STUDIA UBB CHEMIA, LXIV, 1, 2019 (p. 79-92)
(RECOMMENDED CITATION)
DOI:10.24193/subbchem.2019.1.06

INFLUENCE OF TOTAL CHLORIDE, ARSENIC AND ALUMINUM CONTENTS ON MICROORGANISMS OF OPEN WELLS

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ABSTRACT. The paper presents the level and spatial distribution of total chloride, arsenic and aluminum contents, and that of microbiological parameters (coliform bacteria, *E. coli* bacteria, intestinal enterococci) from water wells situated in the Seini area, both on the surface and in depth, using the geostatistical module of ArcGIS. The relations between the levels of microorganisms and the concentrations of chemical parameters (total chlorides, As and Al) were established using Table Curve program for generating 3D mathematical models.

Keywords: *total chloride, arsenic, aluminum, microorganism, drinking water*

INTRODUCTION

Groundwater represents the world's most exploited raw material and supplies around 31.5 % of the global population with drinking and domestic water [1]. Clean water is a quintessential resource that impacts human quality of life across the globe. With 97.5% of water sources being in the form of salty oceans and 2.15% in that of ice caps and glaciers, the remaining 0.65% is of great importance [2]. With the industrial and agricultural expansion, due primarily to a rapid increase in population, fresh, clean water has become a commodity at present.

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Among all contaminants, microorganisms are one of the primary causes of waterborne medical conditions, even if present in low concentrations [2]. However, as it is not economically feasible to test all water sources for every single type of bacteria, the presence of enteric organisms of fecal origin is monitored. Common indicators here come in the form of total coliform bacteria and intestinal enterococci [3]. Microorganisms are relevant markers for the proper health and functioning of the groundwater ecosystem. Thus, by performing microbial analyses such as biomass, activity and diversity of microorganisms, possible organic matter enrichments in groundwater ecosystems could be detected [4].

The spatial distribution characteristics of groundwater presents powerful zonation. This is predominantly caused by the differences in the structures of water storages from mountain to field areas with various scales, water yield properties, and, aquifer permeability [5]. The chemical composition of groundwater and its evolution depend on the mineralogy of the aquifer, overlapping land uses, geochemical processes, the recharge source, as well as the inputs from anthropogenic sources [6]. In terms of its dynamics, groundwater can be classified by the vertical direction (by its depth) into an upper, intense alternation zone, a middle, slow alternation zone, and a bottom, extremely slow alternation zone. In terms of geochemistry and action of microorganisms, aquifers can be classified as high and low-iron content zones [5].

This study is complementary to a previous one about the quality of groundwater in Seini town that focused on heavy metal concentrations [7]. The present study extended the data on the groundwater quality in Seini by the analysis on the microbiological upload of well water samples and spatial distribution of bacterial level in the area [7]. The microbiological data and the chemical parameters, Cl^-, As and Al for groundwater samples was analyzed aiming to find an appropriate mathematical model using a computer program of regression analysis.

RESULTS AND DISCUSSION

Analysis of the 3D distribution of elements and the microbiological parameters in depth and on the surface of the well water

Figure 1 shows the distribution of concentrations of chloride (mg/L), arsenic (μg/L) and aluminum (μg/L) on the surface and in depth. The mean concentration of total Cl^- was 31.83±17.99 in the range of 11.3-63.8. The levels of Cl- are lower than the maximum admissible concentration of 250 mg/L according to drinking water regulations of Romanian Legislation (Law 311/2004).

Inorganic chloride species from water sterilization and the production of fertilizers, along with organic species derived from the production of pesticides and bleaching of fabric have a strong influence on the Cl^- concentration of plants in the vicinity [6]. Total chlorides content in groundwater derives from natural sources such as weathering of rocks, minerals and soils or anthropogenic sources as spreading of deicers on the roads, wastewater infiltrations, runoff from waste dumps and agriculture practice by irrigation, uses of fertilizers or pesticides [8,9]. The presence of chloride increases the corrosivity of water by enhancing electrical conductivity. In metal pipes, chloride reacts with metal ions to form soluble salts, thus, increasing levels of metals in drinking-water [8].

Aluminum is not a heavy metal, it is an element naturally found in the earth's crust in a proportion of 8% and is thus difficult to avoid. The presence of aluminum in natural or anthropogenic water is due to melting and metal foundries, bitumen sand exploits, oil refining, coal extraction and use or construction [10,11]. In regards to its speciation in potable water, literature reports that the total aluminum concentration is the sum of all colloidal, suspended and monomeric forms [12]. The level of aluminum in water can significantly vary depending on physicochemical and mineralogical factors, generally ranging from 1 to 50 µg/L but rising up to 500–1000 mg/L in more acidic water sources or those rich in organic matter. The highest concentrations of Al were found in the middle and eastern part of the investigated area where the Eutricambisol and Gleisols soils are predominant and the pH was more acidic than in the western part of the town, as was previously reported [7]. The major chemical species of Al that can be present in groundwater are hydroxo-aluminum components and those containing silicon, aluminum species with Na^+ and K^+ ions, aluminum free ions, aluminum components with F- ions and aluminum species with SO_4^{2-} ions, but aluminum hydroxide complexes and silicon hydroxide complexes are more widespread [13].

Arsenic in water, at natural pH, is found only in inorganic form at valences III and V. Arsenic can enter the food chain through eating contaminated vegetables and meat, and, drinking water. Generally, for humans, trivalent arsenic compounds are 60 times more toxic than pentavalent ones, leading the WHO to regulate its maximum concentration in drinking water at 10 µg/L [9]. The mean As in groundwater was 0.0132±0.00016 µg/L with a low variability indicating the lack of As pollution sources in the area. Following Figure 1 there is an increase in water surface values in densely populated areas. Arsenic content may be due to soil clay content and anthropogenic activities (such as the use of arsenic pesticides and the burning of fossil fuels) [14].

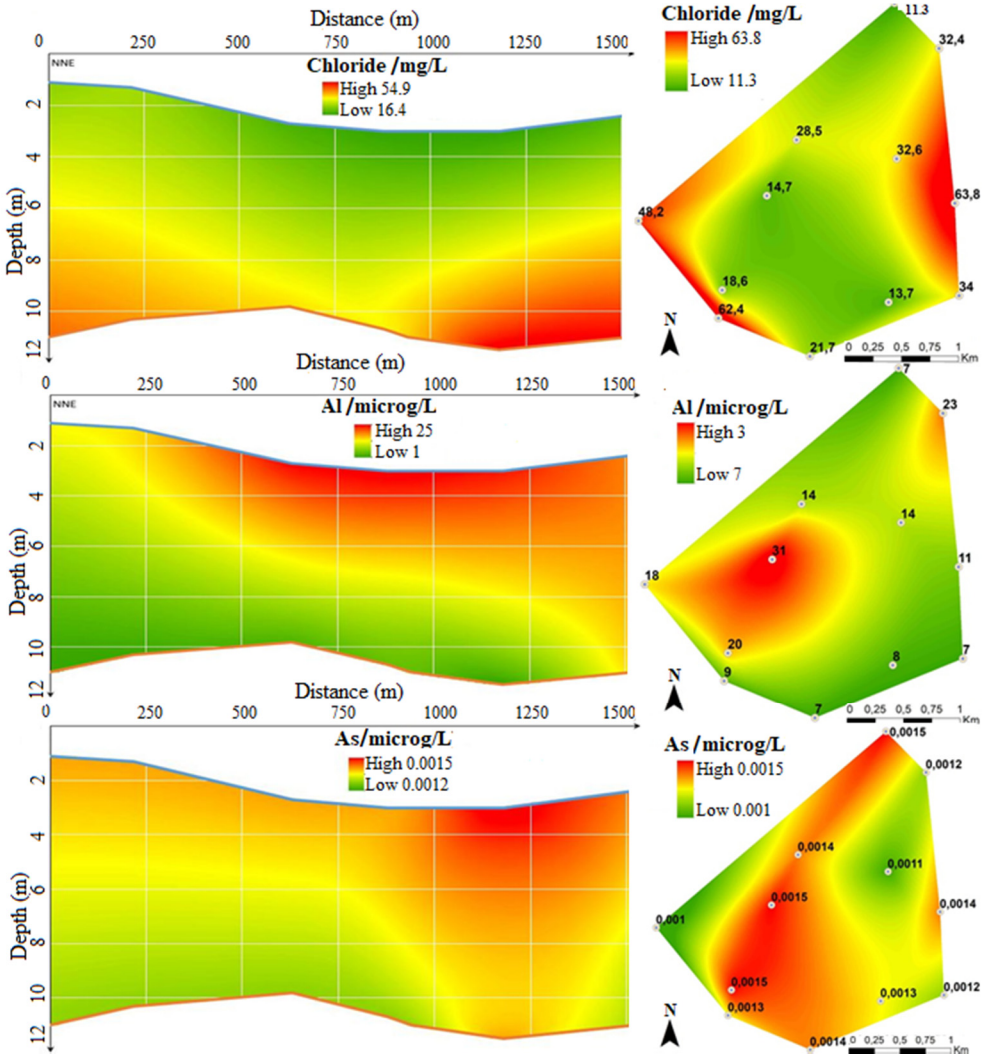

Figure 1. Distribution of total chlorides, aluminum and arsenic contents in depth and on the surface

Figure 2 shows the distribution of microbiological parameters (coliform bacteria, E. coli bacteria, and intestinal enterococci) on the surface and in depth of water at the sampling points. The study is very interesting in the conditions in which fountains are open, there being high chances of contamination

with microorganisms. Coliform bacteria in deep water should be lacking if the water sources are not contaminated. It often happens that water contaminated with coliforms reaches boreholes, springs, when it rains during periods of thawing or cracks of the earth. Contaminated water with coliform bacteria should be boiled for at least one minute before it is consumed. Such bacteria are present in the pig and cow farm areas, but also in the area where a well was affected by the floods.

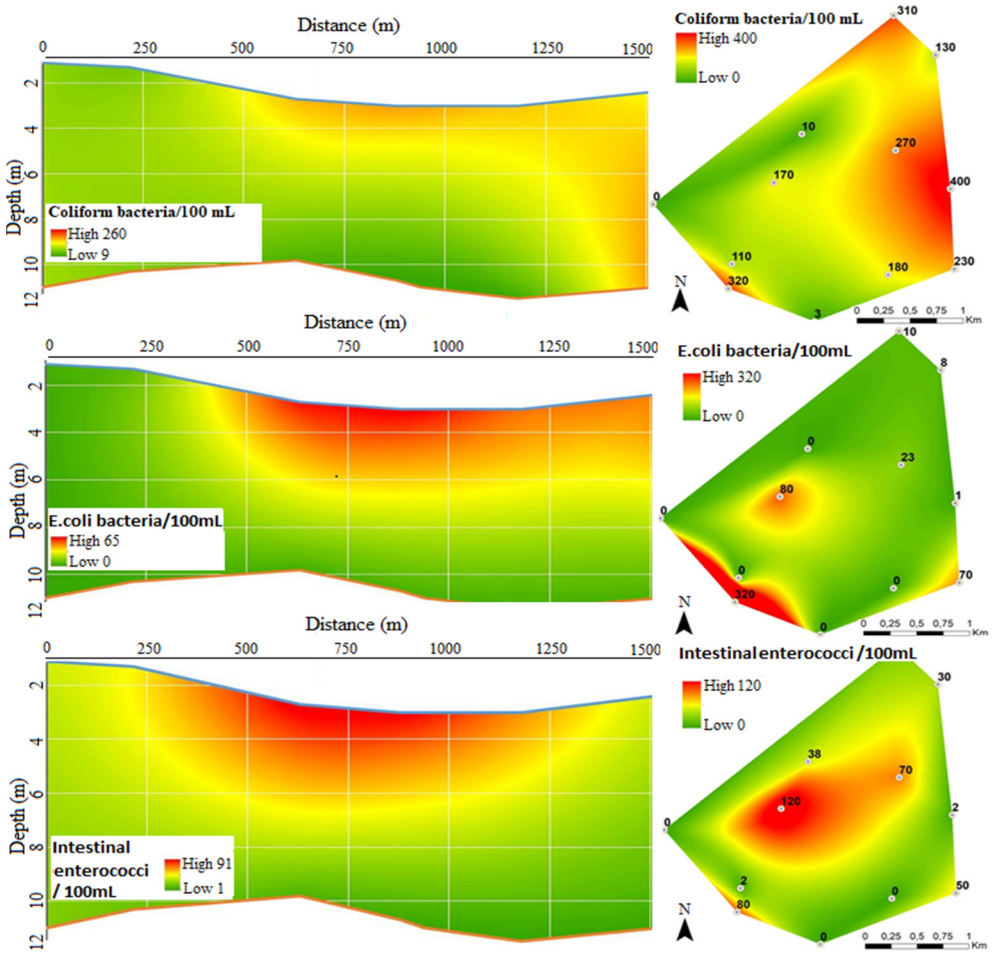

Figure 2. Distribution of coliform bacteria, E. coli bacteria and intestinal enterococci in depth and on the surface

Intestinal enterococci are found in surface areas, especially in places where animals are grown and where anthropogenic activities are advanced, or in low areas, as a result of floods that can affect the septic fosses of citizens. According to Figure 2, intestinal enterococci are found on the surface, respectively in the central area of the town, where the population density is higher.

Escherichia coli bacteria, known as E. coli bacteria, is a specific coliform bacterium of fecal origin that lives in the human intestines and intestines of warm blooded animals, also present in their waste. Each country has established its own indicators, among the limits of which the water is considered to meet the conditions necessary for it to be consumed. E. coli bacteria is usually found in the surface layers of the soil, in the areas where animals are grown, and after the manure infiltrates the soil, and then the drinking water affecting large part of the citizens of the town.

Figures 1 and 2 shows the statistical processing of the data presented in the surface distribution maps. The depth of groundwater influences the probability of E. coli bacteria presence. With the increase in well depth, a decrease in the likelihood of contamination by E. coli bacteria was observed [15]. Groundwater microbial quality may be affected by a large environmental and source-specific risk factors such as well design, location and maintenance, septic system location and maintenance, hydrogeological characteristics, and also climatic events like flooding and snowmelt [1,15].

Figure 3 shows the concentration of microorganisms present in all groundwater samples, in the form of E. coli bacteria, Intestinal enterococci and Coliform bacteria. All samples were contaminated, except for samples P7 and P12. A possible explanation is the lack of the systematic water disinfection by chlorination in the open dug and drilled wells. A free residual chlorine concentration below the maximum value of 0.2 mg/L is recommended for prevention of bacterial growth/regrowth [16]. This is common for open dug/ drilled wells found in more rural areas. The mean sequence count of viable microorganisms increased in the following order: P7<P12<P3<P8<P2<P9< P6<<P10<P1 <P4<P5<P11.

E. coli bacteria was found present in P1, P2, P4, P5, P6, P10, and P11 samples in the range of 1 with a maximum of 320 CFU/100mL. Found in samples P1, P2, P3, P4, P5, P6, P8, P9, P10, and P11, Generic Coliform bacteria registered values of 3 CFU/100mL.

On the other hand, the highest value for Intestinal enterococci was registered for samples P1, P2, P3, P4, P5, P6, P8, P10 and P11 at a 2 CFU/100 mL and a total range of 120. Fecal Enterococci have been reported to be much more resistant when in natural environments than their Coliform bacteria counterparts, their presence indicating a strong fecal matter contamination of water [17]. The highest total microorganism contamination was

registered for sample P11. As per most legislation, including WHO guidelines [18] drinking water should be free from any coliform bacteria, E. coli bacteria and intestinal enterococci.

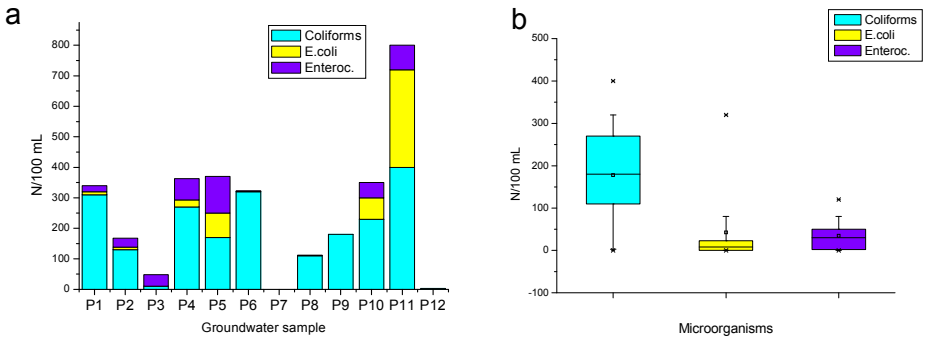

Figure 3. Microorganisms in the groundwater samples a) Microorganisms species in P1-P12 groundwater samples; b) Boxplot representation of groundwater contamination

Cluster analysis

Figure 4 shows the cluster analysis of microorganisms in groundwater samples (a) and the similarities between the indicators of microbiological contamination and the Cl^-, Al and As concentrations (b).

The cluster analysis of groundwater samples showed 2 clusters: C_1 formed by the majority of the samples with a relatively small bacterial load and C_2 where the levels of all the investigated microorganisms are high. C_1 is formed by 2 groups: C_{1a} where the total microorganism load was around 350 /100 mL that comprise more wells located in the central part of the town densely populated and C_{1b} with a lower contamination (<100 microorganisms/100 mL). Cluster analysis of variables: microbiological load of groundwater samples depicted in Figure 4 showed high similarity between E. coli bacteria and intestinal enterococci that are very close linked in the dendrogram at double distance to coliforms and to total chlorides content. Total chlorides confer groundwater with improved dissolution properties for both organic and inorganic compounds and also for the nutrients needed for bacteria growth. Al and As formed a distinct cluster at higher distance.

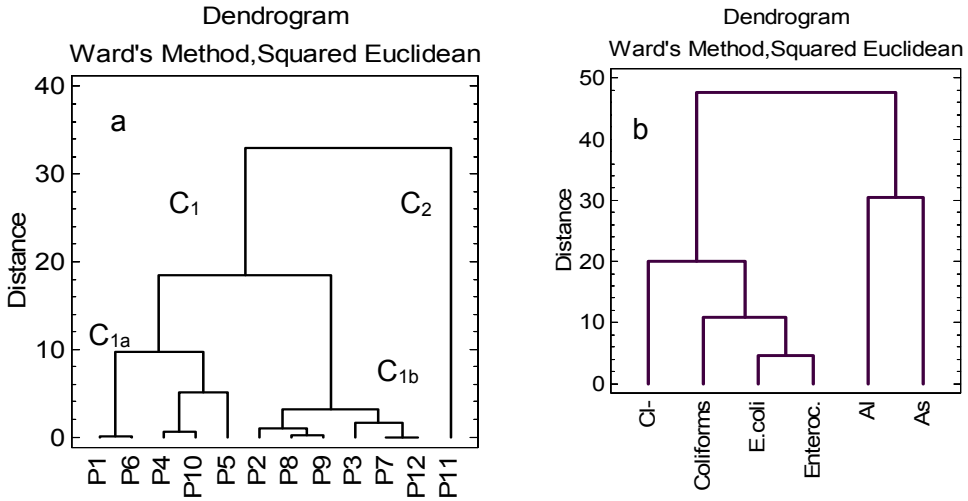

Figure 4. Cluster analysis of groundwater samples from Seini: a) Clusters of groundwater samples based on their microbiological characteristics (Coliform bacteria, E. coli bacteria and intestinal enterococci); b) Cluster analysis of similarities between microbiological characteristics and the studied chemical parameters (Cl^-, As and Al concentrations)

Elaboration of 3D mathematical models

The mathematical models presented in Figure 4 represent the description of the chemical and microbiological properties of the underground water in Seini town. The three elements (chlorine as chloride anions, aluminum, arsenic) give irrelative mathematical models, obtaining low correlation coefficients (R^2 much lower than 0.80). However, better mathematical models are obtained in the case of correlations among microbiological characteristics (R^2 = 0.772), and in the case of correlation of the microbiological parameters with one element (chlorine, arsenic and aluminum) with correlation when coefficients R^2 = 0.95-0.99 are obtained.

The 3D mathematical model follows the dependence of the Intestinal enterococci bacteria on coliform bacteria and E. coli bacteria is given by nonlinear equation (1), with R^2= 0.772 and F = 5.927.

$$z = 17.96 + 0.026(lnx)^2 + 17.87lny - 0.027y \quad (1)$$

Equation (1) shows the relation between the intestinal enterococci level and that of E.coli bacteria and coliform bacteria. The equation (1) is composed of positive terms as logarithm and a negative term (-0.027y). The positive terms show the simultaneous development of more bacteria species in favorable conditions while the negative term could be related to the microbial competition. It is known that within bacteria communities there is a constant competition with their neighbors for space and resources [19,20].

If the coliform bacteria levels are correlated with the concentration of total chloride and the level of E. coli bacteria, the nonlinear equation (2) is obtained, with $R^2 = 0.865$ and $F = 7.705$.

$$z = 325.70 - 10.72x + 11.57lny + 0.17dx^2 \quad (2)$$

The influence of aluminum on microbiological parameters leads to a significant increase in the correlation coefficient ($R^2 = 0.900$ and $F = 15.67$). Thus a 3D mathematical model was obtained showing the dependence of the concentration of E. coli to that of the intestinal enterococci and the concentration of aluminum given by the nonlinear equation (3).

$$\ln z = -27.55 + 30903398x^3 + 0.37y \quad (3)$$

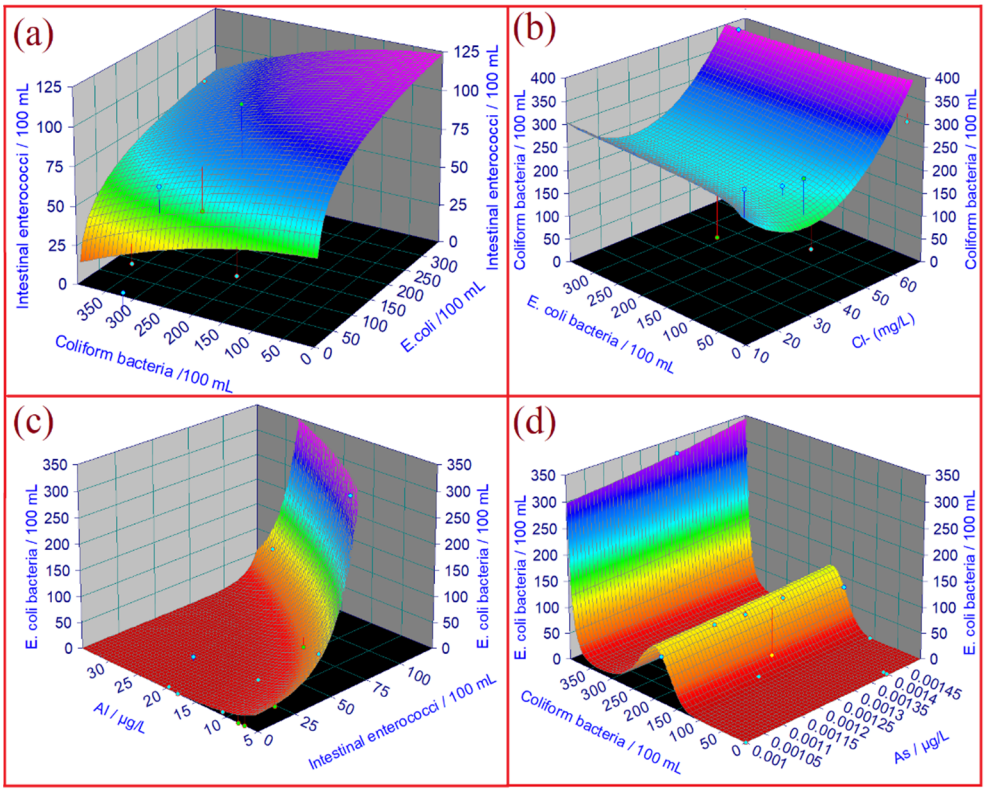

Figure 5. The mathematical models 3D between the microbiological parameters and concentration of total chlorides, Al or As in the samples of groundwater: a) the Intestinal enteroccoci (z) = f (E. coli bacteria (x), Coliform bacteria (y)); b) Coliform bacteria (z)= f(Cl^-(x), E. coli bacteria (y)); c) E. coli bacteria (z)=f (Intestinal enterococci (x), (Al (y)); (d) E. coli bacteria (z)= f(As(x), Coliform bacteria(y))

Similar correlation coefficients are obtained as a result of arsenic use, obtaining a 3D model with the correlation coefficient R^2 = 0.985 and F = 302, due to the dependence between E. coli bacteria, arsenic concentrations and coliform bacteria level according to equation (4).

$$z = 4.72 + 0.00051x^2 - 0.24y \quad (4)$$

CONCLUSIONS

Aluminum, arsenic, E. coli bacteria and intestinal enterococci are distributed in higher concentrations at the surface and in the center of the town, where the population density is higher, while chlorine is distributed at higher concentrations in the deep and at the outskirts of Seini. The distribution maps of the analyzed microorganisms (Coliform bacteria, E. coli bacteria, Intestinal Enterococci) are easy to use and interpret and can be helpful as practical tool for land use and protection zones. Correlations between arsenic, aluminum, and total chlorides contents are very poor (with correlation coefficients below 0.8), without any relevant mathematical model, while correlations between microbiological parameters give a 3D mathematical model with a correlation coefficient of 0.802.

EXPERIMENTAL SECTION

The town of Seini is located at 26 km west of Baia Mare, 42 km east of Satu Mare and 24 km southeast of Negreşti Oas, is crossed by the Seinel stream that runs in the north-south direction flowing into the River Someș.

The sampling was performed on 12 open wells of 8-14 m depth and 20 cm diameter, located in the Seini area and shown in Figure 6. The sampling was carried out according to SR: ISO 5667-3 / 2013 in polyethylene bottles and the average of obtained values was processed in this paper. The aquifer complex, from which the water specimens were harvested, located in the area of Seini (P1-P6, P9, P10), overlaps in particular with the Seini area's glacis area, which is in contact with the sub-mountain area, cantonated in the quaternary deposits, and is usually intercepted at the contact between the quaternary deposits and the pannonian ones, consisting of clay marl. The location of the samples P7, P8, P11, P12 corresponds to the Somes River terraces. The variation of the waterbath level is closely related to the flow of the Somes River. The position of the P1 sample corresponds to the area of development of the regisols of the Protisoil class [19] formed on unconsolidated colluvial deposits. The soil profile is poorly differentiated and

are porous. The positions of samples P2 and P6, P9, P10 overlap with the Eutricambosoil soil type development area in the Cambisoil class and have water retention capacity and ensure good drainage [19]. Samples P3 and P4 are included in the field surface on which the Gleiosoil type of the Hidrisoils class develops, formed with an excess of groundwater which favor a decrease in aeration and an increase in soil density [19]. Sample P5, is located in a Luvosoil gleic soil type development area of the Luvisoil class, are characterized by the presence of eloquent horizons that are strongly leached by clay and organic colloids in association with the illuvial horizons in which these components are deposited. Appropriate samples of P7, P8, P11 and P12 have the wide development of typical luvosoil are acidic soils [19]. Gleiosoil and Luvisoil types were formed on alluvial / proluvial deposits represented by sands and pebbles from the Pannonic Basin. It is possible that the aquifers are accomodated in these quaternary formations which permits good infiltration and accumulation of waters that form important aquatic states [21].

Figure 6. Distribution of sampling points in Seini town

The Al concentrations of samples were measured with the Specord 50 Analytik Jena UV-VIZ spectrophotometer according to the standardized method for low Al concentration (SR ISO 10566 – 2/2001). Al forms a blue color complex with Pyrocatechol Violet reagent at pH 6. The absorbance of this complex was measured at 580 nm in cuvettes of 50 mm. A preliminary step of the analysis was the water sample filtration using a filter membrane

with the pores dimension of 0.45 μm. Then the sample was acidified with HNO_3 until a pH in the range of 1.2 - 1.5. To 25 mL of water sample were added the following reagents: 1 mL mixt reagent to eliminate the effect of interfering substances (25 g $MgSO_4 \cdot 7H_2O$, 5 g ascorbic acid, 0.25 g of 1,10-phenanthroline monohydrate dissolved in distilled water and dilute to 100 mL in a calibrated flask), 1 mL of Pyrocatechol Violet solution (0.050g dissolved in distillated water and dilute to 100 mL in a calibrated flask) and finally a buffer solution of hexamethylenetetramine (210 g in 200 mL of distilled water). Then, the sample was left for 15 minutes until the complex formation was accomplished. The absorbance of the complex was measured against a blank reagent. A calibration curve was established using Al solution in concentrations of 2-100 μg/L prepared by appropriate dilution of the standard solution containing 1000 mg Al/L.

Chloride concentration was measured by precipitation titration with silver nitrate according to ISO 9297-2001. A water sample of 50-100 mL adjusted to pH 8.3 with NaOH 0.1 N was titrated with silver nitrate solution in the presence of potassium chromate as indicator, the color of the sample turning from a yellowish-green to a reddish-brown color.

As was determined by graphite furnace atomic absorption spectrometry (GFAAS) following a standardized protocol (SR EN ISO 15586-2004) using a Perkin Elmer AAnalyst 800 spectrophotometer equipped with a graphite furnace. The quality control and quality assurance of the analysis data was realized by using standard procedures, calibration with standard solutions, analysis of blank reagent and analysis of each sample in triplicate. The results were expressed as mean values. Blanks and control samples were measured in parallel. All the reagents used were of analytical degree (PA). The preparation of standard solutions of As was realized using standard solutions of 1,000 ppm, with certified quality and ultrapure water.

The studied microbiological parameters were: Escherichia coli (according to STAS 3001-91), Intestinal enterococci (STAS 3001-91), and coliform bacteria (ISO 4831/2009 European standard).

The samples were collected in presterilized plastic bottles of 100 mL. The preparation step for all the analyzed microbiological parameters was the same. A water sample of 100 mL was filtered through a membrane filter with a porosity of 0.45 μm, using a weak vacuum pump. For intestinal enterococci, after filtration, the membrane was placed using sterile tweezers, on a Slanetz-Bartley medium with the grill upward, in the incubator at 36 ± 2 ˚C for 24 ± 4h. After incubation, the specific colonies of red-brown or pink color were numbered. The result of the analysis was expressed in colony forming units / 100 mL of sample. The analysis of coliforms bacteria and E. coli bacteria was performed by filtering 100 mL of water sample through a 0.45 μm membrane

filter that was placed with a sterile tweezers on agar chromogenic medium in the incubator at 36 ± 2 ˚C for 21-24 hours. After incubation, the filter membranes were examined. The pink-red color spots were numbered indicating the coliform bacteria, and the blue-violet spots indicated the E. coli bacteria. The results of the analysis were expressed in colony forming units /100 mL of sample.

Spatial distribution maps were created using natural neighbor interpolation employing a GIS system and the delimitation of areas with high and low water quality in wells based on indicator values. To draw maps, the values of the indicators were interpolated using the ArcGIS software.

Cluster analysis has been used to group the groundwater sampling points (wells) by their similarity considering their load with the three species of microorganisms. Clusters were generated by Ward's methods based on Squared Euclidean distance in the multidimensional space of data with Statgraphic program.

The mathematical model has been used to describe a system or process that employs mathematical symbols and functions to optimize the design of experiments and better understand the obtained experimental results. The proposed 3D mathematical models are non-linear regression functions with the highest correlation coefficients for a group of 3 parameters generated by the Table Curve program.

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