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A green approach to milkmaid wastewater: Evaluating up-flow anaerobic sludge blanket-biofilter effluent for fodder shrubs cultivation and drought resistant

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ABSTRACT

The agricultural industry is highly dependent on using water quality due to challenging soil and climatic conditions. Hence, this study investigated the potential reuse of treated effluents from a pilot-scale coupled UASB-Biofilter system for the cultivation of drought-resistant fodder shrubs using wastewater generated from the buffalo milkmaid process. The milkmaid wastewater was treated in a sedimentation tank, with the aim of removing large suspended solids and settleable organic matter, and then in an up-flow anaerobic sludge blanket (UASB). The UASB effluent was treated in a biofilter. A field experiment was carried out at the Experimental Farm of Veterinary Faculty, Suez Canal University, from March to October 2023 to study the effect of different quality of treated buffalo milkmaid wastewater with COD (Null, 1000 ± 28, 130 ± 33, 520 ± 27, 100 ± 30 mg.L^{-1}, respectively)on the growth of *Acacia saligna* and *Moringa oleifera* shrubs. Split plot designed with five Treatments: Control (freshwater) (T1), sedimentation tank effluent (T2), Up-flow Anaerobic Sludge Blanket (modified UASB with rice straw biochar) effluent unit (T3), conventional UASB effluent unit (T4), and biofilter effluent unit (T5), of each species. Vegetative growth, the biomass of shrubs, and the soil NPK, organic matter, and organic carbon were determined. *Moringa oleifera* shrubs showed higher biomass (2330.3 g)and nitrogen content (62.02 mg kg^{-1}) in soil compared to *A. saligna*. UASB treatments (T3 and T4) promoted the best

Abbreviation: A-1, Acacia treated by fresh water Control; A-2, Acacia treated by sedimentation tank effluent; A-3, Acacia treated by Up-flow Anaerobic Sludge Blanket modified UASB with rice straw biochar effluent unit; A-4, Acacia treated by conventional UASB effluent unit; A-5, Acacia treated by biofilter effluent unit; M-1, Moringa treated by fresh water Control; M-2, Moringa treated by sedimentation tank effluent; M-3, Moringa treated by Up-flow Anaerobic Sludge Blanket modified UASB with rice straw biochar effluent unit; M-4, Moringa treated by conventional UASB effluent unit; M-5, Moringa treated by biofilter effluent unit; BOD, Biochemical oxygen demand; CA, Cluster Analysis; Ca^{+2}, Calcium; Cl^{-1}, Chloride; COD, Chemical oxygen demand; EC, Electric conductivity; HCO_3^-, Bicarbonate; K^+, Potassium; LSD, Least significant difference; Mg^{+2}, Magnesium; N, Nitrogen; Na^+, Sodium; NH_3, Ammonia; Nitrite, NO_3; Nitrate, NO_2; NPK, Nitrogen, phosphorus, and Potassium; OC, Organic carbon; OM, Organic matter; P, Phosphorus; PH, Hydrogen ion concentration; PVC, Polyvinyl chloride; SO_4^{-2}, Sulphate; S, Supplementary; SI, Supplementary Information; T, Treatments; TBMW, Treated Buffalo Milkmaid Wastewater; TDS, Total dissolved solids; TK, Total Potassium; TN, Total Nitrogen; TP, Total Phosphorus; TSS, Total suspended solids; UASB, Up-flow Anaerobic Sludge Blanket; VSS, Volatile suspended solids.

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growth, with treated buffalo milkmaid wastewater increasing nitrogen concentrations in the soil. Overall, UASB proved to be the most effective treatment for enhancing growth in *A. saligna* and *M. oleifera* shrubs in sandy loam soil. This study evaluates the performance of a pilot-scale UASB-Biofilter system for treated effluent reuse in drought-resistant fodder shrubs cultivation. It also conducts a bibliometric analysis using VOSviewer and RStudio 4.4.1, analyzing trends in wastewater reuse research from 1999 to 2024 based on data from the Web of Science. The review provides insights into the evolution of wastewater reuse in sustainable agro-hydrology and highlights emerging research areas for further investigation.

1. Introduction

Water scarcity is expected to worsen due to climate change, a growing global population, and increasing water demands and urbanization. Particularly in burgeoning and developing economies, the quality of freshwater resources is a significant concern, alongside issues of quantity (Zinia, Kroeze and Sustainability, 2015). To protect the environment and public health and to preserve freshwater sources for vital potable uses, it is necessary to use treated water with low to intermediate water quality for non-potable uses, such as industrial cooling, recharging aquifers, and irrigation of landscapes and agricultural areas (Maryam and Büyükgüngör, 2019).The ultimate goal of treating wastewater is to create high-quality water that can be reused and utilized again (e.g., for irrigation, potable water, and industry). The conversion of these substances may take place in an environment containing Oxygen (aerobic)or free Oxygen (anaerobic) (Almuktar, Abed and Scholz, 2018).A study by (Razzaghi, Habib and Ghorbani Dashtaki, 2016) examined the long-term effects of wastewater on the soil's hydraulic and physical properties.They compared the effects of several infiltration models. In their study, (Urbano et al., 2017) examined the modifications in the physical, chemical, and microbiological properties of a Dusky Red Latosol soil, as well as the crop yield and lettuce quality, following the use of treated wastewater for irrigation. Numerous factors were analyzed in the irrigation wastewater study, including pH(7.60 $\pm$ 0.11), electrical conductivity (6.3 $\pm$ 0.03dSm^{-1}), magnesium (83.8 $\pm$ 0.02 mg L^{-1}), calcium (95.8 $\pm$ 0.03 mgL^{-1}), potassium (38 $\pm$ 0.02 mgL^{-1}), nitrate (15.9 $\pm$ 0.05 mgL^{-1}), chlorate (1999 $\pm$ 0.04 mg L^{-1}), and chemical and biochemical oxygen demand (73 $\pm$ 0.11 and 22 $\pm$ 0.04 mgL^{-1}, respectively), (Bedbabis et al., 2010). The Up-flow Anaerobic Sludge Blanket UASB reactor, which functions as an anaerobic digester for wastewater treatment, operates using a vertical up-flow pumping mechanism. This mechanism uses a layer of anaerobic sludge to pass the liquid substrate, which may consist of wastewater or growth media. Microorganisms within the sludge layer form a microbial consortium that actively degrades digestible organic components into simpler chemical compounds. Anaerobic digestion, which falls under the purview of wastewater treatment, aims to recover energy and mineralize organic compounds in their entirety simultaneously (Pererva, Miller and Sims, 2020).It is well established that conventional UASB systems do not effectively remove nitrogen or phosphorus compounds. Therefore, post-treatment processes are typically required to meet discharge or reuse standards (Cao et al., 2024; Mainardis, Buttazzoni and Goi, 2020; Mazaheri and Doosti, 2024).In up-flow anaerobic sludge blanket (UASB) reactors,(Wang et al., 2021)evaluated the efficacy of biochar in withstanding high organic input shock. In contrast to the irreversible acidification requirements of conventional UASB reactors, the investigators found that introducing biochar accelerated the growth of an enriched microbiota. It contributed to the rapid recovery of microbial activity, enhancing metabolic and physiological functions while maintaining high levels of organic matter degradation and methane production. (Elbana et al., 2019) showed that small, simple equipment that is easy and cheap to run, maintain, and replace is often used in decentralized systems like UASB. UASB technology faces challenges in meeting environmental regulations with its effluents (Musa et al., 2019). Hence, it is crucial to implement biofilters for post-treatment. Biofilters, also known as biological filters, are a technology that breaks down pollution in air, water, and wastewater treatment plants by attaching biomass to a media (Da Silva and De Campos, 2021). They are models of natural systems that eliminate various types of pollution, including organic matter, suspended solids, natural organic matter, and organic micropollutants. Biofiltration systems are favored in wastewater treatment for their uncomplicated operation, strong durability, and minimal energy use. Common biofiltration tools used in wastewater treatment include membrane bioreactors, trickling filters, and aerated biological filters. The properties, composition, and community structure of microorganisms in the biofilm that forms on the medium determine how well biofiltration systems work (Yogalakshmi, Sharma and Mittal, 2022).A valuable strategy for increasing water resources, using effluent for irrigation can present agricultural difficulties due to its quality and conditions (Habibi, 2019).In contrast, soils in semi-arid regions exhibit a notably low level of nutrient availability and organic matter concentration(García, Hernández and Costa, 1994). In addition to delivering organic matter and vital nutrients, effluent irrigation conserves water and nutrients and reduces water pollution (Khalid et al., 2018). The treated effluent can be distinguished from freshwater based on its salinity, alkalinity, pH, micronutrient density, food items, dissolved organic matter, and total suspended particles. However, it is important to note that the treated effluent is of lower quality compared to freshwater (Halliwell, Barlow and Nash, 2001; Meni Ben-Hur, 2002). Without a doubt, the soil has a dynamic and complex system of many factors, including the use of wastewater for irrigation, which can lead to long-term changes in its biological, chemical, and physical characteristics (Ofori et al., 2021). Research studies have generally shown that the use of treated sewage is widespread globally. However, what is crucial is the implementation of effective solutions, how they are utilized, and their location based on the specific items involved and the physical and chemical characteristics of the soil (Fonseca et al., 2007).According to the United Nations COMTRADE database on international trade, Egypt's Imports of Residues, waste from the food industry, and animal fodder were US$320.13 million in 2022. Several authors have incorporated fodder bushes into diets for ruminants to mitigate forage shortages during dry seasons in marginal fields of semi-arid and tropical regions (Azocar et al., 1996; Devendra, 1989; Guillermo, 1990; Ben Salem, 1998; Nefzaoui, Salem and Ben Salem, 1996). Furthermore, it is common practice to use shrub species as inexpensive substitute feedstuffs, and several publications have documented the animals' favorable reaction to this approach(Khalili and Varvikko, 1992; Muinga et al., 1995; Richards et al., 1994). *Acacia saligna* (Labill.) is a woody species belonging to the family (Fabaceae). It is distributed in the world's warm regions, including Africa; fast-growing shrub produces seeds with a considerable amount of protein (18.25–35.5 %) (Adiamo et al., 2020).In North Africa and the Middle East, *A. saligna* is commonly grown for its many uses, such as fodder, fuel wood, sand stabilization, and windbreak(Turnbull, 1976). As a source of protein, it is fed to sheep and goats in the form of fresh or dried pods, seeds, phyllodes, and young shoots. It is also non-toxic and delicious to these animals (Melgar et al., 1979). According to (Woodward and Reed, 1989), the phyllodes are unsuitable for ruminants. This feed is precious seasonally when other forage is scarce. *Moringa oleifera*Lam., a woody shrub, is a highly valued plant with an impressive range of medicinal uses and high nutritional value, belonging to the family Moringaceae, which comprises 13 species. When farmed, plants with minimal nutrient requirements require little to no fertilizer and little to no insecticides (Tilman, Reich and Knops, 2006). Ghorab et al. (2016) reported that *M. oleifera* yielded the highest values of fruits, seeds, and oil, at 2111, 621, and 82 kg.ha^{-1}, respectively, after two years with a 0.5 m × 0.5 m spacing in the Ismailia region.

Moringa oleifera shrub holds significant promise as a plant with leaves that can improve farmers' incomes in tropical regions (Gedefaw, 2015). Cattle readily consume the leaves, seeds, and foliage of the Moringa plant as part of their daily diet. Supplementing milk with *M. oleifera* has been reported to increase the milk's yield and composition. (Babiker et al., 2017; Brar et al., 2022; Kholif et al., 2018).

This study aims to (i) evaluate the performance of a pilot-scale UASB-Biofilter system in treating buffalo milkmaid wastewater; (ii) assess the effect of treated effluents with varying COD levels on the growth and biomass of *A. saligna* and *M. oleifera* shrubs, as well as on soil nitrogen, phosphorus, potassium, organic carbon, and organic matter content; and (iii) conduct a bibliometric analysis using VOSviewer and RStudio 4.4.1 to explore global research trends in wastewater reuse for sustainable agricultural applications between 1999 and 2024.

2. Materials and methods

2.1. Site of experimental farm

This research was undertaken in the Experimental Farm of the Veterinary Faculty at Suez Canal University. Fig. 1 illustrates the location of the pot experimental farm and UASB. The experimental site is located in the governorate of Ismailia, in the eastern region of the Arab Republic of Egypt (El Shahawy et al., 2024). It is positioned in the middle section of the Suez Canal. At 30° 37'32.9 N latitude and 32° 16'05.3 E longitude from March 2023 to October 2023.

2.2. Experimental setup

The land was cleared of all debris, and the ground was leveled to remove invasive plants, such as *Phragmites australis*. Additionally, lizards and insects were also removed. To link the units later, construction first involved creating a brick wall parallel to the farm's wall. A 1.9 cm-tall fixed base was also constructed for an elevated feeding tank (1 m^3 capacity). A submerged pump (JET HVT-750F, 1 HP) was installed at the bottom center of the ground tank, which has a capacity of 8 m^3 . The tank was repaired and maintained. Moreover, a screen with coarse apertures was added to the tank's influent to catch larger particles and shield the submerged pump.

Fig. 1. Experimental farm location.

2.2.1. Reactors installation

The UASB reactors in this study, seen in Supplementary File (SI) (Figure SI1), were constructed using Cylinders made of polyvinyl chloride (PVC) with an internal diameter of 10 cm and an effective height of 150 cm; one to examine the impact of incorporating supporting media (modified UASB reactor) and the other one (Conventional UASB reactor) will be used as a control. Sewage was transferred to the reactors from a ground storage tank by pumping (using a submerged pump, JET HVT-750F, 1 HP). No external sludge inoculation was applied in the UASB reactors. Instead, the sludge biomass developed naturally during the initial operation phase as the reactors reached a steady state. The microbial consortium originated from the influent wastewater, which facilitated the natural formation of sludge within the system. The flow rate was 30 liters per day, while maintaining a hydraulic retention time (HRT) of 4 h. The supporting media used was rice straw biochar inoculated only at the modified UASB reactor R_2 to enhance the efficiency of the biological process. Rice straws were cleaned and cut into 1–1.5 cm pieces before being oven-dried at 110 °C for 4 h. The pyrolysis test set comprises an oil shower radiator, temperature regulator (thermometer), warming Pyrex jar, three different ways connector, cold water condenser, and biodiesel getting Pyrex flagon. Such a rig was designed and constructed for pyrolysis at a certain temperature (Abu-Elyazeed, 2015). Biochar is prepared by slow pyrolysis since it produces more biochar and has a higher total carbon content (Cai et al., 2020; Tao et al., 2020). The sample was then heated to 400 °C for 1 h in a pyrolysis reactor before being maintained at 700 °C for 1 h (Sangon et al., 2018).

The UASB effluent was then passed into two concrete filters, 2 m × 1 m × 2 m in length and 50:1 slope; 0.3 m width, and 0.5 m thickness. Both filters were filled with limestone and ceramic fragments about 10–15 mm in diameter, reaching a height of 0.3 m. The biofilter was installed with a spacing of 20 cm between each planting. However, to improve biological treatment efficiency, the filter was later inoculated with *Streptomyces*, thereby transforming it into an active biofilter. The inoculation promoted microbial colonization, enhancing the degradation of organic pollutants and contributing to the overall treatment process. The helophyte *Phragmites australis*, often known as the common reed, was used for this purpose (El Shahawy et al., 2024). The mature rhizome-shoot stems of reeds were taken from a nearby surface water farm. The biofilter was initially supplied with fresh water for 3 months, after which it was transitioned to Buffalo Milkmaid Wastewater to allow the plants to adapt and stabilize the bed.

To assess the efficiency of the anaerobic reactors, the concentration of various parameters will be measured in both the influent and effluent samples. These parameters include COD, BOD, TSS, TDS, colour, turbidity, pH, TN, TP, and alkalinity. The measurements will follow the standard techniques established by the American Public Health Association for examining water and wastewater (APHA, 2017; Collivignarelli et al., 2021).

2.2.2. Seedling sources

Acacia saligna and *Moringa oleifera* seedlings, all one year old, were sourced from private nurseries in Ismailia.

2.2.3. Analysis of soil

Physiochemical and mechanical qualities of the soil were measured before the experiment, as shown in Table 1, then after the experiment, available (nitrogen, phosphorus, potassium, organic matter (OM), and organic carbon (OC) were measured based on the procedure of (Alexanderl, 1949).

2.2.4. Treated buffalo milkmaid wastewater

Samples T1, T2, T3, T4, and T5 were collected from various points within the treatment system at the Experimental Farm of the Veterinary Faculty, Suez Canal University. These samples correspond to Tap Water, the Constant Head Tank, the Modified UASB, the Conventional UASB, and the Biofilter Effluent, as illustrated in Fig. 2. Analysis of treated buffalo milkmaid wastewater is shown in Table SI1. The measurements presented were taken at the initial stage of the experiment, specifically before the application of treated wastewater for irrigation, to establish baseline conditions for comparison throughout the experimental period. Table SI2, titled "The geometric and operational conditions of the tested systems," highlights the differences between the conventional UASB, which operates as a control unit, and the modified UASB, which is inoculated with rice straw biochar.

2.2.5. Pots experiment

Seedlings of the two studied shrubs, *A. saligna* and *M.oleifera,* were grown in the field beside UASB units. Fifteen seedlings of each species were planted in fired clay pots (35 cm diameter and 35 cm depth) filled with 10 kg of sandy loam soil mixed with 50 g of peat moss, approximately 2.5 cm from the rim, on 15 March 2023. One seedling was grown in each pot, and surface irrigation was handled with one liter of fresh water at approximate field capacity twice weekly (ALHaithloul et al., 2022). After one month, on 15 April 2023, three replicates of each species were irrigated twice weekly with one liter of four treated buffalo milkmaid wastewater and control (freshwater) for 6 months (till 15 October 2023).

2.2.6. Measurements of growth

Four times during the following months-April, June, August, and October-depositions of shrub height (in meters) and base diameter (in centimeters) were conducted (Abrha, Hintsa and Gebremedhin, 2020). Following this, when the trials were over in October 2023, the total weights of raw and desiccated samples were determined. A meter rule was used to determine the height of the shrubs from the collar region to the apical bud. A vernier caliper was employed to ascertain the collar's diameter (Beji, 2023).

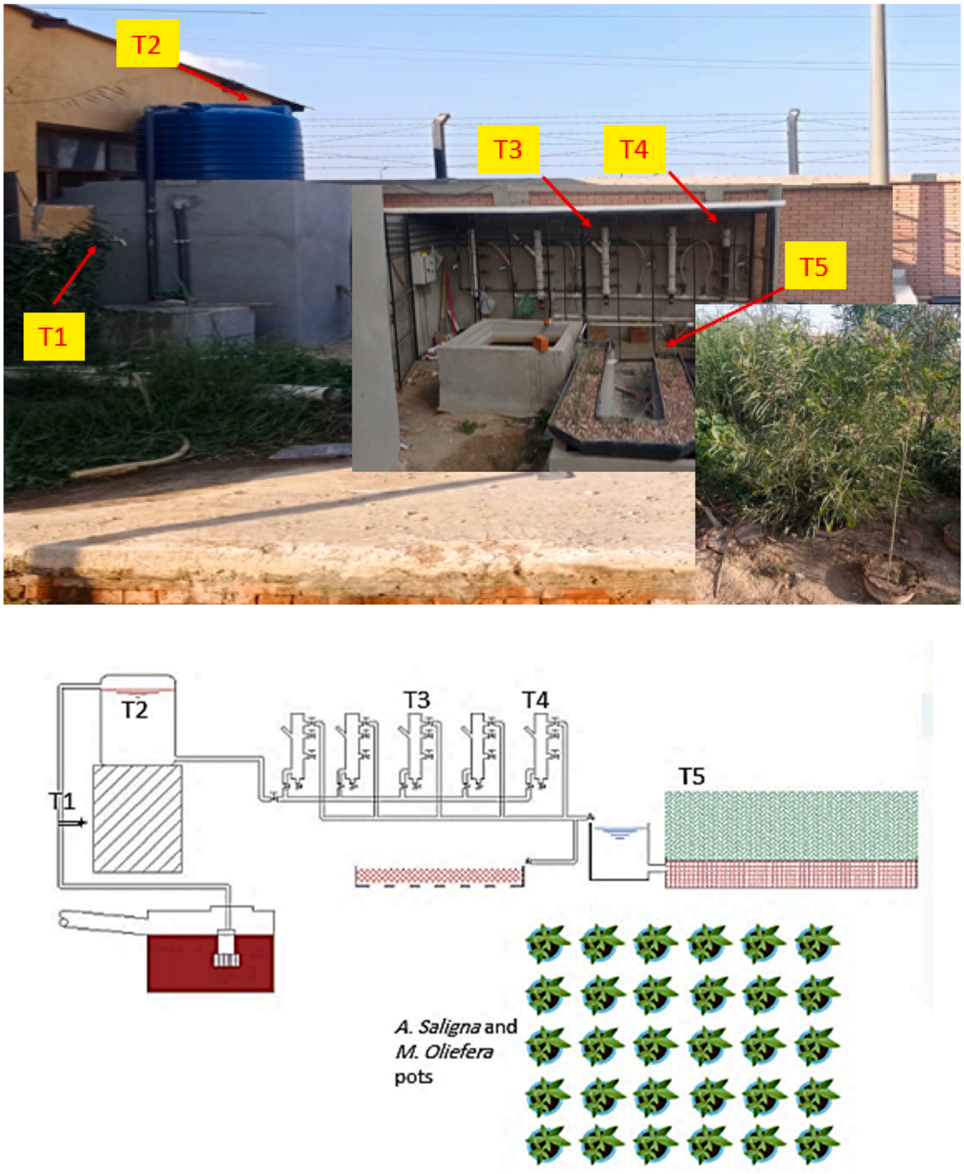
2.2.7. Shrubs Biomass

Three shrubs from each treatment were collected at the end of the experiment to assess the combined fresh weight of the root and

Table 1
Soil characteristics before *A. saligna and M. oleifera* planting in Marsh 2023.

Soil	**pH**	**EC dSm^{-1}**	**Cations (meq L^{-1})**				**Anions (meq L^{-1})**			**OM %**	**OC %**	**Pb ppm**	**Cd ppm**	**Available mg Kg^{-1}**		
			Ca^{+2}	Mg^{+2}	Na^{+}	K^{+}	HCO_3^-	Cl^-	SO_4^{-2}					N	P	k
Paste extract	7.81	2.95	10.2	4.6	14.2	0.5	4.5	17.7	7.3	0.07	0.04	ND	ND	28	20.8	95

Soil texture: Sandy loam [Coarse sand (39.2 %), Fine sand (35.3 %), Loam(7.80 %), and Clay (17.7 %)]



T1: Tap Water **T2:** Sedimentation Tank Effluent

T3: Modified UASB Effluent **T4:** Conventional UASB Effluent

T5: Biofilter Effluent

Fig. 2. a) Experimental farm, and b) Flow chart of the treatment system at the experimental farm.

vegetative sections. During the experiment, it was noticed that *A. saligna* shrubs produced vegetative growth only; however, *M. oleifera* shrubs gave vegetative and reproductive growth, so the weight of the fruits is added to the shoot's fresh and dry weight. The delicate roots were meticulously extracted from the soil substrate and thoroughly washed to maintain their integrity. Every shrub component was subjected to oven-drying at a temperature of 70°C until it reached a consistent weight. Using an electronic weighing scale, the biomass of the shoots, roots, and overall fresh and dry materials was determined in grams per shrub (Achten et al., 2010).

2.2.8. Data analysis with statistics

A split-plot design was employed for the statistical analysis; the main plot included shrub species, while the subplot included freshwater (Control) (T1) and treated buffalo milkmaid wastewater; sedimentation tank (T2), modified UASB unit (T3), conventional UASB unit (T4) and biofilter unit (T5) each treatment had three replicates. Furthermore, the F-test was employed to compare the methods by which each component interacts with the others. According to (Ridgman, 1990), the SAS 9.1 system software was employed to identify the least significant differences (LSD 0.05) with a 95 % confidence level. Figure SI2 shows the experimental process in this study. Minitab software (version 21) was used to determine Correlogram, PCA (Principal Component Analysis), and

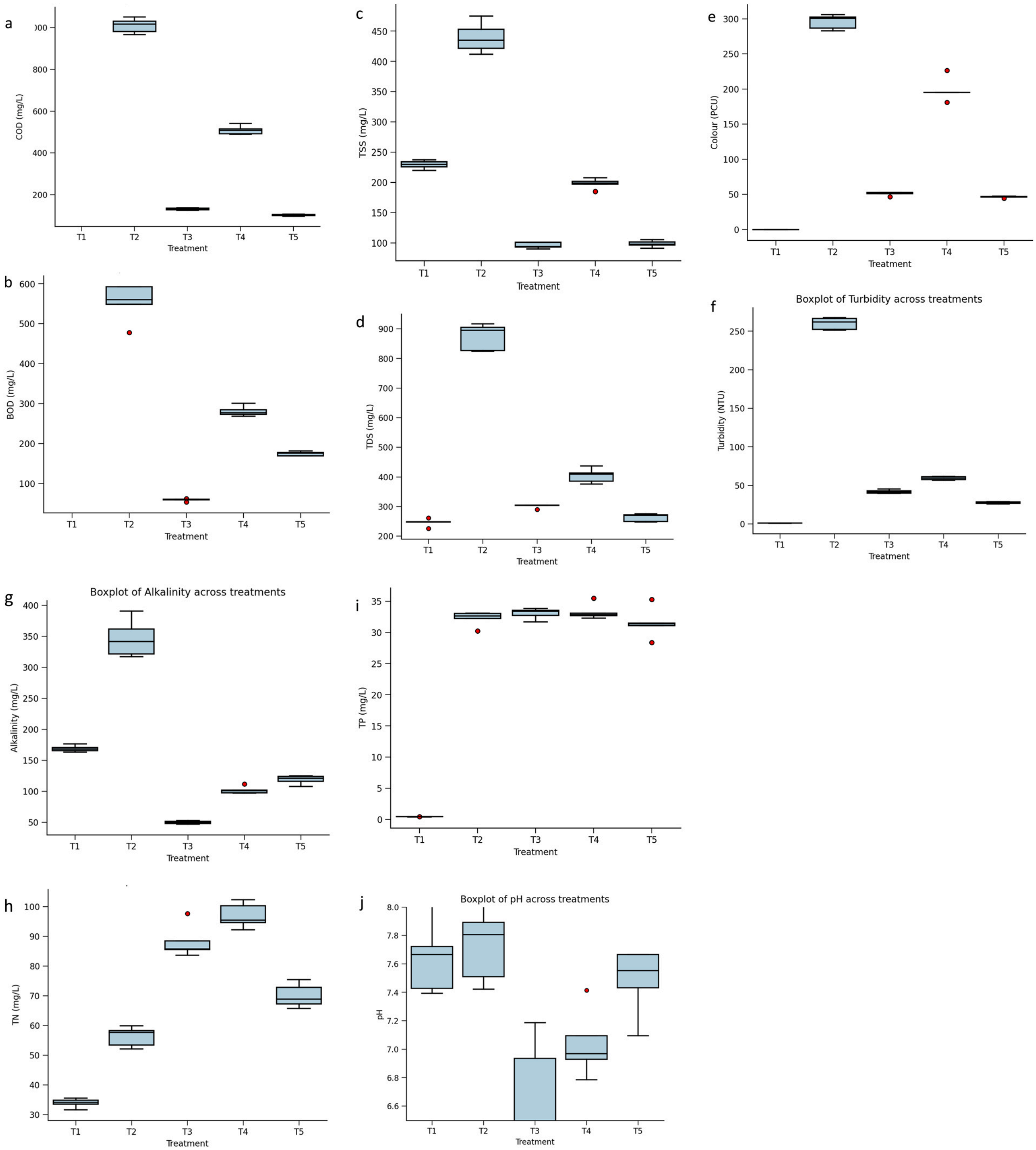


Fig. 3. a) The Distribution of COD treatment, b) The Distribution of BOD treatment, c) The Distribution of TSS treatment, d) The Distribution of TDS treatment, e) The Distribution of Colour treatment, f) The Distribution of Turbidity treatment, g) The Distribution of Alkalinity treatment, h) The Distribution of TN treatment, i)The Distribution of TP treatment, j)The Distribution of pH treatment.

Cluster Analysis (CA).

2.2.9. Bibliometric analysis

A study was conducted using articles accessible on the Web of Science platforms. The following keyword combination for the Web of Science database was created using "AND" and "OR": ("Irrigation" OR "Treated" OR "Buffalo" OR "Milked" OR "Wastewater" OR "Fertilizer" OR "*Oleifera*" OR "*Moringa*" OR "Vegetative" OR "*Acacia*" OR "*Saligna*" OR "Pots" OR "Experiments" OR "Up-flow Anaerobic Sludge Blanket" OR "soil" OR "fresh" OR "water" OR "effluent" OR "Biomass" OR "biofilter" OR "nitrogen" OR "rice straw" OR "biochar" OR "concentrations" OR "shrubs" OR "wetland"). The search phrases that were used were "Topics," "Titles," "Abstracts," and "Keywords."Every search result was assessed separately for its alignment with the main subject of the study. The following information was recorded for each article that was obtained from the Web of Science database: The publication year, annual average of an article's citations, article's publication journal, Each journal's H-index, concentration area, The co-occurrence of keywords, authors and collaborators, authors' and co-authors' national affiliation, highest cited articles, collaboration between journals and affiliations. The program "Bibliometrix R core team 2020" was employed to conduct the study (Aria and Cuccurullo, 2017). The software "RStudio 4.4.1" generated bibliographic maps and visualizations. VOS viewer was also utilized for mapping. Raw data were exported from bibliometric analysis and then redrawn in Microsoft Excel to create line curves and bar charts(Bhat et al., 2023).

3. Results and discussion

3.1. Efficiency of UASB-biofilter system

The efficiency of organic matter removal was assessed across the different treatment units. The modified UASB with rice straw biochar (T3) and the conventional UASB (T4) exhibited the highest COD and BOD removal efficiencies, achieving 93 % and 81 % COD reductions and 91 % and 75 % BOD reductions, respectively. The biofilter unit (T5) further improved the effluent quality, resulting in an additional 90 % reduction in COD and an 88 % reduction in BOD, indicating its role in polishing the wastewater.

In contrast, the sedimentation tank (T2) displayed significantly lower removal rates (COD reduction of 45 % and BOD reduction of 40 %), confirming its primary function in removing suspended solids rather than dissolved organic pollutants. Nutrient contents (NPK) were still within the Environmental Protection Agency (2017) and Nations (2000) levels in each unit of the UASB-Biofilter system, as shown in Table SI1. These results indicated that the modified UASB with rice straw biochar (T3) and conventional UASB (T4) led to the safer reuse of milkmaid wastewater with a high concentration of NPK for irrigation fodder shrubs.

3.2. Water quality analysis

The boxplot in Fig. 3 presents the distribution of ten key wastewater quality parameters COD, BOD, TSS, TDS, Colour, Turbidity, Alkalinity, TP, TN, and pH across four treatment stages: T2 (Sedimentation Tank Effluent), T3 (Modified UASB Effluent), T4 (Conventional UASB Effluent), and T5 (Biofilter Effluent). T2 consistently exhibits the highest values and widest variability for most parameters, indicating poor treatment efficiency and higher pollutant loads. In contrast, T3 and T5 show markedly lower concentrations and narrower interquartile ranges, reflecting more stable and effective treatment. COD and BOD levels drop sharply after T2, with T3 achieving the greatest BOD reduction and T5showing consistent COD removal. TSS and TDS follow a similar trend, with T5 outperforming others in reducing suspended and dissolved solids. While TP shows less dramatic differences, T5 slightly edges others in phosphorus removal. For TN, both T3 and T5 demonstrate significant reductions compared to T2 and T4. Regarding colour and Turbidity, T3 and T5 again achieve superior outcomes, with T5 slightly more effective in color removal and T3 excelling in Turbidity reduction. These results highlight the superior performance of the Modified UASB and Biofilter units, with T5 providing the most balanced and comprehensive contaminant reduction across the monitored parameters.

Table 2
The average shrub height and diameter for the species, treatments, and their interactions.

Species	Height (cm)			Diameter (cm)		
Treatments	***A. saligna***	***M. oleifera***	**Means**	***A.saligna***	***M. oleifera***	**Means**
T1	133 ef	137.5 e	135.25c	2.65 bc	2.41 cd	2.53 b
T2	181.5 cd	219 a	200.25 a	2.82 b	2.48 cd	2.64 b
T3	175 d	213.83 ab	194.41 a	2.44 cd	2.64 bc	2.53 b
T4	114.33 f	215 a	164.66 b	2.69 bc	3.33 a	3 a
T5	132 ef	195 bc	163.5 b	2.3 d	2.33 d	2.31c
Means	147.16 b	196.06 a		2.57 a	2.63 a	
LSD.0.05 Treatments	13.89			0.21		
LSD.0.05 Species	3.9			0.41		
LSD.0.05 Interaction	195			0.3		

For both species and treatments, means that are the same letter do not have any statistical significance.

3.3. Determination of vegetative growth under different treatments

Upon reviewing the statistical analysis, it is evident that there are considerable differences in the height and diameter parameters among the various buffalo milkmaid wastewater treatments and species. Furthermore, the data indicate that the interaction between treatments and species is diverse. The outcomes of the buffalo milkmaid effluent treatments, species, and their interaction as of the end of the experiments in October 2023 are detailed in Table 2. The results showed that the heights of the shrubs varied significantly under different treatments and their interaction (Acacia and Moringa Sp. and Treatments). Treated buffalo milkmaid wastewater (T2 and T3) recorded the highest significant values of height (200.25 cm and 194.41 cm, respectively), followed by T4 and T5 treatment (164.66 cm and 163.5 cm, respectively). However, irrigation by fresh water (T1) was 135.25 cm. These results indicated higher available nutrient content (NPK) and total dissolved solids in T2, T3, and T4 than in T1 and T5. On the other side, the growth limitation of the irrigation by T5 might be caused by the high pH (7.64 $\pm$ 0.1) as the increase of alkalinity 120 $\pm$ 4 mgL^{-1} that affected total dissolved solids, N, P, and K(260 $\pm$ 65, 70 $\pm$ 7, 31.4 $\pm$ 3and 11.73 $\pm$ 0.02 mg.L^{-1}, respectively) as shown in Table SI1. Soil pH affects nutrients available for plant growth. In highly alkaline soil, phosphorus and most micronutrients become less available. (Barrow and Hartemink, 2023)indicated that the effects of pH on nutrient availability are not solely caused by the effects on reaction with soils but are an interaction between these effects and the effects on the rate of uptake by plants. *M.oleifera* gave the highest height value (196.06 cm), followed by *A. saligna* (147.16 cm). Regarding the relationship between treatments and shrub species, *M. oleifera* provided the highest height value, and there was no significant difference between the T2, T3, and T4 treatments, as shown in Table 2.

On the other hand, shrub diameters were significantly different between treatments and the interaction between species and treatment. While there were no significant differences in diameter between the two shrub species, the T4 treatment gave the highest diameter (3 cm) compared to other treatments, and *M. oleifera* gave the highest diameter (3.33 cm) with the same treatment, as shown in Table 2. These results revealed that the T4 treatment is the best compared to other treatments. In addition, the results in Figures SI3

Table 3
Means of the investigated species' leaves, shoot, root, and total fresh and dry weight (g), as well as treatments.

Species	*A. saligna*	*M. oleifera*	Mean	*A. saligna*	*M. oleifera*	mean
Treatments	**Leaves Fresh w. (g)**			**Leaves Dry w. (g)**		
T1	1000 d	455 g	727.5 d	390.93	148.3 f	269.61c
T2	1230 b	850e	1040 b	469.38 b	323.05 d	396.21 a
T3	1565 a	810e	1187.5 a	586.55 a	215.14 e	400.84 a
T4	1272.5 b	780 e	1026.25 b	476.32 b	354.67 cd	415.49 a
T5	1140c	602.5 f	871.25c	400.5c	233.44 e	316.97
Means	1241.5 a	699.5 b		464.73 a	254.92 b	
LSD.0.05 Treatments	50.09			37.81		
LSD.0.05 Species	99.36			0.499		
LSD.0.05 Interaction	70.84			53.47		
	Shoot Fresh w. (g)			**Shoot Dry w. (g)**		
T1	2090 e	2485 d	2287.5 d	897.12 e	1123.99 cd	1010.55c
T2	2385 d	3190c	2787.5c	1034.78 dc	1367.5 b	1201.14 b
T3	4110 a	2307.5 d	3208.75 a	1819.3 a	1050.09 de	1434.69 a
T4	2462.5 d	3612.5 b	3037.5 b	1029.38 dc	1702.64 a	1366 a
T5	2350 d	2335 d	2342.5 d	1016.6 de	1216.45 bc	1116.52 bc
Means	2679.5 b	2786 a		1159.43 a	1292.13 a	
LSD.0.05 Treatments	13.89			111.64		
LSD.0.05 Species	3.9			211.93		
LSD.0.05 Interaction	195			157.90		
	Root Fresh w. (g)			**Root Dry w. (g)**		
T1	917.5 e	1125 b	1021.25c	373.76 f	764.76c	569.25c
T2	954.5 de	1205 a	1079.75 b	503.46 e	880.53 b	691.99 b
T3	1032.5c	1242a	1137.5 a	696.06 cd	862 b	779.02 a
T4	989.5 cd	1205 a	1097.25 b	433.85 ef	989.82 a	711.83 b
T5	967.5 d	1202.5 a	1085 b	639.83 d	904.33 b	772.07 a
Means	972.3 b	1196 a		529.38 b	880.28 a	
LSD.0.05 Treatments	32.74			52.43		
LSD.0.05 Species	1.24			54.21		
LSD.0.05 Interaction	46.31			74.15		
	Total fresh w. (g)			**Total dry w. (g)**		
T1	3007.5 g	3610 d	3308.75 d	1270.88 f	2400.41 bc	1835.64c
T2	3339.5 ef	4395c	3867.25c	1538.24 e	2248.03 cd	1893.13c
T3	5142.5 a	3550de	4346.25 a	2515.36 ab	2189.87 cd	2352.61 a
T4	3452 def	4817.5 b	4134.75 b	1463.23 ef	2692.45 a	2077.83 b
T5	3317.5 f	3537.5 def	3427.5 d	1656.43 e	2120.77 d	1888.59c
Means	3651.8 b	3982 a		1688.82 b	2330.3 a	
LSD.0.05 Treatments	157.11			166.79		
LSD.0.05 Species	89.42			100.6		
LSD.0.05 Interaction	222.19			235.89		

For both species and treatments, means that are the same letter do not have any statistical significance.

(a and b) indicated that monthly height and diameter increments were evident with T2, T3, and T4 compared to other treatments. *M. oleifera* gave the highest monthly increment of height and diameter, followed by *A. saligna*, which means these species adapted to these conditions for a long time.

The results indicated that irrigation with Treated Buffalo Milkmaid Wastewater (TBMW) was the reason for the increased height and diameter of fodder shrubs, which contain a high amount of organic compounds and plant mineral nutrients. This suggests that if cultivated on sandy loam soil and irrigated by (TBMW), there is a potential for achieving enhanced growth as a result of an improved photosynthetic rate. Several researchers have examined the impact of treated wastewater on the vegetative growth traits of seedlings and young plants. After 9 years of research, (Melgar et al., 2009) found no significant changes in shoot length caused by saline irrigation of olive trees with 10 dSm^{-1} water. However, alterations can develop in a single year. Based on these results, extended years of irrigation with TWW would have likely yielded identical results across treatments. Also, this was approved in the experiment of (Bedbabis et al., 2010).

3.4. Shrub biomass

Two shrub species showed the ability to survive under different treatments; this ability was highly different between shrub biomass at the end of the study in October 2023. Table 3 indicates analysis of variance of leaves, shoot, root, and total fresh and dry weight revealed differences ($P < 0.05$) between shrub species, treatments, and their interactions.

Leaves, shoot, root, and total fresh and dry weight of shrub species increased ($P < 0.05$) with the different water treatments (see Table 3). It was clear that *M. oleifera* had the highest significant values for shoot, root, and total fresh and dry weight compared to *A. saligna*. However, *A. saligna* gave the highest significant leaves fresh and dry weight with all treatments (1241.5 g and 464.73 g, respectively). The mean value of *A. saligna* leaves fresh and dry weight is expected to be higher than that of *M. oleifera* because of the morphological disparities between the two kinds, the leaves of *A. saligna* being larger than those of *M. oleifera*. While the shoot fresh and dry weight of *M. oleifera* was higher than that of *A. saligna*, it returned to add the weight of the fruit, which means Moringa has faster phenological stages than Acacia.

On the other hand, the T3 treatment is the most effective compared to other treatments in terms of leaves, shoot, and total fresh and dry weight. However, T4 gave the highest values for root dryness (989.82 g), as shown in Table 3 and Figure SI4. These results may be returned to the pH of each wastewater treatment. It was clear that the pH of T3 was 6.97 ± 0.2, while the pH of T4 and T5 ranged between 7.3 ± 0.19 and 7.64 ± 0.1, respectively; this means that T3 has the best values for growing the shrub species, followed by T4. In addition, the analysis of BOD and COD of T3 gave the lowest values (60 ± 20 and 130 ± 33 $mg.L^{-1}$, respectively), leading to more pure irrigation water, which gave color 50 ± 11 PCU and turbidity 40 ± 8 NTU, as shown in Table SI1. The higher biomass obtained in the UASB, especially with (T3) for irrigated pots, probably resulted from available nutrient elements such as N(88 ± 8 $mg.L^{-1}$), P (34.1 ± 3 $mg\ L^{-1}$), and K (7.82 ± 0.03 $mg.L^{-1}$), Table SI1. Consequently, the irrigation by treated buffalo milkmaid wastewater was a form of fertigation. The results align with those of (Abdulla et al., 2019), who demonstrated that irrigating 3-year-old Jatropha shrubs with treated olive mill wastewater significantly increased fruit yield by up to 1.85 times compared to the control group. This irrigation method did not impact the seed oil content after a 3-month application. On the other hand, the qualitative characteristics of crop productivity were unaffected by the source of irrigation water, according to (Disciglio et al., 2014).

Table 4
Studied species treatments and their interaction for soil means of nitrogen, phosphorus, and potassium ($mg\ kg^{-1}$), organic matter (%), and organic carbon (%).

Species	*A. saligna*	*M.oleifera*	Means	*A. saligna*	*M. oleifera*	Means	*A. saligna*	*M. oleifera*	Means
Treatments	**Nitrogen ($mg\ kg^{-1}$)**			**Phosphorus ($mg\ kg^{-1}$)**			**Potassium ($mg\ kg^{-1}$)**		
T1	47.25 e	56.25 cde	51.75c	26.25 a	25.5 a	25.87 a	175 a	202.5 a	188.75 a
T2	70 ab	73.5 a	71.75 a	28.05 a	25.45 a	26.75 a	170 a	212.5 a	191.25 a
T3	56.9 cde	60.4 bcd	58.65 bc	28.7 a	31.4 a	33.8 a	205 a	190 a	197.5 a
T4	67.4 abc	61.3 abcd	64.35 ab	29.1 a	26.9 a	28 a	220 a	230 a	225a
T5	49.9 de	58.65 bcde	54.27c	28.45 a	26.45 a	27.45 a	197.5 a	192.5 a	195 a
Means	58.29 b	62.02 a		28.11 a	28.11 a		193.5 a	205.5 a	
LSD.0.05 Treatments	8.73			4.59			46.99		
LSD.0.05 Species	2.95			14.48			19.87		
LSD.0.05 Interaction	12.34			6.49			66.45		
	Organic matter (%)			**Organic carbon (%)**					
T1	0.41 b	0.36 b	0.38 b	0.27 b	0.22 b	0.24 b			
T2	0.53 ab	0.82 a	0.67 a	0.31 ab	0.47 a	0.39 a			
T3	0.45 b	0.38 b	0.41 b	0.26 b	0.23 b	0.24 b			
T4	0.45 b	0.43 b	0.43 b	0.26 b	0.24 b	0.25 b			
T5	0.79 a	0.39 b	0.59 ab	0.46 a	0.23 b	0.34 ab			
Means	0.52 a	0.47 a		0.31 a	0.28 a				
LSD.0.05 Treatments	0.21			0.12					
LSD.0.05 Species	0.31			0.15					
LSD.0.05 Interaction	0.29			0.17					

For both species and treatments, means that are the same letter do not have any statistical significance.

3.5. Treated buffalo milkmaid wastewater effect on the soil mineral content

In soil samples from the control and differently treated buffalo milkmaid wastewater (TBMW), NPK was measured. The application of TBMW was noted to enhance the NPK content in the soil after six months of treatment. *M. oleifera* gave the highest significant values of nitrogen (62.02 mg kg^{-1}), followed by *A. saligna* (58.29 mg kg^{-1}); maybe the effectiveness of nitrogen-fixing bacteria of *A. saligna* decreased with the addition of organic nitrogen by irrigation by TBMW.

Based on the results shown in Table 4, the NPK was increased. The two species had available soil nitrogen levels ranging from 51.75 to 71.75 mg kg^{-1}. Sedimentation tank effluent (T2) yields the highest values of available nitrogen, followed by UASB-treated wastewater (T4 and T3), indicating enhanced nutrient availability, which could be attributed to processes associated with the nitrogen cycle or anaerobic digestion. It was clear that the nitrogen content in the conventional UASB effluent unit (T4) and the Up-flow Anaerobic Sludge Blanket (T3) was 100 ± 16 and 88 ± 8 mg. L^{-1}, respectively, compared with the Sedimentation tank effluent (T2), which had a content of 56 ± 6 mg.L^{-1}. Conversely, there was no significant difference in phosphorus and potassium content between species under different treatments in soil, as shown in Table 4 and Figure SI5a. Phosphorus and potassium levels remained relatively stable, potentially due to adsorption-desorption dynamics in the soil matrix. These trends highlight the dynamic nature of nutrient availability under treated wastewater irrigation and underscore the importance of monitoring long-term biological processes in sustainable agricultural systems. According to the results, wastewater treatment increased nutrient availability for shrub species, particularly in areas with poor soil. These results were consistent with (Chaari et al., 2014) results that more organic matter from waste (OMW) led to higher soil concentrations of nitrogen, phosphorus, salt, and potassium. According to (Urbano et al., 2017), Wastewater provides more nutrients than freshwater and can be used as a fertilizer for crops, among other benefits of wastewater utilization.

Irrigation with sewage water can modify the soil's physical and chemical characteristics, such as its structure, texture, porosity, hydraulic conductivity, and mineral content. These changes are crucial for the transfer, storage, and movement of nutrients in wastewater (Habibi, 2019).

3.6. The impact of treated buffalo milkmaid wastewater on the levels of organic matter and organic carbon in the soil

The soil of the experimental site was classified as sandy loam with 0.07 % organic matter (OM) and 0.04 % organic carbon (OC), as shown in Table 1. At the end of the experimental period, soil organic matter (OM) ranged between 0.41 % and 0.67 %, and organic carbon (OC) ranged between 0.24 % and 0.39 %, increasing significantly ($P < 0.05$), as shown in Table 4.

Doubtless, the irrigated buffalo milkmaid wastewater significantly affected the organic matter and organic carbon content of the soil, as evidenced by Table 4 and Figure SI5b (T2), which had the major effect on the organic matter and organic carbon content of the soil (0.67 % and 0.39 %, respectively). No significant difference was found between the two shrub species in terms of organic matter and organic carbon content in the soil.

Regarding organic matter and soil nutrition elements, sandy loam soils tend to be deficient due to the rapid breakdown of organic materials brought about by generally higher temperatures. Subsequently, Soil nutrient availability was enhanced using processed buffalo milkmaid wastewater. These results align with (Habibi, 2019), who found that Soil organic matter (OM) levels are significantly elevated after wastewater treatment. (Abdulla et al., 2019)demonstrated that treated wastewater contains a wealth of plant minerals and useful organic compounds, making it a promising fertilizer option, particularly for areas with low soil quality. The lower removal efficiencies of BOD, COD, TN, and TP in T3 and T5 can be attributed to multiple factors, including the type of biochar used, lack of bioaugmentation, and operational parameters. A related study by (El Shahawy et al., 2024) demonstrated that biochar derived from *Phragmites australis* bio-augmented with actinomycetes significantly improved organic matter degradation in UASB reactors treating buffalo wastewater. In contrast, our study used rice straw biochar without bio-augmentation, which may have resulted in lower microbial synergy and adsorption capacity, leading to reduced COD and BOD removal in T3. Furthermore, UASB and biofilter systems typically exhibit low TN and TP removal efficiencies, as anaerobic digestion primarily converts nitrogen to ammonia rather than removing it, while phosphorus remains largely unaffected without targeted removal strategies.

3.7. Correlation coefficient analysis

The correlation matrix (Figure S1 to Figure S2) visualizes the relationships between variables for Species-Treatment A (1:5) and M (1:5) in terms of vegetable growth, shrub biomass, and Soil Content (Mineral, Organic Matter, and Organic Carbon)(Holland et al., 2024). Species-Treatment A-1 (refer to Figure S1) exhibits coordinated biomass accumulation across plant parts, with a trade-off between stem diameter and overall biomass, indicating an adaptive strategy that prioritizes structural reinforcement over biomass expansion. Species-Treatment A-2 (refer to Figure S1) exhibits coordinated growth across plant parts, characterized by a trade-off between stem thickness and biomass, where thicker stems yield higher fresh biomass but lower dry matter, indicating a strategy that prioritizes structural support over overall biomass accumulation(Chave et al., 2009). Species-Treatment A-3 (refer to Figure S1) reveals a trade-off between stem diameter and overall biomass, with thicker stems correlating with lower fresh and dry weights, and a balance in growth across plant parts, as indicated by strong correlations within the fresh and dry biomass groups (Huang et al., 2019).

The correlation matrix for Species-Treatment A-4 (refer to FigureS1) shows a trade-off between height and fresh biomass, with a unique pattern where dry leaves mass is positively correlated with height but negatively correlated with other traits, indicating a resource allocation strategy prioritizing height and leaves robustness at the expense of other plant parts (Chauhan and Singh, 2024).

Species-Treatment A-5 exhibits strong positive correlations in biomass accumulation, with a trade-off between stem diameter and overall biomass, indicating that plants prioritize structural reinforcement (stem thickness) and vertical growth at the expense of other traits, likely as an adaptive strategy for light competition (refer to Figure S1). The M-1 correlogram reveals perfect positive correlations within biomass categories and negative correlations between size metrics and part masses, indicating a trade-off that prioritizes height and diameter growth over mass accumulation, likely as an adaptive strategy (refer to Figure S1). The M-2 correlogram shows strong positive correlations in biomass scaling and negative correlations between size metrics and individual part masses, indicating a trade-off that prioritizes structural growth over mass accumulation in specific parts (refer to Figure S1). The M-3 correlogram reveals moderate positive correlations in size and biomass, accompanied by strong negative correlations between fresh and dry measurements, suggesting a trade-off between water content and dry matter that optimizes resource use (refer to Figure S1). The M-4 correlogram (refer to Figure S1) shows perfect positive correlations in biomass scaling, with negative correlations between fresh root mass and other fresh metrics, indicating a trade-off that prioritizes root development over above-ground growth under M-4 conditions (Huanca-Nunez et al., 2024). The M-5 correlogram reveals strong positive correlations in growth measurements, with negative correlations between size metrics and specific fresh masses, indicating a trade-off that prioritizes structural growth over mass accumulation in individual parts (refer to Figure S1).

The strong negative correlations in the A-1 treatment suggest inverse relationships between nutrients and organic matter, indicating potential nutrient competition and differing organic compositions, which warrant further investigation into their ecological and agricultural implications (refer to Figure S2). The negative correlations between nitrogen and other nutrients in A-2 may reflect nutrient competition, while the strong positive correlation between phosphorus and potassium suggests a synergistic relationship influenced by soil composition and biological processes (refer to Figure S2). The antagonistic and synergistic relationships between nitrogen and other nutrients underscore the importance of careful nitrogen management, as excessive nitrogen can suppress potassium, reduce organic matter, and alter nutrient uptake dynamics, whereas nitrogen and phosphorus levels tend to increase together (Peñuelas et al., 2013) (refer to Figure S2). The study highlights the complex nutrient relationships under high nitrogen conditions, where nitrogen enhances organic carbon and organic matter but may suppress phosphorus and potassium uptake, emphasizing the need for balanced nitrogen application to maintain soil health and nutrient synergy(refer to Figure S2). The positive correlation between nitrogen, organic carbon, and organic matter highlights nitrogen's role in promoting soil health, while excess nitrogen may suppress phosphorus and potassium, suggesting the need for balanced nutrient application to maximize efficiency (Hirel et al., 2011). The perfect positive correlations under the A-5 treatment suggest co-dependent dynamics, where balanced nutrient availability and organic properties enhance plant growth and soil health, indicating that increasing one nutrient may boost others for sustained fertility (refer to Figure S2). The positive correlations between nitrogen, organic carbon, and organic matter suggest that nitrogen supports organic matter retention, while its high levels may hinder potassium availability due to competitive interactions or chemical imbalances (refer to Figure S2). In the M-2 treatment, phosphorus exhibits a positive correlation with organic carbon and organic matter, indicating its role in supporting the stability of organic matter and carbon sequestration, with all variables showing perfect positive correlations (refer to Figure S2). In the M-3 treatment (refer to Figure S2), nitrogen positively correlates with potassium, organic carbon, and organic matter, indicating a synergistic relationship, while nitrogen and phosphorus exhibit a negative correlation, suggesting competitive interactions that may suppress phosphorus uptake and disrupt the nutrient balance in the soil (Vanzolini et al., 2017). High phosphorus may reduce organic matter and carbon content, while the perfect positive correlations between nitrogen, phosphorus, organic carbon, and organic matter suggest they are closely linked, with potassium negatively correlating with these variables (refer to Figure S2). Phosphorus, potassium, organic carbon, and organic matter are positively correlated, while nitrogen shows a negative correlation with these variables (refer to Figure S2).

3.8. Principal component analysis (PCA)

The purpose of principal component analysis (PCA) was to delve more into the origins of the plant features found in this study. The principal component analysis (PCA) method evaluates the relationships between variables by extracting a small number of latent factors or principal components (PCs). Principal component analysis (PCA) is a popular method for reducing data (Loska and Wiechuła, 2003). Components with eigenvalues greater than one were selected using Kaiser normalization, which determines the optimal number of components to retain. If the eigenvalue of a component is more than 1, then its contribution is large. The Principal Component Analysis (PCA) of this study, which examined twelve Acacia growth traits, was conducted using five types of treated water for irrigation. The analysis generated two PCs with eigenvalues greater than unity, accounting for a cumulative variance of 88 %. The initial component (PC1) accounted for the greatest proportion of variance (76.8 %), with PC2 accounting for 11.2 % of the variance. (Refer to Table S1) displays the factor loadings, which indicate the significance of the variables. For this analysis, loading values > 0.3 were deemed acceptable. Biplot of PCA factors, PC1 and PC2 (refer to Figure S3), for all traits with all tested irrigated water. The highest levels of D (shoot, stem, and total) and F (stem, shoot, leaves, and total) belonged to A-3. Positive loadings were observed for D (shoot, stem, and total) and F (stem, shoot, leaves, and total) in PC1.PC2 included diameter with positive loading. D (shoot, stem, total) and F (stem, shoot, leaves, and total) correlated significantly with A-3, which was confirmed by a correlogram (as shown in S3). The analysis generated three PCs with eigenvalues greater than unity, accounting for a cumulative variance of 91.3 %. The initial component (PC1) accounted for the greatest proportion of variance (49.6 %), with PC2 accounting for 28.8 % and PC3 accounting for 12.8 % of the variance. (Refer to Table S2) displays the factor loadings, which indicate the significance of the variables. For this analysis, loading values > 0.3 were deemed acceptable. Biplot of PCA factors(refer to Figure S3), PC1 and PC2, for all traits with all tested irrigated water. The highest levels of D (shoot, root, and leaves) and F (shoot and total) were significantly correlated with (M-2, M-4)(Figure S3). Positive loadings were observed for D (shoot, root, leaves) and F (shoot and total) in PC1. PC2 included D (fruit) and F (fruit) with positive loadings, while F (stem and root) had negative loadings. PC3 included (diameter, D stem, and D total) with positive loading, while the F leaves had negative loading (refer to Figure S3).

PCA for the five Acacia soil content traits was determined using five types of treated water for irrigation. The analysis generated two principal components (PCs) with eigenvalues greater than unity, accounting for a cumulative variance of 72.9 % (see Table S3). The initial component (PC1) accounted for the greatest variance (45.1 %), with PC2 accounting for 27.9 %. (Refer to Table S3) displays the factor loadings, which indicate the significance of the variables. For this analysis, loading values > 0.5 were deemed acceptable. Biplot of PCA factors, PC1 and PC2, for all traits with all tested irrigated water. The highest levels of O.C% and O.M% belonged to A-5. Positive loadings were observed for O.C% and O.M% in PC1 (refer to Figure S3). PC2 included P and K, which had positive loading. O.C % and O.M% were significantly correlated with A-5(refer to Figure S3). It was confirmed from the correlogram (refer to S2).

PCA for the five Moringa soil content traits was performed using five different types of treated water for irrigation. The cumulative variance of 89 % (refer to Table S4) was accounted for by PC1 (the greatest proportion of variance, 62.4 %), with PC2 accounting for 26.5 % of the variance. The factor loadings indicate the significance of the variables. For this analysis, loading values > 0.5 were deemed acceptable. Biplot of PCA factors, PC1 and PC2, for all traits with all tested irrigated water. The highest levels of O.C%, O.M%, and N were observed in M-2. Positive loadings were observed for O.C%, O.M%, and N in PC1. PC2 included K and P, which had positive and negative loadings. As described in the M-4 correlogram, soil content analysis revealed a notable inverse correlation with K and P. O.C%, O.M%, and N were significantly correlated with M-2 (refer to Figure S3).

3.9. Cluster analysis

Dendrograms display the outcomes of cluster analysis, which were used to verify the PCA findings (refer to Figure S4). Data in two dimensions with values indicated by color allows for quick visual comprehension. The final clustering scheme used to construct the dendrogram consisted of twelve nodes, with a different color indicating each cluster. With a similarity level of 17.18 %, the dendrogram was pruned. A lower degree of trimming would result in more final clusters with a higher degree of similarity, whereas a larger degree of trimming would lead to fewer final clusters with a lower degree of similarity. Data from cluster analyses and hierarchical clustering are presented in a tabular format. By drawing attention to similarities within a class as well as differences across classes, cluster analysis facilitates case (object) classification. When data is clustered, it becomes easier to understand and spot trends (Vega et al., 1998). Twelve variables make up the final product. In the first stage, we merged two previous clusters, denoted as variables 5 and 7 in the spreadsheet, with a similarity level of 99.9819 % and a distance level of 0.00036 (refer to Table S5). This formed eight clusters from the data. In the final step, all variables were joined into a single cluster. F(stem, shoot, and total) and D (stem, shoot, and total) for a tight cluster (refer to Figure S4). Plant growth measurements clustering supported the PCA results. Twelve main categories were used to classify the plant growth measurements. In the A-3 group, visible in the loading plot, F(stem, shoot, and total) and D(stem and shoot) formed a small group. The dendrogram was cut at a similarity level of 28.03 %.

The results comprise fourteen variables; a new cluster was created in step 1 by joining two existing clusters, represented in the worksheet as variables 6 and 8. This partitioned the data into twelve clusters with a similarity level of 99.8176 % and a distance level of 0.00365 (refer to Table S6). In the final step, all variables were joined into a single cluster. F (shoot and total) and D (shoot) for a tight cluster (refer to Figure S4). Plant growth measurements clustering supported the PCA results. Twelve main categories were used to classify the plant growth measurements. In the M-2 group, as visible in the loading plot, F (shoot and total) formed a small cluster.

The dendrogram was cut at a similarity level of 39.62 %. The results comprise five variables; a new cluster was created in step 1 by joining two existing clusters, represented in the worksheet as variables 4 and 5. This partitioned the data into four clusters with a similarity level of 99.0389 % and a distance level of 0.0192 (refer to Table S7). In the final step, all variables were joined into a single cluster.O.C% and O.M% form a tight cluster (refer to Figure S4). Four main categories were used to classify soil content analysis, and clustering supported the PCA results. In the A-5 group, visible in the loading plot, O.C% and O.M% formed a small group (refer to Figure S4).

The dendrogram was cut at a similarity level of 33.4 %. The results comprise five variables; a new cluster was created in step 1 by joining two existing clusters, represented in the worksheet as variables 4 and 5. This partitioned the data into four clusters with a similarity level of 99.9199 % and a distance level of 0.0016 (refer to Table S8). In the final step, all variables were joined into a single cluster. O.C%, O.M%, and N mg. kg-1 form a tight cluster (refer to Figure S4). Four main categories were used to classify soil content analysis, and clustering supported the PCA results. In the M-2 group, visible in the loading plot, O.C% and O.M% formed a small group (refer to Figure S4).

3.10. Summary of key review articles

Certainly, below is a structured Table SI3 summarizing key review articles from the last five years (2019–2024) related to wastewater reuse for agriculture, UASB and biofilter technologies, and fodder or non-edible biomass cultivation using treated effluents. The table includes synthesized keywords, major outcomes, identified gaps, and technological advances based on recent literature.

3.11. Results of bibliometric analysis

3.11.1. Web of science (WOS)

An initial search in the Web of Science database yielded 2309 scientific articles. Out of these, 590 articles were filtered from the Web of Science. The publications evaluated from the Web of Science database are summarized in Table SI4. For the Web of Science database, out of 590 documents, 400 were research publications, whereas just 13 were review articles. A very low number of articles per year is indicated in the WOS database until the 2003 timeframe (Figure SI6a). The number of papers published this year reached its greatest point in 2021, with 20 publications. There was the greatest annual rise in publications from 2014 to 2021. The WOS database has a mean citation per year score of 2.81; the average number of citations per year was highest in 2020 (Figure SI6b).This report, "Wastewater reuse for crop irrigation: Crop yield, soil and human health implications based on giardiasis epidemiology" (Leonel and Tonetti, 2021). This paper was published in the "*Science of the Total Environment*" journal and has 75 citations, followed by "Influence of Regulated Deficit Irrigation and Environmental Conditions on Reproductive Response of Sweet Cherry Trees"(Blanco et al., 2020)with 48 citations published in the “Plants” journal. As the number of articles rises, the total number of citations every year falls. Moving forward, applied technologies such as biochar inoculation in UASB systems demonstrate promising potential in enhancing treated water quality. The increasing research attention toward sustainable and efficient treatment methods suggests a shift towards technologies that improve water quality and align with environmental sustainability. The application of innovative technologies holds significant potential for advancing wastewater treatment and promoting sustainable water management. Biochar, derived from agricultural waste, enhances the adsorption capacity and microbial activity within reactors, leading to improved removal efficiencies for pollutants such as COD, BOD, TSS, TDS, color, and turbidity. This not only results in higher-quality treated water suitable for agricultural irrigation but also contributes to resource sustainability by utilizing waste materials in the treatment process. The enhanced water quality reduces risks of soil contamination and promotes healthier crop growth, supporting sustainable agricultural practices. Furthermore, biochar-based technologies are adaptable and scalable, offering practical solutions for regions facing water scarcity. These advancements align with global efforts to achieve sustainable development goals, particularly those related to clean water, sanitation, and responsible consumption. Future research could focus on optimizing biochar properties, exploring synergistic effects with other materials, and assessing long-term environmental impacts, ultimately contributing to more resilient and efficient water treatment systems. The bibliometric analysis highlights the key sources contributing to water treatment and management advancements. As shown in (Fig. 4a). The "Agriculture Water Management" journal has the most articles, with 55 articles indicating its pivotal role in disseminating research on sustainable water practices, followed by the Journal “Environmental Monitoring and Assessment” with 38 articles, and the journal "Desalination and Water Treatment" was placed third with 33 articles, underscoring the growing interest in technologies enhancing water reuse and treatment efficiency. In terms of research influence, (Fig. 4b) presents the H-index of the top ten journals, regarding the H-index of journals, with Agriculture Water Management achieving the highest score of

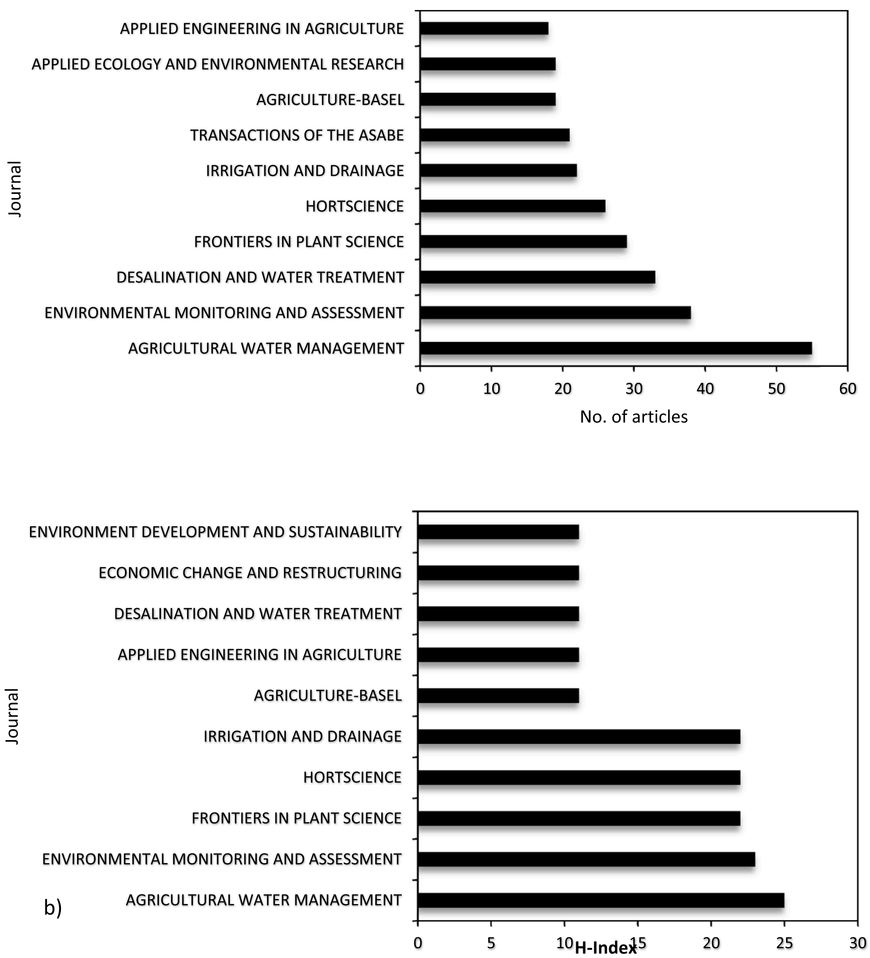


Fig. 4. (a)Top ten journals in terms of the quantity of articles and (b)the top ten journals as determined by the H-index.

25, reflecting its significant citation impact. Environmental Monitoring and Assessment follows with an H-index of 23, and Frontiers in Plant Science with 22. Journals like Hortscience and Irrigation and Drainage also demonstrate strong research contributions, each with an H-index of 22, which suggests that future progress in water treatment will be influenced by innovations featured in these leading journals, especially those emphasizing sustainable and efficient water management approaches.

The most productive affiliation was “The Univerity of Arizona” with 101 articles, followed by “Northwest Aandfd University” with 98 articles, followed by “Colorado State University” with 83 articles, followed by “Gettysburg Coll” with 70 articles, followed by “Henan Agriculture University” with 62 articles, and “Seoul Nat Univ” with 60 articles (Fig. 5a). On the other hand, the dynamics of connections depending on the total number of publications are displayed in (Fig. 5b).

With 284 total publications, the United States of America was the most productive nation; It had the strongest international ties as well as the strongest linkages with practically all periodicals (Figure SI7). India ranked second with 225 publications, while China ranked third with 163 publications. This is clarified in the top fifteen countries’ co-authorship map (Figure SI8a). These countries have strong international collaborations, as evidenced by the numerous connections linking them to other nations. The map reveals dense interconnections between countries, indicating robust international collaborations. The USA and China, in particular, have multiple links to other countries, reflecting their central role in driving global research collaborations. European countries also show strong internal collaboration, as seen with Italy, Spain, and England.

A total of 2410 keywords were utilized in the articles. The visualization presented in (Figure SI8b) offers a detailed overview of the correlation between keywords related to wastewater treatment, irrigation practices, and sustainable agricultural reuse. The map, generated using VOSviewer, highlights several interconnected clusters that emphasize the research’s multidisciplinary nature. The central and most prominent clusters focus on keywords such as "irrigation," "wastewater," and "wastewater reuse," indicating the core research themes. These terms are densely linked to related concepts such as "wastewater treatment," "treated effluents," and "water reuse," demonstrating the strong focus on sustainable water management. The integration of keywords like "UASB," "biochar," and "rice

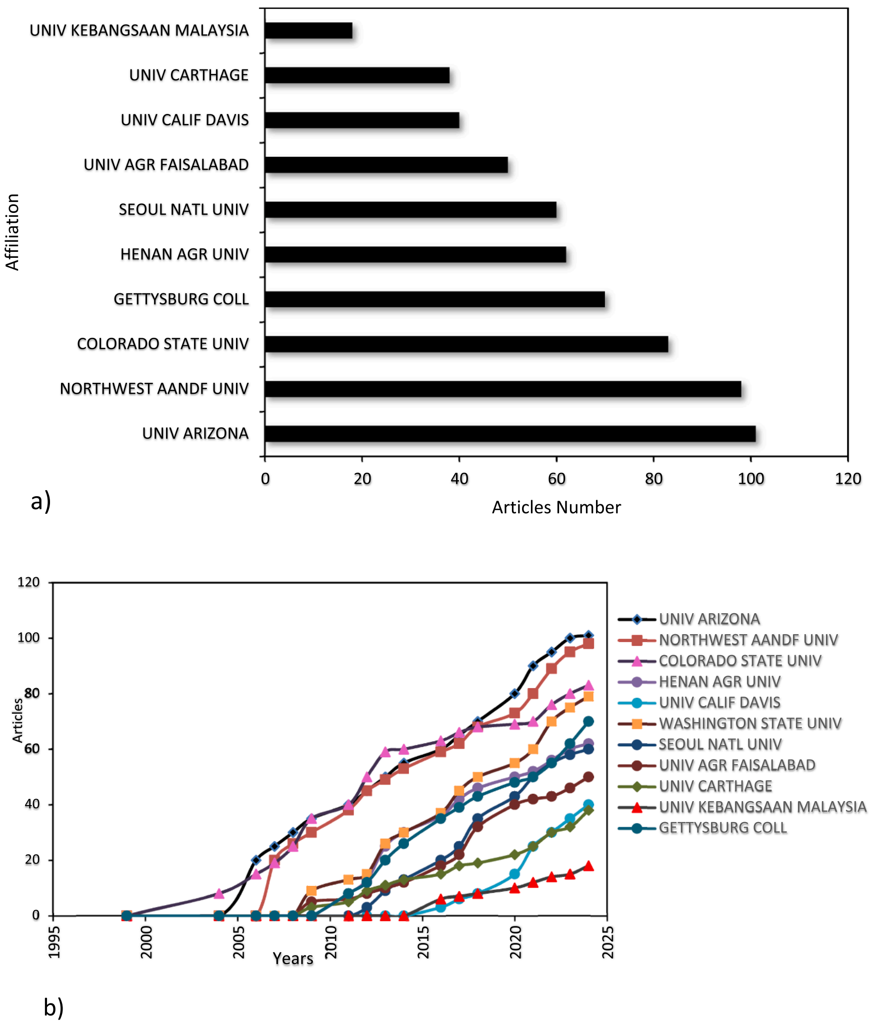


Fig. 5. (a) Top 10 affiliations in terms of articles published. (b) The formation of affiliations over time.

straw" reflects the study’s emphasis on innovative treatment technologies that enhance water quality for reuse in agriculture. The cluster containing "deficit irrigation," "yield," "water use efficiency," and "Moringa" illustrates the interrelationship between water management practices and agricultural productivity. These terms emphasize the importance of optimizing irrigation strategies to enhance crop outcomes, especially in water-scarce environments. Another notable cluster includes keywords like "heavy metals," "health risk," and "wastewater irrigation," highlighting the environmental and health considerations associated with using treated wastewater. The presence of terms such as "soil properties," "soil fertility," and "water scarcity" underscores the broader environmental impacts of these practices. The map also demonstrates emerging research areas, as indicated by less frequently connected terms like "agricultural reuse" and "fodder shrub cultivation," suggesting future directions in optimizing agricultural practices using treated effluents. The publications contained a total of 2510 authors and co-authors. The number of authors of single-authored documents was 26, equivalent to 30 articles that a single author wrote. The Average citation/article was 84.14, and the majority of articles were authored by three to five individuals. "Mal. M." possessed the most articles, with 56. "Ghazi YA" was in second place with 42 articles,

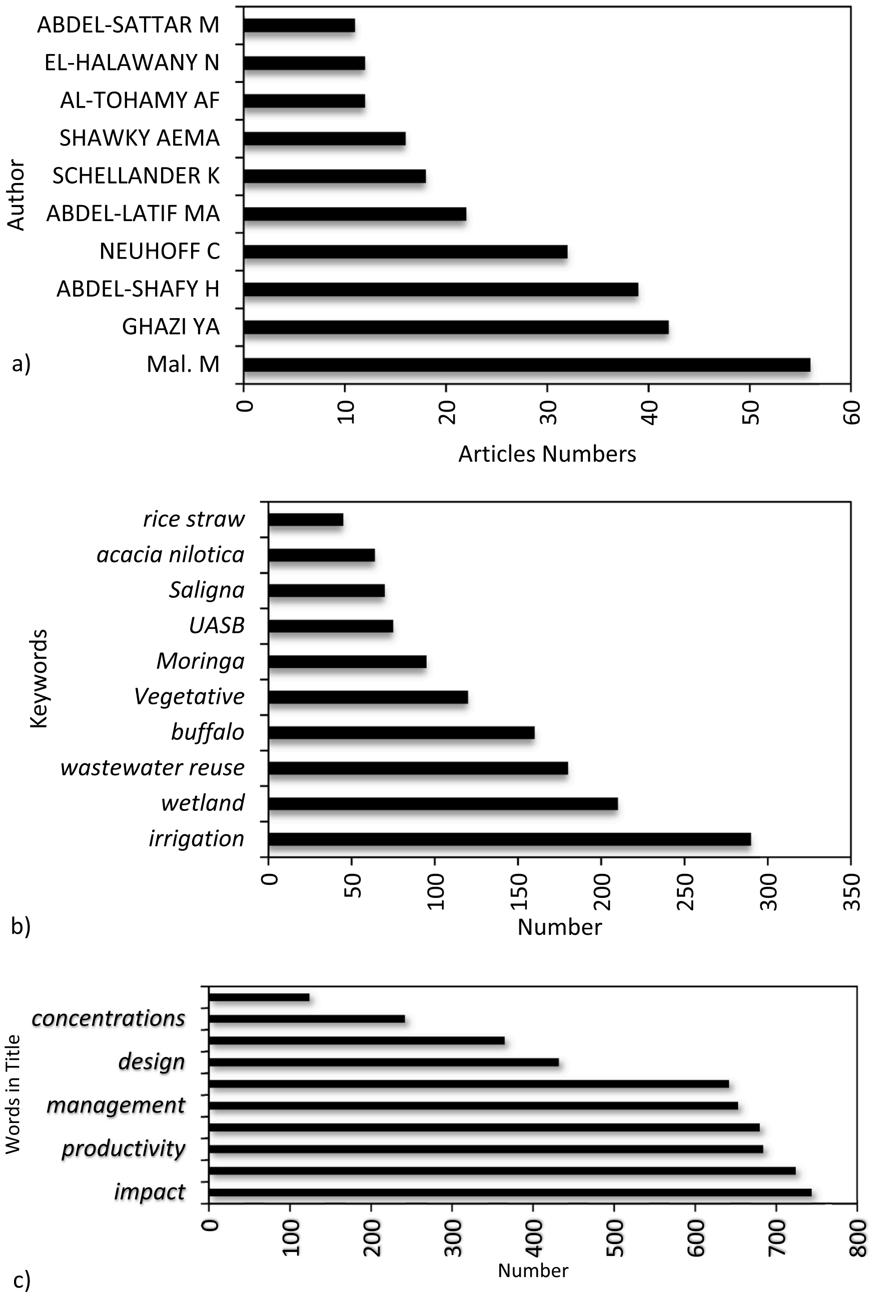


Fig. 6. (a)The top ten authors according to the number of articles,(b)the Top ten used keywords, and **(c)**the Top 10 used words in titles.

after which "Abdel-shafy H" with 39 articles (Fig. 6a).

Their central position also suggests they are well-connected to a wide range of other topics, making them critical focal points in the research field. The term “Irrigation,” which was employed 290 times., then came “Wetland” including 210 instances, followed by “Wastewater reuse” with 180 occurrences, followed by “Buffalo” with 160 occurrences, followed “Vegetative” with 120 occurrences, followed by “Moringa” with 95 occurrences, then came “UASB including 75 instances, and "Acacia" which appears 70 times(Fig. 6b). Regarding the terms used in the article titles, the term "Impact" was used 744 times, while the words "Effect" and "Productivity" were used 724, 684, and 680 times, respectively. “Wastewater” was used 680 times, “Management” was used 653 times, and “Nitrogen” was used 642 times (Fig. 6c).

4. Conclusions

This study demonstrates the potential of treated buffalo milkmaid wastewater, particularly from the modified UASB reactor (T3), to significantly enhance the growth performance of *Acacia saligna* and Moringa *oleifera* shrubs and improve soil fertility indicators such as nitrogen, phosphorus, potassium, organic matter, and organic carbon. Among all treatments, T3 led to the highest biomass and vegetative growth, while sedimentation tank effluent (T2) notably improved soil organic content. Irrigation with T4 increased the diameter of shrubs compared with other treatments. Multivariate analyses (PCA and cluster analysis) further distinguished treatment-specific effects on plant growth and soil chemistry, providing insights for targeted soil management strategies. The findings support the feasibility of integrating anaerobic treatment systems in sustainable agriculture, especially in water-scarce regions. Additionally, bibliometric analysis highlighted growing research interest in wastewater reuse for fodder cultivation, identifying key knowledge gaps for future exploration. Overall, the study underscores a green and practical approach to water reuse that supports both environmental sustainability and agricultural productivity.

5. Recommendations

For reusing buffalo wastewater in planting, *Acacia saligna* and *Moringa oleifera* prefer the Up-flow Anaerobic Sludge Blanket (UASB) system, followed by conventional UASB effluent and maintenance of the pH and other chemical properties of water to reach standard characteristics, the best growth shrubs, and soil sustainability. Future research should also explore the long-term impacts of continuous wastewater irrigation on soil microbiota, heavy metal accumulation, and crop quality. Further investigations into optimizing biofilter media (e.g., different types of biochar or natural zeolites) and scaling up the treatment system for on-farm deployment are also warranted. Additionally, socio-economic assessments, including cost-benefit analyses and farmer adoption studies, would be valuable in facilitating the translation of this green technology into practice.

Ethics approval

(Not applicable)

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CRediT authorship contribution statement

El Shahawy Abeer Abd El Moneam Mohamed: Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Safaa A. Ghorab:** Writing – review & editing, Writing – original draft, Validation, Supervision, Formal analysis, Data curation, Conceptualization. **Ismail Abd-Elaty:** Writing – review & editing, Writing – original draft, Supervision. **Ashraf Ahmed:** Writing – review & editing, Writing – original draft, Visualization. **Aya Mohamed:** Writing – review & editing, Writing – original draft, Supervision.

Consent to participate

(Yes)

Consent for publication

(Yes)

Ethics approval and consent to participate

The manuscript didn’t contain any reporting studies involving human data

Consent for publication

The manuscript doesn't contain any personal data

All authors have read and agreed to the published version of the manuscript. We confirm that all named authors have read and approved the manuscript.

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

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.eti.2025.104397.

Data availability

The data that has been used is confidential.

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