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Characterization and evaluation of waste stabilization pond systems in Namibia

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ABSTRACT

Waste stabilization ponds (WSP) exist worldwide to treat wastewater, especially in warm climates. They are characterized by simple operation and maintenance and over 50 years many WSP were built in urban communities in Namibia. This study characterized and evaluated nine of these WSP systems in terms of their influent and effluent water quality and compared them with the requirements for water reuse in agriculture. In their current state none of them adhered with the Namibian or the new European reuse standards, especially due to tCOD concentrations above 100 mg/L caused by high algal fractions in the pCOD. The algae related chlorophyll-a concentrations correlated linearly with the pCOD and this correlation can therefore be used to fractionate the tCOD for further judgement. Additionally, microbial community analyses determined the composition of pathogens in the WSP influent and effluent, this helped to assess potential risks and distinguish between potentially toxic and non-toxic cyanobacteria. The EU requirement of less than 1,000 *E. coli* per 100 mL for fodder crop irrigation was only achieved with one WSP system which was enhanced with additional pre- and post-treatment. This research delivers a first overview of the current situation and can be used as basis to establish possible enhancement measures for existing WSP as well as to investigate possible effluent application in agricultural irrigation.

Key words: Africa, algae, cyanobacteria, microbial ecology, pathogens, waste stabilization ponds

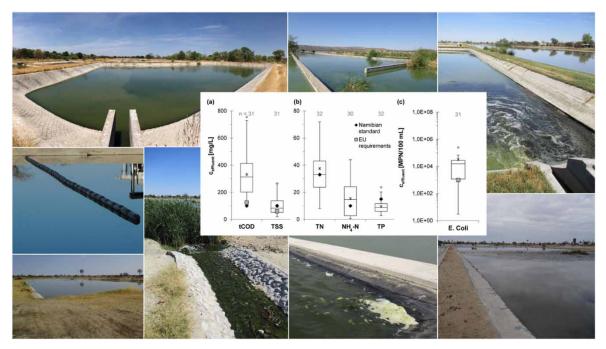
HIGHLIGHTS

- First systematic evaluation of nine pond systems in Namibia.
- All WSP do not fulfill national and international requirements for water reuse with their current design and operation.
- High COD due to algae requires adaptations to meet reuse standards.
- Acinetobacter were more abundant in the influent and Mycobacterium in the effluent.
- EU E. coli-requirement for irrigation of fodder crops is only reached with enhancements.

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GRAPHICAL ABSTRACT



INTRODUCTION

Waste stabilization ponds (WSP) are a cost-effective treatment option for towns in water scarce areas with only few disadvantages such as large land requirements, high methane emissions and high concentrations of chemical oxygen demand (COD) and total suspended solids (TSS) in the effluent due to algae (Mara 2004; Alves *et al.* 2020). Conventional pond system design consists of an anaerobic pond (AP) followed by a facultative pond (FP) and several maturation ponds (MP) (Von Sperling 2007).

In many African countries WSP are most common for wastewater treatment (Nikiema *et al.* 2013; Kihila *et al.* 2014; Bansah & Suglo 2016; Zacharia *et al.* 2019; Janeiro *et al.* 2020; K'Oreje *et al.* 2020; Edokpayi *et al.* 2021). But compared to Latin America (Nelson *et al.* 2004; Hernandez-Paniagua *et al.* 2014; Verbyla *et al.* 2016; Dias *et al.* 2017; Alves *et al.* 2020) and Australia (Buchanan *et al.* 2018; Gruchlik *et al.* 2018; Rose *et al.* 2019) limited information is available about their performance and potential for water reuse in agriculture. Additionally, the occurrence and composition of the algal biomass in WSP has been investigated worldwide (Pham *et al.* 2014; Wallace *et al.* 2015; Eland *et al.* 2018; Liu *et al.* 2020) but not much in Africa.

In regions without perennial receiving water bodies such as Namibia, WSP concepts also include an evaporation pond (EP) (DWAF 2008) to facilitate complete evaporation. Alternatively, reuse of the effluent, e.g. for irrigation, has a twofold benefit: the treated wastewater is put to use and provides an important source of water and nutrients (Mara 2009). But WSP are often overloaded due to high population growth and effluent values often exceed the required quality standards for water reuse (Ho & Goethals 2020).

Water reuse requires certain quality standards, with focus on COD removal and reduction of pathogens. Nutrients such as nitrogen, phosphorous and potassium can remain in the water and add additional value for the irrigation of plants as they complement fertilization. COD threshold values range from 100 mg/L in Namibia (DWAF 2012) to 125 mg/L under the new EU regulation (EU 2020) (Table 1) but are often exceeded due to the formation of algae biomass (Alves *et al.* 2020) that is not well retained in WSPs and thus detected in the effluent. Algae are not necessarily harmful and might even be beneficial for irrigation purposes, with the exception of potentially toxic species (Eland *et al.* 2018).

Depending on the reuse application, i.e. irrigation of green space, fodder crops or vegetables, different levels of reduction for pathogens are required. WSP rely on natural UV disinfection and the efficiency depends on the appropriate hydraulic retention times. Thus effluent quality has to be monitored carefully.

For Africa and particularly in Namibia only limited information about the water quality attained by WSP of different design, state of operation and general condition is available. Therefore, this study aims to provide a

Table 1 | Effluent water quality and performance comparison: load reduction and effluent concentrations judged against the Namibian (DWAF 2012) and EU (EU 2020) standards

System	WSP	#	TSS	tCOD	tCOD (w/o)	TP	TN	NH4-N	EC	E.Coli
FP+MP	D, G1, H	removal:	23 -85%(load)	38 - 77% (load)		17 - 53% (load)	25 - 57% (load)	49 - 99% (load)	0 - 27% increase	2 - 4 log ₁₀ units
	100	effluent value:	57 - 253 mg/L	182 - 568 mg/L	85 -303 mg/L	4.2 - 10.3 mg/L	26 - 89 mg/L	1 - 38 mg/L	440 - 2700 μS/cm	1.0E+03 - 1.7E+05 (MPN/100mL)
AP+FP+MP	A, B, E, F	removal:	23 -91% (load)	21 - 85% (load)	-	16 - 70% (load)	32 - 87% (load)	18 - 99% (load)	2 - 109% increase	2 - 5 log ₁₀ units
		effluent value:	49 - 266 mg/L	198 - 647 mg/L	66 - 327 mg/L	5.1 - 23.7 mg/L	19 -53 mg/L	1 - 31 mg/L	635 - 3150 μS/cm	3.0E+00 - 2.8E+04 (MPN/100mL)
AP+FP+MP	C, I	removal:	no effluent	no effluent	-	no effluent	no effluent	no effluent	0 - 50% increase	2 - 4 log ₁₀ units
w/o effluent		last pond value:	26 - 188 mg/L	116 - 425 mg/L	75 - 95 mg/L	2.8 - 9.7 mg/L	8-41 mg/L	1-20 mg/L	754 - 1352 μS/cm	1.3E+03 - 2.5E+05 (MPN/100mL)
PreT+FP+MP	G2	removal:	48 - 71% (load)	55 -71% (load)	14	0 - 13% (load)	46 - 51% (load)	49 -74% (load)	0	3 - 4 log ₁₀ units
		effluent value:	93 - 144 mg/L	371 - 466 mg/L	131 - 338 mg/L	11.1 - 12.8 mg/L	40 - 56 mg/L	16-36 mg/L	818 - 1062 μS/cm	1.0E+03 - 5.6E+04 (MPN/100mL)
PreT+FP+MP+PostT	G3	removal:	86 - 96% (load)	64 - 84% (load)	.=	64% (load)	45 -85% (load)	55 - 98% (load)	2 - 32% increase	5 - 6 log ₁₀ units
		effluent value:	20-48 mg/L	173 - 202 mg/L	59 - 144 mg/L	4.8 - 13.5 mg/L	15 - 31 mg/L	2 - 22 mg/L	724 - 1072 μS/cm	1.5E+01 - 3.0E+02 (MPN/100mL)
national and international reuse values										
Namibian Reuse Standard (DWAF, 2012)			< 100 mg/L	< 100 mg/L	-	< 15 mg/L	< 33 mg/L	< 10 mg/L	:-	2 5.
EU Reuse Regulatio	n (EU, 202	20)	< 60 mg/L	< 125 mg/L	1-	-	-	-		< 1.0E+03 (MPN/100mL)
FAO (moderate) (Ayers and Westcot, 1985)			< 100 mg/L	•	:-	< 13 mg/L	< 30 mg/L	< 5 mg/L	< 3000 μS/cm	4 2

AP = anaerobic pond, FP = facultative pond, MP = maturation pond, PreT = pretreatment, PostT = post treatment, WSP = w aste stabilization pond system A – I, TSS = total suspended solids, tCOD = total chemical oxygen demand, tCOD (w/o) = tCOD w ithout algae, TP = total phosphorous, NH4-N = ammonia, EC = electrical conductivity, E coli = Escherichia Coli, EU = European Union, FAO = Food and Agriculture Organization

The WSP are grouped according to their system setup. Green indicate WSP that fulfil the requirements, yellow show concentrations up to 20% above the requirements or values for slight to moderate irrigation restrictions (Ayers & Westcot 1985) and in red are all the WSP that are more than 20% above the requirements.

first data set of nine WSP systems in the north of Namibia, focusing on physical-chemical parameters and the microbial community. In particular, we investigated the microbial community composition with a focus on pathogens and cyanobacteria using modern sequencing methods. Such data has not yet been published and therefore adds new insights into the diversity of the microbial community in addition to standard indictor organisms.

Additionally, the WSP systems were compared by means of standard physical-chemical wastewater parameters to generate an overview of inflow and effluent values of mostly overloaded WSP with various configurations. This also includes the calculation of total COD effluent values without algal matter in order to compare the effluents with the national and international COD requirements which could otherwise only be reached with high technological input.

MATERIAL AND METHODS

Data collection at nine WSP in north Namibia

This study characterized and evaluated nine WSP in North Namibia, where the majority of the country's population resides. Background information about each WSP was collected through semi-structured interviews with the local operation managers. The water quality was analyzed between 2017 and 2020. Eight of the WSP (A–F, H and I) remained in their original setup over the whole research period. They were anonymized to ensure confidentiality. One plant (G) has been equipped with a mechanical and anaerobic pretreatment, i.e. a micro sieve and an upflow anaerobic sludge blanket (UASB) reactor, in 2018 to reduce COD and TSS. In 2019 a post-treatment employing a rock filter to further decrease COD, algae and pathogens (Sinn & Lackner 2020) was installed at WSP G. At this full-scale installation further enhancement measures such as sludge removal and baffles were also installed and investigated. G1 refers to samples from 2017 before the enhancement, G2 to samples that were taken after the installation of the pretreatment in 2018 and G3 to the samples after the start-up of the post-treatment in 2019.

The daily evaporation was measured at WSP G with an iMetos SD weather station (Pessl Instruments, Austria) over four years. Due to similar climate conditions at all nine communities, an average evaporation rate of 5.4 mm/d was applied to calculate the theoretical water loss for each WSP. The surface area of the ponds was either provided during the interviews by the responsible person or estimated from aerial images. There were no design documents available, and therefore, no information regarding design values with respect to population or related loading rates are provided.

Sampling and analysis

1 L grab samples were taken during dry weather seasons at the influent of the WSP system and at the outflow of each WSP system once in October 2017 and twice in May and June 2019. Due to the local situation and lab availabilities, only a limited number of samples could be analyzed. Long distances and local road conditions resulted in different sampling times, mostly during morning hours between 10 and 12 am, but some also in the early afternoon (2 till 4 pm). Samples were transported to the laboratory with a cooler box and analyzed within 24 h. COD analyses were performed from homogenized (homogenizer: T 25 digital Ultra-Turrax, IKA, Germany) (total COD (tCOD)) and filtered (0.45 μ m, Whatman membrane filters, ME 25) water samples (soluble (sCOD)). The particulate fractions of the COD (pCOD) were calculated as subtraction of the sCOD from the tCOD. The following parameters were analyzed with Hach cuvette tests using a spectral photometer (DR 2800, Hach Lange, Germany): tCOD, total nitrogen (TN) and total phosphorus (TP) from homogenized samples as well as sCOD, ammonium (NH₄-N), nitrite (NO₂-N), nitrate (NO₃-N), phosphate (PO₄-P) and potassium (K⁺) from filtered samples. Additionally, chlorophyll-a concentrations were determined according to German standard methods for the examination of water, wastewater and sludge (DIN 38409-60) (DIN 2019).

TSS and volatile suspended solids (VSS) were measured by German standard methods (DIN 38409-2, 1987) (DIN 1987) using Whatman 934-AH glass microfiber filters. Electrical conductivity (EC), pH, temperature and dissolved oxygen (DO) were analyzed with a WTW multimeter 3410 (Xylem Analytics, Germany). Additionally, the concentrations of total coliforms and *Escherichia coli* (Colilert-18), Enterococci (Enterolert) and *Pseudomonas aeruginosa* (Pseudalert) were measured with an IDEXX system employing a Quantiy-Tray/2000 (IDEXX, Germany).

16S rRNA amplicon sequencing to determine the composition of cyanobacteria

Samples were collected as biological triplicates at six (A, B, D, G, H and I) of the nine WSP in 50 mL centrifuge tubes to determine the composition and abundance of cyanobacteria and pathogens. These tubes were centrifuged at 8,000 g and 4 °C for 25 minutes. The supernatant was discarded and pellets were stored at 4 °C overnight and brought to Germany for further downstream analysis. Total genomic DNA was extracted using the Fast DNA Spin kit for soil (MP Biomedicals, Germany) according to a modified manufacturer's protocol (Orschler *et al.* 2019). DNA concentration was analysed using Qubit 3.0 Fluorometer with Qubit dsDNA HS kit (Thermo Fisher Scientific, Germany). Further, the DNA was used to perform 16S rRNA amplicon sequencing according to the method by Agrawal *et al.* (2020). Then the raw data was filtered for the sequences associated with the phylum cyanobacteria and the composition of the cyanobacteria was determined in R using ggplot. The abundance of genera associated with pathogenic bacterial species, was determined according to a previous study (Agrawal *et al.* 2020).

Data processing

The collected data was analyzed with conventional statistical methods. Concentrations below the limit of quantification (LOQ) were not considered for evaluation. COD measurements influenced by chloride concentrations above 1,500 mg/L were also discarded. The performance of the WSP systems was evaluated by the water quality at the inflow in comparison to the effluent quality. Additionally, the performance was related to the code of practice for wastewater reuse of Namibia (DWAF 2012), the regulation on minimum water requirements for water reuse in the European Union (EU 2020) as well as the Food and Agriculture Organization (FAO) water quality standards (Ayers & Westcot 1985). In Table 1 values shown in green indicate WSP that fulfil the requirements, WSP in yellow indicate concentrations of not more than 20% above the requirements and all WSP in red are not even close to the standards (>> 20% above the requirements).

Semi-structured interviews

Semi-structured interviews with operation staff in 2017 gave important background information on each WSP system especially with regards to the total population and the population connected to the sewers and wastewater treatment plants. This information is presented in Supplementary Material, Table SI 1. Only in a few towns technical drawings were available. Data from flow meters existed from WSP A, B and G. For the others, values were estimated by the operators (WSP C, D, E, F, H and I).

RESULTS AND DISCUSSION

Waste stabilization pond systems - introduction of the study sites

This is the first comprehensive evaluation of WSP in Northern Namibia. These systems have been built since the 1970s. Almost every ten to fifteen years extensions were implemented to accommodate the constant urbanisation and population growth. The size of the towns connected to these WSP ranged from 2,300 to 50,000 inhabitants; with connection rates from 30 to 70% (Supplementary Material, Table SI 1). Therefore, further upgrades or enhancements would be necessary to accommodate the total population. According to the Namibian code of practice for pond systems, WSP should only be designed for up to 5,000 population equivalents (PE) (DWAF 2008). In consequence, eight out of nine communities would need new treatment systems. This is not possible, neither financially nor in time. Additionally, there is no local experience in operation and maintenance of activated sludge systems or trickling filters. Therefore, WSP remain the only reasonable solution under the given circumstances.

All available details from the operators, inflow quantities and information obtained from satellite images are summarized in Supplementary Material, Table SI 1. The surface areas of most WSP ranged between 7,000 and 50,000 m² while two plants considerably exceeded these values with a surface areas of 200,000 and 280,000 m². At the same time, the per capita land requirements varied from 1 to 26 m²/cap. Von Sperling (2007) recommends values of 3 to 5 m²/cap, which means that six plants were overdesigned and should have the capacity to serve more households. Two WSP were already too small and would need upgrades. The depth and consequently the pond volume was available from five WSP. For the remaining four WSP the volume was calculated with an average depth of 3.5 m for AP and 1.5 m for FP (Von Sperling 2007). The volumes ranged from 8,300 to 360,000 m³. The theoretical hydraulic retention times (HRT) were between 12 and 302 days as calculated from either the measured inflow or estimated values and the corresponding pond volumes. By these

estimates, three of the plants (B, D, H) were already below the 40 days minimum HRT of MP required for water reuse in Namibia (DWAF 2012). This calculation does not take into account short-circuiting and the reduced volume due to sludge accumulation over the years. At one plant, after 15 years of operation, the sludge layer in the primary facultative pond was between 20 and 70 cm thick. So roughly 1/3 was filled with sludge. However, in the subsequent ponds the sludge layers were much thinner. Thus, the available volumes are probably less and the real HRTs shorter particularly in the first ponds. However, as the sludge layers could not be determined for the other pond systems and for comparison purposes the theoretical HRT was calculated as a first estimate.

The nine WSP not only differed by size and HRT but also by the design of the different ponds. Four plants had one treatment train, five had two parallel trains. Three treated the raw sewerage directly in FP without AP. The number of MP varied between one at the smallest plant and up to seven at the oldest plant (D) constructed in the 1970s. All but one WSP had one or two EP at the end (Supplementary Material, Table SI 1).

Influent characteristics

The water quantity entering the different plants was between 83 and 2,160 m³/d depending on the town size and the number of people connected. At three plants the actual inflow was measured with flow meters whilst at the others an average inflow of 120 L/cap/d (Supplementary Material, Table SI 1) was estimated. This inflow was confirmed by the local operators but lay below the average consumption of 163 L/cap/d in Windhoek calculated by Uhlendahl et al. (2010). It, however, reflected the local living standard and accounts for water losses in the systems and also from households with standpipes but no sewer connection. Based on this information the PE loads entering each plant were calculated and compared with typical values in Europe (Germany) and Africa (Uganda) (Tchobanoglous et al. 2014) as well as with a wastewater treatment plant using UASB and rotating biological contactors (RBC) in Outapi, Namibia (Müller 2017) (Figure 1 and Supplementary Material, Table SI 1). The total COD (tCOD) was between 21 and 160 g/(PE·d) with an average of 88 g/(PE·d). These values were lower than in Germany or in Uganda with 123 g/(PE·d) (calculated as twofold the biological oxygen demand (BOD) (Tchobanoglous et al. 2014)) but only slightly higher than the 75 g/(PE·d) of the Namibian comparison. The TSS values of 10 to 50 g/(PE·d) and 27 g/(PE·d) on average, were at a similar level as the study in Namibia with 29 g/(PE·d), but lower than in Uganda (48 g/(PE·d)) and Germany (89 g/(PE·d)). The PE loads for TN and TP were 5 to 13 g/(PE·d) and 0.5 to 1.7 g/(PE·d), respectively. The TN values were lower than in Germany (11-16 g/(PE·d)) but similar to Uganda (8-14 g/(PE·d)) and Namibia with 6 g/(PE·d) on average. The TP load in this study was within the same range as in Germany and Namibia, but more than double the load in Uganda. This indicates that the consumption patterns in Namibia were closer to Germany than to Uganda. Overall the loads were within the given ranges and therefore the inflow estimations were reasonable.

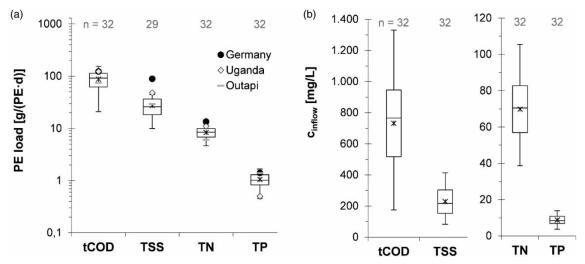


Figure 1 | Inflow parameters as (a) population equivalent (PE) load of the nine waste stabilization ponds (WSP) in comparison with typical values in Germany and Uganda (Tchobanoglous *et al.* 2014) as well as with a biological treatment plant in Namibia (Müller 2017) and (b) inflow concentrations (c_{inflow}) of the nine WSP for total chemical oxygen demand (tCOD), total suspended solids (TSS), total nitrogen (TN) and total phosphorus (TP).

The inflow concentrations of all plants varied considerably (Figure 1 and Supplementary Material, Table SI 2). As all samples were grab samples over a period of two years there were variations within one WSP as well as between all systems. Especially the tCOD of the single samples covered a wide range from 175 mg/L (plant I) to 1,331 mg/L (plant E) with an average of 733 mg/L. The TSS values were 83 to 413 mg/L and 229 mg/L on average. Nutrient concentrations were between 39 and 106 mg/L for TN and between 3.8 and 13.9 mg/L for TP. Inflow concentrations of soluble COD (sCOD), particulate COD (pCOD), NH₄-N, PO₄-P, total coliforms, *E. coli*, Enterococci, pH and EC are presented in the supplementary information (Figure SI 1). This data provides a first glance at the wastewater composition in Namibia and is valuable for further research on WSP.

Effluent characteristics

The tCOD concentrations, taken at the overflows to the EP or from the last MP of each WSP, ranged between 116 and 755 mg/L and were all above the Namibian standard of 100 mg/L and mostly also above the EU standard of 125 mg/L (Figure 2 and Supplementary Material, Table SI 2). This reflected very well the finding of Alves *et al.* (2020) in Bolivia for similar size WSP. The TSS concentrations showed an average concentration of 103 mg/L and thus almost met the local standard of 100 mg/L. They were however above the EU requirements (EU 2020) of 60 mg/L. There are no requirements from the EU (EU 2020) for nutrient concentrations as they pose no harm for water reuse and are even considered necessary for plant growth. On the contrary, in Namibia (DWAF 2012) there are standards for TN, and NH₄-N (33 mg/L and 10 mg/L, respectively) which were just below the measured average effluent values of 38 mg/L and 15 mg/l. The TP concentration of 15 mg/L in the Namibian standard was above the measured values (average 10 mg/L) and not critical. Further removal might be required for TN and NH₄-N though.

In Figure 3, the WSP were grouped according to their system setup: WSP A, B, E and F had the traditional setup of AP, FP and MP and were all overflowing. WSP C and I had the same setup but had no effluent. WSP D, G, and H formed the third group and represented the systems without AP. The systems without AP had higher effluent values for tCOD and TSS, whilst the group without effluent was close to the required standard due to the long HRT. With regards to the volumetric loading rate of the AP all plants were below the design parameters of 0.10–0.35 kg BOD₅/m³/d suggested by Von Sperling (2007) (Supplementary Material, Table SI 1). At two of the plants without AP the surface loading rate of 140 kg BOD₅/ha/d of the primary FP was at the lower end of the design values (Von Sperling 2007). The surface loading rate of plant H was almost double the design value. The concentrations of *E. coli* were at a similar level in all groups. Altogether there was a wide divergence between all nine systems with hardly any compliance to national or international standards. Within the African context soil salinization needs consideration as well as salt acceptance of the irrigated plants. All the WSP

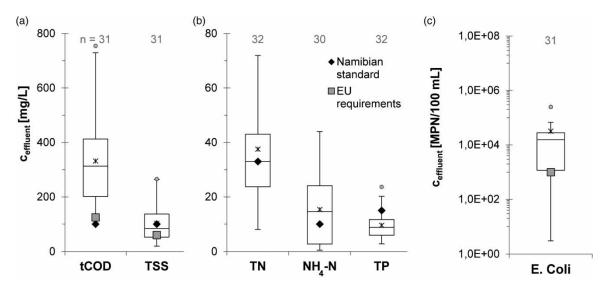


Figure 2 | Effluent quality ($c_{effluent}$) in comparison with the Namibian Reuse Standard (DWAF 2012) and the EU requirements (EU 2020) for (a) total chemical oxygen demand (tCOD), total suspended solids (TSS), (b) total nitrogen (TN), ammonia (NH₄-N), total phosphorus (TP) and (c) *Escherichia coli* (*E. coli*).

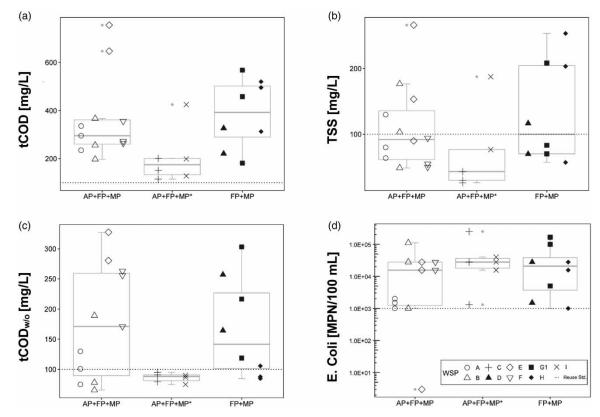


Figure 3 | Effluent quality grouped according to the waste stabilization pond (WSP) system setup: AP = anaerobic pond, FP = facultative pond, MP = maturation pond, P = matu

showed EC values between 440 and 3,150 μ S/cm which were within the slight to moderate restrictions of the FAO (Ayers & Westcot 1985) and therefore allow for fodder irrigation.

Algal biomass

An important aspect to consider with regards to high tCOD effluent concentrations are algae. In WSP they find ideal growth conditions, especially in warm climates, and thus contribute considerable amounts of biomass to the pCOD in the effluent of MP. Measurements of chlorophyll-a can provide a first indication of the algae fraction in the pCOD. For the nine WSP, the chlorophyll-a concentrations ranged from 58 to 1,675 μ g/L and correlated linearly with the pCOD. These values were in the same range as in Bolivia (Alves *et al.* 2020). Based on this relation, the pCOD due to algae was deducted from the tCOD and an adapted tCOD without algae (tCOD $_{w/o}$) was estimated resulting in values between 59 and 339 mg/L and an average of 162 mg/L (Figure 4). These values did not yet reach the required effluent standards of the EU (125 mg/L) and Namibia (100 mg/L), but gave an indication that either algae have to be removed or there is the need for different standards if the reuse water originates from WSP. In situations where chlorophyll-a analysis is not possible, the sCOD can also provide a first approximation. These concentrations will indicate whether the given standards for tCOD are reachable with removal of the algae. Too high values are an indication of an overloaded WSP or of possible non-biodegradable COD. In such cases, further enhancement measures have to be considered. For the nine plants the average sCOD concentration was exactly 100 mg/L with values between 47 and 251 mg/L.

Microbial ecology

Microbiological parameters are also important for water reuse and water quality. Typically, the water quality is judged by cultivation methods capturing indicator bacteria, with total coliforms and $E \, coli$ being the most popular indicators (Liu *et al.* 2020). Total coliforms reached an average concentration of $3.3 \times 10^{+06} \, \text{MPN/100} \, \text{mL}$ at the outflows which resulted in a reduction of only 2 log values. A slightly higher reduction of 3 log values was observed for $E. \, coli$ and Enterococci with average concentrations of $3.2 \times 10^{+04} \, \text{MPN/100} \, \text{mL}$ and

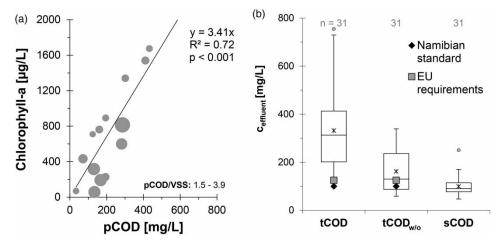


Figure 4 | (a) Effluent ratio chlorophyll-a versus particulate chemical oxygen demand (pCOD) with the size indicating the relation of pCOD to volatile suspended solids (VSS). (b) chemical oxygen demand (COD) effluent concentrations ($c_{effluent}$) as total COD (tCOD), calculated tCOD without algae content (tCOD_{w/o}) and soluble COD (sCOD) in comparison with the Namibian Reuse Standard (DWAF 2012) and the EU requirements (EU 2020).

 $1.3 \times 10^{+04}$ MPN/100 mL, respectively. The EU (2020) stipulates less than 1,000 *E. coli* per 100 mL for the irrigation of fodder crops. Only one plant with a rock filter as post-treatment reached this value. Therefore, it needs to be evaluated if the natural disinfection processes in the MP can be improved or if other measures such as filters or technical disinfection have to be implemented. Further supporting parameters are presented in the Supplementary Material, Figure SI 2.

The determination of fecal contamination through indicator bacteria is a common procedure, it may however not provide an accurate or complete picture of the pathogens present in a water sample. Therefore, a sequencing approach was also used to gain an overview over the diversity and dynamics of pathogenic genera without the bias of culturability. The composition and abundance of the genera consisting of pathogenic species, called 'pathogenic genera', was thus analyzed in the influent and effluent samples of six of the WSPs (Figure 5). Among the influent samples, the relative abundance of these pathogenic species ranged between 8 to 42%, whereas for effluent samples it was between 1 to 20%. In WSP G, the fraction of pathogenic genera was highest in both influent and effluent. Overall, we observed a reduction in the abundance of pathogenic genera in the effluent of all WSP. In total, 79 genera were found across all samples. Eighteen pathogenic genera were shared among the influent samples and four among the effluent samples (Supplementary Material, Figure SI 3). Although the cumulative abundance of low abundant pathogenic genera (i.e. having less than 0.1% abundance) was highest in most samples, Acinetobacter was the most dominant across the influent samples (accounting for up to 16% of the total microbial abundance) and Mycobacterium was dominant (accounting for up to 14% of the total microbial abundance) across the effluent samples (Figure 5).

Additionally to the pathogens, sequencing analysis can also provide information about the diversity of cyanobacteria. All WSP showed higher overall abundance of cyanobacteria in the effluent compared to the influent (Figure 6(a)). But in the effluent their relative abundance (in relation to all bacteria) varied significantly from 0.45 (WSP I) to 25% (WSP H). Figure 6(a) shows the different composition of the biomass samples within the cyanobacteria at genus levels for the six WSP. Overall, *Synechococcus* was most dominant (i.e. up to 45%), followed by *Cyanobium* (approximately. 7%) and *Chlorella* (approximately. 5%); others showed a relative abundance lower than 5%. The identification of the respective genera is particularly important with regard to potential toxicity during the decomposition of these bacteria. A detailed heatmap with potentially toxic and non-toxic genera is presented in Figure 6(b).

Performance efficiency

In order to judge the performance of each WSP not only effluent concentrations needed to be considered but also removal efficiency. This is especially important as all WSP experienced high evaporation due to large surface areas. Three of the plants had water losses below 12%, four between 26 to 49% and two lost 100% of their water (Supplementary Material, Figure SI 4), which is the original aim according to the code of practice in Namibia (DWAF 2008).

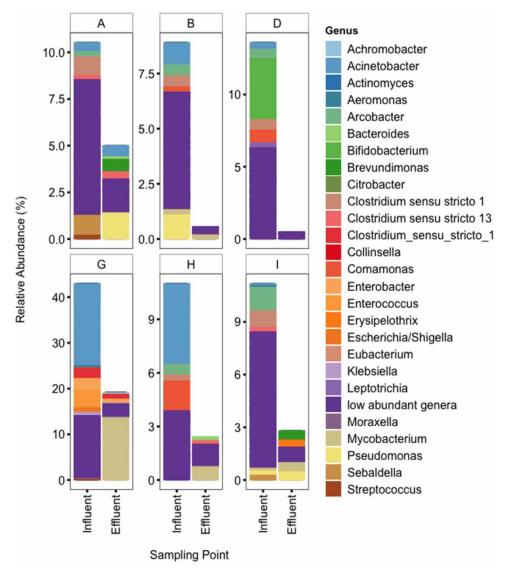


Figure 5 | Bar plot showing the relative abundance of pathogenic genera found in each sample. Pathogenic genera having abundance <0.1% were categorized as 'low abundant genera'.

The calculated load removal is presented in Table 1. The WSP are grouped according to their system setup. WSP D, G1 and H were only composed of FP and MP whilst WSP A, B, E and F were also designed with an AP upfront. WSP C and I evaporated all water and therefore there was no effluent load. Possible enhancements are reflected in WSP G2 with pre-treatment measures, FP and MP. WSP G3 had an additional post-treatment stage.

As mentioned before, none of the configurations reached the effluent requirements for tCOD when algae were included. The plants with the traditional design of FP, MP and some with AP were not able to reach the standards for TSS, tCOD, NH₄-N and *E. coli*. WSP with large surface areas and no effluent reached better values for TSS and tCOD_{w/o}, but still did not meet the required NH₄-N and *E. coli* values. With 100% evaporation they present no viable solution as no water is available for irrigation. The WSP with enhancement measures did not show much improvement when only equipped with pretreatment (WSP G2). In combination with post-treatment (WSP G3) the requirements were met for TSS, TP, TN and *E. coli*. For NH₄-N and tCOD_{w/o} WSP G3 was just above the standards. Especially for the hygienic parameter *E. coli* it was the only plant setup with acceptable values.

Challenges for reuse of WSP effluent

All communities experience an increasing need of irrigation water for year-round fodder production due to changing rainfall patterns. Additionally, solutions are needed to accommodate for fast population growth, reuse of

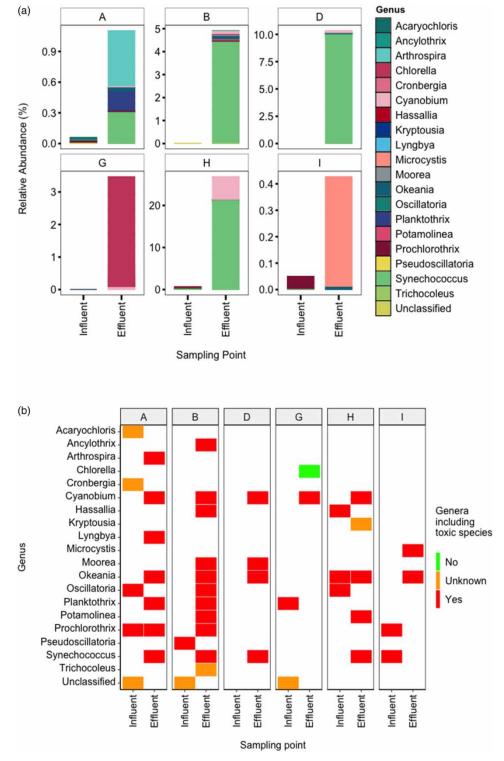


Figure 6 | (a) Bar plot showing the relative abundance of the genera associated with cyanobacteria found in the waste stabilization pond systems (A, B, D, G, H and I). (b) Heatmap showing the composition of the cyanobacteria at genus level. The color designates the difference between genera having toxic and non-toxic species.

water as well as nutrients, production of crops and reduction of greenhouse gas emissions (Hernandez-Paniagua *et al.* 2014; Shelef & Azov 2000). With small improvements WSP can reach a higher capacity and better effluent quality which is particularly essential for fast growing communities with the need of agricultural reuse and without receiving waters (Butler *et al.* 2017).

The results from all WSP showed considerable differences in the effluent quality (Table 1 and Supplementary Material, Table SI 2). In their original stages none of the WSP adhered fully to the required standards for water

reuse. Either all water was evaporated or TSS, tCOD, nutrients and bacteria were not adequately removed. Algae also need consideration as they cause high tCOD. On the other hand, this biomass presents an important soil enhancer that improves the water-holding capacity of the soil (Mara 2004). Other WSP for example in Brazil also reached average COD effluent values of 100 to 150 mg/L even with rock filters (Von Sperling *et al.* 2007) and the water was still recommended for reuse. In the case of agricultural irrigation there was no need to remove algae in coarse filters (Von Sperling & De Andrada 2006), so the COD would be even higher. Juanicó & Milstein (2004) also found high COD effluents during a study in Israel but stated clearly that the organic matter formed by algae growth has no relationship with the solids originally present in the sewage. Also in South Europe countries are struggling to meet the water reuse regulation with constructed wetlands, especially for microbial parameters, and therefore, the reuse purpose has to be adopted to the water quality (Lavrnić & Mancini 2016). A further option is a classification with crop restrictions and obligations for irrigation methods (EU 2020) to ensure a multi-barrier approach.

CONCLUSIONS

According to the feedback of the WSP operators the main purpose of WSP in Namibia is seen in evaporation whilst the wastewater treatment itself is only secondary, especially because so far no reuse projects have been implemented. Therefore, operation and maintenance are mostly neglected. Overall WSP operation and management have to be revised focusing on reuse rather than on evaporation. Especially, as all towns struggle with fast growing populations and their WSP are overflowing into the surrounding environment with no perennial streams. As basis for further in-depth studies on WSP this research presents a first systematic evaluation of nine WSP in North Namibia:

- Pond surface areas vary considerably between 1 to 26 m²/cap and HRT from 12 and 302 days depending on the design, date of construction, current population and surface extensions. Due to a multitude of local influences the effluent values do not correlate with the surface area or HRT.
- PE loads in the raw sewerage are within the literature range and therefore inflow estimations between 83 and 2,160 m³/d are reasonable.
- Removal efficiencies of up to 85% of tCOD, 64% TP and 87% of TN are reached by single WSP.
- The EU requirement of less than 1,000 *E. coli* per 100 mL for the irrigation of fodder crops is only reached with enhancement measures.
- Acinetobacter is the most dominant pathogen across the influent samples (accounting for up to 16% of the total microbial abundance) and Mycobacterium is dominant (accounting for up to 14% of the total microbial abundance) across the effluent samples.
- Relative abundance of cyanobacteria (in relation to all bacteria) varied significantly from 0.45 to 25%. Overall, *Synechococcus* dominated (i.e. up to 45%), followed by *Cyanobium* (approximately 7%) and *Chlorella* (approximately 5%).
- Currently none of the plants fulfills the national reuse standard in their original design and the effluents are not fit for reuse purposes yet, especially due to tCOD concentrations above 100 mg/L.
- Chlorophyll-*a* concentrations provide a first indication of the algal fraction in the pCOD, as they correlate linearly with the pCOD.
- The reuse standards need to be adapted specifically for WSP, either to allow for higher tCOD concentrations caused by algae or to use the sCOD as indicator.

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DATA AVAILABILITY STATEMENT

All relevant data are available from an online repository or repositories. The raw fastq sequences are available in the NCBI Sequence Read Archive under BioProject number PRJNA797533: https://www.ncbi.nlm.nih.gov/bio-project/?term=PRJNA797533.

CONFLICT OF INTEREST STATEMENT

The authors declare there is no conflict.

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