

DENDROINDICATION OF PETROLEUM SOIL CONTAMINATION

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Abstract. The main approaches to the detection of soil contamination by petroleum products using bioindication were analyzed. Necrosis of leaves of woody plants near sludge accumulators was recorded. To assess the possibility of dendroindication of soil contamination, 3 species of trees distributed on the territory of Bytkiv-Babchensky oil and gas field were selected. According to the results of the Fisher-Snedekor test, the influence of the concentration of oil products in the soil on the necrosis of tree leaves was confirmed, which confirms the possibility of using a dendroindication to detect soil contamination with oil products.

Key words: sludge storage, dendroindication, leaf necrosis and chlorosis, oil pollution of soils.

1. Introduction

The vast majority of domestic oil and gas fields are depleted and are at the final stages of development. From the above dependencies, it becomes obvious that a feature of the final stage is that the extraction of formation fluids requires increasing investment. At the same time, the amount of marketable products decreases quite rapidly, and the volumes of extracted liquid as well as the costs of the utilization of associated formation waters and the load on the environment increase. To a greater extent, this applies to the Eastern region of Ukraine. Currently, the situation is further complicated by the fact that world energy prices have fallen significantly and the prospects for their growth in the context of the rapid development of “alternative” and renewable energy are minimal. It should be noted that currently, the of the above types of energy is more promising than the

development of the oil and gas industry (De Fraiture et al., 2008; Petrescu et al., 2016; Kharlamova et al., 2016; Yankiv-Vitkovska et al., 2020).

At the final stage of field development, the influence of so-called “aggravating” factors, which require significant investments, increases significantly industry (Kurbatova, Khlyap, 2015; Knapp, Jester, 2001). The cost of environmental safety is among the major factors. The increase in the probability of environmental risks during the development of oil and gas fields is due to ageing and wear of equipment, buildings and structures, pipelines, etc. In addition, the number of by-products – reservoir water and oil-containing waste, the safe handling of which requires significant costs, grows many times.

It should also be considered that many deposits have a significant number of detected and undetected long-term pollution of various environments, which require significant investment in their elimination.

Thus, an important aspect is the correct forecasting of the situation in the oil and gas industry of Ukraine and the world in the long run. Our analysis of the situation indicates that in a few decades, oil production at most domestic enterprises may be unprofitable (Mandryk et al., 2015; Fyk et al., 2008). At the same time, the issues of withdrawal from the development of oil and gas fields remain unresolved. No other country in the world has such experience. To some extent, such experience is available in Ukraine in deposits developed since the XVIII century (Boryslav, Bytkiv-Babchenske, Podlasie, etc.).

2. Materials and methods of research

It is effective to use such morphological indicators as the height, and length of the plant and the width of leaves as indicator features. In tree species, it is possible to study such morphological features as the height of the trunks, the height of the attachment of the crown, the first living branch, the height of the peeling bark, the state and rarefaction of the crown, dry tops, and so on. The possibility of a quantitative assessment of these parameters in the comparative analysis of the background and anthropogenically disturbed areas using the apparatus of mathematical statistics increases the reliability and probability of the forecast of anthropogenic succession and conclusions about the current state of ecosystems (Glibovyt'ska, Karavanovych, 2018; Baltrėnaitė et al., 2016). The appearance of ugly forms (terats), the appearance of chlorosis and necrosis is an extreme form of stress.

The transition to quantitative characterization is achieved through the use of scales of necrosis, chlorosis or, for example, the scale of life expectancy of needles (leaves) of trees. The intensity of exposure is diagnosed by the degree of damage (% coverage) of leaves by chlorosis or necrosis, presented in the form of appropriate classes. Morphological indicators of the stand may indicate the deterioration of the habitat as a result of disturbances and (or) pollution. Indicators such as height, diameter, closedness, the average distance between trees, the height of dead torn bark, the height of crown attachment, dryness, presence of dryness and stumps are compared with the corresponding background characteristics. Their changes in comparison with the background values indicate unfavourable conditions for the habitat of tree species.

The area of leaves and the area of their necrosis were identified using the ImageJ program. To do this, we processed digital images of leaves, i.e., found the length and width of the scan image using the CorelDRAW program (Fig. 1).



Fig. 1. Scan image of leaf blades of tree species

The values of the length and width of the scan image are located in a special window (highlighted

in red), the size of the scan image is presented in millimeters (Fig. 2).

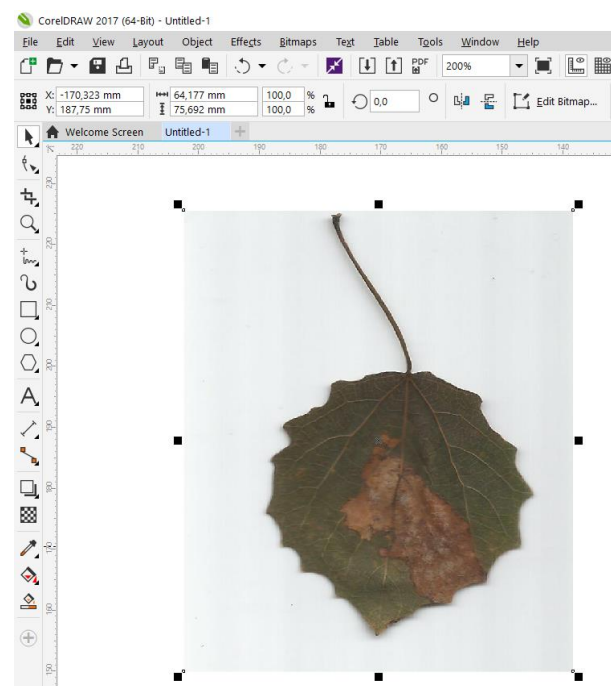


Fig. 2. Measurement of the length and width of the scan image in the CorelDRAW program

After finding the size of the scanned image, we switched to the ImageJ program (Fig. 3).

To calculate the area of necrosis in the ImageJ program window, we clicked the File menu, then the Open button, found the scanned image and opened it (Fig. 3).

To determine the area of leaves and the area of necrosis, scanned images were converted into black and white colours (Fig. 4).

The next step was to calculate the area of leaves and the area of their necrosis. To do this, we clicked Image – Adjust – Threshold. The leaves turned red, then to obtain numerical data, we clicked Analyze – Measure and in a new window in the Area column received the percentage of leaf area from the total area of the scanned image (Fig. 5)

In order to find out the area of necrosis, we dragged the sliders in the Threshold window until the necrosis on the leaf was completely filled with red (Fig. 5).

After obtaining the necessary data, they were substituted into the formula

$$x_{leaf} = a \cdot b \cdot c_{leaf} \quad (1)$$

where x_{leaf} is the leaf plate area in centimetres; a is the length of the scanned image in centimetres; b is the width of the scanned image in centimetres; c_{leaf} is the percentage of the leaf plate area.

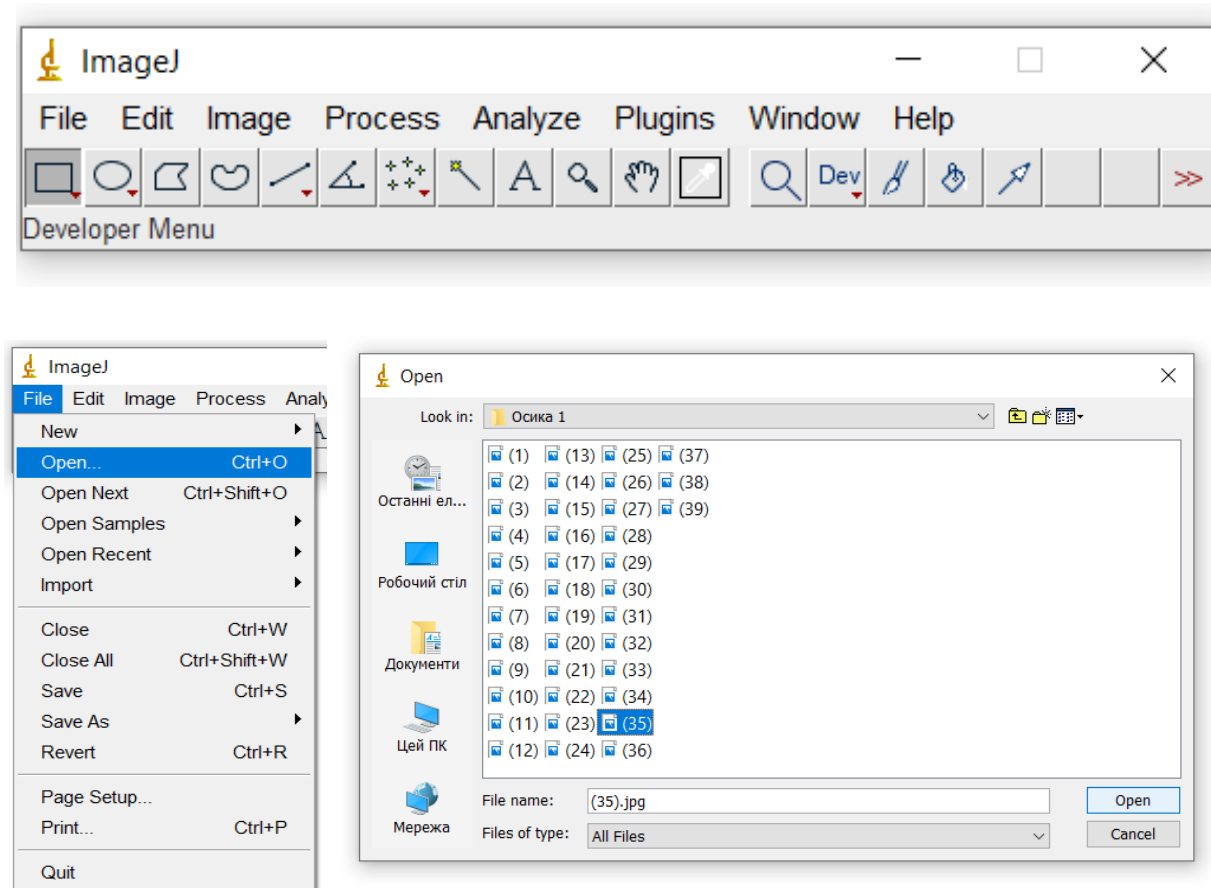


Fig. 3. General view of the ImageJ program window and opening the image

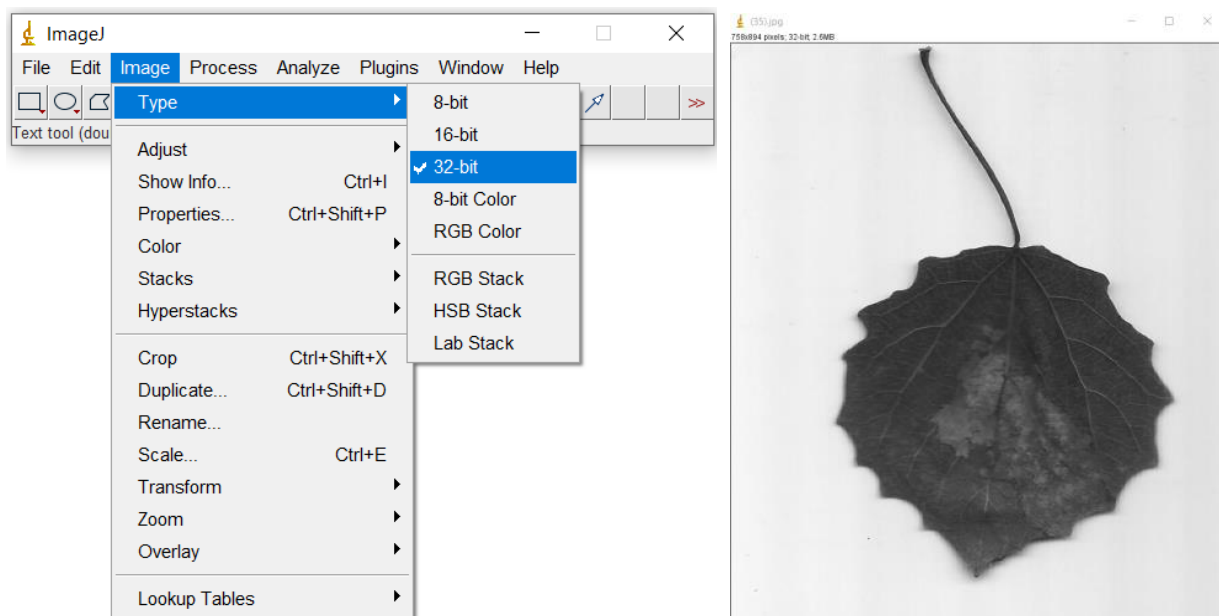


Fig. 4. Converting scanned images into black and white

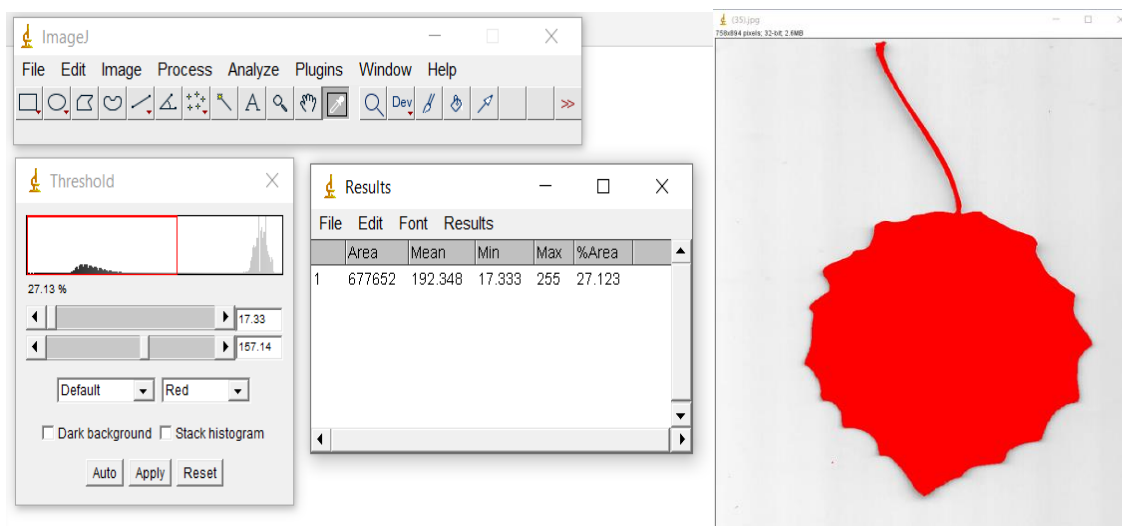


Fig. 5. Determining the percentage of leaf area

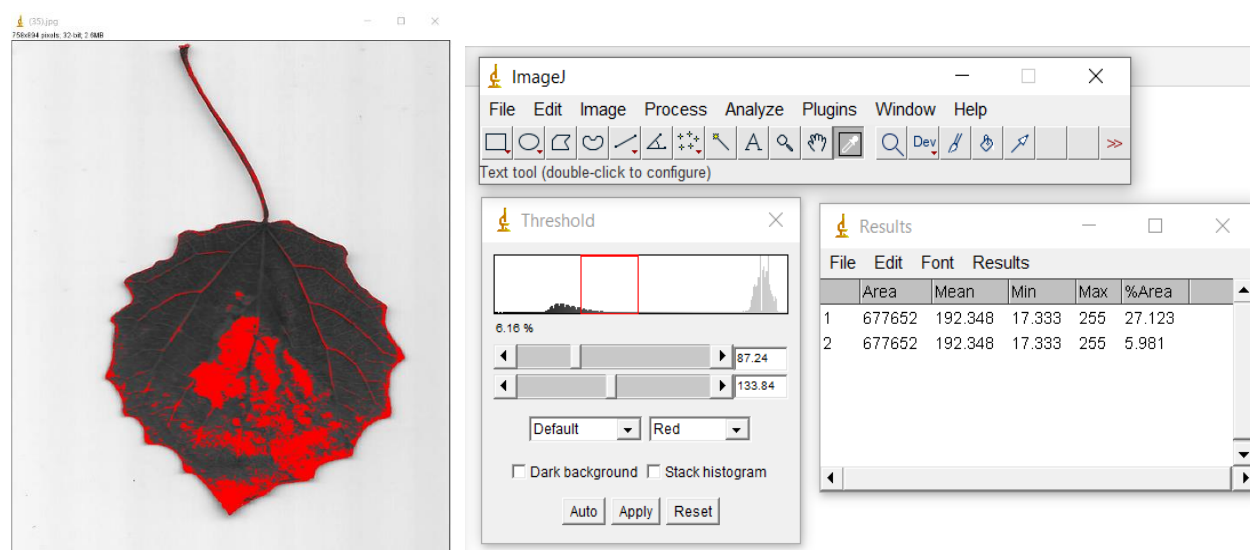


Fig. 6. Determination of the percentage of necrosis area

The area of necrosis was determined by a similar formula with the difference that we substituted the percentage of the area of necrosis

$$x_{necrosis} = a b c_{necrosis} \quad (2)$$

where $x_{necrosis}$ is the area of necrosis in centimeters; a is the length of scan image in centimeters; b is the width of the scan image in centimeters; $c_{necrosis}$ is the percentage of the area of necrosis of the leaf blade.

100 leaf blades of each species were analyzed within the monitoring area. With the help of the above manipulations and calculations, you can calculate the area of leaves and necrosis with great accuracy.

The coefficient of fluctuating asymmetry of plants was determined by the formula:

$$FA = \Sigma (LR) / (L + R) / 2,$$

where L and R are the absolute values of the features considered, respectively, for the left and right parts of the leaf blade.

100 leaf blades of each species were analyzed within the monitoring area.

The presence of necrotic damage to the leaves was established visually. Classification of the detected lesions of the leaf was performed using the following scheme:

- point necrosis – the presence of necrotic lesions in the form of dots on the surface of the leaves;
- marginal necrosis – the death of the edges of the leaf blade;
- interveinal necrosis – the presence of spots on the leaf blade;
- fish skeleton – a combination of interveinal and marginal necrosis.

Table 1

The scale for assessing the deviations of the organism from the conditional norm by the value of the integrated indicator of developmental stability

Mark	The value of the asymmetry index	The value of development stability
I	<0.055	stability of the conditional norm
II	0.055-0.060	slight deviation from the norm
III	0.060-0.065	the average level of deviation from the norm
IV	0.065-0.070	significant deviation from the norm
V	0.07 <	critical condition

Necrotic lesions of 100 leaf blades of each species were examined in the conditions of each monitoring area.

The buffering properties of green leaves are examined on a pH-meter. Leaves weighing 1 g are ground to a homogeneous mass in a porcelain mortar and washed in a glass of 10 ml of distilled water. A day later, the pH of the homogenate is determined and 5 ml of 0.1N of hydrochloric acid is added. Repeated measurements are performed every other day. The difference between the two indicators is the indicator of acid shift (ΔPH), the value of which is used to assess the plant's resistance to man-made conditions. Analytical repetition of experiments on each plant species within the monitoring area is threefold.

3. Results and Discussion

To analyze the dependence of the percentage of necrotization of leaves on the concentration of petroleum products, the experimental and control groups of leaves were considered.

Leaves collected on the territory of Bytkiv-Babchensky deposit were selected for the experimental group. To simplify the calculations, 10 leaf plates of each type were randomly selected from the whole array.

The control group included leaves, which were collected in the background area, i.e. the area that is not contaminated with petroleum products.

To test the hypothesis of the influence of the concentration of petroleum products on leaf necrosis, we use the Fisher – Snedekor test.

Let the sign of the control population X have a normal distribution law with a mathematical expectation μ_x and variance σ_x^2 , and the sign of the experimental set Y obeys the normal distribution law with parameters μ_y and σ_y^2 . For statistical testing of hypotheses, samples are taken from the control population: x_1, x_2, \dots, x_{n_x} , volume n_x and from the experimental population: y_1, y_2, \dots, y_{n_y} , volume n_y .

Statistical test of the hypothesis about the equality of variances of the experimental and control groups (two normal sets):

$H_0: \sigma_y^2 = \sigma_x^2$ is variances of the aggregates are the same;

$H_1: \sigma_y^2 > \sigma_x^2$ is right-hand critical area;

α is the level of significance.

The verification criterion is the value f :

$$f = \frac{S_y^2}{S_x^2} \quad (3)$$

Where

$S_x^2 = \frac{1}{n_x-1} \sum_{i=1}^{n_x} (x_i - \bar{x})^2$ is point estimate of the variance of the control population

$S_y^2 = \frac{1}{n_y-1} \sum_{i=1}^{n_y} (y_i - \bar{y})^2$ is point estimation of the variance of the experimental population

\bar{x}, \bar{y} are sample arithmetic means values of signs X and Y .

Criterion f obeys the Fischer-Snedekor distribution with the numbers of degrees of freedom $\nu_y = n_y - 1$, $\nu_x = n_x - 1$. If the calculated value of the criterion $f > f^*$ (f^* – the critical value of the criterion), the null hypothesis is rejected and it is assumed that the variance of the experimental group is significantly greater than the variance of the control group. Critical value of the criterion $f^* = f^*(1 - \alpha; \nu_y; \nu_x)$ determined according to the Fischer-Snedekor distribution table.

The analysis was performed for birch, willow and linden. In all cases, the value of the Fisher-Snedekor coefficient is within the bilateral critical area, which indicates the effect of the concentration of petroleum products in the soil on the necrotization of leaves.

Experimental data show that the variances of the experimental groups are much larger than the variances of the control groups.

Each object can be described by a set of features. Some of them reflect the state of the object, and the rest – the signs under the influence of which is the object.

Table 2

**Influence of oil products concentration in the soil of Bytkiv-Babchensky field
on necrotization of willow leaves**

No.	Necrosis of leaves of plants in the background	Necrosis of leaves of plants of the deposit
1	0.6494004	1.784016
2	0.63558	0.6850431
3	0.758016	0.8884634
4	0.185313	1.0761696
5	0.1397792	1.9395132
6	0.1612872	1.0593548
7	0.1213446	2.759911
8	0.0360096	5.2291252
9	0.20382	2.8102596
10	0.2579856	2.472218
α	0.05	
Sample size	10	10
Dispersion	0.068106277	1.843095749
The value of the Fisher test	0.03695211	
The lower limit of the Fisher test	0.248385855	
The upper limit of the Fisher test	4.025994158	

Table 3

**Influence of concentration of oil products in the soil of Bytkiv-Babchensky field
on necrotization of linden leaves**

No.	Necrosis of leaves of plants in the background	Necrosis of leaves of plants of the deposit
1	0.0535808	1.9672128
2	0.621954	0.5867056
3	0.5346445	0.6909294
4	0.2019948	0.621954
5	0.4309976	1.7238528
6	0.4845789	3.534996
7	0.4676336	17.823663
8	1.1495484	7.1642363
9	0.4934965	2.5056928
10	0.8100288	2.6637795
α	0.05	
Sample size	10	
Dispersion	0.091346343	27.62378932
The value of the Fisher test	0.0033068	
The lower limit of the Fisher test	0.248385855	
Fisher's upper limit	4.025994158	

Table 4

**Influence of oil products concentration in the soil of Bytkiv-Babchensky field
on necrotization of birch leaves**

No.	Necrosis of leaves of plants in the background	Necrosis of leaves of plants of the deposit
1	2.4822056	1.8543168
2	0.072046	4.79864
3	0.02034	0.17424
4	0.0668312	4.8970404
5	0.1387008	1.1418
6	0.12214	1.7179965
7	0.2370648	2.174436
8	0.0935614	0.5378868
9	0.17424	0.8194095
10	0.1906128	0.5568265
α	0.05	
Sample size	10	10
Dispersion	0.560853918	2.873825651
The value of the Fisher test	0.19515934	
The lower limit of the Fisher test	0.248385855	
Fisher's upper limit	4.025994158	

Table 5

Influence of concentration of oil products on necrosis of birch leaf plates

No.	x_i	y_i	$(x_i - x_c)^2$	$(y_i - y_c)^2$	$(x_i - x_c)(y_i - y_c)$
1	2.482	1.854	4.509	0.000	-0.027
2	0.072	4.799	0.082	8.593	-0.841
3	0.020	0.174	0.115	2.866	0.573
4	0.067	4.897	0.085	9.180	-0.885
5	0.139	1.142	0.048	0.526	0.160
6	0.112	1.718	0.061	0.022	0.037
7	0.237	2.174	0.015	0.094	-0.037
8	0.094	0.538	0.070	1.767	0.353
9	0.174	0.819	0.034	1.098	0.193
10	0.191	0.557	0.028	1.717	0.220
Significance level	0.05				
Sample size	10	10			
Sum			5.048	25.864	-0.254
Arithmetic mean	0.359	1.867			
Correlation coefficient	-0.022			-0.022	
The value of the Student's ratio	-0.063				
The critical value of the Student's ratio	2.306				

In practice, the correlation coefficient is determined by one of the many available samples and thus its numerical value is a random variable. Therefore, before

generalizing the obtained result to the general population, an assessment of the reliability of P or the level of significance $\alpha=1 - P$ correlation is conducted.

To assess the reliability of the correlation coefficient the Z – method or Z – Fisher transformation is used:

$$Z = 0.5 \ln \frac{1+r}{1-r}$$

Since r is a random variable, Z is a random variable too. In contrast to r , the value of Z is practically subject to the normal distribution law (with increasing sample size n , the distribution of the value of Z rapidly approaches the normal distribution law).

The assessment is performed using the Student's ratio:

$$t = \frac{Z}{m_z} = Z\sqrt{n-3},$$

where $m_z = \frac{1}{\sqrt{n-3}}$ is the standard error for the random variable Z .

Using the Student's distribution table by the number of degrees of freedom $v = n - 2$ and the value of t , the level of reliability P or the level of significance $\alpha = 1 - P$ is found according to this scheme.

If $P \geq 0.95$ ($\alpha \leq 0.05$), the correlation between the values of X and Y is considered reliable. At $P < 0.95$ ($\alpha > 0.05$) the correlation between the values of X and Y on the basis of this sample cannot be determined reliably.

After studying samples of willow, linden and birch leaves, correlations were analyzed and it was found that there are nonlinear relationships between them.

Table 6

Influence of concentration of oil products on necrosis of linden leaf plates

No.	x_i	y_i	$(x_i - x_c)^2$	$(y_i - y_c)^2$	$(x_i - x_c)(y_i - y_c)$
1	0.054	1.967	0.222	3.846	0.924
2	0.622	0.587	0.009	11.166	-0.324
3	0.535	0.691	0.000	10.481	-0.032
4	0.202	0.622	0.104	10.932	1.067
5	0.431	1.724	0.009	4.860	0.207
6	0.485	3.535	0.002	0.155	0.016
7	0.468	17.824	0.003	193.081	-0.795
8	1.150	7.164	0.390	10.471	2.021
9	0.493	2.506	0.001	2.024	0.045
10	0.810	2.664	0.081	1.599	-0.361
Significance level	0.05				
Sample size	10	10			
Sum			0.822	248.614	2.769
Arithmetic mean	0.525	3.928			
Correlation coefficient	0.194			0.194	
The value of the Student's ratio	0.558				
The critical value of the Student's ratio	2.306				

Table 7

Influence of oil products concentration on willow leaf necrosis

No.	x_i	y_i	$(x_i - x_c)^2$	$(y_i - y_c)^2$	$(x_i - x_c)(y_i - y_c)$
1	2	3	4	5	6
1	0.649	1.784	0.112	0.082	-0.096
2	0.636	0.685	0.103	1.919	-0.444
3	0.758	0.888	0.196	1.397	-0.524
4	0.185	1.076	0.017	0.989	0.129
5	0.140	1.940	0.031	0.017	0.023
6	0.161	1.059	0.024	1.022	0.155
7	0.121	2.760	0.037	0.475	-0.133
8	0.036	5.229	0.078	9.977	-0.881
9	0.204	2.810	0.012	0.547	-0.082
10	0.258	2.472	0.003	0.161	-0.023
Significance level	0.05				
Sample size	10	10			

Continuation of Table 7

1	2	3	4	5	6
Sum			0.613	16.588	-1.876
Arithmetic mean	0.315	2.070			
Correlation coefficient	-0.588			-0.588	
The value of the Student's ratio	-2.058				
The critical value of the Student's ratio	2.306				

4. Conclusions

1. Morphological and physiological parameters of heart-shaped linden, willow and birch which grow in oil fields were studied. The most informative indicators of the functional state and phytoindication markers of woody plants are singled out and the expediency of their use in assessing the ecological safety of the oil-contaminated environment is substantiated.

2. It is shown that in the conditions of oil pollution of the environment there is a slowdown in the growth and development of woody plants, which is manifested in a decrease in area, linear foliar parameters, the appearance of necrosis and chlorosis.

3. The influence of oil pollution on the necrosis of leaf plates of the studied woody plants was analyzed by the methods of statistical analysis. A clear relationship between leaf necrosis and the level of oil contamination is shown. The results were confirmed by correlation analysis.

4. Morphological and physiological parameters of phytoobjects indicate sufficient adaptability of woody plants, their wide ecological plasticity and the possibility of use as phytoindicators and phytomeliorants of oil-contaminated ecosystems.

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