EFFECT OF NITROGEN TRANSFORMATIONS ON WATER QUALITY IMPROVEMENT

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Abstract. The issue of water quantity and quality is relevant worldwide. The research aimed to determine the quality of water of the Marvelė stream in the area and its pollution by nitrogen compounds entering with drainage water from the adjacent farmland was carried out in the practical training base (Alšėnai eldership) of Kaunas University of Applied Sciences. The research revealed that the concentrations of nitrogen compounds in the stream (during the spring, when the average water temperature is about 12^0 C) are not high, fixed average NO_3-N = 9.58 mgL^{-1}, and is mean – poor water quality by assessment of classes of ecological potential. The amount of dissolved oxygen, dissolved mineral salt concentrations, and changes in water pH indicate that there are intensive biochemical processes and transformations of nitrogen compounds in the water. Under the investigated conditions, chemical changes in water composition were determine due to suitable conditions in the water: optimal temperature and oxygen content (R^2= 0.69; p = 0.05) for nitrification processes to occur. The denitrification process takes place in the water, during which nitrogen is transformed into gaseous form. Was recorded during 11^{th} -12^{th} sampling (when O_2 < 3.5 mgL^{-1}). It can be assumed that active microbiological processes take place at higher temperatures, which is also confirmed by the statistical dependence of dissolved oxygen consumption and nitrate nitrogen concentration (n = 12, p = 0.02), as well as TDS, dynamics of mgL^{-1} values (n = 12, p = 0.04).

Keywords: nitrogen transformations, nitrogen cycle, water quality

Introduction

No life could exist without water. Natural water is a solution of many chemical elements and compounds and, due to its chemical properties, it is the most common compound on the Earth, forming a complex system of dissolved salts, gases, undissolved impurities of mineral and organic origin, as well as bacteria and other plant and animal microorganisms (Sakalauskas et al., 2008).

Water is vital for humans, animals and plants and it is an important resource for the economy. The relevance of water management issues in different parts of the world depends on the relationship between available water resources and water demand. Due to the different distribution of natural water resources in time and space, in addition to intensive human activities and rapid growth of population, many countries and regions of the world are already experiencing significant shortages of freshwater.

It is clear that in the 21st century, the problem of water quantity and quality is one of the most important issues for humanity alongside the problems such as food and energy production. Water, as the most important resource for humanity, which helps to achieve and sustain economic growth and well-being, is an important indicator of climate change and the viability of natural ecosystems.

Criteria for water quality include the properties of water that may affect human health and the existence of other living organisms. Therefore, most countries by legally regulating limit values for pollution concentrations, consider water protection and the quality of natural water as a critical value that must meet certain limits for each area of water consumption: drinking, bathing, domestic use, industry (Povilaitis et al., 2015).

According to L. Bagdžiūnaitė-Litvinaitienė (2005), the quality of surface waters is directly affected by soils, biota, economic human activity, indirectly – by climate, relief, water regime, vegetation, hydrological and hydrodynamic conditions, etc. The issue of the inflow of biogenic substance to surface waters is one of the most important in addressing the problems of the aquatic ecosystem.

While analyzing the quality of water, Lithuanian and foreign authors (Schmalz et al., 2008; Misevičienė, 2013; Husk et al., 2017), assess the impact of diffuse and concentrated pollution from livestock farming facilities, fishery farms, untreated rural residential wastewater on the quality of surface water in order to determine the dynamics of nitrogen compound migration, bacteria breeding and multiplication processes and concentrations.

Studies have shown that the highest concentrations of nutrients are in the water coming from barn areas, yet the highest concentrations of these substances in the surface waters are from crops, especially from accumulative and cereal areas, when there is no plant vegetation (Smalz et al., 2008; Misevičienė, 2013a).

Nitrogen losses from used agricultural and fertilized fields entering surface waters are highest in drainage water, whereas moving away from the sources of pollution, during natural biological purification processes, water pollution by nitrogen compounds decreases. While investigating the efficiency of nitrogen retention in aquatic ecosystems, the following main characteristics are analysed: river basin area, area covered by lakes, ponds and swamps, runoff module as well as derived parameters – hydraulic load, water surface area, water retention time.

Natural water regime in soil depends largely on the precipitation regime. When water enters the soil with precipitation, some of it flows in the direction of the slope of the relief into the ravines and lowlands, simultaneously washing away fine soil particles, sludge, fertilizer residues, etc. from the ground surface. Sedimentation processes take place in soaked loams, some of the transferred nutrients accumulate in plants, aquatic microorganisms, algae - natural biological water purification occur (Novotny, 2003; Gaigalis et al., 2003).

The potential of biological purification is based on cycles of natural elements that depend on the metabolic activity of bacteria. These microbiological processes are very important in the decomposition of chemical compounds in water bodies (Lysovienė, 2013). In the analysis of environmental quality, special attention is paid to the migration of nitrogen compounds in soil and water, the seasonality of changes in the concentrations of these compounds.

The aim of the research is to analyse nitrogen transformations and regularities in water that change water quality**.**

The objectives of the research:

1. To perform the analysis of the nitrogen cycle in water and describe the processes.
2. To determine the influence of abiotic factors on nitrogen transformations that cause changes in water quality.

The current level of economic activity destroys ecological integrity and disrupts the functioning of ecosystems, so by recognizing the importance of ecology and water resources as an economic and social indicator, the issue of water quantity and quality has become especially relevant worldwide; therefore the impact of nitrogen load on water bodies is widely studied not only in Lithuania (Deelstra et al., 2014; David et al., 2016; Povilaitis et al., 2018).

Methods and procedures

The object of research is the transformations of nitrogen compounds in water.

In order to perform the empirical research surface water samples were taken from the ameliorated Marvelė stream, located in the practical training base of Kaunas University of Applied Sciences (Alšėnai eldership) which contain inflowing drainage water from adjacent agricultural areas (see Figure 1).

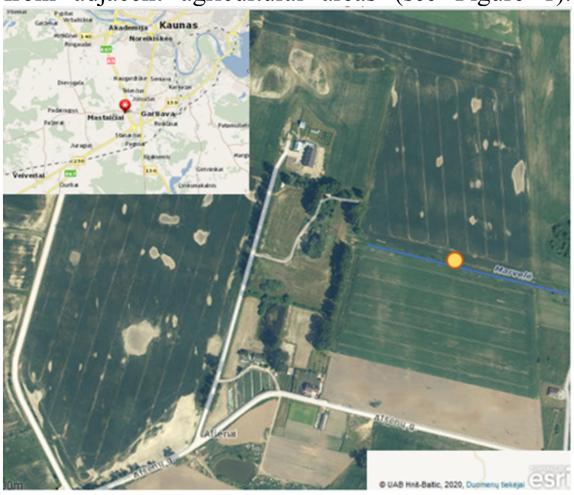

Fig. 1 Water sampling site

The research was conducted in March - May 2019, when the ambient air temperature ranged from +8 to +15 °C. Water temperature (T,°C), pH, total mineralization concentrations (TDS, mgL^{-1}), dissolved oxygen content (O_2, mg L^{-1}) were measured in the laboratory of Agroecology of Kaunas University of Applied Sciences with a portable multifunctional device *WTW Mu WTW Multi 350i.*

The values of all forms of nitrogen concentrations: nitrate nitrogen (NO_3-N, mgL^{-1}), nitrites (NO_2-N, mgL^{-1}) and ammonium nitrogen (NH_4-N, mgL^{-1}) were determined with a *MaxiDirect 600* photometric system by using powder and liquid consistency reagents. The total of 12 water samples ($n = 12$) were tested at a frequency of 1 time per week.

The quality (physico-chemical quality element indicators) of the water body was assessed by order of Environment Minister of Lithuania, on the Methodology for Determining the Status of Surface Water Bodies (2007, current version 01-11-2019) (see Table 1). It should be noted, the dynamics of phosphate concentrations are not discussed in this article, due to lack of data. Further research is needed to investigate water quality parameters for possible pollution of phosphate compounds.

Table 1. Classes of ecological potential of rivers and canals classified as heavily modified water bodies according to physico-chemical quality element indicators

Nr.	**Element of quality**	**Parameter**	**Ecological potential classes criteria of physico-chemical quality elements, values of the parameters**				
			Very good	**Good**	**Medium**	**Poor**	**Very poor**
1.	Nutrients substance	NO_3-N, mg/l N	<1,30	1,30–2,30	2,31–4,50	4,51–10,00	>10,00
2.		NH_4-N, mg/l N	<0,10	0,10–0,20	0,21–0,60	0,61–1,50	>1,50
3.		PO_4-P, mg/l P	<0,05	0,05–0,09	0,09–0,18	0,18–0,40	>0,40

The results obtained during the research were systematized and evaluated by statistical methods using *SPSS Statistics 17* software package (IBM Corporation, USA).

The article analyses the results of the scientific research on the research topics by applying the method of inductive-deductive cognition, as well as elaborates the paradigms of fundamental science related to the nitrogen cycle and draws logical conclusions.

Nitrogen cycle and transformations

Nitrogen is the most common element in the atmosphere – about 78% of the atmosphere is comprised of gaseous nitrogen (N_2). In the nitrogen cycle, nitrogen ions can exist in different chemical forms, each with its own properties and consequences for the ecosystem. Due to biochemical cycles, the nitrogen form changes.

Gaseous nitrogen is sufficiently inert due to the very strong triple bond between two nitrogen atoms and most life forms, including plants and animals, are unable to use this form of nitrogen in life-sustaining biochemical processes (Mažeikienė et al., 2012; Partheeban, 2014).

Most nitrogen compounds are soluble in water; others are gases and solid phases. Nitrogen enters soil through atmospheric precipitation (NOx and NH_3), fertilizers, animal feed and biological nitrogen fixation.

During nitrogen biological fixation, diazotrophs incorporate molecular nitrogen into natural compounds. This process is regulated by the enzyme nitrogenase, which is present in aerobic (*Nitrobacter*) and anaerobic (*Clostridium*) bacteria and algae (Tumas, 2003; Partheeban, 2014).

All nitrogen-fixing microorganisms, except those involved in photosynthesis, require the energy of external carbon compounds for the nitrogen-fixing reaction to take place. The intensity of fixation depends on the light and temperature. At noon, when light radiation is the most active, nitrogen fixation is highest, at night and early in the morning fixation is low (Povilaitis et al., 2015).

Heterotrophic bacteria (which take carbon from organic compounds) while decomposing organic compounds in water or soil, convert organic nitrogen to inorganic, i.e. ammonia or ammonium ion. During ammonification or mineralization, microorganisms release ammonia nitrogen from the organic nitrogen compounds. During immobilization, the nitrogen available to the plants can be converted into the organic form. Immobilization and mineralization occur simultaneously, but their extent varies depending on soil properties, conditions, and the amounts of carbon and nitrogen required for soil microorganisms and plants (Shaffer et al., 2008; Ghane et al., 2018).

Ammonia and ammonium salts are oxidized by bacteria to nitrites and nitrates (*nitrification*). This process takes place under aerobic conditions. Nitrates are the end product of protein breakdown. These are soluble minerals that are easily assimilated by plants. Along with photosynthesis, nitrates are involved in the process of synthesis of organic matter. The nitrification process is performed by nitrifying bacteria. The equation for the nitrification process is presented below (Tchobanoglous 2003; Porter et al., 2011).

$$NH_4^+ + 2O_2 \rightarrow NO_3^- + H_2O + 2H^+ \quad (1.1)$$

The nitrification process is very important in the nitrogen cycle as it converts cations (NH_4^+) into anions (NO_2^- ir NO_3^-). Ammonium cations are sorbed by soil particles, so they stay longer in the soil, the plants carry out bioavailability. Meanwhile, anionic soil particles do not absorb, so they are easily leached into surface or groundwater (Tripolskaja et al., 2002; Warneke et al., 2011).

Bacterial activity in water and soil is very important in the decomposition processes of organic and inorganic compounds. Bacteria gain the energy they need by reducing organic compounds and nutrients during reduction reactions (Shaffer et al., 2008; Haseborg et al. 2010).

Part of the nitrate nitrogen is used by plants; part of the bacteria is reduced to molecular nitrogen and returned to the atmosphere during denitrification. This process is described by the following equation (1.2).

$$4NO_3 + 5C + H_2O \rightarrow 2N_2 + CO_2 + 4HCO_3 \quad (1.2)$$

During the denitrification process, nitrates are successively reduced to nitrites, nitric oxide, and nitrogen dioxide until they are finally converted to gaseous nitrogen. This process is carried out by four enzymes *Nar, Nir, Nor, Nos* responsible for the production of denitrification intermediates (Robertson, Groffman, 2007; Partheeban, 2014).

The activity of heterotrophic bacteria is more intense at high temperatures (Lakha et al., 2009; David et al., 2016). Then the concentration of nitrates in the water can decrease significantly (in the spring and late autumn periods) or they can even be completely consumed (in the summer and autumn periods) (Lysovienė, 2013).

Biotransformation and biodegradation of chemical and biological pollutants with the presence of microorganisms (bacteria, fungi, protozoa and algae) are important processes during which pollutants released into the water are removed from the ecosystem. These processes depend on the molecular structure and concentration of contaminants, the nature of microorganisms, environmental conditions, and temperature (Račys et al., 2012).

Microorganisms involved in biochemical processes firstly absorb airborne organic pollutants, which are subsequently synthesized by microorganisms, i.e. biodegradation of pollutants in the aqueous phase. The microflora most easily decomposes uncomplicated chemical structures, pollutants dissolved in water, and only then chemicals in dispersed and colloidal states. Bacteria, micromycetes, yeast, etc. use chemicals as a source of carbon and energy (Gomez et al., 2011).

The amount of synthetic substances, heavy metals, inhibitors, the influence of abiotic and biotic factors determine the activity of microbiocenosis, i.e. microorganism mass gain and dominance of relevant bacterial species (Souze, 2009; Sapkaitė, 2011; Ghane et al., 2018).

The composition of biocenosis and the ratios of various types of microorganisms in the nitrogen transformation process depend not only on external factors but also on the composition of the nutrient medium and enzymatic degradation – all chemical reactions in cells are catalysed by enzymes (Mažeikienė et al., 2012).

The direction of transformation and kinetics of nitrogen compounds are based on the cycles of natural elements that affect the metabolic activity of bacteria (Souze, 2009).

Biochemical processes are continuous in the environment. Human activities affect natural biochemical processes by disturbing them and their intensity. The quality of air, water and soil depends not only on natural and climatic conditions, but also on anthropogenic load. Under the influence of biological systems, the environment purifies itself in a natural way, but in case of excessive anthropogenic load, there is threat to soil degradation, i.e. loss of soil fertility due to soil erosion, acidification, salinity, inadequate agrotechnical solutions, as well as conservation of water resources due to nutrient pollution (Kroeze et al., 2013).

Results and discussion

Effect of temperature on nitrogen compound transformations

The water temperature values measured during the trials ranged from 8.1 °C to 15.4 °C. This temperature limit is characteristic of spring and autumn water temperatures in surface water bodies in Lithuania.

NO_3-N concentrations fixed in water samples were from 4 to 16 (mgL^{-1}), NO_2-N concentrations were from 0.03 to 0.08 (mgL^{-1}) and NH_4-N concentrations ranged from 0.02 to 0.04 (mgL^{-1}). Fixed nitrate concentrations in the Marvelė stream indicated poor water quality (NO_3-N average 9.58 mgL^{-1}), this may be affected by intensive agricultural activities.

According to many scientists, water temperature is one of the most important factors influencing the biological treatment of water in outdoor conditions (Lysovienė, 2013. Povilaitis et al., 2020).

The research revealed that as the water temperature increases, the concentration of nitrate nitrogen in the water decreases (see Figure 2). Results showed, that NO_2-N leaching from soil to water bodies has an inverse linear dependence on water temperature (R^2= -0,657). This can be explained by the increase in water temperature denitrifying microorganisms more actively. Leaching of nitrogen compounds in different forms (eg. NH_4-N, NO_2-N) allows to judge the complete or partial nitrification occur in water.

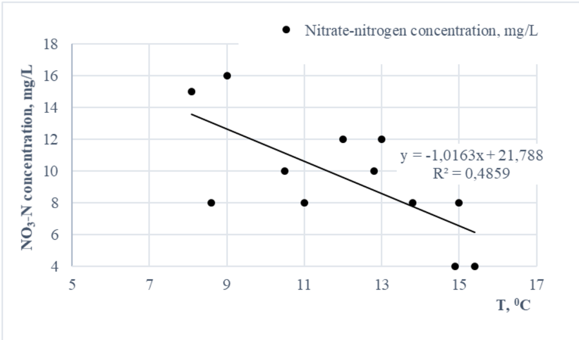

Fig. 2 Change in nitrate nitrogen concentration at different water temperatures

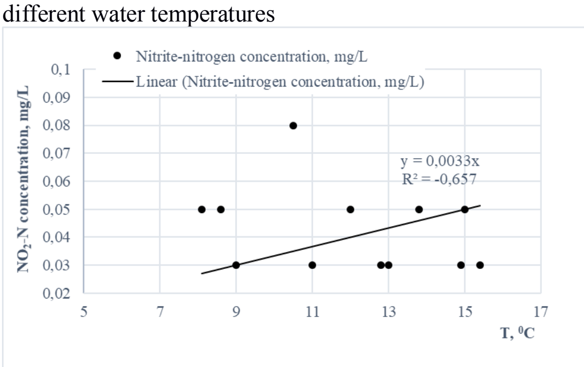

Fig. 3 Change in nitrite nitrogen concentration at different water temperatures

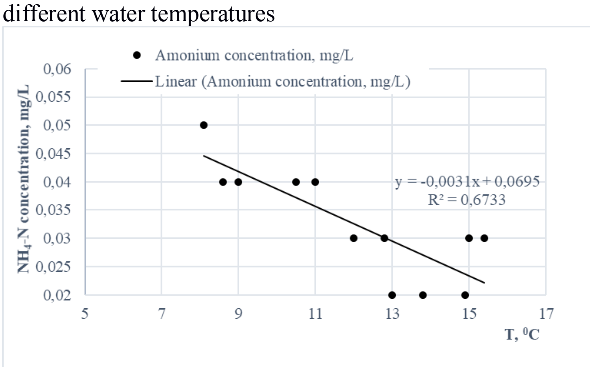

Fig. 4 Change in ammonium nitrogen concentration at different water temperatures

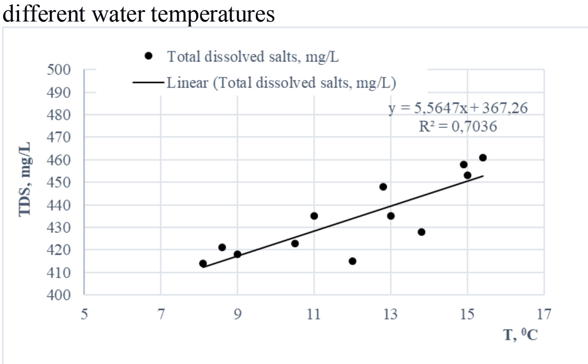

Fig. 5 Change in total dissolved salts concentration at different water temperatures

At temperatures above 13 °C, the values of decomposition of water-soluble minerals (TDS, mgL^{-1}) increase regularly (Fig. 5). Indirectly, in the event of denitrification occur, can be judged from the higher TDS (mg L^{-1}) values in water. The figure is a clear linear relationship between water temperature and TDS values increase (R^2=0,70). This leads to the conclusion, that denitrification is influenced by temperature.

Effect of dissolved oxygen on nitrogen compound transformations

The amount of dissolved oxygen in water is important while analysing the abiotic factors that determine nitrogen transformations. The direction of transformation and kinetics of nitrogen compounds depend on the metabolic activity of the bacteria. The presence of nitrifying bacteria in the aqueous medium can be judged from the appropriate conditions for the growth of microorganisms (i.e. relatively high O_2 concentrations > 3.25 mgL^{-1}, alkaline water pH (7-8) and optimal water temperature of ~ 15 °C).

In the course of the research, an aerobic environment (i.e. O_2 > 2.0 mgL^{-1}) was formed in the Marvelė stream (water sampling site), therefore the transformations of nitrogen compounds could be mostly caused by the nitrification process.

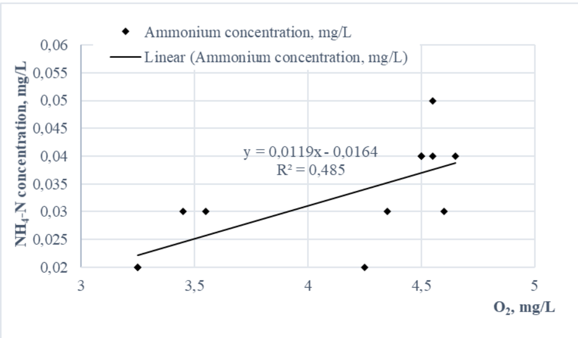

Fig. 6 Change in the concentration of ammonium nitrogen due to the changes in the content of dissolved oxygen in the water

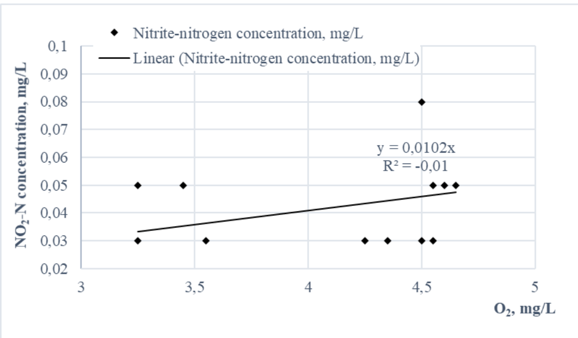

Fig. 7 Change in nitrite nitrogen concentration due to the changes in the content of dissolved oxygen in the water

The nitrification process in water is performed by nitrifying bacteria. This process takes place in several stages: in the first stage ammonium is oxidized to nitrites, in the second stage nitrification is carried out by some autotrophic and heterotrophic bacteria, during photosynthesis, using ammonia or ammonium salts for its protoplasm synthesis, converting them to nitrates. Different stages of nitrification are performed by two different groups of aerobic autotrophic bacteria. Concentrations of nitrogen compounds (e.g. NH_4-N, NO_2-N) of different forms in water imply a complete or partial nitrification in the aqueous medium (see Figure 6 and 7).

Results showed, it can be seen that the process of partial nitrification was recorded during the 4th sampling (when O_2 is > 4.5 mgL^{-1}, temperature is > 10 ^{0}C). The assumption about the nitrification that has taken place (final process product NO_3-N) can be made from the higher concentrations of NO_2-N in the water. A complete nitrification process with decreasing NO_2-N concentrations in the effluent water and increasing NO_3-N (until 16.0 mgL^{-1}) concentrations with enough oxygen in the water to prevent nitrate removal during the denitrification process not occur. Was recorded during 5th -10th sampling (when O_2 > 4.5 mgL^{-1}) (Fig. 8).

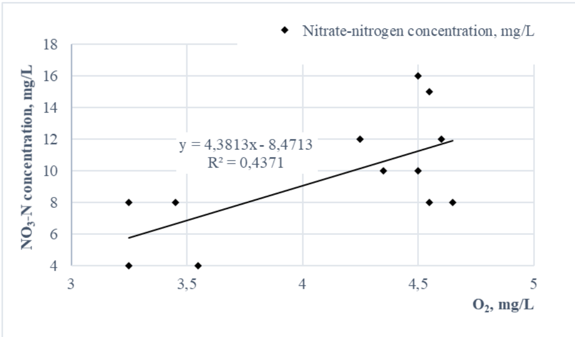

Fig. 8 Change in nitrate nitrogen concentration due to the changes in the content of dissolved oxygen in the water

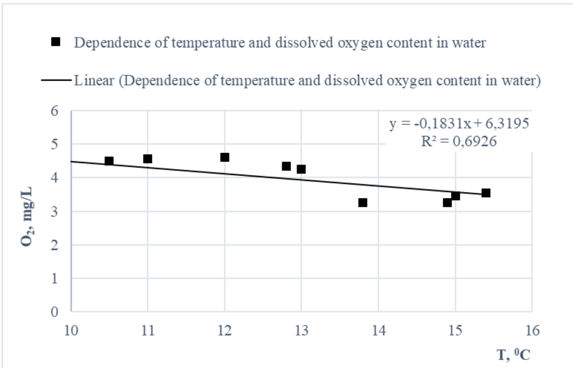

Fig. 9 Dependence of temperature and dissolved oxygen content in water

At the same time, the amount of dissolved oxygen decreases (see Figure 8), which suggests that a denitrification process takes place in the water, during which nitrogen is transformed into gaseous form. Was recorded during 11th -12th sampling (when O_2 < 3.5 mgL^{-1}). It can be assumed that active microbiological processes take place at higher temperatures, which is also confirmed by the statistical dependence of dissolved oxygen consumption and nitrate nitrogen concentration (n =

12, $p = 0.02$), as well as TDS, dynamics of mgL^{-1} values ($n = 12$, $p = 0.04$).

The results of the research showed a strong linear relationship between water temperature and the content of dissolved oxygen (see Figure 9). The coefficient of determination $R^2 = 0.69$ was calculated and this shows strong correlations between the variables when $p = 0.05$.

Rising temperatures stimulate the growth of microbes in a certain range that consume increasing amounts of dissolved oxygen for respiration, but the effective activity of microbial enzymes also depends on pH of the water.

Effect of pH on nitrogen compound transformations

The process of nitrification requires oxygen and a neutral or basic medium i.e. the intensity of nitrification depends on pH.

The results of the research show that a neutral and alkaline aqueous medium (pH mean 7.28) prevailed in the water during the research period. Upon acidification of the aqueous pH medium (pH = 6.9), partial nitrification was recorded, during which relatively higher amounts of nitrite (NO_2-N> 0.05 mgL^{-1}) were determined. There was no statistically significant difference between changes in nitrite nitrogen concentration at different pH ranges ($n = 12$, $p > 0.05$).

According to scientists, nitrifying bacteria cease performing nitrification when water pH drops to 4 (Shaffer et al., 2008). The decrease in pH is thought to be related to hydrogen ions formed during ammonium oxidation, which can lower pH of water. Nitrifying bacteria are very sensitive to changes in pH. The most favourable conditions for the nitrification process occur when pH of the water is between 7.5 and 8.0. When pH drops below 6.8, nitrification rates decrease prominently, and at pH 5.8–6.0, the nitrification rate can reach only 10–20% of the nitrification rate at pH 7.0 (Tchobanoglous, 2003; Almstrand, 2011).

Water acidification occurs due to the decomposition of organic substances in water - the process of hydrolysis. Since the hydrolysis of both cations and anions takes place in water at the same time, pH value is determined by the strength of the acids and alkalis formed in the reactions and their ratio. When pH is > 7.3, nitric oxide is formed. It can be re-absorbed and further denitrified into molecular nitrogen. In general, pH of water is important for the final products of NO_3 (i.e., the nitrogen transformation cycle).

Conclusions

Active biochemical processes take place in aqueous medium. When the water is rich in oxygen, nitrifying microorganisms are active. Under the investigated conditions were determine suitable conditions in the water for nitrification processes occur: optimal temperature is > 12 ºC and oxygen content is > 4.50 mgL^{-1} ($R^2 = 0.69$; $p = 0.05$). Also, was influenced by favourable pH (neutral or alkaline aqueous medium) to acceptable for the activity of microorganisms.

When $O_2 < 3.2$ mgL^{-1}, water temperature is > 15 ^{0}C reduce nitrate concentracion NO_3-N < 4.0 mgL^{-1}. Microorganisms consume dissolved oxygen for respiration, which causes the formation of anaerobic conditions and the activation of the denitrification process. This has also been confirmed by established statistical relationship between the changes in NO_3-N, mgL^{-1} concentrations and O_2, mgL^{-1} content in water ($n = 12$, $p = 0.02$), as well as the dynamics of value of TDS, mgL^{-1} ($n = 12$, $p = 0.04$).

The research revealed that the concentrations of nitrogen compounds in the stream was fixed average of nitrate nitrogen concentration is 9.58 mgL^{-1}. It shows, the poor water quality by assessment of classes of ecological potential.

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