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COMPARISON OF TWO SPECTROPHOTOMETRIC TECHNIQUES FOR NUTRIENTS ANALYSES IN WATER SAMPLES

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Abstract

The aim of this contribution is to compare two common techniques for determining the concentrations of nitrate, nitrite, ammonium and phosphates in surface water and groundwater. Excess of these nutrients in water can directly affect human health (e.g. methemoglobinaemia) or indirectly through the products of secondary pollution – eutrophication (e.g. cyanotoxins, emanation of hydrogen sulphide, mercaptanes, methane...). Negative impact of nutrients excess in surface water often causes the destruction of water ecosystems, and therefore, common substances of these elements must be monitored and managed. For these experiments two spectrophotometric techniques - ultraviolet spectrophotometry and nutrient photometry were used. These techniques are commonly used for quick and simple analyses of nutrients in waste water. There are calibration curves for each nutrient and for determination of their concentration.

Key words

Monitoring of nutrients, surface water, spectrophotometric methods

Introduction

The term "nutrients" refers broadly to those chemical elements essential for life on earth, but more specifically to nitrogen (N) and phosphorus (P) in a water pollution context. Nitrogen is the most abundant element in air, but occurs in a form (N_2) unusable for most life forms. Since nitrogen is an abundant component of biological tissue, any organic matter in water will contain nitrogen. However, until these larger organic molecules are decomposed by bacteria, they are of little or no use for algae or other aquatic plants. Nitrogen is readily utilized by aquatic plants (such as algae) if it is dissolved in the water in an inorganic form:

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chemicals that are combinations of nitrogen and oxygen (nitrates NO_3^- and nitrites NO_2^-) or nitrogen and hydrogen (ammonium cations NH_4^+). It has the potential to adversely affect the health of infants and livestock. Surface water quality is the concern with phosphorus, as runoff and erosion from cropland add nutrients to water bodies that stimulate the excessive growth of aquatic weeds and algae. Of all crop nutrients, it is critical to prevent P from reaching lakes and streams since the biological productivity of aquatic plants and algae in fresh water environments is usually limited by this nutrient. The element phosphorus can occur in nature in many forms, but the most abundant dissolved inorganic form in aquatic environments is as orthophosphate (PO_4^{3-}). Consequences of the increased aquatic plant and algae growth (eutrophication) include reduced aesthetic and recreational value of lakes and streams as well as the seasonal depletion of the water dissolved oxygen content, which may result in fish kills as well as other ecosystem disruptions (1).

Nutrients need to be managed properly to meet the fertility requirements of crops without adversely affecting the quality of water resources. Methods for measuring the P and N forms in natural waters differ largely (Table 1), but the simplest is a chemometric method, which can colour the sample in dependence of nutrient concentration. The intensity of colour can be measured by comparison with standard scale (e.g. striped tests) or using the terrain or laboratory spectrophotometer.

POSSIBILITIES OF METHODS FOR NUTRIENTS DETERMINATION IN WATER SAMPLES (1, 5) Table 1

Analyte	**Methodology**	**Method**
Nitrogen ions	Ion chromatography	Standard method: 4110 Determination of Anions by Ion Chromatography
	UV Spectrometry method	STN ISO 7890-3 (75 7455): Water quality. Determination of nitrate. Part 3: Spectrometric method using sulfosalicylic acid
	UV Spectrometry method	STN EN 26777 (75 7438): Water quality. Determination of nitrite. Molecular absorption spectrometric method
	Titration	STN EN 25663: Water quality. Determination of Kjeldahl nitrogen. Method after mineralization with selenium.
	Colorimetric	EPA 353.1: Nitrate-Nitrite by Colorimetry. Official Name: Nitrogen, Nitrate-Nitrite (Colorimetric, Automated Hydrazine Reduction)
		EPA 353.2: Nitrate-Nitrite Nitrogen by Colorimetry. Official Name: Nitrogen, Nitrate-Nitrite (Colorimetric, Automated, Cadmium Reduction)
Phosphate ions	Colorimetric	EPA 365.1: Phosphorus (all forms) by Semi-Automated Colorimetry. Official Name: Phosphorus, All Forms (Colorimetric, Automated, Ascorbic Acid)
	Ion chromatography	Standard method: 4110 Determination of Anions by Ion Chromatography
	UV Spectrometry method	STN EN ISO 6878 (75 7465): Water quality - Determination of phosphorus - Ammonium molybdate spectrometric method (ISO 6878:2004)
	Gas chromatography-mass spectrometry	EPA Method 507: Determination of Nitrogen- and Phosphorus-Containing Pesticides in Water by Gas Chromatography with a Nitrogen-Phosphorus Detector - Revision 2.1.
	Spectrofluorimetric method	USGS Test Method I-2464-01Organic plus Inorganic Mercury in Filtered Natural Water by Cold-Vapor AFS. Official Name: Methods of Analysis by the U.S. Geological Survey National

		Water Quality Laboratory - Determination of Organic Plus Inorganic Mercury in Filtered and Unfiltered Natural-Water with CV-AFS

Materials and methods

All of the experiments were performed according to the STN standards with solutions prepared from p.a. chemicals, using two spectrophotometers:

- Spectrophotometer TermoSpectronic^{TM}
- HANNAHI 83215 multiparameter bench photometer for Nutrient analyses.

A detailed description of the methods used is reported in the following session.

Fig. 1 *Spectrophotometer TermoSpectronic GENESIS^{TM} and HANNA HI 83215 Spectrophotometer*

Nitrates - The analysis is based on the reaction of nitrate with sodium salicylate in a sulphuric acid medium, which formed yellow coloured salts of nitrosalicylic acid. Interference may be caused by ammonia and amines (as urea and primary aliphatic amines), chloride above 100 mg/L, chlorine above 2 mg/L, copper, iron (III), strong oxidizing and reducing substances. Sulphide must be absent (3, 4).

<u>Technique 1: Spectrophotometric determination of nitrate by sodium salicylate (0.1–20 mg/L) according to the STN ISO 7890-3 (75 7455) standard</u>

- Apparatus: Spectrophotometer TermoSpectronic GENESIS^{TM}, cuvette (5 cm), evaporating dish, boiler
- Reagents: *sodium salicylate* (0.5% water solutions, prepared freshly), sulphuric acid (conc. 96%, CentralChem), *sodium hydroxide* (c(NaOH) = 10 mol/L: 400 g NaOH is dissolved in distilled water in 1000 mL volumetric flask), *potassium nitrate* (c(NO_3^-) = 100mg/L – stock solution: 0.1631 g KNO_3 is dried in temperature 105°C and dissolved in 1000 mL distilled water in volumetric flask).
- Procedure:
  1. 10 mL water samples (solutions in concentration range) with 1 mL sodium salicylate are evaporated in an evaporating dish,
  2. after cooling down, add 1 mL of concentrated H_2SO_4, so that way the entire residue dehumidified and allowed to stand for 10 minutes,

3. quantitatively transfer it to a 50 ml volumetric flask,
4. add 7 mL NaOH and, after cooling to room temperature, adjust the volume to 50 mL with distilled water,
5. after 10 minutes, the stain remains and the absorbance is measured at 410 nm against a blank prepared in the same way (2, 3).

- Data to construct the calibration curves, using a standard solution of KNO_3 100 mg/L are shown in Table 2.

DATA NECESSARY TO CONSTRUCT THE CALIBRATION CURVES Table 2

$c(NO_3^-)$[mg/L]	**2**	**4**	**6**	**8**	**10**	**15**	**20**
V_1[mL]	1.0	2.0	3.0	4.0	5.0	7.5	10.0
V[mL]	50	50	50	50	50	50	50

Technique 2: Determination of nitrate using Nutrient Analysis Photometer

- Apparatus: Spectrophotometer HANNA HI 83215 Spectrophotometer, cuvette
- Reagents: *HI 93728* (cadmium powder <5%, sulfanilic acid <5%)
- Procedure:
  1. Select the "Nitrate LR" method (LR means Low range: 0 – 30.0 mg/L, e.g. irrigation water), "Nitrate MR" method (MR means Medium range: 0 – 150 mg/L, e.g. nutrient solutions, sample must be prepared by mixing 20 mL of testing solution and 80 ml of demineralized water) or "Nitrate HR" method (HR means High range: 0 – 300 mg/L, e.g. nutrient solutions, sample must be prepared by mixing 10 mL of testing solution and 90 ml of demineralized water).
  2. Fill the cuvette with 6 ml of sample up to half of its height, and replace the cap.
  3. Place the cuvette into the holder and close the lid. Press the Zero key. The display shows "-0.0-" when the meter is zeroed and ready for measurement.
  4. Remove the cuvette and add the content of one pocket of HI 93728-0 reagent. Replace the cap and immediately shake vigorously up and down for exactly 10 seconds. Continue to mix by inverting the cuvette gently for 50 seconds, while avoiding to induce air bubbles. Powder will not completely dissolve. The time and way of shaking could sensitively affect the measurement.
  5. Reinsert the cuvette into the instrument, without shaking it. Press Timer and the display will show the countdown prior to the measurement or, alternatively, wait for 4 minutes and 30 seconds and press Read. When the timer ends, the meter will perform the reading. The instrument displays the results in mg/L of nitrate-nitrogen (4).

Nitrites - The analysis is based on the reaction of nitrites present in the sample with sulfanilicacid and α-naphtylamine to form a red-violet colour. The course of the reaction depends on pH (3, 4).

Technique 1: Spectrophotometric determination of nitrite ions by sulphanilic acid andα-naphylamine according to standard STN EN 26777 (75 7438)

- Apparatus: TermoSpectronic GENESIS^{TM}, cuvette (5 cm)
- Reagents: *sulphanilic acid* (0.6 % solution: 6 g of sulphanilic acid is dissoluble in750 mL of hot distilled water and add 250 mL of acetic acid), α – *naphtylamine* (0.6 % solution:

0.6 g of α - naphtylamine is dissolubled in distilled water while heating and stirring, add 25 mL of acetic acid and the solution is made up to 100 mL. Solution is sensitive to light and is stable for 2-3 months.), *sodium nitrite* (c (NO_2^-) = 100 mg/L – stock solution: 0.1497 g of $NaNO_2$ is dried in temperature 105°C and dissolved in 1000 mL; dilutions c (NO_2^-) = 10 mg/L: 10 mL of stock solution is added to a 100 mL volumetric flask with distilled water to final volume).

- Procedure:
  1. to 50 ml of the sample of water (or solution in concentration range supplemented distilled water 50 ml), 1 ml of sulfanilic acid was added and mixed,
  2. after 5 minutes, add 1 ml of α - naphtylamine and mixed,
  3. after 40 minutes the absorbance at 520 nm is measured against a blank prepared in the same way (2, 3).
- Data to construct the calibration curves, using a standard solution of $NaNO_2$ with concentration 10 mg/L are shown in Table 3.

DATA NECESSARY TO CONSTRUCT THE CALIBRATION CURVES Table 3

c(NO_2^-)[mg/L]	**0.1**	**0.15**	**0.2**	**0.25**	**0.4**	**0.45**	**0.5**	**0.6**
V_1[mL]	1.0	1.5	2.0	2.5	4.0	4.5	5.0	6.0
V[mL]	50	50	50	50	50	50	50	50

Ammonium cations - The analysis is based on the reaction of ammonium ions with Nessler reagent, which form a yellow-brown compound. Interference may be caused by: acetone, alcohols, aldehydes, glycine, and hardness above 1 g/L, iron, organic chloramines, sulphide, and various aliphatic and aromatic amines.

Technique 1: Spectrophotometric determination of ammonium cations by Nessler reagent according to the STN ISO 7150-1 (75 7451) standard

- Apparatus: Spectrophotometer TermoSpectronic GENESIS^{TM} Spectrophotometer, cuvette (5 cm)
- Reagents: *Nessler reagent* (mercuryiodide 3%, potassiumiodide 3.5%, sodium hydroxide 12% and water 81.5%), *potassium sodium tartrate* (also called Seignett salt – $C_4H_4KNaO_6.4H_2O$, 50% solution), *ammonium chloride* (c (NH_4^+) = 100 mg/L – stock solution: 0.2965 g of NH_4Cl is dried in temperature 105°C and dissolved in1000 mL; dilutions c (NH_4^+) = 5 mg/L: 5 mL of stock solution is added to a 100 mL volumetric flask with distilled water to final volume).
- Procedure:
  1. to 50 ml of the sample of water (or solution in concentration range supplemented distilled water 50 ml), 1 ml of Seignett salt was added and mixed,
  2. after few minutes, add 1 ml of Nessler reagent and mix,
  3. after 10 minutes, the absorbance at 425 nm is measured against a blank prepared in the same way (2, 3).
- Data to construct the calibration curves, using a standard solution of NH_4Cl with concentration 5 mg/L are shown in Table 4.

DATA NECESSARY TO CONSTRUCT THE CALIBRATION CURVES Table 4

$c(NH_4^+)$[mg/L]	**0.05**	**0.10**	**0.20**	**0.40**	**0.60**	**0.80**	**1.00**	**1.50**	**2.00**	**2.50**	**3.00**	**4.00**
V_1[mL]	0.5	1.0	2.0	4.0	6.0	8.0	10.0	15.0	20.0	25.0	30.0	40.0
V[mL]	50	50	50	50	50	50	50	50	50	50	50	50

Technique 2: Determination of ammonia using Nutrient Analysis Photometer

- Apparatus: Spectrophotometer HANNA HI 83215, cuvette
- Reagents: *HI 93715A-0* (Nessler reagent: mercury iodide <10%, potassium iodide <10%, sodium hydroxide <20%), *HI 93715B-0* (potassium sodium tartrate <50%)
- Procedure:
  1. Select the "Ammonia LR" method (LR means Low range: 0 – 10.0 mg/L, e.g. irrigation water), "Ammonia MR" method (MR means Medium range: 0 – 50 mg/L, e.g. nutrient solutions, sample must be prepared by mixing 20 mL of testing solution and 80 ml of demineralized water) or "Ammonia HR" method (HR means High range: 0 – 100 mg/L, e.g. nutrient solutions, sample must be prepared by mixing 10 mL of testing solution and 90 mL of demineralized water).
  2. Fill the cuvette with 10 ml of sample up to mark, and replace the cap.
  3. Place the cuvette into the holder and close the lid. Press the Zero key. The display shows"-0.0-" when the meter is zeroed and ready for measurement.
  4. Remove the cuvette. Add 4 drops of HI 93715A-0 First Reagent. Replace the cap and mix the solution by inverting the cuvette a couple of times. Add 4 drops of HI 93715B-0 Second Reagent. Replace the cap and mix the solution by inverting the cuvette a couple of times.
  5. Reinsert the cuvette into the instrument. Press Timer and the display will show the countdown prior to the measurement or, alternatively, wait for 3 minutes and 30 seconds and press Read. When the timer ends, the meter will perform the reading. The instrument displays the results in mg/L of ammonia nitrogen (4).

Phosphates - The analysis is based on the reaction of ammonium molybdate with orthophosphate ions to phosphomolybdic acid. This is reduced to molybdenum blue. Interference may be caused by: sulphide, chloride above 150 g/L, calcium above 10 g/L as $CaCO_3$, magnesium above 40 g/L as $MgCO_3$, ferrous iron above 100 mg/L (3, 4).

Technique 1: Spectrophotometric determination of phosphate ion PO_4^{3-} by Molybdenum blue method according to STN EN ISO 6878 (75 7465)

- Apparatus: Spectrophotometer TermoSpectronic GENESIS^{TM}, cuvette (5 cm)
- Reagents: *sulphuric acid* (c (H_2SO_4) = 2.5 mol/L: 140 mL of H_2SO_4 concentrate is dissoluble in 2000 mL flask bank in 800mL of distilled water. Prepare solution on ice to chill, adjust the volume to 2000 mL with distilled water), *ammonium molybdate* (15 g of ammonium molybdate $(NH_4)_6Mo_7O_{24}.4H_2O$ is dissoluble in 500 mL volume flask in distilled water), *potassium antimonyltartrate* (0.68 g of potassium antimonyl tartrate hemihydrate $K(SbO)C_4H_4O_6.1/2H_2O$ is dissoluble in distilled water in500 mL volumetric flask), *ascorbic acid* (2.16 g of ascorbic acid is dissoluble in distilled water in 100 mL volumetric flask), *mixed agent* (mixed agent is prepared only in the required amount before use. It is a mixture of 125 mL of H_2SO_4, 50 mL of ammonium molybdate, 25 mL of potassium antimonyl tartrate hemihydrate and 50 mL of ascorbic acid. Reagent is stable for about 4 hours.), *potassium dihydrogenphosphate* (c(PO_4^{3-}) = 500 mg/L – stock

solutions: 0.7165 g of KH_2PO_4 - dried for two hours to 105°C, is dissoluble in distilled water in 1000 mL volumetric flask).

- Procedure:
  1. to 50 ml of the sample of water (or solution in concentration range supplemented distilled water to 50 ml) was added to 100 mL Erlenmeyer flask,
  2. add 5 ml of mixed agent and mix,
  3. absorbance measured at 690 nm against a blank prepared in the same way (2, 3).
- Data to construct the calibration curves, using a standard solution of KH_2PO_4 with concentration 500 mg/L are shown in Table 5.

DATA NECESSARY TO CONSTRUCT THE CALIBRATION CURVES Table 5

$c(PO_3^{4-})$[mg/L]	**0.01**	**0.02**	**0.40**	**0.10**	**0.20**	**0.40**	**0.80**
V_1[mL]	0.5	1.0	2.0	5.0	10.0	20.0	40.0
V[mL]	50	50	50	50	50	50	50

Technique 2: Determination of phosphorus using Nutrient Analysis Photometer

- Apparatus: Spectrophotometer HANNA HI 83215, cuvette
- Reagents: *HI 93706A-0* (sulphuric acid < 75%, ammonium molybdenate < 7%), *HI 93706B-0* (dimethylformamide < 40%, di-sodium disulphide < 10%)
- Procedure:
  1. Select the "Phosphorus LR" method (LR means Low range: 0 – 10.0 mg/L, e.g. irrigation water), " Phosphorus MR" method (MR means Medium range: 0 – 50.0 mg/L, e.g. nutrient solutions, sample must be prepared by mixing 20 mL of testing solution and 80 ml of demineralized water) or " Phosphorus HR" method (HR means High range: 0 – 100 mg/L, e.g. nutrient solutions, sample must be prepared by mixing 10 mL of testing solution and 90 ml of demineralized water).
  2. Fill the cuvette with 10 ml of sample up to mark, and replace the cap.
  3. Place the cuvette into the holder and close the lid. Press the Zero key. The display show "-0.0-" when the meter is zeroed and ready for measurement.
  4. Remove the cuvette. Add 10 drops of HI 93706A-0. Add the content of one packet of HI 93706B-0 Phosphorus Reagent B to the cuvette. Replace the cap and shake gently until completely dissolved.
  5. Reinsert the cuvette into the instrument. Press Timer and the display will show the countdown prior to the measurement or, alternatively, wait for 5 minutes and press Read. When the timer ends, the meter will perform the reading. The instrument displays the results in mg/L of phosphorus (P) (4).

Results and discussion

Nitrates (NO_3^-) are often reported in all types of water. They are a final biochemical oxidation product of organically bound and nitrogen may be the evidence of contamination of organic origin by their greater concentration in natural waters. The surface water is related to the degree of nitrate eutrophication of these waters. Nitrates are therefore an important indicator of a fundamental analysis of surface water, both for quality control as well as in important assays NO_3^- (5). For spectrophotometric determination of nitrate by sodium salicylate according to standard STN ISO 7890-3 (75 7455), a calibration curve relating

absorbance to concentration of nitrate nitrogen and a calibration curve of absorbance to nitrate concentrations were plotted (Fig. 2). The obtained equations are:

$$N\text{-}NO_3^-: \quad A = 0.6885 \times c \quad R^2 = 0.9930$$
$$NO_3^-: \quad A = 0.1555 \times c \quad R^2 = 0.9930$$

where *A* is absorbance and *c* is concentration (mg/L). The measured values of absorbance of NO_3^-concentration are available in Table 6.

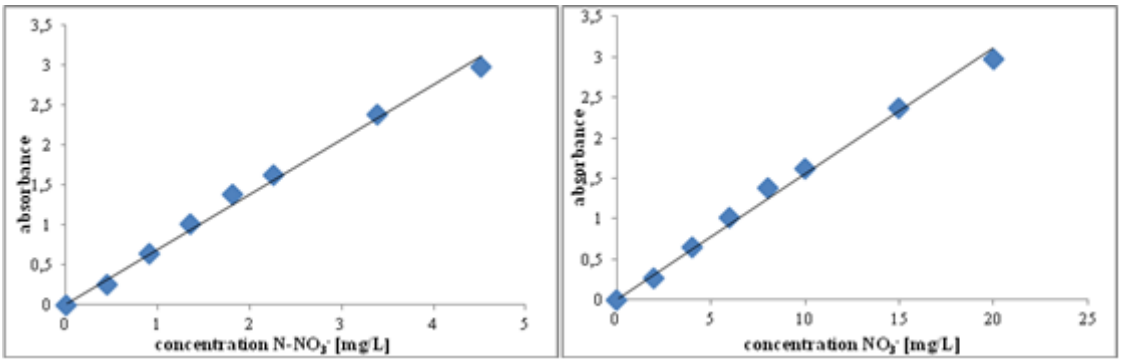

Fig. 2 *The calibration curve of relating absorbance to concentration of N-$NO_3^-$and NO_3^-*

THE MEASURED VALUES RELATING ABSORBANCE OF NO_3^- Table 6

c(N-NO_3^-)[mg/L]	c(NO_3^-)[mg/L]	A_1	A_2	A_3	$A_{average}$
0	0	0	0	0	0
0.452	2.000	0.259	0.259	0.262	0.260
0.904	4.000	0.642	0.649	0.639	0.643
1.356	6.000	1.020	0.957	1.057	1.011
1.807	8.000	1.400	1.342	1.403	1.381
2.259	10.000	1.609	1.633	1.610	1.617
3.389	15.000	2.379	2.392	2.340	2.370
4.518	20.000	2.974	2.963	2.989	2.975

The measurement of nitrates using HI 83215 instrument (Technique 2) is much easier, particularly suitable for the expected higher concentrations (e.g. in eutrophic surface water), which, in using traditional technique 1, would have to be strongly diluted. Technique 2 eliminates time–consuming evaporation of the sample with a mixture of sodium salicylate. On the other hand, classical Technique 1 is more suitable for the accurate determination of the expected lower nitrate concentration (e.g. in groundwater).

Nitrites (NO_2^-) can be found in surface water and in groundwater. Concentrations of nitrite ions are normally low, but high concentration may indicate the presence of pathogenic bacteria. They are therefore used as an indicator that water is not safe to drink (5). For spectrophotometric determination of nitrite ions by sulphanilic acid and α-naphtylamine according to the STN EN 26777 (75 7438) standard, a calibration curve relating absorbance to concentration of nitrate nitrogen and a calibration curve of absorbance to nitrate concentrations were plotted (Fig. 3). The obtained equations are:

$N\text{-}NO_2^-$: $A = 21.6860 \times c$ $R^2 = 0.9766$
NO_2^-: $A = 6.6058 \times c$ $R^2 = 0.9766$

where *A* is absorbance and *c* is concentration (mg/L). The measured values relating absorbance to NO_2^- concentration are available in Table 7.

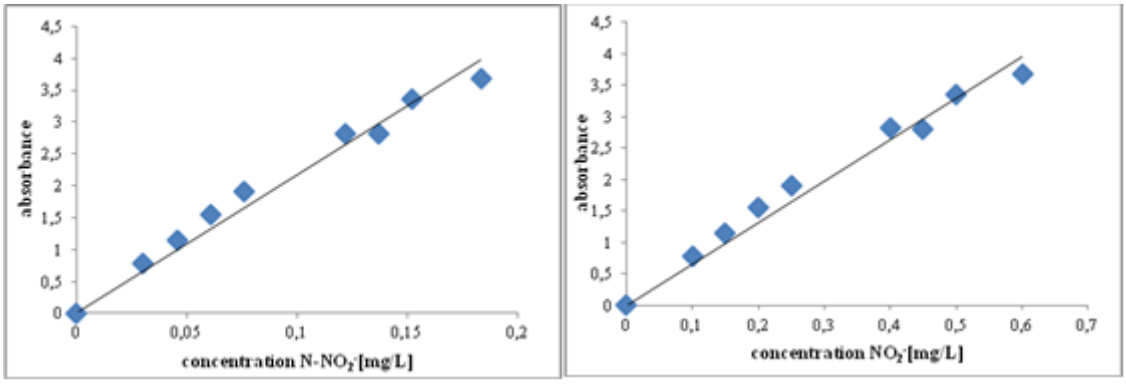

Fig. 3 *The calibration curve relating absorbance to concentration of* $N\text{-}NO_2^-$ *and* NO_2^-

THE MEASURED VALUES RELATING ABSORBANCE NO_2^- Table 7

$c(N\text{-}NO_2^-)$[mg/L]	$c(NO_2^-)$[mg/L]	A_1	A_2	A_3	$A_{average}$
0	0	0	0	0	0
0.030	0.100	0.749	0.789	0.761	0.781
0.046	0.150	1.223	1.081	1.122	1.142
0.061	0.200	1.726	1.473	1.455	1.551
0.076	0.250	1.980	1.905	1.820	1.901
0.122	0.400	2.733	2.873	2.850	2.818
0.137	0.450	2.408	3.011	3.013	2.810
0.152	0.500	3.328	3.342	3.388	3.352
0.183	0.600	3.595	3.621	3.851	3.689

Typical concentration of nitrite is very low, often under the detection limit. Classical Technique 1 is relatively simple and quick. Disadvantage of this method is α-naphtylamine photosensitivity. The HI 83215 instrument does not provide analyses of this parameter.

Ammonium cations ***(NH_4^+)*** occurring in groundwater as a product of microbial activity. NH_4^+ ions are an important indicator of fresh contamination. From health point of view, ammoniacal nitrogen is very important, because it is one of the primary products of the organic nitrogen substances decomposition. Therefore, it is a chemical indicator of water contamination by animal wastes. Ratio of ammonium ions and ammonia in water is pH dependent (5). For spectrophotometric determination of ammonium cations by Nessler reagent according to the STN ISO 750-1 (75 7451) standard, a calibration curve relating absorbance to concentration of ammonium nitrogen and a calibration curve of absorbance to ammonium cations concentrations were plotted (Fig. 4). The obtained equations are:

$N\text{-}NH_4^+$: $A = 0.7195 \times c$ $R^2 = 0.9962$
NH_4^+: $A = 0.5587 \times c$ $R^2 = 0.9962$

where *A* is absorbance and *c* is concentration (mg/L). The measured values relating absorbance of NH_4^+ concentration are available in Table 8.

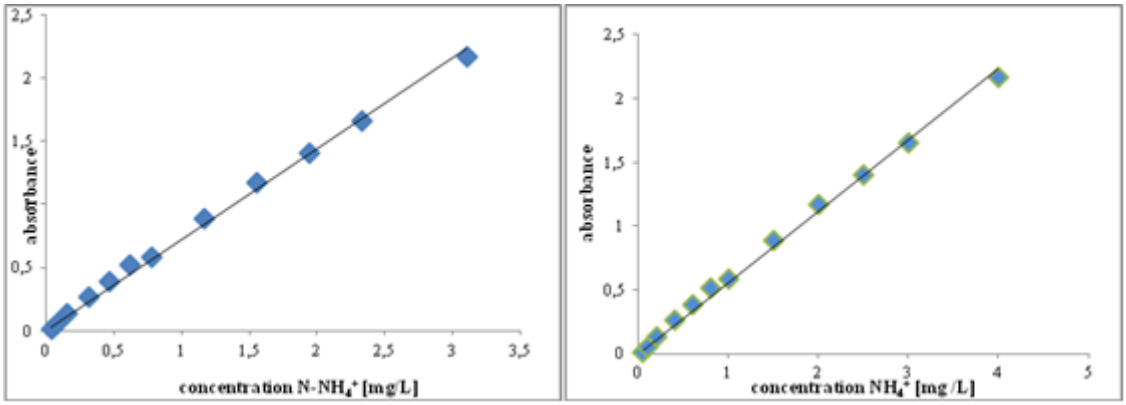

Fig. 4 *The calibration curve relating absorbance to concentration of N-NH_4^+ and NH_4^+*

THE MEASURED VALUES RELATING ABSORBANCE NH_4^+ Table 8

c(N-NH_4^+)[mg/L]	c(NH_4^+)[mg/L]	A_1	A_2	A_3	$A_{average}$
0.039	0.050	0.010	0.013	0.011	0.011
0.078	0.100	0.067	0.044	0.055	0.055
0.155	0.200	0.128	0.128	0.135	0.130
0.311	0.400	0.264	0.265	0.268	0.263
0.466	0.600	0.383	0.383	0.389	0.385
0.621	0.800	0.516	0.515	0.517	0.516
0.776	1.000	0.593	0.598	0.553	0.581
1.165	1.500	0.905	0.886	0.858	0.883
1.553	2.000	1.255	1.101	1.156	1.174
1.941	2.500	1.421	1.396	1.396	1.404
2.329	3.000	1.655	1.646	1.671	1.657
3.106	4.000	2.164	2.172	2.167	2.168

Determination of ammonium cations by both techniques is comparable to demands and the necessary time. Technique 2 allows the determination on the higher concentrations.

Phosphate ions (PO_4^{3}) - the major sources of phosphates in waterways are municipal sewage sludge and fertilizer for crop production on farms. Phosphates themselves are not toxic. Their high content in the river and lake water causes excessive growth of algae. Algae on the surface of the water avoid contact with atmospheric oxygen and substantially interfere with the biological processes in the water. Water contains less dissolved oxygen and becomes unsuitable for the organisms living in it. Eutrophication of water slows down the self-cleaning process, causing the half-life contaminants in waterways extending (5). For spectrophotometric determination of phosphate ion PO_4^{3-} by Molybdenum blue method according to STN EN ISO 6878 (75 7465), a calibration curve relating absorbance to concentration of total phosphorus and a calibration curve of absorbance to phosphates concentrations were plotted (Fig. 5). The obtained equations are:

$P\text{-}PO_4^{3-}$: $A = 1.9499 \times c$ $R^2 = 0.9994$
PO_4^{3-}: $A = 0.6358 \times c$ $R^2 = 0.9994$

where *A* is absorbance and *c* is concentration (mg/L). The measured values relating absorbance to PO_4^{3-} concentration are available in Table 9.

The biggest advantage of using the Technique 2 is stability of the reagents. During the preparation of mixed reagent (Technique 1), many errors can occur (e.g. not well tempered sulphuric acid can decompose ammonium molybdate, potassium antimonyl tartrate must by freshly prepare...). Moreover, the stability of this reagent is restricted to four hours.

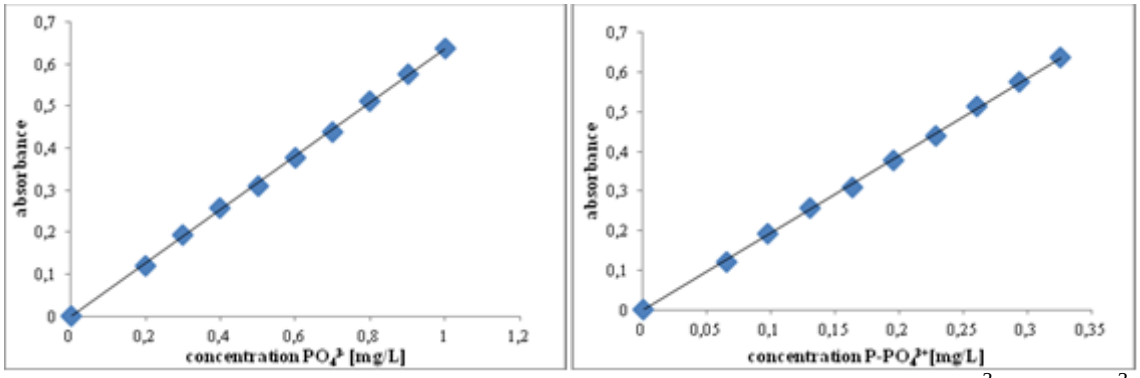

Fig. 5 *The calibration curve relating absorbance to concentration of $P\text{-}PO_4^{3-}$ and PO_4^{3-}*

THE MEASURED VALUES RELATING ABSORBANCE $P\text{-}PO_4^{3-}$ AND PO_4^{3-} Table 9

$c(P\text{-}PO_4^{3-})$[mg/L]	$c(PO_4^{3-})$[mg/L]	A_1	A_2	A_3	$A_{average}$
0	0	0	0	0	0
0.0650	0.200	0.130	0.110	0.122	0.121
0.0978	0.300	0.201	0.180	0.197	0.193
0.1304	0.400	2.275	0,245	0.253	0.258
0.1631	0.500	0.307	0.305	0.316	0.309
0.1957	0.600	0.381	0.371	0.379	0.377
0.2283	0.700	0.436	0.439	0.445	0.440
0.2609	0.800	0.520	0.503	0.515	0.513
0.2935	0.900	0.576	0.564	0.591	0.577
0.3261	1.000	0.645	0.626	0.634	0.638

Conclusions

In this paper, we provide the comparison of usability of laboratory spectrophotometer TermoSpectronic™ and the field photometer HANNA HI 83215 for determination of the selected nutrient concentration. In general, the field photometer is better for higher concentration of nutrition (e.g. in surface water and wastewater), while laboratory spectrophotometer is better for lower concentration (e.g. for groundwater).

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