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Characterization of bird, reptile, and insect community diversity in constructed wetlands and waste stabilization ponds across Tanzania

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A R T I C L E I N F O

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A B S T R A C T

Wastewater treatment systems, such as Constructed Wetlands (CWs) and Waste Stabilization Ponds (WSPs), have untapped biodiversity enhancement and development potential. Birds, insects, and reptiles, which are displaced by human development, might find refuge in these ecosystems. However, the lack of a detailed characterization of the biodiversity status of these wastewater treatment systems hinders their widespread adoption. Point counts, direct observations, and camera traps were used to assess bird diversity across five CWs and three WSPs in Tanzania in 2021. For insects and reptiles, pitfall and pan traps were laid along established transects, in addition, direct observations and fishnets were also used to assess the reptiles dwelling within the WSPs. Abundance, Shannon index, Simpson index, Margalef index, and evenness index were the diversity parameters used to analyze the diversity of birds, insects, and reptiles. Our results show that among the studied groups and between WSPs and CWs, birds had high species abundance ($n = 1132$), richness, Margalef index (D = 4.266), evenness (E = 0.815), Shannon diversity (H = 2.881) and Simpson index ($\lambda = 0.903$). The abundance and diversity of studied groups differed significantly ($P < 0.05$) between WSPs and CWs. Our study also recorded four reptile species belonging to three orders. Molecular analyses confirmed that insect species belong to nine orders and 13 families, with the order Diptera dominating both CWs and WSPs, followed by Orthoptera, Hymenoptera, and Araneae. We conclude that CW and WSP wastewater treatment systems are important for hosting various populations of birds, reptiles, and insect species.

1. Introduction

The rising population densities, industrialization, and growth of human activities have increased the use of fresh water, which on the other hand, has increased the production of wastewater. Wastewater discharge has been shown to negatively impact ecosystems and the environment (Liyanage and Yamada, 2017), particularly in developing countries with inadequate wastewater management systems. Different methods of treating wastewater have emerged as technology has progressed to safeguard the environment and ecosystem from pollution that could harm humans and wildlife (Ambulkar and Nathanson, 2022). A well-designed wastewater treatment system can provide a habitat for various organisms displaced by greater human encroachment in urban ecosystems and can serve as green infrastructure (Stefanakis, 2019). However, establishing a clear understanding of these systems' ranging from social economic, resource recovery, and biodiversity potential could enhance their development, design, and adoption in the modern world.

Previously, waste stabilization ponds (WSPs) were the most common wastewater treatment method utilized in the treatment of sewage generated from households, industrial use, and public and private institutions (Quiroga, 2013). Recently, constructed wetlands have been used to enhance the treatment performance of wastewater stabilization ponds (Vymazal, 2005). Constructed wetlands (CWs) are engineered

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wastewater treatment systems encompassing several treatment modules, including biological, chemical, and physical processes, akin to natural wetlands (Vymazal, 2005). They intercept wastewater and remove various pollutants before discharging them into natural water bodies (Díaz et al., 2012). The CWs constitute of complex integrated systems of water, plants, animals, microorganisms, and the environment (USEPA, 2000). To widen the development of CWs and their associated wastewater treatment systems in specific sub-locations or regions as eco-friendly wastewater treatment systems, there is a need to establish cross-cutting benefits that can be provided to humans and ecosystems.

While natural wetlands are well known to be one of the most productive ecosystems on the planet (Mitsch et al., 2009), little is known about the potential of different types of CWs on the variety of ecosystem services they can offer. Several types of CWs for wastewater treatment have been developed, including surface flow CWs (SF-CWs), subsurface flow CWs (SSF-CWs), and vertical flow CWs (VF-CWs). Constructed wetlands are designed to mimic natural wetland ecosystems, and combine physical, chemical, and biological processes to purify water in more controlled and efficient ways (Scholz et al., 2007; Kadlec and Wallace, 2008). In SF-CWs, wastewater flows as in a natural wetland system and is normally planted with different vegetation types (Hassan et al., 2021). SSF-CWs are specifically designed for treating or polishing wastewater and are typically constructed as beds or channels containing appropriate media, such as sand and gravel, and planted with specific vegetation to enhance removal efficiency (Hassan et al., 2021). In VF-CWs, it is a planted filter bed with a bottom drain. Wastewater is poured or dosed onto the surface from the top using a mechanical dosing system. Wastewater flows vertically down the filter matrix to the bottom of the basin, where it is collected in a drainage pipe (Stefanakis et al., 2014; Tsihrintzis, 2017). CWs can potentially mitigate the negative effects of human activities on biodiversity decline in urban systems, especially when used as wildlife refuges when natural habitats are severely degraded (Hale et al., 2019). However, most CWs research has focused on their purification function and little attention has been paid to their biodiversity value. The lack of knowledge on the potential of CWs for biodiversity conservation in Tanzania has led to a lack of biodiversity-oriented CWs management, which could have a negative impact on urban ecology and biodiversity conservation in growing cities and towns.

On the other hand, waste stabilization ponds (WSPs) are open basins enclosed by earthen embankments and are sometimes fully or partially lined with concrete, compacted clay, or synthetic geofabrics (Verbyla et al., 2017). Natural processes are used to treat domestic wastewater, septage, sludge, and animal and industrial wastes (Verbyla et al.,2017). However, because of the global decline in natural habitats for birds, they have become increasingly reliant on alternative and artificial habitats, such as dumpsites and sewage stabilization ponds (Akinpelu, 2006). These man-made sites are useful for birds with unlimited food sources, and are thought to attract birds to sewage stabilization ponds and dumpsite areas (Anika and Parasharya, 2013). Although some municipal cities in Tanzania have WSPs utilized for treating wastewater generated from households, industries, and public and private institutions, no clear assessment of their biodiversity potential has been performed. Moreover, in some regions, WSPs systems have been integrated with CWs to improve treatment capacities and other ecosystem functions. However, to date, the assessment of the role of integrated constructed wetlands and waste stabilization pond systems on biodiversity potential is not well established, limiting their development, adoption and sustainability as urban infrastructure for wastewater treatment and hotspots for displaced fauna.

In Tanzania, studies on CWs and WSPs have focused on assessing their potential to enhance the removal of organic and inorganic contaminants and integrating different designs to improve the removal efficiency (Njau et al., 2011; Mtavangu et al., 2017). However, no studies have assessed the abundance and diversity of birds, insects, and reptiles in established CWs, and relatively few studies (Massawe, 2017; Salehe, 2021) have assessed bird abundance in waste stabilization ponds. Therefore, the abundance and diversity of different biodiversity groups are poorly understood in CWs and WSPs, attributing to a slow rate of acceptance of these ecofriendly technologies, failure of established systems, and even lack of policy support from the government. This study investigated the biodiversity of birds, insects, and reptiles in CWs and WSPs. Understanding present and future potential of these wastewater treatment systems in supporting the biodiversity of different organisms may be useful for biodiversity conservation managers. These data are needed in urban ecosystems for promoting the adoption of these ecofriendly technologies as well as for treating wastewater and supporting biodiversity. Furthermore, the findings will be useful for developing guidelines for carrying out wastewater projects, such as incorporating wastewater treatment systems into a biodiversity conservation portfolio. The objectives of this study were to (1) document the presence of different types of birds, insects, and reptile species living in different types of CWs and WSPs; and (2) quantify and compare bird, reptile, and insect abundance and diversity across the different types of CWs (surface and subsurface flow CWs) and WSPs in Tanzania. We predicted that WSPs and CWs would host different abundance and diversity of bird, insect, and reptile species as they provide different habitats. We also predicted that birds, insects, and reptiles would have a higher abundance and diversity in WSPs than constructed wetland systems. In addition, we predicted that different types of CWs that is surface flow CW and subsurface flow constructed wetlands, would have different diversity and abundance of bird, insect, and reptile species as they have different configuration designs.

2. Materials and methods

2.1. Study area description

The study was conducted in four regions of mainland Tanzania: Kilimanjaro, Arusha, Iringa, and Dar es Salaam (Fig. 1), where the experimental setup was conducted in two CWs, and two WSPs—experimentally surveyed areas constituted of one surface flow constructed wetland (SF-CWs) and WSPs at the Iringa Urban Water Supply and Sanitation Authority and a subsurface flow CWs (SSF-CW) and a WSPs at the Moshi Urban Water Supply and Sanitation Authority. In addition, an observational recording was performed in one WSPs in Vingunguti, Dar es Salaam and three SSF-CWs at the Nelson Mandela African Institution of Science and Technology, Banana Investment Limited, and Comprehensive Community-based Rehabilitation in Tanzania. Thus, in this study, we examined five different CWs and three WSPs. These locations were selected from the pre-survey and were found to be well-established wastewater treatment systems, including CWs that had been operating for more than three years (Rugaika, 2020; Msaki et al., 2022).

Among the study areas, Dar es Salaam is the largest city with the fastest-growing population; thus, it has a massive sewerage system that collects wastewater from homes and industrial sources (Venkatachalam, 2009; Worrall et al., 2017). Other selected regions have an increasing sewerage system to match the rising human population and industrial development in recent years (Thomas et al., 2013).

2.2. Types of studied wastewater treatment systems and their characteristics

In this study, different types of wastewater treatment systems were assessed: 1) WSPs, which are man-made large impoundments arranged in series treating water using solar radiation and microorganisms living within; 2) SF-CWs, which are man-made wetlands planted with different vegetation and resemble natural wetlands; water treatment is enhanced by the plants and microbial communities as well as animals living within; 3) SSF-CWs designed explicitly for the polishing of wastewater and are typically constructed as a bed or channel containing appropriate

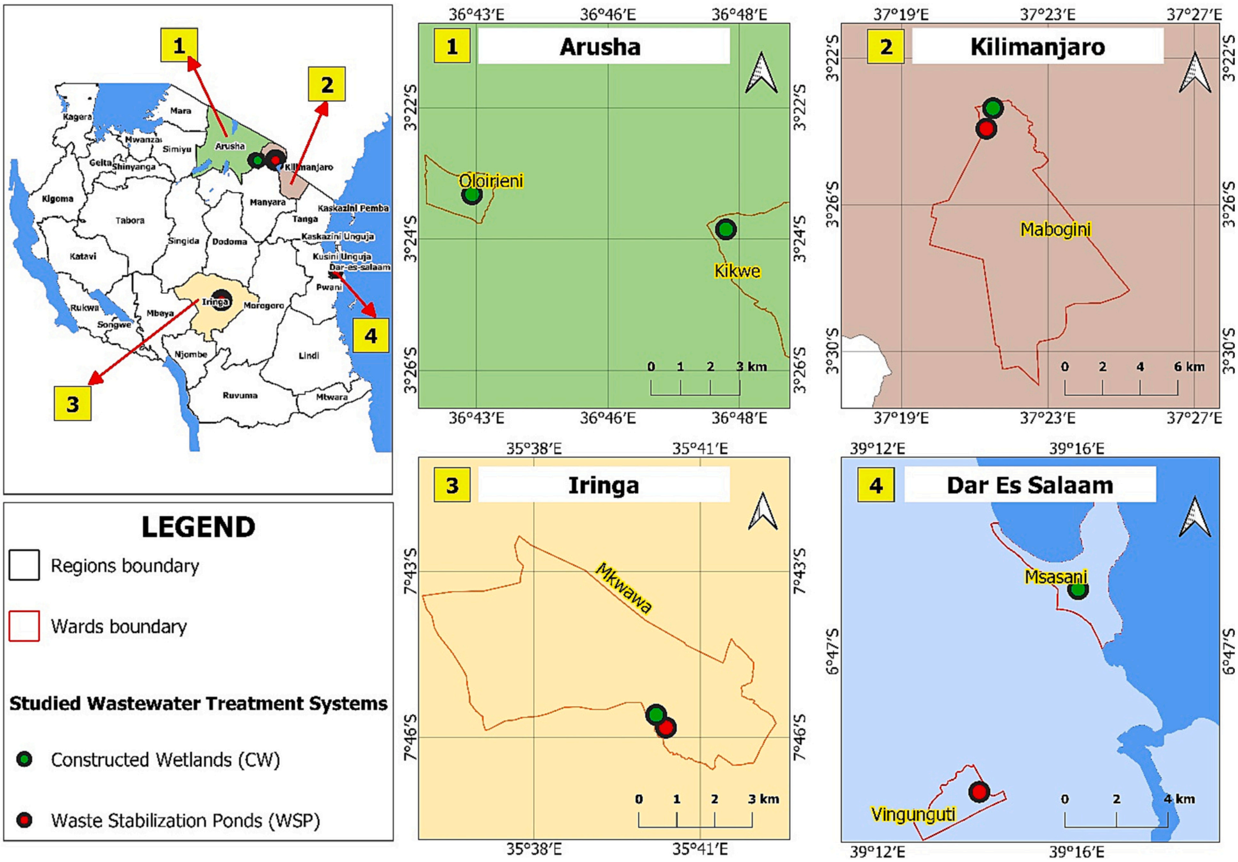

Fig. 1. Map of the study areas selected for sampling during data collection in the year 2020–2021 in Tanzania. CW = Constructed Wetlands; WSP = Waste Stabilization Ponds.

media such as sand and gravels and planted with vegetation to enhance removal efficiency; the treatment is under the influence of gravels (media), microbial communities, and plants, as well as animals that may live within. The area size, methods used in data collection are shown in detail in Table 1.

Different plant species were planted in the constructed wetlands at the study sites: *Cyperus papyrus, Cyperus alternifolius, Canna indica,* and *Phragmites maurtianus*. Two different types of CWs were studied: SF-CW in the Iringa Urban Water Supply and Sanitation Authority Fig. 2 (IRUWASA) and four SFF-CWs in the Moshi Urban Water Supply and Sanitation Authority (MUWSA), Nelson Mandela African Institution of Science and Technology (NM-AIST), Banana Investment Limited (BIL), and Comprehensive Community-Based Rehabilitation in Tanzania (CCBRT). Waste stabilization ponds are arranged in a series of anaerobic, aerobic, facultative, and maturation ponds, and the final treatment point is constructed wetlands (Von Sperling, 2007). In the visited municipal treatment system, the government, and similarly, the private companies, the major physical and chemical characteristics monitored to the effluent discharge standards were *BOD_5 at 20 °C 30 mg/L; COD 60 mg/L; pH ranges 6.5–8.5; Total Suspended Solids (TSS) 100 mg/L and 300 TCU (EWURA, 2014). These chemical and physical characteristics are monitored to ensure the health of these ecosystems is well maintained and, at the same time, they do meet effluent discharge standards.

2.3. Sampling and data collection

2.3.1. Bird species assessment

We used the point count method and applied the BirdLesser app to record every spotted and/or heard bird species, and the eGuide to Birds of East Africa book to identify bird species (Nussbaumer et al., 2021). Four points were laid in two established transects (two/transect) running horizontally along the CWs and WSPs. The points were set at the corner of the WSPs/CWs, covering an area of approximately 80 m (length) by 50 m (width). At each point, the observer stayed for 5 min and all bird species heard or seen were recorded. Double counting was avoided by recording only one species once it was spotted in the area

Table 1
Area size and data collection methods sample size for surveyed Constructed Wetlands and Waste Stabilization Ponds across different regions within Tanzania, sampled from May to December 2021.

Study Region	Type of studied site	WSP/CW surface area (m^2)	Line transects	Point counts	Camera traps
Iringa (IRUWASA)	SF-CWs	3570	4	4	4
	WSP	22,026	4	4	4
Kilimanjaro (MUWSA)	SSF-CWs	972	4	4	4
	WSP	153,900	4	4	4
Dar es salaam	SSF-CWs	71	Direct observations	Direct observations	Direct observations
	WSP	49,500	Direct observations	Direct observations	Direct observations
Arusha	SSF-CWs	372	Direct observations	Direct observations	Direct observations
	SSF-CWs	59	Direct observations	Direct observations	Direct observations

Fig. 2. Google earth satellite map of Iringa urban Water Supply and Sanitation Agency one of study site visited for data collection year 2021; (Source: https://earth.google.com/web/) CW-Constructed Wetland; WSP-Waste Stabilization Pond.

and at a reasonable distance from one count point to another. The counting started each day from 06:30 am to 07:45 am and 05:45 pm to 06:30 pm, three days each week across each month, between May 2021 and December 2021, for different sites. Moreover, four camera traps were used to monitor different bird species in the wetland and were set along the established points with high bird occurrence. The cameras were left for 12 h to allow further capture. The camera was set to capture one image every 15 s.

2.3.2. *Insect species assessment*

Sweep nets, pitfall traps, pan traps, and direct searching were used to collect insects in the study areas. Sweep nets with a diameter of 36 cm were swept at a height of 15 cm above the ground layer and vegetation along four different transects in the wetland, two moving horizontally, *i.e.*, parallel to either side of the wetland at a distance of 80 m long and two in a rectangular way that were 50 m long, the same setup was done for the CWs and the WSPs. All transects were placed close to the wastewater treatment system to account for the diversity within and around the wastewater treatment systems (DiFranco, 2006). Sweep-netting was performed twice daily (07:00 am to 10:00 am and 05:00 pm to 06:30 pm) for three days each week for one month between May 2021 and December 2021 for the visited study sites. The collected insects were then placed in 250 mL plastic sampling containers, preserved in 70% ethanol, and taken to the laboratory for identification (Schauff, 2001). Morphological identification was performed using the guidebook by Martins (2015) and confirmed using molecular analyses.

We also installed 18 pitfall traps and ten pan traps (Fig. 3) along four established transects at each location, spaced 10 m apart (Ward et al., 2001). Four line transects were established around the wetlands, and four other transects were established in the stabilization ponds in a rectangular placement 80 m long horizontally and 50 m vertically. Pitfall traps were allocated to the transect using a systematic random sampling technique and were sunken in the ground to the level at which the top part was equal to the ground surface (Montgomery et al., 2021).

2.3.3. *Reptile species assessment*

To estimate the diversity of reptile species, bucket pitfall traps, fish nets, and opportunistic searches were used to collect data on reptile diversity in the study area. Two pitfall line transects were established on either side of the CWs in a linear layout, and four buckets were placed within a distance of 25 m apart on each transect. Similarly, two line transects were established on either side of the WSPs in a linear placement, and four bucket traps were set at a distance interval of 25 m apart in each transect (Ellis, 2013). In addition, a drift fence made of polythene sheet was set along the transect line (Ellis, 2013). The transect was checked every three hours per day. At each site, selected sites with observed frequent occurrences of reptiles were established, and four camera traps (set at 12 h on a trapping day) were established at a 1.5 m height tilted downward to the ground to monitor the movement of any species. A fish net was used to assess the abundance of reptiles dwelling in the wastewater stabilization ponds. Reptile trapping was performed for three days each week in a month between May 2021 and December 2021. Opportunistic searches were performed every morning from 08:00 to am-10:00. and during the evening from 05:50 to pm-06:50 pm. Reptile species were identified using the field guidebook by Branch (2014). Appendix D summarizes the data collection methods.

2.3.4. *Insect DNA extraction for Sanger COI animal identification*

2.3.4.1. *Sample preparation and DNA extraction.*

Because the collected species were morphologically identified and yielded 14 groups of

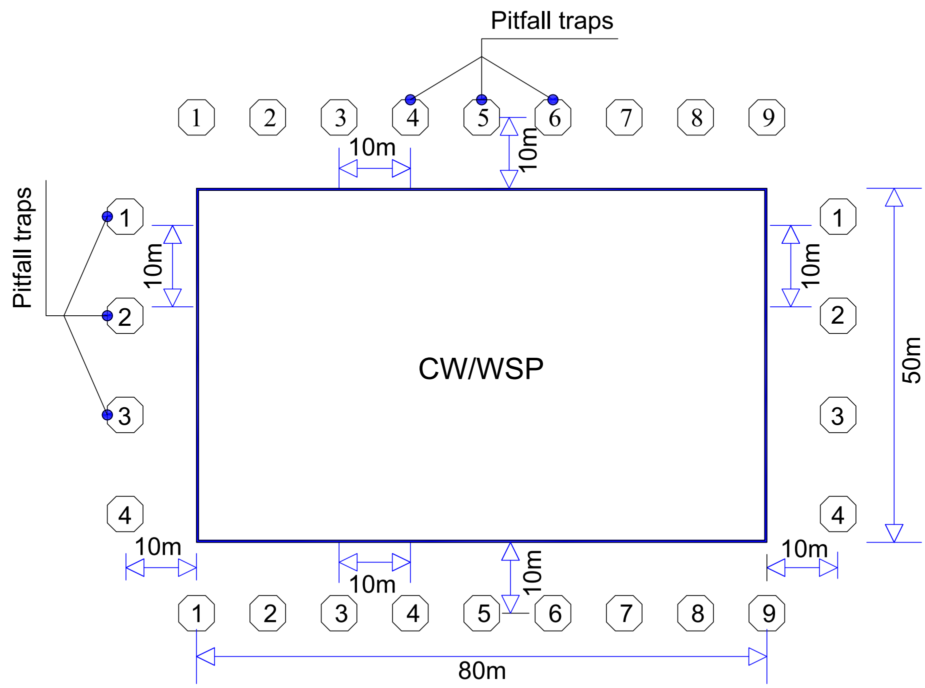

Fig. 3. Schematic Presentation of the layout of pitfall traps across the constructed wetlands and Waste stabilization ponds; 1–9 pitfall traps, 1–4 pan traps (CW-Constructed Wetlands; WSP-Waste stabilization Ponds) during the field survey conducted in Iringa and Moshi Water Supply and Sanitation Authorities wastewater treatment systems between May and December 2021.

morphologically similar individual insects, one individual specimen was picked from each group for molecular confirmation of individual species. DNA extraction from 14 specimens was performed using the PowerSoil DNeasy kit (Qiagen) following the manufacturer's instructions (Qiagen, Germantown, MD, USA). A small part of the specimen, preferably wings and/or thorax, was cut and inserted into the power beads for the initial processes, and the remaining part of the insect was kept as a voucher specimen (Calderón-Cortés et al., 2010).

2.4. Data processing and analysis

2.4.1. Data analysis was conducted to obtain the following parameters

(i). Relative abundance

The relative abundance for different species was determined by using the provided formula as follows;

$$\text{Relative abundance} = n/N \tag{1}$$

Where as n is the total number of a particular specie (bird, insect and reptiles) and N is the total number of all species found (bird, insects and reptiles).

(ii). Diversity indices

Biodiversity indices were calculated using standard formulas. The diversity of bird, insect, and Reptile species at both locations (WSP and CWs) was calculated using the Shannon-Wiener diversity index (H), as described by (Nolan and Callahan, 2006). The Shannon index is given by the following equation:

$$H' = -\sum_{i=1}^{S} p_i \, ln p_i \ (2)$$

Where H' is the species diversity index, s is the number of species, pi is the proportion of individuals of each species belonging to the ith species of the total number of individuals, and ln = logarithm to base e. The proportion of species relative to the total number of species (pi) was calculated and multiplied by the natural logarithm of this proportion (lnpi). The results were summed across species and multiplied by −1.

Evenness is a synthetic measure used to describe the pattern of relative species abundance in a community (Zelený, 2023). The evenness of birds, insects, and reptiles compares the similarity in the population size of each species (Kiros et al., 2018). The evenness index (J′) was calculated using the ratio of observed diversity to maximum diversity, using the following equation:

$$J' = H' / H_{max} \tag{3}$$

where H' is the Shannon Wiener Diversity index and Hmax is the natural log of the total number of species.

Species richness of birds, insects, and reptiles was calculated using the Margalef index (D) (Margalef, 1958). The index measures species richness and its highly sensitive to sample size although it tries to compasate for sampling effects (Magurran, 2004). This index is expressed by the following formula:

$$D = \frac{(S-1)}{lnN} \tag{4}$$

Where S is the total number of species, N is the total number of individuals in the sample, and ln is the natural logarithm (logarithm of base e).

The Simpson index (λ or D) was used to determine the rarity/diversity of different species at the sites (Simpson, 1949). Simpson index is usually a measure of diversity, which considers both species richness and evenness of abundance among the species present. In principle, it measures the probability that two randomly selected individuals from an area belong to the same species. This was calculated using the following equation:

$$\mathrm{D} = \frac{\sum n(n-1)}{N(N-1)} \tag{5}$$

(iii). Analysis of Extracted DNA for COI animal identification

The quantity and purity of DNA was measured using the NanoDrop™ One Microvolume UV–Vis Spectrophotometer (ThermoFisher Scientific, USA). The extracts were subjected to PCR amplification of a 650 bp region near the 5′ terminus of the COI gene following standard protocols. Sanger Sequencing was done using Zymo Research, ZR-96 DNA Sequencing Clean-up Kit™, Catalogue No. D4050. FinchTV (https://finchtv.software.informer.com/1.4/) was used to view the raw chromatogram files (.abi). basic local alignment search tool (BLAST) analysis (with default parameters) (Altschul et al., 1997) was performed on the National Center for Biotechnology Information (NCBI) (NCBI) website (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to enhance animal identification. Molecular confirmation of morphologically identified insects allows us to exactly identify and classify a particular insect species.

2.4.2. Statistical tests

Shapiro-Wilk's test was used to test for normality before analyzing the diversity indices using the Jamovi software version 2.3.18. Average bird, insect, and reptile species abundances were compared between CWs and WSPs using an independent *t*-test for normally distributed data and Mann-Whitney *U* test for non-normally distributed data. Graphs were plotted using Microsoft Excel. Differences among the group categories were assessed at a 5% level of significance ($P < 0.05$).

Table 3
Mann-Whitney U test for statistical test for bird abundance data recorded from field survey conducted in Iringa and Moshi Water Supply and Sanitation Authorities wastewater treatment systems between May and December 2021.

		Mann-Whitney U test				
		Mean	Standard deviation	Standard Error	U	P
Wastewater treatment system	WSPs	32.3	50.9	8.6	246	0.039*
	CWs	34.2	83.4	18.2		

* Significance difference ($p < 0.05$).

3. Results

3.1. Bird species abundance and diversity across CWs and WSPs

We found a total of 46 species belonging to 32 families and 13 orders of birds in the surveyed areas (see Appendices A and B for complete species lists). Waste stabilization ponds were found to host more individuals per species, with overalll abundance of 1132 birds, than constructed wetlands, which had a total of 718 individuals. Statistical analysis revealed significant differences in abundance ($U = 246$, $P < 0.05$). (See Table 3). The order Charadriiformes represented the highest bird species richness of 10 species (32.42%) in the WSPs, whereas the order Passeriformes had the highest species richness of 12 (86.77%) species in the CWs (Table 2). Fig. 4 show the pictures of different bird species captured during data collection in CWs and WSPs wastewater treatment systems.

We found that *Phoeniconaias minor* (Flamingo) represented the highest abundance (individuals) of bird species in the studied WSPs (282 individuals) (Appendix A). They were specifically found to spend time foraging at the Moshi Urban Water and Sanitation Agency WSPs between November and late May. *Actophilornis africanus* (African jacana) was the second most abundant species in the WSPs (112 individuals), followed by *Himantopus himantopus* (Black-winged stilt), and 83 individuals (Appendix A). In contrast, *Amblyospiza albifrons* (Grosbeak weaver) was the most abundant species in CWs (168 individuals), followed by *Lagonosticta rubricate* (African fire finch) with 154 individuals (Appendix B). The diversity of bird species was significantly higher in WSPs compared to CWs ($H = 2.881$; $H = 1.505$); Table 2:Fig. 5. Higher species richness was also found in the WSP with a Margalef Index (D) of 4.266 than in the CWs, which had a Margalef Index (D) of 2.757. Orders that presented only a single species were indicated to have zero diversity (Table 2).

A comparison of bird diversity between the two types of constructed

Table 2
Comparison of bird abundance and diversity indices in studied WSPs and CWs from field surveys conducted in Iringa and Moshi Water Supply and Sanitation Authorities wastewater treatment systems between May and December 2021.

Bird order		Number of family	Number of Species	Abundance	Percentage (%)	Shannon Diversity index (H)	Margalef Index (D)	Evenness Index (J′)	Simpson Index
Overall									
WSPs				1132	99.99	2.881	4.266	0.816	0.903
CWs				718	100	1.505	2.757	0.719	0.682
Charadriiformes	WSPs	4	10	367	32.42	1.828	1.524	0.794	0.805
	CWs	0	0	0	0.00	0.000	0.000	0.000	0.000
Ciconiiformes	WSPs	1	1	5	0.44	0.000	0.000	0.000	0.000
	CWs	0	0	0	0.00	0.000	0.000	0.000	0.000
Pelecaniformes	WSPs	3	7	161	14.22	1.762	1.181	0.906	0.800
	CWs	1	1	2	0.28	0.000	0.000	0.000	0.000
Phoenicopteriformes	WSPs	1	1	282	24.91	0.000	0.000	0.000	0.000
	CWs	0	0	0	0.000	0.000	0.000	0.000	0.000
Anseriformes	WSPs	1	3	91	8.04	1.031	0.443	0.939	0.6217
	CWs	1	1	11	1.53	0.000	0.000	0.000	0.000
Passeriformes	WSPs	4	4	67	5.92	1.023	0.714	0.738	0.580
	CWs	1	12	623	86.77	1.085	1.71	0.437	0.586
Apodiformes	WSPs	1	2	81	7.15	0.691	0.228	0.997	0.498
	CWs	0	0	0	0.00	0.000	0.000	0.000	0.000
Podicipediformes	WSPs	1	1	73	6.45	0.000	0.000	0.000	0.000
	CWs	0	0	0	0.00	0.000	0.000	0.000	0.000
Coliiformes	WSPs	1	1	3	0.26	0.000	0.000	0.000	0.000
	CWs	1	1	47	6.54	0.000	0.000	0.000	0.000
Accipitriformes	WSPs	1	1	2	0.18	0.000	0.000	0.000	0.000
	CWs	0	0	0	0.00	0.000	0.000	0.000	0.000
Gruiformes	WSPs	0	0	0	0.00	0.000	0.000	0.000	0.000
	CWs	1	2	32	4.46	0.000	0.000	0.000	0.000
Columbiformes	WSPs	0	0	0	0.00	0.000	0.000	0.000	0.000
	CWs	1	1	1	0.14	0.000	0.000	0.000	0.000
Coraciiformes	WSPs	0	0	0	0.00	0.000	0.000	0.000	0.000
	CWs	1	1	2	0.28	0.000	0.000	0.000	0.000

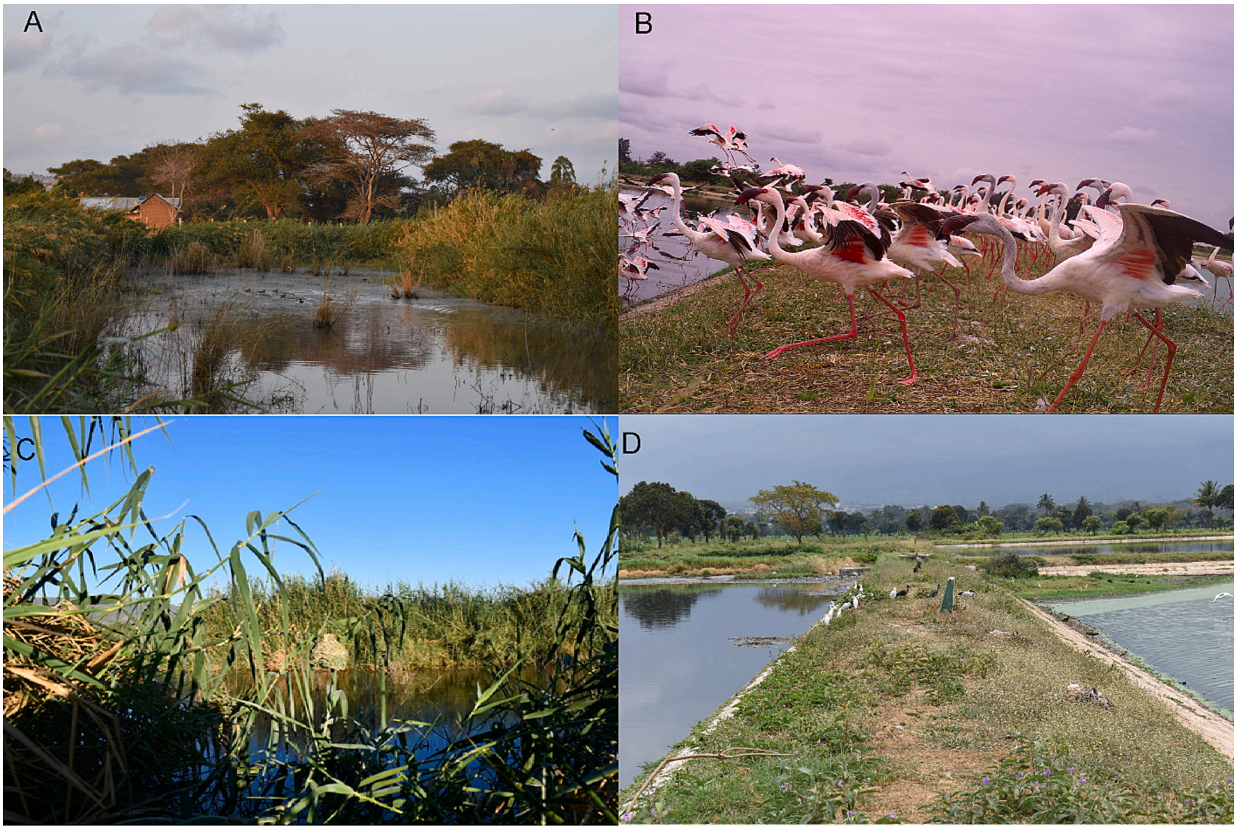

Fig. 4. Pictures of different birds species (A) *Anas undulata* (B) *Phoeniconaias minor* (C) *Lagonosticta rubricata* nests (D) *Egretta garzetta* and *Bostrychia hagedash* spotted during the field survey conducted in Iringa Water Supply and Sanitation Authority and Moshi Water Supply and Sanitation Authority between May and December 2021. (A) and (B) represent Constructed Wetlands (CWs), and (C) and (D) Waste Stabilization Ponds (WSPs).

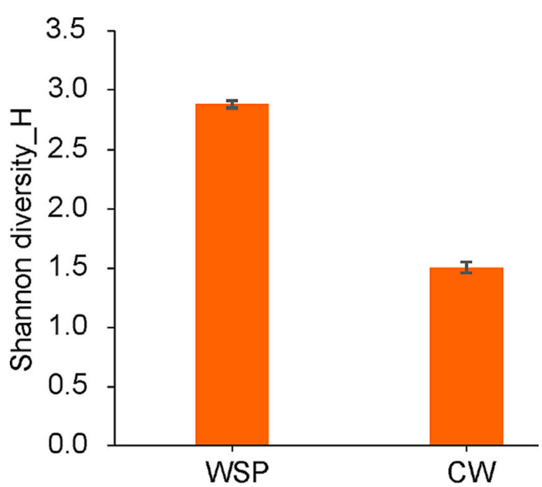

Fig. 5. Average (±SE) Shannon-Wiener diversity index of bird species identified between Wastewater Stabilization Ponds (WSP) and Constructed Wetlands (CW) systems of Moshi and Iringa Water Supply and Sanitation Authority between May and December 2021.

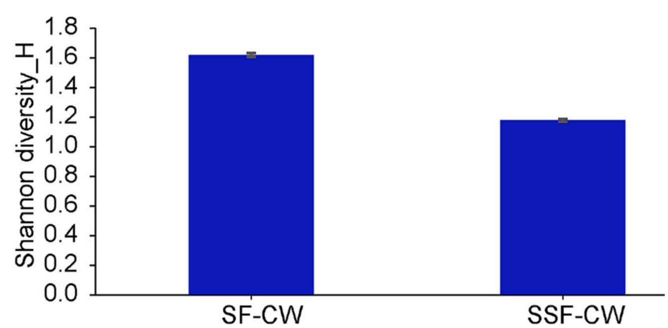

Fig. 6. Average (±SE) Shannon-Wiener diversity index of bird species identified between Constructed Wetlands (CWs) systems of sub-surface flow (SSF) CWs and Surface flow (SF) CWs of Moshi and Iringa Water Supply and Sanitation Authority between May and December 2021.

wetlands showed that surface-flow CWs yielded higher Shannon diversity than subsurface-flow CWs (Fig. 6; ($H = 1.62$; $H = 1.18$; $F_{1,20} = 682$, $p < 0.001$).

The MUWSA wastewater stabilization ponds tend to have more bird species throughout the year than IRUWASA waste stabilization ponds, with African jacana being abundant at all times (Appendix A and B for complete species lists). In addition, observational studies on subsurface flow CWs (BIL, NM-AIST, and CCBRT CWs) showed that there was a high abundance of birds, dominated by weaver birds that made their nests in the wetlands and fed on insects living in these wetlands. Moreover, our study noted that some important palearctic migrant birds tend to visit waste stabilization ponds and constructed wetlands, including *Hirundo rustica* (Barn swallow) and *Tringa nebularia* (Common greenshank). Observational features and conditions noted during the field survey show that bird abundance is high in areas with high food availability (WSPs), followed by the presence of vegetation (wetland plants in CWs) for nest-making birds.

3.2. Reptile species abundance across CWs and WSPs

Four different species belonging to three orders were recorded in this study. Reptile species, including *Varanus* (Monitor lizards), *Trachylepis*

Fig. 7. Reptiles from Wastewater stabilization ponds (WSP) field survey conducted in Moshi Water Supply and Sanitation Authority between May and December 2021, A-using a fisher net to trap reptile's ponds and B- Captured turtles by fishnets.

striata (Common lizards), *Stigmochelys pardalis* (Leopard tortoises) and *Testudines* (Turtles). Turtles were most common (Fig. 7), abundant, and exclusively found in the WSPs (100 individuals), followed by skinks and monitor lizards across both CWs and WSPs (Appendix E). During the direct catch of reptiles in the wastewater of WSPs, there was also an opportunistic catch of *Siluriformes* (catfish), which also dwell in these wastewater treatment systems. Moreover, in opportunistic searches, we found, several unidentified lizard species and tracks of snakes. The results indicate that WSPs had a higher abundance of individual reptile species, with a total abundance of 141 individual reptiles, compared to CWs, with only 25 individual reptiles. But this was not significant (t(6) = −1.29, P = 0.244). Shannon diversity index and Margalef richness index showed that both systems had near similar diversity indices Table 4.

3.3. Insect abundance and diversity across CWs and WSPs

The morphological identification of the specimens revealed that the collected species belonged to 14 morphologically similar insect groups. One individual specimen was picked from each group for sequencing to confirm their identity. All 14 species were differentiated using COI barcoding. Most of the amplified sequences were 860 bp in length. The National Center for Biotechnology Information (NCBI) basic local alignment search tool (BLAST) was used to check the homology between the retrieved sequences and the GenBank library or database of sequences. BLAST analysis revealed that the observed sequences of the four specimens had a similarity threshold ≥95%. The other 10 specimens had an average similarity of 80–94% h with the sequences in GenBank submitted (Appendix F). Molecular identification revealed that the insects belonged to 14 species, nine orders, and 12 families (Appendix C). The order Diptera, largely consisting of flies, was the most dominant in both CWs and WSPs (126 individuals), followed by Orthoptera, which consisted mainly of grasshoppers (114 individuals), followed by Hymenoptera and Araneae. Analysis of abundance and diversity the results indicate that WSP had slightly higher abundance and diversity indices than the CWs (Table 5).

Table 5
Insects abundance and diversity indices in studied WSPs and CWs data recorded from field survey conducted in Iringa and Moshi Water Supply and Sanitation Authorities wastewater treatment systems between May and December 2021.

Diversity Index/Parameter	Wastewater treatent system type	
	WSPs	CWs
Overall insect abundance	422	559
Orders	9	9
Family	12	12
Total number of insect species	14	14
Shannon Diversity Index (H)	2.501	2.419
Margalef Index (D)	2.137	2.065
Evenness Index (*J'*)	0.917	0.948
Simpson Index	0.896	0.907

Although we collected insects from both the CWs and WSPs, there was no statistical difference in the abundance of the collected species (t (26) = 0.884, p = 0.385). The total abundance of captured species varied between CW and WSPs (CW-559 individuals; WSP: 422 individuals).

Observational recording and surveys revealed that most of the visited CWs-WSP systems harbored a significant diversity of mosquitoes and whiteflies at different points in each of the CWs and WSPs and were mostly found to inhabit areas around the inlet and outlet zones and on wetland plant leaves.

4. Discussion

4.1. Bird species abundance and diversity across CWs and WSPs

Integrated wastewater treatment systems with artificial wetlands and waste stabilization ponds supported a diverse range of birds, insects, and reptiles. Our results show that waste stabilization ponds (WSPs) had a higher abundance and diversity of birds than constructed wetlands (CWs), which is consistent with the findings reported by Massawe

Table 4
Reptiles abundance and diversity indices in studied WSPs and CWs data recorded from field survey conducted in Iringa and Moshi Water Supply and Sanitation Authorities wastewater treatment systems between May and December 2021.

Reptiles		Abundance	Shannon Diversity index (H)	Margalef Index (D)	Evenness Index (*J'*)	Simpson Index
	WSPs	141	0.8199	0.6062	0.5914	0.4531
Overall	CWs	25	0.6859	0.3107	0.9896	0.4928

(2017) in study of bird species diversity between waste stabilization ponds and dump sites, which showed higher bird diversity in waste stabilization ponds. We recorded more individuals of birds in WSPs than in CWs, possibly because these sites provide good feeding grounds and contain more food, similar to natural wetland ecosystems, which may attract different bird species (Rajpar and Zakaria; Murray and Hamilton, 2012). Species richness was also higher in WSP than in CWs, which could be attributed by the latter reasons. Moreover, our study found that some bird species encountered in WSPs and associated CWs were of conservation concern, such as the Near-threatened Lesser flamingos (BirdLife International, 2022), which we found particularly after the main rainy season. A study by Rodrigo et al. (2018) on surface flow constructed wetlands conducted in Eastern Spain and Murray and Hamilton (2012) on waste stabilization ponds conducted in Australia also reported that these systems hosted various bird species of conservation concern. We also found that lesser flamingos were more abundant than other bird species in the studied WSPs (282 individuals). We also observed that CWs hosted a high abundance of Grosbeak weavers (*Amblyospiza albifrons*), based on a large number of bird nests. Rodrigo et al. (2018) and Semeraro et al. (2015) reported that surface flow CWs harbored more bird species than subsurface flow CWs in eastern Spain.

Furthermore, the significance of wastewater treatment is likely to increase, as it offers the most realistic means of treating wastewater in developing countries, where the demand for improved sanitation is intense to lift people out of poverty (Murray and Hamilton, 2010). We note that the treatment systems provide habitats for some permanent bird species and important migratory species that are of conservation importance. Generally, studies have shown that, on average, natural wetlands have more species and support higher abundances; however, certain artificial wetlands have the potential to support diverse communities (Mulkeen, 2018; Rajpar and Zakaria, 2013; Ma et al., 2004). Although studies by Massawe (2017) and Salehe (2021) focused on assessing the abundance of birds in waste stabilization ponds and reported a good diversity of birds, our study is the first to report the biodiversity of birds in CWs integrated with WSPs. Our study showed that integrated CWs and WSPs provide more ancillary benefits of refuge for some important bird species, thereby highlighting the ecological importance of these sites.

4.2. Reptile species abundanceand diversity in CWs and SWPs

We found two groups of different species of reptiles, that is, lizards and turtles, which is similar to Semeraro et al. (2015), who reported the presence of three species of reptiles in CWs of Melendugno, province of Lecce, Southern Italy. A high abundance of *Testudines spp.* (turtles) and Varanus *spp.* (monitor lizards) was found to live in WSPs. The *Varanus* and *Testudines spp.* might support the mechanical biodegradation of pollutants in wastewater treatment systems (Bui et al., 2020). Rozkošný et al. (2014) and Semeraro et al. (2015) reported that constructed wetlands provide a significant landscape element that serves as a habitat for reptiles, but their capacity to enhance the abundance of reptiles is much lower than that of natural wetlands because of their limited size and habitat conditions. As shown by the findings of this study, the sites have the potential to host different reptiles, but our study identified only a few species; more studies with diverse methods could be performed to reveal the real picture of varied reptiles from these systems. Moreover, this is the first study to report reptiles in wastewater treatment systems across Tanzania and East Africa.

4.3. Insects species abundance and diversity in CWs and WSPs

We found considerable diversity of insects in WSPs and CWs. Our study found that the orders Diptera, Orthoptera, and Hymenoptera were the most dominant in both CWs and WSPs. Similar results were also reported in a study by Ashfaq et al. (2018) conducted in the Saharo-Arabian region, which found a dominance of Diptera and Hymenoptera. Our observational study showed that most ground insects were found around the surface flow CWs compared to the subsurface flow CWs, possibly due to the lack of an open water area in the latter. Sartori et al. (2015) reported that CWs and constructed ponds for wastewater treatment play a significant role in supporting a great diversity of macroinvertebrates, as did Becerra-Jurado et al. (2010) in their study conducted in the Annestown River catchment, Co. Waterford, Ireland. In contrast, Gucel et al. (2012) reported no statistical significance in terms of insect abundance and diversity between constructed and natural wetlands in Cyprus. In our study, the high numbers of mosquitoes and whiteflies may be attributed to the presence of waterlogged habitats, thereby posing threats to humans and crops. Compared to natural ecosystems, studies have revealed more abundance in natural systems than in artificial wastewater treatment systems. Research reports such as studies by Ojija and Kavishe (2016) reported a natural wetland to have eight orders and 16 species; Wakhid et al. (2021) reported 75 species and ten orders of insects, while our study reported only 14 species and nine orders. On the other hand, findings from previous studies (Knapp et al., 2019; Almuktar et al., 2018; Ruhí et al., 2016; Kadlec and Wallace, 2008) have reported that artificial (constructed wetlands) and other wastewater treatment plants offer a wide variety of niches for various insects, and therefore has a significant abundance of insects which is near similar to those found in natural wetlands. Although this is the first report on the diversity of insects in CWs and WSPs in the country, to the best of our knowledge, we have managed to identify only 14 insect species; improving trapping techniques could yield more results. Since insects are primary pollinators and most of our study areas utilize treated wastewater from these systems for the irrigation of vegetables, we also thought that these sites are important and could be optimized to provide a wider home range of insects and assessed using various methods to establish a complete list of important pollinators at these sites.

To confirm the species that were identified by the morphological approach, molecular techniques were used, and the sequence similarity cut-off used for this study was ≥95%,which has been used to assign species names to different insect groups (Holman, 2004; Gibson et al., 2014; Zenker et al., 2016, 2020). The high number of specimens with a similarity of 79–94 could be explained by three scenarios: i) the obtained OTUs showed high intraspecific variation in the species with the matching sequence in GenBank, resulting in non-redundant BLAST assignments and/or assigned OTUs that can split or agglutinate into the same genus or species (Potter et al., 2017); (ii) the OTUs represent species phylogenetically closely related to the species with the matching sequence in GenBank, but without representative COI barcodes in GenBank;(iii) errors occurred during the sequencing process.

5. Conclusion and recommendation

Based on our findings, WSPs have greater abundance and diversity of birds, insects, and reptiles than CWs, with WSPs hosting bird species of conservation concern, including lesser flamingos. In addition, a comparison of surface flow and subsurface flow CWs revealed that surface flow CWs had a greater diversity of birds, insects, and reptiles than subsurface CWs. We conclude that both types of wastewater treatment systems should be promoted because they both provide a significant landscape element that serves as a habitat for a diverse population of birds, insects, and reptiles.

Our study highlights the potential of both CWs and WSPs in biodiversity development and also creates awareness among wastewater engineers and wildlife managers on the potential of developing modern wastewater treatment systems that can benefit the ecosystem by reducing pollutants and increasing the biodiversity of living organisms devoid of habitats due to human development. Furthermore, the study findings from this study further work as a tool for incorporating wastewater treatment into biodiversity conservation portfolios, as it has been demonstrated that they host different organisms that could have many benefits, including educational and recreational uses. At local and global scales, the information accrued will inform the expert on a better decision to align with the right path towards achieving SDG 6 and 15, as it creates an excellent portfolio for key players to enhance sanitation programs and life on land.

CRediT authorship contribution statement

Gerubin Liberath Msaki: Conceptualization, Methodology, Data curation, Formal analysis, Investigation, Writing – original draft, Project administration, Funding acquisition. **Karoli Nicholas Njau**: Conceptualization, Writing – review & editing, Supervision, Funding acquisition. **Anna C. Treydte**: Conceptualization, Methodology, Writing – review & editing, Supervision, Funding acquisition, Validation. **Thomas J. Lymo**: Conceptualization, Methodology, Writing – review & editing, Supervision, Funding acquisition.

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Declaration of Competing Interest

The authors declare no conflict of interest, as they are not affiliated with any organization or entity with any financial or non-financial interest in the subject matter discussed in this paper.

Data availability

All relevant data are included in the paper.

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Appendix A. Checklists of bird species identified using different methods during the field survey conducted in the Iringa and Moshi Water Supply and Sanitation Authorities waste stabilization ponds (WSPs) between May and December 2021

Order	Family	Scientific Name	Common name	Abundance		Total abundance	Relative abundance
				WSP MUWSA	WSP IRUWASA		
Charadriiformes	Jacanidae	*Actophilornis africanus*	African Jacana	112	0	112	0.099
	Recurvirostridae	*Himantopus himantopus*	Black-winged Stilt	83	0	83	0.073
	Charadriidae	*Vanellus tectus*	Black-headed Lapwing	9	0	9	0.008
	Charadriidae	*Vanellus spinosus*	Spur-winged Lapwing	41	0	41	0.036
	Charadriidae	*Vanellus armatus*	Blacksmith Lapwing	56	0	56	0.049
	Charadriidae	*Charadrius tricollaris*	Three-banded Plover	0	15	15	0.013
	Scolopacidae	*Tringa glareola*	Wood Sandpiper	1	5	6	0.005
	Scolopacidae	*Actitis hypoleucos*	Common Sandpiper	29	11	40	0.035
	Scolopacidae	*Tringa stagnatilis*	Marsh Sandpiper	0	2	2	0.002
	Scolopacidae	*Tringa ochropus*	Green Sandpiper	1	2	3	0.003
Ciconiiformes	Ciconiidae	*Leptoptilos crumenifer*	Marabou Stork	5	0	5	0.004
						0	0.000
	Ardeidae	*Bubulcus ibis*	Western Cattle Egret	23	13	36	0.032
	Ardeidae	*Egretta garzetta*	Little Egret	36	16	52	1.268
	Ardeidae	*Ardea cinerea*	Grey Heron	2	5	7	0.171
	Threskiornithidae	*Bostrychia hagedash*	Hadada Ibis	14	0	14	0.341
	Threskiornithidae	*Threskiornis aethiopicus*	African Sacred Ibis	13	0	13	0.317
	Threskiornithidae	*Plegadis falcinellus*	Glossy Ibis	11	10	21	0.019
						0	0.000
Pelecaniformes	Scopidae	*Scopus umbretta*	Hamerkop	12	6	18	0.016
Phoenicopteriformes	Phoenicopteridae	*Phoeniconaias minor*	Lesser Flamingo	282	0	282	0.249
	Anatidae	*Anas sparsa*	African Black Duck	28	0	28	0.025
	Anatidae	*Alopochen aegyptiaca*	Egyptian Goose	18	0	18	0.016
Anseriformes	Anatidae	*Dendrocygna viduata*	White-faced Whistling Duck	45	0	45	0.040
	Motacillidae	*Motacilla aguimp*	African Pied Wagtail	29	10	39	0.034
	Estrildidae	*Lagonosticta rubricata*	African Firefinch	14	0	14	0.012
	Corvidae	*Corvus albus*	Pied Crow	5	8	13	0.011
Passeriformes	Cisticolidae	*Apalis flavida*	Yellow-breasted Apalis	0	1	1	0.001
	Apodidae	*Tachymarptis melba*	Alpine Swift	38	0	38	0.034
Apodiformes	Apodidae	*Schoutedenapus myoptilus*	African Black Swift	43	0	43	0.038
Podicipediformes	Podicipedidae	*Tachybaptus ruficollis*	Little Grebe	66	7	73	0.064
Coliiformes	Coliidae	*Colius striatus*	Speckled Mousebird	3	0	3	0.003
Accipitriformes	Accipitridae	*Accipiter minullus*	Little Sparrowhawk	0	2	2	0.002
Total Abundance				1019	113	1132	1.000
Relative abundance							
No. of Species				27	15		
No. of Families				18	10		
No. of Orders				10	6		

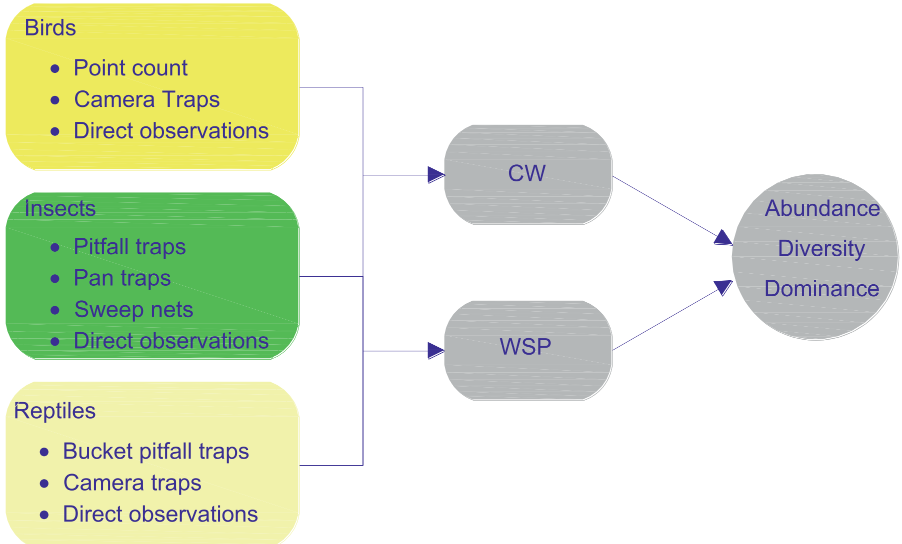
Appendix B. Checklists of bird species identified using different methods during the field survey conducted in the Iringa and Moshi Water Supply and Sanitation Authorities constructed wetlands (CWs) between May and December 2021

Order	Family	Scientific Name	Common name	Abundance			
				CW MUWSA	CW IRUWASA		
Anseriformes	Anatidae	*Anas undulata*	Yellow-billed Duck	0	11	11	0.015
Gruiformes	Rallidae	*Zapornia flavirostra*	Black Crake	0	4	4	0.006
	Rallidae	*Gallinula chloropus*	Common Moorhen	0	28	28	0.039
Passeriformes	Acrocephalidae	*Acrocephalus gracilirostris*	Lesser Swamp Warbler	0	7	7	0.010
	Hirundinidae	*Hirundo rustica*	Barn Swallow	0	2	2	0.003
	Fringillidae	*Crithagra citrinelloides*	African Citril	0	1	1	0.001
	Estrildidae	*Spermestes cucullata*	Bronze Mannikin	0	4	4	0.006
	Cisticolidae	*Cisticola marginatus*	Winding Cisticola	0	9	9	0.013
	Corvidae	*Corvus albus*	Pied Crow	0	1	1	0.001
						0	0.000
	Hirundinidae	*Hirundo smithii*	Wire-tailed Swallow	0	1	1	0.001
	Acrocephalidae	*Iduna natalensis*	African Yellow Warbler	0	1	1	0.001
	Estrildidae	*Lagonosticta rubricate*	African Fire finch	107	154	261	0.364
	Muscicapidae	*Melaenornis microrhynchus*	Grey Flycatcher	0	1	1	0.001
	Pycnonotidae	*Pycnonotus barbatus*	Common Bulbul	24	9	33	0.046
	Ploceidae	*Amblyospiza albifrons*	grosbeak weaver	135	167	302	0.421
Columbiformes						0	0.000
	Columbidae	*Spilopelia senegalensis*	Laughing Dove	0	1	1	0.001
Pelecaniformes	Ardeidae	*Ardea cinerea*	Grey Heron	0	2	2	0.003
Coraciiformes	Meropidae	*Merops pusillus*	Little Bee-eater	0	2	2	0.003
Coliiformes	Coliidae	*Colius striatus*	Speckled Moosebird	36	11	47	0.065
Total Abundance and Relative abundance				302	416	718	1
No. of Species				4	19		
No. of Families				4	18		
No. of Orders				2	7		

Appendix C. Insect species identified in collected specimens, field survey conducted in Iringa and Moshi Water Supply and Sanitation Authorities wastewater treatment systems between May and December 2021

S/N	Order	Family	Scientific Name	Insect Composition		Total Abundance in CWs and WSPs	Relative abundance
				CW	WSP		
1	Orthoptera	Acrididae	*Acrida cinerea*	73	41	114	0.116
2	Diptera	Sarcophagidae	*Sarcophaga tsinanensis*	42	84	126	0.128
3	Coleoptera	Dytiscidae	*Cybister cinctus*	34	24	58	0.059
4	Orthoptera	Gryllidae	*Teleogryllus commodus*	62	45	107	0.109
5	Lepidoptera	Pieridae	*Eurema hecabe*	31	13	44	0.045
6	Odonata	Coenagrionidae	*Ceriagrion fallax*	23	10	33	0.034
7	Coleoptera	Carabidae	*Pheropsophus sp/ Pheropsophus africanus*	42	18	60	0.061
8	Hymenoptera	Formicidae	*Echinopla australis*	86	23	109	0.111
9	Coleoptera	Carabidae	*Harpalinae sp.,*	27	8	35	0.036
10	Blattodea	Blattidae	*Australian cockroach*	21	51	72	0.073
11	Dermaptera	Pygidicranidae	*Challia fletcheri*	17	19	36	0.037
12	Araneae	Lycosidae	*Trochosa aquatica*	49	56	105	0.107
13	Blattodea	Blattidae	*Periplaneta japonica*	18	38	56	0.057
14	Diptera	Drosophilidae	*Drosophila willistoni*	17	9	26	0.027
	Total catch			559	422	981	1.000

Appendix D. Summary of the methods used to study bird, reptile, and insect species distribution across different wastewater treatment areas in Tanzania from May to December 2021



Appendix E. Total number of reptiles captured/spotted using different trapping methods during the field survey conducted in Iringa and Moshi Water Supply and Sanitation Authorities wastewater treatment systems between May and December 2021, CW = Constructed Wetlands, WSP = Waste Stabilization Ponds

			CW					WSP				
		Trapping methods	Pitfall traps	Observation	Camera traps	Fishnet	Total CW	Pitfall traps	Observation	Camera traps	Fishnet	Total WSP
Scientific Name	Common Name	Species type										
Varanus	Monitor lizard	Lizard	3	11	0	0	14	6	14	0	0	14
Stigmochelys pardalis	Leopard tortoise	Lizard	0	0	0	0	0	0	1	0	0	1
Testudines' `	Turtles	Turtles	0	0	0	0	0	0	35	0	65	100
Trachylepis striata	African striped skink	Lizard	8	3	0	0	11	16	10	0	0	26

Appendix F. BLAST analysis of sequences obtained from the molecular analysis of 14 insect specimens collected from field surveys conducted in Iringa and Moshi Water Supply and Sanitation Authorities wastewater treatment systems between May and December 2021

Sample No	Request ID	Predicted Organism	GenBank Accession	*E*-Value	HSP Length	% Identity
1	45DD5WRH013	*Acrida cinerea*	KX673195.1, EU938372.1	0	852 bp	93.31%,
2	45DD9YMX013	*Sarcophaga tsinanensis*	MW415423.1	0	856 bp	83.64%,
3	45DDENVB013	*Cybister cinctus*	DQ813674.1	0	842 bp	96.08%,
4	45DG0T7A013	*Teleogryllus commodus*	MF046167.1, MF046164.1	0	794 bp,	86.02%,
5	45DDRGAW013	*Eurema hecabe*	MT726499.1	0	625 bp	99.36%
6	45DE1PYF016	*Ceriagrion fallax*	NC_054209.1	0	756 bp	79.76%,
7	45DEME6P016	*Pheropsophus sp.*	KU937730.1	0	556 bp	97.84%
8	45DET7CT013	*Echinopla australis*	BK012443.1	0	806 bp	87.22%,
9	45FPAKKV01R	*Harpalinae sp.,*	MH940189.1	0	707 bp	89.82%,
10	45DF2PV7016	*Australian cockroach*	KX640825.1	0	863 bp	89.48%,
11	45DF6X6W016	*Challia fletcheri*	JN651407.1	0	817 bp	79.22%,
12	45DFA3NB013	*Trochosa aquatica*	MF467628.1	0	636 bp	92.45%
13	45DFFJ12013	*Periplaneta japonica*	AB126004.1	0	649 bp	95.7%
14	45DFNZ0R013	*Drosophila willistoni*	JN651407.1	0	825 bp	89.74%

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