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DYNAMICS OF SULPHUR CONTENT IN BIRCH LEAVES DURING VEGETATION PERIOD UNDER THE CONDITIONS OF AIR POLLUTION

SUMMARY. This article is devoted to the study of the sulphur content dynamics reflecting ontogenesis of a leaf during vegetation period. This figure, calculated per unit of surface of light and shadow leaves, fresh and dry mass and water weight, increased under the conditions of pollution during the whole vegetation period, and within control – only until the middle of the vegetation, remaining at this level until it is completed. Most significant increase of sulphur content in light and shadow leaves took place in the middle of vegetation period (in July), when environmental conditions are the best possible. The most significant increase of sulphur content at this time was on the site with average degree of pollution. On the site with high degree of pollution, this indicator was close to control. This can be explained by a more negative impact of sulfur dioxide on photosynthesis on the highly polluted site. This impact reduced the gas exchange rate of leaves with the environment and reduced the absorption of sulfur dioxide. Sulfur content per chlorophyll mass unit during vegetation period (except for its end) remained practically unchanged, both within control and under the conditions of pollution. At the end of the vegetation (September), this figure increased at all sites. The degree of the figure increase clearly reflected the degree of the site pollution. The indicator of sulfur content per chlorophyll mass unit reflected the degree of negative impact of sulfur dioxide, but not the level of its accumulation in the leaves. The quantity of sulfur accumulation in the leaves, with all methods of calculation of its content, during the whole vegetation period, corresponded to the degree of the birches site pollution.

KEY WORDS. Pollution, birch, leaves, sulfur, chlorophyll, water, dry mass.

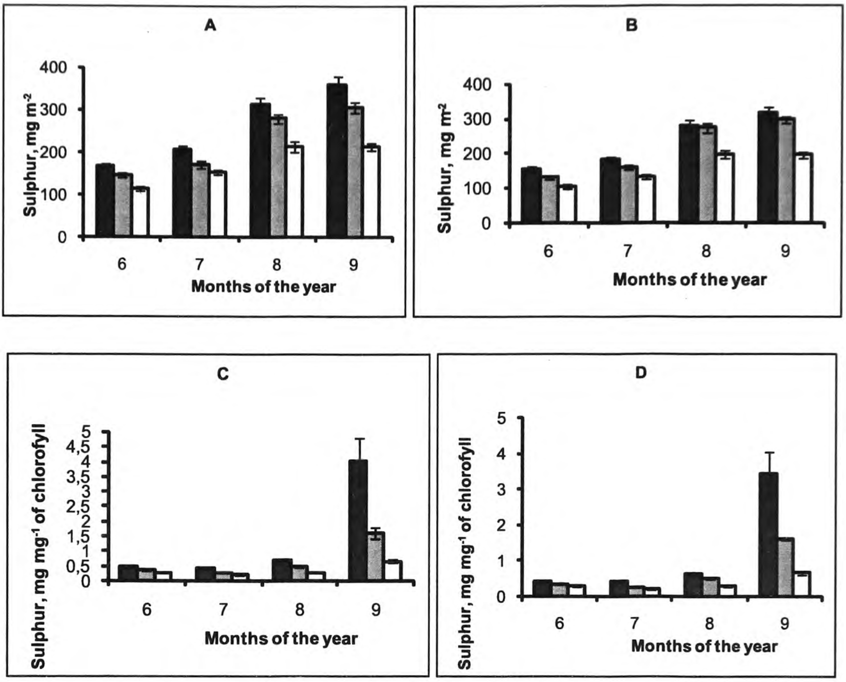
It is generally considered that the level of sulphur in leaves is highly fluctuating even in usual circumstances, that is why this figure serves as a indicator [1]. Other authors claim that the level of sulphur in leaves can be used as an indicator of sulphur dioxide consumption, but not as a criterion for estimation of negative consequences of this fact [2]. Sulphur dioxide consumption by leaves depends on the speed of photosynthesis which changes in leaf ontogeny [3]. The speed of photosynthesis increases from the phase of leaf expansion till the end of its formation and decreases after it. So it can be supposed that the dynamics of sulphur accumulation in leaves in polluted air conditions has the same characteristics as in the usual circumstances: it increases during the maturing of leaf and decreases by the end of a leaf's life. As sunleaves and shadeleaves differ in the speed of photosynthesis they can also differ in the level of sulphur accumulation in the polluted air conditions. The presented research is aimed at the study of sulphur level accumulation dynamics in the ontogeny of sunleaves and shadeleaves of birch trees growing in the conditions of air polluted by sulphur dioxide.

The level of polluting substances in plants is generally measured according to the units of dry weight. But the size of dry weight can due to some reasons vary [4], impacting the figure itself. Two leaves can contain the same amount of sulphur, but one of them can have the higher size of dry weight (e.g. due to amylum level), and because of that the level of sulphur per unit of dry weight in this leaf is lower. In order to have more objective image of sulphur level dynamics in leaves we used several different ways to calculate this figure.

Material and methods of research. For the research we used silver birch (Betula pendula Roth) growing at the distance of 1.5, 3 and 11 kilometers from the pollution source. Annual emission of this source is approximately 70 thousand tons of sulphur dioxide. 15 trees per site were chosen for this research. The site located 11 kilometers from the pollution source was supposed to be a control one, while the sites in 1.5 and 3 kilometers' proximity from the source were called test sites 1 and 2.

The leaves for analysis were taken from the low south west part of a tree crown facing the exposed place. The leaves were collected 4 times per vegetation period from June to September in the beginning of every month. Sunleaves were taken from the outside of a tree crown, and shadeleaves were taken from the inside. Each type of leaves was used for making excisions of prechosen size. Total chlorophyll level was estimated in each excision (a+b) according to Arnon [5]. The total level of sulphur in dry birch and pine leaves was estimated according to Y.I. Maslov [6]. Fresh and dry (after drying at 105°C) masses and total water level were estimated in each excision. The level of sulphur was estimated in *mg per* m^{-2} of a leaf space, *per* m^{-1} of dry and fresh mass, *per* mg^{-1} of chlorophyll and *per* g^{-1} of water. Arithmetical mean and its error were taken for each calculation. Statistical significance was estimated according to Student's test with $p < 0.05–0.001$.

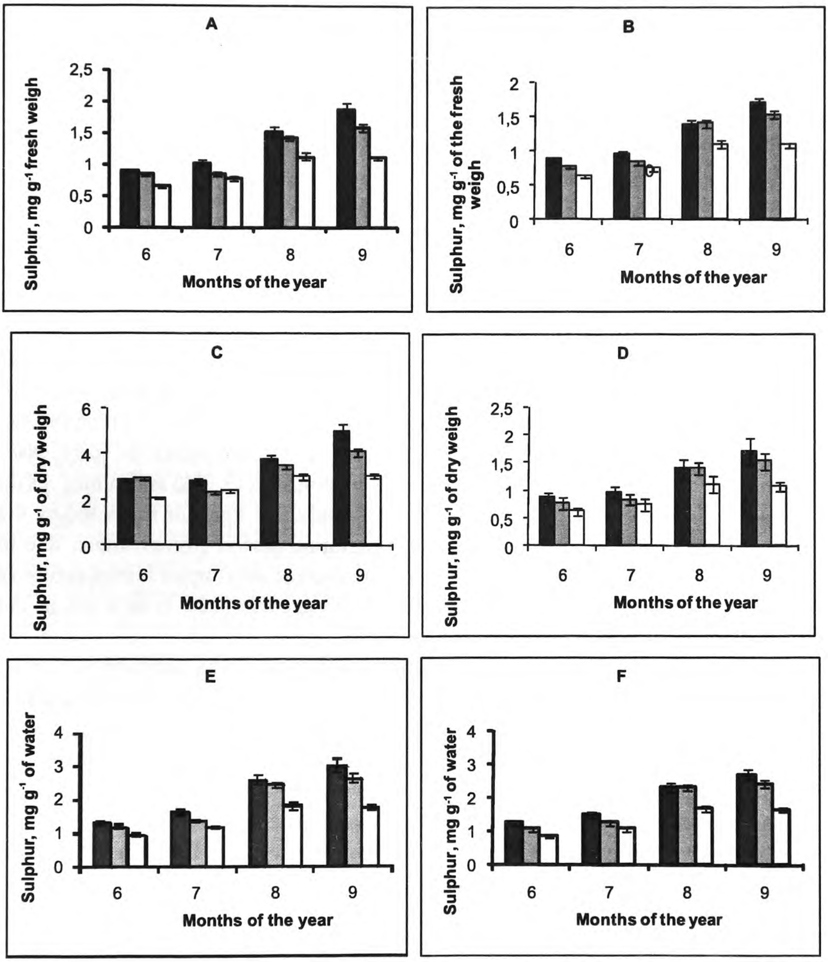
Results and measurement. The level of sulphur per unit of leaf surface increased from June to August in all three sites with both types of leaves (pictures. 1a, b). This increase is connected with the increase of dry mass per unit of leaf area, being connected with its growth. By the beginning of September the figure was increasing only on test sites. It was higher by 27-68% for sunleaves and by 22-63% for shadeleaves. The trend of the figure being higher (by 3-18%) for sunleaves was observed on all the sites. It is connected with the fact that dry weight of area unit of sunleaves is usually bigger than the one of shadeleaves [4]. It concerns birches too [7]. Dry weight per area unit of sunleaves on test sites was verifiably higher by 15-20% than of shadeleaves. This trend was observed on test sites (4-16%). It is connected with spareness of tree crowns on test sites, especially on the first one, increasing lighting of shadeleaves and decreasing the gap between shadeleaves and sunleaves.



Picture 1. Sulphur level per area unit (A, B) and per chlorophyll weight unit (C, D) in sunleaves (A, C) and shadeleaves (B, D) of a birch tree.

Notations. Test site1 (1.5 km from the pollution source) – black bars, test site 2 (3 km from the pollution source) – grey bars, and control site (11 km from the pollution site) – white bars

Sulphur level per weight unit of chlorophyll for June changed only slightly on all the sites (pictures 1c, d). It is connected with the increase of chlorophyll level during leaves growth in June. On the control site the level of sulphur per weight unit of chlorophyll during the ontogeny of leafs was changing varying in both leaf types from 0.2 to 0.3 *mg mg*$^{-1}$ with an increase in September up to 0.66 due to the decrease of chlorophyll level (the beginning of autumn leaf color) (pictures 1c, d). The increase of sulphur level per weight unit of chlorophyll on test sites was observed in the beginning of August, reaching the largest scale in the beginning of September. It is connected with the speeding of leaves aging of plants growing in the conditions of air polluted by high sulfurous gases [8]. Sulfur dioxide combustion leads to the decrease of chlorophyll level in leaves [9]. In our case the decrease of chlorophyll level on the test sites in the end of vegetation period reached the largest scale. It led to the dramatic increase of sulfur level per weight unit of chlorophyll on these sites (pictures c, d). This figure on test site n.2 was 2.4 times higher and on test site n1 it was 5-6 times higher than on control ones. Generally on the test sites this figure is verifiably higher by 30-509% for sunleaves and 21-417 for shadeleaves. On the control site and on site n.2 shadeleaves and sunleaves did not differ significantly according to this parameter. More polluted site n.1 showed the trend of higher rate of this figure (by 12-18%) for sunleaves as compared to shadeleaves. It should also be noted that this figure shows the measure of negative sulphur dioxide impact made on birch trees as compared to the level of sulphur accumulation in leaves.



Picture 2. Sulphur level per fresh (A, B) and dry (C, D) weights and per water weight (E, F) for sunleaves (A, C, E) and shadeleaves (B, D, F) of birch

Same notations as in Picture 1

The character of season dynamics of sulphur level per weight unit (fresh, dry or wet) was identical for both types of leaves (Picture 2). On all the sites these figures were increasing from July to August. But further from August to September on the control sites the levels were stable at August rate, while the test sites showed the increase in sulphur levels. On site n.1 this increase was the highest (Picture 2).

During the vegetation period the verifiable increase of sulphur level per weight unit (fresh, dry or wet) for site n.1 showed for both leaf types 31-34, 39-40 and 26-29% accordingly, and for site n.2 — 15-17, 19-20 and 10-16%. More significant increase of sulphur level per dry weight unit was caused by significant decrease of dry weight unit per leaf area by the beginning of September on the test sites. On test site n.1 the increase in sulphur level in this period was more significant than on test site n.2. It is connected with more fast ageing of leaves on test sites that causes the outflow of products of cells degradation from leaves to other parts of a plant, therefore being connected with a partial loss of dry weight.

The level of sulphur per fresh weight unit on test sites every month was verifiably higher that on control sites by 27-69% for sunleaves and by 12-57% for shadeleaves (Pictures 2a, b). The figures of this parameter for sunleaves and shadeleaves are very close (within the limits of statistical error).

The level of sulphur per dry weight unit on test sites was verifiably higher than on the control site for sunleaves by 15-65% (except July for site n.2), while for shadeleaves it was observed only in the half of all the cases (by 32-48%, Pictures. 2c, d). This figure was, in general, close for sunleaves and shadeleaves. In some cases the trend was observed that shadeleaves on the control sites (3-7%) and sunleaves on test site n.1 (by 9%) show higher numbers. Definitely this figure is impacted by the fluidity of dry weight of a leaf during the vegetation period (for example, due to amylum) [4]. It is known that excessive accumulation of amylum in leaves serves as a symptom of unfavourable conditions for plants growth [10]. It is usual in the conditions of air pollution by sulphur gases [11].

The level of sulphur per water weight unit on test sites was verifiably larger by 15-72% for sunleaves and 22-64% for shadeleaves (Picture 2 e, f). There was also a trend of big importance of this figure (by 4-12%) for sunleaves as compared to shadeleaves. The variability of water constitution per area unit during the vegetation period was quite small. On a more polluted site n.1 both types of leaves had a trend of consequent decreasing of water amount during the vegetation period. But monthly decrease of it was not exceeding standard error of meaning. Test site 2 and the control site observed scarce increase of water level only for the beginning of July with consequent decrease by August (all within the standard error of meaning). As a result of consequent decrease of water level during the vegetation period the level of sulphur per weight unit of water for both types of leaves was verifiably higher on site n.1 as compared to the control side (by 47%), while on site n.2 it was higher only by 31%. This difference can be caused by the negative impact of severe pollution on water regime of a leaf.

The effect of the source of pollution on the level of sulfur in the leaves appeared to be the least remarkable, when it was calculated per a unit of dry weight, and the most – when calculating its mass per a unit of chlorophyll. The latter is connected with a strong negative impact of sulfur dioxide on the level of chlorophyll. Changing the level of sulfur in the leaves of both types during the vegetation was significant with all methods of calculation and at all sites, including the control group (see the table). The greatest increase in the level of sulfur in the leaves in all cases occurred in July, when the formation of leaves finished. This phenomenon can be explained as follows. It is known that gaseous pollutants in the air get in the leaves during gas exchange with environment. The intensity of gas exchange is determined by photosynthesis. The speed of photosynthesis per a unit of area increases with the growth and development of a leaf and reaches its peak during the completion of these processes, and then it decreases [3]. This is also characteristic of birch leaves [12]. Young leaves, during their growth and development, most actively absorb the sulfur dioxide [13, 14].

Change of sulphur level in sunleaves and shadeleaves of birch during the vegetations (a monthly percent; plus — increase, minus — decrease)

Sites	Leaves					
	Sun			Shade		
	June	July	August	June	July	August
Sulphur, mg m^{-2} of leaf area						
Control	+34	+40	-1	+25	+51	-1
Site 2	+18	+63	+10	+22	+72	+8
Site 1	+22	+52	+15	+17	+53	+14
Sulphur, mg g^{-1} of fresh weigh						
Control	+20	+43	-2	+17	+49	-2
Site 2	+1	+66	+12	+6	+69	+9
Site 1	+14	+39	+23	+9	+45	+22
Sulphur, mg g^{-1} of dry weigh						
Control	+14	+27	+1	+10	+31	-2
Site 2	+13	+49	+18	-16	+44	+16
Site 1	-3	+35	+33	-8	+28	+33
Sulphur, mg g^{-1} of water						
Control	+23	+52	-3	+22	+58	-2
Site 2	+14	+78	+8	+18	+83	+5
Site 1	+25	+57	+17	+18	+56	+16

On average, according to the four methods of calculation, the increase in the level of sulfur during July at sites 1, 2, and at the control site in sunleaves and shade leaves was 46, 64, 41 and 46, 67, 48% respectively (see the table). According to this indicator, sunleaves and shade leaves did not only stay similar, but also in the control group and at less polluted site 2 there was a trend for an increase in the level of sulfur in shade leaves compared with sunleaves. This suggests that the speed of photosynthesis in shade leaves was not lower than that in sunleaves.

It is known that the speed of photosynthesis in shade leaves of the birch is lower than that of sunleaves [15], but this speed was calculated per a unit of a leaf area, which in the shade leaves is longer than in sunleaves. Thus, the speed of photosynthesis in a whole shade leaf cannot be lower than that in a whole sunleaf. Since the control group in August practically accumulated very little sulfur in the leaves, unlike the experimental plots (see the table), it can be assumed that accumulation of sulfur in the leaves of control group went mainly through the root system and not through the air. Thus, the degree of sulfur accumulation in the leaves will normally depend on the rate of photosynthesis during the growth and formation of the leaves and on the level of soluble sulfur compounds in the soil. This may explain the significant fluctuations in the level of sulfur in the leaves as a norm [1]. The smaller degree of sulfur accumulation at more polluted site 1 in July, in comparison with site 2 (see the table), can be attributed to the strong negative effect of the sulfur dioxide on photosynthesis [10] at site 1. However, this decline is likely to occur only during the absorption of sulfur dioxide and for some time afterwards. For the rest of time the leaf mesophyll cells, maintaining the vitality, developed the speed of photosynthesis not lower than the control group. This allowed us to increase the level of sulfur (taking the average according to four methods of calculation) in the sunleaves to 69 respective of the control at experimental site 1 and by 43% at site 2. Since the air pollutants like the sulfur dioxide absorbed by the leaf may move in the phloem and xylem [16], then part of the sulfur was, obviously, transported by the phloem in other plant organs. Hence, the determined level of sulfur in the leaves at experimental sites does not reflect the full amount of the absorbed sulfur dioxide, which was the reason for the negative effects in plants. This can serve as a basis for skepticism, concerning the measurement of the level of sulfur in the leaves to assess the extent of the negative action of sulfur dioxide in plants [2].

Thus, during the leaf growth and formation (June – July), the level of sulfur increased, both in the experiment and in the control group of the two types of leaves. The greatest increase was at all sites in July. In general, during the vegetation, at the experimental site the level of sulfur accumulated in the leaves was higher than in the control group. The highest accumulation of sulfur in July was at site 2, while at site 1 it was close to the control group. The decrease of the amount of chlorophyll during vegetation in conditions of pollution, compared to the results in the control group, connected with the increase in the level of sulfur per one leaf area, results in a significantly greater increase in the level of sulfur per a mass unit of chlorophyll.

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