

**Ecosystem research of intensively used surface
waters in a highly vulnerable semi-arid region:
The lishana system in Northern Namibia**



Leona Faulstich

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Ecosystem research of intensively used surface
waters in a highly vulnerable semi-arid region:
The Iishana system in Northern Namibia

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Leona Faulstich

Berlin, den 21. September 2023

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Abstract

In the arid and semi-arid region in northern Namibia, there are three main catchment areas the Kunene, the Kavango, and the Cuvelai-Etoshia Basin (CEB). The Kunene and the Kavango Rivers are perennial and provide the northwestern and northeastern regions with water. In central northern Namibia, the transboundary CEB consists of an ephemeral and endorheic drainage system with channels and depressions, known as the lishana system. High temperatures, high evaporation rates, and low rainfall increase the risk of polluting these waters. Groundwater resources near the surface are heavily salinized and can hardly serve as an adequate water source. Deeper groundwater reservoirs are difficult to access. The high population density in the lishana system area increases the demand upon the surface waters. The geomorphology and surface hydrology of the lishana system favors very slow surface runoff and low infiltration rates. An extreme change in dry and rainy seasons results in an erratic freshwater supply, which can decrease water quantity and water quality and is thus a major challenge. Since there were no data on the quality of the surface waters in this area, the state of the ecosystem and the health risk for the population are completely unclear; therefore, the regional surface waters were examined to determine their suitability as potable water. The aim is to better understand the hydrological systems in northern Namibia. In four field campaigns between 2017 and 2021, the surface waters, suspended solids, and sediments of the three systems (Kunene, Kavango, and CEB) and the local water supply system, the Calueque-Oshakati Canal, were sampled at more than 30 sites. Relevant hydrochemical parameters were analyzed, microplastics were investigated, and bioassays were conducted to gain insights into the status of aquatic ecosystems and the ecological impacts of pollutants. Spatial differences in the water quality relative to the decreasing precipitation gradient from east to west were noticed. Furthermore, metals such as aluminum and iron accumulate around the densely populated region in the eastern part of the study area. These waters were more polluted during the drought events in 2018 and 2019 than in 2017. Microplastics were found in different quantities, in particular PE and PP fragments. Three different trophic levels (algae, daphnia, and zebrafish embryos) were tested for acute toxicity. Mechanism-specific effects, such as estrogenicity (YES), cytochrome potential (micro-EROD), and mutagenicity (AMES) were investigated using in vitro assays. Acute toxicity could be detected for all three systems; in

particular, the fish embryos showed several effects. Estrogenic and mutagenic effects were identified for several sampling sites.

The different methodological approaches facilitate a holistic monitoring. This work is the first study to provide a comprehensive statement on water quality. The ecosystems of the Ilishana, the Kunene, and Kavango Rivers are severely stressed and differ significantly. They show several signs of anthropogenic pollution that can affect human health. The use of water as potable water is not possible without prior treatment. Further investigation of the exact influencing factors, such as pesticides, is necessary to find suitable treatment measures for water use.

Zusammenfassung

In der ariden und semiariden Region im Norden Namibias stellen der Kunene und der Kavango Fluss sowie das Cuvelai-Etосha-Becken (CEB) die drei Haupteinzugsgebiete dar. Der Kunene und der Kavango sind perennierende Gewässer und versorgen die nordwestlichen und nordöstlichen Regionen des Landes mit Wasser. Im zentralen Norden Namibias besteht das grenzüberschreitende CEB aus einem ephemeren und endorheischen Entwässerungssystem mit Kanälen und Senken, dem Iishana-System. Hohe Temperaturen, hohe Verdunstungsraten und geringe Niederschläge sind potentielle Risikofaktoren, die zu einer Verschmutzung dieser Gewässer führen können. Die oberflächennahen Grundwasservorkommen sind stark versalzen und können kaum als ausreichende Trinkwasserquelle dienen. Tiefere Grundwasserreservoirs sind schwer zugänglich. Die hohe Bevölkerungsdichte im Iishana-System erhöht den Nutzungsdruck auf die Oberflächengewässer der Region. Die Geomorphologie und Oberflächenhydrologie des Iishana-Systems begünstigen einen sehr langsamen Oberflächenabfluss und geringe Infiltrationsraten. Ein extremer Wechsel von Trocken- und Regenzeiten führt zu einer unregelmäßigen Frischwasserzufuhr, die die Wassermenge und die Wasserqualität beeinträchtigt und eine große Herausforderung darstellt. Da es bisher keine Daten über die Qualität der Oberflächengewässer gibt, und da der Zustand des Ökosystems und die gesundheitlichen Risiken für die Bevölkerung unklar sind, wurden die regionalen Oberflächengewässer auf ihre Nutzbarkeit untersucht. Das Ziel ist ein besseres Verständnis der hydrologischen Systeme im Norden Namibias. In vier Feldkampagnen zwischen 2017 und 2021 wurden an mehr als 30 Probenahmestellen die Oberflächengewässer, Schwebstoffe und Sedimente der drei Systeme (Kunene, Kavango und CEB) und des lokalen Wasserversorgungssystems, des Calueque-Oshakati-Kanals, beprobt. Verschiedene relevante hydrochemische Parameter wurden analysiert, Mikroplastik untersucht und Biotests durchgeführt, um Erkenntnisse über den Zustand der aquatischen Ökosysteme und die ökologischen Auswirkungen von Schadstoffen zu gewinnen. Es wurden räumliche Unterschiede in der Wasserqualität entsprechend dem abnehmenden Niederschlagsgefälle von Ost nach West festgestellt. Außerdem reichern sich Metalle wie Aluminium und Eisen in der Nähe des dicht besiedelten Gebiets im östlichen Teil des Untersuchungsgebiets an. Die Gewässer waren während der Dürreereignisse in den Jahren 2018 und 2019

stärker belastet als im Jahr 2017. Mikroplastik wurde in unterschiedlichen Mengen gefunden, insbesondere PE- und PP-Fragmente. Drei verschiedene trophische Ebenen (Algen, Daphnien und Zebrafischembryonen) wurden auf akute Toxizität getestet. Mechanismus-spezifische Effekte wie Östrogenität (YES), cytochromes Potenzial (micro-EROD) und Mutagenität (AMES) wurden mit Hilfe von In-vitro-Tests untersucht. Eine akute Toxizität konnte für alle drei Systeme nachgewiesen werden, insbesondere die Fischembryonen zeigten zahlreiche Effekte. Für mehrere Probenahmestellen wurden östrogene und mutagene Wirkungen festgestellt.

Die unterschiedlichen methodischen Ansätze ermöglichen ein holistisches Monitoring. Diese Arbeit ist die erste Studie, die eine umfassende Aussage zur Wasserqualität zulässt. Die Ökosysteme des Ilishana, des Kunene und des Kavango-Flusses sind stark belastet und unterscheiden sich teilweise erheblich. Sie weisen mehrere Anzeichen anthropogener Verschmutzung auf, die die menschliche Gesundheit beeinflussen können. Die Nutzung des Wassers als Trinkwasser ist nicht möglich ohne vorherige Aufbereitung. Weitere Untersuchungen der genauen Einflussfaktoren, wie z.B. Pestizide, sind notwendig, um geeignete Aufbereitungsmaßnahmen für die Wassernutzung zu entwickeln.

Contents

Eidesstattliche Versicherung	I
Danksagung	III
Abstract	IV
Zusammenfassung	VI
Contents	VIII
List of figures	XI
List of tables	XIII
List of abbreviations.....	XIV
1. Introduction	1
1.1. Research background and motivation	3
1.2. The lishana system in the CEB as study area.....	4
1.3. Problems and challenges in the study area.....	10
1.4. The climatic situation during the study period 2017–2021.....	12
1.5. Research questions and aims	14
1.6. Structure of this thesis.....	15
2. Methodological approach	17
2.1. Water quality analytics	17
2.2. Microplastics.....	19
2.3. Ecotoxicology	20
3. Paper 1: Water and sediment pollution of intensively used surface waters during a drought period – a case study in Central Northern Namibia	23
Abstract	23
3.1 Introduction.....	23
3.2 Material and methods.....	25
3.2.1 Study area.....	25
3.2.2 Sampling sites.....	28
3.2.3 Water samples	29
3.2.4 Sediment samples.....	30
3.2.5 Water guidelines	31
3.2.6 Data analysis.....	32
3.3 Results	32
3.3.1 Hydrochemistry and water quality	32
3.3.2 Suspended solids and sediments.....	41
3.4 Discussion.....	44

3.4.1 Calueque-Oshakati canal.....	44
3.4.2 The lishana system	45
3.5 Conclusion and outlook	49
3.6 Acknowledgements	50
3.7 Funding	50
4. Paper 2: Microplastics in Namibian river sediments – a first evaluation.....	51
Abstract	51
4.1 Introduction.....	51
4.2 Material and methods	54
4.2.1 Study area.....	54
4.2.2 Quality assurance and quality control	56
4.2.3 Sampling	57
4.2.4 Density separation with the microplastic sediment separator.....	59
4.2.5 Fractionation, filtration, staining, and microscopy.....	59
4.2.6 FTIR spectroscopy	60
4.2.7 Data analysis.....	61
4.3 Results	62
4.4 Discussion	67
4.5 Conclusion and outlook	73
4.6 Acknowledgements	74
4.7 Funding	74
5. Paper 3: Ecotoxicological evaluation of surface waters in Northern Namibia.....	75
Abstract	75
5.1 Introduction.....	75
5.2 Methods.....	78
5.2.1 Study area.....	78
5.2.2 Sampling and extraction.....	79
5.2.3 Bioassays.....	80
5.2.4 Data analysis.....	85
5.3 Results	86
5.3.1 Fish embryo toxicity test.....	86
5.3.2 Daphnia acute immobilization test.....	87
5.3.3 Algae growth inhibition test	88
5.3.4 AMES fluctuation assay	88
5.3.5 Micro-EROD assay	90
5.3.6 Yeast estrogen screen	90

5.4 Discussion.....	91
5.5 Conclusion and outlook.....	96
5.6 Acknowledgements.....	97
5.7 Funding.....	98
6. Summarizing discussion and synthesis.....	99
7. Conclusions and future perspectives.....	110
7.1. Scientific relevance.....	110
7.2. Sociopolitical context.....	112
7.3. Potentials in applications & practice.....	113
8. References.....	CXV
Supplementary data.....	CLII

List of figures

Figure 1.1: Hydrological map of Namibia with the lishana system as focus area in north-central Namibia (data by Lehner et al. 2008; Lehner & Grill 2013) .	6
Figure 1.2: Aridity index in Namibia (data by Trabucco & Zomer 2022)	8
Figure 1.3: Population density in Namibia (data by NSA 2011)	9
Figure 1.4: Photos of the lishana pans in the study area (photos by Faulstich 2017, 2018, and 2019)	14
Figure 3.1: The Cuvelai Basin with the lishana system, neighboring river systems (Kunene and Kavango catchments), and the study area (red box)	26
Figure 3.2: Map of the study area and sample points at the lishana and the Calueque-Oshakati canal.....	29
Figure 3.3: Piper diagrams of the lishana, the Calueque-Oshakati canal, tap water, and precipitation samples from 2017–2019	40
Figure 3.4: Changes in AI and Fe concentrations in the lishana between 2017 and 2019.....	41
Figure 4.1: The hydrological system of Namibia with the catchments of the four perennial rivers, the Cuvelai Basin, ephemeral catchments (the sampled are colored), and the continental water divide.....	55
Figure 4.2: Locations of the sampling sites in Namibia with the focus region in central northern Namibia	58
Figure 4.3: The focus region of the lishana system with sampling sites, indicating whether the lishana were carrying water at the time of sampling.....	58
Figure 4.4: Amount and polymer type of microplastics found at the sampling sites of the perennial and ephemeral Namibian river systems	63
Figure 4.5: Amount and polymer type of microplastics found at the sampling sites of the focus region, the lishana system.....	64
Figure 4.6: Polymer type differentiated according to river systems	64
Figure 4.7: Shape of microplastics found, differentiated according to the three river systems.....	65
Figure 4.8: Comparison of the amount of microplastics at the three river systems (perennial rivers, ephemeral rivers, and the lishana system).....	67
Figure 5.1: Catchment areas of the three systems with sampling sites and numbers	79

Figure 5.2: Observed effects of a the lishana system and b the perennial rivers.....	86
Figure 5.3: Immobilized <i>Daphnia magna</i> in a lishana and b perennial rivers.....	87
Figure 5.4: Distribution of induction factors (IF) evaluated by exposure of strain TA98 and TA100 +/-S9 to the lishana, and the Kunene and Kavango Rivers in six dilution steps and three replicates.	89
Figure 5.5: EEQ (17 β -Estradiol Equivalents) in ng/l for the lishana system.....	91

List of tables

Table 1.1: Precipitation in the lishana system between 2011 and 2022. Deviation from monthly average values (period 2000–2010) in % (Ondangwa station, WMO Index 68006; data by NOAA 2022; data processed by meteomanz.com). Months outlined in black indicate months in which field visits occurred.....	12
Table 1.2: Floods in the CEB during the study period 2017–2021 (data derived from Atlas of Namibia Team 2022).....	13
Table 1.3: Average runoff of the Kunene and Kavango Rivers in m ³ /s (data 2017 – 2019 derived from Atlas of Namibia Team 2022; data 2020 – 2021 by UNESCO 2021)	13
Table 1.4: Chapters of this thesis, related publications, and their publication status	16
Table 3.1: Range, mean, and standard deviation (sd) of the hydrochemical composition of lishana, Calueque-Oshakati canal, tap water, and precipitation. The temperature in °C, EC in µS/cm, turbidity in NTU (Nephelometric Turbidity Unit), and major ions in mg/l. Limit values of the guidelines Namibian Water Act 54 from 1956 and Guidelines for Drinking-water Quality of the WHO from 2017	34
Table 3.2: Range, mean, and standard deviation (sd) for physio-chemical parameters and major element concentrations of suspended solids and sediments of the lishana, the COC, and the waterworks in Oshakati (WO). EC in µS/cm, concentrations in mg/g, Pb in µg/g.	42
Table 4.1: Particle size of microplastics found, differentiated between perennial rivers, ephemeral rivers, and the lishana system.....	65
Table 4.2: Colors of microplastics found, differentiated according to the three river systems.....	66
Table 4.3: Comparison of microplastics concentrations in different regions	69
Table 5.1: Key data of the bioassays performed.....	81
Table 5.2: The average concentration-dependent induction factors (CDI) for the strains TA98 and TA100 +/- S9 (Salmonella typhimurium) for the lishana system and the Kunene and Kavango Rivers.	90

List of abbreviations

abbreviation	explanation/meaning
ABS	acrylonitrile butadiene styrene
AI	aridity index
Al	aluminum
As	arsenic
ATR	attenuated total reflection
BCA	bicinchoninic acid
BOD	biological oxygen demand
Ca	calcium
CB	Cuvelai Basin
Cd	cadmium
CDI	concentrated-dependent induction factor
CEB	Cuvelai-Etosha Basin
Cl	chloride
Co	cobalt
COC	Calueque-Oshakati canal
COD	chemical oxygen demand
CPR	chlorophenol red
CPRG	chlorophenol red- β -galactosidase
Cr	chrome
Cu	copper
DIN	Deutsches Institut für Normung (German Institute for Standardization)
DLC	dioxin-like chemicals
DMSO	dimethylsulfoxide
EC	electrical conductivity
EDC	endocrine-disrupting compounds
EEQ	estradiol equivalent concentration (17 β -Estradiol Equivalents)
Eh	redox potential
EROD	ethoxyresorufin-O-deethylase
ETX	exogenous substrate 7-ethoxyresorufin
F	fluoride
FAU	Formazine Attenuation Unit
Fe	iron
FET	fish embryo acute toxicity
FTIR	Fourier transform infrared spectroscopy
HCO ₃	hydrogen carbonate
HDAC	histone deacetylase
hER α	human estrogen receptor α
hpf	hours post fertilization
HQI	Hit Quality Index
IF	induction factor
ITCZ	Inter-Tropical Convergence Zone
IWRM	Integrated Water Resources Management
K	potassium
LOD	limit of detection

abbreviation	explanation/meaning
LOQ	limit of quantification
MeOH	methanol
Mg	magnesium
Mn	manganese
MP	microplastics
MPSS	Microplastic Sediment Separator
N ₂	nitrogen
Na	sodium
NaCl	sodium chloride
NC	negative control
NH ₄	ammonium
Ni	nickel
NO ₂	nitrogen dioxide
NO ₂ ⁻	nitrite
NO ₃	nitrate
NOAA	National Oceanic and Atmospheric Administration
NSA	Namibia Statistics Agency
NSDI	National Spatial Data Infrastructure
NTU	Nephelometric Turbidity Unit
O ₂	oxygen
OC	organic contaminant
OD	optical density
OECD	Organisation for Economic Co-operation and Development
ONC	overnight culture
PA	polyamide
Pb	lead
PC	polycarbonate
PC	positive control
PE	polyethylene
PET	polyethylene terephthalate
PFAS	perfluoroalkyl or polyfluoroalkyl substances
PMMA	poly(methyl methacrylate)
PO ₄ ³⁻	(ortho)phosphate
POP	Persistent Organic Pollutants
PP	polypropylene
ProCo	process control
PS	polystyrene
PTFE	polytetrafluoroethylene
PVC	polyvinylchloride
SC	solvent control
sd	standard deviation
SDG	Sustainable Development Goals
SO ₄	sulfate
Sr	strontium
TC	total carbon
TCDD	2,3,7,8-Tetrachlorodibenzo-p-dioxin
TIC	total inorganic carbon
TNb	total nitrogen bound

abbreviation	explanation/meaning
TOC	total organic carbon
TRWP	tire and road wear particles
WHO	World Health Organization
WO	Waterworks Oshakati
WQI	Water Quality Index
YES	yeast estrogen screen
Zn	zinc

1. Introduction

In times of human-induced global warming, climatic conditions are changing all over the world. Rising temperatures increase evaporation rates, precipitation more frequently occurs as heavy rainfall events, and rainy seasons are shifting. Longterm weather extremes, such as droughts and floods occur more often. While individual regions are affected in different ways, arid and semi-arid regions are particularly vulnerable. Arid and semi-arid regions face several challenges regarding the use of soils and water. Salinity, soil degradation, erosion, loss of soil water storage, and decline in water quantity and quality are common problems. In the semi-arid and arid regions in sub-Saharan Africa, droughts are a common natural disaster and are mainly caused by inter-annual variations in rainfall. They have a direct impact on water resources and agriculture (Sheffield et al. 2014; Luetkemeier & Liehr 2018). In structurally weak rural areas in developing countries, there is often an increased water scarcity (WWAP 2019). Water scarcity is defined by the United Nations as the point at which the impact of water users affects the supply or quality of water to the extent that the demand cannot be satisfied (FAO and UN Water 2021). Drought-affected regions suffer particularly from water scarcity and poor water quality (FAO 2016; Garcia-Franco et al. 2018; Lal 2004; Nilin et al. 2019). Water scarcity and water quality are strongly linked. By taking water quality into account, many regions as well as seasons show significantly higher levels of water scarcity (van Vliet et al. 2017). Even in the Sustainable Development Goals (SDGs) of the United Nations, water quality plays a crucial role and is part of several targets (Alcamo 2019). In Addition, it is globally vital to receive good water quality for potable water, domestic use, and industrial use. To ensure the safe use of surface waters, good water quality is as important as its contribution to ecosystem services. In Africa in general, observations and investigations of water quality are limited (van Vliet et al. 2017).

The vulnerability of water resources can be assessed through the analysis of climatic changes and population growth (AghaKouchak et al. 2013). Based on the IPCC report of 2007, vulnerability is defined as a complex of exposure, sensitivity, and adaptive capacities (IPCC 2007). In semi-arid ecosystems, water is an important resource that reacts very sensitively to the intensifying seasonal and annual climatic variability (AghaKouchak et al. 2013). Water accessibility and quality decrease as climatic variability intensifies and extremes such as droughts and floods occur more often

(Kottek et al. 2006; Sommer et al. 2011; van Vliet et al. 2017). The diminishing availability of water related to population growth, changing precipitation patterns, increasing contamination, and inefficiencies in water use increase water scarcity (Emile et al. 2022). Due to shifted and weaker rainy seasons, water resources are insufficiently replenished with fresh water and do not last throughout the entire rain-free dry season. Water scarcity therefore increases especially in the dry seasons, and water storage is challenging due to high evapotranspiration. These physical conditions shape and change ecosystems. The ecosystems of water bodies in particular are affected by climatic changes and anthropogenic pollution. Africa is facing an increasing pollution problem, to which plastics are contributing a major part.

For a long time, aquatic and terrestrial ecosystems were studied separately. However, studies that consider them as connected and examine rivers as a cross-system have lately become more common (Soininen et al. 2015). Aquatic ecosystem research in particular has been given more importance in recent years because the ecosystem services provided by flowing and standing waters are of great significance to humanity (Liao & Huang 2014). The term 'aquatic ecosystem research' describes the integrative analysis of the behavior of aquatic ecosystems such as rivers, lakes, oceans, and groundwater. Natural dynamics and anthropogenic influences are studied in the various catchment areas. The underlying theory calls for an investigation of the interrelationships between ecological stages and different trophic levels. Aquatic ecosystems are home to numerous living organisms of different trophic levels that are dependent on water (Soininen et al. 2015). The ecosystems are often disturbed by pollutants such as pesticides, heavy metals, or microplastics.

Microplastics in aquatic systems are not a new phenomenon, but they are not equally well studied in all parts of the world. In South Africa, plastics are used in most economic sectors, and therefore they play a vital role in the economy (Bouwman et al. 2018). The increasing population generates huge amounts of waste that put pressure on the disposal systems (Hasheela 2009). In many regions, municipal waste management is lacking, and this leads to uncontrolled waste disposal, which in turn increases the microplastic input. Nava and Leoni (2021) have already investigated the connection between microplastics, algae, and the aquatic ecosystem. The goal of aquatic ecosystem research is the integrative consideration of individual aquatic ecosystems while also considering the human factor. One example of these sensitive ecosystems

in a semi-arid region is the lishana system,¹ which is part of the transboundary Cuvelai-Etosha Basin (CEB)² in north-central Namibia.

1.1. Research background and motivation

Namibia is one of the driest countries in Sub-Saharan Africa and faces several challenges regarding the ongoing climatic changes (World Bank Group 2021). The climatic changes—the unevenly distributed and decreasing rainfall across the country and the rainy seasons, which are shifting further back in the year—have been highlighted in several studies (Angula & Kaundjua 2016; Hamunyela et al. 2022; Newsham & Thomas 2011; Turpie et al. 2010; Zeidler et al. 2010). Evaporation, which is already high, is increasing and impeding the water storage and thus the water supply.

Northern Namibia has higher rainfalls than the southern part of the country and therefore offers more water resources. However, this region is also subject to large variations in precipitation and increasing evaporation, which puts pressure on water resources. The region is characterized by three huge catchment areas: the CEB in the center, the Kunene River Basin in the west, and the Kavango River Basin, also called Okavango River Basin,³ in the east. The lishana system, as part of the CEB, is an endorheic system of shallow channels and depressions. These channels and depressions are called lishana (singular Oshana);⁴ they are intensively used and represent an important water resource for the local population. Despite the importance of these surface waters, they have not been sufficiently studied in this area.

Until now there have been no studies on the ecosystem of the lishana, and there are no data regarding the water quality. Only one large-scale and long-term project, the CuveWaters project, has been carried out between 2006 and 2015 by the German Federal Ministry of Education and Research and the German–Namibian cooperation.

¹ The lishana system is also referred to as the Cuvelai drainage system or Oshana system in the literature.

² The CEB is also called the Cuvelai Basin, the Etosha Basin, or the Etosha Cuvelai Basin in the literature.

³ The Okavango River is also called Kavango, Okavango, or Cubango. In this study, the word Kavango is used.

⁴ There exist several names for the lishana, as it is a word in Oshiwambo (also known as Oshivambo, Owambo, or Ovambo), the language spoken by the Ovambo people in the area. In the literature, the names used most often are lishana, shana, iishana, *iishana*, or oshanas. In this study, the word lishana is used.

The aim was to provide long-term access to water for the people in the CEB by implementing an Integrated Water Resources Management (IWRM) (Bischofberger et al. 2015). In addition to this project, several studies on regional groundwater reservoirs have been performed (Hamutoko et al. 2016, 2018, 2019; Lindenmaier et al. 2012). Wanke et al. (2014) analyzed hand-dug wells (artificial shallow wells that expose near-surface groundwater resources) for their usability. The geogenic background load of soils was investigated (Dill et al. 2013; Fujioka et al. 2018), as well as the governmental part of water supply (Ndokosho 2007; Newsham 2011; Sturm 2009). The water quality of the local public water supply in the region, the Calueque-Oshakati canal, was analyzed by Koeniger et al. (2020). The lishana were investigated by Perry et al. (2022) regarding their Environmental-DNA. In the Okavango Delta, a bacterial analysis was carried out, and total coliforms, fecal coliforms, and *E. coli* were calculated (West et al. 2015). In Botswana, numerous elephant deaths occurred in 2020, which were linked to a severe blue-green algae plague and cyanobacteria (Azeem et al. 2020; van Aarde et al. 2021).

All of these studies have examined partial aspects of the lishana, but none have directly looked at water quality and potability. Likewise, no potential sources of pollutants have been investigated. In view of this state of research, there is an urgent need for detailed investigations of water quality and for an examination of possible water-stress situations facing the population. The study of the lishana ecosystem in northern Namibia is the research subject of this thesis.

1.2. The lishana system in the CEB as study area

Namibia's hydrology is characterized by five perennial rivers,⁵ twenty ephemeral rivers, and the Cuvelai-Etoshia Basin (CEB). The perennial rivers Orange, Kunene, Kavango, Kwando, and Zambesi flow at the border regions in the south, northwest, and northeast of Namibia.

The CEB is a transnational catchment area in southern Angola (one-third of the area) and central northern Namibia (two thirds of the area). The Kunene River Basin, which drains into the Atlantic Ocean, borders the western part of the CEB; to the east, the

⁵ In the paper published by Faulstich et al. (2022), which is also a part of this thesis (see Chapter 4), four perennial rivers instead of five were described, as the Kwando was counted as part of the Kavango.

CEB is bordered by the Okavango River Basin, which drains into the Okavango Delta. The northern boundary is Angola's Central Planalto, and the southern border is formed by the Omadhiya Lakes and the Etosha Pan beyond. The entire catchment area of the CEB covers about 20,731 km², making it one of the ten largest catchments in Namibia. The CEB is situated between circa 1,000 and 1,250 m above sea level and is characterized by low gradients, mostly 0.5–1.0 ‰ (Mendelsohn et al. 2013). The only two perennial rivers in the CEB, the Mui and Cuvelai Rivers, are located in Angola. The rest of the CEB is the alluvial Cuvelai drainage system, also known as the lishana system. This endorheic system consists of numerous shallow channels, depressions, and pans, referred to as the lishana (the singular is Oshana). The extent of the lishana differs by anywhere from several meters to several kilometers, and their depth varies from centimeters to meters. This ephemeral drainage system ends into the Oponono Lake as part of the Omadhiya Lakes, and sometimes into the Etosha pan (Atlas of Namibia Team 2022; Mendelsohn et al. 2013). The lishana are mostly independent water bodies, but in the rainy season, when enough rainfall and runoff from Angola occurs, the individual depressions combine to form a dense drainage system. This system of drainage channels and depressions is very different from the rest of the country, where only isolated ephemeral rivers can be found (see Figure 1.1). Due to the watershed, there is a steeper slope of > 1 degree in the western part and a gentle slope of < 1 degree in the eastern part. Therefore, the westward-flowing ephemeral rivers have a strong eroding energy upstream and clear pathways; downstream, they flow gently into wider beds. Several rivers often never reach the ocean. Eastwards-pointed rivers have lower energy and no clear riverbeds due to the sandy ground (Atlas of Namibia Team 2022).

The CEB has an arid and semi-arid climate with a mean annual precipitation of 300–550 mm between November and April and a potential evaporation of 2,200–2,400 mm (Atlas of Namibia Team 2022). The whole area is arid in the west and semi-arid in the east due to the increasing precipitation gradient from southwest to northeast. Rainy and dry seasons with floods and droughts affect the available water resources. During the winter months, there is nearly no rainfall, and in the summer months, there are mostly heavy rainfalls. The rainfall events often do not occur during the whole month, but just within a few hours. Due to the strong differences between the rainy and dry seasons regarding rainfalls, the water in the lishana sometimes remains at a standstill for several months, resulting in an increasing salinity. The elevated evaporation rates desiccate the surface waters.

The aridity index (AI) describes the ratio of total annual precipitation and the potential evaporation. In semi-arid regions, the index is given as a value between 0.20 and 0.50 (Dregne 1983; Glenn et al. 1993; Lal 2004; Reynolds & Stafford Smith 2002; Zomer et al. 2022). In the CEB, the AI is between 0.18 and 0.24 (Atlas of Namibia Team 2022). The aridity indices for Namibia are visualized in Figure 1.2.

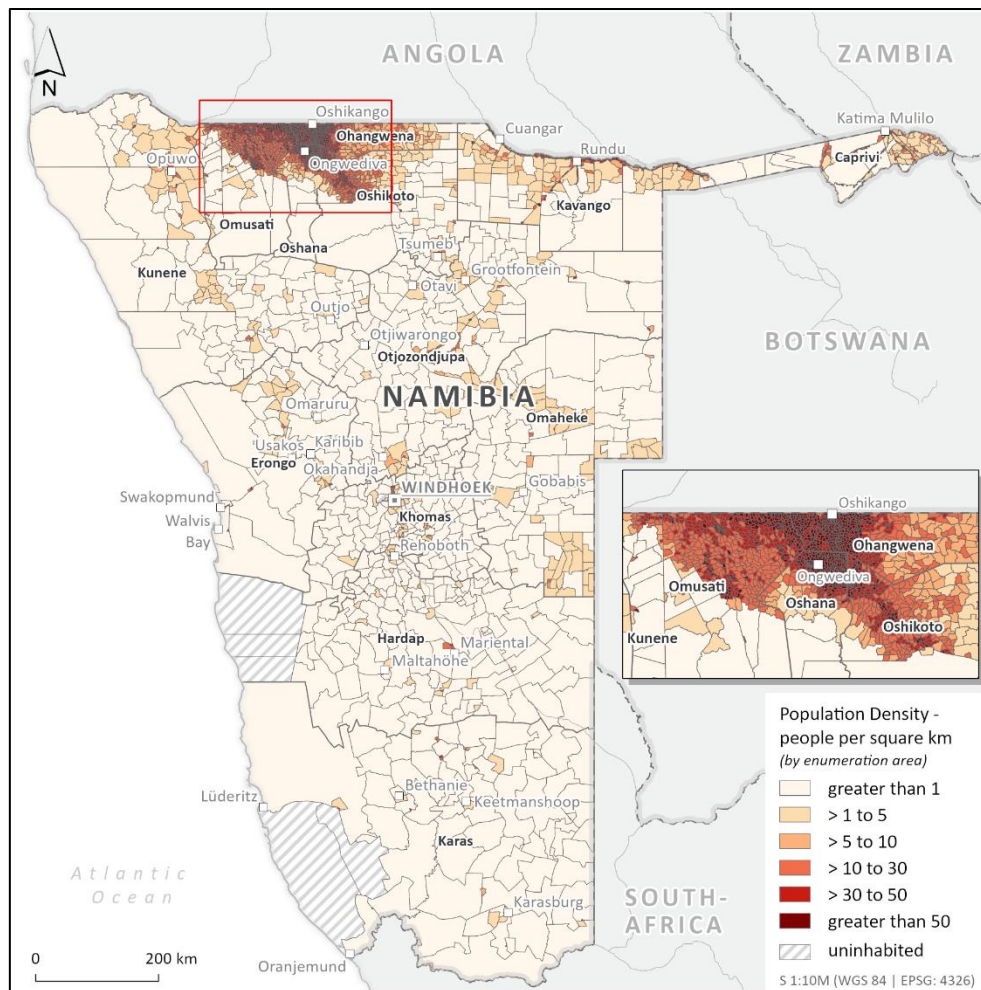


Figure 1.3: Population density in Namibia (data by NSA 2011)

Besides the prevailing aridity and the intensive water use, there are further challenges for the water supply. Clay and saline soils cause low infiltration rates and high surface runoff. Cambisols and calcisols have a water-holding capacity of less than 20 mm (Atlas of Namibia Team 2022). The slow flow velocity due to the flat gradient keeps the surface runoff in the area, where it becomes part of the lishana water bodies.

The water supply in the lishana system is maintained with water from the Kunene River and the surface waters of the lishana system, partially with groundwater from hand-dug wells (Beyer et al. 2018). The public water supply system consists of the Calueque-Oshakati canal which runs from Calueque (Angola) to Oshakati (Namibia). The Olushandja dam with its impoundments is the only reservoir along the canal and the only one in the CEB. The canal transports water that is treated in four Namibian waterworks: Olushandja, Outapi, Ogongo, and Oshakati (Mendelsohn et al. 2000). The public waterworks treat the water from the Kunene and distribute it in the lishana system via pipelines. Several thousand kilometers of rural distribution pipelines

transport the water in north-central Namibia to villages and settlements. Besides the public water supply, the people use lishana water (Atlas of Namibia Team 2022). The waterworks treat the water efficiently, but the region is huge, and not all parts of the population have access to the supply system. The main reason is the limited financial resources to afford the costs for clean water from the supply system for a huge part of the population. In the lishana system, more than 90 % of all households in urban areas have safe access to potable water, whereby in some rural areas less than 20 % have access to potable water (Atlas of Namibia Team 2022). There are no exact numbers on how many people use the lishana's water, but at least the aforementioned 20 % who do not have access to potable water, continue to rely on the lishana's freely available surface water.

Most of the groundwater is saline and therefore it is not suitable as drinking water (Hamutoko et al. 2019). Nevertheless, the regional shallow groundwater, accessed by hand-dug wells, is sometimes used (Wanke et al. 2014). The recently discovered Ohangwena II aquifer, a deep groundwater reservoir, has great potential to fulfill water supply needs in the eastern part of the lishana system (Himmelsbach et al. 2018; Wallner et al. 2017). However, until this groundwater reservoir is developed, the lishana and Calueque-Oshakati Canal will continue to supply the primary water resources in the lishana system.

1.3. Problems and challenges in the study area

As the driest country in Sub Saharan Africa, Namibia faces intense water scarcity (Mapani et al. 2023). During the last ten years, the water stress in Namibia increased due to low rainfalls (McNally et al. 2019). In the CEB, all these aspects combine to create a highly vulnerable region with a low-resilience ecosystem. Intense dry and rainy seasons, high evaporation, time-shifting periods, and several droughts challenge the lishana system. These conditions result in water stress and pressure on highly vulnerable water resources. Schilling et al. (2020) point out that more data are necessary at the sub-national level in order to provide a local, intra-national vulnerability comparison. The flood risk is increased by land-cover change due to expanded urban areas (AghaKouchak et al. 2013). The public water supply in central northern Namibia does not fulfill all the needs of the local population. In particular, during the dry season, the amount of water is not sufficient. The intensively used

surface waters of the lishana must therefore serve as a further water source for a lot of people because they have no access to the public water supply or can not afford to pay for water. Moreover, people also use water from the open canal in an illegal but tolerated manner.

The periodical rivers of the lishana system only flow with sufficient rainfall during the rainy seasons. The water bodies of different sizes cut through the landscape and separate villages and farmland in a random way. After rain falls, floods occur to an increasing degree in Angola and Namibia and endanger the local population there. During the floods in 2008, 2009, 2010, 2011, and 2013, many people lost their lives, the infrastructure was damaged, diseases endangered the population, and supply chains were disrupted (Arendt et al. 2021; Niipare et al. 2020; Shifidi 2016). In particular, the poorer part of the population suffered great economic losses. The local population knows how to adapt and react to extreme situations (Newsham & Thomas 2009). When the rainfall fails, droughts occur. Droughts cost livestock their lives and endanger water supply and harvesting. The leftover water in the lishana cannot be replenished with fresh water, and many lishana desiccate completely. The high evaporation rate increases the water loss and the deteriorating water quality. During the dry season, the ephemeral and shallow surface waters represent the only source of water for agriculture and livestock farming. The surface is dry, with traces of former water bodies, and the lishana retreat further and further into the deeper depressions. The water quality of the intensively used surface water is a big challenge throughout the year. There are no monitoring systems that investigate or control the water quality of the lishana. Several investigations of the groundwater reservoirs and hand-dug wells exist, but not for the surface water of the lishana (Hamutoko et al. 2018 & 2019; Kgabi et al. 2018; Wanke et al. 2014). Intensive anthropogenic overuse—due to fishing, washing, irrigation, watering livestock, garbage disposal, and excrements—affects the water quality. Animals drink the water, the people wash their cars in it, and they pump the water out of the lishana. This leads to several months without freshwater, no water mixing, high evaporation, and a decrease in water volume. All these factors result in poor water quality. The pressure on the resources is huge and the demand for water is enormous. This combination of human activities and the difficult natural conditions makes the lishana system an important study area.

1.4. The climatic situation during the study period 2017–2021

Southern Africa is extremely vulnerable due to climate variability (Archer et al. 2018). The variable precipitation is caused by the seasonal movement of the Inter-Tropical Convergence Zone (ITCZ) (Persendt et al. 2015). In the CEB, increased climate variability has been observed in the last decade. The rainy season shifts back in the year, precipitation decreases, and heavy rainfall events increase. In conjunction with increased temperatures, this results in high interannual climatic variability. The risk of floods has increased, as has the risk of droughts. Future climatic changes will increase temperatures, change rainfall patterns, and cause more extreme weather events (Archer et al. 2018). In general, the study period 2017–2021 coincided with a period of drought in the lishana system. The monthly precipitation was below average every month in 2019–2021 (the average value referred to was calculated over the period 2000–2010; Table 1.1).

Table 1.1: Precipitation in the lishana system between 2011 and 2022. Deviation from monthly average values (period 2000–2010) in % (Ondangwa station, WMO Index 68006; data by NOAA 2022; data processed by meteomanz.com). Months outlined in black indicate months in which field visits occurred.

Deviation from monthly average values in %												
Ondangwa, WMO Index 68006												
Data source: NOAA, 2022; data processed by meteomanz.com and Leona Faulstich												
	October	November	December	January	February	March	April	May	June	July	August	September
2009	163	266	17	38	2	-72	-83	-93	-100	0	-100	-100
2010	-100	-21	108	76	70	27	64	-90	-96	0	-100	-100
2011	-88	-62	-15	46	10	-39	-100	-100	-100	0	-100	-91
2012	-100	23	100	-63	-84	-18	-97	-100	-100	0	-100	-100
2013	-82	-8	28	-66	-52	15	4	-100	-100	0	-100	0
2014	-90	-50	24	-42	-84	-79	1286	-100	-100	0	-100	-100
2015	-73	1011	904	286	308	-68	-100	-97	-100	0	-100	-100
2016	-100	50	-68	-89	-64	-97	-98	-100	-100	0	-100	-100
2017	-92	-100	-67	-68	-78	-53	1283	-97	-100	0	--	-100
2018	-100	-100	-100	-100	-99	-100	-100	-100	-100	0	-100	-100
2019	-98	-100	-88	-53	-66	-100	-100	-100	-100	0	-100	-100
2020	-100	-100	-100	-100	-100	-93	-99	-100	-100	0	-100	-100
2021	-100	-100	-90	-35	-39	-47	49	-100	-100	0	-100	-100
2022	-100	-100	-90	-35	-39	-47	49	-100	-100	0	-100	-100
average [mm]	12	45	41	107	99	97	29	6	5	0	2	7
	October	November	December	January	February	March	April	May	June	July	August	September
	-100	-99 ≤ -75	-75 ≤ -50	-50 ≤ -25	-25 < 0	0	0 ≤ 25	25 ≤ 50	50 ≤ 75	75 < 100	≥ 100	-- no data

Due to low rainfalls, the floods in the CEB strongly varied during the study period (there is no classification of this assessment in the relevant literature; Table 1.2). The annual floods (also called *efundja*) are an integral part of the rhythm of rainy and dry seasons.

Their strength depends on the rainfall conditions in southern Angola and northern Namibia. In 2017 and 2020, the flood level was medium; in 2019 and 2021, no floods occurred in the CEB. The highly variable flow characteristics of the lishana result in uncertain water availability. The prevailing drought provided good study conditions as the many potential risk factors converged and water quality was most at risk.

Whereas the discharges of the two perennial rivers, the Kunene and Kavango, are measurable, and the water availability here continues year-round, such measurements are not possible for the lishana. The runoff of the two rivers is different; most of the years the Kunene River has a higher runoff. The discharge of the Kunene River is controlled at the measuring point at the Kunene waterfalls (Table 1.3).

Table 1.2: Floods in the CEB during the study period 2017–2021 (data derived from Atlas of Namibia Team 2022)

2017	2018	2019	2020	2021
medium	low	none	medium	none

Table 1.3: Average runoff of the Kunene and Kavango Rivers in m³/s (data 2017 – 2019 derived from Atlas of Namibia Team 2022; data 2020 – 2021 by UNESCO 2021)

	2017	2018	2019	2020	2021
Kunene	300	900	150	800	100
Kavango	300	500	200	500	200

The consequences of poor rainfall availability and the affect of drought events on the lishana are illustrated in photographs (Figure 1.4). During the study period, the focus area was characterized by sparse vegetation, dry soils, scattered settlements, and traces of anthropogenic use. The pans are used by people, wildlife, and livestock. These conditions underline the general water scarcity in Namibia, a challenge for the water supply. Unfortunately, not all planned field visits and sampling campaigns could be carried out due to the COVID-19 pandemic. Therefore, the campaigns at the end of the rainy season in spring 2020 and at the end of the dry season in autumn 2020 are missing in the time series from 2017 to 2021.



Figure 1.4: Photos of the lishana pans in the study area (photos by Faulstich 2017, 2018, and 2019)

1.5. Research questions and aims

The lishana are an important water resource in the region. Therefore, it is necessary to analyze their potential as potable water and their water quality, and to investigate the state of the ecosystem. In addition to the factors affecting water quality, the external conditions around the lishana have led to the need to investigate the amount of microplastics and the ecotoxicological potential. The large amount of macroplastic waste has fueled the assumption that microplastic particles will also be found in the waters. Monitoring is intended to provide an overview of the actual quality of the water. In addition to an analysis of ion concentrations, the analysis includes both pollutant and heavy metal contamination. The recording of microbiological activity completes the monitoring, which illuminates the water quality from different angles. Both anthropogenic influences and the existing ecosystem are examined so that a

classification can be made as to where the sources of the respective parameters lie. The focus of this thesis is the lishana system, but for the analyses of microplastics and ecotoxicological effects, the Kunene and the Kavango Rivers were also investigated for the sake of comparison. In the study on microplastics in river sediments, 9 other ephemeral rivers in the country were studied to extend the comparison.

This thesis aims to improve the knowledge concerning the ecosystem of the lishana, particularly in regard to water quality, usability, and potability. The condition of water bodies was evaluated during a drought period. Nutrients, pollutants, sediment loads, and acute toxicity on multiple trophic levels were analyzed to determine possible pollution sources. The number of plastic particles in the main river systems in Namibia was evaluated with a focus on the lishana system.

This is the first time that the state of the water quality of the lishana, the occurrence, abundance, and composition of microplastics in fluvial sediments, and the hazard potential of the surface waters of the lishana and the Kunene and Kavango Rivers on organisms and the environment in Namibia have been investigated. The different approaches provide a holistic monitoring. This work is the first study that offers a comprehensive statement on water quality.

The following main research questions were addressed within this thesis:

1. What is the physicochemical state of the waters of the lishana system and which pollutants and contaminants can be found in them?
2. Are there any spatial or temporal variations regarding the water quality of the lishana and the two neighboring systems, the Kunene and the Kavango Rivers?
3. What is the state of the lishana ecosystem?
4. Is the water of the lishana usable for human consumption without health risks?

1.6. Structure of this thesis

The following chapters (3-5) represent the publications that describe the research and respective results of this thesis (Table 1.4). All three publications were conceptualized, edited, and written by the thesis author, as evidenced by the first authorship listing. The supervisors were involved in all steps of the thesis. The other co-authors participated in the fieldwork, the methodological approach, and the data evaluation, and they contributed by revising the manuscripts. The helpful change of perspective

during scientific conversations was an essential part of the process. The scientific articles have been peer-reviewed by international scientific journals. The articles were originally submitted and published in English.

Table 1.4: Chapters of this thesis, related publications, and their publication status

Chapter	Publication	Status
3	Faulstich, L.; Arendt, R.; Reinhardt-Imjela, Ch.; Schulte, A.; Lengricht, J.; Johannes, P. (2023): Water and sediment pollution of intensively used surface waters during a drought period – a case study in Central Northern Namibia. In: Environmental Monitoring and Assessment 195 (924), https://doi.org/10.1007/s10661-023-11505-1 This article is licensed under a Creative Commons Attribution 4.0 International License .	Published in: July 2023, Environmental Monitoring and Assessment, Springer
4	Faulstich, L.; Prume, J.A.; Arendt, R.; Reinhardt-Imjela, Ch.; Chiffard, P.; Schulte, A. (2022): Microplastics in Namibian river sediments – a first evaluation. In: Microplastics and Nanoplastics 2 (24), https://doi.org/10.1186/s43591-022-00043-1 This article is licensed under a Creative Commons Attribution 4.0 International License .	Published in: September 2022, Microplastics and Nanoplastics, Springer
5	Faulstich, L.; Wollenweber, S.; Reinhardt-Imjela, Ch.; Arendt, R.; Schulte, A.; Hollert, H.; Schiwy, S. (2023): Ecotoxicological evaluation of surface waters in Northern Namibia.	Submitted in: September 2023, Environmental Monitoring and Assessment, Springer

2. Methodological approach

In order to determine the water quality of surface waters and groundwater, the holistic methodological approach includes the investigation of chemical, physical, and biological properties.

2.1. Water quality analytics

The water quality is dependent on physical, chemical, and biological parameters. These parameters are useful for the assessment and monitoring of the waters for specific uses. Available methods differ regarding sample matrix and sampling size (Hossain et al. 2020). Horton (1965) developed a water quality indexing system (WQI) by using 12 common parameters to assess the water quality: pH, electrical conductivity (EC), temperature, turbidity, total solids, dissolved oxygen, coliforms, biochemical oxygen demand (BOD), alkalinity, chloride, total phosphate, and nitrate. This method generates a single numeric result and was widely applied on several continents. Since then, several approaches were developed to integrate water quality parameters into one single index. One important aspect is the purpose of water use, whether for drinking, domestic use, or irrigation (Hossain et al. 2020). There are water quality analysis standards for different purposes to ensure safe water use. The analysis of water quality is mainly used for monitoring purposes (Roy 2019). The assessment includes testing whether the water quality meets the standards. The selection of parameters followed the World Health Organization (WHO) guidelines (WHO 2017). During the last decade, nontarget analysis of water samples has dominated (Richardson & Ternes 2018).

Since no water quality data are available in Northern Namibia, the basic parameters were first investigated by means of target analysis. The methods were chosen to gain an insight into the quality of the waters in particular regarding their potability. All steps were carried out according to the German DIN (Deutsches Institut für Normung; German institute for Standardization) guidelines (DIN 1993; DIN 2009a; DIN 2009b; DIN 2012a; DIN 2012b; DIN 2012c; DIN 2013; DIN 2016; and DIN 2018). The sample design was a combination of on-site, in-situ, and off-site measurements. More than 30 parameters were measured at more than 30 sampling sites. The pH value determines water quality in regard to alkalinity, acidity, chemical speciation, and fluoride solubility.

Electrical conductivity (EC) indicates the degree of mineralization in the water (Hossain et al. 2020). Main and accompanying substances were studied. The main substances are cations (Na, K, Mg, Ca), anions (Cl, NO₃, HCO₃, SO₄), and gases (O₂, N₂) in molecular dispersed form. Accompanying substances are cations (Sr, Fe, Mn, Al, NH₄) and anions (F, NO₂).

Besides the assessment of the water body, the sediments and the total suspended solids were also analyzed for their physicochemical parameters. Total suspended solids are an important aspect in addition to the analysis of the water body and the sediment. Many substances are transported particle-bound and continue to accumulate on the suspended matter. When the suspended matter sink, such as in still waters, a heavily contaminated sediment can be formed. The sediments and suspended solids were sieved and differentiated according to their size (DIN 2009b; DIN 2016; DIN 2018). In an aqua regia digestion (DIN 2012a), the samples are centrifuged to allow the solids to settle and to remove the liquid part of the sample for further analyses. This is followed by an analysis with the ICP-OES (DIN 2009a).

Anthropogenic activities in particular can release toxic substances that feed potentially dangerous microorganisms (Kumar et al. 2012). Bacteria such as cyanobacteria, total coliforms, *Escherichia coli*, *Shigella* spp., *Staphylococcus* spp., and *Klebsiella* spp. are frequently identified as potential pathogens (Egbueri 2019). As water pollution increases, so does the risk of waterborne diseases caused by these bacteria (Wen et al. 2020). Unmonitored surface waters increase the growth of pathogenic microorganisms that endanger their consumers. In central northern Namibia, Perry et al. (2022) investigated fragments of DNA by using an Environmental-DNA approach. They successfully sampled water and soil to identify organisms on different trophic levels. Total coliforms, cyanobacteria, and *Escherichia coli* were also determined in this study. Due to the thematic focus of the individual papers, these results did not find their place in the following published data. However, total coliforms and *E. coli* were found in every sample. According to the WHO, these bacteria should not be present in potable water (WHO 2017).

2.2. Microplastics

Research on microplastics (MP) in the environment has been increasing for more than ten years. Due to the worldwide increase in plastic production and partly uncontrolled disposal, the problem of decomposing plastic particles is growing. In addition, microplastics are entering the aquatic system as primary plastics through their use in clothing, cleaning, and cosmetic products. Macro- and microplastics (> 5 mm and < 5 mm) were found in numerous studies worldwide in different ecosystems (Gerolin et al. 2020; Kundu et al. 2022; Rodrigues et al. 2018; Weber et al. 2021a).

Until now there has been no standardized method for the analysis and identification of microplastics. The large variety of methods complicates the comparison of observations; therefore, the establishment of standards would be highly beneficial (Blettler et al. 2017). The method used in this thesis was a combination of different approaches: density separation, fractionation, manual detection of particles, and Fourier Transformation Infrared (FTIR) spectroscopy. Density separation was performed with the Microplastic Sediment Separator (MPSS) according to the approach of Imhof et al. (2012). The following fractionation and manual detection of particles was developed and adapted to the Namibian sediment samples (Prume et al. 2021). The use of FTIR spectroscopy was chosen as one of the most common methods to identify microplastics (Käppler et al. 2018; Weber et al. 2021). FTIR spectroscopy can only identify a polymeric composition with a size of > 10–20 µm. Working with FTIR spectroscopy can also be very time-consuming and requires a highly experienced operator. Reference databases with spectra of known polymer types are required for the identification of microplastics (Primpke et al. 2015). This study develops its own databank of measured spectra (Faulstich et al. 2022).

In this study, aeolian sediments were excluded because the research questions focused on the river systems and fluvial sediments. Indeed, in dry river beds, aeolian erosion and transport are important and generate aeolian sediments, especially during the dry seasons, and should be further researched. As well as the sinks, such as the Etosha Pan, such sediments should be studied to gain more knowledge about the transport of microplastics.

It is unlikely that plastic production and use, a prioritized sector for economic growth, will decrease. Land use has a big impact on the polymer type (He et al. 2020b). Linking

the sources and sinks of microplastics, especially in rivers and lakes, is one important challenge.

2.3. Ecotoxicology

As micropollutants become more and more important, a comprehensive water quality assessment must also include an ecotoxicological screening. Unlike water analyses, effect-based bioassay tests can deliver substances that were not directly sought. Relevant endpoints are genotoxicity (a change of human genetic information), cytotoxicity (toxic to cells), carcinogenicity (causing cancer), reproductive toxicity (infertility and developmental disorders in the unborn child and in children), and neurotoxicity (damage to the nervous system). For each endpoint several specific test systems exist.

A broad battery of bioassays was chosen to cover several potential effects on different organisms and to provide the necessary overview of different toxicity mechanisms. A multi-taxa toxicity assessment was chosen to link the gap between chemical water quality and ecological status (Di Paolo et al. 2016).

The acute toxicity was tested at different trophic levels using algae, daphnia, and fish embryos. For mutagenicity, the AMES fluctuation assay and the Micro-Erod assay were used. Substances that influence hormone balance, such as endocrine-disrupting compounds (EDC), were analyzed with the yeast estrogen screen (YES) assay. A positive bioassay creates the necessity for chemical analysis to identify the pollutants.

The algae growth inhibition test measures the reduction in the growth rate of primary producers and is representative of aquatic plants. In the natural environment, algae are exposed to various natural but also anthropogenic stressors. Algae groups respond differently to the same pollutant; thus, the ecological consequences vary (Leal et al. 2016). Geis et al. (2000) present test results which show that algae are more sensitive than invertebrates and fish to several detergent surfactants (Geis et al. 2000). Certain substances, depending on their concentration, inhibit the cell growth of the algae. Thus, the response/toxicity is represented by a reduction in growth.

The *Daphnia magna* is representative of aquatic invertebrates (primary consumers). The acute immobilization test using *Daphnia magna* yields swift information on the environmental conditions. The *Daphnia magna*, a water flea, is often used as a “model

organism” (Jansen et al. 2011). It is a well-suited test object and reacts sensitively to different (toxic) pollutants, such as organophosphates, heavy metals, and organochlorines (Carvalho et al. 2019; Tonkopii & Iofina 2008).

The fish embryo toxicity test (FET) determines the acute toxicity of substances in the embryonic stages of fish (secondary consumers). The test is usually applied to zebrafish (*Danio rerio*). For this alternative to classical acute fish toxicity testing, the newly fertilized fish eggs are exposed to the test substance for 120 hours (Kimmel et al. 1995; Johann et al. 2021).

Besides the organisms, the mechanism-specific effects on cells were measured with the AMES mutagenicity test (TA98 and TA100), the micro-EROD test (dioxin-like activity), and the yeast estrogen screen for potential endocrine disruption. The AMES test is highly suitable for the analysis of the mutagenic potential of certain chemicals. The mutagenicity assay uses *Salmonella* bacteria to detect whether a chemical can affect its genetics, causing genetic damage and leading to gene mutations (Mortelmans & Zeiger 2000). The strains TA 98 and TA 100 were used because they respond sensitively to mutagenic compounds (Reifferscheid et al. 2011). The principle of determining the mutagenic effect is based on the ability of the substances present in the test material to induce reversions of simple auxotrophic mutants towards prototrophy, the recovery of the ability to synthesize histidine. The micro-EROD bioassay determines cytochrome activities. In the rat hepatoma cell line H4IIE, the synthesis of ethoxyresorufin-O-deethylase (EROD) is induced by dioxin-like substances (Roy et al. 2002). For this test, the rat hepatoma cell line H4IIE was used. Many substances can cause genotoxic and mutagenic potential (Binelli et al. 2009; Kumar & Dhawan 2013). Polychlorinated dibenzo-p-dioxins, dibenzofurans (“dioxins”), and dioxin-like chemicals (DLCs) are byproducts of industrial or domestic combustion processes (Huang & Buekens 1995). They likely accumulate in sediments and are hardly degraded in the environment (Schiwy et al. 2015b; Weber et al. 2008). One effect of these substances is the activation of the aryl hydrocarbon receptor (AhR) and the induction of cytochrome P450 enzymes (subfamily 1A). The induction of CYP1A-dependent monooxygenases is another one (Dencker 1985). The micro-EROD uses the activation of AhR by agonists present in the respective samples. Differences between different Ah receptors are described by the comparison to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) (Schiwy et al. 2015a). The deethylation of the exogenous substrate 7-ethoxyresorufin (ETX) is catalyzed by the induced CYP1A-

dependent monooxygenases to the fluorescent product resorufin. The quantification is calculated fluorometrically.

The recombinant yeast cell *Saccharomyces cerevisiae* containing the human estrogen receptor α (hER α) is exposed to the sample extracts. The reporter gene lacZ encoding β -galactosidase metabolizes the CPRG (chlorophenol red- β -galactosidase) to CPR (chlorophenol red). This receptor activity results in a color shift from yellow to red (Carvalho et al. 2019). Bistan et al. (2012) introduced the estrogen screening bioassay in the background of the increasing presence of endocrine-disrupting compounds in water bodies, which have a negative effect on wildlife and humans (Bistan et al. 2012). In this study, the chemical analysis and the detection of microplastics were performed beforehand and could be linked to the ecotoxicological results.

3. Paper 1: Water and sediment pollution of intensively used surface waters during a drought period – a case study in Central Northern Namibia

Abstract

Semiarid regions are often affected by water scarcity and poor water quality. Seasonal changes in precipitation and drought events increase the pressure of use on water bodies and their pollution. In Central Northern Namibia a high seasonal intra- and inter-annual variability of precipitation caused a five-year lasting drought period. In the semiarid region, ephemeral channels and water pans represent the main water source, besides the public water supply. No systematic analysis of its quality has been conducted so far. The states of the surface waters at the end of the dry season in 2017 and the end of the rainy seasons in 2018 and 2019 were characterized by the analysis of physical-chemical parameters, focusing on usability. The first results show coarse contamination of the waters, which results in high turbidity values. Salt concentrations, such as Ca^{2+} and Na^+ , greatly increased due to evaporation. Al is present in high concentrations in solid and liquid phases, which indicates direct anthropogenic pollution. Spatial differences are evident in the study area and based on the precipitation gradient, land use, and population density. The waters cannot be used as drinking water without prior treatment.

3.1 Introduction

Occurring droughts have a lasting effect on the quality of water bodies in arid and semi-arid regions (Li et al. 2018a; Mayer et al. 2010; Olds et al. 2011). Long retention times, low water flow, and reduced flushing during dry periods (Caruso 2002; Flanagan et al. 2009) result in high turbidity values and salt accumulation (Yan et al. 1996). Evaporation increases nutrient and pollution concentrations (Valcarcel Rojas et al. 2020). In the Ilishana system in the west of the Cuvelai Basin (CB) in Central Northern Namibia drought events frequently occur, last between 2015 and 2019 (Shikangalah 2020). The semi-arid climate of the region is characterized by high evaporation rates and a strong seasonal variability of precipitation with distinct dry and rainy seasons (Masih et al. 2014; Mufeti et al. 2013; NEWFIU 2015; SADC 2013). In the rainy season,

precipitation is concentrated on a few storm events per month (Kluge et al. 2008; Reason & Smart 2015). The ephemeral drainage system with its depressions called lishana (singular Oshana, see Arendt et al. 2021 for details), provides water for the rural population, though nearly no data exist on the hydrological system and the quality of surface waters (Christelis & Struckmeier 2011). During the drought period, water levels in these water bodies decreased continuously due to evaporation, anthropogenic use, and endangered water supply. In addition, the CB and in particular the lishana system are among the most densely populated areas in southern Africa which further increases the pressure on water resources. The intensive use of water bodies results in various potential sources of chemical pollution. Until now, there are no studies that focus on the conditions of the surface waters in the lishana system, even though it is an important water resource.

In the neighboring Okavango Delta, heavy and light metals were found in surface waters, partially at trace-level concentrations (Dauteuil et al. 2021; Mmualefe & Torto 2011). A concentration effect due to high evaporation was observed. In Nigeria, heavy metals in surface waters caused health risks (Tenebe et al. 2019). Increased light and heavy metal concentrations in the water column cause severe health problems with consumption (Chowdhury et al. 2016). With a decreasing pH the toxicity of metals increases (Campbel & Stokes 1985). In sediments, aluminum for example reacts in an acid environment (Rengel 2004) with a toxic effect on plants and their growth, a major problem in agriculture (DeForest et al. 2018). The toxicity and solubility of aluminum increase in acid and strong alkaline environments, with pH below 6 and above 8 (Wilson 2012).

Previous studies in the area mainly focused on the quality and isotopes of groundwater (Hamutoko et al. 2016, 2018, 2019; Lindenmaier et al. 2012) and hand-dug wells accessing shallow groundwater (Wanke et al. 2014). In these wells, mineralization and turbidity are often high and recharge is low, which decreases the potential use as drinking water (Wanke et al. 2014). All these studies reveal a great need for additional water sources, but none of them examine the lishana. The lishana already cover part of the water demand for households, subsistence agriculture, and livestock farming (Kluge et al. 2008), even if the consequences are unknown. Some studies already point out this grievance and the lack of data (Klintonberg et al. 2007; Liehr et al. 2017). This study aims to improve the knowledge of the water quality and usability of the lishana pans by evaluating the condition of water bodies during a drought period based

on nutrients, pollutants, and sediment loads, to figure out possible pollution sources. Is the water of the lishana usable for human consumption without health risks? Are there any spatial variations in the study area? Without reliable data on the surface waters, structured water use measures cannot be established. Understanding the status of surface waters is also necessary to better understand groundwater reserves. The selection of the measurement parameters is based on the requirements of the World Health Organization (WHO 2017). Samples were taken at the end of the dry season in 2017 and at the end of the rainy season in 2018 and 2019. The results are compared with standards and limit values of the Namibian Water Act 54 (DWA 1990) and international regulations for drinking water quality (Guideline for Drinking-water Quality of the World Health Organization; WHO, 2017).

3.2 Material and methods

3.2.1 Study area

The lishana system, as part of the CB, is a transboundary drainage system that leads from the planalto midlands in Angola through flat areas in northern Namibia and discharges into Lake Oponono. The study area lies in the lishana system with an area of 18,370 km², 8,726 km² on the Angolan site, and 9,644 km² on the Namibian territory (Figure 3.1). The area is located at an elevation between 1100–1200 m.a.s.l. and has a mean slope of 1 ‰ (Calunga et al. 2015; Mendelsohn et al. 2013; Persendt & Gomez 2016), which results in a very slow runoff and increased salinization processes. Saline surface sediments are dominated by fluvial sands, calcretes, and calk crusts that cause low infiltration rates and strong surface runoff (Eitel 1994; Goudie & Viles 2015; Lindenmaier et al. 2014).

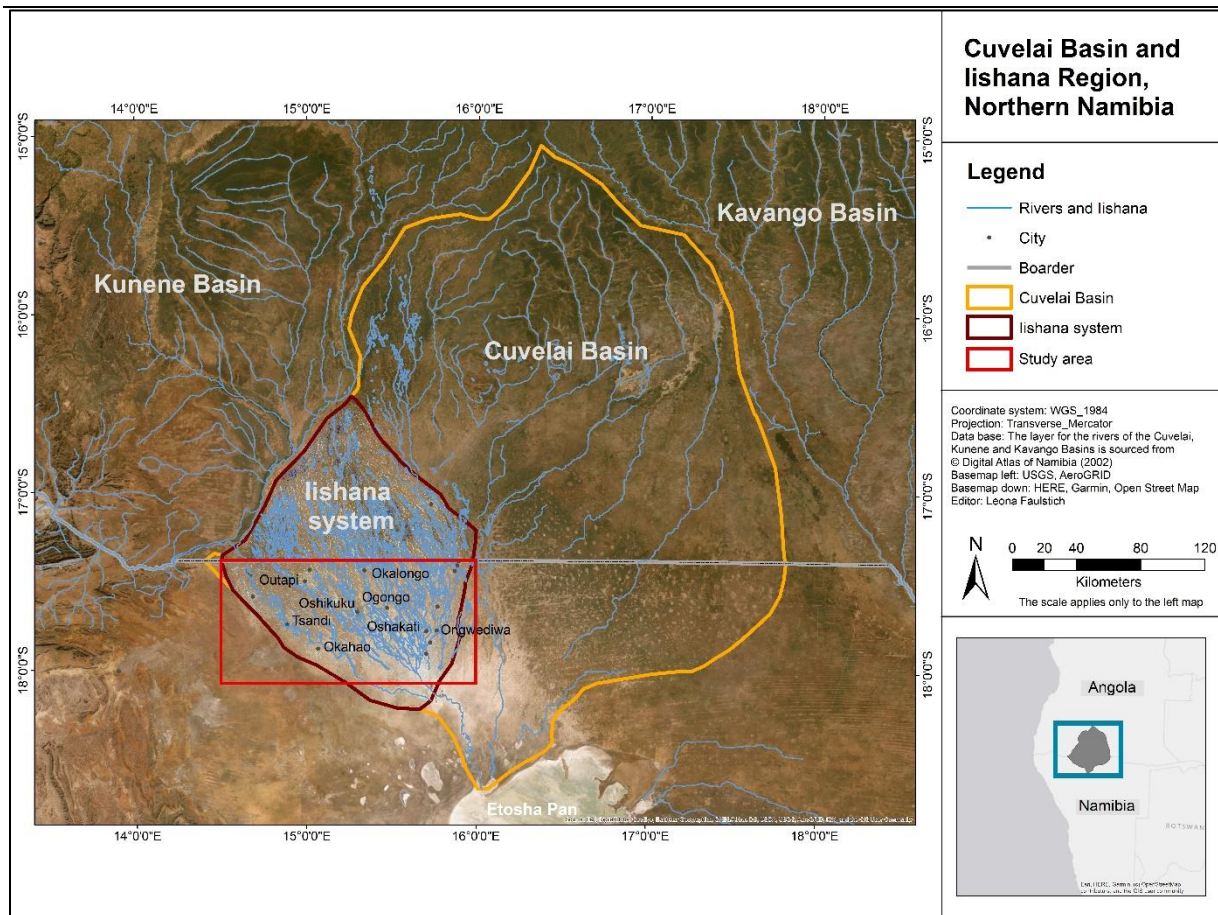


Figure 3.1: The Cuvelai Basin with the Ilishana system, neighboring river systems (Kunene and Kavango catchments), and the study area (red box)

The shallow basin enhances salinization processes in surface water, sediments, and groundwater and is a challenge for the utilization of natural resources. Most of the aquifers in the CB are located at a depth between 250 and 350 m, which impedes abstraction and distribution, so these deep aquifers can only be used to a limited extent, also because the groundwater is strongly over salted (Mendelsohn et al. 2013; Himmelsbach et al. 2018; Seely et al. 2003). Several shallower perched aquifers are in a depth between 10 and 40 m, which facilitates extraction, however, salinity and pollutant concentrations are enormous (Bäumle & Himmelsbach 2018; Christelis & Struckmeier 2011).

In the northern part of the Ilishana system, the perennial rivers Cuvelai and Mui provide water for the Angolan part of the basin (Mendelsohn et al. 2013). On the Namibian side, local, episodic precipitation and surface runoff are the only water sources. This ephemeral and endorheic system (Endorheic basins are systems without an outflow into the ocean, but into an inland body of water, such as a lake, or in this case the

Etosha Pan) consists of channels and embedded natural depressions (Arendt et al. 2021; Miller et al. 2010; Seely et al. 2003). With sufficient rainfall or during large-scale flood events, the depressions fill up and connect to form a large drainage system of thousands of small, branched channels that end in the Etosha Pan (Awadallah & Tabet 2015; Curtis et al. 1998; Hiyama et al. 2014; Hooli 2015; Lindenmaier et al. 2014; Seely et al. 2003; Shifidi 2016).

During the rainy season from October to April, rainfall varies between 350 and 550 mm/a (Mendelsohn et al. 2003 & 2013). The potential evaporation ranges from 2200 to 3500 mm/a (Mendelsohn et al. 2013), which is six times higher than the total rainfall and impedes surface water storage. The decreasing precipitation gradient from east to west affects different small-scale conditions concerning water availability (Mendelsohn et al. 2003). Due to data from the National Weather Service of the National Oceanic and Atmospheric Administration (NOAA) since 2013, the observed precipitation was below average (NOAA 2022), except for the rainy season in 2015/2016.

This complex hydrological system provides water for nearly half of the Namibian population (NSA, 2013) and is under big pressure to meet all supply demands. The main artificial source of water is the 154 km open Calueque-Oshakati canal (COC) that was built as part of the Namibia Water Master Plan in 1974 and transports water from the Kunene Dam by Calueque in Angola over the Olushandja Reservoir in Namibia to Oshakati (Shuuya & Hoko 2014). In the course of the COC, four water treatment plants were built close to urban areas: Olushandjy, Ombalantu, Ogongo, and Oshakati (see Figure 3.2). These plants treat and supply water for the towns and villages of the region. Communities or households that cannot afford charges for treated water use the available surface water from the lishana or hand-dug wells (Liehr et al. 2017; Shifidi 2016). In particular, during drought periods the surface water is usually used for cleaning, irrigation, and livestock. Tap water is rather used as drinking water, for cooking, and washing (Luetkemeier & Liehr 2015). In extreme situations of water scarcity, people use lishana water as drinking water. The high population density increases the pressure on water resources. Recent population projections assume a continuous increase in the future. With a growing rate of 1.4 % per year (NSA 2013) the population in the western CB is projected to be 27 % higher in 2041 than in 2019 (NSA 2014). This will strain the public supply system, and the already heavily used and stressed surface waters will be subject to even greater pressure.

3.2.2 Sampling sites

To analyze the quality of surface waters in the Namibian part of the Iishana system and to identify the influencing factors an evenly distributed measurement grid that would cover the entire investigation area, considering the precipitation gradient, was applied. Sampling sites at the Calueque-Oshakati canal and the Iishana were chosen to compare water sources. Sample points were selected based on areas still containing water at the end of the dry season in 2017 and resampled at the end of the following rainy seasons in 2018 and 2019. Due to the accessibility of potential sites, only those Iishana were selected, which are located within a maximum distance of 300 m from a road. During the campaigns in 2018 and 2019, many of the previously selected Iishana were no longer filled with water. Only dark sediments, partially not even wet depressions were left.

Furthermore, the tap water in Oshakati and Ruacana was analyzed to compare untreated surface water with treated water from the supply system, considering that ailing pipes can influence the water quality and do not necessarily correspond to the quality of the water when it is fed into the network by the public waterworks. During a rainfall event in March 2018, it was possible to collect rainwater for comparative analysis. A table with applied methods, corresponding test protocols, parameters, and accuracies can be found in supplementary data 3.1.

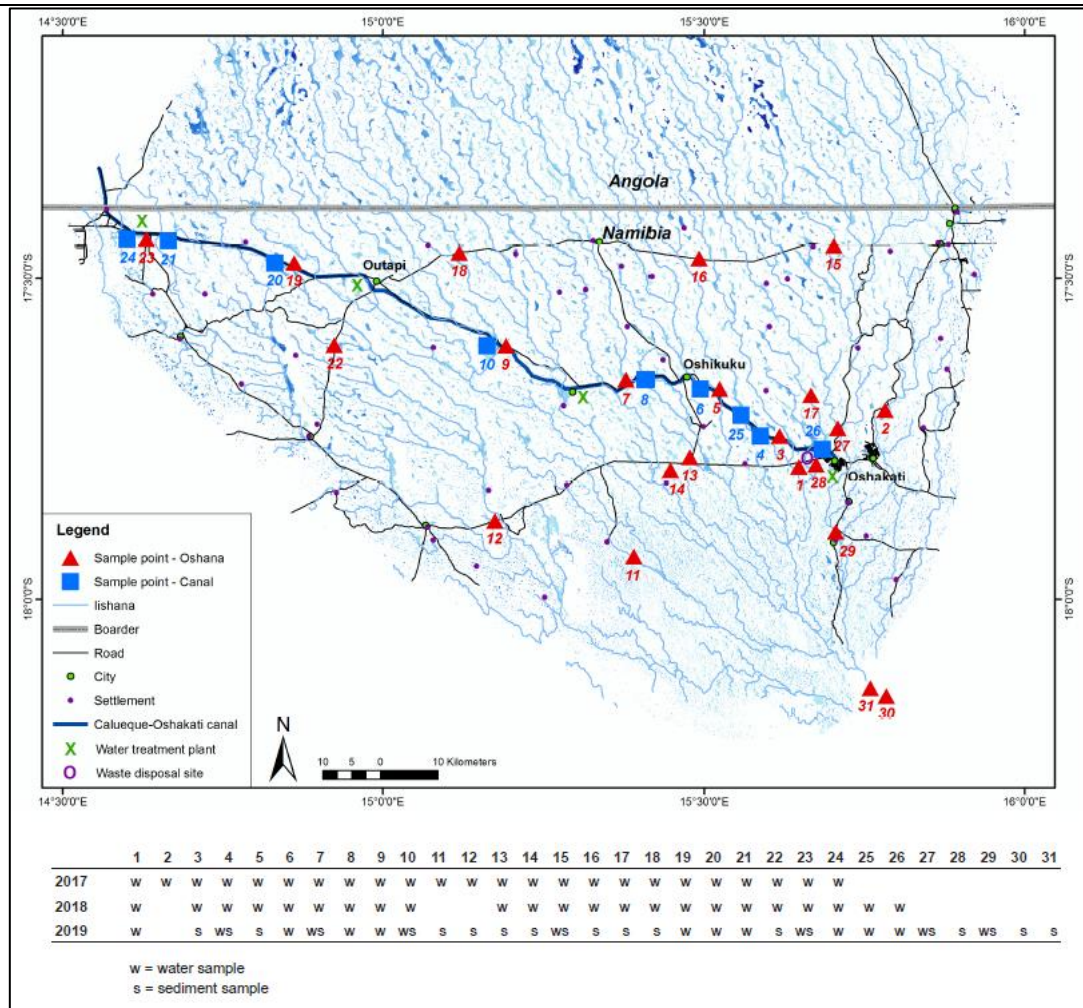


Figure 3.2: Map of the study area and sample points at the lishana and the Calueque-Oshakati canal

3.2.3 Water samples

The parameters temperature, pH value, redox potential (Eh), oxygen content and saturation, electrical conductivity (EC), turbidity, chlorophyll- α , and cyanobacteria were measured in situ with the YSI-multiparameter probe 6600 V2-4 (Xylem Analytics, Germany). Three water samples were taken from the surface water at a depth of about 0.15 m in a PE beaker according to the DIN protocol 5667-3:2013-03 (DIN 2013). The samples were taken in PE bottles (0.1 l; VWR, Germany). One sample was filtrated with a syringe filter holder (0.45 μm ; Hach Lange GmbH, Germany), acidified with 0.1 ml of nitric acid (HNO_3 ; fuming 100 %, Carl Roth GmbH + Co. KG, Germany), and cooled for the transport. One unfiltered sample was taken for the analyses of suspended solids. The last sample was transported in a 0.1 l PE bottle to the field laboratory at the UNAM, Campus Ongwediva, for “on-site” analyses. On the same day,

the samples were centrifugated (EBA 280, Hettich GmbH & Co. KG, Germany) for 20 min at 6000 min^{-1} to facilitate the following filtration with syringe filter holders ($0.45 \mu\text{m}$; Hach Lange GmbH, Germany). Afterwards, the ion concentrations were measured with the portable HACH DR 1900 VIS spectrophotometer (Hach Lange GmbH, Germany) and a cuvette system. Such portable spectrophotometers were previously used in several studies to analyze water samples in the field (Marlow et al. 2000; Pandey et al. 2019; Zhang et al. 2019). Following anions and sum parameters were analyzed: chloride (Cl^-), fluoride (F^-), ammonium (NH_4^+), nitrate (NO_3^-), nitrite (NO_2^-), phosphate and orthophosphate (PO_4^{3-}), sulfate (SO_4^{2-}), chemical oxygen demand (COD), total nitrogen bound (TNb), total carbon (TC), total inorganic carbon (TIC) and total organic carbon (TOC).

At the laboratory at Freie Universitaet Berlin, Germany, the acidified samples were centrifuged with a Multifuge 4KR Heraeus Centrifuge (Thermo Fisher Scientific, USA) for 15 min at $10,000 \text{ min}^{-1}$. The liquid was pipetted and filtrated with a $0.45 \mu\text{m}$ membrane filter (cellulose acetate; VWR, Germany) to exclude all suspended solids. After the preparation, the following cations were analyzed with the ICP-OES 2000 (Perkin Elmer, USA) according to the DIN protocol DIN EN ISO 11885:2009 (DIN 2009a): aluminum (Al), arsenic (As), cadmium (Cd), calcium (Ca^{2+}), chrome (Cr), cobalt (Co), copper (Cu), iron (Fe^{2+}), lead (Pb), magnesium (Mg^{2+}), manganese (Mn), nickel (Ni), potassium (K^+), sodium (Na^+), strontium (Sr) and zinc (Zn).

The unfiltered samples were centrifuged for 15 min at $10,000 \text{ min}^{-1}$ (Multifuge 4KR Heraeus Centrifuge; Thermo Fisher Scientific, USA) and filtered ($0.45 \mu\text{m}$ membrane cellulose acetate filter; VWR, Germany). In total nine lishana left enough suspended solids ($> 0.5 \text{ g}$) for analysis. The solid matter was dissolved with an Aqua Regia digestion (according to DIN EN 16174:2012-11; DIN 2012a). The new solutions were analyzed with the ICP-OES 2000 for the same ions as the water samples.

3.2.4 Sediment samples

The sediment samples were taken from the bottom of the dry lishana, and from the lishana, which were still filled with water, from the water's edge. The first centimeters were discarded to exclude aeolian sediments. The sediments were taken with a wooden spoon and transported in plastic bags. In the canal, the sediments were collected with a plastic beaker on an extension rod.

In the laboratory, the samples were homogenized in evaporation dishes and dried for 24 h at 45 °C. The sieving was done with plastic sieves with a diameter of 150 mm and a mesh width of 2.0 mm and 1.0 mm (Test Sieves; VWR, Germany). The rest of the sample was wet sieved according to the DIN protocol 66165-2:2016-08 (DIN 2016) through a 0.063 mm sieve (Test Sieve, VWR, Germany; diameter of 150 mm). The grain size distribution was performed by using a Beckman Coulter LS 13 320 laser diffractometer (Beckman Coulter Life Sciences, USA) according to the DIN protocols (DIN 2009b; DIN 2018). For determination of the pH value 5 ml of the sample was suspended in a 0.01 mol/l CaCl₂ solution (≥ 99 %, p.a., ACS, Carl Roth GmbH + Co. KG, Germany; DIN 2012b). For the EC measurement, deionized water was used (DIN 1993). Total carbon (TC) was determined using a LECO TruSpec Elemental Determinator (LECO, USA). Total inorganic carbon (TIC) was measured using a Carmograph C16 (Wösthoff; Germany) and total organic carbon (TOC) was calculated. The different carbon contents were determined according to the DIN protocol DIN EN 15936:2012-11 (DIN 2012c). Parts of the fractions < 0.063 mm and 0.063 – 1.0 mm were dried at 50 °C for 24 h and digested with Aqua Regia (DIN 2012a) for analysis with the ICP-OES (DIN 2009a).

3.2.5 Water guidelines

Critical values for the quality of water for domestic use and tap water are not specifically established in Namibia. The only Namibian directive for the quality of different types of water is the Water Act 54 of 1956 (DWAF 1990). It was adopted from South Africa in the 1950s and was updated in 1990 by the Namibian government. The Water Act 54 applies to drinking water and water for domestic use. There are four categories for water analysis that should divide the waters into different groups: i) Group A: Water with excellent quality, ii) Group B: Water with acceptable quality, iii) Group C: Water with low health risk, and iiiii) Group D: Water with a high health risk, or water unsuitable for human consumption (DWAF 1990).

Considering the results and the classification in an international context the critical values for drinking water from the World Health Organization (WHO 2017) are also used. The WHO defines guideline values for parameters, which are harmful to human health (in particular metals like copper, nickel, chrome, lead, cadmium, and arsenic). For other parameters, like ammonium, chloride, iron, manganese, nitrate, potassium,

sodium, sulfate, and zinc no guideline values have been established yet. Usually, these parameters occur in drinking water at concentrations well below those of health concern. Since the water of the lishana is used as drinking water (Neliwa & Kalumbu 2019; Sturm et al. 2009), a combination of these two guidelines guarantees an adequate assessment of local surface waters.

3.2.6 Data analysis

The data analysis was performed by using the scripting language R (Version: 4.2.0) (R Core Team 2019), in an RStudio environment (Version: 2022-04-22 ucrt) with several packages in Rstudio: “compositions”, “psych”, “car”, “dplyr”, “ggplot2”, “pgirmess”, and “PMCMRplus”. Compositional data, as measured concentrations, are non-symmetrical distributed. Since the numerical space is positive, it is necessary to use the geometric mean instead of the arithmetic mean (Aitchison 1994; Greenacre 2021). To perform the statistical tests adequately, the data were log-transformed (centered log-ratio clr transformation) before testing. After performing the tests, the data were retransformed. Each parameter was tested for normal distribution in each campaign using the Shapiro-Wilk test. A Levene test was performed to check the differences in variances. The not normal distributed parameters with unequal variances were tested with a Friedman test to gain knowledge of the differences between sampling sites (lishana and COC) as well as between dry and wet seasons. As a post hoc test, the Wilcoxon test was carried out. The sample sizes of suspended solids of the COC and the lishana in 2019 were too small for statistical analysis. The data of suspended solids in 2017 and 2018 are paired and normally distributed, therefore the paired T-test was performed. Differences between the lishana and the COC were tested with the Mann-Whitney-U-test (95 % confidence interval), because of unrelated and not normally distributed data. The statistical analysis results were defined as significant with a p-value < 0.05.

3.3 Results

3.3.1 Hydrochemistry and water quality

At the end of the dry season in 2017, the sampled lishana were filled with water, after the wet season in 2018 three lishana were dried up, and at the end of the wet season in 2019 eleven more. The rainy seasons in 2018 and 2019 were not strong enough to

fill every lishana with sufficient water. Water levels varied between 0.2 m and > 0.7 m (a detailed table can be found in the supplementary data 3.2). Ranges, means, and standard deviations of physicochemical parameters and major ion concentrations are presented in Table 3.1. Several parameters, like carbon, oxygen, and heavy metal concentrations, are not discussed in the text, but can be found in the supplementary data 3.3.

Table 3.1: Range, mean, and standard deviation (sd) of the hydrochemical composition of lishana, Calueque-Oshakati canal, tap water, and precipitation. The temperature in °C, EC in $\mu\text{S}/\text{cm}$, turbidity in NTU (Nephelometric Turbidity Unit), and major ions in mg/l. Limit values of the guidelines Namibian Water Act 54 from 1956 and Guidelines for Drinking-water Quality of the WHO from 2017

	lishana						Calueque-Oshakati canal					
	n = 19						n = 9					
	range			mean \pm sd			range			mean \pm sd		
	2017	2018	2019	2017	2018	2019	2017	2018	2019	2017	2018	2019
dry	wet	wet	dry	wet	wet	dry	wet	wet	dry	wet	wet	
temp	20.1 - 31.2	23.3 - 33.2	22.4 - 32.1	25.0 \pm 3.3	28.8 \pm 2.2	27.2 \pm 2.8	21.1 - 28.0	25.5 - 29.3	23.3 - 31.7	24.8 \pm 2.1	26.6 \pm 1.2	26.7 \pm 2.9
pH	7.3 - 9.4	6.3 - 8.6	7.0 - 9.2	8.2 \pm 0.6	7.4 \pm 0.8	8.1 \pm 0.7	7.3 - 8.9	6.5 - 8.1	6.4 - 7.7	8.0 \pm 0.5	7.3 \pm 0.4	7.0 \pm 0.5
EC	80 - 2230	90 - 2020	76 - 2935	911.2 \pm 669.9	614.7 \pm 655.4	724.9 \pm 229.3	74 - 99	43 - 98	64 - 109	82.3 \pm 7.8	71.9 \pm 17.7	75.0 \pm 13.2
turbidity	17.5 - 1471.6	47.9 - 1422.3	16.1 - 1615.4	631.5 \pm 551.6	722.2 \pm 545.9	477.6 \pm 496.7	6.5 - 17.6	64.3 - 317.6	53.5 - 290.5	12.7 \pm 3.6	164.8 \pm 96.2	196.5 \pm 83.2
Cl⁻	3.9 - 554.0	9.28 - 678.0	3.9 - 342.0	206.9 \pm 179.0	170 \pm 209.1	71.4 \pm 105.4	1.3 - 2.8	3.3 - 18.1	1.1 - 3.9	1.91 \pm 0.5	8.8 \pm 5.4	2.2 \pm 0.7
SO₄²⁻	72.5 - 707.1	48.7 - 1224.0	47.0 - 309.2	252 \pm 171.1	298 \pm 334.3	126.1 \pm 78.9	< 1.0	47.3 - 96.7	35.5 - 68.6	-	73.1 \pm 19.4	50.1 \pm 14.6
NO₃⁻	0.1 - 3.5	1.6 - 24.1	0.2 - 3.8	1.6 \pm 1.1	8.5 \pm 6.9	1.0 \pm 1.1	0.02 - 0.24	1.8 - 6.0	0.3 - 0.6	0.2 \pm 0.1	3.1 \pm 1.3	0.4 \pm 0.1
NO₂⁻	0.03 - 0.5	0.05 - 7.9	0.03 - 0.1	0.2 \pm 0.2	2.0 \pm 2.6	0.07 \pm 0.05	0.01 - 0.02	0.2 - 0.9	< 0.01	0.020 \pm 0.001	0.5 \pm 0.3	-
NH₄	0.06 - 2.0	0.08 - 12.4	0.02 - 0.2	0.5 \pm 0.5	2.6 \pm 3.5	0.07 \pm 0.1	0.02 - 0.07	0.07 - 8.58	0.02 - 0.06	0.04 \pm 0.02	1.8 \pm 3.1	0.03 \pm 0.01
PO₄³⁻	0.1 - 1.5	0.1 - 37.1	0.05 - 4.5	0.6 \pm 0.4	7.5 \pm 10.8	0.9 \pm 1.4	< 0.05 - 0.3	0.4 - 1.8	0.1 - 0.3	-	0.9 \pm 0.5	0.32 \pm 0.01
F⁻	0.3 - 1.0	0.1 - 0.3	0.2 - 2.8	0.6 \pm 0.3	0.2 \pm 0.1	0.8 \pm 0.9	0.1 - 0.2	< 0.1	0.1 - 0.2	0.15 \pm 0.04	-	0.12 \pm 0.01
K⁺	1.9 - 17.5	3.0 - 26.9	0.3 - 9.6	8.6 \pm 4.6	9.7 \pm 7.2	5.6 \pm 3.6	1.5 - 17.1	1.7 - 4.4	0.1 - 0.5	4.1 \pm 5.3	3.0 \pm 0.8	0.3 \pm 0.2
Na⁺	5.8 - 397.0	10.9 - 429.0	2.1 - 196.5	132.1 \pm 112.3	109.2 \pm 126.9	67.4 \pm 65.4	5.1 - 281.8	4.5 - 15.4	3.7 - 6.8	45.8 \pm 96.4	8.8 \pm 3.7	4.6 \pm 0.9
Mg²⁺	2.0 - 17.7	3.6 - 27.2	2.3 - 17.5	8.6 \pm 4.3	13.1 \pm 6.9	6.8 \pm 4.2	1.9 - 28.7	1.8 - 8.2	1.6 - 2.1	5.9 \pm 9.3	4.7 \pm 2.2	1.8 \pm 0.1
Ca²⁺	6.0 - 37.7	5.8 - 51.4	3.8 - 26.9	17.2 \pm 8.4	28.7 \pm 9.6	15.8 \pm 6.6	5.7 - 42.8	6.2 - 26.4	4.9 - 7.0	11.7 \pm 12.7	16.1 \pm 7.7	5.9 \pm 0.6
Mn	0.0 - 0.7	0.05 - 0.8	0.01 - 0.4	0.2 \pm 0.2	0.3 \pm 0.2	0.1 \pm 0.1	0.004 - 0.620	0.001 - 0.186	0.02 - 0.07	0.1 \pm 0.2	0.08 \pm 0.06	0.05 \pm 0.02
Fe²⁺	0.1 - 3.9	0.3 - 2.8	0.01 - 0.4	1.5 \pm 1.3	1.6 \pm 0.9	0.2 \pm 0.1	0.2 - 3.7	0.3 - 2.0	0.2 - 1.0	0.7 \pm 1.2	1.1 \pm 0.7	0.6 \pm 0.3

Al	0.2 - 16.3	0.7 - 19.3	0.2 - 1.6	5.2 ± 5.5	6.4 ± 5.7	0.7 ± 0.5	0.1 - 18.2	0.3 - 2.4	0.2 - 0.5	2.8 ± 6.3	1.2 ± 0.7	0.3 ± 0.1
Pb [µg/l]	0.0 - 5.5	0.05 - 9.2	0.0 - 3.6	2.5 ± 1.3	2.4 ± 2.5	2.1 ± 1.2	< 0.001 - 2.8	< 0.001 - 3.3	< 0.001 - 1.4	1.9 ± 0.9	1.6 ± 1.2	-

Table 3.1: continued

	Tap water		Precipitation		Limit values				
	n = 2		n = 4		Namibian Water Act 54				WHO
	Ongwediva	Ruacana	range	mean ± sd	A	B	C	D	
	2017	2017	2018	2018					
temp	27.1	27.4	19.2 - 21.8	20.8 ± 1.0	-	-	-	-	-
pH	7.7	8.2	6.4 - 6.6	6.52 ± 0.05	6.0 - 9.0	5.5 - 9.5	4.0 - 11.0	4.0 - 11.0	-*
EC	105	97	17 - 404	152.3 ± 157.7	1500	3000	4000	> 4000	-*
turbidity	1	0	1.4 - 22.2	12.7 ± 9.6	1	5	10	> 10	0.5
Cl⁻	5.2	3.7	26.9 - 72.1	49.5 ± 22.6	250	600	1200	> 1200	-*
SO₄²⁻	< 1.0	< 1.0	< 1.0	-	200	600	1200	> 1200	-*
NO₃⁻	< 0.01	< 0.01	1.3 - 35.2	13.8 ± 13.9	10	20	40	> 40	50
NO₂⁻	< 0.01	< 0.01	0.07 - 0.62	0.3 ± 0.2	-*	-*	-*	-*	3
NH₄	< 0.015	0.04	0.2 - 2.8	1.21 ± 1.06	1	2	4	> 4	-*
PO₄³⁻	< 0.05	< 0.05	0.1 - 0.4	0.3 ± 0.1	-	-	-	-	-
F⁻	0.2	0.2	0.2 - 0.8	0.5 ± 0.3	1.5	2	3	> 3	1.5
K⁺	2.0	1.9	0.08 - 2.80	1.10 ± 1.08	0.2	0.4	0.8	> 0.8	-*
Na⁺	6.0	4.6	0.4 - 63.7	20.8 ± 25.8	0.1	0.4	0.8	> 0.8	-*
Mg²⁺	2.2	1.8	0.2 - 2.7	1.2 ± 1.0	70	100	200	> 200	-*
Ca²⁺	7.0	8.7	1.0 - 17.0	6.3 ± 6.5	0.15	0.2	0.4	> 0.4	-*

Mn	0.0	0.0	0.004 - 0.071	0.03 ± 0.03	0.05	1	2	> 2.0	-*
Fe²⁺	0.0	0.0	0.02 - 0.27	0.14 ± 0.09	0.1	1	2	> 2.0	-*
Al	0.0	0.0	0.04 - 0.56	0.3 ± 0.2	0.15	0.5	1	> 1	0.9
Pb [µg/l]	2.0	9.5	< 0.001 - 1.0	-	50	100	200	200	10

-* = no guideline values have been established yet

The end of the dry season in 2017

Concerning physicochemical parameters temperature and pH, values are slightly higher in the lishana than in the COC. All samples have alkaline pH values and are sorted into category A of the Namibian Water Act (6.00 – 9.00). Only site 17 is strongly alkaline with a value of 9 (category B). EC values of most of the lishana belong to category A, only sites 1, 5, 16, and 17 to category B. The COC always has values < 100 $\mu\text{S}/\text{cm}$ and belongs to category A. The values for turbidity exceed the limit values at each site and are sorted into categories C and D. Only the tap water samples show turbidity values < 1 NTU. For turbidity, the WHO limit is 0.5 NTU and all sites exceed this guideline value.

The major anions of the lishana and the COC can be ranked according to their dominance, based on means, in the order of: $\text{SO}_4^{2-} > \text{Cl}^- \gg \text{NO}_3^- > \text{F}^-$ and the cations in the order of: $\text{Na}^+ > \text{Ca}^{2+} > \text{Mg}^{2+} > \text{K}^+$. Salts and salt compounds are dominant. The Ca^{2+} and Na^+ values are completely in category D of the Namibian Water Act. Most of the element concentrations of tap water were below the detection limits, just Cl^- and F^- were detected in higher concentrations. In the lishana and partially the COC the striking parameters are Al and Fe^{2+} , which occur in high amounts. The observed Fe^{2+} concentrations are considered a health threat for the population in 35.3 % of the cases (14.3 % for the COC, category D). Al is highly concentrated with a maximum of 16.3 mg/l at lishana site 13 and a maximum of 18.2 mg/l at site 4 of the COC. Due to high aluminum levels, 70.6 % of the lishana sites and 5.9 % of the canal-side sites are at increased health risk (category D). Within the study area, the spatial distribution of aluminum is eastbound, with higher values close to urban centers, like Oshakati and Ongwediva.

The end of the wet season in 2018

In 2018, sampling sites 2, 11, and 12 were dried up. The physicochemical parameters slightly changed compared to 2017. The pH value decreased by a value of 1 at the lishana and 0.7 at the COC. Precipitation also has a slightly acid pH value with an average of 6.5. EC values decreased from an average of 911 $\mu\text{S}/\text{cm}$ to 615 $\mu\text{S}/\text{cm}$ at the lishana and 82 $\mu\text{S}/\text{cm}$ to 72 $\mu\text{S}/\text{cm}$ at the COC. The precipitation shows values around 152 $\mu\text{S}/\text{cm}$, with a smaller range. Turbidity values increased on average from 631 to 722 NTU. In the COC, the change is even bigger with an average of 165 NTU

(compared to 13 NTU in 2017). The temperature of the lishana and the turbidity of the COC are significantly different between 2017 and 2018 ($p = 0.04$ and $p = 0.005$).

Like in 2017 the orders of anions in 2018 ($\text{SO}_4^{2-} > \text{Cl}^- \gg \text{NO}_3^- > \text{NO}_2^-$) and cations ($\text{Na}^+ > \text{Ca}^{2+} > \text{Mg}^{2+} > \text{K}^+$) for the lishana and the COC ($\text{Ca}^{2+} > \text{Na}^+ > \text{Mg}^{2+} > \text{K}^+$) show a dominance of salts and salt compounds. Anions in precipitation can be ordered: $\text{Cl}^- \gg \text{NO}_3^- \gg \text{F}^- > \text{NO}_2^-$ and cations: $\text{Na}^+ > \text{Ca}^{2+} > \text{Mg}^{2+} > \text{K}^+$. From 2017 to 2018, the concentrations of SO_4^{2-} , Cl^- , and NO_3^- increased at sites 13, 14, and 16 from category A to B or C and decreased at others (3, 5) from B to A. For the COC, no changes were observed for these parameters. Ca^{2+} and Na^+ are still in category D and most of the sites have increased values. Mn and NO_2^- increased at sites 3, 13, and 14. PO_4^{3-} and NH_4^+ increased at sites 13, 14, and 16 from not dangerous concentrations (categories A and B) to concentrations considered as high health risk (category D). The Mn concentrations of the lishana have been a potential threat to human health in both years (category B and C). Only some sites at the COC included Mn in these amounts (sites 4, 6, 20, 24, and 25). Significant differences were found between the lishana for Ca^{2+} ($p = 0.04$), NH_4^+ ($p = 0.02$), NO_3^- ($p = 0.01$), Al ($p = 0.005$), and Fe^{2+} ($p = 0.03$). For the COC: Cl^- ($p = 0.02$), Pb ($p = 0.01$), NO_3^- ($p = 0.0009$), F^- ($p = 0.002$), and Al ($p = 0.02$). Between the end of the dry season in 2017 and the end of the rainy season in 2018, mixing of the lishana water with rainwater and thus a dilution could be expected. Some parameters showed decreasing concentrations: pH, EC, Na^+ , Cl^- , F^- , however turbidity as well as K^+ , Mg^{2+} , Ca^{2+} , SO_4^{2-} , Fe^{2+} , NO_3^- , NO_2^- and Al increased.

Concentrations of NO_3^- , NO_2^- , PO_4^{3-} , and SO_4^{2-} show great spatial variability. Sites that are close to settlements or cities, in the eastern part of the study area, around the city of Oshakati, or along the COC (3, 17, 27, and 28), show higher values than remote sites in the southwestern part (11, 12, and 22). Sites north of road dams (retention of flood waters and preferred sediment accumulation) also show higher concentrations of metals and nutrients (16, 18).

In 2018 the highest aluminum concentration of 19.3 mg/l and an average of 6.4 mg/l were analyzed, an increase of 23 % compared to 2017. In total 78.6 % of the lishana and 55.5 % of the COC represent a high risk for human health. Especially the lishana close to Oshakati (sites 3 and 5) show high aluminum values. Site 4 at the COC contained more than 18 mg/l in 2017 but decreased in 2018. Concerning Fe^{2+} , 35.7 % of the lishana and 22.2 % of the COC samples are unsuitable for human consumption

and represent a high health risk (category D). Light metals are present in precipitation samples with low concentrations close to the detection limit.

The end of the rainy season in 2019

Due to low rainfall during the rainy seasons of 2018 and 2019 and ongoing high evapotranspiration values, several lishana completely lost their water. The pH values of the lishana increased on average to 8.1, in the COC they dropped to the neutral value of 7. The EC values of the lishana increased on average to 725 $\mu\text{S}/\text{cm}$ and had a broader range from 76 to 2935 $\mu\text{S}/\text{cm}$. The COC also had a wide range from 64 to 109 $\mu\text{S}/\text{cm}$ and raised on average to 75 $\mu\text{S}/\text{cm}$. Turbidity exceeds in every sample the limit of 1 NTU. Over the entire observation period, EC values of the lishana belong to categories A and B and represent water, which still requires prior treatment before consumption.

The order of anions in the lishana is, like in 2017, $\text{SO}_4^{2-} > \text{Cl}^- \gg \text{NO}_3^- > \text{F}^-$ and of cations: $\text{Na}^+ > \text{Ca}^{2+} > \text{Mg}^{2+} > \text{K}^+$. For the COC the cations composition changed: $\text{Ca}^{2+} > \text{Na}^+ > \text{Mg}^{2+} > \text{K}^+$. The high Al and Fe^{2+} values in the lishana water of the last two years were not reached in this campaign. In 2019, 0.7 mg/l were detected on average. The maximum was found at site 7 with 1.6 mg/l. In the center of the study area (northwest to southeast), there are several sites with high Al^{3+} concentrations (sites 18, 9, 13, 14, and 11). The Fe^{2+} concentrations have decreased and samples of the lishana and the COC are in categories A, B, or C. Heavy metals were found in trace concentrations at all sites and fell every year in category A. Significant differences to 2017 and 2018 could be tested for the lishana and the COC for several parameters, like Al ($p = 0.018$), Ca^{2+} ($p = 0.042$), and Fe^{2+} ($p = 0.03$) (more information can be found in the supplementary data 3.3).

Comparison of the three campaigns

To illustrate the ionic composition of the water samples over all three years Piper diagrams were created (see Figure 3a-c). For this presentation, hydrogen carbonate (HCO_3^-) values were calculated via the ion balance. In 2017 (3a) most of the lishana are Na-K dominated. The COC, the tap water, and lishana 5, 19, and 22 contain more calcium. The sites scatter much more in the anion content. Sulfate and chloride contents differ extremely between the sites. In 2018 (3b) the calcium dominance increased, the chloride content decreased, while sulfate and bicarbonate dominated.

Alkaline earths dominate 2018 more than in 2017 and sulfate and chloride prevail slightly more than hydrogen carbonate.

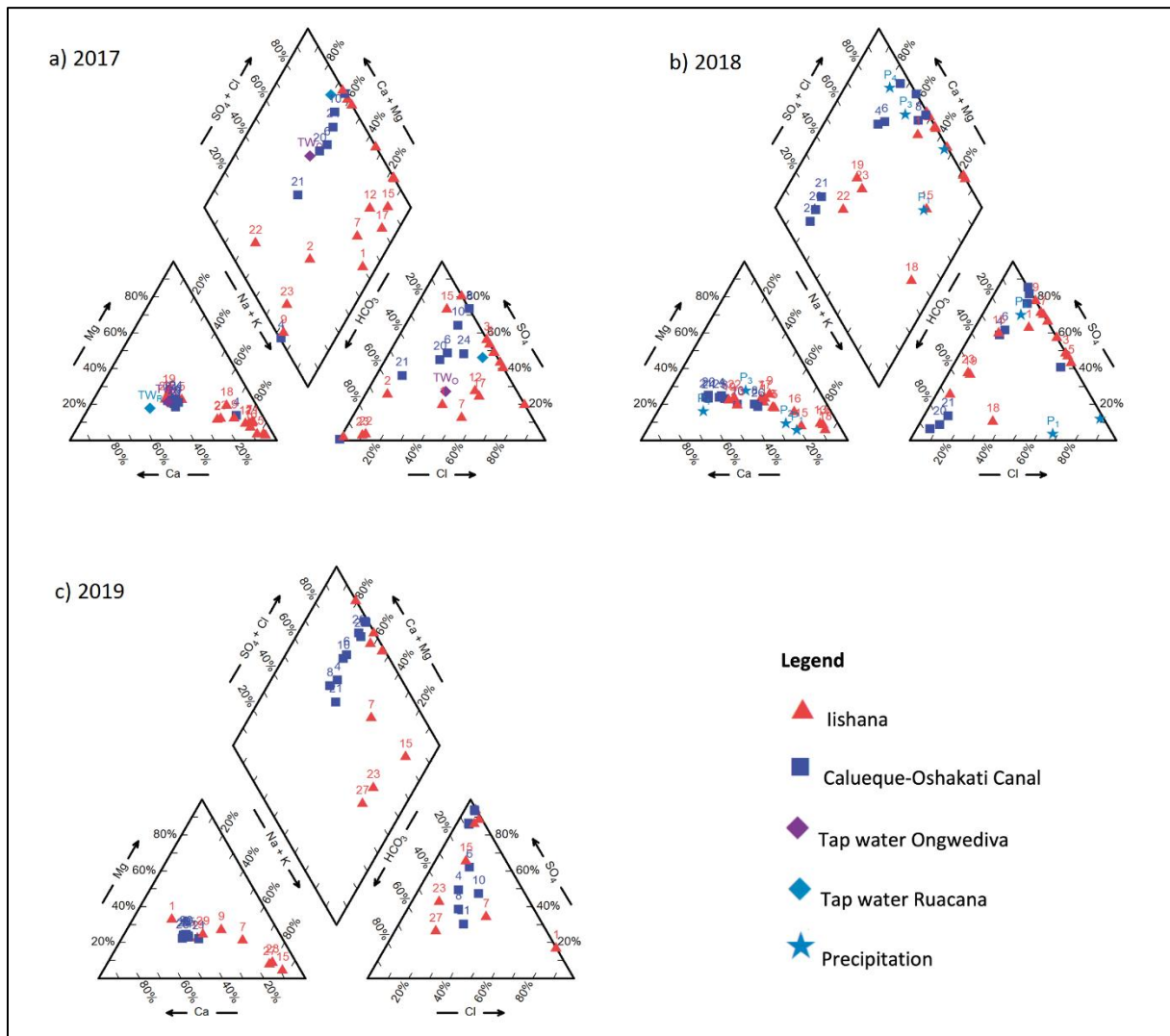


Figure 3.3: Piper diagrams of the lishana, the Calueque-Oshakati canal, tap water, and precipitation samples from 2017–2019

In 2019 (3c) the ratio of the cations has hardly changed and the anions are more dominated by sulfate and hydrogen carbonate. Earth alkaline, sulfate, and chloride prevail, although the sites of the COC are even more homogeneous. Statistically significant effects between the three campaigns exist for the lishana and the COC for almost all parameters, except EC, Mn, and SO_4^{2-} (a detailed table can be found in the supplementary data 3.4).

The aluminum concentrations of the three years are an example of the spatial distribution of the water quality in the study area. High values in the eastern part,

around the urban agglomeration of the city Oshakati are striking. Changes over the three years are particularly visible at the lishana ($p = 0.005$). Except for sites that dried up over the three years, most values increased from 2017 to 2018 and decreased again in 2019. Along the COC, the values increase with the flow direction. Maximum values were measured in 2018 and at site 4 in 2017, and differences were significant ($p = 0.02$). Figure 3.4 shows an example of the changes in Al and Fe²⁺ for the lishana over the study period.

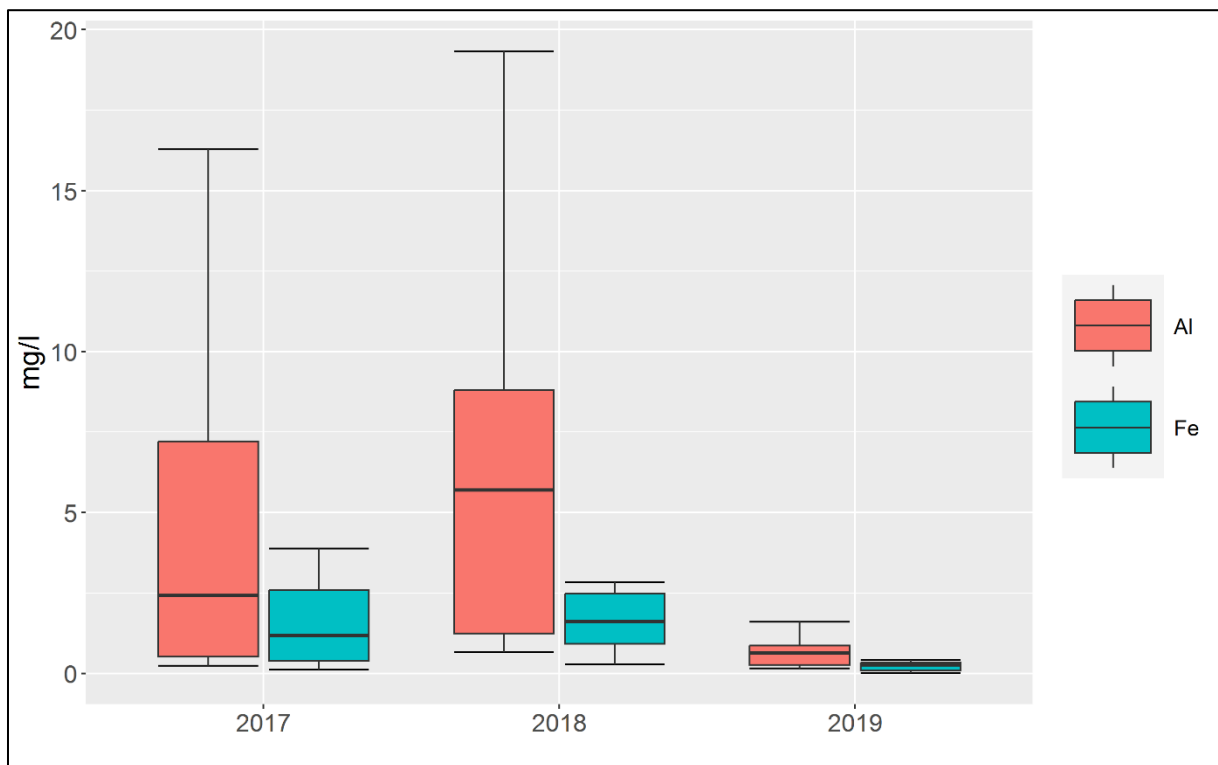


Figure 3.4: Changes in Al and Fe concentrations in the lishana between 2017 and 2019

3.3.2 Suspended solids and sediments

Suspended solids and sediments are presented in Table 3.2. Ranges, means and standard deviations of physicochemical parameters and main concentrations of the lishana and the COC show slight changes over the three years. PH values and EC could only be measured for the bottom sediments of the dried lishana or the canal, not for the suspended solids in the water. The pH values of the lishana are slightly alkaline on average, with a strong alkaline maximum of 9.3. The COC has lower pH values with 7.5. As with the water samples, the sediments of the lishana show up to 14010 $\mu\text{S}/\text{cm}$, much higher EC values than the canal with 104 $\mu\text{S}/\text{cm}$.

Table 3.2: Range, mean, and standard deviation (sd) for physio-chemical parameters and major element concentrations of suspended solids and sediments of the lishana, the COC, and the waterworks in Oshakati (WO). EC in $\mu\text{S}/\text{cm}$, concentrations in mg/g , Pb in $\mu\text{g}/\text{g}$.

	Iishana									
	Suspended solids < 0.45 μm						Sediments < 0.063 mm		Sediments < 1.00 mm	
	n = 8, 9, 5						n = 17		n = 17	
	range			mean \pm sd			range	mean \pm sd	range	mean \pm sd
	2017	2018	2019	2017	2018	2019	2019	2019	2019	2019
pH	-	-	-	-	-	-	6.5 - 9.3	7.4 \pm 0.8	-	-
EC	-	-	-	-	-	-	80 - 14010	2648 \pm 3941	-	-
K⁺	5.5 - 18.0	3.9 - 18.8	4.1 - 10.8	12.8 \pm 4.3	11.5 \pm 5.1	7.0 \pm 2.2	4.6 - 15.9	10.1 \pm 3.5	0.3 - 16.2	5.5 \pm 4.4
Na⁺	0.9 - 5.1	4.4 - 12.5	2.4 - 10.9	2.2 \pm 1.1	8.3 \pm 2.5	5.5 \pm 3.1	0.6 - 62.7	10.4 \pm 14.4	0.2 - 43.4	6.3 \pm 11.4
Mg²⁺	8.1 - 10.6	3.3 - 11.4	7.7 - 9.1	9.4 \pm 0.8	8.3 \pm 2.4	8.4 \pm 0.5	6.2 - 14.1	9.6 \pm 1.9	0.4 - 10.3	5.3 \pm 3.8
Ca²⁺	0.5 - 3.0	1.2 - 3.3	1.8 - 9.7	1.3 \pm 0.7	2.6 \pm 0.7	5.8 \pm 2.5	1.6 - 14.7	6.3 \pm 3.7	0.7 - 7.1	3.1 \pm 1.9
Mn	0.2 - 0.3	0.07 - 0.31	0.2 - 0.3	0.22 \pm 0.03	0.2 \pm 0.1	0.22 \pm 0.04	0.3 - 0.6	0.38 \pm 0.08	0.02 - 0.40	0.2 \pm 0.1
Fe²⁺	27.8 - 44.6	11.0 - 41.2	19.6 - 30.2	39.0 \pm 4.9	30.5 \pm 9.3	26.4 \pm 4.0	12.4 - 38.0	30.8 \pm 7.0	1.0 - 37.8	16.8 \pm 12.1
Al	58.9 - 86.7	25.8 - 85.2	56.6 - 70.4	80.7 \pm 8.9	64.0 \pm 18.3	64.6 \pm 5.2	27.0 - 77.6	63.5 \pm 13.9	1.2 - 75.4	35.2 \pm 26.2
Pb [$\mu\text{g}/\text{g}$]	6.9 - 12.6	4.0 - 9.1	12.5 - 228.2	9.3 \pm 2.0	7.0 \pm 1.3	82.7 \pm 81.6	0.009 - 0.018	0.013 \pm 0.003	0.001 - 0.012	0.007 \pm 0.004

Table 3.2: continued

	Calueque-Oshakati canal					Waterworks Oshakati
	Suspended solids < 0.45 µm	Sediments < 0.063 mm		Sediments < 1.00 mm		Sediment < 0.063 mm
	n = 1	n = 2		n = 2		n = 1
		range	mean ± sd	range	mean ± sd	
	2017	2019	2019	2019	2019	2019
pH	-	6.8 - 7.5	7.1 ± 0.4	-	-	6.1
EC	-	50 - 104	77 ± 27	-	-	423.0
K⁺	11.3	4.1 - 4.4	4.2 ± 0.1	0.1 - 0.2	0.19 ± 0.05	2.4
Na⁺	1.7	0.6 - 1.3	0.9 ± 0.4	0.11 - 0.12	0.118 ± 0.004	0.5
Mg²⁺	9.2	6.8 - 7.2	7.0 ± 0.2	0.3 - 0.4	0.33 ± 0.04	3.4
Ca²⁺	1.3	7.9 - 13.7	10.8 ± 2.9	1.3 - 1.8	1.5 ± 0.2	3.7
Mn	0.2	0.6 - 1.1	0.8 ± 0.2	0.032 - 0.039	0.035 ± 0.004	0.2
Fe²⁺	38.4	25.8 - 28.6	27.2 ± 1.4	1.0 - 1.5	1.3 ± 0.2	31.6
Al	75.6	56.2 - 57.7	56.9 ± 0.8	1.0 - 2.3	1.7 ± 0.7	79.9
Pb [µg/g]	14.7	0.01 - 0.02	0.017 ± 0.003	0.001 - 0.002	0.002 ± 0.000	0.01

The cations for the suspended solids of the lishana water can be ordered: $Mg^{2+} > K^+ > Ca^{2+} > Na^+$. For the COC: $Mg^{2+} > K^+ > Na^+ > Ca^{2+}$. Concentrations of K^+ , Al, and Fe^{2+} decreased from 2017 to 2019, while Na^+ and Mg^{2+} decreased from 2017 to 2018 and increased from 2018 to 2019. Only Ca^{2+} increased from 2017 to 2019. Concentrations of Al vary between 25.8 to 86.7 mg/g and of Fe^{2+} between 11.0 and 44.6 mg/g. In the COC, the value of Al went up to 75.6 mg/g and Fe^{2+} to 38.4 mg/g. Significant differences between 2017 and 2018 were confirmed for Na^+ ($p = 0.007$), Ca^{2+} ($p = 0.003$), and Fe^{2+} ($p = 0.04$).

The ion concentrations of the sediments are similar to those of suspended solids. In the fraction < 0.063 mm, the cations for the lishana can be ordered: $Na^+ > K^+ > Mg^{2+} > Ca^{2+}$. For the COC: $Ca^{2+} > Mg^{2+} > K^+ > Na^+$. The fraction < 1.00 mm shows the same distribution but in lower concentrations. The light metals Al and Fe^{2+} accumulated stronger in the smaller fraction < 0.063 mm than in the fraction < 1 mm (Al: 63.5 mg/g in the lishana and 56.9 mg/g in the COC, Fe^{2+} : 30.8 mg/g in the lishana and 27.2 mg/g in the COC on average).

Almost no organic material could be found in the lishana sediment samples and the water content was low, except for the sample of the sludge from the COC water treatment at the waterworks in Oshakati. This sample contains nearly the same Al and Fe^{2+} concentrations as the sediments of the lishana and the COC: Al with 79.9 and Fe^{2+} with 31.6 mg/g. Further heavy metal concentrations can be found in the supplementary data 3.3.

3.4 Discussion

3.4.1 Calueque-Oshakati canal

The Calueque-Oshakati canal is fed by the Calueque reservoir in Angola, which receives water from the Kunene River. Several parameters (EC, Ca^{2+} , K^+ , Mg^{2+} , and Na^+) are different from the lishana, but comparable to other studies in the region (Koeniger et al. 2020; Shuuya & Hoko 2014). Both studies and the present study discovered an increase in pollution in the course of the COC and found the highest concentrations near the urban area of Oshakati, which is located at the end of the canal. Especially site 4 is striking with very high Al and Fe^{2+} concentrations in the water column, the suspended solids and sediments. Additional samples upstream and

downstream of site 4 contain high Al concentrations as well. Even the Olushandja reservoir contains up to 0.3 mg/l Al, and because of the absence of point sources, a diffuse input has to be assumed. Local waterworks use aluminum hydroxide chloride (aluminum chlorohydrate coagulant Ultrafloc 3200) as a hydrolyzing flocculant in the flocculation process (Shuuya & Hoko 2014). Hydrolysis of the dissolved salts of the trivalent aluminum ions produces metal hydroxides, which are necessary to destabilize the dispersed substances (Cañizares et al. 2008; Lin et al. 2008). After flocculation, the aluminum residues remain in the sludge, which is dried in the waterworks and then passed to local farmers as potential fertilizer. The sample of the sludge is contaminated with Al and Fe^{2+} . As there is no local industry that uses aluminum in high amounts (NAMF 2017) the source could be either illegal waste disposal or pollution sources on fields, like the sludge, from which aluminum might be washed into the canal by surface runoff during rain events. The canal is exposed to various pollutant sources and water-extracting devices, like animals, the use of pumps, washing, and bathing. In addition, farmers take their irrigation water from the canal and the Olushandja dam (Fiebiger et al. 2010). The extensive use of water, in particular during the dry season, causes large water level fluctuations in the reservoir. During floods, the open canal is not protected against flooding water and many pollutants from surrounding settlements are washed in. Its use without treatment is limited due to its hydrochemical properties.

3.4.2 The lishana system

The surface water from the lishana is limited in its quality. Constituents that are striking are EC, turbidity, Al, Fe^{2+} , Ca^{2+} , and Na^+ . Wanke et al. (2014) showed comparable results for EC and turbidity in a study of the quality of hand-dug wells (shallow perched aquifers). They found similar high EC and turbidity values at the end of the rainy season in 2010. In the neighboring Okavango Delta, EC is smaller than 200 $\mu\text{S}/\text{cm}$ (Mmualefe & Torto 2011) and lower than in the CB. Mmualefe and Torto (2011) measured low EC values in the Okavango Delta in 2010, which they explain by the leakage of salts into underground aquifers. High evaporation results in water loss and a concentration of dissolved salts in the remaining water and sediments (Zimmermann et al. 2006). Since near-surface groundwater is very saline in the lishana system, accumulated salts at the surface may leak into underground aquifers (McCarthy & Metcalfe 1990). Endorheic systems are often saline, because of concentration processes due to evaporation (Yapiyev et al. 2017). In hand-dug wells and boreholes, that are

groundwater fed, the elements F^- , NO_3^- , PO_4^{3-} , and SO_4^{2-} were detected in higher concentrations than in lishana in the present study (Wanke et al. 2014; Wanke et al. 2017). A leakage from lishana into aquifers could cause higher concentrations in underlying groundwater horizons than in surface waters. Shanyengana et al. (2004) showed that groundwater and surface water are influenced by seasonal trends. Several processes, like concentration due to evaporation, dissolution of saline sediments (mainly evaporites), mixing with older and more saline groundwater, and precipitation influence the major-ion composition. Rainfall events can refresh surface waters and increase water quality. The rainfall during the study period was low but could cause a dilution and lower concentrations. The precipitation gradient over the study area is depicted in the spatial variations of the results. In the eastern part, with higher rainfalls, less lishana had dried up between 2017 and 2018. Until 2019, even more lishana dried up and the water levels decreased rapidly. Between the dry season in 2017 and the rainy/wet seasons in 2018 and 2019 significant differences for several parameters were proved. The missing dilution by too less rainfall could cause the increased salt contents (Na^+ and Ca^{2+}).

All samples show elevated TC and TOC concentrations, which indicate a high level of organic compounds (supplementary data 3.3). The corresponding high TNb concentrations cause an increased primary production. The high redox potential of the samples acts as an oxidizing agent and favors oxidized compounds, like nitrate, sulfate, Fe, and Mn oxides. Warm water temperatures, around 25 °C in September and 27 °C in March/April favor the spreading of bacteria. The availability of oxygen is largely responsible for the presence of bacteria, low concentrations cause high bacterial counts due to consumed oxygen. Microorganisms adhere to and multiply at the dissolved solids, which offer a suitable environment for organisms (Liu et al. 2016; Luo et al. 2019). The differing oxygen saturation values at several sites suggest the different abundance of consumers. The blue-green algae produce several types of toxins, which can cause health risks for humans. Algae blooms intensify with an increasing eutrophication rate (O'Neil et al. 2012). This increasing eutrophication rate is indicated by temperature, salinity, chlorophyll- α , dissolved oxygen, nutrients, and water transparency (Deggobis et al. 2000). The presented bacteriological risk for human consumption was also identified in hand-dug wells by McBenedict et al. (2017). In their study of hand-dug wells in the lishana region, they found several bacteria of

the *Bacillus* genus. Some of the species are pathogenic and can cause gastrointestinal diseases (McBenedict et al. 2017).

Close to the urban area of Oshakati and Ongwediva anthropogenic influences are prevalent: wastewater is discharged to the surface waters, more litter is distributed and the waste disposal site, without a filtration system, is located close to water sources. Sites 9, 16, and 18 show high concentrations of metals and nutrients although not located near settlements. However, these sites are situated upstream of channel crossing road dams, which could have a water retention effect during flood events (Arendt et al., 2020) resulting in the accumulation and concentration of sediments and pollutants. Some lishana (sites 3, 5, 19) are located close to the canal and show similarly very low concentrations. Residents reported that they pipe water from the canal into the lishana to provide water for their animals. This mixing could result in dilution effects in the lishana.

Further metals, like As, Cd, Co, Cr, Cu, Ni, Sr, and Zn are detectable in low concentrations (see supplementary data 3.3). However, it can be assumed, that some sediments from the planalto in Angola were transported by surface runoff and accumulated in the southern part of the lishana system, as it last happened during the flood in 2011 (Persendt et al. 2015).

High concentrations of Al in the water column, the suspended solids, and the sediments indicate a long-lasting source of anthropogenic influence (Power & Chapman, 1992). Since the free water column also has high concentrations of aluminum, sedimentation processes must have taken place over long periods. As there is only a small metal-processing industry in the region (NAMF 2017) and the geogenic background (concentrations in soil or water that are due to natural processes) does not show elevated aluminum concentrations (Bäumle & Himmelsbach 2018; Dill et al. 2013), there must be other local sources. The high Al concentration in the sample of the sludge of 79.9 mg/g could be a reason for the increased Al content in the lishana and some sections of the canal. It is possible, that aluminum from the sludge on the field was washed out by precipitation, diluted, and accumulated in sediments and water of the lishana. However, there is no information available on when the use of the sludge as potential fertilizers was initialized and whether all four waterworks along the canal hand over the sludge to farmers.

Iron concentrations are also outstandingly high, in all three measurement campaigns. Li et al. (2018b) reported generally large concentrations of Fe^{2+} in the groundwater in northern Namibia, although the geogenic background in Namibia does not contain much iron (Bäumle & Himmelsbach 2018; Dill et al. 2013). Locally, there is no iron processing industry (NAMF 2017), therefore, the iron concentrations measured in this study must come from other local point sources. Similar to aluminum hydroxide chloride or aluminum sulfate, iron chloride or iron sulfate is used for flocculation in waterworks (Aboulhassan et al. 2006).

Sediments and suspended solids show similar values, in particular for Al and Fe^{2+} . It is known that loads in bottom sediments are often higher than in suspended solids or the water column (Power & Chapman 1992). The results from the water column, suspended solids, and sediments indicate that the pollutants have accumulated in the sediments over a long period.

All the samples, except for the tap water, have concentrations that exceed the limits of the Water Act 54 and the WHO guidelines, especially turbidity, Al, Fe^{2+} , Ca^{2+} , and Na^+ . The metals Al and Fe^{2+} do not directly affect human health but can cause intoxication over a long period. Turbidity directly threatens the health of the local population. High turbidity values are an indicator for suspended and dissolved solids, which favors the accumulation of pollutants. If it is unclear, which specific substances cause the high turbidity, it is not recommended to consume this water.

Differences between lishana and the COC are significant for several parameters (see supplementary data 3.4) since water is from different sources. Water from the COC comes from the Kunene River in Angola, i.e. from another hydrological system. As part of a running water system, it has a continuous freshwater supply. Due to the open canal, anthropogenic pollution is present. The water from the lishana is originally rainwater and is only connected to a hydrological system with freshwater supply in case of extreme rainfall events or during floods. This explains the huge differences in terms of water quality and usability. At the end of the dry season in 2017, the surface water has been without any exchange for six months. It has been affected by intensive use and did not have any chance to renew. During the wet seasons of 2018 and 2019, the little amount of rainfall could not cause dilution. These seasons have rather led to a continuation of the drought period that started in 2015 and was only finished with substantial rainfall during the rainy season of 2019/2020.

This study provided much important information about the water quality of the lishana system. As one of the main water sources for the local population in the region it is essential to gain more knowledge about the ecosystem of the lishana, however, the study has some limitations. The little amount of rainfall in 2018 and 2019 that results in a continuation of the drought period since 2015 impedes the assessment of the water quality during that period. Nevertheless, the results indicate the quality of the lishana water and sediments decreases with the increase of electrical conductivity, turbidity, and Al and Fe²⁺ concentrations during a drought period. Missing mixing of water, high temperatures, and evaporation create optimal conditions for bacteria. The accumulation of pollutants and harmful substances is a consequence. Higher temporal and seasonal resolutions are required to consolidate these results. Dried up lishana in 2019 result in low sample numbers, which in turn impedes statistical testing. Therefore, certain statistically significant differences may not have been detected.

3.5 Conclusion and outlook

Drought periods have a lasting effect on the water quality of surface waters, increased salt accumulations, and turbidity values. The results show that the water of the lishana is subject to these processes and is exposed to more pollution sources with years of low precipitation. Critical concentrations could be detected for turbidity, Na⁺, Ca²⁺, Al, and Fe²⁺. Parameters, like Cl⁻, SO₄²⁻, Mn, and NO₃⁻ showed elevated concentrations. Several heavy metals are present in harmless concentrations. The physicochemical conditions, combined with the relatively high carbon and nitrogen levels, indicate organic compounds, and favor primary production. Local characteristics of the individual locations, such as proximity to settlements, strengthen spatial differentiation. Changes over the three years of investigation are evident, partially significant, and can be attributed to different states of water availability, which underlines that prevailing weather conditions are important for the quality of water resources.

Important results on the water quality in the lishana system in the CB are provided and contribute to a better understanding of the ecosystem. The results of the analyses of hydrochemical parameters and ion concentrations of the water show that consumption is not recommended without prior treatment. Further studies on the composition of the waterworks' flocculation sludge and the potential input of Al and Fe²⁺ into the fields are necessary to identify and reduce the sources of the metals. An implementation of

desalination and water treatment methods would be necessary (Lux & Janowicz 2009; McBenedict et al. 2017).

Future climatic projections show a decrease in rainfall and an increasing frequency of drought periods (Kundzewicz et al. 2014; Luetkemeier & Liehr 2015; Masih et al. 2014; Ujeneza & Abiodun 2015). In combination with an increasing potential evaporation these conditions likely result in reduced water availability (Angula & Kaundjua 2016; Archer et al. 2018; Engelbrecht et al. 2009). It is important to find solutions for effective water storage, particularly during the dry seasons (Arendt et al. 2021). People are dependent on the water of the lishana and this dependence will increase in the coming years as the demand for water increases due to projected population growth. Thus, the presented water-related challenges, e.g. water quality and water availability, will further increase in the future and the development of adequate water treatment techniques as well as water resource protection measures need to be understood as a priority task.

3.6 Acknowledgements

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4. Paper 2: Microplastics in Namibian river sediments – a first evaluation

Abstract

The African continent is rarely the focus of microplastics research, although the ubiquity of microplastics in the environment is undisputed and still increasing. Due to the high production and use of plastic products and the partial lack of recycling systems in many parts of the African continent, it can be assumed that microplastic particles are already present in limnic and terrestrial ecosystems. Few studies, mainly from South Africa and the Northern African region, show a contamination with microplastics, especially in marine environments. This study aims to explore the presence and composition of microplastics in fluvial sediments of the major catchments in Namibia with a regional focus on the lishana system in Northern Namibia, as one of the most densely populated areas in the country. In March 2019 and March 2021, at the end of the rainy seasons, sediments from the lishana system and of the largest river catchments were sampled. Extraction was performed by density separation using the Microplastic Sediment Separator (MPSS) with the separation solution sodium chloride (density of 1.20 g/cm³). The particle size was determined by filtration and fractionation, and the polymer type by measurement with ATR–FTIR spectroscopy (minimum particle size 0.3 mm). Microplastics were found in the sediments of each river system, most of the particles in the lishana system (average of 13.2 particles/kg dry weight). The perennial, the ephemeral rivers, and the lishana system are similar concerning polymer type and particle size. Polyethylene and polypropylene were the dominant polymer types. Most of the particles were found in the size fractions 0.3 – 0.5 mm and 0.5 – 1.0 mm. The particles were found mainly as fragments and films, the majority transparent and brown.

4.1 Introduction

In Africa, plastics have been used since the late 1950s, which is long before any recycling policies have been established (Jambeck et al. 2018). Since then production, import, and consumption of different polymers and plastic products increased steadily and caused a high amount of waste (Angnunavuri et al. 2020; Babayemi et al. 2019;

Fobil & Hogarh 2009; Galie 2016). The need for more safety requirements, transport facilitations, and hygiene packaging of food and beverages triggered the increase in plastic use, especially single-use plastics (Fobil & Hogarh 2009; Iroegbu et al. 2020). A growing population causes an increased waste generation, which in turn means the need for a well-functioning waste disposal system (Hasheela 2009). In many parts of the African continent, the management of plastics is insufficient, because of economic and political reasons, such as the lack of financing and investment mechanisms or the absence of producer-consumer responsibility (Angnunavuri et al. 2020). Most of the waste is dumped or burned on disposal sites as there are few measures that allow proper disposal (Fobil & Hogarh 2009; Adebisi-Abiola et al. 2019; Khan et al. 2018). The growing number of people living at short distances from river systems raised the amount of land-based waste that could end up in the aquatic environment. Risk perception and communication about plastic pollution are still insufficient and social awareness within the society towards this problem is lacking (Bouwman et al. 2018).

Although the African continent is not the focus of microplastic research (Jambeck et al. 2018; Khan et al. 2018; Biginagwa et al. 2016), plastic particles have been detected mainly in marine and estuarine areas in South Africa, the northern, western, and eastern African regions (Khan et al. 2018; Abidli et al. 2017; Alimi et al. 2021). Three rivers from Nigeria and Cameroon are among the top 20 polluting rivers, which transport plastic into the ocean (Lebreton et al. 2017). In Arusha, Tanzania, microplastics (MP) were found in surface water and river sediments (Kundu et al. 2022). The relationship between microplastics and macroplastics in irrigated farms and an urban river was demonstrated.

In general, there is a large research gap on MP in freshwater systems in central and southern Africa, except for South Africa (Alimi et al. 2021; Yang et al. 2021). The forecasts for Sub-Saharan Africa assume a doubling of waste production from 2030 to 2050 to 510 million tons per year (Kaza et al. 2018). At the same time, the waste collection rates are in the Sub-Saharan region very low with 44%. Whereby the urban collection rates (43%) are almost five times as high as the rural collection rates (9%). More than 69% of the collected waste is openly dumped in Sub-Saharan Africa (Kaza et al. 2018). Generally, the degree of impact of urban waste on microplastic loads is enormous (Hasheela 2009; Khan et al. 2018). Dealing with waste plays a crucial role when comparing remote and urban areas. Compared to remote areas, urban areas offer more waste collection and sorting infrastructure that might help to reduce the

amount of plastics in the environment (Adebiyi-Abiola et al. 2019). In remote areas the difficulty to handle waste environmentally friendly is enormous. Urban waste, littering, and consumer use generate smaller plastic particles as a breakdown from larger pieces of plastic occurs (Biginagwa et al. 2016). Namibia produces less than 100 tons of plastic waste per day, but its neighboring country Angola produces twice as much (Jambeck et al. 2018). Increased litter on transnational rivers can lead to fluvial transport of plastic particles to Namibia. Neither Namibia nor its neighboring countries have established a plastic waste management system, some of the material is exported to South Africa or overseas (Schraml 2020). On a small scale, the informal sector plays an important role in the recycling industry in Namibia. Collecting, sorting, and dropping off waste products as a small source of income has become established in many places (Nambuli et al. 2021). Due to the insufficient recycling system, many particles enter the (aquatic) environment (Alimi et al. 2021).

Besides the fluvial transport of plastic waste and MP, there is little known about MP in freshwater systems, compared to the knowledge about marine areas. Although about 80% of the plastic is estimated to derive from the terrestrial areas and rivers are dominant pathways for MP (Jambeck et al. 2018; Bouwman et al. 2018; Lebreton et al. 2017; Anderson et al. 2016; Schmidt et al. 2017). So far, most of the studies conducted in the freshwater environment have focused on the occurrence of plastic debris on the water surface, while sediments were hardly examined (Lenaker et al. 2019; Nizzetto et al. 2016a; Matsuguma et al. 2017; Klein et al. 2015). The number of studies focusing on MP in African lakes is scarce, it is not yet possible to draw conclusions about the total extent of microplastic pollution in limnic systems on the African continent (Khan et al. 2018). Hydrodynamic processes, particle size and density influence the fate of MP in sediments. Particles can enter the river water via erosion and resuspension of the riverbed (Kooi et al. 2018). The particles not only remain in the free water column. Due to the different densities of polymer types, some particles settle down and accumulate in bed or bank sediments (Waldschläger & Schüttrumpf 2019; Imhof et al. 2012). The deposition of MP increases in low-flow river segments (Nizzetto et al. 2016a; Akdogan & Guven 2019; Besseling et al. 2017). Plastic and especially microplastic debris have a great ecological and economic impact on freshwater systems (Anderson et al. 2016; Syberg et al. 2015; Akindede et al. 2019). Environmental variables, like water density, temperature, salt content, oxygen, flow velocity, water depth, and sediment types impede measurement and monitoring in

freshwater systems (Bouwman et al. 2018). Plastic poses a health risk to animals living in the aquatic environment and might act as a vector for contaminants, like persistent organic pollutants (POPs) or heavy metals (Driedger et al. 2015; Koelmans et al. 2013), which, however, is still controversially discussed (Koelmans et al. 2013).

This study will investigate the number of plastic particles in the main river systems in Namibia with a focus on the lishana system, which is one of the most densely populated areas in the country and the dominant freshwater system in Namibia. The aim is to make the first survey of microplastic pollution in Namibian freshwater systems.

For the first time, the occurrence, abundance, and composition of MP in fluvial sediments are investigated in Namibia. Polymer type and size fraction were determined to gain knowledge about the origin and distribution of the particles. Research priorities defined by Akdogan and Guven (2019) and Khan et al. (2018) show a lack of catchment-based research in African freshwater systems. Therefore, the central research question is how many plastic particles can be found in the sediments of the main river systems in Namibia. Subsequent questions focus on the different size fractions and polymer types, and whether there are significant differences between river systems.

4.2 Material and methods

4.2.1 Study area

Namibia as an arid and semiarid country with a strong variability of precipitation faces big differences in water availability. The national hydrological system is characterized by four perennial boundary rivers (Kunene, Kavango, Oranje, and Zambesi) and several ephemeral river systems, in Namibia called Rivier (Figure 4.1) (Strohbach 2008).

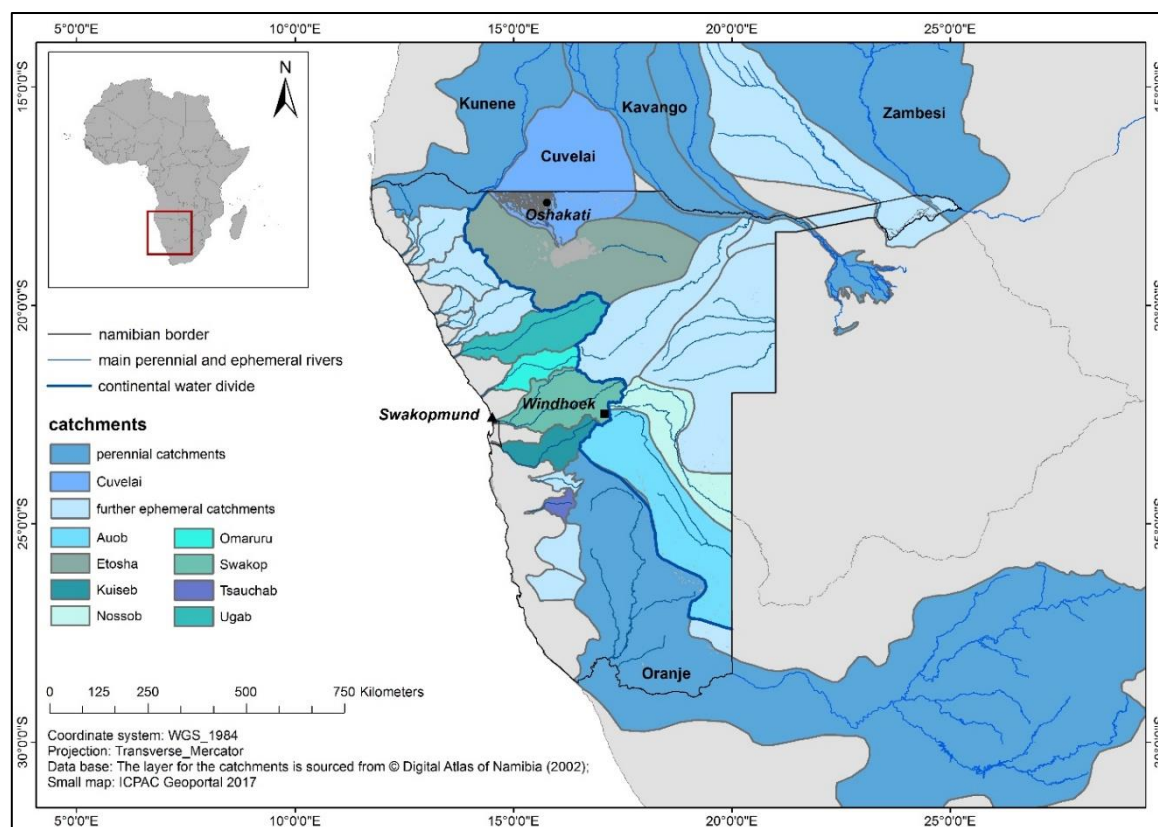


Figure 4.1: The hydrological system of Namibia with the catchments of the four perennial rivers, the Cuvelai Basin, ephemeral catchments (the sampled are colored), and the continental water divide

The transboundary Cuvelai Basin in northern Namibia is the biggest national ephemeral system. The discontinuous occurrence of flow and episodic interruptions by floods characterize ephemeral rivers, water availability is episodically limited (Jacobson & Jacobson 2013). The rivers Ugab, Omaruru, Swakop, Kuiseb, and Tsauchab drain from the western escarpment to the Atlantic Ocean and are part of the ephemeral river catchments within the Namib Desert (Jacobson & Jacobson 2013). The Owambo is a dry river in the eastern part of the Etosha National Park that rarely carries any water and ends in the Etosha pan. The Auob and its tributaries, like Olifants, and the Avis River, are dry/ruderal Riviers in the Kalahari and hardly carry any water (Strohbach 2008).

The focus region is the lishana system as part of the Cuvelai Basin in central northern Namibia. Shallow depressions (natural pans), called lishana (singular Oshana), and thousands of small channels form the hydrological system (Mendelsohn et al. 2013; Persendt et al. 2015; Cunningham et al. 1992). It is an ephemeral river system, episodically coherent only during the rainy season. In the dry season, water levels in

the pans decrease due to evaporation and water use, causing some lishana to dry out completely. Many pans and depressions are connected by narrow channels and only filled with water during the rainy season. The water flows from one pan to the next, and with enough rainfall in southern Angola and northern Namibia, the channels combine, link the pans, and build a broad network of small rivers (Arendt et al. 2021). The very low slope of 1 ‰ causes hardly any flowing movements of the surface runoff. Rather, the waters stand on the surface and cause large-scale flooding. Striking differences in water and sediment quality between urban and remote areas could be identified in a previous study (Faulstich et al. 2018).

The lishana system is densely populated, compared to the rest of the country. Especially in the eastern part around the cities Oshakati and Ongwediva the population density is high with up to 100 people per km² (Mendelsohn et al. 2013). A high population density causes a big amount of waste, which is deposited and burned at a waste dumpsite close to Oshakati. The waste management system is partially lacking, whereby huge amounts of macroplastics were found in the focus area (Kaza et al. 2018). Even if there is no heavy industry, nor packaging industry, the waste is generated by daily consumer products.

4.2.2 Quality assurance and quality control

As there is no standardized method for the determination of MP in sediments so far, the individual steps were derived and adapted from existing studies. Nevertheless, there are published guidelines to standardize reproducibility (Cowger et al. 2020; Hermsen et al. 2018; Rochman et al. 2019) and to assure quality analysis and quality control. In this study, the guidelines from Cowger et al. (2020) were followed (see supplementary data 4.1). The procedure of density separation was derived from Imhof et al. (2012), and further process steps were evolved in the laboratory for microplastics at the Faculty of Physics, University of Marburg (Prume et al. 2021).

The sampling was carried out with wood shovels and spoons that were wet cleaned before. The samples were transported in wet-cleaned aluminum containers, which were additionally wrapped with aluminum foil, to avoid any contamination. For the extraction of the plastic particles different techniques exist on how to proceed, they mainly include density separation, sieving, filtration, microscopic detection, and spectroscopic identification (Blettler et al. 2017; Wang & Wang 2018). During the whole

process in the laboratory, clean, wet wiped surfaces, containers, and tools were used. Contaminations from the air were limited by controlled room ventilation. Contaminations from clothing or other materials were prevented by wearing cotton coats, wet cleaning all surfaces, working with wood or metal tools, and cleaning all tools with compressed air before use. All samples were permanently protected and covered with aluminum foil.

In total four blank samples, empty runs without samples, were taken to identify external plastic contaminations, whereby four potential plastic particles were found. These particles are fragments and have the size of 0.3 mm, three are PP and one is polytetrafluoroethylene (PTFE). The contamination probably occurred from a non-replaceable plastic part of the MPSS. Two blank samples of the air were taken during the process of density separation. The samples of air were taken in the same metal bowls in which the sediment samples were homogenized for preparation. No plastic particles were found in these samples.

4.2.3 Sampling

During the first field campaign in March 2019, sediment samples were taken from dried-up pans in the lishana system. Based on existing sampling locations of previous investigations (Faulstich et al. 2018) sites were selected to provide a representative picture of the focus area. In March and April 2021 river bank and river bed sediments of the major Namibian river systems were sampled to gain knowledge about the amount of MP in the aquatic system in Namibia. The measurement grid was chosen to sample rivers throughout the country. Smaller and larger streams, rivers close and far from settlements were selected (Figure 4.2).

At each site, about 1.4 kg of wet weight material was taken. Sediment samples from three perennial rivers (Kunene, Kavango, and Oranje), nine ephemeral rivers, and 14 lishana were analyzed. At the time of sampling, not all of the lishana were filled with water, some had dried out. At the rivers and lishana filled with water, the samples were taken in the wet part of the riverbanks. At the locations without water, the sediments were taken in the riverbed. At the Kavango, Tsauchab, and Ugab, two samples were taken: Kavango1, Tsauchab1, and Ugab1 at the riverbanks and Kavango2, Tsauchab2, and Ugab2 further in at the river bottom (distance of 5 m from the banks). The Kunene, Kavango, Oranje, Kuiseb, Fish River, and Swakop1 all carried water. The

lishana, on the other hand, all carried water except for sites 3, 12, 16, 18, and 34 (Figure 4.3 and supplementary data 4.2).

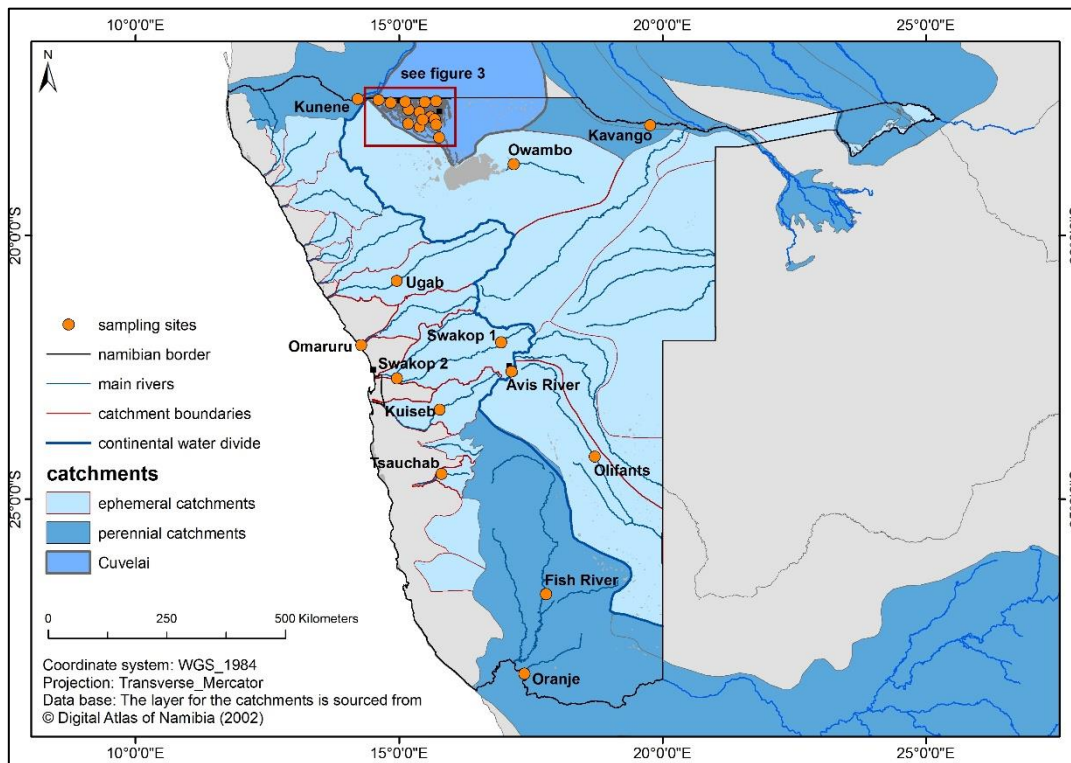


Figure 4.2: Locations of the sampling sites in Namibia with the focus region in central northern Namibia

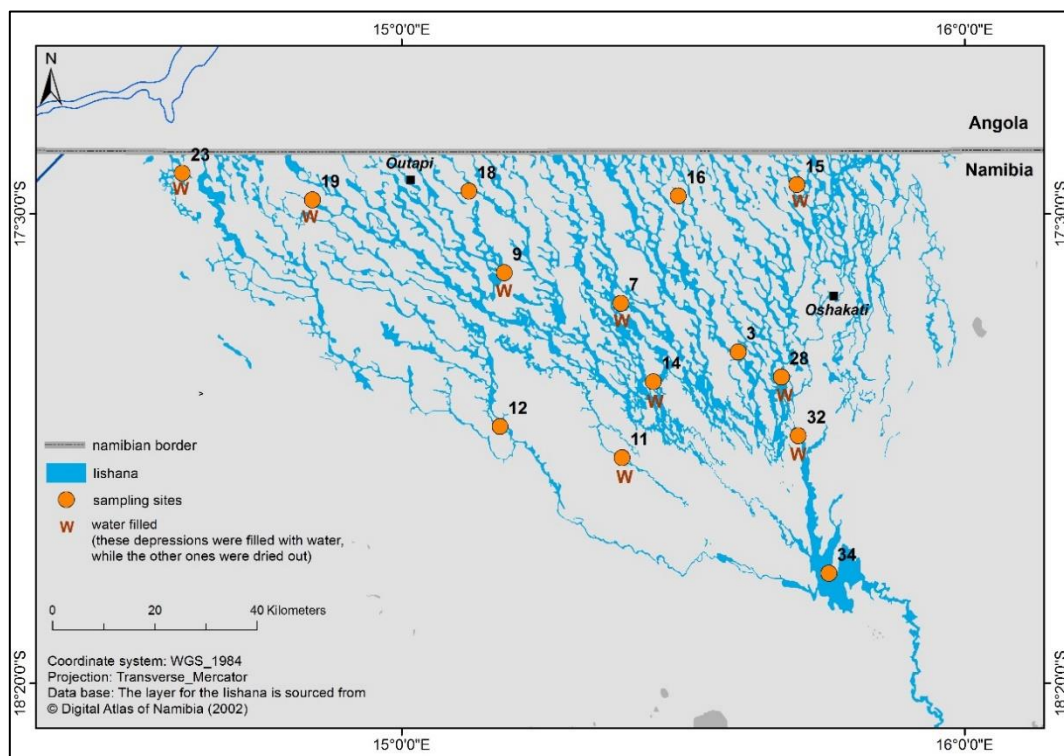


Figure 4.3: The focus region of the lishana system with sampling sites, indicating whether the lishana were carrying water at the time of sampling

4.2.4 Density separation with the microplastic sediment separator

At first, the samples were homogenized with a metal spoon, weighed, and three sub-samples were separated to determine the water content. These three sub-samples were dried at 60 °C for several days and weighed every day at the same time. Once the weight was constant, the water content was calculated. The main samples were dried at 30 °C, ensuring that they were covered and no contamination of the samples could occur.

The Microplastic Sediment Separator (MPSS; Hydro-Bios Apparatebau GmbH, Kiel, Germany) was used for density separation (Imhof et al. 2012; Weber et al. 2021a). Recovery rates of 100% for large particles (> 1 mm) and 95% for small particles (1000 µm – 40 µm) are described by Imhof et al. (2012). The separation liquid was made of filtered tap water (> 50 µm) and sodium chloride. A density of 1.19 g/cm³ was produced by adding about 6 kg of sodium chloride to 15 l of water (solution concentration 6.8 M). The separation liquid was filtered again (> 300 µm) and introduced into the standpipe until a fill height of 85%. Then the sample was added into the MPSS while the motor was rotating. The liquid and the sample were mixed for 60 min. Big organic material was skimmed and stored for further analysis. In the following 14 h the descent process was performed. After this period the sample chamber with ball valve was removed, rinsed and the sample with the ascended material was filled into steep-bottomed glass bottles. About 750 ml were extracted per sample. For the analyses in 2021, the standing time was extended to 20 h to prolong the separation process.

4.2.5 Fractionation, filtration, staining, and microscopy

The size fractionation was done by wet sieving the samples with a metal sieve cascade with a diameter of 150 mm (Test Sieve, DIN ISO 3310-1, stainless steel; VWR, Germany). Mesh sizes of 5 mm, 1 mm, 0.5 mm, 0.3 mm, and 0.1 mm were used. In 2021, sieves with a diameter of 75 mm (Test Sieve, DIN ISO 3310-1, stainless steel; ATECHNIK, Germany) were used to simplify the handling and reduce particle loss. Each sieve was flipped by 180° and rinsed individually with particlefree water (> 50 µm) into a glass filtration unit to ensure that no material remains on the sieves. For the glass vacuum filtration unit 0.45 µm cellulose and pleated filters (LLG-Plain disc filter paper, qualitative, medium/fast, Ø 47 mm; LLG Labware, France) were used. Each fraction was drawn onto several filters. Between 3 and 10 filters were generated per

sample and fraction. All wet filters were stored in glass petri dishes, covered, and dried at about 60 °C. The smallest fraction of 0.1 – 0.3 mm (still in water) was collected in steep-bottomed glass bottles, for further analyses. In 2021 the sieve residues on the filter were directly rinsed into a glass petri dish and dried, to perform microscopic detection on a transparent background. The filter was discarded. The petri dishes were covered and dried at about 60 °C (Prume et al. 2021). In 2019, a Nile Red solution that dyes plastic particles to make them fluoresce under a microscope was used to distinguish plastic from other particles. One milliliter of Nile red solution (1 mg/ml in acetone) was added to the filters and left to stain for 1 – 2 h. Each filter was examined under a microscope (Wild by Heerbrugg; magnification range 6x-50x) and light from a blue LED. The fluorescent plastic particles (highly luminous) were removed from the filter with metal tweezers and collected in well plates before spectroscopic measurements. In 2021, the samples were analyzed under a stereo microscope (SMZ-171; Motic Deutschland GmbH, Wetzlar, Germany; magnification range 11.25x-75x) without staining. Particles without cell structure, which did not break apart under pressure and did not have a glass-like texture were selected manually (Hidalgo-Ruz et al. 2012). The color and shape of the particles were visually detected under the microscope, the polymer type was determined by FTIR spectroscopy.

4.2.6 FTIR spectroscopy

The Fourier-Transform-Infrared (FTIR) spectroscopy is one of the most common analytical methods for the chemical identification of MP in the aquatic environment (Käppler et al. 2018). The ATR (attenuated total reflection) spectroscopy is a non-destructible method to measure single particles after presorting. There are characteristic spectral fingerprints for different chemical structures and thus unknown materials or substances can be identified by comparing their spectrum with the spectra of known materials (Wang & Wang 2018; Tagg et al. 2015). With this method, it is possible to identify the exact polymer type of the MP and also to obtain information about their physiochemical weathering (Wang & Wang 2018).

All particles of the campaign in 2019 were measured with the FTIR spectroscope Tensor 37 (Bruker Optik GmbH, Ettlingen, Germany) in ATR mode (wavenumber range 400 – 4000 cm^{-1} ; 20 scans per measurement). At first, the measured spectra were manually compared with known spectra of the most common polymer types:

acrylonitrile butadiene styrene (ABS), polyamide (PA), polyamide/nylon 6, polyamide/nylon 6.6, polycarbonate (PC), polyethylene (PE), polyethylene terephthalate (PET), poly(methyl methacrylate) (PMMA), polypropylene (PP), polystyrene (PS) and polyvinylchloride (PVC). The reference materials were provided by the University of Bayreuth. For the second data set in 2021 the FTIR microscope Lumos II (Bruker Optik GmbH, Ettlingen, Germany) was used. With the Lumos II spectrometer by mapping it is possible to measure the particles faster, since with the Tensor 37 spectrometer only one particle can be measured per measurement. The measurements were performed in ATR mode (wavelength range 680 – 4000 cm^{-1} ; 30 scans at the beginning, after 15 samples 50 scans), with an open aperture and a low pressure to ensure no particle slips away. The minimum Hit Quality Index (HQI) was 50 and the selected maximum number of hits was 5. An HQI of 700 is not necessarily a characteristic for the correct definition as plastic particles but is often named as a threshold (Weber et al. 2021b; Renner et al. 2019). An atmospheric correction (CO_2 , H_2O , aqueous solutions) was performed in the operating software Opus after every measurement.

In Opus (version 8.5.29), the measured particles were compared to the following commercial data bases: BPAD Bruker Polymer ATR Library, ATF-FTIR LIBRARY KIMW, and BIBL ATR-FTIR FORENSICS Library. The standard or vector normalization search algorithm was applied. The results and the statistical consistency were dependent on comparison with the data bank of spectra. Unambiguously polymer spectra of the samples from Namibia were collected in a new database. This database was used again for matching further, less unambiguously, samples from Namibia. All the spectra from 2019 were compared with the data bases from Bruker and the self-made one to verify the previous manual evaluation.

4.2.7 Data analysis

The data analysis was performed in an RStudio environment (Version: 2021.09.2 Build 382) using the scripting language R (Version: 3.6.1) (R Core Team 2019). The following packages were used in RStudio: “psych”, “car”, and “dplyr”. At first, the Shapiro-Wilk test was done to test the three groups (perennial, ephemeral rivers, and the lishana system) for normal distribution. A Levene test was performed to check the differences in variances. Due to no normal distribution and unequal variances, a

Kruskal-Wallis test was used to gain knowledge of the differences between the three different main river systems. Furthermore, the individual sites were correlated with the classification of urban and rural regions and the water level (dry or water filled) by using multiple regression. The statistical analysis results were defined as significant with a p-value < 0.05. The definition of rural and urban was taken from the Namibia Statistics Agency in Namibia (Digital Namibia, the National Geographic Portal for the National Spatial Data Infrastructure (NSDI), which is coordinated by Namibia Statistics Agency – 2017) (NSA 2017).

4.3 Results

A total number of 703 particles were visually detected as potential plastics under the microscope. During the process, 69 particles got lost due to their form, mostly fibers. Six hundred thirty-four particles were measured with the FTIR spectroscope, whereby 410 particles were clearly identified as polymers (64.7%). The 410 plastic particles were found at 28 of 30 sampling sites with a range of 1 – 66 particles kg⁻¹ dry weight (a detailed table differentiated by sampling site can be found in the supplementary data 4.3). The identified amounts are differentiated between the perennial rivers, the ephemeral rivers, and the lishana system. In the perennial rivers (Kunene, Kavango, Oranje), MP was found at all sites in very small quantities, with an average of 2.5 ± 1.2 particles kg⁻¹ dry weight and a range of 2-7 particles kg⁻¹. In the ephemeral river systems, the average plastic concentration is 12.6 ± 17.2 particles kg⁻¹. The Owambo was found to have the highest content of MP, with 66 particles kg⁻¹ dry weight, followed by the Olifants with 39, Kuiseb with 20, the Avis and Tsauchab1 with 14 particles (Figure 4.4). At Kavango, Tsauchab, and Ugab two samples were taken each. With a ratio of 7:2 (Kavango), 14:2 (Tsauchab), and 1:0 (Ugab) particles kg⁻¹, more particles were found on the riverbank than on the river bottom at each river.

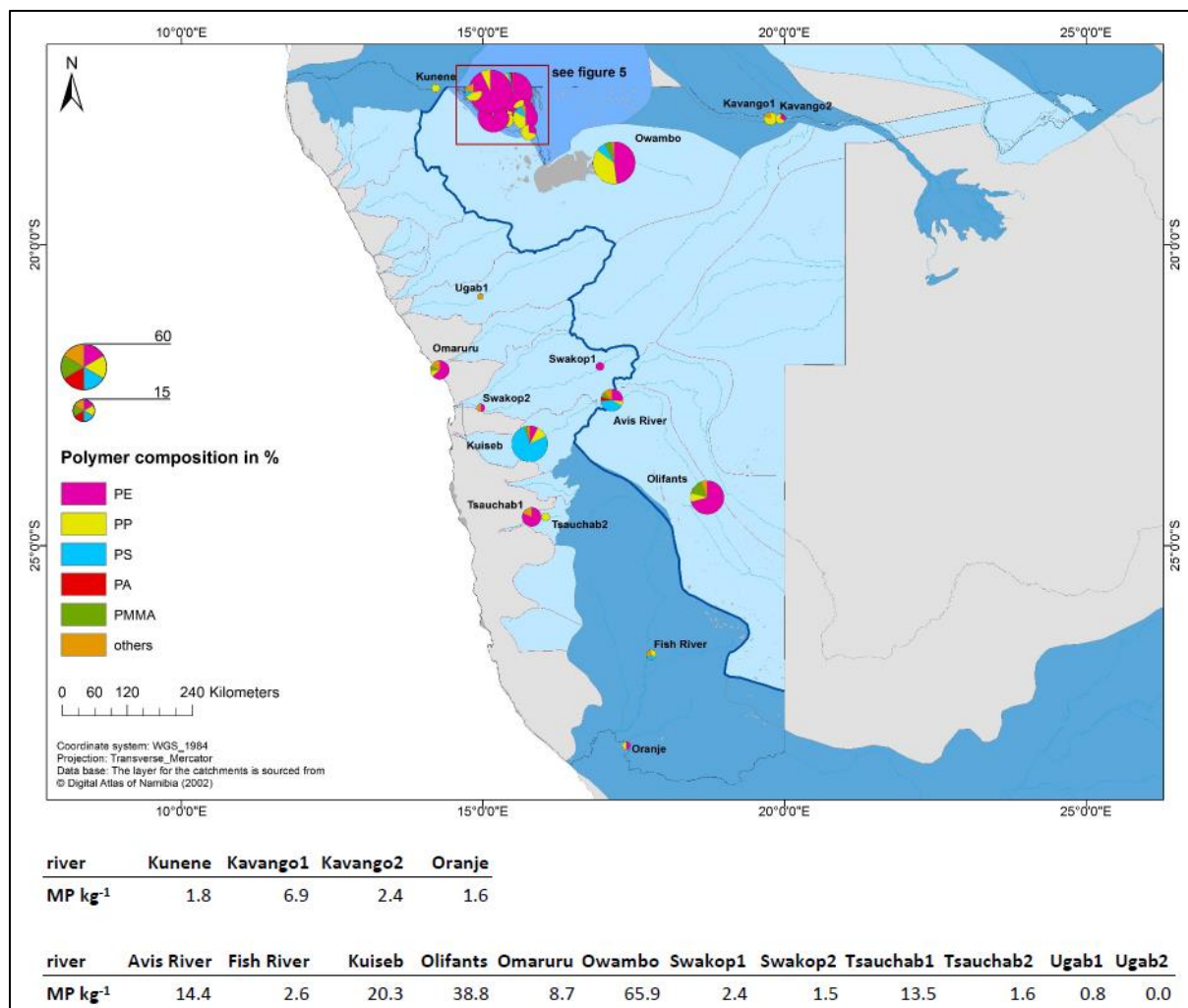


Figure 4.4: Amount and polymer type of microplastics found at the sampling sites of the perennial and ephemeral Namibian river systems

In the lishana system (Figure 4.5) 224 particles were found with an average of $13.2 \pm 16.4 \text{ kg}^{-1}$ dry weight. Most particles were found at sites 18 and 16 in the northern part, close to the border with Angola (53 and $43 \text{ particles kg}^{-1}$ dry weight). Sites 12, 32, 28, 7, and 9 contain between 10 and 26 particles (Figure 4.5). At 50.0% of the sites less than 10 particles were found (3, 11, 14, 15, 19, and 34). No MP was found at site 23. The most common and in this case dominant polymer types are PE (59.3%), PP (20.7%), and PS (11.5%) (Figure 4.6). The polymers at the perennial rivers consist of 75% PP and 16.7% PE. In the ephemeral rivers and the lishana system, the majority of identified polymers is PE (43.7% at the ephemeral rivers and 73.7% at the lishana), followed by PP (18.4% and 19.6%).

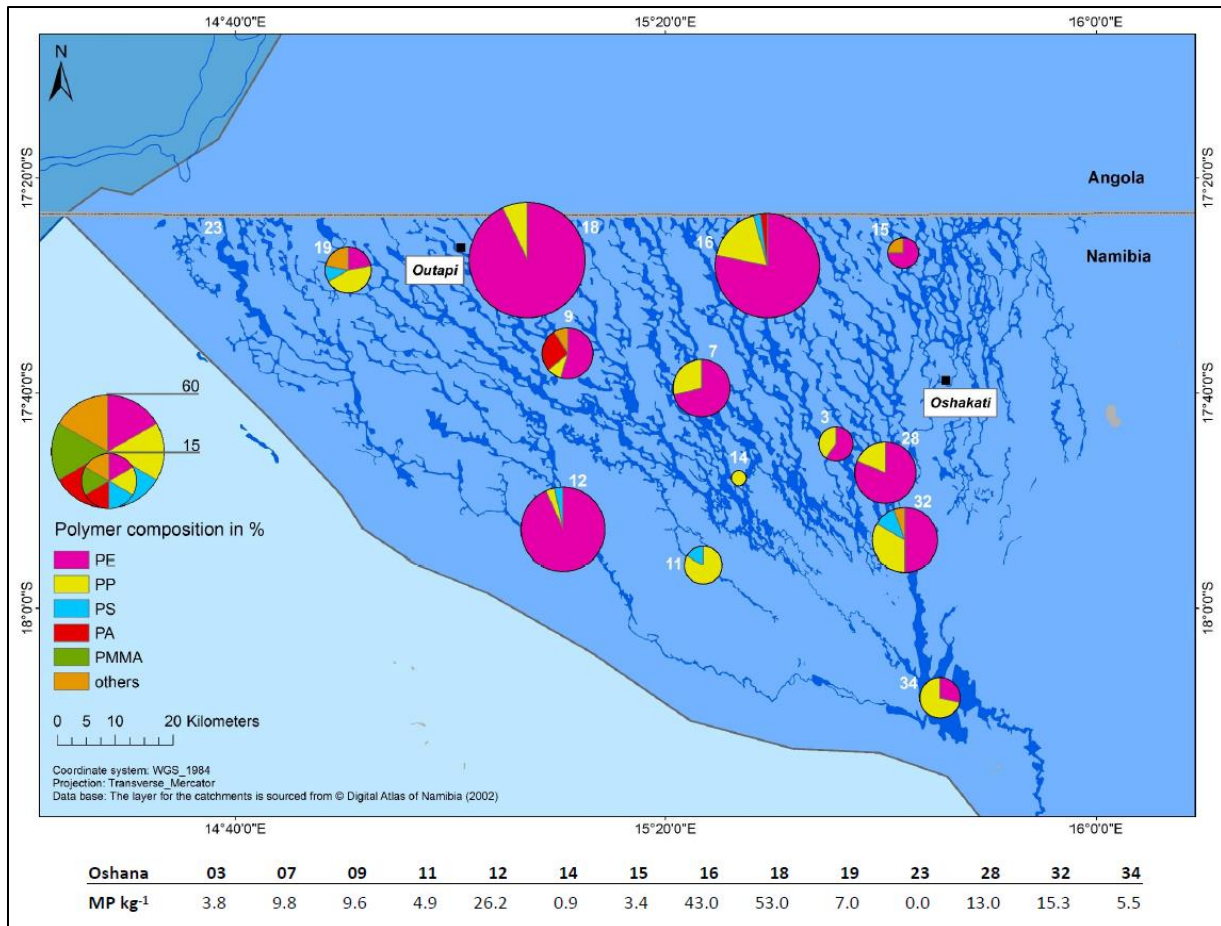


Figure 4.5: Amount and polymer type of microplastics found at the sampling sites of the focus region, the lishana system

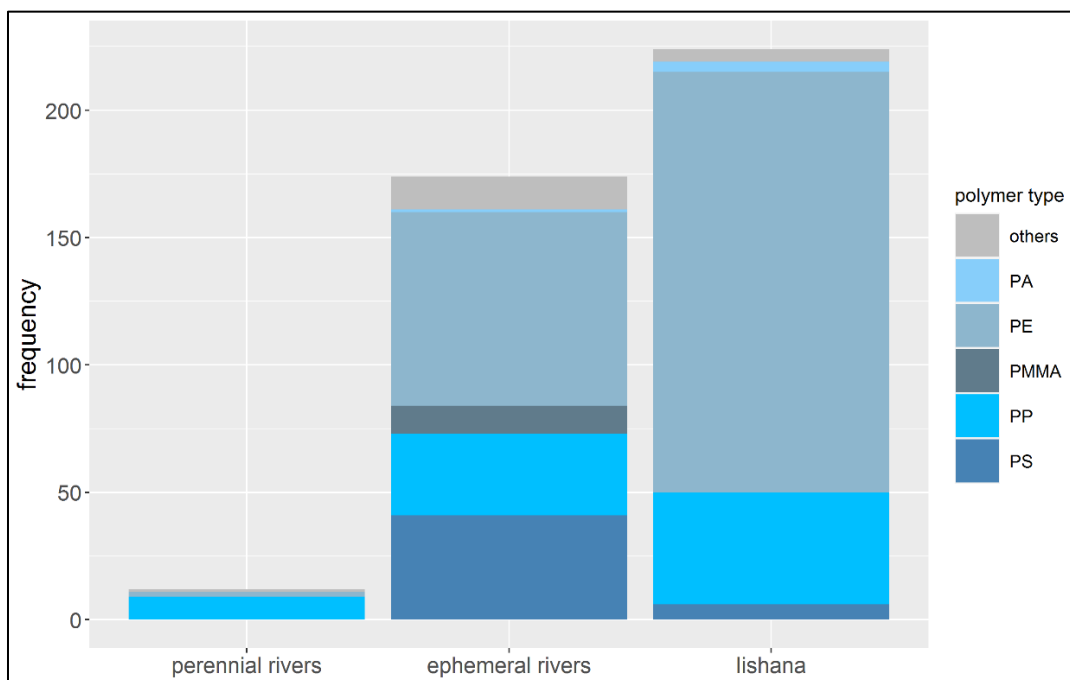


Figure 4.6: Polymer type differentiated according to river systems

The ephemeral rivers contain with 23.6% PS more than the others. The size of the detected and identified particles varies between the fractions 1.0 – 5.0 mm (23.7%), 0.5 – 1.0 mm (42.4%) and 0.3 – 0.5 mm (33.7%) (Table 4.1). In the perennial rivers, particles of the two smallest fractions are equally represented, and the bigger fraction is underrepresented. The ephemeral rivers contain more particles of the size 0.3 – 0.5 mm (39.1%) and the lishana system more of the fraction 0.5 – 1.0 mm (46.9%).

Table 4.1: Particle size of microplastics found, differentiated between perennial rivers, ephemeral rivers, and the lishana system

	5 mm [%]	1 mm [%]	0.5 mm [%]	0.3 mm [%]
perennial rivers	0.0	16.7	41.7	41.7
ephemeral rivers	0.0	24.1	36.8	39.1
lishana	0.4	23.7	46.9	29.0
in total	0.2	23.7	42.4	33.7

The classification of Rochman et al. (2019) was used to categorize the morphology of the particles. Fragments, films, fibers, fiber bundles, and pellets were found. Spheres and foams could not be identified. The particle morphology is composed of 69.7% fragments, 23.3% films, and 6.2% fibers (Figure 4.7). Only one fiber bundle and 3 pellets were found. No significant differences were detected between the three systems.

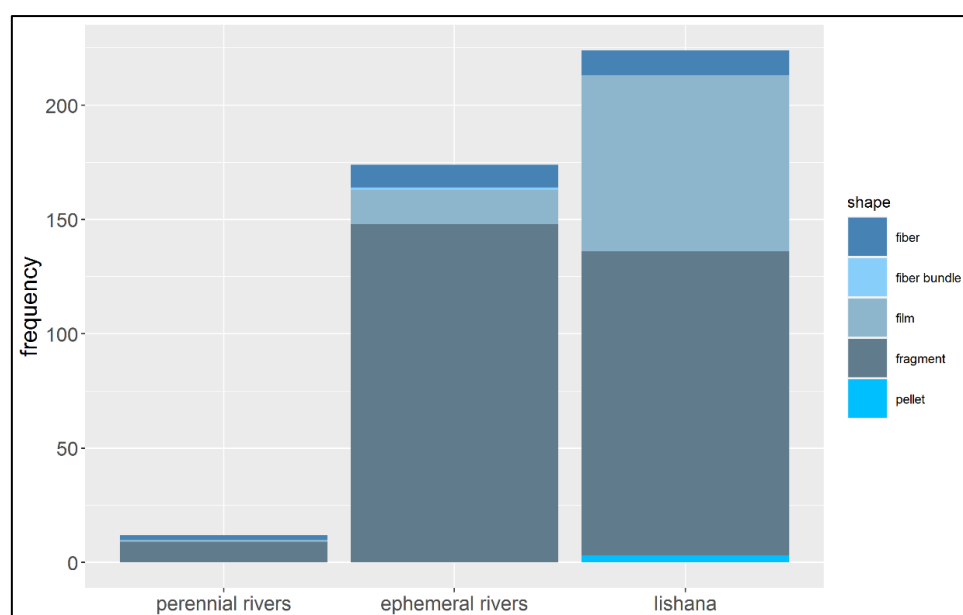


Figure 4.7: Shape of microplastics found, differentiated according to the three river systems

The color of the particles was classified according to Frias et al. (2018) and Wang et al. (2022): transparent, white, yellow, black, blue, brown, grey, green, pink, red, and orange. The color could help to identify the source of the plastic particle and due to that all colors were noted and not merged as “others” (Table 4.2). Most of the particles were transparent (30.5%), brown (23.9%), and black (13.2%).

Table 4.2: Colors of microplastics found, differentiated according to the three river systems

	transparent [%]	white [%]	yellow [%]	black [%]	blue [%]	brown [%]	grey [%]	green [%]	pink [%]	red [%]	orange [%]
perennial rivers	0.0	0.0	25.0	0.0	8.3	66.7	0.0	0.0	0.0	0.0	0.0
ephemeral rivers	27.6	12.6	2.3	28.7	12.1	12.1	0.0	0.6	0.6	2.9	0.6
lishana	34.4	10.7	1.3	1.8	3.6	30.8	14.3	0.4	2.2	0.0	0.4
in total	30.5	11.2	2.4	13.2	7.3	23.9	7.8	0.5	1.5	1.2	0.5

With a p-value of 0.33, the Kruskal-Wallis test showed that there were no significant differences between the three river systems with regard to particle concentrations (Figure 4.8). Although unlike perennial waters, lishana and ephemeral rivers contain similar amounts of MP. The minimum is 0 and the maxima are outliers at 53 and 66, which results in a high range. The first and third quartiles and medians are similar. The perennial rivers have a smaller range between 2 and 7 and no outliers. Microplastic abundance was tested by using multiple regression and the variables rural and urban waterbodies and the water level (dry or water filled). With a p-value of 0.08, there was no significant explanation of the plastic amount by the urban and rural regions or the water level.

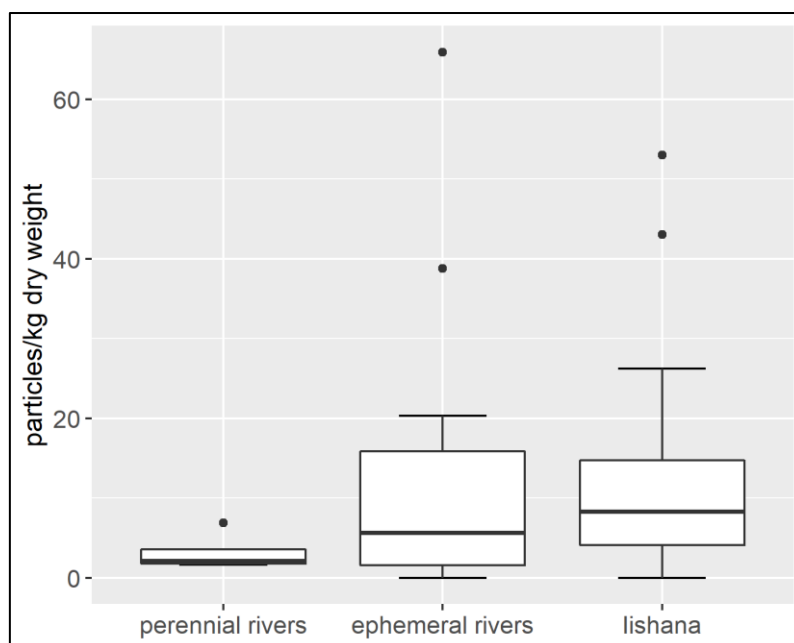


Figure 4.8: Comparison of the amount of microplastics at the three river systems (perennial rivers, ephemeral rivers, and the lishana system)

4.4 Discussion

Methodological approach

The missing standardized method to extract and identify MP in sediments is a lack in all recent studies (Weber et al. 2021b) and results in a few limitations. The density separation (with the MPSS) is commonly applied for the separation of polymer types with a density between 0.8 and 1.70 g/cm³. The most frequently used separation solution is a saturated sodium chloride (NaCl) solution (density of 1.20 g/cm³). It is non-toxic and likely to extract the low-density MP (Wang & Wang 2018). Several polymer types have a density less than 1.2 g/cm³, like PE with a density of 0.92 – 0.97 g/cm³, PP with a density of 0.85 – 0.94 g/cm³ and PS with a density of 1.04 – 1.10 g/cm³ (Crawford & Quinn 2017). These polymers can be separated with the density separation with sodium chloride (Crawford & Quinn 2017; Zobkov & Esiukova 2018). Other polymers, with densities of 1.3 – 1.7 g/cm³ and 1.4 – 1.6 g/cm³ (e.g. polyvinylchloride (PVC) and polyethylene terephthalate (PET)) are rather underestimated (Miller et al. 2021). As the used separation solution NaCl for the density separation has a density of 1.2 mg/l, some polymers, like PET and PVC are denser and cannot be detected. The attachment of organic and inorganic material results in density gain that in turn brings the debris to sink and settle.

Thus, by only measuring the amount of microplastic on the water surface or even in the water column could cause an underestimation of the actual quantity of debris (Imhof et al. 2012). The results presented here probably slightly underestimate the exact MP content, since some particle loss is to be expected during density separation with the MPSS.

The visual-eye identification is well established, but just in combination with another identification step, like the FTIR spectroscopy, it generates trustful results (Renner et al. 2019). The error quote during the identification process is caused by a conservative identification of the selected particles. In some cases, the plastic particles were contaminated with sediment, clay, or silt, which changed the measured spectra. Other particles were degraded and therefore had changed spectra. Their measured spectra had limited quality, were not unique (Primpke et al. 2018), and were therefore conservatively not evaluated as polymers. Using the ATR-FTIR spectroscopy, particles < 300 µm are hard to measure, because of the small surface that is partially too small for the ATR crystal. The manual handling is challenging, which causes some particle damage or loss (1.2% in 2019 and 18.0% in 2021). In particular, fibers are challenging to handle. Some of them are bigger than 300 µm but too slim for the ATR crystal. In 2021 many fibers were found, but not all could be transferred to the measurement plate.

The different spectrometers do not affect the comparability of the results, since both times were measured in ATR mode. The slightly adapted method between 2019 and 2021 should be considered while comparing the results. The longer standing time during the density separation in 2021 caused a higher suspension of microplastic particles and result in higher recovery rates.

The staining with Nile red (applied in 2019) is strongly dependent on the solvent. The used acetone showed good recovery rates, but was not as satisfying as chloroform (Tamminga et al. 2017; Konde et al. 2020). Therefore, some particles may not be recovered and there may be an underestimation of plastic particles. For this reason, in 2021, staining was omitted, resulting in a visual-eye identification and a better selection of plastic particles. The adjustment to the methodology may have caused an underestimation of 2019 results in hindsight.

MP concentrations in Namibia

All investigated Namibian river systems contain MP. At the perennial rivers Kunene, Kavango, and Oranje an average of 2.5 ± 1.2 particles kg^{-1} were found. This abundance of MP is low, compared to rivers with similar dimensions, concerning discharge (Yang et al. 2021). In the Amazon River (Brazil) up to 5725 particles kg^{-1} dry weight were found (Gerolin et al. 2020). In Portugal at the Antuã Rivera between 100 and 629 particles kg^{-1} sediment were identified (Rodrigues et al. 2018). Weber et al. (2021a) used a similar method (MPSS with NaCl, fractionation, staining with Nile red, visual-eye identification, ATR-FTIR spectroscopy) and identified MP in floodplains of the Lahn River (Germany) as temporary sinks. In their study at the Lahn, they found an average of 2.75 MP kg^{-1} . The river Themí in Arusha, Tanzania, contained up to 180 particles kg^{-1} (Kundu et al. 2022) (see Table 4.3 for corresponding data of compared studies).

Table 4.3: Comparison of microplastics concentrations in different regions

country	water body	concentration range [MP kg^{-1}]	dominant polymer type	dominant size [mm]	dominant shape	dominant color	year	study
Brazil	Amazon river	417-8178	--	1 - 3	fibers	white	2020	Gerolin et al.
Portugal	Antuã Rivera	100-629	PE and PP	--	foams and fibers	colored and white	2018	Rodrigues et al.
Germany	Lahn river	0.36-30.46	resin and LDPE	0.3 - 2	films and fragments	--	2021	Weber et al.
Tanzania	Themí river	70-160	HDPE and PP	--	fibers	--	2022	Kundu et al.
Namibia	perennial rivers Kunene Kavango Oranje	2-7	PP and PE	0.3 - 1	fragment	brown	2019/2021	this study
	ephemeral rivers	0-66	PE and PS	0.3 - 1	fragments	black and transparent	2021	this study
	lishana system	0-53	PE and PP	0.3 - 1	fragments and films	transparent and brown	2019/2021	this study

At the ephemeral rivers in Namibia (Avis River, Fish River, Kuiseb, Olifants, Omaruru, Owambo, Swakop, Tsauchab, and Ugab) on average 12.6 ± 17.2 particles kg^{-1} sediment were identified. The lishana system, as an ephemeral system, contains 13.2 ± 16.4 particles kg^{-1} on average. Ephemeral systems face seasonal or episodic flows and floods. These seasonal flow processes mobilize deposited sediments and attached MP. Large pulses of transport and a variable mobilization of MP could occur throughout the year (Horton & Dixon 2018). Concerning the MP concentrations, the riverbed morphology and hydraulic characteristics of the sampling sites are important.

Low flow velocities and in general low energy in the lishana system cause deposition of suspended matter and MP. A high content of suspended material favors the attachment of MP. Previous studies showed a high content of suspended matter in the lishana system that could affect the MP content (Faulstich et al. 2018).

Seasonal conditions, like rain events, water volume, and flow velocity have a strong effect on the deposition and retention of MP in sediments (Rodrigues et al. 2018). In this study, all ephemeral rivers were dry at the time of sampling at the end of March. In 2019 occurred a drought event in the country, in 2021 the rainy season was very well and caused some flooding. It can be assumed that plastic particles were transported and relocated by the rain events in 2021. In the lishana system, nine lishana were filled with water and five lishana were dried-up during sampling. In both years, there was no flow movement at the sampled lishana and the ephemeral rivers, even though sampling occurred at the end of the rainy season. These missing flow movements caused a higher deposition of particles in the depressions. There was no statistical correlation between water level (dry and water filled) and MP concentration in the lishana system. The perennial rivers showed flow movements that could transport the particles downstream and cause low recovery rates of MP in the samples. In Tanzania, it was possible to detect more MP downstream of rivers (Kundu et al. 2022). Rodrigues et al. (2018) identified seasonal changes of MP in sediments of a river in Portugal between March and October with a higher abundance in March. A mobilization of the particles at the time of higher precipitation is given as a reason.

However, it was not possible to identify the exact reason for the higher abundance in March (Rodrigues et al. 2018). There are nearly no studies on MP in ephemeral systems. Eppehimer et al. (2021) investigated the ephemeral Santa Cruz River in the USA. They identified differences between the base flow of the river, caused by water treatment plants, and the post-flood conditions, after rainfall events. After the runoff fewer MP were found in sediments, but more in the water column. In particular, less fibers were found after the flood, they seem to be easily mobilized and get less attached by biofilms than fragments (Eppehimer et al. 2021; Hoellein et al. 2019). Runoff mobilizes plastic particles in sediments and floods are a transport medium while no or low flow conditions cause deposition of MP (Cheng 2002; Nel et al. 2018). With increased surface roughness sedimentation and deposition of MP increase (Weber et al. 2021a). Smaller particles in general are more likely to be resuspended during rainfall than bigger particles (Xia et al. 2021). At the Kavango, Tsauchab, and Ugab more MP

were found at the riverbanks, than at the river bottom. For the Kavango this can be explained by a higher flow velocity in the stream that cause erosion and prevent deposition of MP in the riverbed (Gerolin et al. 2020). At the Tsauchab and the Ugab recently occurred flow movements in 2021 could cause the same effect. Several studies identified similar differences (Yang et al. 2021). Different flow velocities are found in the width of the river and cause areas of erosion and accumulation. Soils, sediments from river deltas, riverbanks and lake bottom sediments are sinks for MP (Yang et al. 2021; Rillig 2012). Lower density MP, like PE or PP, have a high mobility and are transported over larger distances. High density particles, like PA or PET, retain earlier in the sediments and therefore riverbeds, in particular river bottoms, could become sinks for MP (He et al. 2021). This study showed that low density particles can also sink and accumulate in sediments.

Type, morphology and potential sources of Namibian MP

The differences between the three river systems are causal but not statistically significant. This could be due to the small number of samples. MP are mostly more abundant in densely populated areas (Yang et al. 2021; He et al. 2021). Most of the plastic particles were found in the most densely populated region in Namibia, the lishana system as part of the Cuvelai Basin. Almost as many particles were found in the further ephemeral systems in the country. Surrounding population, building density and land use are potential factors influencing the amount of MP in the water bodies (Chen et al. 2020). In this study, no significant correlation was found between rural and urban regions and the MP amount.

Several studies showed no correlation between population density and the occurrence of MP (Klein et al. 2015; Chen et al. 2020). Kataoka et al. (2019) could prove a significant correlation between MP concentration in Japanese rivers and population density. However, the ranges and the spatial differences in this study, are huge. From a small-scale perspective, all sites are located close to bridges or culverts, which indicates a big anthropogenic influence, like plastic waste dumping. It was shown that small-scale differences have a bigger influence on the plastic amount than large-scale differences (e.g. urban or rural regions). Besides the spatial relations, there was no significant correlation between the amount of MP and the condition of the rivers (dry or water filled).

The particle morphology of the Namibian particles is dominated by fragments (69.7%) and films (23.3%). In several studies mostly fibers were found (Abidli et al. 2017). In this study, only 6.2% fibers were found, but due to the used method, the identification of fibers was quite difficult. The shape of the particles is characteristic for secondary microplastics introduced into the environment through anthropogenic use or fragmentation (Rochman et al. 2019). Of primary plastic, such as pellets, three pieces were found in the lishana system. Colorful fibers can come from synthetic clothing, films from agricultural runoff, and fragments from plastic bottles (Rochman et al. 2019). Since the identified MP is mostly secondary plastic, it can be assumed to be degraded MP from locally disposed plastic products.

Most of the particles were transparent, brown, black, or white. Martí et al. (2020) identified white and transparent/translucent as the most common colors in ocean plastics. A discoloration could occur by photo-oxidation and cause a dominance of white and yellow particles (Martí et al. 2020; Pagter et al. 2020). A long exposure time to sunlight can cause fragmentation and discoloration (Martí et al. 2020). The discoloration of original colored particles to transparent, white, and yellow could possibly occur in Namibia, due to high temperatures and intensive sunlight exposure. PE and PP were the most common polymers found in Namibia, which can result from packaging, carrier bags, food wrappers, and beverage bottles (Biginagwa et al. 2016). The results correspond to the mostly used polymer types in southern Africa (Galie 2016; Khan et al. 2018; Egessa et al. 2020). Between 1987 and 2020 most of the studies conducted in Africa identified PE, PP, and PS as the three most common polymer types of MP (Alimi et al. 2021). Furthermore, PE and PP are globally the most common polymers, because of their light density they are easily fluvial transported (Klein et al. 2015). He et al. (2020) indicate a great impact of land use on the plastic type.

Rochman et al. (2014) and He et al. (2020a) detected metals on plastic particles. In the lishana system metals in the water column, suspended solids and sediments already have been detected (Faulstich et al. 2018). It is possible that metals attach to plastic particles and are transported through the rivers, however, this assumption has to be evaluated by further investigations. The application of sewage sludge contributes to MP in soils (Nizzetto et al. 2016b; Zubris & Richards 2005). Surface runoff from urban areas or agricultural lands, wind dispersal, and soil erosion transport MP into the aquatic environment (Horton et al. 2017). This movement of plastics from land to

freshwater systems is dependent on various factors, such as weather conditions and land cover types (Lambert & Wagner 2018). Physical processes, like wind, surface runoff, fluvial transport, and flooding cause a spatial distribution of MP in environmental compartments (Imhof et al. 2012; Zhang 2017).

4.5 Conclusion and outlook

This study was conducted to significantly contribute to the state of research on MP in Namibia, in particular in the lishana system. The main river catchments in Namibia contain MP. Even in rural areas, far away from settlements, MP were found in river sediments. More particles were found in the lishana system than in perennial rivers or ephemeral systems. Most of the particles were found in the smaller fractions between 0.3 and 0.5 mm and the most common polymer types are PE and PP. Secondary plastic, in form of transparent and brown fragments and films, was mainly found. As primary plastic, only three pellets (transparent, grey, and black) could be identified.

The consequences of uncontrolled waste disposal and littering are investigated worldwide (Ferronato & Torretta 2019). Namibia is one of the countries with more than 0.8 kg of mismanaged plastic waste per capita per day (Jambeck et al. 2018). The forecast for mismanaged plastic waste for Namibia will increase up to 10,000 tons per year in 2025. Only by identifying, quantifying, and qualifying the amount of plastic waste on the African continent, adapted measurements and strategies can be developed (Jambeck et al. 2018). Within the last few years plastic production, detection, collection, sorting, recycling, and prevention are being addressed with information technologies and artificial intelligence. The goal is a closed-loop economy for plastic products (Adebiyi-Abiola et al. 2019).

On the African continent, it is just the beginning of studies about MP in freshwater and terrestrial systems (Rochman 2018). This study showed for the first time the occurrence and characteristics of MP in freshwater sediments in Namibia. As an initial study, the focus was on the sinks, in particular the depressions (lishana) and rivers. The influence of floods on the mobilization and deposition of MP in river sediments could be further analyzed by sediment dating (Weber et al. 2021a). To gain more knowledge about the source, transport, and fate of MP in Namibian river systems, aeolian sediments should be investigated. In particular, during a dry episode in

ephemeral systems, aeolian erosion and deposition play a crucial role in the composition of the sediments.

4.6 Acknowledgements

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5. Paper 3: Ecotoxicological evaluation of surface waters in Northern Namibia

Abstract

The main catchment areas are in the arid and semi-arid region in central-northern Namibia, the Kunene and the Kavango Rivers, and the Cuvelai-Etosha Basin. The increasing pressure on freshwater systems due to intensive anthropogenic use is a big challenge. In particular, in the lishana system, an ephemeral part of the Cuvelai-Etosha Basin, the water amount decreases, and the water quality is affected. High evaporation, low precipitation, and intensive anthropogenic use pollute and endanger surface waters. So far, there is no comprehensive knowledge about the ecological status of water bodies, although they represent an important water resource as the groundwater is too saline for use. Therefore, it is crucial to learn about the state of the ecosystem and the ecological effects of pollutants to ensure the safe use of these resources. The surface waters of the three systems were sampled, and three bioassays were applied on three different trophic levels: algae, daphnia, and zebrafish embryos. Additionally, *in-vitro* assays were performed to analyze mutagenicity (Ames fluctuation), dioxin-like potential (micro-EROD), and estrogenicity (YES) by mechanism-specific effects. Acute toxic effects on fish embryos and daphnia and weak effects at the cellular level were detected. Slight mutagenic effects and estrogenic potential have been demonstrated for three lishana in the eastern part of the study area. Algal growth was not inhibited and no cytotoxic effects were found. The systems of the lishana and the perennial rivers differed significantly concerning the studied toxicity.

5.1 Introduction

Freshwater ecosystems provide important ecosystem services and are biodiversity hot spots (Balian et al. 2008; Stendera et al. 2012). However, they are endangered by human activities and climate change, which cause habitat and biotic changes (Chapman 1995; Vörösmarty et al. 2010). The major threats worldwide are (chemical) pollution and overexploitation. Anthropogenic stressors, such as nutrient enrichments, the discharge of toxic metals (Leal et al. 2016), or organic substances (Christensen et

al. 2022), put increasingly high pressure on ecosystems (Jansen et al. 2011). Due to the pollution of surface waters, rivers, and lakes are threatened to lose their biodiversity and are in danger of no longer being able to provide their ecosystem services (Malaj et al. 2014; Vörösmarty et al. 2010). Monitoring and investigating surface waters and their water quality is an increasing challenge. In addition to chemical analysis of water, bioassays are an essential component of water assessment (Di Paolo et al. 2016; Keddy et al. 1995; Methneni et al. 2021) as effect-based bioassays map the effects of the complex sample regardless of the concentration. In this way, mixture toxicity is also included in the assessment. The harmful concentrations of substances in different organisms (bacteria, green algae, small crustaceans, and fish) are studied to determine aquatic toxicity (Chapman 2002; Neale et al. 2017). Several studies investigate aquatic systems for ecotoxicological effects (Aragaw & Mekonnen 2021; Ford et al. 2021; Grund et al. 2011; Hollert et al. 2005; Krein et al. 2012). Most of the studies analyzed aquatic systems of rivers, where active point sources can be identified (Keiter et al. 2006; Pawlowski et al. 2004; Wolf et al. 2022). In addition, it is known that pollutants can induce cytotoxic, endocrine, and mutagenic effects in aquatic systems (Shuliakevich et al. 2022a; Wolf et al. 2022). Furthermore, various relationships, ecosystem responses, interactions, and interdependencies must be considered (Walker et al. 2012).

In particular, river ecosystems in arid and semi-arid regions are stressed due to the alternation of rainy and dry seasons and the associated fluctuating fresh water supply from precipitation (AghaKouchak et al. 2013). Limited and seasonal available water resources are the consequence and pose major challenges to the water supply (Everts 1997; Emile et al. 2022). For this reason, it is essential to use all available water resources, such as groundwater and surface water, as efficiently as possible. The condition of water bodies and their water quality must be known in order to take appropriate treatment measures and use the water bodies as a water resource for different purposes, such as potable water or irrigation water. Particularly intensively used surface waters, exposed to many influencing factors, such as water withdrawal for use as potable water, irrigation water, or drinking water for livestock, input of agricultural runoff, and surface runoff from farmland, must be examined regarding their ecological condition. The hazards are known, but the status quo of the ecosystems is not widely investigated.

In southern Africa, the seasonal precipitation and high evaporation cause a challenging water supply. However, there is a huge research gap on the state of freshwater ecosystems, although they are ecologically important and diverse (Schoenfuss et al. 2022). The region in southern Angola and central-northern Namibia have an increased incident human water security threat (Vörösmarty et al. 2010). In central-northern Namibia, the landscape is characterized by the Cuvelai-Etосha Basin (CEB), and the Kunene and Kavango basins. These three basins are very important for the local water supply but have not been well investigated concerning the potential ecotoxicity of freshwater systems. There are hardly any data on environmental pollution at the Ilishana system, as part of the CEB, and the neighboring river systems Kunene and Kavango. It is necessary to analyze the current state of the freshwater systems to take appropriate protection or renaturation measures.

In previous studies, the water quality and microplastic pollution of the Ilishana were investigated (Faulstich et al. 2022; Faulstich et al. 2023). The Ilishana have high turbidity values and salt concentrations. Aluminum and iron were found in elevated concentrations, particularly in suspended solids and sediments (Faulstich et al. 2023). In order to ensure the safe use of water bodies in the long term and to take appropriate treatment measures, their condition must be examined. In southern Africa (eco)toxicological investigations have only been used since the 1990s (Wepener & Chapman 2012). Potential toxic effects on selected organisms were studied for drinking water (Grabow et al. 1991) or to investigate the consequences of mining activities (Connell et al. 1991). In Namibia, endocrine disruption compounds (EDCs) were investigated in water resources close to densely populated areas (Faul et al. 2013 & 2014). However, no studies on ecotoxicological investigations at the Kavango and the Kunene Rivers were conducted to our current knowledge. The Kunene is the subject of several studies concerning river ecology, water governance, and water management (Hipondoka et al. 2018; Meissner & Jacobs 2016; Schwieger 2019; Schwieger 2020). The Kavango is investigated for discharge, precipitation variability, hydrobiology, and ecology (Bauer-Gottwein et al. 2015; Benitez et al. 2022; Cronberg et al. 1995; Gaughan & Waylen 2012; Jury 2009; Kgathi et al. 2006). Gwenzi and Chaukura (2018) described a lack of ecotoxicological studies in African aquatic systems and the resulting dangers of organic contaminants (OCs), estrogenicity, and acute toxicity. In particular, in Sub-Saharan Africa, except South Africa, investigating OCs in potential drinking water is an important task that has not yet been solved

satisfactorily. In general, ecotoxicological studies in ephemeral systems in arid and semi-arid regions are rare (Lahr 1997). Therefore, this study first gives insight into the hazard potential of the surface waters of the Iishana, Kunene, and Kavango Rivers on organisms and the environment. The main question is if there are any acute toxic or mechanism-specific potential (cytotoxicity, endocrine activity, mutagenicity or dioxin-like potential) in the surface waters of the three basins. After a single application on organisms, the acute toxicity with a toxic effect was measured on three trophic levels: algae, daphnia, and fish embryos. These assays were applied to gain knowledge of the ecotoxicological potential of the surface waters in northern Namibia. Without this data, a final assessment of the waters is not possible.

5.2 Methods

5.2.1 Study area

The Iishana system in central-northern Namibia is a complex hydrological system of drainless depressions and small channels (Iishana, singular Oshana). The Iishana have different sizes, with a depth of 1-7 m (Mendelsohn et al. 2013). When there is enough rainfall, they connect to form a network of narrow drainage ways (Arendt et al. 2021; Faulstich et al. 2022). The Iishana system is part of the Cuvelai-Etосha Basin (CEB) that is neighbored by the Kunene basin in the west and the Kavango basin in the east (Mendelsohn et al. 2013). While ephemeral rivers dominate the endorheic CEB, the Kunene and the Kavango basins are dominated by their namesake perennial rivers that drainage into the Atlantic (Kunene) and the Okavango Delta (Kavango) (Meissner & Jacobs 2016; Steudel et al. 2013). The Kunene River has its source in southwestern Angola, flows to the south, and forms the border between Angola and Namibia. With a discharge of 15 km³/year and a difference in altitude of about 1700 m, it is suitable for hydroelectricity (Meissner & Jacobs 2016). The Kavango originates in the Angolan highlands, flows southwards, and terminates in the Okavango Delta (McCarthy & Ellery 1998). In Namibia, the Cubango and Cuito rivers join, build a wetland strip, and form the Okavango River. At the border between Angola and Namibia, the discharge is about 10 km³/year (Steudel et al. 2013).

Due to the intensive dry (April to September) and rainy seasons (October to March), the water supply is challenging. In the last decade, rainfall was below average in the

rainy seasons, resulting in the lishana not carrying enough water to serve as a water source throughout the dry period (NOAA 2021). Almost half of the Namibian population lives in the Namibian part of the CEB. Water resources are the freely accessible lishana and the Calueque-Oshakati canal as part of the public water supply that transports water from Calueque (Angola) to Oshakati (Namibia) (Shuuya & Hoko 2014).

5.2.2 Sampling and extraction

The lishana, the Kunene, and the Kavango water samples were taken in 2019 (Figure 5.1 shows the catchment areas, sampling locations, and sample numbers; supplementary data 5.1 includes detailed information of the sampling sites).

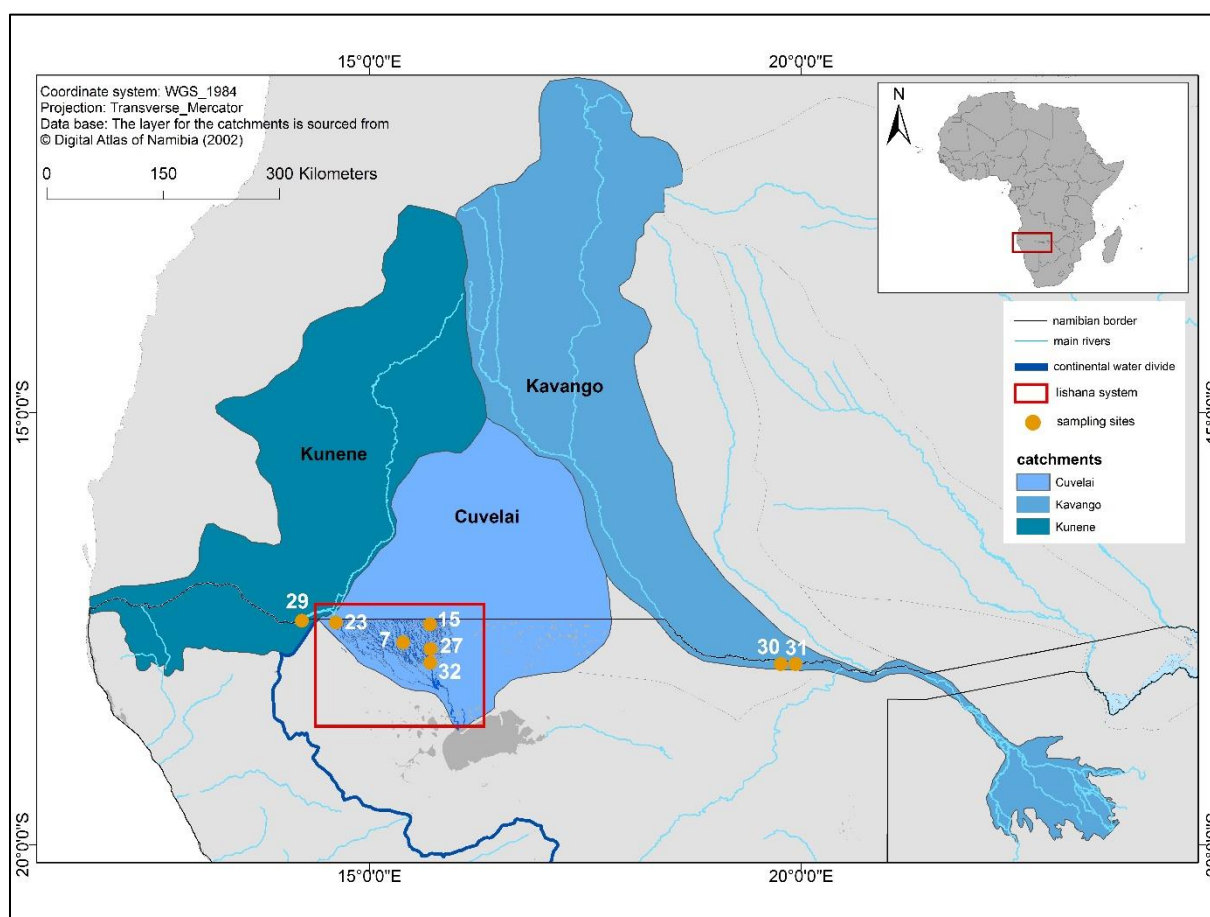


Figure 5.1: Catchment areas of the three systems with sampling sites and numbers

At every site, several aluminum bottles (4 x 60 ml, 8 x 120 ml, and 1 x 600 ml; Bürkle, Germany) were three times pre-rinsed with Acetone (Acetone, HPLC Grade; CarlRoth,

Germany) and filled with water samples. Samples were consistently refrigerated at 4 °C until analysis.

For sample preparation, the individual water samples (1.8 l each) were filtrated with suction through a 0.2 µm fiberglass filter (MN GF-2; Macherey-Nagel, Germany). The samples were concentrated by solid phase extraction (SPE) to extract the per- and poly-fluoroalkyl substances. At first, the columns (CHROMABOND HLB; 60 µm, 15 ml/500 mg; pore diameter 63 Å, particle size 53 µm; Macherey-Nagel, Germany) were conditioned with dichloromethane (Dichloromethane, ROTISOLV HPLC Grade; CarlRoth, Germany), methanol (Methanol, MeOH, HPLC Grade; CarlRoth, Germany), and ultrapure water. Then, the SPE columns were loaded with the samples under a vacuum (-0.3 – -0.4 bar). The columns were eluted with 6 ml MeOH and 6 ml DCM. As a solvent keeper, 50 µl dimethylsulfoxide (Dimethylsulfoxide, DMSO, 99.8%; CarlRoth, Germany) was added. The samples were rotated to a minimum (40°C, slowly decreasing pressure) and transferred to amber glass vials. The vials were evaporated under nitrogen to exclude the remaining MeOH.

The resulting extract of 1 ml is 2000-fold concentrated. The water extracts included a realistic environmental matrix of the water body and were frozen for further bioassay testing. A process control (ProCo) of ultrapure water was treated and tested like a sample to control the purity of the extraction.

5.2.3 Bioassays

In Table 5.1 the key data of the applied bioassays with the information on endpoints, model organisms, exposure time and vessels, medium quantities, and followed guidelines are shown.

Table 5.1: Key data of the bioassays performed

Bioassay	Method title	Endpoint	Model organism	Exposure duration [h]	Exposure vessels	Medium per vessel [ml]	Guideline
FET test	Fish embryo acute toxicity test	Fish embryo lethality and occurrence of morphological sublethal endpoints	<i>Danio rerio</i>	120	24-well plates*	2	ISO 15088:2008 (DIN EN ISO 2008) OECD Test No. 236 (OECD 2013)
Daphnia test	Daphnia acute immobilization test	Immobilization of daphnids	<i>Daphnia magna</i>	48	glass tubes	10	OECD Test No. 202 (OECD 2004)
Algae test	Algal growth inhibition test	Growth inhibition	<i>Raphidocelis subspicata</i> (Formerly: <i>Pseudo-kirchneriella subcapitata</i>)	72	96-well plates*	2	OECD Test No. 201 (OECD 2011)
Ames assay	Ames fluctuation assay	Induction of reverse mutations	<i>Salmonella strains TA100 and TA98</i>	48	24-/384-well plates*	0.5 (+ 2.5)/0.05	ISO 11350:2012-05 (DIN EN ISO 2012), Reifferscheid et al. 2011
Micro-EROD assay	Micro-EROD bioassay	Cytochrome activities	<i>Rat hepatoma cell line H4IIE</i>	72	96-well plates*	0.1	Schiwy et al. 2015a
YES assay	Yeast estrogen screening assay	Estrogen receptor binding activity	<i>Recombinant yeast cells</i>	18-72	96-well plates*	0.2	ISO 19040-1: 2018 (DIN EN ISO 2018), Routledge & Sumpter 1996

*Starlab International GmbH, Germany

5.2.3.1 Fish embryo toxicity test

The zebrafish used were originally from the strain of the Westaquarium (Bad Lauterberg, Germany) and were bred in the Department of Evolutionary Ecology and Environmental Toxicology, Goethe University Frankfurt am Main (Germany). The fish embryo acute toxicity test was applied as a limit test with fertilized eggs of the zebrafish (*Danio rerio*), based on the DIN guideline and the OECD guideline 236 (DIN EN ISO 2008; OECD 2013; Shuliakevich et al. 2022b). As described in Johann et al. (2021),

the experiments were terminated shortly before 120 hours post-fertilization (hpf). According to the EU Directive 2010/63/EU (European Union 2010), testing zebrafish embryos and larvae before 120 hpf does not require animal ethics test approval. After testing, all embryos were euthanized with a benzocaine-ethanol solution (40 g/ml in ethanol). All samples were tested in the highest concentration (Ref 2) of the water sample extracts of the lishana and the perennial rivers. For each sample, ten fertilized eggs in stadium 8-16 cells (collected 2 h after spawning and washed with 0.1 % Methylene blue solution) were added within 22 ml medium (composition of the medium according to guideline, OECD 2013) and 22 μ l sample extract. Eggs were transferred to 24 well plates, one egg in 2 ml per well, including plate control. One positive control (PC; 3,4-dichloranilin, 4 mg/l), one negative control (NC; medium), and one solvent control (SC; DMSO) were included per replicate, with ten eggs per control. A total of three replicates were performed. The embryos were incubated at 26 °C in well plates with gas-permeable foil. They were evaluated every 24 h up to 120 hpf for lethal and sublethal effects. According to the OECD guideline, a test was classified as valid if at least 30 % of the PC showed lethal effects and not more than 10 % of the NC (OECD 2013). All data presented the validity criteria. The effects were defined according to the study of von Hellfeld et al. (2020).

5.2.3.2 Acute immobilization test with *Daphnia magna*

The present study performed the daphnia-immobilization test as a limit test according to the OECD guideline 202 (OECD 2004). The *Daphnia magna* Straus were obtained from the Institute for Environmental Research culture at the RWTH Aachen University (the original daphnids are from stock cultures at the University of Sheffield; Agatz et al. 2012; Simon et al. 2015). At the start of the test, the neonates (younger than 24 h) were sieved and added directly to the test solution. The test medium was prepared according to the guideline (OECD 2004). The experiments were performed in three replicates, with four glass tubes per sample. Five neonates were introduced per glass tube, with 10 μ l sample extract in 10 ml medium. The NC was the respective medium, and the SC was DMSO. The tubes were stored at 20 ± 1 °C and a 16/8 h light/dark photoperiod. After 24 and 48 h, the immobility was examined. Immobilization was considered when individuals did not move after 15 seconds and gentle agitation. The validity criteria (pH value variation of < 1.5 units, dissolved oxygen > 3 mg/l, and

< 10 % of immobilized daphnia in the NC and SC) were fulfilled for all replicates and samples.

5.2.3.3 Algae growth inhibition test

The test was performed according to the OECD guideline 201 (OECD 2011). The green algae (Chlorophyta) *Raphidocelis subspicata* (formerly: *Pseudokirchneriella subcapitata*) was originally obtained from the culture collection of algae (SAG Göttingen, Germany). The short-term algae culture was diluted 1:10, and its density was measured (required cell density: 5000 cells/ml). The highest concentration was Ref 2 with a dilution series 1:6. As SC, 0.1 ml DMSO was prepared and as blank a sample without algae. The fluorescent density of the samples was measured with the multi-well plate reader (Tecan infinite M200; Tecan Group Ltd., Switzerland). All plates were closed with parafilm and incubated for 72 h. Every 24 h, the well plates were mixed for 1 minute at 150 rpm before the fluorescent density was measured. The pH value was measured every 24 h and should be 8.1 ± 0.2 . Further validity criteria regarding growth rate and mean coefficient of variation (OECD 2011) were fulfilled for all experiments.

5.2.3.4 AMES fluctuation assay

The test was performed according to the DIN EN ISO guideline 11350:2012-05 (DIN EN ISO 2012) and the study of Reifferscheid et al. (2011). The amino acid-dependent strains TA 98 and TA 100 of *Salmonella typhimurium* (Strains obtained from the Deutsche Forschungsgemeinschaft, DFG, Bonn, Germany) were tested with and without S9 (rat liver homogenate). The samples were tested in three independent replicates.

Both bacterial strains were grown with sterile growth medium (9.4 g Nutrient broth, 0.6 g NaCl in 0.4 l H₂O) and sterile ampicillin in an overnight culture (Incubator at 12 °C until incubation period, 8.5 h, 37 °C, 150 rpm). With 500 µl total volume, sample extracts and DMSO were diluted 1:50 in the medium. First, the bacteria were diluted according to their measured FAU (Formazine Attenuation Unit; TA 98: FAU 170, TA 100: FAU 45). The plates with bacteria suspension, samples, S9-mix, PC, and NC were incubated for 100 min at 37° C while shaking. After incubation, 2.5 ml of reversion

indicator medium was added. Then, transfer to the 384-well plates with 50 µl/well each and 48 wells per concentration and control.

The highest concentration was Ref 40, and the lowest was Ref 1.75. Several evaluation approaches were combined to achieve the best results (Levy et al. 2019). Revertant colonies were counted, and the induction factor (IF) was calculated by dividing the median result at each concentration by the median result with the corresponding negative control (Keiter et al. 2006; Kosmehl et al. 2004; Seitz et al. 2008). Additionally, the concentration-dependent induction factor (CDI) was calculated to rank the mutagenicity in environmental samples because it is independent of the tested concentrations (Shuliakevich et al. 2022a).

5.2.3.5 *Micro-EROD assay*

The test was performed according to the developed protocol of Schiwy et al. (2015a). The cell suspension (prepared according to the protocol by Schiwy et al. 2015a) was diluted to a cell density of 200,000 cells/ml. Afterwards, 50 µl of the suspension was filled into 96-well plates and incubated for 2 h (37 °C, 5 % CO₂, 95 % humidity). A total of 6 concentrations of the samples (1:2 series) were prepared, with Ref 20 as the highest concentration. TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin) was prepared in medium 1:100, and 50 µl each was added to the cells. The exposed samples were incubated for 70 h (37 °C, 5 % CO₂, 97 % humidity). Subsequently, the medium was aspirated in a dark environment, 100 µl of the ETX stock solution (see protocol) was added, and the samples were incubated for 30 min (37 °C, 5 % CO₂, 95 % humidity). 75 µl methanol was added into each well and shaken for 20 min at room temperature to stop the reaction. The fluorescence of the samples (production of resorufin) was measured with the multi-well plate reader (Tecan infinite M200; Tecan Group Ltd., Switzerland). One protein standard (ETX solution with BSA standard; see Schiwy et al. 2015a) with 7 concentrations was prepared and transferred to the plate. After that, 100 µl of the bicinchoninic acid (BCA) reagent mix (Pierce BCA Protein Assay Kit; Thermo Scientific, Germany) was added to all cells. The plates were incubated for 40 minutes at room temperature. Afterwards, the absorption was measured at 550 nm. The validity criteria (Schiwy et al. 2015a) were achieved for all experiments.

5.2.3.6 Yeast estrogen screen (YES assay)

The Yeast estrogen screen (YES) assay was performed according to DIN EN ISO 19040-1:2018 (DIN EN ISO 2018) and the study of Routledge & Sumpter (1996). The yeasts (cryo culture; originally from Deutsche Forschungsgemeinschaft, DFG, Bonn, Germany) were prepared as an overnight culture (ONC) with 500 µl SD-medium and 500 µl DO-medium (22 h, 30 °C while shaking). The samples were diluted 1:100 in ultrapure water. In 96-well plates, a 1:2 serial dilution with Ref 20 as the highest concentration was performed. The final cell solution (ONC and DO-Medium) was prepared, and the FAU was measured (42.5 – 57.5 FAU). The exposure Medium (SD-Medium, DO-Medium, CuSO₄, Ampicillin, streptomycin) and the cell solution were given in 96-well plates and shaken for 30 seconds at 450 rpm. The optical density (OD) was measured at 600 nm, and the plates were incubated for 18 h at 30 °C (covered with a gas-permeable film). After the incubation, the cell density was measured (600 nm). The lacZ reaction mixture was prepared and transferred to new 96-well plates with the samples. The OD was measured immediately at 580 nm. The plates were incubated for 1 h at 30 °C under agitation. The OD was measured at 580 nm and 540 nm. The validity criteria (DIN EN ISO 2018) were fulfilled for all samples and all replicates.

5.2.4 Data analysis

The data analysis was performed in an RStudio environment (Version: 2022-04-22 ucrt) by using the scripting language R (Version: 4.2.0) (R Core Team 2019). Several packages in RStudio have been used: “compositions”, “psych”, “car”, “dplyr”, “ggplot2”, “pgirmess”, “survival”, and “PMCMRplus”. At first, the three river systems (perennial, ephemeral rivers, and the lishana system) were tested for normal distribution with logarithm transformed data by applying the Shapiro-Wilk test and followed by a Levene test to check the differences of variances, in case of a normal distribution. The not normally distributed data with unequal variances were tested with the Kruskal-Wallis test for significant differences between the three systems. The normally distributed data with unequal variances, i.e. the Ames assay, were also tested with the Kruskal-Wallis-Test. The Friedman test was applied to gain knowledge of the differences between sampling sites. As a post hoc test, the Wilcoxon test was carried out. Differences between the lishana and the perennial rivers were tested with the Mann-

Whitney-U-test (95 % confidence interval), because of unrelated and not normally distributed data. A two-way ANOVA and a post-hoc Tukey test were applied for the Ames assay to test for significant differences. With a p-value < 0.05, the results were defined as significant.

5.3 Results

5.3.1 Fish embryo toxicity test

Several sublethal effects, such as chorion deformations and edema could be observed in the lishana system, the Kunene River, and the Kavango River. The embryotoxic and teratogenic potential was similar in all systems, and the most observed effects were pericardium and yolk sack edema (see supplementary data 5.2). After 48 h, all samples showed more than 10 % effects (10 % to 100 % effects), except samples 15, 23, and 32. All samples showed a decrease in effects after 96 h. Three striking endpoints were an affected, slightly decomposed chorion, pectoral fin underdevelopment, and blood congestion. In particular, at sites 7, 15, and 27 of the lishana, these effects were noted, but at the Kunene and Kavango Rivers, these effects were detected less frequently (10 % to 14 % effects). Embryo malformation and development retardation were the most common effects at the perennial rivers. After 72 hpf 32 % of the development retardations and failures were observed (Figure 5.2). However, there are no statistically significant differences between the lishana system and the perennial rivers regarding the number of observed effects ($p = 0.83$).

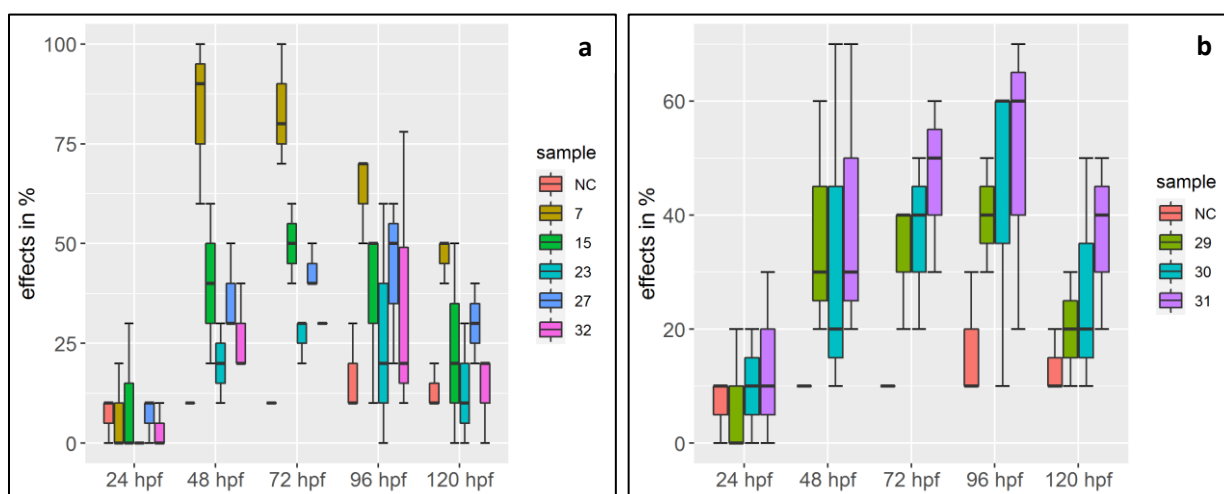


Figure 5.2: Observed effects of **a** the lishana system and **b** the perennial rivers

No embryotoxic effects were detected in the ephemeral and perennial river systems, as the results were not significantly different from those of the negative control. The hatching success was equal in all three river systems compared with control embryos. Of the control group, 96 % hatched until 120 hpf, and 99.6 % of the exposed embryos.

5.3.2 Daphnia acute immobilization test

The negative and solvent controls were identical, with 5 % immobile daphnids in three replicates. An effect of acute immobility is defined as when > 10 % of daphnids are immobilized. At the lishana, sites 7, 15, and 27 and all sites at the perennial rivers show more than 10 % effects. After 24 h, 6 % of the daphnids exposed to lishana water and 8 % of the daphnids exposed to the water of the perennial rivers were immobilized. After 48 h, the immobilized daphnids increased to 9 % at the lishana and 13 % at the perennial rivers (Figure 5.3). Samples from the lishana and perennial rivers differ significantly in the number of immobilized daphnia ($p = 0.049$). The samples of the lishana are not significantly different from the negative control, except for sites 7 ($p = 0.0016$) and 15 ($p = 0.00004$). However, the perennial waters significantly differ from the negative control ($p < 0.05$).

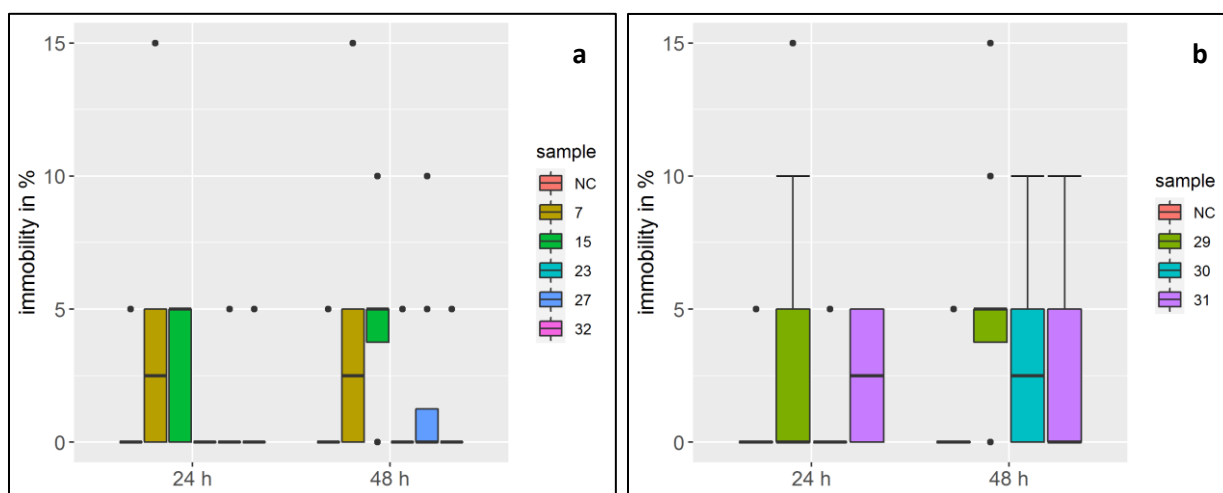


Figure 5.3: Immobilized *Daphnia magna* in **a** lishana and **b** perennial rivers

5.3.3 Algae growth inhibition test

The growth inhibition study showed no effects on the algal species studied. The systems did not differ statistically from each other. Growth rates and inhibition rates can be found in supplementary data 5.3.

5.3.4 AMES fluctuation assay

The investigation of the mutagenicity with the Ames fluctuation with *Salmonella typhimurium* strains TA98 and TA100 (+/- S9) showed effects for both river systems. All samples show an increasing number of wells of revertant growth over the concentration range compared to the NC (two-way ANOVA, $p < 0.05$). The visible reproducible increase in revertant counts compared to the negative control was significant for both strains for the sites 23, 27, and 29. At sites 15 and 32, the strain TA98+ and at sites 30 and 31, the strain TA98- were significant (see supplementary data 5.4). There are no statistically significant differences between the lishana and the perennial rivers ($p > 0.05$).

Figure 5.4 shows the IF of both strains and all samples. A significant potential mutagenic activity is defined with an IF > 1.3 (Kosmehl et al. 2004). The strain TA98 (+/- S9) showed IF values > 1.3 in every sample. In contrast, investigation with the strain TA100- showed at sites 27 and 30 IF values > 1.3 and with strain TA100+ at site 30. The IF of strain TA98 are higher than that of strain TA100 but not significantly different. TA98+ shows slightly higher IFs with a higher range (0.3 – 6) than TA98- (0 – 4.5). TA100- and TA100+ have smaller ranges (both 0 – 4).

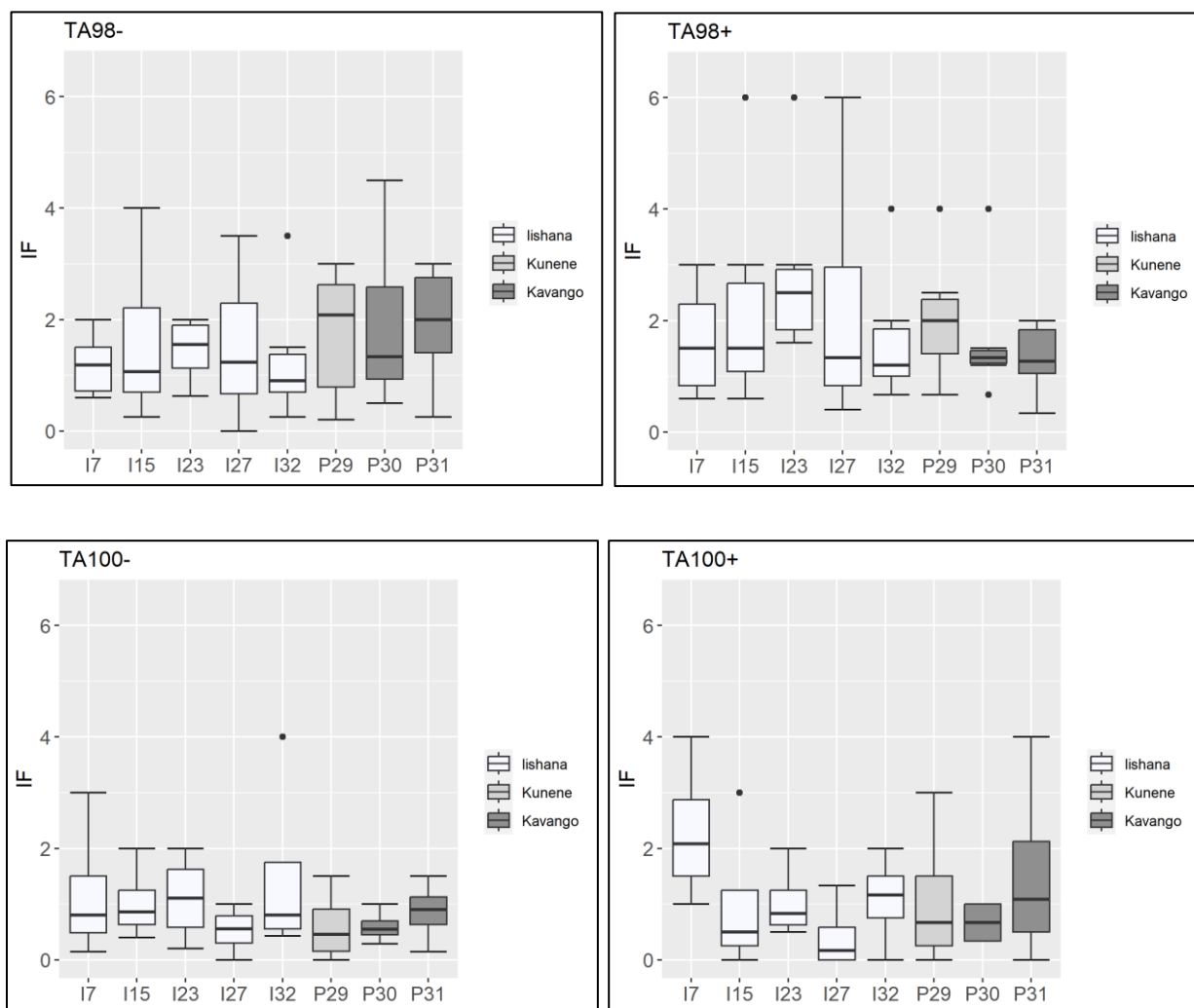


Figure 5.4: Distribution of induction factors (IF) evaluated by exposure of strain TA98 and TA100 +/-S9 to the Iishana, and the Kunene and Kavango Rivers in six dilution steps and three replicates.

Seitz et al. (2008) introduced the CDI as an index value to compare environmental samples regarding their genotoxic potential. In Table 5.2 the concentrated-dependent induction factor (CDI) for the strains TA98 +/- and TA100 +/- for all samples are presented. The results show mutagenic potential in the strain TA98 (+/- S9). In contrast, no mutagenic potential was indicated for the strain TA100 (+/- S9).

Table 5.2: The average concentration-dependent induction factors (CDI) for the strains TA98 and TA100 +/- S9 (*Salmonella typhimurium*) for the lishana system and the Kunene and Kavango Rivers.

	lishana				Kunene		Kavango	
	7	15	23	27	32	29	30	31
TA98-	1.8	2.1	1.8	1.8	1.9	2.1	2.7	2.1
TA98+	3.0	4.3	4.8	3.8	3.6	3.0	3.1	2.4
TA100-	0.8	0.8	0.8	0.5	1.1	0.6	0.4	0.4
TA100+	1.4	0.2	0.5	0.1	0.4	0.6	0.5	0.8

5.3.5 Micro-EROD assay

Prior to the EROD assay, potential cytotoxicity was investigated. Subsequently, only the concentrations that did not show cytotoxicity (>80% cell viability) were used for the EROD assay to exclude the possibility of masking the mechanism-specific effect. No cytotoxic effect could be detected for any samples so that a masking effect can be excluded. In addition, none of the samples tested showed dioxin-like potency in the EROD assay.

5.3.6 Yeast estrogen screen

An endocrine-disrupting potential was found in samples 15, 23, and 32 of the lishana system (Figure 5.5). The concentrations of 10.89 ng/l \pm 0.41 (sample 15), 5.62 ng/l \pm 0.37 (sample 23), and 21.28 ng/l \pm 1.19 (sample 32) were calculated for the two highest concentrations REF 20 and REF 10. The statistical analysis showed no significantly different results ($p = 0.94$) for samples 15 and 23. However, a different result could be observed for sample 32 with p -values of 0.007 and 0.015. In the lower concentrations, the measured values were below the limit of quantification (LOQ). In all the other samples, the estrogenic activity was below the limit of detection (LOD) and the LOQ (for details see supplementary data 5.5).

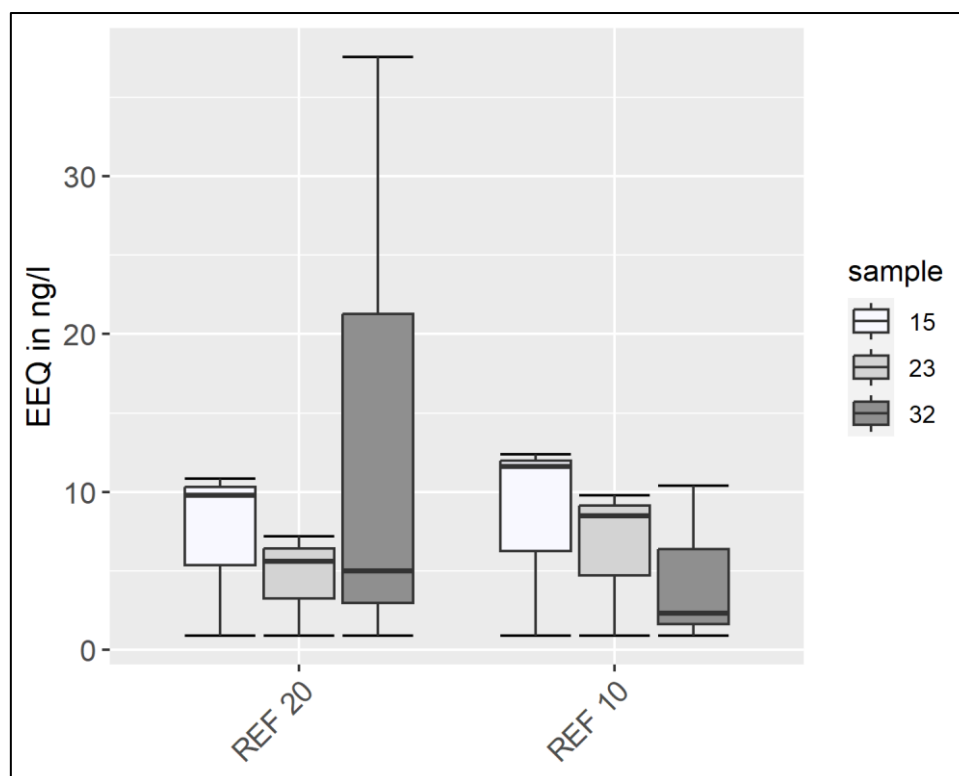


Figure 5.5: EEQ (17 β -Estradiol Equivalents) in ng/l for the lishana system

5.4 Discussion

All samples of the lishana system and the perennial rivers showed > 10 % sublethal and lethal effects per sample in the FET. The observed sublethal effects concerning the blood circulation system, such as edema and slow blood flow, can be caused by substances that bind to the Ah-receptor and act similarly to dioxin (Barron et al. 2004; Kais et al. 2017; Schiwiy et al. 2015b). However, dioxin-like chemicals (DLCs) could not be detected in the micro-EROD assay, so they are probably excluded as possible causative agents. Further compounds, such as pesticides (Awoyemi et al. 2019), flame retardants (Parsons et al. 2019), nanomaterials (Shaw et al. 2016), and heavy metals (Taslina et al. 2022) can also cause edema. In the analyzed samples of the lishana, concentrations of Cd (0.5 $\mu\text{g/l}$), Cr (3.94 $\mu\text{g/l}$), and Cu (37.33 $\mu\text{g/l}$) were detected in previous studies (Faulstich et al. 2023). Muzungaire et al. (2012) could detect 64.0 $\mu\text{g/l}$ Fe and 9.0 $\mu\text{g/l}$ Cu concentrations in the Kavango River. These metals could have caused pericardial edema and slow heart rate, even in small concentrations (Taslina et al. 2022). However, a comprehensive chemical analysis of the substances is to be performed, making it difficult to precisely identify the drivers of the toxic effects.

The pectoral fin underdevelopment could indicate that the fins lack blood supply. Von Hellfeld et al. (2022) observed fin underdevelopment caused by histone deacetylase (HDAC) inhibitors. Different HDACs were linked to a changed skeletal development in mammals, but the underlying functions of HDACs are comparable to the findings in fish embryos in the present study. In this study, the effect of an affected chorion appeared mostly after 48 hpf and 72 hpf at the lishana. The metabolism of zebrafish embryos differs over time, and 72 hpf is the most suitable time for the use of the zebrafish, concerning the stability and viability of the embryos (Schiwy et al. 2015b; Kais et al. 2017; Dhillon et al. 2019). The embryos in affected chorions were mostly able to hatch, suggesting a substance that attacks the chorion but does not cause coagulation of the embryo. The affection and slight decomposition of the chorion can be caused by substances that cannot pass the chorion. During the hours after fertilization, the chorion changes its permeability (Pelka et al. 2017). If effects decrease over time, then the substance has either been used up, or biotransformation has taken place, and the hatched fish have degraded the substance.

Logan et al. (2023) identified several negative impacts of nanofibers, such as increasing apoptosis and the neutrophil response for the embryonic development of fish. Microplastic fragments and fibers have been detected in the lishana system (Faulstich et al. 2023) and the blood stasis as a result of neutrophilic reactions could also be detected in the tested embryos. Therefore, it cannot be excluded that nanofibers may have negatively affected the embryonic development of *Danio rerio*.

In addition to the effects on fish embryos, acute effects on algae growth could also be observed. As mentioned before, heavy metals, such as Cd, Cr and Cu could be detected in the investigated samples (Faulstich et al. 2023). These analyzed heavy metals can also influence the observed toxicological effects on algae. For example, Ni and Cu affect the survival and the photosynthetic energy storage capacities of algae (Muyseen et al. 2004; Bossuyt & Janssen 2004). In the lishana system, 17.7 µg/l of Ni and 37.3 µg/l of Cu were found (Faulstich et al. 2023). In addition to its effects on fish embryos and algae, heavy metals negatively affect daphnia. Zn and Cu, for example, are likely more toxic for algae than daphnia (Ardestani et al. 2014). This study found stronger effects on daphnia than on algae, on the one hand, this could indicate that the metal concentrations are too low to cause effects in algae. On the other hand, interactions between metals and microorganisms could reduce the metal content in the water and cause less effects on algae (Priya et al. 2022).

The lishana system has shown up to 30 % effects on daphnia mobility. The immobility of daphnia could result from disseminated pollutants, for example microplastics (Samadi et al. 2022), heavy metals (Yuan et al. 2020), or antibiotics (Yisa et al. 2023). The perennial Rivers Kunene and Kavango showed no significant toxic effects on daphnia. These findings are in line with other publications. For example, investigations on the Cértima River in Portugal and the Sebou River in Morocco also showed no negative impact on *Daphnia magna* (Serpa et al. 2014; Koukal et al. 2004).

Several studies could prove a negative impact of wastewater treatment plants on the toxicity for the aquatic organisms (Ra et al. 2008; Shuliakevich et al. 2023b). The study area has a wastewater treatment plant near Outapi, a small town between site 23 and site 7 (Liehr et al. 2016). Brooks et al. (2006) describe the influence of effluent discharges on ephemeral systems. Flood events in the CEB can discharge wastewater and pollutants into the lishana system. Major flood events occur regularly in the region, most recently in 2011 and 2013 (Arendt et al. 2021). In 2019, only low rainfall was recorded in the lishana system, which could cause slow flooding. Besides wastewater, open landfill sites could be a source of toxic substances that cause effects in *Daphnia magna*. Wichmann et al. (2006) investigated a landfill site with open combustion and discovered effects < 20 % in the test with *Daphnia magna*. Near Oshakati, between site 27 and site 32, is a landfill site with open combustion (Faulstich et al. 2023), whose effluents could be responsible for the effects found in this study, especially at site 32, which is close to the landfill site.

Besides the water bodies, suspended solids of the lishana are also contaminated with metals: 4.9 µg/g Cd, 83.7 µg/g Cr, 166.9 µg/g Cu, 52.6 µg/g Ni, 82.7 µg/g Pb, 90.2 µg/g Sr, and 122.8 µg/g Zn (Faulstich et al. 2023). Contaminated particles can cause toxic effects on filter feeders such as Daphnia. On the one hand, xenobiotics are continuously dissolved from the particles; on the other hand, the particle-bound fraction can become available within the body of particle-feeding organisms. Consequently, this can lead to unexpectedly high tissue concentrations (Weltens et al. 2000). Therefore, further examining suspended solids for toxicological effects is reasonable. Endocrine-disrupting compounds, such as 17α-ethinylestradiol (EE2) and 17β-estradiol (E2) are hormonally active, even at low concentrations, and are found in surface water and groundwater worldwide (Bistan et al. 2012; Sumpter 2005; Klaic & Jirsa 2022). The lishana system and the perennial rivers Kunene and Kavango showed

EEQ concentrations up to 37.55 ng/l. One reason for these high concentrations of EEQ could be the input of various wastewaters in case of floods and surface runoff from settlements, roads, and agriculture (Burkhardt-Holm 2010). These high concentrations up to 40 ng/l, such as in the Ilishana system, are commonly found in wastewater. Murk et al. (2002) detected with the YES assay up to 317 pmol EEQ/l (~ 141.1 ng/l) in untreated wastewater and 4 pmol EEQ/l in surface water (~ 1.8 ng/l). Kidd et al. (2007) demonstrated that 5-6 ng/l EEQ lead to a collapse of whole fish populations. Wolf et al. (2022) detected EEQ concentrations up to 2.7 ng/l in a small German river and detected that heavy rainfall events influence the input of endocrine compounds into the aquatic system. Kunz et al. (2015) reported concentrations of EEQ up to 9.4 ng/l in European surface waters. In South Africa, in Pretoria and Cape Town, they could not detect estrogenic potential in drinking water samples with the YES assay. Still, the T47D-KBluc bioassay delivered low estrogenic activities with EEQ values between 0.002 to 0.114 ng/l (van Zijl et al. 2017). These measurements from other studies and systems show that the measured concentrations of EEQ in surface waters in this study are unusually high. Aneck-Hahn et al. (2012) observed estrogenic activities in the South African Limpopo province. They discovered a connection between estrogenic activities and metal concentrations, as metals can bind the estrogen receptor alpha (ER α) and block the binding of 17 β -estradiol (Darbre 2006). 17 β -estradiol is released into the environment by agricultural runoff and animal excretions and was found in several studies (He et al. 2022; Liu et al. 2019; Perondi et al. 2020). Also, plastic compounds could be a source of estrogenic potential (Chen et al. 2019). Some studies indicate an accumulation and adsorption of toxic contaminants, such as polychlorinated biphenyls (PCBs), bisphenol A (BPA), and heavy metals, on microplastics (Aragaw & Mekonnen 2021; Vo & Pham 2021; Wang et al. 2019). Microplastics were found in all three systems, and water and sediment samples in the Ilishana system showed elevated metal concentrations (Faulstich et al. 2022 & 2023). It is unlikely that the present microplastics or the elevated metal concentrations caused the high EEQ concentrations up to 37.55 ng/l. Rather, there seems to be a source that releases these estrogenic substances. EDCs have already been found in surface waters in Namibian dams (Faul et al. 2014). Faul et al. show that EDCs were mainly found in water bodies close to urban activities and a high population density, such as Windhoek. Since similar conditions prevail in the Ilishana system, it is possible that EDCs may also be found in surface waters there.

The Calux assay has often replaced the YES assay due to the partially missing transferability of yeasts to the human organism (Luele et al. 2022; Nascimento et al. 2021). Nevertheless, the YES assay is still a good indicator for detecting endocrine potential in environmental samples and is used in aquatic systems in Southern Africa (Aneck-Hahn et al. 2006; Archer et al. 2020; Kasonga et al. 2021).

In addition to evaluating the estrogenic potential of the samples, potential mutagenicity was also investigated with the two strains of *Salmonella typhimurium* (TA98 and TA100). The results showed that the *Salmonella typhimurium* strain TA98 was more sensitive than TA100, resulting in a significant reproducible increase in revertant counts referred to the revertant number in the negative control in all samples of the lishana. The strain TA100 only showed visible differences regarding the revertants. The number of revertants for the strain TA98 +/- is slightly smaller than the spontaneous revertant control values (20-50 revertants) in the literature (Mortelmans & Zeiger 2000; Tejs 2008). For the strain TA100 +/-, the control values (75-200 revertants) are significantly higher than those in this study. However, the measured revertant counts were significantly different from the NC. The strain TA98 is more sensitive and can detect frameshift mutations (Kosmehl et al. 2004). A low cell density of the strain TA100 and fewer targets for base substitution cause a lower sensitivity than the strain TA98 (Reifferscheid et al. 2011). Iji et al. (2021) analyzed the surface water of a stream in South Africa (Mpumalanga Province) with the Ames test to gain knowledge of the genotoxic potential. The IF of strain TA98 was > 1.5 , and of strain TA100 > 1.7 (Iji et al. 2021). The metabolic activation by the rat liver S9-mix had a marginal effect on the mutagenic potential. All tested samples in this study have IF values > 1.3 for the strain TA98. Both strains show slightly higher IF values with the S9 mix and have a higher mutagenic potential. For a quantitative interpretation of genotoxicity and mutagenicity data, reference points are missing, like a benchmark dose. The dose-response relationship still needs to be evaluated (Menz et al. 2023). Therefore, it is difficult to quantitatively assess the genotoxicity and mutagenicity of the investigated river systems. Nevertheless, it is known that heavy metals can cause mutagenicity. In a case study in India, Rajput et al. (2020) described that samples with a higher concentration of heavy metals lead to a higher mutagenicity. As described before, heavy metals were found in the lishana and could cause mutagenic effects.

5.5 Conclusion and outlook

This study is the first study in Namibia that investigated surface waters regarding their ecotoxicological potential. It is an important step towards a complementary water quality assessment of these hardly-researched waters. The main objective of this proof of concept study was to identify the ecotoxicological potential of the lishana system and the two neighboring systems, Kunene and Kavango. Several effects were detected in the investigated samples. Acute toxicity was detected in fish and daphnia, while freshwater algae showed few effects. The investigated river systems differ concerning the observed acute toxicity. The perennial rivers show fewer effects in the FET but more on the immobilization of daphnia than the lishana. Common endpoints included a slightly decomposed chorion, pectoral fin underdevelopment, and blood congestion. Strong endocrine effects up to 40 ng EEQ/l were investigated and a significant mutagenic potential was identified for the strain TA98 +/-.

The demonstrated ecotoxicological effects in the studied aquatic systems seriously affect water bodies and their ecosystems. Toxic substances, EDCs, and microplastics threaten the ecosystem of the lishana. Demonstrated acute toxicity to the daphnia and zebrafish may result in reproductive disruption, affecting the population size of primary and secondary consumers. There are several negative effects of estrogenic and estrogen-like compounds on endocrine systems, reproductive outcomes, and reproductive health of the population (Campbell et al. 2006; Hecker & Hollert 2011; Woodruff 2011). Estrogenicity may cause damage to the reproductive organs, thus also negatively affecting populations. When aquatic pollution affects keystone species, such as daphnia and algae, biodiversity is threatened. A loss of biodiversity in the lishana, the Kunene and Kavango Rivers can reduce fish populations, which are important for the local food supply. The water of the lishana is used as drinking water. If this water has toxic effects and it cannot be excluded that these will be transferred to humans, then this is a high risk for the local population.

Although the approach used in this study, a combination of several bioassays, has already been published in several studies, it delivers results for a region where these analyses have not been documented. To the authors' knowledge, in Namibia, there are no studies concerning the ecotoxic potential of surface waters. Identifying toxic compounds is inevitable to estimate the state of the ecosystem. This study could prove the acute toxicity of the lishana system, the Kavango, and the Kunene Rivers.

Statements on chronic exposure cannot yet be made. Further investigations, for example testing the pre-filtered suspended solids, could help identify the relevant substances causing these effects.

Therefore, more effects may be detectable when using more sensitive test systems or other organisms likely more adapted to arid and semi-arid regions (Lahr 1997). Increasing the exposure times for the endpoints mobility and growth reduction could cause the achievement of more effects. In the environment, organisms are exposed to potentially toxic substances for long durations. These long exposure times can be recreated in the laboratory. Harmful effects often occur after prolonged exposure to pollutants, since the environmental samples are also exposed to the potentially toxic substances for a longer period of time. Long-term toxicity tests, which simulate chronic exposure for up to several weeks or months based on the life cycle of the test organism, can be helpful in assessing long-term effects. For acute toxicity, reproduction can be examined as a "long-term" test for *Daphnia Magna* (OECD 2012) and the early life stage test for *Danio rerio* (OECD 2013b).

One decade ago, ecotoxicology was a relatively new science in South Africa (Wepener and Chapman 2012), but it has developed rapidly, and nowadays, numerous studies can be found on ecotoxicological risk assessment of aquatic systems in the SADC region, with large differences between countries (Eijsackers et al. 2020; Selwe et al. 2022). Future investigations concerning freshwater quality should link chemical and bioanalytical information and quantify cause-effect relationships (Altenburger 2019). For a holistic study of the lishana ecosystem, a chemical analysis, and the analysis of sediments and suspended solids for toxic effects would complete the ecotoxicological assessment.

5.6 Acknowledgements

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6. Summarizing discussion and synthesis

In the previous chapters, the multi-methodological approach was explained, the methods were described in detail, and the results were presented. The aim was to improve the knowledge of the water quality of the lishana system by evaluating the condition of water bodies during a drought period based on nutrients, pollutants, sediment loads, microplastics, and toxic effects, and to assess usability and potability of the waters. The following is a response to the main research questions and a final assessment of the lishana ecosystem.

1. *What is the physicochemical state of the waters of the lishana system and which pollutants and contaminants are found in them?*

The physicochemical state of the waters of the lishana system is poor, as described in Chapter 3.4. The water bodies are subject to a high pressure of use and are therefore exposed to many different influencing factors. The parameters Al, Fe, Ca, and Na are present in high concentrations, above national and international limit values (DWA 1990; WHO 2017). An accumulation of salts and saline compounds is explained by high evaporation values and lacking freshwater input. EC and turbidity were above the limits in every sample of the lishana. This high turbidity values and therefore high amounts of suspended solids decrease the potability of the waters. The suspended solids showed high concentrations of metals—on average more than 60 mg/g of Al and 30 mg/g of Fe—and even Pb is present with more than 0.3 mg/g. Heavy metals, such as As, Cd, Co, and Sr were found in small concentrations, below limit values. The sediment samples also showed high concentrations, with an average of 60 mg/g Al and 30 mg/g Fe in the fraction < 0.063 mm.

TC and TOC concentrations between 20 and 40 mg/l in the lishana are elevated by comparison with clean river systems (Chapman 1996). The high amount of organic material and the elevated TNb concentrations (up to 8 mg/l) indicate a high primary production. This is matched by increased chlorophyll- α and oxygen values and increased algae growth. The high number of blue-green algae intensifies with an increasing eutrophication rate, caused by temperatures over 26 °C, chlorophyll- α , and dissolved oxygen. The algae growth inhibition test showed no effects (see Chapter 5.3.3), which again shows that there are no toxic substances and algae can grow. An increased water temperature promotes biochemical reactions and deepens the

thermocline in standing waters like those of the lishana. Hypoxia in bottom water and the release of pollutants promote the growth of phytoplankton (Xia et al. 2014).

Chapter 4.3 describes the microplastic load for the lishana system and the main ephemeral and perennial streams in Namibia. The microplastic occurrence in the lishana system, just like the water quality parameters, differs very strongly depending on location. While a lot of microplastics are found close to settlements, there are significantly fewer microplastics in locations further away from settlements. With 53 particles kg^{-1} , site 18 in the northern part of the study area was the most polluted. At sampling location 23 no microplastics were found. The most common fraction size was 0.5–1.0 mm (47 %). It is common to find a lot of plastic particles in the smaller fractions. PE and PP were found most frequently. These are polymer types that are often used for food packaging. Most of the particles were shaped as fragments. Due to the methods used, it was difficult to identify fibers. It is assumed that there are many more fibres in the waters that might not have been detected. This is a common challenge in microplastic detection (Haap et al. 2019). The large macroplastic deposits in Namibia and the partially inadequate recycling system explain the large microplastic amount, in particular of PE and PP. The polymer types PA, PS, and PMMA were also detected, but in lower amounts. The 9 analyzed ephemeral streams in Namibia contain fewer microplastics than the lishana system. The rivers are located in sparsely populated regions, without much pressure of use. Although the sampling sites were close to roads and bridges (less than 50 m away), which means that a macroplastic input exists, the pressure of use in the lishana system is significantly greater. In ephemeral rivers, microplastics can also enter the system during dry phases via aeolian input. Only fluvial processes were observed in this study, so the top centimeter of sediment was discarded to exclude aeolian accumulation. However, an additional analysis of the aeolian sediments would be useful to be able to trace the complete spectrum of microplastics input into the water bodies.

Despite being a sparsely populated country, this study found relatively high levels of microplastics in Namibia. Microplastics could be detected in every river system in the country. In freshwater systems, the potential source for microplastics may be human use and waste (Biginagwa et al. 2016). In Africa, an increasing number of people live on the coastlines and within a short distance of river systems. A high amount of mismanaged plastic waste from Namibia and Angola is transported to marine areas via the rivers (Jambeck et al. 2018). These rivers are likely transporting plastic waste

and microplastics to the oceans, as can also be seen from the results of this study. Because it is unclear how much waste is produced per capita, what the plastic content of the waste is, and how much waste is disposed of uncontrolled, it is difficult to estimate the exact amount of plastic that is entering the aquatic environment (Jambeck et al. 2018). Therefore, no estimates can be made as to what the ratio of plastic occurrence to microplastic occurrence is. Nevertheless, the microplastic amounts found can be explained by several aspects. First, there is a common difference in the waste produced depending on the area: In rural areas, organic and biodegradable waste predominate; in urban areas, plastic, glass, metal, and electronics are more prevalent (Hoorweg & Bhada-Tata 2012). The differences in the amount of microplastics between rural and urban regions found in this study can therefore be explained by the type and amount of used plastic products and waste. Second, the application of biosolids from wastewater treatment plants and overflow of open sewers or canals during the rainy season can cause a large microplastic input into the environment. Further input sources are runoff from sludge that is released from industrial processes, rural washing, and runoff from landfills (Bouwman et al. 2018). Tire and road wear particles (TRWP) can be flooded into surface waters and cause microplastic pollution (Horton et al. 2017). Third, an accumulation of waste in certain areas may occur as a consequence of slow water velocity and weak mixing, as it does in the lishana system during a drought period. Moreover, the attachment of organic and inorganic material results in density gain that in turn causes the debris to sink and settle. Thus, only measuring the amount of microplastic and plastic particles on the water surface or even in the water column could result in an underestimation of the actual quantity of debris.

As there is only limited available data on the microplastic emission to soils and rivers, the current assumption is that microplastic transport and storage are equal to natural soil erosion and sediment transport (Nizzetto et al. 2016a). Hydrodynamic processes transport and move pollutants; therefore, far-away impacts occur. But there is still a knowledge gap in the transport and fate of microplastics in streams (Akdogan and Guven 2019).

In Chapter 5.4, the toxic effects of the waters of the lishana system are explained and discussed. The tests with fish embryos and daphnia showed acute toxicity. Heavy metals, such as Cd, Cr, and Cu, can cause malformations in fish (Castillo et al. 2000). Also, cyanobacteria are reported to have harmful effects in fish and could cause the

effects found in this study. Effects on daphnia can be caused by pollutants—for example, the runoff of a waste disposal site. A waste disposal site close to Oshakati may cause these harmful effects on daphnia at sites 27 and 32.

Mutagenic effects were detected with the AMES test, especially for strain TA98. There are weak differences between the sites in the lishana system. Cytotoxic effects and effects on algae could not be detected. A high oestrogenic potential was found for the three sites 15, 23, and 32. This could be caused by algal blooms, as was once reported in Namibian dams (Faul et al. 2014). Why a high oestrogenic potential was found at these three sites still needs to be conclusively investigated. More tests, in particular regarding cytotoxicity, should be carried out to clarify the proven toxic potential. It could be helpful to use other organisms—for example, saltwater organisms—to best recreate the environmental conditions.

2. Are there any spatial or temporal variations regarding the water quality of the lishana and the two neighboring systems, the Kunene and Kavango Rivers?

The spatial differences regarding water quality between the lishana are locally significant and are discussed in Chapter 3.4.2. The individual lishana are independent of each other most of the time, and there is no exchange, except in the case of floods and increased surface runoff. As the local conditions around the lishana are so different, so is the water quality. Land use, population density, and the precipitation gradient influence the spatial differences.

The alternation of rainy and dry seasons causes the water bodies to change from standing to flowing waters, which affects the suspension, transport, and deposition of substances. In a stagnant phase, nutrients and pollutants can accumulate. In the phases where water movements take place, substances can be dissolved and deposited elsewhere. Changes in precipitation and extreme events due to climate change affect water quality.

Droughts increase pollution concentrations and nitrogen mineralization. The low or insufficient water flows weaken the dilution effects of pollutants. Less rain can lead to water loss and an increase in concentrations of suspended solids in surface waters (Bhateria & Jain 2016; Xia et al. 2014). Auer (1997) observed enormously high salt contents in the lishana system, especially before the onset of the rainy season. The precipitation only partially diluted the waters and lowered the salt concentrations. Xia

et al. (2014), by contrast, have found increased concentrations of metals, organic compounds, nitrate, and fecal coliform bacteria during high rainfalls or floods. One reason could be the suspended solids that are washed out and redistribute pollutants. Another reason are contaminants washed out of the sediment and dissolved during strong rainfall events.

As two field campaigns had to be canceled, a comparison of rainy and dry seasons and the evaluation of their influence could, unfortunately, only be made to a limited extent. Although some lishana had desiccated over the years, a concentration and enrichment of nutrients and pollutants could be observed in the remaining water bodies. The temporal differences between the dry season of 2017 and the rainy seasons of 2018 and 2019 were significant regarding water quality. The strong local differences between the lishana can be explained by the independence of the sites from each other. They are only connected in times of rainfall and surface runoff. This alternation of suspension, transport, and deposition becomes very evident in the case of suspended matter and sediments, where similarly high concentrations of nutrients and pollutants occur.

In principle, a gradient can be identified in the lishana system that follows the rainfall gradient. Between 2017 and 2018, fewer lishana desiccated in the eastern part than in the western part of the study area. Precipitation decreases from southwest to northeast, which decreases the mixing of the lishana and the freshwater input. This creates a concentration effect in the sinks and leads to an increase in nutrient and pollutant concentrations. The lishana close to urban centers show higher concentrations of pollutants. This can be observed close to Oshakati and Ondangwa in the eastern part of the study area. Elevated concentrations are also found along the Calueque-Oshakati canal and close to the waste disposal site near Oshakati (sampling locations 3, 17, 27, and 28; see Figure 3.2). The same effect can be observed in the microplastic content of the lishana. The lishana that are close to bridges, culverts, or settlements have a higher number of microplastics. Small-scale effects such as wind, surface runoff, and fluvial transport explain the spatial distribution of microplastics in the lishana system. The ecotoxicological effects are less spatially differentiated. Though here, too, the lishana that are close to settlements show more effects than those that are located in rural areas.

The differences between the three systems are sometimes significant. The perennial waters show different chemical conditions, as the influence of rainwater inflow and the small-scale influencing factors have less of an impact. Nevertheless, acute toxic effects and microplastic contamination are also found in perennial waters. The different origins of the waters and their different local conditions result in different water quality.

The results of the concentration measurements for the Calueque-Oshakati Canal are within WHO limits and only the turbidity shows higher values. The water of the canal is more suitable for human consumption than the water of the lishana. One reason could be the origin of the water. The water of the canal comes from the Kunene River, whose water is transported into the canal through the lishana system. Since the Kunene River is a perennial water source, there is permanent water movement. The evaporation does not affect the concentration of substances as it does in the lishana, which are still waters for a large part of the year.

The water quality of the lishana is worse than that of the perennial waters. It can be seen that the perennial waters, as flowing waters, experience more mixing. The suspended matter content was also lower than in the lishana, which means that fewer pollutants can accumulate. The perennial river systems contained less microplastic than the ephemeral ones. Reasons could be the rapid removal of the microplastics due to the stream flow and the consequent lack of sediment and microplastic deposition opportunities. The two perennial water bodies and neighboring systems of the CEB contain significantly fewer microplastics. At the Kunene, 1.8 particles kg^{-1} were found; at the Kavango, 9.3 particles kg^{-1} . These amounts are significantly less than in other perennial water bodies with similar discharge values, like the Amazon River in Brazil or the Antuã River in Portugal (Gerolin et al. 2020, Rodrigues et al. 2019). The acute toxicity of the perennial waters tested in this study is also much lower than that of the lishana. No acute toxicity on daphnia and algae was found. The AMES test showed IF values of > 3 for the perennial waters, which is significantly higher than the IF values of 1.75 for a river in the Olifantes catchment in the province of Mpumalanga, South Africa, which is in the catchment of a mine (Iji et al. 2021).

In general, it appears that the Kunene and the Kavango Rivers have lower pollutant concentrations, fewer microplastics, and less acute toxicity. The mixing conditions in both perennial rivers are different from those in the lishana.

3. *What is the state of the lishana ecosystem?*

The assessment of ecosystems and corresponding indicators depend on the intention. In this study, the intention is the assessment of usability and potability of the lishana water for humans, as these waters are already an intensively used water resource. In this regard, several trophic levels of the lishana and the water quality were investigated as the focus of the ecosystem research. There is no general assessment scheme for ecosystems, as they are far too diverse. The European Union has developed its own index system for all occurring ecosystems, both terrestrial and aquatic; but this system is directly adapted to the corresponding local conditions and ecosystem services (Maes et al. 2020). However, there are some approaches and models that describe and assess aquatic ecosystems (Allen et al. 2020). The lishana system is an ephemeral river system and ephemeral and intermittent rivers are often not correctly addressed by river-ecosystem conceptual models (Allen et al. 2020). Nevertheless, there are a few approaches that consider ephemeral systems, such as the “Multiple roles of water” by Sponseller et al. (2013) and “The freshwater biome gradient framework” by Dodds et al. (2019).

Sponseller et al. (2013) developed an approach that describes three main categories of the role of water: i) water as a resource and habitat for biota, ii) water as a medium for the exchange of energy, materials, and organisms, and iii) water as a factor of geomorphic change. All these roles depend on precipitation and temperature conditions and can vary regarding the scale of the analysis. In this approach, water availability is included as an important aspect that is often left out in other models of aquatic ecosystems, which is why it is suitable for ephemeral systems. Considering the above aspects in the lishana ecosystem, an evaluation can be made.

Water as a resource and a habitat for biota

The water of the lishana is an intensively used resource. As described in Chapters 3.2.1. and 3.4.2, it serves as drinking water and industrial water, and partly as irrigation water for agriculture. It is used for fishing and as drinking water for animals. It is the habitat for fish, snails, insects, and microinvertebrates. Mammals live along the water bodies and use it as a watering place. However, due to this intensive use, the lishana is also subject to many factors that can disturb the ecosystem. As discussed in Chapter 3.3., at a chemical level, there are several substances in harmful concentrations in the

water body, in suspended solids, and in sediments. Aluminum, iron, and several salt compounds are present in high concentrations above the limit values. High turbidity values and a high number of suspended solids provide a good environment for pollutants and bacteria to accumulate and grow. The ecotoxicological effects described in Chapter 5.3. show that not only humans are affected by poor water quality, but also other trophic levels where acute toxicity has an impact.

The microplastic loads found can be a source for further pollutants that detach. Large-scale pollution with microplastics in the region can also be a focus for the leaching of toxic substances. The link between microplastics and ecotoxicological effects has been demonstrated. Different toxic effects could be identified on different trophic levels of the ecosystem. Primary and secondary consumers show toxic effects. Mutagenic effects in the water can cause diseases when consumed and harm the organisms in the ecosystem.

Water as a medium for the exchange of energy, materials, and organisms

For most of the year, the lishana are standing waters and do not serve as a long-distance transportation medium. Nevertheless, even during this phase they are a transport medium for nutrients and pollutants that attach to suspended solids from the water phase or accumulate in the sediment. This observation was made clear in Chapters 3.4.1. and 3.4.2 for Al and Fe. Above-average concentrations were found in the water phase, suspended solids, and sediment.

Floods and inundations occur repeatedly during the rainy season, and there are simple flow movements of the lishana during weaker rains, transporting water from southern Angola to northern Namibia. As described in Section 4.4, many microplastic particles were found in the sediments of the lishana. Either these originated from macroplastic fragmentation and were directly introduced into the water bodies, or they were introduced into the water bodies through flow movements. Furthermore, spatial analysis of substance concentrations revealed that flow movements arrested at road dams (Arendt et al. 2021) accumulate there and provide higher concentrations than south of the road dams.

Water as a factor of geomorphic change

Due to the flat relief and low flow velocities in the lishana system, there are no major geomorphological changes in this ecosystem. The exception are the floods, which are quite active, carrying a destructive geomorphological force that can shape the landscape. However, the lishana's characteristics are diverse, ranging from narrow drainage channels to wide, almost lake-like shallow waters to deep depressions that carry water through the dry season. The shape of the lishana also shapes the landscape and changes it, depending on the rainy or dry season.

Another factor that shapes the environment is the regular drying of the lishana. Drying is an important factor for the spatial structure of river networks (Allen et al. 2020). The drying of the water bodies during the dry season and the frequent lack of fresh water during the rainy season increases the concentration of pollutants, such as metals (see Chapter 3.4.2). These aspects show how landscape-defining the lishana ecosystem is. Every year, the forms change, which is challenging for the local population because, when water areas change their boundaries, it affects the infrastructure.

The assessment of ecosystems is often connected with the assessment of the ecosystem services (Allison & Brown 2017). There are five main ecosystem services of the lishana system: i) a provider for drinking water, ii) a provider for irrigation water, iii) a provider for water for livestock iv), a provider for domestic water, and v) water for fishing. The ecosystem of the lishana is stressed on the chemical and biological level by various climatic and anthropogenic factors. Pollutants, such as microplastics and metals, climatic extremes, such as drought and floods, and the anthropogenic factor itself disrupt the quality and usability of water. Therefore, the ecosystem services of the lishana system are also disrupted.

4. Is the water of the lishana usable for human consumption without health risks?

The lishana water is not suitable for unrestricted use and should not be used without prior treatment, as discussed in Chapter 3.5. The values of the samples were compared with national and international water quality limits over the entire evaluation period and the guidelines of the WHO (2017) and the Namibian Water Act (DWA 1990) were used to assess the results. The Namibian Water Act 54 of 1956 classifies

water into four categories: i) Group A: Water with an excellent quality, ii) Group B: Water with acceptable quality, iii) Group C: Water with low health risk, and iv) Group D: Water with a high health risk, or water unsuitable for human consumption.

Both guidelines contain limit values for potable water. The WHO Guidelines have no limit values for some parameters, such as pH, ammonia, manganese, potassium, sodium, sulfate, and zinc, as these do not normally occur in drinking water. But they can cause problems with the acceptability of potable water, in regard to taste, odour, and appearance (WHO 2017). Since the lishana water is untreated, several substances are still present, such as ammonia, manganese, potassium, and zinc. However, as long as the untreated water of the lishana is used as drinking water, appropriate limits must also be used as a target for the water quality of the lishana. The Namibian Water Act has limits for all parameters measured in this study, and by classifying them it is easy to understand when health problems may occur. In all three measurement periods, the values for turbidity, Al, and Fe were in the fourth class, unsuitable for human consumption. Ammonia, manganese, phosphate, and sulfate are in the third and fourth class for several sampling sites, depending on the year (DWAf 1990). The measured values of turbidity, Al, and Fe also exceed the international WHO guidelines for potable water (WHO 2017). Metals, such as Al and Fe have already been proven to have health effects when consumed over a longer period (Chowdhury et al. 2016). The high salinity, which is caused by the only temporarily flooded water bodies and the high degree of evaporation, severely restricts the potability of the water at times. Measured salt compounds, such as NaCl or CaCl were also too high to be consumed. The electrical conductivity exceeds the guideline values in the majority of the lishana. The high number of blue-green algae can produce several types of toxins that cause health risks for humans (Al-Hussieny 2022). The lishana water is therefore not suitable for human consumption. It could not be conclusively clarified how water affects livestock or plant organisms.

The reported amount of microplastics can also have negative impacts on the water quality and ecotoxicological consequences. There are assumptions, that microplastics in drinking water can affect human health, provoking endocrine disruption and genetic mutations (Angnunavuri et al. 2022). In some studies, numerous toxic substances, such as persistent organic pollutants (POPs), heavy metals, and perfluoroalkyl or polyfluoroalkyl substances (PFAS) have been found to attach themselves to the plastic particles and cause health risks (Du et al. 2023, Koelmans et al. 2013). In Chapter 5.4

the toxic effects of the lishana water were discussed. The acute toxicity of the waters shows that they have toxic effects on primary and secondary consumers. This means that there are disturbances in these trophic levels and it cannot be ruled out that there are also negative effects in higher trophic levels. Therefore, it is unclear whether the water can be used for domestic or industrial purposes. It is thus not advisable to use the water of the lishana without treatment.

7. Conclusions and future perspectives

This thesis is the first step to an integrative analysis of the hydrological processes and the status of the aquatic ecosystem of the lishana. Valuable data was collected over four years on a system for which there is a lack of data. Field investigations, laboratory analyses, and their integrative analysis have provided useful data for assessing the state of the water bodies. This thesis can be seen as a foundation for further research that could help to achieve a safe water supply in the lishana system. A long-term, sustainable, and year-round monitoring of the changes in water quality, in regard to its physical, chemical, biological, and toxicological characteristics is the essential basis for planning any appropriate treatment measures and for the development of adaptation strategies for the population.

7.1. Scientific relevance

From a scientific perspective, three scientific disciplines were linked together to describe an overall picture of the lishana ecosystem: water analytics, the detection and identification of microplastics, and the analysis of ecotoxicological effects on different trophic levels. This is a new approach that has never been used before to investigate the ecosystem of the lishana. To date, only water-analytical studies on hand-dug wells and the Calueque-Oshakati canal have been conducted in the lishana system (Wanke et al. 2014; Koeniger et al. 2020). Microplastics and the ecotoxicological potential of the water have also never been investigated before. For the first time, microplastics were detected in Namibian waters. So far, there have been no studies that have investigated microplastics in the surface waters of Namibia. In such a sparsely populated country, it is striking to identify so much microplastic content in the rivers. The ecotoxicological studies were carried out for the first time on Namibian waters. Several toxic effects were detected, especially acute toxicity. Together, these pose considerable dangers to the local ecosystem. The combination of the three methodological approaches for the study of surface waters is also new. This thesis is the first comprehensive investigation of the lishana ecosystem. Previously, evidence of the poor water quality of the lishana was purely anecdotal.

Nevertheless, some limitations have to be mentioned in order to classify the results correctly. The application of classical methods of water analysis was challenging in the

terrain, but reliable results were achieved. Considering the size of the study area, the number of selected sites is important for the representativeness of the data. The selected network of sampling sites covers the whole lishana system and strikes a positive balance between different site conditions, such as proximity to the anthropogenic influence factors of settlements, roads, and bridges. Both the size of the study area and the measurement periods presented challenges. Sampling took place at the end of the 2017 dry season and at the end of the rainy seasons of 2018, 2019, and 2021. However, except for the 2021 rainy season, the other rainy seasons were very weak, resulting in no usual freshwater input from rainfall to the lishana. The two field visits that were cancelled due to the COVID-19 pandemic—one at the end of the rainy season in March 2020 and another at the end of the dry season in September 2020—have left a gap in the projected time series. Differences between rainy and dry seasons could therefore not be investigated satisfactorily. The method used for microplastics detection worked efficiently with some limitations. The detection of fibers was only possible to a limited extent. Fractionation with sieves, through which the fibers can slip, made it difficult to capture fibers. Furthermore, it was not possible to detect polymers with a density $> 1.2 \text{ g/cm}^3$ due to the separation solution with a density of 1.19 g/cm^3 . The algae growth bioassay and the micro-EROD assay were not sensitive enough to detect effects in the lishana water.

The insights gained from this study may be of assistance to further research of aquatic ecosystems in arid and semi-arid regions. An investigation of more pollutants, such as pesticides, fertilizers, polycyclic aromatic hydrocarbons, polychlorinated biphenyls, and dichlorodiphenyltrichloroethane should be carried out in order to better assess the health risk of consumption. Further investigation of smaller fractions (i.e. $< 0.3 \text{ mm}$ and $< 0.1 \text{ mm}$) of microplastics would complete the picture, since the toxic potential increases with smaller fractions. The question of whether there are other pollutants or pesticides attached to the plastic particles, which in turn can trigger toxic effects, needs to be further investigated (Syberg et al. 2015). A closer examination of the individual flow systems could lead to an even clearer identification of the sources from which the microplastics come. By choosing a different agent for density separation, heavier polymer types such as PET and PTFE could also be detected. It is to be expected that a further investigation will be able to detect more fibers than those which could only be detected to a limited extent in this investigation. In future studies, a more thorough examination of suspended solids should be performed. The results would show in

greater detail that there are probably still pollutants on them that can trigger toxicity. There should also be an evaluation after a conversion of the suspended matter into the water phase. Whether the microplastics in the sediments of the lishana release substances or accumulate substances that can be pathogenic could not be conclusively clarified. However, the acute toxicity of the waters is a challenge that needs to be further investigated, in particular regarding different concentration levels. If the water has toxic elements, there is a danger to the local population. More pollutant groups and pesticides should likewise be investigated. Finally, although it is difficult to identify concrete sources of pollutants, an attempt must be undertaken to do so in order to conserve water resources.

7.2. Sociopolitical context

Addressing the concept of resilience is important when describing the social implications of climate change and water scarcity. Resilience describes the ability to withstand, adapt, and recover from external disturbances, such as those posed by climate change (Schilling et al. 2017). Resilient aquatic ecosystems for example, can withstand periods of prolonged drought without causing water bodies to eutrophicate. Resilient communities, on the other hand, can adapt to water scarcity by developing strategies. Because water scarcity can be reduced by increasing water quality (van Vliet et al. 2017), such strategies may include efficiently storing water during rainy seasons and maintaining the water quality of existing water resources. Adaptation strategies require a fundamental analysis of the physical conditions of the region—in this case, the lishana system. The findings of this thesis will be of interest to the local politicians who select treatment measures for the surface waters.

The study of the water quality of the lishana is of great importance for the lishana system. As the lishana are a main source of water for the local population, it is important to determine their water quality. With an accurate knowledge of the state of the waters, the resilience of the population faced with water scarcity can be increased. After extreme weather events, such as droughts and floods, the potential for using existing water resources efficiently is much larger if you know how these resources react to such events and what treatment or protection measures you need to take. Access to a public water supply in the lishana system often depends upon income. In the lishana system, 20 % of the population do not have access to a safe water supply.

On the one hand, not all people can afford the connection to the water utilities, and, on the other hand, not all localities are connected to the supply network. The lack of data on this subject impedes an exact description of the water distribution in the lishana system. The waterworks treat and provide water for many people. Further treatment steps—such as coagulation and flocculation, for example, with chlorine—could also be implemented. Wastewater treatment with ozonation or activated carbon stage filtration and the reduction in the emissions of pollutants are big-scale solutions that require more technical resources (van Vliet et al. 2017).

The lishana system has to adapt to climatic changes and water scarcity. The monitoring of surface waters is one important step on the way to sustainable water use. The appropriate treatment of water and water supply will always be a challenging subject in the CEB as an arid and semi-arid region. This work has provided a major contribution to the assessment of the state of the lishana ecosystem, and it can serve as a basis for further studies towards the implementation of safe water resource management. This thesis has also provided a deeper insight into the ecosystem of the lishana, which can help implement water treatment measures to ensure save water use.

7.3. Potentials in applications & practice

Due to the low water availability in semi-arid regions, it is particularly important to have reliable water resources with adequate quality. Only with the appropriate level of knowledge about these resources can plans be made for water treatment, use, and distribution. With the knowledge gained in this thesis, water resource management can be better adapted to local conditions, and the research concept can be transferred to other regions that face similar challenges. Regarding an expansion of ephemeral streams in the future, due to climatic changes, a profound knowledge of their ecosystems is essential (Allen et al. 2020).

The lishana system is a region partially lacking in technical infrastructure. Although the population density is very high compared to the rest of the country, there are still long distances of up to 100 km to cover to reach the nearest city. Therefore, large-scale possibilities are limited, and it is thus essential to find small-scale and low-threshold solutions for water supply and water treatment that can be applied in the small villages and settlements in the rural areas. Following four low-tech and small-scale measures

could improve the water quality significantly: First, the allocation of certain lishana for human use and others for animale use. This separation would reduce the number of pathogen bacteria that were found in this study. Second, protecting the lishana from littering in order to reduce the amount of microplastics and attached pollutants. Third, the protection of existing water resources against evaporation by improving the area-to-depth ratio of the lishana. Fourth, an implementation of water treatment measures for the lishana water. Water treatment facilities for the lishana do not yet exist. An important aim is to provide biologically safe water, because waterborne diseases are one of the main problems in regions with poor water supply (Treacy 2019). The use of filtration systems, such as sand filters or coal filters, could reduce the content of suspended solids and potential pollutants.

The alarming deterioration of water quality on a global scale needs new solutions. The applied approach of field work, laboratoy work, and statistical analyses is time-consuming and cost-intensive. But there are also less cost- and time-intensive approaches, such as using satellite images to assess water quality parameters. An increasing number of studies use these remote sensing measurements to assess water quality parameters (Ross et al. 2019, Sagan et al. 2020). This is a promising field, in particular for rural areas, and it has already been applied in Northern Namibia (Fujioka et al. 2018; Kapalanga et al. 2020). The findings reported there shed new light on the use of machine-learning algorithms. Several studies developed supervised machine-learning algorithms to estimate water quality (Ahmed et al. 2019; Ahmad et al. 2017). However, even remote sensing approaches require measurement data to compare data sets. Therefore, investigations such as those in this thesis are necessary to be able to classify future analyses.

Other trophic levels of the lishana ecosystem can be studied to learn what other toxic effects are visible. Detailed analysis of pesticides in the water will help to find better treatment methods. This thesis is an extensive, yet not exhaustive, insight into the lishana ecosystem. From a scientific perspective, the knowledge acquired here can be implemented in new projects focusing on the ecosystem of the lishana.

8. References

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Supplementary data

Supplementary data 3.1

General information about applied methods.

sample	sample preparation	method	parameters	accuracy	test protocol
water	<i>in situ</i> measurement	YSI-multiparameter probe 6600 V2-4	temperature, pH-value, redox potential, oxygen content, oxygen saturation, electrical conductivity, turbidity, chlorophyll- α , cyanobacteria	± 0.15 °C ± 0.2 units ± 20 mV in standard ± 2 % of value ± 2 % of value ± 0.5 % of value ± 0.3 NTU linearity $R^2 = 0.9999$ linearity $R^2 = 0.9999$	-
water	centrifugation (20 min at 6000 min ⁻¹) filtration (syringe filter holders, 0.45 μ m)	portable HACH DR 1900 VIS spectrophotometer	Cl ⁻ , F ⁻ , NH ₄ ⁺ , NO ₃ ⁻ , NO ₂ ⁻ , PO ₄ ³⁻ , SO ₄ ²⁻ , COD, TNb, TC, TIC, TOC	340 - 800 nm	-
water	filtration (syringe filter holders, 0.45 μ m), acidification (0.1 ml of nitric acid), cooled transport in PE bottles; centrifugation (15 min at 10,000 min ⁻¹) filtration (membrane cellulose acetate filter, 0.45 μ m)	ICP-OES 2000	Al, As, Cd, Ca ²⁺ , Cr, Co, Cu, Fe ²⁺ , Pb, Mg ²⁺ , Mn, Ni, K ⁺ , Na ⁺ , Sr, Zn	1 - 3 % for main elements, 5 - 20 % relative for trace elements	DIN EN ISO 11885:2009
water – suspended solids	cooled transport in PE bottles; centrifugation	digestion with aqua regia ICP-OES 2000	Al, As, Cd, Ca ²⁺ , Cr, Co, Cu, Fe ²⁺ , Pb, Mg ²⁺ , Mn, Ni, K ⁺ , Na ⁺ , Sr, Zn	1 - 3 % for main elements,	DIN EN 16174:2012-11 DIN EN ISO 11885:2009

	(15 min at 10,000 min ⁻¹) filtration (membrane cellulose acetate filter, 0.45 µm) sample of > 0.5 g			5 - 20 % relative for trace elements	
sediment	transport; homogenization, drying for 24 h at 45 °C, sieving: 2 mm, 1 mm, 0.063 mm				DIN EN ISO 66165- 2:2016-08
sediment	transport; homogenization, drying for 24 h at 45 °C, sieving: 2 mm, 1 mm, 0.063 mm	grain size distribution Beckman Coulter LS 13 320 laser diffractometer	grain sizes: clay, silt, sand		ISO 13320:2009-10 DIN EN ISO 14688- 1:2018-05
sediment fraction 1-2 mm	sieving	pH-value and EC measurement	pH EC		DIN EN 15933:2012-11 DIN EN 27888:1993-11
sediment fraction 1-2 mm	sieving	determination of TC LECO TruSpec Elemental Determinator	TC		DIN EN 15936:2012-11
sediment fraction 1-2 mm	sieving	determination of TIC Carmhograph C16	TIC		DIN EN 15936:2012-11
sediment fraction 0.063- 1 mm	wet sieving	digestion with aqua regia ICP-OES 2000	Al, As, Cd, Ca ²⁺ , Cr, Co, Cu, Fe ²⁺ , Pb, Mg ²⁺ , Mn, Ni, K ⁺ , Na ⁺ , Sr, Zn	1 - 3 % for main elements, 5 - 20 % relative for trace elements	DIN EN 16174:2012-11 DIN EN ISO 11885:2009
sediment fraction < 0.063 mm	wet sieving	digestion with aqua regia ICP-OES 2000	Al, As, Cd, Ca ²⁺ , Cr, Co, Cu, Fe ²⁺ , Pb, Mg ²⁺ , Mn, Ni, K ⁺ , Na ⁺ , Sr, Zn	1 - 3 % for main elements, 5 - 20 % relative for trace elements	DIN EN 16174:2012-11 DIN EN ISO 11885:2009

Supplementary data 3.2

General information of the samples: table differentiated by sampling site.

water

name	system	region	location	classification	water level			water depth [m]		
					2017	2018	2019	2017	2018	2019
1	Oshana	Oshana region	0.15 m surface water	rural	water	water	water	0.4	> 0.7	0.6
2	Oshana	Oshana region	0.15 m surface water	rural	water	dry	dry	> 0.7	//	//
3	Oshana	Oshana region	0.15 m surface water	rural	water	water	dry	1.0	1.4	//
4	Calueque-Oshakati canal	Oshana region	0.15 m surface water	rural	water	water	water	0.5	> 0.7	> 0.7
5	Oshana	Omusati region	0.15 m surface water	rural	water	water	dry	> 0.7	> 0.7	//
6	Calueque-Oshakati canal	Omusati region	0.15 m surface water	rural	water	water	water	> 0.7	> 0.7	> 0.7
7	Oshana	Omusati region	0.15 m surface water	rural	water	water	water	> 0.7	> 0.7	0.3
8	Calueque-Oshakati canal	Omusati region	0.15 m surface water	rural	water	water	water	> 0.7	> 0.7	> 0.7
9	Oshana	Omusati region	0.15 m surface water	rural	water	water	water	> 0.7	> 0.7	0.6
10	Calueque-Oshakati canal	Omusati region	0.15 m surface water	rural	water	water	water	1.2	> 0.7	> 0.7
11	Oshana	Oshana region	0.15 m surface water	rural	water	dry	dry	> 0.7	//	//
12	Oshana	Omusati region	0.15 m surface water	rural	water	dry	dry	> 0.7	//	//
13	Oshana	Omusati region	0.15 m surface water	rural	water	water	dry	> 0.7	0.3	//
14	Oshana	Omusati region	0.15 m surface water	rural	water	water	dry	> 0.7	> 0.7	//
15	Oshana	Oshana region	0.15 m surface water	rural	water	water	water	> 0.7	> 1.9	> 0.8
16	Oshana	Omusati region	0.15 m surface water	rural	water	water	dry	> 0.7	> 1.4	//
17	Oshana	Omusati region	0.15 m surface water	rural	water	water	dry	0.5	1	//
18	Oshana	Omusati region	0.15 m surface water	rural	water	water	dry	> 0.7	> 0.7	//
19	Oshana	Omusati region	0.15 m surface water	rural	water	water	water	> 0.7	> 0.7	> 0.7
20	Calueque-Oshakati canal	Omusati region	0.15 m surface water	rural	water	water	water	> 0.7	> 0.7	> 0.7
21	Calueque-Oshakati canal	Omusati region	0.15 m surface water	rural	water	water	water	> 2.5	0.5	> 0.7
22	Oshana	Omusati region	0.15 m surface water	rural	water	water	dry	> 0.7	0.2	//
23	Oshana	Omusati region	0.15 m surface water	rural	water	water	water	> 0.7	> 0.7	0.7
24	Calueque-Oshakati canal	Omusati region	0.15 m surface water	rural	water	water	water	> 1.5	> 0.7	> 0.7

25	Calueque-Oshakati canal	Oshana region	0.15 m surface water	rural	//	water	water	//	> 0.7	> 0.7
26	Calueque-Oshakati canal	Oshana region	0.15 m surface water	rural	//	water	water	//	> 0.7	> 0.7
27	Oshana	Oshana region	0.15 m surface water	rural	//	//	water	//	//	> 0.7
29	Oshana	Oshana region	0.15 m surface water	rural	//	//	water	//	//	> 0.9
TW Ongwediva	water supply	Oshana region	//	urban	water	//	//	//	//	//
TW Ruacana	water supply	Omusati region	//	urban	water	//	//	//	//	//
Precipitation	//	Oshana region	//	urban	//	water	//	//	//	//
Precipitation	//	Oshana region	//	urban	//	water	//	//	//	//
Precipitation	//	Oshana region	//	urban	//	water	//	//	//	//
Precipitation	//	Oshana region	//	urban	//	water	//	//	//	//

// = no measurement possible

sediments

name	system	region	location	classification	water level
3	Oshana	Oshana region	50 - 300 mm of surface	rural	dry
4	Calueque-Oshakati canal	Oshana region	50 - 200 mm of surface	rural	water
5	Oshana	Omusati region	50 - 300 mm of surface	rural	water
7	Oshana	Omusati region	50 - 300 mm of surface	rural	water
10	Calueque-Oshakati canal	Omusati region	50 - 200 mm of surface	rural	water
11	Oshana	Omusati region	50 - 300 mm of surface	rural	water
12	Oshana	Oshana region	50 - 300 mm of surface	rural	water
13	Oshana	Omusati region	50 - 300 mm of surface	rural	dry
14	Oshana	Omusati region	50 - 300 mm of surface	rural	water
15	Oshana	Oshana region	50 - 300 mm of surface	rural	water
16	Oshana	Omusati region	50 - 300 mm of surface	rural	dry
17	Oshana	Omusati region	50 - 300 mm of surface	rural	dry
18	Oshana	Omusati region	50 - 300 mm of surface	rural	dry
22	Oshana	Omusati region	50 - 300 mm of surface	rural	water

23	Oshana	Omusati region	50 - 300 mm of surface	rural	water
27	Oshana	Oshana region	50 - 300 mm of surface	urban	water
29	Oshana	Oshana region	50 - 300 mm of surface	rural	water
30	Oshana	Oshana region	50 - 300 mm of surface	rural	dry
31	Oshana	Oshana region	50 - 300 mm of surface	rural	dry
WO	Oshakati	Oshana region	50 - 200 mm of surface	urban	dry

Supplementary data 3.3

Further parameters of the water samples of the lishana and the Calueque-Oshakati canal (mean \pm standard deviation).

water	lishana			Calueque-Oshakati canal		
	2017	2018	2019	2017	2018	2019
TNb [mg/l]	7.77 \pm 4.58	6.39 \pm 3.05	5.81 \pm 3.31	2.07 \pm 0.86	1.85 \pm 0.69	1.56 \pm 0.39
TC [mg/l]	74.99 \pm 18.33	56.49 \pm 20.08	81.87 \pm 57.78	23.23 \pm 0.51	46.86 \pm 0.34	17.74 \pm 1.29
TIC [mg/l]	37.92 \pm 25.66	28.91 \pm 21.49	44.92 \pm 39.49	12.26 \pm 2.87	24.80 \pm 23.98	12.46 \pm 0.76
TOC [mg/l]	36.90 \pm 25.85	26.42 \pm 21.33	36.96 \pm 27.99	11.04 \pm 2.64	21.95 \pm 24.94	5.27 \pm 0.86
Eh [mV]	217.85 \pm 33.79	233.71 \pm 28.36	938.78 \pm 261.91	237.77 \pm 36.95	236.44 \pm 18.81	981.94 \pm 517.74
dissolved O₂ [mg/l]	7.8 \pm 2.4	5.3 \pm 2.7	9.5 \pm 1.8	8.4 \pm 0.8	6.9 \pm 0.1	7.4 \pm 2.6
dissolved O₂ [sat %]	94.58 \pm 34.36	89.66 \pm 19.95	120.35 \pm 25.35	100.83 \pm 11.26	83.68 \pm 2.69	103.61 \pm 7.45
chlorophyll-α [μg/l]	40.5 \pm 20.2	34.6 \pm 14.7	31.4 \pm 23.2	2.6 \pm 0.7	13.5 \pm 3.8	10.9 \pm 2.7
cyanobacteria / BGA [cells/ml]	//	7879.2 \pm 4395.8	24386.5 \pm 40974.9	//	2063.1 \pm 690.9	2152.3 \pm 591.0

// = not measured

Heavy metal concentrations (in µg/l) of the water samples of the lishana and the Calueque-Oshakati canal (mean ± standard deviation).

water [µg/l]	lishana			Calueque-Oshakati canal		
	2017	2018	2019	2017	2018	2019
As	//	17.60 ± 16.34	0.03 ± 0.04	//	3.60 ± 0.97	0.007 ± 0.003
Cd	0.44 ± 0.11	0.50 ± 0.19	< 0.001	0.40 ± 0.13	0.29 ± 0.09	< 0.001
Co	4.05 ± 3.13	6.86 ± 6.05	2.46 ± 1.38	2.82 ± 6.23	1.01 ± 1.10	0.33 ± 0.20
Cr	2.95 ± 2.67	3.94 ± 2.44	1.26 ± 0.53	1.73 ± 2.88	1.46 ± 0.59	0.86 ± 0.35
Cu	23.04 ± 35.70	7.00 ± 2.68	37.33 ± 17.19	44.67 ± 57.36	7.00 ± 5.11	< 0.001
Ni	10.48 ± 10.45	17.70 ± 16.77	4.37 ± 3.53	7.33 ± 11.22	3.61 ± 1.63	0.98 ± 0.68
Pb	2.51 ± 1.27	2.41 ± 2.52	2.12 ± 1.20	1.88 ± 0.92	1.59 ± 1.19	< 0.001
Sr	//	479.23 ± 285.03	442.84 ± 232.40	//	92.00 ± 54.45	52.90 ± 6.50
Zn	6.55 ± 5.28	16.66 ± 7.52	5.11 ± 4.39	16.51 ± 8.54	13.38 ± 9.69	3.01 ± 3.34

// = not measured

Heavy metal concentrations (in µg/g) of the suspended solids of the lishana (mean ± standard deviation).

suspended solids [µg/g]	lishana		
	2017	2018	2019
As	//	0.09 ± 0.03	0.17 ± 0.02
Cd	0.11 ± 0.02	0.06 ± 0.03	4.88 ± 6.84
Co	11.62 ± 1.09	10.30 ± 2.87	14.15 ± 1.15
Cr	63.82 ± 8.45	50.44 ± 16.96	83.72 ± 14.65
Cu	37.98 ± 10.91	30.07 ± 8.09	166.85 ± 130.95
Ni	41.26 ± 10.62	27.97 ± 7.10	52.64 ± 17.73
Pb	9.32 ± 2.03	7.02 ± 1.33	82.74 ± 81.57
Sr	//	52.97 ± 15.93	90.16 ± 32.85
Zn	111.53 ± 44.61	46.81 ± 6.61	122.79 ± 41.61

// = not measured

Supplementary data 3.4

Results (p-values) for significant differences in the water samples of the lishana and the Calueque-Oshakati canal between dry and rainy seasons (df = 2).

	2017 - 2018		2017 - 2019		2018 - 2019	
	lishana	Canal	lishana	Canal	lishana	Canal
Al	0.005	0.018	0.005	0.018	0.005	0.018
Ca²⁺	0.042	n.s.	0.042	n.s.	n.s.	0.012
Pb	0.309	0.010	0.309	0.010	0.309	0.010
Mn	0.847	0.368	0.847	0.368	0.847	0.368
Fe²⁺	0.030	0.156	0.030	0.156	0.030	0.156
Na⁺	0.513	n.s.	0.513	0.018	0.513	n.s.
K⁺	0.607	n.s.	0.607	0.004	0.607	0.004
Mg²⁺	0.311	n.s.	0.311	0.005	0.311	0.005
temp	0.042	0.102	0.042	0.102	n.s.	0.102
EC	0.847	0.156	0.847	0.156	0.847	0.156
pH	0.115	n.s.	0.115	0.066	0.115	n.s.
turbidity	0.513	0.005	0.513	0.005	0.513	0.005
NH₄⁺	0.016	n.s.	n.s.	n.s.	n.s.	0.004
Cl⁻	0.311	0.018	0.311	0.018	0.311	0.018
NO₃⁻	0.011	0.001	0.011	n.s.	n.s.	n.s.
NO₂⁻	0.115	n.s.	0.115	n.s.	0.115	0.004
PO₄³⁻	0.115	n.s.	0.115	n.s.	0.115	0.004
SO₄²⁻	0.847	0.066	0.847	0.066	0.847	0.066
F⁻	n.s.	0.002	0.015	n.s.	0.015	0.002

* n.s. = not significant

Results (p-values) for significant differences in the water samples between lishana and Calueque-Oshakati canal.

	2017	2018	2019
Al	0.0106	0.0130	0.6532
Ca²⁺	0.0236	0.0034	0.0079
Pb	0.0603	0.1078	0.0281
Mn	0.0195	0.0027	0.4807
Fe²⁺	0.0749	0.2010	0.0079
Na⁺	0.0106	0.0000	0.0079
K⁺	0.0131	0.0001	0.0032
Mg²⁺	0.0160	0.0003	0.0001
temp	1.0000	0.0166	0.5317
EC	0.0000	0.0001	0.0006
pH	0.5675	0.6821	0.0079
turbidity	0.0000	0.0158	0.2359
NH₄⁺	0.0005	0.1227	0.8093
Cl⁻	0.0000	0.0003	0.0001
NO₃⁻	0.0057	0.0472	0.0592
NO₂⁻	0.0018	0.0889	0.0076
PO₄³⁻	0.0009	0.0153	0.0233
SO₄²⁻	0.0025	0.0016	0.0013
F⁻	0.0445	0.4208	0.0093

Results (p-values) for significant differences in the suspended solids of the lishana between 2017 and 2018.

	2017 - 2018
Al	0.075
Ca²⁺	0.003
Pb	0.271
Mn	0.639
Fe²⁺	0.040

Na⁺	0.007
K⁺	0.620
Mg²⁺	0.693

Supplementary data 4.1

Reporting Guidelines Checklist (According to Cowger et al. 2020)

Components to Report in All Procedures

Materials

- All manufacturers of materials and instruments and their calibration: see “Material and methods”
- All software used and their calibration: see “Material and methods”

Quality assurance/quality control

Error propagation

- How instrumental, methodological, and/or statistical error was propagated: see “Discussion”

Replicates

- Number of replicates: 0
- How replicates were nested within samples: 0

Limit of detection

- Quantitative detection threshold: see “Material and methods”
- Plastic morphology, size, color, and polymer limitations of method: see “Discussion”
- Method of accounting for nondetects: see “Material and methods”

Blank controls

- Number of controls: 6
- Characteristics of plastics found in blanks with the same rigor as samples: see “Discussion”
- Potential sources of contamination: see “Discussion”
- Point of entry and exit to method: see “Material and methods”

Positive controls

- Morphology, size, color, and polymer type of positive controls: not applied
- Positive control correction procedure: not applied
- Point of entry and exit to method: not applied

Contamination mitigation

- Clothing policies: see “Material and methods”
- Purification technique for reagents: see “Material and methods”
- Glassware cleaning techniques: see “Material and methods”
- Containment used (e.g., laminar flow cabinet/hoods, glove bags): not applied

Data reporting

- Share raw data and analysis code as often as possible: see “Supplementary data 4.2”

Field Sampling

- Where (e.g., region) and when (e.g., date, time) the sample was collected: see “Supplementary data 4.2”
- Size (e.g., m³, kg) and composition (e.g., sediment, water, biota) of the sample: see “Supplementary data 4.2”
- Location at the site that sample was collected (e.g., 3 cm depth of surface sediment): see “Supplementary data 4.2”
- Sample device dimensions and deployment procedures: see “Material and methods”
- Environmental or infrastructure factors that may affect the interpretation of results: see “Discussion”
- How samples are stored and transported: see “Material and methods”

Sample Preparation

Homogenization

- Homogenization technique: see “Material and methods”

Splitting/subsetting

- Sample splitting/subsetting technique: see “Material and methods”

Drying

- Sample drying temperature and time: see “Material and methods”

Synthesized plastic

- Synthesized plastic polymer, molecular characteristics, size, color, texture, and shape: not applied
- Synthesized plastic synthesis technique: not applied

Fluorescent dye

- Dye type, concentration, and solvent used: see “Material and methods”
- Dye application technique: see “Material and methods”

Sieving strategy

- Sieve mesh size: not applied
- If the sample was wet or dry sieved: not applied

Density separation

- Concentration, density, and composition (e.g., CaCl₂, ZnCl) of solution: see “Material and methods”
- Time of separation: see “Material and methods”
- Device used: see “Material and methods”

Digestion

- Duration and temperature of digestion: not applied

- Digestion solution composition: not applied
- Ratio of digestion fluid to sample: not applied

Filtration

- Filter composition, porosity, diameter: see “Material and methods”

Microplastic Identification

Visual identification

- Imaging settings
 - Image settings (e.g., contrast, gain, saturation, light intensity): not applied
 - Magnification (e.g., scale bar, 50X objective): not applied
- Light microscopy
 - Magnification used during identification: see “Material and methods”
 - Shapes, colors, textures, and reflectance, used to differentiate plastic: see “Material and methods”
- Fluorescence microscopy
 - Magnification used during identification: not applied
 - Fluorescence light wavelength, intensity, and exposure time to light source: not applied
 - Threshold intensity used to identify plastic: not applied
- Scanning electron microscopy (SEM)
 - The coating used (e.g., metal type, water vapor): not applied
 - Magnification used during identification: not applied
 - Textures used to differentiate plastic: not applied

Chemical identification

- Pyrolysis gas chromatography mass spectrometry (py-GC/MS)
 - Pyrolysis reacting gases, temperature, duration: not applied
 - GC oven program, temperature, carrier gas, and column characteristics: not applied
 - MS ionization voltage, mass range, scanning frequency, temperature: not applied
 - Py-GC/MS matching criteria (i.e., match threshold, linear retention indices (LRI), and Kovats index): not applied
 - Py-GC/MS quantification techniques: not applied
- Raman spectroscopy
 - Acquisition parameters (i.e., laser wavelength, hole diameter, spectral resolution, laser intensity, number of accumulations, time of spectral acquisition): not applied
 - Pre-processing parameters (i.e., spike filter, smoothing, baseline correction, data transformation): not applied
 - Spectral matching parameters (i.e., spectral library source, range of spectral wavelengths used to match, match threshold, matching procedure): not applied
- Fourier-transform infrared spectroscopy (FT-IR)

-
- Acquisition parameters (i.e., mode of spectra collection, accessories, crystal type, background recording, spectral range, spectral resolution, number of scans): see “Material and methods”
 - Pre-processing parameters (i.e. Fourier-transformation (FT) parameters, smoothing, baseline correction, data transformation): see “Material and methods”
 - Matching parameters (i.e., FT-IR spectral library source, match threshold, matching procedure, range of spectra used to match): see “Material and methods”
 - Differential scanning calorimetry (DSC)
 - Acquisition parameters (i.e., temperature, time, number of cycles): not applied
 - Matching parameters (i.e., parameters assessed, reference library source, comparison technique): not applied

Microplastic Categorization

- Shape, size, texture, color, and polymer category definitions: see “Material and methods”

Microplastic Quantification

- Units (e.g., kg, count, mm): see “Material and methods”
- Size dimensions (e.g., Feret minimum or maximum): not applied
- Quantification techniques: see “Material and methods”

Toxicology Considerations

- Dosed plastic age, polymer, size, color, and shapes: not applied
- Animal husbandry: not applied
- Exposure concentration, media, and time: not applied
- Effects evaluation metrics (e.g., what markers were evaluated?): not applied
- Biota metrics (e.g., which tissues were analyzed?): not applied

Supplementary data 4.2

General information: table differentiated by sampling site

	date	time	region	composition	weight [kg]	location	classification	waterlevel
perennial rivers								
Kunene	06.04.2019	03:00 pm	Kunene region	sediment	1230.4	50 - 300 mm of surface	urban	water
Kavango1	12.04.2019	11:00 am	Kavango region	sediment	1082.9	50 - 300 mm of surface	rural	water
Kavango2	12.04.2019	11:00 am	Kavango region	sediment	1239.1	50 - 300 mm of surface	urban	water
Oranje	15.04.2021	10:30 am	Karas region	sediment	1225.6	50 - 300 mm of surface	urban	water
ephemeral rivers								
Avis River	20.04.2021	02:30 pm	Khomas region	sediment	1041.7	50 - 300 mm of surface	rural	dry
Fish River	13.04.2021	03:30 pm	Karas region	sediment	1147.7	50 - 300 mm of surface	rural	water
Kuiseb	12.12.2019	03:00 pm	Erongo region	sediment	1926.1	50 - 300 mm of surface	rural	water
Olifantes	13.04.2021	10:30 am	Hardap region	sediment	875.3	50 - 300 mm of surface	urban	dry
Omaruru	04.04.2021	02:00 pm	Erongo region	sediment	1270.9	50 - 300 mm of surface	rural	dry
Owambo	02.04.2021	01:00 pm	Oshikoto region	sediment	820.0	50 - 300 mm of surface	rural	dry
Swakop1	26.03.2021	10:30 am	Otjozondjupa region	sediment	834.3	50 - 300 mm of surface	urban	water
Swakop2	05.04.2021	03:30 pm	Erongo region	sediment	1359.0	50 - 300 mm of surface	rural	dry

Tsauchab1	17.04.2021	03:00 pm	Hardap region	sediment	814.5	50 - 300 mm of surface	rural	dry
Tsauchab2	17.04.2021	03:00 pm	Hardap region	sediment	1228.0	50 - 300 mm of surface	rural	dry
Ugab1	04.04.2021	03:30 pm	Kunene region/Erongo region	sediment	1216.5	50 - 300 mm of surface	rural	dry
Ugab2	04.04.2021	03:30 pm	Kunene region/Erongo region	sediment	1287.7	50 - 300 mm of surface	rural	dry
lishana system								
Oshana 3	30.03.2019	10:30 am	Oshana region	sediment	1322.3	50 - 300 mm of surface	rural	dry
Oshana 7	02.04.2019	11:00 am	Omusati region	sediment	1425.6	50 - 300 mm of surface	rural	water
Oshana 9	30.03.2021	11:00 am	Omusati region	sediment	1145.4	50 - 300 mm of surface	rural	water
Oshana 11	29.03.2021	12:30 pm	Oshana region	sediment	1224.7	50 - 300 mm of surface	rural	water
Oshana 12	30.03.2019	01:30 pm	Omusati region	sediment	1145.4	50 - 300 mm of surface	rural	dry
Oshana 14	29.03.2021	10:30 am	Omusati region	sediment	1159.9	50 - 300 mm of surface	rural	water
Oshana 15	28.03.2021	11:00 am	Oshana region	sediment	1172.3	50 - 300 mm of surface	rural	water
Oshana 16	31.03.2019	11:00 am	Omusati region	sediment	1070.5	50 - 300 mm of surface	rural	dry
Oshana 18	31.03.2019	12:00 pm	Omusati region	sediment	1084.5	50 - 300 mm of surface	rural	dry
Oshana 19	30.03.2021	02:00 pm	Omusati region	sediment	1283.0	50 - 300 mm of surface	rural	water
Oshana 23	30.03.2021	03:00 pm	Omusati region	sediment	1269.6	50 - 300 mm of surface	rural	water
Oshana 28	02.04.2019	02:00 pm	Oshana region	sediment	1233.7	50 - 300 mm of surface	urban	water

Oshana 32	15.04.2019	02:00 pm	Oshana region	sediment	1177.3	50 - 300 mm of surface	rural	water
Oshana 34	09.04.2019	03:00 pm	Oshana region	sediment	1277.6	50 - 300 mm of surface	rural	dry

Supplementary data 4.3

MP data: table differentiated by sampling site

	particles in total	particles / kg	polymer type						fraction				
			PE [%]	PP [%]	PS [%]	PA [%]	PMMA [%]	others [%]	5 mm	1 mm	0.5 mm	0.3 mm	
perennial rivers													
Kunene	2	1.8	0.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	50.0	50.0
Kavango1	5	6.9	0.0	80.0	0.0	0.0	0.0	0.0	20.0	0.0	0.0	40.0	60.0
Kavango2	3	2.4	33.3	66.7	0.0	0.0	0.0	0.0	0.0	0.0	33.3	33.3	33.3
Oranje	2	1.6	50.0	50.0	0.0	0.0	0.0	0.0	0.0	0.0	50.0	50.0	0.0
ephemeral rivers													
Avis River	15	14.4	26.7	6.7	40.0	6.7	6.7	13.3	0.0	6.7	13.3	80.0	80.0
Fish River	3	2.6	0.0	33.3	33.3	0.0	0.0	33.3	0.0	0.0	33.3	66.7	66.7
Kuiseb	39	20.3	7.7	10.3	76.9	0.0	2.6	2.6	0.0	15.4	48.7	35.9	35.9
Olifantes	34	38.8	70.6	8.8	0.0	0.0	14.7	5.9	0.0	23.5	44.1	32.4	32.4
Omaruru	11	8.7	63.6	9.1	0.0	0.0	9.1	18.2	0.0	0.0	63.6	36.4	36.4
Owambo	54	65.9	48.1	37.0	7.4	0.0	5.6	1.9	0.0	38.9	27.8	33.3	33.3
Swakop1	2	2.4	100.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0	0.0	0.0
Swakop2	2	1.5	50.0	0.0	0.0	0.0	0.0	50.0	0.0	50.0	0.0	50.0	50.0
Tsauchab1	11	13.5	81.8	0.0	0.0	0.0	0.0	18.2	0.0	45.5	9.1	45.5	45.5
Tsauchab2	2	1.6	0.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0	0.0	0.0
Ugab1	1	0.8	0.0	0.0	0.0	0.0	0.0	100.0	0.0	0.0	0.0	100.0	100.0
Ugab2	0	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Ishana system													
Oshana 3	5	3.8	60.0	40.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	20.0	80.0
Oshana 7	14	9.8	71.4	28.6	0.0	0.0	0.0	0.0	0.0	28.6	28.6	42.9	42.9

Oshana 9	11	9.6	54.5	9.1	0.0	27.3	0.0	9.1	0.0	18.2	54.5	27.3
Oshana 11	6	4.9	0.0	83.3	16.7	0.0	0.0	0.0	0.0	33.3	16.7	50.0
Oshana 12	30	26.2	93.3	3.3	3.3	0.0	0.0	0.0	3.3	30.0	36.7	30.0
Oshana 14	1	0.9	0.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0
Oshana 15	4	3.4	75.0	0.0	0.0	0.0	0.0	25.0	0.0	50.0	50.0	0.0
Oshana 16	46	43	78.3	17.4	2.2	2.2	0.0	0.0	0.0	23.9	47.8	28.3
Oshana 18	57	53	93.0	7.0	0.0	0.0	0.0	0.0	0.0	24.6	70.2	5.3
Oshana 19	9	7	22.2	44.4	11.1	0.0	0.0	22.2	0.0	22.2	44.4	33.3
Oshana 23	0	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Oshana 28	16	13	81.3	18.8	0.0	0.0	0.0	0.0	0.0	6.3	56.3	37.5
Oshana 32	18	15.3	50.0	33.3	11.1	0.0	0.0	5.6	0.0	33.3	16.7	50.0
Oshana 34	7	5.5	28.6	71.4	0.0	0.0	0.0	0.0	0.0	0.0	28.6	71.4

	shape					color										
	fragments [%]	films [%]	fibers [%]	fiber bundles [%]	pellets [%]	transparent [%]	white [%]	yellow [%]	black [%]	blue [%]	brown [%]	grey [%]	green [%]	pink [%]	red [%]	orange [%]
perennial rivers																
Kunene	50.0	0.0	50.0	0.0	0.0	0.0	50.0	0.0	0.0	0.0	50.0	0.0	0.0	0.0	0.0	0.0
Kavango1	100.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0	0.0	0.0	0.0	0.0	0.0
Kavango2	66.7	33.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	33.3	66.7	0.0	0.0	0.0	0.0	0.0
Oranje	50.0	0.0	50.0	0.0	0.0	0.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
ephemeral rivers																
Avis River	86.7	6.7	6.7	0.0	0.0	33.3	13.3	6.7	26.7	0.0	20.0	0.0	0.0	0.0	0.0	0.0
Fish River	66.7	0.0	33.3	0.0	0.0	66.7	0.0	0.0	33.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Kuiseb	94.9	0.0	5.1	0.0	0.0	2.6	2.6	0.0	92.3	2.6	0.0	0.0	0.0	0.0	0.0	0.0
Olifantes	79.4	8.8	11.8	0.0	0.0	41.2	11.8	5.9	8.8	8.8	20.6	0.0	0.0	0.0	2.9	0.0
Omaruru	36.4	54.5	9.1	0.0	0.0	63.6	27.3	0.0	0.0	0.0	9.1	0.0	0.0	0.0	0.0	0.0
Owambo	98.1	1.9	0.0	0.0	0.0	24.1	16.7	0.0	7.4	31.5	11.1	0.0	1.9	1.9	3.7	1.9

Swakop1	50.0	0.0	50.0	0.0	0.0	50.0	50.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Swakop2	100.0	0.0	0.0	0.0	0.0	0.0	50.0	0.0	50.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Tsauchab1	72.7	27.3	0.0	0.0	0.0	36.4	0.0	9.1	9.1	0.0	36.4	0.0	0.0	0.0	9.1	0.0
Tsauchab2	50.0	0.0	0.0	50.0	0.0	0.0	50.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	50.0	0.0
Ugab1	0.0	100.0	0.0	0.0	0.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Ugab2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
lishana system																
Oshana 3	80.0	20.0	0.0	0.0	0.0	20.0	0.0	0.0	0.0	0.0	80.0	0.0	0.0	0.0	0.0	0.0
Oshana 7	71.4	21.4	0.0	0.0	7.1	7.1	0.0	0.0	0.0	0.0	71.4	14.3	7.1	0.0	0.0	0.0
Oshana 9	9.1	72.7	18.2	0.0	0.0	45.5	45.5	0.0	9.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Oshana 11	83.3	16.7	0.0	0.0	0.0	66.7	33.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Oshana 12	43.3	50.0	6.7	0.0	0.0	40.0	3.3	3.3	0.0	0.0	20.0	20.0	0.0	13.3	0.0	0.0
Oshana 14	100.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0
Oshana 15	100.0	0.0	0.0	0.0	0.0	0.0	50.0	25.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	25.0
Oshana 16	28.3	69.6	2.2	0.0	0.0	71.7	0.0	0.0	0.0	0.0	26.1	2.2	0.0	0.0	0.0	0.0
Oshana 18	82.5	12.3	1.8	0.0	3.5	28.1	8.8	1.8	5.3	5.3	10.5	38.6	0.0	1.8	0.0	0.0
Oshana 19	55.6	44.4	0.0	0.0	0.0	22.2	44.4	0.0	0.0	22.2	11.1	0.0	0.0	0.0	0.0	0.0
Oshana 23	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Oshana 28	81.3	12.5	6.3	0.0	0.0	18.8	0.0	0.0	0.0	6.3	68.8	6.3	0.0	0.0	0.0	0.0
Oshana 32	66.7	22.2	11.1	0.0	0.0	0.0	16.7	0.0	0.0	5.6	77.8	0.0	0.0	0.0	0.0	0.0
Oshana 34	71.4	0.0	28.6	0.0	0.0	0.0	28.6	0.0	0.0	0.0	71.4	0.0	0.0	0.0	0.0	0.0

Supplementary data 5.1

General information of the samples: table differentiated by sampling site

name	system	water depth [m]	depth of view [m]	color	temperature [°C]
site 7	lishana	0.2	0.07	dark grey	22.44
site 15	lishana	> 0.8	0.40	grey	24.52
site 23	lishana	0.5	0.07	green, yellow	32.11
site 27	lishana	> 0.7	0.08	grey	28.37
site 32	lishana	> 0.9	0.09	green	25.98
site 29	Kunene	> 0.7	0.02	grey	32.15
site 30	Kavango	> 0.7	> 0.70	transparent	31.27
site 31	Kavango	> 0.7	> 0.70	transparent	31.14

Supplementary data 5.2

Observed sublethal and lethal effects of zebrafish embryos. Data represent mean and standard deviation of % effects observed during acute fish embryo toxicity test.

	hours post fertilisation (hpf)								
	24 hpf			48 hpf			72 hpf		
	lishana	Kunene	Kavango	lishana	Kunene	Kavango	lishana	Kunene	Kavango
development retardations & failures									
lack of somite formation	--	--	1.7 ± 3.7	--	--	--	--	--	--
lack of tail detachment	0.7 ± 2.5	--	1.7 ± 3.7	--	--	--	--	--	--
missing spontaneous movements	2 ± 5.4	--	--	6 ± 9.5	10 ± 14.1	15 ± 21.4	--	--	--
embryo malformation	--	--	1.7 ± 3.7	--	--	1.7 ± 3.7	--	--	3.3 ± 4.7
impaired/missing heart-beat	--	--	--	--	--	1.7 ± 3.7	--	--	1.7 ± 3.7
impaired/missing blood flow	--	--	--	--	--	3.3 ± 4.7	--	--	3.3 ± 4.7
blood congestion	--	--	--	--	--	--	0.7 ± 2.5	10 ± 8.2	--
pericardium edema	--	--	--	14.7 ± 12.6	10 ± 8.2	10 ± 5.8	24 ± 11.4	23.3 ± 4.7	23.3 ± 12.5
yolk sack edema	--	--	--	5.3 ± 7.2	10 ± 8.2	3.3 ± 4.7	9.3 ± 10.6	--	8.3 ± 6.9
spine deformation	--	--	--	2 ± 5.4	--	--	--	--	--
impaired pectoral fin development	--	--	--	--	--	--	1.3 ± 3.4	--	--
fin deformation (no fin mobility)	--	--	--	--	--	--	--	--	--
damaged chorion	--	--	--	0.7 ± 2.5	--	--	--	--	--
chorion deformation	0.7 ± 2.5	--	--	14.7 ± 30.3	--	--	14 ± 29.6	--	--
unnatural swimming behavior	--	--	--	--	--	--	--	--	--
hatching									
hatched	--	--	--	--	--	--	2 ± 4.0	--	--
not hatched	--	--	--	--	--	--	94 ± 6.1	90 ± 0	90 ± 0
lethality									
coagulation	3.3 ± 4.7	10 ± 8.2	10 ± 11.5	4 ± 6.1	10 ± 8.2	10 ± 11.5	4 ± 6.1	10 ± 8.2	10 ± 11.5

Table continued

	96 hpf			120 hpf		
	lishana	Kunene	Kavango	lishana	Kunene	Kavango
development retardations & failures						
lack of somite formation	--	--	--	--	--	--
lack of tail detachment	--	--	--	--	--	--
missing spontaneous movements	--	--	--	--	--	--
embryo malformation	--	--	3.3 ± 4.7	--	--	3.3 ± 4.7
impaired/missing heart-beat	--	--	3.3 ± 4.7	--	--	3.3 ± 4.7
impaired/missing blood flow	--	--	3.3 ± 4.7	--	--	3.3 ± 4.7
blood congestion	0.7 ± 2.5	6.7 ± 9.4		1.3 ± 5.0	--	--
pericardium edema	20.7 ± 17.3	16.7 ± 17.0	11.7 ± 10.7	10.7 ± 13.9	6.7 ± 4.7	11.7 ± 10.7
yolk sack edema	1.3 ± 3.4		3.3 ± 4.7	2.7 ± 5.7	--	3.3 ± 4.7
spine deformation	1.3 ± 3.4	3.3 ± 4.7	5 ± 7.6	0.7 ± 2.5	--	1.7 ± 3.7
impaired pectoral fin development	4 ± 10.0		3.3 ± 7.5	0.7 ± 2.5	--	1.7 ± 3.7
fin deformation (no fin mobility)	6.7 ± 10.1	3.3 ± 4.7	5 ± 7.6	6.7 ± 10.1	3.3 ± 4.7	5 ± 7.6
damaged chorion	--	--	--	--	--	--
chorion deformation	2.7 ± 10.0	--	--	--	--	--
unnormal swimming behavior	--	--	--	2 ± 5.4	--	--
hatching						
hatched	83.3 ± 13.0	80 ± 16.3	70 ± 19.1	95.3 ± 24.5	90 ± 8.2	88.3 ± 10.7
not hatched	12.7 ± 12.4	10 ± 8.2	20 ± 17.3	0.7 ± 2.5	--	1.7 ± 3.7
lethality						
coagulation	4 ± 6.1	10 ± 8.2	10 ± 11.5	4 ± 6.1	10 ± 8.2	10 ± 11.5

Supplementary data 5.3

Specific growth inhibition in % (mean \pm standard deviation) of the lishana system, the Kunene, and the Kavango Rivers.

lishana							
Conc mg/l	NC	PC	3.2	1.6	0.8	0.4	0.2
7							
growth rate (mean \pm sd) [%]	1.8 \pm 0.1	1.6 \pm 0.6	1.9 \pm 0.1	1.8 \pm 0.1	1.8 \pm 0.1	1.9 \pm 0.1	1.9 \pm 0.1
inhibition [%]	0.0	9.9	-4.9	-2.4	-3.7	-5.3	-4.8
normalized inhibition [%]	0.0	9.9	0.0	0.0	0.0	0.0	0.0
15							
growth rate (mean \pm sd) [%]	1.7 \pm 0.1	1.6 \pm 0.8	1.9 \pm 0.3	2.0 \pm 0.3	2.0 \pm 0.3	2.0 \pm 0.4	2.0 \pm 0.4
inhibition [%]	0.0	5.9	-12.5	-16.4	-14.7	-17.1	-14.8
normalized inhibition [%]	0.0	5.9	0.0	0.0	0.0	0.0	0.0
23							
growth rate (mean \pm sd) [%]	1.8 \pm 0.1	1.7 \pm 0.8	2.0 \pm 0.2	2.0 \pm 0.2	2.0 \pm 0.2	2.0 \pm 0.3	1.9 \pm 0.1
inhibition [%]	0.0	5.8	-9.7	-10.5	-11.0	-12.4	-6.4
normalized inhibition [%]	0.0	5.8	0.0	0.0	0.0	0.0	0.0
27							
growth rate (mean \pm sd) [%]	1.9 \pm 0.1	1.8 \pm 0.7	2.0 \pm 0.2	2.0 \pm 0.2	2.0 \pm 0.3	2.0 \pm 0.3	1.8 \pm 0.2
inhibition [%]	0.0	7.5	-5.4	-4.9	-5.8	-2.2	3.9
normalized inhibition [%]	0.0	7.5	0.0	0.0	0.0	0.0	3.9
32							
growth rate (mean \pm sd) [%]	1.8 \pm 0.1	1.7 \pm 0.6	2.0 \pm 0.3	2.0 \pm 0.3	2.0 \pm 0.4	2.0 \pm 0.4	2.0 \pm 0.4
inhibition [%]	0.0	4.8	-8.1	-9.1	-11.5	-9.9	-9.2

normalized inhibition [%]	0.0	4.8	0.0	0.0	0.0	0.0	0.0
Perennial rivers							
Conc mg/l	NC	PC	3.2	1.6	0.8	0.4	0.2
29							
growth rate (mean ± sd) [%]	1.9 ± 0.1	1.6 ± 0.6	1.9 ± 0.2	1.9 ± 0.3	1.9 ± 0.2	1.9 ± 0.3	1.9 ± 0.3
inhibition [%]	0.0	16.9	-0.3	-0.9	2.2	0.1	0.0
normalized inhibition [%]	0.0	16.9	0.0	0.0	2.2	0.1	0.0
30							
growth rate (mean ± sd) [%]	1.9 ± 0.1	1.7 ± 0.5	1.9 ± 0.2	1.9 ± 0.2	2.0 ± 0.3	1.9 ± 0.3	2.0 ± 0.3
inhibition [%]	0.0	10.3	-3.0	-3.0	-5.7	-3.3	-5.0
normalized inhibition [%]	0.0	10.3	0.0	0.0	0.0	0.0	0.0
31							
growth rate (mean ± sd) [%]	1.9 ± 0.1	1.5 ± 0.7	1.9 ± 0.3	1.9 ± 0.3	1.9 ± 0.3	1.8 ± 0.3	1.8 ± 0.3
inhibition [%]	0.0	17.4	-2.7	-2.2	-1.7	1.3	1.2
normalized inhibition [%]	0.0	17.4	0.0	0.0	0.0	1.3	1.2

Supplementary data 5.4

Mutagenic potential of the samples (Ishana and perennial rivers) based on a visible reproducible increase in revertant counts at single dilution steps referred to the revertant number in the negative control. Significant differences (p-values) are presented in grey and visible differences in white. All samples were tested in six dilution steps and three independent replicates.

	Ishana					Kunene Kavango		
	7	15	23	27	32	29	30	31
TA98-	0.18	0.08	0.003	0.02	0.14	0.02	0.008	0.002
TA98+	0.4	0.02	0.0002	0.03	0.008	0.05	0.06	0.08
TA100-	0.18	0.08	0.29	0.08	0.17	0.08	0.08	0.08
TA10+	0.18	0.18	0.18	0.57	0.58	0.57	0.18	0.18

Supplementary data 5.5

Limit of Detection (LOD) and Limit of Quantification (LOQ) of the YES assay for all samples.

	Replicate 1		Replicate 2		Replicate 3	
	LOD [ng/l]	LOQ [ng/l]	LOD [ng/l]	LOQ [ng/l]	LOD [ng/l]	LOQ [ng/l]
site 7	3.4	4.7	34.6	54.7	4.6	10.2
site 15	0.9	2.8	19.2	31.3	1.6	4.1
site 23	0.9	2.8	19.2	31.3	1.6	4.1
site 27	6.1	8.5	40.9	54.4	2.8	5.7
site 32	27.2	33.3	23.7	40.4	2.5	4.0
site 29	6.1	8.5	40.9	54.4	2.8	5.7
site 30	31.5	42.2	23.1	38.1	4.3	10.6
site 31	31.5	42.2	23.1	38.1	4.3	10.6