Analytical protocols and methods for analysis of TrOCs and heavy metals

Deliverable D5.2

First version (public)
WP5 On-site piloting and performance evaluation
Task 5.2 Identification of the boundary conditions for technology development under Indian conditions
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Dissemination level		
PU	Public	**X**
CO	Confidential, restricted under conditions set out in Model Grant Agreement	
CI	Classified information as referred to in Commission Decision 2001/844/EC)	
Deliverable type		
R	Document, report	
DEM	Demonstrator, pilot, prototype	**X**
DEC	Websites, patent fillings, videos, etc.	
OTHER	Software, technical diagram, etc.	
ETHICS	Ethics	

History			
Version	Date	Reason	
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SUMMARY

This document "**Deliverable D5.2: Analytical protocols and methods for analysis of trace organic compounds and heavy metals**" is the second deliverable of WP5 "On-site piloting and performance evaluation". As laid down in the Description of Action (Annex 1 – Part A) Deliverable 5.2 describes the analytical protocols and methods for analysis of trace organic compounds (TrOCs) and heavy metals (HMs).

The methods for the analysis of TrOCs and HMs are used for the lab studies (WP3), water quality monitoring (WP4) and pilot studies (WP5).

It is the particular **objective to disseminate the methods** established at the labs of the Indian partners, i.e. IIT Delhi and IIT Kanpur through this public deliverable.

- The micropollutant analysis using LC-MS is conducted at the IITD lab (in cooperation with the bilateral Dutch Indian LOTUS HR project) while the sample preparation by solid phase extraction is established at both IITs.
- The HM analysis can be conducted with a range of methods in both IITs. Besides AAS, ICP-MS and ICP-OES as well as MP-AES are available and used.

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CHAPTER 1 DESCRIPTION AND GOAL

This document "**Deliverable D5.2: Analytical protocols and methods for analysis of trace organic compounds and heavy metals**" is the second deliverable of WP5 "On-site piloting and performance evaluation". As laid down in the Description of Action (Annex 1 – Part A) Deliverable 5.2 describes the analytical protocols and methods for analysis of trace organic compounds (TrOCs) and heavy metals (HMs).

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The present first version summarizes the actual state of the work since the LC-MS method is not yet fully implemented due to the late start of the Indian DBT funding and the impact from COVID-19 having led to a complete shutdown of the IIT D lab. It will be updated as soon as the methods are operational.

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CHAPTER 2 WATER QUALITY PARAMETERS

India's water resources are under severe stress resulting from overexploitation, climate change and pollution. Besides the analysis of well-established standard parameters such as COD, BOD, TDS and nutrients, Pavitra Ganga investigates also contaminants less frequently studied in India, i.e. organic micropollutants and heavy metals.

2.1. HEAVY METALS

Heavy metals such as arsenic (As), cadmium (Cd), chromium (Cr), silver (Ag), copper (Cu), iron (Fe), nickel (Ni), mercury (Hg), lead (Pb) and zinc (Zn) are metals with high atomic mass and high density of at least 5 g cm^{-3} (Saleh 2018). Many of them may induce toxicity at low levels, causing cellular disruptions. Some heavy metals like Cr (VI) even show toxicity at mg kg^{-1} body weight (Fishbein 2018). Hexavalent chromium can lead to necrosis or nephritis by just 10 mg kg^{-1} body weight. Other HMs like Pb can cause DNA damage, lead to hypertension and brain damage, or metabolic disorders, while for example Cd can lead to damage in kidneys and the skeleton (Gautam et al. 2014). In table 1, the most prevalent toxic effects of selected heavy metals are summarized, and suggested drinking water standards are displayed.

HM are mostly charged positive, e.g. 2+ or 3+, and have a high adsorption ability (Erdem et al. 2004), which facilitates their sequestration in soils, river sediments and wastewater sludge. Further, they show high bioavailability and biomagnification (Fishbein 2018).

Due to the naturally occurring leaching of heavy metals from soil parent materials into freshwater, e.g. by geogenic washout from the rocks, a natural occurrence is given (Morais et al. 2012). Traces of some heavy metals such as Fe, Se Zn, Cr, and Mg are even essential for human metabolism if they are not exceeding homeostatic levels (Saleh 2018). Due to an increase of anthropogenic emissions from industry, transport vehicles as well as from mining activities (Ahuja 2019), natural systems are disturbed by excess amounts of some HM's. After various research related to their toxicity and harmful effects on living organisms, including human life, the WHO has recommended threshold values for drinking water quality (table 1).

Especially the non-degradability and the sequestering properties of HM in the soil lead to continuous distribution into the environment and make it challenging to remove them once they have entered the atmosphere. Therefore, measures shall be taken to avoid HM spreading into the environment, replacing them by suitable substitutes or install procedures sufficient to prevent their leakage from point sources into the water bodies. Current treatment technologies to extract or remove heavy metals from wastewater flows are mainly based on precipitation and flocculation, through particle size separation, ion exchange, and adsorption (Lenntech 2020). Depending on contaminant concentrations and aimed discharge criteria, applicable processes are applied. Adsorbents and

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especially bio adsorbents, for example, have the benefit of good availability, low costs, regeneration possibility, technically feasible utilization and the affinity for heavy metals to be removed efficiently (Renu et al. 2017).

Table 1: Effects of heavy metals and their concentration guidelines (Gautam et al. 2014)

Metal	Effects	Drinking water standards
Lead	• Toxic to humans, aquatic fauna and livestock	• By the US Environmental Protection Agency maximum concentration: 0.1 mg/l
	• High doses cause metabolic poison	• By the European Commission: 0.5 mg/l
	• Tiredness, irritability anemia and behavioral changes of children	• by the Bureau of Indian standards: 0.1 mg/l
	• Hypertension and brain damage	
	• Phytotoxic	
Nickel	• High conc. can cause DNA damage	• By the US Environmental Protection Agency maximum concentration: 0.1 mg/l
	• Eczema of hands	• By European Commission: 0.1 mg/l
	• High phytotoxicity	• by the Bureau of Indian standards: 0.1 mg/l
	• Damaging fauna	
Chromium	• Necrosis nephritis and death in man (10 mg/kg of body weight as hexavalent chromium)	• By the US Environmental Protection Agency maximum concentration: (hexavalent and trivalent) total 0.1 mg/l
	• Irritation of gastrointestinal mucosa	• By European Commission: 0.5 mg/l
		• by the Bureau of Indian standards: 0.1 mg/l
Copper	• Causes damage in a variety of aquatic fauna	• By the US Environmental Protection Agency maximum concentration: 1.0 mg/l
	• Phytotoxic	• By European Commission: 3 mg/l
	• Mucosal irritation and corrosion	• by the Bureau of Indian standards: 0.01 mg L^{-1}
	• Central nervous system irritation followed by depression	
Zinc	• Phytotoxic	• By the US Environmental Protection Agency maximum concentration: 5 mg/l
	• Anemia	• By European Commission: 5 mg/l
	• Lack of muscular coordination	• by the Bureau of Indian standards: 0.1 mg/l
	• Abdominal pain *etc*.	
Cadmium	• Cause serious damage to kidneys and bones in humans	• By the US Environmental Protection Agency maximum concentration: 0.005 mg/l
	• bronchitis, emphysema, anemia	• By European Commission: 0.2 mg/l
	• Acute effects in children	• by the Bureau of Indian standards: 0.001 mg/l
Mercury	• Poisonous	• By the Environmental Protection Agency maximum concentration: 0.002 mg/l
	• Causes mutagenic effects	• By European Commission: 0.001 mg/l
	• Disturbs the cholesterol	• by the Bureau of Indian standards: 0.004 mg/l
Arsenic	• Causes toxicological and carcinogenic effects	• World Health Organization guideline of 10 µg/l
	• Causes melanosis, keratosis and hyperpigmentation in humans	• By European Commission: 0.01 mg/l
	• Genotoxicity through generation of reactive oxygen species and lipid peroxidation	• by the Bureau of Indian standards: 0.05 mg/l
	• Immunotoxic	
	• Modulation of co-receptor expression	

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In order to be sure that treated wastewater is at an acceptable quality level for reuse, i.e. not to degrade soil quality nor to lead to harmful effects towards organisms and human health, international water reuse guidelines had been introduced in this century. Table 2 shows international guideline values for heavy metals in irrigation water. In India, the first irrigation guideline had been set in order in 1986; updated regulations for maximum permissible concentrations have been set in charge in 2019.

Table 2: Guideline values of heavy metals for irrigations (WHO 2006, NRMMC& AHMC 2006, US EPA 2012)

	WHO (2006)	NRMMC& AHMC (2006)		US EPA (2012)	MoEFCC (2019)	
Metal concentration	root crops	Landscape irrigation	Commercial food crops	Food crops	Inland surface water	On land irrigation
Aluminium (mg/L)	5.00	5.00	20.00	5.00		
Arsenic (mg/L)	0.10	0.10	2.00	0.10	0.2	
Beryllium (mg/L)	0.10	0.10	0.50	0.10		
Boron (mg/L)		0.50	0.5-15	0.75		
Cadmium (mg/L)	0.01	0.01	0.05	0.01	0.05	
Cobalt (mg/L)	0.05	0.05	0.10			
Chromium (VI) (mg/L)		0.10	1.00			
Chromium (mg/L)	0.10			0.10	2	2
Copper (mg/L)	0.20	0.20	5.00	0.20	3	3
Fluoride (mg/L)	1.00	1.00	2.00	1.00		
Iron (mg/L)	5.00	0.20	10.00	5.00	3	3
Lithium (mg/L)	2.50	2.50	2.50	2.50		
Manganese (mg/L)	0.20	0.20	10.00	0.20	2	
Mercury (inorganic) (mg/L)		0.00	0.00		0.01	
Molybdenum (mg/L)	0.01	0.01	0.05	0.01		
Nickel (mg/L)	0.20	0.20	2.00	0.20	3	
Lead (mg/L)	5.00	2.00	5.00	5.00	0.1	
Selenium (mg/L)	0.02	0.02	0.05	0.02	0.05	
Uranium (mg/L)		0.01	0.10			
Vanadium (mg/L)	0.10	0.10	0.50	0.10	0.2	
Zinc (mg/L)	2.00	2.00	5.00	2.00	5	15

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2.2. MICROCONTAMINANTS

Organic microcontaminants, or also called organic micropollutants (OMPs) or trace organic compounds (TrOCs), are those substances, which occur at low nano- to microgram level per litre in natural water bodies and other points along the water cycle. Micropollutants are mostly from organic origin, but also inorganic compounds are accounted for within this group (Umweltbundesamt 2018).

2.2.1. PHARMACEUTICALS AND PERSONAL CARE PRODUCTS

Most of the compounds are from anthropogenic sources, while just a few of them are occurring naturally at small amounts. Among MPs are pharmaceuticals and personal care products (PPCPs), which include a wide range from antibiotics over antipyretic to active hormone substances. Many of those substances do not totally metabolize during medical treatment and are therefore released into the environment through different pathways (Ternes and Joss 2006, Figure 1). The most prevalent entry path is through the sewer system/sewage treatment plant into natural water bodies, as only a small percentage of these MPs are removed effectively during wastewater treatment (Umweltbundesamt 2018).

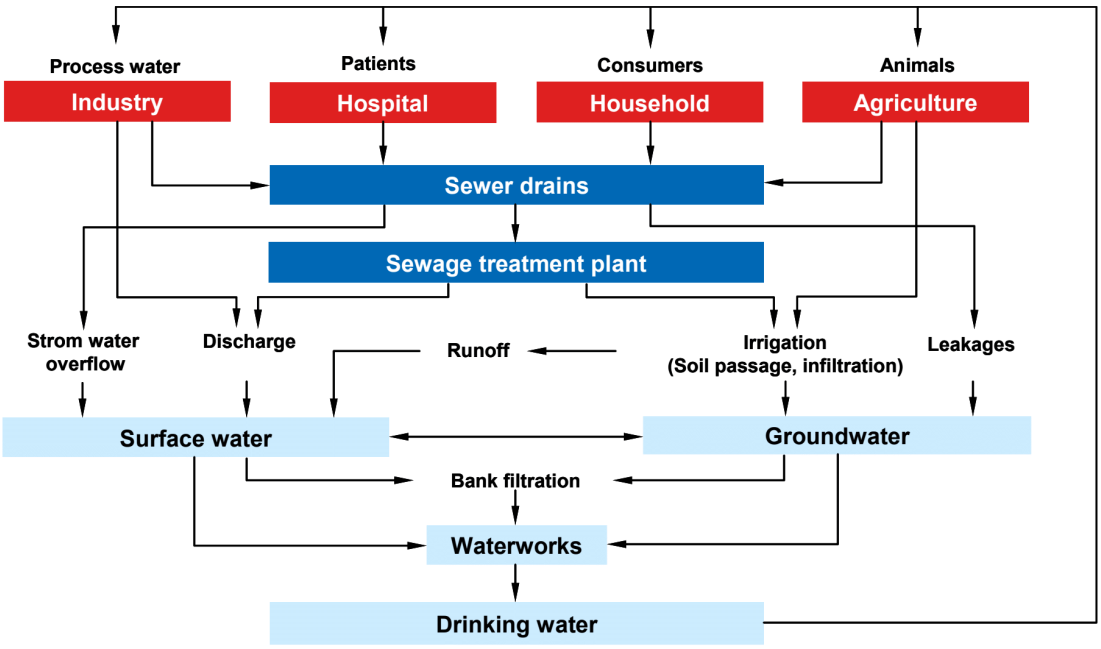

Figure 1: Microcontaminants - pathways into the water cycle (Ternes and Joss 2006)

Through leakages in pipes, overflow, or untreated discharge, which unfavourably is the case in many developing countries, MP's further enter into the environment and lead harmful impact to aquatic ecosystems. Microorganism, macrozoobenthos and fish, and humans have shown significant changes in their metabolism after exposure to certain substances (Fent 2013). Some frequent disorders can be observed in fish through endocrine-disrupting chemicals, changing the hormone

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system and causing infertility of individuals or a drift in sex of populations (Fent 2013). Other MPs induce specific diseases or show an increase of different enzymes in the organisms, indicating a stress factor. The occurrence of chronic diseases from most MP's is not yet verified for human beings, as research of the mixture is very demanding (Fent 2013). Another problem is a rising resistance against antibiotics, which is caused through a steady abundance of antibiotics in the environment that leads target organisms to adapt towards these drugs and lead to their immunity (Gandra et al. 2016).

Nowadays in most parts of the world, a tertiary and quaternary treatment step of the effluent following a two-stage water treatment plant is not obligatory. Lately, the European Union is debating about new standards and Switzerland introduced regulations to remove up to 80 % of the MP's until 2030 (Swiss Confederation 2020). So far, there are only a few WWTP's with a quaternary treatment step, as additional protection for sensitive areas, like endangered wildlife in rivers and lakes. Or when wastewater has to be fit for irrigation and recreational purposes (Gerba & Pepper 2019).

On the other side, most of the drinking water treatment plants are equipped with sufficient measures to remove MPs making use of soil passage, chemical oxidation, or adsorption (Bixio et al. 2006).

2.2.2. PESTICIDES

The intensive use of pesticides in India has led to widespread contamination of the biotic as well as the abiotic environment in India (Yadav *et al.* 2015). Pesticides were found in surface water and groundwater (Lari *et al.* 2014; Mutiyar, Mittal & Pekdeger 2011; Sankararamakrishnan, Sharam & Sanghi 2005; Skiwar *et al.* 2014). A multitude of pesticides have been identified as endocrine disrupting chemicals (EDC). Mnif *et al.* (2011) has compiled an overview of 105 substances and their effect on the hormone system of humans. The combined effects of pesticides are a major health risk to humans. In addition, wildlife is particularly vulnerable to the toxic and endocrine effects of pesticides.

In India, 76% of the used pesticides are accounted for as insecticides, 13% as fungicides and 10% as herbicides. In cotton and paddy cultivation, more than 50% of the pesticides are used. Depending on the climatic conditions and the agriculture in different states in India, the pesticide consumption can vary strongly from one state to another. Uttar Pradesh, Maharashtra and Andhra Pradesh are the states with the highest pesticide consumption (Yadav *et al.* 2015). Based on the precautionary principle, the European Drinking Water Directive (98/83/EC) has set a drinking water limit of 0.1 µg/L for single pesticides (with exception of aldrin, dieldrin, heptachlor and heptachlor epoxide where the limit for the single compound is 0.03 µg/L) and 0.5 µg/L for the sum of all active pesticides detected. India has set individual values for 18 substances in the Indian Standard Drinking Water Specification as shown in the Table below (Table 3). It can be seen that the European standards are much stricter and less compound specific than the Indian threshold values.

In general, the mobility of pesticides and thus their risk of leachability into the groundwater have been correlated with a weak adsorption of the soil matrix quantified in terms of a small soil organic carbon-water partitioning coefficient (K_{OC}) (Arias-Estévez *et al.* 2008). Generally, pesticides with K_{OC} $\leqslant$1,000 are potentially leaching compounds whereas pesticides with $K_{OC} \geqslant$ 1,000 have also been found in the groundwater.

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*Table 3: Pesticide residue limits according to the Indian drinking water guidelines (*Indian Standard Drinking Water - Specification (second revision) *2012)*

Pesticide	Limit [µg/L]
Alachlor	20
Atrazine	2
Aldrin/Diedrin	0.03
Alpha HCH	0.01
Beta HCH	0.04
Butachlor	125
Chlorpyriphos	30
Delta HCH	0.04
2,4- Dichlorophenoxyacetic acid	30
DDT (*o, p* and *p, p* – Isomers of DDT, DDE and DDD)	1
Endosulfan (alpha, beta, and sulphate)	0.4
Ethion	3
Gamma - HCH (Lindane)	2
Isoproturon	9
Malathion	190
Methyl parathion	0.3
Monocrotophos	1
Phorate	2

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CHAPTER 3 ANALYSIS OF TRACE ORGANIC CONTAMINANTS

3.1. SELECTED COMPOUNDS

To assess the adsorption capacity of applicable adsorbents, a set of indicator substances was selected (Table 4). MP's detected in Indian water bodies were evaluated and substances with elevated concentration, distinct polarities and dissociation constants were chosen to be measured with the LC-MS for further adsorbents evaluation. For the ease of the measurement, the used chemicals show distinct dissociation constants, which indicates different retention times on the column and therefore offer proper separation within the chromatogram (Bade et al. 2015). The set of chemicals also links to the LOTUS^{HR} subproject 1b, led by Prof. G. Medema (TU Delft) and Dr. Nagarnaik (NEERI, Nagpur). It is further planned to synchronize the methods used in "Pavitra Ganga" with the parallel EU-India H2020 Project "PANI WATER" where the micropollutant measurement is supervised by Prof. Despo Fatta-Kassinos from the University of Cyprus and links further to the EU NORMAN network.

Table 4: Physiochemical properties of the selected compounds

Category	Core parameter
Antibiotics	• Ciprofloxacin • Erythromycin • Sulfamethoxazole • Trimethoprim
Antiepileptic	• Carbamazepine
Analgesics	• Diclofenac • Naproxen
Antidiabetics	• Metformin (with internal standard)
Betabockers	• Atenolol
Personal care products	• Methylparaben • Triclosan
Pesticides (preliminary selection)	• Atrazine • Butachlor • Dichlorvos • Phorate

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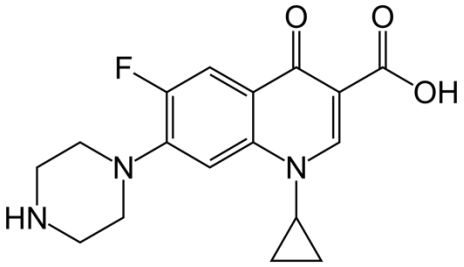
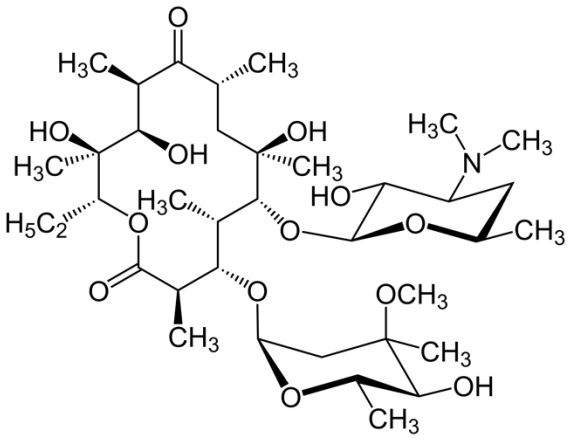
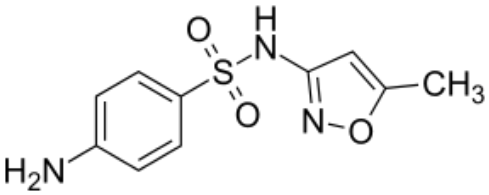
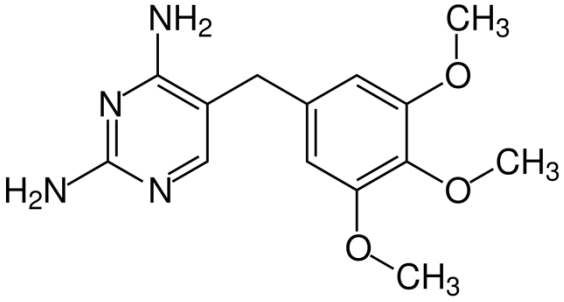
Table 5: Chemical properties of investigated Substances

Substances Formula Application Distribution coefficient	Typ. Conc in Indian wastewater effluent [ng/l]	Mass [g/mol]	Chemical Structure
Ciprofloxacin $C_{10}H_{11}N_3O_3S$ Antibiotic log K_{ow} = 0.28	N/A	331.34	
Erythromycin $C_{10}H_{11}N_3O_3S$ Antibiotic log K_{ow} = 3.06	N/A	733.93	
Sulfamethoxazole $C_{10}H_{11}N_3O_3S$ Antibiotic log K_{ow} = 0.89	400	253.8	
Trimethoprim $C_{10}H_{11}N_3O_3S$ Antibiotic log K_{ow} = 0.91	N/A	290.32	

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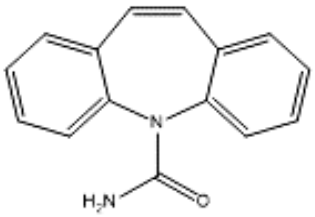
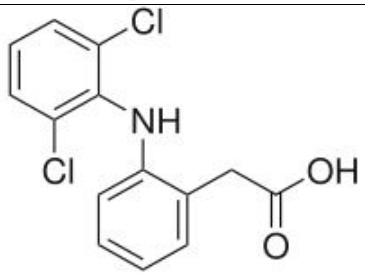
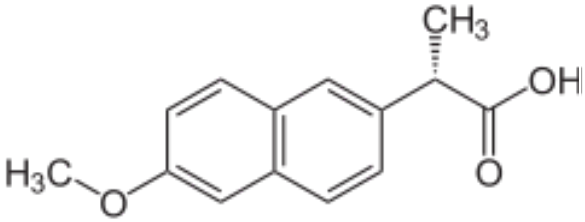
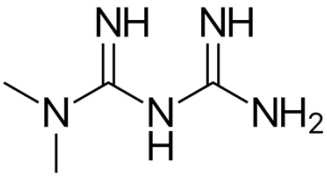
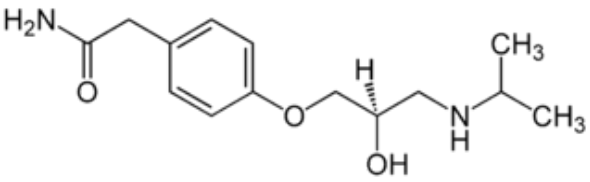
Substances Formula Application Distribution coefficient	Typ. Conc in Indian wastewater effluent [ng/l]	Mass [g/mol]	Chemical Structure
Carbamazepine $C_{15}H_{12}N_2O$ Anticonvulsant log K_{ow} = 1.51	500	236.27	
Diclofenac $C_{14}H_{11}Cl_2NO_2$ anti-inflammatory drug log K_{ow} = 4.51	500	296.15	
Naproxen $C_{14}H_{14}NaO_3$ nonsteroidal anti-inflammatory drug log K_{ow} = 3.18	400	252.25	
Metformin $C_4H_{11}N_5$ antidiabetic log K_{ow} = -2.645	N/A	129.167	
Atenolol $C_{14}H_{22}N_2O_3$ betablocker log K_{ow} = 0.16	1400-2900	266.34	

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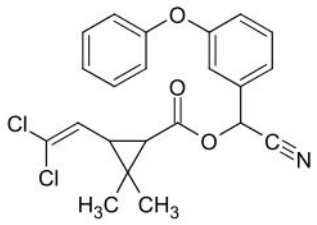
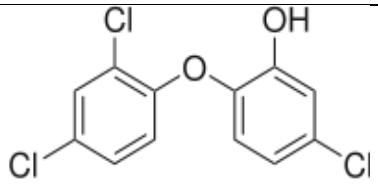
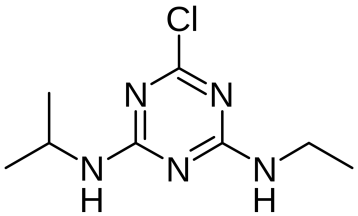
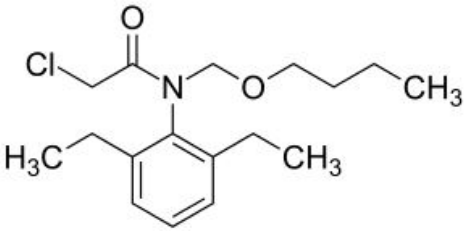
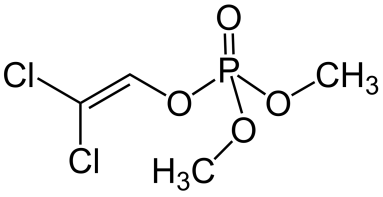
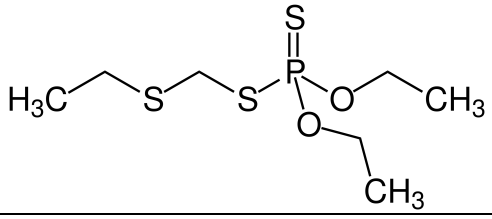
Cypermethrin $C_{22}H_{19}Cl_2NO_3$ insectiside log K_{ow} = 6.6	N/A	416.3	
Triclosan $C_{12}H_7Cl_3O_2$ Antibacterial agent log K_{ow} = 4.76	500	289.53	
Atrazine $C_8H_{14}ClN_5$ herbicide log K_{ow} = 2.61	N/A	215.68	
Butachlor $C_{17}H_{26}ClNO_2$ herbicide log K_{ow} = 4.5	N/A	311.85	
Dichlorvos $C_4H_7Cl_2O_4P$ Insecticide log K_{ow} = 1.43	N/A	220.97	
Phorate $C_7H_{17}O_2PS_3$ Insecticide log K_{ow} = 3.56	N/A	260.38	

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3.2. SOLID PHASE EXTRACTION - SPE

To remove unwanted compounds and minimize their effects, the samples undergo an appropriate **solid-phase extraction** (SPE) with, reducing the background matrix interfering with the measurement of the trace organic compounds. Therefore, the stored samples are prefiltered at room temperature (100 µm glass filter, MN GF-5), pH adjusted with either H_2SO_4 or NaOH (pH = 7,5 ±0.05) and 10 µl of an **internal standard** is added.

The internal standard (IS) is composed of three marked deuterated references that represent the chosen OMPs over the whole range of retention times. These IS were added at a known concentration of 0.1 µg/L and accordingly, OMPs' loss from samples through the SPE can be calculated. The following ISs are selected:

- **(S)-Naproxen-d3**
  - **MW:** 233.28 g/mol
  - $C_{14}H_{11}D_3O_3$
  - 1.0 mg in 1 ml methanol solution
- **Atenolol-d7**
  - **MW:** 273.38 g/mol
  - $C_{14}H_{15}D_7N_2O_3$
  - 1.0 mg in 1 ml methanol solution
- **Carbamazepine-d8 (Major)**
  - **MW:** 244.32 g/mol
  - $C_{15}H_4D_8N_2O$
  - 1.0 mg in 1 ml methanol solution

After adding the standard, the samples are well mixed prior connection to the SPE-System, seen in Figure 2. Before connecting the samples to the system, the cartridges for extraction require conditioning:

The cartridges (HBLC- Oasis HLB 6 cc) are charged with 2 ml heptane, 2 ml acetone, 6 ml methanol and 8 ml Nanopure. All the solvents are HPLC-grade. The cartridge conditioning is conducted without any pressure set up.

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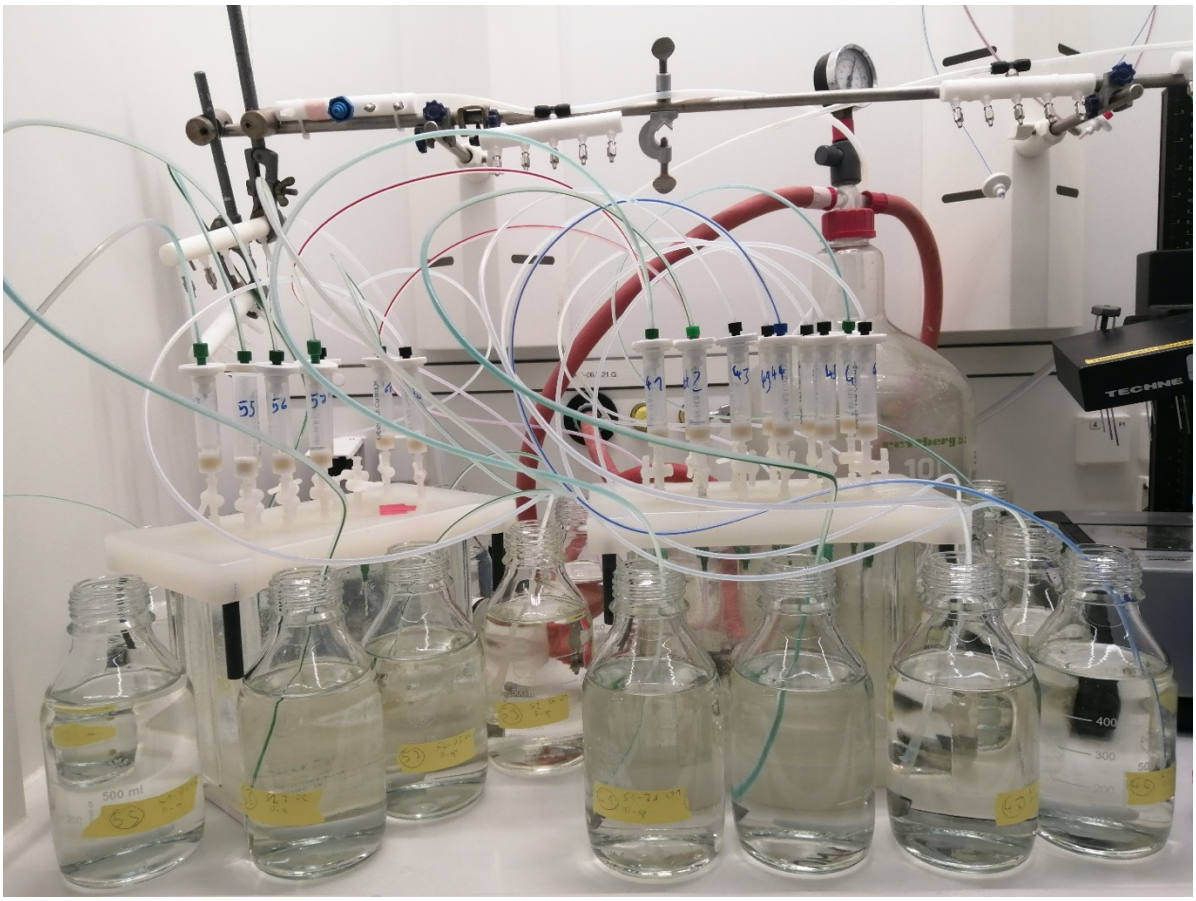

Figure 2: Solid-phase extraction set up with attached cartridges during sample

The samples are extracted by a suction pipe onto the cartridges installed on the SPE-manifold. The attached vacuum pump is set at about 400 mbar to reach a flow of about 10 ml per minute, which relates to about four drops per second. After complete extraction the sample bottles are rinsed with 20ml Nanopure and the cartridges are gently dried with nitrogen at around 1.5 bar.

The dried cartridges are eluted with 4 ml methanol (HPLC-grade) into glass vials and the eluate was vaporized at 32.4 degrees under nitrogen atmosphere. Subsequently, the analytes were redissolved with 300 µl methanol and transferred into vials ready for LC-MS.

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3.3. HPLC WITH MASS SPECTROMETRY (LC-MS)

Based on a literature review of methods used for MP detection in wastewater, a new method following the proceedings of an existing procedure from literature was developed (Vanderford and Snyder 2006; Li et al. 2019). For the measurements of the samples, a liquid chromatography (LC) (Agilent HPLC with a 6495 Triple Quad LCMS) is used (Figure 3).

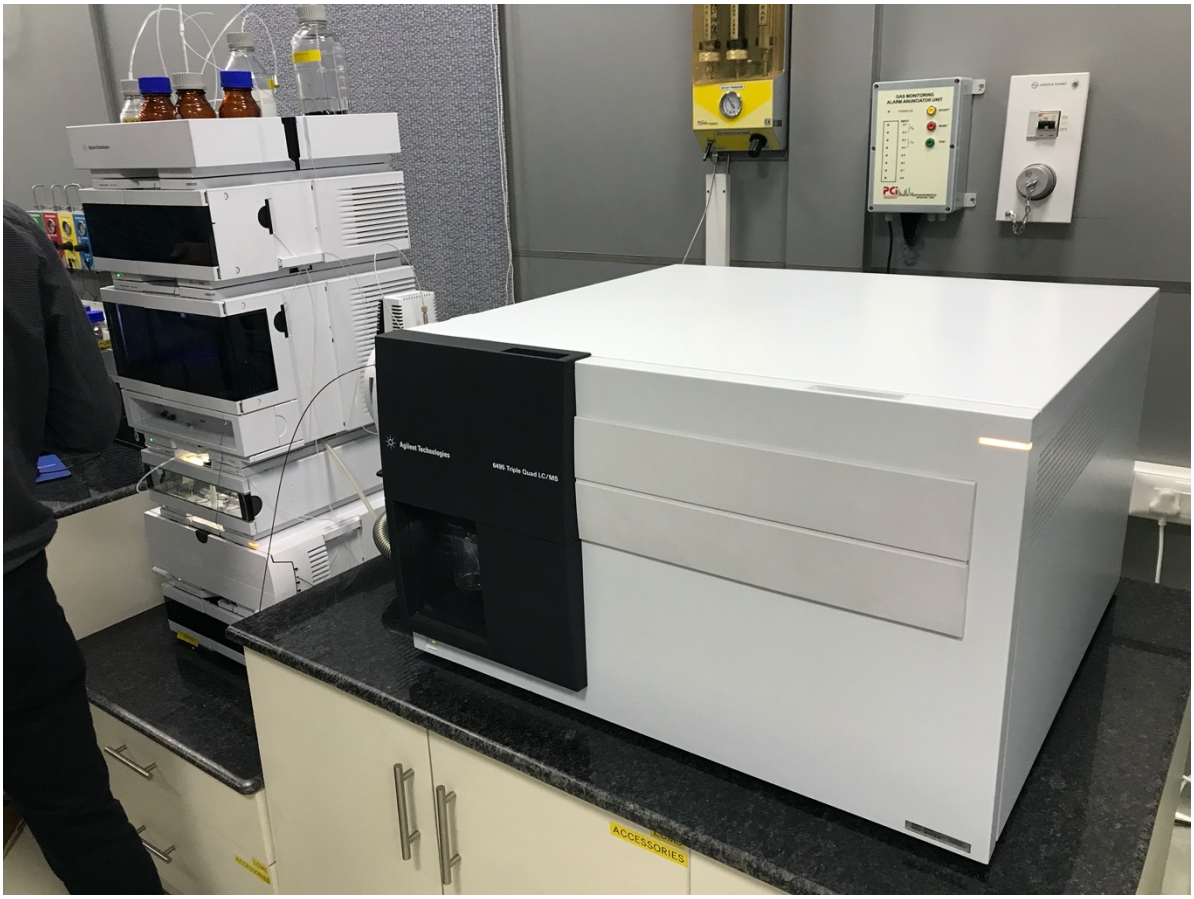

Figure 3: 1 Liquid chromatography-mass spectrometry unit (Agilent HPLC with a 6495 Triple Quad LCMS) with Eluates at the IIT Delhi LOTUS HR laboratory.

The LC is responsible for the separation of the different substances within the samples, while the MS offers identification and quantification of the occurring substances. These instruments are very sensitive; a pre-treatment of the samples had to be conducted, as the matrices of the wastewater samples are highly loaded with organic constituents.

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The selected method relies on the publication from EAWAG, Zurich, CH by Ju et al. (2018) for liquid chromatography triple quad mass spectrometry (LC-MS/MS) with electrospray ionization (verbatim):

"First, all 47 frozen samples were thawed at room temperature (6h) and 5 mL of each sample was transferred into a centrifuge vial for centrifugation at 4000 rpm for 30 min at 22°C. Then, 1000 µL supernatant was transferred into a 1.5 mL sample vial and 40 µL of a mixture with internal standards (IS) (400 ng/L final concentration) was add to each sample. Large volume direct injection of 100 µL sample was thereafter performed on an Agilent 1290 HPLC equipped with a Agilent 6495 triple quad mass spectrometer. The HPLC was operated at a flow rate of 500 µl/min, oven temperature of 30°C, with a gradient of 100% eluent A (nanopure water plus 0.1% Formic acid) to 95% eluent B (MeOH plus 0.1% Formic acid) in 18.5 minutes, hold for 3.5 minutes and rise to 100% eluent B in 0.5 minutes. The average relative recoveries of the pharmaceuticals ranged from 69% to 120% for compounds with labeled IS and 34% to 160% for the ones without labelled IS. The limits of quantification (LOQ) were between 1 and 70 ng/L, except for metformin (between 250 and 400 ng/L).

The 18 pharmaceuticals detected include (I) three macrolides, namely clarithromycin (76-460 ng/L), azithromycin (46-310 ng/L) and erythromycin (8-450 ng/L), (II) one lincosamide, that is clindamycin (13-76 ng/L), (III) five fluoroquinolones including ciprofloxacin (42-1600 ng/L), norfloxacin (7-640 ng/L), levofloxacin (5-170 ng/L), moxifloxacin (1-200 ng/L) and ofloxacin (5-170 ng/L), (IV) four sulfonamides including sulfamethoxazole (20-370 ng/L), N4-acetylsulfamethoxazole (2-1500 ng/L), sulfapyridine (3-180 ng/L) and sulfadiazine (0-18 ng/L), and (V) five other pharmaceuticals, namely vancomycin (0-370 ng/L), trimethoprim (35-300 ng/L), metronidazole (24-290 ng/L), triclosan (49|-740 ng/L), and metformin (380-120000 ng/L)." (Ju et al. 2018)

The method development at IIT D lab is ongoing and was interrupted in March 2020 when the University Campus was evacuated due to Covid-19.

It is foreseen to closely cooperate with experienced analytical partners in Europe such as University Cyprus, FHNW Basel and other reference labs.

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CHAPTER 4 HEAVY METAL MEASUREMENT

Heavy metal measurement is conducted by a range of methods:

- High-resolution inductively coupled plasma mass spectrometry (Agilent Technologies 7900 ICP-MS) available at the IIT Delhi laboratory (Figure 4).
- Microwave plasma - atomic emission spectrometry (Agilent MP-AES 4200), available at the IIT Kanpur laboratory (Figure 5).

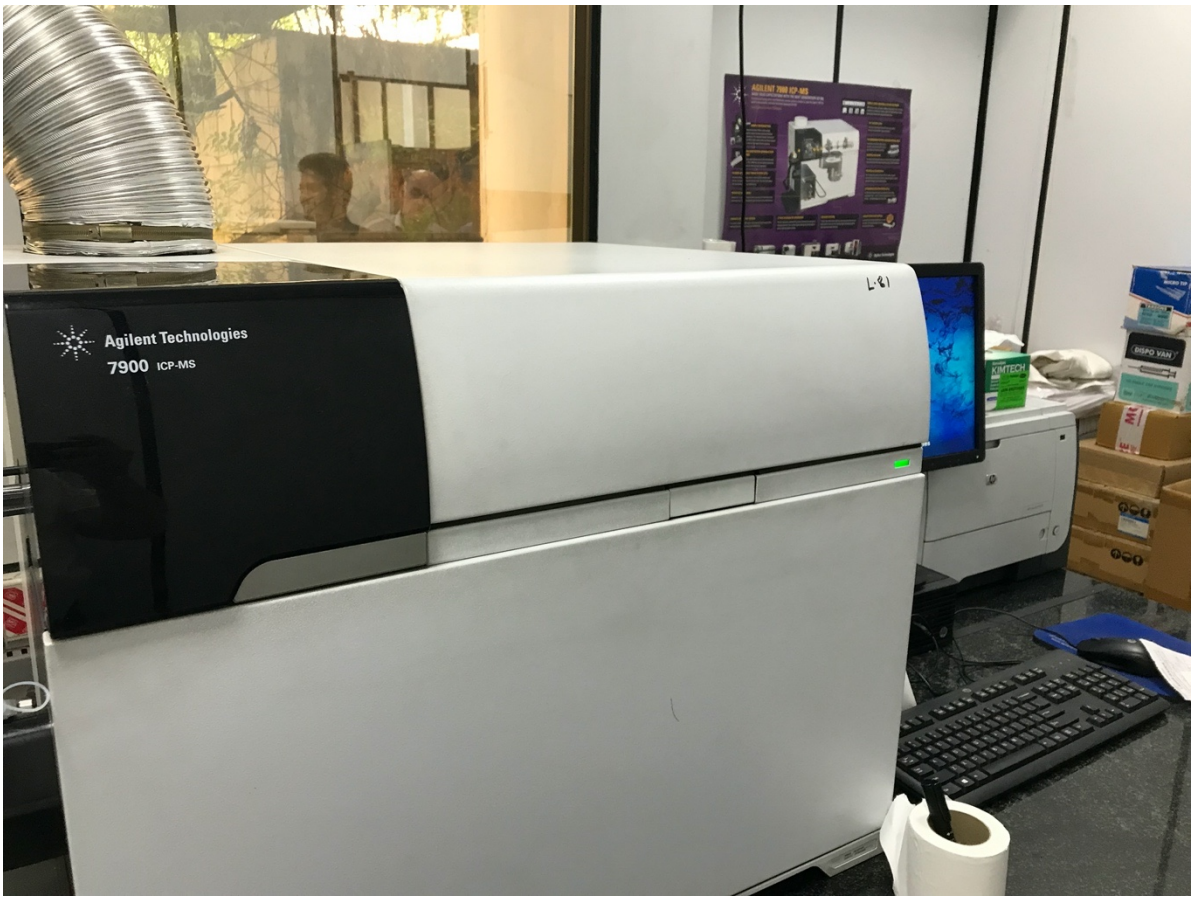

Figure 4: 1 Agilent Technologies 7900 ICP-MS) available at the IIT Delhi laboratory.

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Figure 5: Microwave plasma - atomic emission spectrometry (Agilent MP-AES 4200) at the IIT

Sample preparation and analytical methods follows the established protocols at the respective labs as published earlier.

Details of the adapted methods will be reported in the updated version of 5.2 when running the samples from the project with tailored procedures after re-opening the labs post COVID-19.

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