UNDP/GEF Danube Regional Project

Preparation of Reference Materials for Analytical Quality Control in the Water Laboratories

Report on the Stability Testing and Conclusions

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A. PROJECT OBJECTIVES

Objective: Ensure the reliability and comparability of monitoring results during the implementation of the transnational water quality monitoring (TNMN) in the Danube river basin (DRB) by providing appropriate reference materials (RM) for analytical quality Control (AQC).

Outputs: Ensure availability of homogenous reference samples (RM), as AQC sample, for analyzing specified pollutant characteristics in water and sediment. The RMs shall be available for performance testing (intercalibration) and intra-laboratory quality control.

This project output will assist DRB-countries to control the water quality monitoring results by reference samples in their laboratories and to ensure sustainable quality work as well as to improve their working quality as needed.

Implementation of the project will ensure the continuity of the quality assurance activities in the DRB which have been developed and maintained since 1995 in the frame of different projects supported from different financial sources, e.g. individual countries, PHARE program and the ICPDR.

B. APPROACH OF WORK IN LINE WITH THE REQUIRED SERVICE

In line with the Work Program of the MLIM Expert Groups, there is a need to ensure and maintain the analytical quality control measures in the water laboratories in the DRB as a basic requirement of the quality assurance in the trans-national monitoring. This Project provides significant quantities of water and sediment RMs.

1. Water RMs:

based on earlier experience concerning the problem of determination of the different river quality characteristics the target determinands include nutrients, i.e. nitrogen and phosphorus forms, as well as heavy metals.

The compounds of interest in reference materials present in about the same concentration range (no more than a factor of 5-10) as in the Danube samples which are usually analyzed.

The concentrates for analyses of nutrients contain:

- ammonium-N,
- nitrate-N,
- phosphate-P,
- total P.

The concentrates for analyses of heavy metals contain:

- cadmium,
- copper,
- chromium,
- lead,
- nickel,
- zinc,
- mercury,
- aluminum,
- arsenic

C. REQUIREMENTS FOR REFERENCE MATERIALS

The basic requirements which a reference material must fulfill in order to be of any use, it should be:

- representative,
- homogeneous,
- stable.

Representative:

The RM must represent water and/or sediment sample containing the range of determinands normally expected in the normally tested sample.

Homogeneous:

In order to results obtained with reference samples will be reproducible the distribution of determinands within the material must be homogeneous.

Stable:

The RM must also remain stable over a defined period of time, that is, the concentration of determinands present must remain within defined limits. As water sample would not be stable, so some means of preservation is required.

D. THE PROCEDURE FOR PRODUCING WATER RMs

In the case of water reference materials all the preparation steps and tests were specified. The procedure was the following:

a) preparation of concentrates

b) analyses

c) bottling

d) sterilization + repeated analyses (in the case of nutrients only)

e) storage

f) testing

g) transport

preparation of concentrates:

analytical grade reagents were measured into an appropriate flask and diluted with distilled water. After homogenization – by mechanical stirring – solutions were taken into volumetric flasks and filled to the mark. The solutions were preserved according to ISO 56667-3 Standard.

analyses: were made according to Hungarian and ISO Standards

bottling: reclosable bottles were filled manually

sterilization: bottles for nutrients were placed into autoclave to prevent any change due to microorganisms

analyses: after sterilization analyses were repeated

storage: a/ in refrigerator (≤ 4° C)

b/ in room temperature (22° C)

testing: stability had been tested between bottles. All samples were measured on the same day, by the same technicians.

transport: by courier post

The concentrated solutions should be enough to prepare one litre of water sample ready for analyses.

For practical reasons the first series of nutrients consist of five reclosable bottles for each laboratory to make five dilutions at least.

Both nitrogen and phosphorus form are included in each bottle.

The first series of heavy metals consist of four reclosable bottles for each laboratory to make some dilutions (at least five).

The second series of heavy metals consist of eight reclosable bottles, including five bottles for mercury. As concentrate of mercury solution is rather low, it is advised to use the mercury reference material concentrate once time after opening the small bottles and not to use again the remaining content of the bottles (working instruction was attached to the distributed RMs).

The separated concentrates for heavy metals are the following:

Cd, Cr, Cu

Pb, Ni, Zn

Hg

Al, As

Expiration period of water RMs is one year after the preparation.

Assigned values: in the case of nutrients sub-samples were tested in three replicates per day during a week and the assigned values were calculated from these data. Nominal concentrations were considered as assigned values for the artificially prepared heavy metals samples.

The assigned values of water RMs are attached in **Annex 1**.

E. STABILITY TEST

The most common approach to stability testing is to expose the RM to conditions that are likely to lead to degradation or transformation of the analyte. Such changes in analyte concentration can be due to many sources, e.g., thermal degradation or conversion, microbiological activity, photo-degradation.

For stability testing selected bottles for

> nutrients were sterilized to negligate any degradation due to microbiological activity and stored at room temperature (22 °C), as well as at ≤ 4 °C, where no significant changes are expected.

In parallel not sterilized samples were also storage at ≤ 4 ^{o}C comparing any changes into concentration to sterilized samples.

Heavy metals samples were stored at:

- the normal room temperature (22 ^{o}C)
- at a temperature, where no changes are expected (≤ 4 ^{o}C).

Stability was evaluated by analysing samples stored at different temperatures. The time steps: 0.5, 1, 3 month.

Independent analyses were carried out in triplicate for each storage temperature and time.

The evaluation makes use of the ratio R_T, which is based on the mean values (x_T) of three determination made after storage at room temperature, divide by the mean value for three measurements of samples stored at the safe temperature (X_{-s}):

$$R_T = X_T / X_s$$

.

R_T was calculated for each storage interval.

In order to minimise the day-to day variation, the mean values that were used for the calculation R_T, were obtained from analyses that were performed on the same day.

In the case of "ideal" stability, the ratios R_T should be equal to 1.00, but due to the uncertainty of the measurements there may be some random variations.

The uncertainty (U_T) was obtained from the coefficient of variation (CV) of three measurements obtained at each temperature:

$U_T=(CV_T^2+CV^2{}_4{}^oC)^{1/2} * R_T$

No instability can be concluded when the values 1 are comprised between the respective ranges: $R_T \pm U_T$.

The mean values for the analyses at the different times and for the two storage temperatures are together with the respective standard deviations and variation coefficients presented in **Table 1- 10**.

Results compared sterilized and not sterilized samples are summarized in **Table 11-12.**

Graphical presentations of the stability test results can be seen on **Fig.1-12.**

Table 1. Evaluation of stability testing using the safe temperature ratios

Nutrients (RM-1)

		Storage at + 22 °C				Storage at + 4 °C			
mg/l		NH_4^+-N	NO_3^--N	PO_4^{3-}-P	TP	NH_4^+-N	NO_3^--N	PO_4^{3-}-P	TP
t = 0,5 month		0,462	1,83	0,127	0,230	0,464	1,82	0,131	0,233
		0,463	1,81	0,133	0,228	0,467	1,83	0,131	0,228
		0,461	1,82	0,128	0,229	0,465	1,84	0,130	0,230
	mean	0,462	1,82	0,129	0,229	0,465	1,83	0,131	0,230
	S.D.	0,001	0,010	0,003	0,001	0,002	0,010	0,001	0,003
	CV	0,216	0,549	2,485	0,437	0,328	0,546	0,442	1,093
t = 1 month		0,465	1,80	0,131	0,225	0,464	1,83	0,130	0,228
		0,460	1,82	0,127	0,230	0,465	1,83	0,132	0,231
		0,460	1,80	0,133	0,231	0,465	1,82	0,131	0,232
	mean	0,462	1,81	0,130	0,229	0,465	1,83	0,131	0,230
	S.D.	0,003	0,012	0,003	0,003	0,001	0,006	0,001	0,002
	CV	0,625	0,639	2,344	1,406	0,124	0,316	0,763	0,90
t = 2 months		0,465	1,81	0,131	0,230	0,465	1,82	0,128	0,232
		0,456	1,79	0,129	0,229	0,463	1,84	0,133	0,228
		0,459	1,82	0,129	0,229	0,466	1,83	0,131	0,229
	mean	0,460	1,81	0,130	0,229	0,465	1,83	0,131	0,230
	S.D.	0,005	0,015	0,001	0,001	0,002	0,010	0,003	0,002
	CV	0,996	0,845	0,891	0,252	0,329	0,546	1,926	0,906
t = 0,5 month	R_T	0,99	0,99	0,99	0,99				
	U_T	0,00	0,01	0,02	0,01				
	R_T+U_T	1,00	1,00	1,01	1,01				
	R_T-U_T	0,99	0,99	0,96	0,98				
t = 1 month	R_T	0,99	0,99	0,99	0,99				
	U_T	0,01	0,01	0,02	0,02				
	R_T+U_T	1,00	1,00	1,02	1,01				
	R_T-U_T	0,99	0,98	0,97	0,98				
t = 2 months	R_T	0,99	0,99	0,99	1,00				
	U_T	0,01	0,01	0,02	0,01				
	R_T+U_T	1,00	1,00	1,01	1,01				
	R_T-U_T	0,98	0,98	0,97	0,99				

Table 2. Evaluation of stability testing using the safe temperature ratios

Nutrients (RM-2)

		Storage at + 22 °C				Storage at + 4 °C			
	mg/l	NH_4^+-N	NO_3^--N	PO_4^{3-}-P	TP	NH_4^+-N	NO_3^--N	PO_4^{3-}-P	TP
t = 0,5 month		0,695	2,24	0,214	0,316	0,703	2,35	0,213	0,319
		0,700	2,53	0,213	0,320	0,706	2,43	0,215	0,318
		0,706	2,27	0,217	0,321	0,699	2,28	0,217	0,322
	mean	0,700	2,35	0,215	0,319	0,703	2,35	0,215	0,320
	S.D.	0,006	0,159	0,002	0,003	0,004	0,075	0,002	0,002
	CV	0,786	6,796	0,970	0,829	0,500	3,189	0,930	0,651
t = 1 month		0,697	2,26	0,212	0,317	0,703	2,35	0,212	0,321
		0,706	2,45	0,214	0,322	0,701	2,37	0,216	0,319
		0,703	2,29	0,216	0,319	0,703	2,31	0,214	0,322
	mean	0,702	2,33	0,214	0,319	0,702	2,34	0,214	0,321
	S.D.	0,005	0,102	0,002	0,003	0,001	0,031	0,002	0,002
	CV	0,653	4,378	0,935	0,788	0,164	1,304	0,935	0,476
t = 2 months		0,701	2,27	0,210	0,315	0,703	2,32	0,209	0,323
		0,706	2,39	0,217	0,329	0,704	2,41	0,210	0,318
		0,704	2,27	0,211	0,310	0,699	2,28	0,219	0,321
	mean	0,704	2,31	0,213	0,318	0,702	2,34	0,213	0,321
	S.D.	0,003	0,069	0,004	0,010	0,003	0,067	0,006	0,003
	CV	0,358	2,999	1,780	3,097	0,377	2,849	2,590	0,785
t = 0,5 month	R_T	1,00	1,00	1,00	1,00				
	U_T	0,01	0,07	0,01	0,01				
	R_T+U_T	1,01	1,07	1,01	1,01				
	R_T-U_T	0,99	0,92	0,99	0,99				
t = 1 month	R_T	1,00	1,00	1,00	1,00				
	U_T	0,01	0,05	0,01	0,01				
	R_T+U_T	1,01	1,04	1,01	1,01				
	R_T-U_T	0,99	0,95	0,99	0,99				
t = 2 months	R_T	1,00	0,99	1,00	0,99				
	U_T	0,01	0,04	0,03	0,03				
	R_T+U_T	1,01	1,03	1,03	1,02				
	R_T-U_T	1,00	0,95	0,97	0,96				

Table 3. Evaluation of stability testing using the safe temperature ratios

Heavy metals (RM-1)

		Storage at + 22 ° C			Storage at + 4 ° C		
µg/l		**Cd**	**Cr**	**Cu**	**Cd**	**Cr**	**Cu**
t = 0,5 month		1,97 1,99 2,03	15,50 15,20 15,30	15,00 15,00 14,99	1,99 2,00 2,02	15,40 15,35 15,35	15,02 15,00 14,99
	mean S.D. CV	2,00 0,031 1,530	15,33 0,153 0,996	15,00 0,006 0,038	2,00 0,015 0,762	15,37 0,029 0,188	15,00 0,015 0,102
t = 1 month		1,96 2,04 1,97	15,50 15,10 15,30	15,00 15,00 14,98	1,98 2,03 2,02	15,40 15,25 15,45	15,01 15,00 14,99
	mean S.D. CV	1,99 0,04 2,19	15,30 0,200 1,307	14,99 0,012 0,077	2,01 0,03 1,32	15,37 0,10 0,68	15,00 0,010 0,067
t = 2 months		1,98 2,02 2,00	15,50 15,30 15,40	15,08 15,04 14,96	2,04 2,01 1,96	15,40 15,25 15,45	15,06 15,00 15,00
	mean S.D. CV	2,00 0,02 1,00	15,40 0,10 0,65	15,03 0,061 0,407	2,00 0,04 2,02	15,37 0,10 0,68	15,02 0,035 0,231
t = 0,5 month	R_T U_T	1,00 0,02	1,00 0,01	1,00 0,00			
	R_T+U_T R_T-U_T	1,01 0,98	1,01 0,99	1,00 1,00			
t = 1 month	R_T U_T	0,99 0,03	1,00 0,01	1,00 0,00			
	R_T+U_T R_T-U_T	1,02 0,96	1,01 0,98	1,00 1,00			
t = 2 month	R_T U_T	1,00 0,02	1,00 0,01	1,00 0,00			
	R_T+U_T R_T-U_T	1,02 0,98	1,01 0,99	1,01 1,00			

Table 4. Evaluation of stability testing using the safe temperature ratios

Heavy metals (RM-2)

		Storage at + 22 ^{o} C			Storage at + 4 ^{o} C		
μg/l		**Cd**	**Cr**	**Cu**	**Cd**	**Cr**	**Cu**
t = 0,5 month		1,00	6,06	8,00	1,00	6,00	8,04
		0,99	6,00	7,99	1,01	6,05	7,99
		1,00	6,04	8,00	1,00	6,01	8,00
	mean	1,00	6,03	8,00	1,00	6,02	8,01
	S.D.	0,01	0,03	0,01	0,01	0,03	0,03
	CV	0,58	0,51	0,07	0,58	0,44	0,33
t = 1 month		1,00	6,01	8,10	0,99	5,99	7,90
		0,99	6,10	7,98	1,02	6,15	8,05
		1,00	5,94	8,00	1,00	6,00	8,00
	mean	1,00	6,02	8,03	1,00	6,05	7,98
	S.D.	0,01	0,08	0,06	0,02	0,09	0,08
	CV	0,58	1,33	0,80	1,52	1,48	0,96
t = 2 months		0,98	6,12	8,09	1,01	6,15	7,95
		1,02	5,95	8,00	0,99	5,94	8,02
		1,03	5,96	7,95	1,02	5,99	8,00
	mean	1,01	6,01	8,01	1,01	6,03	7,99
	S.D.	0,03	0,10	0,071	0,02	0,11	0,036
	CV	2,62	1,59	0,885	1,52	1,82	0,451
t = 0,5 month	R_T	0,99	1,00	1,00			
	U_T	0,01	0,01	0,00			
	R_T+U_T	1,00	1,01	1,00			
	R_T-U_T	0,99	1,00	0,99			
t = 1 month	R_T	0,99	1,00	1,01			
	U_T	0,02	0,02	0,01			
	R_T+U_T	1,01	1,01	1,02			
	R_T-U_T	0,98	0,98	0,99			
t = 2 months	R_T	1,00	1,00	1,00			
	U_T	0,03	0,02	0,01			
	R_T+U_T	1,03	1,02	1,01			
	R_T-U_T	0,97	0,97	0,99			

Table 5. Evaluation of stability testing using the safe temperature ratios

Heavy metals (RM-1)

		Storage at + 22 ^{o} C			Storage at + 4 ^{o} C		
μg/l		**Ni**	**Pb**	**Zn**	**Ni**	**Pb**	**Zn**
t = 0,5 month		9,90	9,90	80,10	10,15	10,13	80,00
		9,99	9,70	79,91	9,98	9,89	79,99
		10,03	10,05	79,90	9,98	9,85	80,00
	mean	9,97	9,88	79,97	10,04	9,96	80,00
	S.D.	0,07	0,18	0,11	0,10	0,15	0,01
	CV	0,67	1,78	0,14	0,98	1,52	0,01
t = 1 month		9,92	10,10	81,00	10,05	10,05	80,40
		10,09	9,97	80,02	10,02	10,00	79,59
		9,99	9,80	79,56	9,99	9,97	80,05
	mean	10,00	9,96	80,19	10,02	10,01	80,01
	S.D.	0,09	0,15	0,74	0,03	0,04	0,41
	CV	0,85	1,51	0,92	0,30	0,40	0,51
t = 2 months		9,92	10,06	80,40	10,00	10,35	80,10
		9,98	9,94	81,00	10,00	9,90	80,00
		10,02	9,98	79,50	9,98	9,94	80,00
	mean	9,97	9,99	80,30	9,99	10,06	80,03
	S.D.	0,05	0,06	0,755	0,01	0,25	0,058
	CV	0,50	0,61	0,940	0,12	2,47	0,072
t = 0,5 month	R_T	0,99	0,99	1,00			
	U_T	0,01	0,02	0,00			
	R_T+U_T	1,01	1,02	1,00			
	R_T-U_T	0,98	0,97	1,00			
t = 1 month	R_T	1,00	1,00	1,00			
	U_T	0,01	0,02	0,01			
	R_T+U_T	1,01	1,01	1,01			
	R_T-U_T	0,99	0,98	0,99			
t = 2 months	R_T	1,00	0,99	1,00			
	U_T	0,01	0,03	0,01			
	R_T+U_T	1,00	1,02	1,01			
	R_T-U_T	0,99	0,97	0,99			

Table 6. Evaluation of stability testing using the safe temperature ratios

Heavy metals (RM-2)

		Storage at + 22 ^{o} C			Storage at + 4 ^{o} C		
μg/l		**Ni**	**Pb**	**Zn**	**Ni**	**Pb**	**Zn**
t = 0,5 month		5,06	8,00	10,02	5,00	7,99	10,00
		5,00	7,85	10,00	5,01	7,89	10,00
		5,00	7,90	10,00	5,01	7,95	10,00
	mean	5,02	7,92	10,01	5,01	7,94	10,00
	S.D.	0,03	0,08	0,01	0,01	0,05	0,00
	CV	0,69	0,96	0,12	0,12	0,63	0,00
t = 1 month		5,02	7,93	9,99	5,03	8,02	10,03
		5,04	7,99	9,98	5,04	7,97	10,02
		4,99	7,90	10,00	5,00	7,95	9,98
	mean	5,02	7,94	9,99	5,02	7,98	10,01
	S.D.	0,03	0,05	0,01	0,02	0,04	0,03
	CV	0,50	0,58	0,10	0,41	0,45	0,26
t = 2 months		5,05	7,99	10,15	5,06	8,35	10,08
		5,06	7,85	10,01	5,01	7,86	10,02
		4,98	7,89	10,00	5,01	7,85	9,98
	mean	5,03	7,91	10,05	5,03	8,02	10,03
	S.D.	0,04	0,07	0,084	0,03	0,29	0,050
	CV	0,87	0,91	0,834	0,57	3,56	0,502
t = 0,5 month	R_T	1,00	1,00	1,00			
	U_T	0,01	0,01	0,00			
	R_T+U_T	1,01	1,01	1,00			
	R_T-U_T	1,00	0,99	1,00			
t = 1 month	R_T	1,00	0,99	1,00			
	U_T	0,01	0,01	0,00			
	R_T+U_T	1,01	1,00	1,00			
	R_T-U_T	0,99	0,99	1,00			
t = 2 months	R_T	1,00	0,99	1,00			
	U_T	0,01	0,04	0,01			
	R_T+U_T	1,01	1,02	1,01			
	R_T-U_T	0,99	0,95	0,99			

Table 7. Evaluation of stability testing using the safe temperature ratios

Heavy metals (RM-1)

		Storage at + 22 ° C		Storage at + 4 ° C	
µg/l		**Al**	**As**	**Al**	**As**
t = 0,5 month		9,90	9,89	9,96	10,01
		9,95	9,99	9,99	9,90
		10,10	9,98	10,10	10,00
	mean	9,98	9,95	10,02	9,97
	S.D.	0,10	0,06	0,07	0,06
	CV	1,04	0,55	0,74	0,61
t = 1 month		9,79	10,05	9,98	9,93
		10,12	9,95	9,99	10,10
		10,05	9,95	10,03	9,90
	mean	9,99	9,98	10,00	9,98
	S.D.	0,17	0,06	0,03	0,11
	CV	1,74	0,58	0,26	1,08
t = 2 months		9,94	9,77	10,15	10,10
		10,10	10,15	10,05	10,03
		9,98	9,98	9,99	9,95
	mean	10,01	9,97	10,06	10,03
	S.D.	0,08	0,19	0,08	0,08
	CV	0,83	1,91	0,80	0,75
t = 0,5 month	R_T	1,00	1,00		
	U_T	0,01	0,01		
	R_T+U_T	1,01	1,01		
	R_T-U_T	0,98	0,99		
t = 1 month	R_T	1,00	1,00		
	U_T	0,02	0,01		
	R_T+U_T	1,02	1,01		
	R_T-U_T	0,98	0,99		
t = 2 months	R_T	0,99	0,99		
	U_T	0,01	0,02		
	R_T+U_T	1,01	1,01		
	R_T-U_T	0,98	0,97		

Table 8. Evaluation of stability testing using the safe temperature ratios

Heavy metals (RM-2)

		Storage at + 22 ° C		Storage at + 4 ° C	
µg/l		**Al**	**As**	**Al**	**As**
t = 0,5 month		8,40 8,50 8,50	14,20 14,60 14,50	8,50 8,60 8,52	14,57 15,00 14,00
	mean S.D. CV	8,47 0,06 0,68	14,43 0,21 1,44	8,54 0,05 0,62	14,52 0,50 3,45
t = 1 month		8,60 8,30 8,40	14,49 14,62 13,99	8,65 8,45 8,48	14,43 14,60 14,45
	mean S.D. CV	8,43 0,15 1,81	14,37 0,33 2,32	8,53 0,11 1,26	14,49 0,09 0,64
t = 2 months		8,40 8,55 8,45	14,80 14,20 14,40	8,52 8,49 8,46	14,38 14,54 14,55
	mean S.D. CV	8,47 0,08 0,90	14,47 0,31 2,11	8,49 0,03 0,35	14,49 0,10 0,66
t = 0,5 month	R_T U_T	0,99 0,01	0,99 0,04		
	R_T+U_T R_T-U_T	1,00 0,98	1,03 0,96		
t = 1 month	R_T U_T	0,99 0,02	0,99 0,02		
	R_T+U_T R_T-U_T	1,01 0,97	1,02 0,97		
t = 2 months	R_T U_T	1,00 0,01	1,00 0,02		
	R_T+U_T R_T-U_T	1,01 0,99	1,02 0,98		

Table 9. Evaluation of stability testing using the safe temperature ratios

Heavy metals (RM-1)

		Storage at + 22 ° C	Storage at + 4 ° C
µg/l		Hg	Hg
t = 0,5 month		1,96 1,97 1,99	1,98 1,99 1,98
	mean S.D. CV	1,97 0,02 0,77	1,98 0,01 0,29
t = 1 month		1,94 1,92 1,90	1,95 1,97 1,98
	mean S.D. CV	1,92 0,02 1,04	1,97 0,02 0,78
t = 2 months		1,90 1,92 1,89	1,97 1,95 1,96
	mean S.D. CV	1,90 0,02 0,80	1,96 0,01 0,51
t = 0,5 month	R_T U_T	0,99 0,01	
	R_T+U_T R_T-U_T	1,00 0,99	
t = 1 month	R_T U_T	0,98 0,01	
	R_T+U_T R_T-U_T	0,99 0,96	
t = 2 months	R_T U_T	0,97 0,01	
	R_T+U_T R_T-U_T	0,98 0,96	

Table 10. Evaluation of stability testing using the safe temperature ratios

Heavy metals (RM-2)

		Storage at + 22 ° C	Storage at + 4 ° C
µg/l		Hg	Hg
t = 0,5 month		2,84 2,85 2,79	2,85 2,87 2,82
	mean S.D. CV	2,83 0,03 1,14	2,85 0,03 0,88
t = 1 month		2,84 2,81 2,80	2,85 2,83 2,84
	mean S.D. CV	2,82 0,02 0,74	2,84 0,01 0,35
t = 2 months		2,80 2,84 2,80	2,86 2,80 2,86
	mean S.D. CV	2,81 0,02 0,82	2,84 0,03 1,22
t = 0,5 month	R_T U_T	0,99 0,01	
	R_T+U_T R_T-U_T	1,01 0,98	
t = 1 month	R_T U_T	0,99 0,01	
	R_T+U_T R_T-U_T	1,00 0,98	
t = 2 months	R_T U_T	0,99 0,01	
	R_T+U_T R_T-U_T	1,01 0,98	

Table 11. Comparing results of not-sterilized and sterilized samples stored at 4 °C

Nutrients (RM-1)

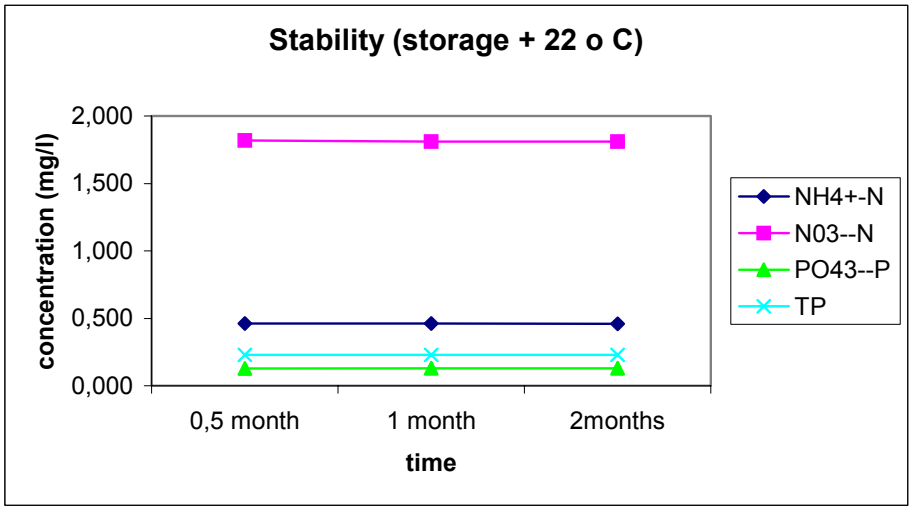
		Without sterilization				Sterilized			
mg/l		NH_4^+-N	NO_3^--N	PO_4^{3-} -P	TP	NH_4^+-N	NO_3^--N	PO_4^{3-} -P	TP
t = 0,5 month		0,466	1,86	0,068	0,232	0,464	1,82	0,131	0,233
		0,470	1,89	0,070	0,230	0,467	1,83	0,131	0,228
		0,471	1,87	0,070	0,229	0,465	1,84	0,130	0,230
	mean	0,469	1,87	0,069	0,230	0,465	1,83	0,131	0,230
	S.D.	0,003	0,015	0,001	0,002	0,002	0,010	0,001	0,003
	CV	0,564	0,815	1,665	0,663	0,328	0,546	0,442	1,093
t = 1 month		0,464	1,85	0,070	0,223	0,464	1,83	0,130	0,228
		0,460	1,80	0,072	0,229	0,465	1,83	0,132	0,231
		0,459	1,84	0,072	0,224	0,465	1,82	0,131	0,232
	mean	0,461	1,83	0,071	0,225	0,465	1,83	0,131	0,230
	S.D.	0,003	0,026	0,001	0,003	0,001	0,006	0,001	0,002
	CV	0,574	1,446	1,619	1,427	0,124	0,316	0,763	0,90
t = 2 months		0,462	1,75	0,059	0,213	0,465	1,82	0,128	0,232
		0,467	1,62	0,063	0,217	0,463	1,84	0,133	0,228
		0,463	1,79	0,061	0,214	0,466	1,83	0,131	0,229
	mean	0,464	1,72	0,061	0,215	0,465	1,83	0,131	0,230
	S.D.	0,003	0,089	0,002	0,002	0,002	0,010	0,003	0,002
	CV	0,570	5,168	3,279	0,970	0,329	0,546	1,926	0,906
t = 0,5 month	R_T	1,01	1,02	0,53	1,00				
	U_T	0,01	0,01	0,01	0,01				
	R_T+U_T	1,01	1,03	0,54	1,01				
	R_T-U_T	1,00	1,01	0,52	0,99				
t = 1 month	R_T	0,99	1,00	0,54	0,98				
	U_T	0,01	0,01	0,01	0,02				
	R_T+U_T	1,00	1,02	0,55	0,99				
	R_T-U_T	0,99	0,99	0,53	0,96				
t = 2 months	R_T	1,00	0,94	0,47	0,93				
	U_T	0,01	0,05	0,02	0,01				
	R_T+U_T	1,01	0,99	0,48	0,95				
	R_T-U_T	0,99	0,89	0,45	0,92				

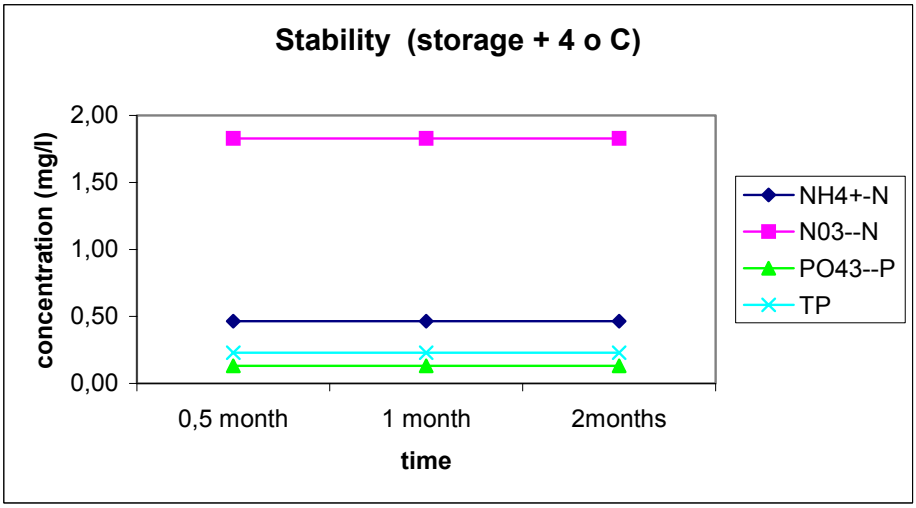
Table 12. Comparing results of not-sterilized and sterilized samples stored at 4 °C

Nutrients (RM-2)

		Without sterilization				Sterilized			
mg/l		NH_4^+-N	NO_3^--N	PO_4^{3-} -P	TP	NH_4^+-N	NO_3^--N	PO_4^{3-} -P	TP
t = 0,5 month		0,654	2,15	0,109	0,290	0,703	2,35	0,213	0,319
		0,701	2,15	0,106	0,290	0,706	2,43	0,215	0,318
		0,680	2,16	0,108	0,291	0,699	2,28	0,217	0,322
	mean	0,678	2,15	0,108	0,290	0,703	2,35	0,215	0,320
	S.D.	0,024	0,006	0,002	0,001	0,004	0,075	0,002	0,002
	CV	3,471	0,268	1,419	0,199	0,500	3,189	0,930	0,651
t = 1 month		0,673	2,33	0,103	0,224	0,703	2,35	0,212	0,321
		0,678	2,28	0,104	0,235	0,701	2,37	0,216	0,319
		0,675	2,33	0,102	0,234	0,703	2,31	0,214	0,322
	mean	0,675	2,31	0,103	0,231	0,702	2,34	0,214	0,321
	S.D.	0,003	0,029	0,001	0,006	0,001	0,031	0,002	0,002
	CV	0,373	1,248	0,971	2,633	0,164	1,304	0,935	0,476
t = 2 months		0,664	2,45	0,100	0,214	0,703	2,32	0,209	0,323
		0,659	2,39	0,101	0,209	0,704	2,41	0,210	0,318
		0,661	2,29	0,103	0,214	0,699	2,28	0,219	0,321
	mean	0,661	2,38	0,101	0,212	0,702	2,34	0,213	0,321
	S.D.	0,003	0,081	0,002	0,003	0,003	0,067	0,006	0,003
	CV	0,381	3,401	1,507	1,360	0,377	2,849	2,590	0,785
t = 0,5 month	R_T	0,97	0,92	0,50	0,91				
	U_T	0,03	0,03	0,01	0,01				
	R_T+U_T	1,00	0,94	0,51	0,91				
	R_T-U_T	0,93	0,89	0,49	0,90				
t = 1 month	R_T	0,96	0,99	0,48	0,72				
	U_T	0,00	0,02	0,01	0,02				
	R_T+U_T	0,97	1,01	0,49	0,74				
	R_T-U_T	0,96	0,97	0,47	0,70				
t = 2 months	R_T	0,94	1,02	0,48	0,66				
	U_T	0,01	0,05	0,01	0,01				
	R_T+U_T	0,95	1,06	0,49	0,67				
	R_T-U_T	0,94	0,97	0,46	0,65				

Figure 1. Graphical presentations of the stability test results
Nutrients (RM-1)





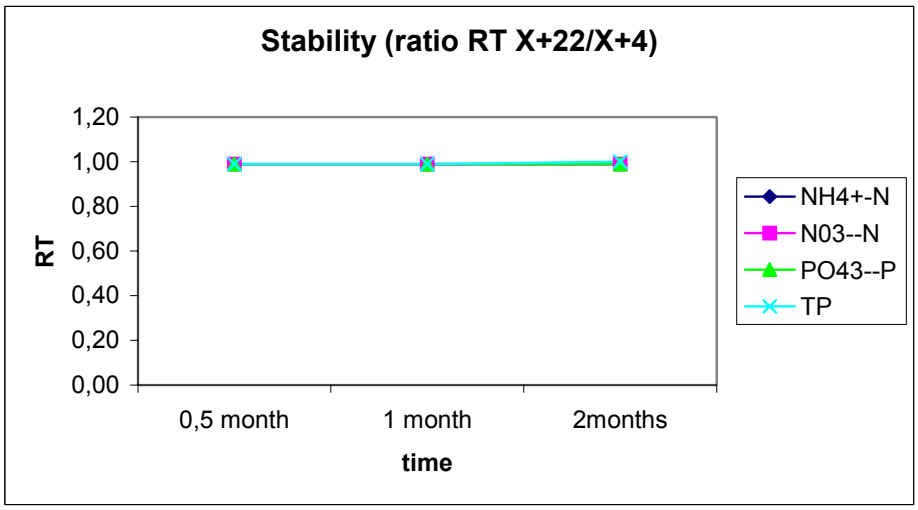
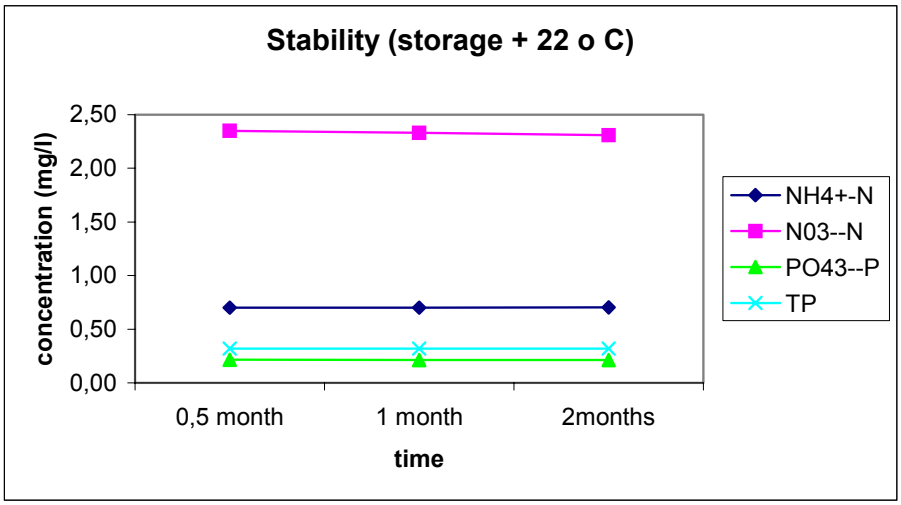
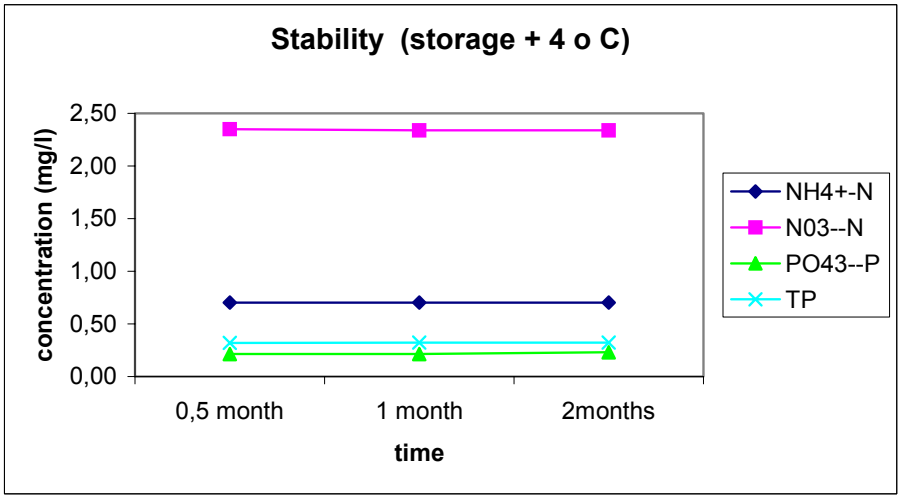


Figure 2. Graphical presentations of the stability test results
Nutrients (RM-2)





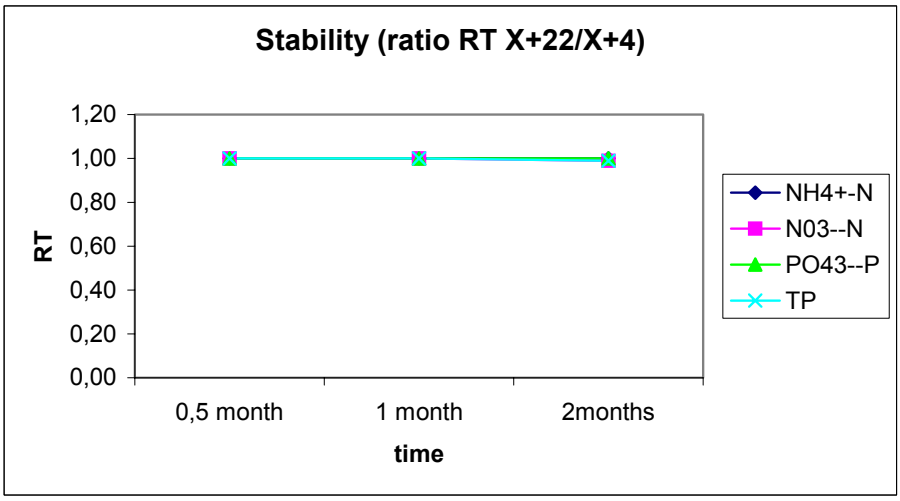
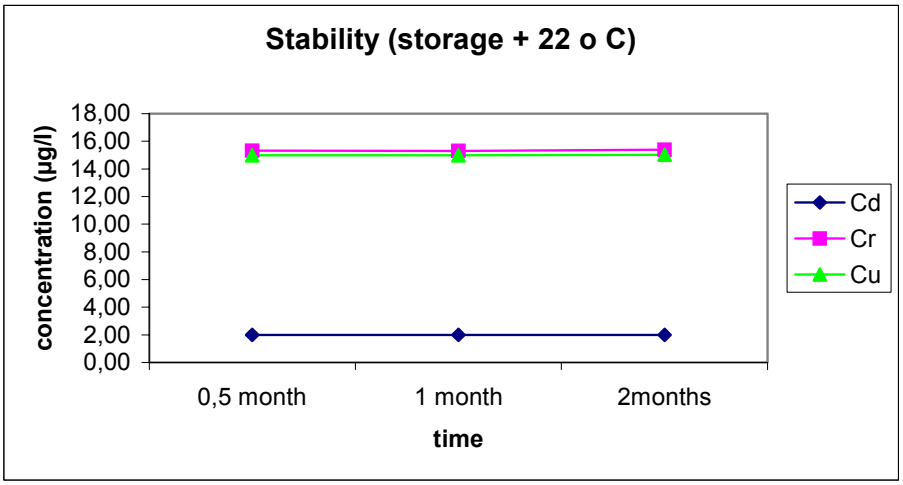
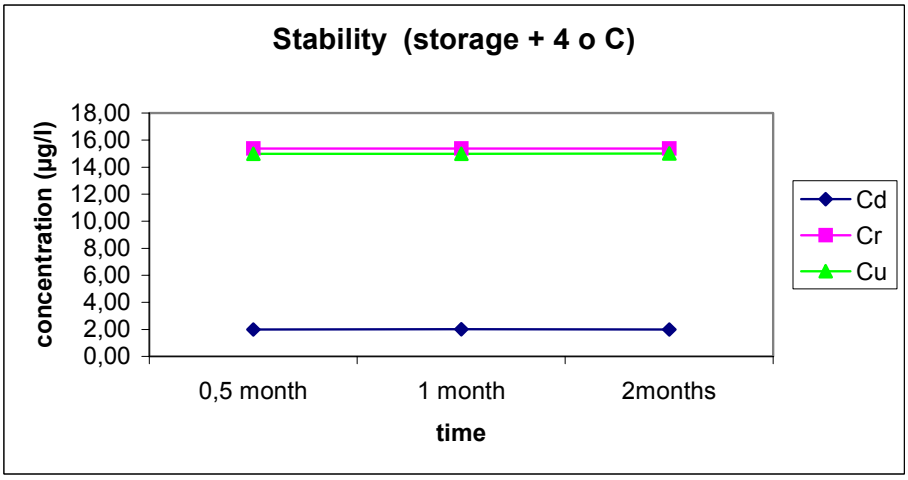


Figure 3. Graphical presentations of the stability test results
Heavy metals (RM-1)





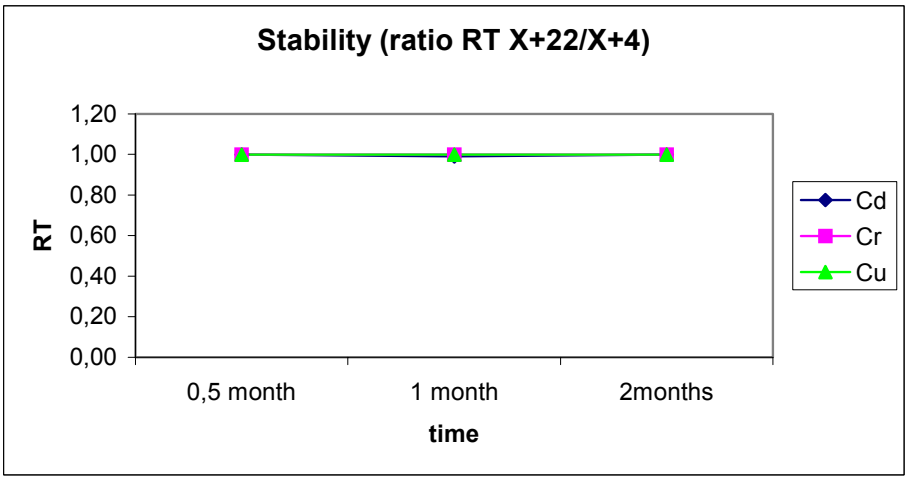
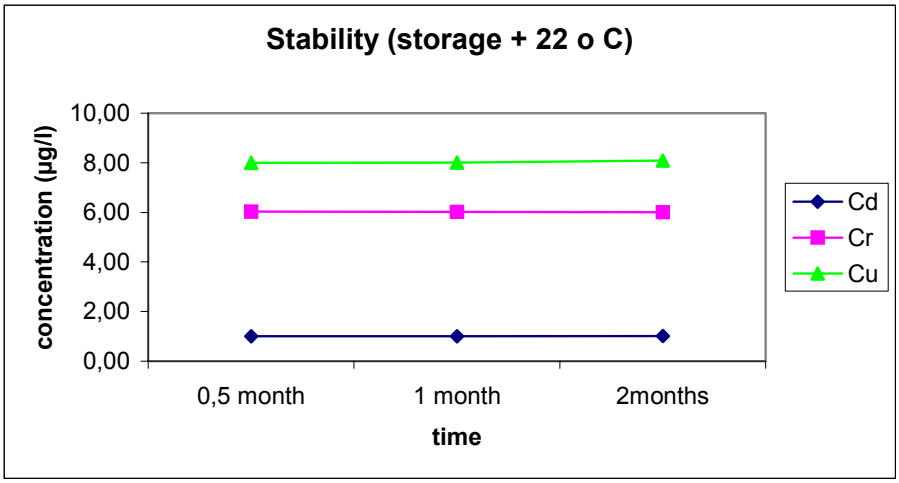
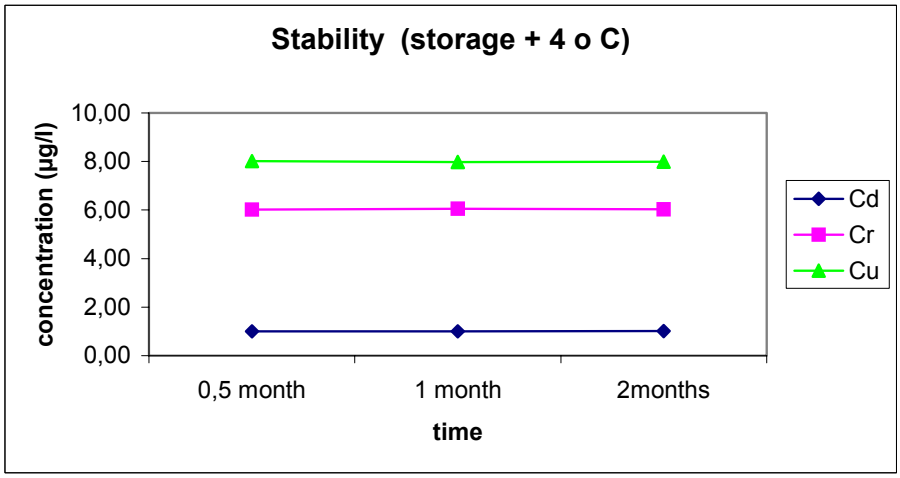


Figure 4. Graphical presentations of the stability test results

Heavy metals (RM-2)





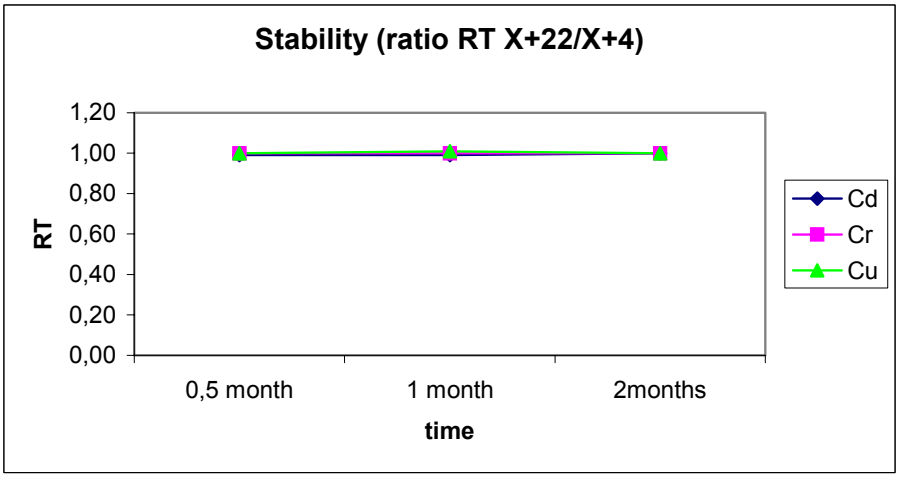
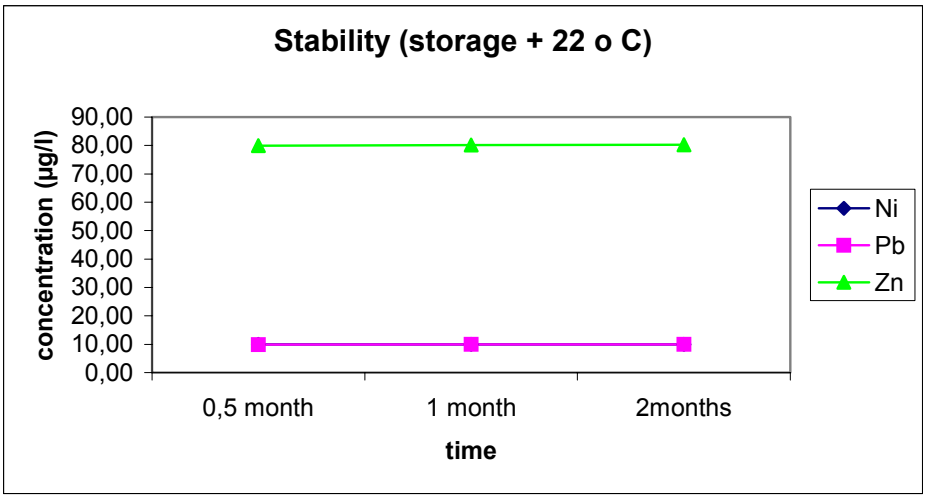
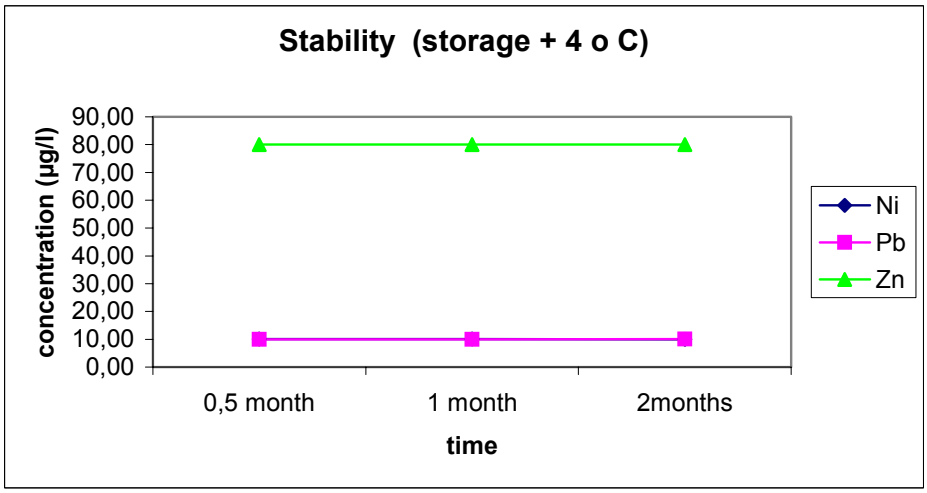


Figure 5. Graphical presentations of the stability test results

Heavy metals (RM-1)





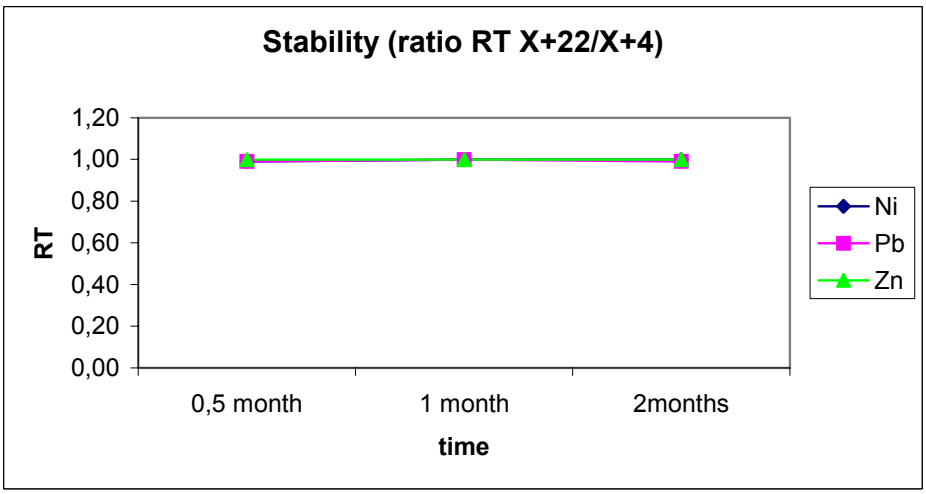
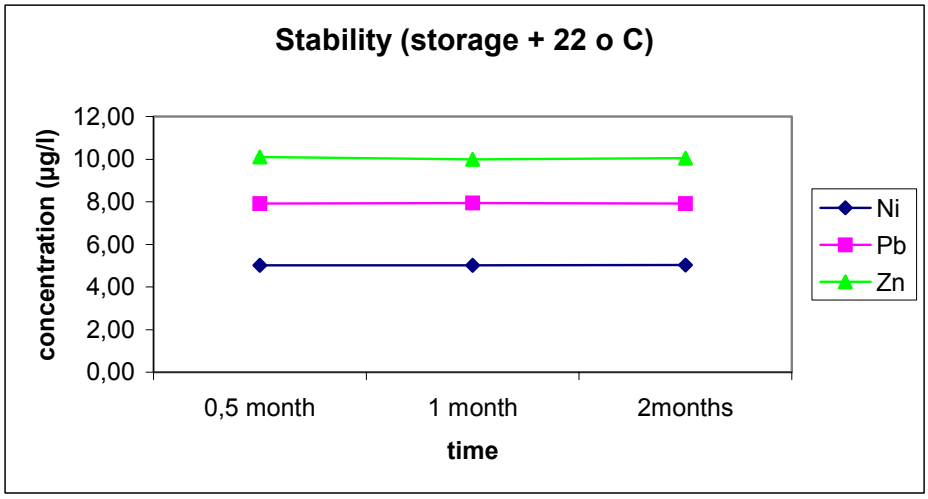
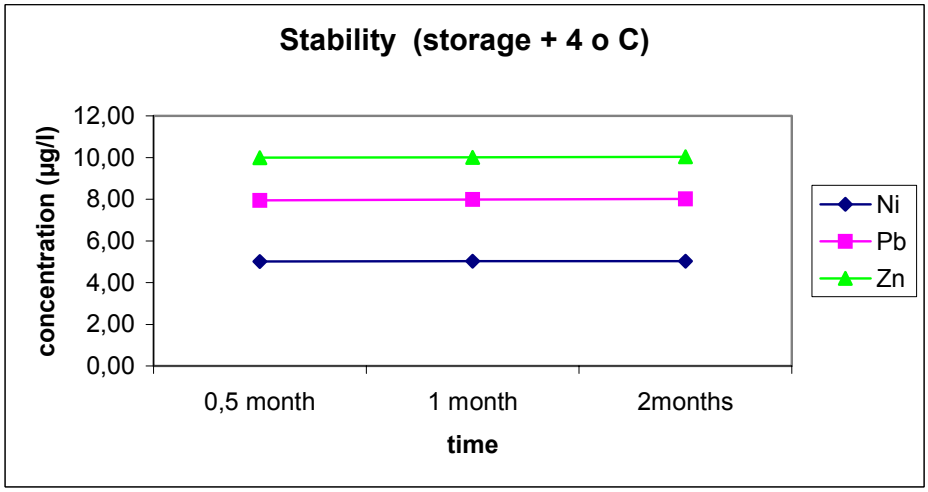


Figure 6. Graphical presentations of the stability test results

Heavy metals (RM-2)





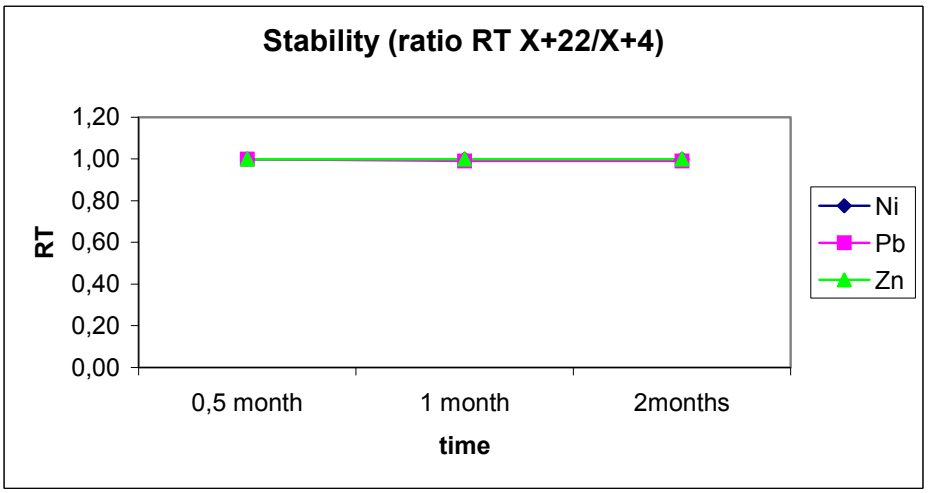
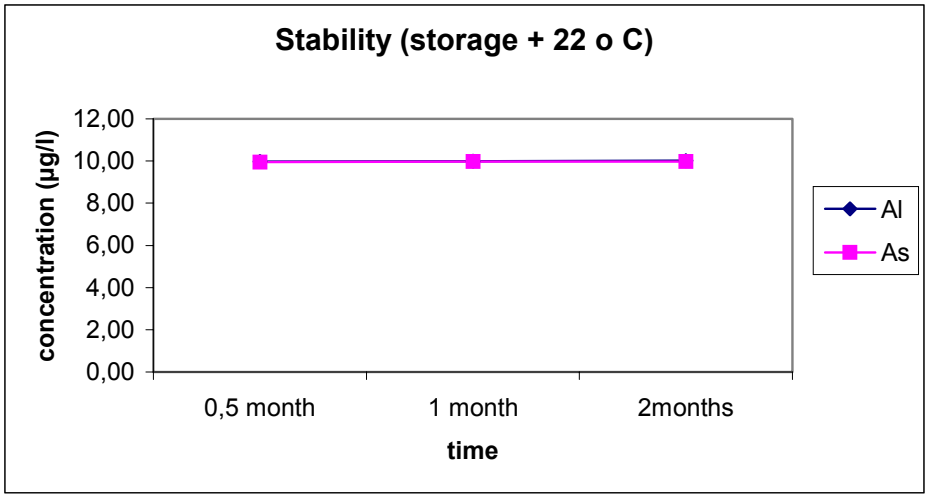
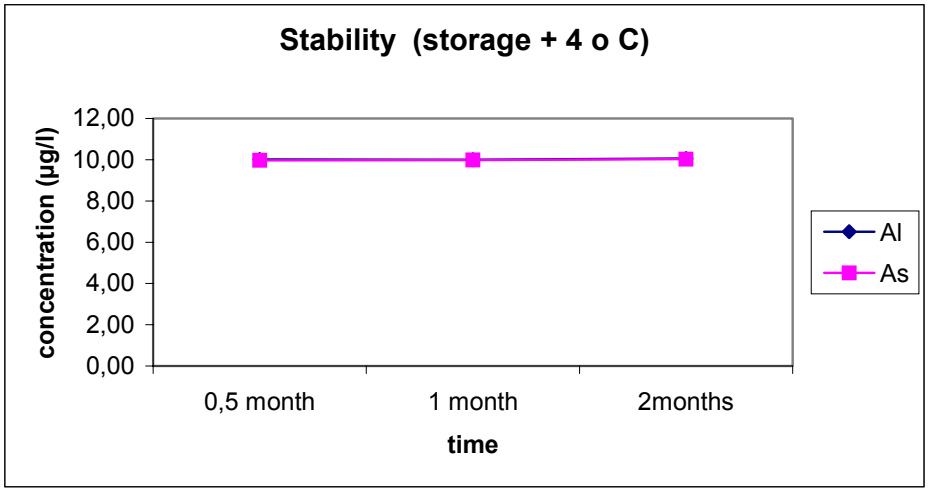


Figure 7. Graphical presentations of the stability test results

Heavy metals (RM-1)





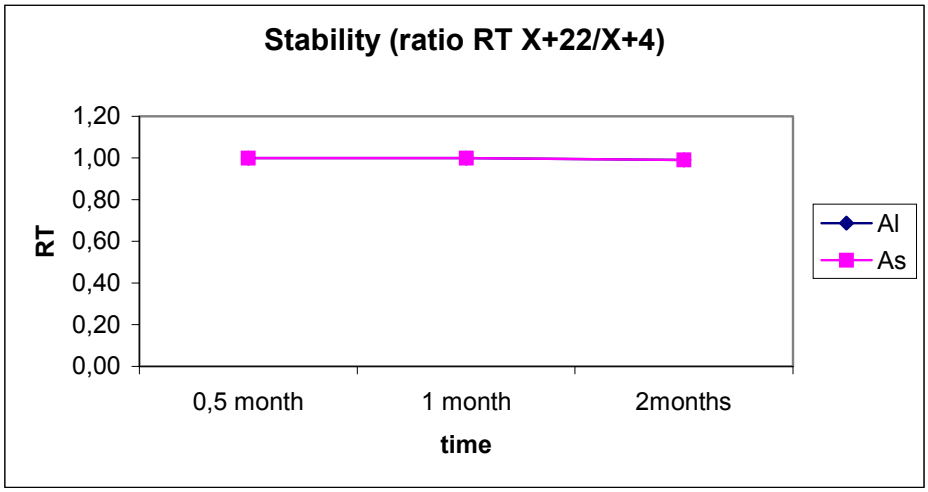
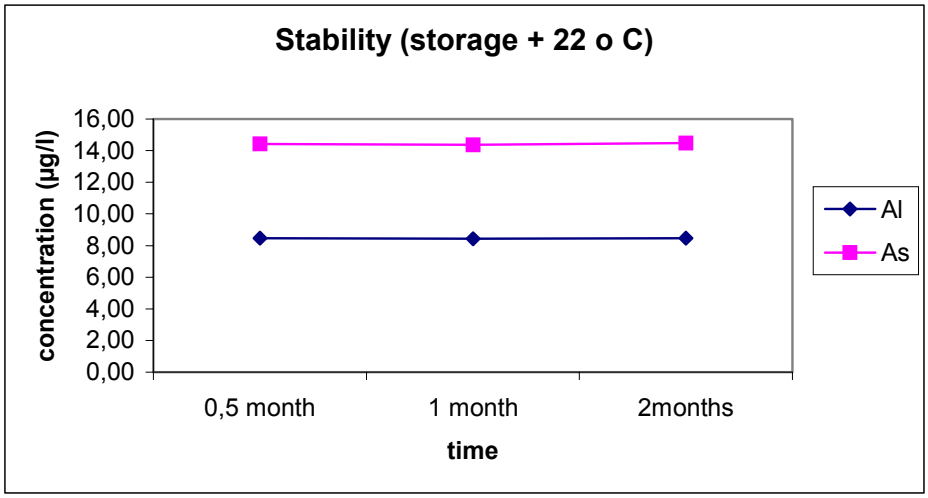
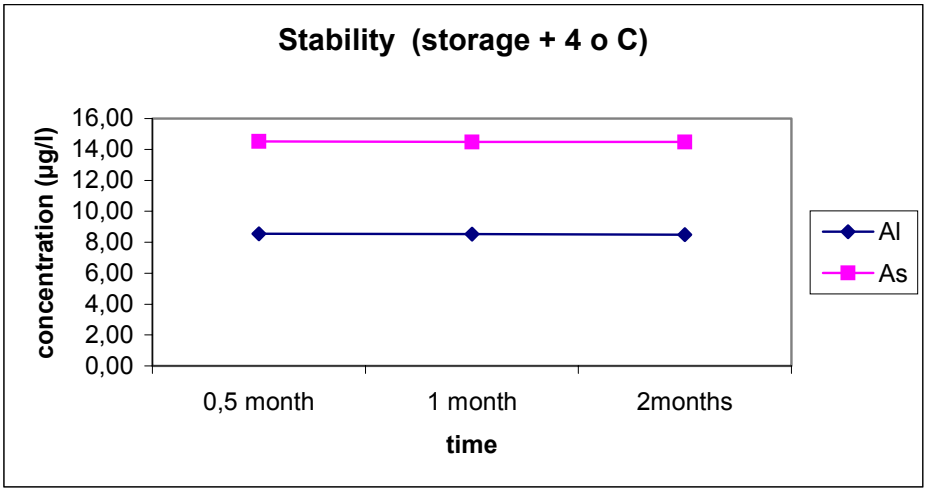


Figure 8. Graphical presentations of the stability test results

Heavy metals (RM-2)





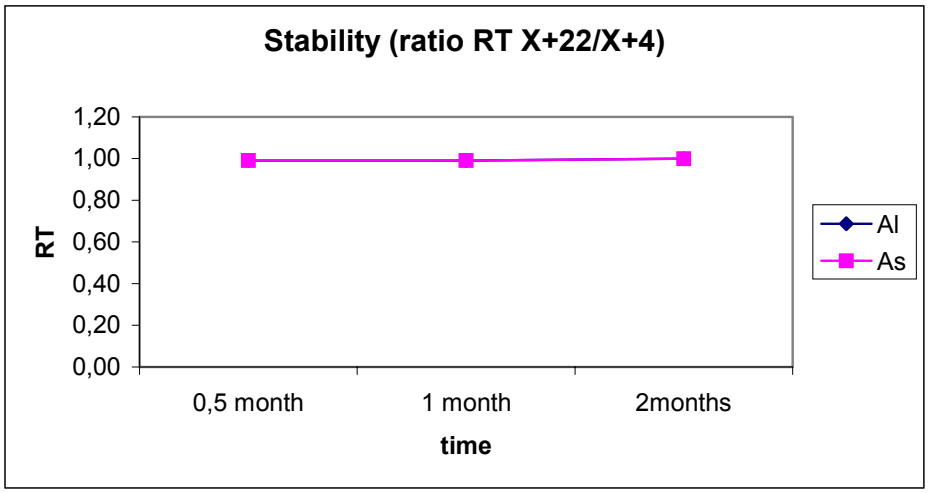
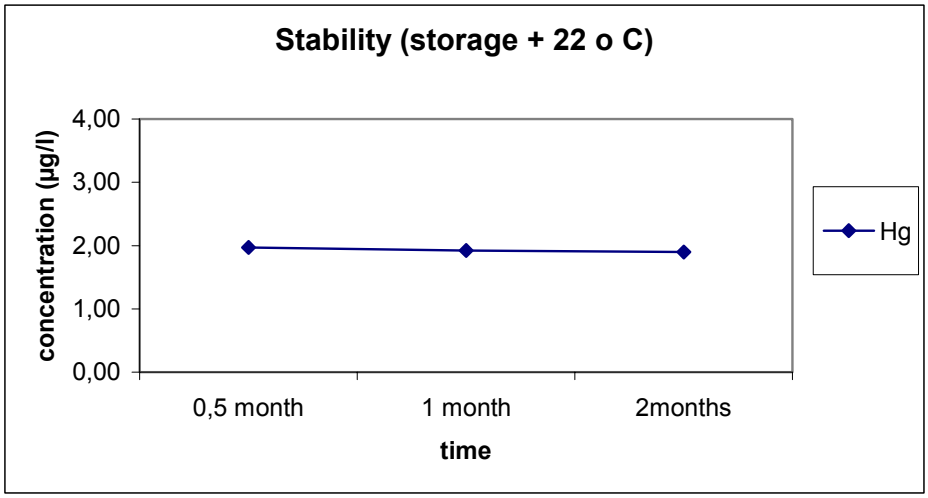
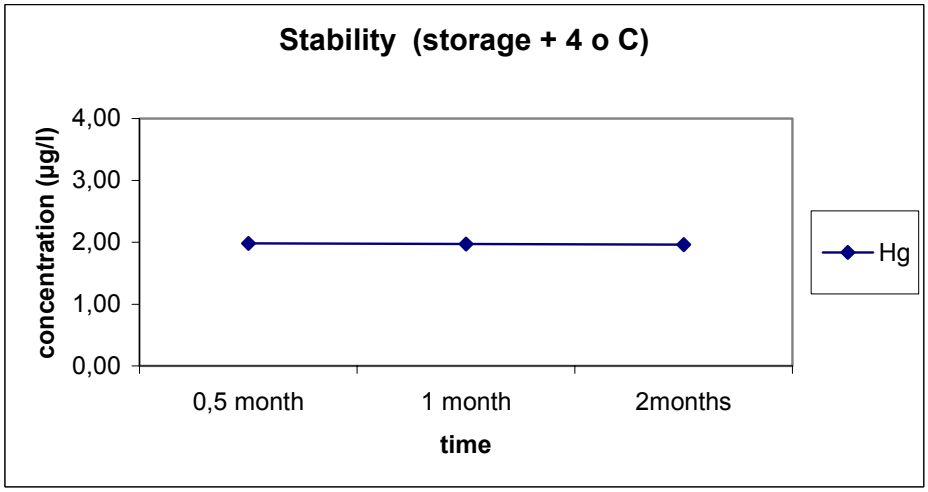


Figure 9. Graphical presentations of the stability test results

Heavy metals (RM-1)





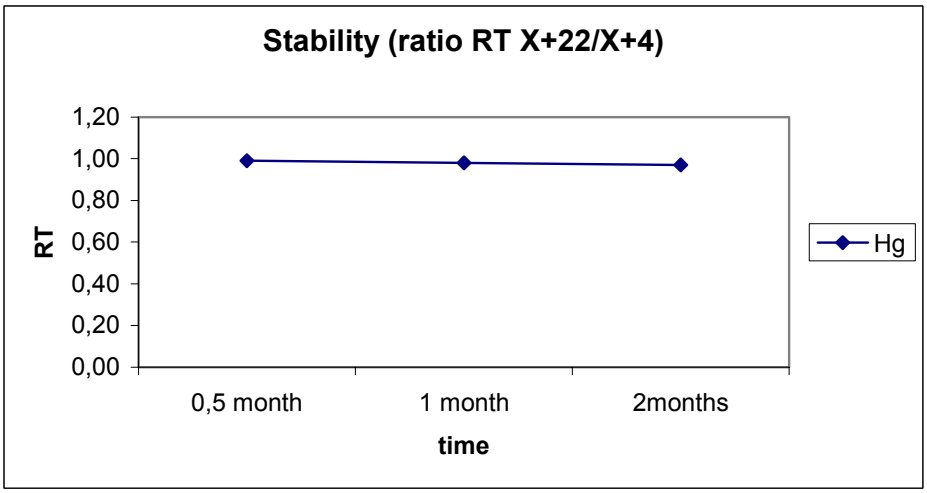
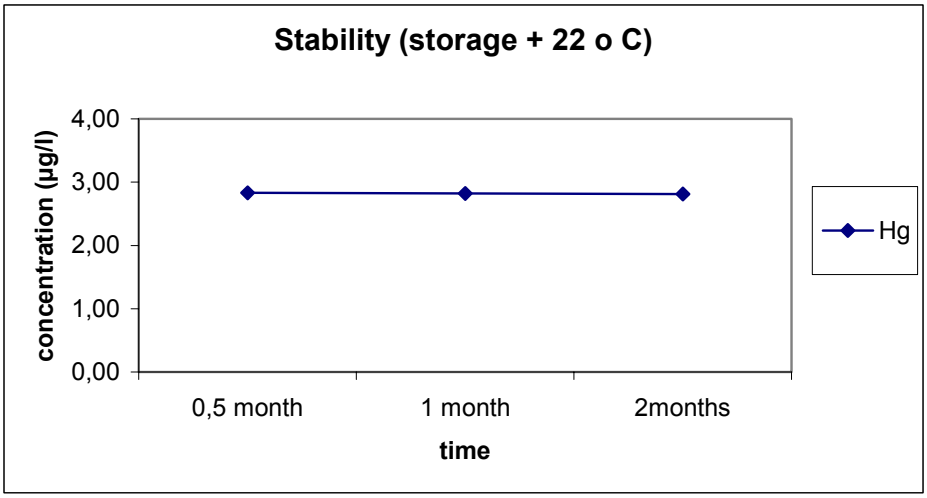
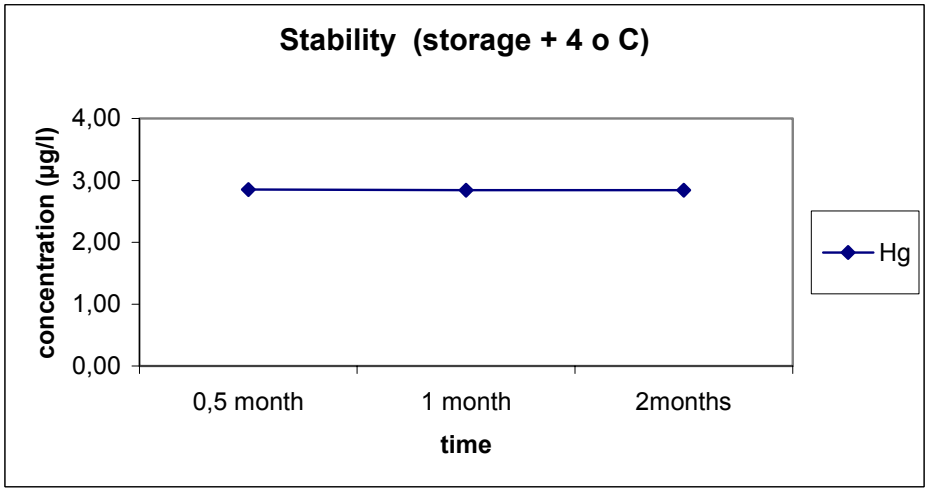


Figure 10. Graphical presentations of the stability test results

Heavy metals (RM-2)





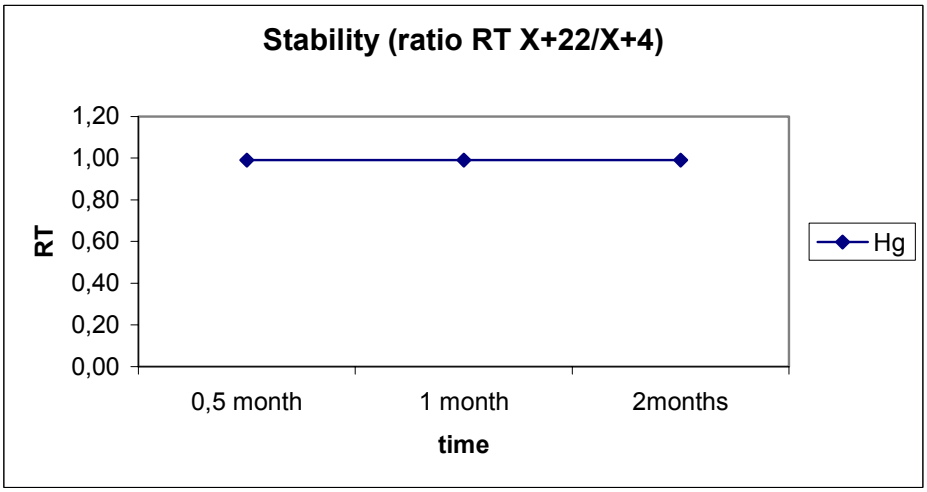
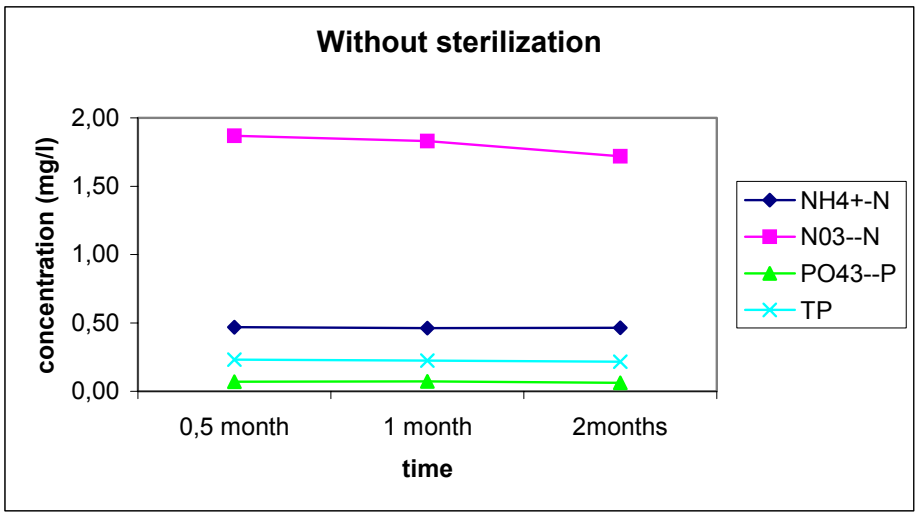
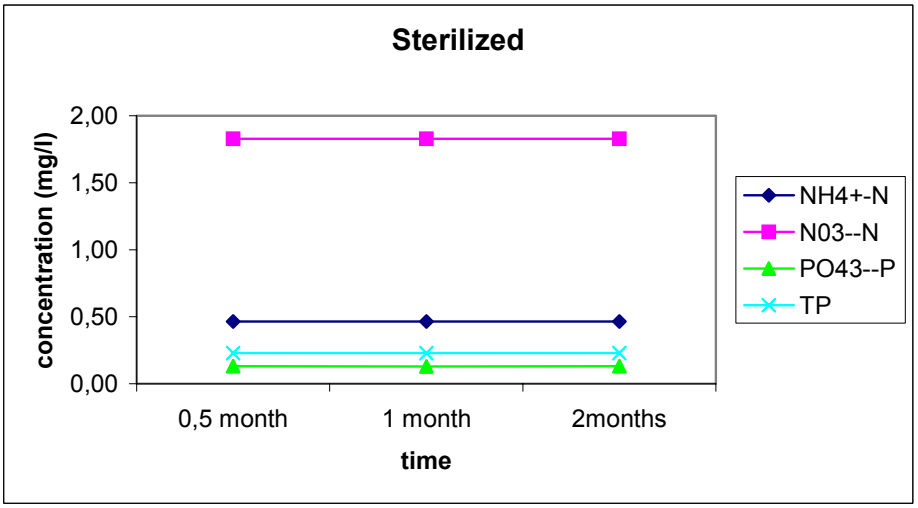


Figure 11. Graphical presentations of the stability test results

Nutrients (RM-1)





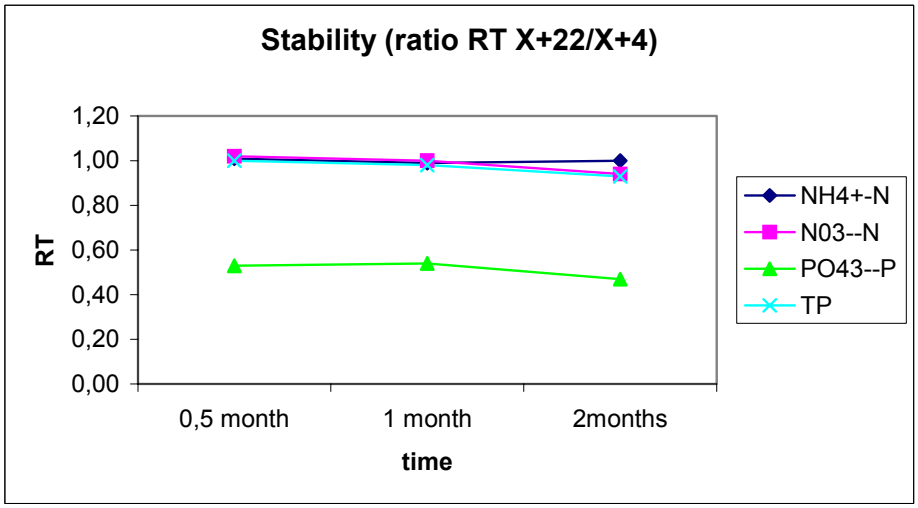
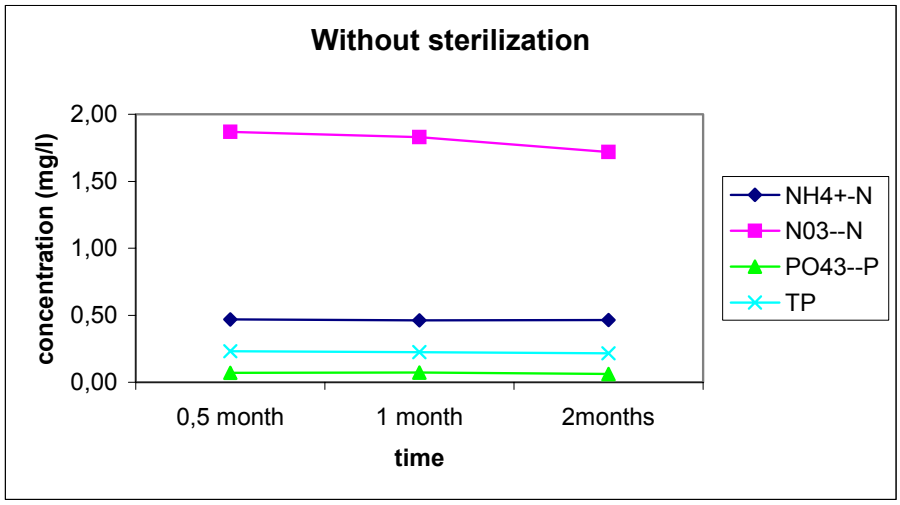
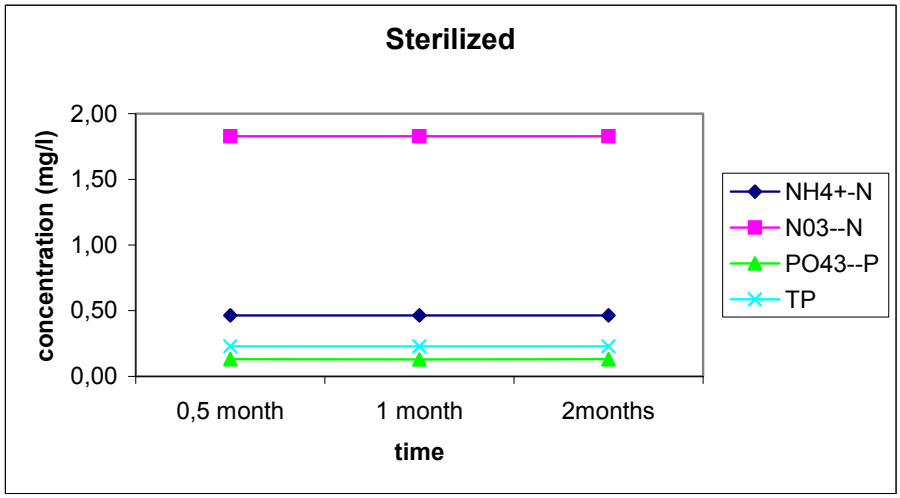
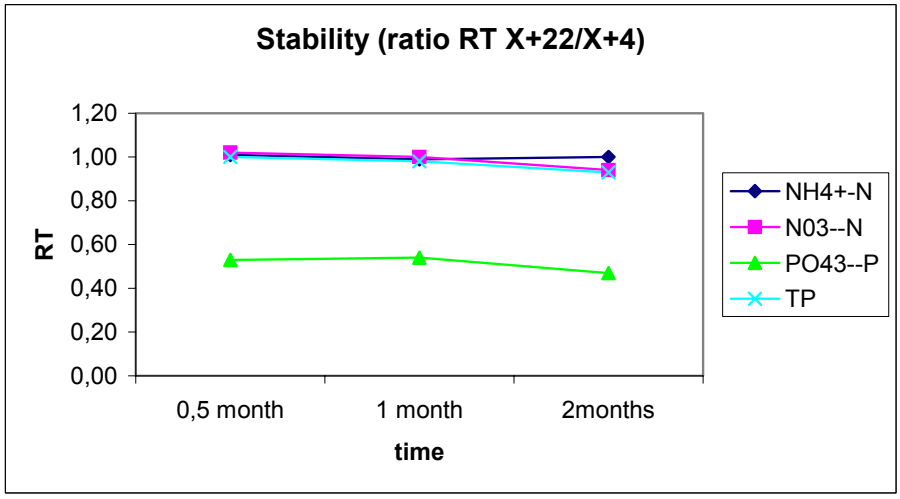


Figure 12. Graphical presentations of the stability test results

Nutrients (RM-2)







CONCLUSIONS AND REMARKS

As the role of certified reference materials (CRMs) is very important in the internal quality controls but most of laboratories cannot bear the costs of CRMs.

Although TNMN laboratories (especially NRLs) do have adequate equipment, the high costs of operating materials, RMs, hamper optimal operation.

To avoid problems with obtaining relevant RMs the present project provided two series of water and sediment reference materials for participating laboratories in the QualcoDanube intercalibration comparison.

Assigned values:

- nominal concentrations were considered as assigned values for the artificially prepared heavy metals samples (and confirmed by analyses)
- measured values were accepted as assigned values not "theoretical" values for nutrients because of concentration changing during sterilization.
- as the sediment RMs are "real-world" materials, before using them for internal quality control the assigned values for the target compounds should be established based on results of intercomparison test.

After preparing of water RMs, stability tests were carried out.

The results obtained from samples stored at the safe temperature of ≤ 4 °C, show a rather similar pattern to the 22 °C data. By calculating the ratios R_T of the means it appears (**Fig. 1-12**) that there is no problems of instability, but there is significant difference in phosphate concentration comparing sterilized samples to not sterilized samples (**Table 11-12**).

Materials, prepared and analysed by the earlier mentioned process, can be used as in-house RMs for a relatively long time.

Remarks:

- period for implementation of present project — to prepare reference materials from two matrices (water and sediment) for the TNMN laboratories, to check homogeneity and stability —was rather short. Sediment preparation – all the steps and tests - takes a long time and it was relatively long in the case of the first sediment RM's preparation, when inhomogeneity was detected and repeated homogenization and tests were necessary,
- evaluation of the analytical data for sediment RMs will be finished over the project as the sediment RMs are "real-world" materials and before using them for internal quality control the assigned values for the target compounds should be established based on results of intercomparison test of a group of experienced laboratories .,
- in the case of water concentrates for nutrients the samples should be sterilized to prevent activity of microorganisms and any changes into concentration.
- it is known from experience and it was confirmed again that concentration of phosphorous forms – inspite of preservation and sterilization – has been changed, as it can be seen in **Table 11-12 and Fig. 11-12.**

  It is expected that date of expiry will be shorter than a year in the case of phosphorous forms. Unfortunately there was not any long term investigation before.

The reference material samples were prepared and tested according to the "Practical Manual for Production of Laboratory Reference Materials" as well as ISO Guide 34,1996. Quality system guidelines for the production of reference materials. Ibid.

Budapest, 26/11/2003

Dr. Jolán Schneider
team leader

Annex I

Assigned value of RM-1 water

Determinands	Unit	Value
Nutrients		
NH_4^+-N	mg/l	0,465
NO_3^--N	mg/l	1,83
PO_4^{3-}P	mg/l	0,13
TP	mg/l	0,23
Heavy metals		
Cd	µg/l	2
Cr	µg/l	15
Cu	µg/l	15
Pb	µg/l	10
Ni	µg/l	10
Zn	µg/l	80
Hg	µg/l	2
Al	µg/l	10
As	µg/l	10

Assigned values of RM-2 water

Determinands	Unit	Value
Nutrients		
NH_4^+-N	mg/l	0,70
NO_3^--N	mg/l	2,35
PO_4^{3-}P	mg/l	0,215
TP	mg/l	0,32
Heavy metals		
Cd	µg/l	1,0
Cr	µg/l	6,0
Cu	µg/l	8,0
Pb	µg/l	8,0
Ni	µg/l	5,0
Zn	µg/l	10,0
Hg	µg/l	2,85
Al	µg/l	8,5
As	µg/l	14,5