

## D 4.2.1

# REPORT OF SEASONAL TREND OF THE CHECKED PARAMETERS ON THE IT COAST

**AdSWiM PROJECT**

<b>Work package:</b>	<b>WP 4 - Innovative solutions in analytical, microbiological controls and to treat urban wastewaters (UWW)</b>
<b>Activity:</b>	<b>4.2 Innovative Analytical Methods/Devices (IAMD) Nutrients and trace elements</b>
<b>Leader:</b>	<b>UNIVPM</b>
<b>Report:</b>	<b>4.2.1. Report of seasonal trend of the checked parameters on the IT coast</b>

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## PART 1: INTRODUCTION

The aim of the work package 4 is to implement technological and control solutions to obtain tools, and so offer services, for the assessment of environmental quality and to ensure that the environment is protected from the effects of the wastewater discharge.

The quality definition of a water body according to the Water Framework Directive provides a broad spectrum of analyses both on chemical- physical and biological parameters. This is because the hygienic quality of the marine environment, the equilibrium of aquatic ecosystems (i.e. the preservation of their biodiversity and productivity) are dependent on the availability of nutrients (phosphorus, nitrogen, organic matter), and their relative relationships. The project aim is to maintain and improve the quality of BW through a controlled use of UWWT. The activities planned were carried out according to the sampling/monitoring plan defined in WP3. To achieve this ambitious purpose the WP was organized into six different but interconnected (Table 1) activities concerning four main topics, stimulating the cross-border cooperation involving all PPs of the project.

The outputs were available to the general public and to governmental organizations that have new analytical tools and new operating control models and the research sector to optimize new devices and new benchmarks. Also managers of DP can benefit from having a more efficient process with lowered impact to the environment.

Table 1. List of different work packages for the technical implementation of the WP4.

### Interreg IT-HR AdSWiM

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#### **wp 4 : technical implementation of the project**

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test of methodologies for microbial load reduction **WP 4.1**

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characterization of treated WW and of the seawater in proximity of the discharging line:  
analysis of nutrients and metal ions **WP 4.2**

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set up of biosensors for nutrient (phosphate) and toxicity (algae biosensors) measurements **WP 4.3 and 4.4**

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mapping of the microbial species at the depuration plants **WP 4.5**

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mapping of the microbial species at the depuration plants **WP 4.6**

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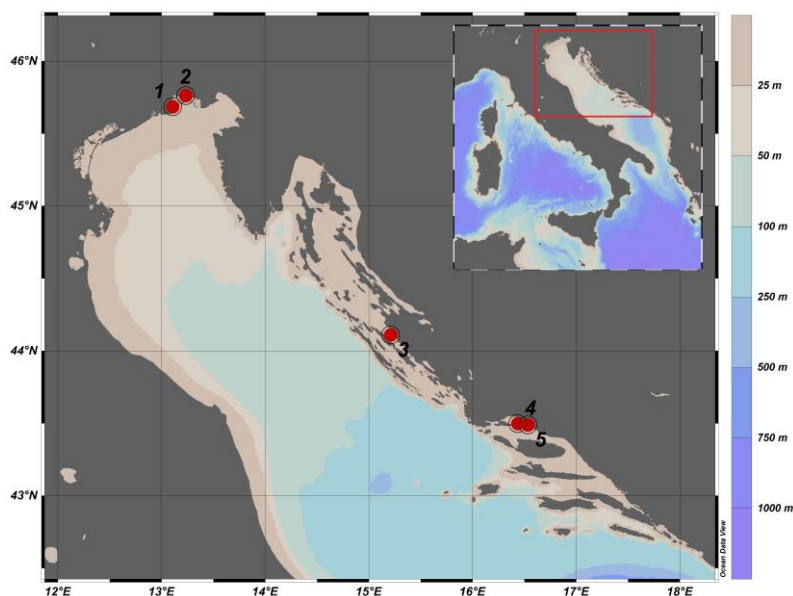
In the framework of WP 4.2 (Innovative Analytical Methods/Devices IAMD) a characterization of treated WW and of the seawater in proximity of the discharging line, concerning nutrients (nitrate, nitrite, ammonium, phosphate and silicate) and metal ions (e.g. Pb, Cd, As, Hg) determination will be performed. In relationship with master hydrological variables (T°, salinity, EC, pH, DO, %Oxygen Saturation, Eh, Chl a, turbidity). To achieve this objective according to WP3 plan we collected samples of treated wastewater in the depuration plants both in Italy than in Croatia and at sea in the proximity of emitting points, with a monthly frequency between April and September (bathing season 2019 and 2020).

In line of this task of Activity 4.2., is this **Report of seasonal trend of the checked parameters on the IT coast (D4.2.1)** 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.

## PART 2: INVESTIGATED SITES

The Adriatic Sea extends in a northwest-southeast direction and is located between the Italian peninsula and the Balkans. The seabed of the northern zone has average bathymetry levels of 35 m, the middle Adriatic of 140 m while the southern zone exceeds 1200 m. The exchanges of water between the Adriatic basin and the Mediterranean Sea take place through the channel of Otranto. Fluvial inputs are particularly important in the northern area and influence its circulation. Runoff phenomena are also important and make the Adriatic basin a dilution basin, since the freshwater gain is about 1 m, as evaporation and precipitation almost compensate each other.

Five sampling stations in three different geographical areas along the Italian-Croatian coastline in the Adriatic Sea were identified, and for each site, two samples were collected: one sampled directly from DP outflow (after the treatment), and one collected in the coastal area, next to the DP discharging pipelines. Samples for nutrients analysis were collected only for the Italian stations (1 - Lignano and 2 - San Giorgio), whereas samples for PTEs determinations were collected both in Italian and Croatian stations. **Figure 1** shows the sampling stations: Gulf of Trieste (Lignano Sabbiadoro and San Giorgio di Nogaro DPs), Zadar (Zadar Upov Centar DP), and Split (Katalinića brig and Stupe DPs). **Table 2** provides information about each DP.



**Figure 1.** Sampling stations in northern Adriatic Sea: Lignano Sabbiadoro (Gulf of Trieste, ITA) (1), San Giorgio di Nogaro (Gulf of Trieste, ITA) (2), Zadar Upov Centar (Zadar, HR) (3), Katalinića brig (Split, HR) (4), and Stupe (Split, HR) (5).

**Table 2.** Depuration Plants description.

DP (Province, State)	ES design capacity <sup>a</sup>	Wastewater input	Wastewater treatment	Distance of discharge from the coast (m) (km)	Depth of diffusors
Lignano Sabbiaodoro (Udine, Italy)	250 000	Households and industry.	Mechanical and biological treatment (primary and secondary settler), and UV and peracetic acid disinfection.	7.5	15
San Giorgio di Nogaro (Udine, Italy)	700 000	Mostly from industry.	Mechanical and biological treatment (primary and secondary settler), and peracetic acid disinfection.	10	14
Zadar Upov Centar (Zadar, Croatia)	100 000	Mostly from households.	Mechanical treatment (rough and fine grid), sand and oil removal, biological treatment.	2	33.8
Katalinića brig (Split, Croatia)	122 000	Households along with rainfall runoff wastewater.	Mechanical treatment (rough and fine grid).	1.3	41.8
Stupe (Split, Croatia)	138 000	Mostly from households.	Mechanical treatment (rough and fine grid) along with sand and oil removal.	2.75	36.0

<sup>a</sup> Number of inhabitants for which the DP was designed for.

In the Gulf of Trieste, samples were collected monthly during the bathing season, from April to October, in 2019 and 2020 (**Table 3**) according to the Italian legislation for seawater environmental quality monitoring. Concerning Croatian sites, a similar sampling strategy was adopted in 2020, whereas in 2019 only a few samples were collected, due to logistical constraints (**Table 3**).

**Table 3.** Sampling activity scheduled for 2019 and 2020 seasons.

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
<b>2019</b> Gulf of Trieste				X	X		X	X		X		
Zadar							X		X		X	
Split							X					
<b>2020</b> Gulf of Trieste				X	X	X	X		X	X		
Zadar				X	X	X	X	X	X			
Split		X			X	X	X		X			

Before collecting water samples at sea, depth profiles of temperature, salinity, pH, turbidity, and oxygen were obtained by means of a CTD Idromar APWIN IP041D (Italian sites) and an SBE 25 Sealogger CTD Sea-Bird Electronics (Croatian sites). Seawater samples were collected with a 2-L Ruttner bottle at Italian sites, and PWS 5-L Niskin bottles Hydro-Bios at Croatian sites. Samples were collected at the bottom (between 13 and 14 m) in the Gulf of Trieste whereas at Croatian stations, samples were collected both in surface (0.5 m) and bottom (from 34 to 42 m) waters. Aliquots destined to be analysed for PTEs were collected into 0.5 L HDPE bottles previously decontaminated with a specific acid-cleaning procedure and stored at -20°C until further analyses.



### PART 3: INVESTIGATED PARAMETERS

All the analytical steps were carried out in a clean room laboratory ISO 14644-1 Class 6, with areas at ISO Class 5 under laminar flow. As for sampling bottles, the same acid-cleaning procedure was performed for all the laboratory materials. Reagents were weighted using the analytical balance AT261 Mettler Toledo (Greifensee, Switzerland, readability 0.01 mg, repeatability SD = 0.015 mg). Variable volume micropipettes and neutral tips were from Brand (Transferpette, Wertheim, Germany). Ultrapure water was produced from a Milli-Q water system from Merck Millipore (Darmstadt, Germany).

PTEs reagents and instruments:  $H_4BNa$  ( $\geq 98.0\%$ ) was from Sigma-Aldrich, NaOH (99.99%, metal basis) was from Alfa Aesar (Thermo Fisher, Kandel, Germany),  $CoCl_2$  (97%) was from Acros Organics (Thermo Fisher, Kandel, Germany), Ascorbic Acid (RPE grade), KI (RPE grade),  $CH_4N_2S$  (RPE grade), and HCl (32.35% RS ultrapure grade) were from Carlo Erba (Milan, Italy). 0.45  $\mu m$  mixed cellulose esters filters (47 mm diam,) were from ChemBio (Camerino, Italy). SLEW-3, NASS-6 (NRC CNRC, Ottawa, Canada) and Trace Metals 1 in Seawater (QC3163, Sigma-Aldrich) were used as certified reference materials. Samples were filtered through decontaminated 0.45  $\mu m$  mixed esters of cellulose filters and acidified with ultrapure grade HCl 2% (v/v). Analyses were carried out with an Atomic Fluorescence Spectrometer (AFS) Titan 8220 (Fulltech Instruments, Rome, Italy). Argon 5.0 (99.999% purity) was used as a gas carrier. Reductant agents to produce metals hydrides were  $H_4BNa$  0.05% in NaOH 0.4% (m/v) for Hg determination,  $H_4BNa$  4.0% in NaOH 0.4%,  $CH_4N_2S$  1.0% (m/v), and  $CoCl_2$  1.0  $\mu g mL^{-1}$  for Cd determination, and  $H_4BNa$  2% in NaOH 0.5% (m/v) for As determination. For As analyses, samples were added with HCl 10% (v/v), 1% KI (m/v), and 0.5% ascorbic acid (m/v) and analysed after 1 hour. AFS instrumental parameters are reported in **Table 4**. **Table 5** shows the instrumental limit of detection (LOD, based on  $3\sigma$ ,  $n = 10$ ) and limit of quantification (LOQ, based on  $10\sigma$ ) [1] and quality control analyses.

**Table 4.** Instrumental parameters of Atomic Fluorescence Spectrometer (AFS) for water analyses.

Instrumental parameter	Cd	Hg	As
PMT (Volts)	280	275	320
Lamp current (mA)	60/30	30/0	60/30
Carrier Gas ( $mL min^{-1}$ )	500	300	300
Shield Gas ( $mL min^{-1}$ )	800	1000	800
Reading time (s)	15	15	15
Delay Time (s)	0.5	0.5	0.5
Blank Judgement Value (if)	10	5	10
Torch height (mm)	8	10	8
IFS Step (s x rpm, a=analyte; c=carrier; r=reading)	(a) 10 x 100 (c) (r) 16 x 120	(a) 10 x 100 (c) (r) 16 x 120	(a) 10 x 100 (c) (r) 18 x 120

Nutrients reagents, instrument and methods:

All reagents used for reagents and standard solutions preparations were analytical grade or higher and purchased from Merck, Germany. Instrumental analyses were performed using an automatic discrete analyser colorimeter EasyChemPlus, SYSTEA, Italy. Samples collected were filtered on site using 0.45  $\mu\text{m}$  GF filters, then conserved at  $-20^{\circ}\text{C}$  until the proceed for laboratory analysis. A washing cycle with a 1:1000 HCl superpure solution was applied between each analysis in order to avoid cross-contamination phenomena.

The following methods were applied for nutrient determinations:

For N-NO<sub>2</sub> EPA #354.1 method was used. Nitrites react with sulphanilamide in acid conditions to give a diazo compound. With N-(1-Naphthyl) ethylenediamine, it becomes a pink coloured complex. Colorimetric measurements are performed at 546 nm (LOD=0.028528  $\mu\text{mol L}^{-1}$ ; 0.40  $\mu\text{g L}^{-1}$ ; LOQ=0.0864  $\mu\text{mol L}^{-1}$ ; 1.21  $\mu\text{g L}^{-1}$ ). For N-NO<sub>3</sub> Ref. National Environmental Methods Index 9171 Nitrate via V(III) reduction was used. Nitrates are reduced to nitrites by an acid solution of vanadium chloride and then determined as nitrite. This vanadium-based method directly measures the nitrate concentration, since the nitrite fraction is subtracted automatically as part of sample blank (LOD=0.0663  $\mu\text{mol L}^{-1}$ ; 0.9286  $\mu\text{g L}^{-1}$ ; LOQ=0.201  $\mu\text{mol L}^{-1}$ ; 2.814  $\mu\text{g L}^{-1}$ ). N-NH<sub>3</sub> was determined with EPA #350.1 method. Ammonia reacts in basic conditions with sodium salicylate and hypochlorite in the presence of nitroprusside salts to form an emerald-green chromatic substance. The pH in the colorimeter cell must approach 12.6. Colorimetric measurements are performed at 670 nm (LOD=0.08  $\mu\text{mol L}^{-1}$ ; 1.45  $\mu\text{g L}^{-1}$ ; LOQ=0.244  $\mu\text{mol L}^{-1}$ ; 4.41  $\mu\text{g L}^{-1}$ ). Inorganic P (P-PO<sub>4</sub><sup>3-</sup>) was determined with EPA #365.1 method. Phosphates are mixed with an acid solution of molybdate to produce  $\alpha$ -Keggin-type heteropolyoxometalates PMo<sub>12</sub>O<sub>40</sub><sup>3-</sup>. In the presence of antimony to catalyse the reaction, this anion is then reduced by ascorbic acid to form the blue coloured  $\beta$ -Keggin ion. Colorimetric measurements are performed at 880 nm (LOD=0.011  $\mu\text{mol L}^{-1}$ ; 0.35  $\mu\text{g L}^{-1}$ ; LOQ=0.034  $\mu\text{mol L}^{-1}$ ; 1.05  $\mu\text{g L}^{-1}$ ). Organic P was evaluated by the difference between total P and Inorganic P. Total P was obtained by adding the oxidant mix of persulfate, boric acid and sodium hydroxide and put samples in autoclave at 120°C for 45 minutes as described by [21]. Total P was then immediately determined with EPA #365.1 method as PO<sub>4</sub><sup>3-</sup>. For Si-SiO<sub>2</sub> APHA Standard Methods for the Examination of Water and Wastewater 4500-SiO<sub>2</sub> method was used. Silicates are mixed with an acid solution of ammonium molybdate to produce  $\alpha$ -Keggin type heteropolyoxometalates SiMo<sub>12</sub>O<sub>40</sub><sup>4-</sup>. In the presence of oxalic acid to mask the interference of phosphates, this anion is then reduced by ascorbic acid to form the blue coloured  $\beta$ -Keggin ion. Colorimetric measurements are performed at 880 nm (LOD=0.0789  $\mu\text{mol L}^{-1}$ ; 2.2  $\mu\text{g L}^{-1}$ ; LOQ=0.2392  $\mu\text{mol L}^{-1}$ ; 6.71  $\mu\text{g L}^{-1}$ ). All the analysis were performed between 12-36 hours after the sample thawing, SD and RSD were calculated to enhance the accuracy of the measurements. In order to avoid instrumental errors, Certified reference material QC3179 - Simple nutrients in seawater, purchased from Merck, was processed during the analysis as Quality Control.

Accuracy control: certified reference materials were processed during the analysis of PTEs and Nutrients as Quality Control. Table 5 shows the results obtained.

**Table 5.** Instrumental LOD and LOQ of Atomic Fluorescence Spectrometer (AFS) and colorimeter EasyChemPlus and accuracy control test.

Element	LOD ( $\mu\text{g L}^{-1}$ )	LOQ ( $\mu\text{g L}^{-1}$ )	CRM		
			Name	Certified value ( $\mu\text{g L}^{-1}$ )	Measured value ( $\mu\text{g L}^{-1}$ )
Hg	0.0006	0.006	QC3163	17.6 $\pm$ 0.314	17.3 $\pm$ 0.2
Cd	0.0005	0.005	NASS-6	0.0311 $\pm$ 0.0019	0.031 $\pm$ 0.008
As	0.01	0.13	SLEW-3	1.36 $\pm$ 0.09	1.47 $\pm$ 0.27
N-NO <sub>3</sub>	0.93	2.81	QC3179	18300 $\pm$ 150	18400 $\pm$ 200
N-NO <sub>2</sub>	0.40	1.21	QC3179	2910 $\pm$ 20	2910 $\pm$ 60
N-NH <sub>3</sub>	1.45	4.41	QC3179	9710 $\pm$ 0.4	9520 $\pm$ 0.35
P-PO <sub>4</sub>	0.35	1.05	QC3179		2920 $\pm$ 40
Si-SiO <sub>2</sub>	2.20	6.71	-	-	-

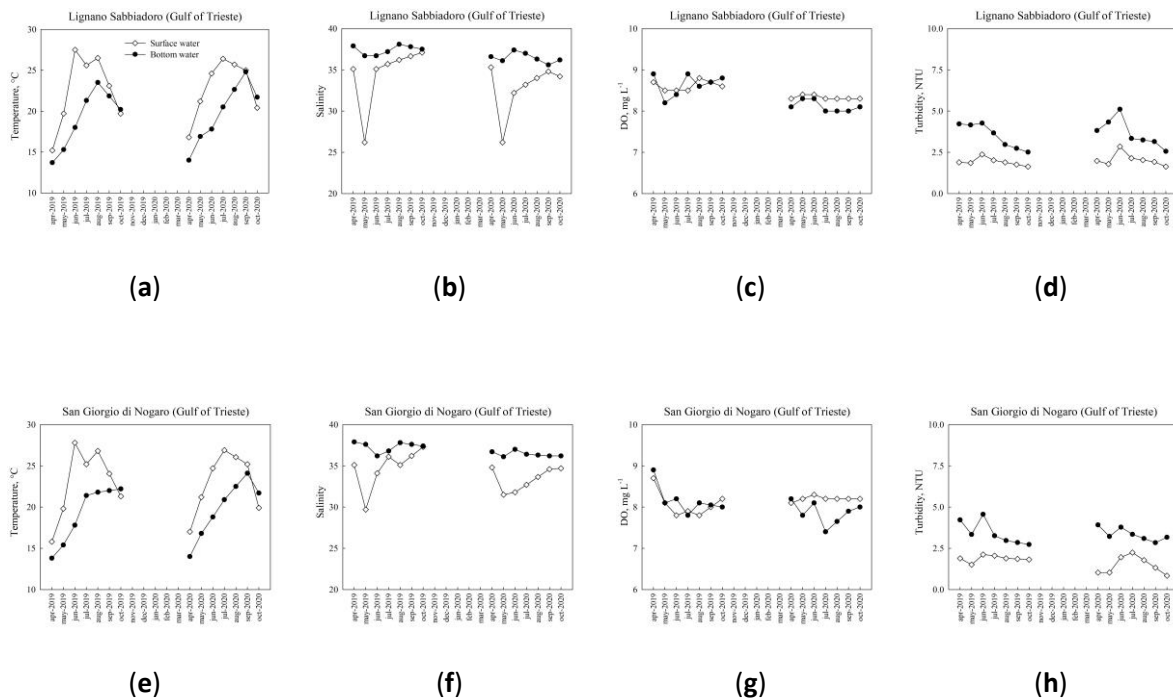
## PART 4: RESULTS AND DISCUSSION

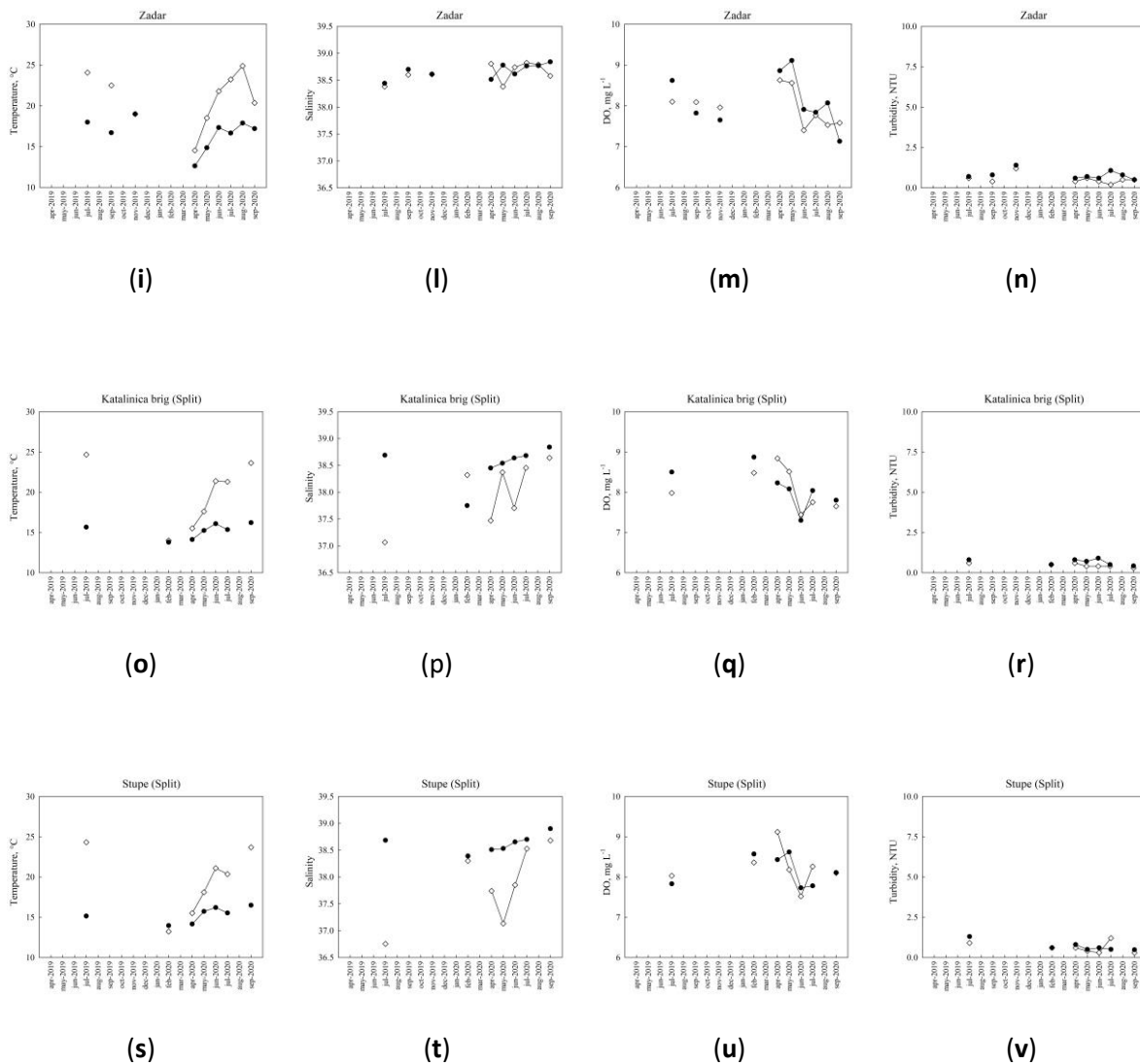
### Chemical Physical Parameters

Temperature (°C), salinity, turbidity, and dissolved oxygen seasonal trends are reported in **Figure 2**. The two stations located in the Gulf of Trieste showed similar temperature trends. Maximum values were reached in June 2019 (27.8 °C) and July 2020 (26.9 °C) in surface waters, while bottom waters showed the highest values in August 2019 (23.5 °C) and September 2020 (24.8 °C). Salinity in bottom water showed varied between 35.6 and 37.9, while in surface water a minimum was recorded in May 2019 (26.2) and May 2020 (26.2). The dissolved oxygen was similar in 2019, while in 2020 bottom water showed lower values in both sites. Turbidity was recorded from 0.8 to 5.1 NTU with a similar trend both in surface and bottom water, but with higher values in bottom waters.

In Zadar, the maximum surface temperature was reached in July 2019 (24.7 °C) and August 2020 (24.9 °C), while in bottom waters it ranged between 12.6 and 18.0 °C. The salinity was stable at 38.0. The dissolved oxygen varied from 7.1 to 9.1 mg L<sup>-1</sup>, while the turbidity was always lower than 1.1 NTU.

As for the Gulf of Trieste, in Slipt sampling stations temperature trend was similar in the site of Katalinića brig and Stupe, with summer temperature maximum values of 24.7 and 23.6°C (surface) and 15.7 and 16.5 °C (bottom), during 2019 and 2020, respectively. Salinity was stable in bottom water (38.6 ± 0.3) while on the surface it varied from 36.7 (February 2019) to 38.6 (September 2020). Dissolved oxygen went from 7.3 to 9.12 mg L<sup>-1</sup>, while the turbidity was lower than 1.3 NTU.





**Figure 2.** CTD parameters recorded in Lignano Sabbiadoro (a, b, c, d); San Giorgio di Nogaro (e, f, g, h); Zadar (i, l, m, n); Katalinića brig (o, p, q, r) and Stupe (s, t, u, v).

### Potential Toxic Elements

Dissolved concentrations of As, Cd and Hg, expressed as mean±sd (ng L<sup>-1</sup>), are shown in **Table 6**.

In the Gulf of Trieste, dissolved Hg ranged between 4 (April 2020, San Giorgio di Nogaro sea) and 30 (August 2019, San Giorgio di Nogaro DP) ng L<sup>-1</sup> with no statistically significant difference ( $p > 0.05$ ) between the overall mean levels of the two years (2019,  $16 \pm 7$  ng L<sup>-1</sup>; 2020,  $14 \pm 6$  ng L<sup>-1</sup>), neither between DP and sea ( $15 \pm 8$  and  $15 \pm 5$  ng L<sup>-1</sup>, respectively). Concerning different DPs, average higher concentrations were found in San Giorgio di Nogaro ( $17 \pm 8$  ng L<sup>-1</sup>) compared to Lignano Sabbiadoro ( $13 \pm 7$  ng L<sup>-1</sup>), while the corresponding seawater samples showed similar values ( $15 \pm 5$  and  $15 \pm 6$  ng L<sup>-1</sup>). Cd lower levels were recorded in April (Lignano Sabbiadoro and San Giorgio di Nogaro DP) and May 2020 (San Giorgio di Nogaro DP) with a value below the LOD (0.5 ng L<sup>-1</sup>), while the maximum concentration was  $98 \pm 4$  ng L<sup>-1</sup>, recorded in Lignano Sabbiadoro sea during April 2019. The difference

between mean concentration in 2019 ( $30 \pm 22 \text{ ng L}^{-1}$ ) and 2020 ( $12 \pm 10 \text{ ng L}^{-1}$ ) was statistically significant ( $p=0.0012$ ). DP showed a general lower mean concentration with respect to seawater samples ( $16 \pm 11$  against  $35 \pm 26 \text{ ng L}^{-1}$  in Lignano Sabbiadoro and  $7 \pm 8$  against  $24 \pm 13 \text{ ng L}^{-1}$  in San Giorgio di Nogaro, respectively). The difference between the two sites was not statistically significant ( $p<0.05$ ). As content ranged between  $< \text{LOD}$  ( $13 \text{ ng L}^{-1}$ ) in San Giorgio di Nogaro DP during October 2019, April, May, and July 2020 and  $1542 \pm 18 \text{ ng L}^{-1}$  in Lignano Sabbiadoro DP (April 2019). Statistically higher ( $p=0.0011$ ) concentrations were evidenced in 2019 ( $748 \pm 486 \text{ ng L}^{-1}$ ) compared to 2020 ( $217 \pm 192 \text{ ng L}^{-1}$ ). Lignano Sabbiadoro DP showed an As mean content of  $763 \pm 538 \text{ ng L}^{-1}$ , higher than San Giorgio di Nogaro DP ( $62 \pm 51 \text{ ng L}^{-1}$ ), while in seawater the concentration in the two sites was similar ( $511 \pm 392$  and  $498 \pm 339 \text{ ng L}^{-1}$ ).

In the Zadar area, Hg lowest levels were found in April and June 2020, with values under the detection limit ( $0.6 \text{ ng L}^{-1}$ ), while the highest concentration was recorded in surface seawater during August 2020 ( $19 \pm 2 \text{ ng L}^{-1}$ ). There was no statistically significant difference ( $p>0.05$ ) between the years, but a higher mean level was recorded in 2019 ( $10 \pm 3 \text{ ng L}^{-1}$ ) than in 2020 ( $7 \pm 6 \text{ ng L}^{-1}$ ). The order of concentration in relation to the type of sample was DP ( $7 \pm 5 \text{ ng L}^{-1}$ )  $<$  sea surface ( $8 \pm 6 \text{ ng L}^{-1}$ )  $<$  sea bottom ( $9 \pm 6 \text{ ng L}^{-1}$ ). Cd level ranged between  $< \text{LOD}$  ( $0.5 \text{ ng L}^{-1}$ ) in all DP samples of 2020 and  $33 \pm 3 \text{ ng L}^{-1}$  in surface seawater during April 2019. Samples collected in 2019 showed a statistically significant higher ( $p=0.0001$ ) content with respect to samples of 2020 ( $23 \pm 6$  and  $2 \pm 2 \text{ ng L}^{-1}$ , respectively). DP samples presented a lower mean Cd concentration ( $5 \pm 8 \text{ ng L}^{-1}$ ) than seawater ones ( $12 \pm 12 \text{ ng L}^{-1}$  and  $9 \pm 11 \text{ ng L}^{-1}$ , in surface and bottom, respectively). As content ranged from  $182 \pm 78 \text{ ng L}^{-1}$  in DP during September 2019 to  $1258 \pm 30 \text{ ng L}^{-1}$  in surface water during June 2020. A statistically significant lower concentration ( $p=0.0014$ ) was recorded in 2019 ( $555 \pm 244 \text{ ng L}^{-1}$ ) than in 2020 ( $910 \pm 228 \text{ ng L}^{-1}$ ). The order of concentration in relation to the type of sample was DP ( $732 \pm 341 \text{ ng L}^{-1}$ )  $<$  sea bottom ( $805 \pm 318 \text{ ng L}^{-1}$ )  $<$  sea surface ( $858 \pm 195 \text{ ng L}^{-1}$ ).

In the Split area, Hg ranged from  $< \text{LOD}$  ( $0.6 \text{ ng L}^{-1}$ ) in Stupe DP during April and September 2020 to  $19 \pm 1 \text{ ng L}^{-1}$  in Katalinića brig bottom water during April 2020. There is not a statistically significant difference ( $p>0.05$ ) between the two sampling years ( $5 \pm 2 \text{ ng L}^{-1}$  and  $7 \pm 4 \text{ ng L}^{-1}$ , respectively), neither between Katalinića brig DP ( $5 \pm 3 \text{ ng L}^{-1}$ ) and Stupe DP ( $5 \pm 4 \text{ ng L}^{-1}$ ). Bottom water samples showed a higher content compared to the surface ( $8 \pm 5$  against  $5 \pm 2 \text{ ng L}^{-1}$  in Katalinića brig and  $9 \pm 5$  against  $8 \pm 3 \text{ ng L}^{-1}$  in Stupe). A statistically significant reduction ( $p=0.0001$ ) of Cd was evidenced in 2020, with a mean value of  $6 \pm 5$  against  $34 \pm 4 \text{ ng L}^{-1}$  of 2019. However, the mean level of different stations resulted similar ( $12 \pm 10$ ,  $10 \pm 13$ , and  $12 \pm 11 \text{ ng L}^{-1}$  in Katalinića brig DP, surface and bottom water and  $14 \pm 8$ ,  $10 \pm 14$ ,  $6 \pm 10 \text{ ng L}^{-1}$  in Stupe DP, surface and bottom water, respectively). Concerning As, the lowest value ( $169 \pm 1 \text{ ng L}^{-1}$ ) was recorded in Katalinića brig bottom water during July 2019, while the maximum ( $2126 \pm 43 \text{ ng L}^{-1}$ ) was recorded in Stupe DP in July 2020. A statistically significant difference ( $p=0.0108$ ) was found between 2019 and 2020 mean levels ( $605 \pm 248 \text{ ng L}^{-1}$  and  $929 \pm 279 \text{ ng L}^{-1}$ , respectively). In the Stupe sampling station, the mean level was higher with respect to Katalinića brig ( $1111 \pm 511$ ,  $1071 \pm 197$ , and  $894 \pm 109 \text{ ng L}^{-1}$  in Stupe DP, water surface and bottom and  $756 \pm 263$ ,  $898 \pm 123$ ,  $740 \pm 308 \text{ ng L}^{-1}$  in Katalinića brig DP, water surface and bottom, respectively).

**Table 6.** Concentration of dissolved Hg, Cd and As found in water sample. DP: Depuration Plant; Sea S.: Sea surface; Sea B.: Sea bottom. Data expressed as mean $\pm$ sd (ng L<sup>-1</sup>)

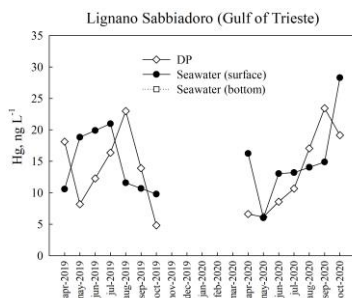
Sampling year	Sampling station			Hg	Cd	As
2019	Gulf of Trieste (ITA)	Lignano	DP	14±7	22±11	1268±214
		Sabbiadoro	Sea	15±5	47±33	857±181
		San Giorgio di Nogaro	DP	21±11	13±9	47±24
			Sea	16±4	34±10	785±239
	Zadar (HR)	Zadar	DP	12±1	18±2	435±358
			Sea S.	8±1	27±7	737±67
		Katalinića brig	Sea B.	12±4	24±3	452±245
			DP	2±1	33±1	626±26
	Split (HR)	Katalinića brig	Sea S.	5±1	40±1	804±24
			Sea B.	5±1	36±3	169±1
		Stupe	DP	5±1	32±1	473±80
			Sea S.	7±1	36±1	751±1
2020	Gulf of Trieste (ITA)	Sea B.	8±1	28±1	804±115	
		Lignano	DP	13±7	10±7	307±229
		Sabbiadoro	Sea	15±8	22±7	203±234
		San Giorgio di Nogaro	DP	14±1	2±3	71±67
	Zadar (HR)	Zadar	Sea	14±9	17±5	256±129
			DP	5±4	<0.5	831±300
		Katalinića brig	Sea S.	7±8	4±2	918±215
			Sea B.	8±6	2±2	982±161
	Split (HR)	Katalinića brig	DP	6±3	9±5	777±282
			Sea S.	5±3	5±3	913±127
		Stupe	Sea B.	9±6	8±5	835±195
			DP	5±4	11±2	1217±467
Stupe	Sea S.	8±3	5±8	923±204		
	Sea B.	9±5	2±2	909±112		

**Figure 3** shows the evolution of dissolved PTEs in different sampling stations, from April 2019 to October 2020.

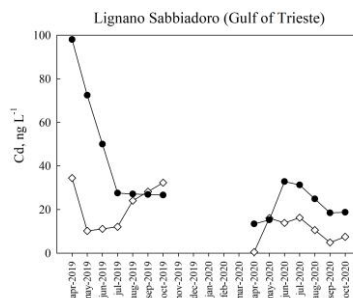
The Gulf of Trieste (**Figure 3a, d**) showed the maximum Hg value in mid-Summer 2019 (between July and August) and the last months of 2020. The dissolved Hg trend showed a similar pattern for DP and sea stations. In Zadar (**Figure 3g**), Hg content remained constant during 2019, decreased at the beginning of Summer 2020, and then reached the peak during August 2020. A similar situation occurred in Split (**Figure 3i, o**), where a minimum was recorded just before the beginning of Summer 2020 (April), and an increase occurred between May and July 2020. Differences with depth were evident only in Katalinića brig during the beginning of summer season and Stupe at the end of it, with higher concentration recorded in bottom water (**Figure 3i, o**).

A particular trend was noticed for dissolved Cd, as a strong decrease occurred in all the sampling stations from 2019 to 2020 year. In particular, in the second sampling year, in the Gulf of Trieste and Zadar (**Figure 3b, e, h**) DP sites showed in most cases lower concentration with respect to the seawater sample, with the site of San Giorgio di Nogaro DP and Zadar DP (**Figure 3e, h**) often showed values below the LOD (0.5 ng L<sup>-1</sup>). In Split sites (**Figure 3m, p**) the seasonal trend was similar in DP and sea, except in the period between February and May 2020, where a clear decrease in Cd concentration was recorded both in surface and bottom seawater.

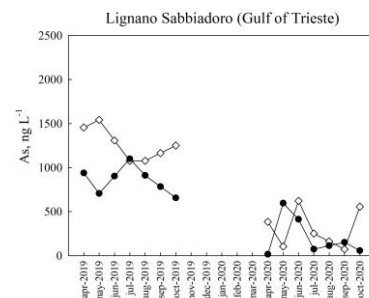
The levels of dissolved As recorded in the Gulf of Trieste (**Figure 3c, f**) were higher in 2019 compared to 2020, except for the site of San Giorgio di Nogaro DP, which showed very low values during all the two years (**Figure 3f**). No particular trend was noticed in this area. In Zadar and Split (**Figure 3i, n, q**) the trend was similar, with higher concentration recorded during March and May 2020, except for DP of Stupe, which reached the maximum level recorded in July 2020 (**Figure 3q**).



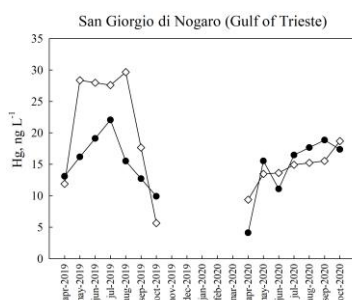
(a)



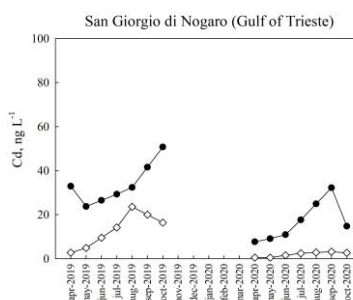
(b)



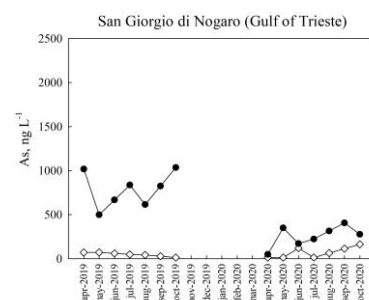
(c)



(d)

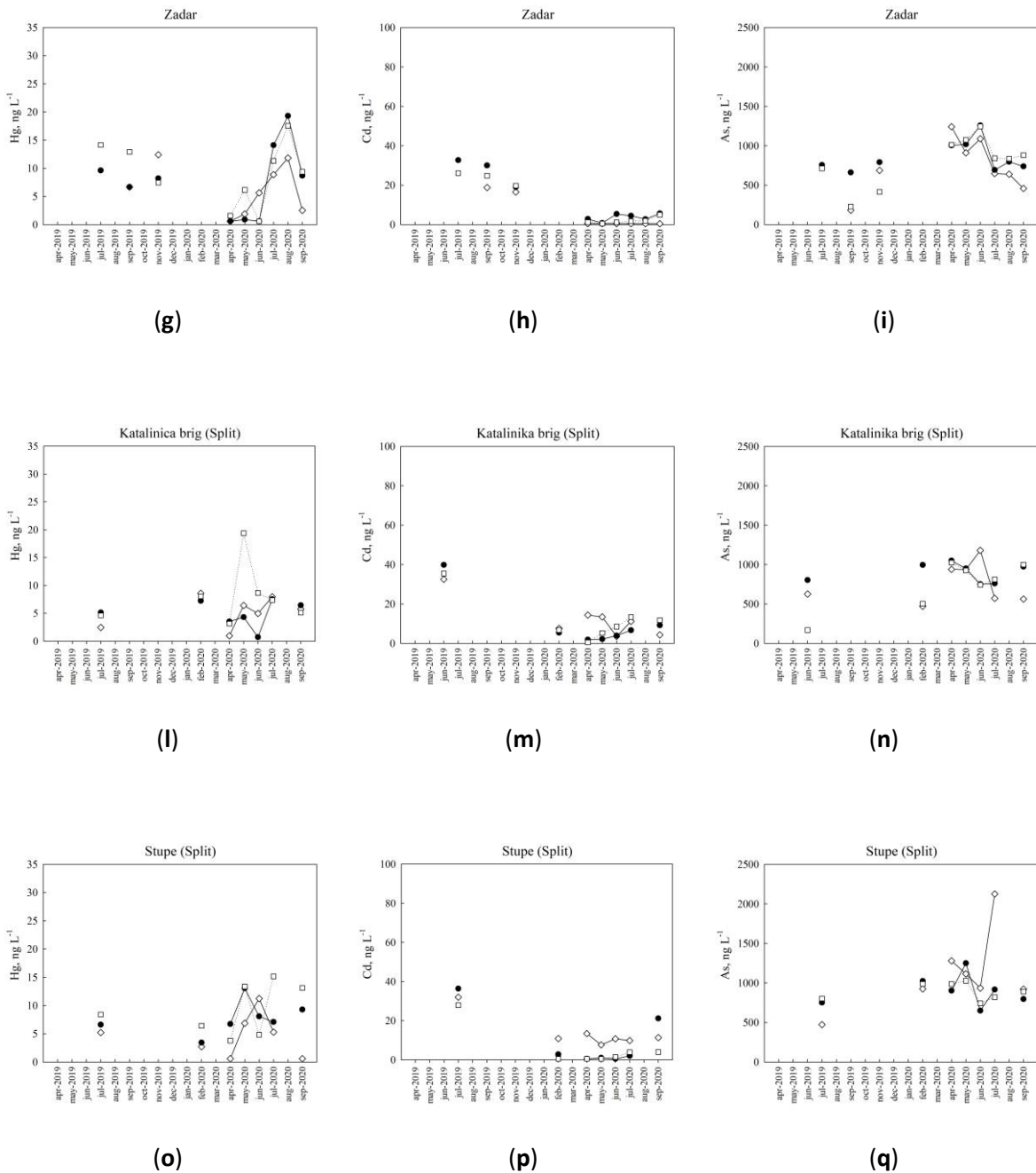


(e)



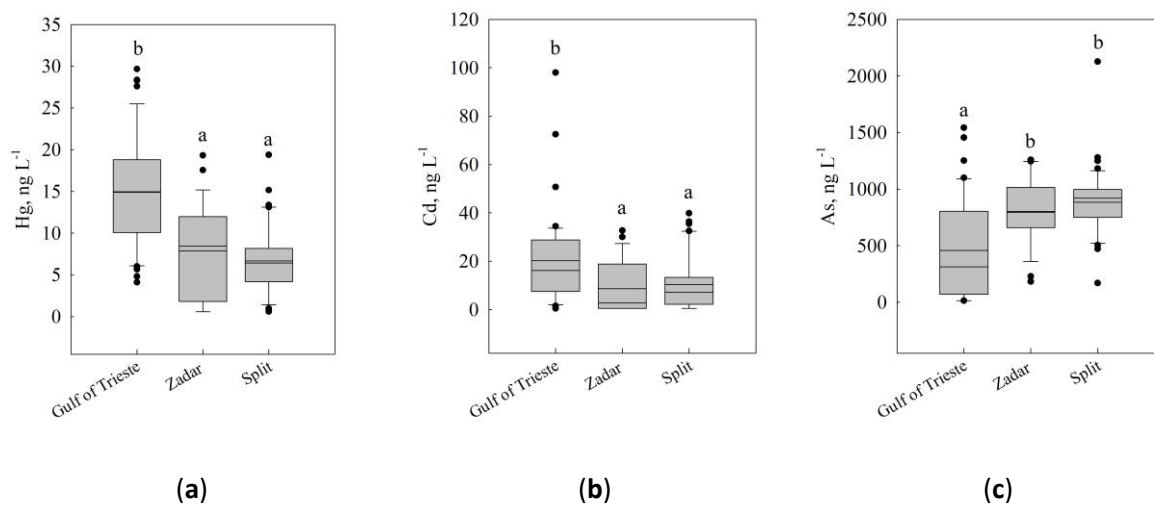
(f)





**Figure 3.** Dissolved Hg, Cd and As (expressed as  $\text{ng L}^{-1}$ ) in: Lignano Sabbiadoro (a) (b) (c); San Giorgio di Nogaro (d) (e) (f); Zadar (g) (h) (i); Katalinika brig (l) (m) (n), and Stupe (o) (p) (q).

In this study, dissolved PTEs were detected with an overall mean concentration of  $15 \pm 7$  (Hg),  $20 \pm 19$  (Cd), and  $458 \pm 442$  (As)  $\text{ng L}^{-1}$  in the Gulf of Trieste,  $8 \pm 6$  (Hg),  $9 \pm 11$  (Cd),  $801 \pm 283$  (As)  $\text{ng L}^{-1}$  in Zadar, and  $7 \pm 4$  (Hg),  $10 \pm 11$  (Cd), and  $883 \pm 295$  (As)  $\text{ng L}^{-1}$  in Split. The Gulf of Trieste presented statistically significant higher levels in Hg ( $p < 0.0001$ ) and Cd ( $p = 0.0013$ ) compared to Croatian samples, while a lower concentration in As ( $p < 0.0001$ ) was detected (Figure 4).



**Figure 4.** Mean concentration of dissolved Hg (a), Cd (b) and As (c) in different sampling areas. Bars bearing different letters indicate a statistically significant difference among PTEs levels in different geographical areas.

Few studies on PTEs levels have been carried out in seawater in this area (**Table 6**), and among them, only Cozzi *et al.* [2] took into consideration wastewater depuration plants as a possible source of input for these elements: however, our data are comparable with those collected from the literature.

Input sources of PTEs into the Adriatic Sea are manifold. The Po River is one of the most important ones, being responsible for metals discharge both in the dissolved [31,40] and in the particulate forms [39]. Sediments on their side are not only an accumulation compartment but can actively represent a source of PTEs in the marine environment [3] as they may be recycled many times across the sediment-water interface before being permanently buried [4]. Moreover, atmospheric depositions driven by wind action are an important contribution that cannot be neglected in the study of biogeochemical cycles and PTE flows in the north Adriatic [5].

In the northern zone of the Adriatic Sea, a high concentration of Hg has been found both in sediments and waters, due to more than 500 years of HgS extraction from the Idrija mine, the second-largest Hg mine in the world, located in the Slovenian sector of the Isonzo River drainage basin [6,7]. During mining operations, mineralised tailings generated by HgS roasting processes were dumped along the banks of the Idrijca River, swept away by floodwaters towards the Isonzo River and finally transported into the Gulf of Trieste [8]. This could explain the higher concentration of dissolved Hg in the seawater samples of the Gulf of Trieste as our data are consistent with the results of studies conducted in the same area [9] or geographical zones located in north Adriatic [10,11]. Moreover, also Kotnik *et al.* [12] reported a concentration gradient in seawater from north to south.

In the only study we found which connected DP and PTEs in the Adriatic Sea, a mean level of Cd of  $0.08 \pm 0.14 \mu\text{g L}^{-1}$  was reported in the proximity of the diffusion system of a DP's pipeline located at 7.5 km from the coast of Trieste (Italy) and the author concluded that this concentration does not indicate a persistent perturbation in the water column of the site [2]. Other studies carried out in northern Adriatic [11] and Italian central coasts [13–15] reported Cd levels similar to our results.

Croatia is known for the high arsenic content in the aquifers coming from natural geological sources present in the eastern area [16–19]. Lazo *et al.* [20] evidenced that anthropogenic activities can influence PTEs levels both in sediments and seawater in the Adriatic eastern coast and a chemometric approach in the area of Montenegro [21] has associated As with both natural and anthropogenic sources. However, As values were consistent with our results, with a slightly higher range of concentrations than that of Split which could explain the growing trend of As from north to south.

As regards the legislative aspect, the levels of PTEs measured in the outflows of the DPs are far below the limits imposed by national regulations [22] (from 2 to 4 orders of magnitude) (Table 7). Furthermore, all the seawater samples analysed fall within the limit values indicated by the European MSFD for Hg and Cd and national legislation for As [23,24] (Table 7).

**Table 7.** Dissolved metals levels in seawater (ng L<sup>-1</sup>) from literature. Data are expressed as mean±sd (min-max). (T: total concentration; nd: not determined).

Location (state), sampling year	Hg	Cd	As	Reference
Gulf of Trieste (Italy), 2019-2020	15±7 (4-30)	20±19 (<0.5-98)	458±442 (<13-1542)	This study
Zadar (Croatia), 2019-2020	8±6 (<0.6-19)	9±11 (<0.5-33)	801±283 (182-1258)	This study
Split (Croatia), 2019-2020	7±4 (<0.6-19)	10±11 (<0.5-40)	883±295 (169-2126)	This study
Gulf of Trieste (Italy) 2002-2003	nd	80±140	nd	[2]
Marano Lagoon (Italy), 2004	4.1-52.4	nd	nd	[9]
Gulf of Trieste (Italy), 2011-2012	0.2-15	nd	nd	[10]
Mljet National Park (Croatia), 2005-2008	0.5-24.2 (T)	6.4-18.7	nd	[11]
Southeast Adriatic coast (Montenegro), 2005-2007	200-2000	0-7400	500-3100	[21]
Durres Bay (Albania), 1999-2002	76.6-101.1 (T)	121-187 (T)	nd	[20]
Ancona (Italy), 2005	nd	14±1	nd	[13]
Central Adriatic Sea, 2004	nd	15±3	nd	[14]
Po Plume (Italy), 2002	nd	14±5	nd	[25]
Mediterranean Sea, 1995	nd	7	nd	[26]
Isonzo River (Italy) Gulf of Trieste (Italy), 2012	<LOD-8.60 1	nd nd	190-2310 1500	[8] [27]
Gulf of Trieste (Italy), 1990-1999	<0.20-4.9	nd	nd	[28]
Venice Lagoon,	nd	nd	nd	[29]

1992				
Adriatic Sea, 1992-1995	nd	nd	nd	[30]
Krka River (Croatia), 1997-2000	0.50-1.10	nd	nd	[31]
Zrmanja River	nd	nd	250-1740	[32]
<b>Limits</b>				
Discharge in surface waters (mg L <sup>-1</sup> )	0.005	0.02	0.5	[22]
MAC-EQS (µg L <sup>-1</sup> )	0.07	0.45 <sup>a</sup>	5	[23,24]

<sup>a</sup> EQS values vary depending on the hardness of the water as specified in five class categories (Class 1: < 40 mg CaCO<sub>3</sub> L<sup>-1</sup>, Class 2: 40 to < 50 mg CaCO<sub>3</sub> L<sup>-1</sup>, Class 3: 50 to < 100 mg CaCO<sub>3</sub> L<sup>-1</sup>, Class 4: 100 to < 200 mg CaCO<sub>3</sub> L<sup>-1</sup> and Class 5: ≥ 200 mg CaCO<sub>3</sub> L<sup>-1</sup>). For this study, the lowest limit value (Class 1) was taken in consideration.

The seasonal trends of the investigated PTEs showed a significant decrease in the Cd content from 2019 to 2020, recorded in all sampling stations, while a decrease in the As content was found only in the northern stations. Although the detected concentrations remain extremely low and are not a signal of environmental contamination, this condition could be ascribable to different factors: i) a reduced touristic activity in these areas (caused by the lockdown period for the covid-19 pandemic in European states during summer 2020 [33]) with a different impact on DPs; ii) natural fluctuations of these concentrations in the marine environment due to changes in environmental parameters such as more intense rain events that can cause a higher intake of freshwater [4,14]. However, more data will be useful to define a precise interpretation of this trend, since only two bathing seasons were available.

The cluster analysis highlighted how the sampling sites are grouped on the basis of the geographical area, regardless of whether they have been sampled near discharging pipelines or in coastal waters. This result leads us to the conclusion that adequately treated wastewater does not compromise the environmental quality of coastal waters in the sites of interest, but it is more the natural characteristics of the area that determine the distribution of PTEs.

The PC1 indicates that the chemical-physical parameters play an important role in the characterization of geographical areas, as warmer and lower salinity waters naturally occur in the northern part of the Adriatic Sea, as well as high turbidity, considering the annual average. However, it has been shown that low levels of salinity, corresponding to high river inputs, can influence the content of PTEs in the Adriatic Sea [25,26]. In our case study, we discovered a statistically significant positive correlation between Cd and As and the salinity parameter only in the Gulf of Trieste.

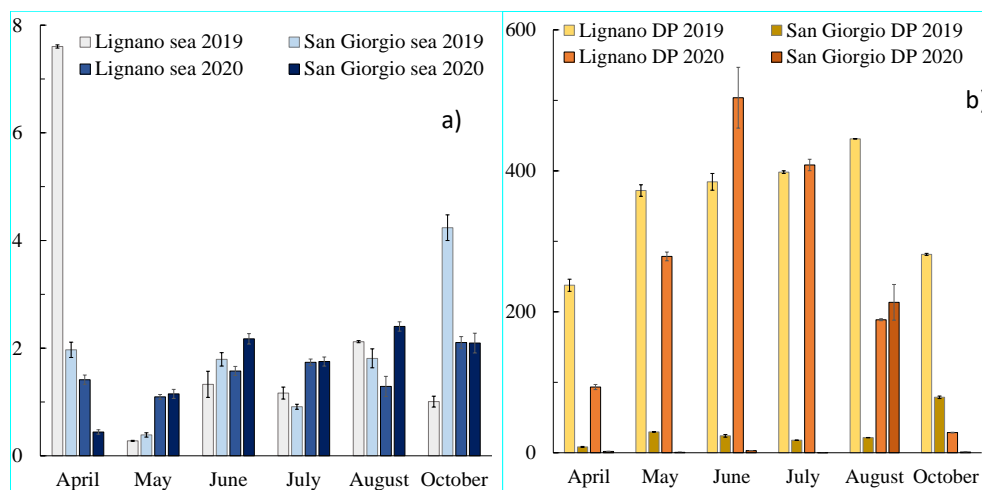
Moreover, further differentiation between sites emerged as considering the site-specific statistical approach applied, a statistically significant correlation was found between the pairs of variables Cd-As (positive in the Gulf of Trieste and negative in Croatian sites) and Hg-As (Zadar only). However, further studies with a higher temporal resolution and with a greater number of elements considered are still needed in order to better understand the behaviour of dissolved PTEs and their relative distribution.

Nutrients:

*Nitrous nitrogen, N-NO<sub>3</sub>:*

*Seawater:* In 2019 N-NO<sub>3</sub> levels ranged from a minimum of 0.28±0.01 (Lignano Sea May) to a maximum of 7.59±0.029 μmol L<sup>-1</sup> (Lignano Sea April), with a mean of 2.1±2 μmol L<sup>-1</sup>. In 2020 N-NO<sub>3</sub> levels ranged from a minimum of values 0.44±0.04 μmol L<sup>-1</sup> (San Giorgio Sea April) to a maximum of 2.40±0.09 μmol L<sup>-1</sup> (San Giorgio Sea August) with a mean of 1.60±0.56 μmol L<sup>-1</sup> (**Figure 5-a**). No particular trend is notable, exception made for the month of may which registered the lowest value.

*DP:* In 2019 N-NO<sub>3</sub> levels ranged from a minimum of 8.3± 0.7 μmol L<sup>-1</sup> (San Giorgio DP April) to a maximum of 445.5±1.5 μmol L<sup>-1</sup> (Lignano DP August), with a mean of 191.6±177.6 μmol L<sup>-1</sup>. In 2020 N-NO<sub>3</sub> levels ranged from a minimum of 0.147±0.012 μmol L<sup>-1</sup> (San Giorgio DP July) to a maximum of 503.80±43.18 μmol L<sup>-1</sup> (Lignano DP June) with a mean of 143.47±176.31 μmol L<sup>-1</sup> (**Figure 5-b**).

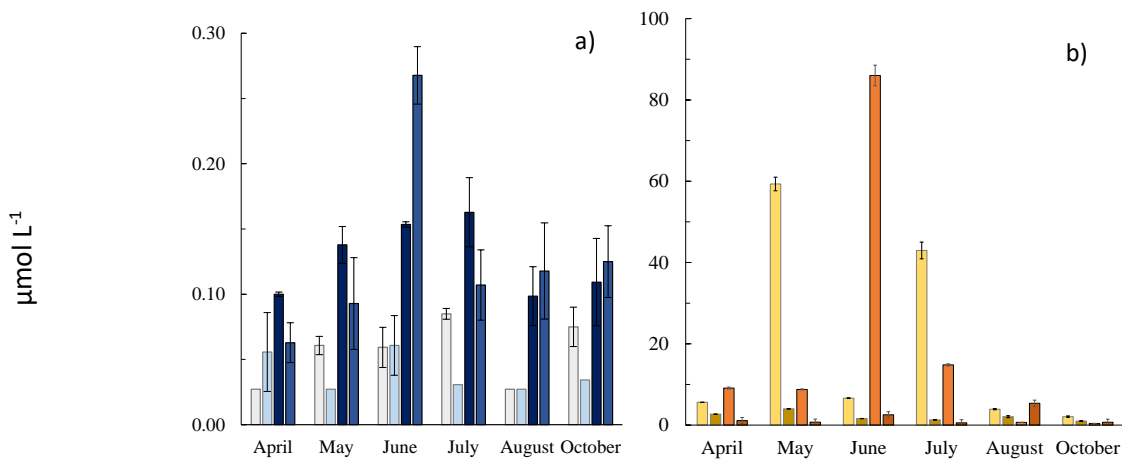


**Figure 5:** Nitrous nitrogen concentrations in **a)** seawater and **b)** deputation plants outflow

*Nitric Nitrogen, N-NO<sub>2</sub>:*

*Seawater:* In 2019 N-NO<sub>2</sub> levels ranged from a minimum of 0.40±0.015 μmol L<sup>-1</sup> (San Giorgio sea July) to a maximum of 0.82±0.08 μmol L<sup>-1</sup> (San Giorgio Sea August) with a mean of 0.53±0.10 μmol L<sup>-1</sup>. In 2020 N-NO<sub>2</sub> levels ranged from a minimum of 0.22±0.005 μmol L<sup>-1</sup> (San Giorgio Sea May) to a maximum of 0.77±0.027 μmol L<sup>-1</sup> (San Giorgio Sea June) with a mean of 0.37±0.19 μmol L<sup>-1</sup> (**Figure 6-a**).

*Depuration Plant:* In 2019 N-NO<sub>2</sub> levels ranged from a minimum of 0.98±0.096 μmol L<sup>-1</sup> (San Giorgio DP October) to a maximum of 59.34±1.686 μmol L<sup>-1</sup> (Lignano DP May) with a mean of 11.10±19.11 μmol L<sup>-1</sup>. In 2020 N-NO<sub>2</sub> levels ranged from a minimum of 0.40±0.004 μmol L<sup>-1</sup> (San Giorgio October) to a maximum of 86.02±2.54 μmol L<sup>-1</sup> (Lignano DP June) with a mean of 10.90±24.11 μmol L<sup>-1</sup> (**Figure 6-b**).

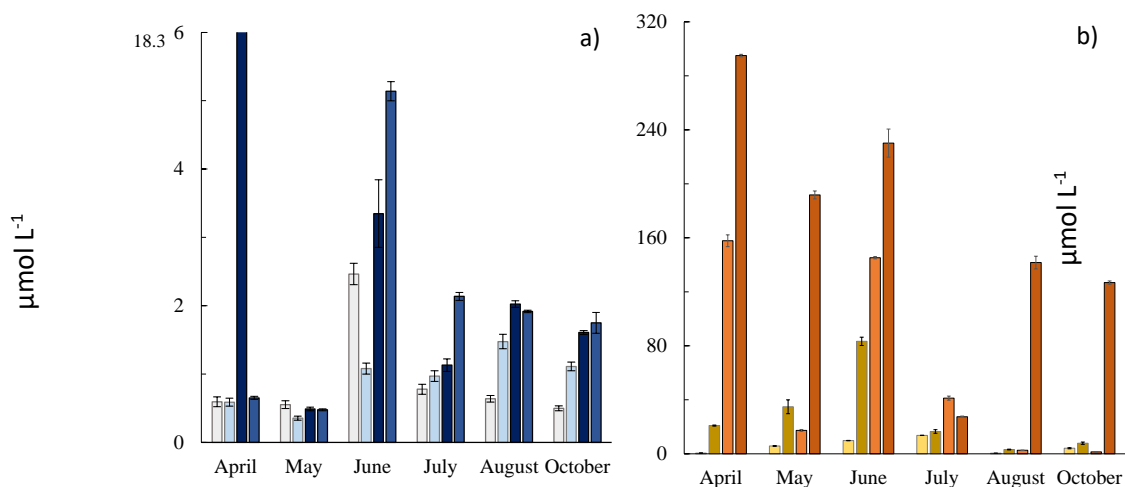


**Figure 6:** Nitric Nitrogen concentrations in **a)** seawater and **b)** depuration plants outflow

Ammoniacal nitrogen, N-NH<sub>3</sub>:

*Seawater:* In 2019 N-NH<sub>3</sub> ranged from a minimum of  $0.4 \pm 0.03$  (San Giorgio Sea, May) to a maximum of  $2.5 \pm 0.16$   $\mu\text{mol L}^{-1}$  (Lignano Sea June) with a mean of  $0.93 \pm 0.58$   $\mu\text{mol L}^{-1}$ . In 2020 N-NH<sub>3</sub> ranged from a minimum of  $0.5 \pm 0.01$   $\mu\text{mol L}^{-1}$  (San Giorgio Sea May) to a maximum of  $18.3 \pm 0.7$   $\mu\text{mol L}^{-1}$  (Lignano Sea April), with a mean value of  $1.9 \pm 1.35$   $\mu\text{mol L}^{-1}$  (Lignano Sea April excluded from mean and dev.st) or  $3.23 \pm 4.93$   $\mu\text{mol L}^{-1}$  (Lignano Sea April included) (**Figure 7-a**).

*Depuration Plant:* In 2019 N-NH<sub>3</sub> ranged from a minimum of  $0.41 \pm 0.19$   $\mu\text{mol L}^{-1}$  (Lignano DP August) to a maximum of  $83.33 \pm 3.1$   $\mu\text{mol L}^{-1}$  (San Giorgio June), following a bell-shape trend with a mean of  $16.74 \pm 23.18$   $\mu\text{mol L}^{-1}$ . In 2020 N-NH<sub>3</sub> ranged from a minimum of  $1.39 \pm 0.04$   $\mu\text{mol L}^{-1}$  (Lignano DP October) to a maximum of  $295.0 \pm 0.9$   $\mu\text{mol L}^{-1}$  (San Giorgio DP April), with a mean value of  $114.9 \pm 96.92$   $\mu\text{mol L}^{-1}$ . Despite the peak in April, N-NH<sub>3</sub> in Lignano DP followed a bell-shape trend as seen in 2019; San Giorgio resulted in more variable data, with a minimum in July. San Giorgio DP showed for both 2019 and 2020 higher value then Lignano DP (**Figure 7-b**).



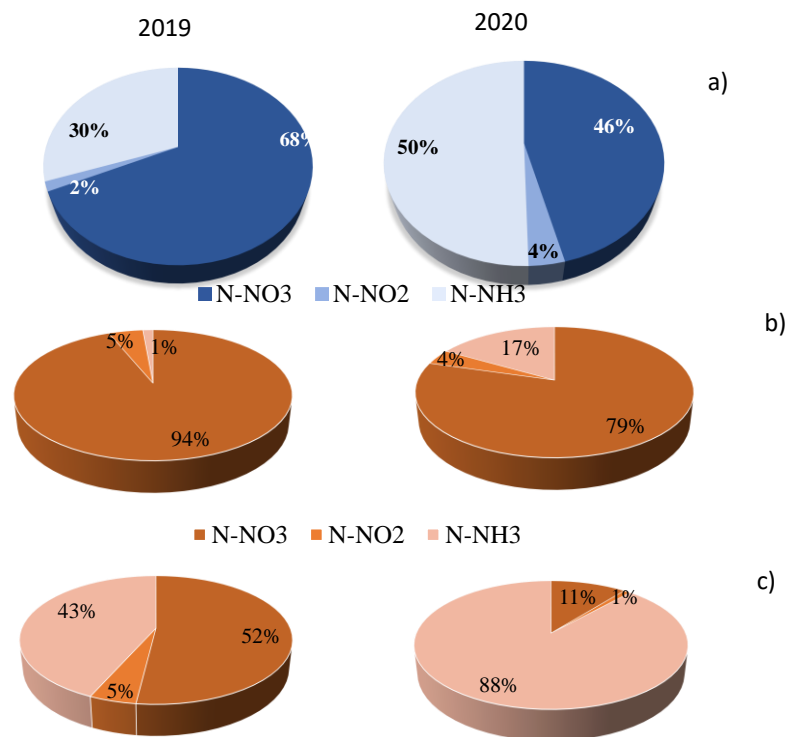
**Figure 7:** Ammoniacal Nitrogen concentrations in **a)** seawater and **b)** depuration plants outflow

DIN distribution:

*Seawater:* The average DIN distribution (in order: N-NO<sub>3</sub>, N-NO<sub>2</sub>, N-NH<sub>3</sub>) is highly comparable between the sites for the same year (2019: Lignano 70%, 2%, 28%, San Giorgio 66%, 1%, 33%; 2020: Lignano 47%, 4%, 50%, San Giorgio 46%, 3%, 51%) but different between the years (2019 average: 68%, 2%, 30%; 2020 average: 46%, 4%, 50%).

*Depuration Plant:* A marked difference between the two DPs is notable by the figure 8, Lignano DP's outflows are mainly composed by nitrous nitrogen (94% in 2019, 79% in 2020), while nitric and ammoniacal nitrogen represented a significantly lower fraction. San Giorgio DP outflows are characterized by higher levels of ammoniacal nitrogen (43% in 2019, 79% in 2020).

An increment on ammoniacal levels between 2019 and 2020 were found both in DPs and sea sites, probably ascribed at the different contribution of the domestic sewages in entrane into the DPs, originated by the suspension of many industrial activities – and so industrial sewages- from the pandemic of covid-19, from march to June-july in Italy.



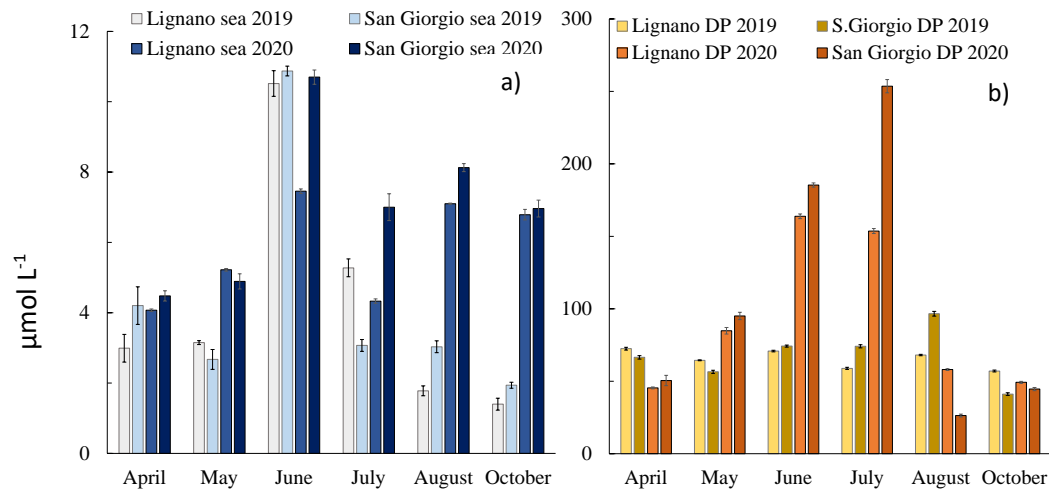
**Figure 8:** average DIN distribution for **a)** average Sea sites, **b)** Lignano DP, **c)** San Giorgio DP

Si-SiO<sub>2</sub>:

*Seawater:* In 2019 Si-SiO<sub>2</sub> ranged from a minimum of 1.4±0.17 μmol L<sup>-1</sup> (Lignano sea October) from a maximum of 10.9±0.14 μmol L<sup>-1</sup> (San Giorgio sea April) with a mean value of 4.21±3.21 μmol L<sup>-1</sup>. In 2020 Si-SiO<sub>2</sub> ranged from a minimum of 4.07±0.04 μmol L<sup>-1</sup> (Lignano sea April2020) to a maximum of 10.7±0.2 μmol L<sup>-1</sup> (San Giorgio sea June2020) with a mean of 6.43±1.93 μmol L<sup>-1</sup> (**Figure 9-a**).

*Depuration Plant:* In 2019 SiO<sub>2</sub> ranged from a minimum of 86.90±0.82 μmol L<sup>-1</sup> (San Giorgio DP October) from a maximum of 158.9±1.48 μmol L<sup>-1</sup> (San Giorgio DP June) with a mean value of

125.1±44.2 μmol L<sup>-1</sup>. In 2020 SiO<sub>2</sub> ranged from a minimum of 26.30±0.99 μmol L<sup>-1</sup> (San Giorgio DP August) to a maximum of 253.59±4.57 μmol L<sup>-1</sup> (San Giorgio DP July) with a mean of 100.9±71.57 μmol L<sup>-1</sup> (Figure 9-b).

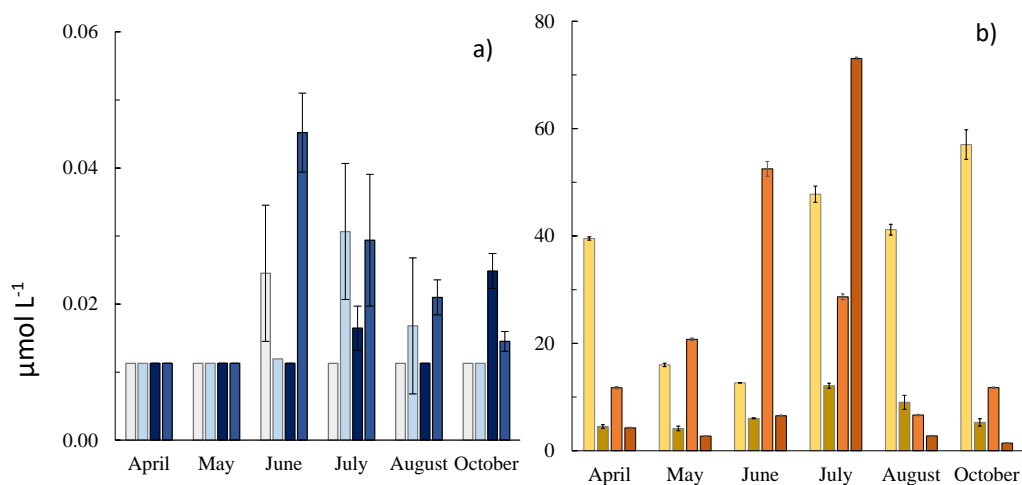


**Figure 9:** Silicates concentrations in a) Seawater and b) Depuration plants

DIP:

*Seawater:* In 2019 most of the DIP values were below LOD (0.01 μmol L<sup>-1</sup>), with a maximum of 0.03±0.01 (San Giorgio Sea, July) with a mean value of 0.015±0.006 μmol L<sup>-1</sup>. In 2020, DIP levels, like 2019, resulted below to LOD in most of the cases, with a maximum of 0.045±0.01 μmol L<sup>-1</sup> (San Giorgio Sea May) and a mean value of 0.018±0.010 μmol L<sup>-1</sup> (Figure 10-a).

*Depuration Plant:* In 2019 DIP ranged from a minimum of 4.15±0.45 μmol L<sup>-1</sup> (San Giorgio DP May) to a maximum of 57.025±2.76 μmol L<sup>-1</sup> (Lignano DP October) with a mean value of 21.274±19.33 μmol L<sup>-1</sup>. In 2020 DIP ranged from a minimum of 1.43±0.06 μmol L<sup>-1</sup> (San Giorgio DP October) to a maximum of 73.06±0.26 μmol L<sup>-1</sup> (San Giorgio DP July) with a mean value of 18.57±22.58 μmol L<sup>-1</sup>. In 2019 DIP in DP samples represents the 72% of the P<sub>tot</sub>. In 2020 DIP % represents the 81.5% (Figure 10-b).



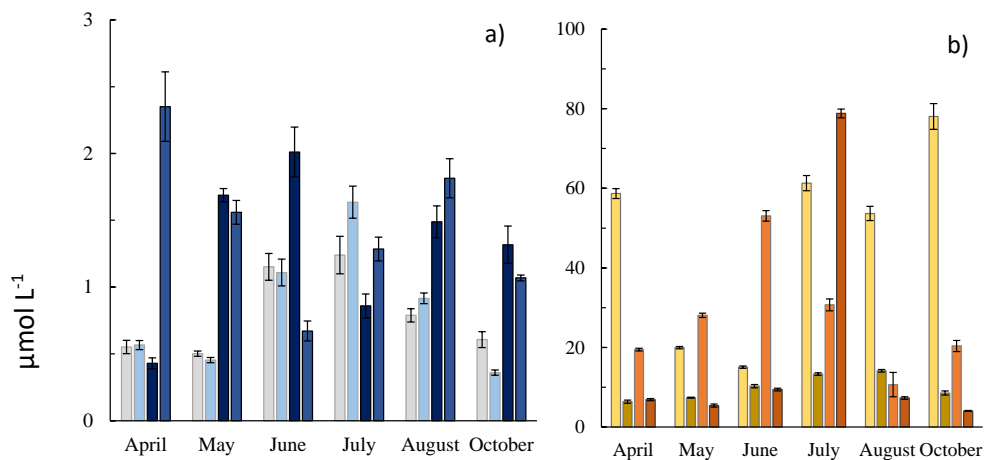
**Figure 10:** Dissolved Inorganic Phosphorous in a) Seawater and b) Depuration plants

TP:



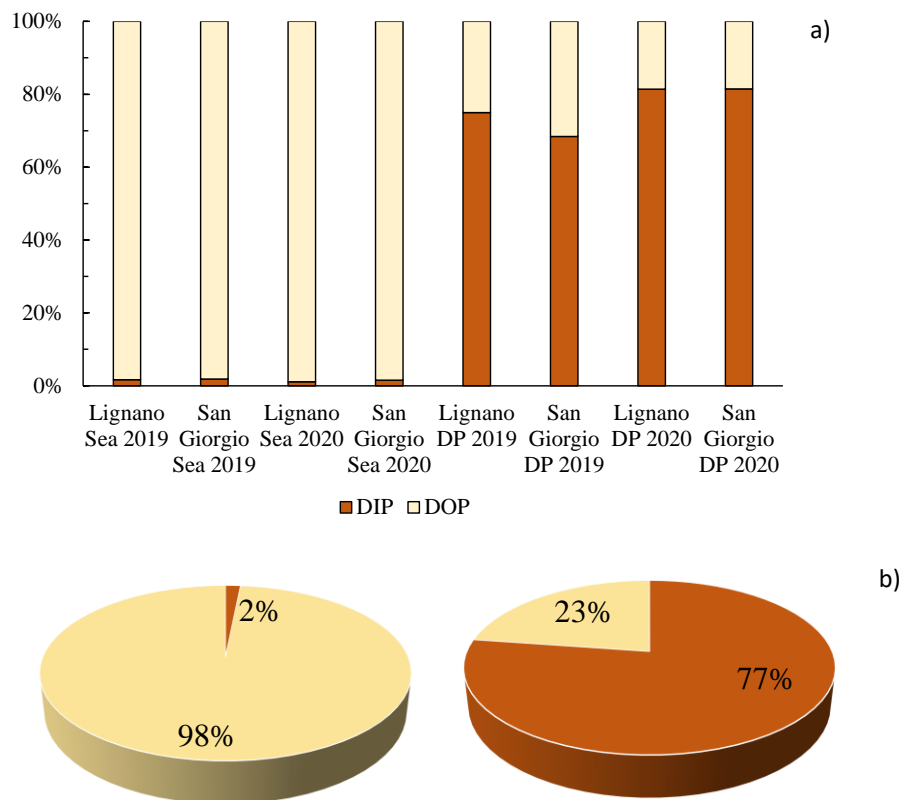
*Seawater:* In 2019 Total Phosphorous ranged from a minimum of  $0.36 \pm 0.02 \mu\text{mol L}^{-1}$  (San Giorgio Sea October) to a maximum of  $1.64 \pm 0.12 \mu\text{mol L}^{-1}$  (San Giorgio Sea July) with a mean value of  $0.82 \pm 0.39 \mu\text{mol L}^{-1}$ . In 2020 P<sub>tot</sub> ranged from a minimum of  $0.43 \pm 0.04 \mu\text{mol L}^{-1}$  (Lignano Sea April) to a maximum of  $2.35 \pm 0.26 \mu\text{mol L}^{-1}$  (San Giorgio Sea April) with a mean value of  $1.38 \pm 0.56 \mu\text{mol L}^{-1}$  (**Figure 11-a**). No significative differences were found between the sites.

*Depuration Plant:* In 2019 TP ranged from a minimum of  $6.39 \pm 0.39 \mu\text{mol L}^{-1}$  (San Giorgio DP April) to a maximum of  $76.57 \pm 2.71 \mu\text{mol L}^{-1}$  (Lignano DP October) with a mean value of  $9.38 \pm 8.39 \mu\text{mol L}^{-1}$ . In 2020 TP ranged from a minimum of  $4.03 \pm 0.16 \mu\text{mol L}^{-1}$  (San Giorgio DP October) to a maximum of  $78.82 \pm 1.11 \mu\text{mol L}^{-1}$  (San Giorgio DP July) with a mean value of  $22.84 \pm 22.64 \mu\text{mol L}^{-1}$  (**Figure 11-b**).



**Figure 11: Total Phosphorous in a) Seawater and b) Depuration plants**

Phosphorus distribution: the contribution to TP incoming from DIP or DOP is strongly comparable for the same typology of site (DP or Sea). Between 2019 and 2020 DIP % increased in both DP sites, respectively: Lignano DP 75% - 81.4%, San Giorgio DP 68.4% - 81.5%. The same increment was not found in Seawater, which remained almost constant between the sites and the years (average 2% DIP, 98% DOP) (**Figure 12**).



**Fig. 12:** Average DIP/DOP distribution in **a)** Sea sites and **b)** DP sites

**Table 8:** Dilution factor, calculated as the relationship between average DP and average Sea concentration.

	2019	2020
N-NO <sub>3</sub>	92.0	83.0
N-NO <sub>2</sub>	222.0	83.8
N-NH <sub>3</sub>	18.1	67.2
DIP	1420.0	1033.3
Si-SiO <sub>2</sub>	33.7	15.7
TP	35.2	16.6

In conclusion:

1) a strong dilution factor from the water body is evident for every nutrient, from one to three orders of magnitude, resumed in **Table 8**. 2) nutrient concentrations found in the DP were in almost every case below the Legal limit (**D.Lgs 152/06**), with an exception for N-NO<sub>2</sub> in one month; anyway, the difference from the sampling methodology than the one described in the active legislation gives them

no legal value. 3) From data comparison, seawater inorganic nutrient levels investigated during this study were generally slightly higher than the normal range of concentrations which can be usually found in the bottom depth of the Gulf of Trieste (Table 8) during the summer, highlighting the efficiencies of the depuration treatments. 4)DPs composition concerning P levels and DIN distribution is quite different, in fact the amount of domestic sewage in entrance in the DPs is highly different; despite these differences in the DPs composition sea sites remained comparable, with no statistical differences between them. The similarities between the sea sites could enforce the thesis that the main nutrient input in the seawater incomes from riverine inputs and from non-proper sewage depuration treatment, so probably the oscillations of nutrient concentrations in our sea sites depends prevalently from the freshwater input in the area.

**Table 8:** Data extracted from EMODnet portal. Data reported refers to the stations nearest to our study area in the Gulf of Trieste, during the summer period, at a depth of 10m from the upper part of the water column. Results on concentrations are expressed in  $\mu\text{mol L}^{-1}$ .

Sampling site (year)	DIN	DIP	Silicates	Reference
2001-2006 average	~0.7-1.6	$\leq 0.03$	<2.6	EMODnet chemistry portal
2006-2011 average	~0.6-1.2	$\leq 0.06$	<3	EMODnet chemistry portal
2011-2016 average	~1-1.5	$\leq 0.03$	<3.6	EMODnet chemistry portal
2015-2020 average	~1-1.9	~0.02-0.026	<1	EMODnet chemistry portal
Sea DP discharge point samples (2019)	2.75 $\pm$ 2.05	0.028 $\pm$ 0.02	4.21 $\pm$ 3.21	This Study
Sea DP discharge point samples (2020)	3.92 $\pm$ 1.96	0.018 $\pm$ 0.010	6.43 $\pm$ 1.93	This Study

Inorganic nutrient levels found in the proximity of the DP discharge points were generally slightly higher than the ones far from the DP's discharge which can be found in the bottom waters of the same region. Anyway, nutrient levels in the seawater remained generally low and not harmful for the environment.

## PART 5: CONCLUSIONS

This study provides for the first time information on some nutrients and dissolved Potentially Toxic Elements (PTEs) in relation to the effect of five depuration plants (DPs) located in the Adriatic Sea during the 2019-2020 Summer period. Our results showed that: (1) all the samples analysed resulted in concentrations below the European and national legal limits for nutrients and PTE; in particular, mean values were 2X below the legal limit for every PTE; (2) for PTEs no particularly DP effect was recorded as concentrations in DP outflow and seawater in the proximity of the DP's discharge pipes were similar; (3) a strong dilution factor from the water body is evident for nutrient, from one to three orders of magnitude passing for DP to the sea(4) a geographical gradient was recorded for Hg and As levels, while an analysis of the seasonal trend has evidenced a strong decrease of Cd levels in 2020 in all sites(5)DPs composition concerning P levels and DIN distribution is quite different whereas sea sites remained comparable.

Concerning the parameters investigated in this project, from our results we can assume that DP discharges do not compromise the environmental quality of the surrounding marine environment in the study area.

However, continuous monitoring and further information (e.g., Urea, Phospaahatesea activity) would be measured to better understand a possible synergic effect of treated wastewater outflow in the marine environment and to ensure the maintenance of a good environmental quality of the Adriatic Sea, as requested by Descriptors 5 and 8 of the Marine Strategy Framework Directive.

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