Bioindication and biomonitoring assessment of the state of atmospheric air and soil in the study area

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Abstract. Monitoring of the natural environment and human habitat must be carried out to compare the ecological state of the contaminated area and places that are not subject to technogenic impact. The main purpose of this work is to assess the state of the atmosphere, hydrosphere and soil using bioindication and biomonitoring methods. Phytotoxicity is the property of the soil to inhibit the growth and development of higher plants due to its contamination with xenobiotics and other toxic substances. In this work, the phytotoxicity of the soil is determined during the experiment on germinating of cucumber and dill seeds. The number of germinated seeds is calculated on differentially polluted soils. A comparative analysis of the of seedlings length and mathematical processing of lengths seedlings data, depending on the level of soil contamination, are carried out.

1. Introduction

Monitoring and assessment of the quality of the environment is the most important component of human activities aimed at using natural resources. There are two approaches to assess the quality of the urbanized environment [1]. The first is to determine the concentration of harmful chemicals in the soil and water using physicochemical and chemical-analytical methods. The second is the assessment of the state of the environment with the help of living organisms: microbes, plants and animals.

Contemporary physicochemical methods do not fully reflect the ecological situation, therefore, it becomes necessary to conduct bioindication studies.

The main purpose of this work is to assess the state of the atmosphere, hydrosphere and soil using bioindication and biomonitoring methods.

Bioindication is the detection and determination of biologically significant anthropogenic loads based on the reactions of living organisms and their communities [2]. An indicator plant is one that shows signs of damage when exposed to a phytotoxic concentration of a pollutant or a mixture of such substances. An indicator plant is a chemical sensor that can detect the presence of a pollutant in the air. Such substances include heavy metals (lead, cadmium, etc.), hydrogen fluoride, ammonia, sulfur oxide, etc. Owing to their...
effect, the plant's growth and maturation rate can change, flowering, fruit and seed formation deteriorate, and, ultimately productivity and yield will decrease.

Phytotoxicity is the property of the soil to inhibit the growth and development of higher plants due to its contamination with xenobiotics and other toxic substances [2].

In this study the phytotoxicity of the soil is determined during the experiment on the germinating cucumber and dill seeds. The number of germinated seeds is calculated on differentially polluted soils. A comparative analysis of seedling lengths is carried out [3]. The following materials were needed to complete the study: 50 g of the soil from each of the four investigated plots; 2 liters of distilled water; saucers with a diameter of more than 10 cm (8 pieces); white cotton cloth; seeds of plants sensitive to soil pollution, i.e. cucumbers and dill in the described version; ruler with 1 mm divisions.

2. Experimental procedure

Soil samples are taken at four sites with varying degrees of pollution. Site # 1 (control) is located in an unpolluted area (in the forest near Lake Abrau), site # 2 - in a residential area in Polynnaia street in the village of Tsemolina, site # 3 – next to the Anapa highway, 50 meters from the road, site # 4 - (dirty site) - is located near the reinforced concrete products plant.

Experimental procedure:
- a suspension of the studied soil is prepared in a glass container with a capacity of 200 ml in a ratio of 1: 5, i.e. 10 g of soil is mixed with 50 ml of distilled water, then the mixture is shaken well;
- the suspension is poured into a saucer in such a way as to cover the bottom with a layer of 3-5 mm. The suspension is covered with cotton cloth in two layers;
- 50 seeds prepared for the experiment are laid out on the surface of the fabric, evenly distributed over the bottom surface;

In the above way, we prepare four saucers with variously contaminated soil for cucumbers and four for dill seeds. We leave them in a dark place at a temperature of 22 ° C.

The first cucumber and dill seedlings begin to sprout within a day. To obtain reliable results, the number of germinated seeds is counted on the third day after the appearance of the first seedlings.

We extend the experiment under the same conditions until the tillering stage. For cucumbers, this period was 15 days, counting from the moment the seeds were placed in suspension, for dill this was 20 days. After this period, seedling lengths were measured and the average length in each dish was calculated. The results obtained are recorded in Table 1.

| Site # | Seedlings (out of 50) | Average seedling length, cm |
|--------|---------------------|-----------------------------|
|        | cucumbers | dill   | cucumbers | dill   |
| 1 control | 41 | 33  | 24,5  | 16,4  |
| 2       | 38 | 30  | 20,1  | 14,2  |
| 3       | 32 | 25  | 16,3  | 11,1  |
| 4       | 24 | 19  | 12,2  | 9,6   |

Let us calculate the percentage of reduction in the number of germinated seeds in contaminated sites relative to the control one according to the formula:

\[ x_i = 100\% - \left( \frac{N_i}{N_4} \times 100\% \right), \quad (1) \]

where:

1. $N_i$ is the number of germinated seeds in the $i$-th contaminated soil site.
2. $N_4$ is the number of germinated seeds in the control soil site.
$N_i$ – the number of germinated seeds in each of three sites,
$N_4$ – the number of germinated seeds in site $\# 4$.

The percentage of seedling length reduction with respect to the control site is determined according to the formula:

$$x_i = 100\% - \left( \frac{N_i}{L_4} \times 100\% \right),$$

where:

$L_4$ – average seedling length in the control site.

We estimate soil phytotoxicity on the basis of the scale given in the methodic aid. The results are recorded in table 2.

### Table 2. Data on soil phytotoxicity based on the percentage of seedling length and number reduction

| Site $\#$ | Percentage of reduction and soil phytotoxicity on the basis of the number of seedlings | On the basis of seedling lengths length |
|-----------|---------------------------------------------|--------------------------------------|
|           | cucumbers                                    | dill                                 |
| 1 control | 9% - weak                                     | 10% - weak                           |
|           |                                             |                                      |
| 2         | 14% - weak                                    | 15% - weak                           |
| 3         | 43% - average                                 | 36% - average                        |
| 4         | 61% – high                                    | 59% - high                           |

Having analyzed the data in the table, we observe a clear dependence of the degree of soil phytotoxicity on the pollution of the studied area.

The highest phytotoxicity is observed in the soil collected on the territory of the concrete products plant (site 4): based on experiments with cucumbers and dill, it is classified as having a high degree of phytotoxicity.

The soil collected at site 3 (along the Anapa highway), according to the results of experiments with cucumbers and dill, is classified as average in terms of phytotoxicity. However, the results of the experiment show that for cucumbers the level of phytotoxicity is 4% lower.

The soil collected at site 2 (Tsemdolina village) is classified as slightly phytotoxic for both studied crops.

Almost similar results are obtained when comparing the average of seedling lengths. Only for the fourth sample of cucumbers the level of soil phytotoxicity is average, while for dill it is high.

Having analyzed the data obtained, we can say that cucumbers are a more sensitive bioindicator, because if we consider variations of soil phytotoxicity levels (both in terms of germination and seedling heights), then its values will turn out to be higher than for dill.

### 3. Mathematical processing of on the seedling lengths data depending on the level of soil contamination

The sprouts of cucumbers and dill separately in each saucer represent a sample. Based on the of seedling lengths in each sample, we will determine the main distribution parameters.

The average of seedling length ($M$) is determined by the formula:
\[ M = \frac{x_1 + x_2 + \ldots + x_N}{N} = \frac{\sum_{j=1}^{N} x_j}{N}, \quad (3) \]

where:
\( x_i \) – length of the seedling \( i \);
\( N \) - the number of sprouted seeds.

The standard deviation (\( \sigma \)) characterizes the degree of the obtained data variation and is determined by the following formula:
\[
\sigma = \sqrt{\frac{\sum_{i=1}^{N} (M - x_j)^2}{N - 1}} = \sqrt{\frac{N \sum_{i=1}^{N} x_i^2 - \left( \sum_{i=1}^{N} x_i \right)^2}{(N - 1)N}}, \quad (4)
\]

The error in arithmetic mean (\( m \)) shows the accuracy of measurements of the obtained data and is determined by the formula:
\[
m = \frac{\sigma}{\sqrt{N}}, \quad (5)
\]

Relative variability of the trait under study is determined using the coefficient of variation (\( K_v \)):
\[
K_v = \frac{\sigma}{M} \cdot 100\% \quad (6)
\]

The calculated basic distribution parameters for each sample are entered into Table 3.

**Table 3.** The main distribution parameters for cucumbers and dill on soils in the studied sites

|     | Cucumbers |          |     | Dill  |          |
|-----|-----------|----------|-----|-------|----------|
| #   | M, cm     | \( \sigma \) | m   | K_v, % | M, cm    | \( \sigma \) | m   | K_v, % |
| 1   | 24,5      | 4,11     | 0,59| 17,3  | 16,4     | 0,89     | 0,16| 8,45  |
| 2   | 20,1      | 3,13     | 0,45| 20,3  | 14,2     | 0,81     | 0,14| 9,56  |
| 3   | 16,3      | 1,83     | 0,38| 20,5  | 11,1     | 0,66     | 0,12| 8,24  |
| 4   | 12,2      | 1,12     | 0,34| 22,1  | 9,6      | 0,41     | 0,09| 6,35  |

Let us determine the reliability of differences in the values of the average seedling lengths, depending on soil contamination based on the Student's criterion (\( t \)-criterion) according to the formula:
\[
t = \frac{M_1 + M_2}{\sqrt{\frac{\sigma_1^2}{N_1} + \frac{\sigma_2^2}{N_2}}}, \quad (7)
\]
where:
\( M_1 \) and \( M_2 \) – arithmetic mean of lengths in two samples;
\( \sigma_1 \) and \( \sigma_2 \) – standard deviations in two samples;
\( N_1 \) and \( N_2 \) – the number of germinated seeds in two samples.

Let us enter the data obtained into table 4.

**Table 4.** The value of Student's criterion (t) for pairs of samples of cucumber and dill seeds germinated on soils with different contamination

| Pairs of samples of cucumber seedlings | Pair of samples of dill seedlings |
|---------------------------------------|----------------------------------|
| 1 and 2                               | 1 and 2                          |
| 1 and 3                               | 1 and 4                          |
| 1 and 4                               | 2 and 3                          |
| 2 and 3                               | 2 and 4                          |
| 3 and 4                               | 3 and 4                          |
| 1 and 2                               | 1 and 3                          |
| 1 and 3                               | 1 and 4                          |
| 1 and 4                               | 2 and 3                          |
| 2 and 3                               | 2 and 4                          |
| 3 and 4                               | 3 and 4                          |

| 48 | 43,3 | 40,8 | 37,8 | 30,8 | 27,1 | 126 | 119 | 120 | 115 | 105 | 99 |

In studies of this type it is assumed that the value of the confidence level (P) is equal to 0.95. The degree of freedom \( V (N_1 + N_2 - 2) \) in all cases under consideration falls within the range from 63 to 175. The standard value of the Student's test (\( t_{st} \)) based on the values of the confidence level and the degree of freedom is 2. Since \( t > t_{st} \), the differences between the mean arithmetic seedling lengths are considered significant with an appropriate degree of probability.

4. **Comparative assessment of the quality of the environment by the state of conifers**

The response of forest ecosystems to unfavorable environmental conditions is manifested in violations of the structure and functions of the entire system and its individual components. These violations are registered by a number of signs that are visible when looking closely at a natural object. The signs are classified visually, without the use of special devices. But in order to notice them and assess the degree of danger, a comparison with the normal state of a tree on a knowingly undisturbed forest area is needed [4].

The following indicators can serve as criteria of the degree of damage to coniferous forests [5]:

- necrosis - necrosis of a site of plant tissue (needles) under the influence of pollutants;
- distal necrosis - early aging of needles and branches under the influence of nitrogen dioxide, ammonia and ethylene;
- top drying - characteristic damage to the upper part of conifers caused by high concentrations of gases, primarily sulfur dioxide;
- chlorosis - early aging of needles under the influence of fluorides, heavy metals and acid precipitation;
- defoliation - lightening, thinning of tree crowns due to the loss of needles in a green state.

5. **Assessment of the state of the environment by the appearance of the Pitsunda pine growing in differentially contaminated areas**

To assess the state of the environment by the appearance of the Pitsunda pine, the same four sites that were tested for phytotoxicity were selected. Pitsunda pine, growing at site # 1, also belongs, according to the visual assessment scale, to category "0" - healthy trees. The bulk of the needles looks healthy and without traces of damage. Pine growing at site # 2, according to the visual assessment scale belongs to "1", a category that indicates the penetration of pollution from the nearest road – the Anapa highway of the Primorsky district, to the residential area. The pine growing at site # 3, according to the visual assessment scale, belongs to category "1" - weakened trees. A loosely transparent crown is noted, damage by insects and diseases covers 32% of the needles. Drying of individual branches in the lower third of the crown is noticeable. Chlorosis and necrosis occupy about 11% of the total area of the tree needles. The tree growing at site # 4, according to the visual assessment scale, belongs to category "2" - severely
weakened trees. Damage and drying of the needles up to 65% are clearly noticeable, especially in the lower part of the crown. Significant damage to the trunk, buttress flares and almost complete absence of growth are clearly visible. Chlorosis and necrosis occupy about 28% of the area of needles of all ages.

Let us determine the reliability of the observed differences in the length of the needles. We will enter the calculation results into table 5.

Table 5. The value of Student's criterion (t) for pairs of shoots of Pitsunda pine growing in the studied sites

| Shoot age | Pairs of compared sites | 1 and 2 | 1 and 3 | 1 and 4 | 2 and 3 | 2 and 4 | 3 and 4 |
|-----------|-------------------------|--------|--------|--------|--------|--------|--------|
| Shoot of 2017 | 112                | 123    | 117    | 98     | 121    | 100    |
| Shoot of 2018 | 110                | 128    | 113    | 87     | 118    | 91     |
| Shoot of 2019 | 89                 | 115    | 102    | 82     | 93     | 88     |
| Shoot of 2020 | 74                 | 97     | 86     | 53     | 65     | 67     |
| Shoot of 2021 | 58                 | 63     | 66     | 46     | 43     | 53     |

It is assumed that the confidence level (P) is equal to 0.95. The degree of freedom (N₁ + N₂ – 2) in all cases under consideration falls within the range from 63 to 175. The standard value of the Student's criterion (tₚ) based on the values of the confidence probability and the degree of freedom is 2. Since t>tₚ, the differences between mean arithmetic needle lengths are considered significant with the corresponding degree of probability.

Based on these data, the data obtained after the experiment with soil phytotoxicity are confirmed. Pitsunda pine is presented here as an indicator, and it can be noted that the dynamics of changes in the results depending on the pollution of the area is similar. The pines, growing near the road, are more damaged than the trees located far from it, and of course the difference between the pines growing in a clean area is more noticeable. It is easy to see from the table that there is a difference both between the needle length and between their width, depending on the place of growth and on the degree of anthropogenic impact on them. The results of processing data on the loss of needles give a complete picture of the dependence of the quantity and quality of needles on the level of pollution. This means that conifers, along with agricultural crops, can serve as good bioindicators of the ecological state of the atmosphere and soil.

6. Conclusion
Monitoring of the natural environment and human habitat must be carried out to compare the ecological state of the contaminated area and places that are not subject to anthropogenic impact.

In this study, biological monitoring method [6] was taken as the basis, as the most effective and visual reflection of the state of the atmosphere, soil on the appearance of plants. As a result, an assessment was made of the state of the atmosphere and soil cover in the Primorsky district of the city of Novorossiyansk using bioindication and biomonitory methods [7]. After carrying out all the experiments and measurements, the following conclusions can be drawn:
- when describing the technogenic load on the study area, it was found that this territory is subject to a fairly strong impact. In this area, there are several hazardous production facilities that affect the environment. Also, vehicles play a significant role in pollution;
- with an integral assessment of the state of the environment for the Pitsunda pine, it can be said that, on average, the state of the vegetation is normal. Trees far from the motorway are healthy. However, near the road, in particular on the Anapa highway street, trees can be classified as weakened;
- when determining the state of their phytotoxicity, we find that, judging by test crops (cucumbers and dill), the soil in the area of the reinforced concrete products plant (research site # 4) is highly
contaminated, since the degree of seed germination is very different compared to the control site (in the forest near Lake Abrau). When determining the seedling lengths of these crops, it turns out that the seedlings of both crops are longer at the control site than at city district cites.

- as a result of mathematical processing, we find that when determining the reliability of differences (t is a criterion) for test cultures, the differences are considered reliable, with the selected confidence level.

Thus, the state of the environment in the Primorskii district raises certain concerns. The large flow of passing freight vehicles in this region causes negative consequences for the environment and vegetation in this district [8]. With a further increase in the cargo flow of trucks, with further development of industrial enterprises in this territory [9], the state of the environment may deteriorate and lead to serious and irreversible consequences.

As recommendations for improving the state of the Primorskii district, the following can be proposed: to build a new bypass road to reduce the flow of cars; to prohibit the construction of new harmful and dangerous enterprises in this area; to increase the number of green spaces along the roads.

References
[1] Alekseenko V A, Maksimovich N G, Alekseenko A V 2017 Geochemical barriers for soil protection in mountainous areas Assessment, restoration and recultivation of soils exposed to mountain influence pp 255-274
[2] Klevtsova M A, Dobrova E A 2019 Bioindication assessment of the dust-collecting ability of Italian poplar leaf blades in the conditions of technogenic urban pollution Assessment and geoinformation mapping of the medical and environmental situation in the territory of the city of Voronezh pp 147-160
[3] Zaikanov V G, Minakova T B, Buldakova E V, Savisko I S 2019 Indices and indicators of geoecological safety of urbanized territories Geocology. Engineering geology, hydrogeology, geocryology 4 94-101
[4] Kurolap S A, Klepikov O V, Prozhorina T I, Klevtsova M A, Vinogradov P M, Umyvakin V M, Sarychev D V 2020 Modern approaches to assessing environmental risks for the population of large industrial cities (urban urban environmental diagnostics) Regional environmental diagnostics of the state of the air environment of industrial cities pp 6-35
[5] Klevtsova M A 2019 Assessment of the ecological state of green spaces in urbanized territories Green infrastructure of the urban environment: current state and prospects of development pp 93-96
[6] Kurolap S A, Klepikov O V, Akimov E L 2017 Assessment of the impact of industrial and transport infrastructure on technogenic pollution of the atmosphere and soil of the city of Voronezh Theory and practice of harmonization of interaction of natural, social and production systems of the region pp 129-132
[7] Buldakova E V, Zaikanov V G, Minakova T B 2020 Ecology and Society: balance of interests Assessment of the geoecological safety of Russian cities for sustainable development pp 285-289
[8] Alekseenko V A, Beh J, Alekseenko A A, Shvydkaya N V, Roka N 2018 Environmental impact of coal mining waste disposal on soils and plants in the Rostov region Russia Journal of Geochemical Exploration 184 261-270
[9] Modina M A, Skoda V V, Kashin Ya M 2018 Ecological status of Tsemesskaya bay Materials and methods of innovative research and development pp 35-37