

Supplementary material

Overview of pipe materials in the study and the reference distribution systems

Table S1. Length of different pipe materials in the study system and the reference system

Material	Study system		Reference system	
	km	% of total length	km	% of total length
Asbestos Cement	1024	37	1210	52
Polyvinylchloride	838	31	575	25
Polyester	606	22	325	14
Ductile cast iron	118	4	99	4
Gray cast iron	45	2	35	2
Concrete	58	2	31	1
Steel	46	2	53	2
Polyester Aluminum composite	4	<1	3	<1
Glass fiber reinforced polyester	2	<1	1	<1
Copper	1	<1	<1	<1

Water quality in the study system and reference system

Table S2. Water quality at the outlets of the treatment plants supplying water to the study system (location A1) and the reference system (location A2).

Parameter	Study system (A1)	Reference system (A2)
TOC (mg/L)	1.1 ± 0.3	1.1 ± 0.2
AOC (µg Ac-C/L)	11.7 ± 3.4	4.3 ± 2.5
Ammonium (mg/L)	<0.02	<0.02
Nitrate (mg/L)	4.6 ± 2.2	2.5 ± 1.2
Ortho-phosphate (µg P/L)	8 ± 10	14 ± 4
Dissolved iron (µg/L)	<10	<10

Table S3. Microbial changes in the study system and reference system. Comparison of microbial measurements at the treatment effluents (A1 and A2) and of average values of measurements at various distribution locations.

Parameter	Study system		Reference system	
	Treatment (A1)	Distribution	Treatment (A2)	Distribution
HPC (CFU/mL)	4 ± 22	41 ± 212	2 ± 16	13 ± 219
<i>Aeromonas</i> (CFU/100 mL)	1 ± 4	442 ± 1039	2 ± 6	65 ± 285
Total cell counts (cells/mL)	3.7×10 ⁵ ± 4.6×10 ⁵	3.2×10 ⁵ ± 2.1×10 ⁵	2.4×10 ⁵ ± 0.5×10 ⁵	2.6×10 ⁵ ± 2.7×10 ⁵
Intact cell counts (cells/mL)	0.6×10 ⁵ ± 1.0×10 ⁵	0.9×10 ⁵ ± 1.0×10 ⁵	2.0×10 ⁵ ± 0.5×10 ⁵	2.1×10 ⁵ ± 2.1×10 ⁵
% intact cells	16% ± 9%	33% ± 19%	83% ± 3%	79% ± 29%
Total ATP (ng/L)	2.0 ± 2.4	3.5±4.3 ng/L	1.8 ± 1.9	1.7±2.4 ng

Conversion of number of organisms to biomass

Table S4. Biomass per individual for a selection of invertebrates observed in sediment collected from two non-chlorinated distribution systems. Only full invertebrates were taken into account for the biomass calculation (parts of dead *Asellus* not taken into account). From: Ketelaars et al., 2022 [42].

Description	Group	Biomass ($\mu\text{g}/\text{individual}$)
Round worms	Nematoda	0.14
Flatworms	Turbellaria	10.1
Water fleas	Chydoridae	10
Copepods larvae	Nauplius larvae of copepods	1.89
Copepods	Cyclopoida	10
Copepods	Harpacticoida	4
Water louse	Isopoda - Asellidae	1680
Water mites	Halacaridae	2.5
Lake flies larvae	Chironomidae	70.6
Ringed worms	Oligochaeta	16.7
Snails	Gastropoda	300

FTIR, XRD and SEM spectra of sediment collected in the study system

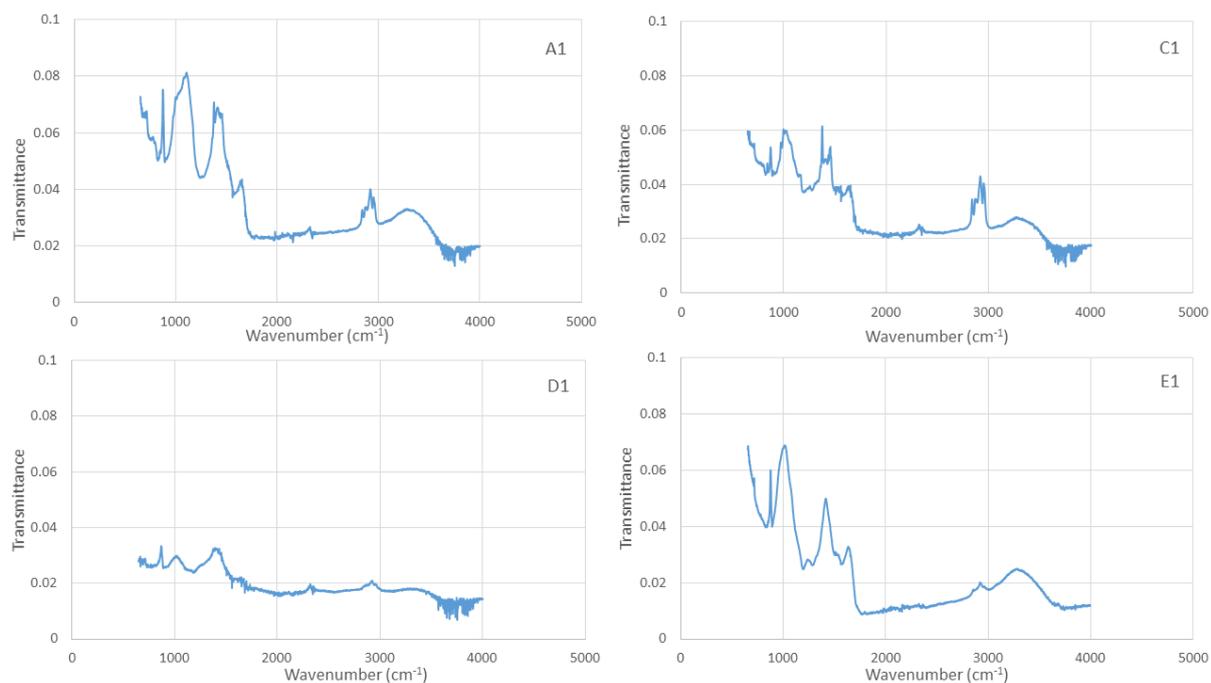


Figure S1. FTIR spectra of sediment samples collected in the study system at locations A1, C1, D1 and E1.

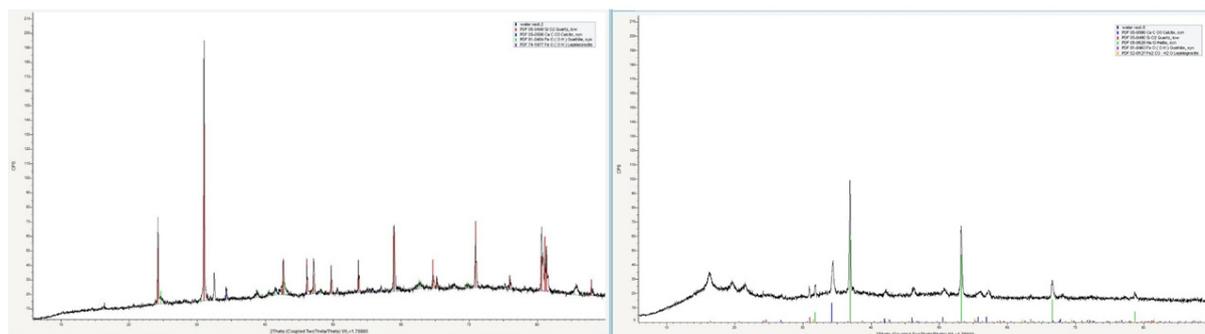


Figure S2. Examples of XRD spectra

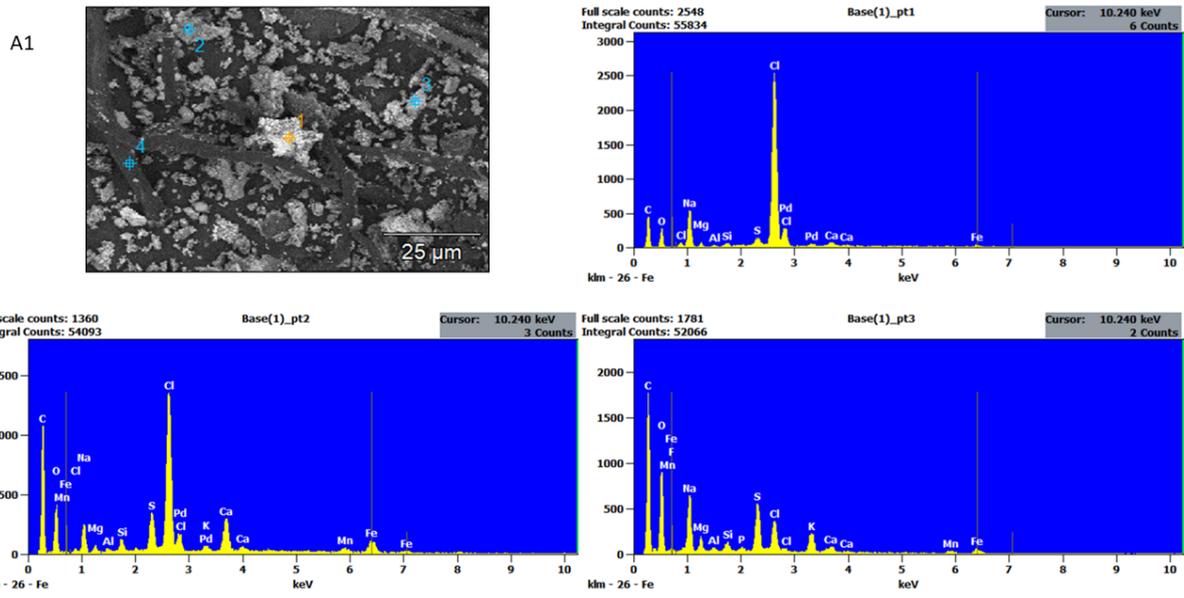


Figure S3. SEM-EDX picture and spectra of sediment collected at location A1 in the study system. The spectra were generated at the points indicated on the microscopic picture.

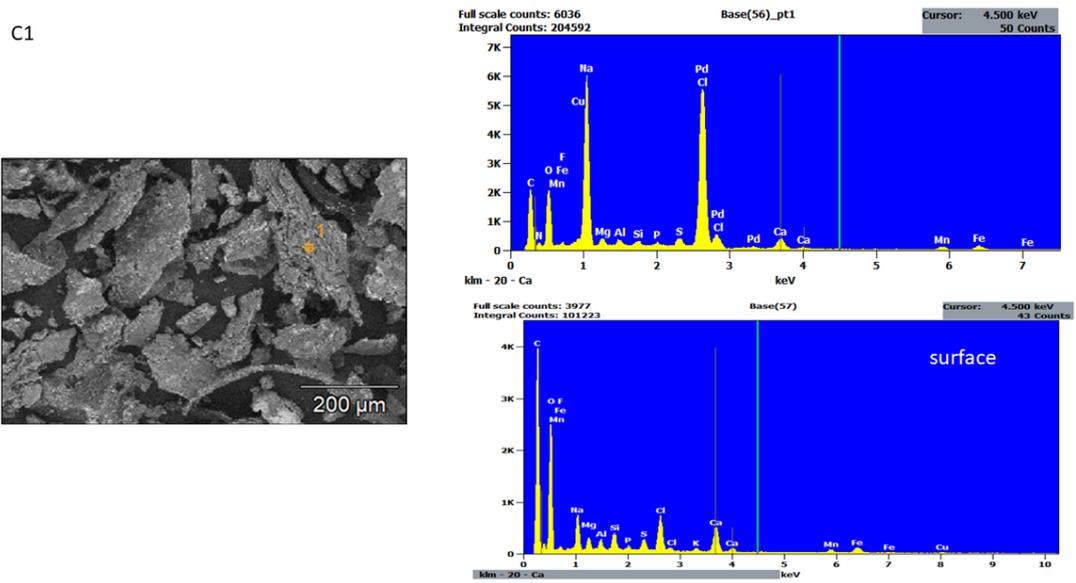


Figure S4. SEM-EDX picture and spectra of sediment collected at location C1 in the study system. The spectra were generated at the points indicated on the microscopic picture.

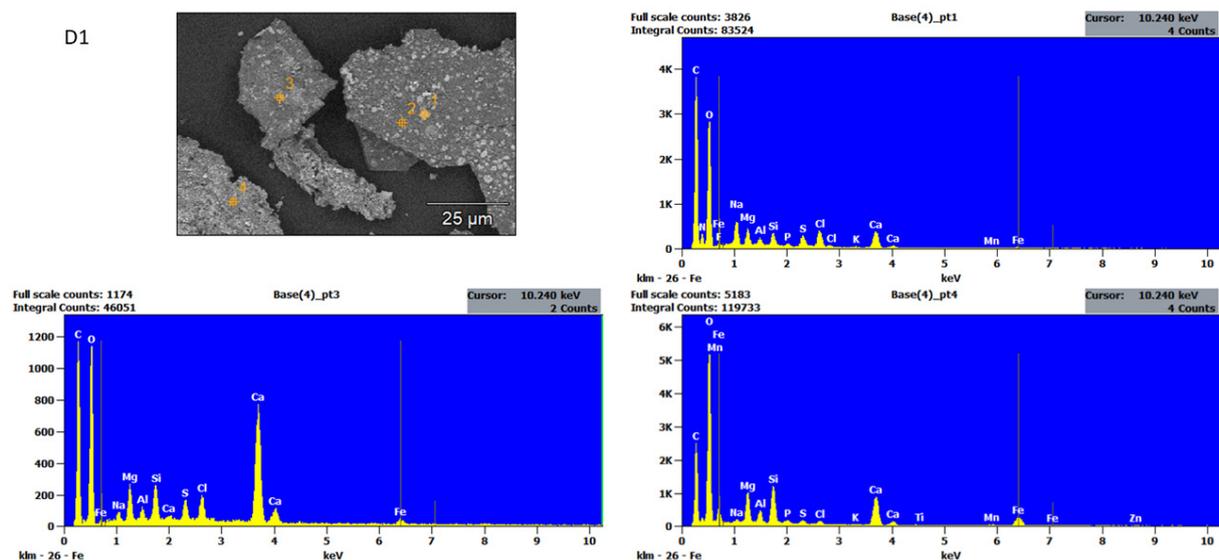


Figure S5. SEM-EDX picture and spectra of sediment collected at location D1 in the study system. The spectra were generated at the points indicated on the microscopic picture.

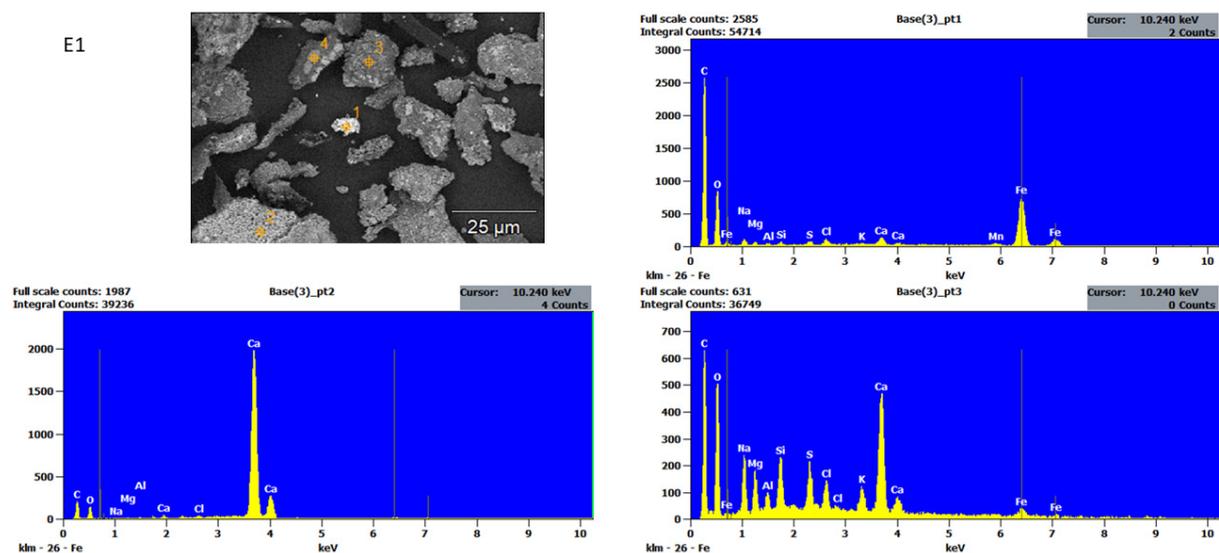


Figure S6. SEM-EDX picture and spectra of sediment collected at location E1 in the study system. The spectra were generated at the points indicated on the microscopic picture.

Sediment analysis of reference system

Detailed analysis of sediment collected in the reference system was performed at one distribution location only (E2). Sediment was collected by pipe flushing (and not with filters as used at other locations). Two fractions were collected over 30 μm and 100 μm filters and analyzed separately.

Table S5. Summary of wavenumbers in FTIR spectrum for samples collected at one location in the reference system by pipe flushing through a 30 μm and a 100 μm filter. Wavenumbers related to organic functional groups are indicated in **bold**; Wavenumbers which are connected to inorganic functional groups are underlined.

Location	Wavenumber (cm^{-1})	Compounds
E2 (30 μm)	3300 , 2648, 1632 , <u>1423</u> , 994 , 874 (very strong)	Proteins, CaCO_3
E2 (100 μm)	3300 , 2928, 1632 , <u>1423</u> , 995 , 873 (very strong)	Proteins, CaCO_3

Table S6. Crystallized minerals in the samples collected in the reference system.

Location	CaCO ₃ %	SiO ₂ %	NaCl %	FeO(OH)+ Fe ₂ O ₃ H ₂ O %
E2 (30 μm)	66.1	21.2	-	4.3+8.5
E2 (100 μm)	7.9	73.4	-	7.5+11.1

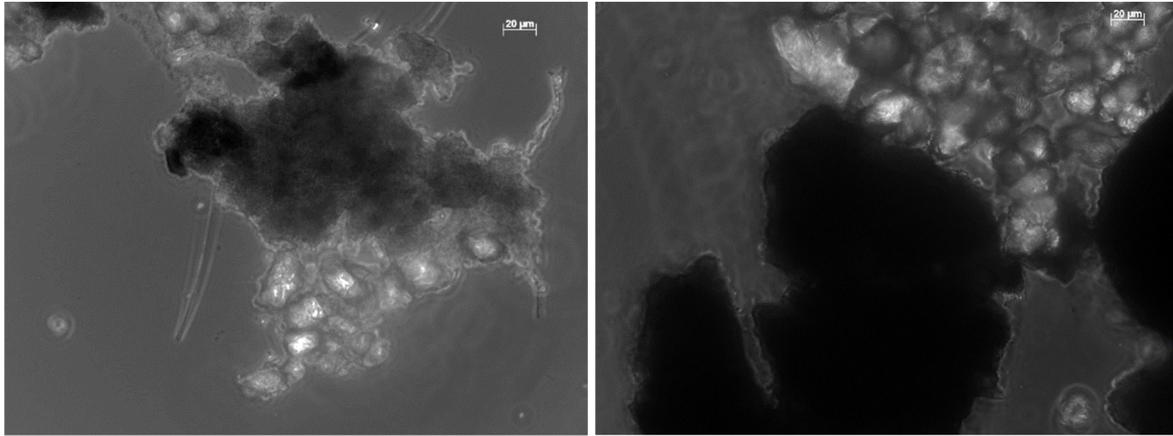


Figure S7. microscope pictures of sediment collected in the reference system through a 30 μm (left) and 100 μm (right) filter after pipe flushing.