Intensity of free radical processes in the leaves of arboreal plants under act of industrial dust borne *extracts*

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SUMMARY

*The influence of industrial pollutants on the intensity of lipid peroxidation in the assimilatory organs of arboreal plant was investigated. The differential changes of the probed indexes are set depending on the species. Information is got can testify to participation of lipid peroxidation products in forming of reactions-answers of arboreal plants on influence of industrial dust borne **extract** with content of heavy metals. Determination of level and rates of accumulation of Zn, Ni, Pb and Cd, in the leaves of arboreal plants in the conditions of different contamination level allowed to take species to two groups. To the first (phytoextraction potential exceeds a base-line level in 10 times) belong Populus bolleana Lauche, P. italica (Du Roi) Moench, Picea pungens Engelm and Sorbus aucuparia L. To the second (exceeds a base-line level from 5 to 10 times) belong Acer negundo L., Aesculus hippocastanum L., Betula pendula Roth and Tilia cordata Mill. The most substantial increase of peroxidation secondary product content (more than in 2.5 times) is peculiar for B. pendula, A. hippocastanum and P. pungens Engelm., that well conforms to the rates of heavy metals translocation, it has however species-specific character.*

INTRODUCTION

Anthropogenic contamination of region in high-urbanized ecosystems results to varied violations of living organism functioning, and above all things plants. Damages show up in changing of anatomical, morphological, physiological and biochemical indexes, which reduce them decorative qualities and biological stability. After the entrance volumes to landscapes heavy metals among, which Zn, Pb, Cd and Ni, have a loan of a leading value and more intensive in all engaged in a biogeochemical rotation (Briat- Lebrun,1999). Among the effects of toxic influence this pollutants on plant organisms displacement of prooxidative-antioxidative equilibrium comes into the special notice in the side of intensification of lipid peroxidation (Bishnoi et al.,1993; Lichtanthaler,1998; Naoharu et al., 2003; Tang - Xi, 2002), in fact exactly activation of lipids peroxidation processes – one of key links between the action of a stress factor and realization of protective reactions of organism (Finkel-Holbrook, 2000; Wang, 2004; Karperts-Kolupaev, 2008).

Coming from that lipid peroxidation products are the determination indexes of character and intensity of a heavy metals stress on living organisms, the purpose of our work was to probe the features of Zn, Pb, Cd and Ni accumulation and dynamics of lipid peroxidation process changes, in the assimilatory agent of arboreal plants at industrial terms.

MATERIALS AND METHODS

The objects of researche were *Populus bolleana* Lauche, *P. italica* (Du Roi) Moench, *Picea pungens* Engelm., *Acer negundo* L., *Tilia cordata* Mill., *Sorbus aucuparia* L., *Betula pendula* Roth, *Aesculus hippocastanum* L., that grow on industrial ground «Red Lead Factory» LTD (in the zone of strong and weak contamination) and in the arboretum of the Kryvyi Rig Botanic Garden of the National Academy of Sciences of Ukraine, which is accepted for conditional control. From ten trees of every species leaves were taken away in the phases of complete isolation of leave (pine-needles) and completion of their growth.

The content of pollutants in plant material was determined on the atomic-adsorption spectrophotometer after the methods accepted in general lines (Feldman, 1974). The indexes of intertissue contamination expected after Kamishnikov (2000). The degree of lipid peroxidation development was estimated after content of TBA-active products determinations of which conducted on a spectrophotometer CF-2000 (Russia) after the generally accepted method (Kamishnikov, 2000). Protein content in plant tissues was determined after the method of Greenberg (Greenberg-Gaddoc,1982).

Experiments conducted in 3-repetition with biological and analytical methods. The statistical processing of experimental data was conducted after the generally accepted methods of self-reactance statistics at 95% levels of meaningfulness after Dospekhov (1985). On figures and in tables the average results and their standard deviations are shown.

RESULTS AND DISCUSSION

Comparative study was made regarding the content of heavy metals in the assimilatory agent of arboreal plants on both phases of leaf (pine-needles) morphogenesis in control and at of technogen exposes at different species and allows to talk about the certain species-specific accumulation of heavy metals.

According to the analysis of pollutant content contained in plants to the arboretum of the Kryvyi Rig Botanic Garden of the National Academy of Sciences of Ukraine (control) in the phase of complete isolation of leaf is more intensive accumulated in the leaves of *Acer negundo* L., while in the *Populus bolleana* Lauche, *P. italica* (Du Roi) Moench and *Tilia cordata Mill.* on 36-45% less (table 1.). In the assimilatory agent of *Sorbus aucuparia* L., *Picea pungens* Engelm., and *Aesculus hippocastanum* L. under the conditions heavy metal content was in 3.3-5.5 times less than, at the *Acer negundo* L. It is interesting to note, that in this phase in the leaves of *Aesculus hippocastanum* L. also least Ni and Pb was accumulated. In the *Populus bolleana* Lauche, *P. italica* (Du Roi) Moench and *Betula pendula* Roth content of the Ni was higher almost in 20 times by comparison to the inter-tissue contamination index (ICI) of *Aesculus hippocastanum* L. The high level of accumulation of Pb (from 1.34 to 1.54 mg kg^{-1} of fresh weight) in the phase of complete isolation of leaves (pine-needles) is fixed at a *Tilia cordata* Mill., *Acer negundo* L. and *Sorbus aucuparia* L. The table of cadmium contents in the assimilatory agent of most species moved between 0.02 to 0.05 mg kg^{-1} of fresh weight.

The analytical results of phytotoxicity index are presented in a table testify that the most of all of investigational metals in the leaves of arboreal plants accumulated in the area of strong contamination. The greatest quantity of zinc accumulation which belongs to the class of high-dangerous elements (Balyuk et al., 2004) was found in the phase of complete isolation of leave where characteristic for a *Sorbus aucuparia* L. (ICI > 30). *Populus bolleana* Lauche and *P. italica* (Du Roi) Moench had considerably less rates of this metal accumulation (ICI from 9.0 to 12.6), while at other species of arboreal plants the inter-tissue contamination index did not exceed 3.2. The similar tendency of specific composition was observed in the zone of weak contamination.

In both industrial affected zones sufficiently high values of inter-tissue contamination index of leaves (ICI > 20) were observed in the phase of complete isolation it was characteristic for a cadmium and nickel. The order Regarding both the absolute and after relative indexes, in the order of accumulation the first most contained was the pine-needle of *Picea pungens* Engelm., while second on absolute indexes – at *Populus bolleana* Lauche, *P. italica* (Du Roi) Moench and *Aesculus hippocastanum* L., and after the rates of accumulation – only in *Aesculus hippocastanum* L. Processes of absorption and translocation to the leaves of Pb in the phase of complete isolation were the least, as compared to other heavy metals. The values of ICI fluctuated from 3.3 (*Populus bolleana* Lauche) to 1.2 (*Picea pungens* Engelm.).

Where as in the phase of completion of leave growth the temps of Pb accumulation grew almost in 3 times, in compared to the above-described phase (*Table 1*.). Thus, both on absolute and after relative, indexes the high level accumulations of this metal are inherent *Populus bolleana* Lauche, *P. italica* (Du Roi) Moench and *Acer negundo* L. (6.69 – 11.64 mg kg^{-1} fresh weight).

In the phase of completion of leave growth among metals investigated zinc had the most often translocated. We have to note that to the group of arboreal plants high this heavy metal content concert *Populus bolleana* Lauche, *P. italica* (Du Roi) Moench and *Sorbus aucuparia* L. The high values of inter-tissue index of contamination of these species have at the same time (in 5.4-10 times more than at other species).

The greatest intensity of cadmium accumulation in the conditions of strong contamination area is observed at Picea *pungens* Engelm (the value of inter-tissue contamination index of pine-needle in 1.8-3.2 times exceeds the index than other species). Together with it at *Populus italica* (Du Roi) Moench and *Betula pendula* Roth, the level of cadmium in an assimilatory agent fluctuated from 0.54 to 0.62 mg kg^{-1} of fresh weight, it is 1.6-2.6 times exceed the cadmium content than other species of arboreal plants.

It worth noticing, that for leafy arboreal plants there is common tendency in relation to the maximal accumulation of certain heavy metals on the areas of strong contamination zone compared to the low contamination zone during the research period. So, for example, in the plants leaves of the first zone during two phases of morphogenesis 1.4-3 times more cadmium accumulated in the leaves than in the second zone.

Similar tendency was experienced after the inter-tissue contamination index of leaves.

Concerning the nickel and zinc content, the above-mentioned index value in the phase of leaf isolation of *Acer negundo* L. in the conditions of strong contamination zone was from 3.77 to 12.62, while in the weak contamination zone it was only between 1.27 and 3.44 at the .

On the basis of Zn, Ni, Pb and Cd accumulation level and determination the assimilatory agent of arboreal plants in the conditions of different contamination level, the species can be divided into two groups. To the first group has the greatest phyto-extraction potential of most heavy metals (exceeds the base level in 10 times), belongs *Populus bolleana* Lauche, *P. italica* (Du Roi) Moench, *Picea pungens* Engelm and *Sorbus aucuparia* L. To the second group has the middle accumulation level (exceeds the base level from 5 to 10 times), belongs *Acer negundo* L., *Aesculus hippocastanum* L., *Betula pendula* Roth and *Tilia cordata* Mill.

The toxic action of the most heavy metals causes oxidative stress development and accompanied varied alterations of plant metabolism, conditioned both direct membrane lipid peroxidation, and accumulation of peroxidation product and their co-operation, with cellular macromolecules. According to studies the finished free-radical reactions products, in particular TBA-active products, including malonyl dyaldehyde, which at cooperating with proteins, amino acid, nucleic acids, lipids can form connection, so called «pigments of senescence» (Finkel-Holbrook, 2000; Kosyk et al., 2006; Wang, 2004).

Table1.

Content of some heavy metals in the leaves of arboreal plants (mg kg^{-1} of fresh weight)

	Zn		Ni		Pb		Cd	
	M±m	ICI	M±m	ICI	M±m	ICI	M±m	ICI
Populus bolleana Lauche								
Control	0.52±0.01 0.70±0.01	—	1.05±0.06 1.55±0.02	—	0.73±0.01 0.93±0.03	—	0.03±0.00 0.05±0.00	—
Zone of strong contamination	4.70±0.18* 6.97±0.04*	9.03 9.97	2.54±0.04* 2.73±0.01*	2.41 1.77	2.39±0.09* 6.96±0.10*	3.30 7.52	0.18±0.02* 0.24±0.00*	6.01 4.80
Zone of weak contamination	0.74±0.08* 6.97±0.04*	1.42 2.69	1.92±0.03* 2.00±0.01*	1.82 1.29	1.35±0.07* 2.14±0.08*	1.86 2.31	0.06±0.00* 0.09±0.01*	1.96 1.77
Populus italica (Du Roi) Moench								
Control	0.46±0.02 0.45±0.00	—	0.72±0.08 0.69±0.01	—	0.82±0.03 1.42±0.05	—	0.05±0.00 0.06±0.00	—
Zone of strong contamination	5.77±0.09* 10.44±0.15*	12.62 23.07	2.73±0.06* 4.03±0.11*	3.77 5.86	2.36±0.07* 11.64±0.10*	2.87 8.19	0.25±0.01* 0.55±0.02*	4.99 8.65
Zone of weak contamination	1.57±0.09* 4.29±0.05*	3.44 9.50	0.92±0.01* 1.32±0.01*	1.27 1.92	1.06±0.03* 5.33±0.04*	1.29 3.75	0.09±0.00* 0.18±0.00*	1.75 2.88
Acer negundo L.								
Control	0.83±0.05 1.07±0.00	—	0.36±0.01 0.56±0.01	—	0.79±0.01 2.22±0.06	—	0.04±0.00 0.05±0.00	—
Zone of strong contamination	2.63±0.17* 3.71±0.09*	3.16 3.48	1.16±0.06* 1.50±0.01*	3.23 2.68	2.00±0.15* 8.08±0.12*	2.53 3.64	0.10±0.00* 0.26±0.01*	2.26 5.52
Zone of weak contamination	1.64±0.01* 1.90±0.04*	1.97 1.79	0.67±0.01* 0.74±0.02*	1.86 1.33	0.56±0.03* 3.10±0.04*	0.71 1.40	0.07±0.00* 0.14±0.00*	1.61 2.99
Picea pungens Engelm								
Control	0.25±0.01 1.01±0.05	—	0.53±0.01 0.53±0.01	—	1.43±0.03 1.17±0.01	—	0.02±0.00 0.04±0.00	—
Zone of strong contamination	0.53±0.04* 2.20±0.05*	2.15 2.18	0.61±0.03* 1.17±0.04*	1.16 2.20	1.86±0.05* 2.16±0.06*	1.30 1.85	0.41±0.01* 0.54±0.00*	22.80 15.14
Tilia cordata Mill.								
Control	0.53±0.02 0.79±0.04	—	0.26±0.02 0.85±0.08	—	1.54±0.19 2.23±0.07	—	0.03±0.01 0.07±0.00	—
Zone of strong contamination	1.24±0.11* 3.03±0.37*	2.36 3.81	1.00±0.03* 2.37±0.04*	3.89 2.78	2.06±0.21 3.03±0.03*	1.34 1.36	0.20±0.01* 0.40±0.01*	6.15 5.37
Sorbus aucuparia L.								
Control	0.15±0.02 0.69±0.01	—	0.41±0.02 0.71±0.04	—	1.34±0.08 1.68±0.00	—	0.03±0.00 0.08±0.00	—
Zone of strong contamination	5.00±0.60* 8.15±0.24*	33.51 11.79	1.79±0.10* 2.12±0.07*	4.33 2.97	2.15±0.09* 4.95±0.07*	1.60 2.94	0.23±0.02* 0.35±0.00*	8.81 4.63
Aesculus hippocastanum L.								
Control	0.20±0.02	—	0.05±0.00	—	0.44±0.02	—	0.05±0.00	—

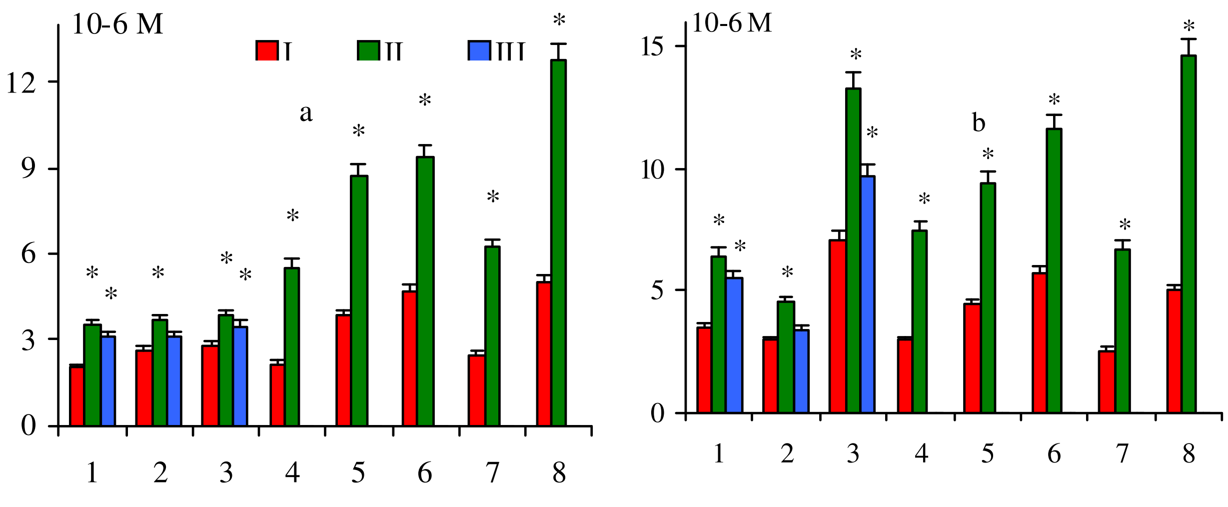
	0.27±0.00		0.30±0.01		1.15±0.16		0.07±0.00	
Zone of strong contamination	0.36±0.02* / 1.21±0.07*	1.79 / 4.55	1.10±0.09* / 2.08±0.01*	23.27 / 7.01	0.93±0.02* / 2.24±0.03*	2.11 / 1.97	0.16±0.02* / 0.37±0.00*	3.47 / 5.55
Betula pendula Roth								
Control	0.39±0.03 / 0.88±0.01	—	0.93±0.04 / 1.06±0.04	—	0.70±0.00 / 1.76±0.08	—	0.08±0.00 / 0.11±0.02	—
Zone of strong contamination	0.75±0.03* / 2.04±0.05*	1.95 / 2.32	1.25±0.11* / 1.52±0.04*	1.34 / 1.43	1.43±0.08* / 4.59±0.22*	2.05 / 2.62	1.16±0.01* / 0.62±0.02*	15.03 / 5.79

Note. ICI is the inter-tissue contamination index of leaves;* - statistically reliable difference in relation to control after $p < 0.05$; where the numerator is the value in the phase of complete isolation of leaf, and the denominator is the phase of completion of leaf growth

Therefore the exact content of TBA-active connections is an important index of different factors influencing degree on the organism. It is interesting to study that which allows to a certain extent to estimate the functional state of organism and its hetero-specific adaptation ability (Bishnoi et al.,1993; Finkel- Holbrook, 2000; Naoharu et al., 2003; Tang-Xi, 2002; Wang, 2004).

The data of the content of TBA-active products testifies the practically identical motion of lipids peroxidation processes (fig.) in the leaves of arboreal plants which grow in the arboretum, that all of the probed species in both phases of leaves morphogenesis. Most intensively lipid peroxidation passes in the assimilative organs of *Betula pendula* Roth, *Sorbus aucuparia* L. and *Tilia cordata* Mill., that, to our opinion, explained the considerable high biological accumulation of Ni and Cd. However necessary to take into account at the discussion of findings, that there are differences in the species-specificity of free-radical peroxidation development.

Figure 1: **Content of TBA-active products in the leaves of arboreal plants (10^{-6} M of malonyl dyaldehyde mg^{-1} proteine) in the phase of complete isolation of leaf (a) and phase of completion of leaf growth (b)**



I – control; II – area of strong contamination on «Red Lead Factory» LTD; III –area of low contamination on «Red Lead Factory» LTD; 1 – *Populus bolleana* Lauche; 2 – *Populus italica* (Du Roi) Moench; 3 – *Acer negundo* L.; 4 – *Picea pungens* Engelm.; 5 – *Tilia cordata* Mill.; 6 – *Sorbus aucuparia* L.; 7 – *Aesculus hippocastanum* L.; 8 – *Betula pendula* Roth;* – statistically reliable difference in relation to control after $p < 0.05$

Information can be presented on figure 1. It testify, that the amount of peroxidation after-products in the assimilatory process of arboreal plants at the terms of strong and weak contamination zone on «Red Lead Factory» LTD exceeds their maintenance in control plants in both phases of leaves' development. For example, in the assimilatory organs of *Populus bolleana* Lauche in the strong contamination zone in the phase of complete isolation of leave TBA-active product content was 70%, and in the weak contamination zone was only 50% compare to control. In the phase of completion of leaf growth this index increased to 83% and 57% accordingly. As compared to control plants for *Populus italica* (Du Roi) Moench the specific increase of lipid peroxidation after-products content was 40% and 17% in leaves (phase of complete isolation of leave) and 52% and 14% (phase of completion of growth) accordingly.

The results show that for species with the greatest phyto-extraction potential, namely *Populus bolleana* Lauche and *Populus italica* (Du Roi) Moench, characteristically low level of free-radical processes are not exceed this index at the control plants more than in 1.9 times (fig.1). To our opinion such results can be explained with the significant intensity of antioxidant systems functioning at the noted species, which is because of the greater stability in the conditions of technogen influence. We have to note that at a certain phyto-extraction potential, next to the considerable inter-tissue contamination index of leaves, there is growth of TBA-active compound content. Regarding the *Tilia cordata* Mill., (species has middle-level coefficient of biological accumulation) characteristically analogical to the above-mentioned tendency, the increase of peroxidation after-products content. However, it is necessary to mark, that at the *Acer negundo* L., which also belongs to this group of arboreal plants, in the phase of complete isolation of leaves there is the insignificant activating of lipid peroxidation processes and, opposite, in the phase of completion of leave growth and ripening – intensification of free-radical processes is swiftly increased.

The most substantial increase of TBA-active content of compounds (more, than in 2.5 times) is peculiar for a *Betula pendula* Roth, *Aesculus hippocastanum* L. and *Picea pungens* Engelm., at a middle level and regards to the Zn, Ni, Pb and Cd accumulation in the different industrial contamination zones.

CONCLUSIONS

The parklands are artificially created area in the zones of industrial enterprise. Because of the substantial anthropogenic influence, several physiological and biochemical changes take place in plants, so it is determined by a certificate what species can be grown. It is set that in the swingeing majority of species of arboreal plants with high heavy metal accumulation level there is low content of peroxidation after-products in assimilatory organs, it is due to their properties in greater adjustment in the conditions of industrial contamination zone. An exception is made by the *Picea pungens* Engelm., what under industrial pollutants significant increase of free-radical processes intensity was measured. From other side it means, that even insignificant maintenance of heavy metals in the assimilatory vehicle of plant with the middle level of this pollutants accumulation results the lipid peroxidation intensification, the growth of TBA-active product concentration was 2.5 times more compare to control plants.

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