

D 4.2.1 Report of seasonal trend of the checked parameters on the IT coast

1

December 2019
Version n.1

PROJECT AdSWiM

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NUTRIENT ANALYSES

The activities carried out in the framework of WP4 (Innovative solutions in analytical, microbiological controls and to treat urban wastewaters) has started with the sampling activities (started on April 2019 on IT coast) for the following determination of nutrients analyses, that is one of the principal task of PP4 in the AdSWiM project. The plan of sampling was delineated during the Kick off Meeting in Udine and successively developed until the “2nd partners, GA and SC Meeting” held in Zadar in November 27-28. The sampling activity of seawater was and will be paralleled by sampling of treated wastewater at the DP level.

The sampling points sampled during the last bathing season are: North Adriatic (①San Giorgio di Nogaro, ②Lignano Sabbiadoro), ③Zadar, Split (④Katalinica brig, ⑤Stobreč), Pescara (⑥Francavilla al mare): in all sampling were collected water at discharge point (at sea BOTTOM) and in Depuration Plant (AFTER water treatment) with monthly frequency of the activities (from April to September).

In this report we will present the results on nutrients (nitrite, nitrate, ammonia, silicates, phosphorous inorganic and total) measured on samples from North Adriatic Sea (Depuration Plants (DP) and seawater collected between April and October 2019 (30 April, 26 May, 27 June, 17 July, 29 August, 08 October) (①San Giorgio di Nogaro, ②Lignano Sabbiadoro)) (Fig. 1).



Figure 1. Sampling points: (a) Lignano Sabbiadoro, (b) San Giorgio di Nogaro

The analytical methodology adopted consisted in performing spectrophotometric instrumental determinations of the nutrients. Nutrients were analyzed by instrumentation Systema EASYCHEM Plus (ANAGNI (RM) Italy) using the following methods:

- Nitrite, Ref. United States Environmental Protection Agency (EPA) Method # 354.1
- Nitrate, Ref. United States Environmental Protection Agency (EPA) Method # 354.1

- Ammonia, Ref. APHA Standard Methods for the Examination of Water and Wastewater 4500 NH₃ G Automated Phenate Method
- SiO₂, Ref. APHA Standard Methods for the Examination of Water and Wastewater 4500 SiO₂
- Orthophosphate, Ref. International Standard Organization 15923

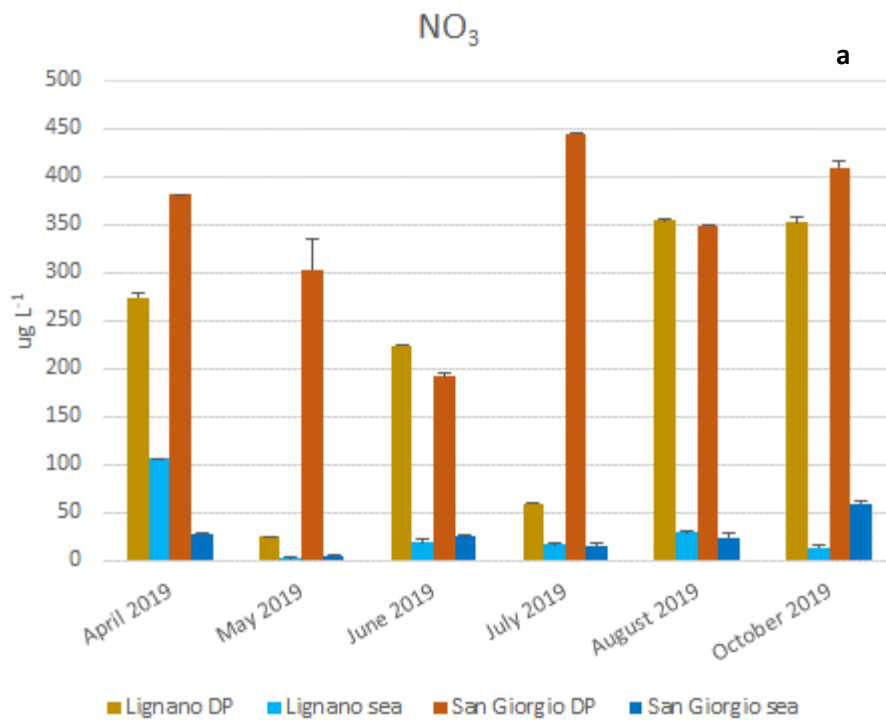
Results

The results are presented in $\mu\text{g L}^{-1}$, as requested by regulation in force for depuration plants.

Nitrates

Concerning the nitrates, the values ranged from 60 – 450 $\mu\text{g L}^{-1}$ in DP (Fig. 2 a, b) with a decrease along the first part of season and an increase in the final part of season (August and October). Data are well below the legal limit (20 mg L^{-1}), whit higher values in San Giorgio than in Lignano DPs. Passing to the seaside a decrease of one order of magnitude was evident (NO_3 ranged from 2 – 106 $\mu\text{g L}^{-1}$) (Fig. 2 a, c) with a no evident trend along the season, except for a decrease in the first part for Lignano samples. No differences between the two sites except for the April month were found and the data obtained are in agreement with literature ones for Adriatic Sea.

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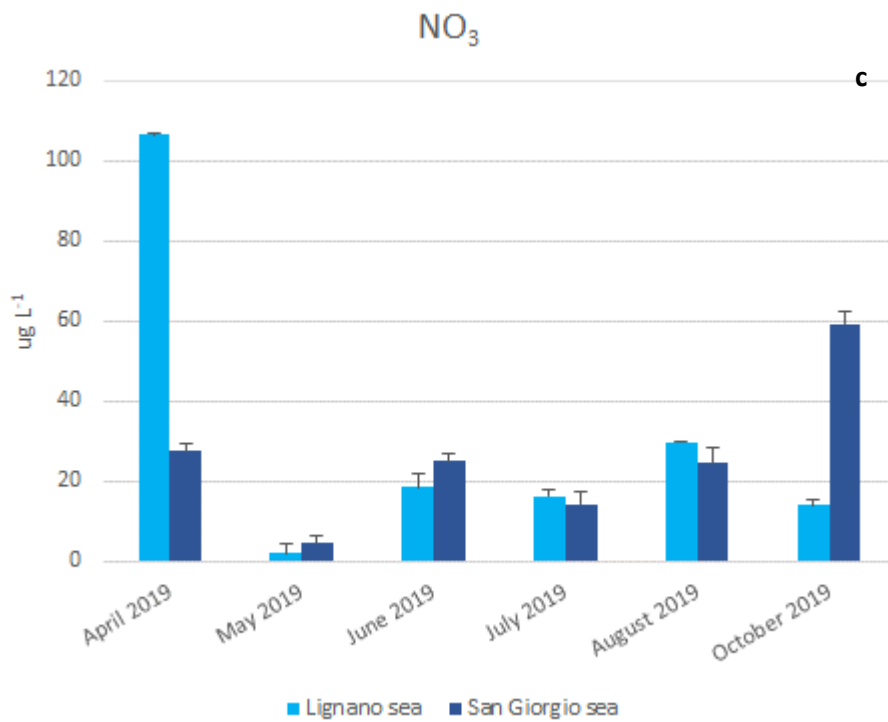
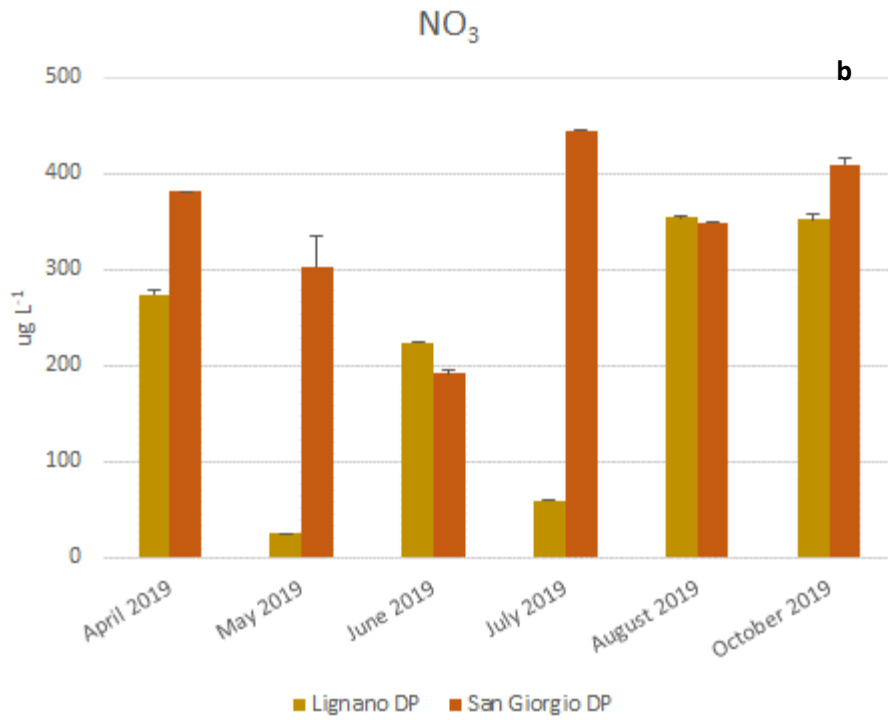
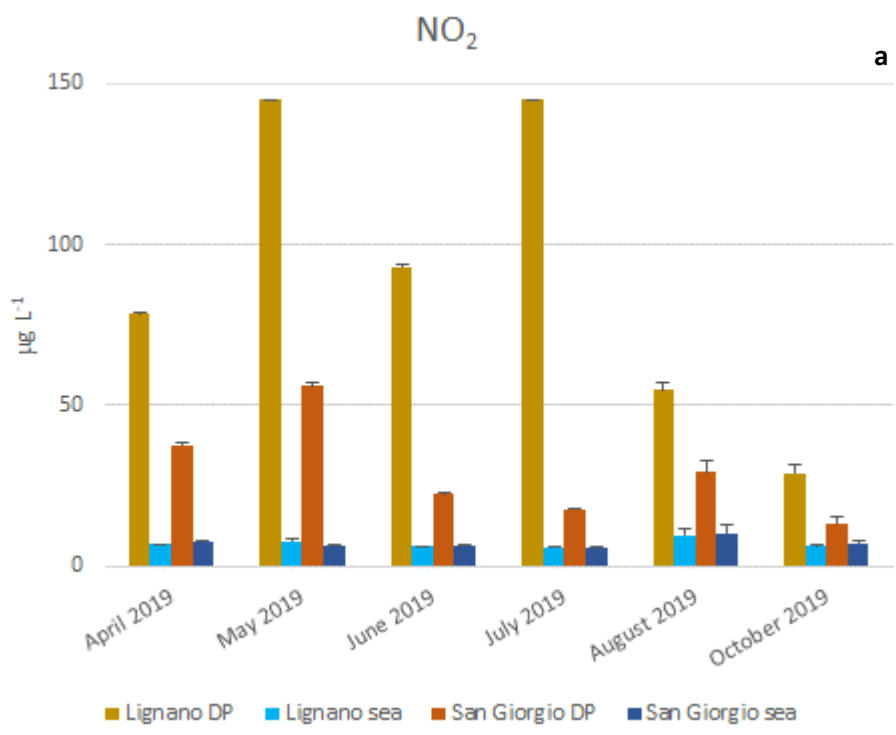


Figure 2. Nitrate concentration in North Adriatic: (a) all stations, (b) Depuration Plant stations, (c) sea stations.

Nitrites

Concerning the nitrites, the values ranged from 20– 140 $\mu\text{g L}^{-1}$ in DP (Fig. 3 a, b) with a substantial decrease along the season and were well below the legal limit fixed (0.6 mg L^{-1}), whit higher values in Lignano than San Giorgio DPs. Passing to the seaside a decrease of one order of magnitude was evident (NO_2 ranged from 6 – 10 $\mu\text{g L}^{-1}$) (Fig. 3 a, c) with no variation along the season and no differences between the two sites. Even in this case the data are in agreement with literature ones.



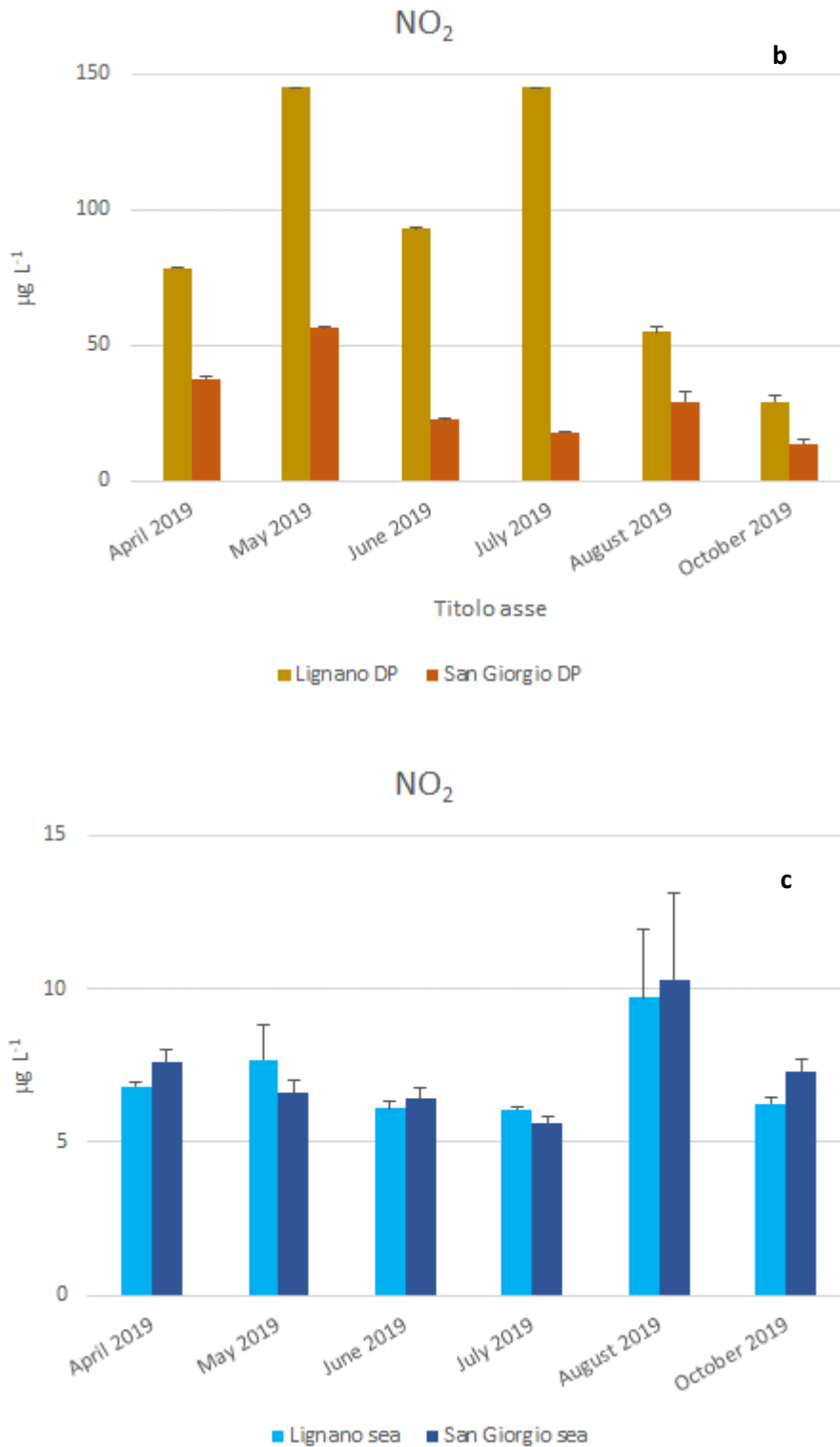
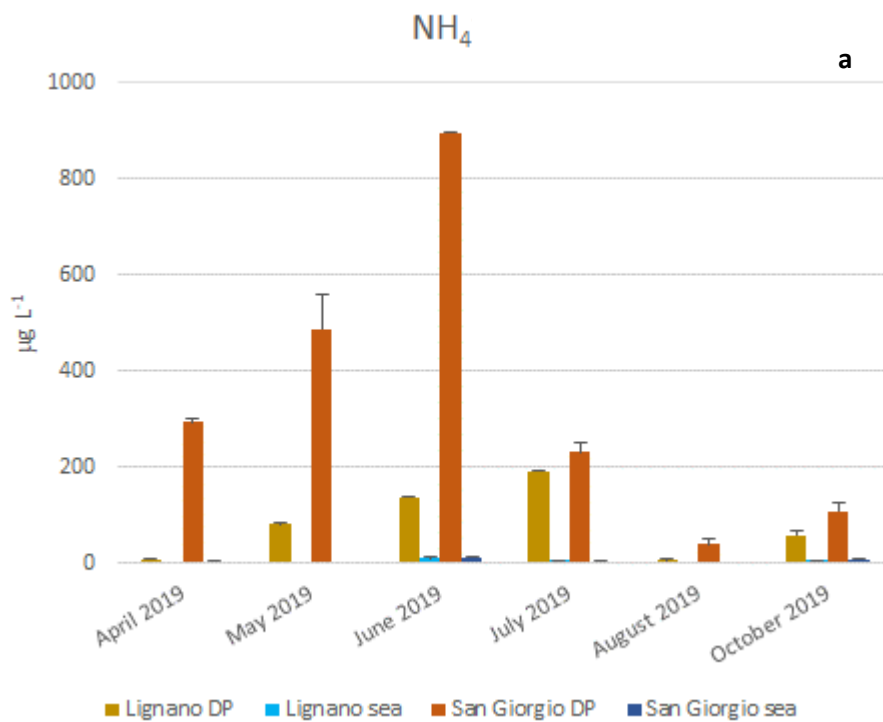


Figure 3. Nitrite concentration in North Adriatic: (a) all stations, (b) Depuration Plant stations, (c) sea stations.

Ammonia

Ammonia values ranged from 7 – 900 $\mu\text{g L}^{-1}$ in DP (Fig. 4 a, b) with an increase trend in the two depuration plant until July and a drastic decrease at the end of the bathing season. Significant differences ($p < 0.05$) were founded at the beginning of the season between San Giorgio DP respect to Lignano, whereas no statistical differences were measured at the end of the sampling season. Passing to the seaside a decrease of one-two order of magnitude was evident (values from 1 – 12 $\mu\text{g L}^{-1}$) (Fig. 4 a, c) with a substantial increase along the season. No differences between the two sites are highlighted and even in this case the data are in agreement with literature ones.



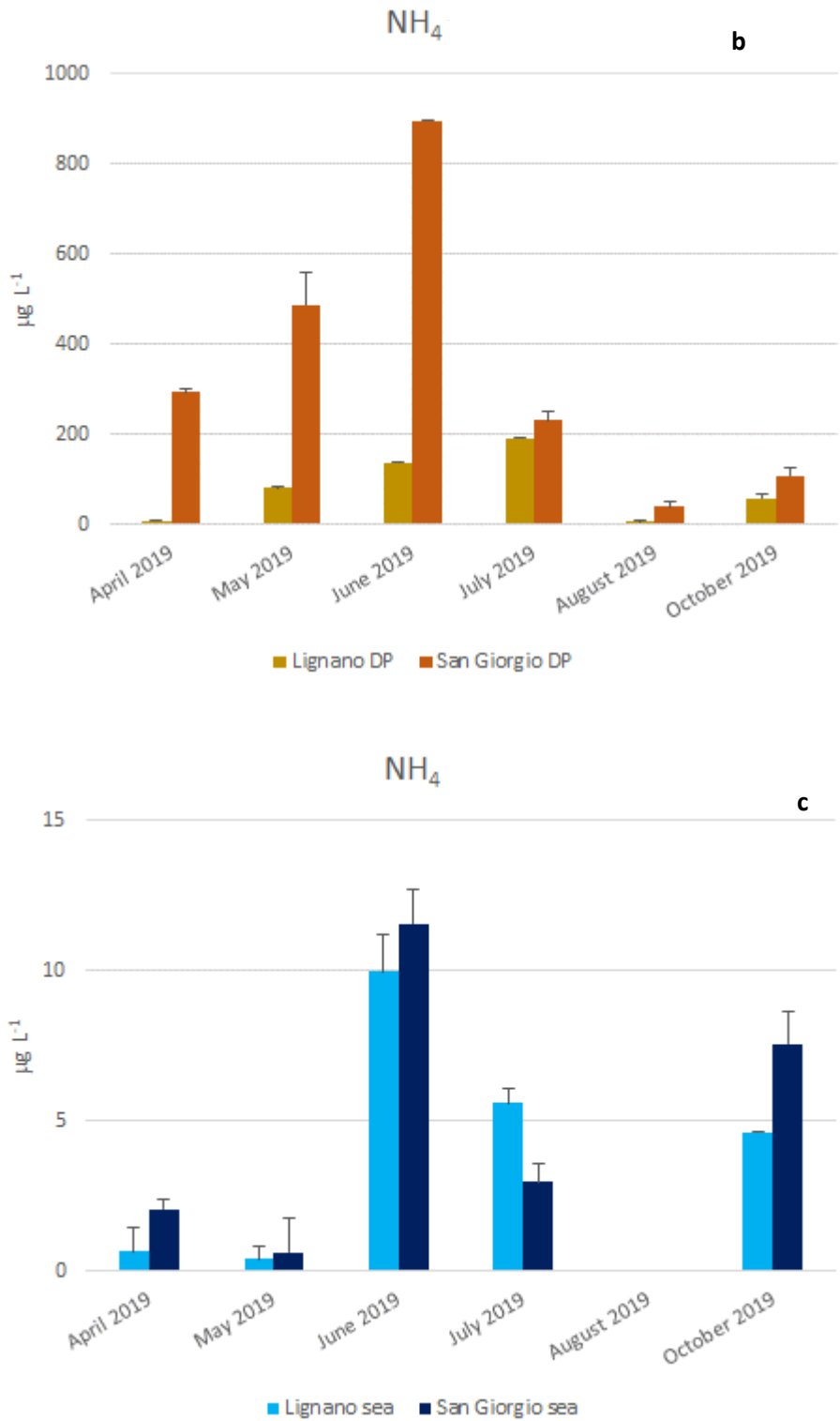


Figure 4. Ammonia concentration in North Adriatic: (a) all stations, (b) Depuration Plant stations, (c) sea stations.

Silicates

Silicates (as SiO_2) showed the same values (about $230 \mu\text{g L}^{-1}$) (Fig. 5 a) in both DPs without variation along the season. We have no explanation of this fact but we will investigate this results, by asking to the responsible of DP the data on silicates of the last years. Passing to the seaside a decrease of one order of magnitude was evident (values from: $3 - 25 \mu\text{g L}^{-1}$) (Fig. 5 b) with a shape bell trend along the season and no differences between the two sites. Even in this case the data are in agreement with literature ones.

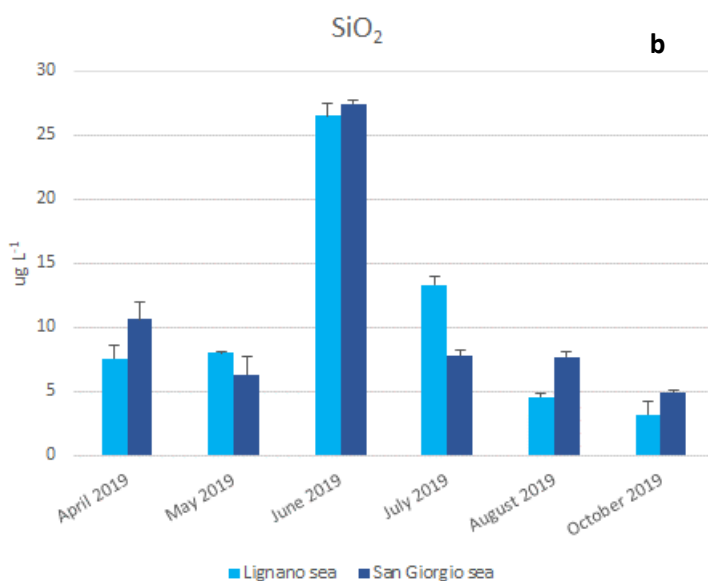
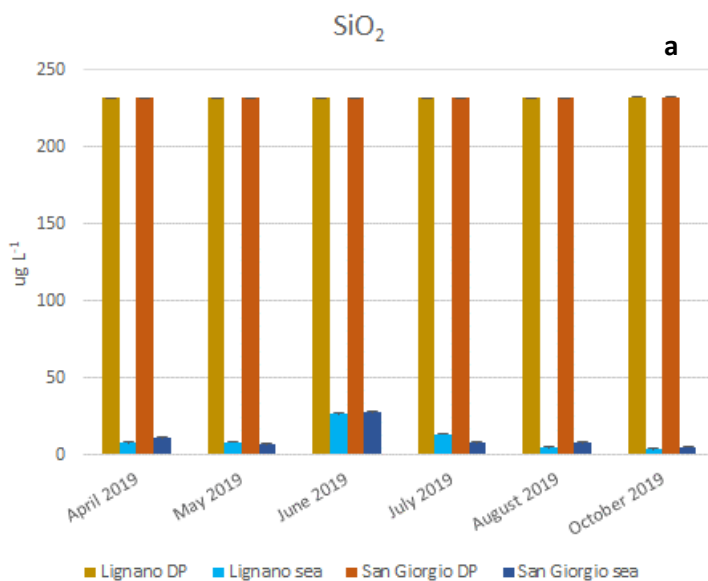
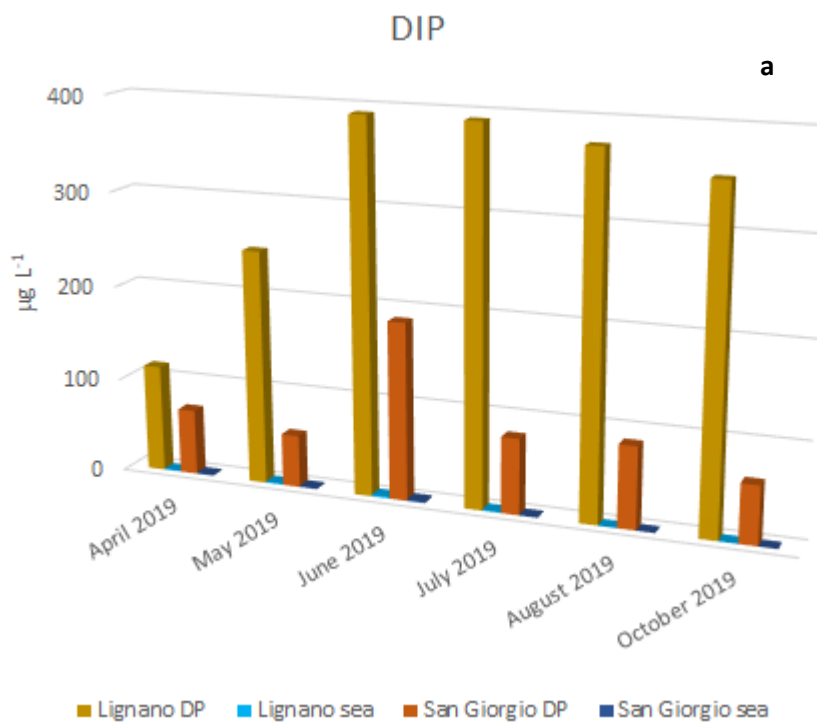


Figure 5. Silicates concentration in North Adriatic: (a) all stations, (b) sea stations.

Phosphorous

Dissolved Inorganic Phosphorous (DIP)

Dissolved Inorganic Phosphorous (DIP) showed values from 60 – 390 $\mu\text{g L}^{-1}$ in DP with an increase along the season in Lignano and lower values in San Giorgio. Passing to the seaside a decrease of two order of magnitude was evident (PO_4 0.1 - 2 $\mu\text{g L}^{-1}$) with a shape bell trend along the season and no differences between the two sites. Even in this case the data are in agreement with literature ones.



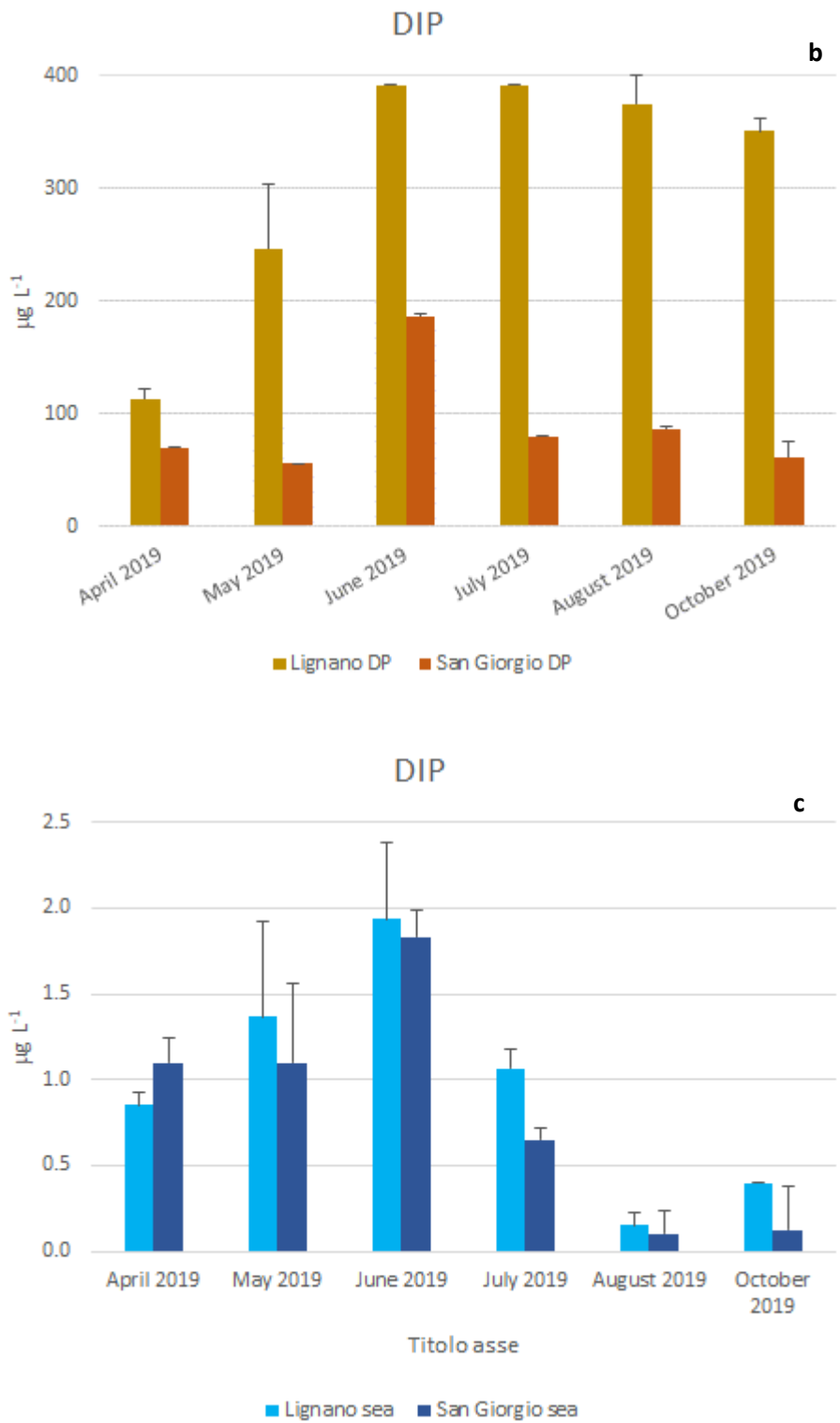
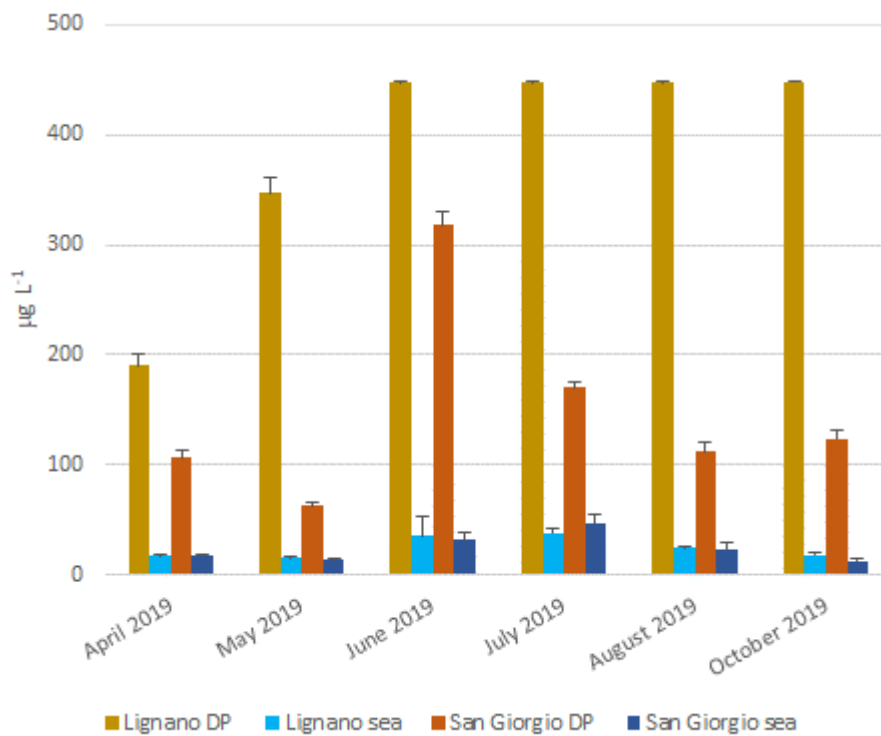


Figure 6. DIP concentration in North Adriatic: (a) all stations, (b) DP stations, (c) sea stations.

Total Dissolved Phosphorous (Dis P_{tot})

Total Phosphorous showed values from 63 – 450 µg L⁻¹ in DP with an increase along the season in Lignano and lower values in San Giorgio. Passing to the seaside a decrease of two order of magnitude was evident (Dis P_{tot} 13 -48 µg L⁻¹) with a shape bell trend with maxima values in June and July: no differences between the two sites were evident. Even in this case the data are in agreement with literature ones.



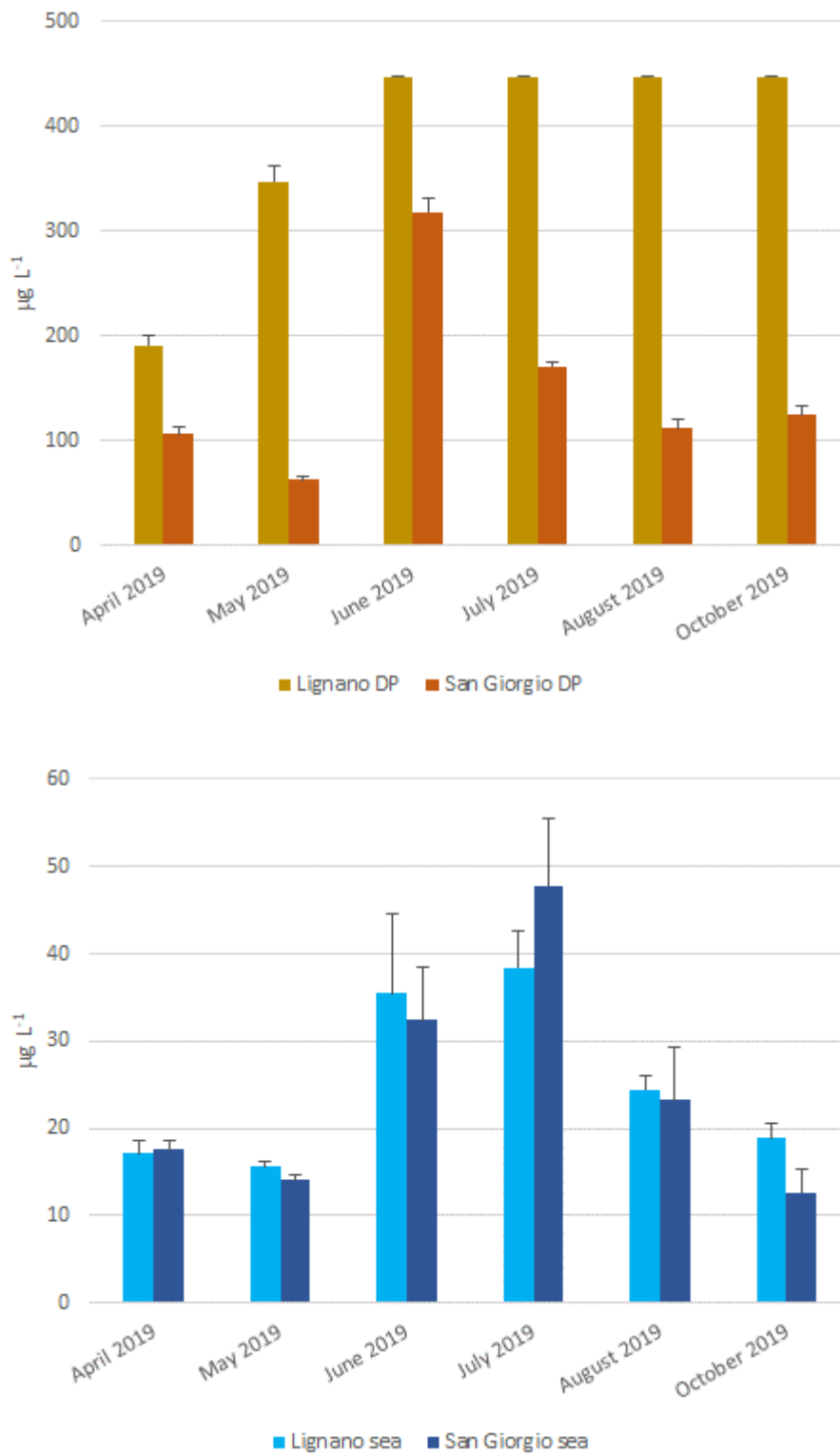


Figure 7. Total Dissolved Phosphorous concentration in North Adriatic: (a) all stations, (b) DP stations, (c) sea stations.

Dissolved Inorganic Phosphorous and Dissolved Organic Phosphorous distribution

Dissolved Organic Phosphorous (DOP) was determined by difference between Dissolved total Phosphorous (Dis P_{tot}) and Dissolved Inorganic P.

The account of DIP to Total Dissolved Phosphorous changes passing from depuration plants to seaside. In fact, in DP the Dissolved Organic Phosphorous (DOP) represents the minor fraction of total dissolved phosphorous %, ranging from 10 to 40% respect to DIP (40-90%). An opposite situation appears in sea stations where DOP accounts the 90 % of total P.

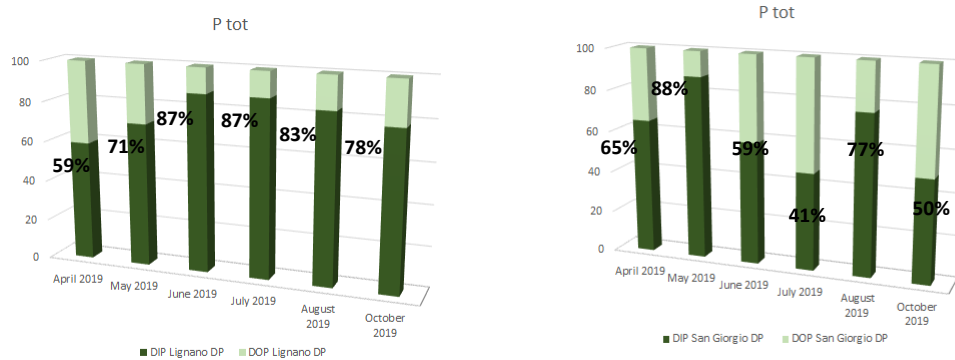


Figure 8. DOP/DIP concentration in North Adriatic North Adriatic Depuration Plants: Lignano (on left), San Giorgio (on right).

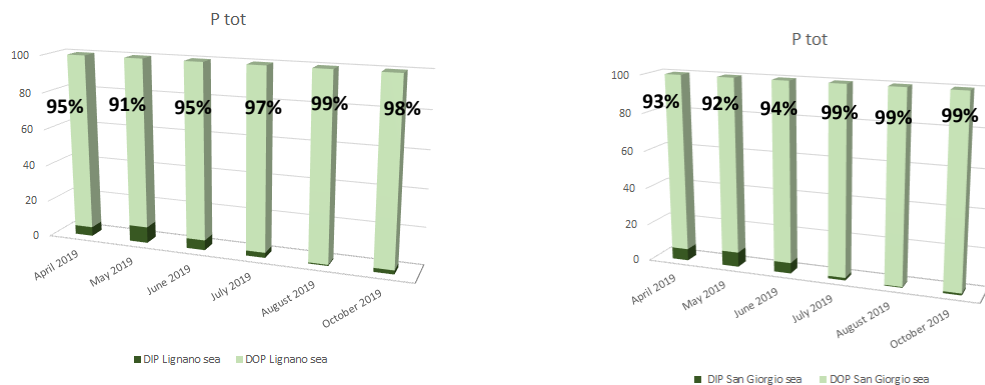


Figure 9. DOP/DIP concentration in North Adriatic Sea: Lignano (on left), San Giorgio (on right).

Redfield Ratio

The ratio of N:P (Nitrogen : Phosphorous) in the water body (referred to as the “Redfield ratio”) is an important indicator of which nutrient is limiting eutrophication. If the Redfield ratio is 16:1, P is most likely the limiting factor for algal growth; lower ratios indicate that N is of great importance. The Redfield Ratio shows values from 20 to 1200 (Fig. 10), underlining that Inorganic phosphorous is the limiting factor, but as seen before the greater contribution of P in sea comes from DOP.

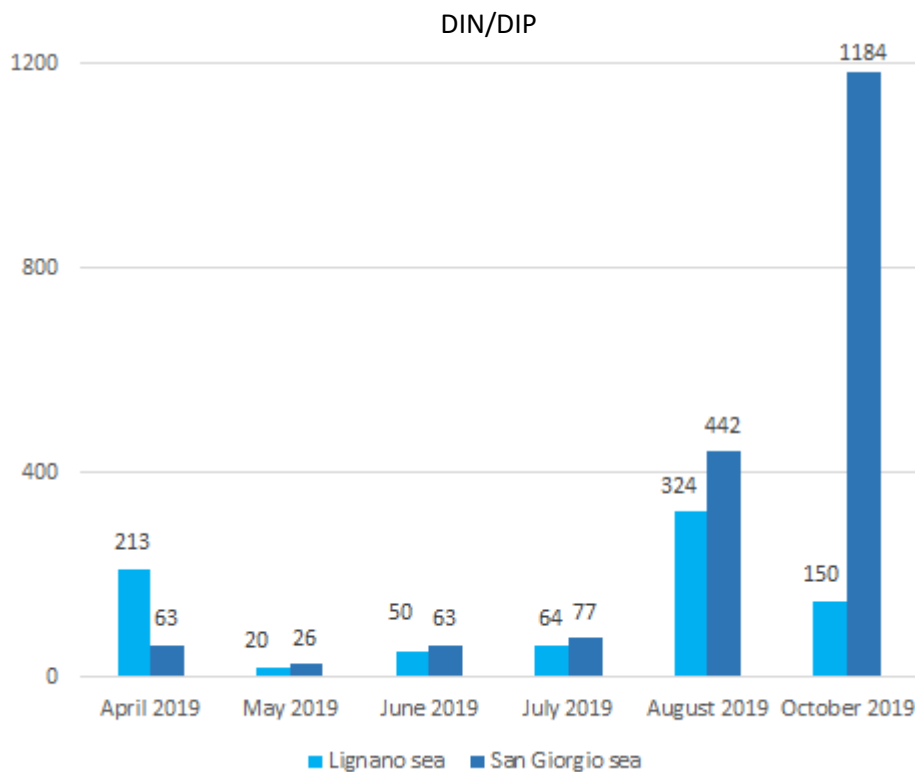


Figure 10. Redfield Ratio in North Adriatic Sea.

CONCLUSION

The first survey on nutrients on IT coast (2019 bathing season) showed that there are differences in nutrient levels between the two Depuration Plants, probably due to the differences in water types input. Passing to the sea no differences between the two sites are recorded and for all nutrients a dilution of 1 or 2 order of magnitude was counted passing from DP to sea. Regarding Redfield ratio the Inorganic phosphorous is the limiting factor, but the greater contribution of P in sea comes from DOP.

D 4.2.2.

REPORT ON THE MONITORING ACTIVITIES IN CROATIA, COMPARISON OF CONVENTIONAL METHODS AND METHODS WITH NEW TECHNOLOGY

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May 2020

Version n.1

PROJECT AdSWiM

Work package:	WP 4 - Innovative solutions in analytical, microbiological controls and to treat urban wastewaters (UWW)
Activity:	4.2 Innovative Analytical Methods/Devices (IAMD) Nutrients and trace elements
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PART 1: INTRODUCTION

According to the description of the **WP 4: Innovative solutions in analytical and microbiological controls of urban wastewater treatment**, determination of water body quality according to the Water Framework Directive (2000/60/EC), produced from a wide range of analysis of chemical-physical and biological parameters (as well as according to Urban Waste Water Directive (91/271/EEC) and the Bathing Water Directive (2006/7/EC). The hygienic quality of the marine environment, the balance of aquatic ecosystems (i.e. conservation of biological diversity and productivity) depends on the availability of nutrients, primarily phosphorus and nitrogen salts as well as their relative relationships. In accordance with the stated goal of WP4, new technological and control solutions have been implemented for the assessment and improvement of the quality of the marine environment and its protection against the effect of wastewater discharges.

As a part of WP4 is **Activity 4.2. Innovative analytical methods / devices (IAMUs), nutrients, and trace elements**). Within this activity, the tasks were: measuring the concentrations of nutrient salts of nitrogen and phosphorus, nutrient salts (nitrate, nitrite, ammonia, phosphate and silicate) and trace elements (Fe, Co, Ni, Mn, Cu, Cr, As, SC, Pb, Zn, Hg) in seawater in relation to the main hydrological variables (T, salinity, EC, pH, dissolved, oxygen saturation, Chla, turbidity). These parameters should be determined by applying uniform analytical methods in the laboratories of project partners from Italy (UNIVPM) and from Croatia (PHI). In addition to physicochemical parameters, microbiological parameters had to be monitored (A. 4.2.2; M8-M24; Comune Pescara, PHI).

In line of this task of Activity 4.2., is this **Report on the monitoring activities in Croatia, comparison of conventional methods and methods with new technology (D4.2.2)** that includes the overview of the monitoring on the parameters at the investigated sites on discharge points locations and in waste waters. All monitored parameters, locations, sampling frequencies and methodologies are presented. The comparison between applied analytical techniques of nutrient analysis with new technologies applied is included.

PART 2: INVESTIGATED SITES

The sites on the Croatian side of the Adriatic where the investigation of the seawater was obtained are located at the discharge points (DPs) of the wastewater treatment plant (WWTP) in the city of Zadar (Center), the WWTP in the City of Split (Katalinića brig) and east of Split near Stobreč (Stupa). The sites were named as ZD-SEA, KB-SEA, and ST-SEA (Figure 1).



Figure 1. Locations of the investigated sites at the DPs of the WWTPs in the city of Zadar, Split and in Stobreč (east from Split) (ZD-SEA, KB-SEA and ST-SEA)

The geographical coordinates of the investigated site ZD-SEA at the discharge point of WWTP Centar in Zadar city, are 44°05,227' N 15°14,178' E, and the depth at the site is 32 meters. Sampling of seawater was performed during 2019 and 2020 in the surface and bottom layer of the water column (0m and 30m depth) using a Nansen bottles (Figure 2, Table 1). In parallel with the field research at



the ZD-SEA site, analysis of waste waters from Zadar WWTP CENTAR were obtained for a number of parameters (at the 24-hour level).

Figure 2. Location of ZD-SEA site at the discharge point of the WWTP in the city of Zadar (Centar) (discharge points are marked with a yellow lines)

Table 1. Geographical coordinates, depth of investigated sites at discharge points with sampling dates

Lokacija	Koordinate	Dubina (m)	Datum uzorkovanja
ZD-SEA	44°05,227' N 15°14,178' E	32	30.07.2019 13.09.2019. 07.11.2019. 22.04.2020. 18.05.2020. 18.06.2020. 27.07.2020. 26.08.2020. 29.09.2020.
WWTP CENTAR Zadar	-	-	30.07.2019.

			13.09.2019. 07.11.2019 22.04.2020. 18.05.2020. 18.06.2020. 27.07.2020. 26.08.2020. 29.09.2020.
KB-SEA	43°30,180' N 16°27,150' E	42	31.07.2019. 27.02.2020. 23.04.2020. 28.05.2020. 26.06.2020. 22.07.2020. 21.09.2020.
WWTP KATALINIĆA BRIG Split	-	-	31.07.2019. 27.02.2020. 23.04.2020. 28.05.2020. 26.06.2020. 22.07.2020. 21.09.2020.
ST-SEA	43°28,60' N 16°31,10' E	36	31.07.2019. 27.02.2020. 23.04.2020. 28.05.2020. 26.06.2020. 22.07.2020. 21.09.2020.
WWTP STUPE Stobreč	-	-	31.07.2019. 27.02.2020. 23.04.2020. 28.05.2020. 26.06.2020. 22.07.2020. 21.09.2020.

The geographical coordinates of the KB-SEA site for the Split area at the discharge point of Katalinića brig, are 43°30,180' N 16°27,150' E, and the depth at the station is 42 meters. Sampling of seawater was performed during 2019 and 2020 in the surface and bottom layer of the water column (0m and 40m depth) using a Nansen bottles (Table 1, Figure 3). In parallel with the field research at the KB-SEA site, analyses of waste waters from WWTP Katalinića brig were obtained for a number of parameters (at the 24-hour level).



Figure 3. Location of KB-SEA site at discharge point of the city of Split (*Kataliniča brig*) and ST-SEA site at discharge point of the *Stupa* located eastern from Split, near the settlement of Stobreč (discharge points are marked with a yellow lines)

The geographical coordinates of the ST-SEA site for the Split area nearby Stobreč at the Stupa discharge points, are 43° 28,60' N and 16° 31,10' E, and the depth at the site is 36 meters. Sampling of seawater was performed during 2019 and 2020 in the surface (0m) and bottom layer of the water column (0m and 34m depth) using a Nansen bottles (Table 1, Figure 3). In parallel with the field research at the ST-SEA site, analyses of waste waters from WWTP Stupe were obtained for a number of parameters (at the 24-hour level).

PART 3: INVESTIGATED PARAMETERS

To estimate the ecological state of the marine environment at Croatian sites of discharge points, a sequence of chemical parameters from the water column were investigated. In line with European guidelines applied into Croatian law, most of these parameters are related with the eutrophication in the marine environment.

According to the tasks of Activity 4.2., examined parameters in seawater at sites of discharge points (ZD-SEA, KB-SEA and ST-SEA) were: concentrations of nutrients (nitrate, nitrite, ammonia, phosphate and silicate) and trace elements (Co, Ni, Mn, Cu, Cr, As, SC, Pb, Zn, Hg) * in seawater as well as the main hydrological variables (T, salinity, conductivity, pH, dissolved oxygen, oxygen saturation, Chla, turbidity) (Table 2, Table 3). * *heavy metal analyzes were obtained by the Italian partners*

Table 2. List of chemical parameters monitored at Croatian sites on DP's locations (ZD-SEA, KB-SEA i ST-SEA) with applied methods and certified reference materials

Parameter	Method	Instrument	CRM
Determination of nitrite Quantification limit 0,005 mg/L	Standard Methods for the Examination of Water and Wastewater, 21st Edition (2005) p 4-118 Method 4500-NO ₂ -	Spectrophotometer UV-2550 PC SHIMADZU UV spectrophotometer Varian Cary 50	Nitrite standard solution 1000 ± 10 mg/L NO ₂ ⁻ (MERCK)
Determination of nitrate (0,001-0,56) mg/L N	In-house method PO – 7.2/84 Edition 01/1 2019-09-02	UV spectrophotometer Varian Cary 50	Nitrate standard solution 1000 ± 10 mg/L NO ₃ ⁻ (MERCK)
Determination of phosphorus Ammonium molybdate spectrometric method Quantification limit 0,0005 mg/L P	EN ISO 6878:2004 (clause 4)	UV spectrophotometer Varian Cary 50 Spectrophotometer UV-2550 PC SHIMADZU Autoclave Inko AV400EN	Phosphate standard solution 1000 mg/L PO ₄ ³⁻ ± 0.4 % (MERCK)
Determination of orthophosphate – Ammonium molybdate spectrometric method Quantification limit 0,0005 mg/L P	EN ISO 6878:2004 (clause 7).	Spectrophotometer Varian Cary 50 Spectrophotometer UV-2550 PC SHIMADZU	Phosphate standard solution 1000 ± 10 mg/L PO ₄ ³⁻ (MERCK)
Determination of dissolved silicon Quantification limit 0,002 mg/L	Methods of Seawater Analysis, 3rd Edition (1999) p10-193	UV spectrophotometer Varian Cary 50	Silicon standard solution 1000 ± 10 mg/L Si (MERCK)

Determination of dissolved anions Quantification limit NO ²⁻ 0,11 mg/L NO ³⁻ 0,1 mg/L SO ₄ ²⁻ 0,4 mg/L	ISO 10304-1:2007; EN ISO 10304-1:2009/AC:2012)	Ion chromatograph Dionex Aquion, Thermo Scientific	Nitrite standard solution, 1000 ± 10 mg/L NO ₂ ⁻ (Merck) , Sulfate standard solution, 1000 ± 10 mg/L SO ₄ ²⁻ (Merck), Nitrate, 1003 ± 4 µg/ml NO ₃ ⁻ (Inorganic ventures)
Determination of total nitrogen Quantification limit 0,01 % mas.	In-house method PO – 7.2/82 Edition 01/1 2019-08-27 /modified EN 12260:2003	TOC-V-CPH + TNM + ASI-V SHIMADZU	-Ammonium sulfate 99.9999 % (MERCK) -Potassium nitrate 99.995 % (MERCK) -Nicotinic acid ≥99.5% (SIGMA-ALDRICH)
Determination of total organic and dissolved organic carbon (0,1-25000) mg C/L	HRN EN 1484:2002 (EN 1484:1997)	TOC-V-CPH + TNM + ASI-V SHIMADZU	Potassium Hydrogen Phthalate 99.8% - 100.2% (NACALAI TESQUE, INC.)
Determination of chlorophyll α by Fluorescence Quantification limit 0,19 µg/L	EPA Method 445.0 (1997)	-Membrane filtration system Sartorius 16831 -Vacuum pump Sartorius 22L -Heraeus Megafuge 8R THERMO SCIENTIFIC -Fluorimeter TD-700 TURNER DESIGNS	-Chlorophyll a from Anacystis nidulans algae (SIGMA ALDRICH) Fluorimeter solid standard (TURNER DESIGNS)
Determination of dissolved oxygen - iodometric method (0,07-20) mg/L O ₂	ISO 5813:1983; EN 25813:1992		

Table 3. List of physical parameters monitored at Croatian sites on DP's locations and in waste waters with applied methods and certified reference materials

Parameter	Instrument	CRM	Method
Determination of pH (2-12) pH	ISO 10523:2008, EN ISO 10523:2012	pH Meter/Conductometer Seven Multi S47 Mettler Toledo	Technical Buffer Solution pH 4.01 ± 0.02; pH 7.00 ± 0.02; pH 10.01 ± 0.02 (METTLER TOLEDO)
Conductivity, Temperature, Salinity, Depth		SEALOGGER SBE 25 CTD - SEA BIRD ELECTRONICS INC	Conductometer, salinity
Determination of water temperature (1-99) °C	Standard Methods for Examination of Water and Wastewater, 21st Edition (2005)p2-61 Method 2550		

Determination of total, suspended and dissolved solids (0,17-200) mg/L	In-house method PO – 7.2/16 Edition 01/3 2019-08-06 modified Standard Methods for the Examination of Water and Wastewater, 21st Edition (2005) p2-55 Method 2540	Heating oven BINDER ED 115	Cellulose microcrystalline (MERCK)
Determination of turbidity Quantification limit 0,1 NTU	ISO 7027-1:2016; EN ISO 7027-1:2016	Turbidimeter 2100N Hach	Gelex secondary standard (HACH)

The upper parameters were monitored in wastewater samples from the WWTPs in Zadar (Center) and in the Split area (Katalinića brig and Stupe). In addition to nutrient salts and some physical parameters, other parameters in wastewater samples were monitored in parallel: biological oxygen demand (BOD), sulfates, phenols, chemical oxygen demand (COD), chlorides, anionic surfactants, dissolved organic carbon (DOC), total oils and fats (Table 4). These analyses are part of regular monitoring of the status of wastewaters.

Table 4 List of chemical parameters monitored in waste waters at WWTP in Zadar (Centar) and Split are (Katalinića brig and Stupe) with applied methods and certified reference materials

Parameter	Method	Instrument	CRM
Determination of the chemical oxygen demand index (STCOD)- Small-scale sealed-tube method (6-1000) mg /L O ₂	ISO 15705:2002	-Thermostat HT200S HACH LANGE -Spectral photometer DR3900 VIS HACH LANGE	-Chemical Oxygen Demand Standard Solution 1000 mg/L COD (HACH) -Cuvettes LCI 400 COD (0-1000 mg/L) ISO 15705 (HACH) -Cuvettes LCI 500 COD (0-150 mg/L) ISO 15705 (HACH)
Determination of biochemical oxygen demand after 5 days Quantification limit 0,27 mg/L O ₂	ISO 5815:1989, modified; EN 1899-2:1998		D-(+)-Glucose anhydrous ≥99.5% (FLUKA) L-Glutamic acid ≥99.0% (FLUKA) N-Allylthioharnstoff 98% (SIGMA-ALDRICH)
Determination of dissolved oxygen - iodometric method (0,07-20) mg/L O ₂	ISO 5813:1983; EN 25813:1992		
Determination of ammonium- Manual spectrometric method Quantification limit 0,05 mg/L	ISO 7150:1:1984	Spectrophotometer UV-2550 PC SHIMADZU	Ammonium standard solution 1000 ± 10 mg/L NH ₄ ⁻ (MERCK)

Determination of nitrite Quantification limit 0,005 mg/L	Standard Methods for the Examination of Water and Wastewater, 21st Edition (2005) p4-118 Method 4500-NO ₂ -	Spectrophotometer UV-2550 PC SHIMADZU	Nitrite standard solution 1000 ± 10 mg/L NO ₂ ⁻ (MERCK)
Determination of nitrate Quantification limit 0,17 mg/L N	In-house method PO – 7.2/77 Izdanje/Edition 01/1 2019-05-26	Spectral photometer DR3900 VIS HACH LANGE	-Nitrate Nitrogen Standard Solution 10.0 ± 0.1 mg/L as N; 44.3 ± 0.4 mg/L as NO ₃ (HACH) -Kivete LCK 339 Nitrat (0.23-13.50 mg/L NO ₃) (HACH)
Determination of phosphorus Ammonium molybdate spectrometric method Quantification limit 0,0005 mg/L P	EN ISO 6878:2004 clause 4.	-UV spectrophotometer Varian Cary 50 -Spectrophotometer UV-2550 PC SHIMADZU Autoclave Inko AV400EN	Phosphate standard solution 1000 ± 10 mg/L PO ₄ ³⁻ (MERCK)
Determination of anionic surfactants Quantification limit 0,055 mg/L	EN 903:1993	Spectrophotometer UV-2550 PC SHIMADZU	Methyl dodecylbenzene sulfonate (MERCK)
Determination of total oils and fats by gravimetric method in wastewater Quantification limit 5,1 mg/L	EPA Method 1664	-Rotavapor R-215 Buchi -Heating oven BINDER ED 115	Hexadecane >98.0% (TCI) Stearic Acid >98.0% (TCI)
Determination of chloride Quantification limit 0,24 mg/L	ISO 9297:1989	-UV spectrophotometer Varian Cary 50 -Spectrophotometer UV-2550 PC SHIMADZU	Chloride standard solution 1000 ± 10 mg/L Cl ⁻ (MERCK)

In addition to physical and chemical parameters, the state of microbiological parameters (abundance of *Pseudomonas aeruginosa*, *Escherichia coli* and enterococci) was monitored, for which determination methodologies are also presented (Table 5).

Table 5. List of microbiological parameters monitored at DPs sites as well as in waste waters

Parameter	Method	Instrument
Enumeration of <i>Escherichia coli</i> and coliform bacteria in water -Membrane filtration method	In-house method PO – 7.2/33 Edition 03/0 2019-09-09 modified EN ISO 9308-1:2014/A1:2017	-Membrane filtration system Sartorius 16831 -Vacuum pump Sartorius 22L - Thermostat 37°C Sutjeska

Detection and enumeration of enterococci by membrane filtration method	ISO 7899-2:2000; EN ISO 7899-2:2000	-Membrane filtration system Sartorius 16831 -Vacuum pump Sartorius 22L -Thermostat 37°C Sutjeska -Thermostat 44°C VELP
Detection and enumeration of <i>Pseudomonas aeruginosa</i> in water by membrane filtration method	ISO 16266:2006; EN ISO 16266:2008	-Membrane filtration system Sartorius 16831 -Vacuum pump Sartorius 22L -UV lamp (254/366 nm) Camag -Vortex mixer VELP 2014 -Thermostat 37°C Sutjeska

PART 4: COMPARISON BETWEEN STANDARD METHODS AND NEW METHODS OF DETERMINATION

4.1. Comparison of methods applied in the seawater samples analysis

In this section the comparison between standard methods and new methods of analytical determination of nutrients in seawater samples is given. For the better interpretation, nutrient concentrations determined in the seawater samples at sites ZD-SEA, KB-SEA and ST-SEA during 2019/2020 are presented in figures that show surface and bottom water concentrations.

In Table 6 descriptive statistics on the whole set of nutrient concentrations obtained by standard (SM) and new methods (NM) of determination is presented.

Table 6 Descriptive statistics (number of valid cases, mean, geometric mean, median, minimum, maximum, lower and upper quartile LQ; UQ, variance and standard deviation) on the nutrient concentration data

Nutrients	Descriptive statistics									
	N	Mean	GeoMean	Median	Min	Max	LQ	UQ	Varian	St.Dev
Nitrite (SM)	138	0,08	0,05	0,05	0,01	0,30	0,02	0,12	0,01	0,08
Nitrite (NM)	132	0,12	0,10	0,10	0,02	0,41	0,07	0,16	0,00	0,07
Nitrate (SM)	138	0,39	0,26	0,35	0,02	1,17	0,12	0,51	0,10	0,31
Nitrate (NM)	132	0,38	0,24	0,25	0,02	1,66	0,11	0,47	0,13	0,37
TP (SM)	138	0,15	0,13	0,13	0,04	0,38	0,10	0,18	0,00	0,07
TP (NM)	132	0,14	0,13	0,13	0,05	0,30	0,09	0,18	0,00	0,06
OrthoP (SM)	138	0,03	0,03	0,03	0,01	0,11	0,02	0,05	0,00	0,02
OrthoP (NM)	132	0,07	0,05	0,06	0,00	0,16	0,03	0,10	0,00	0,04
Silicate (SM)	138	1,44	0,96	0,94	0,11	6,66	0,54	1,64	2,04	1,43
Silicate (NM)	132	1,40	1,01	1,08	0,19	5,04	0,60	1,65	1,30	1,14
Ammonia	138	0,08	0,05	0,05	0,01	0,57	0,04	0,09	0,01	0,09
Ammonia(NM)	132	0,06		0,05	-0,74	1,46	-0,10	0,22	0,13	0,36

In Figure 4 comparison of nitrite concentrations determined with standard method (SM) and new analytical method (NM) applied, shown as nitrite monthly distribution in ZD-SEA site samples investigated during 2019/2020 is presented. The methods of nitrite determination were continuous flow analyzer (CFA method) versus standard spectrophotometric methods (In-house method: PO – 7.2/77 /Edition 01/1 2019-05-26) (Table 2).

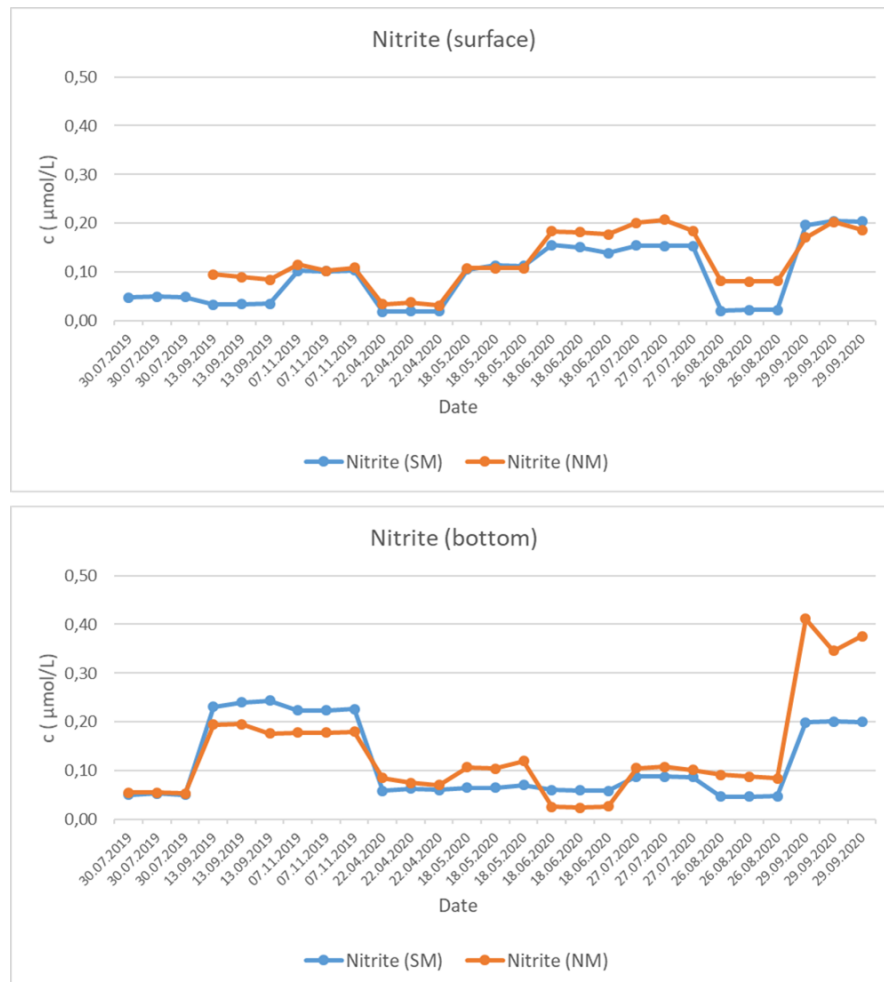


Figure 4. Comparison of nitrite concentrations determined with standard method (SM) and new analytical method (NM), shown as nitrite monthly distribution in ZD-SEA site samples investigated during 2019/2020. (Samples were taken in triplicates per each sampling date.)

Relatively good overlapping between concentrations obtained by new method (NM) and standard method (SM) is visible in most of the surface samples (Figure 4), while the greater disproportion can be seen in the bottom water samples. Furthermore, higher deviations inside the triplicates in CFA samples versus spectrophotometric samples are also visible. Calculated ratio between all nitrite concentrations determined by NM and SM indicated 1,5 times higher values obtained by NM.

In Figure 5 comparison of nitrate concentrations determined with standard method (SM) and new analytical method (NM), shown as nitrite monthly distribution in ZD-SEA site samples investigated

during 2019/2020 is presented. The methods of nitrate determination were continuous flow analyzer method (CFA) versus standard spectrophotometric method (In-house method PO – 7.2/77/Edition 01/1 2019-05-26) (Table 2).

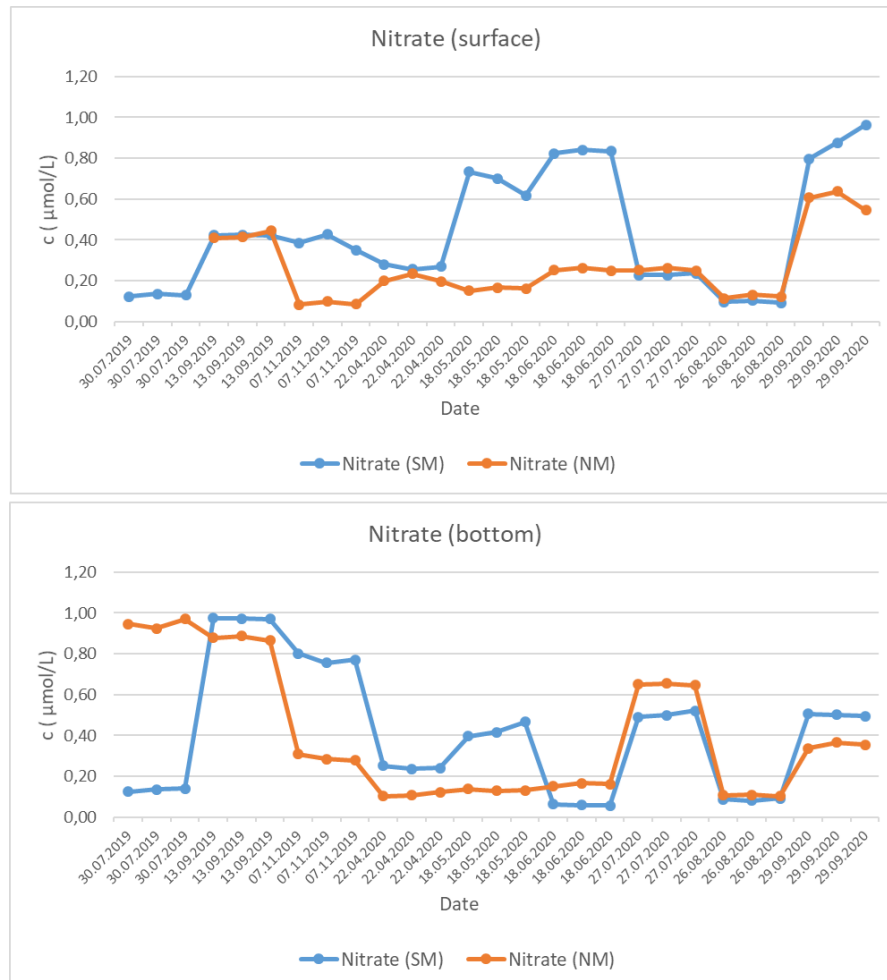


Figure 5. Comparison of nitrate concentrations determined with standard method (SM) and new analytical method (NM) applied, shown as nitrite monthly distribution in ZD-SEA site samples investigated during 2019/2020. (Samples were taken in triplicates per each sampling date.)

Discrepancy between concentrations determined by new method (NM) and standard method (SM) is evident from Figure 5. Concentrations obtained by NM are lower in most of the samples from the surface, while in the bottom water samples, there is no visible pattern in the distribution of concentrations. Furthermore, higher deviations inside the triplicates in spectrophotometric samples versus CFA samples are also visible. Calculated ratio between all nitrate concentrations determined by NM and SM indicated 2,3 times higher values obtained by NM.

In Figure 6 comparison of ammonia concentrations determined with standard method (SM) and new analytical method (NM), shown as nitrite monthly distribution in ZD-SEA site samples investigated during 2019/2020 is presented. The methods of ammonia determination were continuous flow

analyzer method (CFA) versus standard method (spectrophotometric determination of ammonia according to Solorzano method, modified by Ivančić and Degobbi, 1984) (Table 2).

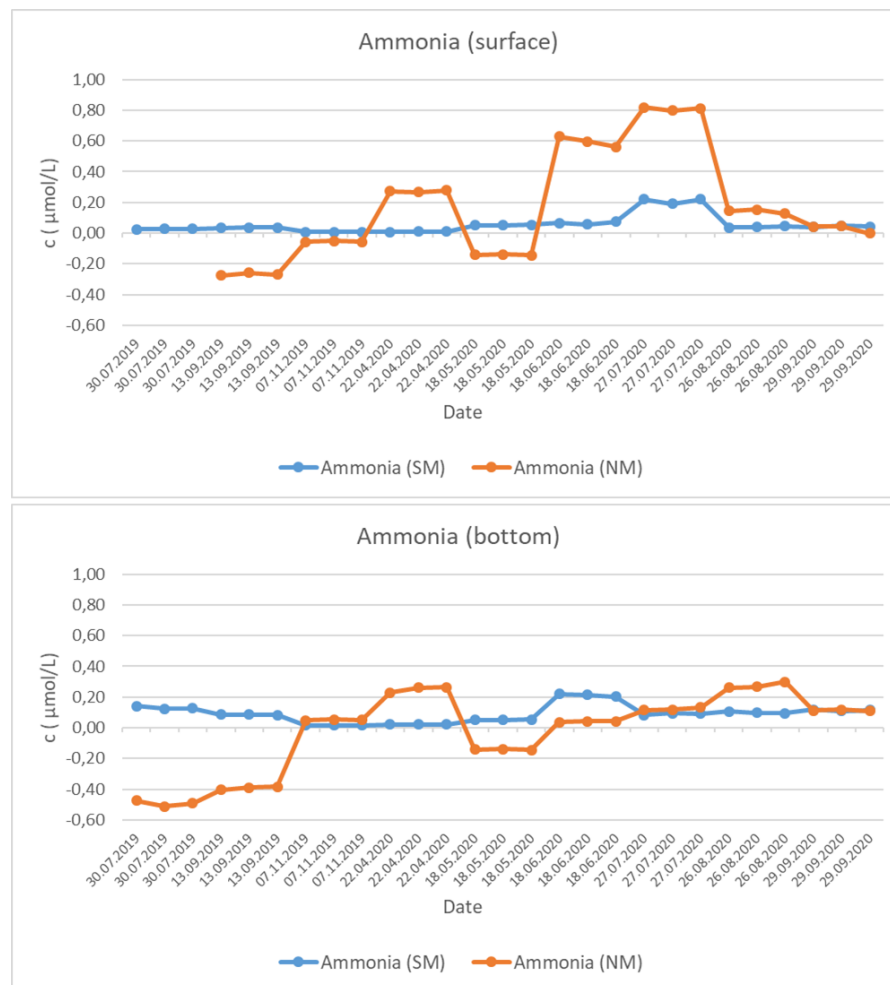


Figure 6. Comparison of ammonia concentrations determined with standard method (SM) and new analytical method (NM) applied, shown as ammonia monthly distribution in ZD-SEA site samples investigated during 2019/2020. (Samples were taken in triplicates per each sampling date.)

The difference between concentrations obtained by new method (NM) and standard method (SM) is evident, even greater in the surface layer samples (Figure 6). Negative ammonia concentrations obtained by NM are also visible that are direct consequence of inadequate laboratory conditions during the analytical determinations of this nutrient by CFA method. Furthermore, higher deviations inside the triplicates in CFA samples versus spectrophotometric samples are also visible. Due to a large number of negative values, ratio between NM and SM concentrations was not taken into consideration.

In Figure 7 comparison of orthophosphate concentrations determined with standard method (SM) and new analytical method (NM), shown as orthophosphate monthly distribution in ZD-SEA site samples investigated during 2019/2020 is presented. The methods of orthophosphate determination were

continuous flow analyzer method (CFA) versus standard spectrophotometric determination of orthophosphate (EN ISO 6878:2004, clause 7) (Table 2).

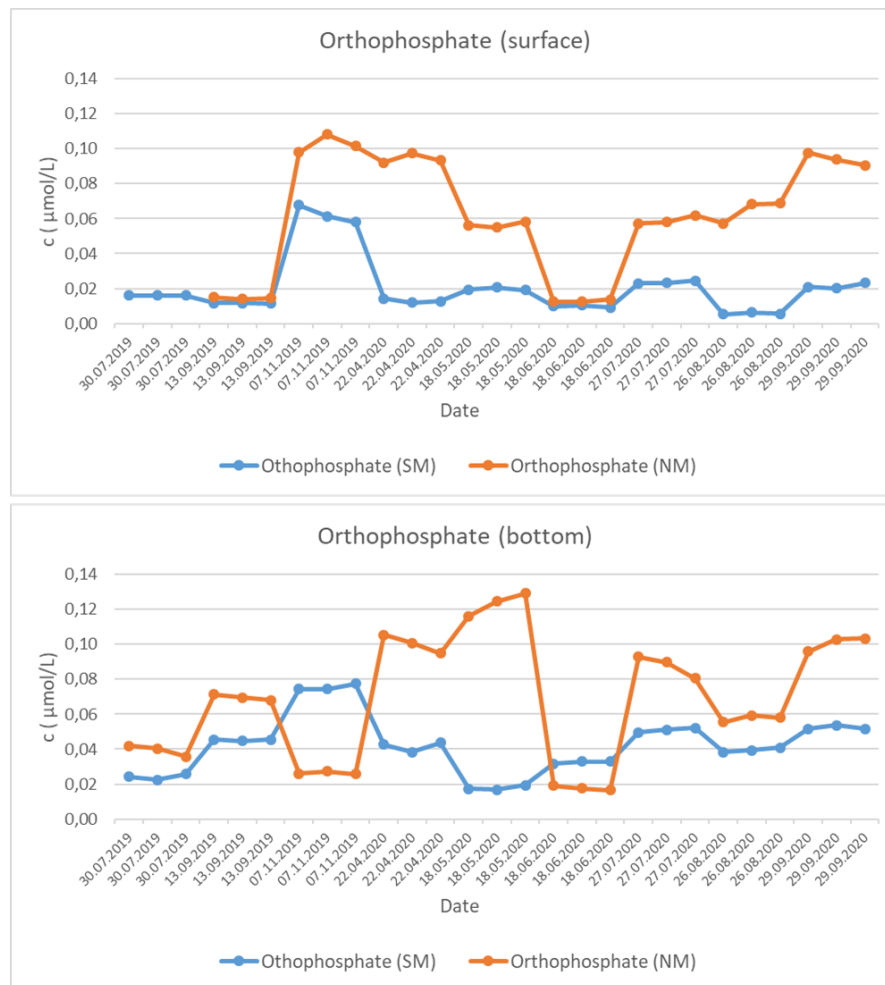


Figure 8. Comparison of orthophosphate concentrations determined with standard method (SM) and new analytical method (NM), shown as orthophosphate monthly distribution in ZD-SEA site samples investigated during 2019/2020. (Samples were taken in triplicates per each sampling date.)

Large discrepancy between concentrations determined by new method (NM) and standard method (SM) is visible (Figure 8). Orthophosphate concentrations obtained by NM are higher in all samples from the surface, while in the bottom water, during two sampling periods, lower values than those obtained by SM are evident. Furthermore, higher deviations inside the triplicates in CFA samples versus spectrophotometric samples are also visible. Calculated ratio between all orthophosphate concentrations determined by NM and SM indicated 3 times higher values obtained by NM.

In Figure 9 comparison of total phosphorus (TP) concentrations determined with standard method (SM) and new analytical method (NM), shown as TP monthly distribution in ZD-SEA site samples investigated during 2019/2020 is presented. The methods of TP determination were continuous flow

analyzer method (CFA) versus standard spectrophotometric determination of TP (EN ISO 6878:2004, clause 4) (Table 2).



Figure 9. Comparison of TP concentrations determined with standard method (SM) and new analytical method (NM), shown as TP monthly distribution in ZD-SEA site samples investigated during 2019/2020. (Samples were taken in triplicates per each sampling date.)

Certain discrepancy between TP concentrations determined by new method (NM) and standard method (SM) is visible (Figure 9), with even higher concentrations obtained by SM in relation to NM concentrations. Furthermore, higher deviations inside the triplicates in spectrophotometric samples versus CFA samples are also visible. Calculated ratio between all TP concentrations determined by NM and SM indicated slightly lower values obtained by NM applied (NM:SM = 0,93).

In Figure 10 comparison of silicate concentrations determined with standard method (SM) and new analytical method (NM), shown as silicate monthly distribution in ZD-SEA site samples investigated during 2019/2020 is presented. The methods of TP determination were continuous flow analyzer method (CFA) versus standard spectrophotometric determination of dissolved silicon (Methods of Seawater Analysis, 3rd Edition (1999) p10-193) (Table 2).

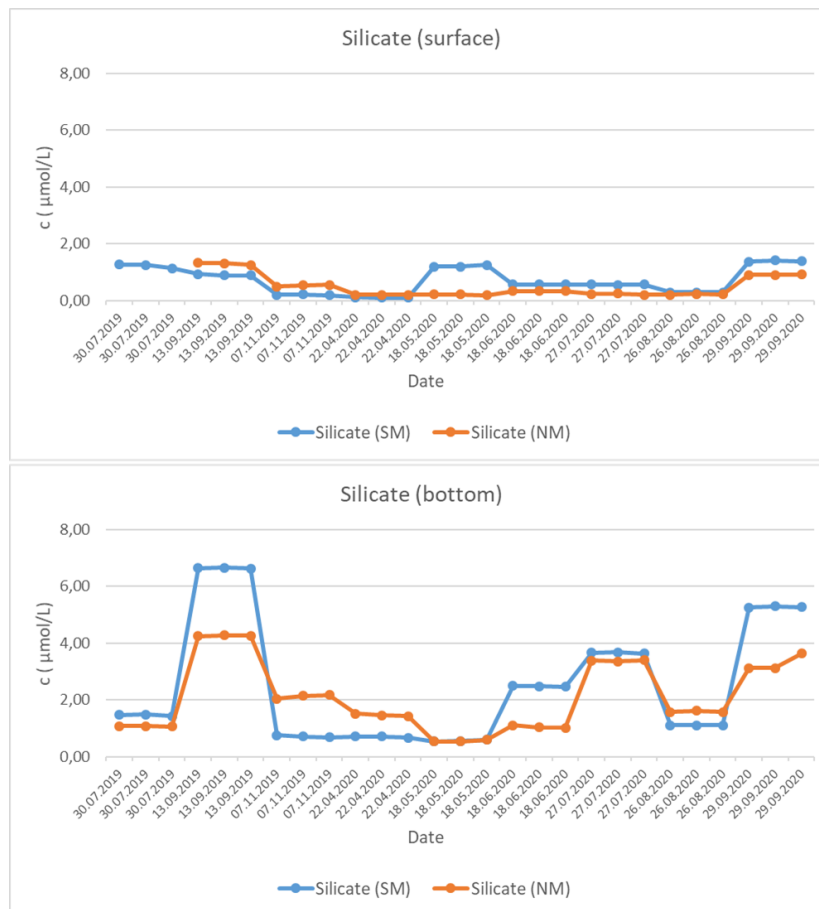


Figure 10. Comparison of silicate concentrations determined with standard method (SM) and new analytical method (NM), shown as silicate monthly distribution in ZD-SEA site samples investigated during 2019/2020. (Samples were taken in triplicates per each sampling date.).

Relatively good overlapping between concentrations obtained by new method (NM) and standard method (SM) is visible in most of the surface samples (Figure 10), while the greater disproportion can be seen in the bottom water samples. Furthermore, higher deviations inside the triplicates in CFA samples versus spectrophotometric samples are also visible. Calculated ratio between all silicate concentrations was NM:SM=1.1, indicated good matching of the methods applied.

In Figure 11 comparison of nitrite concentrations determined with standard method (SM) and new analytical method (NM), shown as nitrite monthly distribution in Split area at KB-SEA site samples investigated during 2019/2020 is presented. Compared methods of nutrient analysis were the same as the methods applied for the ZD-SEA site.

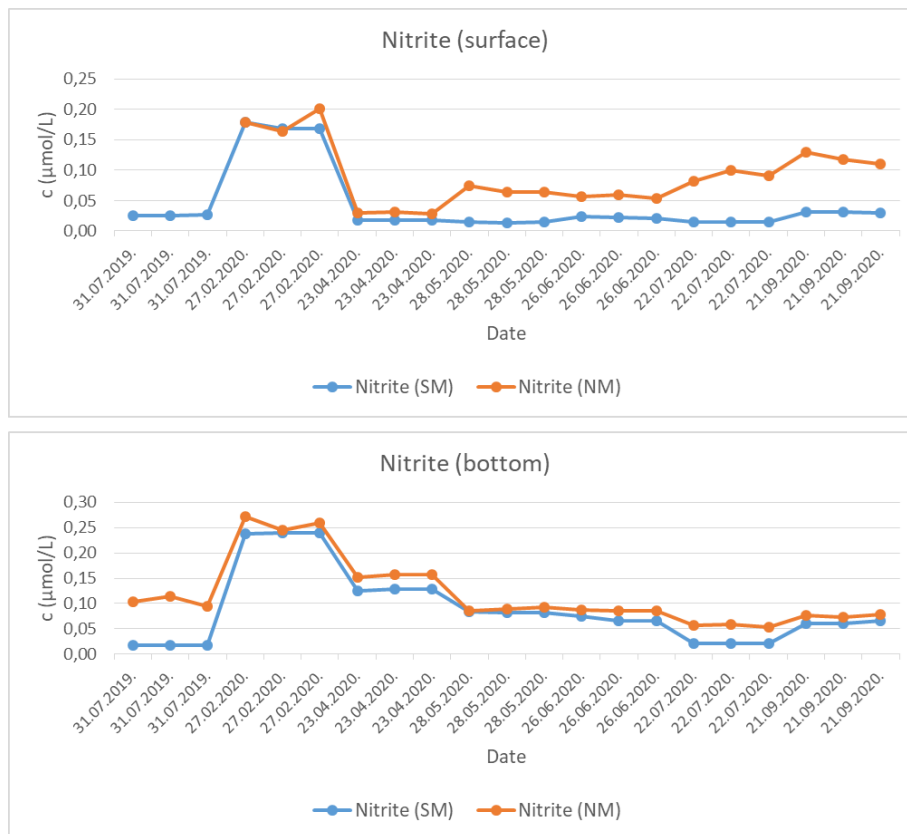


Figure 11. Comparison of nitrite concentrations determined with standard method (SM) and new analytical method (NM), shown as nitrite monthly distribution in KB-SEA site samples investigated during 2019/2020. (Samples were taken in triplicates per each sampling date.)

Relatively good overlapping between concentrations obtained by new method (NM) and standard method (SM) is visible in most of the bottom water samples (Figure 11), while the greater disproportion can be seen in the surface samples. Higher deviations inside the triplicates in CFA samples versus spectrophotometric samples are not visible. Calculated ratio between all nitrite concentrations determined by NM and SM indicated 2,6 times higher values obtained by NM.

In Figure 12 comparison of nitrate concentrations determined with standard method (SM) and new analytical method (NM), shown as nitrate monthly distribution in Split area at KB-SEA site samples investigated during 2019/2020 is presented.

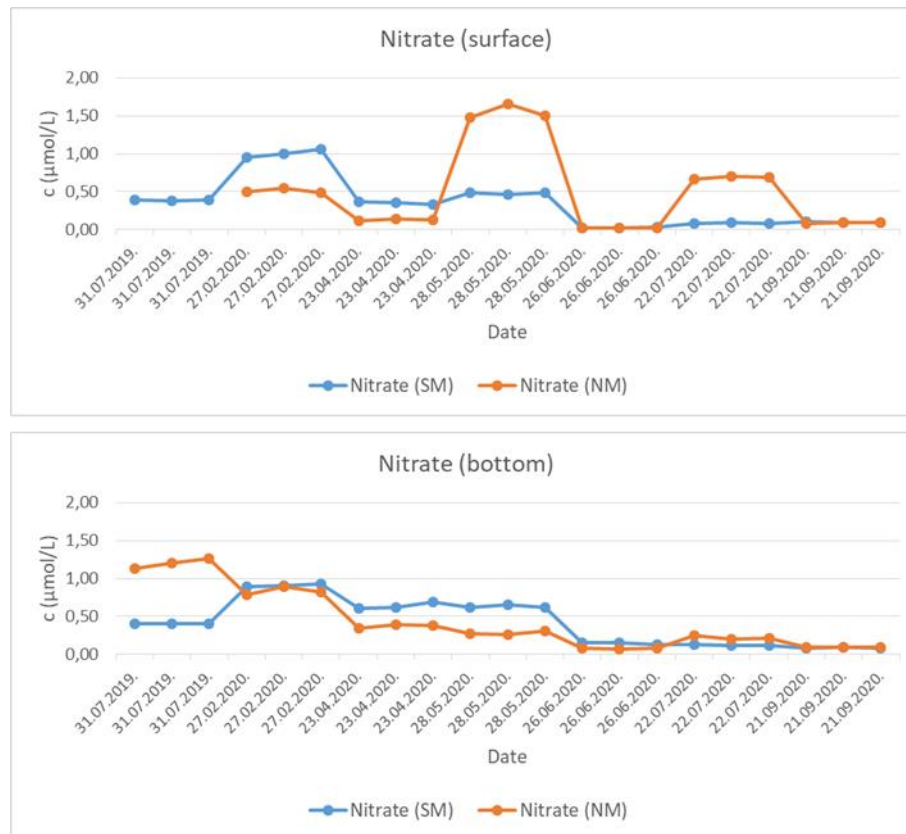


Figure 12. Comparison of nitrate concentrations determined with standard method (SM) and new analytical method (NM), shown as nitrate monthly distribution in KB-SEA site samples investigated during 2019/2020. (Samples were taken in triplicates per each sampling date.)

Relatively good overlapping between concentrations obtained by new method (NM) and standard method (SM) is visible in most of the bottom water samples (Figure 12), while the greater disproportion can be seen in the surface water samples. Furthermore, higher deviations inside the triplicates in CFA samples versus spectrophotometric samples are also visible. Calculated ratio between all nitrate concentrations determined by NM and SM indicated 1,7 times higher values obtained by NM.

In Figure 13 comparison of ammonia concentrations determined with standard method (SM) and new analytical method (NM) applied, shown as ammonia monthly distribution in KB-SEA site samples investigated during 2019/2020 is presented.

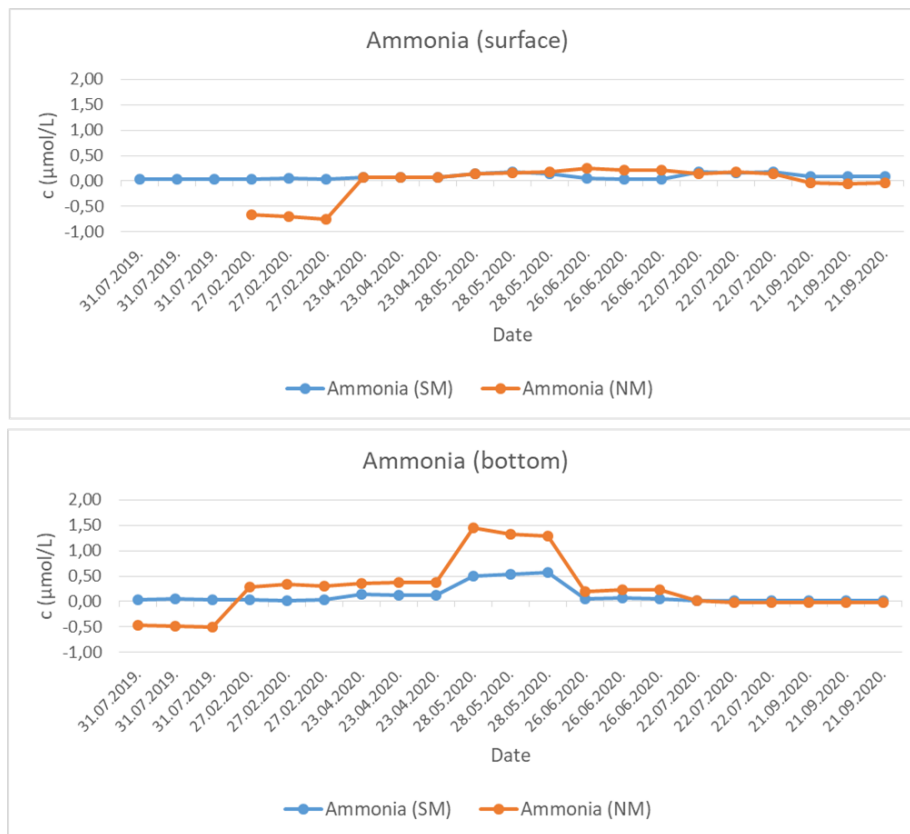


Figure 13. Comparison of ammonia concentrations determined with standard method (SM) and new analytical method (NM) applied, shown as ammonia monthly distribution in KB-SEA site samples investigated during 2019/2020. (Samples were taken in triplicates per each sampling date)

Discrepancy between concentrations obtained by new method (NM) and standard method (SM) is evident, even greater in the bottom layer samples (Figure 6). Negative ammonia concentrations obtained by NM are also visible that are direct consequence of inadequate laboratory conditions during the analytical determinations of this nutrient by CFA method. Due to a large number of negative values, ratio between NM and SM concentrations was not taken into consideration.

In Figure 14 comparison of orthophosphate concentrations determined with standard method (SM) and new analytical method (NM), shown as orthophosphate monthly distribution in KB-SEA site samples investigated during 2019/2020 is presented.

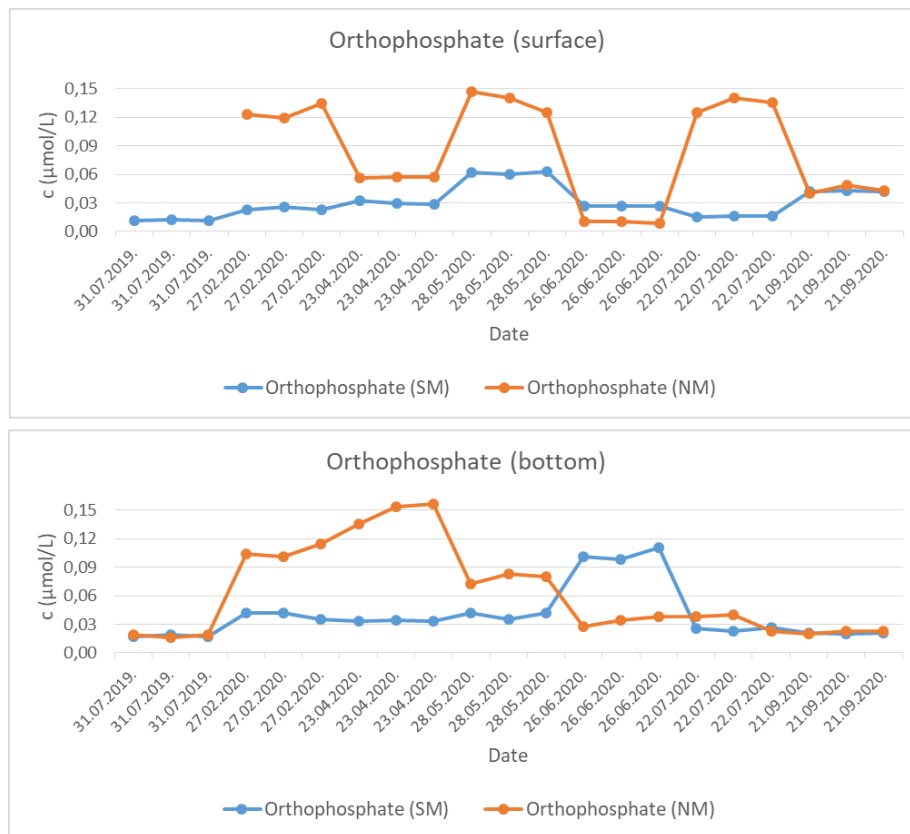


Figure 14. Comparison of orthophosphate concentrations determined with standard method (SM) and new analytical method (NM) applied, shown as orthophosphate monthly distribution in KB-SEA site samples investigated during 2019/2020. (Samples were taken in triplicates per each sampling date)

Large disproportion between concentrations determined by new method (NM) and standard method (SM) is evident (Figure 8). Orthophosphate concentrations obtained by NM are higher in all samples from the surface, while for the bottom water samples in three samplings, lower values than those obtained by SM are evident. Furthermore, higher deviations inside the triplicates in CFA samples versus spectrophotometric samples are also visible. Calculated ratio between all orthophosphate concentrations determined by NM and SM indicated 2,5 times higher values obtained by NM.

In Figure 15 comparison of TP concentrations determined with standard method (SM) and new analytical method (NM) applied, shown as nitrite monthly distribution in KB-SEA site samples investigated during 2019/2020.

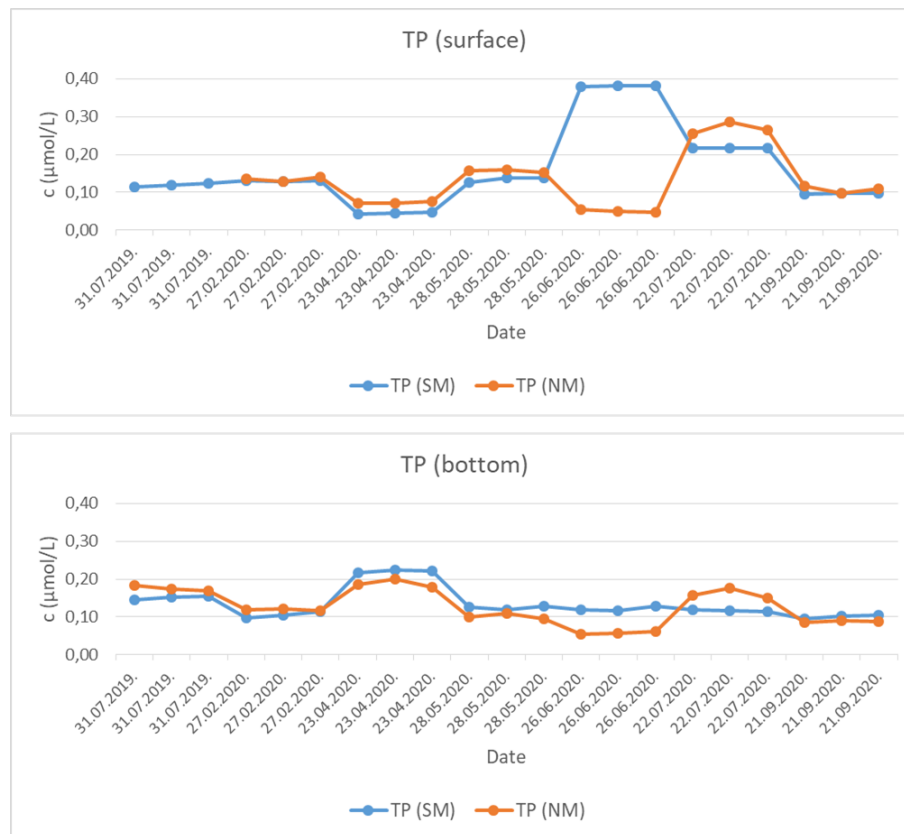


Figure 15. Comparison of TP concentrations determined with standard method (SM) and new analytical method (NM) applied, shown as TP monthly distribution in KB-SEA site samples investigated during 2019/2020. (Samples were taken in triplicates per each sampling date.)

Relatively good overlapping between concentrations obtained by new method (NM) and standard method (SM) is visible in most of the samples (Figure 10), while the greater disproportion can be seen in the surface and bottom water samples during the same period (summer 2020). Furthermore, higher deviations inside the triplicates in CFA samples versus spectrophotometric samples are also visible. Calculated ratio between all silicate concentrations determined by NM and SM was NM:SM=1, indicated good matching of the methods applied.

In Figure 16 comparison of silicate concentrations determined with standard method (SM) and new analytical method (NM) applied, shown as nitrite monthly distribution in KB-SEA site samples investigated during 2019/2020.



Figure 16. Comparison of silicate concentrations determined with standard method (SM) and new analytical method (NM) applied, shown as silicate monthly distribution in KB-SEA site samples investigated during 2019/2020. (Samples were taken in triplicates per each sampling date)

Relatively good overlapping between silicate concentrations obtained by new method (NM) and standard method (SM) is visible in most of the samples (Figure 16), while the greater disproportion can be seen equally in the surface and in bottom water samples. Calculated ratio between all silicate concentrations determined by NM and SM indicated 1,4 times higher values obtained by NM.

In Figure 17 comparison of nitrite concentrations determined with standard method (SM) and new analytical method (NM), shown as nitrite monthly distribution in Split area at ST-SEA site samples investigated during 2019/2020 is presented.

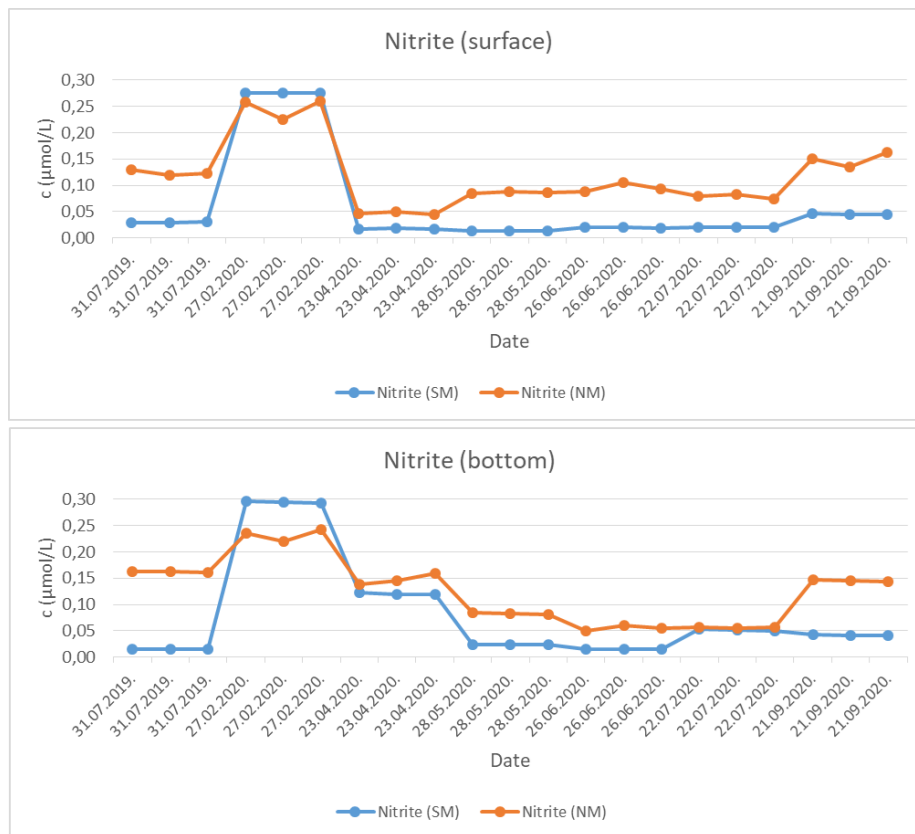


Figure 17. Comparison of nitrite concentrations determined with standard method (SM) and new analytical method (NM) applied, shown as nitrite monthly distribution in ST-SEA site samples investigated during 2019/2020. (Samples were taken in triplicates per each sampling date.)

Discrepancy between concentrations obtained by new method (NM) and standard method (SM) is evident. Higher NM concentrations during most of the samplings are visible for surface and bottom water, except in winter 2020 when SM concentrations were higher than those obtained by NM. Furthermore, higher deviations inside the triplicates in CFA samples versus spectrophotometric samples are also visible. Calculated ratio between all nitrite concentrations determined by NM and SM indicated 3,6 times higher values obtained by NM.

In Figure 18 comparison of nitrate concentrations determined with standard method (SM) and new analytical method (NM), shown as nitrate monthly distribution in Split area at ST-SEA site samples investigated during 2019/2020 is presented.

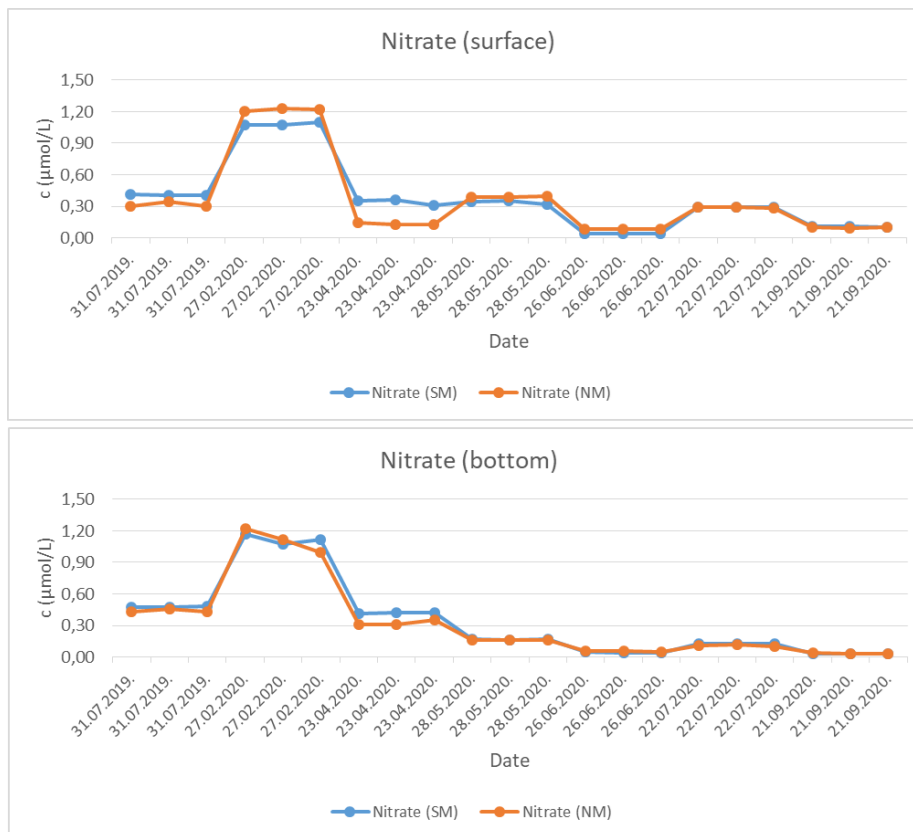


Figure 18. Comparison of nitrate concentrations determined with standard method (SM) and new analytical method (NM), shown as nitrate monthly distribution in ST-SEA site samples investigated during 2019/2020. (Samples were taken in triplicates per each sampling date.)

Very good overlapping between nitrate concentrations obtained by new method (NM) and standard method (SM) is visible in most of the samples (Figure 10), except in February and April 2020. Calculated ratio between all nitrate concentrations determined by NM and SM was NM:SM=1, indicated good matching of the methods applied.

In Figure 19 comparison of ammonia concentrations determined with standard method (SM) and new analytical method (NM), shown as ammonia monthly distribution in Split area at ST-SEA site samples investigated during 2019/2020 is presented.

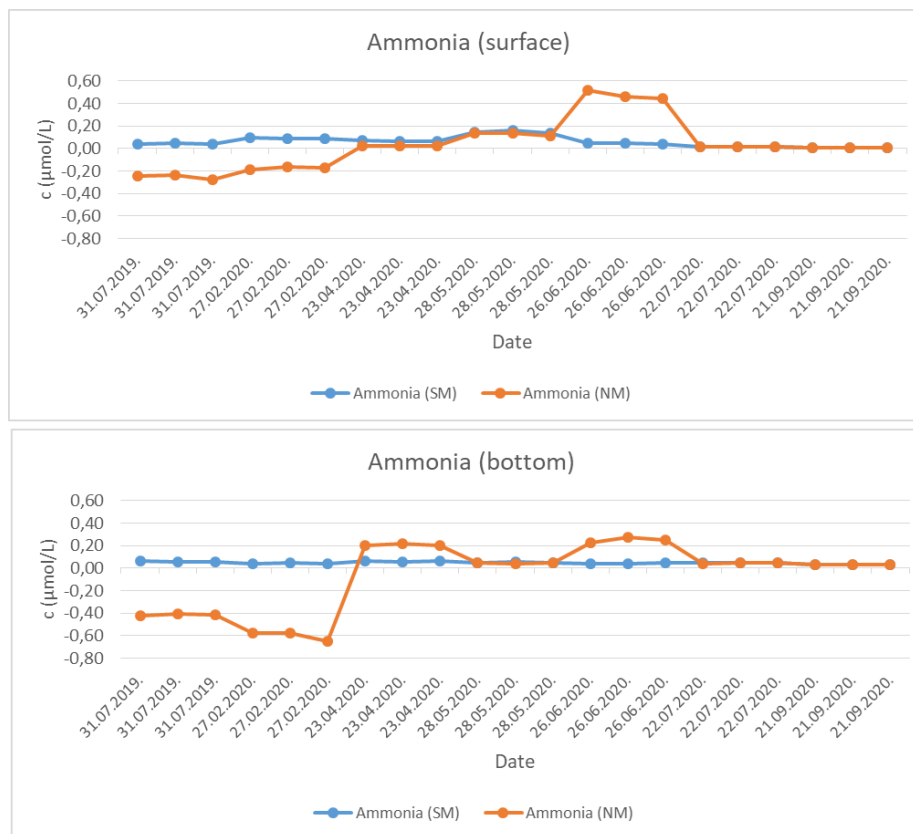


Figure 19. Comparison of ammonia concentrations determined with standard method (SM) and new analytical method (NM) applied, shown as ammonia monthly distribution in ST-SEA site samples investigated during 2019/2020. (Samples were taken in triplicates per each sampling date.)

Disproportion between concentrations obtained by new method (NM) and standard method (SM) is evident, even greater in the bottom layer samples (Figure 19). Negative ammonia concentrations obtained by NM are also visible that are direct consequence of inadequate laboratory conditions during the analytical determinations of this nutrient by CFA method. Due to a large number of negative values, ratio between NM and SM concentrations was not taken into consideration.

In Figure 20 comparison of orthophosphate concentrations determined with standard method (SM) and new analytical method (NM), shown as orthophosphate monthly distribution in Split area at ST-SEA site samples investigated during 2019/2020 is presented.

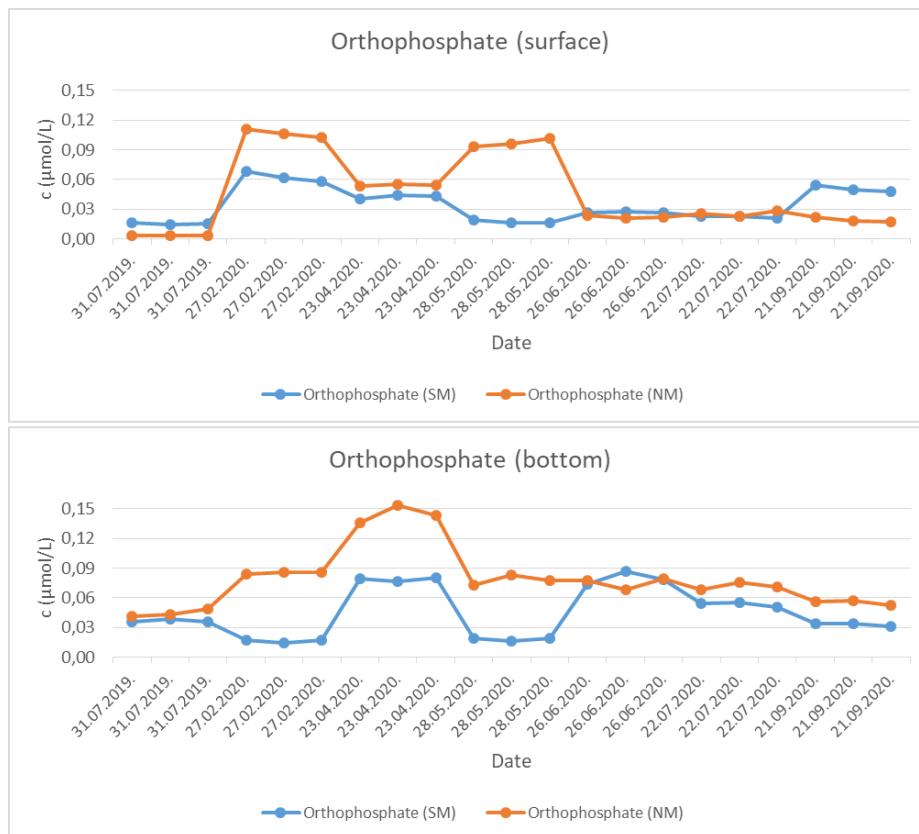


Figure 20. Comparison of orthophosphate concentrations determined with standard method (SM) and new analytical method (NM) applied, shown as orthophosphate monthly distribution in ST-SEA site samples investigated during 2019/2020. (samples were taken in triplicates per each sampling date)

Disproportion between concentrations determined by new method (NM) and standard method (SM) is visible (Figure 20). Orthophosphate concentrations obtained by NM are higher in most of the samples from the bottom water, while in surface, during three sampling periods, lower (or similar) values than those obtained by SM are evident. Calculated ratio between all orthophosphate concentrations determined by NM and SM indicated 2 times higher values obtained by NM.

In Figure 21 comparison of TP concentrations determined with standard method (SM) and new analytical method (NM), shown as TP monthly distribution in Split area at ST-SEA site samples investigated during 2019/2020 is presented.

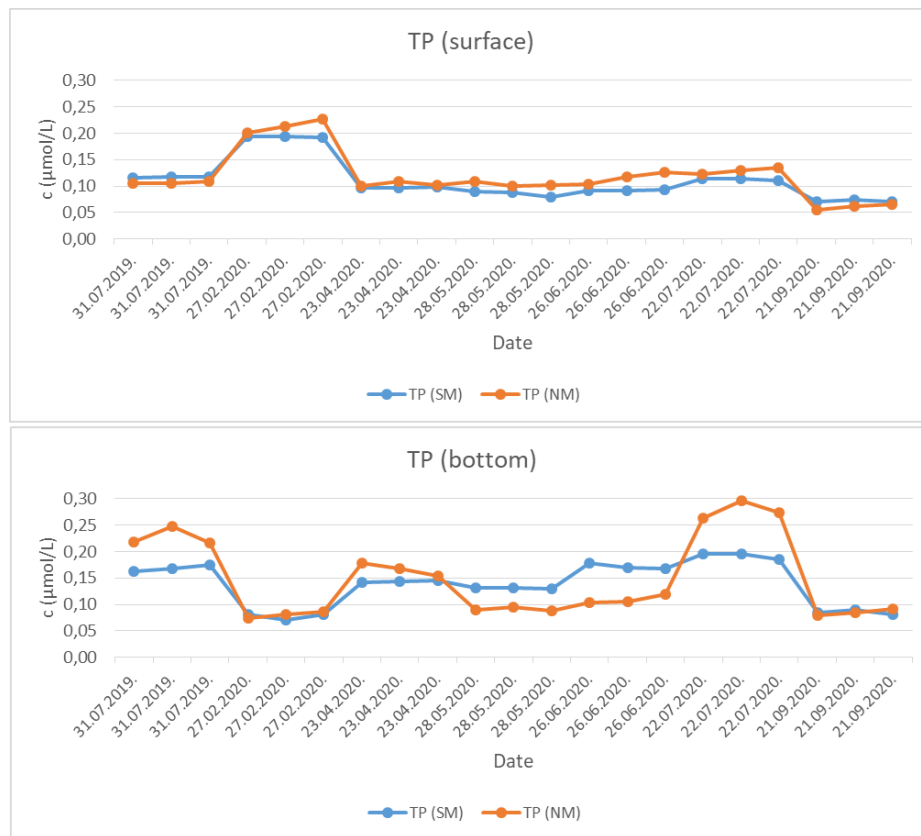


Figure 21. Comparison of TP concentrations determined with standard method (SM) and new analytical method (NM), shown as TP monthly distribution in ST-SEA site samples investigated during 2019/2020. (Samples were taken in triplicates per each sampling date.)

Relatively good overlapping between concentrations obtained by new method (NM) and standard method (SM) is visible in most of the surface samples (Figure 21). Larger disproportion can be seen in the bottom water samples during different periods. Furthermore, higher deviations inside the triplicates in CFA samples versus spectrophotometric samples are also visible. Calculated ratio between all TP concentrations determined by NM and SM was $NM:SM=1$, indicated good matching of the methods applied.

In Figure 22 comparison of silicate concentrations determined with standard method (SM) and new analytical method (NM), shown as silicate monthly distribution in Split area at ST-SEA site samples investigated during 2019/2020 is presented.

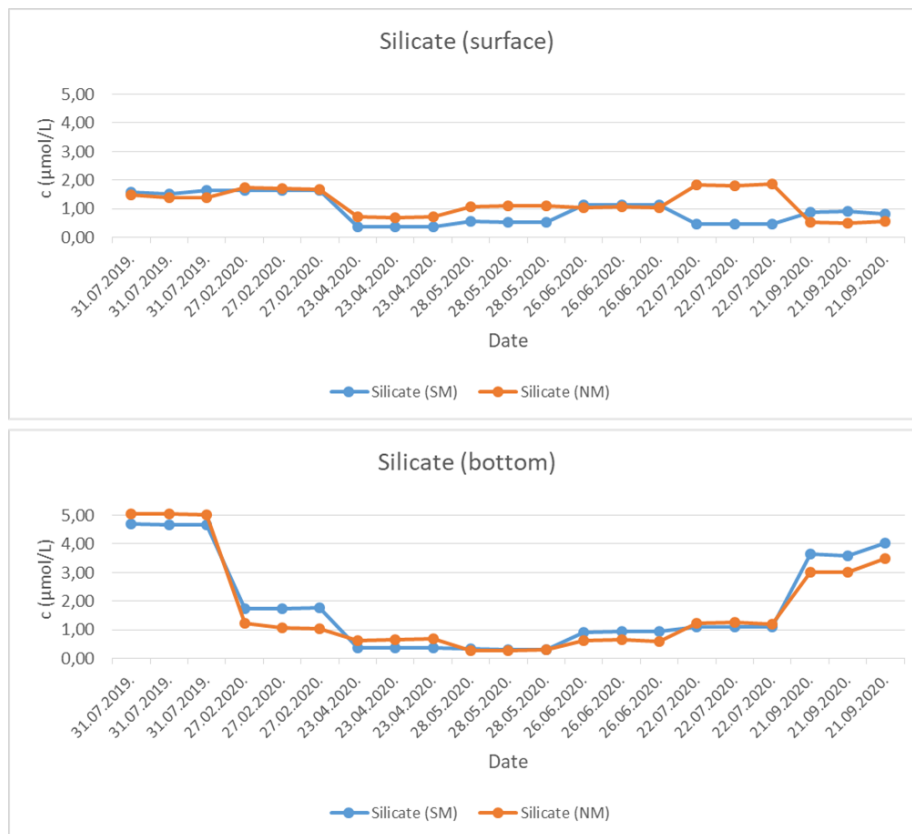


Figure 22. Comparison of silicate concentrations determined with standard method (SM) and new analytical method (NM) applied, shown as silicate monthly distribution in ST-SEA site samples investigated during 2019/2020. (samples were taken in triplicates per each sampling date)

Relatively good overlapping between concentrations obtained by new method (NM) and standard method (SM) is visible in most of the samples (Figure 10), while the greater disproportion can be seen only in surface sample from the summer sampling in 2020. Calculated ratio between all silicate concentrations determined by new and standard method, was $NM:SM=1.3$, indicated good matching of the methods applied.

4.1.1. Statistical data analysis

Correlation analysis of nutrients concentrations determined using standard and new methods was performed in the program Statistica 13, using non-parametric Spearman correlation (Table 7). Statistically significant correlations were found between the concentrations determined by the application of SM and NM for most of the investigated nutrients with the highest coefficients for silicate ($R = 0.705$; $p < 0.01$); nitrate $R = 0.701$; $p < 0.01$), total phosphorus ($R = 0.701$; $p < 0.01$) and nitrite ($R = 0.688$; $p < 0.01$). (A correlation between orthophosphate concentrations determined by SM and NM was not established.

The obtained results are in accordance with the interpretation of comparative measurements of the concentrations of these nutrients in the water column shown in Figures 5-21.

Table 7 Spearman correlation analysis performed on nutrient concentrations data (significant correlations are marked in blue)

Nutrient	Spearman Rank Order Correlations (Marked correlations are significant at $p < 0,01000$)								
	Nitrite (SM)	Nitrite (NM)	Nitrate (SM)	Nitrate (NM)	TP (SM)	TP (NM)	OrthoP (SM)	OrthoP (NM)	Silicate (SM)
Nitrite (NM)	0,688								
Nitrate (SM)	0,589	0,626							
Nitrate (NM)	0,246	0,395	0,701						
TP (SM)	0,252	0,153	0,143	0,168					
TP (NM)	0,190	0,254	0,322	0,411	0,616				
OrthoP (SM)	0,230	0,096	0,053	-0,149	0,277	0,177			
OrthoP (NM)	0,234	0,235	0,383	0,341	0,163	0,345	0,219		
Silicate (SM)	0,343	0,350	0,087	0,167	0,098	0,098	0,131	-0,257	
Silicate (NM)	0,067	0,172	0,000	0,212	0,181	0,181	0,194	-0,186	0,705

*Ammonia concentrations were not taken into account for the reasons explained earlier.

4.2. Comparison of methods applied in the waste water samples

In this section the comparison between standard methods and new methods of analytical determination of nutrients in waste water samples is given.

Comparison of analytical techniques of nutrient determination in the waste waters was possible only for samples from Zadar WWTP, particularly for nitrite and nitrate salts. Nutrients in waste water samples from Split area (Katalinića brig and Stupe WWTP) were analysed only by using standard methods.

In Table 7 descriptive statistics on the whole set of nutrient concentrations data in waste waters obtained by standard (SM) and new methods (NM) of determination is presented.

Table 7 Descriptive statistics (number of valid cases, mean, geometric mean, median, minimum, maximum, lower and upper quartile LQ; UQ, variance and standard deviation) on the nutrient concentration data (and chlorides)

Parameter	Descriptive statistics for WW								
	N	Mean	Geo Mean	Median	Min	Max	LQ	UQ	St. Dev.
Ammonia	67	23,81	9,83	23,12	0,12	67,12	2,48	39,5	20,02
Nitrite (SM)	67	54,98	4,28	102	0,01	102	0,29	102	51,06
Nitrite (NM)	27	11,41	2,17	2,68	0,03	47,75	0,46	19,96	15,16
Nitrate (SM)	67	0,4	0,28	0,27	0,07	2,69	0,18	0,38	0,52

Nitrate (NM)	27	5,21	2,11	2,66	0,15	25,59	0,68	5,46	7,58
TN (SM)	67	35,38	26,87	31	2,67	67,61	24,4	55,09	19,79
TP (SM)	67	4,14	3,23	3,58	0,35	11,12	1,55	6,56	2,75
Chloride	27	3599	3315	3163	1607	6901	3113	3694	1522

On Figure 23 comparison of nitrite concentrations determined with standard method (SM) and new analytical method (NM) applied is shown as nitrite monthly distribution in waste waters at Zadar WWTP investigated during 2019/2020 is presented. Applied technique for nitrite was ion chromatography IC (EN ISO 10304-1:2009/Isp.2016) versus standard spectrophotometric method (Standard Methods for Examination of Water and Wastewater, 21st Edition (2005) p4-118 Method 4500-NO₂⁻).

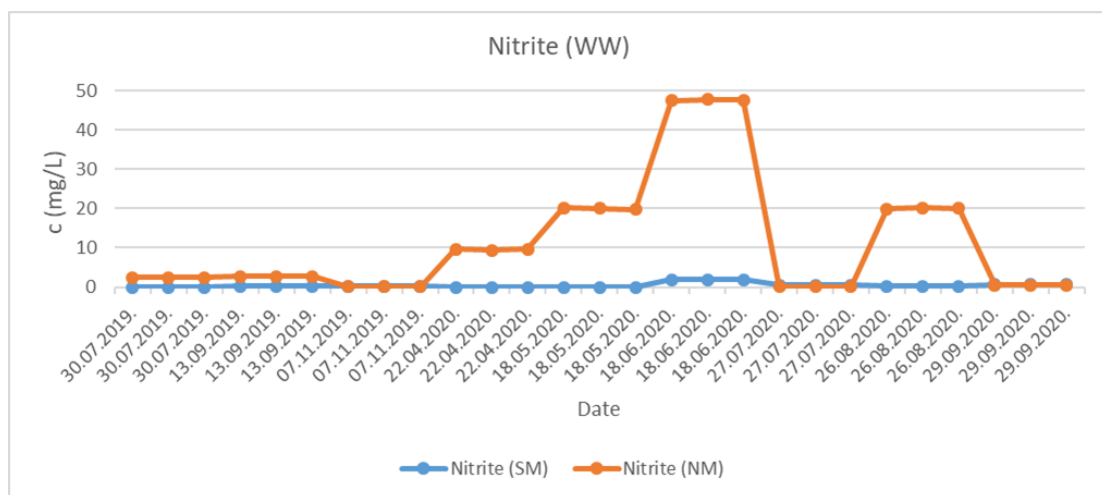


Figure 23. Comparison of nitrite concentrations determined with standard method (SM) and new analytical method (NM), shown as nitrite monthly distribution in waste water samples investigated at Zadar WWTP site during 2019/2020. (Samples were taken in triplicates per each sampling date.)

Extremely large discrepancy between SM and NM is evident, with higher concentrations in samples determined by NM, particularly during 2020 (Figure 23). Calculated ratio between nitrite concentrations determined by NM and SM indicated 350 times higher NM concentrations than those obtained by SM.

On Figure 24 comparison of nitrate concentrations determined by standard method (SM) and new analytical method (NM), is shown as monthly distribution of nitrate concentrations in waste waters at Zadar WWTP investigated during 2019/2020. The techniques applied for nitrates were: ion chromatography method IC (EN ISO 10304-1:2009/Isp.2016) versus standard spectrophotometric method (In house method: PO-7.2/77; Issue 01/1 2019-09-02).

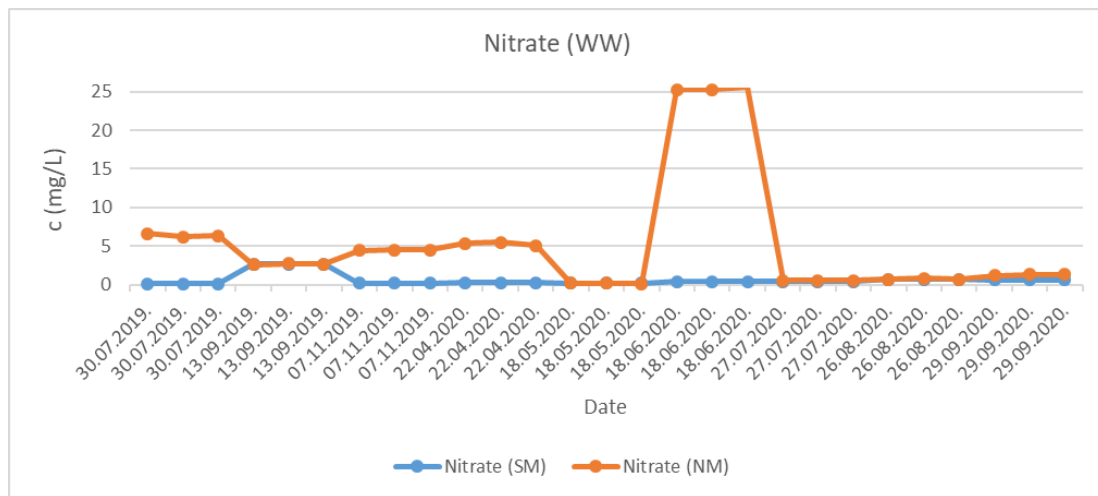


Figure 24. Comparison of nitrate concentrations determined with standard method (SM) and new analytical method (NM), shown as nitrite monthly distribution in waste water samples investigated at Zadar WWTP site during 2019/2020. (Samples were taken in triplicates per each sampling date.)

Extremely discrepancy between SM and NM is evident, with higher concentrations in samples determined by NM, particularly during 2020 (Figure 24). Calculated ratio between nitrate concentrations determined by NM and SM indicated 200 times higher NM concentrations than those obtained by SM.

The explanation of disproportion of results obtained by two methods is in dilution of the samples. Namely, the wastewater samples determined by IC method (nitrites, nitrates and sulphates), must be diluted if the concentrations of chlorides are higher than 100 mg/L. Concentrations of chlorides in the waste water samples ranged between 1000 and 6000 mg/L (Table 7). Accordingly, dilutions ranged from 20 to 100. Furthermore, waste water samples had to be filtered through filter paper to obtain the homogeneity of the sample, as well as through a special filter paper that is used for IC method.

PART 5: CONCLUSIONS

In this report is given the overview of the monitoring on the parameters at the Croatian investigated sites on discharge points located in Zadar and Split area as well as in the waste waters at WWTPs. Detailed sampling locations and sampling frequencies are presented, all monitored parameters and the methodologies applied. The comparison between standard analytical techniques of nutrient analysis and new technologies applied is shown.

A comparison of standard methods (SM) and new analytical methods (NM) in determining the concentration of nutrients in seawater samples showed:

- Generally higher concentrations of nutrient salts are obtained by applying NM compared to SM (CFA method versus spectrophotometric determination)
- Large deviations in NM concentrations in relation to SM concentrations in the determination of orthophosphate
- Large deviations and negative results in the determination of ammonia resulting from inadequate laboratory conditions during the analytical determination
- Relatively good compliance of the concentrations obtained for nitrites and nitrates
- Relatively good compliance of the concentrations obtained for total phosphorus and silicates (with better compliance in samples with higher concentrations)
- No existing of the pattern in the distribution of concentrations' deviation regarding the layer of the water column (surface or bottom) (SITE SPECIFIC PATTERN, IF THERE IS ONE!)
- No existing of the pattern in the distribution of concentrations' deviation regarding the sampling season
- Statistical analysis revealed significant correlation between SM and NM for silicate, nitrate, TP and nitrite, while for orthophosphate, correlation was not found

A comparison of standard methods (SM) and new analytical methods (NM) in determining the concentration of nutrients in waste water samples showed:

- Extremely higher concentrations of nitrites obtained by NM in relation to SM (ion chromatography method versus spectrophotometric determination)
- Extremely higher concentration of nitrates obtained by NM in relation to SM ((ion chromatography method versus spectrophotometric determination)
- These results are directly connected with dilution of samples due to high chloride concentrations in waste water samples