boDEREC-CE
WORKPACKAGE T2

O.T2.5 PILOT ACTION LJUBLJANA BASIN

VERSION 1

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Table of contents

1. Introduction 3

2. Pilot site characteristics 3

2.1. Geographical and hydrological conditions 3

2.2. Water treatment techology .... Error! Bookmark not defined.

2.3. Socio – economic conditions and main end users 6

3. Monitoring methodology and available data 8

3.1. Sampling and laboratory analysis 9

3.2. Objective of monitoring 11

3.3. Sampling 11

4. Monitoring results 13

4.1. Water resource .... Error! Bookmark not defined.

4.2. Water treatment efficiency 16

5. Conclusion 19

6. References 19

1. Introduction

The basic condition for the elimination of any type of pollution is sufficient knowledge of its properties, origin, behaviour in the natural environment and response to various types of technical interventions. The knowledge and findings obtained in the boDEREC-CE project are supported by new data obtained mainly through project monitoring activity, focused on detailed documentation of temporal and spatial changes of PPCP concentrations in the pilot areas. The main objective of the T2 work package was to regularly monitor PPCP levels in drinking water and in raw waters (groundwaters or surface waters) used as a source for its production. Monitoring was performed on 8 pilot sites in different regions and hydrological conditions in Central Europe.

This report summarises the activities of the pilot action in Ljubljana basin in Slovenia. The monitoring system was developed to characterise the conditions in the Ljubljana basin, which represent an important drinking water resource for several cities and other settlements.

A significant part of the monitoring consisted of sampling and laboratory analysis, which were standardised within the boDERED-CE project, in order to better compare the results of the different project pilot actions. The data obtained through monitoring served as input the evaluation of attenuation in the natural environment and the effectiveness of different water technologies. Further processing of the data was done as a part of work packages T3 and T4, which include development of modelling tools, synthesis of results and dissemination activities.

2. Pilot site characteristics

2.1. Geographical and hydrological conditions

The Ljubljana basin with an area of 815 km2 lies in the upper Sava basin and is the largest closed plane in Slovenia (Figure 1). It is bordered on the north by mountains; the Julian Alps, the Kamnik-Savinja Alps and the Karavanke, on the west by the Škofja Loka and Polhov Gradec hills, on the east by the Posavje hills and in the south by the Menišija and Krim hills. The altitude of the basin is between 250 m and 730 m.

The Ljubljana basin is divided into 6 subunits: Dežela and Blejski kot (NW region), Dobrave (NW region), Kranjsko-Sorško polje (central NW region), Kamniško-Bistriška ravan (N region), Ljubljansko polje (central part) and Ljubljansko barje (southern part).

The discharge characteristics of the Sava River in the upper part (north of Radovljica) of the pilot area indicate an Alpine nival-pluvial regime; therefore, the highest discharges occur in the spring due to snowmelt and rain and in the autumn due to heavy rainfall. In the Ljubljana basin, the Sava River and its tributaries' discharge regime changes to Alpine pluvial-nival and remains the same throughout the rest of the flow in Slovenia. The average discharge is 40 m3/s in the upstream part of PA (Radovljica gauging station) and 85 m3/s in the downstream part. Long-term trends in the mean discharge of the Sava River show a slight decrease. The Sava River is a very important hydrodynamic element of surrounding aquifers because it substantially recharges some of them, an example of this is its recharge of Ljubljansko polje.

2.1.1. Geology and hydrogeology

The basin with its central position represents the most important settlement, economy and traffic area in Slovenia where the main roads and rail connections converge. The Ljubljana basin includes 31 municipalities and a total of 40% of Slovenian population lives here. The largest cities in the basin are Ljubljana (largest city in the Republic of Slovenia), Kranj, Kamnik, Domžale, Škofja Loka, Bled and Vrhnika.

According to the guidelines following the Water framework directive the pilot area is positioned in the same area as the groundwater body Savska kotlina – Ljubljansko barje (SIVTPODV1001 – Sava basin and Ljubljana Marsh). Inside of the groundwater body several aquifers are defined, which are predominantly intergranular.

The groundwater body is located inside the tectonic depression with predominantly fluvial-glacial sediments of the Sava River (Figure 2). These deposits consist of Quaternary gravelly sandy sediments, which are in a significant percentage represented as conglomerates. Groundwater body and surface waters are interrelated in several places. In the northern and central part, Quaternary intergranular aquifer consisting of sandy gravel deposits of the Sava River and its surface tributaries predominates. Aquifer is extensive with medium to high yield. Second important aquifer is positioned in Mesozoic carbonate rocks as a karstic-fissured aquifer. It is positioned mainly on the rims of the groundwater body and in some parts extends bellow the Quaternary aquifer. It is extensive, however locally bounded with faults and other hydrogeological barriers. Consequently, it has varying yield, from low to high.

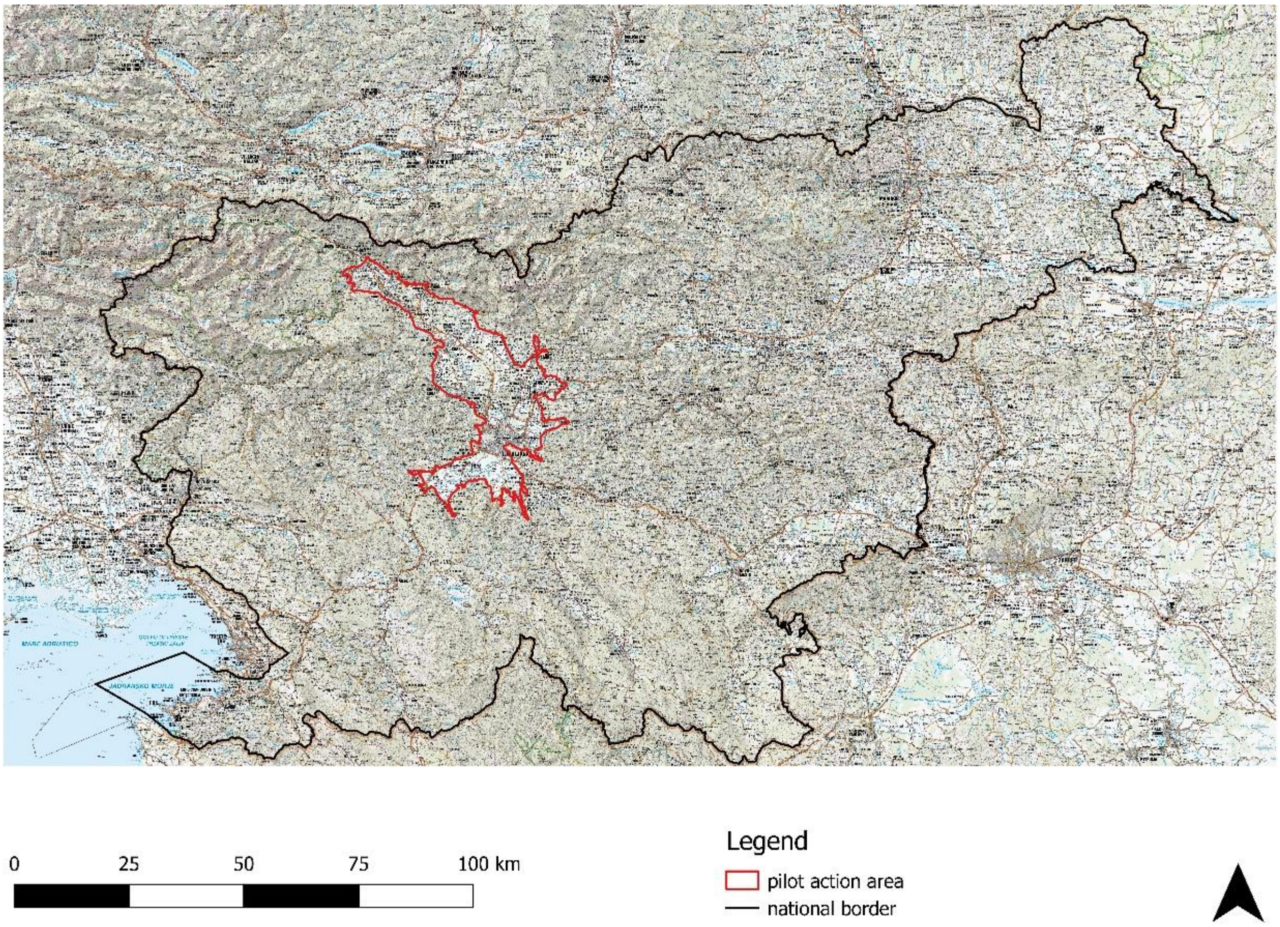

Figure 1: Position of pilot area – PA Ljubljana basin in Slovenia

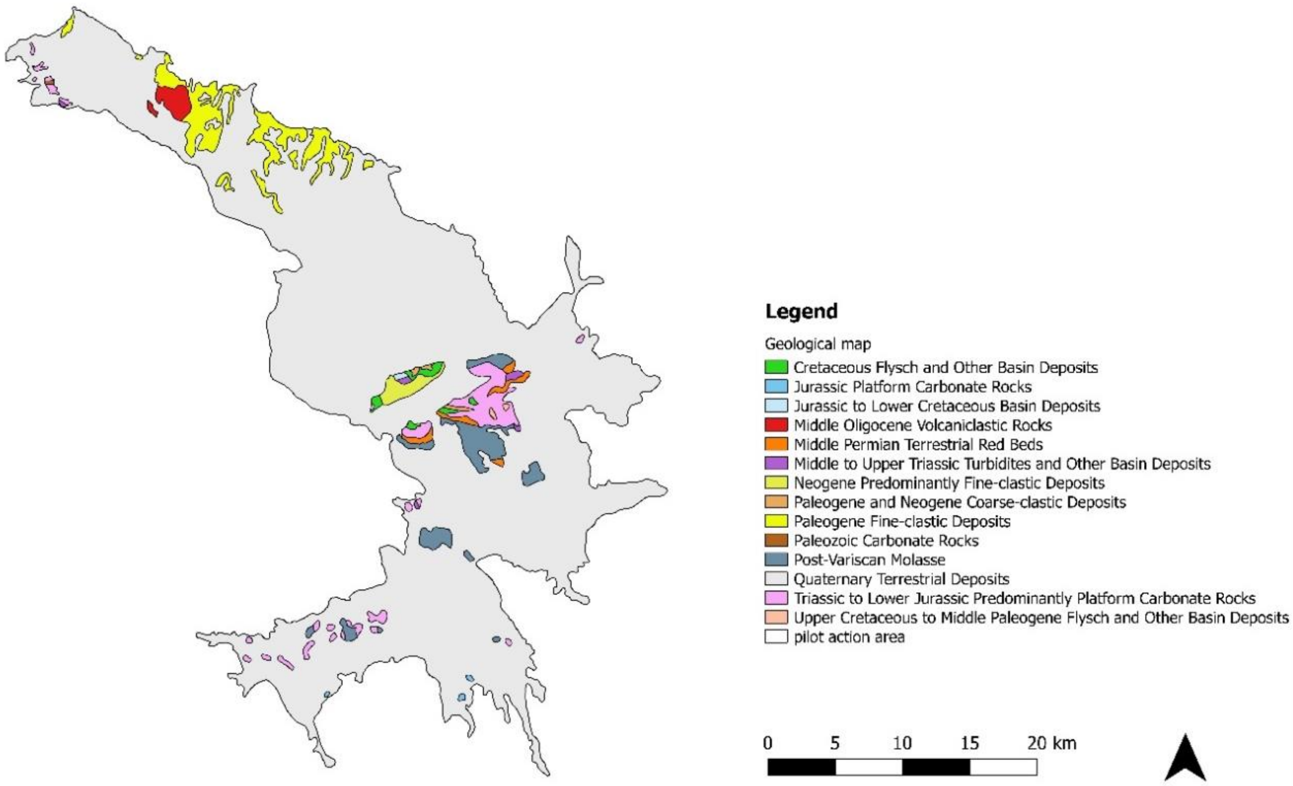

Figure 2: Lithostratigraphical map of the pilot area Ljubljana basin

2.1.2. Drinking water protection zones

Ljubljana basin is an important drinking water resource for several cities and other settlements. Drinking water from the region is provided also from the PA surroundings. Drinking water protection zones are illustrated in Figure 3. Drinking water protection zones in Slovenia are defined at two levels – national and municipal. In the PA, the national level areas are positioned north and south of Ljubljana. There are also drinking water protection zones at the municipality level between Ljubljana and Kamnik to the north-east. Several smaller protection zones are positioned in other parts of PA.

Drinking water protection zones determined on national or municipality level cover almost a quarter (24 %) of Ljubljana basin (Figure 3). On both levels, zone III occupies the largest area- 5 % at the municipal level and 14 % at the national level.

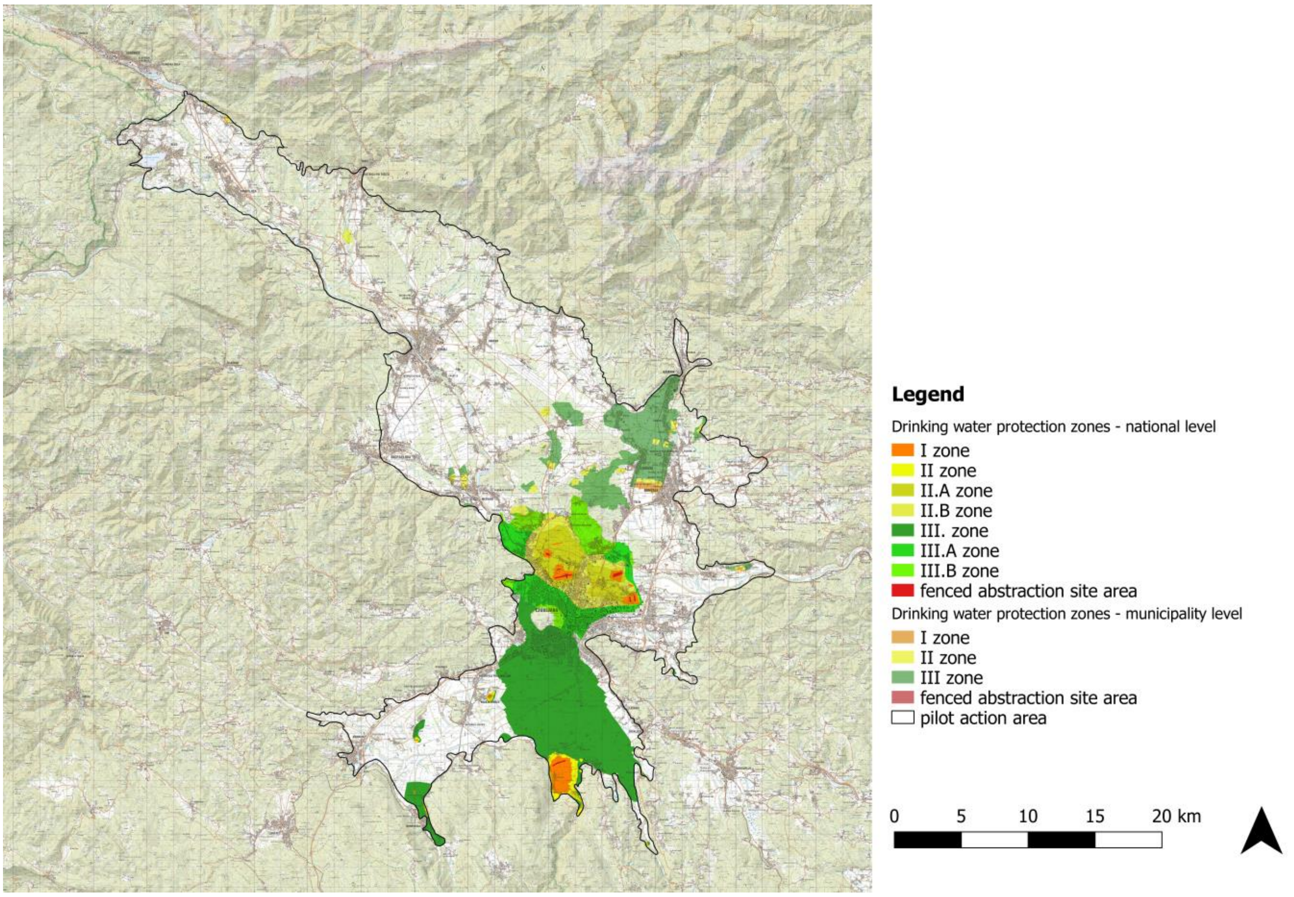

Figure 3: Drinking water protection zones in the pilot area

2.2. Technological conditions

On Mengeško Domžalsko polje water is captured by the water works system Domžale - Mengeš - Trzin which supplied 35.000 inhabitants. On Ljubljansko polje the groundwater is pumped in five water plants: Kleče, Hrastje, Šentvid, Jarški prod and Brest (Figure 4). The water that reaches users in Ljubljana comes from a natural environment, does not undergo technical treatments and is only chlorinated occasionally in water field Jarški prod and Brest. Water never stays in the water supply network for more than a couple of hours.

Central water distribution system includes: Five water field with 44 wells, 1152 kilometers of the water distribution network, 42.835 connections that supply 330.000users. In 2018 30.825.254 m3 of groundwater was pumped and 21.238.550 m3 drinking water was sold.

Drinking water complies with health regulations that are harmonized with European requirements. The water does not contain microorganisms, parasites or their developmental stages that are harmful to people's health. It also does not contain any substances that would be harmful either on their own or in combination with other substances.

Internal control of drinking water is performed in accordance with mandatory plan that is based on HACCP system principles. It enables us to control the entire system and recognize any microbiological, chemical and mechanical parameters that may represent a risk to people's health.

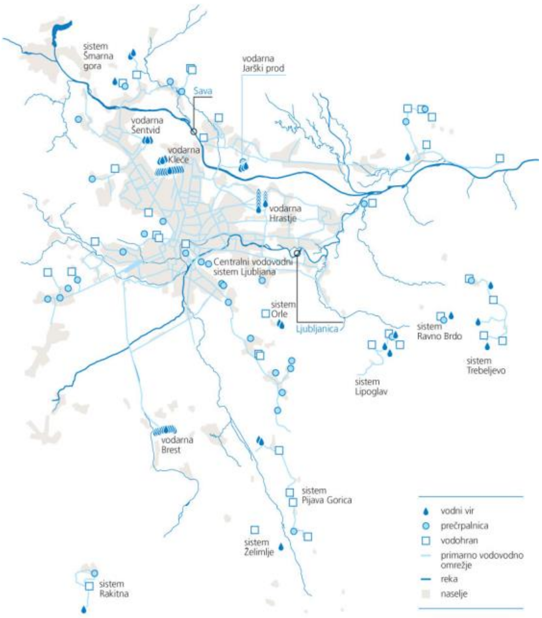

Figure 4: Drinking water supply system in Ljubljana and its surroundings

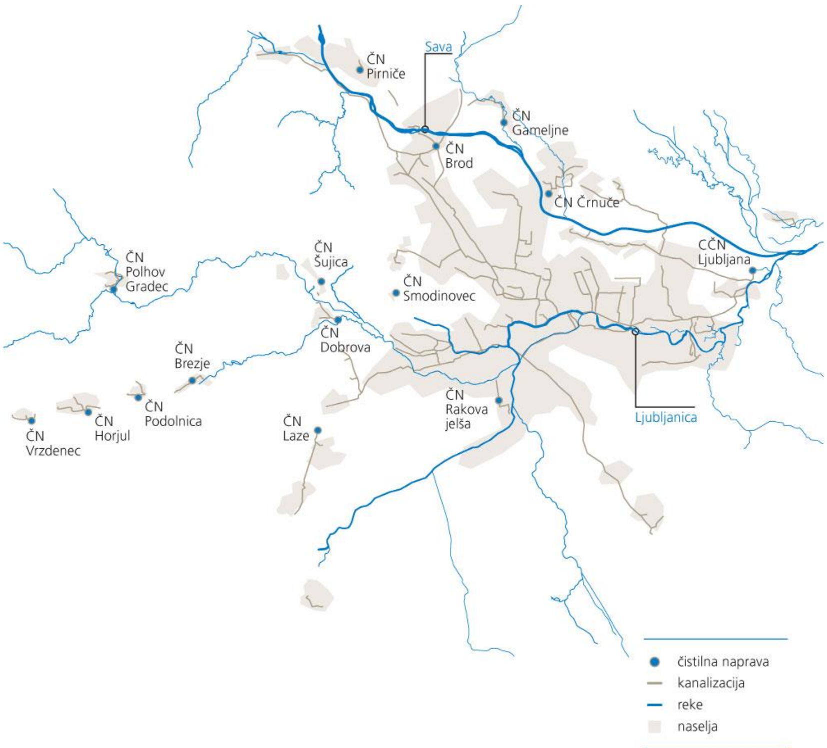

Figure 5: Wastewater system and treatment plants in the Ljubljana area, marked as ČN (WWTP) and CČN (CWWTP).

The central sewage system in Ljubljana terminates with treatment of waste water at the Central wastewater treatment plant Ljubljana, which has the capacity of 360.000 PE. The sewerage system has 27.100

connection and serves 31.600 buildings with 270.000 inhabitants and numerous industrial and commercial buildings (Figure 5).

The Central wastewater treatment plant in Ljubljana (CWWTP) treats 85 % of all wastewater from Ljubljana and its surroundings. CWWTP can treat between 80.000 and 100.000 cubic meters of wastewater per day, which substantially reduces pollution of Ljubljanica and Sava rivers. During the first stage, stone trap and coarse of fine strainers are used to remove physical waste, larger than 6 millimeters. Oil skimmer and sand trap are then used to remove fats and sand. In total, this process removes approximately 1.200 tons of waste annually. During biological treatment, aeration tanks with the volume of 39.000 m3 are pumped with compressed air and microorganisms in activated sludge decompose organic compounds that are dissolved in wastewater. In the following stage, settlers are used to separate activated sludge from treated water. Treated water is discharged into the river Ljubljanica.

2.3. Socio – economic conditions and main end users

JP Prodnik supplies five municipalities in the east of Ljubljana basin; the area known as Mengeško Domžalsko polje; all togheter 36.000 inhabitants.

JP VOKA SNAGA in Ljubljana represents the biggest Public Water Utility in Slovenia and supplies more than 330.000 inhabitants with drinking water. For this reason, the main goal is to maintain the quality of groundwater as it is now.

The drinking water in Ljubljana system is not treated and is directly distributed to the inhabitants. So, therefore the main priority is to continue to distribute clean, natural drinking water to the inhabitants.

Several stakeholders are present in the Pilot Action and among them there are also conflicts. It is and should be in our interest to solve, through communication and education, as many conflicts as possible and to encourage different professions / stakeholders to be cooperating with each other through one-on-one meetings and workshops.

3. Monitoring methodology and available data

3.1. Data availability

For the whole PA complete set of climatic data are available at main climatological stations (Brnik - airport, Ljubljana-Bežigrad) and eight meteorological stations on the Ljubljana basin and several more in the surrounding with at least of 30 years of data. All data are in public domain and freely available through the internet service. Furthermore, data on the river discharges are available at several locations along the Sava River (for example: Radovljica gauging station, 1953-2017 and Šentjakob gauging station 1926-2017). They are also freely available. Data are also available for groundwater measurements as well as data for the chemical status of groundwater. Few years ago, state monitoring started to follow also presence of PCCPs in groundwater. These data are also available.

For Ljubljansko polje, where the biggest water works system is under the operation as a management tool also mathematic model of groundwater flow is available. Also, all management data are at the disposal (pumping discharge, sold and distributed water, treated waste water, etc.).

3.2. Sampling and laboratory analysis

The boDEREC-CE monitoring on all project pilot sites was conducted according to common methodology. The analyses of the collected samples of surface and groundwater were carried out according to valid procedures and EPA method 1694 in the Vltava River Basin Authority laboratory.

Samples were collected in 60 mL amber glass vials (filled only halfway). The samples were stored in a freezer (in an inclined position). They were defrosted at a maximum temperature of 30 °C on the day of analysis. It was necessary to conduct the analysis immediately after defrosting.

One method was developed for the analysis of PPCPs (LC-MS/MS with combinated ESI+ and ESI- mode). The samples of water were centrifuged in headspace vials for 10 min at about 3500 rpm. Subsequently 1.50 g of each sample were weighed in a 2 mL vial on an analytical balance. Then 1.5 µL of acetic acid was added to each sample. An isotope dilution was performed in the next step. Deuterated internal standards of d10-carbamazepine, d6-sulfamethoxazole, d3-iopromide, d3-iopamidol, 13C2-erythromycin, d3-ibuprofen, d4-diclofenac, d3-naproxen, d5-chloramphenicol and others were used.

PPCPs were separated and detected by LC–MS/MS methods based on direct injection of the sample into a chromatograph. A 1290 ultra-high-performance liquid chromatograph (UHPLC) coupled with an Agilent 6495B Triple Quad Mass Spectrometer (MS/MS) of Agilent Technologies, Inc. (Santa Clara, CA, USA) were used.

Method; the separation was carried out on a Waters Xbridge C18 analytical column (100 mm x 4.6 mm, 3.5 µm particle size). The mobile phase consisted of methanol and water with 0.02 % acetic acid and 0.5 mM ammonium fluoride as the mobile phase additives. The flow rate was 0.5 mL/min. The injection volume was 0.050 mL.

The range of analysis and detection limit for each analyte is shown in the table below (Table 1).

Each series of samples were verified by calibration control and by maintaining a clean environment, equipment, and agents. The performance of the analytical system was ensured by blank and spiked samples. The chemicals used for the preparation of calibration solutions had a certified purity of 99%. Calibration solutions were prepared from neat analytes or from solutions with certified concentration. Each fifth sample in a series was processed by the method of standard addition, which was used to control the effect of the matrix of the sample and to reset the actual recovery ratio of a specific analyte. The measuring instruments were under regular control, and measuring vessels were metrologically tested.

The chemicals used were supplied from renowned manufacturers in the EU and USA: Dr. Ehrenstorfer GmbH (Augsburg, Germany), LGC Ltd. (Teddington, Middlesex, UK), Honeywell International Inc. (Morris Plains, NJ, USA), HPC Standards GmbH (Cunnersdorf, Germany), Absolute Standards Inc. (Hamden, CT, USA), CIL Inc. (Tewksbury, MA, USA), Analytika spol s.r.o. (Prague, Czech Republic).

Table 1: Analysed PPCPs

	Pharmaceuticals	unit	Detection limit
1.	1-H-benzotriazole	ng/l	20
2.	4(5)-methyl-1-H-benzotriazole	ng/l	20
3.	4-formylaminoantipyrine	ng/l	10
4.	acebutulol	ng/l	10
5.	acesulfame	ng/l	50
6.	alfuzosin	ng/l	10
7.	atenolol	ng/l	10
8.	atorvastatin	ng/l	10
9.	azithromycin	ng/l	10
10.	bezafibrate	ng/l	10
11.	bisfenol A	ng/l	50
12.	bisfenol B	ng/l	50
13.	bisfenol S	ng/l	50

14.	bisoprolol	ng/l	10
15.	butylparaben	ng/l	10
16.	caffeine	ng/l	100
17.	carbamazepine	ng/l	10
18.	carbamazepine 10,11-dihydro-10-hydroxy	ng/l	10
19.	carbamazepine 10,11-dihydroxy	ng/l	10
20.	carbamazepine 10,11-epoxide	ng/l	10
21.	carbamazepine 2-hydroxy	ng/l	10
22.	celiprolol	ng/l	10
23.	citalopram	ng/l	20
24.	clarithromycin	ng/l	10
25.	climbazole	ng/l	10
26.	clindamycin	ng/l	10
27.	clofibric acid	ng/l	10
28.	cotinine	ng/l	20
29.	cyclamate	ng/l	500
30.	cyclophosphamide	ng/l	10
31.	DEET	ng/l	10
32.	diatrizoate	ng/l	10
33.	diclofenac	ng/l	20
34.	diclofenac-4'-hydroxy	ng/l	20
35.	diltiazem	ng/l	10
36.	erythromycin	ng/l	10
37.	ethylparaben	ng/l	10
38.	fexofenadine	ng/l	10
39.	fluconazole	ng/l	10
40.	fluoxetine	ng/l	10
41.	furosemide	ng/l	50
42.	gabapentin	ng/l	10
43.	gemfibrozil	ng/l	10
44.	hydrochlorothiazide	ng/l	50
45.	chloramphenicol	ng/l	20
46.	ibuprofen	ng/l	20
47.	ibuprofen-2-hydroxy	ng/l	30
48.	ibuprofen-carboxy	ng/l	20
49.	iohexol	ng/l	50
50.	iomeprol	ng/l	50
51.	iopamidol	ng/l	50
52.	iopromide	ng/l	50
53.	irbesartan	ng/l	10
54.	ivermectin	ng/l	50
55.	ketoprofen	ng/l	10
56.	lamotrigine	ng/l	10
57.	lovastatin	ng/l	10
58.	memantine	ng/l	20
59.	metformin	ng/l	20
60.	methylparaben	ng/l	10
61.	metoprolol	ng/l	10
62.	mirtazapine	ng/l	10
63.	naproxene	ng/l	50
64.	naproxene-o-desmethyl	ng/l	20
65.	norverapamil	ng/l	10
66.	octyl methoxycinnamate (OMC)	ng/l	1000
67.	oxcarbazepine	ng/l	10
68.	oxypurinol	ng/l	50
69.	paracetamol	ng/l	10
70.	paraxanthine	ng/l	100
71.	peniciline G	ng/l	10
72.	PFOA (perfluorooctanoic acid)	ng/l	10
73.	PFOS (perfluorooctane sulfonic acid)	ng/l	5
74.	phenazone	ng/l	10
75.	primidone	ng/l	10
76.	propranolol	ng/l	10
77.	propylparaben	ng/l	10
78.	propyphenazone	ng/l	10
79.	ranitidine	ng/l	10
80.	roxithromycin	ng/l	10
81.	saccharin	ng/l	50
82.	salbutamol	ng/l	10
83.	sertraline	ng/l	10

84.	simvastatin	ng/l	10
85.	sotalol	ng/l	10
86.	sucralose	ng/l	500
87.	sulfamerazine	ng/l	10
88.	sulfamethazine	ng/l	10
89.	sulfamethoxazole	ng/l	10
90.	sulfanilamide	ng/l	50
91.	sulfapyridine	ng/l	10
92.	telmisartan	ng/l	20
93.	tiamulin	ng/l	10
94.	tramadol	ng/l	10
95.	triclocarban	ng/l	10
96.	triclosan	ng/l	20
97.	trimetoprim	ng/l	10
98.	valsartan	ng/l	10
99.	valsartan acid	ng/l	10
100.	venlafaxine	ng/l	10
101.	verapamil	ng/l	10
102.	warfarin	ng/l	10

3.3. Objective of monitoring

The obtained results will give us general insight into the spatial distribution of emerging contaminants in the Ljubljana basin and where the possible sources of the contamination are. As well as temporal variability of EC occurrence at the two most important points in the PA. In the pilot area of Ljubljana Basin (Ljubljanska kotlina), surface water samples were collected together at three locations on the Ljubljanica and Sava rivers: Ljubljanica, Sava Medno, and Sava Dolsko (Figure 6). All three sites are located in the central part of the pilot area, near Ljubljana, the largest city in Ljubljana Basin. The Ljubljanica sampling site is located on the southern outskirts of Ljubljana and is an indicator of pollution in the southern part of the pilot action area, which is part of the Ljubljansko barje (Ljubljana marsh). The Sava Medno sampling point is located north of Ljubljana and is an indicator of pollution in the northern part of the pilot action area. The Sava Dolsko sampling point is located east of Ljubljana, at the exit of the pilot area. It is an indicator of the presence of pollutants in the City of Ljubljana and, consequently, in the whole Ljubljana Basin.

3.4. Sampling

Samples were taken one a month from October 2020 to June 2021 at three surface water locations; Ljubljanica in front of Ljubljana, Sava River Medno and Sava River Dolsko and in total 36 samples were taken.

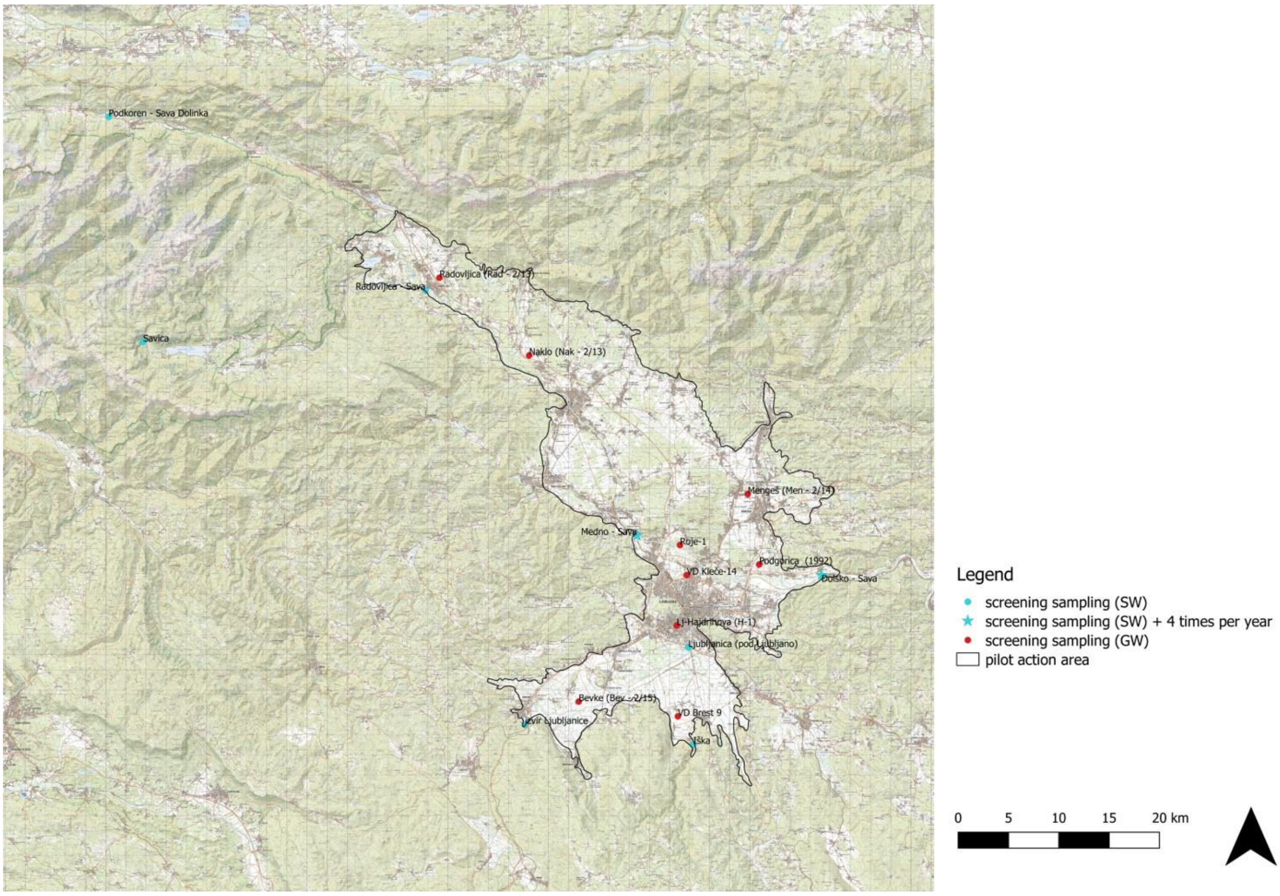

Figure 6: Sampling points in Ljubljana basin

Individual sampling points are characterised as follows:

Location Sava Medno	
Objective: to obtain information on the quality of surface water upstream of the Ljubljana city.	
Method of sampling: sampling directly from surface water	

Location Ljubljanica	
Objective: to get information on surface water quality	
Method of sampling: sampling directly from surface water	

Location Sava Dolsko	
Objective: to get information on surface water quality, where all the water from the pilot area is gathered	
Method of sampling: sampling directly from the surface water	

4. Monitoring results

According to the sampling results, 32 different compounds were detected in the entire area of the pilot action in the Ljubljana Basin. The largest number of different compounds (29) was detected at the Sava Dolsko sampling point, followed by Sava Medno (21) and Ljubljanica (19).

Figure 7 shows all the compounds detected at all three analysed sampling points. It illustrates at which sampling site a single compound occurred and, in addition to the total number of detections, how often it occurred at each sampling site. The most frequently detected was metformin (35 times), which is a drug used to treat type 2 diabetes. It is followed by paracetamol, which was detected 27 times and is used to relieve pain and reduce fever. 4-formylaminoantipyrine, 1H-benzotriazole, and valsartan were also detected more than 20 times.

If we also look at how often a single compound was detected at a single sampling site, we find that compounds are repeated the least frequently at the Ljubljanica sampling site and the most frequently at the Sava Dolsko sampling site. This indicates that the southern part is less polluted than the rest of the pilot area. 10 compounds were detected only at the Sava Dolsko sampling site. These compounds are acesulfame, atorvastatin, clarithromycin, diclofenac, fexofenadine, furosemide, hydrochlorothiazide, ketoprofen, oxypurinol, and telmisartan. Atorvastatin, fexofenadine, and telmisartan were the most common. All ten of these compounds are active ingredients in various pharmaceuticals, thus showing the urban impact or the impact of pollutants on the waters of the City of Ljubljana.

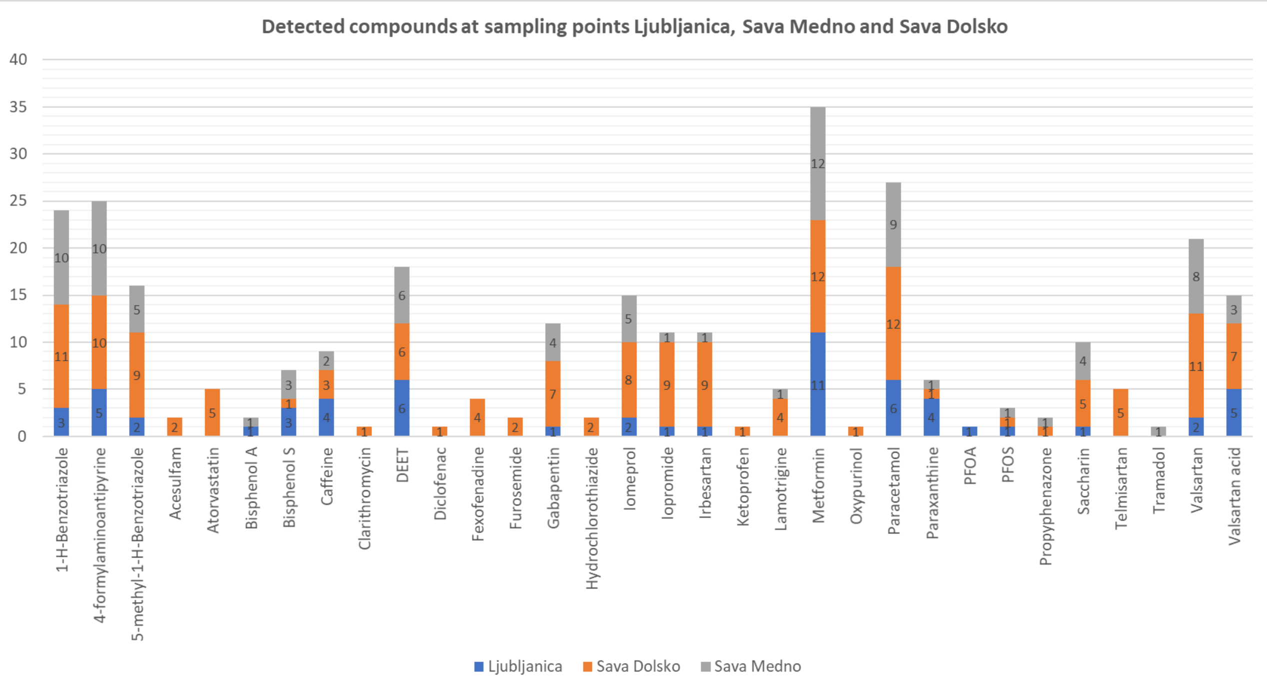

Figure 7: Detected compounds at the sampling points Ljubljanica, Sava Medno, and Sava Dolsko

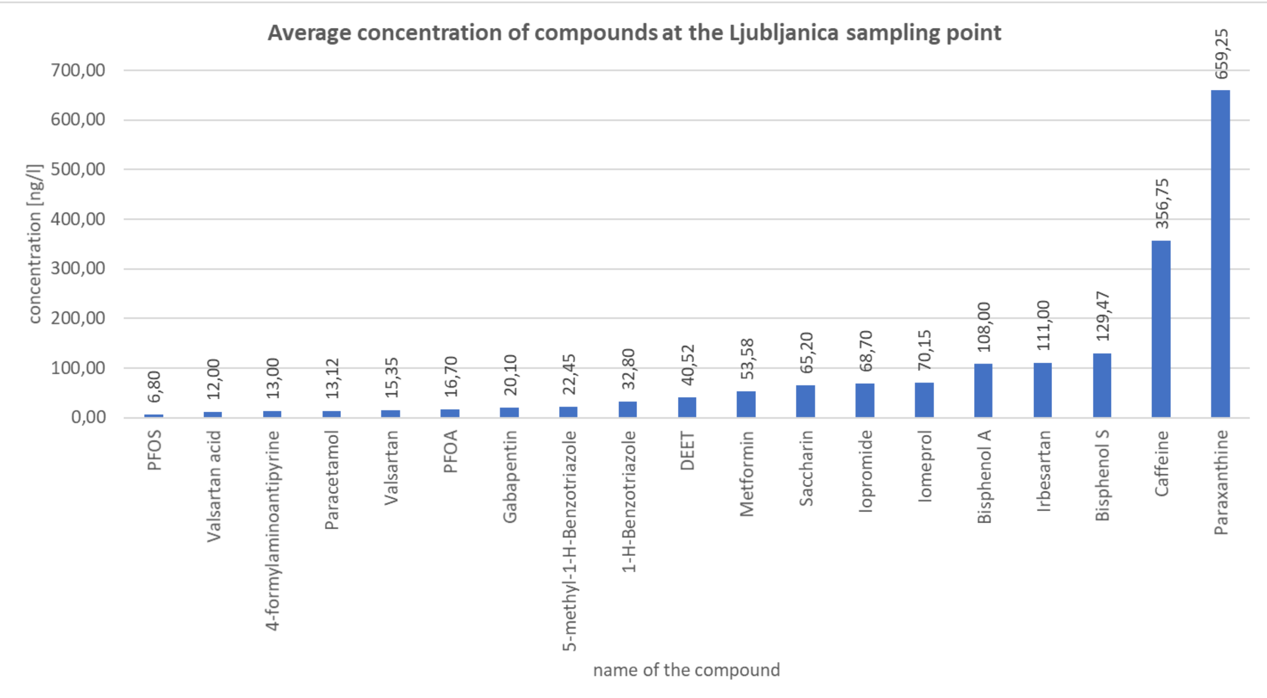

Figure 8: Average concentration of compounds at the Ljubljanica sampling point

Figure 8 shows the average concentration of each compound at the Ljubljanica sampling site. Paraxanthine and caffeine occur in the highest concentrations. The mean concentration for paraxanthine is 659.25 ng/L and for caffeine 356.75 ng/L. Caffeine is a very common substance in the environment due to its widespread use. It is present in the environment at concentrations below 1 µg/L due to its good degradability. It can be used to assess the proximity of a pollution source. The presence of caffeine in the surface or groundwater is a direct evidence of anthropogenic pollution of the environment; its presence may indicate recent pollution from municipal sewage. Paraxanthin is the major metabolite of caffeine (Jamnik et al., 2009). PFOS (6.8 ng/L) has the lowest concentrations at the Ljubljanica sampling site.

As at the Ljubljanica sampling site, paraxanthine and caffeine occur in the highest concentrations at the Sava Medno sampling site (Fig. 9). Paraxanthin was detected only once with a concentration of 1000 ng/L and caffeine twice with an average concentration of 429 ng/L. PFOS (9 ng/L) also appears here with the lowest concentrations.

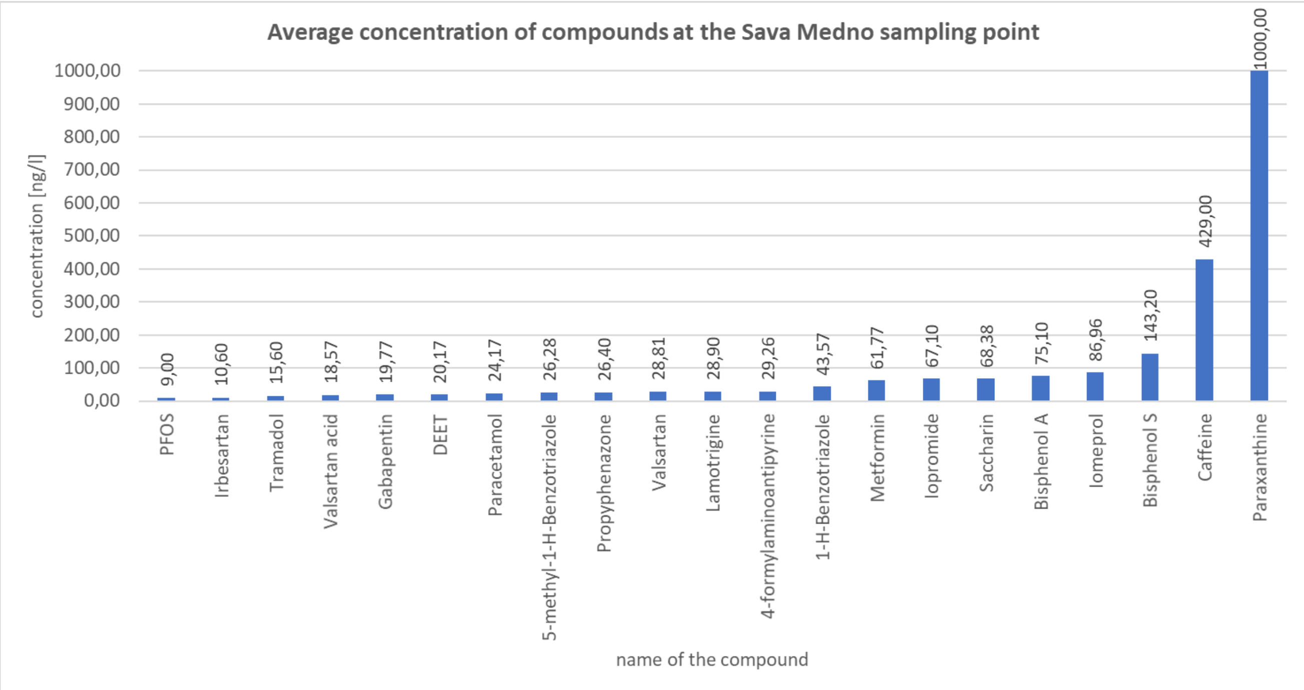

Figure 9: Average concentration of compounds at Sava Medno sampling point

At the Sava Dolsko sampling site, the highest concentrations were found for propyphenazone and iopromide (Fig. 10). Propyphenazone was detected only once at a concentration of 829 ng/L, and for iopromide, the average concentration was 241.11 ng/L. At the lowest concentrations (7 ng/L), PFOS occurs, as it did at the other two sampling sites. Propyphenazone is an active ingredient that is a component of drugs that affect the nervous system and is classified as an analgesic. It is used in conjunction with acetaminophen and caffeine to treat fever and pain. It is much more sustained than caffeine (Jamnik et al., 2009). Iopromide is an X-ray contrast agent used in various types of imaging studies, such as CT (Internet 1).

Trontelj, Klančar, and Roškar (2018) analysed the pollution of some Slovenian rivers with different substances. Valsartan and gabapentin were detected in the highest concentrations (in the range of 40-50 ng/L). Irbesartan, valsartan, and caffeine were present in all samples analysed. As can be seen from the figure 1, all the substances mentioned were also detected quite frequently in the Ljubljana Basin.

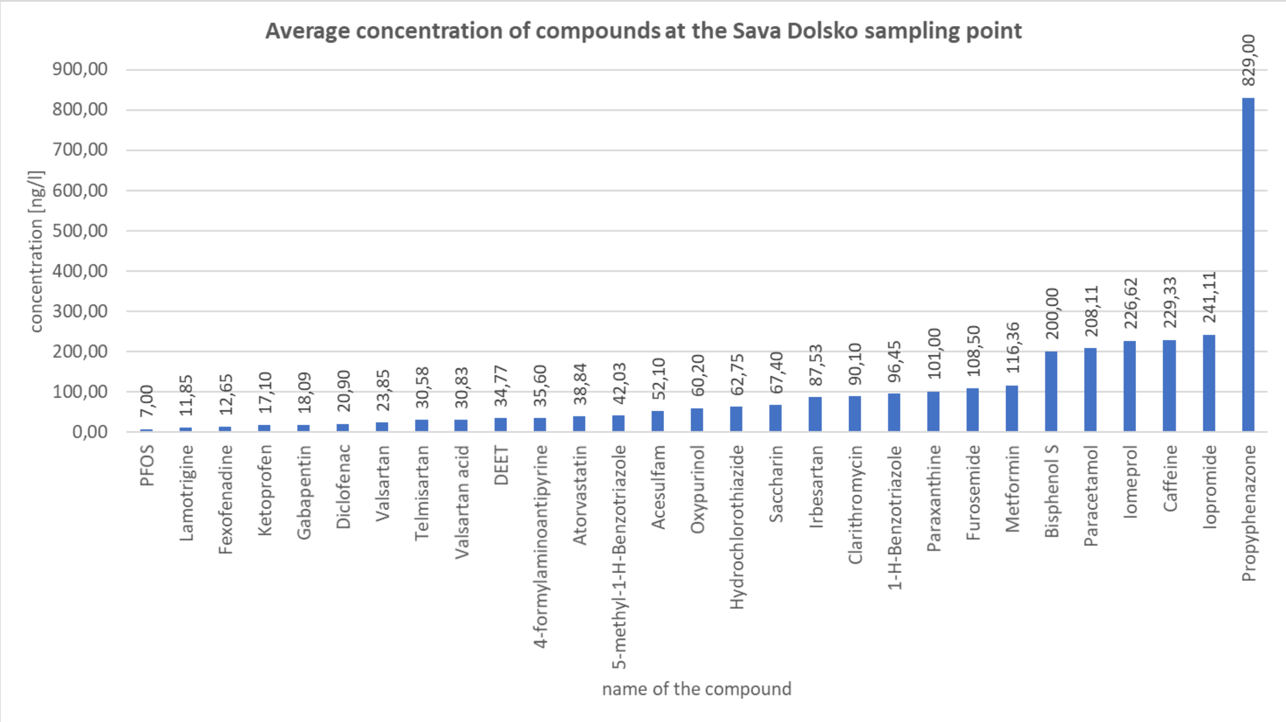

Figure 10: Average concentration of compounds at the Sava Dolsko sampling point

4.1. Water treatment efficiency and natural attenuation efficiency

Within the scope of monitoring we have carried out one-time sampling at the inflow and outflow of Central waste water treatment plant of Ljubljana. This sampling was a part of screening of the entire Ljubljana basin, all together 17 samples in 17 different locations (table 2), whose analyses were performed in laboratory in collaboration with Faculty of Pharmacy.

Table 2: The number of different detected compounds at each sampling point, which were analysed in the laboratory of the Faculty of Pharmacy

Sampling location	No. of substances
CČN Ljubljana outflow	10
CČN Ljubljana inflow	10
Reka Sava, Dolsko	10
Ljubljanica pred Ljubljano	9
Reka Sava, Medno	9
BEV-2/15	8
BEV-1/15	7
Izvir Ljubljanice	7
MEN-1/14	7

MEN-2/14	7
NAK-2/13	7
Radovna pred Vintgarjem	7
Reka Sava, Hidrološka postaja Radovljica	7
Izvir Radovna	6
NAK-1/13	6
Radovna Smešnik	6
Zelenci (izvir)	6

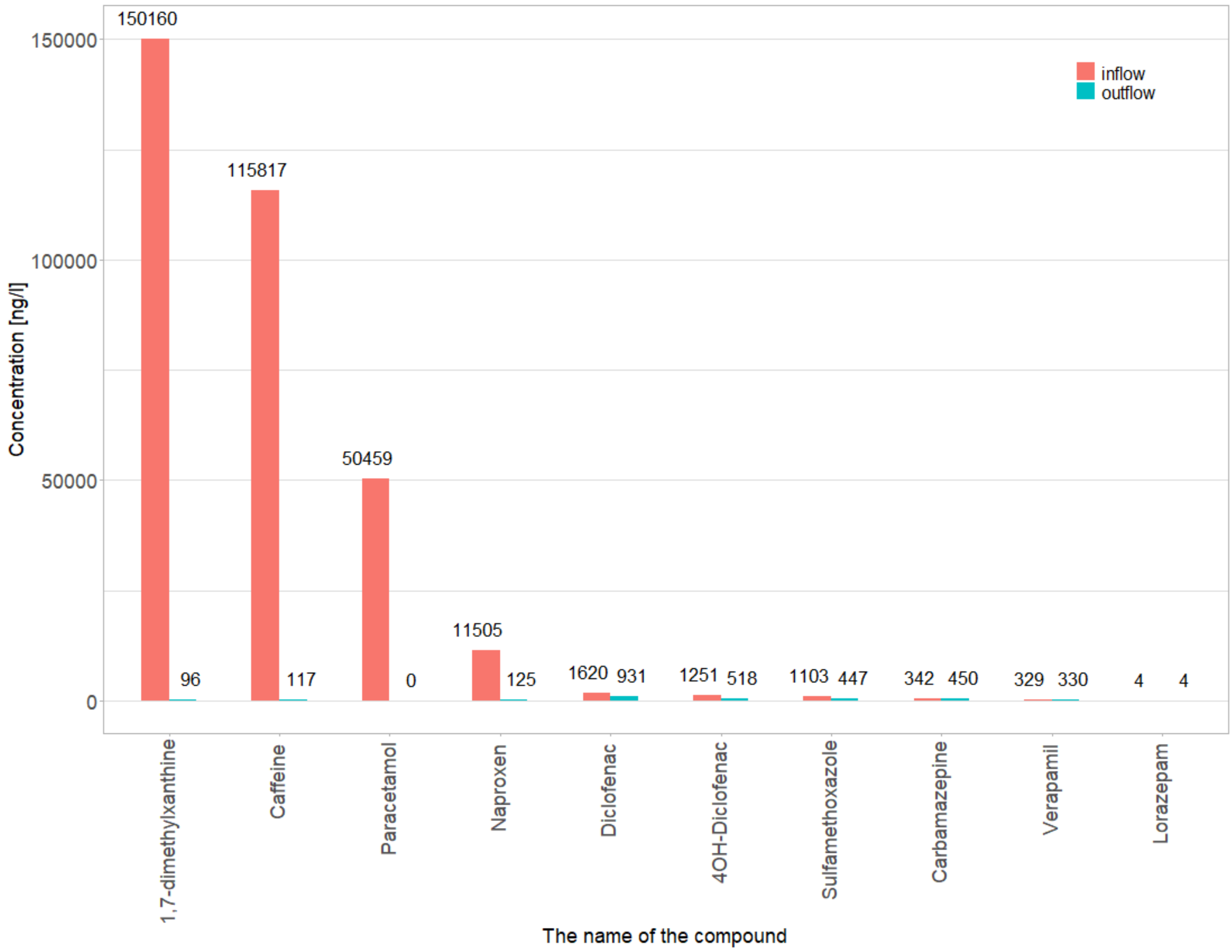

Figure 11: Concentration (ng/l) of detected compounds at the inflow and outflow of the Central wastewater treatment plant Ljubljana

The results in figure 11 shows that 1,7-dimethylxanthine, Caffeine, Paracetamol and Naproxen are efficiently removed by treatment plant. Nevertheless, some of the compounds, for example carbamazepine, lorazepam, and verapamil, higher apparent concentrations were measured in outflow samples compared to the inflow concentrations. This phenomenon is believed to be the consequence of drug-conjugates, for example glucuronides and sulfates cleavage by microorganisms to the corresponding parent compounds (Klančar et al, 2016, Ekpeghere et al, 2018).

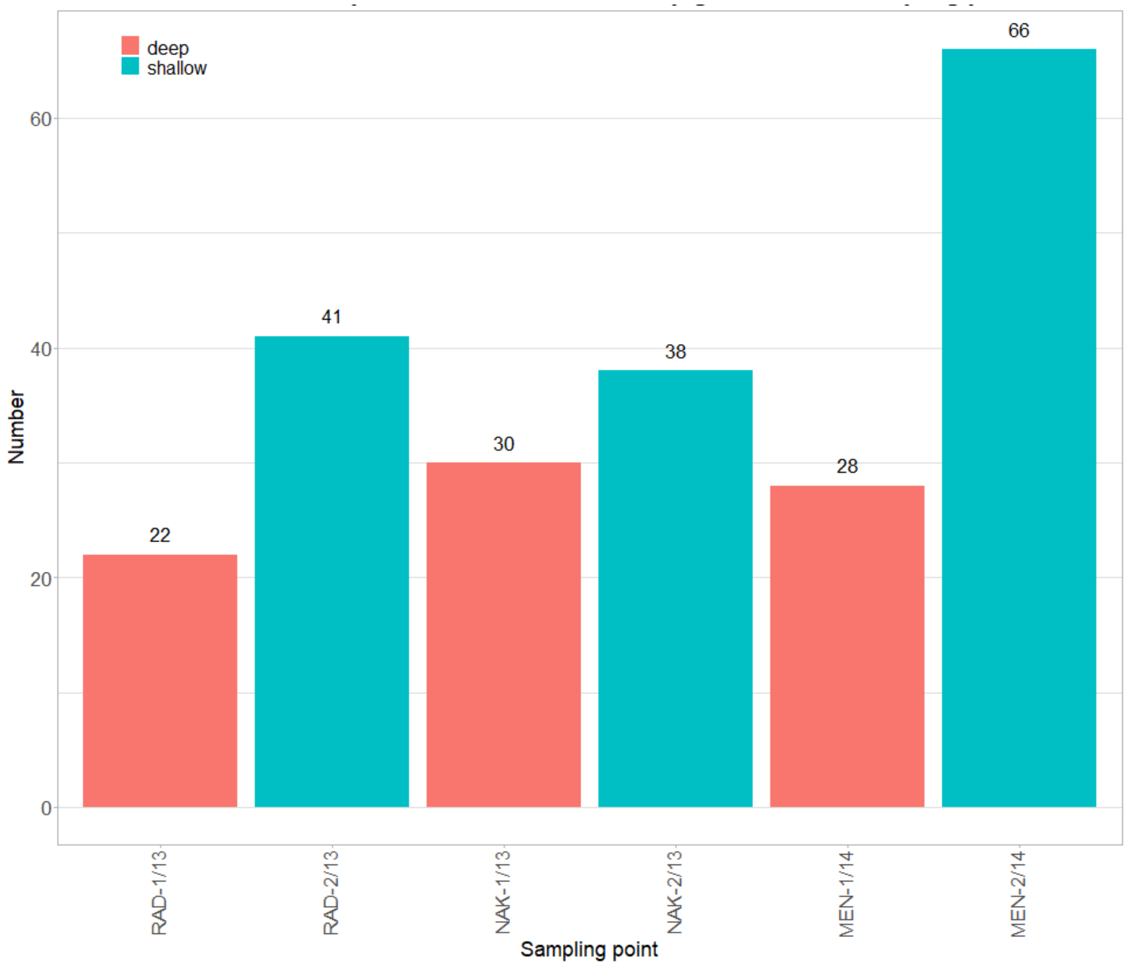

Figure 12: Number of detected compounds at shallow and deep groundwater sampling points

For the natural attenuation process, passive sampling for qualitatively determine the presence of compounds was performed at three locations. At each location passive sampler was at the same time at deep and shallow well. The results in figure 12 show that in all locations less compounds were detected in deep wells than in shallow wells. It could be assumed that this is due to dilution and natural attenuation processes.

5. Conclusion

The monitoring results document occurrence of PPCPs in Ljubljana basin. Most common detected compounds at all sampling points were metformin and paracetamol, followed by caffeine and paraxanthine, indicating the presence of an urban area and presence of anthropogenic impact on the environment.

The results of analyses of samples before and after the treatment plant showed that wastewater treatment for before mentioned compounds is efficient as concentrations of detected compounds drop significantly.

6. References

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