



**Hungary-Croatia**  
Cross-border Co-operation Programme

**Interreg**

Európai Regionális Fejlesztési Alap



EURÓPAI UNIÓ



Daughter of the  
mountains  
Environmental state  
review of Mura River

InterReg HUHR/1901/2.2.1/0128

***A cross-border region where rivers  
connect, not divide***





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connect, not divide***

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## Table of contents

1.	About Mura River (countries, tributaries, catchment area, water quality) .....	15
1.1.	Precipitation.....	15
1.2.	Climatic zones .....	16
1.3.	The effect of climate change in the area.....	18
1.4.	Austria .....	19
1.5.	Slovenia.....	20
1.6.	Mura-Drava-Danube Transboundary Biosphere Reserve, Croatia/Hungary.....	20
1.7.	Quality of surface water .....	22
1.8.	Protected by the presence of the Iron Curtain .....	27
1.9.	Ecological characteristics - The flora and fauna of the Landscape Conservation Area along the Mura River.....	29
2.	Main pollution sources around Mura River.....	34
2.1.	The main sources of pollution .....	36
2.2.	Significant surface water uses .....	39
2.3.	Significant groundwater uses .....	39
2.4.	Diffuse contaminations of agricultural origin .....	40
2.5.	Public water supply and wastewater disposal.....	40
2.6.	Loads caused by wastewater .....	42
2.7.	Other significant land-based pollution .....	43
2.8.	Pollution effects of settlements - Utility water supply and sewage disposal.....	43
2.9.	Sewage loads .....	44
2.10.	Other major land - based pollutions .....	45
2.11.	Groundwater load .....	45
2.12.	Natural loads from climate change - Emergence of hydrological and meteorological extremes	46
3.	Wastewater components and their risk assessment .....	47
3.1.	Microorganisms in wastewater.....	47
3.1.1.	Protozoa and helminths .....	49
3.1.2.	Bacteria.....	49
3.1.3.	Viruses.....	50
3.2.	Aerosols.....	51
3.3.	Indicator organizations .....	51
3.4.	Removal of microorganisms.....	53
3.5.	Risk assessment of microbial contaminants .....	53
3.6.	Chemicals in wastewater .....	54
3.6.1.	Inorganic compounds .....	55

3.6.2.	Organic compounds.....	56
3.6.3.	Trace elements.....	58
3.6.4.	Removal of chemical trace elements.....	59
3.6.5.	Risk assessment of chemical trace elements. ....	60
3.6.5.1.	Potential impact of residual chemical constituents.....	62
4.	Selected substances according to pollution sources – The sources and physiological effects of some substances in water .....	64
4.1.	Anthropogenic materials .....	65
4.2.	Chemical contamination of water and its effects on human health.....	67
4.3.	The major unwanted water pollutants .....	68
4.4.	Phosphorus .....	69
4.5.	Nitrogen.....	70
4.6.	Arsenic.....	73
4.7.	Iron and manganese.....	75
4.8.	Cadmium .....	76
4.9.	Lead.....	78
4.10.	Copper .....	80
4.11.	Mercury .....	81
5.	Sample preparation and measurement possibilities of selected substances in the spotlight of EU directives .....	84
5.1.	Traditional methods in water sampling .....	86
5.2.	Sample preparation and measurement possibilities.....	86
5.3.	Selected components group.....	88
5.3.1.	Metals — total and dissolved metals and metalloids .....	88
5.3.2.	Chemical parameters .....	89
5.3.2.1.	Total nitrogen (TN) .....	89
5.3.2.2.	Total phosphorus (TP).....	89
5.3.2.3.	Total oxidised nitrogen (NO <sub>x</sub> -N) .....	90
5.3.2.4.	Nitrogen as ammonia/ammonium (NH <sub>3</sub> -N/NH <sub>4</sub> -N) .....	90
5.3.2.5.	Total Kjeldahl nitrogen (TKN).....	90
5.3.2.6.	Total organic carbon (TOC) .....	91
5.3.2.7.	Biochemical oxygen demand (BOD) .....	91
5.3.3.	Organic components.....	91
5.3.3.1.	Polycyclic aromatic hydrocarbons (PAHs) and Polychlorinated biphenyls (PCBs) 91	
5.3.3.2.	Trihalomethanes (THM).....	92
5.3.3.3.	Pesticides and herbicides .....	92
5.3.3.3.1.	Determination of pesticides (Maciej Tankiewicz et. al., 2011.).....	94

5.3.4.	Pharmaceuticals.....	96
5.3.4.1.	Liquid-Liquid Extraction Sample Preparation Protocol (Stone, 2017) .....	96
5.3.4.2.	Solid Phase Extraction Sample Preparation Protocol (SPE) (J. Stone, 2017) .....	97
5.3.5.	Microbiological analyses.....	98
6.	Sampling plan of surface water – method and locations .....	100
6.1.	Traditional methods and new approaches in water sampling strategy .....	101
6.1.1.	Sampling and monitoring .....	101
6.2.	Objective of Mura monitoring .....	102
6.3.	Selection of monitoring points .....	103
6.4.	Monitoring frequency.....	108
6.5.	Justification and explanation of the content of the sampling plan.....	112
7.	References .....	114

The rivers Mura and Drava geographically divide the Croatian and Hungarian regions. As both countries have the same problems with surface and groundwater, it is advisable to jointly map the state of the border rivers. The project will help solve problems for professionals by developing a sampling methodology plan using state-of-the-art technologies and best practices, providing an overview of the occurrence of water pollutants in the Mura region, and operating an online platform to disseminate the results more widely. Our aim is to develop a regional research network system through joint cooperation and professional cooperation in the region, thus collecting and sharing information obtained during water sampling in order to protect the aquatic environment of the transboundary region with experts in the water sectors. Our long-term goal is to put the knowledge gained into practice, which in the near future can be extended to the whole of the European region, thus contributing to the achievement of good water status and creating a well-functioning cross-border network to improve water quality.

Expected results of the project:

- exploration of polluted or intermittently exposed river sections
- identification of pollutants in both surface and groundwater
- on-line database of measured parameters
- implementation of joint education and training programs
- newly established cooperation agreements in the field of water and wastewater treatment jointly developed studies (laboratory reports, studies, international publications, etc.)
- common communication materials (roll-ups, flyers, brochures, promotional materials, video clips).

The aim of the environmental state review is

- to characterize the river on the basis of the literature search prior to the start of the work,
- to explore the characteristics and sources of pollution of the studied area,
- to propose / designate the analytes to be examined and
- to develop a sampling plan.

Mura is a cross-border river in Central Europe which connects four countries with its 465 km length. It is a tributary of the Drava and subsequently the Danube. The size of its basin is 14,304 km<sup>2</sup>. The Landscape Conservation Area along the Mura extends from the estuary of the Kerka to the Drava and includes the area from the border to the south-western edge of the Zala Hills. After hundreds of years of constant cultivation, due to military closures it has regained its natural character, however these habitats require further monitoring and management.

The rivers Mura and Drava geographically divide the Croatian and Hungarian regions. As both countries have the same problems with surface and groundwater, it is advisable to jointly map the state of the border rivers. This Interreg project (HUHR/1901/2.2.1/01289) is intended to identify and solve problems for professionals by provide detection of current contaminants in a wide-range. This process consists of developing a sampling methodology plan using state-of-the-art technologies and best practices, providing an overview of the occurrence of water pollutants in the Mura region, and operating an online platform to disseminate the results more widely. Utilizing the principles of Interreg programmes (e.g. efficiency, effectiveness, interregional learning, cooperation) the main goal is to develop a regional research network system through joint cooperation and professional cooperation in the region, thus collecting and sharing information obtained during water sampling in order to protect the aquatic environment of the transboundary region with experts in the water sectors. The most significant aim of this study was 1) to characterize River Mura on the basis of the literature search prior to the start of the work, to explore the characteristics and sources of pollution of the studied area, thus, designate the analytes should be examined, in addition 2) to develop a comprehensive sampling plan that takes into account the most important pollutant sources and to set up a comprehensive monitoring network of Mura River (focused on water quality). Water pollution has a significant impact on the natural renewal of water resources. Pollution does not know the concept of national borders! Pollutants transported in rivers can cross regional and national borders and eventually reach the ocean, where they can spread uncontrollably. Diffuse pollution from landfills or agricultural sources, as wastes, fertilizers and pesticides are mainly non-point sources, however, treated industrial and municipal wastewater discharges are point sources. All of the water is eventually used in the natural cycle and contains detectable amounts of various chemicals. Untreated municipal wastewater contains a number of components, from dissolved metals and organic compounds to large solids such as rags, sticks, floating objects, granules and greases. Inorganic constituents of wastewater include metals, salts, oxyhalides, nutrients and potentially engineered nanomaterials. The organic composition of raw wastewater includes

naturally occurring humic substances, fecal matter, kitchen waste, liquid detergents, oils, greases, consumer goods, industrial waste and other substances that become part of the wastewater. The former wastewater treatment technologies had to be developed and supplemented with new procedures in order to prevent pollution and damage to the environment. Sophisticated analytical tools allow the identification and quantification of extremely low levels of each inorganic and organic constituent. The danger is mainly the dissolved contaminant, which can easily be absorbed into the food chain, accumulate in organisms and have an adverse, harmful effect. In addition, heat is a source of pollution if it changes the properties of the water in an unfavourable direction for the living world.

In European Union the monitoring of river water quality is carried out on the basis of laws and regulations in accordance with the requirements of the Water Framework Directive (WFD - Directive 2000/60/EC) and other guidelines and professional instructions for the establishment and implementation of the monitoring. The Directive determines the limit values of parameters and criteria for the assessment of the chemical status. In recent years, monitoring of anthropogenic pollutants in surface water have been emphasized not only in media but also in legal activity in European Union. Hormones, antibiotics, pesticides, antidepressants, anti-epileptic drugs and analgesics come to spotlight with the EU Priority Substance Watch List (WL, 2018/840) of surface water (under Water Framework Directive, renew in 2018). Monitoring of these substances in surface water is mandatory for each member state.

In general, pollution is made up of municipal, industrial, agricultural and diffuse sources. Industrial activities release significant amounts of wastewater into the environment, almost all of which end up in surface waters. In the agricultural sector, intensive farming, combined with high use of fertilizers and pesticides, has led to the pollution of groundwater with nitrates and pesticides. Large livestock farms are significant point sources of water pollution, with rudimentary wastewater treatment posing a particular risk in karst and groundwater near small streams. In the case of toxic substances (metals, pesticides, organic compounds), industry accounts for more than half of the pollutants, while municipal and agricultural sources account for the rest. Landfills are one of the main sources of untreated leachate, which can have a potentially severe impact on groundwater and surface water.

There are various companies operating in the project area that can be sources of pollution for surface and groundwater. The most important sources of pollution in the Hungarian project area are: abandoned illegal landfills, illegally drilled wells, gas stations, plants, sewage plants, railway lines, thermal baths, and diffuse pollution of intensively cultivated areas, which involves the use of fertilizers and pesticides. The latter is ubiquitous in the project area and, for some parameters, can have a major impact on the quality of surface water and groundwater.



The main sources of pollution in Croatia in the study area are: meat- and textile industry, industrial laundry, hospitals, car maintenance, metal industry, car- and food industry, manufacture of bricks, ceramics and glass.

Taking the above into account, the selected component groups of the monitoring are the following: metals, chemical parameters, organic components, pharmaceuticals, herbicides/pesticides, biological parameters. Currently, the most commonly used method for measuring levels of chemical pollutants is spot (bottle) sampling, followed by extraction and instrumental analysis. This methodology is well established and validated, so it has been accepted for regulatory and legislation purposes. One of the main difficulties in sampling is preservation (handling and storage) of the sample. The initial composition of the sample must be maintained from sampling through to analysis. If this is not the case, the final conclusions will not reflect the initial situation. Water-sampling strategy and select of sampling location are important during status survey of water environment. The sampling site should represent the environment under study. Criteria for section allocation is the two-level point allocation: at a specified river section (around pollution source input) the same number of sampling points (before – after) should be sampled. The primary consideration in designating the surface sampling points was the location of the wastewater treatment plant. When designating the sampling points, we took into account the potential vulnerabilities arising from human interventions and activities on both the Croatian and Hungarian sides. In the examined section (approx. 50 km), sampling points were designated in connection with 7 settlements, mainly in connection with the points of discharge of treated wastewater from a given settlement into the receiver.

By design of the Mura river monitoring, our goal was to monitor the chemical status of surface and groundwater and mapping the examination of the impact of effluent discharges or accidental pollution on the recipient under one-year period. In the case of surface waters, many components are analysed on a monthly basis (chemical and biological parameters, organic contaminants, drug residues) and the frequency of metals and pesticides determined in the other component groups varies. Metals are tested quarterly, while pesticides are also tested four times a year, but at different times. The definition of pesticides is scheduled for the spring months, as their releases to the environment are significant during this period.

A Mura egy határokon átnyúló Közép-Európai folyó, amely 465 km-es hosszával négy országot köt össze. A Dráva, majd a Duna mellékfolyója. Medencéjének mérete 14 304 km<sup>2</sup>. A Mura menti Tájvédelmi Körzet a Kerka torkolatától a Dráváig terjed, és a határtól a Zalai-dombság délnyugati pereméig terjedő területet foglalja magában. Több száz éves folyamatos művelés után a katonai lezárások miatt visszanyerte természetes jellegét, azonban ezek az élőhelyek további felügyeletet és kezelést igényelnek.

A Mura és a Dráva folyók földrajzilag kettéválasztják a horvát és a magyar régiót. Mivel mindkét országnak ugyanazok a felszíni és a felszín alatti vizekkel kapcsolatos problémái, célszerű közösen feltérképezni a határfolyók állapotát. Jelen Interreg projekt (HUHR/1901/2.2.1/01289) hivatott a felmerülő problémák azonosítására és megoldására, az aktuális szennyeződések széleskörű detektálásával a szakemberek számára. Ez a folyamat a legkorszerűbb technológiák és legjobb gyakorlatok felhasználásával egy mintavételi módszertani terv kidolgozásából áll, áttekintést ad a vízszennyező anyagok előfordulásáról a Mura-régióban, valamint egy online platformot hoz létre az eredmények szélesebb körű terjesztésére. Az Interreg programok alapelveit (pl. hatékonyság, eredményesség, régiók közötti együttműködés, tanulás) kihasználva a fő cél egy regionális kutatási hálózati rendszer kialakítása közös szakmai összefogással a térségben, a vízmintavételek során szerzett információk összegyűjtése és megosztása a határokon átnyúló régió vízi környezetének védelme érdekében. A tanulmány legjelentősebb célja az volt, hogy 1) a munka megkezdését megelőző szakirodalmi kutatás alapján jellemezze a Mura folyót, feltárja a vizsgált terület jellemzőit és szennyező forrásait, így kijelölje a vizsgálandó analitokat, továbbá 2) átfogó mintavételi terv kidolgozása, amely figyelembe veszi a legfontosabb szennyezőforrásokat és a Mura folyó átfogó monitoring hálózatára (a vízminőségre) fókuszál. A vízszennyezés jelentős hatással van a vízkészletek természetes megújulására. A környezetszennyezés nem ismeri az országhatár fogalmát! A folyók által szállított szennyező anyagok átléphetik a regionális és országhatárokat, és végül elérhetik az óceánt, ahol ellenőrizhetetlenül terjedhetnek. A hulladéklerakókból vagy mezőgazdasági forrásokból származó diffúz szennyezések (pl. a hulladékok, műtrágyák és növényvédő szerek) főként nem pontszerű források, azonban a tisztított ipari és kommunális szennyvízkibocsátás pontszerű forrást jelent. A víz felhasználódik a természetes körforgásban, és kimutatható mennyiségben tartalmaz különféle vegyi anyagokat. A kezeletlen kommunális szennyvíz számos összetevőt tartalmaz, az oldott fémektől és szerves vegyületektől a nagyméretű szilárd anyagokig (pl. textíliák, lebegő tárgyak, szemcsék és zsírok). A szennyvíz szerves összetevői közé tartoznak a fémek, sók, oxihalogenidek,

tápanyagok és a potenciálisan mesterségesen előállított nanoanyagok. A nyers szennyvíz szerves összetételében megtalálhatók a természetben előforduló humuszanyagok, fekáliák, konyhai hulladékok, folyékony mosószerek, olajok, zsírok, fogyasztási cikkek, ipari hulladékok és egyéb anyagok, amelyek a szennyvíz részévé válnak. A korábbi szennyvíztisztítási technológiákat fejleszteni kellett és új eljárásokkal kiegészíteni a szennyezés és a környezetkárosítás megelőzése érdekében. A kifinomult analitikai eszközök lehetővé teszik az egyes szervetlen és szerves összetevők rendkívül alacsony szintjének azonosítását és mennyiségi meghatározását. A veszélyt elsősorban az oldott szennyeződés jelenti, amely könnyen felszívódhat a táplálékláncba, felhalmozódhat a szervezetekben és káros hatást fejthet ki. Emellett a hó is szennyező forrás, mivel a víz tulajdonságait az élővilág számára kedvezőtlen irányba változtatja.

Az Európai Unióban a folyóvíz minőségének monitorozása a Víz Keretirányelv (VKI - 2000/60/EK irányelv), egyéb irányelvek és szakmai utasítások előírásai szerint törvényi és rendeleti előírások alapján történik a létesítésre és végrehajtásra vonatkozóan. Az irányelv meghatározza a paraméterek határértékeit és a kémiai állapot értékelésének kritériumait. Az elmúlt években a felszíni vizek antropogén szennyezőanyagainak monitorozása nemcsak a médiában, hanem a jogi tevékenységben is hangsúlyt kapott az Európai Unióban. A hormonok, az antibiotikumok, a peszticidek, az antidepresszánsok, az epilepszia elleni szerek és a fájdalomcsillapítók a felszíni vizekre vonatkozó EU Priority Substance Watch List (WL, 2018/840) előtérbe kerültek (VKI, 2018-ban megújult). Ezen anyagok felszíni vizekben való megfigyelése minden tagállamban kötelező.

Általában a szennyezés települési, ipari, mezőgazdasági és diffúz forrásokból tevődik össze. Az ipari tevékenység során jelentős mennyiségű szennyvíz kerül a környezetbe, amelyek szinte mindegyike felszíni vizekbe kerül. A mezőgazdasági ágazatban az intenzív gazdálkodás, valamint a nagy mennyiségű műtrágya és növényvédő szerek használata a talajvíz nitrátokkal és növényvédő szerekkel való szennyezéséhez vezetett. A nagy állattartó telepek a vízszennyezés jelentős pontszerű forrásai, a kezdetleges szennyvíztisztítás különös kockázatot jelent a kis patakok közelében lévő karszt- és talajvízben. A mérgező anyagok (fémek, növényvédő szerek, szerves vegyületek) esetében a szennyező anyagok több mint felét az ipar, a többit a települési és mezőgazdasági források teszik ki. A hulladéklerakók a kezeletlen csurgalékvíz egyik fő forrásai, amely potenciálisan súlyos hatással lehet a talajvízre és a felszíni vizekre.

A projekt területén különböző cégek működnek, amelyek felszíni és felszín alatti vizek szennyező forrásai lehetnek. A magyarországi projektterület legfontosabb szennyező forrásai: felhagyott illegális hulladéklerakók, illegálisan fúrt kutak, benzinkutak, üzemek,

szennyvíztelepek, vasútvonalak, termálfürdők, valamint az intenzíven művelt területek diffúz szennyezése, például műtrágya, ill. rovarirtók által. Ez utóbbi mindenütt jelen van a projekt területén, és bizonyos paraméterek esetén jelentős hatással lehet a felszíni és a felszín alatti vizek minőségére. Horvátország fő szennyező forrásai a vizsgált területen: hús- és textilipar, ipari mosoda, kórházak, autókcarbantartás, fémipar, autó- és élelmiszeripar, téglá-, kerámia- és üveggyártás.

A fentiek figyelembevételével a monitoring vizsgálat kiválasztott komponenscsoportjai a következők: fémek, kémiai paraméterek, szerves komponensek, gyógyszerek, gyomirtó/pesticidok, biológiai paraméterek. Jelenleg a leggyakrabban használt módszer a kémiai szennyező anyagok szintjének mérésére a pontszerű (palackos) mintavétel, amelyet az extrakció és a műszeres elemzés követ. Ez a módszertan jól megalapozott és validált, ezért szabályozási és jogszabályi célokra elfogadott. A mintavétel egyik fő nehézsége a minta tartósítása (kezelése és tárolása). A minta kezdeti összetételét a mintavételtől az elemzésig meg kell őrizni. Ellenkező esetben a végső következtetések nem tükrözik a kiindulási helyzetet. A vízi környezet állapotfelmérése során fontos a vízmintavételi stratégia és a mintavételi hely kiválasztása. A mintavételi helynek reprezentálnia kell a vizsgált környezetet. A vizsgált szakaszon az alábbi kritériumokat alkalmazva kerültek meghatározásra a mintavételi pontok: egy adott ponton a szennyezőforrás bemenet környékén és minden mintavételi ponton további két ponton (előtte-utána) kell mintát venni. A felszíni vizekből történő mintavételi helyek kijelölésénél elsődleges szempont a szennyvíztisztító telepek elhelyezkedése volt. A mintavételi helyek kijelölésénél figyelembe vettük az emberi beavatkozásokból és tevékenységből adódó potenciális sebezhetőséget horvát és magyar oldalon egyaránt. A vizsgált szakaszon (kb. 50 km) 7 településhez kapcsolódóan mintavételi pontok kerültek kijelölésre, elsősorban az adott településről származó tisztított szennyvíz befogadóba történő bevezetési helyeihez kapcsolódóan.

A Mura folyó monitoring tervezésével célunk volt a felszíni és felszín alatti vizek kémiai állapotának monitorozása, valamint a szennyvízkibocsátások vagy véletlen szennyezések befogadóra gyakorolt hatásának feltérképezése egy éves periódus alatt. A felszíni vizek esetében számos komponens havi szinten kerül elemzésre (kémiai és biológiai paraméterek, szerves szennyeződések, gyógyszermaradványok), a többi komponenscsoportban a fémek és növényvédő szerek meghatározási gyakorisága változó. A fémeket negyedévente, a növényvédő szereket szintén évente négyszer, de eltérő időpontokban vizsgálja a partnerség. A növényvédő szerek meghatározását a tavaszi hónapokra tervezve, mivel ebben az időszakban jelentős a környezetbe való kibocsátásuk.

Mura je srednjoeuropska prekogranična rijeka koja je dugačka 465 km i spaja četiri države. Mura je pritok Drave, potom Dunava, a ukupna slivna površina joj je 14 304 km<sup>2</sup>. Prirodno zaštićeno područje uz Muru prostire se od potoka Kerka čak do Drave, a pripada mu i područje koje se prostire od državne granice do jugozapadnog dijela Zalskih brežuljaka. Na ovim prostorima stoljećima se vršila intenzivna poljoprivredna proizvodnja, međutim, zbog zatvaranja pograničnog prostora što je trajalo skoro 50 godina, ovdašnja staništa su se zaštitila u relativno dobrom prirodnom okruženju. Međutim, potreban je strogi nadzor i upravljanje tih staništa.

Mura i Drava su rijeke koje u geografskom smislu razdvajaju hrvatsku i mađarsku regiju. Pošto obje države suočavaju se istim problemima površinskih i podzemnih voda, svrsishodno je pripremiti zajedničku analizu stanja pograničnih rijeka. Za identifikaciju istih i pronalaženje mogućih rješenja, te za detektiranje aktualnih onečišćivača uz pomoć stručnjaka pruža nam mogućnost provedba Interreg projekta (HUHR/1901/2.2.1/01289). Ovaj proces sastoji se od izrade metodičkog plana za monitoring primjenom najsuvremenijih tehnologija i najbolje prakse, daje pregled o pojavljivanju onečišćivača u Mura regiji, odnosno stvaranjem nove online platforme postoji mogućnost za objavljivanje i upoznavanje rezultata šire javnosti. Iskoristivši osnovna načela Interreg programa (kao npr. efikasnost, produktivnost, suradnja između regija, edukacija) glavni cilj nam je stvaranje regionalnog istraživačkog mrežnog sustava u zajedničkoj suradnji sa ekspertima iz regije, uzimanje uzoraka, prikupljanje podataka i podjela informacija u svrhu zaštite prekograničnog vodenog prostora. Najznačajniji cilj studije je bio: 1) prije detaljne analize dati opis o Muri uz pomoć istraživanja stručne literature, identificirati opće karakteristike i izvore onečišćivača istraživanog područja, te na taj način označiti analite, nadalje 2) izraditi opsežan metodički plan uzorkovanja koji uzima u obzir najznačajnije izvore onečišćivača i fokusira na opsežni monitoring sustav (na kvalitetu vode) rijeke Mure. Onečišćenje vode značajno utječe na obnovljivost prirodnih vodenih zaliha. Onečišćenje prirode pak ne poznaje granice! Onečišćene tvari koje rijeke nose sa sobom prelaze regionalne i državne granice, te na kraju stižu do oceana gdje bez kontrole plutaju. Difuzni onečišćivači (npr. otpadi na odlagalištima, umjetna gnojiva i pesticidi) ne spadaju u kategoriju točkastih izvora, međutim, očišćeni industrijski i komunalni kanalizacijski otpad već spada u kategoriju točkastih izvora. Voda je nazočna u prirodnom ciklusu i sadrži u određenoj količini različite kemijske elemente. Neočišćena komunalna kanalizacijska voda sadrži brojne sastojke, od natopljenih metala i organskih spojeva do krutih materijala sve (npr. tekstilije, masti, itd.). Među sastojke anorganskih tvari se ubrajaju metali, soli, aksihalogenidi, hranive tvari i



potencijalno umjetno stvoreni nano materijali. Među sastojcima sirove otpadne vode može se naći humus, fekalija, kuhinjski otpad, sredstva za pranje posuđa, ulje, mast, uporabni predmeti, industrijski otpad i druge tvari koje su dio otpadnih voda. Prethodno korištene tehnologije za čišćenje otpadnih voda trebalo je modernizirati i primjeniti novije tehnologije i procese s ciljem prevencije i daljnjeg onečišćenja okoliša. Profinjena sredstva za analizu omogućuju identificiranje i dokazuju postojanje vrlo malih količina organskih i anorganskih tvari u vodama. Najveću opasnost predstavljaju ipak otopljeni onečišćivači koji lako mogu dospjeti u hranidbeni lanac, tamo se nagomilavaju i stvaraju trajne štetne učinke. Pored toga i toplina spada među izvore onečišćivača, pošto mijenja karakteristiku vode i negativno utječe na prirodu.

U Europskoj uniji monitoring kvalitete prirodnih voda vrši se na temelju Okvirne direktive o vodama (2000/60/EK), nadalje primjenom drugih direktiva, zakona i uredaba. Direktiva definira granice parametara i kriterije pri vrednovanju kemijskog stanja. U proteklim godinama monitoring antropogenih onečišćivača u površinskim vodama dobilo je više prostora ne samo u medijima, nego i u pravnim procesima Europske unije. Hormoni, antibiotici, pesticidi, antidepresivi, lijekovi protiv epilepsije i ublažavanje bolova prema prihvaćenoj Uredbi - EU Priority Substance Watch List (WL, 2018/840) – u svim članicama EU-a trebaju se obvezatno promatrati i istraživati u površinskim vodama. Općenito se može reći da se onečišćivači sastoje od komunalnih, industrijskih, poljoprivrednih i difuznih izvora. Tijekom industrijske proizvodnje značajna količina onečišćivača dopire u prirodu koji pri tome najčešće mogu se naći u površinskim vodama. U poljoprivrednom sketoru intenzivna proizvodnja, odnosno korištenje ogromnih količina umjetnog gnojiva i pesticida može dovesti do onečišćivanja površinskih voda nitratima i drugim pesticidima. Također su veliki zagađivači farme i točkasti izvori onečišćavanja vode što predstavlja problem, ako su u blizini izvori u vapnencima. U slučaju toksičnih tvari (metali, pesticidi, organski spojevi) više od polovine onečišćivača dolazi s područja industrije, a druga polovica s područja poljoprivrede, odnosno iz urbanih sredina. Procjedna voda može biti također jedan od glavnih izvora onečišćenja kod odlagališta otpada što može grubo utjecati na kvalitetu podzemnih i površinskih voda.

Na projektnom području nalaze se različite tvornice i poduzeća koje mogu biti izvori onečišćenja podzemnih i površinskih voda. Na projektnom području u Mađarskoj najčešći potencijalni izvori onečišćenja su sljedeći: ilegalna odlagališta otpada, ilegalno bušeni bunari, benzinske postaje, poduzeća, prošistači otpadnih voda, željezničke linije, termalna kupališta, odnosno intezivno obrađivana zemljišta koja se difuzno zagađuju putem korištenja različitih pesticida i umjetnog gnojiva. Ovo je inače katrateristično za cijelo projektno područje što znatno može utjecati na kvalitetu površinskih i podzemnih voda. Na projektnom području u

Hrvatskoj glavni izvori onečišćenja su sljedeći: - mesna i tekstilna industrija, - industrijska praonica, - bolnice, – servisiranje vozila, - metalna industrija, - automobilska i prehrambena industrija, - proizvodnja opeke, keramike i stakla. Uzimanjem u obzir gore navedeno izabrane komponentne grupe za monitoring su sljedeće: metali, kemijski komponenti, organski komponenti, lijekovi, pesticidi, biološki parametri. Trenutno najčešća metoda za uzimanje uzoraka i mjerenje kemijskih onečišćivača je točkasto (flaširano) uzorkovanje, a nakon toga slijedi ekstrakcija i analiza putem instrumenata. Ova metoda je temeljna i validirana, s toga je prihvaćena i tijekom primjene pravnih i zakonskih postupaka. Jedan od najvećih problema je konzerviranje i skladištenje uzoraka. Početno stanje i strukturu uzorka od početka procesa uzorkovanja do analize treba zadržati. U protivnom konačne konkluzije ne odražavaju početno stanje. Tijekom analize stanja vodenog okoliša važno je definirati strategiju uzorkovanja i označiti lokacije za uzimanje uzoraka. Mjesto uzorkovanja mora reprezentirati okolišno stanje. Na dionici gdje se vrši uzorkovanje definirani su sljedeći kriteriji na temelju kojih su označena mjesta za uzimanje uzoraka: treba identificirati izvor onečišćenja, zatim označiti još dvije točke (prije-poslije) za uzimanje uzoraka. U slučaju uzimanja uzoraka površinskih voda jedan od prioriteta za označavanje mjesta je bio gdje se nalazi pročistač otpadnih voda. Također tijekom označavanja lokacija za uzimanje uzoraka na hrvatskoj i mađarskoj strani uzeli smo u obzir ljudske intervencije i aktivnosti koje utječu na okoliš. Na dionici od 50 km pored 7 naselja označeno je nekoliko mjesta za uzimanje uzoraka, prvenstveno su to lokacije gdje se u površinske vode ulijeva pročišćena otpadna voda.

Planiranjem monitoringa rijeke Mure cilj nam je bio utvrditi kemijsko stanje podzemnih i površinskih voda, odnosno kako utječe na okoliš ispuštena očišćena otpadna voda i drugi onečišćivači u periodu od godinu dana. U slučaju površinskih voda brojni komponenti se analiziraju mjesečno (kemijski i biološki parametri, organski onečišćivači, zaostaci lijekova), a u slučaju drugih komponenata (metala i pesticida) učestalost analize mijenja se tijekom provedbe projekta. Partneri metale kvartalno, a pesticide također godišnje četiri puta analiziraju, ali uvijek u drugim vremenskim terminima. Pesticidi će se analizirati tijekom proljetnih mjeseci, pošto se baš tada koriste u najvećim količinama.

# 1. About Mura River (countries, tributaries, catchment area, water quality)

Mura is a river in Central Europe, 465 km in length, rises in Austria (1898 m above sea-level) Hohe Tauern national park of the Central Eastern Alps. It is a tributary of the Drava and subsequently the Danube. The size of its basin is 14,304 km<sup>2</sup>. More than half of its surface is in Austria, where the River is 326 km. The Slovenian section of the basin is 1,393 km<sup>2</sup> in area, 95 km flow in and around Slovenia (67 km along the borders with Austria and Croatia, 28 km inside Slovenia). The rest forms the border between Croatia and Hungary. The tributary in Croatia is 987 km<sup>2</sup>, and Hungary 1911 km<sup>2</sup>. The largest city on the river is Graz, Austria (Krajnc et al., 2010).

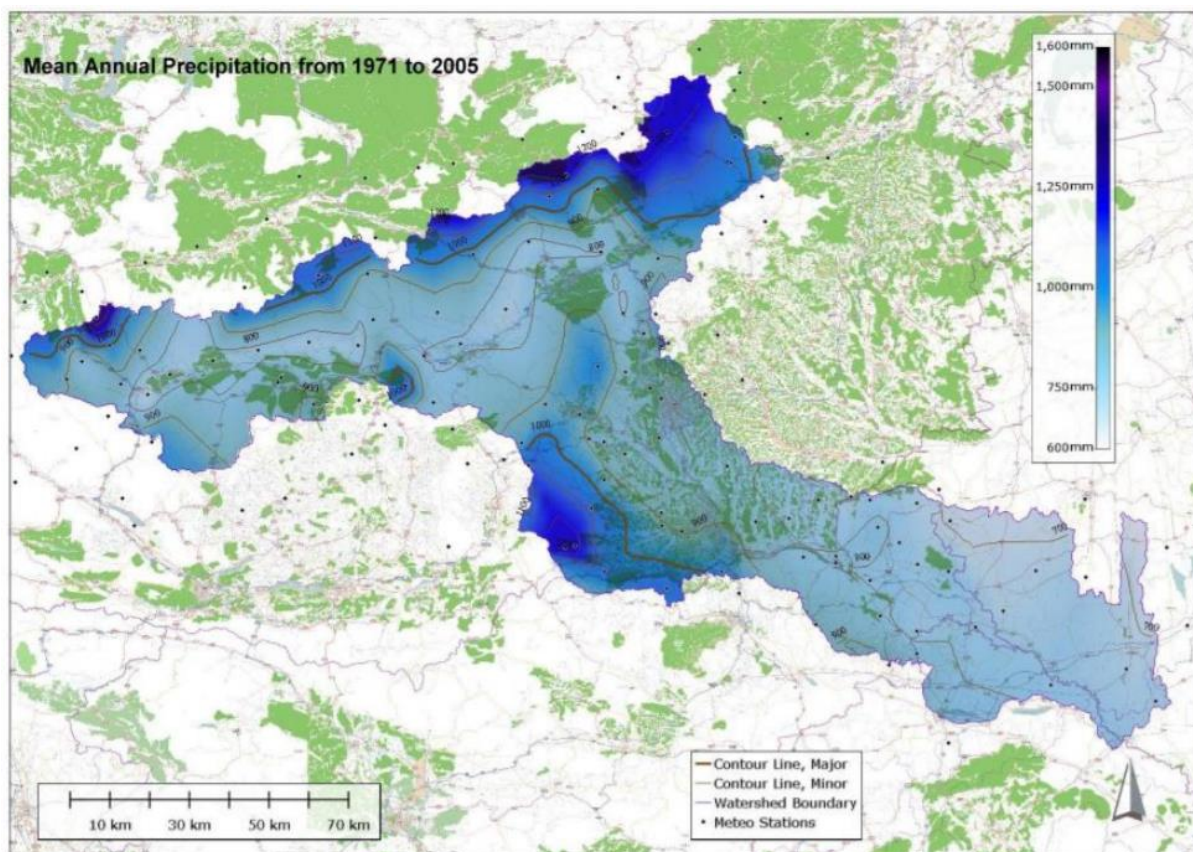
The River Mura can be divided according to the regions through which it flows:

- Mountain section (source – Bruck, Austria) over 216 km (46 %) within a catchment area of 4,700 km<sup>2</sup> (34 %),
- Transitional section (Bruck – Mureck, Austria) over 119 km (26 %) within a catchment area of 5,070 km<sup>2</sup> (36 %), where place the largest city, Graz,
- Lowland section (Mureck – Legrad, Slovenia, Croatia, Hungary) over 130 km (28 %) within a catchment area of 4,255 km<sup>2</sup> (30 %).

Its major left tributaries are Murica and Lendava and right tributaries Ščavnica and Trnava which were, under regulation, redirected from the Drava into the Mura.

## 1.1. Precipitation

Yearly precipitation amount is very various on the basin area of Mura River. An average yearly precipitation map for the period 1971-2005 was produced by Šraj et al. (2011). Maximum yearly amount in this period variates from 1800 mm in headwaters, to 900 mm in southern plains.



Yearly precipitation map for the period 1971-2005 (Source: Šraj et al., 2011)

## 1.2. Climatic zones

The so-called Illyric climate of the Upper Drava Valley is due to the inflow of Mediterranean air masses through the gates of the South Alpine ranges, the movement of Atlantic air from northwestern direction and the protective effect of the High Tauern massif (3,798 m) (Auer et al., 2001). Transitional among Mediterranean, Alpine and the drier Pannonian climates, this zone extends from southern Austria to north-eastern Slovenia. A large number of sunshine hours (more than 2,000 h above 2,000 m elevations) and summer days (10–15 days with above 20 °C daily mean temperature at elevations between 400 and 500 m) are typical of the Upper Drava Valley (ZAMG, 2016). One of the highest absolute maximum temperatures in Austria was recorded in Dellach (Carinthian Drava valley) on 3 August 2013 (39.9 °C). Along the Upper Drava and Gail Rivers Mediterranean influence can be detected in early spring warming (starting in February) and also in the late autumn (November) secondary precipitation maximum (mostly rainfall, 9–11% of annual total).

However, high summer rainfall amounts (30–35% share of annual precipitation, i.e. much higher than under true Mediterranean climate) point to the common occurrence of oceanic cyclones along the Upper Drava. The vegetation at 400–700 m reflects this moderately warm and dry climate: sweet chestnut and fluffy oak forests with periwinkle (*Cotinus coggygia*) and

manna ash (*Fraxinus ornus*) are found in the Upper Drava Valley. The frequent thermal inversions, however, make the (Isel, Möll, Lieser, Gail and Gurk) valleys and basins cold in winter.

The Pannonian (continental) climate zone replaces the Mediterranean climate in the southeast of the drainage basin, downstream the Drava from Slovenia to Croatia and Hungary (Zaninović 2008—Fig. 4.1). The cyclones brought by westerly winds and Mediterranean inflows equally weaken here and anticyclonal weather situations are more common. Therefore, annual precipitation amounts fall below 850 mm and total summer rainfall below 200 mm (ZAMG, 2016; Zaninović, 2008). There are usually less than 110 days with frost, but, because of regular Atlantic air inflows in winter, the number of days with (deeper than 1 cm) snow cover only slightly reduces along the Drava valley floor (from 110 to 80 days—ZAMG, 2016). The moderate relief, lower altitude and openness to easterly winds also contribute to the hot and dry summer and sometimes extremely cold winter weather. The Pannonian basin, however, is not exempt from oceanic and Mediterranean climatic influences either. Intense summer rains can cause flood waves on the lowland tributaries of the Drava, often followed by dry spells (Cindrić, 2006). The share of November precipitation in the annual amount remains relatively high (8–9%) in the Pannonian Basin, too.

The Atlantic climate zone is more extensive in the Upper Mura catchment. Even in January occasional inflow of oceanic air—from northwestern direction, primarily through the Präbichl Pass (1,232 m)—raises temperatures and brings snowfall to the higher regions of the Mura catchment. In February, Atlantic air flows along the southern margin of the North-European anticyclone reach the subcatchments which are open to the north (Lovász, 1972). In May, Atlantic influence is associated with cold spells and in June and July large amounts of rainfall moderates warming, but for the rest of the year it only occasionally predominates in the weather of the catchment. Lovász (1972) claimed that oceanic cyclonic activity had been increasing over the period 1901–1950. Today in valleys of Atlantic climate (in northern Styria and West of Graz) northwestern winds prevail, annual precipitation is above 1,000 mm, rainfall amounts exceed 100 mm in all three summer months, air humidity is high (at 14 h above 55%) and no drought period is observable (ZAMG, 2016). The maximum yearly precipitations are around 1,800 mm in the area of the Mura headwaters. However, the Atlantic climate zone is often heavily influenced by the inflow of Mediterranean air, manifested in 10–13 summer days (daily temperature maxima  $\geq 25.0$  °C) and a minor secondary precipitation maximum (8–10 rainy days in October) (ZAMG, 2016).

The high-mountain (Alpine) environment is located above ca 1,500 m altitude (Fig. 4.1). Here the orographic effect on both temperature and precipitation is more marked. The lowest mean



temperatures are measured on peaks like Sonnblick (3,111 m, annual mean for 1961–1990:  $-3.4$  °C; February mean:  $-10.5$  °C-Schöner et al. 2008). Although there are almost 200 rainy days in a year, more than 2,600 mm annual precipitation and high humidity, the total duration of sunshine is still above 1,700 h (in the Carnic Alps even 2,000 h), almost equally distributed throughout the months of the year (ZAMG, 2016; Lóczy, 2019).



*Climate zones of the Drava-Mura catchment (Source: Lóczy, 2019). 1, Illyric climate; 2, Pannonian climate; 3, Atlantic climate; 4, high-mountain (Alpine) climate. The locations of meteorological stations are shown*

### 1.3. The effect of climate change in the area

The following changes can be confirmed for the Drava-Mura catchment:

- marked rise in annual mean temperature, less pronounced in maximum monthly temperature in mountain environment;
- considerable growth in the number of sunshine hours in winter;
- higher precipitation amounts in mountains, lower in the lowland;
- snow depth increases in the upland section and decreases in the lowland;
- the duration of snow cover has reduced in the lowland (established with high uncertainty);
- the number of sunshine hours has grown in the mountains and in winter;
- relative air humidity has markedly decreased;
- cloud cover has increased in summer, but in winter only increased at higher elevations, reduced in the valleys.

The above changes will probably further enhance the contrasts between the climatic (and land use) subdivisions of the Drava-Mura catchment.

Among the direct impacts of climate change in the Alpine regions the following are usually enumerated (Niederer, 2013):

- increasing heat pressure in urban agglomerations;
- increasing frequency of summer drought affecting crop cultivation;
- higher flood risk;
- reduced slope stability and more frequent mass movements;
- retreat of the lower limit of snowfall to higher elevations;
- deteriorating water, soil and air quality;
- changes in diversity at the levels of Alpine species, habitats and landscapes;
- spreading of pests and alien species.

The modifications of climatic elements are expected to transform the entire physical environment (Lóczy, 2019).

#### 1.4. Austria

Mountain section and Transitional section located at Unterer Murtal Biosphere Reserves in the southeast of Austria and bordering the Slovenian Mura River Biosphere Reserve, which encompasses 13,000 ha. The area is of natural-historical and cross-border importance due to its location along the border with



Slovenia and its participation in the European Green Belt. The river landscapes and the accompanying floodplain forests are unique in the country. The natural area is known as the "Lower Mura Valley". A characteristic feature of the region is the former strong volcanic activity. The Mura River forms the border with Slovenia over a length of approx. 40 km and shapes the landscape together with the adjoining floodplain forests. In interaction between the different abiotic and biotic factors it forms a mosaic of different and valuable land use patterns and habitats. While waterbased sites such as alluvial forests, swamps, ponds and wet meadows can be found mainly along the Mura and its tributaries, agricultural and forestry areas and settlements characterise the more favourable sites for human use. The area is characterized by a high biodiversity, especially with regard to water-bound fauna and flora. The reserve is home to almost 50 fish species, 14 of which are of European importance, and a large number of amphibians and bird species, many of which are also of European importance and can often be

found on the IUCN Red List. Some of the animals to be found include the otter (*Lutra lutra*), Danube salmon (*Hucho hucho*) and kingfisher (*Alcedo atthis*).

## 1.5. Slovenia

Lowland section is located in the eastern part of the country, this biosphere reserve includes the largest preserved complex of floodplains in Slovenia, *Otter (Lutra lutra)* where the interweaving of natural factors and human presence has created an exceptional cultural riverine landscape. The main sources of income for residents are agriculture, industry, forestry and tourism. According to the classification of the EU's Natura 2000 network, the area of the biosphere reserve belongs to the Continental Biogeographic Region. The following Natura 2000 habitat types are represented in significant proportions in the area: rivers with muddy banks; water courses of plain to montane levels; hydrophilous tall herb fringe communities of plains and of the montane to alpine levels; lowland hay meadows; riparian mixed forest; natural eutrophic lakes; molinia meadows on calcareous, peaty or clayey-silt-laden soils; and illyrian oak-hornbeam forest.

It encompasses a large number of habitat types which are important at the national and international levels (in the Continental Biogeographical Region and in Europe), in particular wetlands and floodplain forests; due to the complex ecological conditions, the core area is a mosaic of well-preserved characteristic habitats of the middle sections of dynamic river floodplains, proving its high ecological value; due to complex ecological conditions and low-intensity management; the core area contain very old stands of floodplain forests, in particular white willow, common oak, and black and white poplar stands; the buffer zone is composed of a very wide variety of habitats, in particular: wetlands, agricultural land with significant areas of natural vegetation, transitional woodland-scrubs, natural grasslands, a mosaic of agricultural landscapes with fragmented plots, water bodies, water courses and broad-leaf forests; buffer zone is an area where local communities conduct their economic activities in symbiosis with nature, while supporting social activities; the transition zone is an area with numerous attractions for visitors, created and driven by centuries-old culture and local traditions.

## 1.6. Mura-Drava-Danube Transboundary Biosphere Reserve, Croatia/Hungary

This transboundary biosphere reserve stretches along the Drava, Mura and Danube Rivers, which are separated by flood prevention dykes into an inundation area and a flood-controlled

side. The biosphere reserve provides an important tool in learning different approaches to floodplain management.

Relatively much and large-scale waterworks were carried out on the Mura River in the 1800s and 1830s, manifested in the cutting of the arches of the riverbed. As a result, the river set in motion a huge amount of sediment, the bottom of the riverbed deepened significantly more than expected, and the already built fortifications fell into the water, losing their support. Water interventions took place a hundred years ago and in the 1960s and 1970s, but they did not fundamentally change the nature of the river and the countryside either.

In the days of the Cold War the border area between the two hostile blocks was sealed off and for decades this prevented the “development” of large reaches of the Drava and Mura. The rivers were free to flow in natural style with the current creating islands and steep banks.

These banks can reach a height of 40 metres, great cliffs of clay and sand. From them one can look out for miles over a riverscape of oxbow lakes, islands and riparian forest. The vertical fall of the banks is the chosen breeding spot of sand martins, bee-eaters and kingfishers. Species such as the Little Tern and Little Ringed Plover find their perfect natural breeding grounds on the islands which have formed in the river.

In addition to the positive features of the border zone (it helped to maintain the natural environment), one of the negative consequences is the lack of scientific research (<https://www.euronatur.org/en/what-we-do/project-areas/project-areas-a-z/drava-mura/>)

The Mura River is a daughter of the mountains. The headwaters of the Mura River basin originate in mountainous areas characterised by high rainfall before traversing the foothills of the Alps and lowlands. The stage fluctuation range of Mura is small compared to other rivers. The snow cover of the Alps is listed as a natural reservoir, the melting in the mountains only begins when the flood from the spring rains has already receded. The River Mura is characterized by quick flow and slow subsidence. The major part of the watershed runs over carboniferous rock formations. In its upper sector, the Mura River is a typical alpine lotic system. The tendencies of change in river water quality are difficult to establish since the evaluation systems has changed on several occasions in the past decades. At present, according to the comprehensive assessment system of the EU Water Framework Directive (WFD), on the upper and middle sections (in Austria and Slovenia), the Mura has somewhat worse water quality. Recent data indicate an upgrading in water quality class for the Mura River from a 3rd to a 2nd class river; an improvement partly attributed to actions implemented by upstream riparian countries. However, Mura has two acutely polluted tributaries, the Scavnica (4th class) and the Ledava (3rd to 4th class). On the Drava, Mura and Celje fields, intensive agriculture



and farming with high pesticide and fertiliser use has led to pollution of groundwater (GEF/Slovenia, 2003).



*Ščavnica River*

The lower-flow of the River Mura has managed to preserve many rare parts of its landscape. Conservation of the river's flow, has in addition to exceptional ecological functions and the role of huge water restraint, providing better flood-safety, had a decisive influence on underground water quality and the supply of clean drinking water (Krajnc & Kolar, 2010).

### 1.7. Quality of surface water

The monitoring of river water quality is carried out on the basis of laws and regulations in accordance with the requirements of the Water Framework Directive (WFD - Directive 2000/60/EC) and other guidelines and professional instructions for the establishment and implementation of the monitoring. The Regulation determines the limit values of parameters and criteria for the assessment of the chemical status. There are five classes for inland surface waters (AA, A, B, C, and D), four classes for coastal/marine surface waters (SA, SB, SC, and SD), and four classes for groundwater (GAA, GA, GB, and GC). A WFD compliant ecological assessment includes an ecological typology of water bodies, definition of reference conditions and classification system with five ecological classes. First of all, Member States identify the



location and boundaries of bodies of surface water and carry out an initial characterisation of all such bodies in accordance with the prescribed methodology (Urbanic, 2011). Surface water categories are rivers, lakes, transitional waters or coastal waters, artificial surface water bodies or heavily modified surface water bodies. The following table provides the general definition of ecological quality for rivers, lakes, transitional waters and coastal waters.

<b>Surface water quality</b>	
<b>High status</b>	<p>There are no, or only very minor, anthropogenic alterations to the values of the physico-chemical and hydromorphological quality elements for the surface water body type from those normally associated with that type under undisturbed conditions.</p> <p>The values of the biological quality elements for the surface water body reflect those normally associated with that type under undisturbed conditions, and show no, or only very minor, evidence of distortion.</p> <p>These are the type-specific conditions and communities.</p>
<b>Good status</b>	<p>The values of the biological quality elements for the surface water body type show low levels of distortion resulting from human activity, but deviate only slightly from those normally associated with the surface water body type under undisturbed conditions.</p>
<b>Moderate status</b>	<p>The values of the biological quality elements for the surface water body type deviate moderately from those normally associated with the surface water body type under undisturbed conditions. The values show moderate signs of distortion resulting from human activity and are significantly more disturbed than under conditions of good status.</p>

Waters achieving a status below moderate shall be classified as poor or bad. Waters showing evidence of major alterations to the values of the biological quality elements for the surface water body type and in which the relevant biological communities deviate substantially from

those normally associated with the surface water body type under undisturbed conditions, shall be classified as **poor**. Waters showing evidence of severe alterations to the values of the biological quality elements for the surface water body type and in which large portions of the relevant biological communities normally associated with the surface water body type under undisturbed conditions are absent, shall be classified as **bad**.

In the case of rivers, the evaluated parameters can be high, good or moderate status. These are the following:

- biological quality elements, as phytoplankton, macrophytes and phytobenthos, benthic invertebrate fauna, fish fauna,
- hydromorphological quality elements, as hydrological regime, river continuity, morphological conditions,
- physico-chemical quality elements, as general conditions (nutrient concentrations, salinity, pH, oxygen balance and so on), specific synthetic pollutants, specific non-synthetic pollutants.

According to WFD Member States shall collect and maintain information on the type and magnitude of the significant anthropogenic pressures in each river basin district. These kind of pollutants could be organohalogen compounds, organophosphorus compounds, organotin compounds, endocrine disruptor compounds, carcinogens, mutagens, persistent hydrocarbons, bioaccumulable organic toxins, cyanides, metals, arsenic compounds, biocides and plant protection products, nitrates, phosphates and other substances, which contribute to eutrophication, oxygen balance (COD, BOD) influencers.

The emission limit values and environmental quality standards are regulated in The Mercury Discharges Directive (82/176/EEC), The Cadmium Discharges Directive (83/513/EEC), The Mercury Directive (84/156/EEC), The Hexachlorocyclohexane Discharges Directive and The Dangerous Substance Discharges Directive (86/280/EEC).

Estimation and identification of significant point source pollution, in particular by substances listed in Annex VIII, from urban, industrial, agricultural and other installations and activities, based, inter alia, on information gathered under “Commission Proposal for a Council Directive Establishing a Framework for Community Action in the field of Water Policy” directives.

The Water Framework Directive determines the limit values of parameters and criteria for the assessment of the chemical status. In the future, this regulation will have to be changed as a directive, which will in the course of preparation, determine environmental quality standards for the substances that have, at a European level, been classified as hazardous (priority list of hazardous substances).

The purpose of **Directive 2000/60 EC** is to establish a framework for the protection of inland surface waters, transitional waters, coastal waters and groundwater which: (a) prevents further deterioration and protects and enhances the status of aquatic ecosystems, (b) promotes sustainable water use based on a long-term protection of available water resources; (c) aims at enhanced protection and improvement of the aquatic environment, (d) ensures the progressive reduction of pollution of groundwater and prevents its further pollution, and (e) contributes to mitigating the effects of floods and droughts.

The Member States shall identify the individual river basins lying within their national territory and, for the purposes of this Directive, shall assign them to individual river basin districts. Small river basins may be combined with larger river basins or joined with neighbouring small basins to form individual river basin districts where appropriate. The Member States shall ensure the establishment of a register or registers of all areas lying within each river basin district which have been designated as requiring special protection. The European Parliament and the Council shall adopt specific measures against pollution of water by individual pollutants or groups of pollutants presenting a significant risk to or via the aquatic environment, including such risks to waters used for the abstraction of drinking water.

Annex I regards the information that the Member States shall provide on all competent authorities within each of its river basin districts as well as the portion of any international river basin district lying within their territory, Annex II concerns surface and groundwater, Annex III the economic analysis, Annex IV protected areas, Annex V surface and groundwater status, Annex VI the list of measures to be taken, Annex VII river basin management plans, Annex VIII the list of main pollutants, Annex IX The "limit values" and "quality objectives", Annex X priority substances and Annex XI Ecoregions for rivers and lakes (25 Articles and 11 Annexes) (<https://www.fao.org/faolex/results/details/en/c/LEX-FAOC023005/>).

“Directive 2008/105/EC setting environmental quality standards in the field of water policy” sets environmental quality standards for priority substances and eight other pollutants. These substances include the metals cadmium, lead, mercury and nickel, and their compounds; benzene; polyaromatic hydrocarbons (PAH); and several pesticides. Several of these priority substances are classed as hazardous.

The EQSs in Directive 2008/105/EC are limits on the concentration of the priority substances and 8 other pollutants in water (or biota), i.e. thresholds which must not be exceeded if a good chemical status is to be met. There are 2 types of water standards:

- a threshold for the average concentration of the substance concerned calculated from measurements over a 1-year period. The purpose of this standard is to ensure protection against long-term exposure to pollutants in the aquatic environment;

- a maximum allowable concentration of the substance concerned, i.e. the maximum for any single measurement. The purpose of this standard is to ensure protection against short-term exposure, i.e. pollution peaks (<https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=LEGISSUM%3A128180>).

“Directive 2013/39/EU of the European Parliament and of the Council of 12 August 2013 amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy Text with EEA relevance” was adopted on 12 August 2013. It revises crucial rules on determining the chemical quality of surface water in Europe (e.g. identification of new harmful substances, updating of environmental quality standards, introduction of a new “watch list” mechanism) and establishes new standards for the protection of water in Europe.

### 1.8. Protected by the presence of the Iron Curtain

The stage fluctuation range of Mura is small compared to other rivers. The snow cover of the Alps is listed as a natural reservoir, the melting in the mountains only begins when the flood from the spring rains has already receded. The River Mura is characterized by quick flow and slow subsides.

Relatively much and large-scale waterworks were carried out on the river in the 1800s and 1830s, manifested in the cutting of the arches of the riverbed. As a result, the river set in motion a huge amount of sediment, the bottom of the riverbed deepened significantly more than expected, and the already built fortifications fell into the water, losing their support. Water interventions took place a hundred years ago and in the 1960s and 1970s, but they did not fundamentally change the nature of the river and the countryside either.

In the days of the Cold War the border area between the two hostile blocks was sealed off and for decades this prevented the “development” of large reaches of the Drava and Mura. The rivers were free to flow in natural style with the current creating islands and steep banks.

These banks can reach a height of 40 metres, great cliffs of clay and sand. From them one can look out for miles over a riverscape of oxbow lakes, islands and riparian forest. The vertical fall of the banks is the chosen breeding spot of sand martins, bee-eaters and kingfishers. Species such as the Little Tern and Little Ringed Plover find their perfect natural breeding grounds on the islands which have formed in the river.

In addition to the positive features of the border zone (it helped to maintain the natural environment), one of the negative consequences is the lack of scientific research (<https://www.euronatur.org/en/what-we-do/project-areas/project-areas-a-z/drava-mura/>).

With the construction of flood defense embankments, the level of previously spreading floods increased. The work carried out at the beginning of the last century was also motivated by the fact that an anti-malarial medical office had to be set up in Letenye due to the stagnant water surfaces that remained long after the pourings.

After the Treaty of Trianon, the new border not only split the countryside in two, but also severed many farmers from their own land. Through the dual tenure on both sides of the border, Croatian peasants came to Hungary between the two wars to cultivate their former estates. Due to the tense political relationship between the two countries in the 1950s, this became impossible, and access to the border areas on the Hungarian side was severely restricted. Traces of the border guard (trenches, bunkers, machine gun nests) can still be seen today. As a result of the seclusion, the previously cultivated areas remained homeless, and the countryside began to be forested, naturally afforested.

In addition to the positive features of the border zone (it helped to maintain the natural environment), one of the negative consequences is the lack of scientific research. From the Muraköz region in World War II, Ádám Boros (1944) published valuable data (today the territory of Slovenia). The most significant floristic data can be found in the dissertations exploring the flora of Károlyi-Pócs-Balogh (1954-1975) in Southwestern Transdanubia, but - as the river section was militarily closed - their data also apply only to the hills along the Mura. The phytogeographical research of the Mura and Drava floodplains was started in 1973 by Margit Kovács and István Kárpáti. Due to the lack of the previous exploration, it is not possible to know how extensive the natural habitats may have been, what species may have disappeared permanently.

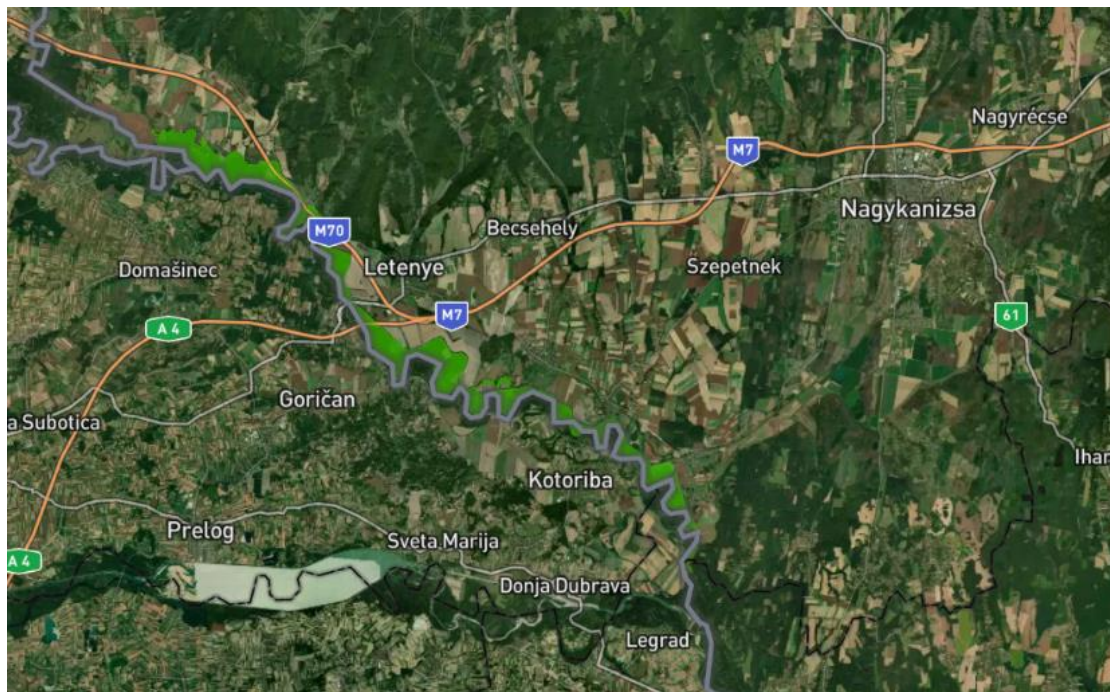
The tendencies of change in river water quality are difficult to establish since the evaluation systems have changed on several occasions in the past decades. At present, according to the comprehensive assessment system of the EU Water Framework Directive (WFD), on the upper and middle sections (in Austria and Slovenia), the Drava has good and the Mura somewhat worse water quality. In 2010, a campaign found the Croatian section of the Drava River in excellent condition based on the Water Quality Index. Regular data collection on water quality of the Hungarian Drava section began in the 1960s at three sampling sites (Órtilos, Barcs and Drávaszabolcs) and soon continued in international cooperation with Yugoslavian authorities. The monitoring and evaluation systems changed in 1981 and again in 1994. In 2001, an automatic Drava Monitor Station (DAM) began to operate at Barcs and complex

(hydromorphological, physico-chemical, biological, and biochemical) monitoring according to the WFD guidelines was introduced.

### 1.9. Ecological characteristics - The flora and fauna of the Landscape Conservation Area along the Mura River

The landscape conservation area extends from the estuary of the Kerka to the confluence of the Mura and the Drava Rivers. These areas have been under constant cultivation, but due to nearly 50 years of military closure, the habitats here have remained relatively natural.

The gravel alluvium that formed its bedrock was accumulated by the ancient Mura River in a 20 km wide bed. There is almost no area in the Mura Valley in Hungary, that does not belong to the Mura riverbed, as it changes with each major flood.



The Landscape Conservation Area along the Mura (source: <https://www.google.hu/maps/@46.3513711,16.8284352,18735m/data=!3m1!1e3?hl=hu>)

In terms of flora, classical vegetation zones are less observable due to the continuous change of the riverbed. The formed plant associations are narrow or not recognizable due to the mosaic nature of the soil. The gravel reefs on the built side of the river are quickly forested by seeds drifting with the river. The most characteristic associations in the floodplain are softwood groves, the main pioneer species is *Salix elaeagnos* (Bódis et al., 2008). Mud vegetation with *Typha latifolia* may also appears at the side of the riverbed.

On the outer bank of the Mura River, *Galanthus* and *Scilla vindobonensis* create a colorful carpet in early spring, meanwhile *Leucojum aestivum* appears scattered across the area from May to



June. Alder groves with *Alnus incana* formed mainly on the banks of backwaters and presumably arrived from the upper reaches of the river. In the associations, *Dryopteris carthusiana* appears as a protected plant (Király, 2020).



*Dactylorhiza incarnata* (source: <https://www.bokortanya.hu/ude-retek-es-legelok-vadviragai/husszinu-ujjaskosbor>)

Hardwood forest have formed in the higher parts of the floodplain, typically with *Fraxinus excelsior*. In these associations, *Epipactis helleborine* and *Vitis sylvestris* are protected plants. *Nymphaea alba* and *Trapa natans* appear as submerged seaweed, while protected *Salvinia natans* appears as emerged seaweed.

Riverside grasslands developed as a result of human activity at the site of the forests. Due to the re-establishment of woody vegetation, they require constant cultivation (mowing, grazing), but this is not common in most cases (Bódis et al., 2008). On the remaining marshes and meadows *Dactylorhiza incarnata* and *Iris sibirica* are still present (Molnár, 2011).

Unfortunately, *Fraxinus pennsylvanica*, *Fallopia*, *Solidago gigantea* and *Amorpha fruticosa* are constantly present as invasive species, although the latter two are beneficial for beekeepers. (Király, 2020).

In terms of fauna, the dragonfly population is the best surveyed. The protected *Ophiogomphus cecilia*, *Onychogomphus forcipatus* and *Epithea bimaculata* can be found, but highly protected *Leucorrhinia caudalis* is also present in the backwaters.

Besides the common butterfly species, protected *Parnassius mnemosyne*, *Zerynthia polyxena* and the highly protected *Apatura metis* are often seen. Among the beetles, it is worth mentioning the xylophagous *Cerambyx cerdo*, *Cucujus cinnaberinus* and *Lucanus cervus*.

More than 50 fish species are present in the Mura River, 13 of them are protected. Among the river fish species, it is important to mention *Romanogobio uranoscopus*, because Mura River is one of the 3 domestic occurrences in Hungary. *Rutilus pigus*, *Zingel zingel*, *Gymnocephalus schraetser*, *Sabanejewia aurata* and *Gobio albipinnatus* also occur in the river. (Sallai & Kontos, 2003). Among the bog fish species, *Umbra krameri* and *Misgurnus fossilis* can be mentioned, which live in backwaters. The backwaters also provide habitats for *Emys orbicularis* and *Bombina bombina*.



*Romanogobio uranoscopus* (source: <http://www.horgasz.hu/page/20/cikkid/379/html/felpillanto-kullo.html>)

In term of birds, the migratory period is the most varied in autumn and spring, when *Ardea cinerea* and *Ardea alba* flocks congregate at the backwaters and they may overwinter in mild winters. On the open water *Anas crecca* and *Bucephala clangula* can be seen in winter. *Pandion haliaetus* is a frequent visitor to fishing lakes and mining lakes in April and September, in addition *Haliaeetus albicilla* is visible all year and also nests along Mura River. Backwaters provide a great nesting ground for *Tachybaptus ruficollis* and *Gallinula chloropus*, while *Charadrius dubius* feels best on plant-free gravel reefs (Bódis et al., 2008).



*Haliaeetus albicilla* (source: <https://www.europamadarai.hu/reti-sas/>)

Traces of otters (*Lutra lutra*) can be found throughout the year, especially in winter. Tall old trees in wooded area provide excellent habitat for bats. Can be found in the area the highly protected *Myotis emarginatus* and *Barbastella barbastellus*, as well as the protected *Myotis daubentonii*, *Myotis alcathoe* and several dwarf bats species (Király, 2020).

The native eurasian beaver (*Castor fiber*) became extinct in Hungary in the 1800s, but due to the repopulation in recent decades, it reappeared (Haarberg, 2007). Repopulation also took place on the Drava River and beaver families presumably migrated from there and then appeared in the Mura River and in its tributaries (Lelkes, 2013).



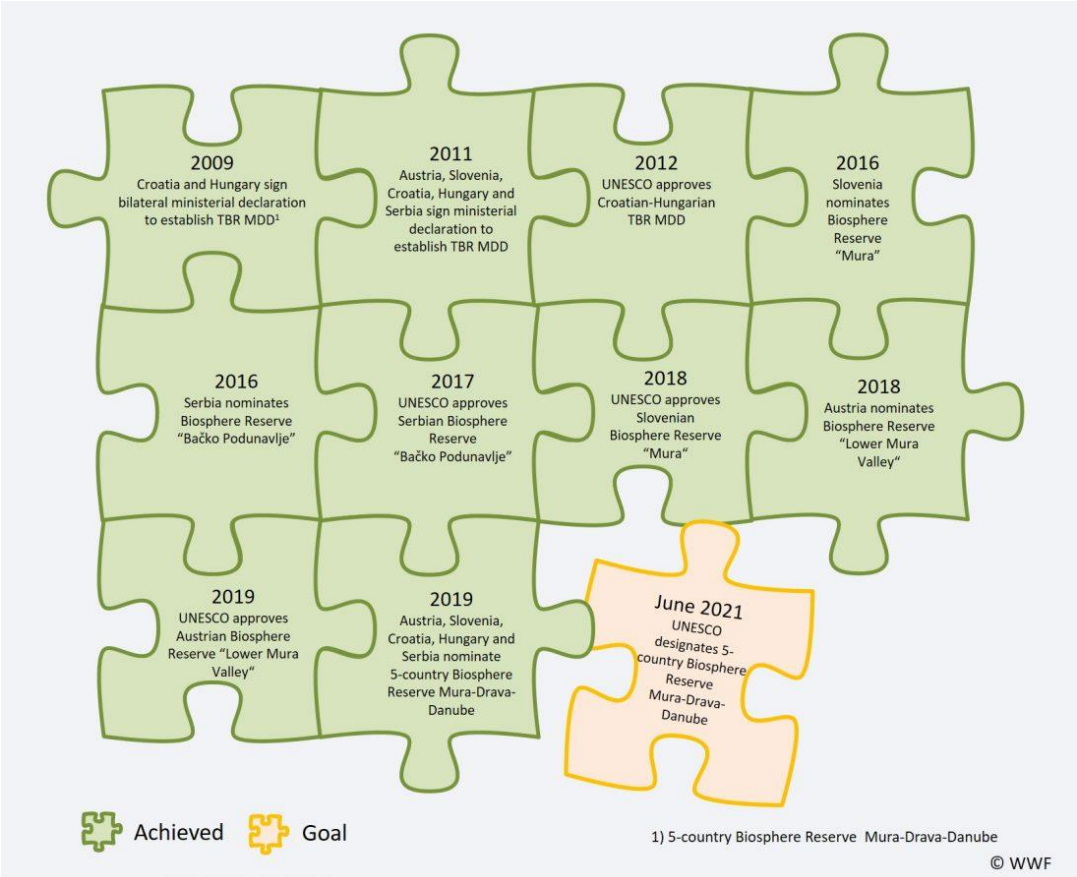
*Castor fiber* (source: <http://voroseszold.eu/index.php/eurazsiai-hod-a-fanyuvo-vizi-emlos>)

According to the classification of the EU's Natura 2000 network, the area of the biosphere reserve belongs to the Continental Biogeographic Region. The following Natura 2000 habitat types are represented in significant proportions in the area: rivers with muddy banks with *Chenopodium rubri p.p.* and *Bidention p.p.* vegetation; Alluvial forests with *Alnus glutinosa* and *Fraxinus excelsior* (*Alno-Padion*, *Alnion incanae*, *Salicion albae*); water courses of plain to montane levels with the *Ranunculion fluitantis* and *Callitricho-Batrachion* vegetation; hydrophilous tall herb fringe communities of plains and of the montane to alpine levels; lowland hay meadows (*Alopecurus pratensis*, *Sanguisorba officinalis*); riparian mixed forest of *Quercus robur*, *Ulmus laevis* and *Fraxinus sp.*; natural eutrophic lakes with Magnopotamion or Hydrocharition-type vegetation; molinia meadows on calcareous, peaty or clayey-silt-laden soils (*Molinion caeruleae*); and illyrian oak-hornbeam forest (*Erythronio-Carpinion*).

It encompasses a large number of habitat types which are important at the national and international levels (in the Continental Biogeographical Region and in Europe), in particular wetlands and floodplain forests; due to the complex ecological conditions, the core area is a mosaic of well-preserved characteristic habitats of the middle sections of dynamic river floodplains, proving its high ecological value; due to complex ecological conditions and low-

intensity management; the core area contains very old stands of floodplain forests, in particular white willow, common oak, and black and white poplar stands; the buffer zone is composed of a very wide variety of habitats, in particular: wetlands, agricultural land with significant areas of natural vegetation, transitional woodland-scrubs, natural grasslands, a mosaic of agricultural landscapes with fragmented plots, water bodies, water courses and broad-leaf forests; buffer zone is an area where local communities conduct their economic activities in symbiosis with nature, while supporting social activities; the transition zone is an area with numerous attractions for visitors, created and driven by centuries-old culture and local traditions.

In 2009, Hungary and Croatia signed a bilateral ministerial declaration on the 5-country Biosphere Mura-Drava-Danube (TBR MDD), which was later followed by Austria, Slovenia and Serbia. Member States have designated sections as biosphere reserves, which have been approved by UNESCO. Their designation and adoption has been a process of many years of work and includes several Natura 2000 and protected areas. Finally, at its meeting on 15 September 2021, UNESCO declared the world’s first five-country biosphere reserve.



*5-country Biosphere Mura-Drava-Danube*

This includes the section between Mura, Croatia and Hungary, where several smaller core areas and buffer zones have been designated. The protection of these areas is particularly important to protect further sections of the TBR MDD.



## 2. Main pollution sources around Mura River

Water pollution has a significant impact on the natural renewal of water resources. In Hungary, diffuse pollution from agricultural sources, as wastes, fertilizers and pesticides (Novotny, 2005), reaches the dimension as point source pollution (HAS, 2017). This is well illustrated by the fact that two-thirds of the diffuse phosphorus load can be derived from soil erosion (both of agricultural and natural origin) and the rest from municipal wastewater treatment plants. Recently, the measurement of pesticides in Hungarian surface waters, such as streams, rivers and lakes, has become increasingly important (Maloschik et al., 2007; Nagy-Kovács et al., 2018). Polluting pesticides can be originated from clearly identifiable point sources (from accidents or inadequate use of pesticides) or from regular plant protection practices on large areas (non-point sources) (Láng et al, 2004). Although neglected for decades, it has become evident that agricultural non-point pollution is the most problematic for the environment (Finizio and Villa, 2002). Almost everything humans do, from growing food to manufacturing products to generating electricity, has the potential to release pollution into the environment. Regulatory agencies charged with protecting the environment identify two main categories of pollution: point-source and nonpoint-source pollution. Point-source pollution is easy to identify. As the name suggests, it comes from a single place. Nonpoint-source pollution is harder to identify and harder to address. It is pollution that comes from many places, all at once. Nonpoint-source pollution is the opposite of point-source pollution, with pollutants released in a wide area (<https://www.nationalgeographic.org/encyclopedia/point-source-and-nonpoint-sources-pollution/>).

Airborne pollutants are major contributors to acid rain. It forms in the atmosphere when sulfur dioxide and nitrogen oxides combine with water. Because acid rain results from the long-range movement of those pollutants from many factories and power plants, it is considered nonpoint-source pollution.

The anthropogenic effect can be divided into two groups: direct and indirect human impacts. The indirect human impacts influence the catchment and the water- and sediment regime (by modifying the runoff), while direct impacts aim to alter the channel and the floodplain (Stover and Montgomery, 2001; Kondolf et al. 2002; Kiss and Andrási, 2017). The hydrology and morphology of the Dráva River are mainly influenced by dams and reservoirs built up the upper section, although other engineering works (such as groins, cut-offs) also influence the development of the channel (Kiss and Andrási, 2017).

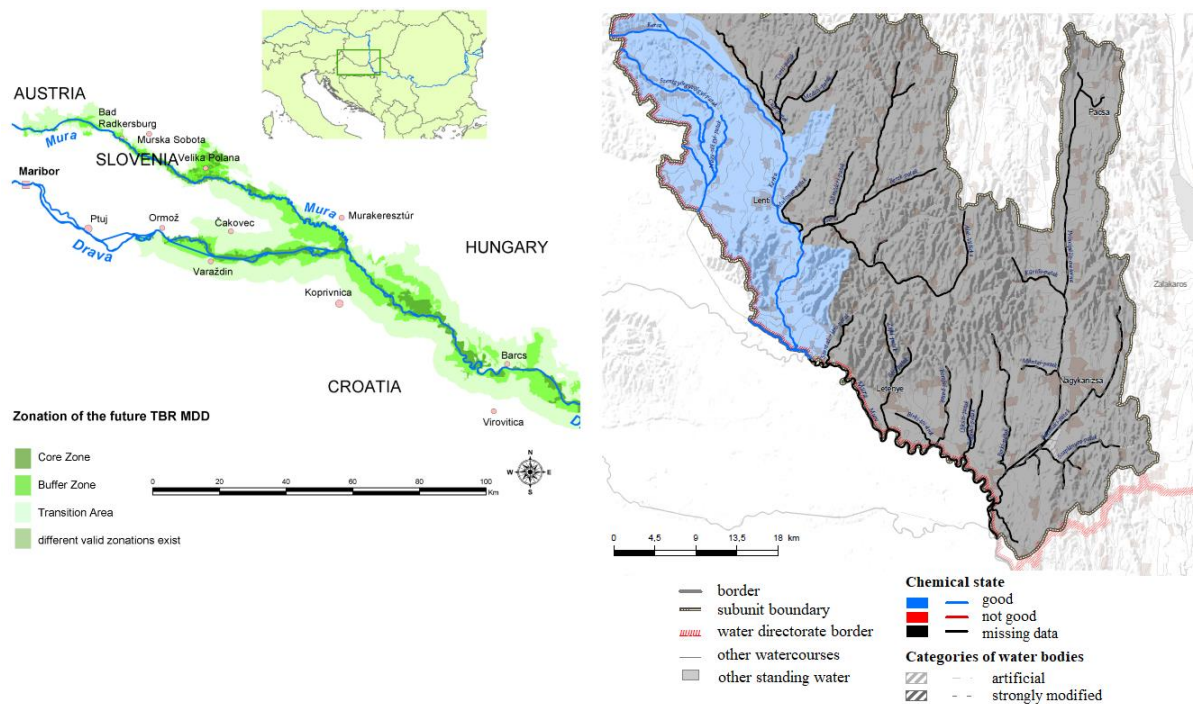
The River Mura, 465 km in length, rises in Austria (1898 m above sea-level) and as a border river between Croatia and Hungary, before it flows into the River Drava. The size of its basin

is 14,304 km<sup>2</sup>. More than half of its surface is in Austria. The Slovenian section of the basin is 1,393 km<sup>2</sup> in area, the Croatian 987 km<sup>2</sup>, and Hungary 1911 km<sup>2</sup>. The average fall of the river is 0.21 %. The average fall in Slovenia is 0.1 % and less than 0.06 % at the border between Croatia and Hungary (Krajnc Galunder & Kolar, 2010).

In the upper part of the Mura, the river is a typical alpine lotus system, but when it enters the territory of Slovenia, it already loses this character and becomes a calm and slow river, making it a typical plain with several meanders. However, during periods of heavy rain and snowmelt, it develops quite differently: it can flood the region along its river and cause significant damage. The forests along the Mura River can be divided into three ecological groups. The first includes forests that appear above the level of floods on the alluvial plain. The second group consists of river forests appearing on the river banks, and the last group consists of forests flourishing in wetlands at a certain distance from the main river (Čarni & Juvan, 2020).

On January 1, 1994, water classification based on Hungarian Standard 12749 “Surface water quality, quality indicators and assessment” came into force in Hungary. The standard includes the sampling method, the national core network sampling points, the sampling frequency, the characteristics to be examined, the classification limits, the characterization of the water quality classes and the way in which each class can be displayed on a map. The standard does not cover the classification of water according to specific water uses and biological characteristics.

After Hungary’s accession to the European Union, The European Parliament and the Council developed and adopted Directive 2000/60/EC establishing a framework for Community action in the field of water policy, which entered into force on 22 December 2000 and appeared in the public domain as the Water Framework Directive (WFD-EC, 2000). Implementation has become mandatory for EC countries and harmonisation for the accession countries. The complexity of the Water Framework Directive, its application in Hungary, the achievement of its objectives and, above all, the way to achieve it, pose new challenges for water management professionals every day. The WFD assesses not only chemical status but also ecological status and aims to ensure sustainable water use. It seeks to gather information on whole water bodies as part of river basin management plans, rather than point sampling (Lóczy, 2019).



*Chemical classification of surface water bodies (Sources: <http://www.interreg-danube.eu/approved-projects/amazon-of-europe-bike-trail>, [http://www.vizugy.hu/vizstrategia/documents/A107DFCF-487D-4A54-8E9B-7A09DC0156BE/VGT2\\_3\\_1\\_Mura\\_vegleges.pdf](http://www.vizugy.hu/vizstrategia/documents/A107DFCF-487D-4A54-8E9B-7A09DC0156BE/VGT2_3_1_Mura_vegleges.pdf))*

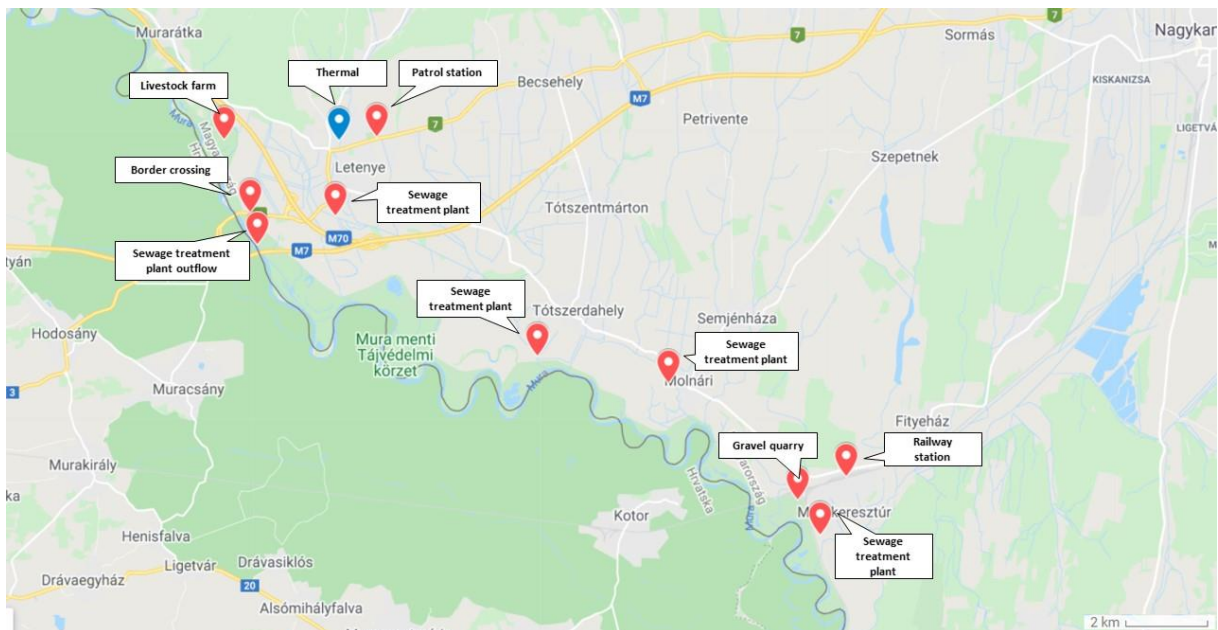
## 2.1. The main sources of pollution

There are various companies operating in the area affected by the project, which can be sources of pollution in terms of surface water and groundwater, as well as from the presumably polluted areas. The following main sources of pollution are to be considered in the project area of Hungary, broken down by settlements:



*The main sources of pollution are to be considered in the project area of Hungary, broken down by settlements*

Tótszerdahely	Molnári	Murakeresztúr	Letenye	In addition, the following may pose a potential threat to the quality of surface water and groundwater in all settlements
abandoned gravel mine (south-east of the settlement)	plants on the site of a former producer cooperative, petrol station there (about 1 km to the east of the settlement)	plants on the site of former producer cooperatives (northeast of the municipality)	livestock farm (gray cattle major)	abandoned illegal landfills (one or two within each settlement)
plants on the site of a former producer cooperative site (on the western edge of the settlement, towards Letenye)	abandoned brick factory, clay pond lakes (on the eastern edge of the settlement)	railway lines passing through the settlement, the total area of railway station	Letenye thermal (inflow into Béci stream)	illegally drilled wells (there are many of them in every settlement, there are no usable records of them, although they are problematic in several respects)
municipal sewage treatment plant	waterworks	operating gravel quarry (west of the settlement)	patrol station	diffuse pollution of intensively farmed areas involving the application of fertilizers and pesticides (typical everywhere in the project area, it may even has a great impact on surface water and groundwater quality for certain parameters)
	municipal sewage treatment plant	the part of the village without a public sewerage system	municipal sewage treatment plant	
		municipal sewage treatment plant	Letenye border crossing	

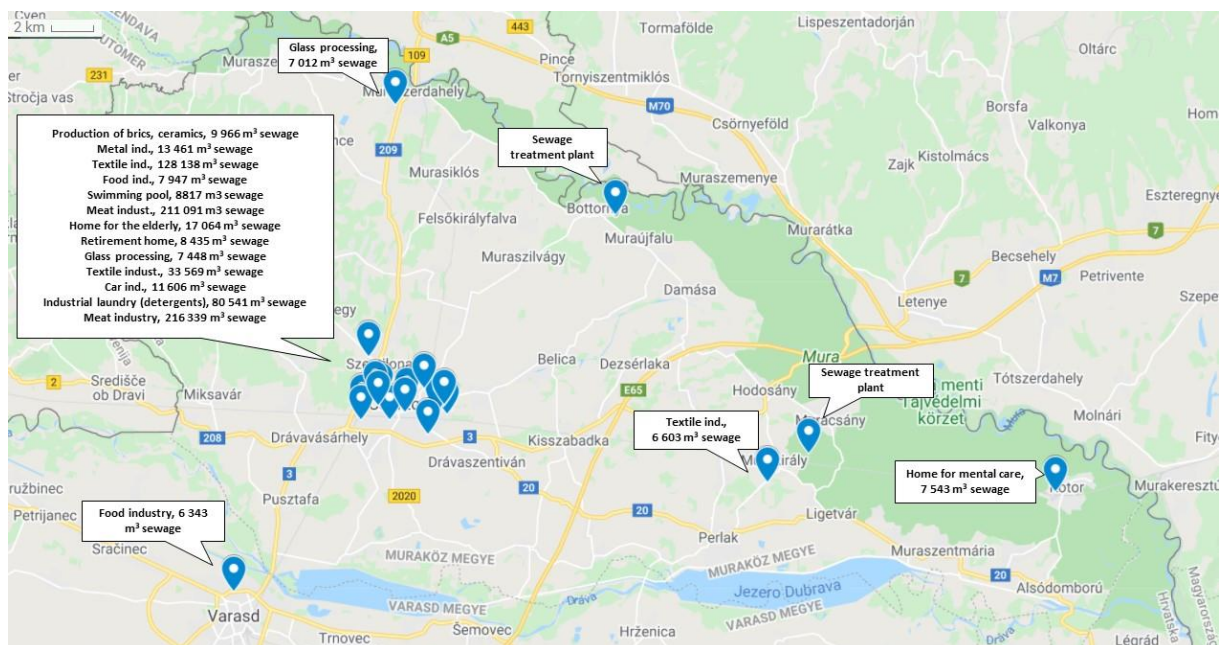


*The main sources of pollution in Hungary*

In the investigated area, in Croatia, the most important companies in sewage consumption, are the following:

- Meat industry - fat
- Textile industry - dye
- Industrial laundry - detergents
- Hospital – chemicals, pharmaceuticals, cytotoxins
- Wagon maintenance – oil
- Metal industry
- Car
- Food
- Production of bricks, ceramics, glass

The Appendix contains data of surface water quality parameters from the last few years.



*The main sources of pollution in Croatia*

Nutrient pollution in Slovenia consists of municipal, industrial, agricultural and diffuse sources. At the turn of the millennium, municipal wastewater discharges were about 126 Mm<sup>3</sup>, of which 71% were treated at wastewater treatment plants. In particular, secondary and tertiary treatments are missing (59% of the treated wastewater mentioned above was treated only with primary treatment). What's more, almost half of the population, especially in rural areas, is not connected to the municipal sewer system, and individual septic tanks often pose a risk to the environment. Industrial activities, in particular the paper, metal and chemical industries, release 701 Mm<sup>3</sup> of wastewater into the environment (1999), almost all of which is discharged into

surface waters. In the agricultural sector, intensive farming, in addition to high fertilizer and pesticide use, has led to contamination of groundwater with nitrates and pesticides through runoff. Large pig farms are a significant point source of water pollution with rudimentary or no wastewater treatment; poses a particular risk in karst and groundwater near small streams. For toxic substances (metals, pesticides, organic compounds), industry is estimated to account for 60% of pollutants, while municipal and agricultural sources account for 10 and 30%, respectively. Landfills are one of the main sources of untreated leachate, which can have a potentially severe impact on groundwater and surface water (GEF / Slovenia, 2003).

## 2.2. Significant surface water uses

There are almost 50 permitted water abstraction sites in the Mura catchment, which are typically lakes. From the point of view of the river basin, the insignificant amount of water abstraction purposes other than the water use of the lakes does not reach 1.5% of the permitted amount of water in the lakes. The water consumption of the lakes is 6.0 million m<sup>3</sup>/year, of which the Móríchely and the water demand of the Magasdi fishponds, which individually exceed 1.3 million m<sup>3</sup>/year. The water inlets in the Mura River Basin can be divided into three major groups. One of them is the municipal wastewater discharge, which is 9.6 million m<sup>3</sup>/year, of which the Nagykanizsa wastewater treatment plant introduces an exceptionally large amount into the Dencsár ditch. The annual volume introduced exceeds 7.7 million m<sup>3</sup>/year. The second is the introduction of industrial waters, which is 1.3 million m<sup>3</sup>/year. The third one is the used water of baths, which load the recipients with 350 thousand m<sup>3</sup> of used water per year.

## 2.3. Significant groundwater uses

With regard to groundwater uses, the production of waterworks providing public drinking water supply is a significant water abstraction. Of these, the territorial waterworks supplying several settlements should be mentioned. In the area of the Mura catchment, the waterworks of Molnári and Lenti produce a significant amount of groundwater. The wells of the Molnári water base, located on the Pleistocene gravel terrace of the Mura River, produce riverbank-filtered water. The amount of water that can be extracted on the basis of the water right permit is 11,235 m<sup>3</sup>/day. The wells producing aquifer below the water base filter Upper Pannonian sand layers, the extractable water volume is 2055 m<sup>3</sup>/day. In addition to public water uses, the industrial water use of the light source factory of GE HUNGARY Ltd. in Nagykanizsa should be mentioned as a significant water abstraction. The wells of the plant were located on the Upper

Pannonian aquifer base, the permitted amount of extractable water is 1,857 m<sup>3</sup>/day. In connection with the utilization of groundwater, thermal water utilization facilities deserve special attention. The wells of the thermal baths established in the Mura catchment tap the Upper Pannonian sand layers for the purpose of obtaining thermal water. The most significant of the thermal baths is the Lenti Thermal Bath, which is operated by Lenti Gyógyfürdő Ltd. The spa has 3 thermal wells, the amount of water that can be extracted is 849 m<sup>3</sup>/day. The beach in Nagykanizsa is operated by Kanizsa Pool Ltd. with 1 thermal well. The amount of water that can be extracted on the basis of the water right permit is 270 m<sup>3</sup>/day. In addition to the above, Letenye and Bázakerettye also have a thermal water base. The thermal baths have 1-1 thermal wells, the amount of water that can be extracted is 15 m<sup>3</sup>/day in Letenye and 28 m<sup>3</sup>/day in Bázakerettye.

#### 2.4. Diffuse contaminations of agricultural origin

In areas under agricultural cultivation (1960-1990) large amounts of fertilizer as well as herbicides and insecticides were used. These fertilizers and sprays are very soluble in water, so they easily get into the groundwater by the infiltration of precipitation. However, their degradation is very slow in an oxygen-poor environment. After 1990, the use of chemicals fell sharply for economic reasons, but after 2000 it showed an upward trend again. Nitrate and pesticide pollution is above or close to the limit value in many places under cultivated areas. The highlighted hilly areas are in a slightly better position, where the thicker cover layer above the deeper groundwater retains some of the pollutions. However, the polluting effect of agriculture on groundwater can be clearly demonstrated here as well. With rare exceptions, groundwater under agricultural land is practically unsuitable for drinking. However, deeper aquifers used for drinking water abstraction are replenished from the surface by contaminated groundwater. The effect of polluted groundwater can already be detected in shallower underground water.

#### 2.5. Public water supply and wastewater disposal

The supply of piped drinking water to the settlements of the Mura catchment area started in the 19th and ended in the 20<sup>th</sup> century. Therefore, today the utility water supply is fully developed throughout the catchment area. In addition to the settlements along the Mura, everyone from Újudvar to Liszó, from Becsehely to Nagyfakos gets the water of Mura River from the Molnári water extraction. Water extraction is special because the wells are on the different side than

usual, they produce surface water, but there is also a type of well suitable for groundwater extraction (drilled wells, horizontal filtering wells, shaft wells).

*The capacity of different types of wells in the area based on data from 2009*

Names of the wells	Capacity		Daily water production capacity [m <sup>3</sup> /24 h]
	[L/min]	[m <sup>3</sup> /h]	
Drilled wells (1-10.)	6 500	390	8 580
No. 1. large diameter drilled well	0	0	0
No. 2. large diameter drilled well	860	52	1 135
C-1 horizontal filtering well	5 240	314	6 917
C-2 horizontal filtering well	5 380	323	7 102
Shaft well	1 340	80	1 769
<b>Total</b>	<b>19 320</b>	<b>1 159</b>	<b>25 502</b>

The treatment of municipal wastewater is provided by 25 wastewater treatment plants with a total capacity of 33,418 m<sup>3</sup>/d. The treatment plants have an artificial biological treatment stage with the exception of one root field and one spring treatment plant. In some places, the water of wastewater treatment plants discharged into intermittent watercourses can cause a problem in the quality of groundwater (eg: Letenye wastewater treatment plant), but such loads are not typical in general. One-third of the water supply systems built were built before the 1980s, while the rest were built in the early 1990s. The water network and their fittings are obsolete, with a network loss of around 30% - very high. The planned, scheduled implementation of the reconstruction tasks of the water supply networks must be started. Out of 135 settlements in the catchment area, 72 are equipped with a network of public sewers, these are mainly settlements with a larger population. The treatment of wastewater from settlements with public sewerage is provided by 31 wastewater treatment plants with a total hydraulic capacity of 29,846 m<sup>3</sup>/d and a biological capacity of 152,896 HP (2018). Out of the wastewater treatment plants, 25 plants have biological treatment stages, 6 wastewater treatment plants have near-natural (spring, root field (reedbed), Organica-SBR) technology. Currently, 18 settlements in the area are planning to solve the sewage drainage and treatment solution by creating a joint agglomeration (Bánokszentgyörgy, Borsfa, Bucsuta, Oltárc, Pusztamagyaród, Szentlisló, Várfölde; and connected to existing agglomerations Alsórajk, Felsőrajk, Kilimán, Pölöskefő,

Mikekarácsonyfa, Szentkozmadombja, Zalatárnok, Pórszombat, Szilvagy, Liszó, Surd). The agglomeration reviews of these settlements have been approved by the Ministry of Interior. In the case of operating wastewater treatment plants, it will also be necessary to develop and modernize the plants. Most of the settlements were built in the '90s or before. Worn mechanical equipment can endanger the operation of the cleaner, ensuring the required cleaning efficiency. The Magyarszombatfa wastewater treatment plant in the Mura catchment will be abandoned due to wear and tear. Wastewater treatment should be provided by another solution.

## 2.6. Loads caused by wastewater

Out of the 31 wastewater treatment plants operating in the area, 22 wastewater treatment plants were fined in 2017. The increased organic matter and nutrient load has a negative effect on the status of the water body. In addition to professional operation, site development may be justified at these sites. 3 sites in the Mura catchment area are biologically overloaded (Gellénháza, Letenye, Sormás). In the case of Gellénháza, the renovation and development of the existing site began, in addition to carrying out reconstruction work. The Letenye wastewater treatment plant was developed, and with the construction of pre-sedimentation, the load on the biological stage was significantly reduced. The receiving wastewater was received by the Mura River, thus relieving the Birkitó ditch, which is a periodic watercourse.

The Lenti agglomeration will also be expanded, connecting Rédic and its co-settlements as well as the settlements of Szilvagy and Pórszombat. Simultaneously with the development, the wastewater treatment plant will be expanded, and a canal reconstruction will be carried out in Lenti. The lower water body of the Principalis canal is loaded by 7 communal wastewater treatment plants. In addition to Nagykanizsa, the wastewater treatment plant of Pacsa also places a greater burden on the water body. The Paks plant typically performs cleaning to the limit values. In the case of the Nagykanizsa site, the decomposition of organic matter works well, in the case of the N forms the limit values ( $\text{NH}_4\text{-N}$ : 5 mg/l; Total N: 15 mg/l) are exceeded in some cases. In the case of Nagykanizsa, the load on the recipient is negatively affected by the large precipitation coming from the combined channel, which can upset the balance of biological treatment. Both organic components and nutrient components exceed the limit in the Principal Channel. Concentrations of total phosphorus, total nitrogen and ammonium nitrogen in some cases significantly exceed the limit value. Settlements contaminating the sections of the Principal Canal should be inspected and the bed of the Principal Canal should be placed in good maintenance. There are also increased wastewater loads in other areas of the Mura River Basin. 3-3 wastewater treatment plants allow the treated wastewater to enter the Alsó-Válicka



and Kebele stream water systems, however, the problems arising from these are not particularly significant. In addition to Bajánsenye, treated wastewater from two larger capacity plants (Lenti and Lovászi) will also be introduced into Kerka. Recently, the two settlements have been fined. The water from wastewater treatment plants discharged into intermittent watercourses can cause a problem in groundwater quality, but such loads are generally not typical.

## 2.7. Other significant land-based pollution

Major industrial plants have public sewer emissions. Most of the discharged wastewater goes to a municipal wastewater treatment plant after pre-treatment. As a result, there is no significant industrial pollution. The storage of on-site dilute and litter manure with adequate technical protection is taking place in more and more places. There is more to safe storage in large livestock farms.

## 2.8. Pollution effects of settlements - Utility water supply and sewage disposal

In the settlements of the Mura catchment area, the supply of piped drinking water dates back to the 20th century, by the end of the century it had become 100%. The public water supply is fully developed throughout the catchment area. One-third of the water supply systems were built before the 1980s, while the rest were built in the early 1990s. The water network and their fittings are obsolete, with a network loss of around 30% (very high). Out of 135 settlements in the catchment area, 72 are equipped with a network of public sewers, these are mainly settlements with a larger population. The treatment of wastewater from settlements with public sewerage is provided by 31 wastewater treatment plants with a total hydraulic capacity of 29846 m<sup>3</sup>/d and a biological capacity of 152896 HP (2018). Out of the wastewater treatment plants, 25 plants have biological treatment stages, 6 wastewater treatment plants have near-natural (spring, root field (reedbed), Organica-SBR) technology. Currently, 18 settlements in the area are planning to solve the sewage drainage and treatment solution by creating a joint agglomeration (Bánokszentgyörgy, Borsfa, Bucsuta, Oltárc, Pusztamagyaród, Szentlisló, Várfölde); and connected to existing agglomerations (Alsórajk, Felsőrajk, Kilimán, Pölöskefő, Mikekarácsonyfa, Szentkozmadombja, Zalatárnok, Pórszombat, Szilvagy, Liszó, Surd). In the case of operating wastewater treatment plants, it will also be necessary to develop and modernize the plants. Most of the settlements were built in the '90s or before. Worn mechanical equipment can endanger the operation of the cleaner, ensuring the required cleaning efficiency. The Magyarszombatfa wastewater treatment plant in the Mura catchment will be abandoned due to amortization. Wastewater treatment should be provided by another solution.

## 2.9. Sewage loads

Of the 31 wastewater treatment plants operating in the area, 22 were treated in 2017. The increased organic matter and nutrient load has a negative effect on the status of the water body. In addition to professional operation, site development may be justified at these sites. 3 sites in the Mura catchment area are biologically overloaded (Gellénháza, Letenye, Sormás). In the case of Gellénháza, the renovation and development of the existing site began, in addition to carrying out reconstruction work. The Letenye wastewater treatment plant was developed, and with the construction of pre-sedimentation, the load on the biological stage was significantly reduced. The recipient of the treated wastewater became the Mura River, thus relieving the Birkitó ditch, which is a periodic water body.

The Lenti agglomeration will also be expanded, connecting Rédics and its co-settlements as well as the settlements of Szilvagy and Pórszombat. Simultaneously with the development, the wastewater treatment plant will be expanded, and a canal reconstruction will be carried out in Lenti. The lower water body of the Principalis canal is loaded by 7 communal wastewater treatment plants. In addition to Nagykanizsa, the wastewater treatment plant of Pacsa also places a greater burden on the water body. The Pacsa plant typically performs the cleaning limit values. In case of the Nagykanizsa site, the decomposition of organic matter works well, in the case of the N forms the limit values ( $\text{NH}_4\text{-N}$ : 5 mg/l; Total N: 15 mg/l) are exceeded in some cases. In the case of Nagykanizsa, the load on the recipient is negatively affected by the large precipitation coming from the combined channel, which can upset the balance of biological treatment. Both organic components and nutrient components exceed the limit in the Principal Channel. Concentrations of total phosphorus, total nitrogen and ammonium nitrogen in some cases significantly exceed the limit value. Settlements contaminating the sections of the Principal Canal should be inspected and the bed of the Principal Canal should be placed in good maintenance. There are also increased wastewater loads in other areas of the Mura River Basin. 3-3 wastewater treatment plants allow the treated wastewater to enter the Alsó-Válicka and Kebele stream water systems, however, the problems arising from these are not particularly significant. In addition to Bajánsenye, treated wastewater from two larger capacity plants (Lenti and Lovászi) will also be introduced into Kerka. Recently, the two settlements have been fined. The water from wastewater treatment plants discharged into intermittent watercourses can cause a problem in groundwater quality, but such loads are generally not typical.

## 2.10. Other major land - based pollutions

Major industrial plants have public sewer emissions. Most of the discharged wastewater goes to a municipal wastewater treatment plant after pre-treatment. As a result, there is no significant industrial pollution. The storage of on-site dilute and litter manure with adequate technical protection is taking place in more and more places. In large livestock farms, safe storage is mostly solved. The problem, however, is the application of the resulting manure to the land. As the incentive scheme for farmers does not favour this, the application of organic manure is often avoided, so that the storage location often becomes a source of pollution. The safe storage of hazardous materials can be considered a solution in the area. Users are under regular official control. There are a large number of out-of-date, disused, unprotected landfills in the catchment area. There is a landfill (category B3) in Nagykanizsa in the catchment area. The disposal of the remaining repositories in private areas is a future unresolved issue. Hazardous waste that is not treated in accordance with the regulations poses an increased risk to the environment, however, due to the strict legal regulations, the best situation for hazardous waste is overall for each waste group. It is only very rarely necessary to take action against the unauthorized treatment or illegal disposal of hazardous waste. Waste management is carried out in accordance with the Territorial Waste Management Plan.

## 2.11. Groundwater load

In the Mura catchment area, drinking water is supplied exclusively from groundwater. A significant part of the waterworks wells tap the Upper Pannonian aquifers between 30 and 150 m. There is a significant riverbank-filtered water abstraction on the gravel terrace of the Mura River in the Molnári area. Shallow aquifers, located between 30 and 50 m, are highly endangered from surface contaminants. In the case of water bases without geological protection or with partial geological protection, the pollutants that reach the surface of the earth pollute the soil and then reach the groundwater, from where they reach the water-producing wells over years and decades. The riverbank-filtered drinking water base located on the Pleistocene gravel terrace of the Mura River represents a significant water abstraction in the catchment. The city of Nagykanizsa is also supplied from this water base. The water base is located in a vulnerable geological environment, so its protection is a priority. In order to ensure the long-term security of public drinking water supply in the mid-1990s, the so-called long-term drinking water bases have been designated. 3 Perspective Water Bases have been designated in the catchment area. Two riverbank-filtered water bases on the Mura gravel terrace (Letenye - South East; Letenye

–Mura bank) and one stratified water base around Lenti. The quality of groundwater is not suitable for drinking water utilization under the settlements and in the agricultural areas, due to the already mentioned agricultural and locally industrial pollutants in the vicinity of the settlements. The utilization of groundwater is thus limited to the use for irrigation purposes, in particular for domestic garden irrigation. The larger agricultural farms and horticultural companies operating in the area already obtain irrigation water from stratified water wells, thus undertaking the application of the water-saving irrigation technology - micro-irrigation - prescribed by law.

## 2.12. Natural loads from climate change - Emergence of hydrological and meteorological extremes

A number of novel natural impacts are affecting the river basin as a result of climate change, highlighting the increase in the frequency of hydrological extremes, which have a strong impact on current and expected future water resources and ecosystems. Extreme, hectic changes in dry-wet periods can be experienced in the catchment, which include long periods of water shortages, lightning floods, and they result in extreme temperature conditions, either annually or intermittently. There have been spatial shifts in hydrological and meteorological characteristics over the last 20 years. Winter precipitation often does not fall in the form of snow, even intensely, while in summer precipitation is accompanied by a downpour, causing an extraordinary runoff ([https://vizeink.hu/wp-content/uploads/2020/04/3\\_1\\_Mura\\_JVK\\_NYUDU\\_JVK\\_2020\\_04\\_22.pdf](https://vizeink.hu/wp-content/uploads/2020/04/3_1_Mura_JVK_NYUDU_JVK_2020_04_22.pdf)).

### 3. Wastewater components and their risk assessment

Untreated municipal wastewater contains a number of components, from dissolved metals and organic compounds to large solids such as rags, sticks, floating objects, granules and greases. All recycling systems require minimal secondary treatment to handle large objects and particles, most dissolved organic matter, certain nutrients, and other inorganic substances. However, there are particles, including microorganisms, dissolved organic and inorganic constituents that remain in the secondary treated wastewater, and most often further treatment is required before it can be reused.

#### 3.1. Microorganisms in wastewater

Microorganisms are ubiquitous and most of them are not pathogenic to humans. Microorganisms, also called microbes, are diverse and critical in the nutrient recycling of ecosystems. In wastewater treatment systems that effectively design ecosystems, they act as useful decomposers of nutrients and organic matter. Concentrations of microorganisms are typically displayed on a logarithmic scale because they may be present in very high concentrations. Similarly, they can be significantly removed, and logarithmic scales help to specify these huge ranges.

In addition to beneficial microorganisms, raw domestic wastewater can contain a wide variety of pathogenic microorganisms that are primarily derived from the feces of infected humans and spread primarily pathogenically. A pathogen is a microorganism that causes diseases in its host. Most pathogens in untreated wastewater are known as “intestinal” microorganisms; they live in the intestinal tract, where they can cause diseases such as diarrhea. The source of human pathogens in wastewater is the feces of infected individuals who show symptoms of the disease, as well as uninfected carriers. Pathogens can also be present in the urine, including pathogens that can cause typhoid, leptospirosis and some sexually transmitted infections. However, the first two diseases show a very low incidence of the disease, and the latter cannot live in wastewater conditions for long. Thus, urinary pathogens pose a low health risk when reusing water.

The following table lists the infectious agents potentially present in raw domestic wastewater. These are classified into three major groups: bacteria, parasites (parasitic protozoa and helminths), and viruses. The table also lists the diseases associated with each pathogen. Concentrations of pathogens in wastewater vary greatly depending on the health status of the population and the season. The concentrations of each organism observed in the research are

reported in a table to provide a general comparison, but available data are scarce due to the lack of funding for this type of study.

*Infectious agents potentially present in untreated (raw) wastewater*

Pathogen	Disease	Numbers in Raw Wastewater (per liter)
<b>Bacteria</b>		
<i>Shigella</i>	Shigellosis (bacillary dysentery)	Up to 10 <sup>4</sup>
<i>Salmonella</i>	Salmonellosis, gastroenteritis (diarrhea, vomiting, fever), reactive arthritis, typhoid fever	Up to 10 <sup>5</sup>
<i>Vibrio cholera</i>	Cholera	Up to 10 <sup>5</sup>
Enteropathogenic <i>Escherichia coli</i> (many other types of <i>E. coli</i> are not harmful)	Gastroenteritis and septicemia, hemolytic uremic syndrome (HUS)	
<i>Yersinia</i>	Yersiniosis, gastroenteritis, and septicemia	
<i>Leptospira</i>	Leptospirosis	
<i>Campylobacter</i>	Gastroenteritis, reactive arthritis, Guillain-Barre syndrome	Up to 10 <sup>4</sup>
Atypical mycobacteria	Respiratory illness (hypersensitivity pneumonitis)	
<i>Legionella</i>	Respiratory illness (pneumonia, Pontiac fever)	
<i>Staphylococcus</i>	Skin, eye, ear infections, septicemia	
<i>Pseudomonas</i>	Skin, eye, ear infections	
<i>Helicobacter</i>	Chronic gastritis, ulcers, gastric cancer	
<b>Protozoa</b>		
<i>Entamoeba</i>	Amebiasis (amebic dysentery)	Up to 10 <sup>2</sup>
<i>Giardia</i>	Giardiasis (gastroenteritis)	Up to 10 <sup>5</sup>
<i>Cryptosporidium</i>	Cryptosporidiosis, diarrhea, fever	Up to 10 <sup>4</sup>
Microsporidia	Diarrhea	
<i>Cyclospora</i>	Cyclosporiasis (diarrhea, bloating, fever, stomach cramps, and muscle aches)	
<i>Toxoplasma</i>	Toxoplasmosis	
<b>Helminths</b>		
<i>Ascaris</i>	Ascariasis (roundworm infection)	Up to 10 <sup>3</sup>
<i>Ancylostoma</i>	Ancylostomiasis (hookworm infection)	Up to 10 <sup>3</sup>
<i>Necator</i>	Necatoriasis (roundworm infection)	
<i>Ancylostoma</i>	Cutaneous larva migrans (hookworm infection)	
<i>Strongyloides</i>	Strongyloidiasis (threadworm infection)	
<i>Trichuris</i>	Trichuriasis (whipworm infection)	Up to 10 <sup>2</sup>
<i>Taenia</i>	Taeniasis (tapeworm infection), neurocysticercosis	
<i>Enterobius</i>	Enterobiasis (pinworm infection)	
<i>Echinococcus</i>	Hydatidosis (tapeworm infection)	
<b>Viruses</b>		
Enteroviruses (polio, echo, coxsackie, new enteroviruses, serotype 68 to 71)	Gastroenteritis, heart anomalies, meningitis, respiratory illness, nervous disorders, others	Up to 10 <sup>6</sup>
Hepatitis A and E virus	Infectious hepatitis	
Adenovirus	Respiratory disease, eye infections, gastroenteritis (serotype 40 and 41)	Up to 10 <sup>6</sup>
Rotavirus	Gastroenteritis	Up to 10 <sup>5</sup>
Parvovirus	Gastroenteritis	
Astrovirus	Gastroenteritis	
Caliciviruses (including Norovirus and Sapovirus)	Gastroenteritis	Up to 10 <sup>9</sup>
Coronavirus	Gastroenteritis	

(Sources: NRC, 1996; Sagik et al., 1978; Hurst et al., 1989; WHO, 2006; Feachem et al., 1983; Mara and Silva, 1986; Oragui et al., 1987; Yates and Gerba, 1998; da Silva et al., 2007; Haramoto et al., 2007; Geldreich, 1990; Bitton, 1999; Blanch and Jofre, 2004; and EPHC, 2008)

Water bodies such as rivers, lakes, streams, planned stormwater drains, groundwater, and swimming pools may be contaminated due to exposure to untreated or improperly treated domestic wastewater and agricultural runoff. Survival of pathogens in the aquatic environment is determined by the distance travelled in the water distribution system, transport rate,



temperature, soil moisture content, humidity, exposure to sunlight, water chemistry (pH, salinity, etc.) and the presence of other organisms.

The main potential routes for the spread of aquatic diseases in connection with water recovery include the consumption of contaminated water or food from vectors or by hand-mouth contact, by inhalation of mist, or by inhalation from aerosol water containing suspended pathogens. Fortunately, treatment technologies are able to remove pathogens from water below detection limits. It is important to know what pathogenic microorganisms are present in wastewater so that proper treatment can be applied.

### 3.1.1. Protozoa and helminths

Parasites can be excreted in feces such as spores, cysts, oocysts that are robust and resistant to environmental stresses such as dehydration, heat, freezing and sunlight. Most parasites have spores, cysts, oocysts and oocytes ranging in size from 1  $\mu\text{m}$  to 60  $\mu\text{m}$  (larger than bacteria). Helminths may be present as an adult organism, larvae or ovum. Eggs and larvae between about 10  $\mu\text{m}$  and 100  $\mu\text{m}$  are resistant to environmental loads. The occurrence of these microorganisms in recovered water has been the subject of a recent research (WRRF, 2012a-e), which confirms that the removal of protozoa and helminth samples from wastewater can be accomplished through either a “removal” or “inactivation” process (WRRF, 2012). In the recovered water, protozoa and helminth samples can be physically removed by sedimentation or filtration due to their relatively large size. Protozoa and helminth samples may be resistant to disinfection with chlorination or other chemical disinfectants, but may be inactivated by UV disinfection, causing mutations in their DNA. Recent research to develop molecular assays that can rapidly differentiate between infectious cysts and are incapable of causing infection in recovered water has confirmed this mode of disinfection (WRRF, 2012).

### 3.1.2. Bacteria

Bacteria are microscopic organisms with a length of about 0.2–10  $\mu\text{m}$ . Many types of harmless bacteria colonize the human intestinal tract and are excreted regularly in the feces. Infected individuals also have pathogenic bacteria in their stools; therefore, municipal wastewater can contain many different concentrations of bacteria, including bacteria pathogenic to humans. Their number and type depend on their occurrence in the animal and human community from which the wastewater originates.

Bacterial levels in wastewater can be significantly reduced by removal or inactivation processes, which typically involve the physical separation of bacteria from wastewater by

sedimentation and/or filtration. Due to density considerations, bacteria do not settle as individual cells or even as colonies. Bacteria can be adsorbed into the particles or flaky particles, and these particles settle during sedimentation, secondary purification or an advanced treatment process such as coagulation/flocculation/sedimentation. Bacteria can also be removed by a filtration process that includes sand filters, disk (fabric) filters, or membrane processes. Bacteria can also be inactivated by disinfection.

### 3.1.3. Viruses

Viruses occur in various forms, ranging in size from 0.01 to 0.3  $\mu\text{m}$ , a fraction of the size of bacteria. Bacteriophages are viruses that infect bacteria; they are not involved in human infections and are often used as indicators. Coliphages are host-specific viruses that infect coliform bacteria. Enteric viruses multiply in the intestinal tract and are released in the feces of infected individuals. Not all types of intestinal soluble viruses have been identified to cause waterborne diseases, but more than 100 different enteric viruses can cause infections or diseases.

In general, viruses are more resistant to environmental stress than many bacteria and some viruses only survive in wastewater for a short time. Like bacteria and protozoan parasites, viruses can be physically removed or inactivated (Myrmel et al., 2006). However, due to the relatively small size of typical viruses, sedimentation and screening processes are less efficient during removal. Significant virus removal can be achieved with ultrafiltration membranes, possibly in the 3-4 log range. However, in the case of viruses, inactivation is generally considered to be the most important of the two main reduction methods and is often performed by UV disinfection. Interestingly, disinfection of viruses requires a relatively higher dose of UV than inactivation of bacteria and protozoa.

While monitoring specific viral pathogens in wastewater samples would provide more reliable information for risk assessment of aquatic viral infections, direct control of many viral pathogens in water is challenging and impractical, despite the recent development of real-time quantitative polymerase chain reaction (PCR) analyzes. (LeCann et al., 2004; Van den Berg et al., 2005). Until more data are available on the detection of active infectious viruses, data from studies to evaluate the effectiveness of wastewater treatment processes should be carefully evaluated in order to develop treatment plans to remove infectious viruses.

### 3.2. Aerosols

Aerosols are particles with a diameter of less than 50 µm that are suspended in air. Viruses, most pathogenic bacteria and pathogenic protozoa are in the respirable size range; therefore, inhalation of aerosols is a possible direct means of human infection. Aerosols are most often a problem when improperly treated recovered water is used with irrigation systems or in urban and agricultural areas, or where it is used to replace water. The infection or disease can be transmitted directly by inhalation or indirectly from aerosols deposited on surfaces such as food, vegetation and clothing. The infectious dose of some pathogens is lower in respiratory infections than in the gastrointestinal tract; Thus, for some pathogens, inhalation is a more likely route of disease spread than either contact or ingestion.

Thus, for occasional spraying of disinfected recovered water, inhalation of occasional accidental contact may pose little health hazard. Cooling towers continuously emit aerosols and can be a bigger problem if the water is not disinfected properly. In both cases, aerosol exposure is limited by design or operational controls, which are discussed in detail in the 2004 Guidelines (EPA, 2004).

### 3.3. Indicator organizations

It is important to distinguish actual pathogens from indicator microorganisms that are used to measure the treatment performance of a particular treatment system, given that pathogens are treated with fecal infection. Indicators are not in themselves dangerous to human health, but they indicate the likelihood of a health risk occurring. The variety and often lower concentrations of pathogenic microorganisms in ambient waters, which, in combination with special analytical methods for the detection of pathogens, make it difficult for a typical wastewater laboratory to perform such tests. Regulatory agencies have historically required routine screening of more abundant and more easily detectable fecal bacteria as an indicator of the presence of fecal contamination. In some countries, whole coliform bacteria are used as indicators; however, in many countries with special regulations, the microbiological safety of recovered water is assessed by monitoring fecal coliform bacteria in disinfected wastewater once a day on a single 100 mL capture sample.

Often indicators containing all coliforms; fecal coliforms, a subset of complete coliforms; *Escherichia coli* (*E. coli*); *enterococcus* and *coliphage* is used to verify the performance of the treatment and the quality of the water quality finally recovered. The main disadvantage of using microbial indicators is that they are somewhat limited in predicting the presence of pathogens.

All current applications of microbial indicators use culture methods that delay results for at least 24 hours. For example, non-pathogenic coliforms may occur in soil, grow in water under certain conditions, leading to positive results that do not necessarily indicate the effect of wastewater. In addition, coliform bacteria do not adequately reflect the presence of pathogens in disinfected recovered water because of their relatively high sensitivity to chemical disinfection and their inability to correlate with protozoan parasites such as *Cryptosporidium* and enteric viruses (Bonadonna, et al., 2002; Havelaar et al., 1993).

Alternative microbiological indicators have been proposed for the assessment of wastewater, drinking water and ambient waters, including *Enterococcus*, *Clostridium* and coliphage. But only a few studies have been performed on recovered water in which the levels of indicator organisms have been directly compared with the levels of viral, bacterial or protozoan pathogens at all stages of treatment, and further research is needed on this topic (Harwood et al., 2005). Analytical methods for actual pathogen monitoring are evolving, and recent studies have relied not only on traditional standard culture methods (Fox and Drewes, 2001; Sloss et al., 1996; Sloss et al., 1999; Yanko 1999). PCR is now commonly used to study pathogens and indicators to detect DNA or RNA in the environment. PCR is useful because the methods are sensitive. In addition, PCR can be much cheaper and more time consuming than conventional pathogen methods, and culture methods are not currently available for some pathogens. Recent studies have reported pathogenic DNA and RNA in ocean water affected by secondary and advanced municipal wastewater, some recycled water, groundwater, and wastewater discharges (Aw and Gin, 2010; De Roda et al., 2009; Hunt et al., 2010; Jjemba et al., 2010; Symonds et al., 2009; da Silva et al., 2008; da Silva et al., 2007; Haramoto et al., 2007). It is important to emphasize, however, that PCR does not determine the viability or infectivity of a pathogen; shows only the presence of DNA or RNA from microorganisms. Research on the use of PCR-based detection methods is ongoing on how this information can be used to assess potential risk. In particular, quantitative PCR can provide data for quantitative microbial risk assessment (QMRA), but it should be borne in mind that indicators only assess “potential” risk. These indicators were not associated with epidemiological risks, with the exception of *E. coli* and *enterococci* in recreational settings. In addition, the evaluation of certain disinfection processes is particularly limited in the use of molecular tools and indicators, although molecular viability methods are emerging.

### 3.4. Removal of microorganisms

Removal of indicator organisms and pathogens is possible through both testing and operational monitoring. The Challenge test allows large-scale log removal by adding inflow concentrations with higher than normal microorganism concentrations. As the detected concentration of actual pathogens approaches or decreases at the lowest detectable concentration of current analytical methods, further research in this area may provide greater confidence in the sensitivity of operational monitoring. The following table shows the indicative range of microbial log reductions described in the literature for the different treatment processes.

*Indicative log removal of indicator microorganisms and enteric pathogens at different stages of wastewater treatment*

Type of Microorganism	Indicator microorganisms			Pathogenic microorganisms				
	<i>Escherichia coli</i> (indicator bacteria)	<i>Clostridium perfringens</i>	Phage (indicator virus)	Enteric bacteria (e.g., <i>Campylobacter</i> )	Enteric viruses	<i>Giardia lamblia</i>	<i>Cryptosporidium parvum</i>	Helminths
Bacteria	X	X		X				
Protozoa and helminths						X	X	X
Viruses			X		X			
Indicative Log Reductions in Various Stages of Wastewater Treatment <sup>1</sup>								
Secondary treatment	1 - 3	0.5 - 1	0.5 - 2.5	1 - 3	0.5 - 2	0.5 - 1.5	0.5 - 1	0 - 2
Dual media filtration <sup>2</sup>	0 - 1	0 - 1	1 - 4	0 - 1	0.5 - 3	1 - 3	1.5 - 2.5	2 - 3
Membrane filtration (UF, NF, and RO) <sup>3</sup>	4 - >6	>6	2 - >6	>6	2 - >6	>6	4 - >6	>6
Reservoir storage	1 - 5	N/A	1 - 4	1 - 5	1 - 4	3 - 4	1 - 3.5	1.5 - >3
Ozonation	2 - 6	0 - 0.5	2 - 6	2 - 6	3 - 6	2 - 4	1 - 2	N/A
UV disinfection	2 - >6	N/A	3 - >6	2 - >6	1 - >6	3 - >6	3 - >6	N/A
Advanced oxidation	>6	N/A	>6	>6	>6	>6	>6	N/A
Chlorination	2 - >6	1 - 2	0 - 2.5	2 - >6	1 - 3	0.5 - 1.5	0 - 0.5	0 - 1

(Sources: Bitton, 1999; EPHC, 2008; Mara and Horan, 2003; NRC, 1998; NRC, 2012; Rose et al., 1996; Rose, et al., 2001; EPA, 1999, 2003, 2004; WHO, 1989)

<sup>1</sup>Reduction rates depend on specific operating conditions, such as retention times, contact times and concentrations of chemicals used, pore size, filter depths, pretreatment, and other factors. Ranges given should not be used as design or regulatory bases—they are meant to show relative comparisons only.

<sup>2</sup>Including coagulation

<sup>3</sup>Removal rates vary dramatically depending on the installation and maintenance of the membranes.

N/A = not available

### 3.5. Risk assessment of microbial contaminants

While most microbes are harmless or beneficial, some are extremely dangerous - these are sometimes referred to as biological agents of concern (BAC). All BACs can cause serious and often fatal disease, but they differ in their physical characteristics, their movement in the environment, and the process of infection. Quantitative microbial risk assessment (QMRA)

measures the behavior of microbes to identify where they may become dangerous and to assess the risk to human health (including risk uncertainty). QMRA has four stages, but is modified to take into account the properties of living organisms such as BAC (NAS, 1983):

- Hazard identification: This process describes a microorganism and the disease it causes, including microbial symptoms, severity, and mortality; identifies particularly susceptible populations susceptible to infection.

- Dose response: Determining the relationship between dose (number of microbes ingested) and the resulting health effects is a critical step in the process. Data sets from human and animal experiments allow the construction of mathematical models to predict dose-response.

- Exposure assessment: This step describes the pathways that allow the microbe to reach individuals and cause infection (through air, drinking water, etc.). The magnitude and duration of exposure should be determined for each route, and the number of people exposed and the categories of people involved should be estimated.

- Risk characterization: The last step in the process integrates information from previous steps into a single mathematical model to calculate risk - the probability of an outcome such as infection, illness, or death. As the first three steps do not provide a single value but offer a range of exposure, dose and hazard values, the risk should be calculated for all values in these ranges. This is done with Monte Carlo analysis and the result is a full range of potential risks, including average and worst cases. These risks are assessed by policy makers when determining regulatory policy, as well as the risks that scientists review to determine where further research is needed to obtain better information.

Further information on QMRA can be found in the 2006 report to the European Commission (Medema and Ashbolt, 2006).

### 3.6. Chemicals in wastewater

All of the water is eventually used in the natural cycle and contains detectable amounts of various chemicals. Rainwater collects chemicals from atmospheric contact; groundwater contains inorganic substances from geology; surface waters collect natural products, possibly pesticides and other chemicals, from runoff and discharges from industrial and other facilities. Wastewater contains chemicals and the number and concentration of components detected depends on a number of factors, including the municipal source, the condition of the collection system and the treatment processes used.



### 3.6.1. Inorganic compounds

Inorganic constituents of wastewater include metals, salts, oxyhalides, nutrients and potentially engineered nanomaterials. The concentration of inorganic constituents in the recovered water depends primarily on the source of the wastewater and the degree of water treatment. The presence of inorganic constituents may affect the acceptability of the recovered water for various reuse purposes. Wastewater treatment using existing technology has generally reduced a number of trace elements below the recommended maximum levels for irrigation and drinking water. The health hazards associated with the ingestion of inorganic constituents directly or through food are generally well established.

The total amount of most inorganic constituents in water is TDS (total dissolved solids) and conductivity, although both may contain some organic constituents. Residential use of water typically represents about 300 mg/L of dissolved inorganic solids, although the amount added can be between about 150 mg/L and 500 mg/L (Metcalf and Eddy, 2003).

- Metals and salts. Regulations for the discharge of treated wastewater and industrial pre-treatment regulations specifically target toxic metals; as a result, most municipal wastewater has toxic metal concentrations lower than public health guidelines and standards. Boron may be present in domestic wastewater, but concentrations are generally well below WHO guidelines. Boron can be toxic to some plants at concentrations that approach levels in recovered water, which can limit the types of plants that can be irrigated with water. Similarly, salts present in recovered water (measured in TDS) do not generally exceed thresholds that are important for human health, but can have an effect on plants. High salinity can cause leaf burns, reduce the permeability of clayey soils, and affect soil structure. Salinity can also cause aesthetic problems (such as taste in drinkable reuse or residues during car wash operations). Salinity can be removed during treatment, but the options are usually costly, and disposal of liquid waste (saline) is an issue.

- Oxyhalides. Oxyhalides of concern for water reuse include bromate, chlorate and perchlorate. Bromate may be formed when effluents containing bromide are ozonated; therefore, treatment facilities should be designed and properly operated to minimize oxyhalide formation during treatment. Bromate, chlorate and perchlorate can be derived from household bleach. The component of the propellants, perchlorate, is able to bioaccumulate in certain plants.

- Nutrients. Nitrogen and phosphorus from human waste products can pose environmental and health problems, but can also be useful in some irrigation applications. Therefore, the need to remove nutrients during treatment for reuse depends on the intended use of the product in water.

- Designed nanomaterials. Nanomaterials are materials that have morphological characteristics on the nanoscale and that often have special properties due to their size. Nanomaterials have one or more dimensions, between 1 and 100 nm: nanofilms (one dimension), nanotubes (two dimensions) and nanoparticles (three dimensions). Larger particles such as zeolites (1000–10,000 nm, or 1–10  $\mu\text{m}$ ) can also be considered nanomaterials because their pores fall in the nanoscale range (0.4–1 nm). Nanomaterials can be organic, inorganic or combinations of organic and inorganic components.

Nanotechnology promises exciting new opportunities in water treatment and water quality control. Nanosorbents, nanocatalysts, bioactive nanoparticles, nanostructured catalytic membranes and enhanced filtration with nanoparticles are categories of new nanotechnologies that can change water treatment and water quality control (Savage and Diallo, 2005). Many consumer products now contain engineered nanomaterials due to their unique surface chemistry, catalytic properties, strength, mass and conductivity compared to their larger-scale counterparts (National Science and Technology Council, 2011; WEF, 2008).

While naturally occurring particles in this range include viruses and natural organic matter, the recent introduction of engineered nanomaterials from consumer products into the environment raises new questions about the fate of these substances and their potential environmental and health impacts. Preliminary studies to determine the health effects of exposure to nanomaterials as well as the risk assessment, toxicity and manageability of nanomaterials show inconsistent results. To date, there is no relationship between trace levels of artificially produced nanoparticles in wastewater and adverse effects on human health (O'Brien and Cummins, 2010). Since most artificially produced nanoparticles in municipal wastewater come from household and personal care products, the direct exposure in the household is likely to be much higher than the potential exposure from water reuse. However, the potential ecotoxicological risk of nanoparticles entering surface waters highlights guidance and restrictions on the use and disposal of commercial products containing nanomaterials (O'Brien and Cummins, 2010). Limited research has been done on their fate in wastewater treatment, but initial results suggest that the designed nanoparticles associate with or remain in the wastewater depending on their size and surface chemistry and the type of treatment applied (Kaegi et al., 2011; Kiser et al., 2009; WEF, 2008).

### 3.6.2. Organic compounds

The organic composition of raw wastewater includes naturally occurring humic substances, fecal matter, kitchen waste, liquid detergents, oils, greases, consumer goods, industrial waste

and other substances that become part of the wastewater. The treatment of these components in the recovered water depends on the end use of the recovered water. Some of the adverse effects associated with organic matter are:

- Aesthetic effects. Organic substances can smell bad and give colour to water.
- Clogging. Particles can clog sprinkler heads or accumulate in the soil and affect permeability.
- Reproduction of microorganisms. Organic matter provides food for microorganisms.
- Oxygen consumption. When decomposed, organic matter depletes the content in dissolved streams and lakes (dissolved oxygen). This negatively affects aquatic life, which depends on oxygen supply for survival.
- Restriction of use. Many industrial applications cannot tolerate water with a high organic content.
- Disinfecting effects. Organic materials can affect chlorine, ozone, and UV disinfection, making them less accessible for disinfection purposes. In addition, chlorination can result in the formation of potentially harmful chlorinated DBPs.
- Health effects. Ingestion of water containing certain organic compounds may cause acute or chronic health damage.

The detection of various organic chemicals in municipal wastewater has raised concerns about the potential presence of chemical contaminants from wastewater in the recovered water and their health effects. For some reuse applications, regulatory agencies and public utilities have tackled the issue of compounds from wastewater, some of which are often present in extremely low concentrations. As many of these compounds are currently unregulated, current research has focused on the composition of highly treated wastewater to identify residual chemicals that may pose a health problem, determine what studies would be needed as a basis for risk assessment, and compile a list of compounds, which require additional information to assess potential human health problems (WRRF, 2012). In addition, the WRRF funded the identification and validation of surrogate pollutant parameters and analytical methods to predict the removal of wastewater contaminants from reclaimed water treatment systems (WRRF, 2008).

Parameters historically used for this purpose, which can serve as an aggregate measure of organic matter, include TOC, dissolved organic carbon (DOC) (the part of the TOC that passes through a 0.45 µm pore size filter), particulate organic carbon (POC) (the part of the TOC that remains on the filter), BOD and chemical oxygen demand (COD). These measures are indicators of treatment efficiency and water quality for many non-stand-alone uses of recovered water.

Previously, the greatest attention was paid to trihalomethane (THM) compounds; a family of organic compounds that typically occur as chlorine or bromo-substituted methane. Chloroform, a commonly used THM compound, plays a role in the development of liver and kidney cancer. Haloacetic acids (HAAs) are other undesirable by-products of chlorination with similar health effects. As a result of better analytical capabilities to detect extremely low levels of chemical constituents in water, a number of health-relevant chemicals and DBPs have been identified in recent years. For example, the carcinogen NDMA is present in wastewater and is also formed when the recovered water is disinfected with chlorine or chloramine (Mitch et al., 2003). As wastewater chlorination is still the most commonly used form of wastewater disinfection, further management of the actual reuse of DBP is critical. In some planned reuse applications, the concentration of NDMA present in the recovered water, even after treatment of the RO, exceeds the action levels set in the drinking water to protect human health.

### 3.6.3. Trace elements

Sophisticated analytical tools allow the identification and quantification of extremely low levels of each inorganic and organic constituent. Examples are gas chromatography/mass spectrometry (GC/MS) and high performance liquid chromatography/mass spectrometry (HPLC/MS). These analyzes are costly and may require extensive and difficult sample preparation, especially for non-volatile organic materials. Advances in these and other analytical chemical techniques have made it possible to quantify chemicals in water in trillion parts (ppt) and even parts in quadrillion levels. With further analytical advances in the future, almost all chemicals will be detectable in ambient waters, wastewater, recovered water and drinking water, but the human and environmental health significance of detecting declining concentrations remains a greater challenge.

With the development of analytical techniques, many anthropogenic chemical compounds that are not water, wastewater or ambient water are usually present in very low amounts. Detection of these compounds does not mean that they are not released into the environment - many are likely to be in the environment for decades. This broad group of individual chemicals and groups of compounds is called trace elements, TrOCs or microcomponents. A wide range of these may include groups of compounds that can be grouped by end use (e.g., drugs, over-the-counter drugs, personal care products, household chemicals, food additives, flame retardants, plasticizers, and biocides), if any (e.g., hormonally active agents, endocrine disruptors, endocrine disruptors (EDs) or endocrine disruptors (EDCs)) or by type of compound (eg. chemical or microbiological, phenolic or polycyclic aromatic hydrocarbons).

Although chemical trace elements are 'pollutants' when present in the environment in concentrations above background levels, they are not necessarily 'pollutants' (ie. they are present in the environment at high enough levels to induce effects on ecological and/or human health). The description of many constituents by subgroup or as individual chemicals is also confusing because they are not well understood by the general public. There is an ongoing debate in the aquatic community about how to trace the traces of chemical compounds, including terminology and relative risk.

#### 3.6.4. Removal of chemical trace elements

As recovered water is increasingly seen as a source of use, including industrial process water or drinking water supply water, the focus of treatment has expanded far beyond secondary treatment and disinfection to include other contaminants such as metals, dissolved solids and trace elements.

The chemical components are suitable for treatment, depending on the physicochemical properties of the compounds and the removal mechanisms of each treatment process. EPA has published a report with a comprehensive literature review of published studies of the effectiveness of various treatment technologies for CECs (EPA, 2010). EPA has developed this information to provide accessible and comprehensive historical information about CEC's current management technologies.

Given the wide range of properties represented by chemical trace elements, there is no single treatment procedure that would impose an absolute barrier on all chemicals. To minimize their presence in treated water, a variety of treatment processes are required that can handle a wide range of physiochemical properties (Drewes and Khan, 2010). Extensive and experimental studies have shown that this can be accomplished by a combination of different processes: biological processes, chemical oxidation or activated carbon adsorption, physical separation (RO) followed by chemical oxidation, or natural processes, chemical oxidation or carbon adsorption. The question is whether these technologies are needed to ensure health protection or whether a particular section is over-treated, especially if the water is returned to the environment through a reservoir or reservoir. Therefore, the water is likely to degrade to some extent before being taken out for further treatment of drinking water.

A recent survey on the fate of medicines and personal care products (PPCP) in wastewater treatment plants revealed that many EDCs are present in mg/l concentrations and are not significantly removed by conventional wastewater treatment processes (Miège et al., 2008). Some removal or chemical transformation is expected during disinfection of drinking water (i.e., sulfamethoxazole, trimethoprim-estrone, 17 $\beta$ -estradiol, 17 $\alpha$ -ethynylestradiol,

acetaminophen, triclosan, bisphenol-A and nonylphenol). Chlorine, chlorine dioxide and ozone disinfection are oxidation processes (Alum et al., 2004; Huber et al., 2005); Among the three oxidizing agents, ozone reacts the most with organic trace elements.

Adsorption of activated carbon can easily remove many organic compounds from water, with the exception of a few polar water-soluble compounds such as iodinated contrast agents and the antibiotic sulfamethoxazole (Adams et al., 2002; Westerhoff et al., 2005). Although very effective, AOP treatment processes are not effective in oxidizing chemical trace elements because they are energy-intensive and involve random reactions with much of the TOC, with only slightly present target compounds. Compared to ozone treatment alone, AOPs only slightly increase removal efficiencies (Dickenson et al., 2009).

The pore size of low pressure membranes such as MF and ultrafiltration (UF) is not sufficient to retain trace elements; however, some hydrophobic compounds are still able to be absorbed on the membrane surface of MF and UF, providing short-term attenuation of hydrophobic compounds and TOC. However, high-pressure membranes such as RO and nanofiltration (NF) are very effective in the physical separation of various drugs and other organic and inorganic substances from water (Bellona et al., 2008). Low molecular weight organic matter is problematic for high pressure membranes, and destruction of the concentrate (saline) at high concentrations can be an issue. Natural processes such as river bank filtration (RBF) can be used as an additional purification step for wastewater recovery or as a pretreatment for subsequent drinking water treatment (Amy & Drewes, 2007; Hoppe-Jones et al. 2010). RBF and SAT are very effective in extracting a wide range of chemicals by subsurface sorption and biotransformation processes, but have limited use in, for example, antiepileptic drugs or chlorinated flame retardants (Drewes et al., 2003).

AOP processes are being researched for their ability to remove organic compounds. For example, although UV photolysis is generally not an effective treatment option for the removal of organic compounds, UV photolysis in combination with H<sub>2</sub>O<sub>2</sub> achieves high removal rates of several potential EDCs, including bisphenol-A, ethinyl estradiol, and estradiol (Rosenfeldt and Linden , 2004).

#### 3.6.5. Risk assessment of chemical trace elements.

Because wastewater treatment plants using conventional treatment methods are not able to completely remove organic chemical traces, wastewater discharges can introduce some of these components into the receiving environment. Thus, during actual reuse, chemical constituents can be introduced into the drinking water supply (Benotti et al., 2009). The detection of



chemical trace elements in drinking water systems and environmental waters raises understandable concerns about the potential consequences for public and ecological health. Research organizations around the world, including the EPA, are investigating these consequences and assessing the risks of acute, chronic diseases and consequences. Although a number of comprehensive studies have been conducted to address potential human health concerns for chemicals in unknown and unidentified traces in recovered water (Nellor et al., 1984; Sloss et al., 1996; Anderson et al., 2010), there is currently no definitive documentation of the on the risks related to chemicals used in the recovery of water for human consumption. Based on the available information, there is no indication that the health risks associated with the use of highly treated recovered water for drinking water would be greater than the risks from the use of existing water resources (NRC, 2012).

A recent report by the Global Water Research Coalition (GWRC) summarized the findings of nine recently published reports on the occurrence of drugs in the drinking water system and their potential impact on human health (GWRC, 2009). The report concludes that drug exposure to drinking water has no known effect on human health and that if a person consumed drinking water at the reported drug level, that person would consume only 5 percent (or less) of one therapeutic procedure per day ( i.e., a single tablet) of a drug throughout its life. In addition, a recent report by the WHO Panel of Experts concluded that exposure to trace levels of drugs in drinking water is unlikely to pose a risk of adverse effects on human health (WHO, 2011); this report did not evaluate non-pharmaceutical trace elements.

The traceability of chemical components used in the reuse of water used for irrigation or other non-irrigated reuse is negligible. The treatment technologies used in the planned potable reuse ensure that the concentration of trace chemicals is extremely low, often below the analytical detection limits.

While the risk associated with chemical constituents in drinking water is indeed very low, the water sector continues to investigate the issue and invest in precautionary treatment technologies. As zero risk to human health cannot be achieved with any level of exposure, there is a need for consensus on upper but minimum risk targets that can form the basis for the design and operation of drinking water reuse facilities.

The greater impact of chemical trace elements may be due to the ecological effects caused by the presence of chemicals and the runoff of rainwater into surface waters. Recent concerns about the ecological effects of released chemical constituents stem primarily from studies in surface waters receiving treated municipal wastewater in the 1990s, where wild fish near release changed their reproductive strategies and the frequency of hermaphroditism (Sumpter & Johnson, 2008). If advanced wastewater treatment is used that includes RO, almost all

microcomponents can be effectively removed, and RO wastewater does not pose a hormonal threat to tissue cultures and live fish (WRRF, 2010). Thus, while many environmental monitoring programs are in progress, toxicological studies at environmentally relevant concentrations are unlikely to provide much information due to the very low hypothetical risk of detected trace concentrations due to the difficulty of performing chronic studies.

In response to uncertainties about the potential risks of potable reuse applications, appropriate treatment technologies have been used to minimize human exposure to chemical trace elements from wastewater. A number of analytical studies have been performed to identify the few residual chemicals that may undergo advanced treatment. Residual TOC levels, which can be considered as a substitute for chemical constituents in the designed ready-to-drink, reusable finished water, are usually a fraction of a milligram/liter.

Treatment technologies for the production of recovered water are well documented to remove traces of chemical constituents to very low concentrations, posing a very low risk to human health. However, the perceived continuous detection of CECs in recovered water has raised public concern about their presence and the consequences of adopting the planned drinkable reuse. Public education to improve the efficiency of available treatment technologies and the safety of highly treated recovered water should be a top priority for scientists and regulators.

#### [3.6.5.1. Potential impact of residual chemical constituents](#)

Most wastewater treatment plants and many water recovery facilities are not designed to remove TrOC. As a result, residual antibiotics and metabolites are accidentally released into the environment. This can lead to the proliferation of antibiotic resistance (AR) in pathogenic or non-pathogenic environmental microorganisms (Pauwels and Verstraete, 2006). However, the proliferation of AR is not limited to the environment and may in fact occur during therapeutic use in which the intestinal flora is exposed to high concentrations of antibiotics or during wastewater treatment, especially secondary biological processes (Clara et al., 2004; Dhanapal and Morse, 2009).

The 2000 WHO report identified AR as a critical challenge for human health in the next century and drew attention to the “need for a global strategy to reduce resistance” (WHO, 2000). More than two million Americans are infected with antibiotic-resistant pathogens each year, and 14,000 die as a result. One potential source of this proliferation of AR is use for human health or animal husbandry, and the subsequent release of antibiotics and metabolites into the environment. It is estimated that up to 75 percent of antibiotics are excreted unchanged or as metabolites (Bockelmann et al., 2009). Nevertheless, few studies have attempted to identify processes that contribute to the selection of AR bacteria. This information will be critical in

developing treatment strategies that reduce the potential for AR proliferation in the environment.

Within a typical wastewater treatment plant, there are a number of critical locations where AR can accumulate or form. AR genes may already be present in the raw wastewater entering the wastewater treatment plant, but there is also significant evolutionary pressure within the wastewater treatment plant to trigger such changes. More specifically, conventional activated sludge (CAS) and MBR processes can be a significant source of AR, as under ideal growth conditions, bacteria are continuously exposed to relatively high concentrations of antibiotics. Despite the direct association between solids retention time (SRT) and decreased antibiotic concentrations, higher SRT also provides sustained exposure of bacterial populations to relatively high antibiotic concentrations present in primary wastewater (Clara et al., 2005; Gerrity et al., 2012; Salveson et al., 2012). Some MBRs operate in the order of 50 days for SRTs, while CAS processes can operate in the range of 1 to 20 days, which is more than sufficient to allow bacteria to adapt given their high growth rate. In both the MBR and CAS configurations, AR bacteria can accumulate in biosolids and enter the environment in finished wastewater or recovered water.

To reduce the potential for AR proliferation, future research should focus on identifying the main source(s) of AR (i.e., raw wastewater, biosolids, or treated wastewater), identify treatment conditions that promote AR development, and characterize the AR environment. Ultimately, this knowledge will help develop mitigation strategies and alleviate environmental and public health concerns.

## 4. Selected substances according to pollution sources – The sources and physiological effects of some substances in water

Water supply has been a major concern for mankind since ancient times. In the initial period, man used water for communal purposes, but there was also significant agricultural water consumption. The first great technical discovery of mankind was the construction of the irrigation system.

More than 2,000 years ago, the Romans used hundreds of miles of aqueducts to supply water to their cities and military garrisons, and built underground treatment plants to treat their wastewater. Mankind has already unknowingly realized that e.g. feces can be used to fertilize the fields. Therefore, in ancient times, methods were developed for the discharge of sewage water (sewerage), for disposal in river waters, where a relatively small amount of wastewater, after dilution, easily degradable materials were disposed of by decomposing organisms. (“Cloaca maxima” canal - the sewage led to the river Tiber.)

The Romans built their canals so well that some sections could still be used today (Cologne). With the fall of the Roman Empire, waterworks were destroyed and the expertise of the Romans was also forgotten.

In the Middle Ages, the sources of water supply were unprotected house wells and contaminated surface waters. As a result, large-scale plague, cholera, typhoid epidemics occurred, which often turned large areas uninhabited.

As a result of the Industrial Revolution, the urban population increased. In order to provide drinking water, waterworks had to be built and the water was delivered to the consumer through a sewer network.

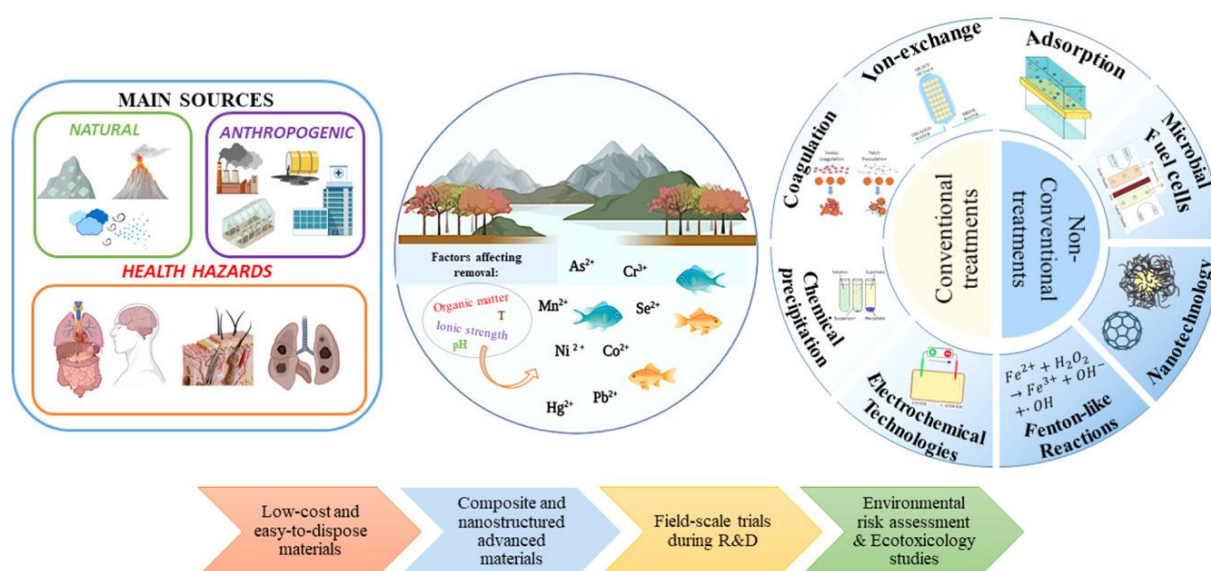
However, no sewage system was built, so the generated sewage (faeces, washing water, sewage from industrial plants) was, of course, only taken to the streets. Later, some of the faecal wastewater was discharged into manure pits, where part of the liquid was leaked (contamination of groundwater, wells).

As a result, epidemics caused by pollution (typhoid, cholera) have occurred in more and more areas, and this has drawn attention to the need to treat wastewater. The first water treatment works date back to the 19th century. They were built in the middle of the 19th century in larger cities (London, Berlin).

Since then, of course, water consumption has multiplied. While before the Industrial Revolution the per capita water consumption was 10-70 l/day, in the second half of the 19th century a specific water consumption of 300-400 l/day could have been expected, which can now be set at only 120 l/day in a modern household.

The development of the industry, the emergence of new industries can be characterized by an ever-increasing amount and often of a purer technological water demand than drinking water, but at the same time the resulting wide variety and large amount of wastewater contained more and more dangerous substances. For this reason, the former wastewater treatment technologies had to be developed and supplemented with new procedures in order to prevent pollution and damage to the environment (Jolánkai et al., 2009).

## HEAVY METAL WATER POLLUTION AND REMEDIATION



Source: [https://ars.els-cdn.com/content/image/1-s2.0-S2352186421001528-fx1\\_lrg.jpg](https://ars.els-cdn.com/content/image/1-s2.0-S2352186421001528-fx1_lrg.jpg)

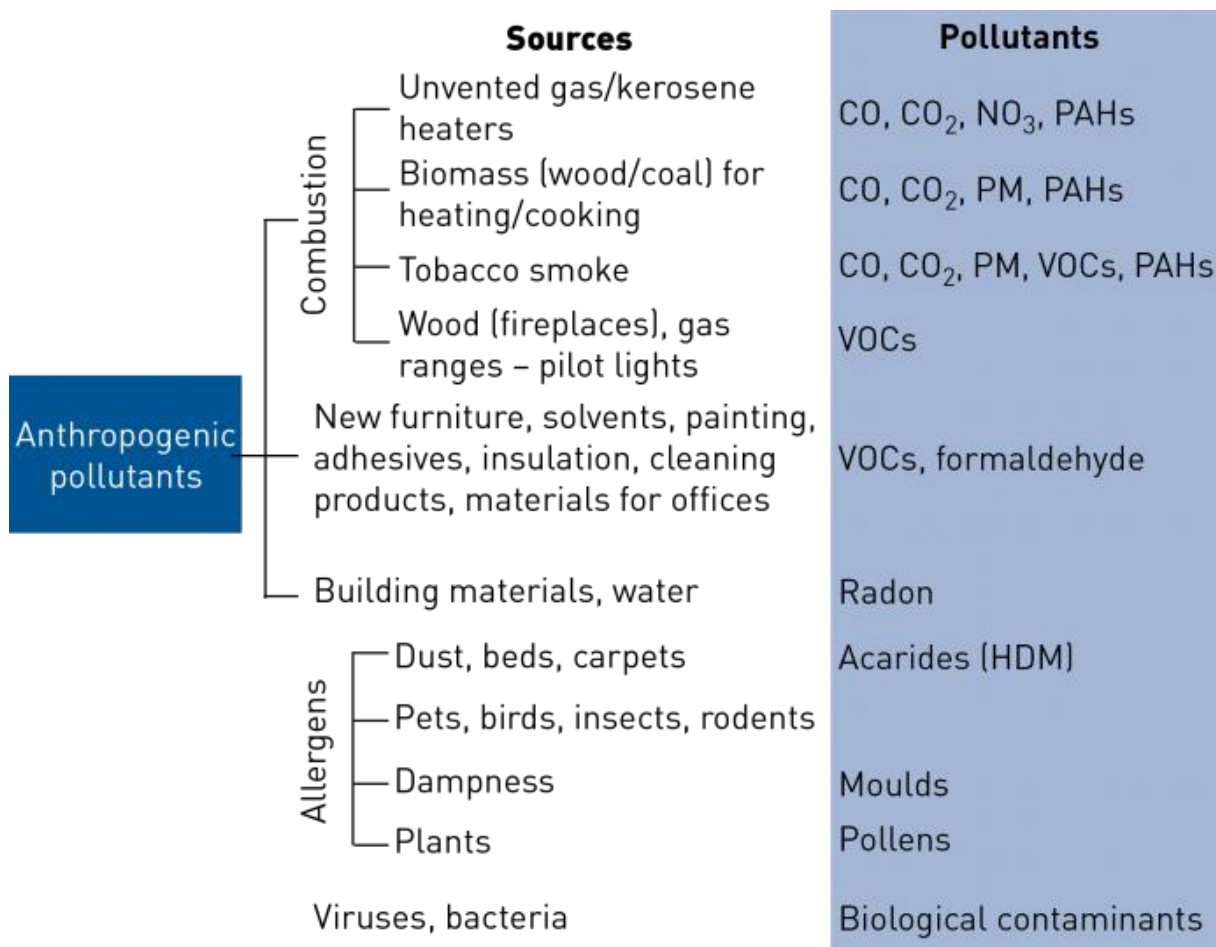
Despite these efforts, larger amounts of industrial, agricultural and municipal wastewater were discharged untreated or only after a simple mechanical treatment.

Its effect is damage to the environment, pollution of the drinking water base. Further pollution should be eliminated sufficiently in the future (construction of canals, water treatment plants, compliance with the protection of protected areas, legislation).

### 4.1. Anthropogenic materials

The term anthropogenic chemicals refers to man-made substances of human origin. The anthropogenic definition encompasses a broader conceptual scope. In the present case, the term anthropogenic means toxic, difficult-to-decompose, and in some cases even non-biodegradable, extraneous matter due to human industrial activity. Municipal wastewater is not included in the term because it contains largely natural pollutants (proteins, carbohydrates, fats). Complex, biodegradable substances (glycoproteins, cellulose and triglyceride derivatives, etc.) are also made up of the constituents of natural materials, but these substances are found everywhere in

nature. Undoubtedly, the products of the modern chemical industry (detergents, surfactants, cosmetics, drug residues, etc.) also appear in municipal wastewater, but overall these substances do not fundamentally change the natural nature of municipal wastewater.



*The main indoor pollutants and their sources. CO: carbon monoxide; CO<sub>2</sub>: carbon dioxide; NO<sub>2</sub>: nitrogen dioxide; PAHs: polycyclic aromatic hydrocarbons; PM: particulate matter; VOCs: volatile organic compounds; HDM: house dust mite (source: <https://www.erswhitebook.org/chapters/indoor-environment/>)*

Among the compounds of the organic synthesis industry, there are more and more compounds that have a specific inhibitory, cytotoxic (cellular poison) and biocidal (harmful effect on living organisms) effects on the cells of living organisms.

Substances of anthropogenic origin pose a threat to the human immune and hormone system, reproductive capacity and in many cases are carcinogenic. There are anthropogenic substances (PCBs; endosulfate) that have all four properties indicated above. In addition to human hazards, anthropogenic materials are generally difficult to biodegrade, in many cases having a toxic effect on activated sludge biology.

In the literature, extraneous substances are also called xenobiotic substances. The names non-biodegradable and refractory; persistent; recalcitrant molecules are also known. The names of hazardous substances are even used to characterize substances in chemical wastewater. This



term usually refers to a source of human hazard (general toxic effect, carcinogenicity, hormonal and immune system damage). The terms described above may be more expressive in some cases because they better highlight the main property or nature of a given group of extraneous matter, and are therefore commonly used in the literature.

As a result of industrial development, the number of new anthropogenic materials used in households, industry and agriculture has been growing unstoppably over the last 30 years. Accordingly, the number and concentration of anthropogenic substances in the generated wastewater is also increasing. A multitude of chemicals for different purposes, many by-products and waste from the chemical industry end up in wastewater, soil, and then from here into the receiving living waters. These compounds include aldehydes, ketones, esters, carboxylic acids and their salts or esters, aliphatic and aromatic alcohols, aromatic nitro and halogen compounds, and other detergents or surfactants of various compositions. Newer disinfectants, insecticides, fungicides, defoliants, rodenticides and various chemicals are being researched and developed. These agents are used to control pathogens of infectious and invasive diseases in humans, animals and plants. A wide variety of organic halogen, organophosphorus and other compounds are used in agricultural chemistry in large quantities. Pesticides and their residues are released into the soil and then into the receiving living waters. Synthetic compounds that are less harmful to wildlife in the form of various materials used in the household and industry also accumulate in soil, landfills, and in the bed of receiving living waters. The chemical industry also produces a number of substances that are very difficult to break down with microorganisms (Öllös, 2006).

#### 4.2. Chemical contamination of water and its effects on human health

Natural waters may initially contain substances that are harmful to health, but the main cause of water pollution can be identified with the consequences of human activity. The source of pollution could be aerosol particles deposited from the air, chemicals washed out of the soil, fertilizers, but the most important source is wastewater. Regarding its generation, wastewater can be:

- household (high organic matter content, detergents, human gut flora),
- agricultural (organic matter content, animal gut flora, fertilizers, pesticides),
- industrial (organic and inorganic chemical products).

In Hungary, about 30% of the generated wastewater goes untreated into the environment (soil, surface waters) which means about 400 million m<sup>3</sup> of wastewater per year. If the contamination does not reach a high degree, the water is capable of self-cleaning, which lasts from a few weeks

to a few months. Sediment dilution, bacteria that break down various substances, as well as bacteriophages that kill pathogens and a vibriol that results in bacteriolysis play a role in this process

([https://regi.tankonyvtar.hu/hu/tartalom/tamop425/0019\\_1A\\_Kornyezetegeszsegtan/ch02s03.html](https://regi.tankonyvtar.hu/hu/tartalom/tamop425/0019_1A_Kornyezetegeszsegtan/ch02s03.html)).

The main sources of chemical pollution of our waters are natural- and artificial pollution.

The main forms of natural pollution are mainly characteristic of groundwater:

- minerals (NaCl, MgCO<sub>3</sub>, CaCO<sub>3</sub>, arsenic),
- nitrate sulphate,
- radioactive substances (radon, radium).

The main source of pollution is chemicals released from waterproofing layers and rocks. Artificial pollution is typical of surface and karst waters, but of course it can also affect groundwater in the case of vulnerable water bodies.

The main forms are:

- oxygen-intensive organic wastes (sewage, manure),
- water-soluble inorganic substances (acids, salts, toxic heavy metals and their compounds),
- inorganic plant nutrients (nitrate, phosphate),
- organic compounds (oil, petroleum derivatives, pesticides, detergents),
- physical contamination (radioactive material, stone).

### 4.3. The major unwanted water pollutants

The water may contain inorganic and organic contaminants, in solid or dissolved forms. The danger is mainly the dissolved contaminant, which can easily be absorbed into the food chain, accumulate in organisms and have an adverse, harmful effect. In addition, heat is a source of pollution if it changes the properties of the water in an unfavourable direction for the living world.

Water pollutants are classified in the literature into the following groups:

- disease-causing agents (bacteria, viruses, parasites, etc.),
- oxygen-demanding wastes (biodegradable organic matter),
- water-soluble inorganic substances (acids, alkalis, salts, heavy metals and their compounds),
- inorganic plant nutrients (nitrogen and phosphorus),
- organic compounds (oils and their derivatives, pesticides, detergents, etc.),
- solid organic or inorganic substances (soil particles, etc., with very different particle sizes),

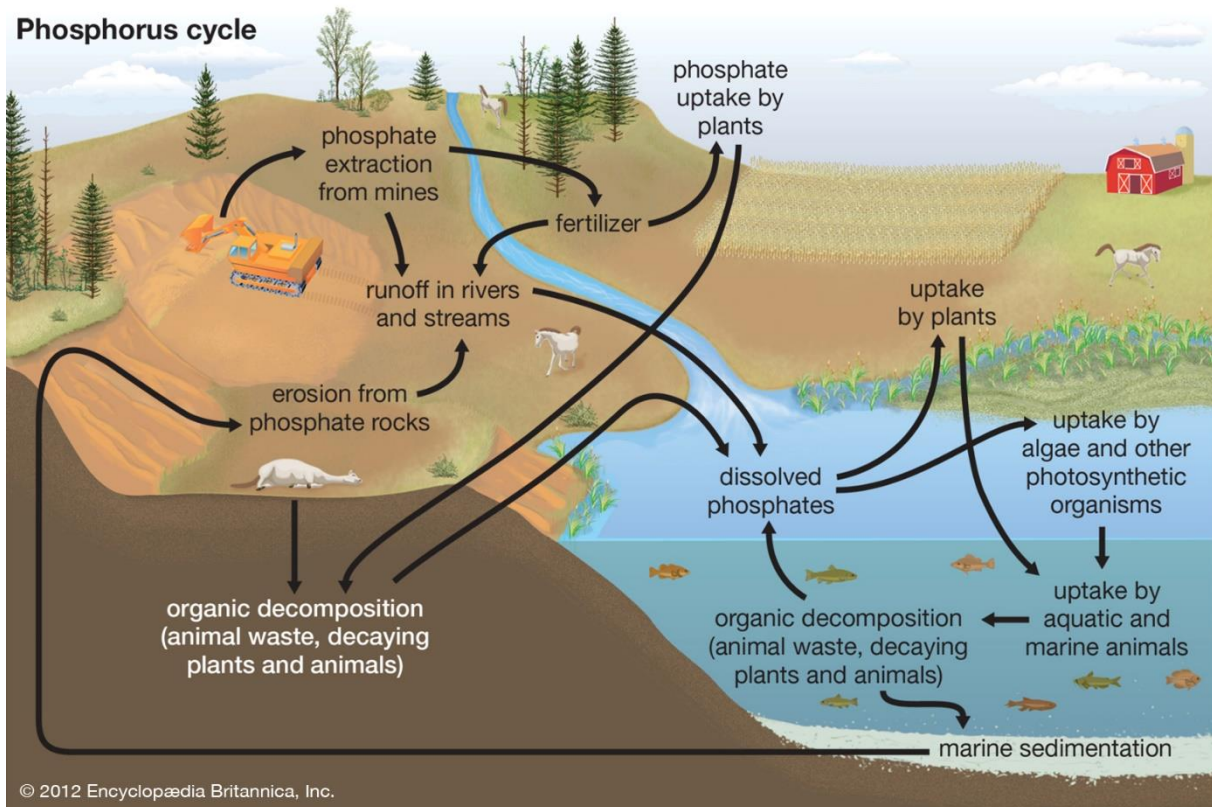
- radioactive materials,
- heat.

#### 4.4. Phosphorus

It is one of the building blocks of living organisms. In addition to calcium, phosphorus also plays a significant role in bone formation. Excessive consumption, however, is harmful if we consume more phosphorus than calcium, the bone-building process is reversed, and much phosphorus extracts calcium. The correct ratio of phosphorus to calcium intake is 1: 1.5. White and dark phosphorus are flammable in air, insoluble in water. It has a toxic modification. Acute effects are nausea, vomiting, pain, diarrhea. The lethal dose in humans is  $LD_{100} = 60-100$  mg. Phosphorus (phosphate ion) is present in natural waters in very small amounts from the weathering of phosphate-containing rocks and from the bone tissue of vertebrates. The soil adsorbs phosphorus to a very large extent.

Appearance in water:  $PO_4^{3-}$ ,  $H_2PO_4^-$ ,  $HPO_4^{2-}$ , the latter two under neutral pH conditions are orthophosphates, which can be absorbed by plants. Forms insoluble compounds with cations in the neutral pH range, eg.  $Fe_3(PO_4^{3-})_2$ , which compounds may redissolve with changing pH.

Phosphorus cycle: the starting material is the orthophosphate ion dissolved in water, which naturally (decomposes rocks, - apatite, etc. -) or artificially (municipal wastewater, agricultural wastewater, detergent, detergent - oxidizes to orthophosphate) gets into surface waters without proper treatment.



(Source: <https://www.britannica.com/science/phosphorus-cycle>)

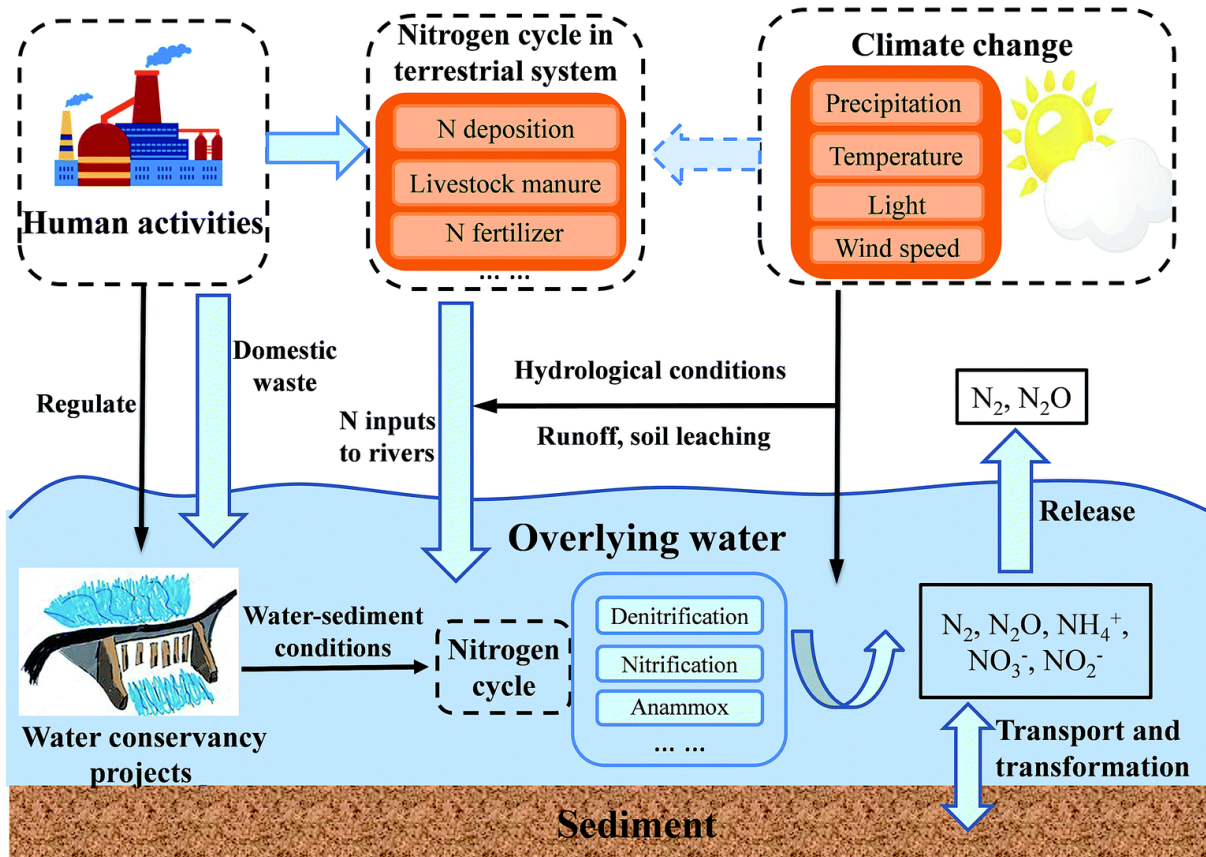
Causes eutrophication at higher concentrations in water. Up to a concentration of  $10 \text{ mg/m}^3$  there is no significant biological production, eutrophication can be prevented. Above  $20 \text{ mg/m}^3$  photosynthesis is accelerated in the presence of sufficient nutrients ( $\text{CO}_2$ ,  $\text{NO}_3$ ,  $\text{PO}_4^{3-}$ ,  $\rightarrow$  protoplasm). The nutrient uptake ratio of the ideal activity of algae is C: N: P = 106: 16: 1. Of the required nutrients, the amount of phosphorus can be controlled (precipitated from water), therefore it is a limiting factor. The presence of large amounts of nutrients in the water causes the rapid growth of algae, the deterioration of the smell, taste and beauty of the water, the reduction of the depth of penetration of sunlight into the water, and the destruction of algae reduces the dissolved oxygen content of the water, unpleasant gas content is produced due to anaerobic decomposition.

#### 4.5. Nitrogen

Nitrogen is one of the most important nutrients that the body requires. In the aquatic environment, 5 forms are typical:

- molecular, elemental nitrogen ( $\text{N}_2$ ) dissolved in water,
- ammonia as ammonium ion ( $\text{NH}_4^+$ ) and free ammonia ( $\text{NH}_3$ ) or ammonium hydroxide ( $\text{NH}_4\text{OH}$ ),

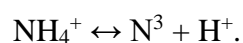
- nitrite, such as  $\text{NO}_2^-$  ion,
- in acidic waters such as nitric acid ( $\text{HNO}_3$ ),
- nitrate, exclusively as  $\text{NO}_3^-$  ion, organic nitrogen compounds, from proteins to simple compounds such as amino acids, urea, methylamines, etc., in dissolved or shaped form.



*The cycle of nitrogen in river systems: sources, transformation, and flux (Source: Environmental Science: Processes & Impacts (RSC Publishing) DOI:10.1039/C8EM00042E)*

Elemental nitrogen is released from the atmosphere or by denitrification of other nitrogen compounds in water, where it is dissolved. The extent of this is a function of temperature and partial pressure (Henry's Law).

Ammonia nitrogen is found in water through the decomposition of organic metabolites and dead organisms. The ammonia content of water characterizes the biodegradation of organic matter. Its appearance in water ( $\text{NH}_3$  or  $\text{NH}_4$ ) depends on the pH of the water. Ammonium ion in water is in equilibrium with ammonia and hydrogen ion:



If the pH of the water rises above 6, the equilibrium shifts to the right. In a strongly alkaline medium, ammonia is released and can be expelled from the water by air.

Free ammonia is a cell poison that crosses the cell membrane. The toxic effect depends on the dissolved O<sub>2</sub> and CO<sub>2</sub> content, the hardness and alkalinity of the water. Maximum permissible value in water for fish: 0.2 - 2 mg/l free NH<sub>3</sub>. As a result of the poisoning, bleeding occurs in the gill, the respiratory epithelium is destroyed and cramps appear in the muscles and floats.

Its detection in drinking water indicates communal contamination, with the exception of abandoned water bases.

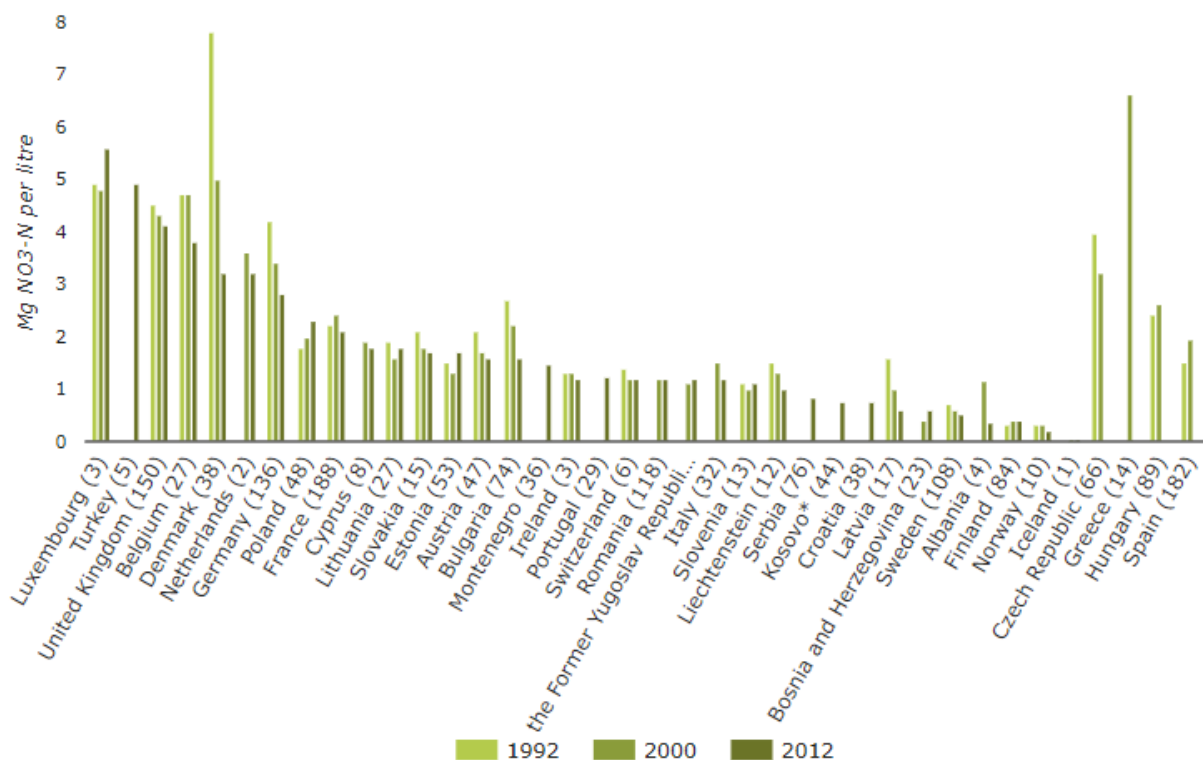
The effects of chlorination: the presence of chlorine and ammonium compounds leads to the formation of chloramines, which have an unpleasant odour and are carcinogenic.

Nitrite, nitrate, organic nitrogen: if ammonia enters the water and sufficient oxygen is present in addition to the appropriate microorganisms, ammonia is nitrified in the first layer (nitrite, nitrate formation).

In the absence of dissolved oxygen, nitrate ion is transformed into elemental nitrogen with the help of special bacteria (*Pseudomonas*, *Denitrobacillus*, *Micrococcus*). This biological process is denitrification. The relative proportions of nitrogen forms are important in determining water quality, and the purification process can be described.

Toxicology of nitrates and nitrites: Nitrate- and nitrite ions formed from them by bacterial reduction - enter the human body naturally through drinking water and food. Contamination of the surface, or near the surface, with fertilizer, urine, feces, or other organic substances can increase the nitrate (or nitrite) content of the waters to such an extent that the intake can multiply. Consumption of meat products also increases intake because nitrates are used in the industrial preservation of meat. Nitrates are directly and indirectly toxic (with the formation of nitrosamines).



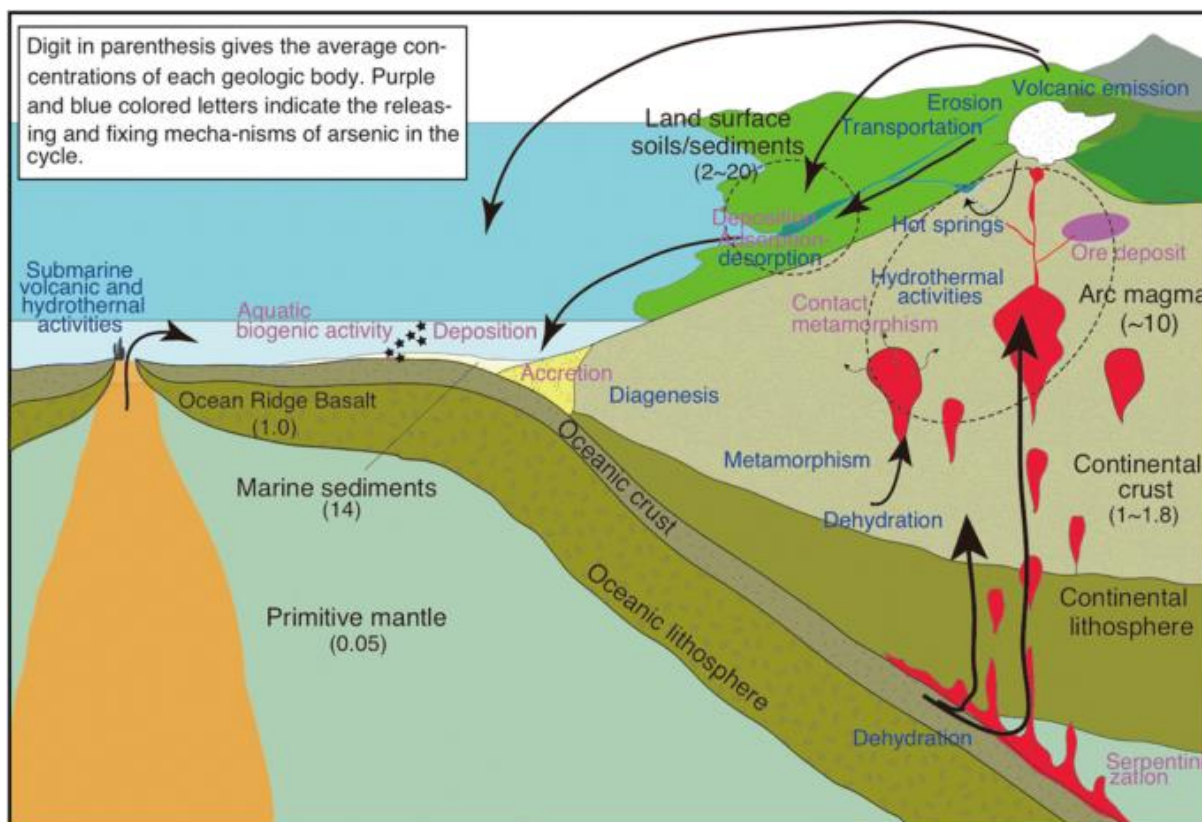


Average concentration of nitrate-nitrogen in rivers in 38 European countries (1992, 2000 and 2012)(Source: <https://www.eea.europa.eu/soer/2015/countries-comparison/freshwater>)

The average nitrate concentration in European rivers has reduced steadily over the period 1992 to 2012, a reduction of 0.5 mg NO<sub>3</sub>-N/l, or 0.03 mg NO<sub>3</sub>-N/l (0.8%) per year. Overall, there has been a decrease at 44% of stations and an increase at 13%. The countries with the highest proportion of stations with significant decreasing trends are Denmark and Germany. Denmark and Germany also had the largest annual decrease, along with Bulgaria and Latvia (<https://www.eea.europa.eu/soer/2015/countries-comparison/freshwater>).

#### 4.6. Arsenic

Arsenic is a semi-metallic element. It often occurs in groundwater and stratified waters even under natural conditions. It is not enriched in the human body in elemental form, but it is in some animals (e.g. rats). It helps the formation of blood cells in the human body, inhibits oxidation processes, influences metabolism.



*Arsenic cycling in the Earth's crust and hydrosphere: interaction between naturally occurring arsenic and human activities (Source: <https://progearthplanetsci.springeropen.com/articles/10.1186/s40645-018-0224-3>)*

In the environment, arsenic and its compounds are mobile and cannot be destroyed. However, interaction with oxygen or other molecules present in air, water, or soil, as well as with bacteria that live in soil or sediment can cause arsenic to change form, attach to different particles, or separate from these particles (Fergusson, 1990). Many common arsenic compounds can dissolve in water, thus arsenic can contaminate lakes, rivers, or underground water by dissolving in rain, snow, or through discarded industrial wastes. Therefore, arsenic contamination in ground water is a serious public health threat worldwide. In addition, the effect of chronic arsenic exposure from ingested arsenic-contaminated food and water or inhaled contaminated air has been investigated in various countries and found to be associated with detrimental health effects (Chung et al., 2014).

Among its compounds, arsenic trioxide is highly toxic, with an LD100 of about 100 mg/person. Death is preceded by severe stomach pain, sore throat, vomiting. Arsenic sulfide is moderately toxic, arsenic halides are highly toxic.

Due to the activity of microorganisms, As (V) is methylated in the soil. These compounds are less toxic.

In some areas of Hungary, the arsenic content of groundwater and drinking water sources is high. The permissible arsenic content of drinking water is 10 µg/l.

The WHO estimates that the human body contains 3-4 mg of arsenic. It is an essential element, but in large quantities it is a strong poison. In acute poisoning, the lethal dose is around 125 mg. Its toxic effects have been known since ancient times (political murders). Chronic poisoning is the result of accumulation in the body. In a small part, it is characterized by a slow elimination from the body (using urine, bile secretions). The critical organs are the liver, spleen, skin, hair, nails, while excretion is mainly through the kidneys. Accumulation in the body (hair, nails, skin) is more common in the case of increased arsenic intake, the arsenic content of hair and nails may be more mg/100g (compared to 50 µg/100g of normal). It causes discoloration of the limbs (poor blood supply), but it also leads to cancer (skin cancer, lung cancer, kidney and bone cancer).

Acute poisoning (severe diarrhea, heart failure, cessation of urinary excretion) is now rare. Chronic poisoning is more common, the most important symptoms of which are: excitatory symptoms of the skin and mucous membranes (conjunctivitis and dermatitis), discolouration in places exposed to light, keratinization of the skin of the hands and feet, anemia, cirrhosis of the liver, neuritis. After consuming waters with a high arsenic content, discoloration and keratinization of the skin of the foot are so characteristic that the symptom ensemble has been termed 'black foot disease'. A particularly serious consequence is skin cancer from the keratinized area. The suspicion of chronic poisoning is confirmed by an increase in the arsenic content of the hair and nails as well as an increase in the amount of arsenic excreted in the urine.

#### 4.7. Iron and manganese

Iron and manganese are regular constituents of natural waters. Their chemical form depends on the pH of the water as well as the oxygen content of the water. Occurs in groundwater in its divalent compounds (bicarbonates, sulfates and, in the case of iron, humates). They are not toxic substances, but they affect the use of water, they give it an unpleasant taste. When oxidized, they can precipitate out of the water, causing it to discolor and become cloudy. The content of 1 mg/l iron and 0.1 mg/l manganese in the pipeline and on the cooling surfaces is dangerous (deposits and blockages may occur) due to the growth of iron and manganese bacteria.

Iron is a constituent of blood and muscle protein, which play a very important role in oxygen transport. Lack of it causes bleeding, weakness, fatigue. Overdose can lead to metabolic disorders and muscle degeneration. Acute iron poisoning should only occur if a large amount of iron is ingested (overdose of an iron-containing medicine) as a symptom of gastritis, intravascular bleeding, or acid overload. No damage to health was observed in aquatic

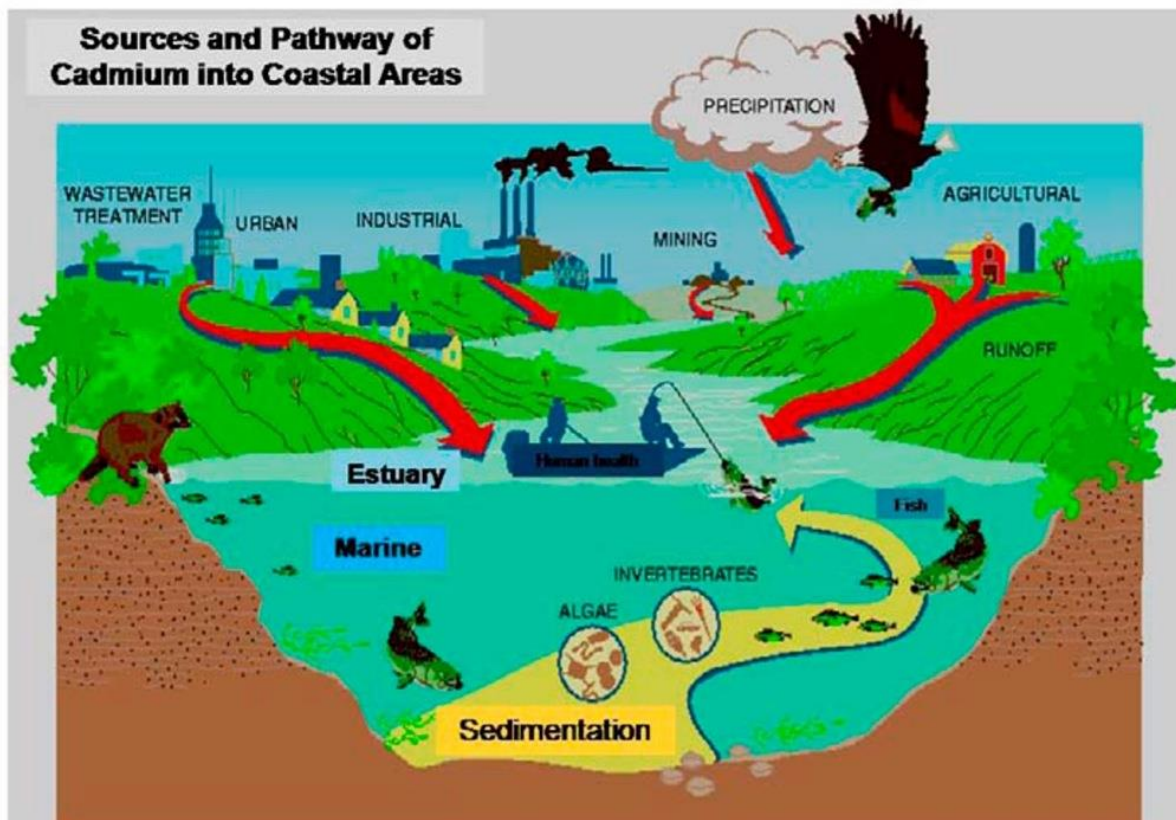
organisms. Contamination above 200 mg/l can be harmful to humans. The maximum concentration recommended by the WHO in drinking water is 100 µg/l.

Manganese promotes enzyme function. Lack of it causes metabolic and growth disorders. Its accumulation in the body is not significant, it accumulates only in a few muscles to a small extent. Its relatively large overdose does not cause any significant damage either. Chronic effects may include nervous system damage, speech, movement disorders. The recommended maximum concentration in drinking water is 0.05 mg/l.

#### 4.8. Cadmium

Cadmium is a member of the zinc group, a rare element in the earth's crust, occurring at an average concentration of 0.2 mg/kg. Cadmium also occurs in the environment under natural conditions. Cadmium occurs in nature as a natural component of rocks, sediments, soils and dusts, air, water, and plant and animal tissues, where it appears to cause no harm either to human beings or to the environment. Its geochemical behaviour is similar to that of zinc because of the similar electron structures and ionization potentials of the two elements (<http://what-when-how.com/mechanisms-of-cadmium-toxicity-to-various-trophic-saltwater-organisms/sources-and-pathways-of-cadmium-in-the-environment-part-1/>). In the air, the annual amount is estimated at 8000 t/year. Its approx. 5-10% comes from natural sources and the rest comes from human activities (metal industry, iron and steel industry, combustion processes).

It occurs as a solid metal in various types of waste and mine waste. The waters of rivers and lakes contain it in a natural concentration of 0.1-5 µg/dm<sup>3</sup>. It precipitates in water at pH > 9.5 and solid water may adsorb to the surface of contaminants. It is concentrated in the sediments of rivers, lakes and reservoirs (0.04 -0.8 mg/kg, but can be up to 30-400 mg/kg or more under human load).



*Different sources and pathway of cadmium into the aquatic environment (Source: <http://what-when-how.com/mechanisms-of-cadmium-toxicity-to-various-trophic-saltwater-organisms/sources-and-pathways-of-cadmium-in-the-environment-part-1/>)*

Cd in the food chain: Humans ingest higher amounts of cadmium through the food chain, but significant amounts are absorbed into the body through smoking as well as occupational hazards. The average cadmium intake is 20-30  $\mu\text{g/day/person}$  (WHO recommends that the daily cadmium intake should not exceed 1  $\text{g/kg}$  body weight), which value can be significantly higher due to lifestyle. This amount is approx. 80% of it enters the body through food and the rest through drinking water.

The rate of elimination from the body is significantly lower ( $\leq 1.18 \text{ mg/day}$ ). The absorbed cadmium leaves the lungs, stomach (intestines) by the blood to different parts of the body, i.e. the kidneys, liver, pancreas, muscle tissues, where it is stored. The biological half-life in the liver and kidney is greater than 10 years, which may lead to a high accumulation in the human body.

Cadmium can be harmful to all living organisms. Plants normally contain less than 0.5  $\text{mg/kg}$  of cadmium. Plant damage (changes in plant tissues) was observed from a cadmium content of 2.5  $\text{mg/kg}$ . Animals react differently to the cadmium load and its extent, depending on their habitat. Standing aquatic and marine animals react differently. Toxic Cd concentration is 1  $\mu\text{g/l}$  in freshwater animals and 7  $\mu\text{g/l}$  in marine animals. Necrosis was observed at concentrations of



2 and 100 µg/l, respectively. The degree of toxicity is alleviated by the increase in salinity, especially the Ca content, and the decrease in temperature.

To date, no cadmium infection has been observed in terrestrial animals that has been associated with toxicity. No signs of toxicity were observed in feeding experiments at a cadmium content of 30 mg/kg.

Acute effects in animals include lung, liver, kidney lesions, cardiological problems, skeletal damage. The symptoms of poisoning are also affected by the presence of other elements, the so-called a combination effect occurs. Eg: Se, Co decrease, Zn partially reduces, Pb enhances toxicity.

The effects of cadmium on humans are mostly based on the results of animal experiments. Acute toxicity (sudden, rapid infection) may occur by inhalation or orally as food.

#### 4.9. Lead

Lead as one part of heavy metals group pose a detrimental risk to human health and the environment due to its toxicity, even exposure to minuscule quantities can be life threatening. Levels of lead are not stable in the environment and vary according to industrial production, urbanization, climate changes and many other factors (Hynek et al., 2011).

Lead and its compounds can burden the environment in a variety of ways. Such opportunities include lead ore mining, metallurgy, processing, use of lead-containing materials, incineration, paper industry, chemical industry, petroleum industry, transportation, agricultural fertilization, waste management, etc.

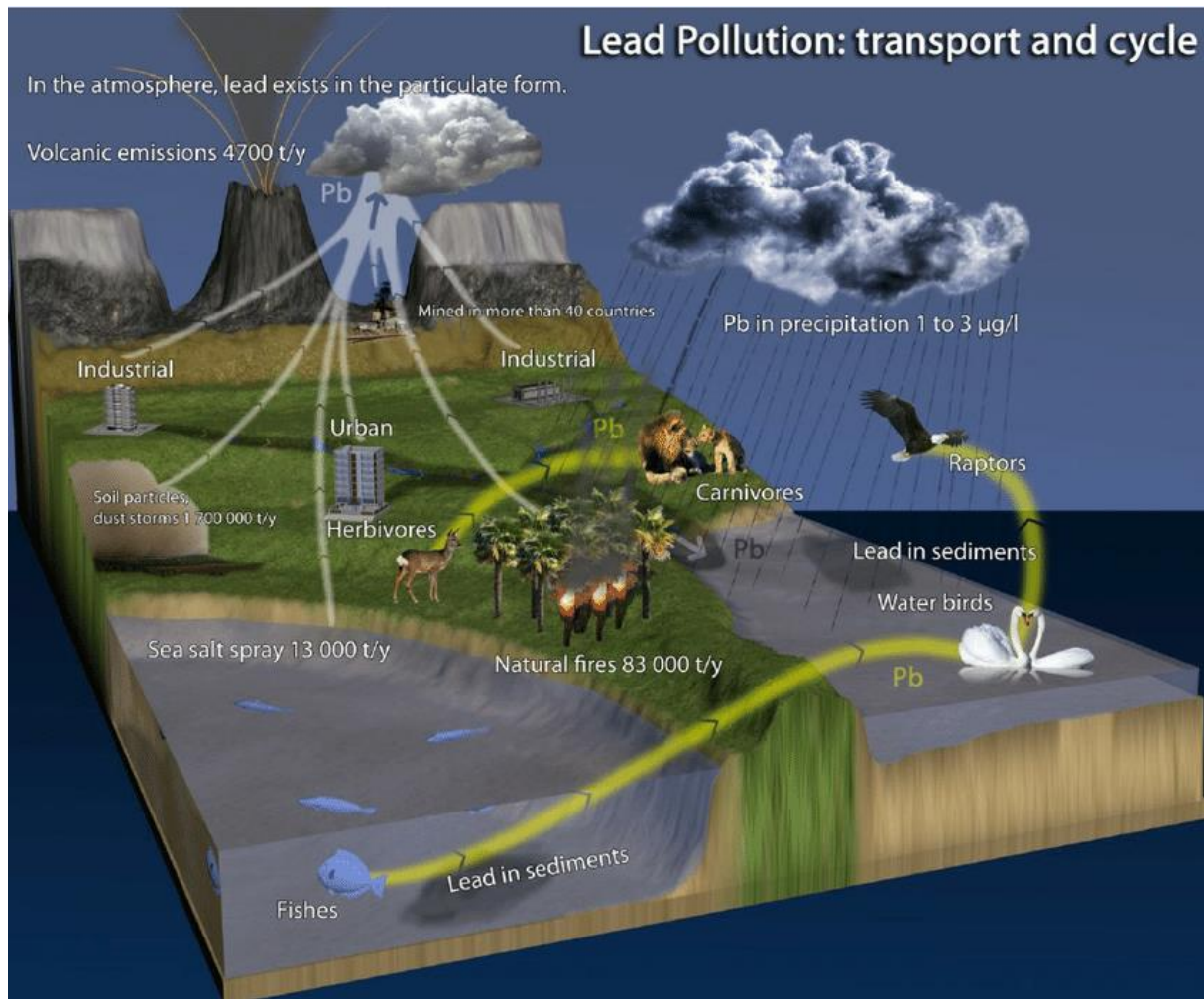
In nature, the amount of natural (dust, volcanic, forest fires) lead pollution is estimated at  $10^4$  t/year, while anthropogenic pollution (metallurgy, firing, transport, etc.) is estimated at  $10^5$  t/year. As a result, lead is found in all environmental factors.

Rivers are important transport media for heavy metals on a national and regional scale. The speciation of lead in the aquatic environment is influenced by many factors, such as: pH, salinity, sorption and biotransformation processes. Lead is typically present in acidic aquatic environments as  $PbSO_4$ ,  $PbCl_4$ , ionic lead, cationic forms of lead hydroxide and ordinary hydroxide  $Pb(OH)_2$ . Through these compounds, thousands of tonnes of lead come to seas annually (Hynek et al., 2011).

The lead content of the atmosphere is higher in large cities and lower in smaller areas with poorer traffic. The use of lead-free fuels significantly reduces this difference. In the latter case, the lead content of the atmosphere is presumably below  $0.1 \mu\text{g}/\text{m}^3$ . Concentrations of 2-200 mg/kg can be measured in the soil, while the lead content of groundwater depends on the lead



content of the soil, 1-60  $\mu\text{g}/\text{dm}^3$  depending on the pH of the water, while the lead content of surface waters depends on the area, what and how much treated wastewater is discharged (0.5-100  $\mu\text{g}/\text{dm}^3$ ).



*Lead pollution - transport and cycle (Source: Hynek et al., 2011)*

Lead is now a permanent participant in the food chain. The load of lead on the elements of the environment is so high that virtually all plant (groundwater, leaf dust deposited on leaves) and animal nutrients (feed) almost always contain lead in higher or higher concentrations.

The WHO recommends that the concentration of lead in drinking water should not exceed 100  $\mu\text{g}/\text{dm}^3$ . Ingested lead can be detected in different parts of the body (blood plasma, urine, hair, teeth, bones). The lead content in the blood is usually 5-30  $\mu\text{g}/\text{dm}^3$  (refers to relatively fresh lead uptake).

Based on the half-life, loaded organizations can be divided into three groups:

- rapid lead halving in the blood, lungs, liver, kidneys, brain, from the intestinal tract,
- has a medium half-life in the muscles, the lead content of the skin,

- has a long half-life (more than 10 years): stored in the bone (about 90% of the lead taken up is found here).

Lead absorbed by humans is excreted in the faeces and urine. It is stored only to a small extent in the hair and nails. Breast milk also contains very little lead.

Effect of lead: lead is not an essential element, it is toxic. In plants, it causes damage to enzymes and other proteins, which causes growth disturbance. In animals, the main symptoms are metabolic disorders, musculoskeletal disorders, visual disturbances - blindness, muscle weakness, weight loss, central nervous system disorder, kidney damage.

Acute illness is rare in humans, with chronic toxicity being more common. The effects of acute poisoning are gastrointestinal problems, acute kidney disease, and only in extreme cases can death.

Chronic effect of chronic disease symptoms:

- weakness, loss of appetite, fatigue, nervousness, headache, gastrointestinal complaints, impotence, etc.,
- weight gain, higher levels of lead in the blood and throughout the body,
- decrease in enzyme activity - blood and hematopoietic disorder (longevity decrease).

The long-term effect of a blood lead content of  $> 70 \mu\text{g}/100\text{ml}$  already causes irreversible renal damage. High long-term lead exposure also damages the heart and circulatory system. The effect of high lead load can also be premature birth.

The carcinogenic effect of lead poisoning has not been clearly demonstrated, but lead-containing As or Cr compounds (due to their arsenic or chromium content) are carcinogenic.

#### 4.10. Copper

Copper is present in the soil (in the crushed state, of which it is the least soluble at pH 5-6), in natural waters ( $<0.01 \text{ mg}/\text{dm}^3$  in fresh water, but ore in water can be significantly higher in concentration), and higher in air ( $80\text{-}90 \text{ mg}/\text{m}^3$ ).

Copper is commonly found in aquatic systems as a result of both natural and anthropogenic sources. Natural sources of copper in aquatic systems include geological deposits, volcanic activity, and weathering and erosion of rocks and soils. Anthropogenic sources of copper include mining activities, agriculture, metal and electrical manufacturing, sludge from publicly-owned treatment works, pesticide use and more. A major source of copper in the marine environment is antifouling paints, used as coatings for ship hulls, buoys, and underwater surfaces, and as a contaminant from decking, pilings and some marine structures that used chromated copper arsenate treated timbers.

Plants pick up and accumulate through the roots. (Aquatic plants can absorb many times as much as terrestrial plants (hundreds of mg / kg can be.) Fish can absorb larger amounts and it accumulates in the liver. Humans absorb some of the copper through food, it is absorbed through the stomach, and accumulates in the liver, kidneys and brain. According to WHO data, an adult needs a copper requirement of 0.03 mg/kg per day.

The effect of copper: an essential element, but it can be just as a dangerous substance as lead or mercury. Copper plays a role in pigment formation, is a component of enzymes required for respiration (plays a role in oxygen transport), is a catalyst for redox reactions in the body, and deficiency can lead to death. In humans, less than 2.5 mg of ingested copper deficiency causes disease, which is caused by malabsorption. In the case of the plant, the lack of copper (below 5 mg) causes a disorder of chlorophyll formation and a change in leaf colour. In the case of an animal, its absence causes a decrease in growth, the formation of pigment-poor hair, and dysfunction in a large number of organs.

Toxic effect: the copper taken in excess is toxic to the plant, many bacteria, viruses, animals, humans. In plants, it inhibits root growth and reduces enzyme activity. Toxic to many viruses and bacteria (pesticide, copper compounds).

Among fish, water with a copper content of more than 0.1 mg/l is already toxic (tolerable value 0.03-0.8 mg/l), and enzyme poisoning can also occur. In some animals, liver damage, changes in blood composition and haemolytic anemia may occur. It is less dangerous for more advanced animals with larger bodies.

Both acute and toxic effects may occur in humans. The acute effect has been recorded as many effects of dissolved copper. The first symptoms are diarrhea, heavy sweating. The effects of mg are those such as diarrhea, intestinal and gastric bleeding, anemia, haemolysis (dissolution of hemoglobin from the red blood cell), cell death, decreased urine output (excretion disorder), accumulation of N compounds in the blood. Ingestion of larger amounts may cause more severe poisoning (convulsions, coma, death). The chronic toxic effect on the eye is visual impairment, loss of vision. As a spray, it can easily enter the lungs where it is stored (this can lead to the formation of cysts, possibly tumors). Storage in the liver is associated with liver disease. The blood also circulates to the brain and can damage it in various ways.

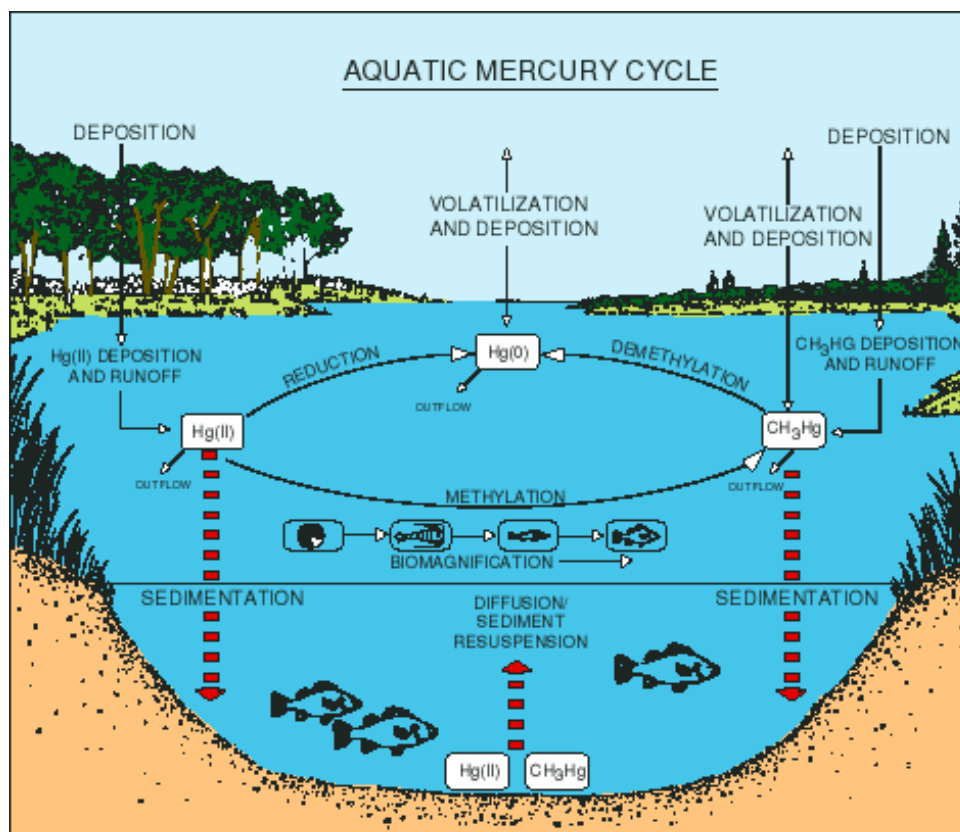
#### 4.11. Mercury

It occurs in the Earth's crust at an average concentration of 0.02 mg/kg, but there are minerals in which higher concentrations are possible (e.g. cinnabar). Relatively large amounts are released into the environment, soil, air and water from natural processes (intermediate decay)

and human activities (incineration, various industrial processes). They have been used in very different ways (in measuring instruments, batteries, switches, lamps, dental fillings, chlor-alkali electrolysis, paint production, catalyst PVC production, etc.), but its use after EU accession is limited and banned in many places.

Virtually insoluble in water, does not come into contact with oxygen in dry air. Evaporates at room temperature. In case of waste incineration and may result from mercury reactions, it primarily pollutes the air. May be chlorinated ( $\text{HgCl}$ ;  $\text{HgCl}_2$ ).  $\text{HgCl}$  is practically insoluble in water. Sublimated, on the other hand, is a strong poison.

Volcanic eruptions, transport, also sources of emissions. It can be washed out of the air by rainwater and get into the soil. Strong accumulation in the food chain is observed and toxic effects may occur.



A schematic drawing of mercury cycling in an aquatic ecosystem (Source: <https://wi.water.usgs.gov/mercury-lab/research/mercury-cycling.html>)

Adverse effects of mercury: it can be both acute and chronic, it is not an essential element. Its effect is mainly due to the appearance of ionic, dissolved forms in the body. A large percentage is absorbed through the nutrient channel, through the intestinal wall (e.g., 95% methylmercuric chloride), and when inhaled, it passes through the nasal mucosa into the brain within a short

time. Getting into the bloodstream into the blood cells it binds and enters various organs of the body, such as the liver (about half), the brain, kidneys, heart, muscle, hair. The half-life in humans is around 75 days.

Symptoms of acute effects:

- metallic taste in the mouth,
- headache, dizziness,
- mucosal stimulation,
- nausea, vomiting,
- stomach pain, diarrhea,
- kidney problems
- gingivitis,
- pneumonia,
- circulatory problems.

Symptoms of chronic poisoning may include:

- gingivitis,
- kidney damage
- cerebrovascular and central nervous system disorders (visual, hearing, psychiatric confusion),
- damage to the baby in the womb before birth,
- weight loss,
- hereditary anomaly (chromosomal aberration, abnormal chromosomal distribution).

## 5. Sample preparation and measurement possibilities of selected substances in the spotlight of EU directives

In recent years, monitoring of anthropogenic pollutants in surface water have been emphasized not only in media but also in legal activity in European Union. Hormones, antibiotics, pesticides, antidepressants, anti-epileptic drugs and analgesics come to spotlight with the EU Priority Substance Watch List (WL, 2018/840) of surface water (under Water Framework Directive, renew in 2018, Loos et al.). Monitoring of these substances in surface water is mandatory for each member state. The current composition of WL supplemented with some other chemicals shown in next table.

*European Union Priority Substances Watch List*

Group	Compound	pKa	logKow	Indicative analytical method	Maximum acceptable method LOD [ng/l]
Hormones	17 $\alpha$ -ethynylestradiol (EE2)		3.67	Large-volume SPE – LC-MS-MS (derivatisation)	0,035
Hormones	17-Beta-estradiol (E2)	10.46 $\pm$ 0.03	4.01	SPE - LC-MS-MS (derivatisation)	0,4
Hormones	estrone (E1)		3.13	SPE - LC-MS-MS	0,4
Macrolide antibiotics	erythromycin	8.88	3.06	SPE - LC-MS-MS	19
Macrolide antibiotics	clarithromycin	8.99	3.16	SPE - LC-MS-MS	19
Macrolide antibiotics	azithromycin	8.05	4.02	SPE - LC-MS-MS	19
Fluoroquinolone antibiotics	ciprofloxacin	6.09	0.28	SPE – LC-MS-MS	89
Aminopenicillin antibiotics	amoxicillin	pKa1 3.2 pKa2 11.7	0.87	SPE – LC-MS-MS	78
Neonicotinoids	imidacloprid	pKa1 1.56 pKa2 11.12	0.57	SPE - LC-MS-MS	8,3
Neonicotinoids	thiacloprid		1.26	SPE - LC-MS-MS	8,3
Neonicotinoids	thiamethoxam		-0.13	SPE - LC-MS-MS	8,3
Neonicotinoids	clothianidin			SPE - LC-MS-MS	8,3
Neonicotinoids	acetamiprid	0.7	0.80	SPE - LC-MS-MS	8,3
Insecticides	metaflumizone			LLE - LC-MS-MS or SPE – LC-MS-MS	65
Pesticide	methiocarb		2.92	SPE - LC-MS-MS or GC-MS	2
Analgesics	diclofenac	4.15	4.51	SPE - LC-MS-MS	0,5
Analgesics	naproxen	4.15	3.18	SPE - LC-MS-MS	0,1



Chemical properties determinates the fate of pollutants in the environment. If released to air, vapore pressure and Henry's constant influence their exist in the vapour and particulate phases in the ambient atmosphere. If released to water, solubility, stability in water and light (UV degradation on the water surface),  $K_d$  value (sludge/water distribution),  $pK_a$  value (acid dissociation constant) are decisive. Distribution of chemical agents between water and solid phase is a unique parameter of each pollutant. If released to soil,  $pK_a$  and  $K_{oc}$  (mobility in soil/sediment) values control the way of pollutants. Bioaccumulation is also an important property, which can be estimated from environmental half-life and  $K_{ow}$  (octanol/water distribution) of each anthropogenic agent. Numerous biological techniques have been developed as qualitative methods to assess the effects of chemical pollutants on the environment. Due to the low cost and improved sensitivity in the last 20 years have these methods been widely integrated into pollution-control programs. (Farré et al., 2005) Using biosensor technology encounter obstacles, like the relatively high development costs, the restricted operational conditions and limited lifetimes for some biorecognition components, the relative complexity of the assay format for many potentially portable systems, and the lack of validation and correlation studies (Sara Rodriguez-Mozaz et al 2006). In contrast, the biggest advantage of analytical techniques in pharmaceutical monitoring is the exact, quantitative measurement, robust methods, validation and existing correlation studies at most instrument. Implementation of standardized experimental toxicity tests (e.g. OECD tests on invertebrates and vertebrates) helps to perform environmental risk assessment with respect to individual chemicals. To estimate the harmful effect of chemicals on an ecosystem, a risk quotient (RQ) is usually applied, which is defined as the ratio of the maximum measured environmental concentration (MEC) to the predicted no effect concentrations (PNEC). Latter, PNEC depends on the available toxicological data (Molnár et al., 2020; Carlsson et al., 2006; Deo, 2014; Ferrari et al., 2004; Hernando et al., 2006; Komori et al., 2013). In general,  $RQ < 0.01$  denotes a negligible risk,  $RQ < 0.1$  reveals a low risk,  $0.1 < RQ < 1$  represents a medium risk, and  $RQ > 1$  indicates a high ecological risk to aquatic organisms (Guzel et al., 2019; Ma et al., 2016; EU Commission, 2003). So the main goal is to support legal decisions over anthropogenic pollutants with a responsible, sensitive and comparable assessment method, what based on chemical properties, standardized ecotoxicology test results, and a novel and reliable analytical technique. From the short summary above we must to strive measuring pollutants using the most sensitive analytical method, what needs suitable sample preparation. Sample preparation is a crucial step, and the used method is determined by the sample type, the target analyte, and the used analytical technique.

## 5.1. Traditional methods in water sampling

Currently, the most commonly used method for measuring levels of chemical pollutants is spot (bottle) sampling, followed by extraction and instrumental analysis. This methodology is well established and validated, so it has been accepted for regulatory and legislation purposes. However, this approach is only acceptable if it is representative of the chemical quality of water at a particular sampling site. We need to consider that spot samples are collected at a given location and time, and that the information obtained is unique to the place and the time selected. The solubility and stability of the analytes in aqueous media as well as other physicochemical properties (eg. density, surface tension, pH, redox potential) affected by the prevailing temperature sample. Larger temperature fluctuations can also have an effect sample integrity. These factors should be considered when selecting sample storage tanks and techniques. Similar behavior applies to other dissolved gases (CO<sub>2</sub>, NH<sub>3</sub>, H<sub>2</sub>S). Also organic materials you can escape the sample if you have enough free space.

## 5.2. Sample preparation and measurement possibilities

One of the main difficulties in sampling is preservation of the sample. The initial composition of the sample must be maintained from sampling through to analysis. If this is not the case, the final conclusions will not reflect the initial situation. For all of that, handling and storage of collected samples are of great importance during sampling.

When collecting surface water samples, special care have to be taken to ensure that the samples are not contaminated. That includes samples that should be stored in a safe place to exclude possible conditions change the properties of the pattern. Samples have to be sealed during this time long-term storage or transport. The collected samples are in the custody of the sampler or sample keeper until the samples are transferred to another party.

During sampling and storage the sample there are several problems that could appear:

- volatilization loss,
- decomposition (chemical reactions with external agents or sample containers walls).

There are several approaches have been applied to preserve sample integrity:

- protecting samples from external agents (using brown-glass containers),
- addition of preservatives,
- storage of the samples at low temperature (for trace-metal analyses, waters are typically stored at 4°C, while sediment and biota have to be frozen).

Storage at higher temperatures can enhance bacterial growth in solution and on the container surfaces, resulting in losses of components. Acidification of water samples will inhibit bacterial growth, but it is only recommended if total acid-soluble metals are being measured, because of the solubilization of particulate metals. Filtration is an important factor that needs to be accounted for during both sampling and subsequent sample-preparation steps.

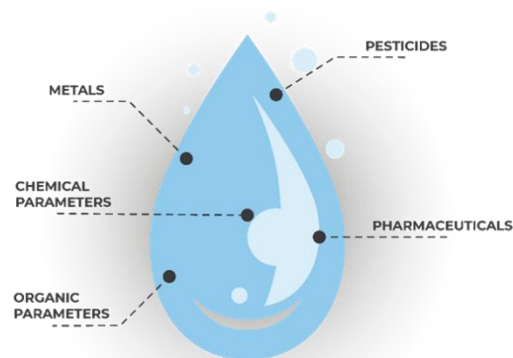
Of special importance in sampling is the nature of the sample containers. The material should be resistant to the preservative conditions and not interact with the analytes as that could lead to sample contamination or losses of analytes. The influence of the container increases as the concentration level decreases. There are several materials: glass, plastic (polyethylene could react with organic solvents and is not suitable for pesticides) and polytetrafluoroethylene (Teflon). The closure should safely seal the container, while remaining inert with respect to the contents. Both container and preservatives are going to depend on the type of analyte and the technique used for further analyses.

Sample preparation is one of the most critical steps in environmental analysis. In this step, the compounds of interest are separated from the matrix and are preconcentrated to improve the selectivity, sensitivity, reliability, accuracy, and reproducibility of the analysis. In recent years there are developed new sample pretreatment techniques. These techniques are faster and more selective and at the same time use lower amounts of solvents and reagents. The current trend in analytical chemistry is to consider the ideology of “green chemistry” and in this sense, “solvent minimised” or “solvent-free” sample preparation methods such as microextraction, membrane extraction and headspace techniques.

It is expected that, for priority metals, monitoring will focus on the dissolved fraction, while, for organic pollutants, the whole water should be considered. Filtration is usually performed with 0.45- $\mu\text{m}$  filter-pore size of different materials (e.g., glass fiber or cellulose acetate). Unless the membrane filter and filtration apparatus used for water samples is rigorously cleaned by soaking in dilute acid followed by distilled water, contamination can be a major problem. For ultratrace analyses, test filtrations of distilled water are recommended to ensure that no contamination is present. Concerning the water volume to be filtered, the effective filter-pore size can change during the filtration of large volumes, especially if there are appreciable amounts of suspended solids. Following filtration, appropriate water-sample-preservation techniques are required to prevent further losses or changes.

### 5.3. Selected components group

1. Metals
2. Chemical parameters
3. Organic components
4. Pharmaceuticals
5. Herbicides/pesticides
6. Biological parameters



*Selected components group in Mura monitoring*

*Sampling parameters and components in Mura River monitoring*

Chemical parameters	Metals	Organic components	Pesticides	Microbiology
<ul style="list-style-type: none"> <li>• <a href="#">water temperature (in field)</a></li> <li>• <a href="#">dissolved O<sub>2</sub> (in field)</a></li> <li>• <a href="#">turbidity</a></li> <li>• <a href="#">sulfate</a></li> <li>• <a href="#">cianide</a></li> <li>• boron</li> <li>• pH</li> <li>• <a href="#">conductivity</a></li> <li>• <a href="#">total P</a></li> <li>• <a href="#">Kjeldal N/ total N</a></li> <li>• <a href="#">nitrate</a></li> <li>• <a href="#">nitrite</a></li> <li>• <a href="#">ammonia</a></li> <li>• BOD</li> <li>• COD</li> <li>• TOC</li> <li>• CH</li> </ul>	<ul style="list-style-type: none"> <li>• <a href="#">iron</a></li> <li>• <a href="#">manganase</a></li> <li>• <a href="#">cadmium</a></li> <li>• <a href="#">copper</a></li> <li>• lead</li> <li>• <a href="#">mercury</a></li> <li>• <a href="#">arsenic</a></li> </ul>	<ul style="list-style-type: none"> <li>• PAH - <a href="#">total PAH</a></li> <li>• total PCBs</li> <li>• THM - <a href="#">total THM</a></li> </ul>	<ul style="list-style-type: none"> <li>• <a href="#">total pesticides</a></li> </ul>	<ul style="list-style-type: none"> <li>• <a href="#">Coliform</a></li> <li>• <a href="#">Escherichia coli</a></li> <li>• <a href="#">Enterococcus</a></li> <li>• <a href="#">Colony count 22°C</a></li> </ul>

#### 5.3.1. Metals — total and dissolved metals and metalloids

Heavy metals can enter the river system from both natural and anthropogenic sources and these are distributed in the water body and sediments. Many metals are very toxic for aquatic animals. They can also bioaccumulate through food chains and this has implications for human health as well as environmental health. Bioaccumulation is the ability of an organism to concentrate an element or a compound from food chain and water to a level higher than that of its environment. Bioaccumulation is the resultant process of many interactions within the compartments of the organisms. Metals uptake and their toxicity in aquatic fauna are influenced by many factors such as pH, hardness of water, alkalinity, temperature etc. Metals exist in a variety of states and their toxicity depends on its nature and chemical forms whether it is in

ionic form or in an oxidized or reduced state in combination with other organic substances and other metals.

Total metals can be analysed by digesting the sample using a concentrated nitric/hydrochloric acid added to an unfiltered water sample prior to analysis. Dissolved metals are determined by analysing those metals in a filtered sample that passes through a 0.45 µm membrane filter. Before analysis of a field-filtered, field-acidified sample, some extra dilute acid is added to the filtered sample, to ensure dissolution of any precipitates formed after filtration. The sample have not to be filtered when determining total metals (which include those metals bound to the particulate matter in the sample); otherwise, the same collection procedure is followed. Slightly different analysis techniques are also required if speciation is necessary to determine concentrations of ferrous iron [Fe (II)] and hexavalent chromium [Cr (VI)].

Metals commonly determined include: aluminium (Al), silver (Ag), arsenic (As), boron (B), barium (Ba), beryllium (Be), calcium (Ca), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), mercury (Hg), potassium (K), lithium (Li), magnesium (Mg), manganese (Mn), molybdenum (Mo), sodium (Na), nickel (Ni), lead (Pb), antimony (Sb), selenium (Se), tin (Sn), titanium (Ti), uranium (U), vanadium (V) and zinc (Zn).

### 5.3.2. Chemical parameters

#### 5.3.2.1. *Total nitrogen (TN)*

Total nitrogen includes all forms of nitrogen, such as (in order of decreasing oxidation state) nitrate, nitrite, ammonia and organic nitrogen. The concentration of nitrogen can be used to assess nutrient status in waterways. Enrichment by nitrogenous compounds may lead to related problems (such as nuisance or toxic algal blooms), although some waterways are naturally high in nitrogen and/or other key nutrients. Some sources of nitrogen enrichment may include fertilizers (in both rural and urban areas), animal wastes (e.g. from farms and feed lots), sewage, nitrogen fixing plants.

#### 5.3.2.2. *Total phosphorus (TP)*

Phosphorus occurs in natural waters and in wastewaters almost solely as phosphates. These are classified as orthophosphates ( $\text{PO}_4^{3-}$ ), condensed phosphates (pyro-, meta-, and other polyphosphates), and organically bound phosphates. They occur in solution, in particle or detritus, or in the bodies of aquatic organisms. Sources of phosphorus enrichment may include some detergents, fertilisers (in both rural and urban areas), animal faeces (e.g. from farms and

feed lots), sewage and some industrial wastes. High levels of phosphorus and other key nutrients may lead to related problems such as nuisance or toxic algal blooms, although some waterways are naturally eutrophic (nutrient enriched).

#### *5.3.2.3. Total oxidised nitrogen (NO<sub>x</sub>-N)*

Total oxidised nitrogen is the sum of the nitrate (NO<sub>3</sub><sup>-</sup>) and nitrite (NO<sub>2</sub><sup>-</sup>) expressed as concentrations in mg/L nitrogen. Additionally, the nitrate and nitrite species can be determined separately. Nitrite is an intermediate form of nitrogen and is generally short-lived as it is rapidly oxidised to nitrate. Nitrate is an essential plant nutrient and its levels in natural waterways are typically low (less than 1 mg/L). Excessive amounts of nitrate can cause water quality problems and accelerate eutrophication, altering the densities and types of aquatic plants found in affected waterways. Some bacteria mediate the conversion of nitrate into gaseous nitrogen through a process known as denitrification, and this can be a useful process reducing levels of nitrate in waterways.

#### *5.3.2.4. Nitrogen as ammonia/ammonium (NH<sub>3</sub>-N/NH<sub>4</sub>-N)*

Ammonia nitrogen and ammonium nitrogen species are determined using the same analytical method. Analytically they are the same species. Ammonia and ammonium exist in equilibrium in aqueous solution. In alkaline solutions the predominant species is ammonia (NH<sub>3</sub>), while ammonium (NH<sub>4</sub><sup>+</sup>) predominates at lower pH. During the analysis the pH is adjusted to alkaline, thereby converting almost all the ammonia to ammonium. Sources of ammonia include fertilizers and the mineralisation (decomposition) of organic matter.

#### *5.3.2.5. Total Kjeldahl nitrogen (TKN)*

Kjeldahl nitrogen is a term used to describe all dissolved nitrogen in the tri-negative oxidation state (e.g. ammonium, ammonia, urea, amines, amides, etc) and therefore comprises all the dissolved nitrogen except for some inorganic species (nitrite and nitrate) and organic compounds (azo- compounds, nitriles, oximes, etc). The Kjeldahl method hydrolyses all the amino nitrogen to ammonium, which is then measured by the ammonium/ammonia method. Assuming that the concentrations of many of the other nitrogen species are very low, the TKN concentration is therefore approximately equal to the TN concentration less the nitrite and nitrate concentrations. Or alternatively the TKN concentration is approximately equal to the sum of the total organic nitrogen and ammonia/ammonium as nitrogen concentrations. Many



analytical laboratories do not actually measure TKN using the Kjeldahl method (unless specifically requested); instead TKN (total) is calculated by subtracting nitrate and nitrite from total nitrogen (TN) on an unfiltered sample. The Kjeldahl determination is rarely used because it is not as precise as the persulphate digestion method used to calculate TN.

#### 5.3.2.6. *Total organic carbon (TOC)*

The total organic carbon (TOC) concentration represents all the carbon covalently bonded in organic molecules and so is not filtered. Total organic carbon does not take into account the oxidation state of the organic matter, and does not measure other organically bound elements, such as nitrogen and hydrogen, and inorganics that can contribute to the oxygen demand measured by biological oxygen demand (BOD). Drinking water TOC concentrations range from less than 100 µg/L to more than 25 mg/L. Wastewaters may contain very high levels of organic carbon (>100mg/L).

#### 5.3.2.7. *Biochemical oxygen demand (BOD)*

Biochemical oxygen demand is a measure of the amount of biologically and/or chemically degradable organic material that is present in the water. It indicates the amount of oxygen that aerobic aquatic organisms could potentially consume in the process of metabolising all the organic matter available to them. The consequence of high BOD is low levels of dissolved oxygen in affected waterways resulting in aquatic organisms becoming stressed and in extreme cases, suffocating and dying.

### 5.3.3. Organic components

#### 5.3.3.1. *Polycyclic aromatic hydrocarbons (PAHs) and Polychlorinated biphenyls (PCBs)*

Polycyclic aromatic hydrocarbons (PAHs) have multiple aromatic rings in their chemical structure. They are also referred to as polynuclear aromatic hydrocarbons. PAHs are found in coal tar, crude oil, creosote, and roofing tar, but a few are used in medicines or to make dyes, plastics, and pesticides. They can be formed during the incomplete burning of coal, oil and gas, garbage, or other organic substances like tobacco or char-broiled meat, and are typical components of asphalts, fuels, oils, and greases. A large number of artificial organic compounds have been found in wastewater and surface waters, such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and pharmaceuticals and personal care products

(PPCPs) (Nelson et al., 1998; Kolpin et al., 2002; Boyd et al., 2004; Chen et al., 2004). PAHs and PCBs are persistent organic pollutants with well-known toxicity and carcinogenic activity whereas PPCPs are regarded as emerging contaminants whose occurrence in the environment was not highlighted until the early 90s (Heberer, 2002). Quantitative evaluation of the environmental fate of these compounds requires efficient analytical methods. As the concentrations of PCBs, PAHs and PPCPs in water are typically very low (ng/l), a concentration step followed by a matrix exchange step before chromatographic determination must be included in the analytical procedure. One widely used extraction method is solid phase extraction (SPE).

#### 5.3.3.2. *Trihalomethanes (THM)*

Trihalomethanes (THM) are byproducts of disinfection the water that are formed when chlorine (or a chlorine based product) is used as a disinfectant. The THMs commonly found in water for human consumption are chloroform, bromoform, bromodichloro-methane (BDCM), dibromochloromethane (DBCM) usually being the main component. Many trihalomethanes are considered to be dangerous for health and suspected as carcinogens. The European Community Drinking Water Directive states that water used for human consumption should not exceed 100 µg/l of total THMs and US regulations state a maximum level of 80 µg/l of total THMs. The determination of THMs in water has mainly been carried out with gas chromatography (GC) followed by electron capture detection (ECD) or mass spectrometry detection (MSD). The concentrations of these compounds in natural and drinking waters is in the order of ng/l to g/l, such that as a general rule it is necessary to perform a preconcentration step of the analytes to achieve a level that can be measured by the analytical method chosen.

#### 5.3.3.3. *Pesticides and herbicides*

As a result of increasingly intensive agricultural activity, increasing amounts of toxic organic and inorganic compounds are released into the environment. It is one of the most commonly used pesticides today among the most dangerous pollutants. Their presence in the environment, in particular dangerous in water. For these reasons, it is essential to monitor pesticide residues in the environment using all available analytical methods. One of the basic ways of limiting the adverse effects of pesticides on human health is monitoring of these compounds. There are numerous monitoring studies, but in Hungary is still no regular monitoring of pesticide contents in waters. These days, they have come to the fore solutions that allow the determination of as many compounds as possible in a small amount of sample at low concentrations. The range of

applications of pesticides is continually expanding, hence their consumption is ever increasing and more of them are getting into the environment. It is estimated that EU countries consume more than 300 000 tons of pesticides per annum on crop protection alone.

The trend at present is to find pesticides that act only in accordance with their intended action, and do not harm humans, or other flora and fauna. Unfortunately absolute selectivity is impossible to achieve in practice. The factor determining whether a compound should be used or not, apart from its selectivity, is its rapid biodegradability, and this criterion applies not only to the pesticides themselves but also to their metabolic products.

Pesticides and herbicides are, by definition, toxic and this provides a potential risk to the ecosystem if natural waterways are polluted. The toxic action can be either direct; by killing similar organisms to that which they were designed to kill, or by bioaccumulation; rendering normal food sources for potential predators and consumers (e.g. fish, mussels and humans) unsafe for consumption. Pesticides and herbicides can be determined in water samples according to the requirements of the sampling program.

These commonly include:

- organochlorine pesticides,
- organophosphate pesticides,
- carbamate pesticides,
- triazine herbicides.

Organochlorines are the first important synthetic organic pesticides that belongs to the class of persistent organic pollutants (POPs). Organochlorine pesticides are highly persistent in the environmental, have high toxicity, bioaccumulative tendency, and induces chronic toxicities through long-term exposure even if their doses are relatively low (Loganathan, 2012).

Organophosphate pesticides are most commonly used in agriculture to control, such as alathion, parathion, profenofos chlorpyrifos, temephos, fenthione and diazinon used against pests. Organoposphate are normally esters, thiol esters, or acid anhydride derivatives of phosphorus containing acids. In humans these act on nervous system by inhibiting acetylcholine esterase enzyme at nerve endings by phosphorylation of the hydroxyl group in the active site of the enzyme (Sogorb & Vilanova, 2002).

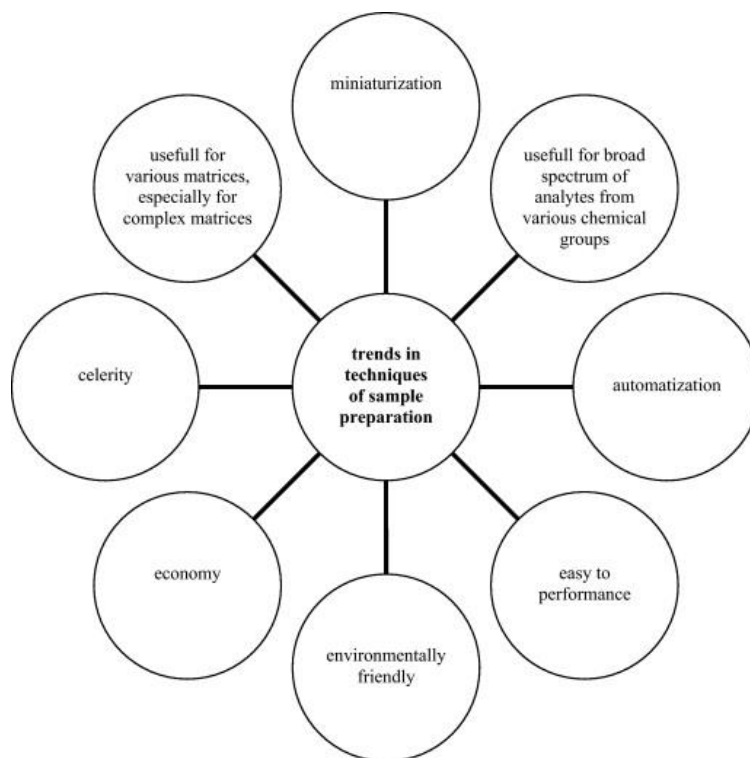
Carbamate pesticides are esters derived from carbamic acid. They are widely used as insecticides, herbicides, fungicides, and nematicides in homes, gardens, and agriculture. Their mode of action is inhibition of cholinesterase enzymes, affecting nerve impulse transmission in a similar fashion as organophosphate insecticides. Carbamates are less persistent than organochlorines and organophosphates. Carbaryl, methomyl, and carbofuran are commonly used carbamates (Urkude, 2019)

The term 'organonitrogen pesticides' usually refers to carbamates and triazines and their derivatives. Carbamates are among the most important chemicals used for protection against agricultural and household pests.

#### 5.3.3.3.1. Determination of pesticides (Maciej Tankiewicz et. al., 2011.)

The monitoring of pesticide residues in water is a matter of urgency. The choice of analytical methodology depends largely on the sample matrix (sample type) and the chemical structure of the target analytes. It also depends on EU and Health Ministers regulations regarding the maximum admissible level of a particular pesticide in water, which usually is 0.10 g/L.

The rapid development of new techniques in analytical chemistry (miniaturization, automation) has meant that the consumption of solvents in the analysis of pesticide residues would be very substantially reduced; very often the use of solvents would be eliminated at all if solvent-free techniques were applied. Proceeding in this direction we can see how extraction techniques have evolved from the classical liquid-liquid extraction (LLE), through liquid-phase microextraction (LPME), solid-phase extraction (SPE) to solvent-free techniques like stir bar sorptive extraction (SBSE) or solid phase microextraction (SPME). The main advantages of these techniques are minimalization of consumption harmful solvents, and typically, the high enrichment factor. The improved sensitivity makes it possible to minimize the amount of sample needed for the analysis. Ideally, sample preparation stage should be as simple as it possible, because it not only reduces the time required, but also decreases the possibility of introducing contaminants. The next figure presents trends in the development of techniques of sample preparation. At present it is common to combine sample preparation technique with chromatographic analysis (either off-line, at-line or sometimes even on-line) e.g. GC with SPE on-line and apply multi-residue methods.



*Trends in the development of techniques of sample preparation*

*Source: Maciej Tankiewicz et. al., 2011.*

The identification of compounds and their quantitative determination using suitable instrumentation. Which technique should be used depends on the properties of the pesticides under scrutiny. One particular method of determination is usually applicable to pesticides with similar properties. In most cases chromatographic techniques are used in combination with suitable detectors, specific to a given group of compounds. The most frequently used are:

- capillary gas chromatography (GC), pesticides determined by GC should be volatile and thermally stable;
- high-performance liquid chromatography (HPLC), usually in reversed-phase mode, for pesticides that cannot be determined by GC, e.g. polar and thermally labile compounds, such as herbicides, carbamates and triazines, and other compounds that require derivatization.

The most commonly used technique is gas chromatography (GC) – equipped with a suitable detector sensitive to the determined analytes (e.g. MS, NPD, ECD, FPD, TSD). Another useful technique for the determination of OPP + ONP is a high performance liquid chromatography (HPLC) – equipped with usually UV and DAD detector.

Nowadays, the trend is to develop analytical methods enabling a broad spectrum of analytes to be determined in a single analytical run (MRM – multiresidue methods). But the problem here is that the compounds to be determined simultaneously, often present at low concentrations, have different physicochemical properties depending on their chemical structure. Such a

methodology, apart from being able to determine a large number of compounds in one run, should:

- ensure maximum removal of interferents from extracts,
- give large recoveries of target compounds, high sensitivity and good precision,
- be environmentally friendly, i.e. require the smallest possible quantities of samples and chemical reagents, especially organic solvents,
- be cheap, quick and easy to carry out.

#### 5.3.4. Pharmaceuticals

Drug residue analysis in surface water has earlier been carried out by gas chromatography, usually in combination with mass spectrometric (MS) detection. Then capillary electrophoresis (CE) was used combined with mass spectrometry (MS) (Ahrer et al 2001), but as it can be seen in Table 1., nowadays, the indicative analytical method is liquid chromatography (LC) followed by MS most of anthropogenic pollutant measurement. In general, the same compound can be prepared in different way for measurement. Cost- and time-efficient when the used pretreatment and the chosen analytical method is multi-compound, selective, precise, and robust. The amount of effort to spend on developing a sample preparation protocol and the acceptable sample preparation cost per measurement can be a controversial topic between chemists who use LC-MS/MS.

Liquid chromatography coupled with MS is widely used in drug discovery and development. Mass spectrometric applications are being used in qualitative analysis, quantitative analysis, and preparative highperformance liquid chromatography (HPLC) analysis where the mass spectrometer serves as a detector to trigger fraction collection.

##### 5.3.4.1. *Liquid-Liquid Extraction Sample Preparation Protocol (Stone, 2017)*

In LLE, an immiscible organic solvent and an aqueous body fluid are mixed. Nonpolar compounds partition to the organic phase, leaving polar moieties in the aqueous phase. In the LLE workflow, samples are mixed with IS and a buffer. The immiscible organic solvent is added, samples are mixed vigorously to transfer analytes from sample to organic phase, and centrifuged to separate the layers. After centrifugation, the organic layer is transferred and evaporated to dryness. A reconstitution solution is added to the evaporated extract, the container is sealed and mixed to solubilize the analytes and provide an injection matrix compatible with the LC method. Advantages of LLE include low cost of materials and high selectivity, the



potential to concentrate analytes while performing extensive cleanup of matrix, including very effective removal of phospholipids. Disadvantages are the complexity of the process, slow throughput with manual LLE, high labor costs, a need for skilled labor, longer and more complex method development, and difficulty in automating with 96-well format. Additionally, as polar compounds are poorly extracted, metabolites may have low recovery or require glucuronide/sulphate hydrolysis prior to LLE.

#### 5.3.4.2. *Solid Phase Extraction Sample Preparation Protocol (SPE) (J. Stone, 2017)*

SPE, whether manual, automated on a liquid handler or online, is essentially a low resolution chromatographic process. Like LLE, SPE was in wide use for HPLC-UV and GC-MS methods prior to the advent of LC-MS/MS. There is abundant literature on SPE sample preparation for LC-MS/MS as well as application notes and extensive support from SPE media vendors. SPE chemistries for use with aqueous matrices are categorized as ion-exchange, reverse-phase, HILIC, or mixed-mode.

Reverse phase SPE is less selective than mixed-mode SPE or LLE and is primarily useful for removing salts and polar matrix components. Nonpolar wash solutions that would remove neutral interferences from reverse phase SPE will also wash analytes to waste, as the only retention mechanism is adsorption to the stationary reverse-phase.

In contrast, mixed-mode SPE becomes highly selective by including an anion or cation exchange moiety in the same bed with the reverse-phase component (nonpolar polymer or C18 bonded to silica). This dual functionality is a powerful tool for removing matrix, because charged analytes can be retained with the ion exchange moiety while matrix is removed from the reverse phase with nonpolar wash solutions.

A vacuum or positive pressure manifold for cartridges or plates is necessary to perform SPE. Positive pressure moves fluids through the SPE bed more reliably than does vacuum. SPE plates with a small bed mass and hold-up volume (e.g., Waters  $\mu$ Elution plate) can be eluted with < 0.5 mL of methanol or acetonitrile such that evaporation is optional. But most SPE protocols require evaporation of water immiscible organic elution solvents so the analytes can be reconstituted in a smaller volume of a solvent:water mixture that is compatible with reverse-phase LC. The first steps for SPE are to mix IS with sample and an application buffer at a pH that maximizes retention of analytes on the stationary phase. Classically, the SPE bed requires conditioning with methanol or acetonitrile to activate the stationary phase. Then the bed is equilibrated with aqueous application buffer.

Most vendors now offer SPE media with a polymer acting as both the structural support and as the nonpolar functionality of the stationary phase, an alternative to C18 bonded to silica.

Polymer SPE does not necessarily require conditioning. This can save both solvent and time. Comparing reproducibility and recovery with and without preconditioning is a good precaution. Another advantage of polymer based SPE is that drying of the bed does not adversely affect analyte retention, unlike silica-based SPE. The diluted sample is loaded onto the cartridge/plate, with attention to the flow rates recommended by the SPE vendor. Analytes adsorb to the stationary phase and the liquid sample flows to waste. In general, slower flow is better with SPE to allow sufficient time for equilibration throughout the bed and interaction with all retention mechanisms. One or more wash solutions to remove matrix and exogenous interferences are applied and eluted through the SPE bed. After washing, the SPE bed is dried with air or nitrogen to remove residual water and solvent. The waste container is replaced with a collection container and elution solvent is applied to flush analytes from the stationary phase into the collection vessel. The eluate is evaporated and a reconstitution solution is added, the containers are sealed, mixed, and introduced to the LC-MS/MS.

The chief advantages of SPE are the capability to concentrate analytes and remove matrix, although SPE that is not specifically designed to remove phospholipids from serum can be less selective than LLE or SLE in this regard. SPE is relatively easy to automate. Disadvantages include cost, complexity of method development and production process, and the longer time required compared to DIL, PPT, PLR, or SLE. SPE has some degree of parallel processing and is often less technically demanding than LLE, so handling large numbers of samples may be easier with SPE than LLE.

#### 5.3.5. Microbiological analyses

For example, total plate count, total coliforms, faecal coliforms (or thermotolerant coliforms), *E. coli* (*Escherichia coli*), *Enterococci*). The sterilized sample bottle should be kept closed until it is ready to be filled. The tank cap should be carefully removed to prevent contamination of the inner surface. The sample should be taken without rinsing by direct collection into the sample bottle.

Application of sampling and analytical method and sample pretreatment in Mura monitoring

Components group	Components	Number of sample	Sampling method	Analytical method	Sample pretreatment	Sample volume	Sampling container	Arrival at the laboratory	Remark
Metals	Iron	100	MSZ 1484-3:2006	Flame atomization (FAAS)	Cooling, 1:1 nitric acid solution (1ml)	100ml	100ml plastic bottle	Within 24 hours of sampling	Take sample without rinsing
	Manganase	100	MSZ 1484-3:2006	Flame atomization (FAAS)	Cooling, 1:1 nitric acid solution (1ml)	100ml	100ml plastic bottle	Within 24 hours of sampling	Take sample without rinsing
	Cadmium	100	MSZ 1484-3:2006	Flame atomization (FAAS)	Cooling, 1:1 nitric acid solution (1ml)	100ml	100ml plastic bottle	Within 24 hours of sampling	Take sample without rinsing
	Copper	100	MSZ 1484-3:2006	Flame atomization (FAAS)	Cooling, 1:1 nitric acid solution (1ml)	100ml	100ml plastic bottle	Within 24 hours of sampling	Take sample without rinsing
	Lead	100	MSZ 1484-3:2006	Flame atomization (FAAS)	Cooling, 1:1 nitric acid solution (1ml)	100ml	100ml plastic bottle	Within 24 hours of sampling	Take sample without rinsing
	Mercury	100	MSZ EN 13506:2002	Cold vapor determining (ETA-AFS)	Cooling, Potassium dichromate nitric acid solution(1%)	200ml	200ml glas or PTFE container	Within 24 hours of sampling	Take sample without rinsing
	Arsenic	100	MSZ 1484-3:2006	Electrothermal atomisation (ETA-AAS)	Cooling, 1:1 nitric acid solution (1ml)	100ml	100ml plastic bottle	Within 24 hours of sampling	Take sample without rinsing
Organic parameters	Bensol	235	MSZ 1484-4:1998	HSS-GC-MS	Cooling, bubble-free sampling, 5mg Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> /vial	2*40ml	40ml EPA VIAL glass	Within 24 hours of sampling	Take sample without rinsing
	THM	235	MSZ 1484-5:1998	HSS-GC-MS				Within 24 hours of sampling	Take sample without rinsing
	Hexabromocyclododecane	100	No specific criterion	HPLC-MS	SPE methode	2x1liter	2x1 liter brown glass	Within 3 days of sampling	Fill the sampling container to 90% with the sample and take sample without rinsing
	Benz(a)pirene+PAH	252	MSZ 1484-6:2003	GC-MS(SIM)	Cooling (2-8°C), Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub>	1liter	1 liter brown glass	Within 5 days of sampling	Fill the sampling container to 90% with the sample and take sample without rinsing
Pesticides + total pesticides	PCB	252	MSZ 1484-11:2003	GC-MS(SIM)	Cooling (2-8°C), Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub>	1liter+40ml	1 literes brown glass + 40ml EPA vial (volatile chlorobenzenes)	Within 5 days of sampling	Fill the sampling container to 90% with the sample and take sample without rinsing
		100	Validated method	GC-MS HPLC-MS	Cooling (2-8°C), Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub>	1liter +40ml+50ml	1 liter brown glas (GC-MS measure); 40ml EPA vial (HPLC-MS measure); 50ml centrifuge tube (Glyphosate + AMPA)	Within 3 days of sampling	Fill the sampling container to 90% with the sample and take sample without rinsing
Chemical parameters	Boron	252	HRN EN ISO 5667-6	ICP-OES	acidify onsite using 4 ml 12,5% HNO <sub>3</sub>	100 ml	HDPE plastic container	Within 24 hours of sampling	Take sample without rinsing
	Dissolved O <sub>2</sub> (laboratory)	252	HRN EN ISO 5667-6	HACH HQ440d-Multi-parameter, LDO101 probe	Cooling	1 liter	Winkler, glass stopper	Within 24 hours of sampling	Take sample without rinsing
	Turbidity	252	HRN EN ISO 5667-6	Portable Turbidimeter 2100Qis	Cooling	1 liter	1 liter glass bottle	Within 24 hours of sampling	
	Sulfate	252	HRN EN ISO 5667-6	IC	Cooling	1 liter	1 liter glass bottle	Within 24 hours of sampling	
	Cyanide	252	HRN EN ISO 5667-6	Spectrometer	Cooling	1 liter	1 liter glass bottle	Within 24 hours of sampling	
	pH	252	HRN EN ISO 5667-6	WTW Multimeter 740, pH-electrode SenTix 81	Cooling	1 liter	1 liter glass bottle	Within 24 hours of sampling	
	Conductivity	252	HRN EN ISO 5667-6	WTW Multimeter 740, TetraCon 325 electrode	Cooling	1 liter	1 liter glass bottle	Within 24 hours of sampling	
	Total P	252	HRN EN ISO 5667-6	Spectrophotometer, Hach, DR 6000	Cooling	1 liter	1 liter glass bottle	Within 24 hours of sampling	
	Kjeldal N/ total N	252	HRN EN ISO 5667-6	Spectrophotometer, Hach, DR 6000	Cooling	1 liter	1 liter glass bottle	Within 24 hours of sampling	
	NO <sub>3</sub>	252	HRN EN ISO 5667-6	Spectrophotometer, Hach, DR 6000	Cooling	1 liter	1 liter glass bottle	Within 24 hours of sampling	
	NO <sub>2</sub>	252	HRN EN ISO 5667-6	Spectrophotometer, Hach, DR 6000	Cooling	1 liter	1 liter glass bottle	Within 24 hours of sampling	
	NH <sub>3</sub>	252	HRN EN ISO 5667-6	Spectrophotometer, Hach, DR 6000	Cooling	1 liter	1 liter glass bottle	Within 24 hours of sampling	
	BOD	252	HRN EN ISO 5667-6	WTW OxiTop - Manometric BOD Measuring Devices, HACH - BOD-System	Cooling	1 liter	1 liter glass bottle	Within 24 hours of sampling	
	COD	252	HRN EN ISO 5667-6	Spectrophotometer, Hach, DR 6000	Cooling	1 liter	1 liter glass bottle	Within 24 hours of sampling	
	TOC	252	HRN EN ISO 5667-6	TOC analyser	Cooling	1 liter	1 liter glass bottle	Within 24 hours of sampling	
CH	252	HRN EN ISO 5667-6		Cooling	1 liter	1 liter glass bottle	Within 24 hours of sampling		
Pharmaceuticals		252	Validated method	UHPLC-MS/MS	Cooling, acidify onsite using 2 ml, 100% HCOOH	2 liter	2.5 liter, darkened, borosilicate glass with Teflon-faced cap	Within 24 hours of sampling	Fill the sampling container with 2 liter
Biological parameters		252	Validated method	Microbiological methode	Cooling	0.5 liter	0.5 liter, sterile glass bottle	Within 24 hours of sampling	Sterile sampling

## 6. Sampling plan of surface water – method and locations

Streams and rivers are the major arteries transporting the earth's freshwater. Many of the world's great cities are located along large streams and rivers, and these waters serve many important functions including habitat for fish and other aquatic life, nutrient transport, drinking water, industrial water supply, irrigation, transportation, power generation and recreation.

Streams and rivers are sensitive ecosystems that are vulnerable to pollution, overuse and climate change causing increased frequency of droughts and flooding. Monitoring these valuable resources is essential to understanding how they are changing and how we can best conserve them.

- Streams and rivers naturally transport nutrients and other solids, but increased loads from agricultural and stormwater runoff can cause harmful algal blooms.
- Discharges from industrial and power generation facilities can introduce pollutants and cause rapid temperature changes that harm ecosystems.
- Overuse for agriculture and other purposes combined with changing weather patterns is causing scarcity in some watersheds.
- Rivers used extensively for transportation may flood, have insufficient water depth or be polluted by the vessels traveling on them.
- Power generation facilities change physical characteristics of streams and rivers and can alter temperature, dissolved oxygen levels and overall water quality (<https://www.nexsens.com/systems/stream-river-monitoring>).

Pollutants transported in rivers can cross regional and national borders and eventually reach the ocean where they can spread uncontrolled. One of the most important pieces of environmental legislation produced in recent years is the Water Framework Directive (WFD) of the European Union. Directive 2000/60 / EC of the European Parliament and of the Council aims to include a framework for the protection of inland surface waters, transitional waters, coastal waters and groundwater.

The aim of the Directive is to increase the protection and improvement of the aquatic environment to progressively reduce emissions and losses of priority substances and to eliminate emissions and losses of priority hazardous substances.

Two decades of EU water policy and law have made a change. The trend of continuous decline of water quality has been reversed. Water quality throughout Europe has improved. Compliance with the Water Framework Directive objectives is increasing gradually. Although in a number of Member States the right policy measures were taken and a number of financial investments made, in many river basins improvements in water quality will still take some time. While a

large majority of groundwater bodies have achieved good status, less than half of surface water bodies are in good status, although some individual underlying trends are more positive. Various EU funds will continue to support these implementation efforts. The path towards full compliance with the objectives of EU water legislation before the end of the third cycle (in 2027) is now very challenging. Reporting shows that, although further measures will be taken until 2021, many others will be needed beyond 2021 (<https://www.ecologic.eu/16371>).

## 6.1. Traditional methods and new approaches in water sampling strategy

### 6.1.1. Sampling and monitoring

Sampling could be defined as a process of selecting a portion of material small enough in volume to be transported conveniently and handled in the laboratory, while still accurately representing the part of the environment sampled. The main difficulties in sampling are representativeness and integrity. Many people think that the analysis starts when the sample arrives in the laboratory. However, sampling is an integral part of the analytical process, so analysis starts with sampling. Sampling is so important that in some cases it represents the main contribution to the error of the whole analytical process, especially when trace contamination are measured. The relative error, as well as the absolute possible error due to sampling, sampling preparation and instrumentation analysis, differs from matrix to matrix and it depends greatly on the range of concentration of analytes. In general, the possible error of instrumental analysis is relatively low.

The development of a sampling plan should always begin with the determination of the purpose of the measurement. As different stages of the workflow are the responsibility of different people, good communication is needed between all parties involved. Sampling planners and analytical scientists need to optimize the entire measurement process (including the sampling step).

After the purpose of the analysis has been determined, a sampling plan should be developed to achieve the purpose. This plan should be used as a protocol (Standard Operating Procedure, SOP) that includes the following aspects:

- exact location and time of sampling,
- sampling equipment,
- sample containers,
- sample containers, including preservative addition and storage,
- sample handling procedures (treatment before measurements),

- sampling procedures,
- sample record (eg. labeling, record information, additional information and chain of custody requirements).

The sampling plan have to be written before the field sampling. Sampling location is important in water-sampling strategy. The sampling site should represent the environment under study. The optimal selection of sampling sites is related to the objective of the program (e.g., whether it is trend detection, regulatory enforcement, or estimation of pollutant loadings).

Representative sampling is one of the most important factors in sampling design. Hydromorphological and hydrological conditions and intermittent chemical releases associated with industrial or urban wastewater effluents, bed-sediment re-suspension and diffuse pollution (e.g., run-off from periodic application of pesticides to agricultural land) lead to spatiotemporal variations in the physico-chemical characteristics of water. Sampling frequency is therefore an important factor in terms of representativeness. Low sampling frequency could underestimate the occasional presence of samples with high analyte concentration. Sampling frequency is subject to influence (e.g., by transport, access to the sampling site, the availability of test organisms, and financial constraints).

In the case of surface waters, samples are often taken by filling the sample bottle directly. For deeper water layers below 0.5 m, these methods no longer work, so designated water samplers are used. In the open state, they are lowered on a rope or steel cable and the closing is initiated remotely. The third option is to use pumps (for example, peristaltic pumps offer the ability to collect larger amounts of water and can be used in conjunction with in-line filtration to avoid contamination (air dust) in the area). For most sampling operations, the measurements are performed on site, possibly even in situ. This is necessary because many parameters (e.g., pH, temperature, and dissolved oxygen) cannot be properly analyzed after delivery to the laboratory. Portable instruments must be properly cleaned and calibrated before measurements begin.

Based on the proposal of Directive and the relevant legislation we created a detailed sampling of water sampling and components analysis. In Hungary, the following standards are available: MSZ EN ISO 5667-3:2018 (Water quality, sampling, preservation and handling of water samples), MSZ EN ISO 5667-1:2007 (sampling techniques), MSZ EN ISO 5667-6:2017 (sampling from rivers and streams).

## 6.2. Objective of Mura monitoring

Streams and rivers offer an above ground glimpse at the health and hydrology of a watershed, and function as a vital resource for human activity, as well as habitat for a host of non-human



animals and plants. Contamination from any foreign source can spell trouble for streams and rivers, but sources that unbalance nutrient levels in the water often have especially far-reaching effects. The most significant aim of this project was 1) to develop a comprehensive sampling plan that takes into account the most important pollutant sources, in addition 2) to set up a comprehensive monitoring network of Mura River (focused on water quality).

By design of the Mura River monitoring, our goal was to monitor the chemical status of surface and groundwater and mapping the examination of the impact of effluent discharges or accidental pollution on the recipient under one year period.

### 6.3. Selection of monitoring points

The primary consideration in designating the surface sampling points was the location of the wastewater treatment plant. When designating the sampling points, we took into account the potential vulnerabilities arising from human interventions and activities on both the Croatian and Hungarian sides. In the examined section (approx. 50 km), sampling points were designated in connection with 7 settlements, mainly in connection with the points of discharge of treated wastewater from a given settlement into the receiver. Three points were designated at each site (under the influence of wastewater, in front of and after it). An exception to this was the Letenye point, where the treated wastewater is not introduced in surface water, but through a discharge pipe. At 14 points, sampling takes place directly from the line of the Mura River.

*The sampling points of surface and underground waters*

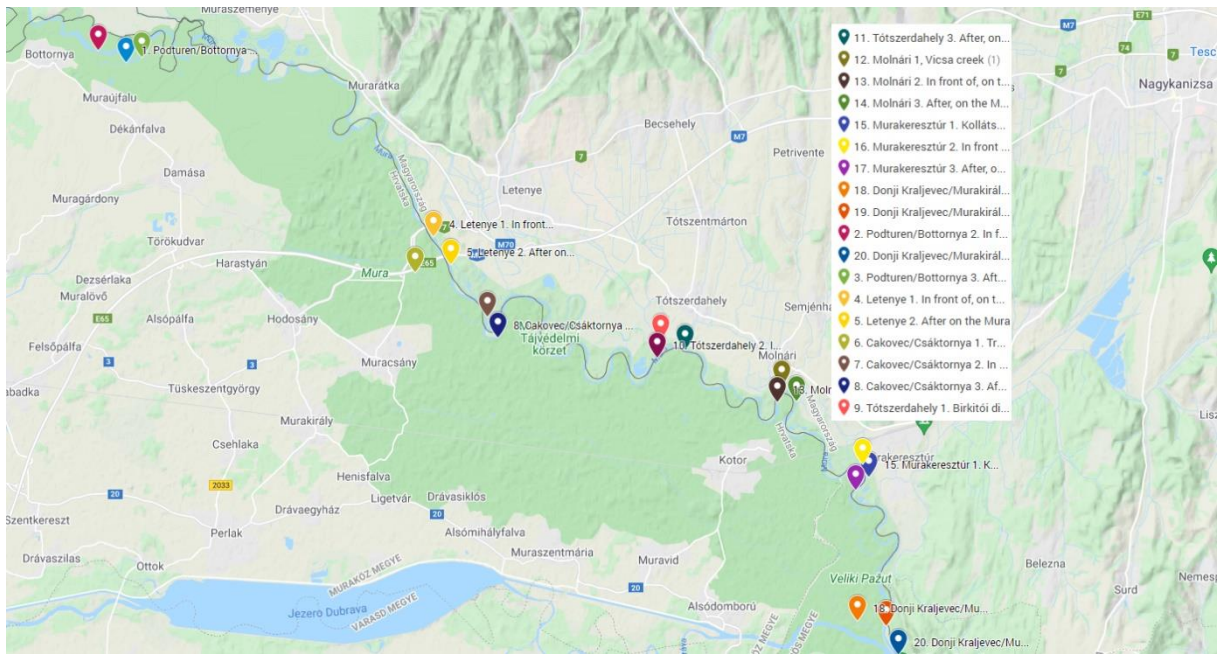
WWTPs from northwest to southeast along the Mura River			Sampling points		
			In front of the inflow point into the Mura River	In front of the inflow point on the Mura River	After the inflow point on the Mura River
A	Croatia	Bottornya / <b>Podturen</b>	1.	2.	3.
B	Hungary	<b>Letenye</b> / Letinja	-	4.	5.
C	Croatia	Csáktornya / <b>Čakovec</b>	6.	7.	8.
D	Hungary	<b>Tótszerdahely</b> / Serdahelj	9.	10.	11.
E	Hungary	<b>Molnári</b> / Mlinarci	12.	13.	14.
F	Hungary	<b>Murakeresztúr</b> / Kerestur	15.	16.	17.
G	Croatia	Murakirály / <b>Donji Kraljevec</b>	18.	19.	20.

Monitoring wells in the nearby environment of the Mura River			Sampling points
H	Croatia	Drávavásárhely / <b>Nedelisce</b>	21.
I	Croatia	Perlak / <b>Prelog</b>	22.
J	Hungary	<b>Letenye</b> / Letinja	23.
K	Hungary	<b>Molnári</b> / Mlinarci	24.
L	Hungary	<b>Murakeresztúr</b> / Kerestur	25.

Sites of surface water sampling					
No.	Sampling point	GPS coordinates	Name	Frequency	Total sampling
1.	Bottornya/Podturen 1	46.46465 16.57511	Rasnic creek	quarterly	4/year
2.	Bottornya/Podturen 2	46.46804 16.56325	Mura	quarterly	4/year
3.	Bottornya/Podturen 3	46.46597 16.58141	Mura	quarterly	4/year
4.	Letenye/Letinja 1	46.41733 16.69656	Mura	monthly	12/year
5.	Letenye/Letinja 2	46.40965 16.70327	Mura	monthly	12/year
6.	Csáktornya/Čakovec 1	46.40763 16.68927	Trnava creek	monthly	12/year
7.	Csáktornya/Čakovec 2	46.39543 16.71777	Mura	monthly	12/year
8.	Csáktornya/Čakovec 3	46.38992 16.72178	Mura	monthly	12/year
9.	Tótszerdahely/Serdahelj 1	46.38915 16.78608	Birkitói-ditch	monthly	12/year
10.	Tótszerdahely/Serdahelj 2	46.38437 16.78487	Mura	monthly	12/year
11.	Tótszerdahely/Serdahelj 3	46.38654 16.79566	Mura	monthly	12/year
12.	Molnári/Mlinarci 1	46.37765 16.83183	Vicsa-creek	monthly	12/year
13.	Molnári/Mlinarci 2	46.37235 16.83215	Mura	monthly	12/year
14.	Molnári/Mlinarci 3	46.37254 16.83975	Mura	monthly	12/year
15.	Murakeresztúr/Kerestur 1	46.34806 16.87252	Kollátszegi railway ditch	monthly	12/year
16.	Murakeresztúr/Kerestur 2	46.35555 16.86568	Mura	monthly	12/year
17.	Murakeresztúr/Kerestur 3	46.34834 16.86289	Mura	monthly	12/year
18.	Murakirály/Donji Kraljevec 1	46.31272 16.8637	Bistrec creek	monthly	12/year
19.	Murakirály/Donji Kraljevec 2	46.31128 16.8754	Mura	monthly	12/year
20.	Murakirály/Donji Kraljevec 3	46.30358 16.87983	Mura	monthly	12/year

Sites of underground water sampling				
No.	Sampling point	GPS coordinates	Frequency	Total sampling
21.	Drávavásárhely/ Nedelisce No.1	46.36595 16.36178	quarterly	4/year
22.	Perlak/Prelog No.1	46.34826 16.62232	quarterly	4/year
23.	Letenye/Letinja K58 (5F)	46.43935 16.68835	quarterly	4/year
24.	Molnári/Mlinarci K34 (13F)	46.36846 16.84406	quarterly	4/year
25.	Murakeresztúr/Kerestur (1F)	46.35247 16.87218	quarterly	4/year

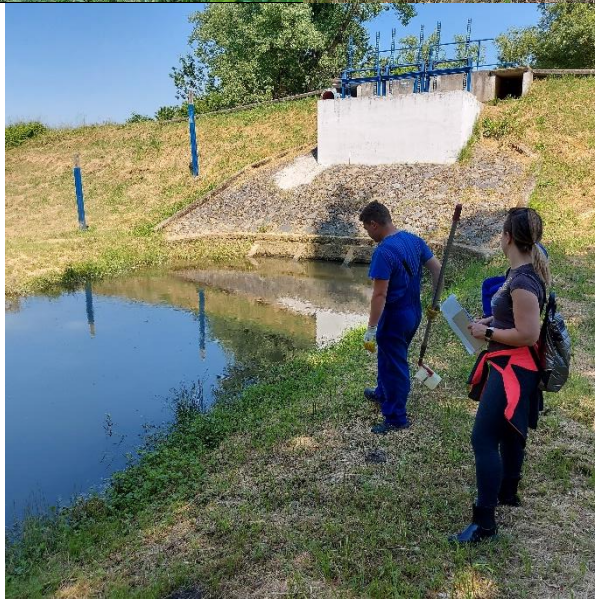






*Sampling from the line of the Mura River (Photos were taken by Nikoletta Méhes)*





*Sampling from the inflows and wells at the Mura River (Photos were taken by Tamás Kucserka)*



#### 6.4. Monitoring frequency

According to the EU Directive, monitoring frequencies shall be selected which take account of the variability in parameters resulting from both natural and anthropogenic conditions. The times at which monitoring is undertaken shall be selected so as to minimise the impact of seasonal variation on the results, and thus ensure that the results reflect changes in the water body as a result of changes due to anthropogenic pressure.”

Measurement almost always involves the sampling process, so sampling is an important step in the water monitoring process. In general, considerable attention is paid to the design of analytical measurement procedures and less to the sampling phase.

The collection of river water quality samples generally necessitates compromise between coverage of spatial and temporal variability and resource limitations (Chapman, 1996).

The variability in water quality in a river cross-section is also often significant because of incomplete mixing of upstream tributary or points-source inputs and groundwater seepage. Variations in velocity and channel geomorphology may also affect distribution and transport of particulates (Horowitz, 1996). Surface grab-sampling methods are commonly employed for stream water sampling, owing to speed and efficiency of sample collection. Good grab-sampling practice necessitates that samples are collected from mid-stream, away from the more quiescent margins of the river (Leeks et al., 1997). However, surface-grab sampling may result in profound underestimation of concentrations of sediment associated water-quality determinants, compared with more time-consuming methods of cross-sectionally integrated sampling (e.g. Martin et al., 1992; Bartram & Ballance, 1996).

River waters are highly heterogeneous in terms of their physical, chemical and biological composition and reactivity. Geology, land use, agriculture and sewage inputs have a major control on river-water quality in terms of matrix chemistry, P and suspended sediment concentrations. The biological status of river waters ranges from ultra-oligotrophic to hyper-eutrophic, and there are large contrasts in sensitivity of river water samples to degradation on storage (Maher & Woo, 1998). The microbial composition and water chemistry of river water often exhibits pronounced seasonal variability, leading to temporal changes in sensitivity to storage and analytical errors (Henriksen, 1969; Jarvie et al., 2002).

The designated sampling dates were as follows: 8nd, June 2021; 6th, July 2021; 3rd, August 2021; 7th, September 2021; 5th, October 2021; 2nd, November 2021; 7th, December 2021; 7th, January 2022; 1st, February 2022; 1st, March 2022; 5th, April 2022; 3rd, May 2022. Information on the sampling time for each component group is provided in the summary table below:



1 - Bottornya/Podturen 1

Components group	Date											
	02/06/2021	06/07/2021	03/08/2021	07/09/2021	05/10/2021	02/11/2021	07/12/2021	07/01/2022	01/02/2022	01/03/2022	05/04/2022	03/05/2022
1. Metals												
2. Chemical parameters												
3. Organic parameters (benzole, benz(a)pyrene, PAH, PCB,THM)												
4. Organic parameters (pesticides,hexabromocyclododecane)												
5. Pharmaceuticals												
6. Biological parameters												

2 - Bottornya/Podturen 2

Components group	Date											
	02/06/2021	06/07/2021	03/08/2021	07/09/2021	05/10/2021	02/11/2021	07/12/2021	07/01/2022	01/02/2022	01/03/2022	05/04/2022	03/05/2022
1. Metals												
2. Chemical parameters												
3. Organic parameters (benzole, benz(a)pyrene, PAH, PCB,THM)												
4. Organic parameters (pesticides,hexabromocyclododecane)												
5. Pharmaceuticals												
6. Biological parameters												

3 - Bottornya/Podturen 3

Components group	Date											
	02/06/2021	06/07/2021	03/08/2021	07/09/2021	05/10/2021	02/11/2021	07/12/2021	07/01/2022	01/02/2022	01/03/2022	05/04/2022	03/05/2022
1. Metals												
2. Chemical parameters												
3. Organic parameters (benzole, benz(a)pyrene, PAH, PCB,THM)												
4. Organic parameters (pesticides,hexabromocyclododecane)												
5. Pharmaceuticals												
6. Biological parameters												

4 - Letenye/Letinja 1

Components group	Date											
	02/06/2021	06/07/2021	03/08/2021	07/09/2021	05/10/2021	02/11/2021	07/12/2021	07/01/2022	01/02/2022	01/03/2022	05/04/2022	03/05/2022
1. Metals												
2. Chemical parameters												
3. Organic parameters (benzole, benz(a)pyrene, PAH, PCB,THM)												
4. Organic parameters (pesticides,hexabromocyclododecane)												
5. Pharmaceuticals												
6. Biological parameters												

5 - Letenye/Letinja 2

Components group	Date											
	02/06/2021	06/07/2021	03/08/2021	07/09/2021	05/10/2021	02/11/2021	07/12/2021	07/01/2022	01/02/2022	01/03/2022	05/04/2022	03/05/2022
1. Metals												
2. Chemical parameters												
3. Organic parameters (benzole, benz(a)pyrene, PAH, PCB,THM)												
4. Organic parameters (pesticides,hexabromocyclododecane)												
5. Pharmaceuticals												
6. Biological parameters												

6 - Csáktornya/Čakovec 1

Components group	Date											
	02/06/2021	06/07/2021	03/08/2021	07/09/2021	05/10/2021	02/11/2021	07/12/2021	07/01/2022	01/02/2022	01/03/2022	05/04/2022	03/05/2022
1. Metals												
2. Chemical parameters												
3. Organic parameters (benzole, benz(a)pyrene, PAH, PCB,THM)												
4. Organic parameters (pesticides,hexabromocyclododecane)												
5. Pharmaceuticals												
6. Biological parameters												

7 - Csáktornya/Čakovec 2

Components group	Date											
	02/06/2021	06/07/2021	03/08/2021	07/09/2021	05/10/2021	02/11/2021	07/12/2021	07/01/2022	01/02/2022	01/03/2022	05/04/2022	03/05/2022
1. Metals												
2. Chemical parameters												
3. Organic parameters (benzole, benz(a)pyrene, PAH, PCB,THM)												
4. Organic parameters (pesticides,hexabromocyclododecane)												
5. Pharmaceuticals												
6. Biological parameters												

8 - Csáktornya/Čakovec 3

Components group	Date											
	02/06/2021	06/07/2021	03/08/2021	07/09/2021	05/10/2021	02/11/2021	07/12/2021	07/01/2022	01/02/2022	01/03/2022	05/04/2022	03/05/2022
1. Metals												
2. Chemical parameters												
3. Organic parameters (benzole, benz(a)pyrene, PAH, PCB,THM)												
4. Organic parameters (pesticides,hexabromocyclododecane)												
5. Pharmaceuticals												
6. Biological parameters												

9 - Tótszerdahely/Serdahelj 1

Components group	Date											
	02/06/2021	06/07/2021	03/08/2021	07/09/2021	05/10/2021	02/11/2021	07/12/2021	07/01/2022	01/02/2022	01/03/2022	05/04/2022	03/05/2022
1. Metals												
2. Chemical parameters												
3. Organic parameters (benzole, benz(a)pyrene, PAH, PCB,THM)												
4. Organic parameters (pesticides,hexabromocyclododecane)												
5. Pharmaceuticals												
6. Biological parameters												

10 - Tótszerdahely/Serdahelj 2

Components group	Date	Date										
		06/07/2021	03/08/2021	07/09/2021	05/10/2021	02/11/2021	07/12/2021	07/01/2022	01/02/2022	01/03/2022	05/04/2022	03/05/2022
1. Metals												
2. Chemical parameters												
3. Organic parameters (benzole, benz(a)pyrene, PAH, PCB,THM)												
4. Organic parameters (pesticides,hexabromocyclododecane)												
5. Pharmaceuticals												
6. Biological parameters												

11 - Tótszerdahely/Serdahelj 3

Components group	Date	Date										
		02/06/2021	06/07/2021	03/08/2021	07/09/2021	05/10/2021	02/11/2021	07/12/2021	07/01/2022	01/02/2022	01/03/2022	05/04/2022
1. Metals												
2. Chemical parameters												
3. Organic parameters (benzole, benz(a)pyrene, PAH, PCB,THM)												
4. Organic parameters (pesticides,hexabromocyclododecane)												
5. Pharmaceuticals												
6. Biological parameters												

12 - Molnári/Mlinarci 1

Components group	Date	Date										
		02/06/2021	06/07/2021	03/08/2021	07/09/2021	05/10/2021	02/11/2021	07/12/2021	07/01/2022	01/02/2022	01/03/2022	05/04/2022
1. Metals												
2. Chemical parameters												
3. Organic parameters (benzole, benz(a)pyrene, PAH, PCB,THM)												
4. Organic parameters (pesticides,hexabromocyclododecane)												
5. Pharmaceuticals												
6. Biological parameters												

13 - Molnári/Mlinarci 2

Components group	Date	Date										
		02/06/2021	06/07/2021	03/08/2021	07/09/2021	05/10/2021	02/11/2021	07/12/2021	07/01/2022	01/02/2022	01/03/2022	05/04/2022
1. Metals												
2. Chemical parameters												
3. Organic parameters (benzole, benz(a)pyrene, PAH, PCB,THM)												
4. Organic parameters (pesticides,hexabromocyclododecane)												
5. Pharmaceuticals												
6. Biological parameters												

14 - Molnári/Mlinarci 3

Components group	Date	Date										
		02/06/2021	06/07/2021	03/08/2021	07/09/2021	05/10/2021	02/11/2021	07/12/2021	07/01/2022	01/02/2022	01/03/2022	05/04/2022
1. Metals												
2. Chemical parameters												
3. Organic parameters (benzole, benz(a)pyrene, PAH, PCB,THM)												
4. Organic parameters (pesticides,hexabromocyclododecane)												
5. Pharmaceuticals												
6. Biological parameters												

15 - Murakeresztúr/Kerestur 1

Components group	Date											
	02/06/2021	06/07/2021	03/08/2021	07/09/2021	05/10/2021	02/11/2021	07/12/2021	07/01/2022	01/02/2022	01/03/2022	05/04/2022	03/05/2022
1. Metals												
2. Chemical parameters												
3. Organic parameters (benzole, benz(a)pyrene, PAH, PCB,THM)												
4. Organic parameters (pesticides,hexabromocyclododecane)												
5. Pharmaceuticals												
6. Biological parameters												

16 - Murakeresztúr/Kerestur 2

Components group	Date											
	02/06/2021	06/07/2021	03/08/2021	07/09/2021	05/10/2021	02/11/2021	07/12/2021	07/01/2022	01/02/2022	01/03/2022	05/04/2022	03/05/2022
1. Metals												
2. Chemical parameters												
3. Organic parameters (benzole, benz(a)pyrene, PAH, PCB,THM)												
4. Organic parameters (pesticides,hexabromocyclododecane)												
5. Pharmaceuticals												
6. Biological parameters												

17 - Murakeresztúr/Kerestur 3

Components group	Date											
	02/06/2021	06/07/2021	03/08/2021	07/09/2021	05/10/2021	02/11/2021	07/12/2021	07/01/2022	01/02/2022	01/03/2022	05/04/2022	03/05/2022
1. Metals												
2. Chemical parameters												
3. Organic parameters (benzole, benz(a)pyrene, PAH, PCB,THM)												
4. Organic parameters (pesticides,hexabromocyclododecane)												
5. Pharmaceuticals												
6. Biological parameters												

18 - Murakirály/Donji Kraljevec 1

Components group	Date											
	02/06/2021	06/07/2021	03/08/2021	07/09/2021	05/10/2021	02/11/2021	07/12/2021	07/01/2022	01/02/2022	01/03/2022	05/04/2022	03/05/2022
1. Metals												
2. Chemical parameters												
3. Organic parameters (benzole, benz(a)pyrene, PAH, PCB,THM)												
4. Organic parameters (pesticides,hexabromocyclododecane)												
5. Pharmaceuticals												
6. Biological parameters												

19 - Murakirály/Donji Kraljevec 2

Components group	Date											
	02/06/2021	06/07/2021	03/08/2021	07/09/2021	05/10/2021	02/11/2021	07/12/2021	07/01/2022	01/02/2022	01/03/2022	05/04/2022	03/05/2022
1. Metals												
2. Chemical parameters												
3. Organic parameters (benzole, benz(a)pyrene, PAH, PCB,THM)												
4. Organic parameters (pesticides,hexabromocyclododecane)												
5. Pharmaceuticals												
6. Biological parameters												

20 - Murakirály/Donji Kraljevec 3

Components group	Date											
	02/06/2021	06/07/2021	03/08/2021	07/09/2021	05/10/2021	02/11/2021	07/12/2021	07/01/2022	01/02/2022	01/03/2022	05/04/2022	03/05/2022
1. Metals												
2. Chemical parameters												
3. Organic parameters (benzole, benz(a)pyrene, PAH, PCB,THM)												
4. Organic parameters (pesticides,hexabromocyclododecane)												
5. Pharmaceuticals												
6. Biological parameters												

21 - Drávavásárhely/Nedelisce No.1

Components group	Date											
	02/06/2021	06/07/2021	03/08/2021	07/09/2021	05/10/2021	02/11/2021	07/12/2021	07/01/2022	01/02/2022	01/03/2022	05/04/2022	03/05/2022
1. Metals												
2. Chemical parameters												
3. Organic parameters (benzole, benz(a)pyrene, PAH, PCB,THM)												
4. Organic parameters (pesticides,hexabromocyclododecane)												
5. Pharmaceuticals												
6. Biological parameters												

22 - Perlak/Prelog No.1

Components group	Date											
	02/06/2021	06/07/2021	03/08/2021	07/09/2021	05/10/2021	02/11/2021	07/12/2021	07/01/2022	01/02/2022	01/03/2022	05/04/2022	03/05/2022
1. Metals												
2. Chemical parameters												
3. Organic parameters (benzole, benz(a)pyrene, PAH, PCB,THM)												
4. Organic parameters (pesticides,hexabromocyclododecane)												
5. Pharmaceuticals												
6. Biological parameters												

23 - Letenye/Letinja K58 (5F)

Components group	Date											
	02/06/2021	06/07/2021	03/08/2021	07/09/2021	05/10/2021	02/11/2021	07/12/2021	07/01/2022	01/02/2022	01/03/2022	05/04/2022	03/05/2022
1. Metals												
2. Chemical parameters												
3. Organic parameters (benzole, benz(a)pyrene, PAH, PCB,THM)												
4. Organic parameters (pesticides,hexabromocyclododecane)												
5. Pharmaceuticals												
6. Biological parameters												

24 - Molnári/Mlinarci K34 (13F)

Components group	Date											
	02/06/2021	06/07/2021	03/08/2021	07/09/2021	05/10/2021	02/11/2021	07/12/2021	07/01/2022	01/02/2022	01/03/2022	05/04/2022	03/05/2022
1. Metals												
2. Chemical parameters												
3. Organic parameters (benzole, benz(a)pyrene, PAH, PCB,THM)												
4. Organic parameters (pesticides,hexabromocyclododecane)												
5. Pharmaceuticals												
6. Biological parameters												

25 - Murakeresztúr/Kerestur (1F)

Components group	Date											
	02/06/2021	06/07/2021	03/08/2021	07/09/2021	05/10/2021	02/11/2021	07/12/2021	07/01/2022	01/02/2022	01/03/2022	05/04/2022	03/05/2022
1. Metals												
2. Chemical parameters												
3. Organic parameters (benzole, benz(a)pyrene, PAH, PCB,THM)												
4. Organic parameters (pesticides,hexabromocyclododecane)												
5. Pharmaceuticals												
6. Biological parameters												

	surface water (WWTP effluent)
	Mura river
	underground water (monitoring wells)

6.5. Justification and explanation of the content of the sampling plan

The sampling frequencies associated with the components defined in the sampling plan will vary from site to site. In the case of surface waters, many components are analysed on a monthly basis (chemical and biological parameters, organic contaminants, drug residues) and the frequency of metals and pesticides determined in the other component groups varies. Metals are tested quarterly, while pesticides are also tested four times a year, but at different times. The definition of pesticides is scheduled for the spring months, as their releases to the environment are significant during this period. Due to the significant agricultural activity on the Hungarian side, the environmental exposure is higher in these months. As a result, we considered it justified to use shifted sampling to monitor surface and groundwater as well, in order to get a realistic picture of the possible spread and maximum detection of pesticide contamination. Bottornya is somewhat outside from the examined section, so sampling is done on the spot with

less frequency. At Letenye the effluent arrives directly into the Mura River via a pressure pipe, so at this sampling point two sampling points are determined on the Mura River.

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