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Biodiversity in wetland+ system: a passive solution for HCH dump effluents

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ABSTRACT

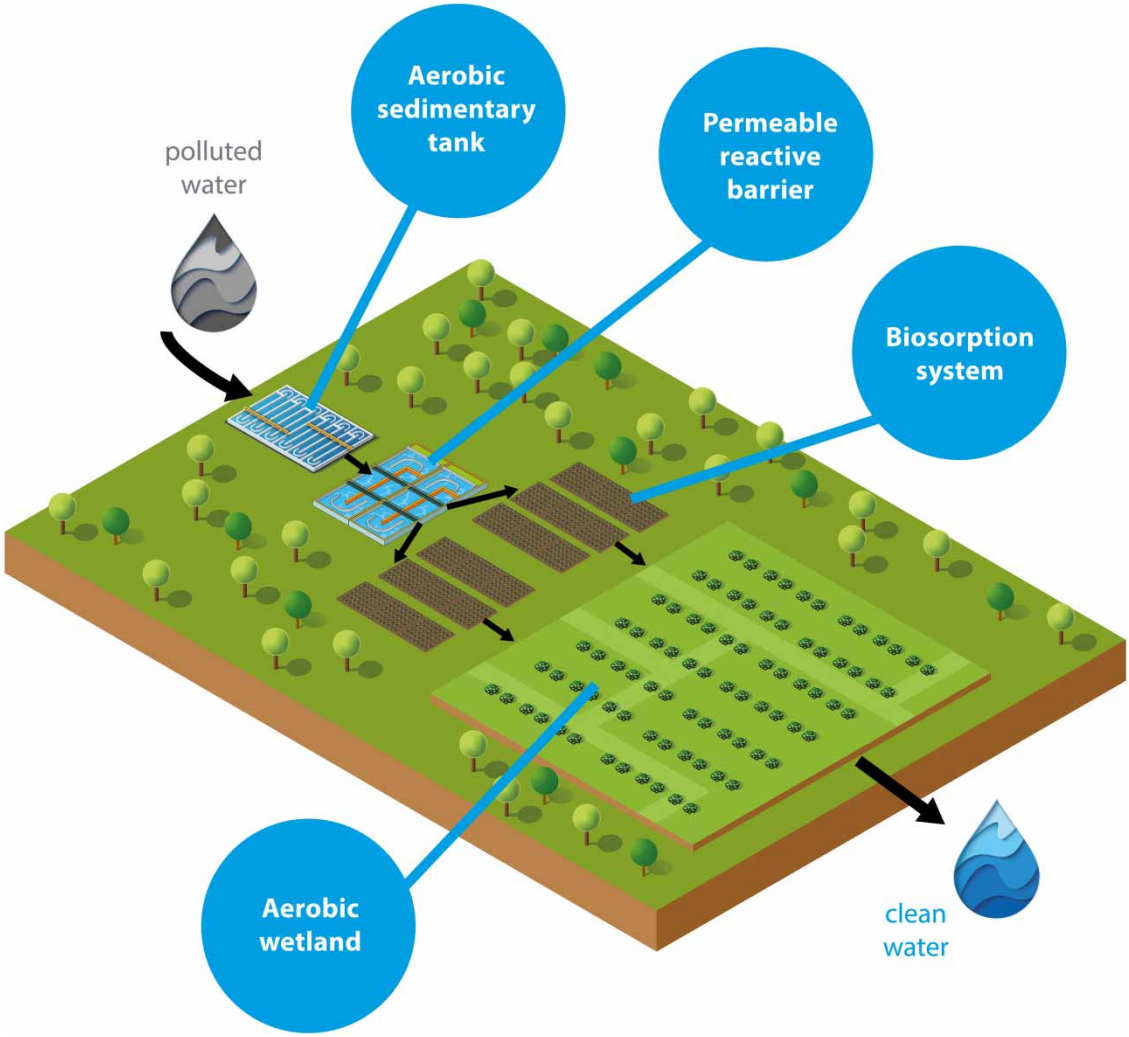
The hexachlorocyclohexane isomers (HCH) are long-banned pesticides. Even though their use has been prohibited for decades, their presence in the environment is still reported worldwide. Wetland + is a registered trademark of the remedial treatment technology consisting of an aerobic sedimentary tank, a permeable reactive barrier, a biosorption system, and an aerobic wetland. This proven method combines a reductive treatment known from PRBs with the natural wetland self-cleaning processes. The average efficiency of the system is 96.8% for chlorobenzenes (ClB) and 81.7% for HCH, during the first 12 months of the system operation. The presence of the genes encoding enzymes involved in the degradation of the HCH compounds indicates that the removal of HCH and ClB occurs not only by chemical removal but also through aerobic and anaerobic combining biodegradation. Changes in abundance and the composition of the diatom community were found to be suitable indicators of the water quality and of the impact of the Wetland + operation on the water ecosystem. The system's annual operation exhibited a markedly higher number of diatom species in the closing profiles of the Ostrovský Creek, the Wetland + effluent recipient.

Key words: biodegradation, bioindicators, constructed wetland, diatoms, environmental biomonitoring, HCH

HIGHLIGHTS

- Wetland+ system achieved high removal rates: 96.8% for ClB and 81.7% for HCH over 12 months.
- Aerobic wetland module had the highest abundance of HCH-degrading microorganisms.
- Diverse microbial community contributed to HCH degradation and pollutant removal.
- Diatom community changes served as reliable water quality indicators.

GRAPHICAL ABSTRACT



INTRODUCTION

Hexachlorocyclohexane (HCH) isomers (α, β, γ, δ, ε) and their transformation products such as chlorobenzenes (ClB) generate severe and persistent environmental problems at many sites worldwide. α, β and γ-HCH are listed under the Stockholm Convention on persistent organic pollutants (POPs) and the use of γ-HCH (lindane) was banned from the end of 2007 under EC Regulation No.850/2004. Many studies suggest that HCH production across Europe has led to about 40 megasites with the total HCH waste exceeding 250,000 tonnes (Vijgen & International HCH and Pesticides Association 2006; Vijgen *et al.* 2019). This is in addition to many smaller sites and diffuse contamination problems.

Commonly, some of the megasites have been treated using physical containment to manage migration pathways and direct contaminated water flow into permeable reactive barriers (PRBs). However, these containments are never perfect and their leakage and subsequent drainage to surface water, or groundwater, remains a potential cause of environmental harm. As HCH bioaccumulates in freshwater food chains (fish, crustaceans) and can enter plant biomass, the potential for cumulative chronic harm in the long term is high (Basu *et al.* 2021; Li *et al.* 2021). Treatment of these leakages can be costly when using conventional wastewater treatment plants (WWTP), particularly for remote locations. Conventional WWTP also has a relatively high associated maintenance and management load, which may also increase the risks of process failure/interruption over the decades that a system is expected to run.

Alternatively, we designed a robust, low maintenance, and sustainable treatment system, cheaper than conventional WWTP that can be deployed in remote locations where access to infrastructure is limited. A full-scale prototype termed 'Wetland+', based on integrated reactive zones with a constructed wetland as a polishing step was installed at Hajek (Czech Republic) within the LIFEPOPWAT project. The water that drains the HCH waste-containing deposit forms the Ostrovský Creek and is the source of the contamination for the surroundings.

To assess the impact of the Wetland+ system on microbial biodiversity in water, we have analyzed benthic diatoms that are well-established bioindicators, sensitive to many different environmental factors such as pH, salinity, oxygen, nutrients, organic matter, pollution by toxic substances, light, and temperature (Stevenson *et al.* 2010; Stevenson 2014; Poikane *et al.* 2016; Salmaso *et al.* 2019). The European Water Framework Directive (European Commission 2000) includes diatoms in an evaluation of water quality in rivers alongside other bioindicators, such as macrophytes, macro-invertebrates, and fish. While physicochemical analysis indicates the water quality only at the time of sampling, diatom analysis assesses water quality within a longer term. Nevertheless, communities of benthic diatoms can be affected by their competitors and predators and also by other disturbances that impair the water quality such as higher flow velocities, turbidity, drying, and sludge cover.

Certain bacteria have been reported to be able to degrade HCH (Phillips *et al.* 2005). They utilize HCH as a carbon source and break it down into less toxic metabolites. Microbial degradation of HCH can occur in various environmental compartments, including soil, water and sediments (Gupta *et al.* 2000; Kumar *et al.* 2006; Kumar & Pannu 2018; Amirbekov *et al.* 2021). The capable bacteria species include *Pseudomonas aeruginosa* (Kumar *et al.* 2005; Lodha *et al.* 2007) and *Pseudomonas putida* strains (Samin *et al.* 2014; Jaiswal *et al.* 2023), which possess the linA gene involved in aerobic HCH degradation. Another species, *Sphingobium* spp. (Nagata *et al.* 1999; Verma *et al.* 2014), *Dehalobacter* spp. (van Doesburg *et al.* 2005), *Dehalococcoides* spp. (Bashir *et al.* 2018; Qiao *et al.* 2020), and *Desulfitobacterium* spp. (El Fantroussi *et al.* 1998) are able to HCH degradation under aerobic or anaerobic conditions.

The primary aerobic degradation pathway of HCH, particularly the γ-HCH (lindane), can be carried out by certain bacteria, most notably by *Pseudomonas* (Imai *et al.* 1989). The initial step involves the conversion of γ–HCH to 1,2,4-trichlorobenzene (1,2,4-TCB) through a dehydrochlorination reaction mediated by the γ–hexachlorocyclohexane dehydrochlorinase (Lindane dehydrochlorinase) enzyme encoded by *linA* gene (Lal *et al.* 2010). The 1,2,4-TCB is further degraded by enzymes such as dioxygenases and monooxygenases, leading to the formation of metabolites like chlorophenols, catechols, and other intermediates. Subsequent steps involve ring cleavage and further degradation of these intermediates, ultimately resulting in the complete mineralization of HCH (Lal *et al.* 2010).

Other genes namely, *linB, linB-RT* encoding the haloalkane dehalogenases have been studied in the context of the aerobic degradation pathway as well. These enzymes are also involved in the initial steps of HCH degradation, specifically the conversion of lindane to 1,2,4-TCB. The exact haloalkane dehalogenases responsible for this transformation may vary among different bacteria, but their activity leads to the removal of chlorine atoms from the HCH molecule. Nagata demonstrated that *linD* gene encoding reductive dechlorinase for 2.5-DCHQ degradation in *Sphingobium japonicum UT26* is an established gene that is expressed only when the substrates of these gene products exist (Miyauchi *et al.* 2002).

Anaerobic degradation of HCH is carried out by certain bacteria such as *Dehalobacter* spp., under low or no oxygen conditions (van Doesburg *et al.* 2005). The initial step involves reductive dechlorination of HCH, where the chlorine atoms are sequentially removed from the HCH molecule. Reductive dechlorination is facilitated by enzymes called reductive dehalogenases, which catalyze the removal of chlorine atoms and produce less chlorinated intermediates, such as pentachlorocyclohexene (PCCH) and tetrachlorocyclohexene (TCCH). Further degradation of these intermediates can occur through various pathways, including fermentation, hydrogenation, and ring reduction reactions, resulting in the formation of simpler and less toxic compounds. Kaufhold *et al.* (2013) reported the transformation of the γ–HCH isomer to monochlorobenzene (MCB) by a highly enriched *D. mccartyi* strain BTF08-containing culture as well as by *D. mccartyi* strain 195 (Kaufhold *et al.* 2013). Dechlorination of the γ–HCH by *Dehalococcoides* species and an enrichment culture achieved by Bashir (Bashir *et al.* 2018). It is important to note that the specific enzymes involved in HCH degradation may vary among different bacterial strains. Furthermore, the complete degradation of HCH can be a complex process involving multiple enzymes and pathways, as well as interactions with co-existing microbial communities in the environment.

The biodegradation potential of each Wetland+ module toward HCH is assessed by identifying functional genes encoding enzymes involved in HCH transformation and determining the relative microbial abundance. The functional genes *linA*, *linB*, *linB-RT*, and *linD* were studied together with the DHC gene anaerobic biomarker of the *Dehalococcoides* spp. The main goal

of this study is to report on the initial performance of the freshly constructed treatment system prior to and during the first vegetation season.

MATERIALS AND METHODS

Site description and design of the system

This study took place in Hajek, Czech Republic (50°17′31.5″ N 12°53′35.2″ E). The Hajek site is located in Western Bohemia near the Karlovy Vary spa. Since the 1960s, the site has been used as a waste repository for the nearby mine of uranium, kaolin, basalt, and bentonite. According to the decision of the state authorities, in 1966–1968, about 3,000–5,000 tonnes of residual ballast isomers of HCH and ClB from the production of lindane (γ-HCH) from the Spolana chemical plant (Neratovice, Czech Republic) were disposed into the Hajek quarry spoil heap. Nowadays, the long-term average concentrations in the outlet drainage channel reach 136, 605, and 28 μg L^{-1} sum of HCH, of ClB and of chlorophenols (ClPh), respectively.

The Wetland+ system was installed in September 2021. It consists of the following parts, connected in series: an aeration and sedimentation module (A), a permeable reactive barrier (B), a biosorption module (C), and an aerobic wetland system (D) (Figure 1). The aeration and sedimentation module (A) consists of three compartments connected in series of an area of 313 m^2 to enhance precipitation and sedimentation of dissolved iron, which is at a relatively high concentration in the inflow water (see below). The next module (B) of an area of 540 m^2, with the total volume of the module of 360 m^3 is filled with zerovalent iron (ZVI) chips. Here, a part of HCH is transformed to ClB by dehalogenation due to strong anaerobic conditions. The B module consists of six compartments B1–B6. The individual compartments are interconnected in three

Figure 1 | Wetland+ modules. The inlet (S), aeration system including sedimentation (a), permeable reactive barrier remedial system (b), biosorption module (c), and aerobic wetland system (d).

parallel branches and each branch consists of two compartments connected in series. The water flows through this system in a longitudinal direction, where the inflow is introduced beneath the surface of the layer of iron chips and the outflow is collected at the bottom by a perforated pipe located in the central longitudinal axis of the B module. Following is the C module, where a mix of gravel, soil, peat, and wooden chips form a bed. This biosorption module consists of two compartments with parallel connections, the total volume of the reactor is 480 m^3. Common wetland species (*Phragmites* sp. and *Phalaris* sp.) were planted in the C module at a density of 4 plants m^{-2} in a total area of 650 m^2. Splitting of the flow in B and C modules enables to refill of the substrate without any break in the system's operation. The final treatment takes place in module D, a surface flow aerobic wetland with an area of 2,669 m^2, and a total aerobic wetland volume: of 1,600 m^3. This stage serves for the final removal of organic substances, suspended solids and the decomposition products of HCH, especially ClB. This aerobic wetland is filled with soil/gravel and planted with common wetland species (*Typha* spp., *Spargamium erectum, Juncus* spp., *Glyceria fluitans*) enriched by rare and protected species supplied by the local botanical garden (Dalovice) and from nearby natural reserves, such as *Hippuris vulgaris, Calla palustris,* or *Menyanthes trifoliata*. Part of the D area was left unplanted for testing purposes to observe the spontaneous succession of wetland plants from natural seed. In contrast to previous modules, in module D, aerobic transformation pathways of HCH and ClB are employed by bacterial enzymes, in addition to adsorption and bioaccumulation.

Sampling

The water samples for chemical analyses and molecular biology analyses (i.e., amplicon 16S rRNA sequencing, qPCR; see below) were collected from outflows of each Wetland+ module. Physical–chemical parameters of water (temperature, pH, electrical conductivity, redox potential, and dissolved oxygen concentration) were measured using Multi 3430 SET C (WTW, Weilheim, Germany) directly on site. All samples were transported to the laboratory in a cooled box, and water samples for molecular biology analysis were immediately filtered through a 0.2 μm membrane filter and stored at −80 °C.

Diatom samples were taken along the Ostrovský Creek (Figure 2), in August 2021 (prior to the Wetland+ system commissioning) and August 2022 (almost a year after commissioning) as well as in modules of the Wetland+ treatment system in

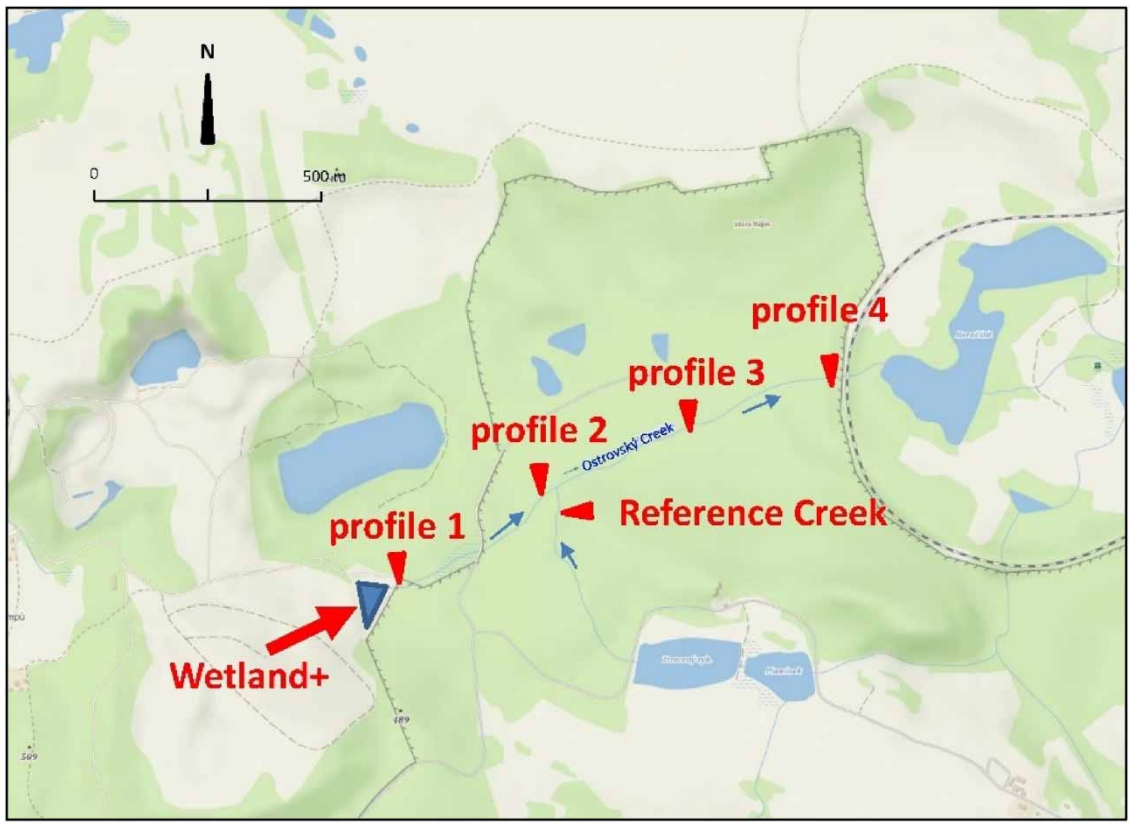


Figure 2 | Profiles of the Ostrovský Creek (the recipient of the Wetland+ outflow) and the Reference Creek sampled for diatom analysis.

August 2022 (Figure 1). The nameless uncontaminated tributary of the Ostrovský Creek was chosen for the sampling of reference samples (Reference Creek, Figure 2). The Reference Creek was sampled only in August 2021, because August 2022 was a dry period. Diatom samples were taken from submerged stones and from various surfaces (submerged plants, leaves, and branches of trees and shrubs) and from the mud surface layer with fine detritus when no stones were present. Samples were taken in 100 mL plastic bottles, and transported to the laboratory under refrigerated and dark conditions. Once in the laboratory, microscopic analysis of live samples was performed and permanent slides of diatoms were prepared. The rest of the samples were preserved with formaldehyde solution to a final concentration of 3% and archived.

Chemical analysis

According to a previous study, the concentration of HCH isomers and their ClB byproducts in water were determined using two GC-MS assemblies (Wacławek *et al.* 2019). The first used an RSH/Trace 1310/TSQ 8000 GC-MS array (ThermoFisher Scientific, USA) with a Scion-5MS column for HCH, ClB, and ClPh (Scion Instruments, Goes, The Netherlands), whereas the other was based on a CombiPal/CP3800/Saturn2200 GC-MS array (PAL, Zwingen, Switzerland; Varian, Palo Alto, CA, USA) using a DB-624 column for benzene and monochlorobenzene determination (Agilent, Santa Clara, CA, USA). Samples were extracted using the headspace SPME technique, either using a polydimethylsiloxane/divinylbenzene (PDMS/DVB) fiber with a coating thickness of 100 μm (Supelco, Bellefonte, USA) or directly injecting the sample in static headspace mode. Prior to extraction, samples were derivatized to form acetylated chlorophenols (following EN 12673). Isotopically labeled compounds (γ-HCH D6, pentachlorophenol 13C6) were used as internal GC-MS/MS analysis standards.

Analysis of benthic diatoms

The determination of the diatom species and counts of diatom valves were processed by permanent slides using a light microscope (Optika, B-383PL, Italy) equipped with a camera (Optika, C-B1, Italy) at a magnification of 1000×. Diatom taxa were identified using standard literature (Lange-Bertalot *et al.* 2017). The relative abundance of diatoms (the proportional representation of different diatom taxa within the community) was evaluated by enumeration of frustules. The number of given species was obtained by calculating individuals in a random sample in the ocular fields of a microscope. A minimum of 300 diatom valves were counted in each sample. In samples with a low number of individuals (<300), all frustules on two permanent slides were counted. The relative abundance data were assigned to seven abundance classes as follows: 0 = 0% (absent), 1 = >0 to <1% (sporadic), 2 = >1 to < 5% (rare), 3 = >5 to < 10% (regular), 4 = >10 to < 30% (common), 5 = >30 to < 60% (frequent), 6 = > 60 (dominant). The Shannon diversity index was used for the evaluation of diversity of the diatom community. This index expresses the diversity of species considering the number of species (richness) and their relative abundance (evenness). The Shannon diversity index values were counted using the OMNIDIA software version 6.1.2 (Lecointe *et al.* 1993).

Molecular biology analysis

Duplicate DNA extractions were performed on all samples using the DNeasy PowerSoil Kit (Qiagen, Netherlands). The DNA yield and quality were evaluated using a Qubit fluorometer (Thermo Fisher Scientific, Waltham, MA, USA) and agarose gel electrophoresis. DNA was assessed for all samples using the primers LinA-F (5′AGCTCAACGGATGCATGAACT3′), LinA-R (5′ GGCGGTGCGAAATGAATG3′), LinB-F (5′ACCACGGGCCGAATGC3′), LinB-R (5′ACCGTGATTTCGGTCTGGTTT 3′), LinB-RT-F (5′GCGATCCGATCCTCTTCCA3′) and LinB-RT-R (5′GCATGATATTGCGCCACAGA3′), LinD-F (5′GAAC TGTTCCACTTCGTGTTCTCA3′), and LinD-R (5′GGTCACGCCCTTCTCCATTA3′) (Suar *et al.* 2004; Bala *et al.* 2010; Gupta *et al.* 2013) DHC-F (5′GGGAGTATCGACCCTCTCTG3′), DHC-R (5′CGTTYCCCTTTCRGTTCACT3′) (Yoshida *et al.* 2005). Total bacterial biomass was assessed by amplification of the 16S rDNA gene using the primers U16SRT-F (5′ACTCCTACGGGAGGCAGCAGT3′) and U16SRT-R (5′TATTACCGCGGCTGCTGGC3′) (Clifford *et al.* 2012). All qPC R reactions were run in the LightCycler 480 Real-Time PCR System (Roche, Basel, Switzerland) using white 96-well plates to increase sensitivity. Triplicate reactions of 20 μL consisted of 1 × SYBR Green I Master (Roche, Basel, Switzerland), 0.5 mM of both primers, and 2 mL of the template (0.4–16 ng). The program consisted of preincubation at 95 °C for 5 min, a melting cycle of 95 °C for 10 s, annealing for 15 s, elongation at 72 °C for 20 s, and melting to 97 °C. Optimized annealing temperatures were 55 and 60 °C with a cycle number of 45. The data were displayed in the form of a heatmap of Ct values, the values of a particular primer being first divided into two sets comprised of values < 36 (low values) and ≥36, the latter being included in the detection boundary group. The second set, values < 36, was divided into three equally wide intervals, and gradually from the lowest values, the intervals were grouped into high quantity, medium quantity, and small quantity.

Ct values equal to 40 were considered below the LOQ. Individual groups (intervals) are presented in heat maps in appropriate colors (Nechanická & Dolinová 2018).

Microbial abundance was assessed by amplifying the V4 region of the bacterial 16S rDNA gene using the primers 530F (5′TGCCAGCMGCNGCGG3′) and 802R (5′TACNVGGGTATCTAATCC3′) in a final volume of 50 μL (White *et al.* 1990; Claesson *et al.* 2010). The amplicons were cleaned using the Agencourt Ampure XP system (Beckman Coulter, USA) and the Ion Torrent platform (Thermo Fisher Scientific, USA) was used for sequencing analysis. Barcoded sequencing adapters were ligated to the PCR products using the Ion Xpress Plus gDNA fragment library kit with Ion Xpress barcode adapters (Thermo Fisher Scientific, USA) and the samples were analyzed using the Ion PGM Hi-Q Sequencing Kit using an Ion 314 Chip (Thermo Fisher Scientific, USA).

The obtained data were processed with QIIME 2 2021.8 software (Bolyen *et al.* 2019). Raw sequence data were demultiplexed and quality filtered using the q2-demux plugin followed by denoising with DADA2 (via q2-dada2) (Callahan *et al.* 2016). Taxonomy was assigned to ASVs (Amplicon Sequence Variant) using the q2-feature-classifier (Bokulich *et al.* 2018), and classified by classify-sklearn naïve Bayes against the Silva 138 database (Quast *et al.* 2013) and then mitochondria and chloroplast were removed. The accuracy of classification was evaluated against an artificial MOCK community sample. QIIME 2 outputs were processed using the phyloseq R package (McMurdie & Holmes 2013).

HCH and ClB removal efficiency

The overall removal efficiency of the Wetland+ treatment system was calculated from the difference in HCH or ClB concentration in inflow water (S) and outflow water (D). The removal efficiency of individual modules of the system was calculated from the difference in pollutant concentrations in inflow and outflow to/from the respective module. The average removal efficiency was calculated as the arithmetic mean of efficiency values from the 12 different sampling campaigns performed in the period September 2021–September 2022.

RESULTS

General characteristics of inflow water and wetland+ water

The inflow drainage water (S) had a long-term average pH value of 6.6, with an average conductivity of 1,719 μS cm^{-1}, redox potential 182 mV (original S water is anoxic with a low redox potential, but due to contact with atmosphere the redox and oxygen content increases), high sulfate concentration 598 mg L^{-1}, high total Fe concentration 24.3 mg L^{-1}, and low F^-, NO_2^- , and NO_3^- concentrations (below LOD). Average concentrations of HCH and ClB in inflow water were 136.2 and 604.6 μg L^{-1}, respectively, with an average flow of approximately. 2 L s^{-1}. The physicochemical parameters of water are given in Table 1.

Pollutant removal efficiency

The average removal efficiencies of individual modules are given in Table 2.

In total, 5.9 kg of HCH and 26.0 kg of ClB were eliminated from the drainage water within the 12-month operation of the system. Individual HCH isomers were not removed evenly but followed the pattern: $\alpha = \gamma = \delta > \varepsilon = \beta$. As a result, while δ-HCH dominated at the inflow to the system covering more than 60% of the total HCH concentration, ε-HCH prevailed at the outflow from the system.

Table 1 | Physicochemical parameters of inflow and outflow water from individual modules of Wetland +

Module	pH (–)	Eh (mV)	O_2 (mg/L)
S (inflow)	6.6	182	2.65
A	6.7	283	4.26
B	7.2	110	2.76
C	7.3	187	7.00
D	7.4	356	9.00

Table 2 | Average removal efficiency of each individual module and of the whole Wetland+ treatment system in the period September 2021 – September 2022

Module	∑HCH (%)	∑ClB (%)
A	3.9	34.3
B	45.0	94.7
C	21.2	53.1
D	37.3	74.0
Whole system	81.7	96.8

Approximately 98% of the inflow total concentration of the ClB group comprised 1.4-di-ClB > 1.3-di-ClB > 1.2.4-tri-ClB > 1.2-di-ClB > 1.3.5-tri-ClB with 1.2.3-tri-ClB, 1.2.4.5 + 1.2.3.5-tetraClB, 1.2.3.4-tetraClB, penta-ClB, and hexa-ClB found at <1% of the total concentration. The removal of individual di-ClB compounds by the treatment system was more intensive than tri-ClB compounds, thus their mutual ratio in the outflow balances out.

Diatom diversity

In the monitored area, 125 diatom taxa were observed in 2021 and 2022 (Supplementary Table S1). For each benthic sample from the Wetland+ treatment system, four profiles of the Ostrovský Creek and, the Reference Creek contained from 0 to 35 species of diatoms and values of the Shannon diversity index ranged from 0 to 4.72 (Table 3). In 2021, the number of species

Table 3 | Composition and classes of relative abundance of diatoms from the Ostrovský Creek, the Reference Creek and Wetland +

	Ostrovský Creek									Wetland+ 2022								
Profile	1		2		3		4		Ref.									
Year	2021	2022	2021	2022	2021	2022	2021	2022	2021	A	C1/1	C1/2	C2/1	C2/2	D1	D2	D3	D4
Species number	0	3	3	14	30	29	35	30	28	1	0	1	0	1	4	12	18	16
Diversity		1.5	1.0	3.5	4.1	3.9	4.7	4.6	4.0	0		0		0	0.4	2.1	3.0	3.6
Species	Class																	
Achnanthidium minutissimum				3	4	4			3						2	2	4	4
Cocconeis placentula						4	2											
Cymbella lange-bertalotii		5		3		3	2			6		6		6	6	5	4	4
Epithemia turgida																		4
Eunotia arcus									4									
Eunotia botuliformis					2				4									
Fragilaria famelica			4															
Gomphonema acuminatum								4										2
Gomphonema micropus							4											
Gomphonema parvulum			6		3	2	2	2	4						2			
Navicula lanceolata					4	2	2	2										
Navicula slesvicensis							4											
Navicula tripunctata									4									
Pinnularia neomajor		4	4															
Pinnularia viridis		4			2													
Rhopalodia parallela																4	5	2
Ulnaria ulna				4		2	2									1	1	

Classes of the relative abundance: 0 = 0% (absent), 1 = >0 to <1% (sporadic), 2 = >1 to < 5% (rare), 3 = >5 to < 10% (regular), 4 = >10 to < 30% (common), 5 = >30 to < 60% (frequent), 6 = >60% (dominant). These classes were determined by enumeration of frustules. The list of species includes only species with a relative abundance > 10% in at least one sample.

increased from profile 1 to 4, where the highest number of diatom species and the highest diversity (35 and 4.72, respectively) was observed. A total of 60 species of diatoms were observed in the four sampled profiles of the Ostrovský Creek and 28 species of diatoms were observed in the Reference Creek in August 2021.

Similarly, a total of 63 diatom species were observed in four sampled profiles of the Ostrovský Creek in August 2022. The most frequently occurring species in the Ostrovský Creek for both monitored years were *Gomphonema parvulum*, *Cymbella lange-bertalotii*, *Navicula lanceolata*, and *Nitzschia linearis* (Supplementary Figures 1–4). The species with the highest relative abundance in the Reference Creek were *Eunotia arcus*, *E. botuliformis*, and *Gomphonema parvulum* (Table 3).

A total of 34 species were found in the Wetland+ system in August 2022. The highest number of species (18) was detected in section D3 of module D and no diatoms were observed in sections C1/1 and C2/1 of module C. The most frequently occurring species in the Wetland+ system were *Cymbella lange-bertalotii, Achnanthidium minutissimum, Rhopalodia parallela*, and *Nitzschia linearis* (Table 3; Supplementary Figures 1, 2). Only one species of diatom (*Cymbella lange-bertalotii*) was observed in very low numbers in modules A and C where the HCH pollution was highest. *Cymbella lange-bertalotii* was also observed in all monitored sites, except for profile 4 of the Ostrovský Creek in 2022.

qPCR results

The functional genes *linA*, *linB*, *linB-RT*, and *linD* encoding enzymes involved in aerobic HCH biodegradation were detected in all samples (Table 4). The highest level of the U16SRT gene, representing the total microbial biomass, was detected in the outlet from the aerobic wetland (module D). The relative abundance of the *linA*, *linB-RT*, and *linD* genes was the highest in the outlet. The quantity of the *lin* genes in the samples from modules S, A, and B was similarly low, but in samples from module C were *lin* genes elevated.

The highest relative abundance of *Dehalococcoides* spp. (DHC), was found in the inflow water (profile S) and in modules A and B. In the direction of water flow (modules C and D), the abundance of DHC was decreasing.

Microbial abundance

The microbial profile demonstrates a high abundance of bacteria belonging to the genus *Galionella* and *Candidatus Omnitrophus* but with a decreasing trend to the end of the wetland system (Figure 3). The D module was rich in bacteria of genera *Massilia, Rhodoferax, Hydrogenophaga, Flavobacterium, Caulobacter*, and *Bacteriovorax* compared to another module. The abundance of bacteria of the sulfate-reducing genera *Desulfovibrio* and *Desulfosporosinus* was found is become highly elevated in all module profiles except for the inlet water S and A modules. The abundance of the family Oxalobacteraceae, family Comamonadaceae and *Pseudarcobacter* increased in modules C1, C2, and D compared to others.

Table 4 | Relative abundance of *Dehalococcoides* spp. and genes indicating total bacterial biomass (16S rDNA), dehydrochlorinase (*linA*), haloalkane dehalogenase (*linB, linB-RT*), and reductive dechlorinase (*linD*) in individual modules of Wetland +

	Total bacterial biomass	**Dehalorespiring bacteria**	**Lindane-degrading bacteria**			
	16S rDNA	*Dehalococcoides* spp.	dehydrochlorinase	haloalkane dehalogenase	haloalkane dehalogenase	reductive dechlorinase
	U16SRT	**DHC**	**linA**	**linB**	**linB-RT**	**linD**
S (inlet)	++	+++	+	+	+	+
A	+	+++	+	+	+	+
B	++	+++	+++	+	++	++
C	++	++	+++	++	++	++
D	+++	+	+++	+++	+++	+++

The color scale indicates the relative quantity of a given marker: red (+++) highest, orange (++) high, yellow (+) intermediate quantity, (+−) low quantity, ND, not detected or below the LOQ.

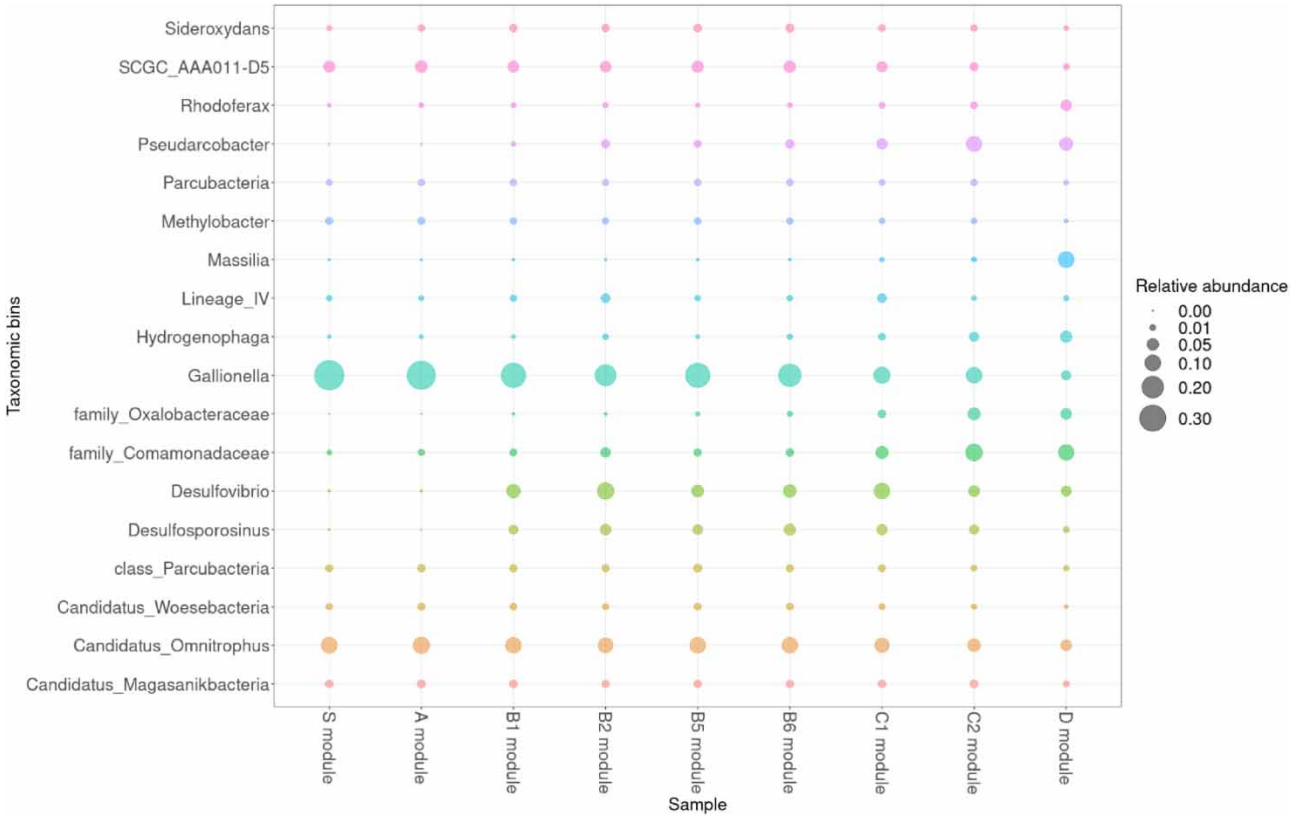


Figure 3 | Relative abundance of taxonomic bins in the individual modules the Wetland+ system.

DISCUSSION

This study assessed the Wetland+ efficiency in removing HCH and ClB from Ostrovský Creek springs in the historically polluted area, the role of bacteria in biodegradation and its effect on the diversity of diatoms. As most of the ClB compounds are volatile and are more readily subject to aerobic degradation (Monferrán *et al.* 2005), a higher ClB removal rate (96.8%) of these compounds was observed. HCH isomers are more persistent compared to ClB; therefore, the average HCH removal rate was 81.7%. This disparity in removal rates emphasizes the need for tailored treatment strategies within the Wetland+ system to effectively address these distinct contaminants.

Analysis of water samples confirmed the presence of functional genes and bacterial genera capable of degrading HCH isomers and their byproducts. Whereas the abundance of *Dehalococcoides* spp., the microorganism capable of anaerobic dechlorination of HCH, was lower in the downgradient profiles C and D, the abundance of the functional genes *linA*, *linB*, *linB-RT*, and *linD* encoding enzymes involved in aerobic HCH biodegradation displayed an opposite trend and in general followed the trend of general increasing concentration of O_2 in water in the direction of water flow. These different patterns indicate prevailing HCH biodegrading processes in the Wetland+ treatment system in addition to chemical reduction in module B and other attenuation processes such as chemisorption, biosorption, bioaccumulation, and evapotranspiration.

The most likely explanation for the co-occurrence of both aerobic and anaerobic biomarkers of HCH degradation is that the inflow drainage water originates in an environment with low redox conditions, but on the way through the system, especially through modules A, C, and D, is exposed to the atmosphere and oxidation – reduction conditions change, see Table 1. Additionally, the flow through individual modules of the Wetland+ system is not homogenous with different pathways of different hydrogeochemical conditions including stagnation zones. Similar cooccurrence of anaerobic and aerobic bacteria degrading chlorinating ethenes was observed in groundwater (Liang *et al.* 2017; Němeček *et al.* 2017), and discrete aquifer soil samples (Richards *et al.* 2019) as well as in surficial riverbed sediment samples (Atashgahi *et al.* 2013).

Understanding the composition and dynamics of microbial communities within the system is essential for its efficient operation. The coexistence of both aerobic and anaerobic bacteria for HCH degradation suggests the need for tailored management strategies. By harnessing this knowledge, it is possible to optimize the conditions that favor specific microbial populations, thereby enhancing the removal of HCH and ClB contaminants.

The analysis of the microbial community revealed a high abundance of iron-oxidizing *Galionella* with a noticeable decreasing trend in the direction of water flow through the system. The presence of these iron-oxidizing bacteria was predicted due to the high iron content of water, and it was reported in our previous study (Amirbekov *et al.* 2021). This trend corresponds with the decreasing content of dissolved (i.e. ferrous) iron (data not shown). The increased abundance of the sulfate-reducing *Desulfovibrio* and *Desulfosporosinus* in module B may be related to the higher content of sulfate and ferrous iron as available electron acceptors and donors, respectively. While sulfate-reducing bacteria do not directly degrade HCH themselves, they contribute to HCH degradation through their metabolic activities and interactions with other microorganisms. Particularly their production of H_2S can stimulate the activity of reductive dechlorinators, including dehalogenases. H_2S can act as a co-substrate, supporting the reductive dechlorination reactions and promoting the stepwise dechlorination of HCH compounds (Boyle *et al.* 1999). Further research could explore how these bacteria can be used to enhance the wetland's performance. For instance, controlling the availability of ferrous iron may be a strategy to modulate the activity of these bacteria and, in turn, enhance HCH degradation.

The D modules were rich in bacteria of genera *Massilia*, *Rhodoferax*, *Hydrogenophaga, Flavobacterium, Caulobacter*, and *Bacteriovorax* compared to other modules. *Massilia* sp. is a common environmental bacterium often associated with plants and roots (Johnston-Monje *et al.* 2021; Li *et al.* 2021; Baek *et al.* 2022; Heo *et al.* 2022) and was described as characteristic of HCH-contaminated rhizospheres (Balázs *et al.* 2021). *Massilia* also has the potential to degrade many pollutants present in the environment, such as *Massilia* sp. WF1 degrades the polycyclic aromatic hydrocarbon phenanthrene (Gu *et al.* 2021).

Fahy *et al.* (2006) states that benzene degradation occurred after a community shift from a *Rhodoferax*-dominated community to a mix of *Rhodococcus* and *Hydrogenophaga* spp. (Fahy *et al.* 2006). Certain species of *Flavobacterium* have been reported to possess the ability to degrade HCH compounds. These bacteria may participate in the biodegradation of HCH and contribute to its removal from the environment (Murthy & Manonmani 2007; Ramteke & Hans 2008; Lata *et al.* 2012; Amirbekov *et al.* 2021). Kaur *et al.* (2021) reported that the abundance of *Caulobacterales* increased with the increasing HCH content in soil). It is important to note that the ability of these bacteria to degrade HCH compounds can vary among species and strains. Further investigation and characterization of the specific strains present in the contaminated samples would provide a clearer understanding of their potential roles in HCH degradation or adaptation.

An increasing number of diatom species was observed in the profiles along the Ostrovský Creek (in the direction of surface water flow: 0, 3, 30, and 35 species in profiles 1, 2, 3, and 4, respectively) in August 2021 (prior to the system commissioning). It fits well with the descending trend of HCH concentration in creek water due to dilution and attenuation processes from 86.53 $\mu g\ L^{-1}$ in profile 1 to 6.91 $\mu g\ L^{-1}$ in profile 4. The absence of diatoms or a low number of species and frustules in profiles 1 and 2 can be explained by the highest HCH contamination and poor environmental conditions. Significantly more diatom species (28) were observed in the Reference Creek in August 2021. The reference profile is close to profile 2 in the Ostrovský Creek, where only three species were found, suggesting that diatom biodiversity in the Ostrovský Creek was suppressed by HCH pollution. Both dominant species (*Eunotia arcus* and *E. botuliformis*) in the Reference Creek prefer undisturbed, oligotrophic and electrolyte-poor freshwater habitats. These species never dominated in the Ostrovský Creek, only *Eunotia bilunaris* which prefers oligotrophic and oligosaprobic habitats were detected in higher numbers in profile 4 in August 2021.

The monitoring campaign carried out after 11 months of the Wetland+ operation exhibited a markedly higher number of diatom species in profiles 1 and 2 of the Ostrovský Creek (3 and 14 species in profiles 1 and 2, respectively). The numbers of diatom species at sites 3 and 4 were similar before and after the start of Wetland+ (30 and 35 species before and 29 and 30 species after in profiles 3 and 4, respectively). Given that the average removal efficiency of the Wetland+ prototype was 81.7% for HCH and 96.8% for ClB, the increase in the number of diatom species detected in profiles 1 and 2 of the Ostrovský Creek was most likely due to improved quality of the creek water.

The findings of this study will help to shape and optimize the design of future Wetland+ systems. Adaptive systems that adjust to changing pollution levels and environmental circumstances may be conceivable. Wetlands might be changed in real-time to solve specific pollution concerns by adding sensors and automated control systems.

CONCLUSIONS

This study assessed the efficiency of the Wetland+ system in treating water heavily contaminated by HCH and ClB after one year of operation. Successful chemical removal of pollutants was supported by biodegradation by the indigenous bacterial community. The improved water quality in Ostrovský Creek was confirmed by increased diatom diversity.

The main findings of this study are:

1. The average efficiency of the system during 12 months of operation was 96.8% for ClB and 81.7% for HCH. The rate of the removal of individual HCH isomers followed the pattern: $\alpha = \gamma = \delta > \varepsilon = \beta$.
2. The highest total microbial biomass was in the outlet from the aerobic wetland (module D). Similarly, the abundance of the *linA, linB, linB-RT*, and *linD* genes was the highest in this outlet along with the highest level of dissolved O_2 in water. Therefore, the best conditions for aerobic biodegradation of HCH isomers and their metabolites were in the aerobic wetland.
3. The highest abundance of *Dehalococcoides*, capable of anaerobic dechlorination of HCH, was found in the inflow water (profile S), in an aeration system with sedimentation (A), and in a permeable reactive barrier remedial system (module B).
4. High abundance of iron-oxidizing *Galionella* corresponded to the level of dissolved iron in water flowing through the system. Similarly, the increased abundance of the sulfate-reducing *Desulfovibrio* sp. and *Desulfosporosinus* sp. in module B may be related to the higher content of sulfate and ferrous iron as available electron acceptor and donor, respectively. The D module exhibited a high abundance of *Massilia, Rhodoferax, Hydrogenophaga, Flavobacterium, Caulobacter,* and *Bacteriovorax* demonstrating a range of metabolic capabilities and interactions that may contribute to the degradation of HCH and other pollutants. Further research is warranted to investigate the specific mechanisms and contributions of these bacteria in HCH degradation.
5. Increased diversity of the diatom community indicated improvement in water quality in Ostrovský Creek after one year of the operation of Wetland+ system. In the Wetland+ system itself, only *Cymbella lange-bertalotii* was identified in modules A and C where the HCH concentrations were the highest, whereas 18 species were found in downgradient section D3 of module D. This indicates a positive effect of improved water quality.

In sum, the trial operation proved Wetland+ system to be a cost-effective successful treatment technology without tackling the source of HCH pollution by excavation. The treatment system sufficiently protects surface waters and enhances biodiversity. In addition, it improves environmental quality such as local climate and water retention.

This study not only sheds light on the Wetland+ system's efficiency in eliminating HCH and ClB pollutants, but it also delves deeply into the delicate microbial and ecological processes involved. The complex relationships within microbial populations, redox conditions, and pollutant removal rates provide potential for wetland design optimization. Furthermore, the observed improvements in diatom species richness highlight the ecological benefits of the Wetland+ system. The research establishes the groundwork for future research and development targeted at improving the efficiency of wetland-based remediation technologies and increasing environmental recovery in historically contaminated regions.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST

The authors declare there is no conflict.

REFERENCES

Amirbekov, A., Mamirova, A., Sevcu, A., Spanek, R. & Hrabak, P. 2021 HCH removal in a biochar-amended biofilter. *Water* **13**, 3396. https://doi.org/10.3390/w13233396.

Atashgahi, S., Maphosa, F., Doğan, E., Smidt, H., Springael, D. & Dejonghe, W. 2013 Small-scale oxygen distribution determines the vinyl chloride biodegradation pathway in surficial sediments of riverbed hyporheic zones. *FEMS Microbiology Ecolog* **84**, 133–142. doi:10.1111/1574-6941.12044.

Baek, J. H., Baek, W., Ruan, W., Jung, H. S., Lee, S. C. & Jeon, C. O. 2022 *Massilia soli* sp. nov., isolated from soil. *International Journal of Systematic and Evolutionary Microbiology* **72**, 005227. https://doi.org/10.1099/ijsem.0.005227.

Bala, K., Sharma, P. & Lal, R. 2010 *Sphingobium quisquiliarum* sp. nov., a hexachlorocyclohexane (HCH)-degrading bacterium isolated from an HCH-contaminated soil. *International Journal of Systematic and Evolutionary Microbiology* **60**, 429–433. https://doi.org/10.1099/ijs.0.010868-0.

Balázs, H. E., Schmid, C. A. O., Cruzeiro, C., Podar, D., Szatmari, P.-M., Buegger, F., Hufnagel, G., Radl, V. & Schröder, P. 2021 Post-reclamation microbial diversity and functions in hexachlorocyclohexane (HCH) contaminated soil in relation to spontaneous HCH tolerant vegetation. *Science of the Total Environment* **767**, 144653. https://doi.org/10.1016/j.scitotenv.2020.144653.

Bashir, S., Kuntze, K., Vogt, C. & Nijenhuis, I. 2018 Anaerobic biotransformation of hexachlorocyclohexane isomers by *Dehalococcoides* species and an enrichment culture. *Biodegradation* **29**, 409–418. https://doi.org/10.1007/s10532-018-9838-9.

Basu, S., Chanda, A., Gogoi, P. & Bhattacharyya, S. 2021 Organochlorine pesticides and heavy metals in the zooplankton, fishes, and shrimps of tropical shallow tidal creeks and the associated human health risk. *Marine Pollution Bulletin* **165**, 112170. https://doi.org/10.1016/j.marpolbul.2021.112170.

Bokulich, N. A., Kaehler, B. D., Rideout, J. R., Dillon, M., Bolyen, E., Knight, R., Huttley, G. A. & Gregory Caporaso, J. 2018 Optimizing taxonomic classification of marker-gene amplicon sequences with QIIME 2's q2-feature-classifier plugin. *Microbiome* **6**, 90. https://doi.org/10.1186/s40168-018-0470-z.

Bolyen, E., Rideout, J. R., Dillon, M. R., Bokulich, N. A., Abnet, C. C., Al-Ghalith, G. A., Alexander, H., Alm, E. J., Arumugam, M., Asnicar, F., Bai, Y., Bisanz, J. E., Bittinger, K., Brejnrod, A., Brislawn, C. J., Brown, C. T., Callahan, B. J., Caraballo-Rodríguez, A. M., Chase, J., Cope, E. K., Da Silva, R., Diener, C., Dorrestein, P. C., Douglas, G. M., Durall, D. M., Duvallet, C., Edwardson, C. F., Ernst, M., Estaki, M., Fouquier, J., Gauglitz, J. M., Gibbons, S. M., Gibson, D. L., Gonzalez, A., Gorlick, K., Guo, J., Hillmann, B., Holmes, S., Holste, H., Huttenhower, C., Huttley, G. A., Janssen, S., Jarmusch, A. K., Jiang, L., Kaehler, B. D., Kang, K. B., Keefe, C. R., Keim, P., Kelley, S. T., Knights, D., Koester, I., Kosciolek, T., Kreps, J., Langille, M. G. I., Lee, J., Ley, R., Liu, Y.-X., Loftfield, E., Lozupone, C., Maher, M., Marotz, C., Martin, B. D., McDonald, D., McIver, L. J., Melnik, A. V., Metcalf, J. L., Morgan, S. C., Morton, J. T., Naimey, A. T., Navas-Molina, J. A., Nothias, L. F., Orchanian, S. B., Pearson, T., Peoples, S. L., Petras, D., Preuss, M. L., Pruesse, E., Rasmussen, L. B., Rivers, A., Robeson, M. S., Rosenthal, P., Segata, N., Shaffer, M., Shiffer, A., Sinha, R., Song, S. J., Spear, J. R., Swafford, A. D., Thompson, L. R., Torres, P. J., Trinh, P., Tripathi, A., Turnbaugh, P. J., Ul-Hasan, S., van der Hooft, J. J. J., Vargas, F., Vázquez-Baeza, Y., Vogtmann, E., von Hippel, M., Walters, W., Wan, Y., Wang, M., Warren, J., Weber, K. C., Williamson, C. H. D., Willis, A. D., Xu, Z. Z., Zaneveld, J. R., Zhang, Y., Zhu, Q., Knight, R. & Caporaso, J. G. 2019 Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nature Biotechnology* **37**, 852–857. https://doi.org/10.1038/s41587-019-0209-9.

Boyle, A. W., Häggblom, M. M. & Young, L. Y. 1999 Dehalogenation of lindane (γ-hexachlorocyclohexane) by anaerobic bacteria from marine sediments and by sulfate-reducing bacteria. *FEMS Microbiology Ecology* **29**, 379–387. https://doi.org/10.1111/j.1574-6941.1999.tb00628.x.

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A. & Holmes, S. P. 2016 DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods* **13**, 581–583. https://doi.org/10.1038/nmeth.3869.

Claesson, M. J., Wang, Q., O'Sullivan, O., Greene-Diniz, R., Cole, J. R., Ross, R. P. & O'Toole, P. W. 2010 Comparison of two next-generation sequencing technologies for resolving highly complex microbiota composition using tandem variable 16S rRNA gene regions. *Nucleic Acids Research* **38**, e200–e200. https://doi.org/10.1093/nar/gkq873.

Clifford, R. J., Milillo, M., Prestwood, J., Quintero, R., Zurawski, D. V., Kwak, Y. I., Waterman, P. E., Lesho, E. P. & Gann, P. M. 2012 Detection of bacterial 16S rRNA and identification of four clinically important bacteria by real-time PCR. *PLOS ONE* **7**, e48558. https://doi.org/10.1371/journal.pone.0048558.

El Fantroussi, S., Naveau, H. & Agathos, S. N. 1998 Anaerobic dechlorinating bacteria. *Biotechnology Progress* **14**, 167–188. https://doi.org/10.1021/bp980011k.

European Union 2000 Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy. *Official Journal of the European Communities Series L*, **327**, 1–73.

Fahy, A., McGenity, T. J., Timmis, K. N. & Ball, A. S. 2006 Heterogeneous aerobic benzene-degrading communities in oxygen-depleted groundwaters. *FEMS Microbiology Ecology* **58**, 260–270. https://doi.org/10.1111/j.1574-6941.2006.00162.x.

Gu, H., Yan, K., You, Q., Chen, Y., Pan, Y., Wang, H., Wu, L. & Xu, J. 2021 Soil indigenous microorganisms weaken the synergy of *Massilia* sp. WF1 and *Phanerochaete chrysosporium* in phenanthrene biodegradation. *Science of the Total Environment* **781**, 146655. https://doi.org/10.1016/j.scitotenv.2021.146655.

Gupta, A., Kaushik, C. P. & Kaushik, A. 2000 Degradation of hexachlorocyclohexane (HCH; α, β, γ and δ) by *Bacillus circulans* and *Bacillus brevis* isolated from soil contaminated with HCH. *Soil Biology and Biochemistry* **32**, 1803–1805. https://doi.org/10.1016/S0038-0717(00)00072-9.

Gupta, S. K., Lal, D., Lata, P., Sangwan, N., Garg, N., Holliger, C. & Lal, R. 2013 Changes in the bacterial community and lin genes diversity during biostimulation of indigenous bacterial community of hexachlorocyclohexane (HCH) dumpsite soil. *Microbiology* **82**, 234–240. https://doi.org/10.1134/S0026261713020185.

Heo, J., Won, M., Lee, D., Han, B.-H., Hong, S.-B. & Kwon, S.-W. 2022 *Duganella dendranthematis* sp. nov. and *Massilia forsythiae* sp. nov., isolated from flowers. *International Journal of Systematic and Evolutionary Microbiology* **72**, 005487. https://doi.org/10.1099/ijsem.0.005487.

Imai, R., Nagata, Y., Senoo, K., Wada, H., Fukuda, M., Takagi, M. & Yano, K. 1989 Dehydrochlorination of γ-hexachlorocyclohexane (γ-BHC) by γ-BHC-assimilating *Pseudomonas paucimobilis*. *Agricultural and Biological Chemistry* **53**, 2015–2017. https://doi.org/10.1080/00021369.1989.10869597.

Jaiswal, S., Singh, D. K. & Shukla, P. 2023 Degradation effectiveness of hexachlorohexane (ϒ-HCH) by bacterial isolate *Bacillus cereus* SJPS-2, its gene annotation for bioremediation and comparison with *Pseudomonas putida* KT2440. *Environmental Pollution* **318**, 120867. https://doi.org/10.1016/j.envpol.2022.120867.

Johnston-Monje, D., Gutiérrez, J. P. & Lopez-Lavalle, L. A. B. 2021 Seed-transmitted bacteria and fungi dominate juvenile plant microbiomes. *Frontiers in Microbiology* **12**, 737616.

Kaufhold, T., Schmidt, M., Cichocka, D., Nikolausz, M. & Nijenhuis, I. 2013 Dehalogenation of diverse halogenated substrates by a highly enriched *Dehalococcoides*-containing culture derived from the contaminated mega-site in Bitterfeld. *FEMS Microbiology Ecology* **83**, 176–188. https://doi.org/10.1111/j.1574-6941.2012.01462.x.

Kaur, I., Gaur, V. K., Regar, R. K., Roy, A., Srivastava, P. K., Gaur, R., Manickam, N. & Barik, S. K. 2021 Plants exert beneficial influence on soil microbiome in a HCH contaminated soil revealing advantage of microbe-assisted plant-based HCH remediation of a dumpsite. *Chemosphere* **280**, 130690. https://doi.org/10.1016/j.chemosphere.2021.130690.

Kumar, D. & Pannu, R. 2018 Perspectives of lindane (γ-hexachlorocyclohexane) biodegradation from the environment: A review. *Bioresources and Bioprocessing* **5**, 29. https://doi.org/10.1186/s40643-018-0213-9.

Kumar, M., Chaudhary, P., Dwivedi, M., Kumar, R., Paul, D., Jain, R. K., Garg, S. K. & Kumar, A. 2005 Enhanced biodegradation of β- and δ-hexachlorocyclohexane in the presence of α- and γ-isomers in contaminated soils. *Environmental Science & Technology* **39**, 4005–4011. https://doi.org/10.1021/es048497q.

Kumar, M., Gupta, S. K., Garg, S. K. & Kumar, A. 2006 Biodegradation of hexachlorocyclohexane-isomers in contaminated soils. *Soil Biology and Biochemistry* **38**, 2318–2327. https://doi.org/10.1016/j.soilbio.2006.02.010.

Lal, R., Pandey, G., Sharma, P., Kumari, K., Malhotra, S., Pandey, R., Raina, V., Kohler, H.-P. E., Holliger, C., Jackson, C. & Oakeshott, J. G. 2010 Biochemistry of microbial degradation of hexachlorocyclohexane and prospects for bioremediation. *Microbiology and Molecular Biology Reviews* **74**, 58–80. https://doi.org/10.1128/MMBR.00029-09.

Lange-Bertalot, H., Hofmann, G. M., Werum, M. & Cantonati, M. 2017 *Freshwater Benthic Diatoms of Central Europe. Over 800 Common Species Used in Ecological Assessment*. (Vol. 942). Koeltz Botanical Books, Schmitten-Oberreifenberg, Germany.

Lata, P., Lal, D. & Lal, R. 2012 *Flavobacterium ummariense* sp. nov., isolated from hexachlorocyclohexane-contaminated soil, and emended description of *Flavobacterium ceti* Vela *et al.* 2007. *International Journal of Systematic and Evolutionary Microbiology* **62**, 2674–2679. https://doi.org/10.1099/ijs.0.030916-0.

Lecointe, C., Coste, M. & Prygiel, J. 1993 'Omnidia': Software for taxonomy, calculation of diatom indices and inventories management. *Hydrobiologia* **269**, 509–513. https://doi.org/10.1007/BF00028048.

Li, H., Jiang, W., Pan, Y., Li, F., Wang, C. & Tian, H. 2021 Occurrence and partition of organochlorine pesticides (OCPs) in water, sediment, and organisms from the eastern sea area of Shandong Peninsula, Yellow Sea, China. *Marine Pollution Bulletin* **162**, 111906. https://doi.org/10.1016/j.marpolbul.2020.111906.

Liang, Y., Liu, X., Singletary, M. A., Wang, K. & Mattes, T. E. 2017 Relationships between the abundance and expression of functional genes from vinyl chloride (VC)-degrading bacteria and geochemical parameters at VC-contaminated sites. *Environmental Science & Technology* **51**, 12164–12174. doi:10.1021/acs.est.7b03521.

Lodha, B., Bhat, P., Kumar, M. S., Vaidya, A. N., Mudliar, S., Killedar, D. J. & Chakrabarti, T. 2007 Bioisomerization kinetics of γ-HCH and biokinetics of *Pseudomonas aeruginosa* degrading technical HCH. *Biochemical Engineering Journal* **35**, 12–19. https://doi.org/10.1016/j.bej.2006.12.015.

McMurdie, P. J. & Holmes, S. 2013 . phyloseq: An R package for reproducible interactive analysis and graphics of microbiome census data. *PLOS ONE* **8**, e61217. https://doi.org/10.1371/journal.pone.0061217.

Miyauchi, K., Lee, H.-S., Fukuda, M., Takagi, M. & Nagata, Y. 2002 Cloning and characterization of linR, involved in regulation of the downstream pathway for γ-hexachlorocyclohexane degradation in sphingomonas paucimobilis UT26. *Applied and Environmental Microbiology* **68**, 1803–1807. https://doi.org/10.1128/AEM.68.4.1803-1807.2002.

Monferrán, M. V., Echenique, J. R. & Wunderlin, D. A. 2005 Degradation of chlorobenzenes by a strain of *Acidovorax avenae* isolated from a polluted aquifer. *Chemosphere* **61**, 98–106. https://doi.org/10.1016/j.chemosphere.2005.03.003.

Murthy, H. M. R. & Manonmani, H. K. 2007 Aerobic degradation of technical hexachlorocyclohexane by a defined microbial consortium. *Journal of Hazardous Materials* **149**, 18–25. https://doi.org/10.1016/j.jhazmat.2007.03.053.

Nagata, Y., Miyauchi, K. & Takagi, M. 1999 Complete analysis of genes and enzymes for γ-hexachlorocyclohexane degradation in *Sphingomonas paucimobilis* UT26. *Journal of Industrial Microbiology and Biotechnology* **23**, 380–390. https://doi.org/10.1038/sj.jim.2900736.

Nechanická M, D. L. & Dolinová, I. 2018 Use of nanofiber carriers for monitoring of microbial biomass. In: *Topical Issues of Rational Use of Natural Resources: Proceedings of the International Forum-Contest of Young Researchers, St.*. Petersburg, Russia, 18–20 April 2018. CRC Press, Boca Raton, FL, USA, p. 361.

Němeček, J., Dolinová, I., Macháčková, J., Špánek, R., Ševců, A., Lederer, T. & Černík, M. 2017 Stratification of chlorinated ethenes natural attenuation in an alluvial aquifer assessed by hydrochemical and biomolecular tools. *Chemosphere* **184**, 1157–1167. doi:10.1016/j.chemosphere.2017.06.100.

Phillips, T. M., Seech, A. G., Lee, H. & Trevors, J. T. 2005 Biodegradation of hexachlorocyclohexane (HCH) by microorganisms. *Biodegradation* **16**, 363–392. https://doi.org/10.1007/s10532-004-2413-6.

Poikane, S., Kelly, M. & Cantonati, M. 2016 Benthic algal assessment of ecological status in European lakes and rivers: Challenges and opportunities. *Science of The Total Environment* **568**, 603–613. https://doi.org/10.1016/j.scitotenv.2016.02.027.

Qiao, W., Puentes Jácome, L. A., Tang, X., Lomheim, L., Yang, M. I., Gaspard, S., Avanzi, I. R., Wu, J., Ye, S. & Edwards, E. A. 2020 Microbial communities associated with sustained anaerobic reductive dechlorination of α-, β-, γ-, and δ-hexachlorocyclohexane isomers to monochlorobenzene and benzene. *Environmental Science & Technology* **54**, 255–265. https://doi.org/10.1021/acs.est.9b05558.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J. & Glöckner, F. O. 2013 The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. *Nucleic Acids Research* **41**, D590–D596. https://doi.org/10.1093/nar/gks1219.

Ramteke, P. W. & Hans, R. K. 2008 Isolation of hexachlorocyclohexane (HCH) degrading microorganisms from earthworm got. *Journal of Environmental Science & Health Part A*. https://doi.org/10.1080/10934529209375844.

Richards, P. M., Liang, Y., Johnson, R. & Mattes, T. E. 2019 Cryogenic soil coring reveals coexistence of aerobic and anaerobic vinyl chloride degrading bacteria in a chlorinated ethene contaminated aquifer. *Water Research* **157**, 281–291. doi:10.1016/j.watres.2019.03.059.

Salmaso, F., Quadroni, S., Compare, S., Gentili, G. & Crosa, G. 2019 Benthic diatoms as bioindicators of environmental alterations in different watercourses of northern Italy. *Environmental Monitoring and Assessment* **191**, 158. https://doi.org/10.1007/s10661-019-7290-x.

Samin, G., Pavlova, M., Arif, M. I., Postema, C. P., Damborsky, J. & Janssen, D. B. 2014 A *Pseudomonas putida* strain genetically engineered for 1,2,3-trichloropropane bioremediation. *Applied and Environmental Microbiology* **80**, 5467–5476. https://doi.org/10.1128/AEM.01620-14.

Stevenson, J. 2014 Ecological assessments with algae: A review and synthesis. *Journal of Phycology* **50**, 437–461. https://doi.org/10.1111/jpy.12189.

Stevenson, R. J., Pan, Y., van Dam, H., 2010 Assessing environmental conditions in rivers and streams with diatoms. In: *The Diatoms: Applications for the Environmental and Earth Sciences* (Stoermer, E. F. & Smol, J. P., eds). Cambridge University Press, Cambridge, UK, pp. 57–85. https://doi.org/10.1017/CBO9780511763175.005.

Suar, M., van der Meer, J. R., Lawlor, K., Holliger, C. & Lal, R. 2004 Dynamics of multiple lin gene expression in *Sphingomonas paucimobilis* b90a in response to different hexachlorocyclohexane isomers. *Applied and Environmental Microbiology* **70**, 6650–6656. https://doi.org/10.1128/AEM.70.11.6650-6656.2004.

van Doesburg, W., van Eekert, M. H. A., Middeldorp, P. J. M., Balk, M., Schraa, G. & Stams, A. J. M. 2005 Reductive dechlorination of beta-hexachlorocyclohexane (beta-HCH) by a *Dehalobacter* species in coculture with a *Sedimentibacter* sp. *FEMS Microbiology Ecology* **54**, 87–95. https://doi.org/10.1016/j.femsec.2005.03.003.

Verma, H., Kumar, R., Oldach, P., Sangwan, N., Khurana, J. P., Gilbert, J. A. & Lal, R. 2014 Comparative genomic analysis of nine *Sphingobium* strains: Insights into their evolution and hexachlorocyclohexane (HCH) degradation pathways. *BMC Genomics* **15**, 1014. https://doi.org/10.1186/1471-2164-15-1014.

Vijgen, J. & International HCH and Pesticides Association 2006 *The Legacy of Lindane HCH Isomer Production: A Global Overview of Residue Management, Formulation and Disposal*. International HCH and Pesticides Association, The Netherlands.

Vijgen, J., de Borst, B., Weber, R., Stobiecki, T. & Forter, M. 2019 HCH and lindane contaminated sites: European and global need for a permanent solution for a long-time neglected issue. *Environmental Pollution* **248**, 696–705. https://doi.org/10.1016/j.envpol.2019.02.029.

Wacławek, S., Silvestri, D., Hrabák, P., Padil, V. V. T., Torres-Mendieta, R., Wacławek, M., Černík, M. & Dionysiou, D. D. 2019 Chemical oxidation and reduction of hexachlorocyclohexanes: A review. *Water Research* **162**, 302–319. https://doi.org/10.1016/j.watres.2019.06.072.

White, T. J., Bruns, T., Lee, S., Taylor, J., 1990 38 - Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: *PCR Protocols* (Innis, M. A., Gelfand, D. H., Sninsky, J. J. & White, T. J., eds). Academic Press, San Diego, CA, USA, pp. 315–322. https://doi.org/10.1016/B978-0-12-372180-8.50042-1.

Yoshida, N., Takahashi, N. & Hiraishi, A. 2005 Phylogenetic characterization of a polychlorinated-dioxin-dechlorinating microbial community by use of microcosm studies. *Applied and Environmental Microbiology* **71**, 4325–4334. https://doi.org/10.1128/AEM.71.8.4325-4334.2005.

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