SLUDGE TREATMENT BY EARTHWORM-ENHANCED REED BEDS TOWARDS SMART-CITIES

Amir Gholipour

Scientific advisors:

Ph.D. Elizabeth da Costa Neves Fernandes de Almeida Duarte

Ph.D. Rita do Amaral Fragoso

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THESIS PRESENTED TO OBTAIN THE DOCTOR DEGREE IN ENVIRONMENTAL ENGINEERING

2024

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Título: Estudo à escala piloto de leitos de macrófitas com minhocas para tratamento de lamas de ETAR em Portugal

RESUMO

Este estudo explorou uma abordagem inovadora para a gestão de lamas de ETAR utilizando leitos de macrófitas para desidratação de lamas (STRB) com minhocas (W-STRB). De acordo com o conhecimento do autor, esta foi a primeira avaliação de STRB com minhocas sob clima temperado (Portugal). O estudo incluiu experiências na estação de tratamento de águas residuais de Beirolas (escala piloto) e na estufa do Horto no ISA (escala de laboratório). A escala piloto de Beirolas foi avaliada durante um ano de alimentação (24 ciclos, taxa média de carga de lamas (SLR): 43,59 ± 14,49 kg de sólidos totais (DS) por m^2.ano) e 132 dias de período de descanso. Testaram-se quatro modalidades, unidade com plantas e minhocas (WP), unidade plantada sem minhocas (P), sem plantas com minhocas (W) e unidade de controlo sem plantas e sem minhocas (C), todas em duplicado. A espécie de planta utilizada foi *Arundo donax* e a minhoca foi *Eisenia fetida*. No ensaio à escala de laboratório no Horto, testaram-se duas espécies de plantas, *Arundo donax* e *Phragmites australis*. As unidades plantadas incluíam minhocas *Eisenia fetida* e usou-se uma unidade sem plantas nem minhocas como controlo. No estudo à escala de laboratório efetuou-se a alimentação durante seis meses (50, 60 e 70 kg.DS.m^{-2}.ano^{-1}) e o período de descanso foi de dois meses. O ensaio em Beirolas mostrou que, o DS de todas as unidades estava acima de 70% enquanto o conteúdo de sólidos voláteis (VS) e a razão VS/DS atingiu o valor mínimo de 53% na unidade WP, indicando que a presença de minhocas levou a maior estabilização. Verificou-se um impacto sinérgico das minhocas, com a produção de biomassa vegetal na unidade WP a ser 30% superior à da unidade P. A unidade WP mostrou uma redução significativa na massa de poluentes no lixiviado. A unidade WP reduziu 10% a massa de metais pesados nas lamas residuais em comparação com a unidade P. O estudo enfatizou o papel positivo das minhocas no STRB e o potencial de reutilização das lamas residuais, contribuindo para a economia circular em cidades inteligentes.

Palavras-chave: desidratação de lamas, minhocas, *Phragmites australis, Arundo donax, Eisenia fetida*

Title: Sludge treatment by earthworm-enhanced reed beds towards smart-cities

Abstract

This study explored an innovative approach for sewage sludge management using sludge treatment reed bed (STRB) enhanced with earthworms (W-STRB). To the authors' knowledge, it was the first evaluation of STRB with earthworms under temperate climate (Portugal). The research included experiments in Beirolas wastewater treatment plant (pilot-scale) and Horto greenhouse at ISA (bench-scale). Beirolas pilot-scale was assessed for one year of feeding (24 cycles, mean sludge loading rate (SLR): 43.59 ± 14.49 kg of dry solids (DS) per m^2.year) and 132 days of final resting. Four types of units were tested including planted unit with earthworms (WP), planted unit without earthworms (P), without plant with earthworms (W) and control unit without plants and earthworms (C), all replicated. In Beirolas pilot-scale, the plant species was *Arundo donax* and the earthworm was *Eisenia fetida*. In Horto bench-scale, two plant species were tested, *Arundo donax* and *Phragmites australis*. Planted units included *Eisenia fetida* earthworms and an additional unit without plants or earthworms was used as control. The bench-scale was studied for six months of feeding (50, 60, and 70 $kg.DS.m^{-2}.year^{-1}$) and two months of resting. Beirolas experiment showed that DS of all units were over 70 % while volatile solid content (VS) reached the minimum content at 53 % in the WP unit indicating higher stabilization in the presence of earthworms. A synergistic impact of earthworms could also increase plant biomass production by 30 % in the WP unit compared to the P unit. The WP unit showed significant reduction in the release mass (g) of pollutants in the drained water. The WP unit reduced 10 % the mass of heavy metal in the residual sludge compared to the P unit. The study emphasized earthworms' positive role in STRB, and potential reuse of the residual sludge contributing to circular economy in smart cities.

Keywords: sludge dewatering, earthworm, *Phragmites australis, Arundo donax, Eisenia fetida*

Título: Estudo à escala piloto de leitos de macrófitas com minhocas para tratamento de lamas de ETAR em Portugal

Resumo alargado

O uso de leitos de macrófitas para o tratamento de lamas (STRB) tem sido utilizado ao longo de várias décadas, demonstrando diferentes eficiências dependendo das condições climáticas. Este estudo de doutoramento explorou uma solução inovadora baseada na natureza (NBS) destinada à desidratação de lamas de ETAR através da tecnologia STRB. Numa primeira fase realizou-se uma revisão bibliográfica abrangente que englobou 73 estudos sobre a tecnologia STRB, revelando quatro tipos de sistema: STRB típico, STRB com minhocas, STEW (wetland eletroquímico para tratamento de lamas) e STEW com minhocas, que estão em uso desde 1990. A meta-análise desses estudos sugeriu taxas médias de carga de lamas de 50, 70 e 101 kg de matéria seca (DS) por m².ano para climas temperados tipo 1 (norte da UE), temperados tipo 2 (Mediterrâneo) e climas tropicais, respetivamente. Com base nesta revisão bibliográfica, fez-se o delineamento experimental dos ensaios a realizar, incluindo um estudo piloto na ETAR de Beirolas e um à escala de bancada no Horto do ISA.

O ensaio em Beirolas foi conduzido ao longo de um ano, abrangendo 24 ciclos de alimentação de lamas mistas ($DS_{média} = 24{,}71 \pm 13{,}67$ $g.L^{-1}$ e $VS_{média} = 19{,}14 \pm 10{,}29$ $g.L^{-1}$) resultantes da mistura de lamas primária e secundária. A alimentação dos leitos foi seguida por uma fase final de repouso de 132 dias e a taxa média de carga de lamas foi de 43,59 $kg.DS.m^{-2}.ano^{-1}$. O ensaio incluiu quatro tipos de unidades: (1) unidades plantadas com minhocas (WP), (2) unidades plantadas sem minhocas (P), (3) unidades não plantadas com minhocas (W) e (4) unidades de controlo, sem plantas e minhocas (C). Cada configuração foi replicada, resultando num total de oito unidades. Foi avaliada a incorporação de *Arundo donax* e da espécie de minhoca *Eisenia fetida*. No ensaio de Beirolas, todas as unidades alcançaram um teor final de DS superior a 70%. A unidade WP demonstrou vantagens significativas, apresentando o valor mais baixo de VS/DS de 53%, indicando uma estabilização melhorada devido às minhocas. Além disso, a unidade WP apresentou uma perda de água 46% superior à unidade P, sugerindo que potencialmente poderá receber taxas de carga de lamas mais elevadas. Esta unidade apresentou também uma produção de biomassa vegetal 30% superior. Por outro lado, o lixiviado da unidade WP continha uma massa de poluentes significativamente inferior às restantes e o teor de metais pesados na lama residual diminuiu em 10% em comparação com a unidade P. A taxa de acumulação de lamas foi de 0,06 cm.ano^{-1} para a unidade WP, indicando um volume de lama residual 33% menor em comparação com a unidade P, que teve 0,09 cm.ano^{-1}. Isto mostra os impactes positivos das minhocas na redução do volume de lama residual, o que potencialmente contribuirá para aumentar a vida útil do sistema em aplicações em grande escala e minimizar os custos de transporte de lama, no final do ciclo de vida do sistema. As variações sazonais influenciaram o desempenho dos leitos, com maior teor de DS durante as estações secas e aumento da espessura da lama residual durante as estações húmidas. As unidades assistidas por minhocas demonstraram maior estabilização, com redução de até 65% no volume de lama residual após o período final de repouso. Esta abordagem sinérgica também resultou em maior produção de biomassa vegetal e taxas de evapotranspiração mais elevadas, especialmente durante as estações secas. As minhocas contribuíram também para a melhoria na qualidade da água drenada do STRB revelou melhorias substanciais, com reduções de 43%, 45%, 75% e 45% nas massas de sólidos suspensos totais, carência química de oxigénio, nitratos e fósforo, respetivamente. Após a fase inicial do ensaio (primeiros seis meses de alimentação), a eficiência de remoção melhorou, com vários parâmetros a atingir os requisitos para reutilização de água. O W-STRB plantado com *Arundo donax* mostrou reduções significativas de sólidos voláteis e de metais pesados, cumprindo os requisitos legais para lamas residuais da UE e de Portugal. A inclusão de minhocas também desempenhou um papel crucial na redução dos níveis de macronutrientes e micronutrientes na lama residual. A lama residual demonstrou uma redução significativa em *Escherichia coli* e *coliformes fecais*, com uma eficiência de remoção superior a 99% em todas as unidades de tratamento. Entre estas, as unidades WP apresentaram maior eficiência de remoção em comparação com as unidades P, W e C. No entanto, no que diz respeito à *Salmonella*, os resultados foram inconsistentes, com deteções frequentes na lama residual. Consequentemente, é necessário efetuar um tratamento adicional para que a lama possa ser reutilizada com segurança. A estimativa de custo para a solução proposta, para 2000 equivalentes populacionais (PE), indicou que os custos iniciais de configuração para cenários de centrifugação e W-STRB foram de 88 e 124 €.PE^{-1}, respetivamente, enquanto os custos operacionais e de manutenção favoreceram os sistemas W-STRB com 3 €.PE^{-1}.ano^{-1} (7 €.PE^{-1}.ano^{-1} para centrifugação), destacando sua viabilidade económica.

No estudo à escala de bancada no Horto, plantaram-se três unidades com *Arundo donax* suplementadas com minhocas (WP1 a WP3), três unidades com *Phragmites australis* e minhocas (WP5 a WP7), e uma unidade serviu como controle (C). O ensaio foi realizado ao longo de seis meses de alimentação com taxas de carga de lamas de 50, 60 e 70 kg.DS.m^{-2}.ano^{-1}, seguido por um período de repouso de dois meses. A uma taxa de carga de 50 kg.DS.m^{-2}.ano^{-1}, as unidades plantadas com *Phragmites australis* alcançaram um maior teor de DS (41%) em comparação com as de *Arundo donax* (25%). Com taxas de carga de lamas superiores a 60 kg.DS.m^{-2}.ano^{-1}, as unidades com *Arundo donax* apresentaram perda de plantas, indicando um limite de tolerância para esta espécie. As taxas de evapotranspiração foram mais altas nas unidades com *Phragmites australis* (5,23 mm.dia^{-1}) em comparação com as unidades com *Arundo donax* (4,24 mm.dia^{-1}), ambas alimentadas a 70 kg.DS.m^{-2}.ano^{-1}. As unidades com *Phragmites australis* exibiram uma perda de água 20% maior do que as unidades de *Arundo donax*. Apesar da melhoria na qualidade da água drenada ao longo do tempo, esta não cumpria os requisitos legais, sendo que as unidades com *Phragmites australis* lixiviaram uma massa de poluentes mais baixa. As camadas de lama residual continham quantidades significativas de macro e micronutrientes, com um elemental significativa de N > Ca > P > S > Mg > K e Fe > Na > B > Mn > Mo para micronutrientes, enquanto os níveis de metais pesados estavam dentro dos valores limites de Portugal. A incorporação de minhocas melhorou a eficiência de desaguamento e reduziu o volume de lodo em 33% na unidade WP, indicando potencial para aumentar a vida útil do sistema e reduzir os custos de transporte de lama. O estudo enfatiza a importância da otimização da taxa de carga de lamas, dos modos operacionais e das características da lama para um desempenho eficaz do STRB, destacando a maior eficiência de desidratação e de redução de poluentes da *Phragmites australis* em relação ao *Arundo donax*, ambas na presença de *Eisenia fetida*. No geral, a integração de minhocas e espécies de plantas específicas no STRB melhorou a eficiência de desidratação e a estabilização da lama residual, melhorou o balanço hídrico e potencialmente reduz os custos, apresentando uma alternativa sustentável para a gestão de lamas residuais dentro do quadro de uma economia circular em cidades inteligentes.

Palavras-chave: desidratação de lamas, minhocas, *Phragmites australis, Arundo donax, Eisenia fetida*

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I would like to express my sincere gratitude to my supervisory team Prof. Elizabeth Duarte, Prof. Rita Fragoso and Prof. Ana Galvão, whose guidance and support were instrumental throughout the entire journey of my doctoral research. Their expertise, encouragement, and invaluable insights significantly contributed to the successful completion of this thesis.

I extend my heartfelt appreciation to the dedicated individuals at the wastewater treatment plant (WWTP), as well as the team at Águas do Tejo Atlântico, for their collaboration and assistance in conducting the Beirolas experiment. Their cooperation and willingness to share their expertise greatly enriched the research process.

A special thanks goes to my colleagues at School of Agriculture at University of Lisbon, who played a crucial role in the experimental study conducted at the Horto greenhouse. Their collaboration and shared commitment to scientific inquiry were essential to the success of this research endeavor.

I would also like to acknowledge the unwavering support of my colleagues in the laboratory, whose assistance facilitated the experimental tests and contributed to the overall quality of the research. During my PhD study, I had the opportunity to participate in short-term scientific missions (STSM) in Iceland, Denmark, and Greece. I worked with Prof. David Finger, Prof. Pedro Carvalho, and Prof. Alexandros Stefanakis. My sincere appreciation goes to these remarkable individuals for sharing their knowledge and hosting me so hospitably. Special thanks to the teams at COST Action 17133 and 20133 for providing the grants that made these STSMs possible.

Heartfelt appreciation is extended to my parents for their enduring support and understanding throughout the four years of my academic journey. Their encouragement, patience, and tolerance of the distance were fundamental in sustaining my pursuit of knowledge.

This thesis is a culmination of collective efforts, and I am grateful to each individual and institution that played a part in making this research possible

Table of Contents

Index of figures

Index of tables

Index of abbreviations

AHTN	6-Acetyl-1,1,2,4,4,7-hexamethyltetraline
ANOVA	Analysis and Variance
ARGs	Antibiotic-Resistant Genes
AZM	Azithromycin
ARGs	Antibiotic resistance genes
A.donax	*Arundo donax*
C	Control unit
CW	Constructed Wetland
COD	Chemical Oxygen Demand
CIP	Ciprofloxacin
CE	Circular economy
DW	Drained water
DE	Dewatering efficiency
DS	Dry Solids
DEHP	Diethyl Hexyl Phthalate
DM	Dry Matter
DB	Drying Bed
EIS	Energy-intensive solutions
E-STRB	Electro- sludge treatment reed bed
E. coli	*Escherichia coli*
EC	Electrical Conductivity
ET	Evapotranspiration
F. coli	Fecal coliform
ISA	Instituto Superior de Agronomia
IPMA	Instituto Português do Mar e da Atmosfera
I-STRB	Intensified- sludge treatment reed bed
ICP	Inductively coupled plasma
GHGs	Greenhouse Gases
GWP	Global Warming Potential
HMs	Heavy Metals
HHCB	2-benzopyran
HLR	Hydraulic loading rate
LCA	Life Cycle Assessment
MA	Meta-Analysis

MES	Microbial Electrochemical System
MS	Mixed Sludge
MFC	Microbial fuel cells
NBS	Nature-based solutions
NO_3^--N	Nitrate nitrogen
NDVI	Normalized Difference Vegetation Index
NH_4^+-N	Ammonium nitrogen
O&M	Operation and Maintenance
OTNE	Octahydro-Tetramethyl-Naphthalenyl-Ethanone
PRI	Photochemical Reflectance Index
P	Planted unit
P_{ry}	Precipitation
P. australis	*Phragmites australis*
PCPs	Personal care products
RS	Residual Sludge
STRB	Sludge Treatment Reed Bed
STEW	Sludge Treatment Electro Wetland
SLR	Sludge Loading Rate
SAS	Surplus Activated Sludge
SD	Standard Deviation
sur	Surface layer
sub	Subsurface layer
SDG	Sustainable development goal
TOC	Total Organic Carbon
TP	Total Phosphorous
TKN	Total Kjeldahl Nitrogen
TN	Total Nitrogen
TK	Total Potassium
TSS	Total Suspended Solid
T	Temperature
TW	Wetland technology
TVS	Total volatile solids
VS	Volatile Solids
VLR	Volumetric Loading Rate
WWTP	Wastewater Treatment Plant

WB	Water Balance
WL	Water Loss
WPR	Water Percolation Rate
W	Worm unit
WP	Worm Planted unit
W-STRB	Worm-Sludge Treatment Reed Bed

Thesis framework

Wastewater generation has surged alongside urbanization and population expansion, prompting the widespread adoption of centralized wastewater treatment plants. However, this approach has led to exorbitant installation, operation, and maintenance costs, exacerbating ecological challenges like global warming and pollution distribution in ecosystems and water bodies. The current wastewater management paradigm, especially concerning technology, requires a more sustainable and cost-effective strategy. Traditional mechanical techniques dominate sludge management practices, despite their costliness, energy intensiveness, and adverse ecological footprint. These methods often demand additional treatment stages and advanced technologies to recover resources embedded in the sludge.

Nature-based solutions (NBS), exemplified by the sludge treatment reed bed, present an eco-friendly alternative to conventional technologies. These low-tech solutions leverage natural materials readily available in local environments. The residual sludge produced within the reed bed system undergoes several biological and chemical transformations facilitated by microorganisms, plants, and filter materials. This process not only aids in resource recovery for land application but also ensures long-term storage with enhanced treatment efficacy.

The application of NBS has garnered significant attention from entities like the European Union and United Nation, with financial support earmarked to promote ecological systems that combat climate change and facilitate resource recovery through cost-effective technologies. While past studies on reed bed systems for sludge management predominantly focused on temperate climates such as those in Denmark, France, and Poland, investigations in Portugal remain scarce. Moreover, experiments involving earthworm inclusion in such systems have primarily been conducted in tropical climates like China, and there is no example within the EU.

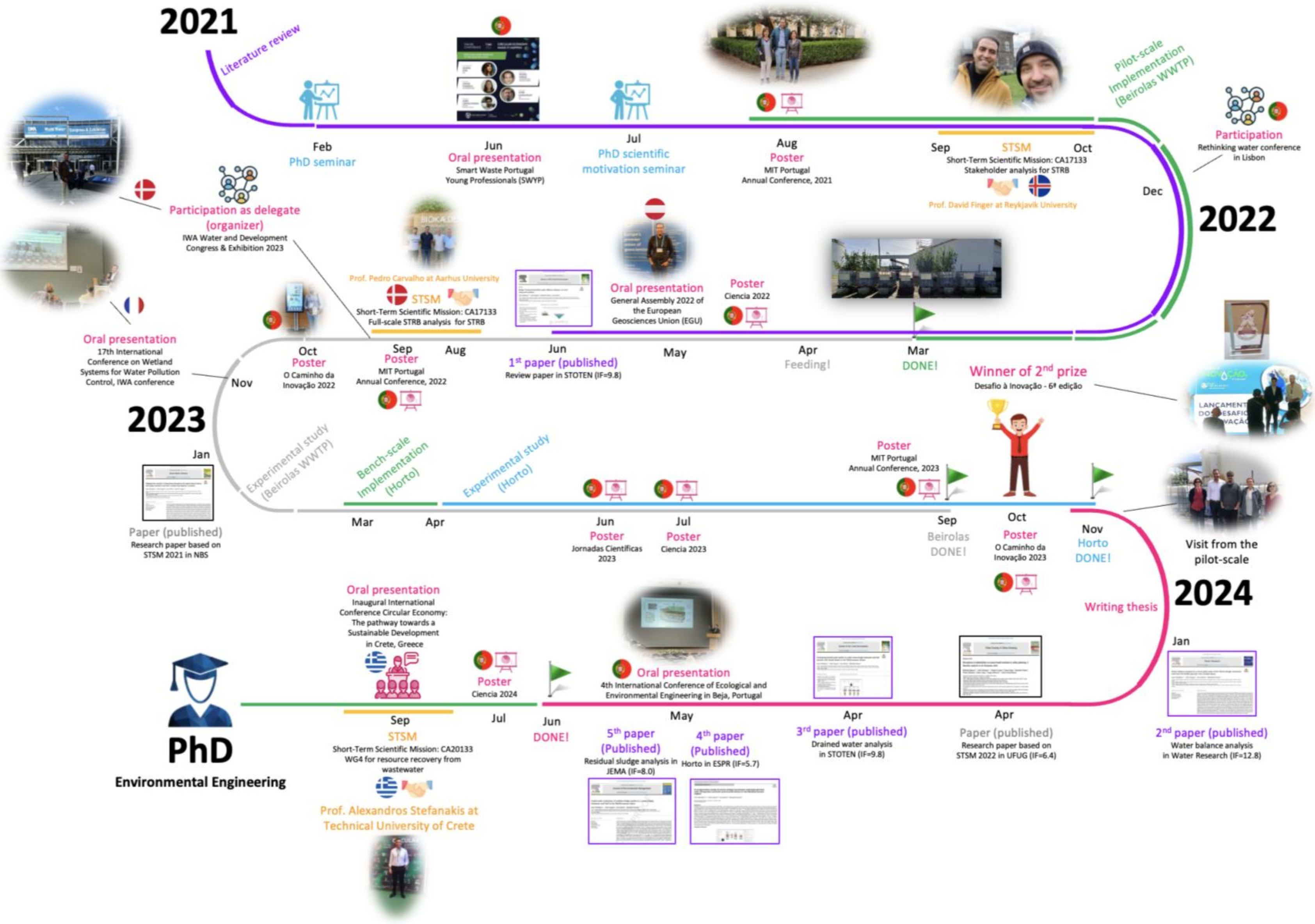
This study ventures into STRB by exploring the integration of earthworms into the sludge treatment reed bed system planted with *Arundo donax* or *Phragmites australis*, with a specific focus on determining the sludge loading rate tailored to the temperate climate conditions of the Mediterranean region.

Preface

This thesis represents the culmination of four years of intensive research and study in the field of environmental engineering. Throughout this journey, I had the privilege of collaborating with esteemed professors and researchers, whose insights and guidance were invaluable. During my study, several significant milestones and tasks were achieved, including:

- Publications**:** Authored seven publications, with five papers resulting directly from my PhD research and two stemming from collaborations within COST Action colleagues (CA17133).
- Scientific missions**:** Completed three short-term scientific missions (STSM) in Iceland, Denmark, and Greece, working with leading experts in the field.
- Presentations**:** Delivered six oral presentations at prestigious international conferences in Austria, France, Portugal, Greece, and Germany along with numerous national and international conference participations, including the IWA conference in Denmark.
- Poster presentations**:** Presented over nine posters at various conferences, sharing research findings, and engaging with the scientific community.
- Pilot and bench-scale Studies**:** Conducted a pilot-scale study at Beirolas WWTP over 1.5 years and a bench-scale study at ISA for eight months, contributing to the PhD objectives.
- Awards**:** Received a prize for innovation, recognized for our innovative projects in a competitive setting.
- Additional activities**:** Engaged in extensive networking and laid the groundwork for future collaborations, further expanding the impact and reach of my research and career.

A graphical representation of the PhD road map journey can be found below:



2021

Literature review

Feb
PhD seminar

Jun
Oral presentation
Smart Waste Portugal
Young Professionals (SWYP)

Jul
PhD scientific
motivation seminar

Aug
Poster
MIT Portugal
Annual Conference, 2021

Sep
STSM
Oct
Short-Term Scientific Mission: CA17133
Stakeholder analysis for STRB
Prof. David Finger at Reykjavik University

Pilot-scale
Implementation
(Beirolas WWTP)

Participation
Rethinking water conference
in Lisbon

Dec

2022

Mar
DONE!

Apr
Feeding!

May

Poster
Ciencia 2022

Oral presentation
General Assembly 2022 of
the European
Geosciences Union (EGU)

Jun
1st paper (published)
Review paper in STOTEN (IF=9.8)

Aug

Prof. Pedro Carvalho at Aarhus University
STSM
Short-Term Scientific Mission: CA17133
Full-scale STRB analysis for STRB

Sep
Poster
MIT Portugal
Annual Conference, 2022

Oct
Poster
O Caminho da
Inovação 2022

Nov

Oral presentation
17th International
Conference on Wetland
Systems for Water Pollution
Control, IWA conference

Participation as delegate
(organizer)
IWA Water and Development
Congress & Exhibition 2023

2023

Jan

Paper (published)
Research paper based on
STSM 2021 in NBS

Experimental study
(Beirolas WWTP)

Bench-scale
Implementation
(Horto)

Mar

Apr

Experimental study
(Horto)

Jun
Poster
Jornadas Científicas
2023

Jul
Poster
Ciencia 2023

Poster
MIT Portugal
Annual Conference, 2023

Sep
Beirolas
DONE!

Winner of 2nd prize
Desafio à Inovação - 6ª edição

Oct
Poster
O Caminho da
Inovação 2023

Nov
Horto
DONE!

Visit from the
pilot-scale

Writing thesis

2024

Jan

2nd paper (published)
Water balance analysis
in Water Research (IF=12.8)

Apr
Paper (published)
Research paper based on
STSM 2022 in UFUG (IF=6.4)

Apr
3rd paper (published)
Drained water analysis
in STOTEN (IF=9.8)

May

4th paper
(Published)
Horto in ESPR (IF=5.7)

5th paper
(Published)
Residual sludge analysis in
JEMA (IF=8.0)

Oral presentation
4th International Conference of Ecological and
Environmental Engineering in Beja, Portugal

Jun
DONE!

Jul
Poster
Ciencia 2024

Sep
STSM
Short-Term Scientific Mission: CA20133
WG4 for resource recovery from
wastewater
Prof. Alexandros Stefanakis at
Technical University of Crete

Oral presentation
Inaugural International
Conference Circular Economy:
The pathway towards a
Sustainable Development
in Crete, Greece

PhD
Environmental Engineering

Chapter 1. Introduction

1.1. Sewage sludge: challenges and management

Wastewater management, an indispensable component of urban infrastructure, exists at the nexus of critical environmental concerns and the challenges posed by growing populations in contemporary cities. Wastewater management is not only important to prevent contagious diseases but also it is important in the context of the environment to protect ecosystems. Its significance is emphasized in Sustainable Development Goal 6, which promotes access to safe drinking water, sanitation, and hygiene (Hák et al., 2016). The treatment and disposal of sewage sludge encounter multifaceted obstacles that necessitate innovative and forward-thinking solutions to ensure sustainable urban development (Cieślik et al., 2015). Central to these challenges is the bulk volume of sewage sludge generated daily by urban centers worldwide (Ayhan Demirbas & Alalayah, 2017). The rapid expansion of urban populations correlates directly with an upsurge in sewage production, placing immense pressure on traditional treatment methodologies. Effectively managing this escalating volume poses a wide range of logistical challenges, ranging from the constraints of storage facilities to the complexities associated with transportation and responsible disposal (Mannina, Barbara, Cosenza, & Ni, 2023).

Moreover, sewage sludge management has drawn attention regarding water reuse and resource recovery. The composition of sewage sludge itself presents a potential challenge due to its high variability, which can complicate the treatment process (Mannina et al., 2023). Additionally, it often contains harmful contaminants, including heavy metals and pathogens, requiring specialized handling and treatment to ensure environmental safety. On the other hand, loaded with valuable nutrients and organic matter, it embodies an untapped resource waiting to be recovered and reused. Yet, intertwined within these sources are contaminants that demand careful treatment for conscious disposal or reutilization (Hušek et al., 2022). Environmental concerns loom large within the domain of sewage sludge management. Inadequate treatment processes carry the high risk of contaminating vital soil and water bodies, threatening delicate ecosystems, and public health. Furthermore, certain energy-intensive treatment methods like mechanical systems, e.g., centrifugation and filter belt presses continue carbon emissions, intensifying the ecological footprint of urban areas and exacerbating climate-related challenges (Mannina et al., 2023). The majority of fertilizing resources are within the solid phase of wastewater treatment plants (WWTP), typically managed in a linear strategy. Traditional sludge management relies heavily on mechanical systems, contributing to the initial goal of wastewater management, sanitation. However, these mechanical techniques are effective for sanitation, but limited in their responsiveness and sustainability for resource recovery and ecosystem protection.

As demands evolve, there is a growing need to explore and implement alternative methods that align with the dual objectives of safeguarding ecosystems and efficiently recovering valuable resources. Incineration and landfilling strategies, while addressing volume concerns to an extent, often raise significant environmental and economic concerns. Concurrently, energy-intensive treatment plants, although effective, strain finite resources and perpetuate reliance on non-renewable energy sources, further exacerbating sustainability issues (Ayhan Demirbas & Alalayah, 2017). Adapting alternatives to climate change and addressing recurrent challenges in urban environments is imperative. Urban development and population growth have heightened the demand for sewer networks and sanitation, leading to increased costs associated with conventional systems. The utilization of mechanical systems becomes economically infeasible in many developing countries due to budget constraints and the unavailability of advanced technologies. The operation and maintenance costs emerge as significant obstacles in the efficient functioning of sludge dewatering system facilities (Cieślik et al., 2015). In the face of recurrent global crises such as energy shortages and climate change, reliance on conventional methods has become unresponsive to evolving needs.

Furthermore, these conventional systems lack sustainability, emphasizing the urgency to explore and adopt alternative approaches that align with the imperatives of resource efficiency and environmental resilience (Zorpas et al., 2021). The reliance on chemicals and additives for the functioning of these techniques introduces a vulnerability, as the relative costs fluctuate due to varying global availability. Consequently, these systems are no longer viable in the present landscape. Given the evolving challenges and demands, there is a pressing need to shift towards alternative methods that are not only cost-effective but also sustainable. Nature-based solutions (NBS) emerge as a promising approach to address these challenges, offering a more resilient and environmentally friendly path forward (Gholipour et al., 2023). NBS are solutions that are inspired and supported by nature, which are cost-effective, simultaneously provide environmental, social, and economic benefits and help build resilience (Frantzeskaki, 2019).

The advent of alternative technologies, exemplified by sludge treatment reed bed (STRB), signifies a paradigm shift towards nature-based, eco-friendly, and resource-efficient approaches (Gholipour et al., 2022). STRB symbolizes the potential of harnessing natural processes to treat sewage sludge, heralding a promising paradigm from conventional methodologies (Brix, 2017). Innovative approaches like STRB represent not just a divergence from conventional methods but also a signal illuminating the potential pathways to effectively address urban sewage challenges. They align with the imperatives of sustainability, resource efficiency, and environmental stewardship, offering promising ways for the future of urban sludge management.

1.2. Reed beds: advancements in sludge treatment

STRB use for sludge management has shown promising outcomes across the globe in various climate conditions especially in temperate climate although there is limited research in arid and polar climates (Gholipour et al., 2022). STRB is one of the applications of treatment wetland (TW) technology which are engineered systems that replicate and enhance the physical, biological, and chemical treatment processes occurring in natural wetlands to remove fine sediments, nutrients, and other pollutants (e.g., pesticides, heavy metals) (Sengupta & Dalwani, 2008; Uggetti et al., 2010). STRB efficiency and their functionality are climate dependent (Gholipour et al., 2022; Koottatep et al., n.d.; Mennerich et al., 2017; Nielsen & Stefanakis, 2020) of which the use of native plants can be an efficient technique and it also enhance the overall efficiency and resiliency of the process (Brix, 2017). Previous studies can be road map in the design of the systems while the need for case studies in different climates still exists contributing to the optimization of system efficiency. In STRB system, sludge loading rate (SLR) is the key factor for system design and performance (Nielsen, 2003). Selecting a specific SLR according to any climate is challenging and can be achieved through onsite studies and seasonal variation analysis (Chen et al., 2016).

In this doctoral study, the focus is to analyze STRB technology in Portugal climate, namely, temperate Mediterranean climate. Despite existing studies on TW technology for wastewater treatment in similar climates, the specific application of STRB system remains unexplored in Portugal until now (Calheiros et al., 2007; Carvalho et al., 2013; Dordio et al., 2007). A review (chapter 2) on STRB technology and variations through 30 years of full and pilot-scale studies was conducted to collect experience and findings (Gholipour et al., 2022). In the Mediterranean region, STRB studies in Greece (Stefanakis & Tsihrintzis, 2012a), Italy (Bianchi et al., 2011) and Spain (Uggetti et al., 2012) showed that engineered wetland systems can be applied in temperate climate areas like Portugal. In addition, these studies have indicated that STRB systems can be operated with higher SLR compared to higher latitudes such as the northern Europe (Kołecka et al., 2018; Kolecka & Obarska-Pempkowiak, 2013; Nielsen, 2007, 2011, 2023; Troesch et al., 2009). In the northern of European countries like Denmark, SLR can be around 50 kg dry solids (DS) per m^2.$year^1$ while in the Mediterranean region, SLR can vary between 50 to 70 kg.DS.m^{-2}.$year^{-1}$ (Gholipour et al., 2022). Denmark is one of the most popular countries for many full-scale STRB systems and through the past decades, many WWTP used STRB for sewage sludge management in which solid contents are being accumulated up to 15 years for further treatment and utilization. In addition to the application of STRB for urban settlements in Denmark (Nielsen, 2003, 2007, 2011), STRB has been applied to treat combined sludge from WWTP and inflow from stormwater basins (SLR: 24.7 ~ 40.9 kg.DS.m^{-2}.$year^{-1}$), a system that has worked in Kallerup WWTP for more than 25 years (Nielsen, 2023). Past STRB studies in the Mediterranean region showed that STRB can also be utilized in Portugal although design SLR ought to be estimated through on-site pilot studies (Stefanakis & Tsihrintzis, 2011; Uggetti et al., 2012).

Literature review studies have also explored STRB technology through the past decades (Bui et al., 2019; Elbaz et al., 2020; Maeseneer, 1997; Pandey & Jenssen, 2015; Uggetti et al., 2010), even specific reviews for Africa (Goussanou et al., 2023), Palestine (Nassar et al., 2009) and Poland (Zwara & Obarska-Pempkowiak, 2000) of which the most recent is the review by Gholipour et al (2022).

Through 2022 and 2024, there have been a few research studies. A recent study in hot and arid climate, Oman, showed the application of STRB planted with *Phragmites australis* can be designed up to SLR of 125 kg.DS.m^{-2}.$year^{-1}$ while it was suggested that SLR should be kept under 75 kg.DS.m^{-2}.$year^{-1}$ (Al-Rashdi et al., 2024) indicating that SLR can be increased in warmer climates. Recently, apart from common applications of STRB for sludge management like septage (Huong et al., 2023), STRB has been used for the dewatering of urban lake sediments in Vietnam (Anh et al., 2022). In Anh's study, *Cyperus alternifolius* with SLR of 180 kg.DS.m^{-2}.year^{-1} and a resting period of 166 days resulted in DS of 63.8 % and VS/DS of 6.5 %. Another emerging application was addressed by Gonzalez-Flo et al. (2023) in Barcelona, Spain that used STRB planted with *Phragmites australis* (28 kg.DS.m^{-2}.year^{-1}) to dewater and stabilize digested microalgal biomass from domestic and agriculture treatment systems achieving a final DS of 12.7 %. Application of STRB for industrial sludge was also novel and reported in the previous studies (Nielsen & Stefanakis, 2020). One of the challenges in the by-product of STRB system is heavy metal (HM) in the sludge residue which remains on the top of reed bed system (Boruszko, 2018; Caicedo et al., 2015; Z. Chen & Hu, 2019; Stefanakis & Tsihrintzis, 2012b). HM distribution and removal pathways have also been studied (Ma et al., 2023). The study of Ma et al. (2023) showed that Zn and Cu accumulated faster than other metal elements in the sludge residue and were removed by 90 and 64 %, while Cd, Cr, Ni, and Pb accumulated slowly and presented a slight reduction over time. In addition, the authors identified plants and substrates as the most important mechanisms in HM removal (Ma et al., 2023).

During the last decade, STRB technology has faced multiple variations in the system design, aiming improvement in general performance and different applications, of which a couple of studies introduced earthworms into STRB system (W-STRB) (Chen et al., 2016; Chen & Hu, 2019; Gutiérrez-López et al., 2016; Hu, Lv, et al., 2020; Hu, Zuo, et al., 2020; Hu & Chen, 2018; Pezzotti et al., 2021; Saeed et al., 2022; Zhong et al., 2021). These studies, which were mostly conducted in China, revealed that incorporating earthworms into the STRB system improved its overall performance, even if sludge composition, climate conditions, and operational parameters vary. In the past, W-STRB studies were conducted under controlled conditions, which may not fully represent real-time W-STRB scale dynamics. W-STRB studies were conducted at preliminary bench and pilot scales, assessing limited dimensions and short-term results, whereas STRB technology is typically operated for 10 years. Consequently, there are still significant gaps and uncertainties in W-STRB research that need to be addressed. Additionally, some research work involved coupling the W-STRB system with microbial fuel cells (MFC) to generate power from the organic matter present in sludge (Saeed et al., 2022; Zhong et al., 2021). STRB was also combined with MFC without worm inclusion, called electro-wetland (STEW) (Wang et al., 2021, 2023). The yielded voltage in these studies ranged between 0.7 and 0.9 V and the power density was from 0.229 to 0.498 W.m^{-2}; however, this application is still in its early stages.

One of the critical factors influencing the performance of the STRB is the choice of plant species, as different climate conditions offer a variety of native plants that are well-suited for the application of STRB technology. Selecting appropriate plant species is essential in optimizing the effectiveness of the STRB system (Brix, 2017). The presence of common reed such as *Phragmites australis* as well as *Arundo donax* (giant reed), are noticeable in Portugal. However, considering *Arundo donax* as an alternative presents a challenge, as this species has not been utilized in previous sewage sludge dewatering experiments. The current thesis aims to assess the performance of *Arundo donax* in a pilot study conducted at Beirolas WWTP. The response of *Arundo donax* in combination with earthworms is also unexplored. Earthworms are sensitive to dryness and bioavailability of organic matter to feed and the dynamic of feeding materials (like sludge load) were additional challenges found within this doctoral thesis. Through the past studies, it is still unclear whether earthworms could survive in STRB system planted with *Arundo donax* or if and how they contribute to the dewatering process. Another complexity arises from the variability in the produced sludge quality in different WWTP like in this study at Beirolas WWTP. The sewage sludge generated at Beirolas WWTP stems from the primary and secondary stages of treatment called “mixed sewage sludge (MS)” in the thesis. This introduces a potential for variability in the sludge characteristics compared to prior studies. The annual dataset from Beirolas wastewater laboratory revealed that the DS content in the mixed sewage sludge averages at 25 mg.L^{-1}, with volatile solid content (VS) constituting 80 % of the DS (VS/DS).

1.3. Sustainable urban ecosystems: STRBs' role in reshaping common urban systems to smart cities

Conventionally, STRB is an engineered system that uses reed beds to treat sewage sludge; however, in smart cities, these systems play a crucial role in sustainable urban planning by addressing multiple challenges of urban development:

- **Sewage sludge management and treatment:** STRBs facilitate the treatment of sewage sludge. As sewage sludge passes through the reed beds, reeds and the microbial community within the bed break down organic matter and filter out pollutants and solids. The solid particles are entrapped on the top of STRB and the liquid fraction percolates along the filtration materials dewatering sewage sludge and treating the drained water before it is released back into the environment. This contributes to managing sewage sludge and maintaining cleaner waterways in the city. On the other hand, dewatered sewage sludge can be recovered for resource reutilization in land applications (Raheem et al., 2018). Many of agricultural lands have been degraded and they lack essential nutrients for plant growth and crop production (Borrelli et al., 2017); therefore, recovered resources from STRB system can replace chemical fertilizers, directly reducing associated costs, and improving crop yields, particularly in urban farming (Kacprzak et al., 2017). There are lands eroded due to urbanization and industrial activities within urban areas threatening urban green infrastructures and contributing to global warming and climate change (Borrelli et al., 2017). Resources can also be reused in landscaping and urban forestry in smart cities (Krajter Ostoić & Konijnendijk van den Bosch, 2015). To achieve a circular economy, fertilization plays an important role in an urban environment in which landscaping is the key to mitigate various urban challenges such as air pollution, noise pollution, water treatment and urban heat island (UHI) (Hák et al., 2016). In the context of a smart city plan, STRB serve as a vital link in establishing a circular system that contributes to urban planning, enhances well-being, and preserves ecosystems.
- **Environmental conservation:** By using natural processes to manage sewage sludge, STRB reduces the need for energy-intensive treatment methods including centrifugation, thermal drying, incineration, chemical conditioning, mechanical presses, vacuum filtration, and electro-dewatering (Hoffmann et al., 2010). This promotes environmental conservation by minimizing energy consumption and greenhouse gas emissions associated with conventional treatment processes. STRB was assessed for global warming potential (GWP) indicating that it contributed negatively while energy-intensive methods showed a positive indication (Uggetti et al., 2011). The reliance on natural processes not only reduces the carbon footprint

associated with conventional treatment but also helps preserve biodiversity by maintaining a more harmonious ecological balance (Vasiliev & Greenwood, 2022; L. Xie & Bulkeley, 2020). By fostering natural treatment processes, STRB contributes to the protection of aquatic ecosystems like rivers and lakes, particularly man-made NBS intervention (Krauze & Wagner, 2019). Unlike some energy-intensive treatment methods that may involve harsh chemicals, the use of STRB minimizes the release of harmful substances into water bodies, preventing potential ecological disruptions and safeguarding the health of aquatic organisms. Furthermore, the reduced dependence on energy-intensive practices translates into lower overall resource consumption, aligning with broader sustainability goals. This holistic approach to environmental conservation underscores the importance of adopting eco-friendly solutions in sewage sludge management, emphasizing the interconnectedness of wastewater treatment practices with broader ecological well-being.

- **Urban agriculture:** Reed beds can also serve as a platform for growing food. In the context of smart cities, these spaces can be utilized for urban agriculture. Soilless farming is one of the systems that has been promoted in urban environment due to its simplicity and higher yields in a small area; however, it is based on nutrient cycle and availability (Gumisiriza et al., 2022). In these systems such as aquaponics, nutrients are frequently added into the cycle to maintain system performance, fish viability and plant growth (Boxman et al., 2018). Soil less systems can use recovered resources from sewage sludge to avoid chemical fertilizers (Wielemaker et al., 2018), in case sludge residue meets regulatory standards or it is treated properly. However, one way to increase nutrient availability is to extract and precipitate nutrients from sewage sludge which can be alternatively reused in soilless systems of farming (Shiba & Ntuli, 2017). Certain plants can thrive in the nutrient-rich environment of treated sludge, potentially allowing for food production within the city although treated sludge might be mixed with soil before reuse as fertilizer. This contributes to local food resilience and reduces the need for transporting produce over long distances.
- **Water conservation:** water scarcity has exacerbated through the past decade and alternative water resources play a crucial role in mitigating water shortage consequences. In sewage sludge, 99 % of the volume is water content and less than

1 % is solid fraction (Nielsen, 2003), this indicates the importance of treating the water content of sludge to protect public health and ecosystems as well as to bring back treated water for reutilization (Hu et al., 2020). Treated drained water from STRB can potentially be reused for non-potable purposes like irrigation in parks, gardens, or other urban green spaces. In a circular system for smart cities, onsite sewage sludge dewatering through STRB can provide an accessible water resource in place minimizing the cost of water transmission (Gholipour et al., 2023). This promotes water conservation by reducing reliance on freshwater sources for non-drinking purposes, helping create a more sustainable water cycle within the city.

- **Energy generation:** While the primary focus of STRB is not energy production, some systems harness the byproducts of the treatment process (like methane gas) for energy generation (Grosser & Neczaj, 2016). Nevertheless, these facilities require improvements in terms of cost-benefit analysis. This biogas can be captured and used as a renewable energy source for various purposes within the city, contributing to energy conservation and reducing dependency on non-renewable sources. E-STRB or STEW for power generation through MFC technology can also directly yield energy (Wang et al., 2021, 2023; Zhong et al., 2021). Energy is an unreplaceable element of sustainability in smart cities and alternative sources of energy contribute to achieving water-energy-food (WEF) nexus (Lahlou et al., 2023). In addition, a key advantage of STRB technology in the energy sector is its low energy dependency, which distinguishes it from conventional technologies like filter belt presses and centrifugation. This reduces energy requirement makes STRB a more sustainable and cost-effective option for sludge management.

In summary, STRB systems use natural processes to treat sewage sludge, making them eco-friendly and efficient. They turn waste into valuable resources, reduce pollution, and lower the carbon footprint of sewage treatment. These systems prevent contaminants from reaching soil and water, protect ecosystems, and produce non-potable water, reducing freshwater demand. Some also support urban agriculture by using nutrient-rich sludge, promoting local food production and resilience. STRB is not merely sewage treatment systems; it is a transformative element in the design of smart cities. Its contributions spans sustainability, resilience, resource efficiency, and environmental conservation, addressing fundamental aspects of urban development and positioning cities on a trajectory towards a more sustainable and resilient future.

1.4. Roadmap to innovation: objectives and structure of the thesis

The main objective of this study was to assess the feasibility of applying sludge treatment reed bed (STRB) technology in Portugal (temperate climate) as an alternative sustainable solution to conventional technologies. It was aimed to improve STRB dewatering efficiency (DE) by the inclusion of earthworms (*Eisenia fetida*) into the system planted with *Arundo donax*. In order to achieve the objective, two studies were developed, one in Beirolas WWTP in Lisbon (pilot-scale) and the other in Horto greenhouse in Instituto Superior de Agronomia (ISA) at University of Lisbon (bench scale).

Hence, the specific objectives of the thesis are to:

I. Investigate the contributions of earthworms within STRB, particularly their impact on water balance through dewatering mechanisms and stabilization processes.

II. Examine the seasonal variations' effects on STRB performance, focusing on how earthworm activities fluctuate and influence system dynamics within a temperate climate.

III. Monitor the quality of drained water from STRB to ascertain its viability for potential reuse purposes, aligning with sustainable water management principles.

IV. Analyze the sludge residue derived from W-STRB in terms of heavy metals and nutrients as well as DS and VS contents, emphasizing its potential as a nutrient-rich resource for applications within smart cities.

V. Investigate the feasibility and performance of introducing *Arundo donax* as an alternative plant species, exploring its suitability and efficacy.

VI. Compare the performance of *Arundo donax* and *Phragmites Australis* regarding their impact and efficacy within W- STRB.

VII. Develop a cost estimation between current conventional sludge management methods like centrifugation and the earthworm assisted STRB (W-STRB) alternative, aiming to evaluate the cost of each scenario for small-scale communities.

It should be mentioned that Beirolas experiment was conducted in cooperation with Águas do Tejo Atlântico, S.A (AdTA). This doctoral project was funded and supported by

MIT Portugal Program (MPP) and Fundação para a Ciència e a Tecnologia (FCT) under sustainable cities theme of MPP scholarship contest in 2020 (SFRH/BD/151361/2021). The thesis is organized in eight main chapters with the aim to address to the proposed objectives, having the following structure:

Chapter 1 presents an introduction in sewage sludge management challenges and the global need for improved approaches. Focusing on reed bed technology, it explores the evolution and effectiveness of STRB in treating sewage sludge. This chapter explores advancements in STRB methodologies delving into the principles, innovations, and state-of-the-art practices. By showcasing the potential and effectiveness of these systems, it underscores their significance as a nature-based and eco-friendly alternative to conventional treatment methods. Highlighting their eco-friendly nature, the chapter emphasizes STRBs' pivotal role in smart cities, reshaping urban landscapes by promoting sustainability, resilience, and resource efficiency across water management, energy conservation, and food production. This thesis objectives and structure are also provided.

Chapter 2 presents a published review in Science of the Total Environment (IF: 9.8) entitled "Sludge Treatment Reed Bed under different climates: A review using meta-analysis," covering studies since 1990 on a global scale. With 73 original papers sourced via web searches, this meta-analysis tracks the technology's evolution over decades, analyzing operational factors across various climates: temperate, Mediterranean, tropical, arid, and polar. The study delves into dewatering efficiency, correlation analyses, plant species used, media characteristics, and compositions. It highlights advancements in heavy metal, nutrient, nitrogen components, and leachate water quality. This inclusive analysis aims to fill the gap in existing reviews, offering a holistic understanding of STRB technology from multiple angles. It serves as a valuable resource for engineers, decision-makers, and users of current and future STRB facilities, addressing the lack of comprehensive systematic reviews in this field. This meta-analysis not only provides a historical perspective on STRB technology but also meticulously details every aspect of previous findings, offering a nuanced understanding for researchers and readers.

Chapter 3 presents a published paper in Water Research (IF: 12.8) entitled "Water balance analysis in a novel pilot-scale of the Worm-Sludge Treatment Reed Bed (W-STRB) planted with *Arundo donax*". This chapter reports pilot trial conducted experimentally in Beirolas WWTP in Lisbon, Portugal, from 2021 to 2023. The primary objective of the study was to explore the feasibility of incorporating earthworms into conventional sludge treatment reed beds (W-STRB) and to investigate the effects of earthworms (*Eisenia fetida*) and *Arundo donax* on water balance and dewatering efficiency in a temperate climate. W-STRB has been partially studied in China with very limited details, in a tropical climate while there is not any data for other climates. The aim was to evaluate the effectiveness of W-STRB as an alternative approach for both dewatering and treating sewage sludge, including a water balance. This research has significant implications for improving sewage sludge management practices to offer an alternative solution which is cost-effective. The findings of this research contribute to our understanding of W-STRB for the dewatering and treatment of sewage sludge, especially in temperate climates. Notably, the positive influence of earthworms in reducing residual sludge volume and the observed enhancement in evapotranspiration rate in the W-STRB underscore the potential of this approach for sustainable wastewater management. Our motivation for conducting a water balance analysis stemmed from the limited information available in the literature regarding W-STRB, as well as the absence of studies specifically examining the application of *Arundo donax* and the synergistic use of earthworms and plants. Additionally, there is a lack of comparative research on W-STRB in temperate climates, and unlike our study, which is in real condition, most previous studies have been conducted under controlled conditions.

Chapter 4 presents a published paper in Science of the Total Environment (IF: 9.8) entitled "Evaluating drained water quality in a pilot worm-sludge treatment reed bed planted with *Arundo donax* in the Mediterranean climate". This chapter presents the other part of the pilot trial on drained water quality conducted in Beirolas WWTP. Past studies inadequately covered the drained water outcomes from a W-STRB system, especially in other climates beyond the limited tropical region. A few studies can be found on W-STRB such as investigations in China, with scarce details and solely within tropical conditions.

This research aimed to fill these gaps by evaluating W-STRB as an alternative method for both dewatering and treating sewage sludge, with a keen focus on the quality of the drained water which was scarcely explored in existing literature. The significance of this investigation lies in its potential to substantially enhance drained water quality in sewage sludge management practices, providing an economically feasible alternative solution. The findings contribute notably to understanding the efficacy of W-STRB, particularly in treating drained water during sewage sludge dewatering, especially in temperate climates. Crucially, our study underscores the positive impact of worms in nitrogen and phosphorous removal, coupled with reductions in chemical oxygen demand and total suspended solids, highlighting the potential of this approach for sustainable wastewater management. Our motivation stemmed from the dearth of comprehensive literature on W-STRB, the lack of specific research on *Arundo donax* application.

Chapter 5 presents a published paper in Journal of Environmental Management (IF: 8.7) entitled " A pilot-scale evaluation of residual sludge quality in a worm-sludge treatment reed bed”. This study reports the other key part of the pilot rial on residual sludge quality in Beirolas WWTP. Previous studies have inadequately covered the residual sludge quality outcomes from W-STRB, especially in climates outside the tropics, which suggests potential for reuse in land applications. This research aimed to fill these gaps by focusing on residual sludge quality and cost estimation. The study's significance lies in its potential to improve residual sludge quality in sewage sludge management, offering an alternative solution. The results highlight the efficacy of W-STRB in temperate climates, emphasizing the positive impact of earthworms in heavy metal removal and sludge volume reduction.

Chapter 6 presents a published paper in Environmental Science and Pollution Research (IF: 5.8) entitled "A comparative study of worm-sludge treatment reed bed planted with *Phragmites australis* and *Arundo donax* in the Mediterranean region”. This bench-scale study was developed in Horto greenhouse, Instituto Superior de Agronomia (ISA) at University of Lisbon. It investigated the integration of earthworms into STRB using *Arundo donax* and *Phragmites australis* in a temperate climate. It extended previous work on dewatering efficiency in Beirolas WWTP. The study increased the sludge loading rate

(SLR) to 50, 60, and 70 kg.DS.m-^{2}.year-^{1} and assessed the impact on plants, earthworms, and dewatering efficiency._It fills gaps in the literature by examining W-STRB systems under various SLRs, focusing on dewatering efficiency, drained water quality, residual sludge quality, and the roles of plants and earthworms. The findings showed that worms enhance evapotranspiration, plant biomass production, and drained water quality, while significantly reducing heavy metals in residual sludge.

Chapter 7 provides a general discussion of the results obtained throughout the study.

Chapter 8 summarizes the main conclusions and future research developments.

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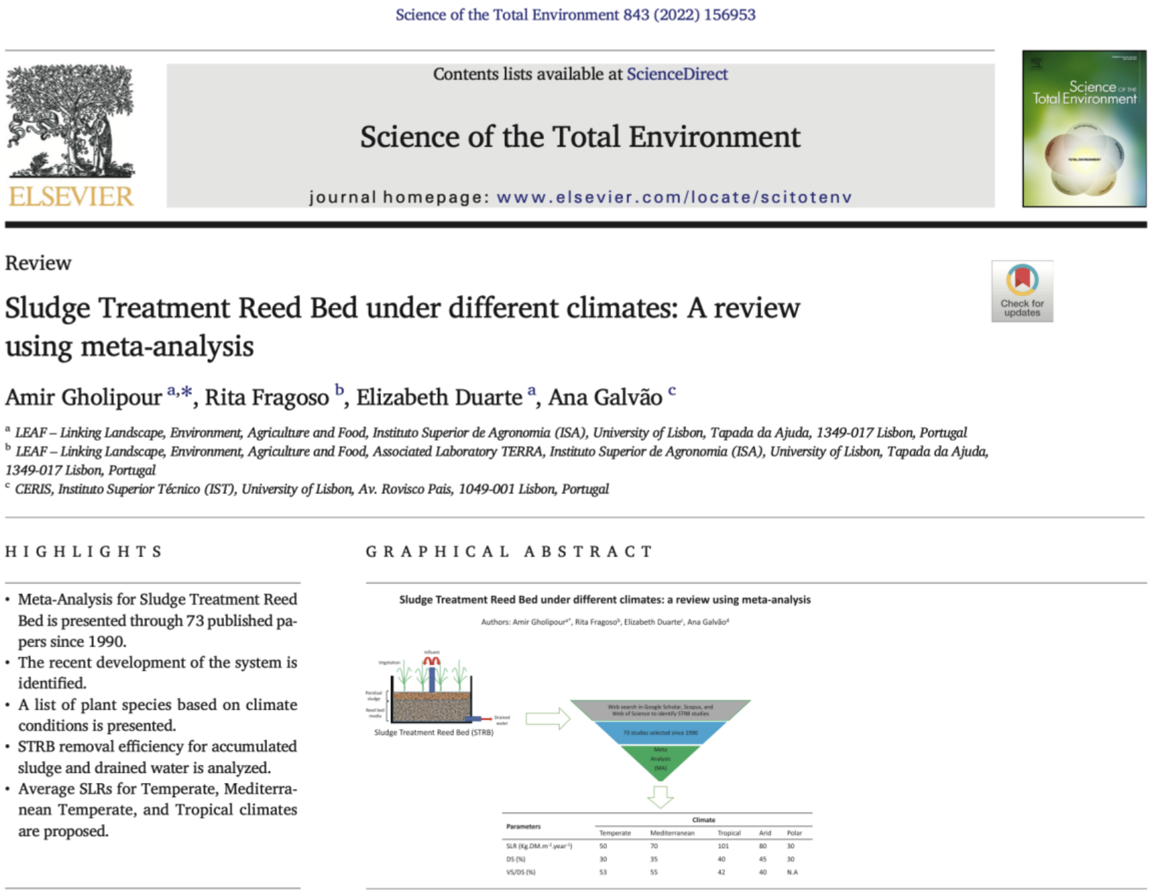
Chapter 2. Sludge Treatment Reed Bed under different climates: a review using meta-analysis



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Review

Sludge Treatment Reed Bed under different climates: A review using meta-analysis

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HIGHLIGHTS

- Meta-Analysis for Sludge Treatment Reed Bed is presented through 73 published papers since 1990.
- The recent development of the system is identified.
- A list of plant species based on climate conditions is presented.
- STRB removal efficiency for accumulated sludge and drained water is analyzed.
- Average SLRs for Temperate, Mediterranean Temperate, and Tropical climates are proposed.

GRAPHICAL ABSTRACT

Sludge Treatment Reed Bed under different climates: a review using meta-analysis

Authors: Amir Gholipour[a*], Rita Fragoso[b], Elizabeth Duarte[c], Ana Galvão[d]

Parameters	Climate				
	Temperate	Mediterranean	Tropical	Arid	Polar
SLR (Kg.DM.m^{-2}.year^{-1})	50	70	101	80	30
DS (%)	30	35	40	45	30
VS/DS (%)	53	55	42	40	N.A

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2.1. Abstract

Sludge Treatment Reed Beds (STRBs) have been used worldwide over the past few decades. This review aims to overarchingly identify and appraise the currently available knowledge of STRB technology and discern climatic patterns through Meta-Analysis (MA). We systematically searched Google Scholar, Scopus, and Web of Science databases (up to Dec 2021) via a combination of keywords to identify English-language studies published in peer-reviewed journals. Of 142 potential articles, 73 studies met the present review objectives and inclusion criteria. Four STRB classifications including typical STRB, earthworm STRB, Sludge Treatment Electro Wetland (STEW), and earthworm STEW were found since 1990. The data and information on STRBs' configuration, operational parameters in terms of location, type of sewage sludge, study scale, Sludge Loading Rate (SLR), Dry Solid (DS), the proportion of Volatile Solid to DS (VS/DS), and their association with the feeding and resting modes were extracted from the selected articles. The analysis was focused on the interconnections between operational parameters and system efficiency for Temperate type 1 (low intensity of solar radiation), Temperate type 2 (high intensity of solar radiation), and Tropical climates. Based on MA, we found the average SLRs of 50, 70, and 101 Kg.DM.m^{-2}. year^{-1} for Temperate type 1, Temperate type 2, and Tropical climates respectively, and DS during the feeding of 33%, 35%, and 40%. A qualitative comparison of Arid and Polar climates was also performed given the reduced number of studies available in these climates. The volume of the sludge reduced was 60% higher and the height of accumulated sludge was annually 2 cm in the earthworm STRBs, and STEWs compared to typical STRBs, which was 6 cm annually in Tropical climates. Correlation analysis, media characterization, list of plant species, and the removal efficiency of STRBs in the residual sludge and leachate are mentioned as well.

Keywords: Sludge Treatment Reed Beds (STRBs); Meta-Analysis (MA); Sludge Loading Rate (SLR); Reed Bed; Sludge Dewatering; Review; Constructed Wetland

2.2. Introduction

In Wastewater Treatment Plants (WWTPs), solid-phase treatment is of paramount importance due to the bulk volume of sludge produced (between 1 to 10% of influent volume), which needs to be addressed (Nielsen, 2003). Sludge extraction and dewatering are currently conducted in most WWTP globally to achieve safe disposal to the environment (Yoshida et al., 2013; Cieślik et al., 2015). Hazardous materials such as toxic components are the main challenges causing threats to the environment and putting human health at risk (Cusidó and Cremades, 2012). Owing to toxic components, sewage sludge has the potential to degrade soil quality, contaminate surface and groundwater resources, and aquifers, threaten aquatic habitats and ecosystem of organisms put biodiversity at risk, and cause diseases outbreak (Kacprzak et al., 2017). On the other hand, the sewage sludge or "bio-solid" is an invaluable resource containing nutrients and beneficial bacteria (gram-positive bacteria) as well as gases, e.g., methane (Epstein, 2000).

A wide range of mechanical solutions for sewage sludge dewatering has already been applied all around the world including belt filter presses, centrifuges, and chamber filter presses among others (Wang et al., 2010; Peeters et al., 2010; Almatin and Gholipour, 2019; Daee et al., 2019). Mechanical techniques typically involve high installation costs, high energy consumption, the need for specialized personnel, high operational and maintenance (O&M) costs, and poor dewatered sludge fertility. Moreover, they are infeasible to apply any locality and climate condition to provide environmental services (Brix, 2017). Nature-Based Solutions (NBS) are cost-effective technologies that can be considered as alternative methods for conventional techniques such as Sludge Treatment Reed Beds (STRBs) and drying beds (Uggetti et al., 2010). STRB technology is one of the applications of Constructed Wetlands (CWs) for wastewater treatment and management (Kadlec, 2000; Vymazal, 2010; Gholipour et al., 2020; Gholipour and Stefanakis, 2021).

STRBs can be easily applied to any scale and climate condition (Vymazal, 2011), and is a sustainable solution, which can benefit the environment in the long-term run. STRB system enriches aesthetically local landscape and provides habitats for a wide range of living organisms particularly migrant birds, contributing to biodiversity preservation and conservation (Lafortezza et al., 2018). STRB technology is an active scrubber in the reduction of air pollution and the mitigation of Greenhouse Gases (GHGs) in comparison with mechanical techniques (Liang et al., 2021). STRBs can also contribute to the alleviation of climate change effects and carbon sequestration by vegetation (Olsson et al., 2014).

To enhance STRB performance and develop the system design, several variations have been applied to the typical STRB design. STRB system has been used with the addition of earthworms (earthworm STRB) to enhance the performance in reed beds (Calderon-Vallejo et al., 2015; Chen et al., 2016; Hu and Chen., 2018; Hu et al., 2020). Energy harvesting through STRB is another application of STRB called Sludge Treatment Electro Wetland (STEW) by Wang et al. (2021) while the addition of earthworms together with STEW systems was tested called earthworm STEW (Zhong et al., 2021). Studies revealed that the highest power output (0.790 V of voltage and 0.229 Wm^{-2} of power density) of STEW can be achieved provided that an SLR of 125 kg $DS.m^{-2}year^{-1}$ is considered (Wang et al., 2021). In another similar study, a combined system of STEW and earthworm was tested which could yield a voltage of 0.832 V and a maximum power density of 94.98 Wm^{-2} on the 5th day (Zhong et al., 2021). In addition, STRB has been coupled with a system of active aeration, intensified STRB, to enhance the dewatering and stabilization mechanisms (Plestenjak et al., 2021).

STRBs have been effective in the removal of different contaminants compared to mechanical systems in terms of final dry and volatile solids obtained (Uggetti et al., 2010). The fate of trace pollutants such as Heavy Metals (HMs), Personal Care Products (PCPs), and pharmaceuticals were reported in previous studies (Nielsen., 2007, Stefanakis and Tsihrintzis, 2012b, Chen et al., 2009, Wang et al., 2018, Kołecka et al., 2019, Arroyo et al., 2018; Kowal et al., 2021). In another study, HMs accumulation in the residual sludge layer was found low and increased with sludge layer depth, and plant uptake was low and less than 16% as well (Stefanakis and Tsihrintzis, 2012a). PCPs such as fragrances HHCB, AHTN, OTNE, and Triclosan were degraded in an STRB system pilot study and reduced up to 20, 30, 70, and 70%, respectively (Chen et al., 2009). Also, antibiotics including roxithromycin, azithromycin, oxytetracycline, and pharmaceuticals (non-steroidal anti-inflammatory drugs - ibuprofen, paracetamol, flurbiprofen, naproxen, diclofenac, and its metabolites) decreased in the STRB system (Wang et al., 2018 and Kołecka et al., 2019). The study by Kołecka et al. (2019) showed that Ibuprofen and naproxen were completely removed, and diclofenac and its metabolites were found as chemicals more persistent to be removed. According to Wang et al. (2018), the reed and the media ventilation structure affect positively the removal of antibiotics. In a study of xenobiotic compounds of the PCPs, it was shown that the bactericide triclosan, fragrance OTNE and DEHP were reduced at least more than 30% of their original concentration in different layers of the accumulated sludge while the polycyclic musk compounds HHCB, AHTN, and the primary metabolite of HHCB, i.e., HHCB-lactone did not degrade in 13 months of the pilot study (Chen et al., 2009). A study by Wang et al, on two antibiotics including ciprofloxacin (CIP) and azithromycin (AZM) showed that STRB had a better removal efficiency of antibiotics compared to the drying bed system. Time was found to be the factor that affects most the concentration of CIP and AZM in the accumulated sludge and after three years of operation, more than half of the antibiotics were removed. A study of Antibiotic Resistance Genes (ARGs) targeted five ARGs such as sul1, sul2, tetC, tetA, and ermB together with intI1, and 16S rRNA showed that STRB was effective at removing more than 73% of all ARGs and intI1 and 16S rRNA were removed 73.5 and 78.6%, respectively.

Helsinge STRB plant in Denmark was effective in the removal of pathogenic microorganisms including *salmonella*, *enterococci,* and *Escherichia coli (E. coli*) in which *enterococci* and E. coli reductions were log 5 and log 6 to 7, respectively (Nielsen, 2007). The bacteria population and biodiversity play important roles in the treatment performance as well (Arroyo et al., 2018; Kowal et al., 2021). In the study of Kowal et al. (2021), 80% of the bacterial community was of the *Bacteroidetes*, *Proteobacteria* and *Firmicutes,* identified in the sequences read through 16S rRNA gene sequencing in domestic wastewater in Gniewino, Poland in an STRB system and a similar result was found in Zhong et al. study on earthworm STEW (2021). The biodiversity of the communities was also found diverse on the surface of the STRB system and on the bottom of a bed equipped with a passive aeration system. The most responsible and abundant organism is *Nitrospira* regulating nitrogen metabolism in the STRBs tested (Kowal et al., 2021). Arroyo et al. (2018) have investigated bacterial community composition, richness, diversity, and ordination in swine slurry fed over an STRB system via 16S rRNA gene high-throughput in which pH value was the crucial indicator of the community response mode.

As the result of the decomposition of organic matter, gases such as CO_2 and CH_4 emit from STRB into the atmosphere. The CO_2 flux of the Drying Bed (DB) was two times higher than that of STRBs and the Global Warming Potential (GWP) of CH_4 emissions in DB was less than that of STRBs. However, the GWP of DB and STRB systems was found to be much lower than values for mechanical dewatering techniques (Cui et al., 2015; Liang et al., 2021). Liang et al. found that annual CO_2-eq CH_4 during the loading period was higher than that during the resting period due to loads of sludge entering the system at once. The gases such as CO_2 generated from wastewater and sludge are considered climatic-neutral (IPCC, 2006).

Most previous STRB reviews defined STRB technology and the design aspects in a general view (narrative reviews) focusing on studies in Europe, as in Denmark and Poland, while other parts of the world were left uncovered (De Maeseneer, 1997; Zwara and Obarska-Pempkowiak, 2000; Nassar et al., 2009; Uggetti et al., 2010; Pandey and Jenssen, 2015). The recent reviews including the study of Bui et al. (2019) narrated a few studies of STRBs to draw a comparison while Elbaz et al. (2020) reviewed sludge filtration beds and Nielsen and Stefanakis (2020) reviewed the application of STRB for the industrial sector. Since STRB is an NBS mimicking natural processes, it is highly dependent on the climate condition, which was not considered thoroughly in the previous reviews. The present study systematically reviewed the variations of STRB technology in terms of design, operation, and dewatering performance through three decades of the published literature in Temperate, Tropical, Arid, and Polar climates classified via Köppen–Geiger climate classification (Beck et al, 2018). A Meta-Analysis (MA) of the extracted data on STRB technology was conducted, which as far as the authors know, is performed for the first time in the literature databases of STRB. Statistical comparisons between different climates and correlation analysis were performed between design and performance factors. Ranges of operating conditions for Sludge Loading Rate (SLR), Dry Solids (DS), and Volatile Solids (VS) were obtained. Feeding and resting modes of STRB, the volume of the sludge accumulated, and the volume reduced will be presented as well. Thus, the result of the MA can be used for the design and operation of STRB systems under each climate. Analysis of the reed bed media compositions, including filtration materials and the list of plant species used, are also documented in this review. A comparison of dewatering efficiencies between typical STRB, earthworm STRB, STEW, earthworm STEW, and intensified STRB are reported for the first time as well.

2.3. Materials and methods

2.3.1. Sludge Treatment Reed Bed (STRB)

Sludge Treatment Reed Bed system consists of several reed beds that are sequentially loaded by surplus sludge of WWTPs (Brix, 2017). Figure 2.1 presents a typical system of STRB, consisting of three layers of natural cobbles such as coarse gravel, sand, and gravel and fine particles including drainage, main, and transition layers planted with the native plant (invasive reeds) (Uggetti, 2010). The raw sludge is fed over the reed bed and most solid particles are retained on the surface of the top layer. In the STRB design, a freeboard space on the top of the bed allows for the accumulation of sludge, which improves dewatering and stabilization through biochemical processes and other treatment mechanisms (Stefanakis, 2014). The liquid part drains down the system as leachate, which is collected through a drainage layer composed of course gravel and is discharged by an outlet pipe connected to a ventilation network.

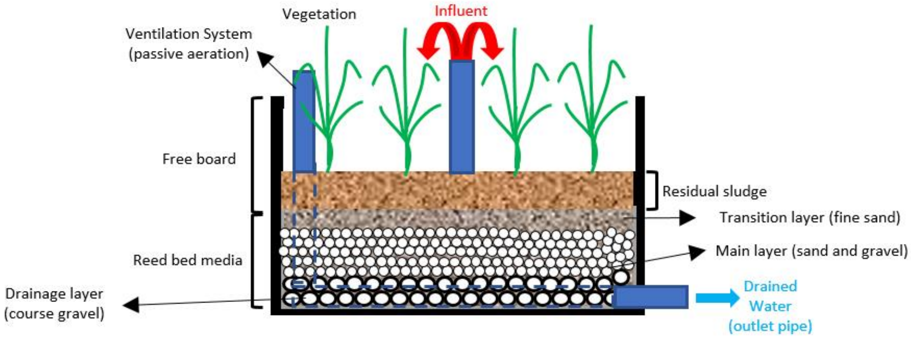


Figure 2. 1. A typical STRB system.

STRB operation requires the application of consecutive loads of sludge with an interval in between along with resting periods. A schematic representation of STRB operation periods for sludge dewatering is shown in Figure 2.2. The selected SLR (Kg, DS/m^2.year) was applied over the whole year of the operation period with each feeding duration consisting of a different load with intervals defined according to climate conditions (Brix, 2017).

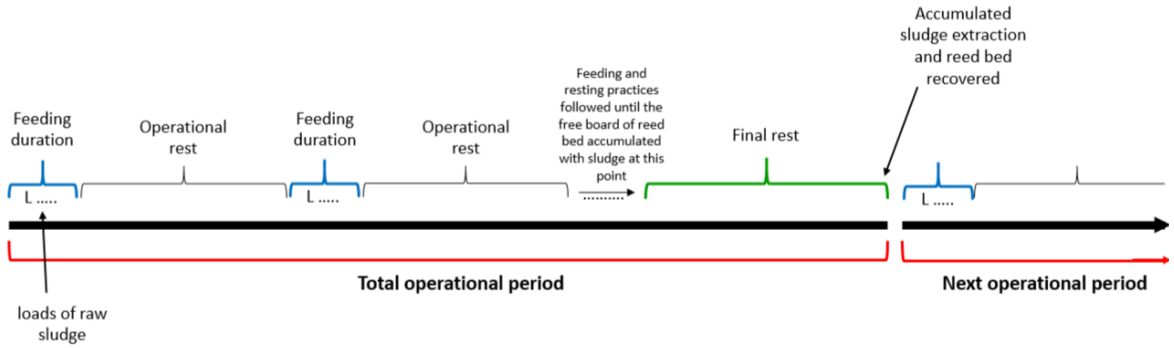
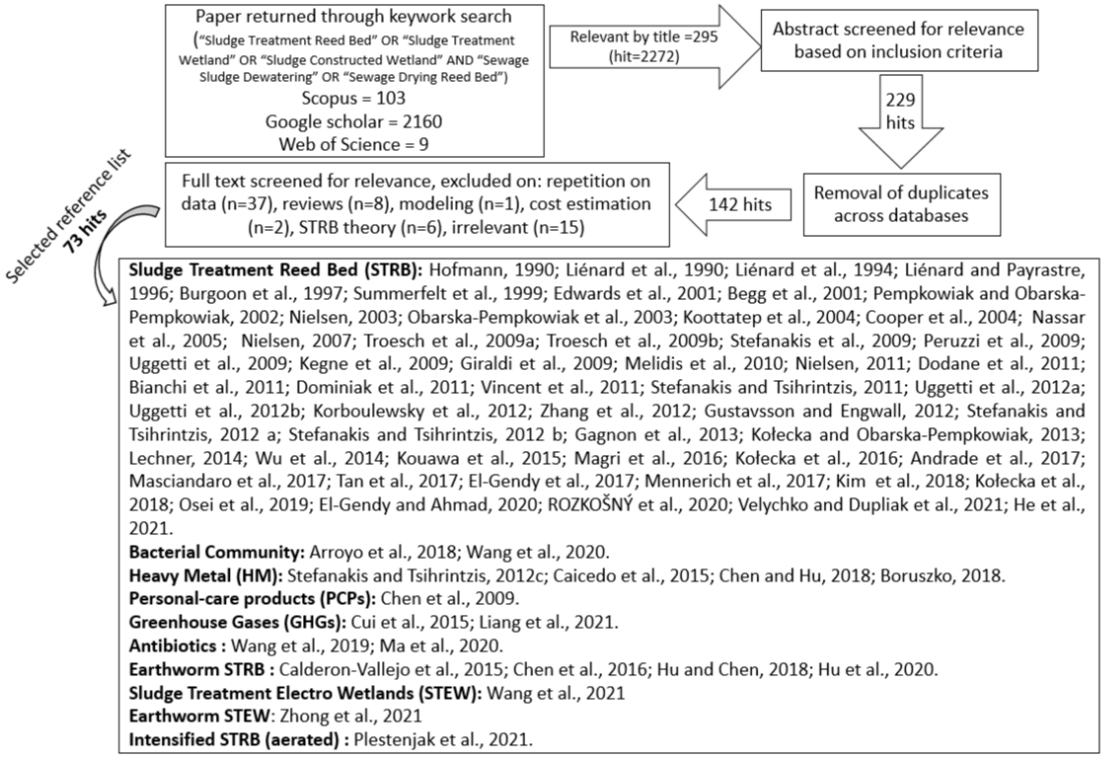


Figure 2. 2. Schematic representation of STRB operation periods

The interval between two consecutive feedings is called operational rest and when the freeboard of STRB is filled with accumulated sludge, a period called final rest is established to minimize sludge volume, increase DS content, and extract residual sludge (Pandey and Jenssen, 2015). Practically, after the extraction of accumulated sludge, the reed bed is ready for the next phase of operation. STRB can be utilized for almost 10 or 15 years depending on the height of the freeboard considered (usually between 0.5 to 1.5 m) and other criteria including the climate condition and the volume of the accumulated sludge determine STRB lifespan (Nielsen and Larsen, 2016; Brix, 2017). By desludging the process, the system can be simply recovered from the first lifespan and operated for the next lifespan (Nielsen, 2007).

2.3.2. Data collection and characterization

The data for Meta-Analysis (MA) was collected from Google Scholar, Scopus, and Web of Science (only English documents were considered) (Schulze, 2004; Field and Gillett, 2010; Hedges and Olkin, 2014). The search was performed through several combinations of keywords as one of the inclusion criteria: “Sludge Treatment Reed Bed” OR “Sludge Treatment Wetland” OR “Sludge Constructed Wetland” AND “Sewage Sludge Dewatering” OR “Sewage Drying Reed Bed.” The search returned 2272 hits including 2160, 103, and 9 hits from Google Scholar, Scopus, and Web of Science, respectively. To either reject or select the hits, inclusion, and exclusion criteria were applied in the meta-analysis of this review (Lozanovska et al., 2018; Kumar et al., 2019; Niu et al., 2020). Inclusion criteria were hits with Sludge Treatment Reed Bed title and content, hits applied to pilot and/or full scale, and hits with English texts. In addition, it is important to highlight that only the studies that presented the result in STRB experiments were taken into consideration to allow for homogeneity in the same database. Second, they should include the following information, including design, operation, and performance criteria. The exclusion criteria are those STRB hits that provided neither the operational parameters nor dewatering performance of STRBs including SLR, DS, VS, and resting periods, STRB hits that have data repetitions and duplication in different databases and journals, STRB hits with reviews and viewpoints, STRB hits with modeling and simulation content, STRB hits with cost assessment purposes, STRB hits with general concepts and theoretical hits. A total of 295 articles were detected within the scope of the study via title checking. Afterward, 66 irrelevant articles were removed through relevance based on inclusion criteria by abstract screening. Therefore, 229 articles remained of which 78 were duplicated through databases, and the remaining 142 papers were selected for full-text reading. Finally, 69 articles were excluded based on exclusion criteria including data publishing repetition (n=37), reviews (n=8), modeling (n=1), cost estimation (n=2), STRB theory (n=6) and irrelevant data to STRB (n=15). Therefore, 73 original papers were selected and 66 studies of typical STRB were considered for the Meta-Analysis (MA) from 1990 until 2021 (30 years of relevant studies). Seven studies had modified designs including earthworm STRB, Sludge Treatment Electro Wetland (STEW), earthworm STEW, and aerated STRB (intensified) were used in this review to draw a comparison with typical STRB studies. Although the excluded articles did not have relevant data for the MA of the STRB design factors, they are often referred to within the review for their scientific findings and contributions to a specific thematic. The process of the search and data collection is shown in Figure 2.3.



Sludge Treatment Reed Bed (STRB): Hofmann, 1990; Liénard et al., 1990; Liénard et al., 1994; Liénard and Payrastre, 1996; Burgoon et al., 1997; Summerfelt et al., 1999; Edwards et al., 2001; Begg et al., 2001; Pempkowiak and Obarska-Pempkowiak, 2002; Nielsen, 2003; Obarska-Pempkowiak et al., 2003; Koottatep et al., 2004; Cooper et al., 2004; Nassar et al., 2005; Nielsen, 2007; Troesch et al., 2009a; Troesch et al., 2009b; Stefanakis et al., 2009; Peruzzi et al., 2009; Uggetti et al., 2009; Kegne et al., 2009; Giraldi et al., 2009; Melidis et al., 2010; Nielsen, 2011; Dodane et al., 2011; Bianchi et al., 2011; Dominiak et al., 2011; Vincent et al., 2011; Stefanakis and Tsihrintzis, 2011; Uggetti et al., 2012a; Uggetti et al., 2012b; Korboulewsky et al., 2012; Zhang et al., 2012; Gustavsson and Engwall, 2012; Stefanakis and Tsihrintzis, 2012 a; Stefanakis and Tsihrintzis, 2012 b; Gagnon et al., 2013; Kołecka and Obarska-Pempkowiak, 2013; Lechner, 2014; Wu et al., 2014; Kouawa et al., 2015; Magri et al., 2016; Kołecka et al., 2016; Andrade et al., 2017; Masciandaro et al., 2017; Tan et al., 2017; El-Gendy et al., 2017; Mennerich et al., 2017; Kim et al., 2018; Kołecka et al., 2018; Osei et al., 2019; El-Gendy and Ahmad, 2020; ROZKOŠNÝ et al., 2020; Velychko and Dupliak et al., 2021; He et al., 2021.
Bacterial Community: Arroyo et al., 2018; Wang et al., 2020.
Heavy Metal (HM): Stefanakis and Tsihrintzis, 2012c; Caicedo et al., 2015; Chen and Hu, 2018; Boruszko, 2018.
Personal-care products (PCPs): Chen et al., 2009.
Greenhouse Gases (GHGs): Cui et al., 2015; Liang et al., 2021.
Antibiotics : Wang et al., 2019; Ma et al., 2020.
Earthworm STRB : Calderon-Vallejo et al., 2015; Chen et al., 2016; Hu and Chen, 2018; Hu et al., 2020.
Sludge Treatment Electro Wetlands (STEW): Wang et al., 2021
Earthworm STEW: Zhong et al., 2021
Intensified STRB (aerated) : Plestenjak et al., 2021.

Figure 2. 3. The process of web search and data collection.

The selected references can be seen in Figure 2.3, categorized into different topics based on the purpose of the investigations, and the differences in reed bed design. Of the studies, 66 cases were on the typical STRB performances, the operational adaption to a certain climate, and the type of raw sludge, of which there are two studies on bacterial communities, four on Heavy Metals (HMs), one for Personal-Care Products (PCPs), two for Greenhouse Gases (GHGs), two for Antibiotics, four for Earthworm STRB, one for Sludge Treatment Electro Wetlands (STEWs), one for Earthworm STEW and one for Intensified STRB (aerated STRB). Based on the structural development over 30 years, STRB technology has been designed in four different classifications including STRB, Earthworm STRB, STEW, and Earthworm STEW. The addition of Earthworm (Calderon-Vallejo et al., 2015; Chen et al., 2016; Hu and Chen, 2018; Hu et al., 2020), power generation (Wang et al., 2021), synchronous earthworm, and power generation (Zhong et al., 2021) and active aeration (Plestenjak et al., 2021) can be considered the state of the art of the technology. The addition of earthworms can accelerate the dewatering and stabilization processes due to the movement of earthworms together with the direct consumption of organic matter (Chen et al., 2016). The introduction of the microbial electrochemical system (MES) was conducted to enhance the conventional STRB efficiency and to generate power out of organic matters (Wang et al., 2021). Zhong et al (2021) have combined two systems of earthworm STRB and STEW and created earthworm STEW that enabled to generate power and enhanced the dewatering and stabilization efficiencies of the typical STRB. A typical STRB has been designed with passive aeration tubes that passively supply air to the reed bed to enhance the process of organic matter decomposition. Another important development was the introduction of active (forced) aeration in STRB media by using a blower (Plestenjak et al., 2021). A statistical analysis of the number of publications by the continent, country, and journal of publication was performed, and the result is shown in Figure 2.4.

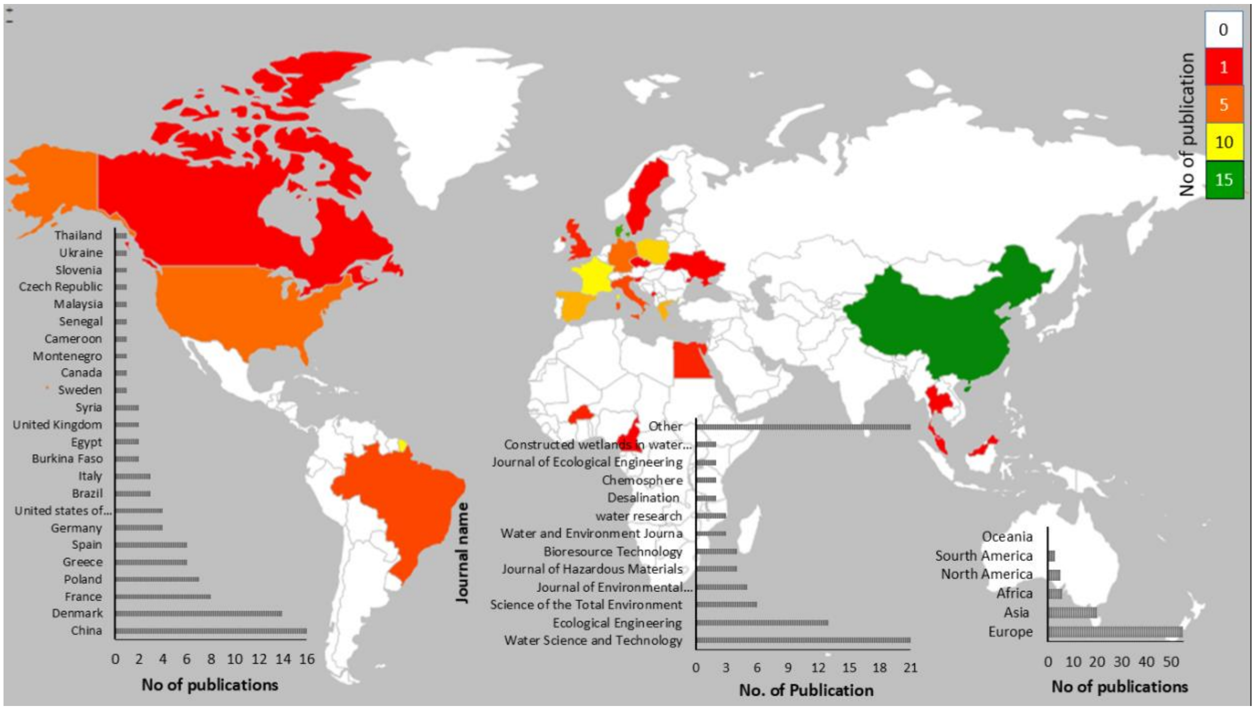


Figure 2. 4. Location of the selected studies and journal of publication.

Based on the world map (Figure 2.4), most investigations were in Europe and Asia, particularly in China with 16 publications. In Europe, there were 14 studies in Denmark and eight in France along with eight investigations in Poland. Although there have been three and four studies in South and North America, respectively, only three studies have been reported from Africa.

We conducted a terminological analysis on the keywords that have been used frequently in previous studies. Through the Meta-analysis, it is found that different terminologies were used to state STRB technology in the literature, which brings difficulty for the research tools to provide relevant studies. The terminology analysis contributes to the identification of common and most widespread terms used in previous studies to harmonize future studies in the application of such keywords. In the analysis, all keywords of the selected studies were collected, and the frequency of each keyword was determined. It showed that keywords like “reed bed”, “dewatering”, “sludge treatment wetland”, “sludge dewatering”, “constructed wetland” and “sludge” have been repeated 21, 17, 14, 12, 11, and 11 times, respectively. Other keywords including “sewage sludge”, “*Phragmites australis*”, “bio-solids”, “sludge treatment reed beds”, “sludge drying”, “sludge drying bed”, “nutrient” and “heavy metals” were also used between five and ten times.

From 1990 to 2021, different milestones in the sludge dewatering science can be identified through reed beds or constructed wetlands. The milestones are defined as whether the technology was used for the first time in a region or a country and in case any variation happened in the technology design or the investigated objectives. Figure 2.5 shows a timeline of the technology since 1990 and the relevant milestones.

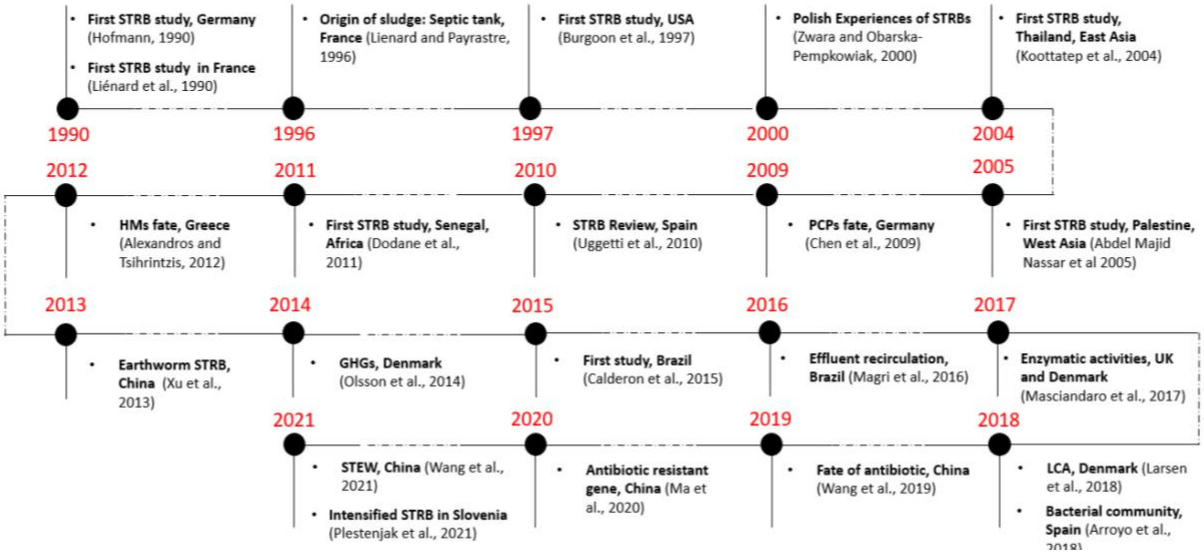


Figure 2. 5. STRB timeline and milestones since 1990.

In 1990, the first STRB studies were conducted in Germany and France followed by a study in 1996, in France, where a reed bed was used for the dewatering of septic tank raw sludge (Hofmann, 1990; Liénard et al., 1990; Lienard and Payrastre., 1996). In 1997, an application of STRB was reported for the first time in the USA (Burgoon et al., 1997) and the experiences of Polish STRB's stated in 2000 (Zwara and Obarska-Pempkowiak, 2000). In Asia, the first STRB study was performed in Thailand (East Asia, in 2004) followed by a study in 2005, in Palestine, West Asia (Koottatep et al., 2004; Abdel Majid Nassar et al., 2005). In 2009, the objective of the studies developed in Germany was to explore the fate of PCPs in accumulated sludge, which is a more specific subject (Chen et al., 2009). The first regional review of the STRB was reported in 2010, in Spain, in which the technology was defined and there was a discussion over the overall performance of STRB (Uggetti et al., 2010). In 2011, the first study of STRB in Africa, Senegal, was documented (Dodane et al., 2011). The fate of heavy metals in accumulated sludge and drainage water quality was investigated in 2012 (Stefanakis and Tsihrintzis, 2012c), and afterward, the STRB system was used together with earthworms in 2013, in China (Xu et al., 2013). The amount of GHGs emitted from STRB was reported in Denmark in 2014 (Olsson et al., 2014). The first study of STRB, which was applied to earthworms as well, was conducted in Brazil, South America in 2015 (Calderon-Vallejo et al., 2015). Magri et al., investigated for the first time the effect of effluent recirculation on STRB in 2016 (Magri et al., 2016), and then the enzymatic activities were explored in UK and Denmark in 2017 (Masciandaro et al., 2017). In 2018, there were two studies on Life Cycle Assessment (LCA) and bacterial communities in Denmark and Spain respectively (Larsen et al., 2018; Arroyo et al., 2018). The fate of antibiotics and Antibiotics Resistant Genes (ARGs) were studied in China in 2019 and 2020 (Wang et al., 2019; Ma et al., 2020). In 2021, studies of STEW and Intensified STRB were reported as well (Wang et al., 2021; Plestenjak et al., 2021).

2.3.3. Data analysis

To find a significant difference between climate conditions ($p\text{-value}<0.05$), Analysis of Variance (ANOVA) was conducted using statistical tools e.g., R between STRB parameters of operation and efficiency. Normality of data was assessed via the Shapiro-Wilk test and homogeneity of variance (Levene’s test) was performed ($p>0.05$). Other statistics such as min, max, mean, standard deviation, and analysis of the level of confidence (upper and lower limits with 95% level of confidence) were calculated as well. Statistical package R was used to conduct all analyses. The operational parameters of STRB considered in the analysis included Sludge Loading Rate (SLR), feeding and resting modes, Dry Solids (DS) and Volatile Solids (VS) obtained from the accumulated sludge, height of the accumulated sludge, and the sludge volume reduced during feeding and after final resting. It has been suggested that climate condition is the most effective criterion influencing the overall performance of STRB technology and the design aspects (Kengne and Tilley., 2014; Gholipour et al., 2015; Nielsen and Larsen., 2016).

In this study, Köppen–Geiger climate classification system is used (Beck et al., 2018), which suggests mainly four climate conditions including Temperate, Tropical, Arid, and Polar. Solar radiation across the Temperate climate varies from relatively high radiation in the Mediterranean region (1700 kW/m^2) to a lower intensity (1200 kW/m^2) in Western, Eastern, British Isles, and Northern Europe regions (Paulescu et al., 2013; Dash et al., 2017; Anas et al., 2021). Solar radiation has been correlated with the evapotranspiration rate (Chatzithomas and Alexandris, 2015; Mokhtari et al., 2018), which was found an effective factor in the dewatering process of sludge in a system of an STRB (Brix, 2017). In this study, Temperate climate was divided into the Temperate type 1 (relatively low-intensity solar radiation) and Temperate type 2 (relatively high-intensity solar radiation). As the solar radiation of the Temperate type 1 and Temperate type 2 is different, we hypothesized that there would be a significant difference between the type 1 and 2 in terms of the operational, design factors, and efficiency factors. Therefore, the hypothesis is assessed in this study to present a more relevant meta-analysis for this region. Temperate type 1 refers to the region with lower intensity of solar radiation which is categorized as humid subtropical (Cfa), oceanic (Cfb), subpolar oceanic (Cfc), dry-winter humid subtropical (Cwa), dry-winter subtropical highland (Cwb), and dry-winter cold subtropical highland climate (Cwc). Temperate type 2 is the region with higher intensity of solar radiation, which refers to a hot and warm (Csa, Csb, and Csc).

Since there is not sufficient data on the latest advances such as earthworm STRB, STEW, earthworm STEW, and intensified STRB to conduct statistical analysis, only the studies of typical STRBs were considered in MA. The results of the climatic categories of the STRB analysis will be later compared with the latest advances though. It contributes to clarifying if there is any enhancement of the STRB technology through the variations in the different types of the latest designs.

2.4. Results and discussion

Our analysis showed that 64% of the studies were pilot scales while 36% were full scales. 90% of the cases were about domestic sludge (WWTPs, pit latrine, or septic tanks) followed by 4% for industrial sludge, and few studies explored the efficiency of STRB for the fishery, aquaculture, and livestock sludge sources (Summerfelt et al., 1999; Gagnon et al., 2013; Arroyo et al., 2018). In the studies on WWTP sludge, 60% of the sludge fed was a combination of primary and secondary sludge (mixed sludge) and 32% was primary sludge while only 8% was secondary sludge. In terms of the origin and the technology of the treatment, the data showed that 51% of the sludge was Surplus Activated Sludge (SAS) followed by 24% from pit latrine and septic tanks. Some studies investigated the sludge from extended aeration systems (12%) while other studies included anaerobic sludge, Imhoff tanks, reflux tanks, trout production, swine slurry, and settled fish farm sludge were conducted (Summerfelt et al., 1999; Melidis et al., 2010; Magri et al., 2016; Arroyo et al., 2018; ROZKOŠNÝ et al., 2020; Wang et al., 2021). Of the total 74 selected studies, 66 cases had an STRB area of less than 5000 m^{2,} and the remaining cases were performed in an area larger than 5000 m^2. In terms of studies' duration, 12 studies were less than one year, and 38 studies had a duration length between one and five years. Only 13 cases were reported having more than a five-year operational period. It is also found that the average Dry Solid (DS) and Volatile Solid to DS (VS/DS) of the raw sludge were 6.64 and 64% in the previous studies, respectively. More information on the 73 selected studies can be found in the supplementary materials (Appendix 1).

2.4.1. Design and Operational aspects

Three parameters in the operation of STRB affect the final performance of a dewatering process, which include Sludge Loading Rate (SLR), mode of sludge feeding, and resting period (Nielsen, 2005). The factors were analyzed for five different climate conditions including a total of 76 STRB facilities from Temperate type 1 (35), Temperate type 2 (15), Tropical (19), Arid (3), and Polar (1) climates. Figure 2.6 presents the statistical analysis of SLR for different climate conditions. For each climate, the maximum and minimum values are presented as well as the upper and lower limits for SLR, which was considered the 95% confidence level. The homogeneity of variance for the data collected was found equal (p-value > 0.05) and, the data were normally distributed (p-value > 0.05). The average SLR values of Temperate type 1, Temperate type 2, and Tropical climates increased with the reduction of latitude with the SLRs values of 50, 70, and 101 Kg.DM.m^{-2}.year^{-1}, respectively. These values were found to be statistically significantly different (p-value < 0.05).

In a Temperate type 1, the upper and lower limits of application were 59.49 and 41.42 Kg.DM.m^{-2}.year^{-1}. In the Temperate type 2, the upper and lower limits of application were 91.21 and 48.88, and for Tropical climates are 130.39 and 71.5 Kg.DM.m^{-2}.year^{-1}, respectively. The reported values of Arid and Polar climates are 80 and 30 Kg.DM.m^{-2}.year^{-1} in Senegal and Canada, respectively (Dodane et al., 2011; Gagnon et al., 2013). Based on this, the highest numbers of SLR occurred in the lower geographical latitudes, which correspond to the warmer climates with fewer days of precipitation. (Although we compare the average values of the meta-analysis, the upper and lower limits of each climate can be used to compare climate more precisely distinction).

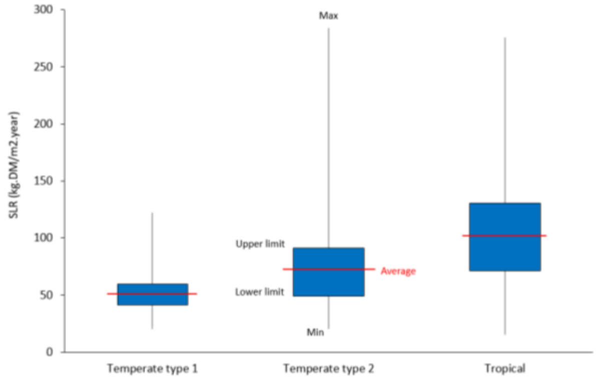
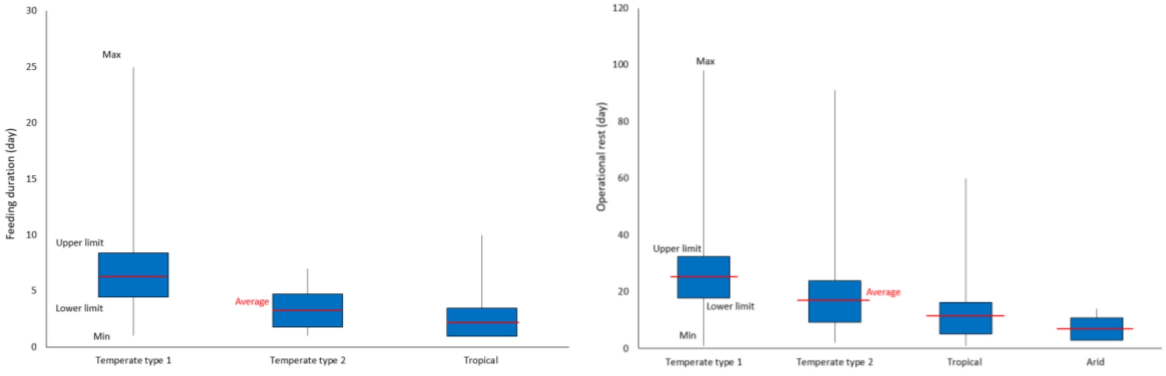


Figure 2. 6. Statistical analysis of SLR for the three different climate conditions

Earthworm STRB studies from the Tropical region (four studies) showed that SLR can be applied up to 120 Kg.DM.m^{-2}.year^{-1} (Calderon-Vallejo et al., 2015; Chen et al., 2016; Hu and Chen, 2018; Hu et al., 2020) while the average SLR for typical STRBs is 101 Kg.DM.m^{-2}.year^{-1}. The SLRs adapted in the systems of STRB assisted with earthworms are within the range of upper and lower limits for typical STRB in Tropical climates; therefore, the average SLR adapted for Earthworm STRB shows an improvement compared to the typical STRBs. SLRs for STEW and earthworm STEW in Tropical climates were 125 and 91.2 Kg.DM.m^{-2}.year^{-1}, respectively (Wang et al., 2021; Zhong et al., 2021). The SLR adapted for intensified STRB in Slovenia was 21.5 Kg.DM.m^{-2}.year^{-1} (Plestenjak et al., 2021). Thus, a system of STEW could dewater the maximum amount of fed sludge within all STRB types in the Tropical region. Due to data insufficiency, a similar comparison for other climates is not addressed.

The operation of STRB was analyzed considering the feeding duration, and operational stages including operational rest, and final rest (Figure 2.7). The distribution of data in each climate was found normal, and the homogeneity of variance was met as well (P-value > 0.05). ANOVA analysis showed that the data obtained for Temperate type 1, Temperate type 2, and Tropical climates are significantly different (p-value <0.05).



a **b**

Figure 2. 7. Statistical analysis: a) feeding duration b) operational rest (data is shown only for climates with 3 or more studies)

According to Figure 2.7 (a), the average feeding durations are 6.44, 3.26 and 2.23 days for Temperate type 1, Temperate type 2, and Tropical climate conditions, sequentially. In addition, average operational rests were 22, 17, 11, and 7 days for Temperate type 1, Temperate type 2, Tropical, and Arid climate conditions, respectively (Figure 2.7.b).

Several factors including precipitation, the season of the final resting, solar radiation, humidity, wind, and evapotranspiration influence the duration of the final resting (De Maeseneer, 1997; Brix, 2017). Higher precipitation during the final resting period increases the duration of the final resting (Kegne and Tilley 2014). In Temperate climate type 1, the final resting duration ranged from 24 days in Germany (Hofmann, 1990) to 365 days in France and in Montenegro (Vincent et al., 2011, Lechner, 2014). In France, Liénard et al. (1994) also applied 70 days and in the UK, 120 days were also considered by Edwards et al. (2001). In Denmark, Uggetti et al. (2012b) and Masciandaro et al. (2017) applied 90 and 60 days, respectively, while in Helsinge facility in Denmark, the duration was 14 months (from June 2004 to August 2005) (Nielsen, 2007). In Temperate climate type 2, the duration of the final resting varies from 25 (in Greece, Stefanakis et al., 2011) to 180 days (in Egypt, Gendy and Ahmad, 2020). In Spain, 90 days were applied by Uggetti et al. (2012a) and Arroyo et al. (2018). In the Tropical climate, the final resting was 30 days in Cameron (Kegne et al., 2009), and in China, the final resting ranged from 30 to 365 days while most studies in China considered 365 days (Zhang et al., 2012; Cui et al., 2015; Chen and Hu, 2018; Wang et al., 2019; Ma et al., 2020; Wang et al., 2020; He et al., 2021; Liang et al., 2021; Zhong et al., 2021). Through the previous studies, there has not been any design guideline on the duration of the final resting; however, the final resting duration depends on the desired degree of final DS and the application of the final dried solid (Brix, 2017). The occurrence of the final resting was recommended in seasons with lower precipitations (Nielsen, 2005).

During feeding, the selected SLR can be fed over the reed bed in one or more loads (Brix, 2017). In the Temperate type 1, Summerfelt et al. (1999) within a one-day feeding, fed the reed bed in six loads while in the facility of Rudkøbing in Denmark, it is reported that one load was considered for a one-day feeding (Nielsen, 2003). Wu et al, (2014) also considered two loads in 3.5 days of feeding duration in a Temperate type 1 while ROZKOŠNÝ et al, (2020) perceived two different modes of loading and feeding I) four loads of raw sludge in 90 days II) 15 loads of raw sludge in 25 days of feeding duration. In the Temperate type 2, one week of daily loading and 7, 14 and 21 days of operational rest were considered by Stefanakis et al, (2009) while Uggetti et al, (2009) had 12 loads during two days of feeding in Alpens and Sant Boi facilities. In addition, one load during a one-day feeding was perceived by Arroyo et al, (2018) in the Temperate type 2. In the Tropical climate, Calderon et al, (2015) fed the pilot reed bed with eight loads in one day while Wang et al, (2021) considered five loads in five days of feeding duration. One load was taken into consideration in a one-day feeding by Kouawa et al, (2015) while Osei et al, (2019) fed the reed bed with two loads in a one-day feeding in an Arid climate condition. In the Polar climate, one load was considered in a one-day feeding by Gagnon et al, (2013).

Our analysis showed that the feeding and resting periods of earthworm STRB (1 to 2 days feeding and 7 to 14 days resting), STEW (5 days feeding and 4 days resting), earthworm STEW (4 days feeding and 4 days resting), intensified STRB (1 day feeding and 40 days resting) have been quite like the typical STRB studies. Table 2.1 presents a summary of the meta-analysis on the design and operational aspects of typical STRB.

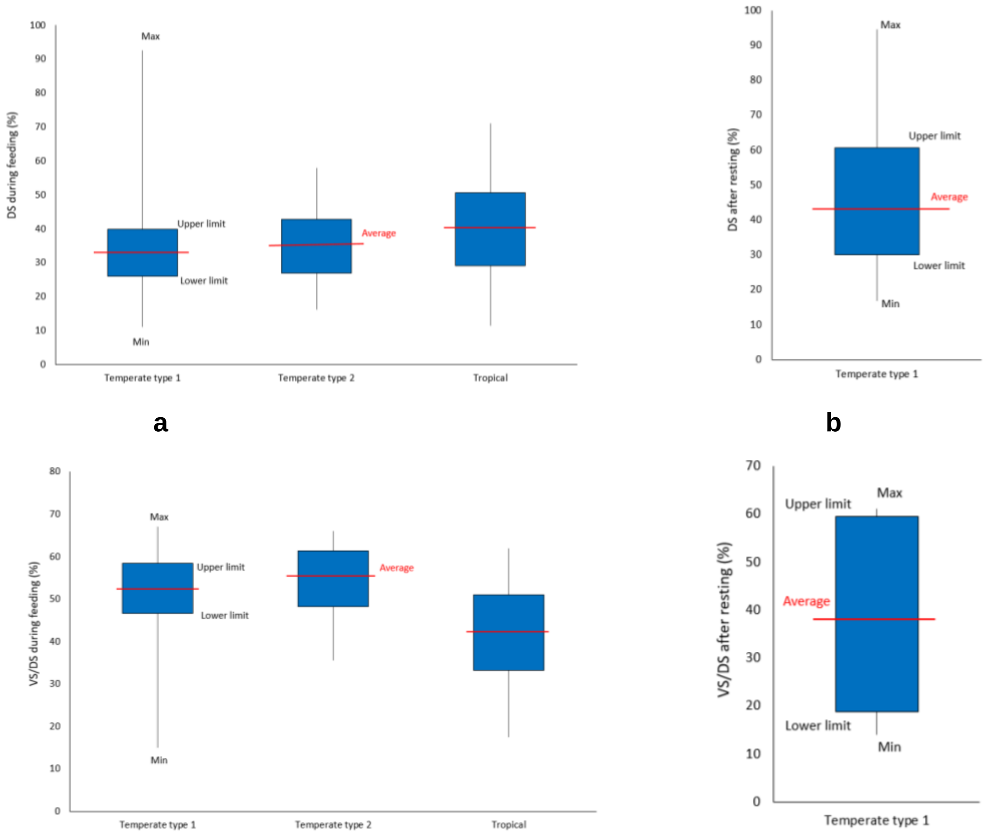
Table 2. 1. A summary of meta-analysis on design and operational aspects of STRB.

Parameters type	Parameters	Statistics	Unit	Climate condition				
				Temperate type1	Temperate type2	Tropical	Arid	Polar
Design and Operational aspects	Sludge Loading Rate (SLR)	Min	kg.DM/m2.year	20	20	15	***	***
		Max		122	284	276		
		Mean		50	70	101		
		Lower limit		41.42	48.88	71.5		
		Upper limit		59.49	91.21	130.39		
		No of studies	Number	28	23	25		
	Number of Loads	Min	Number	1	1	1	1	***
		Max		15	12	8	2	
		Mean		2.18	2	1.73	1.25	
		Lower limit		0.87	0.21	0.71	0.76	
		Upper limit		3.48	3.78	2.75	1.74	
		No of studies		22	12	15	4	
	Feeding duration	Min	Day	1	1	1	***	***
		Max		25	7	10		
		Mean		6.44	3.26	2.23		
		Lower limit		4.45	1.79	0.99		
		Upper limit		8.43	4.74	3.47		
		No of studies	Number	27	13	20		
	Operational rest	Min	Day	1	2	1	3	***
		Max		98	91	60	14	
		Mean		22	17	11	7	
		Lower limit		17.75	9.16	5.16	2.86	
		Upper limit		32.55	24.01	16.26	10.73	
		No of studies	Number	35	22	20	7	

***Less than 3 studies

2.4.2. Performance

The performance of an STRB system in terms of the dewatering process is measured based on dry and volatile solids obtained either during or after resting (Brix, 2017). Several criteria are used to determine the performance of a reed bed including Dry Solids (DS) content of the accumulated sludge, the proportion of Volatile Solids (VS) to Dry Solids (VS/DS), the thickness of the residual sludge (height of accumulated sludge) and the percentage of sludge volume reduced (Nielsen and Larsen, 2019). The present study conducted a statistical analysis of the DS content, VS/DS, the final sludge thickness, and the reduced volume of sludge and categorized them into different climate conditions. Figure 2.8 shows the DS content of the accumulated sludge (Figure 2.8. a and b) and the VS/DS (Figure 2.8. c and d) during feeding and after final resting. The homogeneity of variance and normal distribution of the data was met (p-value > 0.05) and ANOVA analysis showed that the data obtained from different climates are significantly different (p-value <0.05).



a **b**

c **d**

Figure 2. 8. Statistical analysis: a) DS during feeding b) DS after final resting c) VS/ DS during feeding d) VS/ DS after final resting (data is shown only for climates with 3 or more studies)

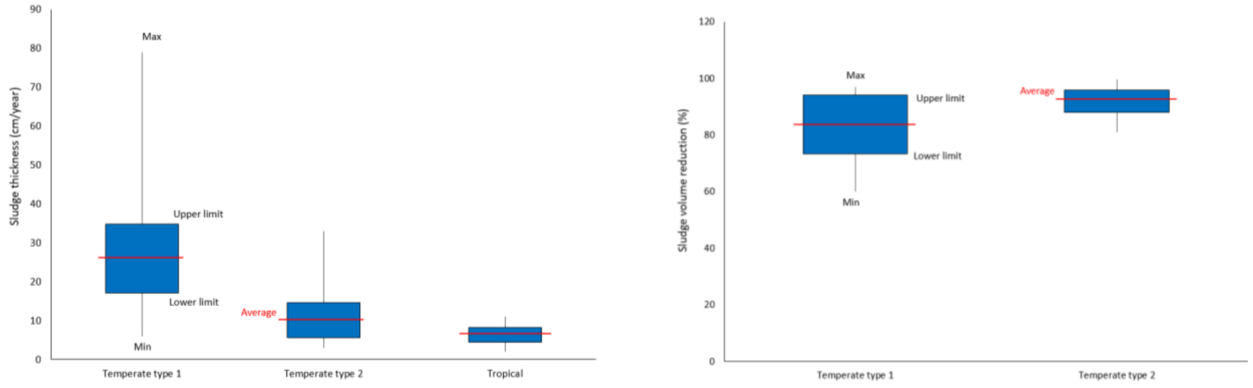
Previous studies showed that the DS during the feeding is always less than the DS obtained after the final resting (Peruzzi et al., 2009; Giraldi et al., 2009; Osei et al., 2019). According to Figure 2.8.a, the average DS during the feeding for Temperate type 1, Temperate type 2, and Tropical climates are 33, 35, and 40%, respectively. Dodane and

Gagnon’s studies showed that 45 and 30% of DS can be obtained in Arid and Polar climates (Dodane et al., 2011; Gagnon et al., 2013). The average DS content of sludge after the final rest is 45 % for Temperate type 1 (Figure 2.8. b), and in Temperate type 2, 80.5 % was obtained (Alexandros and Tsihrintzis, 2012 b). The study by Wang et al. (2020) revealed that DS content after final resting can reach 59% in an STRB system in Tropical climates.

The average DS obtained from earthworm STRBs in Tropical climate after final resting was 63.4% which is higher than that of 59% for the typical STRBs in the Tropical region while this value was 11.5 and 50% for the systems of STEW and earthworm STEW (Calderon-Vallejo et al., 2015; Chen et al., 2016; Hu and Chen., 2018; Hu et al., 2020 Wang et al., 2021; Zhong et al., 2021). A final DS of 62.5% was reported for the intensified STRB (Plestenjak et al., 2021). Therefore, the dewatering performance of earthworm STRBs can potentially be higher than that of typical STRBs in Tropical regions. In a Temperate type 1, the final DS of intensified STRB is higher than that of typical STRB reported in Plestenjak et al., (2021) study. The reason for this could be the lower SLR applied on the intensified STRB (21.5 Kg.DM.m^{-2}.year^{-1}) while the average SLR for typical STRBs in Temperate type 1 was 50 Kg.DM.m^{-2}.year^{-1}. Overall, the final DS obtained in the Temperate type 2 climate was 70%, which is the highest DS of all climates for the typical STRBs and even compared to the latest advances of STRB. Although it is evinced that higher SLRs are adopted in the lower geographical latitudes and warmer climates (Figure 2.6), the highest DS resulted from MA in Figure 2.8 between higher and lower latitudes. Apart from SLR applied, other factors influence the final DS after the final resting including precipitation, wind speed, solar radiation, evapotranspiration, humidity, and seasonal effects (Nielsen., 2007 Arroyo et al., 2018). A Higher DS in the Temperate type 2 climate could be due to the aforementioned factors.

The ratio of VS to DS is a key criterion for showing the amount of decomposable sludge (Brix, 2017). According to Figure 2.8.c, the studies under Temperate type 1, Temperate type 2, and Tropical climates give average values of VS/DS (during feeding) of 53, 55, and 42%, respectively, and for Polar climate, this number is 40% (Gagnon et al., 2013). After the final resting, the average VS/DS is 39 in Temperate type 1 (Figure 2.8. d), and for Temperate type 2 was reported 67.4% (Stefanakis et al., 2009) while 52 % was obtained in Tropical climate by Cui et al. (2015). The difference between the average VS/DS values in Temperate type 1 climates during feeding (53%) and after final resting (39%) can be due to several reasons, like precipitations and the different periods during the feeding and the final resting. Although the final average VS/DS of Temperate type 1 is lower than that of the average value during the feeding period, the range of upper and lower limits of Temperate type 1 after resting (Figure 2.8.d) is larger than the range of upper and lower limits during the feeding in Temperate type 1 (Figure 2.8.c), where the upper limits of both ranges are 59.5 and 58.42%, respectively. The reasons for the difference in Temperate climate should be addressed in future research.

The VS/DS of the studies for the earthworm STRB, STEW, and earthworm STEW in Tropical climates were reported at 33.1, 33.6 and 41.6 %, respectively (Calderon-Vallejo et al., 2015; Chen et al., 2016; Hu and Chen, 2018; Hu et al., 2020; Wang et al., 2021; Zhong et al., 2021). Hence, VS/DS values of these systems are lower than the typical STRBs values, indicating improved stabilization efficiency. The amount of sludge accumulated per year and the sludge volume reduced are also important criteria, which directly influence the size of reed bed, excavation, and transportation costs of sludge for further land applications (Nielsen, 2005). The increment of sludge height per year for each climate condition and the amount of sludge reduced after final resting is shown in Figure 2.9. The distribution of the data was normal, and the variances were homogeneous (p-value >0.05). ANOVA analysis indicated that the data obtained from different climates are significantly different (p-value <0.05).



a **b**

Figure 2. 9. Statistical analysis: a) sludge thickness b) volume reduced (data is shown only for climates with 3 or more studies)

Evapotranspiration (ET) is an effective factor influencing the final DS of sludge (Burgoon et al., 1997; Velychko and Dupliak, 2021). The type of plant species, seasonal effect, climate conditions, atmosphere humidity, wind speed, and the presence of earthworms in the STRB system were found effective on the rate of ET (Burgoon et al., 1997; Vincent et al., 2011; Stefanakis and Tsihrintzis 2011; Chen et al 2016; Mennerich et al., 2017; Hu and Chen., 2018; Velychko and Dupliak, 2021). It is stated that during the growing season, the rate of ET is higher and from April to September is 1.9 mm per day while it can range from 1.23 to 1.78 mm per day in a Temperate type 1 (Velychko and Dupliak, 2021). A study by Burgoon et al. (1997) showed that ET could be 6.4 mm per day between May to Jun in a Temperate type 1 while the ET rate in the study by Vincent et al. (2011) also in a Temperate type 1 reported 2.58 mm per day, resulting in 70% of DS. Other studies found that the ET rate can range from a minimum of 1.34 mm to a maximum of 1.90 mm per day in a Temperate type 1 in which the DS ranged between 20 and 45% (Mennerich et al., 2017). Therefore, the DS obtained in the study of Vincent et al. (2021) is higher than that of the DS obtained by Mennerich et al, in the Temperate type 1 due to a higher rate of ET. A significant difference in the rate of ET was observed between different plant species in which *Phragmites australis, Typha angustifolia, and Scirpus fluviatilis* show 94, 64, and 40 % of ET, respectively (Gagnon et al. 2013). Earthworm STRB system showed a higher ET rate compared to typical STRBs by a 42.9% increase in ET (Chen et al., 2016). This could be since earthworms increase the exchange of water in the STRB system with the atmosphere and then, ET increases while draining can also be enhanced by earthworms creating micro tunnels in the reed bed media. This increases the final DS content of the sludge accumulated as well (Hu and Chen., 2018).

In Figure 2.9.a, the height of the average sludge accumulated annually is 26, 10, and 6 cm for Temperate type 1, Temperate type 2, and Tropical climates, respectively. This number is reported as 30 cm per year for the Arid climate (Dodane et al., 2011). Therefore, the lowest value of accumulated sludge occurs in Tropical climates at 6 cm per year. The average sludge volume reduced was 84 and 92% for Temperate type 1 and Temperate type 2 climates (Figure 2.9.b). In Tropical and Polar regions, volume reduction was averagely reported as 98.55 and 86.5% (Chen et al., 2016; Gagnon et al., 2013).

In comparison to the earthworm STRB, STEW, and earthworm STEW, the height of accumulated sludge is much lower than the typical STRBs, which have been reported as 2 cm per year. Therefore, a system of combined earthworms and typical STRB or MES, or STEW can reduce the amount of sludge accumulated more efficiently (more than 60%). Synchronous earthworms or MES with STRB can enhance the general performance and removal efficiency resulting in less accumulated sludge in reed beds due to the possibility of a higher decomposition rate. Table 2.2 presents a summary of MA analysis on STRB performance.

Table 2. 2. A summary of MA analysis on STRB performance.

Parameters type	Parameters	Statistics	Unit	Climate condition				
				Temperate type1	Temperate type2	Tropical	Arid	Polar
Performance	DS during feeding	Min	%	11	16.25	11.5	***	***
		Max		92.6	57.9	71		
		Mean		33	35	40		
		Lower limit		26.04	26.96	29.12		
		Upper limit		39.83	42.88	50.73		
		No of studies	Number	27	13	14		
	DS after resting	Min	%	16.85	***	***	***	***
		Max		94.5				
		Mean		45				
		Lower limit		30.08				
		Upper limit		60.71				
		No of studies	Number	11				
	VS/DS during feeding	Min	%	15	35.5	17.4	***	***
		Max		67	66	62		
		Mean		53	55	42		
		Lower limit		46.64	48.2	33.27		
		Upper limit		58.42	61.31	50.96		
		No of studies	Number	17	12	11		
	VS/DS after resting	Min	%	14	***	***	***	***
		Max		61.15				
		Mean		39				
		Lower limit		18.81				
		Upper limit		59.5				
		No of studies	Number	4				
	Sludge thickness	Min	cm/year	6	3	2		

	Max		79	33	11		
	Mean		26	10	6		
	Lower limit		17.14	5.67	4.55	***	***
	Upper limit		34.89	14.75	8.33		
	No of studies	Number	18	16	12		
Sludge volume reduction	Min		60	81			
	Max		97	99.64			
	Mean	%	84	92	***	***	***
	Lower limit		73.34	88.01			
	Upper limit		94.12	95.89			
	No of studies	Number	8	8			

***Less than 3 studies

A correlation analysis showed that SLR correlated negatively with DS (-0.64) and VS/DS (-0.53) factors. This may seem contradictory to the trends observed in the comparison between Temperate type 1, Temperate type 2, and Tropical climates, but it most likely shows that there is room to increase SLR in Temperate type 1 and Temperate type 2 climates without affecting performance significantly (Table 2.3). Similar results were found in previous studies of typical STRBs conducting sensitivity analysis on the impact of variation of sludge loading rate on DS content (Stefanakis and Tsihrintzis, 2012b; Kołecka et al., 2019).

Table 2. 3. Correlation between parameters of STRB operation and efficiency (DS and VS/Ds are the values during feeding.)

Parameters		Operational		
		SLR	Feed duration	Operational rest
Efficiency	DS	-0.64	-0.55	0.69
	VS/DS	-0.53	-0.14	-0.20

In addition, feed duration has a negative correlation with DS (-0.55), namely, opting for a longer period of feeding results in lower DS, which was mentioned in pilot studies as well (Caicedo et al., 2015; Boruszko, 2018). On the contrary, operational rest has a positive correlation with DS (0.69); therefore, to achieve higher DS, operational rest should increase (Korboulewsky et al., 2012).

2.4.3. STRB media characterization

One of the key factors in the efficiency of the dewatering process is the filtration material (Brix, 2017). Through the previous studies, various kinds of materials have been used including sand and gravel (used in 89% of the studies). The media configuration in terms of the number of layers is the other factor that influences the overall performance of the drying process (Nielsen and Larsen., 2016). The MA showed that 55% of the previous studies (20 out of 36 studies) applied two layers of filtration material of different sizes (Brix, 2017). The substrates used for the top layers are fine sands while coarse gravels are used in the bottom layers (Nielsen, 2005). Also, 39% of the studies have used three layers of filtration (Kegne and Tilley, 2014). Only three percent of the studies used one layer and three percent applied four layers (Cui et al., 2015; Wang et al., 2019; Wang et al., 2020; Ma et al., 2020; Liang et al., 2021). The size of cobbles used in the previous studies was in the range of 28 to 49 mm (95% level of confidence), with the size of grains between a minimum of 0.2 and a maximum of 400 mm.

Other substrates can be used in the media to influence filtration efficiency, including slag and quartz along with sand and gravel, which were applied in six percent of the studies (Cui et al., 2015; Wang et al., 2019; Wang et al., 2020; Ma et al., 2020; Liang et al., 2021). Other substrates used in combination with sand and gravel are, for instance, peat and crushed pine bark, wood shaving, graphite particles, and granular activated carbon (Gustavsson and Engwall, 2012; Uggetti et al., 2012a; Wang et al., 2021; Zhong et al., 2021). These materials are used to increase the filtration capacity and the rate of organic matter decomposition (Wang et al., 2021). Graphite particles and granular activated carbon can be used alone or with gravel to generate power from the organic matters of the accumulated sludge in the STEW system (Wang et al., 2021; Zhong et al., 2021). A few numbers of studies have also filled the media with earthworms to enhance the dewatering capacity and more particularly to reduce the volume of accumulated sludge and improve the fertilization value of the final residuals (Calderon-Vallejo et al., 2015; Chen et al., 2016; Chen and Hu, 2018; Hu and Chen, 2018; Hu et al., 2020; Zhong et al., 2021). Two types of earthworms were used, including *Lumbricus terrestris* (Calderon-Vallejo et al., 2015) and *Eisenia fetida*, the last was the most applied macro-organisms in previous earthworm STRBs (Chen et al., 2016; Chen and Hu, 2018; Hu and Chen, 2018; Hu et al., 2020; Zhong et al., 2021).

2.4.4. STRB vegetation

The plant species used over the media are another crucial factor influencing dewatering performance and by-product quality (Kolecka et al., 2018). Therefore, selecting the right plant species based on climate conditions, and natural and ecological capacities has been a challenge through experiences. A list of planted species used in previous studies is organized in Table 2.4. It is shown that the application of native plant species causes an improvement in the resilience, performance, efficiency, and sustainability of a reed bed site (Brix, 2017).

Table 2. 4. Plant species used in previous studies, based on climate conditions

Plant species	No of applications					References
	Temperate type1	Temperate type2	Tropical	Arid	Polar	
Bambusa vulgaris				1		Osei et al., 2019
Cymbopogon nardus				1		Osei et al., 2019
Canna indica			3			Chen et al 2016; Wang et al 2021; Zhong et al 2021
Cynodon dactylon pers			2			Calderon et al., 2015; Andrade et al., 2017
Echinochloa crus galli	1					Liénard et al., 1994
Echinochloa Pyramidalis			2			Dodane et al., 2011
Cyperus papyrus			2			Kegne et al., 2009; Magri et al., 2016
Cyperus alopecuroides			1			Gendy and Ahmad., 2020
Eichhornia crassipes			1			Gendy and Ahmad., 2020
Water hyacinth,			1			Gendy and Ahmad., 2020
Phragmites australis	44	21	11		1	Hofmann., 1990; Burgoon et al., 1997; Edwards et al., 2001; Pempkowiak and Obarska-Pempkowiak., 2002; Nielsen., 2003; Nassar et al 2005; Nielsen 2007; Stefanakis et al 2009; Troesch et al 2009a; Dominiak et al., 2011; Gustavsson and Engwall., 2012; Lechner., 2014; Caicedo et al., 2015; Kołecka et al., 2016; Tan et al., 2017; Masciandaro et al., 2017; Kim et al., 2018; Kołecka et al 2018; ROZKOŠNÝ et al., 2020; Wang et al., 2020; Velychko and Dupliak et al., 2021; Plestenjak et al., 2021; He et al., 2021;
Panicum echinochloa		1				Gendy and Ahmad., 2020

Oryza longistaminata				1		Kouawa et al., 2015
Sporobolus pyramidalis				1		Kouawa et al., 2015
Panicum repens		1				Gendy et al., 2017
Typha latifolia	2	3	4			Korboulewsky et al., 2012; Wu et al 2014; Magri et al., 2017; Chen and Hu., 2018; Hu and Chen., 2018; Hu et al 2020; Alexandros et al., 2011; Uggetti et al., 2012a; Alexandros and Tsihrintzis., 2012 a
Typha augustifolia			3		1	Koottatep et al., 2004; Gagnon et al., 2013
Scirpus fluviatilis,					1	Gagnon et al., 2013
Iris pseudacorus	1					Korboulewsky et al., 2012
Vetiveria zizanioides	1					Summerfelt et al., 1999
Zizaniopsis bonariensis			1			Magri et al., 2016

Our statistical analysis reveals that *Phragmities australis* (common reed, *P.a*) was adopted in almost two-thirds of the cases, used in 76 either pilot or large scale of the facilities, and in total 23 different species have been tested in previous studies. *Typha latifolia* (*T.l*) was the second most popular species in previous studies and the more common reeds after *P.a* and *T.l* were *Typha augustifolia*, *Canna indica*, *Cynodon dactylon pers*, *Echinochloa pyramidalis* (*Antelope grass*) and *Cyperus papyrus*. Based on Table 2.4, 13 species were adapted to Tropical climates while five species for Temperate type 1 and four species for Temperate type 2 climates were tested. Four types of plant species were used in the Arid climate while three types of plants were tested in the Polar climate. Some plant species listed in Table 2.4 were used only one time; therefore, future investigations are necessary to find out how these species perform in the dewatering process in different climates.

It is also important to select the right density of reeds for the plantation, especially on the startup day of operation, and in fact, plant density can influence the time of reed bed adaptation to the engineered environment and to the commissioning period (Nielsen., 2005). Provided that the right density of reed is considered, the overall design efficiency of the reed bed maintains from the first day of operation, and the time from plantation to the maximum STRB performance (commissioning period) shortens; however, plants propagate and proliferate by the time and the introduction of raw sewage sludge (Brix, 2017). On the other hand, the increase in planted tufts in the reed bed leads to higher costs, therefore cost/efficiency should be optimized. Our statistical analysis shows that 14 studies used less than five tufts per square meter while 14 studies planted between 5 and 20 tufts per square meter. Three studies used more than 20 tufts per square meter (Osei et al., 2019; Hu et al., 2020; El-Gendy and Ahmad, 2020). The analysis suggests that on average six to ten tufts per square meter are used at the time of construction.

2.4.5. STRB removal efficiency

2.4.5.1. Heavy Metals (HMs)

There have been several studies addressing the removal of Heavy Metals (HMs) from sludge in STRB systems. The main factors studied include vegetation, size of filtration materials, SLR, duration of final resting, and the quality of raw sludge (Stefanakis and Tsihrintzis, 2012c; Zhang et al., 2012). It was found that HMs were accumulated and bounded in residual sludge (Stefanakis and Tsihrintzis, 2012c; Caicedo et al., 2015; Boruszko, 2018). Comparisons between HMs in influent and effluent of STRBs showed that the mean contents of metals in the accumulated sludge are lower compared to the influent (Stefanakis and Tsihrintzis, 2012c). HM concentration can increase by the depth of sludge (Stefanakis and Tsihrintzis, 2012c; Caicedo et al., 2015; Boruszko, 2018; Chen and Hu, 2019) and Uggetti et al. (2012b) reported that the HMs in bio-solids was negligible due to the low concentration in the influent.

The concentration orders of HMs in the residual sludge (fed by domestic raw sludge) were Pb < Ni < Cr < Cd < Fe < Cu < Zn < Mn in the study of Stefanakis and Tsihrintzis, 2012c, while Kegne et al. (2009) Cd < Ni < Cr < Se < Pb < Mn < Cu < Zn. Begg et al. (2001) also found a similar order to Mb < Cd < Cr < Br < Pb < Mn < Ni <Zn < Cu < Fe. Thus, Zn, Cu, and Mn seem to be the predominant elements. The accumulation of Cr, Cd, Pb, and Ni was found slowly, which decreased with time while the accumulation of Cu, Mn, Zn, and Fe was reported faster (Stefanakis and Tsihrintzis, 2012c). The seasonal effect has been shown as an effective factor in increasing the concentration of Cu, Mn, and Fe during the summer due to higher dryness of sludge while Cr, Cd, Zn, Pb, and Ni contents were decreased. It is found that the concentration of all HMs were below legal limits for land applications (Begg et al., 2001; Obarska-Pempkowiak et al., 2003; Uggetti et al., 2009; Kegne et al., 2009; Stefanakis and Tsihrintzis, 2012c; Caicedo et al., 2015; Kołecka et al., 2016; Boruszko, 2018). In STEW systems, the HM removal efficiency was satisfactorily led to the direct use of residual sludge in agriculture, and it is reported that Cd, Ni, Pb, Ti, As, Co, Cr, Cu, and Mn reduced by 81.12, 30.02, 45.70, 23.10, 50.28, 32.39, 39.76, 41.80 and 12.97%, respectively (Wang et al., 2021).

Studies showed that HMs accumulated in upper and below-ground parts of plants and the biomass of the plants would increase year by year (El-Gendy et al., 2017); therefore, the uptake by the plants will gradually increase (El-Gendy and Ahmad, 2020). The bioavailability of HMs in soil and plants depends on several factors including soil pH, plant species and their cultivars, growth stage, bio-solids source, soil condition, and the chemistry of the element (Warmar and Termeer, 2005). It was found that the HM concentrations in plant biomass were, in decreasing order, Cr > Fe > Zn > Mn > Cu > Pb > Ni > Cd (Stefanakis and Tsihrintzis, 2012c). The maximum HMs concentration was in the roots, followed by leaves and stems (Stefanakis and Tsihrintzis, 2012c; Cheng et al., 2020). Caicedo et al. (2015) obtained comparable results, with the highest concentrations of HMs in the roots and then in the rhizomes and aerial parts. In the study of Caicedo et al., (2015) the order of retention was found as Sb < Hg < Cd < Mo < Co < Ni < Pb < Cr < Mn < Cu < Zn < Fe in roots, Sb < Hg < Cd < Mo < Ni < Pb <Co < Cr < Cu < Mn < Zn < Fe in the rhizomes and Sb < Hg < Cd < Mo < Pb < Ni <Co < Cr < Cu < Mn < Zn < in the shoots or aerial parts of the plant biomass. Planted and unplanted units were compared and revealed that vegetation has a profound impact on the reduction and mobility of HMs through the provision of microbial contact surfaces (Stefanakis and Tsihrintzis, 2012c) although the vegetation accounted for only three percent of HMs accumulation (Stefanakis and Tsihrintzis, 2012c). The Bio-Concentration Factor (BCF=HMs content in plants/HMs content in raw sludge) of HMs was found with no statistical difference between P. australis and T. angustifolia ($p > 0.05$) (Chen and Hu, 2019).

Previous studies on earthworm STRB showed that earthworms are effective in the accumulation of HMs (Calderon- Vallejo et al., 2015; Chen et al., 2016; Hu and Chen, 2018; Hu et al., 2020). It is found that the reason for HMs enrichment by earthworms in the accumulated sludge was due to the activities of enzymes in earthworms (Chen and Hu, 2019). Bio-Accumulation Factor or BAFs (HMs content in earthworms / HMs content in raw sludge) were found as Cd > Mn > Ni > Zn > Pb > Cr > Fe (Chen and Hu, 2019). The difference between earthworm STRB and typical STRB in terms of heavy metal contents during the feeding period was not statistically significant although it was significantly different after the resting period (Hu et al., 2020). Earthworm STRB showed a better HMs accumulation in sludge depending on the density of earthworms of which higher density will result in a higher HMs accumulation (Hu et al., 2020). Earthworms provided a positive effect on the accumulated sludge characteristics in STRBs (Hu et al., 2020). Further investigations require to find the role of earthworms in the bioavailability of HMs in residual sludge, plants, and effluent.

2.4.5.2. Nutrients and Nitrogen components

STRB has been shown effective in the removal of different nutrients and nitrogen components in previous studies, although it is highly dependent on the quality of the fed raw sludge (Burgoon et al., 1997; Moss et al., 2002; Bianchi et al., 2011; Uggetti et al., 2012b; Korboulewsky et al., 2012; Andrade et al., 2017; Osei et al., 2019). One of the major nutrients in influent sludge is nitrogen, which is the removal target of most WWTPs, and the order of nutrients was found as Ca > P > S > K > Mg in the accumulated sludge (Caicedo et al., 2015). The concentration of nutrient elements can increase with the time of STRB operation; however, it was found that there is a decreasing tendency along with the layers of the vertical profile of the accumulated sludge due to mineralization, ammonification, and plant uptake (Peruzzi et al., 2009; Melidis et al., 2010; Zhang et al., 2012; Uggetti et al., 2012b; Caicedo et al., 2015; Hu and Chen, 2018). The variation of potassium (k) was found negligible after the treatment (Uggetti et al., 2012b). Depending on the nutrient concentration in the accumulated sludge, its application to agricultural land can be considered (Hu and Chen, 2018).

The concentration of nutrients was found in a common trend with Total Organic Carbon (TOC) concentration along with the vertical profile of bio-solids (Caicedo et al., 2015). Total Kjeldahl Nitrogen (TKN) reduced from the influent to the sludge accumulated (Uggetti et al., 2009). TKN concentration of residual sludge was reduced by up to 66% in previous studies (Stefanakis et al., 2009; Arroyo et al., 2018; Osei et al., 2019). It was observed that there were reductions for NO_2-N and NO_3-N of 86.4 and 28.7% in residual sludge, respectively (Stefanakis et al., 2009). NH_4-N reduction was reported in STRB systems as well (Arroyo et al., 2018).

Low phosphorus concentration was found in treated sludge due to phosphorus retention in plants (Peruzzi et al.,2009). Total phosphorus (TP) reduction of up to 80% was reported in some studies depending on the presence of plants and earthworms (Begg et al., 2001; Stefanakis et al., 2009; Chen et al., 2016; Osei et al., 2019). A study of plant species' effect on P removal in typical STRB showed that *Phragmites, Typha,* and *Iris* were responsible for 17, 12, and 6% of phosphorus removal from the sludge, respectively (Korboulewsky et al., 2012). In the earthworm STRBs, it was shown that the sum of TN, TP, and TK was significantly lower than the STRBs without earthworms (Hu and Chen, 2018). Earthworm movements increase voids in the accumulated sludge, enhancing oxygen availability in the bed, which accelerate TN, TP, and TK conversion and absorption by microorganisms in sludge (Brix, 2017; Chen et al., 2016; Gagnon et al., 2013). Investigations of STEW showed that TN/DS ratio in accumulated sludge after the resting period is between 2.86 to 2.96% (Wang et al., 2021), which is lower than the value obtained for typical STRB of 3.18 to 6.4% TN/DS (Peruzzi et al., 2009; Melidis et al., 2010). Therefore, the TN/DS of residual sludge is low, and the TN/DS of STEW is less than the total Nitrogen of STRB systems. In terms of nutrient concentration, extracted sludge from STEW can be used for agricultural applications (Wang et al., 2021). Also, TP concentration reduced in STEW up to 54.88% while TP/DS of STRBs showed a slightly lower reduction of 50.24% after the resting period (Wang et al., 2021). Among the effective factors of P removal, plant uptake is the major contributor of 68% in the STEW system (Wang et al., 2021).

2.4.5.3. Drain water quality

The water drained out (called also rejected water or leachate) of STRB has been monitored in previous studies as well, including several parameters from HMs, nitrogen components, organic matter, pathogenic organisms, nutrients to emerging pollutants (Lienard and Payrastre, 1996; Edwards et al., 2001; Begg et al., 2001 Cooper et al., 2004; Koottatep et al., 2004; Stefanakis et al., 2009; Gustavsson and Engwall, 2012; Calderon-Vallejo et al., 2015). The removal efficiency of STRB reported for Total Suspended Solids (TSS) and Chemical Oxygen Demand (COD), was higher than 85, 85, 76, and 66% for the mesocosms planted with *Phragmites*, *Typha*, *Iris,* and unplanted one, respectively (Burgoon et al., 1997; Begg et al., 2001; Korboulewsky et al., 2012; Chen et al., 2016;

Magri et al., 2016; Kim et al., 2018; Wang et al., 2021). STRB showed high efficiency in the removal of TKN and TP with more than 95% reduction from the inlet to the outlet points (Kim et al., 2018). The effluent quality of the STRB has been within the regulatory standards of the previous study that needs no further treatment (Begg et al., 2001). Chen et al. (2016) showed that the removal efficiency of the systems with earthworm layer is quite higher than that of STRB systems. It was found that the HM concentration of the drained water is less than 10% of the concentration in influent for the typical STRB (Stefanakis and Tsihrintzis, 2012c; Chen and Hu, 2019). The removal efficiency of HMs in the leachate of STRBs with earthworms was significantly different from that of STRB and unplanted STRB (Chen and Hu, 2019).

To explore whether the dewatered accumulated sludge is safe to reuse for land applications, some studies addressed the presence of fecal pathogens (Koottatep et al., 2004; Kegne et al., 2009; Uggetti et al., 2009; Wang et al., 2021). Kenge et al., (2009) found that *Ascaris Lumbricoides* is the most widespread species of *helminth* in residual sludge followed by *Trichuris Trichiura* varying based on the depth of the sludge, where the most counted *helminth* was detected in the upper layers. The resting time had a negative correlation with the number of fecal species (Kegne et al., 2009). Uggetti et al. found that *E. coli* is present in the residual sludge decreasing with the time of resting while *Salmonella* specie was not detected in the final accumulated sludge (Uggetti et al., 2009). Egg viability was found less than 10% and egg concentrations were less than 6 eggs per gram DS as well (Koottatep et al., 2004). In most studies, it was stated that effluent can be reused without leachate disinfection (Koottatep et al., 2004; Stefanakis et al., 2009; Gustavsson and Engwall, 2012; Calderon-Vallejo et al., 2015).

2.5. Conclusion

In this paper, 73 studies were selected and reviewed of which 66 studies of Sludge Treatment Reed Beds (STRB) were used to conduct a Meta-Analysis (MA) on a global scale since 1990. Most studies have been concentrated in Europe and China, and were mostly pilot-scale studies, and domestic raw sludge was tested stemming from combined primary and secondary surplus sludge. In addition to typical STRB design, seven studies with different designs were identified, including earthworm STRB, STEW, earthworm STEW, and intensified system that can potentially improve dewatering performance and resource recovery as well as power generation. Through the climate categorization of STRB studies in MA, we found the average Sludge Loading Rate (SLRs) for Temperate type 1, Temperate type 2, Tropical, Arid, and Polar climates were 50, 70, 101, 80, and 30 Kg.DM.m^{-2}.year^{-1}, respectively, while for the earthworm STRB, it was 120 Kg.DM.m^{-2}.year^{1}. MA showed that average DS during the feeding of 33, 40, and 45% can be obtained in Temperate type 1, Temperate type 2, and Tropical climates while the average DS after feeding was 45% for Temperate type 1 and 75% for Temperate type 2, with only one study in Tropical climate obtaining 59%. Studies performed on the earthworm STRB, STEW, and earthworm STEW showed that an improvement in the typical STRB was observed. The MA evinced that the height of accumulated sludge can increase in the order of 26, 10, and 6 cm per year for Temperate type 1, Temperate type 2, and Tropical climates, respectively while it is around 2 cm per year in the available studies of earthworm STRB, STEW and earthworm STEW systems (a reduction of 85% of the accumulated sludge volume after final resting and stabilization process). MA showed that in most studies natural cobbles were used for reed bed media with three layers of substrate sizing from 49 to 28 mm while other studies have recently used graphite particles and granular activated carbon to generate power out of organic matters. A total of 23 plant species were identified in the reviewed studies in which *Phragmites Australis* was adapted in two-thirds of the cases. Plants were found effective in the up taking of different pollutants particularly nutrients while they are accelerating the removal process of other pollutants as well. The STRB technology was also found highly effective in removing HMs, nutrients, nitrogen components, pathogens, and emerging pollutants from sludge and drained water.

2.6. References

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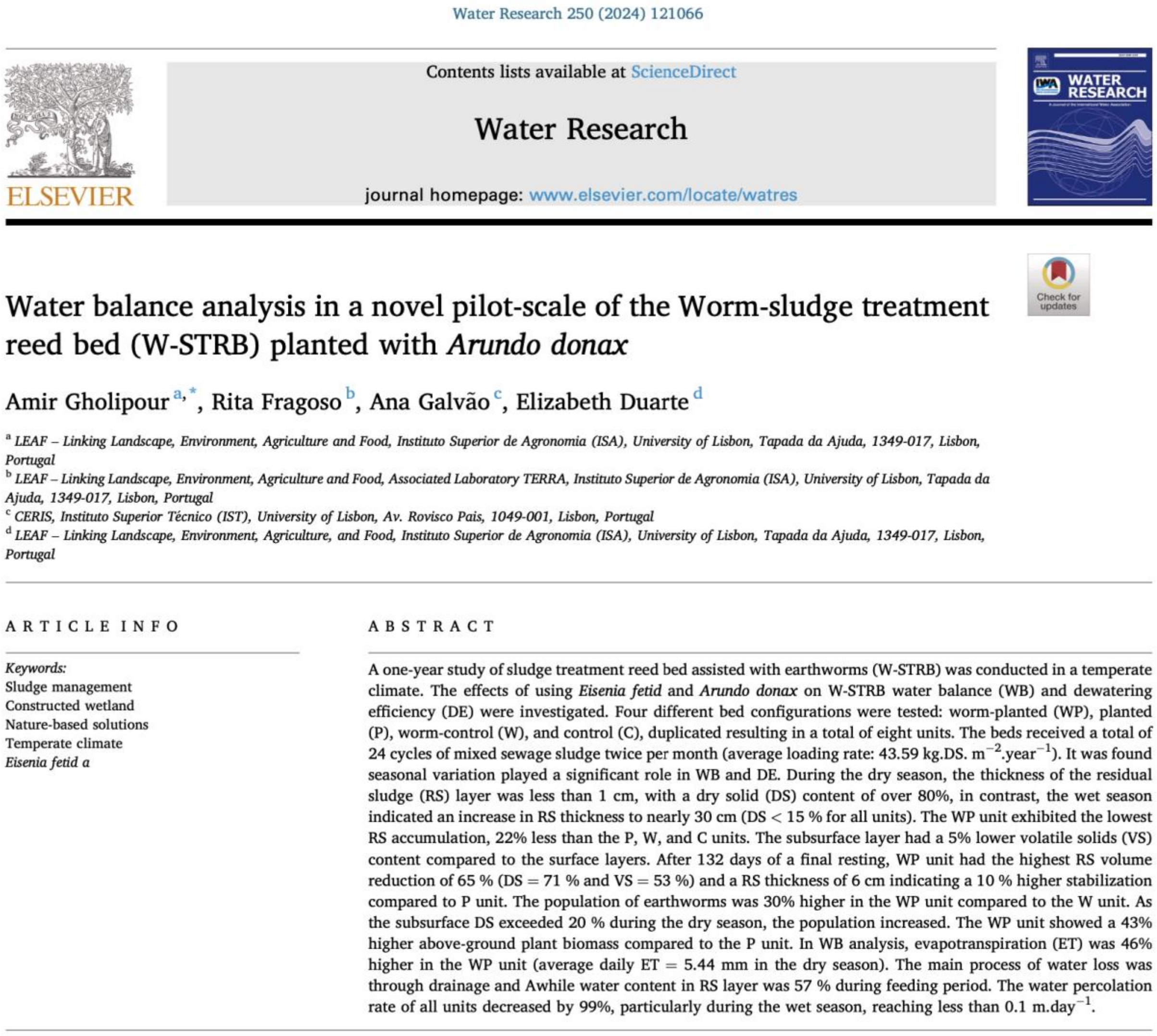
Chapter 3. Water balance analysis in a novel pilot-scale of the Worm-Sludge Treatment Reed Bed (W-STRB) planted with *Arundo donax*



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Water balance analysis in a novel pilot-scale of the Worm-sludge treatment reed bed (W-STRB) planted with *Arundo donax*

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ABSTRACT

A one-year study of sludge treatment reed bed assisted with earthworms (W-STRB) was conducted in a temperate climate. The effects of using *Eisenia fetid* and *Arundo donax* on W-STRB water balance (WB) and dewatering efficiency (DE) were investigated. Four different bed configurations were tested: worm-planted (WP), planted (P), worm-control (W), and control (C), duplicated resulting in a total of eight units. The beds received a total of 24 cycles of mixed sewage sludge twice per month (average loading rate: 43.59 kg.DS. m^{-2}.$year^{-1}$). It was found seasonal variation played a significant role in WB and DE. During the dry season, the thickness of the residual sludge (RS) layer was less than 1 cm, with a dry solid (DS) content of over 80%, in contrast, the wet season indicated an increase in RS thickness to nearly 30 cm (DS < 15 % for all units). The WP unit exhibited the lowest RS accumulation, 22% less than the P, W, and C units. The subsurface layer had a 5% lower volatile solids (VS) content compared to the surface layers. After 132 days of a final resting, WP unit had the highest RS volume reduction of 65 % (DS = 71 % and VS = 53 %) and a RS thickness of 6 cm indicating a 10 % higher stabilization compared to P unit. The population of earthworms was 30% higher in the WP unit compared to the W unit. As the subsurface DS exceeded 20 % during the dry season, the population increased. The WP unit showed a 43% higher above-ground plant biomass compared to the P unit. In WB analysis, evapotranspiration (ET) was 46% higher in the WP unit (average daily ET = 5.44 mm in the dry season). The main process of water loss was through drainage and Awhile water content in RS layer was 57 % during feeding period. The water percolation rate of all units decreased by 99%, particularly during the wet season, reaching less than 0.1 m.day^{-1}.

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3.1. Abstract

A one-year study of sludge treatment reed bed assisted with earthworms (W-STRB) was conducted in a temperate climate. The effects of using *Eisenia fetid* and *Arundo donax* on W-STRB water balance (WB) and dewatering efficiency (DE) were investigated. Four different bed configurations were tested: worm-planted (WP), planted (P), worm-control (W), and control (C), duplicated resulting in a total of eight units. The beds received a total of 24 cycles of mixed sewage sludge twice per month (average loading rate: 43.59 kg.DS. m^{-2}.$year^{-1}$). It was found seasonal variation played a significant role in WB and DE. During the dry season, the thickness of the residual sludge (RS) layer was less than 1 cm, with a dry solid (DS) content of over 80 %, in contrast, the wet season indicated an increase in RS thickness to nearly 30cm (DS<15% for all units). The WP unit exhibited the lowest RS accumulation, 22 % less than the P, W, and C units. The subsurface layer had a 5 % lower volatile solids (VS) content compared to the surface layers. After 132 days of a final resting, WP unit had the highest RS volume reduction of 65% (DS=71% and VS=53%) and a RS thickness of 6cm indicating a 10% higher stabilization compared to P unit. The population of earthworms was 30 % higher in the WP unit compared to the W unit. As the subsurface DS exceeded 20% during the dry season, the population increased. The WP unit showed a 43 % higher above-ground plant biomass compared to the P unit. In WB analysis, evapotranspiration (ET) was 46 % higher in the WP unit (average daily ET=5.44 mm in the dry season). The main process of water loss was through drainage while water content in RS layer was 57% during feeding period. The water percolation rate of all units decreased by 99 %, particularly during the wet season, reaching less than 0.1 m.day^{-1}.

Keywords: sludge management, constructed wetland, nature-based solutions, temperate climate, *Eisenia fetid a*

3.2. Introduction

Sewage sludge management in wastewater treatment plants (WWTPs) is a global challenge due to the large amount of sludge produced in a limited urban space. Worldwide, the annual production of dried sewage sludge is 45 million tons ranging from 18 to 33 million tons in the European Union, China, and the United States (Semblance et al., 2016; Frontino et al., 2023). In Portugal, it varies between 0.12 and 0.42 million tons (>10 million inhabitants) with a sanitation coverage of over 97% (Fade et al., 2015; Ferrentino et al., 2023). Conventional sludge management requiring intensive facilities, advanced technologies, high energy, recurring costs, and skilled personnel is no longer feasible (Cieślik et al., 2015; Zhang et al., 2022). The centrifugation process (consumed 1.1 kW.m^{-3}) resulted in a dry solid (DS) content between 10 and 30 % of which 70 and 80 % is volatile solid (VS) content while DS < 20 % is not economically ponderable imposing extra costs for sludge transportation (Novak, 2006; Ferrentino et al., 2023). Therefore, supplementary stages require to reduce sludge volume through increasing DS content (DS > 30 %) (Rajput et al., 2022). Additionally, linear sludge management (sludge production, thickening, dewatering, and disposal) is unsustainable threatening natural habitats while a circular paradigm offers opportunities for resource recovery (Daee et al., 2019; Gholipour et al., 2023). In contrast, Nature-Based Solutions (NBS), like Sludge Treatment Reed Beds (STRB), offer a promising approach to resource recovery, climate change mitigation, and the addressing of biodiversity issues (Brix, 2017; Gholipour et al., 2020; Gholipour and Stefanakis, 2021). STRB provides multiple benefits contrasting with unsustainable characteristics of conventional systems although an efficient STRB relies on water balance (WB) status to stabilize sludge (Nielsen, 2023).

STRB is effective in enhancing dewatering efficiency (DE), namely increasing DS and stabilization (Nielsen, 2007; Saeed et al., 2022). DS content depends on WB status, Sludge Loading Rate (SLR) and precipitation (P_r), volumes of drained water (DW), and evapotranspiration (ET). High DS content reduces land demand by optimizing designing factors such as SLR. Various ranges of SLR were used based on climate conditions, and for instance, in temperate climates, SLR was between 41 and 59 kg.DS.m^{-2}.year^{-1} (Gholipour et al., 2022), while in an arid climate was 80 kg.DS.m^{-2}.year^{-1} (Dodane et al., 2011; Stefanakis et al., 2022). DE was 33 % in temperate and 45% in arid climates depending on the length of final resting, for the above-mentioned studies, it ranged between 1 and 12 months (Gholipour et al., 2022). P_r, ET, solar power, and plant species vary by climate (Gholipour et al., 2022) as well as hydraulic conductivity and media porosity influencing water percolation and WB. STRB was combined with earthworms (W-STRB), which improved DE between 59 and 63.4% in tropical climates influencing synergistically WB (Calderón-Vallejo et al., 2015; Chen et al., 2016; Hu and Chen, 2018; Hu et al., 2020; Wang et al., 2021; Zhong et al., 2021).

Due to the lack of information on WB in the Mediterranean region and specific examples in Portugal as well as the impact of worms on ET and DW volumes, W-STRB remains largely unexplored. Short-term and small-pilot W-STRBs in China also lacked an overarching assessment of seasonal variations and WWTP conditions (Zhong et al., 2021). Controlled studies in Spain (Uggetti et al., 2012), Greece (Stefanakis and Tsihrintzis, 2012), and Italy (Bianchi et al., 2011) demonstrated the performance of STRB planted with *Phragmites australis* without worms. Moreover, the effect of *Arundo donax* on WB has not been explored, despite its prevalence in Portugal and the Mediterranean region. In this study in Portugal, the performance of the W-STRB system planted with *Arundo donax* and assisted with *Eisenia fetida* was assessed in the Mediterranean region at Beirolas WWTP, while monitoring WB. To bridge the gap between the laboratory and the real world, this study extended over a full year, considering the effects of unexpected events like the 2022 severe storm-caused flood in Lisbon. The study encompasses the influence of worms on water percolation rate (WPR), residual sludge (RS) accumulation, *Arundo donax* development, and changes in ET, contrasting with previous studies.

3.3. Materials and methods

3.3.1. Pilot scale description

The study mesocosm (Figure 3.1) was set up at Beirolas WWTP in Lisbon, Portugal (38°47'18.9"N 9°05'50.0"W) in February 2022. Beirolas receives wastewater from Lisbon and Loures zones (54,500 m^3.d^{-1} for 213,510 inhabitants). Based on the Köppen climate classification, Lisbon has a hot-summer Mediterranean climate (Csa).

Figure 3. 1. The pilot study at Beirolas WWTP

An on-site weather station (EasyWeather-WIFI87CO) was installed at the WWTP receiving meteorological data every five minutes including air Temperature (T, C^o), UV index, solar power (W.m^{-2}), atmospheric pressure (mmHg), humidity (%), wind speed (m.s^{-1}) and wind direction (degree), and P_r (mm) (a summary is available in Table 3.1 in the supplementary materials).

The experiment (Figure 3.2) includes eight units of one cubic meter (Width: Length: Depth=0.95: 1.16: 1 m). Units 1 to 4 are worm-planted (WP), planted (P), worm-control (W), and control (C) units (units 5 to 8 are the replicates of units 1 to 4).

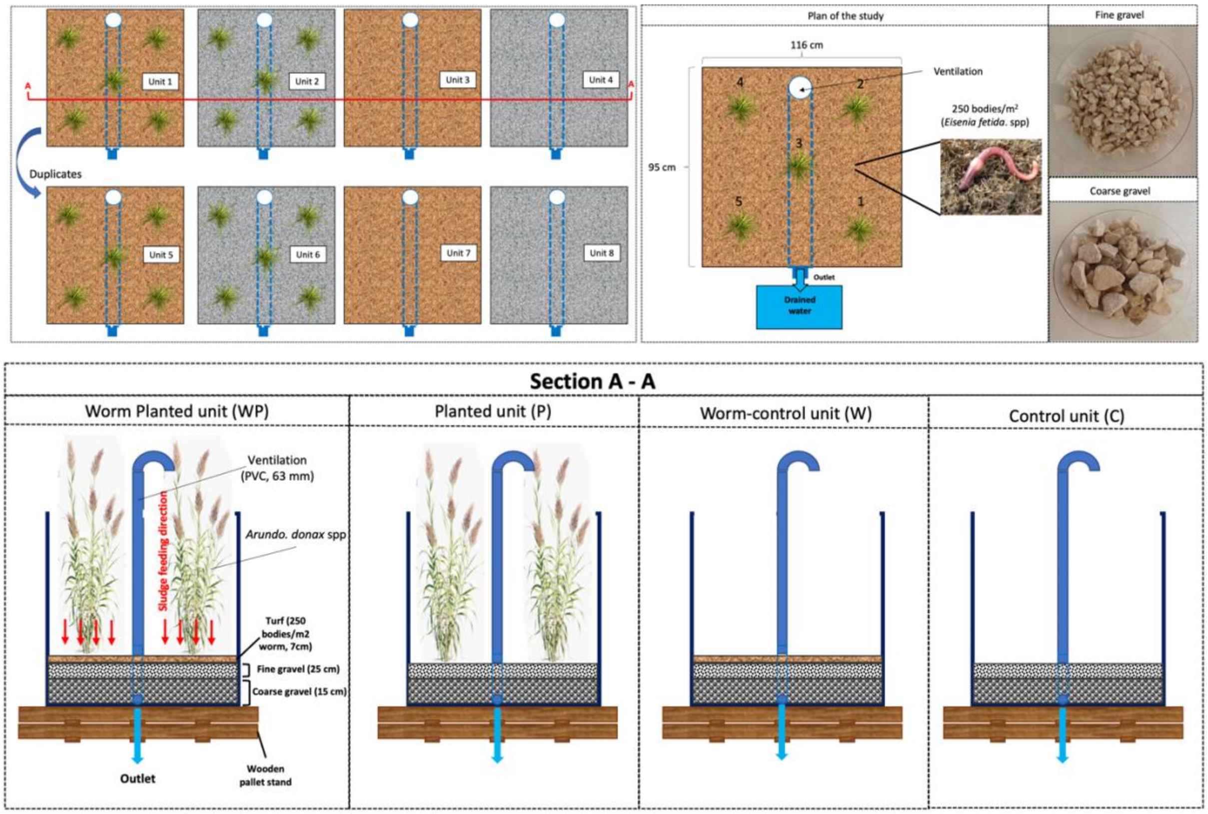


Figure 3. 2. A layout of the experiment

A perforated drainage pipe was installed on the bottom of the beds connected to a storage bucket to collect DW (Figure 3.2). To prevent clogging and to passively aerate the media, the drainage system was connected to a ventilation pipe. The beds were filled with two layers: the drainage layer with 15 cm thickness (coarse gravels, 19 to 25 mm, 38 % porosity) and the transition layer with 25 cm thickness (fine gravels, 4.8 to 9.5 mm, 42 % porosity). Porosities were estimated through the liquid replacement method (Lawrence et al., 2017) of which the total porosity was 39.5%. A layer of turf (Siro 30) was included in WP and W units to accommodate 500 g.*Eisenia fetida*.m^{-2} (250 bodies.m^{-2}). Based on the producer fact sheet, the turf layer had a pH, conductivity, granulometry, and organic matter of 5.5 to 6.5, 40 to 80 µs.cm^{-1}, 0 to 15 mm, and > 70 %, respectively. *Arundo donax* plants were transplanted from a natural wetland located at Instituto Superior Agronomia (ISA), University of Lisbon. The already established habitat from where plants were collected, was considered to ensure the maturity of the transplanted reeds. The collection efforts were aimed to extract plants with a well-developed root system attached to the emerging, thick stems. These transplanted reeds typically exhibited a primary diameter ranging from 1 to 2 cm on average. Transplant was made to WP and P units on April 4th, 2022. To monitor reeds' status, five checkpoints (Figure 3.2) were marked in planted units and the initial status can be found in the supplementary materials (Table 3.1 and Figure 3.1). To establish reeds, between April 4th and May 5th, 2022, planted units were irrigated weekly using Beirolas treated wastewater (10L). On May 13th, a mixed sludge (MS) from Beirolas WWTP was used for feeding regardless of the DS content (mixing the primary and secondary sludges for further processing is a common practice in Portugal). Thus, there was no predefined SLR in contrast with previous studies that measured DS before feeding to achieve a fixed SLR. However, the volumetric loading rate (VLR) of MS was maintained constant (DS content was analyzed after feeding). MS was applied manually in the center of the beds contributing to its uniform distribution and enhancing passive aeration lasting between 5 and 10 min followed by a two-week rest. A total of 24 cycles of MS feeding were applied to each bed in two different stages: 1st stage (May 13th to 29th November 2022) with a VLR of 70 L, and 2nd stage (November 20th, 2022, to May 10th, 2023) with the VLR of 100 L. SLR_1 in the 1st stage with 14 cycles was 40.6 $kg.DS.m^{-2}$. $Year^{-1}$ and SLR_2 in the 2nd stage with 10 cycles was 50.4 $kg.DS.m^{-2}$. $Year^{-1}$ (total SLR = 43.59 ± 14.49 $kg.DS.m^{-2}$. $Year^{-1}$). DS and VS ranged between 11.11 and 45.75 $g.DS.L^{-1}$ (mean = 24.71 ±1 3.67 $g.DS.L^{-1}$), and 8.90-33.65 $g.VS.L^{-1}$ (mean = 19.14 ± 10.29 $g.VS.L^{-1}$), and the average T, pH, and EC of the MS were 22.73 ^{o}C, 5.98, and 1740 $\mu s.cm^{-1}$, respectively (more detail on MS in Table 3.3 of the supplementary materials). From the last feeding day on May 9th, 2023, units were consistently assessed for DS and VS until the first rain event on September 21, 2023. The final assessment took place on September 18, 2023. This meant that from May 9th to September 18th, a span of 132 days, there was a dry period, considered as a resting phase. The final resting was considered to improve stabilization and mineralization of the final residual sludge. An increase of DS and a reduction of VS contents minimize the volume of the residual sludge for further handling (Brix, 2017).

3.3.2. Sampling procedure and lab analysis

Twice a month, MS and RS were sampled to measure DS and VS contents in different RS layers and units. Four samples were taken from the surface (sur) layer and four from the subsurface (sub) layer, which was 5 cm below the surface using a core sampler (Stefanakis and Tsihrintzis, 2011). Through the final resting period, a similar sampling procedure was carried out at four specific time points, namely, 14, 36, 62, and 132 days after May 9th, 2023, to determine DS and VS of the final residual sludge. Samples, equally and randomly distributed from the area of each unit (1.1 m^2), were then mixed to represent the RS of each unit. The thickness of RS was also measured for each unit at the end of the two-week rest multiplied by each unit area to quantify the volume of RS. Having DS content, the volume of water content in RS was estimated. Lab analyses were made in triplicates to determine DS, VS, pH, Electrical Conductivity (EC), and Temperature at the ISA lab. Chemical and physicochemical analyses were conducted according to the Standard Methods for the Examination of Water and Wastewater (APHA, 2017). To estimate the volume of the fed DS for WB estimation, the weight, volume, and DS of MS were firstly measured in a graduated cylinder, and then, DS was subtracted by the weight of MS to determine the weight of water content in MS. Afterwards, the volume of water was obtained (specific weight of water=1000 $g.L^{-1}$), subtracted by the total volume of MS to account DS volume.

Earthworm population was counted through a hand-sorting in the flip and strip test (Gutiérrez-López et al., 2016) in which WP and W units were dug in 20:20:15 cm (width: length: depth). The release of worms either alive or dead bodies together with the date and corresponding unit was registered during the operational period. To measure plant height and number of stems, three plants were marked and monthly measured in height in the five checkpoints. Chlorophyll a, Normalized Difference Vegetation Index (NDVI), and Photochemical Reflectance Index (PRI) were measured using portable devices of CL-01 Chlorophyll Meter, PlantPen NDVI 310, and PlantPen PRI 210, respectively. The plant height and stems as well as the diameter and the leaf length of the harvested reeds were averaged and used in the plant development analysis. On January 13th, 2023, plants were harvested to measure the wet aboveground biomass followed by the dry biomass determination (after drying for three days at 55C^o).

3.3.3. Water balance quantification model

To conduct WB analysis and estimate ET, the inflows and outflows to units were recorded during the two-week rest for the time intervals of 1, 2, 4, 6, and 12 hours as well as 1, 2, 7, 10, and 14 days after feeding. ET was calculated for each cycle according to Eq.1 (Stefanakis and Tsihrintzis, 2011):

$$\mathrm{WL} = \mathrm{P_r} + \mathrm{V_{MS}} + \mathrm{V_{RS\,(A)}} - \mathrm{V_{RS\,(B)}} - \mathrm{V_P} - \mathrm{V_{DW}} \rightarrow \mathrm{ET} = \frac{\mathrm{WL}}{\text{unit area}} \qquad \text{(Eq.1)}$$

Where WL, P_r, and V_{MS} are water loss (L), precipitation volume (L), and water volume (L) in MS. In addition, $V_{RS\,(A)}$ and $V_{RS\,(B)}$ are water volumes in the RS layer before feeding and at the end of each resting period (L). V_P is water volume draining to the mesoporous media (L), and V_{DW} is drained water volume (L) (Stefanakis and Tsihrintzis, 2011). ET is the evapotranspiration rate (mm.year^{-1}). P_r was measured through the onsite weather station. V_{MS}, $V_{RS\,(A)}$ and $V_{RS\,(B)}$ were calculated based on DS content (%) in MS and RS in which the thickness of RS was used to account for V_{RS}. V_{DW} was directly measured by recording the DW volume out of each unit. In the estimation of ET based on Eq.1 and the previous studies, V_P was assumed zero as most of the loss by ET was taken from the RS layer, and at the end of the resting period, V_P stays practically steady and close to zero due to the transpiration and fast drainage.

3.3.4. Statistical analysis

All datasets were assessed for normality through the Shapiro-Wilk test and homogeneity of variance (Bartlett's test). T-test and analysis of variance (ANOVA) were performed via R Studio to check the significant difference between datasets (p-value of 0.05). Correlation analysis and other statistics including min, max, mean, and Standard Deviation (SD) were also conducted.

3.4. Results and discussion

3.4.1. Meteorological analysis

Based on the weather station, the highest and lowest Temperature were 42.2 ^{0}C on July 13th and 3 ^{0}C on January 31st, and the highest and the lowest humidity were 99 and 14 % on October 11th and July 8th, 2022 (Figure 3.3).

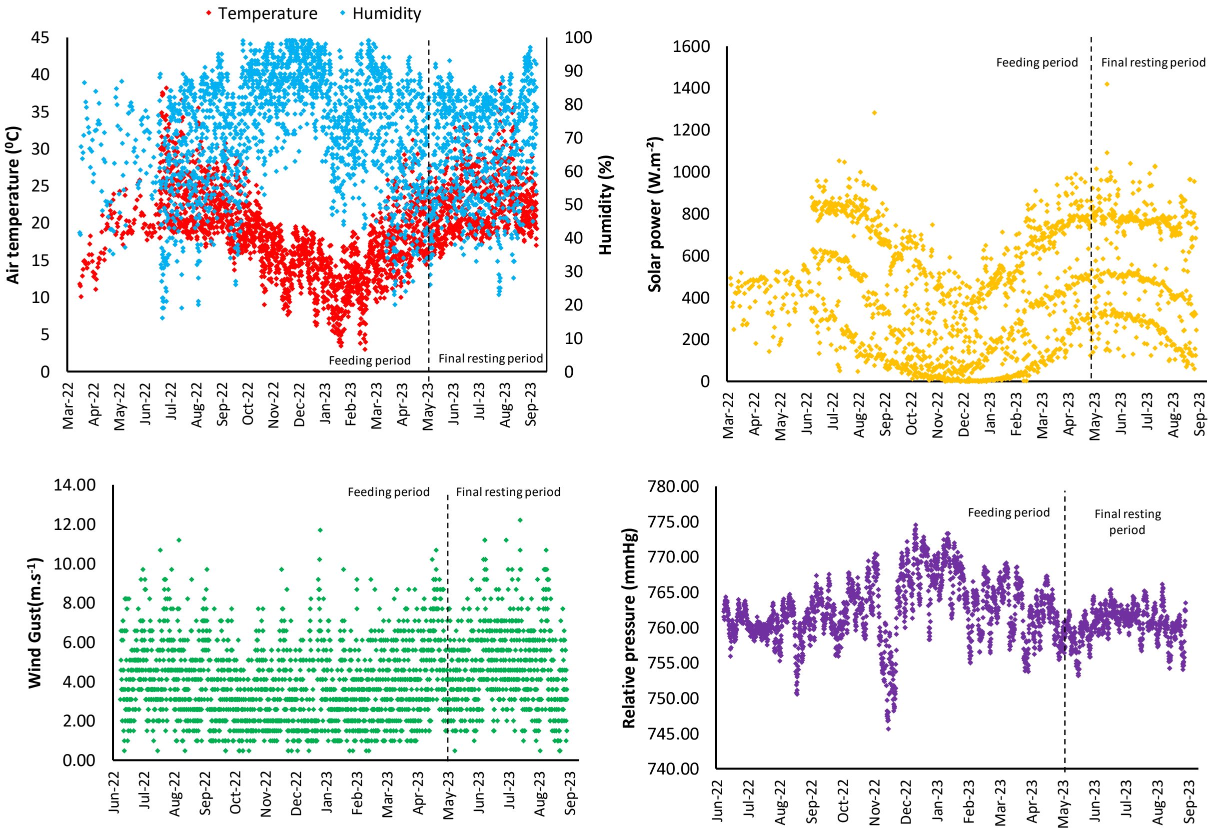


Figure 3. 3. Weather data at Beirolas WWTP

Solar power, wind speed, and gust reached 1283 $W.m^{-2}$ (UV index=10), 15.29 and 12 $m.s^{-1}$ on August 5th, 2022, July 2022, and January 2023, respectively (the average wind direction varied from 177° to 292° to the north direction). The highest and lowest atmospheric pressure were 772.7 (December 27th, 2022) and 745.3mmHg (December 9th, 2022), and the highest daily P_r was on December 13th, 2022, at 130.8 mm in the extreme flood event (total precipitation=906mm during the feeding period).

Accordingly, Lisbon experienced a dry season between April and September 2022, and a wet season between September 2022, and April 2023. The temperature rarely reached extremely low levels, and frosts were infrequent. The dry season can be characterized by long periods of intense solar lights, with minimal rainfall, and low humidity while in the wet season, humidity was higher, solar power was lower and atmospheric pressure was the lowest during the heavy rainfall and highest in January.

Through the final resting period, air temperature was between 12.9 and 35.2 °C with an average of 20.78 °C (±3.42 °C). Humidity levels fluctuated between 23 and 97.2 %, with a mean of 66 % (±15%). The precipitation levels for the months of May, June, July, August, and September were 26, 0, 0, 0, and 48.8 mm, respectively. The maximum solar power reached 1419 W.m^{-2} in June and wind gusts ranged from 0.5 to 12.2 m.s^{-1}, with an average of 4.83 m.s^{-1} (±2.1 m.s^{-1}), while wind speeds varied between 0.2 and 3.8 m.s^{-1}, averaging at 1.54 m.s^{-1} (±0.78 m.s^{-1}). The lowest recorded relative pressure was in June at 753 mmHg, whereas May witnessed the highest at 767 mmHg.

3.4.2. General performance

3.4.2.1. Residual Sludge (RS) analysis

In total, 43588 g.DS.m^{-2}.year^{-1} was fed to each unit through 24 cycles (Figure 3.4.a). The lowest DS was in the wet season (11.11 g.DS.L^{-1} in April 2023) and changed frequently mainly due to seasonal variations and Beirolas WWTP operational condition. For instance, the highest DS of 45.75 g.DS.L^{-1} (on September 19th) dropped to 14.13 g.DS.L^{-1} on November 29th, 2022 (after the extreme rainfall).

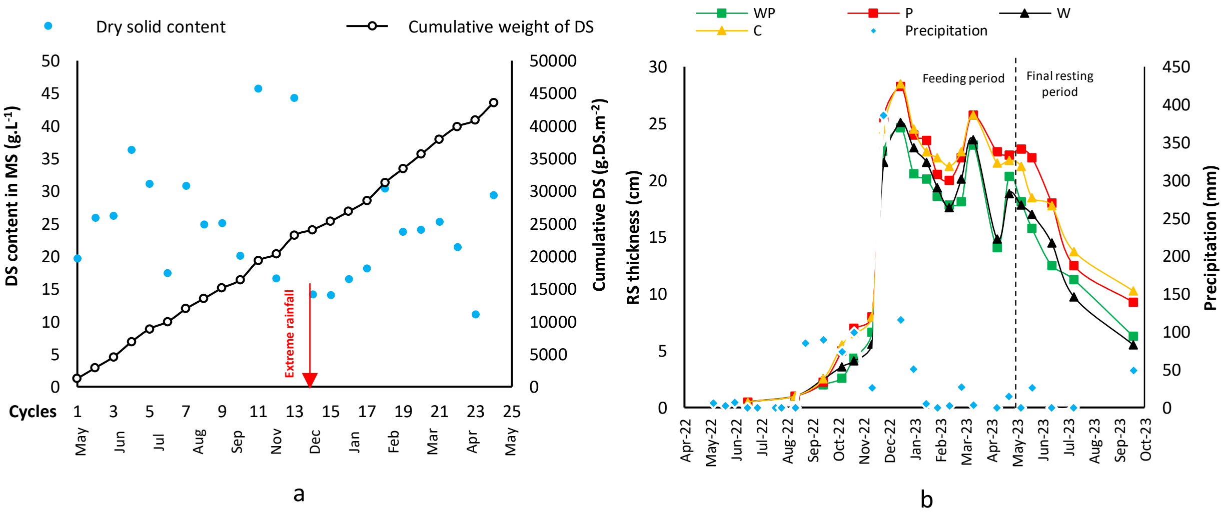


Figure 3. 4. RS formation: a) DS content and cumulative DS, b) RS thickness and precipitation.

The formation of the RS layer by sludge accumulation followed different patterns in dry and wet seasons (Figure 3.4.b). In the dry season, RS thickness was less than 1 cm (DS > 90 % in all units) and increased steadily to 8 cm by cycle 13th (DS < 10 % in all units). RS thickness surged to nearly 30 cm between cycles 14th and 15th (at the extreme rainfall event, DS < 5 % in all units). RS layer expanded and shrank various times due to rain, surface evaporation, and the percolation of RS water content when rain was marginal, for instance, between cycles 17th and 19th or the increase at cycle 21st (the lower DS content was, the more compacted the layer achieved). Worms reduced RS thickness considerably, WP and W units showed a lower thickness compared to P and C units at the end of the feeding period. WP units in comparison with P, and C units had 22 and 15 % less thickness on May 9th, 2023. C Unit also showed 7 % less thickness compared to the P unit due to probably the development of the root system and expansion of the RS layer to an upper position. The above-ground plant development created a shadow on the surface of RS inhibiting surface evaporation and reducing water loss (WL) in the P unit. At the end of the study, WP and W units were not different due to the expansion of RS by the development in the below-ground plant biomass and the shadow on the top of the WP unit limiting the shrinkage. From cycle 1st till cycle 15th, in all units, RS thicknesses are not significantly different while it was different between cycles 16th and 24th. Therefore, it indicates during the dry season probably units were still on the adaptation of the plant root system and worms plus biofilm development in the porous media. On the contrary, WP and W units indicated lower RS thickness in the wet season as worms probably break down organic matter compounds making them available for microorganisms, bacteria, and fungus to feet on it (Suthar, and Singh, 2008). Worms improve microbial activity and mineralization and humification by balancing microbial biomass (Liu et al., 2012; Yang et al., 2013), reducing RS accumulation. Overall, the combined effect of the worms and plants reduced the volume of RS which can increase the lifespan of the system.

Through the final resting period, a consistent reduction in the thickness of the RS was observed, with all units experiencing a decrease of over 52% (Figure 3.4). The reduction rates were more in the WP and W units by 67 and 74 %, respectively (for P and C units were 58 and 52%). The rate of shrinkage was calculated at 0.027 $m.month^{-1}$ for the WP unit and 0.031 $m.month^{-1}$ for the P unit (a higher rate during the initial two months of the resting period). In this study, the accumulation rates of RS for the WP, P, W, and C units were determined to be 0.06, 0.09, 0.05, and 0.1 $m.year^{-1}$, respectively. In temperate climate varied between 0.26 and 0.1 $m.year^{-1}$ (Gholipour et al, 2022). In previous studies in the Mediterranean region, it was 0.09 $m.year^{-1}$ for the STRB unit (or P unit) while the final resting period varying between 25 and 180 days (Stefanakis and Tsihrintzis, 2011, and El-Gendy and Ahmed, 2020). In summary, the WP unit, incorporating both worms and plants, demonstrated a significant reduction in the RS volume compared to previous STRB studies under similar climatic conditions. This synergetic approach improved system performance, reducing the rate of sludge accumulation from 0.1 m.year^{-1} in Mediterranean studies to 0.06 m.year^{-1} in the present study, representing a 40 % enhancement.

The surface DS (sur-DS) and subsurface DS (sub-DS) were significantly different (P-value < 0.05) due to rainfall, evaporation from the surface of RS, plant transpiration from the subsurface layers, solar radiation, and wind (Figure 3.5.a). The sur-DS of the units were not significantly different over the feeding period while the sub-DS of the units were different in which WP (56.14%) and P (56.76 %) units showed higher sub-DS at cycle 9th between January and May 2023. At cycle 24th, WP and P units had sub-DS of 15.55 and 14.54 % while W and C units showed values less than 10 %. Plants extended over the units shadowed the beds inhibiting the penetration of solar lights to the surface layers. As a result, the sur-DS of the units with plants was lower than the unplanted units while the sub-DS in the planted units were higher than the unplanted units. It could be obviously due to the root system and following areal transpiration by leaves. The highest (DS > 90 %) and the lowest (DS < 10 %) DSs for the surface and subsurface layers occurred during the dry and wet seasons. To the end of the wet season, the RS layer got drier slowly (DS > 10 %) as it was observed DW released even after a two-week rest indicating slow infiltration causing lower sub-DS contents.

During the final resting period (Figure 3.5), the sur and sub-DS increased initially in the first month and after rain in May it continued increasing steadily across all units from 21 to 70 %. As the final resting period progressed, sur and sub-DS converged, with the difference becoming marginal, especially in the WP and W units. Hence, after 132 days of resting, the vertical profile of the residual sludge exhibited almost uniform DS.

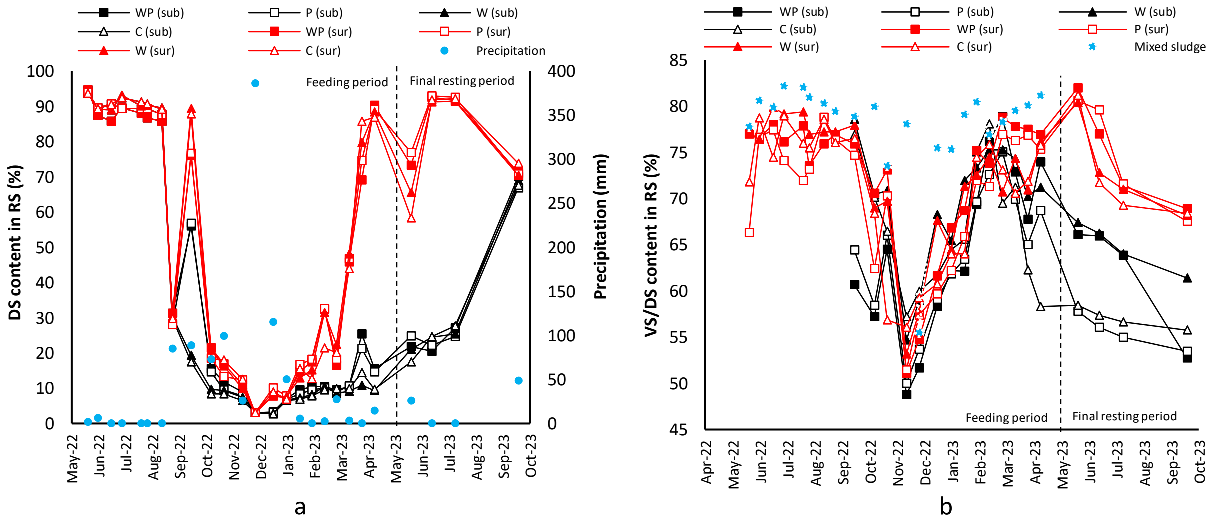


Figure 3. 5. Dewatering efficiency and sludge stabilization: a) DS content for surface (sur) and subsurface (sub) layers, and b) VS/DS content for sur and sub-layers.

In previous studies during feeding period, an annual sur-DS of 33 to 40 % was obtained in temperate and tropical climates (Gholipour et al., 2022) while for this study was higher (49 %). Over feeding period, sur-DS varied between 3 and 94 % (mean = 50 ± 36 %) for P and 3 and 95 % (mean = 49 ± 35 %) for WP units, and sub-DS was between 3 and 57 % (mean = 13 ± 13 %) for P and 3 and 56 % (mean = 14 ± 13 %) for WP units. In the literature after final resting, 45 % of sur-DS were obtained in Dodane et al, (2011) study in arid climates, and 30 % of sur-DS in Gagnon et al, (2013) for polar climates. There is no information available on sur-DS during the feeding period in tropical climates while sur-DS obtained after final resting was 63.4 and 59 % for WP and P units in China (Gholipour et al., 2022). Sur-DS varied between 11.5 and 50 % in WP units according to the previous studies after final resting (Chen et al., 2016; Hu et al., 2020; Zhong et al., 2021). The sur-DS of 62.5 % after final resting was reported by Plestenjak et al, (2021) for an intensified temperate STRB (P unit plus artificial aeration). Based on this study, the DE of a unit with worms and plants can potentially be higher than conventional P units with only plants.

In Figure 3.5.b, VS/DS in the surface (sur-VS/DS) and subsurface (sub-VS/DS) layers were different (p-value < 0.05) with averages of 71 and 66 % for all units respectively. They were lower than VS/DS of MS (78.25 %) over the feeding period indicating sub-VS/DS stabilization. Sur and sub-VS/DS ranged between 65 and 80 % in the dry season and dropped extremely after the occurrence of the wet season (48 to 78 %); however, sur-VS/DS for WP, P, W, and C units were not significantly different likewise for sub-VS/DSs. On average, the lowest sur-VS/DS occurred during November and December 2022 (VS/DS = 55 %) and the highest was in August 2022 (VS/DS = 75 %). In WP units, the sur-VS/DS layer ranged between 51.07 and 78.80 % (mean = 72.48 ± 7.61 %), and for sub-VS/DS, was between 48.79 and 78.86 % (mean = 64.61 ± 9.05 %). Likewise, in P units, the sur-VS/DS layer ranged between 51.45 and 78.77 % (mean = 70.34 ± 7.55 %), and sub-VS/DS was between 49.98 and 75.05 % (mean = 64.13 ± 7.09 %). In W and C units, sur-VS/DS layers ranged between 53.15 and 79.90 % (mean = 72.42 ± 6.91 %) and 56.10 and 78.96 % (mean = 70.80 ± 7.32 %), and for sub-VS/DS, it was between 54.67 and 78.57 % (mean = 69.91 ± 6.48 %) and 57.25 and 78.09 % (mean = 66.92 ± 6.80 %), respectively. Although sub-VS/DS in units were not significantly different, the mean of the sub-VS/DS layer was lower in the WP units (63 %) compared to C units (67 %). On average, WP and P units had 7 % lower sub-VS/DS compared to W and C units. The similarity between units could be due to the continuous MS feeding and RS accumulation without a prolonged resting period. Moreover, plant development in the root system may not extend fully to the RS layer, and the migration of worms to the zones with favorable water content between 10 and 30% of DS, which could limit the effects of worms and plants in the stabilization of RS layer (Suthar, and Singh, 2008; Liu et al., 2012). However, the reduction of VS from MS to sur and sub-layers could be due to various phenomena including the transmission of organic and inorganic matter through worms (Zhou et al., 2020) while worms' presence is dependent on DS, pH, T, and solar power.

During the final resting period, lower VS contents were consistently found on subsurface layers. The difference in sur and sub-VS ranged between 15 and 30 % (P-value < 0.05). The sur-VS/DS contents remained relatively consistent across all units (P-value > 0.05), declining from approximately 81 to 69 %. At the 14-day, the lower content was observed in the P and C units, showing a reduction of 58 %. Conversely, at 132 days, the WP unit displayed the lowest content by 52.78 % reduction. Sub-VS/DS contents at 132 days were 53.5, 61.37 and 55.75 % for P, W and C units. The synergetic effect of worms and plants in the WP unit enhanced the reduction rate, resulting in greater stabilization of the RS compared to the W unit. This could be the utilization of decomposed organic matter by worms and plants and the conversion of organic matter into simpler particles (Makkar et al., 2023). A summary of DE is provided in Table 3.1.

Table 3. 1. A summary of DE

Parameter	Season	Statistics	Mixed sludge	Surface				Subsurface			
				WP	P	W	C	WP	P	W	C
DS (%)	Dry	Min	17	31	28	30	30	**			
		Max	36	95	94	94	94				
		Mean	26	81	82	83	83				
		SD	6	20	22	21	22				
	Wet	Min	11	3	3	3	3	3	3	3	3
		Max	46	90	89	89	88	56	57	30	30
		Mean	24	31	31	32	32	14	13	9	9
		SD	10	28	29	31	31	13	13	4	4
	Final rest	Min	-	71	71	65	58	21	22	21	17
		Max		93	93	92	92	70	67	69	68
		Mean		82	83	80	79	35	35	35	34
		SD		12	11	14	16	24	21	23	23
VS/DS (%)	Dry	Min	14	74	66	76	72	**			
		Max	30	78	79	80	79				
		Mean	21	76	74	78	76				
		SD	5	1	4	1	2				
	Wet	Min	9	51	51	53	56	49	50	55	57
		Max	34	79	77	78	76	79	75	79	78
		Mean	18	70	68	69	68	65	64	70	67
		SD	7	9	8	7	7	9	7	6	7
	Final rest	Min	-	69	68	68	68	53	54	61	56
		Max		82	81	80	81	66	58	67	58
		Mean		75	75	73	73	62	56	65	57
		SD		6	6	5	6	6	2	3	1

** During the dry season, the RS thickness was less than 1cm; therefore, subsurface measurement was not conducted.

Stefanakis and Tsihrintzis (2011) stated, a symbiotic interaction between plants, media, and microorganisms is in the stabilization, mineralization, nutrient cycle, organic matter deposition, and decomposition, which decreases sur and sub-VS layers while worms also contribute to the interaction. Previous studies for P units after final resting showed a sur-VS/DS of 53, 42, and 40 % during feeding in temperate, tropical, and polar climates, respectively (Gagnon et al., 2013; Gholipour et al., 2022) while in the P unit of this study, the sur-VS/DS in a temperate climate ranged between 51 and 79 %. Therefore, the sur-VS/DS of the present study is higher than the similar studies during the feeding period probably due to the continuous feeding without final resting. The sur-VS/DS in the P unit were at 67.4 and 52 % after final resting for other temperate (Stefanakis and Tsihrintzis, 2011) and tropical (Cui et al., 2015) studies, respectively. The values of this study being for the feeding period, sur-VS/DSs in P units average 70.33 ± 7.54 %, which is comparable with Stefanakis and Tsihrintzis's study. The sur-VS/DS of WP units in tropical climates studies was at 33.1, 33.6, and 41.6 % after final resting, respectively (Chen et al., 2016; Hu et al., 2020; Zhong et al., 2021). In this temperate study during the feeding period, the sur and sub-VS/DS in WP units were average 72.48 ± 7.61 % and 64.60 ± 9.05 %, which is higher than the values for tropical studies after final resting. The lower values of tropical climates could be because of precipitation washing VS content and final resting. In previous temperate climate studies after the final resting, VS/DS ratios of 39, 67 and 52 % were reported (Gholipour et al., 2022) with final resting periods of 60, 120 and 365 days. In contrast, this study achieved a sub-VS/DS ratio of 61.37 % after 132 days of resting which aligns closely to the previous studies. In W-STRB unit (WP unit), it was 41.6 % in a tropical climate study by Zhong et al, (2021), whereas it was 52.78 % in this study. Overall, the inclusion of worms in the STRB system appeared to enhance the stabilization by approximately 10 % during the final rest.

3.4.3. Earthworm analysis

The number of worms in units was statistically different in the flip and strip test ($p\text{-value} < 0.05$) and the population of worms varied during the study period, increased in the dry season, and reduced in the wet season (Figure 3.6.a). On the May 6th, 2022, all units contained equally ten bodies (250 bodies.m^{-2}) and then reached the maximum on August 25th with 18, 67, 19, and 41 bodies in WP unit 1, W unit 3, WP unit 5, and W unit 7, respectively. By the wet season, the number of worms decreased till October 3rd, particularly it was less than the initial population (four bodies) in WP units. Due to the high-water content during November 29th, 2022, and January 18th, 2023, RS flipping was not possible as Zhou et al (2020) also stated worms move to the bottom of media based on water content, temperature, and oxygen availability.

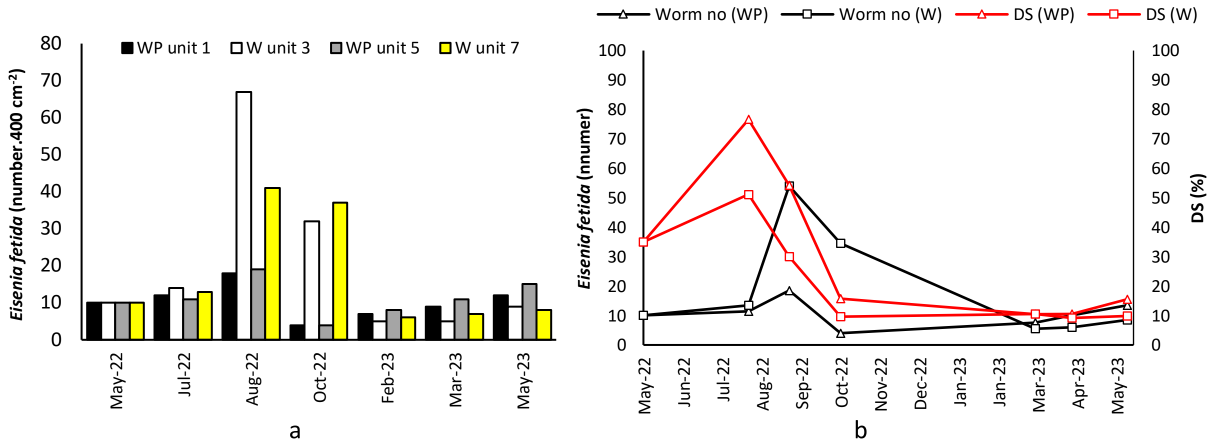


Figure 3. 6. *Eisenia Fetida* a) population, and b) population versus DS content

During the wet season, as observed worms' mortality increased and on October 13th, 2022, the dead and alive bodies were drained from W units 3 and 7, which occurred after six months of feeding and lasted until April 27th, 2023 (Figure 3.4 in the supplementary materials). At the end of the wet season, the population started increasing until May 9th. The growth rates between February and May were 71, 80, 88, and 33% for WP unit 1, W unit 3, WP unit 5, and W unit 7, respectively. The worms' presence in the test (the top 15cm layers) increased (DS>20%) in the dry season while it decreased (DS<20%) in the wet season (Figure 3.6.b), as stated by Suthar and Singh (2008) and Liu et al (2012), it is less proven for the worms to accommodate on the top 15 cm as DS is less than 20 %. However, to the end of the wet season and by higher DS, the presence gradually increased averagely by 79 and 57 % in WP and W units between Feb 28th and May 9th, respectively. There is not any data in worms for the layers deeper than 15 cm since the test was infeasible not to disturb the homogeneity of the media. Accordingly, *Eisenia fetida* adapted to WP units even in a completely buoyant situation, continuous sludge feeding, and seasonal variation increasing WL and DS. However, the accumulation of RS and the conversion to the wet season impose worms to an unfavorable and vulnerable condition (Liesch et al., 2010) in which oxygen is less prone during the wet season in the media due to RS formation. Liu et al (2012) stated worms as detritivores require VS to decay for the gut digestion mechanism and the reduction of VS during the wet season may increase mortality.

3.4.4. Plant analysis

The ultimate reeds' status showed the diameter of stems varied between 1.1 and 3 cm (mean = 2.3 ± 4.1 cm). It was observed that the initial 31 cm leaves grew on average to 70 cm across all units (no significance difference between WP and P units, P value > 0.05). According to established literature, apart from leaf length and the development of the root system, a stem diameter between 2 and 3 cm can be considered an indicative factor of maturity, with leaves reaching nearly 70 cm in length (Spatz et al., 1997; Eid et al., 2016). It is also worth noting that the ultimate height of a mature stem can vary depending on ideal growing conditions. As shown (Table 3.2), between April 2022 and January 2023, *Arundo donax* above-ground wet biomass was 14.6, 18.2, 8.8, and 14.2 kg.m^{-2} (mean water content = 88 %), and based on this, the dry biomass accounts for 1.9, 2.1, 1, and 1.7 kg.m^{-2} in units 1 (WP), 5 (WP), 3 (W), and 7 (W), respectively.

Table 3. 2. *Arundo donax* above-ground biomass

Parameters	Unit 1 (WP)	Unit 5 (WP)	Unit 2 (P)	Unit 6 (P)
Wet biomass (kg.m^{-2})	14,6	18,2	8,8	14,2
Water (kg.m^{-2})	12,7	16,1	7,8	12,5
Water content (%)	87,2	88,7	89,0	87,7
Dry biomass (kg.m^{-2})	1,9	2,1	1,0	1,7

During April 4th and May 13th, plants grew continuously in all units and the growth was not significantly different (P-value > 0.05) when irrigating with treated wastewater (Figure 3.7.a) although new shoots appeared (Figure 3.7.b). The number of stems increased from 3 to 5, 3 to 4, 2 to 4, and 3 to 5 for WP unit 1, WP unit 5, P unit 2, and P unit 6, respectively. After feeding with MS, the plant height increased from the initial plant height by 100, 113, 71, and 154 cm in WP unit 1, WP unit 5, P unit 2, and P unit 6, respectively, indicating a growth between 7 and 15cm.month^{-1} (mean = 11 ± 3.4 cm.month^{-1}) during May 13th, 2022, to Jan 11th, 2023 (Figure 3.7.a). New shoots and stems showed higher growth rate, between 12 and 19 cm.month^{-1} (mean = 15.7 ± 2.9 cm.month^{-1}). In this study, the reeds can be considered mature as their stem diameter reached a maximum of 3 cm, and both the leaves and height exceeded 70 and 200 cm, respectively.

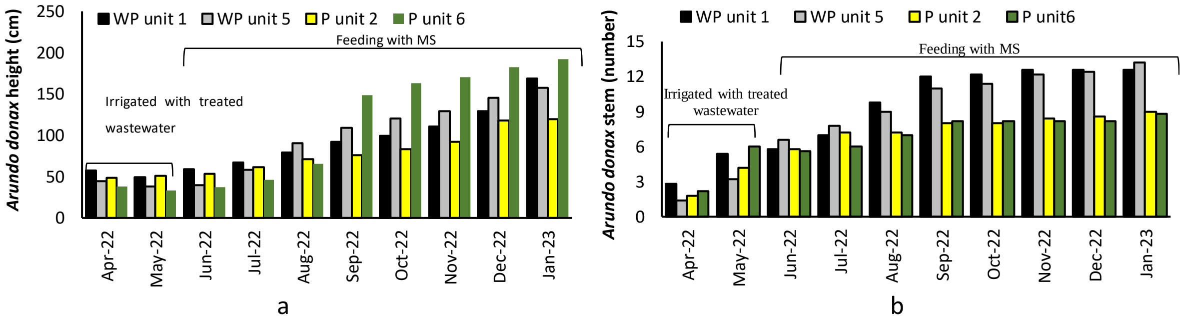


Figure 3. 7. Plant development: a) *Arundo donax* growth, b) number of stems

The growth was higher in the dry season (averagely 100 and 112 cm from April 4th to August 29th, 2022) compared to the wet season (62.3 and 43.8 cm from September 19th to January 9th, 2023) for WP and P units, respectively. New stems appeared rapidly in the dry season from 1 to 10 stems in the WP unit and from 2 to 6 stems in P units while during the wet season, the appearance was marginal with one stem for all WP and P units. WP units had more stems compared to units without worms and had an average of 43% higher biomass production compared to P units.

The *chlorophyll* a content (average annual=19.85 mg.g^{-1}, no significant difference between WP and P units) increased firstly from 15 to 20mg.g^{-1} (April 4th to June 17th, 2022), and decreased to 14 mg.g^{-1} on September 29th, 2022, and then, it reached 37 mg.g^{-1} on January 11th, 2023. The average NDVI and PRI indexes (like chlorophyll a, no significant difference) were 0.3281 and 0.1615 in WP and P units, respectively (Figures 3.2 and 3.3 in supplementary materials). The study showed *Arundo donax* established well into a W-STRB system and the addition of worms affected positively the development of the plant throughout the study period.

3.4.5. Water balance analysis

In the dry season, in total 630 L of MS plus 100L of precipitation entered each unit of which 12.79 L were DS volume from MS fed (Table 3.3) resulting in 717 L of the total influent for each unit. DW volumes were different (143, 288, 274, and 319 L for WP, P, W, and C units) due to plants and worms. WP units showed 50, 47, and 55 % lower DW volumes compared to P, W, and C units. WL were 577, 427, 442, and 397 L for WP, P, W, and C units, and varied among units in which WL in WP was 35, 31, and 45 % higher than P, W, and C units, respectively. Comparing W and C units, WL was 11% higher indicating the contribution of worms. WL in the W unit was marginally higher (1.1 %) than in the P unit. The water content in the RS layer for all units was less than 2L was not significantly different (p-value > 0.05).

In the wet season, the total influent was 2223 L in each unit including 1326 L of water from MS and 897 L of precipitation resulting in 1096, 1464, 1677, and 1903 L of DW. WP units had 22, 35, and 42 % lower DW in comparison to P, W, and C units, respectively, due to plants and worms. The water content in the RS layer was less than 80 L in all units. Moreover, WL in WP units was 53, 125, and 335 % higher than in P, W, and C units, respectively. In the dry season, P and W units had only 1.1 % WL difference while in the wet season, the P unit had 47 % higher WL indicating the effect of plant development from dry to wet seasons. To estimate the effect of worms in WL, comparing W and C unit shows 92 % higher WL.

Table 3. 3. Water balance analysis

Season	Units	MS fed (L)	DS in MS (L)	Influent from MS (L)	Precipitation (L)	Total influent (L)	Total DW (L)	* Water content in RS (L)	Water loss (L)
Dry	WP	630	12,79	617	100	717	143	1,56	577
	P	630	12,79	617	100	717	288	1,35	427
	W	630	12,79	617	100	717	274	1,15	442
	C	630	12,79	617	100	717	319	1,19	397
Wet	WP	1350	24,09	1326	897	2223	1096	74,31	1055
	P	1350	24,09	1326	897	2223	1464	72,03	687
	W	1350	24,09	1326	897	2223	1677	79,68	467
	C	1350	24,09	1326	897	2223	1903	78,40	242
Total (full year)	WP	1980	36,88	1943	997	2940	1239	74,31	1633
	P	1980	36,88	1943	997	2940	1752	72,03	1115
	W	1980	36,88	1943	997	2940	1950	79,68	910
	C	1980	36,88	1943	997	2940	2222	78,40	640

* Water content in RS at the end of dry and wet seasons

In total (full year), the result showed that WP and C units drained the lowest and the highest volumes, and WP had 46, 80, and 155 % higher WL than P, W, and C units. Comparing P and C units showed 74 % WL enhancement by plants and likewise, comparing W and C showed 42 % enhancement by worms. In a study from Stefanakis and Tsihrintzis, (2011), the higher SLR was, the higher ET observed. A similar finding was found in this study once SLR increased from SLR_1 to SLR_2 (40.6 to 50.4 kg.DS.m^{-}

^{2}.year^{-1}). This study ET (1633 L/1.1 m^{2} = 1014 mm.year^{-1}) is comparable with the study of Stefanakis and Tsihrintzis, applying three SLRs in different units including 30, 60, and 75 kg.DS.m^{-2}.year^{1} resulted in the annual ET of 938, 1846, and 2481 mm for the P unit with *Phragmites australis*, respectively. In the present study in the WP unit, ET was 1485 mm.year^{1} (mean SLR = 43.59 ± 14.49 kg.DS.m^{-2}.year^{1}) while ET in the Stefanakis and Tsihrintzis study for the P unit was 1846 mm.year^{1} (SLR = 60 kg.DS.m^{-2}.year^{-1}). Plants, worms, duration of wet season, variations in plant development, and precipitation could be the variables for differences between the two studies.

According to Sun et al (2020), multiple factors increase WL by worms as stated worms require water for continuous metabolic activities to burrow and lubricate burrows with mucus facilitating relocations. Yang et al (2013) stated worm dehydration is due to cutaneous respiration and moisture conservation, gas exchange limitation through the emission of cuticles on their skin, and the closure of spiracles. Sun et al (2020) stated worms require water for the natural defense against desiccation while burrowing. Overall, WL enhanced 46% in a combined unit with plants and worms compared to a unit with only plants; therefore, worm assistance could enhance dewatering efficiency in a sewage sludge dewatering process.

3.4.6. Water percolation analysis

To estimate the water percolation rate (WPR), DW volume was recorded based on the time passing after feeding for cycles 1, 7, 15, and 24 (Figure 3.8.a to e). The result showed 97, 93, 76, and 77 % of the total DW volume percolated after 2hours in the 1st cycle for the WP, P, W, and C units while for the 7th cycle, it was 40, 48, 35, and 38 % after 2hours, respectively (an average 50 % reduction in WPR for all units since cycle 1st). Likewise, 49, 40, 44, and 35 % of the water percolation occurred after one day in the 15th cycle whereas 20, 21, 31, and 33 % of water percolated after two days in the 24th cycle. Thus, WPR reduced 99 % during the wet season in all units regardless of bed configurations. The amount of DW decreased over time (Figure 3.8.e) due to the development in the root system (Brix, 2003), reduction of porosity (Nielsen, 2023), and increase in the worm's population (Wang et al., 2021) and the formation of RS layer (Brix, 2017). The upper plant ground biomass was higher in the WP unit compared to the P unit, probably a higher development in the rhizome, resulting in a lower WPR in the WP unit. El-Nahhal et al

(2014) stated that the porosity of RS is high due to the dust-size particles and its unique structure and has a high capacity like a sponge to store water (causing a low WPR), contributing to DW percolation even after a two-week rest; therefore, RS layer was not completely dry, and the remaining water content drained in the next cycle. WPR has not been often reported in previous studies; however, in the study of Stefanakis and Tsihrintzis, (2011), more than 30 % of DW percolated after two days from feeding time in P units in (over one year of operation). In this study, it was after two days in which 20, 21, 31, and 33 % of DW were obtained for WP, P, W, and C units, respectively.

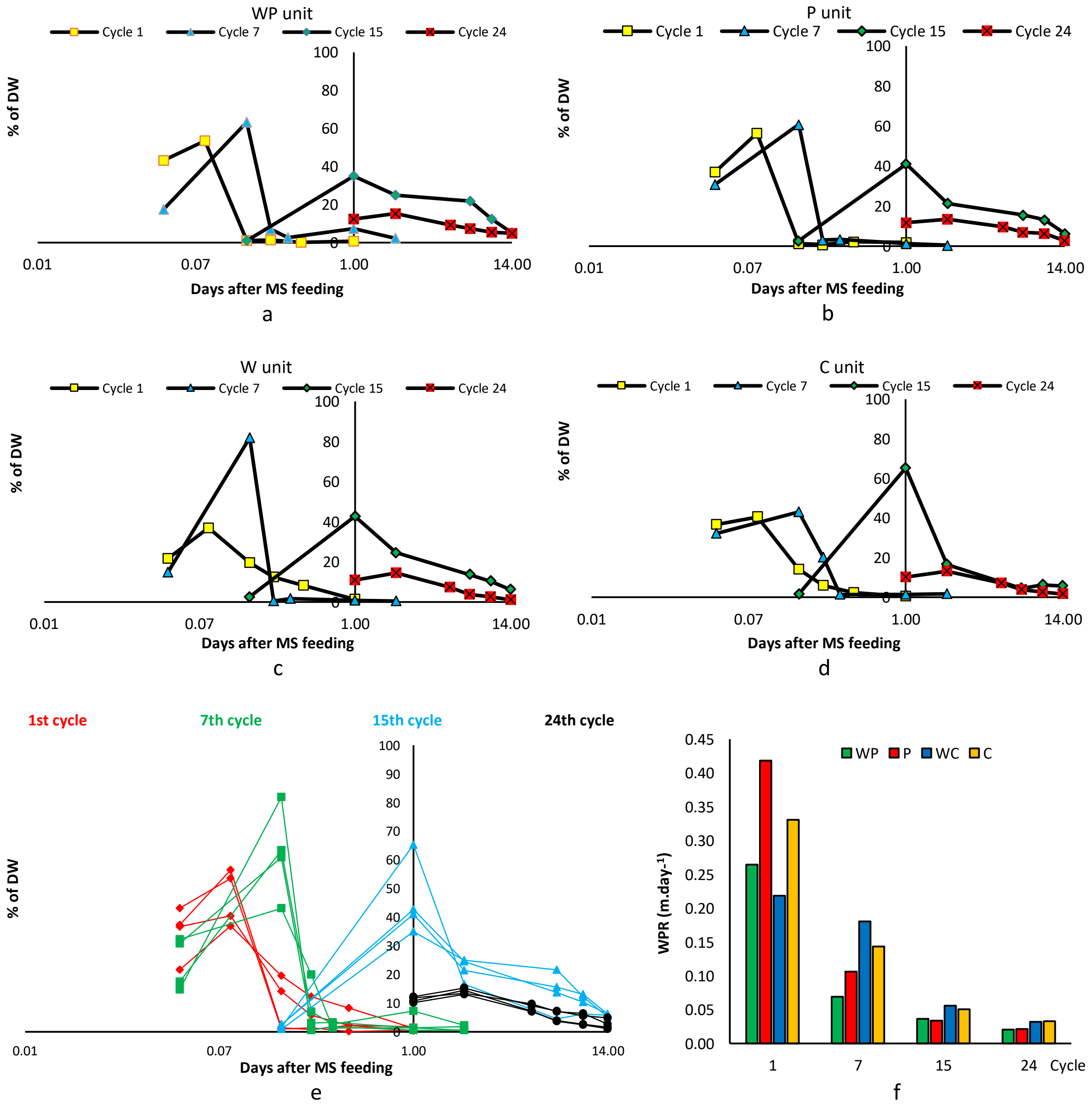


Figure 3. 8. The percentage of DW after MS feeding a) WP, b) P, c) W, d) C units, e) drained water in all units f) HC

From Figure 3.8.f, WPR of beds ranged between 0.2 and 0.5 m.day^{-1} in cycle 1st in which WP and W units varied between 0.2 and 0.3 m.day^{-1}, and P and C units ranged between 0.3 and 0.4 m.day^{-1}. After cycle 7th, WPR of all beds reduced and ranged between 0.1 and 0.2 m.day^{-1}while toward the end of the study period, WPR in all units was almost equal (< 0.1 m.day^{-1}).

3.4.7. Water loss analysis and evapotranspiration estimation

The mechanisms for WL in WP and P units were basically on DW, and ET losses (Figure 3.9), and likewise, for W and C units were through DW, and evaporation. In WB analysis, a fraction of the water content also stored in the RS layer. This fraction increased from 4.82 and 2.17 % to 35.63 and 56.76 % for WP and P units, and from 2.15 and 2.15 % to 38.55 and 46.72 % for W and C units in dry to wet seasons, respectively. This could be due to the role of the RS layer in water retention creating RS thickness differences between dry and wet seasons. Based on onsite observations, DW volumes on WB, and WPR analysis, it can be stated that the flow passed through the RS layer slowly in the wet season and continuously drained even after the two-week resting period. Based on the flip and strip test, the plant root system did not extend fully into the RS layer, and worms did not accommodate fully in the RS layer; therefore, they had a limited effect on the water content in RS layer.

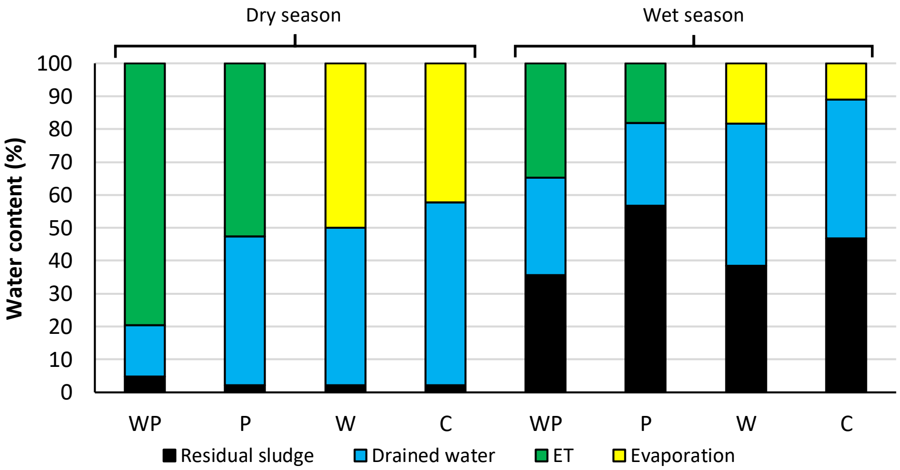
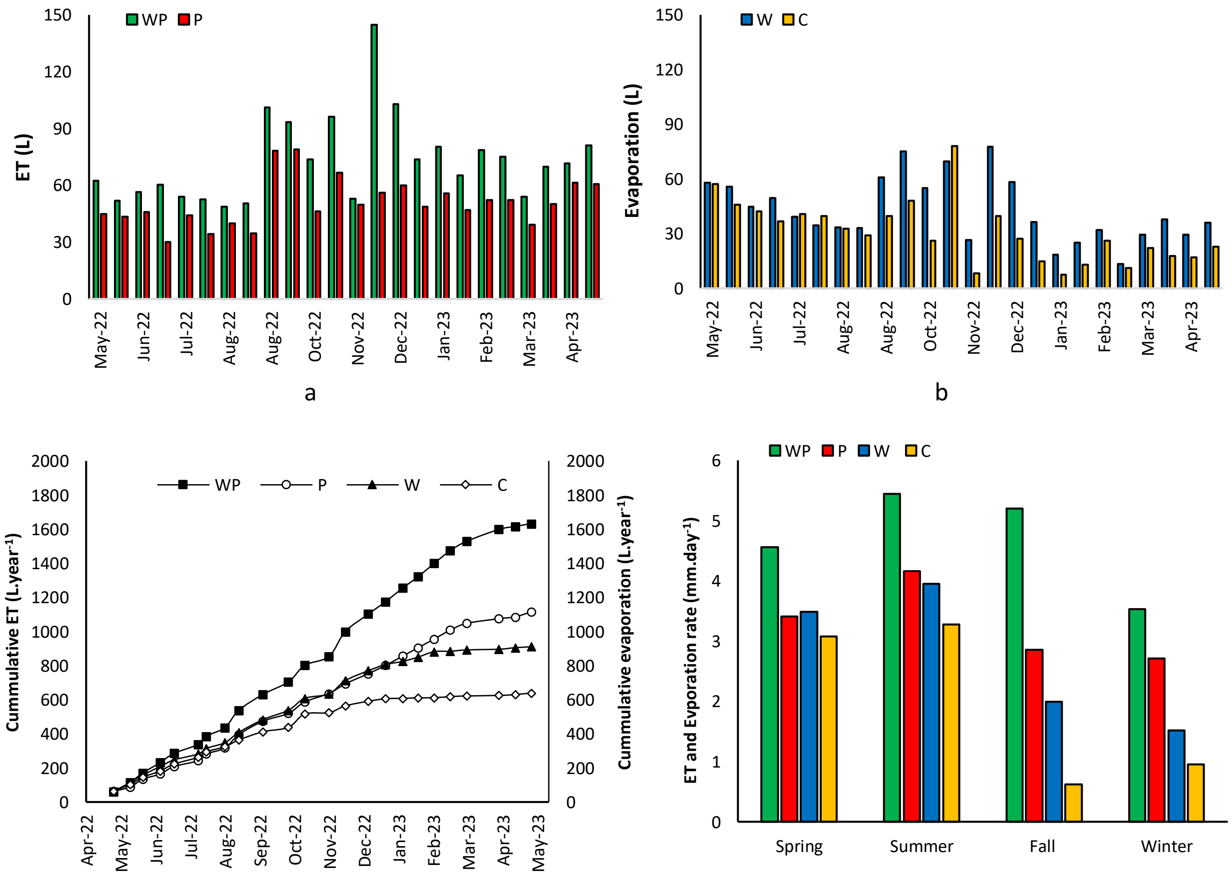


Figure 3. 9. Contribution of each mechanism in water balance

WL through DW increased from 15.59 to 29.70 % in the WP unit and decreased from 45.15 to 25.02 % in the P unit from dry to wet seasons. This indicates the role of worms in the reduction of WL through DW volume during the dry season compared to the P unit. However, in the wet season, WL through DW was quite similar (29.70 and 25.02 % for WP and P units) since units were developed and adapted. WL through DW for W and C units reduced from 47.97 to 43.18 % and from 55.53 to 42.17% for dry and wet seasons. Units of WP and P also lost water through ET which were 79.58 and 52.66 % in the dry season and 34.65 and 18.21 % in the wet season. In addition, WL through evaporation in W and C units were 49.86 and 42.31 % in the dry season, and 18.25 and 11.09 % in the wet season. Overall, WL was higher through the ET mechanism for WP and P units and through DW for W and C units in the dry season while water content in RS was the most dominant fraction for all units in the wet season. In Stefanakis and Tsihrintzis (2011) study in the Mediterranean region (P unit), WL was measured after 1.5 years, which rested for six months without feeding, showed 83 and 15 % of WL were through ET and DW mechanisms, respectively while only 2% of water remained in RS layer. The present study before the final rest in the P unit showed 18 and 25% of WL were through ET and DW mechanisms as well as 57 % was in RS layer. Therefore, the role of final resting is crucial to increase WL through ET. The present study before the final rest in the WP unit showed 35 and 30% of WL were through ET and DW mechanisms (water content of RS layer was 36 %) showing WP system is more effective in the enhancement of WL through ET compared to the P system. Through the final resting in WP unit, DS started at 22 % after 14 days (corresponding to 18 cm thickness of RS with a water content of 155 L) and eventually reached 70 % at 132 days (a water content of 20 L in RS layer with 6 cm thickness). This transition indicates a water loss of 135 L during the resting period (1mm.day^{-1}). This could be due to mainly through evapotranspiration, evaporation, worms, and drainage.

In Figure 3.10.a, the highest and the lowest volumetric ET occurred in wet and dry seasons for WP units by 145 (after heavy rainfall) and 48L (August) while both values of ET for P units by 79 and 30 L were in dry season, August 2022. The highest and lowest evaporations were in the wet season by 78 and 11 L in the W unit and by 79 and 7 L in the C unit, respectively indicating the dependency on the influent amount (through VLR or precipitation). In Figure 3.10.c, cumulative ETs for all units were not different for the first two months of feeding while afterward WP and P units were significantly different, and likewise in W and C units in terms of evaporation. The cumulative ET of P and cumulative evaporation of W units followed a similar pattern until January 2023, and then, the P unit reached 1115 L of cumulative ET compared to the W unit with 910 L of cumulative evaporation. The slope changes of cumulative ET in WP and P units were constantly upward regardless of seasonal changes while the slopes of cumulative evaporations in W and C units were upward in the dry season and flattered during the wet season.



a

b

c

d

Figure 3. 10. WL: a) volumetric ET b) volumetric evaporation c) cumulative ET and evaporation d) daily ET and evaporation

The highest daily ET reached 5.44 mm in summer ranging between 2 and 4 mm during the wet season for the WP unit (Figure 3.10.d) while it was 4.16 mm in the dry season ranging between 2 and 4 mm during the wet season for the P unit. ET rates of WP and P units are significantly different (Figure 3.10.c). Meanwhile, the W unit showed 11 and 93 % higher evaporation (in total showed 43 %) compared to the C unit during dry and wet seasons. In this study, the maximum daily ETs for WP and P units with 5.44 and 4.16 mm in the dry season are higher than the ET obtained by Velychko and Dupliak, (2021), Vincent et al, (2011), Mennerich et al, (2017) studies planted with *Phragmites australis* in a temperate climate (P units), which were 1.78, 2.58, and 1.34 mm, respectively. Chen et al. (2016) study showed WP units planted with *Phragmites* and *Eisenia fetida* increased ET by 42.9 % in tropical climates while in our study, it was 46 % in a temperate climate.

3.5. Conclusions

The application of the sludge treatment reed beds (STRB) system assisted with *Eisenia fetida* (W-STRB) and planted with *Arundo donax* was experimentally tested and fed with a mixed sewage sludge (MS) in a temperate climate. The focus was on dewatering efficiency (DE) and water balance (WB). The performance of the units was influenced by two seasons: dry and wet, as recorded by an onsite weather station. During the dry season, MS had high dry solid (DS) content, which decreased during the wet season. The thickness of the residual sludge (RS) layer was narrower in the dry season but increased during the wet season due to rainfall. DS and volatile solid (VS) contents of the RS layer were higher in the dry season (DS > 80 %) compared to the wet season (DS < 15 %) for all units. The subsurface layers had 5 % lower VS/DS compared to the RS surface, indicating stabilization during the feeding period. The W-STRB unit showed higher DS and lower VS compared to other units, resulting in a 22 % reduction in RS thickness. After 132 days of final resting, W-STRB indicated the highest DS (71 %) and the lowest VS (53 %). As a result, volume of RS layer reduced 65 % leading to a 6 cm sludge residue; therefore, the inclusion of worms in STRB showed 10 % improved stabilization. The population of worms increased during the dry season but decreased during the wet season (DS < 20 %), while the W-STRB unit exhibited increased worm reproduction. The W-STRB unit also demonstrated 43 % higher above-ground plant biomass production. All units experienced an average monthly increase of 16 cm in plant height, with a higher number of stems in the W-STRB unit. Water loss (WL) was influenced by inflow volumes such as rainfall and volumetric loading rate. The W-STRB unit had a higher annual WL of 1014 mm, particularly during the wet season. DE was 46 % higher in the W-STRB unit compared to the STRB, indicating the positive impact of worms. Water percolation rate reduced by 99 % during the study period, with a faster reduction rate in the wet season, reaching less than 0.1 m per day for all units. Three processes of WL were identified: WL through DW, which was higher in the wet season, WL through ET, with the highest ET observed in the W-STRB unit (5.44 mm per day) during the dry season, and WL through evaporation. Overall, the synergistic combination of worms and plants enhanced DE, reduced RS accumulation, increased worm population and plant biomass production, improved WL, and positively influenced WB and the general performance of the W-STRB unit. Therefore, W-STRB presents a sustainable and viable alternative to conventional and intensive technologies for sewage sludge management. It not only enhances DE in STRB but also increases the potential utilization of W-STRB within the circular economy framework in smart cities.

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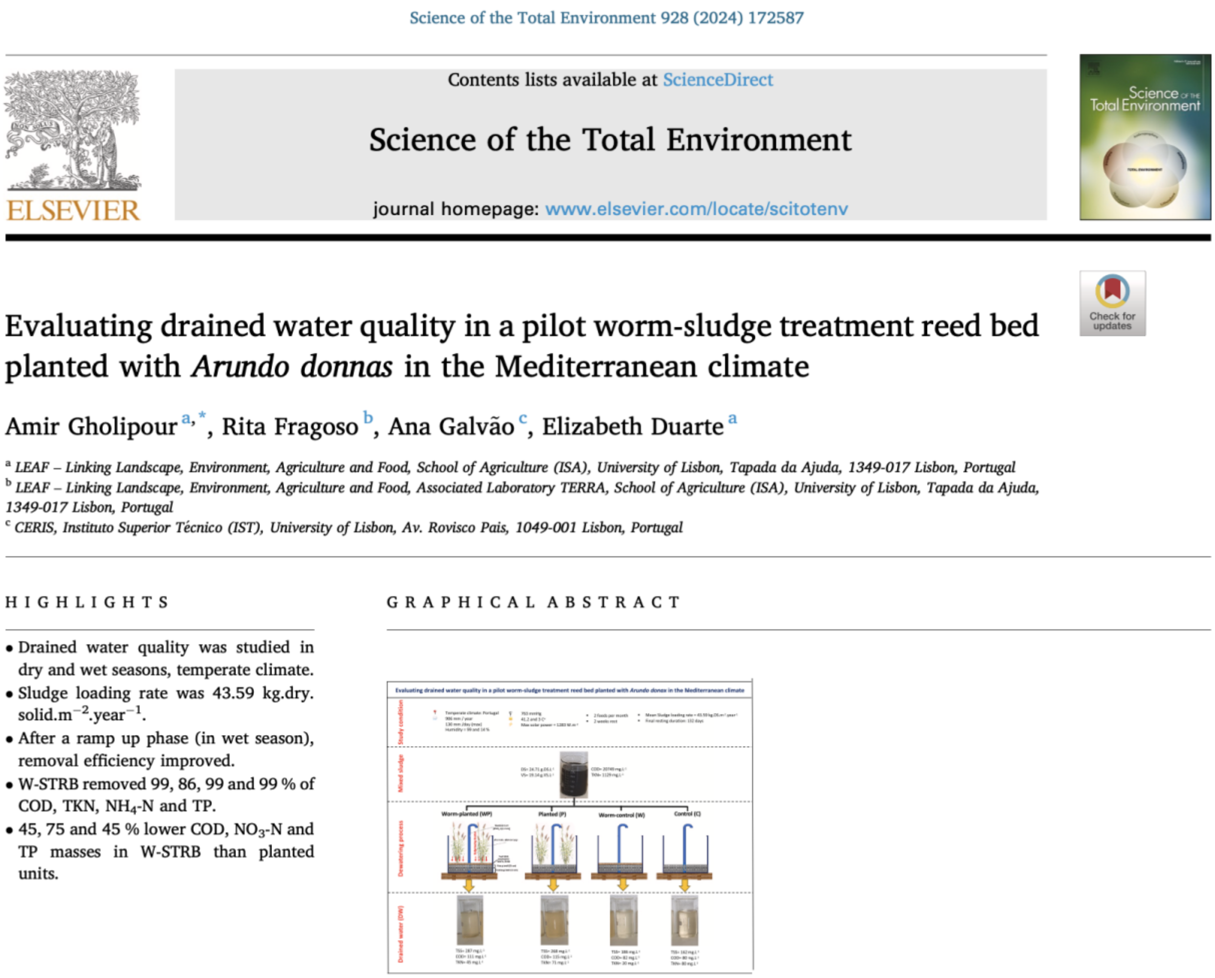
Chapter 4. Evaluating drained water quality in a pilot worm-sludge treatment reed bed planted with *Arundo donax* in the Mediterranean climate



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Evaluating drained water quality in a pilot worm-sludge treatment reed bed planted with *Arundo donnas* in the Mediterranean climate

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HIGHLIGHTS

- Drained water quality was studied in dry and wet seasons, temperate climate.
- Sludge loading rate was 43.59 kg.dry. solid.m^{-2}.$year^{-1}$.
- After a ramp up phase (in wet season), removal efficiency improved.
- W-STRB removed 99, 86, 99 and 99 % of COD, TKN, NH_4-N and TP.
- 45, 75 and 45 % lower COD, NO_3-N and TP masses in W-STRB than planted units.

GRAPHICAL ABSTRACT

Up to date citation: 1

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4.1 Abstract

This study evaluated the impact of incorporating earthworms (*Eisenia fetida*) on the drained water quality from a sludge treatment reed bed. The experiment encompassed four setups of treatment beds in two replicates: planted with *Arundo donax* and addition of earthworms, planted without earthworms, unplanted with earthworms, and treatment bed without plants nor earthworms as control. The units were fed every two weeks with mixed sewage sludge, a blend of primary and secondary sludge over 24 cycles. The mixed sewage sludge had mean dry and volatile solid contents of 24.71 g.DS.L^{-1} (± 13.67) and 19.14 g.VS.L^{-1} (± 10.29) resulting a sludge loading rate of 43.59 kg.DS.m^{-2}.year^{-1} (± 14.49). The inclusion of earthworms in the planted unit reduced release masses of total suspended solids, chemical oxygen demand, nitrate and phosphorous by 43, 45, 75 and 45 % compared to the planted unit. Plant biomass production increased by 43 % with the earthworm presence. The removal efficiency of the units improved after a ramp-up phase (after six months feeding) of which the concentration of TSS, COD and *Escherichia coli* met limits for water reuse while nitrogen components and phosphorous surpassed the limits. The planted unit with earthworms removed 99 and 99 % of TSS and COD, respectively. Overall, water loss namely through evapotranspiration and earthworm hydration need, positively correlated with pollutant concentration, and earthworm-planted unit had 46 % higher water loss compared to control unit.

Keywords: earthworm, sewage sludge, constructed wetland, wastewater treatment, nature-based solution

4.2 Introduction

The world is facing a global risk of diminishing freshwater resources, which hampers water accessibility. Given the pressing concerns related to water scarcity and pollution, particularly in the Mediterranean region, it is vital to introduce sustainable alternatives for safe water reuse. Wastewater treatment plants (WWTPs) could play a fundamental role in addressing water scarcity challenges contributing to meeting water demands; however, WWTPs face challenges such as finding a cost-effective treatment technology (Gholipour *et al.* 2023a). Wastewater treatment concerns treatment of liquid and solid phases and technologies related to the liquid phase have been investigated thoroughly in the literature (Liu and Lipták, 2020). However, the solid phase still needs more attention, especially the process of sewage sludge dewatering. Typically, sewage sludge contains a high proportion of water, with less than 1 to 5% of dry matter (Daee et al. 2019; Gholipour *et al.* 2022); therefore, water content of the sludge could be a considerable unconventional water source although it may need additional treatment. In addition to commonly used conventional technologies for treating sewage sludge, like filter belt press and centrifugation, nature-based solutions (NBS) like sludge treatment reed beds (STRB) can be an alternative approach.

STRB is a type of treatment wetland which is cost-effective in dewatering sewage sludge (Brix, 2017; Gholipour *et al.* 2020; Gholipour *et al.* 2021; Nielsen, 2023). The amount of sewage sludge produced in WWTPs is significant and it includes organic and inorganic matters, nutrients, heavy metals (Stefanakis and Tsihrintzis, 2012), pathogens (Nielsen, 2007), pharmaceuticals (Wang *et al.* 2019; Kołecka *et al.* 2019), antibiotic resistance genes (ARGs) (Chen *et al.* 2009), micropollutant (Dubey *et al.* 2021), personal care products (PCPs) (Chen *et al.* 2009), and hazardous organic compounds (Nielsen, 2023). Hence, choosing an effective method to manage sewage sludge requires particular consideration. This method should not only dewater and treat the sludge but also enhance the quality of the drained water. Managing sludge in WWTPs accounts for a significant portion of operation and maintenance costs, and mechanical dewatering methods often result in drained water that has low quality, necessitating additional treatment with extra expenses (Nielsen, 2023). In contrast, STRB presents an enticing solution for sludge dewatering, cutting down associated expenses, and producing drained water (DW) viable for potential reuse. This hinges on its design accounting for local climatic conditions and its precise operation. To facilitate the potential reuse of drained water from a STRB system in accordance with local standards, several factors such as sludge loading rate (SLR), configuration of the STRB, and duration of resting periods must be considered (Brix, 2017, Nielsen, 2023). The water portion of sewage sludge carries high concentrations of contaminants, which could possibly transfer to drained water. However, drained water is also abundant in macro and microelements, including nutrients, showcasing their potential as valuable fertilizers. STRB research has demonstrated removal efficiencies exceeding 85 % for total suspended solids and chemical oxygen demand from drained water in temperate and tropical climates, typically planted with *Phragmites australis* (Burgoon *et al.* 1997; Begg *et al.* 2001; Wang *et al.* 2021).

Conventional technologies, in contrast, are not structured to yield an acceptable water quality post-sewage sludge dewatering and as a result, additional steps are necessary to channel it into a treatment train for further processing (Cao *et al.* 2021). Furthermore, the combination of earthworms with STRB (W-STRB) has improved overall dewatering performance, although limited information is available regarding drained water quality after a W-STRB (Calderón-Vallejo *et al.* 2015; Chen *et al.* 2016; Hu and Chen, 2018; Hu *et al.* 2020; Wang *et al.* 2021; Zhong *et al.* 2021). A few studies have conducted experiments on W-STRB evaluating drained water quality under controlled conditions; however, these investigations did not assess its performance under varying seasonal conditions (Wang *et al.* 2021; Zhong *et al.* 2021).

Studies conducted in the Mediterranean region have focused on STRBs planted with *Phragmites australis* and did not involve *Arundo donax* in combination with earthworms (Uggetti *et al.* 2012; Stefanakis and Tsihrintzis, 2012; Bianchi *et al.* 2011). Additionally, previous experimental studies were constrained by lab-scale conditions, often of short duration spanning one to three months and protection against rain.

This research is a follow-up study conducted by Gholipour *et al.* (2024) examining drained water quality obtained from a pilot-scale W-STRB enhanced with worms to assess dewatering effect in the Mediterranean region. Considering seasonal and WWTP sludge quality variations, without imposing controlled conditions, this study found that combination of earthworms and plant could increase dry matter content and stabilization of the residual sludge layer while evapotranspiration increased 46 %. The influence of gradually accumulated sludge on top of W-STRB, variations in media and water percolation rate, and the development of plants and earthworms on drained water have not been assessed in previous studies. The primary aims of this study encompass investigating drained water quality across dry and wet seasons, evaluating its potential for water reuse, and scrutinizing the necessity for disinfection and additional treatment. What sets this study apart is its approach, to combine *Eisenia fetida* with *Arundo donax*. The role of *Arundo donax* in the dewatering process and its potentially synergistic impact with earthworms on drained water quality have not been previously documented. These findings hold practical significance for enhancing existing STRB systems. It was assumed that inputs to the studied beds like sludge volume and precipitation as well as outputs like the volume of drained water could be effective factors on the drained water quality. In addition, the gradual accumulation of residual sludge layer over time and the dry solid content of this layer can impact the drained water quality. The study examined these assumptions through a correlation analysis.

4.3 Materials and methods

4.3.1 Study area and setup

This study was conducted in Beirolas wastewater treatment plant (54,500 $m^3.d^{-1}$ for 213,510 inhabitants), Lisbon, Portugal in temperate climate characterized by Köppen classification as a hot-summer Mediterranean (Csa). According to the climate dataset between 1981 and 2010 of Instituto Português do Mar e da Atmosfera (IPMA), Lisbon has an average annual rainfall of 688 mm, and temperature range from -1.5°C (January) to 41.2°C (August). A weather station (Easy Weather - WIFI87CO, Guangdong, China) was installed on the rooftop of the WWTP main building to record meteorological data: air temperature (°C), UV index, solar power ($W.m^{-2}$), atmospheric pressure (mmHg), humidity (%), wind speed ($m.s^{-1}$), wind direction (degree) and precipitation (mm).

The experimental setup (Figure 4.1) consisted of eight one-cubic meter of IBC tanks (Width: Length: Depth = 0.96: 1.16: 1 m). The units were: planted with earthworms (*Eisenia fetida*) (WP), planted (P), unplanted with earthworms (W), and control (C) units without plants and earthworms in which all units were in duplicate.

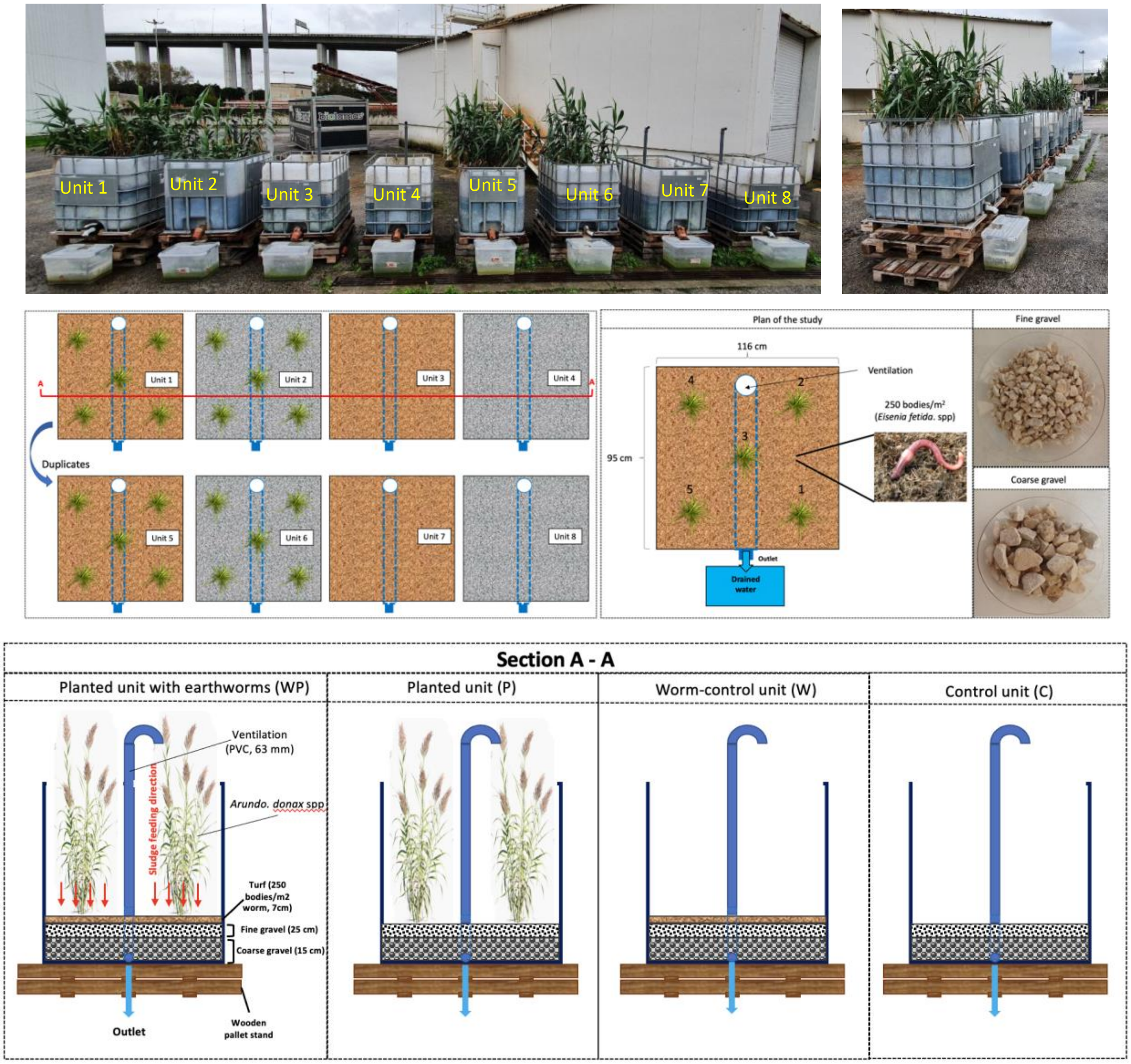


Figure 4. 1. Experimental configuration

Units included drainage (15 cm coarse gravel, 19 to 25 mm, 38% porosity) and transition (25 cm fine gravel, 4.8 to 9.5 mm, 42% porosity) layers. A layer of turf was added to WP and W units (Siro 30: based on the producer fact sheet, the turf layer had a pH, conductivity, granulometry, and organic matter of 5.5 to 6.5, 40 to 80 µs.cm^{-1}, 0 to 15 mm, and > 70%, respectively) hosting 250 bodies.m^{-2} of *Eisenia fetida* (Hu and Chen, 2018; Wang *et al.* 2021; Zhong *et al.* 2021). Regarding drainage system, units were connected to 70 L drums via a perforated drainage (PVC: 63 mm) at the bottom. Each unit had a plastic valve at the outlet for drainage control, remained open throughout the study. The pipe connected to a vent line (1.5 m height) collected drained water and facilitated passive air injection into the media. *Arundo donax* plants with a robust mature root system and stems (additional information regarding plants can be found in the supplementary materials) was obtained from Instituto Superior Agronomia (ISA). It was defoliated, immersed into tap water to prevent desiccation, and planted at density was five tufts.m^{-2} (Brix, 2017). The planted units were weekly irrigated with 10 L treated wastewater from Beirolas WWTP during April 2022. The feeding period with mixed sludge (collected from primary and secondary stages of treatment) started on May 13th, 2022, and ended on May 9th, 2023. Thus, every two weeks, sewage sludge was fed into the beds and then, the beds rested for two weeks (in total, 24 cycles of sludge were fed). Earthworms were added to the units one week prior to the first sludge application. The sludge average temperature, pH, EC, dry solids (DS) and volatile solids (VS) were 22.73°C, 5.98, 1.74 mS.cm^{-1}, 24.71 g.DS.L^{-1} and 19.14 g.VS.L^{-1}, respectively. This study had two sludge loading rate (SLR). From May 13th to November 29th, 2022, to acclimatize the plants gradually, the volumetric loading rate (VLR) was set at 70 L on the area of each bed (SLR$_1$: 40.6 kg.DS.m^{-2}.year^{-1}), called “ramp-up phase”. After November 29th, the load was increased to 100 L (SLR$_2$: 50.4 kg.DS.m^{-2}.year^{-1}) until May, 2023 for a “nominal phase”. The total SLR for the entire period of the study was 43.59 kg.DS.m^{-2}.year^{-1}. After the "nominal phase," the experiment underwent a 132-day undisturbed "final resting phase" without sludge application for stabilization and mineralization (additional information in the supplementary materials).

4.3.2 Observing plant and earthworm dynamics

Morphometric parameters (plant height and plant density) were recorded monthly to assess the growth and development of plants. Plant height was measured with a tape measure from the base of the plants in direct contact with the sludge layer to the apical part of the plants. Plant density was determined by counting the number of stems per bed. Reeds were harvested in January 2023 and dried in an oven at 60°C for three days to achieve a constant weight.

To measure the number of earthworms, a hand sorting process was applied in which earthworms’ population was counted manually by the extraction of a residual sludge layer which was used in the previous studies as well called a “flip and strip test” (Gutiérrez-López et al., 2016). The number of earthworms in WP and W units were registered through a 20:20:15 cm dig (width: length: depth) during the study in the dry and wet seasons.

4.3.3 Physicochemical analyses and sampling

The mixed sludge (100 grams) and DW samples (1 L) (Figure 4.2) were collected in each cycle while turbidity, chemical oxygen demand (COD), total suspended solids (TSS), total volatile solids (TVS), nitrate nitrogen (NO_3^--N), ammonium nitrogen (NH_4^+-N), total Kjeldahl nitrogen (TKN) were determined on a monthly basis. Microbiological analysis (*Escherichia coli*, fecal coliform, *Salmonella* spp) was conducted in ISA lab using Standard Methods for the Examination of Water and Wastewater (APHA, 2017).

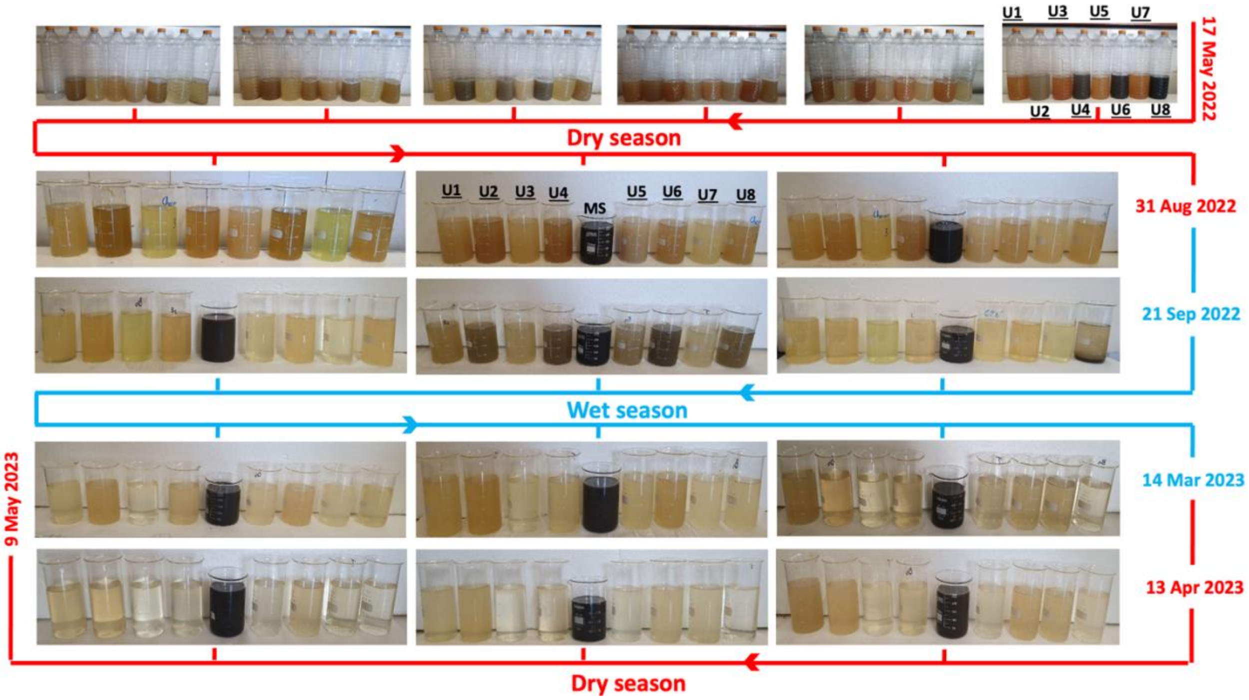


Figure 4. 2. Photo of the samples of the sludge and DW samples

EC and pH were measured using a handheld multi-parameter VWR MU 6100 H. Drained water was collected after feeding completed, and before each feeding, random samples from residual sludge layer were taken to assess DS and VS and measure residual sludge layer thickness. To create representative samples, they were taken from three different locations of the residual sludge layer from the surface of each bed, and they were then mixed. In the lab, they were duplicated to measure DS and VS.

4.3.4 Removal efficiency, water loss and mass balance calculations

The removal efficiency, was calculated according to Eq. 1:

$$\mathrm{Removal\ efficiency} = \frac{(\mathrm{V_{MS}} \times \mathrm{Con_{in}}) - (\mathrm{V_{DW}} \times \mathrm{Con_{iout}})}{\mathrm{V_{MS}} \times \mathrm{Con_{in}}}$$

(Eq.1),

where V_{MS} is the volume of the sludge (L), Con_{in} is the parameter concentration in inlet (mg.L^{-1}), V_{DW} is the volume of drained water (L), and Con_{out} is the parameter concentration in outlet (mg.L^{-1}). To estimate mass balance (g), the concentration of each parameter (mg.L^{-1}) was multiplied by drained water volume (L).

Water loss (WL) was calculated based on Eq.2:

$$\mathrm{WL} = \mathrm{P_r} + \mathrm{V_{MS}} + \mathrm{V_{RS\ (A)}} - \mathrm{V_{RS\ (B)}} - \mathrm{V_P} - \mathrm{V_{DW}}$$

(Eq.2)

Where WL, P_r, and V_{MS} are water loss (L), precipitation volume (L), and water volume (L) in the sludge. In addition, $V_{RS\ (A)}$ and $V_{RS\ (B)}$ are water volumes in residual sludge layer before feeding and at the end of each resting period (L). V_P is water volume draining to the mesoporous media (L), and V_{DW} is drained water volume (L) (Stefanakis and Tsihrintzis, 2011). P_r was measured through the onsite weather station. V_{MS}, $V_{RS\ (A)}$ and $V_{RS\ (B)}$ were calculated based on DS content (%) in the mixed and residual sludge in which the thickness of residual sludge was used to account for V_{RS}. V_{DW} was directly measured by recording drained water volume out of each unit. In the estimation of water loss based on Eq.2 (Stefanakis and Tsihrintzis, 2011), V_P was assumed zero as most of the loss was taken from residual sludge layer, and at the end of the resting period, V_P stays practically steady and close to zero due to the transpiration and fast drainage (Stefanakis and Tsihrintzis, 2011). In the water balance analysis conducted by Gholipour et al. (2024) for the similar experiment in Beirolas, the contribution of various mechanisms into water loss were presented. These included evapotranspiration by plants, evaporation from the surface of residual sludge layers, and the water required for hydration of worms. For detailed insights into the proportion of each mechanism contributing to water loss, readers are directed to a prior water balance analysis paper (Gholipour et al., 2024).

4.3.5 Data analysis

The Kruskal-Wallis one-way analysis of variance test was utilized to evaluate drained water physicochemical parameters among units (significance level: p-value = 0.05). Additionally, duplicated units were compared to ascertain their similarity, facilitating simplification by averaging which reduced overlap in graphical presentations. Spearman method was used to conduct a correlation analysis (strong correlation: $p\text{-value} < 0.05$). All statistical analyses were performed within R Studio. Furthermore, essential statistical metrics, including minimum, maximum, mean and standard deviation (SD) were computed.

4.4 Results

4.4.1 Meteorological analysis

During the study, the minimum and maximum atmospheric temperature were in July 2022 and January 2023 reaching 42.2 and 3 ^{0}C respectively, and humidity reached 99 % in October while the minimum humidity was 14 % in July (Figure 4.3). Solar power reached 1283 W.m^{-2} (UV index=10), and the maximum wind speed (July 2022) was 15.29 m.s^{-1} (wind gust = 1 ~12 m.s^{-1}). The direction of wind was between 177 ^{o} and 292^{o} to the north and atmospheric pressure varied between 773 and 745 mmHg in December 2022. An extreme flood event occurred on December 13th, 2022, when daily precipitation reached 131 mm and the total precipitation during the study was 906 mm.

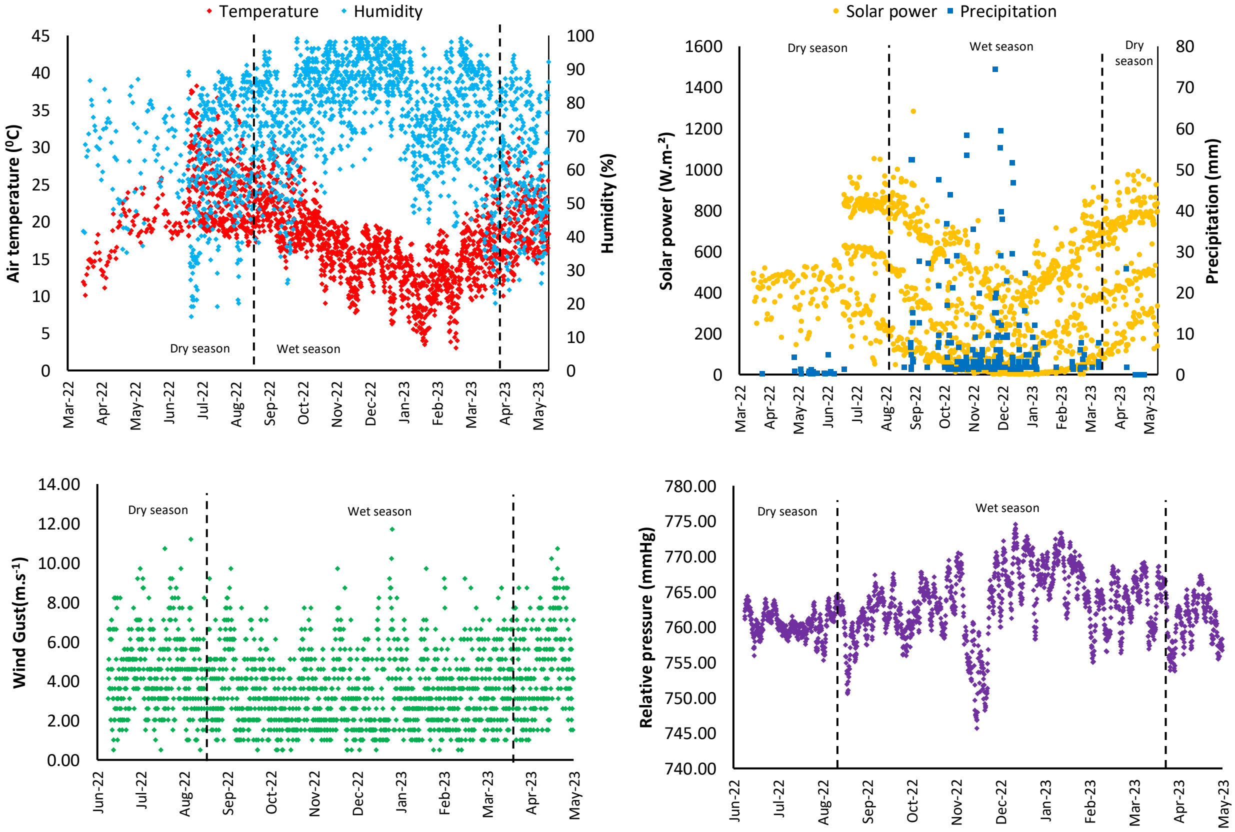


Figure 4. 3. Weather data

Thus, air temperature was rarely below freezing temperature, and the study period can be categorized into two seasons namely a dry season from April to September 2022, and a wet season from September 2022 to April 2023 (Reis *et al.* 2022).

4.4.2 Analysis of plant and earthworm

Plant growth was minimal during the irrigation with treated wastewater in April of which an average of three stems per tuft emerged in the planted units. A substantial surge in the aboveground growth became evident by the beginning of Spring, where sludge was fed into the beds, an 85% increase in the overall height development of the plant. The growth in height (15.7 ± 2.9 cm per month) was consistent across planted units (not significant difference: p-value > 0.05). Throughout the study, *Arundo donax* growth rate was between 12 and 19 cm per month. Table 4.1 shows plant development during the study period in the dry and wet season.

Table 4. 1. Plant development

Season	Date	Height (cm)		Nº of stem	
		WP	P	WP	P
Dry	04-Apr-22	51	44	2	2
	05-May-22	44	42	4	5
	17-Jun-22	49	45	6	6
	18-Jul-22	63	54	7	7
	26-Aug-22	85	69	9	7
Wet	29-Sep-22	101	85	12	8
	15-Oct-22	110	123	12	8
	03-Nov-22	120	131	12	8
	13-Dec-22	137	150	13	8
	13-Jan-23	163	156	13	9

In measurements of the aboveground wet biomass, WP units achieved a higher biomass production (16.4 kg.m^{-2}) while P units had 11.5 kg.m^{-2} (mean water content = 88 %). WP units displayed an average of 43 % higher biomass production compared to P units without earthworms. Table 4.2 shows the average population of earthworm during the study period for WP and W units.

Table 4. 2. Earthworm population and variation

Season	Date	WP	W
Dry	06/05/2022	10	10
	25/07/2022	12	14
	25/08/2022	19	54
Wet	03/10/2022	4	35
	28/02/2023	8	6
	28/03/2023	10	6
	09/05/2023	14	9

By the end of the wet season, the population increased towards May 2023 by 71, 80, 88, and 83 % for WP unit 1, W unit 3, WP unit 5, and W unit 7, respectively.

4.4.3 Drained water (DW) quality

All experimental units showed pH values within neutral range, showing an increase from pH of sludge that varied between 4.88 and 6.98 (mean = 5.98 ± 0.59). pH increased from 6.53 to 8.65 (mean = 7.23 ± 0.5), 6.90 to 8.15 (mean = 7.50 ± 0.4), 6.11 to 7.84 (mean = 7.19 ± 0.4), and 6.82 to 8.47 (mean = 7.52 ± 0.4) for WP, P, W, and C units (Figure 4.4.a), respectively (no significant difference p-value > 0.05).

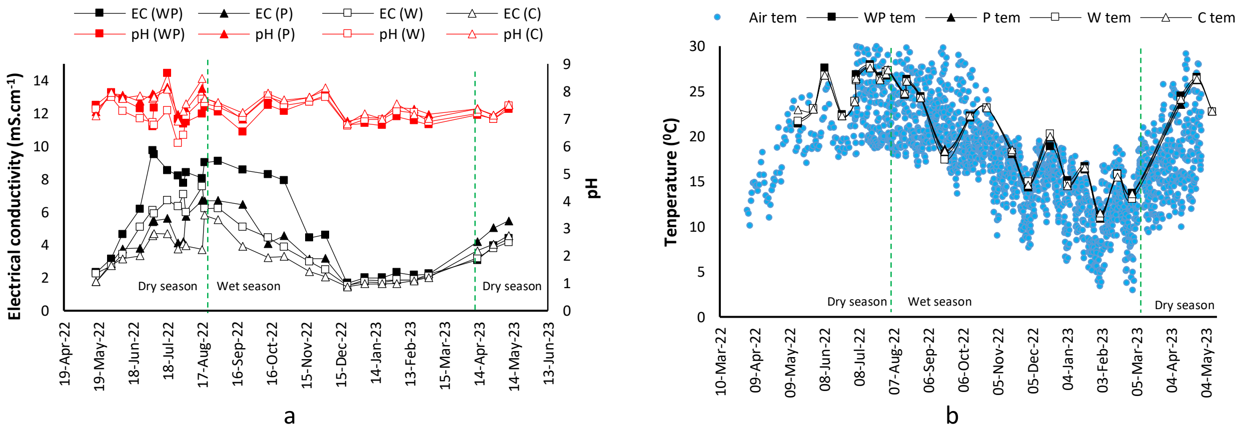


Figure 4. 4. Drained water quality: a) EC and pH, and b) temperature

EC of sludge was between 1.06 and 3.40 mS.cm^{-1} (mean = 1.74 ± 0.55) (additional information can be found in Table 4.3 of the supplementary materials). Yet, EC of drained water for WP, P, W, and C units (significant difference, p-value < 0.05) ranged between 1.67 and 9.74 (mean = 6.64 ± 2.89), 1.52 and 6.71 (mean = 4.08 ± 1.70), 1.54 and 7.55 (mean = 4.16 ± 1.90), and 1.46 and 5.79 mS.cm^{-1} (mean =3.30 ± 1.30), respectively (Figure 4.4.a). WP unit had the highest EC compared to other units while control unit had the lowest EC. The sludge temperature varied between 16 and 28 ^{o}C (mean = 23 ± 4 ^{o}C). The drained water temperature for all units followed atmospheric temperature variations (≈22 ^{o}C) which were not significantly different (p-value > 0.05) (Figure 4.4.b).

TSS and COD in drained water were lower during the dry season in 2022 and higher during the wet season (Figure 4.5.a). Units in terms of TSS and COD were also significantly different throughout the study (p-value < 0.05). WP unit showed consistently lower mass release of TSS and COD compared to other units. Accumulated release rates reached 1.57, 2.75, 2.87 and 3.36 g.TSS and 5.88, 9.72, 7.01 and 7.76 g.COD for WP, P, W and C units by the end of the experiment, respectively. This indicates the combination of earthworms and plants could reduce the released TSS and COD masses in WP unit by 43 and 40 % compared to the planted unit.

TP also reduced to 0.42, 0.73, 0.72 and 0.7 g.P during the dry season in 2022 and increased to 2.62, 3.31, 5.27 and 6.18 g.P in the wet season while the released masses were 0.81, 1.47, 1.64 and 2.75 g.P at the end of the study for WP, P, W and C units, respectively (Figure 4.5.b). WP unit also released 45 % less phosphorous compared to the P unit indicating a synergistic effect of earthworms in the improvement of phosphorous removal.

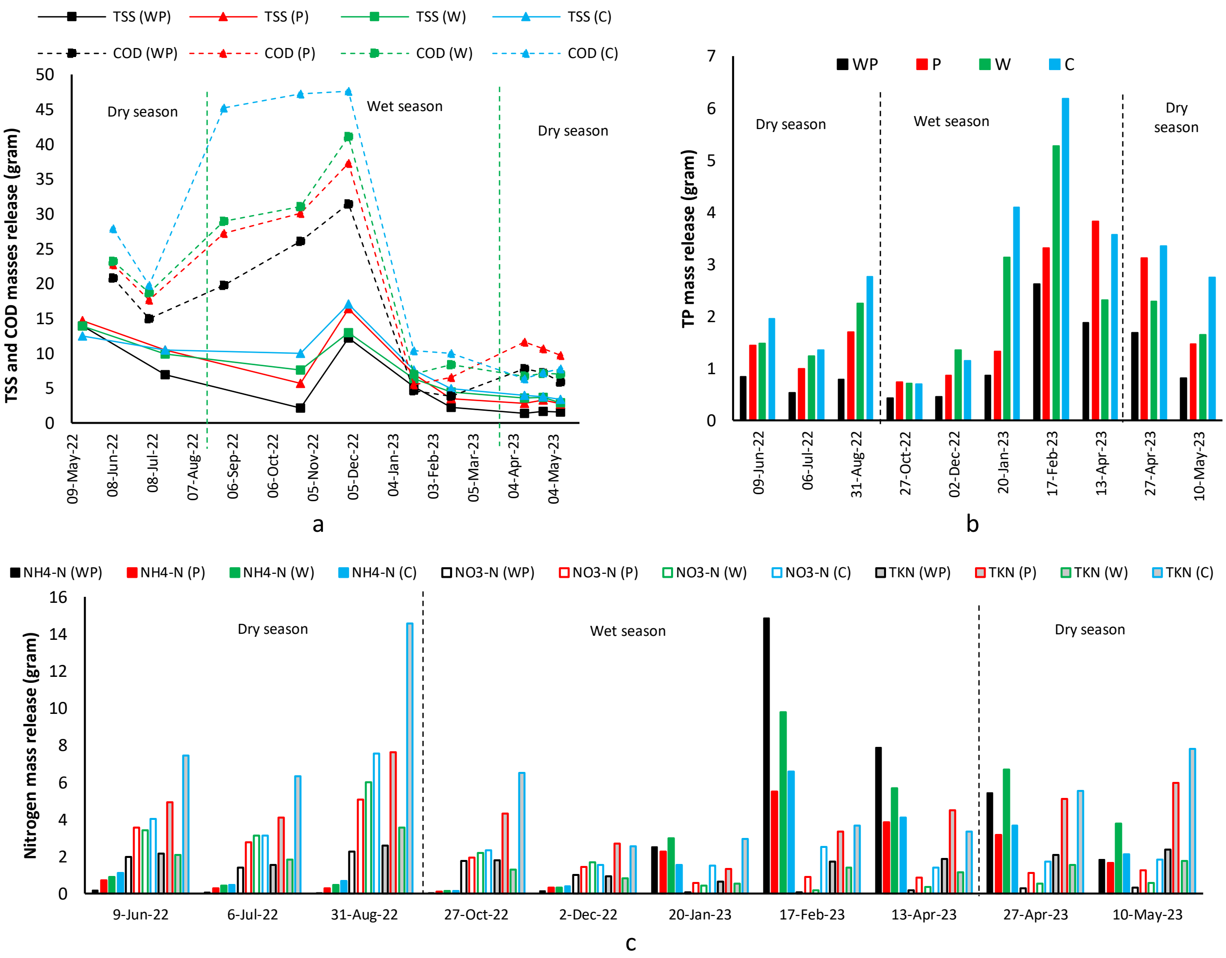


Figure 4. 5. Mass release: a) total suspended solid and chemical oxygen demand, and b) phosphorous c) nitrogen components

NO_3^--N and TKN consistently reduced in the study period while NH_4^+-N initially decreased within the dry season in 2022 and then, increased to the end of the study (Figure 4.5c). Ammonium masses were higher in WP and P units (1.8 and 1.6 g.N) compared to W and C units (3.8 and 2.1 g.N). At the end of study, WP units achieved the least released mass of nitrate by 0.3 g.N which was 75, 50 and 83 % lower masses compared to P, W, and C units, respectively. The mass of TKN was also lower in WP and W units (2 and 1.7 g.N) compared to P and C units (6 and 7.8 g.N). Table 4.5 of the supplementary materials shows variations in physicochemical parameters and masses released during the dry and wet seasons, along with operational factors, water balance components, and atmospheric data.

Residual sludge accumulation rates were 0.06, 0.09, 0.05, and 0.1 m.year^{-1} for WP, P, W, and C units, respectively. Suspended solids drained into drained water was significantly different for all units (p-value < 0.05) (Figure 4.6.a). TSS (mg.L^{-1}) of WP, P, W, and C units were averagely 287 ± 292, 268 ± 200, 186 ± 179, and 162 ± 140, respectively. In addition, lab analysis showed there was an average 80 % (±3.34) proportion between volatile and suspended solids. Removal efficiency of TSS across all units improved over time, particularly after the ramp-up phase (six months after the first feed) in the wet season. TSS of the units after the ramp-up phase was not significantly different (p-value > 0.05) and likewise in the case of TVS. W unit had the highest TVS content compared to other units while the lowest TVS belonged to P units (mean = 77 ± 2.48 %). TSS removal efficiency was also reported to be more than 85% in other studies, both in the planted and unplanted units. The turbidity of the drained water also reduced during the study especially in the wet season while it was 49, 42, 32 and 24 NTU for WP, P, W and C units at the end, respectively.

COD (mg.L^{-1}) of the sludge (Figure 4.6.b) varied between 8802 and 29804 (mean = 20749 ± 6128). All units were effective in removing organic and inorganic contents of which COD in DW for WP, P, W and C units were 98.36, 98.04, 97.89 and 97.27 % lower than COD in the sludge at the end of study period. COD concentrations among units were not significantly different for the study period (p-value > 0.05). The removal efficiency improved after the ramp-up phase in which COD (mg.L^{-1}) decreased from 1147 to 480 (58 %), 842 to 485 (42 %), 776 to 296 (62 %), and 857 to 437 (49 %) for WP, P, W and C units, respectively.

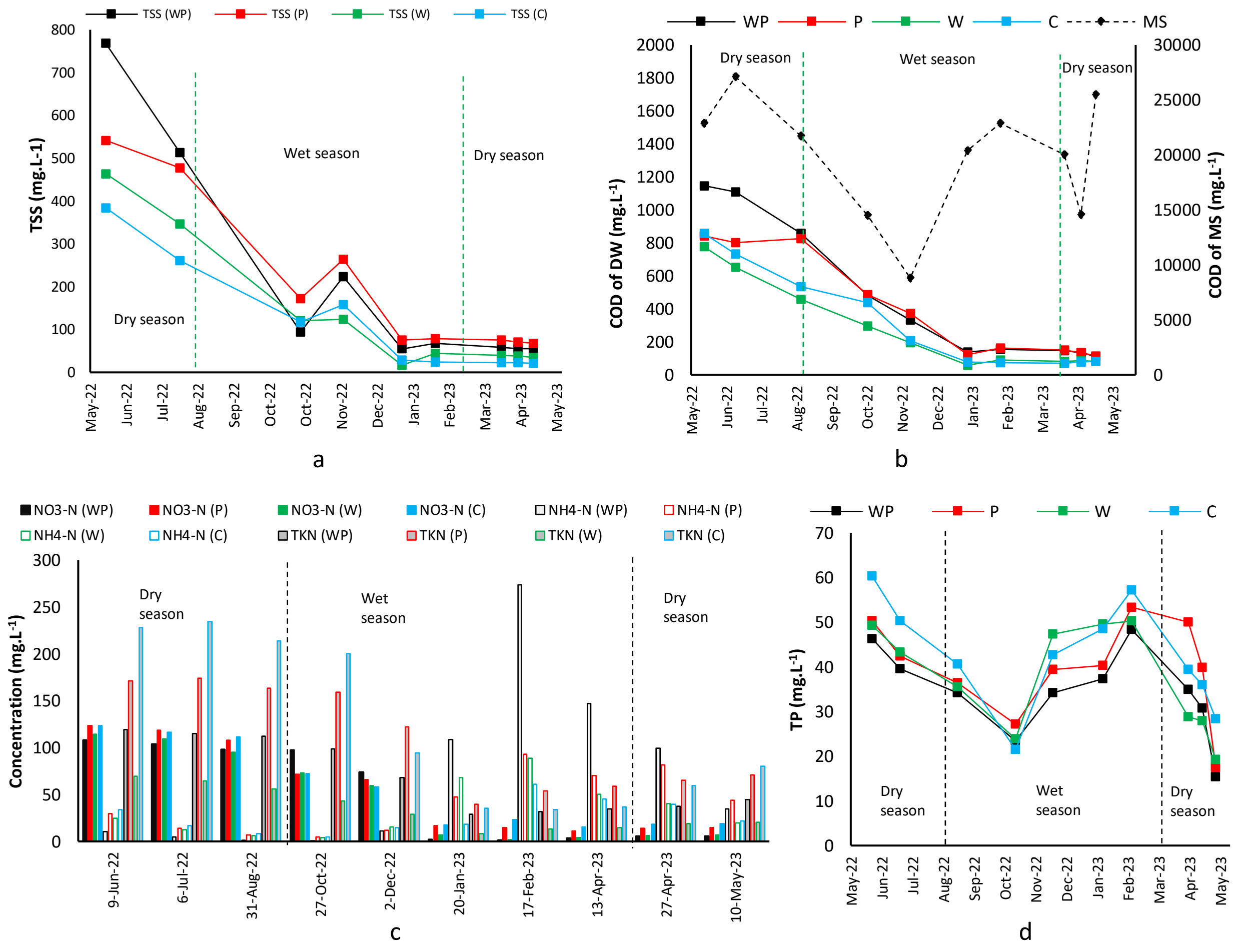


Figure 4. 6. Pollution concentrations: a) total suspended solid b) chemical oxygen demand c) nitrogen components d) phosphorous

Total nitrogen of the sludge was from 807 to 1792 mgN.L^{-1} (mean = 1187 ± 423) throughout the study, with 87% as TKN (mean = 1129 ± 269 mgN.L^{-1}) of which 26 % is ammonium nitrogen (mean = 289 ± 77 mgN.L^{-1}).

Total phosphorous of the sludge was between 2317 and 7580 mgP.L^{-1}. After feeding, units retained efficiently 99% of phosphorous during the study probably due to the sedimentation of particulate phosphorus into media (Figure 4.6.d). At the end of the study, phosphorous was 15, 17, 19 and 29 mgP.L^{-1} for the WP, P, W and C units, respectively, indicating the lowest concentration in WP unit owing to a synergetic effect of earthworms and plants. WP unit improved the removal efficiency by 12, 21 and 48 % compared to P, W and C units, respectively.

All units considerably removed *Escherichia* coli and fecal coliform, but *Salmonella* was observed in some units. *Salmonella* was absent in units 1 (WP), 2 (P), 3 (W), 4 (C) and 7

(W) while it was found in 5 (WP), 6 (P) and 8 (C) units. In addition, total coliform of all studied units was less than 1600 MPN.100mL^{-1} and *Escherichia coli* reduced from 7.8×10^4 in the sludge to less than 15, 830, 410, and 85 CFU.mL^{-1} (6.72, 4.97, 5.28 and 5.96 log reduction) for WP, P, W, and C units, respectively (99 % removal efficiency in all units). Fecal coliform content was 95, 1400, 1700 and 290 CFU.mL^{-1} for WP, P, W, and C units, respectively. Table 4.3 shows a summary of drained water quality.

Table 4. 3. A summary of DW quality

Parameters	Influent	Effluent				Portuguese law for water reuse (Decreto-Lei n.o 119/2019)
	Sludge	WP	P	W	C	
pH	5.98±0.59	7.23±0.5	7.50±0.4	7.19±0.4	7.52±0.4	N.A
EC (mS.cm^{-1})	1.74±0.549	7.78±7.2	4.08±1.7	4.16±1.9	3.3±1.3	N.A
T (^{0}C)	23±4	21.88	21.79	21.75	21.87	N.A
Turbidity (NTU)	N.A	49	42	39	24	N.A
TSS (mg.L^{-1})	N.A	45	50	35	25	≤10 class A ≤35 class B
COD (mg.L^{-1})	20749±6128	111	115	82	80	≤BOD:10 class A ≤BOD:25 class B
TN (mgN.L^{-1})	1187±423	N.A	N.A	N.A	N.A	15
TKN (mgN.L^{-1})	1129±269	45	71	20	80	N.A
NH_4^+-N (mgN.L^{-1})	289±77	35	19	44	22	10
NO_3^-N (mgN.L^{-1})	N.A	6	15	7	19	N.A
TP (mgP.L^{-1})	4097±1390	15	17	19	28	5
Salmonella (Present/Absent: P/A)	P	A&P	A&P	A	A&P	A
Total Coliform (MPN.100mL^{-1})	N.A	<1600	<1600	<1600	<1600	N.A
Escherichia coli (CFU.mL^{-1})	7.8×10^4	12.5	810	405	77.5	≤10 class A ≤100 class B ≤1000 class C ≤10^4 class D
Fecal Coliform (CFU.mL^{-1})	N.A	87.5	1350	1500	250	N.A

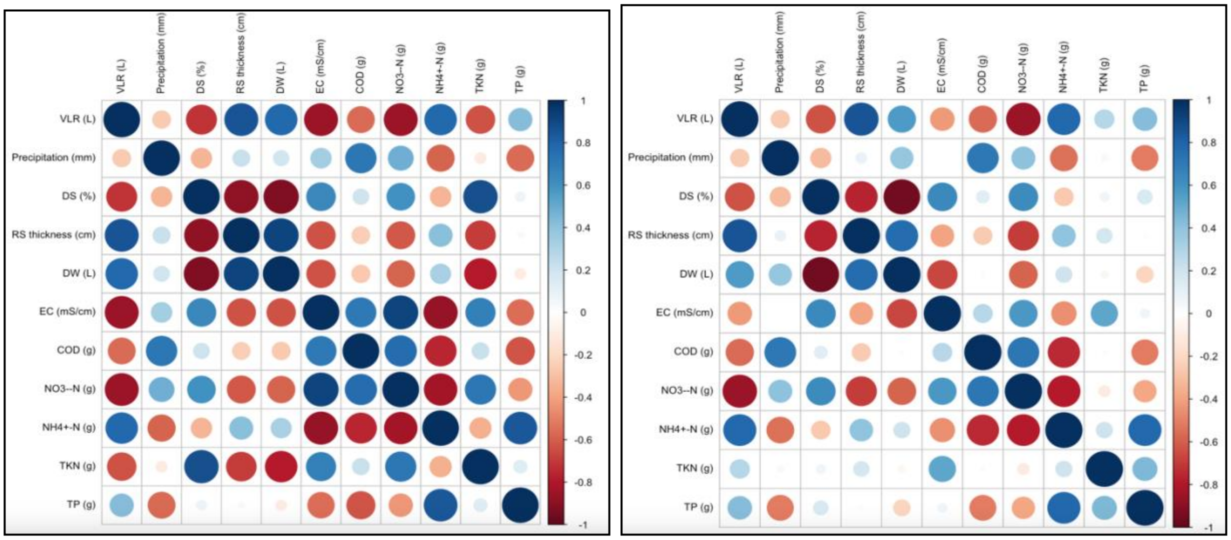
* N.A: Not available

The acceptable TSS level for water reuse in Portugal (Decreto-Lei nº 119/2019) is 10 and 35 mg.L^{-1} for classes A and B. The drained water from W and C units met this criterion at 35 and 21 mg.L^{-1} respectively while WP and P units showed higher values at 45 and 50 mg.L^{-1}, indicating a need for enhancement. Phosphorus should be below 5 mg.L^{-1} and total nitrogen below 15 mg.L^{-1} for water reuse.

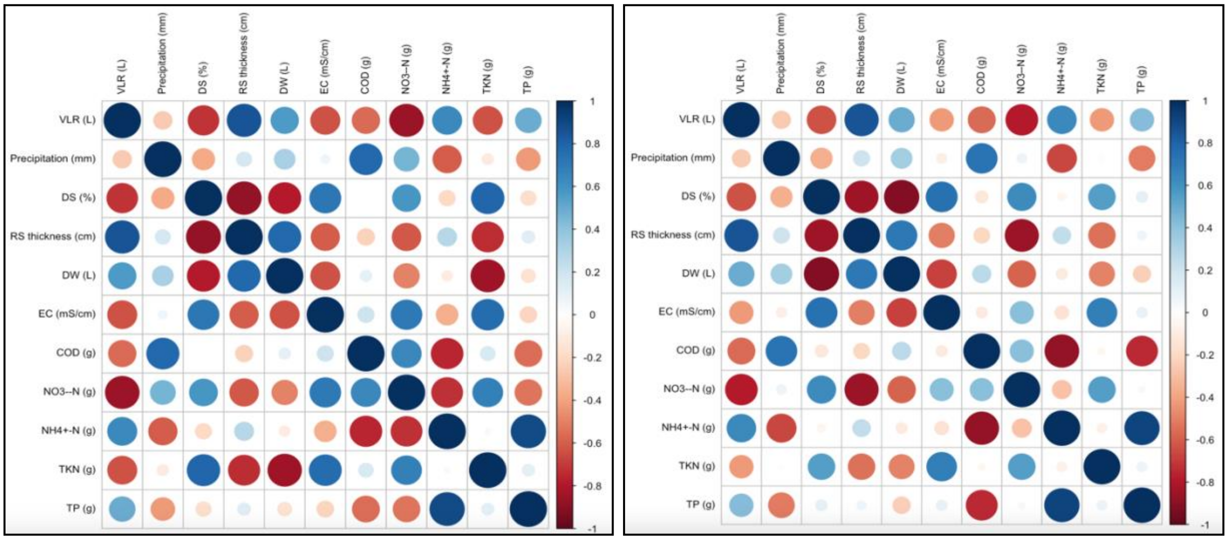
4.4.4 Drained water correlation analysis

Spearman correlation was analyzed exploring the interplay of pollution masses, operational factors, and seasons (Figure 4.7). It was shown that inputs (VLR: volumetric loading rate and precipitation) and outputs (drained water volume) as well as the residual sludge layer condition (DS and residual sludge thickness) could affect mass release of pollutant and drained water quality accordingly. Precipitation showed positive correlation with COD mass across all units, while negatively correlating with VLR (p-value < 0.05). In the wet season, elevated precipitation likely led to a washout of particulate COD from the residual sludge layer, contributing to increased COD mass. Additionally, in WP unit, WL and EC positively correlated with COD mass. Despite increased EC due to earthworms and plants, COD concentration decreased but mass increased, indicating improved removal efficiency in the ramp-up phase.

There were significant positive correlations between nitrate mass and EC for all units (p-value < 0.05). Nitrate mass negatively correlated with VLR, residual sludge thickness, and drained water volume for units (p-value < 0.05), along with ammonium mass. This may be attributed to nitrification in the dry season and denitrification in the wet season. The increase in residual sludge thickness could lead to enhanced anaerobic conditions, resulting in a reduction in nitrate mass release by the end of the study, influenced further by precipitation in the wet season. Despite a significant positive correlation between ammonium mass and VLR (p-value < 0.05), precipitation negatively correlated, leading to increased ammonium mass release in the wet season. In the WP unit, a significant negative correlation existed between ammonium mass and EC (p-value < 0.05). Additionally, correlations were observed between ammonium mass and phosphorous mass, as well as nitrate and COD masses. The rise in ammonium during the dry season could result from nitrification, while in the wet season, the dominance of denitrification might lead to nitrate variations.



WP unit P unit



W unit C unit

Figure 4. 7. Correlation analysis (dark blue: significant-positive correlation and dark red: significant-negative correlation)

TKN mass positively correlated with EC, and DS, while exhibiting negative correlations with residual sludge layer thickness, drained water volume, and VLR ($p\text{-value} < 0.05$). During the dry season in 2022, TKN mass was higher due to elevated DS, possibly indicated ammonium's contribution to TKN increase. Phosphorous mass correlated positively with VLR and negatively with precipitation. A higher phosphorous mass in the wet season (January 2023) could be attributed to the plant harvest diminishing phosphorous uptake as well as low atmospheric temperature. Furthermore, a positive significant correlation existed between ammonium and phosphorous masses and a negative correlation with COD mass (p-value < 0.05), although these correlations may not represent biological or chemical interdependencies.

Despite higher pollution mass release, units with lower water loss (W and C) exhibited improved drained water quality compared to WP and P units, particularly during the wet season with increased precipitation and drained water volume. The dilution was affected by the leaching in WP and P units, the leaching was apparently less, plant roots probably had role in this. The plant and earthworm units showed lower mass discharge, releasing 24 and 55 % less COD mass than the control unit during the dry and wet seasons, respectively. The experimental study, in its six-month ramp-up phase during the dry season, probably influenced drained water quality, the ramp-up phase was also suggested in previous studies (Brix, 2017).

4.5 Discussion

The results of this study showed that a synergistic effect of earthworm inclusion in increased plant biomass production. This could be due to the beneficial interactions between earthworms and residual sludge layer, such as enhanced aeration, nutrient cycling, and soil structure improvement, which collectively promote plant growth and productivity (Jing et al., 2023). *Arundo donax* is rather unexplored in STRB system and past studies without earthworms' inclusion reported the use of various plant species such as *Phragmites australis*, *Bambusa vulgaris*, *Cymbopogon nardus*, *Typha spp, Setaria verticillate, and Oryza longistaminata* (Hardej and Ozimek, 2002; Troesch *et al.* 2009; Zhang *et al.* 2012; Wu *et al.* 2014; Kouawa *et al.* 2015; Osei *et al.* 2017; Osei *et al.* 2019). Table 4.4 presents a summary of the plant development data for different plants across different studies, including height and stem growth rate.

Table 4. 4. Plant development in comparison with previous studies

Specie	Climate	Average stem development (stems.m^{-2})	Average height growth (cm. month^{-1})	Comment	Reference
Phragmites australis	Temperate	250	20 to 200	Full-scale study	Hardej and Ozimek, 2002
			30 to 150	Full-scale study	Troesch *et al.* 2009
	Tropical		120	For 6 weeks	Zhang *et al.* 2012
	Tropical	147	-	Pilot-study	Wu *et al.* 2014
Setaria verticillata	Arid	105	3.61	For 13 weeks	Osei *et al.* 2017
Bambusa vulgaris		6	2.49	For 11 weeks	
		7	1.14	Pilot-study	Osei *et al.* 2019
Cymbopogon nardus		13	2.14	Pilot-study	
Typha spp	Tropical	60	4.5	Pilot-study	Wu *et al.* 2014
Typha latifolia	Arid	4	3.97	For 8 weeks	Osei *et al.* 2017
Oryza longistaminata	Arid		4	Pilot-study	Kouawa *et al.* 2015
Arundo donax	Temperate	- 50 for WP unit and 30 for P unit in the dry season - 5 for WP unit and 5 for P unit in the wet season	- 12 for WP unit and 8.5 for P unit in the dry season - 10.5 for WP unit and 6.5 for P unit in the wet season	- For 4 months in the dry season - For 4 months in the wet season	Present study

* Previous studies are all STRB units (P unit)

This study also found that the population of earthworms was higher in the bed without plants which could be due to the higher availability of organic and inorganic matters as well as the absence of feeding competition between earthworms and plants root (Leroy et al., 2008). Earthworm population also got impacted by the occurrence of wet season indicating a reduction which could be due to increased residual sludge layer moisture levels, which can limit earthworm activity, as well as potential flooding or waterlogging, which can directly affect earthworm survival and habitat suitability. (Uvarov et al., 2011).

In terms of drained water quality, it was found that pH was adjusted from sludge to the drained water. In previous STRB studies, pH neutralization was also found (Stefanakis *et al.* 2014; Chen *et al*. 2016; Hu *et al*. 2020; Zhong *et al*. 2021). As stated by Kadlec *et al.* (2000) and Mayes *et al*. (2009), oxygen provision through plants' rhizome for bacteria, and microbial processes are effective in pH adjustment, along with ion release by earthworms through organic matter decomposition (Heggelund *et al.* 2014). It was also found that EC of drained water was higher than EC of the sludge which is consistent with previous studies (Stefanakis *et al.* 2014; Chen *et al.* 2016; Hu *et al.* 2020; Zhong *et al.* 2021). EC of the planted beds with earthworm was higher than the other beds indicating the influence of plants and earthworms possibly due to a higher dehydration increasing water loss in the system and reducing drained water volume.

The mass release of TSS and COD were lower in the dry season which could be attributed to the effect of dilution by precipitation which was smaller than the effect of increased leaching of the system. This was evident in the previous studies as well (Stefanakis *et al.* 2014). WP units released lower mass of pollutant compared to P units. Several studies stated role of earthworms in the enhancement of water quality in which biodegradation, aeration, mixing and enhanced filtration could be the most important mechanisms (Kadlec *et al.* 2000; Vymazal, 2010). In the wet season, precipitation probably washed particulate phosphorous in residual sludge layer to the drainage across all units increasing phosphorous mass release while plants' phosphorous uptake could reduce in the wet season in WP and P units (Brix, 2017). The result of the flip and strip test also showed earthworms' population was at minimum during the wet season possibly influencing phosphorous release (Xu *et al.* 2015).

Ammonium reduced in January 2023 possibly due to the lower atmospheric temperature and lower ammonification (Zangeneh *et al.* 2021). In addition, nitrate is related to ammonium indicating possible existence of nitrification process in the system while denitrification in sludge dewatering system was stated as a dominant mechanisms of nitrate variation by Brix. (2017). At the end of study period, W unit showed the lowest TKN concentration possibly owing to the ammonium variation via ammonification through earthworms' activity. Higher nitrification rate and plant uptake could happen in the presence of plants in WP and P units. Other removal mechanisms such ammonification, nitrogen fixation, plant uptake and earthworms' effect could be effective (Vymazal, 2010; Brix, 2017; Dotro *et al.* 2017).

Overall, WP unit showed lower released masses compared to the other units underscoring the significant combined role of plants and earthworms in the mitigation of pollution such as TSS, COD, TP, and nitrogen components in DW. Adapting the dewatering system to foster biofilm development and residual sludge accumulation proves effective for enhanced removal efficiency across all units. Additionally, the development in the root system and earthworms' contribution could be other effective mechanisms. Earthworms in W unit would boost organic matter breakdown, leading to higher volatile solids content than other units and subsequent percolation to the drained water. The difference between plant species such as *Phragmites Australis*, *Typha* and

Iris was insignificant (Burgoon et al. 1997; Begg et al. 2001; Korboulewsky et al. 2012; Chen et al. 2016; Magri et al. 2016; Kim et al. 2018; Wang et al. 2021; Saeed et al. 2022).

Drained water quality improved after ramp-up phase. This could be due to various physicochemical processes including the development of biofilm in the porous media, diversification and proliferation of microbial communities, and the filtration of insoluble fraction of COD (Kadlec et al. 2000; Vymazal, 2010). W and C units showed lower concentration of COD compared to WP and P which could be attributed to seasonal variation (dry and wet seasons) and higher water loss in WP and P units by plants and earthworms. Based on this, higher volumes of the drained water would obviously represent lower COD. Additionally, as stated by Kadlec *et al.* (2000), other operational parameters e.g., SLR, feeding and resting periods, natural processes and plant type can affect performance. In previous studies under temperate climate STRB systems planted with *Phragmites australis* showed a COD removal efficiency of more than 85 % (Nielsen, 2007; Kołecka *et al.* 2017), while in the present study, it was more than 94 and 95 % in STRB and W-STRB units, respectively. This fact indicates an improvement in the removal efficiency through the inclusion of earthworms as well as the application of *Arundo donax*. COD removal efficiency was also more than 80 % in tropical and arid climate studies with *Phragmites australis* (Burgoon et al. 1997; Begg et al. 2001; Korboulewsky et al. 2012; Chen et al. 2016; Magri et al. 2016; Kim et al. 2018; Wang et al. 2021).

Variations in nitrogen components in the sludge can be attributed to fluctuations in DS and VS contents in Beirolas WWTP (additional information in the supplementary materials). In nitrogen component variation, the accumulation of the residual sludge layer on the top of units could limit oxygen transfer from atmosphere to the porous media, consequently causing a potential anaerobic condition (He *et al,* 2021). This experiment showed that the residual sludge layer had 2 cm thickness at the end of the dry season in 2022 (DS ≈ 85 %), and it was over 30 cm within the wet season (DS ≈ 3 %) across all units (Gholipour *et al.* 2024). After the final the sludge application, it reduced to 18, 23, 18 and 21 cm for WP, P, W and C units (DS = 22, 25, 21 and 17 %), respectively (Gholipour *et al.* 2024). In the wet season, denitrification and ammonification could be the most dominated mechanisms influencing nitrogen components. In addition, it was observed during the two-week rest, cracks appeared on top of the residual sludge layer, which could oxygenate the media before the proceeding feeding (additional information of the supplementary materials). In this study, TKN concentration removed more than 99% in WP units while it was around 95 % in P units, which is comparable to the previous tropical climate studies planted with *Phragmites* australis showing a removal between 82 and 99 % for P units (Koottatep et al. 2005; Kim et al. 2018; He et *al.* 2021). TKN removal in a temperate study was found 95 % (Stefanakis and Tsihrintzis, 2012, Kołecka *et al.* 2017) resembling the studies in arid climates in Africa with *Phragmites australis* (Goussanou *et al.* 2023).

Phosphorous retainment on the top of the residual sludge layer occurred. Processes like sorption, precipitation, biological uptake, microbial processes, redox reactions could be also effective (Wetzel, 2020). Other factors such as operation and configuration of units, SLR, plant, substrates can influence phosphorous removal (Stefanakis *et al.* 2014). In a tropical climate study by Wang *et al.* (2020) planted with *Phragmites australis*, a higher released mass of phosphorous during wet season was also found. Phosphorous removal efficiency in WP units was >99%, which is comparable to the tropical studies by 95 % for their P units planted with *Phragmites australis* (Kim et al. 2018). Saeed et al. (2022) in tropical climate studied W-STRB planted with *Phragmites australis* and found 99 % phosphorous removal efficiency; yet the raw sludge was from a drinking water treatment plant.

The study also showed improvement in microbiological quality during dewatering process. Removal of microbiological parameters could be due to physical filtration, sedimentation, sun light exposure, natural die-off, competition and predation, chemical reaction, plant uptake, microbial activity, and Redox reactions can be stated (Kadlec *et al.* 2000). Retention time, configuration, monitoring, and maintenance are other influential factors. Overall, the inclusion of plants and earthworms appeared to enhance the removal efficiency, emphasizing the importance of incorporating earthworms into STRB system. However, continuous monitoring and optimization are essential to ensure consistent disinfection and minimize the potential risk of pathogenic contamination in the treated water.

Drained water from all units fell short of meeting these admissible limits, signaling the necessity for improvement for potential water reuse. Microbiological indexes indicated that for class A standard, all units required improvements. However, for class B standard, WP met the required limit. In previous STRB studies, a water reuse was suggested without disinfection (Stefanakis et al. 2014; Calderón-Vallejo et al. 2015). Portuguese water reuse regulation is based on European Union regulation while the quality of the drained water in terms of *Escherichia coli* and fecal coliform should be less than 1000 CFU.mL^{-1} based on WHO recommendations. Therefore, *Escherichia coli* aligned with WHO regulation while fecal coliform needs further improvement for P and W units. In conclusion, while the drained water quality from these units shows potential for water reuse, additional treatment stages are essential to attain the necessary quality standards.

4.6 Conclusions

Sludge treatment reed beds were tested for Portugal temperate climate to assess the effect of *Eisenia fetida* inclusion and planted *Arundo donax* on drained water quality. The earthworms' assistance boosted 30 % plant biomass production while earthworm-control units exhibited a higher earthworm population compared to earthworm-planted units. A ramp up phase improved removal efficiency in earthworm-planted unit of which COD, NO_3^--N, NH_4^+-N, TKN and TP removed 99, 99, 86, 99 and 99 %. Concentrations were higher in earthworm-planted unit compared to the other units; however, the released masses of COD, NO_3^--N, NH_4^+-N, TKN and TP were lower in earthworm-planted unit. COD and TP masses correlated with precipitation; thus, in the wet season, released mass increased. Water loss volume correlated negatively with drained water volume, and a positive correlation between drained water volume and mass release was obtained. Based on this, earthworm-planted unit had lower mass release of COD, NO_3^--N, NH_4^+-N, TKN and TP compared to the other units. The drained water from control unit had lower concentrations indicating a dilution effect from precipitation and increased VLR. Nitrate correlated with EC which was higher during the dry season when the analysis showed potential nitrification. The increase in the residual sludge thickness negatively correlated with TKN. Overall, the inclusion of earthworms into the planted unit indicated 40, 75 and 45 % lower released masses of COD, NO_3^--N and TP compared to the planted unit without earthworms. This study showed that despite some parameters such as TSS, COD, and

Escheriacia coli complied with water reuse limits, a further treatment of drained water is needed.

4.7 References

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Chapter 5. A pilot-scale evaluation of residual sludge quality in a worm-sludge treatment reed bed

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A pilot-scale evaluation of residual sludge quality in a worm-sludge treatment reed bed in the Mediterranean region

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ABSTRACT

A pilot-scale study on sludge treatment reed beds investigated the combined effects of earthworms and *Arundo donax* on sewage sludge dewatering and residual sludge quality. Four units were tested: one planted with earthworms, one planted without earthworms, one unplanted with earthworms, and one control, each unit replicated. Over a year, 24 cycles of sludge (dry and volatile solid contents of 24.71 $g.L^{-1}$, and 19.14 $g.L^{-1}$) were fed onto the units at a sludge loading rate: 43.59 $kg.DS.m^{-2}.year^{-1}$. Afterward, the units experienced 132 days of resting period, increasing dry solids from 21 to 70 % and decreasing volatile solids from 81 to 69 % on average (40 % sludge volume reduction). The bottom layers of the planted unit with earthworms showed a 30 % reduction in volatile solids, indicating improved sludge stabilization. Macronutrient abundance in the residual sludge followed the sequence N > Ca > P > K > S > Mg. The planted unit with earthworms reduced micronutrient concentrations by 22 % compared to the control unit (Fe > Na > Mn > B > Mo). Earthworms also played a key role in reducing heavy metal concentrations by 11 % compared to the planted unit without earthworms (Zn > Cr > Pb > Ni > Cd). Heavy metal levels in the residual sludge met EU and Portugal standards, with a 99.9 % reduction in *Escherichia coli* and fecal coliforms. Cost estimation showed centrifugation and W-STRB scenarios cost 167 and 183 $€.PE^{-1}$ for a ten-year operation, with O&M costs of 7 and 3 $€.PE^{-1}.year^{-1}$, respectively.

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5.1. Abstract

A pilot-scale study on sludge treatment reed beds investigated the combined effects of worms and *Arundo donax* on sewage sludge dewatering. Four units were tested: planted with earthworms, planted without earthworms, unplanted with earthworms, and control, each with four duplicates. Over a year, 24 cycles of sludge (dry and volatile solid contents of 24.71 g.L^{-1}, and 19.14 g.L^{-1}) were fed (sludge loading rate of 43.59 kg.DS.m^{-2}.year^{-1}) to the units. Afterwards, the units experienced 132 days of resting period increasing dry solids from 21 to 70 % and decreasing volatile solids from 81 to 69 % by average (40 % sludge volume reduction). The bottom layers of the planted unit with earthworms showed a 30 % reduction in volatile solids, indicating improved sludge stabilization. Macronutrient abundance in the residual sludge followed the sequence: N > Ca > P > K > S > Mg. The planted unit with earthworms reduced micronutrient concentrations by 22 % compared to the control unit (Fe > Na > Mn > B > Mo). Earthworms also played a key role in reducing heavy metal concentrations by 11 % compared to the planted unit without earthworms (Zn > Cr > Pb > Ni > Cd). Heavy metal levels in the residual sludge met EU and Portugal standards, with a 99.9 % reduction in *Escherichia coli* and fecal coliforms. Cost estimation showed centrifugation and W-STRB scenarios cost 167 and 183 €.PE^{-1} for a ten-year operation, with O&M costs of 7 and 3 €.PE^{-1}.year^{-1}, respectively.

Keywords: *Eisenia fetida*, sewage sludge, constructed wetland, *Arundo donax*, cost assessment

5.2. Introduction

In an era marked by increasing concerns about sustainable wastewater management, sewage sludge management plays a pivotal role. Sewage sludge, a byproduct of wastewater treatment, holds immense promise as a biofertilizer due to its nutrient-rich composition and organic content (Chojnacka et al., 2023; de Barros et al., 2023). It boasts essential plant nutrients like nitrogen, phosphorus, and potassium, readily absorbable by plants, making it a valuable source of nutrients for agriculture (Skrzypczak et al., 2023). Moreover, its organic matter enhances soil structure, water retention, and acts as a slow-release nutrient, fostering long-term soil fertility (J. Xie et al., 2023). On the other hand, when sewage sludge is disposed of without admissible treatment, it can pose a significant threat to both micro and macro biota. Properly treated sewage sludge can reduce the need for synthetic fertilizers, thus curbing nutrient runoff and greenhouse gas emissions.

This approach is not only cost-effective but is also aligned with the circular economy principles in smart cities, where waste is transformed into valuable resources for environmental conservation (Mannina, Barbara, Cosenza, & Wang, 2023a). However, traditional sewage sludge dewatering methods, such as filter belt pressing and centrifugation, pose significant challenges like energy consumption and environmental sustainability. Conventional technologies produce suboptimal sludge in terms of composition and dewatering efficiency, and struggle to remove free water from sewage sludge, which typically contains 65 % water content (Wu et al., 2020). They also yield sludge with high volatile content, heavy metals, and organic and inorganic components (X. Zhang et al., 2022). This makes the dewatering process economically infeasible in the long run, even when coagulants such as polymer flocculants are employed. Hence, alternative approaches are imperative to address challenges of growing urban populations and stringent environmental regulations. Among these emerging technologies, sludge treatment reed bed (STRB) technology has garnered considerable attention for its potential to introduce new techniques to sludge dewatering processes. STRB as a nature-based solution (NBS) technique is one of the applications of treatment wetland technology for sewage sludge dewatering and treatment (Gholipour et al., 2022; Megyesi et al., 2024). Treatment wetland also has many applications including domestic (Daee et al., 2019a; Gholipour & Stefanakis, 2021) and industrial (Gholipour et al., 2020a) wastewater treatment, being considered a sustainable solution when compared with other technologies that have higher energy and construction costs (Galvão et al., 2005).

STRB technology has emerged as a sustainable approach for recycling residual sludge, offering advantages over conventional methods (Brix, 2017; Gholipour et al., 2024a; Gholipour et al., 2024b; Nielsen, 2023; Plestenjak et al., 2021; Stefanakis & Tsihrintzis, 2011; Uggetti et al., 2010). STRB system typically operates for 10 to 15 years, after which the feeding is ceased, and a final resting period (duration varies with climate) is employed to enhance the concentration of dry solids (DS), reduce volatile solids (VS), and minimize residual sludge volume (Nielsen, 2011). In temperate climates, final DS and VS concentrations have been reported between 17 and 94 % (average 45 %), and 14 and 61 % (average 39 %), respectively (Gholipour et al., 2022). In the Mediterranean region in Greece, DS and VS of 85 and 55 % were achieved (Stefanakis & Tsihrintzis, 2012a), while in tropical conditions in China, they were 59 % DS and 42 % VS (Wang et al., 2020). Provided that a STRB is well-operated and monitored, residual sludge can often be potentially reused as a by-product in case of compliance with standard limits (Nielsen, 2023). Quality assessment includes parameters like DS, VS, heavy metal, nutrient content, microbiological contaminants, and emerging pollutants (Brix, 2017; Nielsen, 2023; Uggetti et al., 2009). Previous studies have shown residual sludge stabilized after experiencing final resting of which heavy metals were reduced (Chen & Hu, 2019), nutrients got degraded and bioavailable (Osei et al., 2019), pathogenic organisms disinfected (Calderón-Vallejo et al., 2015a), and emerging pollutants decreased (Chen et al., 2009; Ma et al., 2020). Yet, in some cases, additional stages of treatment may be required to comply with local standards (Brix, 2017). Moreover, innovative variations of STRB such as electro-STRB (E-STRB or STEW) (Wang et al., 2021) and intensified-aerated STRB (I-STRB) (Plestenjak et al., 2021), particularly worm STRB (W-STRB), have demonstrated improvements in residual sludge quality (Gholipour et al., 2024a; Zhong et al., 2021). These innovative systems were used to improve system performance whereas microbial fuel cells (MFC) were also combined with STRB (E-STRB) for power generation, STRB can also be equipped with active aeration to enhance dewatering efficiency and percolation mechanism (I-STRB).

The present study evaluated effectiveness of W-STRB technology as an innovative approach for recovering biosolids, and particularly considering the qualities of the final residual sludge layer. This is a follow-up study of an experimental investigation conducted in the temperate climate of the Mediterranean region, specifically Portugal (Gholipour et al., 2024b). While prior research has demonstrated the efficacy of STRB technology in sludge dewatering, few studies have explored the characteristics of the final residual sludge layer across diverse climatic conditions. Additionally, most of these studies have predominantly utilized *Phragmites australis* (*P.australis*) in STRB, without including earthworms. The limited studies under controlled conditions that incorporated worms did not provide a thorough assessment of the final residual sludge, which could not offer insights into real-world scenarios. This research aimed to increase the knowledge base in NBS applied to sewage sludge dewatering. It was intended to shed light on the synergetic effects arising from the cohabitation of *Eisenia fetida* (earthworms) and *Arundo* *donax* (*A.donax*) in terms of variations in micro and macro nutrients, heavy metal, residual sludge content, and microbiological parameters. Additionally, it was sought to evaluate the potential for land application of the final biosolid. This study also provides insights into the technology costs, and to draw a cost analysis, a hypothetical centrifugation system was compared with W-STRB alternative. The findings were anticipated to make contribution to the ongoing discourse surrounding wastewater treatment practices and their implications for environmental sustainability.

5.3. Materials and methods

5.3.1. Experimental setup

This study was conducted at the Beirolas wastewater treatment plant (WWTP) situated in Lisbon, Portugal, serving to a population of 213,510 individuals and treating 54,500 cubic meters of wastewater daily. The local climate is characterized as hot-summer Mediterranean (Csa) as per the Köppen Geiger classification system. Meteorological data from the Instituto Português do Mar e da Atmosfera (IPMA) for the period spanning 1981 to 2010 reveals that Lisbon receives an average annual rainfall of 688 mm, with temperatures ranging from -1.5°C in January to 41.2°C in August. A weather station (Easy Weather - WIFI87CO, Guangdong, China) was installed on the rooftop of the primary building in Beirolas to gather various meteorological parameters, including air temperature (°C), UV index, solar power (W.m^{-2}), atmospheric pressure (mmHg), humidity (%), wind speed (m.s^{-1}), wind direction (degree), and precipitation (mm).

The experimental setup (Figure 5.1) consisted of eight one-cubic meter Intermediate Bulk Containers measuring 0.96 meters in width, 1.16 meters in length, and 1 meter in depth. These containers were divided into four categories: planted with earthworms (WP), planted without earthworms (P), unplanted but with earthworms (W), and control units (C) lacking both plants and earthworms. Each unit was replicated for the study.

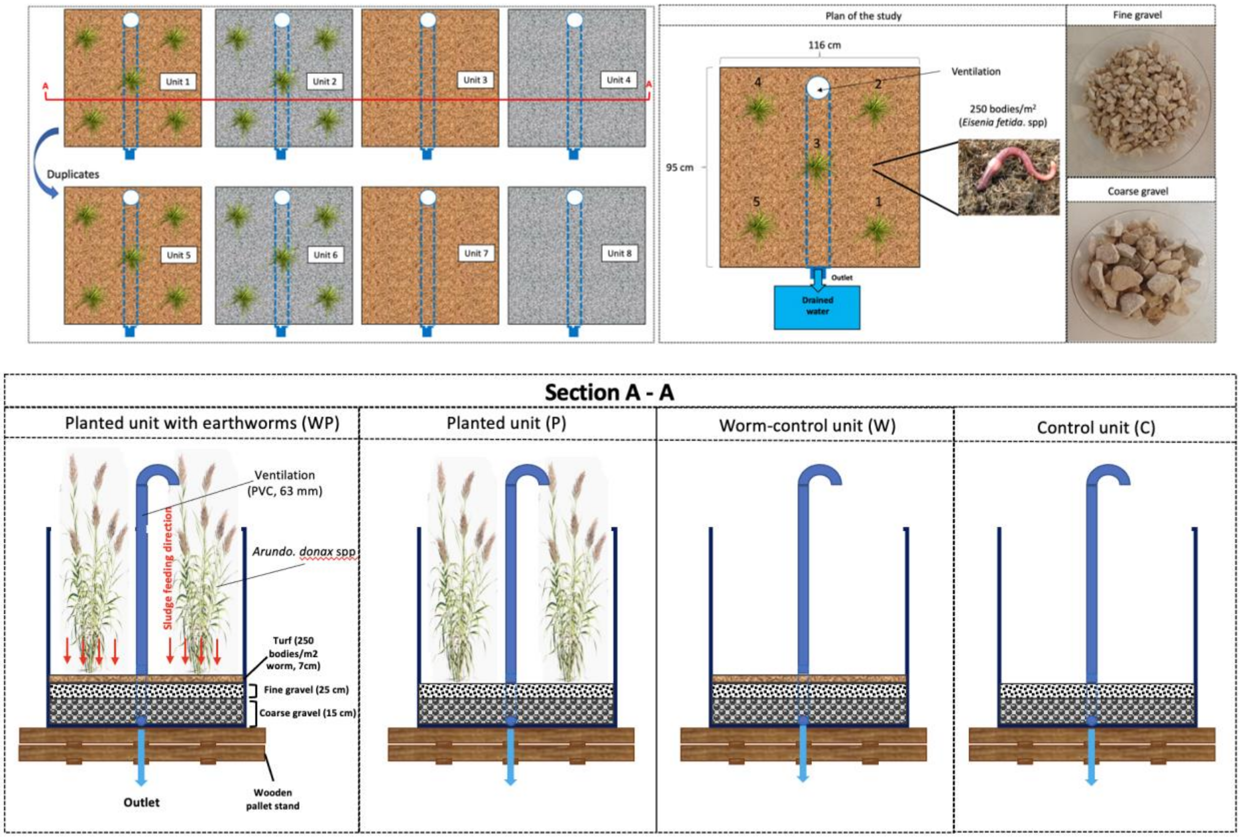


Figure 5. 1. Experimental setup

The units were constructed with drainage and transition layers consisting of coarse gravel (15 cm, 19 to 25 mm, and 38 % porosity) and fine gravel (25 cm, 4.8 to 9.5 mm, and 42 % porosity), respectively. For the WP and W units, a layer of turf (Siro 30) was added, which had specific properties including a pH of 5.5 to 6.5, conductivity of 40 to 80 μs.cm^{-1}, granulometry of 0 to 15 mm, and organic matter content exceeding 70 %. These units hosted 250 *Eisenia fetida* earthworms per square meter. The drainage system of the units was connected to 70-liter drums via perforated PVC drainage pipes (63 mm diameter) at the bottom, with plastic valves for drainage control, which remained open throughout the study. A vent line (1.5 m height) connected to the pipe facilitated passive air injection into the media. *A.donax* plants, obtained from Instituto Superior Agronomia (ISA), were planted at a density of five tufts per square meter after being defoliated and immersed in tap water to prevent desiccation. The planted units were irrigated weekly with 10 liters of treated wastewater from Beirolas WWTP during April 2022. The feeding period with mixed sludge collected from primary and secondary treatment stages started on May 13th, 2022, and ended on May 9th, 2023. This feeding cycle of sludge occurred every two weeks, with a total of 24 cycles. Earthworms were introduced to the units one week prior to the first sludge application. The sludge had average temperature, pH, electrical conductivity (EC), dry solid (DS), and volatile solid (VS) of 22.73°C, 5.98, 1.74 mS.cm^{-1}, 24.71 g.DS.L^{-1}, and 19.14 g.VS.L^{-1}, respectively (additional information can be found on the quality of the sludge and planted reeds in the supplementary materials). The study incorporated two sludge loading rates (SLR). From May 13th to November 29th, 2022, during the ramp-up phase, the volumetric loading rate (VLR) was set at 70 liters per square meter (SLR_1: 40.6 kg.DS.m^{-2}.year^{-1}). Subsequently, from November 29th, 2022, until May 2023, the load was increased to 100 liters per square meter (SLR_2: 50.4 kg.DS.m^{-2}.year^{-1}) for the nominal phase. The total SLR for the entire study period was 43.59 kg.DS.m^{-2}.year^{-1}. Following the nominal phase, 132 days of undisturbed final resting phase without sludge application was implemented for stabilization and mineralization until September 18th, 2023.

5.3.2. Scope of the study

In the study conducted at Beirolas WWTP (Gholipour et al., 2024b), the focus was on analyzing the water balance and the quality of drained water (Gholipour et al., 2024a) within a W-STRB system during feeding period of one year. The findings indicated a considerable increase in evapotranspiration rate and an enhancement in the quality of drained water, attributed to the inclusion of worms in the dewatering process. Throughout the study period, the quality of drained water was closely monitored, revealing an improvement in the WP unit compared to the P unit (Gholipour et al., 2024a).

Building upon the initial pilot study conducted at Beirolas WWTP, this research further investigated the quality of the final biosolids (accumulated residual sludge layer) obtained from the pilot-scale operation during final resting of 132 days. The present study had the following objectives: firstly, to assess the quality of the final biosolids in Beirolas experiment during the final resting period between May and September 2023. This evaluation entailed an examination of the residual sludge quality over 132 days of resting period, focusing on both macro and micro-nutrients as well as heavy metals. Secondly, an economic evaluation was carried out to compare a hypothetical dewatering centrifugation system with the W-STRB technology.

5.3.3. Sampling procedure and lab analysis

To assess characteristics of the feeding sludge from Beirolas WWTP, three sampling campaigns were executed which were carried out in November 2022, January 18th, 2023, and January 31st, 2023. The analysis encompassed both macro and micro-nutrients and heavy metals concentrations classified based on previous research (Kirkby, 2023). In addition, to measure DS and VS contents as well as the micro and macronutrients, microbiological parameters, and heavy metals, samples were collected from both the top and bottom layers of the residual sludge layer within each unit. To ensure representative samples, the residual sludge layer was uniformly and randomly selected from a designated area within each unit and mixed to create a composite sample for each unit.

Sampling activities were carried out at four specific time points, namely, 14, 36, 62, and 132 days after May 9th, 2023. For the elemental analysis, inductively coupled plasma (ICP) was utilized at the laboratory of ISA, following the guidelines outlined in the Standard Methods for the Examination of Water and Wastewater (Baird & Bridgewater, 2017). Microbiological analyses, encompassing the assessment of *Escherichia coli* (E. coli), Fecal coliform, and *Salmonella* spp, were conducted on both 14- and 62-days intervals during the final resting period.

In this study, plants were harvested twice including on January 13th, 2023, and September 22nd, 2023, to measure the wet aboveground biomass followed by the dry biomass determination (after drying for three days at 55C^o). The result of the first harvest on January 13th, 2023, can be found in Gholipour et al., (2024b) in which the dry biomass was 1.90 and 2.10 kg.m^{-2} for the WP units 1 and 5 while it was 1.00 and 1.70 kg.m^{-2} for the P units 1 and 6, respectively. It indicated 88 % water content on average while the planted units with earthworm showed 43 % higher biomass production compared to the planted units without earthworms.

In addition, earthworm population was counted through a hand-sorting in the flip and strip test (Gutiérrez-López et al., 2016) in which the WP and W units were dug in 20:20:15 cm (width: length: depth). In this paper, the population of earthworms analyzed during final resting. During feeding period based on Gholipour et al., (2024b), earthworm population varied, with the highest number between August and September 2022. This study included a wet season (September 2022 and April 2023) and a dry season (April 2023 to

September 2023) according to Gholipour et al (2024b). Based on this, the earthworm population reduced during the wet season and increased within the dry season.

5.3.4. Cost estimation

In this study, cost estimations were conducted based on previous monetary values from literature and online market surveys. To estimate the cost of sewage sludge dewatering, two hypothetical scenarios were considered: 1) centrifugation (S_1), and 2) STRB assisted with earthworms (S_2). To enhance the accuracy of the estimation, transportation, composting, and landfilling costs were excluded due to their variability across different countries since it varies significantly depending on sludge management plan. The boundary of the estimation is shown in Figure 5.2.

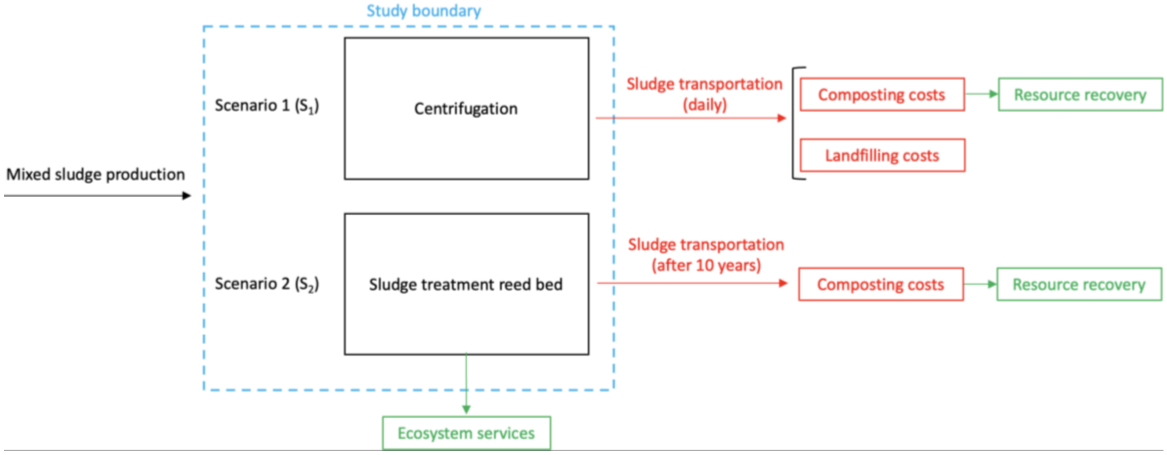


Figure 5. 2. Study boundary

A centrifugation system typically requires daily or weekly sludge transportation for either landfilling or composting depending on the amount of sludge produced and the management plan; therefore, depending on the sludge management strategy, the estimation varies. In contrast, according to Nielsen (2005), STRB can be removed from residual sludge every 10 to 15 years, based on the thickness of the residual sludge and the freeboard of the reed bed for sludge accumulation. The freeboard for the sludge accumulation is generally 1.5 meters, with an accumulation sludge rate of 10 to 15 cm per year (Brix, 2017). This means that sludge residue fills the freeboard after approximately 10 years. In addition, to compare O&M costs between the two scenarios, a ten-year analysis period was used (Nielsen, 2003).

Moreover, a benefit-cost analysis was not performed, as it would require comprehensive evaluation of the benefits from both scenarios, such as resource recovery and ecosystem services. The aim was to evaluate the costs of each scenario separately and provide a comparative analysis, if necessary, to present an overview of their financial implications, aiding engineers, and decision-makers.

To consider scalability, efficiency, and environmental benefits of STRB system, the calculation was conducted for 2000 population equivalents (PE) which represented small community application (Chen et al., 2020). Based on average wastewater production in Portugal, 180 to 220 L per capita (200 L.PE^{-1}.day^{-1}) is daily expected to flow to WWTP (Covas, 2018). The dry and volatile solid contents were 24.71 g.DS.L^{-1}, and 19.14 g.VS.L^{-1}, which were based on Beirolas experiment shown in Figure 5.1. The technical and design specifications of W-STRB systems can be found in Table 5.1.

Table 5. 1. W-STRB technical and design specifications

Specification	Unit	Value	Comment	Reference
Sludge loading rate (SLR)	Kg.DS.m^{-2}.year^{-1}	43.59	Based on Beirolas experiment	(Gholipour et al., 2024b)
Sludge flow (Q)	m^3.day^{-1}	20	(PE × 200L) / 1000	
DS	g.DS.L^{-1}	25	Based on Beirolas experiment	(Gholipour et al., 2024b)
VS	g.VS.L^{-1}	19	Based on Beirolas experiment	(Gholipour et al., 2024b)
Total area required for W-STRB	m^2	4000	(Q × DS × 365) / SLR	(Nielsen, 2003)
Turf layer thickness	m	0.05	Based on Beirolas experiment	(Gholipour et al., 2024b)
Total turf volume	m^3	200	W-STRB area × Turf layer thickness	
Total weight of earthworm	kg	400	0.5g.m^{-2} based on Beirolas experiment	(Gholipour et al., 2024b)

According to Uggetti et al (2011), the electricity consumption of a centrifugation system was 0.28 kWh.year^{-1}.PE^{-1} and for its pumping system required 0.06 kWh.year^{-1}.PE^{-1} which yielded 0.082 m^3 of sludge.year^{-1}.PE^{-1}. Several components were considered to estimate the costs of centrifugation and STRB, e.g. investment costs, which are shown in Table 5.2.

Table 5. 2. Costs assessment specification

Scenarios	Costs component	Cost source	Unit	Cost
Centrifugation	Investment	(Uggetti et al., 2011)	€	160824
	Personnel	(Uggetti et al., 2011)	€/year	9517
	Materials replacement	(Uggetti et al., 2011)	€/year	3496
	Pump electricity	Portuguese power company, (Uggetti et al., 2011)	€/year	239

	Centrifuge electricity	Portuguese power company, (Uggetti et al., 2011)	€/year	1100
W-STRB	Construction	Portuguese technical guide 23 (ERSAR)	€	163000
	Electromechanically and electrical installations	Portuguese technical guide 23 (ERSAR)	€	39000
	Turf layer	SIRO (Portuguese market)	€	14286
	Earthworm	Market search	€	20000
	O&M	(Rizzo et al., 2018)	€/year	6000
	Personnel	(Uggetti et al., 2011)	€/year	4728

To increase the accuracy of the estimation, several literatures were used to obtain the costs for centrifugation and STRB of the previous studies (DiMuro et al., 2014; Gkika et al., 2014; Piao et al., 2016; Rizzo et al., 2018; Tsihrintzis et al., 2007; Uggetti et al., 2011). To update previous studies costs and to predict future cash flows, the interest and discount rate of European Central Bank were used, which were 4.5 and 4.0 %, respectively. Eq.1 was applied for predicting future monetary values (Žižlavský, 2014):

$$\boldsymbol{FV} = \boldsymbol{PV}(\mathbf{1} + \boldsymbol{i})^{\boldsymbol{t}}$$

Eq.1

In which *i* is the discount rate, *t* is time of the cash flow, FV is future value and PV is the present value.

The costs presented in Table 2 are for the year 2024; thus, the respective costs of the following years of operation including personnel, material replacement and electricity until year 2034, were accounted using Eq.1.

5.3.5. Data analysis

Normality (Shapiro-Wilk test) and homogeneity (Bartlett's test) for all datasets were evaluated. In instances where normality and homogeneity criteria were not met, differences among units were assessed using the Kruskal-Wallis’s test for one-way analysis of variance, with statistical significance level set at a p-value = 0.05. All statistical analyses were conducted using R Studio. Additionally, fundamental statistical metrics such as minimum, maximum, mean, and standard deviation (SD) were calculated.

5.4. Results and discussion

5.4.1. Meteorological data analysis

During the final resting, air temperature was between 12.9 and 35.2 °C (Figure 5.3.a), with an average of 20.78 °C (±3.42 °C). Humidity fluctuated between 23 and 97.2 %, with a mean of 66 % (±15 %).

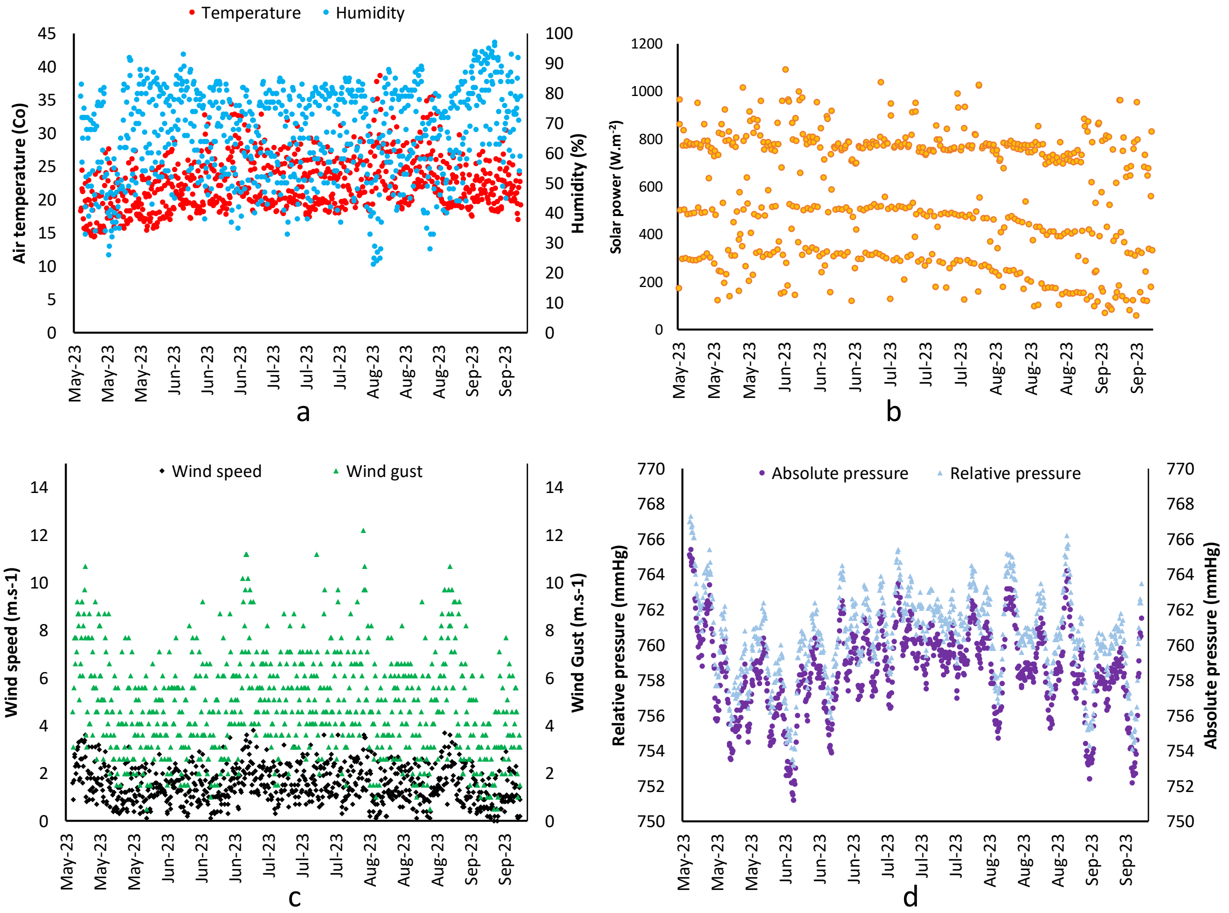


Figure 5. 3. Meteorological data a) air temperature and humidity, b) solar power and precipitation, c) wind speed and gust, and d) relative and absolute pressure

The precipitation levels for May, June, July, August, and until September 22nd, 2023, were 7, 11.8, respectively, and 0 mm, for the other months. The maximum solar power reached 1419 $W.m^{-2}$ in June (Figure 5.3.b), and wind gusts ranged from 0.5 to 12.2 $m.s^{-1}$ (Figure 5.3.c), with an average of 4.83 $m.s^{-1}$ (±2.1$m.s^{-1}$). Wind speeds varied between 0.2 and 3.8 $m.s^{-1}$, averaging at 1.54 $m.s^{-1}$ (±0.78 $m.s^{-1}$). The lowest recorded relative pressure was in June at 753 mmHg, whereas May witnessed the highest at 767 mmHg (Figure 5.3.d). Absolute pressure fluctuated between 751 and 765 mmHg, with an average of 759 mmHg (±2.47 mmHg). Based on previous studies, Lisbon has a Mediterranean climate marked by mild, moist winters and warm, dry summers, featuring relatively low precipitation and observable temperature fluctuations (Reis et al., 2022).

5.4.2. Mixed sludge quality

The highest concentration of macronutrients was observed in November 2022 samples, reaching 122775 mg.kg$_{DS}^{-1}$ in which the DS and VS contents were 14.20 mgDS.L^{-1} and 10.72 mgVS.L^{-1} (Figure 5.4.a). In contrast, January 18th (30.42 mgDS.L^{-1} and 23.40 mgVS.L^{-1}), and January 31st (30.42 mgDS.L^{-1} and 23.40 mgVS.L^{-1}), 2023, exhibited slightly lower concentrations by 106650 and 100827 mg.kg$_{DS}^{-1}$ respectively. The concentrations of micronutrients followed a similar decreasing trend across the three months, with values of 22761, 18989 and 14294 mg.kg$_{DS}^{-1}$ and heavy metal concentrations also displayed variations by 655, 478 and 498 mg.kg$_{DS}^{-1}$ for November, January 18th, and January 31st, respectively. These variations could be attributed to the precipitation during the wet season diluting the mixed sludge tank at Beirolas WWTP.

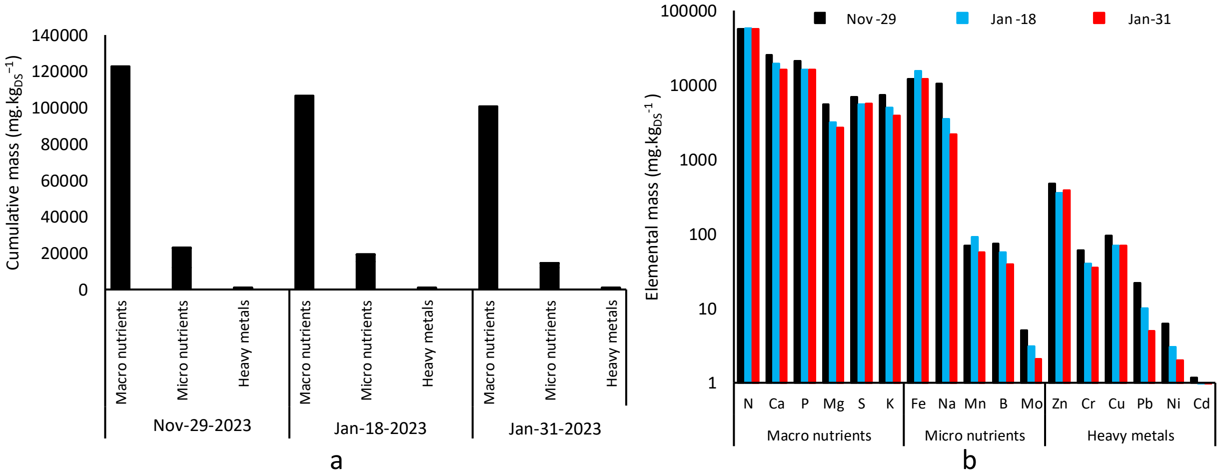


Figure 5. 4. Macro and micronutrients and heavy metals in a) cumulative mass b) elemental mass

The mass of N emerged as the predominant component and the significant concentration order of macronutrients for the three campaigns was N > Ca > P > K > S > Mg (Figure 5.4.b). The proportion of N:P:K was 7:3:1; therefore, the mixed sludge could serve as a nitrogen-rich resource. On the other hand, Fe and Na were dominant in micronutrients (Figure 5.4.b) with a significant order of Fe > Na > Mn > B > Mo. Moreover, Zn was the most abundant element in the cumulative mass of heavy metals (Zn > Cu > Cr > Pb > Ni > Cd). In a study on STRB system (Ma et al., 2023), the order of heavy metals in a raw sludge (from a domestic wastewater) was Zn > Cr > Cu > Ni > Pb > Cd by the concentrations of 492, 166, 95, 92, 84, and 5 mg.kg$_{DS}^{-1}$ which is comparable with the present study. However, the concentration and elemental order of these components are highly dependent on the characteristics of the source of raw wastewater.

5.4.3. Analyzing plant and earthworm dynamics

The above ground plant biomass was higher in the unit with earthworms than in the units without earthworms (Table 5.3). During January and September 2023 (nine months), the dry biomass increased compared to the first harvested (January 2023) and yielded 4.08 and 4.45 kg.m^{-2} for the WP units 1 and 5 while it was 3.20 and 3.20 kg.m^{-2} for the P units 1 and 6, respectively. The WP units showed 24 % higher biomass production than the P units. It could indicate the synergistic effect of earthworms in enhancing plant biomass production. Through the water balance analysis (Gholipour et al., 2024b), namely water loss, through evaporation and evapotranspiration, was also higher in the WP units which could be due to higher biomass production. The difference between the WP and P units biomass production was 43 % from the first harvest in January 2023 and 24 % for the second harvest. This could be attributed to the stabilization of the units after months of feeding and final resting representing more consistency and uniformity in the biomass production (Brix, 2017). In September 2023, the biomass water content reached 78.52 % indicating 10 % reduction compared to the water content from the first harvest in January 2023 (87 %). This could be attributed to the increase in the dry solid content of the residual sludge layer within the final resting period.

Table 5. 3. *A.donax* above-ground biomass (January 2023~September 2023)

Parameters	Unit 1 (WP)	Unit 5 (WP)	Unit 2 (P)	Unit 6 (P)
Wet biomass (kg.m^{-2})	19.90	20.50	14.50	14.80
Water content (%)	79.50	78.30	77.90	78.40
Dry biomass (kg.m^{-2})	4.08	4.45	3.20	3.20

The population of earthworms was monitored during the final resting (Figure 5.5). At the end of the study period in September 2023, the population in the flip and strip test was 26 and 30 worms for the WP units 1 and 5, and 47 and 51 worms for the W units 3 and 7, respectively. This indicated population increase for all units. The units without plants showed a higher number of earthworms which could be due to the higher availability of organic matter in the residual sludge layer (Edwards & Arancon, 2022; Gholipour et al., 2024b).

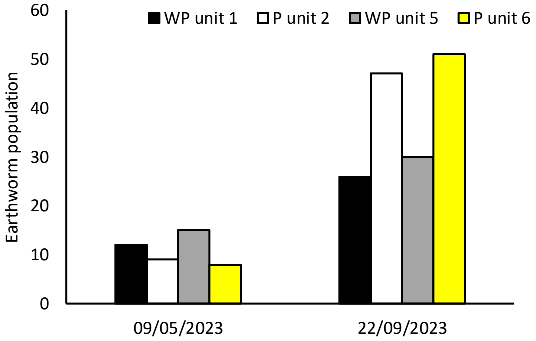


Figure 5. 5. Earthworm population during final resting

5.4.4. Dewatering performance (final resting)

A consistent reduction in the thickness of the residual sludge throughout the resting period was observed, with all units experiencing a decrease of over 52 % (Table 5.4). The reduction was 67, 58, 74 and 52 % for the WP, P, W and C and W units, respectively. This observation suggested the presence of worms and plants in the WP unit likely contributed to a higher reduction in the thickness of the residual sludge. It is plausible that the development of the root system in the WP unit increased belowground biomass, affecting the overall thickness of the residual sludge layer. In contrast, the W unit, which only had worms, resulted in the lowest residual sludge volume.

Table 5. 4. Residual sludge thickness

Time of resting (day)	Thickness of the residual sludge (cm)				Sludge volume reduction (%)			
	WP	P	W	C	WP	P	W	C
14	0.16	0.22	0.17	0.19	13	3	5	13
36	0.13	0.18	0.15	0.18	31	21	19	16
62	0.11	0.13	0.10	0.14	38	45	45	35
132	0.06	0.09	0.05	0.10	65	59	69	52

In this study after 132 days of resting, sludge accumulation rates for the residual sludge layer were 0.06, 0.09, 0.05, and 0.1 m.year^{-1} for in the WP, P, W, and C units, respectively. These rates closely align with temperate climate accumulation rates ranging from 0.26 to 0.1 m.year^{-1}, with the 0.09 m.year^{-1} rate observed in this study being comparable to research in the Mediterranean region, particularly for the STRB (P) unit (Gholipour et al., 2022). Studies in the Mediterranean region have shown residual sludge layer reductions ranging from 81 to 92 %, with resting periods varying between 25 and 180 days (El-Gendy et al., 2017; Stefanakis & Tsihrintzis, 2011). The WP unit, incorporating both worms and plants, demonstrated a significant reduction in residual sludge layer volume compared to previous STRB studies under similar climates, showcasing a 40 % improvement from 0.1

m.year^{-1} in Mediterranean studies to 0.06 m.year^{-1} in the current study through the integration of worms (Gholipour et al., 2022).

The result of sludge dewatering during the feeding period was presented in the water balance analysis study (Gholipour et al., 2024b). In this paper during the final resting, there was an increase in DS content on the top layer during the first month of the resting period, which was later reversed due to September 2023 precipitation, leading to a decrease in top DS content. Meanwhile, DS content in the bottom layer of the residual sludge steadily increased across all units from 21 to 70 %. As the final resting period advanced, DS levels in the top and bottom layers converged, rendering the difference insignificant (P-value > 0.05). Consequently, after 132 days of resting, the vertical profile of the residual sludge demonstrated near-uniform dryness.

Table 5. 5. Residual sludge thickness

Parameter	Time of resting (day)	Top				Bottom			
		WP	P	W	C	WP	P	W	C
DS (%)	14	73	77	65	58	22	25	21	17
	36	93	93	91	92	21	22	24	25
	62	92	93	92	92	27	25	25	28
	132	71	71	70	74	70	67	69	68
VS/DS (%)	14	82	81	80	81	66	58	67	58
	36	77	80	73	72	66	56	66	57
	62	71	72	71	69	64	55	64	57
	132	69	68	68	68	53	54	61	56

Various mechanisms likely contributed to the water loss, including plant evapotranspiration, water use in worms' metabolic processes, surface evaporation, and drainage into the filtration media (Edwards & Arancon, 2022; Gholipour et al., 2024b).

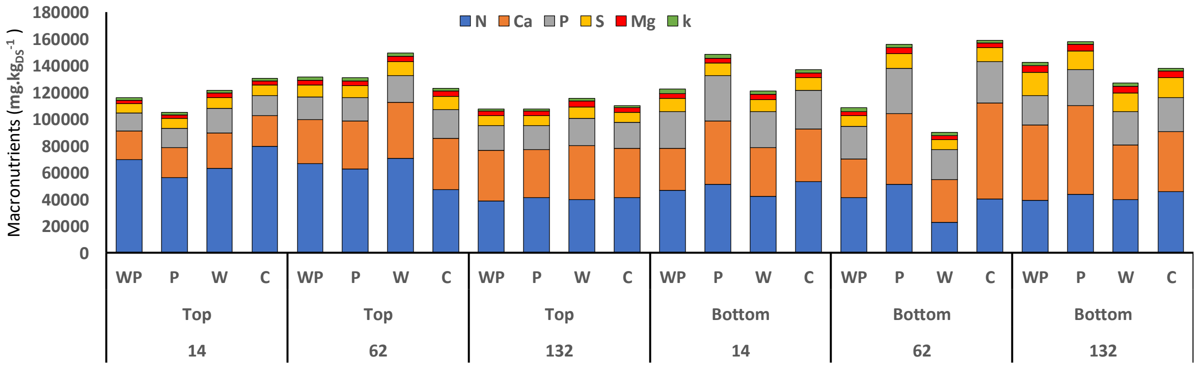
The difference in VS/DS between the top and bottom layers of the residual sludge ranged from 15 to 30 % indicating lower VS content in the bottom layers for all units (P value < 0.05). While the top VS/DS contents remained relatively stable across all units, decreasing from approximately 81 to 69 % during the final resting period. The WP unit exhibited the most significant reduction with 52.78 % at 132 days. At 132 days, the VS/DS contents were 53.5, 61.37, and 55.75 % for the P, W, and C units, respectively. The synergistic effect of worms and plants in the WP units enhanced the reduction rate which could be attributed to the utilization of decomposed organic matter by worms and plants and the conversion of organic matter into simpler particles (Makkar et al., 2023). In comparison with the findings of the STRB studies conducted in temperate and tropical climates, VS/DS ratios ranging from 39 to 52 % were reported in which the final resting periods varied between 60 and 365 days. In contrast, this study achieved a VS/DS ratio of 61.37 % after 132 days of resting, closely aligning with standards for temperate climates, particularly in the Mediterranean region. For the W-STRB unit (the WP unit), a VS/DS content of 41.6 % was reported in a tropical climate (Zhong et al., 2021), whereas this study recorded a higher content of 52.78 %. Overall, the inclusion of worms in the STRB system appeared to enhance stabilization by approximately 10 % compared to this study´s planted unit (Gholipour et al., 2023b).

5.4.5. Final residual sludge quality

The trend across all units was an increase in the cumulative mass of macronutrients in the top layers between days 14 and 62, which could be due to an increase in the dry solid content of the top layer contributing to the reduction of the residual sludge layer (Figure 5.6.a). Subsequently, between days 62 and 132, following a period of rain (57 mm) and a decrease in the top dry solid of the residual sludge layer, macronutrient concentrations experienced an upswing. This increase could be primarily attributed to leaching from the top to the bottom layers, the processes of stabilization and mineralization (Ma et al., 2023). The bottom layers showed a similar trend, but units containing earthworms initially decreased due to rain and water infiltration into the drained water. The bottom layers exhibited higher macronutrient availability, with the unit with plants without earthworms displaying a higher concentration of macronutrients.

At 132-day, the P units showed the highest cumulative mass of macronutrients in the bottom (157868 mg.kg$_{DS}^{-1}$), while the WP, W, and C units presented 142431, 126864, and 138162, mg.kg$_{DS}^{-1}$ respectively. This could be potentially stimulated through various biological and chemical reactions, and possibly facilitated by worms, microbial activities, precipitation, redox processes, and adsorption mechanisms (Edwards & Arancon, 2022). The W unit exhibited higher concentrations compared to the other units (115305 mg.kg$_{DS}^{-1}$), in which the absence of worms in the top layers could lead to this difference. Approaching the end of resting and day 132, the concentrations of macronutrients became more consistent across the vertical profile, potentially due to the increase in the dry solid content and the reduction in volatile solid contents.

The macronutrient composition within the top layers consistently followed a significant elemental order of N > Ca > P > K > S > Mg from day 14 to day 132. In the bottom layers, the significant elemental order was different initially, with Ca taking precedence from day 62 to day 132 (Ca > N > P > K > S > Mg). At day 132, the proportion (N : Ca : P : K : S : Mg) of macronutrient elements in the top was 27:27:13:5:3:1 for the WP units, 27:23:12:5:2:1 for the P units, 20:20:11:4:2:1 for the W units, and 28:25:13:5:2:1 for the C units. In the bottom layers, the proportions differed, with ratios of 15:22:9:7:2:1 for the WP units, 20:30:12:6:2:1 for the P units, 16:16:10:5:2:1 for the W units, and 20:20:11:6:2:1 for the C units. The average proportion for the original mixed sludge was 13:4:3:1:1:1, indicating an accumulation in the proportions of Ca, P, K, and S over time in comparison to the proportions of the final residual sludge layer. This shift could be potentially attributed to processes such as precipitation onto sludge particles, ionic exchange, adsorption, as well as various biological and chemical reactions (Ma et al., 2020). N and Mg decreased, potentially due to transformations into other elemental forms through ammonification and ionic exchange (Antonkiewicz et al., 2020; Nielsen, 2023; Singh et al., 2022).



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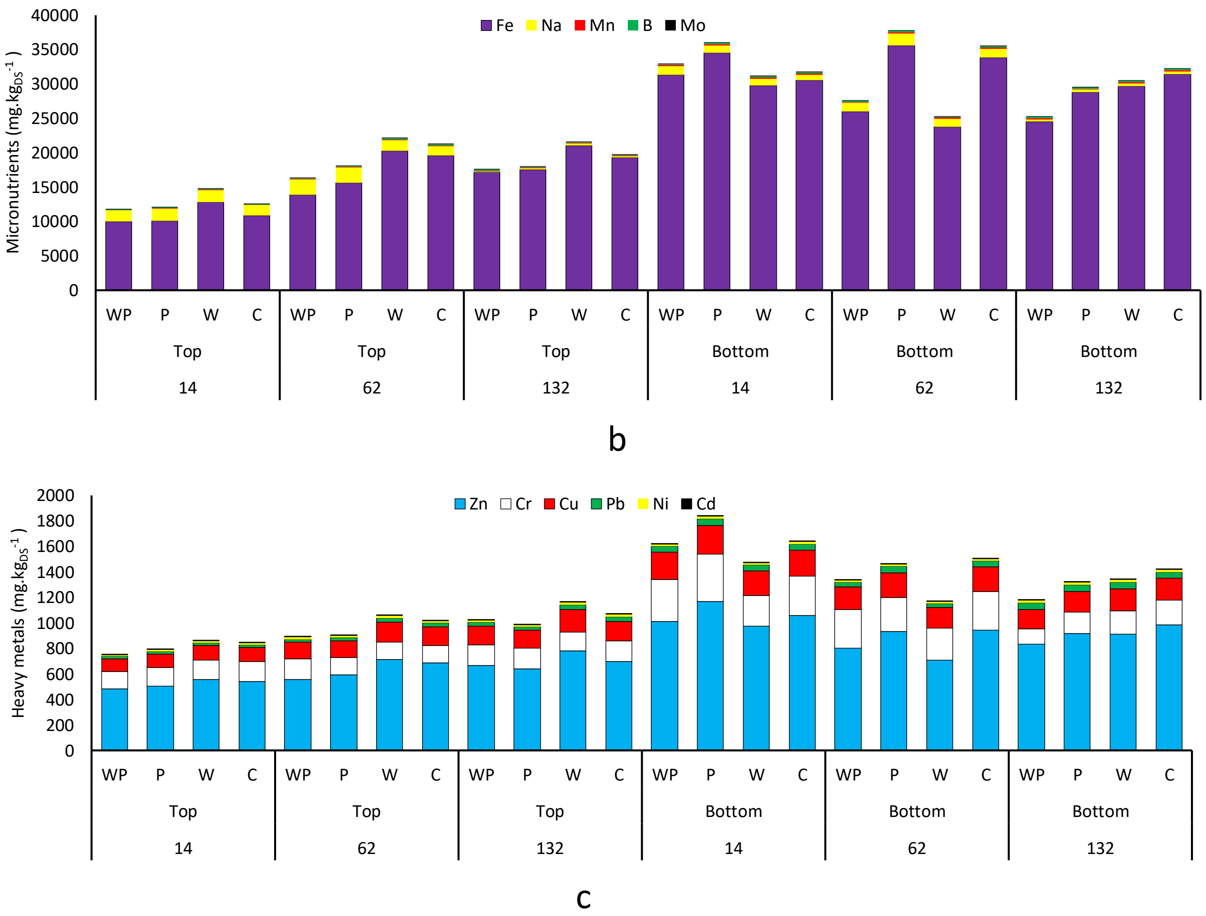

Figure 5. 6. Residual sludge quality in the top and bottom layers a) macronutrients b) micronutrients c) heavy metals

The temporal changes and a similar pattern to that of macronutrients emerged for micronutrient, with bottom layers consistently exhibiting higher cumulative masses compared to the top layers (Figure 5.6.b). In the top layers, the fraction of Fe exhibited an average increase from 12777 mg.kg$_{DS}^{-1}$ on day 14 to 19206 mg.kg$_{DS}^{-1}$ on day 132 across all units. The dominance of Fe could be due to the addition of ferric chloride in Beirolas WWTP for flocculation and H_2S control during biogas production which increased Fe over time. This can be also attributed to multiple mechanisms, including iron precipitation, oxidation adsorption, and reduction and compaction of the residual sludge layer (Ma et al., 2020).

Na exhibited an average decrease, dropping on day 14 toward day 132 across all units, which could be due to various factors, including leaching into the drained water, plant uptake, microbial activity, and chemical reactions (He et al., 2021). Additionally, the coexistence of worms and plants potentially played a role in this decrease which was more intensive in the bottom layer. The cumulative mass of the WP unit (25185 $mg.kg_{DS}^{-1}$) was 22 % lower than that of the C unit (32218 $mg.kg_{DS}^{-1}$). The P and W units exhibited lower final cumulative masses (29531 and 30451 $mg.kg_{DS}^{-1}$), lagging the C unit by 8 and 5 %, respectively. The significant elemental order remained consistent as Fe > Na > Mn > B > Mo.

At day 132, in the top layers, the cumulative mass was 1023, 986, 1165 and 1070 $mg.kg_{DS}^{-1}$ and in the bottom, it was 1182, 1320, 1341 and 1424 $mg.kg_{DS}^{-1}$ for the WP, P, W and C units (Figure 5.6.c). Thus, the WP unit had the lowest availability of heavy metal in the bottom layers, which can potentially highlight the synergistic effect of worms and plants in reducing heavy metal concentrations. The WP unit displayed 17, 12, and 11 % less cumulative mass than the C, W, and P units. Based on previous research bioremediation by earthworms in STRB system likely occurred through several mechanisms, including bioaccumulation, metal binding, improved aeration of residual sludge, stimulation of microbial activity, and biological uptake. The enhancement of residual sludge structure and worms transform toxic elements like heavy metals into less toxic forms and immobilize them within the residual sludge particles (Chen & Hu, 2019; Ma et al., 2020). In both the top and bottom layers, the significant elemental order of heavy metal was consistent, with Zn > Cr > Cu > Pb > Ni > Cd. The elemental order of the mixed sludge was Zn > Cu > Cr > Pb > Ni > Cd showing that copper was the second concentrated element in the sludge while in the residual sludge, Cr took its place. Zn accumulated in the bottom layer potentially due to processes such as aging, precipitation, and chemical bonding (Stefanakis & Tsihrintzis, 2012b). Additional information can be found in the supplementary materials in which a comparative analysis with both national and international standard limits, and insights from previous research is provided. It indicated that the final residual sludge consistently fulfill the standard limits outlined in national and international regulations.

In conclusion, concerning its heavy metal content the residual sludge can be deemed safe for reuse. In this study, Zn and Cr were 832 and 121 $mg.kg_{DS}^{-1}$ in the WP unit while they were 920 and 65 $mg.kg_{DS}^{-1}$ averagely in the previous studies for a planted unit without earthworms (Kolecka & Obarska-Pempkowiak, 2013; Stefanakis & Tsihrintzis, 2012b). The variability in heavy metal masses across different studies can likely be attributed to variations in factors such as study duration, sludge loading rate, dry solid content, sewage sludge source, climatic conditions, plant species, the presence of worms, and the depth from which sewage sludge samples were obtained.

The results of microbiological assessments showed a reduction in *E.coli* and fecal coliform levels after 62 days of the final resting period (Figure 5.7). This reduction was consistent across all units, resulting in an average 2 log decrease (99 %) from the top to the bottom layers. In summary, the concentration of both *E.coli* and fecal coliform dropped to below 1000 CFU.100mL^{-1}. In general, *E.coli* was more prevalent than fecal coliform, and by day 62, in the bottom layers, the WP unit exhibited lower levels of fecal coliform compared to the other units. This indicated that the presence of worms in the residual sludge layer, potentially, led to a more disinfection effect, which can be attributed to several interactions, including improved oxygenation within the residual sludge layer (Wang et al., 2019).

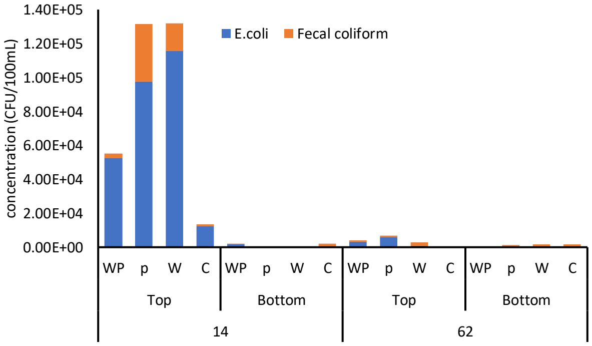

Figure 5. 7. *E.coli* and Fecal coliform in the top and bottom layers of the residual sludge over time

After 62 days of resting, there was an average removal of 3.7 and 2.7 log for *E.coli* and fecal coliform across all units (99.9 % removal). When compared to the Portuguese and EU legislation governing the reuse of sewage sludge (Directive 86/278/EEC), only the final residual from the WP unit meets the prescribed standard limit, which mandates levels lower than 1000 CFU.100mL^{-1} (Additional information in the supplementary materials). *Salmonella* was not detected in the bottom layers of the WP and P units after 62 days of the final resting period while it was present in the W and C units in the same conditions. In the top layers, *Salmonella* was detected at day 62 for all units. The persistence of *Salmonella* in the residual sludge layer may be attributed to several factors. *Salmonella* bacteria can possess survival mechanisms that enable them to endure adverse conditions, such as desiccation and environmental stress (Chaudhari et al., 2023). In fact, they can form spores or persist in protective environments, which may allow them to survive for extended periods and re-emerge when conditions become more favorable. According to Portuguese and EU legislation (Directive 86/278/EEC), the absence of *Salmonella* is required in a 50 g sample. In this study, the bottom layer of the WP unit complied with this regulatory requirement.

5.4.6. Cost assessment

The cost assessment suggested that the investment cost of 160,824 euros dominated the first year, constituting over 91.81 % of the total expenses for the centrifugation system (Figure 5.8.a). In Figure 5.8.b, it was observed that the construction costs for W-STRB system accounted for 68.98% of the total expenses in the initial year (163,000 euros). Together with the additional costs of earthworm (8.46%), turf layer (6.05%) and electromechanically and electrical installations (16.51%), W-STRB for 2000 PE would cost 236,286 euros. This indicates that W-STRB scenario costed 47% greater than the centrifugation scenario in the initial year.

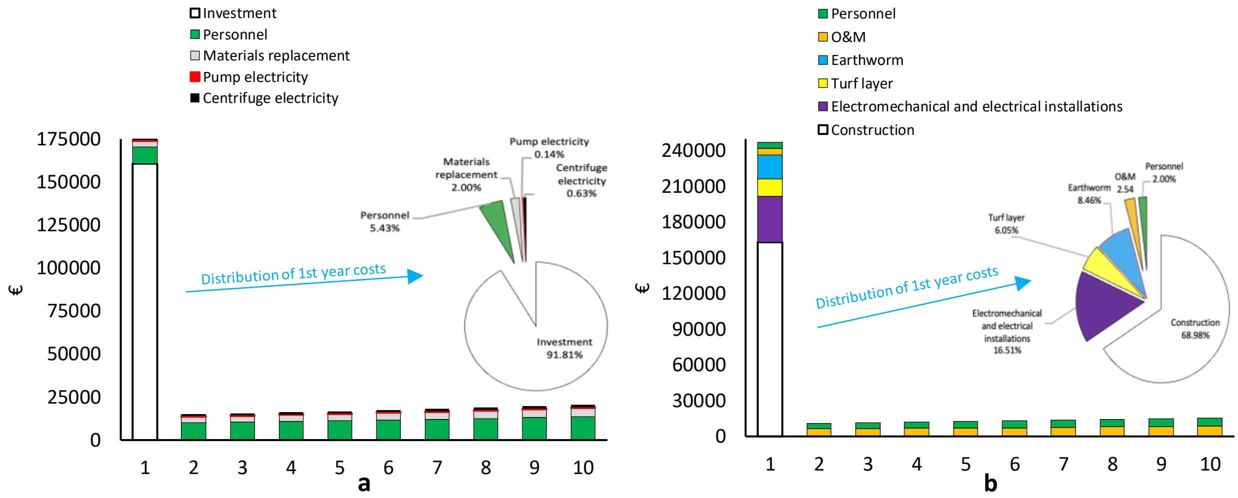

Figure 5. 8. Cost assessment along five years a) Centrifugation b) W-STRB

In the centrifugation system, personnel costs represented 5.43 % of the total expenses, 2.00 % for material replacement costs, 0.14 % for pump electricity and 6.64 % for centrifuge electricity. W-STRB systems bear in total 2.54 % for O&M and 2.00 % for personnel. In addition, the results showed that personnel costs and O&M expenses constituted 66.31 and 33.69 % for the centrifugation (24427 euros) while they were 44.07

and 55.93 % W-STRB scenarios (15269 euros), respectively. This indicates that W-STRB scenario had 37 % lower personnel and O&M costs compared to centrifugation scenario. In comparison, the initial year costs for the centrifugation and W-STRB systems were 88 and 124 €.PE^{-1} of which O&M costs were 7 and 3 €.PE^{-1}.year^{-1}, respectively. However, O&M costs in this study did not include the cost of sludge transportation and further landfilling or possible sludge recycling, which vary across different countries and locations. The study by Uggetti et al. (2011) reported an investment cost of 80 €.PE^{-1} for STRB scenario, which, when adjusted using Eq.1, was estimated at 133 €.PE^{-1} in Spain, aligning with the cost range of the present study (124 €.PE^{-1}). However, the Spanish study did not include earthworm in the system. In an Italian study focusing on constructed wetlands (Rizzo et al., 2018), O&M costs ranged between 6 and 11 €.PE^{-1}.year^{-1}. The result of ten years of both system application showed that centrifugation and W-STRB would cost 167 and 183 €.PE^{-1}, respectively.

5.5. Implication of the study

The study's findings revealed a diverse range of micro and macro nutrients, alongside heavy metals, within residual sludge, suggesting treatment, recovery, and reuse opportunities. Cost assessments comparing centrifugation and W-STRB scenarios emphasized lower maintenance and operation costs with W-STRB, and its potential cost-effectiveness. Based on the literature, centrifugation systems entail high costs for sludge transportation and chemical additives like polymers, highlighting the need for alternative methods such as W-STRB, which offer sustainability and cost efficiency (Tarpani & Azapagic, 2018). Beyond cost savings, W-STRB adoption streamlines sludge management and provides additional benefits like ecosystem services and improved residual quality for repurposing (Cieślik et al., 2015). The concept of sludge recycling, replacing synthetic fertilizers, aligns with sustainable practices and environmental stewardship, marking a shift towards more sustainable and efficient sludge management (Ayhan Demirbas & Alalayah, 2017).

The integration of earthworms into STRB offers a novel approach towards enhancing dewatering efficiency and drained water quality (Gholipour et al., 2024b, 2024a) and potentially balancing economic benefits with environmental considerations (Rizzo et al., 2018). Improved ecosystem services and improved residual quality signify broader implications and opportunities for sustainable waste management practices (Agaton & Guila, 2023). These include water supply, biomass production, water treatment, flood protection, erosion control, habitat formation, nutrient cycling, recreational amenities, and biodiversity preservation (Yang et al., 2008). By reducing carbon footprints, enhancing biodiversity, and promoting soil health, sludge management through nature-based technologies like STRB contributes to overall ecosystem resilience and sustainability (Kacprzak et al., 2017). Its resilience amidst climate change underscores its adaptability and potential to mitigate its impacts (García-Herrero et al., 2022; Gholipour et al., 2023). Quantifying ecosystem services in monetary terms could further increase the cost-effectiveness of the STRB system, despite higher initial investment costs compared to mechanical dewatering techniques (Firth et al., 2020; Gkika et al., 2014; Piao et al., 2016; Tsihrintzis et al., 2007).

Understanding the social implications of implementing such sustainable waste management practices is crucial, requiring community engagement, public health considerations, and stakeholder involvement (Gholipour et al., 2023). Policy implications suggest integrating nature-based solutions into regulatory frameworks to prioritize sustainable waste management practices (Frantzeskaki, 2019; L. Xie & Bulkeley, 2020). Scaling up earthworm-assisted reed bed systems to larger sewage treatment facilities could enhance the general efficiency of the traditional sludge management practices, offering a sustainable and cost-effective solution on a larger scale (Nielsen, 2007, 2023).

5.6. Conclusions

A pilot-scale study conducted in the Mediterranean region explored the use of worms in sludge treatment reed bed to dewater sewage sludge. The study demonstrated the synergistic effects of worms and *A.donax* on the quality of the residual sludge. After 132 days of resting period, the residual sludge thickness decreased 67 % in the planted unit with earthworms and 74 % in the unplanted unit without earthworms. Including earthworms in the system could reduce sludge volume by around 40 %. During the resting period, DS content increased from 21 to 70 %, and VS decreased from 81 to 69 %. The bottom layers exhibited VS levels 15~30 % lower than the top layers, indicating enhanced sludge stabilization over time. The study also examined the impact of feeding sludge on the quality of residual sludge concerning nutrients. It was found that the order of significance accumulation was N > Ca > P > K > S > Mg for macronutrients and Fe > Na > Mn > B > Mo for micronutrients. The study also assessed heavy metal accumulation; the significance order was Zn > Cu > Cr > Pb > Ni > Cd. Over 132 days, the bottom layers accumulated higher masses of these elements than the top layers. The WP unit had a significantly impacted micronutrient masses, with a reduction of 22 % compared to the C unit, with the significance order of Fe > Na > Mn > B > Mo. Worms played a substantial role in reducing heavy metal masses, with the WP achieving a reduction of 17 and 12 % compared to the P and C units, respectively, with the significance order being Zn > Cr > Pb > Ni > Cd. In the cost estimation, the costs of the initial year for centrifugation and W-STRB scenarios were 88 and 124 €.PE^{-1} while the O&M costs were 7 and 3 €.PE^{-1}.year^{-1}, respectively. In conclusion, sludge treatment through reed bed could be practiced based on the residual sludge quality while considering resource recovery, transportation and sludge treatment costs could make the system economically feasible.

5.7. References

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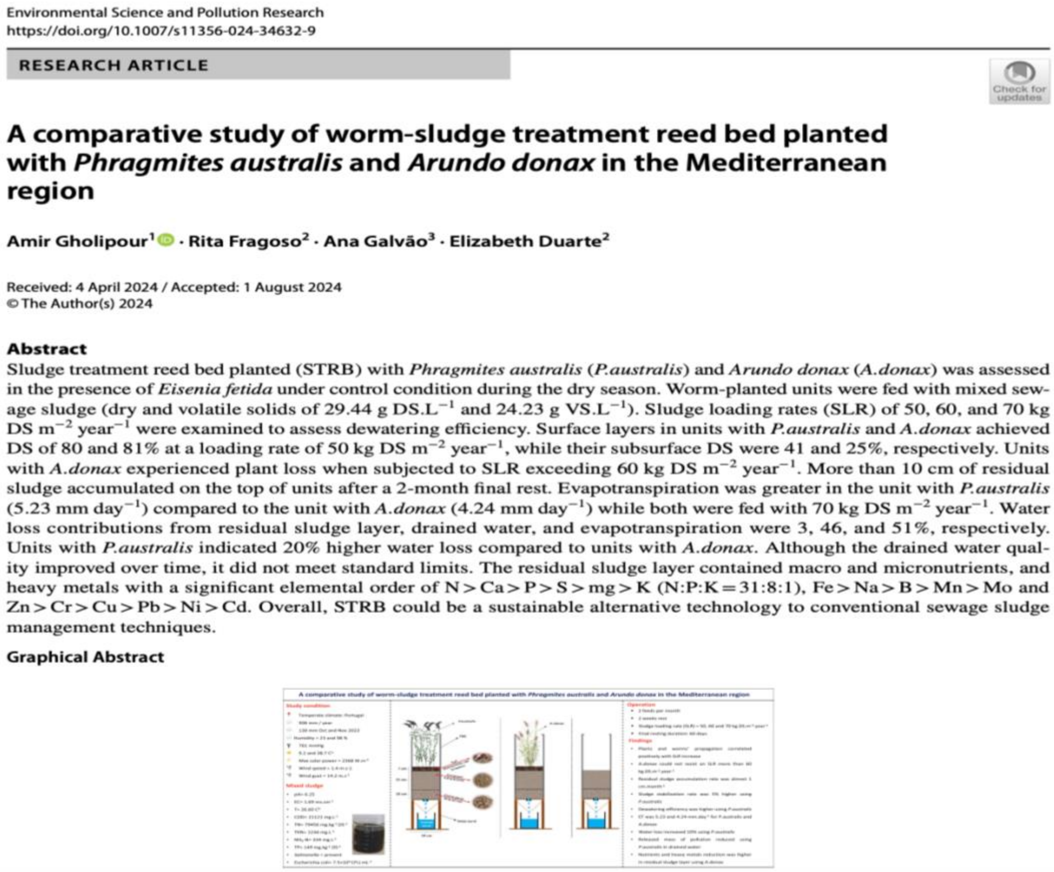
Chapter 6. A comparative study of worm-sludge treatment reed bed planted with *Phragmites australis* and *Arundo donax* in the Mediterranean region



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RESEARCH ARTICLE

A comparative study of worm-sludge treatment reed bed planted with *Phragmites australis* and *Arundo donax* in the Mediterranean region

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Abstract

Sludge treatment reed bed planted (STRB) with *Phragmites australis* (*P.australis*) and *Arundo donax* (*A.donax*) was assessed in the presence of *Eisenia fetida* under control condition during the dry season. Worm-planted units were fed with mixed sewage sludge (dry and volatile solids of 29.44 g $DS.L^{-1}$ and 24.23 g $VS.L^{-1}$). Sludge loading rates (SLR) of 50, 60, and 70 kg DS m^{-2} $year^{-1}$ were examined to assess dewatering efficiency. Surface layers in units with *P.australis* and *A.donax* achieved DS of 80 and 81% at a loading rate of 50 kg DS m^{-2} $year^{-1}$, while their subsurface DS were 41 and 25%, respectively. Units with *A.donax* experienced plant loss when subjected to SLR exceeding 60 kg DS m^{-2} $year^{-1}$. More than 10 cm of residual sludge accumulated on the top of units after a 2-month final rest. Evapotranspiration was greater in the unit with *P.australis* (5.23 mm day^{-1}) compared to the unit with *A.donax* (4.24 mm day^{-1}) while both were fed with 70 kg DS m^{-2} $year^{-1}$. Water loss contributions from residual sludge layer, drained water, and evapotranspiration were 3, 46, and 51%, respectively. Units with *P.australis* indicated 20% higher water loss compared to units with *A.donax*. Although the drained water quality improved over time, it did not meet standard limits. The residual sludge layer contained macro and micronutrients, and heavy metals with a significant elemental order of $N > Ca > P > S > mg > K$ (N:P:K = 31:8:1), $Fe > Na > B > Mn > Mo$ and $Zn > Cr > Cu > Pb > Ni > Cd$. Overall, STRB could be a sustainable alternative technology to conventional sewage sludge management techniques.

Graphical Abstract

Environmental Science and Pollution Research

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6.1. Abstract

Sludge treatment reed bed planted with *Phragmites australis* and *Arundo donax* was assessed in the presence of *Eisenia fetida* under control condition during dry season. Worm-planted units was fed with mixed sewage sludge from primary and secondary treatment stages with dry and volatile solids of 29.44 $g.DS.L^{-1}$ and 24.23 $g.VS.L^{-1}$. Sludge loading rates of 50, 60 and 70 $kg.DS.m^{-2}.year^{-1}$ were examined to assess dewatering efficiency and plant survival. Surface layers in units with *Phragmites australis* and *Arundo donax* achieved dry solid content of 80 and 81% at a loading rate of 50 kg.DS.m^{-2}.year^{-1}, while their subsurface dry solids were 41 and 25%, respectively. Units with *Arundo donax* experienced plant loss around last cycles subjected to loading rates exceeding 60 kg.DS.m^{-2}.year^{-1}. More than 10 cm of residual sludge accumulated on the top of units after a two-month of final resting due to precipitation. Evapotranspiration was greater in unit with *Phragmites australis* (5.23 mm.day^{-1}) compared to unit with *Arundo donax* (4.24 mm.day^{-1}) while both were fed with 70 kg.DS.m^{-2}.year^{-1}. Water loss contributions from residual sludge layer, drained water, and evapotranspiration were 3, 46, and 51%, respectively. Units with *Phragmites australis* indicated 20% higher water loss compared to units with *Arundo donax*. Although the drained water quality improved over time, it did not meet standard limits. Units with *Phragmites australis* released a lower mass of pollutants compared to those with *Arundo donax*. The residual sludge layer contained macronutrients, micronutrients, and heavy metals with a significant elemental order of N > Ca > P > S > mg > K (N:P:K=31:8:1), Fe > Na > B > Mn > Mo and Zn > Cr > Cu > Pb > Ni > Cd.

Keywords: sludge management, constructed wetland, nature-based solutions, sludge dewatering, *Eisenia fetida*

6.2. Introduction

The significance of sanitation in modern cities, extremely underscored during the pandemic, stands as a critical priority (Hannah et al., 2020). The sixth sustainable development goal, focusing on clean water and sanitation, is pivotal not just as a fundamental right but also as a critical action, especially in the face of resource scarcities such as water and nutrients (Tortajada & Biswas, 2018). Urban development has magnified the urgency of sanitation, especially concerning wastewater treatment (van der Merwe & Simha, 2023). The current scarcity of resources, particularly food and water, demands an exploration of alternative resources. Nutrient depletion stands as a prominent global challenge exacerbating concerns for productive agriculture. Dwindling phosphorus availability in agricultural lands poses a threat to soil fertility, directly impacting crop yield and quality (Marschner & Rengel, 2023). The prevailing linear economy, starting from raw material extraction and culminating in disposal, is no longer a sustainable approach; therefore, embracing a circular economy offers a viable alternative paradigm (Morseletto, 2023). In circular economy, resources are recovered and reused, which is illustrated in sanitation by the treatment of wastewater aiming a recovery of valuable resources in sewage sludge (Mannina, Barbara, Cosenza, & Wang, 2023b). Sewage sludge holds high amount of organic and inorganic contents (Gholipour et al., 2022); however, there are still challenges in its recovery, primarily due to high costs and environmental impacts of conventional techniques (Daee et al., 2019b; Tarpani & Azapagic, 2018). Conventional processes contribute to climate change, prompting the need for a paradigm shift (Chang et al., 2023a). Global warning potential of technological processes were found positive in previous studies compared to nature-based solutions (NBS) (Uggetti et al., 2011; Zhuang et al., 2022). NBS, like sludge treatment reed red (STRB), offer an eco-friendly alternative to energy-intensive solutions such as centrifugation (Nielsen, 2023). STRB, as an application of treatment wetland, employs sand and gravel media planted with common reeds like *Phragmites australis* (*P.australis*) (Gholipour et al., 2020; Gholipour and Stefanakis, 2021). STRB significantly reduces energy consumption compared to energy-intensive solutions, requiring minimal energy input. NBS proves high performance in reducing operation, maintenance, and capital costs, outshining energy-intensive solutions (Chang et al., 2023b). Energy-intensive solutions also fails to provide a reusable by-product and often needs further treatment for reuse purposes, contributing to environmental degradation and a positive global warning potential (Zhuang et al., 2022). While STRB stands as a promising NBS, offering numerous advantages over conventional techniques, it requires improvement, particularly in reducing land demand and enhancing the quality of residual sludge (Gholipour et al., 2023).

Previous studies have explored advancements in typical STRB, introducing variations like electro-STRB (E-STRB or STEW), worm-STRB (W-STRB), and intensified-STRB (I-STRB) (Z. Chen et al., 2016; Z. Chen & Hu, 2019; Hu, Lv, et al., 2020; Hu & Chen, 2018; Plestenjak et al., 2021; Wang et al., 2021; Zhong et al., 2021). E-STRB aimed not only at sludge dewatering but also at generating power from organic matter via microbial fuel cells which yielded 60 mW.m^{-3} in previous studies (Saeed et al., 2022). W-STRB primarily addresses land demand through an enhancement in sludge loading rate (SLR) up to 120 kg.dry solid.m^{-2}.year^{-1} in tropical climate (Z. Chen et al., 2016; Hu, Lv, et al., 2020). Worms were introduced also to enhance residual sludge stabilization and mineralization (Calderón-Vallejo et al., 2015b). Meanwhile, I-STRB involves aeration system injecting air into the media to augment dewatering efficiency and organic matter decomposition (Plestenjak et al., 2021). In contrast, typical STRB relies on passive oxygen intake through plant root or ventilation systems (Kołecka et al., 2018; Nielsen, 2007; Stefanakis & Tsihrintzis, 2011; Uggetti et al., 2009). Moreover, the development of STRB variants has involved testing different plant species in dewatering process of which *P.australis* was the most common specie used while *Typha latifolia, Typha angustifolia*, *Canna indica*, *Cynodon dactylon pers*, *Echinochloa pyramidalis* and *Cyperus papyrus* were the other species tested (Gholipour et al., 2022). Native plant species were anticipated to exhibit high efficiency, especially concerning dewatering efficiency, reduction in contaminants in STRB drained water and higher stabilization (Nielsen, 2011). STRB played crucial roles in effectively removing various pollutants from residual sludge and drained water including nitrogen components, phosphorus, heavy metals, pharmaceuticals, personal care products, organic compounds, micropollutants and greenhouse gases (Z. Chen & Hu, 2019; Liang et al., 2021; Ma et al., 2020; Nielsen, 2023; Stefanakis & Tsihrintzis, 2012b; D. Zhang et al., 2016).

The typical STRB has been tested across different climates, whereas E-STRB, W-STRB, and I-STRB have mostly undergone controlled testing in tropical (E-STRB and W-STRB) and temperate (I-STRB) climates (Hu & Chen, 2018; Plestenjak et al., 2021; Wang et al., 2021). The process of sewage sludge dewatering with STRB relies on evapotranspiration (ET), and drainage which are highly influenced by climate (Brix, 2017). Consequently, there is a pressing need to explore STRB variants in various climates. SLR commonly serves as a design parameter, and previous studies have showcased varying SLRs for E-STRB, W-STRB, and I-STRB, often enhanced in drier climates (Gholipour et al., 2022). Previous studies in China and Brazil have employed *P.australis* in controlled conditions for W-STRB without assessing the impact of seasonal variations on dewatering efficiency (Calderón-Vallejo et al., 2015a; Hu & Chen, 2018). On the contrary, our preliminary study in Portuguese temperate climate analyzed the effect of seasonal variations on W-STRB efficiency (Gholipour et al., 2024b). W-STRB was assessed for the dewatering of mixed sludge from an urban wastewater treatment plant (WWTP) where an SLR of 43.5 kg.DS.m^{-2}.year^{-1} was applied. This paper serves as a follow-up of Gholipour *et al.* (2024), focusing on assessing the cooperative effect of worms and plants under elevated SLR ranges of 50, 60, and 70 kg.DS.m^{-2}.year^{-1}. This study was conducted in Horto greenhouse at School of Agriculture of University of Lisbon (ISA) and compares W-STRB system planted with *P.australis* and *Arundo donax* (*A.donax*) concerning dewatering efficiency, water balance, drained water quality, and final residual sludge quality. The findings from this study can assist researchers and engineers who are considering the adoption of this NBS as a diversion technology applied to mixed sludge dehydration in similar climate conditions.

6.3. Materials and methods

6.3.1. Study area and experimental setup

The experiment was conducted in Horto greenhouse facility, ISA, University of Lisbon, Portugal, 2023 (38°42'28.9"N 9°11'06.4"W). The research area was without protective cover for the bench-scale mesocosm, resulting in the mesocosms being subjected to precipitation and ambient temperature. The experiment proceeded to evaluate the W-STRB system in Portugal, building upon our previous investigation conducted in Beirolas over the course of one year, which accounted for seasonal variations (Gholipour et al., 2024). Our findings from the Beirolas experiment indicated that the W-STRB system reached maturity after six months of operation. In the current study conducted in Horto, we specifically focused on the initial six-month period to avoid redundant repetitions, considering Horto as a follow-up to the Beirolas experiment. Meteorological data were recorded through an onsite weather station including maximum, minimum, and average of air temperature (°C), solar power (W.m^{-2}), humidity (%), wind speed (m.s^{-1}), and precipitation (mm).

The experiment included seven units built in 150 mm diameter (60 cm height) PVC pipe (Figure 6.1). Units 1 to 3 were planted with *P.australis* (WP1, WP2 and WP3), units 5 to 7 with *A.donax* (WP5, WP6 and WP7), and all units included worms. Unit 4 served as a control, without plants and worms. All units were filled with two layers of gravel containing a drainage layer (15 cm of coarse gravels, 19 to 25 mm, and 38% porosity) and a transition layer (25 cm of fine gravels, 4.8 to 9.5 mm, and 42% porosity). Except for the control unit, a turf layer (Siro 30) was added on the top to host 20 bodies of *Eisenia fetida*.

One tuft of reeds was planted in WP units (Brix, 2017) and worms were inserted a week before feeding to acclimatize into the turf layer (Hu and Chen, 2018; Wang et al., 2021; Zhong et al., 2021).

Transplanted *P.australis* (*Poaceae*) and *A.donax* (*Arundineae*) were taken from a natural wetland in ISA and were weekly irrigated during April 2023 using tap water of 1.5L for all units (photos can be found in the supplementary materials). The PVC pipes were placed on a metal stand above the ground to collect drained water and were connected to five-liter buckets for the measurement of drained water volume and sampling.

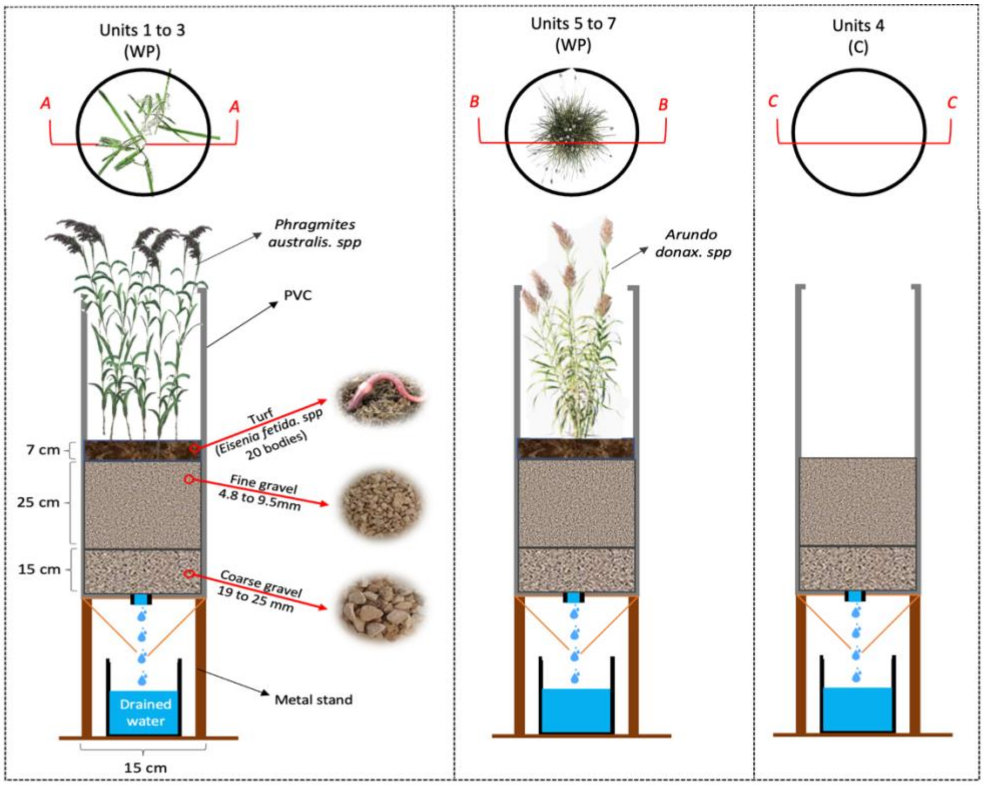


Figure 6. 1. Pilot study configuration

An urban sewage sludge was collected from Beirolas WWTP, Lisbon (213,510 inhabitants) from both primary and secondary stages flowing into a mixed tank. The mixed tank receiving the primary sludge after thickening and, the surplus activated sludge after air flotation thickening, designated by mixed sludge. Before the feeding phase, 180L of mixed sludge were transferred to Horto facility and stored into two 100-liter plastic drums under the greenhouse condition. The drums were capped and placed in a shadow to avoid direct exposure of sun lights used for feeding. Dry and volatile solids (DS and VS) of the sludge were 29.44±1.02 $g.DS.L^{-1}$ and 24.23±0.84 $g.VS.L^{-1}$ and measured before each feeding, which reduced to 26.58 $g.DS.L^{-1}$ and 21.56 $g.VS.L^{-1}$ during the study in the drums. The feeding phase was between May and September 2023 in 10 cycles in which units were fed every two weeks followed by a two-week rest. The sludge was characterized before the storage and its average temperature, pH, and electrical conductivity (EC) were 26.6°C, 6.25, and 1.69 $mS.cm^{-1}$, respectively (additional information in the supplementary materials). Following SLRs were tested: 70 $kg.DS.m^{-2}.year^{-1}$ for WP1 and 5 (SLR_1), 60 $kg.DS.m^{-2}.year^{-1}$ for WP2 and 6 (SLR_2), and 50 $kg.DS.m^{-2}.year^{-1}$ for WP3, WP7 (SLR_3) and control units. The unit area was 170 cm^2 and based on DS content, 70, 60 and 50 $kg.DS.m^{-2}.year^{-1}$ corresponded to 1.766, 1.513 and 1.261L of mixed sludge; thus, hydraulic loading rate was 10.4, 8.9 and 7.4 cm. Gholipour et al., (2024) tested an SLR of 43.5 $kg.DS.m^{-2}.year^{-1}$ for a configuration similar to WP7 (*Eisenia fetida* and *A.donax*) in which feeding was between May 2022 and May 2023 and then, 132 days of final resting until September 2023 was considered. The tested configurations could compare two plants species under higher SLRs in Portugal climate as well. To increase the dryness and stabilization, feeding was ceased in September 2023, and the units passed two months of final resting as suggested in previous studies (Gholipour et al., 2022). To compare the effects of final resting periods in both dry and wet seasons, this study aimed to conduct experiments during different climatic conditions. While our previous study conducted at Beirolas examined the final resting period during the dry season, the current study sought to investigate the same parameter during the wet season in Horto. This deliberate choice allowed for a comparative analysis of how varying seasonal conditions impact the effectiveness of the final resting phase in our experiments and how different weather affects final dry solid and residual sludge quality. Therefore, the final resting period in the present study was specifically scheduled during the wet season to facilitate this comparative analysis between Horto and Beirolas experiments.

6.3.2. Plant growth and earthworm dynamics

Detailed morphometric parameters, encompassing both average sizes and densities, were documented to evaluate plant development. A tape measure was used to gauge the distance from the plants' base, directly in contact with the sludge layer, to their apical part.

Plant density was counted the number of stems in each unit, which is crucial in reflecting the plant population. The measurements were conducted four times during the study including at the time of transplantation, after two months of resting, at last cycle of feeding, and at the end of study, providing a comprehensive understanding of plant growth and progression over time. Additionally, to measure the aboveground plant biomass accurately, reeds were harvested at the end of the final resting period in November 2023 and dried in a 60°C oven for three days until dry biomass was achieved a constant weight. To study the effect of worms on the systems, a manual sorting process was conducted following a flip and strip test to count the worm population in the whole layers of residual sludge and turf (Gutiérrez-López et al., 2016). The number of worms was counted through a dig in the PVC pipe. In addition, onsite observations like cocoons were registered.

6.3.3. Physicochemical analyses and sampling

Twice a month, residual sludge was collected to measure DS and VS contents, taken from the top and bottom layers using a core sampler (Stefanakis and Tsihrintzis, 2011). A tape measure was sticked to the inner side of each unit to measure the thickness of residual sludge, recorded at the end of the two-week rest multiplied by each unit area to quantify the volume of residual sludge.

Lab analyses for residual sludge, mixed sludge and drained water were conducted to determine DS, VS, pH, EC, and temperature. Additionally, pH and EC of the mixed sludge and drained water samples were measured, in situ using a handheld multi-parameter VWR MU 6100 H. Dewatering efficiency was interpreted based on DS, VS, and residual sludge thickness (Gholipour et al., 2022). Samples were collected from drained water on each cycle after feeding completed. Physicochemical analyses of drained water were conducted for turbidity, chemical oxygen demand (COD), total suspended solids (TSS), total volatile solids (TVS), nitrate nitrogen (NO_3^--N), ammonium nitrogen (NH_4^+-N), total kjeldahl nitrogen (TKN), total phosphorous (TP) as well as microbiological parameters such as *Escherichia coli* (E. coli), Fecal coliform (F. coli), and *Salmonella* spp. It was according to the Standard Methods for the Examination of Water and Wastewater (APHA, 2017).

In addition, to assess the content of micro (Fe, Na, B, Mn, and Mo) and macronutrients (N, Ca, P, S, mg and K), microbiological parameters, and heavy metal (HM: Zn, Cr, Cu, Pb, Ni and Cd) in residual sludge, samples were collected from both top and bottom layers after final resting representing the surface and subsurface layers of the accumulated residual sludge quality. To ensure representative samples, residual sludge was uniformly, and randomly selected from a designated area and mixed to create a composite sample for each unit in November 2023. For the elemental analysis, inductively coupled plasma (ICP) was utilized (APHA, 2017). Microbiological analyses, encompassing the assessment of E. coli, F. coli, and *Salmonella* spp, were conducted in November 2023 (photos of drained water samples can be found in the supplementary materials).

6.3.4. Mass balance, removal efficiency and water loss estimation

To conduct water balance analysis and estimate evapotranspiration (ET: $mm.day^{-1}$), inflows and outflows were recorded. ET was calculated for each cycle according to Eq.1 and 2 (Gholipour et al., 2024):

$$\mathrm{WL} = \mathrm{P_r} + \mathrm{V_{MS}} - \mathrm{V_{RS\,(A)}} - \mathrm{V_{RS\,(B)}} - \mathrm{V_P} - \mathrm{V_{DW}} \qquad \text{(Eq.1)}$$

$$\mathrm{ET} = \frac{\mathrm{WL}}{\text{unit area}} \qquad \text{(Eq.2)}$$

Where WL is water loss (L), P_r is precipitation volume (L), V_{MS} is water volume (L) in mixed sludge, $V_{RS\,(A)}$ is water volume in residual sludge layer before feeding (L), $V_{RS\,(B)}$ is water volume in residual sludge layer at the end of each resting period (L), V_P is water volume draining to the mesoporous media (L), and V_{DW} is drained water volume (L). P_r was measured through the onsite weather station. V_{MS}, $V_{RS\,(A)}$ and $V_{RS\,(B)}$ were calculated based on DS content (%) in mixed sludge and residual sludge. The thickness of residual sludge was used to estimate V_{RS} in which an average DS of top and bottom was considered. V_{DW} was measured by directly recording drained water volume out of each unit. In the estimation of water loss based on Eq.1, V_P was assumed zero as most of water loss was taken from residual sludge layer, and at the end of the resting period, V_P stays practically steady and close to zero due to the transpiration and fast drainage (Stefanakis and Tsihrintzis, 2011). During each two-week rest, drained water volume was recorded at different time intervals of 1, 2, 4, 6, and 12 hours as well as 1, 2, 7, 10, and 14 days after feeding to account water percolation rate. In addition, to calculate the removal efficiency, Eq. 3 was used:

$$\text{Removal efficiency (\%)} = \frac{(\mathrm{V_{MS}} \times \mathrm{Con_{in}}) - (\mathrm{V_{DW}} \times \mathrm{Con_{iout}})}{\mathrm{V_{MS}} \times \mathrm{Con_{in}}} \times 100$$

(Eq.3)

Where V_{MS} is the volume of mixed sludge (L), Con_{in} is the parameter concentration in inlet (mg.L^{-1}), V_{DW} is the volume of drained water (L), and Con_{out} is the parameter concentration in outlet (mg.L^{-1}). To estimate mass release (g) in the inlet, each parameter concentration (mg.L^{-1}) was multiplied by mixed sludge, and in the outlet, each parameter concentration (mg.L^{-1}) was multiplied by drained water volumes (L).

6.3.5. Data analysis

The normality and homogeneity of variance for all datasets were evaluated using the Shapiro-Wilk test and Bartlett's test, respectively. In instances where normality and homogeneity criteria were not met, differences among units were assessed using the Kruskal-Wallis’s test for one-way analysis of variance, with statistical significance set at a p-value of 0.05. Statistical significance was conducted where the minimum number of samples were five. All statistical analyses were performed using R Studio. Statistical analysis including minimum, maximum, mean and standard deviation (SD) were computed.

6.4. Results and discussion

6.4.1. Meteorological analysis

Based on the historical dataset (1981-2010), the average annual rainfall of Lisbon is 688 mm, and the absolute temperature varies between 41.2°C in Augusst and -1.5°C in January reported by Instituto Português do Mar e da Atmosfera (IPMA) (Reis et al., 2022). On-site weather station recorded air temperatures ranging from 9.2 to 38.7°C, accompanied by humidity levels spanning 23 to 98% during the study period. Solar power peaked at 2368 W.m^{-2}, while average wind speed measured 1.4 m.s^{-1} (±0.5), with gusts reaching 12.2 m.s^{-1} (±4.6) (Figure 6.2).

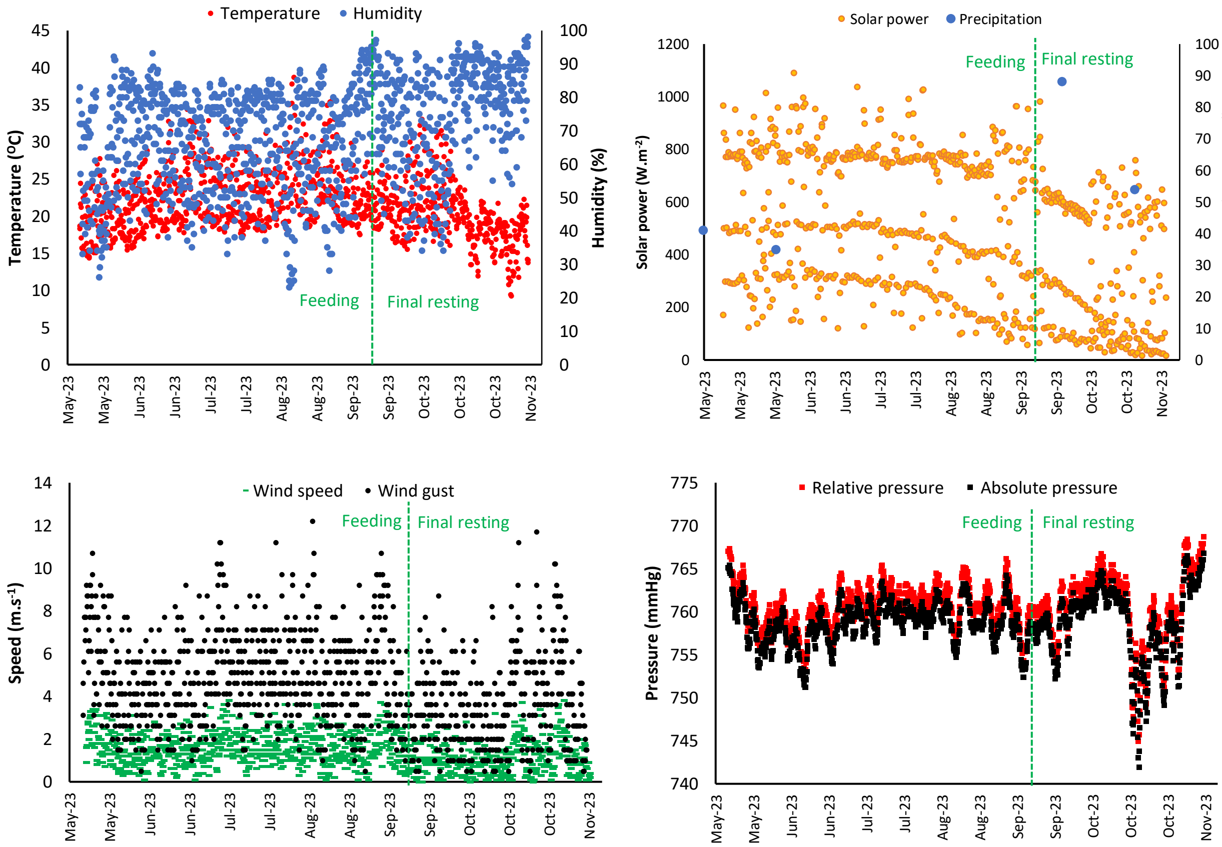


Figure 6. 2. Weather data

The relative atmospheric pressure measured 761 mmHg, slightly differing from the absolute atmospheric pressure, which was 758 mmHg. Throughout the study period, a total of 218 mm of precipitation was recorded, with 130 mm falling between October and November 15th, 2023. There was no recorded rainfall during June, July, and August. Previous studies also identified a comparable weather data range within the Lisbon district (Andrade & Alcoforado, 2008; Reis et al., 2022). According to Schleussner *et al.* (2020), Lisbon has Mediterranean climate with mild wet winters and warm dry summers, with relatively low precipitation and temperature variations.

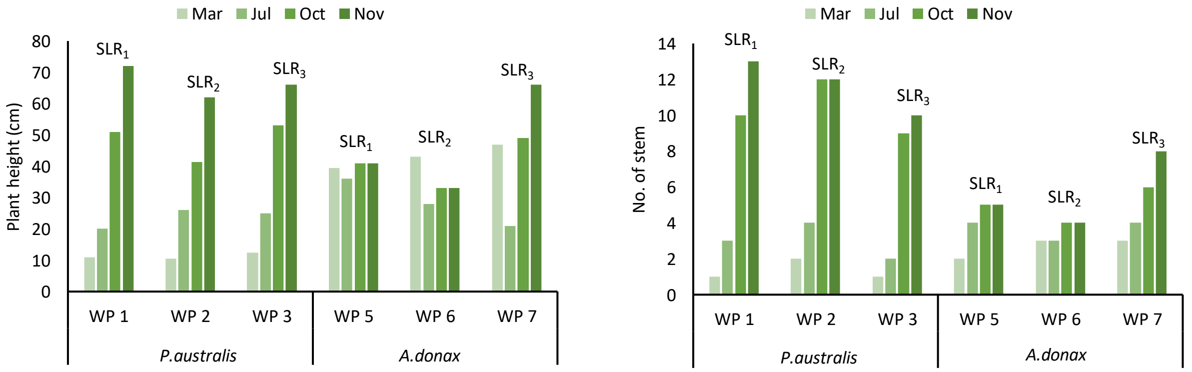
6.4.2. Plant and earthworm analysis

Plant analysis showed *A.donax* produced higher wet biomass compared to *P.australis*. For SLR_1 to SLR_3, the total wet biomass production of *P.australis* was 372, 306 and 265 grams in each unit of which 66% was water content by average (8023, 6600 and 5715 gram.dry.m^{-2}). *A.donax* wet biomass was 410, 390 and 550 grams in each unit with 90% water content by average (2679, 2549 and 3595 gram.dry.m^{-2} for SLR_1 to SLR_3).

Moreover, higher SLRs resulted in a higher biomass production, for instance, SLR_1 in WP1 produced 31% higher *P.australis* biomass compared to SLR_3 in WP3.

Onsite observation showed by the late August 2023, plants in WP5 and WP6 started getting stressed and dry for the final cycle. Then, they experienced a two-month period of final rest while the plants exhibited chlorosis. This could show SLRs of 70 and 60 kg.DS.m^{-2}.year^{-1} were above *A.donax* capacity in this specific configuration. The formation of residual sludge layer on the top of the media possibly created an anaerobic condition minimizing oxygen availability for *A.donax* survival, and hypoxia might increase by the accumulation of sludge in SLRs (Gholipour et al., 2024b). Moreover, water stagnation on the top of the units due to precipitation in early September 2023, could also accelerate the plant stress promoting hypoxia in residual sludge layer (Loreti & Striker, 2020). On the contrary, WP7 with 50 kg.DS.m^{-2}.year^{-1} continued growing until the end of experiment. This result is in agreement with Beirolas pilot, in which *A.donax* was tested for 43.59 kg.DS.m^{-2}.year^{-1}.

Regarding plant development, an average plant growth rate of 12 and 11 cm.month^{-1} was registered for *P.australis* and *A.donax*, respectively with higher growth during the feeding period (Figure 6.3). The average number of stems in each unit also increased, from two to 11 for *P.australis* and from three to seven stems for *A.donax* (Figure 6.3). The higher SLR had a positive effect on the stem number, for example *P.australis* showed 13 stems for SLR_1 and 10 stems for SLR_3. The effect of *A.donax* plant stress is observable for WP5 and WP6 of which the produced biomass decreased with SLR_1 and SLR_2. Yet, WP7 planted with *A.donax* had higher biomass production although it was fed with SLR_3.



a

b

Figure 6. 3. Plant development: a) height growth, b) number of stems

The number of worms varied in units, higher SLR resulted in higher number of worms in which six worms was in WP1 with SLR_1 compared to WP2 and WP3 (SLR_2 and SLR_3) that had four and two worms. The number of worms taken was lower than the initial value of 20 worms possibly due to the natural die-off and the effect of seasonal variation (Pezzotti et al., 2021). However, visual observations showed several cocoons were found in the vertical profile of the media and as stated in previous studies, worm reproduction can be ceased during a cold season in which worms leave cocoons for the next dry season reproduction (Pezzotti et al., 2021).

As plant got dried in WP5 and WP6, the number of worms declined to zero, whereas in WP7 planted with *A.donax* had four worms (Figure 4, supplementary materials). The absence of worms in WP5 and WP6 could also be due to oxygen deficiency and exposure to higher SLR. We experimented the effect of seasonal variation on the number of worms in Beirolas experiment (Gholipour et al., 2024b). It showed that the number of worms increased during the dry season and declined in the wet season especially in October, which is comparable with the current study.

6.4.3. Dewatering efficiency analysis

Residual sludge thickness did not increase until fifth cycle of feeding (May and July 2023), possibly due to the accumulation of residual sludge within filtration material (Figure 5, supplementary materials). By the end of feeding period in September 2023, residual sludge thickness reached around 1 cm for WP1 to WP3, and around 3 cm for WP5 to WP7, while control unit showed 1.5 cm (DS content for all units ≈ 47%). After the final rest, residual sludge thickness was 13, 12.5, 10.5, 14.5, 14 and 14 cm for WP1 to WP7, respectively while it was 16.5 cm for control unit. The accumulated thickness was like WP unit achieved in Beirolas experiment in November 2022, around 10 cm in WP with *A.donax* (Gholipour et al., 2024b). This could be due to water absorption by residual sludge layer from the precipitation in September for both Horto and Beirolas experiments after dry season. Overall, units with *P.australis* resulted in lower residual sludge accumulation compared to units with *A.donax*. It can be attributed to higher ET for *P.australis* and higher decomposition rate of organic matter. In addition, higher SLR led to higher residual sludge accumulation. WP3 had 36% less accumulation compared to control unit due to plants and worms while it was 25% for WP7.

In Beirolas study, an accumulation rate of 0.06, 0.09, 0.05, and 0.1 m.year^{-1} was found for WP, planted, worm and control units, respectively in which DS was 70% while 132 days of final rest was between May to September 2023 (Gholipour et al., 2024b). This was 0.09 m.year^{-1} for STRB unit without worms with a final rest between 25 and 180 days in Mediterranean studies (Stefanakis and Tsihrintzis, 2011, and El-Gendy and Ahmed, 2020) while it varied between 0.26 and 0.1 m.year^{-1} in temperate climate (Gholipour et al, 2022). This suggests for minimizing residual sludge volume, the duration of final rest needs to be longer within a dry season.

Dewatering efficiency indicated DS (Figure 6.4.a) and VS/DS (Figure 6.4.b) contents were lower in the subsurface (sub) layers compared to the surface layers (sur) and were significantly different (p-value < 0.05). Several factors including rainfall, evaporation, plant transpiration, solar radiation, and wind could influence in DS content. During feeding, sur-DS contents of all units were statistically different (p-value < 0.05) ranging between 50 and 95% while during the final rest, they were not statistically different (15 ~ 86%). During feeding, sub-DS contents were different (23 ~ 85%) while after the final rest, it ranged from 9 to 46%. DS contents of control unit were consistently lower in comparison with the other units while VS/DS content was higher in control unit. This indicates the coexistent of plants and worms could increase dewatering efficiency and enhance stabilization.

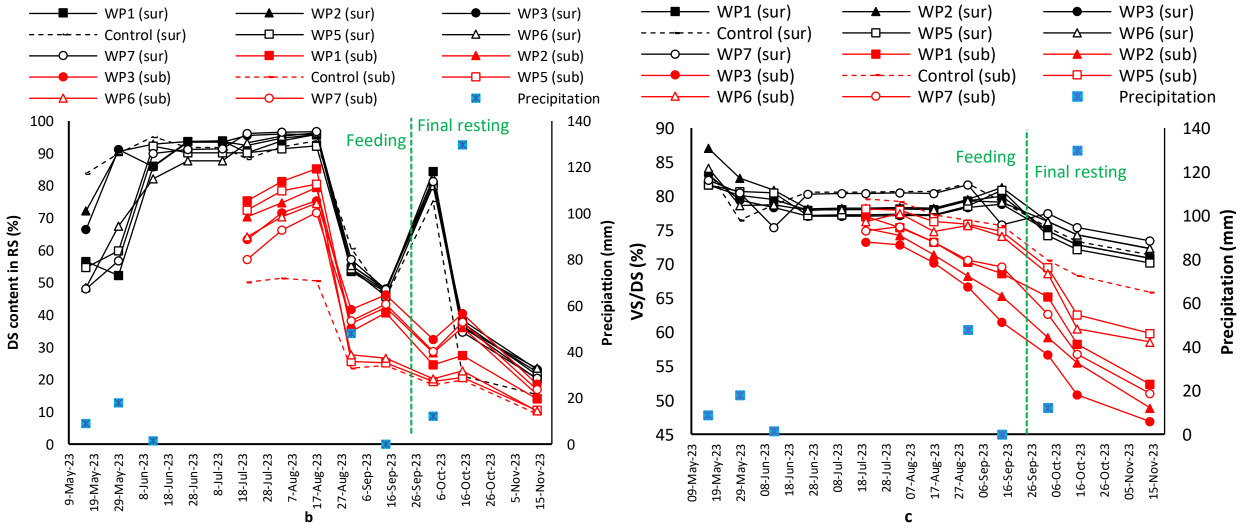


Figure 6. 4. Dewatering efficiency: a) DS content versus precipitation, b) VS/DS content versus precipitation.

As it can be seen, the final sub-DS contents of WP1 to WP7 after two months of rest were 14, 15, 18, 10, 10 and 17% (control = 9%) while they were 20, 21, 23, 22, 23 and 20% on the surface (control = 15%), respectively. In terms of sub-VS/DS, WP units showed 16% reduction (control = 10%) during the final rest; therefore, worms and plants could enhance stabilization rate by 6%. In addition, higher SLRs like SLR_1 resulted in lower DS content due to obviously higher feed. In WP5 and WP6 due to plant wilting, sub-DS contents were like control unit. Sub-VS/DS of WP5 and WP6 units was around 59 and 59% which were higher than other units (52, 49, 47 and 51 for WP1, WP2, WP3 and WP7) and close to control unit (65%) (a summary of dewatering efficiency was presented in the supplementary materials).

Overall in the final resting, in units planted with *P.australis* in average, there was 5% higher sub-DS and 3% lower sub-VS/DS values compared to units planted with *A.donax*. The highest DS and lowest VS/DS was achieved in WP3, planted with *P.australis*, with 50 $kg.DS.m^{-2}.year^{-1}$(SLR_3). Furthermore, for units planted with *P.australis* and SLR_3 resulted 5% higher stabilization comparing to SLR_1.

Final sur-DS obtained in this study for planted units with *P.australis* (41 ~ 46%) is comparable with previous studies applied units without worms planted with *P.australis*. In temperate and tropical climates, sur-DS was from 33 to 40% (Gholipour et al., 2022), was 45% in arid climates (Dodane et al., 2011), and 30% for a study in polar climates (Gagnon et al., 2013). In tropical climates, sur-DS ranged between 63 and 59% for WP and planted units in China (Chen et al., 2016; Hu et al., 2020). In another Chinese study, it also varied between 12 and 50% in WP units (Zhong et al., 2021). For an I-STRB in temperate climate, sur-DS of 62.5% was reported (Plestenjak et al., 2021).

Final sub-VS/DS of this study varied between 47 and 52% for planted units with *P.australis*, which is comparable with previous studies, which were reported 53, 42, and 40% in temperate, tropical, and polar climates, respectively. In a temperate climate, sub-VS/DS ranged between 51 and 79% (Gagnon et al., 2013; Gholipour et al., 2022). In other temperate climates, it was 67% while it was 52% for tropical studies (Cui et al., 2015). For WP units in tropical climate studies, sub-VS/DS varied between at 33 and 42% (Z. Chen et al., 2016; Hu, Lv, et al., 2020; Zhong et al., 2021). In previous temperate climates, 39, 67 and 52% were reported (Gholipour et al., 2022) with final resting of 60, 120 and

365 days, respectively. In this study, sub-VS/DS of 46.87% achieved by 60 days resting which aligns closely to the previous studies mentioned. Overall, the inclusion of worms in the system could potentially enhance stabilization.

6.4.4. Water balance analysis

There was a total influent volume (including water from mixed sludge and precipitation) of 18.78L into WP1 and WP5, 16.56L to WP2 and WP6, and 14.35L to WP3, WP7, and control (Table 6.1). A part of the influent volume underwent to drained water, and a marginal volume remained in residual sludge layer.

Table 6. 1. Water balance parameters

Units	mixed sludge fed (L)	DS (L)	Influent from mixed sludge (L)	Precipitation (L)	Total influent (L)	Total drained water (L)	Water content in residual sludge (L)	Water loss (L)
WP1	17,66	0,43	17,23	1,56	18,78	9,57	0,31	8,91
WP2	15,38	0,38	15,01	1,56	16,56	8,66	0,22	7,69
WP3	13,12	0,32	12,79	1,56	14,35	8,88	0,18	5,30
Control	13,12	0,32	12,79	1,56	14,35	10,86	0,33	3,17
WP5	17,66	0,43	17,23	1,56	18,78	11,34	0,32	7,13
WP6	15,38	0,38	15,01	1,56	16,56	9,50	0,24	6,82
WP7	13,12	0,32	12,79	1,56	14,35	8,92	0,28	5,15

The result of water balance analysis showed water loss accounted 47.42, 46.40, 36.89, 37.95, 41.15 and 35.88% of the total influent for WP1 to WP7 units, while it was only 22.05% for control unit. Thus, units planted with *P.australis* had higher water loss compared to units planted with *A.donax*, for instance, WP1 had 10% (1.77 L) higher water loss compared to WP5 with a similar SLR of 70 kg.DS.m^{-2}.year^{-1}. This could be due to the higher ET rate of *P.australis* and a higher number of worms enhancing dewatering. In term of drained water, WP5 released the highest amount (11.34 L) and WP2 the lowest (8.66 L).

Water percolation rate was around 0.4 m.day^{-1} at first cycle for WP1 to WP7 while it was 0.6 m.day^{-1} for control unit. At the last cycle in September, it reduced to 0.073, 0.081, 0.085, 0.067, 0.072 and 0.078 m.day^{-1} for WP1 to WP7, respectively, and control unit had a water percolation rate of 0.1 m.day^{-1}. This indicates unit with *P.australis* had lower water percolation rate due to higher water loss and DS, plant root system and media biofilm development.

The mechanisms for water loss in WP units were basically through residual sludge, drained water, and ET (Figure 6.5.a), and likewise, for control units were through residual sludge, drained water, and evaporation. Contribution of residual sludge in water loss in units planted with *P.australis* was lower than units planted with *A.donax* of which 2.88, 2.39 and 1.68% were for WP1 to WP3 and 5.16, 4.12 and 3.23% for WP5 to WP7 (control unit = 4.14%).

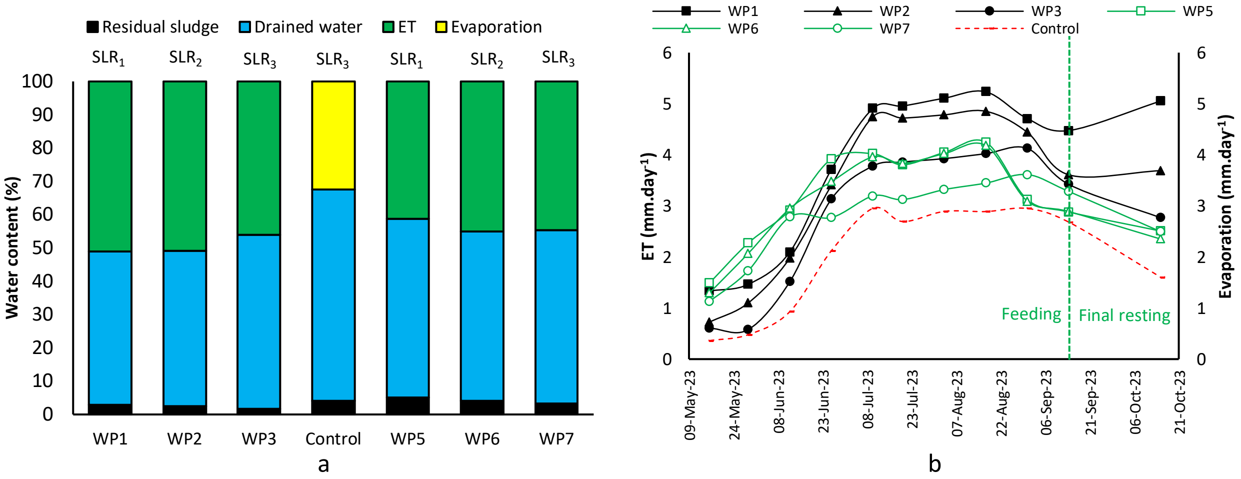


Figure 6. 5. Water balance a) contribution of each mechanism in water content b) daily ET and evaporation

This behavior can be attributed to the higher DS content in units planted with *A.donax* indicating different ET using *P.australis* and *A.donax* while SLRs were similar. The higher SLR also increased residual sludge contribution in water loss. The most important pathway of water loss was through drained water for all units, with the control unit showing the highest value of 63%, and the lowest was for WP1 by 46%. Thus, units with *P.australis* showed lower drained water contribution in water loss compared to units with *A.donax*. Water loss through ET was 51, 50, 46, 41, 45 and 45% for WP1 to WP7, while in control unit showed 33% contribution. In the Beirolas experiment, a notable increase in drained water contribution to water loss was observed during the dry season. However, this contribution decreased during the wet season, possibly as a result of the accumulation of a residual sludge layer and accumulation of increased water content on the surface.

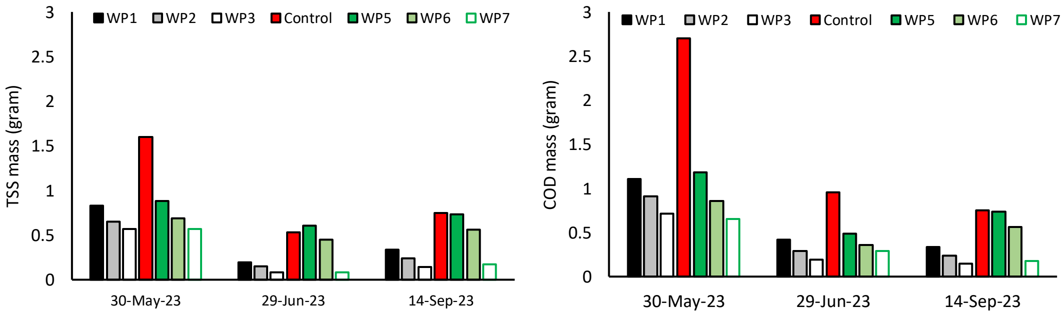
In terms of ET (Figure 6.5.b), within May and June, WP5 to WP7 units planted with *A.donax* showed higher ET rate compared to WP1 to WP3 planted with *P.australis*.

Afterwards, ET of WP1 to WP3 surpassed ET of WP5 to WP7 indicating *P.australis* development after two months of feeding. WP1 with SLR_1 showed the highest ET rate, which was 5.23 $mm.day^{-1}$ in August whereas it was 4.24 $mm.day^{-1}$ (SLR_1) for WP5. The effect of plant loss in WP5 and WP6 during August is significant, illustrated by a sudden drop in ET from 4.24 to 2.98 $mm.day^{-1}$. At the end of study, WP1 indicated 68 and 27% enhancement in ET rate compared to control unit and WP2, respectively. This reveals that plants, worms and SLR were effective factors. In Beirolas experiment (Gholipour et al., 2024b), the contribution of residual sludge layer in water loss was 4.82 and 2.17% for WP and planted units (with *A.donax*) which is comparable with the present study with 5.16, 4.12 and 3.23% for WP5 to WP7. Water loss through drained water was also 16 and 45% for WP and planted units in Beirolas study while it was 52, 51 and 52% for WP5 to WP7 in the present study.

In total, cumulative ET for WP1 to WP7 were 10.95, 9.41, 7.85, 8.71, 8.44 and 7.64 L, respectively (control unit = 5.58 L). Reduction in SLR from 70 to 60 $kg.DS.m^{-2}.year^{-1}$ decreased cumulative ET by 14% for WP1 and WP2 (*P.australis*), while reducing from 60 to 50 $kg.DS.m^{-2}.year^{-1}$ between WP2 and WP3 led to a 17% reduction. Whereas, for units planted with *A.donax*, the same change in SLR decreased ET only by 3 and 9%. Maximum ET rate fell in August for Horto, which was 5.23, 4.85, 4.02, 4.24, 4.18 and 3.44 $mm.day^{-1}$ for WP1 to WP7 while evaporation rate was 2.88 $mm.day^{-1}$ (Figure 5.b). This is comparable with ET for Beirolas study in August (dry season), which was 5.44, 4.16 and 3.27 $mm.day^{-1}$ for WP, planted (with *A.donax*) and control units, respectively (Gholipour et al., 2024b). After last feed for Horto, ET reduced and then during the final resting in October, it increased by the increase in precipitation. Higher precipitation also triggered higher ET in Beirolas experiment (Gholipour et al., 2024b).

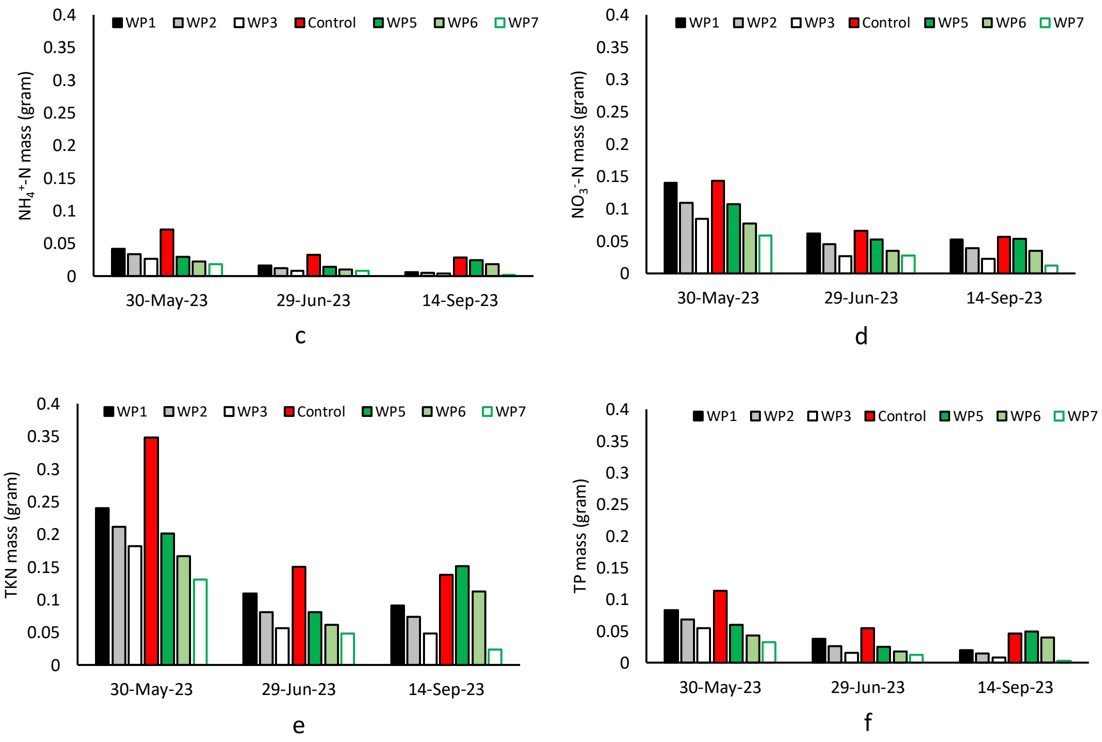
6.4.5. Drained water analysis

EC of drained water was 5.82, 6.02, 5.70, 6.07 and 5.68 $mS.cm^{-1}$ for WP1 to WP7 by average (control unit: 6.50 $mS.cm^{-1}$) while EC of mixed sludge was 1.69 $mS.cm^{-1}$; therefore, an increase in EC of mixed sludge to drained water was observed. Likewise, pH of drained water was higher than pH of mixed sludge from 6.25 to 7.4 in all units (no significant difference between units: $p\text{-value} > 0.05$) indicating pH neutralization from mixed sludge to drained water. Drained water temperature followed atmospheric temperature variation and was averagely 25^{o}C for all units (no significant difference between units: p-value > 0.05). Drained water quality in all units improved over time and in terms of released masses including TSS, COD, TKN, NH_4^+-N, NO_3^--N and TP parameters, planted units with *P.australis* consistently showed lower mass release (Figure 6.6). Lower SLRs resulted in lower mass release, as expected. Control unit indicated higher mass release compared to other units. The released mass of TSS in May was 0.83, 0.65, 0.57, 0.88, 0.69 and 0.57 g for WP1 to WP7 (control unit: 1.60 g) while in September, it reduced to 0.19, 0.15, 0.08, 0.61, 0.44 and 0.08 g (control unit: 0.53 g), respectively (Figure 6.6.a). This corresponded to 77, 77, 85, 31, 35 and 85% reduction for WP1 to WP7 (control unit: 67%).



a

b



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e

f

Figure 6. 6. Mass release of a) TSS b) COD c) TKN d) NH_4^+-N e) NO_3^--N and f) TP

In addition, COD mass released reduced 70, 74, 80, 38, 34 and 73% (control unit: 72%) from May to September (Figure 6.6.b) and reached 0.34, 0.24, 15, 0.73, 0.56 and 0.18 g for WP1 to WP7 (control unit: 0.75 g), respectively. Ammonium mass release reduced from May to September by 85, 84, 85, 17, 17 and 90% in WP1 to WP7, while control unit showed 16% reduction (Figure 6.6.c). The higher release masses of ammonium in WP5 and WP6 by 0.02 and 0.018 g could be due to the plant death. Nitrate mass release reduced during the study by 62, 64, 73, 50, 55 and 80% in WP1 to WP7 (control unit: 60%), respectively (Figure 6.6.d). WP7 (SLR$_3$ and *P.australis*) showed lower release of nitrate compared to WP3 with similar SLR and plant, indicating a potential decrease in nitrate availability in drained water using *A.donax* and worms. The general trend of TKN variation was downwards from May to September, but in WP5 and WP6, there was an increase from June to September attributed to the plant witling in these units (Figure 6.6.e). The reductions in TKN mass release for WP1 to WP3 were 62, 65 and 73% while they were 25, 36 and 82% for WP5 to WP7 (control unit: 60 %), respectively. This shows 13% difference between WP3 planted with *P.australis* and control unit and 21% between WP7 and control unit, although TKN mass release increased by the increase in SLR.

A summary of drained water quality from the last cycle of feeding (September 2023) of the present study and from Beirolas experiment (September 2022) is presented in the supplementary materials, expressed in concentration, and compared with the quality requirements for water reuse.

In terms of Nitrate removal efficiency from mixed sludge to drained water, WP7 (planted with *A.donax*) showed 9% higher efficiency compared to WP3 with similar SLR planted with *P.australis*. A similar trend also occurred in case of phosphorous although all units showed over 99% reduction owing to the entrapment of particulate phosphorous on the top of units and filtration mechanism (Tan et al., 2017). It can be stated *A.donax* was more effective in the removal of nitrogen and phosphorous components compared to units planted with *P.australis*.

Nevertheless, WP units were effective to improve drained water quality particularly in comparison with control unit, drained water would require a post-treatment stage to comply with the standard limits for water reuse (Decreto-Lei nº 119/2019). Although, NH_4^+-N concentration of WP1, WP2, WP3 and WP7 were under the limit value of 10 mg.L^{-1}. *E.coli* limit for class B is 100 CFU.mL^{-1}, which was met by WP1, WP2, WP3, WP6 and WP7 units. Overall, units with lower SLRs showed lower concentrations.

6.4.6. Residual sludge analysis

Macro and micro-nutrients and heavy metal (HM) were detected in the final residual sludge. Macro-nutrient concentration was greater in top layers for WP1 to WP7 by 524, 645, 569, 633, 504, and 546 mg.kg^{-1}.DS^{-1} compared to bottom layers by 215, 171, 236, 177, 165, and 175 mg.kg^{-1}.DS^{-1} (Figure 6.7.a). This was 509 and 527 mg.kg^{-1}.DS^{-1} for the top and bottom layers in control unit, respectively. This could be due to the combined effect of plants and worms reducing elements on bottom layers while the entrapment of particulate matters on the top of the units increased the availability of the elements. Cumulative mass of macronutrients was 60, 73 and 59% lower than in the bottom for WP1 to WP3 planted with *P.australis* while it was 72, 68 and 68% for WP5 and WP7. WP units planted with *P.australis* indicated potentially lower macronutrients percentage compared to WP units planted with *A.donax*. Moreover, WP5 and WP6 lost plants and worms, which could also influence macronutrients availability in bottom layers. Elemental order of macro-nutrient significance was N > Ca > P > S > Mg > K of which nitrogen was more abundant while calcium was another significant fraction. The proportion of NPK was averagely 29:8:1 on the top and 23:4:1 on the bottom for WP units (31:8:1 on the top and 34:8:1 on the bottom for control unit).

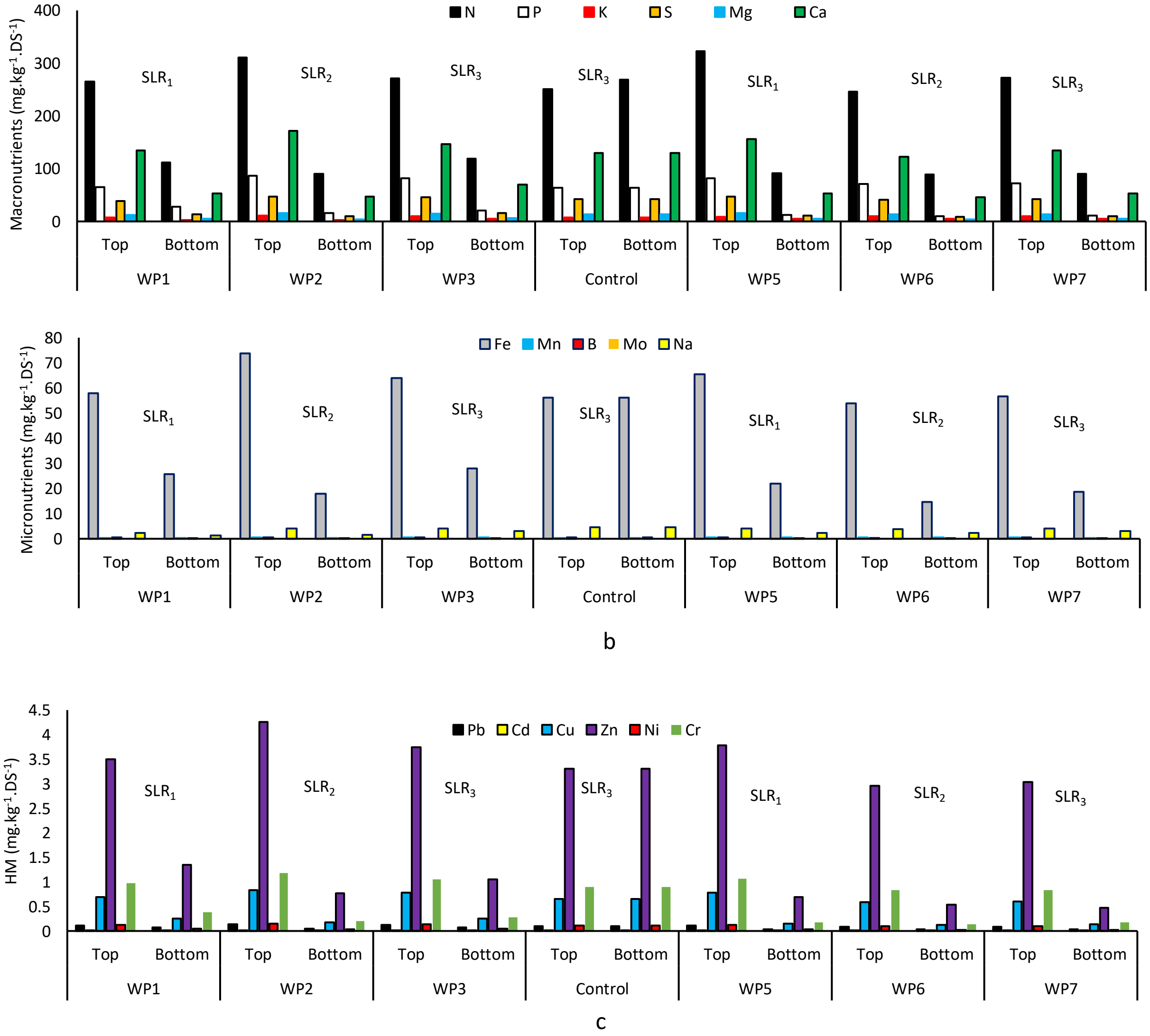

Figure 6. 7. Residual sludge a) macronutrients b) micronutrients and c) HM

The effect of higher SLR in WP1 did not make any clear difference in the cumulative mass of macronutrients compared to the lower SLRs in WP2 and WP3; however, WP5 with SLR1 showed higher macronutrient availability compared to WP6 and WP7 units.

In addition, the top layers of residual sludge for WP units had more micronutrients than the bottom layers (Figure 6.7.b) except in control unit. This indicates the influence of worms and plants reduced micronutrients availability in the bottom layers. The elemental order of micronutrients significance was Fe > Na > B > Mn > Mo in which Fe represented

95% of the cumulative mass, which could be due to the addition of ferric chloride in Beirolas WWTP for flocculation. In terms of SLR effect, the behavior of micronutrients availability was like macronutrient variations, namely, higher SLR did not increase cumulative mass particularly in WP1 to WP3 while WP5 indicated higher presence of macronutrients compared to WP6 and WP7. Plants also did not make significant increase or decrease in macronutrients availability.

Based on Figure 6.7.c, HM elements were also present in residual sludge by a significant order of Zn > Cr > Cu > Pb > Ni > Cd. Top layers had also higher availability of HM compared to bottom layers possibly due to plants and worms. This indicates HM potentially varied due to various mechanisms in the presence of plants and worms. Bioaccumulation, metal binding, improved aeration of residual sludge, stimulation of microbial activity, biological uptake, and enhancement of residual sludge structure could be other factors (Chen & Hu, 2019). Worms transform toxic elements like HM into less toxic forms and immobilize them within the residual sludge particles (Ma et al., 2020) (summary of HM in bottom layers and insights from previous research was presented in the supplementary materials).

Looking into the previous studies shows that a longer duration of final resting would increase the availability of HM due to residual sludge layer compaction during dehydration. The operational conditions of this study are comparable with Chen and Hu, (2019) in tropical climate while the results are different in which 60 days of final resting in units with plants (*P.australis*) and worms were assessed.

Regarding microbial contamination, it was found after 60 days of final resting, *E.coli* and Fecal coliform reduced 99.9% across all units (4 and 3 log removals; *E.coli* and Fecal coliform of mixed sludge were 7.5E+04 and 5.3E+04 and *Salmonella* was present). Both *E.coli* and Fecal coliform values dropped below 1000 CFU.100mL^{-1} for WP units while in control unit, they were 4200 and 1600 CFU.100mL^{-1}, respectively (national and international standard limits were shown in the supplementary materials).

HM concentration fell below all standard limits especially for Portugal (Decreto Lei 276/2009), which could be due to shorter period of the study and lower accumulation of HM in comparison with previous studies. Thus, WP units met the Portuguese limits (Decreto Lei 276/2009) mandating less than 1000 CFU.100mL^{-1}. Enhanced oxygenation by worms within residual sludge layer and accelerated competition for nutrients and organic matter affecting the microbial community could improve disinfection efficiency in WP units and the effect of plant root system (Wang et al., 2019). *Salmonella* was present in control, WP5 and WP6 units while it was absent in WP1, WP2, WP3 and WP7 units. This could be due to the plant and worm losses in WP5 and WP6. Thus, the presence of plants and worms could potentially be effective in *Salmonella*. According to the Portuguese law (Decreto Lei 276/2009), the absence of *Salmonella* is required in a 50 g sample. Therefore, WP1, WP2, WP3 and WP7 units complied with this regulatory requirement. Overall, residual sludge obtained from WP1, WP2, WP3 and WP7 units met the standard limits; however, it should be checked for other contaminations, particularly emerging pollutants; thus, additional disinfection stage for a safe reuse is suggested.

6.5. Conclusions

Two plant species, namely *P.australis* and *A.donax* combined with *Eisenia fetida* were tested in STRB technology under control condition in Portugal. SLR of 50, 60 and 70 kg.DS.m^{-2}.year^{-1} was studied in WP and C units fed with mixed sludge. Units with *A.donax* lost their plants and worms around last cycle when SLR was above 60 kg.DS.m^{-2}.year^{-1}. Higher SLR yielded higher biomass and elevated numbers of worms, particularly for *P.australis*. Unit with *P.australis* fed by 70 kg.DS.m^{-2}.year^{-1} produced 31% more biomass compared to 60 kg.DS.m^{-2}.year^{-1}. Precipitation during the final rest caused an increase in the thickness of residual sludge to more than 10 cm for all WP units (C unit: 16.5 cm) due to the reduction in DS content. Residual sludge accumulation increased by higher SLRs. Sur-DS content was 7% greater than sub-DS (no significant difference between units) while sub-VS/DS was averagely 15% less than sur-VS/DS. VS/DS reduced during a two-month of final resting by 13%, averagely. Water balance analysis showed water loss increased with higher SLR, and units with *P.australis* showed 20% higher water loss compared to units with *A.donax*. Water loss through absorbance in residual sludge layer was 2.88% for *P.australis* and 5.16% for *A.donax*, both with SLR of 70 kg.DS.m^{-2}.year^{-1}. Furthermore, water loss was 46 and 53% through drained water, and 51 and 41% through ET for *P.australis* and *A.donax*, respectively. A 10 kg.DS.m^{-2}.year^{-1} greater SLR resulted in 14% higher ET for both plants and SLRs. The unit with *P.australis* and SLR of 70 kg.DS.m^{-2}.year^{-1} showed the highest ET by 5.23 mm.day^{-1} while the unit with *A.donax* and similar SLR showed 4.24 mm. drained water quality ought to be analyzed in terms of both mass release and pollution concentration allowing a correct assessment of total pollutants discharged. Drained water improved over time and units with *P.australis* released lower mass of COD, TSS, TKN, NH_4^+-N and TP compared to units with *A.donax* and in addition, higher SLR resulted in greater masses release. Drained water quality did not meet standard limits for water reuse. Residual sludge quality showed top layers had higher availability of nutrients and HM compared to bottom layers while availability of them were not significantly different between units. The elemental order of macro-nutrients showed a notable hierarchy with N > Ca > P > S > Mg > K, while for micro-nutrients, the sequence was Fe > Na > B > Mn > Mo. Heavy metal availability adhered to standard limits, ranking Zn > Cr > Cu > Pb > Ni > Cd. The incorporation of worms into STRB has the potential to improve dewatering efficiency, significantly. However, it is crucial to analyze carefully factors such as SLR, operational modes, and the specific characteristics of the sludge when selecting the appropriate season and plants for optimal results.

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Chapter 7. General discussion

In this doctoral study, an alternative nature-based solution for sewage sludge dewatering was tested. To achieve research objectives, two distinctive studies were set up in Beirolas WWTP in Lisbon (pilot-scale) and Horto greenhouse in Instituto Superior de Agronomia (ISA), at University of Lisbon (bench-scale). This study assessed STRB technology for the first time in the Portuguese climate. Additionally, this doctoral project introduced the inclusion of earthworms (*Eisenia fetida*) in the STRB system, marking the first exploration of earthworms' presence within the sewage sludge dewatering process in the European and Mediterranean regions. Although, there have been previously a few studies in China. Another innovative aspect was the use of giant reed (*Arundo donax*), applied for the first time in STRB based on the literature. These innovations aimed to enhance sewage sludge dewatering efficiency and improve sludge stabilization.

In this research, various analyses including dewatering effect, plant, and earthworm influence, drained water quality, sludge loading rate, residual sludge and cost analyses were conducted. A meta-analysis (chapter 2) was conducted to summarize and appraise previous studies (Gholipour et al., 2022) showing that STRB technology has been applied in various climate conditions. STRB has been designed with different configurations like E-STRB to generate power, I-STRB to increase oxygen availability and W-STRB to enhance dewatering process, all contributing to the minimization of land demand and to the enhancement of sludge stabilization. STRB was also operated at different sludge loading rates, feeding intervals, operational and final resting durations. In term of sludge type, most of studies dewatered surplus activated sludge in which *Phragmites australis* was frequently used. Previous research also used various plant species while the number of layers of reed bed media were widely two and three layers of substrates (Gholipour et al., 2022).

In the present study, an experimental setup in Beirolas WWTP was directly fed after mixed sludge production; therefore, the sludge dry and volatile solids varied. Nonetheless, DS and VS in the previous pilot studies were measured before feeding which was practically impossible in real time and WWTP operational parameters applied. In this study, *Arundo donax* was tested with a two-layer of drainage and transition layers for STRB configuration while in W-STRB system, a layer of turf was considered hosted earthworms, namely, *Eisenia fetida*.

In Beirolas experiment, water balance analysis revealed that evapotranspiration rate increased 46 % by the synergistic effect of earthworms in W-STRB system (chapter 3). The presence of earthworms could significantly increase plant growth of which they boosted the biomass production of *Arundo donax* plants by an average of 30 % compared to the planted unit without earthworms. This was likely due to earthworms help distribute organic matter evenly throughout the reed bed, which potentially provided essential nutrients supporting plant growth.

Final dry solid content obtained from W-STRB system was higher than that of STRB particularly in the wet season where the difference was frequently 15 %. The presence of earthworms in the system could be the primary reason for the occurrence of higher dry solids as they need water for multiple mechanisms in their lives like lubrication and hydration needs. Earthworms also increased the plant biomass production which directly influences on the water demand in the reed bed for plant need. Therefore, W-STRB system indicated an expedited dehydration efficiency compared to STRB system (chapter 3). This could contribute to the potential increase in sludge loading rate, which is the main parameter used for STRB design (Gholipour et al., 2022). Higher SLR contributes to the reduction of land demand and W-STRB system with enhanced SLR can decrease the associated capital, operation and maintenance costs making this NBS technology more feasible and competitive when it comes to decision making between conventional technologies and STRB (Uggetti et al., 2011).

On the other hand, Beirolas experiment showed that the sludge accumulation rate was 0.06 cm.year^{-1} for W-STRB while it was 0.09 cm.year^{-1} for STRB indicating 33 % lower rate for W-STRB. This implicates that the inclusion of earthworms in the system could potentially reduce the volume of residual sludge produced which directly impacts the cost of sewage sludge management. Furthermore, in case of onsite measurements confirm the compliance with reuse standard, it can be reused without further treatment stage. Thus, associate costs of sludge management would decrease making it more feasible solution compared to STRB.

Drained water quality of W-STRB and STRB systems were also monitored in this study (chapter 4) indicated that W-STRB released lower pollution mass compared to STRB although the concentration of water quality parameters, e.g., COD and TSS was better in STRB system because of intensified water loss through ET in W-STRB. It is common to send drained water to a post treatment train after dewatering in STRB system while the use of W-STRB system showed that drained water restored less pollution mass (for instance: g.COD); therefore, it can burden less pollution to the treatment train.

Additionally, results in the W-STRB system showed lower levels of volatile solid content compared to typical STRB which can also improve final residual sludge quality for further land applications. The final residual sludge can potentially be reused and, in some case, extracted residual sludge would require a post treatment stage like composting which would be less intensive for the residual sludge obtained from W-STRB when compared to typical STRB (chapter 5). The assessment on the residual sludge quality showed that it contains a wide range of nutrient elements and heavy metals (chapters 5 and 6). Macronutrients were available on the order of N > Ca > P > S > mg > K and micronutrients had the order of Fe > Na > B > Mn > Mo showing that the residual sludge is a valuable resource and can be potentially recovered. The analysis also showed that it contained heavy metals which was in the order of Zn > Cr > Cu > Pb > Ni > Cd. Interestingly, W-STRB system showed lower concentration of heavy metals compared to STRB owing to the synergistic effect of worms and plants. This indicates that sewage sludge management through NBS can contribute to the farm to fork EU strategy and create a circular economy for a sustainable food production in smart cities (Mannina, Barbara, Cosenza, & Wang, 2023b; Morseletto, 2023).

Complementary to the 2-year study in Beirolas, a Horto study was developed to assess the potential to increase SLR and compare with a different plant species (chapter 6). In this study**,** SLR was increased to 50, 60 and 70 kg.DS.m^{-2}.year^{-1} to evaluate the effect of higher SLR on *Arundo donax* and *Phragmites australis* dewatering performance under controlled condition. *Arundo donax* could not sustain SLR higher than 60 kg.DS.m^{-2}.year^{-1} while *Phragmites australis* performed well with SLR up to 70 kg.DS.m^{-2}.year^{-1}. In Horto experiment, DS and VS were not significantly different after final resting for units planted with *Arundo donax* and *Phragmites australis* (chapter 6). However, in water balance analysis, ET was higher for *Phragmites australis* and higher SLR resulted higher ET. *Phragmites australis* resulted in lower VS content compared to *Arundo donax*, enhancing sludge stabilization. This contributed to a lower sludge accumulation rate in units planted with *Phragmites australis*; thus, these units showed a reduced volume of residual sludge (Chapter 6). This finding could be potentially adapted in the design of STRB systems to reduce the volume of residual sludge, thereby minimizing sludge production and associated handling costs.

The effect of SLR variation on drained water volume was significant in which higher SLR released higher pollution mass (chapter 6). It was found that the removal efficiency for nitrogen components was higher in the unit planted with *Arundo donax* compared to the units planted with *Phragmites australis* with similar SLR (chapter 6). Yet, *Arundo donax* resulted in a higher concentration of COD and TSS. Based on Horto experiment, *Arundo donax* showed higher nitrogen removal efficiency. The concentration of nutrients and heavy metal in the residual sludge showed no significant difference for *Phragmites australis* and *Arundo donax* in (chapter 6).

The overall assessments for Beirolas and Horto experiments showed that W-STRB system can be an alternative solution for sludge dewatering which is a cost-effective and sustainable nature-based technology. In addition, W-STRB can be applied in Portugal climate particularly in the case of *Phragmites australis* utilization while the use of *Arundo donax* can be another option provided that the objective would be higher production of plant biomass and higher nitrogen and phosphorous removal. To operate and design W-STRB system, sludge loading rate can vary between 50 and 70 kg.DS.m^{-2}.$year^{-1}$ based on the plant species.

Furthermore, STRB technology offers significant advantages over conventional techniques for sewage sludge dewatering such as centrifugation. STRB has lower energy requirements and lower operation and maintenance costs, making them more sustainable and cost-effective. Additionally, they provide improved sludge stabilization and dewatering efficiency through natural processes involving plants and earthworms. Conventional techniques commonly dewater sludge of which dry solid content is mostly less than 30 % (Wu et al., 2020), and the mechanical process does not include sludge stabilization. Higher dry and lower volatile solid contents result in reduced residual sludge volumes minimizing associated handling and disposal costs. The integration of ecological components like plants and earthworms not only enhances the treatment process but also contributes to a circular economy by promoting the potential reuse of treated sludge in agricultural applications while conventional techniques required post treatment stage for full resource recovery.

Chapter 8. Main conclusions and future research developments

8.1. Conclusion

This study contributed to a better understanding of the potential of STRB system for sewage sludge dewatering in the Portuguese climate. Initially, the meta-analysis review showed that STRB can be used in different climate like in the Mediterranean region with temperate condition and Portugal could be a good example for STRB implementation.

Afterwards through experimental studies, the prospect of enhancing STRB overall efficiency via the inclusion of earthworms also showed promising results on water balance, dewatering efficiency and residual stabilization. W-STRB provided enhanced dry solid contents and degraded volatile solid contents. In addition, earthworms increased water loss in the dewatering process (namely, evapotranspiration and earthworm hydration demand) indicating potential contribution to enhance sludge load. In dry season, this technology was more effective in sewage sludge dewatering than in wet season, it should be carefully loaded and maintained to avoid sewage sludge flooding on the top of the reed bed.

The study showed that W-STRB technology contributed to the treatment of the drained water although the quality of the drained water still needed improvement to meet the local standard limits. Therefore, the drained water of W-STRB technology may require additional treatment stages. In fact, a W-STRB technology by essence is a sewage sludge dewatering system, and drained water is a leachate impacted by evaporation and evapotranspiration mechanisms contributing to a lower volume discharge of drained water. In addition, the final residual sludge showed a great potential of different nutrients which are essential for plant needs and soil quality. W-STRB system showed a minimize availability of heavy metals complied with the standard limits in this study. This indicates that the use of W-STRB can be considered in a circular economy system to smartly distribute recovered resources in sustainable-smart cities. In terms of tested plant species, both *Phragmites australis* and *Arundo donax* can be planted in W-STRB system in the presence of worm in the Portuguese climate. However, sludge loading rate should be carefully adjusted according to plants and seasonal variations. The study of two different plant species also allowed to understand the effect that they have in STRB technology exposure to earthworms. The simplicity of STRB containing local materials and plants together with lack of need to energy-intensive structure could promote this technology in the Portuguese climate. This technology could be a viable sustainable solution to conventional technologies, which is cost-effective, and the by-product can be reused to link within smart urban environment.

Additionally, W-STRB could be more efficient than STRB configuration for sewage sludge dewatering and stabilization enhancements. This technology could be an interesting solution for locations with available land since it can achieve desired dry solid content, with simple operation and low energy and maintenance costs. Overall, W-STRB technology showed a good performance in dewatering efficiency and pollutant removal from the residual sludge and the drained water. Including earthworms also presented an effective strategy for enhancing sludge loading rate, particularly when combined with *Phragmites australis*. It could be a viable alternative solution for sewage sludge dewatering in Portugal climate and in an era with current environmental, economic, societal, and political challenges and crisis, can contribute to the achievement of the circular economy paradigm. Hence, employing of these tested components could deliver better dewatering efficiency and should be further explored.

8.2. Future recommended developments

Along the present thesis, specific gaps in the research were revealed and should be further addressed. Firstly, innovative solutions to enhance general system performance should be studied. For instance, in this study, the focus was to test STRB system in a temperate climate in the presence of earthworms while the system did not include active aeration. Therefore, further research could be led on W-STRB with active aeration, assessing system performance towards enhancing dewatering efficiency. This might increase earthworms' movements affecting final residual sludge quality with a higher decomposition rate of organic matter. In addition, there should be more investigation into earthworms' population variations in W-STRB system, and different earthworms' species should be tested to assess their performance in the dewatering process. Another important research could be seasonal monitoring of earthworms' dynamics in the media, under different feeding and resting periods. In this study, power generation through microbial fuel cells was not researched, this gap can be explored in future experimental studies to achieve intensified sewage sludge dewatering and power generation. This might lead to the design of an off-grid W-STRB lowering the negative environmental impact of the technology. In terms of cost analysis, this study estimated the cost of the current management using mechanical systems (centrifuge) compared to W-STRB, however, it could be extended not only to system costs but also to a complete life cycle cost. In terms of drained water quality, which has not been thoroughly investigated in previous studies, W-STRB did not comply with standard limits. Thus, drained water quality can be addressed and improved through innovative approaches. The system materials in this study included gravel in different sizes and based on this, the reed bed material can be altered to various nature-based materials to search for improved pollution removal from residual sludge and drained water. Another important aspect was the quality of the final residual sludge and possible improvement of the by-product obtained, which must be treated for compliance with local standards. Research can be directed to this topic to connect WWTP to smart city needs.

Finally, there is also a huge gap in the literature regarding greenhouse gas (GHG) assessment in W-STRB. There has not been any research on GHG monitoring and W-STRB climate impact and global warming potential, therefore this should be included in future research. Moreover, the impact of STRB as a nature-based solution in combating climate change should be investigated, particularly regarding its potential for carbon sequestration and reduction. Extensive studies are needed to evaluate the ecosystem services provided by such solutions, quantifying them in monetary terms to highlight their cost-effectiveness and additional co-benefits. Future research should include sustainability assessments that compare conventional dewatering techniques with STRB, including a comprehensive analysis of their relative advantages.