Influence of Anthropogenic Pressures on Groundwater Quality from a Rural Area

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During the past few decades, the anthropogenic activities induced worldwide changes in the ecological systems, including the aquatic systems. This work analysed the contamination level of groundwater resources from a rural agglomeration (Central-Western part of Prahova County) by biological and physico-chemical approaches. The study was performed during the autumn of 2016 on several sampling sites (four drilling wells, depth higher than 100 m supplying three villages; two wells lower than 10 m depth and one spring). The water quality was evaluated by comparison with the limit values of the drinking water quality legislation (Law no.458/2002) and the Order 621/2014 (applicable to all groundwater bodies of Romania). The results showed that phenols and metals (iron and manganese) exceeded the threshold values in all sampling sites. Moreover, the anthropogenic factors including agriculture, use of fertilizers, manures, animal husbandry led to an increase of the bacterial load, particularly at wells sites.

Keywords: groundwater quality, metals, microbiological contamination, rural agglomeration

Anthropogenic activities (industrial development and agriculture) have had a major impact on all ecological systems around the world. In spite of the fact that measures were initiated to monitor and to reduce contaminants from these systems, unfortunately still there are gaps for an appropriate management of the natural capital components. The groundwater systems represent the world's largest freshwater reservoir, being the main drinking water source (more than 75% of the European Union (EU) population depends on groundwater for water supply) and an important resource for industry (cooling water) and agriculture (irrigation) [1]. Moreover, the groundwater systems play an essential role in the hydrological cycle maintaining the wetlands by acting as a buffer reservoir during periods of drought and raining [2]. The major pollutants which could affect the groundwater quality were identified such as petroleum products, products from industrial processes, chemical products used in agriculture (fertilizers, pesticides) [3], household products, heavy metals, pharmaceuticals products [4], personal care products and food products [5].

However, groundwater aquatic ecosystems have a tremendous potential to naturally mitigate and degrade aerobically and anaerobically [6] a wide range of discharged pollutants [7 - 9] due to a large diversity of microbial communities identified at this level [10 - 14]. In spite of their benefic effects, the microorganisms may be pathogenic and they have become another class of contaminants together with viruses. Potentially pathogenic microorganisms reach groundwater levels, usually by infiltration of surface wastewater or rainwater from the chemical fertilized areas [15, 16]. The presence of microorganisms with pathogenic potential at groundwater level has been a significant health hazard issue because these *reservoirs* are intended for human consumption, too.

In this context, this paper assessed by biological and physico-chemical methods the contamination degree of groundwater resources, as main raw water source for human consumption, from a rural area (Cocorastii-Mislii, Goruna and Tiparesti, Prahova County).

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Experimental part

Sampling sites

Samples from eight sampling points were monthly collected during September and November 2016: i) from five drilling wells (F1, F2, F3, F5 and F4 which was not in use during the study), ii) from a spring (I) - 9 km in Bustenari region, upstream Cocorastii-Mislii village and iii) from two wells (depth of 10 m well at Tiparesti - P_T and depth of 8 m well at Goruna-P_G) (fig.1). The water from the drilling wells was discharged in two storage tanks of 300 cubic meters (cm) capacity each. From the water tanks, the water was gravitationally distributed through a 18 km lenght main network, covering about 95% of the streets from the villages. Almost 98% of the households were connected to the water supply network.

Pysico-chemicals assessment of the groundwater quality parameters was performed according to the drinking water quality Law no. 458/2002 [17] and the Order 621/2014 (in which it mentioned the threshold values applicable to all groundwater bodies in Romania) [18]. In this particular case, the limit values were set for the groundwater body ROIL15.

Several physical and chemical quality parameters such as *p*H, turbidity, conductivity, permanganate index (CCOMn), total hardness, ammonium (NH_4^+), nitrates (NO_3^-), nitrites(NO_2^-), phosphates (PO_4^{3-}), chlorides, sulphates (SO_4^{2-}), chromium (Cr), nickel (Ni), copper (Cu), zinc (Zn), cadmium (Cd), mercury (Hg), arsenic (As), iron (Fe), manganese (Mn), phenol index, petroleum products were characterized throughout electrochemical, volumetric, UV-Vis spectrometry, inductively coupled plasma atomic emission spectrometry (ICP-EOS), respectively high-performance liquid chromatography (HPLC) methods. All analyses were performed in accreditation system according to SR EN ISO 17025:2015 reference standard [19].

Microbiological assessment of the total number of bacterial colonies grown at 22 and at 37°C or total number of coliform bacteria, *Escherichia coli*, enterococci and *Pseudomonas aeruginosa* were previously described Gheorghe et. al [11]. In addition, the identification of the bacterial microflora structure was conducted based on their specific metabolism by the OmniLog (Biolog Inc., USA) automated system.

Results and discussions

The studied area (fig. 1) was chosen based on the oil exploitations that occurred during 1857-1933 in Bustenari region [20]. Unfortunately, at the present, there still is a high contamination level with petroleum products and phenolic compounds due to the groundwater aquifer from the Prahova-Teleajen alluvial cone. In addition, there were important levels of chemicals from the agriculture fertilization (nitrogen and phosphates compounds etc.) and a significant residual level of heavy metals due to anthropogenic impact.

The hydrogeological informations of the drilling wells (Table 1) and the lithological information showed that only clays was present up to 10 m of depth creating an impermeable soil that constitute a real protector screen against the penetration of the surface contamination.

Characterization of physical and chemical parameters

The *p*H values of the water samples varied between 6.9 - 7.4 *p*H units, which was acceptable for drinking water quality according to the Law no. 458/2002. Turbidity values were also below the limit imposed by law (lower than 5 NTU), but a few exceptions appeared such as: i) 5.35 NTU in September and 5.36 NTU in November at the F1 sampling site, or 8.9 NTU at F2 and 7.13 NTU at F5, both sites in November 2016.

The electrical conductivity, chlorine and SO_4^{2-} concentrations were similar at the four drilling wells (F1, F2, F3 and F5) and under the legal threshold. Overall, the values of the electrical conductivity, chloride concentrations and SO_4^{2-} from all sampling sites during the entire monitoring period were within the limit values required by Law 458/2002 and Order 621/2014. The highest concentrations of electrical conductivity (770 ìS/cm), chlorides (47.7 mg/L to 55.6 mg/L) and SO_4^{2-} (25.4 mg/L - 33.02 mg/L) were measured at P_G sampling site followed by spring I and drilling wells sampling sites.

The nutrients values of NH_4^+, NO_3^-, NO_2^-, PO_4^{3-} calculated at F1, F2, F3, F5, I and P_T did not exceed the limit values

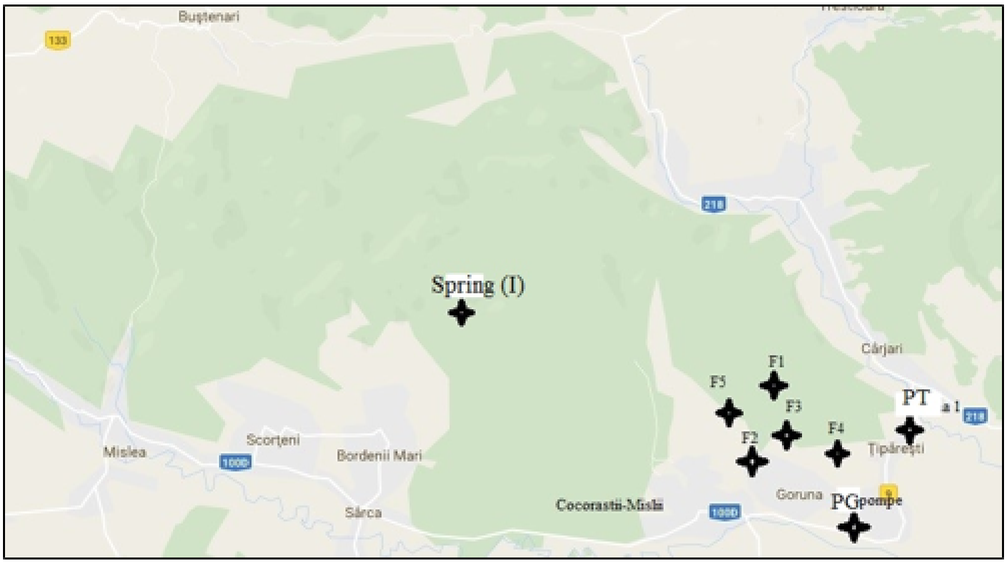

Fig. 1. Map of the sampling sites – in Cocorastii-Mislii village, Prahova county. F1, F2, F3, F4, F5 – drilling wells; I, spring; P_G, P_T, wells

Table 1

HYDROGEOLOGICAL INFORMATIONS OF THE DRILLING WELLS [21] (ACCORDING TO MURARESCU, 2015)

Drill name	*Year of construction*	*Depth (m)*	*Aquifer intervals captured by filters (m)*	*Hydrostatic level (m)*	*Hydrodinamic level (m)*	*Extract flow (cm/h)**
F1	1979	100	35.5-38,5 52.5-57 68-71 79-83.5	41	47.5	14
F2	1979	100	58.5-64.5; 76.5-79.5; 89-95	41,5	45	20
F3	1983	100	51,5-55,5 66-68 76-77,5 79-81	44,5	50	9
F4	1988	100	53-56 79-83.5	52	57.6	9
F5	2010	144.5	75.5-80 89-94 101.5-104.4 131.5-134.5	44	56	31/s

**cm/h- cubic meter per hour*

Table 2
THE VARIATION OF Fe (μg/L) AND Mn (μg/L) CONCENTRATIONS ALONG GROUNDWATER SAMPLING SITES DURING SEPTEMBER AND NOVEMBER 2016

Sampling site / Law no. 458/2002 Order no. 621/2014	*Period / metals*							
	Fe (μg/L)				**Mn (μg/L)**			
	IX-16	*X-16*	*XI-16*	*Avg±SD#*	*IX-16*	*X-16*	*XI-16*	*Avg±SD#*
F1	**276**	**211**	**306**	264.33±48.6	**171**	**158**	**173**	167.3±8.14
F2	**207**	**263**	**825**	431.7±341.8	**142**	**165**	**167**	158±13.9
F3	33.8	22.6	7.5	21.30±13.2	26.2	11.3	15	17.50±7.76
F5	58.8	56.9	**224**	113.2±95.9	42.3	**54.1**	**52.4**	49.6±6.38
I	19.3	12.1	13.5	14.9±3.82	41	39	38.7	39.6±1.25
P_T	23.6	84.4	69	59±31.6	3.6	**69.9**	38.6	37.4±33.2
P_G	32.2	71.3	61.2	54.9±20.3	29.7	9.7	7.9	15.8±12.1
Threshold value	**200**				**50**			

 # Average ± Standard Deviation

Table 3
THE DYNAMICS OF MICROBIOLOGICAL QUALITY PARAMETERS DURING SEPTEMBER – NOVEMBER 2016 AT P_G

Bacteria	*M.U.*	*Period*			*Law no. 458/2002*	*Avg.± SD*
		IX-16	X-16	XI-16		
Number of bacteria at 22°C	CFU/mL	**955**	**6100**	**609**	100	2555±3075
Number of bacteria at 37°C	CFU/ mL	**310**	**609**	**673**	20	531±194
Total coliforms	CFU/100 mL	**2525**	**417**	**704**	0	1215±1143
Escherichia coli	CFU/100 mL	**145**	**135**	**409**	0	230±155
Enterococci	CFU/100 mL	**27**	**545**	**318**	0	297±260
Pseudomonas aeruginosa	CFU/100 mL	0	**54**	**45**	0	33±29

during the study period. An exception was the P_G site where the phosphate concentration 0.73 mg/L in September, 0.96 mg/L in October and 0.75 mg/L in November exceeded the threshold value (0.7 mg/L) [18]. Phosphate inputs attributed to the anthropogenic impact such as household waste and / or stored manure used as fertilizer in agriculture, explained the significant number of bacteria detected at this sampling site (table 3).

The phenol concentrations exceeded the limit value imposed by the groundwater quality norm and it was correlated to the historical oil pollution and phenolic compounds previously reported in the Prahova-Teleajen area [22] due to PetroBrazi, Astra Romana, Petrotel Ploiesti, Vega refineries.

The concentration of the most tested metals (Na, Cr, Ni, Cu, Zn, Cd, Hg, Pb, As), with the exception for Fe and Mn (table 2), was below the limit values of drinking water and groundwater quality regulations.

Fe and Mn were naturally present in soil, rocks, and minerals from the groundwater bodies and a higher concentration of 0.2 mg/L Fe or 0.05 mg/L Mn could seriously affect the water quality [23]. During the study period, the Fe values calculated at P_T, P_G and I were below the limit value according to regulations [17, 18]. Overall, the average Fe concentrations at F3 and F5 at all four drilling wells were lower than the drinking water quality threshold. The concentration of Mn at I sampling site was increased by 4-folds compared to the threshold value and overall the average Mn values were above the maximum admissible value [17, 18]. Although Mn could be eliminated from water by treatment technologies, this treatment was costly and not efficient because all the water supply infrastructure (not only the treatment area) pipelines, pumps and drilling could be continuously affected by the accumulation of Mn oxides [24, 25]. The occurrence and concentration of Mn in the groundwater aquatic systems were modulated by several factors such as geochemical composition of the rock, chemical composition of the water and the microbiological activity. Certain types of rock, such as mafic rocks, marl and limestones, contain high Mn concentrations, which can lead to massive accumulation in soil and sediment. The chemical composition of water, especially pH, redox potential, dissolved oxygen and dissolved organic carbon play an essential role in mobilizing Mn, controlling its specificity and concentration in the aquatic environment. Mn was mainly produced by reducing the soluble Mn^{2+} in low pH conditions and redox potential, and oxidized to form precipitates in the presence of oxygen at a higher *p*H [26]. Complexation with humic substances [27] inhibited Mn oxidation and precipitation, although complexation with inorganic ligands such as sulphate or acid carbonate had only a limited effect on Mn concentration, with the exception of large concentrations of ligand [28]. At the same time, micro-organisms could play an important role in mobilizing Mn from the medium which could modulate Mn concentrations in the groundwater. The effects of Mn mobilisation can be – direct – by enzymatic catalysis of Mn oxidation and reduction, and specific binding to cell-associated materials, or indirectly by changing pH and redox conditions, thereby influencing Mn concentration. The impact of microbiological activity on Mn behavior in water was proved by the oxidized Mn accumulation in biofilms from the supply infrastructure surfaces [24].

Microbiological characterization

The microbiological analysis of the groundwater systems showed a link between bacterial populations at sampling sites and the type of pollution.

P_T sampling site. Throughout the study period, the density of bacteria grown at 22°C and 37°C as well as the presence of *Escherichia coli*, enterococci and *Pseudomonas aeruginosa* at P_T site were above the limit value of Law 458/2002. Additionally, we were able to identify the following bacteria species: *Serratia liquefaciens, Klebsiella pneumoniae, Enterococcus mundtii, Paenibacillus sanguinis, Enterococcus galinarum.* At this sampling site, the groundwater was connected to a surface water source (Cosmina River) which could explain the presence of a high number of potentially pathogenic bacteria.

Enterobacteria present at this point (*Enterococcus mundtii and Enterococcus galinarum*) were considered as indicators of animal and / or human faecal contamination [29, 30]. It was also known that enterococci have pathogenic-potential causing disease in hosts where the immune compromised hosts [31]. Unfortunately, these bacteria species have been previously reported in groundwater systems from rural areas [32].

P_G sampling site. High bacterial load was observed at P_G during the study period where 6100 colonies forming units (CFU) / mL were present at 22°C in October 2016, a 60-fold higher than the limit value of drinking water quality law. In addition, at 37°C total coliform bacteria, *Escherichia coli*, enterococci, *Pseudomonas aeruginosa* densities increased, too. The increased number of total coliform bacteria could be explained by well maintenance (corrosion, superficial membranes) (table 3) and / or by the infiltration of residual waters from surrounding households, rainfall water leaching from fertilized agricultural crops (gardens) or manure storage. Coliform bacteria could persist in biofilms and we assume that mechanical disturbances during pumping can cause biofilm dislocation, leading to the release of coliform bacteria to the wells. Among the released bacteria, we were identified potential pathogen bacteria *Enterococcus hirae, Paenibacillus wynnii, Enterococcus casseliflovus, Enterococcus mundtii, Routella planticola* (gram-negative aerobe species, it is representative for the environmental samples that do not cause invasive infection in humans [33]).

Furthermore, a positive correlation was found between the density of total coliform bacteria, *Escherichia coli*, enterococci and phosphate concentrations at this sampling site. These bacteria are usually found attached to suspended matter, and their dynamics depend on the presence of particulate matter which was observed (data not shown).

Spring (I) sampling site. During the study period, high numbers of total coliforms bacteria (13 CFU/100 mL in September, 51 CFU/100 mL in October and 81 CFU/100 mL in November), *Escherichia coli* (3 CFU/100 mL average density) and enteroccoci (4 CFU/100 mL) were recorded at spring sampling site. Moreover, the number of bacteria developed at 22 °C (179 CFU/ mL in November 2016) was above the limit value for drinking water.

Drilling wells sampling sites. The dynamics of numerical densities of microbiological quality indicators of all the four boreholes (F1, F2, F3, F5) which supplies water for the three villages (Cocorasii-Mislii, Goruna and Tiparesti) were shown in figures 2 and 3.

Total coliforms bacteria were above the limit value for drinking water quality in October (5 CFU/100 mL) and November (47 CFU/100 mL) at F1 and September (4 CFU/

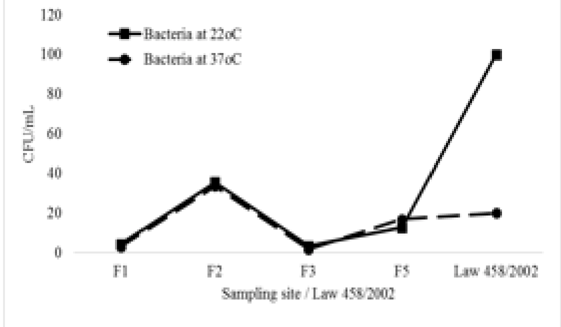

Fig. 2. The variation of number of bacteria at 22°C and 37°C (reported as the average value of the three-sampling period)

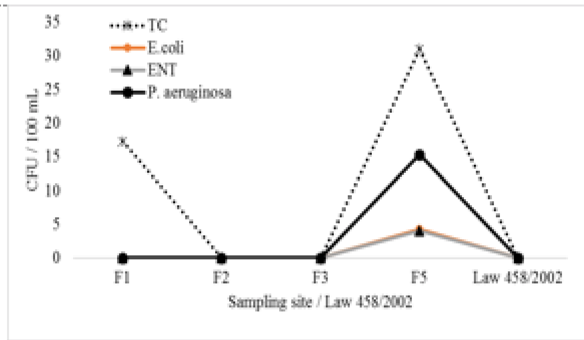

Fig. 3. The variation of numerical densities of total coliforms (TC), *Escherichia coli* (*E. coli*), enterococci (ENT), *Pseudomonas aeruginosa* (*P. aeruginosa*) (reported as the average value of the three-sampling period)

100 mL), October (1 CFU/100 mL) and November (88 CFU/ 100 mL) at F5. *Escherichia coli* (2 CFU/100 mL in September and 11 CFU/100 mL in November), enterococci (6 CFU/100 mL in October and November), *Pseudomonas aeruginosa* (46 CFU/100 mL in November) and number of bacteria at 37°C exceeded the limit value of Law 458/2002 at F5. Overall, it was found that F3 did not record overtaking of the microbiological quality indicators throughout the study period, which allows us to appreciate that F3 meets the drinking water quality conditions.

Conclusions

This study monitored the groundwater aquatic systems from a rural area from September to November 2016. Turbidity, phosphates (PO_4^{3-}), phenols, iron (Fe) and manganese (Mn) values were above the limit values for drinking water quality according to the Law no. 458/2002 and Order 621/2014, regardless of the sampling sites and the period of time.

The microbiological quality parameters (total number of bacteria grown at 22, at 37°C as well as total coliform bacteria, *Escherichia coli*, enterococci and *Pseudomonas aeruginosa*) were also above the limit values for drinking water quality, at all sampling sites with the exception of F3.

Several pathogenic microorganisms were identified at the wells sites: *Serratia liquefaciens, Klebsiella pneumoniae, Enterococcus mundtii, Paenibacillus sanguinis, Enterococcus galinarum, Routella planticola, Enterococcus hirae, Paenibacillus wynnii, Enterococcus casseliflovus, Enterococcus mundtii.*

Future researches will focus on the distribution patterns of groundwater associated fauna in various environmental conditions, including their structure and composition in order to pinpoint various bioindicators for groundwater quality assessment. In addition, the drinking water (after chlorination) quality which is distributed to rural consumers will be considered for evaluation.

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References

1.DALY, D., Biology and Environment: Proceedings of the Royal Irish Academy, JSTOR, 2009, p. 221.

2.SCRADEANU, M., IORDACHE, V., PALCU, M., SCRADEANU, D., International Symposium The Environment and the Industry, 2016, p. 292.

3.STANESCU, B., DUMITRACHE, R., KIM, L., International Symposium "The Environment and the Industry", **1**, 2013, p. 234.

4.GHEORGHE, S., PETRE, J., LUCACIU, I., STOICA, C., NITA-LAZAR, M., Environ. Monit. Assess., **188**, no. 6, 2016, 379.

5.RICHARDSON, S.D., TERNES, T.A., Anal. Chem., **83**, 2011, p. 4614.
6.PATROESCU, I.V., DINU, L.R., CONSTANTIN, L.A., ALEXIE, M., JINESCU, G., Rev. Chim. (Bucharest), **67**, no.8, 2016, p. 1433.
7.DIAZ, E., JIMENEZ, J. I., NOGALES, J., Curr. Opin. Biotechnol., **24**, 2013, p. 431.
8.JEON, C.O., MADSEN, E.L., Curr. Opin. Biotechnol., **24**, 2013, p. 474.
9.GIEG, L.M., FOWLER, S.J., BERDUGO-CLAVIJO, C., Curr. Opin. Biotechnol., **27**, 2014, p. 21.
10.GRIEBLER, C., LUEDERS T., Freshwater Biol., **54**, 2009, p. 649.
11.GHEORGHE, S., CATRANGIU, A., LUCACIU, I., TIRON, O., COSMA, C., STOICA, C., DINU, L., Water Sci Tech-W Sup, **15**, no.1, 2015, p. 66.
12.VAN DER MEER, J.R., Front Ecol Environ, **4**, 2006, p. 35.
13.KOLVENBACH, B.A., HELBLING, D.E., KOHLER, H.-P.E., CORVINI, P.F.X., Curr. Opin. Biotechnol., **27**, 2014, p. 8.
14.LAZAR, M.N., GHEORGHE, S., ANGHELACHE, A., BANCIU, A., STOICA, C., LUCACIU, I., Rev. Chim. (Bucharest), **67**, no.8, 2016, p.1454.
15.LUCENA, F., RIBAS, F., DURAN, A.E., SKRABER, S., GANTZER, C., CAMPOS, C., MORON, A., CALDERON, E., JOFRE, J., J. Appl. Microbiol., **101**, 2006, p. 96.
16.POPESCU, L.R., IORDACHE, M., BUICA, G.O., UNGUREANU, E.M., PASCU, L.F., LEHR, C., Rev. Chim. (Bucharest), **66**, no. 12, 2015, p. 2060.
17.*** LAW 458/2002 on drinking water quality.
18.*** ORDER 621/2014 (the threshold values applicable to all groundwater bodies in Romania, the groundwater body ROIL15).
19.***SR EN ISO/CEI 17025:2005. General requirements for the competence of testing and calibration laboratories.
20.NICULESCU, G., VELCEA, I., Editura Academiei Republicii Socialiste România, 29, 1973.
21.MURARESCU, M., Studii geologice - geotehnice si hidrogeologice - si consultanta in domeniu, 2015 (*in Romanian*).
22.SERBAN, P., GALIE, A., Tipored, 2006, p.293.
23.HOMONCIK, S.C., MACDONALD, A.M., HEAL, K.V., DOCHARTAIGH, B.E.O., NGWENYA, B.T., Sci Total Environ, **408**, 2010, p. 2467.
24.SLY, L.J., HODGKINSON, M.C., ARUNPAIROJANA, V., Appl Environ Microbiol, **56**, 1990, p. 628.
25.VASILE, G.G., DINU, C., ENE, C., CRUCERU, L., DAMIAN, N., STANCU, A., Rev. Chim. (Bucharest), **66**, no. 1, 2015, p. 92.
26.HEM, J.D., Department of the Interior, US Geological Survey, **2254**, 1985.
27.GRAHAM, M.C., GAVIN, K.G., FARMER, J.G., KIRIKA, A., BRITTON, A., Appl Geochem, **17**, 2002, p. 1061.
28.HEM, J.D., Geol Soc Am Bull, 83, 1972, p. 443.
29.VALENZUELA, A.S., OMAR, N.B., ABRIOUEL, H., LÓPEZ, R.L., ORTEGA, E., CAÑAMERO, M.M., GÁLVES, A., Food Chem Toxicol, 46, 2008, p. 2648.
30.NITA-LAZAR, M., GALAON, T., BANCIU, A., PAUN, I., STOICA, C., LUCACIU, I., J. Environ. Prot. Ecol., **17**, no. 1, 2016, p.237.
31.FISHER, K., PHILLIPS, C., Microbiol, **155**, 2009, p. 1749.
32.ATEBA, C.N., MARIBENG, M.D., Afr. J. Microbiol. Res., **5**, no. 23, 2011, p. 3930.
33.NADA, B., AREEJ, M., J. Taibah Univ. Med. Sci., **9**, 2014, p. 158.

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