**Supplementary Materials**

**Fate and impact of wastewater-borne micropollutants in lettuce and the root-associated bacteria**

Yvonne Bigott1‡, Sara Gallego2‡, Nicola Montemurro3, Marie-Christine Breuil2, Sandra Pérez3, Antonios Michas1, Fabrice Martin-Laurent2, Peter Schröder1\*

1Research Unit for Comparative Microbiome Analysis, Helmholtz Zentrum München GmbH, Ingolstädter Landstraße 1, 85764, Neuherberg, Germany

2AgroSup Dijon, INRAE, Univ. Bourgogne, Univ. Bourgogne Franche-Comté,Agroécologie, Dijon, France

3ENFOCHEM, IDAEA-CSIC, c/Jordi Girona 18-26, 08034 Barcelona (Spain)

‡ Equal first author

\*Corresponding author: Peter Schröder,

1Research Unit for Comparative Microbiome Analysis, Helmholtz Zentrum München GmbH, Ingolstädter Landstraße 1, 85764, Neuherberg, Germany. Tel: +49 89 3187-4056. Fax: +49 89 3187-3376. Email: peter.schroeder@helmholtz-muenchen.de

**1. Supplementary Materials**

**1.1 Soil and wastewater characteristics**

Soil (loam, pH 8.2, soil organic matter 3.68%, total organic carbon 2.13%, total nitrogen 0.201%) was collected from the experimental fields of IRSTEA at Montpellier (Lavalette, France, 43.64682 N, 3.87418 E). Secondary treated domestic wastewater (pH 7.1, WHC 71.91%; COD 200 mg/L, total suspended solids 58 mg/L, total organic carbon 56.8 mg/L, N-NH4 29 mg/L, N-NO3- <0.22 mg/L) was collected from the wastewater lagoon at Murviel-les-Montpellier (Hérault, France, 43.605034 N, 3.757292 E) and stored at 4ºC until use. Additional details on soil and wastewater physico-chemical characteristics are described in Gallego et al., 2021.

**1.2 Chemicals**

Highly pure analytical reference standards (acesulfame (ASF), benzotriazole (BNZ), carbamazepine (CBZ), carbamazepine-10,11-epoxide (CBZ-EPX), ciprofloxacin (CIP), citalopram (CTP), clarithromycin (CLT), climbazole (CLB), diclofenac (DCF), 4'‑hydroxydiclofenac (4’-OH-DCF), hydrochlorotiazide (HCT), irbesartan (IRB), metropolol (MTP), sucralose (SUC), sulfamethoxazole (SMX), 4‑nitro‑sulfamethoxazole (4-nitro-SMX), valsartan (VAL), valsartan acid (VAL-AC) were obtained from Sigma Aldrich (St. Louis, MO, U.S.). Whereas, isotopically labelled standards for quantitation purpose (acesulfame-d4, benzotriazole-d4, carbamazepine-d10, ciprofloxacin-d8, citalopram-d6, climbazole-d4, diclofenac-13C6, hydrochlorothiazide-d2, irbesartan-d6, metoprolol-d7, sucralose-d6, sulfamethoxazole-d4, valsartan acid-d4, and valsartan-d3) were purchased from Cerilliant (Sigma Aldrich, St. Louis, MO, U.S.) and Toronto Research Chemicals (Toronto, ON, Canada). LC-MS grade solvents (Acetone, acetonitrile (≥ 99.9%), methanol (≥ 99.9%), dimethyl sulfoxide (≥ 99.9%), and HPLC water were purchased from Merck (Darmstadt, Germany). All the above-mentioned reference standards were prepared individually in either LC-MS grade acetone, acetonitrile (ACN, ≥ 99.9%), methanol (MeOH, ≥ 99.9%), dimethyl sulfoxide (DMSO, ≥ 99.9%), or HPLC water according to compounds solubility and stored at −20°C. The most relevant physicochemical properties of each compound are reported elsewhere (Montemurro et al., 2021). Commercially available Original QuEChERS (OR) extraction salts kit (4 g MgSO4 + 1 g NaCl) and dispersive solid phase extraction (dSPE) clean-up mixture (150 mg PSA (primary secondary amine), 150 mg of C18-bonded silica, and 900 mg MgSO4) were obtained from BEKOlut GmbH & Co. KG (Hauptstuhl, Germany). Disodium hydrogenphosphate dihydrate (Na2HPO4·2H2O), citric acid monohydrate and anhydrous ethylenediamine tetraacetic acid (EDTA) (≥99%) for preparation of the EDTA-McIIvaine buffer (pH 4) (Montemurro et al., 2021) were obtained from Sigma Aldrich (St. Louis, MO, U.S).

**1.3 Soil chemical extraction**

For the extraction of the fourteen compounds and their main metabolites and transformation products from soil, 10 g of air-dried soil sample were added to a 50-mL polypropylene centrifuge tube and 3 mL of acetone were added followed by 50 μL of isotopically labeled compounds mixture (2 μg/mL). The tubes were then vortexed for 2 min at 2500 rpm using a BenchMixer XLQ QuEChERS Vortexer (Benchmark Scientific, Sayreville NJ, US) and left overnight under the hood at room temperature. Next, 8 mL of EDTA-Mcllvaine buffer were added to rehydrate the samples and then vortexed and left to stand for one hour prior to the extraction step. When 80% hydration was achieved, 10 mL of acetonitrile was added to the sample and vortexed again. To promote salting out, the QuEChERS original salt kit was emptied into the extraction tube and the resulting mixture was immediately shaken by hand for one minute to avoid salt agglomeration and then vortexed for another minute. Finally, the tube was centrifuged for 10 min at 4000 rpm and 4 ⁰C and 1 mL of the obtained supernatant was evaporated under a gentle nitrogen stream at room temperature to total dryness. Lastly, the samples were reconstituted with 1 mL of water/10% methanol solution and injected for LC-MS/MS analysis (Manasfi et al., 2022. in preparation).

**1.4 Lettuce chemical extraction**

Pharmaceuticals from lettuce leaves were extracted according to Montemurro et al., 2020. Briefly, 1 g of freeze-dried leaves were placed in a 50-mL centrifuge tube and hydrated with 9 mL HPLC water. After 1-hour rest, the tubes were spiked with a proper volume of deuterated mix, vortexed and extracted by adding 10 mL of acidified acetonitrile (0.5% formic acid) followed by the OR QuEChERS salts content. The tubes were immediately hand shaken, vortexed, and centrifuged for 10 min at 4°C and at 4000 rpm. Before the clean-up step, the obtained supernatants were frozen overnight at -20°C to allow fatty acids and lipids precipitation. On the following day 6 mL of the organic phase supernatant were transferred to the dSPE tube for further extract clean-up. The tube was immediately hand shaken, vortexed, and centrifuged for 10 min at 4°C and at 4000rpm. Finally, 1 mL of the supernatant was evaporated under gentle nitrogen flow at room temperature until total dryness and reconstituted with 1 mL of water/MeOH (90/10, v/v) for injection. For the extraction of the PPCPs in roots, 1 g of freeze-dried plant material were hydrated with 9 mL of EDTA-Mcllvaine buffer (See Manasfi et al. 2022 in preparation) and extracted by adding 10 mL of acetonitrile followed by the OR QuEChERS salts kit. The tubes were immediately hand shaken, vortexed, and centrifuged for 10 min at 4°C and at 4000 rpm. No clean up step was performed for root extracts.

**1.5 Statistical analyses of sequencing data**

Originally, 4,466,919 reads (Table S2) were present in all samples. After denoising, rarefying and the removal of negative and blank extraction controls and two samples, which were not considered in downstream analysis due to contamination with *Enterobacteriaceae*, 45.34% of the reads were remaining. The rarefaction curves (Fig. S1) reached a plateau before the cutoff of 34,920 reads per sample, confirming that the sampling depth was sufficient. In total 22,961 different ASVs were detected and used for bacterial diversity and community composition analysis. To compensate the unequal sampling depth the datasets were subsampled to the minimum number of reads per sample (34,920 reads) using the vegan package (version 2.5-6; Oksanen et al., 2018).

Statistical data analysis and visualization was performed using the statistical program R (version 3.6.2). The normality of the data and residuals was checked using Shapiro Wilk’s test (p>0.05) and the homogeneity of variance was verified using Levene’s test (p>0.05). Inverse, root and log-10 transformations of the data were performed when necessary. For parametric distributions two-way ANOVA followed by Tukey’s test (using time and treatments as factors) and pairwise Student’s t-test were used to determine differences. For non-parametric distributions, data was compared using Kruskal Wallis test. The sample ordination by non-metric multidimensional scaling (NMDS) on the Bray-Curtis dissimilarity and the stacked barplot were generated with the phyloseq package (version 1.28.0; (McMurdie and Holmes, 2013). A PERMANOVA was then performed on the Bray-Curtis dissimilarity using the Adonis function also from the vegan package. The mvabund package (version 4.0.1; (Wang et al., 2012) was used for calculating significant differences among the relative abundance of ASVs between the treatments. ASVs with significantly different relative abundance were graphically displayed using boxplots and heatmaps. All data was visualized by phyloseq and ggplot2 (version 3.2.1; Wickham, 2009). Differences were considered as significant in all tests when the p-value was ≤ 0.05.

**Supplementary Table S1.** Source sequence of the qPCR linear standard.

|  |  |  |
| --- | --- | --- |
| Gene | Sequence | Originating organism |
| 16S rRNA | 5’‑CAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGAAAGCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGTCTTCGGATTGTAAAGCACTTTAAGTTGGGAGGAAGGGTAGTAACCTAATACGTTGCTACTTTGACGTTACCGACAGAATAAGCACCGGCTAACTTCGTGCCAGCAGCCGCGGTAATACGAAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCGCGTAGGTGGTTCAGTAAGTTGGAAGTGAAATCCCCGGGCTCAACCTGGGAACTGCTTTCAAAACTGCTGAGCTAGAGTACGGTAGAGGGTGGTGGAATTTCCTGTGTAGCGGTGAAATGCGTAGATATAGGAAGGAACACCAGTGGCGAAGGCGACCACCTGGACTGATACTGACACTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATA‑3’ | *Pseudomonas anguilliseptica* strain YSH-14 |

**Supplementary Table S2**. Number of 16S rRNA sequences obtained per sample through Illumina MiSeq from lettuce roots planted in soil microcosms irrigated with either water, 10μg/L or 100μg/L spiked water, wastewater or 10μg/L or 100μg/L spiked wastewater and collected at the end of the first and second cultivation campaign.

| **Sample** | **Timepoint** | **Treatment** | **Sequences** | **Replicate** | **Number of different ASVs** |
| --- | --- | --- | --- | --- | --- |
| 1 | 1st campaign | water | 66584 | 1 | 1414 |
| 2 | 1st campaign | water\* | 56343 | 2 | 822 |
| 3 | 1st campaign | water | 54325 | 3 | 998 |
| 4 | 1st campaign | water | 115872 | 4 | 1432 |
| 5 | 1st campaign | water | 52741 | 5 | 1103 |
| 6 | 1st campaign | water 10μg/L | 67709 | 1 | 1358 |
| 7 | 1st campaign | water 10μg/L | 58818 | 2 | 1402 |
| 8 | 1st campaign | water 10μg/L | 51893 | 3 | 968 |
| 9 | 1st campaign | water 10μg/L | 48311 | 4 | 1211 |
| 10 | 1st campaign | water 10μg/L | 62011 | 5 | 1089 |
| 11 | 1st campaign | water 100μg/L | 56679 | 1 | 1172 |
| 12 | 1st campaign | water 100μg/L | 94687 | 2 | 1585 |
| 13 | 1st campaign | water 100μg/L | 76072 | 3 | 1477 |
| 14 | 1st campaign | water 100μg/L | 84228 | 4 | 1540 |
| 15 | 1st campaign | water 100μg/L | 94910 | 5 | 1449 |
| 16 | 1st campaign | wastewater | 92711 | 1 | 1362 |
| 17 | 1st campaign | wastewater | 85556 | 2 | 1358 |
| 18 | 1st campaign | wastewater | 189565 | 3 | 1669 |
| 19 | 1st campaign | wastewater | 102616 | 4 | 1335 |
| 20 | 1st campaign | wastewater | 86489 | 5 | 1366 |
| 21 | 1st campaign | wastewater 10μg/L | 87398 | 1 | 1433 |
| 22 | 1st campaign | wastewater 10μg/L | 84075 | 2 | 1192 |
| 23 | 1st campaign | wastewater 10μg/L | 86460 | 3 | 1181 |
| 24 | 1st campaign | wastewater 10μg/L | 109081 | 4 | 1515 |
| 25 | 1st campaign | wastewater 10μg/L | 98604 | 5 | 1499 |
| 26 | 1st campaign | wastewater 100μg/L | 100424 | 1 | 1560 |
| 27 | 1st campaign | wastewater 100μg/L\* | 74981 | 2 | 63 |
| 28 | 1st campaign | wastewater 100μg/L | 73791 | 3 | 1251 |
| 29 | 1st campaign | wastewater 100μg/L | 71084 | 4 | 1343 |
| 30 | 1st campaign | wastewater 100μg/L | 97388 | 5 | 1157 |
| 31 | 2nd campaign | water | 66832 | 1 | 1060 |
| 32 | 2nd campaign | water | 49912 | 2 | 1162 |
| 33 | 2nd campaign | water | 73786 | 3 | 1132 |
| 34 | 2nd campaign | water | 63847 | 4 | 1183 |
| 35 | 2nd campaign | water | 80986 | 5 | 1426 |
| 36 | 2nd campaign | water 10μg/L | 80290 | 1 | 1670 |
| 37 | 2nd campaign | water 10μg/L | 79083 | 2 | 1541 |
| 38 | 2nd campaign | water 10μg/L | 99483 | 3 | 1755 |
| 39 | 2nd campaign | water 10μg/L | 76950 | 4 | 1392 |
| 40 | 2nd campaign | water 10μg/L | 65630 | 5 | 1326 |
| 41 | 2nd campaign | water 100μg/L | 81266 | 1 | 1278 |
| 42 | 2nd campaign | water 100μg/L | 70610 | 2 | 1154 |
| 43 | 2nd campaign | water 100μg/L | 62981 | 3 | 1184 |
| 44 | 2nd campaign | water 100μg/L | 88302 | 4 | 1154 |
| 45 | 2nd campaign | water 100μg/L | 77847 | 5 | 1542 |
| 46 | 2nd campaign | wastewater | 74199 | 1 | 1153 |
| 47 | 2nd campaign | wastewater | 43076 | 2 | 772 |
| 48 | 2nd campaign | wastewater | 34948 | 3 | 613 |
| 49 | 2nd campaign | wastewater | 56681 | 4 | 790 |
| 50 | 2nd campaign | wastewater | 51852 | 5 | 877 |
| 51 | 2nd campaign | wastewater 10μg/L | 63727 | 1 | 1126 |
| 52 | 2nd campaign | wastewater 10μg/L | 63209 | 2 | 1106 |
| 53 | 2nd campaign | wastewater 10μg/L | 63922 | 3 | 1261 |
| 54 | 2nd campaign | wastewater 10μg/L | 64864 | 4 | 1125 |
| 55 | 2nd campaign | wastewater 10μg/L | 64694 | 5 | 976 |
| 56 | 2nd campaign | wastewater 100μg/L | 59167 | 1 | 794 |
| 57 | 2nd campaign | wastewater 100μg/L | 56508 | 2 | 1200 |
| 58 | 2nd campaign | wastewater 100μg/L | 56284 | 3 | 908 |
| 59 | 2nd campaign | wastewater 100μg/L | 50820 | 4 | 1025 |
| 60 | 2nd campaign | wastewater 100μg/L | 61048 | 5 | 975 |

\*Samples not considered in downstream analysis



**Supplementary Figure S1**. Rarefaction curves of 16S rRNA sequences obtained from the sequencing analysis (n=60) before subsampling and removal of samples, which were not considered in downstream analysis.

**Supplementary Table S3**. Total nitrogen and carbon (in percentage) in lettuce roots and leaves planted in soil microcosms irrigated with either water and wastewater collected at the end of the first and second cultivation. Each value is the mean of five replicates. ANOVA followed by Tukey’s test was performed. Values indicated by different letters are significantly different.

|  |  |  |  |
| --- | --- | --- | --- |
|  |  | **roots** | **leaves** |
|  |  | **carbon** | **nitrogen** | **carbon** | **nitrogen** |
| **1st campaign** | **water** | 43.24 ± 0.16ab | 0.48 ± 0.04a | 39.97 ± 0.87ab | 1.12 ± 0.09a |
| **wastewater** | 43.66 ± 0.14a | 0.57 ± 0.02b | 39.62 ± 0.31b | 1.17 ± 0.08a |
| **2nd campaign** | **water** | 43.00± 0.51b | 0.63 ± 0.14b | 41.47 ± 0.31c | 1.13 ± 0.10a |
| **wastewater** | 42.31± 0.87c | 0.68 ± 0.06b | 40.60 ± 0.68ac | 1.26 ± 0.15a |

**Supplementary Table S4**. Concentration of the spiked products and major metabolites [ng/g d.w.] in soil from lettuce planted soil microcosms irrigated with either spiked water (100μg/L) or spiked wastewater (100μg/L) and collected at the end of the first and second cultivation campaign. BLOQ: Bellow limit of quantification; N.D.: Not detected.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Timepoint** | **Treatment** | **ASF** | **BNZ** | **CBZ** | **CBZ-EPX** | **CIP** | **CTP** | **CLT** | **CLB** | **DCF** | **4`OH- DCF** | **HCT** | **IRB** | **MTP** | **SUC** | **SMX** | **4-nitro-SMX** | **VAL** | **VAL-AC** |
| **1st campaign** | **water** | 0.28 | 0.57 | 3.65 | 0.72 | 0.16 | 4.02 | 230.35 | 2.57 | N.D. | N.D. | 25.80 | 1.17 | BLOQ | BLOQ | BLOQ | BLOQ | BLOQ | N.D. |
|  |  | 0.60 | 0.47 | 3.29 | 0.69 | BLOQ | 5.35 | 193.17 | 2.90 | N.D. | N.D. | 23.46 | 0.87 | 0.07 | BLOQ | BLOQ | BLOQ | BLOQ | BLOQ |
|  |  | 0.28 | 0.56 | 3.55 | 0.76 | 0.15 | 4.93 | 180.52 | 2.61 | N.D. | N.D. | 29.56 | 0.86 | BLOQ | 2.13 | BLOQ | BLOQ | BLOQ | BLOQ |
|  |  | 0.15 | 0.58 | 3.42 | 0.63 | 0.32 | 5.06 | 189.87 | 3.11 | N.D. | N.D. | 33.09 | 0.72 | 0.11 | BLOQ | 0.23 | BLOQ | BLOQ | N.D. |
|  |  | 0.32 | 1.09 | 3.83 | 0.76 | 0.25 | 7.09 | 299.47 | 3.31 | N.D. | N.D. | 21.35 | 1.30 | 0.10 | BLOQ | BLOQ | BLOQ | BLOQ | N.D. |
|  | **wastewater** | 0.40 | 1.01 | 4.24 | 0.92 | 1.20 | 9.10 | 316.72 | 5.90 | BLOQ | N.D. | 29.60 | 1.42 | 0.22 | BLOQ | 0.23 | BLOQ | BLOQ | N.D. |
|  |  | 0.14 | 0.85 | 3.76 | 0.65 | 0.38 | 5.32 | 211.32 | 4.04 | N.D. | N.D. | 26.41 | 1.09 | 0.08 | BLOQ | 0.31 | BLOQ | BLOQ | N.D. |
|  |  | 0.21 | 0.66 | 3.59 | 0.59 | 1.06 | 5.52 | 181.19 | 3.22 | N.D. | N.D. | 27.48 | 1.20 | 0.17 | BLOQ | 0.24 | BLOQ | BLOQ | N.D. |
|  |  | 0.23 | 0.91 | 4.12 | 0.85 | 0.51 | 8.74 | 286.56 | 5.10 | N.D. | N.D. | 32.80 | 1.19 | BLOQ | 1.09 | BLOQ | BLOQ | BLOQ | BLOQ |
|  |  | 0.24 | 0.78 | 3.69 | 0.61 | 0.60 | 5.23 | 181.54 | 4.11 | N.D. | N.D. | 25.30 | 0.84 | 0.10 | BLOQ | BLOQ | BLOQ | BLOQ | N.D. |
| **2nd campaign** | **water** | 0.47 | 0.98 | 5.55 | 1.70 | BLOQ | 10.92 | 396.70 | 5.50 | N.D. | N.D. | 31.30 | 1.18 | 0.12 | BLOQ | 0.37 | BLOQ | BLOQ | BLOQ |
|  |  | BLOQ | 1.23 | 5.74 | 2.25 | 5.50 | 10.58 | 348.70 | 5.85 | N.D. | N.D. | 33.39 | 1.08 | 0.10 | BLOQ | 0.26 | BLOQ | BLOQ | BLOQ |
|  |  | BLOQ | 1.11 | 5.81 | 2.62 | N.D. | 12.59 | 359.30 | 6.53 | N.D. | N.D. | 30.98 | 1.16 | 0.08 | BLOQ | 0.34 | BLOQ | BLOQ | N.D. |
|  |  | 0.67 | 1.08 | 5.72 | 2.63 | 0.81 | 14.65 | 307.14 | 6.29 | N.D. | N.D. | 36.77 | 1.11 | 0.19 | BLOQ | 0.24 | BLOQ | BLOQ | N.D. |
|  |  | 0.46 | 1.35 | 6.10 | 2.04 | 2.01 | 10.69 | 373.08 | 5.22 | N.D. | N.D. | 31.67 | 1.13 | 0.20 | BLOQ | 0.33 | BLOQ | BLOQ | N.D. |
|  | **wastewater** | BLOQ | 1.22 | 6.73 | 2.30 | 1.67 | 12.32 | 319.70 | 8.59 | 0.14 | N.D. | 33.14 | 1.81 | 0.23 | BLOQ | BLOQ | BLOQ | BLOQ | 0.09 |
|  |  | BLOQ | 0.87 | 6.28 | 1.74 | 2.34 | 10.70 | 369.30 | 8.88 | 0.29 | N.D. | 34.94 | 1.85 | 0.22 | BLOQ | 0.42 | BLOQ | BLOQ | 0.14 |
|  |  | BLOQ | 0.96 | 6.38 | 2.77 | 0.94 | 13.57 | 324.10 | 8.28 | 0.14 | N.D. | 28.15 | 2.17 | 0.29 | BLOQ | 0.30 | BLOQ | BLOQ | 0.27 |
|  |  | BLOQ | 0.90 | 6.64 | 2.41 | 0.59 | 13.40 | 294.69 | 8.16 | 0.13 | N.D. | 27.92 | 2.03 | 0.14 | BLOQ | 0.33 | BLOQ | BLOQ | 0.15 |

The MQL (minimum quantification level) [ng/g d.w.] are ASF= 0.01, CIP= 0.03, DCF= 0.16, MTP= 0.01, SUC= 0.05, SMX= 0.03, 4-nitro-SMX= 0.07, VAL= 0.03 and VAL-AC= 0.12.

**Supplementary Table S5**. Concentration of the spiked products and major metabolites [ng/g d.w.] in lettuce roots planted in soil microcosms irrigated with either spiked water (100μg/L) or spiked wastewater (100μg/L) and collected at the end of the first and second cultivation campaign. BLOQ: Bellow limit of quantification; N.D.: Not detected.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Timepoint** | **Treatment** | **ASF** | **BNZ** | **CBZ** | **CBZ-EPX** | **CIP** | **CTP** | **CLT** | **CLB** | **DCF** | **4`OH- DCF** | **HCT** | **IRB** | **MTP** | **SUC** | **SMX** | **4-nitro-SMX** | **VAL** | **VAL-AC** |
| **1st campaign** | **water** | 14.54 | BLOQ | 7.45 | BLOQ | N.D. | 6.40 | 5.85 | 4.22 | N.D. | N.D. | 12.16 | 1.57 | 1.87 | BLOQ | 28.77 | N.D. | N.D. | N.D. |
|  |  | 33.78 | BLOQ | 21.20 | BLOQ | N.D. | 14.95 | 68.74 | 8.18 | N.D. | N.D. | 31.71 | 2.87 | 4.29 | 139.54 | 6.09 | N.D. | N.D. | N.D. |
|  |  | 29.58 | BLOQ | 24.44 | BLOQ | N.D. | 14.79 | 36.84 | 9.71 | N.D. | N.D. | 15.37 | 0.68 | 1.62 | 601.70 | 32.13 | N.D. | N.D. | N.D. |
|  |  | 31.11 | BLOQ | 17.48 | BLOQ | N.D. | 7.06 | 7.93 | 3.70 | N.D. | N.D. | 11.73 | N.D. | BLOQ | 113.59 | 3.87 | N.D. | N.D. | N.D. |
|  |  | 18.91 | BLOQ | 8.40 | BLOQ | N.D. | 5.64 | 9.24 | 2.57 | N.D. | N.D. | 4.55 | 1.17 | 0.60 | 98.70 | 9.78 | N.D. | N.D. | N.D. |
|  | **wastewater** | 53.53 | BLOQ | 21.76 | BLOQ | N.D. | 13.10 | 8.65 | 5.99 | N.D. | N.D. | 17.77 | 2.06 | 0.76 | 44.02 | 10.59 | N.D. | N.D. | N.D. |
|  |  | 193.6 | 48.49 | 138.08 | 14.07 | N.D. | 163.3 | 643.44 | 32.90 | N.D. | N.D. | 141.75 | 12.22 | 63.44 | 734.69 | 18.43 | N.D. | N.D. | N.D. |
|  |  | 109.2 | 0.65 | 73.73 | BLOQ | N.D. | 30.75 | 92.60 | 11.47 | N.D. | N.D. | 51.26 | 4.38 | 2.11 | 711.55 | 41.94 | N.D. | N.D. | N.D. |
|  |  | 113.5 | BLOQ | 68.84 | BLOQ | N.D. | 22.46 | 28.03 | 8.89 | N.D. | N.D. | 60.13 | 5.41 | 0.82 | 175.20 | 13.45 | N.D. | N.D. | N.D. |
|  |  | 134.5 | BLOQ | 96.89 | 1.53 | N.D. | 101.0 | 95.85 | 28.48 | N.D. | 7.94 | 69.32 | 11.34 | 54.48 | 160.83 | 5.95 | N.D. | N.D. | 9.50 |
| **2nd campaign** | **water** | 11.13 | 3.86 | 36.46 | BLOQ | N.D. | 29.93 | 48.66 | 8.96 | N.D. | 11.80 | 33.77 | 5.16 | 6.38 | 280.53 | 228.81 | N.D. | N.D. | 9.23 |
|  |  | 34.42 | 0.66 | 21.63 | BLOQ | N.D. | 18.09 | 17.59 | 7.13 | N.D. | 5.94 | 15.65 | 3.46 | 4.70 | 125.02 | 131.25 | N.D. | N.D. | 4.37 |
|  |  | 4.04 | BLOQ | 7.20 | BLOQ | N.D. | 5.05 | 2.27 | 2.08 | N.D. | 0.66 | 5.19 | 1.18 | 0.42 | BLOQ | 51.43 | N.D. | N.D. | 1.57 |
|  | **wastewater** | 25.50 | 20.67 | 137.28 | 3.74 | N.D. | 265.4 | 191.71 | 37.65 | N.D. | 115.61 | 200.66 | 35.38 | 34.37 | 2137.0 | 955.08 | N.D. | N.D. | 53.35 |
|  |  | 45.96 | 23.61 | 198.51 | 5.79 | N.D. | 325.0 | 209.90 | 54.30 | 11.67 | 83.21 | 173.95 | 38.38 | 32.40 | 1945.8 | 738.99 | N.D. | N.D. | 59.36 |
|  |  | 19.50 | 6.01 | 129.28 | 2.37 | N.D. | 183.5 | 210.18 | 22.80 | N.D. | 52.45 | 122.82 | 20.03 | 35.55 | 1054.7 | 666.24 | N.D. | N.D. | 29.41 |
|  |  | 4.17 | 0.67 | 28.94 | BLOQ | N.D. | 33.97 | 13.57 | 9.85 | N.D. | 2.08 | 23.37 | 5.02 | 4.70 | 197.03 | 254.97 | 0.22 | N.D. | 8.13 |
|  |  | 18.13 | 0.08 | 85.69 | BLOQ | N.D. | 197.5 | 108.47 | 26.52 | 10.51 | 110.15 | 73.77 | 20.78 | 40.66 | 1343.4 | 526.08 | 0.36 | N.D. | 44.72 |

The MQL (minimum quantification level) [ng/g d.w.] are BNZ= 0.35, CBZ-EPX= 0.45, MTP= 0.23, SUC= 0.68.

**Supplementary Table S6**. Concentration of the spiked products and major metabolites [ng/g d.w.] in lettuce leaves planted in soil microcosms irrigated with either spiked water (100μg/L) or spiked wastewater (100μg/L) and collected at the end of the first and second cultivation campaign. BLOQ: Bellow limit of quantification; N.D.: Not detected.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Timepoint** | **Treatment** | **ASF** | **BNZ** | **CBZ** | **CBZ-EPX** | **CIP** | **CTP** | **CLT** | **CLB** | **DCF** | **4`OH- DCF** | **HCT** | **IRB** | **MTP** | **SUC** | **SMX** | **4-nitro-SMX** | **VAL** | **VAL-AC** |
| **1st campaign** | **water** | 312.20 | 0.52 | 83.03 | 25.26 | 3.46 | 11.87 | 99.56 | 3.64 | N.D. | N.D. | 29.93 | 0.38 | 2.60 | 305.70 | N.D. | N.D. | 1.11 | N.D. |
|  |  | 291.00 | BLOQ | 92.06 | 27.42 | N.D. | 6.36 | 48.53 | 1.91 | N.D. | N.D. | 36.06 | BLOQ | 1.61 | 399.80 | N.D. | N.D. | N.D. | N.D. |
|  |  | 166.60 | 1.43 | 75.42 | 17.40 | N.D. | 4.05 | 62.79 | 1.83 | N.D. | N.D. | 29.21 | BLOQ | 0.84 | 187.60 | 0.32 | N.D. | 0.79 | N.D. |
|  |  | 275.18 | 1.57 | 81.37 | 22.40 | 2.57 | 7.57 | 80.86 | 2.25 | N.D. | N.D. | 38.52 | 0.90 | 2.76 | 370.76 | N.D. | N.D. | 0.72 | N.D. |
|  | **wastewater** | 748.80 | 0.80 | 100.60 | 30.18 | 1.90 | 8.72 | 23.28 | 1.63 | N.D. | N.D. | 36.41 | 0.22 | 1.71 | 378.70 | 0.32 | N.D. | 0.63 | N.D. |
|  |  | 312.63 | 0.56 | 84.00 | 21.65 | 1.16 | 7.03 | 62.16 | 1.59 | N.D. | N.D. | 34.20 | 0.88 | 0.98 | 396.68 | N.D. | N.D. | N.D. | N.D. |
|  |  | 357.84 | 0.85 | 88.12 | 19.25 | 1.82 | 4.89 | 38.95 | 0.47 | N.D. | N.D. | 24.36 | 0.21 | 0.53 | 144.53 | N.D. | N.D. | N.D. | N.D. |
|  |  | 415.17 | BLOQ | 90.80 | 20.67 | 2.11 | 5.63 | 16.51 | 0.77 | N.D. | N.D. | 24.29 | 0.49 | 1.03 | 402.47 | 3.38 | N.D. | 0.57 | 0.98 |
|  |  | 305.63 | 0.55 | 90.75 | 22.85 | 1.28 | 4.14 | 13.27 | 0.56 | N.D. | N.D. | 20.55 | 0.24 | 0.77 | 174.12 | 0.51 | N.D. | N.D. | N.D. |
| **2nd campaign** | **water** | 209.44 | BLOQ | 127.88 | 89.30 | N.D. | 12.27 | 9.88 | 1.34 | N.D. | N.D. | 34.77 | BLOQ | 2.31 | 317.69 | 0.80 | N.D. | N.D. | 2.97 |
|  |  | 210.92 | 0.56 | 119.46 | 56.22 | N.D. | 12.55 | 19.00 | 2.22 | N.D. | N.D. | 29.72 | 0.35 | 2.83 | 166.12 | 0.22 | N.D. | N.D. | N.D. |
|  |  | 220.58 | 0.95 | 104.13 | 44.60 | 1.14 | 9.64 | 50.55 | 2.12 | N.D. | N.D. | 34.01 | 0.40 | 2.35 | 545.60 | N.D. | N.D. | N.D. | N.D. |
|  |  | 816.08 | 0.50 | 143.22 | 84.70 | N.D. | 21.76 | 31.63 | 1.54 | N.D. | N.D. | 56.97 | 0.25 | 6.81 | 383.90 | N.D. | N.D. | N.D. | 2.70 |
|  |  | 324.56 | BLOQ | 107.92 | 42.91 | 1.03 | 9.45 | 54.03 | 2.69 | N.D. | N.D. | 36.87 | 0.72 | 2.11 | 397.19 | 1.04 | N.D. | 0.84 | 2.63 |
|  | **wastewater** | 165.85 | 0.87 | 185.03 | 99.29 | 0.84 | 55.28 | 53.70 | 2.55 | N.D. | N.D. | 69.18 | 0.27 | 11.04 | 818.45 | 1.62 | N.D. | N.D. | 8.22 |
|  |  | 127.68 | 2.01 | 176.93 | 100.01 | 0.74 | 45.11 | 24.14 | 1.45 | N.D. | N.D. | 55.50 | BLOQ | 10.01 | 797.90 | 1.07 | N.D. | N.D. | 0.99 |
|  |  | 94.31 | 2.44 | 154.40 | 78.34 | N.D. | 29.27 | 52.87 | 1.70 | N.D. | N.D. | 47.32 | 0.16 | 8.02 | 678.70 | 0.61 | N.D. | 0.90 | 2.54 |
|  |  | 95.59 | BLOQ | 150.79 | 77.93 | N.D. | 24.47 | 21.23 | 2.40 | N.D. | N.D. | 37.83 | 0.16 | 3.90 | 343.91 | 0.13 | N.D. | N.D. | 2.26 |
|  |  | 91.53 | BLOQ | 153.61 | 74.31 | N.D. | 32.59 | 26.02 | 2.95 | N.D. | N.D. | 34.74 | 0.10 | 5.93 | 543.86 | 0.15 | N.D. | 0.55 | 3.92 |

The MQL (minimum quantification level) are [ng/g d.w.] BNZ= 0.18, IRB = 0.08.

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**Supplementary Figure S2.** Difference [%] in the concentration of PPCPs in soil/plant tissue, derived from samples irrigated with wastewater 100μg/L PPCPs compared to water 100μg/L PPCPs. Significant differences between the concentration of PPCPs measured in water compared to wastewater irrigated samples are indicated as "\*" for p–value ≤ 0.05.



**Supplementary Figure S3.** Copy numbers of bacterial 16S rRNA. "W", "W10", and "W100" refer to samples irrigated with water, or water spiked with 10μg/L or 100μg/L PPCPs; "WW0", "WW10", and "WW100" indicate the corresponding wastewater-irrigated samples. No significant difference was detected by ANOVA and Tukey’s post hoc testing.



**Supplementary** **Figure S4.** Alpha-diversity and richness of root-associated bacteria in fresh soil (1st campaign, **A**) and contaminated soil (2nd campaign, **B**) irrigated with water or treated wastewater and different concentrations of PPCPs. "W", "W10", and "W100" refer to samples irrigated with water, or water spiked with 10μg/L or 100μg/L PPCPs; "WW0", "WW10", and "WW100" indicate the corresponding wastewater-irrigated samples. Standard deviations are indicated by error bars. ANOVA followed by Tukey’s test was performed. Significant differences is indicated as “\*\*” for N.D.1 ≤ p–value ≤ 0.01. For 1st campaign water without PPCPs, and 1st campaign wastewater 100μg/L, n=4.

**Supplementary Table S7**. Permutation analysis for the homogeneity of multivariate dispersions among samples (permutations = 1000).

|  |  |  |  |
| --- | --- | --- | --- |
| **Dataset** | **Factor(s)** | **Df** | **p-value** |
| 1st Campaign | WaterType | 1 | 0.2218 |
|  | PPCP Concentration | 2 | 0.4885 |
| 2nd Campaign | WaterType | 1 | 0.3207 |
|  | PPCP Concentration | 2 | 0.6993 |



**Supplementary Figure S5**. Relative abundance [%] of bacterial phyla based on the taxonomic assignments of the amplified 16S rRNA genes. Only phyla with abundance higher than 1% are shown. For 1st campaign water without PPCPs, and 1st campaign wastewater 100μg/L, n=4.



**Supplementary Figure S6**. Heatmap showing relative abundance of significantly different bacterial families in the first (A) and in the second (B) campaign. Grey bars indicate bacterial families significantly affected by the WaterType, green bars the significantly affected by the Concentration of pharmaceuticals and personal care products. For 1st campaign water without PPCPs, and 1st campaign wastewater 100μg/L, n=4.

**Supplementary Figure S7**. Heatmap showing relative abundance of significantly different bacterial genera in the second campaign. Grey bar indicates bacterial genera significantly affected by the WaterType, green bar the significantly affected ones by the concentration of PPCPs and the orange bar the ones affected by the interaction of WaterType:Concentration. For 1st campaign water without PPCPs, and 1st campaign wastewater 100μg/L, n=4. *Allo.-Neo.-Para.-Rhizobium* = *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium*.

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