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# Studies on tolerance of some ornamental plants to soil pollution with some combinations of heavy metals.
# I. The European black elderberry (*Sambucus nigra* L.)

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## ABSTRACT

This investigation was performed under the full sun at Orman Botanical Garden, Giza, Egypt during 2015 and 2016 seasons to reveal the response of one-year-old transplants of black elderberry (*Sambucus nigra* L.) grown in 20-cm-diameter polyethylene black bags filled with about 3 kg/bag of an equal mixture of sand and clay (1:1, v/v) to toxicity of lead (Pb), cadmium (Cd) and nickel (Ni) when added as acetates to the soil mixture in combinations at concentrations of 00.00 ppm for each metal as a control, 500 ppm Pb + 50 ppm Cd + 25 ppm Ni for combination number one (T1) and 2-, 3- and 4- fold of these concentrations for combinations number two (T2), three (T3) and four (T4), respectively. The results of this experiment showed that no mortality was occurred among the plants, as the survival % was 100 % for plants cultivated in unpolluted or polluted soil. However, a progressive reduction in the means of top and root growth traits was observed as the concentration of the three heavy metals was increased to reach the minimal values by T3 and T4 combinations, with the inferiority of T4 one which gave the least records at all in both seasons. The only noticed exception in such study was the improvement of some growth parameters induced by T1 combined treatment which was non-significant in most cases . The pollution resistance index (%), as a real indicator for tolerance of heavy metals toxicity took a similar trend, as it was slightly improved in the first season and tenuously decreased in the second one by T1 treatment, but greatly reduced by T2, T3 and T4 ones to be more than 50 % in the first season and more than 55 % in the second one by the highest metallic combinations (T3 and T4) indicating ability of *Sambucus nigra* plants to tolerate the high levels of toxic metals. It was noticed also that the content of photosynthetic pigments, total soluble sugars, nitrogen and phosphorus% in the leaves were gradually decreased with increasing heavy metals concentration, with few exceptions in the two seasons, meanwhile the opposite was the right concerning potassium% in the leaves, as well as Pb, Cd and Ni contents in the leaves and roots, as these constituents were progressively increased as a result of increasing heavy metals concentrations but were generally less than the upper critical toxicity levels. Concentration of the three toxic elements was higher in the leaves than in the roots. From the foregoing results , it is clear that black elderberry (*Sambucus nigra* L.) trans of one year old plants can tolerate toxicity of Pb, Cd and Ni at medium and high concentrations plus uptaking considerable amounts from such metals, and this render them to be used in phytoremediation of heavy metal – contaminated soil.

***Key words:*** Black elderberry (*Sambucus nigra* L.), heavy metal pollution, lead (Pb), cadmium (Cd) and Nickel (Ni), pollution resistance index.

## Introduction

Contamination of agricultural soils with several heavy metals including lead (Pb), cadmium (Cd) and nickel (Ni) and others is still one of the growing crisis in different parts of the world. It becomes the most serious problem facing all countries, even the non-industrial and poor ones (Adrees *et al*., 2015). These heavy metals not only decrease plant growth and production, but also cause serious threat to human health via the food chain crops (Keller *et al*., 2015). So, it is urgent to explore an active and potentially cheap method to solve such dilemma. This can be greatly achieve through using the non-food chain plants (ornamentals) which may remove these pollutants from the soil or render them harmless (Tauqeer *et al*., 2016). However, the use of local plant species for this purpose

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is meaningful because these plants could survive well under local environmental conditions (Luttus and Lefevre, 2015). Among ornamental trees that may serve in this concern, the black elder or European black elderberry (*Sambucus nigra* L.) which is native to North Africa.

It is a deciduous shrub or small tree growing up to 6-9 m tall and wide, belongs to Fam. Adoxaceae (formerly, Fam. Caprifoliaceae) and native to Europe, North Africa and West Asia. The bark is light grey when young, changes to a coarse grey, the outer bark with length-wise furrowing. The leaves are arranged in opposite pairs, 10-30 cm long, pinnate with 5-7 leaflets, elliptic, with 12 cm long, of disagreable odour when bruised; flowers yellowish-white, in umbel-like cymes, of heavy odour, appear in late spring to mid summer; fruits are glossy dark purple to shining black, edible propagated by seeds and cuttings (Bailey, 1976). It is used as ornamental when planted in mass. The berry fruits can be eaten when fully ripe and can be used to make jelly, jams and sauce. The flowers contain mainly flavonoids (min. 0.8 %), organic acids and essential oils, while fruits have anthocyanins, flavonoids and phenolic acids (Polish Pharmacopoeia, 2008). Stem bark, leaves, flowers, fruits and root extracts and preparations have antiviral properties, reducing duration of flu symptoms to 3-4 days, stimulate the immunology system by increasing production of inflammatory cytokinens and are probably immunoprotective for cancer and AIDS patients. These extracts may act as antioxidants, neutralizing free radicals and inhibiting the co-oxidation reactions of linolenic acids and B-carotene (Barak *et al*., 2001; Dawidowicz *et al*., 2006; Simonovik *et al*., 2007). Elderberry fruits have been used for making preserves, wines, winter cordials and for adding flavour and color to other products (The ABC, 2003). The wild elderberry shrubs often grow in areas polluted by vehicles, mostly in ruderal habitats (landfill and rubble), in thickets, on roadsides and along busy streets (Atkinson and Atkinson , 2002).

In this regard, Kolodziej *et al*., (2012) found that *Sambucus nigra* stands near to transportation roads had a lower total content of flavonoids than those further away from roads. Closer proximity and heavier road traffic significantly increased the content of either Cr, Fe, Cd and Cu in fruits or Fe, Zn, Cu, Cr, Cd, Pb and Mg elements in flowers. Fernandes and Henriques (1989) detected that holm-oak trees growing in substrate affected by the mining showed pronounced stunting, reduced leaf size and extensive necrotic and chlorotic spotting, and had concentrations more than 50 times higher for Cu and 20 times higher for Pb and Zn. Similar observations were also obtained by Schenk and Bucher (2000) on Petumia, Dissanayake *et al*., (2002) on *Lantana camara and Wedelia trilobata,* Abbaas (2002) on *Casuarina glauca, Taxodium distichum* and *Populus nigra*, Wang and Zhou (2005) on *Tagetes erecta,Salvia splendens* and *Abelmoschus manihot,* Nogales and Benitez (2006) on *Dittrichia viscosa*, Shahin *et al*., (2007) on *Matthiola incana and Dimorphotheca ecklonis*, as well as Abdalla and Mahmoud (2008 a, b) on *Acalypha wilkesiana, Asclepias curassavica, Dodonaea viscosa* and *Tabernaemontana divarigata,* Erdogan *et al*., (2011) on *Aptenia cordifolia, Carpobrotus edulis* and *Bryophyllum tubiflorum,* Shivhare and Sharma (2012) on Dahlia, Wang *et al.*, (2012) on *Chlorophytum comosum*, Teixeira da Silva (2014) on hybrid Cymbidium, Ramana *et al*., (2015) on *Euphorbia milii* and Ehsan *et al*., (2016) who indicated that plant height and fresh and dry weights of *Vinca rosea* plants were increased at low concentration of chromium (10-30 ppm), but were decreased with high levels (50-60 ppm). Chromium concentration in plant tissues gradually increased from 10 to 30 ppm treatments and became almost same for higher concentration levels (50-60 ppm).

Recently, Forte and Mutiti (2017) reported that both *Helianthus annuus and Hydrangea paniculata* accumulated significant amounts of Cu and Pb to be classified as hyperaccumulator species. *H. annuus* took up significant amounts of Cu in the shoots, specifically the leaves and easily translocated it from stem to leaf. Pb was not as easily taken up and translocated as Cu was by this species. *H. paniculata* took up Cu and Pb in high concentrations, but preferentially stored more metals in the stems than in the leaves. However, the current work aims to reveal the tolerance of the black elderberry transplants to toxicity of lead, cadmium and nickel when applied together in gradual concentrations.

## Materials and Methods

This study was undertaken in the open field at Orman Botanical Garden, Giza, Egypt during the two consecutive seasons of 2015 and 2016 to find out the impact of lead (Pb), cadmium (Cd) and

nickel (Ni) in combinations at various levels on survival, growth and chemical composition of the black elderberry transplants of one year old, as non-food chain ornamentals.
Therefore, one-year-old transplants of the European black elderberry (*Sambucus nigra* L.) at a length of 18 ± 2 cm and carry about 8 ± 1 leaves were planted on April, 1st for each season in 20-cm-diameter black polyethylene bags (one transplant/bag) filled with about 3 kg/bag of sand and clay mixture at equal parts for each by volume (1:1, v/v). The physical and chemical properties of the sand and clay soil used in the two seasons were determined and listed in Table (a).

**Table a:** The physical and chemical properties of the sand and clay soil used in 2015 and 2016 seasons.

| Soil type | Season | Particle size distribution (%) | | | | S.P. | E.C. (dS/m) | pH | Cations (meq/l) | | | | Anions (Meq/l) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Coarse sand | Fine sand | Silt | Clay | | | | $Ca^{++}$ | $Mg^{++}$ | $Na^{+}$ | $K^{+}$ | $HCO_3^{-}$ | $Cl^{-}$ | $SO_4^{--}$ |
| Sand | 2015 | 18.72 | 71.28 | 4.76 | 5.34 | 21.83 | 1.58 | 8.20 | 2.65 | 2.48 | 21.87 | 0.78 | 3.85 | 13.00 | 10.93 |
| | 2016 | 79.76 | 9.30 | 2.50 | 8.44 | 23.10 | 1.76 | 7.90 | 19.42 | 8.33 | 7.20 | 0.75 | 1.60 | 7.80 | 26.30 |
| Clay | 2015 | 7.46 | 16.75 | 34.53 | 40.89 | 41.67 | 2.10 | 8.33 | 16.93 | 9.33 | 20.44 | 0.37 | 3.82 | 1.46 | 41.79 |
| | 2016 | 7.64 | 22.50 | 30.15 | 39.71 | 53.36 | 2.23 | 7.92 | 7.50 | 2.21 | 15.49 | 0.75 | 6.28 | 8.12 | 11.05 |

Thawing salts of Pb, Cd and Ni (acetates), manufactured by Aldrich Chemical Co., Inc., 1001 West Saint Paul Avenue, Milwaukee, Wisconsin 53233, USA were mixed well in combinations through the particles of the used soil mixture before filling the plastic bags at concentrations of 00.00 ppm for each metal as a control, 500 ppm Pb + 50 ppm Cd + 25 ppm Ni for treatment number one (T1) and 2-,3- and 4-fold of these concentrations for treatments number two (T2), three (T3) and four (T4), respectively above the background levels of these metals in the used mixture. The plastic bags (without drain holes to keep the metals from leaching) were immediately irrigated after planting with 250 ml of fresh water/bag, but thereafter the irrigation was done once day by day with only 200 ml of water/bag till end of the experiment. The other agricultural practices needed for such plantation were done as usually grower did. The plants were set out for every season in a complete randomized design and replicated thrice with five plants per replicate (Mead *et al*., 1993).

At the end of each season (on October, 1st), the following data were recorded: survival percentage (by dividing number of the survived plants in the treatment/total number of plants in it x 100), plant height (cm), stem diameter at the base (cm), number of branches/plant, number of leaves/plant, leaf stalk length (cm), mean root length (cm), number of root branches/plant, as well as aerial parts and roots fresh and dry weights (g), while the pollution resistance index as a percent (PRI %) was calculated from the equation previously used by Wilkins (1957):
PRI (%) = mean root length (cm) of the polluted plants/mean root length of control ones x 100

In fresh leaf samples taken from the middle part of the plants, photosynthetic pigments (chlorophyll a, b and carotenoids, mg/g f.w.) and total soluble sugars (mg/g f.w.) were determined according to the methods of Yadava (1986) and Dubois *et al*., (1966), respectively, while in dry samples, nitrogen (Black, 1956), phosphorus (Luatanab and Olsen, 1965) and potassium (Jackson, 1973) were measured as percentages. Besides, content of Pb, Cd and Ni in dry samples of leaves and roots (ppm) were assessed in the second season only using a Perkin Elmer 403 atomic absorption spectrophotometer (Jackson, 1973).

Data were then tabulated and the only morphological ones were statistically analyzed using program of SAS Institute (2009), which was followed by Duncan's New Multiple Range Test (Steel and Torrie, 1980) for means comparison.

## Results and Discussion

### Effect of lead (Pb), cadmium (Cd) and nickel (Ni) combinations on:

*1. Survival percentage and vegetative and root growth traits:*

It is obvious from data presented in Table (1) that no mortality was observed among Sambucus plants although the top and root growth were severely reduced by growing them in polluted soil, especially at the medium and high concentrations of heavy metals (T2, T3 and T4). So, survival % was 100 % for control and all combined treatments employed in this study with non-significant differences between themselves in the two seasons. This may indicate the high ability of Sambucus plants to tolerate heavy metal toxicity due to their ability to produce metallothioneins, which are low molecular weight cystein-rich proteins that have great ability to sequester heavy metals such as Cd, Cu, Ni, Pb and Zn (Obertello *et al*., 2006). In this regard, Coupe *et al*., (1995) noted that a metallothionein-like protein has been isolated from a cDNA library from the abscission zones of ethylene-treated *Sambucus nigra* leaflets. The precise function of this group of proteins is that they bind heavy metals. Moreover, *Sambucus nigra* has several advantages as a model system on which to study the mechanisms associated with organ shedding as the leaflet abscission zone comprises approximately 30 rows of cells, which facilitate the isolation of mRNAs and proteins that may be the centrel for this process. Thus, Atkinson and Atkinson (2002) affirmed that wild elderberry shrubs successfully grow in areas polluted by vehicles, on roadsides and along busy streets due to its high tolerance for air pollution.

On the other hand, data presenter in Tables (1, 2 and 3) exhibited that means of all vegetative and root growth parameters; expressed as plant height (cm), stem diameter (cm), No. branches and leaves/plant, petiol length (cm), root length (cm), No. root branches/plant, as well as aerial parts and roots fresh and dry weights (g) were descendingly decreased with increasing concentration of heavy metals to reach the minimal values by T3 and T4 combined treatments compared to control means in both seasons, with the inferiority of T4 one which recorded the least means at all. This may be attributed to the higher accumulation of toxic metals in plant tissues (as indicated in Table 6), which usually leads to depression of vital processes, such as photosynthesis, inhibition of some enzymatic systems and blocking the formation of proteins and chlorophylls (Adrees *et al*., 2015). The organic Pb was found to derange the spindle fiber mechanism of cell division in plants (Foy *et al*., 1978). A reduction in glutathione reductase activity in relation to Cd and Ni stress was detected by Schickler and Capsi (1999) on *Alyssum argentium*.

In this concern, Abbaas (2002) found that Pb, Cr and Ni combinations declined all vegetative growth characters of *Casuarina glauca*, *Taxodium distichum* and *Populus nigra* trees relating positively with raising the concentration. Defoliation of *P. nigra* leaves occurred by all rates of Cr when added individually and by high concentrations of the three elements when added in combination. Manousaki and Kalogerakis (2009) noticed that Cd and Pb markedly decreased biomass and changed water relations in *Atriplex halimus* plants. Further, Hossain *et al*., (2012) suggested that the common consequence of heavy metal toxicity is the excessive accumulation of reactive oxygen species (ROS) and methyl glyoxal (MG), both of which can cause peroxidation of lipids, oxidation of protein, inactivation of enzymes, DNA damage and/or interact with other vital constituents of plant cells. On Dahlia, Shivhare and Sharma (2012) revealed that as the concentration of Ni and Pb in soil increases, the overall length of plant decreases with respect to control. Teixeira da Silva (2014) postulated that all heavy metals tested (Al, As, Cd, Cr, Cu, Hg, Ni and Zn) fully reduced neo-protocorm growth of hybrid *Cymbidium* at/or greater than 10 μM. Ramana *et al*., (2015) pointed out that *Euphorbia milii* plant could tolerate applied Cr well up to 75 mg/kg soil and beyond that, the plants were died.

An exception was noticed regarding the effect of the first combined treatment (T1), which caused either a significant increment in the means of some traits over control or a slight reduction in some others with non-significant differences relative to control in most cases of the two seasons. This may be ascribed to that some heavy metals, such as Cd at low concentration may activate some enzymatic systems, which consequently promote vital processes in plant tissues. In this respect, Tauqeer *et al*., (2016) clarified that the activities of superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) and ascorbate peroxidase (APX) in *Alternanthera bettzickiana* plant tissues increased under lower levels of Cd and Pb (0.5 and 1.0 mM), while decreased at higher levels (2.0 mM). Ehsan *et al*., (2016) indicated that plant height and herb fresh and dry weights of *Vinca rosea were* increased at low concentration of Cr (10-20 ppm) because translocation factor of Cr was found to be lower than 1 at low level of concentration, but was higher than 1 for higher level of concentration (50-60 ppm).

**Table 1:** Effect of heavy metals combinations on survival % and some vegetative growth parameters of *Sambucus nigra* L. plants during 2015 and 2016 seasons.

| Heavy metals combinations (Pb + Cd + Ni, ppm) | Survival (%) | | Plant height (cm) | | Stem diameter (cm) | | No. branches per plant | | No leaves per plant | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 2015 | 2016 | 2015 | 2016 | 2015 | 2016 | 2015 | 2016 | 2015 | 2016 |
| 0.0 + 0.0 + 0.0 Cont.) | 100.00 | 100.00 | 40.50b | 43.75a | 0.75a | 0.81a | 2.33ab | 2.50a | 15.00a | 15.67a |
| 500 + 50 + 25 (T1) | 100.00 | 100.00 | 44.33a | 44.50a | 0.68a | 0.78a | 2.69a | 2.70a | 12.33b | 14.31a |
| 1000 + 100 + 50 (T2) | 100.00 | 100.00 | 28.76c | 32.61b | 0.68a | 0.76a | 1.67b | 1.85b | 9.81c | 10.64b |
| 1500 + 150 + 75 (T3) | 100.00 | 100.00 | 24.68d | 26.78c | 0.52b | 0.55b | 1.67b | 1.80b | 9.67c | 9.33b |
| 2000 + 200 + 100 (T4) | 100.00 | 100.00 | 19.33e | 20.17d | 0.45b | 0.51b | 1.67b | 1.65c | 9.33c | 9.00b |

*Means followed by the same letter in a column do not differ significantly according to Duncan`s New Multiple Range t-Test at P = 0.05*

**Table 2:** Effect of heavy metals combinations on petiole length, root length, No. root branches and PRI of *Sambucus nigra* L. plants during 2015 and 2016 seasons.

| Heavy metals combinations (Pb + Cd + Ni, ppm) | Petiole length (cm) | | Root length (cm) | | No. root branches per plant | | Pollution resistance index (PRI %) | |
|---|---|---|---|---|---|---|---|---|
| | 2015 | 2016 | 2015 | 2016 | 2015 | 2016 | 2015 | 2016 |
| 0.0 + 0.0 + 0.0 (Cont.) | 9.07a | 9.80a | 60.67a | 58.76a | 10.33a | 9.51a | 100.00a | 100.00a |
| 500 + 50 + 25 (T1) | 7.58b | 8.65b | 63.33a | 58.40a | 9.67a | 9.33a | 104.38a | 99.39a |
| 1000 + 100 + 50 (T2) | 7.17b | 7.78bc | 31.50b | 34.03b | 8.69b | 8.00b | 51.92b | 57.91b |
| 1500 + 150 + 75 (T3) | 6.00c | 6.59c | 30.83b | 33.71b | 8.33bc | 7.46b | 50.82b | 57.37b |
| 2000 + 200 + 100 (T4) | 4.97d | 5.60d | 30.78b | 32.50b | 7.67c | 7.00c | 50.73b | 55.31b |

*Means followed by the same letter in a column do not differ significantly according to Duncan`s New Multiple Range t-Test at P = 0.05*

**Table 3:** Effect of heavy metals combinations on aerial parts and roots fresh and dry weights of *Sambucus nigra* L. plants during 2015 and 2016 seasons.

| Heavy metals combinations (Pb + Cd + Ni, ppm) | Aerial parts | | | | Roots | | | |
|---|---|---|---|---|---|---|---|---|
| | Fresh weight (g) | | Dry weight (g) | | Fresh weight (g) | | Dry weight (g) | |
| | 2015 | 2016 | 2015 | 2016 | 2015 | 2016 | 2015 | 2016 |
| 0.0 + 0.0 + 0.0 (Cont.) | 30.78a | 33.25a | 14.24a | 15.38a | 26.46a | 24.61a | 10.65b | 9.89a |
| 500 + 50 + 25 (T1) | 31.51a | 34.00a | 13.42a | 14.69a | 27.50a | 24.58a | 12.24a | 9.98a |
| 1000 + 100 + 50 (T2) | 29.00b | 31.33b | 11.16b | 11.88b | 23.01b | 21.67b | 10.45b | 8.83b |
| 1500 + 150 + 75 (T3) | 27.81c | 28.91c | 9.50c | 9.37c | 22.60b | 20.50bc | 10.00b | 8.51b |
| 2000 + 200 + 100 (T4) | 18.79d | 21.17d | 9.30c | 9.50c | 19.57c | 18.16c | 8.16c | 7.33c |

*Means followed by the same letter in a column do not differ significantly according to Duncan`s New Multiple Range t-Test at P = 0.05*

### *2. Pollution resistance index percentage (PRI %):*

The resistance index of pollution, as a real indicator for tolerance of heavy metal toxicity (Table, 2) was 100 % for unpolluted control plants in the two seasons, but it was slightly increased in the 1$^{st}$ season by T1 treatment to 104.38 % and decreased in the 2$^{nd}$ one by the same treatment to 99.39 % with non-significant differences compared to control in both seasons. However, a great reduction in the values of such index was attained by T2, T3 and T4 combinations to be 51.92 % by T2 combination and trivially more than 50 % by T3 and T4 ones in the first season, while in the second season, it was reduced to be more than 55 % by the three combinations mentioned before. Reducing PRI % to more than 50 % coupled with 100 % survival percentage clear that black elderberry plants can tolerate toxicity of the higher concentrations of Pb, Cd and Ni, when applied together. This may be referred to that elderberry plants produce metallothioneins which bind heavy metals (Coupe *et al*., 1995) and to occurrence of antagonism between the 3 used metals (Foy *et al*., 1978). In this connection, Wang and Zhou (2005) found that Cd had significant inhibitory effects on root elongation of *Tagetes erecta*, *Salvia splendens* and *Abelmoschus manihot* plants, so tolerance indices of these plants (the ratio of maximum root length in an experimental group to that in a control group) were greatly reduced. Likewise, Wang *et al*., (2012) showed that the tolerance indicies of

*Chlorophytum comosum* plants were all above 100 % indicating tremendous value in the remediation of Cd-contaminated soils up to 200 mg/kg soil.

Analogous results were also explored by Shahin *et al*., (2007) on *Matthiola incana* and *Dimorphotheca ecklonis*, Abdalla and Mahmoud (2008 a) on *Acalypha wilkasiana*, *Asclepias curassavica*, *Dodonaea viscosa* and *Tabernaemontana divaricata*, Wang *et al*., (2012) on *Chlorophytum comosum* and Ramana *et al*., (2015) on *Euphorbia milii*.

*3. Chemical composition:*

According to data presented in Table (4), it can be concluded that leaf content of chlorophyll a, b and carotenoids (mg/g f.w.) was linearly decreased as a result of increment in heavy metals concentrations in the different combinations used in this work. Hence, the least content was scored by T4 combined treatment, which gave the utmost minimum values compared to control means in the two seasons. A similar trend was also obtained regarding total soluble sugars content (mg/g f.w.), except for T1 treatment that induced a slight increment in such constituent in the leaves of contaminated plants.

This may be due to the indirect effects of heavy metals on photosystems related to the disturbances caused by the metals in Calvin cycle reactions and down regulation or even feedback inhibition of electron transport by the excessive amounts of ATP and NADPH (Krupa *et al*., 1993). Moreover, Droppa *et al*., (1996) suggested that Cd in greening leaves interferes with chlorophyll biosynthesis, acts mainly by inhibiting the LHC synthesis into stable complexes required for normal functional photosynthesis activity. In this concern, Schenk and Bucher (2000) observed that lower levels of Cd, Cu and Zn induced chlorosis of Petunia leaves, which became chlorotic at plant heavy metal concentrations (dry weight basis) of 160 mg/kg Zn, 12.3 mg/kg Cu and 3 mg/kg Cd. In addition, Erdogan *et al*., (2011) manifested that *Carpobrotus edulis* plants turned to brown by heavy metals of sewage sludge at the end of the $10^{th}$ week from planting, and then died. On hybrid *Cymbidium*, Teixeira da Silva (2014) elicited that thin cell layers of the neo-protocorm-like body necrosed at 50 μM of Cd or Ni.

Analogas response occurred as well in respect of the percentages of nitrogen and phosphorus in the leaves (Table, 5), which were gradually decreased as the concentration of heavy metals was increased, with the exception of N % in the leaves of plants polluted with T1 combined treatment, which was noticeably increased over control content in the two seasons. The opposite was the right in relation to K % in the leaves, as well as Pb, Cd and Ni content (ppm) in the leaves and roots (Tables, 5 and 6), as the content of these constituents was progressively increased in response to the gradual rising of heavy metals concentration in the polluted soil. Thus, the highest content of such metals was maximum by T3 and T4 combinations with the prevalence of the latter one, which elevated content of these metals to the maximal values. However, the concentrations of such metals (especially Pb and Cd) did not reach the upper critical toxicity levels in tissues of elderberry plant, as it ranged in various plant tissues between 35-50, 60-120 and 6-8 ppm for Pb, Cd and Ni, respectively (Macnicol and Peckett, 1985), except for Ni content in the leaves, which surpassed such critical levels by all pollution treatments applied in this investigation. In general, content of Pb, Cd and Ni in the leaves was higher than their content in the roots.

Absorption of metals by roots of plants grown in heavy metal- contaminated soil may be necessary for keeping the equilibrium between their concentrations in soil solution and nutrients content in plant tissues (Foy *et al*., 1978). In this respect, Dissanayake *et al*., (2002) suggested that the high ability of ion uptake by *Albizia odoratissima*, *Lantana camara* and *Wedelia trilobata* can be attributed to the high amount of parenchyma in their tissues. On the same line, were those results revealed by Abbaas (2002) on *Casuarina glauca*, *Taxodium distichum* and *Populus nigra*, Abdalla and Mahmoud (2008 b) on *Acalypha wilkesiana*, *Asclepias curassovica*, *Dodonaea viscosa* and *Tabernaemontana divarigata* , and Wang *et al*., (2012) who found that Cd content in *Chlorophytum comosum* roots and aboveground part reached 1.522 and 865.5 ppm, respectively in the soil contaminated with Cd up to 200 mg/kg soil. On *Vinca rosea*, Ehsan *et al*., (2016) implied that Cr in plant increased gradually from 10-30 ppm concentration levels, but became almost same for higher levels (40-60 ppm).

**Table 4:** Effect of heavy metals combinations on pigments and total soluble sugars content in the leaves of *Sambucus nigra* L. plants during 2015 and 2016 seasons.

| Heavy metals combinations (Pb + Cd + Ni, ppm) | Pigments (mg/g f.w.) | | | | | | Total soluble sugars (mg/g f.w.) | |
|---|---|---|---|---|---|---|---|---|
| | Chlorophyll (a) | | Chlorophyll (b) | | Carotenoids | | | |
| | 2015 | 2016 | 2015 | 2016 | 2015 | 2016 | 2015 | 2016 |
| 0.0 + 0.0 + 0.0 (Cont.) | 1.219 | 1.329 | 0.382 | 0.443 | 0.898 | 0.979 | 10.76 | 11.74 |
| 500 + 50 + 25 (T1) | 1.168 | 1.287 | 0.379 | 0.431 | 0.810 | 0.883 | 10.78 | 12.03 |
| 1000 + 100 + 50 (T2) | 0.890 | 0.981 | 0.301 | 0.328 | 0.698 | 0.765 | 08.94 | 10.01 |
| 1500 + 150 + 75 (T3) | 0.842 | 0.893 | 0.285 | 0.291 | 0.577 | 0.633 | 08.47 | 09.80 |
| 2000 + 200 + 100 (T4) | 0.732 | 0.796 | 0.251 | 0.263 | 0.480 | 0.536 | 08.29 | 0.0 |

**Table 5:** Effect of heavy metals combinations on nitrogen, phosphorus and potassium % in at the leaves of *Sambucus nigra* L. plants during 2015 and 2016 seasons.

| Heavy metals combinations (Pb + Cd + Ni, ppm) | N (%) | | P (%) | | K (%) | |
|---|---|---|---|---|---|---|
| | 2015 | 2016 | 2015 | 2016 | 2015 | 2016 |
| 0.0 + 0.0 + 0.0 (Cont.) | 3.139 | 2.920 | 0.498 | 0.461 | 0.708 | 0.765 |
| 500 + 50 + 25 (T1) | 3.409 | 3.113 | 0.457 | 0.430 | 0.825 | 0.894 |
| 1000 + 100 + 50 (T2) | 1.734 | 1.822 | 0.376 | 0.314 | 0.833 | 0.907 |
| 1500 + 150 + 75 (T3) | 1.656 | 1.705 | 0.365 | 0.389 | 0.967 | 1.048 |
| 2000 + 200 + 100 (T4) | 1.149 | 1.263 | 0.375 | 0.263 | 0.880 | 1.112 |

**Table 6:** Effect of heavy metals combinations on lead, cadmium and nickel content in the leaves and roots of *Sambucus nigra* L. plants during 2016 season.

| Heavy metals combinations (Pb + Cd + Ni, ppm) | Pb (ppm) | | Cd (ppm) | | Ni (ppm) | |
|---|---|---|---|---|---|---|
| | Leaves | Roots | Leaves | Roots | Leaves | Roots |
| 0.0 + 0.0 + 0.0 (Cont.) | 18.704 | 13.007 | 1.839 | 1.083 | 7.545 | 3.119 |
| 500 + 50 + 25 (T1) | 19.829 | 14.501 | 2.648 | 1.195 | 8.906 | 3.878 |
| 1000 + 100 + 50 (T2) | 22.737 | 16.050 | 3.676 | 1.331 | 10.186 | 4.437 |
| 1500 + 150 + 75 (T3) | 26.869 | 17.867 | 3.831 | 1.436 | 11.369 | 5.343 |
| 2000 + 200 + 100 (T4) | 33.095 | 19.255 | 4.078 | 1.485 | 12.348 | 5.831 |

From the previous gains, it is concluded that black elderberry (*Sambucus nigra* L.) trans plants can tolerate toxicity of Pb, Cd and Ni at medium and high concentrations with uptaking considerable amounts from these metals, and this render them for using in phytoremediation of heavy metal-contaminated soil.

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