

Parameters Affecting Microbiological Groundwater Quality: Statistical Modelling

G.A. Dumitrel

POLITEHNICA University of Timisoara, Industrial Chemistry and Environmental Engineering Faculty, 300006, 2 Victoriei Square, Timisoara Romania, alina.dumitrel@chim.upt.ro

Abstract: Water, essential element of life and human activities is an important indicator for assessing the degree of civilization and development opportunities that a city offers to its inhabitants. Drinking water quality standards exist for a long time. As science progressed and pollution has increased and diversified, the demand and complexity of standards, analysis and control methods increased too. The presence of nitrogen-containing compounds in waters indicated the existence of a pollution source (municipal and industrial wastewater, septic tanks, feed lot discharges, animal wastes, runoff from fertilized agricultural field). Excessive concentrations of nitrogen in water alter the water quality and cause negative effect on human body. This paper presents the influence in time of nitrogen-containing compounds from groundwaters on the microbial load of groundwaters. The values of model adequacy indicators shows a good correlation between experimental data and those obtained based on mathematical equations of model developed.

Keywords: groundwater, nitrogen cycle, microbial load, experimental modelling.

1. Introduction

Importance of drinking water for the body can be summarized as follows: vital element, environment for absorption, diffusion and excretion, helping to maintain the basic constants of the body.

The population from rural areas, who is not benefiting from the centralized water, is supplied from wells. These wells are sources of groundwater used untreated for different purposes. In most cases the water is subject to chemical and microbiological pollution from anthropogenic sources. Water quality assessment is made by evaluation of certain parameters (physical, chemical, microbiological) which limits are legally specified [1-3]. In Romania, drinking water quality is regulated by Law no. 311/2004 who completes the Law no. 458/2002 both transposing the European Directive 98/83/EC of drinking water quality.

However water pollution is continuing. Water biological pollution is caused by domestic wastewater, liquid wastes discharge from human hospitals and veterinary laboratories, abattoirs, etc. Polluted water is a source of animal and humans infections and parasitic infestations. Bacteria that are transmitted through water causes human and animal bacteriosis [4].

Escherichia coli can be spread by water consuming and cause gastroenteritis and septicemia at children and youth animal species. The incidence of *Escherichia coli* is 17.5% in well waters. It survives 6 months in drinking water from wells and 4 months in centralized water.

Drinking water from the wells is a new situation that has been generated by the idea that it is healthier than water from public system and it is for free [5].

To emphasize the link between microorganisms presence in drinking water and nitrogen quantity, in this paper aspects of nitrogen circuit in water were presented. Living organisms, dead organic matter, mineral and organic compounds dissolve in an aquatic ecosystem are not in an inert state. They are in a continuous change and movement that condition the existence of living systems. Thus, between ecosystems components, complex relationships are created that ensure the evolution of these components.

Nitrogen, very important nutrient in aquatic ecosystems, enters in several ways and is found in water in many forms: molecular nitrogen, nitrogen oxides, ammonia, ammonium, nitrites and nitrates [6]. In the ecosystem, nitrogen enters in biogeochemical cycle, as a result of a complex factor interaction from aquatic ecosystem. Algae can use the free nitrogen from salt water, as well as ammonia salts (NH_3) and after their depletion, nitrates (NO_3^-).

Bacteria have an important role in spreading nitrogen into specific cycle of aquatic ecosystems. Bacterial transformations phases of nitrogen compounds are reversible, the way of processes evolution being dependent on the concentration of dissolved oxygen. Nitrogen cycle in aquatic ecosystems was presented by Beaupied [7] (Figure 1).

Nitrites appear from organic substances pollution by partial oxidation of amino radicals or by reducing nitrates. Their presence indicates older water pollution.

The purpose of this study was to develop a series of mathematical models that demonstrate the time dependences between the water microbial load and the nitrogen forms from water.

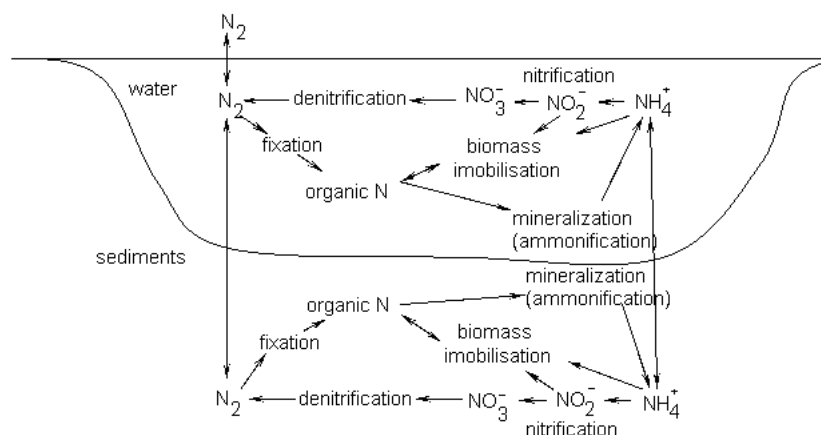


Figure 1. Nitrogen cycle in aquatic ecosystems

2. Experimental

Evolution in time of nitrites, nitrates and ammonium concentrations as well as microbial load in waters collected from a well located in Alba Iulia city was studied.

A Spectroquant® NOVA 60 UV-Visible spectrophotometer was used for the absorbance measurements of samples.

Nitrites determination

The method is based on the fact that in acid solution, nitrites ions reacts with sulphanilic acid to form diazonium salt which then reacts with the dihydrochloride of N-1-azo naftiletilendiamine forming a violet red compound which is measured spectrophotometrically.

Two Spectroquant® test kits were used for analysis: one covering the measurement range from 0.02-1.00 mg/dm³ NO₂-N or 0.07-3.28 mg/dm³ NO₂ for a 10 mm cuvette; 0.10-0.50 mg/dm³ NO₂-N or 0.03-1.64 mg/dm³ NO₂ for a 20 mm cuvette; 0.002-0.200 mg/dm³ or 0.07-0.657 NO₂ for a 50 mm cuvette. The measuring range for the other kit was 0.01-0.700 mg/dm³ NO₂-N or 0.03-2.30 NO₂.

Water samples are analyzed immediately after collection. Their pH must be in the range 2-10. If necessary, adjust with sodium hydroxide or sulfuric acid solution. Turbid samples are filtered through filter paper. 5 ml of sample are pipetting into a reaction tube and NO₂-1 reagent is added. After 10 minutes of rest, the sample is transfer into spectrophotometer cuvette and measured.

Nitrates determination

The method is based on the fact that in sulfuric acid and phosphoric acid solutions, nitrate ions react with 2,6-dimethylphenol(DMP) to form 4-nitro-2,6-dimethylphenol, an orange complex. In sulphuric acid solution, nitrate ions react with a benzoic acid derivative and form a red nitro compound.

The kit used for analysis had the following measuring ranges: 0.5-25.0 mg/dm³ NO₃-N or 4-221 mg/dm³ NO₃⁻; 1.0-50.0 mg/dm³ NO₃-N or 2.2 - 79.7 mg/dm³ NO₃⁻.

1 mL of sample (kit: 0.5-25.0 mg/dm³ NO₃-N) or 0.5 mL (Kit: 1.0-50.0 mg/dm³) is transferred into a reaction tube. 1 ml of reagent NO₃-1K is added and the mixture is mixed for 1 minute. After 10 minutes of rest the reaction tube is inserted in SQ and the concentration is read.

Ammonium determination

Ammonium nitrogen is presented partly as ammonium ions and partly as ammonia. There is a pH dependent equilibrium between the two forms. In strongly alkaline solutions, ammonia nitrogen is present almost entirely as ammonia, which reacts with hypochlorite ions to form monochloramine, which reacts with substituted phenol to form a blue indophenol derivative.

The kit used for analysis had the following measuring ranges: 0.010 to 2.000 mg/dm³ NH₄-N or 0.01 to 2.58 mg/dm³ NH₄ respectively 5.0 to 16.0 mg/dm³ NH₄-N and 0.013 to 3.86 mg/dm³ NH₄-N.

1 mL of sample is transferred into the reaction tube. A dose of NH₄-1K reactive is added and the mixture is mixed for 1 minute. After 10 minutes of rest, the sample is transfer into spectrophotometer and measured.

Determination of waters microbial load at 37°C (CFU/ mL)

Drinking water should not contain pathogenic microorganisms. Analysis methods are approved and standardized methods, criteria for water quality being specified by STAS 1342/91, law 458/2002 amended by law 311/2004.

Quality of measurements is assured by the culture medium quality (SR ENV ISO 11133-1/2002; ISO/TS 11133-2 /2004) and the calculus algorithm used.

Presence of mesophilic bacteria is highlighted by seeding sample (water) or the decimal dilutions by the incorporation process in a solid nutrient medium (nutrient agar) incubated at 37°C for 48 h. This procedure is followed by counting the colonies developed. The result is expressed as the number of colony forming units per cubic centimetres (CFU/cm³).

3. Results and Discussion

Table 1 presents the evolution of chemical and microbiological parameters of water samples collected between 2006 – 2010 from a well located in Alba Iulia city. Sampling was done with a periodicity of 3 months. In this way it was possible to follow the evolution of parameters in all seasons (cold, warm and rainy seasons).

Graphically representing the time evolution of nitrate, nitrite and ammonium concentration, figures 2-4 were obtained.

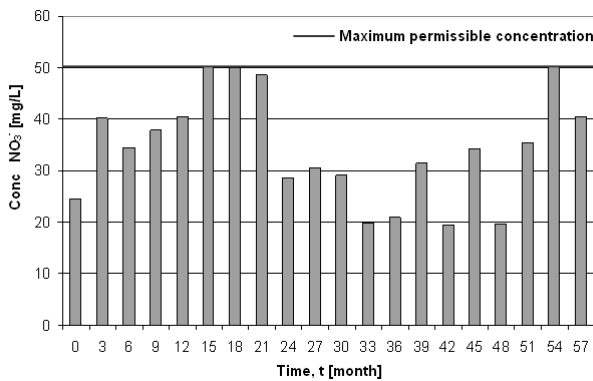


Figure 2. Evolution in time of nitrate concentration from Alba Iulia groundwater

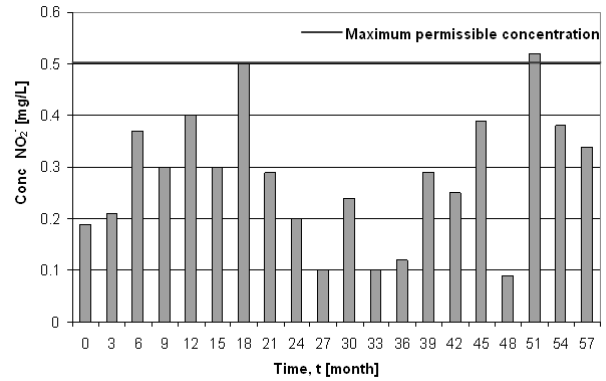


Figure 3. Evolution in time of nitrite concentration from Alba Iulia groundwater

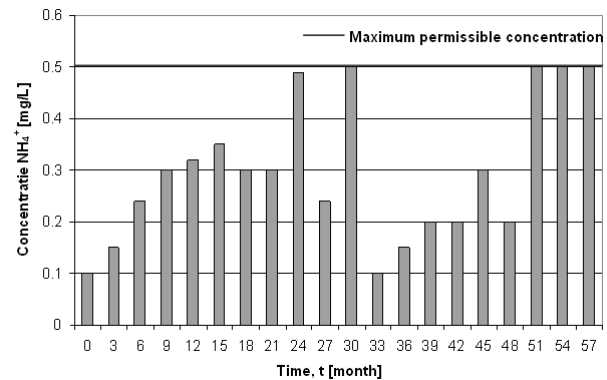


Figure 4. Evolution in time of ammonium concentration from Alba Iulia groundwat

TABLE 1. Evolution of chemical and microbiological parameters of groundwater from Alba Iulia city

Time, [month]	NO ₃ ⁻ [mg/L]	NO ₂ ⁻ [mg/L]	NH ₄ ⁺ [mg/L]	Aerobic bacteria, [ufc/mL]	Aerobic bacteria, log [ufc/mL]
0	24.51	0.19	0.1	58	1.76
3	40.2	0.21	0.15	98	1.99
6	34.5	0.37	0.24	162	2.21
9	37.9	0.3	0.3	207	2.32
12	40.6	0.4	0.32	330	2.52
15	50.12	0.3	0.35	425	2.63
18	50.04	0.5	0.3	460	2.66
21	48.52	0.29	0.3	250	2.40
24	28.51	0.2	0.49	111	2.05
27	30.45	0.1	0.24	164	2.21
30	29.15	0.24	0.5	103	2.01
33	19.89	0.1	0.1	51	1.71
36	20.9	0.12	0.15	82	1.91
39	31.5	0.29	0.2	94	1.97
42	19.5	0.25	0.2	73	1.86
45	34.2	0.39	0.3	201	2.30
48	19.59	0.09	0.5	95	1.98
51	35.4	0.52	0.5	369	2.57
54	50.22	0.38	0.5	562	2.75
57	40.61	0.34	0.5	618	2.79

Figures analysis shows that the concentration of nitrate and ammonium in groundwater from Alba Iulia city does not exceed the legally limits. One exception could be seen for nitrite concentration.

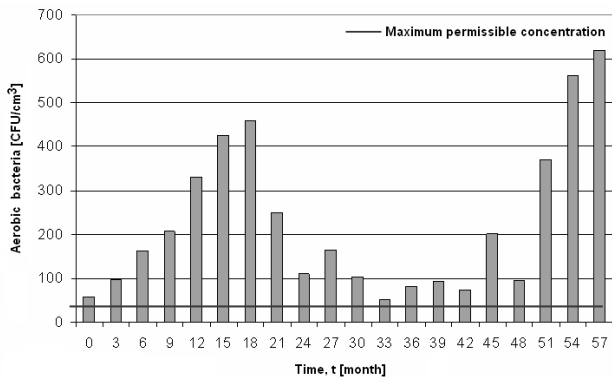


Figure 5. Evolution in time of aerobic bacteria from Alba Iulia groundwater

In terms of microbiological parameters of studied groundwater for Alba Iulia city, the measured values were greater than the legal limit (Law 458 and 311) during the entire period of study (figure 5).

In many practical cases the connection between two or more parameters that describe a particular process is sufficiently close so that the variation of a parameter can be controlled and expressed in terms of changes in other parameters. Functional links of this type are called stochastic or probabilistic links. Study of this kind of links has led to the development of multiple correlation theory.

In order to develop statistical mathematical models the effect of two parameters influence was evaluated through a multiple correlation analysis between microbial loads (aerobic bacteria) as dependent variable and nitrogen

content (nitrate, nitrite and ammonium) and time, as independent variables.

For accurate description of microbial load dependence of nitrogen content (nitrate, nitrite) or ammonium and time, a second order polynomial equation was used.

Equation (1) is the general form of mathematical models describing the second order polynomial correlation.

$$y = a_0 + a_1 \cdot x_1 + a_2 \cdot x_2 + a_3 \cdot x_1 \cdot x_2 + a_4 \cdot x_1^2 + a_5 \cdot x_2^2 \tag{1}$$

where: a_i – equation coefficients; x_1 - NO_3^- , NO_2^- , NH_4^+ concentration in groundwater [mg/L]; x_2 – time, [month]; y – total number of aerobe bacteria, log [CFU/mL].

For second-order polynomial regression, the values of coefficients correspond to the minimum of the following objective function:

$$S = \sum_{j=1}^m (\hat{y}_j - (a_0 + a_1 \cdot x_{1j} + a_2 \cdot x_{2j} + a_3 \cdot x_{1j}^2 + a_4 \cdot x_{1j} \cdot x_{2j} + a_5 \cdot x_{2j}^2))^2 \tag{2}$$

To minimize S , the following condition must be satisfied: $\frac{\partial S}{\partial a_i} = 0$. This lead to a system of linear equations

for which the coefficient matrix (A) and the vector of free coefficients (B) are given by relations (3):

By using MATLAB software, the system of equations was solved and the experimental data were processed and analyzed.

Experimental data together with the surfaces generated by mathematical models are presented in Figures 6, 7 and 8.

$$A = \begin{bmatrix} \sum_{j=1}^m 1 & \sum_{j=1}^m x_{1j} & \sum_{j=1}^m x_{2j} & \sum_{j=1}^m x_{1j}^2 & \sum_{j=1}^m x_{1j} \cdot x_{2j} & \sum_{j=1}^m x_{2j}^2 \\ \sum_{j=1}^m x_{1j} & \sum_{j=1}^m x_{1j}^2 & \sum_{j=1}^m x_{1j} \cdot x_{2j} & \sum_{j=1}^m x_{1j}^3 & \sum_{j=1}^m x_{1j}^2 \cdot x_{2j} & \sum_{j=1}^m x_{1j} \cdot x_{2j}^2 \\ \sum_{j=1}^m x_{2j} & \sum_{j=1}^m x_{1j} \cdot x_{2j} & \sum_{j=1}^m x_{2j}^2 & \sum_{j=1}^m x_{1j}^2 \cdot x_{2j} & \sum_{j=1}^m x_{1j} \cdot x_{2j}^2 & \sum_{j=1}^m x_{2j}^3 \\ \sum_{j=1}^m x_{1j}^2 & \sum_{j=1}^m x_{1j}^3 & \sum_{j=1}^m x_{1j}^2 \cdot x_{2j} & \sum_{j=1}^m x_{1j}^4 & \sum_{j=1}^m x_{1j}^3 \cdot x_{2j} & \sum_{j=1}^m x_{1j}^2 \cdot x_{2j}^2 \\ \sum_{j=1}^m x_{1j} \cdot x_{2j} & \sum_{j=1}^m x_{1j}^2 \cdot x_{2j} & \sum_{j=1}^m x_{1j} \cdot x_{2j}^2 & \sum_{j=1}^m x_{1j}^3 \cdot x_{2j} & \sum_{j=1}^m x_{1j}^2 \cdot x_{2j}^2 & \sum_{j=1}^m x_{1j} \cdot x_{2j}^3 \\ \sum_{j=1}^m x_{2j}^2 & \sum_{j=1}^m x_{1j} \cdot x_{2j}^2 & \sum_{j=1}^m x_{2j}^3 & \sum_{j=1}^m x_{1j}^2 \cdot x_{2j}^2 & \sum_{j=1}^m x_{1j} \cdot x_{2j}^3 & \sum_{j=1}^m x_{2j}^4 \end{bmatrix} \tag{3}$$

$$B = \begin{bmatrix} \sum_{j=1}^m \hat{y}_j \\ \sum_{j=1}^m x_{1j} \cdot \hat{y}_j \\ \sum_{j=1}^m x_{2j} \cdot \hat{y}_j \\ \sum_{j=1}^m x_{1j}^2 \cdot \hat{y}_j \\ \sum_{j=1}^m x_{1j} \cdot x_{2j} \cdot \hat{y}_j \\ \sum_{j=1}^m x_{2j}^2 \cdot \hat{y}_j \end{bmatrix}$$

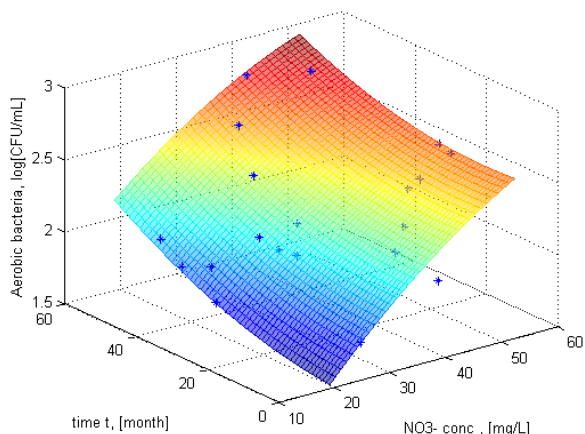


Figure 6. Variation of groundwater microbial load as a function of nitrates content and time - Alba Iulia City

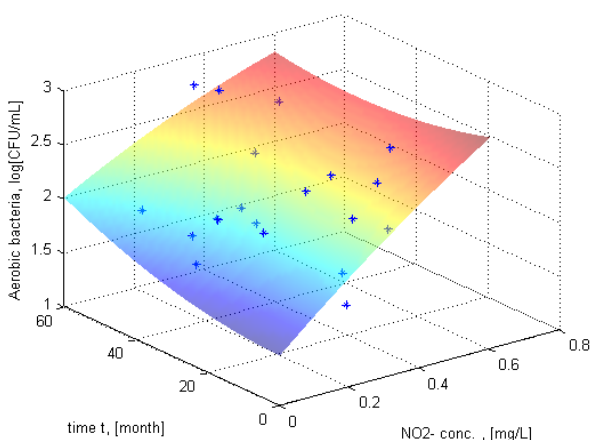


Figure 7. Variation of groundwater microbial load as a function of nitrites content and time - Alba Iulia City

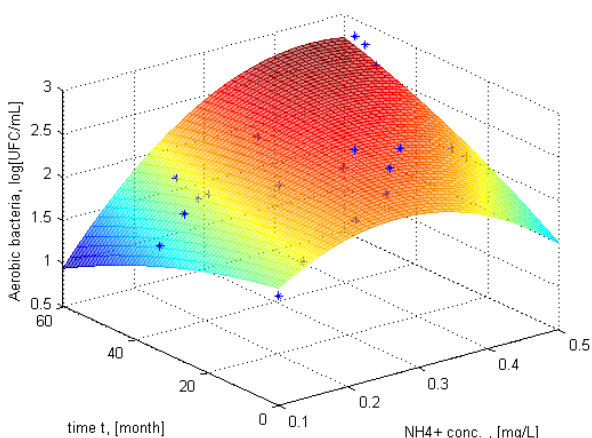


Figure 8. Variation of groundwater microbial load as a function of ammonium content and time - Alba Iulia City

Equations derived from statistical mathematical models are presented in Table 2. They are valid on the range values studied.

After model coefficients determination, a comparison between model predictions and data from the real process is necessary to make. As indicators of model adequacy

were used: dispersion, standard deviation, correlation coefficient R , coefficient of precision R^2 (Table 3).

TABLE 2. Equations of statistical mathematical models

Model	Equations of statistical mathematical models
Alba Iulia (NO_3^-)	$y = 0.5927 - 0.0553 \cdot x_1 - 0.005 \cdot x_2 + 1.4073 \cdot 10^{-4} \cdot x_1 \cdot x_2 - 3.1875 \cdot 10^{-4} \cdot x_1^2 + 1.2548 \cdot 10^{-4} \cdot x_2^2$
Alba Iulia (NO_2^-)	$y = 1.4588 + 2.8943 \cdot x_1 + 9.6276 \cdot 10^{-4} \cdot x_2 - 0.0186 \cdot x_1 \cdot x_2 - 0.6864 \cdot x_1^2 + 1.3502 \cdot 10^{-4} \cdot x_2^2$
Alba Iulia (NH_4^+)	$y = 1.2000 + 8.0253 \cdot x_1 - 0.0160 \cdot x_2 + 0.0895 \cdot x_1 \cdot x_2 - 14.9085 \cdot x_1^2 - 1.3681 \cdot 10^{-4} \cdot x_2^2$

TABLE 3. Indicators of model adequacy

Model	dispersion σ^2	standard deviation, σ	coefficient of precision, R^2	correlation coefficient, R
Alba Iulia (NO_3^-)	0.0164	0.1280	0.8565	0.9255
Alba Iulia (NO_2^-)	0.0474	0.2178	0.5846	0.7646
Alba Iulia (NH_4^+)	0.0212	0.1456	0.8144	0.9024

The values of model adequacy indicators shows the correlation between data obtained experimentally and those obtained based on mathematical equations that describe dependencies between parameters.

The correlations parameters calculate argue a good predictive capacity of statistical mathematical model.

4. Conclusions

Following experimental determinations a series of data have resulted. It can be used to evaluate the comparative effect of the measured parameters by developing a multiple correlation analysis.

Correlative analysis shows that between the total number of aerobic bacteria present in water and the other physico-chemical parameters are appreciable correlations.

The equations of statistical models can approximate the hygienic water quality (microbial load) if usual physico-chemical parameters of waters are known. Prediction models can also be a criterion in assessing the quality of ground waters.

REFERENCES

- Nieto P., Custodio E. and Manzano M., *Environmental Science & Policy*, 8(4), **2005**, 399-409.
- Zacheus O.M. and Martikainen P.J., *Science of The Total Environment*, 204(1), **1997**, 1-10.
- Mihelcic J.R., *Fundamentals of Environmental Engineering*, Wiley, **1999**.
- Fields M.W., Yan T., Rhee S.-K., Carroll S. L., Jardine P. M., Watson D.B., Criddle C.S. and Zhou J., *FEMS Microbiology Ecology*, 53(3), **2005**, 417-428.
- Troja R., Prifti D. and Sini K., *Official Journal of the Balkan Environmental Association*, 9(3), **2008**, 159-167.
- Rivett M.O., Buss S.R., Morgan P., Smith J.W.N. and Bemment C.D., *Water Research*, 42(16), **2008**, 4215-4232.
- Widdison P.E. and Burt T.P., *Encyclopedia of Ecology, Nitrogen Cycle* **2008**.

Received: 11 October 2012

Accepted: 18 December 2012