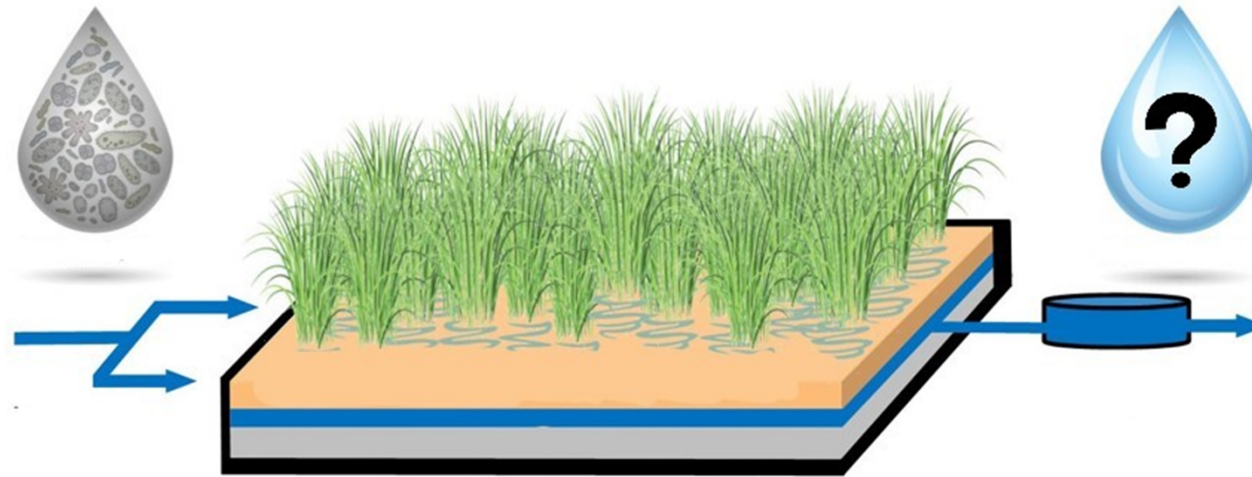


*Quantifying the log reduction of pathogenic microorganisms by
constructed wetlands: A literature review*



Outline

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WWTPs

- Efficient pathogen removal ✓
- Reliable ✓
- Environmental degradation ✗
- High energy consumption ✗

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Constructed Wetlands

- Cost-efficient system ✓
- Easily operated ✓
- Additional ecological and recreational value ✓
- Low nutrient removal efficiency ✗
- Seasonal variations and temperature ✗
- Extensive land area to function properly ✗

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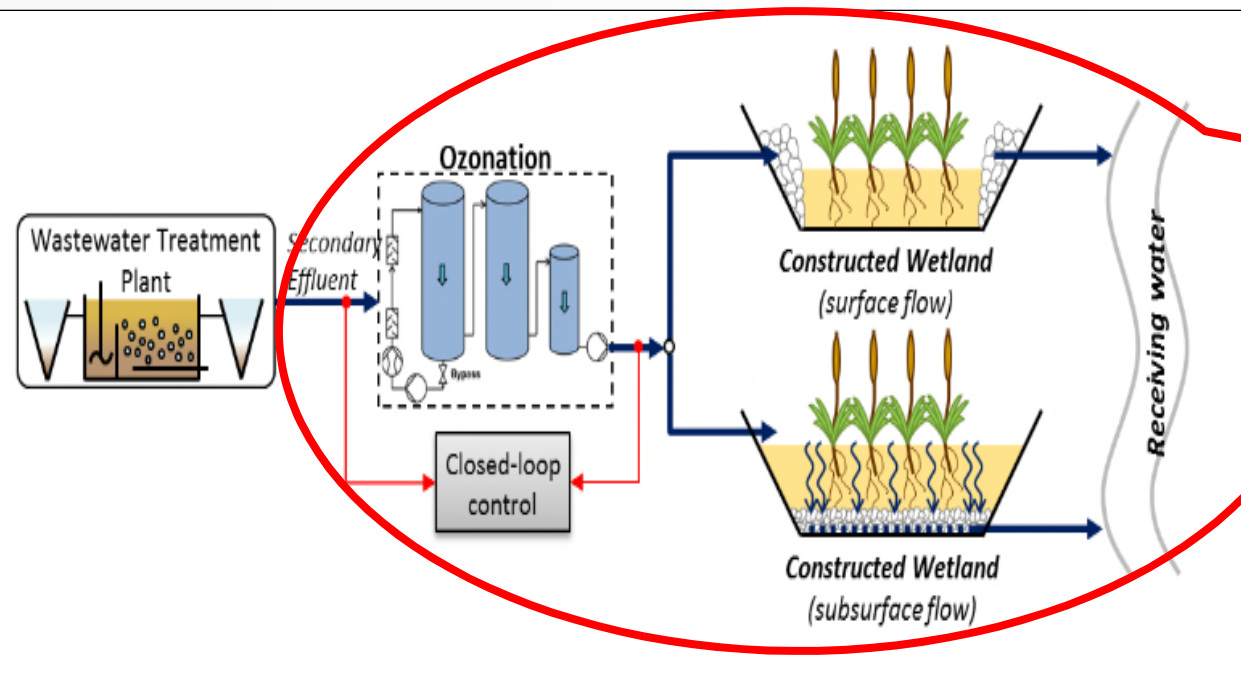
Discussion/
Conclusion

- Water can contain various microbial contaminants and can cause detrimental health effects if a specific dose is consumed

Wastewater effluent



Imperative need for effective removal of pathogens



cNES: combined Natural and Engineered Systems

Need for investigation on Constructed Wetlands by performing a systematic literature review in order to create a comprehensive dataset that:

- Provides a complete overview of CWs performance
 - Under various conditions
- Highlights potential knowledge gaps and opportunities for meta-analysis

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- Classification of human pathogens in five groups



Bacterium



Virus



Protozoan



Fungus



Helminth



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- Classification of human pathogens in five groups
- Enumeration of human pathogens can be an expensive and time-consuming process
- Numerous methods have been developed that first quantify groups of indicator organisms that are easy and inexpensive to monitor, and then correlate them with their respective index pathogenic organisms

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- Classification of human pathogens in five groups
- Enumeration of human pathogens can be an expensive and time-consuming process
- Numerous methods have been developed that first quantify groups of indicator organisms that are easy and inexpensive to monitor, and then correlate them with their respective index pathogenic organisms
- The most common indicators are:
 - Coliforms (total and fecal)
 - *Escherichia Coli*
 - *Fecal Streptococcus*

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Definition: *A man-made system designed to replicate the operation of a natural wetland (Nuttall et al., 1998).*

- Constructed wetlands are alternative engineered systems that are based on the use of emerging plants for the purification of wastewater
- Series of physical, chemical and biological mechanisms for the removal of pathogens

Physical



- Filtration
- Sedimentation

Chemical



- Solar radiation
- Oxidation

Biological



- Predation
- Natural die-off

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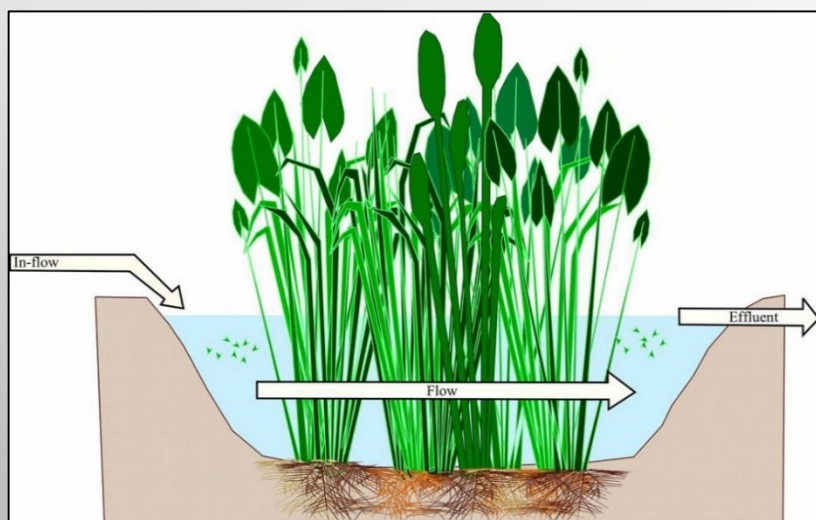
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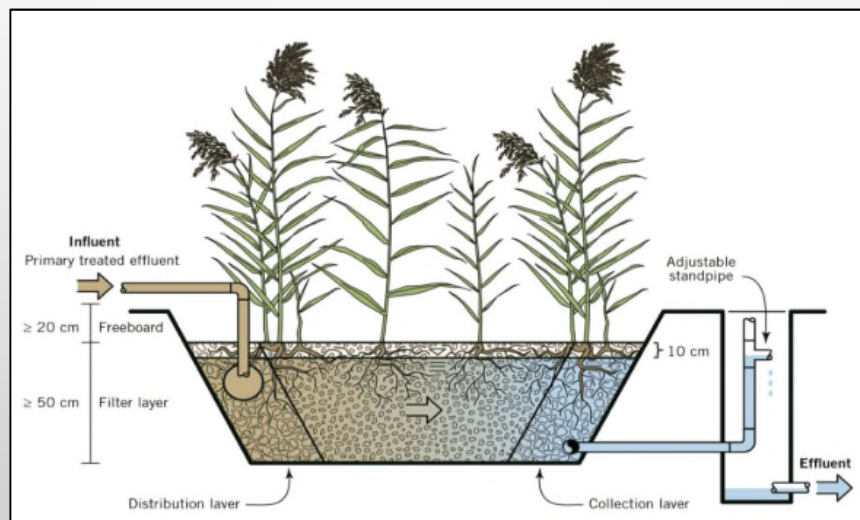
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Types of Constructed wetlands

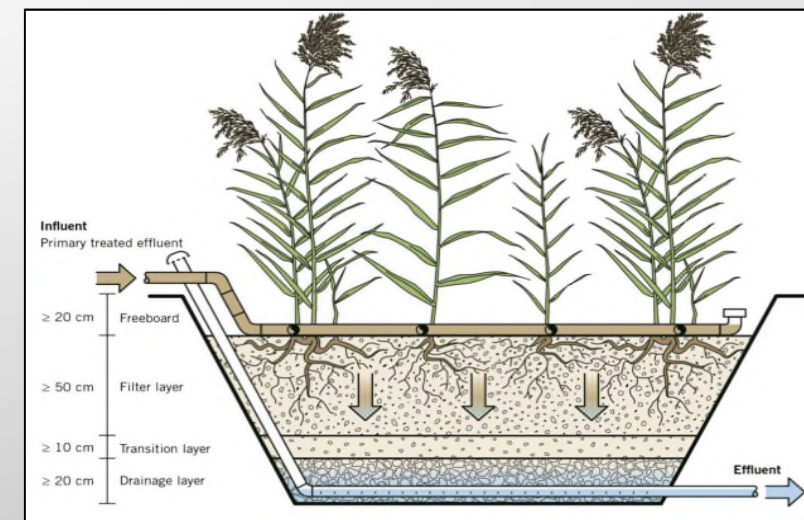
Free Water Surface (FWS)



Subsurface Horizontal Flow (SSHFCW)

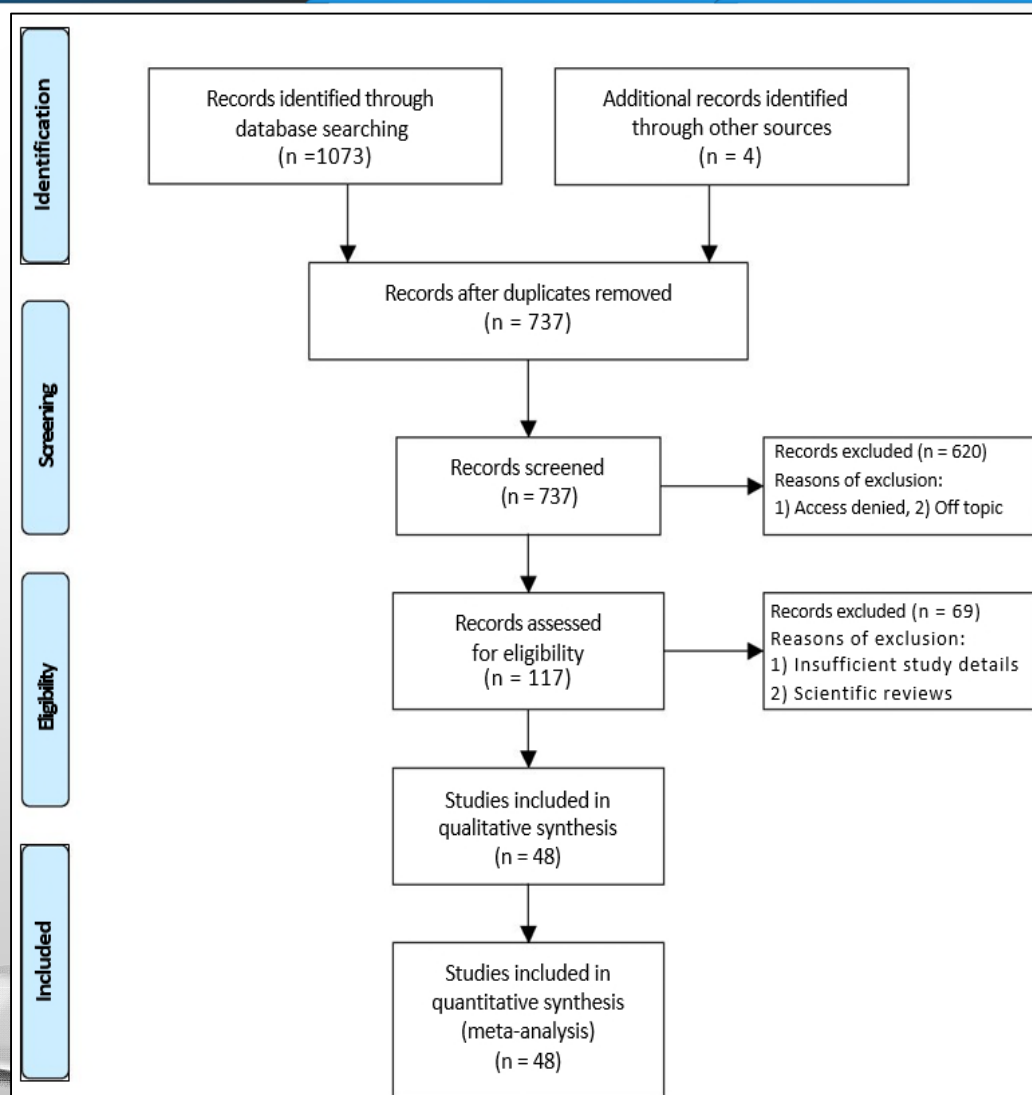


Vertical Flow (VFCW)



Systematic literature review

- Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines
- Preferred search engines “Scopus and “PubMed”
- After two series of screening, a total of 48 case studies qualified for both qualitative and quantitative analyses



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Conclusion***Inclusion criteria of case studies*****Technical features**

- The dimensions of the constructed wetland
- Hydraulic Loading Rate (HLR)
- Hydraulic Retention Time (HRT)
- Porosity (n) of the media grains
- A detailed description of CWs

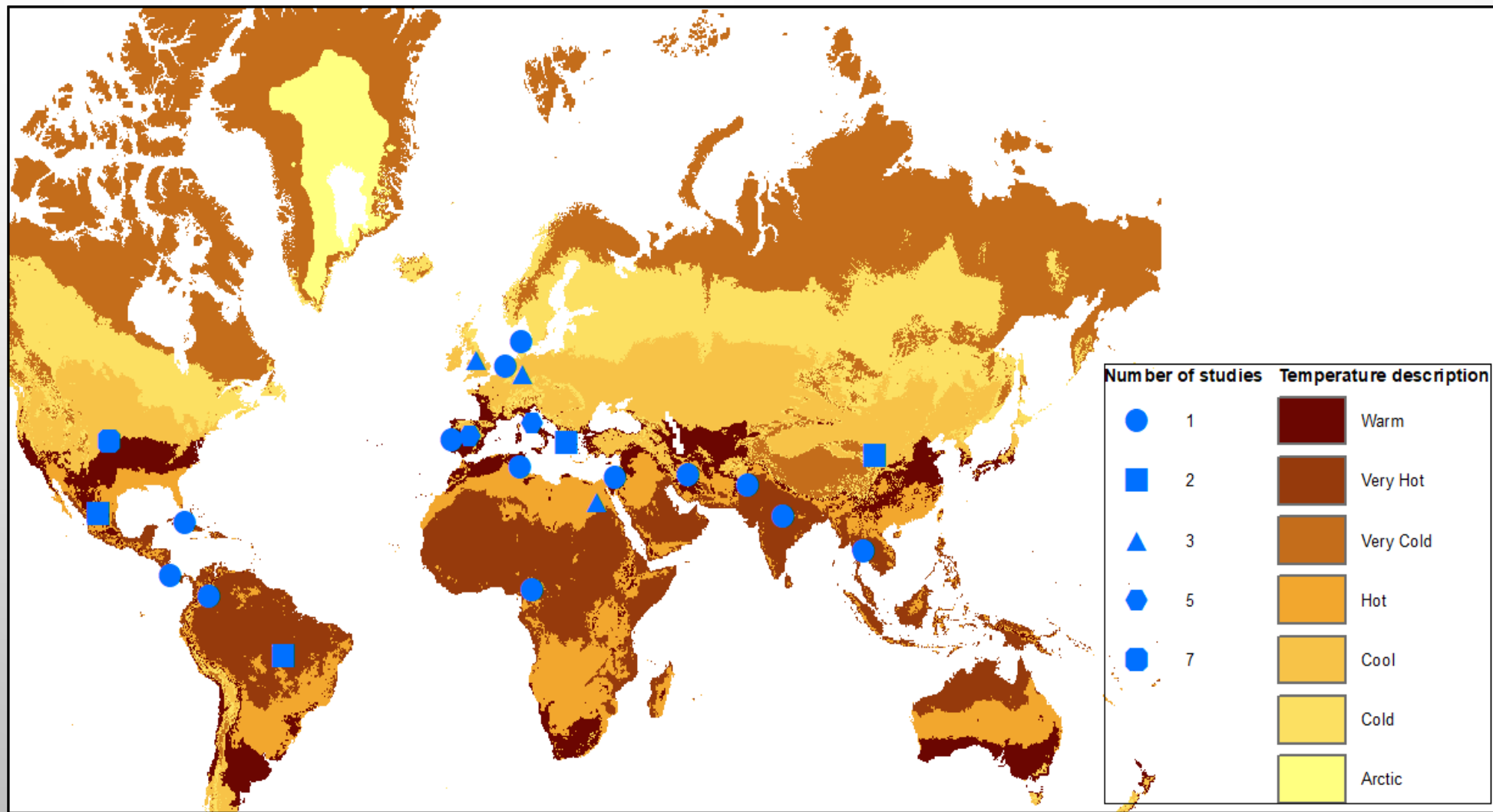
Experimental conditions

- Temperature (T)
- Type of influent wastewater
- Method of enumeration
- Physicochemical parameters like
 - Biological Oxygen Demand (BOD)
 - Chemical Oxygen Demand (COD)
 - Total Suspended Solids (TSS)

Spatial distribution of case

studies:

- Shows great variability
- Has different temperature/climatic zones
- Strengthens the validity of the results
- A relationship between temperature/climatic zones and log removal was examined



Classification of pathogens:

- Three main categories
- Largest differences in removal between categories, and rather smaller within categories

	Gram-negative bacteria	Viruses	Protozoan parasites
Indicator	Escherichia coli Total/fecal coliform Fecal streptococci/ enterococci Intestinal enterococci	Coliphages F-RNA specific phages Bacteriophages infecting GB124 MS2 bacteriophages	Clostridia Clostridium perfringens spores
Pathogen	Pseudomonas aeruginosa Campylobacter Salmonella Aeromonas	Adenovirus Aichi virus 1 BG/JC polyomavirus Enteric virus Norovirus GII	Giardia/giardia lamblia Cryptosporidium

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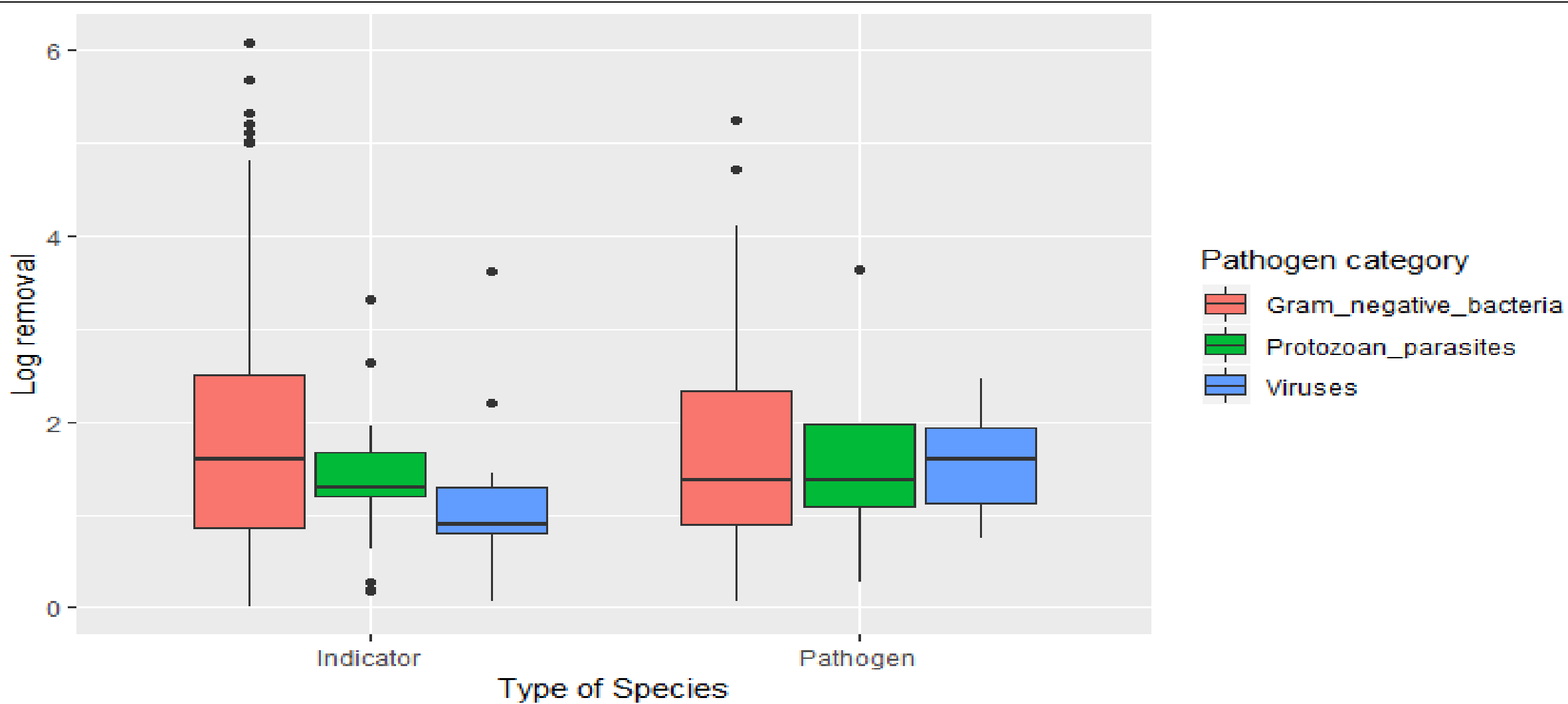
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Conclusion***Preliminary exploration between parameters:***

The goal of this preliminary exploration was to identify potential patterns between parameters by plotting them to each other regarding either different types of wetland or different pathogen categories. Regarding parameters, the C_{in} , C_{out} , HRT, and HLR were assessed since those were the only parameters that were consistently reported in the literature review.

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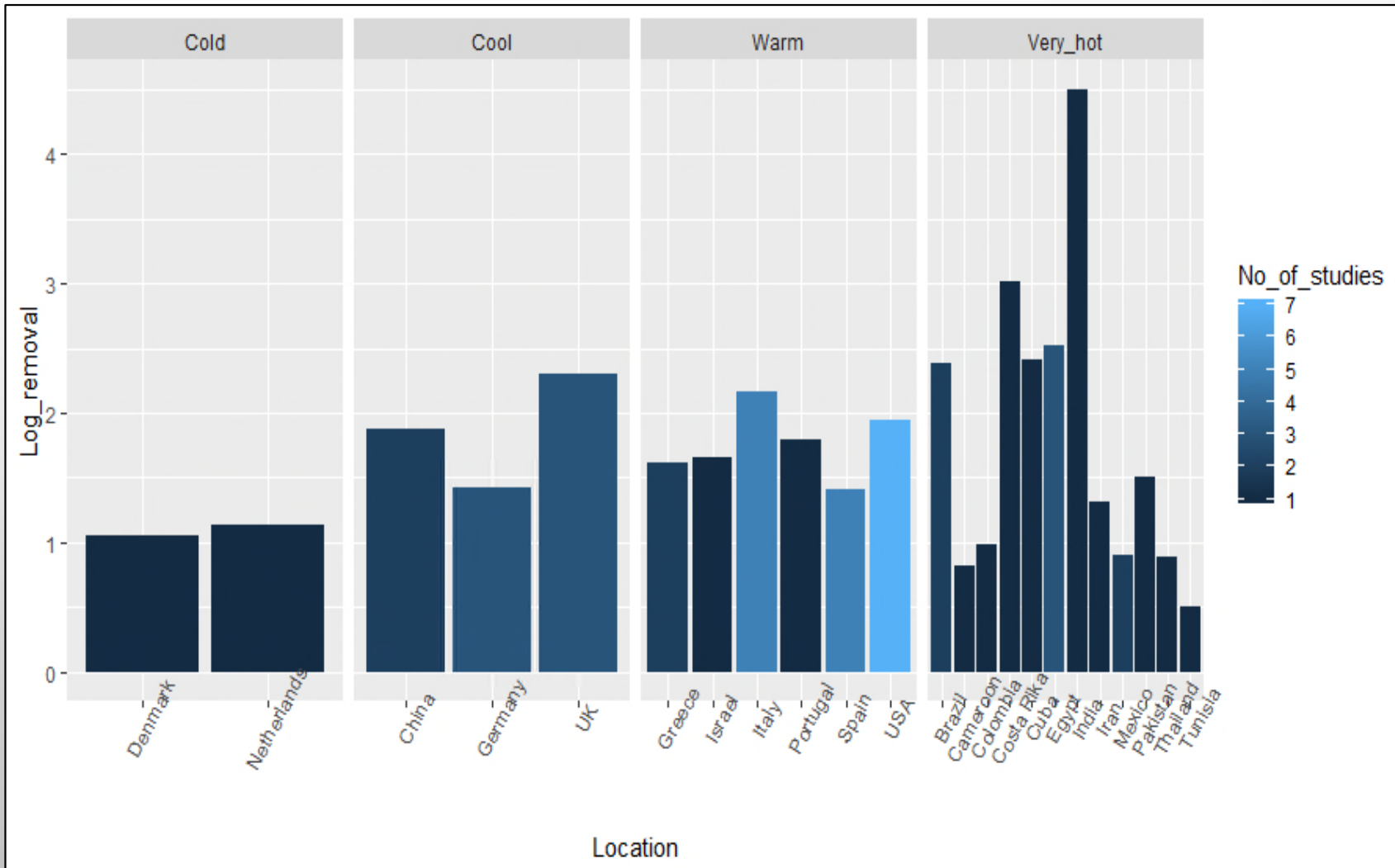
A sample of created dataset

Title	Type_of_C/w	HLR	HRT	[BOD5_remc	COD_removal	TSS_total	Medium	Dimensions_of_	Water_type	Group_Genus	Group	Type_of_s	Method_of_quan	Conce	Concer	Units	Concer	Conce	Units	sign	Log_removal		
Presence of	VFCW		1.08	0.83	98.034996	92.66247379	0.32954	gravel	L= 29 m,w= 40	SST effluent	Escherichia	Gram_negat	indicator	TBX medium	=	2.44	CHU/100 mL	=	1.15	CFU/100 mL	=	1.29	
Presence of	VFCW		1.08	0.83	98.034996	92.66247379	0.32954	gravel	L= 29 m,w= 40	SST effluent	Faecal Coliform	Gram_negat	indicator	membrane filtratio	=	2.86	CHU/100 mL	=	1.61	CFU/100 mL	=	1.25	
Presence of	VFCW		1.08	0.83	98.034996	92.66247379	0.32954	gravel	L= 29 m,w= 40	SST effluent	Intestinal Enter	Gram_negat	indicator	SB agar	=	1.79	CHU/100 mL	=	0.48	CFU/100 mL	=	1.31	
Presence of	VFCW		1.08	0.83	98.034996	92.66247379	0.32954	gravel	L= 29 m,w= 40	SST effluent	Somatic coliph	Viruses	indicator	triplicates by enur	=	4.61	CHU/100 mL	=	4.01	CFU/100 mL	=	0.6	
Presence of	VFCW		1.08	0.83	98.034996	92.66247379	0.32954	gravel	L= 29 m,w= 40	SST effluent	F-RNA specific	Viruses	indicator	triplicates by enur	=	3.2	CHU/100 mL	=	2.2	CFU/100 mL	=	1	
Presence of	VFCW		1.08	0.83	98.034996	92.66247379	0.32954	gravel	L= 29 m,w= 40	SST effluent	phages infectir	Viruses	indicator	triplicates by enur	=	2.57	CHU/100 mL	=	2	CFU/100 mL	=	0.57	
Comparative	VFCW		0.0028	4		91.82222222	0.01307	sand+ gravel	25-cm diamete	raw wastewa	Escherichia	Gram_negat	indicator	membrane filtratio	=	6.47	CFU/mL	=	2	CFU/mL	=	4.47	
Comparative	VFCW		0.0028	4		91.82222222	0.01307	sand+ gravel	25-cm diamete	raw wastewa	Total Coliform	Gram_negat	indicator	membrane filtratio	=	7.41	CFU/mL	=	2.4	CFU/mL	=	5.01	
Comparative	VFCW		0.0028	4		91.82222222	0.01307	sand+ gravel	25-cm diamete	raw wastewa	Faecal Coliform	Gram_negat	indicator	membrane filtratio	=	6.08	CFU/mL	=	0	CFU/mL	=	6.08	
Comparative	VFCW		0.0028	4		89.22222222	0.01103	sand+ gravel	25-cm diamete	raw wastewa	Escherichia	Gram_negat	indicator	membrane filtratio	=	6.47	CFU/mL	=	3.04	CFU/mL	=	3.43	
Comparative	VFCW		0.0028	4		89.22222222	0.01103	sand+ gravel	25-cm diamete	raw wastewa	Total Coliform	Gram_negat	indicator	membrane filtratio	=	7.41	CFU/mL	=	3.6	CFU/mL	=	3.81	
Comparative	VFCW		0.0028	4		89.22222222	0.01103	sand+ gravel	25-cm diamete	raw wastewa	Faecal Coliform	Gram_negat	indicator	membrane filtratio	=	6.08	CFU/mL	=	2.52	CFU/mL	=	3.56	
Comparative	VFCW		0.0028	4		91.84444444	0.01865	sand+marble c	25-cm diamete	raw wastewa	Escherichia	Gram_negat	indicator	membrane filtratio	=	6.47	CFU/mL	=	2.54	CFU/mL	=	3.93	
Comparative	VFCW		0.0028	4		91.84444444	0.01865	sand+marble c	25-cm diamete	raw wastewa	Total Coliform	Gram_negat	indicator	membrane filtratio	=	7.41	CFU/mL	=	2.59	CFU/mL	=	4.82	
Comparative	VFCW		0.0028	4		91.84444444	0.01865	sand+marble c	25-cm diamete	raw wastewa	Faecal Coliform	Gram_negat	indicator	membrane filtratio	=	6.08	CFU/mL	=	0	CFU/mL	=	6.08	
Comparative	VFCW		0.0028	4		92.59259259	0.01861	sand+marble c	25-cm diamete	raw wastewa	Escherichia	Gram_negat	indicator	membrane filtratio	=	6.47	CFU/mL	=	2.66	CFU/mL	=	3.81	
Comparative	VFCW		0.0028	4		92.59259259	0.01861	sand+marble c	25-cm diamete	raw wastewa	Total Coliform	Gram_negat	indicator	membrane filtratio	=	7.41	CFU/mL	=	3.18	CFU/mL	=	4.23	
Comparative	VFCW		0.0028	4		92.59259259	0.01861	sand+marble c	25-cm diamete	raw wastewa	Faecal Coliform	Gram_negat	indicator	membrane filtratio	=	6.08	CFU/mL	=	1.26	CFU/mL	=	4.82	
Halophytes a	VFCW		0.095	3.5		78.125		2 layers of grav	L= 1.20 m, W=0.	primary treat	Total Coliform	Gram_negat	indicator	IDEXX Quanti-Tra	=	6.2	MPN/100mL	=	5	MPN/100mL	=	1.2	
Halophytes a	VFCW		0.095	3.5		78.125		2 layers of grav	L= 1.20 m, W=0.	primary treat	Escherichia	Gram_negat	indicator	IDEXX Quanti-Tra	=	4.2	MPN/100mL	=	2.1	MPN/100mL	=	2.1	
Halophytes a	VFCW		0.095	3.5		79.01785714		2 layers of grav	L= 1.20 m, W=0.	primary treat	Total Coliform	Gram_negat	indicator	IDEXX Quanti-Tra	=	6.2	MPN/100mL	=	5.5	MPN/100mL	=	0.7	
Halophytes a	VFCW		0.095	3.5		79.01785714		2 layers of grav	L= 1.20 m, W=0.	primary treat	Escherichia	Gram_negat	indicator	IDEXX Quanti-Tra	=	4.2	MPN/100mL	=	2.4	MPN/100mL	=	1.8	
Emerging org	VFCW		0.044	6.34		91.2	82.94573643	0.09649	1layer of sand	D=0.8	combined se	Escherichia	Gram_negat	indicator	Chromogenic Mer	=	6.6	CFU/100 mL	=	5.95	CFU/100 mL	=	0.65
Emerging org	FWS		0.06	2.3		94.4	88.75368932	0.11404	siliceous gravel+stones		combined se	Escherichia	Gram_negat	indicator	Chromogenic Mer	=	5.95	CFU/100 mL	=	3.47	CFU/100 mL	=	2.48
Emerging org	FWS		0.02	5.1		95.2	81.78294574	0.05263	siliceous gravel		combined se	Escherichia	Gram_negat	indicator	Chromogenic Mer	=	3.47	CFU/100 mL	<	1.6	CFU/100 mL	<	1.87
Integrated tre	VFCW		0.044	6.34		97.455471	91.46567718	0.02787	1layer of sand	D=0.8	combined se	Escherichia	Gram_negat	indicator	Chromogenic Mer	=	6.6	CFU/100 mL	=	5.18	CFU/100 mL	=	1.424
Integrated tre	FWS		0.06	2.3		98.727735	94.43413729	0.02787	gravel		combined se	Escherichia	Gram_negat	indicator	Chromogenic Mer	=	5.176	CFU/100 mL	=	3.55	CFU/100 mL	=	1.626
Integrated tre	FWS		0.02	5.1		98.21883	90.72356215	97.9034	gravel		combined se	Escherichia	Gram_negat	indicator	Chromogenic Mer	=	3.55	CFU/100 mL	=	4.5	CFU/100 mL	=	-0.95

Log removal and climatic zones

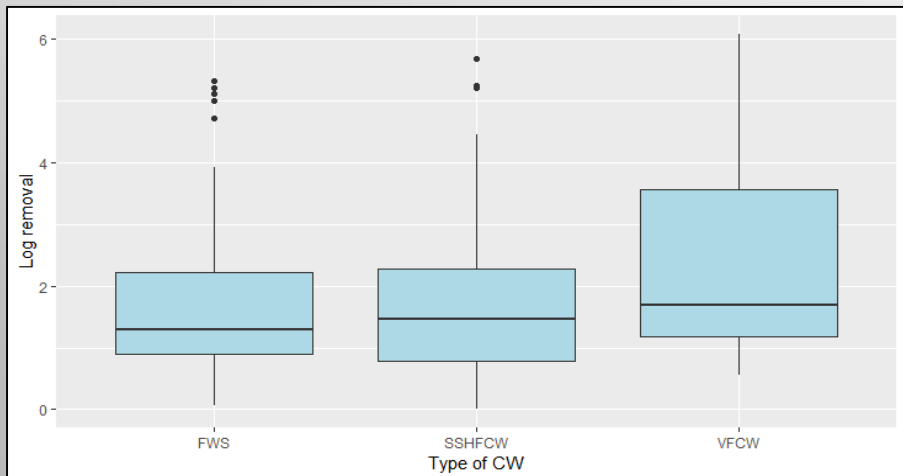
Although there seems to be a trend where higher removal values can be found in hot climatic zones, and rather low removal values can be found in cold and cool climatic zones, a clear correlation was not found since there is a significant variation within different climatic zones.

Additionally, the log removal values are averaged per country and in many cases there is only one case study per country





Overall performance of CW types

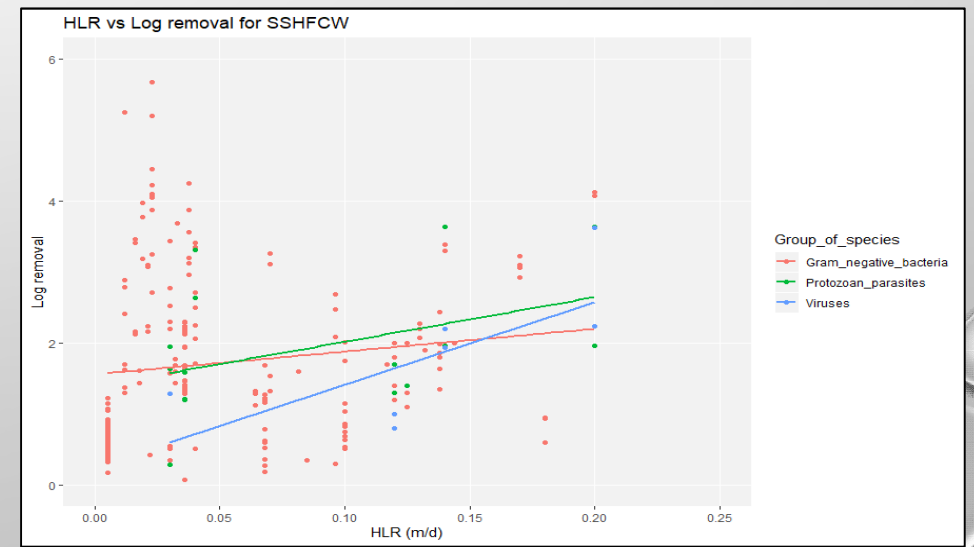
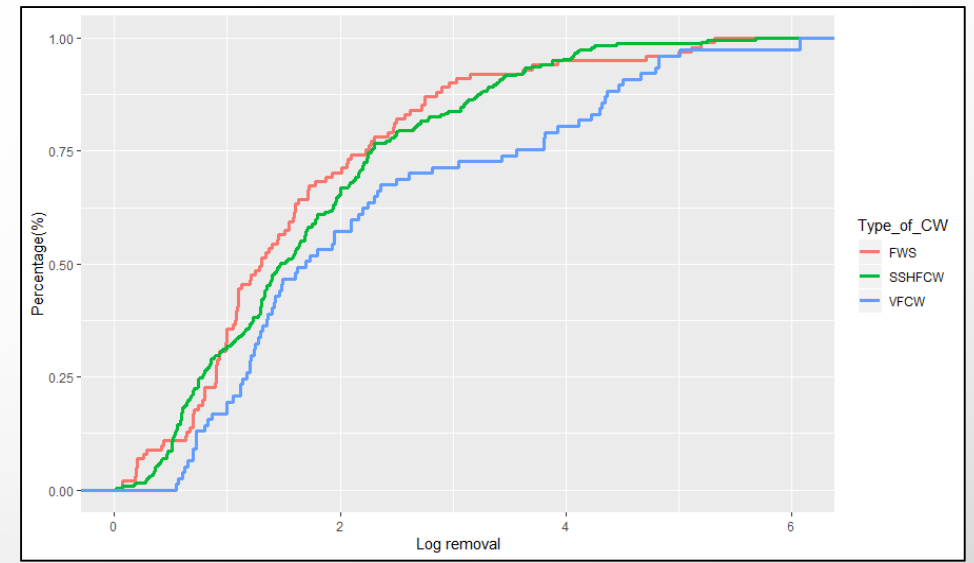


	Overall performance	FWS	SSFHCW	VFCW
General info	Number of papers	21	31	19
	Number of pilot-scale	11	22	16
	Number of full-scale	10	9	3
Pathogen removal	Log removal	1.54 (n=106)	1.72 (n=241)	2.26 (n=77)
	Percentage removal	97.1	98.09	99.45
	Minimum Log removal	0.07	0.011	0.35
	Maximum Log removal	5.3	5.68	6.08
	Standard Deviation	0.74	0.77	1.01
Physicochemical characteristics	Air temperature °C	20 (n=34)	19.2 (n=95)	19.4 (n=31)
	Water temperature °C	17.3 (n=47)	21.6 (n=121)	17.9 (n=63)
	PH	7.43 (n=73)	7.03 (n=1223)	7.35 (n=57)
	COD removal (mg/L) %	66.6 (n=57)	69.5 (n=169)	83.4(n=58)
	BOD removal (mg/L) %	72.6 (n=32)	68.2 (n=83)	92.5 (n=24)
	BOD5 removal (mg/L) %	65.03 (n=11)	83.01 (n=88)	93.7 (n=18)
	TSS removal (mg/L) %	71.6 (n=56)	65.6 (n=136)	82.8 (n=50)
	HLR range (m/d)	0.058-5.1 (n=107)	0.005-2.59 (n=255)	0.0028-1.36 (n=86)
	HRT range (d)	0.29-7 (n=107)	0.028-13 (n=255)	0.01-9.1 (n=86)

Overall performance of CW types

Based on the CDF:

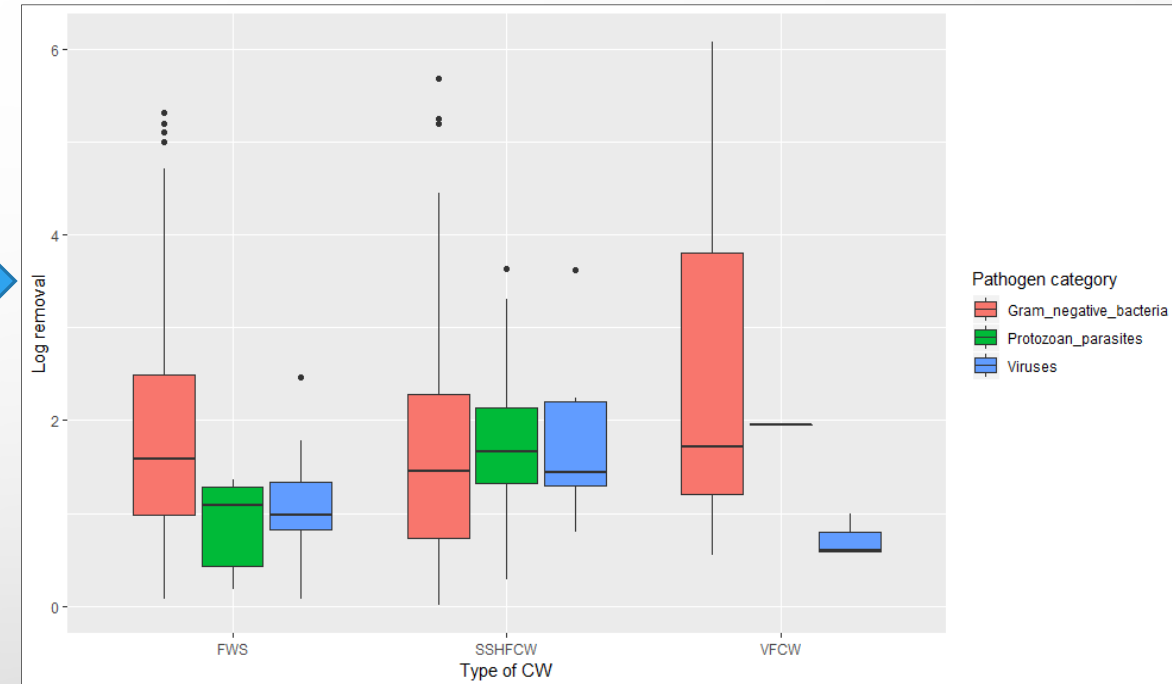
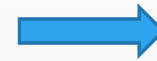
- All CWs show an adequate removal since 50% of the distribution has log removal >1.5 log.
 - FWS and SSHFCW have an identical distribution where 75% is between 0-2 log and the rest is between 2-4 log.
 - VFCW shows a slightly different profile since 75% of the distribution is between 0-4 log and the rest between 4-6 log, indicating the higher capacity in removal compared to the other 2 systems.
-
- Potential relationship between log removal and HLR and/or HRT
 - Observed variation can be attributed to:
 - Different applications (full-scale vs pilot-scale) plotted together
 - Different technical features/experimental conditions of each study leads to different removal efficiency



Removal of pathogen categories

Comparison of pathogen categories per CW type:

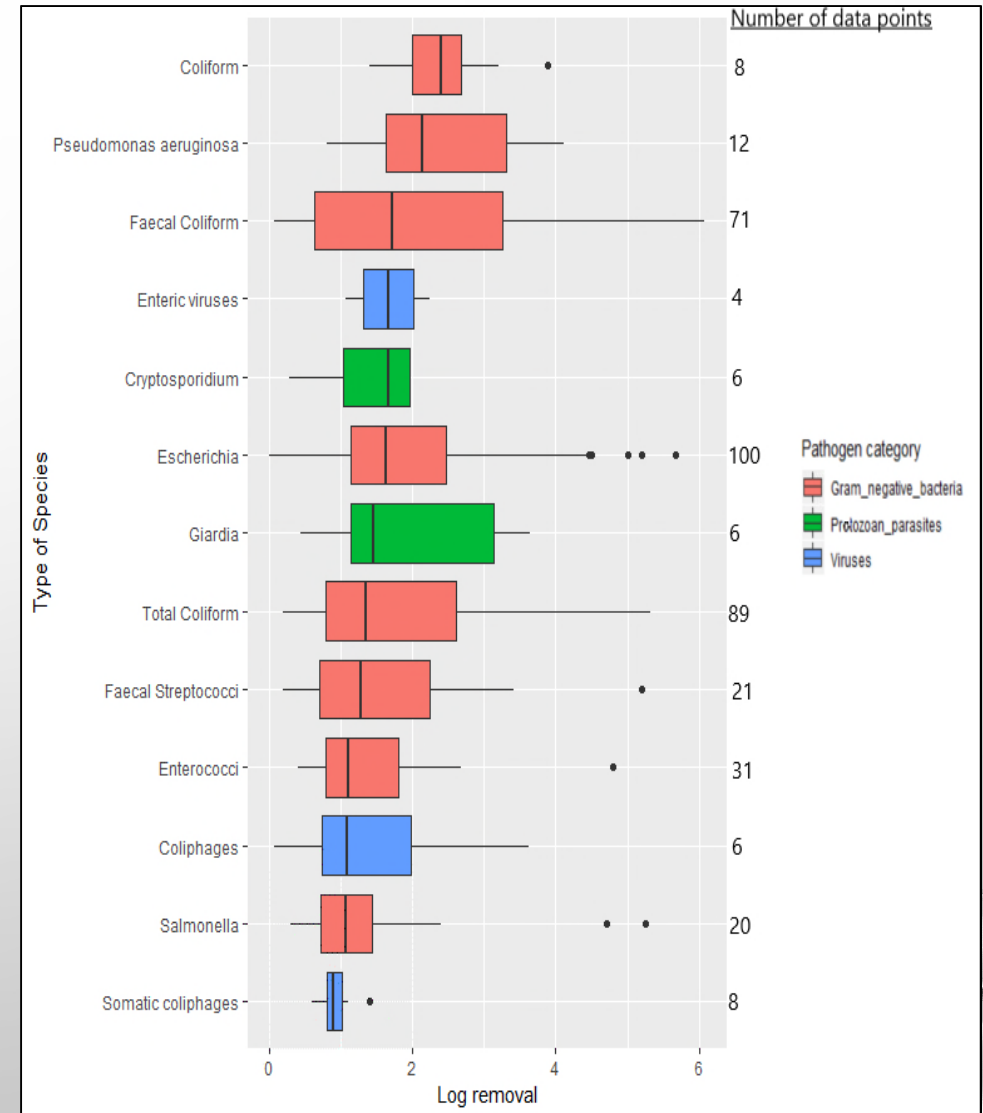
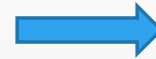
- Gram-negative bacteria show great variability in their removal (0.01-6.08 log) compared to viruses and protozoan parasites categories where the ranges are between 0.02-3.62, and 0.18-3.63 log respectively.
- Average removal values ranging between 1 and 2 log for the three categories in all of the CW types except the viruses' category in VFCW where the average log removal is less than 1.



Removal of pathogen categories

Removal range for the most representative indicators and index pathogens ranked based on average removal:

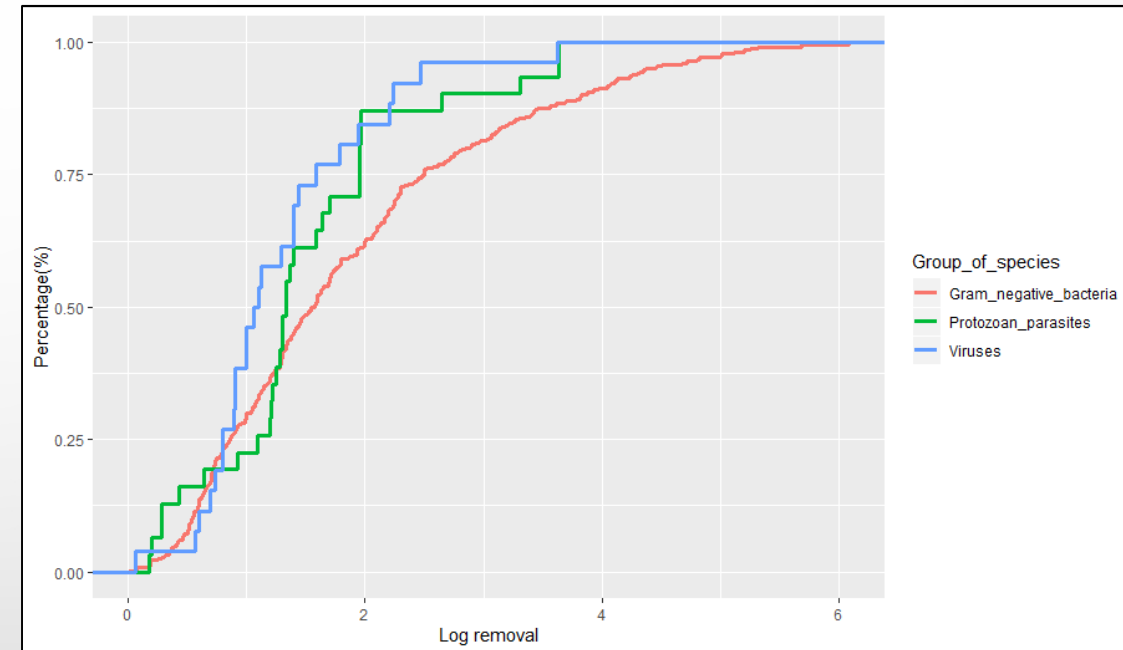
- Group of coliforms are those with the greatest variability possibly due to difference in data points.
- Variability of pathogen log removal within their categories.
 - Important observation since it was expected for the different pathogen categories to formulate clusters regarding log removal.



Removal of pathogen categories

Based on the CDF:

- 75 % of both protozoan parasites and viruses categories have a removal lower or equal to 2 log while their peak lies just before 4 log.
- Gram-negative bacteria category exhibits a smooth curve along the distribution where 60% of the observed data points have a removal lower or equal to 2 log while the rest 40% lies between 2 and 6 log.



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- Access to overall performance of CWs.
- Spatial variability of case studies.
- Benchmark for any new relevant research.

Pathogen categories

- Gram-negative Bacteria category has the highest log removal in all 3 types of CW.
- Difficult to draw conclusions due to small number of data points.

Constructed Wetlands

- Credible choice for WW polishing.
- The CDFs of different types of CWs and different pathogen categories simply provided an initial mapping of the situation (in terms of performance and removal capacity) according to the literature review and can be used as a reference point.
- Various patterns were observed between hydraulic characteristics and influent/effluent concentrations which gives room for further investigation.
- A potential meta-analysis of this database using statistical analysis can provide additional and insightful information on the significance of these parameters on pathogen removal.



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Overall, the final outcome does provide an efficient approach to the scientific community by taking a step closer to a better understanding of these “black boxes” and pointing out where future research needs to focus, in order to fine-tune and quantify the factors that influence the performance of constructed wetlands.

A photograph of a pond with tall green and yellow reeds. The reeds are reflected in the water. The text "THANK YOU!" is overlaid in the center of the image.

THANK YOU!