

The Impact of Man-made Environment on the Ecological and Biological Characteristics of Drooping Birch

Irina Leonidovna Bukharina, Ajgul Muhametnagimovna Sharifullina,
Petr Anatolyevich Kuzmin, Natalya Valentinovna Zakharchenko and
Ilzira Ilurovna Gibadulina

¹Udmurt State University, Russian Federation, 426034, Izhevsk, 1 Universitetskay str.

²Izhevsk State Agricultural Academy, Russian Federation, 426069, Izhevsk, 11 Student str.

³Kazan Federal University, Yelabuga Institute, Russia Federation, Elabuga, 423600, 89 Kazan str.

(Received: 02 March 2015; accepted: 04 April 2015)

The article analyzes the data on characteristics of phenological phases of development, the content of ascorbic acid and tannins, and peroxidase activity of drooping birch in the environment of the city of Naberezhnye Chelny. The specific reaction of drooping birch on a complex of negative conditions of the built environment was defined. A special feature is the increase in the growing season in species under anthropogenic stress of buffer zones, in-main plantations, as compared to CCA (conditional control area). The content of tannins in the leaves of drooping birch grows in the course of growing season, reaching the highest values in August. The increased anthropogenic impact leads to an increase in the content of ascorbic acid in the leaves of drooping birch in the buffer zone plantations of industrial enterprises and the in-main plantations in the early stages of active vegetation. In technological terms the peroxidase activity in leaves increases over the entire period of active vegetation.

Key words: Drooping birch, Urban environment, Phenological observations, Ascorbic acid, tannins, peroxidase activity.

Major industrial centers are the artificial ultra-opened systems created by man and entirely dependent on it, both in terms of maintaining the ecological balance, and in terms of the possible ways to improve man-made habitats. Such issues are of particular relevance in view of the need of selection of species of living organisms capable both to survive in the extreme conditions of the urban environment, and positive influence thereon, helping to optimize and improve it (T.K.

Goryshina, 1991; I.L.Bukharina and others, 2013; Cheeseman J.M., 2007). The works on the study of possibilities of plants as a factor for improving the quality of urban and man-made habitats are continuing.

Different secondary metabolites, including those of low-molecular and high-molecular nature which are important indicators of well-being of plants vegetation, are involved in adaptive reactions. Such metabolites are ascorbic acid, tannins, peroxidase activity dynamics of which is changing throughout the vegetation under the influence of anthropogenic stress. Ascorbic acid is involved in numerous life processes of plants: growth, flowering, vegetative and

* To whom all correspondence should be addressed.
E-mail: petr.kuzmin84@yandex.ru

reproductive differentiation, in water exchange, regulation of enzyme activity, stimulation of metabolic reactions, involving the exchange of nucleic substances and protein synthesis, and in plant defense reactions (M.E. Balandaikin, 2014; Wyrwicka., 2006; Horemans N., 2007; Gill S.S., 2010; Delia-Gabriela Dumbrav0, 2012). Peroxidase, together with catalase and superoxide dismutase, are involved in protecting the organism from oxidative stress, control the growth of plants, their differentiation and development. Since the substrates of peroxidase can be phytohormones (abscisic acid, gibberellic acid, auxin), the enzyme may regulate the composition of physiologically active substances in the plant tissues. However, this enzyme is sufficiently sensitive to external influences, prompting suggestion on the possibility of using indicators of its activity as test characteristics to determine the living condition of woody plants (V.V. Rogozhin., 2004; Schofield J.A., 1998). Tannins are an important element in shaping the responses of woody plants to the rising level of environmental pollution. Upon entering into the state of dormancy, the phenol concentration therein increases and decreases at its end. Elevated level of easily hydrolyzable phenols in the litter inhibits the rate of loss of the total mass and promotes the immobilization of nutrients (Foyer C.H., 2005; Mishra S., 2006; John R., 2008).

Various metabolites, including phenolic compounds (FC), the representatives of which are tannins (tanstuff), are involved in adaptive reactions. Phenolic compounds affect the growth and development processes. The mechanism of their action on plant growth is not clear, and it is often associated with exposure to auxin metabolism, by regulation of the amount of auxins. Monophenols used in the experiments increase the activity of IAA-oxidase and auxin degradation, but di- and polyphenols inhibit degradation of indoleacetic acid. Phenols share with IAA the precursor in the biosynthesis, therefore the synthesis of phenols may be associated with slowing of auxin biosynthesis and vice versa. It is assumed that the tannins are the main antagonists and regulators of gibberellins activity. Phenolic compounds can also play an important role in the buds dormancy. Some of the

phenols violate oxidative phosphorylation. In case of mechanical damage, an intensive new formation of phenolic compounds, which is accompanied by an oxidative condensation in the surface layers, the products of which have protective function, begins in tissues. A number of phenolic compounds (carotenoids, flavonoids, phenolcarboxylic acids) have a strong antioxidant effect (I.L. Bucharina, 2014; Galves-Valdivieso, 2010).

The drooping birch (*Betula pendula* Roth.) is widespread in planting of the city of Naberezhnye Chelny and buffer zones of JSC "Kamaz". The drooping birch is forced to adapt to hostile conditions of the built environment. Negative factors of the man-made environment cause premature aging of plants. The purpose of our work was to study the phenology and dynamics of the content of ascorbic acid, tannins and peroxidase activity in leaves of drooping birch growing in plantations of different environmental categories.

Methods

Naberezhnye Chelny is a part of the Republic of Tatarstan, which is located on the territory of the Middle Volga. The annual rainfall reaches an average of 555 mm. The average annual temperature is about 2 ... 3.1 ° C. Naberezhnye Chelny is a major industrial center with a population of 530 thousand people. Major industries in the city are mechanical engineering, electric power, construction industry, and food processing industry. The key (city-forming) company of the city is Kama Automobile Plant. Characteristics of the degree of air pollution in areas of growing of woody plants were carried out by us on the basis of the "Report on the environmental condition of the Republic of Tatarstan" for 2013. The complex air pollution index (API = 15.3) describes the condition of air pollution in the city as very high. There was established the excess of maximum permissible concentration levels of benzo (a) pyrene, formaldehyde, phenol, carbon oxides and nitrogen (State report "On the condition of Natural Resources and Environmental Protection of the Republic of Tatarstan in 2013", 2014).

The research object is the drooping birch (*Betula pendula* Roth.). The studied plant species grows in the city as part of plants of different

ecological categories: main landings (large main Auto 1 and Mira Avenue) and sanitary and protection zones (SPZ) of the industrial enterprises JSC “Kamaz”, plants “Liteiniy” and “Kuznechniy”, which are the main polluters of the city. The following zones were chosen as the conditional control zones (CCZ): the area of Chelny forestry (forest-steppe zone of 9,539 hectares, forest-steppe regions of the European part of the Russian Federation).

The trial plots were laid in a regular way (by 5 pcs. in each area, with the size of not less than 0.25 ha). In order to examine the contents of the physiological and biochemical indexes in leaves of plants within the trial plot (TP), the selection (10 plants of each species) and numbering of accountable woody plants, and the estimation of their living condition were made. The accountable species had a good living condition and middle-aged generative ontogenetic state (g2). During the active growing season, i.e., June, July and August, the samples of leaves of the middle formation at the annual vegetative growth were taken from the accountable species (with the lower third of the tree crown of southern exposure). The selection of soil samples (composite sample made up of individually taken samples by the envelope method) was made within the TP. The exposure was determined by compass and corresponded to the location of the crown part against the north and south. In main plantation the southern exposure directly faced the avenue.

During phenological observations, we recorded the following stages of seasonal changes: Pb2 – budding (the appearance of a cone of leaves), C2 - the beginning of flowering, C3 - the end of flowering, Pl3 - ripening of fruits, seeds, L3 - colorization of dying leaves, and P4 - leaf fall (phenological observations of trees and shrubs: Guidelines for dendrology, 1990; N.E. Bulygin, 2001).

Under laboratory conditions, the content of ascorbic acid was determined according to GOST 24556-89 (titrimetric method). The content of condensed tannins in the leaves of woody plants was defined using permanganometric method (Leventhal method within Kursanov modification), peroxidase activity - using colorimetric method according to A.M.Boyarkin (D.P.Viktorov, 1991,

V.S.Nikolaevskiy, 1999).

The mathematical processing of materials was made using the statistical package «Statistica 5.5». For the interpretation of the obtained materials, the methods of descriptive statistics and dispersive multivariate analysis (by cross-hierarchical scheme, upon the subsequent assessment of differences by multiple comparing LSD-test) was used.

RESULTS AND DISCUSSION

Features of the phenology and soils in the study areas

The conducted agrochemical analysis showed that the soil in plantations of the conditional control zones had slight alkaline reaction ($\text{pHKCl} = 7.1-7.2$), the average content of organic matter (5.3-5.8%), from high to very high content of mobile phosphorus (115.4-291.3 mg/kg) and from high to very high - exchangeable potassium (210-314 mg/kg). In the soils a high content of nitrate forms of nitrogen (210-405) and a low content of ammonium forms of nitrogen was observed (8.3-19.3 mg/kg) (Table 1).

In plantations of sanitary protection zones of industrial enterprises the soils were characterized by a slightly acid and slightly alkaline reaction ($\text{pHKCl} = 6.7-7.5$), organic matter content from medium to high (5.5-6.2%), the content of the nitrate forms of nitrogen at the level of 247-300 mg/kg, and ammonium forms of nitrogen at the level of 6.1-14.9 mg/kg. In main plantations the soils had: the exchange acidity of 7.4-7.7 ($\text{pHH}_2\text{O} = 8.4-8.6$), characterizing the slightly alkaline reaction of soils; low organic matter content (1.7-3.1%); low to medium content of ammonia nitrogen (6.4-8.1 mg/kg) and available phosphorus ($\text{P}_2\text{O}_5 = 29.8-53.5$ mg/kg); the average content of nitrate nitrogen (164-175); and high to very high content of exchangeable potassium ($\text{K}_2\text{O} = 210-325$ mg/kg).

The earliest vegetation in the CCZ noted in 2012 - at the end of the second and the beginning of the third decade of April the appearance of the green cone of leaves (22.04) was observed; conversely, in 2014, the emergence of green leaves cone was pointed at the end of the third decade of April (26.04) (Table 2).

Throughout the study period, we

observed differences in the occurrence of certain phenophases in sanitary and protection zones (SPZ) of industrial enterprises and main plantations in comparison with the CCZ, as the green cone of leaves appeared 4-6 before in 2012; 3-6 days before in 2013, and 2-5 days before in 2014. Also, in the data environmental plantings the flowering phase occurred earlier than in the CCZ. Duration of flowering in the conditions of

the main plantation declined throughout the study period.

In all the studied sample trees growing in the conditions of intensive technogenic load, the earlier colorization of dying leaves as compared to the CCZ plants was observed. The longest vegetation was typical for the trees of Liteiniy plant, 187-190 days, for SPZ of Kuznechniy plant - 186-191 days, for the main

Table 1. Agrochemical characteristics of soils in the study area (Naberezhnye Chelny)

Study area	Indicators		content of available forms of compounds in mg/kg				organic matter, %
	pH _{KCl}	pH _{H₂O}	NH ₄ ⁺	NO ₃ ⁻	P ₂ O ₅	K ₂ O	
Conditional control zones							
Chelninsky forestry	7.2	7.8	8.3	405	115.4	210	5.3
Sanitary and protection zones of industrial enterprises							
Liteiniy Plant	7.5	8.5	6.1	300	400.0	267	6.2
Kuznechniy plant	6.7	7.3	14.9	247	326.0	245	5.5
In-main landings							
Road «Auto 1»	7.4	8.4	8.1	175	29.8	325	1.7
Mira Avenue	7.7	8.6	6.4	164	22.7	210	3.1

Table 2. The average dates of occurrence of phenological phases in drooping birch in (Naberezhnye Chelny, 2012 - 2014)

Functional zones		Phenological stage						
		Pb ²	C ²	C ³	Pl ³	L ³	L ⁴	Pb ² -L ⁴
2012								
CCZ*	Chelninsky forestry	22.04	28.04	04.05	30.05	13.09	17.10	179
SPZ ** of								
industrial enterprises	Liteiniy Plant	16.04	21.04	27.04	19.05	03.09	22.10	190
main landings	Kuznechniy plant	16.04	21.04	26.04	19.05	04.09	20.10	186
	Mira Ave.	18.04	24.04	29.04	21.05	04.09	25.10	191
	Auto 1	17.04	22.04	27.04	19.05	02.09	24.10	191
2013								
CCZ	Chelninsky forestry	24.04	30.04	08.05	31.05	18.09	26.10	186
SPZ of	Liteiniy Plant	21.04	29.04	04.05	29.05	14.09	27.10	190
industrial enterprises	Kuznechniy plant	20.04	27.04	03.05	27.05	11.09	27.10	191
Main landings	Mira Ave.	19.04	23.04	02.05	25.05	12.09	28.10	193
	Auto 1	18.04	23.04	29.04	23.05	11.09	25.10	191
2014								
CCZ	Chelninsky forestry	26.04	30.04	10.05	31.05	18.09	27.10	185
SPZ of	Liteiniy Plant	24.04	29.04	07.05	29.05	14.09	27.10	187
industrial enterprises	Kuznechniy plant	24.04	27.04	06.05	27.05	11.09	27.10	187
Main landings	Mira Ave.	22.04	25.04	05.05	27.05	12.09	28.10	190
	Auto 1	21.04	25.04	04.04	27.05	11.09	26.10	189

* CCZ – conditional control zone

** SPZ – sanitary and protection zone

plantings of Auto1 and Prospect Mira - 189-191 days, and the shortest growing season in the species was observed in the area of conditional supervision. The increase in the growing season duration was due to the large number of artificial light sources and a higher air temperature. A special feature is the increase in the growing season in species under anthropogenic stress of buffer zones, in-main plantations, as compared with CCA (conditional control area).

In 2012, the weather conditions of the growing season of woody plants showed increased air temperature, the excess of mean annual data within +3 ... +7% C and precipitation

at the normal level. In 2013 and 2014 more arid conditions were observed as compared to 2012, the increase in the temperature above the long-term average annual data was +7 ... +11% C, and the precipitation was below normal.

Analysis of the content of low molecular weight compounds in plants

In 2012 the dispersive multivariate analysis of the results of research in 2012 revealed a significant effect of the complex conditions of the locus (significance level = 8.37×10^{-5}), period of vegetation ($P = 1.16 \times 10^{-3}$), as well as the interaction of these factors ($<10^{-5}$) on the content of tannins in the leaves of birch (Table 3).

Table 3. Dynamics of tannins content in the leaves of birch growing in plantations of different environmental categories, Naberezhnye Chelny, mg/g of dry substance

Year	Plantings of different categories								
	of conditional control zones			of sanitary and protection zones of industrial enterprises			of mains		
	June	July	August	June	July	August	June	July	August
2012 ($HCP_{05} = 0.02$)	5.85	8.25	11.89	5.25	8.92	11.87	5.26	8.57	11.88
2013 ($HCP_{05} = 0.03$)	4.21	7.63	10.22	3.93	8.26	9.78	3.81	6.83	8.34
2014 ($HCP_{05} = 0.03$)	4.03	7.17	9.81	3.34	7.83	9.42	3.38	6.44	8.00

Table 4. The dynamics of the content of ascorbic acid in the leaves of birch growing in plantations of different environmental categories of Naberezhnye Chelny, mg% (2012 - 2014)

Year	Plantings of different categories								
	of conditional control zones			of sanitary and protection zones of industrial enterprises			of mains		
	June	July	August	June	July	August	June	July	August
2012 ($HCP_{05} = 2.4$)	372.8	222.5	188.6	457.6	218.6	256.2	142.8	118.8	78.6
2013 ($HCP_{05} = 1.7$)	382.5	179.1	220.9	492.9	383.1	153.3	507.6	278.1	141.4
2014 ($HCP_{05} = 3.4$)	349.2	216.2	172.3	346.6	241.0	146.1	347.4	227.6	131.1

Table 5. Dynamics of peroxidase activity in leaves of birch growing in plantations of different environmental categories, Naberezhnye Chelny, units. act

Year	Plantings of different categories								
	of conditional control zones			of sanitary and protection zones of industrial enterprises			of mains		
	June	July	August	June	July	August	June	July	August
2012 ($r_{05}^! = 0,03$)	1.36	2.00	2.21	1.75	2.94	1.97	1.55	3.17	1.65
2013 ($r_{05}^! = 0,02$)	1.27	2.45	1.64	1.52	2.87	3.32	1.86	3.66	2.65
2014 ($r_{05}^! = 0,01$)	1.11	1.64	1.45	1.41	1.67	1.62	1.48	1.64	1.88

The results of research in 2012 showed that all the plants and in all types of plantings the tannins content in the leaves increases during the growing season, reaching the highest values in August. The drooping birch growing in the CCZ was characterized by the greatest values of tannins (11.87-11.89 mg /g of dry substance).

This can be explained either by a reduction in the synthesis of this metabolite, or its attrition rate in the protective reactions of plants, because according to long-term data, these months differ by the highest levels of air pollution in the city.

In main plantations and in sanitary zone plantings the plants had a lower tannin content as compared to CCZ. In July the results were different from those of June. In the leaves of plants in main plantings the tannin content was significantly higher as compared to the CCZ. The species of drooping birch in this observation period in different types of plantations had no significant differences with the control by the content of tannins.

In 2013-2014 in SPZ plantations of industrial enterprises the nature of changes in the content of tannins of the drooping birch was similar to the growing season of 2012. In main plantations in conditions of intensive technogenic influence during more complex years due to meteorological conditions a significant reduction of tannins content in leaves as compared with control plants was observed during the whole observation period.

Thus, we can conclude that the plant response to the growing conditions depended not only on the degree of anthropogenic impact, but also on the prevailing weather conditions during the growing season.

The dispersive multivariate analysis of research results showed that a set of conditions of the locus ($<10^{-5}$), vegetation phase ($<10^{-5}$) as well as the interaction of these factors ($<10^{-5}$) had a significant impact on the content of ascorbic acid in leaves of birch. (Table 4).

In all plants, regardless of the growth conditions, the amount of the metabolite was reduced with varying degrees of intensity throughout the growing season, indicating a decrease in the activity of redox processes in plants.

In the drooping birch growing in the conditions of anthropogenic stress, the ascorbic acid content increased as compared with the control in the early stages of the active growing season (June - July): 84.8; 110.4 – 125.1 and 11.4 – 24.8 mg%, then its level decreased in August: by 110; 67.6 – 79.5; 26.2 – 41.2 mg % , respectively in 2012, 2013 and 2014.

The increased anthropogenic impact leads to an increase in the ascorbic acid content in the leaves of birch in SPZ plantings of industrial enterprises and in the main plantations in the early stages of the active growing season, except for 2012, where the ascorbic acid content was less than the control options at the early stages of the growing season. Perhaps this is due to more arid microclimatic conditions of the study area. In our view, this indicates the presence of other physiological processes, offsetting the negative impact of man-made load in urbanistic area.

Features of peroxidase activity in plants

The dispersive multivariate analysis of the research results showed that peroxidase activity in leaves of woody plants was under a considerable influence of the complex of growth conditions ($<10^{-5}$), the growing season ($<10^{-5}$), and the interaction of these factors ($<10^{-5}$) (Table. 5).

The results of biochemical studies for the three years showed that the drooping birch had a maximum level of peroxidase activity in July, regardless of the zone of growth. In technological terms the peroxidase activity in leaves increased over the entire period of active vegetation: in June 0.31 - 0.38 units. act; in July, 0.46 - 0.79; and in August 0.37 - 0.13, respectively, in the sanitary zones and main plantations, as compared to CCZ with NSR05 = 0.05 units. act. The fact of an increase in peroxidase activity in the leaves of birch in the man-made environment shows the sensitivity of the species to various air pool pollutants. Presumably, the most part of the toxic substance includes acidic gases form peroxide that form peroxide in the light, and they in their turn caused the activation of peroxidase substrate. Thus, it can be assumed that the level of air pollution within the main plantations is higher than in sanitary protection zones, since the degree of activation of the enzyme in the leaves of birch is higher. Moreover, the maximum level of pollution is in July.

CONCLUSION

The longest vegetation is characteristic for trees of SPZ plants, Lineiniy and Kuznichniy for in-main plants Auto 1 and Mira Avenue. The content of tannins in the leaves of birch increases during the study period, and reaches the maximum value in August, and the content of ascorbic acid is reduced by the end of the growing season, it shows the various functions in the metabolism of plants. In birch the condensed tannins and ascorbic acid are active participants in the processes of adaptation to the conditions of the built environment. Peroxidase activity in leaves of birch shall increase during the period of active vegetation in technological terms, indicating an important function of this metabolite to ensure adaptation to hostile environmental conditions. The drooping birch has a high degree of adaptive capacity in terms of man-made stress, so it can be offered for wider use in the creation of green shields of large industrial cities. In addition it can be used for bioindicative research for the purpose of environmental quality monitoring. As scientists and researchers, we are concerned about environmental degradation in the large Kama region of Tatarstan, the Russian Federation due to the rapid development of the free economic zone Alabuga, petrochemical production in Nizhnekamsk, chemical plants and refineries Mendeleevsk KAMAZ in Naberezhnye Chelny. Our next task is to examine other native and introduced tree species in order to develop local plans to create a multi-species phytocenoses that will ensure the quality performance of environmental functions.

REFERENCES

1. Bulygin, N.E., & Yarmishko, V.T., *Dendrology* "oscow: MSFU. 2001; 18-26.
2. Balandaykin, M.E., Correlating the content of ascorbic acid in the assimilation apparatus *Betula pendula* Roth. With the influence of the pathological agent. *Chemistry of the Plant Raw Materials*, 2014; **1**: 153-157.
3. Bukharina, I.L., Sharifullina, A.M., & Kuzmin, P.A., An analysis of the dynamics of the content of low molecular and high molecular compounds in the leaves of woody plants in urban medium. *News of the Samara Scientific Center of the Russian Academy of Sciences*, 2013; **3**(4): 1236-1240.
4. Bukharina, I.L., Sharifullina, A.M., & Kuzmin, P.A., The content of low-molecular organic compounds in the leaves of trees in the technogenic loads. *Forest Science*, 2014; **2**: 20-26.
5. State report "On the condition of Natural Resources and Environmental Protection of the Republic of Tatarstan in 2013" (2014, pp. 429). Kazan.
6. Goryshina, T.K., *The plant in the city* . Leningrad: LSU, 1991; 152.
7. Viktorov, D.P., *Workshop on Plant Physiology* Voronezh: Ed. of Voronezh State University, 1991; 60.
8. Nikolaevskiy, V.S., *Environmental assessment of pollution and condition of ecosystems using phytoindication methods* "oscow: MSFU, 1999; 193.
9. Rogozhin, V.V., *Peroxidase as a component of the antioxidant systems of living organisms*. St Petersburg: GIOR, 2004; 240.
10. *The phenological observations of trees and shrubs: Guidelines for dendrology*. "oscow: MLTI Publishing house, 1990; 17.
11. Cheeseman, J.M., Hydrogen Peroxide and Plant Stress: A Challenging Relationship. *Plant Stress. Global Scientific Books*, 2007; **3**: 4-15.
12. John, R., Ahmad, P., Gadgil, K., & Sharma, S., Effect of cadmium and lead on growth, biochemical parameters and uptake in *Lemna polyrrhiza* L. *Plant Soul Environ*, 2008; **54**(6): 262-270.
13. Galves-Valdivieso, & Mullineaux, P.M.G., The role of reactive oxygen species in signalling from chloroplasts to the nucleus. *Physiology of Plant*, 2010; **138**: 430-439.
14. Gill, S.S., & Tuteja, N., Reactive oxygen species and antioxidant machinery in Abiotic stress tolerance in crop plants. *Plant Physiological Biochemistry*, 2010; **48**: 909-930.
15. Foyer, C.H., & Noctor, G., Redox homeostasis and antioxidant signaling: a metabolic interface between stress perception and physiological responses. *Plant Cells*, 2005; **17**(7):1866-1875.
16. Horemans, N., Raeymaekers, T., Beek, K.V., Nowocin, A., Blust, R., Broos, K., Cuypers, A., Vangronsveld, J., & Guisez, Y., Dehydroascorbate uptake is impaired in the early response of *Arabidopsis* plant cell cultures to cadmium. *J. Exp. Bot.*, 2007; **58**: 4307-4017.
17. Delia-Gabriela Dumbrav0, Camelia Moldovan, Diana-Nicoleta Raba, & Mirela-Viorica Popa., Vitamin C, chlorophylls, carotenoids and xanthophylls content in some basil (*Ocimum*

- basilicum* L.) and rosemary (*Rosmarinus officinalis* L.) leaves extracts. *Journal of Agroalimentary Processes and Technologies*, 2012; **18**(3): 253-258.
18. Mishra, S., Srivastava, S., Tripathi, R.D., Kumar, R., Seth, C.S., & Gupta, D.K., Lead detoxification by coontail (*Ceratophyllum demersum* L.) involves induction of phytochelatins and antioxidant system in response to its accumulation. *Chemosphere*, 2006; **65**: 1027-1039.
 19. Schofield, J.A., Hagerman, A.E., & Harold, A., Loss of tannins and other phenolics from willow leaf litter. *Journal of Chemical Ecology*, 1998; **24**: 1409-1421.
 20. Wyrwicka, A., & Sklodowska, M., Influence of repeated acid rain treatment on antioxidative enzyme activities and on lipid peroxidation in cucumber leaves. *Environmental and Experimental Botany*, 2006; **56**: 198-204.