Babeş-Bolyai University
Faculty of Physics

PhD Thesis Summury

MONITORING STUDY OF BIOLOGICAL AND ECOLOGICAL COMPOUNDS

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CONTENT

Numbering of tables, figures and references are the same as in PhD thesis.

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Mass spectrometry, Gas chromatography, GC-MS, quantitative analysis
Validation parameters, uncertainty, control charts
Volatile organic compounds, BTEX, ambiental level (ppb)
Monitoring parameters, fish tissues, sediment, water
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Introduction

The aim of the thesis was to determine the biological and ecological compounds that may affect the environment and human health, by testing and development of analytical methods of air, water, soil, food samples and their monitoring.

The thesis contains seven chapters which describe the theoretical and experimental results using several methods of analysis.

The first and second chapter presents theoretical aspects using two analytical techniques, mass spectrometry and gas chromatography. Mass spectrometry is a spectroscopic technique that offers many possibilities of analyses for environmental and biological compounds.

Gas chromatography - mass spectrometry (GC-MS) method is particularly advantageous because gas chromatography is an ideal introduction system that provides the separation of components from a mixture of biological and environment samples, and mass spectrometer is an ideal detector, which offers the possibility of identification.

Gas chromatography is an analytical technique efficient for the separation of components from a mixture and also for qualitative and quantitative analysis of separated substances. Mass spectrometry has known a rapidly development in recent decades, and today is an important instrument for environment, biological and medical analyses. It is an outstanding technique in the fields of investigation and finds applications that can identify unknown structures. In comparison with conventional analytical methods that are appliable to components in large quantity, GC-MS can identify chemical species in small quantities (nanogramelor and picogramelor).

Chapter 3 presents the analytical aspects that are necessary for laboratory accreditation. To demonstrate the quality of analytical results is required continuous control, which is achieved by validation of methods used. By checking the validation parameters (limit of detection, limit of quantification, precision, accuracy, linearity of response), is shown that the method chosen is correct and correspond to the intended purpose. Recognition of laboratory capability, which includes alongside human factors, environmental conditions, methods validation, equipments, metrological inspection, use of reference materials, traceability, all complet the accreditation process, which involves official recognition by an authority and give permission to the laboratory to perform specified services.

Chapter 4 presents GC-MS method applications to determin volatile organic compounds from environment and their influence on the human body.

Chapter 5 contain studies related to monitoring of water, air and soil samples and details about investigated geographical area, testing of some methods and validation procedure.

In **chapter 6** are described methods and measurements for quality control of food (drinking water), implementation of quality management system in accordance with U.E. reglementations and adapted to Romanian standards, and also the benefits of laboratory accreditation.

The final chapter summarizes the conclusions obtained from experimental results [1-12].

CAP. IV. GC-MS ANALYSIS OF VOLATILE ORGANIC COMPOUNDS FROM AMBIENTAL AIR

Benzene, toluene, ethyl benzene and xylene (BTEX) are widespread pollutants, main source in the outside environment being vehicle traffic and indoor, the cigarette smoke. They are also present in small quantities in drinking water and food, in painting substances or adhesives. The benzene outdoor maximum limit recommended by the European Community is 10 µg/m^3 [24]. Volatile organic compounds (VOCs) are a major class of contaminants of indoor air where levels may be up to 10 times higher than those outdoors. Benzene was classified as a Group A, human carcinogen [29].

Gas chromatography-mass spectrometry (GC/MS) is the most used quantitative methods for VOCs levels detection in the indoor air [19-48].

Results

The GC/MS method developed for BTEX determinations in indoor air is simple and rapid.

Fig. 2.7. Trace DSQ ThermoFinnigan MS coupled with Trace GC-system equipped with autosampler

The mass spectrometer was operating in the SIM mode, with high selectivity and specificity, which allowed the peak deconvolution of benzene and its deuterated analogue internal standard, as they were coeluting in the total ion chromatogram. SIM mode offers higher sensitivity than scan mode.

A 7.5 minute temperature program was used: 33^{o}C (2 min), 10^{o}C/min to 70^{o} C then 50^{o}C/min to 200^{o}C, SIM mode.

The limit of detection for BTEX was of 0.01µg.

The linearity parameters obtained for BTEX in the range 0–80 µg by using deuterated benzene and 0-100 µg by using 1mg pyridine as internal standards are presented. Coefficients of regression when deuterated benzene (r>0.99) and pyridine were used as internal standards (r>0.90) showed good values. The validation of the method gave good values for precision, when deuterated benzene was used, and showed for 40 and 80 µg aliquot samples, R.S.D.<18.75% and respectively <6.43%. The accuracy R.S.D. calculated was <17.7% for samples of 40 µg and 17.8% for samples of 80 µg.

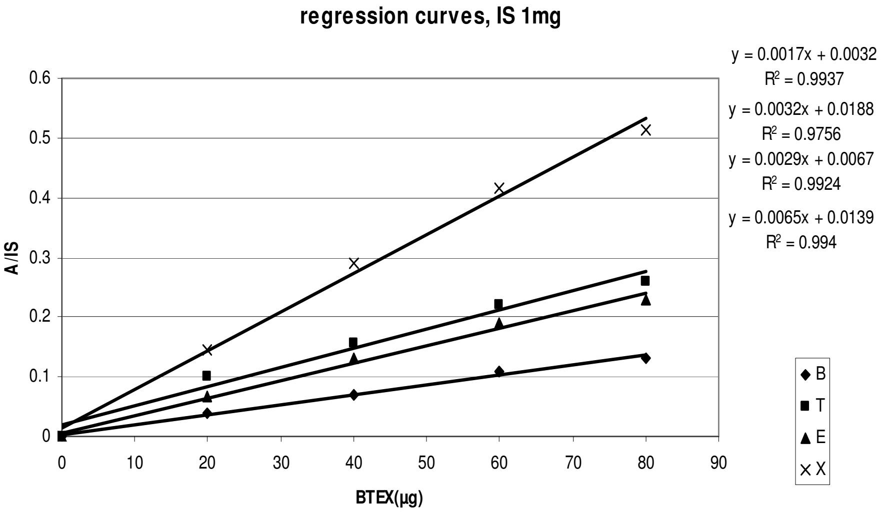


Fig.4.2. Regression curves for BTEX by using deuterated benzene as the internal standard

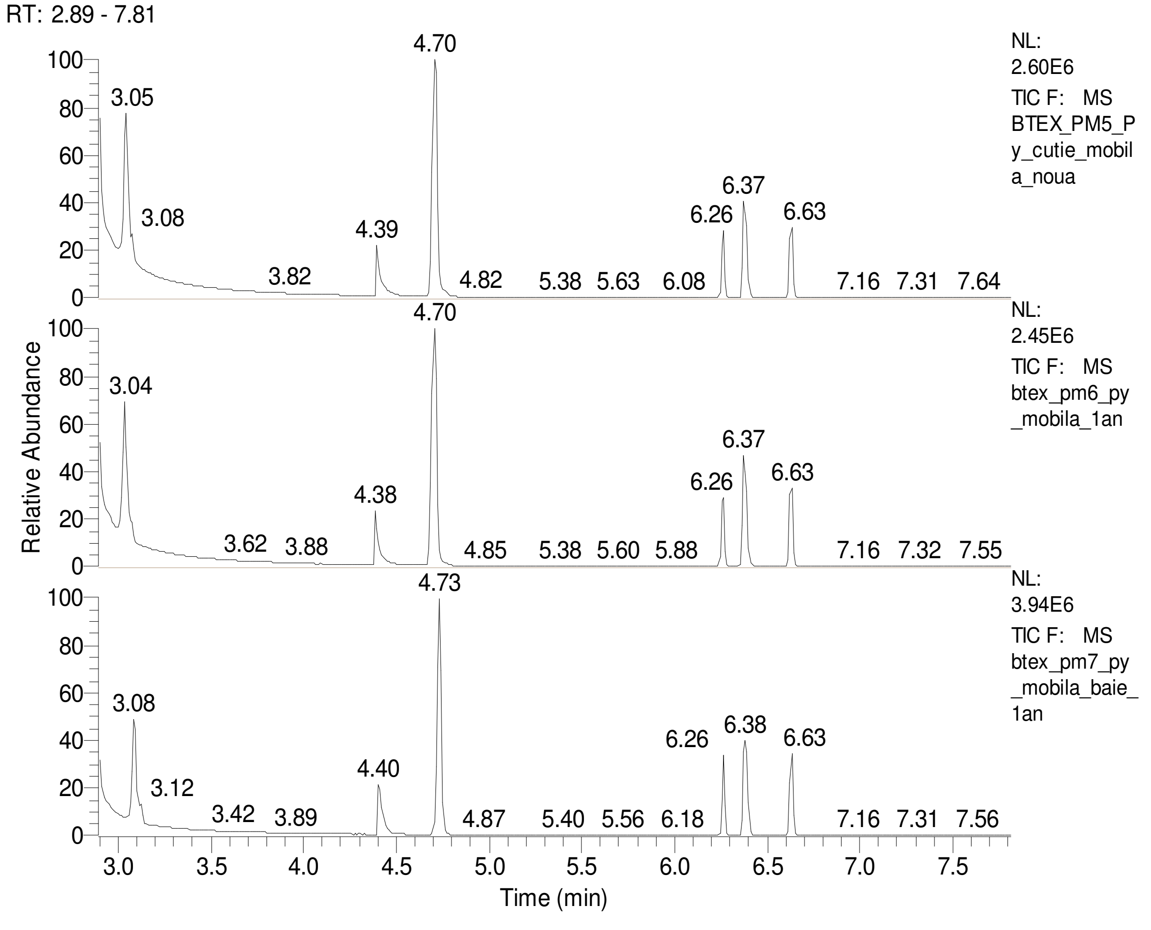


Fig.4.4. Chromatographic separation of BTEX from indoor air extracts near a new box furniture, from an apartment room of 15 m2 with new furniture (1 year old) and into a bathroom cabinet (SIM mode)

The BTEX levels measured in the air contaminated when new furniture (1 year old) is bought into a room of an apartment, as well as the indoor pollutants found in the case of a vacuum mattress placed into the room with the new furniture. The indoor air contaminated in the first days, when the furniture was placed in the room, has been kept into the mattress. By jumping on the mattress, similar BTEX values were obtained as in a new furniture box. No jumping on the mattress, the indoor air extract BTEX values were smaller in the room with the furniture bought one year ago; the levels for indoor air were obtained also when the cartridge was placed into a bathroom cabinet, one year old.

We have compared indoor air values for BTEX measured inside a parked old car (4 years) under sunlight conditions, with their engines turned on and air conditioner opened and after then minutes of ventilation (opened windows). The BTEX values represented were obtained from two posters plastic boxes from laboratory.

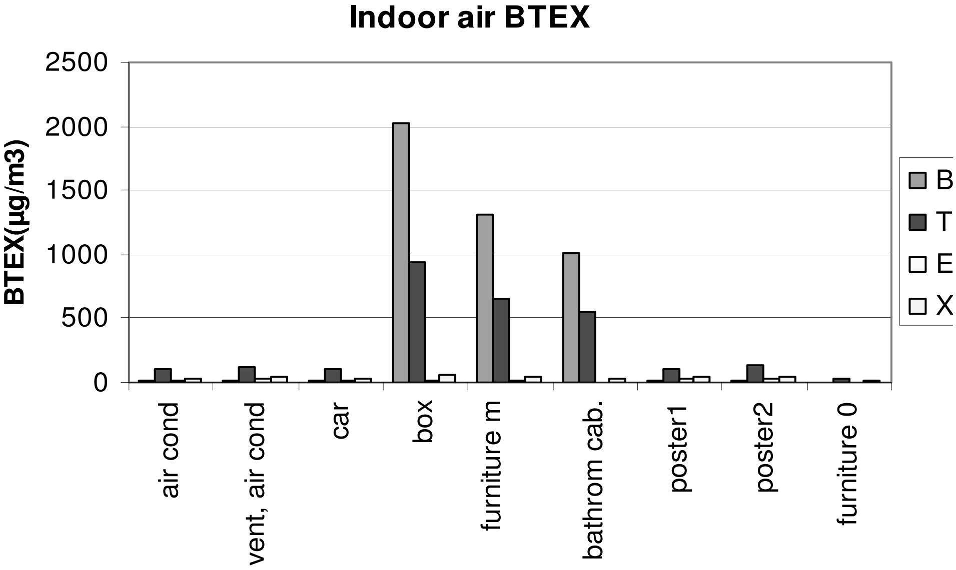


Fig.4.5. Comparison of BTEX values (room indoor air, poster boxes and car indoor air; furniture m: jumping on the mattress; furniture 0: no jumping)

Conclusions

The GC/MS developed method for BTEX determinations in indoor air is simple and present high sensibility, selectivity and specificity.

The results obtained were higher than BTEX measured in the ambient air in the vicinity of industrial area, were in addition to the traffic emissions, industrial activity enhanced the concentration levels of VOCs [29, 33, 35, 37-40]. Linearity gave better coefficients of regression in the case of deuterated benzene, as internal standards in comparison with pyridine [42].

The values for BTEX measured showed higher concentrations values then outdoor maximum limit recommended by the European Community, almost the occupational maximum exposure limit. Attention should be paid to room’s ventilation and indoor air when inflate a new mattresses vacuum packed. Exposure of the sensitive groups as children or pregnant women should be avoided [43-48], [49-50].

CAP. V. ANALYSIS OF SEVERAL ENVIRONMENTAL COMPOUNDS FROM WATER, AIR AND SOIL

5.1. POLLUTION MONITORING INDICATORS TO CHARACTERIZE THE QUALITY OF SURFACE WATER FROM SOUTHERN PART OF ROMANIA

The surface water quality depends of some conditions regarding the aquatic life of the water stream. Keeping the water clean, increase of earth inhabitants, resources exploitation and pollution, remain an important issue to solve.

Human activities such as agriculture, mining and urban development may have impact on water quality. Pollution from these sources can include sediments, nutrients and toxic contaminants. Such pollutants can be transported long distances due to atmospheric processes and stored in soil or even in drainage basin of water.

The monitoring program was based on analysis performed of surface waters originating from Southern of Romania (Arges, Olt and Jiu rivers) and they were studied in order to assess the effects of atrophic pollution.

Water quality indicators like pH, conductivity, turbidity and dissolved oxygen, chloride, calcium and flour ions, nitrates and ammonia ions, total organic carbon and heavy metals content have been analyzed in 3 different points/rivers thereby before, after and from a main city on the rivers. The main water quality parameters were determined by potentiometric methods, less the particulate matter that was determined by gravimetric method and the heavy metals by inductively coupled plasma mass spectrometry measurements.

In polluted water, varied products can appear, as C and S compounds, a special importance being accorded to N compounds. Nitrogen may be in the form of nitrate, nitrite, ammonia or ammonium salts. The differing forms of nitrogen are relatively stable in most river systems with nitrite transforming into nitrate in well oxygenated rivers and ammonia transforming into nitrite/nitrate. High levels of nitrogenous compounds tend to lead at extreme variations in parameters which in turn can degrade the ecological worth of the watercourse.

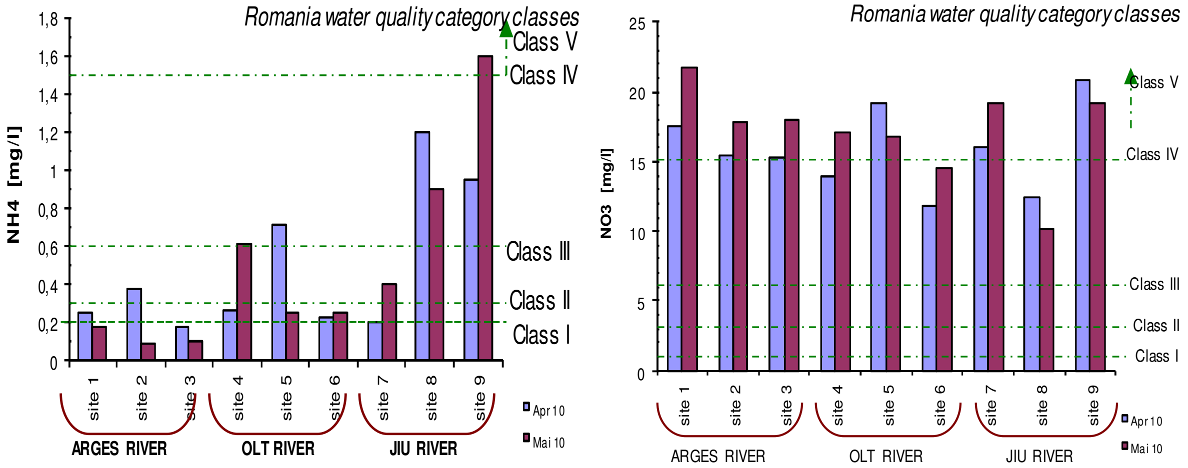


Fig. 5.1.6. Nitrates and Ammonium concentrations

A high amount of nitrates in rivers water (Figure 5.1.6) can be observed along the investigated route as a consequence of urban settlements, agricultural emissions and/or industry and traffic.

Ammonium is a nutrient for plants, but can also have toxic effect, especially on fish, when its concentration is higher than 0.2 mg/l. The toxicity of ammonia is dependent on pH and temperature and added the buffering effect which masks any additional toxicity over pH=8. To avoid ecological damage we have to consider the pH fluctuation caused by photosynthesis which is difficult in the case of ammonia as a wide range of parameters such as concentration, pH and temperature. The surface water with a concentration of NH_4^- over 1.5 mg/l belongs to the V class quality.

Metals - depending on the metal concentration, its form (dissolved or particulate), and the hardness of the water, trace metals can be toxic to aquatic life. Metals in dissolved form are generally more toxic than metals in the particulate form. The dissolved metal concentration is dependent on pH, as well as the presence of solids and organic matter that can bind with the metal to render it less toxic.

Fig 5.1.7. Inductively coupled plasma mass spectrometer ICP-MS, Varian 820

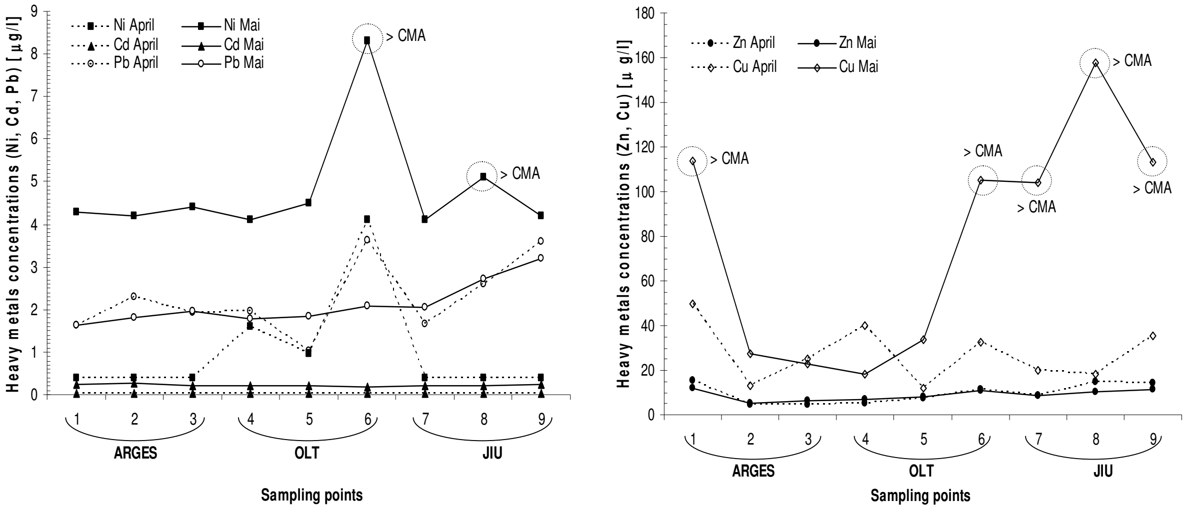


Fig. 5.1.8. Heavy Metals concentrations

An increase of Ni and Cu can be observed on Jiu River, as a concequence of mining activities in the area.

Water can also be polluted with zinc, due to the presence of large quantities of zinc in the wastewater of industrial plants. This wastewater is not purified satisfactory, one of the consequences being that rivers are depositing zinc-polluted sludge on their banks.

Regarding of Cd and Pb concentrations, the investigated waters rivers are almost all included in Class II and III, according with the Romanian Standards.

Conclusions

The water quality for all three investigated rivers is affected by both natural and anthropogenic factors. The measured parameters from analysed water samples show a slight variation depending from the collecting sites.

The river pollution indicators exceed the admissible limits for various classes of quality if in environmental area is evolve communal economy activities, chemical industry and agriculture activities followed by the economic units within the ore mining and metal processing.

As regard of Cd and Pb concentrations, the investigated waters rivers are almost all included in Class II and III, according with the Romanian Standards; and for ammonium, nitrates and chloride, classified the surface water in III and IV.

The presence in water of nutrients, like nitrogen and ammonium ions, can be associated with the biogeochemical cycles, but also with the industrial (chemical industry and mining) and agricultural activities. The concentration of ammonium ions in water can be also correlated with the water temperature, concentration of dissolved oxygen and pH [56, 57].

5.2. VALIDATION PROCEDURE FOR ASSESSING THE TOTAL ORGANIC CARBON IN WATER SAMPLES

The total organic carbon (TOC) is an index of the total amount of organic substances in water, with role in assessing the efficiency of a wastewater purification process.

It becomes extremely important to choose a fast and efficient analytical method that gives us the best results in determining the amount of TOC in the wastewater [58].

If the oxygen consumed is highest, then the organic carbon content is larger. To have efficiency in purification process of wastewater, it is important to determine total carbon organic content [59].

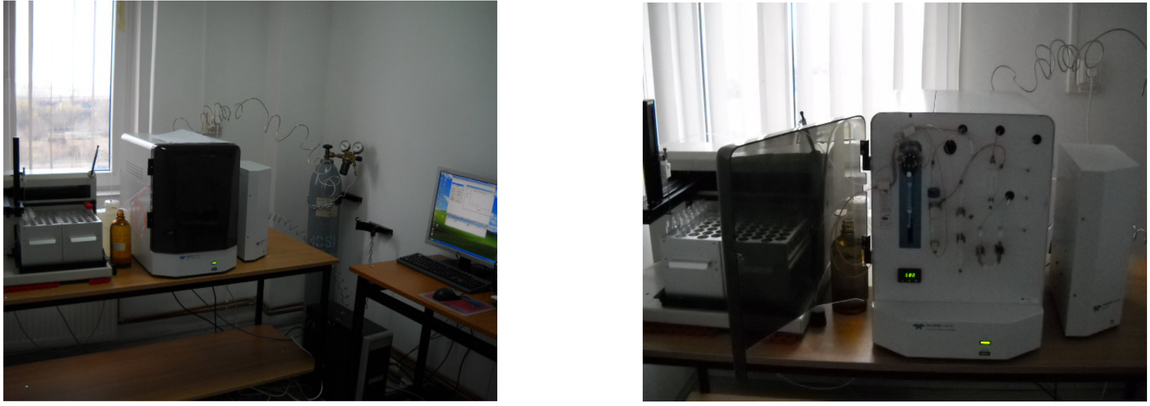


Fig.5.2.1 – Total Organic Carbon Analyzer TOC – Apollo 9000 - Teledyne Instruments

Determination of TOC is done by Total Organic Carbon Analyzer TOC – Apollo 9000 made by Teledyne Tekmar Instruments.

The standard measurement procedure, described in Water quality – guidelines for the determination of total organic carbon (TOC) and dissolved organic carbon (DOC) (ISO 8245:2000), was followed [60], which provides guidelines for the determination of TOC in wastewater.

It was used levels of TOC from wastewater samples, range between 5÷200 ppm for the present investigation.

Potassium hydrogen phthalate (KHP) was manufactured by Teledyne Tekmar, USA and it was used as Certified Reference Material (CRM).

From the reference solution, the working reference solutions were prepared by further dilutions using volumetric flask and deionized water.

Validation is the process of proving that an analytical method is acceptable for its intended purpose.

The correlation coefficient was 0.996 and proved a good linearity of the analytical method.

Mathematical equation which defines the calibration curve is: $y = 1422x + 302985$

The limit of detection, LOD, is the lowest quantity of a substance that can be distinguished with a stated confidence limit.

$LOD = 0 + 3\ s = 0 + 3 \times 0.405$ ppm $= 1.215$ ppm

The limit of quantification, LOQ, is the limit which can reasonably give the difference between two different values.

$LOQ = 0 + 10\ s = 0 + 10 \times 0.405$ ppm $= 4.05$ ppm

To determine the precision of method must be calculated a series of measurements obtained from multiple sampling under the same conditions. Repeatability expresses the precision under the same operating conditions over a short interval of time.

Tabel 5.2.2. Data for determination of method repeatability

i	***x (ppm)***	***c (ppm)***	***s***	***RSD (%)***
1	25	25.73	0.40	1.55
2	50	48.89	0.78	1.60
3	75	76.22	0.45	0.59

were:

i- serial number of concentration step (experimental point); x-expected concentration of prepared standard sample; c-average value for concentration (analyzed values) of ten analyses of the same sample; s-standard deviation; RSD-relative standard deviation.

The average value for standard deviation of all measurements was 0.54 and the average value for relative standard deviation of all measurements is gave 1.25 %. That means that this method is repeatable.

The accuracy represent the closeness between the ’’true’’ value (reference value) and the value obtained from analysis.

Tabel 5.2.3. Data for determination of method accuracy

i	***x (ppm)***	***c (ppm)***	***s***	***bias (%)***	***R_f (%)***
1	10	9.88	0.06	1.2	98.8
2	100	99.12	0.55	0.88	99.12

were:

R_f - recovery factor calculated as a percentage which includes ratio between analyzed values and standard addition values.

Conclusions

The method gave good validation parameters for linearity, the correlation coefficient being 0.996 and for precision and accuracy showed coefficients of variation lower than 1.6 % respectively lower than 1.2 %. LOD and LOQ were also good.

The measurement of TOC was evaluated in a range between 5÷200 ppm. To obtain calibration curves it was used the ratio between the peak area of standard solution and sample TOC concentration.

The validation procedures have proved that analytical process is applicable to determination of TOC content in water by Apollo 9000 Total Organic Carbon Analyzer, and that results of TOC content are valuable [64].

5.3. TRACE OF HEAVY METALS IN FISH TISSUES, SEDIMENT AND WATER FROM DANUBE RIVER IN ROMANIA

The aim of this study was to determine the heavy metals concentrations (Cd, Cu, Mn, Ni, Pb, and Zn) in water, sediments and in tissue of fish species *Gobius cephalarges*. This fish is an important component of the human food in this area.

Heavy metals such as copper, manganese and nickel play an important role in biological systems, whereas cadmium and lead are toxic, even in small quantity. For the normal metabolism, the metals must be taken up from water, fish and sediment.

These metals can have toxic effects, if metal intake is excessive. Heavy metals are non-degradable and very harmful to plants, aquatic organisms and human health at certain levels of exposure [72]. Fish accumulates metals in tissues through absorption and human can be exposed to metals via food.

The monitoring area was chosen to be Danube River because we want to investigate the heavy metals contamination levels from Cernavoda – Topalu area [74].

a) b)

Fig.5.3.2 – a) A microwave digestion system CEM Mars 5 and b) spectrophotometer 650 Zeenit, from Analytik Jena

A microwave digestion system (CEM Mars 5) was used to prepare the samples for analyses (Figura 5.3.2. a).

Determinations of the metals concentrations from all samples were performed using graphite furnace atomic absorption spectrophotometer 650 Zeenit, from Analytik Jena (Figura 5.3.2. b).

The accuracy of analytical procedure was checked by analyzing the standard reference materials (water: ICP Multi Element Standard Solution X, CertiPur; sediment: Metals in Sewage Sludge, SQC 001S; fish: DORM-2, National Research Council). Recovery rates ranged from 95 - 110 % for all elements investigated.

All data were measured in μg/kg dry weight, but for water samples the unit is μg/L.

Results

The Cd, Cu, Zn, Ni, Pb concentrations in river water in the sampling sites were compared with national standards, Order 1146/10.12.2002. The obtained results for water samples showed that, the concentrations of copper exceed to Oltina, Rasova, Topalu and Capidava.

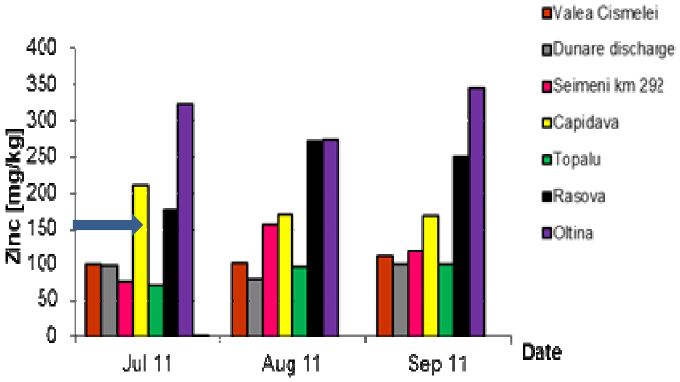
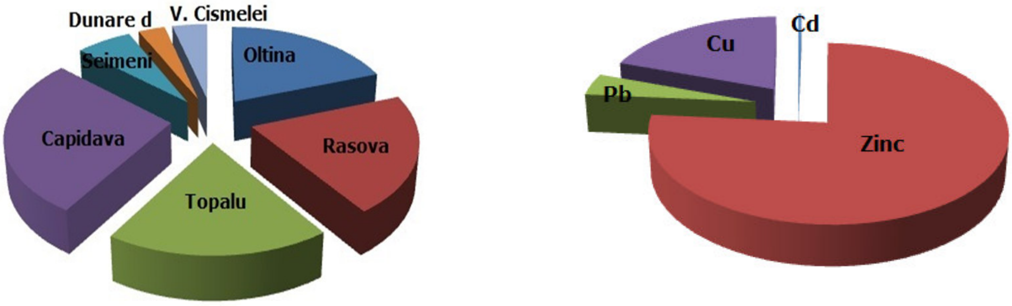


Fig.5.3.3. Concentraţia de zinc din probele de sediment

The metal concentrations obtained from the sediment samples were compared with Sediment Quality Guideline and showed a slightly exceed the maximum admissible limit, for Cd in Oltina and Topalu site, then in Rasova, Capidava and Topalu was found the Cu concentration exceeds and in Seimeni, Capidava and Rasova point prelevation, was found Zn with concentration up to limit recommended.



a) b)

Fig.5.3.3. The heavy metal concentrations from samples of a) water and b) fish

When the values obtained in the Gobius cephalarges sample were compared with the tolerable values, it has been determined that the pollution has reached to hazardous levels for the health of human (Table 5.3.4). Cd, Cu and Pb values were higher than tolerable values. The results confirmed the differences of accumulation of heavy metals tissues.

Metals concentrations in fish could be used as an index to estimate level of pollution especially in aquatic bodies [81].

As the Danube River is also used for agricultural irrigation purposes, performance of pollution researches at certain periods is significancant for both environment and public health.

Conclusions

According to Romanian Standard Classification of Water Quality, Danube River can be classified between classes I to IV which is suitable for aquatic organisms growth.

Heavy metal concentrations in the water were decreased in the sequence of Cu > Zn > Ni > Pb > Cd.

Heavy metals concentration in sediments ascertain that Danube River is free from metal contamination, although Cu, Cd and Zn were exceeded slightly the higher concentration, but this is because of anthropogenic factors.

Heavy metal concentrations in the sediments decreased in the sequence of Zn > Cu > Pb > Ni > Cd.

Cd, Cu and Pb detected in fish were upper then permissible limit provided by national law.

Heavy metal concentrations in the fish sample decreased in the sequence for the tissue as Zn > Cu > Ni > Mn > Cd > Pb [82].

5.4. SOIL SEDIMENTS ASPECTS FROM AN INDUSTRIAL PLANT OF SOUTHERN AREA OF ROMANIA

The most common chemicals involved are petroleum hydrocarbons, solvents, pesticides, and heavy metals [83, 84, 85].

Chemical and metallurgical industries are the most important sources of heavy metals in soils and sediments [86].

Soil pollution can lead to water pollution if toxic chemicals leach into groundwater or if runoff reaches streams, lakes or oceans [87].

The heavy metal accumulation in soils can affect the food quality, undergrounded water, microorganisms and plants growing [88].

Thus, representative soils from Turceni industrial area, sampled from eight (2m, 3.5m, 5.5m, 6.3m, 7.5m, 8.9m, 13m, 14.5m) different depths were selected to be measured.

Results

The pH measurements were done using normal pH-meter. The concentrations of the organic carbon were determined in aqueous solutions using an instrument with NDIR (non dispersive infrared) detector; and heavy metals (Mn, Cu, Pb and Zn) were determined in aqua regia extractant and then measured with Atomic Absorption Spectrometer.

Regarding heavy metals, the excess accumulation in soils becomes toxic to both people and animals. Due to food chain transfer, an exposure to heavy metals is normally chronic meeting with less cases of acute poisoning through ingestion or dermal contact. The chronic problems associated with long-term heavy metal exposures could be mental lapse caused by lead (Pb) poisoning, headaches, stomachaches, dizziness, vomiting caused by copper (Cu), fatigue, weakness, fever, nausea caused by zinc (Zn), or neurological problems caused by manganese (Mn).

Table 5.4.1. Concentrations of heavy metals, pH and organic carbon in industrial soils

Nr.	Soil Sample Depth (m)	pH (upH)	Plumb (Pb) (mg/kg)	Zinc (Zn) (mg/kg)	Cupru (Cu) (mg/kg)	Mangan (Mn) (mg/kg)	Carbon organic total (TOC) (%)
1	2	6.82	7.31	26.34	29.92	723.3	1
2	3.5	7.10	7.83	28.17	67.97	951.6	0.85
3	5.5	7.06	11.23	21.54	21.14	877.2	0.81
4	6.3	7.09	6.66	27.3	29.8	733.0	0.79
5	7.5	6.71	7.28	28.02	32.83	421.6	0.72
6	8.9	6.95	9.30	26.12	37.16	394.4	0.7
7	13	6.62	8.5	22.98	23.88	310	0.65
8	14.8	6.59	7.68	22.08	21.5	311.6	0.65

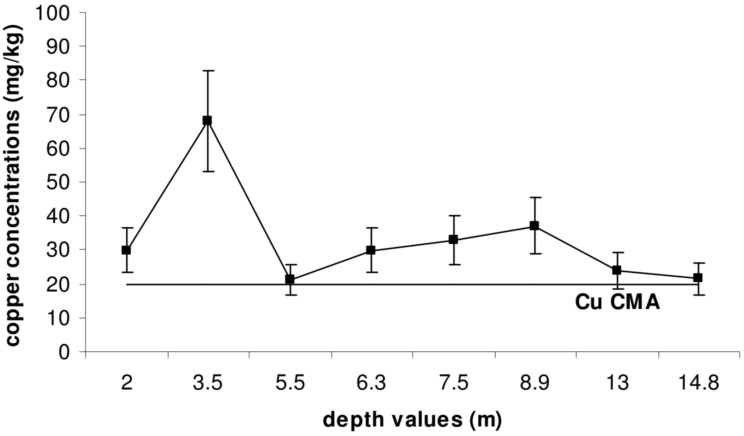


Fig. 5.4.3. The amount of Cu on different soil depths

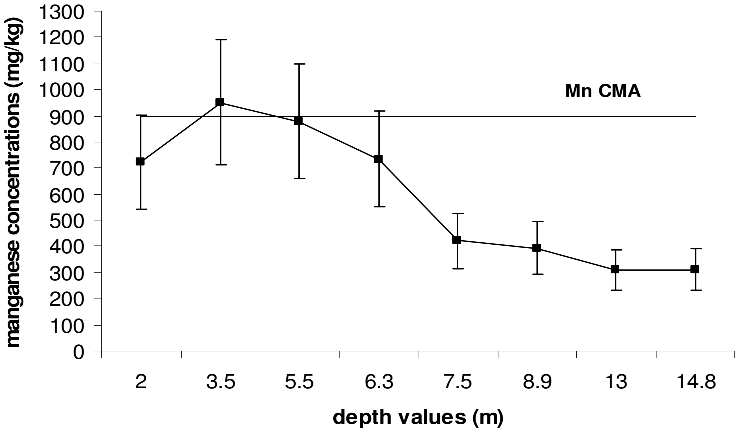


Fig. 5.4.4. The amount of Mn on different soil depths

Conclusions

The heavy metals concentration (Pb and Zn) in soils of the measured area Turceni did not exceed the limited values recommended, by the standards for soils, law nr. 104/15.06.2011 [89]. All the concentrations of Cu for all depths values exceed the recommended limits and only at 3.5 m depth the values of Mn concentration exceed the recommended limits. Load of metals is generally not critical, although the continuous exposure to heavy metals does require attention.

In all of the investigated locations, the study found that concentrations decreased with increasing of soils depth. The results indicate that there is not real environmental risk of contamination from area Turceni.

This monitoring is effective and inexpensive and may encourage polluting industries to be more environment-protective and carefully with all rumors because can affect the Romanian industry image [90, 91].

5.5. INVESTIGATION AND MONITORING OF ''IN SITU'' ATMOSPHERE POLLUTANTS FROM INDUSTIAL COLLECTED SAMPLES

Anthropogenic sources can release substances into the air, which can cause problems for humans, plants, and animals. Anthropogenic primary organic aerosols are emitted by fossil fuel, biofuel and agricultural burning [92]. The main types of pollution and the effects of pollution which we discussed, include smog, acid rain, the greenhouse effect, and "holes" in the ozone layer. Each of these problems has serious implications for our health and as well as for the whole environment.

Another type of pollution is the release of noxious gases, such as sulfur dioxide, carbon monoxide, nitrogen oxides, and chemical vapors. The long term disadvantage is the buildup of carbon dioxide and its contribution to the greenhouse effect, referring as global warming [96].

Results

The area of collecting samples is situated in the north-west part of the Calarași County and the main purpose of industrial plant is the electrical steel-melting concern.

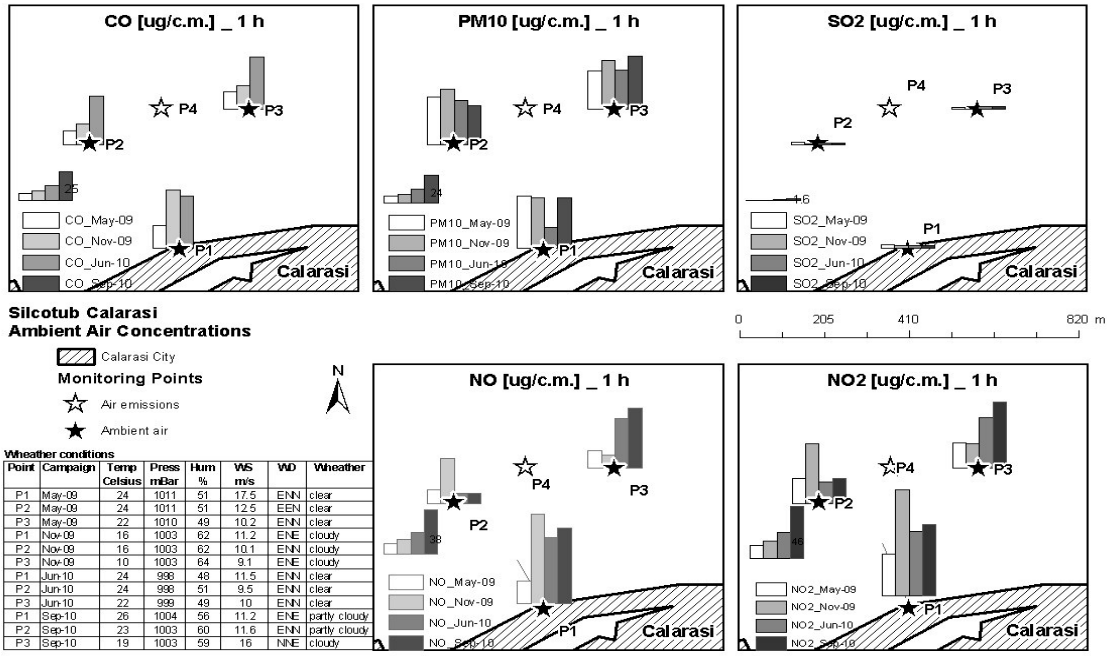
The ambient air samples were collected from 4 points of the industrial plant, at different weather conditions. Sampling locations were kept constant: at the access gate area of electrical steel-melting plant (I), borderline area between electrical steel-melting point and Calarași-Bucharest highway (II) and dust storage area (III). For the determinations on the furnace, we climbed the stairs up to the tower and this it was the last point (IV).

Geo-climatic conditions, like temperature, humidity, wind and altitude, are a significant factor that can influence the air quality monitoring program. That why, the maximum elevation of CO, SO_2, NO, NO_2 monitoring was 1-1.5 meters and for PM10, it was necessary furnace determination.

All these measurements were collected from 30 minutes to 1 hour.

All these portable analyzers are part from a complex system of a mobile laboratory, used for in situ monitoring.

Fig. 5.5.2. A complex system of ICIT Rm.Valcea mobile laboratory, used for in situ monitoring



Wheather conditions

Point	Campaign	Temp Celsius	Press mBar	Hum %	WS m/s	WD	Wheather
P1	May-09	24	1011	51	17.5	ENN	clear
P2	May-09	24	1011	51	12.5	EEN	clear
P3	May-09	22	1010	49	10.2	ENN	clear
P1	Nov-09	16	1003	62	11.2	ENE	cloudy
P2	Nov-09	16	1003	62	10.1	ENN	cloudy
P3	Nov-09	10	1003	64	9.1	ENE	cloudy
P1	Jun-10	24	998	48	11.5	ENN	clear
P2	Jun-10	24	998	51	9.5	ENN	clear
P3	Jun-10	22	999	49	10	ENN	clear
P1	Sep-10	26	1004	56	11.2	ENE	partly cloudy
P2	Sep-10	23	1003	60	11.6	ENN	partly cloudy
P3	Sep-10	19	1003	59	16	NNE	cloudy

Fig. 5.5.3. CO, SO2, NO, NO2, SOx, PM10 variation in time

Conclusions

The data of air monitoring demonstrates that the investigated area has made significant progress in reducing pollution levels. Nevertheless, the fine particulate matter remains of serious health concern in the area, even their level of concentration were kept below the allowed limit.

Using these portable/automatic equipments for on-site monitoring creates a great advantage against the classic equipment from analysis laboratory, namely:

- Real time measurements, appropriate for accidental alarming in case of special events of dangerous components release, even if the intensity is of reduced extension;
- Reduced price for calibration and maintenance comparative to classical instruments;
- Appropriate for urban area air quality measurements, not sensible to air temperature fluctuation;
- Good correlation for the repeatability of the signals [104, 105].

CAP. VI. ANALYSIS OF CHEMICAL AND BIOLOGICAL COMPOUNDS FOR FOOD CONTROL

Romanian legislation on drinking water is regulated by Law No. 458 from July 8, 2002 – concerning the quality of drinking water, being supplemented and amended by Law No. 311 from 28 June 2004. In the European Union, the bodies have to submit regulations by Directive 98/83/EC regarding the quality of water intended for human consumption.

Water must be present in significant quantities, and also it must accomplish certain quality criteria [107].

Drinking water is used for human consumption, and should accomplish certain properties and using physic-chemical analysis can be made determinations even from place of sampling, in order to establish the quality.

For checking the quality of drinking water in Romania we monitored about 26 parameters, such as: aluminum, ammonia, coliform bacteria, color, pH, electrical conductivity, free residual chlorine, Clostridium perfringens, Escherichia coli, iron, taste, smell, nitrites, oxidability, Pseudomonas aeruginosa, sulfur and hydrogen sulfide, turbidity, number of developed colonies (22°C and 37°C) [108].

6.1. UNCERTAINTY ESTIMATION FOR ELECTRICAL CONDUCTIVITY IN DRINKING WATER

Demineralized water has electrical conductivity value between 0÷80 µS/cm, distilled water has value 0.5 µS/cm, drinking water has a value between 0.5÷1 mS/cm and for ultrapure water the values of conductivity is 0,055 µS/cm.

Electrical conductivity was determined using conductometers HORIBA U-23, in which 8 sensors are connected simultaneously to analyze aqueous solution parameters of water quality.

Electrical conductivity carries current depending on the amount of ions present in the water and depends on the nature and concentration of ions, temperature and viscosity of the solution [110].

This parameter is monitored according to SR EN 27888:1997 standard that refers to drinking water quality for determining electrical conductivity.

In order to evaluate the uncertainty of measurement must be taken into account to establish the analyte [8] for which it will be calculated the uncertainty of measurement, will be identified sources of uncertainty, then they will be quantified and finally, it will be calculate the combined uncertainty.

Uncertainty can give the information about quality of result providing the dispersion of values which can be attributed to the analyte.

Calibration standard solution was KCl with a concentration of 71.8 mS/m, with a purity of 98%.

Identification of uncertainty sources: type A uncertainties is uncertainty due to repeatability and type B uncertainty are uncertainty due to calibration of device; uncertainty due to standard certification, uncertainty due to bias, uncertainty due because of standard solution used (purity reagents, instrumental effects, measurement conditions, effects of sample, calculation effects, etc.), uncertainty due to the resolution of the equipment.

Table 6.1.1. Uncertainty sources to determine water conductivity

NR. CRT.	SURSELE DE INCERTITUDINE	CALCUL	INCERTITUDINEA	INCERTITUDINEA RELATIVA
1.	Repetabilitate	Media aritmetica: 72,37 mS/m. Abaterea standard experimentala: s = 0,48 mS/m = 4,8 $\mu S\ /\ cm$	$u_1 = 4{,}8\ \mu S\ /\ cm$	$u_{r_1} = \frac{u_1}{C_1} = \frac{4.8\,\mu S/cm}{723.7\,\mu S/cm} = 0{,}0066$
2.	Etalonarea aparatului	Este ± 2.82 mS/m, calculata cu factor de acoperire k = 2, corespunzator unui nivel de incredere de 95 % $u_2 = 2{,}82/2 = 1{,}41$ mS/m	$u_2 = 14{,}1\ \mu S\ /\ cm$	$u_{r_2} = \frac{u_2}{C_2} = \frac{14.1\,\mu S/cm}{723.7\,\mu S/cm} = 0{,}019$
3.	Componenta la deplasare	$\text{Bias} = \frac{\lvert 71.8 - 72.37 \rvert}{71.8} * 100$ $u_3 = 0{,}79\ \text{mS/m} / \sqrt{3} = 0{,}46\ \text{mS/m}$	$u_3 = 4{,}6\ \mu S\ /\ cm$	$u_{r_3} = \frac{u_3}{C_3} = \frac{4.6\,\mu S/cm}{718\,\mu S/cm} = 0{,}0064$
4.	Etalonul preparat	a) *puritatea materialelor de referinta:*	$u_a = 1\ \%$	$u_{r_a} = \frac{u_a\%}{p\%} = \frac{1}{99} = 0{,}01$
		b) *limita de exactitate a balantei* Sartorius CP 2250-OCE,	$u_b = 0.2/2 = 0{,}1$ mg	$u_{r_b} = \frac{u_b}{0.373} = \frac{0.1\,mg}{373\,mg} \approx 0{,}0027$
		c) *repetabilitatea la volumetrie,*	$u_c = 2x\ s = 2x\ 2.028 = 4{,}056$g	$u_{r_c} = \frac{u_c}{V} = \frac{4.056\ g}{494.056\ g} = 0{,}0082$
		d) *etalonarea cilindrului gradat din CE*	$u_d = 5/2 = 2{,}5$ ml	$u_{r_d} = \frac{u_d}{V} = \frac{2.5\ ml}{500\ ml} = 0{,}005$

Extended uncertainty is 5% calculated using a coverage factor k = 2 which gives a confidence level of approximately 95% and it must be calculate for every obtained result.

Uncertainty parameter can be considered standard deviations, but it is recommended to evaluate uncertainty of all sources, to characterize a good spreading of the obtained values.

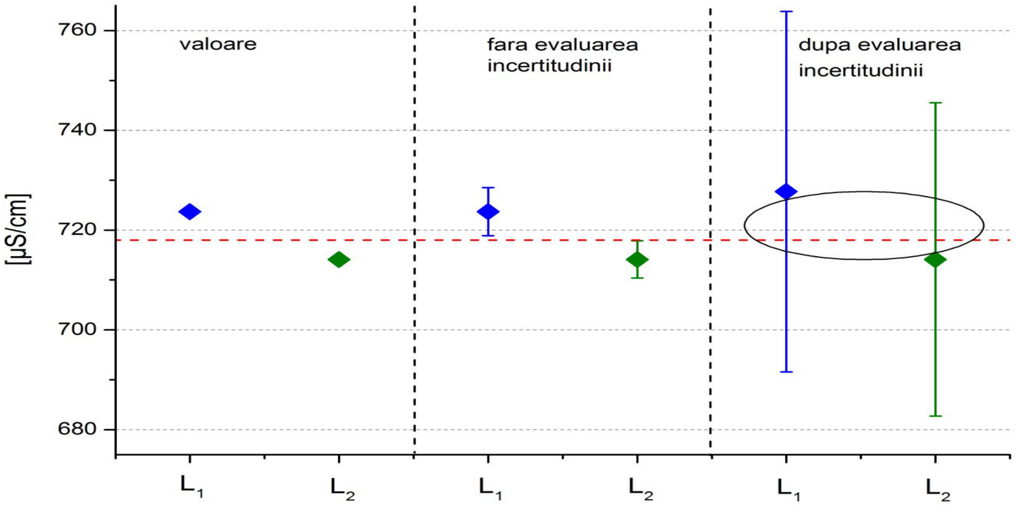


Figure .6.1.1. Test de intercomparare cu două aparate diferite pentru măsurarea conductivităţii cu soluţia standard de KCl

6.2. METHOD VALIDATION FOR DETERMINATION OF BIOCHEMICAL OXYGEN DEMAND IN WASTEWATER

Requirements from quality of wastewater depend from the place in which are discharged this waters. Romanian orders, No. 352/2005 [112], prescribe technical regulation, by NTPA 001 wastewaters qualities that are discharge in natural receivers (rivers, lakes) and NTPA 002 for sewage network or wastewaters treatment plants.

A valuable parameter regarding biodegradability who indicates the type of treatment process is biochemical oxygen demand.

Biochemical oxygen demand after n days, 5 and 7, is defined as mass concentration of dissolved oxygen consumed under specified conditions by biochemical oxidation of organic and/or inorganic substances in water [113, 114].

In order to determine biochemical oxygen demand (BOD_5), it was used a spectrophotometer DR HACH 2800 purchased from Analytic Jena Company from Romania. Accessories and specific reagents have been delivered by the Analytic Jena Company as well, LTV073 thermostat, set for water dilution and cuvette test for determination of BOD_5 manufactured by the HACH Lange Company from Germany.

Figure.6.2.1. Spectrophotometer DR HACH 2800, LTV073 thermostat, set for water dilution and cuvette test for determination of BOD_5

Our analytical method showed very good validation parameters and proved to be acceptable for the intended purpose [12].

Table 6.2.3. Validation parameter and obtained decisions

Analytical principle	Validation parameters	Laboratory Requirements	Realized parameters	Decision
Biochemical Oxygen Demand to 5 days	Range of measurement	3÷25 mg/L	3÷25 mg/L	Admis
	Liniarity	$R^2 > 0.995$	$R^2 = 0.997$	Admis
	Limit of detection	1.5 mg/L	0.921 mg/L	Admis
	Limit of quantification	3 mg/L	3.07 mg/L	Admis
	Precision	2 mg/L	1.75 mg/L	Admis
	Accuracy	1 mg/L	0.95 mg/L	Admis

Conclusions

Although the differences between biochemical oxygen demand (BOD_5) values stipulated in NTPA001, normative for wastewater discharged into natural receivers, where maximum admissible concentration is 25 mg/L and biochemical oxygen demand (BOD_5) values stipulated in Order no. 161 from 16/02/2006 [25], which classify surface waters, where the range is 3÷20 mg/L, is considered small, it can be concluded that validation described below can be used for both types of water.

For chemical analysis of surface water it is important to demonstrate the impact of oxygen on aquatic life.

The method showed good parameters for linearity, the correlation coefficient is 0.997, and for precision and accuracy, coefficients of variation are less than 2.5%. Limits of detection and quantification have also given good results. Biochemical oxygen demand (BOD_5) measurements were assessed in the range 3÷25 mg/L.

6.3. DEVELOPMENT OF CONTROL CHART FOR DETERMINATION OF CHEMICAL OXYGEN DEMAND FROM WATER

Control charts are called statistical process control or Shewhart charts (were developed by Shewhart in 1920). The control charts is a graph used to study how a process changes over time [115]. Control charts contribute to establish norm for each analysis and to discover process variation (in or out of control) and to predict events which can affect it (detection of possible errors).

The instrument use to determine chemical oxygen demand was VIS Spectrophotometer DR 2800 from HACH Lange Gmbh. The MRC (Certified Reference Material) with concentration of 1000 mg/dm3 (chemical oxygen demand standard solution) was acquired also from HACH Lange Gmbh.

According to SR ISO 6060: 1996, chemical oxygen demand (COD) from water measure water capacity to consume oxygen by oxidation of inorganic substances and decomposition of organic matter. Increasing the amount of organic substances from water encouraged water pollution with bacteria.

In accordance with quality systems provided by ISO/CEI 17025:2005 [116], a laboratory must have control procedures to demonstrate the quality of performed analysis by using control charts [117].

To build, maintain and supervise control charts there are two stages, a preliminary one, when are performing multiple analysis of samples control and will be determin the limits of attention and control and the routine stage, when are performing analysis of single or double samples control.

To build charts control of the chemical oxygen demand, was analyzed a certified reference material (CRM), prepared with a concentration of 125 mg/L O_2, 20 times, on different days, by different operators.

x was average calculated by summing the data and dividing by the number of results:

$$\bar{x} = \sum_{i=1}^{n} \frac{x_i}{n} \qquad \bar{x} = 126{,}3$$

Standard deviation of sample *s* was calculated:

$$s = \sqrt{\frac{\sum_{i=1}^{n} (x_i - \bar{x})^2}{n-1}} \qquad s = 2{,}178846$$

The chart control can be realized using data of average:

- Upper limit of control LCS = $\bar{x}$ + 3s; LCS=132,8365
- Upper limit of alert, LAS = $\bar{x}$ + *2s;* LAS=130,6577
- Middle line (target) = average; $\bar{x}$ = 126,3
- Lower limit of alert, LAI = $\bar{x}$ - *2s;* LAI=121,9423
- Lower control limit, LCI = $\bar{x}$ – 3S; LCI=119,7635

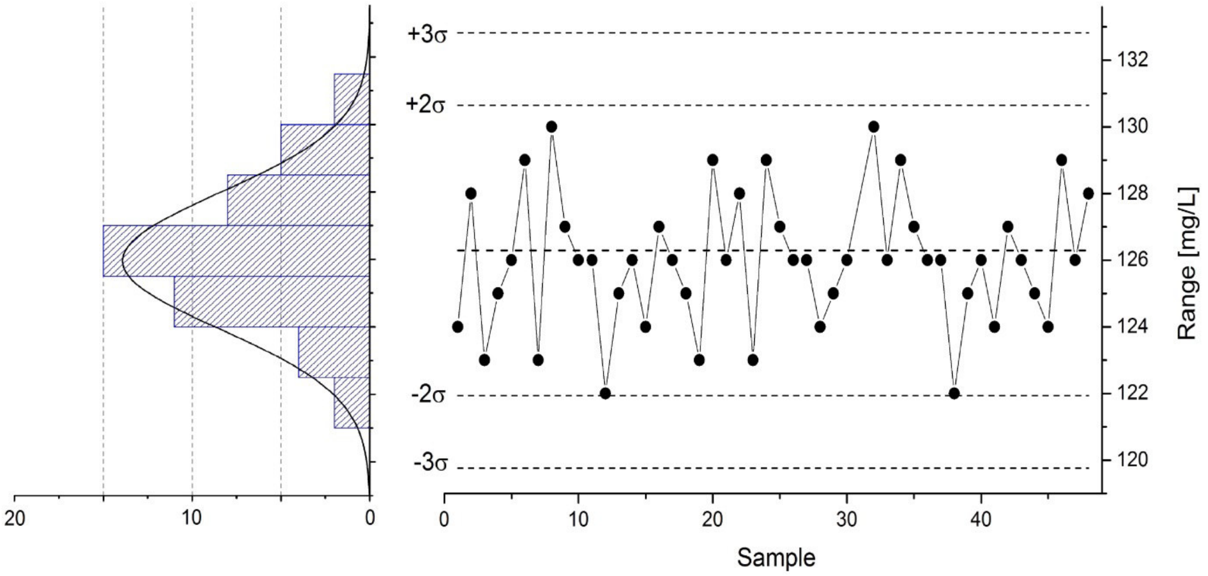


Fig. 6.3.1. Stewhart control charts for chemical oxygen demand of surface waters

According with theory of probability and also with normal distribution, a chart control must have the following characteristics: half of the points must be above the centerline and the other half must be below; points should not follow an increasing or decreasing trend; approximately 68% of points must be in range ± 1s; about 95% of points should be between the two warning limits (range ± 2s); 99.73% (virtually all points) should be between the two lines of control (range ± 3s).

In the case when one of these situations is violated, the analysis should be stopped and certified reference material one more time analyzed. The obtained results will be not reported. If there is no problem with the reference material, then it should be investigated other sources of error, the problem is corrected and the analysis is retaked.

A series of multiple control rules were introduced by Westgard and can help user to take a decision about the control process with criteria of acceptance or rejection.

To identify if the process is or not *under control*, Levey - Jennings charts are used. With this you can identify the values that are not between acceptable limits and corrective action will be taken.

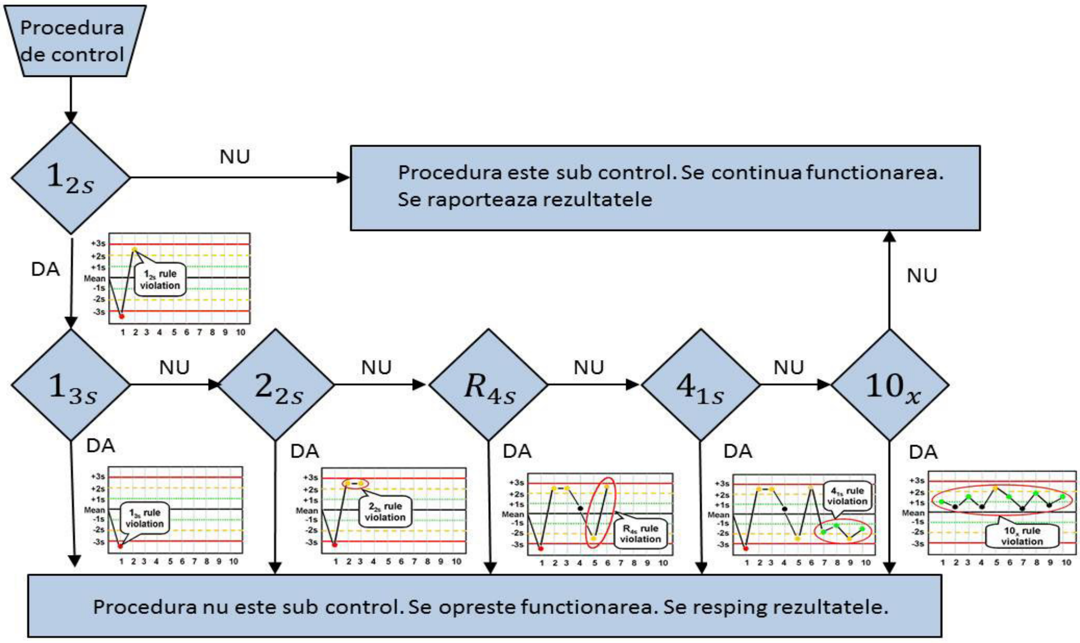


Fig. 6.3.2. Diagram of control process according to the Westgard rules

The performance characteristics of the analytical method for proper quality control procedures are precision and accuracy.

The application of control charts is a laborious and time consuming process. The use of computers is recommended because of precision and facility of this work.

An exact choice of quality control of parameters and then implementation in the process of measurement is based on laboratory experience.

As control samples can be used the certificate reference materials, if they doesn't involve suplimentary cost.

CAP. VII. CONCLUSIONS

The experimental results lead to the following conclusions:

We have determined biological and ecological compounds from water, air, soil, and food samples by testing several methods of analysis and there was estimated their hazard on the environment and human health.

Several techniques of instrumental analysis have been used, such as gas chromatography-mass spectrometry (GC-MS), inductively coupled plasma mass spectrometry (ICP-MS), atomic absorption spectrometry (AAS), UV-VIS spectroscopy, NDIR detector methods, potentiometric and gravimetric, portable analyzers measurements being performed for *in situ* samples.

The validation parameters were tested for the analytical methods used, as: precision, accuracy, linearity, limit of detection, limit of quantitation, specificity and selectivity.

The value of uncertainty of measurement was calculated and control charts were built, procedures that assure the quality of results.

The analyses performed for all types of samples were made in accordance with the U.E. recommendations, adjusted to Romanian requirements standards.

Satisfactory results have been obtained after interlaboratory measurements and other testing program.

Almost all measurements from water, air and soil samples were accredited RENAR, Romanian national accreditation body.

Accreditation certified the conformity of analyses and the accreditation certificate was the assureance of quality results.

The obtained results in this thesis were disseminated as follows:

- 7 original articles published in journals with impact factor;
- 5 original articles published in journals indexed in national and international data bases;
- 34 participation at national and international conferences;
- 3 research research stage at the University of Osnabrück, Germany.

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