

DETERMINATION OF NITRATE NITROGEN IN SURFACE WATER: COMPARISON OF DISTILLATION-TITRATION AND SPECTROPHOTOMETRIC METHODS

^aJAKUB ELBL¹, ^bKATEŘINA ZÁKOUTSKÁ², ^cJAROSLAV ZÁHORA¹, ^dPETRA OPELTOVÁ², ^eANTONÍN KINTL¹, ^fLUKÁŠ PLOŠEK¹

¹Department of Agrochemistry, Soil Science, Microbiology and Plant Nutrition, Faculty of Agronomy, Mendel University in Brno, Zemědělská 1, 613 00 Brno 13, Czech Republic

²Department of Applied and Landscape Ecology, Faculty of Agronomy, Mendel University in Brno, Zemědělská 1, 613 00 Brno 13, Czech Republic

email: ^ajakub.elbl@mendelu.cz, ^bkaterina.zakoutska@mendelu.cz, ^cjaroslav.zahora@mendelu.cz, ^dpetra.opeltova@mendelu.cz, ^eantonin.kintl@mendelu.cz, ^flukas.plosek@mendelu.cz

This work was supported by the IGA – Internal Grant Agency Faculty of Agronomy MENDELU reg. No. IP 23/2013. And this work was supported by the program “Excellence of doctoral studies” registration no.: CZ.1.07/2.3.00/20.005 and National Agency for Agricultural Research (NAZV), project: *The possibilities for retention of reactive nitrogen from agriculture in the most vulnerable infiltration area of water resources*, registration no.: QJ 1220007.

Abstract: This work deals with comparison of two methods for determination of mineral nitrogen in surface water. The distillation-titration and spectrophotometric methods were compared. From January to September 2013, mineral nitrogen was determined by both methods in samples, which were taken from seven sites. These sites are located in CHKO Jizera Mountains. Water sampling was carried out every month (from April to August 2013). Based on the statistical analysis of results from each method, distillation-titration method is more accurate than spectrophotometric method.

Keywords: nitrogen, surface water, distillation-titration method, spectrophotometric method

1 Introduction

Nitrogen is a key element for all living organisms, because it is an essential component of proteins and nucleic acids. Aber et al (1989), Nohel et al. (2008) and Donatela et al. (2010) point out dangers of high input of reactive nitrogen (N_r).

Although the element nitrogen is extremely abundant making up 78% of the Earth's atmosphere, it exists mainly as unreactive form as di-nitrogen (N_2). In contrast, reactive nitrogen forms are needed to be useable by most plants and animals. These include oxidized and reduced nitrogen compounds, such as nitric acid, ammonia, nitrates, ammonium and organic nitrogen compounds. Each of it is normally scarce in the natural environment (Sutton, 2011). Reactive nitrogen is defined here as all other nitrogen forms in our system apart from N_2 . This includes oxidized nitrogen, mainly NO , NO_2 , NO_3 ; reduced forms of nitrogen: NH_4^+ , NH_3 and organic nitrogen: proteins, amines, etc., with different stages of oxidation (Erisman, 2011).

The major threat to the quality of surface water is mineral nitrogen (N_{min}). N_{min} is a reactive nitrogen consisting of ammonia (NH_4^+-N) and nitrate (NO_3^--N) nitrogen.

Nitrogen may occur in surface water by direct runoff or by infiltration through the root zone and discharge to surface water through seepage or tile drainage systems Zebarth et al. (1999).

Nitrogen is a fundamental component of organic and mineral agricultural fertilizers, natural substances and fossil fuels. Nitrogen is essential for the functioning of all living ecosystems. Anyway, if there is too much nitrogen in surface water, it is a great problem, because nitrogen can accelerate eutrophication. An increased concentration of mineral nitrogen in surface water presents negative impact on biodiversity and quality of such water (Sutton, 2011).

The most dangerous are nitrates, because they are very mobile in soil. They have a negative charge (NO_3^--N) and soil sorption complex has minimal affinity for negatively charged particles. Leaching of nitrate nitrogen from arable soil is a major threat to the quality of drinking water from underground reservoirs in the Czech Republic (Záhora & Mejzlík, 2007; Elbl et al., 2013).

Therefore, the adequacy of methods for NO_3^--N determination was investigated. Hypothesis, if those differences between distillation-titration method and spectrophotometric methods exist, was tested. Realized terrain experiment has been

investigated for the research of minerals forms in soil and water, which has already been carried out for a long time in the Department of Microbiology and in the Department of Landscape Ecology.

2 Materials and Methods

2.1 Experimental Sites

Experiment was carried out in CHKO Jizera Mountains. This area is on the north of Czech basin at the borders of Poland (see the Figure 1). Six experimental sites (Rynoltice – R, Smědá – SM and Jizerka - J) were selected from this area. Individual sites were always chosen above (R1, SM1, J1) and below (R2, SM2, J2) the municipalities:

Rynoltice - Panenský potok (Virgin Stream) springs 0,5 km to the east far from Jitřava in 650 m a.s.l., it empties into the Ploučnice River in Mimoň. Its watershed is 133,2 km² wide, the length of the flow is 28,8 km. Average flow in the river is 1,10 m³·s⁻¹. It's a significant water flow with trout water. The part of river up to Jablonné v P. is continuously regulated with a fringe planting of Canadian poplar. The rest of the river has comparatively natural flow with a preserved floodplain with rests of wet meadows.

Smědava is a name of area around chalet of the same name in Jizera Mountains in 847 m a.s.l. It is a place of confluence of rivers Bílá, Černá and Hnědá Smědá (White, Black and Brown Smědá) and therefore of emergence of the river Smědá. The chalet Smědava, with a larger part of parking lots around, belongs to the municipality Bílý Potok (White Stream). In north of the chalet, there is a group of houses, which belong to the Hejnice town.



Fig. 1 Area of our interest

The first mention of **Jizerka** settlement dates in 1539, when it used to be the site of fowlers. Later, lumberjacks and collectors of precious stones came. In the mid-19th century, two glassworks were established there. One of them is still working. The stream of the same name flows through the settlement as well as a stream Saphir, where deposits of precious stones were found (sapphire, ruby, chalcedony, topaz). About 10 inhabitants permanently live there. Jizerka is exceptional by its altitude, which is 850 – 900 m a.s.l. The combination of the altitude, relief of the valley and the river Jizerka supports the extreme weather in winter time. For example, the maximum snow depth (29. 4. 1944) was 315 cm. Instantaneous measured value -36 °C appeared there several time. Temperature under the freezing-point occurs even in the summer (Bercha, 2006, Zákoutská et al., 2013).

2.2 Design of the experiment

Nitrate nitrogen (NO_3^--N) was measured by spectrophotometric and distillation-titration methods in water samples taken from the area of our interest. The water sampling was performed once a month from April to August 2013. These values were used to determine the influence of the intensity of settlement on the

quality of surface water and to identify possible differences between the individual methods.

Accuracy of each method was determined using standard solutions. Solutions of known NO_3^- -N concentration were prepared. Concentration of NO_3^- -N was determined by both methods in individual solutions. The measured values were statistically evaluated and used to determine the accuracy of each method.

2.3 Spectrophotometric determination of the nitrate nitrogen concentration (in surface water)

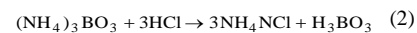
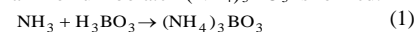
Spectrophotometric method was performed according to Hach-Lange Method 10071 – Persulfate Digestion Method for spectrophotometer DR/400. An alkaline persulfate digestion converts all forms of nitrogen to nitrate. In well aerated water, the most of the mineral nitrogen is in the form of nitrate (Tyson, 2011). Sodium metabisulfite is added after the digestion to eliminate halogen oxide interferences. Nitrate then reacts with chromotropic acid under strongly acidic conditions to form a yellow complex of absorbance maxima at 410 nm (Hach-Lange Methodology; Zákoutská et al., 2013).

2.4 Distillation-titration determination of the nitrate nitrogen concentration (in surface water)

Water samples were collected into the 120 ml plastic bottles and transported to the laboratory. Samples were stored at 4 °C before the determination. Nitrate nitrogen was determined together with ammonium nitrogen, because nitrate nitrogen is determined after ammonium nitrogen using distillation-titration method.

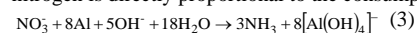
Concentrations of NO_3^- -N and NH_4^+ -N were measured using distillation-titration method by Peoples et al. (1989). Principle of this method is given in Eq. (1) – (3) and calculation is shown in Eq. (5).

NH_4^+ -N was determined by distillation-titration method in an alkaline solution after the addition of MgO. During heating of sample, NH_4^+ -N is released from the sample in the form of ammonia (NH_3). Subsequently during cooling of the sample, NH_3 is taken into boric acid (H_3BO_3 with indicator). Thus, ammonium borate - (NH_4)₃ BO_3 is formed.



Consumption of HCl during titration indicates the amount of ammonium nitrogen, which was displaced from sample.

Nitrate nitrogen was determined in the same manner using Devard's alloy (mixture of 50% Cu, 45% Al and 5% Zn). This alloy reduces NO_3^- -N to NH_3 (Eq. 3). Subsequently NH_3 is determined according to Eq. 1 and 2. The amount of nitrate nitrogen is directly proportional to the consumption of HCl.



Concentration of NO_3^- -N was calculated according Eq. (4):

$$\text{mg NH}_4^+ - \text{N or NO}_3^- - \text{N} = \left(\frac{\text{normality of standart HCl}}{0.03571} \right) \times 0.5 \times \text{titration} \quad (4)$$

This method of determination of nitrogen in water solutions, soil solutions and chemical extracts have already been published (Záhora & Mejzlík, 2007; Novosadová et al., 2011; Elbl et al., 2013a; 2013b; Plošek et al., 2013) and described by Peoples et al. (1989) and Weaver et al. (1994). Moreover, determination of nitrate and ammonium nitrogen after reduction by Devard's alloy is specified in ČSN EN 15476.

2.5 Statistical Analysis

The measured values of nitrates nitrogen from individual methods were analyzed by one-way analysis of variance (ANOVA; $P < 0.05$) in combination with post-hoc Tukey's HSD test ($P < 0.05$). All data were analyzed in Statistica CZ 10 software. Graphic processing of measured data was performed in Microsoft Excel 2010 and Statistica 10 software.

3 Results and Discussion

3.1 Validation of the measurement methodology for the determination of nitrate concentration in surface water – Comparison of Spectrophotometric and Distillation-titration methods

Comparison was made from the results obtained applying both methods to several standard samples covering a range of concentration (NO_3^- -N) from 0.25 mg/l to 7.5 mg/l. Samples of known concentration were prepared from the calibration solution (reg. no.: CZ 970761H5, primary substance: NH_4NO_3 99.999%, manufacturer: ANALYTIKA). Concentration of nitrate nitrogen was determined using the Spectrophotometric (SPM) and Distillation-titration methods (DTM). Three replicates of each sample were analyzed by these methods. The mean of the differences between the real values (standards) and the measured values (by individual methods) are shown in the Table 1.

Table 1 Comparison of SPM and DTM accuracy

Standard solution (mg/l)	Methods	Differences (%)	P
S1	SPM	-5.93	0.026148
3.75	DTM	2.07	0.019373
S2	SPM	6.72	0.016065
3.125	DTM	6.39	0.014396
S3	SPM	-8.70	0.000832
2.5	DTM	2.96	0.104029
S4	SPM	-5.49	0.001967
1.25	DTM	2.01	0.079200
S5	SPM	6.72	0.121690
0.625	DTM	5.06	0.033756
S6	SPM	-8.70	0.074180
0.25	DTM	5.63	0.059712
S7	SPM	-6.25	0.129612
0.125	DTM	4.05	0.319096

Comment for the Table 1: Weighted average (\bar{x}) of differences between standard solution (ST) and individual methods (SPM or DTM) are presented there. Individual differences were analyzed using T-test. The means of differences are significant at the level 0.05 ($P < 0.05$). These differences are shown in **bold numbers** (red for SPM and black for DTM).

The Table 1 shows significant differences between concentration of standard solution and concentration that were determined by individual method. The largest differences were found when using SPM. These results indicate that DTM is more accurate than SPM. If we compare the total average efficiency, the difference will be minimal. The average efficiency of each method was: SPM = 93.07 % and DTM = 95.97 %, these results are not significant.

Beda & Nedospasov (2005) state, that the accuracy of the spectrophotometric methods may be affected by increasing concentrations of the analyte. The above information shows that optical methods have a limited range of applicability depending on the concentration of nitrate. Consider the Table 1, data presented here show significant differences were found in variant with highest concentrations of nitrogen (1.25 mg/l – 3.75 mg/l of NO_3^- -N). The second factor that could affect accuracy of the measurement is the quality of the reagents and the optical purity of the cuvettes. Sensitivity of optical methods, for instance, is confirmed by Moorcroft et al. (2001).

3.2 Concentration of nitrate nitrogen in surface waters

Nitrate nitrogen is an important indicator of pollution of surface waters. Amount of nitrate nitrogen in samples taken from April to August 2013 were determined by both methods. Data are shown in the Table 2.

The Table 2 presents data of cumulative concentration of NO_3^- -N for five months (from April to August 2013) in surface water at individual locations. These data indicate differences between SPM and DTM in determination of NO_3^- -N at variants R1, R2, anyway, significant difference was found only at variant R2. The highest concentrations of pollutants (NO_3^- -N) were measured at

variant R1 and R2, thus at the locations with the most developed settlement. Negative effects of human settlement on water quality are confirmed by (Coote & Gregorich, 2000 and Sutton, 2011).

Table 2 Concentrations of nitrate nitrogen determined by SPM and DTM in surface water at individual locations

Experimental site	SPM		DTM	
	NO ₃ ⁻ -N (mg/l)	±SE	NO ₃ ⁻ -N (mg/l)	±SE
R1	19.53	10.01	1.83	0.40
R2	13.48	4.58	4.03	0.50
SM1	2.10	1.28	0.39	0.09
SM2	2.61	0.94	0.79	0.29
J1	3.82	1.23	0.78	0.56
J2	3.29	1.42	0.29	0.02

Comment for the Table 2: Weighted average (\bar{x}) of nitrate nitrogen with SE (standard error) are presented there. These parameters were calculated from five measurement (n = 5) by Statistica 10 software for each experimental site.

The above Table 2 shows great differences between individual experimental sites (this fact is real for the results obtained from the SPM and DTM). Reason of this: water quality differs depending on the season and on geographic area. The background chemistry of river and lake water is determined by soil, geologic formations, terrain, and vegetation in the drainage basin and also by human activities such as agriculture, industry, transport etc. (Coote & Gregorich, 2000). Experimental sites SM1, SM2, J1 and J2 are located in the protection zone of CHKO Jizera Mountains, but another sites R1 and R2 are situated outside this protection zone. Moreover, experimental sites R1 and R2 are the most populated. And therefore, there is the increased leakage of pollutants into the watercourse.

Differences between individual variants of the experiment were not analyzed, only differences between methods of determination concentration of NO₃⁻-N at individual variants were analyzed by ANOVA with post-hoc Tukey's HSD test (P<0.05). This analysis is divided in two parts and presented in the following Tables 3 and 4.

The values of NO₃⁻-N concentration determined at individual sites are presented in the Table 2. These data indicate large differences between SPM and DTM, although these differences are significant only at variant R2 (consider Table 4). Large differences between individual methods in variants R1, R2 could be caused by improper water sampling. Silva et al. (2000) point to the fact that processing and sampling has crucial influence on the precision of the determination of nitrogen compounds in the water.

Table 3 Analysis of variance for individual experimental sites

Experimental site	Methods	95% Confidence interval		F	P
		Lower bound	Upper bound		
R1	SPM	-8.27	47.33	3.118	0.115
	DTM	0.71	2.95		
R2	SPM	-0.04	6.45	5.619	0.045
	DTM	0.02	0.80		
SM1	SPM	-0.33	1.52	0.722	0.420
	DTM	-0.05	0.64		
SM2	SPM	0.32	2.57	0.833	0.388
	DTM	-0.60	2.29		
J1	SPM	-32.07	71.21	0.0005	0.982
	DTM	-33.07	71.02		
J2	SPM	-0.29	1.98	2.279	0.169
	DTM	-0.31	0.66		

There is a presumption that samples of water, which were collected for SPM, were of a different composition. This situation was due to character of watercourse. Samples were collected at the point where the flow was directly affected by sewage water. Sampling was performed for each method

separately and because sampling was complicated by approach to watercourse, time intervals were created between individual sampling (about 10 minutes). During this time, waste water from the surrounding buildings contaminated water flow and thus subsequently collected samples.

Table 4 Detailed comparison of SPM and DTM by Tukey's HSD test

Number of cell	Experimental site	Methods	Mean difference	
			(1)	(2)
1	R1	SPM	-	0.1155
		DTM	0.1155	-
2	R2	SPM	-	0.0453
		DTM	0.0453	-
1	SM1	SPM	-	0.4203
		DTM	0.4203	-
1	SM2	SPM	-	0.3820
		DTM	0.3820	-
1	J1	SPM	-	0.9825
		DTM	0.9825	-
1	J2	SPM	-	0.1697
		DTM	0.1697	-

Comment for the Table 4: The means of differences are significant at the level 0.05 (P<0.05). These differences are shown **in bold**. Methods (SPM and DTM) were always compared for one experimental site.

The used methods differ one from other by principle and method of determination. Therefore, both methods have different specifics which are the reason for their advantages and disadvantages for determination of nitrate nitrogen in surface water. The main advantages of SPM are low cost, compactness and speed of execution. Conversely, the main disadvantages are maintenance of the equipment, necessity of using original reagents and distortion of the measurement by contamination with solid material (lightproof material).

DTM is suitable for the determination of mineral nitrogen in the soil solution, extracts (KCl, NaCl), samples of water and solution from organic matter (for example compost). DTM is very fast, accurate and affordable analytical method, but for its successful implementation, quality analytical chemicals are necessary. Moreover, the analysis (DTM) must be performed by trained person in order to minimize inaccuracies caused by incorrect settings of device or inaccurate dosing of chemicals (Peoples et al., 1989 and Weaver et al., 1994).

Conclusions

This work presents results of DTM and SPM comparison. These results confirm our hypothesis. It is obvious that results from sampling and determining of water quality indicators set by spectrophotometric method have the same predictive value as distillation-titration one.

Literature

- COOTE, D. & L. GREGORICH. *The health of our water: toward sustainable agriculture in Canada*. Ottawa: Research Branch, Agriculture and Agri-Food, Canada, 2000, 173 p. ISBN 06-622-8489-5.
- ELBL, J., L. PLOŠEK, A. KINTL, J. ZÁHORA, J. PŘICHYŠTALOVÁ & J. HYNŠT. Effect of organic-waste compost addition on leaching of mineral nitrogen from arable land and plant production. *World Academy of Science, Engineering and Technology*. 2013a, no. 78, pp. 2858-2863. ISSN 2010-376X.
- ELBL, J., L. PLOŠEK, J. ZÁHORA, A. KINTL & M. STROBLOVÁ. Effect of increased doses of compost to

- prepare reclamation substrate on soil respiration and content of mineral nitrogen in the soil. *Ad Alta*. 2013b, vol. 3, no. 2, pp. 88-91. ISSN 1804-7890.
4. BEDA, N. & A. NEDOSPASOV. A spectrophotometric assay for nitrate in an excess of nitrite. *Nitric Oxide: Biology and Chemistry*. 2005, vol. 13, no. 2. ISSN: 1089-8603.
 5. BERCHA, Š. Experimentální hydrologická základna Jizerské hory. *Journal of Hydrology and Hydromechanics*, 2006, vol. 54, no. 2, pp. 163-182. ISSN 0042-790X.
 6. MOORCROFT, M. J., J. DAVIS & R. G. COMPTON. Detection and determination of nitrate and nitrite: a review. *Talanta*. 2001, vol. 54, no. 5, pp. 785-803. ISSN: 0039-9140.
 7. NOVOSADOVÁ, I., J. ZÁHORA & J. D. R. SINOGA. The availability of mineral nitrogen in Mediterranean open steppe dominated by *Stipa tenacissima* L. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis*. 2011, vol. 59, no. 5, pp. 187-192. ISSN 1211-8516.
 8. PEOPLES, M. B., A. W. FAIZAH, B. RERKASEM & D. F. HERRIDGE. *Methods for evaluating nitrogen fixation by modulated legumes in the field*. Canberra: Australian Centre for International Agricultural Research, 1989, 81 p. ISBN 09-495-1190-0.
 9. PLOŠEK, L., F. NSANGANWIMANA, B. POURRUT, J. ELBL, J. HYNŠT, A. KINTL, D. KUBNÁ & J. ZÁHORA. The Effect of Compost Addition on Chemical and Nitrogen Characteristics, Respiration Activity and Biomass Production in Prepared Reclamation Substrates. *World Academy of Science, Engineering and Technology*. 2013, vol. 7, no. 11, pp. 363-369. ISSN 2010-376X.
 10. SILVIA S. R., C. KENDALL, D. H. WILKISON, A. C. YIEGLER, C. C. Y. CHANG & R. J. AVANZIO. A new method for collection of nitrate from fresh water and the analysis of nitrogen and oxygen isotope ratios. *Journal of Hydrology*. 2000, vol. 228, no. 1-2, pp. 22-36. ISSN: 0022-1694.
 11. SUTTON, M. A. *The European nitrogen assessment: sources, effects, and policy perspectives*. New York: Cambridge University Press, 2011, 612 p. ISBN 978-80-89128-96-9.
 12. WEAVER, R. W., S. ANGLES, P. BOTTOMLEY, D. BEZDICEK, S. SMITH, A. TABATABAI & A. WOLLUM, Eds. *Methods of Soil Analysis, Part 2: Microbiological and Biochemical Properties*. Madison: SSSA Book series, 1994, 1097 p. ISBN: 978-0-89118-865-0.
 13. ZÁHORA, J. & L. MEJZLÍK. The leaching of mineral nitrogen into underground water from soil environment of different ecosystems. In: *Ekológia Travného Porastu VII, Banská Bystrica, Slovakia, 28-30 November 2007*. Banská Bystrica, 2007, pp. 170-174. ISBN 978-8088872-69-6.
 14. ZÁKOUTSKÁ, K., F. TOMAN, P. OPELTOVÁ, J. ELBL. Wastewater Treatment and Its Influence on Surface Water in Upper Parts of Jizera Mountains. In *Agrosym Jahorina 2013*. Banja Luka: Národní a univerzitní knihovna Republika Srbská, Banja Luka, 2013, pp. 904-911. ISBN 978-99955-751-3-7.
 15. ZEBARTH, B. J., J. W. PAUL & R. V. KLEECK. The effect of nitrogen management in agricultural production on water and air quality: evaluation on a regional scale. *Agriculture, Ecosystems and Environment*. 1999 vol. 72, no. 1, pp. 35-52.

Primary Paper Section: D, G

Secondary Paper Section: EH, DA