Elements in River Water
UME EnvCRM 02

Report Prepared By

Alper İŞLEYEN
Süleyman Z. CAN
Oktay CANKUR
Betül ARI
Murat TUNÇ
Fatma Gonca COŞKUN
Zehra ÇAKILBAHÇE
Gökhan AKTAŞ
Hatice ALTUNTAŞ
Elif TURAN

Milena HORVAT
Radojko JACIMOVIC
Tea ZULIANI
Vesna FAJON
Maria OCHSENKUHN-PETROPOULOU
Fotios TSOPELAS
Klaus-Michael OCHSENKUHN
Theopisti LYMPEROPOULOU
Lamprini-Areti TSAKANIKA
Olga SERIFI
Teemu NAYKKI
Timo SARA-AHO

Jochen VOGL
Maren KOENIG
Aida JOTANOVIC
Luka GAZEVIC
Marija PAUNOVIC
Agnieszka ZON
Beata WARZYWODA
Ewa BULSKA
Anna TOMIAK
Eliza KUREK

Date
22/03/2019

M. Cetintas

Dr. Mustafa ÇETİNTAŞ
Director

TABLE OF CONTENTS

ABBREVIATIONS

ANOVA	analysis of variance
ASV	anodic stripping voltammetry
α	significance level
BAM	Bundesanstalt für Materialforschung und -prüfung (BAM), Germany
CRM	certified reference material
DMDM	Directorate of Measures and Precious Metals, Serbia
ET-AAS	electrothermal atomic absorption spectrometry
GUM	Central Office of Measures, Poland
HDPE	high density polyethylene
HR ICP-MS	high resolution inductively coupled plasma mass spectrometry
ICP-MS	inductively coupled plasma mass spectrometry
ICP-OES	inductively coupled optical emission spectrometry
ID ICP-MS	isotope dilution ICP-MS
IJS	Jožef Stefan Institute, Slovenia
IS	internal standard
ISO	International Organization for Standardization
$MS_{between}$	mean square between-bottle from ANOVA
MS_{within}	mean square within-bottle from ANOVA
n	number of replicates per unit
NTUA	National Technical University of Athens, Greece
PTFE	polytetrafluoroethylene
QC	quality control
QQQ-ICP-MS	Triple quadrupole ICP-MS
RSD	relative standard deviation
s	standard deviation
s_{bb}	between-bottle standard deviation
SGT	single Grubbs' test
SI	International System of Units
s_{wb}	within-bottle standard deviation
SYKE	Finnish Environment Institute, Finland
u_{bb}	standard uncertainty related to possible between-bottle heterogeneity
u^*_{bb}	standard uncertainty of heterogeneity that can be hidden by method repeatability
UME	TÜBİTAK National Metrology Institute of Turkey, Turkey
u_{char}	standard uncertainty related to characterisation
u_{lts}	standard uncertainty related to long term stability
UW	University of Warsaw, Poland

The subscript "rel" is added when a variable is expressed in relative terms (e.g. as percent)

ABSTRACT

The need for quality assessment of anthropogenic impact on environmental pollution is increasing due to discharge from various industries, the use of chemicals in agriculture and the consumption of fossil fuels. Diminishing resources such as natural waters used for the cultivation of agricultural products, plant and animal habitats are under severe pollution pressure and are at constant risk. The EU has stipulated the maximum allowable concentration of priority pollutants in different classes of surface water under the Water Framework Directive in Directive 2008/105/EC Annex I “Environmental quality standards for priority substances and certain other pollutants”, and Annex II “List of priority substances in the field of water policy”. Within the scope of the joinr research project in the framework of European Metrology Programme for Innovation and Research (EMPIR) “Matrix Reference Materials for Environmental Analysis”, it was aimed to develop capacity to produce certified reference materials (CRMs) for environmental analysis by transferring know-how between the partners and combining their skills to focus on environmental CRM production. The project is co-funded by the EU Horizon 2020 research and innovation programme and the EMPIR Participating States. As one of the outputs of this project, the production of a river water reference material in compliance with requirements of ISO Guide 34:2009 and ISO 17034:2016 has been performed. Homogeneity, stability an characterisation of the material were assessed in accordance with ISO Guide 35. The material was characterized by an interlaboratory comparison among competent laboratories. This reference material is intended to be a reference point for method development and validation for analysis of elements in river water. Selected target parameters (Pb, Cd, Ni, Hg) were listed in the priority substances and Cd and Hg were further identified as priority hazardous substances. Arsenic is also added to the target parameters list because of its potential toxicological and carcinogenic effects. Furthermore selenium is included in the investigation although it appears to be an essential trace element, as well as it shows evidence of toxicity at levels which are regarded as normal for many trace elements.

INTRODUCTION

Reliable analysis of chemical indicators in water samples for the purpose of environmental pollution assessment poses one of the greatest analytical challenges, due to the complexity of sample matrix and low concentrations of pollutants. Heavy metals and metalloids (As, Cd, Hg, Ni, Pb and Se) in river water represent target parameters for the candidate certified reference material (CRM). Laboratories performing sampling and tests in this field regulated by respective EU directives [1], need matrix CRMs enabling appropriate quality control. National metrology institutes and designated institutes with proven metrological capabilities for the production and certification of such materials are necessary for the provision of quality data. The EMPIR joint research project ENVCRM [2] is aiming to develop capacity to produce CRMs for environmental analysis by transferring the theoretical and practical know-how between the partners and combining their skills to focus on environmental CRM production according to ISO Guide 34:2009 [3] and ISO 17034 [4]. Homogeneity, stability an characterisation of the material were assessed in accordance with ISO Guide 35 [5].

The matrix CRMs available in the market are starting points in the validation of analytical methods, ensuring accuracy and traceability of the measurement results.

UME ENVCRM 02, the production of which was carried out by a project consortium described in this report, is intended to be used as a quality assurance and quality control tool especially by the laboratories involved in the mandatory monitoring prescribed by relevant environment legislations.

The parameters aimed to be certified in UME ENVCRM 02 are the following: mass fractions of As, Cd, Hg, Ni, Pb and Se. The values are stated as mass concentrations in micrograms per kilogram (µg/kg). The target concentration levels for all elements were decided to reflect laboratories' needs.

PARTICIPANTS

The laboratories /organisations involved in production of the CRM and their contribution is summarized in Table 1.

Table 1. Institutes/organisations involved in activities

Activity	Laboratory / Organization
Project management and data evaluation	TÜBİTAK UME, Ulusal Metroloji Enstitüsü, Gebze - Kocaeli, TURKEY
Preliminary measurements	TÜBİTAK UME, Ulusal Metroloji Enstitüsü, Gebze - Kocaeli, TURKEY UW-University of Warsaw, Warsaw, POLAND
Sampling and processing	TÜBİTAK UME, Ulusal Metroloji Enstitüsü, Gebze - Kocaeli, TURKEY
Sterilization	GAMMA PAK Sterilizasyon Sanayi ve Ticaret A.Ş., Çerkezköy - Tekirdağ, TURKEY

Activity	Laboratory / Organization
Homogeneity study	TÜBİTAK UME, Ulusal Metroloji Enstitüsü, Gebze, Kocaeli, TURKEY IJS, Jožef Stefan Institute, Ljubljana, SLOVENIA
Stability studies	TÜBİTAK UME, Ulusal Metroloji Enstitüsü, Gebze, Kocaeli, TURKEY IJS, Jožef Stefan Institute, Ljubljana, SLOVENIA
Characterization study (in alphabetical order)	BAM, Bundesanstalt für Materialforschung und prüfung, Berlin, GERMANY DMDM, Directorate of Measures and Precious Metals, Belgrade, SERBIA GUM, Central Office of Measures, Warszawa, POLAND IJS, Jožef Stefan Institute, Ljubljana, SLOVENIA NTUA, National Technical University of Athens, Athens, GREECE SYKE, Finnish Environment Institute, Helsinki, FINLAND TÜBİTAK UME, Ulusal Metroloji Enstitüsü, Gebze, Kocaeli, TURKEY UW, University of Warsaw, Warsaw, POLAND
Collection and combination of characterization results and preliminary evaluation	IMBIH, Institute of Metrology of Bosnia and Herzegovina, Sarajevo, BOSNIA and HERZEGOVINA

MATERIAL PROCESSING

The river water used as raw material was collected from a creek feeding an artificial lake (Darlık Dam), which is one of İstanbul's water supplies in Turkey. Prior to collection, carboys (10 L, HDPE) were cleaned with deionized water (Resistivity: > 15 MΩ·cm) and dried at ambient temperature. Water samples were collected with beakers (polypropylene) and transferred to cleaned carboys. Then, collected samples (total of 200 L in 20 carboys) were transported to TÜBİTAK UME for further processing.

Upon arrival at TÜBİTAK UME, one carboy of sample was kept at +4 °C as unacidified for preliminary density, pH and ion chromatography measurements and 2 x 100 mL of this sample were transferred to University of Warsaw, Poland. Results of measurements on these raw samples are summarized in Table 2.

All the other samples were acidified in carboys to have a 2% (v/v) HNO_3 (65 %, for analysis EMSURE® Reag. Ph Eur, ISO, Merck, Germany) and stored at +4 °C.

Table 2. Preliminary analysis results of river water by UW

Parameter	Preliminary Measurement Result, *n* = 2
Density (at 20 °C)	1.004 ± 0.002* g/cm^3
pH (at 20 °C)	8.13 ± 0.01*
Ca^{2+}	86.5 ± 4.3** mg/L
Cl^-	16.1 ± 0.8** mg/L
Mg^{2+}	8.3 ± 0.4** mg/L
Na^+	10.9 ± 0.5** mg/L
K^+	2.0 ± 0.1** mg/L
SO_4^{2-}	9.5 ± 0.5** mg/L

* Values written with "±" sign represents standard deviation
**Values written with "±" sign represents expanded (k=2) measurement uncertainty

For preliminary measurements, subsamples from 10 different carboys were selected and analyzed with HR-ICPMS (Thermo Element 2, Germany) for 6 target elements by TÜBİTAK UME. Results of this measurement are summarized in Table 3.

Table 3. Natural and target concentration levels of elements in river water

Parameter	Preliminary Measurement Result (µg/kg, *n* = 20)	Target Level (µg/kg)
As	1.0 ± 0.1	15
Cd	< 0.1	0.5
Hg	< 0.05	0.1
Ni	1.4 ± 0.2	15
Pb	0.3 ± 0.1	5
Se	< 0.1	5

Results showed that the candidate raw material has lower element content than the target level, thus it was decided to spike this material to reach the target level for all elements. For mercury, it was decided to spike the material with an organo-mercury compound: thiomersal (2-(Ethylmercuriomercapto)benzoic acid sodium salt, CAS No: 54-64-8) to improve the stability. HDPE Bottles with 125 mL volume in low particulate narrow-mouth series (Nalgene, Cat No: 382099-0125, USA) were selected as product container. These bottles are produced in a controlled environment and certified by the producer. Blank levels of target elements were monitored in 20 different bottles from 20 different boxes. Bottles were filled with 2% HNO_3 and were stored for a week before analyzing with ICP MS. Slight deviations were observed between the tested bottles for the elements of interest. In order to minimize the possible deviation between bottles, 1250 bottles were filled with deionized water (Resistivity >18 MΩ·cm, Elga Water Purification System Purelab Flex 2, Germany) in an ISO Class 6 clean laboratory, poured after three days and dried in laminar air flow cabinet in the ISO Class 6 clean laboratory.

The raw material from the 10-L carboys was combined in HDPE homogenization tanks (2 x 110 L, Nalgene, USA) by filtering through first cloth filter and then 0.8/0.45 µm filter (Pall Corp., AcroPac 1000, USA). Spike mixture (250 mL, prepared from NIST and SCP Science standards for As, Cd, Ni, Pb, Se) and Thiomersal for Hg (100 mL solution prepared from salt, Alfa Aesar, J61799) were added. Homogenization performed by circulating the content between tanks (HDPE) for total 6 hours (4 hours first day and 2 hours second day). A pressurized air driven circulation pump (DEBEM, SDB 71722, Italy) was used to transfer the content between the tanks via PVC pipes.

Filling and capping were conducted using an automated filling machine (FARMATEK, FTED 1-150, Turkey). A total of 1250 units, each containing approximately 100 mL, were filled and capped within 4 hours. The candidate CRM was sterilized by γ-irradiation with a ^{60}Co source at a minimum dose of 25 kGy. The irradiation caused the expected change in color of the medium transparent HDPE bottles to pale yellow. After this step, the bottles were stored at 4 °C in the dark. All stages of processing is summarized and given as a flow diagram in Annex 1.

HOMOGENEITY

Homogeneity study between the units is performed to show that the assigned values are valid for all units within the stated uncertainty. Homogeneity study between the units is performed with a number of samples representing the whole batch. In this project, 12 units were selected by using random stratified sampling for each of the two participant laboratories. Homogeneity tests were carried out by measuring three sub-samples under repeatability conditions. The samples to be analyzed were introduced to the instruments by random order to find out any trend arising from analytical and/or filling sequences.

Measurement results showed inconsistency with the spiked amount for Hg, as a consequence Hg was not further investigated, and tests were performed on the remaining five elements. The data obtained by both laboratories were evaluated statistically by regression analysis for the presence of any trend in analytical and filling sequence at 99% confidence level. Only analytical sequence trends were found for As (IJS), Cd (UME) and Ni (UME) and data was not reprocessed to correct for these trends.

Grubbs test (one sided) was applied to all data for the presence of outlier at 99% confidence level and no outlier was detected. Data was visually checked whether all individual data follow a unimodal distribution using histograms and normal probability plots. It was found that the distribution was normal and unimodal. Minor deviations from unimodality of the individual values do not significantly affect the estimate of between-unit standard deviations. The results of all statistical evaluations are presented in Table 4.

Table 4. Statistical evaluation results of homogeneity for river water CRM

Parameter (Lab)	**Any trend?**		**Any outlier?**		**Distribution**
	Analytical sequence	**Filling sequence**	**All data**	**Unit averages**	**All data**
As (UME)	No	No	No	No	Normal/unimodal
As (IJS)	Yes	No	No	No	Normal/unimodal
Cd (UME)	Yes	No	No	No	Normal/unimodal
Cd (IJS)	No	No	No	No	Normal/unimodal
Ni (UME)	Yes	No	No	No	Normal/unimodal
Ni (IJS)	No	No	No	No	Normal/unimodal
Pb (UME)	No	No	No	No	Normal/unimodal
Pb (IJS)	No	No	No	No	Normal/unimodal
Se (UME)	No	No	No	No	Normal/unimodal

The ANOVA allowed the calculation of the within- (s_{wb}) and between-unit homogeneity (s_{bb}), estimated as standard deviations, according to the following equations:

$$\mathrm{s}_{\mathrm{wb}} = \sqrt{MS_{within}} \qquad (1)$$

MS_{within} : Mean squares within-unit,

s_{wb} is equivalent to the *s* of the method, provided that subsamples are representative for the whole unit.

$$s_{bb} = \sqrt{\frac{MS_{between} - MS_{within}}{n}} \qquad (2)$$

$MS_{between}$: Mean squares between-unit,

n : Number of replicates per unit.

When MS_{between} is smaller than MS_{within}, s_{bb} cannot be calculated. Instead, u^*_{bb}, the heterogeneity that can be hidden by the method repeatability, is calculated, according to the following Equation [6]:

$$u^*_{bb} = \frac{s_{wb}}{\sqrt{n}} \sqrt[4]{\frac{2}{\nu_{MSwithin}}} \qquad (3)$$

$\nu_{MSwithin}$: Degrees of freedom of MS_{within}

The occurrence of $MS_{between} < MS_{within}$ can be seen, if material heterogeneity is smaller than that can be detected by the analytical methodology used.

For the parameters for which ANOVA was applied, the larger value of s_{bb} or u^*_{bb} is taken as uncertainty contribution for homogeneity, u_{bb} (Table 5). As a conservative approach, the uncertainty contribution for homogeneity, $u_{\mathrm{bb,rel}}$ is accepted as the highest value of the two institute's results (except for Se which is only measured by UME).

Table 5. Results of the homogeneity study

Parameter (Lab)	$s_{\mathrm{wb,rel}}$, %	$s_{\mathrm{bb,rel}}$, %	$u^*_{\mathrm{bb,rel}}$, %	$u_{\mathrm{bb,rel}}$, %
As (UME)	1.15	$MS_{between} < MS_{within}$	0.36	**0.36**
As (IJS)	0.90	$MS_{between} < MS_{within}$	0.28	0.28
Cd (UME)	1.73	$MS_{between} < MS_{within}$	0.54	**0.54**
Cd (IJS)	1.72	$MS_{between} < MS_{within}$	0.53	0.53
Ni (UME)	2.92	$MS_{between} < MS_{within}$	0.90	**0.90**
Ni (IJS)	1.13	$MS_{between} < MS_{within}$	0.35	0.35
Pb (UME)	0.97	0.15	0.30	0.30
Pb (IJS)	2.17	$MS_{between} < MS_{within}$	0.67	**0.67**
Se (UME)	2.53	$MS_{between} < MS_{within}$	0.79	**0.79**

The between-unit variation for all elements is found to be low. Maximum allowed target uncertainty for homogeneity was 2 % and it was achieved.

The plotted data used for the evaluation of homogeneity can be found in Annex 2.

STABILITY

The stability studies were carried out using an isochronous design [7]. In this approach, samples are stored for a certain time at different temperature conditions. Afterwards, the samples are moved to conditions where further degradation can be assumed to be negligible ("reference conditions"), effectively "freezing" the degradation status of the materials. At the end of the isochronous storage, the samples are analyzed simultaneously under repeatability conditions. Analysis of the material (after various exposure times and temperatures) under repeatability conditions greatly improves the sensitivity of the stability tests.

For the Short Term Stability (STS) test, two different temperatures (18 ˚C and 60 ˚C) and 4 time points (0, 1, 2, and 4 weeks) were tested. 14 samples were randomly selected for each of the participating laboratories and 12 samples were subjected to the test temperatures for the specified time intervals.

Test samples were moved to +4 ˚C (reference temperature) after completion of the test time. All samples were analyzed at the same time. Three replicate measurements for each unit were performed by each laboratory under the repeatability conditions.

The data for each temperature were first examined by single Grubbs test for both 95% and 99% confidence intervals to find out outliers. Number of detected outliers are given in the Table 6. Since no technical reason can be found to reject these data, all outliers were included in the STS calculations.

Values calculated for each time point were plotted against the time for the assessment of short term stability. The relationship between variables were analyzed in order to determine if any significant change exists with the testing time (regression analysis). It was found that the slopes were not significantly different than zero for all parameters in the 95% and 99% confidence intervals. Short term stability plots are presented in Annex 3.

Short Term Stability Results:

The results obtained from isochronous measurements were first grouped according to the time period and then evaluated for each time point. These evaluations were carried out for both temperatures, separately.

The results were screened for outliers by applying the single Grubbs' test at confidence levels of 95% and 99%. The measured concentration values were plotted against time and the regression lines were calculated to check for significant trends indicating possible changes in the concentrations of the analytes by time. The calculated slope values were tested for significance using a t-test, with $t_{\alpha,df}$ being the critical t-value (two-tailed) for a significance level α = 0.05 (95% confidence level). The graphs are presented in Annex 3.

Outliers for As, Ni, Pb and Se were identified in the statistical evaluation (Grubbs' test) of the data. Nevertheless, as there was no technical reason to exclude them from evaluation, they remained in the data set. The data points were plotted against storage time at the test temperature and the regression line was calculated. In all cases the slope of the regression line was not found to be significantly different from zero. The data evaluation results for the short-term stability at +18 °C and +60 °C are summarised in Table 6.

Uncertainty calculations are performed using Equation (4). Maximum time for transfer is chosen as four weeks.

$$u_{sts,rel} = \frac{RSD}{\sqrt{\Sigma\left(t_i - \bar{t}\right)^2}} \times t \tag{4}$$

where,

RSD : relative standard deviation of the points on the regression line,
t_i : time point for each replicate,
$\bar{t}$: mean of all time points,
t : maximum time suggested for transfer (4 weeks).

Results obtained from short term stability tests are given in Table 6.

Table 6. Results of short term stability tests

Parameter (Lab)	**18 °C** $u_{sts,rel}$ (%)	**60 °C** $u_{sts,rel}$ (%)	Number of outliers in 95% confidence interval*		Number of outliers in 99% confidence interval*		Is there a significant trend in 95% confidence interval?		Is there a significant trend in 99% confidence interval?	
			18 °C	**60 °C**	**18 °C**	**60 °C**	**18 °C**	**60 °C**	**18 °C**	**60 °C**
As (UME)	0.91	**0.97**	1	-	-	-	No	No	No	No
As (IJS)	0.51	0.61	-	1	-	-	No	No	No	No
Cd (UME)	**0.79**	0.78	-	1	-	-	No	No	No	No
Cd (IJS)	0.58	0.75	-	-	-	-	No	No	No	No
Ni (UME)	0.94	**0.98**	1	1	1	1	No	No	No	No
Ni (IJS)	0,54	0.79	1	-	1	-	No	No	No	No
Pb (UME)	0.59	**0.60**	1	-	-	-	No	No	No	No
Pb (IJS)	0.32	0.57	1	-	-	-	No	No	No	No
Se (UME)	3.19	**3.55**	1	1	1	1	No	No	No	No

*SGT: Single Grubbs' Test

As a conservative approach, the uncertainty contribution for short term stability, $u_{sts,rel}$ is accepted as the highest value obtained for the two test temperatures by two institute's (shown in bold, Table 5) except Se which was analyzed only by TÜBİTAK UME.

The material is found to be stable at 18 °C and 60 °C for up to four weeks. Thus, the samples can be safely dispatched under conditions where the temperature do not exceed 60 °C for up to four weeks, i.e. at ambient temperature without applying any cooling elements.

Long Term Stability Results:

Shelf life of the CRM has been determined through long term stability measurements. For the measurements, four units for each of the months of 0, 3, 6 and 12 have been stored at +18 °C and transferred to reference temperature (+4 °C) after each period of time to be measured isochronously afterwards. Four units, designated as reference units, of the month 0 (zero) was stored at +4 °C.

The data for each time point has been calculated by three replicate measurements for each of two units. Thus, the average of 6 measurements for each time point is given in Annex 4. The error bars on each time point are calculated as the standard deviation of 6 measurement results.

No outlying data was detected by Grubbs' test. The graphs were plotted against time and the regression lines were calculated. The relative long term stability uncertainty, $u_{\mathrm{lts,rel}}$ for each parameter is then calculated using Equation (5) for the required shelf life as [6]:

$$u_{lts,rel} = \frac{RSD}{\sqrt{\Sigma(t_i - \bar{t})^2}} \times t \qquad (5)$$

where;

RSD : the relative standard deviation of the points on the regression line,
t_i : being the time point for each replicate,
$\bar{t}$: being the average of all time points,
t : being the proposed shelf life at 18 °C (12 months).

The uncertainty contribution u_{lts} was calculated for 12 months (*t*) at 18 °C. As a conservative approach, the relative uncertainty contribution for short term stability, $u_{\mathrm{lts,rel}}$ is accepted as the highest value obtained by two institute's (shown in bold, Table 7). This uncertainty was one of the four parameters contributing to the overall uncertainty of the certified values. The results are given in Table 7. The graphs for long term stability are given in Annex 4.

Table 7. Results of the long-term stability tests for 12 months

Analyte	Is the slope significantly different from zero at 18 °C?*	$u_{\mathrm{lts,rel}}$ [%] for shelf-life of 12 months at 18 °C
As (UME)	No	**1.48**
As (IJS)	No	0.98
Cd (UME)	No	0.73
Cd (IJS)	No	**0.95**
Ni (UME)	No	**0.90**
Ni (IJS)	No	0.88
Pb (UME)	No	0.52
Pb (IJS)	No	**1.39**
Se (UME)	No	1.35
Se (IJS)	No	**2.22**

*Data are evaluated at confidence level of 95%.

Based on the results obtained, 18 °C was found to be suitable to keep the samples for up to 12 months. In addition, to ensure stability beyond the initially determined shelf life, stability will be re-evaluated in certain periods, based on the results of post-certification monitoring.

CHARACTERIZATION

According to ISO 17034, the characterization and the value assignment can be carried out in different ways. In this project, the characterization of the material was carried out by an interlaboratory comparison exercise. The participating laboratories were partners of the EnvCRM project consortium [2]. The detailed information about the laboratories is given in the participants section. The participating laboratories used validated methods, including potentially primary methods such as ID-ICP-MS.

Each laboratory received two bottles of samples which were selected from the whole set of samples to represent the whole produced batch. The samples were selected randomly from the set of samples by the random stratified sampling technique. Each laboratory was asked to report at least 6 independent measurement results for two sample units, together with their associated measurement uncertainty values and the approach used for the estimation of measurement uncertainty. In the reports, the details of the reference materials used in the calibration were also requested from the laboratories in order to assure the traceability of the reported results.

If the laboratory provided results using more than one method, all results were taken into account during the assignment of the certified value. A list of laboratories with their abbreviations and corresponding methodologies used for the measurements are summarized in Table 8. More details about the measurement methods are presented in Annex 5.

Table 8. Techniques used by participating laboratories

Parameter	BAM	DMDM	GUM	IJS	NTUA	SYKE	UME	UW
As	-	ICP-MS	ICP-OES	ICP-MS	ICP-MS	ICP-MS	ICP-MS	ICP-MS
Cd	ID ICP-MS	ICP-MS	ICP-OES	ICP-MS	1-ICP-MS 2-ASV	ICP-MS	ID ICP-MS	ICP-MS
Ni	ID ICP-MS	ICP-MS	ICP-OES	ICP-MS	ICP-MS	ICP-MS	ICP-MS	ICP-MS
Pb	ID ICP-MS	ICP-MS	ICP-OES	ICP-MS	1-ICP-MS 2-ASV	ID ICP-MS	ID ICP-MS	ICP-MS
Se	-	-	-	ICP-MS	ICP-MS	-	ID ICP-MS	ICP-MS

ASV : Anodic Stripping Voltammetry
ICP-MS : Inductively Coupled Plasma Mass Spectrometry
ICP-OES : Inductively Coupled Plasma Optical Emission Spectrometry
ID ICP-MS: Isotope Dilution ICP-MS

Characterization standard uncertainty (u_{char}) is calculated using Equation (6) by taking into account the uncertainties and the standard deviation of the means reported by the participating laboratories except Se. Characterization standard uncertainty for selenium is accepted as the uncertainty reported by TÜBİTAK UME for the ID-ICP-MS method. Graphs for characterization data are presented in Annex 6, where error bars represent expanded uncertainties reported by the laboratories.

$$u_{\mathrm{char}} = \sqrt{\bar{u}^2_{\mathrm{labs}} + \left(\frac{SD}{\sqrt{n}}\right)^2} \qquad (6)$$

where,

u_{char} : Standard uncertainty arising from characterization,

$\bar{u}_{labs}$: Arithmetic mean of standard uncertainties reported by the participating laboratories,
SD : Standard deviation of accepted means of participating laboratories,
n : Number of laboratories with accepted results.

PROPERTY VALUE AND UNCERTAINTY ASSIGNMENT

Datasets from participants were collected and robust statistics were applied.

Summary of the results can be found in Table 9. The certified values are given in Table 9 as obtained from the characterization study as explained above. All the certified values reported are calculated as the median of the laboratory means of all the accepted data sets (except Se). Since there are only four available datasets for Se, assigned value is calculated as the arithmetic mean of the data set obtained by ID-ICP-MS which is a primary method carried out by a single laboratory (TÜBİTAK UME). Absence of bias for the assigned value was further confirmed by comparing the values with the median of a larger dataset obtained from the supplementary comparison EURAMET.QM-S11 [8]. Graph of the results reported by the participating laboratories to the supplementary comparison study is presented in Annex 7.

The uncertainty of the certified values contains contributions of the characterization u_{char}, the homogeneity u_{bb}, the long-term stability u_{lts}, and the short term stability u_{sts}.

The different contributions to the CRM uncertainty are combined using equation (7):

$$U_{\mathrm{CRM}}=k\,.\,\sqrt{u_{\mathrm{char}}^2+u_{\mathrm{bb}}^2+u_{\mathrm{lts}}^2+u_{\mathrm{sts}}^2} \qquad (7)$$

The expanded uncertainty of the certified value U_{CRM} is calculated with a coverage factor of $k = 2$, representing a confidence level of approximately 95%. U_{CRM} values are presented as dashed lines in Annex 6.

The certified values and uncertainties are summarized in Table 9 and uncertainty contribution percentages are given in Table 10. Certified values are found to be in good agreement with the target values (Table 2) except for Pb. An unforeseen lead contamination might have caused a 8.6 µg/kg higher value than the targeted value. Since there were no sign of inhomogeneity and the assigned value with its uncertainty is still useful for the laboratories, Pb was decided to be certified.

Table 9. Summary of certified values and their uncertainties

Element	**Certified Value (µg/kg)**	U_{CRM} **(µg/kg, *k* = 2)**	$U_{\mathrm{CRM,rel}}$ ***(%, k = 2)***	$u_{\mathrm{char,rel}}$ **(%)**	n_{char}	$u_{\mathrm{bb,rel}}$ **(%)**	$u_{\mathrm{lts,rel}}$ **(%)**	$u_{\mathrm{sts,rel}}$ **(%)**
As	15.1	1.6	10.6	4.65	7	0.36	1.48	0.97
Cd	0.52	0.06	11.5	4.98	9	0.54	0.95	0.79
Ni	14.4	1.1	7.6	3.27	8	0.9	0.9	0.98
Pb	13.6	1.0	7.4	3.30	9	0.67	1.39	0.60
Se	5.0	0.5	10.0	1.00	1	0.79	2.22	3.55

Table 10. Percent contribution of each uncertainty component to U_{CRM}.

Element	u_{bb} (%)	u_{lts} (%)	u_{sts} (%)	u_{char} (%)
As	5	20	13	62
Cd	7	11	13	69
Ni	15	16	15	54
Pb	11	10	23	56
Se	10	30	47	13

INFORMATIVE VALUES

Density and pH were measured on 4 different units by UW, results for these measurements are given in Table 10.

Table 11. Informative values for river water measured by UW

Parameter	Measurement Result (n = 4)
Density (at 20 °C)	1.015 ± 0.001* g/cm^3
pH (at 20 °C)	0.70 ± 0.03*

* Values written with "±" sign represents standard deviation

TRACEABILITY

The metrological traceability of the CRM was ensured by using SI traceable calibration standards and/or using reference methods i.e. ID-ICP-MS by the laboratories participating to the interlaboratory comparison. The laboratories were asked to provide detailed information about the calibration standards and reference methods used in the measurements. The details about the measurement methods, calibration standards and quality control materials used by the participating laboratories are given in Annex 5.

INSTRUCTIONS FOR USE

Storage conditions

The material should be stored at (18 ± 4) °C in dark and clean environment. The bottle should be shaken before opening the cap to avoid a bias due to condensed water at the bottleneck. In order to prevent contamination, it is recommended that the bottle should be opened in a clean environment and pipette should not be inserted into the bottle. TÜBİTAK UME cannot be held responsible for changes that might happen to the material at customer's premises due to noncompliance with the instructions for use, and the storage conditions given.

Safety precautions

The material contains 2% (v/v) nitric acid and elements spiked into solution. Therefore, the usual laboratory safety measures apply as in the case of similar solutions. It is strongly recommended that the material must be handled and disposed according to the safety guidelines where applicable. Please refer to the Safety datasheet (SDS) before any use of the material.

Minimum sample intake

During the measurements performed for homogenization, characterization, long term and short term stability studies, the lowest volume used was 250 µL for certified elements and no significant problem of heterogeneity have been observed. Therefore, this can be considered as the minimum sample volume to be used in the analysis.

Use of Certified Value

For assessing the method performance, the measured values of the CRM are compared with the certified values [9]. The procedure can be described briefly as:

- Calculate the absolute difference between mean measured value and the certified value (Δ_m).
- Combine measurement uncertainty (u_{meas}) with the standard uncertainty of the certified value (u_{CRM}) using following equation:

$$u_{\Delta}=\sqrt{u_{\mathrm{meas}}^{2}+u_{\mathrm{CRM}}^{2}} \qquad (8)$$

- Calculate the expanded uncertainty (U_{Δ}) from the combined uncertainty (u_{Δ}) using a coverage factor of two ($k = 2$), corresponding to a confidence level of approximately 95%.

If $\Delta_m \leq U_{\Delta}$, then it is assumed that there is no significant difference between the measurement result and the certified value at a of about 95% confidence level.

An online application: CRM Result Evaluation-CRM RE to evaluate measurement results and automatically create quality control charts is available through the link: https://rm.ume.tubitak.gov.tr/en/crm_re/

ACKNOWLEDGEMENTS

The work of this study is part of the project EnvCRM, which was funded within the framework of the EMPIR. The EMPIR initiative is co-funded by the European Union’s Horizon 2020 research and innovation programme and the EMPIR Participating States.

Authors would like to thank Ülkü Adıgüzel, Cengiz Yağar, Okan Özkan and Hayri Dalga (DSI, General Directorate of State Hydraulic Works, Turkey) for their support for the supply of river water material.

REFERENCES

[1] Water Framework Directive 2008/105/EC – Annex II: Priority substances and other pollutants; http://ec.europa.eu/environment/water/water-framework/priority_substances.htm

[2] Matrix reference materials for environmental analysis project homepage; http://www.envcrm.com

[3] International Organization for Standardization, ISO Guide 34, General requirements for the competence of reference material producers, ISO, Geneva (2009)

[4] International Organization for Standardization, ISO 17034, General requirements for the competence of reference material producers, ISO, Geneva (2015)

[5] ISO Guide 35:2017. Reference materials — Guidance for characterization and assessment of homogeneity and stability

[6] T. P. J. Linsinger, J. Pauwels, A. M. H. Van der Veen, H. Schimmel, A. Lamberty, Homogeneity and stability of reference materials, Accred. Qual. Assur. 6:20-25 (2001)

[7] Lamberty, H. Schimmel, J. Pauwels, The study of the stability of reference materials by isochronous measurements, Fres. J. Anal. Chem. 360:359-361 (1998)

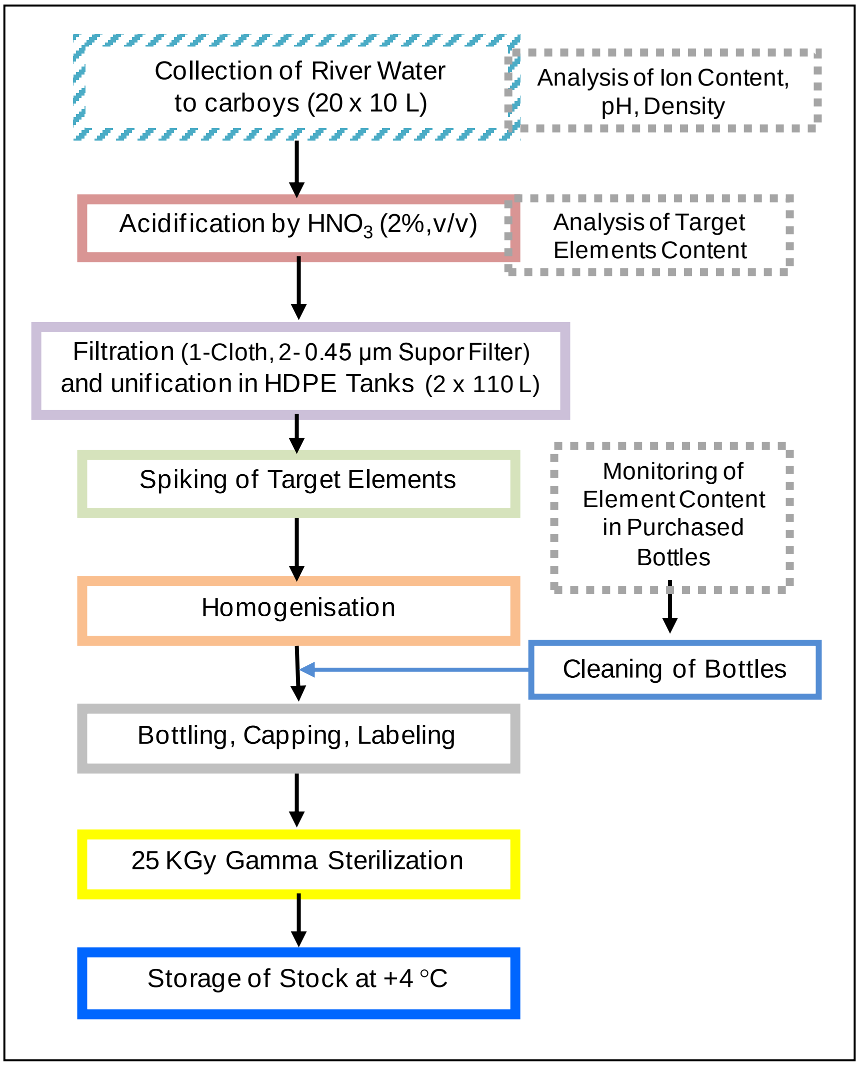
[8] EURAMET.QM-S11 Supplementary comparison & pilot study on determination of Elements in River Water; https://www.bipm.org/kcdb/comparison?id=558

[9] ERM Application Note 1: Comparison of a measurement result with the certified value, (2010) https://ec.europa.eu/jrc/sites/jrcsh/files/erm_application_note_1_en.pdf

REVISION HISTORY

Date	Remarks
22.03.2019	First issue.

Annex 1. Flow Diagram for the Preparation of the River Water CRM



Annex 2. Graphs for Homogeneity Studies

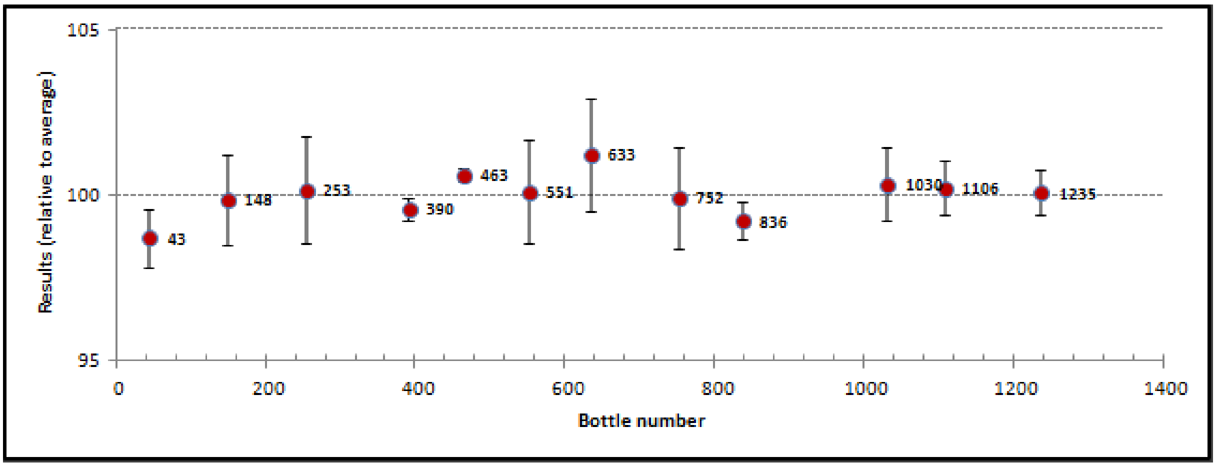


Figure A2.1. Homogeneity plot for As by UME

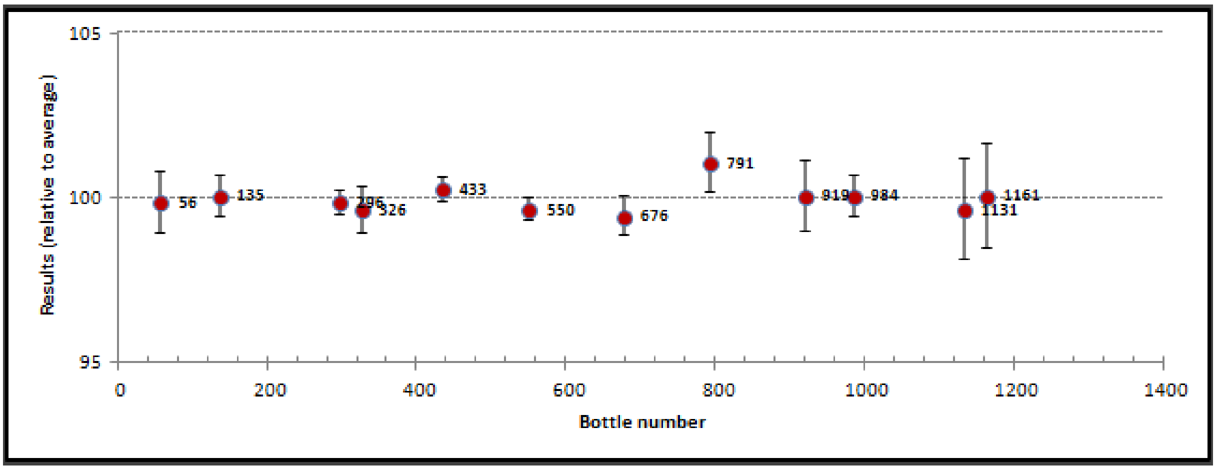


Figure A2.2. Homogeneity plot for As by IJS

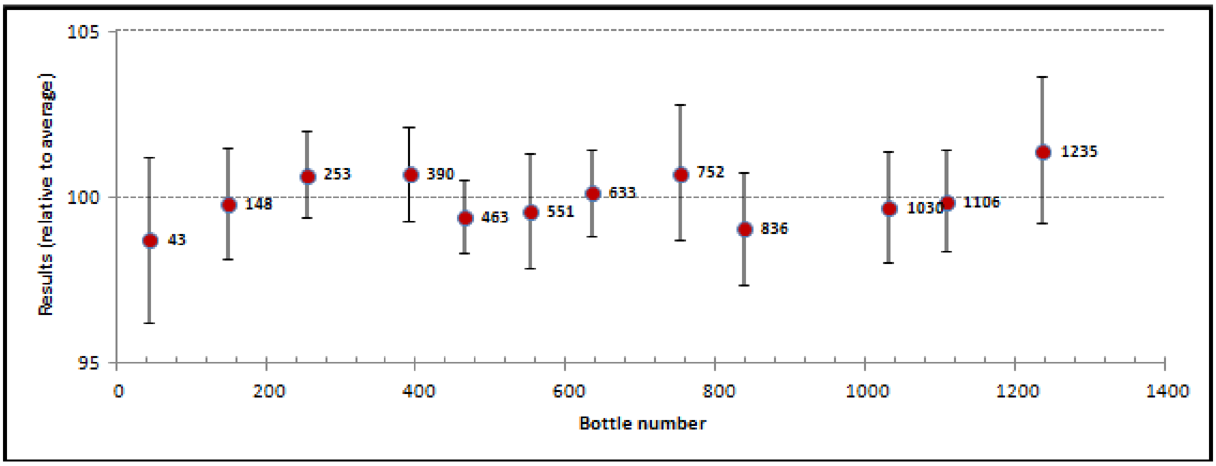


Figure A2.3. Homogeneity plot for Cd by UME

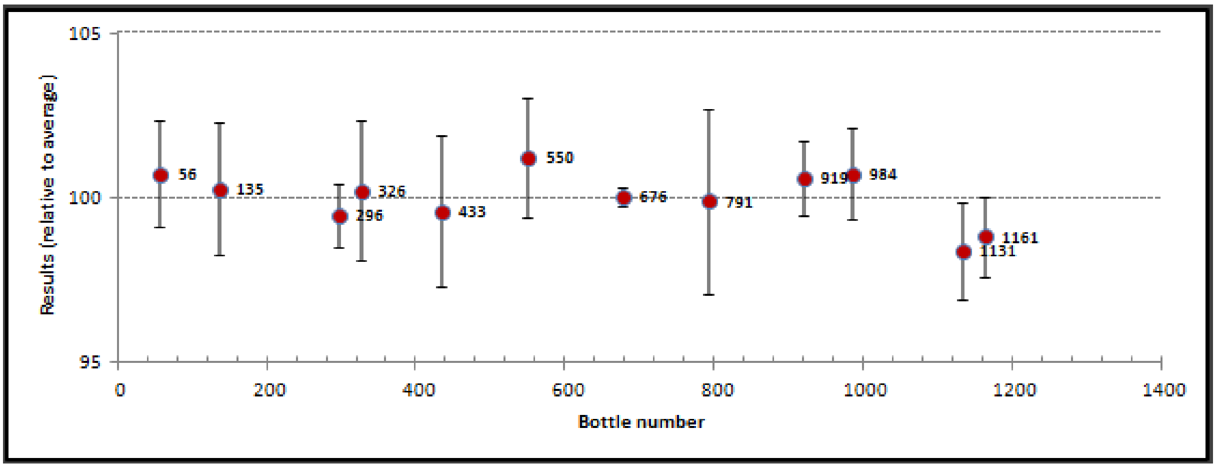


Figure A2.4. Homogeneity plot for Cd by IJS

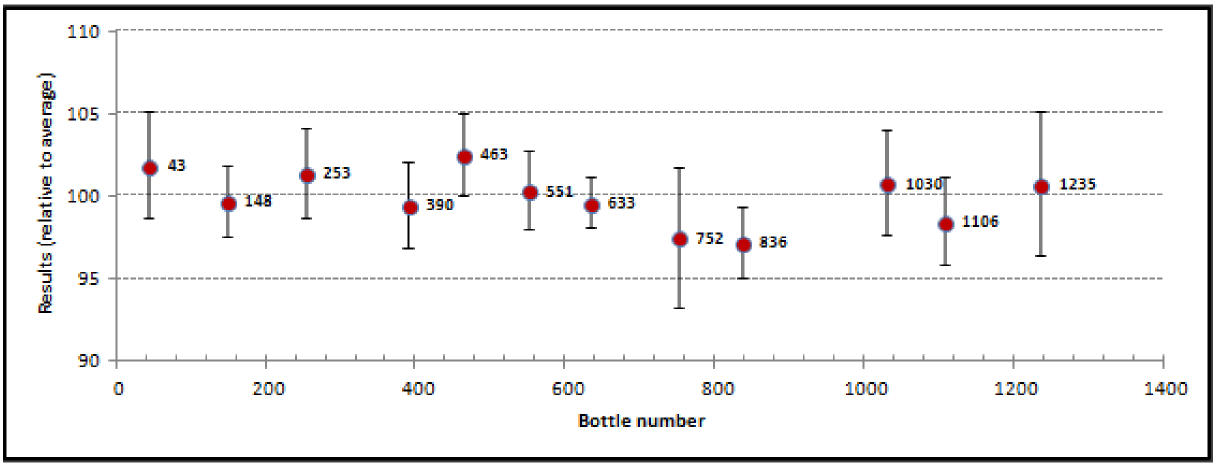


Figure A2.5. Homogeneity plot for Ni by UME

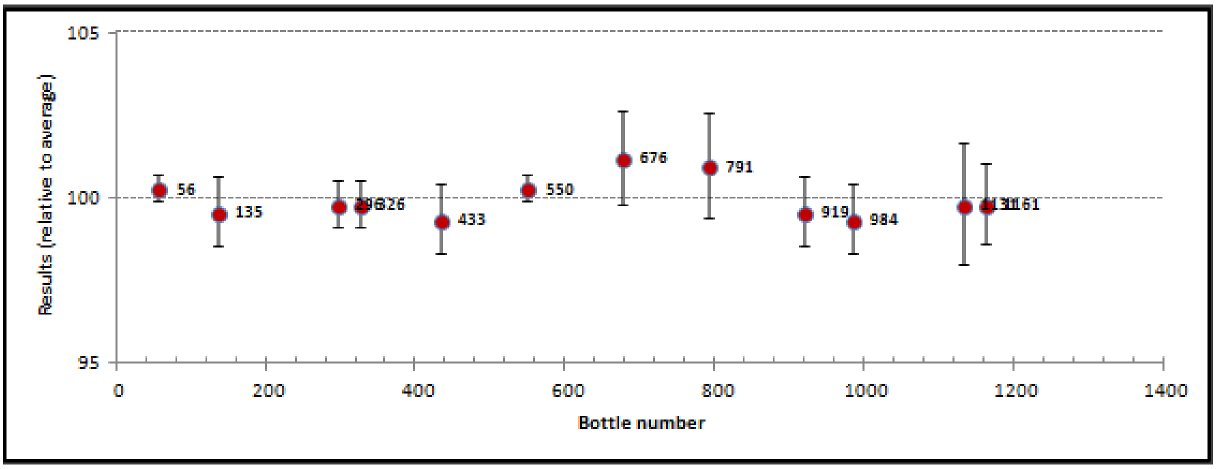


Figure A2.6. Homogeneity plot for Ni by IJS

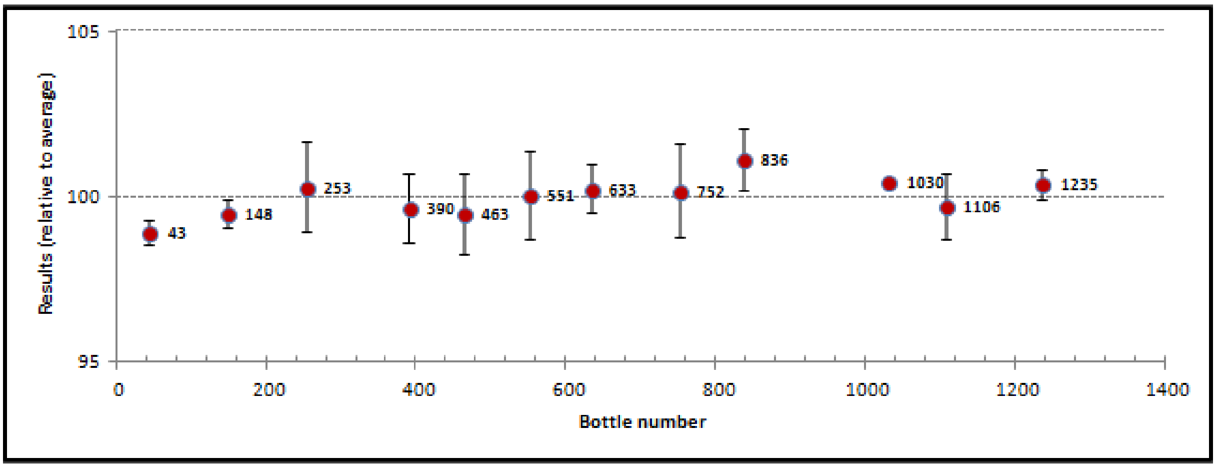


Figure A2.7. Homogeneity plot for Pb by UME

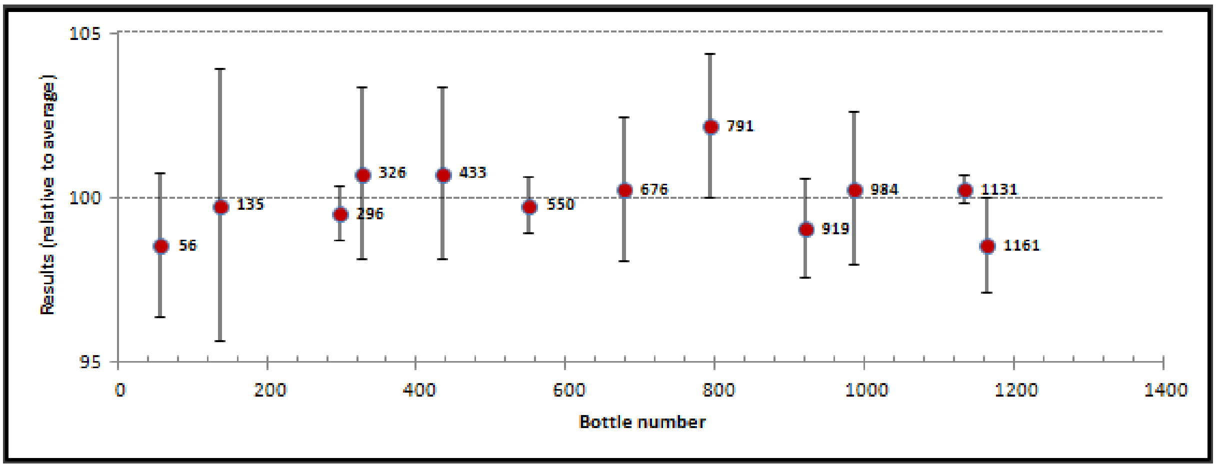


Figure A2.8. Homogeneity plot for Pb by IJS

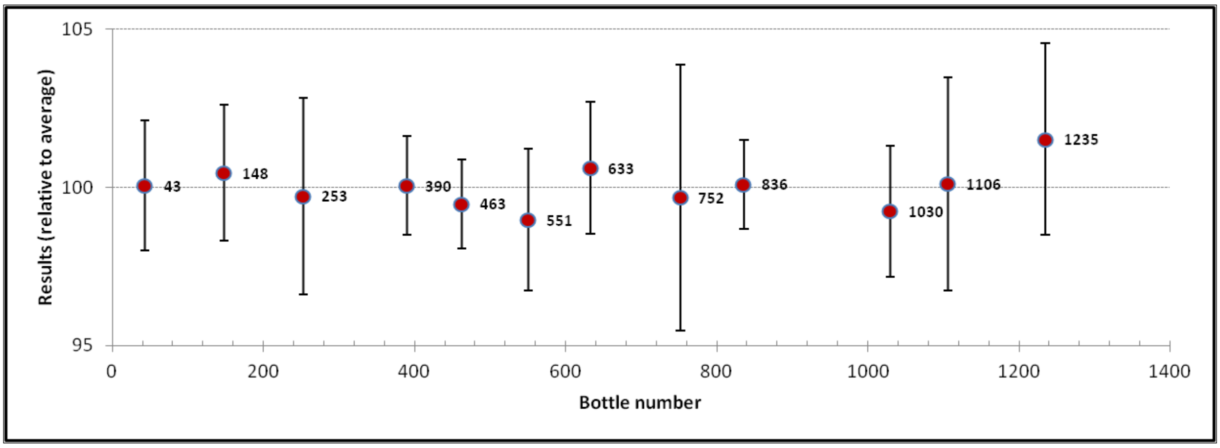


Figure A2.9. Homogeneity plot for Se by UME

Annex 3. Graphs for Short Term Stability Studies

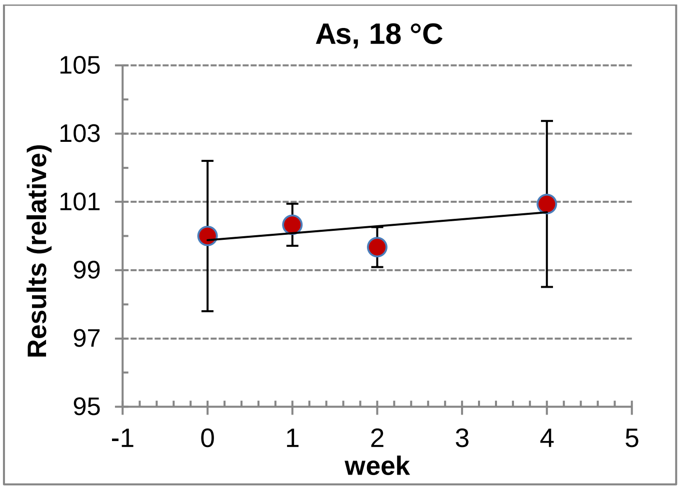


Figure A3.1. STS plot for As at 18 °C by UME

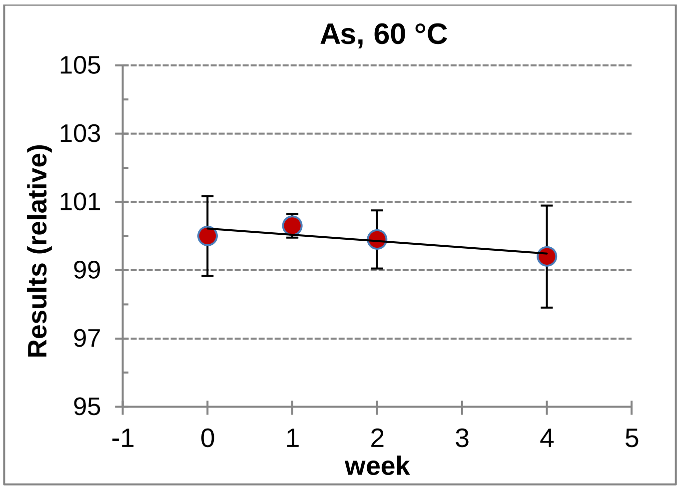


Figure A3.4. STS plot for As at 60 °C by IJS

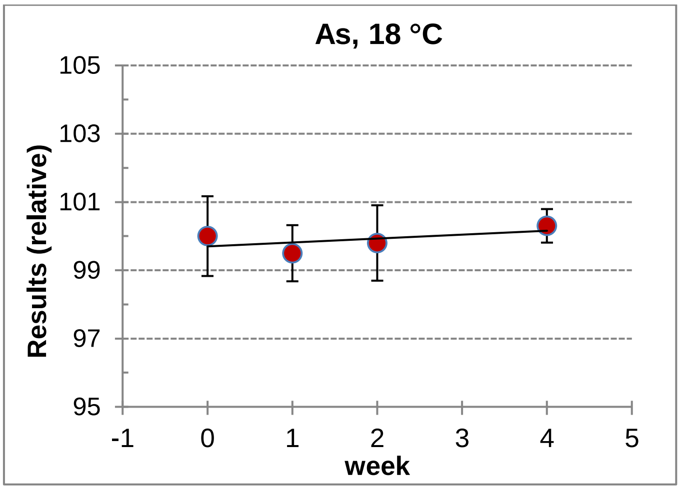


Figure A3.2. STS plot for As at 18 °C by IJS

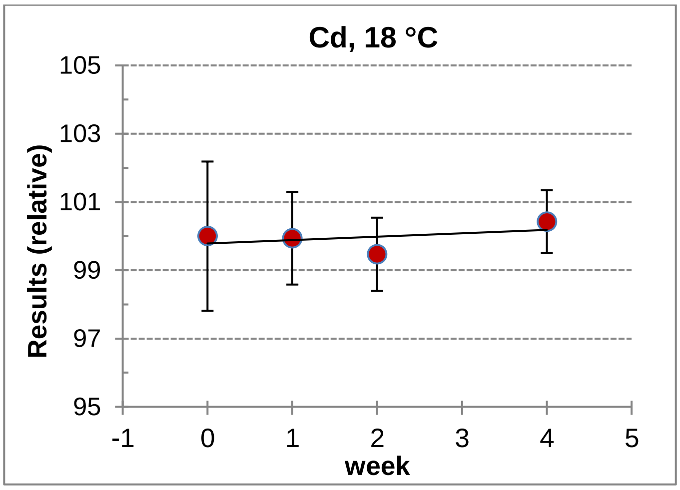


Figure A3.5. STS plot for Cd at 18 °C by UME

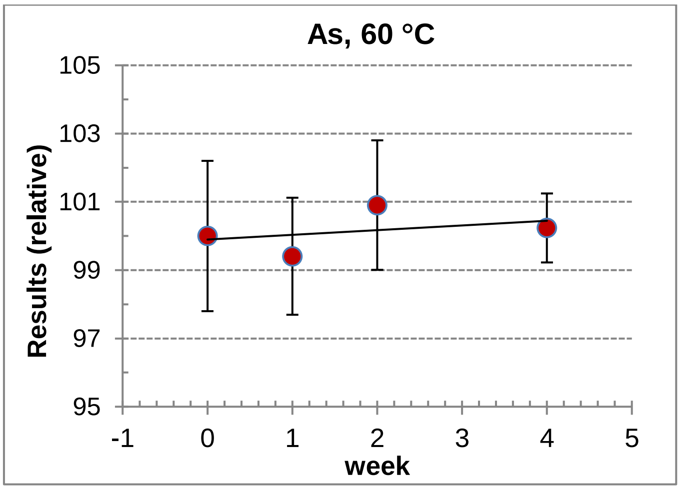


Figure A3.3. STS plot for As at 60 °C by UME

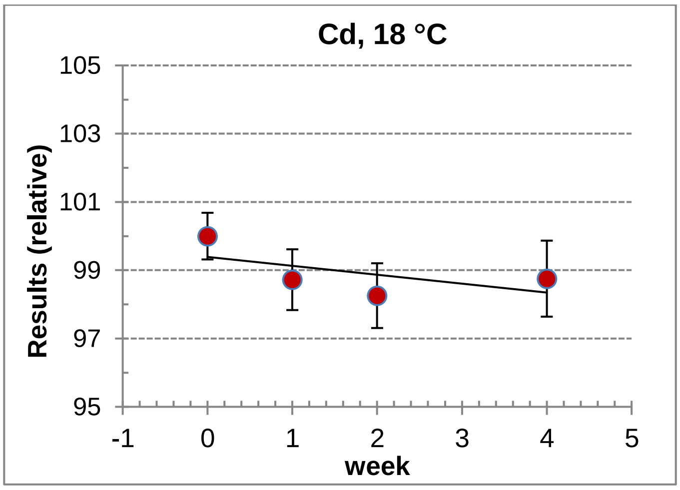


Figure A3.6. STS plot for Cd at 18 °C by IJS

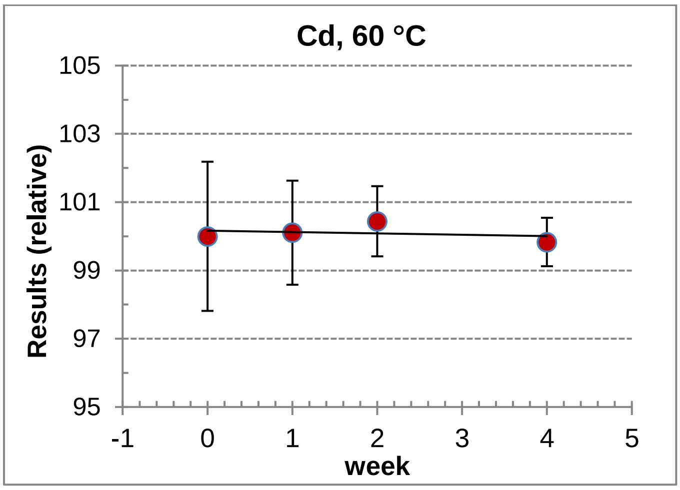


Figure A3.7. STS plot for Cd at 60 °C by UME

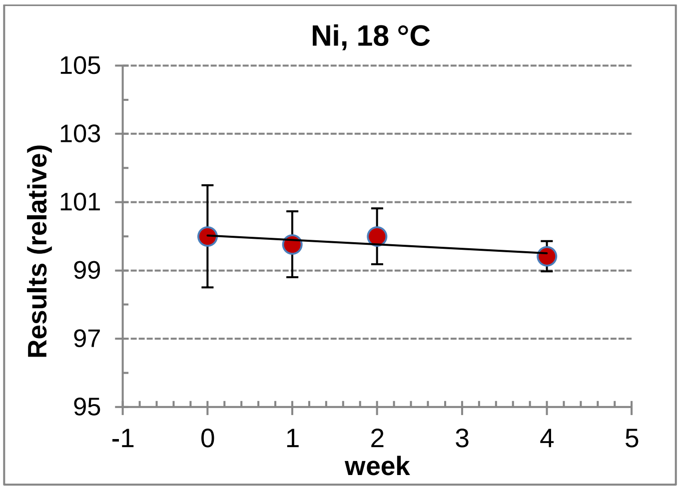


Figure A3.10. STS plot for Ni at 18 °C by IJS

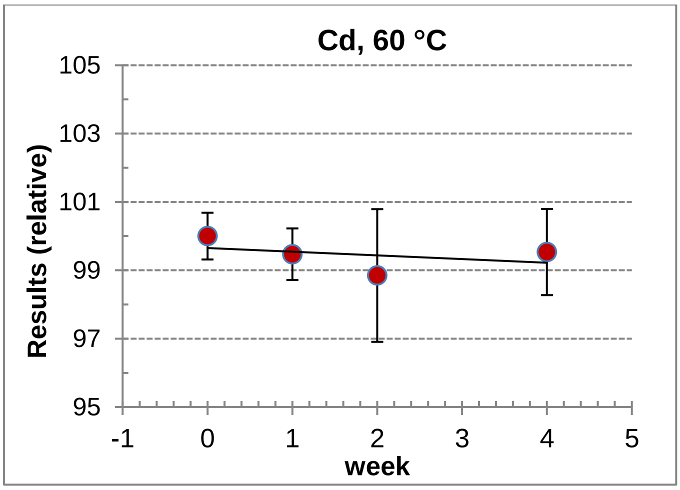


Figure A3.8. STS plot for Cd at 60 °C by IJS

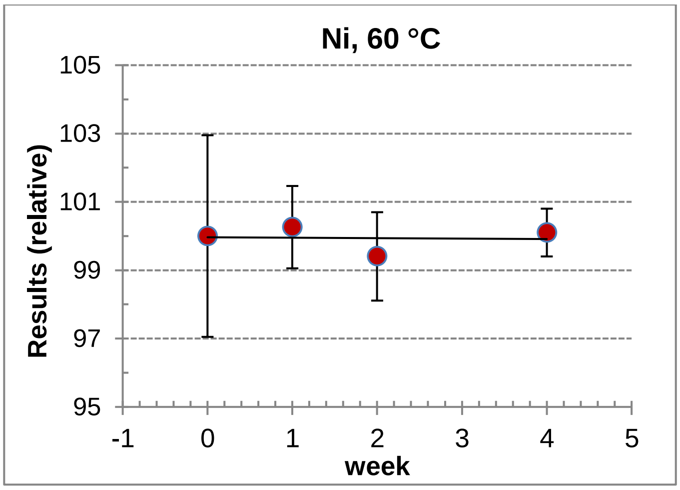


Figure A3.11. STS plot for Ni at 60 °C by UME

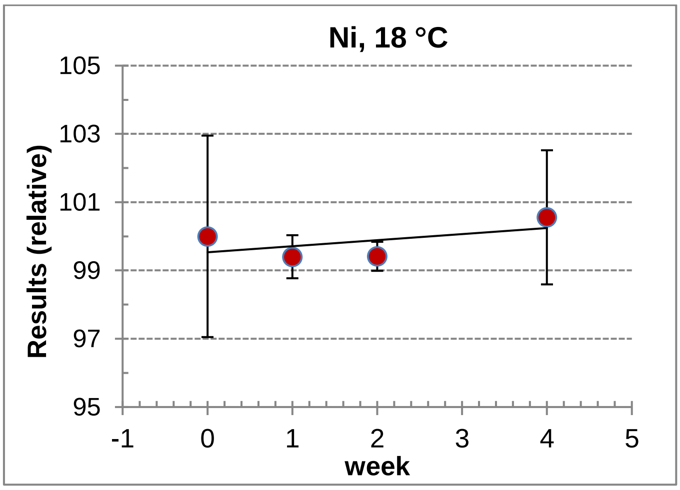


Figure A3.9. STS plot for Ni at 18 °C by UME

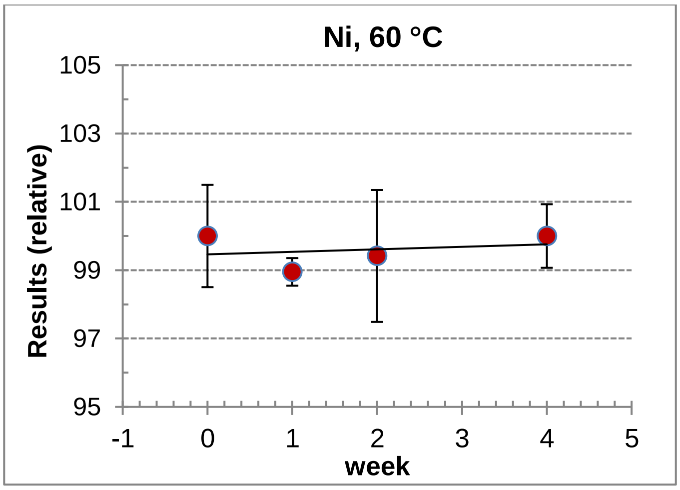


Figure A3.12. STS plot for Ni at 60 °C by IJS

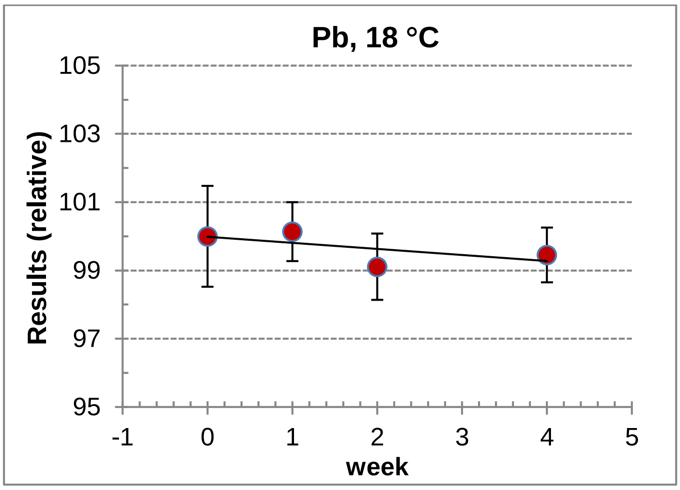


Figure A3.13. STS plot for Pb at 18 °C by UME

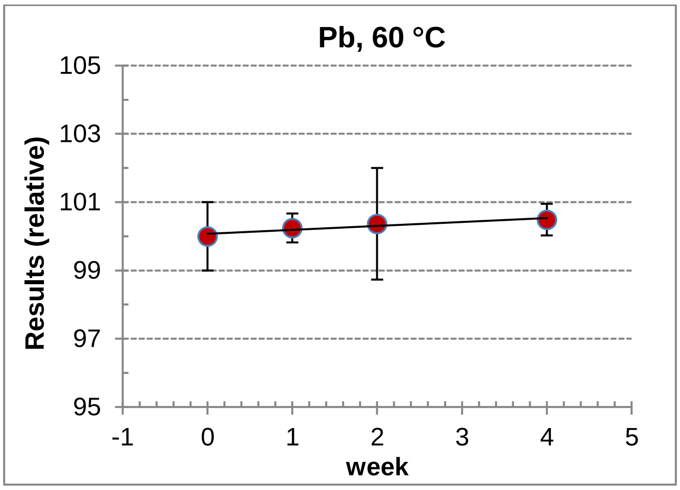


Figure A3.16. STS plot for Pb at 60 °C by IJS

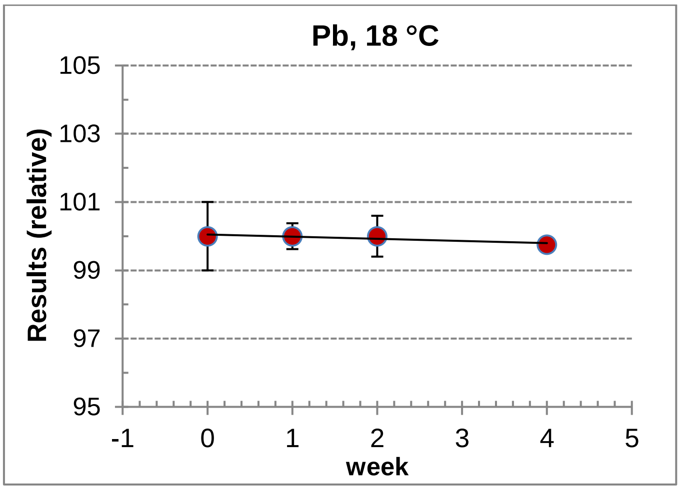


Figure A3.14. STS plot for Pb at 18 °C by IJS

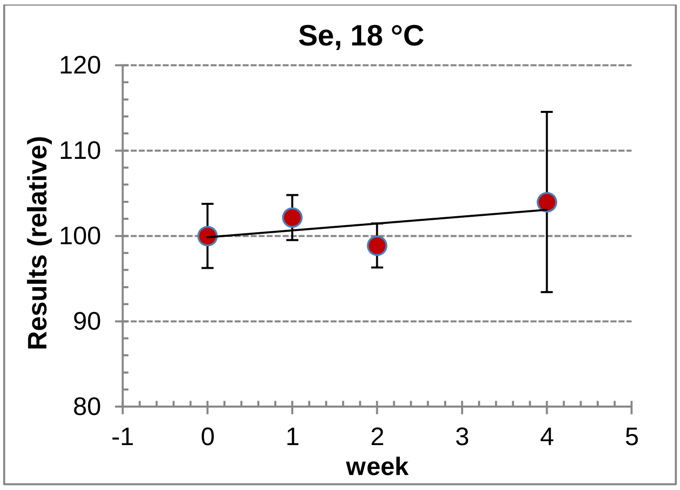


Figure A3.17. STS plot for Se at 18 °C by UME

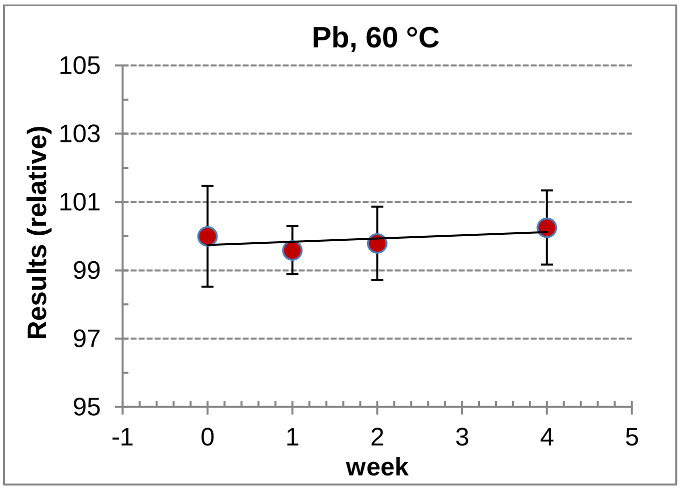


Figure A3.15. STS plot for Pb at 60 °C by UME

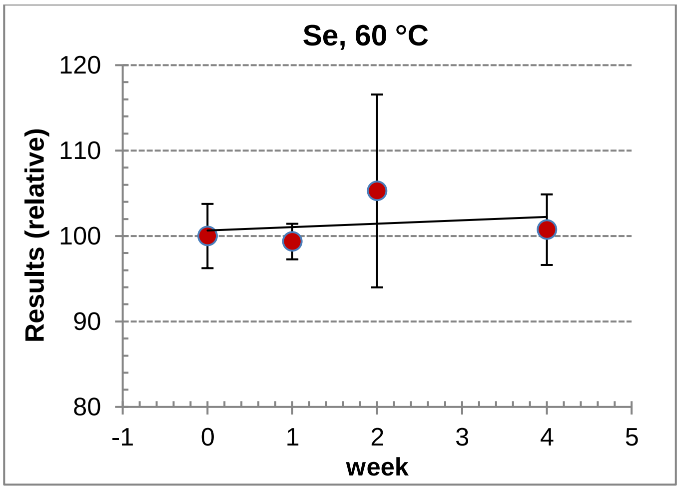


Figure A3.18. STS plot for Se at 60 °C by UME

Annex 4. Graphs for Long Term Stability Studies

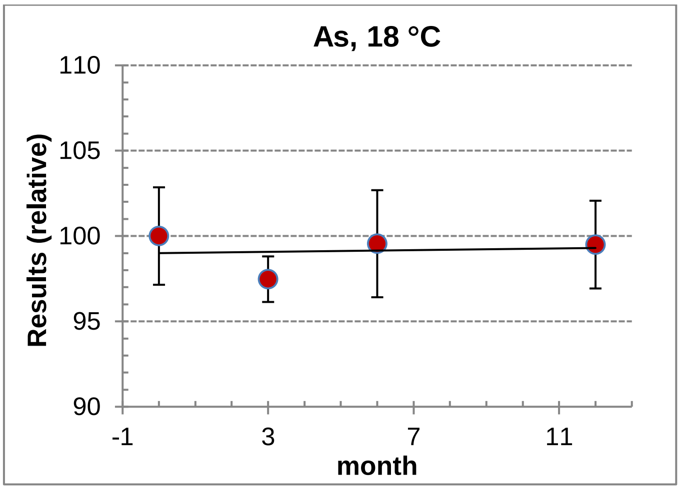


Figure A4.1. LTS plot for As at 18 °C by UME

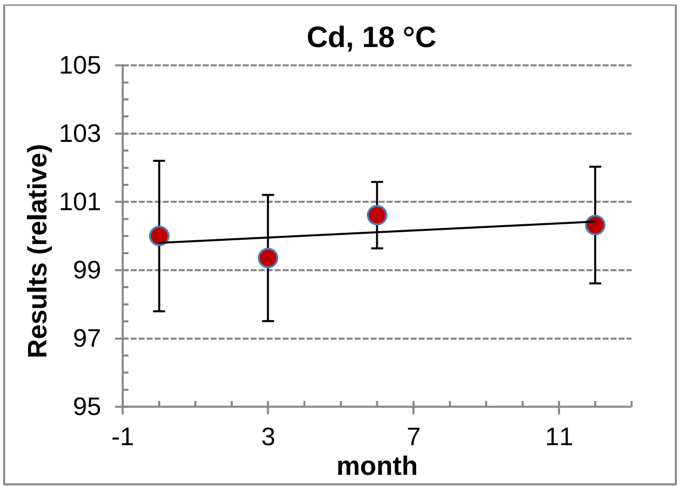


Figure A4.4. LTS plot for Cd at 18 °C by IJS

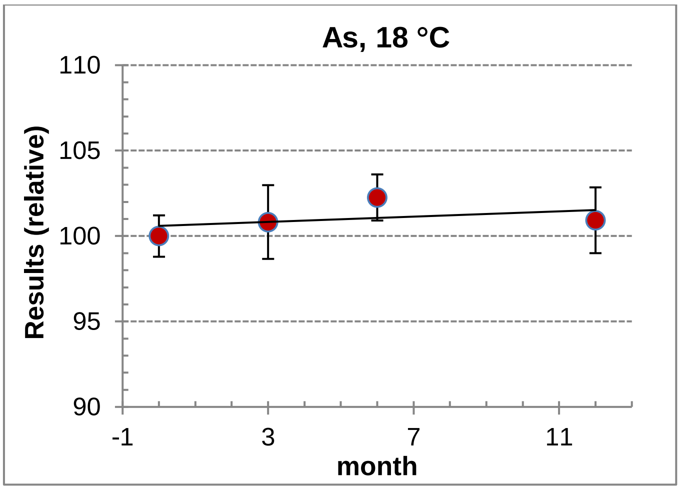


Figure A4.2. LTS plot for As at 18 °C by IJS

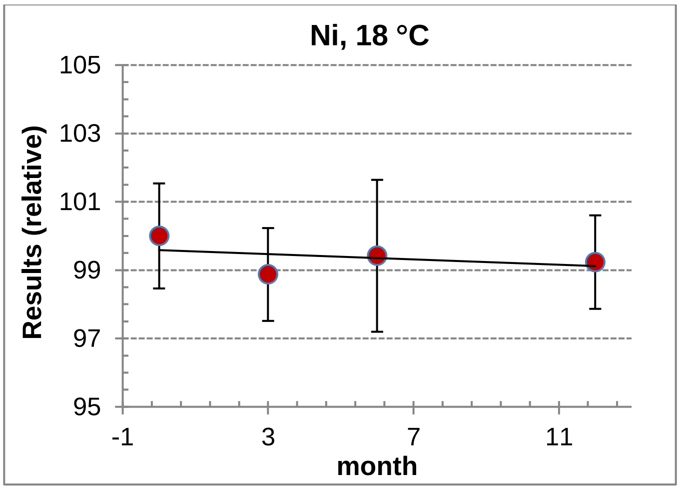


Figure A4.5. LTS plot for Ni at 18 °C by UME

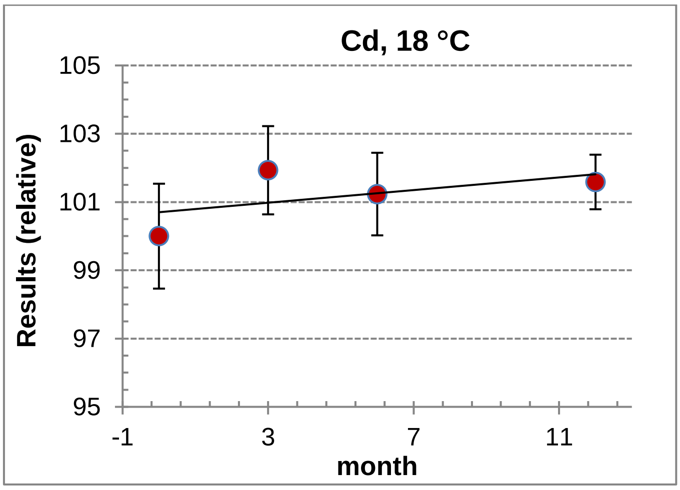


Figure A4.3. LTS plot for Cd at 18 °C by UME

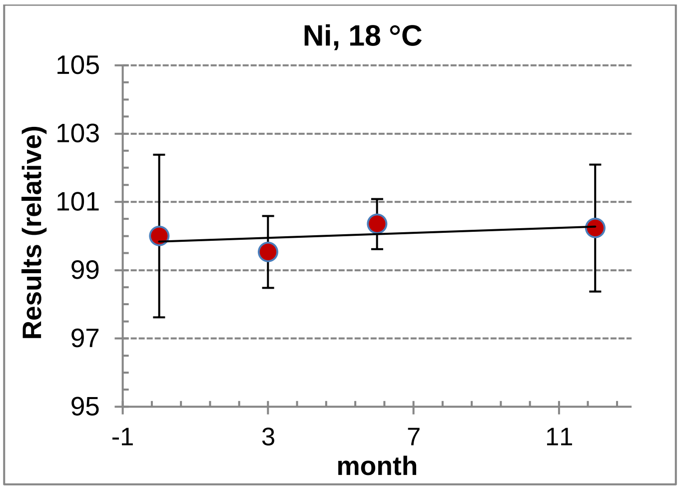


Figure A4.6. LTS plot for Ni at 18 °C by IJS

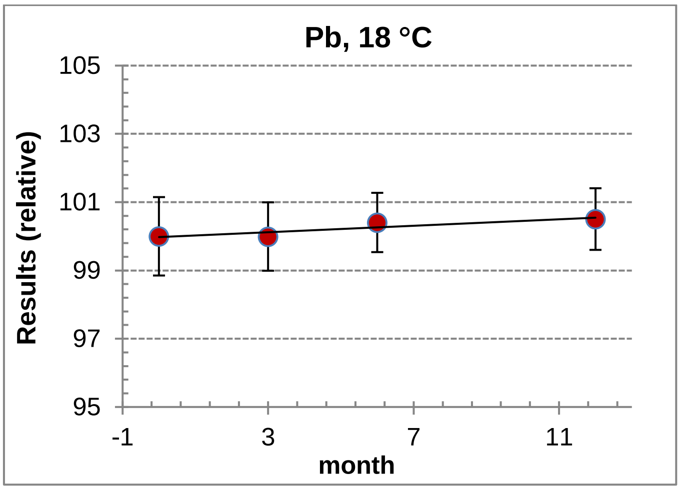


Figure A4.7. LTS plot for Pb at 18 °C by UME

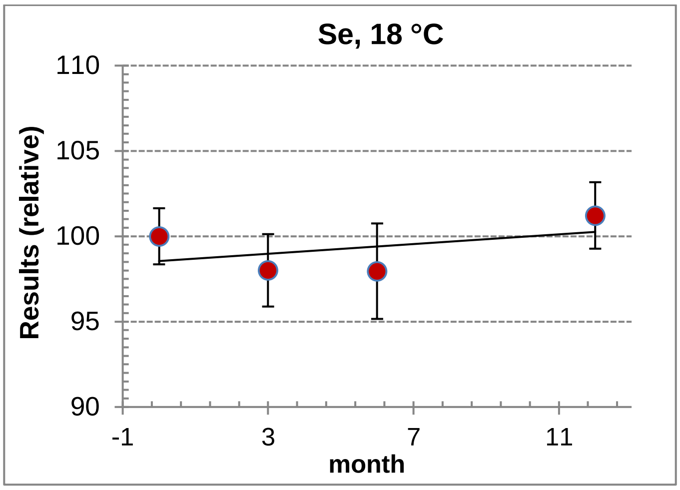


Figure A4.9. LTS plot for Se at 18 °C by UME

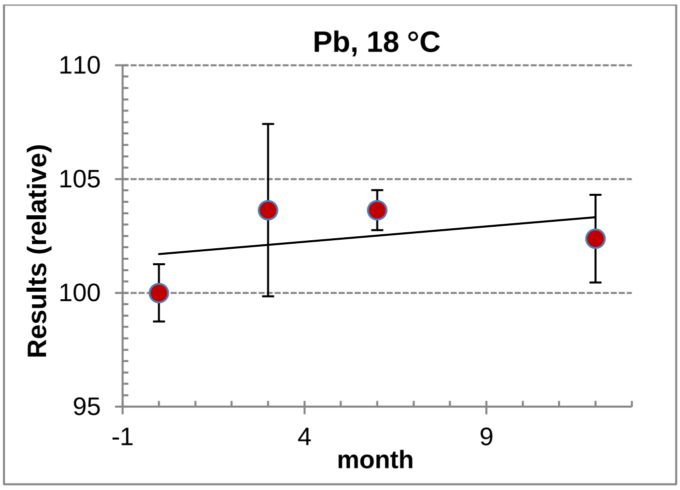


Figure A4.8. LTS plot for Pb at 18 °C by IJS

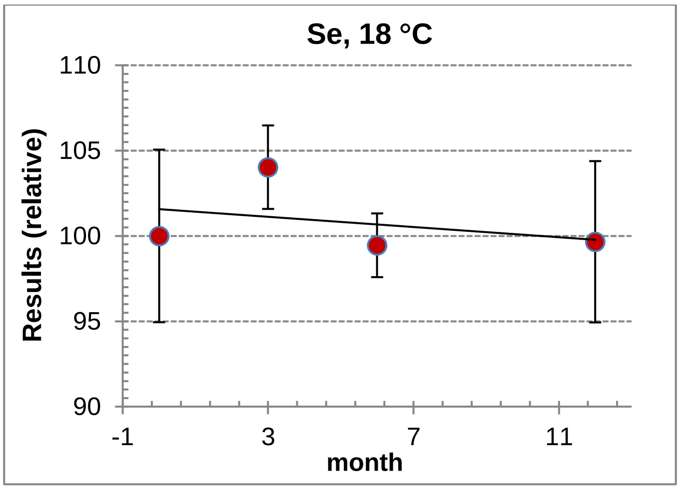


Figure A4.10. LTS plot for Se at 18 °C by IJS

Annex 5. Information About the Methods Used for the Characterization Study

Laboratory	Analytes	Sample Preparation	Calibration Strategy	Method / Technique	CRM(s) used for Calibration and Quality Control
BAM	Cd, Ni, Pb	Sample weighing and spiking with enriched isotope; single spiking with Pb-207, Ni-61 and Cd-111; separate samples were also spiked with Cd-113, which provided identical results	Double IDMS, each element was spiked and measured separately Cd: Cd-111/Cd-113; Cd-112/Cd-111 with correction for Sn-interference Ni: Ni-60/Ni-61 Pb: Pb-208/Pb-207, Pb-206/Pb-207, Pb-204/Pb-207 NIST SRM 981 was used for correction of instrumental mass discrimination	ID-ICP-MS	SI traceability was established with BAM back-spikes, which were inhouse prepared primary calibration solution from the following materials: Ni: BAM RS4; Cd: high-purity Cd, 99.999 %, from Johnson Matthey; Pb: BAM-Y004
DMDM	As, Cd, Ni, Pb	No sample preparation	Using gravimetric method for preparation of multielemental standard for calibration, with single elemental ICP standard solutions. Internal standard used: LGC VHG-LIS2-100 Internal standard Multielement mix 2 in 2% HNO_3.	ICP-MS	ROTH Single element standard solutions of As, Ni, Cd, Pb
GUM	As, Cd, Ni, Pb	None sample preparation procedure. The sample masses stated are the masses of the solutions used for the analysis with the solution flow specific for the equipment.	External calibration method; Standards solutions were prepared by gravimetric dilution of the CRMs. Used wavelength: As = 189,0 nm, Cd = 214,4 nm, Ni = 231,6 nm, Pb = 220,3 nm	ICP-OES	SMU certified reference materials: As-monoelemental aqueous solution B03; Cd-monoelemental aqueous solution B08; Ni-monoelemental aqueous solution B24; Pb - monoelemental aqueous solution B26. NIST SRM 1640a Trace Elements in Natural Water - matrix CRM

Laboratory	Analytes	Sample Preparation	Calibration Strategy	Instrument / Technique	CRM(s) used for Calibration and Quality Control
IJS	As, Cd, Ni, Pb, Se	No sample preparation	External calibration, calibration curve. ^{60}Ni, ^{111}Cd, ^{208}Pb, ^{75}As, ^{78}Se internal standards ^{103}Rh, ^{115}In, ^{209}Bi	ICP-MS	NIST standards; NIST SRM 3136 Ni, NIST SRM 3108 Cd, NIST SRM 3128 Pb, NIST SRM 3103a As, NIST SRM 3149 Se, Reference Materials for Measurement of Elements in Surface Waters (SPS-SW1, Spectrapure Standards AS, Oslo, Norway)
NTUA	As, Cd, Ni, Pb, Se	The samples were analyzed directly without any sample preparation	External calibration with internal standard: ^{75}As (IS ^{72}Ge), ^{111}Cd (IS ^{115}In), ^{59}Ni (IS ^{72}Ge), ^{206}Pb, ^{207} Pb, ^{208}Pb (IS ^{191}Ir), ^{88}Se (IS ^{72}Ge)	ICP-MS	Multi element standard, traceable to NIST standards
	Cd, Pb	Ammonium acetate buffer was added to the samples as supporting electrolyte	Standard addition method according Metrohm VA Application Note No. V-83	ASV	
SYKE	As, Cd, Ni	No preconcentration or matrix separation	As: ICP-MS ^{75}As(IS: ^{103}Rh) Cd: ICP-MS, ^{111}Cd (IS: ^{103}Rh) Ni: ICP-MS ^{60}Ni (IS: ^{103}Rh)	ICP-MS	As: NIST SRM3103a Cd: NIST SRM 3108 Ni: NIST SRM 3136
	Pb		Exact matching double (reverse) IDMS is used for Pb. Both the sample and a certified pure reference material are spiked with an enriched isotope solution. The target isotope ratio of the reference isotope and the enriched isotope is 1 in both blends.	ID-ICP-MS	Pb: NIST SRM 981

Laboratory	Analytes	Sample Preparation	Calibration Strategy	Instrument / Technique	CRM(s) used for Calibration and Quality Control
UME	As	No sample digestion, direct dilution of samples with dilute (1% (v/v)) HNO_3 prior to analysis	Standard addition method	HR ICP-MS	NIST SRM 31XX series CRMs were used for calibration UME CRM 1201 (Spring Water CRM) was used for method development and validation
	Ni		Standard addition method	QQQ-ICP-MS	
	Cd, Pb, Se		Isotope dilution mass spectrometry with isotopically enriched standards	ID-ICP-MS	
UW	As, Cd, Ni, Pb	Samples were filtered before analysis	External calibration curves were used. For the preparation of standard solutions multielemental stock solution was appropriate diluted by 1% HNO3	ICP-MS	SPS-SW1 Batch 114 and SPS-SW2 Batch 118: Reference materials for measurement of elements in surface waters.

Annex 6. Graphs for Characterization Study

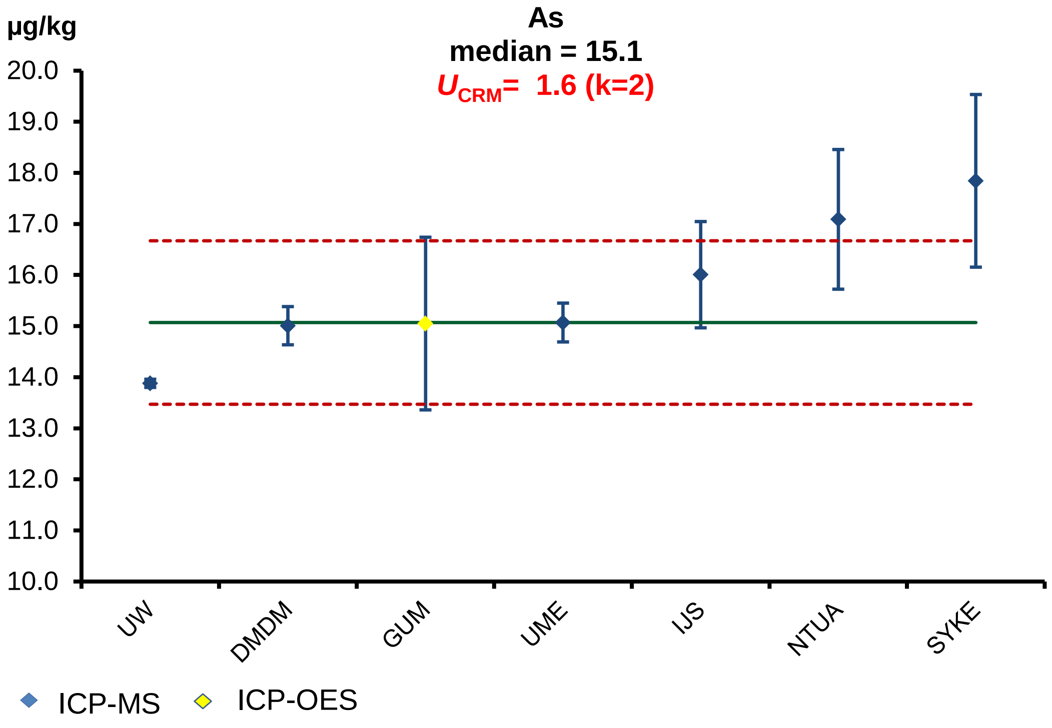


Figure A6.1. Characterization Study Plot for As

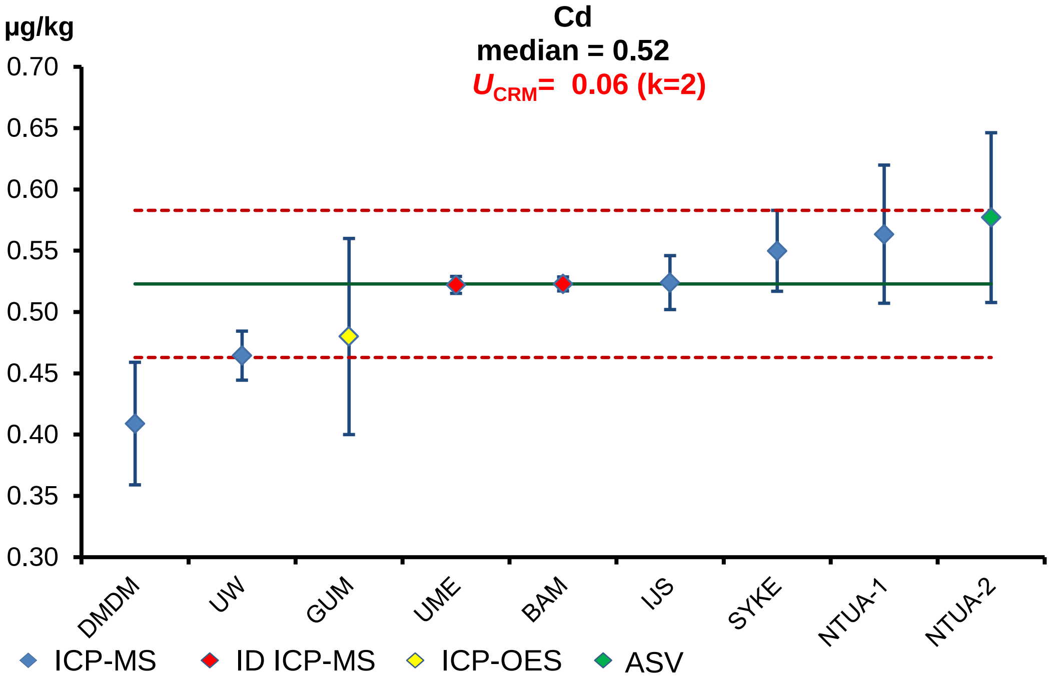


Figure A6.2. Characterization Study Plot for Cd

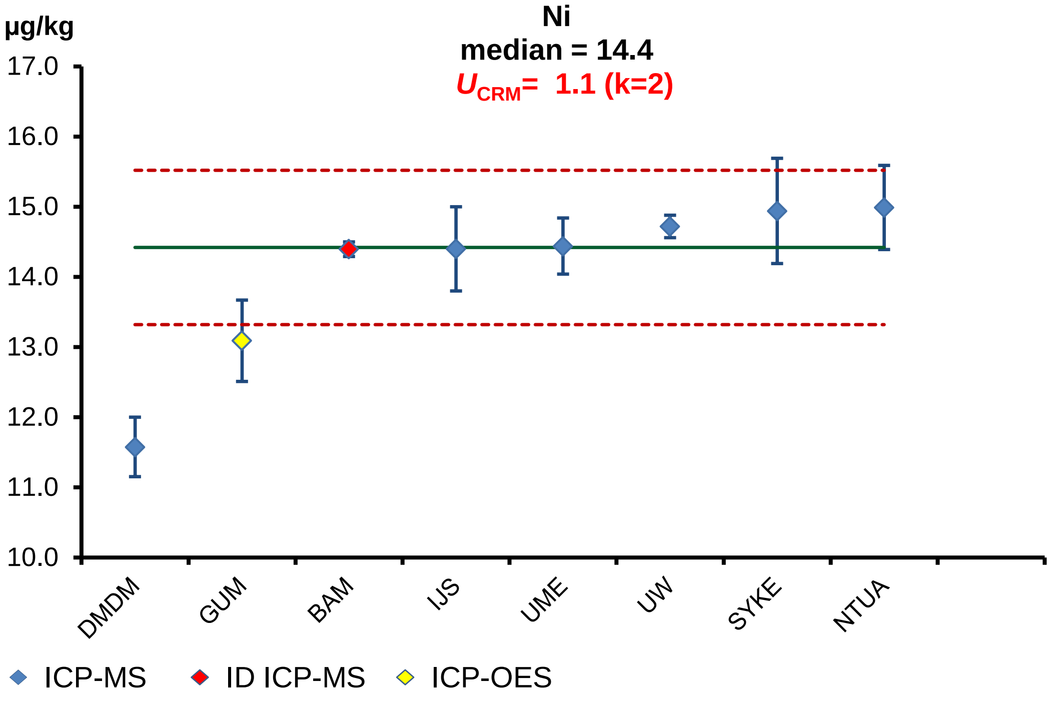


Figure A6.3. Characterization Study Plot for Ni

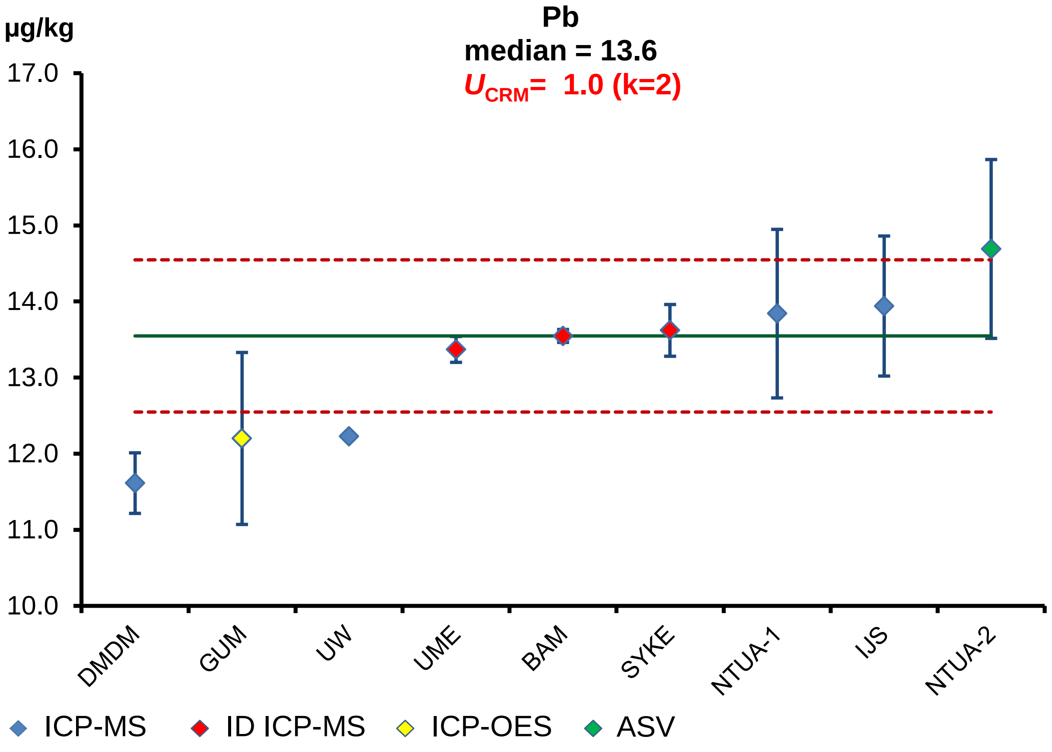


Figure A6.4. Characterization Study Plot for Pb

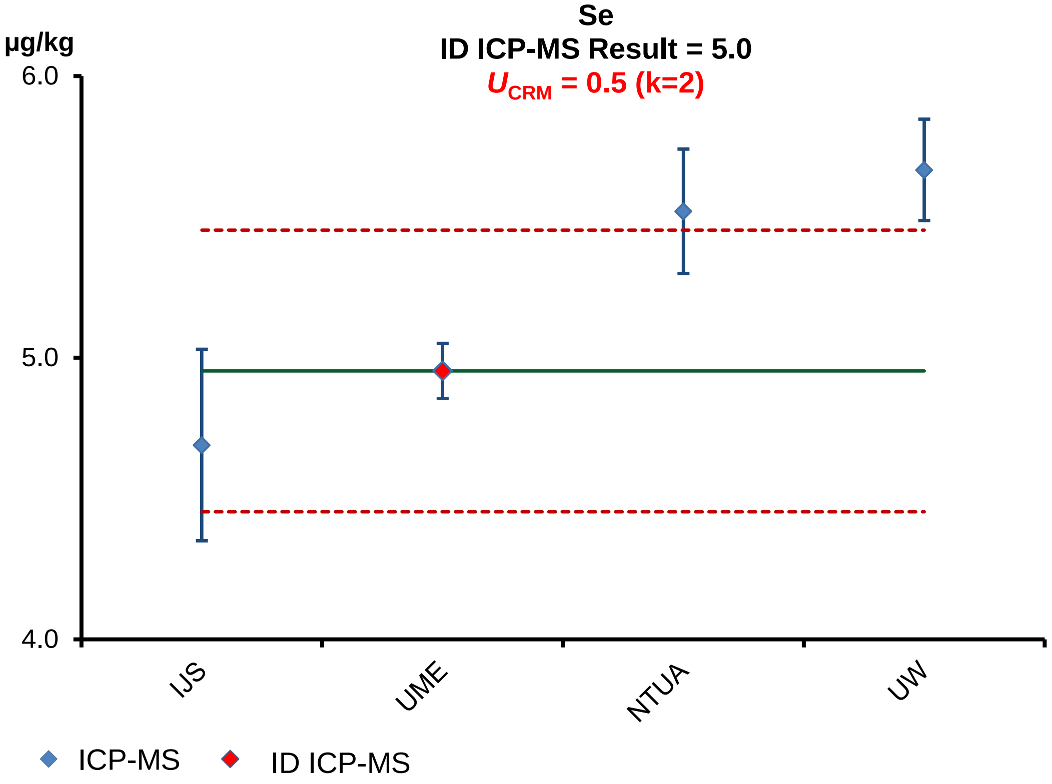


Figure A6.5. Characterization Study Plot for Se

Annex 7. Graph for Supplementary Comparison EURAMET.QM-S11 Study

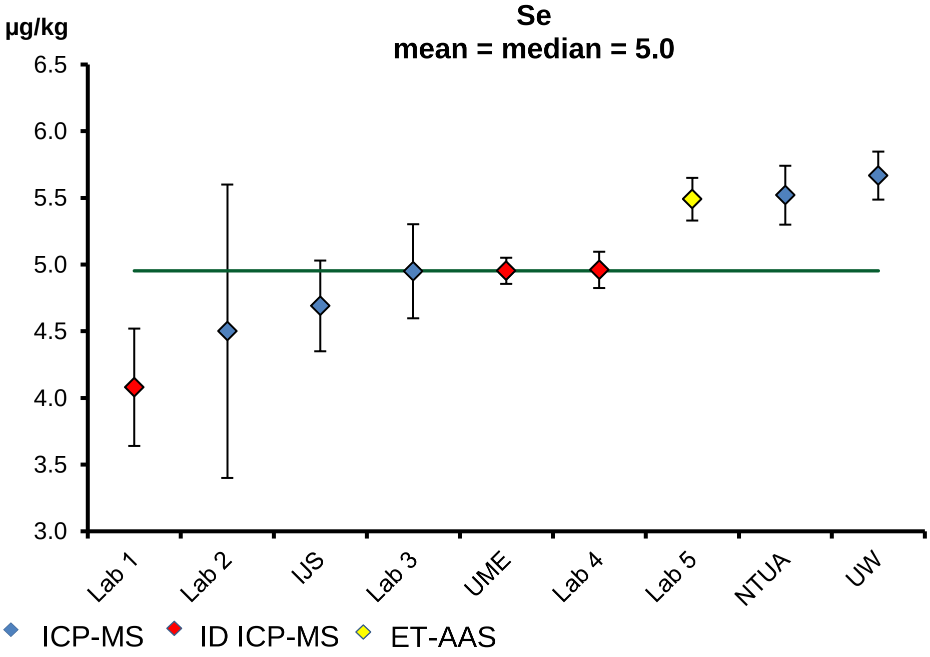


Figure A7.1. Supplementary Comparison Study Plot for Se