Artículo de investigación

Development of Regional Maximum Permissible Concentrations of Oil, Lead, Chromium, Nickel, and Copper in the Ordinary Black Soils of Central Ciscaucasia

Разработка региональных предельно допустимых концентраций содержания нефти, свинца, хрома, никеля и меди в черноземах обыкновенных Центрального Предкавказья

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Abstract

Contamination of ordinary chernozems of the Central Ciscaucasia with oil, lead, chromium, nickel and copper leads to a deterioration in their biological indicators. A significant decrease in the number of microflora, enzymatic activity and inhibition of the state of plants was established. The ecotoxicity sequence of heavy metals for ordinary black soils of Central Ciscaucasia is the following: Cr > Pb ≥Cu ≥ Ni. Ordinary black soils of Central Ciscaucasia, compared to similar black soils of Western Ciscaucasia, are somewhat less resistant to pollution with chromium, but are more resistant to pollution with copper and nickel. Resistance to pollution with lead and oil is the same. Regional maximum permissible concentrations of oil, lead, chromium, nickel and copper have been set for ordinary black soils of

Аннотация

Загрязнение черноземов обыкновенных Центрального Предкавказья нефтью, свинцом, хромом, никелем и медью приводит к ухудшению их биологических показателей. Установлено достоверное снижение численности микрофлоры, ферментативной активности и угнетения состояния растений. Ряд экотоксичности тяжелых металлов для черноземов обыкновенных Центрального Предкавказья следующий: Cr > Pb ≥Cu ≥ Ni. Черноземы обыкновенные Центрального Предкавказья по сравнению с аналогичными черноземами Западного Предкавказья несколько менее устойчивы к загрязнению хромом, но более устойчивы к загрязнению медью и никелем. Устойчивость к загрязнению свинцом и нефтью одинаковая.

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Central Ciscaucasia, based on disruptions of the environmental and the agricultural functions of the soil.

Keywords: Central Ciscaucasia, ordinary black soils, pollution, heavy metals, lead, chromium, nickel, copper, oil, sustainability, biological parameters.

Установлены региональные предельно допустимых концентрации содержания нефти, свинца, хрома, никеля и меди в черноземах обыкновенных Центрального Предкавказья на основе нарушения экологических и сельскохозяйственных функций почвы.

Ключевые слова: Центральное Предкавказье, черноземы обыкновенные, загрязнение, тяжелые металлы, свинец, хром, никель, медь, нефть, устойчивость, биологические показатели.

Introduction

Humanity receives 80% of the food using the soil. The normal functioning of the soil (the fulfillment of ecological and agricultural functions) is the key to the ecological and food security of mankind. However, the ever increasing anthropogenic impact, including chemical pollution, leads to disruption of the ecological and agricultural functions of the soil (Brouwer et al., 2013; Hester & Harrison, 2010; Ruhl et al., 2007). Correspondingly, environmental, economic, social and other kinds of damage from the reduction of soil fertility and the lack of harvests, pollution of soils and agricultural products are growing.

Currently, in science and practice, a significant reserve has been created on the problem of rationing chemical pollution of soils and ecosystems (Kabata-Pendias, 2010; Reimann & De Caritat, 1998; Sherameti & Varma, 2015). However, many problems are still not solved. For many polluting substances (elements), the environmentally safe norms for their content in soil have not been developed. At the same time, for those substances, the standards for which have been established, the values of these standards are of general (“global”) nature. They have been developed, as a rule, either for the “soil in general” (maximum permissible concentration – MPC), or for “large groups of soil” (approximate permissible concentration – APC), similar in basic soil properties, determining pollution resistance (particle size, pH, etc.). However, the values of these standards are often unsuitable for both objective and subjective reasons, such as soil polyfunctionality, soil heterogeneity, diversity of soil types, diversity of chemical compounds of pollutants, phenomena of synergy and antagonism between atoms of elements, the ability of living organisms to adapt, and the ability of soil to self-healing, etc. (Kolesnikov, Kazeev et al., 2017).

It is relevant to develop standards for the quantity of polluting substances (elements) in soil at both “regional” and “local” levels, taking into account the geochemical and environmental characteristics of soils. At the moment, the assessment of the state of the environment and its standardization is dominated by the ecological approach (Kolesnikov, Kazeev et al., 2017; Brouwer et al., 2013; Hester & Harrison, 2010; Ruhl et al., 2007; Kolesnikov et al., 2002; Reimann & De Caritat, 1998).
Since biological indicators of soil condition are the most sensitive to chemical pollution (Martinez-Salgado et al., 2010; Kolesnikov et al., 1999; Zvyagintsev et al., 1997), they should be used in normalizing chemical pollution of the soil.

Black soils are the most fertile soils in the world, and play an indispensable role in providing food for men (Fridland, 1985). However, as a result of intensive agricultural use, they are subjected to significant anthropogenic loads (Shcherbakov & Vasenev, 2000), chemical contamination in particular (Vodyanitsky, 2012). With that, not all black soils have the same resistance to pollution (Kolesnikov et al., 2013). Various black soils require determining regional maximum permissible concentrations (rMPC) of pollutants in the soil. Earlier, rMPC for oil and heavy metals were calculated for ordinary (Kolesnikov et al., 2010) and Southern (Kolesnikov, Vernigorova et al., 2017) black soils in Western Ciscaucasia.

This work was aimed at determining rMPC of oil, lead, chromium, nickel and copper for ordinary black soils of Central Ciscaucasia, based on disruptions of the environmental and the agricultural functions of the soil.

Methods

Black soil pollution was simulated at the laboratory. The correctness of transferring the results of laboratory simulation of chemical foiling of soils to natural conditions has been proved earlier (Kolesnikov et al., 2014).

Ordinary black soil was sampled in the vicinity of village Kochubeevskoye in the Stavropol territory (44°36'12.48"N, 41°50'26.30"E). Topsoil 0-20 cm deep was used, as it retains most of soil contaminating substances (Kabata-Pendias, 2010).

In accordance with the international classification of soils a.k.a. World Reference Base for Soil Resources (WRB), ordinary black soils (Egorov et al., 1977) are referred to as Chernozems Calcic (IUSS Working Group WRB, 2006).

Presumably, such genetic properties of the soil should determine high resistance of black soils in Central Ciscaucasia to chemical contamination. However, no research devoted to assessing the resistance of ordinary black soils in Central Ciscaucasia to contamination with oil and heavy metals has been performed until now.

Lead, chromium, nickel and copper were chosen as pollutants, since these were these metals that greatly polluted soils in the South of Russia (D'yachenko & Matasova, 2016).

Oil MPC in the soil has also not been developed, therefore its content in the soil was expressed in percent.

Heavy metals were introduced into soil in the quantities of 1, 10, 100 MPC (100, 1,000 and 10,000 mg/kg, respectively), oil - in the quantities of 1, 5, 10% by weight of the soil.
Oxides of heavy metals were used: lead oxide (II), chromium oxide (VI), copper oxide (II), nickel oxide (II). Firstly, a significant share of heavy metals comes to the soil in the form of oxides (Kabata-Pendias, 2010). Secondly, the use of heavy metal oxides allows excluding the effect of accompanying anions on soil properties, as it occurs in case of introducing salts of metals. The biological properties of the soil were determined 30 days after infection. This period is the most informative in assessing the chemical effects on the biological state of the soil. (Kolesnikov et al., 19).

The laboratory and analytical studies were performed with the use of the methods generally accepted in soil biology (Zvyagintsev, 1991) in modification (Kazeev et al., 2016). The total bacterial count, abundance of Azotobacter bacteria, catalase and dehydrogenase activity, celluloselytic activity, phytotoxic properties of soils, and other indicators were determined. The total bacteria count in the soil was found by the method of fluorescent microscopy, Azotobacter- using the method of fouling lumps in the Ashby medium, catalase activity — by the rate of hydrogen peroxide decomposition, activity of dehydrogenase — by the speed of triphenyl tetrazolium chloride transformation into triphenyl formazan, cellulose lytic activity — by the speed of decomposition of cotton cloth, and soil phytotoxicity was assessed by germination of radish.

To combine many indicators, the method of the integral indicator of the biological state of the soil (IIBS) was previously determined (Kolesnikov et al., 2000). This method allows assessing ecological functions of the soil by soil biological state.

Results and Discussion

As a result of the research, it has been found that contamination of ordinary black soil of Central Ciscaucasia with oil, lead, chromium, nickel, and copper deteriorates its biological characteristics (Table 1).

Table 1. Resistance of ordinary black soils of Central Ciscaucasia to contamination with oil, lead, chromium, nickel, and copper

Element (substance)	Contaminant dosage				
	Reference	1 MPC (1%)	10 MPC (5%)	100 MPC (10%)	LSD05
The total bacteria count, billion per 1 g of soil					
Cr	4.7	1.9	1.4	0.9	0.3
Cu	4.7	3.1	2.2	1.5	0.2
Ni	4.7	2.4	1.9	1.3	0.2
Pb	4.7	1.7	1.8	1.3	0.2
Oil	4.7	3.2	1.2	0.7	0.3
LSD05		0.2	0.2	0.1	
Catalase activity, ml of O_2 per 1 g soil for 1 min					
Cr	11.4	11.6	4.2	2.9	1.0
Cu	11.4	11.6	11.1	10.5	0.8
Ni	11.4	11.9	11.2	11.1	0.8
Pb	11.4	11.6	10.4	9.2	0.8
Oil	11.4	10.6	5.2	2.3	1.0
LSD05		1.0	0.8	0.6	
Dehydrogenase activity, mg of TTF per 10 g of soil in 24 hours					
Cr	16.7	13.5	12.8	9.6	1,8
Cu	16.7	16.7	15.1	12.1	2,0
Ni	16.7	16.1	15.8	15.1	2,1
Pb	16.7	16.1	15.2	14.5	2,1
Oil	16.7	17.3	16.6	13.1	2,1
LSD05		1.9	2.1	2.3	
Cellulosolytic activity, % from the reference					
Cr	100	53	35	9	10
Cu	100	95	80	61	6
Ni	100	95	88	84	4
Pb	100	80	73	72	7
Oil	100	62	35	3	12
LSD05		8	9	11	
Abundance of Azotobacter bacteria, % of fouling lumps					
Cr	100	44	0	0	10
Cu	100	100	99	99	13
Ni	100	100	98	93	9
Pb	100	100	99	91	13
Oil	100	97	95	91	7
LSD05		11	12	15	
Radish root length (phytotoxicity), % of the reference					
Cr	100	96	28	1	9
Cu	100	100	97	97	4
Ni	100	102	100	91	5
Pb	100	101	96	95	6
Oil	100	101	92	85	9
LSD05		13	14	17	
Integral indicator of soil biological status (IIBS), % of the reference					
Cr	100	69	34	19	
Cu	100	94	85	76	
Ni	100	91	87	81	
Pb	100	86	81	76	
Oil	100	84	64	49	

In most cases, bacteria count and abundance of Azotobacter bacteria decreased, activity of catalase and dehydrogenase, and cellulolytic activity deteriorated, radish root length decreased, and IIBS reduced. Changes in biological characteristics depended on the nature of the pollutants and their concentration in the soil.

However, there were individual cases of heavy metals' and oil catalytic action on enzymatic activity of black soil — activity of catalase in the variants with 1 MPC of heavy metals, and of dehydrogenases in the variant with 1 MPC of oil (Table 1) (Kabata-Pendias, 2010; Kolesnikov et al., 1999; Zvyagintsev et al., 1997). They are called "hormesis" or the "effect of small dosages".

By the degree of negative effect on the biological characteristics of ordinary black soils of Central Ciscaucasia, heavy metals form the following sequence (the sequence has been averaged by the dosages of contaminant): Cr > Pb ≥ Cu ≥ Ni.

Chromium had the most significant negative effect, while lead, copper and nickel had weaker effects. Similar regularity was observed in the studies performed according to the same method with the soils in the South of Russia: leached, typical, compacted, montane (Kolesnikov et al., 2013), chestnut, brown semi-desert, saline, sandy (Kolesnikov et al., 2011), brown forest (Kolesnikov, Kuzina et al., 2016), meadow (Kolesnikov, Myasnikova et al., 2017), salt marshy (Kolesnikov, Vernigorova et al., 2016), and other soils.

The resulting sequence of heavy metals in their ecotoxicity in most cases does not coincide with the results of other studies obtained on northern soils, which are more acidic, with a lack of humus, under conditions of recovery (Crommentuijn et al., 1997; Van de Plassche & De Bruijn, 1992; Vodyanitsky, 2012). The reasons may be as follows. High toxicity of chromium in chernozems is expressed by the fact that chromium is more mobile in oxidizing and alkaline conditions (Zachara et al., 1989). Low toxicity of lead is expressed by a high content of humic acids in chernozems, which bind lead more strongly than, for example, copper (Morin et al., 1999; Manceau et al., 2002).

It has been previously found (Kolesnikov et al., 2009) that with reducing soil IIBS by less than 5%, ecological functions of the soil are not disrupted, with reduction of IIBS by 5-10%, information ecofunctions change, with reduction of IIBS by 10-25%, chemical, physico-chemical, biochemical and holistic functions change, and with reduction of IIBS by 25% and more, physical functions change.

Using the results of these studies, regression equations were built, which reflected the dependence of IIBS decrease on the content of contaminants in the soil. Based on the obtained data, a scheme of regional ecological standardization of black soil pollution of the Central Ciscaucasia has been proposed (Table 2).

Table 2. The scheme of environmental rationing of oil, lead, chromium, nickel, and copper content in ordinary black soils of Central Ciscaucasia according to the degree of ecological functions' disruption

Soils	Not polluted	Weakly polluted	Moderately polluted	Heavily polluted
Degree of reducing Integral index	< 5%	5 – 10%	10 – 25%	> 25%
Disrupted ecological function	–	Informational	Chemical, physico-chemical, biochemical; holistic	Physical
Substance	Oil content in soil, %			
oil	< 0.50	0.50-1.00	1.0-2.50	> 2.50
Element	Content of heavy metals in soil, mg/kg			
Cr	< 100	100-130	130-160	> 160
Cu	< 65	65-150	150-450	> 450
Ni	< 65	65-150	150-450	> 450
Pb	< 65	65-130	130-400	> 400

Comparative analysis has shown that ordinary black soils of Central Ciscaucasia, compared to similar black soils of Western Ciscaucasia (studied earlier (Kolesnikov et al., 2013)), are somewhat less resistant to contamination with chromium, but are more resistant to contamination by copper and nickel. The

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resistance to contamination with lead and oil is the same.

The higher resistance to contamination with copper and nickel is probably due to slightly higher content of organic matter, higher pH values, heavier particle-size composition of ordinary black soils in Central Ciscaucasia, compared to the ordinary black soils in Western Ciscaucasia. These properties determine the reduced mobility, and, therefore, lower toxicity of copper and nickel.

The lower resistance to contamination with chromium may be due to the slightly higher pH value of black soils in Central Ciscaucasia. Since chromium oxide and hydroxide are amphiprotic, compounds of chromium retain high mobility and toxicity in neutral and alkaline soils (Zachara et al., 1989).

Conclusions

Contamination of ordinary black soils in Central Ciscaucasia with oil, lead, chromium, nickel, and copper results in the deterioration of their biological indicators. In most cases, a significant reduction of the studied indicators - the total number of bacteria, activity of enzymes, cellulose lytic ability, oppression of indicators of germination and initial growth of radish - was observed, along with the abundance of bacteria of genus Azotobacter.

The ecotoxicity sequence of heavy metals for ordinary black soils of Central Ciscaucasia is the following: $Cr > Pb \geq Cu \geq Ni$.

Ordinary black soils of Central Ciscaucasia, compared to similar black soils of Western Ciscaucasia, are somewhat less resistant to contamination with chromium, but are more resistant to contamination with copper and nickel. The resistance to contamination with lead and oil is the same.

The rMPC of oil, lead, chromium, nickel and copper have been set for ordinary black soils of Central Ciscaucasia, based on disruptions of the environmental and the agricultural functions of the soil.

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References

Brouwer, R., Brander, L., Kuik, O., Papyrakis, E., & Bateman, I. (2013). A synthesis of approaches to assess and value ecosystem services in the EU in the context of TEEB. University of Amsterdam.
Crommentuijn, T., Polder, M., & Van de Plassche, E. (1997). Maximum Permissible Concentrations and Negligible Concentrations for metals, taking background concentrations into account. RIVM Report 601501001. Bilthoven, Netherlands, pp. 260.
D'yachenko, V., & Matasova, I. (2016). Regional clarkes of chemical elements in soils of southern European Russia. Eurasian Soil Science, 10, 1091-8.
Egorov, V.V., Fridland, V.M., Ivanova, E.N., & Rozov, N.N. (1977). Classification and diagnosis of soil of USSR. Moscow: Kolos, 223 pp.
Fridland, V.M. (Ed.). (1985). Chernozemy SSSR (Predkavkazyei Kavkaz) [Chernozems of the USSR (Ciscaucasia and Caucasus)]. Moscow: Agropromizdat, 262 p.
Hester, R.E., & Harrison, R.M. (Eds.). (2010). Ecosystem Services. In Issues in Environmental Science and Technology. Royal Soc. Chem. Publishing (RSC Publishing).
IUSS Working Group WRB. (2006). World reference base for soil resources 2006. World Soil Resources Reports No. 103. FAO, Rome.
Kabata-Pendias, A. (2010). Trace Elements in Soils and Plants. Boca Raton: Crc Press.
Kazeev, K., Kolesnikov, S., Akimenko, Yu., & Dadenko, E. (2016). Methods of bio-diagnostics of terrestrial ecosystems. Rostov-on-Don: Publishing Southern Federal University, pp. 260.
Kolesnikov, S.I., Aznaurian, D.K., Kazeev, K.Sh., & Val'kov, V.F. (2010). Biological Properties of South Russian Soils: Tolerance to Oil Pollution. Russian Journal of Ecology, 41(4), 398-404.
Kolesnikov, S.I., Evreinova, A.V., Kazeev, K.Sh., & Val'kov, V.F. (2009). Changes in the Ecological and Biological Properties of Ordinary Chernozems Polluted by Heavy Metals of the Second Hazard Class (Mo, Co, Cr, and Ni). Eurasian Soil Science, 42(8), 936-42.
Kolesnikov, S.I., Kazeev, K.S., Denisova, T.V., Minkina, T.M., & Akimenko, Y.V. (2017). Development of regional and local norms for chemicals in the soil using biological parameters. In 17th International Multidisciplinary Scientific GeoConference Surveying Geology and Mining Ecology Management (pp. 125-130). SGEM.
Kolesnikov, S.I., Kazeev, K.Sh., & Valkov, V.F. (1999). The Effect of Heavy Metal Contamination on the Microbial System in

Chernozem. Eurasian Soil Science, 4, pp. 459-65.
Kolesnikov, S.I., Kazeev, K.Sh., & Val'kov, V.F. (2000). Effects of Heavy Metal Pollution on the Ecological and Biological Characteristics of Common Chernozem. Russian Journal of Ecology, 31(3), 174-81.
Kolesnikov, S., Kazeev, K., & Valkov, V. (2002). Ecological Functions of Soils and the Effect of Contamination with Heavy Metals. Eurasian Soil Science, 12, pp. 1335-1340.
Kolesnikov, S.I., Kuzina, A.A., Kazeev, K.Sh., Evstegneeva, N.A., & Akimenko, Yu.V. (2016). Assessment of resistance of brown forest sour soils of the black sea coast of the Caucasus to the chemical pollution. Ecology, Environment and Conservation, 22(3), 519-23.
Kolesnikov, S.I., Myasnikova, M.A., Minnikova, T.V., Ter-Misakyants, T.A., Kazeev, K.Sh., & Akimenko, Yu. V. (2017). Assessment of meadow soil resistance of the Azov Sea Region to pollution with heavy metals and oil. Ecology, Environment and Conservation, 23(4), 2346-51.
Kolesnikov, S., Spivakova, N. & Kazeev, K. (2011). The Effect of Model Soil Contamination with Cr, Cu, Ni, and Pb on the Biological Properties of Soils in the Dry Steppe and Semidesert Regions of Southern Russia. Eurasian Soil Science, 9, 1001-7.
Kolesnikov, S.I., Vernigorova, N.A., Kazeev, K.Sh., Tishchenko, S.A., Dadenko, E.V. & Akimenko, Yu.V. (2016). Biodiagnostics of the Azov Sea Solonchaks Condition at Chemical Contamination. Oriental Journal of Chemistry, 32(6), 3015-9.
Kolesnikov, S.I., Vernigorova, N.A., Kuzina, A.A., Kazeev, K.Sh., Akimenko, Yu.V., Dadenko, E.V., & Tyshchenko, S.A. (2017). Standardization of heavy metals and oil content in the chernozems of the southern Taman. Journal of Experimental Biology and Agricultural Sciences, 5, 706-12.
Kolesnikov, S.I., Yaroslavtsev, M.V., Spivakova, N.A., & Kazeev, K.Sh. (2013). Comparative Assessment of the Biological Tolerance of Chernozems in the South of Russia towards Contamination with Cr, Cu, Ni, and Pb in a Model Experiment. Eurasian Soil Science, 46(2), 176-81.
Kolesnikov, S.I., Zharkova, M.G., Kazeev, K.Sh., Kutuzova, I.V., Samokhvalova, L.S., Naleta, E.V., & Zubkov, D.A. (2014). Ecotoxicity Assessment of Heavy Metals and Crude Oil Based on Biological Characteristics of Chernozem. Russian Journal of Ecology, 45(3), 157-66.
Manceau, A., Marcus, M.A., & Tamura, N. (2002). Quantative speciation of heavy metals in soils and sediments by synchrotron X-ray techniques. Applications of Synchrotron Radiation in Low-Temperature Geochemistry and Environmental Science. Reviews in Mineralogy and Geochemistry. Washington, DC, 49, 341-428.
Martinez-Salgado, M.M, Gutierrez-Romero, V., Jannsens, M., & Ortega-Blu, R. (2010). Biological soil quality indicators: a review. In A. Méndez-Vilas (Ed.), Current research, Technology and education topics in applied microbiology and microbial biotechnology (pp. 319-326).
Morin, G., Ostergren, J.D., Juillot, F., Ildefonse, P., Calas, G., & Brown, J.E. (1999). XAFS determination of the chemical form of lead in smelter-contaminated soils and mine tailings: Importance of adsorption process. Am. Mineral., 84, 420-34.
Reimann, C., & De Caritat, P. (1998). Chemical elements in the environment factsheets for the geochemist and environmental scientist. New York: Springer.
Ruhl, J.B., Kraft, S.E., & Lant, C.L. (2007). The Law and Policy of Ecosystem Services. Island Press.
Sherameti, I., & Varma, A. (Eds.). (2015). Heavy Metal Contamination of Soils: Monitoring and Remediation. Springer.
Shcherbakov, A.P., & Vasenev, I.I. (Eds.). (2000). Antropogennaya evolyutsiya chernozemov [Anthropogenic evolution of black soils]. Voronezh: Voronezh State University, 409 p.
Van de Plassche, E., & De Bruijn, J. (1992). Towards integrated environmental quality objectives for surface water, sediments and soil for nine metals. RIVM Report 679101005. Bilthoven, Netherlands, pp. 130.
Vodyanitsky, Y. (2012). Standards for the contents of heavy metals and metalloids in soils. Eurasian Soil Science, 3, 321-8.
Zachara, J.M., Ainsworth, C.C., Cowan, C.E., & Resch, C.T. (1989). Adsorption of chromate by subsurface soil horizons. Soil Sci. Soc. Am. J., 53, 418-28.
Zvyagintsev, D.G. (Ed.). (1991). Soil Microbiology and Biochemistry Methods. Moscow: Mosk. Gos. Univ., pp. 304.
Zvyagintsev, D.G., Kurakov, A.V., Umarov, M.M., & Filip, Z. (1997). Microbiological and biochemical indicators of contamination of lead sod-podzolic soil. Eurasian Soil Science, 9, 1003-9.