Affected by Cd, Cu, and Pb Concentrations in the Soil

Treatment (mg/kg soil)	**Bioconcentration Factor (BCF)**			**Translocation Factor (%)**	**Tolerance Index Biomass (TI_b)**	**Tolerance Index Root (TI_r)**
	Leaves	**Stem**	**Root**			
$Cd Cl_2$						
20	0.02±0.011c	0.11±0.019b	0.53±0.020a	23.74±5.18d	0.89±0.002a	0.92±0.021a
40	0.12±0.003b	0.12±0.005b	0.43±0.003b	57.19±1.68c	0.80±0.003b	0.85±0.015b
60	0.14±0.002b	0.13±0.005b	0.37±0.001c	74.15±0.71b	0.75±0.003c	0.79±0.008c
80	0.23±0.003a	0.17±0.004a	0.43±0.012b	91.82±1.71a	0.69±0.005d	0.72±0.003d
$Cu Cl_2$						
Cont.	0.19±0.017d	0.07±0.002d	0.30±0.0200d	86.77±1.81b	-----	-----
50	0.48±0.003a	0.36±0.002a	0.59±0.0010c	141.01±1.47a	0.92±0.004a	0.96±0.014a
100	0.30±0.009c	0.31±0.001b	0.93±0.0210a	66.63±2.32d	0.83±0.001b	0.83±0.006b
150	0.36±0.006b	0.29±0.007c	0.82±0.0030b	79.69±1.22c	0.77±0.007c	0.74±0.011c
200	0.46±0.001a	0.30±0.003b	0.92±0.0002a	82.63±0.39bc	0.71±0.002d	0.69±0.023d
$(CH_3COO)_2Pb$						
250	0.32±0.0003a	0.11±0.001c	0.51±0.003a	85.35±0.29b	0.93±0.001a	0.96±0.017a
450	0.31±0.0100a	0.19±0.008ab	0.49±0.003a	102.73±2.80a	0.87±0.002b	0.84±0.009b
650	0.28±0.0061b	0.18±0.004b	0.45±0.004b	102.70±1.49a	0.75±0.004c	0.78±0.013c
850	0.27±0.0100b	0.20±0.003a	0.44±0.012b	108.79±3.10a	0.70±0.005d	0.69±0.024d

Means followed by a similar letter within a column are not significantly different at 0.05 level probability by Duncan's Multiple Range Test (P≤0.05).

CONCLUSIONS

1. *Populus nigra* had high survival rate (100%) under Cd, Cu, and Pb treatments up to 15.6, 63.6, and 173.3 mg kg^{-1} soil, respectively.
2. The used levels of Cd, Cu, and Pb had an adverse effect on both vegetative traits and leaf N, P, K, and carbohydrates (%).
3. The stimulating effect occurred for the activities of PPO, POD, and CAT enzymes. Moreover, EL increased compared to the control after applications of the different levels of used HMs.
4. There was greater content and uptake of Cd, Cu, and Pb in roots than in the aerial parts.
5. The risk of contamination of the wider environment through leaf fall can thus be considered as minimal.
6. According to BCF, TF, TI_b, and TIr, *Populus nigra* can use as a phytostabilizator for Cd and Cu and a phytoextractor for Pb contaminated slightly alkaline soil at the used levels in this study.
7. *Populus nigra* can be regarded as a suitable alternative plant to hyperaccumulator plants for Cd, Cu and Pb contaminated slightly alkaline soil.

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