

EFFECT OF LONG-TERM STRESS WITH HEAVY METALS COMBINATIONS ON GROWTH AND CHEMICAL COMPOSITION OF SOME ORNAMENTAL SHRUBS

I. EFFECT ON VEGETATIVE AND ROOT GROWTH

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ABSTRACT

Four separated pot experiments were carried out in the open field at the Experimental Station of Vegetable and Floriculture Department, Faculty of Agriculture, Mansoura University during the two successive seasons of 2006/2007 and 2007/2008, as each season contained two periods of growth, to find out the response of six-months-old transplants of *Acalypha wilkesiana* Müll. Arg., *Asclepias curassavica* L., *Dodonaea viscosa* (L.) Jacq. and *Tabernaemontana divaricata* (L.) R. Br. ex Roem & Schult. to long-term stress of lead (Pb), cadmium (Cd) and nickel (Ni) combinations added as thawing acetate salts to the soil mixture at the rates 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-, 4- and 5-fold of these concentrations for treatments number two (T₂), three (T₃), four (T₄) and five (T₅), respectively. Planting was in 25-cm-diameter black polyethylene bags filled with 3 Kg of a mixture of sand and loam at 1:1 (v/v).

The obtained results indicated that no mortality was observed among *Acalypha* and *Asclepias* transplants, as they recorded 100% survival during the two periods of growth in the two seasons, but in case of *Tabernaemontana* and *Dodonaea* transplants, such parameter was reduced, especially for the transplants which were exposed to T₄ and T₅ combinations in the second period of growth. However, survival % of *Tabernaemontana* transplants was higher than that of *Dodonaea*. In general, top and root growth of the four shrubs was declined with various significant differences, specially with prolonging growth period under high level of toxic elements, except for *Acalypha* transplants which gave longer roots than those of control transplants under low and medium concentrations of heavy metals (T₁, T₂ and T₃). However, under high concentrations (T₄ and T₅), the length of their roots was quite similar to that of control. So, it gave the highest pollution resistance index percentage (PRI %) compared to the other shrubs.

In brief, according to the aforementioned results (in particular survival % and PRI %), ornamental shrubs undergo long-term stress of Pb, Cd and Ni combinations under conditions of the present work should be arranged in the following descending order: *Acalypha wilkesiana* > *Asclepias curassavica* > *Tabernaemontana divaricata* > *Dodonaea viscosa*.

INTRODUCTION

Air, soil and water pollution with some toxic metals, as an ever growing crisis in different parts of the world, is still the most serious problem all over the world. It is difficult to solve the problem completely, but we can only reduce the excessive increment in these toxic pollutants and protect the environments from their hazards through planting more ornamental plants, which are not food chain crops. Among shrubs may serve in this concern, Jacob's coat, blood flower, hop bush and crepe jasmine ornamental shrubs.

Acalypha wilkesiana Müll. Arg., Jacob's coat (Fam. Euphorbiaceae), is a monocious shrub with attractive foliage, as their profuse, elliptic or ovate, serrate leaves are bronzy green mottled with copper, red or purple, mostly used for bedding, hedging and as lawn specimens (Bailey, 1976). *Asclepias curassavica* L., blood flower (Fam. Asclepiadaceae), is an annual or short-lived evergreen subshrub, sometimes developing tubers; leaves to 15 cm, elliptic-lanceolate, acuminate and opposite; flowers in axillary or subterminal cymes; corolla cinnabar red, sometimes white or yellow, reflexed; hoods erect and yellow-orange. Native to South America, occur on dry or perfectly drained soils, grown for its nectar-rich, sometimes fragrant and often glowing red-flowered umbels, for the herbaceous border and the wild gardens (Huxley *et al.*, 1992). *Dodonaea viscosa* (L.) Jacq., hop bush (Fam. Sapindaceae), is a very widespread tropical and subtropical shrub, used in gardens for hedges and as a solitary specimen on turf. It reaches up to 15 feet and usually has sticky shoots carry undivided, oblongish leaves 10-12 cm long and about 1.5-2.5 cm wide, and greenish flowers usually appearing in terminal clusters, propagated by seeds (Bailey, 1976). Another important one is *Tabernaemontana divaricata* (L.) R. Br. ex Roem & Schult., crepe jasmine or crepe gardenia (Fam. Apocynaceae), a shrub to 1.75 m, branching dichotomous; leaves 7-10 cm, elliptic-oblong, unequal, narrowed to base, thin-textured; flowers nocturnally fragrant, borne 4-6 in short and paired axillary cymes, corolla white. Native to North India and North Thailand. Grown for their decorative blooms and the glossy-foliage in borders or as informal hedging. It may be grown in large pots or tubs to be easily moved out-of-doors for the summer months, and kept in warm glasshouse or conservatory for the winter. The blooms making a perfect substitute in corsage and button holes. Propagated by semi-ripe cuttings in summer rooted in a closed case with bottom heat, or by simple layering in spring (Huxley *et al.* 1992).

The different effects of metallic pollution for plants were last reviewed by Kumar (1999) who indicated that the lowest concentrations of Pb, Cd, Ni and Hg (10^{-6} M) slightly increased vegetative and root growth of *Vinca rosea*, whereas higher ones (10^{-3} and 10^{-2}) greatly reduced it. Similarly, Schenk and Bucher (2000) mentioned that high level of Cd (100 ppm) in soil or in atmosphere induced a significant reduction in top growth, root length and number of flowers/plant of Petunias. On *Salvia splendens* and *Vinca rosea* cvs. Alba and Major, Shahin *et al.* (2002) found that high concentrations of Pb, Cd and Hg in combinations reduced top and root growth, delayed flowering and depressed floret diameter and stalk length, while low concentrations slightly improved them. No mortality was observed among the elemental contaminated plants except for the two cultivars of periwinkle, as the high levels of heavy metals slightly raised the percent of death.

Similar observations were also recorded by Abbaas (2002) on *Casuarina glauca*, *Taxodium distichum* and *Populus nigra*; Salgare and Palathingal (2002) on *Allamanda cathartica*; Bush *et al.* (2003) on *Betula nigra* and *Ulmus parvifolia*; Laypheng *et al.* (2004) on *Bougainvillea spectabilis*, *Ixora coccinea* and *Heleconia taxa*; Rossini-Oliva and Rautio (2004) on *Duranta repens*; and Wang and Zhou (2005) on *Tagetes erecta*, *Salvia splendens* and *Abelmoschus manihot*. Similarly, Shahin and El-Malt

(2006) revealed that survival % of sant and oak transplants was declined when grown in polluted sandy soil collected from the farm of Abou-Rawash Treatment Plant, while tipu transplants gave 100% survival. Vegetative and root growth of the three trees were improved during the first stage of growth, but depressed afterwards, especially for sant and oak. Pigments content in the leaves, as well as N, P and K % in the leaves and roots were increased through the first period of growth, but declined afterwards. However, Pb, Cd and Cu were markedly increased irrespective of the growth period.

Recently, Shahin *et al.* (2007) concluded that stock (*Matthiola incana*) is more tolerant to Pb, Cd and Ni toxicity than Cape marigold (*Dimorphotheca ecklonis*) plants, as it gave higher percentages of survival and pollution resistance index.

Such trial, however aims to detect the tolerance of some ornamental shrubs to toxicity of lead, cadmium and nickel combinations.

MATERIALS AND METHODS

Four separate pot experiments were conducted in the open field at the Experimental Station of Vegetable and Floriculture Department, Faculty of Agriculture, Mansoura University during 2006/2007 and 2007/2008 seasons to study the effect of long-term stress of Pb, Cd and Ni in combinations at various levels on growth of Jacob's coat, blood flower, hop bush and crepe jasmine transplants.

So, six-months-old transplants of Jacob's coat (55-60 cm long with 2 branches carrying about 15-17 leaves), blood flower (40-45 cm long with one branch which carries about 32-35 leaves), hop bush (25-27 cm long with one branch which carries about 7-9 leaves) and crepe jasmine (43-45 cm long with 2 branches carrying about 16-18 leaves) were planted on April, 1st for both seasons in 25-cm-diameter black polyethylene bags (one transplant/bag) filled with 3 kg of a mixture of sand and loam (1:1 v/v), which its some physical and chemical properties are shown in Table (a).

Thawing salts (acetates) of Pb, Cd and Ni produced by Aldrich Chemical Co. Inc., USA, were thoroughly mixed in combinations with the used soil mixture before planting at the 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-, 4- and 5-fold of these concentrations for treatments number two (T₂), three (T₃), four (T₄) and five (T₅), respectively above the background levels of these metals in the used soil mixture. The bags (without drainage holes to prevent leaching of metals) were immediately irrigated after planting with 250 ml of fresh water/bag, but thereafter the irrigation was done once every 3 days with only 200 ml of water/bag. The transplants were not fertilized throughout the course of study, but received the usual agricultural practices recommended for such plantation. They were set out in a complete randomized design (Mead *et al.*, 1993) for each plant and replicated 3 times with 6 transplants per replicate. The experiments began in the first season on April, 1st 2006 and lasted till the end of October 2007, whereas for the second season, it commenced on April, 1st 2007 and

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lasted till the end of October 2008, which means that each season contained two periods of growth. The first period for both seasons commenced on April 1st till the terminal of October, whereas the second one lasted from November 1st till the end of the followed October. Therefore, data were recorded two times for each season, which were at the terminal of October of both 2006 and 2007 years in the first season, and the terminal of October of both 2007 and 2008 years in the second season. Such registered data were: survival (%), plant height (cm), stem diameter at the base (cm), number of leaves and branches/plant, leaf area (cm²) from the middle part of plant, the longest root length (cm), number of root branchlets/main root and fresh and dry weights of aerial parts and roots (g). The rooting method for metal tolerance testing has been used previously by Wilkins (1957), and will be used in this study as an actual index for pollution resistance using the following equation: Pollution resistance index (PRI) % = Mean root length (cm) of the polluted transplant / Mean root length (cm) of the control transplant x 100.

The data were subjected to analysis of variance and the method of L.S.D. was used to differentiate the means (Mead *et al.*, 1993).

RESULTS AND DISCUSSION

Effect of Pb, Cd and Ni combinations on survival %, vegetative and root growth and PRI % of the different transplants:

1. *Acalypha wilkesiana* Müll. Arg., Jacob's coat transplants

According to data presented in Table (1), it is clear that pollution with heavy metals at various concentrations had no lethal effect on Jacob's coat transplants during the two periods of growth in both seasons. Plant height (cm), however was slightly improved in the first growth period due to the low and medium treatments of toxic metals (T₁, T₂ and T₃ treatments), but in the second period, such improvement was only observed in the first season for transplants treated with only T₁ combination. The shortest transplants, in general, were gained from those subjected to the highest level of toxic metals (T₅), which non-significantly reduced such parameter in the first growth period to 68.00 and 71.33 cm comparing to 121.00 and 123.42 cm for control transplants in the first and second seasons, respectively. In the second growth period this reached 80.00 and 87.16 cm with highly significant differences when compared to 153.33 and 160.26 cm for control transplants in the first and second seasons, respectively. Likewise, stem diameter (cm), number of leaves and branches/transplant, leaf area (cm²) and aerial parts fresh and dry weights (g) were greatly decreased in response to the different heavy metals combinations, especially the medium and high ones (T₂, T₃, T₄ and T₅), with highly significant differences in most cases for both growth periods in both seasons. However, an exception was obtained for leaf area trait, which was increased in the tow periods of growth with various significance levels due to T₁ treatment in the first season, and to both T₁ and T₂ in the second one, as well as aerial parts dry weight that was raised in both seasons during the first growth period, while during the second one, it was raised in the second season only.

Data in Table (2) reveal that the root length (cm) was pronouncedly increased as affected by T₁ treatment with highly significant differences compared to the root length of control transplants during both periods of growth in the two seasons. A similar trend was also obtained concerning roots dry weight (g) in both seasons during the second growth period only. Moreover, the same treatment (T₁) induced a slight increment in number of root branchlets/main root and roots fresh weight (g) in the two periods of growth in both seasons. Other treatments, however decreased all the previous parameters in polluted transplants, specially T₅, which gave in general, the least means with significant or highly significant differences in most cases of both growth periods and seasons.

The pollution resistance index (%), as a real indicator for tolerance of toxic metals, indicated that Jacob's coat transplants can tolerate metallic toxicity at various levels as they registered PRI means higher than 90% even at the highest concentrations and regardless of growth period, i.e. the long-term toxicity did not cause an excessive stress. The highest value of PRI %, however was recorded by transplants exposed to the lowest level of toxic metals (T₁) throughout the two periods of growth in both seasons. That may be due to that transplants under such treatment gave the longest root at all.

The reduction in vegetative and root growth may be due to accumulation of toxic metals in plant tissues, which usually leads to depression of vital processes, such as photosynthesis, inhibition of some enzymatic systems and blocking the formation of proteins and chlorophylls (Mengel and Kirkby, 1979). In addition, 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 also attained by Schenk and Bucher (2000) in *Petunias*.

Similar observations were also noticed by Abbaas (2002) on *Casuarina glauca*, *Taxodium distichum* and *Populus nigra*; Bush *et al.* (2003) on *Betula nigra* and *Ulmus parvifolia*; Laypheng *et al.* (2004) on *Bougainvillea spectabilis*, *Ixora coccinea* and *Heleconia taxa*; and Shahin *et al.* (2007) on *Matthiola incana* and *Dimorphotheca ecklonis*.

2. *Asclepias curassavica* L., blood flower transplants:

As previously shown in case of Jacob's coat transplants, data in Table (3) exhibit that no mortality was observed among blood flower transplants due to either of heavy metals treatments used in the present work at the end of both growth periods in the two seasons giving survival percentage equal 100% in all cases of both seasons. Top growth, however was reduced, but the reduction was more pronounced in the parameters of stem diameter (cm), number of leaves/plant and aerial parts fresh and dry weights (g) with various significancy levels in most cases of both periods of the two seasons. As for number of branches per plant, it was declined during the first period of growth with non-significant differences in all cases of the two seasons, while in the second period, the decrement was highly significant in most cases of both seasons. The opposite was the right regarding leaf area trait (cm²), as treatments T₁ and T₂ caused a highly significant increasing in such character in both seasons throughout the first period of

growth, while in the second period, the rate of elevation was non-significant. In general, the most pronounced reduction was noticed in number of leaves/transplant, which consequently led to a great decrement in aerial parts fresh and dry weights in all cases of the two seasons.

Similarly, the results of root growth (Table, 4), as root length (cm), No. root branchlets/main root and roots fresh and dry weights (g), were progressively decreased with increasing heavy metals concentration with highly significant differences in most cases of both growth periods in the two seasons. Concerning PRI %, it was greatly decreased in the first period of growth to less than 50% for transplants undergo T₃, T₄ and T₅ treatments in both seasons, while in the second period, it was raised to become more than 50% for the same treatments indicating the ability of blood flower transplants to tolerate heavy metals toxicity with prolonging growth period due to increasing the rate of root growth of polluted transplants in the second period to more than that of control transplants.

The previous results, however may be interpreted and discussed as previously stated in case of Jacob's coat transplants.

3. *Dodonaea viscosa* (L.) Jacq., hop bush transplants:

From data shown in Table (5), it is evident that means of survival percentage exhibited a highly significant decrement in response to T₃, T₄ and T₅ treatments during the first period of growth in both seasons. An addition decrement was observed in such means with prolonging growth period except for control transplants and those ones subjected to T₁ treatment. However, the highest lethal effect was found due to the highest concentration of toxic metals (T₅), as this treatment reduced such parameter to less than 50% in all cases of the two seasons. In addition, top growth parameters of plant height, stem diameter, No. leaves and branches/transplant, leaf area, as well as, aerial parts fresh and dry weights were gradually depressed with various significant differences by elevating heavy metals concentrations throughout the first growth period. An exception was obtained for T₁ treatment, which slightly improved stem diameter, No. leaves/transplant and aerial parts fresh and dry weights measurements in the two seasons, as well as No. branches/transplant in the first season only. However, the depression in the second period of growth was more pronounced with highly significant differences in most cases of both seasons.

A similar trend was also gained with regard to root growth parameters (Table, 6), as all traits were declined with different significance levels in most cases of both periods in the two seasons. The treatment number 1 (T₁) is the only treatment that caused a significant increase in roots fresh and dry weights (g) during the first period of growth in the first season, but in the second one the rate of increase was non significant. Moreover, PRI % during the first growth period surpassed the percent of 80% in the first season and 70% in the second one for the treatments from 1 to 4, but was decreased for the treatment No.5 to less than 50% in both seasons. However, this character registered a percent higher than 70% during the second growth period in the two seasons, even for transplants subjected to the highest combination of heavy metals (T₅).

These findings, however could be explained and discussed as mentioned before in case of Jacob's coat transplants.

4. *Tabernaemontana divaricata* (L.) R. Br. ex Roem & Schult., crepe jasmine transplants:

Data in Table (7) show that survival % was significantly declined in the first period of growth as a result of treatment with only the highest level of toxic metals (T₅) in both seasons, whereas in the second period such parameter was depressed in the two seasons with significant differences due to T₄ treatment only and with highly significant ones due to T₅ treatment. In general, survival % of crepe jasmine transplants in both growth periods of the two seasons was more than 75% showing the ability of such ornamental shrub to tolerate medium and high levels of heavy metals in polluted soils. However, top growth was reduced, especially stem diameter and aerial parts fresh and dry weights traits at different periods of growth in the two seasons. Plant height, number of leaves and branches/transplant and leaf area, on the other hand, were the traits less affected by the various pollution treatments, except for T₄ and T₅ combinations, which caused a significant and highly significant decrements in plant height and leaf area parameters in both growth periods of the two seasons. Similarly, only root length was decreased during the first period of growth in response to T₄ and T₅ treatments with highly significant differences in the two seasons, whereas number of root branchlets/main root and roots fresh and dry weights (g) exhibited highly significant decreases in all cases of both seasons (Table, 8). In the second growth period, however such parameters decreased in only transplants treated with T₄ and T₅ combinations, with the exception for No. root branchlets/main root character that was increased with various significant differences in the two seasons. Concerning PRI %, it was higher than 58% during the first period of growth, but raised afterwards to become more than 75% for both seasons during the second period of growth.

Such gains, however could be interpreted and discussed as done before in case of Jacob's coat transplants.

In conclusion, ornamental shrubs undergo long-term stress of Pb, Cd and Ni combinations in this study should be arranged in the following descending order: *Acalypha wilkesiana* > *Asclepias curassavica* > *Tabernaemontana divaricata* > *Dodonaea viscosa* according to their survival % and the pollution resistance indices (PRI %).

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تأثير الإجهاد طويل المدى بتوليفات من بعض المعادن الثقيلة على النمو والتركيب الكيميائي لبعض شجيرات الزينة

١- التأثير على النمو الخضري والجذري

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أجريت أربع تجارب أصص منفصلة تحت ظروف الحقل في محطة تجارب قسم الخضر والزينة - كلية الزراعة - جامعة المنصورة خلال الموسمين المتتاليين ٢٠٠٧/٢٠٠٦ و ٢٠٠٨/٢٠٠٧ حيث اشتمل كل موسم على فترتين للنمو، وذلك لدراسة إستجابة شتلات الأكاليفيا (*Acalypha wilkesiana* Müll. Arg.)، الدفلة التركي (*Asclepias curassavica* L.)، الدودونيا (*Dodonaea viscosa* (L.) Jacq.)، والتابريانا (*Tabernaemontana divaricata* (L.) R. Br. ex Roem & Schult.) عمر ستة أشهر للإجهاد طويل المدى بتوليفات من عناصر الرصاص والكاديوم والنيكل والتي إضيفت لمخلوط التربة قبل الزراعة في صورة أملاح الخلات سريعة الذوبان بالتركيزات التالية: صفر جزء في المليون لكل عنصر من العناصر الثلاثة (كمقارنة)، ٥٠٠ + ٥٠ + ٢٥ جزء في المليون للعناصر الثلاثة على الترتيب للمعاملة الأولى (T₁)، بالإضافة إلى ٣، ٤، ٥ أضعاف هذه التركيزات للمعاملات الثانية (T₂)، الثالثة (T₃)، الرابعة (T₄) والخامسة (T₅) على الترتيب. وقد تمت الزراعة في أكياس بلاستيك سوداء غير مثقبة قطرهما ٢٥ سم ملئت بحوالي ٣ كجم من مخلوط متساوي من الرمل والطيني (بالحجم).

ولقد أوضحت النتائج المتحصل عليها عدم حدوث موت لأي من شتلات الأكاليفيا والدفلة التركي حيث أعطيتا نسبة حياة ١٠٠% خلال مرحلتى النمو لكلا الموسمين، بينما فى حالة شتلات التابريانا والدودونيا إنخفض هذا القياس، خاصة للشتلات التى تعرضت للتركيزات العالية من العناصر السامة (T₄، T₅) فى الفترة الثانية من النمو، إلا أن نسبة الحياة لشتلات التابريانا كانت أفضل منها فى الدودونيا. وبصفة عامة، إنخفض معدل النمو الخضري والجذري للأنواع الأربعة من شجيرات الزينة المستخدمة فى هذه الدراسة بمستويات معنوية مختلفة، خاصة عند إطالة فترة النمو أو التعرض للتركيزات المرتفعة من العناصر السامة، بإستثناء شتلات الأكاليفيا التى أعطت جذورا أطول من جذور شتلات المقارنة عند معاملتها بالتركيزات المنخفضة والمتوسطة من العناصر الثقيلة (T₁، T₂، T₃) بينما عند تعرضها للتركيزات المرتفعة (T₄، T₅) كانت أطوال جذورها متقاربة مع أطوال جذور شتلات المقارنة، لذلك أعطت أعلى نسب مئوية لمعامل مقاومة التلوث (PRI %) مقارنة بالأنواع الأخرى.

وطبقا للنتائج سالفة الذكر (خاصة النسبة المئوية للنباتات الحية ولمعامل مقاومة التلوث)، فإن شجيرات الزينة التى تعرضت فى هذه الدراسة لإجهاد طويل المدى بتوليفات من عناصر الرصاص، الكاديوم والنيكل يمكن ترتيبها تنازليا كما يلى:

الأكاليفيا < الدفلة التركي < التابريانا < الدودونيا

