UNIVERZITET U NOVOM SADU
FAKULTET TEHNIČKIH NAUKA
DEPARTMAN ZA INŽENJERSTVO ZAŠTITE
ŽIVOTNE SREDINE I ZAŠTITE NA RADU

MSc. Maja Sremački

Modules of emerging xenobiotics detection in mixed urban wastewater

– PhD thesis –

Mentor:

Prof. emeritus Mirjana Vojinović Miloradov

Novi Sad, 2017.

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Moduli određivanja emergentnih ksenobiotika u mešovitim otpadnim vodama

– doktorska disertacija –

Mentor:

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Novi Sad, 2017.

УНИВЕРЗИТЕТ У НОВОМ САДУ ● **ФАКУЛТЕТ ТЕХНИЧКИХ НАУКА**
21000 НОВИ САД, Трг Доситеја Обрадовића 6

КЉУЧНА ДОКУМЕНТАЦИЈСКА ИНФОРМАЦИЈА

Редни број, **РБР**:		
Идентификациони број, **ИБР**:		
Тип документације, **ТД**:		Монографска публикација
Тип записа, **ТЗ**:		Текстуални штампани материјал
Врста рада, **ВР**:		Докторска дисертација
Аутор, **АУ**:		М.Сц. Маја Сремачки
Ментор, **МН**:		Др Мирјана Војиновић Милорадов, професор емеритус
Наслов рада, **НР**:		Модули одређивања емергентних ксенобиотика у мешовитим отпадним водама
Језик публикације, **ЈП**:		Енглески
Језик извода, **ЈИ**:		Енглески/Српски
Земља публиковања, **ЗП**:		Република Србија
Уже географско подручје, **УГП**:		Аутономна Покрајина Војводина
Година, **ГО**:		2017
Издавач, **ИЗ**:		Ауторски репринт
Место и адреса, **МА**:		Нови Сад, Трг Доситеја Обрадовића 6
Физички опис рада, **ФО**: (поглавља/страна/ цитата/табела/слика/графика/прилога)		8/189/159/18/38/-/4
Научна област, **НО**:		Инжењерство заштите животне средине и заштите на раду
Научна дисциплина, **НД**:		Инжењерство заштите животне средине
Предметна одредница/Кључне речи, **ПО**:		Мешовите комунална отпадна вода, емергентни ксенобиотици, хроматографија, процеси третирања отпадне воде
УДК		
Чува се, **ЧУ**:		У библиотеци Факултета техничких наука у Новом Саду
Важна напомена, **ВН**:		
Извод, **ИЗ**:		Емергентне супстанце (ЕмС) припадају широј групи хемикалија које се фреквентно користе, а нису обухваћене законски прописаном мониторингу, као приоритетне и хазардне приоритетне супстанце. Емергентни ксенобиотици (ЕК), а посебно супстанце које ометају ендокрини систем (ЕД), су група једињења које припадају и приоритетним и емергентним супстанцама. Модули одређивања ксенобиотика су интерактиван, сложен и динамичан процес, подложан променама, детаљно описан у тези. Аналитичка метода, ТТЕ праћене ГЦ-МС, успешно је прилагођена за скрининг идентификацију емергентних и приоритетних супстанци у отпадној води. За евалуацију добијених резултата коришћене су статистичке методе (ПЦА, ХЦА и ПК) у сврху корелације хемијских карактеристика са предвиђеним третманима отпадних вода, као и процена ризика за животну средину.
Датум прихватања теме, **ДП**:		24.08.2015.
Датум одбране, **ДО**:		
Чланови комисије, **КО**:	Председник:	Др Срђан Колаковић, ред. проф.
	Члан:	Др Ивана Михајловић, доцент
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Потпис ментора

UNIVERSITY OF NOVI SAD ● **FACULTY OF TECHNICAL SCIENCES**
21000 NOVI SAD, Trg Dositeja Obradovića 6

KEY WORDS DOCUMENTATION

Accession number, **ANO**:			
Identification number, **INO**:			
Document type, **DT**:			
Type of record, **TR**:		Monographic Publication	
Contents code, **CC**:		Textual Printed Material	
Author, **AU**:		Ph.D Dissertation	
Mentor, **MN**:		Maja Sremački, M.Sc	
Title, **TI**:		Modules of emerging xenobiotics detection in mixed urban wastewater	
Language of text, **LT**:		English	
Language of abstract, **LA**:		English/Serbian	
Country of publication, **CP**:		Serbia	
Locality of publication, **LP**:		Autonomus Provance of Vojvodina	
Publication year, **PY**:		2017	
Publisher, **PB**:		Author's reprint	
Publication place, **PP**:		Novi Sad, Trg Dositeja Obradovića 6	
Physical description, **PD**: (chapters/pages/ref./tables/pictures/graphs/appendixes)		8/189/159/18/38/-/4	
Scientific field, **SF**:		Environmental Engineering and Occupational Safety	
Scientific discipline, **SD**:		Environmental Engineering	
Subject/Key words, **S**/**KW**:		Mixed urban wastewater, emerging xenobiotics, chromatography, wastewater treatment processes	
UC			
Holding data, **HD**:		Library of Faculty of technical sciences, Novi Sad	
Note, **N**:			
Abstract, **AB**:		Emerging substances of concern (EmS) are wide groups of chemicals frequently used, that are not included in legislation nor mandatory monitored like priority and hazardous priority substances. Group of substances overlapping in priority and emerging substances list provided by legislation are Endocrine Disruptive Compounds (EDCs), referred to as emerging xenobiotics (EXs). The modules of emerging xenobiotics detection and identification presents an interactive, complex and dynamic process shown in the research. Analytical method, LLE followed by GC-MS was successfully adapted and applied to screening of emerging and priority substances in wastewater. The screening and target analyses results were evaluated via risk assessment (ERA) and detected substances chemical properties were statistically evaluated (PCA, HCA an PC) for the purpose of correlation of chemical properties and predicted treatment possibilities from wastewater.	
Accepted by the Scientific Board on, **ASB**:		24.08.2015.	
Defended on, **DE**:			
Defended Board, **DB**:	President:	Dr Srđan Kolaković, full prof.	
	Member:	Dr Ivana Mihajlović, assist. prof.	
	Member:	Dr Jelena Radonić, assoc. prof	
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	Member:	Dr Ivan Spanik, full prof.	Menthor's sign
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Acknowledgment and appreciation

The most appreciation and acknowledgement I would like to give to my mentor Prof. emeritus Mirjana Vojinović Miloradov for her selfless support and understanding during all my years of study and research and particularly during the PhD research. I admire her spirit, strength and outmost respect and patience for younger collages.

The special appreciation goes to my second adviser and foremost a friend in need, Prof. Ivan Špánik who helped me tremendously during the experimental part of PhD research. It was great honour and most of all fun working alongside Prof. Ivan Špánik, and his associates, particularly PhD Olga Vyviurska, on the Institute of Analytical Chemistry, Faculty of Chemical and Food Technology, Slovak University of Technology in Bratislava.

For most, I owe gratitude and appreciation to all my professors and colleges on Department for Environmental Engineering and Occupational Safety for the amazing and friendly working atmosphere that always makes an individual grow and work with great inspiration.

The particular thanks to collages Doc. Ivana Mihajlović, MSc. Maja Đogo, MSc. Boris Obrovski from Department for Environmental Engineering and Occupational Safety, and PhD Maja Milanović, from Department of Pharmacy, Faculty of Medicine, University of Novi Sad.

This thesis was developed under the scope of several projects and institutions:

- *Ministry of education, science and technological development,*
- *University of Novi Sad, Faculty of technical sciences, Department of environmental engineering and occupational safety,*
- *Slovak University of Technology in Bratislava, Slovakia, Faculty for food and chemical technology, Department of Analytical Chemistry,*
- *SCORE-ES1307 COST Action, NIVA (Norwegian Institute for Water research) and EWAG (Swiss Federal Institute of Aquatic Science and Technology) from 2013 to 2017,*
- *International Visegrad Fund,*
- *Project "Drinking Water Quality Risk Assessment and Prevention in Novi Sad municipality, Serbia" Programme "The Science for Peace and Security" P. -no. ESP.EAP.SFP 984087,*
- *National Project: "Treatment and quality of meat industry wastewater and determination of "emerging" substances with the goal of water bodies contamination reduction", Sub-Project: "Development of hygienic and technological process for production of animal origin viands for the purpose of*

manufacturing products with better quality and safety for the competitive world market" no. III 46009.

And last but not least, for all the unconditional love, support, and motivation during the research and work I have to thank to my family – mum, dad, fiancé and especially my sister Marina for putting it all into perspective.

Abstract

Emerging substances of concern (EmS) are wide groups of chemicals recognized by global scientific and technical societies as pollutants in environment that should be monitored. In these groups there are substances widely used all over the world in different branches of industry, agronomy, science, research and everyday life. Such substances are industrial chemicals (ICs), pharmaceuticals (PhACs), personal care products (PCPs), plasticizers, wood preservatives (WPs), pesticides and many others.

Priority substances and hazardous priority substances (PhPSs) have designed and audited monitoring plan with defined maximum allowable concentration and doses in different environmental media, but emerging substances do not, and the fact that EmS present the frequently and continuously used and detected substances in low concentration levels, there is a question of chronic effect that they might have on environment and living organisms. Group of substances that is overlapping in priority and hazardous priority and emerging substances lists provided by legislation, environmental standards and guidelines of research institutes is Endocrine Disruptive Compounds (EDCs), referred to as emerging xenobiotics (EXs). Groups of substances most commonly known as endocrine disruptors are pesticides, polychlorinated biphenyls, dioxins, phenols, phthalates and estrogens, that have en masse unknown fate in environment.

Xenobiotics are any foreign chemical species detected within the organism that is not naturally occurring within or produced by the observed organism. The term is also used for chemical species naturally present in the organism but in much higher concentrations than normal. Explicitly, licit and/or illicit drugs in humans are treated as xenobiotics, because the human body does not produce them itself, nor they are part of a food or water sources. EXs are a group of biologically highly active molecules that are found in environment compartments but are not naturally produced or occurred. EXs in highly contaminated urban wastewater can be EDCs, pharmaceuticals (pharmaceutically active compounds – PhACs), illicit drugs (IDs), nano-compounds (NCs), flame retardants (FRs), PCPs, steroids (natural or synthetic) and others.

The medium selected for the research is urban wastewater generated in the city of Novi Sad which is defined as mixed urban wastewater (MUWW) to emphasize the specific segment of urban wastewater defined by *Council Directive 91/271/EEC on urban waste-water treatment as*" domestic wastewater or the mixture of domestic wastewater with industrial wastewater and/or run-off rain water". Mixed urbane wastewater represents the problem of developing countries as it is a mixture of all urban effluents – industrial, domestic, communal, road wash-out. This mixture can be extremely difficult for analysis and treatment.

The research and studies of mixed urban wastewater, showed there is a high possibility of different types of xenobiotic compounds detection of diverse groups and concentrations.

In recent studies different pharmaceuticals and illicit drugs have been detected, 9 and 5 different substances, respectively, in the samples of mixed urban wastewater of research area.

The screening analysis makes a powerful analytical tool for predetermination, identification and prioritization of organic substances and pollutants in surface water, as well as in any other water body or system. The analysis of surface water samples should be modified to suit the specific analyte and adapted to extract and obtain the most important and valuable information. This is why every module of emerging xenobiotics research is significant and requires separate and specific planning and consideration.

The concentrations of detected and identified substances are in micro and nano world, which is why it is necessary to precisely construct and perform every module, so the relevant information for the design and decision making processes is obtained. The screening analysis, as the common analytical procedure, is a process that comprises of extraction, isolation, detection, identification and registration of selected substance or a group of substances within a minimum number of steps and relatively short period of time. For the purpose of screening analyses, gas chromatography coupled with mass spectrometry was selected as an analytical method. During the adaptation of the preparation method for screening analyses, different solvents have been utilised during extraction process to obtain the optimum solvent for specific sample.

The chemical species that have been detected in surface water during the year of 2012 belong to emerging and priority groups of substances – flammables, irritants, toxic and cancerogenic compounds, EDCs, industrial chemicals, plasticizers, aliphatic and fatty alcohols and acids, higher alkanes, wood preservatives, flavour and fragrances, personal care products, pesticides, antifoaming agents, additive residues and others. The registered substances during the screening analyses expose the possibility for specification of target analyses, selection of target substances, and better organisation of surface water research and control monitoring system specific for the selected location.

The research goals are detection environmental emerging xenobiotics that can infiltrate food chain and water sources used by humans - endocrine disruptive compounds (organochlorine pesticides and natural steroids) and illicit drugs. The results of the research are adapted sampling methodology and location selection, preparation and analytical method for screening analysis, selection of detected substances for target analysis, specific guidelines for monitoring (early warning system) and recommended treatment process possibilities.

Analytical method, based on combination of liquid-liquid extraction followed by gas chromatography-mass spectrometry was successfully adapted and applied to detection and identification of possible emerging and priority substances in wastewater samples collected during the period 2012/13. The data obtained during research thought analytical tools and methods and literature overview are requiring further statistical evaluation and risk assessment for the deduction of comprehensive conclusions.

Key words: Mixed Urban Wastewater, Emerging Substances and Pollutants, Liquid-liquid Extraction, GC-MS, HPLC-MS2, HPLC-HPMS, Wastewater Treatment Processes, Endocrine Disruptors, Illicit drugs.

Abbreviations

AA– Annual Average

AA EQS – Annual Average Environmental Quality Standards

ACP – Activated Carbon Processes

ADD - Attention Deficit Disorder

ADHD -Attention Deficit Hyperactivity Disorder

AOEL – Acceptable Operator Exposure Level

AOPs – Advanced Oxidation Processes

ASP – Activated Sludge Processes

BBP - Butyl-Benzyl Phthalate

BCF – BioConcentration Factor

BE - Benzoylecgonine

BOD_5 – Biological Oxygen Demand in 5 days

BPA – Bisphenol A

CECs – Contaminants of Emerging Concern

CEPT – Chemically Enhanced Primary Treatment

CFR – Code of Federal Regulations

CMR - Carcinogenicity, Mutagenicity or Reproductive toxicity

CNS – Central Nervous System

COD – Chemical Oxygen Demand

D9-THC - D9-tetrahydrocannabinol methanolic solution

DBP - Di-n-butylphthalate

DDD - Dichlorodiphenyldichloroethane

DDE - Dichlorodiphenyldichloroethylene

DDT - Dichlorodiphenyltrichloroethane

DDA - Dichlorodiphenylacetic acid

dEfOM – Dissolved Effluent Organic Matter

DEP – Diethyl Phthalate

DEHP – Bis-(2-ethylhexyl) Phthalate

DIDP - Diisodecyl Phthalate

DIOP – Diisooctyl Phthalate

DnOP - Di-n-Octyl Phthalate

DOM – Dissolved Organic Matter

DRB – Danube river Basin

E1 – Estrone

E2 - 17β-Estradiole

E2-17α - 17 α-Estradiole

E3 - Estriol

EC_{50} – Half maximal effective concentration

ECOSAR – Ecological Structure Activity Relationship

EDCs – Endocrine Disruptive Compounds

EDDP - 2-Ethylidine-1,5-Dimethyl-3,3-Diphenylpyrrolidine Perchlorate

EE2 - 17α-Ethinylestradiole

EfOM – Effluent Organic Matter

EQS – Environmental Quality Standards

EQSD - Environmental Quality Standards Directive

EmS – Emerging Substances

EXs – Emerging Xenobiotics

EmP – Emerging Pollutants

ERA - Ecotoxicology and Environmental Risk Assessment

EWS – Early Warning System

FRs – Flame Retardants

GCMS – Gas Chromatography coupled with Mass Spectrometry

GHGs – GreenHouse Gasses

HCB –Hexachlorobenzene

HL – Half-Life

HPLC – High Pressure Liquid Chromatography

HRBTP – High Rate Biological Treatment Process

HRT – Hydraulic Retention Time

ICs – Industrail Chemicals

IR - InfraRed

JDS 3 – Joint Danube Survey 3

KDC – Kuderna Danish concentrator

LC_{50} – Lethal Concentration

LD_{50} – Median Lethal Dose

LLE – Liquid - Liquid extraction

LOAEL – Lowest Observed Adverse Effect Level

LOD – Limit of Detection

LOQ – Limit of Quantification

LSD - Lysergic acid Diethylamide

LV – Limit value

MAC EQS – Maximum Annual Concentration EQS

MDMA - Methylenedioxy Derivative of Methamphetamine

MEC - *Maximum Environmental Concentrations*

MES - Morpholinoethane sulfonate

MEHP - 1,2-benzenedicarboxylic acid, mono(2-ethylhexyl) ester

MF – Micro-Filtration

MUWW – Mixed Urban Wastewater

NEC – No Effect Concentration

NCs – Nano-Compounds

NF – Nano-Filtration

NORMAN – Network of reference laboratories, research centres and related organizations for monitoring of emerging environmental substances

NOM – Natural Organic Matter

NOAEL –No Observed Adverse Effect Level

NOEC – No Observed Effect Concentration

OVAT – One Variable At a Time

PAHs – Polycyclic Aromatic Hydrocarbon

PBTs- Persistent, Bioaccumulative and Toxic substances

pEfOM – Particulate Effluent Organic Matter

PCP – Phencyclidine

PCPs – Personal Care Products

PCB – Polychlorinated Biphenyl

PC unite – Personal Computer unite

PeCB - Pentachlorobenzene

PhPPs - Priority and hazardous Priority Pollutants

PhACs – Pharmaceutically Active Compounds

PNEC – Predicted No Effect Concentration

PPCPs – Pharmaceuticals and Personal Care Products

PTV – Programmed Temperature Vaporization

PUC WWS – Public Utility Company Waterworks and Sewerage

PPC – Potassium Permanganate Consumption

QMI – Quality Match Index

QS – Quality Standards

RAS – Return Activated Sludge

RBC – Rotating Biological Contractors

RBM – Regression Base Model

RC – Rate Constant

REACH - Registration, Evaluation, Authorisation and Restriction of Chemicals

ROS – Reactive Oxygen Species

RQ – Risk Quotient

RT – Retention Time

SA – Screening Analysis

SPE – Solid Phase Extraction

SMPs - Soluble Microbial Products

SS – Suspended Solids

TA – Target Analysis

TBD – Total Biodegradation

TBLa – Triple Bottom Line analysis

TDS – Total Dissolved Solids

THC – Tetrahydrocannabinol

TOC – Total Organic Carbon

TRIS - Tris(hydroxymethyl)aminomethane

TR – Total removal

TSA – Total Sludge Adsorption

TSS – Total Suspended Solids

TtA – Total to air

TU – Toxic Units

UF – Ultra-Filtration

US EPA – United States Environmental Protection Agency

VF – Vacuum Filtration

VOCs – Volatile Organic Compounds

vPvB - Very Persistent and Very Bioaccumulative

VSS – Volatile Suspended Solids

WAS – Waste Activated Sludge

WFD – Water Frame Directive

WMPs – Water Management Plans

WPs - Wood Preservatives

WWTp – Wastewater Treatment Process

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1. Overview of the thesis

"If there is magic on the planet, it is contained in Water."

Loren Eiseley, Immense Journey, 1957.

"All the water that will ever be, is right now!"

Greenpeace International

"The amount of moisture on Earth has not changed. The water the dinosaurs drank millions of years ago is the same water that falls as rain today. But will there be enough for a more crowded world?"

NGM

Water is the most essential and powerful substance on Earth, a substance that is responsible for the origin, development and stainability of life. In the hydrosphere, the water is circulating, making the dynamic and reversible hydrological cycle a system of processes necessary for the continued existence of life.

The structure of liquid water is related to the 14-molecule tetrahedral structures of hexagonal and cubic ice. Water clusters, shown in Figure 1.1, appear to be relatively stable in liquid water, forming curved surfaces when bound together by three potential hydrogen bonds. Twenty of the 14-molecule tetrahedral units may form a 3 nm in diameter of cosahedral structure. The icosahedral $(H_2O)_{280}$ network of water cluster shows increased stabilization as the shells increase in the order *(Loboda and Goncharuk 2010).*

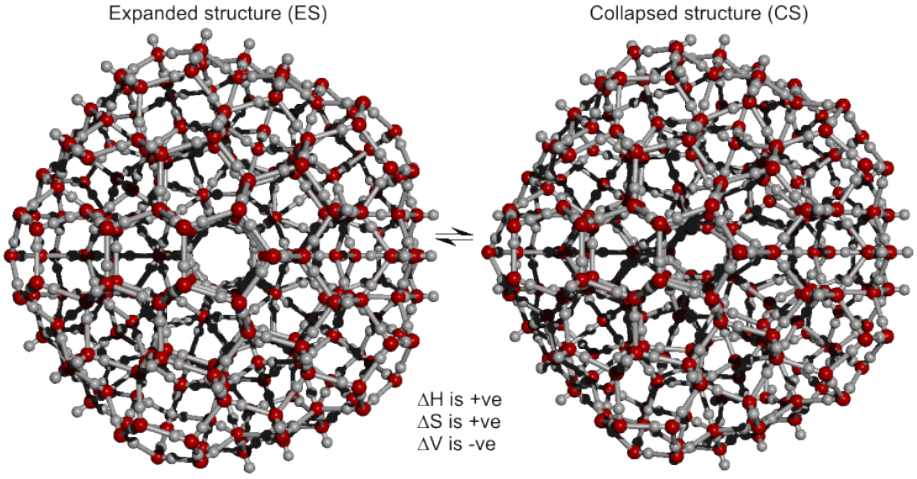


Figure 1.1. Water cluster (Chaplin 2017)

Many unique properties and anomalies of water (41) are the result of the hydrogen bonds. In the field of environmental research and protection engineering, each chemical compound is enfolded in a water cloud. The icosahedral and network structure of H_2O_n is responsible for the protective water buffer "scafander" of all polar compounds, ionic species, organic molecules of sugars, proteins, DNA, emergent substances and

xenobiotics (pesticides, pharmaceuticals and others) *(Chaplin 2017).* Water from various sources contains dissolved gases, minerals, organic and inorganic substances that are transferred to water during transformation, passage and water cycle.

The steady growth of human population and technology development has resulted in an exponential increase of demand and consumption of natural water resources. The issue of the availability and quality of water is one of the most important but inadmissible neglected problem of modern society. Temperature rise, floods, changes of relief, biodiversity, and conditions of life, at both global and local level remain the essential important issues and activities for further development of society and civilization. Anthropogenic sources of water pollution are numerous and expanding.

While the developed world is researching how to preserve and stabilize the quality of natural water and make better use of technology to treat and purify liquid waste and wastewater, developing countries have the problems of water availability, efficient use of sources and the quality of natural water, while the wastewater receives an insignificant amount of attention. What is the difference in thinking between the developed and developing world is the fact that the developed part of the world deals with the detection and treatment/neutralization of micropollutants in wastewater, while developing countries are still dealing with independent systems of drinking water delivery, accessible to all residents and canalling/disposal of wastewater. The main priority goal for the sustainable water management should be the development of integrated management system on the national level as the quality of water is strategic issue and must be harmonized with other developed countries *(Dimkić et al. 2008).* Integrated water resource management is a complex, complicated and essential mission, which encompasses a set of procedures, measures and activities, securing the quantities of water of the optimal quality for different purposes, protection of water from pollution and protection against harmful effects of polluted water. Water management in Serbia is carried out through the development and implementation of key planning documents: the Strategy for Water Management of the Republic of Serbia (Strategy) and the Water Management Plan for the Danube River Basin, Water Management Plans for water areas (WMPs), as well as Plans that govern protection from the harmful effects of water (Plan of flood risk management, General and Operational Plan for flood protection), as well as Plans for governing the protection of water (Plan for water pollution protection and monitoring program).

The national legislation is still developing and transponning from EU legislation. Serbia has a developed and amended Law of Water (Official Gazette 30/10, 93/12, 101/16), and developed some bylaws by the end of 2017, nonetheless there are still a significant number of bylaws and official documents to be developed and transponned.

According to opening of Chapter 27: Environment and Climate Change, in EU process of admission, Serbia is obligated to make significant progress in the areas of air quality and climate change, waste management, water management, nature and biodiversity protection and so forth.

Hydrospheric processes are phases in which water cycles on the planet Earth. These processes include precipitation, sublimation, evaporation, evapotranspiration and other processes of transport. All these processes are related to the physical, chemical, biochemical and hydrological properties of water. The freshwater resources are extremely limited, and it is of most importance to carefully consider the design and management of water supply and protection systems. The priority of natural water system protection is the minimization of anthropogenic impact, achieved through multidisciplinary, interdisciplinary and transdisciplinary approach to protection system design.

The shift of paradigm from exploitation to the protection of environment and resources happened during the industrial revolution in XVIII century, when the sense of impact onto the environment was much sensible to human population. From the XIX century to today focus of pollution and protection shifted from global-scale to local-scale and from macropollutants to micropollutants.

Wastewater represents water that has been influenced or altered by anthropogenic or natural source in sense of its quality or characteristics. Water consumption of human population for diverse use has a by-product in variety of wastewater types and pressures on environment.

In the scope of thesis hypothesis, goals and objectives the research was conducted on municipal wastewater and industry wastewater, agricultural and urban run-off, that mixed represent mixed urban wastewater. Organic loads of water sample are significant and it is relevant to emphasise that organic substances present in water and wastewater can be difficult to separate, detect, identify and eliminate. Although there are significant difficulties in the systems of collection and treatment of wastewater in Serbia, there is a quality solution in the best available technologies, principles of environmental protection and sustainable development, and smart planning. When solving problems related to water and sewage systems in Serbia, there are solutions that are recommended and can be very efficient, considering best available techniques and technologies (BATs).

The thesis presents a critical review of the systems for collection and treatment of wastewater, especially in field of emerging xenobiotics (endocrine disruptive substances and illicit drugs).

Great agricultural potential of Vojvodina is the basis of processing industry development, primarily food industry, petrochemical and general chemical industries, followed by metals and building materials. Large industrial facilities have the problem of WWTP installation, while most urban areas in rural parts of Vojvodina are not connected to the sewage system. All existing sewage collection systems in urban areas are mixed type, collecting industrial, domestic and urban effluents. The greatest distress about the mixed urban wastewater collection systems is the reality of untreated mixture released into the natural water bodies, which are further used as a water sources for production of drinking water.

Due to the already high organic load of domestic effluents some industries can have a significant direct impact on the points of entering into the natural environment. Organic compounds and their metabolites that occur in water may pose a risk to human health and environmental, even if they are present in low concentrations with an unknown contribution to toxicity effects of mixture.

Traditional water monitoring using priority lists or specific compounds is progressively supplemented by new approaches multi-target, non-target and bio-analytical techniques, which aspire to unveil effects and connect them to a compound in a non-deterministic manner. Therefore, future potentially increasing pollution of the Danube and its tributaries with persistent compounds, especially when they are harmful, must be prevented (JDS3, 2015). Emerging substances (EmS) are wide variety of families newly recognized and received by global researchers and scientists as substances in environment widely used all over the world in different branches of industry, agronomy, science and research or everyday life with the pathways of entry into the different environmental media – air, water, soil and biota. There is a significant number of possible emerging contaminants, from industrial chemicals, antiseptic and anti-microbial agents, flame retardants, detergents and derivatives, and plasticizers and derivatives, pharmaceuticals and personal care products (PPCPs) (e.g., cosmetics, cleaning products, and fragrances) *(Janna, 2011).*

Furthermore, natural substances, hormones, cyanotoxins, inorganic compounds, preservatives for wood and others are also classified as EmS. The U.S Geological Survey introduces emerging substances as "any synthetic or naturally occurring chemical or any micro-organism that is not commonly monitored in the environment but has a potential to enter the environment and cause known or suspected adverse ecological or human health effects" *(Janna, 2011).* Network of Reference Laboratories for Monitoring of Emerging Environmental Pollutants (NORMAN) provided the open access list of the most frequently detected emerging substances of concern (around 1 200) *(Milić et al. 2013).* The presence of emerging contaminants in the aquatic environment represents a potential concern to wildlife *(Barceló and Kettrup, 2004)* and it may also have an significant impact on human health *(Barceló and Petrović, 2006).* Family of substances overlapping in priority and emerging substances list provided by legislation, environmental standards and guidelines research institutes is EDCs or Endocrine Disruptive Compounds. Groups of substances most commonly known as endocrine disruptors are pesticides, polychlorinated biphenyls, dioxins, phenols, phthalates and estrogens. The previous studies and screenings of mixed urban wastewater showed there is a high possibility of endocrine disruptive compounds detection, diverse groups and concentrations. In original studies pharmaceuticals and illicit drugs have been detected, 9 and 5 different substances, respectively.

Environmental risk assessment (ERA) approach for pharmaceuticals must also advance beyond historical paradigms. Though engineering solutions to improve technologies that reduce pharmaceuticals from discharges to surface waters have received much attention over the past decade *(Verlicchi et al 2012)*. Environmental monitoring of illicit

drugs (IDs) in aquatic systems could define the spatial extent of IDs usage in urban and rural regions. In fact, identifying urban sewer sheds with elevated biomarkers of disease *(Daughton 2012)* or IDs usage *(van Nuijs et al 2011, Jurado et al 2012)* presents a robust approach to target areas for delivery of public health services and allocation of law enforcement resources.

According to the conceptual idea of the thesis, it is necessary to obtain preliminary and up-to-date information about the environmental status, which can only be achieved through screening analyses and monitoring of physicochemical and biological parameters of water in the area of interest. For the purpose of wastewater monitoring on-line in-situ and standardized laboratory analyses were applied. For the screening analyses detection and identification of emergent substances methods of gas chromatography coupled with mass spectrometry (GC-MS) were used, and for the target analyses GC-MS (for pesticides and phthalates) and high pressure liquid chromatography coupled with tandem mass spectrometry or high resolution mass spectrometry (HPLC-MS^2 or HPLC-HRMS) (for estrogens and illicit drugs) were used.

2. State of the art in the field of research, current literature and legislation overview

2.1. Wastewater

"Water that has been used and contains dissolved or suspended waste materials."

EPA's Office of Wastewater Management (OWM)

"The Directive defines urban, domestic, and industrial wastewater as follows:

Article 2 "Urban wastewater" means domestic wastewater or the mixture of domestic wastewater with industrial wastewater and/or run-off rain water;

Article 2(2) "Domestic wastewater" means wastewater from residential settlements and services which originates predominantly from the human metabolism and from household activities;

Article 2(3) "Industrial wastewater" means any wastewater which is discharged from premises used for carrying on any trade or industry, other than domestic wastewater and run-off rain water."

Urban Wastewater Treatment Directive 91/271/EEC

Every industry and settlement produces both liquid and solid wastes and air emissions.

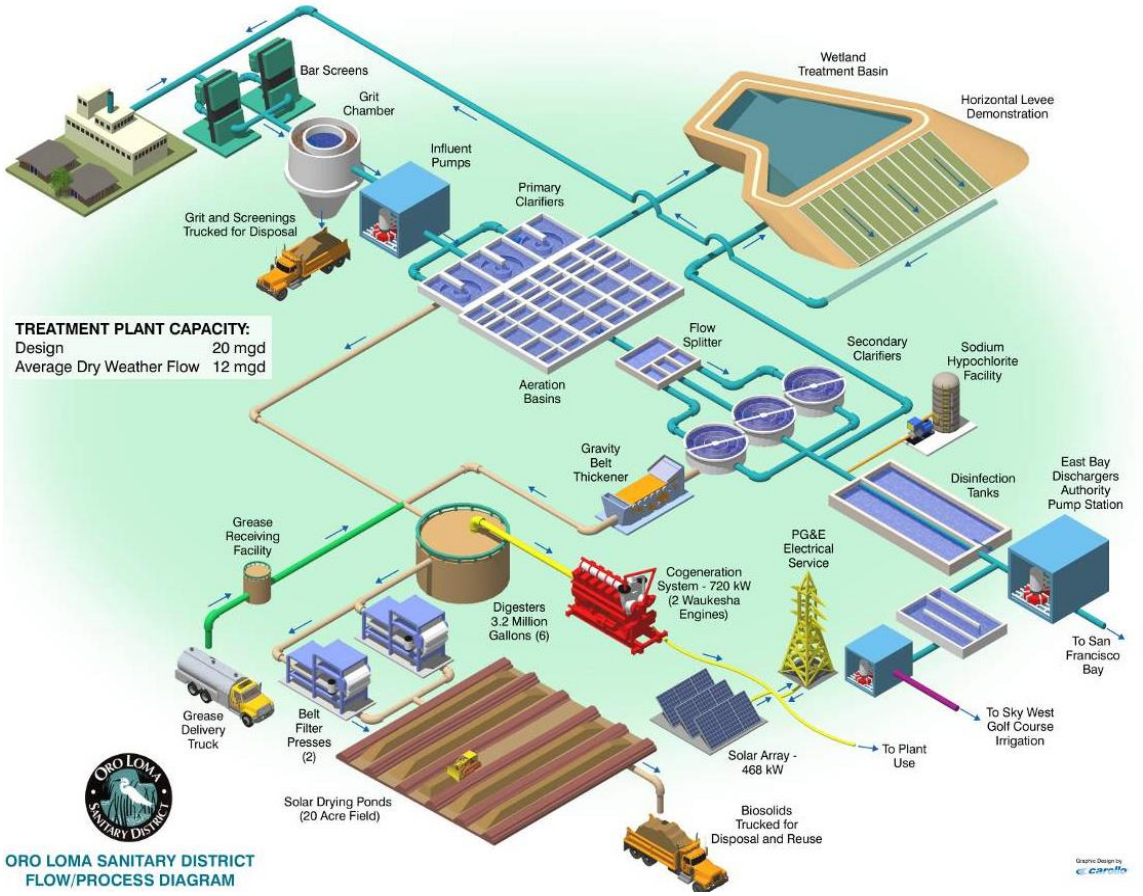


Figure 2.1 Schematic diagram of WW management infrastructure for 380 000 PE

In the Figure 2.1 schematic diagram of WW management infrastructure for 380 000 PE is shown.

Mixed urban wastewater is medial liquid phase fluid that is used by general population in every-day life, industrial and/or agricultural activities in urban or sub-urban areas, and thus has altered chemical, physical and/or biological properties.

As a direct consequence of anthropogenic impact wastewater is submitted to regulations, limitations and special requirements, for the purpose of environmental impact minimization *(EMA, Canada 2014 and Directive 91/271/EEC).*

2.1.1. Mixed urban wastewater – municipal wastewater, urban effluent

"Municipal wastewater means domestic wastewater or municipal liquid waste, including contributions from holding tanks in recreational vehicles, boats and houseboats, commercial, institutional and industrial sources, inflow and infiltration, septic tank pumpage, holding tank solids, and sludge from wastewater facilities"

Environmental Management Act, Canada, 2014

"Wastewater from residential settlements and services which originates predominantly from the human metabolism and from household activities"

"Article 2(1) "Urban wastewater" means domestic wastewater or the mixture of domestic wastewater with industrial wastewater and/or run-off rain water;"

Council Directive 91/271/EEC concerning urban waste-water treatment

"Article 3 (11) - Wastewater from household wastewater from residential settlements stemming mainly from the human metabolism and from household activities"

National regulation on limit values for emissions of pollutants in water and deadlines for their achievement ("Off. Gazette of RS", no. 67/11 and 48/12 and 1/16);

Urban settlements contaminate surface water through municipal liquid waste (sewage effluents) that is directly released into natural water bodies.

The sewage wastewater consists of a variety of organic matter and other chemical constituents, detergents and numerous chemicals, diversity of microorganisms, which can result in the appearance of waterborne disease outbreaks. The largest number of bacteria in waste and surface water originates from human faeces and excretions (*Naidoo and Olaniran 2014*).

In the EU legislation there is a specific definition of effluent that is comprised of domestic and pre-treated industrial wastewater, as well as run-off, but in Serbian national legislation there is no such definition.

It is necessary to explicitly define this type of effluent as it is a spatially complex mixture that can pose a problem in further analyses.

In Serbia most of the urban effluent is mixture of industrial, domestic and agricultural wastewater as well as run-off form streets which is why in this dissertation this water type will be referred as mixed urban wastewater (MUWW).

The MUWW is a significant source of organic pollution in natural water bodies, especially priority, hazardous priority and emerging substances, that are and objective of this research.

Priority pollutants from wastewater are an exciting research topic due to the hazardous nature and characteristics, on the other hand emerging substances and pollutants are intriguing, due to the frequent consumption, adverse and possible chronically effects that are not yet sufficiently investigated *(Sremački et al. 2016a).*

Sources of emerging compounds (illicit drugs and pharmaceuticals, personal care products, pesticides, grease and oil, endocrine disruptive compounds and others) in natural water bodies in urban areas are domestic and industrial effluents, agricultural and urban runoff.

For these reasons it is very important to investigate entry sources, behaviour and spatial distribution of possible contaminants. Hence, there is a need to study the interaction of different groups of substances (organochlorine pesticides and estrogens, licit and illicit drugs) as well as metabolisation processes of chemical species in wastewater and natural recipient.

Due to the variations of wastewater type and quality, effects onto the natural aquatic system can be adverse.

Wastewater containing high load of organic matter (OM), impacts aquatic life instantly, disturbs the natural balance and is drastically decreasing the number of biospecies. The number of units of remaining few species is increasing exponentially by process of proliferation, until the dilution and regeneration mechanisms manage to reverse the process.

If the wastewater is rich with suspended matter it will exponentially reduce the number of species and density of different organisms.

In Figure 2.2 and 2.3 is shown the effect that wastewater from urban areas can have on bio-species and natural conditions in aquatic environment and river systems.

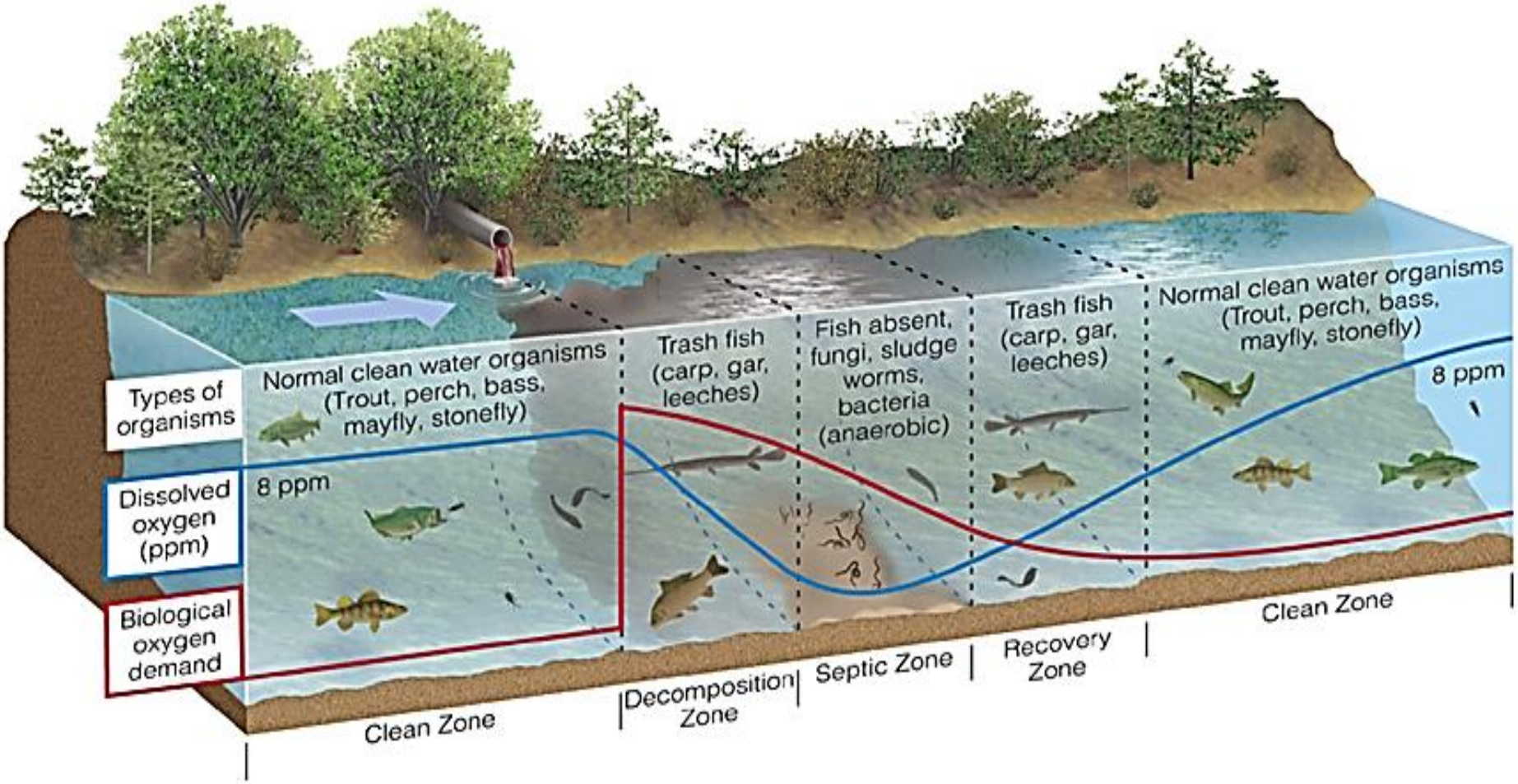


Figure 2.2 Wastewater effect on bio-species in river (©Brook/Cole, Thomason 2005)

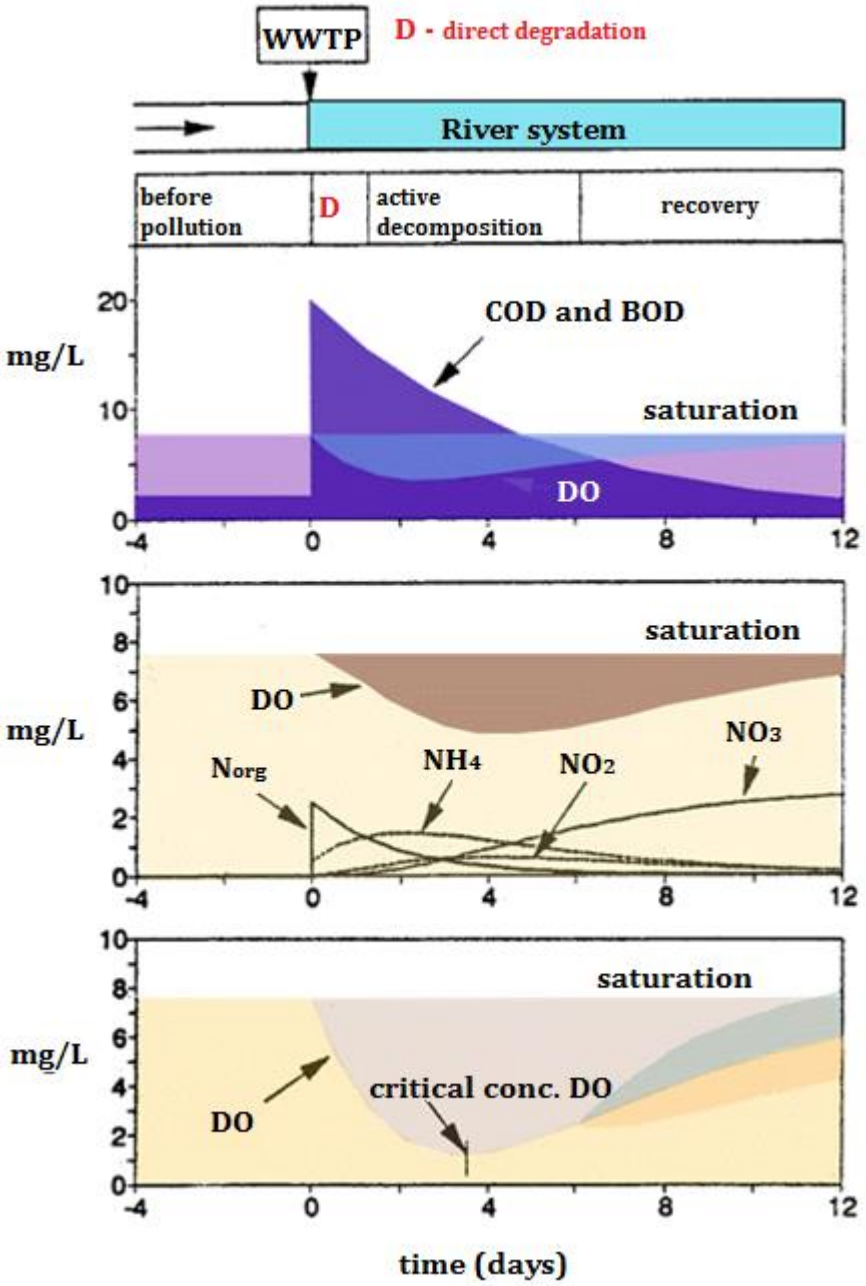


Figure 2.3 Trends of carbon, nitrogen and oxygen affected by a WWTP discharge into a river (http://echo2.epfl.ch/VICAIRE/mod_2/chapt_6/main.htm)

2.1.1.1. Quality and requirements

The population growth and especially urban population growth has a significant impact onto the surface water bodies. Insufficiently treated urban wastewater, with its dynamic physical, chemical and biological properties, has an adverse negative effect on the natural balance of the recipient. The causes of surface water pollution during the last two centuries were changing alongside development of industry and society.

Pressure on water resources is constantly increasing, as the consumption is directly in correlation with growth of population and economy, industrial and agricultural production, households, etc.

The used water purification technologies aren't sufficiently developed to treat all used or contaminated water, especially when it is mixed urban wastewater. Pollution of this kind, continual and divers is persistently contributing to the reduction of drinking water resources by reducing the security and safety of water (*Grant et al. 2012, Pimentel et al. 2014, Gleick 2014*).

Pollution of water resources is a significant limiting factor in the development of society, which demands constant economic growth. Population and anthropogenic activities have the crucial role in environmental pollution. The important concentrated and scattered sources of anthropogenic pollution are:

- *industrial facilities, power plants and supporting facilities,*
- *agricultural facilities,*
- *human settlements,*
- *unsanitary open dumps.*

Concentrated pollution can be controlled, but scattered sources of pollution can be difficult for prevention, monitoring and elimination processes.

2.1.1.2. Monitoring of basic physical and chemical parameters – predicted quantity and load onto the environment

Monitoring of basic physical and chemical parameters of wastewater is indispensable for any wastewater study. During the research pH, conductivity, dissolved oxygen, permeability, ammonia, chemical oxygen demand (COD) and biological oxygen demand (BOD_5) were followed for all water samples. The concentration ranges for values of basic physicochemical parameters in wastewater samples on specific locations are shown in Table 2.1 as well as legislation requirements *(Mihajlović et. al. 2014).*

Table 2.1 Concentration ranges of basic physicochemical parameters in wastewater samples and national legislation requirements (NLR) (modified Mihajlović et. al. 2014)

Parameter	Unite	NLR	Minimum	Maximum	Mean ± SD
Conductivity	**[µS/cm]**	-	568	4 450	1 362 ± 746
Dissolved oxygen	**[mg/L]**	-	0.07	3.57	0.90 ± 0.72
BOD_5	**[mgO_2/L]**	25	100	614	346 ± 139
COD	**[mgO_2/L]**	125	196	862	534 ± 195
NO_3-N	**[mg/L]**	10	0.1	0.5	0.3 ± 0.1
NO_2-N	**[mg/L]**		0.01	0.17	0.05 ± 0.04
NH_4-N	**[mg/L]**		13.7	60.4	37.8 ± 11.1
Total phosphorus	**[mg/L]**	2	1.6	8.7	4.6 ± 1.2
PPC	**[mg/L]**	-	10.1	81.1	48.1 ± 16.8

Most studies that have been done for urban wastewater are usually focused separately on domestic, industrial and agricultural effluents, observing specific quality of water discharging from industrial facilities, domestic sewerages, agricultural or urban run-off.

Even the treated urban effluent has shown the significant amount of organic matter – dissolved and particulate effluent organic matter, dE_fOM and pE_fOM, respectively.

As the effluent type in selected location is untreated mixed urban effluent, suggesting the mixture of urban, industrial and agricultural effluents, it is possible to correlate to the literature research and conclude which detected pollutants are introduced to wastewater from which source of wastewater.

Eliminating the industrial and agricultural effluent pollutants, can give an idea about the pollutants originating from households and runoffs in urban wastewater effluent.

The Figure 2.4 shows the wastewater inputs and the residual pollutants in the effluent after treatment, showing the pollutants that have to be removed by advanced technique.

The E_fOM is consisted of two fractions – dissolved and particulate effluent organic matter *(Michael-Kordatou et al. 2015).*

A vast component of pEfOM is cellulose insoluble in water with high molecular mass *(Pettit 2004).*

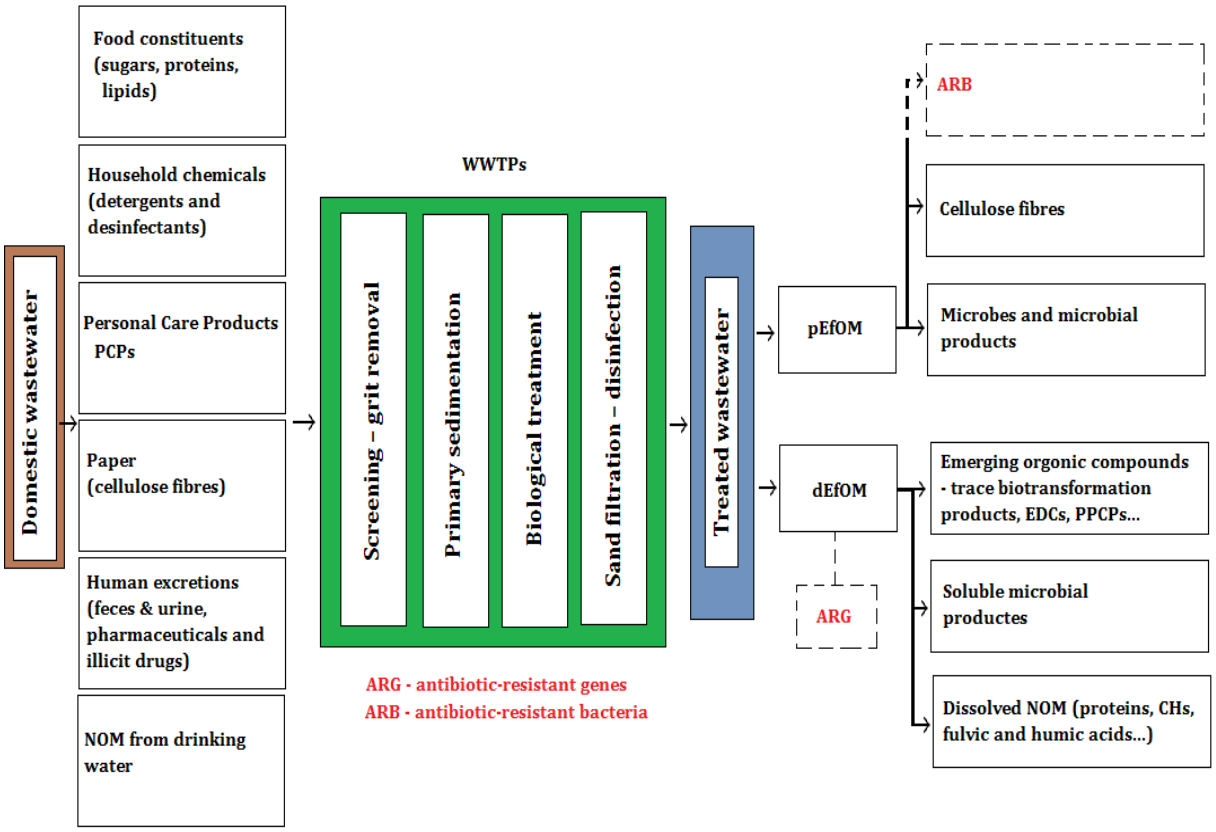


Figure 2.4 The origin of effluent organic matter in domestic wastewater (Modified from Michael-Kordatou et al. 2015)

The dE_fOM consisted of dissolved natural organic matter (dNOM) from drinking water sources, soluble microbial products (SMPs) formed during the biological wastewater treatment, trace organic compounds produced during domestic and/or industrial use (e.g., EDCs, PhAC and PCP residues, etc.), disinfection by-products (DBPs) and transformation products resulting from the various biotic and abiotic processes that can take place during treatment.

The characteristics and the composition of dEfOM are highly dependent on the sources of wastewater, the processes of wastewater treatment and their operating conditions (*Uyguner-Demirel and Bekbolet 2011*).

Particles of dNOM with <0.45mm in diameter emerge as a significant fraction for urban wastewater effluents (*Filloux et al. 2012; Yu et al. 2015*).

dNOM is a complex mixture of aromatic and aliphatic molecules with a widespread spectrum of structures and molar mass distribution (MMD), highly affected by origin and the bioclimatic-geochemical cycles of the environment (*Fabris et al., 2008*).

The chemical structure and physicochemical characteristics can have significant seasonal variations on the same location, due to the meteorological and environmental conditions (*Matilainen et al., 2010*).

It could be concluded that seasonal variations such as changes in precipitation and changes in air and water temperatures have a substantial impact on the quality of wastewater.

Correlation matrix with the values of the Pearson coefficients with highlighting the significant correlations with the probability of 95 % and 99 % are shown in Table 2.2 *(Mihajlović et. al. 2014).*

Tabel 2.2 Correlation matrix of the influence of meteorological parameters on the quality of wastewater

	EC	**D.O.**	**NH_3**	**Total P**	**PPC**	**COD**	**BOD_5**
T_{air}	-0.252	-0.262	0.019	0.035	0.397	-0.147	-0.254
Humidity	0.424	**0.555***	-0.460	-0.372	-0.005	-0.147	-0.133
Precipitation	**0.779****	**0.718****	**-0.614***	**-0.549***	-0.217	**-0.554***	-0.452
T_{water}	-0.453	-0.460	0.376	0.244	0.204	0.087	-0.035

*Bold values indicate statistically significant correlations - * Correlation is significant at the level p=0.05 and ** Correlation is significant at the level p=0.01*

Although the presence of soluble microbial products (SMPs) formed during the biological WWT has been recognized, there is lack of information regarding their formation and composition. SMP have been identified as humic and fulvic acids, polysaccharides, proteins, nucleic acids, organic acids, amino acids, antibiotics, steroids, exocellular enzymes, siderophores, structural components of cells and products of energy metabolism *(Barker et al. 2000).*

The conclusions were diverse depending on the technology used for the treatment, but nevertheless very informative and universal conclusions were derived:

- The experimental results showed that ~ 85 % of the effluent DOC consisted of SMPs, which contained mainly organic compounds with high molecular mass and
- SMPs production was found to decrease with decreasing the HRT and the increase of temperature results in higher SMP production (*Barker et al. 2000*).

In the last decade productive development of analytical techniques have enabled the identification and essentially quantification of a wide variety of micropollutants, widely known as "contaminants of emerging concern (CECs)" or "emerging substances (EmS)" in treated wastewater effluents (*Fatta-Kassinos et al., 2011*).

According to the NORMAN Network the term 'contaminants of emerging concern' does not necessarily refer to 'new substances', i.e., newly introduced chemicals and their transformation products and/or metabolites, but also refers to chemicals (both synthetic and naturally occurring compounds) with previously unrecognized adverse effects on the environment.

EmS include endocrine disrupting compounds (EDCs), pharmaceuticals and personal care products residues (PPCPs), licit and illicit drugs, hormones and many other complex compounds and families of substances(plasticisers, surfactants, pesticides, detergents, nanoparticles, etc.) (*Nikolaou et al. 2007; Fatta-Kassinos et al. 2011*).

The emerging micropollutants have been detected in the dEfOM at the ng/L concentrations (*Heberer 2002*). This indicates that the conventional treatment technologies do not completely remove micro-contaminants and this leads to their subsequent release into the aquatic environment through discharge, leachate from dumpsites and via accidental situations. Hydrophobic micro-contaminant residues are accumulated in pEfOM, while hydrophilic ones are expected to occur at higher concentration in dEfOM.

A comprehensive review on the occurrence of these micro-contaminants in treated urban wastewater has been provided in literature (*Watkinson et al. 2009; Fatta-Kassinos et al. 2011; Michael et al., 2013*).

The dEfOM is generally quantified as surrogate parameter (*Michael-Kordatou et al. 2015)*, parameters of bulk organic load characterisation for water samples, which are Biological Oxygen Demand (BOD_5), Chemical Oxygen Demand (COD), Potassium Permanganate Consumption (PPC) and Total Organic Carbon (TOC).

BOD_5 – the amount of oxygen consumed for the decomposition of organic substances under the influence of aerobic bacteria in water samples. Complete decomposition of organic matter depends on many factors such as type of matter that decomposes, temperature, oxygen, pH and other. The oxygen consumption tests carried out for a period of 5 days is performed (BOD_5) as the 40 to 80 % of the biodegradable organic matter is decomposed *(Penn et al. 2009).* BOD can be divided into two parts:

- Carbonaceous BOD is the result of organic molecules degradation (cellulose and sugars), as the first stage of oxidation.
- Nitrogenous Oxygen Demand represents the second stage of oxidation and breakdown of protein molecules.

BOD can be represented by variety of chemical reactions, such as degradation of sugars and ammonia in water in redox reactions. Ammonium is highly soluble in water, therefore in water NH_3 is present as a cation NH_4^+ (if pH is lower than 7) and as a $NH_4OH_{(aq)}$ dominantly as non-ionised molecular system. The process of nitrification, oxidation of NH_3 in water to nitrate is shown trough reactions 1 trough 3:

$$NH_{4\,\text{absorbed}}^{+} \rightarrow NH_{4\,\text{solution}}^{+} \rightarrow NH_{3\,\text{solution}} \rightarrow NH_{3\,\text{soil}} \rightarrow NH_{3\,\text{atmosphere}} \qquad \text{r.1}$$

$$2NH_{4(aq)}^{+} + 3O_{2(g)} \rightarrow 4H_{(aq)}^{+} + 2H_2O_{(l)} + 2NO_{2(aq)} \qquad \text{r.2}$$

$$2NO_{2(aq)}^{-} + O_{2(g)} \rightarrow 2NO_{3(aq)}^{-} \qquad \text{r.3}$$

Potassium permanganate consumption (PPC) – The amount of $KMnO_4$ consumed depends on the amount of organic matter in the water, but also their chemical structures. Some inorganic substances (nitrites, Fe^{2+} ions and H_2S) may be oxidized by $KMnO_4$ under certain conditions; therefore, consumption of $KMnO_4$ can only conditionally be considers the criteria of organic matter in the water.

COD – measure of the mass concentration of oxygen equivalent to the amount of dichromate consumed by organic matter when a WW sample is treated with oxidant under defined conditions. COD is used to assess the degree of water organic load. Biodegradation of organic matter in water can be determined from the BOD_5 to COD ratio (BOD_5/COD).

TOC – measure of contamination by the organic matter and the degree of biodegradation of organic matter present on the surface and in wastewater. Determination of TOC in the water is based on the oxidation of organic molecules to one-carbon molecular form or carbon dioxide (CO_2). Oxidation of organic molecules can be generally display by model chemical equation 4 (*Kašpar et al. 2003*):

$$4C_xH_y + (4x+y)O_2 \rightarrow 4xCO_2 + 2yH_2O \qquad \text{r.4}$$

2.2. Wastewater management

2.2.1. EU and national legislation and regulations

On 23 October 2000, the "Directive 2000/60/EC of the European Parliament and of the Council establishing a framework for the Community action in the field of water policy" or, in short, the *EU Water Framework Directive* (WFD) was finally adopted. The Directive was published in the Official Journal (OJ L 327) on 22 December 2000 and entered into force the same day, Council Directive of 12 December 1991 concerning the protection of waters against pollution caused by nitrates from agricultural sources (91/676/EEC). Some amendments have been introduced into the Directives since 2000.

National legislation about the subject has been developing and amending over the last five years, to be co-related and adapted to EU legislation. The most significant regulatory documents for the purpose of research:

- National Law "Water Law", "Official Gazette of RS", no. 30/2010 and 93/2012 (25th September 2014);
- National by-law "Minimum number of wastewater quality examination, "Official Gazette SRS," no.47/83, 13/84; (25th September 2014);
- National by-law "Parameters of the ecological and chemical status of surface waters and the parameters of the chemical and quantitative status of groundwater "Official Gazette of RS", no. 74/2011; (25th September 2014);
- National by-law "Limit values of pollutants emission in water and deadlines", "Official Gazette of RS", no. 67/2011and48/2012); (25th September 2014);
- National by-law "Limit values for pollutants in surface and ground water and sediments, and deadlines", "Official Gazette of RS", no. 50/2012; (25th September 2014);
- National by-law "Regulation of limit values for priority and hazard priority pollutants for surface water and deadlines", Republic of Serbia, "Official Gazette of RS", no. 35/2011.

2.2.2. Management and monitoring systems in research area

In the research area of Novi Sad there is an existing wastewater canalling system dating from 1953, and it is separated onto the 2 main canalling segments – south and north. The south sewerage canalling system is ending with the GC1 collector and direct discharge to Danube, without treatment, and the north segment is ending with the GC2 collector and direct discharge to Danube, with the exception of newly installed grid for the separation of mechanical wastes.

The sewerage system is designed and constructed as a mixed wastewater canalling system, and collects communal (domestic) and industrial wastewater mixed with the

urban, sub-urban and rural runoff, making it very difficult to maintain the specific and regular water quality.

The proper and official monitoring system for wastewater discharged to Danube River from the city of Novi Sad is basically non-existent. There have been some sporadic scientific research and monitoring during the cases of extremely visible pollution, but nothing permanent, constant and thorough, which cannot lead to the conclusions on quality, periods of change in quality and/or necessary treatment.

The research activities were planned within NATO International Project in order to gain more insight into occurrence, concentration levels and sources of emerging and priority pollutants sources in this area.

Some progress has been made during the NATO International Project ESP.EAP.SFP 984087, which was based on the premises that the wastewater in Novi Sad has to be continuously monitored for basic physicochemical parameters, as well as, specific and trance organic pollutants, resulting in preparation for development of early warning system (EWS). The open access list of organic pollutants created by the NORMAN network of the most frequently detected emerging substances of concern (around 1036), as well as, EU Directive 2008/105/EC determining the list of priority and priority hazardous substances were consulted and used during the research. In the NORMAN list emerging substances are provided and divided into 26 families of chemicals according to the structure and effect (*Milić et al. 2014*).

The NORMAN list of emerging substances and pollutants is being continuously changed and amended, so it can be up-to-date. The last modification was made in February 2016.

2.2.3. Wastewater treatment

Wastewater treatment by itself is defined by wastewater, and represents technological and technical solutions aimed at removal of pollutants from wastewater, depending on the wastewater quality. For the purpose of planning and selection of processes for wastewater treatment, it is not enough to consider only production of high quality effluent. Nowadays, it is particularly important to consider the optimization of efficiency and minimization of operation and maintenance cost (labour, energy, by-product stabilisation and disposal or reuse) *(Metcalf and Eddy, 5th edition).*

As research progress, especially in the field of defining and analysing wastewater constituents that may cause adverse negative effects, greater levels of treatment will be needed. The degree to which wastewater must be treated varies, depending on domestic environmental conditions and governmental standards and guidelines. Guidelines and standards are designed to prevent the deterioration of existing water quality, set limits on the amounts of specific pollutants allowed in streams, rivers, and lakes.

The limits depend on a classification of the "maximum beneficial use" of the water *(WHO 1997 and US EPA 2004).* Water quality parameters that are regulated by standards include dissolved oxygen, coliforms, turbidity, acidity, and toxic substances.

Standards for effluent, on the other hand, are directly regarding the quality of the treated wastewater discharged from a sewage treatment plant. The factors of effluent standards are biochemical and chemical oxygen demand (BOD, COD), suspended solids, acidity, and coliforms.

Three common levels of wastewater treatment are preliminary (removal of coarse material), primary and secondary. Primary treatment removes about 40 – 60 % of total suspended solids (TSS), depending on efficiency, and about 35 % of BOD; dissolved impurities are not removed. Secondary treatment removes 85 – 95 % of TSS and BOD *(FAO Document Repository).*

When it is obligatory to remove more than 95 % of TSS and BOD must be removed, or when dissolved nitrate and phosphate levels must be reduced, advanced treatment methods are necessary.

Advanced processes remove more than 98 % of all the impurities from sewage, producing an effluent of near drinking-water quality, depending on a wastewater treatment process (WWTp). It is used only under specific circumstances.

For all levels of wastewater treatment (WWT), the last step prior to discharge of the sewage effluent into a body of surface water is disinfection, which eliminates remaining pathogens in the effluent and protects public health. Due to chlorine residuals, effluent may have adverse effects on aquatic life.

Ultraviolet radiation, which can disinfect without leaving any residual in the effluent, is becoming more competitive with chlorine as a wastewater disinfectant. In the Figure 2.5 size of impurities in water are shown.

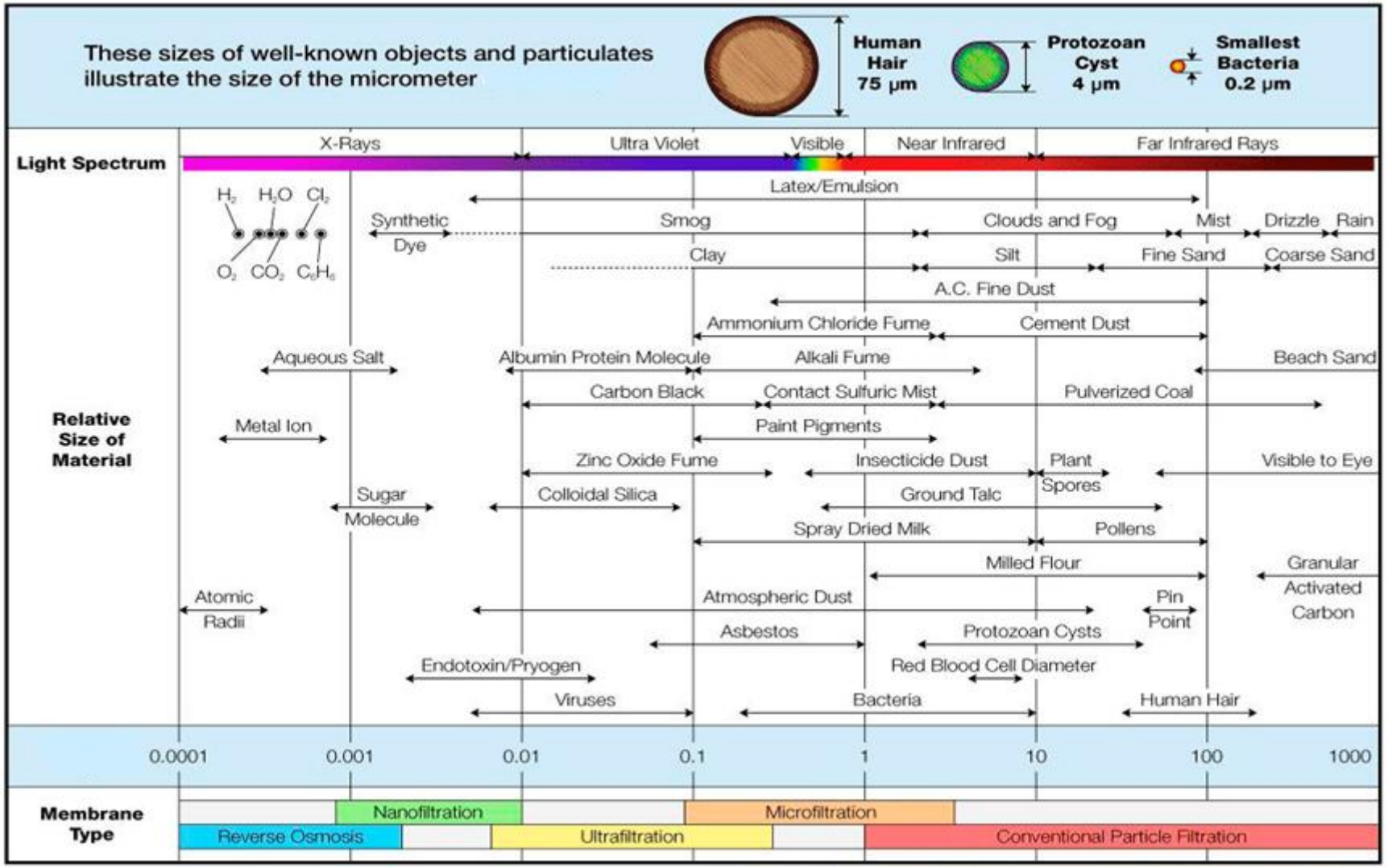


Figure 2.5 Size of water impurities particles (McGowan 2001)

Table 2.3 Advanced techniques and efficiency of wastewater treatment (Chang et al. 2008, Raucher et al. 2008)

	Inorganic ions	Dissolved gasses	Organics	Particles	Bacteria	Pyrogens	Nucleases	Efficiency of impurities removal	Cost of treatment	Energy consumption	Total
Distillation	*3*	*2*	*1*	*3*	*3*	*2*	*1*	*2.14*	*1*	*1*	*1.38*
Reverse osmosis	*2*	*1*	*2*	*3*	*3*	*3*	*1*	*2.14*	*3*	*1*	*2.05*
(Electro) Deionization	*3*	*3*	*1*	*1*	*1*	*1*	*1*	*1.57*	*1*	*2*	*1.14*
Filtration	*1*	*1*	*1*	*3*	*3*	*1*	*1*	*1.57*	*3*	*3*	*2.52*
Ultrafiltration	*1*	*1*	*1*	*3*	*3*	*3*	*2*	*2*	*2*	*2*	*2*
Adsorption	*1*	*2*	*3*	*1*	*1*	*2*	*2*	*1.71*	*3*	*3*	*2.57*
Ozonization	*2*	*1*	*2*	*1*	*3*	*3*	*3*	*2.14*	*2*	*2*	*2.04*
UV oxidation	*1*	*1*	*3*	*1*	*3*	*1*	*1*	*1.57*	*3*	*1*	*1.86*
UV/F oxidation	*1*	*1*	*2*	*3*	*3*	*3*	*2*	*2.14*	*2*	*1*	*1.71*

**3-excelent; 2-good; 1 - poor*

In Table 2.3 are shown advanced techniques of wastewater treatment processes and the efficiency for removal of pollutants.

Serbia is a developing country that aims to make a "acceleration of improvement" in environmental protection segment in an attempt to develop rapidly certain segments of the society and systems, based on technologies that have been developed and used in the developed countries of Europe and the World.

Conventional treatment of urban wastewater is significantly dependant on wastewater quality and quantity. It is not enough to design a specific wastewater treatment plant (WWTP) according to only physical data used in the past – number population, density, type of settlement, sewerage type and meteorological conditions.

Therefore, it is necessary to design with high capacity, optimal and energy efficient WWTP, for this new approach it is of most importance to have as much data about specific location as possible - about the type and quality of wastewater, details of sewerage system, possible natural risks and dangers, possible different types of influents and their characteristics (hospitals and pharmaceutical laboratories, industry and agricultural effluents) and other.

The environmental considerations and impacts of a proposed WWTP are more important than economic considerations. In addition to impact of discharged effluent on the aquatic environment in the natural recipient, it is important to address the emission of greenhouse gases (GHGs) from the WWTP into the atmosphere.

Wastewater is a substantial in the water-energy correlation, as wastewater collection and treatment require significant amount of energy, but can also be a source of energy *(WWAP 2017).* The energy contained in wastewater can be recovered in the form of biogas, heating/cooling and electricity generation through on-site and off-site processes *(Meda et al. 2012).* Energy recovery has significant potential of reducing the amount of used energy, operational costs and carbon footprint of WWTP. Reducing the carbon footprint of WWPT can increase revenue streams through carbon credits and carbon trading programs *(Drechsel et al. 2015).* All of the considerations can be covered if the triple bottom line (TBLa) analysis is used in the process of design and planning, considering and evaluating economic, environmental and social aspects of the project as part of decision making process.

Conventional wastewater treatment plant has 3 to 4 steps of treatment according to Figure 2.5 – Preliminary, primary, secondary and nutrient removal is optional, and usually annexed after the construction of the first three phases. First three phases of urban wastewater can be divided as mechanical (preliminary), physicochemical (primary) and biological (secondary).

Advanced treatment is a possible stage if needed, especially according to wastewater quality, overall cost of design, construction and operation of the WWTP.

Nowadays, according to development of science and technology, practical approach and newly acquired data about the quality of wastewater, especially a change of the premise of doses to response and toxicity, discovery and proofing of toxicity, hazardless of substances, acknowledgement of emerging substances and pollutants, advanced treatment becomes a necessity if not requirement. Following the new approach, tertiary and advanced treatment can be separated, as the tertiary, represents nutrients removal (nitrogen and phosphorus) and advanced treatment is recognized as any other extra

process necessary to reach water quality provided by national legislation, not necessary following the tertiary treatment in wastewater treatment process of WWTP.

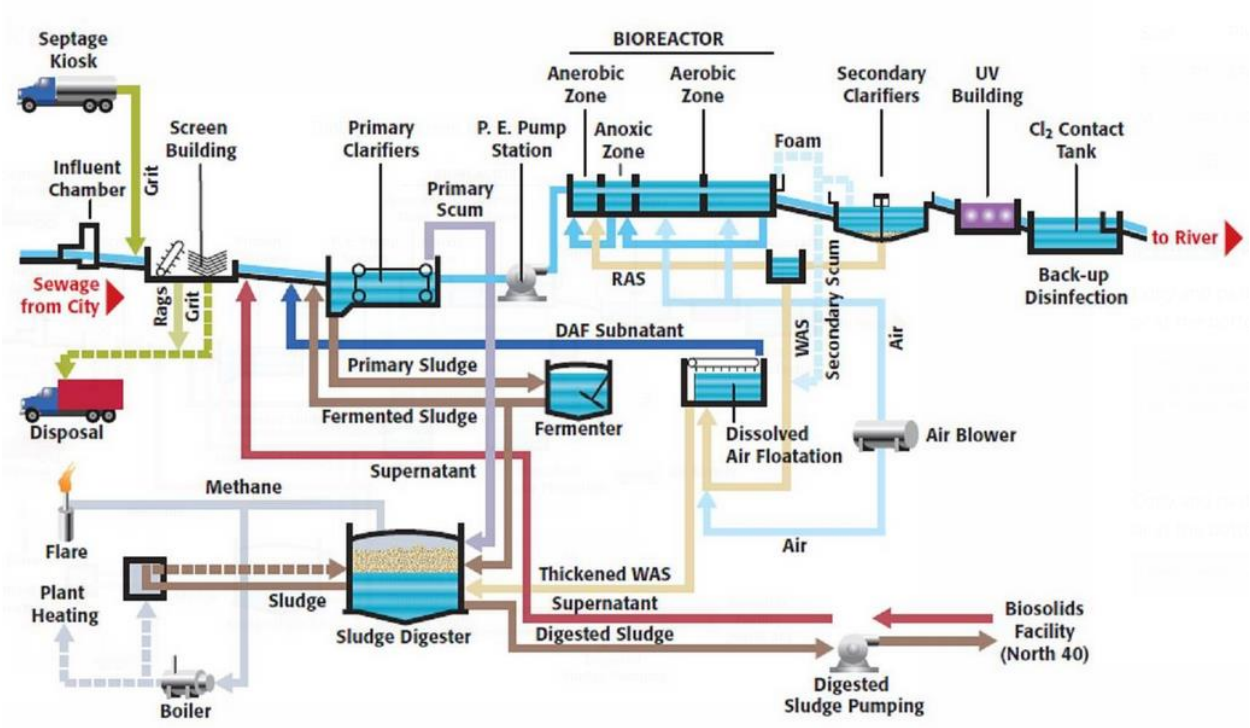


Figure 2.6 General scheme of WWTP via ASP with sludge digestion

Figure 2.5 shows general scheme of wastewater treatment via conventional activated sludge process.

2.2.3.1. Preliminary treatment (pre-treatment)

The goal of preliminary treatment unite is to remove coarse and gross solids and materials found in wastewater, in order to regulate, shield and enhance the operation and maintenance of following plant treatment phases *(WWAP 2017).* This section of treatment includes coarse screening and grit removal, where the rate of the water through the chamber is maintained sufficiently high to prevent the settling of solids. The figure 2.5 shows a general scheme of WWTP.

2.2.3.2. Primary treatment (prim-treatment)

The objective of primary treatment unit is to remove settleable solids thought process of sedimentation and flotation by skimming. During prim-treatment approximately 25 to 40 % of the BOD_5, 50 to 70% of TSS, and 65% of the oil and grease are removed *(Metcalf and Eddy, 5th edition, 2014).*

A certain amount of organic nitrogen and phosphorus, as well as cations of heavy metals associated with solids, is also removed, but colloidal and dissolved constituents are not affected.

Characteristics of wastewater changes due to the settling process, by settling of large "non-biodegradable" suspended solids, thus leaving volatile fraction in effluent.

Characterization of primary influent and effluent is necessary to determine the transformation of wastewater, which is mainly influenced by original characteristics and quality of wastewater. In large WWTP, primary sludge is processed biologically by anaerobic digestion.

In large sewage treatment plants, primary sludge is processed biologically by anaerobic digestion. In the digestion process, anaerobic and facultative bacteria metabolize the organic matter in sludge, thereby reducing the volume requiring ultimate disposal, making the sludge stable and improving its dewatering characteristics. Digestion is carried out in anaerobic digesters producing biogas used for energy-efficient and independent WWTp.

The HRT in a digester may vary from a minimum of about 10 days to 60 days or more. Gas containing about 60 to 65 % methane is produced during digestion and can be recovered as an energy source. In small sewage treatment plants, sludge is processed in a variety of ways including: aerobic digestion, storage in sludge lagoons, direct application to sludge drying beds, in-process storage (as in stabilization ponds), and land application *(FAO Document Repository).*

Considering all the "newly recognized" pollutants the new approach to primary treatment is chemically enhanced primary treatment (CEPT). The level of purification in the process of CEPT depends on type of chemicals added, mixing times and monitoring and control of the process. Primary settling enhanced with chemical precipitation can result in higher removal rates for suspended solids (80 – 90 %), including some colloidal particles and significantly higher BOD removal in range of 50 to 80 %. Enhanced removal of solids and BOD in primary treatment is a crucial issue for energy management in WWTPs, as the solids have a high energy value, which can be re-used trough anaerobic digestion or other thermal conversion process and used for energy purposes.

Higher BOD removal considerably lowers the energy demand for aeration processes during secondary treatment. In current practice, chemical precipitation is used to improve the primary settling process, for the removal of phosphorus and/or heavy metals and for improving the quality of water to be reused. For the purpose of chemical precipitation several agents can be used – aluminum sulfate (alum) ($AL_2(SO_4)_3 \cdot (14\text{-}18)H_2O$), aluminum chloride ($AlCl_3$), calcium hydroxide (lime) ($Ca(OH)_2$), iron(III)chloride ($FeCl_3$), iron(III)-sulfate $Fe_2(SO_4)_3$, iron(II)-sulfate $FeSO_4$, and other, highly dissolved chemical molecular formulas and ionic species forming voluminous aquatic systems *(Metcalf and Eddy, 5th edition, 2014).*

Mass of removed total suspended solids can be calculated trough equation 1:

$$M_{TSS}\left[\frac{\text{kg}}{\text{day}}\right] = \frac{\%\text{ of removal}[-] \cdot \text{TSS of untreated WW}\left[\frac{\text{g}}{\text{m}^3}\right] \cdot \text{WW flowrate}\left[\frac{\text{m}^3}{\text{day}}\right]}{\frac{1000\text{ g}}{1\text{ kg}}} \qquad \text{e.1}$$

Mass of formed substance can be calculated via equation 2:

$$M_{\text{formed substance}}\left[\frac{\text{kg}}{1000\text{m}^3}\right] = \frac{m_{\text{ agens}}\,[\text{kg}] \cdot \left(\frac{n_{\text{formed substance}}[\text{mol}] \cdot M_{\text{formed substance}}\left[\frac{\text{g}}{\text{mol}}\right]}{n_{\text{agens}}[\text{mol}] \cdot M_{\text{agens}}\left[\frac{\text{g}}{\text{mol}}\right]}\right)}{1000\text{m}^3} \quad \text{e.2}$$

Total sludge volume of formed during chemical precipitation can be calculated trough equation 3:

$$V_{\text{sludge}}\left[\frac{\text{m}^3}{\text{day}}\right] = \frac{M_{TSS}\left[\frac{\text{kg}}{\text{day}}\right] + M_{\text{formed sludge}}\left[\frac{\text{kg}}{\text{day}}\right]}{\text{specific gravity} \cdot (1 - \text{moisture content}) \cdot 1000\frac{\text{kg}}{\text{m}^3}} \quad \text{e.3}$$

2.2.3.3. Secondary treatment

The objective of secondary treatment is to remove the residual organics and suspended solids. In most cases, secondary treatment follows primary treatment and represents the removal of biodegradable dissolved and colloidal organic matter using aerobic biological treatment processes. Aerobic biological treatment is performed in the presence of oxygen by aerobic microorganisms that metabolize the organic matter in the wastewater, thereby producing more microorganisms and inorganic end-products (principally CO_2, NH_3, and H_2O).

Several aerobic biological processes are used for secondary treatment differing primarily in the manner in which oxygen is supplied to the microorganisms and kinetic correspondence in the rate at which organisms metabolize the organic matter *(FAO Document Repository).*

Common HRBTP include the activated sludge processes, trickling filters or bio-filters, oxidation ditches, and rotating biological contactors (RBC). A combination of two processes in series can be installed for the treatment of domestic wastewater containing a high concentration of organic material from industrial sources.

Activated Sludge

The dispersed-growth reactor is an aeration tank or basin containing a suspension of the wastewater and microorganisms as the mixed liquid. Aeration devices include submerged diffusers that release compressed air and mechanical surface aerators that introduce air by agitating the liquid surface. HRT in the aeration tanks ranges from 3 to 8 h depending of wastewaters BOD_5 values.

The surplus of microorganisms is separated from the liquid by sedimentation and the clarified liquid is secondary effluent. A portion of the biological sludge is recycled to the aeration basin to maintain a high level of mixed-liquor suspended solids (MLSS).

According to literature assumptions biomass yield and oxygen consumption can be calculated in theory, for the purpose of design estimation, following the stoichiometry of organics *(Metcalf and Eddy, 5th edition, 2014).*

Neglecting nutrients other than nitrogen, according to literature *(Al-Kayiem et al. 2014) is shown as* reaction 5 for oxidation of organics and the yield of biomass can be calculated trough equation 4:

$$3C_6H_{12}O_{8(s)} + 8O_{2(g)} + 2NH_{3(aq)} \rightarrow 2C_5H_7NO_{2(s)} + 8CO_{2(g)} + 14H_2O_{(l)} \quad \text{r.5}$$

$$Y = \frac{2M(C_5H_7NO_2)}{3M(C_6H_{12}O_8)} = 0.418 \quad \text{e.4}$$

Where Y is yield of biomass and M – molecular mass.

Oxidation of glucose is shown trough reaction 6 and COD for glucose can be calculated via equation 5:

$$C_6H_{12}O_{8(s)} + 6O_{2(g)} \rightarrow 6CO_{2(g)} + 6H_2O_{(l)} \quad \text{r.6}$$

$$COD_{\text{glucose}} = \frac{6\,M(O_2)}{M(C_6H_{12}O_8)} = 1.07\,\frac{\text{g } O_2}{\text{g glucose}} \quad \text{e.5}$$

So yield of biomass is expressed in terms of COD, for the portion of the substrate converted into new cells can be calculated trough equation 6:

$$Y = \frac{2M(C_5H_7NO_2)}{3M(C_6H_{12}O_8)\cdot COD_{\text{glucose}}} = 0.391\ \frac{\text{g cells}}{\text{g } COD} \text{ or } \frac{\text{g } VSS}{\text{g } COD} \quad \text{e.6}$$

It should be noted that the actual yield in biological treatment process will be less than calculated *(Metcalf and Eddy, 5th edition, 2014).* Oxygen needed for degradation of newly formed cell can be calculated via equation 7, knowing the reaction 7 of oxidation of $C_5H_7NO_2$:

$$C_5H_7NO_{2(s)} + 5O_{2(g)} \rightarrow NH_{3(aq)} + 5CO_{2(g)} + 2H_2O_{(l)} \quad \text{r.7}$$

$$COD_{\text{cell tissue}} = \frac{5M(O_2)}{M(C_6H_{12}O_8)} = 1.416\,\frac{\text{g } COD}{\text{g } VSS} \quad \text{e.7}$$

Consumed oxygen can be further calculated via equations 8 and 9:

$$O_{2\text{ consumed}} = (COD \cdot n \cdot M)_{\text{gucose}} - (COD \cdot n \cdot M)_{\text{cells}} = 257.78\text{ g } O_2 \quad \text{e.8}$$

$$\frac{O_{2\ \text{consumed}}}{COD_{\text{glucose}}} = \frac{257.78}{577.8} = 0.446 \frac{\text{g } O_2}{\text{g } COD} \qquad \text{e.9}$$

Trickling Filters

A trickling filter or bio-filter is a basin or tower filled with support media for microorganisms in the form of stones, plastic forms or wooden slats. Microorganisms become attached to the media forming a biological layer or fixed film, where organic matter is metabolized *(FAO Document Repository).*

Oxygen is normally supplied to the film by the natural flow of air either up or down through the media, depending on the relative temperatures of the wastewater and ambient air. Controlled stream of air can also be supplied by blowers but is rarely necessary. The thickness of the bio-film increases is directly proportional to new organisms grow. Periodically, portions of the film slough off the media, and can be separated from the liquid in a secondary clarifier and discharged to sludge processing. An amount of secondary effluent is often recycled to the bio-filter to improve hydraulic distribution of the wastewater over the filter.

Since filtration is the flow of a liquid through a porous medium, it is governed by Darcy's law for the rates usually applied in water treatment, shown in equation 10 *(Vojinović Miloradov et al. 2014a)*:

$$V = \frac{K}{\eta}\frac{\Delta P}{\Delta H} = \frac{1}{R\eta}\frac{\Delta P}{\Delta H} \qquad \text{e.10}$$

Where
V - filtration rate,
K - permeability of the filtering layer,
ΔP - head loss through the filtering layer (loss of pressure),
ΔH - depth of considered layer,
η - dynamic viscosity of water,
R - resistance to filtration of the filtering

Rotating Biological Contactors

Rotating biological contactors (RBCs) are fixed-film reactors similar to bio-filters in that organisms are attached to support media. In the case of the RBC, the support media are slowly rotating discs that are partially submerged in flowing wastewater in the reactor. Oxygen is supplied to the attached bio-film from the air when the film is out of the water and from the liquid when submerged, since oxygen is transferred to the wastewater by surface turbulence created by the discs rotation. HRBTP in combination with primary sedimentation, typically remove 85 % of the BOD_5 and SS originally present in the raw wastewater and some of the heavy metals ions. Activated sludge generally produces an effluent of slightly higher quality, in terms of these constituents, than bio-filters or RBCs. When coupled with a disinfection step, these processes can provide substantial but not

complete removal of bacteria and virus. However, the removal efficiency for nutrients, non-biodegradable organics or dissolved minerals is very low.

2.2.3.4. Advanced treatment

Advanced wastewater treatment is employed when specific wastewater constituents cannot be removed by secondary treatment, but must be eliminated. Individual treatment processes are necessary to remove nitrogen, phosphorus, additional suspended solids, emerging or refractory organics[1], xenobiotics and cations of heavy metals and dissolved solids. Because advanced treatment usually follows high-rate secondary treatment, it can be referred to as tertiary treatment. However, advanced treatment processes can be combined with primary or secondary treatment (e.g. chemical addition to primary clarifiers or aeration basins to remove phosphorus) or used in place of secondary treatment (e.g. overland flow treatment of primary effluent). Effluent from primary clarifiers flows to the biological reactor, which is physically divided into five zones by barriers and dams.

In sequence these zones are:

- anaerobic enzyme reaction (digestion) zone - characterized by very low dissolved oxygen levels and the absence of nitrates;
- anoxic zone - low dissolved oxygen levels but nitrates present;
- aerobic zone - aerated;
- secondary anoxic zone and
- final aeration zone.

Advanced oxidation processes (AOP)

Process of advanced oxidation are used when it is necessary to eliminate trace substances that are not been able to be removed by previous or conventional wastewater treatment process, like endocrine disruptive substances, xenobiotics or other emerging substances and/or pollutants *(Rosenfeldt and Linden 2004).* During the conventional oxidation process, there is an uncertainty regarding the formation of toxic by-products and other trace organic chemical species. The benefit of advanced oxidation is the formation of high concentration of hydroxyl radicals ($^{\cdot}HO$), that are capable of oxidizing almost all organics to carbon dioxide, water and mineral acids. The most potent of all oxidants are hydroxyl radical ($^{\cdot}HO$) and O_3, due to characteristics, but also to their effects in oxidation of inorganic and organic substances, improvement of coagulation processes, as a biocide to control algae and disinfectant to control growth in distribution pipes.

Advanced oxidation processes differ from other advanced treatment processes, such as ion exchange, adsorption, striping, nanofiltration, as organic substances are degraded rather than concentrated, transformed or deposited. However, the presence of background organic and inorganic substances can be interference for the process

[1] A variety of organic compounds are classified as refractory when they're poorly biodegraded and/or exhibit a low value for the ratio of biological oxygen demand to chemical oxygen demand (BOD/COD)

efficiency, therefore bench and/or pilot studies are necessary for the specific wastewater *(Silva et al. 2017).*

The time needed for removal of a substance by AOP technique can be calculated by equation 11:

$$t = \frac{1}{K_r \cdot c_{\cdot OH}} \ln\left(\frac{c_{0\,\text{target compound}}}{c_{1\,\text{target compound}}}\right) \qquad \text{e.11}$$

Where

Kr – reaction constant for hydroxyl radical and target constant $\left(\frac{dm^3}{mol \cdot s} \text{ or } \frac{L}{mol \cdot s}\right)$

$c_{\cdot HO}$ - concentration of hydroxyl radical

c_0 i c_1 – concentration of target substance in influent and in effluent

Photolysis

Photolysis processes is initiated by the chemical absorption of infrared (IR), visible (VIS), or ultraviolet (UV) light by at least one component of a reaction mixture. An atom, molecule or other chemical species absorb a quantum of light energy from a photon, which increases the electronic, vibrational and rotational energy states of the atom or molecule above its normal level.

Photo-degradation has a potential as important segment in the environmental fate and degradation of emerging substances. In the direct photo-degradation of EmS, the molecule absorbs radiation, which leads to a break-up of the molecule. It can occur when the absorption spectrum of the target compound overlaps with that of the sunlight.

Indirect photo-degradation involves naturally occurring molecules (photosensitizers) such as nitrate, dissolved organic matter (DOM); generating strong reactive oxygen species (ROS) - hydroxyl radical (·OH), or peroxyl radicals (·OOR). Humic acids can reduce the rate of photo-transformation by absorbing light and acting as an inner filter voluminous mass. The presence of DOM might be of critical importance for photochemical reactions of EmS in surface waters *(Vojinović et al. 2015).*

However, in all cases the EDCs were more effectively degraded utilizing UV/H_2O_2 advanced oxidation as compared to direct UV photolysis treatment *(Rosenfeldt and Linden 2004).*

Membrane filtration

There are four basic types of membrane filtration process depending on the applied pressure - microfiltration (MF), ultrafiltration (UF), nanofiltration (NF) and reverse osmosis (RO). MF and UF process are used to remove suspended particles sizes range from 0.1 to 10 μm, colloidal particles, viruses and bacteria greater than 0.01 μm, and take place on microporous membranes.

The processes operate on the principle of filtration through a sieve, and substance removal efficiency depends only on the size of particles *(Kukučka and Kukučka 2016).* Depending on the required quality of filtered water, microfiltration and ultrafiltration can be installed individually or as a pre-treatment for nanofiltration processes or reverse osmosis. Nanofiltration is a process is designed for the removal of the solute particle size of 1 to 2 nm, and the reverse osmosis for the solute size 0.1-1.0 nm.

Reverse osmosis and nanofiltration are the technologies that use semipermeable membranes to perform separation of the dissolved substances, based on the principle of diffusion. Diffusion is described by *Fick's first law* which postulates that the flux goes from regions of high concentration to regions of low concentration, with a magnitude that is proportional to the concentration gradient (spatial derivative). In one (spatial) dimension, the law is shown in equation 12 *(Miller et al. 2009)*:

$$J=-D\frac{\mathrm{d}c}{\mathrm{d}x} \qquad \text{e.12}$$

Where J is the diffusion flux $\left[\frac{\mathrm{mol}}{\mathrm{m^2 s}}\right]$, D is the diffusion coefficient $\left[\frac{\mathrm{m^2}}{\mathrm{s}}\right]$, c (for ideal mixtures) is the concentration $\left[\frac{\mathrm{mol}}{\mathrm{m^3}}\right]$, x is position (length) [m].

The Fick's law is further transformed into equation 13:

$$J=\frac{K_\mathrm{w}}{\mu}\left(\left(\frac{p_\mathrm{f}+p_\mathrm{c}}{2}-p_\mathrm{p}\right)-\Delta\pi\right) \qquad \text{e.13}$$

Where J is the diffusion flux $\left[\frac{\mathrm{m}}{\mathrm{s}}\right]$, Kw is permeability of water through the membrane[m], μ is absolute viscosity [Pa·s], p_f is pressure of influent [Pa], p_c is pressure of concentrate [Pa], p_p is backpressure [bar], $(\frac{p_\mathrm{f}+p_\mathrm{c}}{2}-p_\mathrm{p})$is transmembrane pleasure (TMP) [Pa], $\Delta\pi$ is difference of osmotic pressure of membrane and permeate [Pa].

Although ultrafine membranes can also partially remove suspended material, their pores are susceptible to clogging, and this membrane is never used without proper pre-treatment, as a advanced or tertiary treatment process. Exposing the water to a pressure higher than the natural osmotic pressure, water passes through the molecular structure of the membrane wall, while the dissolved solids remain on the surface of the membrane.

Disinfection

Effective disinfection of viruses is inhibited by suspended and colloidal solids in the water; therefore, these solids must be removed by modern advanced treatment before the disinfection step. Disinfection usually involves the injection of a chlorine solution at the head end of a chlorine contact basin. The chlorine dosage depends upon the strength of the wastewater and other factors, but dosages of 5 to 15 mg/L are common. Ozone and UV irradiation can also be used for disinfection. Chlorine contact basins are usually

rectangular canals, with barriers to prevent short-circuiting, designed to provide a contact time of about 30 minutes. However, to meet wastewater treatment requirements, a chlorine contact time of as long as 120 minutes is required for specific irrigation uses of reclaimed wastewater. The bactericidal effects of chlorine and other disinfectants are dependent upon pH, contact time, organic content, and effluent temperature.

Treatment processes typically considered for the removal of EmS from wastewater include adsorption, AOPs, NF and RO membranes *(Bolong et al. 2009).* However, the limitations of these methods are high investment and maintenance costs and/or formation of secondary pollution (generation of toxic sludge, known and unknown by-products etc.) and/or complicated procedure. Physicochemical treatments such as coagulation/flocculation processes were generally found to be unable to remove EDCs. For adsorption processes several materials can be used as adsorbents that successfully remove different types of emerging compounds, but their use is restricted due to high cost, especially for activated carbons. The operating costs of ACs procedures have resulted in attempts by various researchers to utilize low cost alternative adsorbents *(Gupta et al. 2009; Pap et al 2016).* Therefore, natural materials that are available from agricultural operations have been evaluated as possible low cost and environmental friendly adsorbents *(Khattri and Singh 2009).* A growing number of studies that utilization the waste materials as adsorbents have been carried out, especially for removal of emerging substances and pollutants, and show significant positive results.

2.3. Analytical methods of separation and detection of volatile xenobiotics

Only a few chemical analytical methods are specifically designed to correspond to a particular analyte, and it is necessary to separate the analyte of interest from the multitude of compounds present in a sample. The second step, after separation of analytes of interest from secondary compounds it is necessary to perform analysis to detect, identify and quantify selected analytes.

The chromatographic techniques can provide both methods of separation and analysis in one – depending on chromatographic method detection, identification and quantification can be performed. During chromatographic procedure a sample extract is dissolved in a mobile phase (gas or liquid depending on technique selected), and transported through an immobile, immiscible stationary phase. The mobile and stationary phases are specifically selected that analytes of interest have differing solubility in each phase; a result of mobility differences components will separate as they pass through the stationary phase *(Poole 2003).*

Techniques that use elution of sample trough column can be HPLC (High Performance Liquid Chromatography) and GC (Gas Chromatography). There is another technique using supercritical fluid but it is rarely used in commercial and research laboratories, due to complexity of procedures.

The equilibrium constant of a reaction (K_r) is the partition coefficient, representing molar concentration of analyte in the stationary phase divided by the molar concentration of the analyte in the mobile phase. The time between sample injection and an analyte peak reaching a detector at the end of the column is termed the retention time (RT), which is significant and specific for every separated substance. Each analyte in a sample will have a different retention time.

Sharp and symmetrical chromatographic peaks have to be obtained for optimal separation and analysis of selected analytes (*Poole 2003*).

2.3.1. Screening analysis

A screening analysis is a process that extracts, separates and identifies substances in a sample with the minimum number of sample manipulation phases. It is important to emphasise that the analytical approach taken should be adapted to correspond to the purpose and that this criteria must be reflected in the analytical method proposed *(Muñoz-Olivas 2004)*.

Screening analysis is a semi-quantitative technique of yielding an approximation of the quantity of a substance.

The "screening" analysis is defined as:

- methods that show if target analytes are present above or below a threshold; and
- a prompt acquisition of semi-quantitative data about all substances in the sample.

The specific goals of the screening analysis are to avoid processing a large number of samples in order of making timely and adequate decisions, or to obtain overall composition of pollutants; to optimize a conventional analytical process, which can be tedious, time-consuming, and sources of systematic errors; and, to optimize the need for permanent use of instruments with great procurement and maintenance costs (only samples with a positive response would need such instrumentation to be used) *(Muñoz-Olivas 2004)*.

Results of screening analysis for Danube River identified 159 substances Annex I.

2.3.2. Target analysis

Target analysis is the analytical approach where target analyte or group of substances is selected to be analysed via analytical technique and method. In cases where a relatively small number of analytes can be defined, target analytical methods can be applied. In environmental research it should be the module that follows screening analysis, when the selection and prioritisation of identified pollutants is carried out.

The target analysis is defined according to process of targeting one ore a group of substances to qualitatively and quantitatively determine. Such methods can achieve great accuracy and precision particularly where stable isotope labelled internal

standards are available. In such targeted analyses, signals from all other components are ignored *(Halket et al. 2004).*

2.3.3. Gas and liquid chromatography

Environmental samples of every medium are complex mixtures of different substances. Even if the substance of interest is successfully isolated, a sample mixture is still an unknown and it is important factor for understanding the behaviour of an isolated substance in naturally occurring matrix. Chromatography technique involves the partitioning of substances between mobile and stationary phase, providing the necessary separation of analytes. The longer the separation process takes the better the separation of analytes will be and thus the detection via detector. Chromatographic methods are categorized as methods based on separation in a two-phase system, with the repetitive establishment of equilibrium. In the chromatographic system, one of the phases is physically immobilised - *stationary phase*, and the other is mobile, therefore, called a *mobile phase.* The sample is dissolved or dispersed into mobile phase which could be a liquid, a gas or a supercritical fluid, which forces the sample through the column coated with stationary phase, thus enabling the separation of analytes in sample. Separation leads to formation of zones containing molecules of particular chemical species, differentiating less retained components that reach the end of the column faster than more retained components. Due to interactions of components with stationary phase, after sufficient analysis time, the sample components are distributed into the separated zones. Separation and retention processes progress simultaneously and continuously, resulting in formation of chromatogram *(Vojinović Miloradov et al. 2014a)*.

In chromatographic techniques of separation the especially important factor for identification and detection of separated substances is retention time of the substance. The interval between the injection and the detection of the component is known as the retention time. Because retention time varies with the molecular mass of the analyte, it is utilized as one of the parameters for qualitative analysis. The retention time of substances is a specific time of elution of substance from column during the analyses. In gas chromatography (GC) the mobile phase is an inert gas, usually helium. The stationary phase is liquid of high molecular mass, usually silica gel, which is chemically bonded to the inner walls of a long capillary column. For environmental analyses of complex heterogenic mixtures the column should be at least 30 m in length and have an internal diameter of about 0.2 mm.

The analysis of effluents for organic compounds requires extraction of the organics from the water matrix, concentration of the extract, separation of individual components of the organic extract by a GC column and detection of the separated components as they are eluted from the GC column. The High Performance Liquid Chromatography (HPLC) is a modern culmination of LC development. In this technique

the automatic sampler is a necessity, where the sample is introduced into the column by a stream of solvent, usually acetonitrile *(Snyder et al. 2010).*

LC enables separation of almost all types of compounds: non-dissociable/dissociable, polar/non-polar, organic/inorganic compounds with low or high molecular weight. The precondition for separation is that substances must dissolvable in common organic solvents, water or diluted inorganic acids *(Vojinović Miloradov et al. 2014a)*.

2.3.4. Mass spectrometry

Gas chromatography coupled with mass spectrometry (GC-MS) is frequently used in environmental analysis due to possibility to record mass spectra of eluted analytes, which are then used for identification. The analytes are transferred from the gas chromatography column by carrier gas to ionization chamber, via interface. Separated molecules enter the ion source chamber of the mass spectrometer, maintained under high vacuum, where they are bombarded by electrons, and fragmented to ions. These ions are accelerated in electric field, separated by analyser, detected by ion multiplier and processed by central processor unit *(Vojinović Miloradov et al. 2014a)*. The principle of MS is shown in Figure 2.6.

The energy transferred to molecules in this process causes them to ionize and dissociate into various fragment ions. Ions may be singly or multiply-charged. The positive ions formed are made to cross an analyser section, maintained at $1.33 \cdot 10^{-3}$ to $1.33 \cdot 10^{-5}$ Pa. After the ions pass the analyser section where they are separated according to their mass-to-charge ratio (m/z), they are detected by an extremely sensitive electron multiplier *(Clement and Taguch, 1991).*

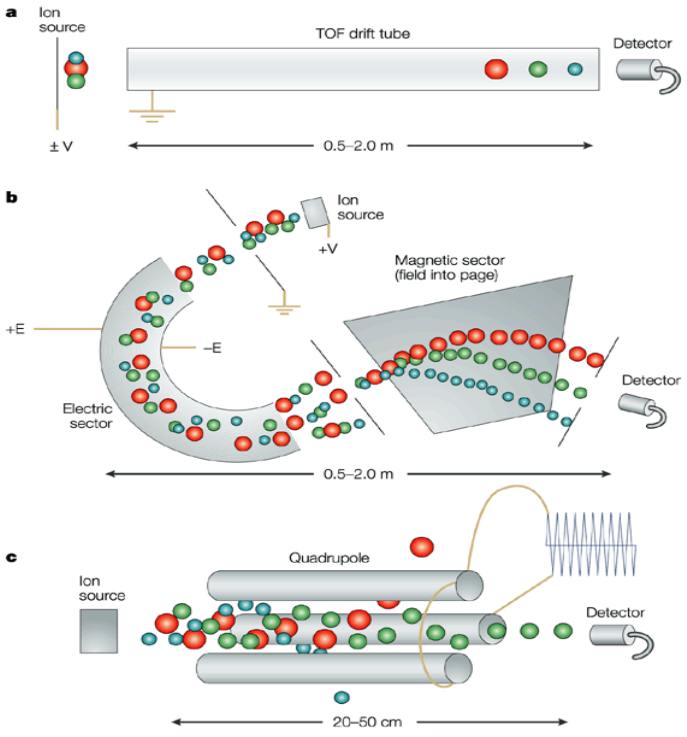


Figure 2.7 Principle of mass spectrometry (Glish and Vachet 2003)

By plotting the abundance of ions detected versus their m/z, a mass spectrum is obtained. The mass spectrum of a compound can be used to identify the original organic structure. It consists of a bar graph representation of the m/z of the ions and their abundances normalized to the most abundant ion (base peak).

Mass spectrum of a compound is the unique characteristic, and can be called a "fingerprint of a compound". Comparing the retention time of an analyte and its mass spectrum with provided mass spectrum libraries, a positive identification of the sample component is possible.

2.4. Priority and emerging substances – xenobiotics

Priority and hazardous priority pollutants (PhPPs) are a set of chemical pollutants that are regulated, and for which there are developed analytical test methods.

In EU legislation PhPPs are recognized and selected from pollutants that present a significant risk to or via the aquatic environment, using the approaches outlined in Article 16 of the WFD, and introduced in Annex X "List of priority substances" in WFD. The Annex X of WFD developed into the Annex II of the Directive on Environmental Quality Standards (Directive 2008/105/EC) (EQSD)**,** known as the Priority Substances Directive, posing environmental quality standards (EQS) for the substances in surface waters (river, lake, transitional and coastal). Via Directive 2008/105/EC substances were defined as priority or priority hazardous substances, the latter being a subset of particular concern.

The current list of 126 Priority Pollutants is presented in Appendix A to 40 CFR Part 423. The Priority Pollutant list makes the list of toxic pollutants more usable, in a practical way, for the purposes assigned to EPA by the Clean Water Act. The Priority Pollutant list is more practical for testing and for regulation in that chemicals are described by their individual chemical names *(Clean Water Act, 1972*).

According to the international and national laws and by-laws all priority and priority hazardous substances have designed and audited monitoring plan with defined maximum allowable concentration and doses in different environmental media, but emerging substances do not, and the fact that EmS present the frequently and continuously used substances in low doses, there is a question about chronic effect that they might have on environment and living organisms.

Emerging substances of concern are wide groups of substances recognized by global scientific and technical association as substances in environment that should be monitored. In these groups there can be found substances widely used all over the world in different branches of industry, agronomy, science and research or everyday life. Such substances are industrial chemicals, pharmaceuticals, personal care products, plasticizers, wood preservatives, pesticides and many others. Emerging contaminants are ubiquitous, persistent/pseudo-persistent and biologically very active molecules that

occur in the environment as a result of natural, industrial and human activities. Emerging substances in low doses with pseudo-persistency effects are recognized as powerful chemical eco stressors on biosphere with unknown eco toxicology implication, fate, behaviour, distribution and partitioning as well as the transport through all environmental media *(Vojinović Miloradov et al., 2014a).*

EmS are, in best case, just partially removed during the conventional chemical, physical, and biological treatment processes, a considerable amount of EDCs (synthetic hormones, hormone-like and benzothiazoles) are released to the recipient *(Jones et al., 2007).*

Group of substances overlapping in priority, priority hazardous pollutants and emerging substances list provided by legislation, environmental standards and guidelines research institutes is EDCs or Endocrine Disruptive Compounds. EDCs can be chemicals from different categories – household products ingredients, personal care products and cosmetics ingredients, food additives, flame retardants, plastics and rubber, pesticides ingredient, antimicrobials, biogenic compounds, industrial additives, solvent, breakdown products of other chemicals, as well as chemicals used:

- in the extraction, processing, or manufacturing of a metal or metal-containing product, including welding,
- in the synthesis of other compounds and/or unwanted by-products such as impurities and contaminants, including combustion by-products,
- in hospitals, medical supplies, and equipment, in laboratories as reagents, and pharmaceuticals,
- in hydraulic fracturing and those associated with the process (including drilling) that are released into water, air, and soil.

Groups of substances most commonly known as endocrine disruptors are pesticides, polychlorinated biphenyls, dioxins, phenols, phthalates and estrogens.

The phenomenon of low concentrations is of particular interest, especially in EmS that interferes with the functions of the endocrine system (EDCs) and represents risk to human health and the environment. Disorder of the endocrine glands can be a result in the continuous exposure to EmS, especially EDCs. Pico and nano concentrations of EmS correspond to the concentrations of the natural hormones in the biological organism. A large number of polar functional groups provide a good solubility and the ability of the various chemical transformations. Polar and/or non-polar nature of the whole or part of the individual EmS molecules often prevents diffusion of the compound across the boundary surface of a heterogeneous two phase system, and hence the dispersion of the water molecules in the other media of the environment *(Gilbert 2012).*

Emerging substances and pollutants are “new old” chemicals of concern, presented as chemicals widely used every day that can have diverse influence on health and environment in different low doses. NORMAN list has defined emerging substance as a chemical that has been detected in the environment, but which is currently not included

in routine monitoring programs and whose fate, behaviour and (eco)toxicological effects are not well understood. As well the emerging pollutants have been defined as substances currently not included in routine environmental monitoring programs and which may be candidate for future legislation due to its adverse effects and/or persistency *(NORMAN list 2016).* The Norman list of substances contains 1036 substances under review, divided into 30 categories. The list is updated every year; ad was updated last in February 2016.

After the prioritization process the list is showing additional 20 pollutants of certain and potential basin-wide concern is presented in the JDS3 Report, from which 11 are suspected or proven endocrine disruptive compounds.

In table 2.4 prioritisation of pollutants in surface water of Danube and tributaries form JDS3 is shown.

Therefore, different emerging substances can be detected in wastewater, according to literature - flame retardants, alkyphenols (APs) and polycyclic aromatic hydrocarbons (PAHs), phthalates *(Vojinović Miloradov 2014c)* and pesticides *(Vojinović Miloradov 2014c, Sremački et al. 2015)*, caffeine and pharmaceuticals *(Grujić-Letić et al. 2015, Fernandez et al. 2010, Kasprzyk-Hordern et al. 2008)*, hormones and EDCs *(Kasprzyk-Hordern et al. 2008, Sremački et al. 2015)*, illicit drugs *(Kasprzyk-Hordernet al. 2008, Ort et al. 2014)* and other.

Most emerging substances cannot be removed completely or sufficiently enough by the conventional wastewater treatment processes, and therefore they are released into natural recipients. Great number of EmS are highly water soluble and poorly degradable, hence, they can pass through all the natural filtrations and reach groundwater and ultimately drinking water *(Milić et al. 2014).*

Table 2.4 Prioritisation of pollutants in surface water (Modified from JDS3 Report 2015)

	Substance	No. of sites	C_{max}	MEC_{95}	Lowest PNEC/EQS	Type	EoE	FoE	Score
1.	2,4 Dinitrohenole	68	0.06	0.04	0.001	AA-EQS	40	1	1.2
2.	Perfluorooctansulfonate	63	0.026	0.02	0.00065	AA-EQS	31	0.93	1.13
3.	Chloroxuron	65	0.04	0.02	0.0024	PNEC acute	8.3	0.93	1.03
4.	Desethylterbutylazine	54	0.028	0.01	0.0024	AA-EQS	4.2	0.79	0.89
5.	2-hydroxy atrazine	53	0.06	0.02	0.002	AA-EQS	10	0.76	0.86
6.	Bromacil	31	0.19	0.14	0.01	AA-EQS	14	0.46	0.66
7.	Dimefurone	58	0.041	0.04	0.008	AA-EQS	5	0.56	0.36
8.	Bisphenol A	30	1.94	1.03	0.1	AA-EQS	10	0.16	0.36
9.	Benzo(h,g,i)perylene	65	0.029	0.003	0.002	AA-EQS	1.5	0.26	0.36
10.	Diazinone	21	0.009	0.01	0.001	PNEC acute	10	0.12	0.22
11.	Indeno(1,2,3-c,d)perylene	15	0.005		0.002	AA-EQS	4.3	0.19	0.19
12.	Linuron	32	1.42	1.12	0.26	AA-EQS	1	0.07	0.17
13.	Amoxicillin	33	0.28	0.08	0.078	PNEC acute	1.1	0.03	0.13
14.	Methayachlor	30	0.03	0.02	0.019	AA-EQS		0.03	0.13
15.	Diclofenac	51	0.318	0.036	0.05	AA-EQS		0.04	0.04
16.	Bentazone	61	0.1	0.02	0.06	PNEC acute		0.01	0.01
17.	Fluoranthene	58	0.02	0.006	0.0063	AA-EQS		0.01	0.01

C_{max} – Maximum concentration in µg/L reported in case the substance has been measured by several JDS3 laboratories, MEC_{95} – 95 % of C_{max}, calculated only if the analyte has been found above LOQ at minimum 20 sites, EoE – Extent of Exceedance, FoE – Frequency of Exceedence

2.4.1. Illicit drugs

Illicit drugs are defined by United Nations as follows "The United Nations drug control conventions do not recognize a distinction between licit and illicit drug, they describe only use to be licit or illicit. The term illicit drugs is used to describe drugs which are under international control (and which may or may not have licit medical purposes) but which are produced, trafficked and/or consumed illicitly" (*UNODC, 2016*).

Illicit drugs are the latest group of emerging compounds identified in the aquatic environment which are drawing much attention, and included into the NORMAN list of EmS in 2016. These compounds and their primary metabolites, as already stated for pharmaceuticals *(Daughton and Jones-Lepp, 2001),* reach surface waters unaltered predominantly through treated or non-treated wastewater discharged into the recipient. In the recent years there is a growing concern related to the presence of illicit drugs and their metabolites in rivers ecosystems, which triggered the wave of new studies and research worldwide, as well as in Europe. Drugs that enter the environment from clinical and/or illicit applications, account for approximately 60 % of wastewater treatment demand in Europe *(Pal et al. 2013).*

The estimation of drug consumption is rather complicated and unreliable, due to the voluntary participation of the consumers in specific analytical, medical and/or psychological studies. A new approach to obtain significantly reliable data for environmental monitoring of illicit drugs of a population was proposed in 2001 and for the first time put into practice in 2005. This approach, that is considering the determination of illicit drugs through urban wastewaters samples, has gained worldwide attention by the media and has been supported by various scientists *(Daughton and Jones-Lepp, 2001***,** *Ort. et al. 2014).* Obtaining data is of extreme interest, especially in environmental studies.

The principal difficulty for researchers is the low concentration levels of drugs in combination with the complexity of the matrix. Developed analytical methods are based on solid-phase extraction (SPE), for sample pre-treatment and pre-concentration, and the analytical technique of choice is liquid chromatography coupled to tandem mass spectrometry (LC–MS/MS).

2.4.1.1. Cocaine and benzoylecgonine (BE)

Cocaine hydrochloride is a white to light brown crystalline powder, and cocaine base is white to beige in colour. Naturally derived CNS stimulant and local anaesthetic extracted and refined from the leaves of the coca plant (*Erythroxylon coca*). Cocaine is a strong CNS stimulant that interferes with the reabsorption process of dopamine, a chemical messenger associated with pleasure and movement. Cocaine is metabolized to a variety of compounds: benzoylecgonine, ecgonine, and ecgonine methyl ester are the major inactive metabolites. BE is produced upon loss of the methyl group and is the major urinary metabolite. The

apparent half-life for cocaine is short, approximately 0.8 ± 0.2 hours, while the half-life of BE is 6 hours. Unchanged cocaine when excreted remains in less than 2 % of introduce dose, while primary metabolites benzoylecgonine and ecgonine methyl ester can be detedted in ranges of 26-39 % and 18-22 %, respectively. Most of cocaine and it's metabolites, 64-69% of the initial dose, are excreted within 3 days, while BE persists in urine at detectable concentrations from 2-4 days. Chronic, heavy use of cocaine can result in detectable amounts of BE in urine for up to 10 days *(NHTSA Raport 2004).* In Figure 2.9 structural formula of cocaine and benzoylecgonine is shown.

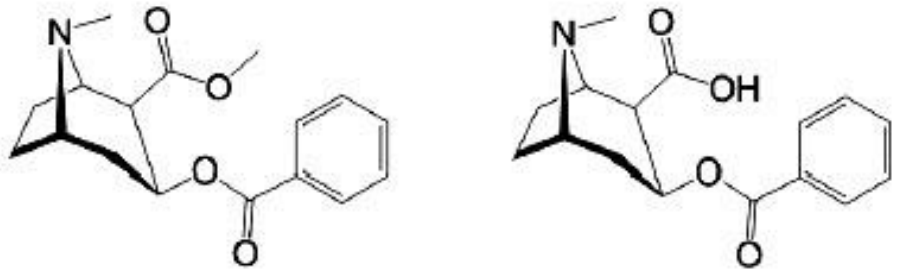


Figure 2.8 Structural formula of cocaine and benzoylecgonine (Bravo et al. 2012)

2.4.1.2. Methamphetamine and amphetamine

Methamphetamine hydrochloride is CNS stimulant, appetite suppressant; a white to light brown crystalline powder, or clear crystals resembling ice. Methamphetamine base is a liquid. Methamphetamine's effects are similar to cocaine but its onset is slower and the duration is longer. Methamphetamine is infrequently used in the treatment of obesity, overeating disorders, and mass loss due to its abuse potential. Amphetamine is also used in ADD, narcolepsy, and mass control. Following oral administration, peak methamphetamine concentrations are seen in 2.6-3.6 hours and the mean elimination half-life is 10.1 hours (range 6.4-15 hours). The amphetamine metabolite peaks at 12 hours. Following intravenous injection, the mean elimination half-life is slightly longer (12.2 hours). Methamphetamine is metabolized to active amphetamine, and inactive p-OH-amphetamine and norephedrine. Several other drugs are metabolized to amphetamine and methamphetamine and include benzphetamine, selegeline, and famprofazone. Detection in urine is indicative of use within 1-4 days, however this period can be prolonged to 7 days due to heavy chronic use, and as the rate of excretion is greatly depended on pH of urine. Initial oral dose is excreted as unchanged methamphetamine and amphetamine in range of 30-54 % of and 10-23 %, respectively, while intravenous dose application changes the distribution significantly to 45 % of methamphetamine and 7% amphetamine *(NHTSA Raport 2004).* In Figure 2.10 structural formula of amphetamine and methamphetamine is shown.

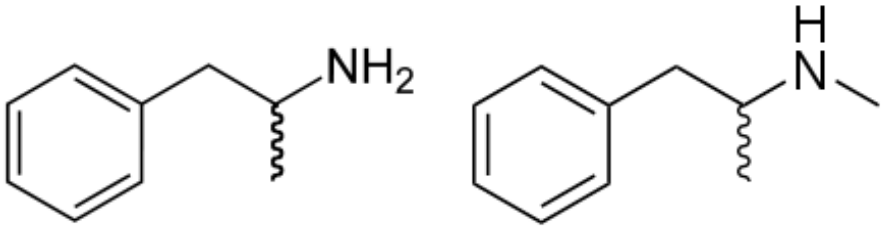


Figure 2.9 Structural formula of amphetamine and methamphetamine (Brunt and Niesink 2011)

2.4.1.3. MDMA – Ecstasy

MDMA is mild CNS stimulant, mild hallucinogen and psychedelic, appetite suppressant; a white, tan or brown powder. MDMA is the methylenedioxy derivative of methamphetamine.

Originally patented as an appetite suppressant and used as a possible adjunct to psychotherapy, there is currently no legitimate medical use. MDMA is a phenylethylamine that has stimulant as well as psychedelic effects.

MDMA is rapidly absorbed and half-life of MDMA is ~ 7 hours. MDMA is metabolized to MDA which is the only metabolite reported in blood and plasma. Additional MDMA metabolites include 3-hydroxy-4-methoxymethamphetamine (HMMA) and 3,4-dihydroxymethamphetamine (HHMA). These polar hydroxylated metabolites are conjugated prior to their excretion in urine *(NHTSA Raport 2004).* In 2.11 structural formula of MDMA is shown.

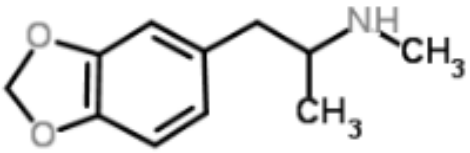


Figure 2.10 Structural formula of MDMA (http://www.chemspider.com/Chemical-Structure.1556.html?rid=4267e7ab-ba4a-41a5-9db8-d34f45152ea1)

2.4.1.4. THC-COOH – Cannabis (Δ ^{9} -Tetrahydrocannabinol, THC)

Marijuana is a green or gray mixture of dried shredded flowers and leaves of the hemp plant *Cannabis sativa*. Hashish consists of resinous secretions of the cannabis plant.

Dronabinol (synthetic THC) is light yellow resinous oil. Cannabis contains chemicals called cannabinoids, including cannabinol, cannabidiol, cannabinolidic acids, cannabigerol, cannabichromene, and several isomers of tetrahydrocannabinol (THC). One of these isomers, Δ ^{9}-THC, is believed to be responsible for most of the characteristic psychoactive

effects of cannabis. Marijuana refers to the leaves and flowering tops of the cannabis plant; the buds are often preferred because of their higher THC content. The substance is indicated for the treatment of anorexia associated with mass loss in patients with AIDS, and to treat mild to moderate nausea and vomiting associated with cancer chemotherapy. Marijuana is used for its mood altering effects, euphoria, and relaxation. Marijuana is the most commonly used illicit drug throughout the world. Correspondingly, THC produces alterations in motor behaviour, perception, cognition, memory, learning, endocrine function, food intake, and regulation of body temperature. Absorption is slower following the oral route of administration with lower, more delayed peak THC levels.

Bioavailability is reduced following oral ingestion due to extensive first pass metabolism. Smoking marijuana results in rapid absorption with peak THC plasma concentrations occurring prior to the end of smoking. Concentrations vary depending on the potency of marijuana and the manner in which the drug is smoked, however, peak plasma concentrations of 100-200 ng/mL are routinely encountered. THC is highly lipid soluble, and plasma and urinary elimination half-lives are best estimated at 3-4 days, where the rate-limiting step is the slow redistribution to plasma of THC sequestered in the tissues. THC is rapidly and extensively metabolized with very little THC being excreted unchanged from the body.

THC is primarily metabolized to 11-hydroxy-THC which has equipotent psychoactivity. The 11-hydroxy-THC is then rapidly metabolized to the 11-nor-9-carboxy-THC (THC-COOH) which is not psychoactive. A majority of THC is excreted via the feces (~65 %) with approximately 30 % of the THC being eliminated in the urine as conjugated glucuronic acids and free THC hydroxylated metabolites *(NHTSA Raport 2004).* In the Figure 2.12 structural formula of THC-COOH is shown.

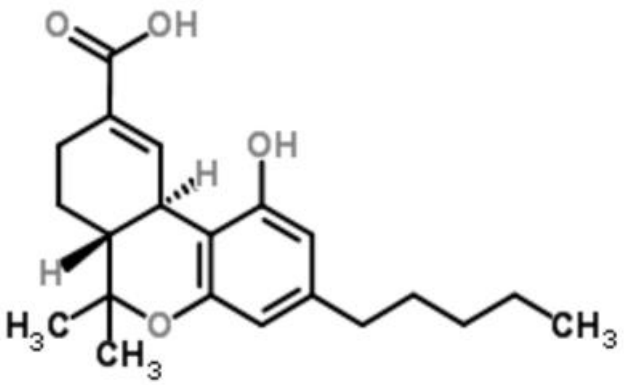


Figure 2.11 Structural formula of Tetrahydrocannabinol (http://www.chemspider.com/Chemical-Structure.97282.html?rid=d42e8755-20b4-4beb-81ad-d76da2d182ce)

2.4.2. Endocrine disruption substances

The organochlorine pesticides and hormones, as well as plasticizers, are targeted as specific and "of interest" for the research due to their possible effects of mimicking and overlapping, or enhancing one another.

Several EU regulatory documents and bodies related to regulatory safety testing refered to the need of endocrine disrupters (EDs) identification, such as:

- Regulation 1907/2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH),
- Regulation (EC) 1107/2009 concerning the placing of plant protection products on the market (PPPR),
- Regulation (EU) 528/2012 concerning the making available on the market and use of biocidal products (BPR), and
- Regulation (EC) 1223/2009 on cosmetic products.

Uncertainty and lack of information about the overall effects and interactions of organochlorine pesticides and hormones, especially estrogens, poses a great unease in technical, professional and science communities *(Milić et al. 2013, Vojinović Miloradov et al. 2014b).*

2.4.2.1. Estrogens

Natural and synthetic estrogens are some of the most potent endocrine disrupting compounds found in municipal wastewater. Much research has been conducted on the source and fate of estrogens in wastewater treatment plants. Sorption and biodegradation are the primary removal mechanisms for estrogens in activated sludge systems, which are widely used biological treatment techniques for municipal wastewater treatment *(Khanal et al. 2006).*

Among the numerous trace organic contaminants in wastewater effluent, hormones and hormone mimics may be of greatest concern to human and environmental health. Estrogens and estrogen mimics are among the most relevant sources of concern in waters intended for reuse.

In recent years a new problem has emerged in our water environment, namely, endocrine disrupters (EDs) that may affect the reproductive functions of human beings and wild life. In Japan, contamination of water with EDs poses new and potential environmental problems. Naturally occurring estrogens tend to have higher estrogenic potentials than synthetic, industrial chemicals.

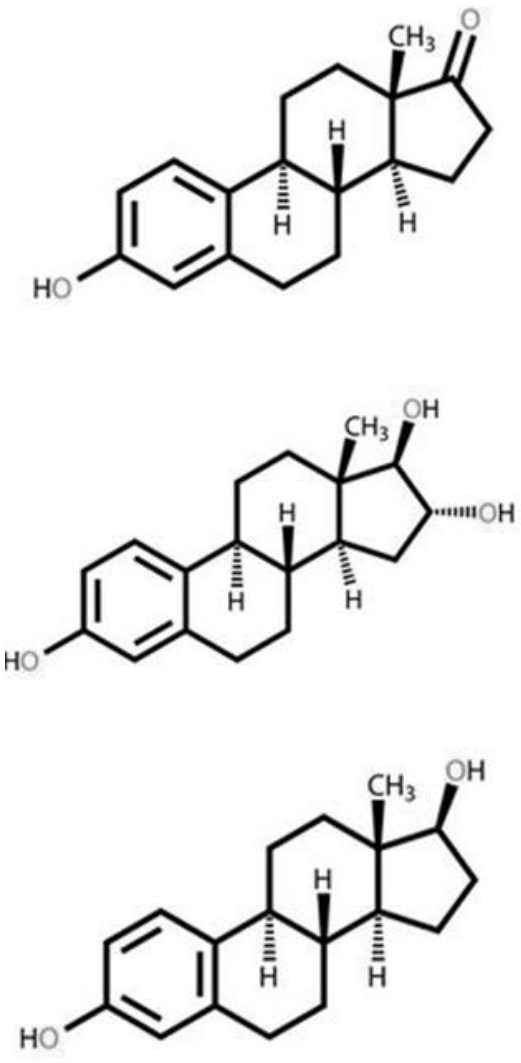


Figure 2.12 Structural formula of estrone, estriol and estradiole (https://www.kingsrxandwellness.com/know-your-estrogens-and-your-body/)

Effluents from municipal and industrial wastewater treatment plants, and agricultural run-off and drainage add numerous exogenous compounds to the aquatic system. Research has shown that the main substances causing these effects are the natural compounds estrone (E1), 17β-estradiole (E2) and estriol (E3) and the synthetic estrogen, 17α-ethinylestradiole (EE2). A few other steroid estrogens are also a reason for concern. The primary source of these substances in the environment has previously been attributed to human release through sewage treatment however, the question of whether wastes from farm animals (cattle and pigs) (17 α-estradiole (E2-17α) is a significant source for the observed effects remains unanswered.

Many chemical substances display estrogenic activity and may be suspected of causing adverse effects in humans and/or environmental organisms. However, only a few examples

provide evidence that the presence of chemicals released to the environment by human activities is causing adverse effects on environmental organisms. Recently evidence was presented showing that steroid estrogens released from humans are the main causal agents for the feminisation of fish in an aquatic environment impacted by sewage *(Bachmann Christensen et al. 2002).*

Understanding the basis of process-dependent differences will set the stage for process design or operation for efficient removal of estrogens and estrogen mimics. Republic of Serbia is in the midst of planning efforts that will lead to selection of a water plan and wastewater treatment facilities that should serve for decades. Wastewater reclamation and reuse will be a major part of both water supply and wastewater treatment planning. The fate of trace organics during wastewater treatment or, from another perspective, facilities design/operation for control of trace organics should be an important factor in facilities planning.

Regarding the hormones, 17β-estradiole is showing high percentile of removal during the biological treatment (47 %), resulting in concentrations below the LOQ in both the effluent of this unit and of the overall plant. On the contrary, estrone concentrations increase over the course of treatment, illustrating the fact that under oxidizing conditions, 17b-estradiole is quickly converted into estrone, which is slowly degradable. The overall removal efficiencies within the WWTP ranged around 65% for 17β-estradiole. However, the concentration of estrone increased along the treatment due to the partial oxidation of 17β-estradiole in the aeration tank (*Carballaa et al. 2004*).

The behaviour of 17β-estradiole, estrone, estriol, estrone-3-sulfate (E1-S), β-estradiole 3-sulfate (E2-S), estriol 3-sulfate (E3-S), 17-disulfate estradiole-3 (E2-diS), in aerobic wastewater treatment process was studied in detail. Concentrations of target substances in influent and effluent were determined by LC/MS^2. Concentrations of free estrogens were declining in aerobic wastewater treatment process, while estrogenic metabolites remained in the effluent. Moreover it was found that estrone-3-sulfate, β-estradiole 3-sulfate and estriol 3-sulfate were degraded to some extent, while E2-diS was stable in the aerobic wastewater treatment process.

Concentrations of all selected estrogens were measured every 90 minutes in the 9-hour batch degradation experiment. Free estrogens (17β-estradiole, estrone) were found in the primary effluent, however, they were not found in the final effluent. It indicated that free estrogens were immediately degraded in the aerobic reactor. Concentrations of estrone-3-sulfate, β-estradiole 3-sulfate and estriol 3-sulfate were declined to some extent, but concentrations of estradiole-3, 17-disulfate were increasing throughout the treatment process *(Komori et al. 2004, Okayasu et al. 2005).*

2.4.2.2. Organochlorine pesticides

The historical background of pesticides use in agriculture is dated back to the beginning of agriculture itself and it became more pronounced with time due to increased pest population paralleled with decreasing soil fertility. The first generation of pesticides involved the use of highly toxic compounds, arsenic (calcium arsenate and lead arsenate) and a fumigant hydrogen cyanide in 1860's for the control of such pests like fungi, insects and bacteria. Their use was prohibited due to toxicity to the environment and ineffectiveness. The second generation used synthetic organic compounds - the first key synthetic organic pesticide was dichlorodiphenyl-trichloroethane (DDT) first synthesized in 1873 *(Kirk and Othmer 1996)* and its insecticidal effect discovered in 1939. In its early days DDT was hailed as a miracle because of its broad-spectrum activity, persistence, insolubility, inexpensive and simple application process. *P, p'*-DDT in particular was so effective at killing pests and thus boosting crop yields and was so inexpensive to make its use quickly spread over the globe. DDT was also used for many non-agricultural applications as well. For example, it was used to desinsect soldiers in the World War II and in the public health for the control of mosquitoes which are the vectors for malaria *(Zacharia 2011).*

p,p'-DDT is in the European countries and in Serbia forbidden since 1972 and 1989, respectively. However, p,p'-DDT and metabolites, as high persistent chemicals with long half-lives, are detected in water samples, biota and human material. p,p'-DDE and p,p'-DDA are polar compounds excreted from the human organisms by physiological liquids, urine and faeces. But, metabolite p,p'-DDD is highly soluble and bio-acumulative in fat tissue. The concentration ratio of p,p'-DDD : p,p'-DDT is an indicator for the resident time of p,p'-DDT in the environment. Residual quantities of p,p'-DDD indicate historical contamination of p,p'-DDT. In wastewater in the vicinity of Novi Sad and surface water of Danube, surprising unexpected relatively high concentrations of p,p'-DDT, p,p'-DDD and p,p'-DDE are identified and detected by screening and target analyses. According to the most cited literature references, p,p'-DDT and metabolites generally show decreasing trend *(Vojinović Miloradov et al. 2015).* Estrogenic pesticides, such as DDT, generate negative reproductive effects. An "in culture" bioassay was used to assess the estrogenic-effects of several pesticides. Among the organochlorine pesticides tested, toxaphene, dieldrin, and endosulfan had estrogenic properties comparable to those of DDT and chlordecone; the latter are known to be estrogenic in rodent models. It has also been revealed that estrogenic chemicals may act cumulatively; when mixed together they induce estrogenic responses at concentrations lower than those required when each compound is administered alone *(Soto et al. 1994).*

Solubility is measure of how easily can substances dissolve in a solvent. Unless stated otherwise, the unit for solubility in water is given in ppm (mg/L). When the solubility is too

low, the units are given in ppb (µg/L). Measurements of solubility are influenced by temperature, pH, polarity of the substance, hydrogen bonding, molecular size and the method used. The significance in environment fate of solubility of pesticides is that, a pesticide which is very soluble in water will tend not to accumulate in soil or biota, due to its strong polar nature. This suggests that it will degrade via hydrolysis which is a favoured reaction in water. Degradation of pesticides is the breakdown or chemical transformation of pesticide molecules into other forms that are not necessarily simpler and less toxic compared to the parent molecule. In some cases the degradation products are also toxic and have some pesticide effects as well. A good example is the degradation of DDT to DDD. The rate of pesticides degradation is usually measured in terms of half-life ($t_{1/2}$), which is the time required for the depletion of half (or 50 %) of the amount of pesticide present initially, these characteristics can be seen in section 5.7, Table 5.10. The pesticides degradation processes can be categorized into three major groups:

- Physical – thermal or mechanical processes.
- Chemical – takes place in water or atmosphere following the reactions of oxidation, reduction, hydrolysis and photolysis.
- Biological – takes place in soil and in living organisms following the reactions of oxidation, reduction, hydrolysis and conjugation.

The type of the reaction in which a pesticide undergoes is largely determined by the pesticide inherent physiochemical properties and the environmental compartment (water, soil, air, biota) in which it is hosted (*Zacharia, 2011*). Furthermore, for ratio of DDT and its metabolites can show if the detected pollution is historic or recent, taking into account the detected concentration, ratio of DDT/DDE or DDT/DDD and the value of half-life in environment compartment *(Vojinović Miloradov et al. 2015).*

2.4.2.3. Plasticizers

Phthalates are a group of substances widely used as plastic additives in various industrial and consumer products.

Despite the fact that some phthalates are banned, many other phthalates are still used in cosmetics, paints, food packaging, cleaning agents and medical devices such as tablet coatings, blood bags and tubes. Phthalates are not accumulating in the body, but are metabolized and mainly excreted in the urine within hours or few days. However, their ubiquitous use leads to inevitable constant exposure *(Boas et al. 2012).*

In the Figure 2.14 Structural chemical formulas of dominant phthalate molecules are shown.

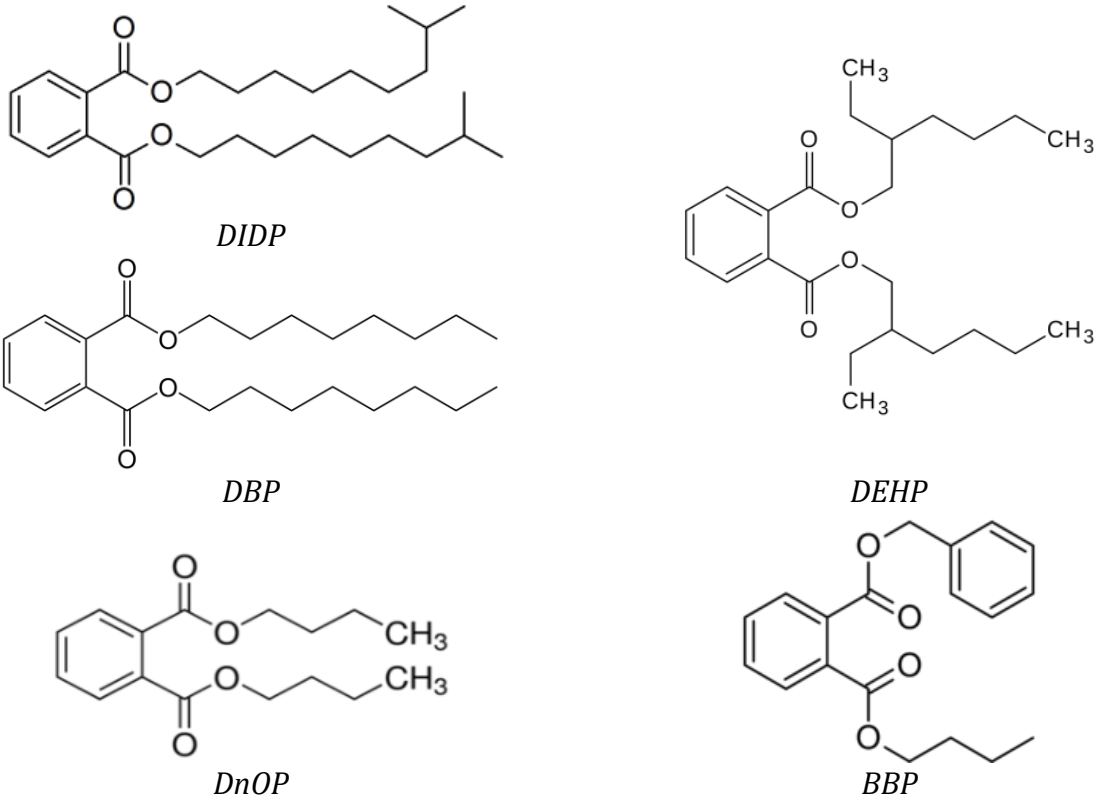


Figure 2.13 Structural chemical formulas of dominant phthalate molecules (Chen et al. 2014)

A considerable relationship between the metabolite of DBP and free and total tiroxin was found during the study involving pregnant women *(Huang et al., 2007),* as well as, DEHP-exposure and free thyroxine serum (T4) and total triiodothyronine have been reported in adult men, and serum levels of triiodothyronine (T3) and height in children *(Meeker et al., 2007, Boas et al., 2010).* Experimental studies suggest adverse mechanisms of phthalate effects on the thyroid homeostasis. Diisodecyl phthalate (DIDP), butyl-benzyl phthalate (BBP) and di-n-octyl phthalate (DnOP) have been shown to interfere with the activity of the natural immune system *(Breous et al., 2005),* dibutyl phthalate (DBP) and BBP inhibit T3 uptake in cells *(Shimada and Yamauchi, 2004).* DEHP and DBP have shown antiandrogenic activity. DEHP is the most widely used and its concentration in influent and effluent treatment plant is the highest. The rate of removal of phthalates is greater than 90 % for most compounds studied *(Deblondea et al. 2011).* Among phthalates only six analytes are regularly monitored. Phthalates in general show high potential for estrogen-like behavior, which is why in recent years these substances are of the most interest and human exposure to them *(Nollet 2005).*

2.4.3. Sorption of selected EDCs and illicit drugs in conventional wastewater treatment

During the conventional treatment processes it is highly probable that most of emerging substances, especially the once researched in the thesis will sorb to sludge or pass through the system into the recipient.

DDT and related compounds are very persistent in the environment and as much as 50 % can remain in the soil up to 30 years after application. This persistence, combined with a high partition coefficient provides the necessary conditions for DDT to bio-concentrate in organisms.

Bio-concentration factors of 154 100 and 51 335 have been recorded for fathead minnows and rainbow trout, respectively. It has been suggested that higher accumulations of DDT at higher trophic levels in aquatic systems results from a tendency for organisms to accumulate more DDT directly from the water, rather than by biomagnification.

The chemical properties of DDT (low water solubility, high stability and semi-volatility) favour its long range transport and DDT and its metabolites have been detected in Arctic air, water and organisms. In Figure 2.15 solubility of DDT and metabolites is shown.

DDT has also been detected in virtually all organochlorine monitoring programs and is generally believed to be ubiquitous throughout the global environment.

DDT degrades to DDE and DDD and the ratio of DDE/DDT or DDD/DDT could be used as a rough estimate of the period of its application: in areas where DDT exposure has been recent, the DDE/DDT ratio is low, while in areas where substantial time since exposure has passed, the DDE/DDT value is higher *(Ying et al. 2003).*

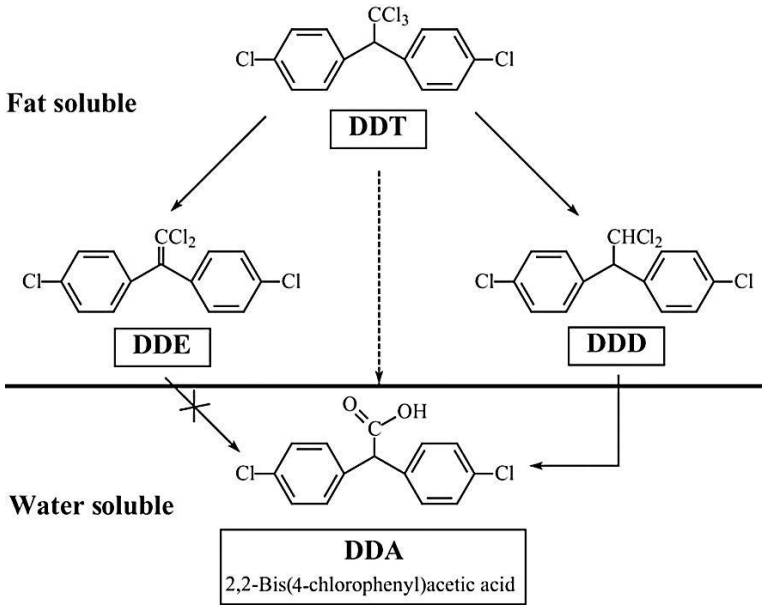


Figure 2.14 Solubility of DDT, DDD, DDE, DDA (Chen et al. 2009)

Hydrolysis is a pH dependent reaction in which pesticides react with water. Hydrolysis is one of the most common reactions that most pesticides undergo in the environment. Most organophosphates are particularly known to be highly responsive to hydrolysis reaction under alkaline condition.

The distribution of an organic solute between sorbent and solvent phases results from its relative sorption affinity for each phase, which in turn relates to the nature of forces which exist between molecules of the solute and those of the solvent and sorbent phases. The type of interaction depends on the nature of the sorbent as well as the physicochemical features of the sorbate (hydrophobic or polar at various degrees) *(Delle Site 2001).* Sorption of polar and ionizable compounds depends at various degrees on moisture content in sorbing system, the presence of exchangeable cations electrolyte concentration and pH.

Sorption of a chemical on a solid sorbent occurs when the free energy of the sorptive exchange is negative. The change in enthalpy represents the difference in binding energies between sorbent and the sorbate (solute) and between solvent and the solute *(Delle Site, 2001).*

Thus, sorption may occur as the result of two types of main forces: enthalpy-related and entropy-related forces *(Rounak 2011).* Hydrophobic bonding is an example of an entropy-driven process; associated with large entropy changes resulting from the removal of the sorbate from the solution. For polar chemicals, the enthalpy-related forces are greater, due to the additional contribution of electrostatic interactions. Generally sorption coefficients decrease with increasing temperature. However, some examples of increasing equilibrium sorption with increasing temperature and of no effect of temperature on sorption equilibrium were also found. Inverse relationship exists for organic compounds between sorption coefficients and solubility. Lower Kd values are found at higher temperatures for most compounds for which solubility increases with temperature, while increased sorption at higher temperatures can be expected for compounds for which solubility decreases with temperature *(Weiner 2012).*

In Table 2.5 are shown effects of pH of soil on herbicide activity and environmental risk.

Table 2.5 Effects of pH of soil on Herbicide Activity and Environmental Risk

Herbicide	pH	Influence	Result	Risk
Atrazine and Simazine	> 7	Slow degradation	Increased residue	Great
	<5.5	Increased retention, intensified hydrolysis	Reduced weed control	Decreased

Detailed properties concerning sorption and solubility of selected substances are shown in Table 5.10.

For different substances bioaccumulation in the organisms, especially aqua-organisms, depends mainly on octanol-water partition coefficient K_{ow}, where if log K_{ow} has equal or lower value of 3 the bioaccumulation study is not necessary *(REACH 2014).* Octanol-*water* partition coefficients for 611 organic compounds can be browsed in the NIST document "Octanol-Water Partition Coefficients of Simple Organic Compounds" by James Sangster following the link https://www.nist.gov/sites/default/files/documents/srd/jpcrd367.pdf.

2.5. Ecotoxicity and risk assessment of analytes

Toxicology studies effects of chemicals on living organisms, especially relationship between dose and effect, as all chemicals can be toxic under the right conditions and high enough dose. Toxicity of a chemical can be acute (sudden and severe exposure with rapid onset symptoms) and chronic (continuous, long-term exposure, relatively low dose with severe effects on health). Assessing ecological risk from pollutants requires preliminary research into the emission and transport of contaminating substances in the environment and the exposure to which live organisms may be subjected. The results are used to calculate the likelihood of ecosystems being affected by the use of potentially toxic substances. Figure 2.16 shows typical logarithmic dose-response curve with NOEL value.

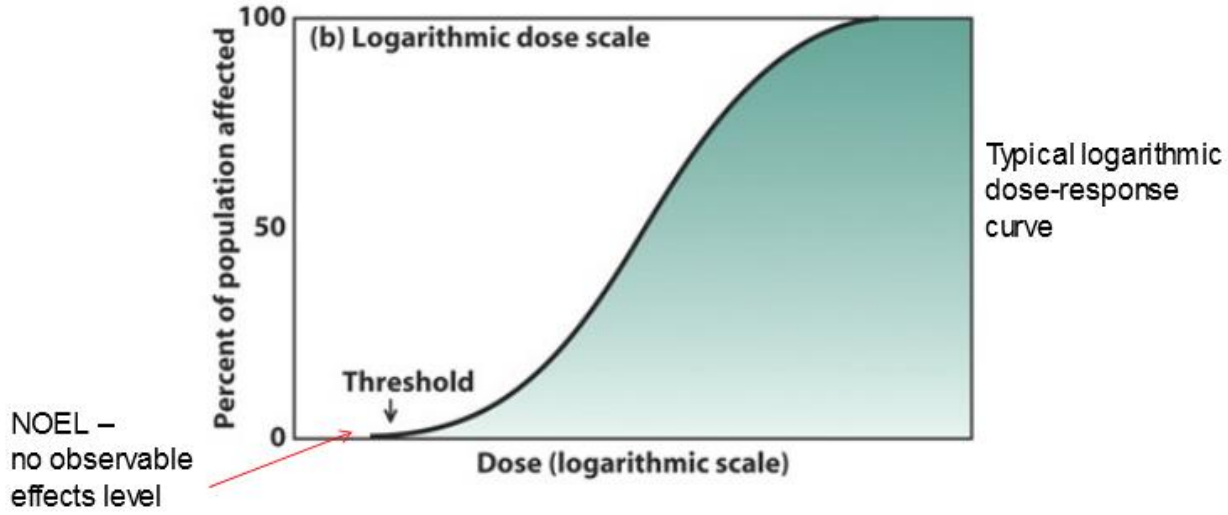


Figure 2.15 Typical logarithmic dose-response curve with NOEL value

Ecotoxicology and environmental risk assessment (ERA) can be applied in various fields:

- Recording and evaluating chemical substances - every substance must undergo ecotoxicological assessment, for which the criteria and guidelines are provided by regulations (e.g. REACH).

- Environmental monitoring and ecological water status assessment
- Calculating ecological risk and development of predictive models

The analysis examines two major parts of risk, exposure and effects, and the possible connection and interaction. The process of examining effects is ecological effects characterization, whereas the process of examining exposure is called exposure characterization. For the evaluation of risk the main relationship to examine is stressor-response relationship *(USEPA, 2016).*

Toxicity units (TU) for each assayed substance can be calculated dividing the measured environmental concentrations (MEC) by the lowest acute toxicity value (for algae, invertebrates and fish) *(Fernandez et al. 2010).* The other method for risk assessment is calculation of risk quotient (RQ) via MEC and PEC, measured environmental concentration and predicted environmental concentration, respectively, and PNEC values (predicted no-effect concentration) for every substance selected *(Milanović et al. 2016).* PNEC values for selected illicit drugs according to the literature are PNECs derived by the Ecological Structure Activity Relationships (ECOSAR) modeling were given for amphetamine (2.3 μg/L), MDMA (2.70 μg/L), cocaine and BE (both 4.90 μg/L) *(van der Aa et al. 2013).* The PNEC value for atrazine is 0.6 μg/L *(Guérita et al 2008)*, lindane 2 ng/L, endosulfane α 0,5 ng/L, PeCB 0,001 ng/L, HCB 13 ng/L, heptachlor 0,03 ng/L, chlorpyriphos 33 ng/L, DBP 10 μg/L, DEPH 1,3 μg/L, estradiole 0,1 ng/L, estrone 2 ng/L, estrone 6 ng/L and estriol 60 ng/L. PNEC values for DDT and its metabolites DDE and DDD, are 0.18, 0.6 and 0.64 ng/L, respectively[2].

According to literature the RQ is than calculated via equation 14:

$$RQ = \frac{MEC\ (PEC)}{PNEC} \qquad \text{e.14}$$

Where:

RQ – risk quotient

MEC – measured environmental concentration

PEC – predicted environmental concentration

PNEC – predicted no-effect concentration

[2] References for PNEC values - http://www.ineris.fr/substances/fr/substance/, https://circabc.europa.eu/, https://circabc.europa.eu/webdav/CircaBC/env/wfd/Library/framework_directive/thematic_documents/priority_substances/supporting_substances/monitoring-based/07_Annex%20VII_PNEC_Candidate-substances.pdf, Hester and Harrison2015

If the RQ is calculated via MEC values it represents real risk ratio, and if the PEC values are used it signifies estimated risk ratio *(Bouissou-Schurtz et al. 2014).*

Industry is continually manufacturing new chemicals, which requires evaluation of the potential danger for human health and risk to the environment. Risk assessment is nowadays considered essential for making decisions on a scientifically sound basis. To perform risk assessment it is important to know hazard evaluation information (acute, chronic), quantitative dose-response information and an estimation of the potential human exposure.

3. Hypothesis, objectives and aims of research

As most frequent and predominant source of surface water pollution mixed urban wastewater (MUWW) samples were selected for the research to acquire relevant and up-to-date data of effluent and input into the natural recipient, as well as to gain a new perspective in the light of emerging xenobiotics. MUWW represents the problem of developing countries as it is a mixture of all urban effluents – industrial, domestic, communal, road wash-out.

The chemical cocktails can be extremely difficult for analysis and treatment. In Serbia the Law predicts the treatment of industrial wastewater before it is released to urban sewerage but until today this regulations are not entirely respected, only about 5 % of industrial wastewater is treated before disposed into the natural recipient or municipal sewerage system.

The MUWW is an important source of organic pollution in raw water and natural water bodies. Sources of emerging xenobiotics in urban areas are mostly defined trough sewerage effluent, industrial and municipal effluent.

Priority pollutants from wastewater are an provocative research topic because of their illicit nature and characteristics, on the other hand emerging substances are interesting for their consumption, adverse and possible chronically effects that are not sufficiently investigated.

It is significant to investigate possible entry sources and behaviour of detected emerging and priority contaminants. Hence, there is a need to investigate their spatial distribution in samples and the possible input sources, for the purpose of detection of possible reaction, metabolisation and chemical cocktail formation.

It is important to investigate the possibilities of treatment and deposition of illicit drugs and EDCs to sludge during the conventional wastewater treatment. As the research was conducted on municipal wastewater it was realized that priority and emerging substances are the substances that need to be more closely monitored in Danube River Basin (DRB), where the first set of samples was obtained. Wastewater is one of the main contributors to depleting status of water quality in Republic of Serbia, as well as Vojvodina region.

The analyses of surface water before and after the municipal wastewater discharge were also performed during the research period.

The analyses can directly demonstrate what type of impact municipal wastewater from mixed sewerage system can have on the aquatic environment. During the research screening method for surface and wastewater samples preparation and analysis was

revised and adapted in the Laboratory of Faculty for Chemical and Food Technology, STU in Bratislava.

Detection and identification of organic pollutants via target analyses was performed in the Laboratory AQ-BIOS, Bratislava, Slovakia.

The basic physical-chemical analysis for mixed WW for urban area of Novi Sad, were performed in Accredited Laboratory for wastewater, soil and landfill gas of Department of environmental engineering and occupational safety. The selected groups of emerging xenobiotics are selected in accordance with results obtained by screening analysis performed during stay in the Laboratory of Analytical Chemistry Department of Faculty of Chemical and Food Technology of STU, Bratislava, Slovak Republic to proceed to the target analyses.

Municipal wastewater treatment plant (WWTP) effluents are considered to be a key source of pollution, concerning the occurrence of endocrine disrupting compounds (EDCs) in the environment.

The efficiency of the removal of emerging xenobiotics - pharmaceuticals and personal care products (PPCPs), EDCs, and illicit drugs was found to be strongly dependent on the technology implemented in the WWTP and precision and detail of collected data on composition and concentration levels of wastewater.

Although removal potential of many EDCs by conventional WWTPs is documented, literature data are not easily comparable. In order to reach acceptable and required concentrations, a further treatment is required. The recommended results can be achieved by advanced processes of adsorption or chemical oxidation; yet, techno-economic applicability is still to be fully investigated *(Bertanza et al. 2010).*

There are suspected substances that can be characterized as hormones, phthalates, and pesticides in the chromatograms obtained during *screening* analysis, but without high level of certainty due to the low doses and non-monotonic response, which is the reason for selection of emerging xenobiotics as target for further research and analyses.

The objective of the study are the low doses and low removal efficiency in different wastewater treatment methods and technologies, or simple sorption to sludge. Pesticides and estrogens in low doses show very low removal efficiency in commercial wastewater treatment, usually only sorption onto sludge.

In this thesis the main goal of research will be MUWW and its impact onto the natural water bodies, essentially surface water, as a direct recipient of effluents. From the main goal the hypothesis emerges as premision of detection and quantification of emerging xenobiotics and illicit drugs in MUWW of Novi Sad and surface water of River Danube.

The specific aims of the research are adaptation of sampling methodology, preparation and analysis method for screening analysis for highly polluted MUWW and surface river water samples for specific location, detection and quantification of target xenobiotics, investigation of treatment options and elimination possibilities for selected substances, and removal and sorption potential according to polarity and K_{ow} characteristics, calculation of environmental eco-toxicological risks of selected analytes.

The secondary goal of the research is a review of possible and optimal advanced or tertiary wastewater treatment process if needed for selected research location and specific wastewater sample. And as a significant specific benefit the research should have the impact onto the design of a monitoring plan and new guidelines for emerging xenobiotics.

3.1. Hypotheses of thesis

The detail literature research and the experimental conceptualization performed during the development of the study lead to the postulation of several essential hypotheses that were investigated during the PhD research. The hypotheses were emerging during the evolution of research, opening new visions and presumptions in the scope of the doctoral study.

- The first hypothesis of the thesis is the premise of successful identification and detection of emerging substances, priority and hazardous priority pollutants, particularly endocrine disruptive substances (EDCs) in mixed urban wastewater and surface water in Novi Sad.
- The second hypothesis is the requirement for the development of the newly adopted methodology (modules algorithm) for the detection of organic pollutants in the environmental water samples.
  - Sub-hypothesis emerged during the research is the need for the adaptation of the screening analysis, as a significant phase of research, for the selected type of water samples and specific research area.
- The third hypothesis was established as development of the specific sampling strategy which will enable the advanced stream of conclusions on the impact of urban area wastewater onto the primary aquatic recipient.
- The fourth hypothesis emerged, according to the physical-chemical characteristics of the detected analytes as the questionable behaviour in the aquatic environment and transport to other media, in the context of wastewater treatment and accumulation processes.

- The fifth hypothesis is the toxicological and eco-toxicological risks assessment according to the EU recommendations and measured environmental concentrations of the detected emerging xenobiotics and their interaction, primary in the aquatic environment.
- The last hypothesis is the proposition that the quality and quantity of mixed urban wastewater of Novi Sad evaluated throughout the PhD research would require innovative advanced techniques and technologies for wastewater treatment.

4. Concept, framework and methodology of research

Experimental part of the thesis is consisted of five stages:

1. Establishment and analyses of the research area and research
2. Development of sampling methodology for screening and target analyses of specific group of pollutants – emerging xenobiotics.
    a. sampling protocols (grab and composite samples)
3. Analyses
    a. Analyses of basic physicochemical parameters of wastewater from discharge point GC2 in Novi Sad during the period of December 2012 to April of 2013
        i. pH, BOD_5, COD, dissolved oxygen, PPC.
    b. Adaption of screening analyses of surface and wastewater samples which would be the first step towards selection of the target groups of emerging xenobiotics for further steps of examination:
        i. sample preparation (extraction and evaporation)
        ii. sample analysis (GC-MS program).
        iii. detected emerging substances during screening and target analysis – evaluation of obtained data.
4. Calculation of organic load, RQ for Novi Sad during the selected research period, and specific load of detected emerging xenobiotics.
5. Statistical evaluation of obtained data about selected analytes and possibilities of removal from wastewater matrix, and derivation of conclusions.

The screening analysis methodology for surface water samples was modified and adapted to fit the specific purpose and type of analysed samples. During the screening analysis adaptation of sample preparation and analytical method was performed using the traditional "one variable at a time" (OVAT) methodology to increase organic compounds recoveries during chromatography in screening procedure *(Engineering Statistics, NIST/SEMATECH, 2005).* Emerging xenobiotics, especially EDCs had a high certainty of detection by screening analyses.

The adaptation of screening analyses of surface and wastewater samples which is the first step towards selection of the target groups of emerging substances was conducted in Laboratory of Analytical Chemistry Department, Faculty for Chemical and Food Technology, STU in Bratislava.

Target analyses for detection of pesticides and hormones were conducted in Laboratory AQ-BIOS, Bratislava, Slovakia, and detection of estrogens in Laboratory of Analytical department, Faculty of Technology and Metallurgy.

The detection and quantification of selected illicit drugs in wastewater samples, was done in Laboratories of Swiss Federal Institute of Aquatic Science and Technology and Toxicological Centre, University of Antwerp.

The possibilities of emerging contaminants deposition to sludge are great, especially in controlled operating conditions, corresponding to the wastewater treatment plant. Furthermore, emerging xenobiotics are not usually removed during treatment processes in conventional WWTP, but are accumulated in aquatic organism and/or returned to the food chain trough food or water *(Bolong et al., 2009; Jones et al. 2005; Jones et al., 2007; Weiss and Reemtsma, 2005).*

4.1. Research area

The city of Novi Sad hugs the S-shaped meander of the river Danube. The main part of the city lies on the left bank of the Danube, in Bačka region, while smaller parts Petrovaradin and Sremska Kamenica lie on the right bank, in Srem (Syrmia) region. A section of the Danube-Tisa-Danube Canal marks the northern edge of wider city centre, and merges with the Danube, introducing a vast industrial effluent into the river. The total land area of the city is 699 km^2, while the urban area is 129.7 km^2.

The city of Novi Sad is a typical Central European town with the population of 250 439 inhabitants *(Census of population, 2011),* with a density of 487 inh. per km^2. The planned Central WWTP is foreseen to be designed for capacity of 450 000 p.e. taking into account the most of the Municipality (comprised of 3 urban settlements and 13 rural settlements, while some of the furthest settlements (Begeč, Stepanovićevo, Kovilj, Sremska Kamenica and Sremski Karlovci) may be predicted for the independent small capacity WWTP.

As it is important to obtain enough data and information for development of environmental status of surface water sources it is important to collect previous information on overall quality of media. The year 2011 was selected as a staring year for simultaneous begging of data collection for two segments of natural water body that is important for overall environmental status –surface water, mixed urban wastewater.

The sampling strategy is equally important segment of the research the analysis itself. The correct and precise organisation of sampling and sample preparation is securing the precise data that can be evaluated and then used for making of crucial decisions about the potential treatment or differentiation treatment process in order to obtain the highest possible efficiency and environmental safety of the treatment system. Sampling for the research was conducted during the period of 2011 to 2016.

Water samples were collected from Danube on its flow through city of Novi Sad, municipal wastewater of Novi Sad collected on outlets from the municipal system, and from the wastewater collectors. Samples of surface and wastewater were used for screening analyses during the sampling period of 2012 and 2013.

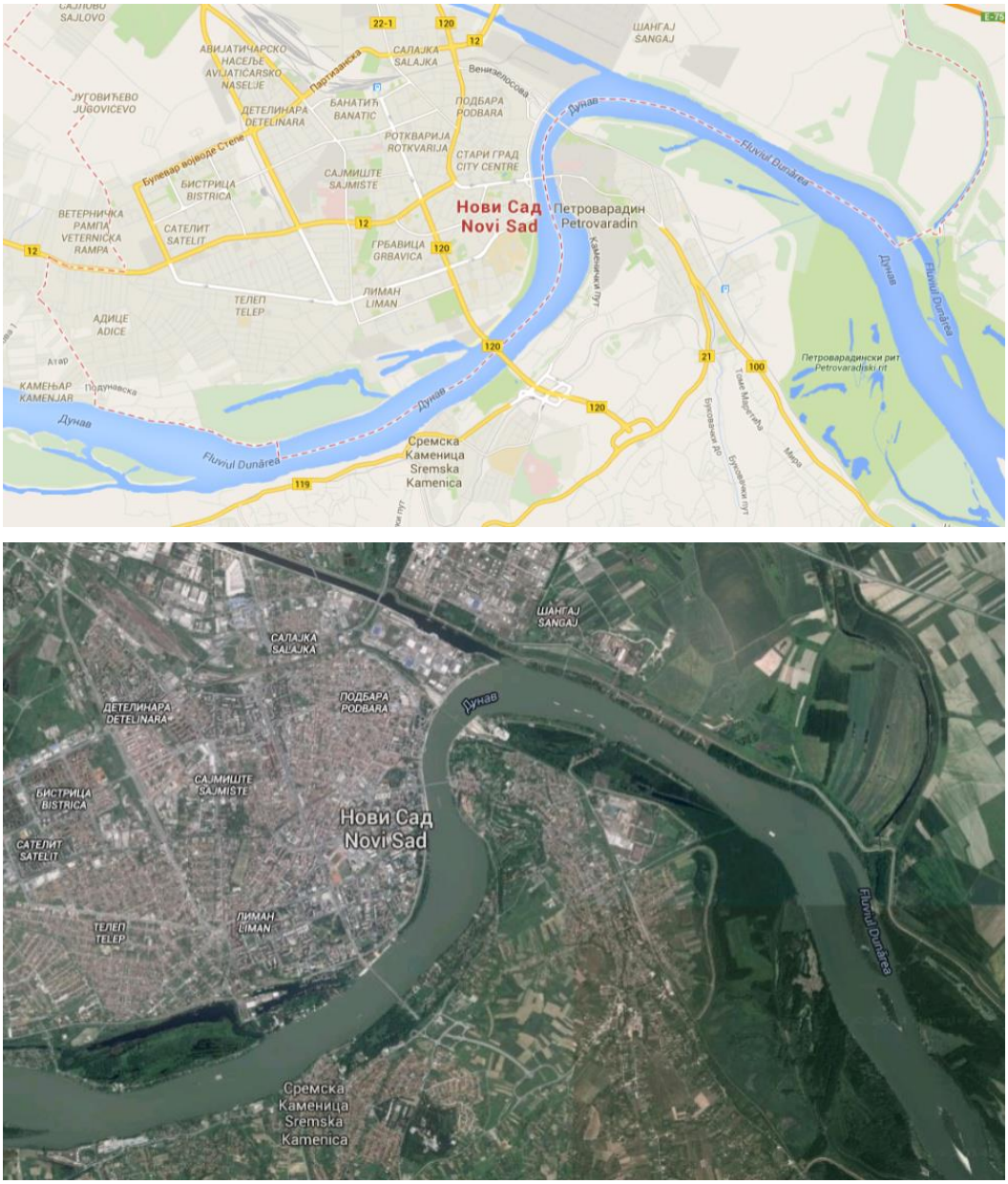


Figure 4.1 Research area

For this research area, shown in Figure 4.1, technical and planning documentation is in the procedure of development. The Plans of General and Detailed Regulation are developed, and the selection for the spatial positioning of WWTP is selected. This is the reason to obtain up-to-date and location specific detailed data of WW quality, for the purpose of adaptation and selection of WWTPs that are location specific.

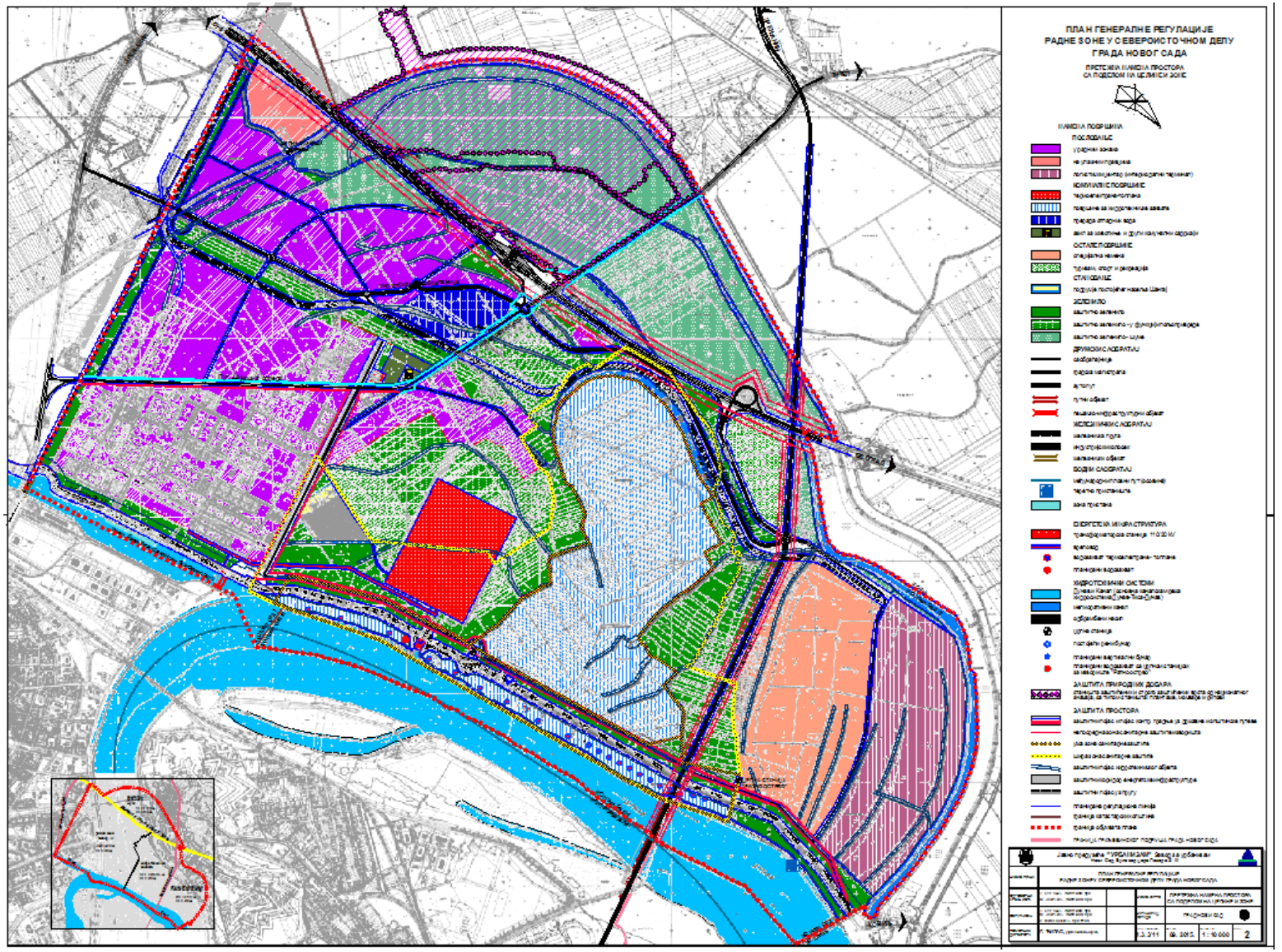


Figure 4.2 Plan of General Regulation for the Work Zone North-East (http://www.nsurbanizam.rs/pgr?page=1)

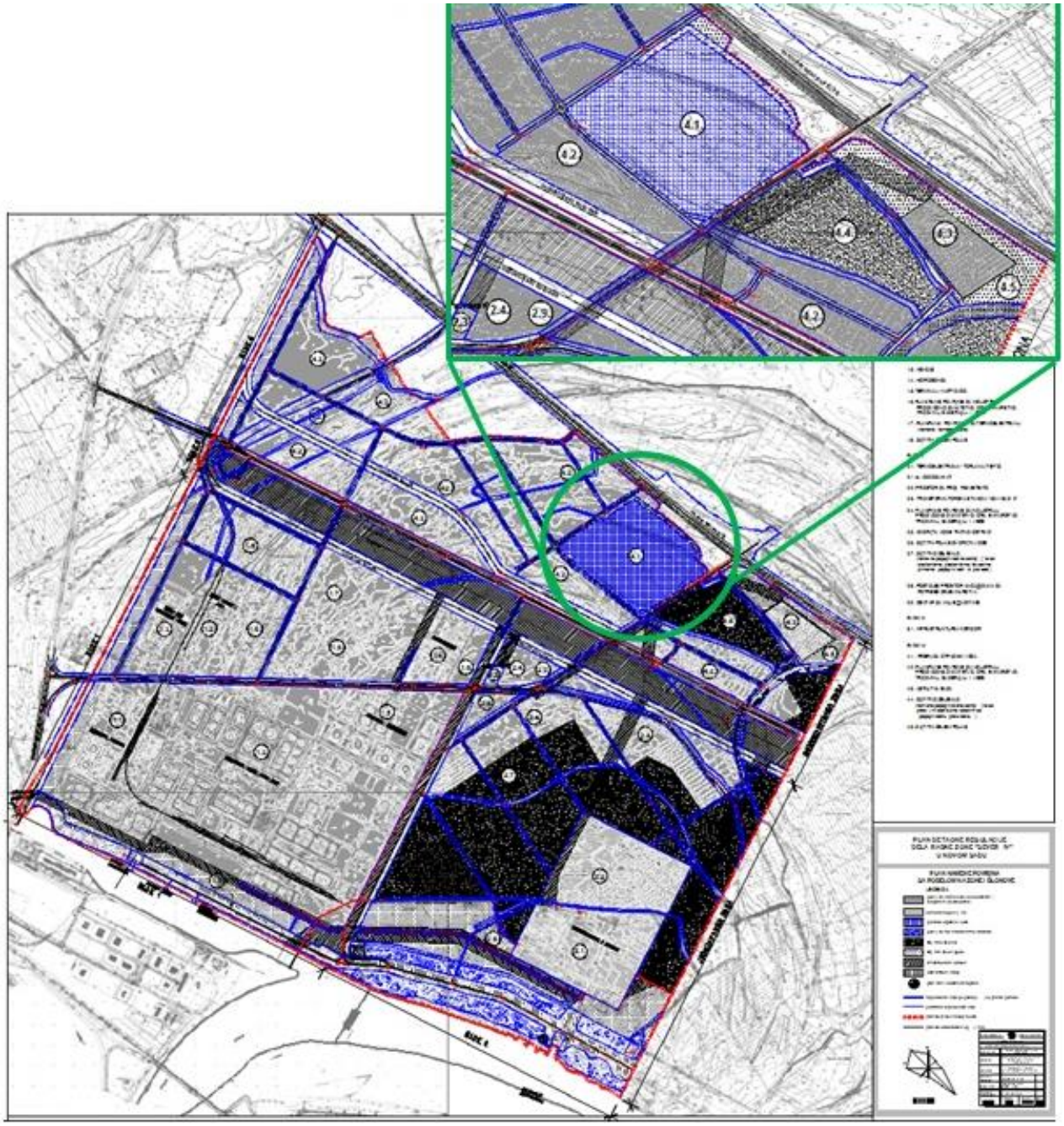


Figure 4.3 Plan of Detail Regulation for the Work Zone North IV and detail of space regulated for WWT (http://www.nsurbanizam.rs/pdr?page=4)

The Figures 4.2 and 4.3 are showing the spatial plans for the selected placement of the future WWTP, and are publically available on the web site of PUC Urbanizam, Institute for Urbanism of Novi Sad.

4.2. Sampling methodology

4.2.1. Sampling strategy

The year of 2011 was a starting year for sampling of two segments of natural water body that is important for overall environmental status – surface water and mixed urban wastewater. To obtain enough data and information for development of environmental status of water sources it is significant to collect previous information on overall quality of media.

The precise organisation of sampling and sample preparation is securing the precise data that can be evaluated and then used for making of crucial decisions about the potential treatment or differentiation treatment process in order to obtain the highest possible efficiency and environmental safety of the treatment system.

Sampling for the research was performed during the period of 2011 to 2016. Water samples were collected from Danube River surface water on the banks of Novi Sad, municipal wastewater of Novi Sad collected from the wastewater collectors.

Samples of surface and wastewater during the sampling period of 2011 and 2013 were used for screening analyses. Samples obtained during the 2013, 2014 and 2016 were obtained for target analysis of selected analytes (pesticides, phthalates, estrogens and illicit drugs *(NIVA Collaboration, 2013, 2016).*

4.2.2. Sampling locations

Sampling locations were selected to obtain the most reliable and representative information about the previous contamination and the impact of mixed urban wastewater onto the recipient, in this case of Danube River.

In the case of Novi Sad there is a specific situation where some of the water wells for acquiring the raw water for production of drinking water are downstream of urban wastewater discharge, which is shown in the Figure 4.4.

The position of water wells and discharge points emphasizes the need for closer, continuous and permanent monitoring of wastewater quality until the realisation of designed wastewater treatment plant is possible.

The location of water well and wastewater discharges are shown in Figure 4.4.

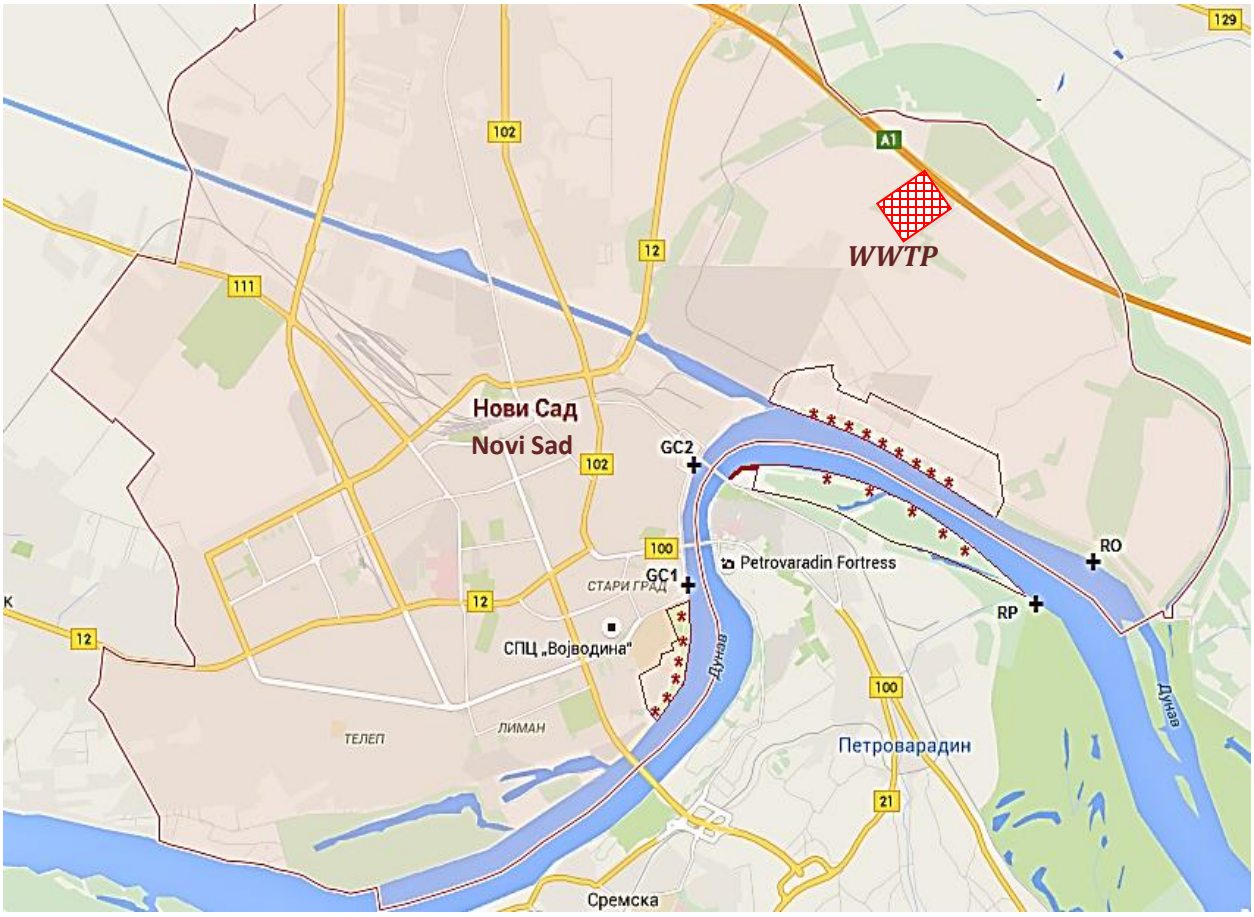


Figure 4.4 Locations of wastewater discharge (GC1, GC2, RP and RO), underground water wells for acquisition of raw water for drinking water production (*) and selected location for WWTP (—)

In order to create relevant monitoring network of the surface Danube water in the vicinity of Novi Sad, selection of sampling points and defining of monitoring dynamic have been chosen. Selected sampling points were wastewater collectors of mixed urban wastewater effluent discharges GC1, GC2, War Island (RO) and Roko's Creak (RP), while samples of surface water from Danube River were taken 100 meters downstream of the each discharge: 45°15'5,42"N, 19°51'22,95"E (100 m downstream of the discharge GC1), 45°15'44,4"N, 19°51'28,46"E (100 m downstream of the discharge GC2), 45°15'11,84"N, 19°54'40,18"E (100 m downstream of the discharge War Island) and 45°15'2,2"N, 19°54'9,92"E (100 m downstream of the discharge Roko's Creak) (Figure 4.4). For the purpose of comparison and comprehensive conclusions the samples were taken from location upstream of Novi Sad discharges near Alas Island.

In the Table 4.1 GPS coordinates of sampling locations are shown, as well as description of sample types and sampling location names.

Monitoring was performed downstream the surface water station at 1254.98 km distance from the Danube mouth (staff gauge from 1919). In Table 4.1 are shown sampling locations.

Table 4.1 Sampling locations for water samples

No.	Location	Label	Northern latitude	Eastern longitude	Type of sample
1.	*Collector Cepelin*	GC1´	45°15'3,704"N	19°51'18,329"E	WW*
2.	*Cepelin*	GC1˝	45°15'5.40"N	19°51'22.53"E	SW*
3.	*Collector Belgrade Quay*	GC2´	45°15'44.19"N	19°51'22.16"E	WW
4.	*Belgrade Quay*	GC2˝	45°15'43.03"N	19°51'27.09"E	SW
5.	*Discharge War Island*	RO´	45°15'22.95"N	19°54'39.94"E	WW
6.	*War Island*	RO˝	45°15'13.39"N	19°54'38.48"E	SW
7.	*Collector Roko's creak*	RP´	45°14'56.65"N	19°53'43.673"E	WW
8.	*Roko's creak*	RP˝	45°15'0.47"N	19°54'11.33"E	SW
9.	*Alas Island*	RI	45°13'54.25"N	19°50'44.62"E	SW

** WW – wastewater; SW – surface water of river Danube*

4.2.3. Sample categories

Two types of sample were taken and analysed during the research. As it is shown in previous segment of the thesis two types of water selected for sampling are mixed urban wastewater and surface water of the recipient, on several locations. A grab or catch sample, is represented as a sample taken from selected location at a specific point in time. This is the most common type of sample and sampling technique. Essentially, a grab sample can be observed as a snapshot of the water characteristics at a specific point and time, so it may not be completely representative of the entire flow *(SMWWE, 1999).* But grab samples are acceptable for gathering an insight to scope of pollutants in specific selected aquatic environment, as a necessary basis for further research, and are thus to be preferred for some tests, especially screening analyses. Specifically, pH, dissolved oxygen, and total residual chlorine can change very rapidly in water once the sample is removed from the flow, so grab samples are preferred for these tests. Grab samples must be collected carefully to make them as representative as possible of the water as a whole.

The grab sample was used for laboratory analyses of physicochemical parameter and on-line monitoring system was set on the location of GC2 to compare the real time fluctuations. The samples for laboratory analyses were taken in 6, 14 and 22 h 3 times a month during the research period from December of 2012 to April of 2013.

A composite sample is a sample consisted of several individual grab samples and mixed into one, each sample taken in proportion to the flow rate at that time. Composite sample gives a more representative sample over a longer period of time. The greatest strength of composite samples is the possibility to take into account changes in flow and other characteristics of the water over time. This is important for overall sense of the total effects that the influent will have on the wastewater treatment process and/or that the effluent will have on the receiving water. However, composite samples cannot be used for tests of water characteristics which change during storage (such as dissolved gases) or of water characteristics which change when samples are mixed together (such as pH) *(SMWWE, 1999).*

4.2.3.1. Surface water samples

Samples of surface water were collected in 2.5 L brown glass bottles. Prior to sampling, glass bottles were washed according to standard procedure and rinsed with ultrapure water and methanol, respectively, and then heated at 110 °C. Surface water samples were collected from a boat, on given locations as a grab sample 2 m under the surface of River. Samples were taken from 5 different locations as a grab sample for screening and target analyses *(SMWWE, 1999).*

4.2.3.2. Mixed urban wastewater

Mixed urban wastewater samples were collected as a grab sample and a composite sample, depending on the type of analysis that was conducted – screening or target analysis. Urban wastewater as grab sample was collected from 4 outlets from sewerage system of Novi Sad – GC1, GC2, RO and RP. Mixed urban wastewater samples were collected in 2.5 L brown glass bottles. Samples were collected directly from a collector, on given locations as a grab sample 2 m under the surface. For target analyses of mixed urban wastewater 24-h composite samples were collected, over a 7 day period of time for illicit drugs, as well as one sample per season over a 4 year period for pesticides and hormones. The locations GC1 and GC2 were selected as representative for target analysis of illicit drugs, as the 2 largest outlets of mixed urban wastewater into the recipient, Danube River in city of Novi Sad.

4.3. Analysis of surface and mixed urban wastewater samples

For the analyses of waste and surface water in the vicinity of Novi Sad integral approach was selected, to contribute to detailed results and information about the quality of water samples in question.

The organic content of wastewater was analysed through the basic physicochemical parameters, screening analyses and, in the end target analyses for specific substances, considered priority, hazardous priority and/or emerging.

4.3.1. Evaluation of basic physical-chemical water characteristics

Samples for detection of emerging pollutants have been conserved before preparation and analysis by freezing and preserved in frozen state. Before freezing, the analysis of the basic parameters of wastewater has been done in the accredited Laboratory for monitoring of landfills, wastewater and air, on the Department of Environmental engineering, Faculty of Technical Sciences, University of Novi Sad.

The basic parameters that have been performed are temperature, pH, conductivity, COD, BOD_5, N_{tot}, P_{tot}, dissolved oxygen, PPC, TSS, TDS. Standard methods for determination of selected parameters were obtained from Standard Methods for the Examination of Water and Wastewater.

4.3.2. Semi-quantitative screening analyses – adaptation

Screening analyses are carried out to identify and register the chemical components and properties of a certain water sample. Preparation method of samples for GC-MS analysis was adapted. Different methods were used and one was selected as the optimal. Methods utilised for treatment and preparation of sample before evaporation was different types of filtration, rotary evaporation and liquid liquid extraction, solid phase extraction and stir-bar.

Filtration – the filter paper 125 mm Ø, 100 g/m^2 and particle retention 2.5 μm was used during gravity filtration of sample. After gravity filtration, the vacuum and active carbon filtration were performed, respectively. The vacuum filtration was performed on Sartorius apparatus for vacuum filtration with filter paper of 42.5 mm Ø, 92 g/m^2 and particle retention 20 - 25 μm was used during preparation of sample.

Solid phase extraction (SPE) is a technique designed for fast and selective sample preparation and purification preceding the chromatographic analysis. By controlling the selectivity, SPE provides clean-up, recovery, and concentration of the sample, which is essential for accurate quantitative analysis *(Żwir-Ferenc and Biziuk 2006).*

Solid Phase Microextraction (SPME) is a solvent-free sample preparation technique, uses a fibre coated with a polymer and/or sorbent. The fibre coating extracts the substances from sample and the fibre is then inserted into the chromatograph for desorption and analysis. SPME has many applications including analysis of flavours and fragrances, forensics and toxicology (*Vas and Vekey 2004).*

Stir bar sorptive extraction (SBSE) is an extraction technique for extraction of volatile and semi-volatile organic compounds from aqueous and gaseous media. After exposure to a sample, the stir bar, which is covered in a layer of a polydimethylsiloxane is subsequently removed and the sorbed compounds are then either thermally desorbed, and analysed by GC-MS or desorbed by means of a liquid, for improved selectivity or for interfacing to an LC system. The technique has been applied successfully to trace analysis in environmental, biomedical and food applications *(Soini et al. 2005).*

Liquid-liquid extraction - A 500 mL aliquot of water sample was spiked with an internal standard (benzophenone) and extracted with three 30 mL portions of solvent for 30 minutes using automatic shaker device at 700 rpm. Liquid-liquid extraction method is used for preparation of samples for chromatographic techniques. It is used for extraction of organic compounds from the sample matrix, so it can be further prepared for chromatography, depending on a technique gas or liquid. Liquid-liquid extraction is used for the extraction of organic compounds from aqueous solutions by using solvents which are water immiscible. The process is carried out in a funnel for extraction. Shaking the solution with immiscible solvent, allows the formation of a large contact area between the two liquid phases which increases the performance of extraction. In the case that the process of stirring doesn't result with the formation of emulsions or differentiation does not occur, insulation should be performed. Some organic substances have a relatively high solubility in water, and must be isolated from the aqueous solution by the addition of inorganic salts, which solubility in water is greater. For isolation, the most commonly used is sodium chloride (NaCl). During extraction of organic compounds from the aqueous solution, a certain amount of water is always dissolved in an organic solvent, and part of it is emulsified. Removing water from the organic part of the solution is carried out by addition of a drying agent, and water uptake. This agent must be soluble in the organic solvent and must not react with the dissolved substance. The parameters which characterize the drying agent are volume, efficiency and speed of drying.

LLE is based on the principles of differential solubility and partitioning equilibrium of analyte molecules between aqueous (the original sample) and the organic phases. Liquid-liquid extraction generally involves the extraction of a substance from one liquid phase to another liquid phase.

The extracted organic phase is evaporated to dryness and re-suspended with mobile phase or a similar solvent system and then injected onto the column (*Devanshu et al.* 2010). Most

organic compounds are more soluble in water-insoluble organic solvents such as dichloromethane. Therefore a compound in water will readily partition between solvent and water when the two liquids are mixed together in a separatory funnel.

Liquid-liquid extraction has a large linear sample capacities, as well as the possibility of direct injection of organic extract by gas chromatography are considered as major advantages of the sample treatment *(Pawliszyn 2002).* Technique itself can be directly subjected to the quantitative analytical measurement step such as gas chromatography. Liquid-liquid extraction method is used for extraction of organic compounds from the sample matrix, so it can be further prepared for chromatography, depending on a technique gas or liquid.

The solvents of different polarity were used to determine the optimal procedure for meat processing industry sample matrix. Solvents used were n-pentane (≥ 99.8 % purity), dichloromethane (≥ 99.5 % purity) and methanol (≥ 99.9 % purity), sigma Aldrich *(Sremački et al 2016a).*

The liquid-liquid extraction into dichloromethane favours the transport of hydrophobic organic compounds from water to an extraction solvent, while the extraction efficiency depends on the compound partitioning coefficient. In the Figure 4.5 step by step adaptation of sample preparation method for screening analysis is shown.

Figure 4.5 Step by step adaptation of sample preparation method for the screening analysis of surface and mixed urban wastewater

Kuderna Danish concentrator - Jacketed Kuderna-Danish concentrator tubes circulate hot water through the jacket to boil the solvent dry during extraction. The temperature of evaporation was set according to boiling point of solvent used during preparation. All collected extracts were evaporated to final volume 1 mL.

Kuderna Danish concentrator is used for enhancement of extract, precisely, evaporation of excess solvent *(Motteran et al. 2013).* Jacketed Kuderna-Danish concentrator tubes circulate hot water through the jacket to boil the solvent dry during extraction/concentration. The bottom portion of the tube is never heated, so samples won't boil dry. The concentration automatically stops when the solvent sinks below the jacket. Tubes have either serrated tabulations or screw thread connections. The temperature of evaporation was set according to boiling point of solvent used during preparation. After the extraction, the extract was evaporated using the Kuderna Danish apparatus to final volume of 1 mL. The results indicate that the properties of the raw wastewater do not comply with the regulatory discharge standards for the industrial wastewater into the sewerage network *(Wahaab and El-Awady 1999).* According to national legislation (*Water Law*, 2010and *By-law on Hazardous Substances in Water*, 1982 and By-law on emission limits for priority and hazard priority substances in surface water and deadlines, "Official Gazette, 35/11) the maximum allowable concentrations of hazardous substances in waters results obtained during sampling period exceed maximum allowable concentrations. This apparatus is used to concentrate analytes from volatile solvents. Apparatus consist of a 3-ball Snyder distilling column, flask and concentrator tube. The flask and receivers are held together by ST joints and the included poly joint clamp. Concentrator tube is graduated. Evaporation of extracts in KDC is widely used to concentrate samples, particularly pesticides and other pollutants prior to the instrumental analysis. Complete apparatus consists of a Snyder column 150 mm long (with Standard Taper 24/40 joints), a flask (with Standard Taper 24/40 tops and Standard Taper 19/22 lower joint) a graduated tube (with Standard Taper 19/22 joints) and two joint springs. The 250 mL and 500 mL completes include a 10mL tube. The 1000 mL complete includes a 25 mL tube.

Preparation involves filling the flask up to 60 % and no less than 40 % of flask capacity. Initially, for prevention of sample loss, the column should be pre-wet with about 1 mL of the solvent used in the process of extraction.

The apparatus should be placed over a vigorously boiling water bath; set to the temperature up to boiling temperature of used solvent, depending on the room temperature it can be increased up to 15 % of boiling temperature, just to start the evaporation process.

The water level should be maintained just the lower joint and the apparatus mounted so that the lower rounded surface of the flask is bathed in steam. The final sample remains in

the lower tube for further analysis. Lower tube is not graduated. Solvent Recovery Apparatus may be added to this unit.

4.3.2.1. Adaptation of GC-MS analysis method

The principal function of the gas chromatograph is to provide those conditions required by the stationary phase for achieving a separation without adversely affecting its performance in any way. Operation of the column with stationary phase requires a regulated flow of carrier gas; an inlet system to vaporize and mix the sample with the carrier gas; a thermostatted oven to optimize the temperature for the separation; an on-line detector to monitor the separation; and associated electronic components to control and monitor instrument conditions, and to record, manipulate and format the chromatographic data (*Poole 2003*).

A chromatogram provides information about the complexity (number of components), quantity (peak height or area) and identity (retention parameter) of the components in a mixture. A mass spectrometer produces a mass spectrum, a fingerprint of the molecule, which is a histogram of the relative abundance of the ions generated by ionization of the sample and their subsequent separation, based on their mass-to-charge ratio (m/z) (*Poole 2003*).

A 500 mL aliquot of water sample was spiked with an internal standard (benzophenone) to achieve the final concentration of 1 μg/L and extracted with three 30 ml portions of dichloromethane for 30 minutes. A 2 μL of the extract was injected into the gas chromatography system. The GC-MS screening analysis was performed using Agilent 6890 gas chromatograph coupled to the Agilent 5973 mass spectrometric detector.

The GC system was equipped with the programmed temperature vaporization (PTV) injector, known to be suitable for large volume samples, that was raised from 60 °C to 230 °C at the rate of 2 °C/min. The capillary GC analysis was done on a 30 m x 250 mm I.D., 0.25 mm df DB-FFAP column. The oven programme was formed accordingly to utilized solvent, from 40 °C to 60 °C, with hold time for 10 minutes for solvent delay. Helium was used as a carrier gas. The mass selective detector (MSD) was used in the scan mode (m/z 45-600) for all the samples. The identification of compounds was done using Wiley7n and NIST08 mass spectral libraries.

Several parameters during extraction and sample preparation were optimised using the "one variable at a time" (OVAT) methodology to increase organic compounds recoveries during chromatography in screening procedure.

GC-MS, Agilent 7890N GC, in scan mode was used for analysis of prepared extracts. One of the research goals was screening and identification of organic compounds and pollutants content in meat industry wastewater, with emphasis on hazardous and priority pollutants within the Water Frame Directive (*Directive 2000/60/EC*, 2000) and compounds that are

on NORMAN list of emerging substances (*NORMAN*, 2014) but it can only be developed after the adaptation of analysis method. Only peaks shown higher reproducibility were taken for data analysis *(Mudiam et al. 2013).*

Development and adaptation of this procedure was carried out by utilisation of solvents with different polar properties, to observe the qualitative and quantitative properties of extraction. Solvents used for this part of the research were pentane, dichloromethane (DCM) and methanol, due to their different polar properties, but similar boiling points or dipolar moment.

Methanol showed problems in preparation of sample, during evaporation as it has the highest boiling point (65 °C), the evaporation procedure with this solvent lasted for 4 – 6 h depending on a room temperature, and it was disregarded as an optimal solvent for selected method. The high boiling point is assuring significant loss of volatile organic compounds - VOCs.

Evaluation of obtained data was performed with Agilent ChemStation software. Databases used in this section of investigation were Wiley 7n mass spectrum libraries and NIST08 mass spectrum libraries.

Processing of chromatographs obtained during analyses was performed using Chemstation and Origin 8.6 was used to redraw chromatograms.

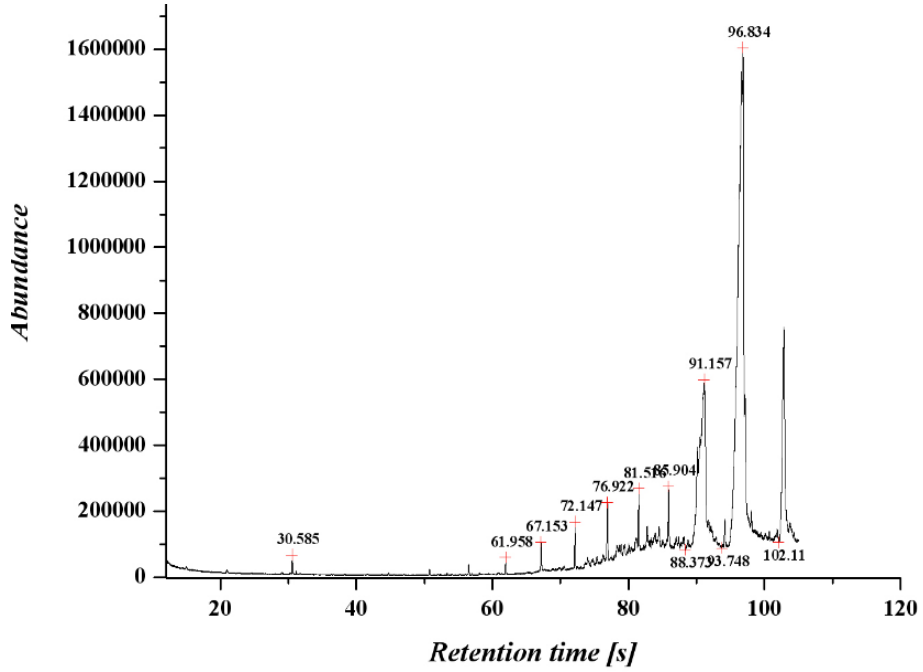


Figure 4.6 Chromatograms for samples prepared with extraction solvent DCM

Chromatograms showing extraction performed via different solvents is shown in Figures 4.6 and 4.7.

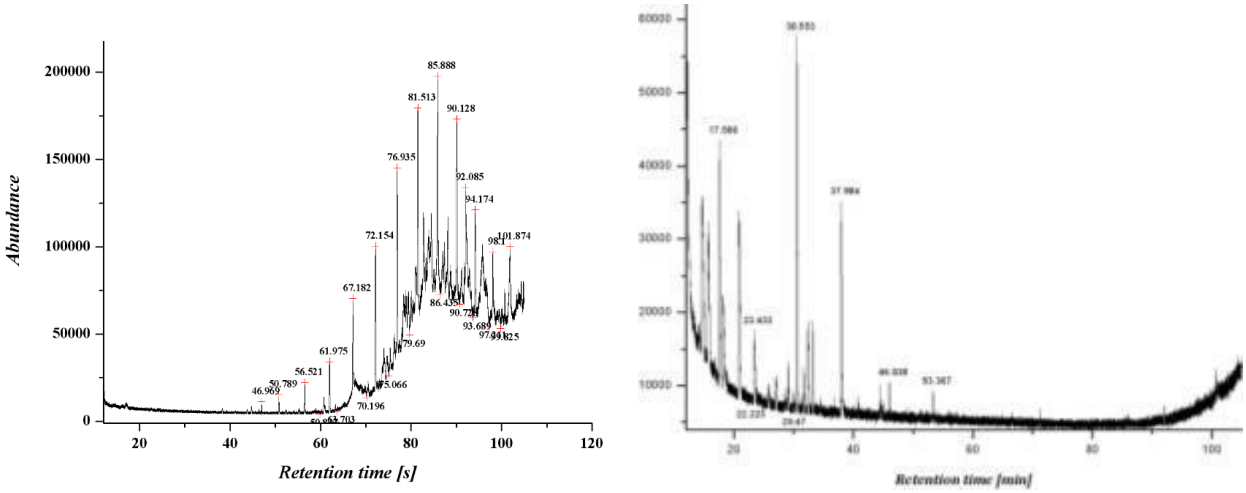


Figure 4.7 Chromatograms for samples prepared with extraction solvent n-pentane

4.3.3. Detection and identification of emerging substances – target analysis

The most frequently compounds detected were alkanes, fatty acids, industrial and lubricant oils and defoaming agents, phthalates, PAHs, terpenes, pesticides and hormones. Selected emerging substances for further target analysis were from class of pesticides and hormones (estrogens), as it was a high possibility of confirmation. From the screening analysis phenol and phenolic derivates are confirmed to be present in wide variety, as well as benzene and its derivates.

Within NATO Project during screening and target analysis using the Agilent GC-MS system detected 19 organic components above the LOD value. PAHs, phthalates, phenols and pesticides have been detected in nearly every sample during the project period. Formal monitored organic compounds, organochlorine pesticides are most frequently detected in the highest concentrations.

The analysis of sewage for urine biomarkers of illicit drugs is a promising and complementary approach for estimating the use of these substances in the general population. Illicit drugs were selected as target compounds of analysis as the most exciting substances to track and analyse. In mixed urban wastewater matrix the urinary biomarkers of cocaine, amphetamine, ecstasy, methamphetamine and cannabis were analysed using in-house optimized and validated analytical methods *(Data source SCORE 2017, Ort et al. 2014).*

This study shows that a standardized analysis for illicit drug urinary biomarkers in sewage can be applied to estimate and compare the use of these substances at local and international scales. This approach has the potential to deliver important information on drug markets (supply indicator).

Pesticides and plasticizers

Pesticides were analysed using GC-MS system employing large volume injection according to modified ISO 6468 procedure. All samples were stored at 4 °C until analysis. An aliquot of water sample, volume 800 mL, were spiked with internal standard propazine or cis-chlordane. Substances of interest were extracted during auto-shaking from water samples using two 50 mL portions of dichloromethane for 20 minutes. Small aliquots of copper powder were added into obtained extracts to remove elementary sulphur. After filtration, the combined extract was evaporated using KDC to final volume of 1 mL. A 50 µL of extract was injected into Agilent 6890 gas chromatograph with Agilent 5973 mass spectrometric detector. The GC system was equipped with PTV injector that was programmed from 60 °C to 260 °C (5 minutes) at a rate of 40 °C/min. Capillary GC analysis was performed on a 30 m x 250 mm I.D., 0.25 mm df DB-XLB and HP-5MS column. Helium was used as carrier gas. The MSD was used in SIM mode for all samples. Each target compound was qualified by two qualifying ions and quantified by one specific or base ion.

The group of pesticides and some industrial pollutants such as PeCB, HCB, DEHP, other phthalates were analysed using GC-MS employing large volume injection according to modified ISO 6468 procedure. Volatile organic compounds were analysed using GCMS according to ISO 10301 procedure. Water samples (10 ml) were placed in 20 ml gas-tight vials. No special sample preparation was required for analysis. Vials filled with samples were directly transferred from the heated cells of the headspace device to the gas chromatograph equipped with the ECD and FID detectors.

A five-point internal standard calibration curve was used for the quantification of the detected compounds. All standards of individual analytes used to produce the standard calibration curves were of a quality given in *'Reference Materials for Residue Analysis obtained from Dr. Ehrenstorfer (Seelze, Germany).*

The LOQ for monurone, simazine, atrazine and propazine was 0.01 ng/L, for diurone 0.05 ng/L and for linurone 0.02 ng/L *(Sremački et al. 2015).*

More than 130 different organic compounds were found in the wastewater and the Danube river samples at the sampling point of Zeppelin. The compounds belonging to the group of hormones were detected in both surface and wastewater samples. The significant quantity of the hormones came from the wastewater into the Danube at the sampling point of Belgrade Quay. Approximately 150 different organic compounds were detected in the wastewater and the Danube surface water samples at the sampling point of War Island. Nearly 130 different organic compounds were analysed in the wastewater and the Danube samples at the sampling point of Roko's Creak. The presence of the hormones androstane-17-one, 3-hydroxy-, androstan-17-one, 3-hydroxy-, (3.α, 5.β.), cholest-5-en-3-ol, cholestan-3-one and stigmast-5-en-3-ol and coprostanol highlighted the great impact of faecal pollution due to the lack of the WWTP *(Vojinović Miloradov et al. 2014c).*

Estrogens

Composite 24-h mixed urban wastewater samples are collected from the main collector - GC2. Samples were collected from a depth of 2 m. Frozen, composite samples of wastewater Novi Sad volume of 1 L was submitted for analysis.

A water sample was prepared by the method of solid phase extraction (SPE). Before extraction the sample is passed through a paper filter pore size of 125 mm ø and then through a filter paper with glass pores, pore size of less than 1 mm ø, with the aim of removing physical impurities that could affect the process of analysis. A 100 mL of filtered sample with set pH value was passed through the pre-conditioned SPE column. Column was dried and the process of the analyte elution with an organic solvent started. Thereafter the eluate was evaporated to dryness under a stream of nitrogen. The evaporated sample was dissolved in an organic solvent, homogenized and filtered through a nylon filter with a pore size of 0.45 um ø, directly into vials for HPLC-MS analysis. Prepared extracts were analysed by high performance liquid chromatography - tandem mass spectrometry (HPLC-MS^2). For the analysis the system Surveyor HPLC and mass spectrometer LTQ XL, Thermo Fisher Scientific U.S., was used. In order to obtain mass spectra of steroids chemical ionization at atmospheric pressure was used as ionization technique. Each analyte was identified on the basis of the characteristic reactions of fragmentation of precursor ions in the most intense and most stable fragment ion (MS^2 analysis).

Quantification were performed using standard addition method, comparing the signal intensities of the analyte in the studied sample with the intensity of the signal in sample containing a known concentration of selected pesticides and steroids ("spiked" sample).

Selected estrogens for detection and quantification were 17β and α estradiole, mestranol, estriol and estrone, with the LOQ of 0,1 ng/L for all analytes *(Sremački et al. 2015).*

Illicit drugs

The composition of mixed urban wastewater effluent is a complex mixture and includes large loads of suspended particulates as well as the presence of relatively high concentrations of compounds that can potentially interfere with the analysis of the target substance. Therefore, the mixed urban wastewater effluent samples were filtered (filter type GFC, 0.45 μm), concentrated and cleaned-up using solid phase extraction (SPE) prior to analysis using polymeric cartridges (e.g. Oasis HLB) in off-line or on-line mode. Details of analytical methodology can be found in literature *(Castiglioni et al., 2006; Kasprzyk-Hordern et al., 2008; Postigo et al., 2008; Hogenboom et al., 2009; van Nuijs et al., 2009; Vazquez-Roig et al., 2010; González-Mariño et al., 2011).* Highly sensitive methods and analytical tools, with preparation steps and clean-up of samples enable analysis at a low concentration level (ng/L) in mixed urban wastewater effluent.

The amount (daily mass load) of each target residue that was excreted by a population was calculated by multiplying measured mixed urban wastewater effluent concentrations (ng/L) by corresponding daily flow rates of mixed urban wastewater effluent (L/day) during the sampling campaigns.

The samples of wastewater for illicit drugs detection and quantification were spiked with isotope-labelled internal standards, either filtered and extracted immediately on SPE cartridges or frozen at −20 °C until analysis.

For detection of and quantification selected analyses HPLC-MS^2 (high pressure liquid chromatography coupled with tandem mass spectrometry) or HPLC-HRMS (high pressure liquid chromatography coupled with high-resolution mass spectrometer).

During the analysis 11 illicit drugs were selected for monitoring (cocaine, benzoylecgonine, amphetamine, methamphetamine, MDMA, THC-COOH, 6-MAM, heroin, morphine, ketamine and mephedrone). It has been confirmed the presence of 5 illicit drugs on selected location. The LOQ values for cocaine is 12 ng/L, BE 10 ng/L, amphetamine 25 ng/L, methamphetamine 18 ng/L, MDMA 20 ng/L and THC-COOH 10 ng/L.

4.3.4. Chemicals, standards and methods

Internal standards, solvents and other chemicals used during screening and target analyses: Benzophenone, Phenanthrene D10, Propazine, Cis-chlordane, individual standards and standard mixtures for detection of target analytes, Dichloromethane (SupraSolv® MS, Merck Millipor Chemicals, USA), Pentane (SupraSolv® MS, Merck Millipor Chemicals, USA), Hexane (SupraSolv® MS, Merck Millipor Chemicals, USA), Acetone (SupraSolv® MS, Merck Millipor Chemicals, USA), Methanol (SupraSolv® MS, Merck Millipor Chemicals, USA), sodium sulphate, concentrated sulphuric acid, 11N sulphuric acid, ammonium persulphate, antimony, ascorbinic acid. Dichlorodimethylsilane, ammonium formate, morpholinoethane sulfonate (MES), tris(hydroxymethyl)aminomethane (TRIS), ammonium acetate, acetic acid and formic acid were also purchased from Aldrich, (all ACS reagent grade). BDH AnalaR grade hydrochloric acid was used for sample pH adjustment (Poole, UK). Ammonium hydroxide solution, (LC–MS additive grade) was obtained from Fluka (Steinheim, Germany). Cocaine hydrochloride, morphine sulfate salt pentahydrate, methadone hydrochloride, ketamine hydrochloride, heroin and D9-tetrahydrocannabinol methanolic solution (D9-THC) were purchased under license from Sigma–Aldrich (St. Louis, MO, USA). Benzoylecognine hydrate, cocaethylene, D-amphetamine sulfate salt, Tempazepam, diazepam, fluoxetine hydrochloride, lysergic acid diethylamide (LSD), 3,4-methylenedioxymethamphetamine hydrochloride (MDMA) papaverine hydrochloride, and 2-ethylidine-1,5-dimethyl-3,3-diphenylpyrrolidine perchlorate (EDDP) were purchased under license from Sigma–Aldrich (Poole, UK). Individual 100 mg L_1 stock solutions of

each chemical were prepared in methanol and were stored at 4 °C in the dark. Working solutions were prepared from the individual stock standards using water as a diluent.

Reagent water used throughout, unless otherwise stated, was obtained from a Millipore MilliQ water purification system (Millipore, Bedford, MA, USA) and was 18.2 MO or greater. The use of mass labelled internal standards is obligatory as a method of compensation for the potential analytical errors of sample manipulation and matrix interferences. Internal standards are added to the samples prior to sample treatment.

The helium and acetonitrile were used as GC and HPLC carrier gasses.

The filter papers used during filtration of samples for pre-treatment were Whatman® qualitative filter paper, Grade 5, 125 mm Ø, 100 g/m^2, particle retention 2.5 μm, and Grade 4, 42.5 mm Ø, 92 g/m^2 and particle retention 20 - 25 μm.

Methods used during experimental research: Temperature measurement, pH, concentration of dissolved oxygen, BOD_5, COD, suspended and dissolved solids (TSS and TDS), gravity (GF) and vacuum (VF) filtration, rotary evaporation (RE), liquid-liquid extraction (LLE), solid phase extraction (SPE), gas chromatography (GC), high pressure liquid chromatography (HPLC), mass spectrometry (MS), tandem mass spectrometry (MS^2), high performance mass spectrometry, triple quadrupole mass spectrometry, hybrid quadrupole-linear ion trap mass spectrometry. All methods used were validated priory to analysis.

Procedures used: EPA 170.1, EPA 150.1, HACH LCK 114, BODTrak™ Manual, EPA 360.1, HACH LCK 303, HACH LCK 349, EPA 160.2, ISO 10301, modified ISO 6468.

Evaluation of obtained data from screening analyses was performed in Agilent software for data assessment and registered substances in chromatograms were compared to databases NIST08 и Wiley7. Statistical methods of data evaluation were performed with statistical analysis in software Origin 8.6 trail version (OriginLab Corporation, Northampton, MA, USA), IBM SPSS statistics 20 and Microsoft Excel 2010.

4.4. Calculation of organic load and risk quotient

The evaluation of mixed urban wastewater overall and specific organic load is a significant for selection of adequate wastewater treatment process. Thus, the evaluation of provided results will be also be performed as a calculation of overall organic matter in wastewater samples and specific load of specific selected substances, considered priority, hazardous priority and/or emerging detected during the research period.

Total or overall organic content of mixed urban effluent was measured as BOD_5, COD and PPC. For every measured parameter, overall and specific xenobiotics, calculation of load

was conducted for dry and wet weather, the lowest and the highest flow through the collector GC2, 560 L/s and 4080 L/s, respectively. The number of inhabitants for this collector is 133 245.

The calculation of load [ng/day/inh] for selected parameters was conducted with the equation 15.

$$X_{\text{parameter load}} = \frac{\text{measured concentrations } \left[\frac{\text{mg}}{\text{L}}\right] \cdot \text{daily flow rates } \left[\frac{\text{L}}{\text{day}}\right]}{\text{number of inhabitants}} \qquad \text{e.15}$$

The BOD_5 to COD ratio was calculated for samples analysed in laboratory and in real time measurement (Equation 16). This parameter can indicate the biodegradability of wastewater, which is a particularly significant for wastewater treatment.

$$\frac{BOD_5}{COD}\,\text{ratio} = \frac{\text{measured value of } BOD_5 [\text{mg } O_2/\text{L}]}{\text{measured value of } COD\ [\text{mg } O_2/\text{L}]} \qquad \text{e.16}$$

Following the EU guidelines, the ERA is performed by calculation of the $PEC/PNEC$ or $MEC/PNEC$ ratio, known as the risk quotient (RQ) shown in equation 17 or 18, and RQ should not exceed 1, otherwise, a risk to the aquatic environment is predicted.

$$\text{Risk Quotient (RQ)} = \frac{\text{Measured environmental concentration (MEC)}}{\text{Predicted no} - \text{effect concentration (PNEC)}} \qquad \text{e.17}$$

$$\text{isk Quotient (RQ)} = \frac{\text{Predicted environmental concentration (PEC)}}{\text{Predicted no} - \text{effect concentration (PNEC)}} \qquad \text{e.18}$$

If the equation e.16 is used for calculation of RQ, the information about the real risk ratio of a substance is obtained. This equation can be used if there is information about the real concentrations on the specific location. In the other case if there is no measured concentration of a substance and the prediction has to be made, predicted environmental concentration is used, and the equation e.17.

4.5. Statistical evaluation of analyte characteristics and predicted behaviour in the WWTPs

Physicochemical properties, eco-toxicological characteristics and wastewater treatment and removal possibilities have been selected for further statistical analysis by Person correlation, Multivariate Analysis - Principal Component Analysis (PCA) and Hierarchal Cluster Analysis (HCA). Results are further discussed in Chapter 5.8.

5. Results and discussion

In the results and discussion segment of the thesis the results of basic physicochemical parameters and specific pollutants (PhPP and EmS) will be elaborated.

Furthermore, obtained data are evaluated according to the national and international (EU) requirements and processed via statistical and graphical tools to extract conclusions needed for the purposes of this thesis.

5.1. Results of basic physicochemical parameters

The first sampling campaign of all selected localities has been conducted under equal hydro meteorological conditions. The hydro meteorological data were obtained from the Republic Hydro meteorological Service of Serbia. Both sampling campaigns in July and September 2012 were performed under similar weather conditions with no precipitation and average daily air temperatures of 29 and 26 °C, respectively. The water height levels were from 190 to 157 cm, measured water temperatures were from 25.7 to 16.5 °C, and river flows were from 2702 to 2274 m^3/s.

In Tables 5.1, 5.2 and 5.3 are shown physical, chemical and meteorological conditions for illicit drugs sampling campaign in 2013, estrogens in 2015 and results of laboratory analyses for COD, BOD_5 and PPC, respectively.

Table 5.1 Target campaign for illicit drugs in March of 2013 – physical, chemical and meteorological conditions

Parameter [Unit]			14th	15th	16th	17th	18th	19th	20th
Precipitation		***Descriptive***	*snow*	*snow*	*rain*	*rain*	*clear*	*clear*	*clear*
Average temperature	***Daily***	***[°C]***	*5.1*	*-0.2*	*2.9*	*4.2*	*7.8*	*11.9*	*11.6*
	WW		*7.1*	*9.3*	*10.7*	*12.7*	*13.6*	*15.2*	*15.1*
pH		***-***	*6.8*	*6.9*	*7.1*	*6.9*	*7.1*	*7.1*	*7.1*
BOD_5		***[mg/L]***	*448*	*241*	*286*	*478*	*682*	*322*	*307*
COD			*598*	*534*	*571*	*709*	*800*	*648*	*661*
N_{tot}			*71*	*79*	*71*	*74*	*84*	*79*	*74*
P_{tot}			*2*	*4*	*4*	*4*	*4*	*4*	*6*
WW flow	***Average***	***[m^3/s]***	*2.9*	*1.5*	*1.3*	*1.4*	*1.6*	*1.7*	*1.6*
	Max.		*4.1*	*1.7*	*2.4*	*1.7*	*1.7*	*1.7*	*1.7*
	Min.		*1.7*	*0.6*	*0.6*	*0.6*	*1.1*	*1.7*	*0.6*

Table 5.2 Target campaign for estrogens in 2015 – physical, chemical and meteorological conditions

Parameter	Unit	25.01.
Precipitation	Descriptive	*Clear*
Average daily temperature	°C	*7.1*
WW temperature	°C	*10.1*
pH	-	*7.76*
Dissolved O_2	[mg/L]	*3.95*
COD	[mg/L]	*339*
BOD_5	[mg/L]	*147*
TSS	[mg/L]	*73*
TDS	[mg/L]	458

Table 5.3 Results of laboratory analyses for COD, BOD_5 and PPC

Date	PPC	COD	BOD_5
18.12.	27.6	395	242
	46.9	561	394
	52.8	746	506
19.12.	10.1	196	100
	43.5	501	296
	56.2	636	442
22.01.	23.1	401	220
	45.6	602	392
	51.8	739	586
28.01.	39.3	277	154
	66.4	703	436
29.01.	37.5	391	266
	39.3	751	414
04.02.	61.9	736	484

	69.2	729	502
05.02.	54.3	508	368
	70.8	699	512
	65.3	722	614
21.02.	27.8	291	158
	70.6	787	468
22.02.	24	264	141
	56.2	601	354
	43.5	637	414
26.03.	32.5	302	244
	67.3	678	434
	39.2	421	336
27.03.	27.1	285	202
	62.8	726	490
	59.7	689	448
02.04.	33.2	357	252
	65.4	749	487
	37.8	420	313
19.04.	26.5	270	181
	65.2	720	443
22.04.	23.6	230	152
	51.1	689	486
	156.2	839	430
23.04.	81.1	316	223
	35.8	636	416
	41.4	611	352

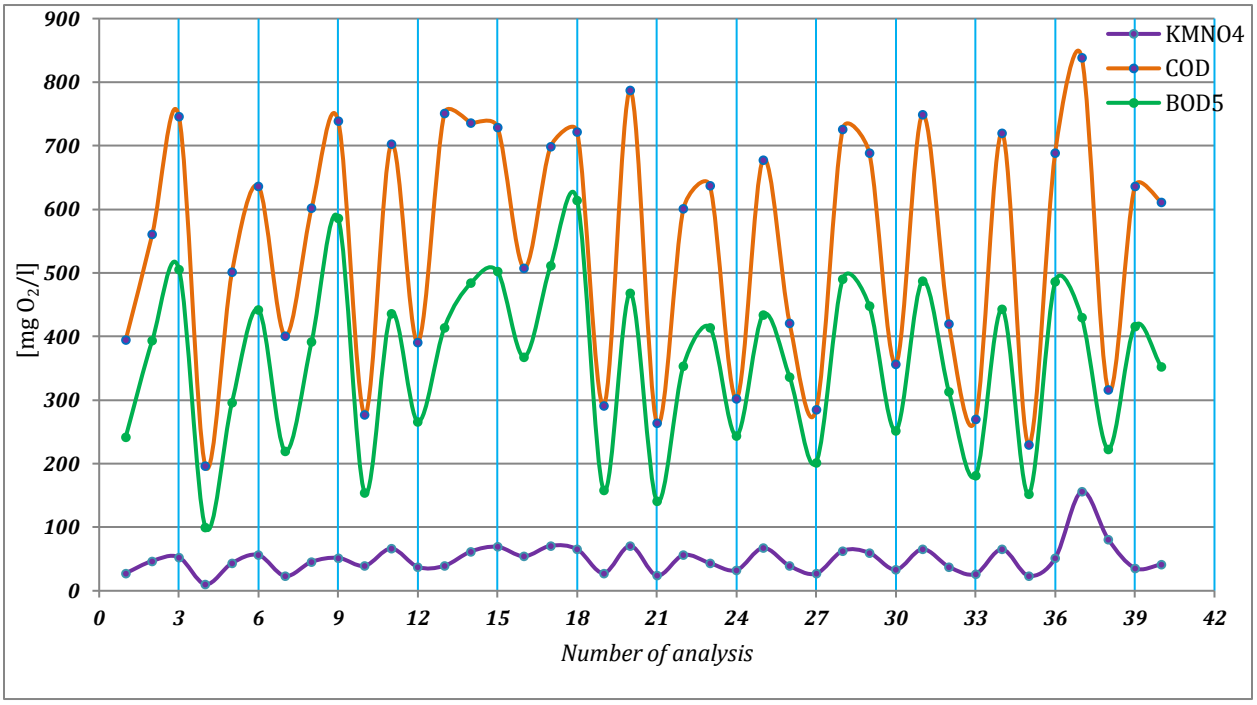


Figure 5.1 Results of COD, BOD_5 and PPC in wastewater of discharge point GC2 during research period

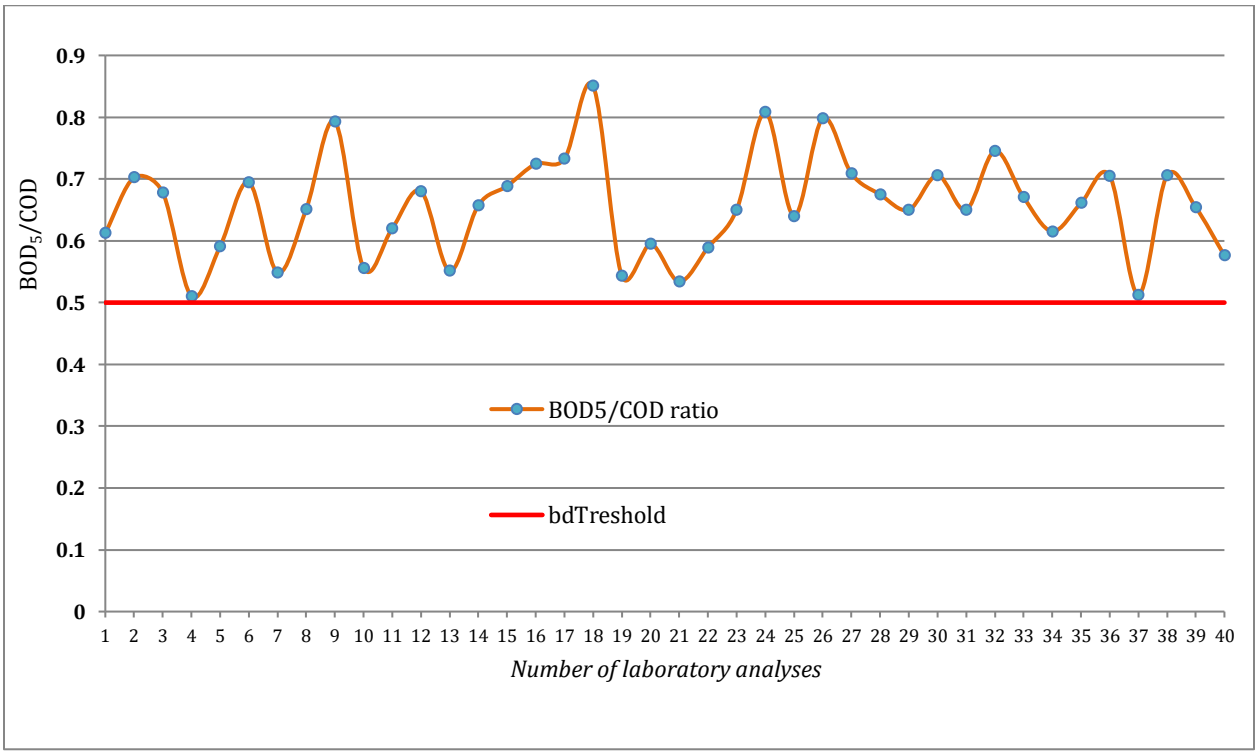


Figure 5.2 BOD_5 to COD ration according the laboratory analyses by 3 daily samples

In Figures 5.1 and 5.2 results of Laboratory analyses for COD, BOD and PPC were shown. Laboratory measures of BOD_5/COD ratio were in range from 0.51 to 0.85, indicating the presence of more easily degradable organic matter which could be easily removed using aeration treatment process.

The untreated mixed urban wastewater BOD/COD ratio is in the range of 0.3 to 0.8, ranging from extremely low biodegradability of 0.3 value to 0.8 and over for highly biodegradable wastewater. If the BOD/COD ratio for untreated wastewater is 0.5 or greater, the waste is considered to be easily treatable by biological means. If the ratio is below 0.3, either the waste may have some toxic components or adapted microorganisms may be required in its stabilization *(Sremački et al. 2016b).* As a wastewater is oxidized through a wastewater treatment plant, the BOD_5/TOC ratio will drop. A treatment plant effluent may have a BOD_5/TOC ratio of as low as 0.5 since the effluent wastewater is much less biodegradable (it has already been largely degraded) *(Sremački et al. 2016b).*

Over the period of 5 months s::can spectro::lyser made 2272 scans of COD (range: 196 – 810 mg/L) and BOD_5 (range: 100 – 614 mg/L) concentrations were also determined in laboratory in the highest concentrations in samples collected at 22 h, indicating the highest wastewater pollution, which could be influenced by human activities during evening hours.

According to results of BOD_5 to COD ratio 446 of all measures were under 0.5, representing 19.63 %, some as low as 0.1, indication low biodegradability of wastewater in 20 % of time during the research period.

The results of BOD/COD ratio obtained during on-line real time measurements by Spectro::lyser are shown in Annex I as Figures I.1 and I.2.

5.2. Results of screening analysis adaptation

Due to the physical characteristics of the environmental samples, high content of floatables, suspended and dissolved solids and other, for the use of SPE, SPME and SBSE pre-treatment, of samples was necessary. The filtration processes were extremely time-consuming, for volume of 1L of sample. The first 50 mL of sample were filtrated trough all diameter type of filter paper, and the fastest period of filtration was 40 minutes.

Approximate time for the filtration of 1 L sample volume would be at least 8 hours, which is neither optimal nor practical for organic analysis sample to be exposed to room temperature and open space for such a long period of time, without changes in organic content. The filter paper 125 mm Ø, 100 g/m^2 and particle retention 2.5 μm was used during preparation of sample.

After gravity filtration, the vacuum and active carbon filtration were performed, respectively. The vacuum filtration was performed on Sartorius apparatus for vacuum

filtration with filter paper of 42.5 mm Ø, 92 g/m^2 and particle retention 20-25 μm was used during preparation of sample. The pre-treatment method showed as inefficient for many samples, as 50 mL of sample was filtered for approximately an hour. Vacuum filtration and filtration on carbon filter were more or less of the same efficiency as the gravity filtration. The vacuum filtration method showed as inefficient, as 50 mL of sample was filtered for approximately an hour. Filtration was effective procedure only after extraction was conducted, and it was applied only if it was necessary.

As the filtration of samples wasn't shown to be efficient, more sophisticated sample preparation techniques (SPE, SPME and SBSE) were not probable for optimal sample preparation. Due to the results from another relevant study during the NETREL Project conducted during the year of 2015, it has been concluded that LLE, as a preparation technique for environmental samples shows highest response for gas chromatographic determination.

The following procedure was used for the evaporation of the environmental sample in 500 mL of roto-vapor at a temperature of 50 °C, and 0.2 bar. It is necessary, on average, about 8 hours of active evaporation of the sample, in order to the 250 mL of the aqueous solution was evaporated to dryness. The process was further disturbed by the presence of large amounts of detergents and proteins in WW, which are caused due to the heating of the occurrence of large amounts of foam which jeopardized the precision of the capacitors and success of the methods as a whole. Since it is evaporated in this manner, the method is designated a semi-successful. Under the conditions selected it was concluded that it is necessary to perform the solvent extraction priory to evaporation, to diminish the possibility of analyte loss. Rotary evaporation was unsuccessful method; the process was not 50 % completed even after 3 times 8 h of evaporation, as the equipment could not be adjusted to effective evaporation. Rotary evaporation was shown to be a good selection after the LLE.

The LLE was the only method of preparation for this sample matrix that was fully successful and optimal in sense of time, efficiency and sample contamination. The filtration of water samples was shown to be unnecessary when LLE was used, only in the samples with high content of floating solids and with filters. Water samples (500 mL) were placed in a 1000 mL glass separatory funnel and extracted with three 30 mL portions of solvent for 30 minutes using automatic shaker device at 700 rpm. All collected extracts were concentrated in Kuderna-Danish apparatus to final volume of 1 mL.

During liquid/liquid extraction adaptation of extraction solvent system was conducted on the first three samples collected in October 2012.

Extraction solvent should cover a broad range of chemical properties of metabolites to enable extraction of all metabolites in high yields with good reproducibility *(Liebeke and*

Bundy 2012), and solvent system should not affect the stability of metabolites extracted *(Rubin and Evert 2006).*

According to real operation time, n-pentane has shown to be the most efficient, as it's boiling point is the lowest, with the average of 1 h needed for concentration of extract, but the difference of half an hour between n-pentane and dichloromethane is acceptable in correspondence with the higher quality of peaks obtained during analyses of samples prepared with dichloromethane.

Methanol was disregarded after conclusion that the concentration procedure was not optimal in relation to time consumption, as the average concentration process for selected solvent was at least 4 hours.

The solvents of different polarity were used to determine the optimal procedure for meat processing industry sample matrix. Solvents used were n-pentane (≥ 99.8 % purity), DCM (≥ 99.5 % purity) and n-methanol (≥ 99.9 % purity), sigma Aldrich, due to their different polar properties, but similar boiling points or dipolar moment.

According to operation time of sample preparation, n-pentane has shown to be the most efficient, as it's boiling point is the lowest, 36 °C, with the average of 1 hour and 15 minutes needed for concentration of extract, but the average difference of 15 minutes between n-pentane and dichloromethane is acceptable in correspondence with the higher quality of peaks obtained during analyses of samples prepared with dichloromethane. Boiling point of dichloromethane is 40 °C, and methanol 60 °C.

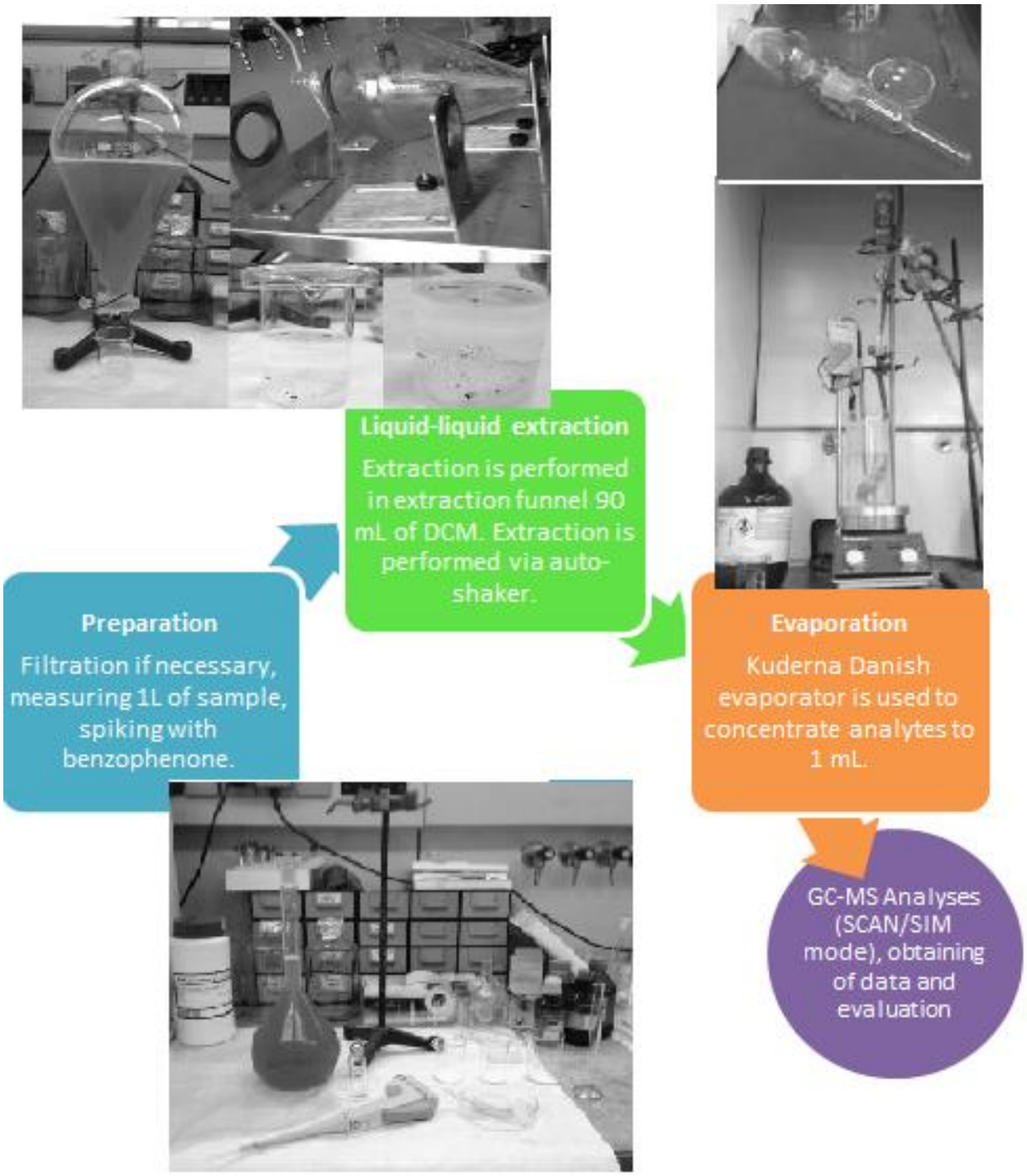
Another disadvantage of n-pentane and methanol is their density of 0.626 g/mL and 0.791g/mL respectively, in relation to surface water sample, which reflects as a technical difficulty during the extraction process, if there is no special glassware for this extraction it can lead to substantial complications and losses.

As the whole volume of sample fluid has to be removed from the extraction funnel first, there is a higher possibility of extract volume lose. As the dichloromethane has a density of 1.327 g/mL, which is higher density than water, this is not the case whit the dichloromethane as solvent.

Dichloromethane (DCM) has the higher density, so the extract is concentrated on the bottom of extraction funnel and is removed first, which prevents loss of extracts and time.

During this phase DCM has shown as the optimal solvent for this type of samples, being the solvent which made possible to extract the largest quantity of compounds, had the cleanest baseline with low level of interference, the least amount of peak distortion, and clearest peak separations in chromatogram out of all 3 solvents used.

Figure 5.3 Algorithm of surface water and mixed urban wastewater screening analysis – adapted procedure



In Figure 5.3 Algorithm of surface water and mixed urban wastewater screening analysis – adapted procedure is shown.

The liquid-liquid extraction into dichloromethane favours the transport of hydrophobic organic compounds from water to an extraction solvent, while the extraction efficiency depends on the compound partitioning coefficient.

According to the overall shape of chromatograms and peaks, chromatograms obtained from surface water samples prepared for analysis with dichloromethane as solvent have cleaner baseline which facilitates the calculation of the peak areas and the concentration of the components can be calculated with greater accuracy.

Samples prepared with dichloromethane have better peak separation with less peak slopes and shape distortion which suggests that dichloromethane is the optimal and best solution for screening analyses of selected surface water sample matrix.

N-pentane as solvent has shown as more suitable solvent for extraction of alkanes and higher alkanes, which is shown by the chromatogram shape, which suggests a high content of crude oil pollution. N-methanol is disregarded as it has shown to be most time-consuming and the lowest extraction capacity, confirmed with significantly minimized number of peaks.

A 2 µL of the extract was injected into the gas chromatography system. The GC-MS screening analysis was performed using Agilent 6890 gas chromatograph coupled to the Agilent 5973 mass spectrometric detector.

The GC system was equipped with the programmed temperature vaporization (PTV) injector, known to be suitable for large volume samples. The capillary GC analysis was done on a 30 m x 250 mm I.D., 0.25 mm df DB-FFAP column.

The oven programme was formed accordingly to utilized solvent, from 40 °C to 60 °C, with hold time for 10 minutes for solvent delay, that was raised to 230 °C with temperature gradient of 2 °C/min. Helium was used as a carrier gas. The mass selective detector (MSD) was used in the scan mode (m/z 45-600) for all the samples, during screening analyses.

According to real time operation, n-pentane has shown to be the most efficient, as it's boiling point is the lowest, with the average of 1 h needed for concentration of extract, but the difference of half an hour between n-pentane and DCM is acceptable because of the higher quality of peaks obtained during analyses of samples prepared with dichloromethane.

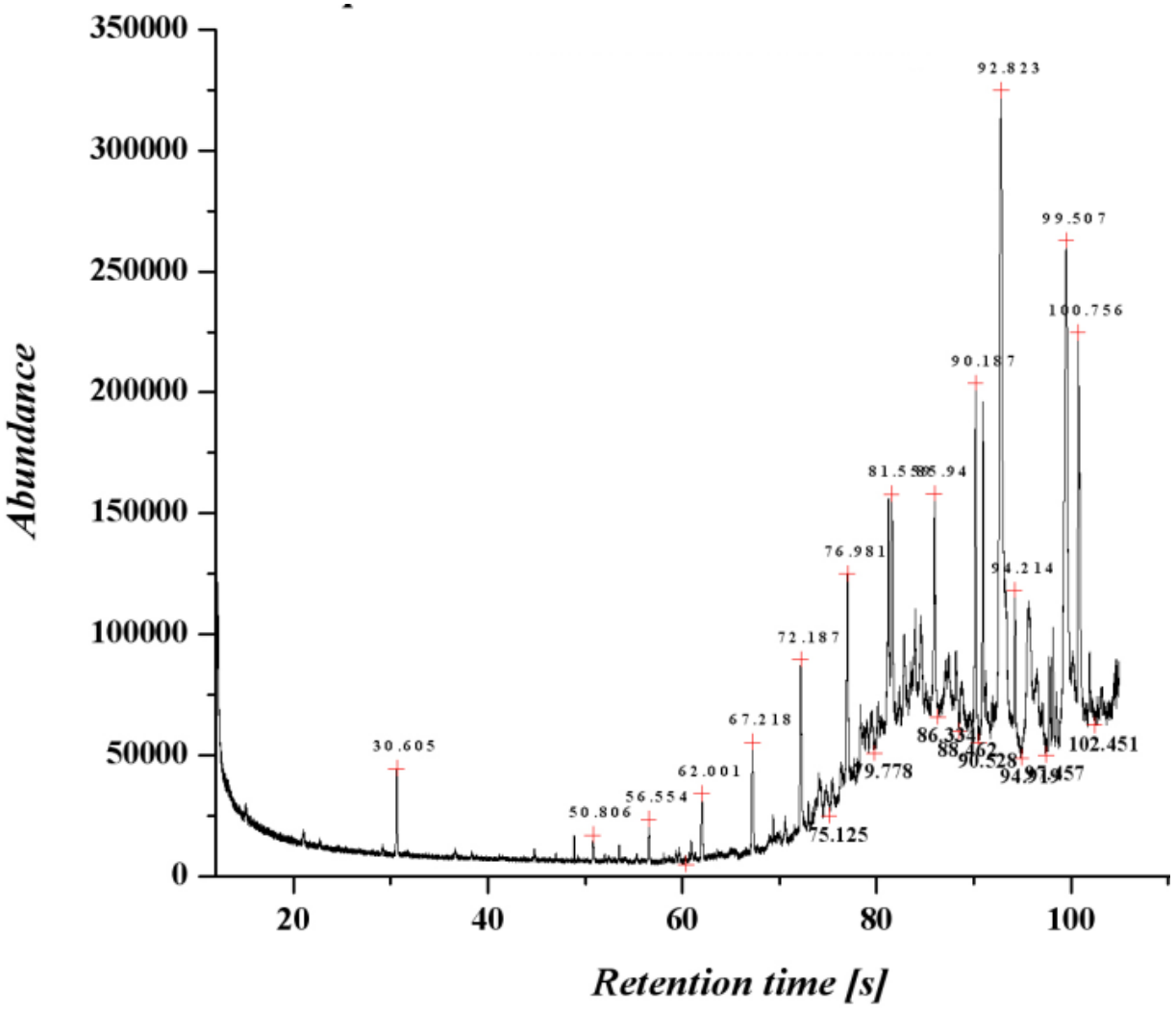
Methanol showed problems in preparation of sample, during evaporation as it has the highest boiling point (65 °C), the evaporation procedure with this solvent lasted for 4 – 6 h depending on a room temperature, and it was disregarded as an optimal solvent for selected sample.

In some occurrences, after LLE, concentrated and prepared sample had to be filtered through fine micro filters, due to the high content of particles, foaming agents, detergents or fat.

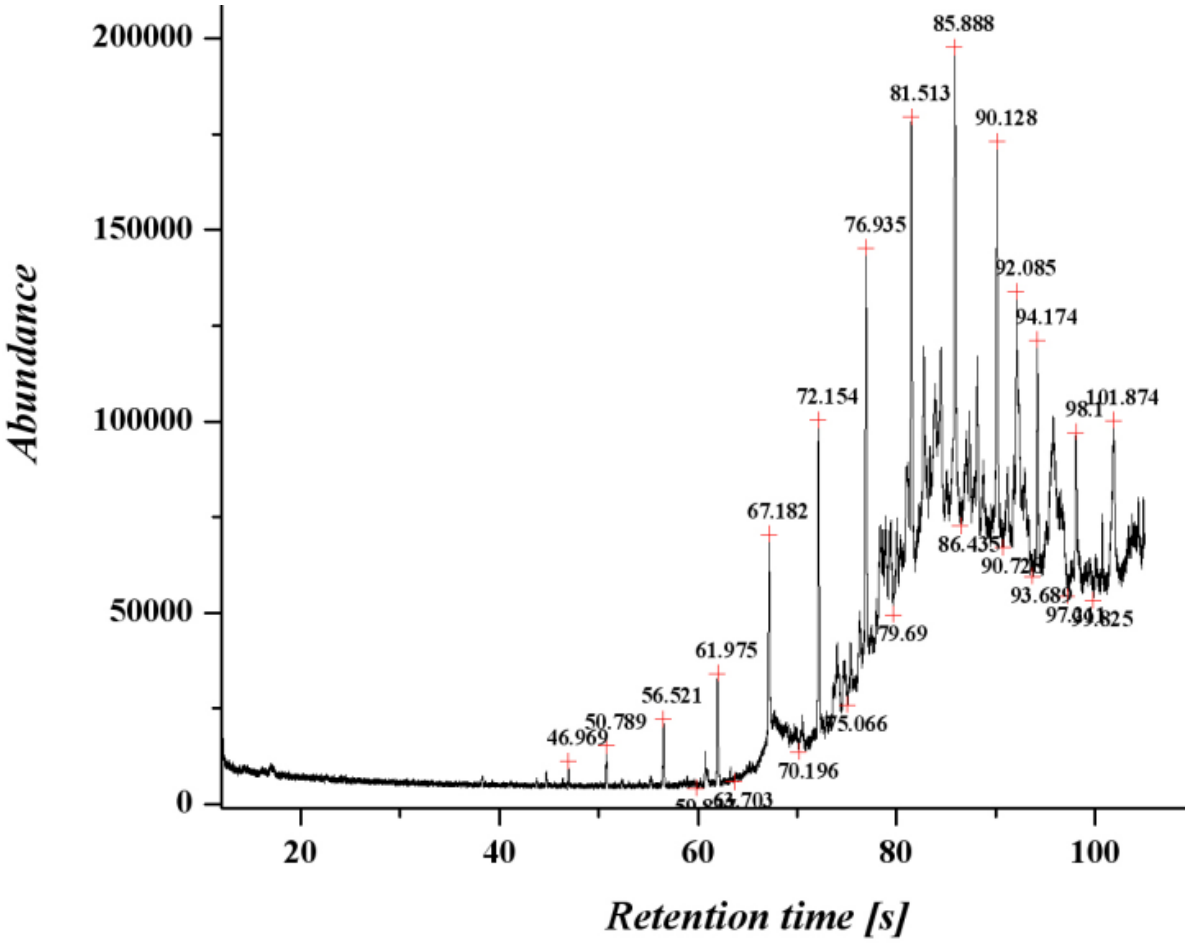
During the data evaluation the extraction of compounds for the selected solvents was significantly different, up to 38.15 % in favour of DCM in comparison with n-pentane.

Solvent to solvent extraction ratio DCM/n-pentane for sample 1 was 1.25, sample 2 1.62, and sample 3 1.82. The results were expected as the research was conducted on a complex sample matrix.

In the Figure 5.4 chromatograms of samples prepared via different solvent are shown.



a)



b)

Figure 5.4 Chromatograms of samples prepared with a) DCM and b) n-pentane

The peak shape or peak symmetry is the other important parameter related to the distribution of substance between phases in the column.

For the same sample, different polarity solvents have shown different profiles of chromatograms, shown in figure 2. DCM has higher quality extraction, with significantly less pick deterioration and distortion than pentane.

Overall around 1125 peaks were detected by GC-MS, out of those 313 substances that have shown quality match index (QMI) greater than 65 % during spectral search using relevant spectral library.

The highest number of peaks were detected by DCM average difference in pick acquisition was 38.34 % in favour of samples prepared with DCM. Identification percentage for both solvents was in the range of 62 to 67 %, slightly higher in favour of n-pentane.

About 20 % of obtained peaks are substances that show QMI in range 65 % to 50 % during spectral search using relevant library. According to the peak shapes and peaks of chromatograms it is concluded, that the solvent DCM for LLE is the best solution for studied sample matrix.

The identification of compounds was done using Wiley7n and NIST08 mass spectral libraries.

Overall around 1146 peaks were detected by GC-MS in all 9 samples during 3rd screening analysis, and that summers up to about 129 peaks per sample.

Throughout the evaluation process 417 peaks were identified, and 69 were selected for further analyses.

5.3. Results of surface water and mixed urban wastewater screening analyses

After a notable number of trial and error processes and analysis of three samples from first sampling campaign the difficulties were resolved during 1st screening analyses.

The most frequently occurring compounds in studied water samples during all screening analyses were phthalates, phenols, PAHs and other aromatics, esters of fatty acids and alkanes.

Phthalates are used as plasticizers, industrial and lubricating oils, defoaming agents, cosmetics and insect repellents.

In all samples of wastewater and Danube River water diethyl phthalate (DEP), diisobutylphthalat (DIBP), dibutyl phthalate (DBP) and diisooctyl phthalate (DIOP) have been detected and were selected as one of the subject of target analysis.

Some of the detected phthalates are already on the NORMAN list of emerging substances (DEP and DBP).

Table showing the identified substances in all 9 samples during first screening analyses Appendix II as Table II.1.

All identified substances during screening analyses are in concentration levels from mg/L to ng/L.

The chemical species that have been detected in surface water during the year of 2012 belong to emerging and priority groups of substances – flammables, irritants, toxic and cancerogenic compounds, EDCs, industrial chemicals, plasticizers, aliphatic (fatty) alcohol, higher alkanes, wood preservatives, flavour and fragrances, terpens and terpenols, personal care products, pesticides, PAHs, antifoaming agents, additive residues and others.

The registered substances render the possibility for specification of target analytes and enable better organisation and prioritisation of surface water monitoring system designed for the specific location.

This specific property of screening analysis is esspetialy important for the location where, as in Novi Sad, it is extremely important to monitor wastewater quality as there is a high possibility of infiltration to water well used for drinking water production.

Phenols were identified practically in all studied samples of urban effluent. Considering activities performed in vicinity of sampling areas, identified phenols could originate from the biodegradation processes of higher phenols (octylphenols, nonylphenols or alkylphenoletoxylates), as well as from processing of coal and wood or crude oil.

PAHs and other aromatics, as well as alkanes and alkenes, could originate from petrol industry. Polycyclic aromatic hydrocarbons (PAHs) like Anthracene have been found in almost all water samples.

Anthracene is included in the list of Priority Substances as hazardous substance and Certain Other Pollutants according to Annex II of Directive 2008/105/EC.

Various esters of fatty acids were found in almost all studied samples, as a product of degradation process of organic matter, as easily soluble organic compounds, in this study were not of particular interest.

The sources of the detected fatty acids in the aquatic environment are mainly degradation processes of petroleum hydrocarbons and/or animal and vegetable fats.

In Table 5.4 are shown meteorological conditions during sampling for screening analysis.

Table 5.4 Meteorological conditions during sampling for screening analysis

	Survey I	**Survey II**	**Survey III**
Date	13th June 2011	26th October 2011	12th December 2011
Mean temperature	20 °C	13 °C	8 °C
Precipitation	No	No	No
Wind speed	8 km/h	30 km/h	10 km/h

Table 5.5 Excerpt from results of 2nd screening analysis of Danube water at the location GC2

No.	Compound	Detection
1.	Alpha-isomethyl ionone	Detected in wastewater and Danube water
2.	1,2-benzenedicarboxylic acid, diethyl ester (DEP)	
3.	Dihydro methyl jasmonate	
4.	N,N,N',N'-tetraacetylethylenediamine	
5.	Benzofuran derivat	
6.	Tetradecanoic acid	
7.	1,2-benzenedicarboxylic acid, bis(2-methylpropyl) ester (DIBP)	
8.	Caffeine	
9.	Hexadecanoic acid	
10.	1,2-benzenedicarboxylic acid, dibutyl ester (DBP)	
11.	1,2-benzenedicarboxylic acid, mono(2-ethylhexyl) ester (MEHP)	
12.	Cholestan-3-ol, cholest-5-en-3-ol, cholestan-3-one, cholestane, 3-ethoxy-, (3.beta, 5.alpha)-, pregnane and stigmast-5-en-3-ol	
13.	Benzothiazole, 2-(methylthio)-	Detected in Danube surface water
14.	Cyclopenta[g]-2-benzopyran (Galaxolide)	
15.	1,2-benzisothiazole (BIT)	
16.	Biphenyl	

In Table 5.5 is shown excerpt from results of 2nd screening analysis of Danube water at the location GC2. The comprehensive results of the screening analyses and chromatograms are shown in Annex II in Table II.1 and II.2.

5.4. Results of target analyses

For the target analyses it is recommended the LLE or SPE preparation of samples and GC-MS and GC-MS^2, LC-MS^2, for pesticides and hormones or illicit drugs, respectively.

A significant number of the detected compounds in surface and wastewater can be found on the NORMAN list of emerging substances and on the list of WFD priority hazardous substances.

According to screening analysis in sampling campaigns in research period from 2011 to 2013, seventy five organic compounds were selected for target analysis. In the first sampling campaign in 2012, 21 organic compounds were detected in water samples from selected compounds. The target campaign for illicit drugs was performed in 2013 – eleven were targeted, and five were detected. The next 2 target campaigns for illicit drugs were performed in 2015 and 2016 for cocaine, benzoylecgonine, amphetamine and MDMA. The target campaign for estrogenic compounds was performed during 2015.

The surface water samples and the sample before discharge of wastewater of Novi Sad (Alas Island – RI) were obtained for the purpose of deduction of pollutants already in surface water, for the purpose of specific selection of wastewater specific pollutants. According to groups of substances most frequently found in screening analyses - phthalates, hormones, indols, PPCPs, nicotine, pesticides, indigo and other, the substances that require special attention. Emerging xenobiotics are of extreme importance due to their effect onto the living organisms, especially aquatic and then humans. The other substances detected during analysis, that can also be of interest are caffeine, PAHs, 1,2-benzisothiazole, alpha-isomethyl ionone, and butylated hydroxytoluene (p-cresol), specified in the NORMAN list of the emerging substances; furthermore, diethyl, dimethyl propyl and dibutyl phthalates, (bio)- pesticides 6a-β,12a-β-rotenolone, and a WFD pollutant benzo(a)pyrene; nicotine and its metabolite cotinine that came from the wastewater to the Danube surface water.

The target compounds of interest are selected from lists of priority, priority hazardous and emerging substances – xenobiotics from the groups of pesticides, hormones and illicit drugs, such as DDT and metabolites, trifluralin, endosulfan, dieldrin, endrin, cholestan-3-ol, cholest-5-en-3-ol, cholestan-3-one, cholestane, 3-ethoxy-, (3.β, 5.α)- , stigmast-5-en-3-ol,17α- i 17β-estradiole, estriol, estron, mestranol, cocaine and metabolite benzoylecgonine, amphetamine, methamphetamine, THC-COOH and ecstasy.

Some of the detected concentrations of target pollutants in the Danube surface water samples exceeded proposed annual average environmental quality standard (AA EQS) values for inland surface waters, which is a reason for concer particularly if the wastewater discarges are positioned close to the water wells used for drining water production.

The target analysis is the basis for selection of the most adequate early warning system (EWS) for monitoring of the quality of raw water used for drinking water production in the city of Novi Sad, and indispensable information for WWTP in sense of capacities, operation and design.

Pollutant detected during the target analyses represent the substances included into the monitoring plan of a EWS. The emerging substances with the high RQ or the substances already implemented into the regulation guidelines are the substances that should be incorporated into the selected location EWS.

5.4.1. Pesticides and plasticizers

The research showed that from all targeted analytes, organochlorine pesticides are detected in the highest concentrations. The substances detected on the location site RI present the previous pollution of settlements and population activities before the city of Novi Sad. In this context, it can be called previous pollution. The pollution detected in samples GC1", GC2", RO" and RP" represent the direct pollution from the city of Novi Sad and urban activities, and the GC1', GC2', RO', RP' represent the direct impact of the city onto the River Danube.

Pesticides were the substances detected in the highest concentrations, particularly in the urban effluents indicating pollution from agricultural activities, household and farms in the vicinity of the sampling points.

Endosulfan, dieldrin and endrin were detected in significantly higher concentrations in sampling location RO' and also in Danube River downstream of RO discharge. As the RO' sampling location is located in agricultural area, pesticide pollution can be expected.

Dieldrin was also detected in Danube river water 100 meters downstream of discharges GC1, GC2, which can be a result of infiltration of agricultural runoff. Endrin was primarily used as an insecticide and rodenticide onto the soil, with the high partition coefficients as a great potential to bioaccumulated and persist in soil for over 10 years *(UNDP, FAO UN 2005).* Pentachlorobenzene (PeCB), hexachlorocyclohexane gamma (Lindane) and hexachlorobenzene (HBC) were detected in wastewater at sampling site RO'. PeCB was also detected at GC1'', while HCB was found in concentrations up to 5 times higher than AA EQS in Danube surface water near discharges GC1 and GC2. A predominant source of the PeCB released into the environment is a result of backyard and garden waste and wastewater. PeCB was used as an intermediate product for production of pesticides, for viscosity reduction of products containing polychlorinated biphenyls and as a fire retardant (US EPA). HCB is extremely toxic to aquatic organisms, and it may cause long-term adverse negative effects in the aquatic ecosystem. Chemical and toxicological properties show that HCB has high potential for biomagnification and bioaccumulation in aquatic organisms.

In 2009, an international ban on the use of Lindane in agricultural activities was applied through the Stockholm Convention on Persistent Organic Pollutants, with a specific exclusion for the purpose of the head lice and scabies treatment *(Report on POPs, Geneva 2009).* In Serbia usage of Lindane is allowed in shampoo used to treat lice *("Official Gazette of RS" no. 50/12).*

For the purpose of results discussion, in the case of DDT and its metabolites, there is a third category of pollution that can be calculated from obtained concentration levels, and that is historic pollution. DDT degrades to DDE and DDD (previously shown in Chapter 2.4.3. Figure 2.15) and the ratio of metabolites to DDT can be used as an estimate of the period of application: if DDT exposure has been recent, the ratio should have value lower than 1, while in areas where substantial time since exposure has passed, the DDE/DDT value is higher than 1. p,p'-DDE detected at sampling site Alas Island (RI), upstream of all discharge points, indicated the ecotoxicological status of the river Danube prior to city of Novi Sad. Unexpectedly p,p'-DDD (Rhothane), metabolite of DDT, was detected in almost all samples with extremely high values at sampling location RO' and GC1''. Increased concentration of DDT and its metabolites could suggest the illegal use of DDT, which is a reason for concern. The most lipophilic metabolite p,p'-DDD, with the lowest K_{OW} value, was detected in almost all the samples, with particularly high values at GC1'' and RO', which confirms historical contamination, but also recent contamination upstream of the city of Novi Sad *(Vojinović Miloradov et al. 2014c).* According to the concentration ratio of p,p'-DDD and p,p'-DDT at sampling points with the highest concentrations indicated significant historical contamination in addition to recent pollution with p,p'DDT. p,p'-DDD, p,p'-DDE and p,p'-DDT at GC1' and RO' were determined in concentrations more than 8 times higher than AA EQS values in EU. p,p'-DDE were detected at sampling site RI, and the concentrations did not amplify further downstream the Danube near Novi Sad. Although DDT was banned the detected concentrations could be the evidence of historical contamination and illegal use of DDT *(Vojinović Miloradov et al. 2014c).*

DEHP concentrations were the highest and have been found in all analysed waste and surface water samples. The higher concentration in samples of wastewater was recorded in industrial parts of the sewerage system (GC2' and RO'). In surface waters samples collected 100 meters downstream of sewage discharges GC2, RP and RO concentrations of DEHP were 3 times lower than at the discharges indicating the dilution in River flow. DEHP is the most commonly used plasticizer, due to its suitable properties and low cost. It can be used as hydraulic and dielectric fluid in capacitors, solvent in glow sticks and plasticizer in medical devices. DEHP is highly soluble in oil, but not in water. DEHP is potential endocrine disruptor, androgen antagonist and can cause extreme oligospermia in men. The most probable source of contamination is from illegal dump sites and unsanitary landfills. DBP was detected only in surface water sample after the discharge point GC1. The highest concentration of DBP and DEHP in the sampling locations RO" and GC1'', respectively, downstream of collector GC1 were detected during the second target analysis.

The results for pesticides and phthalates are shown in Table 5.6 and Figures 5.5 and 5.6.

Table 5.6 Results of 1st and 2nd target analysis for pesticides and phthalates measured above LOQ

Compound		AA-EQS	RI	GC1'	GC1''	GC2'	GC2''	RO'	RO''	RP'	RP''
		[ng/L]									
Lindane		20	<20	<20	<20	<20	<20	<20	<20	<20	<20
Heptachlor		200	<50	<50	420	<50	<50	70	<50	<50	<50
Dieldrin		10	<10	<10	270	<10	100	70	30	<10	<10
Endrin		10	<10	<10	<10	<10	<10	20	100	<10	<10
Chlorpyrifos		30	<30	<30	<30	<30	<30	40	<30	<30	<30
Endosulfan α		5	<5	<5	230	<5	<5	60	<5	<5	<5
Endosulfan β		5	<5	<5	<5	<5	<5	80	40	<5	<5
p,p'-DDD		10	<25	230	400	240	<25	620	220	220	<25
p,p'-DDE		10	80	80	25	80	<25	110	80	80	<25
p,p'-DDT		10	<10	260	310	<10	<10	500	<10	<10	<10
PeCB	1st	7	<7	<7	<7	40	30	<7	<7	<7	<7
	2nd		15	10	26	11	9	9	34	14	7
HCB	1st	10	10	<3	<3	50	30	30	<3	<3	<3
	2nd		7	<3	8	<3	<3	<3	8	<3	<3
DBP	1st	800	<10	<10	<10	426	<10	<10	<10	<10	<10
	2nd		215	920	1250	860	1040	1220	1840	1160	1160
DEHP	1st	1300	150	152	670	2170	646	220	530	270	117
	2nd		1340	750	2630	760	550	830	1670	390	770

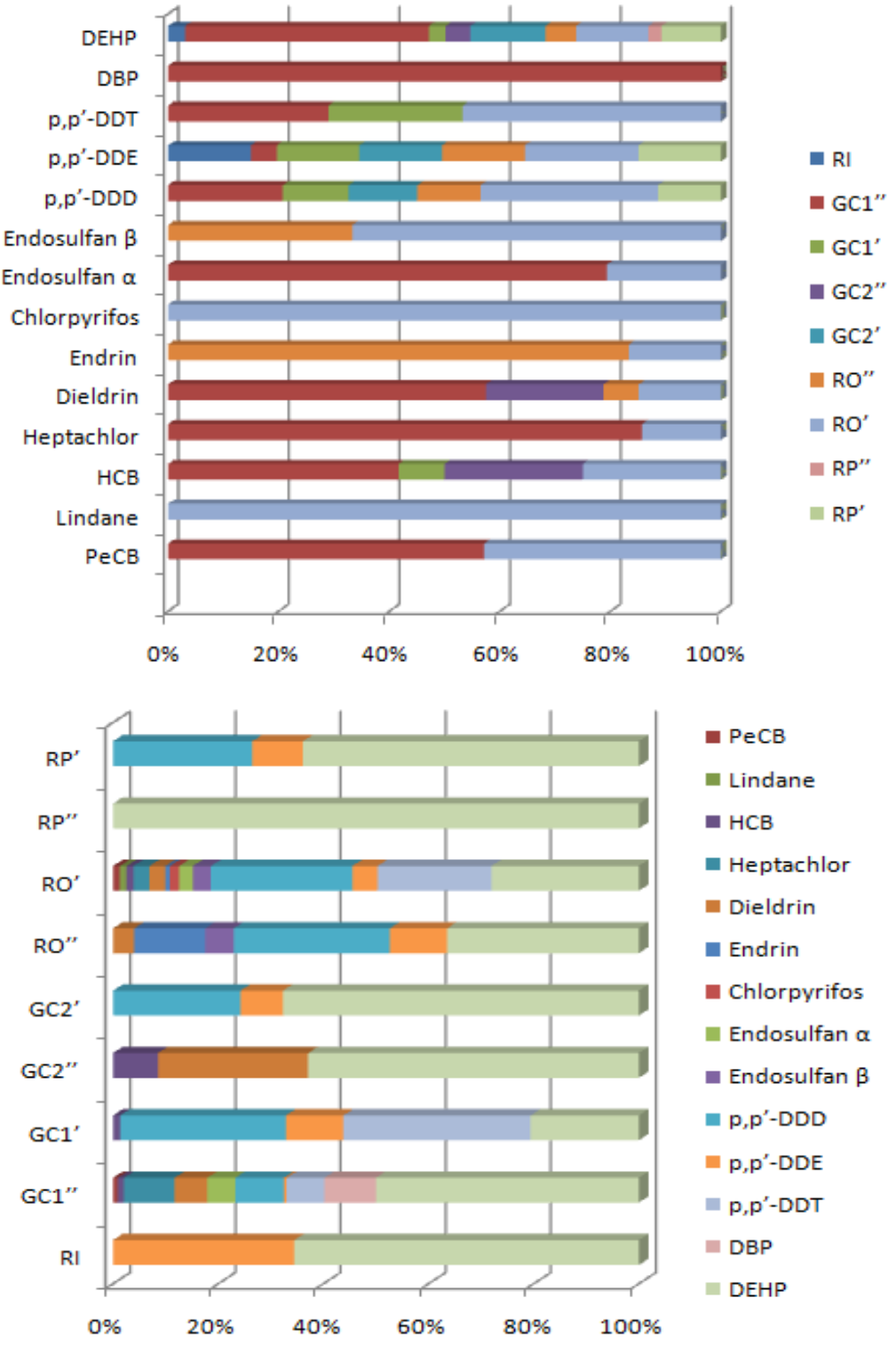


Figure 5.5 Concentration in percentiles of detected substances on locations

In Figure 5.5 concentration of detected substances in percentiles is shown, and in Figure 5.6 in ng/L.

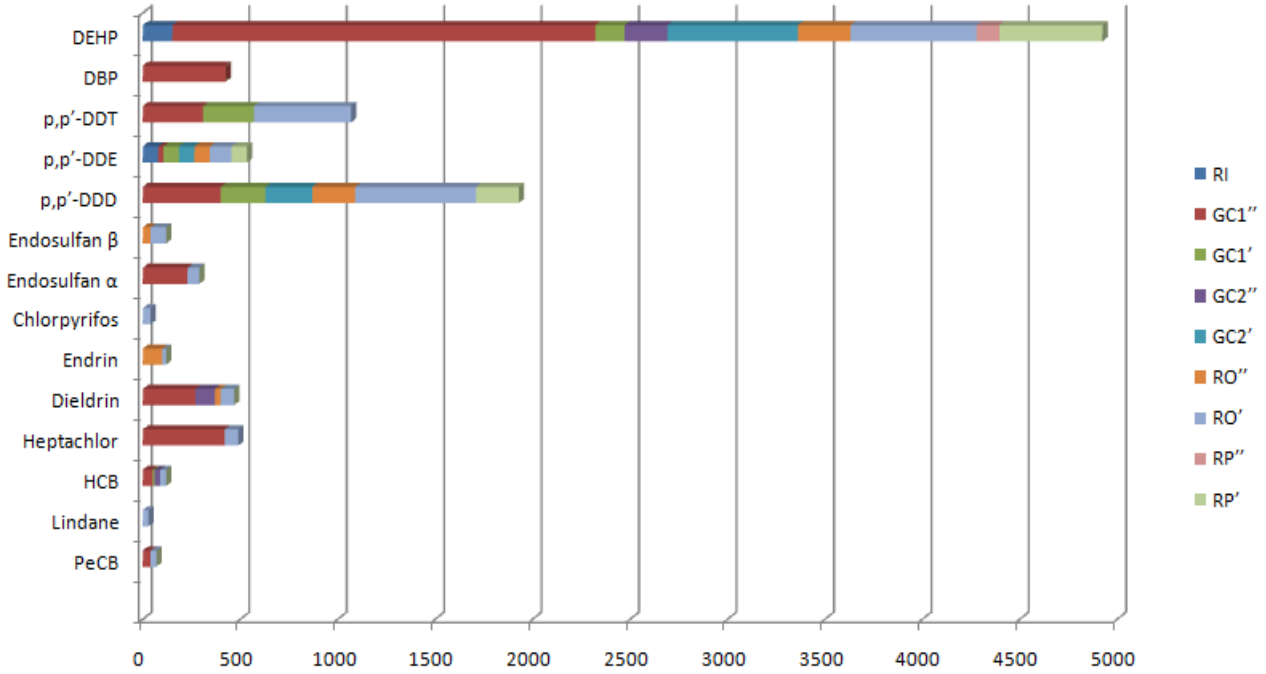


Figure 5.6 Concentrations in ng/L of detected substances on locations

5.4.2. Hormones

During the 3rd Joint Danube Survey several samples were found to be cytotoxic in the MELN assay at a REF of 300 and above.

The MELN test is an estrogen receptor transactivation assay, assessing the activity of (anti)estrogenic compounds. Therefore only effects observed at a REF of 100 or below were considered as positive in the assessment of estrogenic activity.

Estrogenic activity was found in several of the JDS samples, at non cytotoxic concentrations. The most active samples were JDS 22 (downstream of Budapest), 27 (Hercegszanto), 29 (Drava (rkm 1.4)), 30 (Downstream Drava (Erdut/Bogojevo)), 32 (Upstream Novi-Sad), 41 (Velika Morava), where concentrations of estradiol-equivalents (E2-EQ) are in the 0.01-0.1 ngE2-EQ/L range. However, the values are considered preliminary *(Modified from JDS3 Report 2015)*.

The significant quantity of the hormones androstane-17-one, -cholestan-3-ol, cholest-5-en-3-ol, cholestan-3-one,cholestane, 3- hydroxy-,(3.β, 5.α)-,stigmast-5-en-3-ol and coprostanol were detected in urban effluent at the sampling points of GC1 and GC2. The presence of hormones is mainly highlighting the impact of faecal pollution due to the lack of the WWTP. The hormones were only detected above the LOD but it was not possible to quantify them *(Sremački eta al. 2015).*

In sampling point GC2' estriol has been detected in concentration of 4.1±0.5 ng/mL. For the detection and quantification of estrogens standard mixture was used, consisting of 36

analytes, 16 pesticides, 9 hormones and 11 sterols. Chromatograms with results of estrogens are in Annex III as a Figure III.1.

5.4.3. Illicit drugs

The research for detection of illicit drugs started in 2013 with the target of 13 compounds, from which 5 substances emerged above the limits of quantification – cocaine and benzoylecgonine (BE), amphetamine, MDMA and THC-COOH. In Table 5.7 Measured concentration levels of selected illicit drugs in Novi Sad in 2013 and 2016.

Table 5.7 Measured concentration levels of selected illicit drugs in Novi Sad in 2013 and 2016 (Data source SCORE 2017, Ort et al. 2014).

Illicit drug	**Measured concentrations in [ng/L] 2013.**						LOQ
	Thu	**Fri**	**Sat**	**Sun**	**Mon**	**Tue**	
Cocaine	4.4	17	14	5.3	n.d.	16	12
BE	14	25	60	43	43	39	10
Amphetamine	31	50	61	60	52	95	25
MDMA	n.d.	n.d	16	24	14	22	20
THC-COOH	442	368	435	500	345	284	10
	Measured concentrations in [ng/L] 2016.						LOQ
	Wed	**Thu**	**Fri**	**Sat**	**Sun**	**Mon**	**Tue**	
Cocaine	24	41	46	66	62	51	35	12
BE	92	113	157	242	302	231	114	10
Amphetamine	80	70	108	172	180	159	103	25
MDMA	36	42	106	190	448	318	87	20

During the determination of the concentration levels of 5 selected analyte, THC COOH analyte singled out as very interesting for the site of Novi Sad. As for analyte THC-COOH was recorded significantly higher concentration at the site of Novi Sad in all samples, compared to the region, but also in relation to all selected sites in Europe that were selected as part of the research.

Significantly higher concentration of all selected analytes was detected from Friday through Sunday, in range of 2 to 4 times higher concentrations were detected during the weekend, which can be expected result as most of the population is using recreationally, just over the weekend.

The results of target analyses for ID are shown in Table 5.7, Figures 5.7 and 5.8.

Figure 5.7 Measured concentration levels of selected illicit drugs in Novi Sad in 2013 and 2016

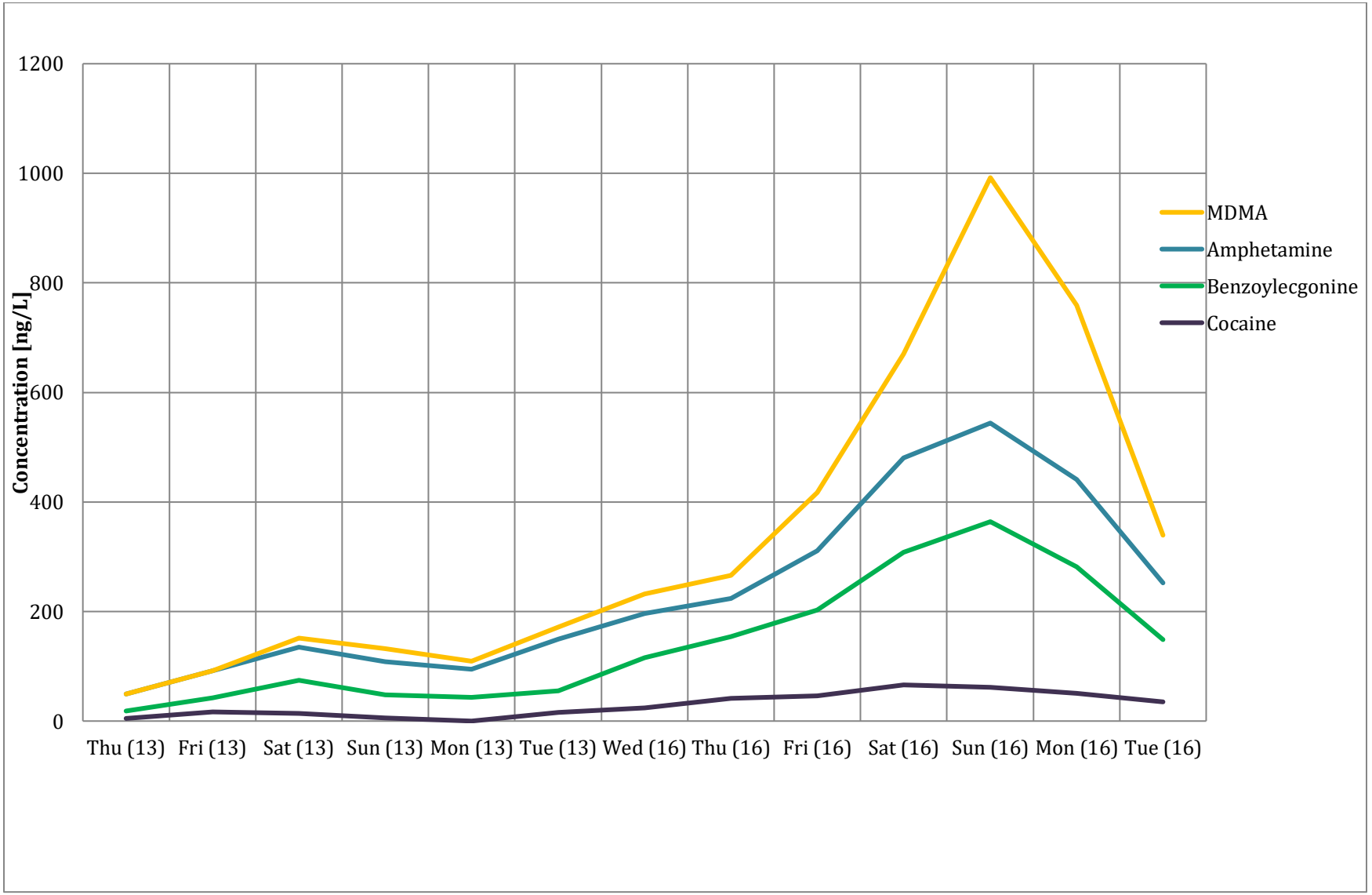
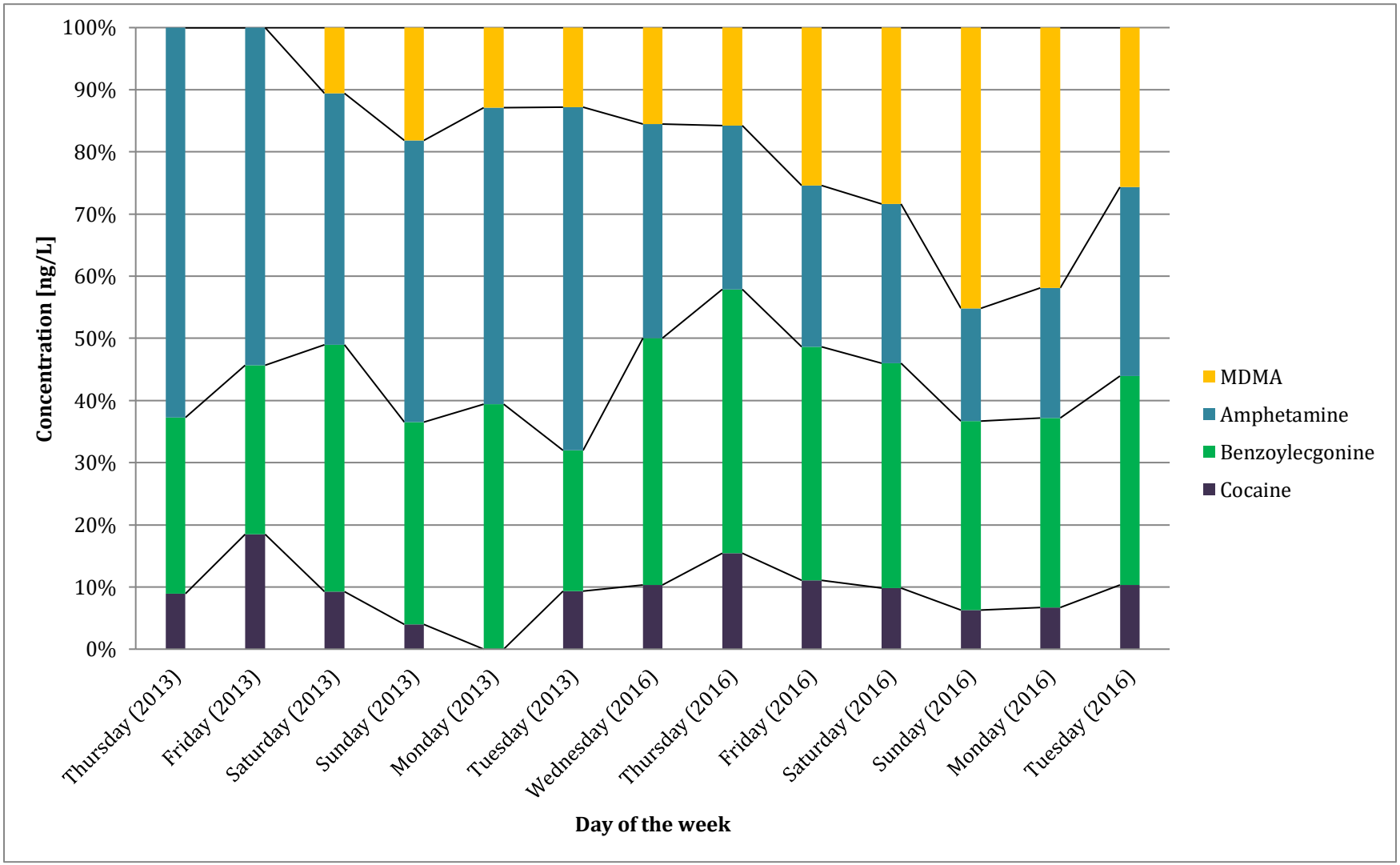


Figure 5.8 Measured concentration levels of selected illicit drug



5.4.4. Summary results of target analyses

During the research period 69 compounds from screening analyses results were selected for target analysis, 21 were found in concentrations above LOQ in surface water and wastewater collected on 9 sampling sites. PeCB, HCB, endosulfan α and γ, DEHP, DDT, dieldrin and endrin, are in Annex II of Directive 2008/105/EC for Priority Substances and Certain Other Pollutants. Dieldrin and endrin, are in the Annex A, DDT is in Annex B and lindane is on the list of nine new chemicals of the Stockholm Convention. DBP is included as plasticizer in the list of NORMAN emerging substances; therefore deserves special attention during monitoring and research. In the Figure 5.9 summary of detected concentrations for selected analytes is shown.

In abovementioned Serbian national legislation, there are limits for some of the selected and detected substances, but not for all. For aldrine, dieldrine, endrine and isodrine there is a summary AA concentrations 10 ng/L and no LV. For the atrazine and endosulfane, the LV is 2000 and 10 ng/L, respectively.

Total DDT and p,p'-DDT have no designated limiting values, just AA concentration, 25 and 10 ng/L, respectively. The term total DDT (tDDT) refers to the sum of the concentrations of p,p'-DDT, o,p'-DDT, p,p'-DDE, o,p'-DDE, p,p'-DDD, and o,p'-DDD, from which o,p'-DDE (non hydrosolubile)and p,p'-DDE are the most abundant, non-hydro soluble and persistent in the environment. For HCHs the LV is 40 ng/L. For phthalates, hormones, illicit drugs and many other emerging substances there are no limiting values in legislation or in any other documents.

Endosulfan, dieldrin and endrin were detected in significantly higher concentrations in sampling location War Island and also downstream of discharge, in ranges of 0 to 230 ng/L, 270 ng/L and 100 ng/L, respectively. Dieldrin was also detected in surface water sample downstream of discharges GC1, GC2. PeCB, lindane and HBC were detected in wastewater at discharging location of War Island, while PeCB was also detected in surface water downstream of GC2, and HCB was found in concentrations 5 times higher than AA EQS in surface water downstream of discharges GC1 and GC2. All the detected pesticides downstream of discharges of wastewater, if not detected in wastewater can be the consequence of agricultural runoff priory to Novi Sad area. PeCB, lindane and HCB were detected in ranges of 0 to 40 ng/L, 30 ng/L and 50 ng/L, respectively. Concentrations of organochlorine pesticides (p,p'-DDD, p,p'-DDE, p,p'-DDT, Endosulfan α and β) were higher in wastewater collected at discharge RO' compared to Danube surface water 100 meters downstream, while the opposite ratio was found at discharge form collector GC1. These pesticides were determined in concentrations over 10 times higher than annual average values in EU countries. The concentrations of DDT and its metabolites varied from 0 to 620 ng/L, where the highest concentrations very detected for metabolite p,p'-DDD.

DEHP concentrations were the highest and have been found in all analysed waste and surface water samples. The higher concentration in samples of wastewater was recorded in industrial parts of the sewerage system (GC2' and RO'). In surface waters samples collected 100 meters downstream of sewage discharges GC2, RP and RO concentrations of DEHP were 3 times lower than at the discharges indicating the dilution in River flow. DEHP concentration varied from 117 to 2170 ng/L.

In sampling point GC2' estriol has been detected in concentration of 4.1 (±0.5) ng/mL.

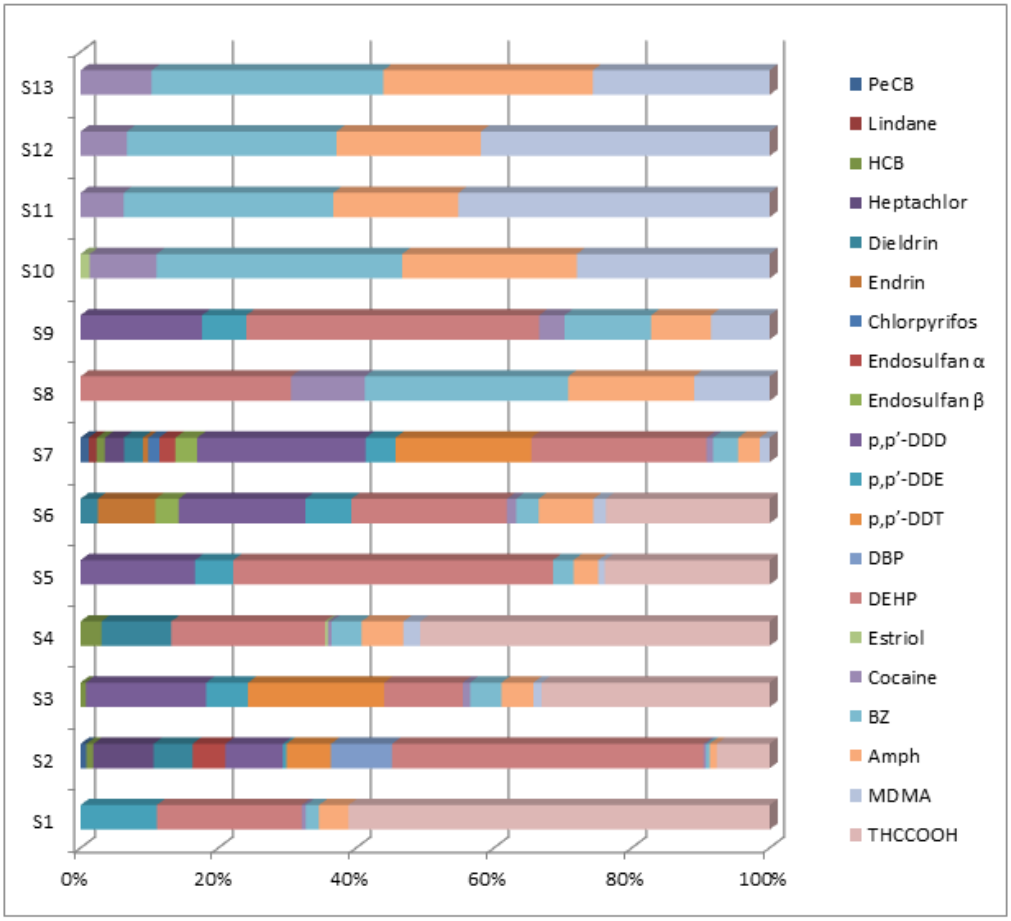


Figure 5.9 Summary of concentrations for selected analytes for Novi Sad

Significantly higher concentration of illicit drugs was detected from Friday through Sunday, in range of 2 to 4 times higher concentrations were detected during the weekend, which can be expected result as most of the population is using as the leisure activity during the weekend. Cocaine and BE were identified in ranges of 0 to 66 ng/L and 14 to 302 ng/L, respectively. AMPH and MDMA were detected in the ranges of 31 to 108 ng/L and 0 to 448 ng/L, respectively. THC-COOH, as the least expensive drug and the most widely consumed of all, had a range from 284 to 500 ng/L, and had the most constant concentration.

5.5. Modules of emerging xenobiotics detection in urban wastewater

The research and detection of emerging xenobiotics is a complex process that requires methodical and structural approach to obtain relevant results and conclusions.

Figure 5.10 Modules of emerging xenobiotics detection in urban wastewater

According to all obtained results and after finalization of the research on emerging substances, especially xenobiotics in urban wastewater it is clear that there is optimal algorithm for analyses of EmS. Figure 5.10 represents the modules of emerging xenobiotics detection in mixed urban wastewater. The modules of emerging xenobiotics detection and identification presents an interactive process, subjected to the changes throughout the process, but the planning and research, as well as preparation is an important segment that is interacting with all the other modules and affecting the process. Every module has to be evaluated before, throughout and after the process to be objective and representative, as the results can alter decisions and procedures of higher instances and processes.

5.6. Calculation of emerging xenobiotics load and risk assessment (RQ index) around 1255 km of Danube River flow

In almost all samples pesticides and hormones were detected, and with the high possibility of interrelation and interaction between this two groups of substances, could pose a problem during the wastewater treatment process. Merely the frequency of allopregnane identification in almost all the samples (6 out of 8) in every sampling campaign, suggested the bothersome fact that it can be used as an indicator of presence of hormones. Mass discharge loads/emissions of persistent chemicals from the Danube River to the Black Sea is calculated with the flow average rate in both sampling campaigns of 2488 m^3/s at the 1255 km of Danube in Novi Sad focused on phenolic compounds, pesticides and trace metals measured above AA EQS in Danube surface water. Total organic content of MUWW was measured as BOD_5, COD and PPC. The highest mass loads of 91 and 60.4 t/year were obtained for p,p'-DDE and di-n-butyl phthalate, respectively. For every measured parameter, overall and specific xenobiotics, calculation of load was conducted for dry and wet weather, the lowest and the highest flow through the collector GC1 and GC2, 560 L/s (48 384 m^3/day), real flow (at the time of sampling) and 4080 L/s (352 512m^3/day), respectively. The number of inhabitants for GC2 is 133 245, and the whole system has 321 282 inhabitants connected to the sewerage system according to the PUC's estimate of connected households. The loads of illicit drugs and hormones are shown in Table 5.8 in ng/day/inhabitant, and for pesticides and phthalates it is represented in µg/day/inhabitant as the concentrations were higher. Also, the concentration of illicit drugs and hormones was followed on sampling points at collectors GC1 and GC2 as mainly domestic parts of sewerage system of Novi Sad. In Figures 5.11 and 5.12 the load of illicit drugs and calculated risk quotient, respectively.

For the risk assessment and calculation of RQ the equation 4.3 was selected, as the measured environmental concentrations (MEC) for selected pollutants were obtained. The RQ greater than 1 is indicating substances with potential eco-toxicity and need for frequent monitoring.

Table 5.8 Load of organochlorine pesticides, estrogens and illicit drugs

Compound	Flow	Load [ng/inh/day]												
		day 1	day 2	day 3	day 4	day 5	day 6	day 7	day 8	day 9	day 10	day 12	day 13	day 14
E3	dry	n.d.	n.d.	n.d.	0.06	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	wet	n.d.	n.d.	n.d.	10.85	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Cocaine	dry	1.6	6.17	5.08	1.92	n.d.	5.81	3.61	18.633	20.91	29.97	28.18	23.18	15.91
	real	8.36	16.42	12.07	0.48	n.d.	17.43	10.91	18.633	20.91	29.97	28.18	23.18	15.91
	wet	11.64	44.98	37.04	14.02	n.d.	42.33	26.33	44.99	50.47	72.42	68.03	55.96	38.4
BE	dry	5.08	9.08	21.79	15.61	15.61	14.16	13.86	51.36	71.35	109.98	137.25	104.99	51.81
	real	26.6	24.15	51.75	3.9	44.33	42.49	41.81	51.36	71.35	109.98	137.25	104.99	51.81
	wet	37.04	66.14	158.74	113.76	113.76	103.18	100.94	123.98	172.26	265.52	331.36	253.45	125.08
Amphetamine	dry	11.26	18.16	22.15	21.79	18.88	34.5	12.05	31.81	49.08	78.17	81.81	72.26	46.81
	real	58.9	48.31	52.61	5.45	53.61	103.49	36.36	31.81	49.08	78.17	81.81	72.26	46.81
	wet	82.01	132.28	161.38	158.74	137.57	251.33	87.78	76.8	118.5	188.72	197.5	174.46	113.01
MDMA	dry	n.d.	n.d.	5.81	8.76	5.08	7.99	5.42	19.09	48.18	86.35	203.61	144.52	39.54
	real	n.d.	n.d.	13.8	2.18	14.43	23.97	16.36	19.09	48.18	86.35	203.61	144.52	39.54
	wet	n.d.	n.d.	42.33	63.5	37.04	58.2	39.5	46.08	116.3	208.47	491.55	348.91	95.46
THC-COOH	dry	160.5	133.63	157.96	181.56	125.28	103.13	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	real	839.76	355.55	375.15	45.39	355.7	309.38	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	wet	1169.4	973.58	1150.83	1322.8	912.73	751.35	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Compound		Sampling point - load [µg/ inh/day]								
		RI	GC1'	GC1''	GC2'	GC2''	RO'	RO''	RP'	RP''
Lindane		0.00	0.00	0.00	0.00	0.00	33.95	0.00	0.00	0.00
Heptachlor		0.00	0.00	475.23	0.00	0.00	79.21	0.00	0.00	0.00
Dieldrin		0.00	0.00	305.51	0.00	113.15	79.21	33.95	0.00	0.00
Endrin		0.00	0.00	0.00	0.00	0.00	0	0.00	0.00	0.00
Chlorpyrifos		0.00	0.00	0.00	0.00	0.00	45.26	0.00	0.00	0.00
Estragol		0.00	0.00	260.25	0.00	0.00	67.89	0.00	0.00	0.00
Endosulfan α		0.00	0.00	0.00	0.00	0.00	90.52	45.26	0.00	0.00
Endosulfan β		0.00	260.25	452.61	271.562	0.00	701.54	248.93	248.93	0.00
p,p'-DDD		90.521	90.52	28.29	90.521	0.00	124.47	90.52	90.52	0.00
p,p'-DDE		0.00	294.19	350.77	0.00	0.00	565.76	0.00	0.00	0.00
p,p'-DDT		0.00	0.00	482.02	0.00	0.00	0.00	0.00	0.00	0.00
HCB	**1st**	0.00	56.58	11.32	33.95	0.00	0.00	0.00	33.95	0.00
	2nd	0.00	60.89	0.00	69.59	0.00	0.00	0.00	69.59	0.00
PeCB	**1st**	0.00	45.26	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	2nd	1.13	1.70	1.24	2.94	1.02	1.02	1.58	3.84	0.79
DBP	**1st**	171.99	2455.38	169.73	0.00	758.11	305.51	599.7	730.96	132.39
	2nd	104.04	24.31	97.25	141.35	137.96	117.61	131.18	208.07	131.18
DEHP	**1st**	147.1	0.00	192.36	0.00	418.66	305.51	214.99	565.76	113.15
	2nd	65.24	116.56	66.11	228.78	72.20	47.84	33.93	145.27	66.98

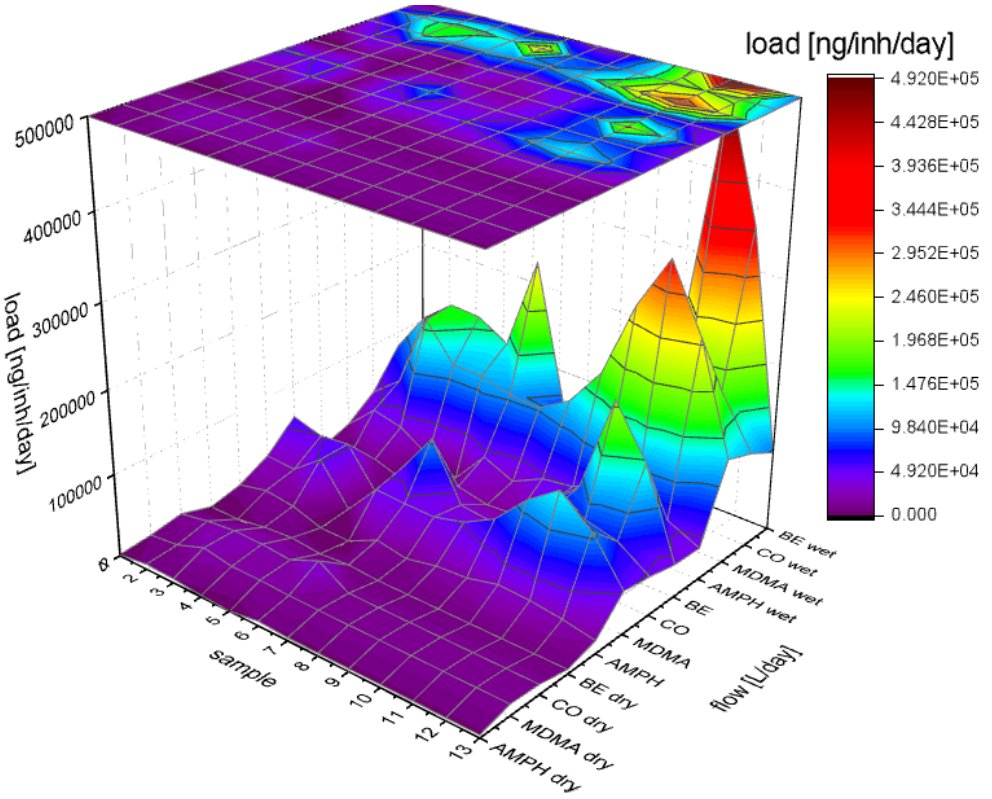


Figure 5.11 Load of illicit drugs for dry, wet weather capacities of sewerage system (minimum, real and maximum flow)

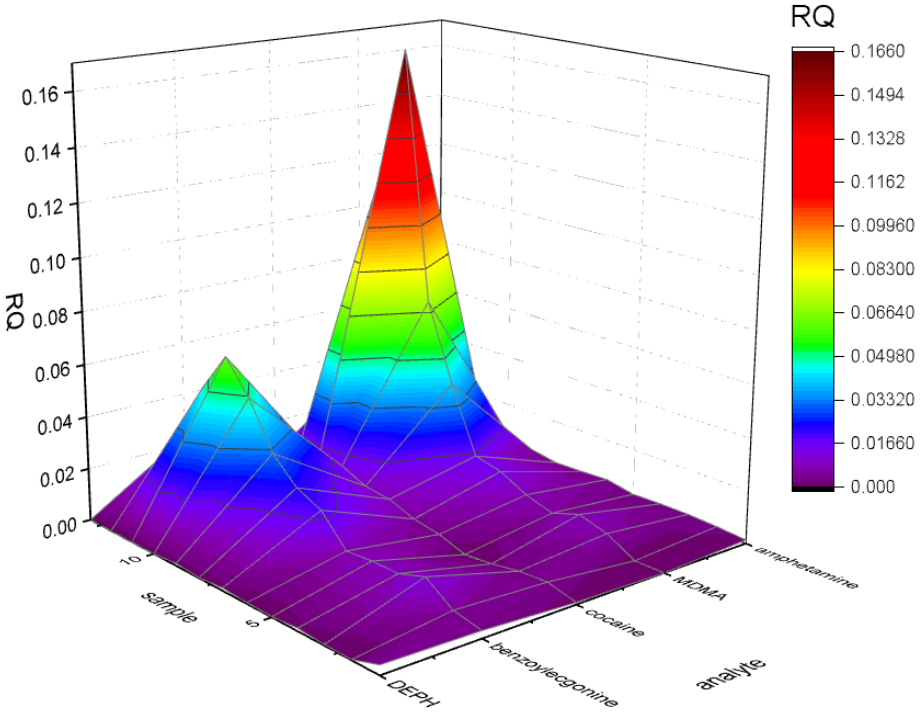


Figure 5.12 Risk quotient for illicit drugs per sample

Significantly higher load of illicit drugs was observed during the 2016 campaign which can be observed in Table 5.8. In the Figures 5.11 and 5.12, as well as in the Table 5.8 loads of illicit drugs are shown in measured values and real flow, as well as for the maximum flow for wet weather days and minimum flow for dry weather days.

Amphetamine values ranged from 11.26 to 251.33 ng/inh/day, MDMA 5.08 to 491.55 ng/inh/day, cocaine from 1.6 to 72.42 ng/inh/day and BE from 5.08 to 331.36 ng/inh/day. Based on reported excretion values of CO and BE after cocaine consumption (1–9 % and 35–54%, respectively) *(Postigo et al., 2008)* and their molar mass relation, the excreted CO/BE ratio should range from 0.02 to 0.27.

For the results of Novi Sad in 2013 this range is significantly varying from 0.12 to 0.68, in 2016 the range is more stable and shows significant correlation with recommendation form literature with values ranging from 0.21 to 0.36.

THC-COOH was detected only in the first campaign from 2013, the cannabis metabolite was not detected in 2016 campaign, as the identification of THC-COOH loads in wastewater poses some sampling and analytical challenges, the analyte is excluded from comments for further calculations and conclusions.

Load for the other emerging xenobiotics, pesticides and phthalates was calculated only for real measured values, and it ranges from 28.29 μg/inh/day for p,p'-DDD to 2455.38 μg/inh/day for DBP.

Illicit drugs did not show potential for environmental toxicity, during the research, but the illicit drugs show a significant increase in concentrations form 2013 to 2016. The average concentration of every analyte increased for one order of magnitude, 2 times for amphetamine, 5 times for cocaine and BE, and 14 times for MDMA, showing significant directly proportional growth of RQ. In the Table 5.9 calculated values of RQ are shown.

Tables are structured for the purpose of better visibility of location sampling, detection and quantification of selected analytes, and risk quotient manifestation.

Table 5.9 Calculation of risk quotient

Compound	Risk quotient - RQ												
	Sample												
	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12	D13
Amphetamine	0.001	0.002	0.003	0.003	0.002	0.004	0.003	0.003	0.005	0.007	0.008	0.007	0.004
MDMA	0.000	0.000	0.006	0.009	0.005	0.008	0.013	0.016	0.041	0.07	0.166	0.118	0.032
Cocaine	0.001	0.003	0.003	0.001	0.000	0.003	0.005	0.008	0.009	0.013	0.013	0.01	0.007
BE	0.003	0.005	0.012	0.009	0.009	0.008	0.019	0.023	0.032	0.049	0.062	0.047	0.023

		RI	GC1'	GC1"	GC2'	GC2"	RO'	RO"	RP'	RP"
Lindane		0.000	0.000	14000.000	0.000	0.000	2333.333	0.000	0.000	0.000
Endrin		0.000	0.000	108.000	0.000	40.000	28.000	12.000	0.000	0.000
Dieldrin		0.000	0.000	0.000	0.000	0.000	8.000	40.000	0.000	0.000
Endosulfane α		0.000	0.000	0.000	0.000	0.000	1.212	0.000	0.000	0.000
Heptachlor		0.000	0.000	460.000	0.000	0.000	120.000	0.000	0.000	0.000
p',p'-DDD		0.000	0.359	0.625	0.375	0.000	0.969	0.344	0.344	0.000
p',p'-DDE		0.133	0.133	0.042	0.133	0.000	0.183	0.133	0.133	0.000
p',p'-DDT		0.000	1.444	1.722	0.000	0.000	2.778	0.000	0.000	0.000
Chlorpyriphos		0.000	0.000	0.000	0.040	0.030	0.000	0.000	0.000	0.000
E3		0.015	0.010	0.026	0.011	0.009	0.009	0.034	0.014	0.007
HCB	1st	0.769	0.000	0.000	3.846	2.308	2.308	0.000	0.000	0.000
	2nd	0.538	0.000	0.615	0.000	0.000	0.000	0.615	0.000	0.000
PeCB	1st	0.000	0.000	0.000	0.040	0.030	0.000	0.000	0.000	0.000
	2nd	0.015	0.010	0.026	0.011	0.009	0.009	0.034	0.014	0.007
DBP	1st	0.000	0.000	0.000	0.426	0.000	0.000	0.000	0.000	0.000
	2nd	0.215	0.920	1.250	0.860	1.040	1.220	1.840	1.160	1.160
DEPH	1st	0.115	0.117	0.515	1.669	0.497	0.169	0.408	0.208	0.090
	2nd	1.031	0.577	2.023	0.585	0.423	0.638	1.285	0.300	0.592

** The RQ index was calculated for every substance that PNEC value was available in literature.*

According to the obtained concentrations and PNEC values, amphetamine is not a reason of concern for the environment in these levels, as the concentration has to be about 130 times higher in water to represent a risk factor. The cocaine is a specific substance as it is excreted in low concentrations from the body in the first place, and the focus will be allocated to its metabolite BE.

The concentrations of MDMA and BE are really close to posing the risk. Average concentration of MDMA has a trend of growth of 14 times in 3 years, from 0.13 ng/L in 2013 to 1.76 ng/L in 2016, and the RQ over 1 is obtain by 6 times higher concentration than measured in 2016. For the BE is the similar case, but it is necessary for the concentration to grow at least 16 times than measured in 2016 which was 0.18 ng/L, while in 2013 it was 0.04 ng/L.

Pesticides lindane, endrin, dieldrin, endosulfane α, heptachlor, heptachlor, p',p'-DDT, hexachlorobenzene show a significantly high calculated RQ on almost every location where these substances were detected, which is a reason for concern due to direct endocrine disrupting effects, bioaccumulation characteristics and toxicity in aquatic systems.

Hexachlorobenzene shows significant RQ during the first target analysis, but during the second one it doesn't show any potential risk. This substance should be subjected to further research, if the decision on its priority should be established.

The calculated RQ for DDT was over 1 on every location it was detected GC1', GC" and RO'. The metabolites of p',p'-DDT, p',p'-DDE and p',p'-DDD do not show high RQ on any location were identified, but their presents is significant for the determination of pollution time, making a conclusion is the pollution recent or historic.

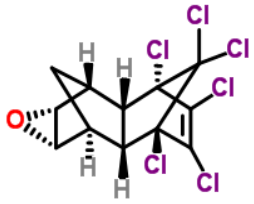
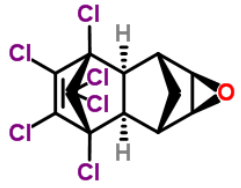
Phthalates, for the most part in the first target campaign did not show high risk potential, except DEHP on the location GC2, in wastewater samples, as the AA EQS and PNEC values are still high for detected concentrations. These substances are frequently detected in aquatic systems, so the further research was necessary.

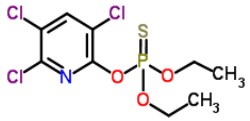
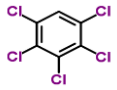
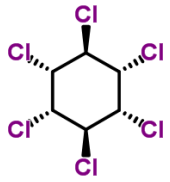
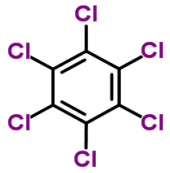
The second target campaign showed a significantly different image, where DEHP showed a high RQ on the locations of RI, GC1" and RO", for surface water of River Danube, which is the reason for concern, as the concentration is peaking after the GC1 discharge of wastewater and downstream after the RO discharge. DBP shows a high RQ in every surface water sample except on the location RI, suggesting there is no previous contamination. DBP is also showing the high risk factor in wastewater samples on location sites RO' and RP', and on the locations GC1' and GC2' is just below the limit for significant RQ.

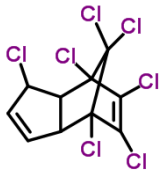
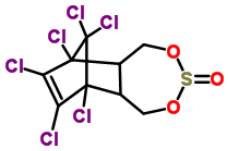
5.7. Characteristics and treatment possibilities of selected emerging xenobiotics

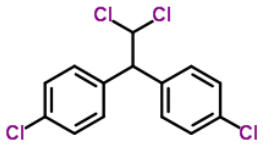
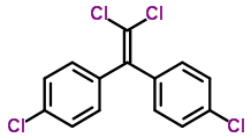
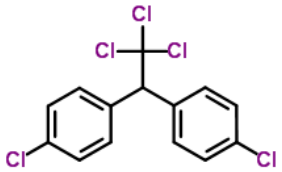
The key physicochemical properties and treatment possibilities for targeted and detected pollutants are shown in Table 5.10.

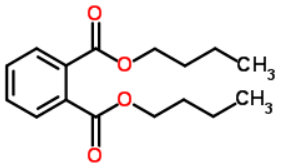
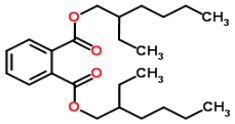
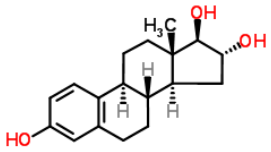
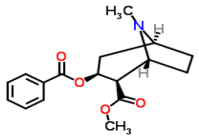
Table 5.10 Characteristics and treatment possibilities of selected emerging xenobiotics (www.chemspider.com)

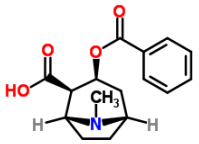
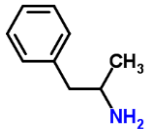
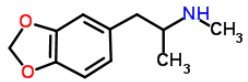
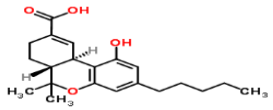
Name	Structural formula	Group	Properties	Chemical oxidation
Dieldrin		Organochlorine pesticide, Annex "List of Other pollutants" Directive 76/464/EEC, amended by Directive 88/347/EEC,90/415/EEC	Reported estrogenic and cancerogenic effects. Water Solubility at 25 °C is 0.1455 mg/L, log K_{ow}= 5.20; Aqueous Base/Acid-Catalyzed Hydrolysis (25 °C) [HYDROWIN v1.67]: Total Ka at 25 °C: 1.713E-002 L/mol·s, Ka Half-Life[6] at pH 7: 12.823 years; Log BCF from RBM[5] = 3.304 (BCF = 2014); Removal WWt: TR[1]: 83.13 %, TBD[2]: 0.71 %; TSA[3]: 82.35 %; TtA[4]: 0.07 %	Hydroxyl Radicals Reaction: HL[6] = 1.163 days (12-hr day; 1.5· 10^6 OH/cm^3); HL = 13.950 h; Ozone Reaction: HL = 320.239 days (at 7· 10^{11} mol/cm^3);
Endrin			Swallowing large amounts can cause convulsions and lead to death. The US EPA does not classify endrin as a carcinogen (not enough information), but the regulations maximum level contamination in drinking water is 0.0002 mg/L. It doesn't break down easily in water, and accumulates in the tissues of aquatic organisms. Endrin has not been produced or available in the United States since 1986. Log Kow ranges from 3.21 to 5.34, making this substance bioaccumulative and proven to be highly toxic to aquatic organisms.	

Chlorpyrifos		Organochlorine pesticide	Water Solubility at 25 °C is 0.357 mg/L, log K_{ow}= 4.96; Log BCF from RBM= 3.119 (BCF = 1316); Removal WWt: TR[1]: 76.41 %, TBD[2]: 0.67 %; TSA[3]: 75.71 %; TtA[4]: 0.03 %	Hydroxyl Radicals Reaction: HL = 0.117 days (12-hr day; $1.5 \cdot 10^6$ OH/cm^3); HL = 1.400 h;
PeCB		Organochlorine fungicide	Banned via Stockholm Convention on POPs (SCPOPs) in 2011. Water Solubility at 25 °C is 0.831 mg/L, log K_{ow}= 5.18 (https://pubchem.ncbi.nlm.nih.gov/)	
Lindane		Organochlorine insecticide (acaricide) – vector control	Banned via SCPOPs in 2011, can be produced only for the use in treating lice. Water Solubility at 25 °C is 4.044 mg/L, log K_{ow}= 4.14; Log BCF from RBM[5] = 2.488 (BCF = 307.5); Removal WWt: TR[1]: 36.98 %, TBD[2]: 0.37 %; TSA[3]: 36.43 %; TtA[4]: 0.18 %	Hydroxyl Radicals Reaction: HL = 18.659 days (12-hr day; $1.5 \cdot 10^6$ OH/cm^3)
HCB		Organochlorine fungicide	Banned via SCPOPs in 2011. Water Solubility at 25 °C is 0.1922 mg/L, log K_{ow}= 5.73; Log BCF from RBM[5] = 3.712 (BCF = 5153); Removal WWt: TR[1]: 91.09 %, TBD[2]: 0.73 %; TSA[3]: 88.03 %; TtA[4]: 2.33 %	Hydroxyl Radicals Reaction: HL = 633.112 days (12-h day; $1.5 \cdot 10^6$ OH/cm^3); The sorbed fraction to airborne particles may be resistant to atmospheric oxidation

Heptachlor (HPC)		Organochlorine insecticide	Banned via SCPOPs in 2011. Water Solubility at 25 °C is 0.0276 mg/L, log K_{ow}= 6.10; Log BCF from RBM[5] = 3.997 (BCF = 9931); Removal WWt: TR[1]: 92.57 %, TBD[2]: 0.77 %; TSA[3]: 91.59 %; TtA[4]: 0.22 % The US EPA has limited the sale to the specific application. The amount present in different foods is regulated *(Metcalf 2002).*	Hydroxyl Radicals Reaction: HL = 0.221 days (12-h day; $1.5 \cdot 10^6$ OH/cm^3), 2.649 h; Ozone reaction: HL = 0.06 days (at $7 \cdot 10^{11}$mol/cm^3) HL = 1.375 h; The sorbed fraction to airborne particles may be resistant to atmospheric oxidation
Endosulfan		Organochlorine pesticide POPs) Annex II "List of PaPHs" Directive 2008/105/EC	Reported estrogenic and cancerogenic effects. Water Solubility at 25 °C is 1.487 mg/L, log K_{ow}= 3.83; Log BCF from RBM[5] = 2.249 (BCF = 177.5);Removal WWt: TR[1]: 25.20 %, TBD[2]: 0.26 %; TSA[3]: 22.37 %; TtA[4]: 2.57 %	Hydroxyl Radicals Reaction: HL = 1.182 days (12-hr day; $1.5 \cdot 10^6$ OH/cm^3); HL = 14.179 h; Ozone Reaction: same as Dieldrin

p,p'-DDD		Organochlorine pesticide, Annex "List of Other pollutants" Directive 76/464/EEC, amended by Directive 88/347/EEC,90/415/EEC	Reported estrogenic and cancerogenic effects. Water Solubility at 25 °C is 0.068 mg/L, log K_{ow}= 6.02; Log BCF from RBM[5] = 3.935 (BCF = 8618); Removal WWt: TR[1]: 92.24 %, TBD[2]: 0.77 %; TSA[3]: 91.46 %; TtA[4]: 0.01 %	Hydroxyl Radicals Reaction: HL= 2.462 days (12-hr day; $1.5 \cdot 10^6$ OH/cm^3); HL = 29.546 h;
p,p'-DDE			Reported estrogenic and cancerogenic effects. Water Solubility at 25 °C is 0.026 mg/L, log K_{ow}= 6.51; Log BCF from RBM[5] = 4.313 (BCF = 0.0002); Removal WWt: TR[1]: 93.45 %, TBD[2]: 0.78 %; TSA[3]: 92.66 %; TtA[4]: 0.01 %	Hydroxyl Radicals Reaction: HL = 1.440 days (12-hr day; $1.5 \cdot 10^6$ OH/cm^3); HL = 17.275 h; Ozone Reaction: HL = 2.224 days (at $7 \cdot 10^{11}$ mol/cm^3); HL = 53.373 h
p,p'-DDT			Reported estrogenic and cancerogenic effects. Water Solubility at 25 °C is 0.0073 mg/L, log K_{ow}= 6.91; Log BCF from RBM[5] = 4.621 (BCF = 0.0004); Removal WWt: TR[1]: 93.80 %, TBD[2]: 0.78 %; TSA[3]: 93.02 %; TtA[4]: 0.00 %	Hydroxyl Radicals Reaction: HL = 3.114 days (12-hr day; $1.5 \cdot 10^6$ OH/cm^3); HL = 37.365 h;

DBP		Plasticizer – insect attractant	The use in cosmetics and nail polishes, is banned in the EU under Directive 76/768/EEC 1976. The use of DBP has been restricted in the EU for use in children's toys since 1999. Water Solubility at 25 °C is 2.351 mg/L, log K_{ow}=4.5, Log BCF from RBM = 2.765 (BCF = 582.1). Removal In WWT: TR: 56.06 %; TBD: 0.52 %; TSA: 55.49 %; TtA: 0.04 %	Hydroxyl Radicals Reaction: HL = 1.153 days (12-hr day; 1.5· 10^6 OH/cm^3); HL = 13.836 h; The sorbed fraction to airborne particles may be resistant to atmospheric oxidation
DEHP		Plasticizer and acaricide	EU banned the use of DEHP along with several other phthalates in toys for young children in 2004.Water Solubility at 25 °C is 0.27 mg/L, log K_{ow}=7.6 (https://pubchem.ncbi.nlm.nih.gov/)	Possible high removal rate via ozonisation or other chemically enhanced oxidation process.
E3		Natural estrogen, NORMAN list	Water Solubility at 25 °C is 440.8 mg/L, log K_{ow}= 2.45; Log BCF from RBM[5] = 1.187 (BCF = 15.36); Removal WWT: TR[1]: 2.96 %, TBD[2]: 0.10 %; TSA[3]: 2.86 %; TtA[4]: 0.00 %	Hydroxyl Radicals Reaction: HL = 0.083days (12-hr day; 1.5· 10^6 OH/cm^3); HL = 0.999 h;
Cocaine		Illicit drug, NORMAN list	Water Solubility at 25 °C (mg/L): 1298, log K_{ow} used: 2.30; Log BCF from RBM = 1.071 (BCF = 11.78) Removal in WWT - TR: 2.64 %; TBD: 0.10 %; TSA: 2.54 %; TtA: 0.00 %	Hydroxyl Radicals Reaction: HL= 0.202 days (12-hr day; 1.5· 10^6 OH/cm^3); HL = 2.421 h;

BE		Illicit drug, NORMAN list	Water Solubility at 25 °C (mg/L): 1605, log K_{ow} used: -1.23; Log BCF from RBM = 0.500 (BCF = 3.162) Removal in WWT - TR: 1.85 %; TBD: 0.09 %; TSA: 1.75 %; TtA: 0.00 %	Hydroxyl Radicals Reaction: HL = 0.196 days (12-hr day; $1.5 \cdot 10^6$ OH/cm^3); HL = 2.350 h;
Amphetamine		Illicit drug, NORMAN list	Water Solubility at 25 °C (mg/L): 0.00028, log K_{ow} used: 1.76; Log BCF from RBM = 0.500 (BCF = 3.162) Removal in WWT - TR: 2.14 %; TBD: 0.09 %; TSA: 1.98 %; TtA: 0.06 %	Hydroxyl Radicals Reaction: HL = 0.217 days (12-hr day; $1.5 \cdot 10^6$ OH/cm^3); HL = 2.601 h;
MDMA		Illicit drug, NORMAN list	Water Solubility at 25 °C (mg/L): 5413, log K_{ow} used: 2.28; Log BCF from RBM = 1.058 (BCF = 11.43) Removal in WWT - TR: 2.60 %; TBD: 0.10 %; TSA: 2. 15 %; TtA: 0.00 %	Hydroxyl Radicals Reaction: HL = 0.079 days (12-hr day; $1.5 \cdot 10^6$ OH/cm^3); HL = 0.943 h;
THC-COOH		Illicit drug, NORMAN list	Cannabinoids, a1,s amphetamine-like drugs, showed significant differences between compounds, with elimination rates above 90% in the case of THC and OH-THC, and poor removal (48%) in the case of THC-COOH that presented often higher concentrations at the STP outlet than at the inlet *(Postego C. et al. 2010).*	

1 –TR is total removal,
2 – TBD is total biodegradation,
3 – TSA is total sludge adsorption,
4 – TtA is total to air,
5 –RBM regression/based model
7 – HL is half-life ($t_{1/2}$)

5.8. Statistical evaluation of obtained data

Physicochemical properties, eco-toxicological characteristics and wastewater treatment and removal possibilities have been selected for further statistical analysis by Person correlation, Multivariate Analysis - Principal Component Analysis (PCA) and Hierarchal Cluster Analysis (HCA). The PCA was conducted to evaluate the correlation and the relationship of eco-toxicological (PNEC) and chemical parameters (half-life, K_{ow}, BCF) to treatment possibilities , removal or transportation from wastewater to other environmental media (TR, TSA, TtA, TBD). The analyses were conducted on the bases of Table 5.11 in which is shown the excerpt of Table 5.10.

The Pearson correlation is +1 in the case of a direct linear correlation, -1 in the case of a inverse linear relationship, and some value in the open interval (-1, 1) in all other cases, indicating the degree of linear dependence between the variables. As it approaches zero there is weak or no relationship. The closer the coefficient is to either −1 or 1, the stronger the correlation between the variables. In this case it is evident that the parameters show high correlation, except PNEC and TtA. In Table 5.12 and in Figure 5.13 the results of correlation are shown. PNEC is showing negative correlation, meaning invers linear relationship, but TtA is showing the lowest correlation factors in relation to all other characteristics.

PCA analysis is used for the reduction of a large number of data streams in which selected variables based on similar characteristics are categorized in factors. Original set of variables is transformed to new set of reduction variables. PCA analysis was detected a group of variables that are similar and therefore have great interdependence. Used measured of sampling adequacy in this paper is 0.7 and determines the strength of the correlation between variables. Two factors are obtained on the basis of which we observe the impact of significant physicochemical parameters of selected analytes. These factors explain around 89 % of total variance, and therefore the most important information contained in these data. The analyses has shown 2 principal components, where in the first characteristics of pesticides showed significant correlation and in the second illicit drugs, which was expected. Unexpected result was the significantly higher, but still lover than 0,7 vales was the correlation of E3 to organic pesticides (for example endosulfan α), showing the possible interaction or mimicking effects in the environment.

Hierarchical cluster analyses were conducted to group characteristics according to similarity between the selected most important physicochemical parameters. The results are shown in Annex IV in Table IV.1.

Table 5.11 Excerpt of significant characteristics of analytes prepared for statistical analyses

	PNEC	HL	Kow	BCF	TR	TSA	TtA	TB
AMPH	0.023	0.001	1.76	0.5	0.2	0.19	0.01	0.09
MDMA	0.0027	0.001	2.28	1.058	0.3	0.2	0.01	0.1
CO	0.0049	0.001	2.3	1.071	0.3	0.25	0.01	0.1
BE	0.0049	0.001	-1.23	0.5	0.2	0.18	0.01	0.09
Endrin	2.50E-06	5	5.34	3.34	8.3	8.2	0.07	0.71
Lindane	2.00E-06	4	4.14	2.488	3.7	3.6	0.18	0.37
Ealpha	5.00E-07	0.08	3.83	2.249	2.5	2.2	2.57	0.26
HCB	1.30E-05	6	5.73	3.712	9.1	8.8	2.33	0.73
Heptachlor	3.00E-08	0.1	6.1	3.997	9.3	9.2	0.22	0.77
Chlorpyrifos	3.30E-05	0.2	4.96	3.119	7.6	7.6	0.03	0.67
DBP	0.001	0.5	4.5	2.765	5.6	5.5	0.04	0.52
Estriol	6.00E-05	0.02	2.45	1.187	3	0.3	0.01	0.1
DDD	6.40E-04	5	6.02	3.935	9.2	9.2	0.01	0.77
DDT	1.80E-04	5	6.91	4.621	9.4	9.3	0.01	0.78
DDE	6.00E-04	5	6.51	4.313	9.3	9.26	0.01	0.78

Table 5.12 Results of correlation analysis of physicochemical properties and WWT possibilities and removal for selected analytes

	PNEC	**HL**	**Kow**	**BCF**	**TR**	**TSA**	**TtA**	**TB**
PNEC	1	-0.3389	-0.3389	-0.5715	-0.5314	-0.484	-0.1928	-0.4936
HL	-0.3389	1	0.6633	0.71289	0.6972	0.7041	0.1365	0.6977
Kow	-0.4892	0.6633	1	0.9533	0.9113	0.9062	0.1352	0.9094
BCF	-0.5715	0.71289	0.9533	1	0.9659	0.9707	0.1219	0.9719
TR	-0.5314	0.6972	0.9113	0.9659	1	0.986	0.0649	0.9842
TSA	-0.484	0.7041	0.9062	0.9707	0.986	1	0.0609	0.9994
TtA	-0.1928	0.1365	0.1352	0.1219	0.0649	0.0609	1	0.0573
TB	-0.4936	0.6977	0.9094	0.9719	0.9842	0.9994	0.0573	1

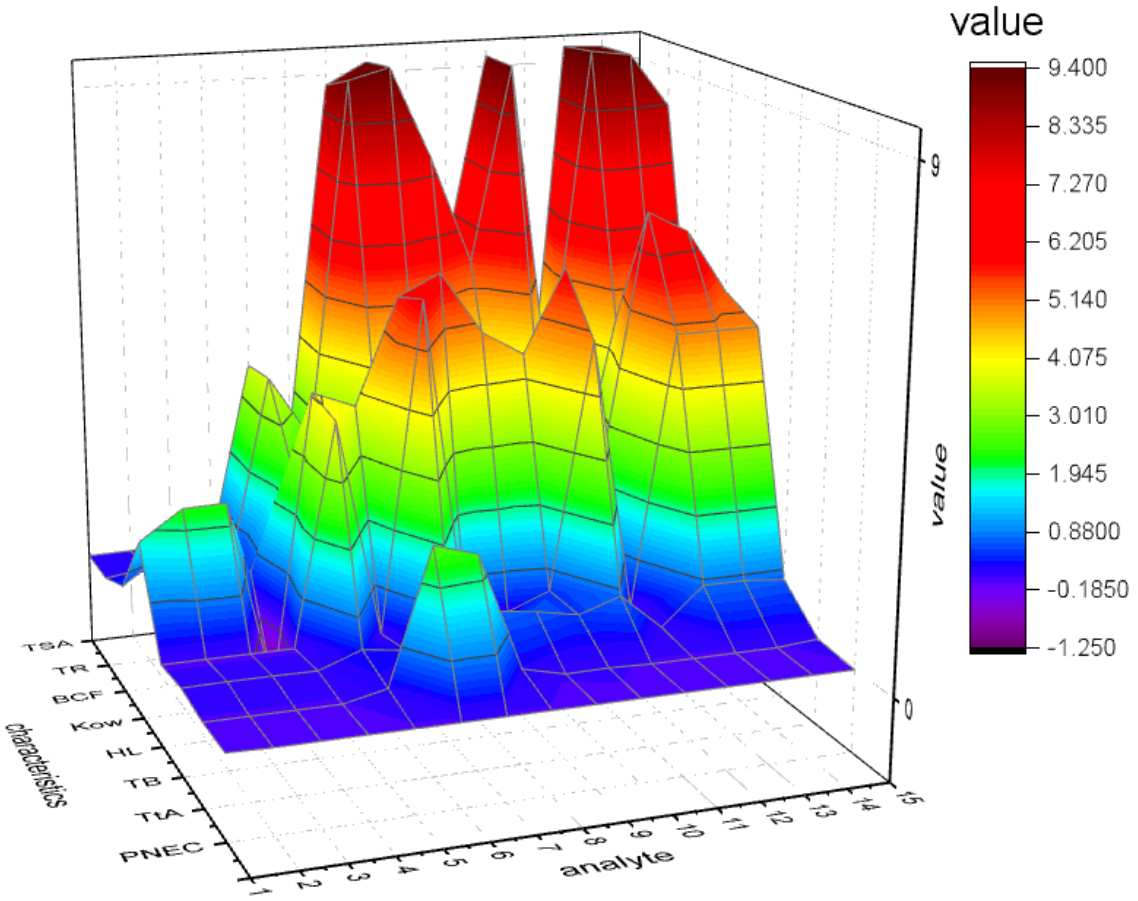


Figure 5.13 3-D representation of analyte characteristics from Table 5.10

5.9. Propositions for WWTPs suitable for MUWW of Novi Sad

According to obtained results of wastewater quality – basic physicochemical parameters and specific pollutants (emerging xenobiotics – endocrine disrupters (pesticides, hormones) and illicit drugs) there are some indications of the best available technologies and techniques for wastewater treatment in Novi Sad.

The obtained data of wastewater quality generated in city of Novi Sad suggests the need for specific processes of treatment. Detection and concentration of emerging xenobiotics in mixed urban effluent imply that an adequate advanced treatment process will be necessary to incorporate in WWTP.

The literature research shows that emerging xenobiotics, especially EDCs can be degraded in the environment through processes of adsorption, UV photolysis, chemical oxidation or hydrolysis. The following treatment techniques may be employed in wastewater and drinking water treatment facilities to actively reduce emerging xenobiotics concentrations prior to human consumption or discharge to the environment. The most effective treatment methods in terms of maximum possible removal were granular activated carbon and advanced UV oxidation with hydrogen peroxide *(Castiglioni et al. 2006; Jones et al. 2007; Bolong et al. 2009; van Nuijs et al.*

2009;Bertanza et al. 2010; Postigo et al. 2010; Janna 2011;). Biological removal as second stage removal has shown to be partially effective for the removal of emerging xenobiotics, so the advanced processes have to be incorporated.

The detection of specific emerging substances leads to selection of ASP as the secondary biological treatment. For the purpose of better removal of specific priority, priority hazardous and emerging substances and a wider scope of specific organic non-biodegradable pollutants it is to enhance the treatment phases.

Considering all the priority and "newly recognized" pollutants in wastewater, there are two the best available techniques to improve primary treatment. The first approach is advanced oxidation processes (AOP) using Fenton processes via ferrous iron and hydrogen peroxide or hydroxide radicals. The second approach is chemically enhanced primary treatment (CEPT) including coagulation or flocculation during primary settlement.

The Fenton's oxidation process is very potent and cost-effective method for the removal of PhPPs and EmS from wastewaters. It is also an effective form of pre-treatment is it transforms parent compounds into biodegradable and less toxic metabolites. As a suggested AO process it can also be a form of CEPT, as the Fenton's reagent has not only oxidation function but also coagulation by the formation of ferric-hydroxy complexes, and removal of the remaining pollutants after oxidation *(Benatti and Tavares 2012).* Transfer of electrons enables formation of $^{\cdot}OH$, that can interact with reagents instead of target pollutants, which indicates that the optimal molar ratio of iron ion to H_2O_2 has to be experimentally determined. Fe^{3+} can form sludge at a specific treatment conditions, therefore it has to be separately disposed of, increasing the treatment complexity and operational costs. Also the formation of $OH\cdot$ radicals is the most effective in an acidic conditions, as a result, the application of Fenton reaction for wastewater treatment is modified in practice to the Fenton-like, photo-Fenton and electro-Fenton process. During the photo-Fenton process, UV light is applied coupled with the classical Fenton reaction system. Higher efficiency and lower cost WWTP for removal of many recalcitrant organic contaminants can be developed by integrating photo-Fenton system of AOP with biological treatment (ASP) and utilization of solar energy for higher energy efficient and independent system (*Barbusinski 2009, Pouran et al. 2015)*.

Enhanced removal of solids and BOD in primary treatment is a crucial issue for energy management in WWTPs, as the solids have a high energy value, which can be re-used trough anaerobic digestion or other thermal conversion process and used for energy purposes.

Adsorption process is recommended as a BAT for advanced treatment of secondary effluent for the purpose of removal residual persistent organic PhPP and emerging substances and their metabolites. Filtration via ACP results in the removal of the non-biodegradable organic compounds, toxins, colour compounds, aromatic compounds, chlorinated/halogenated organic compounds, and pesticides from WW. Activated carbon can also be economically and environmentally sustainable option as the new techniques of production of AC from waste biomass is a feasible option for utilisation in Novi Sad, as Vojvodina Region is an agricultural region of the country. In the Figure 5.14 the diagram of the location specific design of WWTP for Novi Sad is shown.

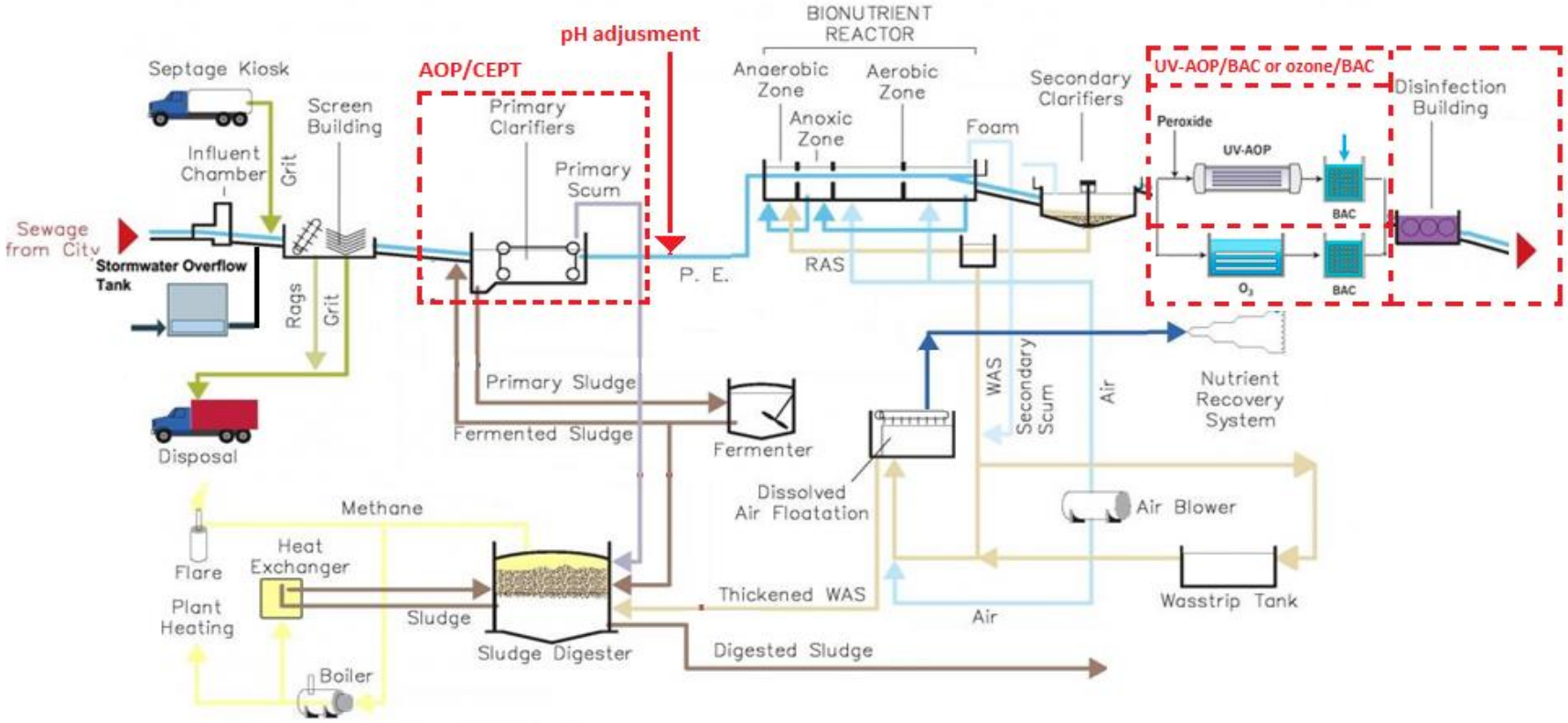


Figure 5.14 Diagram of the recommended processes for the selected location specific type and quality of WW with the accent on detected pollutants (Modified http://www.mybusinessprocess.net/wp-content/uploads/2013/01/Wastewater-Treatment-Process-002.jpg)

6. Comprehensive conclusions and implementation possibilities of research results with future research

The field of water protection is grounded on acquired specific data, for the purpose of precise and location-specific design of water monitoring and treatment systems. With the development of science and technology predicted limitations and allowable concentrations of primary and specific pollutants that are discharged into the natural recipients are continuously and steady lowering. Requirements for treatment and removal of PhPPs and even EmS from wastewater are becoming more rigorous and inflexibile. If detected, PhPPS and EmS, pose an obligation for the treatment, an in the best possible scenario, removal from generated wastewater.

One of the significant conclusions within the doctoral research is the correct and optimal methods and methodological procedures that have to be used and implemented in temporal segments and modules that have to be followed in order to obtain relevant data. It is significant to follow the modules algorithm so it is secured the representable sample and precise analysis, which are the two most important premises for obtaining the relevant data needed for accurate design of the WWTP. Environmental sample analysis, water samples, has to start with the detailed sampling planning, selection and investigation of representative sampling location and any special equipment needed for sampling, for the benefit of developing an optimal sampling strategy. Procedures on the sampling locations include measurements on the location, environmental conditions on site and transportation of the samples to the Laboratory, which is followed by stabilisation and/or preparation and storage of the samples. After the proper preparation of the samples depending on the analysis that has to be performed and regulation and/or EPA standard requirements analysis can be performed immediately after or the samples can be stored for a period of time. The first parameters measured are basic physicochemical parameters, followed by screening analysis via sensitive and modern technique (GC-MS, HPLC-MS). After the screening analysis, basic selective prioritization process and selection of target analytes was performed. The final phase within the last module is publication and presentation of the results which is important segment for further development of the field and decision making process. This phase was only possible after tedious evaluation of data obtained through screening and target analysis, risk assessment and statistic evaluation.

Screening analysis is the predictive part of the water and wastewater studies, in environmental research field. It is the first step toward a selection of significant target pollutants. It should be well planned and programmed. Best available technology for performing the screening analysis is GC-MS or HPLC-MS. For this research GC-MS was used, ad screening analysis was adapted to suit the specific needs of selected samples, locations and purpose. During the adaptation of screening analyses method the conclusion emerged that the contaminants contained in “dirty samples” from mixed

urban wastewater interfere with subtle preparation techniques for analytical chromatographic determination. It was necessary to prepare the sample for GC-MS with the lowest possible loss of analytes and high extraction efficiency. The LLE is confirmed to be the optimal preparation technique for selected type of sample, and can be completed in optimal time range, from 2-3 hours. During the research activities, other sample preparation techniques (SPE and SPME) were shown to be less effective for screening in terms of pick height, width and sharpness. The most suitable solvent for high organic content wastewater sample is dichloromethane, considering the physicochemical characteristics (boiling point, polarity and extraction efficiency) and lipophilic characteristics of analytes. In the case of DCM the peaks of chromatograms developed were "cleaner" and better separated with minimal interferences.

Target analysis of selected substances presents the important data about the quality and quantity of wastewater that is directly, without any treatment, being discharged into the natural recipient, which is the typical example for Serbia. The information and data obtained from target analysis should be used for key prioritisation processes, and design of regulatory limiting values that should be incorporated into the laws, by-laws and conventions. Different techniques were used to obtained concentration data for selected emerging xenobiotics – illicit drugs (cocaine, benzoylecgonine, MDMA, methamphetamine, amphetamine, THC-COOH), organochlorine pesticides (DDT and it's metabolites, atrazine, dieldrin, edndrin, chlorpyrifos, endosulfan α and β, pentachlorobenzene, trifluralin, hexachlorobenzene, heptachlor), plasticizers (DBP and DEHP) and estrogens (17β-estradiole, 17α-estradiole, mestranol, estriol, estrone). Results obtained through target analyses were used to calculate daily load and RQ, for the purpose of risk assessment. The target analysis enables better perception of complex wastewater quality and impact onto the natural recipient, allowing load calculation and assumption, risk assessment and by identification of pollutants better selection and design of conventional and advanced technological wastewater treatment processes.

Most of the emerging substances are persistent in environment, if not parent substances then their metabolites. The ones that are not characterized as typical persistent, have newly recognized pseudo-persistency, namely the kinetic rate of input is significantly higher than the rate of the output. The main problem if those chemicals are not removed from wastewater before discharge into natural recipient is that they can easily enter food chain, trough accumulation in aquatic organisms and agricultural use of surface water from recipient. This is why the location specific advanced treatment of wastewater is a serious and eminent stage of wastewater treatment. It is concluded that some form of advanced chemical oxidation process should be employed as a part of primary treatment, so the high degradation and transformation of emerging and priority substances should be reached. In this sense, it is also confirmed that ASP should be employed with the possibility of enhancing the process with sorption via alternative adsorbents.

Endosulfan, dieldrin and endrin were in significantly higher concentrations in sampling location War Island and also downstream of discharge, in ranges from 0 to 230 ng/L, 0 to 270 ng/L and 0 do 100 ng/L, respectively.

Dieldrin was also detected in surface water sample downstream of discharges GC1, GC2, which can suggest infiltration of agricultural runoff priory to Novi Sad area.

PeCB, Lindane and HBC were identified in wastewater at discharging location of War Island, while PeCB was also in surface water downstream of GC2 and HCB was found in concentrations up to 5 times higher than AA EQS in surface water downstream of discharges GC1 and GC2. All the detected pesticides downstream of discharges of wastewater, if not detected in wastewater can be the consequence of agricultural runoff priory to Novi Sad area. PeCB, lindane and HCB were detected in ranges of 0 to 40 ng/L, 0 to 30 ng/L and 0 to 50 ng/L, respectively. Concentrations of organochlorine pesticides (p,p'-DDD, p,p'-DDE, p,p'-DDT, Endosulfan α and β) were higher in wastewater collected at discharge RO' compared to Danube surface water 100 meters downstream, while the opposite ratio was found at discharge from collector GC1. These pesticides were determined in concentrations over 10 times higher than annual average values in EU countries.

The concentrations of DDT and its metabolites varied from 0 to 620 ng/L, where the highest concentrations were detected for metabolite p,p'-DDD (Rothane). According to the concentration ratio of p,p'-DDD and p,p'-DDT at sampling points with the highest concentrations indicated significant historical contamination in addition to recent pollution with p,p'DDT. p,p'-DDD, p,p'-DDE and p,p'-DDT at GC1' and RO' were determined in concentrations more than 8 times higher than AA EQS values in EU. p,p'-DDE were detected at sampling site RI, and the concentrations did not amplify further downstream the Danube near Novi Sad.

DEHP concentrations were the highest and have been found in all analysed waste and surface water samples. The higher concentration in samples of wastewater was recorded in industrial parts of the sewerage system (GC2' and RO'). During the first target campaign in surface waters samples collected 100 meters downstream of sewage discharges GC2, RP and RO concentrations of DEHP were 3 times lower than at the discharges indicating the dilution in River flow. DEHP concentration varied from 117 to 2 170 ng/L.

During the second target campaign phthalates showed significantly higher concentrations, on all sampling sites, DBP in range from 250 to 1840 ng/L and DEHP in range from 550 to 2 630 ng/L.

In sampling point GC2' hormone estriol has been detected in concentration of 4.1 (±0.5) ng/mL. Significantly higher concentration of illicit drugs was detected from Friday to Sunday; the detected concentrations were 2 to 4 times higher during the weekend, which can be expected as most of the population is using IDs jus for the relaxation and festivity time during the weekend.

Cocaine and its metabolite BE were identified in ranges of 0 to 66 ng/L and 14 to 302 ng/L, respectively. Amphetamine was detected in the range of 31 to 108 ng/L, and ecstasy (MDMA) in 0 to 448 ng/L. THC-COOH, as the least expensive drug, and the most widely consumed of all had a range from 284 to 500 ng/L, and had the most constant concentration.

For the risk assessment and calculation of RQ the real risk ratio was selected. The RQ values lower than 1 is indicated that illicit drugs do not have high potential for environmental toxicity for selected locations. However RQ value higher than 1 for most of the pesticides and estrogen (Lindane, endosulfane α, hexachlorobenzene, heptachlor, chlorpyriphos, p',p'-DDD, estriol), shows the reason for concern, need for frequent monitoring and appointment of limiting values, especially due to the mimicking effect.

Most of the detected pesticides (DDT and metabolites, endrine, lindane and others) are banned for use by either Stockholm Convention or other regulatory body (EPA, EEA, WHO) for their toxicity, cancerogenity or other hazardous and health risk characteristics, which is the reason why the PNEC values are low, and all the calculated RQ values are significantly higher the detected illicit drugs.

The high concentrations of organochlorine pesticides alongside estrogens detected provides one more layer to the risk assessment as the organochlorine pesticides are known estrogenic mimics, which represents another aspect of environmental and health concern.

Although, phthalates have a short half-life in surface water due to rapid photo and microbial degradation, which is favoured in the summer, due to the higher temperatures, higher photo-catalytic and microbiological activities, the overload of the contamination tended to resist degradation in the surface water. Pollutant detected during the target analyses represent the substances included into the monitoring plan of an early warning system (EWS). The EmS with the high RQ or PhPPs that are already implemented into the regulation guidelines are the substances that should be incorporated into the selected location EWS.

In the case of Novi Sad those are organochlorine pesticides selected during this research, as all were detected in concentration higher than AA EQS on at least one location. The most thought-provoking are DDT and its metabolites, their high residual (historic) concentrations, but particularly the more recent inputs.

Phthalates, for the most part in the first target campaign did not show high risk potential, except DEHP on the location GC2, in wastewater samples, as the AA EQS and PNEC values are still high for detected concentrations. As these substances are frequently detected in aquatic systems further research was necessary.

The second target campaign showed a significantly different situation where DEHP showed a high RQ on the locations of RI, GC1" and RO", for surface water of River Danube. DBP shows a high RQ in every surface water sample except on the location RI, suggesting there is no previous contamination. DBP is also showing the high risk factor

in wastewater samples on location sites RO' and RP', and on the locations GC1' and GC2' is just below the limit for significant risk factor.

The suggested processes for the MUWW from Novi Sad is foreseen as a advanced technological treatment process and estimated to be installed as the second phase of a CWWTP due to the obtained data of quality of WW on the specific location. As the plans for the WWTP propose the connection of other more agricultural and rural settlements to the sewerage system the concentration of selected emerging xenobiotics studied in the thesis can only be enhanced, therefore verifying the necessity of the recommended advanced WWTp.

The primary recommendation is installation of the pilot plants on the CWWTP to experimentally confirm the optimal process and conditions for AOP/CEPT of primary effluent and ACP of secondary effluent. Considering all the priority and "newly recognized" pollutants in wastewater, there are two the best available techniques to improve primary treatment. The first approach is advanced oxidation processes (AOP) using Fenton processes via ferrous iron and hydrogen peroxide or hydroxide radicals. The second approach is chemically enhanced primary treatment (CEPT) including coagulation or flocculation during primary settlement. The Fenton's oxidation process is very potent and cost-effective method for the removal of PhPPs and EmS from wastewaters. It is also an effective form of pre-treatment is it transforms parent compounds into biodegradable and less toxic metabolites.

As a suggested AO process it can also be a form of CEPT, as the Fenton's reagent has not only oxidation function but also coagulation by the formation of iron-hydroxy complexes and removal of the remaining pollutants after oxidation. Sorption process is recommended as a BAT for advanced treatment of secondary effluent for the purpose of removal residual persistent organic PhPP and emerging substances and their metabolites. Filtration via ACP results in the removal of the non-biodegradable organic compounds, toxins, colour compounds, aromatic compounds, chlorinated/halogenated organic compounds, and pesticides from WW. Activated carbon can also be economically and environmentally sustainable option as the new techniques of production of AC from waste biomass is a feasible option for utilisation in Novi Sad, as Vojvodina Region is a fertile agricultural region of the country.

Enhanced removal of solids and BOD in primary treatment is a crucial issue for energy management in WWTPs, as the solids have a high energy value, which can be re-used trough anaerobic digestion or other thermal conversion process and used for energy purposes.

The results of the thesis could enable and facilitate the decision making process for the selection of the suitable advanced engineered wastewater treatment needed for specific location of Novi Sad, as the second phase of WWTP that is already in the process of planning. The results acquired during this doctoral research, identification and concentrations of toxic substances, as well as the review of possible and optimal

advanced technologies for further treatment will represent the crucial data for further development of the WWTP. The advanced technologies for treatment of wastewater discharged from Novi Sad Municipality are shown pressingly obligatory due to the fact that spatial positioning of water wells for drinking water productions are exceedingly close to the points of discharge, and the provided information on the wastewater quality during the research period.

Throughout the research, all provided hypotheses, objectives and goals were completed, realised and implemented.

Results of detected persistent pollutants and emerging xenobiotics and conclusions are clear, that on the specific location of Novi Sad it will be necessary to consider the advanced technological treatment processes for generated mixed urban wastewater. Future research should be concentrated to pilot plants (CEPT/AOP and sorption on alternative material activated carbon) on the site examining the efficiency of removal of emerging substances and priority and hazardous priority pollutants, and their operational and maintenance cost before application of selected process onto the wastewater. The results and conclusions of the doctoral research on PHPPs and EmS are opening the new visions, strategies and directions for further research activities, particularly in Serbia.

In the light of opening the Chapter 27 of Serbia acceptance negotiation with EU, Republic of Serbia will have great obligations to fulfil in the area of environmental protection, and the most important and mostly neglected area is protection of natural water, drinking water and wastewater treatment.

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8. Annex

Annex I

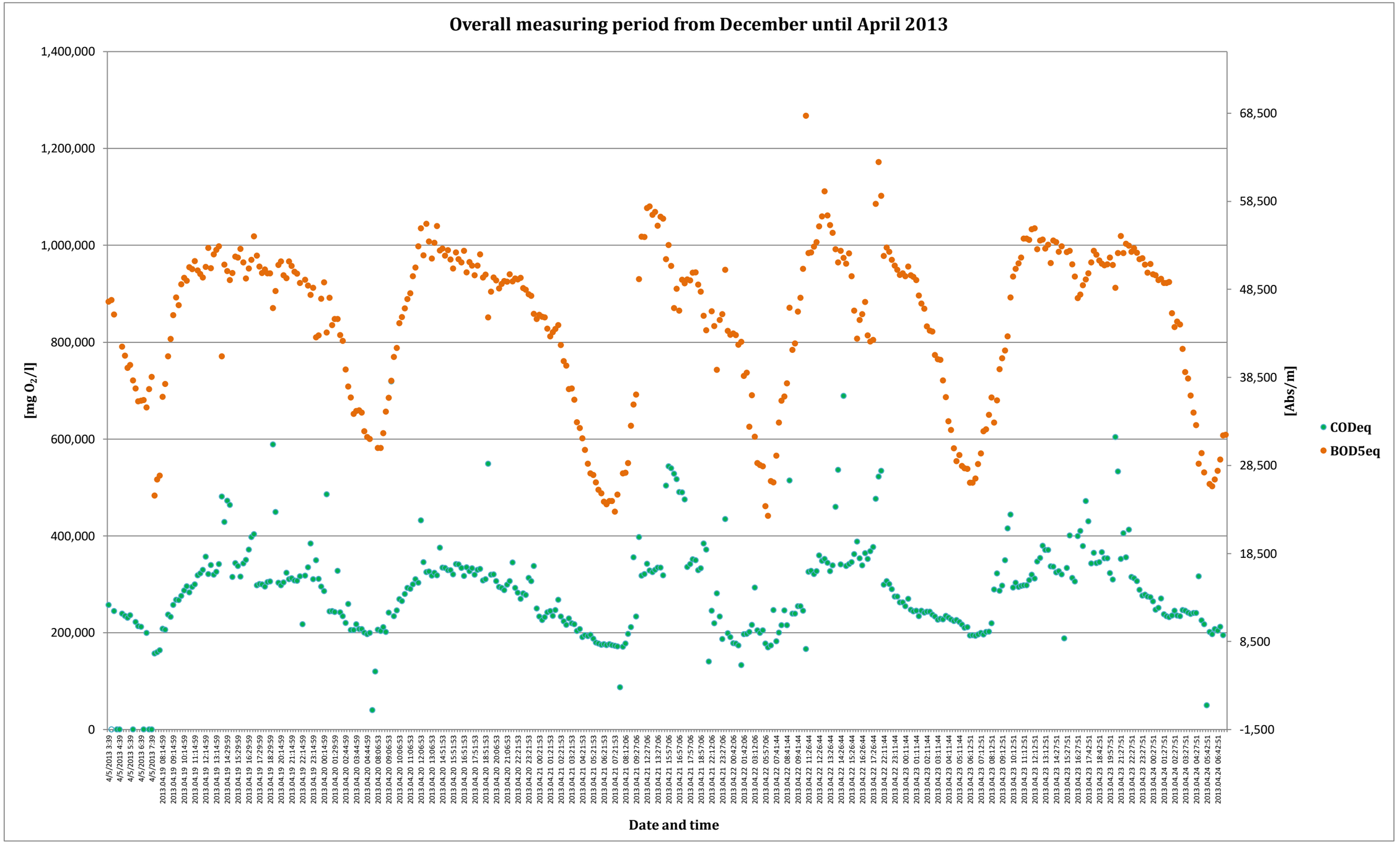


Figure I.1 Spectrolyzer data for BODeq and CODeq in [mg O_2/L]

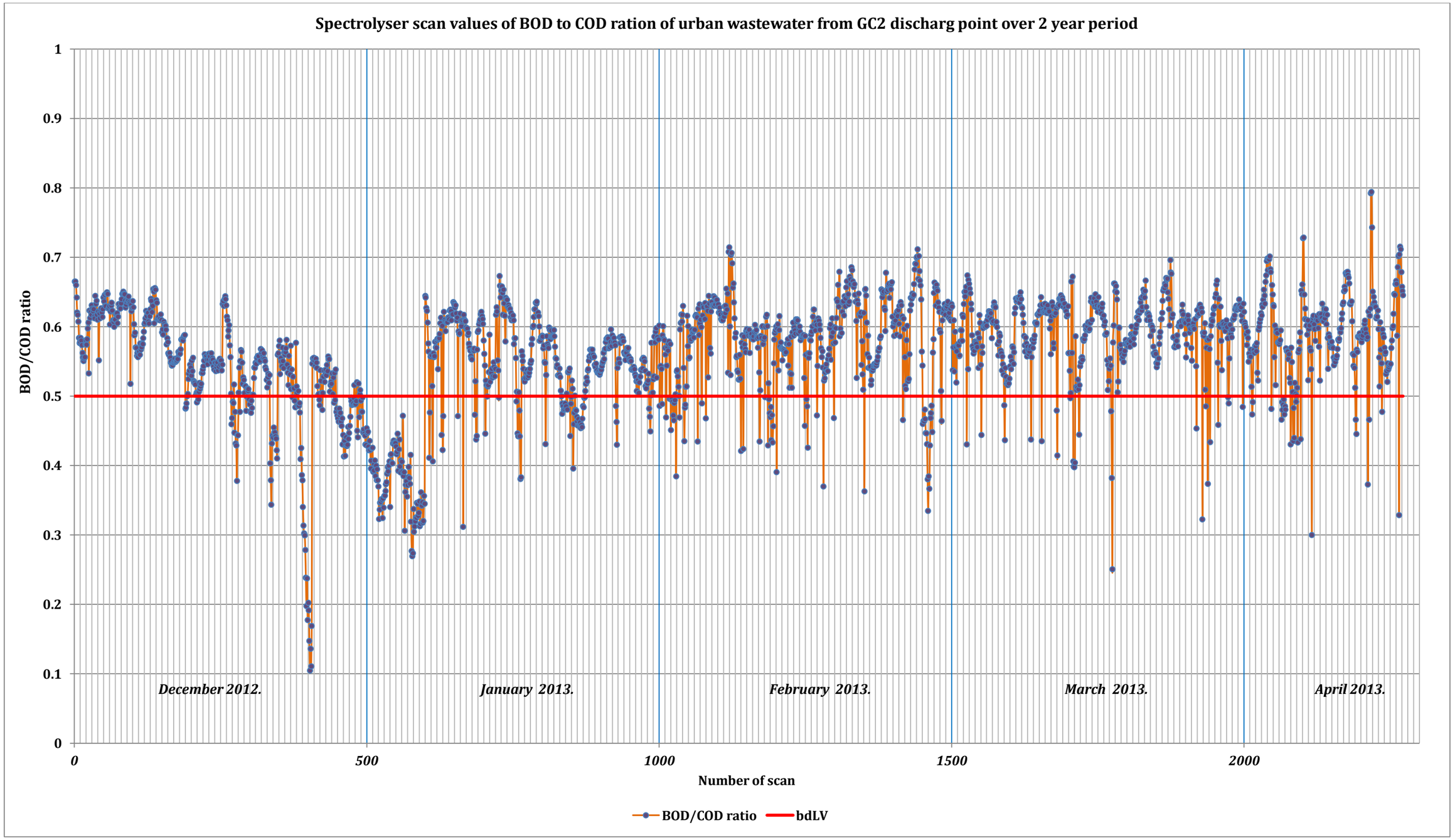


Figure I.2. Spectrolyzer analyses results interpreted as BOD/COD ratio with the limit line of 0.5 representing biodegradability threshold

Annex II

Table II.1 Results of identified substances and their calculated concentrations according to internal standard concentration in all 9 samples during the screening analyses

No.	Retention time	Name of compound	Sample	Calculated concentration
1.	**12.421**	Benzene, methyl-	GC1'	**5.48386E-06**
2.	**14.264**	Butane, 2,3-dichloro-2-methyl-	GC1'	**2.60787E-06**
3.	**15.058**	3-Octanol, 2,3-dimethyl-	GC1'	**6.57186E-07**
4.	**15.864**	2-Butenal, 2-methyl-	GC1'	**9.72675E-07**
5.	**16.888**	Undecan	GC1'	**5.78509E-07**
6.	**17.753**	Benzene, ethyl-	GC1'	**9.78993E-07**
7.	**18.337**	Benzene, 1,x-dimethyl-	GC1'	**1.01993E-06**
8.	**18.757**	Benzene, 1,x-dimethyl-	GC1'	**8.80883E-07**
9.	**21.102**	3-Penten-2-ol	GC1'	**6.13454E-06**
10.	**24.043**	dodecane	GC1'	**4.97146E-07**
11.	**27.000**	Styrene	GC1'	**5.46529E-07**
12.	**27.949**	Dodecane, 2,6,10-trimethyl-	GC1'	**6.85966E-07**
13.	**28.654**	benzene, 1,2,x-trimethyl-	GC1'	**5.53582E-07**
14.	**30.680**	2-pentanol, 4-methyl-	GC1'	**0.000125258**
15.	**31.385**	Acetic acid, chloro-, ethyl ester	GC1'	**1.30449E-06**
16.	**31.746**	2-Buten-1-ol,2-methyl-	GC1'	**9.74116E-07**

17.	**32.891**	2-Propanol, 1-butoxy-	GC1'	**1.99008E-07**
18.	**35.376**	Trisulfide, dimethyl	GC1'	**3.86228E-07**
19.	**37.813**	phenol, x,y-derivative	GC1'	**5.13435E-07**
20.	**38.006**	Tetradecane	GC1'	**1.33938E-06**
21.	**39.479**	linalool oxide	GC1'	**5.52284E-07**
22.	**40.768**	Pentane, 3-chloro-3-methyl-	GC1'	**4.60117E-07**
23.	**41.358**	linalool oxide	GC1'	**5.9497E-07**
24.	**41.561**	dihydromyrcenol	GC1'	**3.60264E-07**
25.	**42.742**	1-hexanol, 2-ethyl-	GC1'	**1.28645E-07**
26.	**44.447**	Pentadecane	GC1'	**2.14259E-06**
27.	**45.978**	4-hexen-3-ol, 2-methyl-	GC1'	**6.16978E-07**
28.	**47.005**	1-Octanol	GC1'	**3.60452E-07**
29.	**51.655**	Menthol	GC1'	**4.71778E-07**
30.	**52.530**	Acetophenone	GC1'	**2.83375E-07**
31.	**52.701**	Acetophenone	GC1'	**2.20563E-07**
32.	**55.849**	2-heptadecanal	GC1'	**2.66783E-07**
33.	**56.243**	Heptadecan	GC1'	**1.08154E-05**
34.	**58.220**	Benzeneethanol, α,α-dimethyl-	GC1'	**3.28436E-07**
35.	**58.427**	Benzeneethanol, α,α-dimethyl-	GC1'	**2.60297E-07**
36.	**61.699**	Octadecane	GC1'	**1.69168E-05**

37.	**63.047**	6-methyl-gamma-ionone	GC1'	**1.32726E-07**
38.	**66.582**	Phenol, 2,6-bis(1,1-dimethylethyl)-4-methyl- (CAS); Vianol	GC1'	**2.22121E-05**
39.	**66.943**	Nonadecane	GC1'	**3.14995E-05**
40.	**75.236**	Eicosane, 3-methyl-	GC1'	**8.72777E-06**
41.	**76.876**	heneicosane	GC1'	**9.44693E-05**
42.	**77.84**	Cyclohexane, tetradecyl-	GC1'	**9.51351E-06**
43.	**78.277**	Eicosane, 10-methyl-	GC1'	**2.72773E-05**
44.	**80.359**	oxalic acid ester	GC1'	**1.56332E-05**
45.	**83.963**	eicosane, alkyl-	GC1'	**2.89532E-05**
46.	**85.035**	Sulfurous acid, cyclohexylmethyl octadecyl ester	GC1'	**2.01864E-05**
47.	**86.068**	Tetracosane	GC1'	**0.000104841**
48.	**86.871**	cyclopentane alkyl	GC1'	**5.55772E-06**
49.	**87.419**	cyclohexane alkyl	GC1'	**1.81248E-05**
50.	**88.193**	heneicosane alkyl-	GC1'	**2.31444E-05**
51.	**89.632**	Sulfurous acid, cyclohexylmethyl heptadecyl ester	GC1'	**8.48582E-06**
52.	**90.285**	Hexacosane	GC1'	**9.03195E-05**
53.	**91.879**	cyclohexane alkyl	GC1'	**1.32655E-05**
54.	**92.135**	Benzophenone	GC1'	**0.0000016**

55.	**100.057**	cyclohexane, alkyl-	GC1'	**1.10135E-05**
56.	**100.776**	1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester;	GC1'	**2.36132E-05**
57.	**101.799**	Triacontane	GC1'	**1.90818E-05**
58.	**103.301**	9-Octadecanoic acid	GC1'	**2.15669E-06**
59.	**103.989**	cyclohexane, alkyl-	GC1'	**6.94525E-06**
60.	**104.376**	Hexanedioic acid, dioctyl ester	GC1'	**7.38897E-06**
61.	**15.055**	3-Octanol, 2,3-dimethyl-	GC1''	**8.94305E-08**
62.	**21**	3-Penten-2-ol	GC1''	**6.18362E-07**
63.	**30.622**	2-pentanol, 4-methyl-	GC1''	**7.02332E-07**
64.	**31.405**	Acetic acid, chloro-, ethyl ester	GC1''	**1.40209E-07**
65.	**31.704**	2-Buten-1-ol,2-methyl-	GC1''	**6.81494E-08**
66.	**38.213**	Tetradecane	GC1''	**9.22997E-08**
67.	**46.982**	1-Octanol	GC1''	**8.05098E-08**
68.	**53.449**	Decane, 1-chloro-	GC1''	**9.3994E-08**
69.	**56.42**	Heptadecan	GC1''	**1.0589E-06**
70.	**60.791**	Geraniol	GC1''	**3.17157E-07**
71.	**61.87**	Octadecane	GC1''	**1.82284E-06**
72.	**63.267**	6-methyl-gamma-ionone	GC1''	**1.06421E-07**
73.	**67.081**	Nonadecane	GC1''	**3.63094E-06**

74.	**74.735**	Eicosane, 2-methyl-	GC1''	**9.42018E-07**
75.	**75.322**	Eicosane, 3-methyl-	GC1''	**1.29328E-06**
76.	**76.889**	Heneicosane	GC1''	**1.01196E-05**
77.	**77.873**	Cyclohexane, tetradecyl-	GC1''	**1.0854E-06**
78.	**78.253**	Eicosane, 10-methyl-	GC1''	**3.38592E-06**
79.	**80.359**	oxalic acid ester	GC1''	**1.9498E-06**
80.	**83.913**	eicosane, alkyl-	GC1''	**5.29072E-06**
81.	**85.042**	Sulfurous acid, cyclohexylmethyl octadecyl ester	GC1''	**2.28001E-06**
82.	**85.914**	Tetracosane	GC1''	**1.32066E-05**
83.	**87.39**	cyclohexane alkyl	GC1''	**2.02975E-06**
84.	**88.108**	heneicosane alkyl-	GC1''	**3.7806E-06**
85.	**90.141**	Hexacosane	GC1''	**2.31932E-06**
86.	**91.777**	cyclohexane alkyl	GC1''	**1.43349E-06**
87.	**92.102**	Benzophenone	GC1''	**0.0000016**
88.	**100.044**	cyclohexane alkyl	GC1''	**7.12481E-07**
89.	**100.7**	1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester;	GC1''	**1.43244E-10**
90.	**101.825**	Triacontane	GC1''	**3.46942E-06**
91.	**103.297**	9-Octadecanoic acid	GC1''	**3.19581E-07**
92.	**103.724**	Docosanoic acid	GC1''	**2.61246E-07**

93.	**103.983**	cyclohexane alkyl	GC1''	**6.38273E-07**
94.	**104.311**	Hexanedioic acid, dioctyl ester	GC1''	**1.05305E-06**
95.	**14.53**	Butane, 2,3-dichloro-2-methyl-	GC2'	**5.25969E-05**
96.	**15.169**	3-Octanol, 2,3-dimethyl-	GC2'	**6.68315E-08**
97.	**21.17**	3-Penten-2-ol	GC2'	**6.46241E-07**
98.	**22.823**	Limonene	GC2'	**4.45501E-07**
99.	**23.243**	1,8-Cineole	GC2'	**1.19844E-07**
100.	**30.772**	2-Pentanol, 4-methyl-	GC2'	**3.78878E-06**
101.	**32.979**	2-Propanol, 1-butoxy-	GC2'	**7.2601E-07**
102.	**35.432**	Trisulfide, dimethyl	GC2'	**1.5227E-07**
103.	**37.137**	Cyclohexanol	GC2'	**1.52984E-07**
104.	**39.59**	Pentane, 3-chloro-3-methyl-	GC2'	**1.85645E-07**
105.	**41.679**	dihydromyrcenol	GC2'	**1.2229E-06**
106.	**42.952**	1-hexanol, 2-ethyl-	GC2'	**1.54166E-07**
107.	**43.955**	Pentadecane	GC2'	**1.83511E-07**
108.	**47.159**	1-Octanol	GC2'	**6.59013E-08**
109.	**50.983**	Cyclohexanol, 2-(1,1-dimethylethyl)-	GC2'	**3.6733E-07**
110.	**51.196**	Cyclohexanol, 2-(1,1-dimethylethyl)-	GC2'	**1.25116E-07**
111.	**51.419**	Cyclohexanol, 2-(1,1-dimethylethyl)-	GC2'	**1.09763E-07**
112.	**51.835**	Menthol	GC2'	**1.41185E-07**

113.	**52.724**	Acetophenone	GC2'	**4.1268E-07**
114.	**53.288**	(2-(2-butoxyisopropoxy)-2-isopropanol	GC2'	**1.10982E-06**
115.	**57.699**	Cyclohexanol, 4-(1,1-dimethylethyl)-, trans-	GC2'	**1.19724E-06**
116.	**58.466**	Benzeneethanol, α,α-dimethyl-	GC2'	**3.70799E-07**
117.	**60.916**	1-Decanol, 2-methyl-	GC2'	**2.92556E-07**
118.	**61.67**	Benzenemethanol, methyl-	GC2'	**1.37749E-07**
119.	**63.283**	6-methyl-gamma-ionone	GC2'	**3.08675E-07**
120.	**64.087**	phenol, 2-methoxy-	GC2'	**5.54896E-07**
121.	**66.576**	Phenol, 2,6-bis(1,1-dimethylethyl)-4-methyl- (CAS); Vianol	GC2'	**2.68923E-07**
122.	**67.389**	Nonadecane	GC2'	**2.80074E-06**
123.	**69.803**	dicyclopentenyl alcohol	GC2'	**1.82135E-06**
124.	**75.063**	Phenol, 4-methyl-	GC2'	**1.1815E-06**
125.	**75.617**	Eicosane, 3-methyl-	GC2'	**1.11981E-06**
126.	**77.178**	Heneicosane	GC2'	**9.12235E-06**
127.	**78.224**	Eicosane, 10-methyl-	GC2'	**7.68184E-07**
128.	**80.306**	oxalic acid ester	GC2'	**1.67141E-06**
129.	**80.359**	oxalic acid ester	GC2'	**1.71597E-07**
130.	**85.36**	Sulfurous acid, cyclohexylmethyl octadecyl ester	GC2'	**2.04941E-06**

131.	**86.189**	Tetracosane	GC2'	**1.13647E-05**
132.	**87.308**	cyclohexane alkyl	GC2'	**2.79318E-06**
133.	**88.459**	heneicosane alkyl-	GC2'	**2.16566E-06**
134.	**90.42**	Hexacosane	GC2'	**1.0966E-05**
135.	**92.381**	Benzophenone	GC2'	**0.0000016**
136.	**100.995**	1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester;	GC2'	**3.21795E-06**
137.	**102.199**	Triacontane	GC2'	**5.34406E-06**
138.	**103.15**	9-Octadecanoic acid	GC2'	**1.86003E-05**
139.	**103.661**	Docosanoic acid	GC2'	**5.4962E-07**
140.	**104.042**	cyclohexane alkyl	GC2'	**2.82975E-07**
141.	**104.639**	Hexanedioic acid, dioctyl ester	GC2'	**1.0332E-06**
142.	**12.49**	benzene, methyl-	GC2''	**6.90948E-06**
143.	**14.491**	Butane, 2,3-dichloro-2-methyl-	GC2''	**3.55372E-06**
144.	**15.061**	3-Octanol, 2,3-dimethyl-	GC2''	**7.9677E-07**
145.	**15.878**	2-Butenal, 2-methyl-	GC2''	**1.45679E-06**
146.	**16.93**	Undecan	GC2''	**8.12256E-07**
147.	**17.76**	Benzene, ethyl-	GC2''	**1.18783E-06**
148.	**18.35**	Benzene, 1,x-dimethyl-	GC2''	**1.23074E-06**
149.	**18.76**	Benzene, 1,x-dimethyl-	GC2''	**1.23315E-06**

150.	**24.069**	dodecane	GC2''	**7.89177E-07**
151.	**27.116**	Styrene	GC2''	**7.17975E-07**
152.	**28.008**	Dodecane, 2,6,10-trimethyl-	GC2''	**8.10124E-07**
153.	**28.638**	Benzene, 1,2,x-trimethyl-	GC2''	**6.74296E-07**
154.	**30.658**	2-pentanol, 4-methyl-	GC2''	**0.000174431**
155.	**31.363**	Acetic acid, chloro-, ethyl ester	GC2''	**2.01222E-06**
156.	**31.759**	2-Buten-1-ol,2-methyl-	GC2''	**1.449E-06**
157.	**32.835**	2-Propanol, 1-butoxy-	GC2''	**8.05463E-07**
158.	**35.449**	Trisulfide, dimethyl	GC2''	**3.09043E-07**
159.	**37.852**	phenol, x,y-derivative	GC2''	**6.38666E-07**
160.	**38.085**	Tetradecane	GC2''	**1.65718E-06**
161.	**40.738**	Pentane, 3-chloro-3-methyl-	GC2''	**4.89126E-07**
162.	**41.637**	dihydromyrcenol	GC2''	**4.29109E-07**
163.	**42.788**	1-hexanol, 2-ethyl-	GC2''	**4.56955E-07**
164.	**44.496**	Pentadecane	GC2''	**3.26027E-06**
165.	**45.956**	4-hexen-3-ol, 2-methyl-	GC2''	**9.34349E-07**
166.	**50.966**	Cyclohexanol, 2-(1,1-dimethylethyl)-	GC2''	**8.20111E-07**
167.	**56.302**	Heptadecan	GC2''	**1.62613E-05**
168.	**57.505**	Cyclohexanol, 4-(1,1-dimethylethyl)-, trans-	GC2''	**7.41605E-07**

169.	**58.263**	Benzeneethanol, α,α-dimethyl-	GC2''	**6.22338E-07**
170.	**61.765**	Octadecane	GC2''	**2.54337E-05**
171.	**63.06**	6-methyl-gamma-ionone	GC2''	**3.21927E-07**
172.	**64.064**	Phenol, 2-methoxy-; Guaiacol	GC2''	**1.3197E-07**
173.	**66.573**	Phenol, 2,6-bis(1,1-dimethylethyl)-4-methyl- (CAS); Vianol	GC2''	**1.77372E-05**
174.	**67.002**	Nonadecane	GC2''	**3.34398E-05**
175.	**74.784**	Eicosane, 2-methyl-	GC2''	**1.87196E-05**
176.	**75.351**	Eicosane, 3-methyl-	GC2''	**1.41943E-05**
177.	**77.07**	Heneicosane	GC2''	**0.000109801**
178.	**77.945**	Cyclohexane, tetradecyl-	GC2''	**1.10824E-05**
179.	**85.301**	Sulfurous acid, cyclohexylmethyl octadecyl ester	GC2''	**2.22526E-05**
180.	**86.107**	Tetracosane	GC2''	**0.000147445**
181.	**87.557**	cyclohexane alkyl	GC2''	**2.27138E-05**
182.	**88.449**	heneicosane alkyl-	GC2''	**2.97481E-05**
183.	**90.495**	Hexacosane	GC2''	**0.000106598**
184.	**92.118**	Benzophenone	GC2''	**0.0000016**
185.	**100.182**	cyclohexane alkyl	GC2''	**2.45877E-05**
186.	**100.759**	1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester;	GC2''	**2.22604E-05**

187.	**101.953**	Triacontane	GC2''	**2.38871E-06**
188.	**103.386**	9-Octadecanoic acid	GC2''	**5.46426E-06**
189.	**103.796**	Docosanoic acid	GC2''	**9.63715E-06**
190.	**104.071**	cyclohexane alkyl	GC2''	**5.47253E-06**
191.	**104.383**	Hexanedioic acid, dioctyl ester	GC2''	**1.21022E-06**
192.	**12.435**	benzene, methyl-	RI	**4.6067E-07**
193.	**20.994**	3-Penten-2-ol	RI	**8.70703E-07**
194.	**22.650**	Limonene	RI	**3.86766E-07**
195.	**24.135**	dodecane	RI	**5.16004E-08**
196.	**30.618**	2-pentanol, 4-methyl-	RI	**4.06615E-06**
197.	**31.382**	Acetic acid, chloro-, ethyl ester	RI	**2.63883E-08**
198.	**31.691**	2-Buten-1-ol,2-methyl-	RI	**9.36125E-08**
199.	**38.203**	Tetradecane	RI	**2.69713E-07**
200.	**46.989**	1-Octanol	RI	**9.30362E-08**
201.	**56.413**	Heptadecan	RI	**1.99354E-06**
202.	**61.854**	Octadecane	RI	**3.5921E-06**
203.	**67.068**	Nonadecane	RI	**7.00507E-06**
204.	**74.712**	Eicosane, 2-methyl-	RI	**1.86153E-06**
205.	**75.318**	Eicosane, 3-methyl-	RI	**1.76808E-06**
206.	**76.886**	Heneicosane	RI	**1.77523E-05**

207.	**77.876**	Cyclohexane, tetradecyl-	RI	**1.86496E-06**
208.	**78.24**	Eicosane, 10-methyl-	RI	**6.13774E-06**
209.	**80.316**	oxalic acid ester	RI	**3.49335E-06**
210.	**83.891**	eicosane, alkyl-	RI	**6.35718E-06**
211.	**85.028**	sulforous acid, cyclohexyl alkyl ester	RI	**4.35166E-06**
212.	**85.94**	Tetracosane	RI	**2.27588E-05**
213.	**86.819**	cyclopentane, alkyl-	RI	**1.02392E-06**
214.	**87.386**	cyclohexane, alkyl-	RI	**3.56507E-06**
215.	**88.203**	heneicosane, alkyl-	RI	**4.9476E-06**
216.	**89.557**	sulfurous acid, cyclohexylmethyl alkyl ester	RI	**1.99023E-06**
217.	**90.154**	Hexacosane	RI	**1.91768E-05**
218.	**91.797**	cyclohexane alkyl	RI	**2.7175E-06**
219.	**92.118**	Benzophenone	RI	**0.0000016**
220.	**100.058**	cyclohexane alkyl	RI	**2.02061E-06**
221.	**100.733**	1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester;	RI	**3.05585E-06**
222.	**101.819**	Triacontane	RI	**6.7116E-06**
223.	**103.294**	9-Octadecanoic acid	RI	**4.92153E-07**
224.	**103.730**	Docosanoic acid	RI	**9.92637E-07**
225.	**104.003**	cyclohexane, alkyl-	RI	**1.20838E-06**

226.	**104.334**	Hexanedioic acid, dioctyl ester	RI	**1.88803E-06**
227.	**14.458**	Butane, 2,3-dichloro-2-methyl-	RO'	**1.60147E-07**
228.	**15.101**	3-Octanol, 2,3-dimethyl-	RO'	**1.42694E-06**
229.	**21.000**	3-penten-2-ol	RO'	**1.31377E-06**
230.	**30.615**	2-pentanol, 4-methyl-	RO'	**1.1234E-05**
231.	**38.197**	Tetradecane	RO'	**3.35721E-07**
232.	**47.294**	(S)-3,4-Dimethylpentanol	RO'	**1.5709E-07**
233.	**56.371**	Heptadecan	RO'	**3.1835E-06**
234.	**61.329**	2,4-Decadienal	RO'	**1.92099E-06**
235.	**61.818**	Octadecane	RO'	**5.42915E-06**
236.	**67.019**	Nonadecane	RO'	**1.10425E-05**
237.	**75.063**	Phenol, 4-methyl-	RO'	**1.77579E-06**
238.	**75.250**	Eicosane, 3-methyl-	RO'	**3.13323E-06**
239.	**75.476**	Eicosane, 3-methyl-	RO'	**6.00016E-07**
240.	**76.820**	heneicosane	RO'	**2.87225E-05**
241.	**77.653**	Cyclohexane, tetradecyl-	RO'	**5.02061E-07**
242.	**77.847**	Cyclohexane, tetradecyl-	RO'	**3.1198E-06**
243.	**78.175**	Eicosane, 10-methyl-	RO'	**3.72779E-06**
244.	**80.237**	oxalic acid ester	RO'	**5.1284E-06**
245.	**82.156**	cyclopentane, decyl-	RO'	**1.35643E-06**

246.	**83.031**	sulforous acid, butyl octadecyl ester	RO'	**3.24869E-06**
247.	**83.832**	eicosane, alkyl-	RO'	**5.99175E-06**
248.	**84.946**	Sulfurous acid, cyclohexylmethyl octadecyl ester	RO'	**6.8092E-06**
249.	**85.822**	Tetracosane	RO'	**3.32138E-05**
250.	**87.294**	cyclohexane alkyl	RO'	**6.36641E-06**
251.	**90.036**	Hexacosane	RO'	**3.0778E-05**
252.	**91.712**	cyclohexane alkyl	RO'	**5.11923E-06**
253.	**92.089**	Benzophenone	RO'	**0.0000016**
254.	**99.995**	cyclohexane alkyl	RO'	**2.1334E-06**
255.	**100.707**	1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester;	RO'	**6.89916E-06**
256.	**101.753**	Triacontane	RO'	**9.94667E-06**
257.	**103.252**	9-Octadecanoic acid	RO'	**1.16078E-06**
258.	**103.717**	Docosanoic acid	RO'	**1.75867E-06**
259.	**103.924**	cyclohexane alkyl	RO'	**2.09932E-06**
260.	**104.294**	Hexanedioic acid, dioctyl ester	RO'	**3.77683E-06**
261.	**11.815**	2-Butene, 1-chloro-2-methyl	RO''	**5.72109E-06**
262.	**12.419**	benzene, methyl-	RO''	**2.62564E-05**
263.	**14.419**	Butane, 2,3-dichloro-2-methyl-	RO''	**2.34516E-06**
264.	**15.022**	3-Octanol, 2,3-dimethyl-	RO''	**5.11568E-07**

265.	**15.796**	2-Butenal, 2-methyl-	RO''	**6.97996E-07**
266.	**16.806**	Undecan	RO''	**5.73049E-07**
267.	**17.682**	Benzene, ethyl-	RO''	**9.70539E-07**
268.	**18.272**	Benzene, 1,x-dimethyl-	RO''	**8.89573E-07**
269.	**18.695**	Benzene, 1,x-dimethyl-	RO''	**1.00591E-06**
270.	**20.955**	3-Penten-2-ol	RO''	**6.52846E-06**
271.	**22.984**	1,8-Cineole	RO''	**5.87338E-07**
272.	**23.994**	dodecane	RO''	**2.82955E-07**
273.	**27.034**	Styrene	RO''	**4.5999E-07**
274.	**27.749**	Benzene, 1-methyl-2-(1-methylethyl)-	RO''	**1.26026E-06**
275.	**27.880**	Dodecane, 2,6,10-trimethyl-	RO''	**5.46556E-07**
276.	**28.605**	Benzene, 1,2,x-trimethyl-	RO''	**5.02517E-07**
277.	**30.592**	2-Pentanol, 4-methyl-	RO''	**9.71107E-05**
278.	**31.291**	Acetic acid, chloro-, ethyl ester	RO''	**1.01956E-06**
279.	**31.665**	2-Buten-1-ol,2-methyl-	RO''	**8.76947E-07**
280.	**32.773**	2-Propanol, 1-butoxy-	RO''	**1.51055E-06**
281.	**37.751**	phenol, x,y-derivative	RO''	**3.63422E-07**
282.	**37.948**	Tetradecane	RO''	**3.13367E-07**
283.	**39.112**	3-Octanol, 3,7-dimethyl-	RO''	**3.31682E-06**
284.	**39.427**	linalool oxide	RO''	**4.38182E-07**

285.	**40.552**	Pentane, 3-chloro-3-methyl-	RO''	**3.70573E-07**
286.	**41.283**	linalool oxide	RO''	**4.18363E-07**
287.	**41.486**	dihydromyrcenol	RO''	**1.21308E-05**
288.	**42.322**	p-Menthone	RO''	**2.05606E-07**
289.	**42.736**	1-hexanol, 2-ethyl-	RO''	**1.17593E-05**
290.	**43.677**	Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-	RO''	**2.29483E-08**
291.	**44.349**	Pentadecane	RO''	**3.60186E-07**
292.	**45.913**	4-hexen-3-ol, 2-methyl-	RO''	**3.87778E-07**
293.	**46.943**	Dihydroterpineol	RO''	**5.44007E-07**
294.	**49.045**	Cyclohexanol, 5-methyl-2-(1-methylethyl)-	RO''	**3.1818E-07**
295.	**51.150**	Cyclohexanol, 2-(1,1-dimethylethyl)-	RO''	**2.56431E-06**
296.	**51.593**	Menthol	RO''	**6.82989E-06**
297.	**51.770**	Menthol	RO''	**4.4933E-07**
298.	**52.472**	Acetophenone	RO''	**8.21071E-07**
299.	**53.046**	Isoborneol	RO''	**1.92685E-06**
300.	**53.777**	(2-(2-butoxyisopropoxy)-2-isopropanol	RO''	**1.54048E-06**
301.	**54.935**	α-terpineol	RO''	**2.39186E-06**
302.	**56.135**	Heptadecan	RO''	**1.95885E-06**

303.	**56.496**	Cyclohexanol, 4-(1,1-dimethylethyl)-, trans-	RO''	**4.32654E-07**
304.	**57.450**	Cyclohexanol, 4-(1,1-dimethylethyl)-, trans-	RO''	**1.41254E-06**
305.	**61.438**	Benzenemethanol, methyl-	RO''	**4.1968E-07**
306.	**61.592**	Octadecane	RO''	**3.56934E-06**
307.	**62.992**	6-methyl-gamma-ionone	RO''	**6.32286E-07**
308.	**66.494**	benzeneethanol	RO''	**5.05618E-06**
309.	**66.802**	Nonadecane	RO''	**8.42272E-06**
310.	**67.711**	3-Cyclohexene-1-ethanol, 4-dimethyl-	RO''	**4.39314E-07**
311.	**71.325**	Phenol	RO''	**3.93676E-05**
312.	**74.984**	Phenol, 4-methyl-	RO''	**0.000413714**
313.	**76.673**	heneicosane	RO''	**2.93398E-05**
314.	**77.683**	Cyclohexane, tetradecyl-	RO''	**2.92862E-06**
315.	**78.021**	Eicosane, 10-methyl-	RO''	**6.66462E-06**
316.	**82.064**	cyclopentane, nonyl-	RO''	**1.10538E-06**
317.	**82.943**	sulforous acid, butyl octadecyl ester	RO''	**3.9287E-06**
318.	**83.773**	eicosane, alkyl-	RO''	**1.1837E-05**
319.	**87.278**	cyclohexane alkyl	RO''	**7.63214E-06**
320.	**90.079**	Hexacosane	RO''	**4.42036E-05**
321.	**92.076**	Benzophenone	RO''	**0.0000016**

322.	**99.930**	cyclohexane alkyl	RO''	**6.96673E-06**
323.	**100.707**	1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester;	RO''	**1.59233E-05**
324.	**103.193**	9-Octadecanoic acid	RO''	**1.77719E-06**
325.	**103.872**	cyclohexane alkyl	RO''	**3.49828E-06**
326.	**106.712**	2H-Indol-2-one,1,3-dihydro-	RO''	**2.13425E-05**
327.	**14.297**	Butane, 2,3-dichloro-2-methyl-	RP'	**5.18914E-05**
328.	**21.184**	3-Penten-2-ol	RP'	**6.24654E-09**
329.	**23.207**	1,8-cineole	RP'	**2.03877E-07**
330.	**30.772**	2-Pentanol, 4-methyl-	RP'	**2.03406E-06**
331.	**32.963**	2-Propanol, 1-butoxy-	RP'	**5.67791E-07**
332.	**35.413**	Trisulfide, dimethyl	RP'	**2.9781E-07**
333.	**37.115**	Cyclohexanol	RP'	**3.08754E-07**
334.	**39.322**	3-Octanol, 3,7-dimethyl-	RP'	**1.54095E-06**
335.	**40.801**	Pentane, 3-chloro-3-methyl-	RP'	**1.12985E-07**
336.	**41.332**	linalool oxide	RP'	**8.92395E-08**
337.	**41.673**	dihydromyrcenol	RP'	**8.60333E-06**
338.	**42.935**	1-hexanol, 2-ethyl-	RP'	**2.4085E-07**
339.	**43.929**	Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-	RP'	**9.13641E-07**
340.	**46.595**	α-terpinolene	RP'	**2.92653E-07**

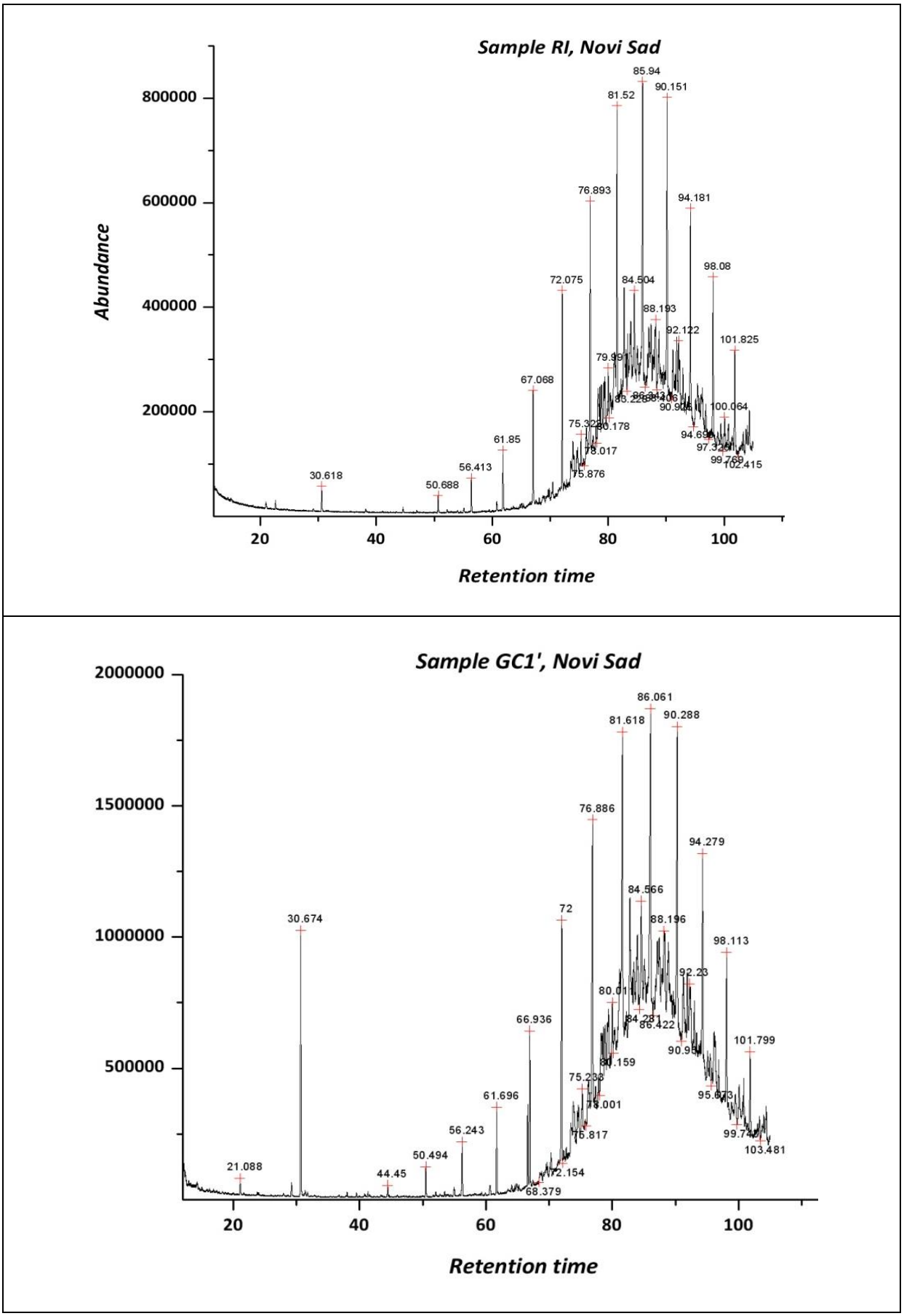
341.	**47.169**	1-Octanol	RP'	**2.48051E-07**
342.	**50.960**	Cyclohexanol, 2-(1,1-dimethylethyl)-	RP'	**3.1988E-07**
343.	**51.170**	Cyclohexanol, 2-(1,1-dimethylethyl)-	RP'	**7.92122E-08**
344.	**51.380**	Cyclohexanol, 2-(1,1-dimethylethyl)-	RP'	**8.18784E-08**
345.	**51.829**	Menthol	RP'	**3.16756E-06**
346.	**52.685**	Acetophenone	RP'	**5.45487E-07**
347.	**53.285**	Isoborneol	RP'	**9.16461E-07**
348.	**55.174**	Fenchyl alcohol	RP'	**1.57876E-06**
349.	**57.686**	Cyclohexanol, 4-(1,1-dimethylethyl)-, trans-	RP'	**9.56615E-07**
350.	**58.456**	Benzeneethanol, α,α-dimethyl-	RP'	**3.69832E-07**
351.	**61.644**	Benzenemethanol, methyl-	RP'	**1.62259E-07**
352.	**63.277**	6-methyl-gamma-ionone	RP'	**2.21568E-07**
353.	**66.766**	Phenol, 2,6-bis(1,1-dimethylethyl)-4-methyl- (CAS); Vianol	RP'	**1.73212E-07**
354.	**67.366**	Nonadecane	RP'	**2.56233E-06**
355.	**69.793**	dicyclopentenyl alcohol	RP'	**1.87505E-06**
356.	**70.764**	4-phenyl-2-butanol	RP'	**3.67812E-07**
357.	**75.023**	Phenol, 4-methyl-	RP'	**1.13271E-06**
358.	**75.597**	Eicosane, 3-methyl-	RP'	**5.61633E-07**
359.	**77.129**	Heneicosane	RP'	**7.76247E-06**

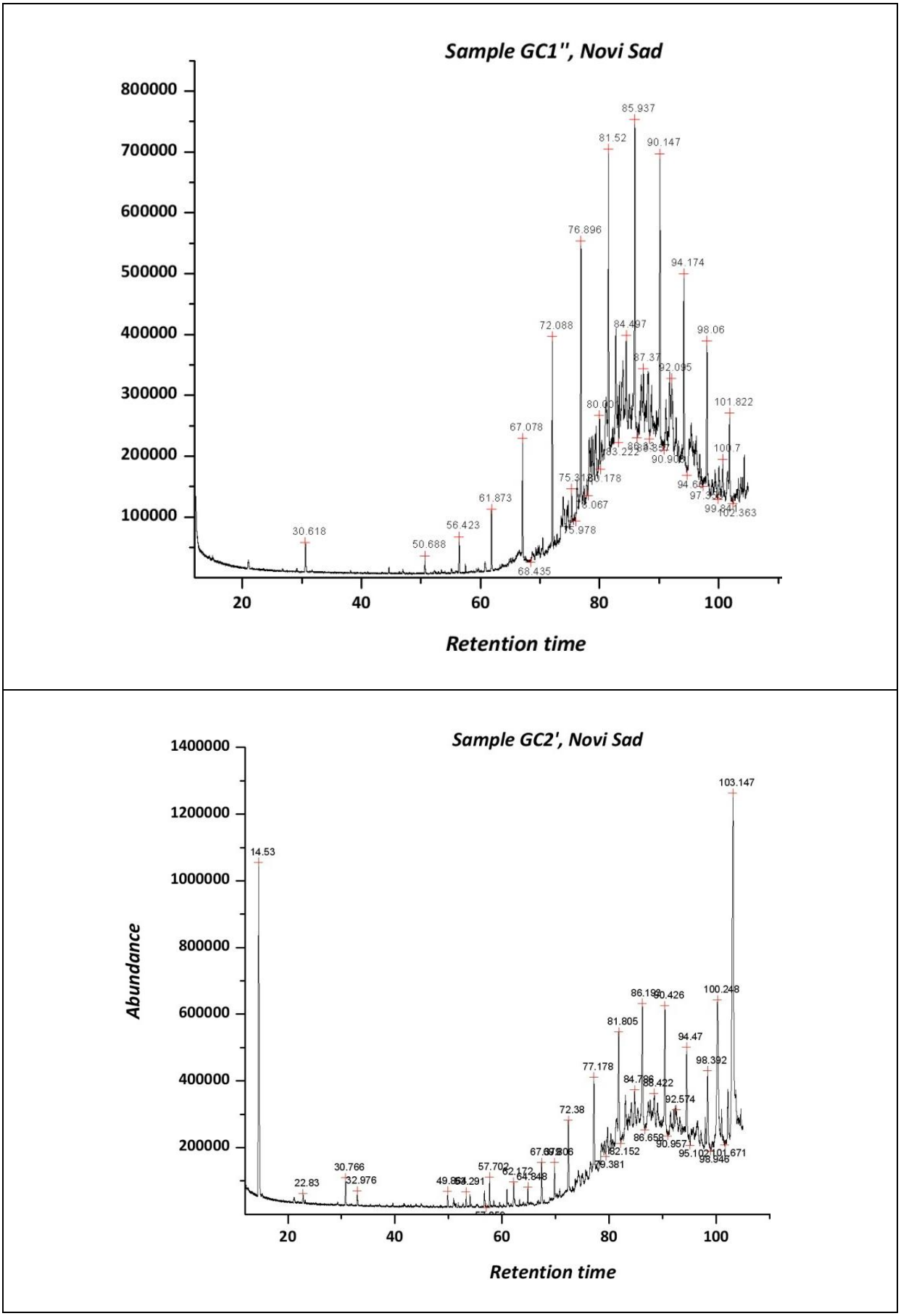
360.	**78.208**	Eicosane, 10-methyl-	RP'	**7.62319E-07**
361.	**80.270**	oxalic acid ester	RP'	**1.4025E-06**
362.	**81.428**	hexadecanoic acid, methyl ester	RP'	**5.4664E-07**
363.	**83.005**	sulforous acid, butyl octadecyl ester	RP'	**1.98072E-06**
364.	**85.288**	Sulfurous acid, cyclohexylmethyl octadecyl ester	RP'	**1.70072E-06**
365.	**86.140**	Tetracosane	RP'	**8.57166E-06**
366.	**88.380**	heneicosane alkyl-	RP'	**2.3339E-06**
367.	**90.374**	Hexacosane	RP'	**9.0006E-06**
368.	**92.046**	cyclohexane alkyl	RP'	**1.14901E-06**
369.	**92.368**	Benzophenone	RP'	**0.0000016**
370.	**100.982**	1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester;	RP'	**3.98551E-06**
371.	**102.176**	Triacontane	RP'	**3.61751E-06**
372.	**104.586**	Hexanedioic acid, dioctyl ester	RP'	**7.79381E-07**
373.	**12.454**	benzene, methyl-	RP''	**1.0622E-06**
374.	**14.464**	Butane, 2,3-dichloro-2-methyl-	RP''	**2.26953E-07**
375.	**15.055**	3-Octanol, 2,3-dimethyl-	RP''	**1.304E-07**
376.	**17.750**	Benzene, ethyl-	RP''	**8.98532E-07**
377.	**18.337**	Benzene, 1,x-dimethyl-	RP''	**5.12115E-07**
378.	**18.747**	Benzene, 1,x-dimethyl-	RP''	**1.06203E-06**

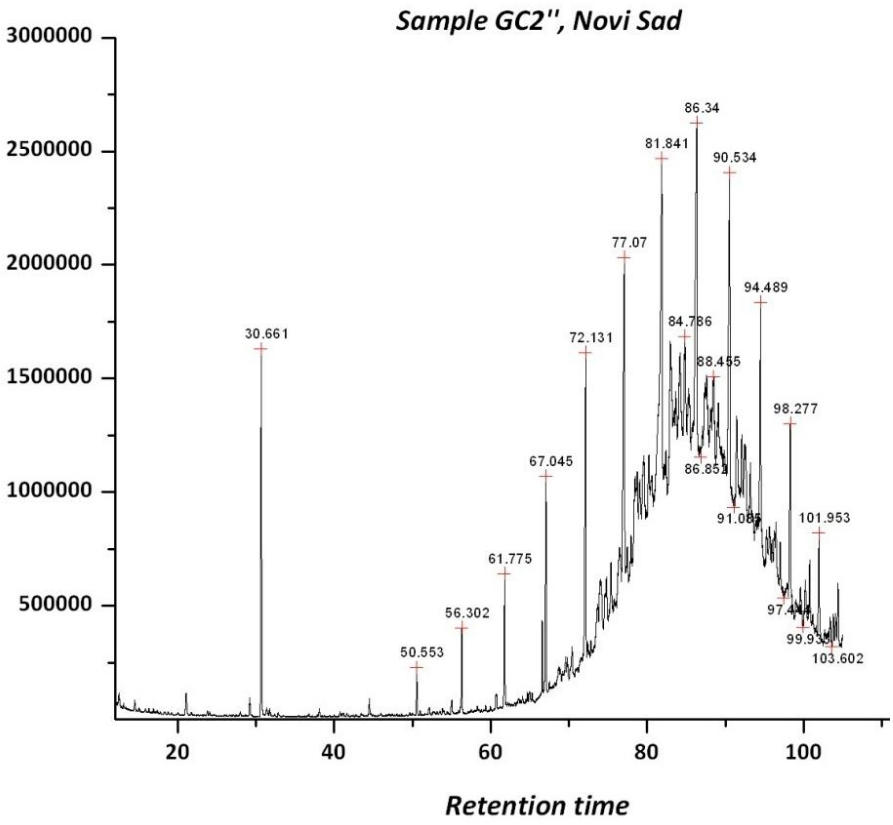
379.	**21.020**	3-Penten-2-ol	RP''	**1.07063E-06**
380.	**22.656**	Limonene	RP''	**6.46224E-07**
381.	**30.625**	2-Pentanol, 4-methyl-	RP''	**4.95127E-06**
382.	**31.713**	2-Buten-1-ol,2-methyl-	RP''	**1.30981E-07**
383.	**38.174**	Tetradecane	RP''	**1.40018E-07**
384.	**46.998**	1-Octanol	RP''	**5.67051E-08**
385.	**56.384**	Heptadecan	RP''	**1.30728E-06**
386.	**58.453**	Benzeneethanol, α,α-dimethyl-	RP''	**9.68118E-08**
387.	**61.847**	Octadecane	RP''	**2.07924E-06**
388.	**66.549**	benzeneethanol	RP''	**1.54303E-07**
389.	**67.054**	Nonadecane	RP''	**3.84194E-06**
390.	**75.072**	Phenol, 4-methyl-	RP''	**5.26543E-07**
391.	**75.282**	Eicosane, 3-methyl-	RP''	**8.32267E-07**
392.	**76.827**	heneicosane	RP''	**8.98822E-06**
393.	**77.863**	Cyclohexane, tetradecyl-	RP''	**1.03202E-06**
394.	**78.194**	Eicosane, 10-methyl-	RP''	**3.1078E-06**
395.	**80.277**	oxalic acid ester	RP''	**1.40032E-06**
396.	**83.044**	sulforous acid, butyl octadecyl ester	RP''	**1.10443E-06**
397.	**83.841**	eicosane, alkyl-	RP''	**1.56404E-06**
398.	**84.982**	Sulfurous acid, cyclohexylmethyl	RP''	**2.0144E-06**

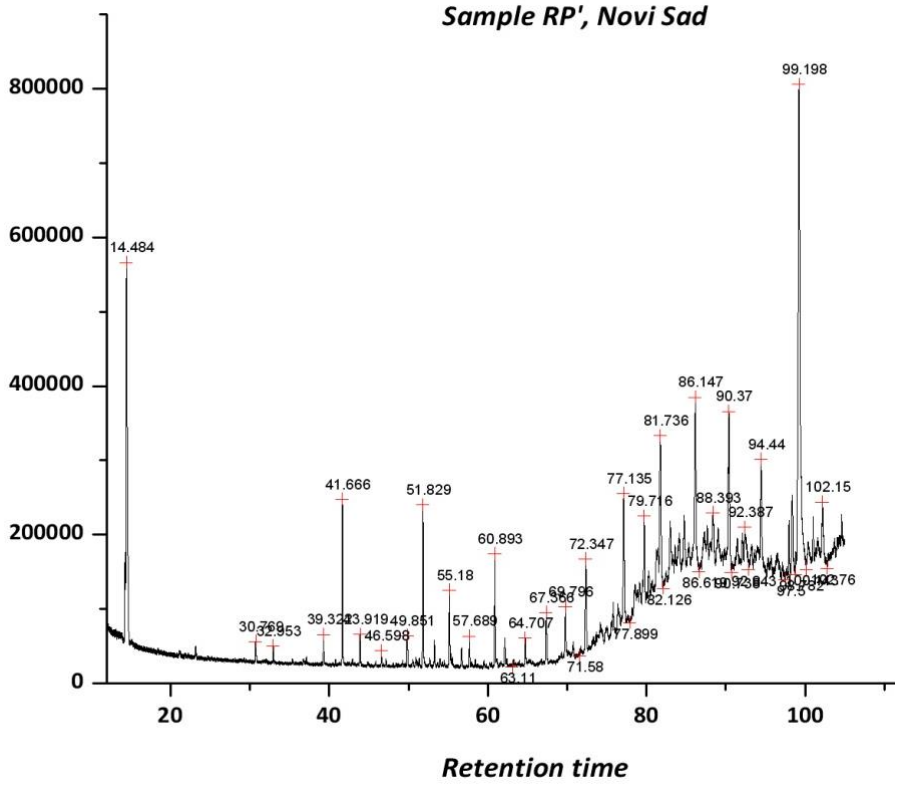
		octadecyl ester		
399.	**85.845**	Tetracosane	RP''	**1.03326E-05**
400.	**87.314**	cyclohexane alkyl	RP''	**1.8853E-06**
401.	**90.052**	Hexacosane	RP''	**9.93075E-06**
402.	**91.721**	cyclohexane alkyl	RP''	**1.41543E-06**
403.	**92.089**	Benzophenone	RP''	**0.0000016**
404.	**99.995**	cyclohexane alkyl	RP''	**6.45758E-07**
405.	**100.726**	1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester;	RP''	**3.0178E-06**
406.	**101.763**	Triacontane	RP''	**2.76944E-06**
407.	**103.238**	9-Octadecanoic acid	RP''	**4.08471E-07**
408.	**103.714**	Docosanoic acid	RP''	**5.95247E-07**
409.	**103.960**	cyclohexane alkyl	RP''	**5.23688E-07**
410.	**104.314**	Hexanedioic acid, dioctyl ester	RP''	**9.34861E-07**

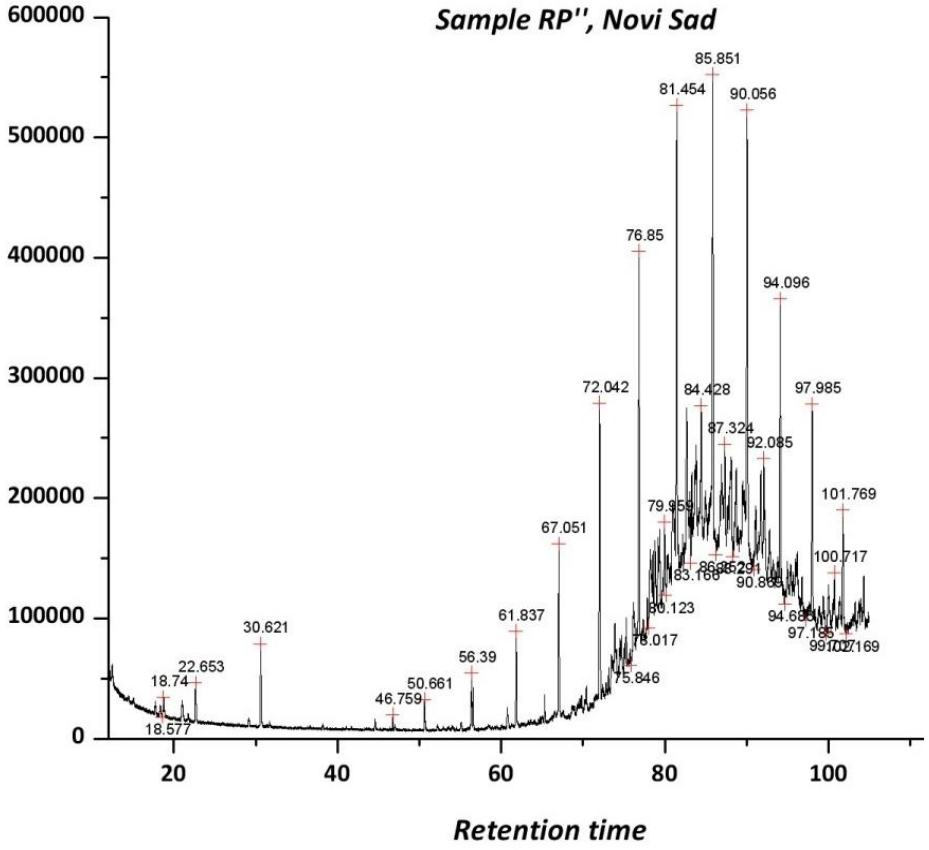
Table II.2 Chromatograms representing the 3rd screening analyses of all 9 samples

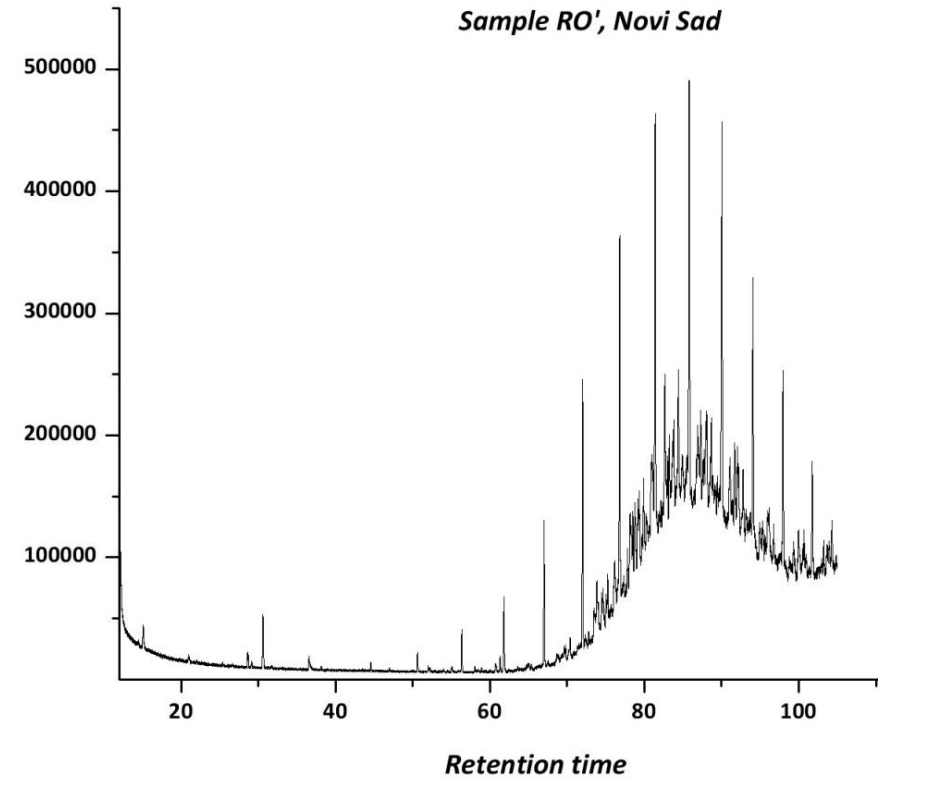


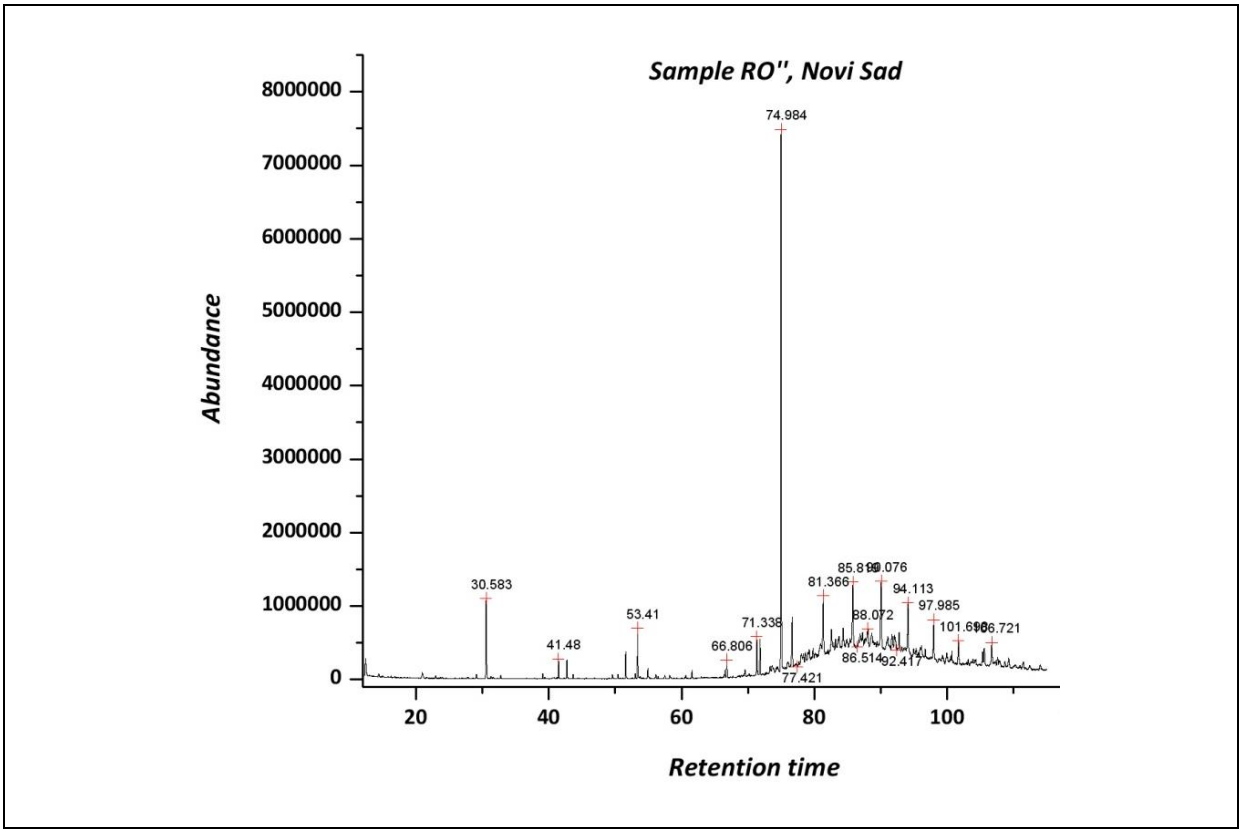

Sample GC1'', Novi Sad
800000
700000
600000
500000
400000
300000
200000
100000
85.937
81.52
90.147
76.896
94.174
72.088
84.497
98.06
87.37
92.095
101.822
80.00
67.078
83.222
90.904
100.7
75.31
80.178
61.873
75.067
56.423
75.978
30.618
50.688
68.435
20
40
60
80
100
Retention time
Sample GC2', Novi Sad
Abundance
1400000
1200000
1000000
800000
600000
400000
200000
103.147
14.53
100.248
86.198
90.426
81.805
94.47
98.392
77.178
84.786
88.422
92.574
72.38
86.658
90.957
82.152
95.102
101.671
79.381
98.946
30.766
57.702
62.172
64.848
22.83
32.976
20
40
60
80
100
Retention time


Sample GC2'', Novi Sad
3000000
2500000
2000000
1500000
1000000
500000
30.661
50.553
56.302
61.775
67.045
72.131
77.07
81.841
84.786
86.34
88.455
90.534
94.489
98.277
101.953
103.602
20
40
60
80
100
Retention time


Sample RP', Novi Sad
800000
600000
400000
200000
0
14.484
30.769
32.953
39.324
41.666
43.919
46.598
49.851
51.829
55.18
57.689
60.893
63.11
64.707
67.366
68.796
71.58
72.347
77.135
77.899
79.716
81.736
82.126
86.147
88.393
90.37
92.387
94.44
97.5
99.198
102.15
20
40
60
80
100
Retention time

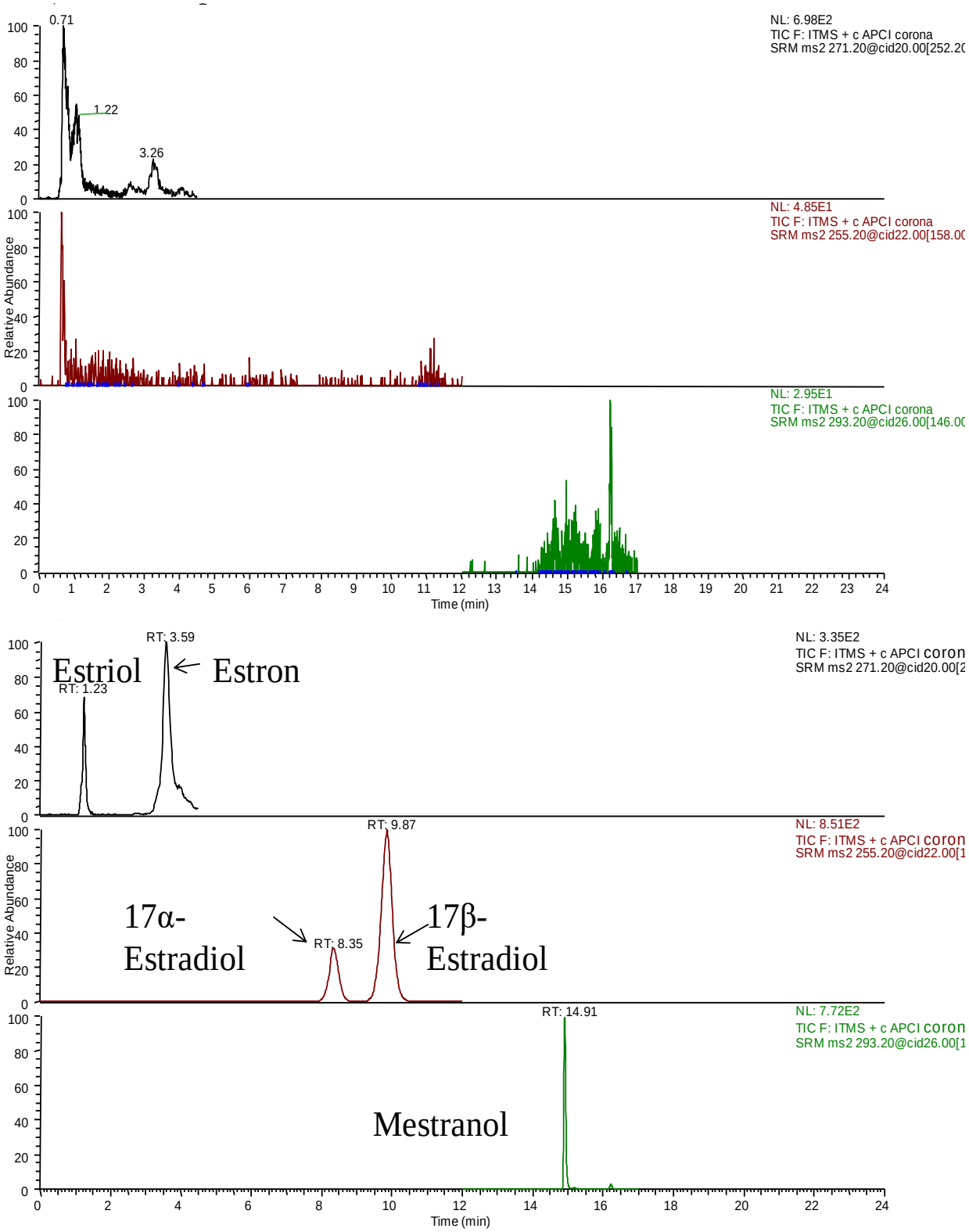

Sample RP'', Novi Sad
600000
500000
400000
300000
200000
100000
0
20
40
60
80
100
Retention time
85.851
81.454
90.056
76.85
94.096
72.042
84.428
97.985
87.324
92.085
101.769
79.959
67.051
100.717
30.621
61.837
56.39
22.653
18.74
50.661
46.759
18.577
75.846


Sample RO', Novi Sad
500000
400000
300000
200000
100000
20
40
60
80
100
Retention time

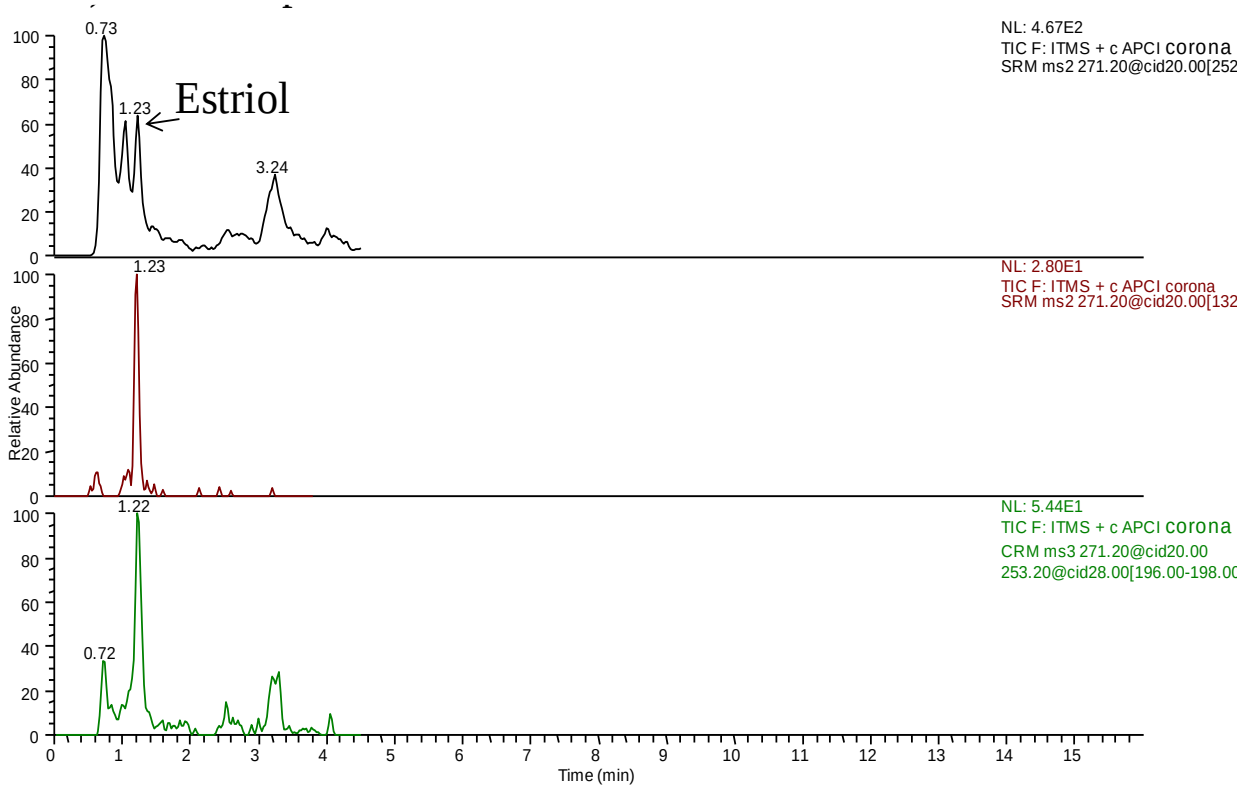

Sample RO'', Novi Sad
Abundance
Retention time
8000000
7000000
6000000
5000000
4000000
3000000
2000000
1000000
0
20
40
60
80
100
74.984
30.583
41.48
53.41
66.806
71.338
77.421
81.366
85.899
89.076
94.113
97.985
88.072
86.514
92.417
101.696
106.721

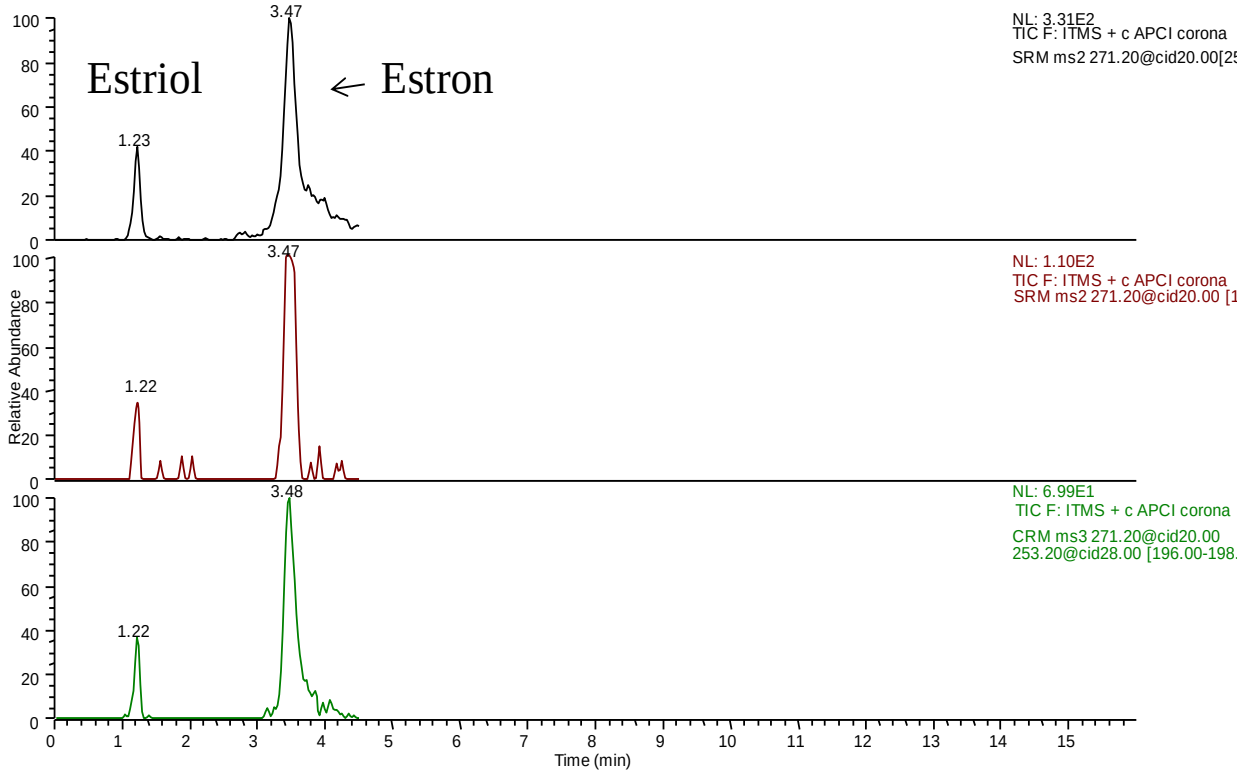
Annex III

Figure III.1 Chromatograms of Estriol confirmation



a) Mixed wastewater sample; b) Standard mixture 200 ng/mL

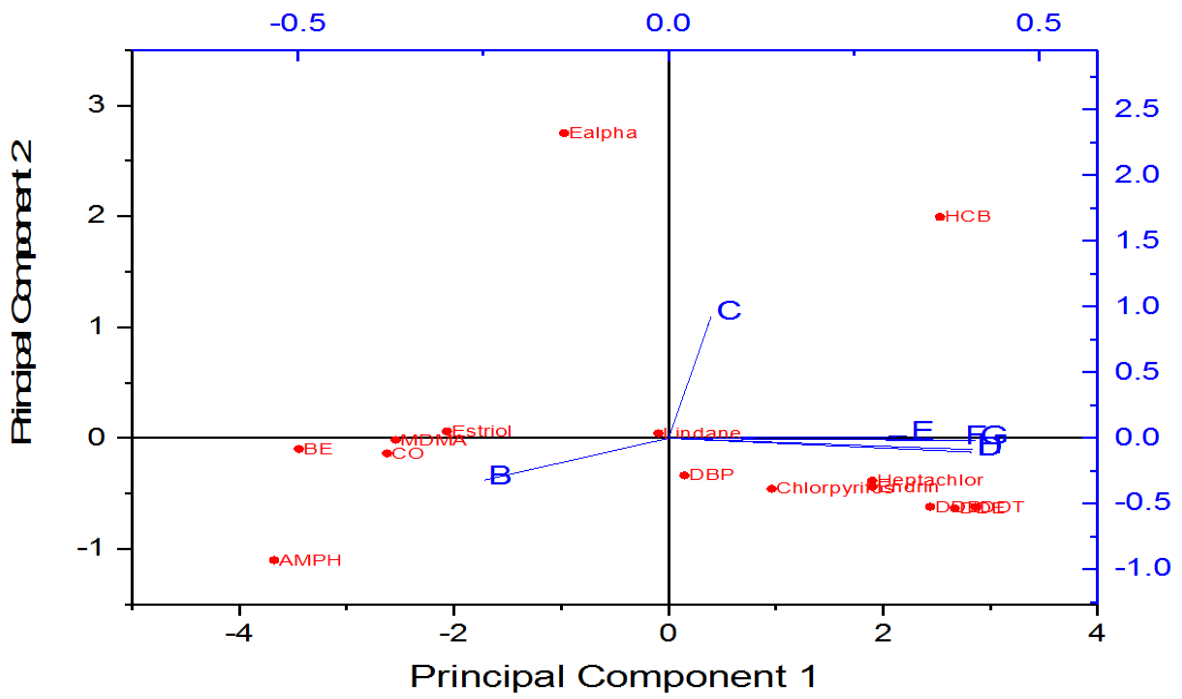




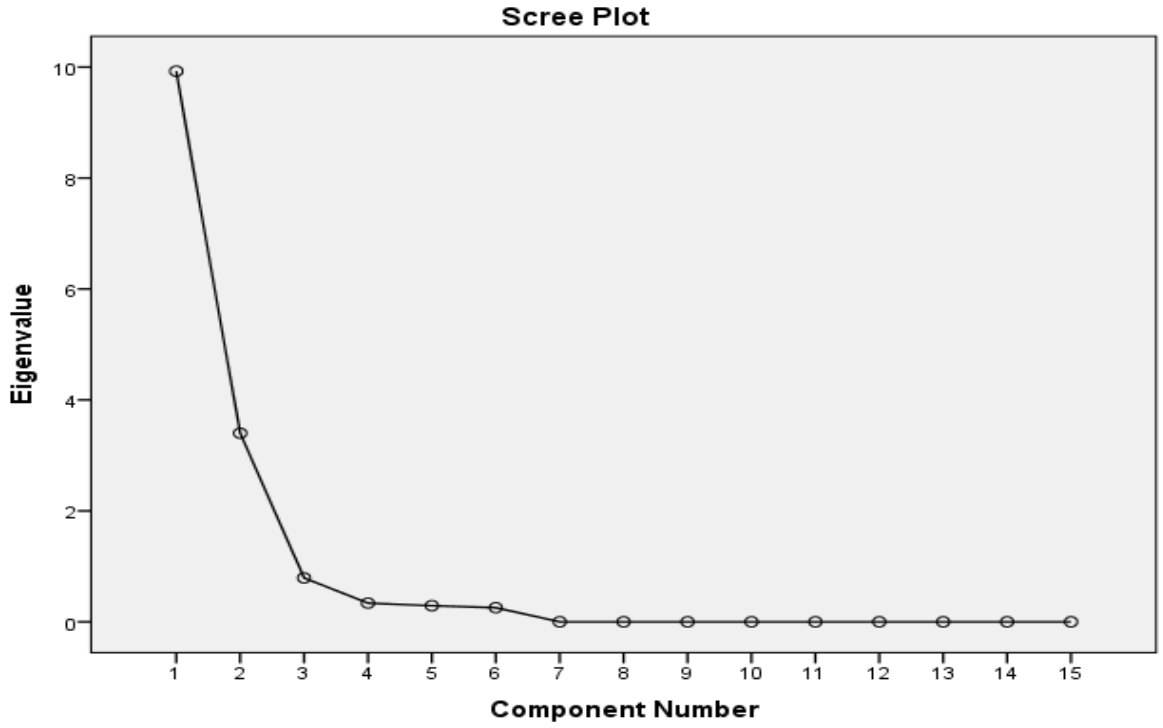
a) Mixed wastewater sample; b) Standard mixture 200 ng/mL

Annex IV

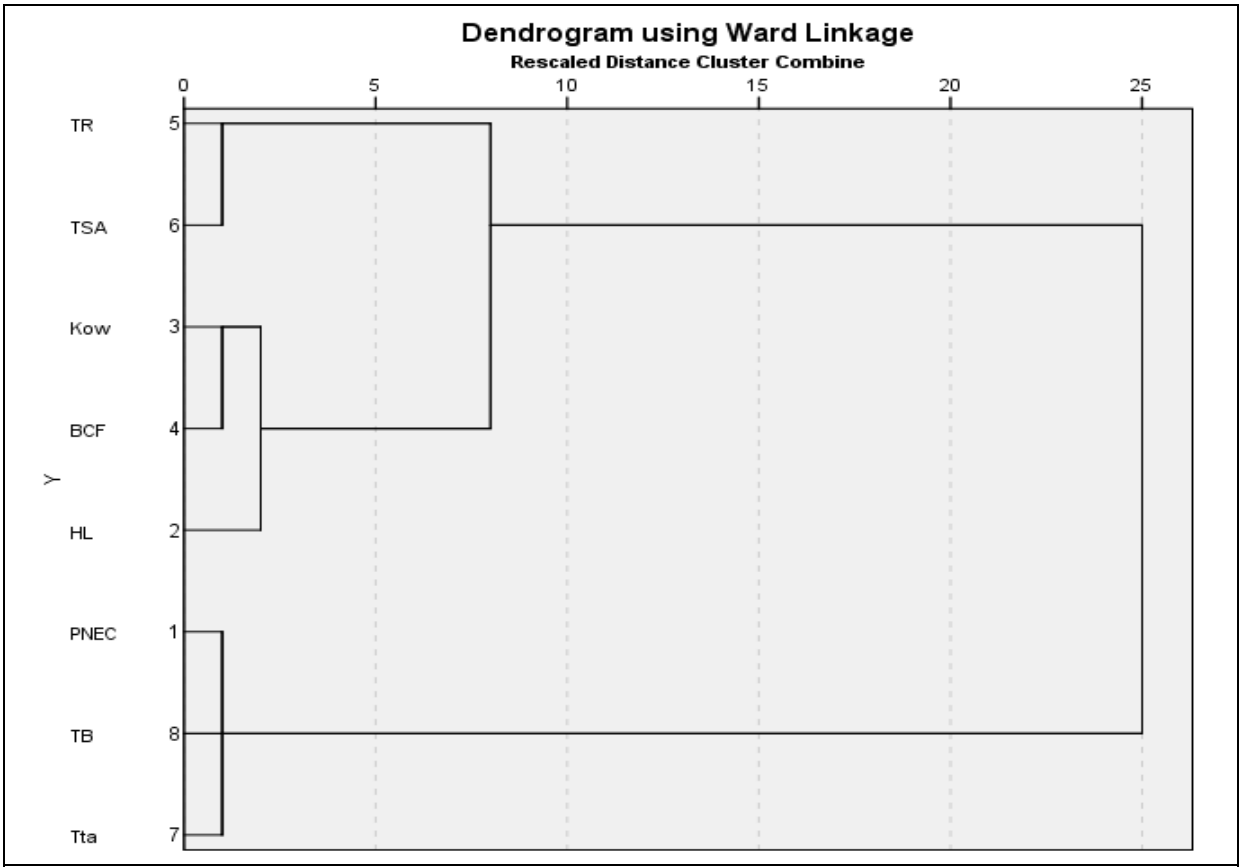
Table IV.1 Results of the statistical processing of obtained data via PCA and HCA



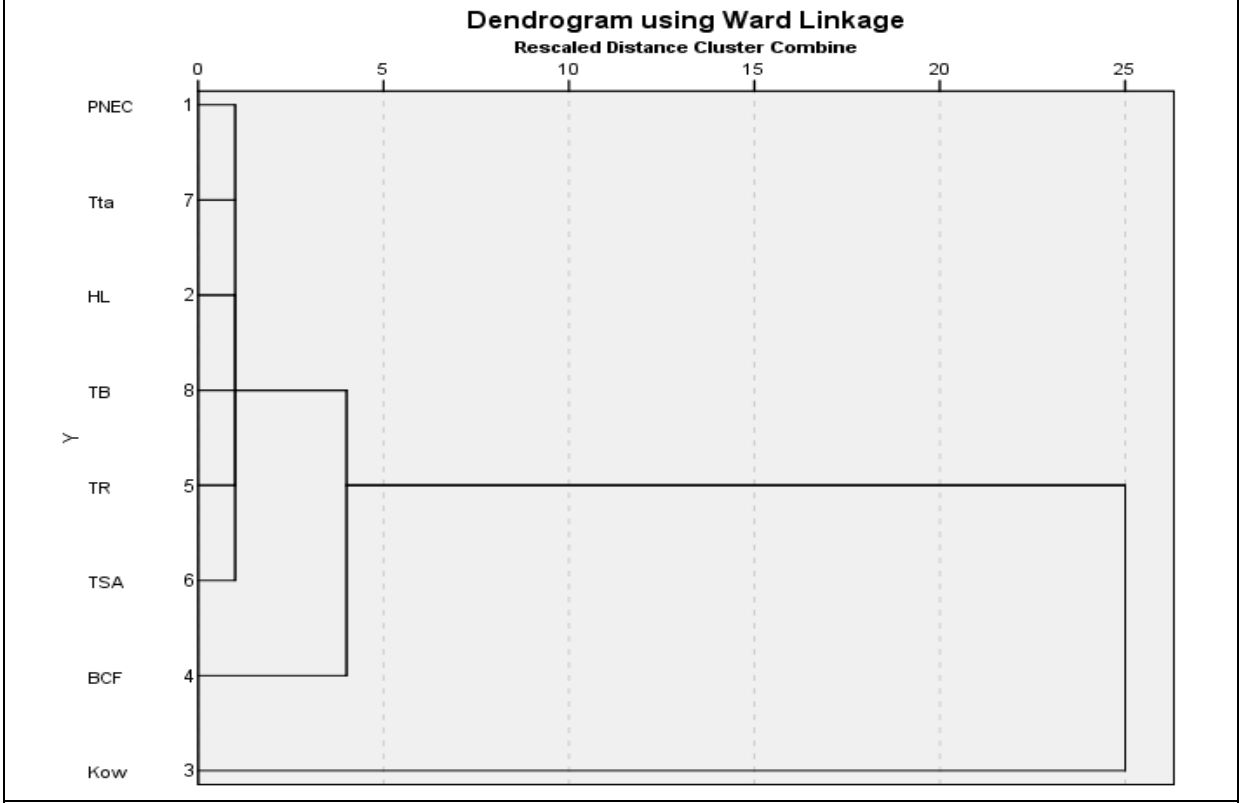
Biplot of PCA analysis for selected parameters



Scree plot of PCA analysis



Dendogram for organochlorine pesticides and phthalates



Dendogram for illicit drugs