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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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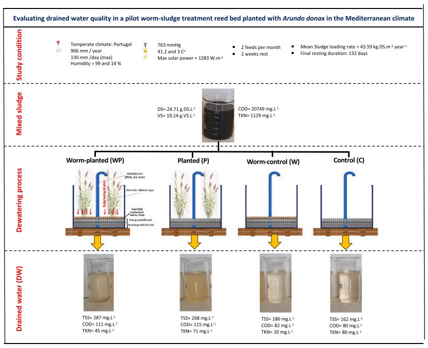
HIGHLIGHTS

- 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



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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.

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Abbreviations

ARGs	Antibiotic resistance genes
NH_4^+-N	Ammonium nitrogen
C	Control unit
COD	Chemical oxygen demand
DW	Drained water
DS	Dry solid
EC	Electrical conductivity
ISA	Instituto Superior de Agronomia
IPMA	Instituto Português do Mar e da Atmosfera
NBS	Nature-based solution
NO_3^--N	Nitrate nitrogen
PCPs	Personal care products
P	Planted unit
STRB	Sludge treatment reed bed
SLR	Sludge loading rate
SD	Standard deviation
TSS	Total suspended solids
TVS	Total volatile solids
TKN	Total Kjeldahl nitrogen
VS	Volatile solids
VLR	Volumetric loading rate
WWTP	Wastewater treatment plant
W	Unit with earthworms
W-STRB	Earthworm sludge treatment reed bed
WL	Water loss
WP	Planted unit with earthworms

1. 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., 2023). 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 <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 and Stefanakis, 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.

2. Materials and methods

2.1. 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 (Fig. 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.

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

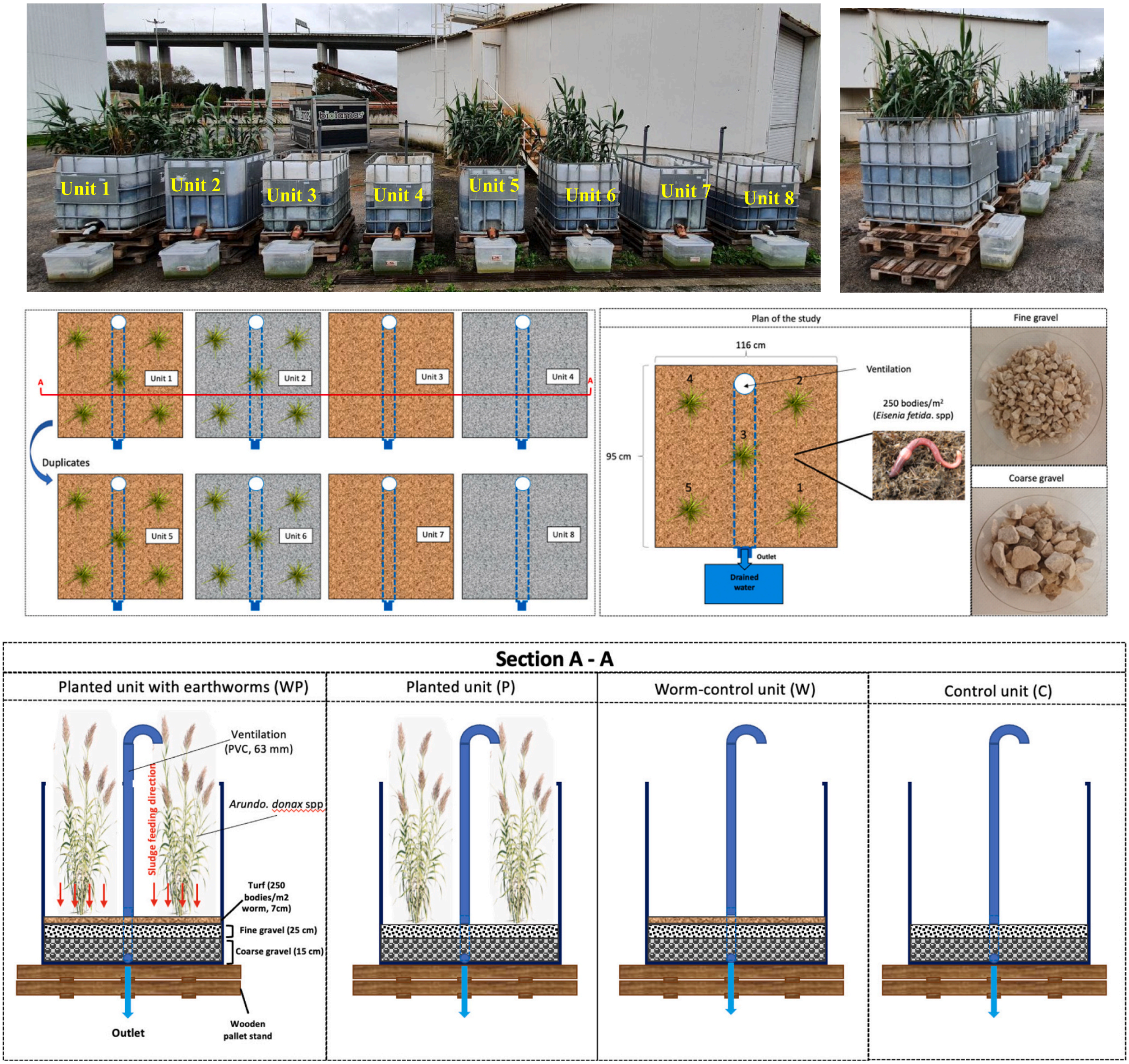


Fig. 1. Experimental configuration.

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}), 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).

2.2. 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.

2.3. Physicochemical analyses and sampling

The mixed sludge (100 g) and DW samples (1 L) (Fig. 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).

EC and pH were measured using a handheld multi-parameter VWR MU 6100H. 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.

2.4. Removal efficiency, water loss and mass balance calculations

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

$$\text{Removal efficiency} = \frac{(V_{MS} \times Con_{in}) - (V_{DW} \times Con_{iout})}{V_{MS} \times Con_{in}} \tag{1}$$

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):

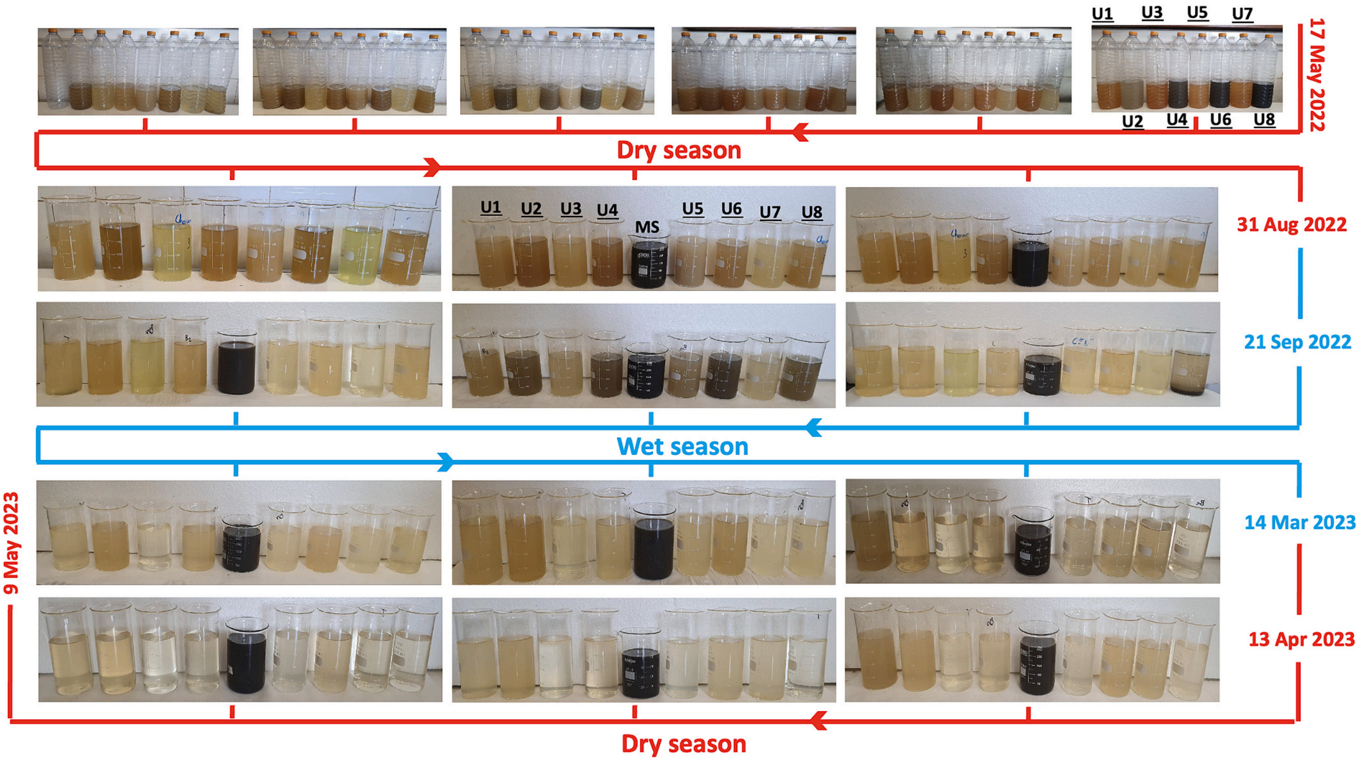


Fig. 2. Photo of the samples of the sludge and DW samples.

$$WL = P_r + V_{MS} + V_{RS\ (A)} - V_{RS\ (B)} - V_P - V_{DW} \tag{2}$$

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).

2.5. 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-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.

3. Results

3.1. Meteorological analysis

During the study, the minimum and maximum atmospheric temperature were in July 2022 and January 2023 reaching 42.2 and 3 °C respectively, and humidity reached 99 % in October while the minimum humidity was 14 % in July (Fig. 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 ° and 292° 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.

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).

3.2. 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

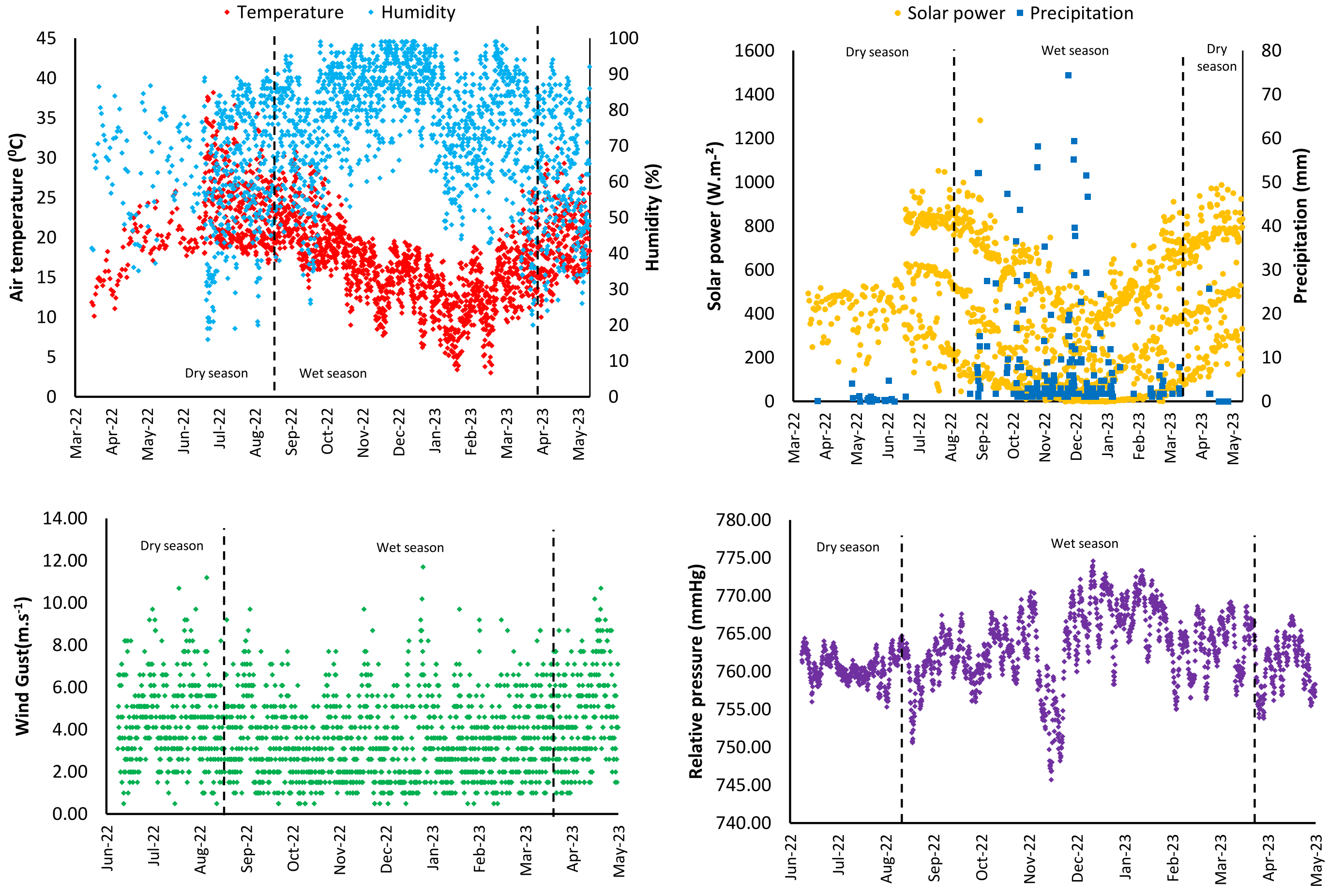


Fig. 3. Weather data.

Table 1
Plant development.

Season	Date	Height (cm)		N° 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
	29-Sep-22	101	85	12	8
Wet	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

Table 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

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 1 shows plant development during the study period in the dry and wet season.

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 2 shows the average population of earthworm during the study period for WP and W units.

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.

3.3. 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 (Fig. 4a), respectively (no significant difference *p*-value >0.05).

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 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 (Fig. 4a). 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 °C (mean = 23 ± 4 °C). The drained water temperature for all units followed atmospheric temperature variations (≈22 °C) which were not significantly different (p-value >0.05) (Fig. 4b).

TSS and COD in drained water were lower during the dry season in 2022 and higher during the wet season (Fig. 5a). 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 (Fig. 5b). WP unit also released 45 % less phosphorous compared to the P unit indicating a synergistic effect of earthworms in the improvement of phosphorous removal.

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 (Fig. 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 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) (Fig. 6a). 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 >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 (Fig. 6b) varied between 8802 and 29,804 (mean = 20,749 ± 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.

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 (Fig. 6d). 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.

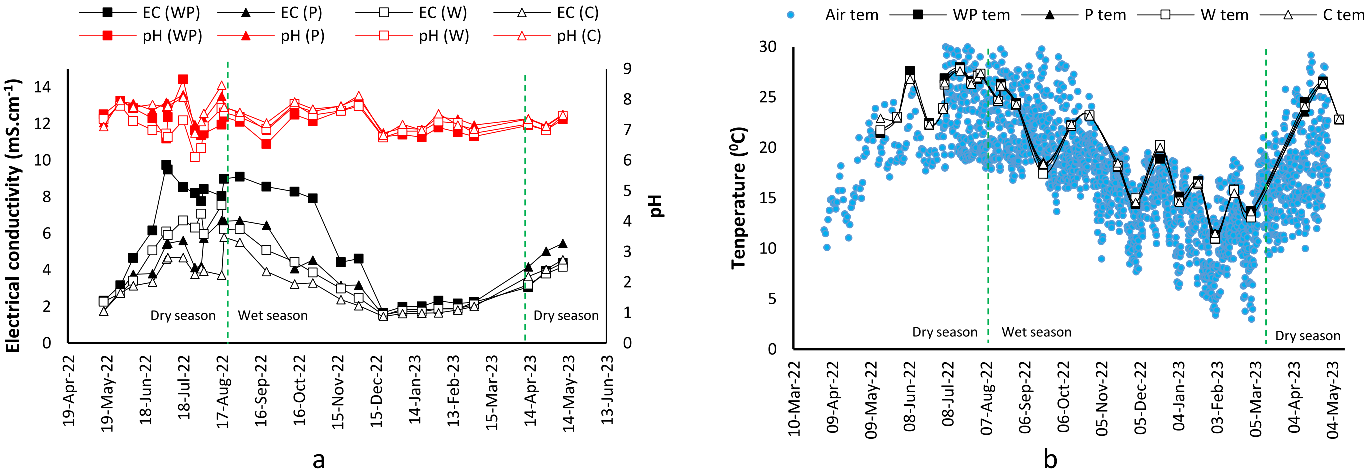


Fig. 4. Drained water quality: a) EC and pH, and b) temperature.

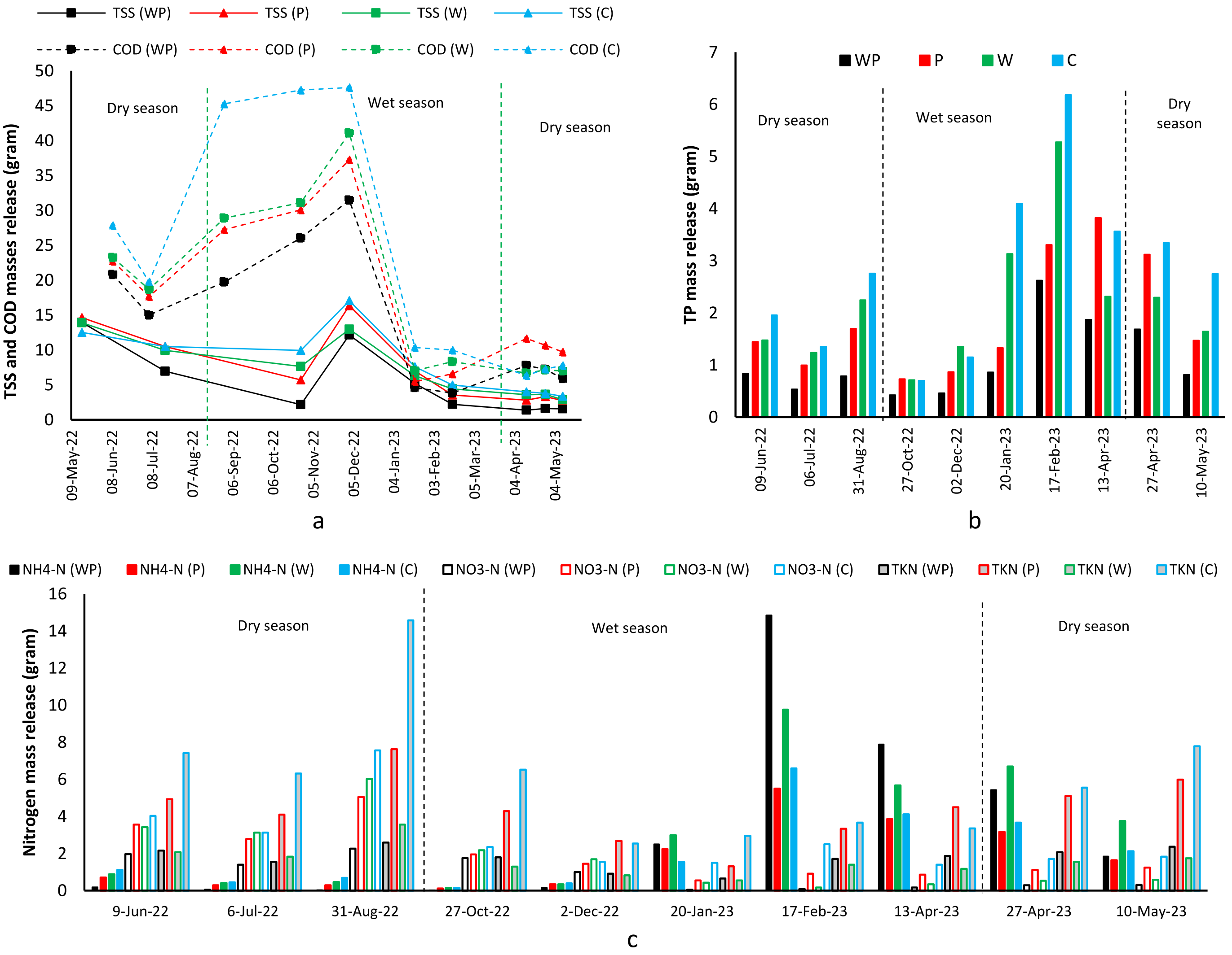


Fig. 5. Mass release: a) total suspended solid and chemical oxygen demand, and b) phosphorous c) nitrogen components.

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 <1600 MPN.100 mL^{-1} and *Escherichia coli* reduced from 7.8 × 10^{4} in the sludge to <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 3 shows a summary of drained water quality.

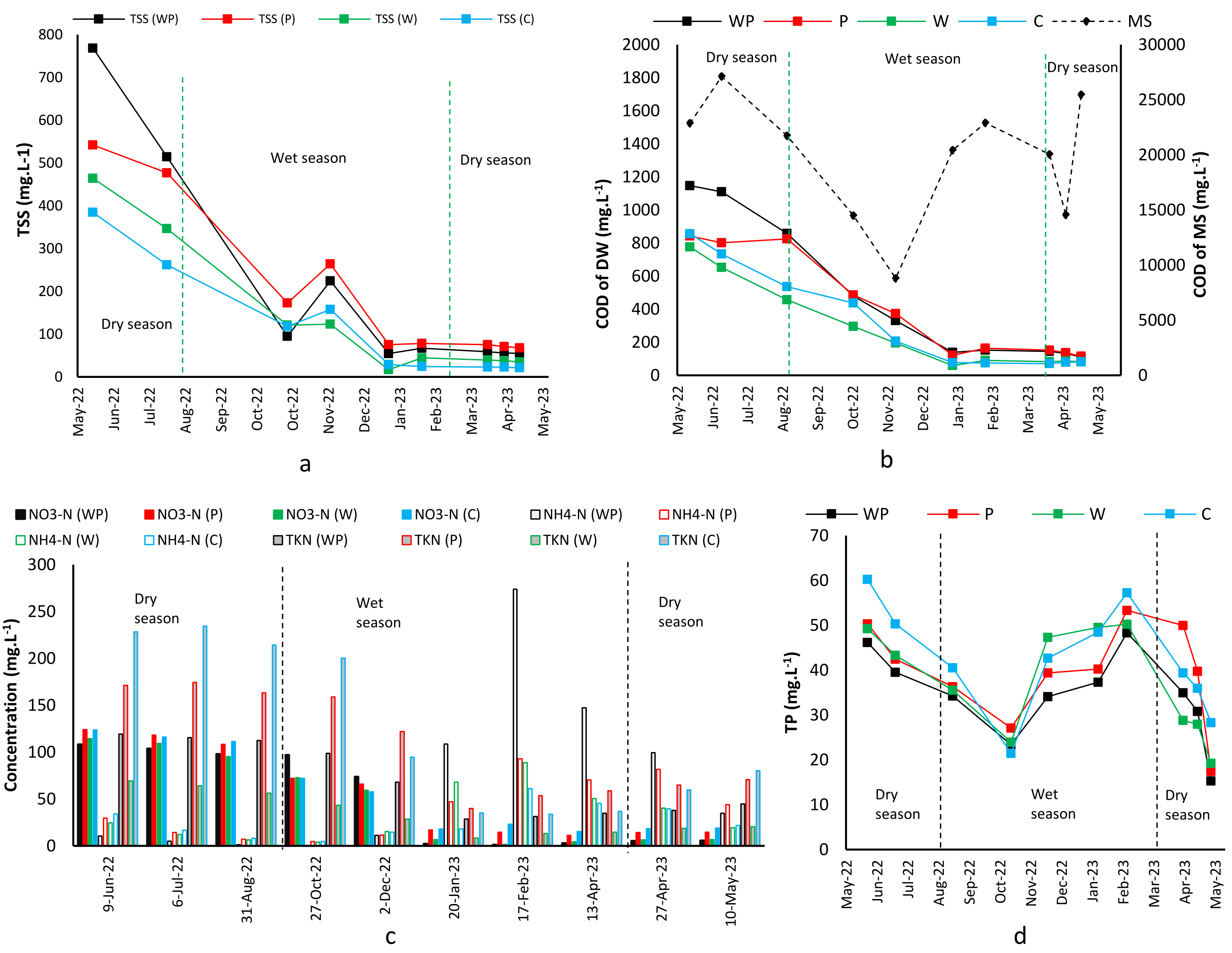


Fig. 6. Pollution concentrations: a) total suspended solid b) chemical oxygen demand c) nitrogen components d) phosphorous.

Table 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 (°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})	20,749 ± 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.100 mL^{-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[a]

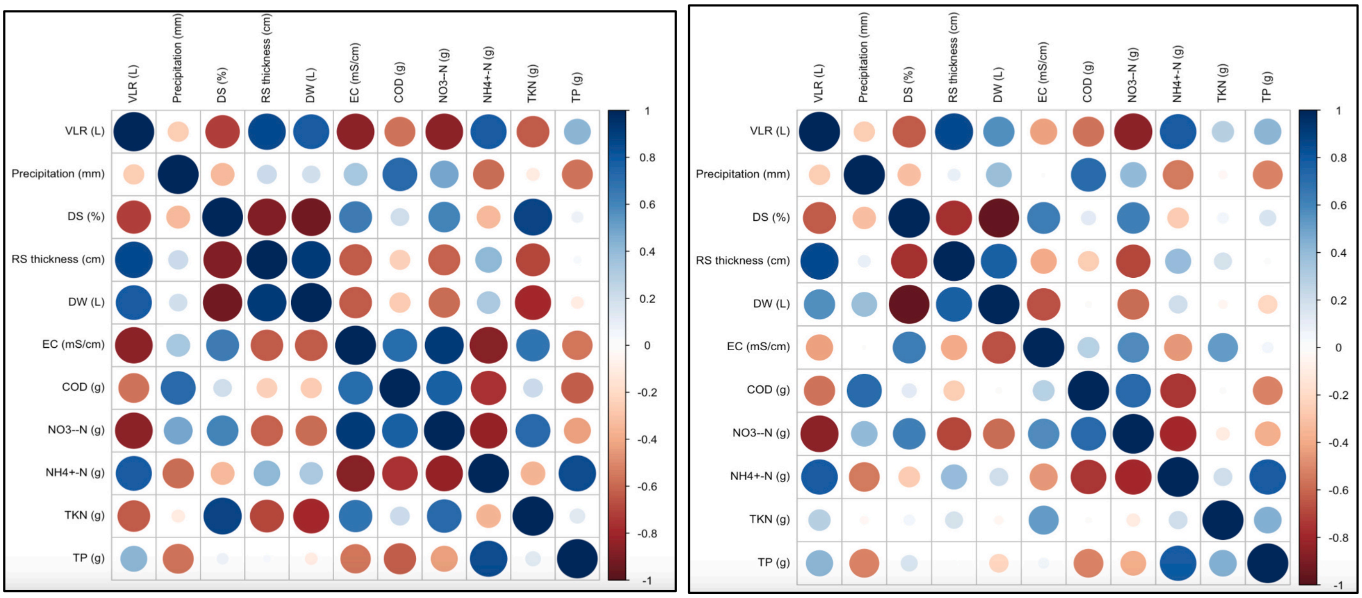
[a] N.A: Not available

The acceptable TSS level for water reuse in Portugal (Decreto-Lei n° 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.

3.4. Drained water correlation analysis

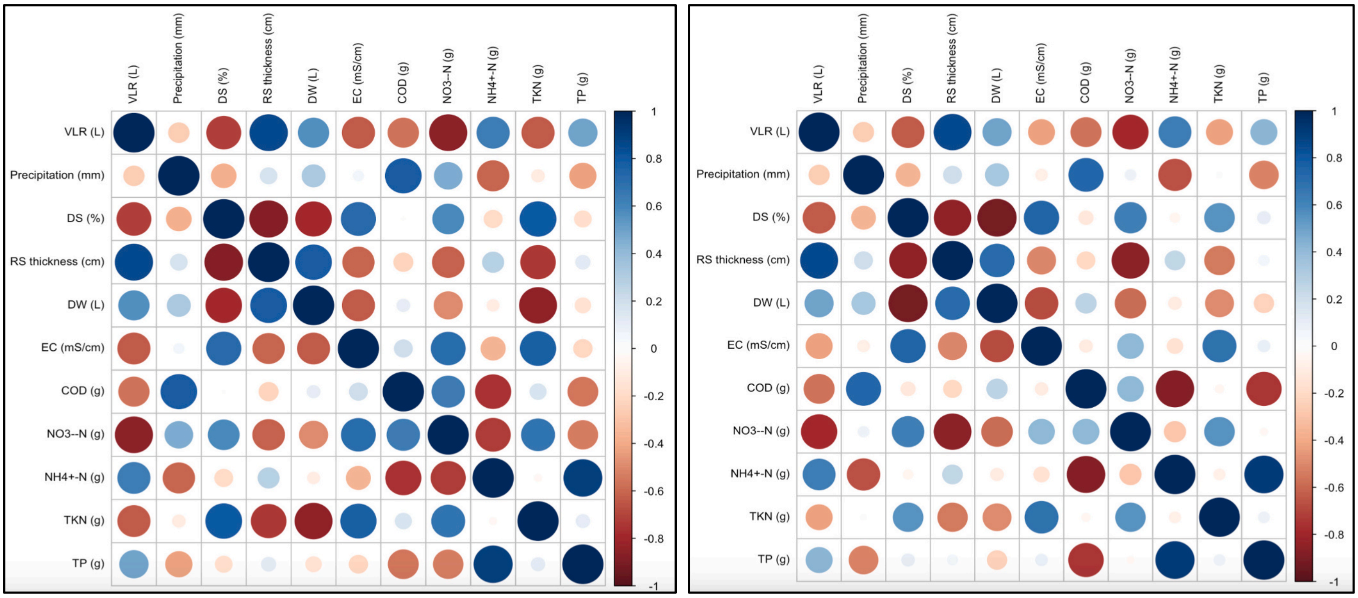
Spearman correlation was analyzed exploring the interplay of pollution masses, operational factors, and seasons (Fig. 7). It was shown that inputs (VLR: volumetric loading rate and precipitation) and outputs (drained water volume) as well as the residual sludge layer condition (DS and residual sludge 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



WP unit

P unit



W unit

C unit

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

in ammonium during the dry season could result from nitrification, while in the wet season, the dominance of denitrification might lead to nitrate variations.

TKN mass positively correlated with EC, and DS, while exhibiting negative correlations with residual sludge layer thickness, drained water volume, and VLR (p-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. 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, 2014; Kouawa et al., 2015; Osei et al., 2017). Table 4 presents a summary of the plant development data for different plants across different studies, including height and stem growth rate.

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

Table 4
Plant development in comparison with previous studies[a].

aSpecie	Climate	Average stem development (stems.m^{-2})	Average height growth (cm. month^{-1})	Comment	Reference
Phragmites australis	Temperate		20 to 200	Full-scale study	Hardej and Ozimek, 2002
		250	30 to 150	Full-scale study	Troesch et al., 2009
	Tropical		120	For 6 weeks	Zhang et al., 2012
	Tropical	147	–	Pilot-study	Wu, 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, 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

[a] Previous studies are all STRB units (P unit).

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 >85 % (Nielsen, 2007; Kołecka et al., 2017), while in the present study, it was >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 >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 >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 <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.

5. 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.

CRediT authorship contribution statement

Amir Gholipour: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Rita Fragoso:** Writing – review & editing, Visualization, Validation, Supervision, Methodology, Formal analysis, Conceptualization. **Ana Galvão:** Writing – review & editing, Visualization, Validation, Supervision, Methodology, Formal analysis, Data curation, Conceptualization. **Elizabeth Duarte:** Writing – review & editing, Visualization, Validation, Supervision, Methodology, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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