

## **Morpho-anatomical and physiological effects of the industrial polluting agents from Moldavian industrial areas – Romania, on *Populus tremula* L.**

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**Abstract**— We have studied one specie of wooden angiosperms from the areas next to the industrial platforms in Borzesti (Bacau country) and in Bicaz and Tasca (Neamt country). In the first case, the noxious substances are mainly gaseous (sulphur dioxide, chlorine, ammoniac), but also solid (carbon black, soot); in the other two cases, the noxious substances are mainly solid ones (lime or cement powders prone to sedimentation). Our investigations have focused on observations concerning the *Populus tremula* L. specie, *Salicaceae* family, in various phenophases, the state of the leaves surfaces and physiological changes of the leaves under the influence of the above-mentioned atmosphere pollutants.

The polluting agents involved favor the installation of a specific micro flora on the leaves (fungi and algae), micro flora that affects their general functions. The necrosis and the inducted foliar chlorosis by polluting agents represent the clear materialization of some profound physiological modifications which disturb the photo-assimilating structures, the medium content of water, dry substance and assimilator pigments. In agreement with the results of the experiences performed by various researchers, using various concentrations of several noxious substances on herbal and ligneous species, whatever the type of noxious substance may be, the cyto-histological changes in the leaves are almost the same, phenotypising as chlorotic and necrotic spots; in the later case, the structure of all leaves is severely altered, which leads to the degradation of the photo-assimilating structures, and ultimately to the death of the entire plant.

**Keywords**— wooden angiosperms; polluting agents; foliar surface; chlorosis; necrosis; physiological modifications

### I. INTRODUCTION

The negative influence of atmospheric pollution agents on vegetation was remarked, mainly, by total and partial defoliation, chlorosis, foliar necrosis processes, and different teratology cases, obvious for those species situated at the outskirts of polluted areas.

In our country, the vegetation pollution by solid substances from cement and lime factories, as well as from superphosphatic fertilizer factories, was studied at Bicaz, Valea Calugarească, Medgidia, Barsesti – Tg. Jiu [1]-[2] - [3] - [4] - [5] - [6] - [7] - [8] - [9] - [10] - [11] - [12] - [13] - [14]. Most of the factories are situated in the neighborhood of forests on rocky terrain, which favors the vegetation's cement

and lime pollution.

The dust covers the surface of the plants forming a crust with devastating effects on the leaf because of its high alkalinity; it covers the stomata preventing respiration and perspiration processes; it interferes with the photosynthesis process. From the analysis carried out at Bicaz area the dust eliminated by the factory's chimney has the following composition: 42 – 45% CaO, 0,4 – 0,8 % MgO, 12 – 14 % SiO<sub>2</sub>, 3,5 – 4,5 % Al<sub>2</sub>O<sub>3</sub>, 2 – 3,5 % Fe<sub>2</sub>O<sub>3</sub>, with calcinations losses of up to 34%.

The physic-geographical conditions favor the dust dispersion and settlement; an important part is played by the terrain configuration, atmospheric circulation and in some part by the present vegetation.

In the study of vegetation pollution effect it is not easy to generalize a phenomenon of air pollution; the atmospheric pollutants, by their chemical diversity, their possibility to connect with other atmospheric agents, which often results in new and unknown agents, the multiple transformations that they go through from the moment of emission to the moment of interaction with the vegetal substrate, the manner in which the species react to this allergen by their metabolic and structural response, are only some of the aspects that are taken into account by researchers.

Industrial polluting agents, gas or solid, are considered permanent aggression factors for air, soil and water quality; this way the plant's way of life is subjected to a generalized stress, which, most often, materializes through an ecological misbalance.

The 'survival' of species, in these conditions represents a multitude of transformations in the continuous and desperate attempt to adapt to a more and more hostile environment; as a consequence, some species restrain their specific area of growth, or the autochthonous vegetation is replaced with the industrialized one – poor in individuals and species numbers – that become permanent as a industrial-climax.

Even if the involved pollution agents are chemically special, the placement of the two industrial sites that we have investigated in depression areas, the presence of valley isles and atmospherically circulation, centered along them, thermo inversions, atmospherically precipitations, absolute calm percentage, contribute to the core stability with the highest vegetation concentrations along the valley isles. It is the reason because the most powerful impact between pollution agents and vegetations take place in the coterminous areas of these industrial platforms.

Our investigations focused on the observations of wooden

specie in different phenophases, on the surface area condition, as well as the physiological and biochemical changes that take place in the leaves under the influence of atmospheric polluting agents, agents that are specific for the areas taken under investigation.

Our observations allowed us to concur that these plants react in most of the cases to the aggression of pollution agents regardless of their chemical nature. Of course, the 'reactions' are conditioned by a great number of factors (genetic, pedo-climatically, natural habitat, acclimatizer conditions, the distance to the pollution source, age, etc.); that is why the data will only be reunited under the form that completes the clinical picture of foliar symptoms.

The present study continues the series of our investigations [16]-[17]-[18] concerning the ill-fated effects of pollutants from the industrialized areas of Moldavia - Romania upon the protective vegetation, hoping to make the proper authorities well aware of the importance of keeping, in the investigated areas, the normal natural life conditions.

## II. MATERIAL AND METHODS

The analyzed material consists of leafs belonging to woody specie of plants: *Populus tremula* L., *Salicaceae* family situated in the Bicaz – Tasca industrial perimeter and on the Borzesti industrial platform. The agents involved in this perimeter are mostly solid for the Bicaz industrial area (cement and lime dust), and gas or solid for the Borzesti (sulphur dioxide, chlorine, ammoniac, and carbon black).

The vegetal material was collected between 1997 and 2000, each year from May to the end of September, with parallel terrain observations in orders to asses the vegetation status around the pollution source.

The material was processed in the Morphology and Vegetal Anatomy Laboratory from the Biology Faculty of "Alexandru Ioan Cuza" University, Iasi.

The tests were carrier out on dry and fresh material according to the specific methodology of the two laboratories.

To investigate the foliar surfaces, the vegetal material was examined with scavenging microscope.

For physiological investigations we elected individuals that were situated at different distances from the source. The analysis tried to achieve:

- the water amount and dry foliar substance dosage by the gravimetric method, of bringing the weight to a constant value for the material that was dried up at 105 °C;
- assimilating foliar pigments dosage by spectrophotometer method, after acetone 80% extraction.

For each foliar test we used 3 parallel probes, and the data was selected as the average value of the results

## III. RESULTS AND DISCUSSION

We shall now present a series of common situations of the investigated specie, occurring as a general response to the action of (gaseous and solid) noxious industrial substances:

### The assimilating pigments amount:

In Bicaz industrial area (fig. 1, 2) the average amount of chlorophyll a is minim in May (9.037 mg/g) and maxim in August (10.714 mg/g) and September (10.46 mg/g). In the polluted leafs this amount is, in all cases, lower than the control, and not necessary related to the distance to the polluting source.

For the leaves collected from the individual closest to the polluting source, (around 80 m), the chlorophyll an amount was 85.60% from the control in May, 66.48% in August and 64.78% in September. For the leafs situated farthest from the source (about 900 m) this amount was 79.35% from the control in May, 66.20% in August and 69.86% in September.

The average b chlorophyll amount from the control leafs is minim in May (3.292 mg/g) and maxim in August (4.172 mg/g) and September (4.271 mg/g). In the polluted leafs this amount exceeds the control by 13% in May, and in August and September the values are significantly lower compare to the control, and not necessary correlated to the distance between the individual and the source. The lowest values are for the individual located farthest from the source 75.31% in August and 59.68% in September.

The average amount of carotenoidic pigments from the control leafs in May is minim (1.736 mg/g) and maxim in August (2.067 mg/g) and September (2.178 mg/g). In the polluted leafs this amount exceeds lightly the control values for May (104.36% and respectively 102.88%), and in August and September the values are lower than the control (86.21% and 86.84% in August; 72.22% and 73.41% in September).

For this specie we can say that all the assimilating pigments for the control have a minim in May and a maxim in August and September. For the polluted leafs a chlorophyll has values lower than the control, and the b chlorophyll and carotenoidic pigments exceed lightly the control in May and in August and September the values are smaller.

Total average amount of assimilating pigments (a+b+c) in the control leafs is minim in May (14,065 mg/g) and maxim in August (16,953 mg/g) and September (16.995 mg/g). In the polluted leafs this amount drops significantly from May to September (from 94.08% in May to 65.74% in September). The drops in August and September are mostly caused by the two chlorophylls that have significantly low values, but also by the low carotenoidic pigments amount.

The chlorophyll a + b, carotenoidic pigments, (a+b/c) ratio from the polluted leafs, generally has lower values compared to the control, the chlorophyll having significantly lower values compared to the control.

In Borzesti industrial area (fig 3, 4) the average amount of chlorophyll from the polluted leafs of *Populus tremula* (see analyzed control from Bicaz) is lower than the control, being strictly related to the distance from the pollution source. So, in leafs from the individual closest to the source (about 100 m) this amount drops progressively from May to September: 69.39% in Mai, 49.21% in August and 48.52% in September. We can also observe this for the individual situated the farthest from the source (about 800 m): 87.38% in Mai, 76.45% in August and 68.45% in September.

The average b chlorophyll amount in the polluted leaves is significantly lower than the control, in relation to the distance from the source. The lowest values are presented for the individual situated closest to the source (about 100 m) 79.37% from the control in May, 51.03% in August and 48.84% in September. Less significant drops occur for the individual situated farthest from the source; a maximum is 98.69% from the control in May, and a minimum of 72.98% in September.

The average amount of carotenoidic pigments has in all the cases a spectacular increase compared to the control. So, for leaves closest to the source this amount is 122.69% in May, 159.40% in August and 154.68% in September. For the individual situated farthest from the source the values are higher, 123.79% in May, 181.03% in August and 162.67% in September.

Total average amount of assimilating pigments (a+b+c) from the polluted leaves is lower than the control, its minimum reading being for the individual situated closest to the source (about 100 m): 78.30% in May, 62.94% in August and 62.20% in September. The low values registered in August and September are influenced by the drop of both chlorophyll amount by more than 50% from the control and by the growth of the carotenoidic pigments.

The chlorophyll a and chlorophyll b (a/b) from the polluted leaves has values close to the control, but it results from extremely low amounts of chlorophyll, so it is not an accurate index for the given situation; for example in August its value was 2,56 (10,714 mg/g chlorophyll a and 4,172 mg/g chlorophyll b), and in the polluted leaves for the individual closest to the source the value was 2,47 (5,273 mg/g chlorophyll a, which is 49,21% from the control, and 2,129 mg/g chlorophyll b, which is 51,02% from the control).

The chlorophyll a + b, carotenoidic pigments, (a+b/c) ratio has extremely low values compared to the control because of significant drop of both types of chlorophyll, as well as the increase of carotenoidic pigments.

#### The water and the dry substance amount:

In Bicz industrial area (fig. 5, 6) the water and dry substance average amount are very close to the control, without significant drops in May, August or September. One explanation can be that the defoliation observed at some individuals were caused by excessive amount of solid deposits, or an exaggerated sensibility to freezing associated with the one produced by the impact of solid pollution agents.

In Borzesti industrial area (fig. 7, 8) for the polluted leaves the water amount varies compared to the control between 80.56% and 83.23% in May, and in August drops to 75.67%. The average amount of dry substance increases, compared to the control, in May by 44.99%, and in August up to 49.87%; these extremely high values for dry substance phenotypical indicates extensive foliar necrosis, as well as extended defoliations, observed along summer months. In September the dry water amount drops down to 29.80% of the control, while the dry substance average amount exceeds the control by 51.15%. For these species, we also observed on the foliar surface the presence of some galls produced by insects, a sign

that the general vitality weakening is not only caused by pollution factors, although these ones play a major part in this process.

The appearance of chloroses and necroses in the early stages of the development of leaves, the accumulation of physiological disorders due to nutrition deficiencies, the systematic aggression of the noxious substances, the existence of unfavourable stational factors have repercussions on the degree of development of the photo-assimilating apparatus of plants; the small average surface of the leaf (due to deposits and to necroses and/or chloroses) is the main cause of the 'chronic starvation' of the individuals which stop growing, fructify more and more rarely and consume their own smaller and smaller organic reserves.

The degree of occupation with chloroses and necroses of the leaf surfaces of the plants under investigation varies very much and reaches very high values both in the species in Borzesti area and in those in the areas of Bicz and Tasca; for example, in the *Populus* leaves in Borzesti area, the afflicted surface may reach 76.98%, and, in some cases, the presence of necroses in young leaves influences their symmetry.

In the case of the analyzed species of deciduous plants with leaf burns and necroses, we have noticed changes of the general shape of the cells by their contraction due to the absence of the state of turgescence, and the presence of algal and fungal "colonizers" in extremely large numbers on the surfaces of leaves showing premature signs of senescence (full yellowing in the month of June).

The existence of partial or total defoliation phenomena with no phenotypisation by necroses and/or chloroses doubtlessly constitutes a special aspect of the fact that, in the areas where the vegetation is subject to chronic aggressions from atmosphere pollutants, the responses are various, unexpected and cannot always be placed into clear categories, but rather in that of 'possible responses'. During our observations, it is not seldom that we contested the veracity of the chosen witness due to physiological or histo-anatomical changes observed on the investigated material, without the existence of any suspicion when the 'phenological' observations were made:

- ◆rare foliage in Bicz area; the main causes are the defoliations, but also the general critical state of the individuals, which is worse every year, due to the cumulated effects of the action of the noxious substances over time;

- ◆the second sprouting during the same vegetation season after serious defoliation episodes in Borzesti area.

The histo-anatomical changes induced by the (gaseous and solid) atmosphere pollutants on the lamina have been observed in protecting tissues, assimilator tissues (the palisade layer and the lacunose tissue), vascular and mechanical tissues. Some leaves have presented disorders in all the component tissues, while in others, the structure changes have only appeared in part of the lamina.

Referring to the foliar surface for the investigated material, we shall now present:

The massive deposits of lime and cement dust on the

surfaces of leaves in the investigated plants in Bicaz area close the ostiola of the stomata, change the characteristic cuticular relief, and favour the development of a micro-flora (fungi and algae) which, once in place, covers photo synthetically active and may release a series of toxic substances influencing the general state of the leaves.

Although some authors [19] consider the presence of a micro-flora on the leaves surfaces to be normal, there are also opinions according to which its presence is a warning sign for important physiological disorders, which are not manifest phenotypically in time; therefore, this is another possible answer to the controversial problem of the massive defoliations of trees.

Similar, but less widespread phenomena have been observed on the leaves of *Populus* plants in Borzesti industrial area; the soot and carbon black deposits are generally less adherent than those made of lime and cement, but they favor the settlement of a micro-flora made of 'colonizers' in permanent competition among themselves.

So, in Borzesti industrial area (Photo 1 – 4) the control leaves for *Populus tremula* have on the adaxial face epidermal cells with a polygonal contour, with straight lateral walls; in different places we can see different anomocytic stomata. On the abaxial face the epidermal cells have an irregular contour, with slightly undulated lateral walls; the stomata are more than on the adaxial face, are still anomocytic and a little prominent over the epidermal cells. The cuticle has fine striations, only visible on the external walls of the annex cells.

At the old leaves one can see deposits in June in the form of crusts on the adaxial face (Photo 1); these deposits give the leaf a breakable character and an increased rigidity which is represented by a combination of solid dusts (smoke dust, PVC) and elements that suggest the presence of epiphyll micro flora. The stomata are masked by this crust. On the abaxial face we can see a micro flora represented by mycelia hypha, formations of a conidial and algal aspect displayed directly on the cuticular surface or on the solid particle "substrate" (Photos 2, 3). This kind of micro flora settlement on the leaf's surface even from the June indicates general 'suffering' for the plant, 'suffering' noticed by the colonizers.

For the leaves formatted at the end of August as a result of the second sprout, from the individuals who had lost almost all their leaves in July, one can see on the adaxial face of the foliar lamina a deformation of the epidermal cells due to a local hypertrophy (in this category we do not include insect actions), as well as the loss of contour for the epidermal cells, fact that can be correlated to their turgescencia; other times we can observe an abundant micro flora, that translates the individual status of a almost dried up plant (Photo 4); on the abaxial face one can notice the incipient installation of the small "colonizers", epidermal cells that maintain their contour, as well as areas invaded by mycelia hypha, conidial and algal form, that can clog or not the stomata of the lamina.

As effect of the previous presented modifications, we notice some morpho-anatomical changes in the lamina structure of the studied plants.

Among the deviations from the normal structure, we mention that many spongy parenchyma cells contain tannin. Also, the tannin is present in small quantities in the palisade parenchyma cells of the adaxial layer and in the fundamental parenchyma of the median nerveure. Thus, the lamina has a fawn nuance on its entire surface, without the necrosis taking place. Some of the mesophyll cells from the neighbourhood of the vascular bundles contain simple crystals and rarely ursine of calcium oxalate.

In some sites of the lamina, the structure is in process of disorganization. The palisade tissue layers consist of lower cells, the adaxial one being entire tanniferous. Some lateral nervures are entire parenchymatous, comprising any vascular elements. Some epidermis cells form veritable promoters between them and the palisade tissue, remaining visible aeriferous cavities. In several sectors of the lamina, especially at its margins, the lower epidermis and the spongy parenchyma are completely exfoliated; where the epidermis persists, its component cells have thicker walls. In some portions, the lamina consists of 2-3 layers of atypical cells, full of tannin, with completely perturbed structure.

On vast sectors from the adaxial face of the lamina, the epidermis is profoundly modified, the component cells being more tangentially elongated, with very thick walls and lumen of slot shape. Thus, it is difficult to distinguish the epidermis layer, the palisade tissue seeming to be cover by the thick cuticle.

#### IV. CONCLUSION

In this study we investigated a woody plant specie situated at the neighborhood area of two industrial platforms: Borzesti (Bacau district) and Bicaz (Neamt district); in the first case the polluting agents are mostly gasses (sulphur dioxide, chlorine, ammoniac), but also solid (smoke black, and smoke dust); in the second case we encounter mostly solid deposits (lime and cement dust). Although the polluting agents are different we can notice similarities regarding the depression location of the two industrial zones, the presence of valleys and the air current circulation, with the greatest concentrations affecting the vegetations around the industrial platforms.

From the correlated interpretation of the obtained data, one can conclude that, beside particularly manifestations of interactions between atmospherically pollutants (gas and solid) and vegetations, there is a common series of manifestations, as a general response to the stress caused by pollutants aggressions, regardless of their chemical nature: episodes of partial or total defoliation; partial and total dry up phenomenon, chlorosis and / or foliar necrosis; a second sprout period during the same vegetation season after prior defoliations; flower and fruits affections, etc.

The "response" of each individual to industrial pollutant aggression, regardless of their chemical nature, is conditioned by: a multitude of genetic factors, pedo-climatic conditions, natural habitat, acclimatization degree, location and distance

from the pollution source, etc., and it cannot be generalized for the representatives of the same family, not even for the same genus. Because of this the 'resistance scales' that can be promoted by our observations on the tested specie, as a member of a more ample group of woody and grassy angiosperms have an orienteer character.

The analysis of the foliar surfaces using a scavenging microscope showed the role of industrial solid deposits on the phenomenon of individual partial or total defoliation, which often occurs in full vegetative season. These deposits deactivate large portions of photosynthesis active areas, stop respiration and perspiration processes by clogging the stomata of the stomata: they modify the characteristic texture by disorganizing the cubical striations pattern; they alter the ratio between the amorphous and crystallized wax in the favor of the amorphous one, which can contribute in some part to the stomata clogging; sometimes one can notice the small quantity or the absence of the epicuticular wax. The foreign deposits favor the micro flora colonization of the foliar surfaces (funguses and seaweed), which can affect the general status of the leaf. Regardless of the chemical nature of the pollutant agent, this micro flora is a sign of early senescence of the leaf and a possible defoliation cause in full vegetative season. It is obvious that beside leaves, the micro flora is also affected by the industrial agents, which can inhibit or stimulate their activity. The phenomenon was noticed at the angiosperm species at both investigated industrial sites.

Foliar necrosis and chlorosis are a clear proof of profound physiological modifications that affect the average amount of water, dry substance and assimilating pigments.

We also state that not always the dry substance amount in the leaves is related to the distance to the pollution source, and that not always this amount is strictly related to the microscopic visible necrosis; in other words the alteration of different physiological functions is directly followed by dry up or defoliation. In other situations the defoliations occur after the moment when the phenotype necrosis has occupied a large portion of the foliar surface.

Under the influence of solid and gas polluting agents the average water and chlorophyll amount drops, the values being most often correlated to the distance from the pollution source. The most spectacular drops occurred for the individuals situated at Borzesti industrial platform, this fact states that the gas polluting agents are more destructive than the solid ones. The average chlorophyll amount is in almost all cases lower than the control. For the carotenoidic pigments the individuals from Borzesti have higher values and the ones from Bicaz have smaller ones.

Further investigations are necessary, in order to study the variation of these pigments in the control leaves along all vegetative season and to anticipate more accurately the possible 'answers' that the vegetation might have when subjected to a chronic aggression from atmospheric pollution agents.

When dealing with the response reactions of plants to the action of noxious substances, we cannot consider any noticed

change to be absolute; there is no uniformity among the individuals belonging to the same species, let alone among species belonging to different genera or families. The responses are individual ones; therefore, the studies concerning the impact of pollutants on vegetation should comprise a large variety of investigations for each individual in a polluted environment.

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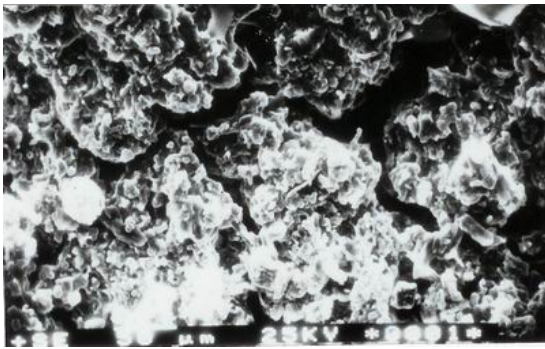


Photo 1 *Populus tremula* Borzesti polluted leaf adaxial face

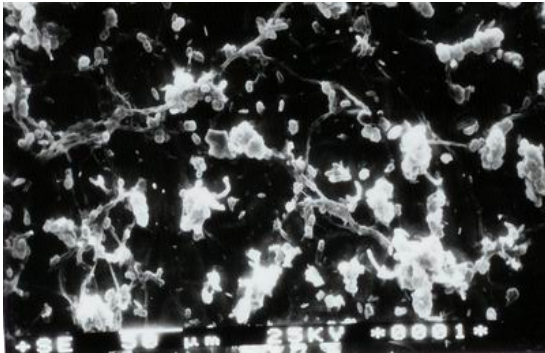


Photo 2 *Populus tremula* Borzesti polluted leaf abaxial face

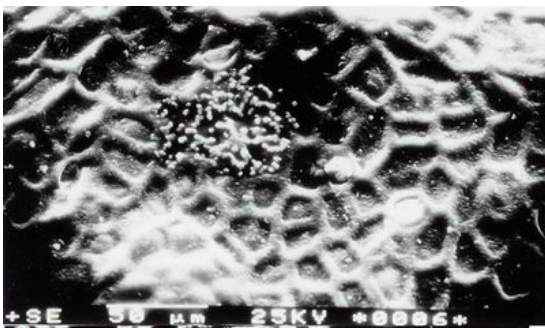


Photo 3 *Populus tremula* Borzesti polluted leaf adaxial face



Photo 4 *Populus tremula* Borzesti polluted leaf abaxial face

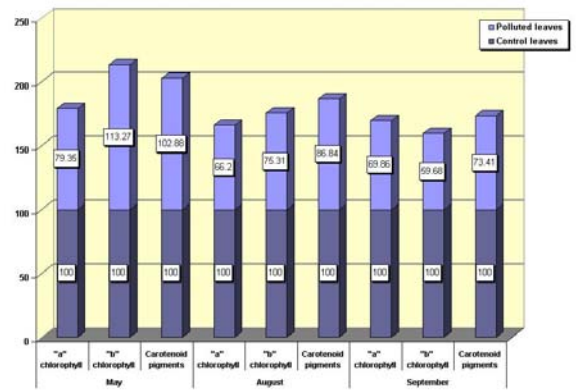


Fig. 1 The medium content of assimilating pigments in leaves of *Populus tremula* in Bicaz area at 80 m distance from pollution source

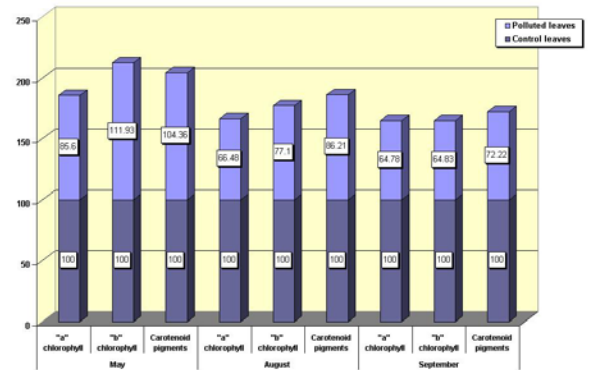


Fig. 2 The medium content of assimilating pigments in leaves of *Populus tremula* in Bicaz area at 900 m distance from pollution source

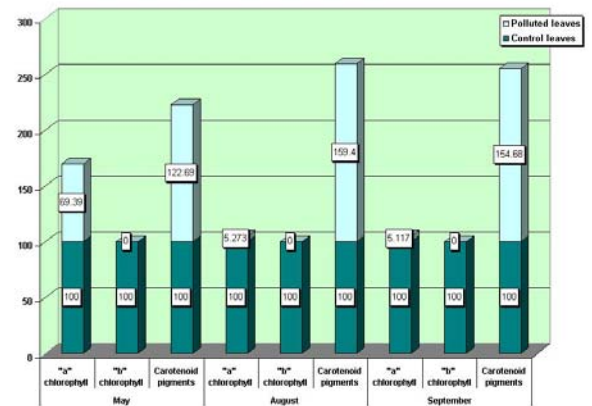


Fig. 3 The medium content of assimilating pigments in leaves of *Populus tremula* in Borzesti area at 100 m distance from pollution source

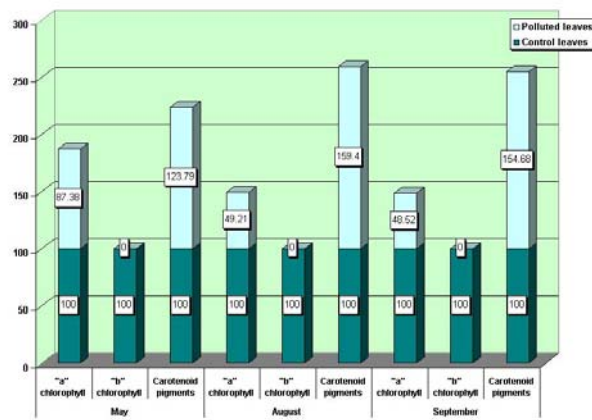


Fig. 4 The medium content of assimilating pigments in leaves of *Populus tremula* in Borzesti area at 900 m distance from pollution source

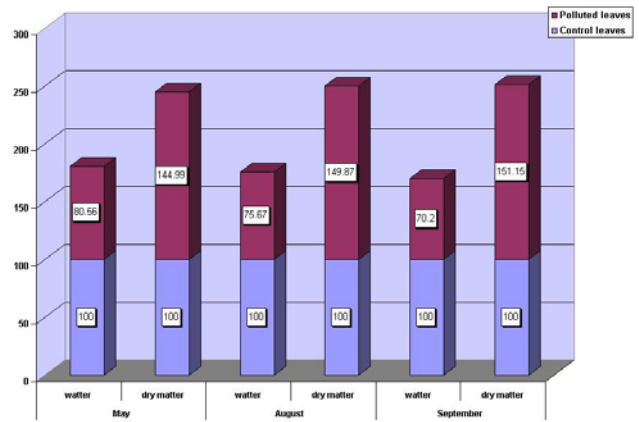


Fig. 7 The medium content of water and dry matter in leaves of *Populus tremula* in Borzesti area at 100 m distance from pollution source

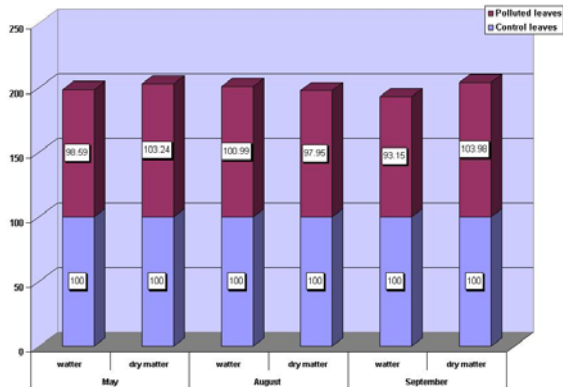


Fig. 5 The medium content of water and dry matter in leaves of *Populus tremula* in Bicz area at 80 m distance from pollution source

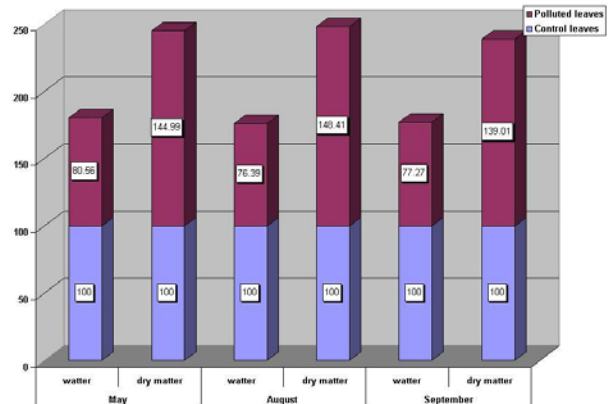


Fig. 8 The medium content of water and dry matter in leaves of *Populus tremula* in Borzesti area at 900 m distance from pollution source

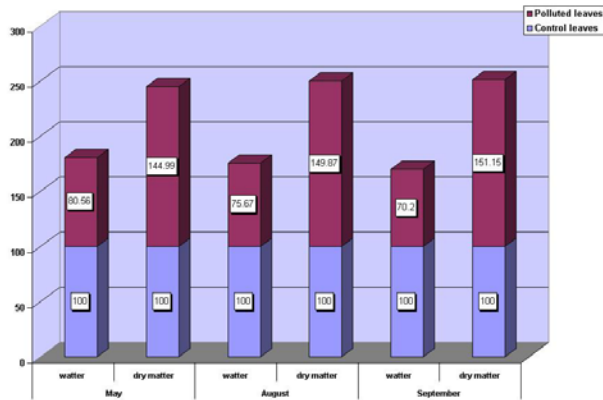


Fig. 6 The medium content of water and dry matter in leaves of *Populus tremula* in Bicz area at 900 m distance from pollution source

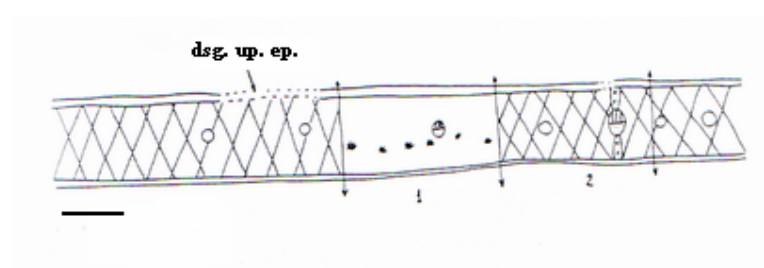


Fig. 9. Cross sections through the *Populus tremula* L. lamina (diagram) scale bars= 100µm

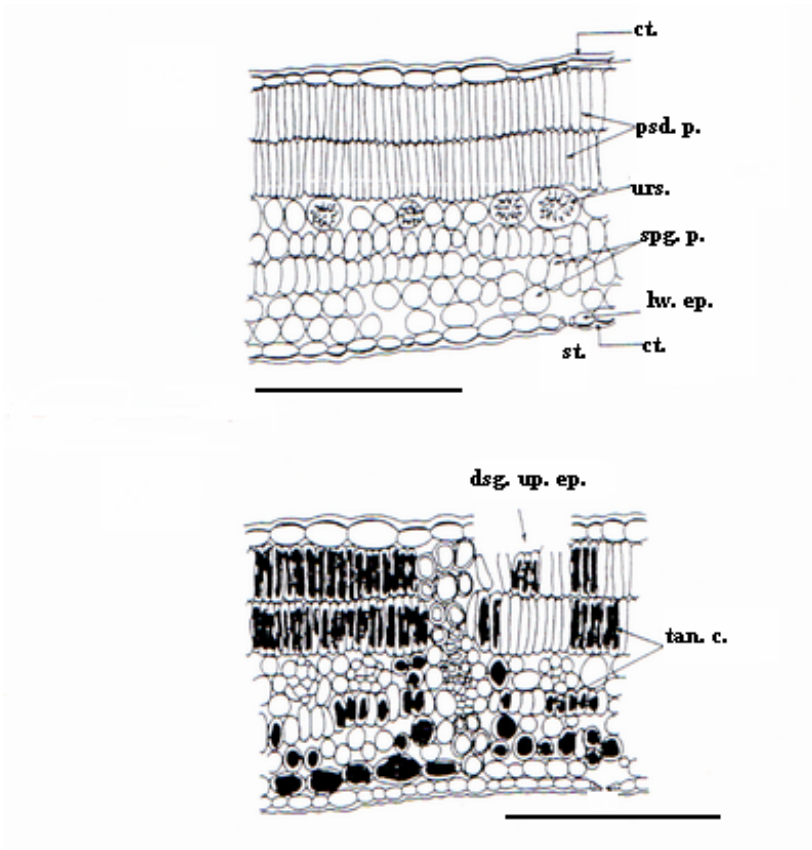


Fig. 10, 11. Cross sections through the *Populus tremula* L. lamina: 11. unaffected sector; 12. affected sector (details) scale bars= 100µm