Original Paper

Evaluation of concentrations of inorganic forms of nitrogen in the Bocegaj watercourse and Kolínany pond

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Article Details: Received: 2022-12-05 | Accepted: 2023-02-06 | Available online: 2023-06-30

https://doi.org/10.15414/afz.2023.26.02.179-185

The aim of the study was to determine concentrations of inorganic forms of nitrogen (N-NO_3^-, N-NH_4^+, N-NO_2^-) in the Bocegaj creek and Kolínany pond, the impact of the wastewater treatment plant on the water quality in the pond, to identify the sources of pollution in the creek during the year of 2014. Water sampling was carried out at regular monthly intervals periodically from January to October, from six sampling points. During the time horizon, all four seasons alternated, which was also reflected in the change of the concentrations of individual forms of nitrogen. It was found that the water in the Kolínany pond did not meet the recommended value (N-NH_4^+, N-NO_2^-) and the limit value (N-NH_4^+) specified by the government regulation of national low. The main source of pollution was insufficiently purified water discharged from the Kolínany wastewater treatment plant.

Keywords: ammonium, brook, nitrate, nitrite nitrogen, water

1 Introduction

Nitrates (NO_3^-), ammonium nitrogen (NH_4^+) and nitrites (NO_2^-) belong to the most reactive ionic forms of inorganic nitrogen in the water environment. They can enter aquatic ecosystems naturally through atmospheric deposition, surface and underground water runoff, dissolution of nitrogen-rich geological deposits, N_2 fixation by some prokaryotes, and biological degradation of organic matter (Rabalais, 2002; Camargo et al., 2005). Nitrate nitrogen is present in low concentrations in almost all waters, where it forms the last segment of nitrogen compounds. Higher concentrations of nitrate nitrogen in water are related to its use in agriculture in the form of fertilizers and pollution from sewage or agricultural wastewater. Nitrogen from industrial fertilizers is the cause of the eutrophication of surface waters (Hornáčková et al., 2008). The primary product of microbial decomposition of organic nitrogenous substances is ammonium nitrogen. In natural waters, it is very unstable in the presence of oxygen. Biochemical oxidation (nitrification) changes to nitrites or nitrates. Ammonium ions are an important indicator of fresh faecal pollution (Samešová et al., 2006). In surface waters, its concentrations usually do not exceed 1 mg.dm^{-3}. Its sources are mainly sewage wastewater and waste from agricultural production. Nitrogenous fertilizers are also an important source, which, through washing and infiltration from agriculturally cultivated areas, reach the surface and underground waters. Ammonia is also the main metabolic product of fish, zooplankton and other aquatic organisms, and its increased concentrations should be expected, especially in intensive fish farming in ponds and in recirculation systems. It is removed from water in the process of nitrification (Adamec et al., 2016). Nitrites are formed in water as a transitional compound in the nitrogen cycle, most often during the biochemical reduction of nitrates or the biochemical oxidation of ammonium nitrogen. As a rule, they are accompanied by nitrates and ammonium nitrogen. The concentration of nitrites in underground and surface waters is generally very low (hundredths to tenths of mg.dm^{-3}), but higher in waste sewage (single units to tens of mg.dm^{-3}). The sources of water pollution by nitrites, as well as nitrates,

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are agriculture (natural and artificial fertilizers) and industrial waste. Some industrial wastewaters are very rich in nitrites (e.g. wastewater from the production of paint, from engineering plants where they are used to cool machine tools) and are also part of some de-icing fluids. The main source of nitrites in surface waters is the increased concentration of ammonia, especially in the summer (Lenghartová et al., 2015).

The aim of the work is to evaluate the concentrations of inorganic forms of nitrogen in the Bocegaj stream and to determine their impact on the ichthyofauna.

2 Material and methods

The village of Kolínany is located in the southeastern part of the Tríbeč Mountains (48° 21′ 33″ S 18° 11′ 28″ V), in the Žitavská Pahorkatina, southwest of the city of Nitra at an altitude of 170 to 356 m MSL (Regional planning of Kolíňany village, 2010). The area of the village is 1,250 ha. The border of the village is the Bocegaj watercourse from the north and the road from the south No. 1669. Neighbouring villages are Žirany, Jelenec, Pohranice, Čeľadice and Hosťová.

The main crops grown in the area of interest are cereals, mainly winter wheat and barley, and there is also widespread cultivation of sunflowers and fodder crops. From the geological point of view, the area of interest is made up of gray, calcareous clays, dusts, sands, gravels, there are also layers of lignite and freshwater limestones.

The territory of the municipality of Kolínany lies in the basin of the river Nitra. The Bocegaj watercourse flows through the village, which springs above the village of Žirany. A small water reservoir with an area of 13 ha is built on the watercourse, used for experimental fish breeding. There is a wastewater treatment plant nearby (Magová, 2004).

The area of interest belongs to a warm climate region with long, warm and dry summers, short and mild winters and short snow cover. The average annual air temperature is 10 °C and the average annual rainfall is 580 mm.

There is a farm in the eastern part of the village, which is managed by the university farm of SUA in Nitra (Regional planning of Kolíňany village, 2010).

2.1 Characteristics of sampling points

The sampling sites were chosen in order to evaluate the effect of nitrate, ammonium and nitrite nitrogen concentration on fish life as optimally as possible. A total of 6 collection points were determined:

1. Sampling point: located about 50 m before the wastewater treatment plant, with a depth of about 0.3 m. The banks are covered with grass, *Rosa canina* and willows (*Salix*).
2. Collection point: it was located at the exit from the wastewater treatment plant. The banks are lined with willow (*Salix*) and *Rosa canina*.
3. Sampling point: it was situated at the mouth of the watercourse into the lake. Reed (*Phragmites australis*), willows (*Salix*) and poplar (*Populus*) dominate the vegetation here.
4. Sampling site: situated on the northwestern edge of the pond, with growth of common reed (*Phragmites australis*).
5. Collection point: located at the end of the pond. The banks are lined with poplars (*Populus*) and willows (*Salix*).
6. Sampling point: located below the mouth of the pond.

2.2 Sample collection and processing

Water samples were taken in 2014, once a month, from January to October. Nitrate nitrogen was determined in the water samples spectrophotometrically (using WTW nitrospoectral in concentrated sulfuric acid). Ammonium nitrogen was determined spectrophotometrically (using indophenol blue – Bertholot reaction), and nitrite nitrogen was determined spectrophotometrically (using sulfanilic acid and 1-naphthylamine).

The detected concentrations of the monitored indicators were compared with the limit and recommended values specified in the Regulation of the Government of the Slovak Republic no. 269/2010 Coll., in Annex no. 2, part C for the carp zone. According to this regulation, the values of the monitored indicators should not be exceeded, because the water would be unsuitable for the breeding and reproduction of fish.

3 Results and discussion

Nitrate nitrogen was the most represented form of all monitored forms of nitrogen. In Regulation of the Government of the Slovak Republic no. 269/2010 Coll., in Annex no. 2, part C, the limit and recommended value for life and reproduction of fish for this indicator are not set. The average concentration of nitrate nitrogen during the investigated period was 1.96 $mg.dm^{-3}$.

According to seasonal dynamics, the highest average concentrations of nitrate nitrogen were measured in spring (March, April, May) and the lowest during the winter months (January, February) (Figure 1). It can be concluded that the decrease in the concentration of $N\text{-}NO_3^-$ in the winter period is probably related to the low water temperature in the pond, when in January

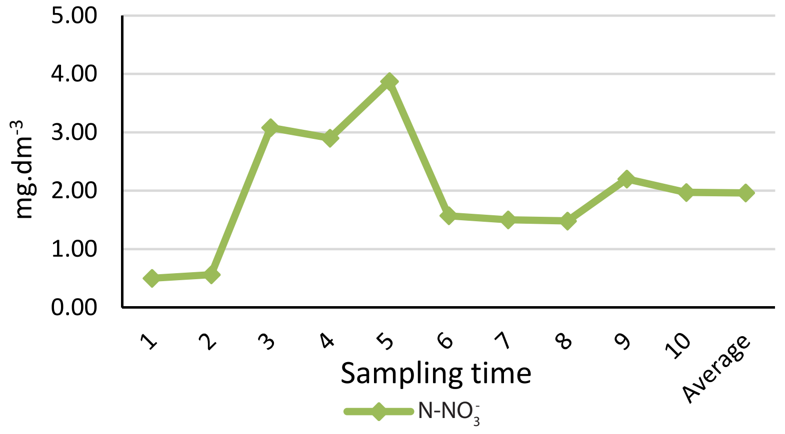

Figure 1 Average concentrations of N-NO_3^- (mg.dm^{-3}) depending on the sampling time

the average water temperature was 0.5 °C, as a result of which the nitrification processes were inhibited. Average water temperatures in the Bocegaj watercourse are reported by Babošová et al. (2022). Brooks et al. (2013) justifies the decrease of nitrate nitrogen concentrations in the summer season due to the fact that nutrient concentrations in surface waters drop significantly in the summer season because of their removal by phytoplankton.

According to the sampling location, the highest average concentration (3.86 mg.dm^{-3}) was found in sampling location no. 2, which was located behind the wastewater treatment plant. Other sampling points recorded a decrease in the concentration. The greatest decrease in its concentration was recorded at the mouth of the watercourse into the pond (sampling point no. 3). It can be assumed that the decrease in the concentration of N-NO_3^- at sampling points no. 3 to no. 6 is related to the lower concentration of oxygen in the water reservoir, which resulted in the inhibition of nitrification (Figure 2). According to Camargo et al. (2005), the toxic effect of nitrates is caused by the transformation of oxygen-carrying pigments into non-oxygen-carrying forms. For aquatic animals, the toxicity of nitrates increases with their increasing concentration in the water and the length of their exposure. Conversely, nitrate toxicity may decrease with increasing body size, water salt content, and adaptation to environmental conditions. Compared to marine animals, freshwater animals are much more sensitive to the nitrate content in the water. It was found that a nitrate concentration of 10 mg N-NO_3^- dm^{-3} can negatively affect freshwater invertebrates, fish and amphibians with long-term exposure. For the most sensitive freshwater organisms, their maximum concentration in water is 2 mg N-NO_3^- dm^{-3}. In the case of marine animals, the maximum nitrate concentration in water is up to 20 mg N-NO_3^- dm^{-3}, but some developmental stages of some marine invertebrates

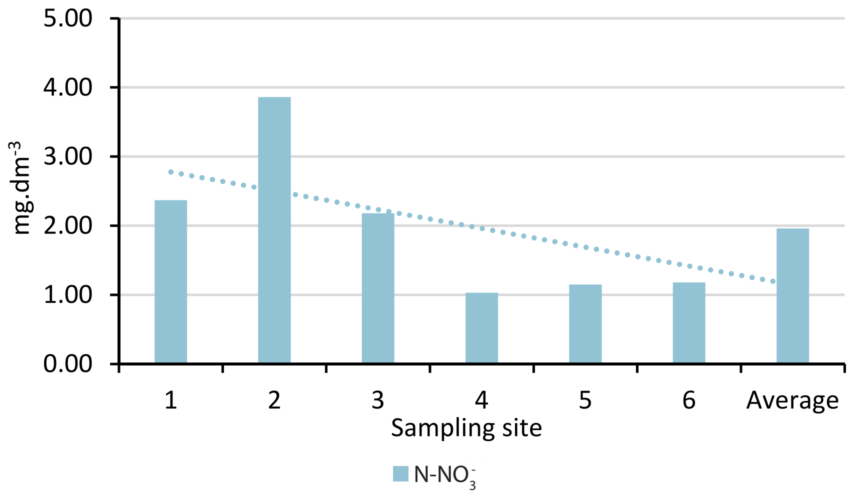

Figure 2 Average concentrations of N-NO_3^- (mg.dm^{-3}) depending on the sampling site

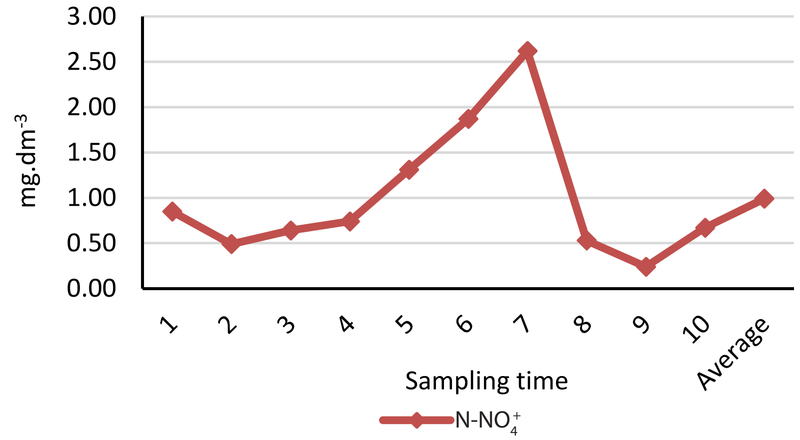

Figure 3 Average concentrations of N-NH_4^+ (mg.dm^{-3}) in water depending on the sampling time

can be similarly sensitive to nitrates as freshwater invertebrates.

During the observed period, the average concentration of N-NH_4^+ represented 0.99 mg.dm^{-3}. For surface waters intended for the life and reproduction of fish, the regulation of the Government of the Slovak Republic No. 269/2010 Coll. Specified a limit value for ammonium nitrogen 0.8 mg.dm^{-3}.

According to the sampling time, the highest average concentrations of N-NH_4^+ were measured in the months of May, June and July, when a gradual increase in their average concentration was detected until the month of July. Its highest average concentration was measured in this month (2.62 mg.dm^{-3}). According to the figure no. 3, the lowest average concentrations were measured in autumn and winter. It can be concluded that the increase in the concentration of ammonium nitrogen in the summer season was probably related to the rising water temperature and lower oxygen content, which does not create optimal conditions for the nitrification of ammonium nitrogen to nitrite and nitrate (Noskovič et al., 2011; Babošová et al., 2018). Adamec et al. (2016) and Sytar et al. (2016) state that the danger of fish poisoning with ammonia is particularly high in spring in highly eutrophic ponds, when ammonia is not yet consumed by green organisms, the water temperature rises, and phytoplankton develops. As a result of photosynthetic assimilation, there is an increase in the pH values of water, which, together with the rising water temperature, creates conditions for an increase in proportion of the free form of ammonia.

According to the sample collection site, the result was that the highest average concentration of N-NH_4^+ (1.71 mg.dm^{-3}) was determined in collection point no. 3 (mouth of the watercourse into the pond), where the most significant rise was also detected (Figure 4). The recommended value of this indicator in the government regulation for the carp fish zone is 0.15 mg.dm^{-3}

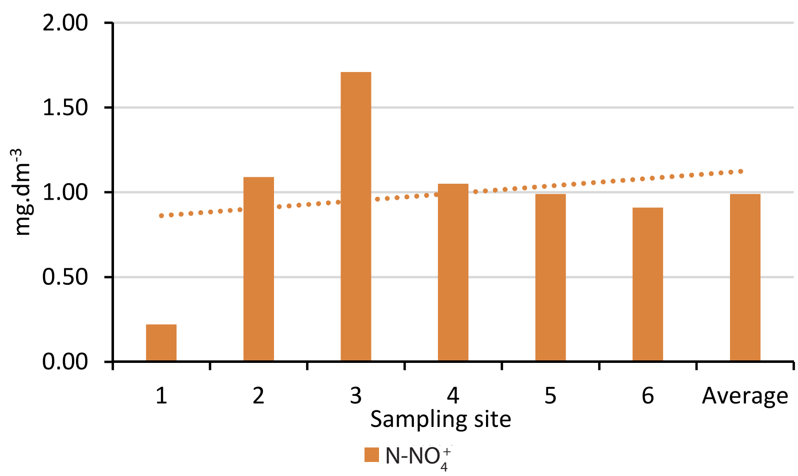

Figure 4 Average concentrations of N-NH_4^+ (mg.dm^{-3}) depending on the sampling site

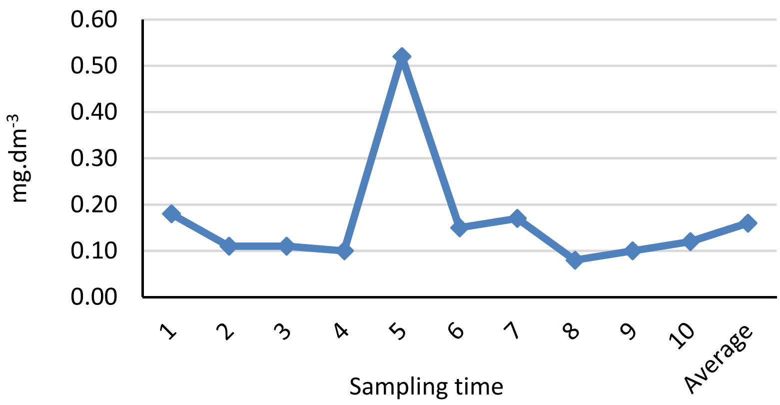

Figure 5 Average concentrations of N-NO_2^- (mg.dm^{-3}) depending on the sampling time

(Regulation of the Government of the Slovak Republic no. 269/2010 Coll., in Annex no. 2, part C). A decrease in the concentration of ammonium nitrogen was detected in the northwestern part of the pond (sampling point no. 4), where there was a large number of hydrophytes, after death of which a large amount of organic matter accumulated on the bottom, and decomposition of which had resulted in the release of N-NH_4^+. Therefore, it can be stated that generally in all the water samples taken, the concentrations of ammonium nitrogen exceeded both the recommended and the limit value stated in the government regulation for surface waters that are suitable for the life and reproduction of carp species of fish, with an exception of the water sample taken above the wastewater treatment plant (sampling point No. 1). Despite the fact that the concentrations of free ammonia in the water did not reach critical values, damage to the fish could occur due to poisoning of the organism with its own ammonia. This process occurs, for example, with a sudden drop in water temperature and dissolved oxygen concentration or with high water pH values, when fish are unable to excrete the metabolic product – ammonia (Hyánek et al., 1991).

In water, ammonium nitrogen can be found in two forms, dissociated – bound (NH_4^+), which is not significantly toxic to fish, and undissociated – free (NH_3), which is highly toxic. Which of these forms prevails in the water is determined by the pH value and water temperature. The higher the water pH value, the greater the proportion of NH_3 in relation to the ionized form (NH_4^+). From a fish farming point of view, concentrations of the free (toxic) ammonia (NH_3) are important. For carp fish, the highest permissible concentration is 0.05 mg.dm^{-3} (Adamec et al., 2016). Damage to fish occurs during their prolonged exposure to the free ammonia in concentrations as low as tenths of mg.dm^{-3}. In terms of the toxic effect, it is important that the cell wall is essentially impermeable to the dissociated ammonia – NH_4^+, while the free ammonia NH_3 penetrates tissue barriers very easily and is thus poisonous to fish (Adamec et al., 2016; Shin et al., 2016).

During the observed period, the average concentration of nitrite nitrogen detected by us was 0.16 mg.dm^{-3}.

Depending on the sample collection time, the maximum average concentration was found in the month of May (0.52 mg.dm^{-3}) and the minimum in the month of August (0.08 mg.dm^{-3}) (Figure 5). The increase in the concentration in the month of May was probably related to the fact that during this period nitrite-oxidizing bacteria growth was slower than ammonia-oxidizing bacteria and production of nitrites prevails over their consumption (Kabelková-Jančárová, 2002). The limit value for this indicator is not stated in the government regulation, Annex no. 2, part C.

Its maximum average concentration (0.20 mg.dm^{-3}), depending on the sampling location, was measured in sampling locations no. 2 and 3. It can be stated that there were higher concentrations of N-NO_2^- detected in individual sampling points, than the limit concentration stated in NV SR no. 269/2010 Coll., Annex No. 2, Part C – for the carp fish zone. Higher concentrations of nitrites in water are the cause of increased intake at the expense of chlorides in fish. Higher concentrations of chlorides in water protect fish from nitrate intake and its toxic effects (Jansen, 2003). Nitrites penetrate into the blood plasma of fish, then into red blood cells, where they bind to haemoglobin to form methemoglobin, which is not able to transport oxygen (Kroupova et al., 2005; Králinský, Mečiaková, 2014). According to Kocour Kroupová et. al. (2018), the most significant factor affecting the toxicity of nitrites in water can be considered the concentration of chlorides, where it can be assumed that they have a positive effect on reducing the toxicity of nitrites in all aquatic animals (including their early stages) that breathe with gills. The minimum average concentration (0.07 mg.dm^{-3}) was found in the sampling point located

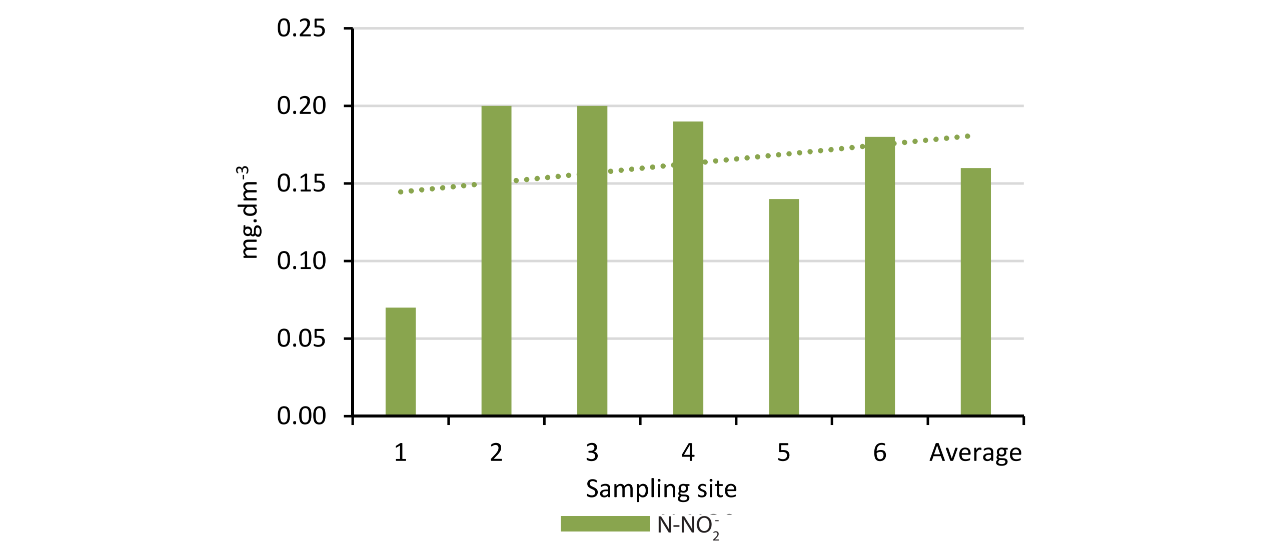

Figure 6 Average concentrations of N-NO_2^- (mg.dm^{-3}) depending on the sampling site

in front of the wastewater treatment plant (sampling point no. 1). These values show that the increase in N-NO_2^- concentration was probably caused by insufficient water purification from the Kolínňany WWTP. It is also documented in Figure 6, from which the result is that the lowest average concentration of N-NO_2^- was detected before the wastewater treatment plant, and subsequently it significantly increased in the following sampling points.

4 Conclusions

Concentrations of nitrate, ammonium and nitrite nitrogen were evaluated during 2014 in the Bocegaj watercourse and Kolínany pond. In the experimental period, the average concentration of nitrate nitrogen was 1.96 mg.dm^{-3}. The maximum average values were found in the spring and the lowest in the winter, which was probably caused by the low temperature of the water in the pond and the subsequent inhibition of nitrification processes. Depending on the sample collection location, the maximum average concentration was found below the outlet of the wastewater treatment plant. The average concentration of ammonium nitrogen in the observed period was 0.99 mg.dm^{-3}. The lowest average concentrations were in winter and the lowest in summer. When evaluating the sample collection location, the highest average concentrations were at the mouth of the watercourse to the pond, which was probably related to the presence of a large number of hydrophytes. The average concentration of nitrite nitrogen represented 0.16 mg.dm^{-3}. The highest average concentrations were found in spring and summer and the lowest in winter and autumn. The highest average concentration recorded was on the surface of the pond, and the lowest was before the wastewater treatment plant. It can be concluded that the water in the Kolínany pond did not meet the recommended (N-NH_4^+, N-NO_2^-) and limit value (N-NH_4^+) specified in NV SR no. 269/2010 Coll. The main source of pollution was insufficiently purified water discharged from the Kolínany Wastewater Treatment Plant.

Acknowledgements

The research was carried out as part of a grant project VEGA 1/0604/20.

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