

RESEARCH ARTICLE

Features of the morphological and anatomical characteristics of the leaves of *Taraxacum officinale* Wigg. in conditions of rock dump

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The morphological and anatomical features of the assimilation apparatus *Taraxacum officinale* Wigg., growing under the conditions of a rock dump of the Kedrovsky coal mine in the Kemerovo Region, were studied. It was experimentally established that the specific environmental conditions of the rock dump cause morphological and anatomical changes in the leaves of the dandelion. At the level of leaf morphology, there is a decrease in the average leaf area, thickening of leaf blades, the formation of a trend towards a decrease in the number of leaves in the outlet. At the level of the anatomical structure, there is a significant increase in the thickness of mesophyll, a tendency to a decrease in the thickness of the tissues of the upper and lower epidermis, a decrease in the number of cells of the upper and lower epidermis by 1 mm² and an increase in the size of stomata in the tissues of the lower and upper epidermis, a decrease in the number of stomata by 1 mm² and stomatal index on the upper epidermis. Under the conditions of the rock dump, an increase in the number of reliable correlations between the anatomical parameters of the leaf of *Taraxacum officinale* Wigg., as well as anatomical signs with indicators of atmospheric pollution, was established, to a greater extent due to the formation of positive correlations that are known to control the development of biosystems and are critical for body periods.

Keywords: Morphology; Anatomy; Leaf; *Dandelion officinalis*; Environmental Conditions; Rock Dumps; Opencast coal mine

Introduction

As a result of coal mining in Kuzbass, the area of disturbed lands exceeds 100 thousand hectares. Along with reclamation, man-made soils are naturally overgrown as a result of seed drift from the surrounding territories. Rock dumps formed as a result of an open coal mining method are characterized by specific environmental conditions-topography, formation of borrow cut, depression funnels, environmental pollution by chemical elements and their compounds, especially air slack. The technogenic change of the relief on the waste dumps leads to a lack of productive moisture, failure of water permeability, contrasting temperature conditions on different elements of the relief; low potential fertility of embryozems (Manakov, 2012). In this regard, waste dumps are a classic object for studying the adaptive mechanisms of plants settling on them. A high degree of resistance of some species of higher plants is ensured by a combination of morphological, physiological, anatomical adaptive reactions, which makes these plants potentially capable of improving the quality of the natural environment-urban areas, industrial zones, and man-made disturbed soils. Plant adaptations are associated, first of all, with rearrangements of their assimilating organs, which, due to their gas exchange function, are highly sensitive to external influences (Neverova et al., 2013). It is known that morphological and anatomical rearrangements alter the physiological status of a plant under extreme conditions of existence for a more efficient use of environmental resources (Menshakova et al., 2008). The aim of the work is to study the morphological and anatomical features of the assimilation apparatus *Taraxacum officinale* Wigg., growing in the conditions of the rock dump of the Kedrovsky opencast coal mine.

Materials and methods

The studies were carried out at the Yuzhniy rock dump of the Kedrovsky opencast coal mine. The spoil hear has a flat-sloping relief with a height of 58 m, an area of 599.3 hectares. The dump rocks are represented by sandstone (60%), siltstones (20%), mudstones (15%), loams and clays (5%) (Figure 1). The experimental site is located at the top of the planned refuse dump. The duration of the biocenosis is 7-10 years. The technical and biological stages of soil reclamation were carried out at the dump (planting *Pinus sylvestris* L. and *Onobrychis arenaria* (Kit.) DC., the initial stages of the formation of meadow communities are observed, there are separate sections of rocky placers with an area of up to 3 m² from a different format of the dump rock, completely devoid of vegetation. Foliage cover is up to 50%, in rocky areas less than 10%. There is no turfness. *Onobrychis arenaria* (Kit.) DC., *Taraxacum officinale* Wigg., *Melilotus officinalis* (L.) Pall., *Artemisia sericea* Web. Ex Stechm., *Picris hieracioides* L. are prevailing in the grass. Embryozems are characterized by alkaline reaction (pH of water extract 7.1–7.7), average availability of humus (3.5%), low availability of mobile forms of phosphorus (10–50 mg/kg) and nitrogen (3.6–6.0 mg/kg), high availability of potassium (100–140 mg/kg), lack of productive moisture (19–22 mm in the 0-20 cm layer). The control site was located 15 km north-west from the spoil hears on the border of the birch forest. A large area (about 80%) of the plot is occupied by a gramineous-herb meadow. The projective cover is 95–100%, the soil is not sod. *Centaurea scabiosa* L., *Hieracium umbellatum* L., *Lathyrus pratensis* L., *Achillea Asiatica* Serg., *Taraxacum officinale* Wigg. predominate in meadow communities. The soils of the site are represented by chernozem-meadow medium-thick heavy loam with a high supply of humus (9.65%), a relatively high content of phosphorus (83.0 mg/kg) and potassium (171.0 mg/kg), a slightly acidic soil solution (pH 6, 1 ... 6.3) and a good supply of productive moisture (50-60 mm in a 0-20 cm layer).

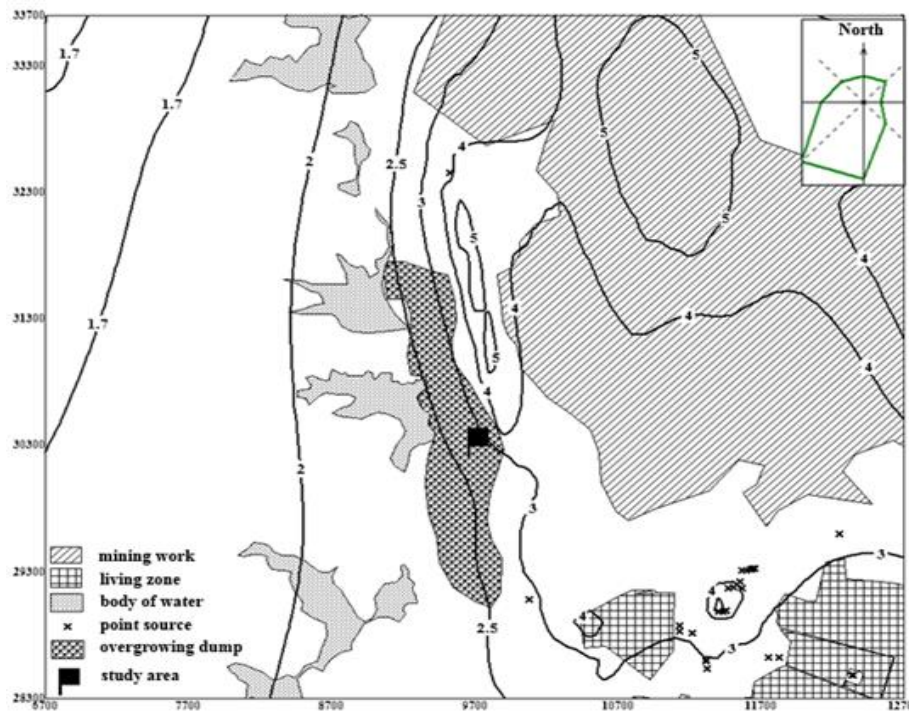


Figure 1. Surroundings of the research site with isolines of the calculated comprehensive indicator of average annual air pollution.

To calculate the impact of industrial emissions into the atmosphere on vegetation in the research area, the ERA-AIR software package (<http://www.lpp.ru>) was used, which implements the normative models for calculating maximum single and average annual concentrations adopted in Russia from 01.01.2018 and given in the order (Order of the Ministry of Natural Resources of Russia of 06.06.2017 No. 273). The models make it possible to calculate the single and average annual atmospheric pollution by both gaseous and precipitating emissions of impurities from industrial sources from data on emissions of sources of atmospheric pollution and characteristic meteorological parameters for a given territory. Obviously, the condition of plants depends primarily on the long-term effects of pollutants, which is determined by the average annual concentrations in the surface layer of the atmosphere and the annual fall of aerosols on the soil cover. The module for calculating the precipitation of particles on the underlying surface (Bykov et al., 2002), which is a research supplement to the ERA-AIR SC, was repeatedly used in the Kuzbass conditions and showed good agreement between the calculation results and field observations (Bykov et al., 2006).

The composition of the initial data for calculating the average concentration over a long period of time, in addition to source parameters, includes three distribution densities: wind direction $p_1(\varphi)$; wind speed $p_2(u)$; dimensionless parameter λ of turbulent mixing intensity $p_3(\lambda)$. For normative calculations, these distributions, which determine the long-term atmospheric pollution regime for the study area, are requested from the Main State Hydrometeorological Observatory n.a. A.I. Voeykova (St. Petersburg, Russia). The function $p_1(\varphi)$ (smoothed wind rose) for a specific averaging period can be obtained from the 8th wind rose compiled from standard meteorological observations. However, to assess the effect of pollutants on plants for a long time, it is preferable to use a climatic (average of at least 5 years) wind rose, which is contained in a special meteorological file sent from the Main State Hydrometeorological Observatory.

The research area is affected by sources of air pollution not only from the Kedrovsky opencast coal mine, but also from the cities of Kemerovo, powerful sources of energy and chemical enterprises located in the southwest 20 km from the experimental site, i.e. from the direction of the prevailing winds. The parameters of the sources of atmospheric pollution of urban enterprises and roads are taken from the inventory materials of the consolidated volume of MPE of the city of Kemerovo, and the Kedrovsky open pit sources based on departmental volume of MPE, developed a little later.

In the process of modeling atmospheric pollution, the conditional dimensionless complex indicator (CI) of the total average annual atmospheric pollution $CI = C_1/MPC_{c1} + C_2/MPC_{c2} + \dots + C_n/MPC_{cn}$, where C_i , $i=1, 2, \dots, n$, was calculated total annual average surface concentration of impurities over all sources, MPC_{ci} corresponding average daily hygienic MPC_s , and indices 1, 2, ..., n relate to pollutants whose average annual CI concentrations exceed the level of $0.1MPC_{ci}$. When calculating the CI, the following substances and their compounds were taken into account: manganese and its compounds, lead and its inorganic compounds, nitrogen dioxide, nitrogen oxide, sulfuric acid, soot, sulfur dioxide, hydrogen sulfide, carbon oxide, gaseous fluoride compounds, chlorine, ammonium carbonate, benzene, dimethylbenzene, methylbenzene, benz(a)pyrene, naphthalene, butan-1-ol, cyclohexanol, butyl acetate, formaldehyde, cyclohexanol, dimethylaniline, nitrobenzene, kerosene, petroleum-based mineral oil, hydrocarbons saturated C12–C19, inorganic dust containing silicic acid anhydride, abrasive dust, coal dust. This indicator is not a normative hygienic criterion, since not all the substances considered have the effect of unidirectional effects on humans. It bears the meaning of the «total manmade load» created by industry through the atmospheric transfer of pollution to a particular territory of the city. Table 1 shows the atmospheric emissions at the observation site.

When calculating the estimation of soil pollution by atmospheric precipitation, the dispersed composition of emissions and the deposition rate of various particle size fractions were additionally set as was done in Modeling of soil pollution by atmospheric emissions from industrial facilities in a coal-mining region (Bykov et al., 2006). The term dust in Table 1 means the sum of all the solid particles emitted into the atmosphere of the city of Kemerovo and the Kedrovsky open pit.

An analysis of the calculated atmospheric emissions in the study area shows that the contribution of the Kedrovsky section to the amount of dust loss dust fall, which is 5.68 g/m^2 per year or 84% of the total dust loss to the study area, taking into account urban industrial facilities, is most significant. The contribution of the strip mine to the value of the complex indicator of air pollution in the research area is 49.8% (the

contribution of the city is 50.2%). The contribution of the Kedrovsky coal pit to the total amount of precipitation of nitrates and sulfates to the study area is less significant and makes 36% and 16%, respectively, while the contribution of the city is 64 and 84%, respectively.

The object of botanical research was the medicinal dandelion (*Taraxacum officinale* Wigg.), as the most common species of perennial herb growing on rock dumps. Harvesting of raw materials was carried out in dry sunny weather, in accordance with generally accepted rules during the period of maximum development of the vegetative mass (end of May 2016). Raw materials were collected without visible signs of damage from 15 accounting sites of 1 m² in size from the experimental and control plots. For morphological studies, the sample of plants from each accounting site was 10 plants, in total from the experimental and control plots - 150 plants. Leaf area (S) was determined by scanning them with subsequent image processing using the Image Tools computer program. For anatomical studies, plant material was fixed in a 60% solution of ethyl alcohol. Cross sections were made on the microtome from the middle part of the leaf blade and placed in glycerin. The anatomical parameters of the leaves (the thickness of the sheet and its tissues, the number of cells in 1 mm² of the lower and upper epidermis, stomata sizes) were measured using an Axioscope-2+microscope, model ZEISSN HBO103 and N XBO75 (Germany) with an ocular micrometer, camera, and software. The stomatal index was calculated by the formula: $Si = N_{stom} \times 100\% / (N_{stom} + N_{ce})$, where N_{stom} is the number of stomata per 1 mm², N_{ce} is the number of main cells of the leaf epidermis per 1 mm².

Table 1. Indicators of the long-term impact of atmospheric emissions on the study area.

Composite indicator (CI), integer /%	Total	Contribution of coal mine	Contribution of the city
Dust fallout, g/m ² per year/%	6.79/100	5.68/84	1.11/16
Nitrate fallout, g/m ² per year/%	0.31/100	0.11/36	0.20/64
Sulfates fallout, g/m ² per year/%	0.16/100	0.03/16	0.13/84

Results and discussion

A comparative analysis of the morphology of dandelion growing under various environmental conditions showed that the average leaf area on waste dumps in plants decreases (by 24%), the number of leaves in the outlet decreases, the leaf blade thickens (by 15%) (Table 2 and Figure 2).

Indicators	Control	Experience
Leaf area, cm ²	48.02 ± 4.82	36.43 ± 3.46*
Number of leaves on one plant, piece	5.80 ± 0.45	4.92 ± 0.42
Leaf thickness, μm	118.10 ± 3.22	136.25 ± 6.22*
Tissue thickness, μm		
- Upper epidermis	19.61 ± 1.24	18.66 ± 1.19
- Lower epidermis	16.81 ± 0.65	15.37 ± 0.42
- Mesophyll	84.00 ± 2.12	103.11 ± 3.24*
Number of mesophyll layers, piece	4–6	4–6

Table 2. Anatomical and morphological characteristics of the leaf blade *Taraxacum officinale* Wigg.

Note: * - significant differences from the control when $p < 0.05$.

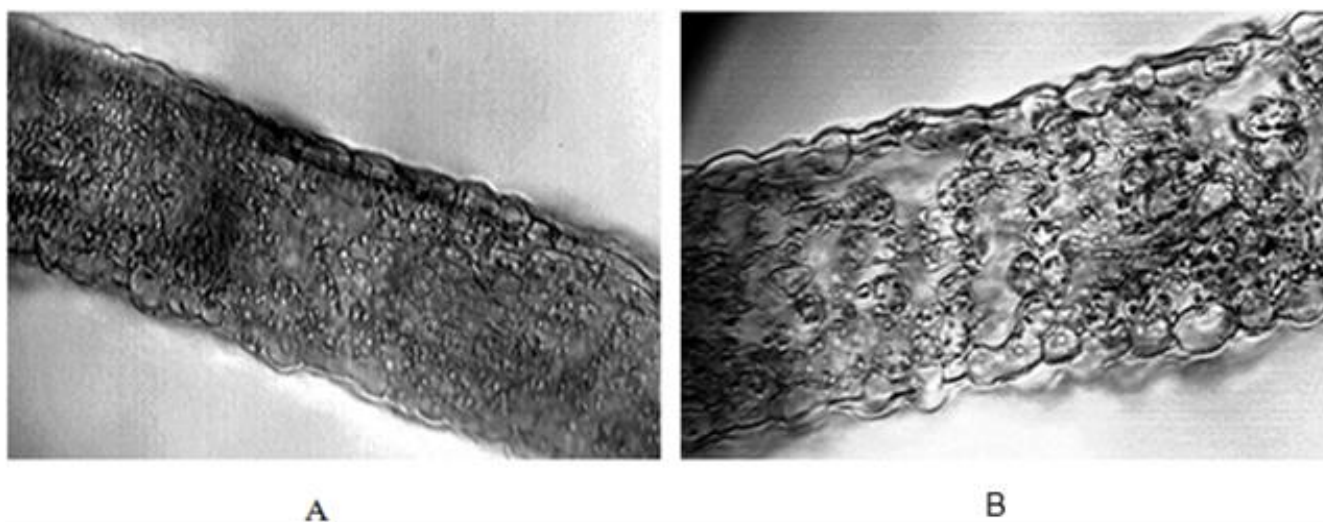


Figure 2. Cross cut leaf *Taraxacum officinale* Wigg. (A – Control, B – Experience).

An analysis of the anatomical structure of the leaves showed that under the conditions of a rock dump, dandelion significantly increases the thickness of mesophyll (by 55%), there is a tendency to a decrease in the thickness of the tissues of the upper and lower epidermis (Table 2). From literary sources it is known that morphological changes in the leaf are a functional response to environmental factors (Zhongqiang & Dan, 2009; Scheepens et al., 2010). As noted earlier, waste dumps are characterized by a lack of productive moisture. It is known that when growing under arid conditions and high insolation in herbaceous plants, an increase in the thickness of the leaf plate and assimilation tissue occurs (Ivanova, 2014). P.E. Lauri et al., 2014 found that the lack of moisture leads to a decrease in the area of the leaf blade and its mass in absolute values, which can be considered as an adaptive reaction to adverse environmental factors. It is known that adaptive reactions of plants to

environmental factors are non-specific. So according to T.G. Akatieva 2014, near the industrial enterprises of Iset district of the Tyumen region, *dandelion officinalis* showed a decrease in the number of leaves per 1 plant, plant height, leaf length and width. A.K. Frolov 1998 notes in urban conditions an increase in the thickness of the sheet and mesophyll in the *dandelion officinalis*, a decrease in the size (length and width) of spongy tissue cells. Anatomical and morphological changes towards xeromorphism are observed in lawn grass under conditions of anthropogenic pollution of Yoshkar-Ola: thickening of the leaf blade due to an increase in the height of the epidermis and mesophyll, reduction of the intercellular spaces, decrease in the length and width of the leaf blade (Polovnikova, 2007).

Under the influence of adverse environmental factors, the most obvious responses of plants are manifested in the structure of the leaf epidermis. The obtained experimental data testify to the differences in the anatomical features of the epidermis of the leaf of the *dandelion officinalis* growing under the conditions of a rock dump (Table 3).

Table 3. Anatomical characteristics of the epidermis of the leaf *Taraxacum officinale* Wigg.

Studied parameters	Lower epidermis		Upper epidermis	
	control	experience	control	experience
Number of cell in 1 mm ²	978.80 ± 24.32	924.45 ± 16.23	1042.37 ± 25.62	940.44 ± 20.08
Number of stomata in 1 mm ²	142.02 ± 1.29	125.01 ± 1.34*	81.01 ± 1.14	60.02 ± 0.82*
Stomata length, μm	24.36 ± 0.47	26.12 ± 0.58	23.65 ± 0.52	24.28 ± 0.46
Stomata width, μm	19.32 ± 0.41	20.54 ± 0.67	17.93 ± 0.36	18.44 ± 0.92
Stomatal index, %	12.67 ± 0.25	11.87 ± 0.11	7.21 ± 0.28	6.21 ± 0.36*

Note: * - significant differences from the control when $p < 0.05$.

In particular, medicinal dandelion growing on pit tips tends to decrease the number of cells in 1 mm² and increase the size of stomata in the tissues of the lower and upper epidermis, the number of stomata significantly decreases by 1 mm² (12 and 26%, respectively); on the upper epidermis there is a significant decrease in the stomatal index (by 14%). Given the low supply of productive moisture of embryozems on rock dumps, a decrease in the stomatal index is a natural response of plants to survival. There is information in the literature that the low frequency of stomata in combination with their large sizes contributes to a more effective control of water exchange (Ceulemans et al., 1978; Bissing, 1982). Our findings are also consistent with I.I. Krokmal 2015, who established that in *Campanula sibirica* L. at the level of rosette leaves, adaptation to radical growing conditions is achieved by reducing the number of stomata and increasing their size.

Based on the correlation analysis, the dependence of the anatomical and morphological characters of the leaves of *Taraxacum officinale* Wigg. on the indicators of atmospheric emissions in the territory of the waste dump was revealed (Table 4).

The results of the correlation analysis show that the value of the complex indicator of atmospheric pollution (CI) and dustfall are significantly correlated with some anatomical and morphological characteristics of the leaves of *Taraxacum officinale* Wigg. In particular, the leaf area, the thickness of the upper and lower epidermis, the number of cells and stomata in 1 mm² of the lower and upper epidermis negatively correlate with the index of CI; while positive correlations are formed between the CI and such indicators as: sheet thickness, mesophyll thickness, length and width of stomata of the lower and upper epidermis; the number of stomata is 1 mm² and the stomatal index of the upper epidermis is inversely related to the amount of dust fallen. Thus, the experimentally established success rate of the growth of *Taraxacum officinale* Wigg. in conditions of a rock dump, including a certain correlation of «+» and «-», reliable correlations between the indicators of technogenic pollution of the waste dump and the anatomical and morphological characteristics of the leaves are provided.

Table 4. Significant correlations between the anatomical and morphological features of the leaves of the *Taraxacum officinale* Wigg. and indicators of the long-term effects of air emissions on the territory of the waste dump.

Depending studied traits	Significant Correlations, (r), n=10, p<0,05
CI – Leaf area	-0,68
CI – Leaf thickness	+0,75
CI – Thickness of the upper epidermis	-0,69
CI – Thickness of the lower epidermis	-0,70
CI – Mesophyll thickness	+0,74
CI – The number of cells in 1 mm ² of the lower epidermis	-0,69
CI – The number of stomata in 1 mm ² of the lower epidermis	-0,72
CI – The length of the stomata of the lower epidermis	+0,70
CI – Stomata width of the lower epidermis	+0,70
CI – The number of cells in 1 mm ² of the upper epidermis	-0,70
CI – The number of stomata in 1 mm ² of the upper epidermis	-0,76
CI – The length of the stomata of the upper epidermis	+0,71
CI – Stomata width of the upper epidermis	+0,70
Dust fallout – The number of stomata in 1 mm ² of the upper epidermis	-0,81
Dust fallout – Stomatal index of the upper epidermis	-0,75

Under the conditions of the waste dump, the dandelion has a fundamentally different system of reliable correlations between the anatomical and morphological characters in comparison with the plants of the control plot (Table 5). This is expressed in an increase in the total number of correlations, and to a greater extent due to positive correlations.

So, in a dandelion, under conditions of a pit hill, close correlations arise between the thickness of the upper and lower epidermis (+0.84); the thickness of the mesophyll positively correlates with the width of the cells and the number of stomata of the lower epidermis (+0.84, +0.86, respectively), the thickness of the lower epidermis positively correlates with the length of stomata of the lower epidermis (+0.81) and negatively with the width of stomata of the upper epidermis (-0.87); there is an inverse close relationship between the number of cells of the upper epidermis and the number of cells of the lower epidermis (-0.89) and a direct correlation with the length of the stomata of the lower epidermis (+0.81). The number of cells of the lower epidermis is closely related by feedback with the length of the stomata of the upper

epidermis (-0.97) and a direct relationship with the stomatal index of the lower epidermis (+0.86). There is a direct positive relationship between the number of stomata of the upper and lower epidermis (+0.82) (Table 5).

Table 5. Correlations between the anatomical indicators of the leaves of the *Taraxacum officinale* Wigg.

Depending studied traits	Significant Correlations, (r), n=150, p<0,05	
	control	experience
Leaf thickness–stomata width of the lower epidermis	-0,83	–
Thickness of the upper epidermis–Thickness of the lower epidermis	–	+0,84
Thickness of the upper epidermis–Mesophyll thickness	+0,89	–
Thickness of the upper epidermis–width of the stoma of the upper epidermis	–	+0,97
Mesophyll thickness–number of cells in the lower epidermis	+0,84	–
Mesophyll thickness–cell width of the lower epidermis	–	+0,84
Mesophyll thickness– number of stomata of the lower epidermis	–	+0,86
Thickness of the lower epidermis–width of the stomata of the upper epidermis	–	-0,87
Thickness of the lower epidermis–length of the stomata of the lower epidermis	–	+0,81
Number of cells in the upper epidermis–number of cells in the lower epidermis	–	-0,89
Number of cells of the upper epidermis–number of stomata of the upper epidermis	-0,94	–
Number of cells in the upper epidermis–stomatal index of the lower epidermis	+0,96	–
Number of cells of the upper epidermis–Length of the stomata of the lower epidermis	–	+0,81
Number of stomata of the upper epidermis–stomatal index of the lower epidermis	+0,96	–
Number of stomata of the upper epidermis–number of stomata of the lower epidermis	–	+0,82
Number of cells in the lower epidermis–length of the stomata of the upper epidermis	–	-0,97
Number of cells in the lower epidermis–stomatal index of the lower epidermis	–	+0,86
Stomatal width of the upper epidermis–stomatal index of the upper epidermis	+0,86	–
Total correlations:	7	11
r+/r-	3,5	2,66

Positive correlations are known to control the development of biosystems and arise during periods critical for the organisms. Therefore, a coordinated increase in the number of correlations and more positive between the anatomical parameters of the dandelion leaf can be considered as a specific reaction aimed at survival and a stable existence in the conditions of a rock dump.

Conclusion

The specific environmental conditions of the Kedrovsky coal mine rock dump cause morphological and anatomical changes in the leaves of *Taraxacum officinale* Wigg. It was revealed that the responses in the conditions of the waste dump are: at the level of leaf morphology - a decrease in the average leaf area, thickening of lamina, the formation of a trend towards a decrease in the number of leaf rosette; at the level of the anatomical structure - a significant increase in the thickness of the mesophyll, a tendency to a decrease in the thickness of the tissues of the upper and lower epidermis, a decrease in the number of cells in 1 mm² and an increase in the size of stomata in the tissues of the lower and upper epidermis, a decrease in the number of stomata by 1 mm², a decrease in the stomatal index on the upper epidermis.

Reliable correlations between the morphological and anatomical parameters of the leaves of *Taraxacum officinale* Wigg. were established and a comprehensive indicator of atmospheric pollution and dust: leaf area, thickness of the upper and lower epidermis, the number of cells and stomata in 1 mm² of the lower and upper epidermis negatively correlate with the index of CI; leaf thickness, mesophyll thickness, length and width of stomata of lower and upper epidermis positively correlate with CI; the number of stomata is 1 mm² and the stomatal index of the upper epidermis is inversely related to the amount of dust fallout.

Thus, the experimentally established success rate of the growth of *Taraxacum officinale* Wigg. in conditions of a rock dump, including a certain correlation of «+» and «-», reliable correlations between the indicators of technogenic pollution of the pit heap and the anatomical and morphological characteristics of the leaves are provided. Under the conditions of a rock dump at *Taraxacum officinale* Wigg. a fundamentally different system of reliable correlations between anatomical and morphological characters in comparison with plants of the control plot is being formed. This is expressed in an increase in the total number of correlations, and to a greater extent due to positive correlations, which are known to control the development of biosystems and occur during periods critical for the body. A coordinated increase in the number of correlations between the anatomical parameters of the leaf of *Taraxacum officinale* Wigg., as well as the indicators of atmospheric pollution can be considered as a specific adaptive reaction aimed at survival and stable existence in conditions of a rock dump.

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