INVENTORIES OF CAUSES OF LEAF SCORCH OF LINDEN TREES UNDER ANTHROPOGENIC CONDITIONS IN KYIV

Oleksandr V. Sovakov[1], Maria O. Sovakova[1], Oleksandr S. Ostapchuk[2], Dmytro I. Bidolakh[3*], Svitlana M. Pidkhovna[3], and Oleg I. Kytaiev[4]

[1]Forest Restoration and Meliorations Department, National University of Life and Environmental Sciences of Ukraine, 19 Heneral Rodimtsev Str., Kyiv, Ukraine. E-mail: sovakov@nubip.edu.ua
[2]Forestry Department, Uman National University of Horticulture, 1 Institutska Str., Uman, Cherkasy region, Ukraine. E-mail: ostapchyk1958@gmail.com
[3]Department of Forestry and Landscape-park Management, Separated Subdivision of National University of Life and Environmental Sciences of Ukraine, 20 Academichna Str., Berezhany, Ternopil region, Ukraine. [*]E-mail: dimbid@ukr.net
[4]Institute of Horticulture of the National Academy of Agrarian Sciences of Ukraine, 23 Sadova Str., Kyiv-27, Ukraine. E-mail: o_kitaev@i.ua

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Abstract

Linden species (*Tilia* spp.) are the most spread woody species in the street side plantings in Kyiv. It is determined that all linden species that grow along streets or sidewalks are in a poor state. The visual signs of scorch varied from severe to minimal damages (leaves collected in different parts of city). The control samples were collected in the conditions with minimal level of anthropogenic pressure (stands in the city outskirts). These were healthy leaves, without any visual signs of biotic or abiotic damages. In the leaves with maximal level of necrosis and chlorosis damages (to 90 % of leaf blade area) we found very high level of sodium ions and minimal level of potassium ions. Consequently, there is ionic imbalance in plant tissues – potassium ions are substituted for sodium ions. These chemical elements are monovalent and in the process of nutrient uptake the plant does not differentiate them. Accumulation of Na^+ ions and decrease of K^+ ions is typical to all the linden species growing along streets. Little-leaf linden is the most susceptible to salt contamination; however, it is an aboriginal species in Kyiv city conditions. Absence of biotic origin of necrosis and chlorosis on linden leaves is confirmed by luminescent analysis of photosynthetic apparatus – photo- and thermoinduction of chlorophyll fluorescence. Consequently, the results of conducted research allow to state that lindens are not suitable for growing in salt contaminated soils. In anthropogenically transformed (urban) conditions it is reasonable to select salt-tolerant wood species.

Key words: chlorosis, fluorescence, necrosis, photosynthesis, salt, urban environment.

Introduction

Planting in cities has a number of obvious advantages. Positive influence on the health of city residents is considered to be the main one. Species of linden tree genus (*Tilia*) have become common in urban landscaping in a lot of big cities within their natural area. Linden trees are widely spread in landscapes of large cities in Europe and the USA (Borowski and Pstrągowska 2010, Stravinskienė et al. 2015). Ukraine is of no exception and its capital city of Kyiv in particular (Nebesnyi and Grodzynska 2015, Karpyn and Zayika 2017).

Urban plantations decrease the level of air pollution, absorb large amounts of carbonic oxide, serve as a protective shield from noise and dust, cool down the surrounding areas, provide shade and coziness in hot seasons, make urban landscapes aesthetically attractive (Tyrväinen et al. 2005, Willis and Petrokofsky 2017). On the other hand, the growth and the development of a plant body depends on various environmental factors, which interact with each other as well as with the plant itself. The abnormality of physiological processes in a plant can be caused by a large number of stress factors that have a negative influence. They include both natural and anthropogenic factors (Nebesnyi and Grodzynska 2015). The first ones include the extremums in the conditions that influence plant life, namely temperature, humidity, oxygen and light. Nutritional deficiency or excess of nutrients (water, oxygen and mineral substances) negatively affects plants as well. The anthropogenic factors include air pollutants and soil contaminants. Unsatisfactory state of plant bodies, which grow in complicated urban environments, caused by abiotic factors can be further enhanced by biotic ones (Larher 1978).

Urban environment is quite complicated for the growth and the development of woody plants. Those plants that grow along the streets and pavements, particularly on paved territories, face both atmospheric pollution and unsatisfactory soil conditions, namely soil contamination and soil deficiency. Under such conditions woody plants often suffer from browning and death (necrosis) and yellowing (chlorosis) of leaf edges. International researches refer such damage to abiotic diseases and call it marginal leaf scorch (Appleton et al. 2009). There are several causes of it. Dehydration of leaf tissues is one of them. In a hot season plants that grow on paved and concreted surfaces are affected by solar radiation. Building walls and roadway pavements slowly give up heat, it is especially intensive from a south and a western side. This process continues even after sunset. This effect is called ‘urban heat island’ by urban ecologists (Kucheriavyi 1999, Appleton et al. 2009). High temperatures, which have a disastrous effect on leaves and roots, cause enhanced vegetal discharge. In order to decrease the temperature of leaf blades, a plant begins to cool down, that is to say, to transpire water through its stomas. It is known that one big tree can transpire 400 L of water into the environment. However, the same amount of water is not always available for a plant. It can cause the dieback of leaf blade edges, that is to say, marginal necrosis and chlorosis (Appleton et al. 2009). The above mentioned indicates the expediency of conducting a series of additional laboratory tests in order to prove the anthropogenic nature of linden leaf necrosis and chlorosis.

Accumulation of toxic elements is another reason for leaf blade damage. Under the conditions of heavy technogenic pollution in cities, street plants accumulate harmful phytotoxic elements in excess concentrations, which disturb cation-anion balance and the processes of mineral nutrition, suppress the activity of enzyme and hormonal systems, cause abnormalities in the permeability of cell membranes and inhibit photosynthetic processes

(Kondratyuk et al. 1980). Salt is one of such pollutants. In order to deal with icy roads and snow, sodium chloride and calcium dichloride are used (Douglas 2011). Although $CaCl_2$ is less harmful than NaCl, last is most commonly used both abroad and in Ukraine. There are two main ways of damaging woody plants by salt: transfer of salt grains from the air into plant tissues or saline spray and accumulation of ions rhizosphere area of soil (Douglas 2011). In the first case, salty water immediately contacts the leaves or the needles, salt penetrates into plant cells and intercellular area, and influences the vitality of buds and shoots. In a lot of cities in the USA, coniferous plantations along highways suffer from saline spray. It becomes evident as needle browning and scorch. Damage by salt can be usually seen from the road side and at 9–15 m from it. In the second case, the accumulated salt is dissolved in the soil and is broken down into Na^+ and Cl^- which influence both soil and plant. They change the structure chemically and, to a certain extent, physically, since sodium causes the adhesion of soil particles. The ions of Na^+ and Cl^-, which are dissolved in water, are easily absorbed by roots and are transported to plant active growth parts, namely to leaf blade edges and root tips, where they are accumulated in toxic concentrations (Delahaut and Hasselkus 1996, Craul 2010). This process results in ionic imbalance – sodium blocks the enrichment of a plant by necessary macro- and microelements, namely cations K^+, Ca^{2+} and Mg^{2+}. The displacement of potassium ions by sodium ions results in the so called potassium deficiency in a plant, the typical symptoms of which are necrosis and chlorosis of leaf blade edges and interveinal chlorosis (Douglas 2011). In addition, soil salinization results in the change of osmotic potential, which is caused by high concentrations of interstitial water and low water potential. There is a decrease in the amount of water, which is available for roots, therefore even the soil that is moist enough becomes dehydrated under the action of large amounts of salt (Slabu et al. 2009). It is obvious that salt affects plants at various times, but its use at the end of winter and the beginning of spring is more harmful, since there is less time left for washing out due to rainfall and snow melting compared to the use of salt at the beginning of winter.

Some researchers (Sinclair and Lyon 2005) think that browning of linden leaf edges can be microbially-derived. Having assumed that the possible cause of marginal necrosis and chlorosis of linden trees is bacterial affection, the detailed analytical survey of this problem has been considered as well. Thus, the bacterium called *Erwinia amylovora* (Burrill) Winslow et al., which causes bacterial blight, damages fruit trees only. This is a rather major illness that severely affects species of Rose family (Rosaceae; Juss.). Another bacterium called *Xylella fastidiosa* Wells et al. has been found on the leaves of ornamental woody plants in the southern and the eastern states of the USA (Gould and Lashomb 2007). It typically affects various species of oaks, elm trees and planes. Such leaf damage closely resembles the one that can be observed in the urban landscapes of Kyiv, namely brown edges and a compulsory yellow line between the brown died off part of a leaf and its green part. However, American scientists mentioned that *X. fastidiosa* has not been diagnosed on linden trees yet (Hartman et al. 1991).

The aim of the conducted research was to analyze the state of various linden tree species leaf apparatus and to determine possible reasons for its worsening.

Material and Methods

The paper presents the materials of the original investigation of photosynthetic apparatus of various species of *Tilia* genus, which was conducted in the plantations of Kyiv in 2010–2016. The subject of the research were model trees aged 10–40 of 4 species and 1 hybrid linden tree, namely *T. cordata* Mill, *T. platyphyllos* Scop, *T. begoniifolia* Stev., *T. tomentosa* Moench., and *T.* × *europaea* L. in three ecological-phytocoenotic zones or eco clines, according to Kucheriavyi classification (Kucheriavyi 1999) that represents the reduction of negative urban factors: I – suburban forests, II – garden squares and III – street plantations. The age of plants was determined according to the inventory data provided by a public utility company that manages community landscapes called Kyivzelenbud. The location of the model trees was plotted on a map. The plants, which grow in the landscapes where there is minimum anthropogenic impact, that is to say, in the plantations in the suburbs of the city (Fig. 1), served as control samples. The leaves were chosen from the southern-western side of a tree crown after a leaf blade finished its growth and after the end of a blossoming period of the trees aged 10–40-year-old.

The diagnostics of trees' functional state by the level of dechromatization or

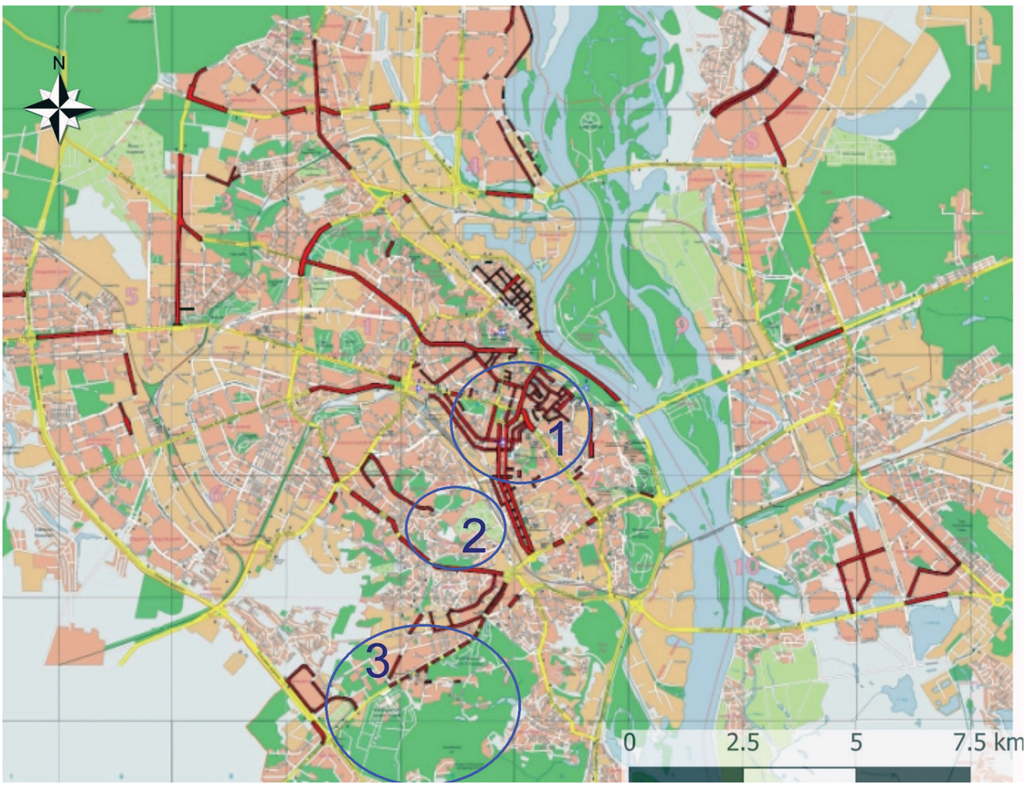

Fig. 1. Research places in Kyiv.

Note: Inspected street lining plants in Kyiv (marked in red); places of sampling (marked in blue): 1 – streets, 2 – parks, 3 – outside the city.

the change in colour included both field and laboratory investigations. During field studies were used: observation, analysis, synthesis, systemic approach scientific methods. The length of the streets according to the map on the Figure 1 is 80 km. The general information about observed trees is indicated in the Table 1.

Table 1. General characteristics of the observed linden plantings alongside Kyiv streets.

Length of observed streets, km	Average distance between trees, m	Number of trees	Number of trees per sample plot	Number of trees by species, %	Status / condition of them	Trees per km
80	4	20000	From 10 to 300	*T. cordata* – 40; *T.* × *europaea* – 58; *T. begoniifolia*, *T. platyphyllos*, *T. tomentosa* – 2	From 5 (wee tree) to 1 (dead tree)	250

Laboratory methods included analysis of macroelement content and biophysical or spectral research. Laboratory investigations were conducted with the equipment of the Laboratory of Plant Physiology and Microbiology of the Institute of Horticulture of the National Academy of Agrarian Sciences of Ukraine.

Since a definite indicator of the negative effect of the anthropogenic environment in plant bodies is a leaf blade, it has been decided to take leaves of woody plants as test objects. Various species of linden trees, which are the most common in the street plantations in Kyiv, have been chosen. In order to diagnose the cause of leaf tissue damage and dieback, it was necessary to verify the hypothesis about their abiotic nature. As opposed to the diseases caused by biotic factors, abiotic diseases cannot be passed from tree to tree, that is to say, they are noninfectious diseases. It is rather difficult to diagnose them, since there are various reasons for giving rise to the disease or the damage with similar manifestation markers.

The analysis of macroelements' content (Na^+ and K^+) in linden tree leaves was conducted according to modified Dekster procedure (Kirillov et al. 1981). The number of the free ions transferred into the solution, was determined using spectral micro fluorometer SMF-2. The functional state of photosynthetic apparatus was diagnosed with photo- and thermoinduction of chlorophyll fluorescence with the use of a luminescent image, according to modified procedure (Makarova and Kytaiev 2008). The measurement was conducted using laboratory scale spectral micro fluorometer SMF-2 (a luminescent microscope connected to a monochromator) in duplicate, according to the modified procedure (Makarova and Kytaiev 2008). The device made it possible to excite and collect fluorescent spectra at certain parts of a leaf surface, the individual changes in fluorescence, which are the results of light or heat effect. The emission of fluorescence was registered in 500–800 nm region of the spectrum. Luminous induction of chlorophyll fluorescence was measured at the length of waves being 680 and 740 nm with time interval from 0.5–1.0 s to 300 s. The changes in fluorescence induced by temperature were registered at the stationary level of luminous fluorescence induction when the leaves were heated from 20 to 80 ºC at a speed from 8–10 to 20–30 ºC per minute.

Luminous induction and thermoinduction of chlorophyll fluorescence of a leaf blade was excited in a continuous mode by a blue-violet light (λ_{max} = 435.8 nm). Exciting light of various intensity was used in the research (from 5 to 300–600 W/m^2). Thus, it was possible to reach the saturation of the light stages of photosynthetic process, which were responsible for producing luminous- and thermoinduction changes in chlorophyll fluorescence. Prior to the analysis, the leaves had been kept in darkness (darkness adaptation) during 30 min. Here, the experiment was replicated 10 times for every species under each condition (street, park and outside the city).

According to the results of the conducted investigation (Weinert et al. 1988), linden trees are intolerant to salinization and, in its turn, salt accumulation is the cause of marginal leaf injuries, which visually resemble similar problems with water supply or root damage. On the other hand, the above mentioned data could be outdated, thus, we set the task to verify the data.

In addition, we studied linden plantations under the condition of being loaded by the influence of urban environment and not in their natural ecosystem, in contrast to our predecessors. As for the other scientists (Borowski and Pstrągowska 2010, Douglas 2011), although they conducted their research under the condition of urban environment (Warsaw, Poland), they studied only 1 species of linden trees (*T. cordata*). Hence, the problem of salt accumulation and the state of other linden tree species that grow in urban environment remains uninvestigated. Thus, we aimed at investigating these processes through the example of 5 linden tree species, which are represented in Kyiv street plantations.

Results

The data of our survey in 2010–2016 has been verified the same results as the latest inventory in 1999, conducted in Kyiv street plantations, that linden trees are the most common among the woody plants that grow there. Having conducted the inventory of 300 streets (1/5 of their overall number in Kyiv) with the average number of trees 250 per kilometer. It has been determined that there are 4 species and 1 hybrid of linden trees that are common in street plantations – *T. cordata*, *T. platyphyllos*, *T. begoniifolia*, *T. tomentosa* and *T.* × *europaea*. *T. cordata* and *T.* × *europaea* prevail in terms of quantity and take up to 90 % of all the surveyed plantations. Most of the main streets are landscaped with trees planted in lines on paved territories, where every single tree is designed by a tree grill that is several square meters in area. Woody plants that are planted according to this principle suffer from insufficient watering and lack of micro- and macroelements. The soil near a tree trunk is very consolidated, especially if close to public transport stops, small architectural forms, etc. This inhibits plant breathing and moisture supply to the roots. Urban soil, which consists of sand, construction rubbish, etc. does not meet the needs of proper amount of nutrients. Besides, during tree planting, new fertile soil very often is not provided in planting holes, instead, a died out tree is substituted and nothing more. As a result, a tree can just stay in a hole without rooting, 30–40 % of newly planted linden plantings were this way. Another critical conclusion concerning the state of linden trees in street plantations is that fungal fruits of *Schizophyllum commune* Fr. could be seen on young trees everywhere. It is known that in the wild they are found only on decaying trees. Thus, the trees, which had been planted along the streets only several years ago, died out and should be replaced by the new ones.

Street plantations survey, which was conducted during three vegetative seasons, demonstrated the fact that leaf scorch causes serious damage to linden tree plantations. According to the state of a leaf blade, it can be said that marginal necrosis and chlorosis damages all the species. Linden trees that grow along the main streets are in the worst state. In some cases, necrosis and chlorosis covers up to 90 % of a leaf surface. The damage can be divided into interveinal chlorosis and interveinal necrosis, marginal chlorosis and marginal necrosis (Fig. 2). The browning of leaves begins in May just after their unfolding. The tree part, which is near a roadway or a pavement, is the most damaged, the same is stated by international researches as well (Delahaut and Hasselkus 1996, Craul 2010, Douglas 2011). The extension of marginal necrosis reaches its maximum in midsummer. Damaged leaves do not recover and remain during the whole vegetative season, which is curtailed in this case – the trees are characterized by premature defoliation. As a result, even at the end of the summer it is possible to find trees with completely fallen off leaves.

Our research shows, that all the species, the leaf blades of which had visual signs of necrosis and chlorosis damage (50–90 % of a leaf area), contained phytotoxic sodium in the amount from 1.0 % (*T. begoniifolia*) to 3.6 % (*T. cordata*) (Fig. 3). In control trees without any signs ofleaf damage those indices reached 0.01–0.02 %. The highest level of Na^+ in the leaves was found in June. Obvious monthly reduction in the content of sodium ions in linden tree leaves was observed.

So, the large damage of linden leaves by necrosis and chlorosis occurs in the middle or at the end of the summer and the initial disease signs, which are evident at the beginning of the vegetation period, are intensified. In midsummer this process is expanded by insufficient moisture

a b

Fig. 2. Condition of *T. cordata* leaf blades in the central streets: a) marginal necrosis and interveinal chlorosis in midsummer; b) marginal and interveinal necrosis and interveinal chlorosis in the beginning of autumn.

and high temperatures of soil and air.

It has been determined that all the species of linden trees with damaged leaves are characterized by displacement of potassium ions by sodium ones (Fig. 4).

The lowest potassium content in the

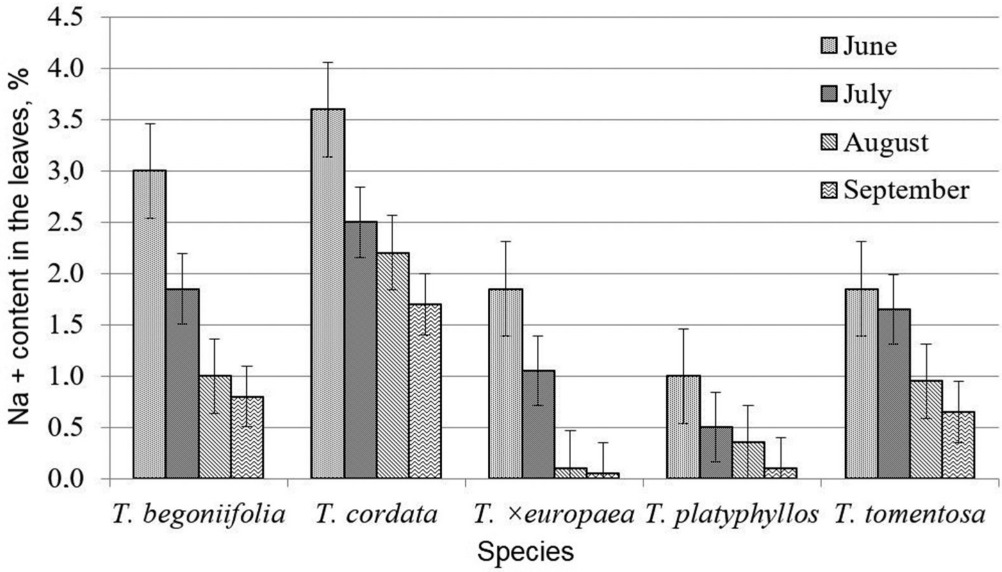

Fig. 3. Seasonal dynamics of sodium content.

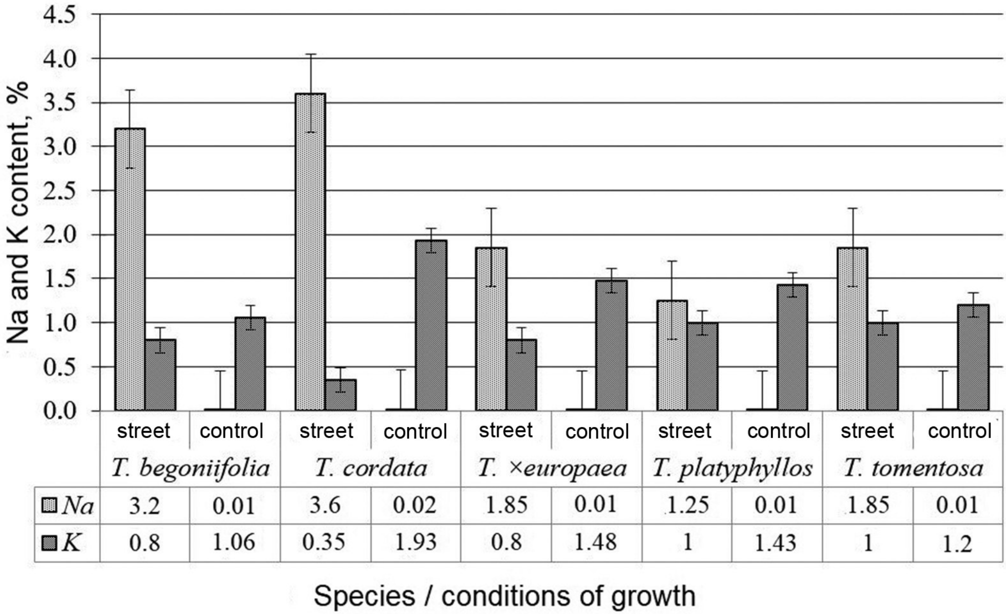

Fig. 4. Ionic imbalance in leaves of lindens from different ecological phytocoenotic belts.

leaves of *T. cordata* was found when the level of sodium was the maximum compared to other species. It is to be noted that young trees aged 10–40 prevail in street plantations and they are highly sensitive to salinization, since salt resistance improves with aging (Udovenko 1977). The 'potassium deficiency' in plants can be visually identified by necrotization of leaf edges. The small-leaved linden tree (*T. cordata*), namely its leaves as the center of variability and flexibility of a plant body, can be used as a test-object for indication of saline environmental pollution.

By means of fluorescence photoinduction, which is widely used in ecological monitoring for the assessment of the toxic influence of anthropogenic pollutants on plant test-organisms, the disruption of photosynthetic reactions has been recorded in the damaged plants of all the objects under study. The shape of the induction curve depends on the state of the photosynthetic apparatus, which is greatly affected by environmental factors (Krajicek and Vrbova 1994, Makarova and Kytaiev 2008). For example, Figure 5 presents the obtained data concerning the most widespread linden tree species – small-leaved.

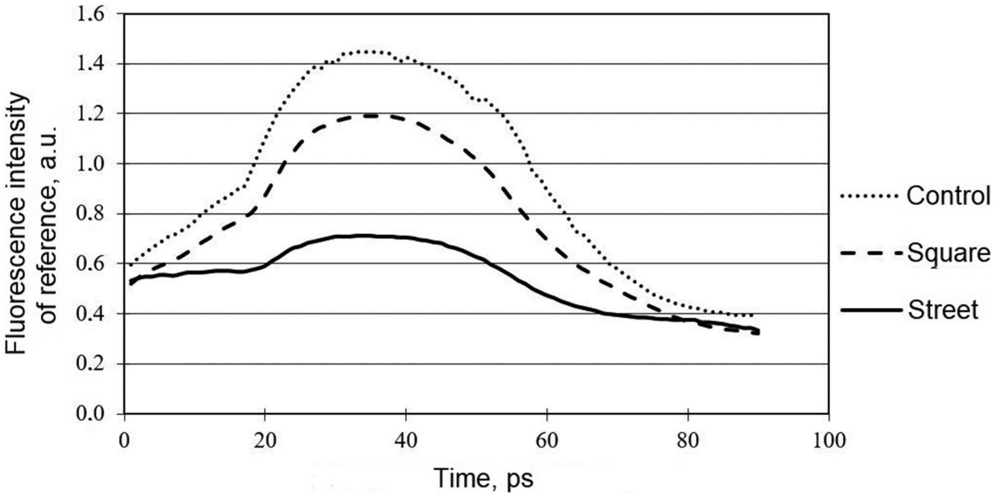

Fig. 5. Changes in fluorescence intensity of *T. cordata* leaves.

Comparative analysis of the functioning of leaf chloroplasts has been conducted and the results have been compared to the ones obtained under control conditions.

It is to the point to note that a linden tree forms its root system gradually and older trees have roots in deeper soil layers that are less saline. Young trees with roots in a shallow soil layer risk to receive larger amounts of sodium. Newly planted trees are at greater risk, which can be traced in rapid leaf blade decay and can be evident in their dechromatization and premature defoliation.

Discussion

It has been determined that under street conditions linden plants show greater changes in photosynthetic processes, which are more apparent is case of a dark photosynthesis phase and are caused by negative urban environmental factors (Fig. 5). The obtained data are prove the investigations of other scientists (Oleksiichenko et al. 2009, Hallik et al. 2012). It is worth mentioning that, according to indices of the rapid changes in chlorophyll fluorescence at the so called plateau level, virus infection has not been found in the experimental samples.

By means of fluorescence thermoinduction it has been determined that dark photochemical processes are the most intolerant of salt contamination. It has become evident in the increase of the stationary level of chlorophyll fluorescence induction as a result of the decrease in ribulose bisphosphate carboxylase activity and general attractive capacity of a plant body due to salinity stress. With the help of a microspectral analysis, the increase of yellow-green leaf fluorescence (λ_{max} = 530 nm) has been determined to be several times higher even in the green parts of damaged leaves compared to the healthy leaves. It is necessary to note that in yellow leaf parts fluorescence intensity increases by ten folds at the wave length being λ_{max} = 530 nm. The latter is caused by the accumulation of oxidized lipids in cell membranes and is followed by the increase of their permeability, which is also controlled by the changes in the conductance of plant cells. According to instrumental control data, it has been determined that salinity stress causes catabolic changes in plant cells, which are permanent and are followed by premature plant defoliation.

Besides the determination of virus plant damage, microspectral express-methods make it possible to conduct bacterial damage testing. Thus, the determination of bacterial infection of the leaf samples with large leaf blade areas damaged by chlorosis and necrosis, as well as of dark green healthy leaves gathered in control environments has been conducted. Having analyzed the samples, no signs of bacterial damage has been found.

Conclusions

Our research demonstrated the fact that leaf scorch of linden trees causes serious damage to plantations under anthropogenic conditions along the streets of Kyiv. The results of inventory survey determined that the most of them are in an unsatisfactory state. That is to say, they are characterized by drying of tree branches, top drying, crown density loss, leaf damage in the form of marginal and interveinal necrosis and chlorosis and abnormal refrondescence. Constant man-made load breaks the physiological resistance of woody plants. The main factor of unsatisfactory state of various species of linden trees is saline soil contamination.

The accumulation of sodium ions in linden leaves causes the displacement of the elements that are necessary for a plant, namely, the displacement of potassium. Dark photochemical processes have proved to be the most sensitive to saline contamination, which is caused by the decrease in ribulose biphosphate carboxylase activity and general attractive capacity of a plant body due to salinity stress. It is to the point to choose drought- and salt-resistant species of wooden plants in order to create street plantations.

If linden trees are continued to be planted, we propose to use species such as *T. begoniifolia* or *T. caucasica* and *T. tomentosa* as more drought resistant. Express and informative biophysical methods, which include photo- and thermoinduction of chlorophyll fluorescence, can be used for ecological monitoring of environmental conditions.

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