

MONITOOL PROJECT

Relationship between metal concentrations obtained by DGTs and by "classical" spot (water) sampling. WP nr. 4, action nr. 2.

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Report/Deliverable by

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Disclaimer

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Glossary

ASV: Anodic Stripping Voltammetry. In graphs refers to the mean value of concentration of a metal measured in spot samples covering an exposure period.

CSV: Cathodic Stripping Voltammetry. In graphs refers to the mean value of concentration of a metal measured in spot samples covering an exposure period.

DGT: Diffusive Gradients in Thin films. In graphs refers to the mean value of concentration of a metal measured in DGT by ICP-MS.

ICP-MS: Inductively Coupled Plasma Mass Spectrometry. In graphs refers to the mean value of concentration of a metal measured in spot samples covering an exposure period.

Volt: Voltammetry. It refers to CSV or ASV.





Executive Summary

In this deliverable we explore the relationships in the metal concentration variability derived from total dissolved fraction and chemically labile fraction in "classical" spot (water) samples and labile fraction in DGTs.

It was found that in general there is a log-log linear relationship between the three methodologies (DGT, ICP-MS in spot samples, and voltammetry in spot samples). The strength of these relationships variated among metals.





1. Introduction

According to the EU Water Framework Directive (WFD, 2000/60/EC), the good chemical status of water bodies is achieved when the concentrations of priority substances do not exceed the relevant Environmental Quality Standards (EQS) established by Directive 2008/105/EC (subsequently amended by Directive 2013/39/EU). Regarding metals, the EQS refers to the dissolved concentration, i.e. the metal concentrations measured in a water sample previously filtered through a 0.45 μ m filter or subjected to any equivalent pre-treatment.

In water, metals are present in different chemical forms, i.e., free ions, complexes with inorganic and organic ligands and/or adsorbed on the surface of particles or colloids. This implies that different methodologies may measure different fractions (or chemical forms) of the total metal content. The most commonly used approach, to comply with the requirements of the WFD for metals, relies on water samples obtained by spot sampling, followed by filtration (dissolved metal), preconcentration and instrumental analysis (CIS, 2009). The limitations of low-frequency spot sampling, such as the lack of representativeness in dynamic systems have been discussed elsewhere (e.g., Allan et al., 2006). Thus, the inclusion of complementary methodologies, which integrate the environmental metal fluctuations and/or measure the metal speciation that can be more easily related to ecotoxicological effects, might improve the quality of the assessment (CIS, 2009). Hence, passive samplers have been used for measuring labile metal concentrations in waters. The Diffusive Gradients in Thin-films (DGT; Davison and Zhang, 1994) is the most extensively used sampler for in situ labile metal measurements (Menegário et al., 2017). DGT samplers accumulate metals continuously during the deployment time, usually ranging from several days to weeks. This provides time-weighted average metal concentrations and enables the achievement of lower limits of quantification compared with low-volume water samples. In addition, DGT samplers only accumulate free metal ions and easily dissociable metal complexes, operationally known as DGT-labile concentration, which has been related to observed toxicity in different types of organisms (e.g., Koppel et al., 2019). These advantages might favour the inclusion of DGT technique within monitoring programmes.

In this deliverable we will deal on the one hand with the "classical" strategy of spot sampling but repeated in time during a specific period. On the other hand, simultaneously, during that period we will expose DGT in water. In this way we can evaluate whether there is a global relationship between the two approaches for different metals.

2. Scope

The scope of this deliverable is to explore the relationships in the metal concentration variability derived from total dissolved fraction and chemically labile fraction in "classical" spot (water) samples and labile fraction in DGTs.



3. Material and Methods

3.1 Origin of data

Sampling campaigns were carried out by MONITOOL partners (AZTI, CEFAS, DCU, IFREMER, IPMA, ITC, MSS, SEPA and UNICA) covering eight countries (England, France, Ireland, Italy, Northern Ireland, Portugal, Scotland and Spain) located in five European sub-regions. Sampling campaigns were carried out in 2018, consisting of the simultaneous deployment of DGT samplers, collection of discrete water samples and measurement of physico-chemical parameters. A total of 36 sites were sampled and when possible, the same stations were sampled in different seasons.

At each sampling site three different methodologies were used to measure metal content in waters: i) Inductively Coupled Plasm-Mass Spectrometry on (IPC-MS) in Diffusive Gradient in Thin films (DGTs), ii) Inductively Coupled Plasm-Mass Spectrometry (IPC-MS, with seaFAST preconcentration system) on water spot sampling and, iii) voltammetry (Volt) on water spot sampling. Details on the laboratory and field methodologies are given in deliverables from WP5 and in Rodríguez et al. (2021).

Some remarks on data and statistical analysis:

- 1) The main data are provided from WP5. Only data obtained following the Monitool protocols have been considered.
- 2) At some sampling sites the DGTs were collected in two sampling dates. In those cases, the mean values of spot water samples associated at each DGT collection data were calculated including only the values of the dates in which the DGT were deployed. This implies that from the same sampling site and season can be two data, e.g., one DGT from Day #0 to Day #2, and other DGT from Day #0 to Day #4 (Table 1). This implies that these data cannot be considered as independent data from a statistical point of view. Nevertheless, for the exploration of results, these data were considered as independent, and therefore, the statistical results should be taken into account carefully.
- 3) The voltammetry method was used for measuring three metals: Cd, Ni and Pb. Hence the determination of conditional labile Pb and Cd concentrations in filtered and acidified water samples was done by Anodic Stripping Voltammetry (ASV). For the determination of total Ni dissolved concentrations, Cathodic Stripping Voltammetry (CSV) was carried out after UV irradiation of water samples to guarantee the oxidation of organic matter. For Cd and Pb determination the samples were not UV-irradiated before the analysis. It should be noted that (ASV) is typically used for the measurement of labile metal species in water samples at natural pH, instead of acidified water samples. Because the determination of ASV-labile concentrations at natural pH must be performed within a reasonable time after sample collection to minimize loss of metal species due to adsorption on vessel walls, samples were acidified on site in this study. Immediate acidification of the water samples once filtered can, however, give information about the labile fraction at a lower pH than the natural one, in the presence of a still inert fraction that may comprise metals strongly bound to natural organic molecules or bound to colloids.
- 4) The total dissolved concentrations of trace metals Cd, Cu, Ni, Pb and Zn in filtered and acidified water samples were determined by an online pre-concentration seaFAST system (Elemental Scientific, Nebraska, USA) coupled with an ICP-MS.





- 5) The determination of trace elements in DGTs by ICP-MS was done by binding resin gel was removed from the sampler and eluted in 1.22 mL of 1M HNO₃ acid solution for at least 24 hours. The resulting acid extracts were analysed by ICP-MS for the determination of trace elements Cd, Cu, Ni, Pb and Zn after dilution 5 times with ultrapure water (Milli-Q, Millipore).
- 6) The values below the quantification limit were transformed as the half of the quantification limit. In those cases where the mean value of concentrations is below the quantification limit, the values were not included.
- 7) The laboratories provided a list of possible anomalous results to the partners. Each partner, based in their experience and knowledge of the studies areas, checked the obtained data and decided which values should be removed o included for the statistical analysis.
- 8) The relationships between the metal concentration analysis methodologies (ICP-MS in DGTs, ICP-MS on water spot sampling and voltammetry on water spot sampling) were explored based upon arithmetic mean values. Hence, for ICP-MS in DGTs, the mean value among replicates was used. For data based upon spot sampling at each sampling the mean value among replicates was used, and as whole mean the mean values of mean values at each sampling was used as representative of the sampling period.
- 9) There is no available metal data from the three methods of analysis in all water samples. In other words, it is possible that from a sampling site only results from one of two methods of analysis is available (e.g., due of problems in the transport to laboratories or problems in the sampling).
- 10) Outliers were identified using linear modelling of the data. The objective was to identify outliers samples whose standardized residuals from the linear model were greater than 3 (rejection of values above 99.73% of the total values, assuming that their distribution follows a normal distribution). A detailed procedure is given in the Supplementary material of Rodríguez et al. (2021). Values identified as outliers were excluded.

Partner	Label (name)	Two days collection data	Label (number)
AZTI	DEBA_DS		1
AZTI	LEZO_DS		2
AZTI	MUSEO_DS_D2	X	3
AZTI	MUSEO_DS_D4	X	4
AZTI	PRACTICOS_DS		5
AZTI	DEBA_WS		6
AZTI	LEZO_WS		7
AZTI	MUSEO_WS_D3	X	8
AZTI	MUSEO_WS_D5	X	9
AZTI	PRACTICOS_WS		10
CEFAS	BELFAST_DS		11
CEFAS	FAL_DS_D2	Х	12
CEFAS	FAL_DS_D4	X	13
CEFAS	LIVERPOOL_DS		14

Table 1. List of samples.





Partner	Label (name)	Two days collection data	Label (number)
CEFAS	X38A_DS		15
CEFAS	BELFAST_WS		16
CEFAS	FAL_WS_D2	Х	17
CEFAS	FAL_WS_D4	Х	18
CEFAS	LIVERPOOL_WS		19
CEFAS	X38A_WS		20
DCU	ABW_DS_D2	Х	21
DCU	ABW_DS_D4	Х	22
DCU	M69_DS		23
DCU	M70_DS		24
DCU	DUBLINBAYBUOY2_DS		25
DCU	DUBLINBAYBUOY4_DS		26
DCU	ABW_WS		27
DCU	M69_WS		28
DCU	M70_WS		29
DCU	DUBLINBAYBUOY2_WS	Х	30
DCU	DUBLINBAYBUOY4_WS	Х	31
IFREMER	FONTENELLE_DS		32
IFREMER	TERENEZ_DS		33
IFREMER	BESSIN_DS		34
IFREMER	LAZARET_EIL		35
IFREMER	SAINTNAZAIRE_DS_D2		36
IFREMER	SAINTNAZAIRE_DS_D4		37
IFREMER	SAUMONARD_DS		38
IFREMER	FONTENELLE_WS		39
IFREMER	ANTIFER_WS_BAF		40
IFREMER	BESSIN_WS		41
IFREMER	LECROISIC		42
IFREMER	SAINTNAZAIRE_WS_D2	Х	43
IFREMER	SAINTNAZAIRE_WS_D4	Х	44
IFREMER	SAUMONARD_WS		45
IFREMER	SAUMONARD_WS_BAF		46
IFREMER	SILLONDESANGLAIS_WS_BAF		47
IPMA	AVEIRO_DS		48
IPMA	PORTO_DS		49
IPMA	SESIMBRA_DS		50
IPMA	TAGUS_DS		51
IPMA	AVEIRO_WS_D3		52
IPMA	AVEIRO_WS_D5		53
IPMA	PORTO_WS		54
IPMA	SESIMBRA_WS_D2	X	55
IPMA	SESIMBRA_WS_D4	Х	56
IPMA	TAGUS_WS_D2	X	57
IPMA	TAGUS_WS_D4	X	58
ITC	GANDO_DS		59





Partner	Label (name)	Two days collection data	Label (number)
ITC	JINAMAR_DS		60
ITC	LUZ_DS_D2	X	61
ITC	LUZ_DS_D4	X	62
ITC	LUZ_WP4_2_D2	Х	63
ITC	LUZ_WP4_2_D4	X	64
ITC	TALIARTE_DS		65
ITC	TALIARTE_WP4_2_D2	Х	66
ITC	TALIARTE_WP4_2_D4	Х	67
ITC	GANDO_WS		68
ITC	JINAMAR_WS		69
ITC	LUZ_WS_D3	Х	70
ITC	LUZ_WS_D7	Х	71
ITC	TALIARTE_WS		72
MSS-SEPA	BRAEHEAD_WS		73
MSS-SEPA	MONTROSE_WS		74
MSS-SEPA	NEWHAVEN_WS		75
UNICA	MOLODOGANA_DS		76
UNICA	MOLOINCHUSA_DS		77
UNICA	MOLORINASCITA_DS		78
UNICA	SANTELMO_DS_D2	Х	79
UNICA	SANTELMO_DS_D5	Х	80
UNICA	MOLODOGANA_WS		81
UNICA	MOLOINCHUSA_WS		82
UNICA	MOLORINASCITA_WS		83
UNICA	SANTELMO_WS_D3	Х	84
UNICA	SANTELMO_WS_D5	Х	85

3.2 Statistical approach

Exploration was carried out on untransformed data and on log-transformed data (i.e., logarithm with base 10). For modelling the relationships, linear model II regression analyses were carried out. It was used the ranged major axis (RMA) approach with GRAPHER software (version 13). It should be noted that this kind on regressions cannot be used for predicting Y values from X values (Warton et al. 2006). However, these methods are useful for modelling the functional linear relationships between variables that are random and measured with error (Legendre and Legrendre, 2012).





Type I regression: fits the line by minimizing the sum of the squares of the y-offsets (residuals). E.g., ordinary least squares regression.



Type II regression: fits the line by minimizing the sum of the squares of the offsets measured along a line perpendicular (or normal) to the regression line.



Figure 1. Type I vs. type II regression models.

4. Cadmium

The values of Cd measured with the three different approaches (DGT, IPC-MS, Volt) showed a relatively close log-normal distribution. This fact difficulties to model correctly the relationships in a linear way.

The linear relation between DGT and ICP-MS accounts a R^2 value of 0.90. The log-log relationship between both methods accounts a R^2 value of 0.80 (Figure 2).

On the other hand, several concentration values analysed by voltammetry were below quantification limit. The linear relation between DGT and Volt accounts a R² value of 0.55. The log-log relationship between both methods accounts a R² value of 0.55 (Figure 3).

Finally, the R^2 value was 0.48 in the linear relation between Volt and IPC-MS, whereas the log-log relationship between both methods has a R^2 value of 0.44 (Figure 4).





Equation Y = 0.9413407219 * X + 2.429713368 R-sq'd = 0.902628 P-Value = Less than 0.0001



Figure 2. Relationship between mean concentration of Cd measured in spot sampling (ICP-MS) and DGT.





Figure 3. Relationship between mean concentration of Cd measured in spot sampling (Anodic Stripping Voltammetry, ASV) and DGT.





P-Value = 0.0485117122947524

Figure 4. Relationship between mean concentration of Cd measured in spot sampling by Anodic Stripping Voltammetry (ASV) and by ICP-MS.





5. Nickel

The values of Ni measured with the three different approaches (DGT, IPC-MS, Volt) showed a relatively close log-normal distribution. This fact difficulties to model correctly the relationships in a linear way.

The linear relation between DGT and ICP-MS accounts a R^2 value of 0.53. The log-log relationship between both methods accounts a R^2 value of 0.56 (Figure 5).

The linear relation between DGT and Volt accounts a R² value of 0.58. The log-log relationship between both methods accounts a R² value of 0.48 (Figure 6).

Finally, the R^2 value was 0.68 in the linear relation between Volt and IPC-MS, whereas the log-log relationship between both methods has a R^2 value of 0.61 (Figure 7).





Equation Y = 1.10081161 * X - 82.94542108 R-sq'd = 0.529786 P-Value = Less than 0.0001



Figure 5. Relationship between mean concentration of Ni measured in spot sampling (ICP-MS) and in DGT.





Equation Y = 1.010785869 * X - 37.98058853 R-sq'd = 0.579447 P-Value = Less than 0.0001



Equation Y = 1.078966764 * X - 0.245210234 R-sq'd = 0.481187 P-Value = 0.000210586935434964

Figure 6. Relationship between mean concentration of Ni measured in spot sampling (Cathodic Stripping Voltammetry, CSV) and in DGT.







Figure 7. Relationship between mean concentration of Ni measured in spot sampling by Cathodic Stripping Voltammetry (CSV) and by ICP-MS.





6. Lead

The values of Pb measured with the three different approaches (DGT, IPC-MS, Volt) showed a relatively close log-normal distribution, but some sampling sites have values considerably higher than the mean values (with the three methodologies). This fact difficulties to model correctly the relationships in a linear way or in a log-log way.

The linear relation between DGT and ICP-MS accounts a R² value of 0.52. The log-log relationship between both methods accounts a R² value of 0.56 (Figure 8). It should be noted that at concentrations lower than 50 ng/L measured by DGT the relationship between DGT and spot sampling is weaker.

The linear relation between DGT and Volt accounts a R² value of 0.43. The log-log relationship between both methods accounts a R² value of 0.43 (Figure 6). It should be noted that at concentrations lower than 25 ng/L measured by DGT the relationship between DGT and spot sampling is weaker.

Finally, the R^2 value was 0.63 in the linear relation between Volt and IPC-MS, whereas the log-log relationship between both methods has a R^2 value of 0.60 (Figure 7).





Equation Y = 4.31253146 * X - 197.0411321 R-sq'd = 0.521662 P-Value = Less than 0.0001



Equation Y = 1.132002399 * X - 0.01930857905 R-sq'd = 0.558358 P-Value = Less than 0.0001

Figure 8. Relationship between mean concentration of Pb measured in spot sampling (ICP-MS) and in DGT.





Equation Y = 1.756537532 * X - 56.57184713 R-sq'd = 0.428514 P-Value = 0.0113247081379637



Equation Y = 1.756537532 * X - 56.57184713 R-sq'd = 0.428514 P-Value = 0.0113247081379637

Figure 9. Relationship between mean concentration of Pb measured in spot sampling (Anodic Stripping Voltammetry, ASV) and DGT.









Equation Y = 0.9613207583 * X - 0.14249011 R-sq'd = 0.602578 P-Value = Less than 0.0001

Figure 10. Relationship between mean concentration of Pb measured in spot sampling by Anodic Stripping Voltammetry (ASV) and by ICP-MS.





7. Cobalt

The values of Co measured with the two different approaches (DGT, IPC-MS) showed a relatively close log-normal distribution. This fact difficulties to model correctly the relationships in a linear way.

The linear relation between DGT and ICP-MS accounts a R^2 value of 0.50 (Figure 11). The log-log relationship between both methods accounts a R^2 value of 0.41 (Figure 11). Both in the linear relationship and in the log-log relationship there is a high dispersion in the relationship between the two methods. It should be noted that at concentrations lower than 100 ng/L measured by DGT the relationship between DGT and spot sampling is weaker.

8. Copper

The values of Cu measured with the two different approaches (DGT, IPC-MS) showed a relatively close log-normal distribution. This fact difficulties to model correctly the relationships in a linear way.

The linear relation between DGT and ICP-MS accounts a R^2 value of 0.76 (Figure 12). The log-log relationship between both methods accounts a R^2 value of 0.62 (Figure 12).

9. Manganese

The values of Mn measured with the two different approaches (DGT, IPC-MS) showed a relatively close log-normal distribution. Some sampling sites have values considerably higher than the mean values. This fact difficulties to model correctly the relationships in a linear way.

The linear relation between DGT and ICP-MS accounts a R^2 value of 0.92 (Figure 13). The log-log relationship between both methods accounts a R^2 value of 0.73 (Figure 13).

10.Zinc

The values of Zn measured with the two different approaches (DGT, IPC-MS) showed a relatively close log-normal distribution. This fact difficulties to model correctly the relationships in a linear way.

The linear relation between DGT and ICP-MS accounts a R^2 value of 0.41 (Figure 14). The log-log relationship between both methods accounts a R^2 value of 0.44 (Figure 14).





Equation Y = 0.6109370282 * X - 30.62247223 R-sq'd = 0.496098 P-Value = Less than 0.0001



Figure 11. Relationship between mean concentration of Co measured in spot sampling (ICP-MS) and in DGT.





Equation Y = 1.073549595 * X + 157.4827267 R-sq'd = 0.758816 P-Value = Less than 0.0001



P-Value = Less than 0.0001

Figure 12. Relationship between mean concentration of Cu measured in spot sampling (ICP-MS) and in DGT.





Figure 13. Relationship between mean concentration of Mn measured in spot sampling (ICP-MS) and in DGT.





Equation Y = 0.793884869 * X - 863.7856664 R-sq'd = 0.406561 P-Value = 0.0207946868409477



Equation Y = 1.095540387 * X - 0.5468783692 R-sq'd = 0.435844 P-Value = 0.00220304220964651

Figure 14. Relationship between mean concentration of Zn measured in spot sampling (ICP-MS) and in DGT.





11.DGT vs spot sampling: Cd, Ni, Pb

The main objective of this deliverable is to explore the relationship between metal concentrations obtained by DGTs and by "classical" spot (water) sampling. In the case of Cd, Ni and Pb apart from DGTs, two different measurements were carried out at laboratory (ICP-MS and Volt). In this chapter, these relationships among them are explored. For this purpose, in the same figure there are represented the log-log regressions (DGT vs Volt -in blue-, and DGT vs ICP-MS -in red-), and with a line of hypothetical 1:1 (in black).

In the Figure 15 results for Cd are shown. Due to the high amount of data below the quantification limit in the Volt, comparisons should be taken carefully. There is an important difference in the slope of the regression lines. In the case of the ICP-MS fit line (in red) it is observed that it crosses the hypothetical 1:1 line so that at lower concentrations higher values are observed for the same sampling point with spot sampling than with DGT. In the case of the adjustment for voltammetry (blue line) it is in the highest values where the value measured with spot sampling is higher than that measured with DGT.

In the Figure 16 results for Ni are shown. Both relationships (DGT-Volt and DGT-ICP-MS) are relatively similar and very close to a 1:1 relationship. The similarity between the two regressions results is consistent with the fact that the methodology used to prepare the sample prior to voltammetric analysis theoretically approximates a total dissolved fraction determination result.

In the Figure 17 results for Pb are shown. It is observed that the slope is relatively similar in both regressions and that the values determined by voltammetry are on average lower than those determined by ICP-MS. Additionally, the regression line for DGT vs. spot sampling (ICP-MS) is located in the area above the 1:1 line indicating that, in general, the values determined by spot sampling (ICP-MS) are higher than those determined by DGT. On the other hand, the regression line for DGT vs. spot sampling (voltammetry) is relatively similar to 1:1.



Figure 15. Relationship between mean concentration of Cd measured in spot sampling (by Anodic Stripping Voltammetry and ICP-MS) vs. DGT. Type II regressions are shown.



Figure 16. Relationship between mean concentration of Ni measured in spot sampling (by Cathodic Stripping Voltammetry and by ICP-MS) vs. DGT. Type II regressions are shown.



Figure 17. Relationship between mean concentration of Pb measured in spot sampling (Anodic Stripping Voltammetry and ICP-MS) vs. DGT. Type II regressions are shown.

12.DGT vs spot sampling: Co, Cu, Mn and Zn

The concentration of Co, Cu, Mn and Zn was not measured by voltammetry, and hence only a laboratory method was used for determination of spot samplings (IPC-MS). The relationships between spot sampling and DGT differ among metals. The concentrations of Co, Mn and Zn determined by spot sampling (ICP-MS) are in averaged, lower that those determined by DGT (Figure 18). On the other hand, the relationship between DGT and spot sampling for Cu is relatively very close to a 1:1 relationship.







Figure 18. Relationship between mean concentration of Cu, Co, Mn and Zn measured in spot sampling (ICP-MS) vs. DGT. Type II regressions are shown.

13.Main conclusions

1) DGT vs. spot sampling (concentration measured by ICP-MS)

Cd, Mn and Cu: there is a strong and direct log-log relationship between the concentration measured by DGT and the mean concentration measured by spot sampling (by ICP-MS). R² values: 0.61-0.80 (Table 2).

Ni and Pb: there is a medium-strength and direct log-log relationship between the concentration measured by DGT and the mean concentration measured by spot sampling (by ICP-MS). R^2 value: 0.56 (Table 2).





Co and Zn: there is a weak and direct log-log relationship between the concentration measured by DGT and the mean concentration measured by spot sampling (by ICP-MS). R² values: 0.41-0.44 (Table 2).

2) DGT vs. spot sampling (concentration measured by voltammetry)

Cd and Ni: there is a medium strength and direct log-log relationship between the concentration measured by DGT and the mean concentration measured by spot sampling (by voltammetry). R² values: 0.48-0.55 (Table 2).

Pb: there is a weak and direct log-log relationship between the concentration measured by DGT and the mean concentration measured by spot sampling (by voltammetry). R² value: 0.43 (Table 2).

3) Spot sampling (concentration measured by ICP-MS) vs. spot sampling (concentration measured by voltammetry)

Cd and Ni: there is a strong and direct log-log relationship between the concentration measured by spot sampling by ICP-MS and by voltammetry. R^2 values: 0.60-0.61 (Table 2).

Pb: there is a weak and direct log-log relationship between the concentration measured by spot sampling by ICP-MS and by voltammetry. R² values: 0.43 (Table 2).

Table 2. R-squared of lineal and log-log regressions between mean concentration measured in waters by DGT and by spotsampling.

Metal	X	Y	Regression	R ²
Cd	DGT	spot sampling (ICP-MS)	lineal	0.90
Cd	DGT	spot sampling (ICP-MS)	log-log	0.80
Cd	DGT	spot sampling (Volt)	lineal	0.55
Cd	DGT	spot sampling (Volt)	log-log	0.55
Cd	spot sampling (ICP-MS)	spot sampling (Volt)	lineal	0.48
Cd	spot sampling (ICP-MS)	spot sampling (Volt)	log-log	0.44
Ni	DGT	spot sampling (ICP-MS)	lineal	0.53
Ni	DGT	spot sampling (ICP-MS)	log-log	0.56
Ni	DGT	spot sampling (Volt)	lineal	0.58
Ni	DGT	spot sampling (Volt)	log-log	0.48
Ni	spot sampling (ICP-MS)	spot sampling (Volt)	lineal	0.68
Ni	spot sampling (ICP-MS)	spot sampling (Volt)	log-log	0.61
Pb	DGT	spot sampling (ICP-MS)	lineal	0.52
Pb	DGT	spot sampling (ICP-MS)	log-log	0.56
Pb	DGT	spot sampling (Volt)	lineal	0.43
Pb	DGT	spot sampling (Volt)	log-log	0.43
Pb	spot sampling (ICP-MS)	spot sampling (Volt)	lineal	0.63
Pb	spot sampling (ICP-MS)	spot sampling (Volt)	log-log	0.60





Metal	Х	Y	Regression	R ²
Со	DGT	spot sampling (ICP-MS)	lineal	0.50
Со	DGT	spot sampling (ICP-MS)	log-log	0.41
Cu	DGT	spot sampling (ICP-MS)	lineal	0.76
Cu	DGT	spot sampling (ICP-MS)	log-log	0.62
Mn	DGT	spot sampling (ICP-MS)	lineal	0.92
Mn	DGT	spot sampling (ICP-MS)	log-log	0.73
Zn	DGT	spot sampling (ICP-MS)	lineal	0.41
Zn	DGT	spot sampling (ICP-MS)	log-log	0.44

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MONITOOL PROJECT

Relationship between metal concentrations obtained by DGTs and by "classical" spot (water) sampling. WP nr. 4

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Report/Deliverable by

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Glossary

ASV: Anodic Stripping Voltammetry. In graphs refers to the mean value of concentration of a metal measured in spot samples covering an exposure period.

CSV: Cathodic Stripping Voltammetry. In graphs refers to the mean value of concentration of a metal measured in spot samples covering an exposure period.

DGT: Diffusive Gradients in Thin films. In graphs refers to the mean value of concentration of a metal measured in DGT by ICP-MS.

ICP-MS: Inductively Coupled Plasma Mass Spectrometry. In graphs refers to the mean value of concentration of a metal measured in spot samples covering an exposure period.

Volt: Voltammetry. It refers to CSV or ASV.





Executive Summary

This deliverable can be considered an extension or a complementary work to the previous one exploring the relationships in the metal concentration variability derived from total dissolved fraction and chemically labile fraction in "classical" spot (water) samples and labile fraction in DGTs with 2018 data. Taking into account 2018 and 2022 data, it was found that in general there is a log-log linear relationship between the three methodologies (DGT, ICP-MS in spot samples, and voltammetry in spot samples).





1. Introduction

According to the EU Water Framework Directive (WFD, 2000/60/EC), the good chemical status of water bodies is achieved when the concentrations of priority substances do not exceed the relevant Environmental Quality Standards (EQS) established by Directive 2008/105/EC (subsequently amended by Directive 2013/39/EU). Regarding metals, the EQS refers to the dissolved concentration, i.e. the metal concentrations measured in a water sample previously filtered through a 0.45 μ m filter or subjected to any equivalent pre-treatment.

In water, metals are present in different chemical forms, i.e., free ions, complexes with inorganic and organic ligands and/or adsorbed on the surface of particles or colloids. This implies that different methodologies may measure different fractions (or chemical forms) of the total metal content. The most commonly used approach, to comply with the requirements of the WFD for metals, relies on water samples obtained by spot sampling, followed by filtration (dissolved metal), preconcentration and instrumental analysis (CIS, 2009). The limitations of low-frequency spot sampling, such as the lack of representativeness in dynamic systems have been discussed elsewhere (e.g., Allan et al., 2006). Thus, the inclusion of complementary methodologies, which integrate the environmental metal fluctuations and/or measure the metal speciation that can be more easily related to ecotoxicological effects, might improve the quality of the assessment (CIS, 2009). Hence, passive samplers have been used for measuring labile metal concentrations in waters. The Diffusive Gradients in Thin-films (DGT; Davison and Zhang, 1994) is the most extensively used sampler for in situ labile metal measurements (Menegário et al., 2017). DGT samplers accumulate metals continuously during the deployment time, usually ranging from several days to weeks. This provides time-weighted average metal concentrations and enables the achievement of lower limits of quantification compared with low-volume water samples. In addition, DGT samplers only accumulate free metal ions and easily dissociable metal complexes, operationally known as DGT-labile concentration, which has been related to observed toxicity in different types of organisms (e.g., Koppel et al., 2019). These advantages might favour the inclusion of DGT technique within monitoring programmes.

In a previous deliverable (Rodríguez et al., 2021a) it was found that in general there was a log-log linear relationship between the three methodologies (DGT, ICP-MS in spot samples, and voltammetry in spot samples). This previous research was carried out with sampling data from 2018. In 2022, new samplings were carried out, and in this deliverable, this new data is considered to improve the knowledge of the relationship between methods for Cd, Ni and Pb.

2. Scope

The scope of this deliverable is to explore the relationships in the metal concentration variability derived from total dissolved fraction and chemically labile fraction in "classical" spot (water) samples and labile fraction in DGTs. This deliverable can be considered an extension or a complementary work to the previous one (Rodríguez et al., 2021a), and it is advisable to review the previous one if better understanding of the methodological details is desired.



3. Material and Methods

3.1 Origin of data

Sampling campaigns in 2018 and 2022 were carried out by MONITOOL partners (AZTI, CEFAS, DCU, IFREMER, IPMA, ITC, MSS, SEPA and UNICA) covering eight countries (England, France, Ireland, Italy, Northern Ireland, Portugal, Scotland and Spain) located in five European sub-regions. Sampling campaigns were carried out in 2018, consisting of the simultaneous deployment of DGT samplers, collection of discrete water samples and measurement of physico-chemical parameters. A total of 36 sites were sampled and when possible, the same stations were sampled in different seasons.

At each sampling site three different methodologies were used to measure metal content in waters: i) Inductively Coupled Plasm-Mass Spectrometry on (IPC-MS) in Diffusive Gradient in Thin films (DGTs), ii) Inductively Coupled Plasm-Mass Spectrometry (IPC-MS, with seaFAST preconcentration system) on water spot sampling and, iii) voltammetry (Volt) on water spot sampling. Details on the laboratory and field methodologies are given in deliverables from WP5 and in Rodríguez et al. (2021b).

Some remarks on data and statistical analysis:

- 1) The main data are provided from WP5. Only data obtained following the Monitool protocols have been considered.
- 2) At some sampling sites the DGTs were collected in two sampling dates. In those cases, the mean values of spot water samples associated at each DGT collection data were calculated including only the values of the dates in which the DGT were deployed. This implies that from the same sampling site and season can be two data, e.g., one DGT from Day #0 to Day #2, and other DGT from Day #0 to Day #4 (Table 1, Table 2). This implies that these data cannot be considered as independent data from a statistical point of view. Nevertheless, for the exploration of results, these data were considered as independent, and therefore, the statistical results should be taken into account carefully.
- 3) The voltammetry method was used for measuring three metals: Cd, Ni and Pb. Hence the determination of conditional labile Pb and Cd concentrations in filtered and acidified water samples was done by Anodic Stripping Voltammetry (ASV). For the determination of total Ni dissolved concentrations, Cathodic Stripping Voltammetry (CSV) was carried out after UV irradiation of water samples to guarantee the oxidation of organic matter. For Cd and Pb determination the samples were not UV-irradiated before the analysis. It should be noted that (ASV) is typically used for the measurement of labile metal species in water samples at natural pH, instead of acidified water samples. Because the determination of ASV-labile concentrations at natural pH must be performed within a reasonable time after sample collection to minimize loss of metal species due to adsorption on vessel walls, samples were acidified on site in this study. Immediate acidification of the water samples once filtered can, however, give information about the labile fraction at a lower pH than the natural one, in the presence of a still inert fraction that may comprise metals strongly bound to natural organic molecules or bound to colloids.
- 4) The total dissolved concentrations of trace metals Cd, Ni and Pb in filtered and acidified water samples were determined by an online pre-concentration seaFAST system (Elemental Scientific, Nebraska, USA) coupled with an ICP-MS.





- 5) The determination of trace elements in DGTs by ICP-MS was done by binding resin gel was removed from the sampler and eluted in 1.22 mL of 1M HNO₃ acid solution for at least 24 hours. The resulting acid extracts were analysed by ICP-MS for the determination of trace elements Cd, Ni and and Zn after dilution 5 times with ultrapure water (Milli-Q, Millipore).
- 6) The values below the quantification limit were transformed as the half of the quantification limit. In those cases where the mean value of concentrations is below the quantification limit, the values were not included.
- 7) The laboratories provided a list of possible anomalous results to the partners. Each partner, based in their experience and knowledge of the studies areas, checked the obtained data and decided which values should be removed o included for the statistical analysis.
- 8) The relationships between the metal concentration analysis methodologies (ICP-MS in DGTs, ICP-MS on water spot sampling and voltammetry on water spot sampling) were explored based upon arithmetic mean values. Hence, for ICP-MS in DGTs, the mean value among replicates was used. For data based upon spot sampling at each sampling the mean value among replicates was used, and as whole mean the mean values of mean values at each sampling was used as representative of the sampling period.
- 9) There is no available metal data from the three methods of analysis in all water samples. In other words, it is possible that from a sampling site only results from one of two methods of analysis is available (e.g., due of problems in the transport to laboratories or problems in the sampling).
- 10) Outliers were identified using linear modelling of the 2018 data. The objective was to identify outliers samples whose standardized residuals from the linear model were greater than 3 (rejection of values above 99.73% of the total values, assuming that their distribution follows a normal distribution). A detailed procedure is given in the Supplementary material of Rodríguez et al. (2021b). Values identified as outliers were excluded. Outliers of the 2022 data were removed based on standardized residuals tanking into account 2018 and 2022 together.

Partner	Label (name)	Two days collection data	Label (number)
AZTI	DEBA_DS		1
AZTI	LEZO_DS		2
AZTI	MUSEO_DS_D2	X	3
AZTI	MUSEO_DS_D4	X	4
AZTI	PRACTICOS_DS		5
AZTI	DEBA_WS		6
AZTI	LEZO_WS		7
AZTI	MUSEO_WS_D3	X	8
AZTI	MUSEO_WS_D5	X	9
AZTI	PRACTICOS_WS		10
CEFAS	BELFAST_DS		11
CEFAS	FAL_DS_D2	X	12
CEFAS	FAL_DS_D4	X	13
CEFAS	LIVERPOOL_DS		14

Table 1. List of samples (2018).





Partner	Label (name)	Two days collection data	Label (number)
CEFAS	X38A_DS		15
CEFAS	BELFAST_WS		16
CEFAS	FAL_WS_D2	Х	17
CEFAS	FAL_WS_D4	Х	18
CEFAS	LIVERPOOL_WS		19
CEFAS	X38A_WS		20
DCU	ABW_DS_D2	Х	21
DCU	ABW_DS_D4	Х	22
DCU	M69_DS		23
DCU	M70_DS		24
DCU	DUBLINBAYBUOY2_DS		25
DCU	DUBLINBAYBUOY4_DS		26
DCU	ABW_WS		27
DCU	M69_WS		28
DCU	M70_WS		29
DCU	DUBLINBAYBUOY2_WS	Х	30
DCU	DUBLINBAYBUOY4_WS	Х	31
IFREMER	FONTENELLE_DS		32
IFREMER	TERENEZ_DS		33
IFREMER	BESSIN_DS		34
IFREMER	LAZARET_EIL		35
IFREMER	SAINTNAZAIRE_DS_D2		36
IFREMER	SAINTNAZAIRE_DS_D4		37
IFREMER	SAUMONARD_DS		38
IFREMER	FONTENELLE_WS		39
IFREMER	ANTIFER_WS_BAF		40
IFREMER	BESSIN_WS		41
IFREMER	LECROISIC		42
IFREMER	SAINTNAZAIRE_WS_D2	Х	43
IFREMER	SAINTNAZAIRE_WS_D4	Х	44
IFREMER	SAUMONARD_WS		45
IFREMER	SAUMONARD_WS_BAF		46
IFREMER	SILLONDESANGLAIS_WS_BAF		47
IPMA	AVEIRO_DS		48
IPMA	PORTO_DS		49
IPMA	SESIMBRA_DS		50
IPMA	TAGUS_DS		51
IPMA	AVEIRO_WS_D3		52
IPMA	AVEIRO_WS_D5		53
IPMA	PORTO_WS		54
IPMA	SESIMBRA_WS_D2	X	55
IPMA	SESIMBRA_WS_D4	Х	56
IPMA	TAGUS_WS_D2	X	57
IPMA	TAGUS_WS_D4	X	58
ITC	GANDO_DS		59





Partner	Label (name)	Two days collection data	Label (number)
ITC	JINAMAR_DS		60
ITC	LUZ_DS_D2	X	61
ITC	LUZ_DS_D4	X	62
ITC	LUZ_WP4_2_D2	Х	63
ITC	LUZ_WP4_2_D4	X	64
ITC	TALIARTE_DS		65
ITC	TALIARTE_WP4_2_D2	X	66
ITC	TALIARTE_WP4_2_D4	X	67
ITC	GANDO_WS		68
ITC	JINAMAR_WS		69
ITC	LUZ_WS_D3	X	70
ITC	LUZ_WS_D7	Х	71
ITC	TALIARTE_WS		72
MSS-SEPA	BRAEHEAD_WS		73
MSS-SEPA	MONTROSE_WS		74
MSS-SEPA	NEWHAVEN_WS		75
UNICA	MOLODOGANA_DS		76
UNICA	MOLOINCHUSA_DS		77
UNICA	MOLORINASCITA_DS		78
UNICA	SANTELMO_DS_D2	Х	79
UNICA	SANTELMO_DS_D5	X	80
UNICA	MOLODOGANA_WS		81
UNICA	MOLOINCHUSA_WS		82
UNICA	MOLORINASCITA_WS		83
UNICA	SANTELMO_WS_D3	X	84
UNICA	SANTELMO_WS_D5	X	85

Table 2. List of samples (2022).

Partner	Label (name)	Two days collection data	Label (number)	
IFREMER	B1		86	
IFREMER	B2		87	
CEFAS	NEYLAND MARINA (HT)	Х	88	
CEFAS	NEYLAND MARINA (LT)	Х	89	
AZTI	HERRERA		90	
AZTI	LEZO_2		91	
UNICA	MOLO SABAUDO (MS)		92	
UNICA	PARCO DI MOLENTARGIUS (PM)		93	
DCU	DUN LAOGHAIRE HARBOUR		94	
DCU	POOLBEG MARINA		95	





3.2 Statistical approach

Exploration was carried out on untransformed data and on log-transformed data (i.e., logarithm with base 10). For modelling the relationships, linear model II regression analyses were carried out. It was used the ranged major axis (RMA) approach with GRAPHER software (version 13). It should be noted that this kind on regressions cannot be used for predicting Y values from X values (Warton et al. 2006). However, these methods are useful for modelling the functional linear relationships between variables that are random and measured with error (Legendre and Legrendre, 2012).



Figure 1. Type I vs. type II regression models.

4. Cadmium

The values of Cd measured with the three different approaches (DGT, IPC-MS, Volt) showed a relatively close log-normal distribution. This fact difficulties to model correctly the relationships in a linear way.

The linear relation between DGT and ICP-MS accounts a R^2 value of 0.90. The log-log relationship between both methods accounts a R^2 value of 0.79 (Figure 2).

On the other hand, several concentration values analysed by voltammetry were below quantification limit. The linear relation between DGT and Volt accounts a R² value of 0.72. The log-log relationship between both methods accounts a R² value of 0.67 (Figure 3).

Finally, the R^2 value was 0.99 in the linear relation between Volt and IPC-MS, whereas the log-log relationship between both methods has a R^2 value of 0.77 (Figure 4). The R^2 value for the linear relationship should be considered with caution due to the distribution of the data.





Figure 2. Relationship between mean concentration of Cd measured in spot sampling (ICP-MS) and DGT.





Figure 3. Relationship between mean concentration of Cd measured in spot sampling (Anodic Stripping Voltammetry, ASV) and DGT.







Figure 4. Relationship between mean concentration of Cd measured in spot sampling by Anodic Stripping Voltammetry (ASV) and by ICP-MS.





5. Nickel

The values of Ni measured with the three different approaches (DGT, IPC-MS, Volt) showed a relatively close log-normal distribution. This fact difficulties to model correctly the relationships in a linear way.

The linear relation between DGT and ICP-MS accounts a R^2 value of 0.69. The log-log relationship between both methods accounts a R^2 value of 0.59 (Figure 5).

The linear relation between DGT and Volt accounts a R^2 value of 0.75. The log-log relationship between both methods accounts a R^2 value of 0.52 (Figure 6).

Finally, the R^2 value was 0.99 in the linear relation between Volt and IPC-MS, whereas the log-log relationship between both methods has a R^2 value of 0.80 (Figure 7). The R^2 value for the linear relationship should be considered with caution due to the distribution of the data.







Figure 5. Relationship between mean concentration of Ni measured in spot sampling (ICP-MS) and in DGT.







Figure 6. Relationship between mean concentration of Ni measured in spot sampling (Cathodic Stripping Voltammetry, CSV) and in DGT.







Figure 7. Relationship between mean concentration of Ni measured in spot sampling by Cathodic Stripping Voltammetry (CSV) and by ICP-MS.





6. Lead

The values of Pb measured with the three different approaches (DGT, IPC-MS, Volt) showed a relatively close log-normal distribution, but some sampling sites have values considerably higher than the mean values (with the three methodologies). This fact difficulties to model correctly the relationships in a linear way or in a log-log way.

The linear relation between DGT and ICP-MS accounts a R^2 value of 0.53. The log-log relationship between both methods accounts a R^2 value of 0.58 (Figure 8).

The linear relation between DGT and Volt accounts a R² value of 0.44. The log-log relationship between both methods accounts a R² value of 0.43 (Figure 6).

Finally, the R^2 value was 0.80 in the linear relation between Volt and IPC-MS, whereas the log-log relationship between both methods has a R^2 value of 0.67 (Figure 7).





Figure 8. Relationship between mean concentration of Pb measured in spot sampling (ICP-MS) and in DGT.







Figure 9. Relationship between mean concentration of Pb measured in spot sampling (Anodic Stripping Voltammetry, ASV) and DGT.





Figure 10. Relationship between mean concentration of Pb measured in spot sampling by Anodic Stripping Voltammetry (ASV) and by ICP-MS.





7. Main conclusion

As mentioned above, this study should be considered complementary to that of Rodriguez et al. (2021a). In this sense, it has been found that the relationship between methods of the 2022 sampling adjusts the information of the 2018 campaign. Said in other words, in the figures above it can be seen how the 2022 data falls within the 2018 data. It can be concluded that the 2022 sampling confirms the 2018 observation, i.e., that there is a log-log relationship between the methodologies (Table 3).

Table 3. R-squared of lineal and log-log regressions between mean concentration measured in waters by DGT and by spotsampling.

Metal	X	Y	Regression	R ²
Cd	DGT	spot sampling (ICP-MS)	lineal	0.90
Cd	DGT	spot sampling (ICP-MS)	log-log	0.79
Cd	DGT	spot sampling (Volt)	lineal	0.72
Cd	DGT	spot sampling (Volt)	log-log	0.67
Cd	spot sampling (ICP-MS)	spot sampling (Volt)	lineal	0.99
Cd	spot sampling (ICP-MS)	spot sampling (Volt)	log-log	0.77
Ni	DGT	spot sampling (ICP-MS)	lineal	0.69
Ni	DGT	spot sampling (ICP-MS)	log-log	0.59
Ni	DGT	spot sampling (Volt)	lineal	0.75
Ni	DGT	spot sampling (Volt)	log-log	0.52
Ni	spot sampling (ICP-MS)	spot sampling (Volt)	lineal	0.99
Ni	spot sampling (ICP-MS)	spot sampling (Volt)	log-log	0.80
Pb	DGT	spot sampling (ICP-MS)	lineal	0.53
Pb	DGT	spot sampling (ICP-MS)	log-log	0.58
Pb	DGT	spot sampling (Volt)	lineal	0.44
Pb	DGT	spot sampling (Volt)	log-log	0.43
Pb	spot sampling (ICP-MS)	spot sampling (Volt)	lineal	0.80
Pb	spot sampling (ICP-MS)	spot sampling (Volt)	log-log	0.67

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