Journal of Environmental Management 370 (2024) 122772

Contents lists available at ScienceDirect

Journal of Environmental Management

journal homepage: www.elsevier.com/locate/jenvman

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

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ARTICLE INFO

Keywords:
Eisenia fetida
Sewage sludge
Constructed wetland
Arundo donax
Cost assessment

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.

1. 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 an immense source 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, which are readily absorbable by plants, making it a valuable source of nutrients for agriculture (Skrzypczak et al., 2023). Moreover, its organic matter enhances soil structure and water retention, and acts as a slow-release nutrient, fostering long-term soil fertility (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 reduces the need for synthetic fertilizers, curbing nutrient runoff and greenhouse gas emissions. This approach is cost-effective and aligned with the circular economy principles in smart cities, where waste is transformed into valuable resources for environmental conservation (Mannina et al., 2023). 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 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 (Zhang et al., 2022). This makes the dewatering process economically infeasible in the long run, even when coagulants such as polymer flocculants are used. Hence, alternative approaches are imperative to address the challenges of growing urban populations and stringent environmental regulations. Among these emerging technologies, sludge treatment reed bed (STRB) technology has drawn

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https://doi.org/10.1016/j.jenvman.2024.122772
Received 29 July 2024; Received in revised form 16 September 2024; Accepted 29 September 2024
Available online 2 October 2024

considerable attention for its potential to introduce new techniques to sludge dewatering processes. The 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 wetlands also have many applications, including domestic (Daee et al., 2019; Gholipour and Stefanakis, 2021) and industrial (Gholipour et al., 2020) 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, 2024b; Nielsen, 2023; Plestenjak et al., 2021; Stefanakis and Tsihrintzis, 2011; Uggetti et al., 2010). STRB system typically operates for 10–15 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 of Denmark, 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; Nielsen, 2011). In the temperate climates of Portugal, 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 and 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 (Decreto-Lei n.o 276/2009 of Portuguese regulation and 86/278/EEC of EU) (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 that residual sludge stabilizes after a final resting period. During this period, heavy metals are transformed from a more bioavailable state to a less bioavailable state (Chen and Hu, 2019), nutrients become less bioavailable (Osei et al., 2019), pathogenic organisms are disinfected (Calderón-Vallejo et al., 2015), and emerging pollutants decrease (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 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). Earthworms can act as natural aerators by burrowing through the sludge, which improves drainage and root growth (Gholipour et al., 2024c). By decomposing organic matter, converting it into rich humus, known as vermicomposting, accelerates the breakdown of complex organic materials, making nutrients more accessible to plants (Edwards and Arancon, 2022). Their activities lead to improved sludge structure, increased water retention, and reduced erosion (Chen and Hu, 2019).

The present study evaluated the effectiveness of W-STRB technology as an innovative approach for recovering biosolids particularly considering the qualities of the final residual sludge layer. This study provided an examination of earthworms in combination with *Arundo donax* in a STRB system, which has not been studied yet in the literature (Gholipour et al., 2022). 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 in different climatic conditions. Additionally, most of these studies have predominantly utilized *Phragmites australis* (*P.australis*) in a STRB without including earthworms. The limited studies under controlled conditions that incorporated worms did not provide a thorough assessment of the final residual sludge. This research aimed to increase the knowledge base in NBS applied to sewage sludge dewatering by examining the effects of earthworms and *Arundo donax* (*A.donax*). It aimed to shed light on the synergetic effects of *Eisenia fetida* (earthworms) and *A. donax* in terms of variations in micro and macronutrients, heavy metals, 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 the W-STRB alternative. The findings were anticipated to contribute to the ongoing discourse surrounding wastewater treatment practices and their implications for environmental sustainability.

2. Materials and methods

2.1. Experimental setup

This study was conducted at Beirolas wastewater treatment plant (WWTP) located in Lisbon, Portugal, serving a population of 213,510 individuals and treating 54,500 cubic meters of wastewater daily. The local climate is characterized as a 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 shows that Lisbon has 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 (Fig. 1) consisted of eight one-cubic meter Intermediate Bulk Containers, each measuring 0.96 m in width, 1.16 m in length, and 1 m 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) without plants and earthworms. Each unit was replicated for the study.

The units were constructed with drainage and transition layers consisting of coarse gravel (15 cm, 19–25 mm, and 38 % porosity) and fine gravel (25 cm, 4.8–9.5 mm, and 42 % porosity), respectively. For the WP and W units, a layer of turf (Siro 30) had specific properties including a pH of 5.5–6.5, a conductivity of 40–80 μs cm^{-1}, a granulometry of 0–15 mm, and an organic matter content exceeding 70 %. These units hosted 250 *Eisenia fetida* earthworms per square meter. The units were connected to 70-L drums via perforated PVC drainage pipes (63 mm diameter) at the bottom, with plastic valves for drainage control that 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 L 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 (each cycle took 1 h: 5 min per unit). Earthworms were introduced to the units one week before 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

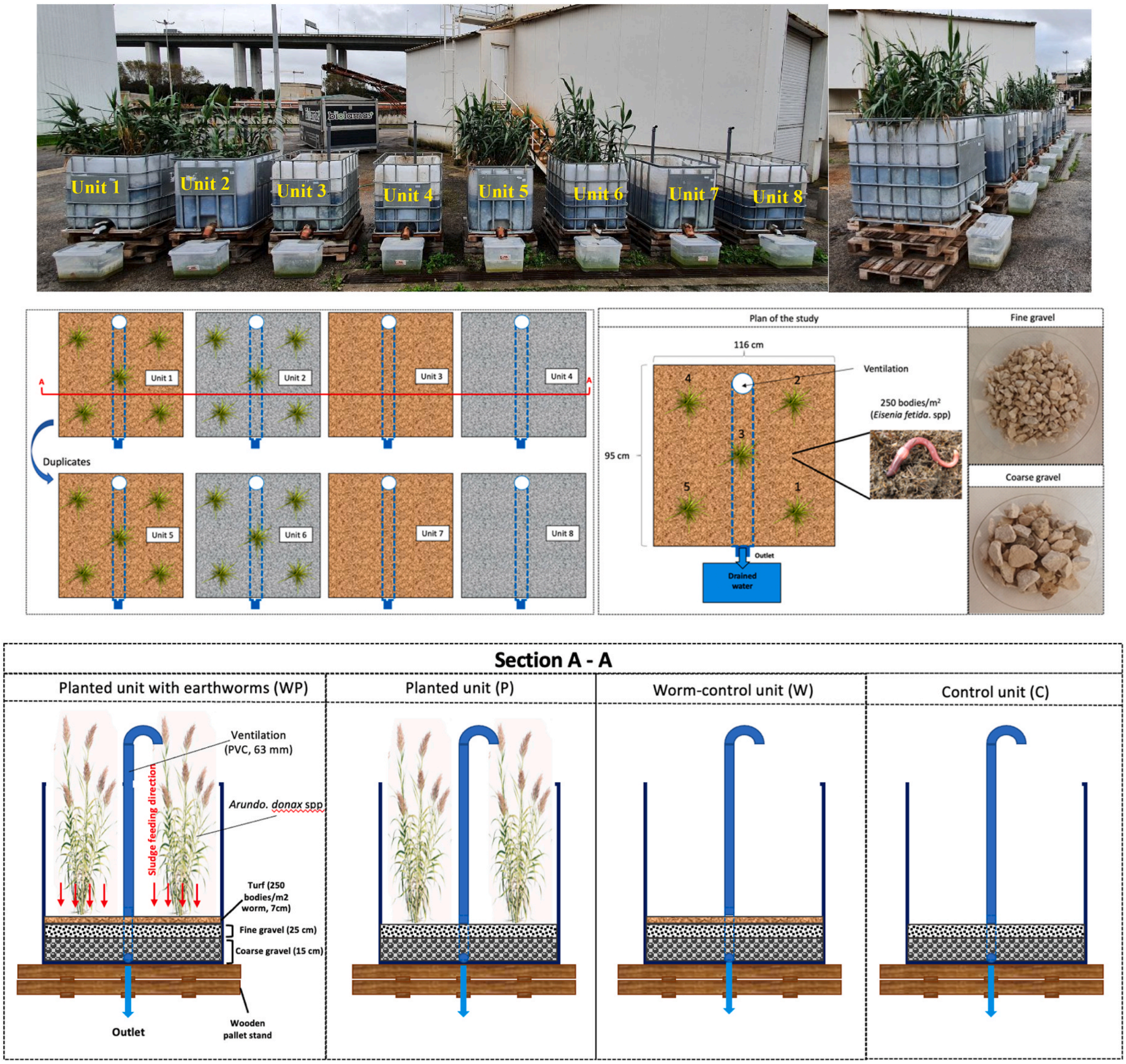

Fig. 1. Experimental setup at Beirolas WWTP and detailed layouts.

used 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 L per square meter (SLR_1: 40.6 $kg.DS.m^{-2}.year^{-1}$). From November 29th, 2022, until May 2023, the load increased to 100 L 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. This study reports the results of the experiment along the 132 days of the final resting.

2.2. *Scope of the study*

In the study conducted at Beirolas WWTP, the focus was on analyzing the water balance and the quality of drained water within a W-STRB system during a feeding period of one year (Gholipour et al., 2024a, 2024b). 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, the results of the study are detailed in Gholipour et al. (2024a).

The analytical methodology employed in the pilot-scale study on STRB involved a systematic approach to assess the impact of earthworms and *Arundo donax* on sewage sludge dewatering and residual sludge quality. 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 a final resting of 132 days. The study utilized four distinct units: planted with earthworms, planted without earthworms, unplanted with earthworms, and a control unit. The present study had the following objectives: firstly, to assess the quality of the final biosolids in the Beirolas experiment during the final resting period between May and September 2023. This evaluation entailed an examination of the residual sludge quality over the 132-day 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.

2.3. *Sampling procedure and lab analysis*

This study had two sections of sludge analysis, including feeding sludge, and residual sludge that remained on the top of mesocosms. The analysis encompassed both macro and micro-nutrients, as well as heavy metals, classified based on previous research (Kirkby, 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 and Bridgewater, 2017).

To assess the characteristics of the feeding sludge from Beirolas WWTP, three sampling campaigns were executed in November 2022, January 18th, 2023, and January 31st, 2023. Afterward, DS and VS contents together with micro and macronutrients, microbiological parameters, and heavy metals of the feeding sludge, were measured.

Sampling activities for the residual sludge layers were continued and carried out at four specific time points, namely, 14, 36, 62, and 132 days after May 9th, 2023. Samples were collected from both the top and bottom layers of the residual sludge within each unit to measure DS and VS contents, micro and macronutrients, microbiological parameters, and heavy metals. 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. Microbiological analyses, encompassing the assessment of *Escherichia coli* (*E. coli*), Fecal coliform, and *Salmonella* spp, were conducted at both 14- and 62-day intervals during the final resting period. *Salmonella* detection was done according to the International Organization for Standardization (ISO) 6579–1:2017. The enumeration of *E. coli* was according to ISO 16649–3:2015 using TBX medium (Biokar, Beauvais, France) and incubated at 44 °C ± 1 °C for 24 h.

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}) (Jagodziński et al., 2020). 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 earthworms showed 43 % higher biomass production compared to the planted units without earthworms.

In addition, the earthworm population was counted through a hand-sorting method in the flip and strip test (Gutiérrez-López et al., 2016) where the WP and W units were dug to dimensions of 20 cm width, 20 cm length, and 15 cm depth. In this paper, the population of earthworms is analyzed during final resting. During the feeding period based on Gholipour et al. (2024b), earthworm populations 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 earthworms' population reduced during the wet season and increased during the dry season.

2.4. Cost estimation

In this study, cost estimation was 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 and depending on the sludge management plan. The boundary of the estimation is shown in Fig. 2.

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, the estimation varies depending on the sludge management strategy. In contrast, according to Nielsen (2003), STRB can be removed from residual sludge every 10–15 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 m, with an accumulation sludge rate of 10–15 cm per year (Brix, 2017). This means that sludge residue fills the freeboard after approximately ten years. In addition, to compare O&M costs between the two scenarios, a ten-year analysis period was considered (Nielsen, 2003).

Moreover, a benefit-cost analysis was not performed because it would require a 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 the scalability, efficiency, and environmental benefits of the STRB system, the calculation was conducted for 2000 population equivalents (PE) which represented small community applications (Chen et al., 2020). Based on average wastewater production in Portugal, 180–220 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 the Beirolas experiment (shown in Fig. 1). The technical and design specifications of W-STRB systems can be found in Table 1.

According to Uggetti et al. (2011), the electricity consumption of a centrifugation system was 0.28 kWh.year^{-1}.PE^{-1} and 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, including investment costs, were considered to estimate the costs of centrifugation and STRB, as shown in Table 2.

To increase the accuracy of the estimation, several works of literature 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.,

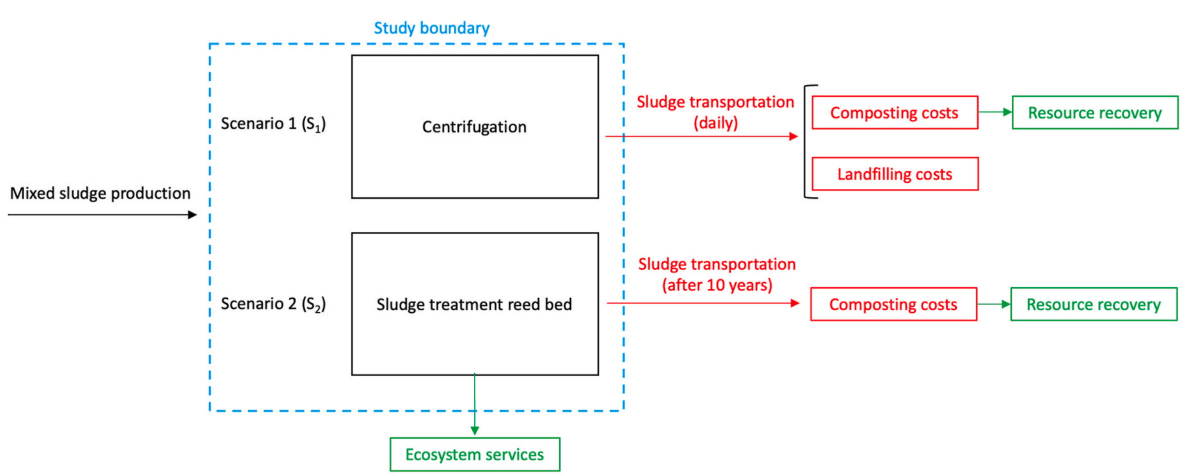

Fig. 2. Study boundary for cost estimation.

Table 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	0.5 g m^{-2} based on the Beirolas experiment	Gholipour et al. (2024b)

Table 2
Costs assessment specification.

Scenarios	Costs component	Cost source	Unit	Cost
Centrifugation	Investment	Uggetti et al. (2011)	€	160,824
	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)	€	163,000
	Electromechanically and electrical installations	Portuguese technical guide 23 (ERSAR)	€	39,000
	Turf layer	SIRO (Portuguese market)	€	14,286
	Earthworm	Market search	€	20,000
	O&M	Rizzo et al. (2018)	€/year	6000
	Personnel	Uggetti et al. (2011)	€/year	4728

2016; Rizzo et al., 2018; Tsihrintzis et al., 2007; Uggetti et al., 2011).

To update previous studies' costs and predict future cash flows, the interest and discount rates of the European Central Bank were used, which were 4.5 and 4.0 %, respectively. Eq.1 was applied for predicting future monetary values (Žižlavský, 2014): $FV = PV(1 + i)^t$ Eq.1

In which i is the discount rate, t is the time of the cash flow, FV is the 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 the year 2034, were accounted for using Eq.1.

2.5. Data analysis

Normality (Shapiro-Wilk test) and homogeneity (Bartlett's test) for all datasets were evaluated. 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 a statistical significance level of 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.

3. Results and discussion

3.1. Meteorological data analysis

During the final resting, the air temperature ranged from 12.9 to 35.2 °C (Fig. 3a), with an average of 20.78 °C (±3.42 °C). Humidity fluctuated between 23 and 97.2 %, with a mean of 66 % (±15 %).

The precipitation levels for May and June were 7, and 11.8, and it was 0 mm for the other months, respectively (July, August, and September 22nd, 2023). The maximum solar power reached 1419 W m^{-2} in June (Fig. 3b), and wind gusts ranged from 0.5 to 12.2 m s^{-1} (Fig. 3c), 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 (Fig. 3d). 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).

The study was conducted in a real wastewater treatment plant setting, leveraging a period of stable environmental conditions. Specifically, the experiment took place during the dry season from May to September 2023, which is characterized by minimal precipitation and temperature variability. These conditions allowed for a focused assessment of the sludge treatment reed bed's impact on residual sludge quality, minimizing the confounding effects of external environmental factors. This approach ensures that the observed outcomes are more directly attributable to the treatment processes, providing a clearer understanding of the system's performance under controlled conditions.

3.2. Mixed sludge quality

The highest concentration of macronutrients was observed in November 2022 samples, reaching 122,775 $mg.kg_{DS}^{-1}$, with DS and VS contents being 14.20 $mgDS.L^{-1}$ and 10.72 $mgVS.L^{-1}$ (Fig. 4a). 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 106,650 and 100,827 $mg.kg_{DS}^{-1}$ respectively. The concentrations of micronutrients followed a similar decreasing trend across the three months, with values of 22,761, 18,989, and 14,294 $mg.kg_{DS}^{-1}$ 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.

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 (Fig. 4b). The N:P:K ratio was 7:3:1, indicating that the mixed sludge could serve as a nitrogen-rich resource. On the other hand, Fe and Na were dominant in micronutrients (Fig. 4b), with Fe > Na > Mn > B > Mo. Moreover, Zn was the most abundant of heavy metals followed by Cu, Cr, Pb, Ni, and Cd. In a study on the STRB system (Ma et al., 2023), the order of heavy metals in a raw sludge (from 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

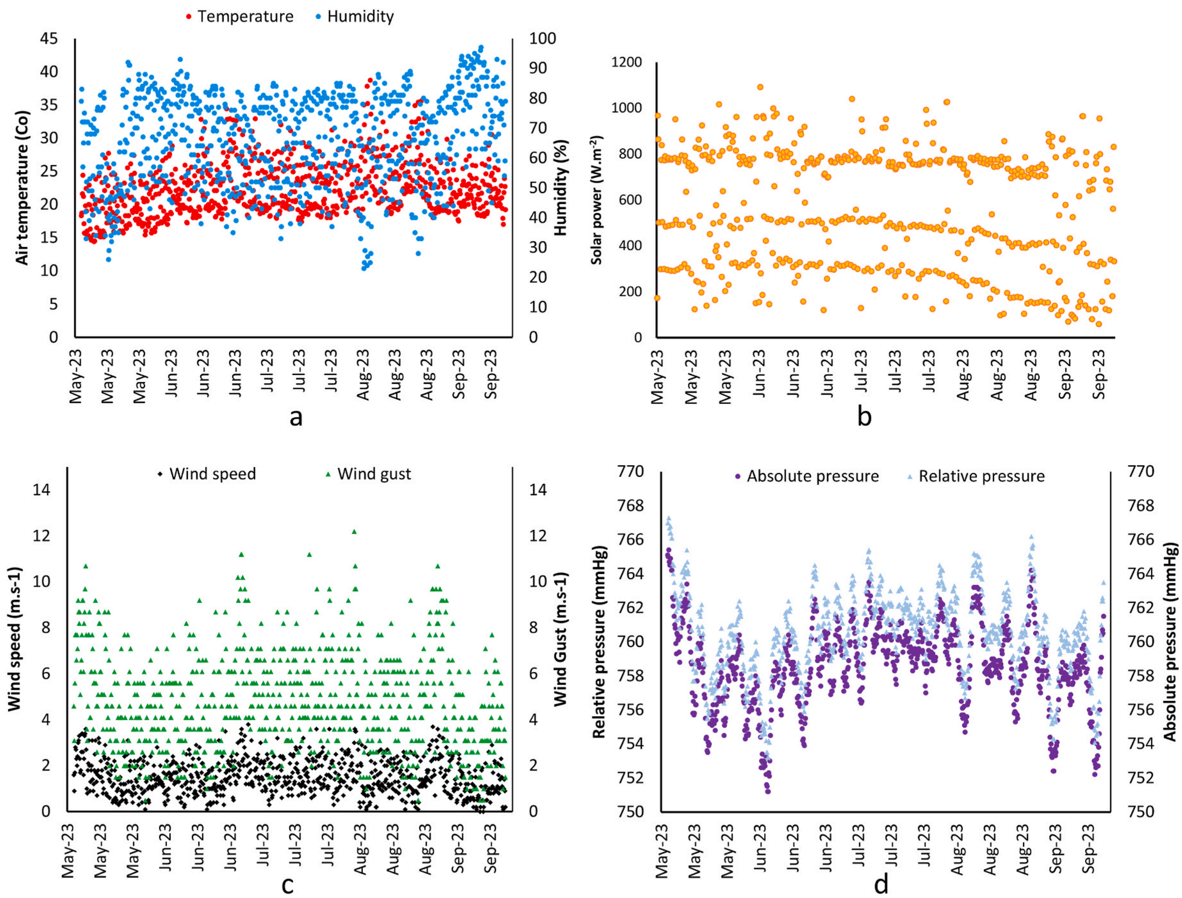

Fig. 3. Meteorological data a) air temperature and humidity, b) solar power and precipitation, c) wind speed and gust, and d) relative and absolute pressure.

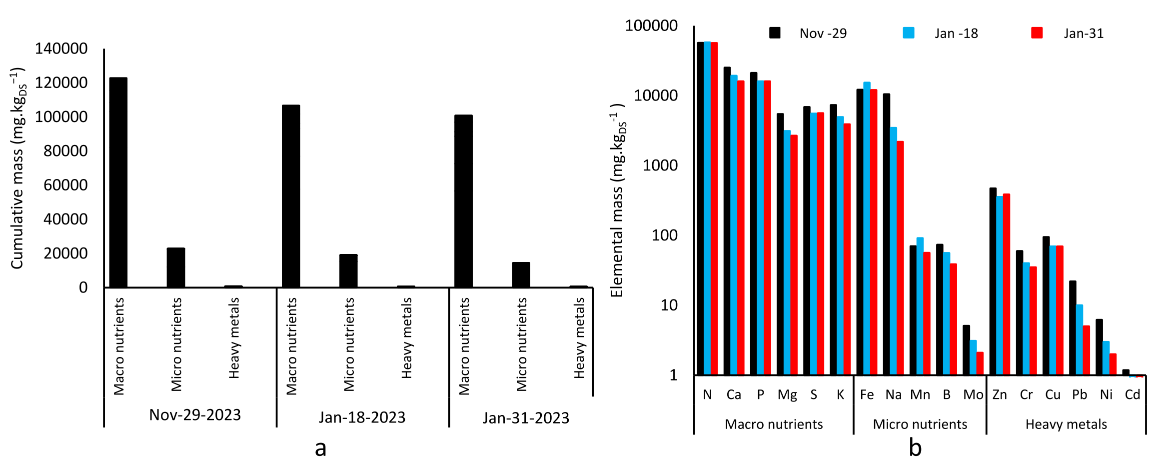

Fig. 4. Macro and micronutrients and heavy metals in a) cumulative mass and b) elemental mass of the mixed sludge.

source of raw wastewater.

3.3. Analyzing plant and earthworm dynamics

The above-ground plant biomass was higher in the units with earthworms than in the units without earthworms (Table 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 WP units 1 and 5 while it was 3.20 and 3.20 kg m^{-2} for 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

Table 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

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 WP and P units of 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 biomass production (Brix, 2017). In September 2023, the biomass water content reached 78.52 %, indicating a 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.

The population of earthworms was monitored during the final resting (Fig. 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 a 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 and Arancon, 2022; Gholipour et al., 2024b).

3.4. 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 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. The development of the root system in the WP unit increased below-ground 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 (see Table 5).

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 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 and 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 rest, the vertical profile of the residual sludge demonstrated near-uniform dryness.

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 and 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).

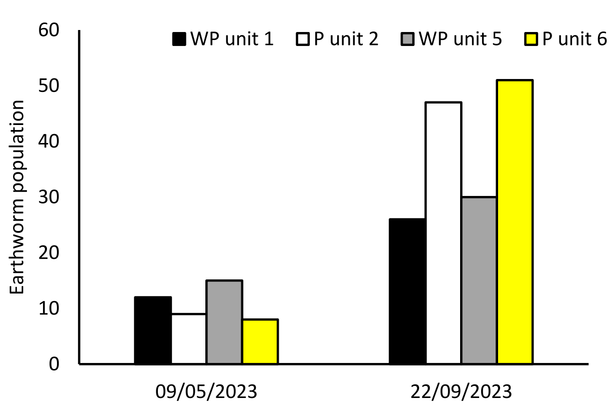

Fig. 5. Earthworm population during final resting in the residual sludge layer.

Table 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

Table 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

3.5. 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 (Fig. 6a). 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 increased. 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 higher macronutrient concentrations.

At 132-day, the P units showed the highest cumulative mass of macronutrients in the bottom (157,868 $mg.kg_{DS}^{-1}$), while the WP, W, and C units presented 142,431, 126,864, and 138,162, $mg.kg_{DS}^{-1}$ 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 and Arancon, 2022). The W unit exhibited higher concentrations compared to the other units (115,305 $mg.kg_{DS}^{-1}$) which could be due to the absence of worms in the top layers e. By 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 the 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).

The temporal changes and a similar pattern to that of macronutrients emerged for micronutrients, with the bottom layers consistently exhibiting higher cumulative masses compared to the top layers (Fig. 6b). In the top layers, the fraction of Fe increased from 12,777 $mg.kg_{DS}^{-1}$ on day 14–19,206 $mg.kg_{DS}^{-1}$ on day 132 across all units. The dominance of Fe

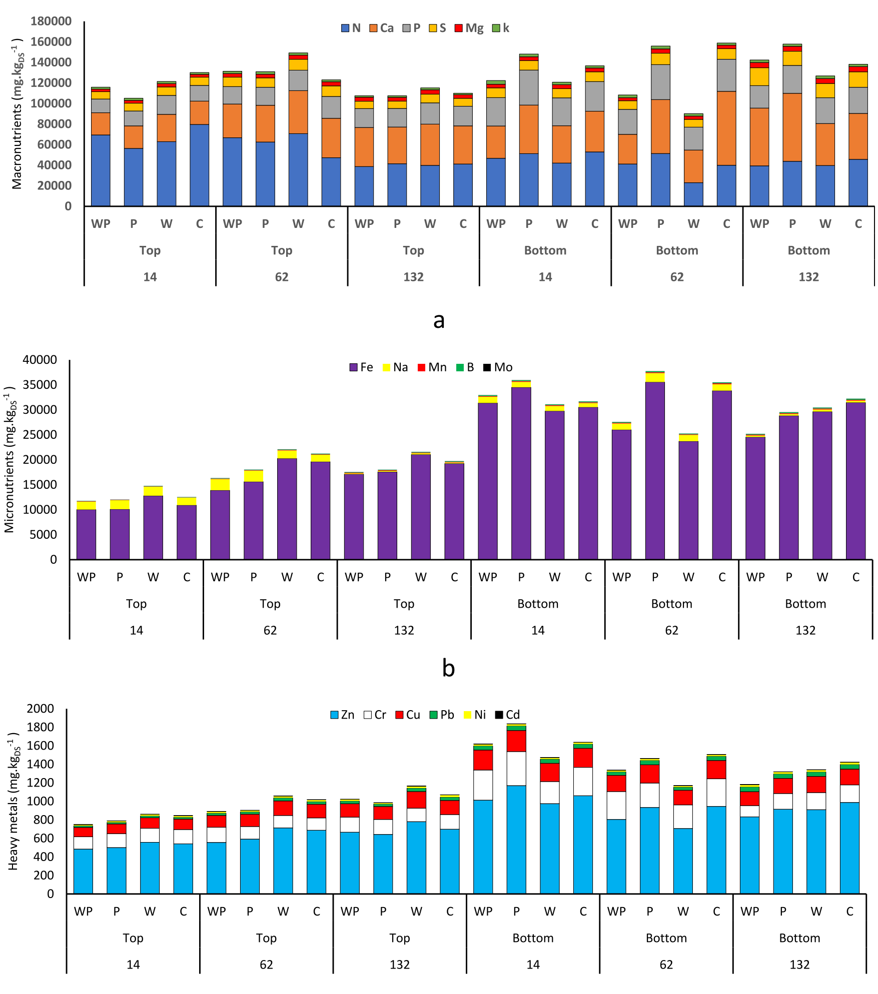

Fig. 6. Residual sludge quality in the top and bottom layers for 14, 62, and 132 days of final resting a) macronutrients b) micronutrients c) heavy metals.

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 also be 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 from day 14 to 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 (25,185 mg.kg_{DS}^{-1}) was 22 % lower than that of the C unit (32,218 mg.kg_{DS}^{-1}). The P and W units exhibited lower final cumulative masses (29,531 and 30,451 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, the cumulative mass in the top layers 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 (Fig. 6c). Thus, the WP unit had the lowest availability of heavy metal in the bottom layers, potentially highlighting 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, respectively. Based on previous research, bioremediation by earthworms in the STRB system likely occurred through several mechanisms, including bio-accumulation, metal binding, improved aeration of residual sludge, microbial activity, and biological uptake. The enhancement of residual sludge structure and worms transforms toxic elements like heavy metals into less toxic forms and immobilizes them within the residual sludge particles (Chen and 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 and Tsihrintzis, 2012b).

Furthermore, the integration of a turf layer to accommodate earthworms within the STRB system could potentially influence the quality of the residual sludge. The physical properties of the turf, including its porosity, texture, and material composition, could create a favorable environment for earthworm habitation and activity. The turf layer had 70% organic matter, which not only supports a thriving earthworm population but also could enhance the biodegradation of the residual sludge. Gholipour et al. (2024b) have highlighted another beneficial aspect of the turf layer, noting its effectiveness in increasing evapotranspiration by 46%. This increased water loss through evapotranspiration could be advantageous for sludge dewatering and stabilization processes. However, it is important to consider that a potential shortage of water within the turf layer could have implications for the earthworms' metabolism and overall activity. Such changes in earthworm behavior could, in turn, affect the quality of the residual sludge.

During the one year of feeding, it was found that seasonal variation, including precipitation and temperature, influenced the dewatering efficiency of which DS and VS of the residual sludge layer changed from 3 to 90% and 49–79%, respectively (Gholipour et al., 2024b). These variations correlated with precipitation with DS and VS reduced during the wet season and increased in the dry season. The results of the drained water quality analysis indicated that the drained water varied with precipitation, improving water quality due to dilution with rainfall while the amount of drained water increased (Gholipour et al., 2024a). These changes in the residual sludge and drained water quality could potentially followed by changes in the nutrients and heavy metal compositions of the residual sludge during the feeding period. The final resting was without precipitation; thus, there was no effect of that on the residual sludge. However, several mechanisms during the feeding period, including absorption, chemical precipitation, infiltration, percolation, and leaching, could change the nutrients and heavy metal compositions of the residual sludge. In addition, seasonal variation could also influence microorganisms, flora and fauna, and abiotic components, impacting the residual sludge layer compositions. The transformation of elements and heavy metals could involve changes in the speciation and concentration of metals, influenced by the reed bed's microbial communities, plant uptake, and abiotic conditions (Ma et al., 2020). Heavy metals could undergo valence state changes, affecting their solubility and bioavailability (Stefanakis and Tsihrintzis, 2012b). The reed bed ecosystem, including plants, microorganisms, and abiotic components, plays a pivotal role in immobilizing and detoxifying heavy metals, thereby improving the sludge's suitability.

On the other hand, the pH of the system could also influence the solubility and bioavailability of heavy metals, which in turn affects their mobility and potential for uptake by plant roots (Ma et al., 2020). Changes in pH could alter the speciation of metals, affecting their availability for plant absorption and microbial interaction (Chen and Hu, 2019). This could have important implications for the overall treatment efficiency, as it can either enhance or impede the removal of heavy metals from the sludge. Higher concentrations of heavy metals could lead to toxicity symptoms in the biomass, reducing the plants' capacity for metal uptake and affecting their role in the treatment process (Stefanakis and Tsihrintzis, 2012b). The root systems of the reeds provide a substantial surface area for microbial colonization, crucial for the biodegradation of organic pollutants and the transformation of heavy metals (Ma et al., 2020). The biomass can serve as both a reactor for these microbial processes and a sink for heavy metals, aiding in their long-term stabilization within the system (Ma et al., 2020).

Additional information including a comparative analysis with both national and international standard limits and insights from previous research can be found in the supplementary materials. It indicated that the final residual sludge consistently fulfills the standard limits outlined in national and international regulations.

In conclusion, the residual sludge can be deemed safe for reuse because of its heavy metal content. 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} average in the previous studies for a planted unit without earthworms (Kolecka and Obarska-Pempkowiak, 2013; Stefanakis and Tsihrintzis, 2012b). The variability in heavy metal masses across different studies can 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 of sewage sludge samples.

The results of microbiological assessments showed a reduction in *E. coli* and fecal coliform levels after 62 days of the final resting period (Fig. 7). This reduction was consistent across all units, resulting in an average 2-log decrease (99 %) from the top to the bottom layers. In

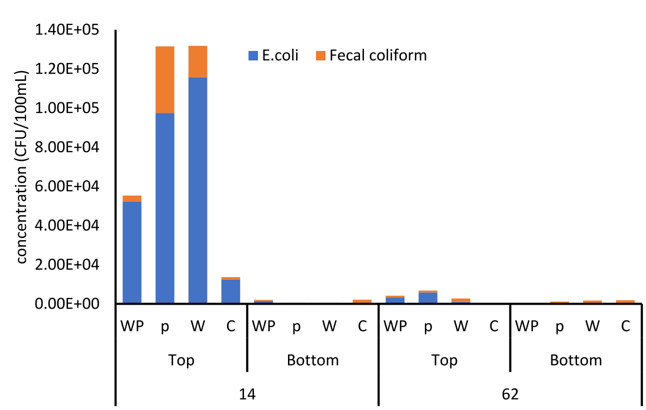

Fig. 7. *E.coli* and Fecal coliform in the top and bottom layers of the residual sludge for the 14 and 62 days of final resting.

summary, the concentration of both *E.coli* and fecal coliform dropped to below 1000 CFU.100 mL^{-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).

After 62 days of resting, there was an average removal of 3.7 and 2.7 logs 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.100 mL^{-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. *Salmonella* was detected in the top layers at day 62 for all units. The persistence of *Salmonella* in the residual sludge layer may be attributed to several factors including survival mechanisms that enable them to endure adverse conditions, such as desiccation and environmental stress (Chaudhari et al., 2023). They can form spores or persist in protective environments, allowing 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.

3.6. 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 (Fig. 8a). In Fig. 8b, construction costs for the 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 electromechanical and electrical installations (16.51%), W-STRB for 2000 PE would cost 236,286 euros. This indicates that the W-STRB scenario cost 47% greater than the centrifugation scenario in the initial year.

In the centrifugation system, personnel costs represented 5.43 % of the total expenses, material replacement costs were 2.00 %, pump electricity was 0.14 %, and centrifuge electricity was 6.64 %. W-STRB systems bear 2.54 % for O&M and 2.00 % for personnel in total. In addition, the results showed that personnel costs and O&M expenses constituted 66.31 and 33.69 % for the centrifugation (24,427 euros) and 44.07 and 55.93 % for W-STRB scenarios (15,269 euros), respectively. This indicates that the W-STRB scenario had 37 % lower personnel and O&M costs compared to the 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 the 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 earthworms 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 applications showed that centrifugation and W-STRB would cost 167 and 183 €.PE^{-1}, respectively.

4. Implication of the study

The study's findings revealed a diverse range of micro and macronutrients, 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 and the potential cost-effectiveness of W-STRB. 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 and Azapagic, 2018). In addition, the STRB system necessitates energy input for the feeding process, which is executed biweekly. The duration of each feeding depends on the sludge load and the area of the STRB. (Brix, 2017). STRB system offers a more energy-efficient alternative to traditional centrifugation methods for sludge dewatering. Unlike centrifugation, which requires continuous energy for both dewatering and operating process equipment daily, STRB requires significantly less energy, as it is primarily needed for the biweekly feeding process. This reduced energy consumption, coupled with the STRB's high dewatering efficiency, increases DS, and decreases VS, promoting enhanced sludge stabilization. The operational strategy of the STRB system, involving continuous loading, dewatering, and residual sludge storage, is effective in managing and optimizing the sludge treatment process. Gholipour et al. (2024a, 2024b) have conducted comprehensive analyses of the water balance and the treatment of drained water within the STRB system, providing valuable insights into its functionality and efficiency. Furthermore, the STRB has demonstrated resilience in handling high sludge loads in previous full-scale systems, as reported by Nielsen

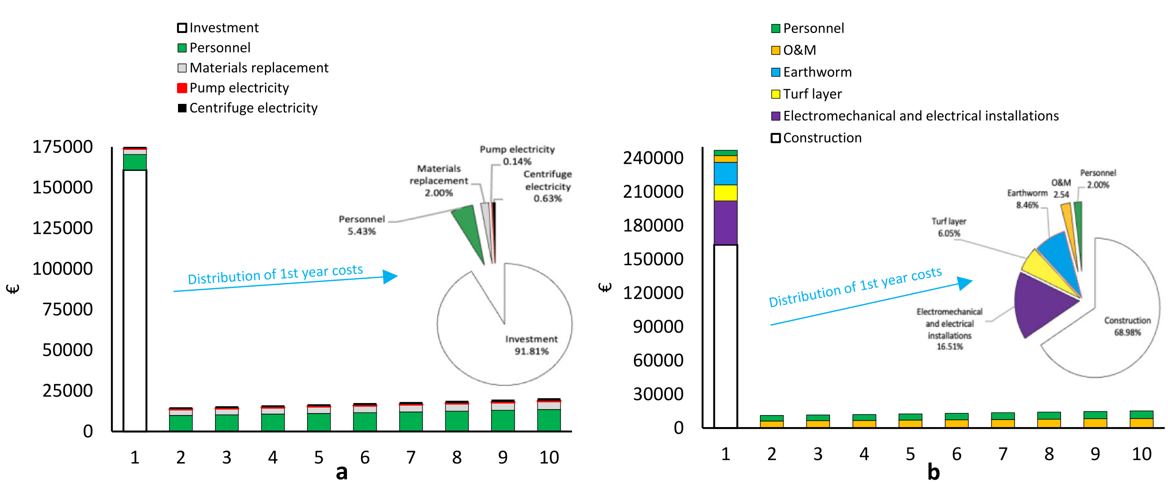

Fig. 8. Cost estimation along five years of operation for a) Centrifugation, b) W-STRB scenarios.

(2023). It has shown the capability to effectively manage hydraulic shocks caused by stormwater and increased sludge generation without compromising its dewatering efficiency, as observed by Uggetti et al. (2010). This robustness underscores STRB's potential as a reliable and sustainable solution for sludge treatment and resource recovery in various operational conditions (Uggetti et al., 2010). Beyond cost savings, adapting W-STRB 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 and Alalayah, 2017).

Integrating earthworms into STRB offers a novel approach to enhance dewatering efficiency and drained water quality (Gholipour et al., 2024a, 2024b) 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 and 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 and Bulkeley, 2020). Scaling up earthworm-assisted reed bed systems to larger sewage treatment facilities could enhance the general efficiency of traditional sludge management practices, offering a sustainable and cost-effective solution on a larger scale (Nielsen, 2007, 2023).

5. Conclusions

A pilot-scale study conducted in the Mediterranean region explored the use of worms in sludge treatment reed beds 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. The order of significance for nutrient accumulation was found to be N > Ca > P > K > S > Mg for macronutrients and Fe > Na > Mn > B > Mo for micronutrients. The study also assessed heavy metal accumulation, with the significance order being 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 being 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 initial year costs 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, the application of sludge treatment reed beds is supported by the improved residual sludge quality and offers potential for resource recovery. Economic feasibility depends on balancing transportation, treatment costs, and the initial investment against long-term operational savings. Future work should explore the system's scalability and sustainability, focusing on optimizing the W-STRB system for efficiency, investigating various earthworm species, and feeding regimes, and elucidating the microbial mechanisms underlying sludge stabilization and heavy metal immobilization. This comprehensive approach will pave the way for the widespread adoption of sludge treatment reed beds as a sustainable waste management solution.

CRediT authorship contribution statement

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

Acknowledgment

This Ph.D. study (doctoral Grant SFRH/BD/151361/2021) was funded by national funds through FCT – Fundação para a Ciência e a Tecnologia, I.P., under the projects UIDB/04129/2020 of LEAF-Linking Landscape, Environment, Agriculture and Food, Research Unit and LA/P/0092/2020 of Associate Laboratory TERRA.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jenvman.2024.122772.

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