UDC 502.7

Analysis of the Content of Nitrate and Ammonium Ions at Bioremediation of Ground Water Polluted by Oil Products

I. V. TRUSEY[1], YU. L. GUREVICH[2], V. P. LADYGIN[2], YU. P. LANKIN[3], and S. V. FADEEV[3]

[1]*Astafiev Krasnoyarsk State Pedagogical University,*
Krasnoyarsk, Russia

E-mail: trusey@list.ru

[2]*Krasnoyarsk Scientific Centre, Siberian Branch, Russian Academy of Sciences,*
Krasnoyarsk, Russia

E-mail: btchem@mail.ru

[3]*Hydrogeological Party Ltd.,*
Minusinsk, Russia

E-mail: gidropartiya2009@yandex.ru

(Received February 9, 2016; revised March 25, 2016)

Abstract

An opportunity for bioremediation of ground water polluted by oil products through a system of observation wells was studied. The activity of bioremediation processes was assessed by a change in the content of nitrogen (ammonia, nitrate, and nitrite) in water, the number of aerobic and anaerobic microorganisms and oil products. Microbial growth was stimulated by the introduction of mineral fertilizers into ground water as sources of N and P. Prior to treatment, the number of ammonifying and hydrocarbon oxidizing microorganisms in water collected from wells at polluted sites did not exceed 10^5 CFU/mL and $10^3 \cdot 10^5$CFU/mL, respectively. In response to nutrients feeding that limits the growth, the number of aerobic microorganisms increased by 3–4 orders. The number of ammonifying microorganisms increased to $1.8 \cdot 10^8$ CFU/mL, hydrocarbon oxidizing – $2.3 \cdot 10^7$ CFU/mL. An increase in the concentration of ammonia nitrogen (to the values above 50 mg/L) happened in ground water with delay of 2–6 weeks. Dynamics analysis of the chemical composition of ground water by the data of all wells demonstrated that a concerted fluctuation of the activity of nitrification and denitrification processes proceeded in oil products biodegradation in the active phase, and accordingly, of ammonium and nitrate concentrations. Predicting the chemical composition of ground water using a neural network confirmed the same. The content of oil products in ground water decreased by 65–97 %.

Key words: ground water, oil products, nitrate, ammonium, microorganisms, denitrifiers

INTRODUCTION

Petroleum hydrocarbons relate to common pollutants of surface and underground ecosystems. This type of pollution is typical for ground in the region of large cities and industrial objects. In comparison with soils, the application of a number of efficient measures, such as hoeing, aeration, surface treatment with sorbents, microbial preparations, *etc.* for purifying polluted ground and ground water is strictly limited. Research in this area is relevant due to a lack of knowledge in substance transformation processes in soils and in the practice of holding reconstruction. Among the whole number of reconstruction methods of polluted natural objects, bioremediation methods based on using microorganisms are widespread. Biodegradation intensification of organic pollution is reached by the introduction into these objects of nutrients (as a rule, nitrogen and phosphorus) limiting the development of destructors microorganisms, as well as microbial preparations, in the composition of which selectively selected strains are included. The whole range of physiological groups of microorganisms capable of degrading hydrocarbons at low temperatures under aerobic and anaerobic conditions is observed in the composition of the natural microflora of ground water polluted by hydrocarbons. Herewith, the contribution of microorganisms into pollution degradation is quite considerable (to 60 %) [1], which should be considered when planning and holding bioremediation measures. In many instances, when pollutants are natural compounds, and there are destructors in the composition of the natural microflora, their activity stimulation without microbial preparations may be efficient enough [2]. This is a less costly, at the same time, a more available and technological approach to ground cleaning.

The oxygen transfer rate in ground is limited. Therefore, the space distribution of areas of aerobic and anaerobic bioremediation processes happens in the zone of aeration and the zone of saturation depending on the concentration of organic pollutions, soil permeability, the filtration rate of ground water, and the activity of microorganisms [3]. In the anaerobic region, depending on the red-ox potential of the medium, bacteria use nitrate, iron (III), sulphate and carbonic acid as electron acceptors for decomposition of organic compounds in nitrification, iron- and sulphate-reducing, methanogenesis, respectively [2, 4]. Thus, the chemical composition of ground water and the activity of various physiological groups of microorganisms are interrelated [5]. Cleaning of soils and ground water in the whole volume of the polluted underground medium can be carried out under condition of the active growth of all microorganisms, aerobic and anaerobic.

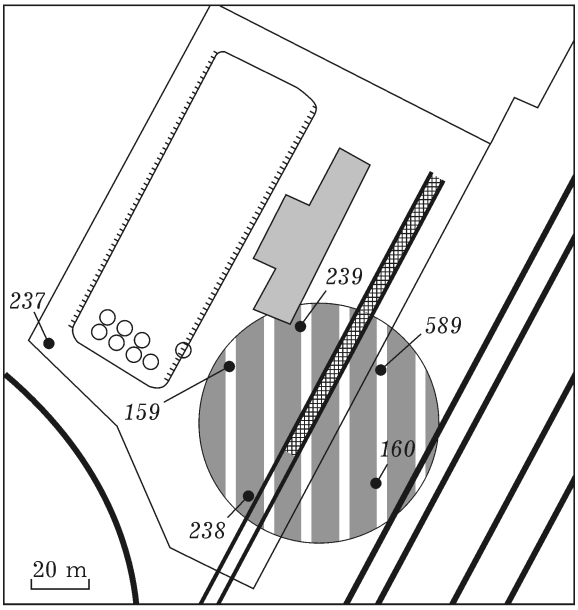
Oxidation of organic compounds by anaerobic microorganisms is associated with a decrease in the concentration of dissolved oxygen and the release of bound nitrogen into a soluble form. Appearance of ammonium stimulates the growth of nitrifying bacteria that oxidize it to nitrate. The latter initiates the growth of denitrifiers that decrease the medium potential and contribute to the formation of growth zones of iron and sulphate reducing bacteria at low oxygen concentrations. Decomposition intensity of contaminations in these interrelated processes is low because of the imbalance of feed supply of the microflora (ratio of C_{org}, N and P). By a change in the chemical composition of ground water and the number of various physiological groups of microorganisms, one can judge their activity [6, 7]. The results of this analysis may serve as a basis for carrying out these or those specific actions on the recovery of polluted objects.

The work purpose is the study of bioremediation of ground water polluted by oil products by introducing nutrients (nitrogen- and phosphorus-containing fertilizers) through a system of observation wells and by stimulating the development of the aboriginal microflora.

EXPERIMENTAL

Groundwater of combined heat and electric power plant (HEPP) fuel oil facilities polluted by oil products (mixtures of fuel oil, diesel fuel and process oils) was a research object. Ground is built from gravel-pebble sandy rocks, on the surface of which the technogenic layer (0.5 m) is found. The level of ground water during a year varies from 4 to 7 m. The temperature in winter is 4 °C, in summer +7–9 °C. By the chemical composition, groundwater relates to sulphate-hydrocarbonate and hydrocarbonate sodium-potassium and a mixed cationic composition with mineralization of 0.37–1.5 g/L. The type of groundwater may change to the side of hydrocarbonate-sulphate. At the site of fuel oil facilities, pH of ground water is changed from 6.6 to 8.5. Soils have low moisture content (2–7 %), moreover, it decreases with the increase in depth.

Ground water samples for analysis were collected from observation wells; the layout scheme of the wells is given in Fig. 1. Wells Nos. 159, 160, 238, 239, 589 are located in the area with high levels of contamination (highlighted by shading); the content of oil products in ground water at the initial stage reached 350–400 mg/L of water. Well No. 237 is located on the border of the polluted area, and wells Nos. 161 and 240 – outside its limits.



Scale 1:2000

Fig. 1. Location of wells at the territory of HEPP fuel oil facilities.

From 2005 to 2008 at the territory of the HEPP fuel oil facilities, the authors jointly with the Minusinsk Hydrogeological Party carried out tests of an opportunity to stimulate the microflora and bioremediation of the geological medium polluted by oil products. Biogenic elements (as nitrogen and phosphorus fertilizers) in relatively small amounts were introduced through observation wells to exclude migration of the former outside the limits of the contaminated site. Wells Nos. 237 and 159 were selected to treat soils. Well No. 237 is located at the borderline of the petroleum product plume in contaminated soil and above along the current of ground water relatively to well No. 159. The latter is located in the central part of the problem site. The removal from the lower borderline of the spot and the maximum rate of ground water movement (0.17–0.27 m/day in the area of the well No. 159) minimized the risks for groundwater outside the pilot site.

The activity of bioremediation processes was assessed by a change in the chemical composition of ground water, the number of aerobic and anaerobic microorganisms and the content of oil products in water. Ground water samples for chemical and microbiological analysis were collected after washing wells. Chemical analysis of ground water was carried out by the standard techniques using a KFK-3 Photometer; the permitted value limit of the basic absolute error is 0.5 % [8, 9]. The content of oil products in water was determined by fluorometric techniques (Fluorate-02-2M); the measurement error for the studied ranges is 25 %. Samples for microbiological analysis were stored at a low temperature (not higher than 10 °C). For accounting the number of aerobic microorganisms, the method of limited dilutions with seeding on agarised medium was used [10]. Determining the number of denitrifiers was carried out using Mac-Kredy's tables ($P_{0.95}$). Denitrifiers were isolated on the medium of the following composition: peptone agar (1.35 %), KNO_3 (0.5 %) and an indicator (bromthymolblau). Accounting for the number of microorganisms was performed at a temperature of 20 °C.

Processing of microbiological data was carried out using Poissonian statistics [10] and the Excel 2007 package for statistical analysis. The neural network implemented as a Microsoft Excel expansion pack was used to analyze a large volume of data of the hydrochemical composition in ground water [11, 12]. The chain was studied on the base of the algorithm of dual functioning to detect the dynamics in a change of the content of ammonia and nitrate nitrogen that is typical for the denitrification process.

RESULTS AND DISCUSSION

The nitrogen content in samples of ground water collected from wells during bioremediation works is demonstrated in Fig. 2. The con-

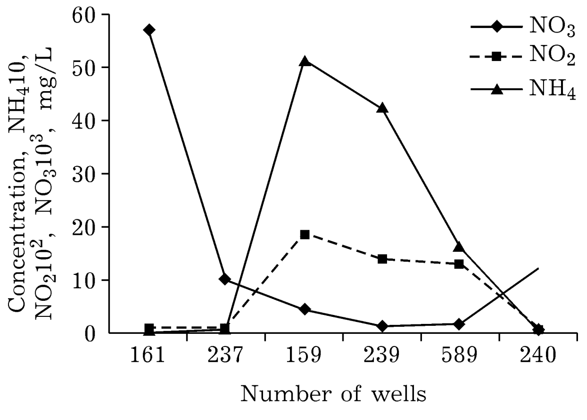


Fig. 2. Content of ammonium, nitrite and nitrate forms of nitrogen in groundwater prior to the introduction of mineral fertilizers.

TABLE 1

Scheme of the introduction of fertilizers into wells

Time of the introduction	Fertilizer	Mass, kg		
		Number of wells		
		159	237	238
July of 2005	NPK	15.5	3.0	–
	Ammonium sulphate	11.0	2.0	–
September of 2005	NPK	6.0	–	–
	Ammonium sulphate	4.5	–	–
August–September of 2006	NPK	3.75	–	3.75
	Ammophos	1.5	–	1.5

tent of ammonia nitrogen is much higher in water of the polluted site (wells Nos. 159, 239, 589) than outside its limits (wells Nos. 161, 240). The availability of three forms of mineral nitrogen and their ratio indicates the fact that nitrification and denitrification processes proceed in the underground medium, but their intensity is low. The concentration of dissolved oxygen in ground water outside contamination spots (wells Nos. 161, 240) is sufficient for oxidation of organic compounds, including hydrocarbons at low concentrations and of ammonium by nitrifying bacteria. Under these conditions, nitrification maintains the low concentration of ammonium that is oxidised it to nitrate. Since, oxygen supply rate into the underground medium is limited, oxidation of aromatic compounds by aerobes, including hydrocarbon oxidizing, in polluted water leads to exhausting of dissolved oxygen. The nitrification process is reduced, herewith, accumulation of nitrite happens (wells Nos. 159, 239 and 589) that inhibits the second phase of nitrification (its oxidation to nitrate). Electron donors for denitrifying bacteria are organic compounds, but their biodegradation does not happen, since the nitrate concentration is very low.

There is much more organic carbon available to microorganisms than nitrogen and phosphorus in water polluted by oil product. It is known that petroleum contains about 1–2 % of nitrogen [13]. It is mainly found as heavy petroleum fractions. Upon biodegradation, they can act as nitrogen source for microorganisms. Sources of additional arrival of phosphorus into water polluted by oil products are absent except for the background content in ground water. On the assumption of analysis of the nutritional balance of microorganisms, additional sources of nitrogen and phosphorus nutrition were introduced. Fertilizers were introduced by the scheme given in Table 1.

Based on analysis of the initial data (see Fig. 2) and transformation regularities in soils of oxygen, carbon and biogenic elements, the accent in backfeed was made for phosphorus, since the phosphorus concentration in ground water have a pronounced trend of reduction in the direction of water flow and was 0.1 mg/L. This is a limiting concentration for the growth of microorganisms. Control measurements performed in a month after the introduction of fertilizers into wells did not detect the exceeding limit values of the content of ammonia and nitrate nitrogen in ground water.

Carried out treatment influenced the number of natural microorganisms. Preliminary microbiological analysis demonstrated that there are aerobic (ammonifying, hydrocarbon oxidizing) and anaerobic (denitrifiers, sulphate reducer, iron reducer) microorganisms in water of the ground polluted with oil products. The number of ammonifiers varied in the limits of 10^5–10^6 CFU/mL, hydrocarbon oxidizing – 10^4–10^5 CFU/mL [14]; and that of denitrifiers relatively to other groups of microorganisms was by 2–3 orders of magnitude higher (10^3–10^5 CFU/mL) than that of other anaerobes, which points out at their dominating part upon anaerobic degradation of organic compounds in this ecosystem.

The most intensive treatment was carried out in well No. 159. The maximum increment

TABLE 2

Number of aerobic and anaerobic microorganisms in the water of wells

Physiological groups of microorganisms	Well No.	Sampling dates					
		2005		2006		2007	
		June	October	May	October	March	September
Aerobic		$n \cdot 10^5$ CFU/dm^3					
Ammonifiers	159	15±0.05	21±6.5	1790±904	208±133	0.23±0.04	–
	237	0.1±0.04	380±22	6.7±2.7	12.2±10.2	0.18±0.06	0.24±0.02
	239	0.34±0.02	–	3.4±1.9	171±98	0.6±0.2	0.1±0.03
	589	0.5±0.05	–	1.1±0.96	0.8±0.5	0.22±0.16	0.1±0.04
Hydrocarbon oxidizing	159	1.26±0.25	31.6±18	231±140	141±90	0.16±0.09	–
	237	0.044±0.02	31.6±18	3.4±1.4	102±89	0.17±0.07	0.13±0.07
	239	0.082±0.02	–	1.42±0.69	15.5±6.5	0.16±0.12	0.37±0.09
	589	0.028±0.004	–	0.24±0.14	0.43±0.3	0.0021±0.002	0.005±0.003
Anaerobic		$n \cdot 10^4$ CFU/dm^3					
Denitrifiers	159	n/d	4.5	0.007	9.5	–	–
	237	n/d	20	0.02	0.15	0.02	0.25
	239	n/d	–	H. o.	0.006	0.35	1.1
	589	n/d	-	H. o.	0.004	0.75	0.007

Note. n/d – not detected, dash – not determined.

in the number of all groups of microorganisms (ammonifying, hydrocarbon oxidizing denitrifiers) was observed precisely in ground water samples (Table 2). Already in two months after remediation actions, the number of ammonifiers increased by an order of magnitude from $1.45 \cdot 10^5$ to $20.5 \cdot 10^5$ CFU/mL; hydrocarbon oxidizing – by two orders of magnitude. The number of ammonifiers in water was higher in two orders of magnitude already in three months, and differed from the initial number by three orders in spring of 2006. Herewith, a change in the number of microorganisms was also noted in samples of ground water from wells, in which mineral fertilizers were not introduced. For example, the number of ammonifiers in water of well No. 239 increased by two orders of magnitude. This points out at gradual distribution of the introduced biogenic elements with water current.

As for anaerobic degradation processes, denitrifiers were not registered in ground water at the initial stage of studies, before treatment. As noted earlier (see Fig. 2) nitrate ions became exhausted in ground water, their concentration was insufficient for maintaining denitrification processes. The introduction of nitrate with fertilizers stimulated the development of denitrifiers (see Table 2). Anaerobes were registered in ground water samples from wells Nos. 159 and 237, in which biogenic nutrients were introduced directly, right after the first introduction of nitrate fertilizers. Denitrifiers were detected in all the investigated wells of the polluted site in a year after the second treatment.

Carried out treatment simultaneously affected the chemical composition of water. Chemical composition indicators of the composition of ground water (concentrations of ammonium, sulphate, alkalinity, etc.) can act as biodegradation indicators of oil products under anaerobic conditions [15]. In particular, the ammonium concentration in ground water (over 50 mg/L) significantly increased through 2–3 months after the introduction of fertilizers. Since these changes happened a sufficiently long time, it is impossible to consider nitrogen-containing fertilizers introduced to wells Nos. 159 and 237 as the source of elevated concentrations of ammonium. An increase in the ammonium concentration in ground water testifies biological processes of oil products degradation.

Figure 3 demonstrates the dynamics of the content of ammonium and nitrate ions in well No. 589. The dynamics is of antiphase nature and suggests that intensive enough nitrification

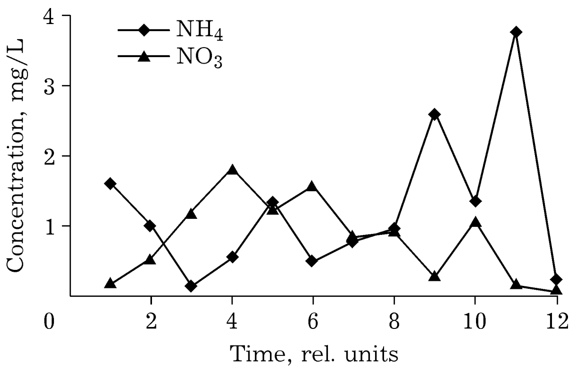


Fig. 3. Dynamics of concentrations of ammonium and nitrate in groundwater from well No. 589 (period from April of 2004 to October of 2010).

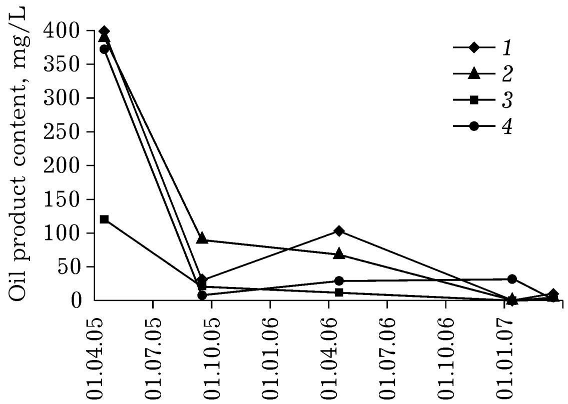


Fig. 5. Dynamics of decline in the concentration of oil products in ground water of fuel oil facilities. Wells Nos.: 159 (*1*), 238 (*2*), 239 (*3*), 589 (*4*).

and denitrification processes proceed in well No. 589. A similar picture was also observed in well No. 239.

To confirm this suggestion a part of data of the ground water composition was used to teach the neuron network to recognize the dynamics in concentrations of ammonium and nitrate forms of nitrogen typical for these processes. Analysis results of the dynamics in concentrations of nitrogen in ground water are demonstrated in Fig. 4.

Compliance of predictions of the neuronet with actual data (see Fig. 4) testifies high a activity of nitrification and denitrification and their determinative role for concentrations of ammonium, nitrite and nitrate in ground water of well No. 589, as well as No. 239.

Thus, despite the fact that biogenic elements were not introduced into well No. 589, intensification of nitrification and denitrification processes occurred in water. The results of studying the neuron network also confirm this conclusion. A high quality of studying driven by the availability of stable regularities is demonstrated in Fig. 4 demonstrating that nitrification and denitrification processes proceed in the vicinity of borehole. Deviations from the detected consistency of nitrification and denitrification indicate that the balance between carbon, nitrogen and phosphorus nutrition of the microflora is violated in ground water of these wells. Factors of the medium that violate this interrelation become dominating. First of all, concentrations of dissolved oxygen, phosphorus and nitrate nitrogen relate to them.

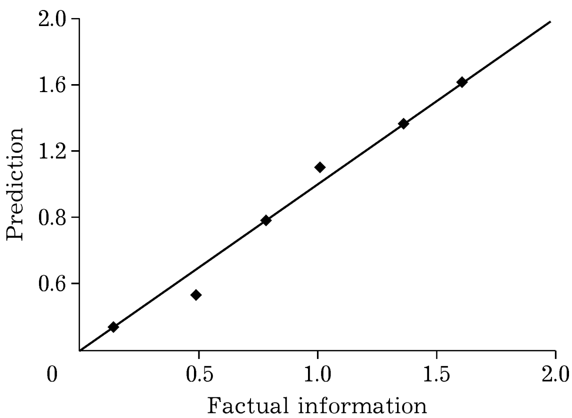


Fig. 4. Results of studying the neuronet to predict the concentrations of ammonium/nitrate in groundwater (by data of well No. 589, 2004–2007).

The efficiency of an increase in the bioremediation intensity of the polluted ground water by the introduction of biogenic elements is demonstrated in Fig. 5. The content of oil products in ground water of all the investigated wells decreased from 350–400 to 1.5–10 mg/L. The disappearance of an oil film on the surface of water was registered visually.

CONCLUSION

A stable decrease in the concentration of oil products in water in the whole problem site was observed resulting from stimulation of microflora activity of the polluted ground water during three years. Thus, determining the leading factors of a change in the content of all forms of nitrogen in ground water enables to select most important for recovery of the entire object of the well and an option of their treatment. It was demonstrated that efficient bioremediation processes of ground water polluted by organic compounds could be intensified by stimulating the natural microflora without using commercial microbial preparations.

REFERENCES

1 Vodyanitskiy Yu. N., *Pochvovedeniye,* 11 (2011) 1364.

2 Margesin R., Schinner F., Marx J.-C., Gerday C., Psychrophiles: from Biodiversity to Biotechnology, in Margesin R., Schinner F., Marx J.-C., Gerday C. (Eds.), Springer, Berlin, 2008.

3 Trusey I. V., Ozerskiy A. Yu., Ladygina V. P., Gurevich Yu. L., *Sib. Ekol. Zh.*, 1, 16 (2009) 29.

4 Kraynov S. R., Ryzhenko B. N., Shvets V. M., Geokhimiya Podzemnykh Vod. Teoreticheskiye, Prikladnye i Ekologicheskiye Aspekty, in N. P. Laverov (Ed.), Nauka, Moscow, 2004.

5 Franzmann P., Robertson W., Zappia L., Davis G., *Biodegradation*, 13, 1 (2002) 65.

6 Kondakova G. V., Bakterial'nye Protsessy Transformatsii Soyedineniy Ugleroda i Azota v Termal'nykh Mineralizovannykh Podzemnykh Vodakh s Razlichnoy Stepen'yu Kontaminatsii (Abstract of Candidate's Dissertation in Biology), Moscow, 2001.

7 Maksimovich N. G., Khmurchik V. T., *Vestn. Perm. Un-ta*, 3, 16 (2012) 47.

8 Metodicheskiye Rekomendatsii po Vyyavleniyu, Obsledovaniyu, Pasportizatsii i Otsenka Ekologicheskoy Opasnosti Ochagov Zagryazneniya Geologicheskoy Sredy Nefteproduktami, GIDEK, Moscow, 2002.

9 Goldberg V. M., Mel'kanovitskaya S. G., Luk'yanchikov V. M., Metodicheskiye Rekomendatsii po Vyyavleniyu i Otsenke Zagryazneniya Podzemnykh Vod, VSEGINGEO, Moscow, 1988.

10 Gerhardt P. (Ed.), Manual of Methods for General Bacteriology, American Society for Microbiology, American Society for Microbiology, Washington, D.C., 1981.

11 Okhonin V., Okhonin S., Ils A., Ilegemres M., *Neural Network World*, 11, 2 (2001) 145.

12 Lankin Yu. P., Lobova T. I., Popova L. Yu., *Optical Memory and Neural Networks,* 15, 2 (2006) 65.

13 Yanovskaya S. S., Sagachenko T. A., *Izv. TPU*, 3 (2009) 132.

14 Ladygina V. P., Trusey I. V., Gurevich Y. L., Bioremediation of the Fuel Oil Contaminated Subsurface, The 2nd Int. Conf. on Bioinformatics and Biomedical Engineering (iCBBE 2008), May 16–18, 2008, Shanghai, China, vol. V: Environmental Pollution and Public Health (2). URL: http://www.icbbe.org (accessed January 21, 2016).

15 Eriksson S., Ankner T., Abrahamsson K., Sjöling Esa, *Geomicrobiol. J.*, 23 (2006) 45.