UDC 504.054:543.95

DOI: 10.15372/CSD2019148

Evaluation of the Acute and Chronic Toxicity of Reagents for the Treatment of Oil-Contaminated Soils and Sludge

O. A. KULIKOVA[1], E. A. MAZLOVA[1], V. A. TEREKHOVA[2,3], M. V. AGADZHANYAN[1], P. V. UCHANOV[4]

[1]*Gubkin Russian State University of Oil and Gas (National Research University), Moscow, Russia*

E-mail: kulikovaoa@bk.ru

[2]*Lomonosov Moscow State University, Moscow, Russia*

[3]*Plekhanov Russian University of Economics, Moscow, Russia*

[4]*Severtsov Institute of Ecology and Evolution, Russian Academy of Sciences, Moscow, Russia*

(Received December 08, 2018; revised February 10, 2019)

Abstract

The acute and chronic toxicity of several chemical reagents based on surfactants and used to treat oil-contaminated soils and sludge was evaluated. Biotesting based on the response of invertebrates and higher plants allowed us to conclude that the tested chemical reagents can be applied with effective concentrations that are safe for ecosystems. The effective concentrations were established for the phytoeffects of inhibiting the growth and development of two types of test plants (oil radish – *Brassica rapa CrGC* syn. Rbr, and cultivated oat – *Avena sativa* L.) and for the survival ability of the standardized test culture of *Paramecium caudatum* Ehrenberg.

Keywords: surfactants, reagent treatment, oil-contaminated soils, oil sludge, biotesting, ecotoxicity

INTRODUCTION

Environmental pollution with oil and oil products remains an essential problem for ecological safety and sustainable development of ecosystems and natural complexes. Among diverse methods of purification of natural media and technogenic substrates, much attention is paid to washing oil-polluted soil and ground with reagents – hot solutions of surfactants (SAS). This method is implemented in agreement with GOST R 57447–2017 "The best technological achievements. Recultivation of land and plots polluted with oil and oil products. General provisions". Among known and widely used reagents, and new compounds and substances recommended by manufacturers for the purification of soil and ground from oil products, entering the market nowadays with a high rate, there are many compounds that may turn out to be rather dangerous for ecosystems in the environment. Possible secondary pollution with hot reagents based on SAS is extremely rarely discussed during substantiation of their application for the purification of sludge, ground and soil from oil and oil products.

Evaluation of chemical reagents on the basis of their ecotoxicity parameters for ground systems is not provided in the legislation, while for water-based systems there are state standards GOST 32419–2013, GOST 32425–2013. Few documents regulating the use of potentially toxic reagents may include the Technical Regulations of the Eurasian Economic Union "About the safety of chemical products" (TR EEU 041/2017), and the project of the Technical Regulations of the Customs Union "About the safety of synthetic cleansing agents and the household chemical goods" (TR CU__/2013), in which the use of reagents referred to the 1st and 2nd classes of danger is not allowed.

Since June 1, 2007 a specialised Regulation is valid in the countries of Europe: REACH (Registration, Evaluation, Authorisation of CHemicals). This regulation formulates the requirements to the ecotoxicological safety of chemical products and obliges the manufacturers to carry out ecological and toxicological tests of the reagents under production. However, this practice is not duly applied in the Russian Federation yet.

As a consequence, at present, there are no direct standard requirements for the evaluation of the ecological safety of reagents used to purify oil-polluted grounds (OPG) and to process oil sludge (OS). However, it is evident that the ecological safety of the use of definite concentrations of reagents based on SAS involved in these processes should be reasoned.

In the world practice, such evaluation of SAS is made using different versions of biotesting [1–4]. Test results give the criteria for the choice of a reagent for processing OPG which are essential for the conservation of life [5]. The difficulty is connected with the fact that test organisms in different test systems do not always provide a synchronous and unambiguous response to the actions of a similar type.

The criteria of the determination of waste toxicity valid in the Russian Federation imply the use of two species of hydrocole test organisms belonging to different taxonomic groups (Order of the Ministry of Natural Resources and Ecology of the RF, December 4, 2014, No. 536 "On the approval of the Criteria for the assignment of wastes to the I–V classes of danger according to the degree of the negative action on the environment"). We think that the efficiency of the toxicity evaluation for oil-containing substrates according to the reaction of hydrocoles alone, which characterizes the effect of the aqueous extract from oil-containing samples on water-based organisms, is low. With these substrates, it is reasonable to analyze the interaction of solid samples and test organisms in direct contact.

Comparison between the responses of test systems has never been carried out in the analysis of ecotoxicity of reagents containing SAS of different nature on the basis of applicate (when added into soil) and eluate (into aqueous solutions) biotesting methods.

The goal of the work was to characterize the ecotoxicity of reagents recommended for the treatment of OPG and OS in the versions of acute and chronic phytotesting, as well as on the basis of the response of hydrocoles – a test culture of protozoa (infusoria). The results of these tests will allow us to elaborate an approach to the choice of efficient concentrations of ecologically safe reagent providing the efficiency of its application at different stages of the use of oil-containing substrates.

EXPERIMENTAL

Materials

A number of grades of the reagents containing various SAS were chosen as the material for investigation (Table 1). The individual reference substance was NaOH, used as the defatting reagent in many technological processes.

Methods and procedures of investigation

Phytotoxicity of SAS-containing reagents was evaluated on the basis of the responses of two plant species: rapid-growing oil-yielding radish (*Brassica rapa CrGC* syn. Rbr) and cultivated oat (*Avena sativa* L.) in two versions of phytotesting.

Chronic phytotoxicity was determined according to the State Standard GOST R ISO 22030–2009 and ISO 11269-2:2012 in vegetation vessels. As test functions, we analysed the parameters of growth and development of plant seedlings (the green mass of plants, seedling length, root length). The duration of the experiment was 14 days, according to ISO 11269-2:2012. Initial parameters of the germinating power of the plants chosen for the experiment corresponded to GOST 12038–84 and were 97.5 % for rapid-growing radish and 94.5 % for cultivated oat.

To establish the phytotoxic concentrations of reagents using the applicate method, the Zhirnozem ground of PeterPeat grade (Russia) based on high-moor and valley peat with the addition of agroperlite, river sand, limestone powder and a complex mineral fertilizer was used in vegetation vessels. Peat ground was dried preliminarily, then sieved to remove the coarse fraction and inclusions larger than 5 mm.

Acute phytotoxicity was determined using a plate method in the Phytoscan test system ac-

TABLE 1

Characteristics of reagents under investigation

SAS No.	Grade of SAS-containing reagent	SAS class*	Characterisation of the substantial composition
1	Sulphonol NP-1 [6]	ASAS	Alkylbenzenesulphonate (C_{12}–C_{14}), mixture of the isomers of sodium salts of alkylbenzene sulphonic acids
2	Neftenol ML [7]	ASAS + NSAS	Multicomponent composition
3	BOK-6	NSAS	Mixture NSAS (≤5 %), soda ash (≥80 %), sodium sulphate (≤10 %), sodium metasilicate (≤5 %)
4	Vega ChM [8]	NSAS	The same
5	OP-10 [9]	NSAS	Products of the treatment of a mixture of mono- and dialkylphenols with ethylene oxide
6	AddiMax PV01 [10]	ASAS	Glycerylsulphonates (alkylated alcohols)
7	AddiMax PV02 [10]	ASAS	The same
8	Stenor 25P15E10 [11]	NSAS	PPG-15/PEG-10 Ethoxylated, propoxylated alcohols of C_{12}–C_{15} fraction
9	Sintanol ALM-7 [12]	NSAS	Mixture of primary oxyethylated higher fatty alcohols of C_{12}–C_{14} fractions

* ASAS is anion SAS, NSAS is non-ionogenic SAS.

cording to the regulations FR.1.31.2012.11560 "Procedure for the measurement of biological activity of humic acids by means of phytotesting". The seeds were placed in transparent plastic plates on the substrate made of filtering paper treated with the solution under test with the reagent or distilled water (reference).

After exposure for 96 h, the length of roots and seedling was measured, and the degree of toxicity was established from the ratio of test parameters in the experimental (SAS-containing) and reference versions.

Determination of the ecotoxicity of the chosen SAS-containing reagents was carried out in the test system with fresh-water infusoria *(Paramecium caudatum* Ehrenberg) according to Regulations FR.1.39.2006.02506 "Procedure for the determination of the toxicity of wastes, soil, sediments in waste, surface and ground waters by means of biotesting using holotrichs". The viability of individuals in the samples after exposure for 24 h was evaluated as the test function.

RESULTS AND DISCUSSION

During phytotesting carried out according to GOST R ISO 22030–2009, the toxic effects of the analyzed reagents were determined by comparing the biomass of fresh-cut and dried plants grown in the test vessels with polluted soil to the data of reference experiment.

Results of the evaluation of chronic phytotoxicity of SAS-containing reagents on the basis of the response of oil-yielding radish are shown in Fig. 1. One can see that the plant possesses a rather weak sensitivity to the reagents under test. It should be stressed that hormesis was observed in the majority of experiments, which is the stimulation of tested parameters by relatively low reagent concentrations.

The activating effect of two SAS-containing reagents (Sintanol ALM-7 and AddiMax PV01) possessing similar chemical compositions based on alkylated alcohols on the biomass was established.

The effects of VEGA ChM reagent and the reference substance NaOH on the plant biomass are comparable, which agrees with the alkaline nature of the reagent. The solutions of BOK-6 are also characterized by high alkalinity, (pH 11.72), however, their negative effect is less clearly pronounced.

Figure 2 shows the results of the evaluation of the chronic phytotoxicity of SAS-containing reagents on the basis of the response of cultivated oat.

In comparison with oil-yielding radish, oat demonstrated higher sensitivity to the reagents. This is especially clearly illustrated by the plot with fresh-cut seedlings (see Fig. 2, *a*). Reagents BOK-6, VEGA ChM in view of the alkaline nature of component composition (see Table 1) show similar dependences as the reference substance NaOH and similarly to Neftenol and Sulfonol NP-1 exhibit suppressing action.

Quite the contrary, reagents AddiMax PV01, Sintanol ALM-7 and OP-10 caused stimulating action.

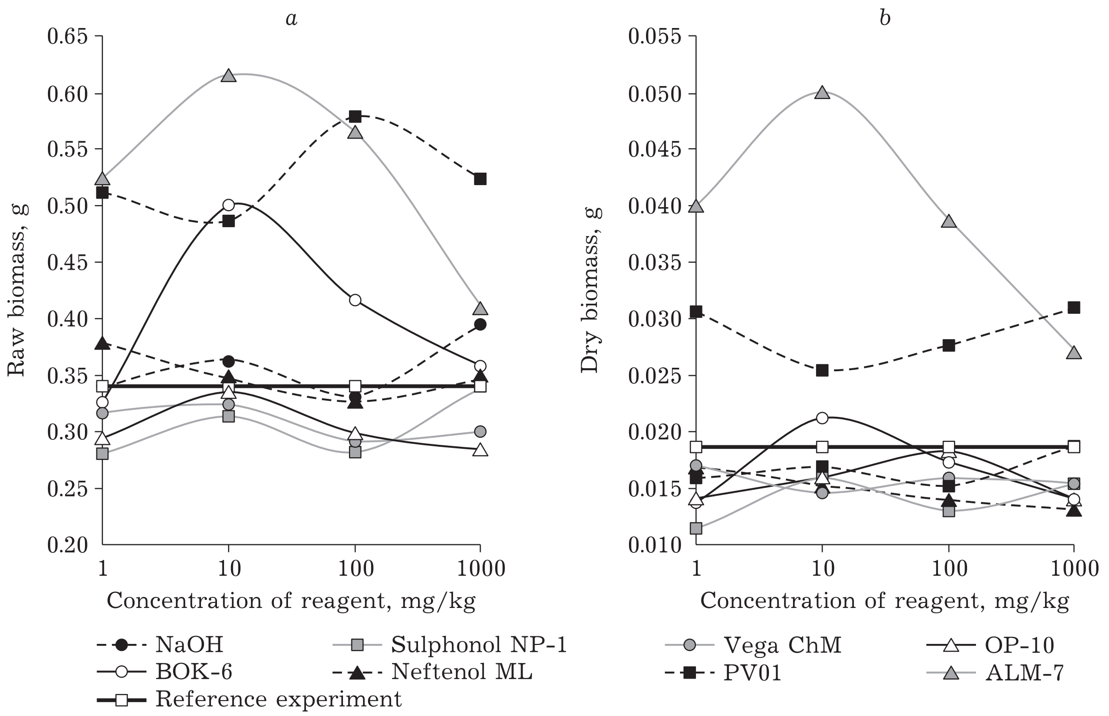

Fig. 1. Dependence of the raw (*a*) and dry (*b*) biomass of oil-yielding radish on reagent content in the ground.

On the basis of the results of the vegetation experiment, it may be stated that the high sensitivity to SAS-containing reagents is characteristic of cultivated oat. Nevertheless, a similarity in the dynamics of the biomass response of radish and oat was discovered for the same concentrations of a number of reagents. The stimulating action on both test species was expressed by AddiMax PV01 and Sintanol ALM-7, and in the experiment with oat – OP-10.

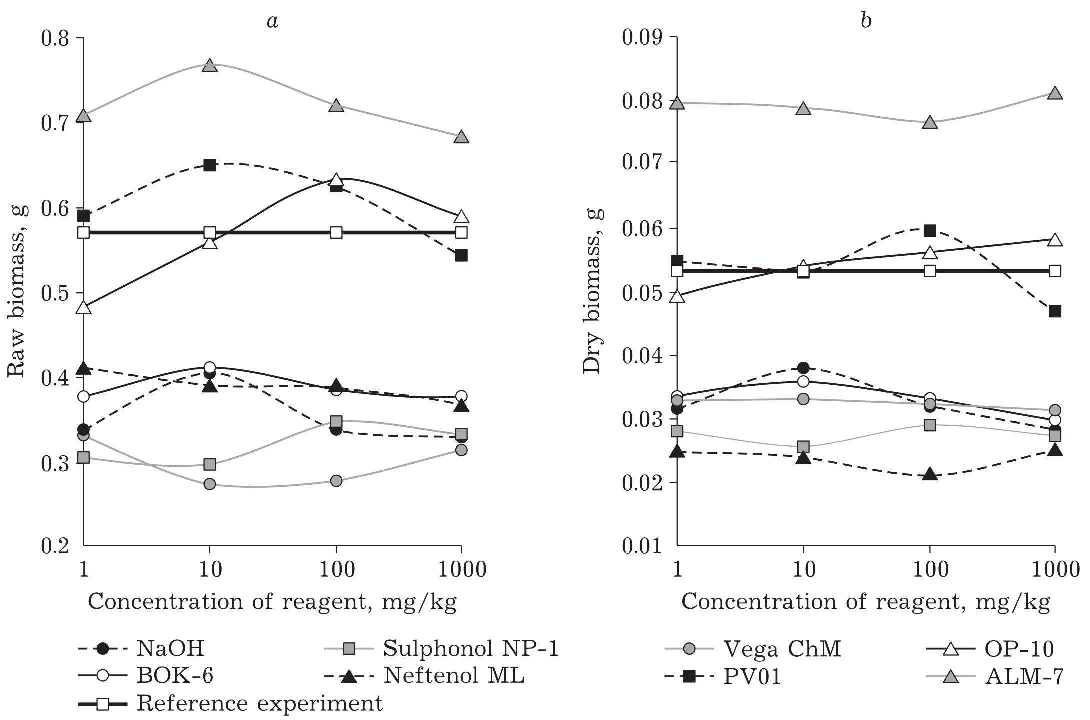

Fig. 2. Dependence of raw (*a*) and dry (*b*) biomass of cultivated oat on reagent content in ground.

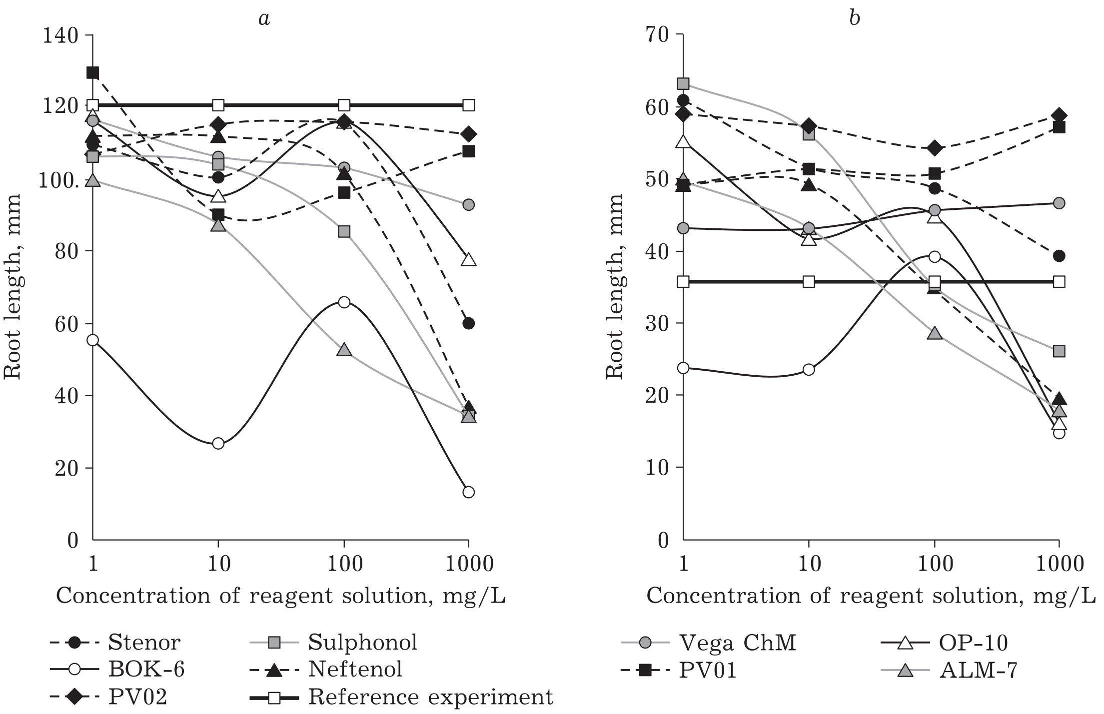

Fig. 3. Results of the evaluation of acute phytotoxicity in the Phytoscan test system, test plant – oil-yielding radish: *a* – raw, *b* – dry biomass.

Results of the investigation of acute phytotoxicity in the test system Phytoscan are shown in Fig. 3 and 4.

Analysis of the average root length as the test parameter for oil-yielding radish revealed the inhibiting effect of all the used reagents, though

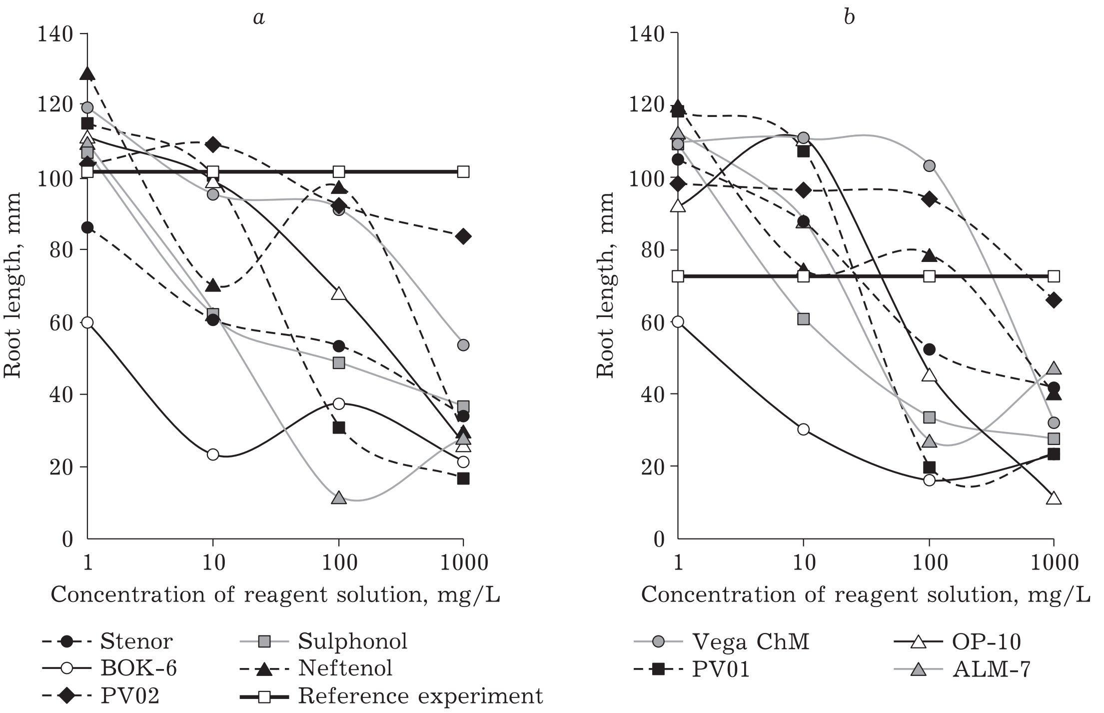

Fig. 4. Results of the evaluation of acute phytotoxicity of the Phytoscan test system, test plant - cultivated oat: *a* – root length, *b* – seedling length.

TABLE 2

Results of the determination of reagent toxicity against fresh-water infusoria

Grade of SAS-containing reagent	Average lethal concentration LC_{50-24}, mg/L	Harmless concentration HC_{10-24}, mg/L
BOK-6	0.1	N/d
Vega ChM	0.1	N/d
Sulphonol NP-1	1	0.1
OP-10	0.1	N/d
AddiMax PV01	1000	100
AddiMax PV02	100	0.1
Nephthenol ML	100	0.1
Sintanol ALM-7	1	N/d
Stenor 25P15E10	N/d (above 1000)	N/d

Примечание. N/d – not determined within the tests.

with different extent of action. The weakest effect was caused by the reagents AddiMax PV01, PV02 and OP-10. The dependencies are not monotonous. This means that testing according to this procedure also demonstrates hormesis. Sintanol ALM-7 reagent, unlike for the studies of its chronic phytotoxicity according to GOST 22030–2009 and ISO 11269-2:2012, revealed clearly pronounced negative phytoeffect. This may be connected with different effects of the reagent on the parameters of plant growth and development, namely suppression of some parameters with simultaneous stimulation of others.

The strongest inhibiting action was that caused by BOK-6 reagent.

The average length of seedlings as the test parameter turned out to be less sensitive. In the majority of experiments, a positive phytoeffect was revealed with the conservation of the trend of reagent action on a plant.

The responses of oat and radish seedlings in the plate version are similar in many respects. BOK-6 reagent has the maximal inhibiting effect, AddiMax PV02, Vega ChM cause the smallest negative phytoeffect.

It is important to stress that the plants of cultivated oat turned out to be more sensitive with respect to all test functions than oil-yielding radish. This confirms the advisability of the choice of *Avena sativa* L. as a test culture in the methodical recommendation MR 2.1.7.2297–07 "Substantiation of the class of danger of industrial and consumption wastes on the basis of phytotoxicity".

Investigations of the ecological toxicity of reagents using the test cultures of protozoa were carried out evaluating the average lethal concentration (LC_{50-24}) causing the death of 50 % of test organisms within exposure for 24 h, and the harmless concentration (HC_{10-24}) causing the death of not less than 10 % test organisms within the same period. The results of tests are presented in Table 2.

It was established that the AddiMax PV01 reagent is one of the most harmless ones on the basis of infusoria survival. The threshold concentrations for the characterization of the toxicity of Stenor 25P15E10 reagent could not be established because all its concentrations turned out to be harmless for infusoria. At the same time, other reagents under analysis are characterized by rather high toxicity parameters.

CONCLUSION

Results of the evaluation of ecotoxicity of SAS-containing reagents in different biological tests showed that the test culture of cultivated oat expresses higher sensitivity to the stimulating effects both in the acute eluate and in chronic applicate experiments. This plant species may be recommended as the test plant to establish effective non-acting concentrations of SAS-containing reagents for the use of solid oil-containing substrates treated with these substances, which are assumed to be arranged in the environments, including the vicinity of water objects.

According to the criteria of waste danger class determination accepted in our country, for the wastes that may under definite conditions act as the substrates for plants, it is sufficient to have the results of toxicity evaluation on the basis of the response of two test organisms. In our work, we used two test plant species and a standardized test culture of protozoa. Based on the results obtained in the study, it appears possible to choose less toxic grades of SAS-containing reagents and to use then to purify oil-polluted natural ground. Natural ground purified with the help of these substances to the acceptable level of residual content of oil products may be returned to the environment for subsequent bioremediation, in particular with the participation of plants. High ecotoxicity (LC_{50-24} = 0.1–1.0 mg/L) of some SAS-containing reagents showing at the same time high efficiency [13] points to the fact that these preparations should be used on a limited basis – for example, to purify oil sludge that is not planned to be returned into the environment.

The results obtained in the studies provide evidence of the necessity to control the residual content of reagents in ground to be returned into the environment, or ground the subsequent use of which is connected with the potential possibility of washing toxic chemical compounds out into the environment.

REFERENCES

1 Ying G.-G., *Environ. Int.*, 2006, Vol. 32, P. 417–431.
2 Cserhati T., Forgacs E., Oros G., *Environ. Int.*, 2002, Vol. 28, P. 337–348.
3 Parr J. F., Norman A. G., *American Society of Plant Biologist,* 1964, P. 502–507.
4 Poklonov V .A., Kotelevtsev S. V., Ostroumov S. A., *Uspekhi Nauk o Zhizni,* 2013, No. 6, P. 71–78. (in Russ.).
5 Zubair A., Design and Optimization of Surfactant Based Enhanced Remediation of Bunker Fuel oil Contaminated Soil, Canada, Newfoundland: St. John's, 2015.
6 Sheykhet F. I., Materials Science of Chemicals, Dyes and Cleansers, Moscow: Legkaya Industriya, 1969. (in Russ.).
7 TU 2481-056-17197708–2000, Cleanser surfactant NEFTENOL ML, 2005. (in Russ.).
8 Technical cleanser VEGA-CHM-01 [submitted by manufacturer LLC Chistiy Mir M]. (in Russ.).
9 GOST 8433–81, Auxiliary materials OP-7, OP-10. Specifications, 1981. (in Russ.).
10 Certificate No. 014, Surfactants AddiMax PV01, AddiMax PV02 [submitted by manufacturer LLC Zavod Sintanolov]. (in Russ.).
11 Certificate No. 1, Stenor 25R15E10 [submitted by manufacturer LLC Zavod sintanolov]. (in Russ.).
12 TU 2483-004-71150986–2012, Sintanol ALM-7, Sintanol ALM-8 (SintaNorm TM ALM-7 i ALM-8). URL: http://docs.cntd.ru/document/437155836 (accessed 6 June 2018). (in Russ.).
13 Kulikova O. A., Mazlova E. A., Bradik D. I., Kudrova E. P., *Chemistry and Technology of Fuels and Oils*, 2018, No. 6, P. 47–52. (in Russ.).