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THE QUANTITATIVE DETERMINATIONS OF NITRATES AND NITRITES IN WATER SAMPLES USING SPECTRAL METHODS

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Abstract: *Nitrates and nitrites are the most common inorganic nitrogen compounds in the environment, and are present in waters, soils, foods and biota. Nitrite and nitrate are etiologic agents of methaemoglobinaemia or 'blue baby syndrome'. If large quantities of nitrogen compounds are ingested in drinking water, the ability of the blood to carry oxygen is impaired, causing headache, fatigue, breathing difficulties, diarrhea and vomiting and, in extreme cases, loss of consciousness and death.*
The study presents the spectral analysis for nitrates and nitrites monitoring for water samples-drinking water, underground water and surface water. This overview on the most common inorganic nitrogen compounds (nitrates and nitrites) helps us understand their toxic effects on human health.

Key words: *water, health, spectral analysis, nitrate, nitrite.*

1. Introduction

Nitrogen is one of the essential elements of some functional biomolecules (peptides, proteins, nucleic acids), is a common component of living organisms and is the most abundant of the Earth. During the last five decades, human have altered the global cycles of majority of chemical elements, as well as the global nitrogen cycle [3].

For lawn, garden care and crop production the nutrient applied in the largest quantities is nitrogen. Nitrogen in organic forms also occurs naturally in the soil from decaying plant and animal residues. Bacteria are responsible for converting various forms of nitrogen to nitrate, a nitrogen/oxygen ion (NO_3^-), in the soil. Nitrate form is much more useful as plants absorb nitrogen in this form. However, because nitrate is very leachable can readily move with water through the soil profile. Nitrate can drain below the plant's root zone after excessive rainfall or over-irrigation and may eventually reach groundwater.

Nitrate-nitrogen (NO_3-N) in groundwater may result from point sources such as sewage disposal systems and livestock facilities or non-point sources such as fertilized cropland, parks, golf courses, lawns, and gardens, or naturally occurring sources of nitrogen. Standard water

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treatment practices, such as sedimentation, filtration, chlorination or pH adjustment with lime application, do not affect nitrate concentrations in the water. Nitrates from water can be removed by specialized water treatment technologies, such as ion exchange, biochemical denitrification, and reverse osmosis [20].

For humans, sources of ***nitrate*** intake could be:

- vegetables (30-90% of total anion intake);
- meat products and as a result, it enter the human food chain;
- medications and volatile nitrite inhalants (quinone derivatives, nitroglycerine, bismuth, subnitrite);
- accidental exposures to nitrites in chemical laboratories [6].

Nitrate from water and foods is recirculated in human body between saliva and gastrointestinal tract, via blood plasma (saliva – esophagus – stomach – small intestine – plasma – saliva) and it was demonstrate that humans excrete more nitrate than they consume [2].

Some epidemiological studies demonstrate that the nitrate can be advantageous to human health because it protects the intestine from bacterial infections, and nitric oxide (NO) as nitrate reduced product is an important signal molecule for regulating many physiological functions, or act as a defense effectors against pathogens. These epidemiological studies cause a controversy because generally it is considered that nitrate increase the risk of hypertension, children polyuria, methemoglobinemia and cancer [1].

In the mouth, nitrate is rapidly reduced to nitrite in presence of nitrate reducing bacteria, and nitrite will reduce to NO (nitric oxide) and N-nitroso compounds (nitrosamines, nitroamides) in the acidic stomach (in the absence of nitrite reductase in the mouth) in presence of reducing substances (thiols and ascorbic acid). These reactions are advantageous because the normal range of pH in the stomach of healthy human is 1-3, and nitrate reducing bacteria will be inhibited and no exogenous nitrate can be transform in nitrite [2].

Nitrites are the degradation products of nitrates. Nitrites are regulated in feed and water as undesirable substances. Nitrite, the anion of inorganic nitrite salts, such as sodium nitrite, is formed naturally by the nitrogen cycle during the process of nitrogen fixation and is subsequently converted to nitrate, a major plant nutrient and constituent.

Most common - sodium nitrite - is widely used as a preservative, anti-microbial agent, color fixative and flavoring in cured meats and other products [5].

The nitrous acid (HNO_2) and nitrite anion (NO_2^-) are in equilibrium, and this is dependent on environment pH. As the values of hydronium concentration tends to increase, the concentration of nitrite decrease, but the concentration of nitrous acid increase, and within the pH range 7-8 (near of physiological range) the nitrous acid concentration is 4-5 orders of magnitude less than the nitrite concentration. In these conditions nitrite anion is considered to be major responsible for human health [3].

In human body, nitrite enters the red blood cells and it induce oxidation of hemoglobin iron [ferrous iron (Fe^{2+}) in hemoglobin (Hb) transformed in the ferric form (Fe^{3+})], functional hemoglobin being converted into non-functional methemoglobin [14]. Methemoglobin (MetHb) is unable to transport and release molecular oxygen to the tissues (as hemoglobin) because oxygen and methemoglobin forms a stable complex with high dissociation constant and induces hypoxia or death [12]. Symptoms of ***methemoglobinemia*** include an unusual bluish gray or brownish gray skin color, excessive crying and irritability in children with moderate MetHb levels. At higher levels symptoms are drowsiness and lethargy. Diagnosis is established through the observation of chocolate-colored blood or a laboratory test showing the presence of elevated MetHb levels [8].

The condition of methemoglobinemia is characterized by cerebral anoxia, cyanosis and stupor. Under normal conditions, less then 2% of the total Hb circulates as MetHb. Signs of methemoglobinemia appear at 10% MetHb or more [13].

In infants less than four months of age, methemoglobin reductase activity (enzyme that convert methemoglobin back to hemoglobin) is lower than adult methemoglobin reductase, and fetal hemoglobin is readily oxidizable than adult hemoglobin. For this instance, infants are more susceptible at nitrite (nitrate) toxicity than adults.

After ingestion of water high in nitrates methemoglobinemia may occur in an infant especially if the infant was suffering from a gastrointestinal disorder. Enteric infections, potentially caused by fecal bacteria contamination of water sources, may lead to the endogenous production of nitrite, if the exposure to exogenous MetHb-forming agents is excluded [4].

Microbially poor water, high drinking-water nitrate levels, and also gastro-intestinal illness, as a result of exposure to poor water quality, may play a role in methemoglobinemia.

Cancer. *N*-nitroso compounds (NOC) are a class of genotoxic compounds, most of them animal carcinogens. In the human body, nitrate is a stable, inert compound and it cannot be metabolized by human enzymes. Even so, the commensal bacteria with their nitrate-reducing activity may convert nitrate into nitrite and other bioactive nitrogen compounds that can interfere with physiological processes and cause health problems. Nitrate intake can be related with endogenous formation of NOC. High intake of drinking water nitrate (above the MCL) is associated with an increased endogenous capacity to nitrosate proline [10, 18].

It seems that populations with high rates of esophageal and gastric cancer excrete high levels of *N*-nitrosoproline. For the acceptable daily nitrate intake level (3.67 mg/kg body weight, 0.84 mg/kg as nitrate-N) the urinary excretion of NOC increases, often associated with an increased intake of dietary nitrosatable precursors [17].

The role of *N*-nitroso compounds and nitrite in the promotion of cancer appears to be undeniable, the evidence for the role of nitrates is less clear [7, 9].

In addition, there have been found the following toxic effects for human body:

- electrolyte imbalance through depletion of intracellular and extracellular chloride anion;
- membrane potential changes through depletion of intracellular potassium cation and elevation of extracellular potassium cation, causing hearth function, neuro- transmissions or muscle contraction;
- mutagenic and carcinogenic action through N-nitroso compounds formation;
- decreasing of immune system [21, 23, 25].

2. Material and Methods

In this work, there were used 1 liter of 13 water samples originating from central and southern areas of Romania. Water samples analyzed were freshly harvested, acidified. Water samples were divided in four categories.

For the first category- *drinking water*- there were used 5 water samples: drinking water Pietrosani (1), unfiltered drinking water Ploiesti (2), filtered drinking water

Ploiesti (3), drinking water Buzau (4), drinking water Bucuresti (5).

For the second category there were used 3 *underground water* samples: Noua Brasov spring (1), fountain 1 Sita Buzaului (2), fountain 2 Sita Buzaului (3).

The third category-*river water*-included samples from Sipoia -7 Scari (1), Tarlung river (2), Buzaul de Jos river (3) and Timis river (4).

For the forth category-*lake water*- the sample analysed was collected from Noua Brasov lake.

Domain of nitrate analysis is from 0.00 to 30.0mg/L, with a resolution of 0.1mg/L and there were obtained data with an accuracy of ±10% of reading, which recommend their use for such rapid screening tests.

Spectral analysis were made using HANNA Photocolorimeter, C99 & C200 Series.

The national admission limit for nitrate in drinking and surface waters is 50 mg / L. For nitrite, the national admission limit in drinking water is 0 mg/L in underground water, 0.004 mg/L , in river water 0.03 mg/L and for lake water the limit is 0.01 mg/L [16].

The U.S. Environmental Protection Agency (EPA) established the maximum contaminant level (MCL) for nitrate in drinking water at 10 mg/L nitrate-nitrogen (nitrate-N) (equivalent to 45 mg/L as nitrate). World Health Organization (WHO) guideline sets the limit at 50 mg/L for nitrate (equivalent to 11 mg/L as nitrate-N). These levels are known to prevent methemoglobinemia, or "blue baby syndrome," especially in infants who are the most susceptible [24].

3. Results and Discussions

Using HANNA Photocolorimeter there were detected the amounts of nitrate and nitrite present in each water samples. The results for nitrate in drinking water samples are shown in Figure 1.

The results for nitrate in drinking water were below the maximum allowed limit.

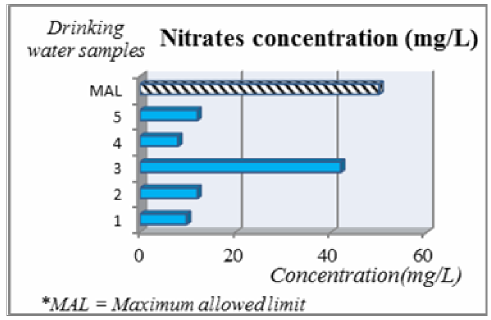

Fig. 1. *The concentration of nitrate in drinking water samples*

The concentration of nitrite present in drinking water samples is illustrated in Figure 2.

As we can see, the nitrite concentration in water sample Pietrosani was higher then maximum allowed limit. This could be risky for human health, as nitrite tends to accumulate and cause adverse effects.

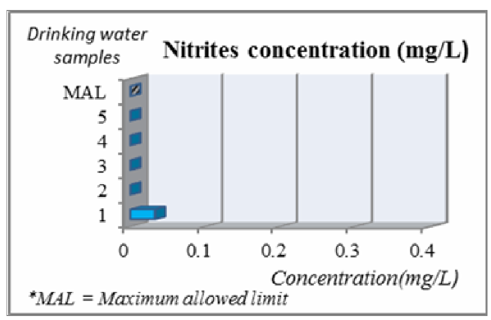

Fig. 2. *The concentration of nitrite in drinking water sample*

Nitrate in water is colorless, odorless, and tasteless and this is why is undetectable without testing. Households with infants, pregnant women, nursing mothers, or elderly people should test the water for nitrate. Nitrate-nitrogen occurs naturally in groundwater, usually at concentrations far below a level of concern for drinking water safety. To determine the baseline nitrate concentration of a new water supply an initial test is needed [15].

The amount of nitrate present in underground water samples was also analysed and the results are shown in Figure 3.

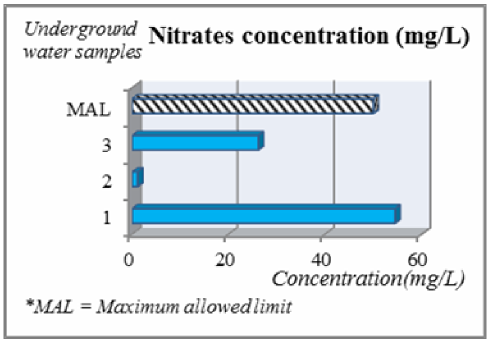

Fig. 3. *The concentration of nitrate in underground water samples*

For Noua spring water sample, we have found a high concentration of nitrate.

The amounts of nitrite present in underground water samples are shown in Figure 4.

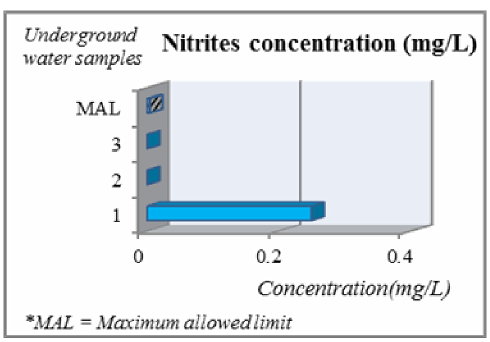

Fig. 4. *The concentration of nitrite in underground water samples*

Analysis for underground water (potable water also) showed a high concentration for both nitrates and nitrites, that could indicate a permanent contamination in the area of that source (Noua spring). This could be dangerous for the health

In Romania, 20% of 2,000 wells had nitrate levels higher then 23 mg/L as nitrate-N, as other studies showed. Also, in other eastern European countries were reported high levels of nitrate contamination in a large proportion of private wells. Countries like China, Botswana, Turkey, Senegal and Mexico reported private well water levels above the WHO guideline, in some instances at levels higher then 68 mg/L nitrate-N [22].

The amounts of nitrate present in river water samples were analysed and the results are shown in Figure 5.

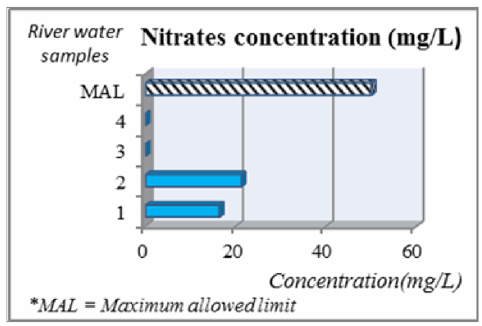

Fig. 5. *The concentration of nitrate in river water samples*

For all the samples, the nitrate concentration was below the MAL.

For river water, the concentration for nitrite in Tarlung water sample (2) is higher then the MAL. That could suggest an old contamination (fertilizer, waste) with nitrate, knowing that nitrite is the degradation products of nitrates (Figure 6).

There could be another cause for high nitrate concentration in rivers. The rivers associated with catchments dominated by permeable bedrock show a smaller range in nitrate concentrations than those associated with clay and mixed sedimentary bedrock of lower permeability. Nitrate concentrations often increase in a gradual way as a function of flow for the rivers draining the permeable catchments, although there is usually a minor dip in nitrate concentrations at low to intermediate flow due to (1) within-river uptake of nitrate during the spring and the summer when biological activity is particularly high and (2) a seasonal fall in the water table and a change in preferential flow-pathway in the Chalk [19].

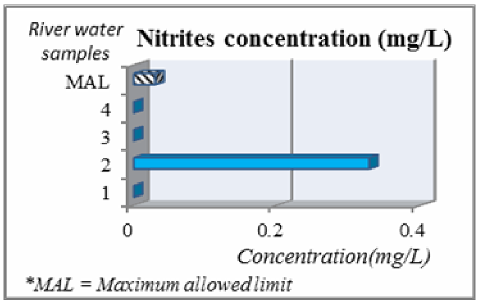

Fig. 6. *The concentration of nitrite in river water samples*

The amounts of nitrate present in lake water samples was also measured and the results are shown in Figure 7.

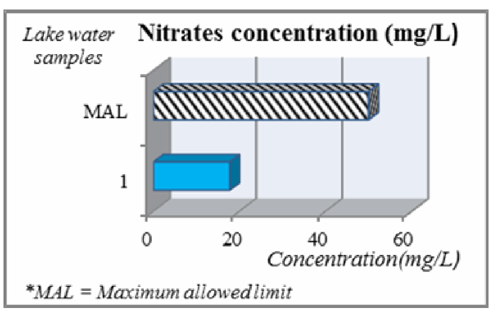

Fig. 7. *The concentration of nitrate in lake water samples*

The results for lake water showed that nitrate concentration was below the maximum allowed limit.

Also, nitrite concentrations were below the maximum allowed limit (Figure 8).

If the concentration of nitrate or nitrite was increased, we could think of several causes. The origin of the soluble nitrogenous compounds may be attributed to the inflowing water, the decomposition of the insoluble organic material, and the excretions of aquatic animals.

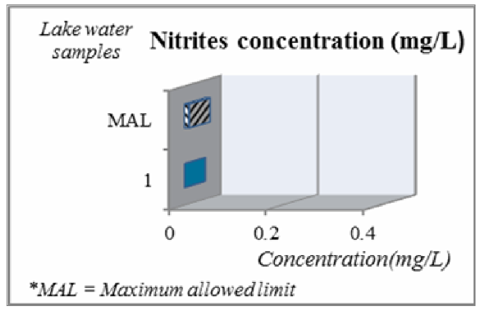

Fig. 8. *The concentration of nitrite in lake water samples*

Also, the plankton (plant material) nitrogen increases as the soluble nitrogen decreases because nitrates are absorbed by plants and are needed for their growth [11].

3. Conclusions

The presence of nitrate in human body comes from food, water or medication or could be intrinsic (self synthesis of nitrogen compounds). As we have seen, excessive nitrogen environment pollution can cause ecological and toxicological effects and adverse effects on human health.

In our study, for drinking water there were obtained high levels only for nitrites in only one water sample that could suggest an old pollution with nitrate in the studied area.

For underground water samples (also drinkable water), the concentration was high for both nitrate and nitrite in the Noua spring. This could be a reason of concern for consumers in that area and some measures should be taken. Although the fountain water is nitrate and nitrite-free, some rules to avoid this kind of pollution can be applied. Proper site selection for the location of domestic water wells and proper well construction can reduce potential nitrate contamination of drinking water source.

The high nitrite concentration that was found in river water sample (2) could be linked to agriculture activity, such as fertilizing, in the area.

Also, as the concentration for both nitrate and nitrite was below MAL in lake water samples, we can assume the plankton was not that abundant.

The contribution from nitrogen-containing fertilizers, commercial fertilizers, as well as human and animal waste to high levels of nitrate in food and drinking water could be the cause for our results.

Adverse health effects from drinking-water with nitrates are most likely the result of a complex interaction between the amounts of nitrate ingested the concomitant ingestion of nitrosating cofactors and precursors, and medical conditions of the host that may increase nitrosation. These effects may be attenuated by inhibitors of endogenous nitrosation such as vitamin C and alpha-tocopherol [22].

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