Biotesting in the assessment of vineyard soil toxicity

Marina Modina[1,*], *Eugene* Heckert[1], *Valentin* Shkoda[2] and *Julia* Kuznetsova[1]

[1] Admiral Ushakov Maritime State University, 93, Lenin Ave., Novorossiysk, 353924, Russian Federation
[2]A.K. Serov Krasnodar Higher Military Aviation School of Pilots

Abstract. The data on contamination of vineyard soil with heavy metals, pesticides, macro- and microelements in the system were reviewed. Biological methods allow establishing the degree of general pollution and general toxicity of environmental objects for living organisms and expediency of their further detailed analysis by chemical, physico-chemical and physical methods. Biochemically active substances, as a rule, in large concentrations are highly toxic for living organisms (Hg, Cd, Pb, Ni, Cr, Cu, etc.). The factors causing soil contamination are presented, the application of biotesting for different soil contamination is analysed. The results of the impact of soil pollution on the environment are described. In connection with the ever-increasing technogenic load, the solution of the most important problem of vineyard soil contamination and increase of ecological safety of the grape-growing products obtained afterwards require a complex approach, which is urgent.

Keywords: biotesting, phytotesting, toxicity, heavy metals.

1 introduction

Myskhako is a village within the municipal formation of Novorossiysk, Krasnodar Krai. It is the centre of the Myskhako Rural District. The village is located on the Black Sea coast, 6 kilometres from the centre of Novorossiysk. Myskhako includes the eastern slopes of Mount Koldun.

The climate in Myskhako is subtropical Mediterranean. Summers in Myskhako are hot: the average temperature is 28 degrees above zero. Winter is mild and moderate: the average temperature is about 3 degrees above zero. In winter there is often a nord-ost (north-east wind). The bathing season lasts from June to October. The average water temperature is 20 degrees in summer and 6 degrees above zero in winter. Winemaking and tourist business are developed in Myskhako. Myskhako is home to one of the oldest winemaking companies in Russia: Agrofirma Myskhako CJSC.

Predominant soils of the farm are sod-carbonate soils of different thickness and composition. These soils are located on slopes of various directions and thus, there is no confinement to slopes of certain exposures.

The area of vineyards of Agrofirma Myskhako is 266 ha. Plans to realise the planting of 304 hectares of vineyards remain unchanged, the terms have been increased due to the study of the unique varietal composition.

Fig. 1. Area of vineyards of Agrofirm Myskhako

Industrial vine plantations are a complex agrobiological system, where the risk of socio-ecological effects due to contamination of agro-land by pollutants of various origins increases annually.

The issues of studying the existing ecological problem are currently receiving serious attention. At the same time, out of the entire volume of scientific literature on environmental studies, only a small part of it is devoted to perennial plantations. At the same time, they are the most exposed to toxic substances [1–3]. Absorption capacity of soil, carbonate content in it, close to neutral reaction of the environment and a number of other features to different degrees create special "specific" conditions for the "existence" of its pollutants. This leads to the fact that the soil, and especially its upper arable layer, acquires the characteristic status of a "bank of accumulator" of toxic substances [4–7]. In soil, as one of the main eco-objects, processes of synthesis

* Corresponding author: marishamodina@yandex.ru

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and destruction of organic matter occur continuously. Along with them, there is a cycle of elements of ash and nitrogen nutrition of plants in conjunction with incomplete detoxification and removal of toxic substances. As a result, the undesirable impact of hazardous chemicals on agro-lands of vine plantations is aggravated, which causes their contamination of grape products [8, 9].

2 Materials and methods

The research was carried out in viticultural zones of the south of Kuban (Myskhako settlement) on industrial plantations of specialised farms. Objects of research are vine plantations of table and technical varieties, vineyard soil, grape berries. Toxic residues were determined in soil samples.

One of the main problems of the food industry is heavy metal pollution. Among chemical elements, heavy metals are the most toxic and are second only to pesticides in terms of their level of danger. The toxic ones include Co, Ni, Cu, Zn, Sn, As, Se, Te, Rb, Ag, Cd, Au, Hg, Pb, Sb, Bi, Pt. They get from the soil into various types of agricultural plants, distinguished by a specific ability to accumulate certain heavy metals, and then into food products, causing various forms of chronic poisoning and harm national health [5, 8]. The most appropriate method for determining the integral toxicity of vineyard soil is biotesting [17–20].

3 Results and discussion

The purpose of this work was to determine the toxicity of soils of vineyards of Agrofirma "Myskhako" and the territory next to it by the phytotesting method using sown oats (Avena sativa L.) and barley (Hordeum vulgare L.) of CV Triumph variety as test crops. In the process of work, we determined the level of soil contamination at the selected sites and assessed phytotoxicity of soils of the selected sites by seed germination energy and morphological parameters of seedlings and roots of sown oats and barley [2–4].

Nowadays, a large number of methods can be found to prevent environmental pollution, including those used in the field of agro-industrial complex. One such method is biotesting, which establishes the presence of toxicity in the environment. The essence of biotesting is to establish the effects of toxicants on the organisms selected for testing under laboratory conditions, recording indicators such as behavioural, physiological or biochemical. This method focuses on analysing the full toxic effects of all types of pollutants using test subjects.

Phytotoxicity is the property of contaminated soil to inhibit seed germination, growth and development of seedlings. The onset of phytotoxicity is correlated with MAC [18–22].

The objects of the study were soils experiencing different intensities of transport and industrial impact.

In the process of work, the biotesting of substrate toxicity was carried out on seedlings of indicator plants based on the soil surface survey technique. This method has high sensitivity, versatility, integrality and simplicity.

Equipment, materials for testing solid substrates are 1) plastic cups; 2) tweezers; 3) watering tubes; 4) film; 5) test object; 6) test-plant shoots [6].

Solid substrates (soil, crushed peat) are tested as follows. The substrate is placed in a cup, moistened with an equal amount of water. Seeds of test plants are preliminarily soaked in steadfast and purified tap water, spread on two layers of filtered paper in a large cuvette, placed in a thermostat for germination at a temperature of +25–+26^{0}C.

When the length of coleoptiles reaches 10-15 mm, and roots appear, shoots are divided into fractions according to their length and 5 plants of each fraction are planted in cups on the test substrate. The control is a substrate taken in a relatively clean area. Watering is done through a tube with tempered water and purified tap water. When sprouts reach a length of 6–10 cm (after 1–2 weeks), they are measured and weighed. Sprouts are divided into parts (above-ground part, roots) and each part is measured and weighed separately. Almost all seeds can be used as test plants. The data are processed statistically [7, 8, 15, 16].

The proposed method of biological evaluation of substrates or solutions is carried out in the following way: growing plants on substrates whose toxicity is to be evaluated (soil, water).

This method gives very good results in assessing the toxicity of certain substrates.

Soil samples were taken from a depth of 20 to 40 cm from 1m× 1m plots using the envelope method over the entire area (except for paved areas). Control sampling is 3–5 % of the total number of samples.

Soil samples for analysis were collected from 6 different sites.

All soil samples were collected and prepared according to standard sampling and sample preparation techniques [3].

The content of mobile forms of Pb, Cd, Cu and Zn was determined in each soil sample (extracted with acetate-ammonium buffer, pH=4.6). The analysis was carried out in triplicate, in three parallel samples on the voltammetric analyser TA-Lab, which is designed for highly sensitive measurements of the content of toxic impurities in aqueous solutions of soil samples [4, 9].

The analyses are summarised in Table 1.

Table 1. Content of mobile forms of heavy metals in Novorossiysk city soils (extracted with acetate-ammonium buffer, pH=4.6) (mg/kg)

No.	Depth of selection, cm	Metal concentration, mg/kg			
		Pb	Cd	Cu	Zn
1	0–20	2.6	0.044	1.06	2,6
2	0–20	4.6	0.048	0.064	6,6
3	0–20	4.44	0.026	1.64	6,4
4	0–20	2.56	0.014	0.66	6,2
5	0–20	5.8	0.126	0.266	6,04
6	0–20	6.4	0.126	1.46	4,4
MPC		6.0	–	3.0	23.0

Therefore, it was established that the content of mobile forms of Pb, Cd, Cu, and Zn in sample No. 1 did not exceed the MPC, so we consider it as an environmental control. In sample No. 3–6 no exceedance was recorded. Currently, to determine the toxicity of soils by biotesting methods, toxicity indicators of aqueous extracts from soils are used [10–12].

The shorter length of shoots and roots of seedlings in the studied soil indicates their growth retardation, and therefore, possible toxicity. Decrease in the number of seedlings in contaminated soil, compared to the control, of more than several times, indicates significant degradation of soil, reduction of its productivity, loss of soil self-purification ability.

Sprout root lengths and seed germination energies are summarised in Table 2.

Table 2. Mean values of considered test functions in soil biotesting

Platforms	Sprout length, cm		Root length, cm		Energy germination, %	
	Oats	Barley	Oats	Barley	Oats	Barley
1	6.0	6.2	8.00	6.6	86	86.6
2	5.66	6.0	8.86	7.6	86	86.4
3	8.46	8.04	7.06	8.8	96.6	86.2
4	8.6	6.88	7.6	8.4	96.2	80.6
5	6.46	8.68	8.2	8.2	94	94
6	8.6	7.26	10.6	6.88	96	88

4 Conclusion

Nowadays it is necessary to have such methods of soil contamination assessment, which could give an objective idea of soil condition, i.e. to what extent it is able to fulfil its assigned functions. The considered methods, such as biotesting and biodiagnostics of contaminated soils fulfil the requirements of modern times for the study of contaminated soils.

Soil productivity primarily depends on the composition of soil solution (its acidity, content of heavy metals, pesticides, synthetic detergents, petroleum products and nutrients) and can be assessed by the phytotoxicity value. Phytotoxicity is the property of contaminated soil to inhibit seed germination, growth and development of seedlings. The onset of phytotoxicity is correlated with MAC and EPC. Reduction in the number of seedlings in contaminated soil in comparison with the control of more than 2 times indicates significant degradation of soil and reduction of its productivity, loss of soil self-purification ability, as well as changes in the composition, number and structure of microflora and mesofauna.

Heavy metals are protoplasmic poisons whose toxicity increases with increasing atomic mass. Many heavy metals inhibit enzyme activity, form complex compounds with organic substances that easily penetrate cell membranes, bind sulfates, phosphates, and common metabolites, disrupting metabolism, and enhance degradation of some important compounds, such as ATP [17–22, 24, 25].

Phytotoxicity is usually determined when monitoring chemically contaminated soils, identifying areas of ecological disasters and ecological catastrophes, assessing the possibility of using chemically contaminated soils for agricultural production. It allows assessing the possibility of using various kinds of wastes as ameliorants or fertilisers: sewage sludge, various kinds of composts, hydrolysed lignin. Currently, various methods are used to determine the phytotoxicity of soils: the method of seedlings, the method of determining the depression of guttation, or the release of moisture by the tips of plant leaves when the growth of roots outpaces that of leaves, etc. [17–22].

Hygienic indicators based on norms are often alternative for diagnosing the condition of territories and provide more information about the degree and structure of chemical pollution of soils [17].

Diagnostics of the state of territories provides great informativeness about the degree and structure of chemical pollution of soils. And quantitative analysis of the level of pollution of the selected objects has shown that if the content of each, exceeding MAC, a polluting agent is considered individually, the analysed soils can be considered polluted.

To prevent negative consequences of soil and agro-land contamination with various toxicants (e.g., toxic metals, nitrates, nitrites, nitrosoamines, pesticides, mycotoxins, etc.), it is necessary to know the specifics of the impact of chemicals on biological processes in the soil and to study the mechanisms of soil and plant resistance to such contamination. In particular, it concerns perennial plantations of monoculture: vineyards [5, 23]. Modern agrochemicals directly affect agrocenoses (agroecosystems) and their productivity, and they include chemical plant protection products, mineral fertilisers, plant growth regulators. It is necessary to change the current agrochemical concept of farming to a biotechnological one, as the use of agrochemicals in farming causes a number of undesirable consequences. These are, first of all, deterioration of soil properties (structure, water permeability, aeration, reduction in the amount of mobile forms of nitrogen, phosphorus and potassium), contamination of the environment with harmful substances, reduction in the quality of agricultural products due to the accumulation of toxic elements [17–22, 25].

Hence, the solution of such important problem as soil contamination and improvement of ecological safety of winery products requires a comprehensive approach and is urgent. In connection with the ever-increasing anthropogenic load, it is necessary to update and improve the developments to reduce it, to carry out the constant ecological and toxicological monitoring of the soil of vineyards, grown grapes and subsequently obtained products.

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