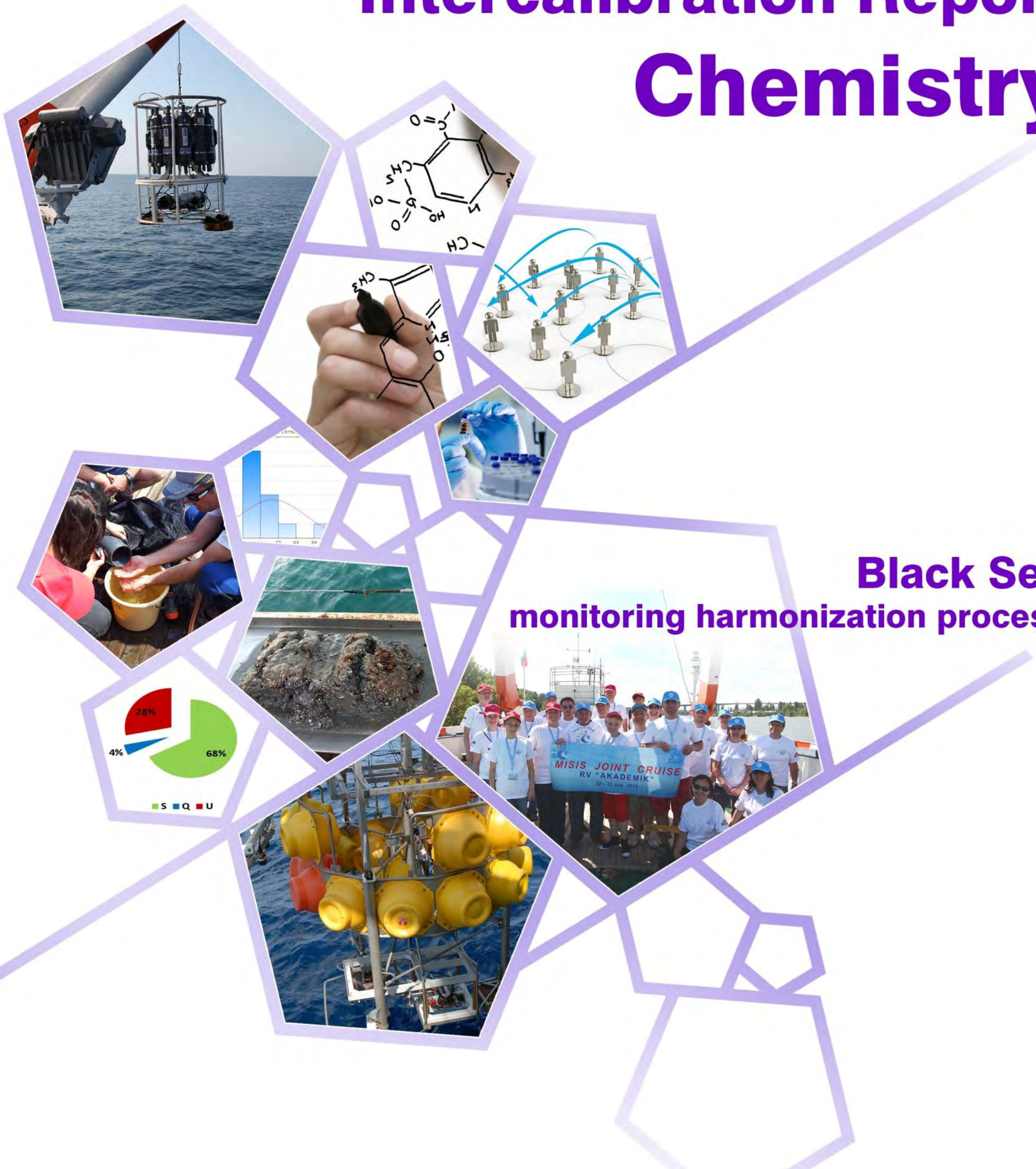
Intercalibration Report
Chemistry



Black Sea
monitoring harmonization process

Intercalibration Report

Chemistry

Black Sea monitoring harmonization process

This document on the MISIS Cruise Intercalibration Report: Chemistry is based on the activities of the MISIS project (MSFD Guiding Improvements in the Black Sea Integrated Monitoring System) with the financial support from the EC DG Env. Programme "Preparatory action – Environmental monitoring of the Black Sea Basin and a common European framework programme for development of the Black Sea region/Black Sea and Mediterranean 2011".

Contributing authors:

Chapter I. Nutrients - under the coordination of dr. Luminita Lazar (NIMRD) with the contribution of dr. Galina Shtereva (IO-BAS), dr. Colpan Beken and Hakan Atabay (TUBITAK), dr. Dan Vasiliu (GEOECOMAR), Cerasela Caraula (RWNA-WADL), cited as:

Lazar L., Shtereva G., Vasiliu D., Beken C., Atabay H., Caraula C., 2014. *Report on the MISIS cruise Intercalibration Exercise: Nutrients*, 9-31.

Chapter II. Heavy Metals in sediments - under the coordination of dr. Andra Oros (NIMRD) with the contribution of dr. Dan Secrieru (GEOECOMAR), dr. Colpan Beken, dr. Leyla Tolun and Hakan Atabay (TUBITAK) cited as

Oros A., Secrieru D., Beken C., Tolun L., Atabay H., 2014. *Report on the MISIS cruise Intercalibration Exercise: Heavy Metals*, 32-63.

Chapter III. Persistent Organic Pollutants in sediments - under the coordination of Daniela Tiganus and dr. Valentina Coatu (NIMRD), Neguta Rusu (RWNA) cited as

Tiganus D., Coatu V., Rusu N., 2014. *Report on the MISIS cruise Intercalibration Exercise: Persistent Organic Pollutants*, 64-76.

Chapter IV. Chlorophyll a - under the coordination of dr. Dan Vasiliu (GEOECOMAR) with the contribution of dr. Laura Boicenco (NIMRD), dr. Snejana Moncheva and Natalia Slabakova (IO-BAS), Oana Culcea (NIMRD) cited as

Vasiliu D., Boicenco L., Moncheva S., Slabakova N., Culcea O., 2014. *Report on the MISIS cruise Intercalibration Exercise: Chlorophyll a*, 77-84.

For bibliographic purpose the entire document should be cited as:
Report on the MISIS cruise Intercalibration Exercise: Chemistry, 2014. MISIS project - MSFD Guiding Improvements in the Black Sea Integrated Monitoring System, Ed. ExPonto, 85 pp.

Design and layout: Tudor Fulga

Number of pages: 85

ISBN: 978-606-598-358-8

Contents

List of Figures

List of Tables

Executive Summary

This report presents the results of from the intercalibration exercise (chemistry) done within the Western Black Sea Joint Survey (done under MISIS project coordination). In the exercise participated up to five laboratories (depending on parameters) from three countries (Bulgaria, Romania and Turkey). During the cruise seawater (for nutrients and chlorophyll a) and sediments for contaminants (heavy metals, PAHs, OCPs and PCBs) were sampled from common stations as follows: open waters (nutrients), shelf and coastal stations (contaminants), open and coastal waters (chlorophyll a).

The aim of the report is to compare results obtained with different methods for the same parameter in order to have an overview of the Western Black Sea datasets comparability.

The results for Nutrients showed satisfactory agreements for the most measurements of and for the participating laboratories in the MISIS State of the Environment report (75%). Phosphate and nitrite results were comparable($|z| \leq 2$) in all reported measurements, followed by TNOx with 75%, ammonium with 63% and silicate with 43% satisfactory results.

The results for Heavy Metals outlined satisfactory agreements for the most measurements of the participating laboratories (80 – 81%). Cd and Co results were comparable in all reported measurements, followed by Mn, Ni and Zn with 83% satisfactory results, Cr and Pb (75%) and Cu (63%).

In case of Persistent Organic Pollutants, due to lack of data the statistical analysis had no significance and the report is only informative.

For Chlorophyll a were found satisfactory agreements for all measurements of the participating laboratories in the MISIS intercalibration exercise. Generally, the precision (ability to reproduce the measurements) within laboratories were found to be high.

Generally, despite of the methodological differences the analyzed samples were subject of lack of homogeneity which definitively contributes to the results and assessment.

Chapter I. Nutrients

I.1. Description of the intercalibration

This chapter presents the results from the MISIS cruise intercalibration, on nutrients in seawater. There are reported results from 5 laboratories from three countries. The participating laboratories are listed in Annex I.1. Not all laboratories have reported data for all components. Following components are included in the intercalibration: PO_4, SiO_4, NH_4, NO_2, TNO_x. For TP and TN only three laboratories results were compared and for TOC only two laboratories thus the results are indicative.

The raw data from the laboratories can be found in Annex I.2. For ease of representations and discussions, each laboratory has been given a code number. The results from the laboratories were delivered in different units and setup of the reporting spreadsheet. For the statistical evaluation, data from one laboratory, for the nutrients reported in mg/L, have been transformed to the same units, µM (µmoles/L)(for PO_4, SiO_4, NH_4, NO_2, TNO_x).

Data below detection limits (reported as "<value") are not included in the statistical analysis.

The statistical evaluation is dealing with the score z calculated from an assigned value established as consensus value according to ***The International Harmonized Protocol for the Proficiency Testing Of Analytical Chemistry Laboratories (IUPAC Technical Report)*** (IUPAC, 2006) recommendations and a target standard deviation established as 20% from the relative standard deviation (RSD).

I.2. Sampling

Samples were taken during the MISIS cruise on 28th of July 2013 from station MO13int, longitude East 29.3433, latitude North 42.7372, bottom depth 2015.5m (Fig.0) from surface (0m) and water column (46m), each batch in three replicates.

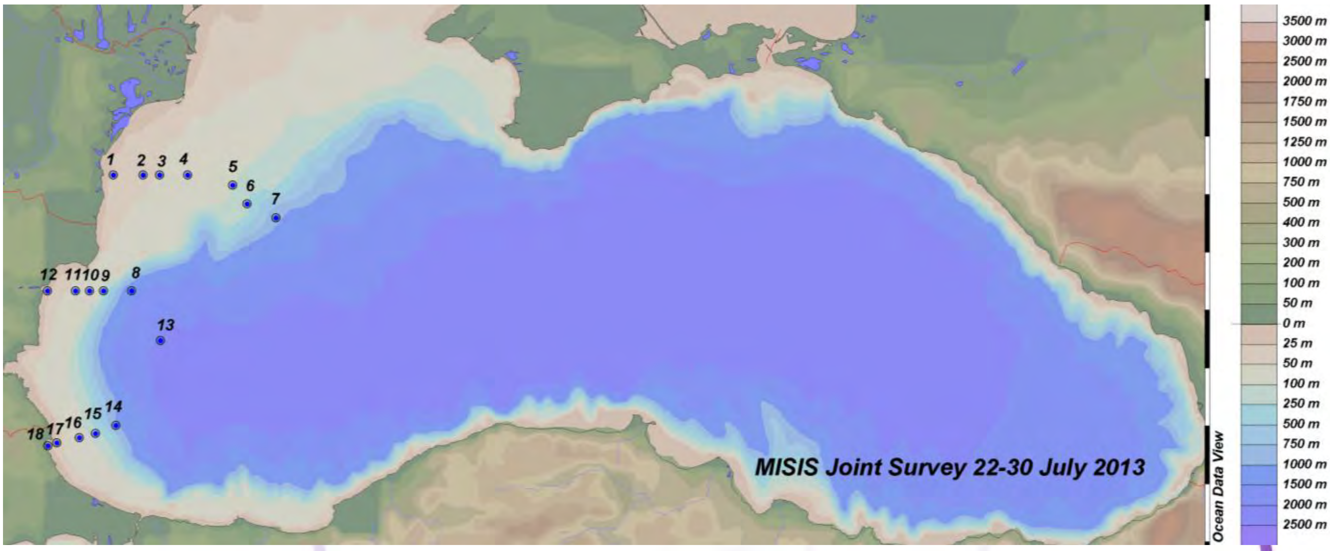


Figure 1. Map of the MISIS cruise stations – intercalibration, station 13.

The water samples (volumes within 0.25 – 0.5 L) were collected by Seabird CTD-Rosette system in 5 L plastic bottles during the up-cast at different depths according to CTD profiles. All the samples for the exercise were frozen (at – 20 ÷ - 24 °C) until their subsequent analysis in laboratories. No additional steps for assurance of samples homogeneity were done. Samples were analyzed with methods described in Annex I-I.3 (Cruise report, MISIS Joint Cruise, 2013).

I.3. Evaluation of the results

Scope and field of application:

• the principal aim is the assessment of laboratory performance against established criteria based on fitness for a common purpose;

• compliance with these criteria may be judged on the basis of the deviation of measurement results from assigned values.

Assessment of performance:

Laboratories will be assessed on the difference between their result and the assigned value. A performance score will be calculated for each laboratory, using the *z*-score based on a fitness-for-purpose criterion.

For each component the following statistical analysis has been performed:

A. Establishment of the consensus value

The consensus value was calculated according to ***The International Harmonized Protocol for the Proficiency Testing Of Analytical Chemistry Laboratories (IUPAC Technical Report)*** (IUPAC, 2006) recommendations.

According to the IUPAC's technical report, an assigned value is an estimate of the value of the measurand that is used for the purpose of calculating scores and shall be determined by one of the following methods:

- measurement by a reference laboratory,
- the certified value(s) for a CRM used as a test material,
- direct comparison of the proficiency testing test material with CRMs,
- consensus of expert laboratories,
- formulation (i.e., value assignment on the basis of proportions used in a solution or other mixture of ingredients with known analyte content),
- a consensus value (that is, a value derived directly from reported results).

In the absence of the assigned values we chose to establish the consensus value following next steps:

- Visualization of data and exclusion of data reported as "<"
- Calculate median and the range median ±50%
- Exclude the values which are not included in the range median ±50%
- Recalculate the median which is assumed to be a consensus value.

B. Assignment of z-scores

The IUPAC (2006) Harmonized Protocol recommended the conversion of participants' results into *z*-scores, and experience in the intervening years has demonstrated the wide applicability and acceptance of the *z*-score in proficiency testing. A participant's result *x* is converted into a *z*-score according to the equation

$$z = (x - x_a)/ \sigma$$

where x is the individual laboratory result as average of replicates, x_a is the "Exercise Assigned Value," and σ is the fitness-for-purpose-based "standard deviation for proficiency assessment", that underline the importance of assigning a range appropriate to a particular purpose (ISO Guide 43; Statistical Guide ISO 13528). In the equation defining, the term ($x - x_a$) is the error in the measurement. The parameter σ describes the standard uncertainty that is most appropriate for the application area of the results of the analysis, in other words, "fitness-for-purpose". The uncertainty that is fit for purpose in a measurement result depends on the application. For example, a relative standard uncertainty [i.e., $u(x)/x$] of 10 - 20% is probably adequate for many environmental measurements. As described in the IUPAC guidelines, the choice of σ is dependent upon the data quality objective of a particular program. The fixed performance criterion is more useful in the comparison of a laboratory's performance on different materials. In instances, where the concentration of the analytes varies over a wide range, the fitness-for-purpose criterion has to be specified as a function of concentration. The most common approaches are as follows:

- Specify the criterion as a relative standard deviation (RSD). Specific σ values are then obtained by multiplying the selected RSD by 20%.

C. Interpretation of the z-scores

According to IUPAC, the interpretation of *z*-scores is *not* generally based on summary statistics that describe the observed participant results. Instead, it uses an assumed model based on the scheme provider's fitness-for-purpose criterion, which is represented by the standard deviation for proficiency assessment σ.

- A score of zero implies a perfect result. This will happen rarely even in the most competent laboratories.
- *z*-scores fall between –2 and +2. The sign (i.e., – or +) of the score indicates a negative or positive error respectively. Scores in this range are commonly designated "acceptable" or "satisfactory" ($|z| \le 2$ *Satisfactory*).
- Scores in the ranges –2 to –3 and 2 to 3 are designated as "questionable" ($2 \le |z| \le 3$ *Questionable*).
- A score outside the range from –3 to 3 indicate that the cause of the event should be investigated and remedied. Scores in this class are commonly designated "unacceptable" or "unsatisfactory" ($|z| \ge 3$ *Unsatisfactory*).

I.3.1. Phosphate

The raw data distribution is represented in the histogram, without results reported as "<".

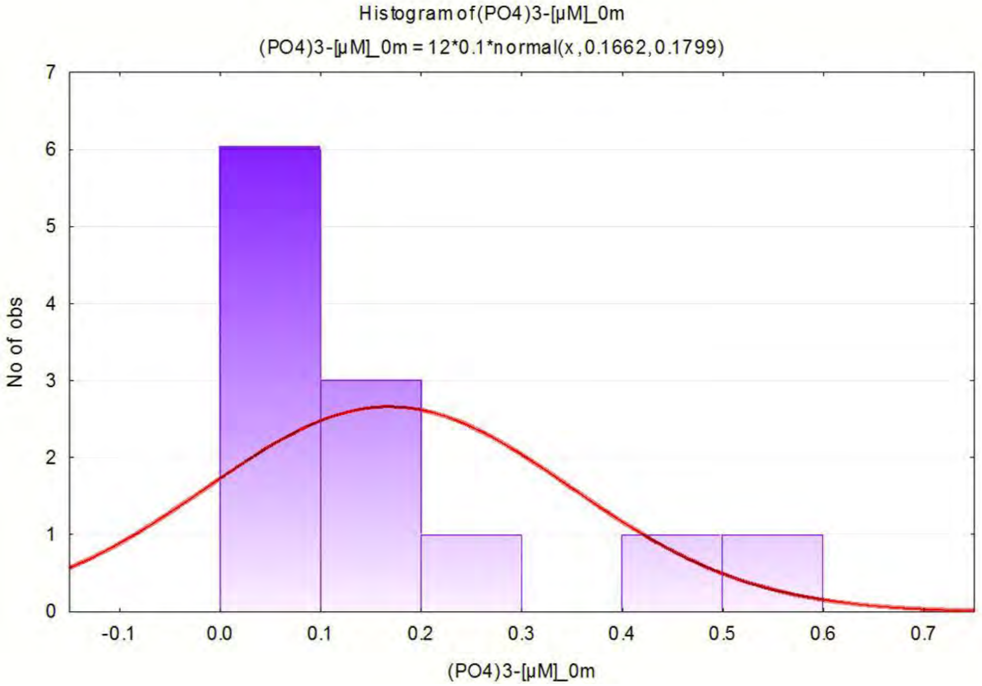


Figure 2. Raw data distribution – phosphate (0m).

- The range (median±50%): 0.04 – 0.13µM
- Consensus value: 0.06µM
- Z-score (RSD=0.93; σ=0.19):

Lab code				
1	2	3	4	5
-0.1	0.4	0.0	2.0	n.a.

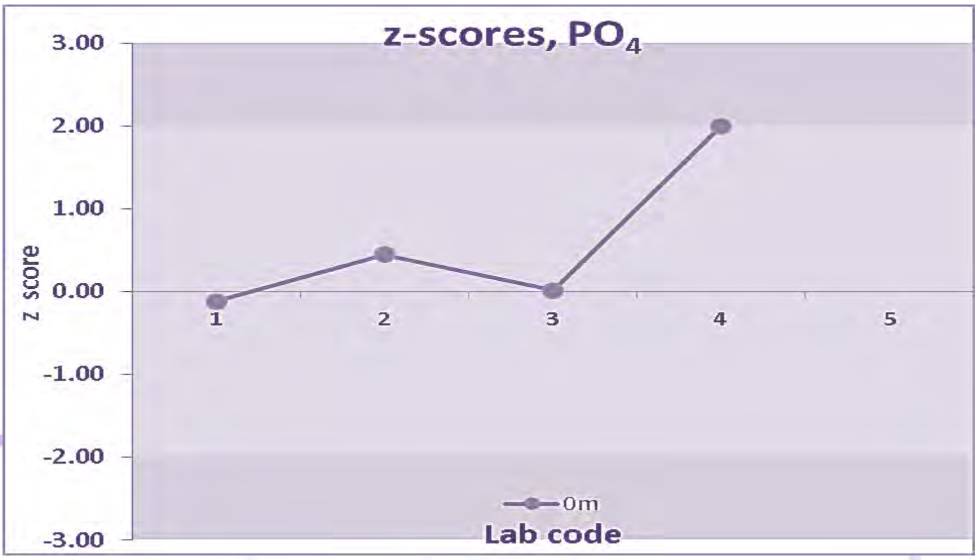


Figure 3. Z-scores for phosphate (0m).

The raw data distribution is represented in the histogram.

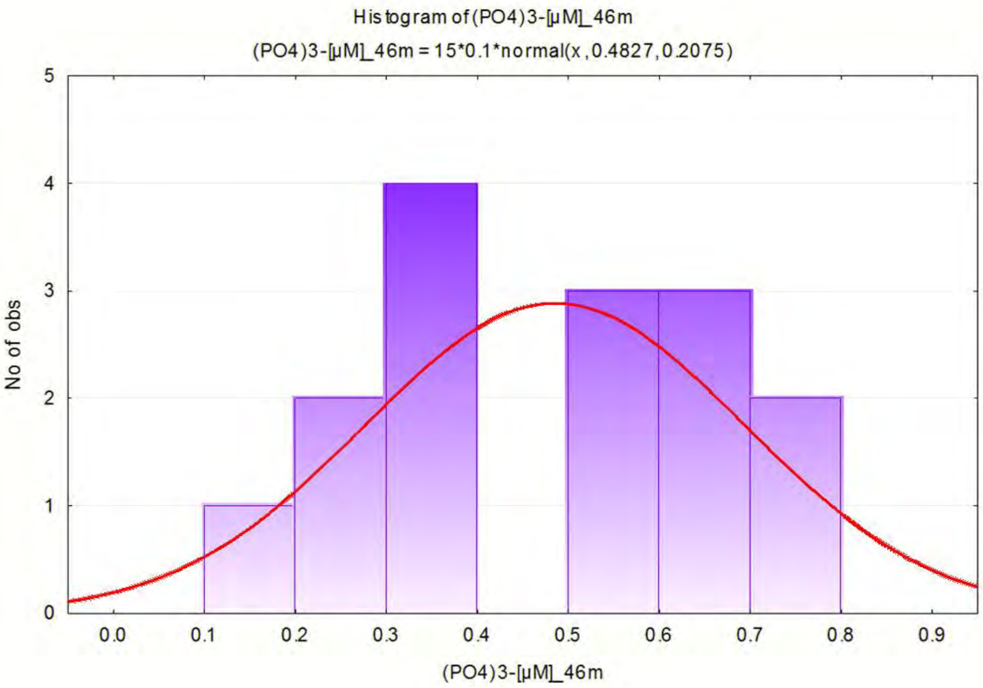


Figure 4. Raw data distribution – phosphate (46m).

- The range (median±50%): 0.26 – 0.79µM
- Consensus value: 0.58µM
- Z-score (RSD=2.32; σ=0.46):

Lab code				
1	2	3	4	5
-0.5	-0.1	0.0	0.4	-0.8

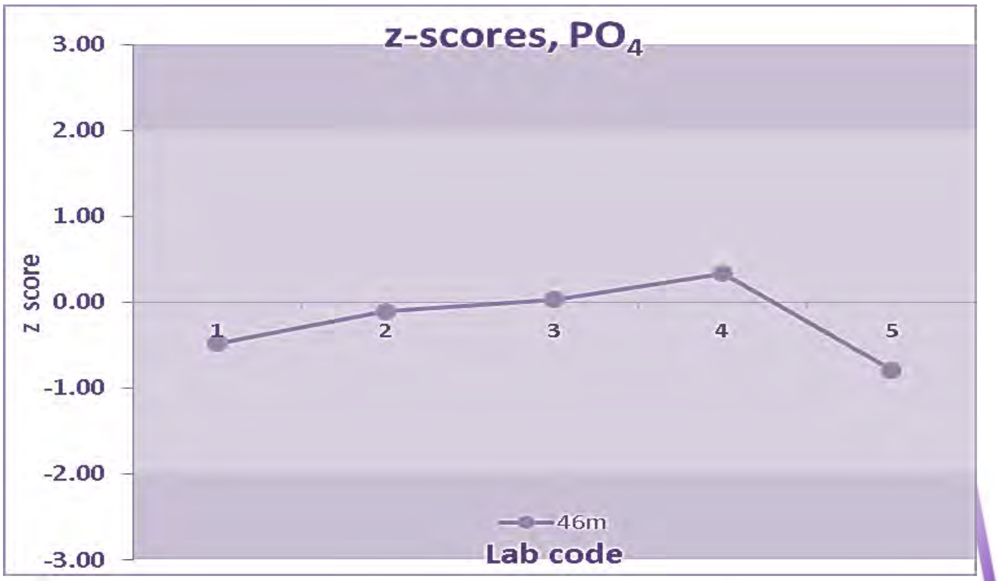


Figure 5. Z-scores for phosphate (46m).

I.3.2. Silicate

The raw data distribution is represented in the histogram, without results reported as "<".

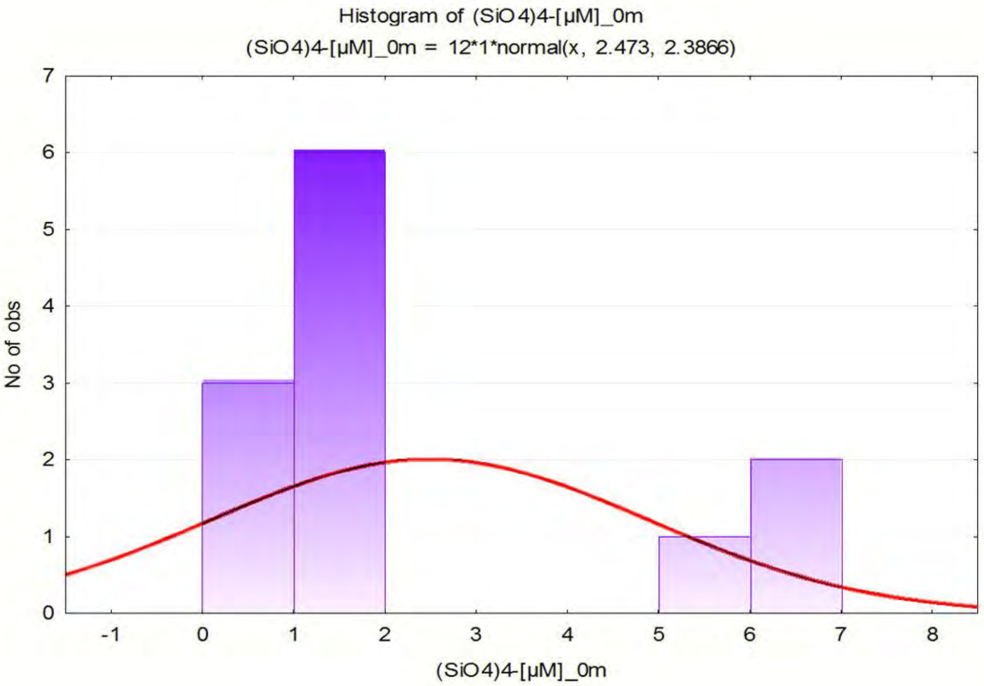


Figure 6. Raw data distribution – silicate (0m).

- The range (median±50%) values: 0.80–2.39µM
- Consensus value: 1.59µM
- Z-score (RSD=1.04; σ=0.21):

Lab code				
1	2	3	4	5
0.5	22.5	-5.4	-0.8	n.a.

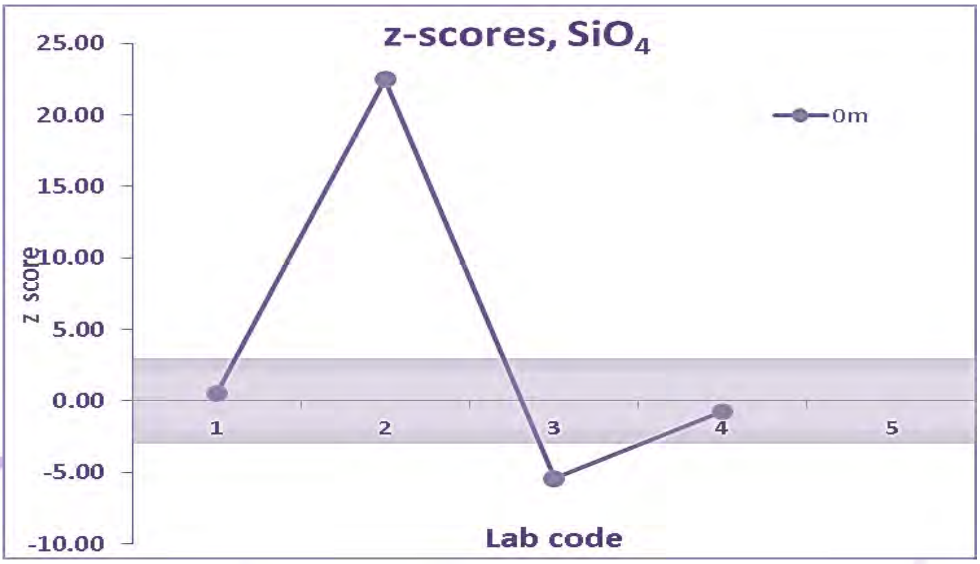


Figure 7. Z-scores for silicate (0m).

The raw data distribution is represented in the histogram.

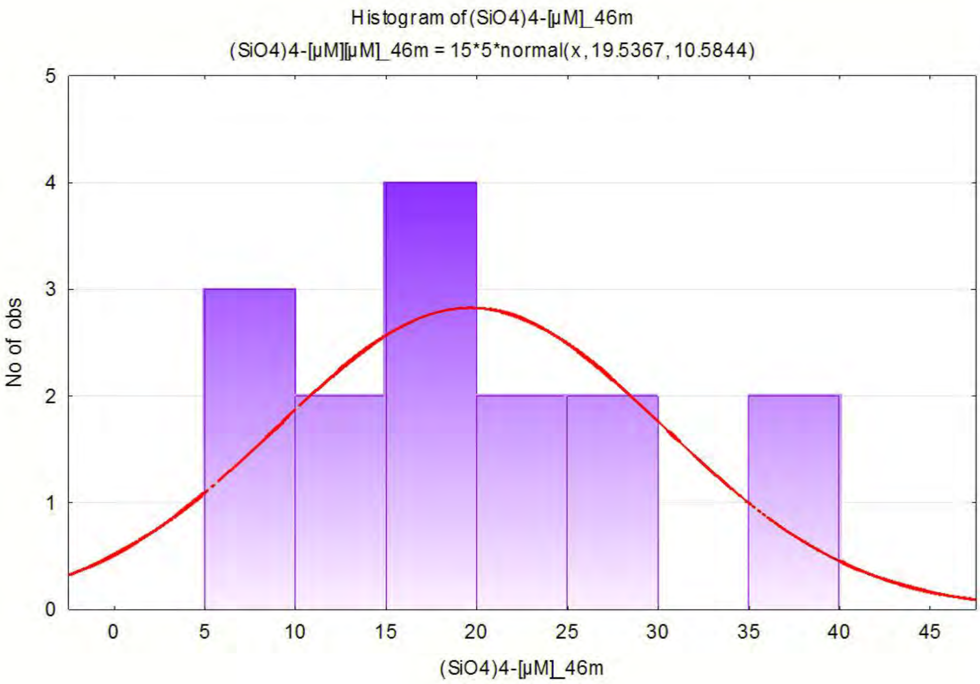


Figure 8. Raw data distribution – silicate (46m).

- The range (median±50%) values: 8.50–25.50µM
- Consensus value: 17.00µM
- Z-score (RSD=1.85; σ=0.37):

Lab code				
1	2	3	4	5
-9.9	20.5	-27.2	0.1	50.9

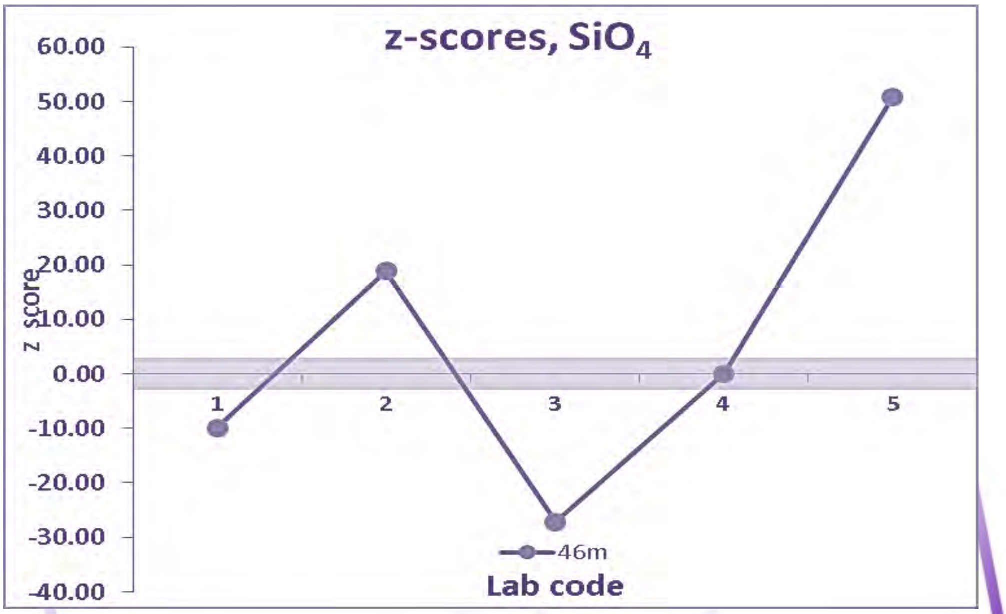


Figure 9. Z-scores for silicate (46m).

I.3.3. Nitrite

The raw data distribution is represented in the histogram.

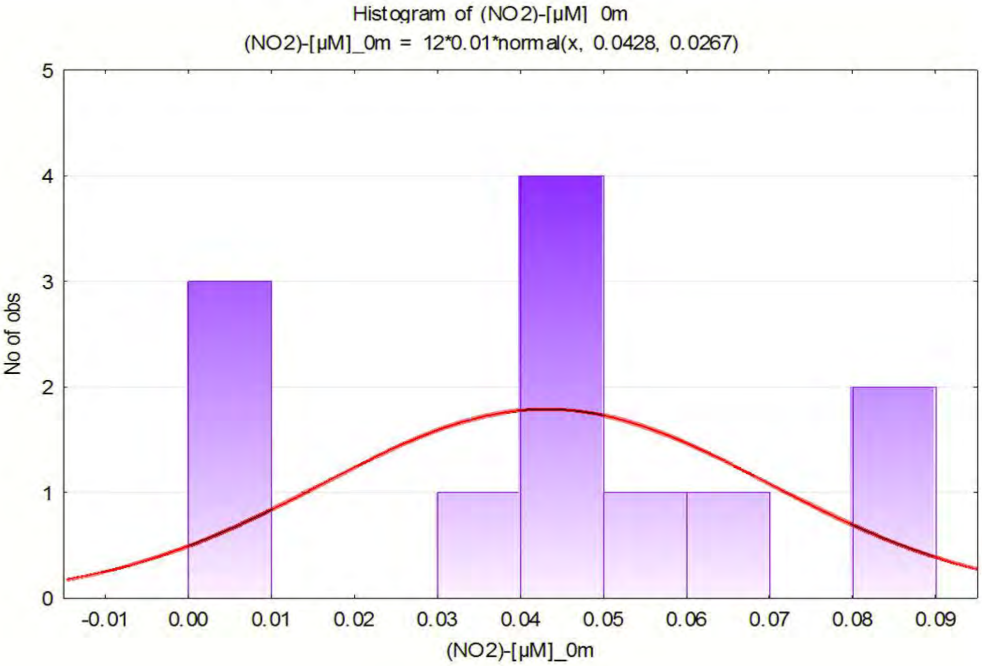


Figure 10. Raw data distribution – nitrite (0m).

- The range (median±50%) values: 0.02 – 0.07µM
- Consensus value: 0.05µM
- Z-score (RSD=1.57; σ=0.31):

Lab code				
1	2	3	4	5
0.0	0.0	-0.1	0.1	n.a.

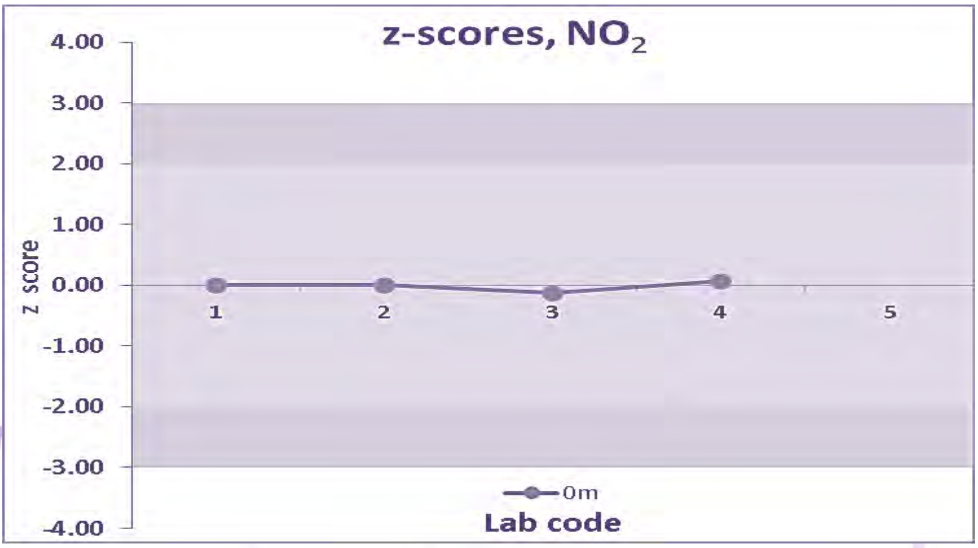


Figure 11. Z-scores for nitrite (0m).

The raw data distribution is represented in the histogram, without results reported as "<".

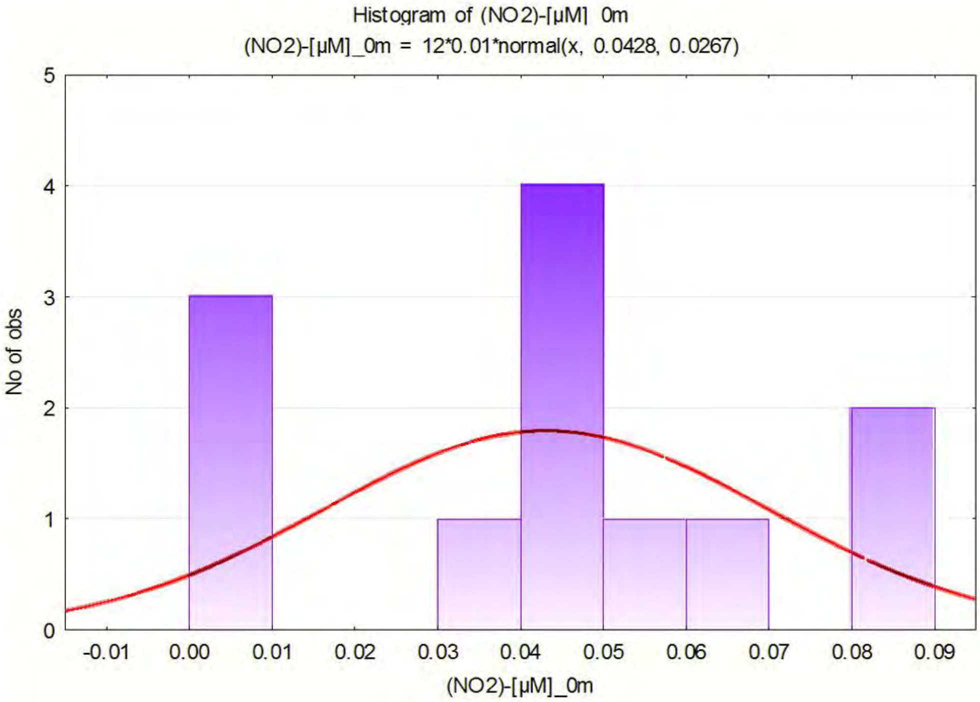


Figure 12. Raw data distribution – nitrite (46m).

- The range (median±50%) values: 0.04–0.12µM
- Consensus value: 0.08µM
- Z-score (RSD=1.99; σ=0.40):

Lab code				
1	2	3	4	5
0.0	0.0	-0.2	0.1	n.a.

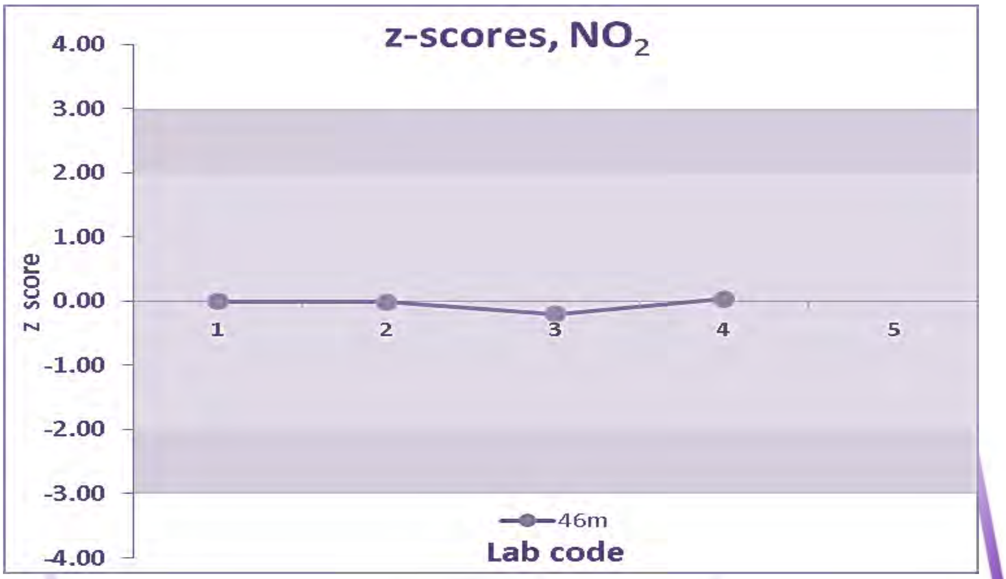


Figure 13. Z-scores for nitrite (46m).

I.3.4. TNOx

The raw data distribution is represented in the histogram, without results reported as “<”.

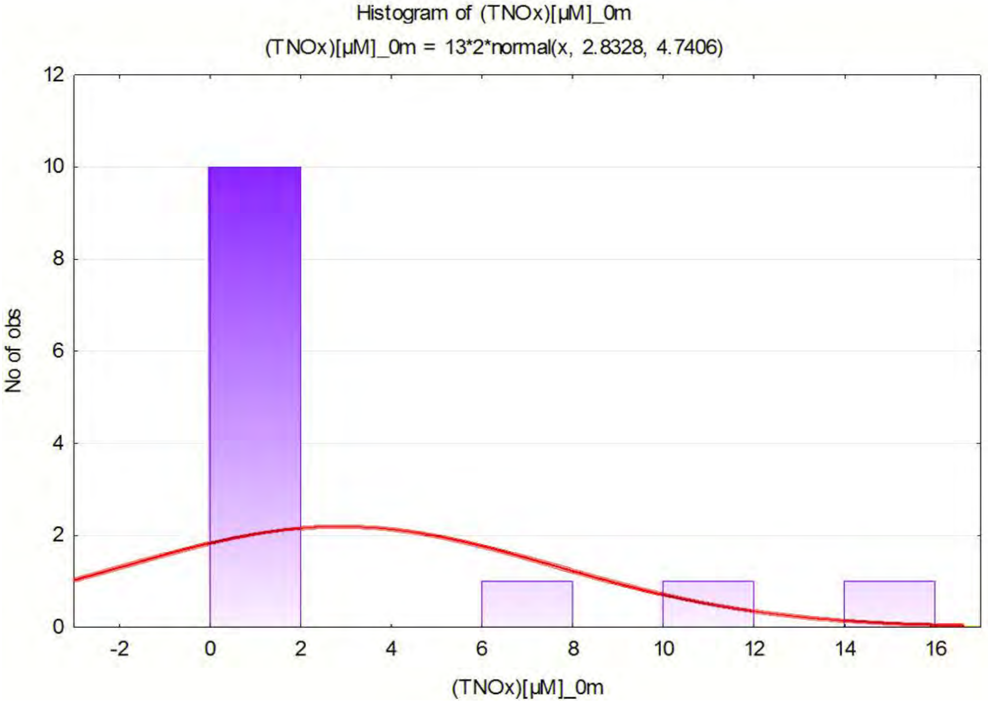


Figure 14. Raw data distribution – TNOx (0m).

- The range (median±50%) values: 0.32 – 0.96µM
- Consensus value: 0.33µM
- Z-score (RSD=0.60; σ=0.12):

Lab code				
1	2	3	4	5
-1.3	86.7	-0.1	5.5	-1.2

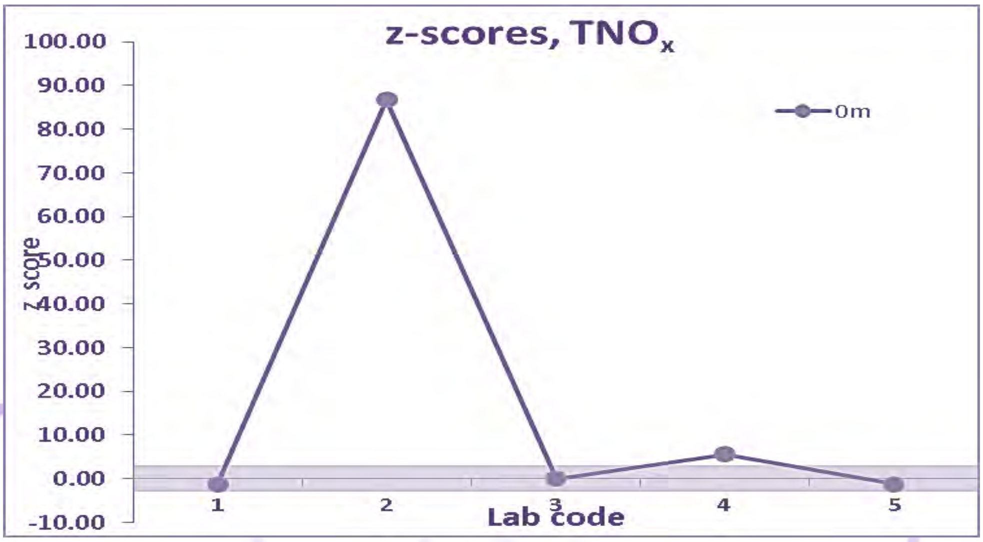


Figure 15. Z-scores for TNOx (0m).

The raw data distribution is represented in the histogram.

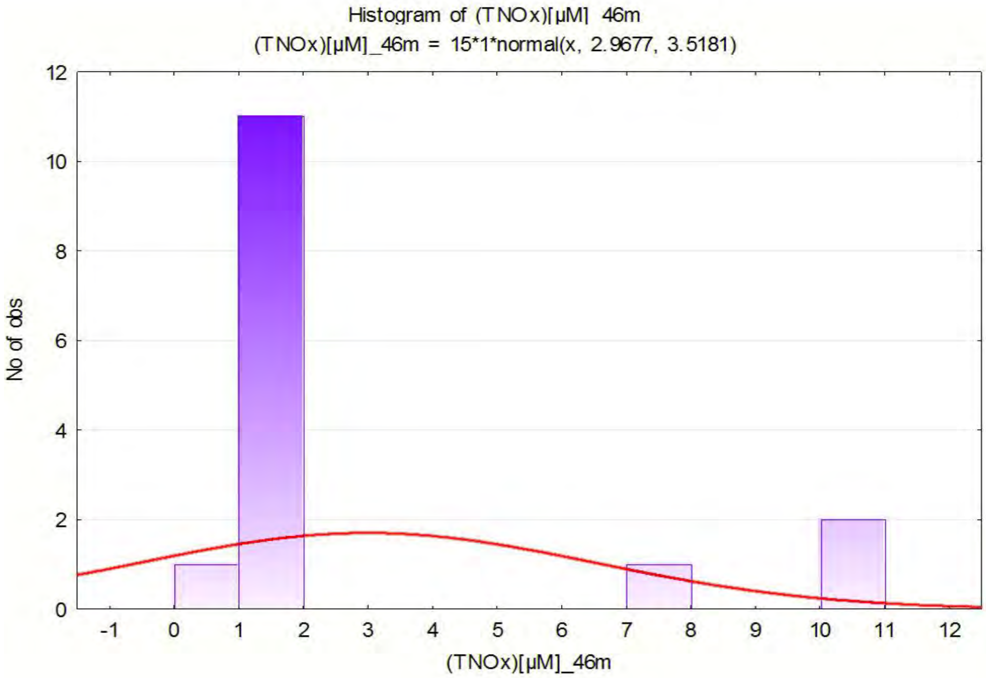


Figure 16. Raw data distribution – TNOx (46m).

- The range (median±50%) values: 0.65–1.95µM
- Consensus value: 1.26µM
- Z-score (RSD=0.84; σ=0.17):

Lab code				
1	2	3	4	5
-1.6	49.0	0.1	0.2	2.6

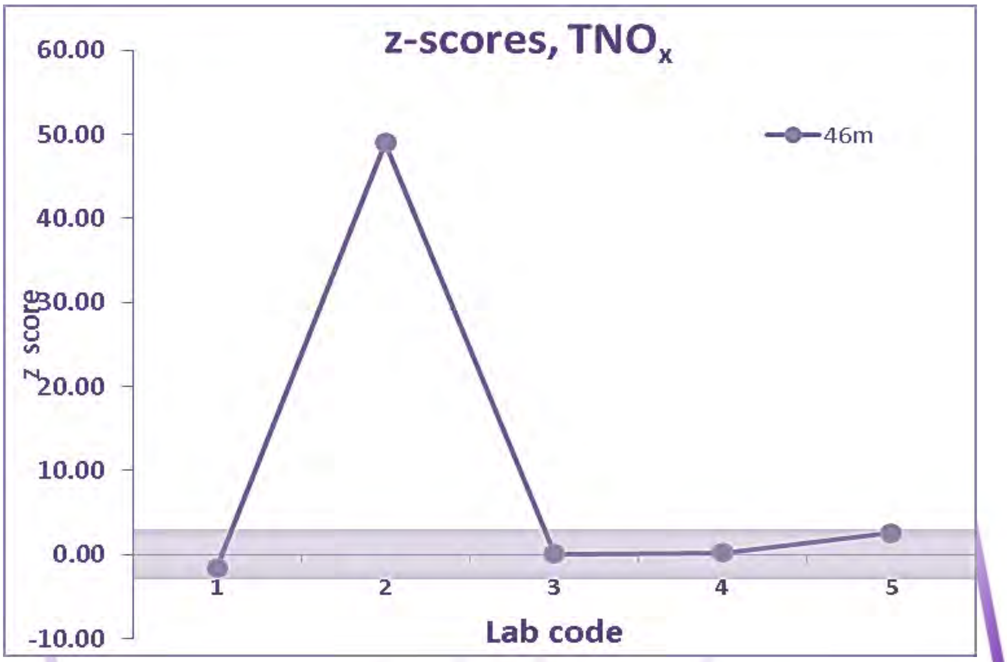


Figure 17. Z-scores for TNOx (46m).

I.3.5. Ammonium

The raw data distribution is represented in the histogram.

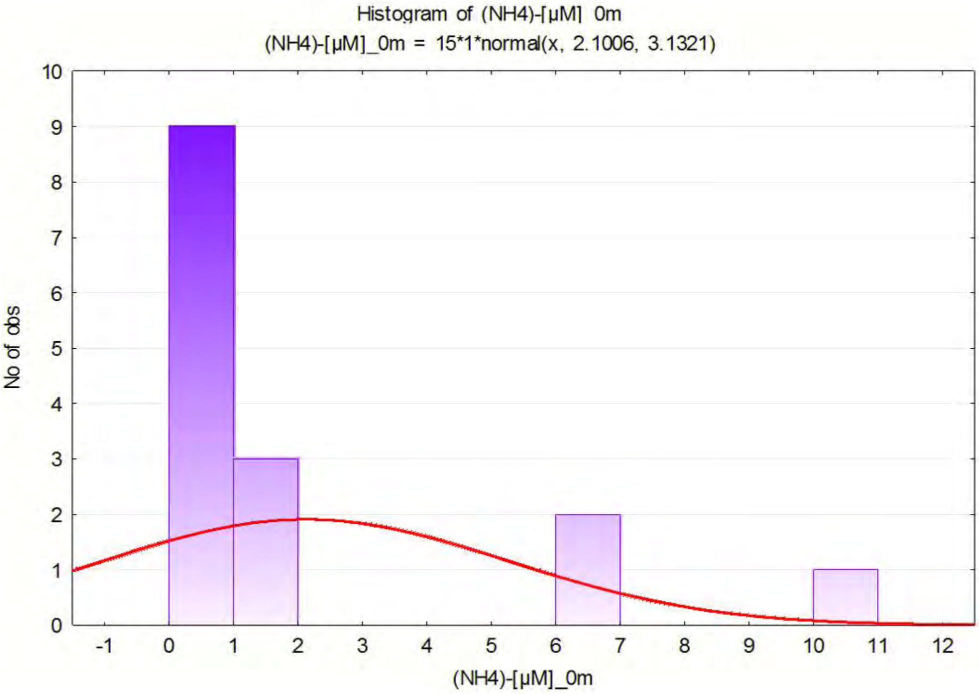


Figure 18. Raw data distribution – ammonium (0m).

- The range (median±50%) values: 0.46 – 1.39µM
- Consensus value: 0.59µM
- Z-score (RSD=0.67; σ=0.15):

Lab code				
1	2	3	4	5
-2.7	48.6	4.8	0.2	-0.5

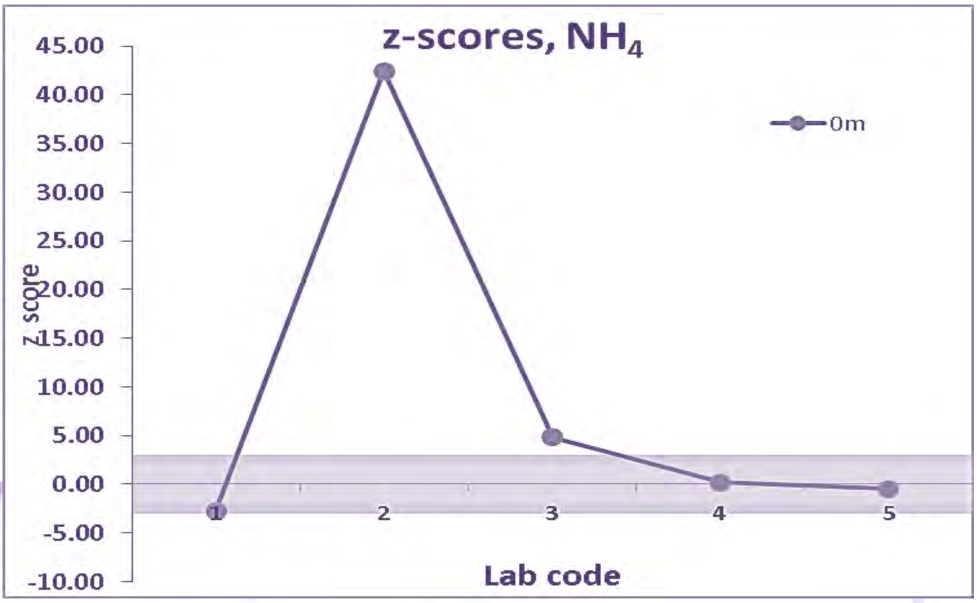


Figure 19. Z-scores for ammonium (0m).

The raw data distribution is represented in the histogram, without results reported as "<".

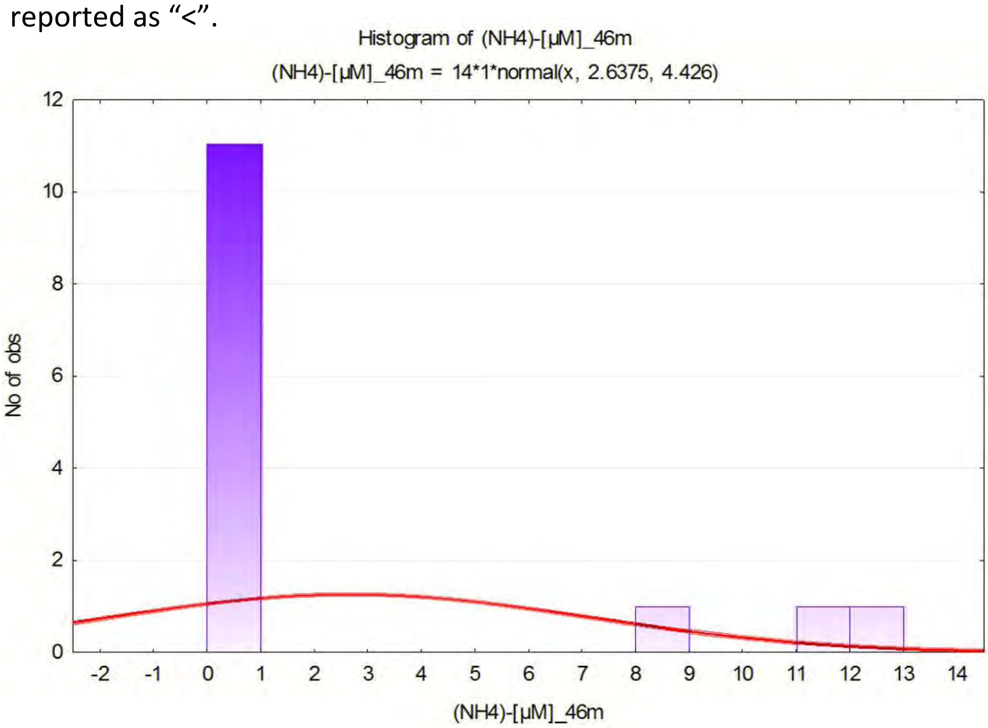


Figure 20. Raw data distribution – ammonium (46m).

- The range (median±50%) values: 0.46–1.39µM
- Consensus value: 0.59µM
- Z-score (RSD=0.67; σ=0.15):

Lab code				
1	2	3	4	5
-2.7	48.6	4.8	0.2	-0.5

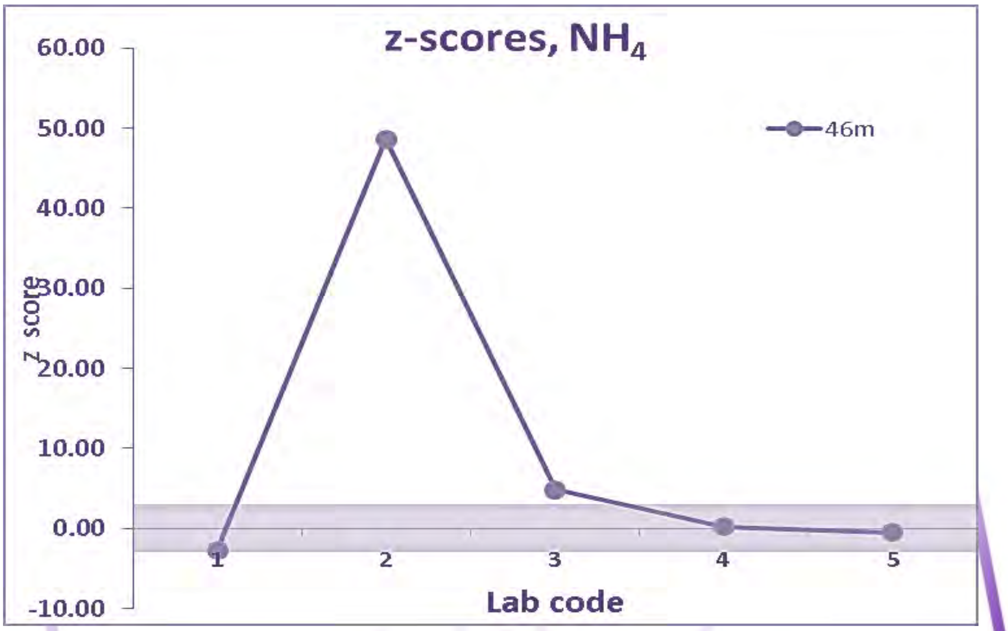
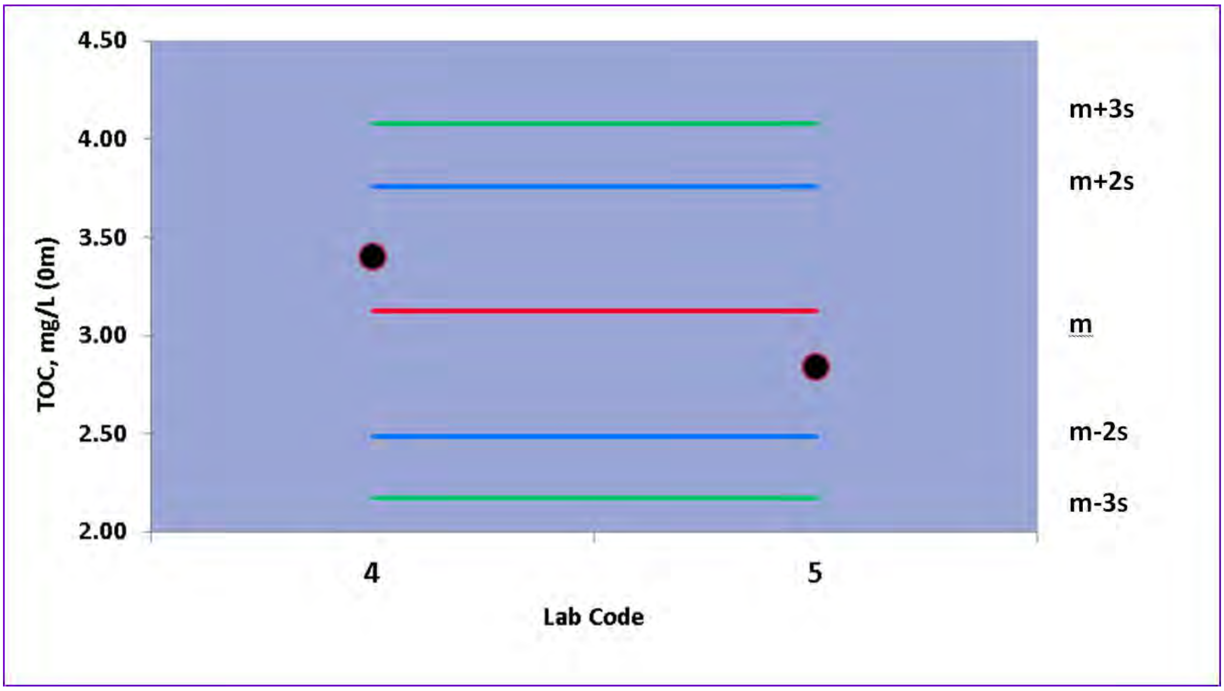
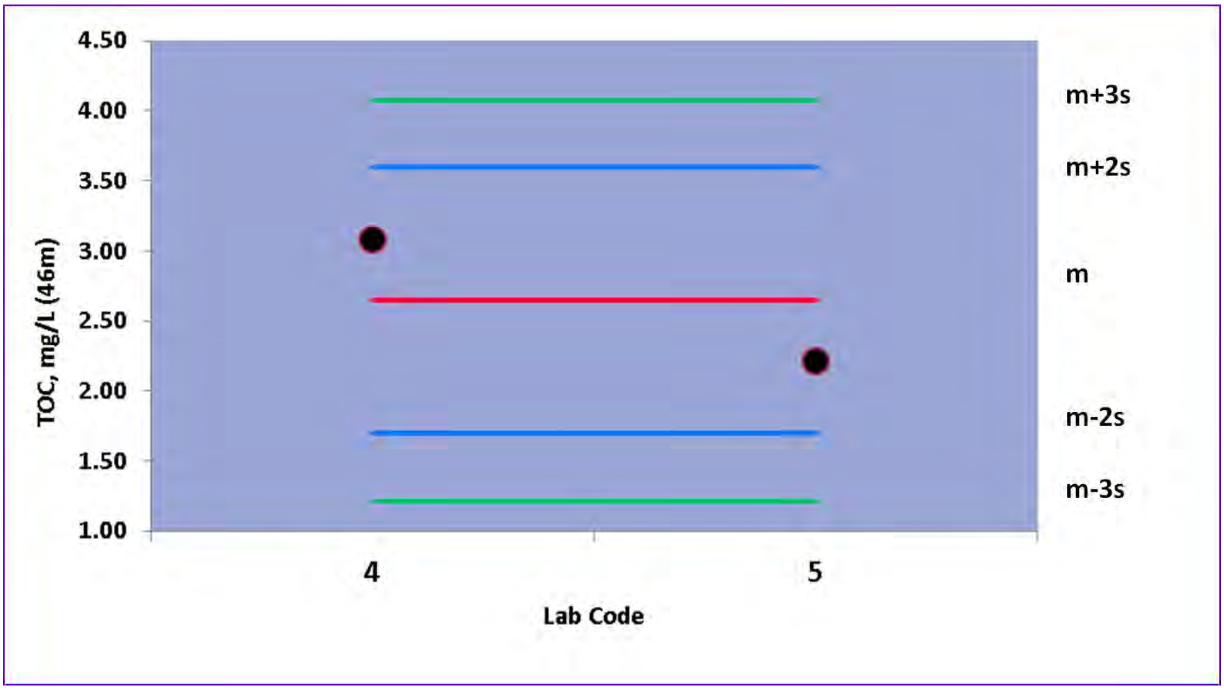


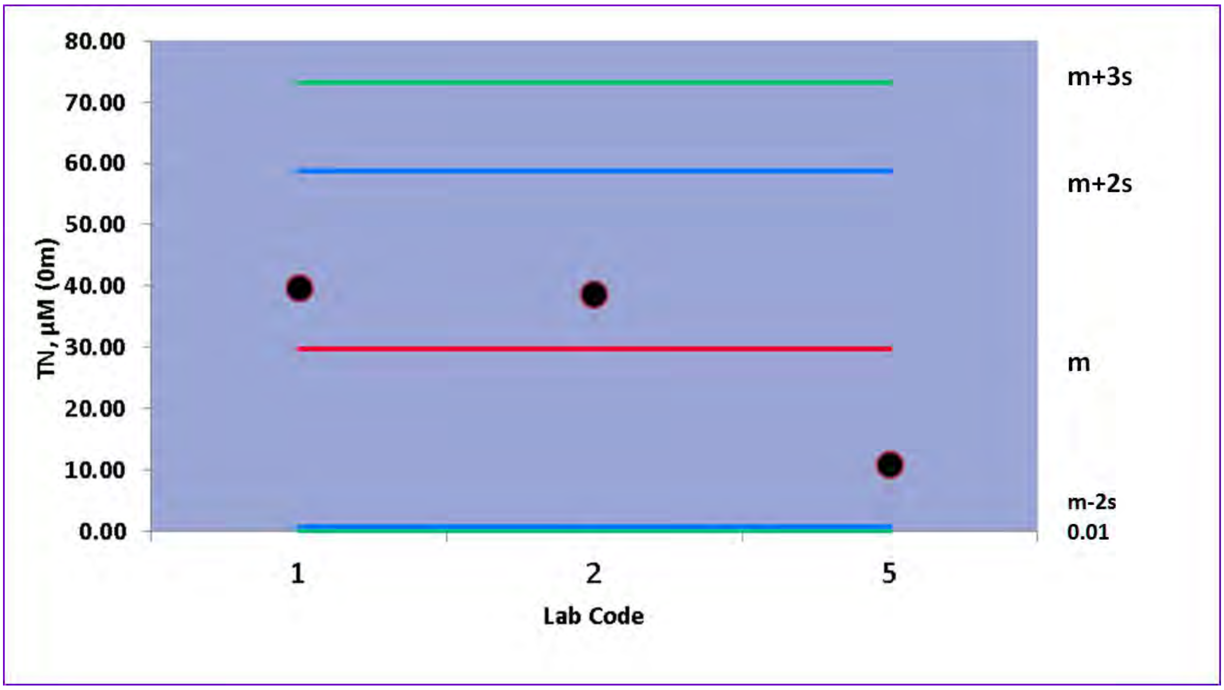
Figure 21. Z-scores for ammonium (46m).

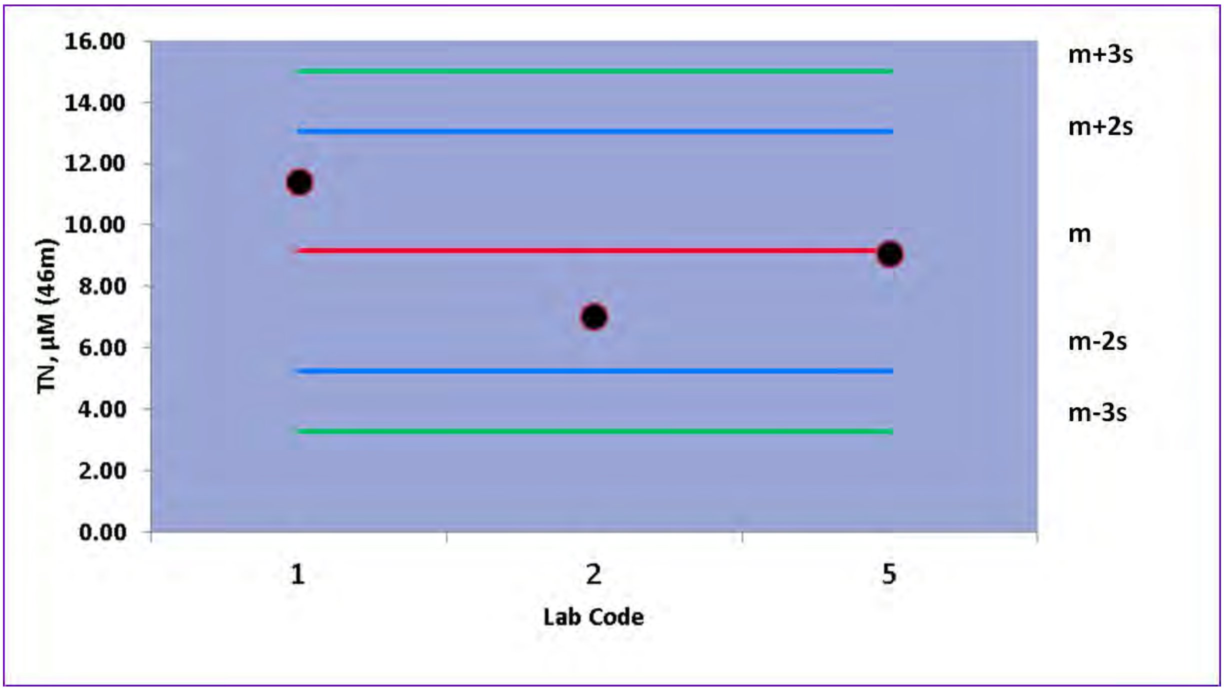
I.3.6. Total Organic Carbon (TOC)



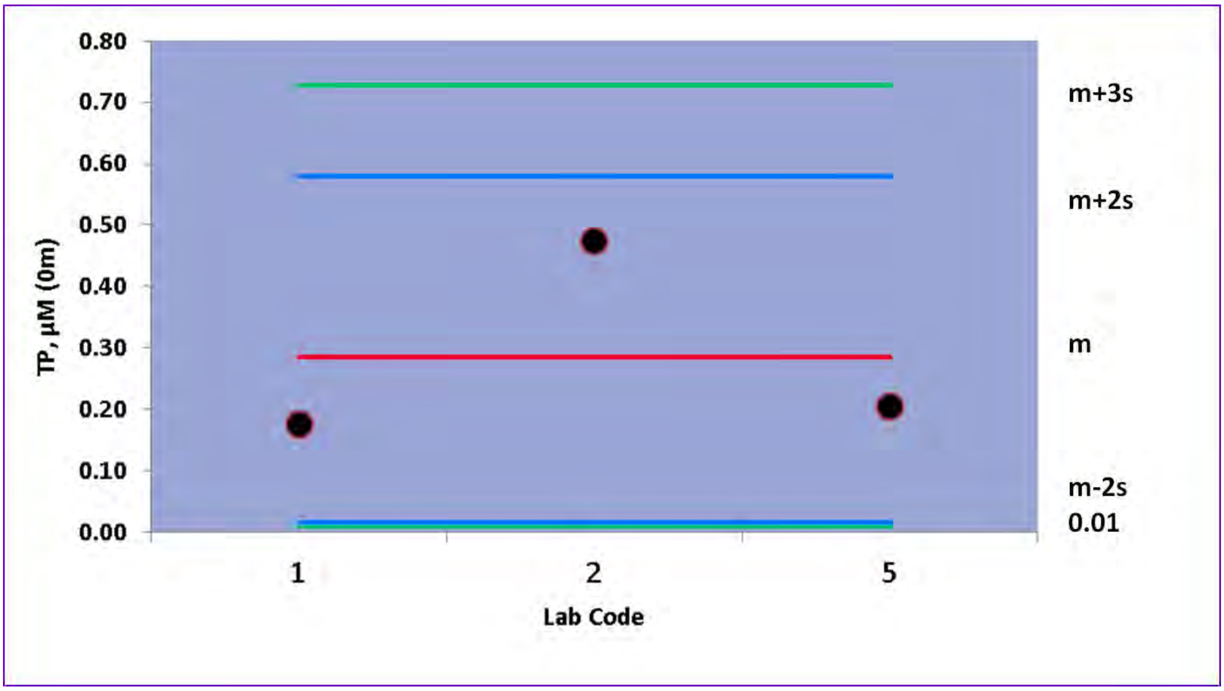


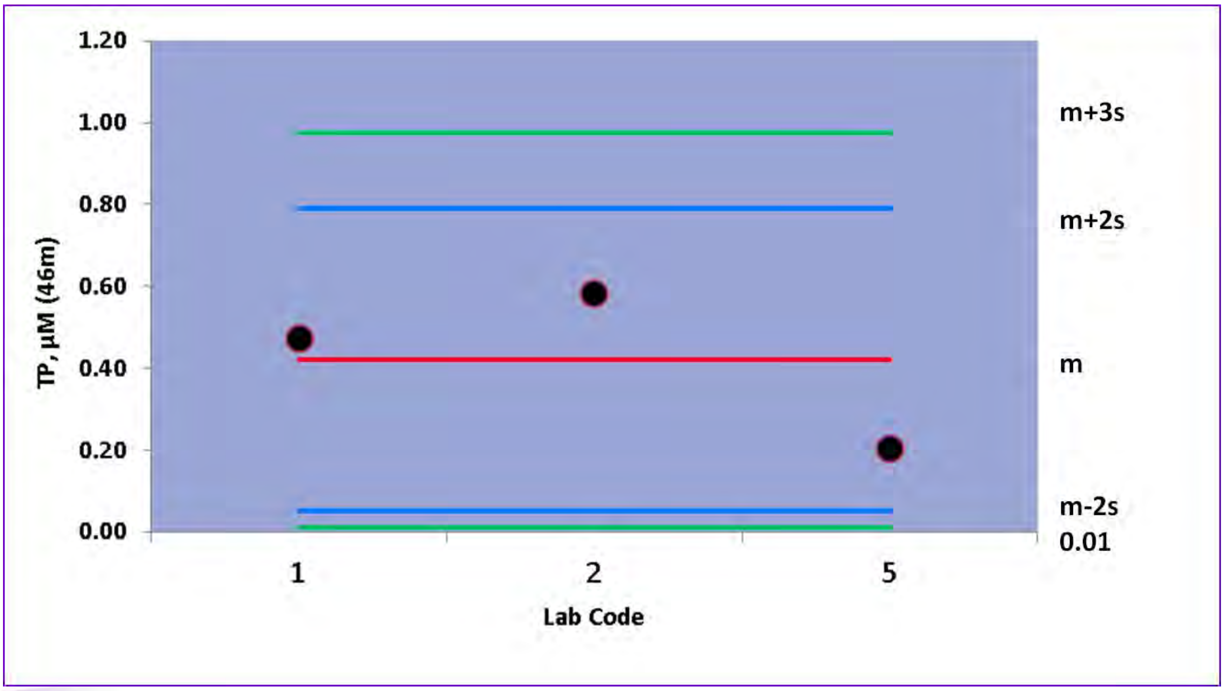
I.3.7. Total Nitrogen (TN)





I.3.8. Total Phosphorus (TP)





I.4. Conclusions - Nutrients

Satisfactory agreements (|z| ≤ 2) were obtained for the most measurements of and for the participating laboratories in the MISIS State of the Environment report (75%), questionable results (2 ≤ |z| ≤ 3) in 6%, whereas unsatisfactory agreements (|z| ≥ 3 in 19 % of cases (Fig. 23).

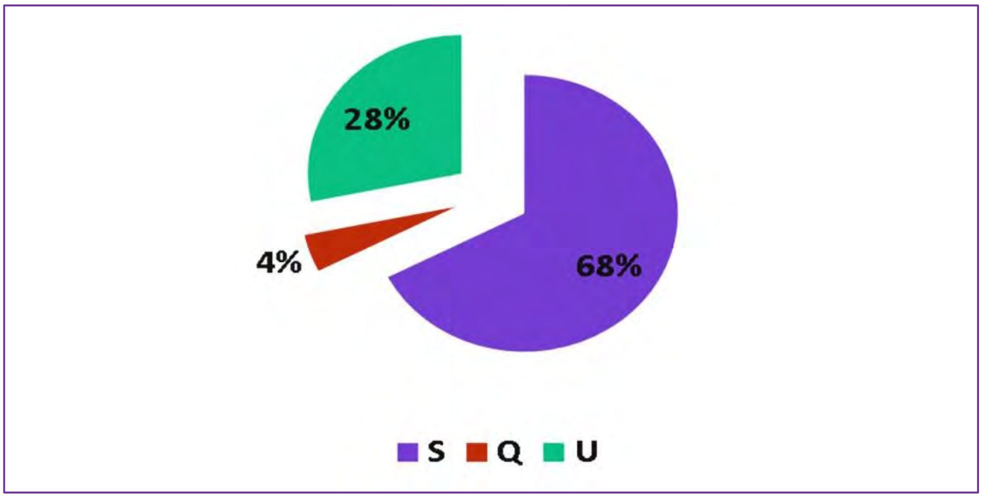


Figure 22. Classification of the performance based on z- scores for the all reported results (S – satisfactory; Q – questionable; U – unsatisfactory).

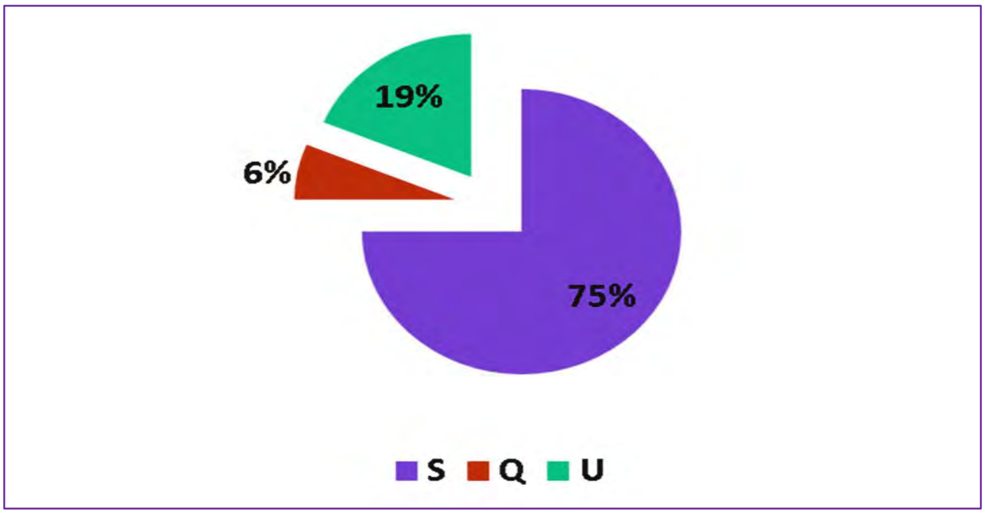


Figure 23. Classification of the performance based on z- scores for the reported results participating in MISIS SoE report (S – satisfactory; Q – questionable; U – unsatisfactory).

Phosphate and nitrite results were comparable(|z| ≤ 2) in all reported measurements, followed by TNOx with 75%, ammonium with 63% and silicate with 43% satisfactory results.

Despite of the methodological differences (Annex I - I.3) the analyzed samples were subject of lack of homogeneity which definitively contributes to the results and assessment.

Annex I

I.1. List of the participating laboratories - nutrients

Laboratory code	Institution	Country	Responsible person	Parameters reported
1	Institute of oceanology-Bulgarian Academy of Sciences IO-BAS	BUGARIA	Galina SHTEREVA g.shtereva@io-bas.bg	PO_4, TP, SiO_4, NO_2, NO_3, TNOx, NH_4, TN
2	Romanian Water National Administration - Water Administration Dobrogea Littoral RWNA-WADL	ROMANIA	Cerasela CARAULA cerasela.caraula@dadl.rowater.ro	PO_4, TP, SiO_4, NO_2, NO_3, TNOx, NH_4
3	National Research and Development Institute for Marine Geology and Geoecology GEOECOMAR	ROMANIA	Dan VASILIU dan.vasiliu@geoecomar.ro	PO_4, SiO_4, NO_2, NO_3, TNOx, NH_4
4	National Institute for Marine Research and Development NIMRD	ROMANIA	Luminita LAZAR llazar@alpha.rmri.ro	PO_4, SiO_4, NO_2, NO_3, TNOx, NH_4, TOC, TN
5	TUBITAK Marmara Research Center	TURKEY	Hakan ATABAY hakan.atabay@tubitak.gov.tr	PO_4, SiO_4, TNOx, NH_4, TOC

I.2. Raw data reported - nutrients

I.2.1 Laboratory 1

Depth m	PO_4 µM	TP µM	SiO_4 µM	NO_2 µM	NO_3 µM	TNOx µM	NH_4 µM	TN µM
0	0.05	0.18	1.83	0.04	0.15	0.19	0.20	39.13
	0.03	0.18	1.68	0.07	0.09	0.16	0.20	39.97
	0.04	0.18	1.58	0.04	0.13	0.17	0.15	39.83
46	0.35	0.48	12.87	0.09	0.96	1.05	0.32	45.37
	0.32	0.46	11.58	0.08	0.76	0.84	0.30	33.57
	0.37	0.48	15.59	0.09	1.00	1.09	0.35	36.66

I.2.2 Laboratory 2 (each batch in 3 replicates)*

Depth m	PO_4 mg/L	TP mg/L	SiO_4 mg/L	NO_2 mg/L	NO_3 mg/L	NH_4 mg/L
0	0.00978	0.007	0.653	0.0019	0.442	0.1205
	0.018	0.008	0.528	0.0029	0.442	0.1298
	0.013	0.008	0.590	0.0023	0.442	0.12515
	0.018	0.008	0.538	0.0019	0.442	0.109
	0.012	0.006	0.482	0.0026	0.884	0.117
	0.012	0.004	0.510	0.00098	0.663	0.113
	0.0067	0.003	0.626	0.00098	0.884	0.196
	0.0097	0.006	0.656	0.00065	0.884	0.179
	0.0128	0.006	0.641	0.0019	0.884	0.1875
46	0.055	0.020	2.217	0.0036	0.442	0.237
	0.046	0.020	2.191	0.0042	0.884	0.217
	0.049	0.019	2.204	0.0032	0.663	0.227
	0.065	0.022	2.368	0.0022	0.442	0.182
	0.068	0.020	2.253	0.0022	0.884	0.219
	0.065	0.021	2.311	0.0026	0.663	0.2005
	0.037	0.013	2.263	0.0046	0.442	0.147
	0.034	0.014	2.268	0.0029	0.442	0.1456
	0.037	0.014	2.265	0.0059	0.442	0.1463

*the assessment used each batch average transformed in µM

I.2.3 Laboratory 2 – data used for assessment

Depth m	PO_4 µM ARD*10.5263	TP µM ARD*32.2581	SiO_4 µM ARD*10.8696	NO_2 µM ARD*21.7391	NO_3 µM ARD*16.1290	TNOx µM (NO2+NO3)	NH_4 µM ARD*55.5556
0	0.14	0.25	6.42	0.05	7.13	7.18	6.95
	0.15	0.19	5.54	0.04	10.69	10.73	6.28
	0.10	0.16	6.97	0.03	14.26	14.29	10.42
46	0.53	0.63	23.96	0.08	10.69	10.77	12.61
	0.69	0.68	25.12	0.06	10.69	10.75	11.14
	0.38	0.44	24.62	0.10	7.13	7.23	8.13

*ARD=average reported data *factor
(calculated as 1000/molecular weight)

I.2.4 Laboratory 3

Depth m	PO_4 µM	SiO_4 µM	NO_2 µM	NO_3 µM	TNOx µM	NH_4 µM
0	0.065	0.472	0.005	0.310	0.322	1.408
	0.056	0.442	0.006	0.320	0.328	1.345
	0.063	0.432	0.008	0.300	0.307	1.179
46	0.579	7.275	0.005	1.298	1.303	0.938
	0.606	6.905	<0.005	1.242	1.242	0.800
	0.600	6.680	0.007	1.265	1.272	0.599

I.2.5 Laboratory 4

Depth m	PO_4 µM	SiO_4 µM	NO_2 µM	NO_3 µM	TNOx µM	NH_4 µM	TOC mg/L	TN mg/L
0	0.28	1.5	0.08	0.87	0.95	0.54	3.458	11.364
	0.59	1.2	0.06	0.99	1.05	0.62	3.405	11.700
	0.44	1.6	0.08	0.90	0.98	0.68	3.348	11.121
46	0.72	17.5	0.10	1.13	1.23	0.42	3.024	6.924
	0.70	17.0	0.12	1.13	1.25	0.47	3.063	7.037
	0.80	16.6	0.08	1.32	1.40	0.38	3.153	7.016

I.2.6 Laboratory 5

Depth m	PO_4 µM	TP µM	SiO_4 µM	TNOx µM	NH_4 µM	TN µM	TOC mg/L
0	<0.08	0.166	<0.61	<0.17	0.51	9.74	2.777
	<0.08	0.148	<0.61	<0.17	0.56	11.21	2.775
	<0.08	0.293	<0.61	0.18	0.47	11.31	2.975
46	0.235	0.428	39.35	1.60	0.27	8.14	2.248
	0.141	0.445	39.64	1.63	<0.14	9.48	2.181
	0.217	0.530	28.36	1.86	0.20	9.56	2.213

	Lab.1	Lab.2	Lab.3	Lab.4	Lab.5
PO_4	Spectrophotometric (Grasshoff et al., 1999)	Spectrophotometric SR EN ISO 6878:2005	Spectrophotometric (manual) (Grasshoff et al., 1999)	Spectrophotometric (manual) (Grasshoff et al., 1999)	S.M. 4500-P : 2005 G (Method of Determination of orthophosphate) (Skalar Autoanalyzer)
TP	Spectrophotometric (Grasshoff et al., 1999)	Spectrophotometric SR EN ISO 6878:2005	-	-	S.M. 4500 P J:2005 (Persulfate Method for Simultaneous Determination of Total Nitrogen and Total Phosphorus) (Skalar Autoanalyzer)
SiO_4	Spectrophotometric (Grasshoff et al., 1999)	Spectrophotometric STAS 9375-73	Spectrophotometric (manual) (Grasshoff et al., 1999)	Spectrophotometric (manual) (Grasshoff et al., 1999)	SM 4500-SiO2- :2005 F (Colorimetric method) (Skalar Autoanalyzer)
NO_2	Spectrophotometric (Grasshoff et al., 1999)	Spectrophotometric SR EN 26777:2002/C91:2006	Spectrophotometric (manual) (Grasshoff et al., 1999)	Spectrophotometric (manual) (Grasshoff et al., 1999)	-
TNO_x	Spectrophotometric heterogeneous reduction through Cd column (Grasshoff et al., 1999)	Spectrophotometric heterogeneous reduction through Cd column	Spectrophotometric (manual), homogenous reduction with hydrazine, (Mullin and Riley, 1955; Strickland and Parsons, 1960)	Spectrophotometric (manual), homogenous reduction with hydrazine, (Mullin and Riley, 1955; Strickland and Parsons, 1960)	S.M. 4500-NO3-I:2005 (Cadmium reduction method) (Skalar Autoanalyzer)
NH_4	Spectrophotometric (Grasshoff et al., 1999)	NA	Spectrophotometric (manual) (Grasshoff et al., 1999)	Spectrophotometric (manual) (Grasshoff et al., 1999)	S.M. 4500-NH3 H:2005 (Flow injection method) (Skalar Autoanalyzer)
TOC	-	-	-	Automated by NPOC method (Shimadzu analyzer)	SM 5310 B:2005 High-Temperature Combustion Method (Shimadzu analyzer)
TN	Spectrophotometric method after alkaline oxidation (Grasshoff et al., 1999)	-	-	Automated (Shimadzu analyzer)	S.M. 4500- P A:2005 (Persulfate Method for Simultaneous Determination of Total Nitrogen and Total Phosphorus) (Skalar Autoanalyzer)

Chapter II. Heavy Metals in sediments

II.1. Sampling

One of the shelf stations from Bulgarian waters (M 10 – bottom depth of 75 m) was chosen for inter-comparison exercises for contaminants in sediments. Another inter-comparison station was select on the Turkish transect (M18 – in the coastal waters, bottom depth of 27 m).

Station	**Transect**	**Lat, °N**	**Long, °E**	**Bottom depth, m**	**Type**	**Date**
M 10	Galata, inter-comparison	43°10.000	028°.30.000	76.1	shelf	27.07.13
M 18	Igneada, inter-comparison	41°49.795	028°.00.275	27.2	coastal	30.07.13

II.2. Sediments Description

Station: M10	Date: 27. 07. 2013
Depth into the sediment (cm)	Grab description
0-1	Light gray mud, fine, very soft, sticky, with a milimetric layer of yellowish brown mud, semiliquid, gelatinous
1-4	Darker gray mud, soft, sticky, slightly greasy
4-5	Muddy very coarse sand, with small corroded shells and shell fragments
5-10	Sandy mud, gray, corroded shells and shell fragments
10-14	Muddy sand, gray, sticky, semi plastic, with many corroded, fragile shells and shell fragments
Station: M18	Date: 30. 07. 2013
Depth into the sediment (cm)	Grab description
0-4	Gray mixture of terrigenous and biogenous medium coarse sand with some silt fraction; very few small shells: *Spisula*, *Chamelea*, *Mytilus*
4-8	Coarse to very coarse biogenous sand (carbonated sand – sand sized shell fragments), brownish, with very few shells: *Spisula*, *Chamelea*, *Mytilus*

(*Photos: Dan Secrieru*)

Sediment samples for trace metals in stations M10 and M18 were collected using a Van Veen grab sampler. The undisturbed surface layer was carefully collected with a spatula, mixed and homogenized on board, and immediately divided among participants, samples being placed in polyethylene containers (1 for each laboratory), sealed, labeled and transported into the laboratory. The samples were stored frozen (at – 20 ÷ -24 °C) and analyzed subsequently in the participant laboratories: GEOECOMAR (1), NIMRD (2), RWNA-WADL (3) and TUBITAK (4).

Participants determined metal concentrations in three replicate digests of each of the two sediments samples: M10 (shelf) and M18 (coastal).

II.3. Inventory of analytical methods for trace metals analysis reported by the participating laboratories

	GEOECOMAR (1)	**NIMRD (2)**	**RWNA-WADL (3)**	**TUBITAK (4)**
Drying procedure	Air drying	Freeze-drying	Drying 105^{0}C/24h	Freeze-drying
Digestion technique	Wet mineralization; Acids used: nitric and hydrochloric acid (HNO_3 + HCl); Open vessels; 150^{0}C, evaporation to dryness + 2 ml HCl 37% to 50 ml;	Wet mineralization; Acid used: nitric acid (HNO_3); Microwave oven, 175^{0}C,30 min;	Wet mineralization; Acids used: nitric and hydrochloric acid (HNO_3 + HCl); Closed vessels; 220^{0}C.	Wet mineralization; Acids used: Nitric acid (HNO3) and hydrofluoric acid (HF);
Instrumental techniques	Atomic absorption spectrometry (AAS): flame technique for Ni, Co, Cu, Pb, Zn; graphite furnace technique (GF-AAS) for Cd; Deuterium background correction; WDXRF technique for Fe, Mn, Cr, V, Sr, Rb, Ba, Zr;	Graphite furnace - atomic absorption spectrometry (GF-AAS) for Cr, Cu, Pb, Cd, Ni; Zeeman background correction;	Graphite furnace - atomic absorption spectrometry (GF-AAS) for Cr, Cu, Pb, Mn, Zn; Deuterium background correction;	ICP-MS for Al, As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Zn; DMA-80 for Hg;
Quality control procedure	CRM	CRM	Control charts	CRM

Various dissolution/extraction procedures for the determination of trace metals in marine sediments were reported: two laboratories (labs 1 and 3) used aqua regia (HNO_3 + HCl), one used nitric acid (HNO_3) (lab 2) and one used nitric acid and hydrofluoric acid (HNO_3 + HF) (lab 4).

Strong acid digestions, using nitric acid or aqua regia, commonly used to decompose marine sediments, result in incomplete digestion because silicates and other refractory oxides are not dissolved. Thus, the proportion of metals dissolved is variable and depends on the sample type, matrix and element. Total decomposition methods, using hydrofluoric acid (HF) in combination with concentrated oxidizing acids, release the total metal content from sediments into solution, as HF is the only acid that completely dissolves the silicate lattices and release all the associated metals (UNEP, 1995).

Various instrumental techniques were used: flame or graphite furnace Atomic Absorption Spectrometry (AAS), Wavelength Dispersive X-ray Fluorescence (WDXRF), Inductively Coupled Plasma Mass Spectrometry (ICP-MS), Direct Mercury Analyzer (DMA).

All four laboratories reported results for Cr, Cu, Pb, three laboratories reported results for Cd, Ni, Mn, Zn and two laboratories for Co and Fe.

Al, As and Hg were reported only by one laboratory (4), and Sr, Rb, Ba, Zr, Ti and V were reported only by laboratory no.1.

II.4. Evaluation of the results

The statistical evaluation is dealing with the score z calculated from an assigned value established as consensus value according to *IUPAC Technical Report* (IUPAC, 2006) recommendations and a standard uncertainty that is most appropriate for the application area of the results, established as 20%.

Scope and field of application:

- the principal aim is the assessment of laboratory performance against established criteria based on fitness for a common purpose;
- compliance with these criteria may be judged on the basis of the deviation of measurement results from assigned values;

Assessment of performance:

Laboratories will be assessed on the difference between their result and the assigned value. A performance score will be calculated for each laboratory, using the *z*-score based on a fitness-for-purpose criterion.

A. Establishment of the consensus value

The consensus value was calculated according to ***The International Harmonized Protocol for the Proficiency Testing Of Analytical Chemistry Laboratories (IUPAC Technical Report)*** (IUPAC, 2006) recommendations.

According to the IUPAC's technical report, an assigned value is an estimate of the value of the measurand that is used for the purpose of calculating scores and shall be determined by one of the following methods:

- measurement by a reference laboratory,
- the certified value(s) for a CRM used as a test material,
- direct comparison of the proficiency testing test material with CRMs,
- consensus of expert laboratories,
- formulation (i.e., value assignment on the basis of proportions used in a solution or other mixture of ingredients with known analyte content),
- a consensus value (that is, a value derived directly from reported results).

For the establishment of the consensus value we followed the next steps:

- Visualization of data and exclude data reported as "<"
- Calculate median and the range median ±50%
- Exclude the values which are not included in the range median ±50%
- Recalculate the median which is assumed to be a consensus value.

B. <u>Assignment of z-scores</u>

The IUPAC (2006) Harmonized Protocol recommended the conversion of participants' results into *z*-scores, and experience in the intervening years has demonstrated the wide applicability and acceptance of the *z*-score in proficiency testing. A participant's result $\boldsymbol{X}$ is converted into a *z*-score according to the equation

$$z = (x - x_a)/\sigma_p$$

where x_a is the "assigned value", the scheme provider's best estimate of the value of the measurand, and σ_p is the fitness-for-purpose-based "standard deviation for proficiency assessment", that underline the importance of assigning a range appropriate to a particular purpose (ISO Guide 43; Statistical Guide ISO 13528).

In the equation defining, the term $(x - x_a)$ is the error in the measurement. The parameter σp describes the standard uncertainty that is most appropriate for the application area of the results of the analysis, in other words, "fitness-for-purpose".

The uncertainty that is fit for purpose in a measurement result depends on the application. For example, a relative standard uncertainty [i.e., $u(x)/x$] of 10 - 20% is probably adequate for many environmental measurements. As described in the IUPAC guidelines, the choice of σ is dependent upon the data quality objective of a particular program. The fixed performance criterion is more useful in the comparison of a laboratory's performance on different materials.

In instances where the concentration of the analytes varies over a wide range, the fitness-for-purpose criterion has to be specified as a function of concentration. The most common approaches are as follows:

- Specify the criterion as a relative standard deviation (RSD). Specific σp values are then obtained by multiplying the selected RSD by the assigned value.

For the purpose of the present exercise, we choose to aim for an RSD of 20 %.

Interpretation of the z-scores

According to IUPAC, the interpretation of *z*-scores is *not* generally based on summary statistics that describe the observed participant results. Instead, it uses an assumed model based on the scheme provider's fitness-for-purpose criterion, which is represented by the standard deviation for proficiency assessment σp.

- A score of zero implies a perfect result. This will happen rarely even in the most competent laboratories.
- *z* - scores fall between –2 and +2. The sign (i.e., – or +) of the score indicates a negative or positive error respectively. Scores in this range are commonly designated "acceptable" or "satisfactory".
- A score outside the range from –3 to 3 indicate that the cause of the event should be investigated and remedied. Scores in this class are commonly designated "unacceptable" or "unsatisfactory".
- Scores in the ranges –2 to –3 and 2 to 3 are designated as "questionable".

$|z| \leq 2$ Satisfactory

$2 \leq |z| \leq 3$ Questionable

$|z| \geq 3$ Unsatisfactory

Histograms of all reported metals concentrations (see also Annex II.1 – Intercomparison Exercise Data Reporting Forms)
- sediment M10 (Fig. 24 - 31);

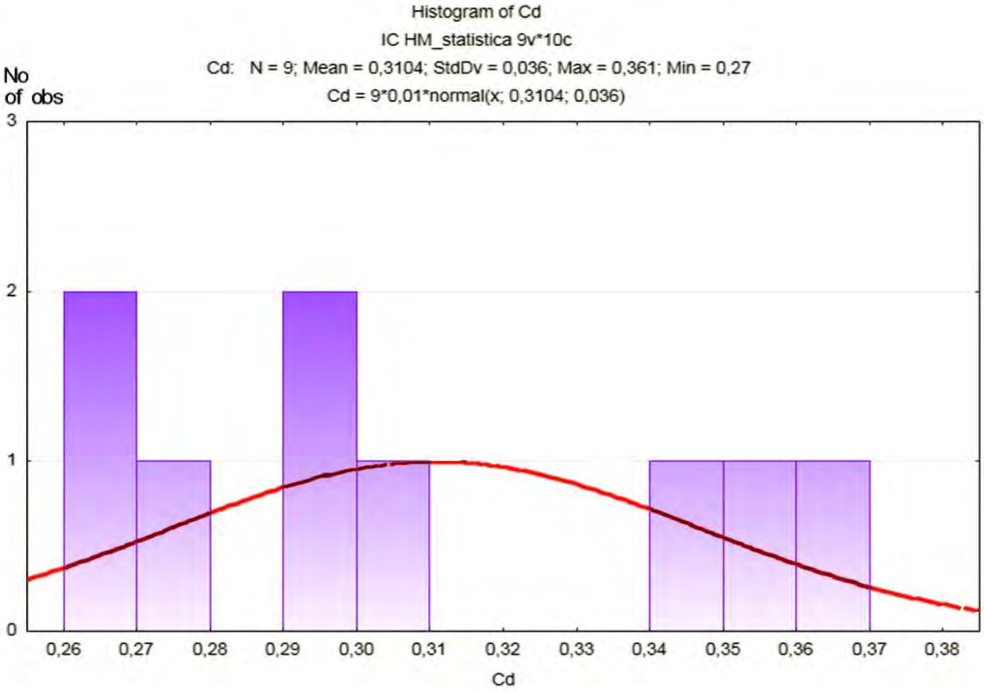


Figure 24. Histogram of all reported Cd concentrations (mg/kg) in the sediment M10.

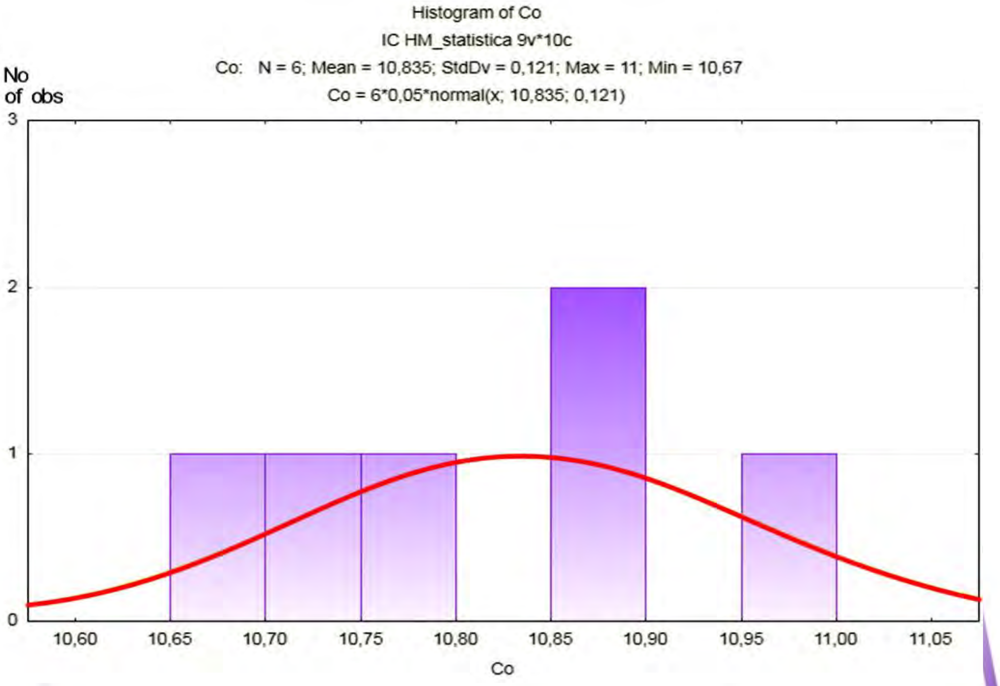


Figure 25. Histogram of all reported Co concentrations (mg/kg) in the sediment M10.

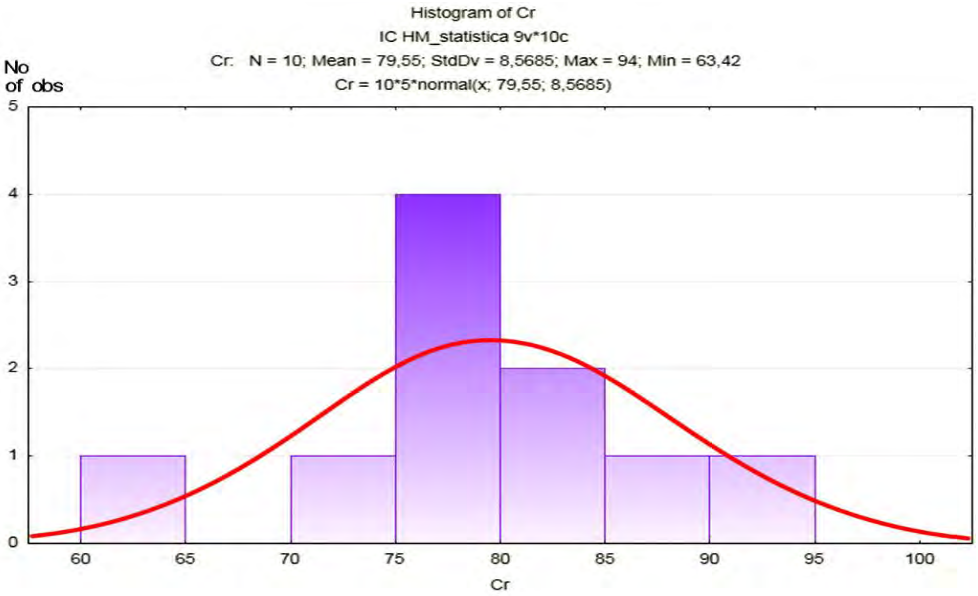


Figure 26. Histogram of all reported Cr concentrations (mg/kg) in the sediment M10.

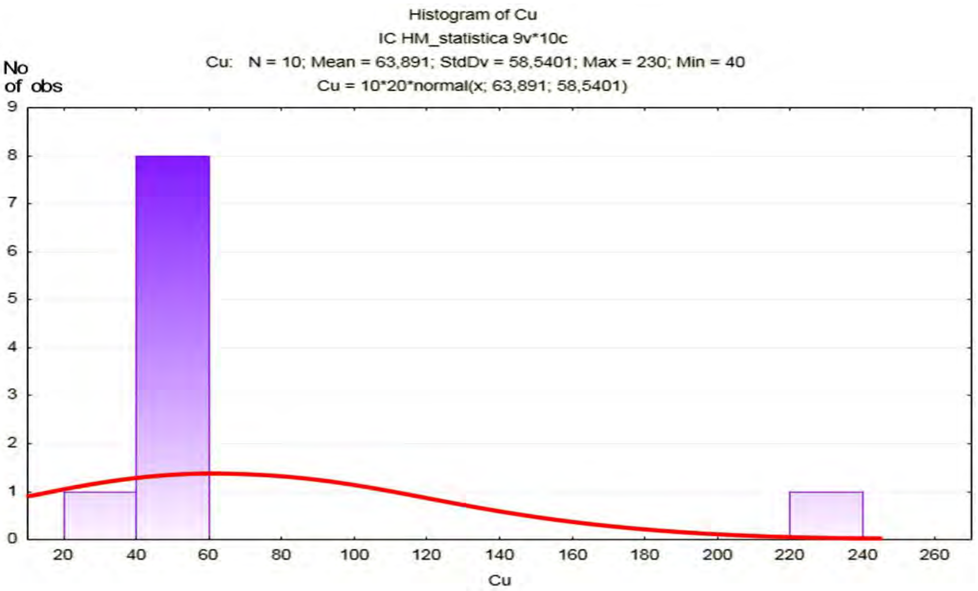


Figure 27. Histogram of all reported Cu concentrations (mg/kg) in the sediment M10.

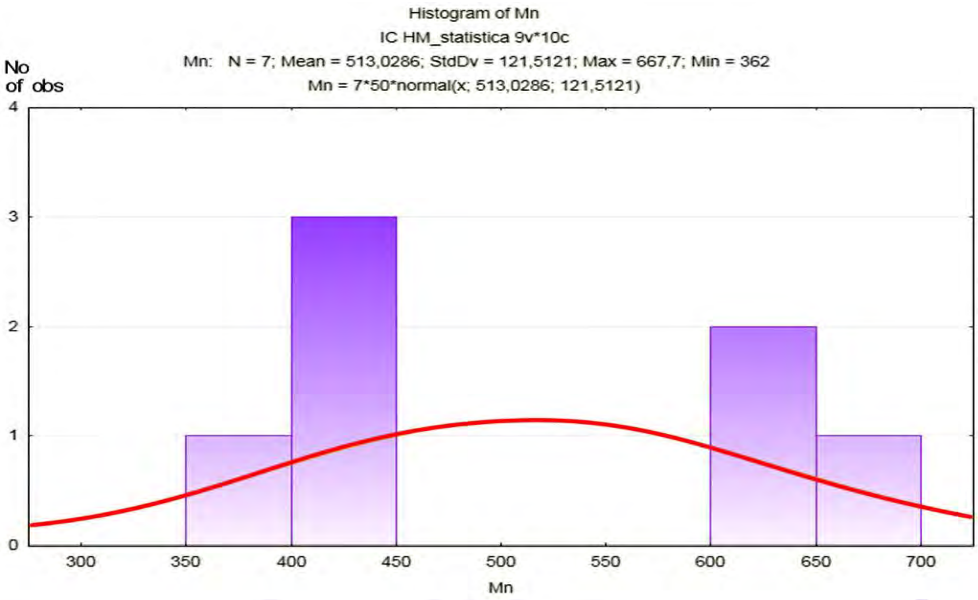


Figure 28. Histogram of all reported Mn concentrations (mg/kg) in the sediment M10.

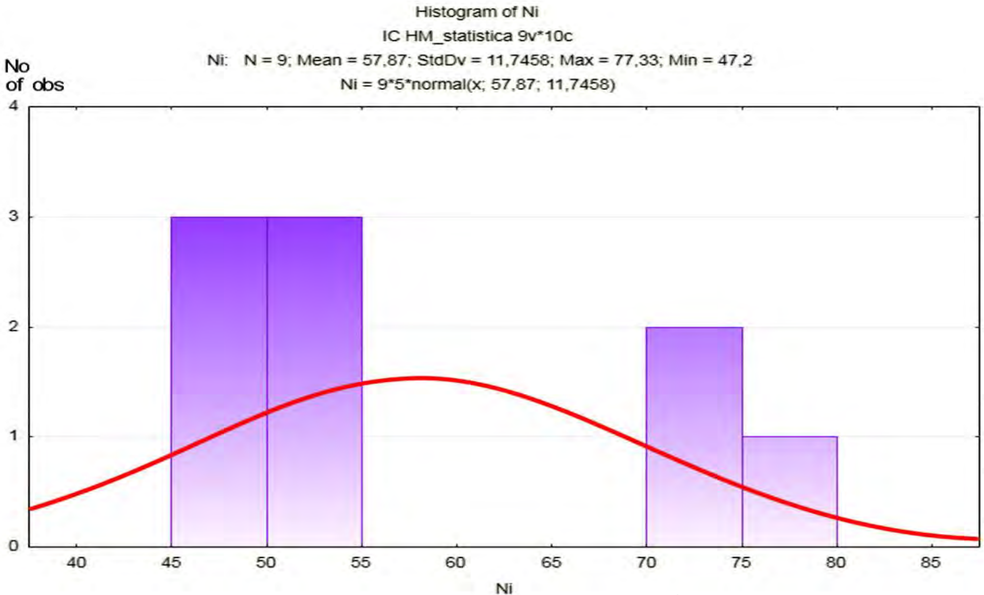


Figure 29. Histogram of all reported Ni concentrations (mg/kg) in the sediment M10.

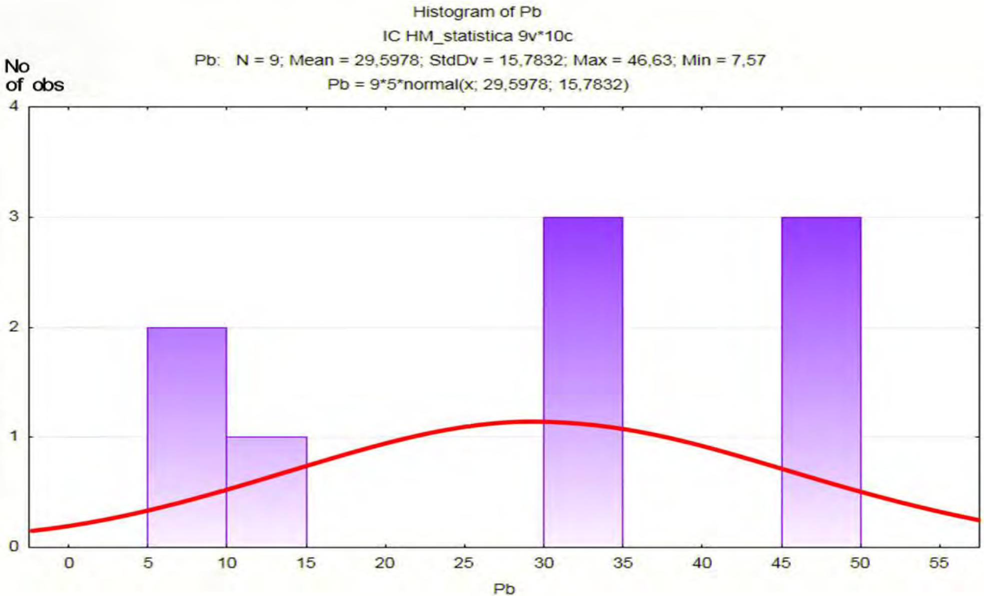


Figure 30. Histogram of all reported Pb concentrations (mg/kg) in the sediment M10.

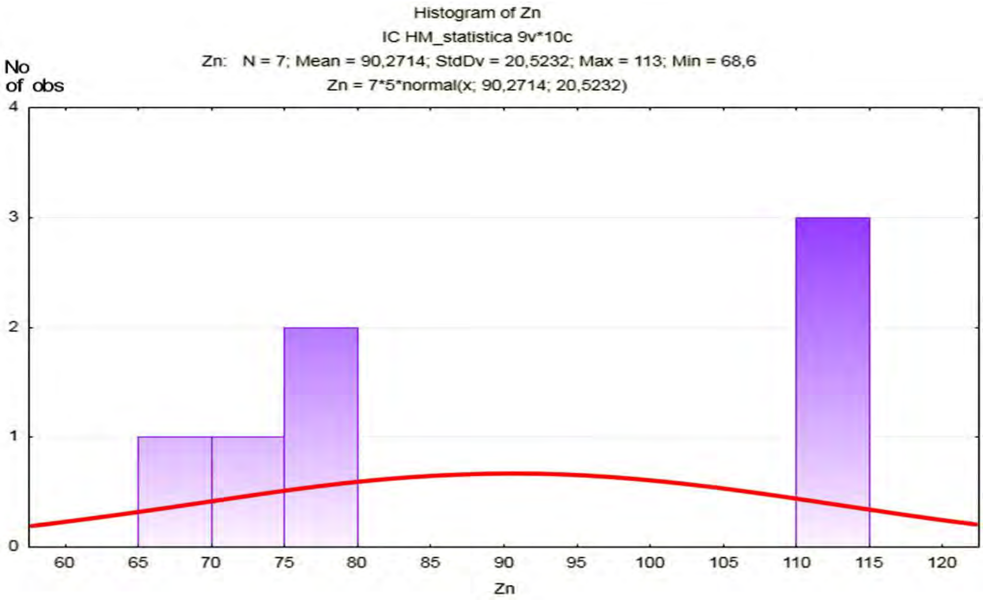


Figure 31. Histogram of all reported Zn concentrations (mg/kg) in the sediment M10.

- Sediment M18 (Fig. 32 - 39);

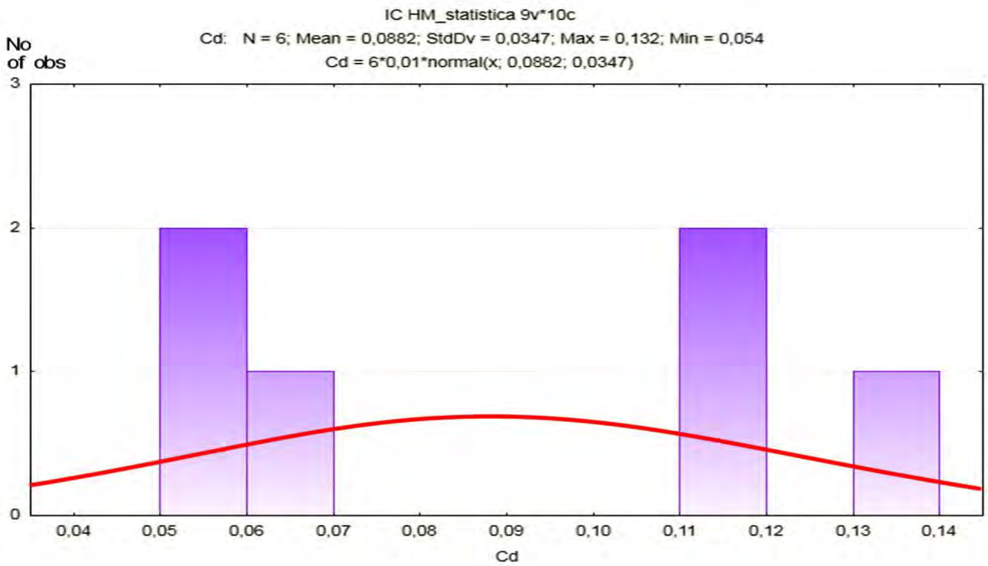


Figure 32. Histogram of all reported Cd concentrations (mg/kg) in the sediment M18.

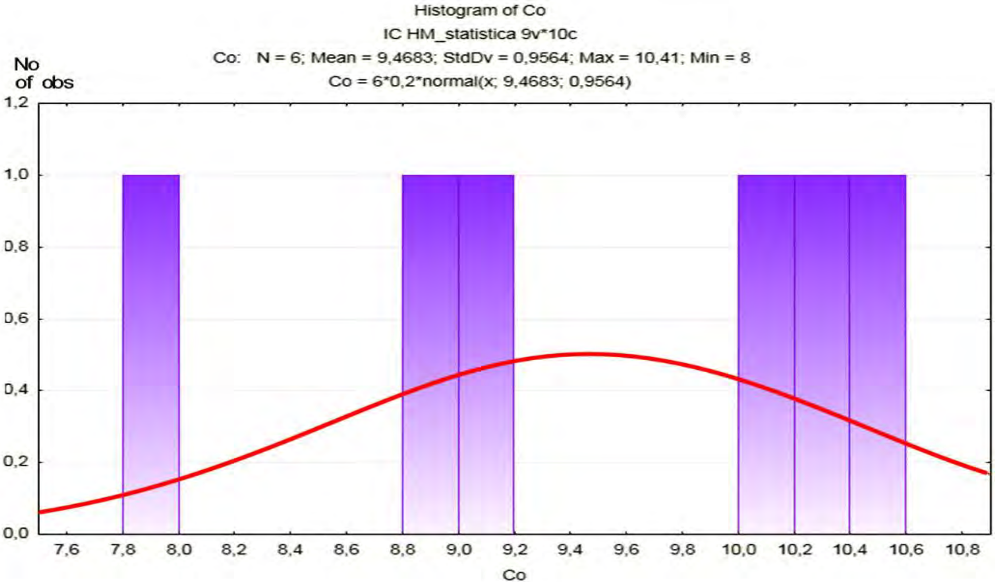


Figure 33. Histogram of all reported Co concentrations (mg/kg) in the sediment M18.

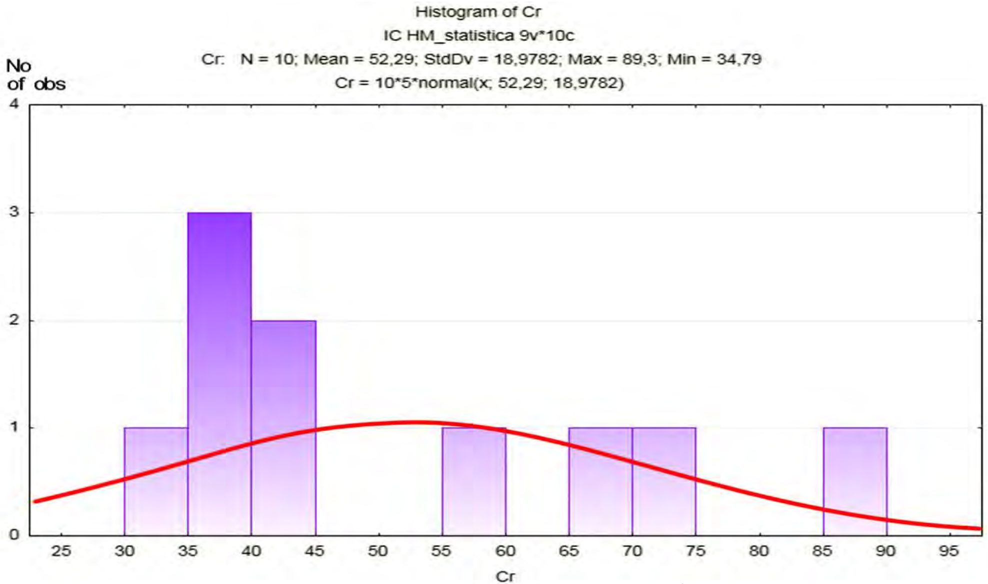


Figure 34. Histogram of all reported Cr concentrations (mg/kg) in the sediment M18.

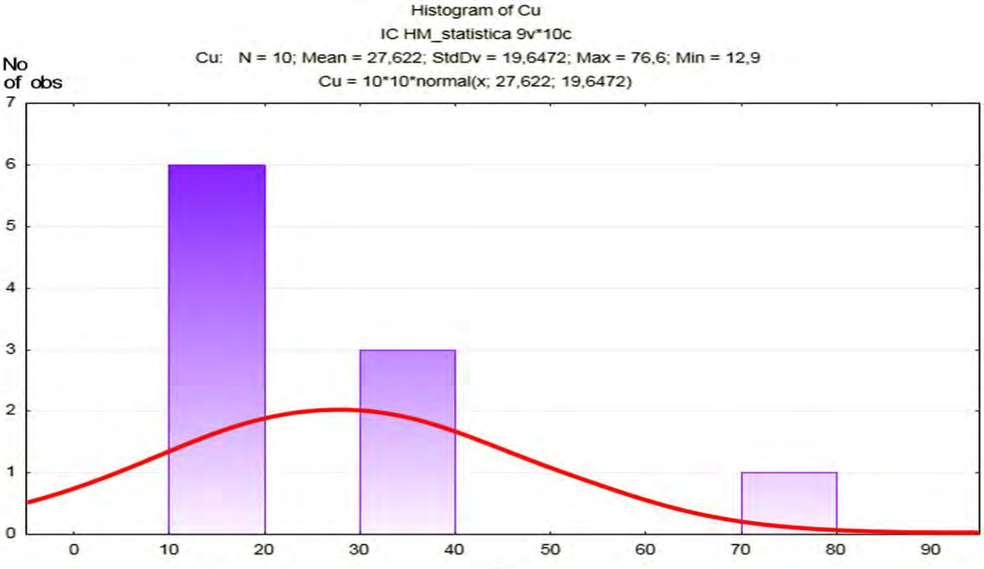


Figure 35. Histogram of all reported Cu concentrations (mg/kg) in the sediment M18.

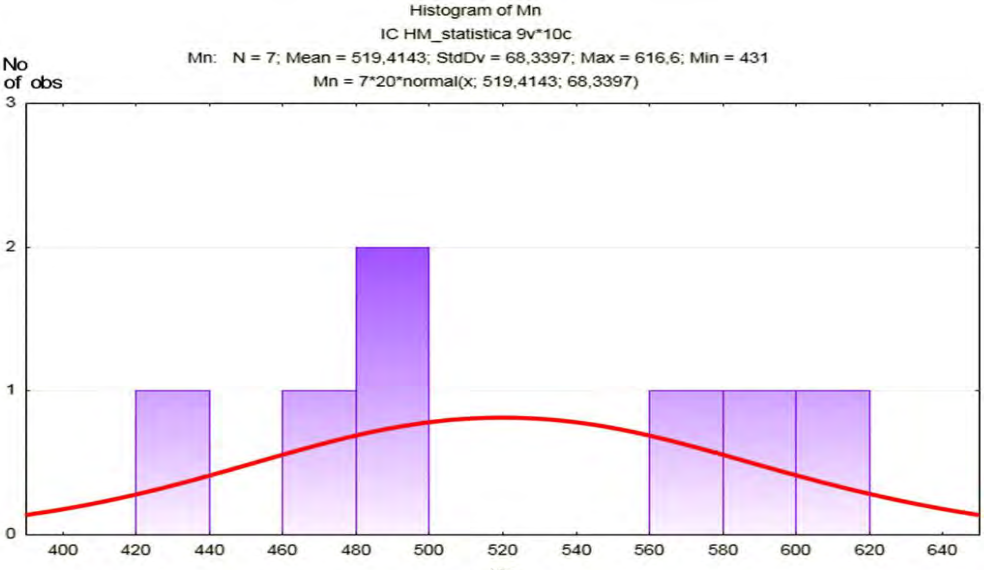


Figure 36. Histogram of all reported Mn concentrations (mg/kg) in the sediment M18.

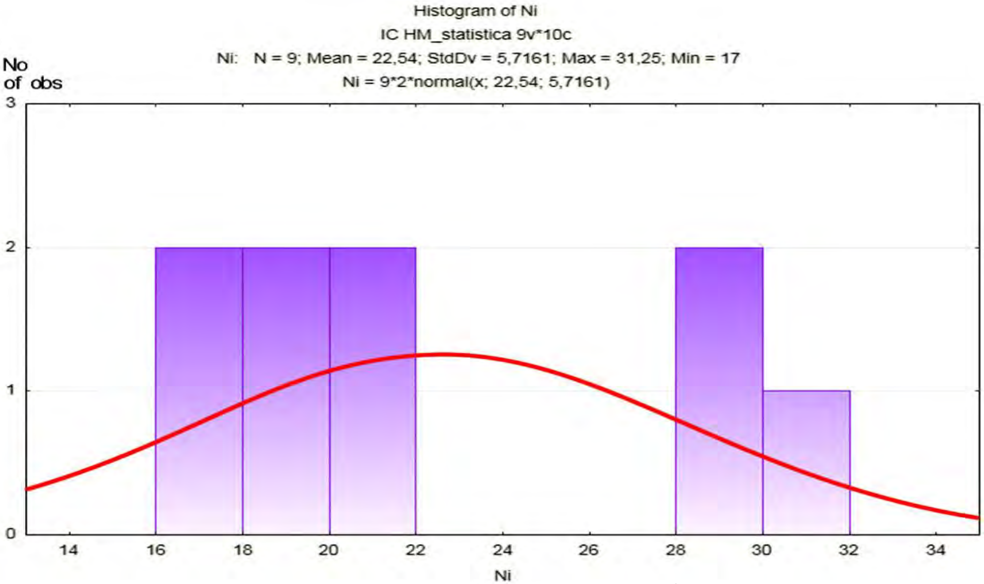


Figure 37. Histogram of all reported Ni concentrations (mg/kg) in the sediment M18.

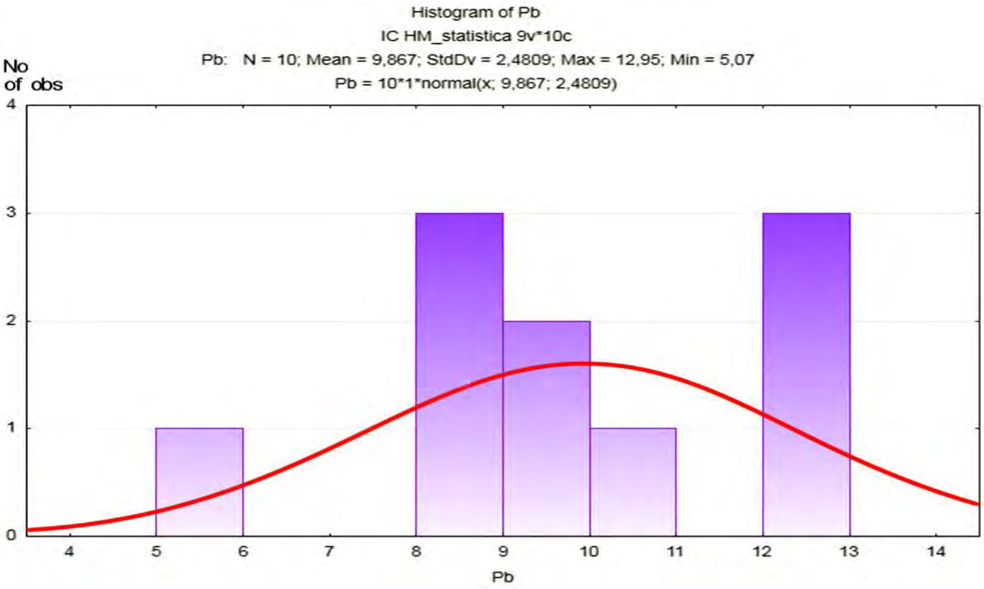


Figure 38. Histogram of all reported Pb concentrations (mg/kg) in the sediment M18.

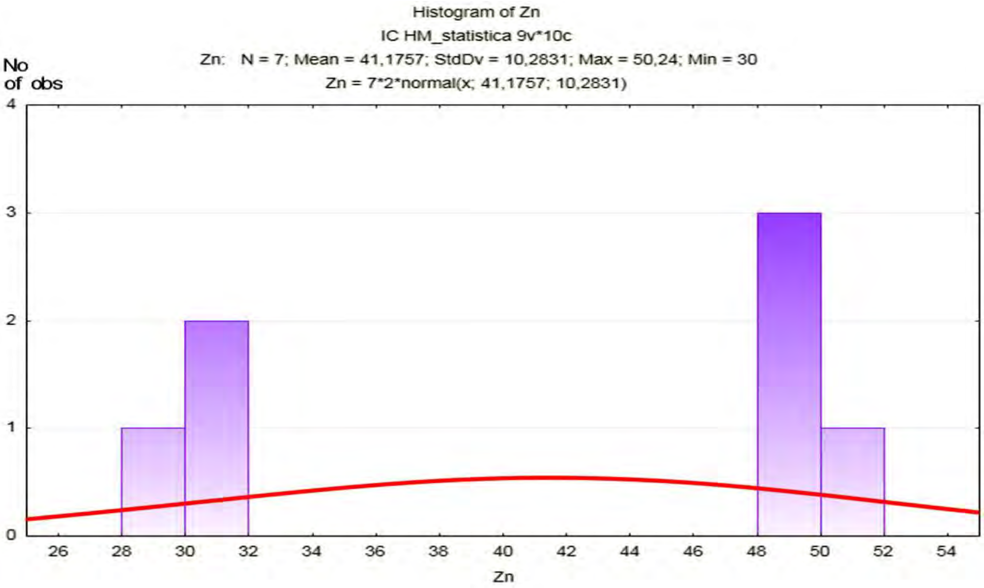
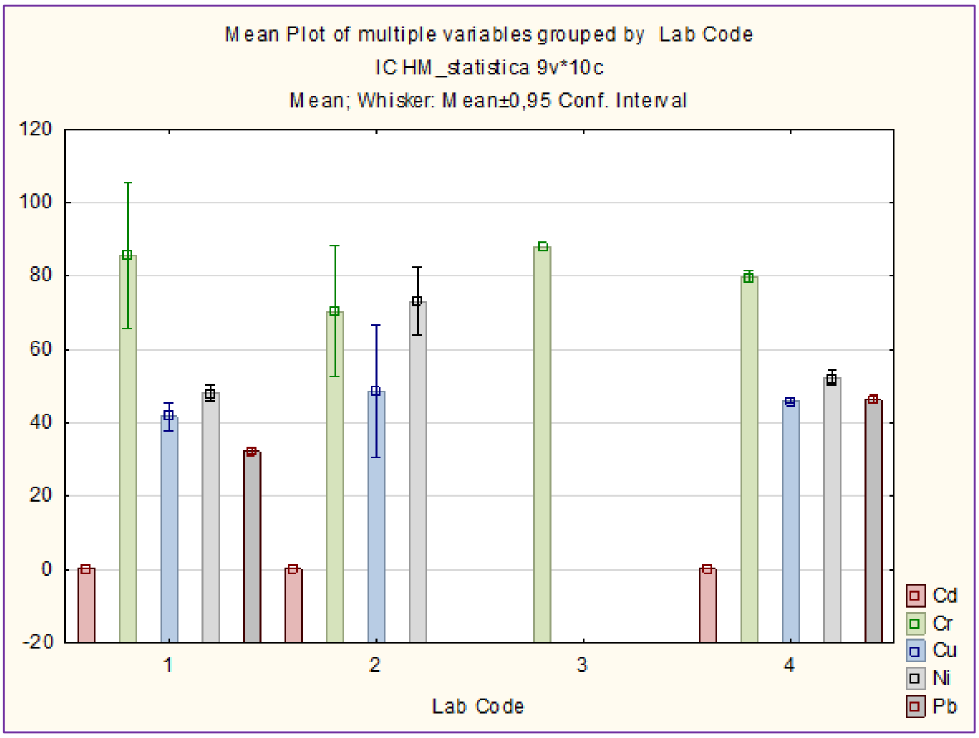


Figure 39. Histogram of all reported Zn concentrations (mg/kg) in the sediment M18.

For the establishment of the **consensus value** we followed the next steps:

- Visualization of data and exclude data reported as "<" ;
- Calculate median and the range median ± 50%;
- Exclude the values which are not included in the range median ± 50% (Fig. 40, Fig. 41);
- Recalculate the median which is assumed to be a consensus value (Fig. 42, Fig. 43);



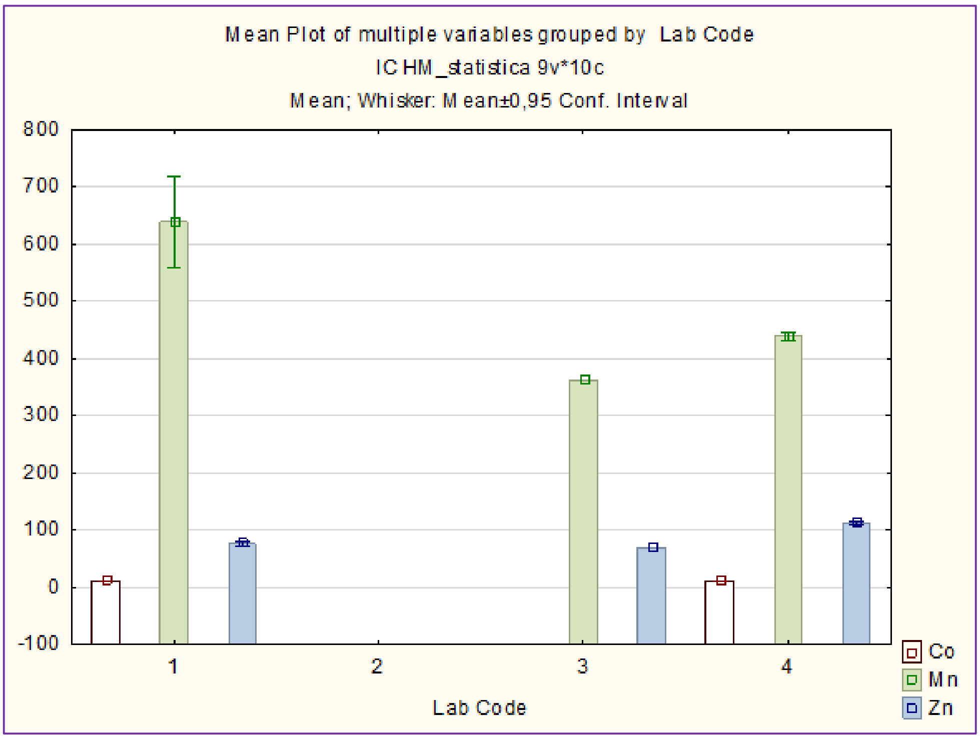
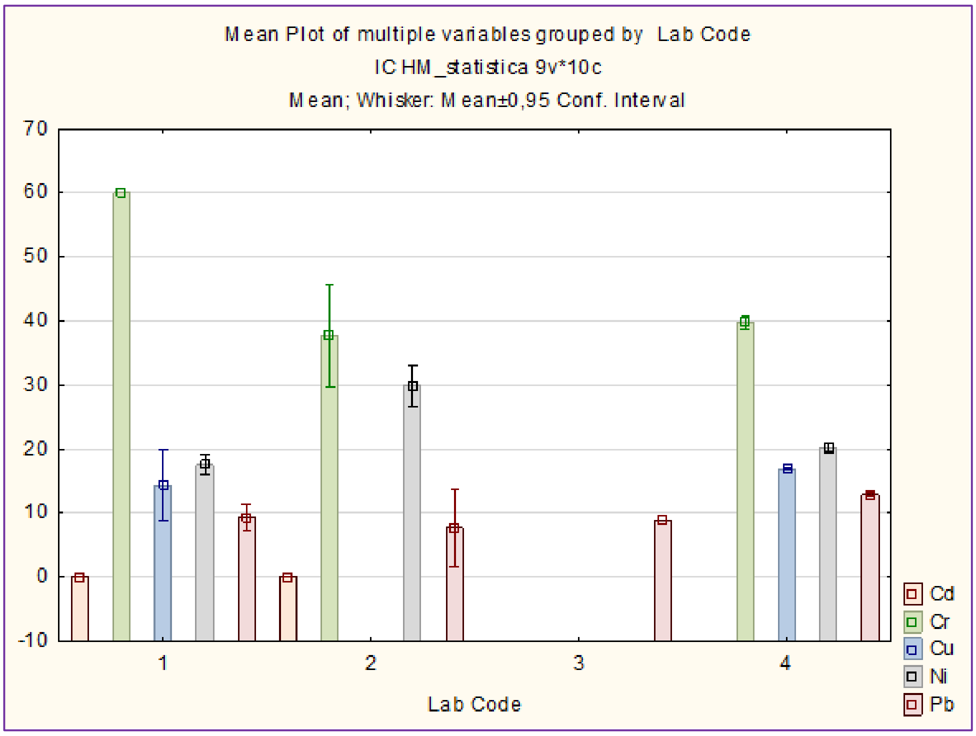


Figure 40. Metals concentrations (mean ± 0.95 Conf. interval) in sediment M10 reported by participating laboratories, after exclusion of the few values which are not included in the range median ± 50%



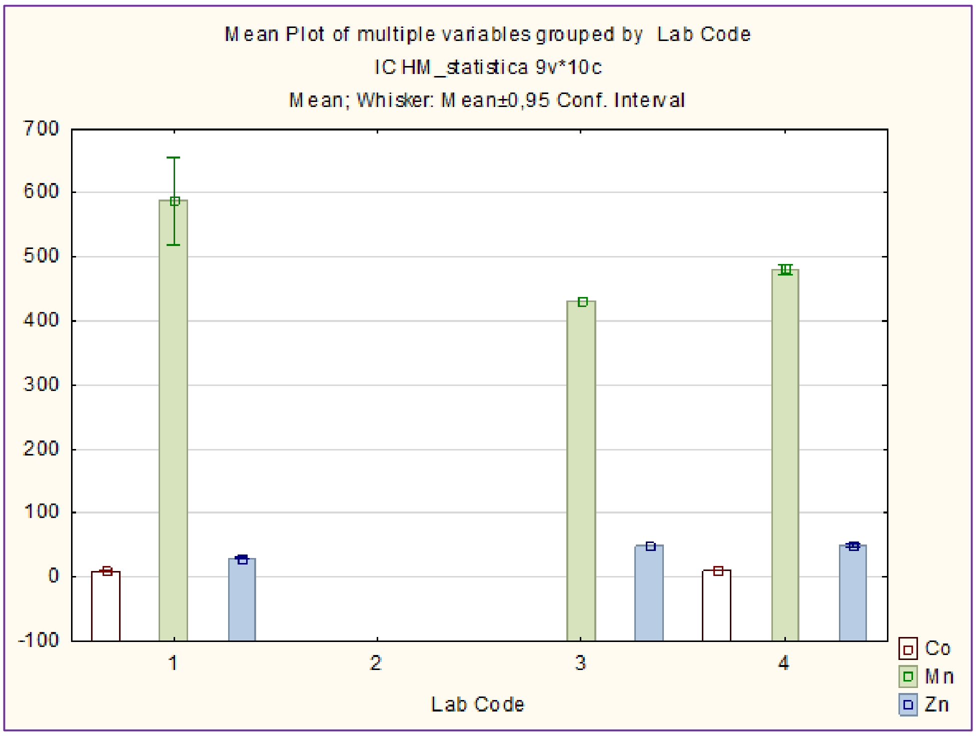


Figure 41. Metals concentrations (mean ± 0.95 Conf. interval) in sediment M18 reported by participating laboratories, after exclusion of the few values which are not included in the range median ±50%.

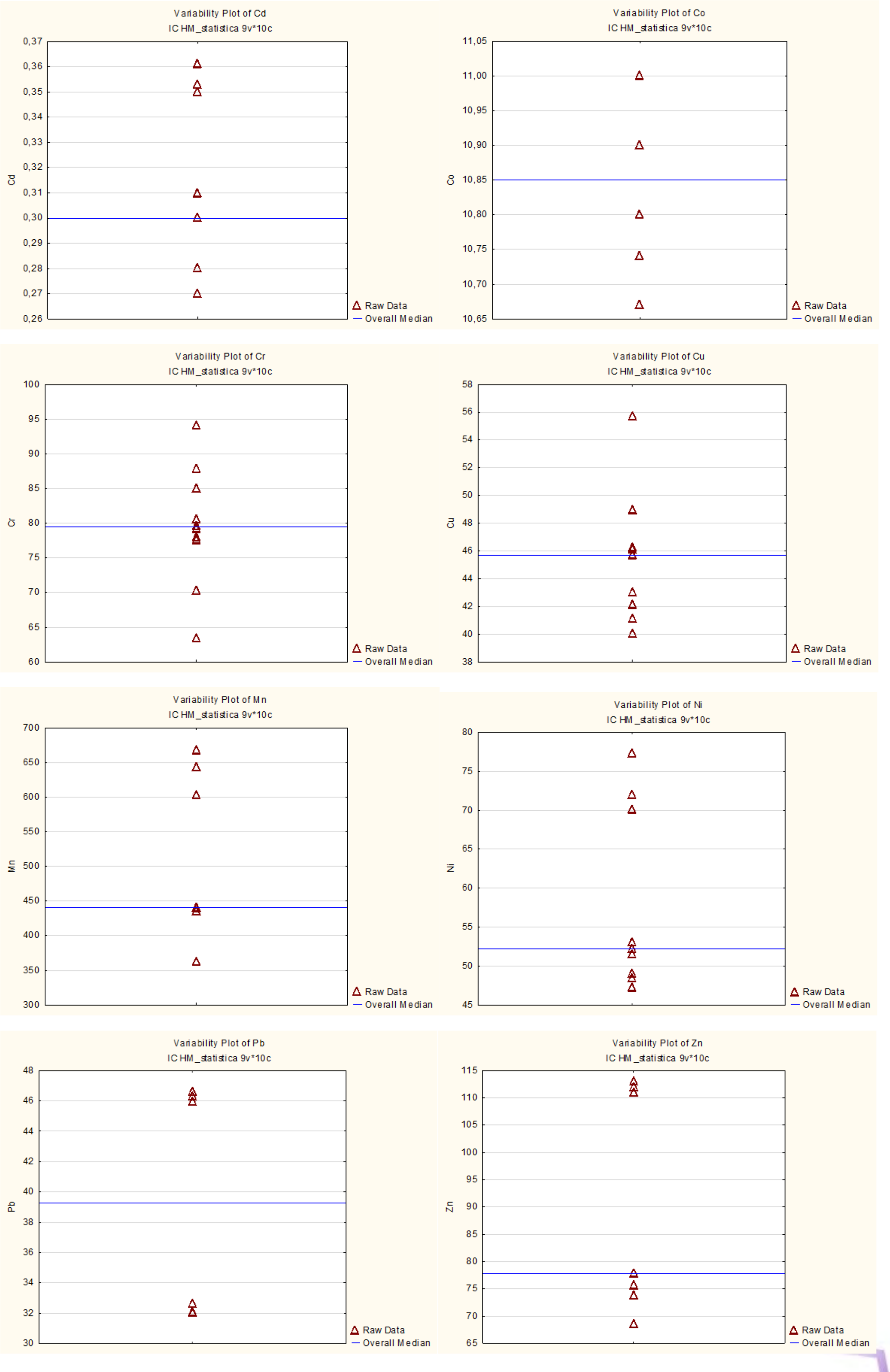


Figure 42. Variability plot of individual concentrations (outliers excluded), and recalculated median for sediment M10.

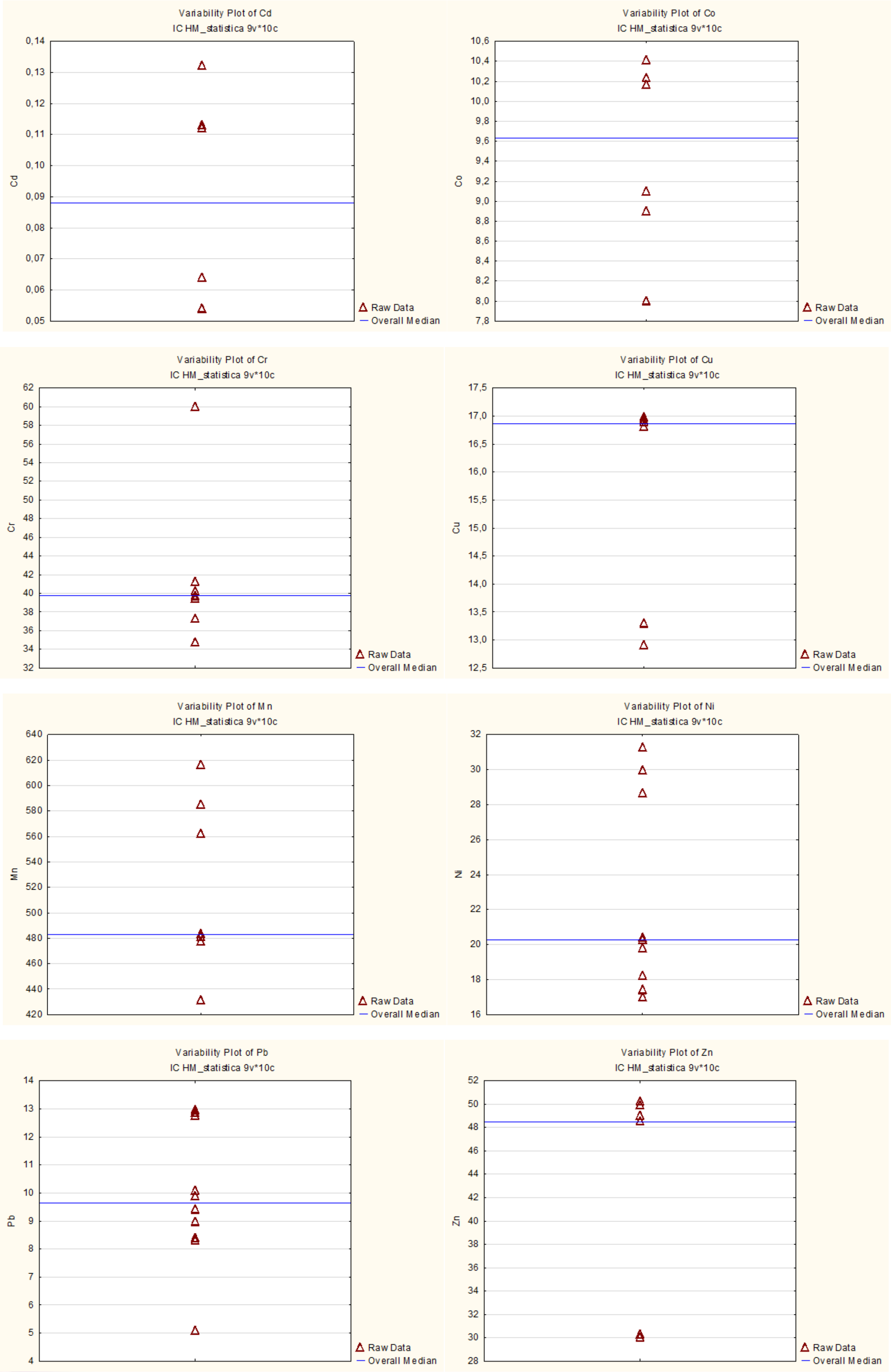
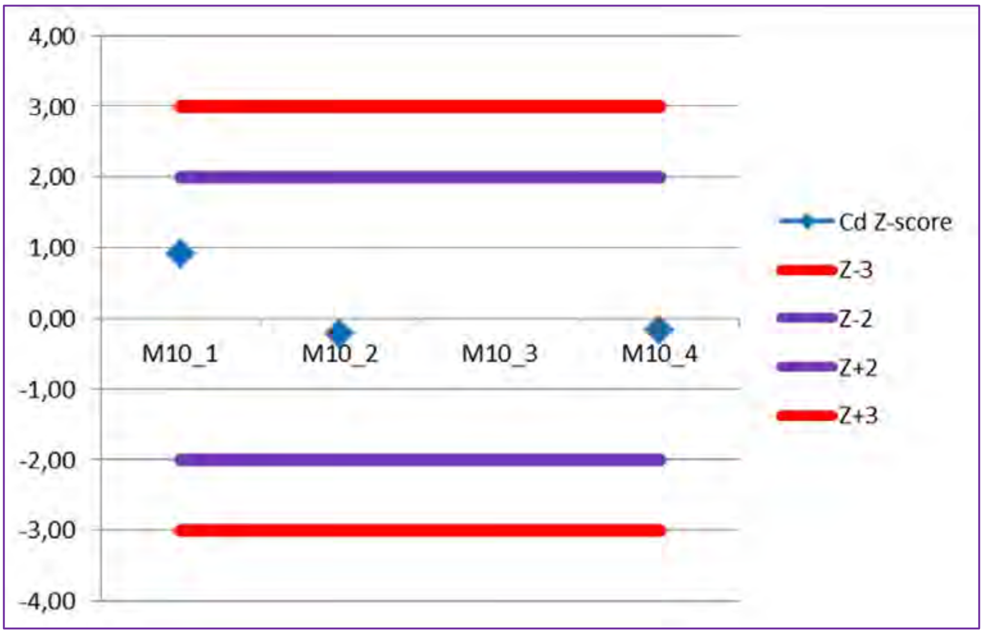


Figure 43. Variability plot of individual concentrations (outliers excluded), and recalculated median for sediment M18.

For each reported element, a participant's result *x* (average of three replicates) was converted into a *z*-score according to the equation:

$$z = (x - x_a)/\sigma_p$$

where x_a is the "assigned value", and σ_p is the fitness-for-purpose-based "standard deviation for proficiency assessment" (specified as a function of concentration, obtained by multiplying the selected RSD by the assigned value).



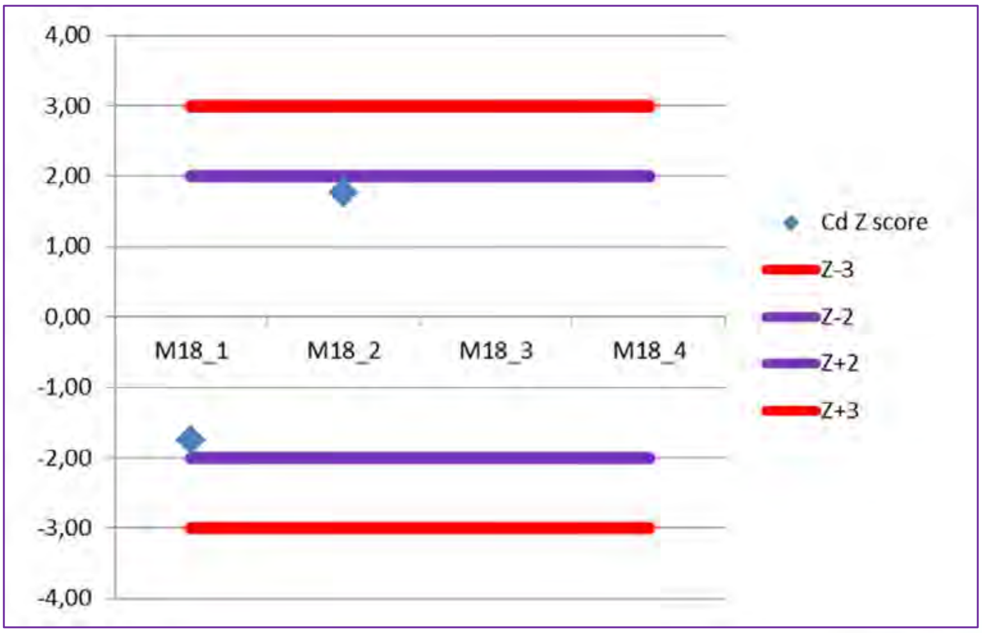
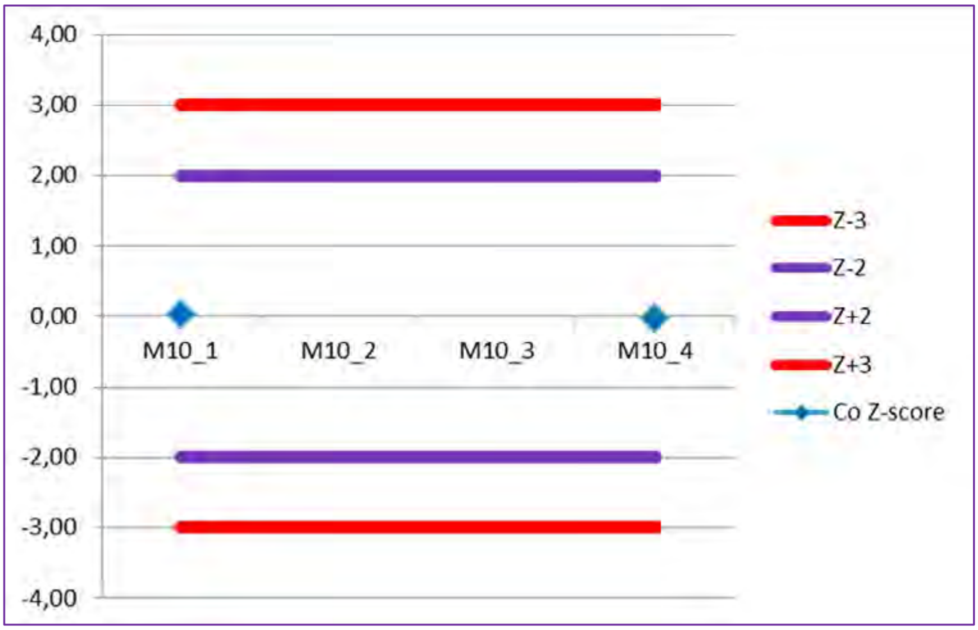


Figure 44. Z-scores of participating laboratories for reported Cd concentrations in sediment samples M10 and M18.



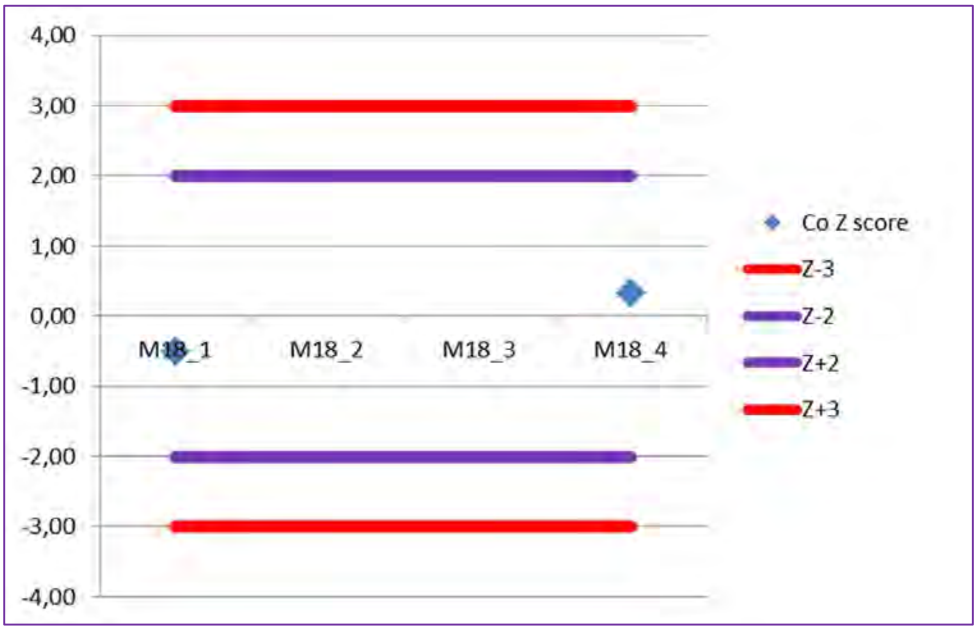
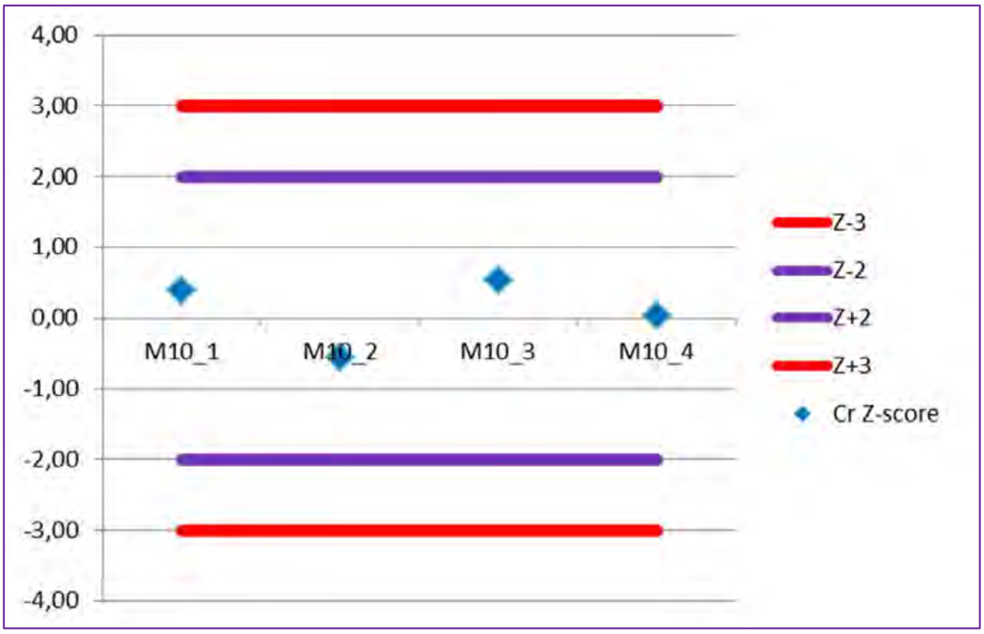


Figure 45. Z-scores of participating laboratories for reported Co concentrations in sediment samples M10 and M18.



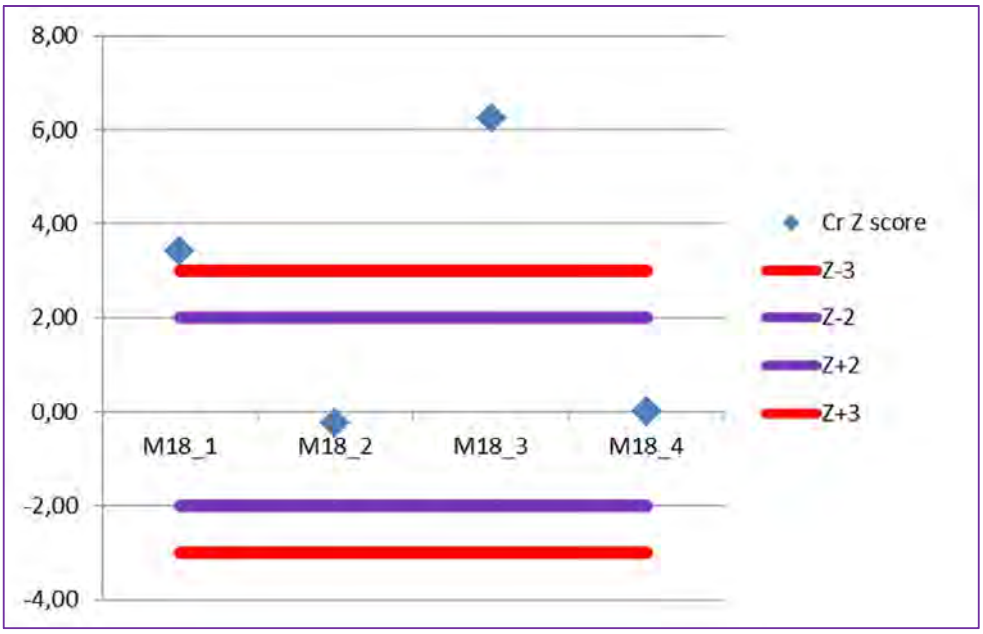
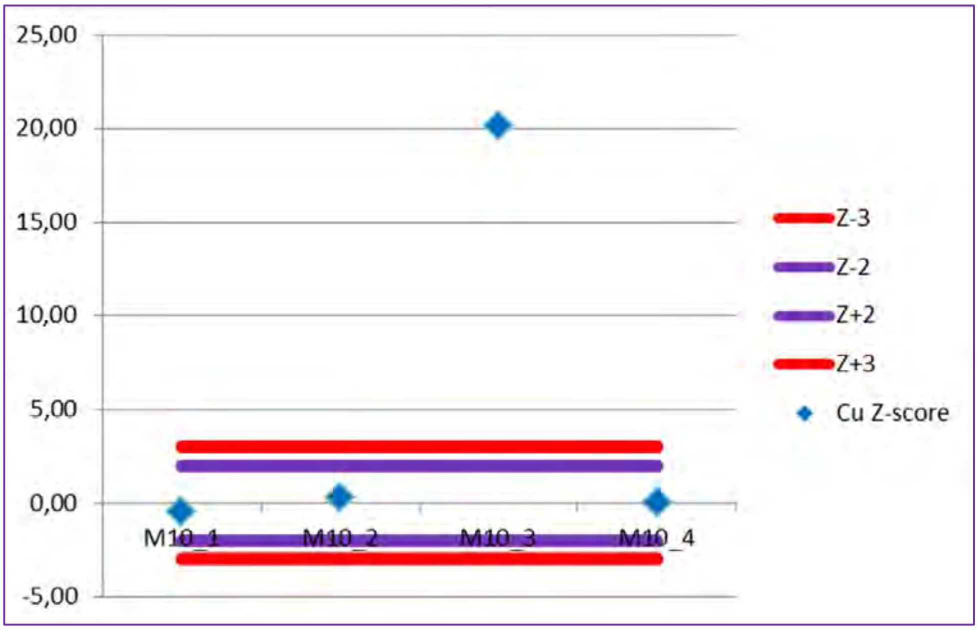


Figure 46. Z-scores of participating laboratories for reported Cr concentrations in sediment samples M10 and M18.



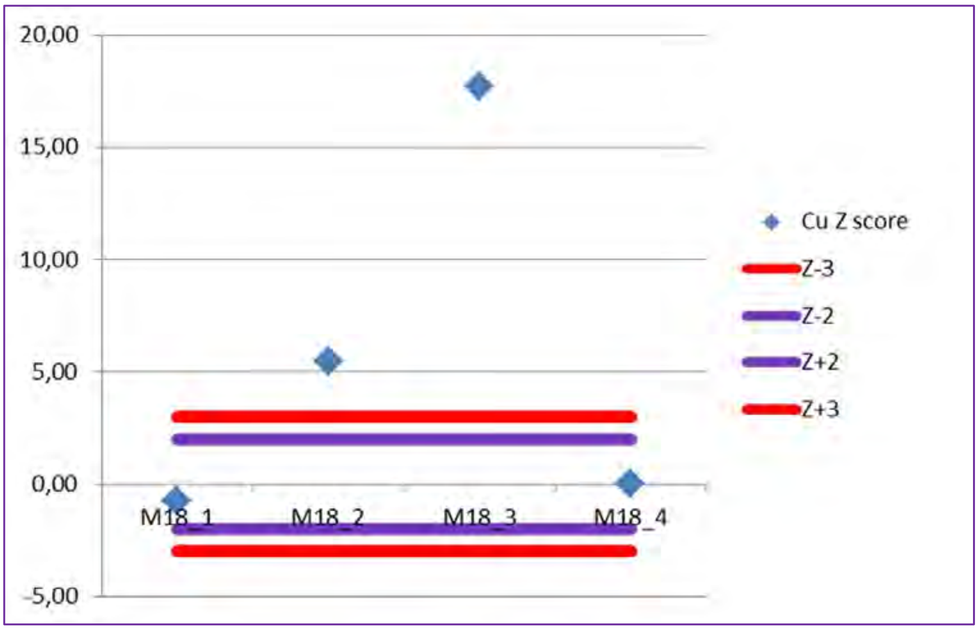
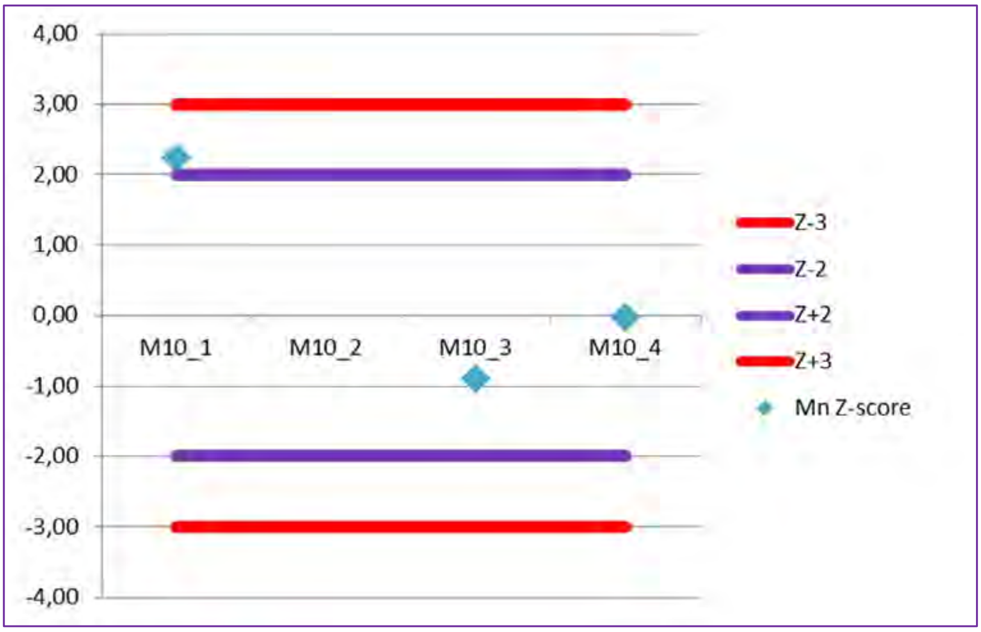


Figure 47. Z-scores of participating laboratories for reported Cu concentrations in sediment samples M10 and M18.



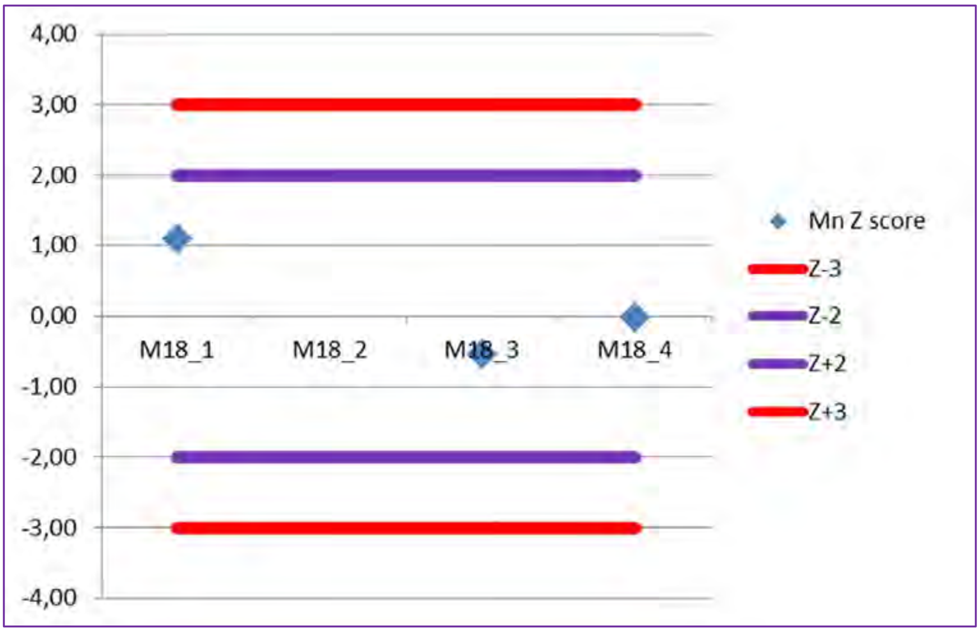
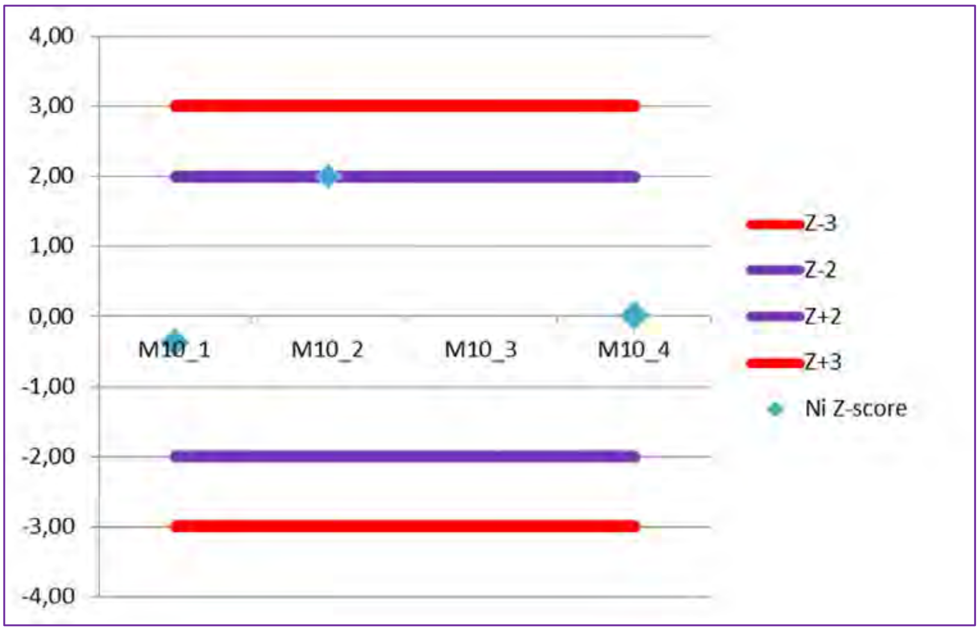


Figure 48. Z-scores of participating laboratories for reported Mn concentrations in sediment samples M10 and M18.



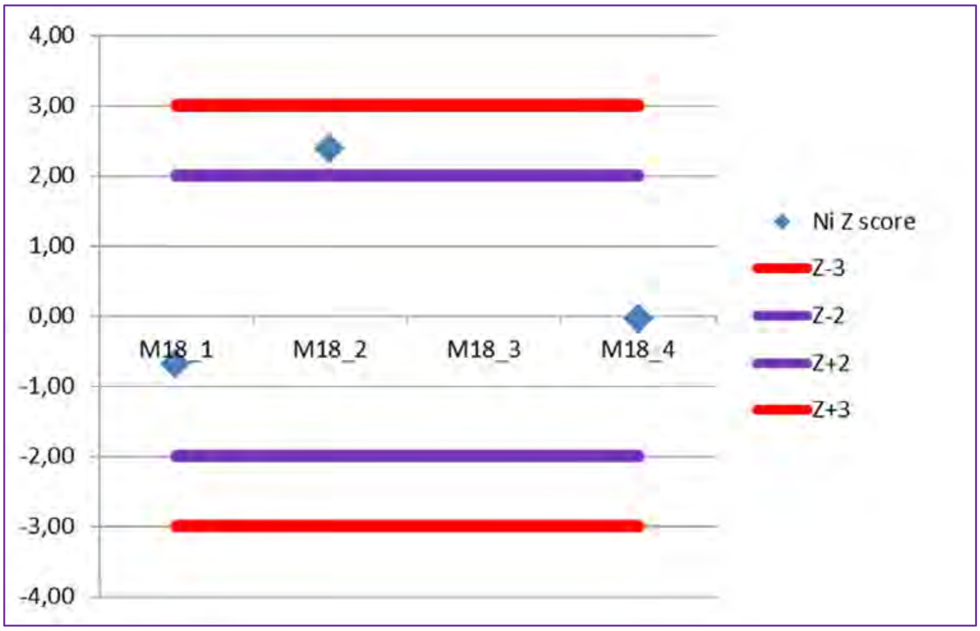
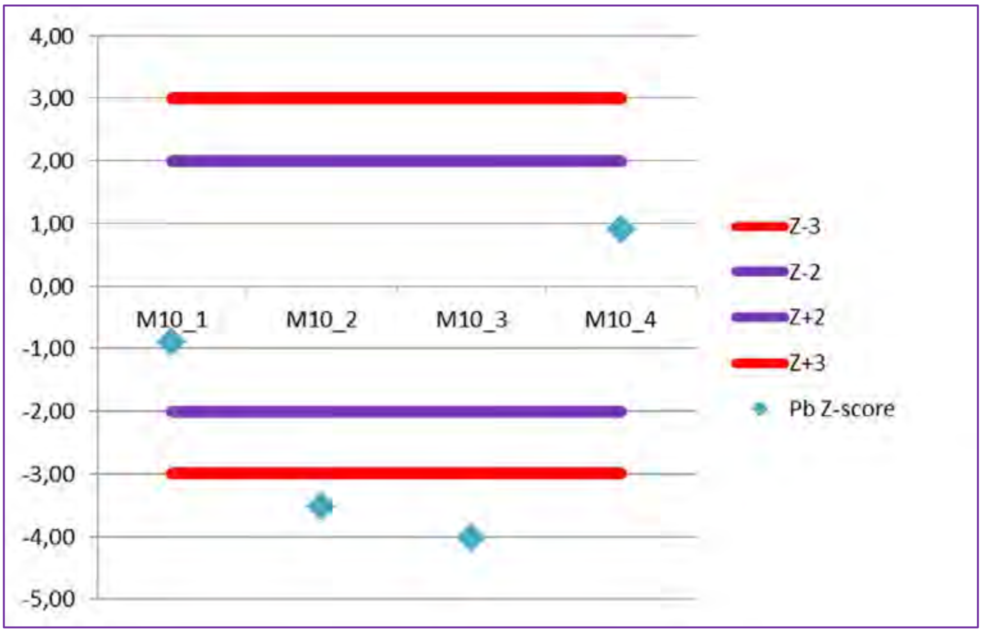


Figure 49. Z-scores of participating laboratories for reported Ni concentrations in sediment samples M10 and M18.



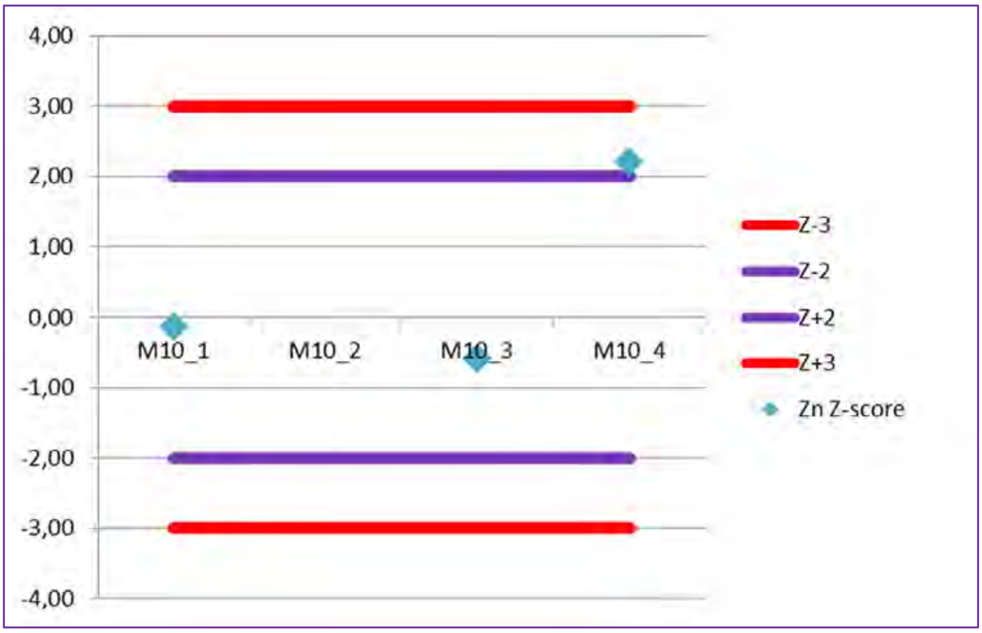
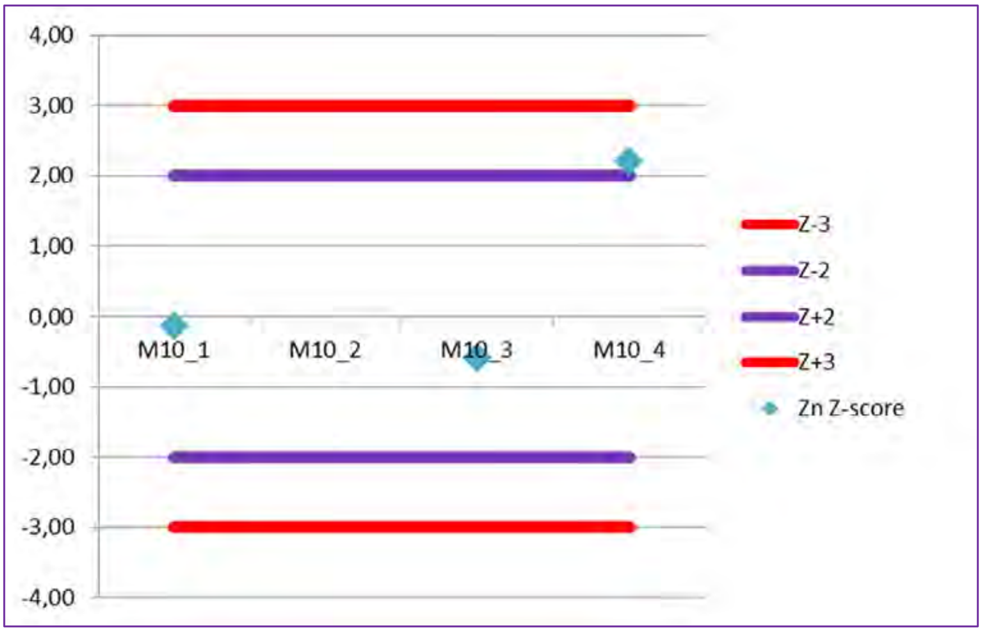


Figure 50. Z-scores of participating laboratories for reported Pb concentrations in sediment samples M10 and M18.



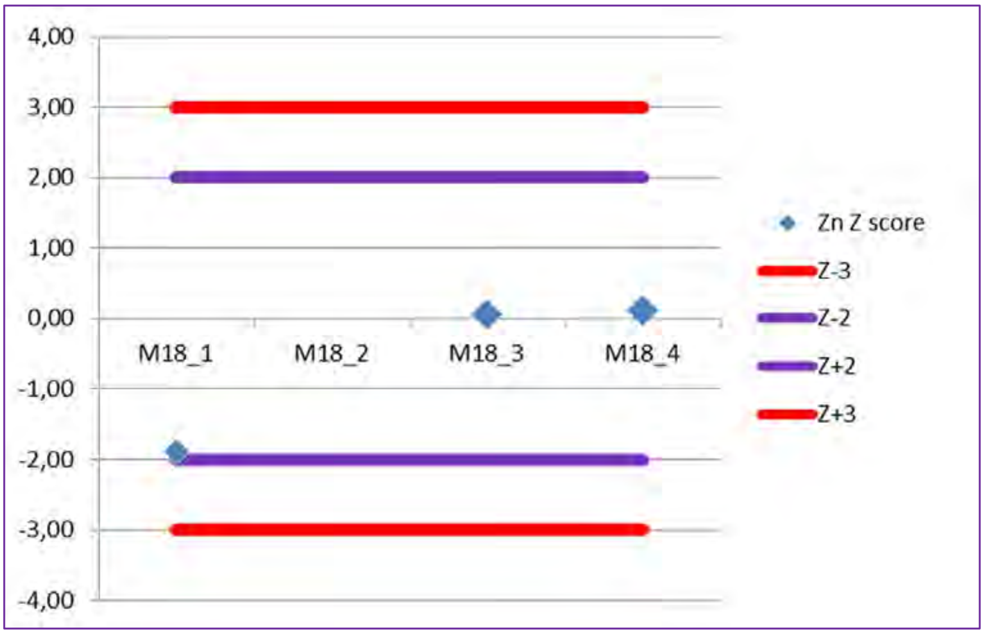


Figure 51. Z-scores of participating laboratories for reported Zn concentrations in sediment samples M10 and M18.

II.6. Conclusions – Heavy Metals

Satisfactory agreements ($|z| \le 2$) were obtained for the most measurements of the participating laboratories (80 - 81%), in both samples, questionable results ($2 \le |z| \le 3$) ranged from 4% (in M18) to 8% (in M10), whereas unsatisfactory agreements ($|z| \ge 3$) ranged from 11 % (M10) to 16 % (M18) (Fig. 29).

Cd and Co results were comparable ($|z| \le 2$) in all reported measurements, followed by Mn, Ni and Zn with 83% satisfactory results, Cr and Pb (75%) and Cu (63%).

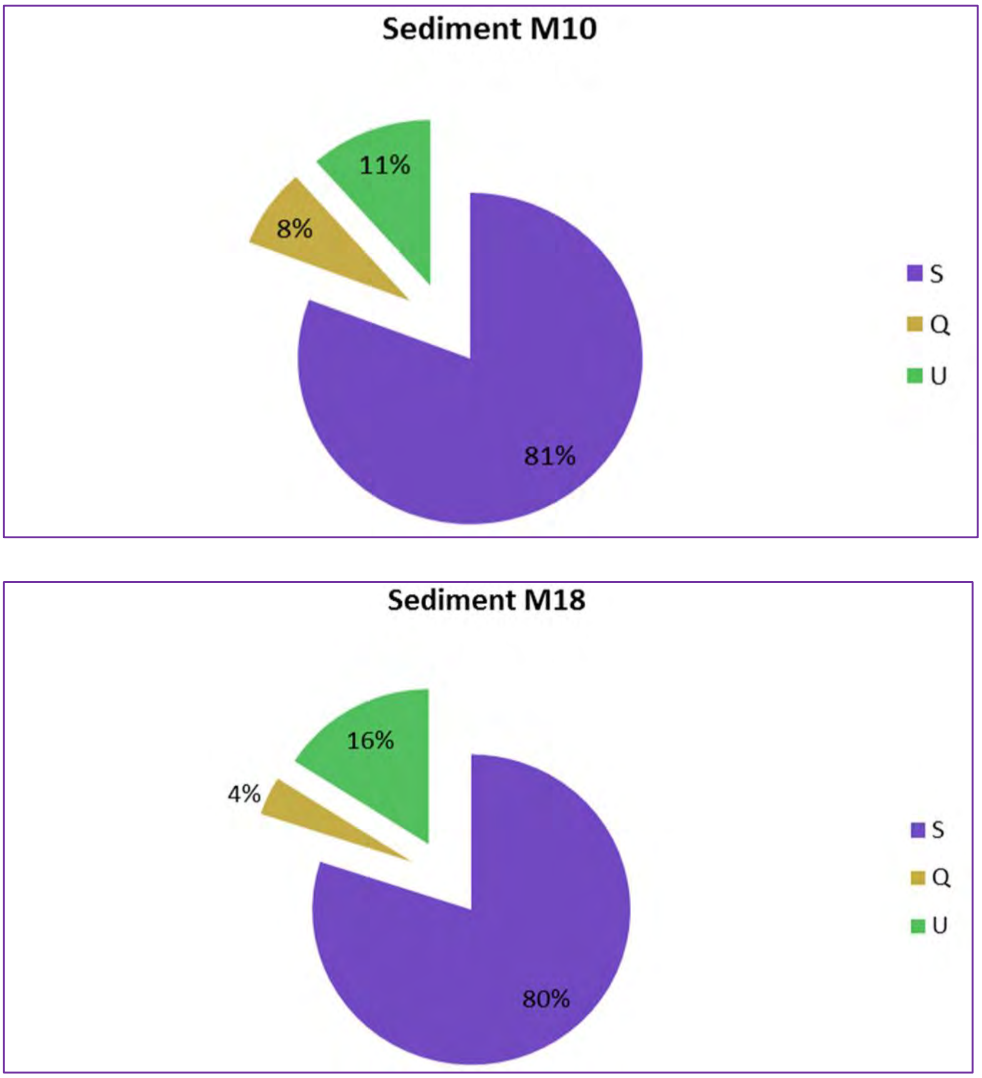


Figure 52. Classification of the performance based on z- scores for the all reported results (S – satisfactory; Q – questionable; U – unsatisfactory).

Differences between results could be attributed not only to the lack of homogeneity of the composite sediments samples, but also to the differences between digestion methods and instrumental techniques used by laboratories.

Annex II

II.1 – Intercomparison Exercise Data Reporting Forms – Heavy Metals

Laboratory 1 - GEOECOMAR, sample M10.

Elements	Unit	Average sample weight (mg)	Concentration Individual determination (reported on dry weight basis)			Mean value	Standard deviation	Combined uncertainty	Detection limit (µg/L)
			Value 1	Value 2	Value 3				
Al	**mg/kg**								
As	**mg/kg**								
Cd	**mg/kg**	1052	0.353	0.350	0.361	0.355	0.0057	0.015	0.0005
Co	**mg/kg**	1052	10.9	11.0	10.8	10.9	0.10	0.257	0.01
Cr	**mg/kg**	3000	78	94	85	85.7	8.0	20.6	10
Cu	**mg/kg**	1052	42.1	40.0	43.0	41.70	1.54	4.0	0.01
Fe	**mg/kg**	3000	41248	40522	41103	40957.7	384.20	987	500
Hg	**mg/kg**								
Li	**mg/kg**								
Mg	**mg/kg**								
Mn	**mg/kg**	3000	603.2	643.3	667.7	638.1	32.6	83.8	0.1
Ni	**mg/kg**	1052	49.0	48.4	47.2	48.20	0.92	2.4	0.015
Pb	**mg/kg**	1052	32.6	32.1	32.0	32.23	0.32	0.8	0.5
Sr	**mg/kg**	3000	314	320	317	317.0	3.0	7.7	10
Zn	**mg/kg**	1052	75.7	73.8	77.8	75.77	2.00	5.1	0.1
Rb	**mg/kg**	3000	131	130	132	131.0	1.0	2.6	2
Ba	**mg/kg**	3000	442	478	424	448.0	27.5	70.7	50
Zr	**mg/kg**	3000	132	134	128	131.3	3.06	7.9	50
Ti	**mg/kg**	3000	4045	3174	3486	3568.3	441.30	1134	300
V	**mg/kg**	3000	53	55	51	53.0	2.0	5.1	10

Laboratory 1 - GEOECOMAR, sample M18.

Elements	Unit	Average sample weight (mg)	Concentration Individual determination (reported on dry weight basis)			Mean value	Standard deviation	Combined uncertainty	Detection limit (µg/L)
			Value 1	Value 2	Value 3				
Al	**mg/kg**								
As	**mg/kg**								
Cd	**mg/kg**		0.054	0.054	0.064	0.0573	0.0058		0.0005
Co	**mg/kg**		8.9	9.1	8.0	8.66	0.58		0.01
Cr	**mg/kg**	3000	74	67	60	67.0	7.0		
Cu	**mg/kg**		16.9	13.3	12.9	14.37	2.20		0.01
Fe	**mg/kg**	3000	33268	32563	32492	32774.	429.00		300
Hg	**mg/kg**								
Li	**mg/kg**								
Mg	**mg/kg**								
Mn	**mg/kg**	3000	585.4	561.9	616.6	587.96	27.44		0.1
Ni	**mg/kg**		17.4	18.2	17.0	17.53	0.61		0.015
Pb	**mg/kg**		9.4	10.1	8.4	9.30	0.85		0.5
Sr	**mg/kg**	3000	739	734	732	735.0	3.6		10
Zn	**mg/kg**		30.3	30.0	30.3	30.2	0.17		0.1
Rb	**mg/kg**	3000	55	53	50	52.7	2.5		2
Ba	**mg/kg**	3000	333	399	373	368.3	33.2		50
Zr	**mg/kg**	3000	181	173	180	178.0	4.4		50
Ti	**mg/kg**	3000	2658	2598	2900	2718.7	159.88		
V	**mg/kg**	3000	53	56	54	54.3	1.5		

Laboratory 2 - NIMRD, sample M10.

Elements	Unit	Average sample weight (mg)	Concentration Individual determination (reported on dry weight basis)			Mean value	Standard deviation	Combined uncertainty	Detection limit (µg/L)
			Value 1	Value 2	Value 3				
Al	**mg/kg**								
As	**mg/kg**								
Cd	**mg/kg**	100	0.31	0.27	0.28	0.29	0.02	0.07	0.08 µg/L
Co	**mg/kg**								
Cr	**mg/kg**	100	77.64	63.42	70.23	70.43	7.11	14.08	0.30 µg/L
Cu	**mg/kg**	100	41.11	48.93	55.72	48.59	7.31	9.71	0.70 µg/L
Fe	**mg/kg**								
Hg	**mg/kg**								
Li	**mg/kg**								
Mg	**mg/kg**								
Mn	**mg/kg**								
Ni	**mg/kg**	100	70.07	71.98	77.33	73.13	3.76	14.62	0.30 µg/L
Pb	**mg/kg**	100	9.88	13.39		11.63	2.48	2.90	0.50 µg/L
Zn	**mg/kg**								

Laboratory 2 - NIMRD, sample M18.

Elements	Unit	Average sample weight (mg)	Concentration Individual determination (reported on dry weight basis)			Mean value	Standard deviation	Combined uncertainty	Detection limit (µg/L)
			Value 1	Value 2	Value 3				
Al	**mg/kg**								
As	**mg/kg**								
Cd	**mg/kg**	100	0.113	0.132	0.112	0.119	0.011	0.03	0.08 µg/L
Co	**mg/kg**								
Cr	**mg/kg**	100	41.20	37.28	34.79	37.76	3.23	7.55	0.30 µg/L
Cu	**mg/kg**	100	38.57	32.63	34.61	35.27	3.02	7.05	0.70 µg/L
Fe	**mg/kg**								
Hg	**mg/kg**								
Li	**mg/kg**								
Mg	**mg/kg**								
Mn	**mg/kg**								
Ni	**mg/kg**	100	28.65	29.94	31.25	29.94	1.30	5.98	0.30 µg/L
Pb	**mg/kg**	100	9.88	8.31	5.07	7.75	2.46	2.93	0.50 µg/L
Zn	**mg/kg**								

Laboratory 3 – RWNA-WADL, sample M10.

Elements	Unit	Average sample weight (mg)	Concentration Individual determination (reported on dry weight basis)			Mean value	Standard deviation	Combined uncertainty	Detection limit (µg/L)
			Value 1	Value 2	Value 3				
Al	**mg/kg**								
As	**mg/kg**								
Cd	**mg/kg**								
Co	**mg/kg**								
Cr	**mg/kg**	500	87.8	87.8	87.8	87.8			0.287µg/l
Cu	**mg/kg**	500	230	230	230	230			0.386µg/l
Fe	**mg/kg**								
Hg	**mg/kg**								
Li	**mg/kg**								
Mg	**mg/kg**								
Mn	**mg/kg**	500	362	362	362	362			6.1µg/l
Ni	**mg/kg**								
Pb	**mg/kg**	500	7.57	7.57	7.57	7.57			1.025µg/l
Zn	**mg/kg**	500	68.6	68.6	68.6	68.6			2.7µg/l

Laboratory 3 – RWNA-WADL, sample M18.

Elements	Unit	Average sample weight (mg)	Concentration Individual determination (reported on dry weight basis)			Mean value	Standard deviation	Combined uncertainty	Detection limit (µg/L)
			Value 1	Value 2	Value 3				
Al	**mg/kg**								
As	**mg/kg**								
Cd	**mg/kg**								
Co	**mg/kg**								
Cr	**mg/kg**	500	89.3	89.3	89.3	89.3			0.286µg/l
Cu	**mg/kg**	500	76.6	76.6	76.6	76.6			0.386 µg/l
Fe	**mg/kg**								
Hg	**mg/kg**								
Li	**mg/kg**								
Mg	**mg/kg**								
Mn	**mg/kg**	500	431	431	431	431			6.1 µg/l
Ni	**mg/kg**								
Pb	**mg/kg**	500	8.96	8.96	8.96	8.96			1.025 µg/l
Zn	**mg/kg**	500	49	49	49	49			2.7 µg/l

Laboratory 4 – TUBITAK, sample M10.

Elements	Unit	Average sample weight (mg)	Concentration Individual determination (reported on dry weight basis)					Mean value	Standard deviation	Combined uncertainty	Detection limit (µg/L)
			Value 1	Value 2	Value 3	Value 4	Value 5				
Al	**mg/kg**	502.2	17453	17573	17822	17851	17692	17678	168	-	-
As	**mg/kg**	502.2	11.79	11.71	11.77	11.86	12.06	11.84	0.1	9.0 %	1.092
Cd	**mg/kg**	502.2	0.30	0.27	0.30	0.30	0.30	0.30	0.01	9.0 %	0.066
Co	**mg/kg**	502.2	10.74	10.77	10.67	10.88	10.90	10.79	0.1	9.0 %	0.004
Cr	**mg/kg**	502.2	79.39	79.68	79.63	80.55	79.23	79.70	0.5	9.0 %	0.067
Cu	**mg/kg**	502.2	46.26	46.10	46.01	46.10	45.69	46.03	0.2	6.6 %	0.236
Fe	**mg/kg**	502.2	17433	17513	17672	17692	17523	17567	111	-	-
Hg	**mg/kg**	101.53	0.2068	0.1923	0.1951	-	-	0.198	0.008	17.5 %	0.00023
Li	**mg/kg**	-	-	-	-	-	-	-	-	-	-
Mg	**mg/kg**	-	-	-	-	-	-	-	-	-	-
Mn	**mg/kg**	502.2	438	435	439	441	435	438	2.6	-	-
Ni	**mg/kg**	502.2	52.22	51.53	51.68	53.10	52.43	52.19	0.6	14.0 %	0.037
Pb	**mg/kg**	502.2	45.93	46.42	46.28	46.63	46.32	46.31	0.3	9.0 %	0.109
Zn	**Mg/kg**	502.2	111	112	112	112	113	112	0.7	6.5 %	0.974

Laboratory 4 – TUBITAK, sample M18.

Elements	Unit	Average sample weight (mg)	Concentration Individual determination (reported on dry weight basis)					Mean value	Standard deviation	Combined uncertainty	Detection limit (µg/L)
			Value 1	Value 2	Value 3	Value 4	Value 5				
Al	**mg/kg**	501.5	5203	5267	5210	5332	5365	5276	71.9	-	-
As	**mg/kg**	501.5	9.72	10.26	10.25	10.19	9.17	9.92	0.5	9.0 %	1.092
Cd	**mg/kg**	501.5	<0.066	<0.066	<0.066	<0.066	<0.066	<0.066	-	9.0 %	0.066
Co	**mg/kg**	501.5	10.17	10.20	10.19	10.23	10.41	10.24	0.1	9.0 %	0.004
Cr	**mg/kg**	501.5	39.90	39.76	40.21	40.44	39.36	39.93	0.4	9.0 %	0.067
Cu	**mg/kg**	501.5	16.98	16.92	16.64	16.81	16.76	16.82	0.1	6.6 %	0.236
Fe	**mg/kg**	501.5	10229	10259	10199	10289	10399	10275	76.8	-	-
Hg	**mg/kg**	101.53	0.0091	0.0173	0.0116	0.0124	-	0.0126	0.003	17.5 %	0.00023
Li	**mg/kg**	-	-	-	-	-	-	-	-	-	-
Mg	**mg/kg**	-	-	-	-	-	-	-	-	-	-
Mn	**mg/kg**	501.5	475	477	475	481	483	478	3.6	-	-
Ni	**mg/kg**	501.5	20.29	20.01	19.77	19.67	20.36	20.02	0.3	14.0 %	0.037
Pb	**mg/kg**	501.5	12.84	12.95	12.76	12.97	12.81	12.87	0.1	9.0 %	0.109
Zn	**Mg/kg**	501.5	49.90	49.81	48.49	50.05	50.24	49.70	0.7	6.5 %	0.974

Chapter III.
Persistent Organic Pollutants in sediments

III.1. Sampling

There were two intercalibration samples: one was collected from the shelf area in the Bulgarian waters (M 10 – bottom depth of 75 m), the other one from the coastal area in the Turkish waters (M18 – bottom depth of 27 m).

Station	Transect	Lat, °N	Long, °E	Bottom depth, m	Type	Date
M 10	Galata	43°10.000	28°.30.000	76.1	shelf	26.07.13
M 18	Igneada	41°49.795	28°.00.275	27.2	coastal	30.07.13

Samples were collected using a Van Veen grab sampler. The undisturbed surface layer was carefully collected with a spatula, mixed and homogenized on board, and immediately divided in subsamples for the institutions involved in the intercalibration exercise (one for each laboratory). The samples were packed in aluminum foil, placed in plastic bags, sealed, labeled, frozen (at – 20 ÷ - 24 °C), and transported to the participant laboratories:

- NIMRD (1) and RWNA (2) for organochlorine pesticides and polychlorinated biphenyls and
- NIMRD (1), RWNA (2) and TUBITAK MRC (3) for polycyclic aromatic hydrocarbons.

Participants analyzed persistent organic pollutants in three replicate of each of the two sediments samples: M10 (open sea) and M18 (coastal).

III.2. Polycyclic Aromatic Hydrocarbons (PAHs)

The participants were requested to analyze polycyclic aromatic hydrocarbons (PAHS) using their usual technique. They were also requested to make at least one, but preferably three separate determinations for each compound, and to report the results together with a short description of the method used (e.g. extraction, clean-up, gas/liquid chromatographic conditions). A suitable reporting form was attached to the information sheet for this purpose. It was stated that concentrations were to be reported as net values (i.e. after correction for blank, moisture content, etc.), leaving as many significant figures as justified by the precision of the method used.

For each group of compounds the participants were requested to report:

1. The average weight of the sample taken for analysis.
2. Methods used to confirm identity of the compounds.
3. Calculation of the results.
4. Summary of the quality control procedures routinely employed within the laboratory.
5. The arithmetic mean value and the relative standard deviations of determinations.

Results of the analysis were reported by three laboratories, from two countries: Romania (Lab. 1 - NIMRD, Constanta, Lab. 2 - RWNA, Bucharest) and Turkey (Lab. 3 –TUBITAK MRC). The data sets reported by the laboratories and the statistical evaluation of results for each determinant is given in this report. Only one laboratory (no. 2) has reported 95% of the results as "not detected". The final results from this interlaboratory comparison have no statistical significance due to the small number of data for an evaluation of robust values of the average and standard deviation and z-scores of the data to which it is applied.

This guidance document is being issued for the purpose of providing a brief description of the statistical method used in determining the unacceptable results of interlaboratory comparison and the statistical methods used in determining the laboratory performance using *z*-scores in accordance with ISO 13528.

III.2.1. Evaluation of the results – PAHs

Data tables

Results are presented in Annex III.1 (Tables III.1 and III.2).

Analytical methods

The pretreatment of the samples for polycyclic aromatic hydrocarbons (PAHs) is described, for each laboratory, in the Table III.3 of Annex III.1 and the analytical instrument conditions are reported in the Table III.4.

A. <u>Assignment of consensus values</u>

The assigned values for organic contaminants in the sediment samples were established from the robust means of the participants' results, as suggested by ISO standard 13528:2005. The robust mean was calculated using Huber's method according to Algorithm A described in the cited standard Annex III.3 (normative) (ISO, 2005).

The estimates of robust standard deviation and robust average are derived by an iterative calculation by updating the values several times (iteration 0 - 6) using the modified data, until the process converges. Convergence may be assumed when there is no change from one iteration to the next in the third significant figure of the robust standard deviation and the equivalent robust average. (ex. Statistical analysis to calculate z-score and assigned the target value in compliance with ISO 13528:2005 for naphthalene ($ng \cdot g^{-1}$) in sediment code M10). The assigned values and the standard deviations for proficiency assessment of polycyclic aromatic hydrocarbons ($ng \cdot g^{-1}$ *dry mass*) in sediment code **M10** are presented in the Annex III.2, Table II.1.

B. Performance indicator and target standard deviation

The performance of an individual laboratory *i* was expressed by the z_i-score, which was calculated according to equation:

$$\boldsymbol{z_i = (x_i - x^*) / \sigma_P}$$

Where:

- z_i is the *z*-score of laboratory *i* for the respective sample;
- x_i is the reported result of laboratory *i* for that sample, expressed as the mean of multiple determinations;
- x^* is the assigned value for the respective analyte;
- σ_P is the target standard deviation for proficiency assessment.

The laboratory performance was evaluated using *z*-scores in accordance with ISO 13528 (Annex III.2, Table II.2.) The acceptability of a laboratory's performance was evaluated according to the following generally accepted limits (IAEA, 2013):

- $|z| \leq 2.0$ satisfactory
- $2.0 < |z| < 3.0$ questionable
- $|z| \geq 3.0$ unsatisfactory

Method: Robust analysis, Algorithm A, by Annex III.3 (ISO 13528:2005)

Example - Statistical analysis to calculate z-score and assigned the target value in compliance with ISO 13528:2005 for naphthalene (ng·g^{-1}) in sediment code M10

1. Denote the p items of data, sorted into increasing order, by: x_1, x_2, ..., x_i, ..., x_p

Briefly, individual results (Table 1), set containing 5 values were put in an increasing order: (**0.81; 10.10; 11.00; 11.60; 13.40**)

Table 1. The results for naphthalene in one round intercalibration between three laboratories.

No. crt.	Participant	Lab. code	Concentration (ng·g^{-1} dry weight)		Mean Value (ng·g^{-1} dry weight)	Standard Deviation (ng·g^{-1} dry weight)
1.	NIMRD - Romania	1	10.10	11.00	10.55	0.63
2.	RWNA - Bucharest Romania	2	0.81	-	0.81	-
3.	TUBITAK- MRC-Turkey	3	11.60	13.40	12.5	1.3

2. Denote the robust average and robust standard deviation of these data by x and s*.*

The initial values for *x**, the median of the data and *s**, the normalized median of absolute deviations (Table) was calculated as:

$$x^* = \text{median of } x_i \ (i = 1, 2, ..., p)$$

$$s^* = 1,483 * \text{median of } |x_i - x^*| \ (i = 1, 2, ..., p)$$

Table 2. The initial values of robust average and robust standard deviation obtained for iterative calculation-iteration 0.

	x_i	x^*	$\lvert x_i - x^* \rvert$
Lab.2	0.81	*11.00*	10.19
Lab.1	10.10	*11.00*	0.90
Lab.1	11.00	*11.00*	0.00
Lab.3	11.60	*11.00*	0.60
Lab.3	13.40	*11.00*	2.40
Mean	9.38		
Std.dev.	4.94		
Initial x*=the median of x_i	***11.00***		
Median of $\lvert x_i - x^* \rvert$			0.90
Initial s*=1.483*median of $\lvert x_i - x^* \rvert$	***1.33***		

3. Update the values of x^* and s^* as follows- iterative calculation (iteration 1-2)

According to the Huber's method the normalized median of absolute deviations should be multiplied by factor between 1 and 2 with most frequently used value of 1.5. The asset limit (IAEA, 2013) was estimated as $\delta = 1.5s^*$.

Calculate $\mathbf{x^*} - \delta$ and $\mathbf{x^*} + \delta$

$\delta = 1.5 * 1.33 = \mathbf{2.00}$

$\mathbf{x^*} - \delta = 11.00 - 2.00 = \mathbf{9.00}$

$\mathbf{x^*} + \delta = 11.00 + 2.00 = \mathbf{13.00}$

The initial values x_i, were transformed as follows (iteration 1):

$x_i^* = x^* - \delta$ if $x_i < x^* - \delta$

$x_i^* = x^* + \delta$ if $x_i > x^* + \delta$

$x_i^* = x_i$, otherwise

New value for the robust mean x^* was calculated as: $x^* = \Sigma\, x_i^*/p$

New x^* = $\Sigma\, x_i^*/p$ = 10.94

New value for robust standard deviation s^* was calculated as:

New s^* $= 1.134$ * Sqrt of $\Sigma(x_i^* - x^*)^2 / (p - 1)$ **= 1.72**

Convergence may be assumed when there is no change from one iteration to the next of the robust standard deviation and of the robust average. The first and second iterations show the same values of 10.94 and 1.72 for the robust average standard deviation (Table 3).

Table 3. The estimates of robust standard deviation and robust average derived from the iterative calculation by updating the values two times (iteration 1 –2).

	Iteration				
	0	**1**	**$(x_i^* - x^*)^2$**	**2**	**$(x_i^* - x^*)^2$**
$\delta = 1.5s^*$		2.00		2.57	
$x^* - \delta$		9.00		8.37	
$x^* + \delta$		13.00		13.51	
Lab.2	0.81	**9.00**	3.764	9.00	3.764
Lab.1	10.10	10.10	0.706	10.10	0.706
Lab.1	11.00	11.00	0.004	11.00	0.004
Lab.3	11.60	11.60	0.436	11.60	0.436
Lab.3	13.40	**13.00**	4.244	13.00	4.244
$\Sigma(x_i^* - x^*)^2$			9.152		9.152
$\Sigma(x_i^* - x^*)^2 / (p - 1)$			2.288		2.288
Sqrt of $\Sigma(x_i^* - x^*)^2 / (p - 1)$			1.513		1.513
Initial x^*	***11.00***				
Initial s^*	***1.33***				
New x^*= $\Sigma\, x_i^*/p$		***10.94***		***10.94***	
New s^*= 1.134 *Sqrt of $\Sigma(x_i^* - x^*)^2 / (p - 1)$		***1.72***		***1.72***	

4. Evaluation laboratory performance using z-score

The laboratory performance was evaluated using *z*-scores in accordance with ISO 13528:2005 (Table 4).
The performance of an individual laboratory *i* was expressed by the z_i-score, which was calculated according to equation:

$$z_i = (x_i - x^*) / \sigma_P$$

Table 4. ***Z-scores*: assessment of laboratory performance on the determination of Naphthalene.**

Lab. code	Mean of lab.	Robust mean	Robust sd	z score	
Lab.1	10.55	10.94	1.72	-0.23	Satisfactory
Lab.2	0.81	10.94	1.72	-5.89	Unsatisfactory
Lab.3	12.50	10.94	1.72	0.91	Satisfactory

III.3 Organochlorine Pesticides and Polychlorinated Biphenyls

Inventory of the analytical methods for organochlorine pesticides and polychlorinated biphenyls analysis reported by the participating laboratories is presented in the Table 5.

Table 5. Inventory of analytical methods for organochlorine pesticides and polychlorinated biphenyls analysis.

	NIMRD	ANAR/ABADL
Description of methods used:		
Extraction procedure	Soxhlet	-
Solvent used	Hexane	-
Clean-up procedure	Copper	-
Fractionation procedure	Florisil column	-
Gas Chromatographic Conditions:		
Apparatus (type)	CLARUS 500 - PERKIN ELMER	6890N - AGILENT
Injector (type)	Split	-
Carrier gas	Helium	-
Column (type):	ELITE 35 MS	-
Detector type:	ECD	MS
Recorder/Integrator (type):	Soft (TOTALCHROM)	-
Quality control procedure	CRM	-

The instrumental techniques were different: GS-ECD and GS-MS. The sensitivity of the detectors in respect with organochlorinated compounds is different, ECD being much sensitive for this kind of compounds. The compounds reported by participating laboratories are listed in Table 6.

Table 6. List of the compounds reported by participating laboratories.

OCPs/PCBs reported	Laboratory 1	Laboratory 2
HCB (Hexaclorbenzen)	✓	✓
Lindan	✓	✓
Heptaclor	✓	
Aldrin	✓	
Dieldrin	✓	
Endrin	✓	
p,p'DDE	✓	
p,p'DDD	✓	
p,p'DDT	✓	
PCB28	✓	
PCB52	✓	
PCB101	✓	
PCB118	✓	
PCB153	✓	
PCB138	✓	
PCB180	✓	
1.2.4 triclor benzen (QE 32)		✓
1.2.3 triclor benzen (QE 32)		✓
Triclor benzeni (suma) (QE 32)		✓
Pentaclorbenzen (QE 32)		✓
alfa-HCH (QE 33)		✓
beta-HCH (QE 33)		✓
Suma izomeri HCH		✓

III.3.1. Statistics

The compounds reported by both participating laboratories were HCB and Lindane.

Results reported by Laboratory 1 for the two compounds in M 18 sample were: “<value”.

All results reported by Laboratory 2 for the two compounds were: “<LD” (Table 7).

Table 7. Results reported for organochlorine pesticides and polychlorinated biphenyls in the intercalibration sediment samples - mean value (ng·g^{-1}).

Compound	Laboratory 1		Laboratory 2	
	M 10	M 18	M 10	M 18
HCB (Hexaclorbenzen)	4.349	<0.300	<LD	<LD
Lindan	4.661	<0.300	<LD	<LD
Heptaclor	2.577	0.506		
Aldrin	0.287	0.526		
Dieldrin	2.401	0.903		
Endrin	0.397	1.544		
p,p'DDE	7.401	0.788		
p,p'DDD	2.533	3.170		
p,p'DDT	15.679	1.854		
PCB28	0.402	0.400		
PCB52	1.617	1.772		
PCB101	1.087	0.600		
PCB118	0.598	0.400		
PCB153	0.796	0.600		
PCB138	4.790	0.700		
PCB180	2.495	0.452		
1.2.4 triclor benzen (QE 32)			<LD	<LD
1.2.3 triclor benzen (QE 32)			<LD	<LD
Triclor benzeni (suma) (QE 32)			<LD	<LD
Pentaclorbenzen (QE 32)			<LD	<LD
alfa-HCH (QE 33)			<LD	<LD
beta-HCH (QE 33)			<LD	<LD
Suma izomeri HCH			<LD	<LD

Data below detection limits (reported as “<” value) are not included in the statistical analysis (HELCOM, 2013), because if many participants are working close to their detection limits, regardless of whether they provide a default result, it becomes difficult to estimate a valid consensus for the assigned value (IUPAC, 2006).

Considering the above information, our report is only informative.

ANNEX III

III.1 Data Report (Tables III.1.1 – III.1.4)

Table III.1.1. Results for PAHs in the M10 sample sediment - mean value (ng·g^{-1}).

Lab. No.	**Naphthalene**	**Acenaphthylene**	**Acenaphthene**	**Fluorene**	**Phenanthrene**	**Anthracene**	**Fluoranthene**	**Pyrene**	**Benzo[a]anthracene**	**Crysene**
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
	(ng·g^{-1})	(ng·g^{-1})	(ng·g^{-1})	(ng·g^{-1})	(ng·g^{-1})	(ng·g^{-1})	(ng·g^{-1})	(ng·g^{-1})	(ng·g^{-1})	(ng·g^{-1})
1	10.6	5.7	3.0	8.4	74.1	7.4	124.9	110.8	30.2	86.4
2	0.8	N.D.	N.D.	N.D.	1.3	N.D.	2.2	N.D.	N.D.	N.D.
3	12.5	N.D	N.D	9.8	38.2	4.7	30.6	28.9	8.3	32.7

Table III.1.1. Results for PAHs in the M10 sample sediment (cont.) - mean value (ng·g^{-1}).

Lab. No.	**Benzo[b]fluoranthene**	**Benzo[k]fluoranthene**	**Benzo[a]pyrene**	**Benzo (g,h,i)perylene**	**Dibenzo(a,h)anthracene**	**Indeno(1,2,3-c,d)pyrene**	**$\sum_{16}$PAH**
	11	**12**	**13**	**14**	**15**	**16**	
	(ng·g^{-1})	(ng·g^{-1})	(ng·g^{-1})	(ng·g^{-1})	(ng·g^{-1})	(ng·g^{-1})	**(ng·g^{-1})**
1	139.9	21.0	40.6	72.2	4.1	0.4	**738.7**
2	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	**4.3**
3	17.2	12.6	50.7	97.0	20.8	9.4	***373.2***

Table III.1.2. Results for PAHs in the M18 sample sediment - mean value (ng·g^{-1}).

Lab. No.	Naphthalene	Acenaphthylene	Acenaphthene	Fluorene	Phenanthrene	Anthracene	Fluoranthene	Pyrene	Benzo[a]anthracene	Crysene
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
	(ng·g^{-1})	(ng·g^{-1})	(ng·g^{-1})	(ng·g^{-1})	(ng·g^{-1})	(ng·g^{-1})	(ng·g^{-1})	(ng·g^{-1})	(ng·g^{-1})	(ng·g^{-1})
1	6.1	2.6	3.1	3.8	3,8	3.5	1.0	1.6	0.4	0.4
2	0.4	N.D.	N.D.	N.D.	N.D.	N.D.	1.2	N.D.	N.D.	N.D.
3	4.1	N.D	N.D	10,0	39.3	0.5	9.7	3.3	2.9	7.5

Table III.1.2. Results for PAHs in the M18 sample sediment (cont.) - mean value (ng·g^{-1}).

Lab. No.	Benzo[b]fluoranthene	Benzo[k]fluoranthene	Benzo[a]pyrene	Benzo (g,h,i)perylene	Dibenzo(a,h)anthracene	Indeno(1,2,3-c,d)pyrene	Σ_{16}PAH
	11	**12**	**13**	**14**	**15**	**16**	
	(ng·g^{-1})	(ng·g^{-1})	(ng·g^{-1})	(ng·g^{-1})	(ng·g^{-1})	(ng·g^{-1})	**(ng·g^{-1})**
1	1.3	0.9	0.6	0.6	0.6	0.4	**17.0**
2	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	**1.6**
3	5.0	2.9	11.0	2.5	1.6	5.4	**99.6**

Table III.1.3. PAHs – Treatment of samples.

Lab. No.	Extraction procedure used			Internal standard	Clean-up procedure	Fractionation procedure	Method for confirmation
	Instrument	Solvent	Time				
1	Soxhlet	Hexane / MeCl2	8 hrs.	9,10 Dihydroanthracene	Florisil	3 fractions	confirmation ions (m/z) retention times
2	-	-	-	-	-	-	-
3	Soxhlet	Hexane / MeCl2	8 hrs.		Silica	2 fractions	HPLC, Std. retention times

Table III.1.4. PAHs – Instrument conditions.

Lab. No.	Instrument type	Detector type	Injection type	Injector temp. (°C)	Injection volume	Splitter closing time
1	GC/MS, Clarus 500 - PERKIN ELMER	MS	Splitless	300	2 µl	1 min.
2	-	-	-	-	-	-
3	Agilent 1200 series HPLC	Variable Wavelength Detector-254nm	-	-	10 µl	-

Table III.1.4. PAHs – Instrument conditions (cont.1).

Lab. No.	Carrier gas / Mobile Phase		Column				
	Type	Flow rate	Type	Length	I. diameter	Phase	Film thickness
1	Helium	1 ml/min	ELITE 35 MS	30 m	0.32 mm	Dimethylpolysiloxane (35% Diphenil)	0.25 µm
2	-	-	-	-	-	-	-
3	Acetonitrile-Distile Water	1.5ml/min.	ZORBAX Eclipse	250 mm	4.6 mm	PAH C18	5 µm particles

Table III.1.4. PAHs – Instrument conditions (cont.2).

Lab. No.	Temperature programme (°C)							
	Init. temp (°C)	Isoth. (min.)	1st rate	to: (°C)	Isoth. (min.)	2nd rate	to: (°C)	Isoth. (min.)
1	100	5	6	250	0	10	330	10
2	-	-	-	-	-	-	-	-
3	**Gradient Programme:**							
	Time (min.)	**% ACN**	**%DW**					
	0	40	60					
	30	100	0					
	32	100	0					
	35	40	60					

III.2 Consensus value expresses on a dry mass basis and target standard deviation, z-scores (Tables III.2.1. – III.2.2)

Table III.2.1. Assigned values for the interlaboratory comparison - sediment code - M10.

Polycyclic aromatic hydrocarbons -PAHs (ng·g^{-1} dry mass)

Analyte	Unit	Concentration*	Target S.D.**	N***	SD%
Naphthalene	ng·g^{-1}	10.94	1.72	3	15.73
Fluorene	ng·g^{-1}	8.69	3.67	3	42.24
Phenanthrene	ng·g^{-1}	56.15	23.55	2	41.95
Anthracene	ng·g^{-1}	6.24	1.63	2	26.13
Fluoranthene	ng·g^{-1}	77.73	63.08	2	81.16
Pyrene	ng·g^{-1}	69.80	53.87	2	74.18
Benzo[a]anthracene	ng·g^{-1}	19.23	15.47	2	80.45
Crysene	ng·g^{-1}	59.50	36.35	2	61.10
Benzo[b]fluoranthene	ng·g^{-1}	78.55	81.70	2	104.01
Benzo[k]fluoranthene	ng·g^{-1}	16.78	9.71	2	57.87
Benzo[a]pyrene	ng·g^{-1}	45.60	7.33	2	16.08
Benzo (g,h,i)perylene	ng·g^{-1}	80.10	12.07	2	15.07
Dibenzo(a,h)anthracene	ng·g^{-1}	12.45	11.41	2	91.65
Indeno(1,2,3-c,d)pyrene	ng·g^{-1}	4.90	5.90	2	120.41
$\sum_{16}$PAHs	**ng·g^{-1}**	**555.93**	**246.34**	**2**	**44.32**

*Consensus value expressed on a dry mass basis;
** Target standard deviation
***Number of laboratory means which were used for calculation of the assigned values.

Table III.2.2. *Z*-scores: assessment of laboratory performance on the determination of PAHs in the sediment - M10.

Lab.No.	1	2	3
Naphthalene	-0.23	-5.89*	0.91
Acenaphthylene	-	-	-
Acenaphthene	-	-	-
Fluorene	-0.08	-	0.31
Phenanthrene	0.76	-	-0.76
Anthracene	0.71	-	0.94
Fluoranthene	0.75	-	-0.75
Pyrene	0.76	-	-0.76
Benzo [a] anthracene	0.74	-	-0.74
Crysene	0.75	-	-0.75
Benzo [b] fluoranthene	0.75	-	-0.75
Benzo [k] fluoranthene	0.43	-	-0.43
Benzo [a] pyrene	-0.68	-	0.70
Benzo (g,h,i) perylene	-0.65	-	1.40
Dibenzo (a,h) anthracene	-0.76	-	0.76
Indeno (1,2,3-c,d) pyrene			
PAHs total	0.33	-	0.51

* Shaded areas represent unacceptable results

III.3 (normative, ISO 13528:2005) - Robust analysis

Robust analysis: Algorithm A

This algorithm yields robust values of the average and standard deviation of the data to which it is applied.

NOTE 1
Algorithms A and S given in this annex are reproduced from ISO 5725-5.

NOTE 2
Robustness is a property of the estimation algorithm, not of the estimates it produces, so it is not strictly correct to call the averages and standard deviations calculated by such an algorithm robust. However, to avoid the use of excessively cumbersome terminology, the terms “robust average” and “robust standard deviation” should be understood in this International Standard to mean estimates of the population mean or of the population standard deviation calculated using a robust algorithm.

Denote the p items of data, sorted into increasing order, by:

$$x_1, x_2, \ldots, x_i, \ldots, x_p$$

Denote the robust average and robust standard deviation of these data by x^* and s^*.

Calculate initial values for x^* and s^* as:

$$x^* = \text{median of } x_i \ (i = 1, 2, \ldots, p) \tag{C.1}$$

$$s^* = 1{,}483 * \text{median of } |\, x_i\, x^* | \ (i\ 1, 2, \ldots, p) \tag{C.2}$$

Update the values of x^* and s^* as follows. Calculate:

$$\delta\ 1{,}5s^* \tag{C.3}$$

For each x_i (i =1, 2, ..., p), calculate:

$$x_i^* \quad = \quad \left\{ \begin{array}{ll} x^* - \delta & \text{if } x_i^* < x^* - \delta \\ x^* + \delta & \text{if } x_i^* > x^* + \delta \\ x_i, & \text{otherwise} \end{array} \right\} \tag{C.4}$$

Calculate the new values of x^* and s^* from:

$$x^* = \Sigma\, x_i^* / p \tag{C.5}$$

$$s^* = 1{,}134 * \text{Sqrt of } \Sigma(x_i^* - x^*)^2 / (p - 1) \tag{C.6}$$

where the summation is over i.

The robust estimates x^* and s^* may be derived by an iterative calculation, i.e. by updating the values of x^* and s^* several times using the modified data, until the process converges. Convergence may be assumed when there is no change from one iteration to the next in the third significant figure of the robust standard deviation and of the equivalent figure in the robust average. This is a simple method to program on a computer.

Chapter IV. Chlorophyll a

IV.1. Sampling

For the chlorophyll *a* inter-comparison exercise, two stations were chosen; the first one, in the deep waters, station M 13, was selected for low Chl *a* concentration, while the second one, in the coastal waters, station M 18, was selected for Chl *a* higher concentrations.

Station	**Transect**	**Lat, °N**	**Long, °E**	**Bottom depth, m**	**Type**	**Date**
M 13	Inter-comparison	41°49.795	028°.00.275	20.18	open sea	27.07.13
M 18	Igneada, inter-comparison	41°49.795	028°.00.275	27.2	coastal	30.07.13

Four laboratories attended the exercise: IO-BAS (Laboratory code - 1), NIMRD (Laboratory code - 2), GEOECOMAR (Laboratory code - 3) and TUBITAK (Laboratory code - 4). Each team sampled three water replicates (using separate Niskin bottles for each replicate) from Depth Chlorophyll Maximum Layer (DCM) at both inter-comparison stations (43 m and 16 m depth, respectively).

The water samples (volumes within 1 – 5 l) were collected by Seabird CTD-Rosette system in 5 l plastic bottles during the up-cast. Immediately after collection, the samples were filtered onboard using two types of filters: Whatman GF/F, 0.7 µm pore size (IO-BAS and TUBITAK), and nitrocellulose membrane Millipore, 0.8 µm pore size (GEOECOMAR and NIMRD). Then, the filters were frozen at -22 ÷ -24 °C until their subsequent analysis.

IV.2 Inventory of analytical methods for Chlorophyll *a* analysis reported by the participating laboratories

90% Acetone was used by all teams as extraction solvent, but the extraction techniques differed. The simplest technique was used by NIMRD; the filters were immersed in acetone, then were shacked strongly and left for 24 hours at temperature of 0 – 4°C for pigment extraction. IO-BAS and TUBITAK used grinding of Whatman GF/F filters in 90 % acetone, while GEOECOMAR used stirring of the filters immersed in 90 % acetone followed by sonication (Table 8).

The extracts were transferred into centrifuge tubes and were subject to centrifugation.

Trichromatic method was used by all teams for absorbance readings (at the three maximum wavelengths of the chlorophyll *a*, plus a blank wavelength) and Chl *a* computation was done according to equations used (SCOR-UNESCO (1966) for NIMRD and Jeffrey-Humphrey (1975), for the rest of participants).

Table 8. Inventory of analytical methods for Chlorophyll *a* analysis.

IO-BAS (1)	NIMRD (2)	GEOECOMAR (3)	TUBITAK (4)
GF/F filter 0.7 µm pore size	nitrocellulose membrane, 0.8 µm pore size	nitrocellulose membrane, 0.8 µm pore size	GF/F filter 0.7 µm pore size
90 % acetone, homogenization (grinding)	90 % acetone, soaking overnight	90 % acetone, homogenization (stirring) and sonicating	90 % acetone, homogenization (grinding)
Trichromatic method	Trichromatic method	Trichromatic method	Trichromatic method
Jeffrey-Humphrey	SCOR-UNESCO	Jeffrey-Humphrey	Jeffrey-Humphrey

IV.3 Statistics

The overall results of the chlorophyll-a intercalibration exercise are given in Table 9 as raw data, means, standard deviation and coefficients of variation for each laboratory and series of subsample.

Table 9. Raw data, means, standard deviations and coefficients of variation.

Station/ sampling depth		Lab. 1	Lab. 2	Lab. 3	Lab. 4
Station M13 **Sampling depth of 43 m**	C_1 (µg/L)	0.98	0.869	1.02	0.65
	C_2 (µg/L)	0.98	0.973	1.09	0.98
	C_3 (µg/L)	1.06	0.822	1.00	1.04
	Mean (µg/L)	1.007	0.888	1.04	0.89
	St.dev (µg/L)	0.046	0.077	0.046	0.21
	CV (%)	4.6	8.7	4.4	23.6
Station M18 **Sampling depth of 16 m**	C_1 (µg/L)	0.99	1.102	1.39	1.36
	C_2 (µg/L)	1.03	1.3	1.50	1.77
	C_3 (µg/L	1.08	1.21	1.47	1.20
	Mean (µg/L)	1.033	1.204	1.46	1.443
	St.dev (µg/L)	0.045	0.099	0.056	0.294
	CV (%)	4.4	8.2	3.9	20.4

The standard deviations and coefficients of variation are generally low (CVs less than 10%, except Laboratory 4 ~20%). This shows that the precision (ability to reproduce the measurements) within the laboratories generally is generally high, but tells nothing about the accuracy (ability to get close to the true concentrations) of the measurements.

As regarding the means, it could be observed, in case of Laboratories 2 and 3 (used the same filter type), higher values (for the both station) determined by GEOECOMAR. This is due to, most probably, the differences either between extraction techniques, or spectrophotometer used (the bandwidths differ: 2 nm - GEOECOMAR and 4 nm NIMRD, respectively).

The statistical evaluation is dealing with the score z calculated from an assigned value established as consensus value according to *The International Harmonized Protocol for the Proficiency Testing Of Analytical Chemistry Laboratories (IUPAC Technical Report)* (IUPAC, 2006) recommendations and a standard uncertainty that is most appropriate for the application area of the results, established as 20%.

Scope and field of application:

- the principal aim is the assessment of laboratory performance against established criteria based on fitness for a common purpose;
- compliance with these criteria may be judged on the basis of the deviation of measurement results from assigned values;

A. Assessment of performance:

Laboratories will be assessed on the difference between their result and the assigned value. A performance score will be calculated for each laboratory, using the *z*-score based on a fitness-for-purpose criterion.

The assessment of results based on standardized deviations of laboratory values from the assigned value (target value), which are measured by so-called *Z*-scores [=(*analysis result – target value*)/*standard deviation*]. If the analysis results are normally distributed, the probability of the absolute amount of the *Z*-score not exceeding a value of 2 is approximately 95%. Therefore, a *Z*-score of 2 is usually fixed as quality limit and the tolerance limit in this interlaboratory study was set to two.

B. Establishment of the consensus value

The consensus value was calculated according to *The International Harmonized Protocol for the Proficiency Testing Of Analytical Chemistry Laboratories (IUPAC Technical Report)* (IUPAC, 2006) recommendations.

According to the IUPAC technical report, an assigned value is an estimate of the value of the measurand that is used for the purpose of calculating scores and shall be determined by one of the following methods:
- measurement by a reference laboratory,
- the certified value(s) for a CRM used as a test material,
- direct comparison of the proficiency testing test material with CRMs,
- consensus of expert laboratories,
- formulation (i.e., value assignment on the basis of proportions used in a solution or other mixture of ingredients with known analyte content),

- a consensus value (that is, a value derived directly from reported results).
For the establishment of the consensus value we followed the next steps:

- Calculate median and the range median ±50%
- Exclude the values which are not included in the range median ±50%
- Recalculate the median which is assumed to be a consensus value.

C. Assignment of z-scores

The IUPAC (2006) Harmonized Protocol recommended the conversion of participants' results into *z*-scores, and experience in the intervening years has demonstrated the wide applicability and acceptance of the *z*-score in proficiency testing. A participant's result *x* is converted into a *z*-score according to the equation

$$z = (x - x_a)/\sigma_p$$

where x_a is the "assigned value", the scheme provider's best estimate of the value of the measurand, and σ_p is the fitness-for-purpose-based "standard deviation for proficiency assessment", that underline the importance of assigning a range appropriate to a particular purpose. (ISO Guide 43; Statistical Guide ISO 13528).

In the equation defining, the term $(x - x_a)$ is the error in the measurement. The parameter σ_p describes the standard uncertainty that is most appropriate for the application area of the results of the analysis, in other words, "fitness-for-purpose".

The uncertainty that is fit for purpose in a measurement result depends on the application. For example, a relative standard uncertainty [i.e., $u(x)/x$] of 10 - 20% is probably adequate for many environmental measurements. As described in the IUPAC guidelines, the choice of σ is dependent upon the data quality objective of a particular program. The fixed performance criterion is more useful in the comparison of a laboratory's performance on different materials.

In instances where the concentration of the analytes varies over a wide range, the fitness-for-purpose criterion has to be specified as a function of concentration. The most common approaches are as follows:

- Specify the criterion as a relative standard deviation (RSD). Specific σ_p values are then obtained by multiplying the selected RSD by the assigned value.

For the purpose of the present exercise, we choose to aim for an RSD of 20 %.

D. Interpretation of the z-scores

According to IUPAC, the interpretation of *z*-scores is *not* generally based on summary statistics that describe the observed participant results. Instead, it uses an assumed model based on the scheme provider's fitness-for-purpose criterion, which is represented by the standard deviation for proficiency assessment σ_p.

- A score of zero implies a perfect result. This will happen rarely even in the most competent laboratories.
- *z*-scores fall between –2 and +2. The sign (i.e., – or +) of the score indicates a negative or positive error respectively. Scores in this range are commonly designated “acceptable” or “satisfactory”.
- A score outside the range from –3 to 3 indicate that the cause of the event should be investigated and remedied. Scores in this class are commonly designated “unacceptable” or “unsatisfactory”.
- Scores in the ranges –2 to –3 and 2 to 3 are designated as “questionable”.

IV.4 Results

The raw data distribution is represented in the histograms below (Fig. 53 for station M13 and Fig. 54 for station M18).

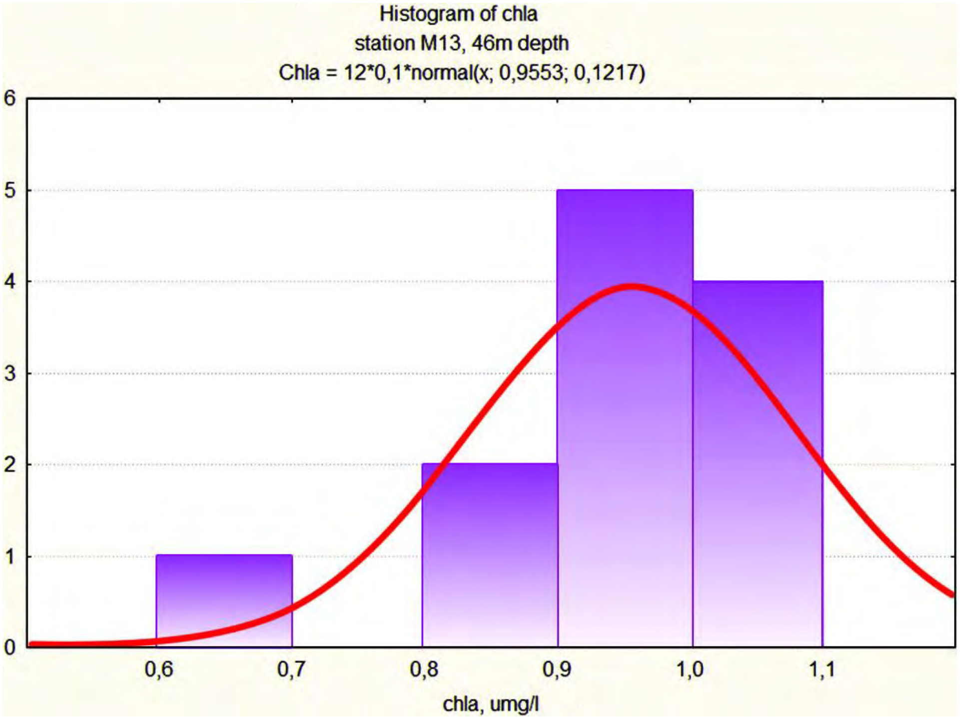


Figure 53. Raw Chl a data distribution for station M13.

Station M13 (deep sea waters)

- Median: 0.98 µg/L
- The range (median ± 50%) values: 0.49 – 1.47 µg/L.
- No values were ranged outside the interval 0.49 – 1.47 µg/L
- Consensus value: 0.98 µg/L
- Z-scores (RSD=7.87; σ=1.57) are shown in the Table 10

Table 10. Z-Scores – station M13 (sampling depth of 43 m).

Laboratory Code	1	2	3	4
Z score	0.017	-0.058	0.035	-0.057

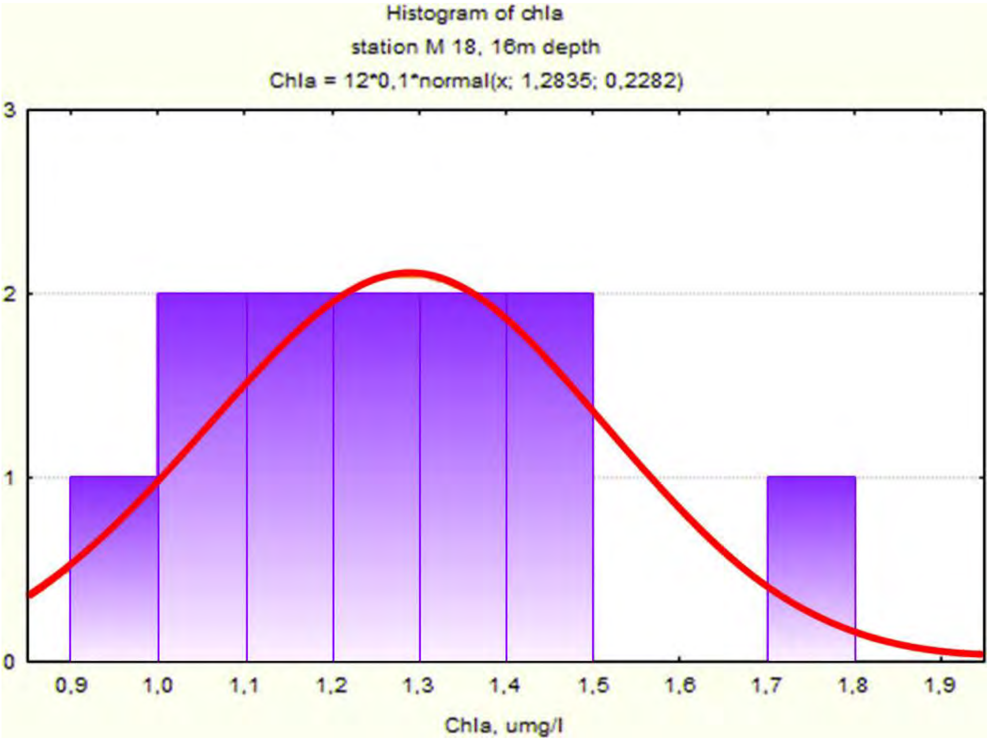


Figure 54. Raw Chl a data distribution for station M18.

Station M18 (coastal waters)

- Median: 1.25 µg/L.
- The range (median ± 50%) values: 0.63 – 1.88 µg/L.
- No values were ranged outside the interval 0.63 – 1.88 µg/L
- Consensus value: 1.25 µg/L
- Z-scores (RSD=5.62; σ=1.12) are given in the Table 11

Table 11. Z-Scores – station M18 (sampling depth of 16 m).

Laboratory Code	1	2	3	4
Z score	-0.197	-0.045	0.178	0.167

IV.5. Conclusions – Chlorophyll a

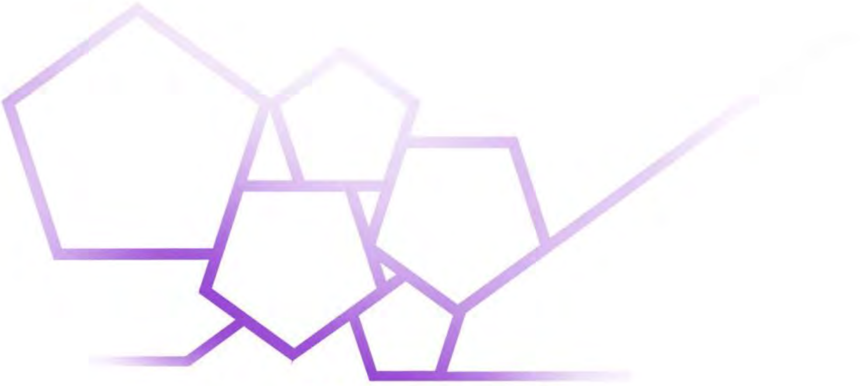
- All z-scores were found to be ranged between -2 and 2, thus suggesting satisfactory agreements for all chlorophyll a measurements of the participating laboratories in the MISIS Intercalibration exercise.

- Generally, the precision (ability to reproduce the measurements) within laboratories were found to be high.

References

- **COMMISSION DIRECTIVE 2009/90/EC of 31 July 2009** laying down, pursuant to Directive 2000/60/EC of the European Parliament and of the Council, technical specifications for chemical analysis and monitoring of water status, Official Journal of the European Union.

- **Cruise report**, MISIS Joint cruise, 2013.
- **Grasshoff K., Kremling K., Ehrhardt M.,** 1999. *Methods of Seawater Analysis*, Wiley-VCH, pp. 599.
- **International Atomic Energy Agency, 2013**. *Worldwide laboratory comparison on the determination of organochlorine compounds, polybrominated diphenyl ethers and petroleum hydrocarbons in IAEA - 459 sediment sample*, Vienna
- **International Organization for Standardization, 2005**. *Statistical methods for use in proficiency testing by interlaboratory comparisons,* ISO 13528:2005, ISO, Geneva
- **IUPAC, 2006.** Thompson M., Ellison S.L.R., Wood R. *The International Harmonized Protocol for The Proficiency Testing Of Analytical Chemistry Laboratories (IUPAC Technical Report),* Pure Appl. Chem., Vol. 78, No. 1, pp. 145–196, 2006.
- **Jeffrey, S. W. & Humphrey, G. F., 1975.** *New spectrophotometric equation for determining chlorophyll a, b, c1 and c2,* Biochem. Physiol. Pflanz., 167, 194-204.
- **Lassen, P. & Larsen, M.M. 2013**. *Report on the HELCOM PLC6 intercalibration*. Aarhus University, DCE – Danish Centre for Environment and Energy, 114 pp. Technical Report from DCE – Danish Centre for Environment and Energy No. 2, http://dce2.au.dk/pub/TR27.pdf
- **Standard Methods, 2005:** *Standard Methods for the Examination of Water and Wastewaters.* Contennial Edition (21st Edition) Edited by: Lenore S. Clescer l., Arrold E. Greenberg, Andrew D.Eaton, Eugene W. Rice
- **Strickland J.D.H, Parsons T.R.,** 1960. *A Manual of Sea Water Analysis.* Fisheries Research Board of Canada, Bulletin no.25.
- **UNEP, 1995**. *Manual for the geochemical analyses of marine sediments and suspended particulate matter.* Reference Methods for Marine Pollution Studies no. 63.
- **UNESCO, 1966.** *Determinations of photosynthetic pigments in seawater.* Monographs on Oceanographic Methodology 1: 11 – 18.
- http://www.quasimeme.org/



Black Sea
monitoring harmonization process

Intercalibration Report
Chemistry

MSFD Guiding **I**mprovements in the
Black **S**ea **I**ntegrated Monitoring **S**ystem

www.misisproject.eu