Quantifying the Log Reduction of Pathogenic Microorganisms by Constructed Wetlands: A Literature Review

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Abstract: Over the last 30 years, constructed wetlands (CWs) have been used as an alternative, cost-efficient way of treating wastewater, often in combination with conventional wastewater technologies. When CWs are attached at the end of conventional wastewater treatment plants, they treat the effluent and thus provide a polishing step. However, recent studies have shown that when CWs are used as a main wastewater treatment method for agricultural reuse of effluents, they perform poorly on meeting the accepted limit of microbial contamination. Moreover, CWs are increasingly used within the scope of circular economy and water reuse applications. Therefore, there is a need for a comprehensive exploration of the performance of CWs on pathogen removal. This paper explores relevant case studies regarding pathogen removal from constructed wetlands in order to create a comprehensive dataset that provides a complete overview of CWs performance under various conditions. After performing a systematic literature review, a total of 48 case studies qualified for both qualitative and quantitative analyses. From the dataset, the general performance, optimal conditions, and knowledge gaps were identified. The review confirmed that constructed wetlands (as a standalone treatment) cannot meet the accepted limits of pathogen removal. However, they can be a credible choice for wastewater polishing when they are combined with conventional wastewater treatment systems. Regarding the most common indicators that were recorded, the removal of Escherichia coli ranged between 0.01-5.6 log, the removal of total and fecal coliforms was 0.2-5.32 and 0.07-6.08 log respectively while the removal of fecal streptococci was 0.2-5.2 log. The great variability on pathogen removal indicates that the complexity of CWs makes it difficult to draw robust conclusions regarding their removal efficiency. Potential correlations were identified between influent and effluent concentrations, as well as between log removal and hydraulic characteristics. Additionally, no correlations between pathogen removal and temperature/climatic zones were found since average pathogen removal per country showed high variation throughout the various climatic zones. The dataset can be used as a benchmark of CWs performance as a barrier against the spreading of pathogens in the environment. The knowledge gaps identified in this review can provide direction for further research. Finally, a potential meta-analysis of the dataset using statistical analysis can pave the way for a better understanding of the design and operational parameters of CWs in order to fine-tune and quantify the factors that influence the performance of these systems.

Keywords: Constructed wetlands; Microbial pathogens; Log removal; Literature review; Dataset

1. Introduction

Constructed wetlands (CWs) are alternative, easily operated and cost-efficient systems that can be applied to wastewater (WW) purification, municipal sewage [1], or polishing of a wastewater treatment plant (WWTP) effluent [2]. Moreover, the use of CWs provides an additional ecological value as well as social acceptance through the creation of habitats, preservation of wildlife and recreational added value respectively, making them preferable compared to conventional engineered systems in terms of ecosystem services [3]. However, CWs can have disadvantages like low nutrient removal efficiency [1] as well as limitations regarding microbiological degradation processes with

several factors affecting the process like temperature (T) and seasonal variations [4]. While conventional engineered wastewater treatment has already proven to be efficient in the removal of the majority of pollutants, they come with the disadvantage of environmental degradation and high energy consumption amongst others [5]. Therefore, a potential combination of these natural systems with engineered treatments like oxidation, MBR (membrane bio-reactor) or anaerobic reactors as a pre or post-treatment, can overcome these disadvantages and even enhance the efficiency of these combined systems as it combines the removal mechanisms of both types [6].

Constructed wetlands can be classified in three main categories depending on their water flow regime and these are:

- Free water surface (FWS) constructed wetlands that have areas of open water and behave similarly to natural wetlands.
- Subsurface horizontal flow constructed wetlands (SSHFCW), which typically consist of a gravel bed covered with wetland vegetation. The water flows constantly below the surface of the bed in a horizontal direction.
- Vertical flow constructed wetlands (VFCW), where the main difference compared with SSHFCWs is that water percolates vertically through sand or a gravel bed planted with vegetation. The influent enters through perforated pipes which are distributed over the surface in the form of a grid.

Each of these categories demonstrates different layouts, media, efficiency and flow patterns [7].

When the end product of wastewater treatment is destined for water reuse applications like drinking water or irrigation, effective removal of pathogenic bacteria that can be found in the effluents, is of great importance. CWs can remove the remaining micro pollutants of conventional WW treatments adequately when they are used as a polishing step. However, recent studies have shown that when they are used as the main wastewater treatment method for agricultural reuse of effluents, they perform poorly on meeting the accepted limit of microbial contamination [8,9]. Moreover, the frequency of their usage (mainly in combination with conventional treatment) is increasing under the scope of circular economy and water reuse applications that continuously come to light [10]. Therefore, the need for a comprehensive exploration of the performance of CWs on pathogen removal as a standalone wastewater treatment method or as a combined treatment is imperative.

During the last 30 years, a lot of scientific papers and books have been published regarding the characteristics of CWs and their pathogen removal performance [7,11,12]. What is common in these publications is the complexity and heterogeneity that characterizes these natural systems, having rightfully earned the title "black box". That is because there are various processes of pathogen removal that take place simultaneously and sometimes act in combination. These processes include physical (sedimentation, filtration, adsorption), chemical (UV radiation, oxidation) and biological (predation, natural die-off) processes and it can be quite challenging to specify the main factors that are responsible for the removal of pathogens [13]. The focal point regarding pathogenic microorganisms will include viruses, bacteria, and protozoa with Escherichia *coli*, total and fecal coliforms, and fecal streptococci drawing the most attention since they are the most common indicators.

The aim of this paper is to explore relevant studies regarding pathogen removal from constructed wetlands and their respective characteristics. To do that, a systematic literature review will be performed in order to create a comprehensive dataset that provides a complete overview of CWs performance under various conditions, and highlights potential knowledge gaps and opportunities for meta-analysis.

2. Materials and Mmethods

2.1. Sustematic Literature Review

The systematic review of the literature was performed adopting the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [14]. The preferred search engines, ''Scopus'' and ''PubMed'' were used. The combination of keywords, as well as the results from the databases regarding the total number of papers, are shown in Appendix A.

2.1.1. Inclusion criteria

The primary objective of this systematic literature review was the record of case studies and not scientific reviews as well as the extraction of results associated with the removal of pathogens such as viruses, bacteria, and protozoa. Furthermore, the inclusive criteria were about the optimal availability and quality of the technical features such as:

- 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

From the above criteria, the included studies should contain at least the hydraulic characteristics.

Additionally, it was preferred to include studies who described at least part of the experimental conditions (if not all) such as:

- Temperature (T)
- Type of influent wastewater
- Method of enumeration such as Colony Forming Units (CFU/100 ml) and Most Probable Number (MPN)
- Physicochemical parameters like
  - Biological Oxygen Demand (BOD)
  - Chemical Oxygen Demand (COD)
  - Total Suspended Solids (TSS)

Figure 1, demonstrates the search process of the systematic review, the exclusion reasons for papers, as well as the number of case studies where, after two series of screening, a total of 48 case studies qualified for both qualitative and quantitative analyses.
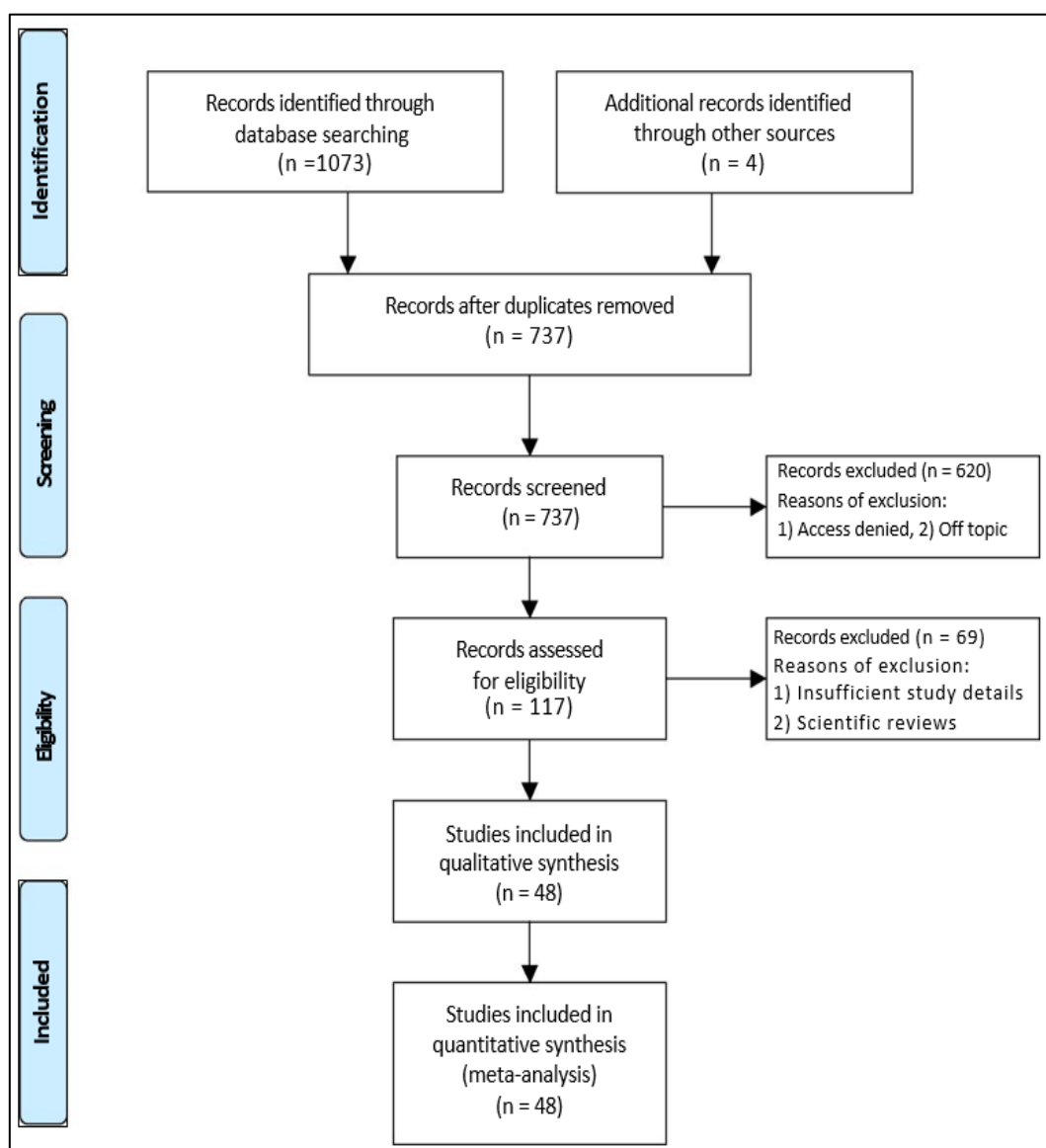
Figure 1. Flow diagram on the selection of studies adopted by the PRISMA guidelines. Source: Liberati et al., (2009).

2.1.2. Spatial distribution of case studies

After the selection of the case studies, a spatial distribution map was created to show the dispersion and the number of studies on a country level throughout the world. Figure 2 shows that the selected studies come from various regions of the world with different temperature/climatic zones[1], which strengthens the validity of the database as it was expected that there would be variability on the performance of different CW types between different climatic zones. A relationship between temperature/climatic zones and log removal was examined by looking at potential corellations.

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[1] More information about the climatic zones can be found in :

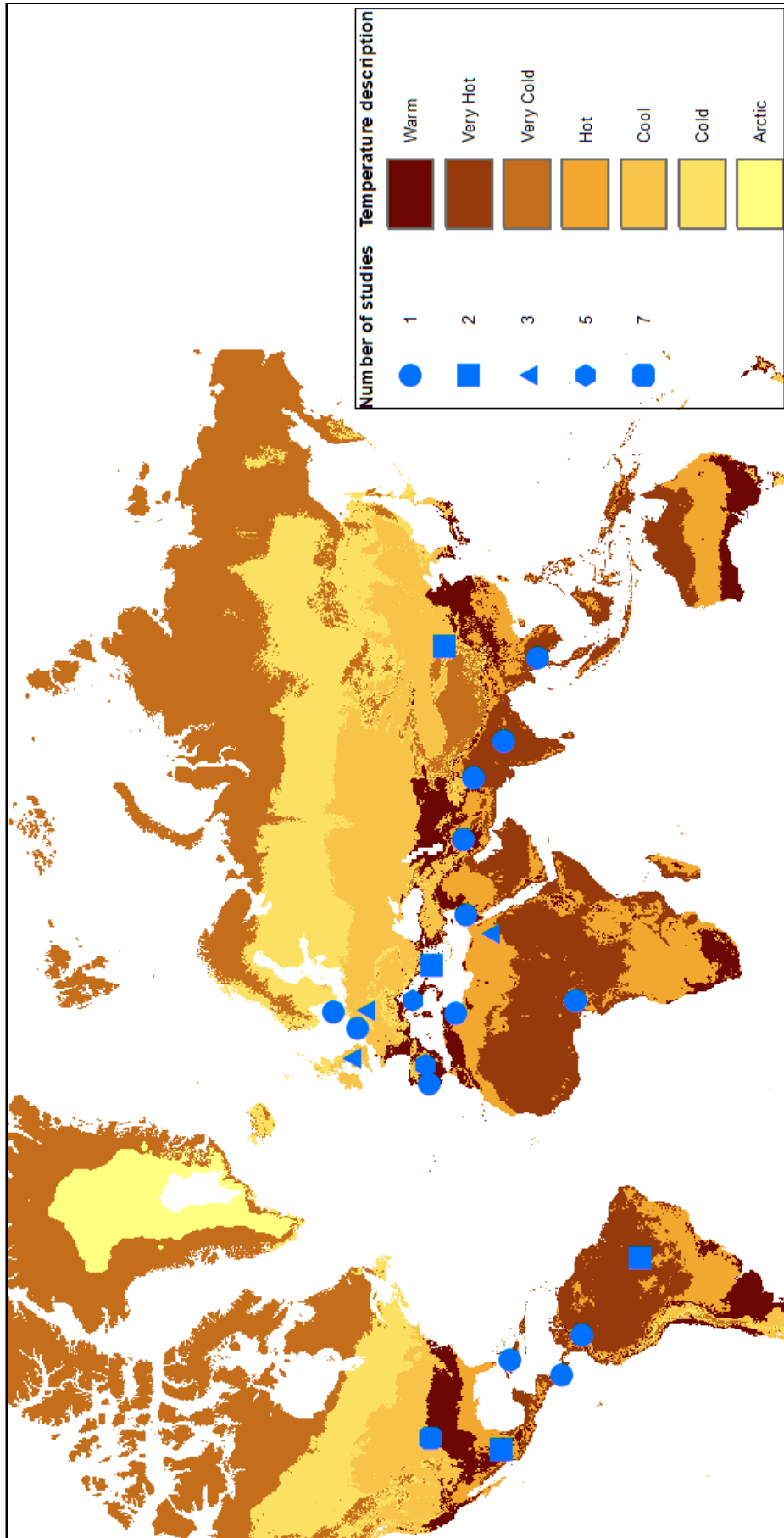https://www.arcgis.com/home/item.html?id=5826b14592ab4ebc99574919165bd860

Figure 2. Spatial distribution of selected case studies throughout the different temperature/climatic zones.

2.2. Data Eextraction Pprocess

During the extraction process, a classification between the case studies under consideration was made in order to ease up the process of data extraction. The classes "Include 1", "Include 2" were created, with the numbering system indicating the degree of difficulty on extracting data as well as the quantity of descriptive information of case studies. The second class was decided to be incorporated only after realizing that the first class did not provide enough information. Moreover, in cases where important information was missing, it was decided to calculate them while many times default values were used. Briefly, in studies where the HRT value was missing, it was decided that for the calculation of this value, the addition of porosity had to be included to the numerator in order to get a more representative value of the retention time. In times were the porosity value was not indicated, a default value of 0.35 for sand and 0.4 for clay was used [15].

2.3. Creation of Dataset and Classification of Ppathogens

After the data extraction was over, a comprehensive dataset with all the necessary values was created as can be seen in Appendix B. This dataset includes information about HRT, HLR, type of CW, temperature and concentration of influent (*Cin)* and effluent (*Cout*) for various pathogens. This information helped create a complete, up to date overview of the performance of constructed wetlands.

The next step was the classification of pathogenic microorganisms into three main categories named gram-negative bacteria (GNB), viruses, and protozoan parasites (Table 1). As can be seen, the systematic literature review identified various microorganisms, both indicators, and pathogens for each category but not on the same frequency. Briefly, although E. *coli* (which is a common indicator) was found in 33 of the 48 total papers, other indicators such as fecal coliforms, total coliforms, and fecal streptococci, did not have the same frequency (23, 25, and 9 respectively). Furthermore, from the viruses' category, coliphages were found in only 5 papers in total, whereas from protozoan parasites category, Giardia had considerably less information, with only 3 papers. Therefore, the grouping between indicators and pathogens for every category was necessary, in order to have a better understanding of their removal from CWs since it was expected to observe largest differences on removal between categories, and rather smaller within categories.

Table 1. Grouping of pathogenic microorganisms found in the literature review in three main 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

Figure 3 demonstrates the range and average log removal for each category between indicators and pathogens. It can be seen (for GNB and protozoa) that indicators and pathogenic microorganisms have relatively similar levels of removal, which increases the reliability of the decision to group

indicators with pathogens. For viruses, the indicators appear to be removed to a lesser extent, therefore they should be regarded as conservative indicators.
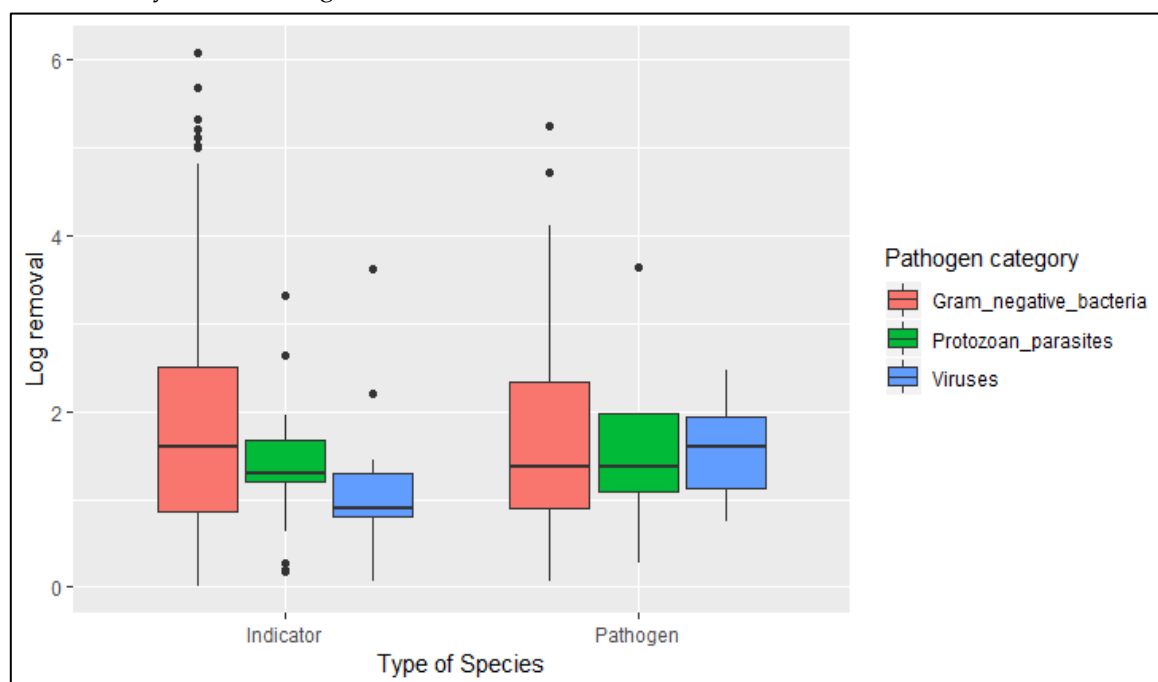

Figure 3. Average log removal comparison between indicators and pathogens for the three categories.

2.4. 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 *Cin, Cout*, HRT, and HLR were assessed since those where the only parameters that were consistently reported in the literature review. The reason behind this preliminary plotting was the fact that multiple studies have been conducted for the removal of pollutants and pathogens from CWs where they perform regression analysis and try to determine if there are significant relationships between the aforementioned parameters [16-22]. These studies found that there seems to be a linear/nonlinear relationship that is expressed through an equation that is using the *Cout* as a function of *Cin* and/or HRT, HLR. Having the above information in mind, a simplified expression of these relationships was performed.

3. Results

3.1. Log Removal and Temperature/Climatic Zzones

The following figure demonstrates the average log removal of case studies per country and the respective temperature/climatic zones throughout the world. Although there seems to be a trend where higher removal values can be found in hot temperature zones, and rather low removal values can be found in cold and cool zones, a clear correlation was not found since there is a significant variation within different zones. Additionally, the log removal values are averaged per country and in many cases there is only one case study per country.
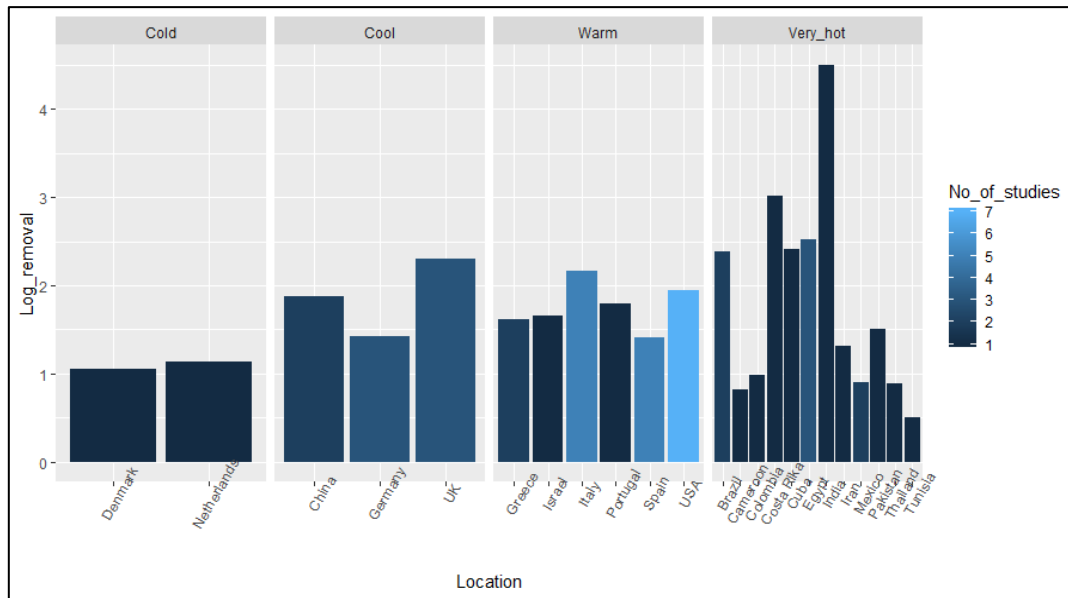
Figure 4. Average log removal per country and their respective climatic zone.

3.2. Overal Performance of CW Types

Figure 5 shows that the log removal for the three types of CWs ranges between 1 and 6 log, whereas the average value ranges between 1 and 2 log. Table 2 demonstrates the overall performance of CWs. The majority of papers in the systematic literature review used SSHFCW for the removal of pathogens, whereas all types seem to have a potential of pathogen removal to a certain level. Briefly, the FWS types have log removal values ranging from 0.07 to 5.3, the SSHFCW from 0.01 to 5.68 and finally the VFCW type from 0.35 to 6.08.
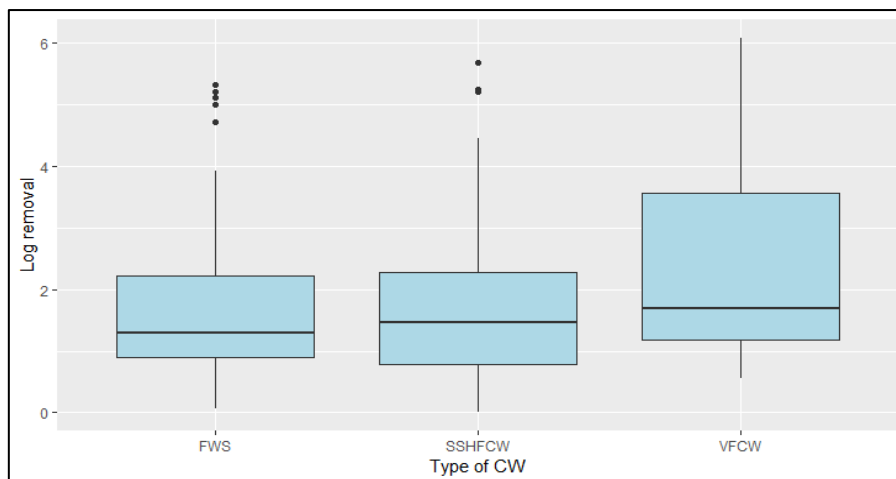

Figure 5. Log removal comparison between types of CW.

Table 2. Overall performance of CW types. Log removal of pathogens is calculated on average.

	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)

N = number of data points.

Moreover, by plotting the influent versus the effluent concentrations of pathogens there seems to be a positive correlation and potentially a non-linear relationship between the two parameters (the example of FWS is shown in Figure 6 while the rest can be found in Appendix C). That means that other parameters (HRL and/or HRT) also influence the removal of pathogens.
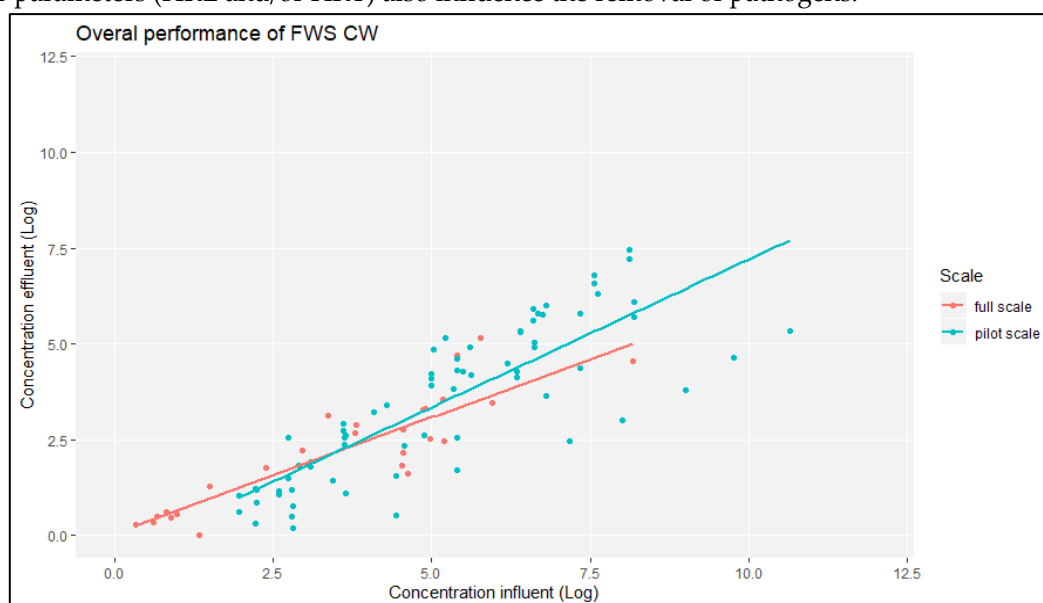

Figure 6. Relationship between influent and effluent concentration in FWS.

Therefore, plotting log removal values [LRV=*log (Cin/Cout)*] vs HLR and HRT gives a first estimate of the significance of those parameters. SSHFCW plot will be used as an example while the rest can be found in Appendix D. Figures 7, and 8 demonstrate the relationship between log removal

and HLR/HRT for the 3 pathogen categories in a SSHFCW. The observed variation can be explained by the different applications (full-scale vs pilot-scale[2]), the complexity and variability in performance for the system itself, but also due to the different technical features/ experimental conditions of each study (porosity, dimensions of CW, type of influent wastewater). Regardless, an important information that can be extracted from these plots, is the positive correlation between removal of pathogens and HLR/HRT for all 3 pathogen categories[3] indicating that both hydraulic characteristics play a significant role in the removal of pathogens.

Figure 7. Positive correlation between HLR and log removal in FWS CW for the 3 different pathogen categories.

Figure 8. Positive correlation between HRT and log removal in FWS CW for the 3 different pathogen categories.

[2] Pilot-scale applications have different dynamics (lower loading rates) and experimental conditions (dimensions, vegetation etc.) which lead to a huge variation regarding removal.

[3] The positive correlation could not be observed in all types of wetlands as can be seen in the Appendix C.

Additionally, Figure 9 demonstrates the Cumulative Distribution Function (CDF) of the different types of CWs regarding their removal. It can be observed that all 3 wetlands show an adequate removal since 50% of the distribution has a removal higher than 1.5 log. The FWS and SSHFCW have an identical distribution where 75% of them have log removal between 0-2 log and the rest is between 2-4 log. The 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.
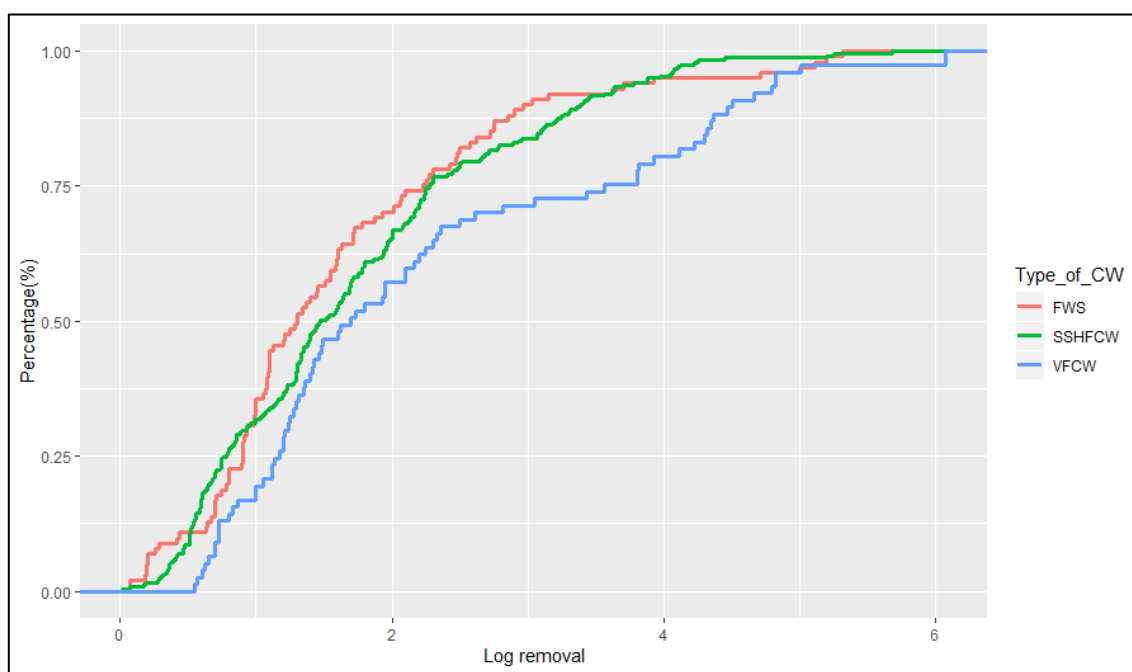

Figure 9. Cumulative distribution of the 3 types of wetlands as found in the literature review.

3.3. Removal of Pathogen Categories

Table 3 shows that the category of GNB can be found in the majority of the papers collected (47 studies) while the protozoan parasites and viruses were found in 8 and 7 studies respectively. This comes as no surprise since the specific category includes the most common indicators and index pathogens such as E. *Coli*, total coliforms, fecal coliforms, campylobacter and salmonella. Furthermore, as it was expected the same category is responsible for the minimum and maximum log removal values overall, when compared with the viruses and protozoan parasites categories. Table 4 gives an overview of the three pathogen categories regarding their removal per CW type.

Table 3. Gram-negative bacteria removal performance for all three types of wetlands.

	Pathogen category	Gram-negative Bacteria		
General information	Average log removal		1.84	
	Number of papers		47	
	Number of pilot-scale		33	
	Number of full-scale		14	
Constructed wetlands	Types of CWs	*FWS*	*SSHFCW*	*VFCW*
	Number of data points	n=77	n=216	n=72
	Average log removal	1.75	1.70	2.33
	Minimum log removal	0.07	0.01	0.55

	Maximum log removal	5.32	5.68	6.08
	Standard deviation	1.19	1.14	1.45
	Average percentage removal	98.22	98	99.5
Physicochemical characteristics	Air temperature °C	19.6 (n=27)	19.1 (n=84)	19 (n=27)
	Water temperature °C	15.6 (n=29)	21.6 (n=108)	18.1(n=54)
	PH	7.5 (n=52)	7.03 (n=108)	7.4(n=48)
	COD removal (mg/L) %	66.2 (n=50)	67.5 (n=151)	82.6 (n=52)
	BOD removal (mg/L) %	72.5 (n=26)	67.6 (n=81)	92.2 (n=22)
	BOD5 removal (mg/L) %	64.8 (n=10)	84.6 (n=71)	92.46 (n=14)
	TSS removal (mg/L) %	71.1 (n=49)	64.7 (n=127)	83.5(n=45)
	HLR range (m/d)	0.058-5.1 (n=75)	0.005-2.59 (n=219)	0.0028-1.36 (n=72)
	HRT range (d)	0.29-7 (n=74)	0.28-15 (n=219)	0.01-9.1 (n=72)

Table 4. Average log removal range for all three types of wetlands.

	Type of CW	**FWS (log)**	**SSHFCW (log)**	**VFCW (log)**
Pathogen Category	Gram-negative bacteria	0.07-5.32	0.01-5.68	0.55-6.08
	Viruses	0.06-2.88	0.02-3.62	0.57-1.75
	Protozoan parasites	0.18-2	0.28-3.63	0.35-2.72

Furthermore, Table 3 depicts the overall performance of the GNB while the other 2 categories can be found in Appendix 4. These Tables include information regarding the number of pilot and full-scale applications as well as physicochemical characteristics such as air and water temperature, HRT and HLR range. In addition to that, there are values of COD, BOD and TSS removal percentage that can be indicators of pollutant removal for each type of CW and pathogen category. What can be extracted from these tables is the difference and the small number of data points between types of CWs regarding their reported physicochemical characteristics which makes it difficult to draw conclusions and compare the different types of wetlands. Only the HRT and HLR were consistently reported. Figure 10 shows that 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. Again, it can be seen that the average log removal values ranging between 1 and 2 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. Furthermore, Figure 11 illustrates the average log removal of the most representative indicators and index pathogens as recorded during the systematic review. The group of coliforms are those with the greatest variability. While the variability can be explained due to the different number of data points, another possible explanation is again the fact that both pilot and full-scale applications are included in the boxplots. The outliers in many of the species strengthen this assumption. Additionally, there is great variability of pathogen log removal within their categories. This is an important observation since it was expected for the different pathogen categories to formulate clusters regarding log removal.
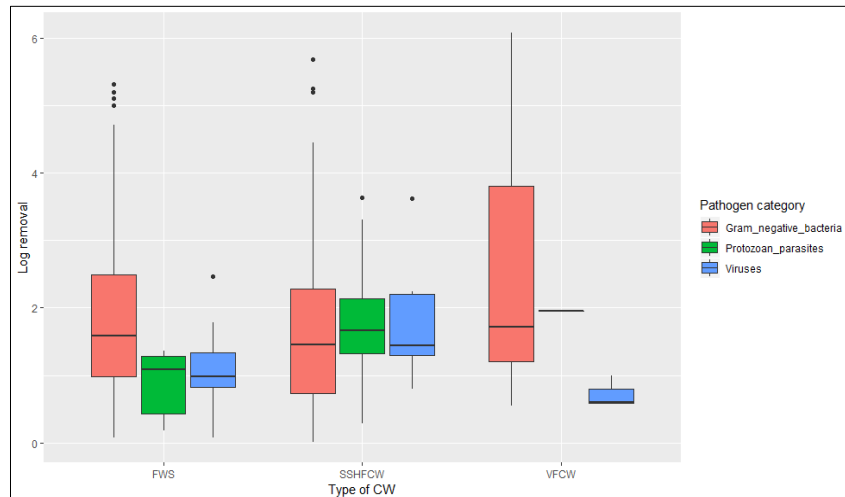
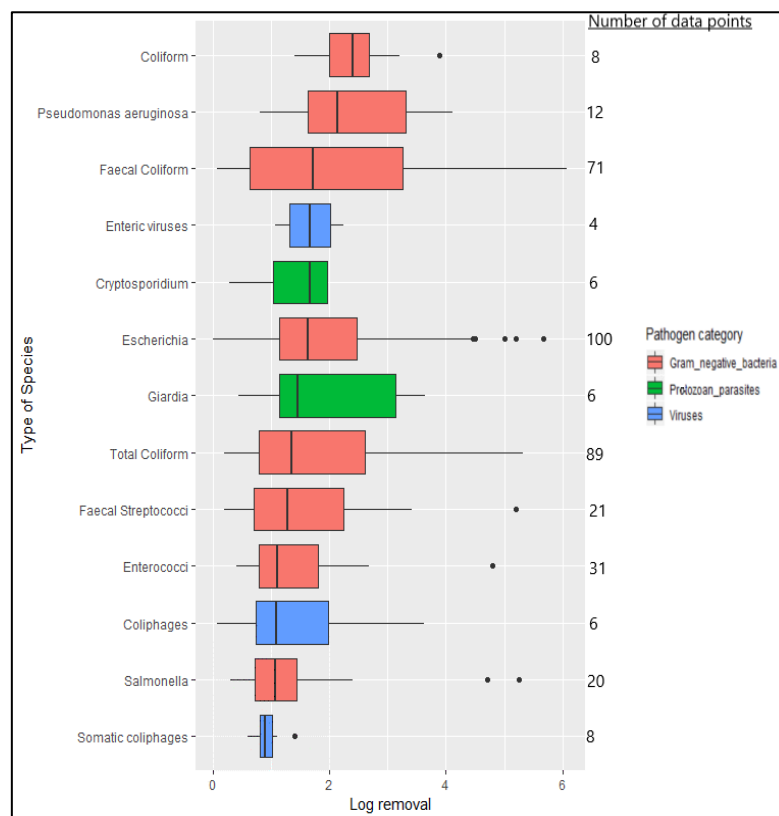**Figure 10.** Comparison of pathogen categories per CW type.

Figure 11. Log removal range for the most representative indicators and index pathogens, ranked based on average log removal.

Finally, the CDF (Figure 12) of the different pathogen categories regarding their removal indicates that 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. The sharp increase in log removal (straight lines) between 2 log onwards can be attributed to the low density of data points in these categories. In contrast, the 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. Regarding the most representative indicators, Figure 13 shows that all 4 indicators follow a rather smooth curve along the distribution and have an adequate removal since 50% of all indicators show a removal higher than 1.5 log.
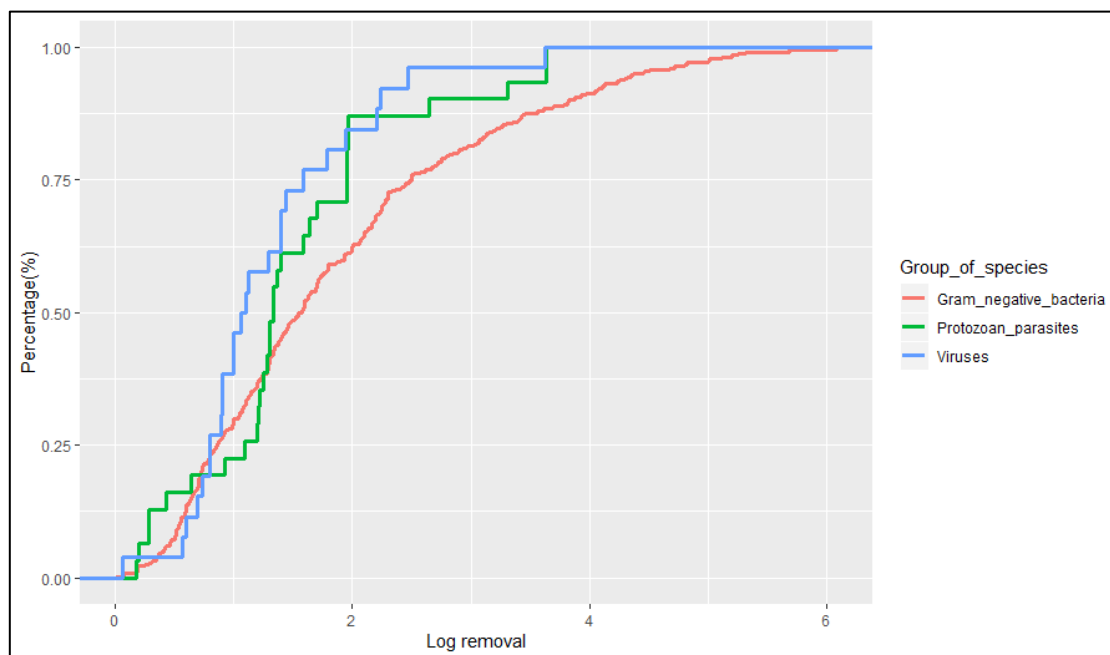
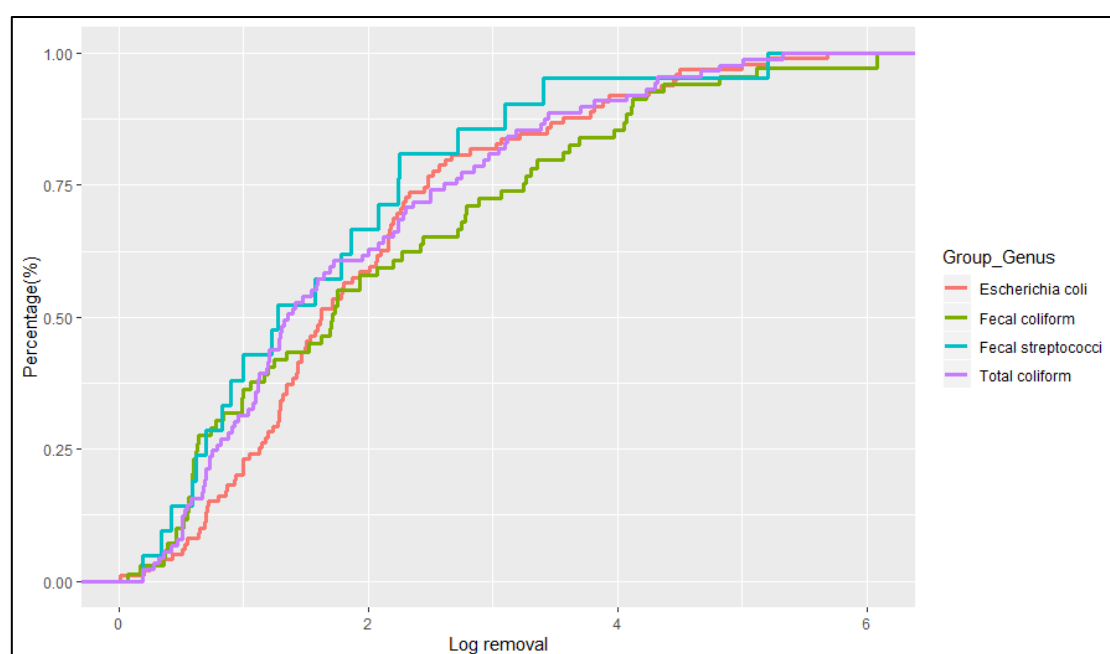**Figure 12.** Cumulative distribution of the three pathogen categories as found in the literature review.

Figure 13. Cumulative distribution of the most representative indicators as found in the literature review.

4. Discussion

This study brings all current quantitative data in a dataset giving access to any end user to the overall performance of CW types and their respective pathogen removal efficiency. More importantly, the systematic review included case studies from all over the world, therefore the collected information can be considered representative. Pilot-scale applications have been reported the most, mainly because the majority of them were experimental case studies with an objective of investigating the removal efficiencies of pollutants and pathogens. Full-scale applications although used in many cases, they are not reported in scientific papers to the same extent. Furthermore, the

initial attempt to try to identify trends and possible correlations between log removal and temperature/climatic zones was unsuccessfull since no robust conclusions could be drawn from the graph.

The average pathogen removal of all three types of CWs was between 1 and 2 log, indicating that CWs cannot be a reliable option for pathogen removal as a standalone wastewater treatment method, although some case studies showed potential for further research since they achieved a removal as high as 6 log. However, they can be a credible choice as a wastewater polishing step when they are combined with conventional wastewater treatment systems. Regarding the removal of the three pathogen categories, Table 4 shows that the gram-negative bacteria category have the highest log removal in all types of CW. On the other hand, the same group shows great variability which makes it difficult to draw tangible conclusions about the ease of removal of this category. This can best be seen in Figure 13 where the variability of all categories is shown using boxplots. Protozoan parasites show the less variability in FWS whereas the viruses' category is the one with the smallest range in SSHFCW. Conclusions could not be drawn for the last two categories in VFCW types since their number of data points was small. In addition, the huge difference in variability between the three categories can be attributed to the huge database of the gram-negative bacteria category where, to some extent, it is expected to see scattered removal values. On the other hand, although the other categories show less variability and it is easier to draw conclusions about their removal, their databases are quite small which should make any decision-maker skeptical about the reliability of the results. 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.

Regarding limitations, one of the major difficulties encountered in this literature review was the lack of important and relevant data from the studies that were included in the dataset. Therefore, a need for more descriptive information from papers is considered imperative especially on studies that are directly related to constructed wetlands and pathogen removal. Additionally, a lot of studies did not provide extractable information which meant that a lot of assumptions had to be made (e.g. default value of porosity, calculation of missing parameters using default values). Therefore, it is recommended that the scientific community should focus on adequate reporting of extractable data and information to better facilitate future research.

Finally, a relationship was identified by plotting the influent versus effluent concentration as well as the log removal versus HLR/HRT between different types of CWs. Various patterns were observed which gives room for further investigation in these parameters. Therefore, a potential meta-analysis of this database using statistical analysis can provide additional and insightful information on the significance of C_{in}, HLR, and HRT on pathogen removal. If enough data can be provided for all types of CWs and pathogen categories, then a model that can predict the removal of pathogens under the influence of specific parameters can be created. That can potentially help the scientific community gain a better insight on the removal capacity of these systems.

5. Conclusions

The purpose of this literature review was to shed more light in the performance of CWs regarding pathogen removal. That was achieved as the final outcome of the review includes a comprehensive database that covers a wide range of information about constructed wetlands and log removal values from specific pathogen categories. Special attention must be paid on better reporting of extractable information to be able to draw tangible conclusions regarding different pathogen categories and their respective removal from CWs and improve the current dataset. Moreover, the plotting of different parameters indicated potential relationships that can lead to the creation of a predictive model if a meta-analysis of this dataset is performed. 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.

Conflicts of Interest: "The authors declare no conflict of interest."

Abbreviations

The following abbreviations are used in this manuscript:

BOD: Biological Oxygen Demand

C in: Concentration influent

C out: Concentration effluent

CDF: Cumulative Distribution Function

CFU: Colony-Forming Unit

COD: Chemical Oxygen Demand

CW: Constructed Wetland

FWS: Free Water Surface

GNB: gram-negative bacteria

HLR: Hydraulic Loading Rate

HRT: Hydraulic Retention Time

LRV: Log Removal Value

MPN: Most Probable Number

n: Porosity

SSHFCW : Subsurface Horizontal Flow Constructed Wetland

T: Temperature

TSS: Total Suspended Solids

VFCW: Vertical Flow Constructed Wetland

WW:Wastewater

WWTP:Wastewater Treatment Plant

Appendix A

Search engine	Search terms	Searched fields	Number of papers	New papers
Schopus	'constructed wetlands" AND "pathogen removal"	TITLE-ABS-KEY	50	50
	'constructed wetlands" AND "pathogen index"	TITLE-ABS-KEY	1	0
	"constructed wetlands" AND "pathogen indicator"	TITLE-ABS-KEY	7	6
	'constructed wetlands" AND "microbes removal"	TITLE-ABS-KEY	1	0
	'constructed wetlands" AND "micro organism" OR "microorganism" AND removal	TITLE-ABS-KEY	611	551
	'constructed wetlands" AND "micro organism" OR "microorganism" AND inactivation	TITLE-ABS-KEY	17	6
	"constructed wetlands" AND "micro organism" OR "microorganism" AND immobilization	TITLE-ABS-KEY	12	3
	"constructed wetlands" AND "micro organism" OR "microorganism" AND "microbial removal"	TITLE-ABS-KEY	10	0
	"constructed wetlands" AND "pathogen inactivation"	TITLE-ABS-KEY	3	1
	"constructed wetlands" AND inactivation AND bacteria	TITLE-ABS-KEY	17	5
	"constructed wetlands" AND inactivation AND virus	TITLE-ABS-KEY	8	3
	"constructed wetlands" AND inactivation AND protozoa	TITLE-ABS-KEY	3	1
	"constructed wetlands" AND index AND bacteria	TITLE-ABS-KEY	54	33
	"constructed wetlands" AND "pathogen removal" AND bacteria	TITLE-ABS-KEY	25	0
	"constructed wetlands" AND "pathogen removal" AND virus	TITLE-ABS-KEY	7	0
	"constructed wetlands" AND "pathogen removal" AND protozoa	TITLE-ABS-KEY	4	0
	"constructed wetlands" AND index AND bacteria OR virus OR protozoa	TITLE-ABS-KEY	57	3
Pubmed	(constructed wetland and pathogen removal)	All fields	72	50
	((constructed wetland* AND (immobilization OR inactivation) AND water AND (microorganism OR bacteria OR protozoa OR virus OR spore)))	All fields	20	8
	((constructed wetland) AND log removal) AND bacteria	All fields	28	11
	((constructed wetland) AND log removal) AND virus*	All fields	5	0
	((constructed wetland) AND log removal) AND microorganism*	All fields	6	2
	((constructed wetland) AND log removal) AND bacteria OR constructed wetland AND log reduction	All fields	24	4
	(constructed wetland*) AND pathogen inactivation AND treatment	All fields	4	0
Sum of Schopus				662
Sum of Pubmed				75
Total number of papers				737

Figure A1. Total number of papers and the combination of keywords that was used at Scopus and PubMed databases.

Appendix B

Title	Type_of_Cw	HLR	HRT	tBOD5_remc	COD_removal	TSS_total	Medium	Dimensions_of_	Water_type	Group_Genus	Group	Type_of_s	Method_of_quan	Conce	Conce Units	sign	Concer	Concer Units	sign	Log_removal
Presence of	VFCW	1.08	0.83	98.034996	92.66247379	0.32954	gravel	L=29m,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=29m,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=29m,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=29m,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=29m,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=29m,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		92.53253253	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		92.53253253	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		92.53253253	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 gra	L=1.20 m, w=0,	primary treat	Total Coliform	Gram_negat	indicator	IDEXX Quanti-Tr	6.2	MPN/100 mL	=	5	MPN/100 mL	=	1.2
Halophytes a	VFCW	0.095	3.5		78.125		2 layers of gra	L=1.20 m, w=0,	primary treat	Escherichia	Gram_negat	indicator	IDEXX Quanti-Tr	4.2	MPN/100 mL	=	2.1	MPN/100 mL	=	2.1
Halophytes a	VFCW	0.095	3.5		73.01785714		2 layers of gra	L=1.20 m, w=0,	primary treat	Total Coliform	Gram_negat	indicator	IDEXX Quanti-Tr	6.2	MPN/100 mL	=	5.5	MPN/100 mL	=	0.7
Halophytes a	VFCW	0.095	3.5		73.01785714		2 layers of gra	L=1.20 m, w=0,	primary treat	Escherichia	Gram_negat	indicator	IDEXX Quanti-Tr	4.2	MPN/100 mL	=	2.4	MPN/100 mL	=	1.8
Emerging org	VFCW	0.044	6.34	91.2	82.945739643	0.09649	1 layer 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	88.75368992	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	81.78234574	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	1 layer 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	97.9094	gravel		combined se	Escherichia	Gram_negat	indicator	Chromogenic Mer	3.55	CFU/100 mL	=	4.5	CFU/100 mL	=	-0.95

Figure B1. A sample of the complete dataset after the systematic literature review was complete.
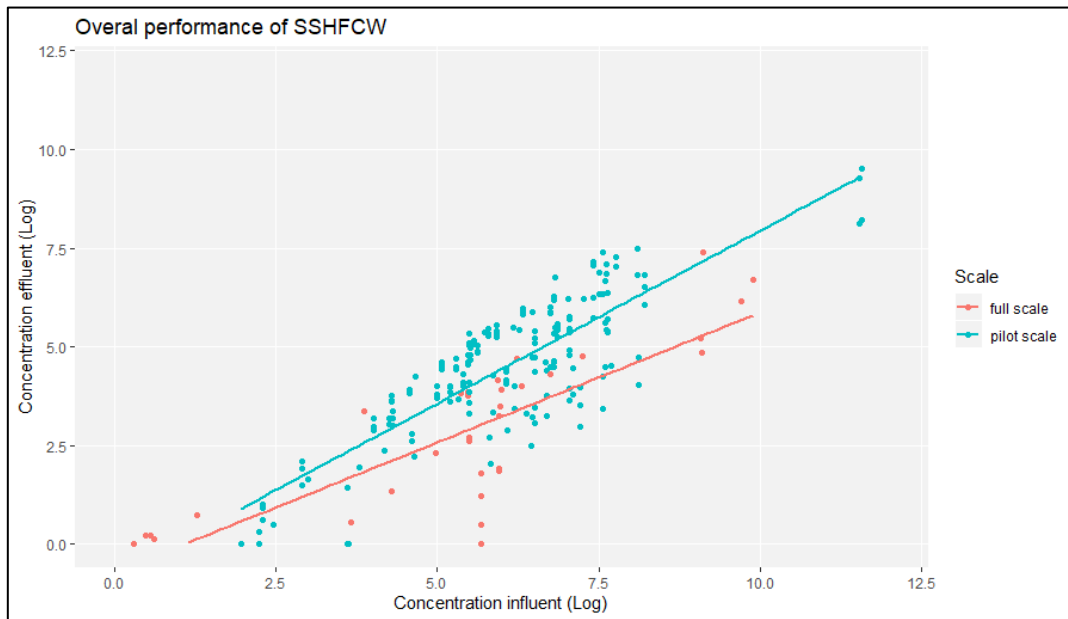
Appendix C

Figure C1. Relationship between influent and effluent concentration in SSHFCW.
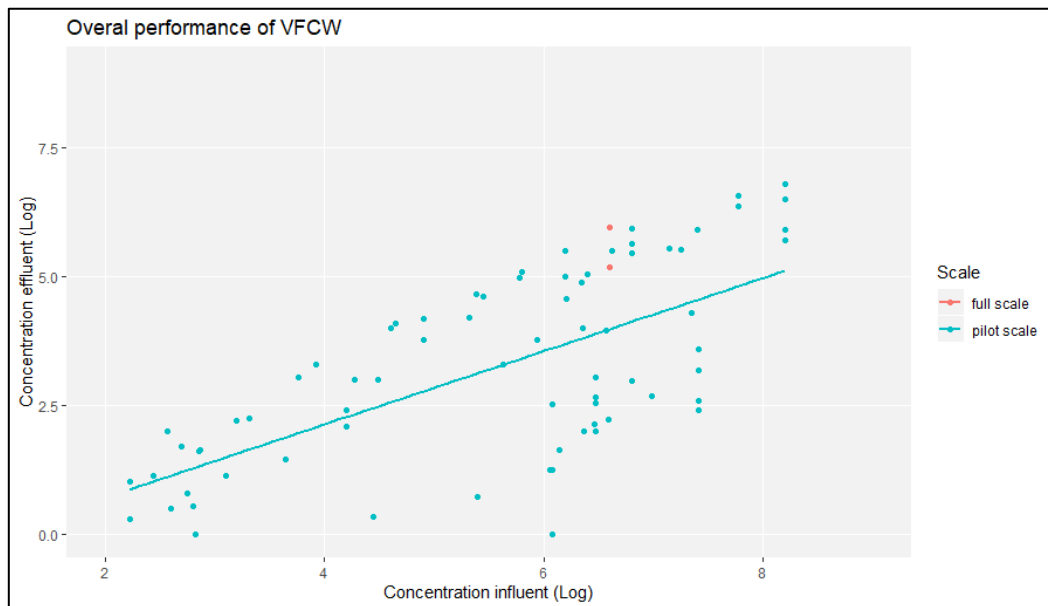

Figure C2. Relationship between influent and effluent concentration in VFCW.
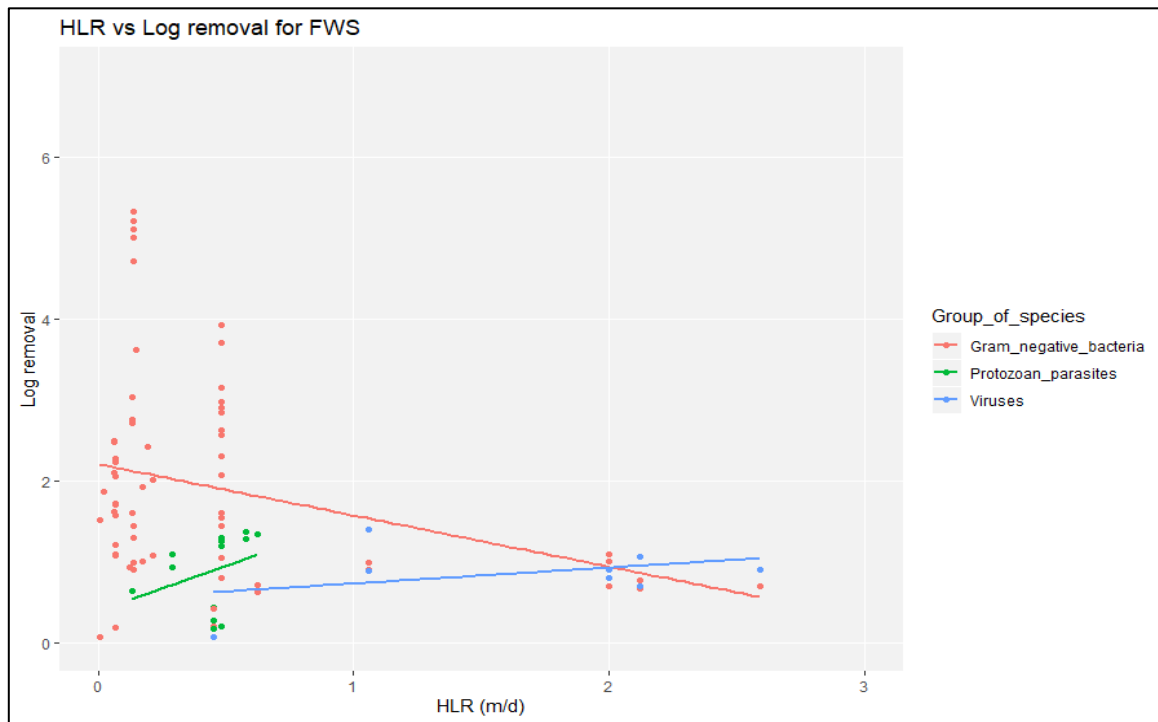
Appendix D

Figure D1. Positive and negative correlations between log removal and HLR for different pathogen categories.
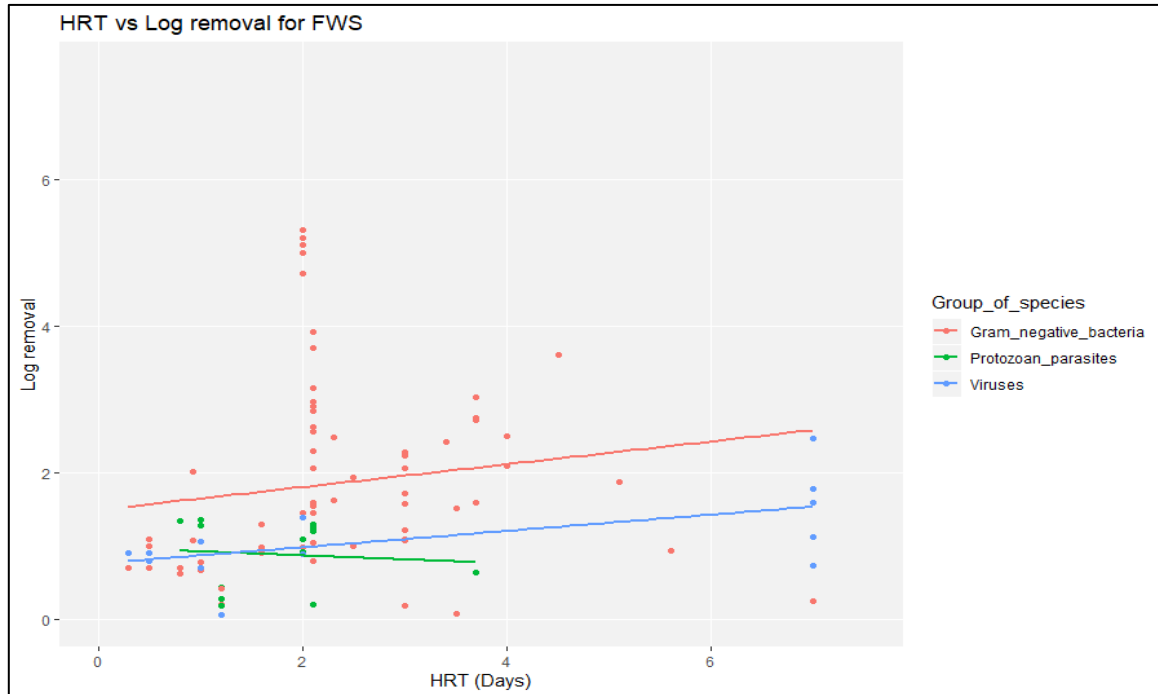

Figure D2. Positive and negative correlations between log removal and HRT for different pathogen categories.
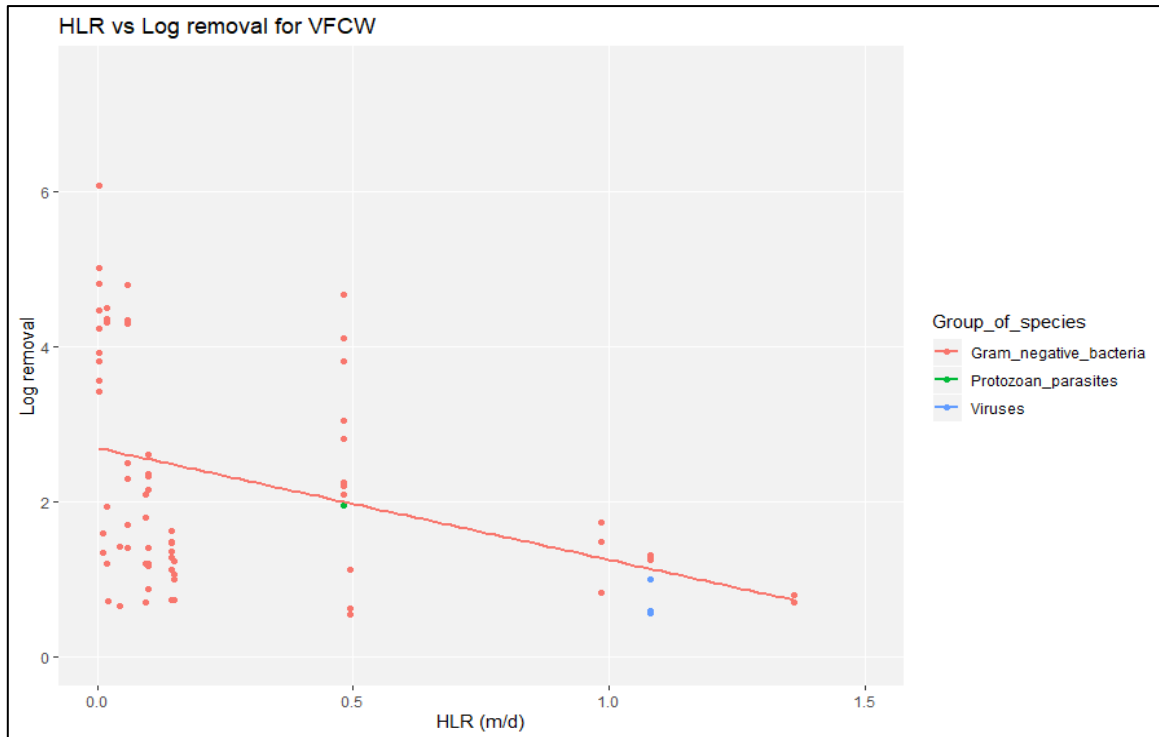
Figure D3. Negative correlation between log removal and HLR for gram-negative bacteria. The remaining categories did not have enough data points.
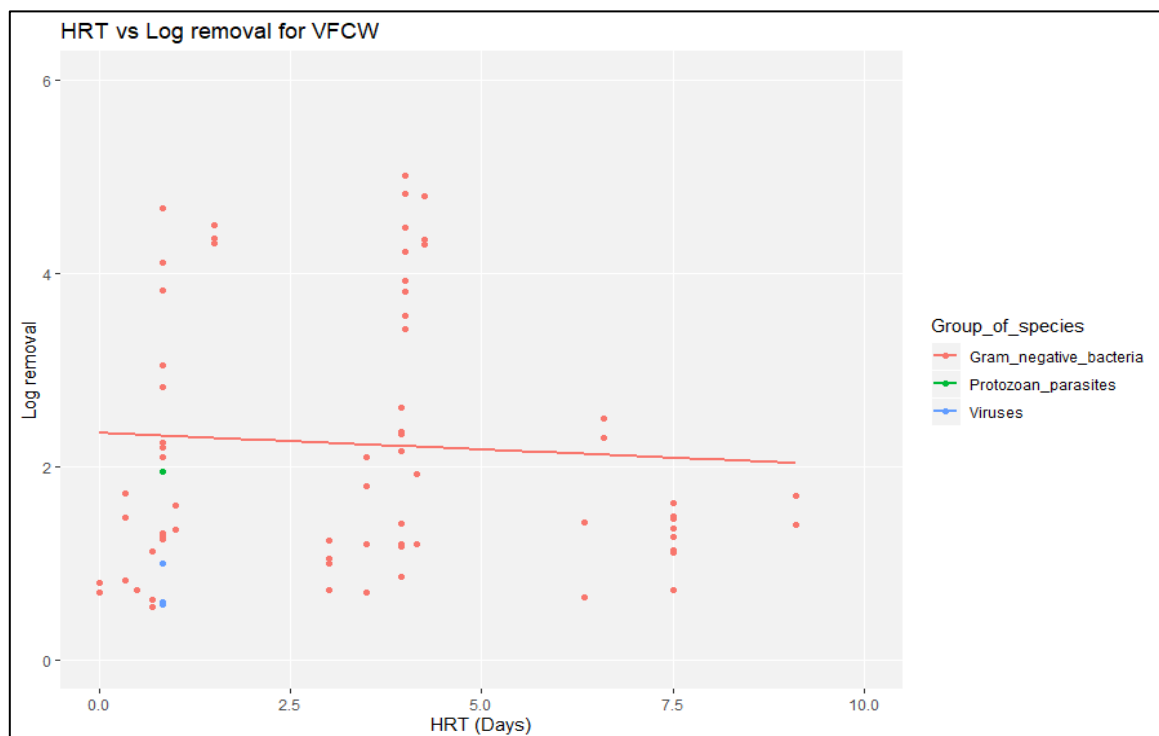

Figure D4. Somewhat negative correlation between log removal and HRT for gram-negative bacteria. The remaining categories did not have enough data points.

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