Bioindication of Soil Based on Differences in Parameters of Development of the Indicator Species in Soils of Different Territories of Vladivostok

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Abstract. In the process of interaction of the man with the habitat he is an integral part of the natural environment. To assess the anthropogenic impact upon the environment, living bioindicators are used which have several advantages over chemical methods of environmental assessment. They can respond even to any minor changes and enable to avoid the use of expensive and labor-intensive physical and chemical methods for measuring biological parameters. The assessment of a degree of soil contamination using bioindication, in places of oil products storage and active contamination from motor transport (central city), enables to determine a soil toxicity. Environmental toxicity can be determined by a level of response of the test object used. Reliability of results is confirmed by environmental statistical methods or biometrics. In this study, we obtained data that, taking into account the geographical location of the sampling point, the results demonstrated both a low toxicity of the soil and a stimulating effect of man-made environmental factors upon its state.

1. Introduction
The man deals with various activities throughout the entire life cycle being in various spheres of existence: natural, industrial, social, domestic [1-7]. In so doing, the man constantly interacts with the habitat being an integral component of the natural environment. In the course of his activity, the man changes the environment, often negatively affects its natural dynamic balance [8].

2. Relevance, scientific importance of the problem with a brief review of literature
Bioindication is an assessment of the state of the environment using living organisms, i.e its biota [9]. Relevance of the study of the environmental status by bioindication method is due to the fact that it enables to judge about a status of the entire ecosystem at that time by several specific parameters, predict a direction of changes occurring in the ecosystem, and timely prevent any negative effects. Bioindication is based on monitoring the composition and number, as well as biological parameters of indicator species [10-12].

Currently, the assessment of soil contamination and its toxicity is carried out using various biotesting methods which are widely used to determine toxic properties of the environment (air, water, soil, industrial waste, etc.).

The ability to protect against the action of adverse environmental factors is a mandatory feature of any organism. The latter, depending on extreme conditions of the habitat, impact of adverse factors,
responds to them manifesting in certain specific and nonspecific reactions. Biological systems try to maintain the functional stability of the organism when environmental conditions change, i.e. adapt it to the particular environmental conditions. An individual achieves this through physiological mechanisms, and populations of organisms achieve the same due to genetic variation and heredity. Since the vital functions of an organism or a community of organisms are very closely correlated with certain environmental factors, they can be used to assess a status of ecosystems, i.e. can serve as bioindicators [13].

Indicator’s functions are performed by the species that has a narrow amplitude of environmental tolerance with respect to any factor. Most of the indicators are plants - organisms that are not capable of active movement [14].

Living bioindicators have some advantages over chemical methods for assessing the environmental status that are widely used at present:

1. They summarize each and all biologically important data on the environment and reflect its state as a whole.
2. Under chronic anthropogenic stress, bioindicators can react to very weak exposures due to dose accumulation.
3. Exclude a need to record physical and chemical environmental parameters.
4. Make it unnecessary to use expensive and time-consuming physical and chemical methods for measuring biological parameters; living organisms are constantly present in the human environment and react to short-term and burst releases of toxicants that can not be recorded using an automatic control system with periodic sampling.
5. Record a speed of changes taken place in the environment.
6. Indicate the ways and places of accumulations of various contaminations in ecological systems and possible ways for these substances to get into human food.
7. Allow to judge a degree of harmfulness of substances synthesized by the man for the nature and the man, and allow to control the effect of these substances.
8. Help to normalize a permissible load on ecosystems that differ in their resistance to anthropogenic impact since the same composition and volume of contaminations can result in different reactions of natural systems in various geographical areas.

All these advantages also determine a commensurability of using bioindication methods and relevance of studying this method of assessing the ecosystem status [15].

3. Task definition
To assess a soil state based on differences in developmental parameters of indicator species in soils of two different territories of Vladivostok, by means of classification of soil samples by toxicity classes.

4. Theoretical part
Soil toxicity can be determined by a level of response of the test object used.

Biotesting of soils was conducted using “Growth test”. Tests were conducted in Petri dishes [16]. Frequency of the test [17] and temperature conditions of testing [18-19] are taken according to R.R. Kabirov (1997).

To assess the factor’s effect on the bioindicator, a classification developed by R. R. Kabirov (1997) was used, which is presented in Table1 [17].

Calculation of a level of the factor’s effect on the bioindicator was made from the formula [18]:

\[ ITF = \frac{TF_d}{TF_k} \]

where \( TF_d \) – values of recorded test function in the experiment;
\( TF_k \) – values of recorded test function in control.
Table 1. Classification of a level of the factor’s effect in the bioindicator.

| Toxicity class                | ITF value | Explanations                                                                                   |
|-------------------------------|-----------|-----------------------------------------------------------------------------------------------|
| VI (stimulation)              | >1.10     | Factor produces a stimulating effect on bioindicator. Test function value exceed the control value. |
| V (norm)                      | 0.91-1.09 | Does not produce essential effect on bioindicator development. Test function value is on the control level |
| IV (low toxicity)             | 0.71-0.90 | Different degree of decrease of test function in the experiment in comparison with control      |
| III (average toxicity)        | 0.51-0.70 |                                                                                               |
| II (high toxicity)            | 0.31-0.50 |                                                                                               |
| I (extremely high toxicity)   | <0.30     | Death of bioindicators                                                                      |

Mathematical processing of the results obtained in the study of selected biological parameters (length of seedlings and roots) of the testing culture.

For mathematical processing of the results obtained in the study of selected biological parameters of the testing culture using biological methods, biological statistics or biometrics is used. According to data by L. A. Vasileva (2007) this area of scientific knowledge covers the classification, systematization and processing of experimental data in biology, medicine and agriculture by mathematical statistical methods [20].

The arithmetic mean value is a very important parameter characterizing the sample multitude. This value is a generalized characteristic of the multitude.

The arithmetic mean value is calculated from the formula:

$$\bar{x} = \frac{\sum x_i}{n}$$  \hspace{1cm} (1)

where $x_i$ – value of i indicator;

n – quantity of indicators of i value.

A more exact indicator characterizing a variation or scattering of variants around the arithmetic mean value is a sum of squares of deviations of the multitude from the mean. This characteristic of the multitude is referred to as a dispersion and is denoted by $S$ symbol.

Dispersion is calculated from the formula:

$$S = \sum (x_i - \bar{x})^2$$  \hspace{1cm} (2)

Dispersion accumulates as a count of multitude increases just as $\sum x_i$ accumulates when estimating the arithmetic mean value. In two multitudes of different volume with the same variation, a value of dispersion will be higher in that multitude in which a count is larger. Therefore, dispersion should be averaged, namely, should be divided into the number of variations in the multitude.

The averaged value of dispersion is referred to as the variance and is calculated from the formula:

$$\sigma^2 = \frac{S}{n}$$  \hspace{1cm} (3)

$\sigma$ is referred to as the mean-square deviation. Value $\sigma$ is the basic indicator characterizing a variability of the analyzed sample multitude [20].

The analysis includes uniform signs (live weight in kg, length in cm) and it is necessary to know by what sign the variability is higher. The variability should be expressed in relative measure. This measure is called a coefficient of variation,% (CV) and is calculated from the formula:

$$CV = \frac{\sigma \times 100}{\bar{x}}$$  \hspace{1cm} (4)
F-ratio test (F-distribution) is in existence to verify a hypothesis for equality of general dispersions of general multitudes, is calculated from the formula:

\[ F = \frac{s_1^2}{s_2^2} \]  

providing that \( s_1^2 \geq s_2^2 \).

It is customary to take the ratio of greater dispersion variance to lesser one, therefore, in this case, ratio \( F \geq 1 \).

The greater the inequality between the sample dispersions, the greater the value \( F \) will be, and vice versa, the smaller the difference between the dispersions, the smaller the value \( F \) will be. The value \( F \) has a continuous distribution function and depends only on the degrees of freedom, which are calculated from the following formulas:

\[ k_1 = n_1 - 1, \]

\[ k_2 = n_2 - 1, \]

where \( k_1, k_2 \) – numbers of degrees of freedom.

F-ratio is completely determined by sample dispersions.

5. Practical significance, suggestions and results of implementations, results of experimental studies

In our study, we monitored over plants in a seedling phase (12-day barley seedlings) [21]. Spring barley (Galactic grade) was used as a testing culture.

Biological parameters were a length of seedlings and roots. Soil samples for biotesting were taken by envelope method in places of storage of oil products and active contamination from motor transport (central city). As a control sample in the study, soil samples were taken from the Botanic Garden territory of the Far Eastern Branch of the Russian Academy of Sciences located in Vladivostok (sample №6).

6 test soil samples were taken in the territories:

1-4 sample: area of OJSC Primornefteproduct, 44, Ostryakov Ave.

5 sample: territory of Vladivostok State University of Economics and service, 41, Gogol Str.

The obtained values of test functions are presented in table 2, and a toxicity index of the assessed factor is calculated on their basis (table 3).

| Table 2. Values of test functions on soils from different sampling points. |
|-----------------------------|----------------|----------------|----------------|----------------|----------------|----------------|
|                            | 1              | 2              | 3              | 4              | 5              | 6 (sample)    |
| Root length                | 4.95±0.08      | 9.54±0.19      | 5.98±0.11      | 4.43±0.22      | 4.44±0.16      | 5.98±0.10      |
| Seedling height            | 15.10±0.14     | 14.91±0.15     | 16.95±0.22     | 17.08±0.16     | 17.69±0.15     | 16.94±0.14     |
| Average value              | 10.02          | 11.46          | 11.57          | 10.76          | 11.06          | 11.46          |

| Table 3. Toxicity index of the assessed factor for soils from different sampling points. |
|---------------------------------|---------------|---------------|---------------|---------------|---------------|
| Toxicity index                  | 0.86          | 1.1           | 0.96          | 0.78          | 0.87          |
Based on the tabulated data we can distribute soil samples by toxicity classes:
1 point - IV (low toxicity)
2 point – VI (stimulation)
3 point - V (norm)
4 point - IV (low toxicity)
5 point - IV (low toxicity)
F-ratio test is within 1.02 to 1.45.
Variation is considered average and varies within 11–25 %.

6. Conclusions
As a result of mathematical processing of the results obtained in the study of selected biological parameters of the testing culture, we concluded that the soil was toxic, the samples of which were taken in different districts of Vladivostok.

Distribution of soils as to toxicity degree:
1.4 point - IV (low toxicity). Located in the area actually protected against wind from two or three sides. Only in one case, natural objects act as protection, and in the other case, these are structures that allow toxic substances to accumulate in these points.
2 point – VI (stimulation). Stimulation is noted mainly due to changes in the root length (with a standard length of the seedling), which is associated with a different type of soil (typical technosol). In view of a difficulty of obtaining nutrients from an indicator plant, an excessive development of the root system occurs.
3 point – V (norm). Associated with a geographical location of the sampling point (located on a hill windswept from all sides).
5 point – IV (low toxicity). Samples were taken along Krasnogo Znemeni Ave, on the hill. The main source of contamination includes motor vehicles. Due to hilly terrain, a concentration of pollutants decreases.

7. References
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