D 4.2.1.

REPORT ON STATISTICAL PROCESSING OF DATA ON SEASONAL TRENDS OF NUTRIENTS, PHYSICO-CHEMICAL PARAMETERS AND TRACE ELEMENTS IN CORRELATION WITH MAIN HYDROLOGICAL CHARACTERISTICS

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**
Phase Leader:	**UNIVPM**
Deliverable:	**4.2.1. Report on statistical processing of data on seasonal trends of nutrients, physicochemical parameters and trace elements in correlation with main hydrological characteristics**

Version:	**01**	**Date:**	
Type:	**Report**		
Availability:	Confidential		
Responsible Partner:	UNIVPM		
Editor:	Anna Annibaldi (UNIVPM)		
Contributors:	UNIVPM, UNIST-FGAG, OGS, UNIUD		

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PART 1: INTRODUCTION

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., this **Report (D4.2.1)** provides statistical processing of data on seasonal trends of nutrients, physicochemical parameters and trace elements in correlation with the main hydrological characteristics. The document deals with the parameters investigated in seawater and in waste waters from WWTP in IT and HR area.

PART 2: INVESTIGATED SITES AND PARAMETERS

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), while 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). All the details on this part are extensive explained in D 4.2.1. I part.

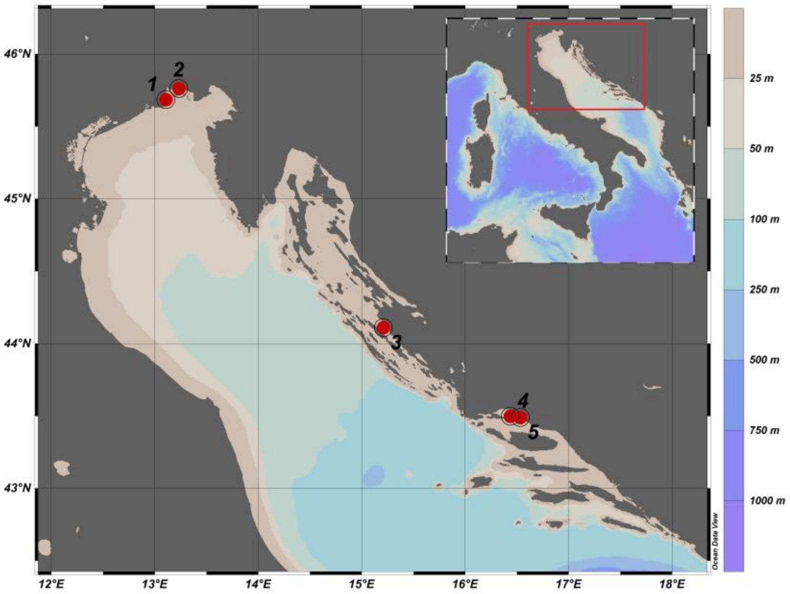


Figure 1. Sampling stations in northern Adriatic Sea: Lignano Sabbiadoro (Gulf of Trieste, ITA) (**1**), San Giorgio di Nogaro (Gulf of Trieste, ITA) (**2**), Zadar Upov Centar (Zadar, HR) (**3**), Katalinića brig (Split, HR) (**4**), and Stupe (Split, HR) (**5**).

PART 3: STATISTICAL ANALYSIS

To investigate the similarities between the sampling sites, a cluster analysis taking into account the values of dissolved Hg, Cd and As was applied (**Figure 2a**).

A clear similarity was confirmed for all the sites in Croatia, as they were grouped with a distance <1, while the sampling sites in the Gulf of Trieste are similar to each other, with the exception of the DP of San Giorgio di Nogaro.

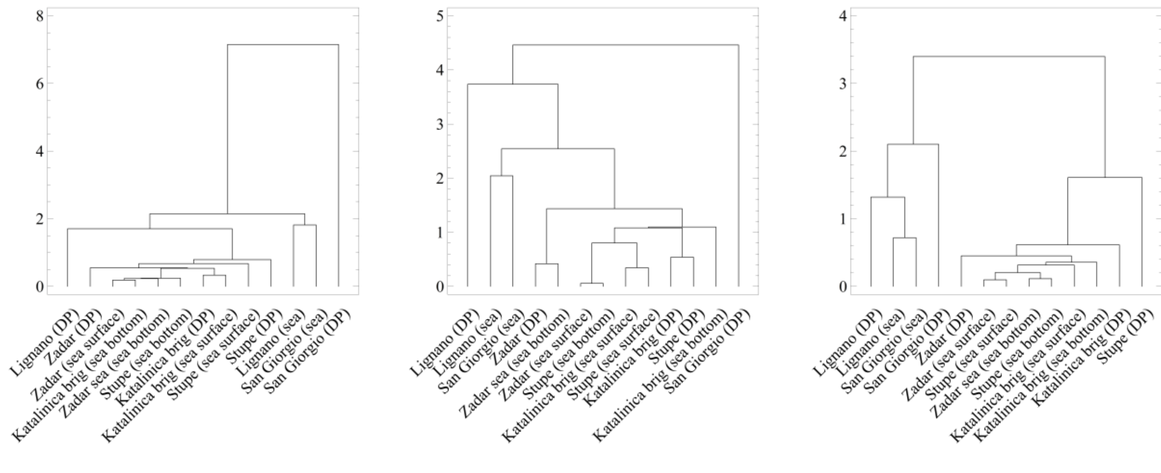
Since statistically significant differences were found in the concentrations recorded between the two sampling years with the same site for both As and Cd, we applied a second and third cluster analysis for both years. This was necessary to verify whether these fluctuations in the levels of PTEs could influence possible similarities between the sampling stations.

In 2019 (**Figure 2b**), the analysis associates the two DP sites and the two surface seawater sites of Split (Katalinića brig and Stupe). This is probably due to the proximity of the two sites, both located in the city of Split, but also due to the similarity of the treated wastewater (households waste) and the similar treatment applied to the wastewaters by the two DPs. Zadar DP is associated mainly with the bottom water sampled in the proximity of the Zadar discharge pipe (**Figure 2b**). In the Gulf of Trieste, the more similar sites are the seawater of Lignano Sabbiadoro and San Giorgio di Nogaro, while a consistent difference was found for the DP sites, which could be due to the fact that the type of treated wastewater differs greatly as the Lignano Sabbiadoro plant manages also household wastewater, while

San Giorgio di Nogaro DP purifies only industrial waste. In addition, extremely low concentrations of Cd and As at the San Giorgio di Nogaro plant and high levels of As at the Lignano Sabbiadoro plant were detected.

The cluster analysis applied to samples of 2020 shows a situation similar to 2019 (**Figure 2c**), with Croatian sites grouped except for Stupe DP, mainly due to a higher average value of As compared to the other sites. The sites of the Gulf of Trieste are far from those of Croatia, and the most similar sites are still the seawater ones, as in 2019.

The cluster analysis highlighted how the geographical component has a greater influence than the type of sample collected (DP or seawater), as the sites are grouped mainly according to the sampling area, in particular showing a clear division between the samples collected in Italy and Croatia, both in the 2019 and 2020 sampling years. This result is strengthened by the fact that Hg and As have geographical peculiarities, as mentioned in the previous paragraph since the first is found at higher concentrations in the northern part of the basin while the second is known to show higher levels in the southern part of the Adriatic Sea.



(**a**) (**b**) (**c**)

Figure 2. Cluster analysis on PTEs mean concentrations results as a function of sampling stations: overall mean (**a**), 2019 (**b**), and 2020 (**c**).

To better understand the main factors influencing the distribution of PTEs in the various investigated areas, a multivariate analysis (PCA) was applied to the overall dataset. The PCA extracted 2 significant, cross-validated principal components which account for 93.6% of the variability in the original data (**Table 1**).

Table 1. Principal Component Analysis. Eigenvalues, explained and cumulative variance, loadings of the variables for the first two PCs.

	Principal Components	
	1	**2**
Variance explained		
Eigenvalues	5.482	1.072
% of variance	78.312	15.318
Cumulative %	78.312	93.630
Factor loadings		
Lignano Sabbiadoro	2.970	1.045
San Giorgio di Nogaro	2.108	-1.193
Zadar Upov Centar	-1.511	-0.998
Katalinića brig	-1.674	-0328
Stupe	-1.892	-0.818

Figure 3 shows the results of the PCA in terms of loading plot and score plot of PC1 vs. PC2.

The first principal component (PC1, 78.31%) highlighted the difference between two different groups of samples: i) the Croatian sites (negative scores) far from Cd and Hg as they showed a very low concentration of them, but associated with the salinity and As parameters; ii) the Italian sites (positive scores), associated with a higher concentration of Cd and Hg, but also the temperature and turbidity parameters (**Figure 3**). The second principal component (PC2, 15.32%) separated the sampling stations of Split (Katalinića brig and Stupe) and Lignano Sabbiadoro (positive scores) from San Giorgio di Nogaro and Zadar sites (negative scores), with dissolved oxygen and temperature parameters as the main driver (**Figure 3**). In our opinion, this component is mainly influenced by the different hydrodynamic behaviour of the water masses in the sampling areas.

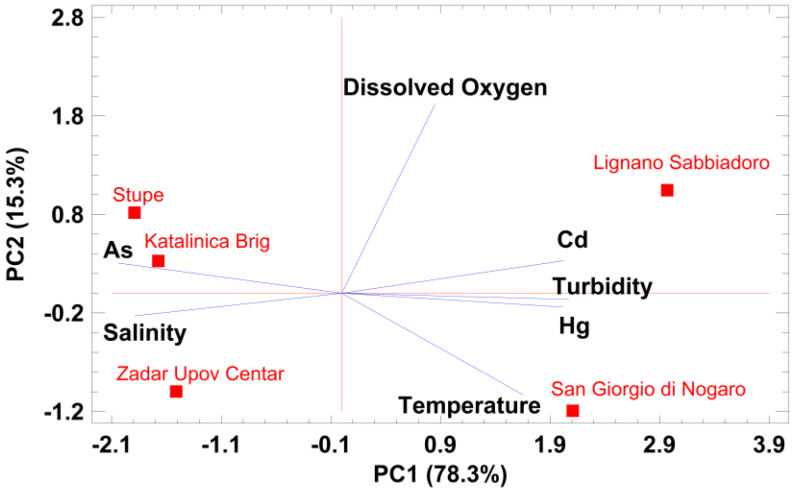
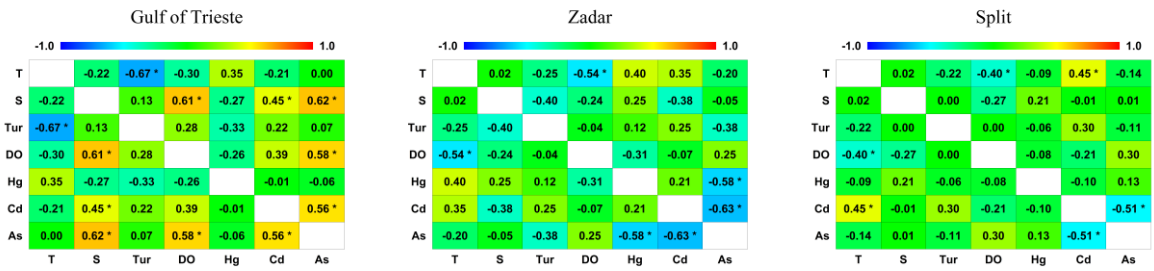


Figure 3. 2D biplot of PC1 vs PC2.

The environmental conditions vary greatly from site to site, as in the north there is a thermocline that goes under the bottom in the warmer months and this, therefore, leads to higher average temperatures than in Croatian coastal waters, where instead of the deeper samples, which are therefore colder, have a significant weight in the annual average. Similarly, for oceanographic reasons and the influence of freshwaters, naturally less salty waters are found in the northern area of the basin.

To reduce this environmental effect and normalize the weights of the CTD variables, we reported the statistical correlation matrices applied to each sampling area, considering all the monthly samples collected (**Figure 4**).



(**a**) (**b**) (**c**)

Figure 4. Site-specific statistical correlation matrices applied in the Gulf of Trieste (**a**), Zadar (**b**), and Split (**c**). T: temperature; S: salinity; Tur: turbidity; DO: dissolved oxygen. The color scale indicates the Pearson product moment correlations between each pair of variables (from -1 to 1). *Statistically significant correlation ($p<0.05$) between the pair of variables.

In the Gulf of Trieste, a statistically significant correlation was found between the following pairs: temperature-turbidity ($p=0.0007$), dissolved oxygen-salinity ($p=0.0025$), Cd-salinity (0.0371), As-salinity (p=0.0021), dissolved oxygen-As (p=0.0044), and Cd-As (p=0.0066) (**Figure 4a**). In Zadar, the pairs of variables showing a statistically significant relationship are temperature-dissolved oxygen (p=0.0196), Hg-As (p=0.0119), and Cd-As (0.0048) (**Figure 4b**). In Split, a statistically significant correlation was found between the following pairs: temperature-dissolved oxygen (p=0.0327), Cd-As (p=0.0055), and Cd-temperature (p=0.0170) (**Figure 4c**).

To better understand the main factors influencing the distribution of nutrients in the various investigated areas, a multivariate analysis (PCA) was applied to the data obtained from Italian site (overall mean 2019-2020). The PCA extracted 2 significant, cross-validated principal components which account for 99.99% of the variability in the original data (**Table 2**). The chemical physical parameters were not included since these data are available only for seawater samples.

Table 2. Principal Component Analysis. Eigenvalues, explained and cumulative variance, loadings of the variables for the first two PCs.

	Principal Components	
	1	**2**
Variance explained		
Eigenvalues	5.63	1.37
% of variance	80.41	19.60
Cumulative %	80.41	99.99
Factor loadings		
Lignano Sabbiadoro DP	3.21	-075
San Giorgio di Nogaro DP	0.37	1.75
Lignano Sabbiadoro sea	-1.79	-0.49
San Giorgio di Nogaro sea	-1.79	-0.50

The first principal component (PC1, 80.41%) highlighted the difference between two different groups of samples: i) the Depuration Plants (positive scores) near to all nutrients as they showed a very high concentration of them; ii) the Sea sites (negative scores), associated with a lower concentration of nutrients (**Figure 5**). The second principal component (PC2, 19.6%) associated the San Giorgio di Nogaro sampling sites (positive scores), with ammonia parameters as the main driver (**Figure 5**). This component remarked the difference between the two DPs ; 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). On the other side (negative score) the two Sea sites are very close, to highlight their similarity in nutrient contents.

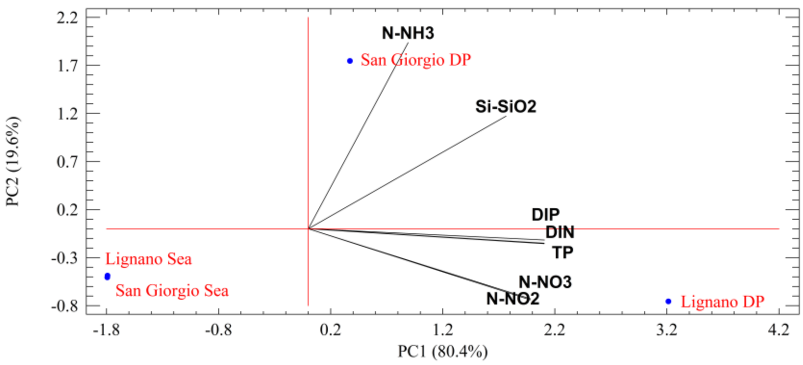


Figure 5. 2D biplot of PC1 vs PC2

For a deeper investigation a multivariate analysis (PCA) was applied to the data obtained from Italian site, considering the annual mean 2019 and 2020 separately. The PCA extracted 2 significant, cross-validated principal components which account for 99.99% of the variability in the original data (**Table 3**).

Table 3. Principal Component Analysis. Eigenvalues, explained and cumulative variance, loadings of the variables for the first two PCs.

	Principal Components	
	1	**2**
Variance explained		
Eigenvalues	5.41	1.16
% of variance	77.28	16.51
Cumulative %	77.28	93.79
Factor loadings		
Lignano Sabbiadoro DP 2019	4.15	-1.04
Lignano Sabbiadoro DP2020	2.61	-0.20
San Giorgio di Nogaro DP2019	-0.27	0.54
San Giorgio di Nogaro DP DP2020	0.76	2.43
Lignano Sabbiadoro sea 2019	-1.83	-0.45
Lignano Sabbiadoro sea DP2020	-1.79	-0.40
San Giorgio di Nogaro sea2019	-1.83	-0.45
San Giorgio di Nogaro sea DP2020	-179	-0.43

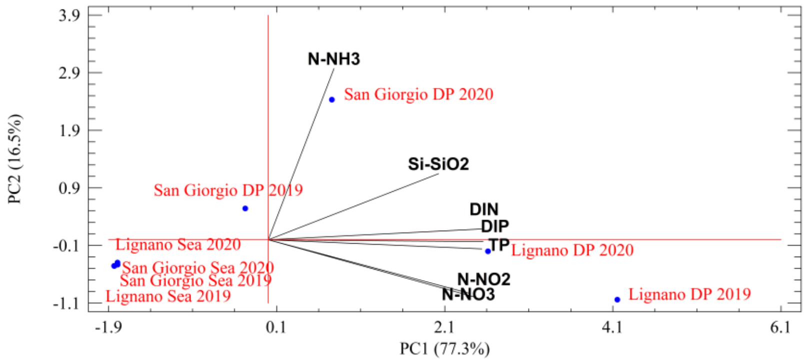


Figure 6. 2D biplot of PC1 vs PC2.

Principal Component Analysis (PCA) performed on nutrients' mean values for every site and year, confirm the evidence highlighted in the previous analysis which account for 94% of the variability in the original data. In fact, even in this case the main component (PC1, 77.28%) applies a distinction from DPs and Sea site for the content on TP, DIP, DIN and Silicates mainly. As evident from the figure, all Sea sites are on the left side of the graph, indicating their low content on nutrients in respect to DPs, thanks to the high dilution effect of the water body. No differences between years are evident for seaside. The second component (PC2, 16.5%) shows a separation on N-NH_3 and N-NO_2, N-NO_3 concentrations; in facts the two DPs are extremely different concerning the DIN composition, as result of the different typology sewage input in the plant. In this case San Giorgio DP outflows are characterized by higher levels of ammoniacal nitrogen, especially in 2020, well clear in PC2.

PART 4: CONCLUSION

This project provides for the information on nutrients and some 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:

Nutrients:

i) A strong dilution effect between one to three orders of magnitude from DP to Sea was evident for every nutrient. (ii) Phosphorus levels, both Inorganic and Total, are different in the DPs and are generally higher about 2.6 times in Lignano than in San Giorgio. (iii) There is a strong difference between Lignano and San Giorgio DP's outflow composition, because of the different typology of sewage inputs.

PTEs:

i) all the samples analysed resulted in concentrations below the European and national legal limits, in particular, mean values were 2X below the legal limit for every PTE; ii) no particularly DP effect was recorded as concentrations in DP outflow and seawater in the proximity of the DP's discharge pipes were similar; iii) 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.

Concerning the parameters investigated in this study, 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., faecal indicator bacteria and nutrient levels) are required 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.