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JOINT DANUBE SURVEY 4

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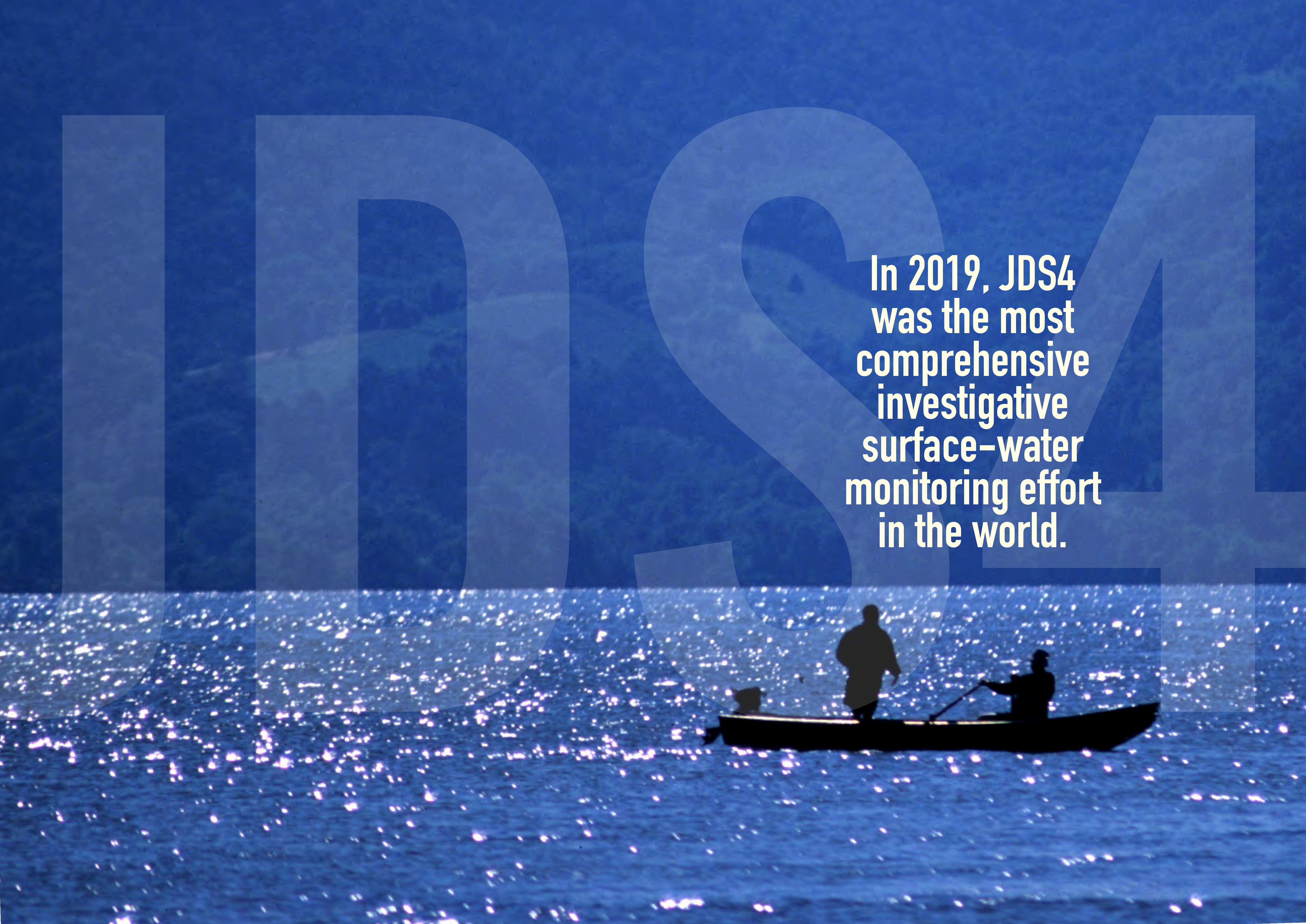
A SCIENTIFIC SELFIE OF THE DANUBE RIVER

ICPDR IKSD

International Commission for the Protection of the Danube River

Internationale Kommission zum Schutz der Donau



In 2019, JDS4 was the most comprehensive investigative surface-water monitoring effort in the world.

This report presents the public with a snapshot of the scientific findings of the Joint Danube Survey 4 (JDS4).

For more detailed information and data, please consult www.danubesurvey.org/jds4/scientific-report

A map showing the locations of all JDS4 sampling sites can be found at the end of this report.

The authors wish to thank all those who made JDS4 possible and carried out this unique international survey – including national delegations to the ICPDR from throughout the Danube River Basin, core team members, national coordinators and national teams, supporting experts and laboratories, as well as donors and sponsors.

Although it's not possible to list every individual who contributed to JDS4, they will recognise themselves within the pages of this report, and we acknowledge their efforts and expertise.

danubesurvey.org/jds4/publications/public_report
danubesurvey.org/jds4/publications/scientific_report
danubesurvey.org/jds4/publications/full_report

https://www.youtube.com/watch?v=iI1Xw58kQ94

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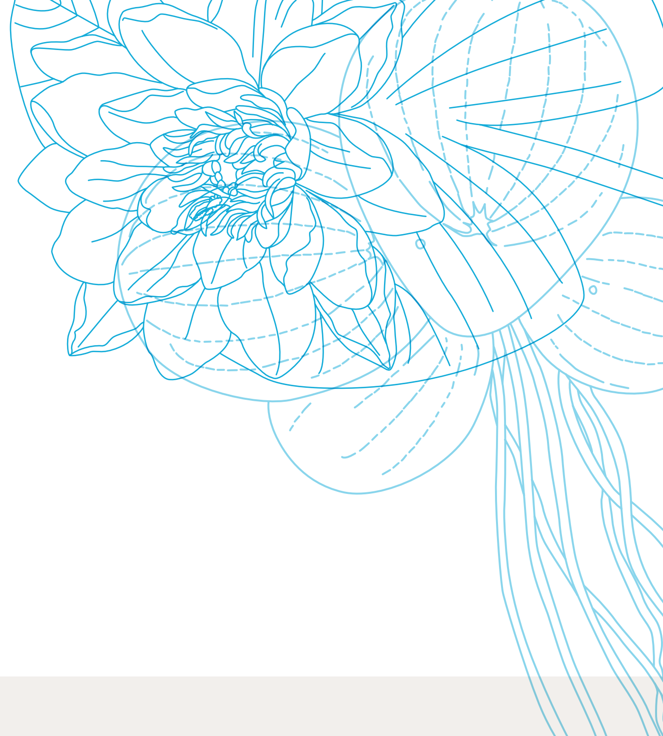


Joint Danube Survey 4

What is JDS4?

With the aim of obtaining a complex picture of the water quality in the Danube and its major tributaries, the annual assessment of water quality – published every year in the ICPDR TransNational Monitoring Network's (TNMN) Yearbooks – has been supplemented by periodic investigations: Joint Danube Surveys. These are carried out every six years in sync with the river basin management planning period according to the EU Water Framework Directive (WFD). It requires that Danube River Basin countries periodically assess certain water characteristics, while the ICPDR sets out processes and goals for the cooperation. An assessment must include determining the quality and quantity of waterbodies, the effects of human activity on the surface and groundwaters, and the economic effects of water use.

The Joint Danube Survey – the most comprehensive investigative surface water monitoring effort in the world – harmonises water monitoring practices across the Danube countries, in support of the WFD committing member states to achieving good water status.

> *The outcome of JDS4 helps to cover the information gaps deemed necessary for the planned 2021 update of the Danube River Basin Management Plan. http://icpdr.org/main/wfd-fd-plans-published-2021*

Three JDSs were previously conducted in 2001, 2007 and 2013, and the fourth of its kind, JDS4, started in mid-2019 at sampling sites in 13 countries across the Danube River Basin. The widened scope of this fourth survey focused on 51 sites nominated by the ICPDR experts. The sites comprised TNMN sites, JDS3 sites and sites for national surveillance monitoring in 2019, plus 7 additional groundwater sites in the Danube aquifer and 11 urban wastewater treatment plants (WWTPs).

The ambitious program of JDS4 necessitated the inclusion of additional specific sampling sites for passive sampling, eDNA analysis of fish and microbiological as well as microplastics monitoring.

With JDS4, the ICPDR seized the opportunity to create an inclusive motto, **Discover Danube**, and connect with a larger audience. This motto engaged with and gave a sense of purpose and unity to everyone involved in the survey, raising awareness of the cooperative approach to protecting and monitoring the Danube River Basin.

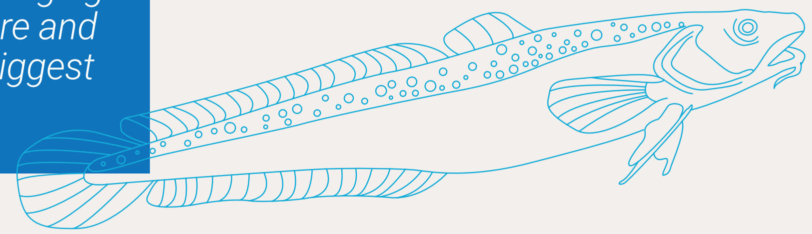
A river monitoring exercise of this size and scope **naturally raises a wide variety of questions** along the way. The following chapter attempts to answer some of those questions!

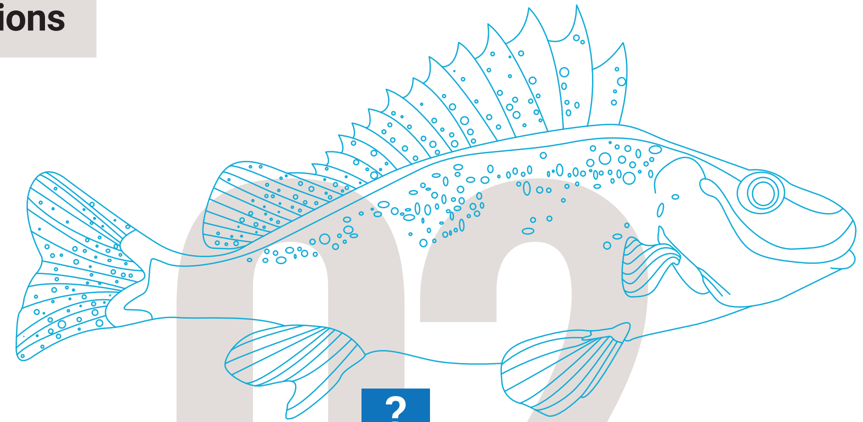
> *All operations of JDS4 took place under the motto "Discover Danube", encouraging Danubians to get out there and take part in the world's biggest river basin survey!*

As with all issues relating to the environment and to safety, there aren't always easy or simple answers. With this scientific selfie we are attempting to give you a better understanding of the situation in and around the Danube River and its major tributaries.

This situation, however, is constantly changing and we would advise all Danubians to always exercise curiosity and caution around their waters.

Many terms used in this report are very technical in nature. Please refer to the Glossary on page 32, which provides readers with helpful explanations and definitions of key JDS4 terminology.





01

How many fish species are there in the Danube?

▶ JDS4 found 72 out of the 100 fish species that are known to inhabit the Danube (from all past studies and historic data). Fish are well adapted to the specific environment of aquatic ecosystems. Some species in the Danube live near the bottom of the river, while others prefer to be in the shelter of water plants near the banks, swim in fast-flowing waters, or prefer slow-flowing zones.

▶ Fish are ideal indicators of the ecological quality of the ecosystem – the diversity and biomass of the total fish community show the ecological quality of the waterbody. For this reason, fish are one of the biological quality elements defined in the Water Framework Directive (WFD) together with invertebrates, water plants, algae and plankton. Overall, the fish biodiversity remains high along the Danube, and in all the regions the typical species ("reference species") are still present. The biomass of fish however, is too low – pointing at a number of problems (e.g. loss of habitats and connection to surrounding wetlands, river regulations, impoundments, migration barriers and overfishing).

02

Is it safe to swim in the Danube?

▶ Swimming in the Danube – like any river – always comes with a risk. Currents and flows of water, flash flooding, concealed obstacles, river vessels and more can always pose a risk to swimmers.

▶ When it comes to the quality of the water though, JDS4 is able to provide some answers, in the form of a snapshot of faecal pollution analysis showing it to be mostly safe in regions with state-of-the-art wastewater treatment. However, while this snapshot shows us that 70-80% of the Danube has 'good microbiological status', the EU Bathing Water Directive requires an even larger number of samples per site than taken in JDS4 to make a conclusive statement about safe swimming in the Danube.

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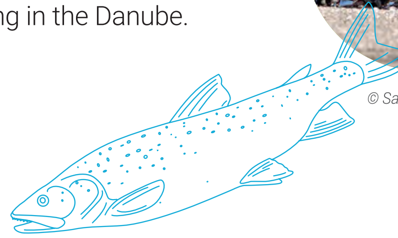


03

Have they really found salmon in the Danube?

▶ Yes, the Danube actually has its own native species of salmon: the Huchen, aka Danube Salmon (Hucho hucho).

▶ However, via the groundbreaking use of eDNA monitoring methods, our experts detected DNA from additional salmon species 'exotic' to the Danube during JDS4. Such 'exotic' fish species' DNA can enter the river from a variety of sources, mostly originating from sewage coming from wastewater treatment downstream of large cities. In short, wastewater can contain DNA from fish you eat at home.

eDNA (aka "Environmental DNA"). Animals and plants living in the Danube leave their traces in the water in the form of microscopic particles from their body. The DNA in those particles (eDNA found in the environment in contrast to the "organismic DNA" found directly in the body) can be analysed and compared to "barcodes" in a database. (e)DNA = environmental DNA- and DNA-based approaches

▶ So the answer to this one is a yes *and* a no! DNA from non-Danubian fish consumed in the basin area – like Atlantic salmon, sardines, ocean perches, tuna and herring – was discovered in the Danube River and its tributaries during JDS4.

04

Is there really caffeine in the Danube?

▶ Yes, but the results indicated that caffeine is not currently posing a threat to the ecosystem. Caffeine was detected in many samples taken during JDS4, including all wastewater and groundwater samples. The highest concentration levels were detected in wastewater samples reaching up to 3.94 µg /L*, whereas its main metabolites (theophylline and theobromine) were also present in all influent samples.

An average person would have to drink almost 50,000 cups of Danube water to consume the same amount of caffeine as you get in an average cup of coffee or cappuccino.

▶ The occurrence of caffeine is actually fairly widespread in water bodies around the world. We all drink a lot of coffee… Therefore, it's even been proposed as a representative indicator for anthropogenic (human) activities in aquatic ecosystems.

▶ While JDS4's results indicate no imminent dangers from caffeine, its frequent detection in groundwater indicates increased mobility of the compound, which could be of future concern.

* µg is the unit symbol for microgram.
1 milligram (mg) is equal to 1000 micrograms (µg).

05 ? Are there really party drugs in the river's waters?

▶ The terms "illicit drugs" and "drugs of abuse" are often interchangeable in the literature. Illicit drugs, including opioids, cocaine, cannabis, amphetamine-type and ecstasy-group compounds, are highly addictive substances for which non-medical use is prohibited by national or international laws and they are illegal to make, sell and/or use.

▶ While 87 illicit drugs, drugs of abuse and their "transformation products" (TPs) were detected in JDS4 samples, none of them are present in any amount that poses an environmental or health risk.

▶ The most prominent from the group of illicit drugs monitored was benzoylecgonine – the main metabolite of cocaine – detected in all 11 tested samples from wastewater treatment plants in the basin.

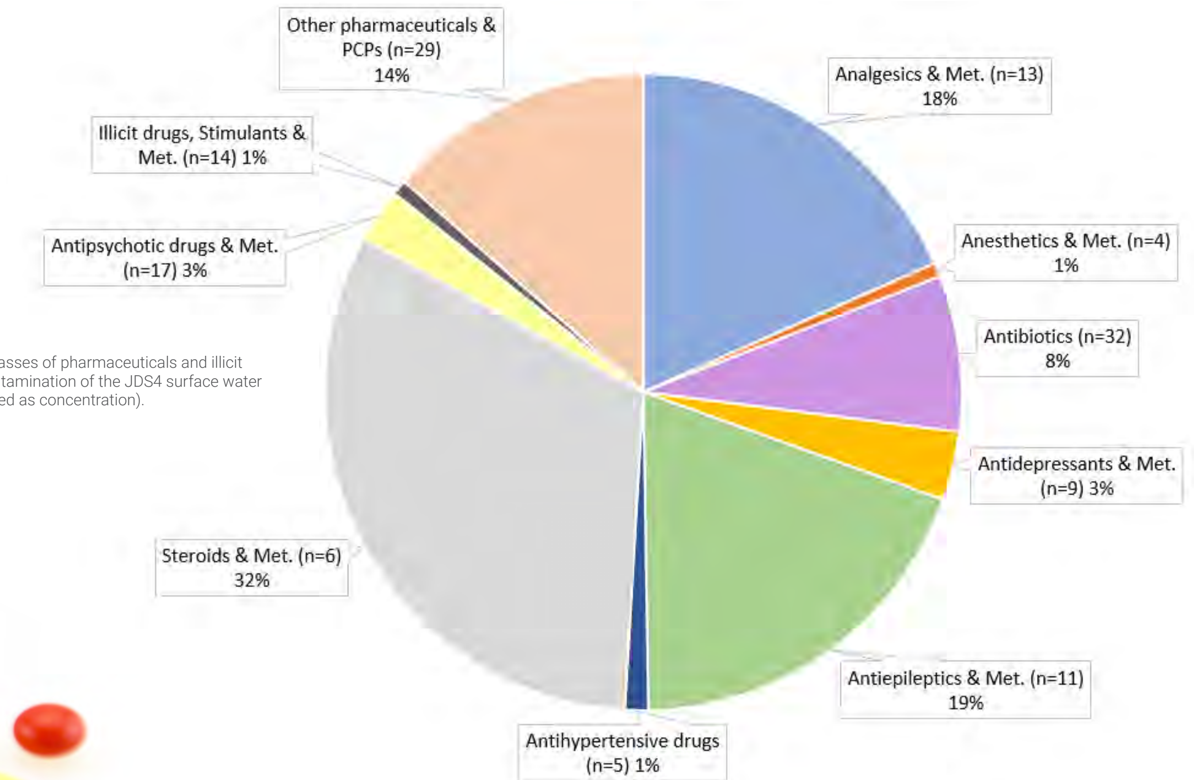
▶ The best news? The majority of illicit drugs and drugs of abuse that were detected in surface water in JDS3 were determined at significantly lower concentration levels in JDS4 samples.

06 ? Is faecal matter really present in the Danube River?

▶ Faecal pollution is present in all rivers – as is natural, for example from water birds – including the Danube. Certain pollution levels are completely to be expected, even for rivers with state-of-the-art wastewater management.

▶ While state-of-the-art wastewater treatment is going a long way towards reducing pollution in water, it is not designed to completely eliminate communal (human) faecal pollution emissions.

▶ No site with excessive levels of pollution from faecal matter was observed during JDS4, taking into account over 35 sites from throughout the Danube River Basin.



%Contribution of the different classes of pharmaceuticals and illicit drugs to the overall cumulative contamination of the JDS4 surface water samples (expressed as concentration).

07 ? Is antibiotic resistance an issue in river waters?

▶ Technically, this is an issue which could have impacts upon the human population, though it isn't something of concern to the aquatic life in the Danube and other water bodies.

▶ Due to a myriad of man-made factors, and the heavy use of antibiotics, bacteria have become increasingly resistant to antibiotics. This phenomenon can also be observed in surface waters. As a result, antibiotic-resistant bacteria, including pathogenic strains, are able to spread over long distances. The analysis of antibiotic resistant bacteria in the Danube River during JDS4, showed a significant increase in multi-resistance (acquired resistances to antibiotics from three or more tested antibiotic classes) in comparison to JDS3. The accumulation of resistance mechanisms in the Danube's *Escherichia coli* population, (more commonly known as *E. coli*), for example, has continued over the last six years.

▶ Unfortunately, resistance to last-line antibiotics was also detected. Last-line antibiotics are antibiotics of last resort, used when all other antibiotics have failed. Such last resort antibiotics are used only rarely to minimize the development of resistance which could lead to infections no longer being treatable.

▶ The European Commission (EC) has recognised the importance in addressing the issue of antimicrobial resistance since 2011, when the first "Action Plan" against it was adopted (EC, 2011). Subsequently, in 2017, the "One Health Action Plan" reinforced the previous document by encompassing the environmental contribution to the spread of such resistance (EC, 2017).

08

?

How much microplastics is there in the Danube?

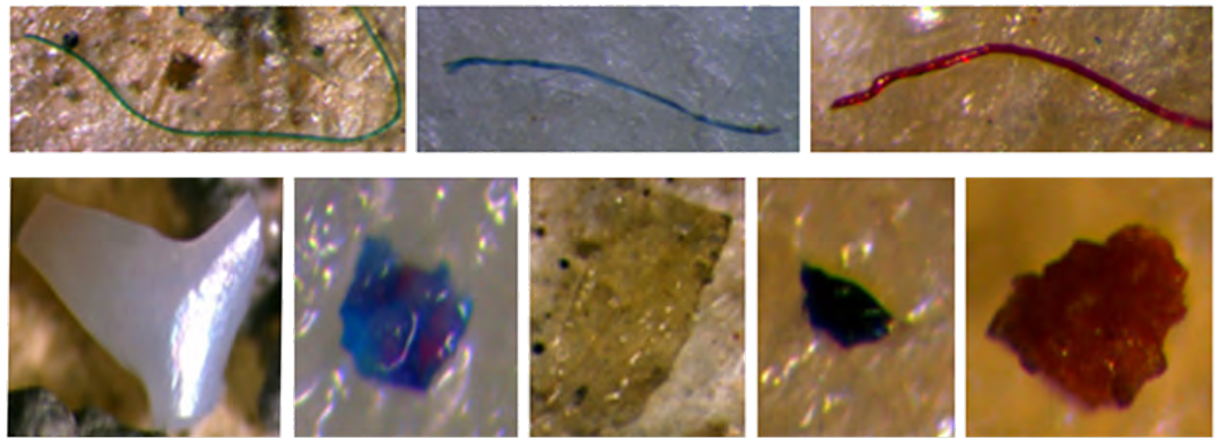
▶ While plastic pollution has long been a focus of environmental concern worldwide, nowadays, the presence of very small microplastic particles invisible to the naked eye is an increasingly important subject for scientific and regulatory discussions. Their inputs from land ultimately end up in accumulation areas and the oceans, where they remain for a long time. River systems represent an important path of microplastics' entry into the oceans.

▶ According to JDS4, microplastics are unfortunately to be found everywhere in the Danube – but in rather low concentrations.

▶ JDS4 comprised the first ever comprehensive screening of microplastics along the whole Danube, establishing a baseline of pollution by microplastics.

Make smart choices! Microplastic particles come from a myriad of sources, including fragmented larger plastic refuse, cosmetics, tyres, and clothing. Be mindful to dispose of your plastic waste responsibly, and avoid products that can lead to more microplastics entering into the environment whenever possible. To make more informed choices, visit: https://eurlex.europa.eu/eli/dir/2019/904/oj

In all water samples plastic polymers were detected and polyethylene was detected as the most abundant component of microplastics in almost all water samples. The screening of mussels discovered the presence of microplastics at all sites and revealed polyethylene terephthalate (commonly known as PET plastics) - as the dominant plastic pollutant. It's used for plastic bottles, all kinds of food-packaging and coffee to-go cups.



Photographs of microplastic particles isolated from clams tissue. Green, blue, and red fibres.
Row below: Hard plastic, nylon or rubber particles (white, transparent, blue, red or black particles).

09

?

Is it true that there are thousands of harmful chemicals in Danube waters?

▶ Yes – like in all rivers, but a great many of them are highly diluted and the vast majority are at safe levels. The extremely accurate up-to-date methods used in JDS4 mean that we could screen for more substances than ever.

▶ While there is a huge number of both natural and manmade chemicals present in our environment, only some are toxic or pose a danger to the environment due to their elevated concentrations. JDS4 aimed to look at the chemical river pollution posing the most immediate threat, including some chemicals listed by the EU as so-called Priority Substances, plus emerging chemicals from the EU Watch List.

▶ A handful of diverse target screening methods were applied during JDS4 focusing on several thousand of compounds in total. Hundreds of compounds were detected, and it was possible to make a priority list of pollutants in water, biota, sediment, wastewater and groundwater. In the course of this process, JDS4 could specify dozens of substances with the most immediately adverse effects on the Danube River's ecosystem.

10

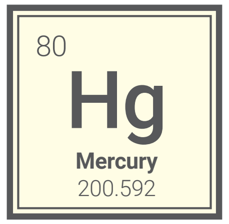
?

Is there a mercury problem in the Danube?

▶ Mercury is a metal element considered as a ubiquitous, persistent, bioaccumulative, and toxic substance to both humans and animals. This toxic substance can gradually accumulate up the food chain, making it a potential hazard, which requires close monitoring.

▶ The results from monitoring of fish species in JDS4 show that mercury is still present throughout the Danube's and its tributaries' fish, and higher than WFD EQS (Environmental Quality Standards) allow. But it was noted that the maximum levels for foodstuffs were only partly exceeded.

▶ However, concentrations show some potential signs of having decreased since JDS3. It's too early to tell though, and mercury will have to remain closely monitored in future surveys.



The Survey in Numbers

13

13 countries of the Danube River Basin

51

51 sites nominated by ICPDR experts

140

140 laboratories

7

7 groundwater sites

>2,600

> 2,600 chemical substances were looked for by target screening

11

11 urban wastewater treatment plants (WWTPs)

Hydromorphology

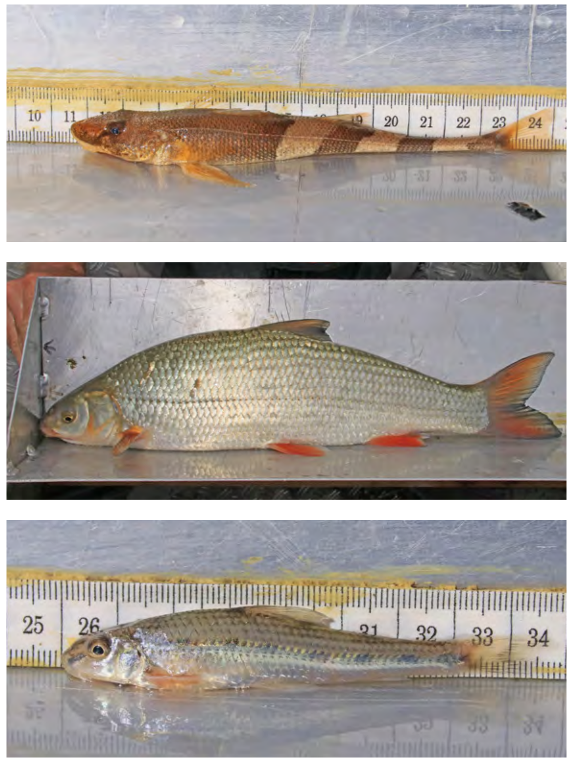
- 73 observed changes – 54 improvements and 19 deteriorations – within 55 monitoring segments.
- 4 segments have profited from fish passes in Austria, reconnecting seven segments in total (70 km) for fish migration.

Hydromorphology segment

Fish

Bleak asp

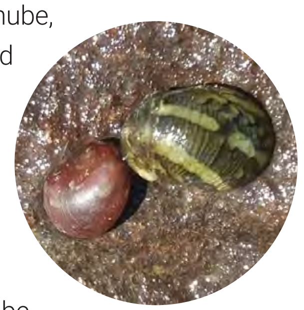
- 76,265 specimens of 72 fish and 3 jawless species were detected during JDS4, which underlines the importance of the Danube as a substantial source of fish biodiversity in Europe.
- Only 17% of the sites indicated good ecological status.



From above: Streber, Nase, White-fin gudgeon
all fish photos ©Vinzenz Bammer

Macroinvertebrates

- 484 taxa were found belonging to 19 higher taxonomic groups.
- 81% of sites show an indication of good or high status in respect to organic pollution across the whole Danube.
- However, high status decreases downstream – 91% of sites in the Upper Danube, 80% in the Middle Danube and 67% in the Lower Danube.
- The multi-metric index (MMI) shows a quite different picture: only 37% of sites reach an indication of good status, the situation is better in the Upper Danube (45%) and in the Lower Danube (50%) compared to the Middle Danube (20%). The MMI is an indicator for habitat degradation and these results show hydromorphological deficits of the ecosystem in habitat quality caused by a variety of pressures.



Theodoxus fluviatilis

> *Macroinvertebrates are key indicators for oxygen depletion due to pollution by degradable organic substances as well as for habitat degradation.*

Sponge colony ©Béla Csányi

385

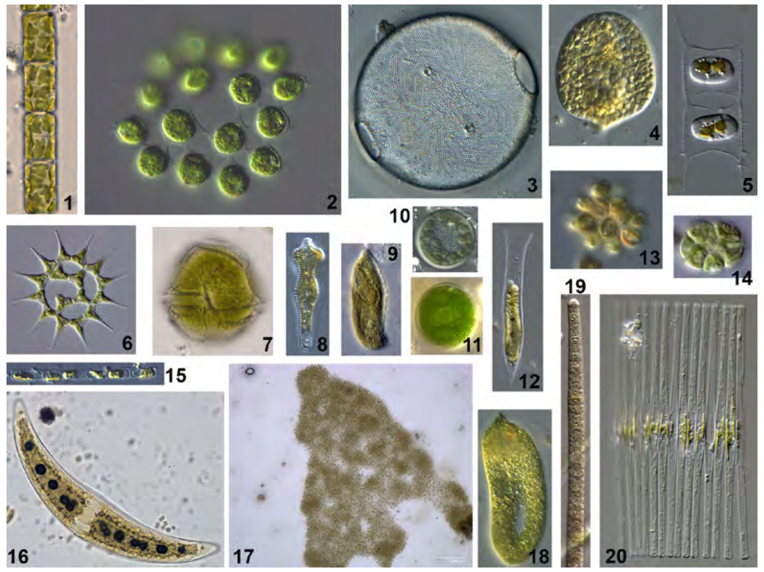
Phytobenthos

- 385 diatom species belonging to 78 groups (genera) were identified.
- Growth and prosperity of phytobenthos responds to variables of the river water.
- Indicative status of the phytobenthos samples generally gets less good travelling downstream. In the Upper Danube 61% of the sites indicate good status, in the middle section of the Danube 20% of the sites and in the Lower Danube none.
- In tributaries, however, this indicative status was found to be better in comparison to the Danube itself.

©Miroslav Ocladic

Phytoplankton

- 682 taxa were identified.
- Phytoplankton-based ecological status assessment ranged from high to low status, but it was deemed good on most of the sampling sites.
- Among all Danube tributaries, the Morava, Ipeľ and Rackevei-Soroksari Danube Arm had the highest values of chlorophyll a concentration and total biomass of phytoplankton, indicating increased eutrophication and nutrient pressure.



©Igor Stankovic

The phytoplankton of the Danube is very diverse in shape, size, taxonomic classification and ecology. Diatoms (1, 3, 5, 8, 10, 15, 20) are the dominant component of the river phytoplankton, with representatives clearly indicating anthropogenic pressure (3, 10). Green algae (2, 6, 11, 14) are the most diverse group in shape and size, readily taking up nutrients by increasing their surface area with a spherical shape, and often dominating in warm summer waters. Cyanobacteria are prokaryotes that occur in colonies of varying shape and size and often have toxic representatives (17, 19). Chrysophytes (12, 13), dinoflagellates (7), cryptophytes (9) and euglenophytes (4, 18) have the potential for mixotrophy (feeding as autotrophs and heterotrophs), while desmids (16) are a more benthic group of algae that prefer acidic environments and are represented by only a few species in the Danube.

Phytoplankton diversity of the Danube: 1. Melosira varians, 2. Gonium pectorale, 3. Pleurosira laevis, 4. Phacus sp., 5. Acanthoceras zachariasii, 6. Pediastrum simplex, 7. Peridinium sp., 8. Gomphonema acuminatum, 9. Cryptomonas curvata, 10. Actinocyclus normanii, 11. Chlamydomonas sp., 12. Dinobryon cylindricum, 13. Synura sp., 14. Pandorina morum, 15. Skeletonema potamos, 16. Closterium moniliferum, 17. Microcystis aeruginosa, 18. Euglena ehrenbergii, 19. Planktothrix agardhii, 20. Fragilaria crotonensis. Individual cells are large from 5 – 200 µm (0,005 – 0,2 mm).

132

Macrophytes

- 132 taxa were identified.
- The composition of macrophytes was found to be stable in terms of richness and diversity across several years.

Macrophytes, or aquatic plants visible to the naked eye, are an important part of the aquatic ecosystems. They are well known indicators for hydromorphological alterations

Zooplankton

- 157 taxa identified.
- General variation observed in results across the Danube in comparison to previous JDSs, most likely explained by the high water-levels before and during sampling.

© Katalin Zsuga and Anita Kiss

Invasive Alien Species

- JDS4 results reconfirmed that the Danube River and its main tributaries are under considerable influence from a growing number of alien species.
- Despite a higher number of alien species being recorded in the Danube, the data analysis shows that pressures caused by non-indigenous species remain unchanged.
- eDNA-based methods enabled detection of a non-indigenous snail species, *Bulinus umbilicatus*.

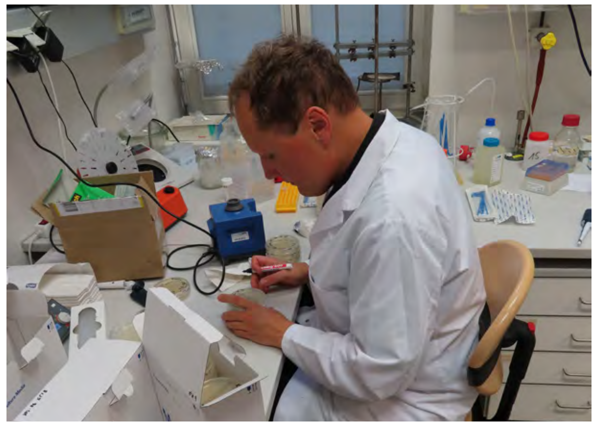
Used for the first time in JDS4, the Invasive Alien Species in Europe app enables the general public (amateurs and professionals) to receive and share information about Invasive Alien Species (IAS) in Europe. It provides details about 66 different IAS that are considered to be of interest to the European Union. Users can record pictures of possible Invasive Alien Species together with complementary information about their observation. The app, developed by the European Commission's Joint Research Centre (JRC), facilitates greater public access to records on invasive species. Visit: https://www.eea.europa.eu/data-and-maps/indicators/invasive-alien-species-in-europe

72

Microbiology

- In total, 72 samples were collected at 36 sites and tested for standard faecal indicator bacterium *E. coli* and genetic microbial source tracking markers.
- 56 samples (78%) displayed little or moderate pollution levels as can be expected for rivers with state-of-the-art wastewater management.
- 14 samples (19%) showed critical and 2 samples (3%) strong pollution levels.
- No site with excessive pollution level was observed during JDS4.

Filtering water samples



Processing samples

Groundwater

- 7 groundwater monitoring sites along the Danube were sampled, and results compared to concentrations detected at the closest Danube sites to identify any kind of interaction.
- In total 286 pesticide substances, pharmaceuticals, drugs, artificial sweeteners, industrial substances, isotopes, and more were detected in either groundwater or in a Danube monitoring site closest to a monitored groundwater-site.
- A considerable number of substances (23%) were only detected in a groundwater site and not found in any of the adjacent Danube sites, which indicates that pollution of groundwater is being caused by local or regional polluting activities.

Sampling groundwater

2,600

Chemical Screening

- An advanced wide-scope target screening of more than 2,600 chemicals and their transformation products was carried out in samples of influent and effluent wastewater, groundwater, river water, sediments and biota, collected within JDS4 as a collaborative study of three reference laboratories of the NORMAN network.
- In total, 580 contaminants were detected in the samples. As expected, influent wastewater samples were the most contaminated in terms of both number of compounds and concentration levels. Next came treated wastewater followed by surface water, biota and finally groundwater.
- Suspect (non-target) screening demonstrated its feasibility to reveal the presence of toxic substances and their transformation products, which would otherwise stay unnoticed. Out of the more than 65,000 substances analysed in JDS4 samples by suspect screening, ca. 2,000 were detected in at least one sample.

The raw data with mass spectra ('chemical fingerprints') of all detected pollutants stay stored for future retrospective screening, without the need for additional investments in sampling and analysis campaigns.

- The JDS4 target and non-target screening exercise was supported by the NORMAN network and this intensive cooperation resulted in the fact that the Danube is probably the best monitored river in terms of organic micro-pollutants in the world.

The NORMAN network is a network of reference laboratories, research centers and related organizations for monitoring of emerging environmental substances

Rare Earth Elements

- For the first time, JDS4 included several of those lesser-known elements from the Periodic Table (aka Mendeleev's Periodic Table) in its monitoring. Scandium (Sc), yttrium (y), and the lanthanides (along the bottom of the Periodic Table) were all monitored along the Danube River and its tributaries.
- A notably large positive anomaly was observed for gadolinium (Gd), used as a contrasting element in MRI (Magnetic Resonance Imaging) machines. There is currently no European quality standard established for elements such as this – but studies such as JDS may pave the way forward.

Microplastics

- JDS's first ever microplastics study was conducted during JDS4.
- A comprehensive screening of microplastics in the Danube and its tributaries was carried out on 15 sites. Sampling was performed by means of deploying sedimentation boxes into the river for 14 days; followed by thermo-analytical detection for determination of the total content of various plastic polymers in the collected suspended particulate matter samples. In all samples, plastic polymers were detected.
- A microplastic study was also conducted in 216 Asian clam specimens (Asian clam is also an IAS), collected from 23 sites along the Danube River and main tributaries.

Asian clam

- A total of 1,998 microplastic particles were collected with an average of 9.25 particles per organism.
- The fact that PET was not detected in water but was dominant in biota (such as mussels) showed that any future comprehensive monitoring of microplastics in rivers will require the very broad analysis of all relevant matrices.

Now that you've learned some new facts and what we've achieved during JDS4, come along and meet the team!

Meet the Team!

The ICPDR

The International Commission for the Protection of the Danube River (ICPDR) is a transnational body working to ensure the sustainable and equitable use of waters in the Danube River Basin. The work of the ICPDR is based on the Danube River Protection Convention (DRPC), the major legal instrument for cooperation and transboundary water management in the Danube River Basin.

In 2000, the ICPDR was mandated by its contracting parties as the platform for the implementation of all transboundary aspects of the EU Water Framework Directive (WFD). The successful implementation of the WFD is therefore clearly high on the political agendas of the countries of the Danube River Basin. In 2007, the ICPDR also took responsibility for coordinating the implementation of the EU Floods Directive (FD) within the Danube River Basin.

Science & Communication

JDS4 is coordinated by the ICPDR's Monitoring and Assessment Expert Group (MA EG) and the ICPDR Secretariat. Communication activities are coordinated by the ICPDR Public Participation Expert Group (PP EG) and the ICPDR Secretariat.

Even though science and communication are separate fields, both have much in common. Ideally, science and communication go hand in hand to serve each other's purpose, sharing obtained information with the public. The Joint Danube Surveys are part of a broader trend towards "Open Science", the idea that scientific knowledge should be shared, transparent and accessible. Raising awareness of the Danube's water quality, ongoing protection efforts and full implementation of the EU Water Framework Directive (WFD) are vital to the project.

JDS4: A New Approach

JDS4 took a novel and innovative approach to keep local scientists and local communicators in close touch. National teams were more involved than ever before, creating a synergy that increased the relevance, level of detail of the survey and the usefulness of its results.

This more active deployment of national experts put a higher burden on countries but resulted in a very intense monitoring exercise, which not only generated another vast amount of data but also significantly strengthened both cooperation and coordination between countries in the Danube River Basin.

Due to the active engagement of national teams and its extremely wide scope JDS4 mobilised the largest amount of actively cooperating experts in the history of the ICPDR.

Active involvement of all participants led to a high spirit of cooperation and was a mobilizing factor for the ICPDR Contracting Parties

JDS4's opening ceremony was held in Budapest, with the mayor of the city in attendance, and saw unprecedented local media coverage in Slovakia, the Czech Republic, and Serbia. Furthermore, above average public turnout was noted at events in Romania and Croatia. An exhibition to take place in the Danube-Swabian Central Museum in Ulm, Germany is planned to cover the survey and its findings, while piles of social media posts have spread the word about JDS4 in Austria, Slovenia, and Ukraine. Contact points were present in all participating countries too, including Montenegro, Bosnia & Herzegovina, Moldova, and Bulgaria.

It is a testament to just how mutually beneficial closer synergies between science and communication staff can be.

Technical Report

Have we awakened your interest? Want to know more about the science behind JDS4?

You can read more, and even gain access to the full and thoroughly detailed scientific results of JDS4 in the Reports section of the Danube Survey website: *danubesurvey.org/jds4/scientific-report*

What Did We Want to Achieve with JDS4?

To collect all of this data in a way that is readily comparable across the region's countries

To ensure experts could provide comparable results, training workshops were organized prior to JDS4 to harmonize methodologies. This was the first time ever when the experts on all EU WFD biological quality elements from all ICPDR Contracting Parties met to discuss monitoring and assessment harmonization issues. Already during this overture to JDS4, the significant benefit of the new concept could be seen. Making sure data was gathered in a way that could be compared was essential to JDS4. This way, we could work out what happens on the Danube's journey from Bratislava to Belgrade for example, and usefully measure changes and shifts in water status and chemical makeup in such a way as to make results comparable between all sampling teams.

To collect data on parameters not normally analysed

The goal of JDS4 is to collect data on a variety of parameters, including several which are not normally analysed, and certainly not in such a comparable way across the Danube River Basin.

All kinds of data get measured every day across the Danube, but JDS4 is about getting all the extra information on a lot of issues, which are not normally in focus or under the microscope. It's about going the extra mile, and about putting all of this knowledge into the wider context. JDS4 captured a detailed snapshot of the biology, chemistry, hydromorphology, pollution, and much more throughout the entire Danube River Basin. As important as measuring the broad state of the river is though, the survey focused on going in deep and gaining some insight into hitherto unmonitored substances and phenomena, such as:

- Rare Earth Elements
- Rare species (now discernible via eDNA)
- Trace amounts of a myriad of substances (including party drugs)
- First ever comprehensive screening of microplastics
- Radioactivity of river sediment
- Analysis of antibiotic resistance in bacteria

Additional ambitions of JDS4 stem from the parallel use of classical monitoring methods in biology and chemistry, with novel approaches such as eDNA and non-target screening. This parallel application of standard and new monitoring techniques at the large scale of the Danube River offered an opportunity to assess the potential of these new approaches.

To raise awareness of the Danube's water quality and ongoing protection efforts

Getting the public in the Danube River Basin on board with the project was vital. This involved an intensive public outreach effort, disseminating JDS4 news to the stakeholders and wider public. We needed them to know – and still do – why their river matters, and we want them to feel welcome to get involved in JDS in any way they can, and to share the findings of this unique **'scientific selfie'**.

To fill the gaps in WFD implementation

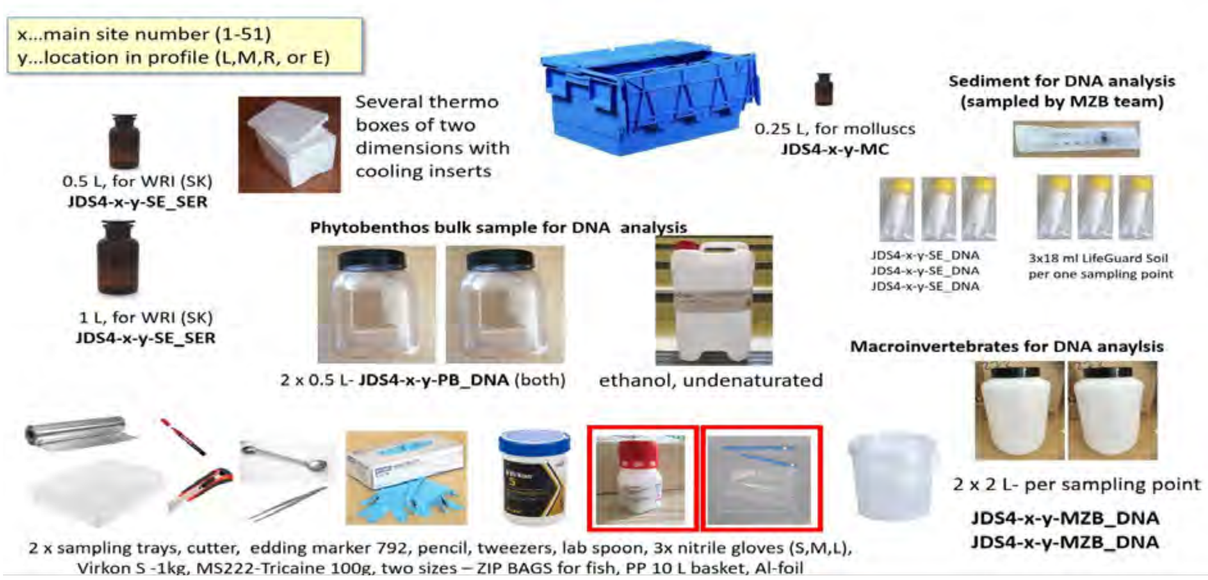
The EU Water Framework Directive (WFD) is stimulating higher standards for water status across the continent. JDS4 focused on the Danube Region specifically though, filling in all the gaps the broader WFD implementation might miss. There may be important issues at the Danube level, which may not apply to the entire neighbourhood of Europe and the surrounding area. The WFD is an invaluable tool for the future of the EU and Europe in general. Projects like JDS4 however, enable us to be even more focused at our basin level and to go a step beyond the requirements of the WFD.

What Methods Did JDS4 Put into Practice?

2,600 substances
searched for via wide-scope target screening,
>65,000 substances
used for suspect/non-target screening and
altogether >300,000
target screening results obtained.

Six years ago at the end of JDS3, the key conclusions began with the statement that it had provided a unique opportunity to assess the water quality in the whole Danube and provided the largest ever amount of knowledge about Danube water pollution collected within a single scientific exercise. Following the conclusion of JDS4, this statement can be repeated.

JDS4 sampling paraphernalia



Various Screening methods

Current chemical river pollution monitoring is focused on target analysis of Priority Substances and on River Basin Specific Pollutants. This means JDS4 was trying to work out how much of the Danube and its tributaries contained substances, pollutants and other specific substances defined as 'Priority Substances'.

In addition to those, a few **emerging chemicals** from the EU Watch List were investigated.

A handful of diverse target and non-target screening methods were applied during JDS4 focussing on thousands of compounds. Hundreds of compounds were ultimately detected by target screening while about 2,000 compounds were detected as suspects by non-target screening. This comprehensive use of screening techniques enabled their comparison to be made, and interlaboratory trials and training to be completed. Acquiring this huge dataset from screening methods made it possible to perform prioritisation of pollutants in water, biota, sediment, wastewater and groundwater. This led to the specification of dozens of substances proven to have the most adverse effects on the Danube ecosystem.

> *Two emerging chemicals:*
> ***Imidacloprid** is an insecticide broadly applied throughout the Danube River Basin in both horticulture and agriculture. It was detected in 50 out of 51 samples.*
> ***Diclofenac** is a widely used pharmaceutical, detectable at 48 sampling sites.*

eDNA & Microplastics: Groundbreaking new methods

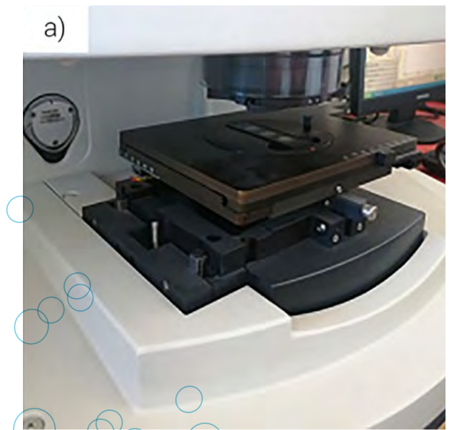
Of the many methods and techniques deployed alongside traditional biological assessment techniques in JDS4, one demonstrated its huge potential for the first time at such a magnitude anywhere in the world. **Environmental DNA and DNA-based approaches** were used to assess the biodiversity and to indicate WFD ecological status class in the Danube and its tributaries.

Thanks to PCR testing (the PCR testing here predates the now famous COVID-19 PCR testing) and DNA sequencing testing we could for example identify canned salmon consumption or identify rare species untraceable by standard assessment techniques.

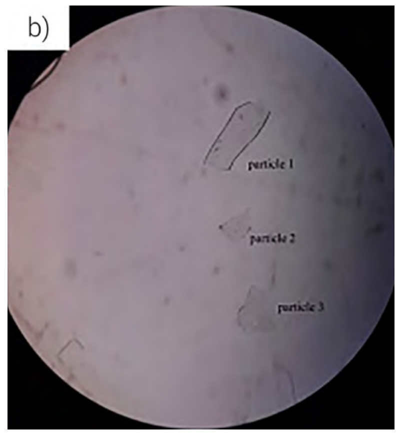
eDNA water analysis of fish revealed most of the taxa also detected by the traditional fish survey – however, it was particularly effective in detecting the hard-to-capture benthic taxa of fish (including endangered sturgeon species), plus a plethora of additional non-biting midges and worms. Indication of status by the traditional biological assessment techniques and by modern molecular methods showed a promising correlation for fish and macrozoobenthos – plus proved the value of the young and pioneering new method of eDNA monitoring for use in future river screening projects around the world.

Additionally, the first ever comprehensive screening of **microplastics** at 15 sites along the Danube established a baseline of pollution by microplastics in the Danube River Basin (DRB). Nowadays, the presence of microplastics in the environment is subject of scientific and regulatory discussions. Their inputs from land ultimately end up in the oceans, where they remain for a long time. River systems represent an important path of microplastics entry into the oceans. The results from JDS4 represent a first set of quantitative data, establishing a baseline of occurrence of microplastics in the DRB.

a)



b)



MicroATR spectroscopy (a) and particles prepared for analyses (b).

What the Danube Told Us…

The ICPDR's ultimate purpose in implementing the various directives and strategic plans shaping its work such as the Water Framework Directive is also to achieve the key goals of the 2016 Danube Ministerial Declaration.

Key goals of the 2016 Danube Ministerial Declaration

- Assessment of **faecal pollution** showed that 78% of samples displayed little or moderate pollution levels as it can be expected for rivers with state-of-the-art wastewater management. Only 19% of samples showed critical and a mere 3% of samples showed strong pollution levels. **No site** with an excessive pollution level was observed during JDS4.
- We analysed 19 priority substances regulated by the WFD, and only 2 (cypermethrin and cybutryne) showed concentrations above the Environmental Quality Standards (EQS). **All others** showed concentrations below the respective EQS.
- The results for mercury and brominated diphenylethers in biota showed concentrations higher than the EQS for biota at all sites. Whether the existing mitigation measures for these compounds are effective has to be shown in future monitoring programs.
- JDS4 monitored microplastics in the Danube River Basin for the first time, establishing a baseline of pollution by microplastics in the area. This is the first step towards tackling this vital issue.
- The results of the radiometric analysis showed that there continues to be no indication of hazardous manmade radioactive contamination in the Danube's ecosystem compartments.
- The analysis of groundwater showed that in many cases the bank-filtration process contributes to a smaller number of substances and lower concentrations being detected in groundwater than in the river itself.
- None of the pesticide substances and metabolites for which European quality standards for groundwater and drinking water exist, have exceeded these standards in the analyzed groundwater samples.
- Biological quality elements such as phytoplankton, macrophytes, phytobenthos and macrozoobenthos, considered indicators of pressure from nutrients and oxygen depletion, showed good status at many sites and pointed at local pressures only.
- Despite ongoing pressures on fish species in the Danube and its tributaries, JDS4's results showed that most of the Danube's fish species could still be found at nearly all sites. This is even true for strongly altered hydromorphological stretches in the Upper Danube section. In total 76,265 specimens of 72 fish and three jawless species were detected. This underlines the importance of the Danube as a substantial source of fish biodiversity in Europe.
- JDS4 results reconfirmed the considerable influence from invasive alien species in the Danube River and its main tributaries as previously shown.
- The analysis of antibiotic resistant bacteria showed a significant increase in resistance. The accumulation of resistance mechanisms in the Danube River's *E. coli* population has continued over the last six years.
- Hydromorphological reassessment showed the benefits of intensified restoration efforts in the Upper and Middle Danube reaches. The Lower Danube reach showed only slight to moderate alterations.

The River Surveys of Tomorrow

This scientific selfie left 'no stone unturned' in the Danube River and its tributaries. Besides the key findings about the health of the waters, JDS4 has also left behind a wider scientific legacy, testing new methods and shaping future efforts in the region and beyond.

The fingerprints of climate change can also be seen throughout the Danube River and its tributaries. How such effects fit into the results of JDS4 however, remains a subject for future scrutiny. As a survey, JDS4's job was to observe the state of the Danube River Basin's waters, rather than to ask 'why' they are the way they are.

For example, data from the JDS4 zooplankton investigation indicated that the observed increased frequencies of species preferring higher temperatures could be linked to climatic changes in the catchment area.

Likewise, the high abundance and species diversity of invasive alien macrozoobenthic species at many sites may have been supported by climate change effects and decreasing fish abundances. Disturbed age distributions could be partly linked to changes in the temperature regime.

In general, though, significant statements and analyses of climate change effects will have to be based on longer-term data series. In any case, the JDS4 data offer a valuable basis for further investigations in this field.

JDS4 Teams

JDS4 was organized in a different way when compared to previous surveys. The major part of the sampling during JDS4 was accomplished by the national experts while the Biology Core Team and Chemistry Experts focused on methodological coordination and advisory to ensure the coherence between the approaches used by the national experts. The Management and Support and Administrative Teams took care of the project management, political backup, data collection and public awareness. The National Coordinators organised the national sampling activities. The involvement of the ICPDR Expert and Task Groups ensured wide participation of Danube experts in planning and reporting on JDS4.

JDS4 Management

JDS4 Manager	Igor Liška	JDS4 project management
Technical Coordinator	Jaroslav Slobodnik	Logistical support and sampling coordination

JDS4 Support and Administrative Team

ICPDR Executive Secretary	Ivan Zavadsky	Political backup of the JDS4 project
Information Management Expert	Alexander Höbart	Data collection and data management
Public Awareness Expert	Hélène Masliah-Gilkarov	Public awareness
GIS Expert	Zoran Major	Map preparation
Financial Officer	Martina Noitzmüller	Financial accounting support
Editorial Support	Tristan Bath Ivo Monnerjahn	
Administration Support	Jelena Krstajic Olexandra Lohunova	

JDS4 Biology Core Team

JDS4 Core Team leader for biology	Momir Paunović
Fish expert	Vinzenz Bammer (supported by Predrag Simonovic as the Lower Danube expert)
Macrozoobenthos expert	Miroslav Očadlík
Phytobenthos expert	Dana Fidlerová and Jarmila Makovinská
Phytoplankton expert	Igor Stankovič
Macrophytes expert	Kateřina Bubíková and Igor Stanković
IAS expert	Béla Csányi
eDNA	Jonas Astrin and Alexander Weigand
Microbiology	Alexander Kirschner

JDS4 Chemistry Experts

Manfred Sengl, Karin Deutsch, Carmen Hamchevic, Zoran Stojanović, István György Tóth, Peter Tarábek, Hana Hudcová

JDS4 National Coordinators

The ICPDR Heads of Delegations nominated the following JDS4 National Coordinators:

Country	National Coordinator	Deputy National Coordinator
Germany	Manfred Sengl, Benno Kügel	
Austria	Karin Deutsch	Helena Mühlmann
Czech Republic	Ivana Bedĕrková	
Slovakia	Emília Mišíková Elexová	Soňa Ščerbáková
Hungary	Tünde Andrea Zagyva, György Istvan Tóth	
Slovenia	Irena Cvitanič, Tjaša Zimšek Muc	
Croatia	Draženka Stipaničev	
Serbia	Marta Mihailović	
Romania	Monica Mainerici	Florentina Soare
Bulgaria	Mina Assenova, Valeriya Gyosheva	
Ukraine	Iurii Nabyvanets	Sergiy Afanasiev
Moldova	Arcadie Leahu, Petru Prodan, Victor Bujac	

Supporting ICPDR Expert and Task Groups

Group	Chairperson
Monitoring and Assessment Expert Group (JDS4 organiser)	Franz Wagner
Groundwater Task Group	Andreas Scheidleder
Hydromorphology Task Group	Petra Repnik-Mah
Public Participation Expert Group	Susanne Brandstetter
Information Management and GIS Expert Group	Dragana Ninković
Pressures and Measures Expert Group	Elena Tuchiu

Special Longitudinal Survey Teams (SLST)

SLST 1	Peter Oswald, Zoran Stojanović
SLST 2	Nikiforos Alygizakis, Jörg Ahlheim
SLST 3	Michal Kirchner, Martin Hanuska

eDNA Survey Teams

eDNA Team 1	Didier Pont, Michael Schabuss
eDNA Team 2	Emre Keskin, Aysegul Er, Esra Mine Unal, Elena Stoica, Mihaela Tanase

Microbiology Team

Alexander Kirchner, Clemens Kittinger, Gernot Zarfel, Michael Koller, Daniela Toplitsch, Rita Baumert, Stefan Jakwerth, Erika Toth, Stoimir Kolarević, Mary Craciun, Cristina Dumitru

Passive Sampling Team

Branislav Vrana, Roman Prokeš, Jakub Vinkler

JDS4 Reference Laboratories

Next to national laboratories directly involved in the ICPDR activities, there was also a significant contribution from numerous specialised laboratories contributing specific analyses:

01 Biological Quality Elements

- Hungarian Academy of Sciences, Centre for Ecological Research, Danube Research Institute, Budapest, Hungary
- Institute for Biological Research "Siniša Stanković", University of Belgrade, Serbia
- National Museum of Natural History Luxembourg
- EC Joint Research Centre, Ispra, Italy
- WWF Slovakia, Bratislava, Slovakia
- Nature Conservation Agency of the Czech Republic, Prague, Czech Republic
- Hrvatske vode, Zagreb, Croatia
- Danube Research Institute, Budapest, Hungary
- Agrint Ltd., Gödöllő, Hungary
- University of Zagreb, Croatia
- Danube Research Institute, Debrecen, Hungary
- Technical University Zvolen, Slovakia
- Water Research Institute, Slovak National Water Reference Laboratory, Bratislava, Slovakia

02 DNAquaNet COST Action (CA15219)

- Université de Genève, Geneva, Switzerland
- IDGene ecodiagnostics, Geneva, Switzerland
- ECOSSA (Ecological Sediment & Soil Assessment), Starnberg, Germany
- Institute of Oceanology, Polish Academy of Sciences, Sopot, Poland
- National Museum of Natural History Luxembourg
- Botanischer Garten und Botanisches Museum Berlin, Freie Universität Berlin, Germany
- Center for Natural Science, University of Pannonia, Veszprém, Hungary

> In total, more than 140 laboratories from all over Europe participated in the JDS4 analytical programme.

- Hungarian Academy of Sciences, Budapest, Hungary
- UMR CARRTEL, INRAE, Université de Savoie MontBlanc, ThononlesBains, France
- INRA, UMR CARRTEL, Thonon les Bains cedex, France
- Water Research Institute, Slovak National Water Reference Laboratory, Bratislava, Slovakia
- Aquatic Ecosystem Research, University of DuisburgEssen, Germany
- Slovak Academy of Sciences, Bratislava, Slovakia
- University of Belgrade, Serbia
- Danube Research Institute, Budapest, Hungary
- University of Natural Resources and Life Sciences, Vienna
- Evolutionary Genetics Laboratory (eGL), Ankara University, Ankara, Turkey
- Bundesamt für Wasserwirtschaft, Institut für Gewässerökologie und Fischereiwirtschaft, Abteilung Gewässerökologie, Scharfling, Austria
- SPYGEN, Le Bourget du Lac, France
- Centre for Ecological Research, Tihany, Hungary
- Technical University of Munich, Germany
- Trnava University, Slovakia
- PRO FISCH OG Ecological Consultants, Vienna, Austria
- National Institute for Marine Research and Development "Grigore Antipa", Constanța, Romania
- Zoological Research Museum Alexander Koenig (ZFMK), Bonn, Germany

03 NORMAN network

- UFZ Leipzig, Germany
- University of Athens, Greece
- Environmental Institute, Kos, Slovakia
- RECETOX, Brno, Czech Republic
- University of Lorraine, CNRS, France
- TU Munich, Germany
- Water Research Institute, Slovak National Water Reference Laboratory, Bratislava, Slovakia

04 Widescope target and suspect screening survey and bioassays

- LW Langenau, Germany

05 Polarityextended non-target screening

- AFINTS, Augsburg, Germany

06 Target analyses of chemical parameters

- EC Joint Research Centre, Ispra, Italy
- NLZOH, Maribor, Slovenia
- PM, Brno, Czech Republic
- Umweltbundesamt GmbH, Vienna, Austria
- WRI, Bratislava, Slovakia

07 Bioassays survey

- BDS, Amsterdam, the Netherlands
- University of Belgrade, Serbia
- National Institute of Biology, Ljubljana, Slovenia

08 Microbiology survey

- EC Joint Research Centre, Ispra, Italy
- Karl Landsteiner University of Health Sciences, Krems, Austria
- Technical University Vienna, Austria
- Medical University Vienna, Austria
- Medical University Graz, Austria
- University of Insubria, Varese, Italy
- Interuniversity Cooperation Centre Water & Health, Austria
- Eötvös Loránd University, Budapest, Hungary
- Institute for Biological Research "Siniša Stanković", University of Belgrade, Serbia

09 Microplastics survey

- German Federal Environment Agency and BAM, Berlin, Germany
- Institute for Biological Research "Siniša Stanković", University of Belgrade, Serbia
- University of Comenius, Bratislava, Slovakia

10 Stable isotopes of water and nitrate and radiology survey

- IAEA Vienna and BOKU, Vienna, Austria

11 Nanoparticles survey

- University of Vienna, Austria

> An interlaboratory study to increase capacities of Danube laboratories in widescope target, suspect and nontarget screening was organised by UFZ Leipzig with involvement of Croatian Waters, Zagreb, Croatia, WRI Bratislava, Slovakia, SEPA Belgrade, Serbia, University of Athens, Greece, Environmental Institute, Kos, Slovakia, LfU Augsburg and BfG Koblenz, Germany.

JDS4 National Laboratories

- Regional office for water management, Donauwörth, Germany
- Regional office for water management, Ingolstadt, Germany
- Regional office for water management, Landshut, Germany
- Regional office for water management, Deggendorf, Germany
- State Office for Water Management, section biology, Donauwörth, Germany
- State Office for Water Management, section biology, Ingolstadt, Germany
- State Office for Water Management, section biology, Landshut, Germany
- State Office for Water Management, section biology, Deggendorf
- Bavarian Environment Agency, unit 83, Ecology of Rivers and Lakes, Hof, Germany
- Bavarian Environment Agency, unit 54, Fish and Freshwater Ecology Wielenbach, Germany
- DWS Hydro-Ökologie GmbH, Vienna, Austria
- Systema, Bio-Management Consulting GmbH, Vienna, Austria
- Institute of Hydrobiology and Aquatic Ecosystem Management, Vienna, Austria
- Environmental Agency, Vienna, Austria
- Institut für Gewässerökologie und Fischereiwirtschaft, BAW, Scharfling, Mondsee, Austria
- Fa. Synlab Analytics & Services Austria GmbH / Eurofins Umwelt Österreich GmbH, Vienna, Austria
- ESW Consulting Wruss ZT GmbH, Vienna, Austria
- National Water Reference Laboratory, Water Research Institute, Bratislava, Slovakia
- Budapest Waterworks, Budapest, Hungary
- DMRV Danubian Regional Waterworks Corporation, Vác, Hungary
- Pest County Government Office, Érd, Hungary
- Wessling Hungary Ltd., Budapest, Hungary
- Hrvatske vode, Central Water Management Laboratory, Zagreb, Croatia
- Department of Biology, University of J. J. Strossmayer, Osijek, Croatia
- Department of Biology, Faculty of Science, University of Zagreb, Croatia
- Eurofins Croatiakontrola d.o.o., Zagreb, Croatia
- Slovenian Environment Agency, Ljubljana, Slovenia
- National laboratory of Health, Environment and Food, Novo mesto, Slovenia
- Institute for Biological Research "Siniša Stanković" – National Institute of the Republic of Serbia, University of Belgrade
- University of Belgrade, Faculty of Biology, Serbia
- Faculty of Sciences, University of Novi Sad, Department of Biology and Ecology, Serbia
- Serbian Environmental Protection Agency
- Department of Biology and Ecology, Faculty of Sciences and Mathematics, University of Niš, Serbia
- Institute of Chemistry, Technology and Metallurgy, National Institute, Belgrade, Serbia
- Jaroslav Černi Water Institute, Belgrade, Serbia
- University of Kragujevac, Faculty of Science in Kragujevac, Department of Biology and Ecology, Serbia
- Institute of Public Health of Serbia "Dr Milan Jovanović Batut"
- Regional Laboratory Montana, Executive Environment Agency, Sofia, Bulgaria
- Regional Laboratory Pleven, Executive Environment Agency, Sofia, Bulgaria
- Regional Laboratory Ruse, Executive Environment Agency, Sofia, Bulgaria
- Regional Laboratory Varna, Executive Environment Agency, Sofia, Bulgaria
- Institute of Biodiversity and Ecosystem Research at the Bulgarian Academy of Science, Sofia, Bulgaria
- Faculty of Biology, Plovdiv University, Bulgaria
- Water Quality Laboratory SGA Mehedinti, Turnu Severin, Romania
- Water Quality Laboratory ABA Jiu, Craiova, Romania
- Water Quality Laboratory SGA, Tulcea, Romania
- Water Quality Laboratory ABADL, Constanta, Romania
- National Water Quality Laboratory, Bucharest, Romania
- Water Quality Laboratory SGA, Calarasi, Romania
- Water Quality Laboratory SGA, Giurgiu, Romania
- Water Quality Laboratory SGA Mehedinti, Turnu Severin, Romania
- Water Quality Laboratory ABA Jiu, Craiova, Romania
- Water Quality Laboratory SGA, Tulcea, Romania
- Water Quality Laboratory ABADL, Constanta, Romania
- National Water Quality Laboratory, Bucharest, Romania
- Water Quality Laboratory SGA Ialomita, Slobozia, Romania
- Regional Water Quality Laboratory ABAST, Cluj Napoca, Romania
- Water Quality Laboratory ABA Buzău-Ialomita, Romania
- Water Quality Laboratory SGA, Arad, Romania
- Water Quality Laboratory SGA, Bucharest, Romania
- Water Quality Laboratory SGA Caras-Severin, Resita, Romania
- Water Quality Laboratory SGA Gorj - Tg. Jiu, Romania
- Water Quality Laboratory ABA Banat, Timisoara, Romania
- Water Quality Laboratory ABA Siret, Bacau, Romania
- Water Quality Laboratory SGA, Calarasi, Romania
- Water Quality Laboratory ABAPB, Iasi, Romania
- Water Quality Laboratory ABAC, Oradea, Romania
- Water Quality Laboratory SGA, Braila, Romania
- Water Quality Laboratory SGA Vrancea - Focsani, Romania, Monitoring Department ABA Jiu - Craiova, Romania
- Monitoring Department ABA Siret -Bacau, Romania
- Executive Environment Agency, Water Basin Administration Arges-Vedea, Water Quality Laboratory, Giurgiu, Romania
- „IWA": „Institut für Wasseraufbereitung, Abwasserreinigung und –forschung", Austria
- Vodovody a kanalizace Hodonín a.s., Czech Republic
- Laboratory of Bratislavská vodárenská spoločnosť, a.s., Slovakia
- PANNON-VÍZ Zrt. Minőségvizsgáló Laboratórium, Hungary
- Komunala Novo mesto d.o.o., Laboratorij na CČN Novo mesto, Slovenia
- Internal laboratory of the WWTP Županja, Croatia
- Plant laboratory at the Central Wastewater Treatment Plant of Sabac, PUC "Vodovod Sabac", Serbia
- Stația de Epurare Giurgiu (SC APA SERVICE SA GIURGIU), Romania
- "Regional Laboratory Vratsa", Directorate "Laboratory and Analytical Activity" at the Executive Environmental Agency, Bulgaria
- Wastewater control laboratory of the Uzhorod utility company "Vodokanal", Ukraine.

Aa

Glossary: Name Description

Abundance (species) The number of individuals per species

Assay A procedure measuring the presence or amount or the functional activity of a target entity

Atmospheric deposition Chemicals or other substances that are deposited from the atmosphere onto the surface (e.g. land, water)

Benthic A term referring to anything associated with or occurring on the bottom of a body of water. The animals and plants that live on or in the bottom are known as the benthos

Bioaccumulation The accumulation of substances, such as pesticides, or other organic chemicals in an organism

Bioassay Involves the use of live animal or plant or tissue or cell to determine the biological activity of a substance

Biocide A biocide is a chemical substance or microorganism which can deter, render harmless, or exert a controlling effect on any harmful organism by chemical or biological means

Biodiversity The variation of life forms within a given ecosystem, biome or for the entire Earth. Biodiversity is often used as a measure of the health of biological systems

Biological sampling Animals and plants are collected with specific devices and analysed regarding species composition and individual density. This data gives information about how natural the ecosystem is at a certain sampling site – i.e. how far away the ecosystem is from being in a completely undisturbed state.

Biomarker A measurable indicator of a biological condition

Biomass Biological material derived from living or recently living organisms

Biota Plants and animals

BQE Biological Quality Elements, as outlined in the EU WFD

Bulinus Tropical genus of small sized snail species having sinistral shell opening like Physa. Most of the species have importance due to their role in the distribution of schistosomiasis

Carcinogenic Capable of causing cancer

Chemical sampling Water, sediment, or organism tissue is collected and analysed for chemical substances. Some of those substances are natural and good for the ecosystem, e.g. nutrients like nitrogen and phosphorus, or a variety of organic substances stemming from biodegradation. These only cause problems when their concentration is above or below certain limits.

Chemical screening The process of assessing the presence of pre-defined chemicals in the environment.

Chlorophyll-a A green pigment found in plants and cyanobacteria

Composition (species) The identity of all the different organisms that make up an ecological community

Confluence The meeting of two or more bodies of water

Crustacean This large group of species includes various familiar animals such as crabs, lobsters, crayfish, shrimp and barnacles. The majority of them are aquatic

Cyanobacteria A type of bacteria that obtains its energy through photosynthesis (cyano means blue)

Danube River Basin Management Plan The WFD requires all EU countries to have River Basin Management Plans, including a Programme of Measures, by 2009 and to update them in 2015 and 2021. The DRBM Plan Part A (Basin-wide overview) is coordinated by the ICPDR and based on the national RBM Plans

Danube River Protection Convention Signed in 1994 by Danube countries and the EU, it is the major legal instrument for cooperation and transboundary water management in the Danube River Basin

Density (species) The number of individuals of a species in an area

Diatoms A major algae group and one of the most common types of phytoplankton

Diversity (species) The number of species within a biological community (also known as "richness")

DNA Deoxyribonucleic acid is a molecule that encodes the genetic instructions used in the development and functioning of all known living organisms and many viruses

Dominance (species) The species that predominates in an ecological community, particularly when they are most numerous or form the bulk of the biomass

E. Coli (Escherichia coli) A coliform bacterium commonly found in the lower intestine of warm-blooded organisms. Most strains are harmless, but some can cause serious food poisoning in their hosts.

eDNA (aka "Environmental DNA"). Animals and plants living in the Danube leave their traces in the water in the form of microscopic particles from their body. The DNA in those particles (eDNA found in the environment in contrast to the "organismic DNA" found directly in the body) can be analysed and compared to "barcodes" in a database. Such a "barcode" is the genetic information for a single species that is necessary for its identification. With this revolutionary method, the presence of animals and plants can be detected in a water sample without catching or collecting them – even without observing them directly! During JDS4, brand new eDNA methods are being tested in comparison to conventional methods of aquatic species identification. In the future, the use of eDNA could revolutionize biological research and biological monitoring for the assessment of ecological quality

Electric fishing The act of using an electric field in water to stun fish so they can be collected with a net, assessed and then released, usually unharmed

Emerging substances Chemicals discovered in water which have not been detected previously, or those detected at levels that may be significantly different than expected

Endocrine disrupting compounds Organic compounds which can significantly impact the hormones of animals such as humans, fish and snails

Environmental quality standards (EQS) Under the WFD, EQS refer to commonly agreed concentration levels that are acceptable for "good chemical status", used by scientists as toxicity indicators

EU Watch List A proposal designed to allow targeted EU-wide monitoring of substances of possible concern

Eutrophication Elevated production of biomass in waters mainly due to an overload of nutrients (typically nitrogen or phosphorus)

Faeces Excrement; or waste expelled from an animal's digestive tract

Fauna A typical collection of animals found in a specific time or place

Fish Aquatic vertebrates (having a backbone) that are typically cold-blooded and covered with scales

Flame retardant Compounds added to manufactured materials to prevent the spread of fire

Floodplain Any land area susceptible to being inundated by floodwaters from any source

Flora A typical collection of plants found in a specific time or place

Food chain (or web) Shows how organisms are related with each other by the food they eat

Good biological and ecological status The quality required for a water body to meet WFD requirements

Groundwater 'Groundwater' refers to all water which is below the surface of the ground in the so-called "saturation zone" and in direct contact with the ground or subsoil. It is all of the water that is stored in spaces – pores, fissures, cracks and cavities – in soil, rock, gravel and sand. Being 'stored' does not mean that groundwater always stays in one place; in fact, it rises and falls with the water level of rivers, and flows at variable rates through 'aquifers' – any underground formation which can contain groundwater.

Habitat The physical and biological environment on which a given species depends for its survival

Glossary: **Name** Description

Helophytes Plants that grows in a marsh, partly submerged in water

Hydromorphology As defined by the WFD, the physical characteristics of the shape, boundaries and content of a water body

Hydrophytes Free-floating or submerged plants

Immunotoxicity Toxicity to the immune system

Impoundment A reservoir formed by a dam

International Commission for the Protection of the Danube River (ICPDR) The international organisation which has been established to implement the Danube River Protection Convention

Invasive alien species Non-indigenous species (e.g. plants or animals) that affect the habitats they invade economically, environmentally or ecologically

JDS1 The first Joint Danube Survey coordinated by the ICPDR in 2001

JDS2 The second Joint Danube Survey coordinated by the ICPDR in 2007

JDS3 The third Joint Danube Survey coordinated by the ICPDR in 2013

Joint Program of Measures Part of the DRBM Plan Part, this is a summary of the national Programmes of Measures and some of the common activities of the Danube Basin countries in the ICPDR

Macroinvertebrates Aquatic insects, worms, clams, snails and other animals without backbones that can be determined without the aid of a microscope and that live in or on sediments

Macrophytes Aquatic plants, either free-floating or attached to the bottom, which can be determined by the naked eye without the need for a microscope

Macrozoobenthos (Also Macroinvertebrates): Small animals without backbones that live on or in the sediments underwater and can be seen without the help of a microscope (i.e. larger than 0.5 mm). A huge variety of aquatic insects, worms, snails, clams, crabs and other animals belong to this group

Metabolism Includes all the things your body does to turn food into energy and keep you going

Microbiology The study of microscopic organisms that are unicellular or exist in cell clusters

Microplastics Plastic particles between 1 μm and 1 mm made from the disintegration of plastics including polymers, pellets and fibres made out of polyethylene (PE), polypropylene (PP), polystyrene (PS), polyethylene terephthalate (PET), polyamide (PA), natural rubber (NR) and styrene- butadiene rubber (SRB).

Mutagenic Can damage genes and possibly cause cancer

Nutrient Substances such as nitrogen and phosphorus, used by organisms to grow

Nutrient pollution Contamination of water resources by excessive inputs of nutrients. In surface waters, excess algal production is a major concern

Nutrient retention Plants are able to absorb and retain nutrients, and therefore reduce nutrient pollution

Order of magnitude An amount equal to ten times a given value

Organic compounds Natural or synthetic substances based on carbon

Organic pollution Occurs when an excess of organic matter, such as manure or sewage, enters the water

Parameter A characteristic, feature, or measurable factor that can help in defining a particular system

Passive Sampling "Passive sampling" is a technique used to monitor an environment, whereby a medium is collected over time in something, such as a man-made device or biological organism. This is in contrast to "grab sampling", which involves taking a sample directly from the media of interest at a single point in time. In passive sampling, average chemical concentrations are calculated over a device's deployment time, which avoids the need to visit a sampling site multiple times to collect multiple representative samples.

Pathogens Bacteria, viruses, parasites or fungi that can cause disease

Pelagic Any water in a sea or lake that is neither close to the bottom nor near the shore

Perfluorinated acids Chemicals that repel water and oil and are resistant to heat and chemical stress

Persistent organic pollutants (POPs) Chemicals that remain intact in the environment for long periods, become widely distributed geographically, accumulate in the fatty tissue of living organisms and are toxic to humans and wildlife

Pesticide A substance, usually chemical, used to kill unwanted plants and animals

Phytobenthos Microscopic plants such as algae that live in the bottom layers of the river and seabed

Phytoplankton Plants, mainly microscopic, existing in water bodies

Point source A well defined source of pollution from a single point, such as a pipe. Non-point sources of pollution enter water from a dispersed (or "diffuse") and uncontrolled source, such as runoff from land or from the atmosphere, rather than through a pipe

Priority substances The EU's 'Priority Substances' or groups of substances which have been shown to be of major concern for European waters. Priority Substances include organic compounds and heavy metals

Radioactivity The spontaneous discharge of radiation from atomic nuclei

Rare Earth Elements A series of metallic elements that rarely occur naturally, and whose oxides are classed as 'rare earths'.

Reach The Danube is split into three "reaches" (see map for more info)

Rip-rap Large boulders that have been artificially placed to fix riverbanks, especially at channelized and impounded river sections

Rkm Distance in the river upstream from the river's mouth (for the Danube River, distance from the Danube Delta)

Sediment Material that was suspended in water and that settles at the bottom of a body of water

Species abundance The number of individuals per species. Relative abundance species is the species abundance relative to the abundances of other species represented in the community

Species diversity The number of species within a biological community (also known as "species richness")

Sturgeon The flagship family of fish species in the Danube River Basin

Substrate The surface on which a plant lives. Suspended sediment refers to the solid particles, suspended within the water column, which the water is carrying. Also known as suspended particulate matter (SPM)

Taxon (sg), Taxa (pl) A group or category of living organisms

Toxicity The degree to which a substance can damage an organism

Toxicology Study of the effects of chemicals on living organisms

Trans-National Monitoring Network (TNMN) Coordinated by the ICPDR, it comprises over 75 monitoring stations and provides a regular overview of the main chemical and physical parameters important for assessing water quality

Tributary A river that flows into a larger river or other body of water

Turbidity The cloudiness or haziness of a fluid caused by large numbers of individual particles that are generally invisible to the naked eye

Vascular plants Having tissues for conducting water and minerals throughout the plant

Water Framework Directive (WFD) Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy

Zooplankton Tiny invertebrates (animals without backbones) that float freely in water bodies

Map of Route and Sampling Sites

THE DANUBE HAS THREE 'REACHES'

The Danube River is divided into three main sections or 'reaches' – Upper, Middle and Lower.
The Upper Danube runs from the Danube's source in Germany to 'Porta Hungarica', where the Alps' eastern foothills connect with the Carpathian Mountains below the confluence of the Danube and Morava rivers east of Vienna.

The Middle Danube flows from 'Porta Hungarica' to the start of the southern Carpathian and Balkan mountains before the Iron Gates hydro-electric power plant.

The Lower Danube runs through the Romanian and Bulgarian lowlands including the catchments of the Prut and Siret rivers and their surrounding mountainous landscapes.

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ICPDR IKSD
International Commission for the Protection of the Danube River
Internationale Kommission zum Schutz der Donau

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