DEVELOPING TAXONOMIC AND TRAIT-BASED APPROACHES FOR ASSESSING AND PREDICTING MACROINVERTEBRATE RESPONSES TO ELEVATED FINE SEDIMENTS IN THE TSITSA RIVER AND ITS TRIBUTARIES, SOUTH AFRICA

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PHINDIWE NTLOKO

RHODES UNIVERSITY
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ABSTRACT

Sedimentation of freshwater systems is one of the leading causes of water quality deterioration. The Mzimvubu River catchment, which includes the Tsitsa River and its tributaries, in the Eastern Cape is prone to elevated sediment impact due to dispersive soils that are easily erodible. In this study, taxonomy and trait-based approaches were used to assess the responses of macroinvertebrates to fine sediments in the Tsitsa River and its tributaries. Macroinvertebrates and environmental variables were sampled seasonally in winter, spring, summer and autumn of 2016 to 2018 in six selected sites, using the South African Scoring System version 5 as a collecting protocol.

The sites were selected to represent a decreasing gradient of sediment influence from the highly impacted Sites 1 (Tsitsa upstream) 2 (Tsitsa downstream), and 3 (Qurana River) to moderately impacted Sites 4 (Millstream upstream) and 5 (Millstream downstream) and the least impacted Sites 6 (Pot River upstream), 7 (Little Pot River) and 8 (Pot River downstream), which were collectively referred to as the control sites. Analysis of basic physico-chemical variables, dissolved oxygen, pH, electrical conductivity, turbidity, total suspended solids, temperature and nutrients were undertaken seasonally over the study period. Sediments grain sizes were also analysed. All collected data were subjected to appropriate statistical tests – univariate and multivariate techniques. A fine-sediment-specific multimetric index was developed to monitor the impact of fine sediments on macroinvertebrate assemblages of the Tsitsa River and its tributaries. A total of 12 traits, resolved into 48 trait attributes, were selected to explore their distribution in relation to a fine-sediment stress gradient, and identify the trait-based signature of fine-sediment impact. A trait-based approach was then developed to classify South African macroinvertebrates into two groups: taxa that are potentially vulnerable to fine-sediment impact and those potentially resilient, based on the combination of traits possessed.

Two-way analysis of variance (ANOVA) indicated that electrical conductivity, turbidity, embeddedness and total suspended solids were statistically significantly different between the sites. Apart from Dissolved oxygen, the remaining variables were statistically significantly lower at the control sites ($P < 0.05$). The two-way multivariate analysis of variance (MANOVA) indicated global significant differences between sites and seasons. The two-way MANOVA also revealed that the interaction between the sites and seasons were statistically significant. The MANOVA indicated global combined interactive effects across the sites for suspended fine-sediment grain sizes, two-way ANOVA, followed by a Tukey's post-hoc test,

was carried out to indicate where the significant differences lay. The one-way ANOVA results indicated that very fine sand, very coarse silt, medium silt, and fine silt were significantly higher at Tsitsa upstream, Tsitsa downstream, Qurana tributary that is at Millstream upstream, Millstream downstream and Control sites. The rest of the grain sizes did not differ statistically between the sites. In terms of the settled sediment grain sizes, the volumetric analysis did not show considerable differences across the sites. Settled fine-sediment grain sizes were evenly distributed across the sites. Statistically, MANOVA results indicated no significant differences across sites or across seasons.

The developed Sediment Multimetric Index indicated that the sites in the Tsitsa River and those in the Qurana River were highly sedimented during the wet season, but became moderately sedimented during the dry season, indicating that the index responded to seasonality. The sediment multimetric index indicated that the control sites were less sedimented during both the wet season and dry seasons, suggesting minimal seasonal effects at the control sites.

Traits such as an exposed and soft body, collector-filterers, shredding, feeding on coarse particulate organic matter and a high sensitivity to dissolved oxygen were identified as fine-sediment-sensitive indicator traits. Identified fine-sediment-tolerant traits and ecological preferences included complete sclerotisation, a cased/tubed body, a preference for fine particulate organic matter, a high tolerance to dissolved oxygen depletion, and climbing and skating behaviours.

Regarding the trait-based approach followed for classifying macroinvertebrates into vulnerable taxa and resilient taxa, the results revealed that the relative abundance and richness of the vulnerable taxa decreased predictably along the increasing gradient of sediment impact. However, the relative abundance and richness of resilient taxa showed no marked response to the impact of an increasing gradient of fine sediments. Overall, the present study makes a contribution to the complementary application of trait-and taxonomy-based approaches to freshwater biomonitoring. The trait-based approach enables predictions to be made and tested based on the mechanistic understanding of the mediating roles of traits in organism-environment interaction.

A fundamental challenge, which showcases the limitation of the current study, is the sparse trait data on Afrotropical macroinvertebrates at the species or generic levels. In this regard, the trait-based approaches developed here were the family level instead of species or genus. This is the first study in South Africa to develop explicit trait-based indicators of elevated fine sediments as well as an approach for predicting macroinvertebrate vulnerability and resilience to fine-sediment effects, thus advancing the science and practice of freshwater biomonitoring.

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ANOVA	Analysis of variance
APHA	American Public Health Association
ASPT	Average score per taxon
AUSRIVAS	Australian River Assessment System
BMWP	Biological monitoring working for the party
CA	Correspondence Analysis
CCA	Canonical correspondence analysis
CLS	Control sites
CPOM	Coarse particulate organic matter
DCA	Detrended Canonical Analysis
DO	Dissolved oxygen
DWAF	Department of Water Affairs
DWS	Department of Water and Sanitation
EC	Electrical Conductivity
EPOT	Ephemeroptera, Plecoptera, Odonata and Trichoptera
EPT	Ephemeroptera, Plecoptera, and Trichoptera
EWQ	Environmental Water Quality
FPOM	Fine Particulate Organic Matter
FR	Functional redundancy
FTD	Functional Trait Diversity
GSM	Gravel Sand and Mud
HBI	Hilsenhoff's Biotic Index
HFC	Habitat template concept Index
HIS	Highly Impacted Site
HTC	Habitat Template Concept
IHAS	Integrated Habitat Assessment System Interquartile
IBI	Index of Biotic Integrity

IWRM	Integrated water resources management
LIS	Least Impacted Site
MANOVA	Multivariate analysis of variance
MIS	Moderately Impacted Site
MM1	Multimetric index
MLU	Millstream upstream
MLD	Millstream downstream
NWA	National Water Act
NWRS	National Water Resource Strategy
PCA	Principal Component Analysis
PSI	Proportion of Sensitive invertebrates Index
QHR	Qurana River
RLQ	Environmental variable: (R), macroinvertebrates taxa (L) and traits (Q)
RDA	Redundancy Analysis
RDM	Resource Directed Measures
RIVPACS	River Invertebrate Prediction and Classification System
RQOs	Resource Quality Objectives
RT	Resilient Taxa
SASS5	South African Scoring System Version 5
SDC	Source-directed controls
SIC	Stone-in-current
SOOC	Stone-out-of-current
SOMI	Serra dos Orgaos Multimetric Index
SMMI	Sediment Multimetric Index
SWMMI	Semi-wadeable Rivers in the Mid-Atlantic Region
TBA	Traits-based approach
TIN	Total inorganic nitrogen

TSU	Tsitsa upstream
TSD	Tsitsa downstream
VS	Vulnerability Score
VT	Vulnerable Taxa

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DEDICATION

This thesis is dedicated to my late mother, Fanelwa Flora Nosiseko Ntloko, and to my beautiful daughters, Amvuyele Ntloko and Ithandile Ntloko.

CHAPTER 1: GENERAL INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

Degradation of freshwater ecosystems and biodiversity loss are areas of major concern worldwide (Grzybowski and & Glińska-Lewczuk, 2019; Di Lorenzo *et al.,* 2020). In particular, rivers are among the world's most threatened ecosystems (Sendzimir & Schmutz, 2018). Rivers suffer increasingly; human-induced factors such as urbanisation, industrialisation, and a growing human population are the main drivers of the degradation of freshwater ecosystems (Vörösmarty *et al.,* 2010; Kopf *et al.,* 2017). As a result, freshwater ecosystems experience a decline in biodiversity more than terrestrial and marine systems (UN, 2020; Van Rees *et al.,* 2021). The human population is estimated to grow to 8.6 billion by 2030, 9.8 billion by 2050, and 11.2 billion by 2100 (UN, 2017). This projected growth implies an increased demand for food and consequent increased pressure on water resources, which may manifest in the form of increased freshwater abstraction and pollution, impacting both water quantity and quality (Strayer & Dudgeon, 2010; Steffen *et al.,* 2015; Zhang *et al.,* 2017).

A critical water quality stressor is excessive input of fine sediments into freshwater ecosystems from anthropogenic activities in catchments (Larsen *et al.,* 2011; Tiecher *et al.,* 2017), primarily agricultural activities (de Castro *et al.,* 2018). Reportedly, agriculture uses about 70% of freshwater and is the largest contributor to pollution of both surface and groundwater (FAO, 2018). Countries such as the United States of America (USA), Canada, Australia and South Africa have experienced increased fine-sediment surface runoff into freshwater systems from crop production in floodplains (Le Roux & Summer, 2013; Neal & Anders, 2015). Deteriorating freshwater quality due to elevated fine-sediment inputs (Van der Merwe-Botha, 2009), impacts on ecosystem services that benefit humankind, which are essential for socio-economic development and prosperity (Whiles & Dodds, 2002; Sobota *et al.,* 2015).

Fine sediments are a natural component of river systems (Wood & Armitage, 1997) and are important for substrate composition as they play a part in the integrity of macroinvertebrate microhabitats (Wood & Armitage, 1997; Mathers *et al.,* 2019). However, levels beyond the natural backgrounds can be deleterious to freshwater biota (Walling & Fang, 2003; Conroy *et al.,* 2016; Doretto *et al*., 2017; Vercruysse *et al.,* 2017). For example, elevated levels of fine sediments in freshwater ecosystems change channel morphology, impact water flow, and increase turbidity, so limiting light penetration, decreasing habitat complexity and potentially reducing primary productivity. Consequently, they affect primary producers at the base of the food chain (Wood & Armitage, 1997; Mathers *et al.,* 2017), thereby affecting biological assemblages and ecosystem functioning (Jones *et al.,* 2012; Morwenna *et al.,* 2019).

Elevated levels of fine sediments can potentially lead to several biological and ecological effects, including clogging of gills, smothering of eggs, filling up interstitial spaces, disrupting fine feeding organs, burying less motile species, and depleting dissolved oxygen, while associated increases in turbidity can cause visual impairment and reduce light penetration (Dallas & Day 2004; Extence *et al.,* 2013; Gordon *et al.,* 2013; Turley *et al.,* 2016). Elevated fine-sediment inputs can have severe effects on biological communities, including drift due to unstable substrates, reduction of suitable habitat of some species (Schalchi, 1995; Richards & Bacon, 1994; Jones *et al.,* 2012), reduction of respiration due to silt deposition on breathing structures such as gills (Lemly, 1982), resources (i.e., food) availability (Graham, 1990), thereby affecting biological communities in freshwater systems (Yadamsuren *et al.,* 2020). Consequently, macroinvertebrate taxa decrease in density and community diversity (Yadamsuren *et al.,* 2020), due to elevated fine sediments. Fine sediments have been reported to differentially affect macroinvertebrate groups. Some studies have revealed certain taxa belonging to the orders Ephemeroptera, Plecoptera and Trichoptera (EPT) are vulnerable to sedimentation, and their species richness is reduced in fine-sediment-impacted habitats (Jones *et al.,* 2012; Beermann *et al.,* 2018). However, the abundance and richness of some species of Chironomidae have been observed to increase in fine-sediment-impacted systems because they have adaptive traits that allow them to withstand sediment impact (Kreutzweiser *et al.,* 2005).

Poor land management practices, such as cropping practices and over-grazing (Zhang *et al*., 2014; Collins *et al*., 2016), forestry operations (Nizzetto *et al*., 2016; Seiwa *et al*., 2021), road construction (Fu *et al.,* 2010; Jaafari *et al.,* 2015)) and mining (Hudson *et al*., 1997) are among the main contributors of fine sediments to river and stream systems. Landscape degradation can accelerate the input and delivery of fine sediments into stream and riverine ecosystems (Zhang *et al.,* 2017). For example, landscape degradation resulting from agricultural activities accounts for 48% of stream pollution from excessive fine-sediment loads in the USA (Sutherland *et al.,* 2012). In South Africa, for example, landscape degradation is one of the major causes of fine-sediment delivery into rivers and streams (Le Roux & Summer, 2013).

South African rivers deliver excessive amounts of fine sediment into the ocean, with the Orange River delivering the most (Gordon *et al.,* 2012). Sedimentation of South African rivers is one of the leading causes of water quality degradation (Le Roux & Summer, 2013), exacerbated by other interacting factors, such as soil erosivity, slope steepness, flow, and rainfall variabilities (Msadala *et al*., 2010). The major sources of fine-sediment load into South African rivers can be classified into in-channel and non-channel sources, where channel sources are those derived from the beds and banks of rivers and streams (Russel *et al.,* 2017). Non-channel sources originate from the wider landscape and may include activities such as logging, agricultural activities, and urban development (Le Roux *et al.,* 2008; Gordon *et al*., 2013). Over 70% of South Africa's surface area has been affected by various degrees of soil erosion (Le Roux *et al.,* 2007; Le Roux *et al.,* 2008; Collins *et al.,* 2016), making most riverine systems in South Africa, particularly in the northern part of the Eastern Cape province, vulnerable to fine sediment loads.

The Eastern Cape province of South Africa, where this study was undertaken, is currently classified as being among the areas most severely impacted by soil erosion in the country (Le Roux *et al.,* 2007; Foster *et al.,* 2017). The Mzimvubu River catchment, which includes the Tsitsa River and its tributaries, consists of highly erodible duplex soils, placing it among the highest sediment-yielding regions in South Africa (Msadala *et al*., 2010). The Tsitsa River is subject to excessive fine sediment inputs through the effects of gully erosion (Le Roux, 2013). Sediment loads derived from gully erosion and other forms of erosion, such as rill and sheet erosion, could possibly affect the overall integrity and ecosystem health of the Tsitsa River system. Although elevated fine-sediment input into the Tsitsa River and its tributaries in the Mzimvubu catchment has been suggested as the major cause of water quality and biodiversity impairment, only a few studies, such as Madikizela and Dye (2010), Gordon *et al*. (2013) and Akamagwuna *et al.* (2019) have explicitly investigated the impacts of elevated fine sediments on water quality and macroinvertebrates in this catchment

Despite elevated fine sediments being a major water quality stressor in South Africa, the extent to which fine sediments affect the taxonomic and trait-based responses of macroinvertebrates remain largely unexplored. Macroinvertebrate-based biomonitoring in South Africa relies primarily on the South African Scoring System version 5 (SASS5), which was developed based mainly on organic pollution (Dickens & Graham 2002). The SASS5 has proved ineffective in assessing the effects of fine sediments on macroinvertebrates (Gordon *et al.,* 2013), necessitating the need to develop tools responsive to and effective for monitoring the effects of fine sediments. Given that the persistence of macroinvertebrates in any environment is partly determined by the adaptive features and traits possessed, it is also important to explore and develop tools based on traits. This is important for a number of reasons: i) traits mediate the interactions between macroinvertebrates and their environments (Poff *et al.,* 2006; Kuzmanovic *et al.,* 2017), ii) the possession of the appropriate combination of traits may confer resilience on such macroinvertebrates, and the opposite may be true for other macroinvertebrates, iii) traits allow the exploration of the mechanistic basis for predicting macroinvertebrate responses to fine sediment stress, and potentially permitting the development of predictive tools based on theoretical insights. Therefore, the primary aim of this study is to investigate the taxonomic- and trait-based responses of macroinvertebrates to elevated fine sediments effects in the Tsitsa River and its tributaries. In the process, a novel trait-based predictive tool is developed, and fine-sediment trait indicators are identified in addition to taxonomic indicators for routine biomonitoring of rivers and streams impacted by elevated fine sediments. The rest of this chapter is a literature review, beginning with water resource management in South Africa and environmental water quality with a focus on fine sediments, taxonomy- and trait-based biomonitoring approaches. The Chapter concludes with the study rationale, study aim and objectives, and a description of the thesis structure.

1.2 Water resource management in South Africa

The Department of Water and Sanitation oversees water resource management and policy implementation in South Africa (Department of Water Affairs, 2016). It has a regulatory and oversight role on all matters related to water resource development, protection, conservation, management and use. This regulatory power is derived from the provisions of the National Water Act (NWA, Act No. 36 of 1998) (Republic of South Africa, 1998). The Act is the primarily legal framework that provides for the protection, use, management, conservation, control and development of water resources in South Africa. The Act is underpinned by three priority values – equity, sustainability and efficiency – that guide the protection, management, development and conservation of water resources in South Africa.

The newly developed water quality policy and strategy document identifies eutrophication, salinisation, acid mine drainage, acidification, **sedimentation** and urban runoff as priority water quality stressors in South Africa, needing urgent attention (Department of Water Affairs, 2016). These priority water quality stressors have extensively impacted South African water resources, leading to impaired ecosystem health conditions and a decline in the supply of ecosystem services (Department of Water & Sanitation, 2013).

In terms of freshwater ecosystem impairments in South Africa, of the 223 river ecosystem types, 60% are threatened, with 25% of these extremely endangered and less than 15% of river ecosystems found only within protected areas (Le Roux & Nel, 2012). Many of the threatened and endangered river systems are impaired and degraded by upstream anthropogenic activities that influence water quality (Department of Water Affairs, 2012). Furthermore, of the 792 wetland ecosystems, 65% are identified as threatened, and 48% as critically endangered (IUCN, 2009; Department of Environmental Affairs, 2014).

Because of the escalating rate at which impairment of South Africa's freshwater resources is occurring, there is a need for a concerted management effort to halt and/or reverse the current trajectory. South Africa's National Water Act (Act No. 36 of 1998) (Republic of South Africa, 1998) provides the legal basis for managing water resources in South Africa, including water quality and ecosystem health. To effect coordinated management of water resources, the Act provides for the development of a National Water Resource Strategy (NWRS).

1.2.1 The National Water Resource Strategy (NWRS)

The second edition of the National Water Resource Strategy (NWRS2) provides essential strategies and guidelines in order to fulfil the objectives of the National Water Act (NWA, Act No 36 of 1998; Department of Water Affairs, 2013). In fulfilling the objectives of equity and sustainability that are enshrined in the NWA, two complementary strategies have been designed: Resource Directed Measures (RDM) and Source Directed Controls (SDC) (Department of Water Affairs and Forestry, 2013). The RDMs and SDCs are the strategies used in the management of water resources in South Africa (Department of Water Affairs & Forestry, 2013). They provide different tools and approaches for monitoring water quality to realise the objective of balancing water resource protection and use.

Resource Directed Measures (RDM)

Resource directed measures (RDMs) are directed towards the water resources and are focused towards protecting water resources by managing the quality, quantity, habitats, geomorphology and riparian vegetation of water resources needed for both human and aquatic environments while allowing for socio-economic growth and development (Department of Water Affairs, 2004). The RDMs recognise the increasing anthropogenic activities that threaten the aquatic environment and, thus they provide necessary tools and approaches to balance the protection and use of water resources. The RDMs offer a classification system and procedures for determining the classes of every significant water resource in South Africa, for determining the ecological Reserve and for setting Resource Quality Objectives (RQOs). Water resources are classified into three major management classes, reflecting the expected levels of use as well protection: Class I are water resources that are less used, where the ecological condition has been minimally altered from its pre-development condition; Class II are water resources that are moderately used, where the ecological condition has been moderately altered from its pre-development condition, and Class III are water resources that are profoundly used, and where the ecological condition has been significantly altered from its pre-development condition (Department of Water Affairs, 2011).

The Ecological Reserve aims to ensure water resource protection so that the development and use of water resources in South Africa is ecologically sustainable and responsible. The Reserve provides for both water quality, quantity and supply assurance that is required for human basic consumption (the Human Need Reserve) as well as to protect aquatic ecosystems (ecological Reserve; King & Pienaar, 2011; Department of Water & Sanitation, 2013). The Reserve is the only water right specified as inviolable in the Act, making it a legally guaranteed right that ensures the supply and delivery of water for both basic human rights in terms of access to water, protection and functioning of the aquatic ecosystem. The ecological Reserve thus sets out a sound ecological basis for managing water resources in South Africa.

Resource quality objectives (RQOs) provide quantitative and qualitative descriptions of the physical, biological and chemical attributes that characterise the desired level of protection of a water resource, as defined by its management class (Department of Water & Sanitation, 2011b; 2013). The RQOs capture the management class and the ecological needs determined in the reserves into measurable objectives that give direction on how a water resource should be managed (Department of Water & Sanitation, 2011b; King & Pienaar, 2011).

Source Directed Controls (SDC)

The SDCs are the second strategy in the NWRS2 (Department of Water Affairs, 2016) for water resource protection. The regulation of water uses in South Africa is supported by SDCs, ensuring that the primary purpose that has been identified for the water resource is achieved. The SDC tools comprise regulatory mechanisms such as water quality standards for wastewater discharges, pollution prevention measures, waste-discharge charge systems, water-use licences and general authorisation. The Department of Water and Sanitation progressively encourages implementation of self-regulation using both disciplinary and/or punitive measures and economic incentives.

Environmental water quality (EWQ) comprises of tools that are used to implement both RDM and SDC. The tripod system of EWQ include physico-chemical monitoring, biomonitoring, and ecotoxicology, all of which contributes to the development of RDM and SDC tools (Department of Water & Sanitation, 2013). For example, physico-chemical monitoring contributes information about the physico- chemical conditions of water resources, enabling an assessment of the direction of the resource either towards or away from the set desired RQOs. Similarly, through biomonitoring, biological data are collected to assess the biological conditions of the resource, while ecotoxicology provides an opportunity to evaluate the cause-effect relationship between a toxicant/stressor and the biological agent. Overall, EWQ is the holistic approach to water quality management and is often applied in South Africa (Department of Water Affairs, 2013). The primary focus of the current study is physico-chemical monitoring, mainly fine sediments and related basic chemical variables, and biological monitoring, combining taxonomic and trait-based approaches.

1.3 Environmental Water Quality (EWQ)

Environmental water quality (EWQ) is an integrated approach that has been applied in several studies; it links water quality chemical variables to the actual responses of instream biota, ecosystem function and processes (Sherman *et al.,* 2003; Vellemu, 2017; Odume, 2017). The EWQ approach links water physico-chemistry, biomonitoring and ecotoxicology information to monitor instream water quality (Odume, 2014). Water physico-chemistry involves measuring and analysing physical and chemical variables of water to determine the state of the water resources (Odume, 2014). Physico-chemical variables include temperature, turbidity, dissolved oxygen, total suspended solids and pH, as well as nutrient variables, such as nitrate-nitrogen, nitrite-nitrogen and phosphate-phosphorus (Palmer *et al* 2004; Odume, 2017). Biomonitoring uses the response of aquatic biota to provide integrated information on the condition of the aquatic ecosystem. In assessing environmental degradation, resident biota has proved to provide robust information about the integrated state of the resource (Bremmer *et al.*, 2006a; Arimoro & Muller, 2010). The last approach of EWQ, ecotoxicology, provides information on the cause-effect relationship of specific concentrations of toxicants on individual organisms, thereby providing a bridge between the physico-chemical and biomonitoring approaches (Vellemu, 2017). Biomonitoring tools are very important for freshwater system monitoring (Bonada *et al.,* 2013), and this study focuses on physico-chemical monitoring and biomonitoring, which are further reviewed.

1.3.1 Water physico-chemistry

Water quality assessment is generally about evaluating the physical, chemical and biological characteristics of water (Govenor *et al.,* 2019). Physico-chemical variables, such as nutrients, pH, and dissolved oxygen, have been well studied in monitoring and assessing rivers and streams (Rasifudi *et al.,* 2018). Physical disturbance of the river can result from both natural conditions and human interventions, either within the catchment or the channel (Akamagwuna, 2018; Mathers *et al.,* 2019) or even beyond, as in the case of climate change affecting aquatic temperatures (Dallas & Rivers-Moore, 2013). The purposes of water quality assessment are: to validate whether the observed water quality is appropriate for intended use, to determine trends in the quality of the aquatic environment, and to establish how water quality is affected by water stressors due to anthropogenic activities and natural conditions (Mwangi, 2014; Ochieng *et al.,* 2020).

The physico-chemical approach to and method of measuring water quality variables is the most widely used in managing environmental water quality. Measuring physico-chemical variables allows managers to keep track of concentrations of water quality variables in freshwater ecosystems for management purposes and to set goals and guidelines. The presence of high concentrations of specific chemicals has a variety of implications for aquatic ecosystem health and functionality (Akamagwuna, 2018). High loads of both settled and suspended sediments can absorb contaminants, change the geomorphology of the stream channel, and affect aquatic biota (Jones *et al.*, 2012). Given that the rivers where this study was undertaken are subject to high fine-sediment input from the catchments, elevated fine sediments as freshwater stressors are therefore further reviewed.

1.3.2 Elevated fine sediments as freshwater ecosystem stressor

Fine sediments are natural components of freshwater ecosystems and are important for substrate composition and heterogeneity as they play a part in the integrity of the microhabitats where macroinvertebrates inhabit (Wood & Armitage, 1997; Mathers *et al.,* 2019). Fine sediments are organic or inorganic particulate matters that can be transported and deposited in aquatic environments (Waters, 1995; Logan 2007; Mahoney, 2017). Fine sediments can be classified based on whether they are settled or suspended. Suspended fine sediments are defined as the grain sizes that remain in solution in the aquatic ecosystem (Waters,1995; Martinez *et al.,* 2020). Settled fine sediments refer to the grain sizes covering the streambed. Settled fine sediments on stream bottom moves by sliding, rolling, or slating along the substrate surface. However, depending on water velocity and turbulence, intermediate size particles may be suspended or bedload (Waters, 1995; Davies-Colley *et al.,* 2015; Akamagwuna & Odume, 2020). One measure of the degree of settled fine sediments is embeddedness, which refers to the extent to which gravel, cobble, and boulders are buried by silt, sand, or mud in the stream bottom (Barbour *et al.,* 1999, Govenor *et al.,* 2019). An increase in bedded sediments has been associated with changes in community composition and reduced macroinvertebrate abundance (Jones *et al.,* 2012; Davies-Colley *et al.,* 2015; Akamagwuna, 2018).

Fine sediments can also be classified based on their grain sizes (fine sand, fine silt, coarse silt, coarse sand, very fine silt, very fine sand) (Akamagwuna, 2018; Martinez *et al.,* 2020). It has been stated that sediments less than 63 µm in size are the most significant fraction for contaminant adsorption and transport due to their comparatively larger surface area (Akamagwuna *et al.,* 2019). For example, silt and clay transport heavy metals in fluvial systems, and elevated concentrations of smaller sediment grain sizes are acknowledged to be more harmful to macroinvertebrates because of their sizes, which accumulate high concentrations of contaminants and clog fine biological structures (Wood & Armitage, 1997, Zhang *et al.,* 2014).

Sources of fine sediments are well-defined as either channel sources, regarded to be derived from within the stream channel, or non-channel sources, which originate outside of the stream channel. Channel-derived fine sediments are sourced from banks and channel margins, point bars, fines stored in interstitial spaces or sequestered in vegetation, and pools or backwater areas (Wiitala, 2013). Outside of the stream channel, sources of sediments include leaf and litter fall, unvegetated soils, landslides, gullies, particles from atmospheric deposition, and in general, anthropogenic activities such as land degradation (Moore, 2016; Martinez *et al.,* 2020). Transport of non-channel sediment into the water column is determined by the source of sediments as well as the path of transport, which are highly complex. Stream derived sediment transport is less varied and depends on hydrological and hydraulic characteristics, such as stream discharge and streambed stability (Govenor *et al.,* 2019). Sediments varies spatially or temporally (Bilotta *et al.,* 2012a; Odume *et al.,* 2018), depending on the number of fine sediments delivered to and mobilised in rivers. Fine sediments variability in streams is also influenced by catchment land cover, catchment geology, riparian vegetation, topography, hydro-geomorphology, hydro-meteorology, and human management interventions (Allan, 2004, Vercruysse *et al.,* 2017).

The increase in the amount of fine sediments in the river channel adversely impacts aquatic habitat and the associated biological communities (Doretto *et al.,* 2018; Akamagwuna *et al.,* 2019). Fine sediments disrupt in-stream biota including macroinvertebrates in different ways, and these may include impairment of body parts, clogging of respiratory and filtering organs. Indirect effects are usually through habitat loss due to filling of interstices between substrates, the burial of bottom-dwelling macroinvertebrates, oxygen depletion, changes in quantity and quality of food, and drift because of sediments deposition or substrate instability (Jones *et al.,* 2012; Ding *et al.,* 2016; Edegbene *et al.,* 2020).

Downstream drifting of macroinvertebrates can occur in large numbers because of increased flow and sediments discharge (Gibbins *et al.,* 2007; Giesiwein *et al.,* 2019). Drifting of macroinvertebrates depends on the flow rate as well as how much sediments are discharged. Fine sediments do not impact all macroinvertebrates to the same extent. Some macroinvertebrates can survive in river channels impacted by sediments because of their morphological, physiological, or behavioural characteristics (Extence *et al.,* 2013; Mathers *et al.,* 2019). For example, a study by Wood *et al.* (2005), investigated the effect of burial by different sediments grain sizes on four taxa of macroinvertebrates, which showed that the nymphs of the Plecoptera *Nemoura cambrica* freed themselves from the different sediments grain sizes at different burial depth, but the nymphs of *Baetis rhodani* remained buried. The results from the Wood *et al.* (2005) study highlighted the biological implication of differences in fine sediment grain sizes. For this reason, the current study pays particular attention to assessing grain sizes across the study sites in the Tsitsa River and its tributaries. In addition, the current study applies both taxonomic and trait-based approaches to better understand the effects of fine sediments on macroinvertebrate assemblages in the Tsitsa River and its tributaries.

1.4 Biomonitoring

Biomonitoring is an approach that is used to assess the ecological health of freshwater systems (Parmar *et al.,* 2016) using aquatic biota such as plants, algae, fish, and macroinvertebrates to monitor freshwater environments (Li *et al*., 2010; Friberg *et al.,* 2011; Odume, 2017). It has been widely used in South Africa to manage freshwater resources (Palmer *et al.,* 2004; Bremmer *et al.,* 2006a). The application of biomonitoring is based on the idea that instream biota responds predictably to stressors, and such responses provide an indication of the impairment of ecosystem health (Bonada *et al.,* 2006; Yadamsuren *et al.,* 2020). Biota that can successfully respond along a gradient of water quality impact are referred to as biological indicators (Bonada *et al.,* 2006; Rasifudi *et al.,* 2018). The biological indicators most commonly used in assessing river health include phytoplankton, fish, and macroinvertebrates (Parmar *et al.,* 2016; Kefford *et al.,* 2020; Wang *et al.,* 2021).

1.4.1 Macroinvertebrate-based biomonitoring

Macroinvertebrates are among the most widely used biological indictors in biomonitoring freshwater ecosystems (Bonada *et al.,* 2006; Friberg *et al.,* 2011). Macroinvertebrates have been extensively used as bioindicators of water quality owing to their sensitivity, ubiquity, ease of identification, and the availability of standardised collection protocols and techniques, which are quick and cost effective (Masese *et al.,* 2009, Friberg *et al.,* 2011). Macroinvertebrates can be monitored at various organisational levels from species to community (Akamagwuna, 2021) and can be found in a wide array of substrates (Altermatt *et al.,* 2013), for example, vegetation, stones, gravel, sand, and mud (Dickens & Graham, 2002; Bonada *et al.,* 2006). As bioindicators, macroinvertebrates are used for monitoring long-term environmental changes (Odountan *et al.,* 2019) and have an important role in the aquatic food web, playing vital roles in processing organic matter and the flow of energy (Masese *et al.,* 2009; Akamagwuna, 2021). As biological indicators, macroinvertebrates have been found to respond to fine-sediment effects through drifting, changes in community structure, as well as functional diversity (Mohammed, 2018; Akamagwuna *et al.,* 2019). Most studies analysing macroinvertebrate community response to fine sediments are taxonomy-based, that is, the taxonomic compositions of impacted sites are compared with those of less impacted, control or reference sites (Gordon *et al.,* 2013; Odume, 2013). However, in recent years, there has been a growing interest in a complementary approach: the trait-based approach (TBA) (e.g., Gieswein *et al.,* 2019). In this study, macroinvertebrates were used as biological indicators to assess fine-sediment effects in the Tsitsa River and its tributaries.

The macroinvertebrate-based approach has been widely used in South Africa: the Department of Water and Sanitation uses the approach for sustainable management of freshwater resources (DWAF, 2008). Biological indicators such as fish, vegetation and macroinvertebrates are used to assess the ecological responses of riverine ecosystems to environmental stressors (DWAF, 2008). Biomonitoring tools such as the South African Scoring System version 5 (SASS 5) and Macroinvertebrate Response Assessment Index (MIRAI) are used to collect data. The SASS 5 is a well-developed single biotic index that uses a scoring system (Dickens & Graham, 2002), and it is widely used in South Africa because it is easy to use and to interpret results. Additionally, the biomonitoring tool depends on the presence or absence of tolerant and sensitive taxa to deteriorating water quality in freshwater environment (Dickens & Graham, 2002; Dallas, 2007). In the present study, macroinvertebrate taxonomy- and trait-based analyses at family level were used to assess fine-sediment effects on freshwater systems.

1.4.2 Taxonomic approaches

A taxonomy-based approach in biomonitoring is used to compare biological information (e.g., community structure, species richness and abundance measures**)** and relate them to environmental conditions (Culp *et al.,* 2011). Taxonomy-based approaches use several metrics, such as single biotic metrics and diversity indices, multimetric indices and multivariate models, and have been used for biomonitoring programmes in different countries (Hilsenhoff, 1988; Barbour & Yoder, 2000; Turak *et al.,* 2004; Jorgensene *et al.,* 2005). Changes in taxonomic composition of macroinvertebrates have been used to detect changes in the quality and health of riverine ecosystems. Tools that have been developed based on taxonomic analysis of macroinvertebrate communities include single biotic indices such as the Biological Monitoring Working Party (BMWP) in the UK and the South African Scoring System Version 5 (SASS5) in South Africa (Walley & Hawkes, 1996; Dickens & Graham, 2002. The South African Scoring System (SASS) is used in South Africa as a biomonitoring tool for assessing freshwater ecosystems and is currently in its fifth version, known as SASS5. In SASS5, macroinvertebrate families are given scores of 1–15, according to their sensitivity to water quality impact. Taxa that are tolerant are awarded lower scores, and those that are sensitive are awarded higher scores (Dickens & Graham 2002, Odume, 2012). In this study, the SASS5 protocol was only used as a macroinvertebrate collecting technique.

The multimetric approach mainly focuses on pooling together metrics that represent structural, functional aspects of macroinvertebrate ecology, together with those of ecosystem level processes to determine river health (Doretto *et al.,* 2018; Giesiwein *et al.,* 2019, Edegbene, 2020). The Benthic Index of Biological Integrity (BIBI) was first developed in the United States of America for assessing fish assemblages in freshwater streams (Kerans & Karr, 1994; Yazid *et al.,* 2014; Shull *et al.,* 2019), and since then, the approach has been widely used across the globe (e.g., Camargo, 2017; Shull *et al.,* 2019; Giesiwen *et al.,* 2019; Edegbene, 2020). Carmago (2017) developed a macroinvertebrate-based multimetric index for assessing the ecological conditions of polluted streams in Spain, which proved to be effective for monitoring the responses of macroinvertebrates to freshwater pollution.

Similar indices have been developed and applied in assessing the health and ecological conditions of lakes, wetlands, streams, and rivers in Chile (Fierro *et al.,* 2018), in China (Lu *et* *al.,* 2019), in South Africa (Odume *et al.,* 2012, Akamagwuna, 2018), in Ethiopia and Kenya (Mereta *et al.,* 2013; Lakew & Moog, 2015; Aura *et al.,* 2017), and in Nigeria (Edegbene *et al.,* 2019; Edegbene, 2020). In the present study, a stressor-specific multimetric index was developed for assessing the effects of fine sediments in the Tsitsa River and its tributaries.

In South Africa, biomonitoring relies primarily on taxonomic analysis of assemblage composition. Although there has been a growing interest in using traits for freshwater biomonitoring (e.g. Akamagwuna *et al.,* 2019, Odume, 2020), the trait-based approach is not yet well-developed in the country. The use of trait information in assessing freshwater systems has recently gained popularity because traits provide an indirect measure of functional responses and predict instream biota response to the environmental impact by understanding the trait-environmental connection (Akamagwuna, 2021). Therefore, in addition to the taxonomic approaches applied in this study, a trait-based approach was developed for predicting the potential vulnerability and resilience of macroinvertebrates to fine sediments effects.

1.4.3 Trait-based approach (TBA) to biomonitoring

The trait-based approach (TBA) is an emerging approach that uses traits to analyse and predict the effects of a stressor on macroinvertebrate communities (Menezes *et al.,* 2010). Traits are defined as inherent characteristics possessed by organisms at the individual level of biological organisation (McGill *et al.,* 2006; Odume *et al.,* 2018). Traits can be categorised into morphological (e.g., body form), reproductive (e.g., number of offspring per reproductive event), biochemical (e.g., DNA make-up) (Kuzmanovic *et al.,* 2017; Krynak & Yates, 2018; Desrosiers *et al.,* 2019), behavioural (e.g., locomotion) and physiological (e.g., respiration). However, this study adopts the definition of traits by Violle *et al.* (2017) who define a trait as a measurable feature of an organism without reference to the environment, while Odume *et al.* (2012) argues; a trait is the biological feature of an organism, and thus the product of the direct interaction of the organism with its external environment cannot be termed a trait. For example, from the viewpoint of applied ecology, an organism's preference for particular environmental conditions such as flow velocity, sensitivity to an environmental stressor (i.e., sediments) or its functional role in relation to ecosystem functioning cannot be regarded as traits since these are all products of the organism-environment direct or indirect interactions.

The application of traits in freshwater biomonitoring is rooted in the habitat template concept (HTC) which postulates that organisms survive and thrive in an environment for which they have the appropriate trait combination (Southwood, 1977; Townsend & Hildrew, 1994). The HTC is based on the idea that environment characteristics filter species with suitable combinations of traits able to adapt to the environmental requirement, and such traits can be predictive and diagnostic of the prevailing stressors (Culp *et al.,* 2011; Verberk *et al.,* 2013; Akamagwuna *et al.,* 2019; Odume, 2020). Species possessing trait combinations that do not allow them to adapt to specific environmental conditions become eliminated (Verberk *et al.,* 2013; Yadamsuren *et al*, 2020). For example, environmental conditions such as elevated fine-sediment deposition act as filters for traits and shape community composition by selecting well-adapted species with an appropriate combination of traits (Statzner *et al.,* 2004; Hamilton *et al.,* 2020). Therefore, the environmental conditions act as a filter (Poff *et al.,* 2006), and only taxa possessing adaptive traits pass through; such traits can predict and diagnose the prevailing environmental stressor (Culp *et al.,* 2011; Webb *et al.,* 2010).

TBA studies have often used two approaches (Verberk *et al.,* 2008): an analysis of trait combination, and their interaction and life-history strategies as underlying mechanisms responsible for individual species responses to prevailing environmental conditions (Verberk *et al.,* 2013; Piliere *et al.,* 2016; Libala *et al.,* 2020). Underlying the approach that combines traits is the assumption that an environmental stressor impacts not only a single trait but a combination of traits that characterise a particular species. Therefore, the relationship between a particular trait and specific environmental conditions is determined by the interactions among traits, which collectively determine and influence species behaviour, resilience, sensitivity and response to environmental conditions (Verberk *et al.,* 2013; Piliere *et al.,* 2016). The second approach involves predicting the responses of macroinvertebrate taxa based on the type of traits, trait interactions and the combinations possessed (e.g., Liess & Von de Ohe 2005; Extence *et al.,* 2011). The second approach has been used to develop the proportion of sediment-sensitive invertebrates index (PSI) in the United Kingdom (Extence *et al.,* 2011). Therefore, in this study the approach is applied in assessing sediment impact in freshwater ecosystems.

A few studies elsewhere have used the trait-based approach to explore the impact of fine sediment stress on macroinvertebrates (Herrera *et al.,* 2016; Mathers *et al.,* 2017; De Castro *et al.,* 2020). The approach offers an opportunity to identify indicator traits based on the specific environmental stressor, which in turn, can be beneficial to the development of a standard trait-based tool. For example, Collins & Fahrig (2020) used the approach to assess and identify indicator traits of 27 farmland ditches in Eastern Ontario, Canada, and the application of multiple trait-based approaches has gained popularity in assessing multiple environmental stressors (Kuzamanovic *et al.,* 2017). Mondy & Usseglio-Polatera (2013) used life-history traits of macroinvertebrates to assess the effects of multiple stressors like urbanisation and sedimentation on shallow rivers in France.

The application of the TBA in freshwater biomonitoring is promising since trait responses are less constrained by space (Verberk *et al.,* 2013; Odume *et al.,* 2018). However, the trait-based approach is yet to gain popularity in Africa. In this study, the approach was used to complement a taxonomy-based approach to assess fine sediments and identify potential indicator-tolerant traits, taxa and ecological preferences of macroinvertebrates in the Tsitsa River and its tributaries.

Although the trait-based approach is useful, challenges remain. These challenges include i) the difficulty of distilling the complex relationship between different trait attributes and the external environment, which may confound the interpretation of trait-based responses; ii) the difficulty in linking traits to community response; iii) the availability of appropriate statistical techniques for analysing trait data (Murphy, 2020); iv) dealing with a deficiency in regional information on traits, particularly in Africa.

1.5 Rationale and significance of the study

The effects of elevated fine sediments on water quality are a global challenge that has impacted both developed and developing countries, hindering socio-economic development and livelihoods (Beeckman, 2017; Food & Agricultural Organisation, 2018). The continued deterioration of water quality of rivers and streams in many catchments of the world receives little attention with regard to water quality problems associated with elevated levels of fine sediments; instead, solutions to the global water crisis focus mainly on water quantity, water-use efficiency and allocation issues (Biswas & Tortajada, 2012). However, the continued decline in water quality in many rivers of the world greatly contributes to global water scarcity by reducing the quantity of clean, potable water (Akamagwuna, 2021). South African rivers, such as the Tsitsa River, like many rivers in the developing countries, experience deterioration in water quality due to human activities (Gordon *et al.,* 2013; Akamagwuna *et al.,* 2019), especially sedimentation and poorly managed agricultural activities (DWAF, 2011). Assessing elevated fine-sediment effects can bring us closer to achieving the provision of clean water and sustainable livelihoods, thereby contributing to achieving the United Nations Sustainable Development Goals (SDGs). Elevated levels of fine sediments in the Tsitsa River catchment severely impair freshwater communities (e.g., directly clogging filter-feeding organs and indirectly, by reducing stable habitats), contributing significantly to the loss of biota (Akamagwuna *et al.,* 2019). It is essential to thoroughly investigate water quality deterioration and achieve a clear understanding of the link between fine sediments and freshwater ecosystem structure and function.

To ensure ecosystems services provided by freshwater ecosystems are not hampered by elevated sediments, it is essential to use integrative approaches to develop a sediment-specific biomonitoring tool for assessing the impact of elevated sediment on freshwater ecosystems. A combination of taxonomic and trait-based approaches to assess the potential response of macroinvertebrate communities to sediment may provide further insights into such impacts.

Researchers have developed several approaches to assess anthropogenic pollution; however, considerable gaps remain in our knowledge. Firstly, the most frequently used approaches, which include the taxonomic approach, are based on assessing structural indices that mainly describe taxonomic assemblage at the family-level resolution with little indication of the effects of environmental stressors on function. It has been argued that functional indicators such as traits can indicate disturbance (e.g., sediment impact) before shifts in taxonomic structure occur, providing an early indication of anthropogenic stressors. However, there is a poor characterisation of both structure and function of ecosystems; the environmental stressor can cause changes to the structure but not function, or vice versa, or both (Akamagwuna, 2021). Also, the application of traits and functional diversity provides essential metrics to elucidate the links between community structure and ecosystem functioning. Yet, changes in functional diversity and traits along stress gradients remain less studied. Most trait-based studies pay little attention to identifying indicator traits that can be helpful in developing predictive trait-based tools (Murphy *et al.,* 2020)

Because sedimentation contributes significantly to the deteriorating water quality of many rivers, including the Tsitsa River, paying attention to the knowledge gaps will help us understand how fine sediments affect both water quality and the biological assemblages of freshwater ecosystems. Such research will also provide an opportunity to identify indicator traits of fine-sediment pollution and for the potential development of robust biomonitoring tools to monitor and manage stream ecosystems impacted by fine sediments. This study applied taxonomic and trait-based approaches to assess the effects of elevated fine sediments on macroinvertebrates, paying critical attention to identifying taxonomic and trait-based indicators of sediment impact in the Tsitsa River and its tributaries.

1.6 Aim and objectives

1.6.1 Aim

The overall aim of this study was to develop novel taxonomic and trait-based approaches for assessing macroinvertebrate responses to elevated fine sediments in the Tsitsa River and its tributaries in the Eastern Cape, South Africa.

1.6.2 Objectives

I. To characterise suspended and settled fine-sediment grain sizes and their distribution in the Tsitsa River and its tributaries.

II. To develop and validate a macroinvertebrate-based, sediment-specific, multimetric index suitable for monitoring the effects of elevated fine sediments in the Tsitsa River and its tributaries.

III. To explore macroinvertebrate traits and ecological preferences with a view to identifying possible trait-based indicators of fine-sediment impact in the Tsitsa River and its tributaries.

IV. To develop and apply a novel trait-based approach for assessing and predicting the potential vulnerability and resilience of South African macroinvertebrate families to fine sediments.

1.7 Thesis structure

Chapter 1 provides a general introduction and an extensive review of existing literature and outlines the rationale of the study. It concludes with the aim, objectives, and thesis structure.
Chapter 2 is a general materials and methods chapter describing the study area, sampling sites and protocols, methods and approaches used, and providing statistical analyses.
Chapter 3 is the first results chapter. In this chapter suspended and settled fine sediments are characterised and a multimetric index developed.
Chapter 4 explores the pattern of ecological preferences and traits in relation to fine-sediment impacts in the Tsitsa River and its tributaries.

Chapter 5 provides a description of the development of a novel TBA for assessing and predicting macroinvertebrate potential vulnerability and resilience to sediment impact.
Chapter 6 presents a synthesis of the findings of this research and an integrated discussion of the results, with recommendations for further studies and draws general conclusions.

CHAPTER 2: STUDY AREA DESCRIPTION, GENERAL MATERIALS AND METHODS

2.1 Introduction

The purpose of this chapter is to describe the study area and the factors influencing the delivery of fine sediments into the selected sampling sites. The selected sampling sites and the methods, approaches, and protocols used in this study are described. The chapter closes with a description of the traits selected and a general description of the statistical methods employed.

2.2 Study area description

The Mzimvubu River catchment is bounded in the south by the Mthatha and Mbashe river catchments, in the west by the Orange River catchment, in the north-east by the Umzimkhulu and Mtamvuna river catchments, and in the east by the Pondoland coastal catchments. Although the catchment shares an international border with Lesotho, there are no shared rivers between them. The Tsitsa River and its tributaries form part of the broader Mzimvubu River catchment. The Tsitsa River rises in the Drakensberg 15 km to the southeast of Rhodes, a small town close to Maclear about 80 km west of Mount Frere, and flows eastwards. The Tsitsa Falls are in the upper course of the Tsitsa River, in a mountainous area of great beauty. Flowing southwards for a few miles, the river passes east of Maclear, before it meanders eastwards again. Finally, it empties into the Mzimvubu River, passing through deep river gorges about 36 km southeast of Qumbu. The main tributaries of the Tsitsa River include the Inxu, Mooi and Pot rivers.

The Mzimvubu catchment falls within the quaternary catchment T35A–E. The Mzimvubu River flows mainly from the eastern escarpment of the Drakensberg Mountains near the town of Matatiele and discharges into the Indian Ocean at Port St. Johns, after passing through hills and forming tributaries with the Tina, Kinira and Mzintlava Rivers, as well as the Tsitsa River and its tributaries (Le Roux *et al.,* 2013; Akamagwuna, 2018). The Tsitsa catchment, within the Mzimvubu, covers an area of 4 924 km^2. The Tsitsa River connects the Mzimvubu River after a flow length of approximately 200 km from northwest to southeast. The upper reaches of Tsitsa River are situated in a confined channel between two steep valleys with narrow floodplains, while most of the study area is formed by a hilly landscape.

2.2.1 Climate and rainfall

The climate in the Tsitsa River catchment ranges from temperate in the northern altitude to sub-tropical along the coastal belt with summer rainfall (Bäse *et al.,* 2006); approximately 75% of the mean annual precipitation falls between November and March (Bäse *et al.,* 2006; Moore, 2016). Maclear receives an average of 700 mm of rain per year, with a low of 14.5 mm in July and a high of 132.9 mm in January. Maclear has an average midday temperature of 17˚C in June and 26˚C in January (SA Explorer, 2014). The study area is strongly seasonal, with a wet summer and autumn and dry winter and spring.

2.2.2 Geology and soils

The Tsitsa River catchment comprises mudstone and sandstones of the Karoo Sequence (DWA, 2005), which are underlain, mainly by highly erodible Beaufort series of sandstones. The geology is characterised by basalt material in the upper alpine zone, which is increasingly dominated by sandstones combined with shales and mudstones and with deep alluvial deposits in the lower lying areas (Wepener *et al.,* 2015). Soils in the catchment are duplex and are easily erodible, ranking among the top sediment-yielding catchments of South Africa (Madikizela & Dye, 2003; Wepener *et al.,* 2015). The high runoff and erosion in the catchment are related to the melanic and vertical content of clay that characterises the soils in the area (Wepener *et al.,* 2015). These components of the soils increase from topsoil to the subsoil and prevent infiltration, and the soil properties have resulted in a large section of the catchment being affected to varying degrees by deep gullies and elevated fine-sediment deposition into the Tsitsa River and its tributaries.

2.2.3 Topography, land cover and vegetation

The Drakensberg Highlands and Table Mountain are the two prominent escarpments in the Tsitsa River catchment. The watercourses in the catchment generally have steep slopes; however, slopes in the Pot River are steeper than the rest of the sites in the Tsitsa, Qurana and Millstream rivers. The coastal regions are dominated by valley, bush forest, and Table Mountain sandstone with steep sea cliffs (Rutherford & Mucina, 2006). The vegetation in the Tsitsa catchment is influenced by altitude, soils, and by grazing and grassland burning. Montane, sub-alpine and alpine belts with pockets of shrub and woodland or savannah dominate the grassland in the catchment (Rutherford & Mucina, 2006; Moore, 2016) (Figure 2.1). Natural vegetation, which includes indigenous forest, covers approximately 3400 km^2 (70%) of the catchment area (Bäse *et al.*, 2006). Changes in land use, such as trampling by grazing livestock and other farming activities, contribute to the transport of sediments into the Tsitsa River and its tributaries. Vegetation in the area is extensively degraded and clearing of trees for house-use purposes has aggravated sediment flux into the Tsitsa River and its tributaries.

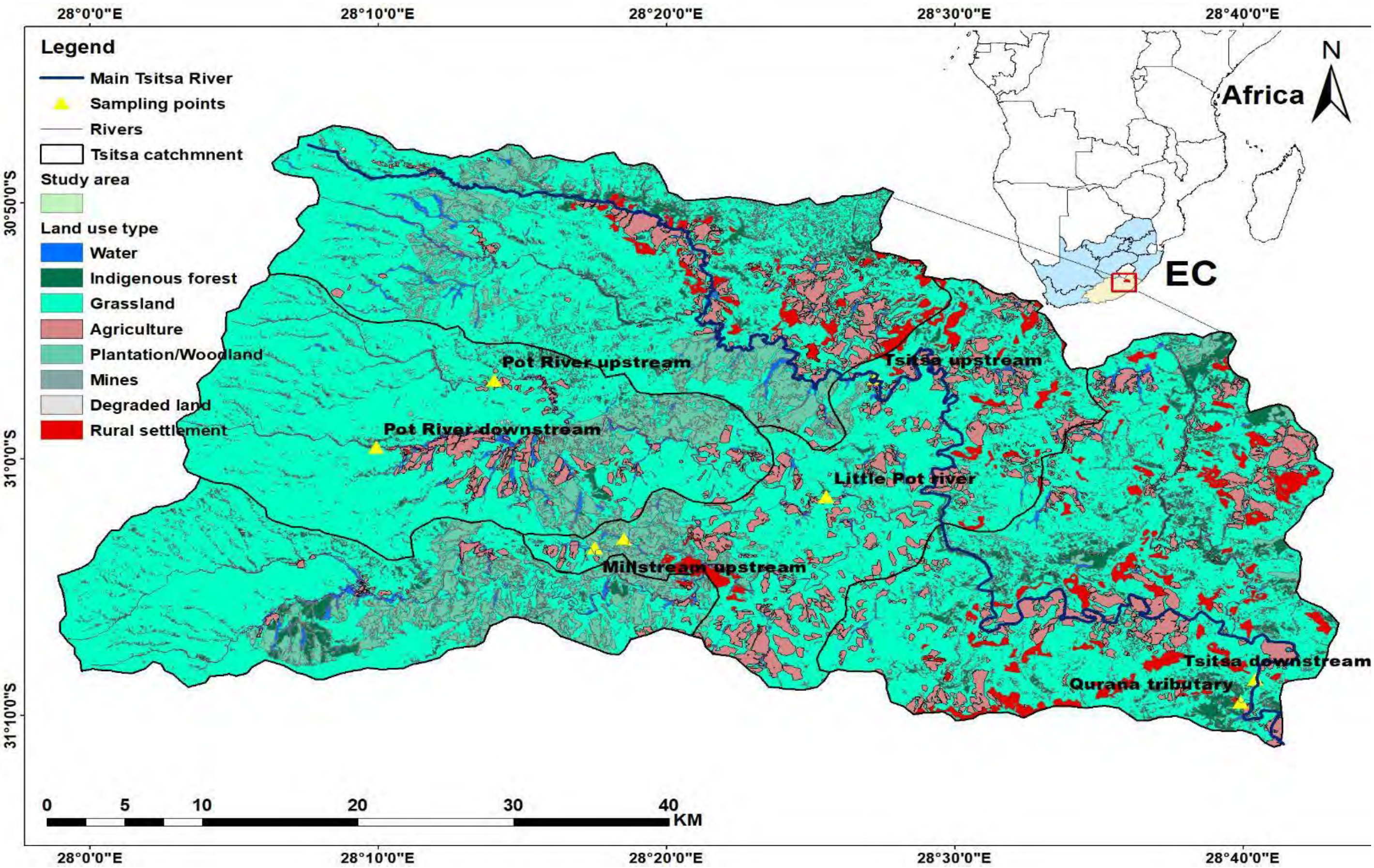


Figure 2. 1: Land use map of the sampling sites on the Tsitsa River and its tributaries showing the land cover around each site**.**

2.2.4 Anthropogenic influences in the catchment

Poor grazing activities, cultivation of farms and fields, and forestry activities combined with the duplex, easily erodible soils are the major contributing factors of fine-sediment input into the Tsitsa River and its tributaries. These factors are the main anthropogenic drivers of water quality change in the river (DWA, 2015; van Tol *et al.,* 2016). Urban developments within the catchment are minimal and do not constitute a principal source of water quality impact (Akamagwuna, 2018).

It has been reported that the Tsitsa catchment, including the greater Mzimvubu catchment, develops approximately 12 265 new gullies per year, affecting an area of 3970 m^2. An impressive statistic, underlining the need for this work and better management of soils. The high number of gullies contributes significantly to fine-sediment delivery into the river system, delivering about five tonnes/hectare/year of sediment (Le Roux *et al.,* 2015). While the Pot and Little Pot Rivers catchments are in comparatively good condition regarding land cover and potential sediment delivery into these river systems, the Tsitsa and Qurana are not because they are situated within communal lands where poor grazing practices are common. Conditions in the Millstream catchment are similar to the Tsitsa, even though the Millstream is much better managed (Akamagwuna, 2018).

2.2.5 Sampling sites

The study was conducted seasonally at eight selected sampling sites over a period of two years, beginning in late winter (August 2016) and ending in late autumn (March 2018). The selected sites with coordinates (Table 2.1) included two sites in the Tsitsa River (Tsitsa upstream and Tsitsa downstream), one site in the Qurana River, two sites in the Millstream (Millstream upstream and Millstream downstream), two sites in the Pot River (Pot River upstream and Pot River downstream) and one site in the Little Pot River (Figure 2.2). Sites were selected to indicate a gradient (i.e., analysing sediment particle sizes) of fine-sediment impact based on turbidity, total suspended solids, and land use practices (privately, well-maintained catchment versus communal, poorly maintained landscape).

Other factors that were considered when selecting the sites were the availability of macroinvertebrate biotopes and microhabitat diversity: stones, vegetation, and gravel, sand, and mud. Sites 1 and 2 (i.e., Tsitsa upstream (TSU) and Tsitsa downstream (TSD)) are situated in the Tsitsa River, and Site 3 is situated in the Qurana River (QHR), a small tributary of the Tsitsa River. Sites 4, 5 and 6 (i.e., Pot upstream, Pot downstream, and Little Pot) are situated in the Pot River and Little Pot, respectively, while Sites 7 and 8 (i.e., Millstream upstream (MLU) and downstream (MSD)) are situated in the Millstream River. The sites in the well-maintained catchment (i.e., Pot River and Little Pot River sites) were collectively referred to as the control sites (CLS). The Millstream upstream and Millstream downstream sites were regarded as moderately sedimented, and Tsitsa upstream, Tsitsa downstream sites, as well as the site in Qurana River (QHR), were regarded as highly sedimented, based on the extent of erosion, land use practices and previous studies (Akamagwuna *et al.,* 2019).

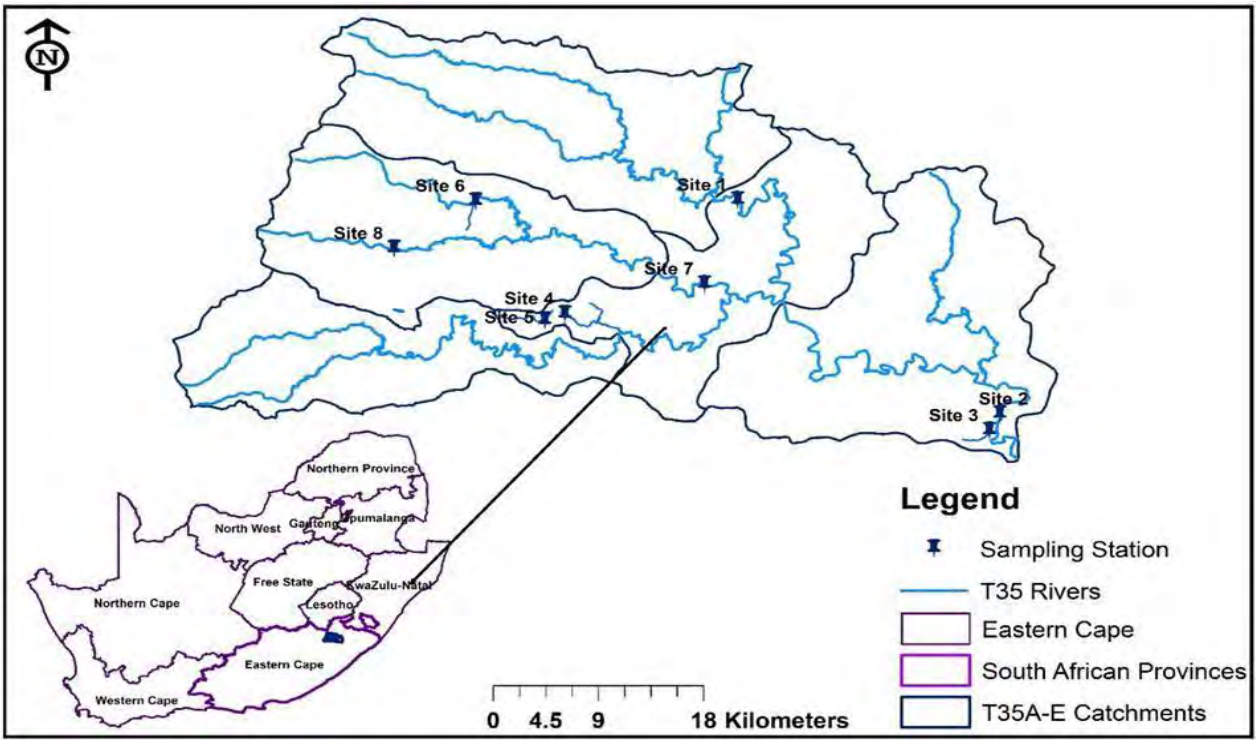


Figure 2. 2: Map of the study area showing the location of the sampling sites in the Tsitsa, Pot, Little Pot, Millstream and Qurana rivers. The location of the study area within South Africa is shaded grey in the Eastern Cape Province on the map of South Africa, and the relative position of South Africa is marked on the map of Africa. Sites: Site 1 (Tsitsa upstream site), Site 2 (Tsitsa downstream site), Site 3 (Qurana River), Site 4 MLU (Millstream upstream site), Site 5 (Millstream downstream site) and Sites 6–8 (control sites)

Site 1 (TSU) S 30° 56' 51.5" E 28° 27' 16.2" with an elevation of 1232 m, is situated in the upper reaches of the Tsitsa River. The site's catchment is subject to a combination of private and communal land practices such as agricultural and cattle farming. There was little evidence of soil erosion nearer the site (Figure 2.3), which was chosen as an example of a sediment-impacted site. Abandoned agricultural ploughing fields, cattle grazing and removal of sand, creating exposed soil were all noticeable around the site. During the wet season, this site can become turbid owing to the influx of fine sediments (Figure 2.3).

Figure 2. 3: Tsitsa upstream (TSU) during the dry season (right) and wet season (left) showing sampling sites and water turbidity. [Photo credit: Siphokazi Tantsi]

Site 2, (TSD) S 31° 8' 34.69", E 28° 40' 26.29" with an elevation of 887 m is located at the lower reaches of the Tsitsa River between the town of Maclear and the Qumbu villages of Cekwayo, Singungweni and Ngqongweni. Sediment delivery to this site is influenced by human activities in the TSU, Pot and Little Pot Rivers, Millstream Rivers and Qurana River – all of which connect to the Tsitsa River upstream of the site. Other contributing factors to the influx of sediments in this site are poor communal grazing practices of livestock farming. Evidence of gully erosion was noticeable on the riparian area of the site, causing both habitat modification and influx of sediments into the river. The major occupation of the rural dwellers is subsistence agriculture, and poor ploughing practices are profound. All three of the sampling biotopes were represented in this site (Figure 2.4).

Figure 2. 4: Tsitsa downstream (TSD) during the dry season (right) and wet season (left) showing sampling sites and water turbidity. [Photo credit: Siphokazi Tantsi]

Site 3, Qurana S 31° 9' 29.16", E 28° 39' 55.22" with an elevation of 895 m, is situated in the lower reaches of the Tsitsa and surrounded by trees in the riparian area. The site is within a community of the Didi rural area. The Qurana River is a little tributary to the Tsitsa River, and as with the Tsitsa River, its catchment is degraded owing to extensive grazing and soil erosion (Figure 2.5).

Figure 2. 5: Qurana River (QHR) during the dry season (left) and wet season (right) showing sampling habitats and water turbidity. [Photo credit: Notiswa Libala]

Site 4, Pot River upstream (CLS) S 30°56'56.62", E 28° 14'1.72" with elevation of 1322 m, is situated in the upper reaches of Tsitsa River catchment close to the Pot River Pass. The site is within private land, away from rural homesteads. There was no evidence of riverbank erosion or landscape degradation. The site is one of the control sites (CLS) indicating minimal sediment influence (Figure 2.6).

Figure 2. 6: Pot River upstream (CLS) during the dry season (left) and wet season (right) showing sampling habitats and water turbidity. [Photo credit: Siphokazi Tantsi]

Site 5, Little Pot River (CLS), S 31 01’28.4”, E 28 25’33.4” with an elevation of 1160 m, is situated on privately owned land. The river at the site flows through privately owned farmland with no surrounding homesteads (Figure 2.7). Cattle grazing is well controlled, and no evidence of gully erosion was noticeable around the site. The site is one of the control sites (CLS) indicating minimal sediment influence.

Figure 2. 7: Little Pot River (CLS) during the dry season (left) and wet season (right) season showing sampling habitats and water turbidity. [Photo credit: Siphokazi Tantsi]

Site 6, Pot River downstream (CLS), S 30 59’32.9”, E 28 09’ 55” has an elevation of 1380 m. The river at the site flows through a privately owned farmland with limited grazing activity. The site is located on the Woodcliffe farm with little evidence of gully erosion and was chosen as a control site (CLS) (Figure 2.8). The sampling biotopes are all well-represented.

Figure 2. 8: Little Pot River (CLS) during the dry season (left) and wet season (right) season showing sampling habitats and water turbidity. [Photo credit: Siphokazi Tantsi]

Site 7, Millstream upstream S 31 3' 28.04", E 28 17' 30.91" with an elevation of 1413 m, is situated near the town of Maclear. The PG Bison tree plantations are on the riparian zone of the river at the site and may thus contribute to sediment delivery into the river (Figure 2.9). Other catchment-related activities, such are timber and wood processing, as well as livestock grazing were the contributing factors to sediment in the river at the site.

Figure 2. 9: Millstream upstream (MLU) during the dry season (left) and wet season (right) season showing sampling habitats and water turbidity. [Photo credit: Notiswa Libala]

Site 8, Millstream downstream S 31 3' 6.91", E 28 18' 31.46" with an elevation of 1386 m, is situated near the PG Bison plantations and is a few kilometres away from the town of Maclear. It was selected as an example of a moderately impacted site (Figure 2.10).

Figure 2. 10: Millstream downstream (MLD) stream during the dry season (left) and the wet season (right) showing sampling habitats and turbid water. [Photo credit: David Gwapedza]

Table 2. 1: Summary of geospatial information of the sites

Site name	Abbreviation	Latitude	Longitude	Elevation (m)
Tsitsa upstream	TSU	S 30° 56' 51.5"	E 28° 27' 16.2"	1232
Tsitsa downstream	TSD	S 31° 8' 34.69"	E 28° 40' 26.29"	887
Qurana River	QHR	S 31° 9' 29.16",	E 28° 39' 55.22"	895
Pot River upstream	CLS	S 30° 56'56.62"	E 28°14'1.72"	1322
Pot River downstream	CLS	S 31° 01'28.4"	E 28° 25'33.4"	1160
Pot River	CLS	S 30° 59'32.9"	E 28° 09' 55"	1380
Millstream upstream	MLU	S 31° 3' 28.04"	E 28 17' 30.91"	1413
Millstream downstream	MLU	S 31° 3' 6.91"	E 28° 18' 31.46"	1386

2.3 Measurement of physico-chemical variables

Physico-chemical variables were measured seasonally in winter (August 2016), spring (October 2016), summer (December 2016), autumn (March 2017), winter (June 2017), spring (September 2017), summer (November 2017) and autumn (March 2018) at all the sampling sites. For each sampling event, the selected physico-chemical variables measured on site include dissolved oxygen (DO), electrical conductivity (EC), temperature, and pH using the multiparameter meter probe; model H198. Turbidity was measured on site using the portable turbidity Orbeco-Hellige 966 Meter. Samples for suspended solids (TSS) were collected for one year (August 2017–March

2018); TSS was measured according to the protocol described in (APHA, 1997; Sabri *et al.,* 1993).

Embeddedness was assessed as a proxy indicator for settled sediment according to the protocol described by Platts *et al.* (1983). The Platts/Bain Visual Method was used to visually estimate the fraction of the streambed within a reach covered by fine sediments. The visual estimate describes embeddedness as one of five embeddedness classes: 0 to 5%, 5 to 25%, 25 to 50%, 50 to 75%, or 75 to 100% (Platts *et al.,* 1983). A high percent corresponds to low embeddedness and low percent to high embeddedness.

2.3.1 Collection and preservation of water samples for nutrient analysis

Polyethene acid-washed bottles of 250 ml were used to collect water samples. The water samples were then transported to the laboratory at the Institute for Water Research, Rhodes University. In the laboratory, water samples were preserved in a refrigerator at a temperature of 4°C until samples were analysed within 24 hours. Water samples were analysed for nitrate-nitrogen (NO_3-N), nitrite-nitrogen (NO_2-N), orthophosphate-phosphorus (PO_4-P), ammonium-nitrogen (NH_4-N), and total inorganic nitrogen (TIN). Orthophosphate-phosphorus and NH_4-N were analysed using Merck spectroquant® phosphate and ammonium concentration test kits, catalogue number 1.14752.0001 and 1.14848.0001, respectively according to the manufacturer's instructions. Nitrate-nitrogen and nitrite-nitrogen were analysed according to APHA *et al.* (1971) on a Biotek microplate reader at 540 nm. Total inorganic nitrogen (TIN) concentration was calculated by adding the concentrations of nitrate, nitrite and ammonium (Palmer *et al.,* 2004).

2.3.2 Fine-sediment sampling and analysis

Fine sediments were collected from the riverbed using a disturbance technique (Collins & Walling, 2007; Duerdoth *et al.,* 2015; Jones *et al.,* 2015). To sample suspended sediments, an open-ended, cylindrical polyethylene bucket (height 75 cm; diameter 48.5 cm) was carefully inserted into the water column. The water column within the cylindrically shaped container was then agitated using a wooden pole about 15 cm long. The agitation of the water column was done to avoid disturbing the stream bed. While the water was still vigorously in motion, fine-sediment samples were collected and then filtered through a 2000 µm-pore size sieve into 250 ml acid-washed sampling bottles. Filtration removed particles larger than 2000 µm, such as debris.

To sample the settled fine sediment, the same protocol was followed, but the streambed was agitated to mobilise settled fine sediments into suspension. Once in suspension, samples were scooped and filtered, as described above. Samples of suspended and settled sediment were transported to the laboratory and refrigerated until analysed.

Fine-sediment (suspended and settled) grain sizes were characterised using the Mastersizer 3000 laser diffraction particle size analyser (Figure 2.11) designed to measure particle sizes in the range of 0.02 µm to 2000 µm. Prior to fine-sediment-particle size analysis, the samples were transferred from the 250 ml sampling bottle into a 500 ml beaker. The sediment samples were then left to stand for 24 hours to allow the sediments to settle, after which part of the water solvent on the sediments samples was gently emptied, leaving only the sediment particles remaining. Thereafter, the sediments samples were put in a hot plate stove to dry for a maximum period of 48 hours at a temperature of 55°C (Figure 2.11).

The hot plate-dried sediment samples were crushed with a mortar and pestle to ensure homogeneity. About 0.3–0.5 g of the crushed samples was transferred into a 40 ml beaker and then filled up to 30 ml; 10 ml of dispersant (sodium hexametaphosphate) was added to the beaker to disperse fine-sediment grain sizes evenly. An empty 500 ml beaker was filled with tap water and placed onto the hydro EV unit of the Mastersizer, spinning at 3000 rpm. The prepared fine-sediment grain sizes were slowly added using a teaspoon (taking small portion of sediments) into the 500 ml beaker containing water until an obscuration range was reached. Once the obscuration range was reached, no further grains were added, but spinning continued for about 5–6 minutes until fine-sediment grain sizes were distributed and displayed in µm units. The fine-sediment grain sizes output from the Mastersizer 3000 was further analysed for particle distribution using the GRADISTAT version 8.0 (Blott, 2010) and separated into size fraction as shown in Table 2.2.

Figure 2. 11: Sediment samples left to settle out of solution (left) and those placed on a hot plate for evaporation (right).

Table 2. 2 : Grain-size classes as used in this study (adapted from Blott, 2010).

Particle size description	**Size range (µm)**
Sand	
Very coarse sand	>2000 –1000
Coarse sand	1000 – <2000
Medium sand	500 – <1000
Fine sand	250 – <500
Very fine sand	125 – <250
Silt	
Very coarse silt	63 – <125
Coarse silt	31 – <63
Medium silt	16 – <31
Fine silt	8 – <16
Very fine silt	4 – <8
Clay	<4

2.4 Macroinvertebrate sampling

Concurrent with physico-chemical sampling, macroinvertebrates were collected using a kick net (dimension 300 x 300 mm frame and 1000 µm mesh) in accordance with the South African Scoring System version 5 protocol (SASS5) (Dickens & Graham, 2002). Three different biotopes inhabited by macroinvertebrates were sampled per site and on each sampling event. The biotopes were stones (stones-in-current (SIC) and stones-out-of-current (SOOC)), vegetation (marginal and aquatic vegetation), and sediments (gravel, sand and mud (GSM)). The SIC comprised pebbles and cobbles (2–25 cm), and boulders greater than 25 cm located in current that prevents the deposition of fine sediments. The SOOC included pebbles and cobbles, and boulders in pools that allowed fine sediments to settle. The marginal vegetation comprised vegetation growing on the edges of the riverbank and eventually fringing into the river, whereas aquatic vegetation was frequently submerged in the main river channel. The GSM consisted of small stones, ranging between 2 cm and 0.06 mm in diameter and fine sediments e.g. sand, silt and clay. Sampling the three biotopes was to ensure all microhabitats used by macroinvertebrates were taken into account.

During each sampling period, three replicate samples representing each biotope (vegetation, stone and GSM) were collected, making a total of nine samples per site on each sampling occasion. Throughout the study, a total of 144 samples was collected per site, making 1152 samples for the eight sites for the study period. Collected macroinvertebrate samples were preserved in 70% ethanol, transported to the laboratory. In the laboratory, samples were sorted and identified to the family level using keys described by Gerber & Gabriel (2002).

2.5 Statistical analysis

Data in this study were subjected to appropriate univariate and multivariate statistical tests. Data used in different statistical software were first arranged as data matrices in Microsoft Excel (2010 version) and afterward exported, depending on the statistical packages used. The purpose of this subsection is to briefly describe the statistical tests employed in this study in Chapters 3, 4 and 5.

2.5.1 Multivariate analysis of variance (MANOVA) and Analysis of variance (ANOVA)

Multivariate analysis of variance (MANOVA) is a parametric statistic that compares the means between two or more samples. MANOVA simultaneously uses multiple independent variables to compare the sites in terms of the sediment particle size distribution and the physico-chemical variables. When using MANOVA, the basic assumptions of normality and homogeneity of variance need to be examined using the Shapiro-Wilk test and Levene's test, respectively. If assumptions were not met, then data were transformed logarithmically, but normalised if assumptions were still not met. The one-way analysis of variance (ANOVA) was undertaken to compare the sites in terms of proportions of grain sizes as well as physico-chemical variables**.** When ANOVA indicated a globally significant difference, a post-hoc test, the Tukey's Honestly Significant Difference (HSD) test was used to indicate the sites that differed. The MANOVA and ANOVA were employed in Chapter 3 as statistical test methods for the sediment particle size and the physico-chemical variables. The MANOVA, ANOVA and Turkey's HSD tests were conducted using the Statistica software package version 13.

2.5.2 Kruskal-Wallis test

The Kruskal-Wallis multiple comparison test was used to explore the significant differences between the sites (TSU, TSD, QHR, MLU, MLD and CLS) to assess the discriminatory potential of selected metrics between the sampling sites (Chapter 3). The test was also applied in Chapter 5 to ascertain differences between the sites in terms of the relative abundance of macroinvertebrate groups classified as either vulnerable or resilient to fine-sediment impacts. The Kruskal-Wallis test was conducted using the Statistica software package version 13.

2.5.3 Spearman's rank and Pearson correlation coefficients

Spearman's rank correlation coefficient (r) was performed on the seasonally stable metrics to explore co-linearity between the metrics. Spearman's rank correlation is employed to describe the simultaneous change of random variables that are not functionally dependent on each other (Marques de Sá & Frias, 2007). Spearman's rank correlation is the non-parametric counterpart of the Pearson correlation coefficient (Ogbeibu, 2005). In Chapter 3, it was used to test for metric redundancy. When two or more metrics were highly correlated ($r > 0.65$), only one of such metrics was retained for integration into the multimetric index developed. The Pearson correlation was used to evaluate the relationship between the macroinvertebrate metrics, the sediment particle sizes, turbidity and physico-chemical variables (Chapter 3). Prior to correlation analysis, the data were transformed using a natural logarithm (log x + 1) to meet the assumption of normality. The analysis was undertaken using Statistica software version 13.

2.5.4 Fourth-corner

A fourth-corner test is a multivariate test that offers a global picture of the traits-environment relationships (Dray *et al.,* 2014; Akamagwuna *et al.,* 2019, Odume, 2020). The fourth-corner test reveals the traits and ecological preferences that either negatively or positively correlate with given physico-chemical variables. The test was applied in Chapter 4 to examine the correlations between traits and environmental variables using the ade4 package in R software version 3.4.1 (Dray & Dufour, 2007; R Core Team, 2017).

2.5.5 Ordinations

Detrended correspondence analysis (DCA)

A detrended correspondence analysis (DCA) is a test suitable for exploring the distribution of data to determine whether they are unimodally or linearly distributed (ter Braak, 1995; Xu *et al.,* 2012). Detrended correspondence analysis is relevant for choosing one of the two ordination tests (CCA or redundancy analysis). If a DCA returns a gradient length >3, a CCA is more suitable, and when DCA returns a gradient length of <3 standard deviation (SD), an RDA is more appropriate (ter Braak & Verdonschot, 1995). A DCA was computed for macroinvertebrate metrics selected in Chapter 3 to determine the gradient length of macroinvertebrate data sets prior to selecting either a CCA or an RDA. Detrended correspondence analysis was plotted using Vegan package version 2.5.4 in the R programming environment (Oksanen *et al.,* 2015; R Core Team, 2019).

Redundancy analysis (RDA)

A redundancy analysis (RDA) is a multivariate ordination analysis that elucidates the relationship between biological community structure and environmental variables for linear data sets (ter Braak & Verdonschot, 1995; Legendre & Legendre, 2012). Redundancy analysis was used in Chapter 3 in relating the selected metrics with the physico-chemical variables. To explore the spatial distribution of suspended and settled fine-sediment concentrations as well as the grain sizes, a redundancy analysis (RDA) was undertaken. Two separate RDA ordination plots were constructed, one for suspended fine sediments and the other for settled fine sediments. The decision to use RDA instead of a CCA was informed by a DCA, which returned a gradient length <3 suggesting that the data met the linear assumption of an RDA (Akamagwuna *et al.,* 2019). RDA analyses were run separately for the dry and wet seasons data sets.

RLQ test

The RLQ test is a multivariate statistical analysis that performs multiple interactive ordinations and permutations on three matrices: environmental variables, taxa abundance, and trait data (Dolédec *et al.,*1996). It was used in this study in Chapter 4 to relate environmental variables (physico-chemical variables) (R) to macroinvertebrate taxa (L) and the traits and ecological preferences (Q).

2.5.6 Linear regression

To assess the predicted responses of macroinvertebrate taxa to sediment impact, the relative abundance and richness of macroinvertebrates belonging to designated groups (Chapter 5) were regressed against TSS, turbidity and embeddedness using linear regression analysis. Linear regression analyses were undertaken using the STATISTICA software package version 13.3.

CHAPTER 3: CHARACTERISING FINE-SEDIMENT DISTRIBUTION AND DEVELOPING A SEDIMENT-SPECIFIC MULTIMETRIC INDEX (SMMI) FOR ASSESSING THE EFFECTS OF FINE SEDIMENTS IN THE TSITSA RIVER AND ITS TRIBUTARIES

3.1 Introduction

Elevated fine sediments are known to be freshwater ecosystem stressors (Jones *et al.,* 2012). Fine-sediment accumulation in rivers may result in changes to channel morphology, microhabitat modification, and alteration of biological assemblages, causing both structural and functional impacts (Owens, 2005; Jones *et al.,* 2012; Wilkes *et al.,* 2017). Elevated fine-sediment inputs can originate from several anthropogenic sources, such as agriculture (Benoy *et al.,* 2012; Burdon *et al.,* 2013), deforestation, clear-cut practices (Couceiro *et al.,* 2010), road construction (Kaller & Hartman, 2004; Cocchiglia *et al.,* 2012), mining activities (Smolders *et al.,* 2003; Pond *et al.,* 2008), damming and river flow regulation (Dunbar *et al.,* 2012). Natural processes that may contribute to elevated fine-sediment input into freshwater systems may include easily erodible duplex soils (Marzen *et al.,* 2019) and a steep elevation that accelerates sediment influx into nearby riverine systems (Macklin & Woodward, 2009).

Elevated concentrations of suspended sediments may impair fine biological structures such as gills and filter-feeding apparatus of organisms through clogging. Impairment of biological structures, such as gills, may have a devastating effect on respiration and the overall metabolic performance of affected organisms (Jones *et al.,* 2012; Akamagwuna, 2018). Furthermore, the impact on feeding structures may severely impair feeding behaviour or rate of food uptake, which may constrain growth and functional performances, such as energy transfer and material fluxes in the ecosystems. Elevated concentrations of settled fine sediments have also been alleged to result in the burial of less motile organisms (Mckenzie & Jackson, 2020). Elevated concentrations of settled fine sediments can indirectly impact biological organisms by modifying microhabitats, for example, by filling interstitial spaces in bed substrates and reducing food availability by smothering periphyton (Buendia *et al.,* 2013; Herrera *et al.,* 2016). Many freshwater organisms, such as macroinvertebrates, prefer interstitial spaces as habitats, mainly during their early developmental stages; thus, changes to interstitial spaces may affect the assemblage distribution of such organisms (Jones *et al.,* 2012).

Several fine-sediment-related factors may mediate their effects on organisms and overall ecosystems. These factors include sediment geochemical composition, concentrations in the impacted environments, fine-sediment shapes, and grain sizes (Guagliardi *et al.,* 2012). Sediment grain sizes are particularly important because experimental studies have demonstrated that the severity of the effects of fine sediments are mediated by their sizes (Yang *et al.,* 2019). For example, Herrera *et al.* (2016) have demonstrated that smaller grain sizes affect organisms more severely because the grains can easily clog fine biological structures, have greater surface area for adsorption of toxic chemicals, and organisms, such as macroinvertebrates, buried in them take longer to escape from the burial. What these experimental studies demonstrate is the importance of characterising the distribution of fine-sediment grain sizes in field studies, while also measuring their concentrations, a task fulfilled in the current study.

In South Africa, the effects of water quality impairment on macroinvertebrate assemblage structure are usually assessed using the South African Scoring System version 5 (SASS5), reviewed in detail in Chapter 1. However, when the SASS5 method was applied to study the effects of elevated sediments, it was found to perform poorly (Gordon *et al.,* 2013); it did not indicate any significant results on sediment effects in freshwater systems. Its poor performance could be attributed to the fact that SASS5 was originally developed to assess the effects of organic pollution, even though it is now widely applied to several other water quality impairments, as well as flow regime and geomorphological alteration (Dickens & Graham, 2002) Mokgoeba, 2019; Feio *et al.,* 2020). The complexity of interactions between elevated fine-sediment and biological communities, as well as the growing instances of fine-sediment depositions in freshwater ecosystems globally, has led to the development of sediment-specific biomonitoring tools (e.g., Extence *et al.,* 2013; Turley *et al.,* 2016; Gieswein *et al.,* 2019). In South Africa, despite several riverine systems suffering from impacts of elevated fine-sediment deposition (Gordon *et al.,* 2013), no sediment-specific biomonitoring tool has been developed for effective monitoring and management of fine-sediment effects. Since SASS5 has been demonstrated as ineffective for monitoring the effects of fine sediments, particularly of inorganic sources, it is argued that the development of a sediment-specific biomonitoring tool will accelerate efforts towards a better understanding of the extent to which elevated fine sediments shape biological assemblage structure in South African riverine systems.

This study follows a macroinvertebrate-based multimetric approach in developing a sediment-specific biomonitoring tool using the Tsitsa River and its tributaries as case studies. A multimetric approach brings together multiple criteria of biological information such as diversity, richness, abundance, and composition to better understand stressor-induced structuring of biological assemblages (Odume *et al.,* 2013, Doretto *et al.,* 2018; Gieswein *et al.,* 2019). Thus, the objectives of this chapter are to i) characterise suspended and settled fine- sediment grain sizes and their distribution in the Tsitsa River and its tributaries, and ii) to develop and validate a macroinvertebrate-based, sediment-specific, multimetric index suitable for monitoring effects of elevated fine sediments in the Tsitsa River and its tributaries. This chapter thus addresses objectives 1 and 2 of this study as stated in Chapter 1, Section 1.7.2.

3.2 Materials and methods

3.2.1 Sampling sites

Eight sampling sites in four river systems were selected for the study and were sampled seasonally: dry season (winter; June, July and August; spring: September, October and November) and wet season (summer: December, January and February; autumn: March, April and May) for two years (August 2016–March 2018). The selected sites included two sites in the Tsitsa River (upper and lower reaches), one site in the Qurana River, two sites in the Millstream, two sites in the Pot River (upper and lower reaches), and one site in the Little Pot River. The Tsitsa River is the main stem, and other rivers are its tributaries. Sites in the Tsitsa and Qurana Rivers were selected as sites that receive a high influx of fine sediments, whereas sites in the Millstream were selected as those that are moderately sedimented. The sites in the Pot and Little Pot Rivers were selected as examples of sites less influenced by fine-sediment deposition as fully described in Section 2.2.5, Chapter 2.

3.2.2 Physico-chemical and macroinvertebrate sampling

Water physico-chemical variables, including dissolved oxygen (DO), electrical conductivity (EC), temperature, and pH were measured on site using the Hanna multiparameter probe (model H198). Turbidity was also measured on site using a portable probe, the Orbeco-Hellige 966 meter. Water samples were collected and transported to the laboratory for analysis of nitrate-nitrogen (NO_3-N), nitrite-nitrogen (NO_2-N), ammonium-nitrogen (NH_4-N), orthophosphate-phosphorus (PO_4-P), and total inorganic nitrogen (TIN) as described in Chapter 2, Section 2.3 (APHA *et al.,* 1971; Odume & Mgaba, 2016). Suspended fine-sediment concentration was measured as total suspended solids (TSS), whereas embeddedness was measured as a surrogate for settled fine sediments. Sediment grain sizes were also analysed for each site per sampling occasion. Fine-sediment grain sizes, both suspended and settled, were classified following Blott (2010), as described in Chapter 2, Section 2.3.2. All physico-chemical measurements were taken seasonally for two years, making it a total of eight occasions between August 2016 and March 2018. A full description of the sampling and analysis regimes is given in Chapter 2, Section 2.3.

Macroinvertebrate samples were collected at each sampling site per sampling occasion, following the collection protocol described by Dickens & Graham (2002). At each site per sampling event, three replicate samples were collected from stones, vegetation, and GSM, making a total of nine replicate samples per site per sampling occasion. A detailed description of the macroinvertebrate sampling strategy is provided in Section 2.4, Chapter 2. The multivariate analysis of variance (MANOVA) was used to test the significant differences ($P < 0.05$) in terms of the means of the physico-chemical variables and sediment particle sizes across the eight sites. Prior to MANOVA, the basic assumption of normality and homogeneity of variance was tested, and normality applied when necessary, using square root transformation. The analysis was carried out as described in Chapter 2, Section 2.5.1. and ANOVA was used to compare the sites in terms of grain sizes as well as physico-chemical variables as fully described in Chapter 2, Section 2.5.1.

3.2.3 Spatial distribution of suspended and settled sediment grain sizes

To explore the spatial distribution of suspended and settled fine-sediment concentrations as well as the grain sizes, a redundancy analysis (RDA) was undertaken. Two separate RDA ordination plots were constructed, one for suspended fine sediments and the other for settled fine sediments, as described in Section 2.5.5 in Chapter 2. The decision to use RDA instead of canonical correspondence analysis (CCA) was informed by a detrended correspondence analysis (DCA), which returned a gradient length <3, suggesting that the data met the linear assumption of an RDA. The RDA analyses were run separately for the dry and wet seasons.

3.2.4 Development of a sediment-specific multimetric index (SMMI) for the Tsitsa River and its tributaries

The SMMI was developed following a five-step approach: i) selection of candidate metrics; ii) testing selected metrics for their potential to discriminate the control sites from the rest of the sampling sites; iii) testing metrics for redundancy; iv) integrating selected metrics into a unified multimetric index, and v) testing and validating the developed SMMI. The SMMI was developed using the first-year data and then tested and validated using data collected during the second year.

3.2.5 Selection of candidate metrics

A total of 21 candidate metrics was selected based on the review of literature on macroinvertebrate responses to fine-sediment impact (Giesiwen *et al.,* 2019, Doretto *et al.,* 2018). The selected candidate metrics are summarised in Table 3.1.

Table 3. 1: Candidate metrics selected for the development of the sediment-specific multimetric index at family level for the Tsitsa River and its tributaries. Unless otherwise stated, metrics are defined according to Odume *et al.* (2012).

Candidate metrics	**Definition**	**Metric codes**	**Predicted response to sediment impact**
Abundance measures			
Chironomidae abundance	The absolute number of individuals in Chironomidae family	Chi Abun	+
Chironomidae/Diptera abundance	The absolute number of individuals in Chironomidae family divided by those of Diptera taxa	Chi/Dip Abun	+
Gastropoda abundance	The absolute number of individuals in Gastropoda taxa	Gastr Abun	+
Crustacea abundance	The absolute number of individuals in Crustacea taxa	Cru Abun	+
Composition measures			
%1- GOLD (Gastropoda, Oligochaeta, and Diptera)	1-GOLD describes the relative proportion of Gastropoda, Oligochaeta, and Diptera in the community/sample (Buffagni *et al.*, 2008)	%GOLD	+
%Ephemeroptera, Plecoptera and Trichoptera/%Chironomidae	%EPT ratio divided by the ratio of %Chironomidae in the sample (Hieber *et al.*, 2005; Fenoglio *et al.*, 2015; Doretto *et al.*, 2018) −	%EPT/%Chiro	-

%Diptera	Percentage of individuals in Diptera relative to the entire sample	%Dip	+
%Ephemeroptera, Trichoptera, Odonata, and Coleoptera	Percentage of individuals in Ephemeroptera, Trichoptera, Odonata and Coleoptera relative to the entire sample.	%ETOC	-
%Gastropoda	Percentage of individual Gastropods relative to the entire sample.	%Gas	+
%Chironomidae	Percentage of individual Chironomids relative to the entire sample.	%Chi	+
Richness measures			
Ephemeroptera, Plecoptera and Trichoptera richness	The absolute number of taxa in Ephemeroptera, Trichoptera, and Coleoptera	EPT Rich	-
Ephemeroptera richness	The absolute number of taxa in Ephemeroptera	Eph Rich	-
Trichoptera richness	The absolute number of taxa in Trichoptera	Tri Rich	-
Diptera richness	The absolute number of taxa belonging to Diptera	Dip Rich	+
Ephemeroptera, Trichoptera and Odonata richness	The absolute number of taxa belonging to Ephemeroptera, Trichoptera and Odonata.	Hem Rich	-
Gastropoda richness	The absolute number of taxa belonging to Gastropoda	Gas Rich	+
Plecoptera richness	The absolute number of taxa belonging to Plecoptera	Plec Rich	-
Diversity measures			
Evenness index	Measures the relative even distribution of the abundance of taxa	Eve Ind	-

	within a sample (Clarke and Warwick, 1994).		
Margalef's index (Taxa diversity index)	Accounts for both the number of taxa and individuals and is independent of sample size (Ogbeibu, 2005).	Mar Ind	-
Shannon diversity index	Information statistical index which takes account of the contribution of individual taxa to the diversity while assigning greater weight to most dominant taxa (Ogbeibu, 2005).	Sha Ind	-
Simpson diversity	Weighted towards the abundance of commonest families (Ogbeibu, 2005).	Sim Div	-

3.2.6 Testing selected metrics for their potential to discriminate the control sites from the rest of the sampling sites

The 21-candidate metrics were tested for their potential to differentiate between the control sites and the elevated fine-sediment-influenced sites. The discriminatory test was carried out using box plots (Baptista *et al.,* 2007; Odume *et al.,* 2012). A metric was considered to have a satisfactory discriminatory potential if the interquartile ranges of the control sites did not overlap with those of the sediment-influenced sites; or the interquartile ranges overlapped, but the means did not. Similar criteria are widely selected to determine a metric's discriminatory potential in the literature (e.g., Baptista *et al.,* 2007; Odume *et al.,* 2012; Gieswein *et al.,* 2019, Edegbene, 2020). Metrics that were considered to have satisfactory discriminatory potential were further subjected to a Kruskal-Wallis multiple comparison test for confirmation at $P > 0.05$. Only metrics that were considered satisfactory in terms of their discriminatory potential based on the results of the box plots and Kruskal-Wallis test were retained and subjected to further testing.

3.2.6 Testing metrics for redundancy

Metrics that scaled through the test of discrimination were subjected to a redundancy test using the Spearman's rank correlation test ($r > 0.65$; $P < 0.05$). When two or more metrics were redundant, only one of such redundant metrics was retained for integration into the multimetric index.

3.2.7 Integration of metrics into the multimetric index

The retained metrics were integrated into the SMMI. A multimetric index requires standardisation of numeric values of each metric before integration because the different metrics, for example EPT richness and Shannon diversity, have different numeric scales (Odume, 2013). The numeric values of the retained metrics were standardised by dividing the metric values into three possible scores for each metric according to the method described by Baptista *et al.* (2007). To standardise the numeric values of the metrics, the minimum, lower quartile (25%), mid-quartile (50%), upper quartile (75%) and maximum values per metric for the control sites (CLS) during the dry season were calculated and used as the basis for scoring the numeric values of metrics at all other sites. Only the assemblages during the dry season at the CLS were used because fine-sediment influx is seasonally mediated, with the wet season likely to contribute higher sediment deposition into the receiving river systems.

For metrics expected to increase in numeric value with higher sediment impact, if the numeric value at TSU, TSD, MLU, MLD sites (sediment-impacted sites) was lower than the upper quartile (75%) of the CLS assemblage distribution, it was scored 5, and if it was between the upper quartile and maximum value of the CLS assemblage, it was scored 3. A score of 1 was awarded to the metric value if it was greater than the maximum value of that metric for the CLS assemblage (Odume, 2013). For metrics predicted to decrease in numeric value with increasing sediment impact, if the numeric value at the impacted sites was greater than the lower quartile (25%) of that metric for the CLS assemblage distribution it was scored 5, and if it was between the minimum and the lower quartile of the metric for the CLS assemblage, it was scored 3. A score of 1 was awarded if the value was less than the minimum value for the CLS assemblage for that metric (Baptista *et al.,* 2007). Therefore, subject to the predictable response of the metric to impact, thresholds based on the appropriate quartiles were established for each metric, using the CLS assemblages so that a score 5 shows that the numeric value of the metric does not differ from those at the CLS; a score 3 indicates a moderate deviation from that at the CLS, and a score 1 indicates the greatest deviation from the numeric value at the CLS (Odume, 2013).

Validation and application of the SMMI

After standardising the metric values using the scoring system, the SMMI was then calculated by summing scores of the component metrics and the final index value range divided into five categories, corresponding to A (naturally sedimented), B (minimally sedimented), C (moderately sedimented), D (seriously sedimented), and E/F (critically sedimented).

3.3 Results

3.3.1 Physico-chemical variables

The means, standard deviations, and ranges of the basic physico-chemical variables including temperature, dissolved oxygen (DO), pH, nutrients (NO_2-N-, NO_3-N, NH_4-N, PO_4-N, and TIN) and electrical conductivity (EC) recorded in the Tsitsa River and its tributaries for the current study are presented in Table 3.2. With the exceptions of electrical conductivity which was significantly different across the sites, the rest of the basic physico-chemical variables were not statistically significantly different (Table 3.2). A two-way MANOVA indicated a significant difference between the sites, but not between the seasons and between sites and season (Table 3.3. $P > 0.05$) Generally, the results indicate that the values for physico-chemical variables across the sites were similar and were also similar between the seasons (Table 3.3).

Table 3. 2: Mean ± standard deviation and range (in brackets) of physico-chemical variables measured in the Tsitsa River and its tributaries for two years (August 2016–March 2018). P-value as indicated by ANOVA is provided only for EC, being the only variable that was statistically significantly different. Different superscript letters for EC across sites indicate significant differences ($P < 0.05$) revealed by Tukey HSD post-hoc test. The same superscript letter between sites for EC indicates no significant differences ($P > 0.05$).

Water quality variables	**TSU**	**TSD**	**QHR**	**MLU**	**MLD**	**CLS**	***P*-value**
Electrical Conductivity (mS/m)	66.3 ± 20.4^{a} (43.0-93.0)	108.8 ± 64.2^{a} (38.0-246.0)	88.9 ± 41.8^{ba} (49.0-175.0)	64.9 ± 35.3^{bc} (39.0-146.0)	66.3 ± 21.8^{ab} (35.0-105.5)	53.1 ± 14.2^{bc} (39.0-74.0)	0.003
Dissolved Oxygen (mg/L)	8.8 ± 6.9 (3.03 - 11.09)	9.0 ± 18.0 (4.6 - 21.0)	6.7 ± 5.8 (2.7-10.0)	6.4 ± 4.6 (2.5 -14.2)	6.8 ± 5.2 (8.7 - 15.0)	6.9 ± 5.8 (4.3 - 17)	
pH	7.3±0.4 (6.7-7.9)	7.6±0.5 (6.7-8.1)	7.3±0.7 (6.4-8.3)	8.5±4.3 (4.5-18.7)	7.2±0.8 (5.1-8.2)	7.5±0.7 (5.9-8.2)	
Nitrate-Nitrogen (NO_3-N) (mg/L)	1.35±1.50 (0.01-3.80)	1.9±1.5 (0.0-3.9)	2.4±2.8 (0.0-8.1)	2.9±2.3 (0.2-7.2)	1.8±2.5 (0.3-7.2)	2.8±3.5 (0.-10.1)	
Nitrite-Nitrogen (NO_2-N) (mg/L)	1.12±0.99 (0.08-2.30)	1.1±1.1 (0.0-3.2)	1.2±1.8 (0.0-5.2)	0.8±0.9 (0.0-2.6)	0.9±0.9 0.0-0.2	1.1±2.1 (0.0-6.2)	
Ammonia-Nitrogen (NH_3-N) (mg/L)	0.13±0.15 (0.02-0.73)	0.5±0.5 (0.1-1.1)	0.3±0.4 (0.1-0.7)	0.2±0.3 (0.1-0.9)	0.6±0.8 (0.2-1.2)	0.1±0.2 (0.1-0.7)	
Orthophosphate –Phosphorus (PO_4-P) (mg/L)	0.5±1.27 (0.2-3.6)	0.5±1.1 (0.2-3.1)	0.5±2.1 (0.1-3.1)	0.4±0.6 (0.2-1.6)	0.4±1.0 (0.1-2.9)	1.0±1.4 (0.9-1.6)	
Total inorganic nitrogen (TIN) (mg/L)	0.7±1.21 (0.02-3.66)	2.2±2.0 (0.6-6.6)	1.4±1.3 (0.0-3.7)	1.6±2.0 (0.0-6.1)	1.5±1.1 (0.1-3.3)	2.4±1.9 (0.1-5.6)	
Temperature (^{0}C)	16.43±6.38 (6.3-24.0)	18.4±5.4 (11.3-28.0)	18.5±4.1 (12.3-24.0)	17.3±5.8 (6.3-24.5)	17.8±6.0 (5.8-24.0)	19.2±6.8 6.7-28.8	

Table 3. 3: MANOVA results for the physico-chemical variables between the sampling sites and the seasons, indicating a significant difference between the sites but not between the seasons, and interaction between sites and seasons during the study period (August 2016–March 2018).

Effect	Test	Value	F	Effect df	Error df	*P*-value
Intercept	Wilks	0.01	223.6	10	23.0	0.00
Sites	Wilks	0.01	6.56	30	68.18	**0.00**
Season	Wilks	0.677	0.981	10	13.00	0.67
Sites*season	Wilks	0.178	0.762	41	3200	0.45

3.3.2 Spatio-temporal variation in suspended and settled fine-sediment concentrations

In this study, total suspended solids (TSS) and turbidity were used as proxy measures for the concentrations of suspended fine-sediment concentrations, whereas embeddedness was used as a proxy measure of settled fine-sediment concentrations as shown in Figure 3.1. As expected, TSS and turbidity were consistently higher during the wet seasons than in the dry seasons (Figure 3.1). The exception was at the control sites (CLS) where these variables did not display a noticeable seasonal trend. When viewed across the sites, TSS and turbidity were consistently higher at the two sites within the Tsitsa River than in the sites in the remaining rivers. The TSS value at the sites in the Tsitsa River reached an average maximum value of 10 000 mg/L during the wet season. Embeddedness was higher at the Tsitsa River and Qurana showing a low percent of uncovered substrates, signifying that the Tsitsa and Qurana rivers were highly embedded, while the control sites were less embedded and showed a high percent of uncovered substrates.

The two-way MANOVA indicated global significant differences between the sites, and between the seasons (Table 3.5). The two-way MANOVA also revealed that the interaction between the sites and seasons were statistically significant. Following MANOVA, One- way ANOVA was conducted to identify the variables responsible for the differences between the sites and to identify which of the sites differed. The ANOVA results revealed that all three sediment-related variables were significantly different between the sites (Table 3.4).

Table 3.4: MANOVA results for total suspended solids, turbidity, and embeddedness between the sampling sites and seasons, indicating significant difference ($P < 0.05$) during the study period (August 2017–March 2018).

Effect	Test	Value	F	Effect df	Error df	P
Intercept	Wilks	0.006	813.79	2	11	0.000
Sites	Wilks	0.015	15.644	10	22	**0.000**
Season	Wilks	0.926	0.433	2	11	**0.000**
Sites*season	Wilks	0.280	1.955	10	22	**0.003**

Table 3.5: Mean standard deviation and range (in brackets) for embeddedness, turbidity and total suspended solids for the Tsitsa River and its tributaries for a period 2 year (August 2017-March 2018). P values are indicated by Two-way ANOVA. Different superscript letters for embeddedness, total suspended solids and Turbidity across sites indicate significant differences (P < 0.05) revealed by Turkey's HSD post-hoc test. The same superscript letter between sites per variable indicates no significant differences (P > 0.05).

Sediment indicator variables	TSU	TSD	QHR	MLU	MLD	CLS	P Value
Embeddedness (%)	4.6±5.38 (39.0-74.0)^{ab}	2.6±4.58 (35.0-105.5)	4.5±5.55 (4.00-5.000)	2.9±3.68 (1.98-3.45)	1.5±2.57 (1.00-200)	1.0±2.00 (1.00-1.00)^{ac}	0.000
Total suspended solids (mg/L)	10883 ± 11220 (1333-34567)^{ab}	2095 ± 2353 (198.3-9268)^{b}	9120 ± 10656 (2310-34567)^{b}	5404 ± 9883 (198 -34618)^{b}	5727 ± 8286 (231.0 - 19604)^{ab}	2265 ± 3456 (1988-16946)^{ad}	0.019
Turbidity (NTU)	4.10 ± 8.04 (2.40 -10.03)^{a}	20.04 ± 20.11 (1.38-8.05)^{ab}	17.8 ± 6.0 (5.8 - 24.0)^{ab}	0.8 ± 0.24 (0.25 - 0.99)^{b}	0.4 ± 1.0 (0.0 - 2.9)^{ab}	0.1 ± 0.2 (0.0 - 0.6)ab	0.001

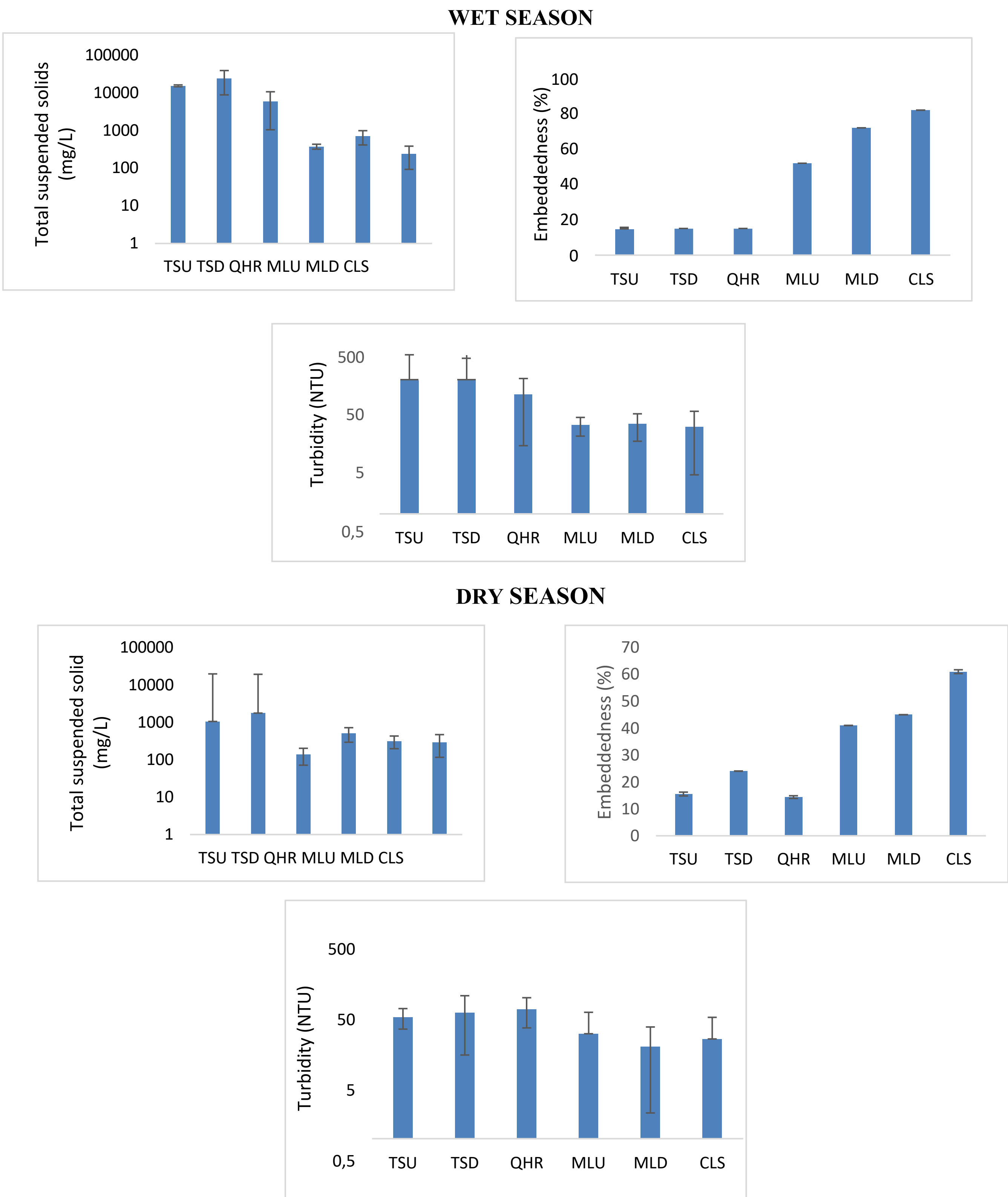


Figure 3. 1: Means and standard deviations (bars) for total suspended solids, embeddedness and turbidity for the Tsitsa River and its tributaries during the study period. Results are represented for wet and dry seasons across sites. Abbrevations: TSU (Tsitsa upstream), TSD (Tsitsa downstream), QHR (Qurana tribituary), MLU (Millstream upstream), MLD (Millstream downstream) and CLS (control site).

3.3.3 Spatio-temporal distribution of suspended and settled grain sizes

The distribution of suspended fine-sediment grain sizes was analysed for the Tsitsa River and its tributaries over the study period. The sites (i.e., TSU, TSD, and QHR) situated in the Tsitsa and Qurana rivers, which were highly impacted by fine sediments, were mainly dominated by very fine sand, very fine silt, medium silt, very coarse silt, and clay as shown in Table 3.6, whereas the two sites situated in the Millstream (i.e. MLU and MLD) were primarily dominated by clay, constituting more than 60% of the volumetric grain size at the two sites. Grain sizes within the control sites seemed to be evenly distributed, with the dominant grain size (i.e., coarse silt) constituting only 15% of the volumetric grain size within the control sites (Table 3.6). The combined interactive effects of the grain sizes were statistically significant (P <0.05) across the sites, but not across the two seasons as shown in (Table 3.7). The interactions between the sites and seasons in terms of the suspended sediment particle sizes were not statistically significant. Because MANOVA indicated global combined interactive effects across the sites for suspended fine-sediment grain sizes, one-way ANOVA, followed by a Tukey's post-hoc test was carried out to indicate where the significant differences lay. The one-way ANOVA results indicated that very fine sand, very coarse silt, medium silt, and fine silt were significantly higher at TSU, TSD, QHR than at MLD, MLU, and CLS. The rest of the grain sizes did not differ statistically between the sites.

In terms of the settled sediment grain sizes, the volumetric analysis did not show considerable differences across the sites (Table 3.8). Settled fine-sediment grain sizes were evenly distributed across the sites. Statistically, MANOVA results indicated no significant differences across sites or across seasons. The interactive effect between seasons and sites was also not statistically significant (Table 3.9).

Table 3. 6: Means, ± standard deviations, and ranges (in parentheses) for distribution of suspended fine-sediment grain sizes across sites in the Tsitsa River and its tributaries. Different superscript letters for very fine sand, very coarse silt, medium silt and fine silt across sites indicate significant differences ($P < 0.05$) revealed by Tukey HSD post-hoc test. The same superscript letter between sites per variable indicates no significant differences ($P > 0.05$).

Suspended sediment grain size (µm) (Volumetric fraction of 1)	TSU	TSD	QHR	MLU	MLD	CLS	p-value
Coarse sand	0.15 ± 0.19 (0.02–0.49)	0.13 ± 0.18 (0.01–0.47)	0.02 ± 0.05 (0.04–0.14)	0.13 ± 0.17 (0.03–0.38)	0.01 ± 0.02 (0.04–0.04)	0.04 ± 0.01 (0.02–0.05)	
Medium sand	0.07 ± 0.09 (0.03–0.15)	0.06 ± 0.08 (0.01–0.18)	0.17± 0.033 (0.07–0.19)	0.07 ± 0.05 (0.03–0.15)	0.04 ± 0.02 (0.03–0.13)	0.01 ± 0.02 (0.03–0.07)	
Fine sand	0.13 ± 0.19 (0.03–0.39)	0.4 ± 0.10 (0.07–0.37)	0.02 ± 0.08 (0.073–018)	0.13 ± 0.23 (0.07–0.15)	0.04 ± 0.07 (0.09–0.36)	0.09 ± 0.03 (0.69–0.13)	
Very fine sand	0.77 ± 0.89 (0.44–0.96)^{ab}	0.21 ± 0.23 (0.14–0.67)^{a}	0.06 ± 0.08 (0.08–0.57)^{a}	0.09 ± 0.30 (0.23–0.92)^{a}	0.26 ± 0.35 (0.44–0.96)^{ad}	0.25 ± 0.28 (0.39–0.86)^{ac}	0.038
Very coarse silt	0.06 ± 0.08 (0.04–0.11)^{b}	0.06 ± 0.09 (0.01–0.26)^{a}	0.4 ± 0.23 (0.07–0.19)^{a}	0.06 ± 0.11 (0.08–0.36)^{bd}	0.03 ± 0.06 (0.17–0.37)^{ad}	0.03 ± 0.07 (0.17–0.33)^{a}	0.032
Coarse silt	0.05 ± 0.11 (0.08–0.9)	0.04 ± 0.05 (0.01–0.17)	0.06 ± 0.08 (0.70–018)	0.10 ± 0.14 (0.03–0.14)	0.11 ± 0.23 (0.67–0.14)	0.15 ± 0.11 (0.68–0.29)	
Medium silt	0.02 ± 0.01 (0.01–0.04)^{ac}	0.02 ± 0.04 (0.03–0.08)^{a}	0.4 ± 0.08 (0.04–0.15)^{a}	0.18 ± 0.19 (0.04–0.51)^{ac}	0.04 ± 0.09 (0.01–0.06)^{ac}	0.06 ± 0.04 (0.034–0.11)^{b}	0.027
Fine silt	0.27 ± 0.15 (0.48–0.59)^{a}	0.04 ± 0.06 (0.02–0.10)^{a}	0.2 ± 0.11 (0.09–0.36)^{bc}	0.02 ± 0.03 (0.01–0.08)^{bc}	0.02 ± 0.04 (0.01–0.22)^{b}	0.02 ± 0.06 (0.01–0.04)^{bc}	0.046
Very fine Silt	0.13 ± 0.03 (0.07–0.15)^{b}	0.23 ± 0.26 (0.01–0.69)^{bd}	0.69± 0.26 (0.25–0.99)^{b}	0.15 ± 0.20 (0.01–0.47)^{b}	0.04 ± 0.11 (0.005–0.38)^{b}	0.02 ± 0.06 (0.07–0.17)^{bc}	0.043

Table 3. 7: Multivariate analysis of variance MANOVA results for suspended sediment grain-size distribution between sites and seasons, indicating significant difference ($P < 0.05$) between sites during the study period (August 2016–March 2018).

Effect	Test	Value	F	Effect df	Error df	*P*-value
Intercept	Wilks	0.000	32727.6	11	8.000	0.000
Sites	Wilks	0.001	2.362	55	40.61	0.002
Season	Wilks	0.482	0.969	13	12.000	0.558
Sites*season	Wilks	0.740	2.361	55	8.000	0.255

Table 3. 8: Means, ± standard deviations, and ranges (in parentheses) for the distribution of settled fine-sediment grain sizes across sites in the Tsitsa River and its tributaries. Volumetric fraction of 1.

Settled fine-sediment grain sizes (µm)	TSU	TSD	QHR	MLU	MLD	CLS	*P*-value
Coarse sand	0.02 ± 0.07 (0.00–0.27)	0.01 ± 0.01 (0.00–0.05)	0.02 ± 0.04 (0.00–0.14)	0.01± 0.04 (0.00–0.13)	0.05 ±0.08 (0.00–06)	0.03±0.06 (0.00-14)	
Medium sand	0.04 ± 0.06 (0.00–0.18)	0.04 ± 0.06 (0.00–0.16)	0.02 ± 0.05 (0.00–0.22)	0.02 ± 0.07 (0.00–0.17)	0.04± 0.6 (0.006–18)	0.02±0.04 (0.00-0.14)	
Fine sand	0.06 ± 0.04 (0.00–0.13)	0.08 ± 0.04 (0.01–0.18)	0.09 ± 0.06 (0.03–0.20)	0.15 ± 0.02 (0.04–0.25)	0.08 ±0.09 (0.002–13)	0.02±0.04 (0.00-0.14)	
Very fine sand	0.21 ± 0.27 (0.00–0.51)	0.20 ± 0.37 (0.01–0.49)	0.31 ± 0.14 (0.09–0.56)	0.11 ± 0.12 (0.00–0.21)	0.08± 0.02 (0.02–12)	0.02±0.04 (0.00-0.14)	
Very coarse silt	0.08 ± 0.06 (0.00–0.20)	0.14 ±0.16 (0.02–0.23)	0.13 ± 0.04 (0.06–0.22)	0.04 ± 0.03 (0.01–0.14)	0.14 ±0.16 (0.02–0.23)	0.02±0.04 (0.00-0.14)	
Coarse silt	0.04 ± 0.03 (0.00–0.12)	0.06 ± 0.02 (0.02–0.12)	0.06 ± 0.02 (0.03–0.09)	0.08 ± 0.04 (0.01–0.18	0.06± 0.02 (0.03-0.09)	0.02±0.04 (0.00-0.14)	
Medium silt	0.05 ± 0.12 (0.00–0.50)	0.04 ± 0.03 (0.01–0.14)	0.03 ± 0.02 (0.01–0.09)	0.06 ± 0.04 (0.00–0.13)	0.03 ± 0.02 (0.01–0.09)	0.02±0.04 (0.00-0.14)	
Fine silt	0.01 ± 0.00 (0.00–0.02)	0.01 ± 0.01 (0.00–0.04)	0.02 ± 0.04 (0.00–0.18)	0.14 ±0.16 (0.02–0.23)	0.02 ± 0.04 (0.00–0.18)	0.02±0.04 (0.00-0.14)	
Very fine silt	0.05 ± 0.08 (0.01–0.33)	0.02 ± 0.02 (0.00–0.10)	0.03 ± 0.04 (0.00–0.12)	0.03 ± 0.02 (0.01–0.09)	0.03 ± 0.04 (0.00–0.12)	0.02±0.04 (0.00-0.14)	

Table 3. 9: Multivariate analysis of variance MANOVA results for the settled fine-sediment grain-size distribution between the sampling sites and seasons, indicating no significant difference ($P > 0.05$) between the sites, seasons and interaction between sites and seasons during the study period (August 2016–March 2018).

Effect	**Test**	**Value**	**F**	**Effect df**	**Error df**	***P*-value**
Intercept	Wilks	0.00	17201.64	10	23.000	0.00
Site	Wilks	0.03	1.53	70	140.928	0.57
Season	Wilks	0.495	0.834	12	14.000	0.683
Sites*Season	Wilks	0.345	0.754	40	28.000	0.813

3.3.4 Sites clustering based on fine sediments

To investigate the structure of the sites in terms of the settled fine-sediment grain sizes, an RDA ordination was undertaken separately for the dry (winter and spring) and wet (summer and autumn) seasons for the study period. Results of the RDA tri-plot revealed the control sites (i.e., CLS_w, CLS_s) and Millstream downstream (MLD_s) were positively associated with clay during the winter and spring periods, as indicated on Axis 1 of the RDA plot (Figure 3.2). On the same Axis 1, the Tsitsa River downstream and Qurana River (i.e., TSD_w and QHR_w) were associated with very coarse silt, very fine sand, fine sand and coarse silt. Tsitsa upstream (TSU_s) and Qurana River (QHR_s) on Axis 2 were associated more closely with coarse sand, medium silt, embeddedness, fine silt and very fine silt. Millstream downstream (i.e., MLD_w), Tsitsa downstream (i.e., TSD_s) and Tsitsa upstream, on the same axis, were associated with medium sand. The RDA analysis undertaken on settled sediment during the dry season revealed that the first three axes accounted for 97% cumulative variance. The first RDA axis explained 47% variance with an eigenvalue of 0.20; the second axis explained 32% variance with an eigenvalue of 0.16, and the third axis accounted for 21% variance with an eigenvalue of 0.01 (Table 3.10).

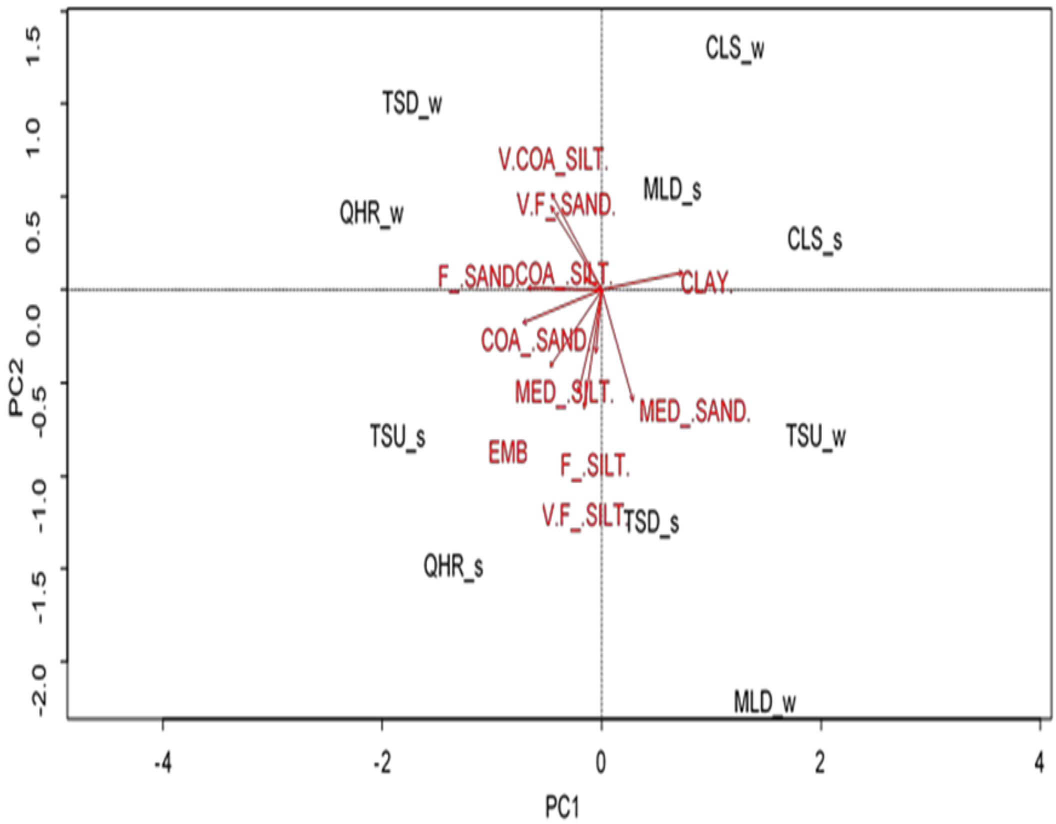


Figure 3. 2: RDA analysis during the dry season showing the pattern of clustering of the sampling sites in relation to the settled fine-sediment grain sizes. RDA analysis during the dry season for settled sediment variables across six sites. Abbreviations: TSU_w (Tsitsa upstream_winter), TSU_s (Tsitsa upstream_spring), TSD_w (Tsitsa downstream_winter), TSD_s (Tsitsa downstream_spring), QHR_w (Qurana tributary, winter), QHR_s (Qurana tributary_spring), MLU_w (Millstream upstream_winter), MLU_s (Millstream upstream_spring), MLD_w (Millstream downstream_winter), MLD_s (Millstream down_spring, CLS_w (Control sites_winter) and CLS_s (Control sites_spring). Settled fine-sediment variables: F_sand (Fine_sand), Med_sand (Medium sand), Coa_sand (Coarse_sand), VF_sand (Very fine sand), COA_silt (coarse_silt), Med_silt (Medium silt), VF-silt (Very fine silt), EMB (Embeddedness) and Clay.

Table 3. 10: Properties of the RDA ordination tri-plot showing eigenvalues, percent variance explained and percent cumulative explained during the wet and dry seasons for the settled sediment grain sizes.

Canonical properties	**Dry season settled sediments**			**Wet season settled sediments**		
	Axis			Axis		
	1	2	3	1	2	3
Canonical eigenvalue	0.20	0.16	0.01	0.29	0.10	0.03
% Variance explained	47	32	18	48	34	16
% Cumulative variance explained	47	79	97	48	82	98

During the wet season, the sites in the Qurana River, the control site, and the Tsitsa River downstream were found to be associated with very coarse silt and clay on Axis 1. On the same axis, the control sites and Millstream sites were positively associated with the very coarse silt, fine sand and very fine sand. On Axis 2, Millstream sites and Tsitsa River sites were associated with embeddedness and medium sand (Figure 3.3). On the same axis, Tsitsa River upstream and Qurana River were associated with medium silt, fine silt, and very fine silt. Overall, the results during the wet season suggest that the highly impacted sites (i.e., QHR, TSU, and TSD) were mainly associated with various sediment grain sizes, whereas the control sites were mainly associated with the smaller grain sizes, such as clay. The RDA analysis undertaken on settled sediment during the wet season (Table 3.10) revealed that the first three axes accounted for 98% cumulative variance, with the first RDA axis explaining 48% with an eigenvalue of 0.29. Axis 2 accounted for 82% cumulative variance with an eigenvalue of 0.10. The third RDA axis accounted for 98% cumulative variance and with an eigenvalue of 0.0.3, as shown in Table 3.10.

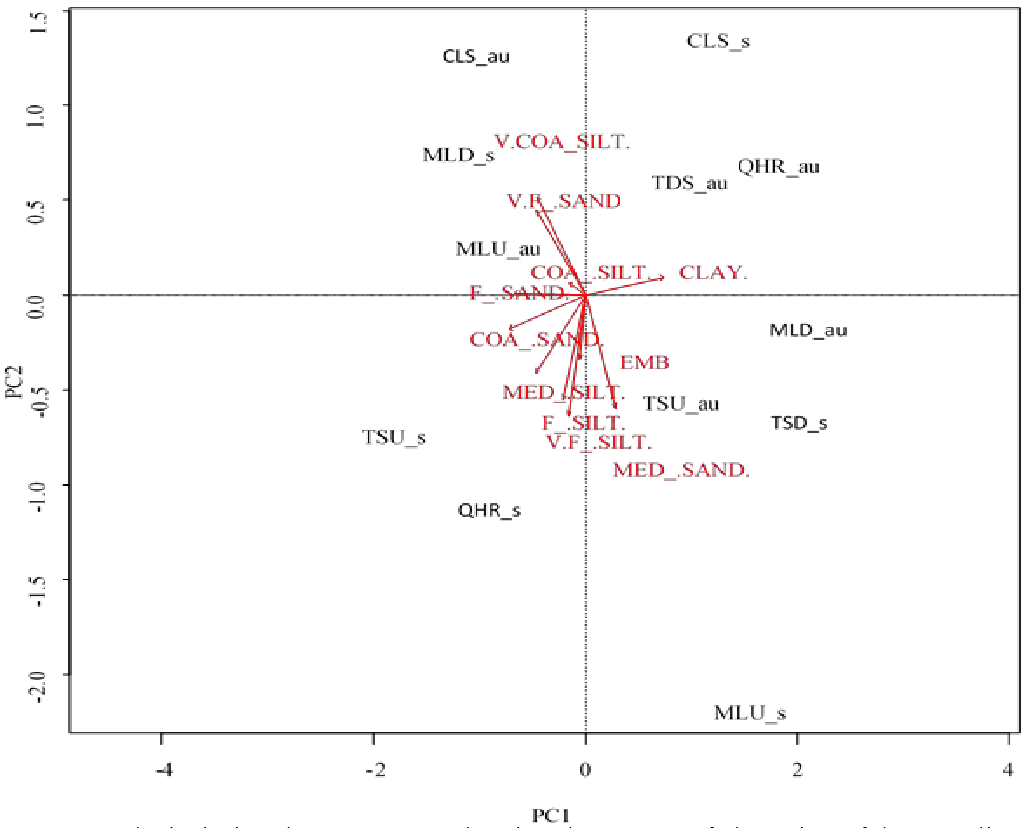


Figure 3. 3: RDA analysis during the wet season showing the pattern of clustering of the sampling sites in relation to the settled fine-sediment grain sizes. RDA analysis during the wet season for settled sediment varaibles across six sites. Abbreviations: TSU_au (Tsitsa upstream_autumn) TSU_su (Tsitsa upstream_summer), TSD_au (Tsitsa downstream_autumn), TSD_su, QHR_au (Qurana tributary_autumn), QHR_su (Qurana tributary_summer) MLU_au (Millstream upstream_autumn), MLU_su (Millstream upstream_ summer) MLD (Millstream downstream) and CLS_au (Control sites_autumn) and CLS_su (Control sites_summer). Settled sediment variables: F_sand (Fine_sand), Med_sand (Medium sand), Coa_sand (Coarse_sand), VF_sand (Very Fine sand), COA_silt (coarse_silt), Med_silt (Medium silt), VF-silt (very fine silt), EMB (embeddedeness) and Clay.

An RDA ordination for the suspended fine sediments was undertaken separately for the dry and wet seasons to elucidate the site clustering. During the dry season, sites within the Tsitsa upstream and control sites during winter and spring were mostly clustered together on Axis 1 and positively correlated with very coarse silt, very fine sand, medium silt, and coarse silt (Figure 3.4). Still on Axis 1, the Qurana river negatively correlated with fine sand, turbidity, very fine silt, coarse sand and TSS. Further sites within the Tsitsa River and Millstream River were closely clustered during the spring and winter seasons and negatively correlated with fine silt (Figure 3.4). On the same axis, Tsitsa downstream and Millstream sites were associated with medium sand and clay. The RDA analysis undertaken on suspended sediments across sites during the dry season revealed that the first three axes accounted for 92%. The RDA for the first axis accounted for a 42% variance with a 0.38 eigenvalue; the second axis revealed 77% of the cumulative variance and an eigenvalue of 0.23. The last axis (Table 3.11) accounted for a 99% cumulative variance with a 0.18 eigenvalue.

During the wet season, the RDA results for the suspended fine sediments revealed that the Tsitsa River sites and Qurana site were correlated with clay and coarse sand on Axis 1 during the summer season. On the same axis, the Tsitsa River sites, Qurana River, and Millstream sites were associated with fine sand, TSS, and turbidity. The control sites and Millstream sites during summer were associated with very coarse silt, coarse silt, and medium silt on Axis 2. The RDA analysis undertaken on settled sediment during the wet season revealed that thefirst three axes accounted for 79% cumulative variance. The first RDA axis explained 35% of the cumulative variance with a 0.13 eigenvalue. The second axis revealed a 61% cumulative variance with a 0.08 eigenvalue, while the third axis (Table 3.11) explained 77% cumulative variance and a 0.03 eigenvalue.

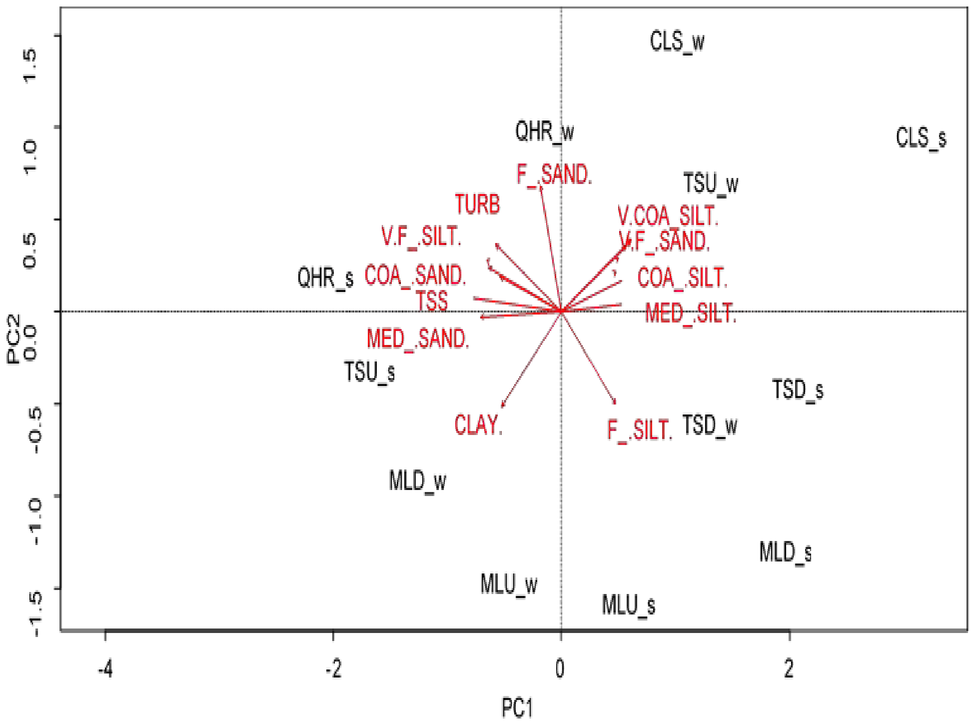


Figure 3. 4: RDA analysis during the dry season showing the pattern of clustering of the sampling sites in relation to the suspended fine-sediment grain sizes. RDA analysis during the dry season for suspended sediment variables across six sites. Abbrevations: TSU_au (Tsitsa upstream_autumn) TSU_su (Tsitsa upstream_summer), TSD_au (Tsitsa downstream_autumn), TSD_su, QHR_au (Qurana tributary_autumn), QHR_su (Qurana tributary_summer) MLU_au (Millstream upstream_autumn), MLU_su (Millstream upstream_ summer) MLD (Millstream downstream) CLS_au (Control sites_autumn) and CLS_su (Control sites_summer). Settled sediment variables: F_sand (fine_sand), Med_sand (medium sand), Coa_sand (coarse_sand), VF_sand (very fine sand), COA_silt (coarse_silt), Med_silt (medium silt), VF-silt (very fine silt), Turb (turbidity), TSS (total suspended solids) and Clay.

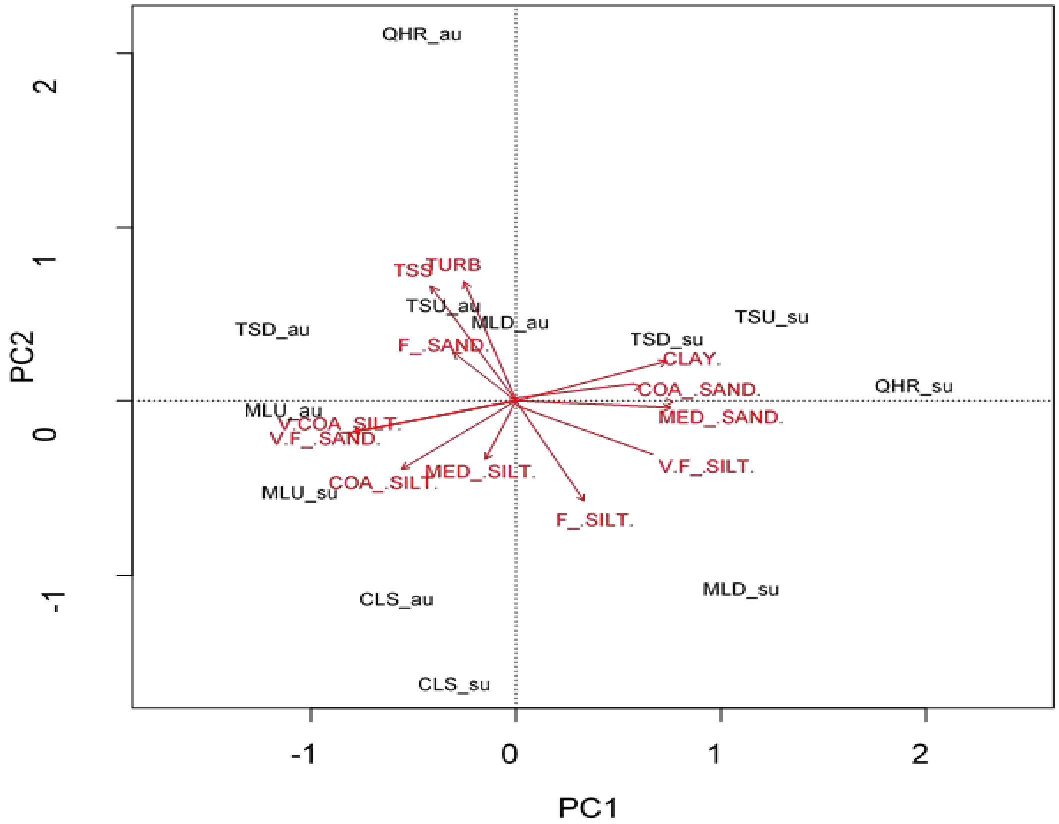


Figure 3. 5: RDA analysis during the wet season showing the pattern of clustering of the sampling sites in relation to the suspended fine-sediment grain sizes. RDA analysis during the wet season for suspended sediment variables across six sites. Abbrevations:TSU_au (Tsitsa upstream_autumn) TSU_su (Tsitsa upstream_summer), TSD_au (Tsitsa downstream_autumn), TSD_su, QHR_au (Qurhana tributary_autumn), QHR_su (Qurhana tributary_summer) MLU_au (Millstream upstream_autumn), MLU_su (Millstream upstream_ summer) MLD (Millstream downstream) and CLS_au (Control sites_autumn) and CLS_su (Control sites_summer). Settled sediment variables: F_sand (Fine_sand), Med_sand (Medium sand), Coa_sand (Coarse_sand), VF_sand (Very Fine sand), COA_silt (coarse_silt), Med_silt (Medium silt), VF-silt (very fine silt), Turb (Turbidity), TSS (total suspended solids) and Clay.

Table 3. 11: Properties of the RDA ordination tri-plot showing eigenvalues, percent variance explained and percent cumulative explained during the wet and dry seasons for the suspended sediment grain sizes.

Canonical properties	**Dry season suspended sediments**			**Wet season suspended sediments**		
	Axis			Axis		
	1	2	3	1	2	3
Canonical eigenvalue	0.38	0.23	0.18	0.13	0.08	0.03
% Variance explained	52	25	22	35	26	16
%Cumulative variance explained	52	77	99	35	61	77

3.3.5 Developing a sediment multimetric index (SMMI) for the Tsitsa River Catchment

Of the 21 metrics that were selected to assess fine-sediment impact in this study, six proved to be sensitive to fine-sediment impact. These six metrics are Shannon diversity, Simpson diversity index, Evenness, Margalef's richness, %EPT/%Chiro and EPT richness (Figure 3.6). These six sensitive metrics were subjected to a redundancy test, the results of which indicated that the Shannon diversity index, Simpson index, Evenness, and Margalef's richness index were redundant ($r > 0.65$, $P < 0.05$) (Table 3.12). Of these four redundant metrics, the Shannon diversity index was retained for integration into the final SMMI. Although EPT richness and %EPT/%Chiro were significantly correlated, the r-value was less than 0.6, thus both metrics were retained. Overall, the three metrics that were retained for integration into the SMMI were the Shannon diversity index, EPT richness, and %EPT/%Chiro. The metrics that were not sensitive are shown in Appendix A (Figure A1–Figure A4).

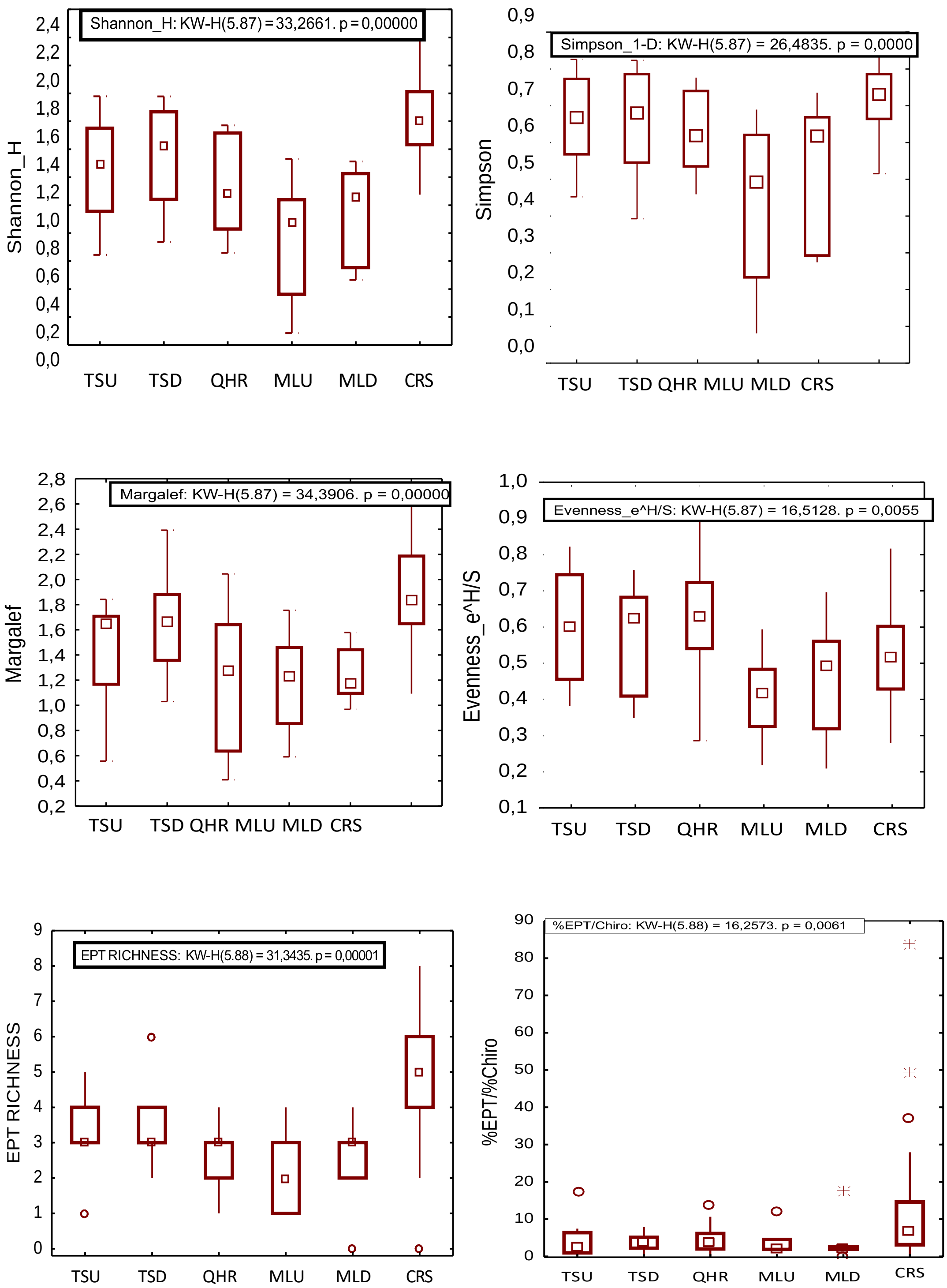


Figure 3. 6: Box plot showing macroinvertebrate metrics that discriminated the control sites from the rest of the sampling sites in the Tsitsa River and its tributaries over the study period (August 2016–March 2017). Site abbreviations: TSU (Tsitsa upstream), TSD (Tsitsa downstream), QHR (Qurana tributary), MLU (Millstream upstream), MLD (Millstream downstream), and CLS (Control sites).

Table 3. 12: Spearman's rank correlation coefficient showing redundant macroinvertebrate metrics (r >0.65, P <0.05) analysed for the Tsitsa River macroinvertebrate communities during the first-year collection events (August 2016–March 2017).

Metrics	Simpson	Shannon	Evenness	Margalef	%EPT/Chiro	EPT richness
Simpson	1,000	0,962	0,621	0,672	0,051	0,129
Shannon	0,962	1,000	0,488	0,813	0,021	0,110
Evenness	0,621	0,488	1,000	0,097	0,081	0.100
Margalef's	0,672	0,813	0,097	1,000	0.012	0,033
%EPT/%Chiro	0,051	0,021	0,081	1.00	1,000	0,436
EPT richness	0,129	0,110	0.013	0,033	0,436	1,000

Integration of the final metrics into the fine-sediment multimetric index (SMMI)

The three metrics selected were integrated into the SMMI, concluding the development process. For each metric, the minimum value, lower quartile (25%), mid-quartile (50%), upper quartile (75%), and maximum values for the control site assemblages were calculated and used as threshold metric scores (Table 3.13). The SMMI was computed by summing the scores of the three metric components, and the index value range (5–15), as only three metrics were used (5 X 3=25). The potential degree of sedimentation as indicated by the developed SMMI is illustrated in Table 3.14.

Table 3. 13: Score of metric thresholds of the selected metrics for integration into the SMMI to assess fine-sediment impact in the Tsitsa River and its tributaries.

Metrics	**Statistics**					**Scores**		
	Minimum value	**Lower quartile**	**Mid-quartile**	**Upper quartile**	**Maximum value**	**5**	**3**	**1**
Shannon diversity index	0.620	1.042	1.284	1.400	1.933	≥1.042	0.620 -1.042	<0.620
%EPT/%Chiro	2.793	3.394	5.718	7.659	27.956	≥3.394	2.793 - 3.39	<2.793
EPT richness	3	4	6	6	8	≥4	3.0 -4.0	<3

Table 3. 14: Thresholds of SMMI values corresponding to the potential degree of sediment impact (A–E/F) in the Tsitsa River and its tributaries.

Sediment impact categories	**E/F**	**D**	**C**	**B**	**A**
Descriptive condition	Critically sedimented	Seriously sedimented	Moderately sedimented	Minimally sedimented	Naturally sedimented
SMMI score range	3-5	6-8	9-11	12-14	15

Application of the Sediment Multimetric Index (SMMI) for the Tsitsa River and its tributaries

When the newly developed SMMI was applied to assess the potential degree of sediment impact in the Tsitsa River and its tributaries, the index proved to be effective, using data that was collected during the second year. As expected, during the dry season (winter and spring) the index indicated that most of the sites, including those in the Tsitsa River, were only moderately to minimally sedimented (Figure 3.7), but sedimentation increased during the wet season (summer and autumn). The SMMI indicated that sites in the Tsitsa River were seriously sedimented during the wet season, compared to the dry season where the index indicated moderate to minimal levels of sedimentation for the Tsitsa River. Similar results were obtained for sites in the Qurana and Millstream. However, the index indicated that the control sites were minimally sedimented throughout the period, except in autumn (wet season) when the index indicated a moderate level of sedimentation. Overall, the newly developed SMMI showed that sedimentation was seasonally mediated, indicating higher levels of sedimentation during the wet season than during the dry season (Figure 3.7).

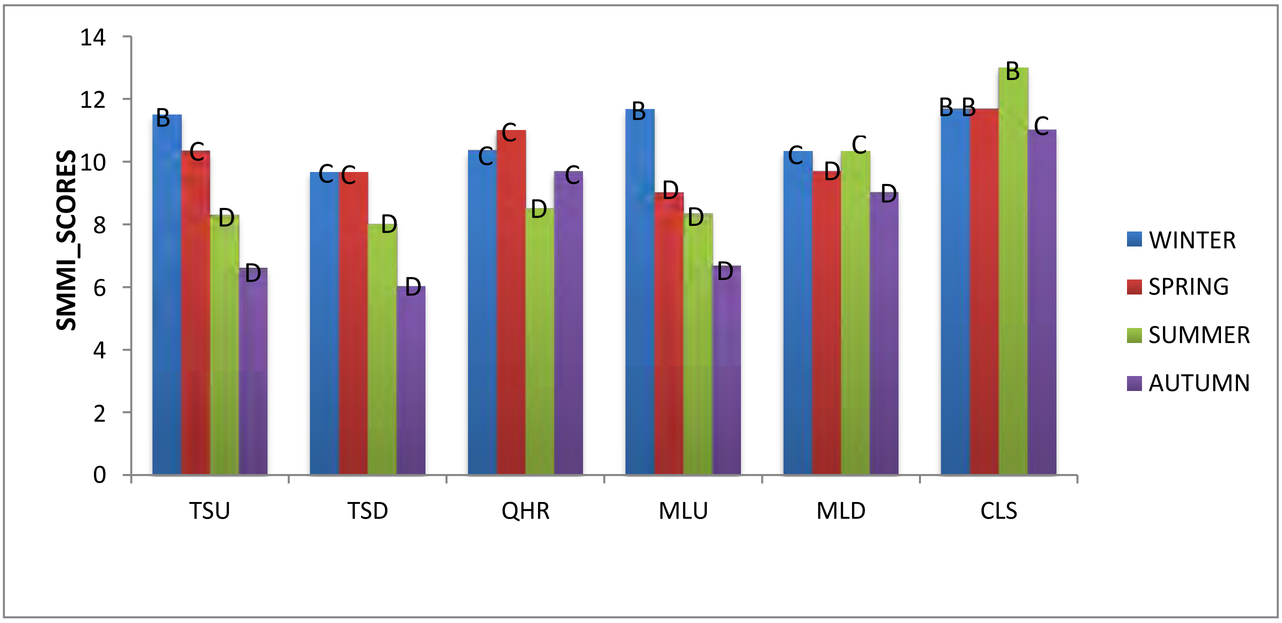


Figure 3. 7: The newly developed SMMI applied, showing the degree of sedimentation in the Tsitsa River and its tributaries. Sediment impact categories: B (minimally sedimented), C (moderately sedimented), D (seriously sedimented) E/F (critically sedimented). Sites: Tsitsa upstream (TSU), Tsitsa downstream (TSD), Qurana river (QHR), Millstream upstream (MLU), Millstream downstream (MLD), Control site (CLS).

3.4 Discussion

3.4.1 Characterisation of fine sediments and physico-chemical variables

The results of this study showed that suspended grain sizes were differentially distributed across sites in selected streams in the Tsitsa River catchment. For example, the distribution of suspended fine grain sizes of very fine sand, very coarse silt, medium silt and fine silt were significantly higher at TSU, TSD, QHR than at the control sites (CLS) (Table 3.7), while the distribution of settled sediment grain sizes was not significantly different between the sites (Table 3.9). The distribution of these fine suspended grain sizes within the Tsitsa River catchment might have been contributed by different anthropogenic activities, such as sand mining and animal grazing, which contribute to the transport of different grain sizes. Finer grain sizes are commonly recognised as having more harmful effects on aquatic biota than coarser sediments (Bryce *et al.*, 2010; Conroy *et al.*, 2016). Conroy *et al.* (2018) found smaller sediment to be most harmful to species studied, with *Baetis rhodani* the only species among the EPT taxa that was able to escape from smaller grain sizes compared with coarser grain sizes. A previous study by Akamagwuna (2019), in the same catchment also implicated suspended grain sizes as the main water quality stressor affecting the Tsitsa River. They noted that the large-grain sediment impacted the functional feeding groups (FFG) of EPT taxa.

The high proportions of very fine sand at TSD, TSU and QHR sites might be attributed to subsistence agricultural activities in the river systems. The predominance of fine sand may be attributed to relatively scarce cultivation and grazing activities at these sites. Studies have shown that agricultural activities contribute smaller particles of sediments to rivers and streams. Fine sediment particles can affect stream ecosystems with particular impact on macroinvertebrates. Study findings elsewhere, reported that the degrees of impacts depend on the sediment particle classes and properties of particles and other confounding factors such as level of exposure and presence of multiple stressors (Jones *et al.,* 2016; Akamagwuna, 2019)

Total suspended solids and turbidity were used as proxy measures of suspended sediment, and the results indicated the variables were higher in the highly disturbed sites during the wet season than in the dry season. The levels of sedimentation of riverine systems are often controlled by temporal-mediated changes such as rainfall, run-off events, hydrological regimes and flow. These changes are important factors shaping the community structure of macroinvertebrates and overall functional diversities. Freshwater ecosystems with increased total suspended solids and turbidity impact aquatic biota, possibly affecting the feeding and respiration of macroinvertebrates (Mathers *et al.,* 2019).

3.4.2 Developing a sediment multimetric index

A sediment-specific multimetric index was developed to assess the degree of sedimentation of the Tsitsa River and selected tributaries. The results showed six metrics that enabled satisfactory discrimination of the highly sedimented sites (TSU, TSD and QHR) from the control sites (CLS). The metrics included Margalef's index, %EPT/%Chiro, EPT richness, Evenness index, Simpson and Shannon indices. This study's findings are similar to those of Akamagwuna *et al.* (2019) who also found a decline of Shannon index in the highly sedimented sites within the Tsitsa River catchment.

The significant decrease in the Shannon index values in the highly sedimented sites in the Tsitsa River suggests that fine sediments may severely affect the presence or absence of macroinvertebrate communities in sediment-impacted rivers. The Shannon index also reflects the heterogeneity of biological communities as it considers the relative abundance of macroinvertebrates (Buendia *et al.,* 2013). The homogenisation effects of fine sediments on biological diversity have been observed in a previous study by Buendia *et al.* (2013) who noted the decline in the relative number of numerous taxa in highly impacted sites in the Isábena River, in northeast Spain.

In terms of EPT metrics, the findings of this study are similar to those of Akamagwuna *et al.* (2019), who observed a decline in EPT richness in severely sedimented sites in the Tsitsa River. Most EPT species are filter feeders and obtain DO via external gills, which are highly vulnerable to clogging by fine sediments. The clogging effects of fine sediments may suggest the significant reduction in the numbers of EPT metrics observed in this study. Overall, some of the selected metrics proved useful in discriminating between sites based on the level of sediment impact, thus providing more evidence for their inclusion in developing a sediment multimetric index (SMMI).

Three metrics: the Shannon index, %EPT/%Chiro and EPT richness, were integrated into the SMMI in finalising the development of the index. The integration of these three metrics into the SMMI provided an opportunity to represent three aspects of community distribution in the SMMI for the Tsitsa River. However, the Shannon index was the only diversity index that was integrated into the final index, and it has been a useful diversity metric in the development of SMMI for biomonitoring sediment pollution (Doretto *et al.,* 2018; Giesiwen *et al.,* 2019). Similar studies by Doretto (2018), Giesiwen *et al.* (2019) and Edegbene *et al.* (2019) integrated diversity measures into SMMI development. Integration of diversity measures into SMMI suggests they can serve as an important tool in biomonitoring sediment impact.

The newly developed SMMI indicated that the effects of sediments were more deleterious in the wet season than in the dry season. Temporal changes associated with hydrological and rainfall regimes are a critical factor in structuring biological assemblages of streams and river ecosystems (Mathers *et al.,* 2017; Akamagwuna *et al.,* 2019). For example, peak rainfall in the Tsitsa River occurs in the summer months of the wet season, with a consequent increase in sediment input through surface runoff and erosion. These inputs are exacerbated by the highly erodible riverbank, and extensive overgrazing in the Tsitsa River catchment. The effects of season on sediment entrainment in the Tsitsa River have been observed in a study by Akamagwuna *et al.* (2019), who investigated the responses of taxa in the EPT orders to sediment impact in the Tsitsa River and its tributaries. The reduced numbers (low index values) of SMMI in the wet period observed in this study suggest that seasonality played a significant role in mediating the inputs and consequent effects of sediment on macroinvertebrate distribution in in the Tsitsa River and its tributaries.

3.5 Conclusion

The results of this study revealed that elevated sediments input into the studied river system is the primary stressor of water quality. This is supported by the fact that the majority of the water quality variables, including sediment grain sizes measured during the study period largely indicated the sites to be distinct based on sediment grain sizes and basic water quality parameters. Based on basic water quality parameters (i.e., EC, turbidity, TSS and embeddedness) were statically significant different across sites. While, the distribution of suspended fine grain sizes of very fine sand, very coarse silt, medium silt and fine silt were significantly higher at TSU, TSD, QHR than at the control sites (CLS).

This study has developed a multimetric index based on macroinvertebrates communities in Tsitsa catchment. Twenty-one metrics have been incorporated into the final index, which can determine the attainment of ecological integrity in the study area. This is accomplished by distinguishing between the reference and test sites and significantly correlating the sediment grain sizes in this study. The results showed six metrics that enabled satisfactory discrimination of the highly sedimented sites (TSU, TSD and QHR) from the control sites (CLS). The newly developed SMMI indicated that the effects of sediments were more deleterious in the wet season than in the dry season. Thus, the SMMI performed well in distinguishing highly sedimented sites (TSU, TSD, QHR) from less sedimented sites (CLS).

CHAPTER 4: EXPLORING MACROINVERTEBRATE ECOLOGICAL PREFERENCES AND TRAIT-BASED INDICATORS OF FINE-SEDIMENT EFFECTS IN THE TSITSA RIVER AND ITS TRIBUTARIES

The following paper has been published from this chapter:

Ntloko P., Palmer CG., Akamagwuna FC and Odume ON (2021) Exploring macroinvertebrates ecological preferences and trait-based indicators of suspended fine-sediment effects in the Tsitsa River and its tributaries, Eastern Cape, South Africa. Water 2021, 13, 798 https://doi.org/10.3390/w13060798

4.1 General introduction

Sedimentation is among the most common freshwater ecosystem stressors impacting macroinvertebrate communities (Larsen *et al.,* 2011; Hubler *et al.,* 2016). Fine-sediment impacts on aquatic biota are wide-ranging and can be profound because their effects can be complex and are mediated by a range of factors, including exposure duration, grain-size distribution, sediment load, sources, geomorphological setting, and the vulnerability of resident biota (Kaller & Hartman 2004; Akamagwuna, 2018; Odume *et al.,* 2018). Sediment effects on macroinvertebrates can be direct, for example, clogging of fragile and exposed gills and filter-feeding structures, burial, abrasion; or indirect effects, for example, alteration of the physical and chemical condition of streams, such as reduction of dissolved oxygen and increased turbidity (Larsen *et al.,* 2011; Jones *et al.,* 2017; Mathers *et al.,* 2017; McKenzie and Jackson, 2020).

Macroinvertebrate assemblages have been reported to change in response to human-induced stressors in river and stream ecosystems. Globally, the taxonomy-based approaches to analysing the effects of water quality impacts on macroinvertebrates are widely used (Odume *et al.,* 2012; Naden *et al.,* 2016; Gieswein *et al.,* 2019). The taxonomic approach compares macroinvertebrate communities across a stress gradient, and the degree of impact is inferred by assessing the deviation of the assemblages at the impacted sites from those at the control or reference sites/conditions (Akamagwuna, 2018; Doretto *et al.,* 2018). Metrics, tools, and methods developed based on the taxonomy-based approaches have found routine application in many countries such as European WFD (Filipe *et al.,* 2019), South Africa, for example, the South African Scoring System version 5 (Dickens & Graham, 2002), the Biological Monitoring Working Party System in the United Kingdom (BMWP, 1978), Hilsenhoff's Biotic Index

(HBI) in the United States of America (Hilsenhoff, 1988), and the Australian River Assessment System, AUSRIVAS in Australia (Jorgenson *et al.,* 2005).

The taxonomy-based approach is useful because, apart from its use in inferring water quality impact, it provides important biological information needed for biodiversity conservation and protection. Such important information about biodiversity may include the occurrence and distribution of rare, endangered, dominant, keystone, or vulnerable species, which may be impacted by stressors (Gieswein *et al.,* 2019; IUCN, 2021). Further, because of the widespread application of the taxonomy-based approach, key water quality indicators have been established. For example, the richness and diversity of macroinvertebrate taxa such as Ephemeroptera, Plecoptera, and Trichoptera (EPT) have been known to decrease in response to water quality stressors like organic pollution and acid mine drainage as well as sedimentation (Akamagwuna *et al.,* 2019; Musonge *et al.,* 2020). On the other hand, the compositions and abundances of taxa such as chironomids, and many other dipterans are usually reported to increase in relation to stressors such as organic pollution and sediment stress.

Like the taxonomic indicators, it is asked in this chapter whether trait-based indicators can also be identified to monitor effects of sedimentation in impacted river and stream ecosystems. The approach followed in this chapter offers an opportunity to identify indicator traits based on the specific environmental stressor. The Tsitsa River and its tributaries are subject to an elevated fine- sediment inputs from the surrounding landscape as a result of, for example, animal grazing and crop production. The river is situated in the rural part of the Eastern Cape Province of South Africa, where duplex, dispersive, and easily erodible soils have caused the influx of fine sediments into the Tsitsa River, impacting both the structure and function of biological communities (Akamagwuna *et al.,* 2019). In an earlier study, elevated fine sediments were found to impact EPT community structures in the Tsitsa River and its tributaries (Akamagwuna, 2018). Because these rivers are situated in rural catchments, water quality is relatively good, but elevated sediments remain a critical challenge, particularly during the wet seasons.

Given that these rivers are mainly impacted by elevated fine sediments, they provide an opportunity for exploring macroinvertebrate traits responses and identifying trait-based indicators of settled and suspended fine-sediment effects, without the confounding effects from other water quality stressors such as urban pollution. Identifying trait-based indicators of elevated, settled, suspended fine-sediment effects is useful because traits mediate organism-environmental interaction, potentially providing mechanistic insights for predicting assemblage response to a given environmental filter (Statzner *et al.,* 2001; Odume *et al.,* 2018). Environmental filters such as fine sediments favour particular suites of traits (Larsen *et al.,* 2011; Jones *et al.,* 2017; Akamagwuna, 2018).

The trait-based approach (TBA) is informed by the habitat template theory (Southwood, 1977; Townsend & Hildrew, 1994), which is based on an autecology, which predicts that a correspondence is expected between prevailing habitat conditions and traits (Webster *et al.,* 2012). The TBA has been used to explore the impact of fine-sediment stress on macroinvertebrates (e.g., Larsen *et al.,* 2011; Herrera *et al.,* 2016; Akamagwuna *et al.,* 2019; Yadamsuren *et al.,* 2020). Further, it has been argued that, unlike the taxonomy-based indicators, trait-based indicators can link macroinvertebrate response to ecosystem function, for example, material fluxes are directly related to feeding behaviour and body size (Dolédec *et al.,* 2006; Menezes *et al.,* 2010; Liu *et al.,* 2013; Bolam *et al.,* 2017). However, only a few studies (e.g., Akamagwuna *et al.,* 2019; Odume, 2020; Edegbene *et al.,* 2020) have explored the TBA in Africa, especially in sediment-impacted rivers, so it remains unclear how trait-based studies from other regions apply to highly sedimented rivers in the Afrotropical region. In the Afrotropical region where taxonomic expertise is sparse, identifying useful trait-based indicators of fine-sediment stress can help contribute to and accelerate the science and practice of freshwater biomonitoring without the necessity of species identification as not all traits are constrained by taxonomy, for example, body shape, body size, and many ecological preferences of macroinvertebrates at family level (Ochieng *et al.,* 2019).

Thus, in the present study, the effects of settled and suspended fine sediments on macroinvertebrate communities by means of multivariate RLQ (Environmental variables, R; macroinvertebrate taxa, L; traits, Q) and fourth-corner analyses were examined. Six sites that represent an increasing fine-sediment concentration were selected for this study. This chapter thus fulfils the objective of exploring macroinvertebrate ecological preferences and traits with a view to identifying possible trait-based indicators of fine sediment impact in the Tsitsa River and its tributaries (Chapter 1, Section 1.7.2).

4.2 Materials and methods

4.2.1 Selected sampling sites

Eight sampling sites in four river/tributary systems were selected for the study, and macroinvertebrate samples were collected seasonally over two years (August 2016–March 2018). The selected sites included Tsitsa upstream (TSU), Tsitsa downstream (TSD), Qurana

River (QHR), Millstream upstream (MLU), Millstream downstream (MLD), sites in the Pot River upstream, Pot River downstream, and the Little Pot River, making the control sites (CLS). Details of the rivers and sites are fully described in Chapter 2, Section 2.2.5.

4.2.2 Sampling fine-sediment grain sizes and selected water quality variables

Fine-sediment grain sizes (settled and suspended) were sampled seasonally for two years and analysed as described in Chapter 2, Section 2.3.2. Water quality variables such as turbidity, total suspended solids (TSS), dissolved oxygen (DO), electrical conductivity (EC), and embeddedness were analysed seasonally, as described in Chapter 2, Section 2.3.

4.2.3 Macroinvertebrate sampling

Macroinvertebrates were sampled using a 30 cm x 30 cm, 1000 µm mesh net following the South African Scoring System version 5 (SASS5) protocol (Dickens & Graham, 2002). Samples were collected from gravel, sand, and mud (GSM), vegetation (marginal and aquatic), and stones (stone-in-and-out-of-current) as fully described in Chapter 2, Section 2.4.

4.2.4 Selected macroinvertebrate traits and ecological preferences

A total of 12 traits and ecological preferences were selected for the study and further resolved into 47 attributes (Table 4.1). The selection of trait and ecological preferences was informed by the literature and a mechanistic link between the fine-sediment mode of impact and the particular traits. For example, respiration is selected for analysis because fine sediments have been hypothesised to clog respiratory trait attributes such as gills (Jones *et al.,* 2012). Feeding was also selected because, like respiration, organisms which feed by filtering particulate organic matter have been shown to have their feeding apparatus clogged by elevated fine sediments. Accumulation of fine sediments has also been shown to alter food quality, covering stable surfaces on which many macroinvertebrates feed (Jones *et al.,* 2012). Concerning velocity preference, for example, Jones *et al.* (2012) and Odume *et al.* (2018) argued that velocity mediates fine-sediment impact on macroinvertebrates because, at a higher velocity, the frictional force between organisms' body surfaces and the moving fine sediments is likely to be aggravated, causing increased abrasion of soft and exposed body surfaces. Overall, the selection of traits and ecological preferences were informed by i) mechanistic relationships between the trait and fine-sediment modes of impact, ii) availability of trait and ecological preference data, and iii) ease of measurement and observation. Information on trait and ecological preferences was retrieved from the newly compiled trait database for South African macroinvertebrates (Odume *et al.*, 2018) and supplemented by other sources (Akamagwuna, 2019; Odume, 2020).

Table 4. 1: Selected macroinvertebrate ecological preferences and traits and their respective attributes. Abbreviations: FPOM (fine particulate organic matter), CPOM (coarse particulate organic matter) and GSM (gravel, sand, and mud) and Code – an abbreviation of trait attribute (Table adapted from Odume, 2020)

Traits and ecological preferences	**Code**
Maximum body size (mm)	
Very small (≤5)	A1
Small (>5 to 10)	A2
Medium (>10 to 20)	A3
Large (>20 to 40)	A4
Very large (>40)	A5
Respiration	
Gills	B1
Tegument	B2
Aerial; spiracles	B3
Aerial vegetation: breathing tube, straps/other apparatuses e.g., elytra	B4
Aerial lung	B5
Mobility	
Climber	C1
Crawler	C2
Sprawler	C3
Swimmer	C4
Skater	C5
Burrower	C6
Body shape	
Streamlined	D1
Flattened	D2
Spherical	D3

Cylindrical	D4
Preferred food	
FPOM (fine particulate organic matter)	E1
CPOM (coarse particulate organic matter)	E2
Feeding habit	
Shredder	F1
Collector-gatherer	F2
Collector-filterer	F3
Scraper (grazer, brushers)	F4
Predator	F5
Preferred biotope	
Sediment (gravel, sand, and mud)	G1
Stones	G2
Vegetation	G3
Attachment mechanism	
Free living	HI
Temporarily attached	H2
Permanently attached	H3
Respiratory type	
Aerial	I1
Aquatic	I2
Oxygen sensitive/ tolerance	
Highly sensitive	J1
Moderately sensitive	J2
Low sensitivity	J3
Not sensitive	J4
Body protection	
Exposed and soft	K1
Cased/tubed	K2

Exposed but sclerotised	K3
Completely sclerotised	K4
Velocity preference (m/s)	
Very fast flowing (>0.6)	L1
Moderately flowing (0.3-0.6)	L2
Slow flowing (0.1-0.3)	L3
Very slow flowing (< 0.1),	L4

4.2.5 Exploring the pattern of distribution of ecological preferences and traits and identifying trait-based indicators of fine-sediment impact (suspended and settled).

Before analysing for the pattern of ecological preferences and traits distribution, taxa at each site were pooled, resulting in a taxon-site matrix. A second matrix was then created containing trait-taxon with trait data fuzzy-coded (Appendix B) and the abundance of taxa log (x+1) transformed. Fuzzy coding was used to describe the association of each taxon to each trait attribute. Affinity scores ranging from 0 to 5 were used for the fuzzy coding system, taking into account potential functional variation between species within a family and life stages within a taxon (Chevenet *et al.,* 1994). Each taxon per trait attribute was assigned a score of 0, indicating no affinity of a taxon to the given trait attribute, a score 1 indicates low affinity, 3 moderate affinity, and 5 high affinity to a trait attribute (Odume, 2020). The fuzzy coding (Appendix B) was particularly useful because working at the family level, it allowed for accounting for the potential plasticity, variability, and functional diversity that exist within a given family. The approach has been widely used in other studies (Archambault *et al.,* 2010; Gonza´lez-Trujillo, 2016).

To analyse the pattern of distribution of ecological preferences and traits, the RLQ analysis was applied. The RLQ is a three-step ordination method developed by Dolédec *et al.* (1996) that routinely performs three separate ordination analyses on three data sets – environmental data (R), taxa data (L), and trait data (Q). In an RLQ, the first ordination (correspondence analysis, CA) is undertaken on the taxa data set L-table, second ordination (principal component analysis, PCA) on the environmental data sets, in this case, the sediment grain sizes, turbidity, EC, embeddedness, TSS and DO, R-table, which link the taxa data set to the physico-chemical variable data set by using the sample scores result of the CA as row weights. A third ordination (Hill-smith analysis, HS) links the L data set to the trait data set by using the taxon score results of the CA as row weights. A final ordination (combined RLQ) analysis simultaneously conducts ordination on the three separate ordinations (CA, PCA, and HS) by searching for a linear combination of traits-taxon scores in the traits-taxon scores in Q-HS ordination and physico-chemical variables sample scores in R-PCA ordination, by maximising the covariance between Q and R through L ordination. The significance of the RLQ analysis was tested using the Monte Carlo permutation test with 999 permutations at *alpha* = 0.05. Two separate RLQ ordinations were carried out, one for suspended sediments and the other for settled sediments. The RLQ ordination thus allows the spatial visualisation of the distribution of traits and ecological preferences with the sediment grain sizes and selected physico-chemical variables.

To identify potential trait-based indicators of fine sediment impact, ecological preferences and trait attributes associated with the TSU, TSD, and QHR were designated as tolerant traits, being associated with sites with elevated sediment impact, whereas those associated with the CLS were designated as potential fine-sediment sensitive traits. The fourth-corner analysis (Dolédec *et al.,* 1996; Dray & Legendre, 2008) was then further conducted to confirm designated trait-based indicators of fine-sediment impact. The fourth-corner analysis is a multivariate permutational test that concurrently searches for significance between multiple traits and water quality variables. In this study, it was used to test the association between fine-sediment grain sizes that showed significant difference between the sites (Table 3.4 and Table 3.5 Chapter 3) TSS, turbidity, embeddedness, EC and the selected traits. A trait was deemed fine-sediment tolerant if it was positively associated with the TSU, TSD, QHR and was significantly positively correlated with at least one sediment grain size or increasing TSS, turbidity (for suspended sediment), or embeddedness (for settled sediments) following the approach by Odume *et al.* (2020). A trait was confirmed sensitive if the correlation was significantly negative to grain sizes (suspended or settled), including embeddedness, electrical conductivity, total suspended solids, turbidity, but significantly positively correlated with dissolved oxygen.

4.3 Results

4.3.1 Spatial configuration of suspended and settled fine sediments and distribution of traits and ecological preferences

The results of the RLQ analysis for suspended grain sizes during the dry season revealed that the first two axes explained 99.1% cumulative variance of the data set. The first axis accounted for 81.94% and the second axis 17.16% of the total variance. The ordination plot revealed that medium silt, coarse sand, and medium sand were clustered together and were positively associated with Site 3, (QHR), Site 4, (MLU), Site 5, (MLD), and Sites 6–8 (CLS). During the dry season, except on a few occasions, the control sites and the moderately sedimented sites, that is, 4 and 5 (MLU and MLD) were closely clustered together and were mainly influenced by increasing DO and clay. The traits that were mainly associated with the control sites were a preference for climbing, aerial respiration, temporary attachment and skating. These traits were associated with taxa such as Dyticidae, Oligonuridae, Baetidae, Syphidae and Muscidae (Figure 4.1). The highly sedimented Sites 1 (TSU) and 2 (TSD) and Site 3, (QHR) were clustered together. Cerapogonidae, Baetidae, Caenidae, Leptophlabidae and Helodidae taxa were found to be associated with these sites. Traits associated with these taxa include a preference for very fast-flowing waters, predation and scraping. Clustering of these sites was mainly influenced by increasingly fine sand, very fine silts and TSS (Figure 4.1). For the wet season data, the first two axes of the RLQ ordinations explained 92.87% cumulative variance of the data set. The first axis accounted for 81.87% variance, and the second axis, 11% total variance. The ordination plot revealed that Site 4, (MLU), Site 3, (QHR), Site 2, (TSD), and Site 1, (TSU) were clustered together and showed a positive association with fine sand, coarse silt, fine silt, turbidity, and TSS. Collector-filterers, shredders, CPOM, and a preference for slow-flowing waters were associated with fine sand, coarse silt, fine silt, turbidity, and total suspended solids (Figure 4.1-4.2). These traits were associated with taxa such as Oligonuridae, Syphidae, Dyticidae and Muscidae.

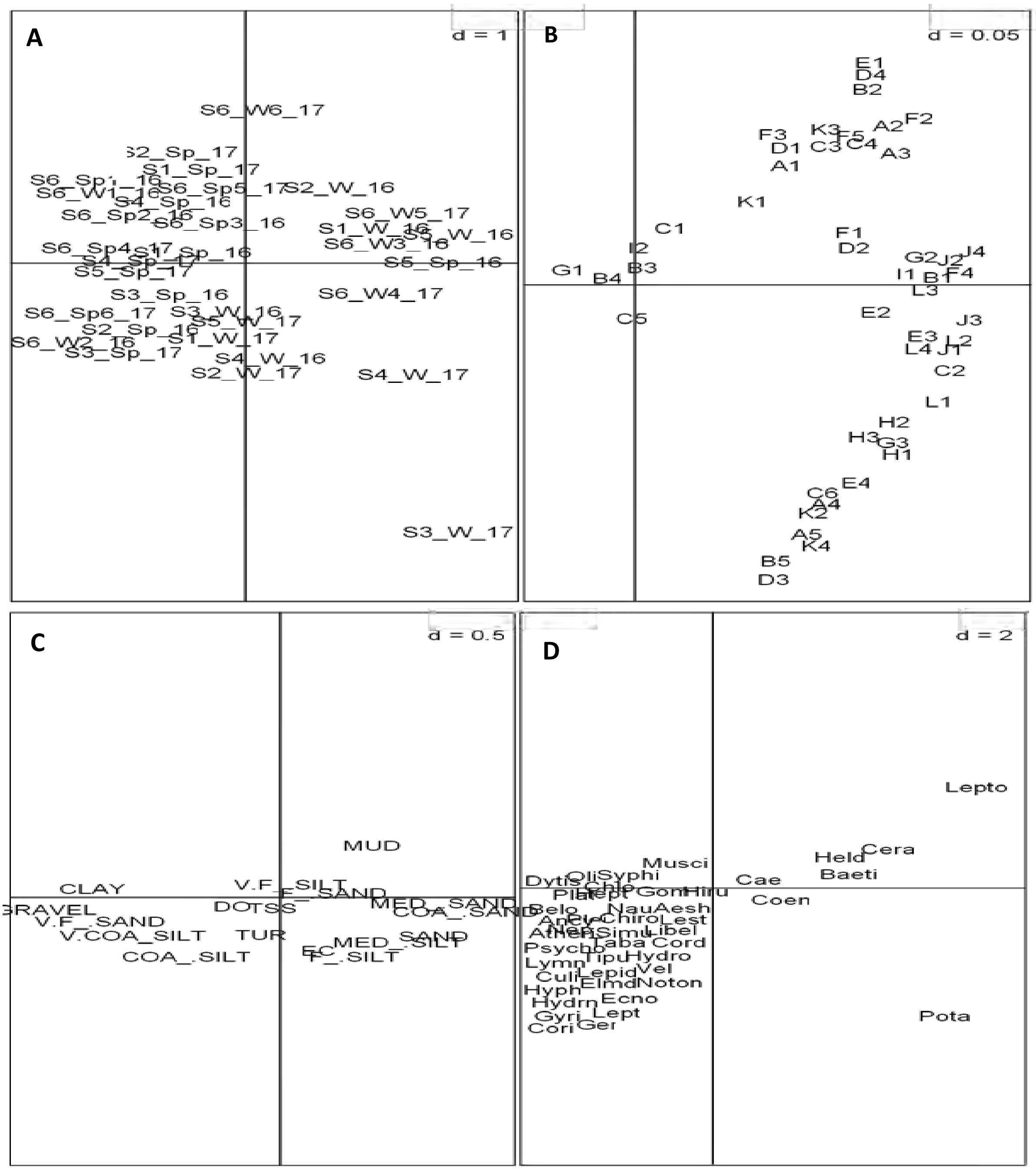


Figure 4. 1: RLQ plot showing the site clustering (A) during the sampling seasons, based on traits (B), environmental variables (C) and macroinvertebrates (D) during the dry seasons over the study period (August 2016–March 2018) in the Tsitsa River and its tributaries. Abbreviations: traits are as in **Table 1,** for traits and ecological preferences, physico-chemical variables and suspended grain sizes in **Table 2**; taxa: Oli: Oligonuridae; Baeti: Baetidae; Coen: Coenogranidae; Gom: Gomphidae; Lepto: Leptophlabidae; Held: Helodidae; Dyti: Dytiscidae; Pota: Potamonautidae, Cera: Ceratopogonidae; Syph: Syrphidae, and Caen: Caenidae sites: S6_W4_17; Control site_winter_year 2017, Control site_winter_year 2017, S2_W_16;Tsitsa downstream_winter_year 2016, S4_W_16; Millstream upstream_winter_year 2016, S5_W_17; Millstream downstream_winter_year 2017 and S3_W_16; Qurana River_winter_year 2016, S3_Au_17: Qurana River_autumn_year 2017; S1_Su_16: Tsitsa upstream_summer_year 2016 and S2_Su_16: Tsitsa downstream_summer_year 2016.

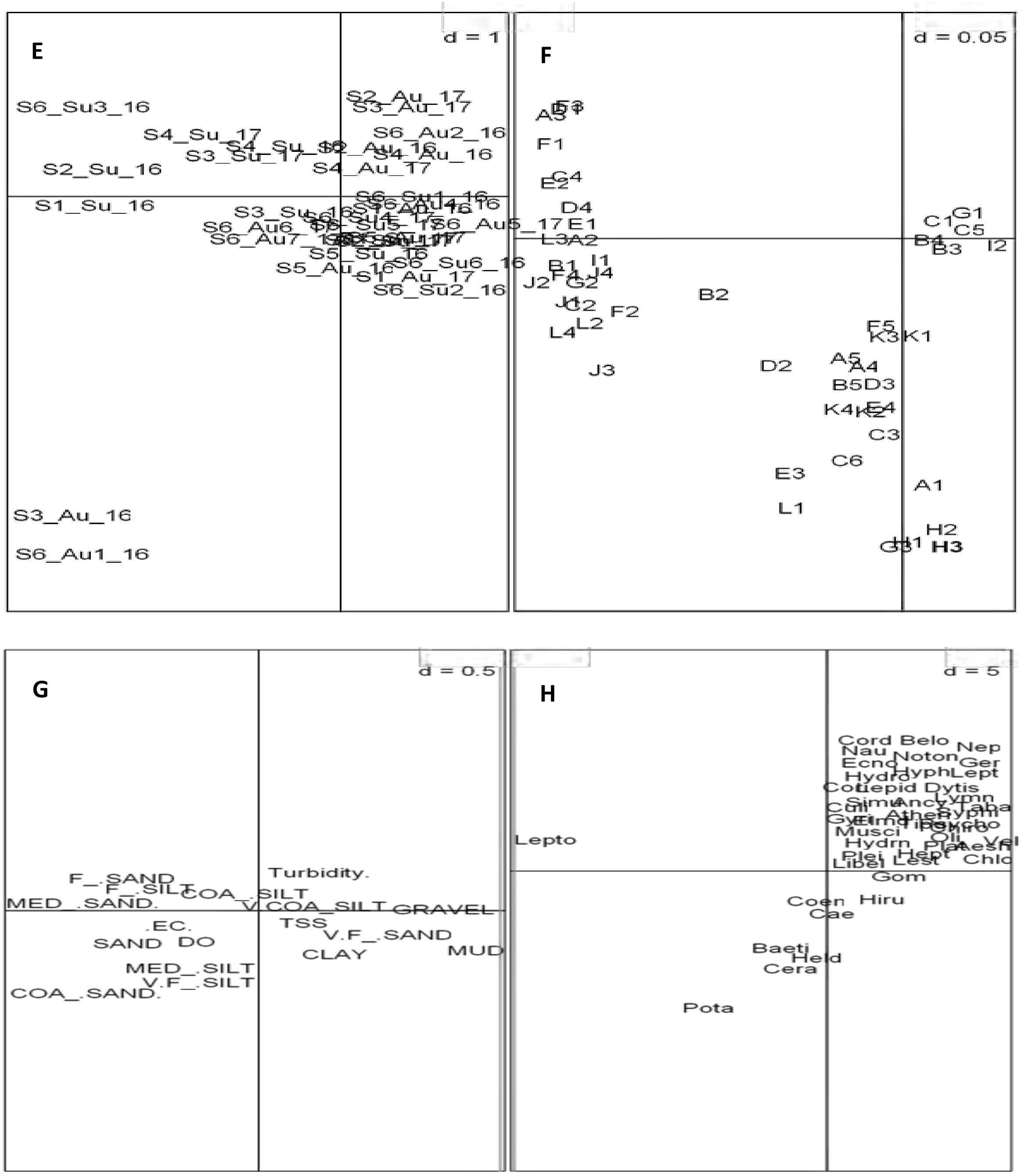


Figure 4. 2: RLQ plot showing the site clustering (E) during the sampling seasons, based on traits(F), environmental variable (G) and macroinvertebrates (H) during the wet seasons over the study period (August 2016–March 2018) in the Tsitsa River and its tributaries. Abbreviations: traits are as in **Table 4.1,** for traits and ecological preferences, physico-chemical variables and suspended grain sizes in **Table 2;** taxa : Musc: Muscidae; Coen: Coenogranidae; Lepto: Leptophlabidae; Baeti: Baetidae; Cera; Ceratopogonidae; Pota: Potamonautidae, and Caen: Caenidae, Gom:Gomphidae, Hiru: Hirudea sites: S6_SP_17; Control site_spring_year 2017, Control site_spring_year 2016, S1_W_16;Tsitsa upstream_winter_year 2016, S4_W_16; Millstream upstream_winter_year 2016, S5_W_17; Millstream downstream_winter_year 2017 and S3_au_16; Qurhana River_autumn_year 2016, S3_Au_17: Qurhana River_autumn_year 2017; S1_Su_16: Tsitsa upstream_summer_year 2016 and S5_su_16: Millstream upstream_summer_year 2016.

The RLQ analysis results for settled fine sediments that was undertaken during the dry season revealed that the first two axes explained 91.98% cumulative variance. The first axis accounted for 56.94% variance and the second axis 35.04 % variance. The ordination plot during the dry season showed that the proportion of medium silt and clay were clustered together and were positively associated with the CLS (Sites 6–8). Coarse sand, coarse silt and fine silt were clustered together and influenced the structuring of assemblages at the MLD (Site 5), QHR (Site 3) and CLS (Sites 6–8). The trait attributes and ecological preferences that were associated with the MLD, QHR and CLS included aerial vegetation, collector-gatherer and exposed and soft body protection. These traits were associated with Muscidae (Figure 4.3-4.4) The highly (TSU) and moderately (MLU) sediment-influenced sites and, on few occasions, the less sediment-influenced sites (CLS) were clustered together and showed a positive association with mud. Trait attributes, and ecological preferences associated with the mud included very fast, slow, very slow-flowing waters, skaters and permanently attached macroinvertebrates into a substrate; taxa that were associated with mud included a combination of sensitive and tolerant taxa: Leptophlabidae, Caenidae, Chironomidae, Coenogranidae, and Heptoginidae (Figure 4.2).

For the wet season, the ordination result of the combined RLQ analysis showed that settled sediment grain sizes during the wet season explained 96.82% cumulative variance. The first axis accounted for 82.5% and the second axis 14.32% of the total variance. The ordination result of the combined RLQ analysis showed that settled sediment grain sizes – medium sand, fine silt, and coarse silt – were clustered together and were associated with Site 3 (QHR), Site 4 (MLU), and Site 5 (MLD). These proportions of settled grain sizes were associated with trait attributes and ecological preference such as spherical shape, very small body size, cased, tubed and completely sclerotised. The macroinvertebrate taxa associated with the sites included Baetidae, Ceratopogonidae, Potamonautidae, and Leptophlabidae. The proportion of embeddedness, very fine sand, and fine sand were clustered together and associated with less and moderately sedimented sites; Site 6 (CLS) and Site 4 (MLU). This proportion of settled sediment particle classes was associated with trait attributes and ecological preferences such as exposed and soft body parts, exposed but sclerotised body parts and a preference for stones as a biotope. The following taxa, Muscidae, Gomphidae, Hirundinae, were associated with Site 4 (MLU) and Site 6 (CLS) (Figure 4.2).

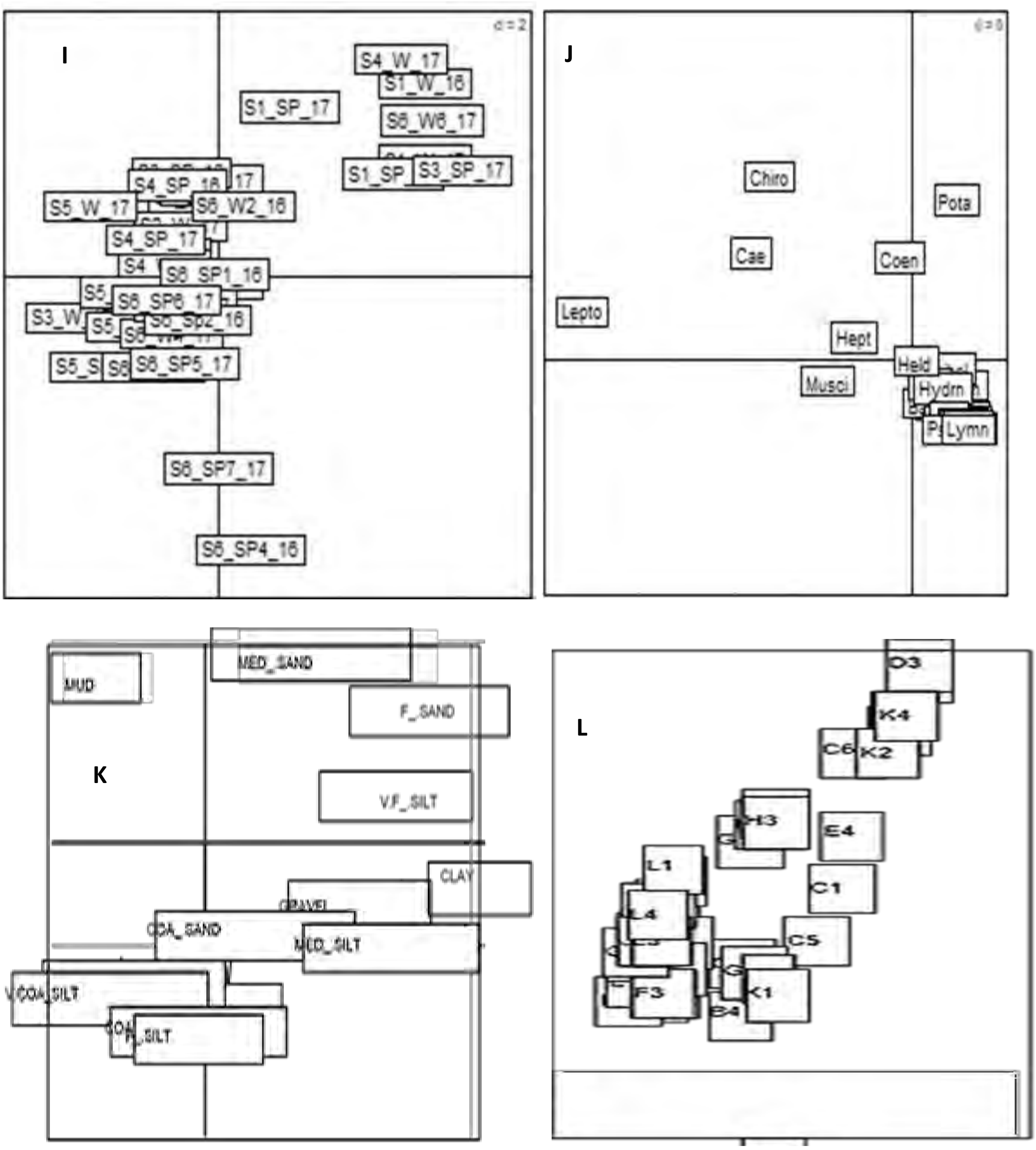


Figure 4. 3: RLQ plot showing the site clustering (I) during the sampling seasons, based on environmental variables (J), traits (K) and macroinvertebrates (L) during the dry and wet seasons over the study period (August 2016–March 2018) in the Tsitsa River and its tributaries. Abbreviations: traits are as in Table 4.1, for traits and ecological preferences, physico-chemical variables and suspended grain sizes in Table 2; taxa : Musc: Muscidae; Coen: Coenogranidae; Lepto: Leptophlabidae; Baeti: Baetidae; Cera; Ceratopogonidae; Pota: Potamonautidae, and Caen: Caenidae sites: S6_SP_17; Control site_spring_year 2017, Control site_spring_year 2016, S1_W_16;Tsitsa upstream_winter_year 2016, S4_W_16; Millstream upstream_winter_year 2016, S5_W_17; Millstream downstream_winter_year 2017 and S3_au_16; Qurhana River_autumn_year 2016, S3_Au_17: Qurhana River_autumn_year 2017; S1_Su_16: Tsitsa upstream_summer_year 2016 and S5_su_16: Millstream upstream_summer_year 2016.

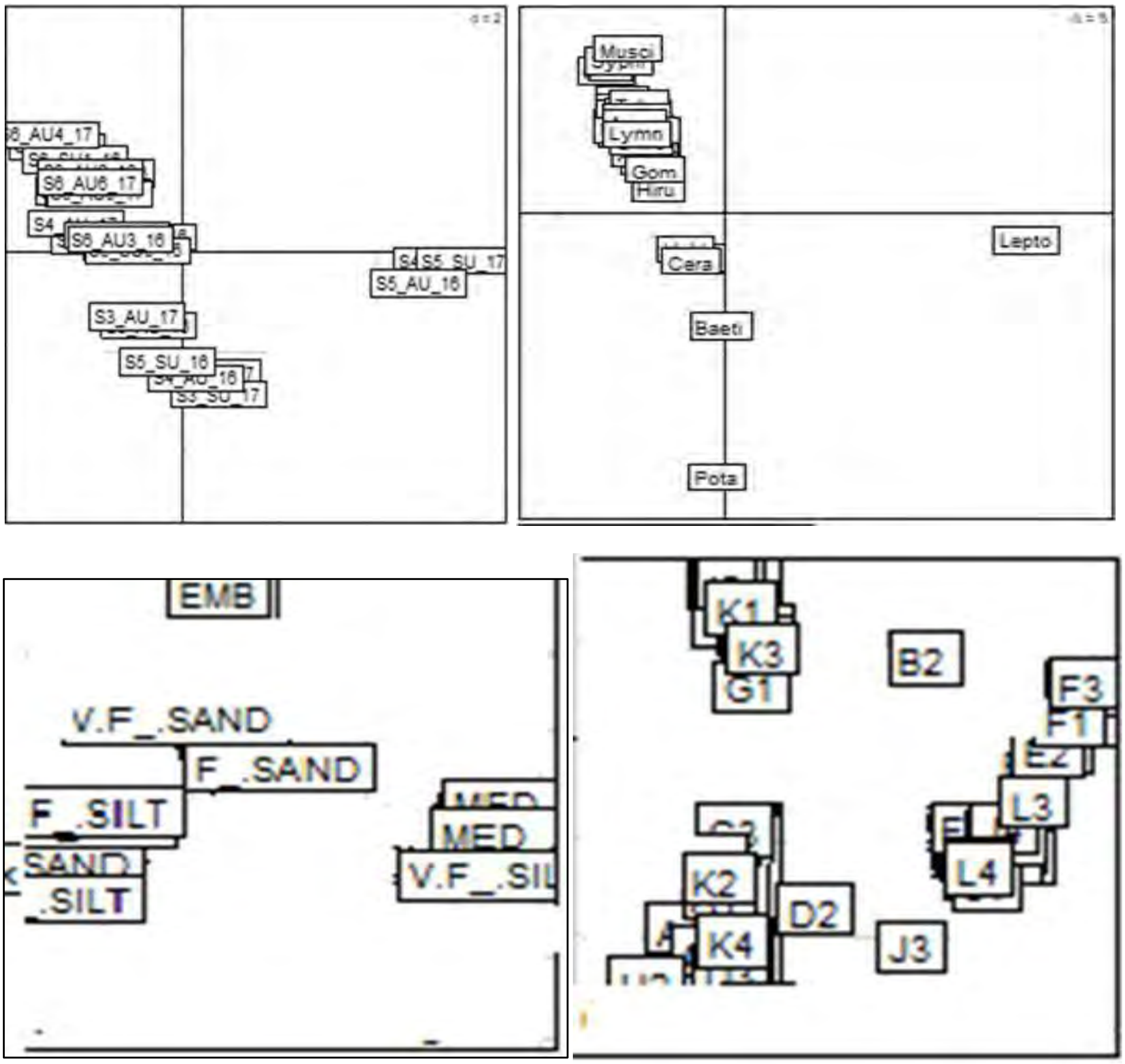


Figure 4. 4: RLQ plot showing the site clustering (i and m) during the sampling seasons, based on environmental variables (k and o), traits (l and p) and macroinvertebrates (j and n) during the dry and wet seasons over the study period (August 2016–March 2018) in the Tsitsa River and its tributaries. Abbreviations: traits are as in Table 4.1, for traits and ecological preferences, physico-chemical variables and suspended grain sizes in Table 2; taxa : Musc: Muscidae; Coen: Coenogranidae; Lepto: Leptophlabidae; Baeti: Baetidae; Cera; Ceratopogonidae; Pota: Potamonautidae, and Caen: Caenidae sites: S6_SP_17; Control site_spring_year 2017, Control site_spring_year 2016, S1_W_16;Tsitsa upstream_winter_year 2016, S4_W_16; Millstream upstream_winter_year 2016, S5_W_17; Millstream downstream_winter_year 2017 and S3_au_16; Qurhana River_autumn_year 2016, S3_Au_17: Qurhana River_autumn_year 2017; S1_Su_16: Tsitsa upstream_summer_year 2016 and S5_su_16: Millstream upstream_summer_year 2016**.**

4.3.2 Identifying trait-based indicators of fine-sediment impact

The fourth-corner analysis was used to test the significance of individual trait-environment association. Specifically, it was used to explore the association between the individual trait attribute/ecological preference and suspended sediment grain sizes, turbidity, EC, DO and TSS, (Figure 4.3). During the dry season, only coarse sand, fine silt, and clay correlated significantly with macroinvertebrate traits and ecological preferences (Table 4.2). A positive correlation was detected between fine silt, and large (>20 to 40 mm) and very large (>40 mm) body sizes, possession of lungs and spherical body shape, whereas clay was negatively correlated with crawling, a high tolerance of DO depletion and all attributes of velocity preference (m/s), except slow flowing waters (0.1–0.3 m/s). Coarse sand indicated significant positive correlations with gills, crawling, CPOM, scraping, a preference for stone biotope, aerial respiration, and all attributes of sensitivity/tolerance to dissolved oxygen depletion and velocity preferences, except very fast flowing waters (>0.6 m/s) (Table 4.2).

During the wet season, a total of 36 traits and ecological preferences were significantly correlated with suspended fine-sediment grain sizes. Of the 36 traits/ecological preferences that were significantly correlated, 18 trait attributes and ecological preferences, such as small body size, medium body size, gills, crawling, swimming, a preference for FPOM, CPOM as food sources, collector-filtering, scraping, a preference for the stone biotope, high sensitivity to DO depletion and a moderate sensitivity to DO depletion were negatively significantly correlated with gravel and mud. Conversely, 18 trait attributes and ecological preferences were significantly positively correlated with coarse sand and medium sand. These traits include small to medium body size, gills, crawling, swimming, streamlined body shape, cylindrical body, a preference for FPOM, CPOM, predation, permanent attachment, and aquatic respiration. Of the traits that negatively correlated with coarse sand and fine sand, CPOM, collector-filtering, high sensitivity to DO depletion were associated with the control sites. These traits were therefore deemed sensitive traits to elevated suspended fine sediments (Table 4.2). Of the traits that showed a positive correlation with medium sand and very fine sand, a high tolerance to DO depletion, skating, and a preference for FPOM were associated with the highly sedimented sites. These traits were thus deemed tolerant traits (Table 4.2).

The fourth-corner analysis undertaken to assess the significant association between environmental variables and biological variables for settled sediment characteristics, including embeddedness, indicated no significant association between settled grain sizes and trait attributes/ecological preferences during the dry season (Figure 4.4). However, during the wet season, a total of eight trait attributes/ecological preferences indicated significant association; clay was negatively correlated with small body shape, streamlining and collector-gatherers, while shredders were significantly negatively correlated with coarse sand, medium silt, and clay. Small body size was negative and significantly correlated with coarse silt. Trait attributes and ecological preferences of small body size, collector-filterer, and streamlining were significantly positively associated with coarse sand, medium sand, and medium silt, while the aerial type of respiration was significantly positively correlated with fine sand and medium silt, and shredders were only significantly positively correlated with medium silt. The traits that showed positive correlation to settled sediment grain size were deemed sensitive traits, and traits that were negatively correlated to settled sediment fine sediments were deemed tolerant (Table 4.3).

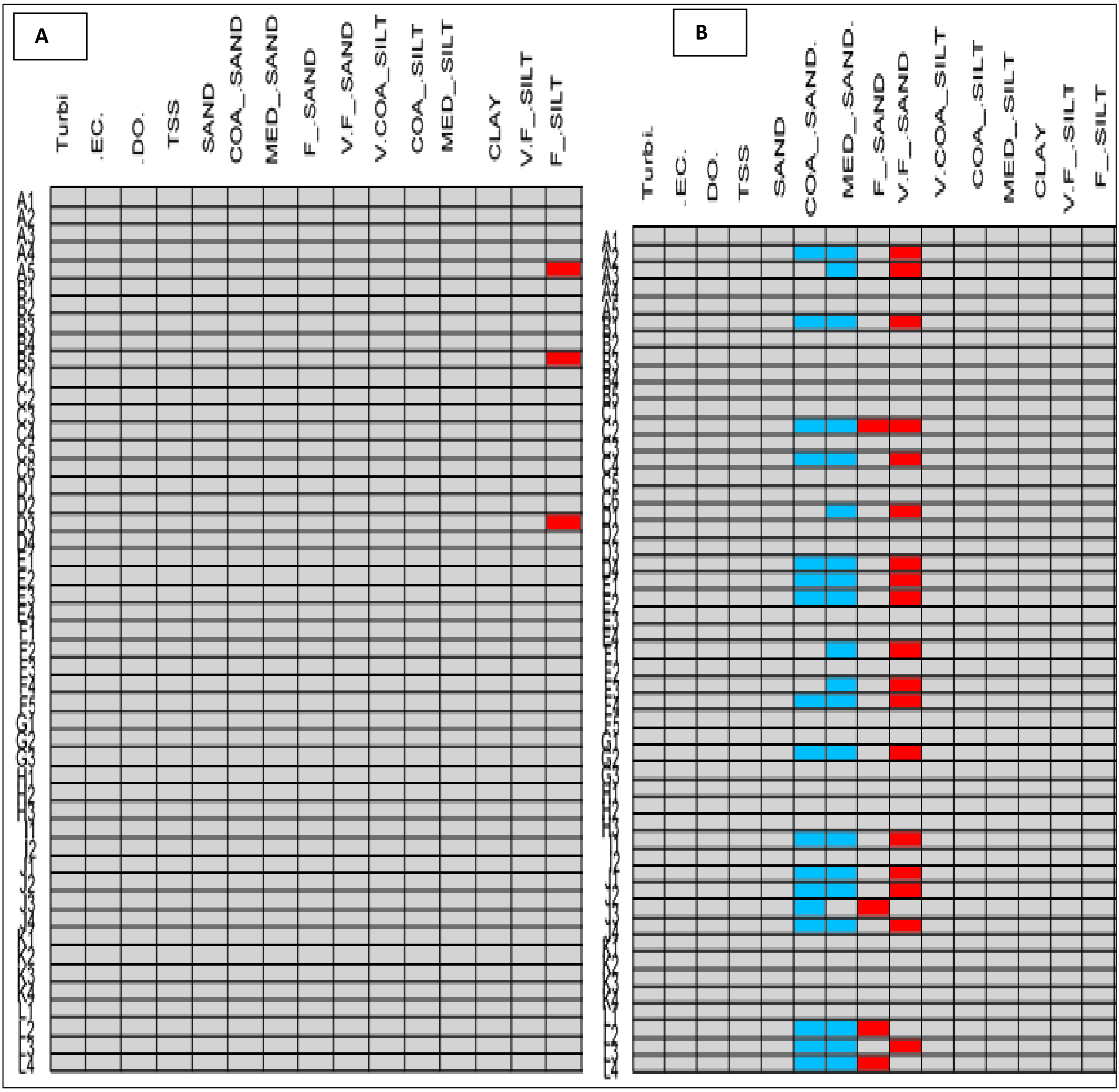


Figure 4. 5: Results of the fourth-corner analysis showing the correlations between macroinvertebrate traits/ ecological preferences, and the fine suspended sediment grain sizes as well as selected physico-chemical variables in the Tsitsa River and its tributaries during the dry season (A) and wet season (B). Red indicates a significant positive correlation ($P \leq 0.05$) and blue indicates a significant negative correlation ($P \leq 0.05$); grain sizes and physico-chemical variables: Turb: turbidity; EC: electrical conductivity; TSS: total suspended solids; F_SAND: fine sand; COA_SAND: coarse sand; MED_SAND: medium sand; VF_SAND: very fine sand; V.COA_SILT: very coarse silt; COA_SILT: coarse silt; MED_SILT: medium silt; F_SILT: fine silt, and VF_SILT: very fine silt.

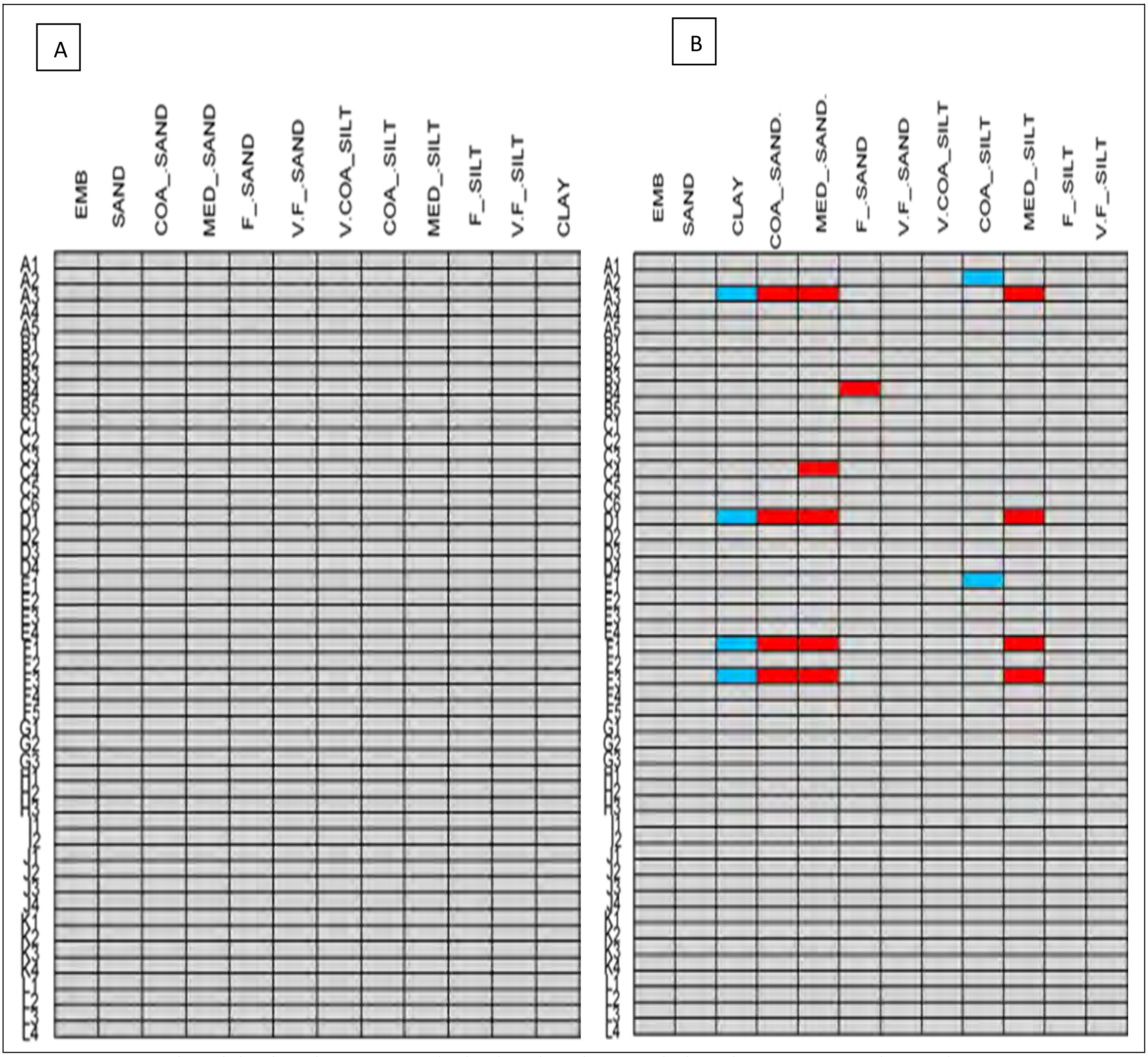


Figure 4. 6: Results of the fourth-corner analysis showing the correlations between macroinvertebrate traits/ ecological preferences, and the fine settled sediment grain sizes as well as selected physico-chemical variables in the Tsitsa River and its tributaries during the dry season (A) and wet season (B). Red indicates a significant positive correlation ($P \leq 0.05$) and blue indicates a significant negative correlation ($P \leq 0.05$); grain sizes and physico-chemical variables: Embed: Embeddeddness; Clay F_SAND: fine sand; COA_SAND: coarse sand; MED_SAND: medium sand; VF_SAND: very fine sand; V.COA_SILT: very coarse silt; COA_SILT: coarse silt; MED_SILT: medium silt; F_SILT: fine silt, and VF_SILT: very fine silt

Table 4. 2: Fourth-corner statistics after 4999 permutations showing correlation coefficient and level of probability of statistical significance for correlation between traits and environmental variables during the dry and wet seasons for suspended sediments. *($P \leq 0.05$). Only significant correlations are shown**.** Grain size abbreviation: DO (Dissolved oxygen), COA_sand (Coarse_Sand), Med_sand (Medium Sand), Coa_Silt (Coarse_Silt), Med_Sil (Medium Silt) and F_Silt (Fine_Silt).

Traits	DO	SAND	COA_SAN	MED_SAN	COA_SIL	MED_SIL	F_SILT	CLAY
				Dry season				
A4							0.042 *	
B1			0.034 *				0.040 *	
B5								-0.045 *
C2			0.041 *				0.039 *	
D3								
E2			0.049 *					
E3			0.045 *					
F4			0.035 *					
G2			0.042 *					
I1			0.034*					
J1			0.031*					
J2			0.040 *					
J3			0.029 *					
J4			0.044 *				0.040 *	
L1								-0.049 *
L2			0.039 *					
L3			0.043 *					
L4			0.035*					-0.048 *
				Wet season				
A2	-0.035 *	-0.032 *				0.034 *		
A3		-0.023 *		0.022 *				
B1	-0.027 *	-0.024 *		0.035 *				
C2	-0.041 *	-0.040 *	0.037 *	0.046 *				
C4	-0.037 *	-0.017 *		0.020 *				
D1				0.024 *		0.020 *		
D4	-0.037 *	-0.023 *		0.026 *				
E1	-0.036 *	-0.031 *		0.038 *				

E2	-0.041 *	-0.020 *		0.015 *
F1		-0.018 *		0.019 *
F3		-0.023 *		0.027 *
F4	-0.024 *	-0.030 *		0.040*
G2	-0.044 *	-0.034 *		0.040 *
I1	-0.027 *	-0.024 *		0.035 *
J1	-0.027 *	-0.035 *	0.05 *	0.044 *
J2	-0.023 *	--0.025 *	0.074	0.033 *
J3	-0.044 *		0.0436 *	
J4	-0.028 *	-0.024 *	0.079	0.029 *
L2	-0.039 *	0.052	0.032 *	
L3	-0.020 *	-0.017 *		0.019 *
L4	-0.034 *		0.034 *	

Table 4. 3: Fourth-corner statistics after 4999 permutations showing correlation coefficient and level of probability of statistical significance for correlation between traits and environmental variables during the dry and wet seasons for settled sediments. *(P ≤ 0.05). Only significant correlations are shown. Abbreviviations: F_Sand (Fine Sand) COA_sand (Coarse_Sand), Med_sand (Medium Sand), Coa_Silt (Coarse_Silt), Med_Sil (Medium Silt) and F_Silt (Fine_Silt).

Traits	F_SAND	COA_SAND	MED_SAND	COA_SILT	MED_SILT	F_SILT	CLAY
			Wet season				
A1		0.022 *	0.025 *	0.038 *	0.024 *		
A2		0.029 *	0.028 *		0.040 *		-0.025 *
B3	0.025 *				-0.019 *		
D1		0.035 *	0.025 *				-0.015 *
F1		-0.035 *			-0.038 *		-0.018 *
F2					0.025 *		-0.035 *

4.4 Discussion

This study explored the influence of elevated settled and suspended sediments on the distribution patterns of macroinvertebrate with particular traits and ecological preferences in selected streams in the Tsitsa River Catchment, Eastern Cape Province of South Africa. The results showed that elevated settled and suspended sediments differentially affected macroinvertebrate traits and ecological preferences, consistent with other freshwater studies (e.g., Firmiano *et al.,* 2021; Wilkes *et al.,* 2017), which have demonstrated significant effects of fine sediments on macroinvertebrate traits. Traits such as feeding on CPOM, and high sensitivity to DO depletion were positively associated with the control sites; they were identified as potentially sensitive traits, revealing ecological preferences related to suspended sediments. These ecological preferences exhibited significant negative correlations with any of suspended sediments grain sizes (Figure 4.1). Conversely, a high tolerance to DO depletion, skating and a preference for FPOM were associated with the highly sedimented sites and were deemed tolerant indicator traits of suspended sediments.

Macroinvertebrate feeding activities and preferences for food are commonly reported to be affected by elevated fine-sediment concentrations as they are linked to nutritional quality or impaired access to food resources (Mathers *et al.,* 2017). In the present study, CPOM was associated with less sedimented sites, indicating that elevated sediments may have affected macroinvertebrates that feed on coarse detritus, CPOM, through the reduction in food quality. Settled sediments that are deposited on the stream bottom or substrates, including CPOM, may cover food items, thereby reducing the quality/palatability and access to food for macroinvertebrate shredders feeding on CPOM (Wilkes *et al.,* 2017). The present study findings also revealed shredders to be negatively associated with settled coarse silt. Doretto *et al.* (2016), who investigated the effects of fine sedimentation on CPOM availability and shredder abundance in Alpine streams in the Pellice River, Italy, found elevated fine sediments significantly reduced the amount of coarse particulate organic matter, affecting the abundance of invertebrate shredders. Further, the palatability of CPOM for macroinvertebrate shredders depends on the initial actions of microbes on leaf litter (Bo *et al.,* 2014).

With regard to collector-filterers, it is likely that suspended sediment in the water column negatively affects filterers by clogging their respiratory and feeding apparatus; thus, their predominant association with the less sedimented sites. The sensitivity of CPOM and collector-filterers is consistent with other studies that have observed a significant decline in macroinvertebrates that prefer CPOM as food and feed by collecting food particles from the water column (Descloux *et al.,* 2014; Doretto *et al.,* 2016).

Dissolved oxygen plays a critical role in the distribution of macroinvertebrates, particularly with regard to those species that have high sensitivities to DO depletion. In this study, the association of taxa with high sensitivity to DO depletion and a high tolerance for DO depletion provided support for the importance of DO in structuring stream communities (Calapez *et al.,* 2018). Sediment delivery from catchment areas that are rich in organic materials is likely to stimulate microbial activities that can cause DO depletion. Moreover, increased sediment concentration which includes both settled and suspended grain sizes can also impact the vertical distribution of oxygen, thereby influencing the depth to which organisms may burrow (Jones *et al.,* 2012). Thus, taxa (e.g., the EPTs) that have a high sensitivity to DO, can be severely affected, and tolerant species of Chironomidae and Oligochaeta are favoured, explaining the distribution pattern of sensitivity/tolerance to DO depletion observed in this study. Further, infiltration of settled fine sediment into the riverbed has been reported to modify macroinvertebrate community structure and functioning (Descloux *et al.,* 2014). Taxa with low DO requirements frequently dominate substrates characterised by a high proportion of fine sediment (see Rabení *et al.,* 2005) with an absence of taxa vulnerable to fine sediment through damage to gills (Murphy *et al.,* 2017; Wood & Armitage, 1997).

Macroinvertebrate filter-feeding structures are usually prone to clogging, particularly when levels of suspended sediments are elevated (Jones *et al.,* 2012). Collector-filterers feed on suspended FPOM from water column have been considered the most intolerant macroinvertebrate functional feeding group, as respiration and filter-feeding structures are clogged by fine particles (Henley *et al.,* 2000; Wood & Armitage, 1997). Although fine sediments in the water column can clog delicate organs such as filter-feeding apparatus, leading to a reduction in macroinvertebrates that feed on FPOM (Descloux *et al.,* 2014; Rabení *et al.,* 2005), fine sediments likely serve as an important source of organic food particles. In the present study, macroinvertebrate preferring FPOM proved tolerant of suspended sediments; thus, it is likely that suspended sediments increased the food availability for macroinvertebrates feeding on FPOM, thereby increasing their occurrence in the highly sedimented sites. However, traits are unlikely to respond to environmental impact in isolation, but a combination of traits is more likely to determine the response of an individual species to a stressor (Piliere *et al.,* 2016). Since collector-filterers that feed on FPOM were earlier identified as sensitive indicators of suspended sediments in this study, it is possible that the observed response of FPOM was mediated by the interactive and correlative effects of other traits (Menezes *et al.,* 2010; Poff *et al.,* 2006).

The tolerance of the skaters to suspended sediments was expected as they are active surface swimmers that live mainly on the water surface and can escape the effects of suspended sediments in the water. Most actively swimming taxa are able to escape from danger and seek refuge (Wood & Armitage, 1997). Thus, the significant association of skaters with the highly sedimented sites suggests that skaters can actively move out of highly sedimented areas and return when conditions normalised, and thus, their tolerance in this study. The tolerance of actively mobile taxa such as skaters observed in the Tsitsa River has been demonstrated in other studies investigating the effects of fine sediments on macroinvertebrate traits (Buendia *et al.,* 2013; Akamagwuna *et al.,* 2019).

4.5 Conclusion

The results of this study showed that macroinvertebrate traits are affected differentially by fine sediments. For suspended sediment grain sizes, it was revealed that traits such as aquatic respiration, predation, a preference for very fast flowing waters, a preference for low DO, skating, and a preference for FPOM were associated with the highly sedimented sites. The Tsitsa river sites were mainly influenced by increasing turbidity, TSS and sediment grain sizes such as fine sand and very fine silt. In the case of settled sediments, traits such as small body size and streamlined body shape were negatively correlated with coarse silt and coarse sand and were more closely associated with the highly sediment-influenced sites. Shredding and aerial respiration were positively correlated with medium silt and were associated with the less sedimented sites. These traits, that is, collector-filtering, shredding and an exposed and soft body were deemed sediment-sensitive to fine silt, coarse silt, fine sand. Traits such as a preference for low DO, skating and a preference for FPOM were regarded as sediment-tolerant traits. On the other hand, traits/ecological preferences such as CPOM, collector-filtering, and a high sensitivity to DO were deemed sensitive to suspended sediments. Overall, the present study, which identifies trait-based indicators provides insights into the potential utility of the TBA in freshwater biomonitoring of sediment effects on macroinvertebrates.

CHAPTER 5: DEVELOPING A TRAIT-BASED APPROACH FOR ASSESSING AND PREDICTING THE POTENTIAL VULNERABILITY AND RESILIENCE OF SOUTH AFRICAN MACROINVERTEBRATES TO FINE-SEDIMENT IMPACT IN THE TSITSA RIVER AND ITS TRIBUTARIES

5.1 Introduction

Fine sediments, usually less than 2 mm in diameter, are a natural component of aquatic ecosystems where they provide microhabitats for aquatic organisms (Bilotta & Brazier, 2008). Although fine sediments provide habitats for aquatic organisms, levels beyond natural backgrounds can cause deleterious effects on aquatic organisms, with potentially serious consequences for biodiversity and overall ecosystem health (Jones *et al.,* 2012). Thus, water quality impairment caused by elevated fine-sediment levels has increasingly become a global concern.

Increased fine-sediment deposition of organic and inorganic sources in rivers and streams can have several biological and ecological effects: clogging gills, smothering eggs, filling up interstitial spaces, disrupting fine feeding organs, burying less motile species, depleting dissolved oxygen, and reducing visual clarity and light penetration (Extence *et al.,* 2011; Gordon *et al.,* 2013; Turley *et al.,* 2016). These effects have been thoroughly reviewed in Chapter 1.

Macroinvertebrate taxa can be differentially vulnerable to fine-sediment effects, depending on their adaptive features. Statistical modelling approaches (e.g., Paillex *et al.,* 2017) have been used to model the potential responses of macroinvertebrate taxa to water quality stressors. Such statistical modelling approaches can also be applied to fine-sediment impacts. However, to successfully apply statistical models with some degree of certainty and reliability, long-term historical data are required. In situations where such long-term data are scarce or near non-existent, as in South Africa, the use of biological traits with mechanistic linkages to the stressor of interest offer an ecologically sound alternative approach for predicting the potential vulnerability and resilience of macroinvertebrate taxa to water quality stressors. Biological traits are inherent features of organisms that influence their adaptive capacity and mediate the organism's relationships with their external environment. Thus, organisms survive and thrive when they possess the appropriate combinations of traits, allowing them to adapt to the prevailing external environmental conditions. The principle that traits mediate species-environment relationship, and those traits may determine species-adaptive capacity and extent of vulnerability and resilience, has led to the use of biological traits to predict the potential responses of macroinvertebrates to sediment impact (Murphy *et al.,* 2017).

Two main approaches to the use of biological traits exist in the literature (Extence *et al.,* 2011; Verberk *et al.,* 2013; Desrosiers *et al.,* 2019). The first approach, which was adopted in Chapter 4 of this thesis, involves analysing and predicting the distribution of multiple biological trait classes across a gradient of impact in order to determine trait-impact association (Dolédec & Statzner 2008; Descloux *et al.,* 2014). This approach is the commonest and has been widely used to study the distribution of traits in relation to fine-sediment impact in riverine ecosystems (e.g., Descloux *et al.,* 2014). The second approach, which is followed in this chapter, involves predicting the responses of macroinvertebrate taxa based on the type of traits, trait interactions and the combinations possessed (e.g., Liess & Von de Ohe 2005; Extence *et al.,* 2011). The second approach has been used to develop the proportion of sediment-sensitive invertebrates index (PSI) in the United Kingdom (Extence *et al.,* 2011) and also the species at risk (SPEAR) model (Liess & Von de Ohe 2005).

Verberk *et al.* (2013) developed a robust open-ended framework allowing taxa predictions based on the traits, trait interactions and combinations possessed. The usefulness of the second approach is that the outcomes of prediction are macroinvertebrate taxa rather than the traits themselves. Trait-environment interactions are thus seen as mediating the vulnerability and resilience of the macroinvertebrate taxa. This is a particularly useful approach because it allows for identification and protection of vulnerable species.

In South Africa, despite sedimentation of rivers being one of the leading causes of water quality impairment, the potential vulnerability and/or resilience of riverine macroinvertebrates to sediment impact is poorly studied. Given the complexity of factors associated with the impact interactions between macroinvertebrates and sediment, predicting the vulnerability of macroinvertebrates to sediment impact is complex. Nevertheless, in this study, a novel TBA is developed to attempt such a complex task. The objectives of this chapter are: i) to develop a novel TBA for assessing and predicting macroinvertebrate potential vulnerability and resilience to fine-sediment impact, and ii) to test the predictions arising from the developed approach in the Tsitsa River and its tributaries in Eastern Cape, South Africa. It was hypothesised that families designated as potentially vulnerable to sediment impact following the TBA developed, would decrease with increasing levels of fine sediment. This chapter thus fulfils Objective 4 of the study as stated in Chapter 1.

5.2 Materials and methods

5.2.1 Developing the trait-based approach for prediction

To predict macroinvertebrate responses to disturbances such as elevated fine sediments in freshwater ecosystems requires an understanding of the mechanistic relationship between species-environmental interaction, mediated by traits (Lavorel & Garnier, 2002; Libala *et al.,* 2019). Therefore, a rule-based approach involving six steps following the approach developed by Odume *et al.* (2018) and applied by Libala *et al.* (2019) was adopted. The steps are: i) reviewing published literature for reported modes of fine-sediment impact on macroinvertebrates; ii) on the basis of the reported sediment modes of impact, identifying traits that are mechanically linked to the sediment modes of impact; iii) identifying potentially vulnerable trait attributes per taxon; iv) identifying non-redundant vulnerable trait attributes per taxon; v) quantifying the measure of functional trait diversity (FTD) per trait and per taxon; vi) calculating potential macroinvertebrate vulnerability based on the combination of trait attributes possessed, functional trait diversity (FTD), and non-redundant vulnerable trait attributes. Macroinvertebrates were classified as either potentially vulnerable or resilient to fine-sediment impact.

Step 1: Reviewing fine-sediment modes of impact on macroinvertebrates

Fine-sediment modes of impact on macroinvertebrates were extensively reviewed in the literature to identify documented mechanisms by which sediments influence macroinvertebrates. Sediment modes of impact could be through direct or indirect mechanisms and are summarised in Table 5.1.

Table 5. 1: A summary of fine-sediment modes of impact on aquatic macroinvertebrates (adapted from Odume *et al*., 2018).

Fine-sediment mode of impact	**Effect on aquatic macroinvertebrates and their environment**
Clogging	Inputs of elevated fine sediments into freshwater ecosystems can clog sensitive organs such as gills and filter-feeding apparatus. Gill clogging can result in respiratory impairment, and when sediments remain elevated over a long time, may result in mortality and perhaps removal of affected species from the environment (Jones *et al.,* 2012). Efficiency of food intake can be impacted when filter-feeding apparatus are clogged by fine sediments, even though some groups of macroinvertebrates, e.g., molluscs, are able to cleanse their feeding organs regularly.
Abrasion	Excessive inputs of fine sediments, particularly those moving at high velocity in riverine ecosystems, are likely to abrade exposed body parts (Wilkes *et al.,* 2017). Organisms with exposed, fleshy body parts are thus prone to abrasion. Macroinvertebrate species that can retract into cases, or whose bodies are partly or fully sclerotised are likely to be less vulnerable to the effect of abrasion (Kurtak, 1978).
Burial	Increased deposition of fine sediments can bury less motile and sessile/attached organisms (Jones *et al.,* 2012; Bona *et al.,* 2016). Slow-moving taxa are also vulnerable if they cannot keep pace with the rate of sediment accretion. Further, as a direct consequence of burial, the chemical composition of the microhabitats may be impacted through oxygen reduction and other chemical processes. Taxa that are attached either permanently or temporarily, or are sedentary, may be particularly vulnerable to burial effects.

Fine-sediment mode of impact	Effect on aquatic macroinvertebrates and their environment
Substrate modification	Gradual and sustained accretion of fine sediments may alter the stability of the riverbed, modify substrate surfaces and fill up interstitial spaces, thereby impacting those taxa with a preference for stable substrates such as stones and vegetation. Substrate modification may also impact on the chemical conditions of microhabitats, with potential consequent effects for organisms that are sensitive to changes in physico-chemical conditions.
Physico-chemical effects (oxygen depletion)	A primary mode of physico-chemical impact by organic sediments on aquatic organisms is by depleting dissolved oxygen (Billota & Brazier 2008). Sediment delivery from catchments rich in organic materials is likely to stimulate microbial activity, depleting dissolved oxygen. Increased sediment loads may also impact on vertical distribution of oxygen, influencing the depth to which organisms burrow (Jones *et al.,* 2012).
Physico-chemical effects (increased turbidity)	Increased turbidity may result from elevated concentrations of suspended sediments, which may impact visibility, with implications for predators that rely on visual clarity to search for prey (Billota & Brazier 2008). Increased turbidity can reduce light penetration, which in turn, may affect growth of periphyton. Reduced periphyton growth has implications for food availability, particularly for grazers (Danger *et al.*, 2008).

Fine-sediment mode of impact	**Effect on aquatic macroinvertebrates and their environment**
Food availability and quality	The potential effects of elevated fine-sediment deposition on food availability and quality are complex and involve a range of interacting factors. For example, increased sediment deposition may cover the surfaces of substrates, reducing the growth of periphyton and the overall quality of the available food resources (Buendia *et al.,* 2013). On the other hand, depending on the accretion rate, increased fine sediments can increase particulate organic matter, an important food resource for the filter feeders, which may lead to the blossoming of filter-feeding organisms (Jones *et al.,* 2012). However, sustained deposition of fine sediments over an extended period may result in negative overall effects on filter feeders. Shredders can be negatively affected as fine sediments accumulate over leaf litter.

Step 2: Trait selection

A total of ten trait categories resolved into 45 trait attributes deemed mechanistically linked to fine-sediment modes of impact were selected. The selected trait categories include respiration, respiratory type, mobility, attachment, habitat preference, dominant food type, feeding mode, oxygen sensitivity/tolerance, body protection and velocity preference (Table 5.2). The trait information was retrieved from the recently compiled South African macroinvertebrate trait database (Odume *et al.,* 2018). Sensitivity scores awarded each macroinvertebrate taxon in the South African Scoring System version 5 (SASS5) were used as surrogates for oxygen tolerance and sensitivity, as the two metrics of SASS5 score and ASPT value have been found to be very responsive to and correlated with dissolved oxygen depletion (Dickens & Graham 2002; Odume *et al.,* 2012). Habitat and velocity preferences were derived from the Macroinvertebrate Response Assessment Index (MIRAI) version 2 (Thirion, 2016).

Step 3: Vulnerable trait attributes

For each trait category, trait attributes likely to confer vulnerability on the taxon in relation to one or more modes of sediment impact were identified and termed "vulnerable trait attributes" (Table 5.2). Vulnerable trait attributes are those trait features possessed by an organism that increase its likelihood of vulnerability to a particular environmental stressor. The identification of vulnerable trait attributes was based on: i) results obtained in Chapter 4, ii) ecological reasoning, and iii) the predicted response of specific trait attributes to sediment impact in the literature, and those whose predicted responses have been confirmed in empirical studies (e.g., Murphy *et al.,*2017; Wilkes *et al.,* 2017). The rationale for identifying vulnerable trait attributes for each trait category is that, depending on the trait functional redundancy and diversity, a taxon possessing greater numbers of vulnerable trait attributes would be likely to be more vulnerable to a stressor of interest than another taxon with fewer vulnerable trait attributes.

Table 5. 2: Macroinvertebrate traits, trait attributes, vulnerable trait attributes and associated sediment modes of impact, selected to develop the TBA for assessing potential vulnerability of macroinvertebrates at family level in the studied river systems.

Trait category	Trait attribute	Sediment mode(s) of impact	Vulnerable trait attributes	Rationale
Respiration	Gills, tegument, spiracles, lung, plastron, respiratory pigment (e.g., haemoglobin), breathing tubes and other similar apparatus e.g., elytra, straps.	Clogging, change in water chemistry, and abrasion.	Gills.	Increased fine sediments can easily clog exposed gills and cause abrasion, particularly if sediments are moving at a relatively high velocity. This effect is likely to be more detrimental for taxa relying chiefly on exposed gills for respiration.
Mobility	Burrowers, crawlers, climbers, swimmers, skaters, sprawlers.	Burial, change in water chemistry, and substrate modification.	Burrowers, crawlers.	Mobility determines whether an organism can escape from impending danger. Elevated fine sediments modify river substratum, water chemistry, and may cause burial. Less motile taxa, such as burrowers and crawlers, have been predicted to be potentially vulnerable to sediment effect through burial, change in microhabitat water chemistry and substrate modification (Wilkes *et al.*, 2017).

Attachment	Free living, temporary attachment, permanent attachment.	Burial, change in water chemistry, and substrate modification.	Temporary attachment, permanent attachment.	Sessile and attached taxa (whether permanent or temporary) are less likely to escape from impending danger; sustained elevated sediment input may bury attached taxa and may also influence their microhabitat chemistry, particularly in relation to DO availability and substrate stability (Kaufmann *et al.,* 2009). Temporary/permanent attachments have been predicted to decrease with increasing sediment loads (Wilkes *et al.,* 2017).
Habitat preference	Stones, vegetation, sediments, and surface water column.	Burial, change in water chemistry, change in substrate/substrate modification.	Stone and vegetation.	Gradual and sustained sediment accretion modifies the stability of substrates, the chemistry of microhabitats, and fills up interstitial spaces between stones, making taxa with a preference for stable substrates, such as stone and vegetation, more likely to be vulnerable than those with a natural preference for sediments and an open water column.

Dominant food type	Algae, animal parts, living macrophytes, leaf litter and detritus.	Food availability and quality.	Algae and leaf litter.	Although the mode by which sediments affect food availability and quality is complex, its effect is more likely to be pronounced for the availability of periphyton growing on stable substrate and leaf litter, which are more likely to be covered by sediment accretion (Buendia *et al.*, 2013).
Feeding mode	Filter feeders, grazers/scrapers, shredders, predators, deposit feeders, collector/gatherers.	Clogging, burial, change in water chemistry, food availability and quality, substrate modification.	Grazers, shredders, filter feeders and predators.	The potential influence of elevated sediments on feeding mode/habit is complex. However, elevated fine sediments are likely to negatively influence food sources and quality of food for grazers and shredders through burial of food materials and substrate modification, particularly for taxa that graze on periphyton. Although input of fine sediments with high organic materials is likely to make more food available for filter feeders, sustained sediment accretion may cause clogging of

				feeding organs. Predators are also likely to be affected by visual impairment.
Respiratory type	Aquatic respiration, aerial/non-aquatic respiration.	Change in water and microhabitat chemistry (particularly dissolved oxygen).	Aquatic respiration.	Sediment accretion depletes dissolved oxygen in both open water and in microhabitats, causing obligate aquatic breathers to be more vulnerable than non-aquatic or facultative aquatic breathers.
Oxygen sensitivity/tolerance	Highly sensitive to oxygen depletion (SASS5 score 12–15), sensitive to oxygen depletion (SASS5 score 7–11), tolerant of oxygen depletion (SASS5 score 4–6), highly tolerant of oxygen depletion (SASS5 score 1–3).	Change in water and microhabitat chemistry (particularly dissolved oxygen).	Highly sensitive and sensitive to oxygen depletion.	Accretion of deposited fine sediments with high organic matter content can lead to depletion of DO concentrations in both open water and within microhabitats. Taxa are likely to respond in a manner similar to oxygen depletion occasioned by organic pollution. Highly sensitive and sensitive taxa derived mostly based on a gradient of organic pollution are therefore more likely to be vulnerable than non-sensitive taxa.

Body protection	Exposed and soft, cased/tubed, exposed but sclerotised.	Abrasion.	Exposed and soft.	Fine sediments are likely to cause abrasion of fine, soft, and exposed body structures. The degree of abrasion is likely to be related to the velocity at which deposited and suspended sediments are moving. Tube-builders and cased taxa are less likely to be affected as they would retract into cases or tubes to protect delicate structures. Taxa whose bodies are protected with a shell or carapace or are heavily sclerotised are also less likely to be affected by abrasion.
Velocity preference (m/s)	Standing water (< 0.1), slow flowing (0.1–3), fast flowing (0.3–0.6), very fast flowing (>0.6).	Abrasion.	Fast flowing and very fast flowing.	Fine sediments moving at a relatively high velocity are more likely to cause abrasion of delicate organs or soft and fleshy body surfaces/structures (Jones *et al.*, 2012). Taxa preferring fast/very fast-moving water are particularly at risk.

Step 4: Functionally redundant trait attributes

Functional redundancy (FR) is a key determinant of the stability and/or persistence of an ecosystem function as multiple species can perform similar functions in ways such that their roles can be interchangeable (Schemera *et al.,* 2017). Ecosystems with high FR are functionally more stable and resilient than those with low redundancy. In this study, FR describes vulnerable trait attributes that are functionally redundant with non-vulnerable trait attributes per trait category for each taxon. For example, in terms of the trait 'mobility', crawling is identified as a vulnerable trait attribute, whereas swimming is not. However, many macroinvertebrates taxa crawl and also swim, making these two traits functionally redundant. Crawling would be described as a functionally redundant vulnerable trait attribute. In the same way, for many taxa, gills and tegument may be considered functionally redundant although the efficiency of oxygen uptake is higher for gills. It is postulated that the impact of a stressor on an organism via a functionally redundant vulnerable trait attribute is likely to be moderated by the non-vulnerable trait attributes that can perform the same or similar function. On the other hand, when no other trait attribute for a trait category can perform the function of the vulnerable trait attributes, such a trait attribute is referred to as a functionally non-redundant vulnerable trait attribute. For example, where the respiratory type is resolved into aquatic and air breathing, the former is a functionally non-redundant trait attribute for taxa that are obligate aquatic breathers, whereas it is functionally redundant for taxa that are facultative aquatic breathers such as pulmonate snails.

Step 5: Functional trait diversity (FTD) (plasticity)

Functional diversity (FD) describes what organisms do in communities or ecosystems, and it is measured and assessed using various methodologies (Petchey & Gaston 2006; Schemera *et al.,* 2017). Functional diversity enhances resource use and niche complementarity. It is therefore a critical determinant of biodiversity persistent in an ecosystem. Functional trait diversity (FTD) is here applied to qualitatively describe the range of trait attribute diversity that exists for a taxon per selected trait category. The rationale is that macroinvertebrate families comprise many species and genera, each with a range of environmental requirements, behaviour, tolerance and sensitivity. In addition, within some taxa, different aquatic life stages differ in their environmental requirements and are therefore functionally diverse. The implication is that a taxon with a wider range of FTD is more likely to withstand perturbation than those with a narrower range of FTD. For example, in the family Chironomidae, all the feeding modes are represented, and it is therefore more likely to withstand food scarcity than a specialist feeding family. In terms of respiration, species of the family Chironomidae are also functionally diverse, relying on both gills and tegument for respiration, as well as haemoglobin to enhance respiratory efficiency, making them more tolerant of oxygen depletion than families that rely on gills only. Therefore, in developing the TBA, FTD is applied to scale the perceived FD of each family per selected trait category.

A scale of 1–5 was chosen where 1 indicates a low FTD for the specific trait category, 3 a moderate FTD, and 5 a high FTD. For example, a family in which all the feeding groups are represented, that is, shredders, predators, grazers/scrapers, collectors/gatherers and filter feeders, would be awarded an FTD of 5 for the trait category, feeding mode; whereas a family whose members feed only by shredding and grazing would be awarded an FTD of 1 for the same trait category. A score of 3 would be awarded to a family with more than two feeding modes but not having all the described feeding modes represented. This logic was applied to all trait categories except for oxygen sensitivity/tolerance derived from the SASS5 scores. For oxygen sensitivity, a score of 5 was awarded to taxa in the categories tolerant and highly tolerant. These taxa were awarded a higher FTD score as they are perceived to have a wider requirement for dissolved oxygen. Taxa belonging to the categories ‘highly sensitive’ and ‘sensitive’ were awarded a score of 1 and 3, respectively.

Step 6: Classifying taxa into potentially vulnerable groups

Vulnerability scores for macroinvertebrate families were calculated based on the vulnerable trait attributes possessed, non-redundant vulnerable trait attributes, and FTD per trait category using the equation below:

$$\text{Taxon vulnerability score (VS)} = \mathrm{N} + \sum_{\mathrm{n1}}^{\mathrm{i}} \left(\frac{\mathrm{ni}}{\mathrm{FTDi}} \right) NRi$$

where N = total number of vulnerable trait attributes possessed, n = vulnerable trait attribute per trait category, FTD = functional trait diversity per trait category, NR = total numbers of non-functionally redundant vulnerable trait attributes possessed, n1 = trait category number 1..n^{th} trait category for the particular taxon.

Using the calculated scores, macroinvertebrates were grouped into two distinct groups: taxa designated as potentially vulnerable, and those designated as resilient. The percentile distribution of the VS calculated for all the macroinvertebrates families were used to designate potential vulnerability as follows: vulnerable: (70^{th}–100^{th} percentile) or resilient: ($<70^{th}$percentile). An illustration of the approach for specific taxa is provided in Appendix C.

5.2.2 Predicting macroinvertebrate responses

Following the developed approach, the predictions below were made:

i. that the relative abundance and richness of the potentially vulnerable macroinvertebrate families would decrease with increasing total suspended solids, turbidity, and embeddedness. It was also predicted that these biological metrics would increase with increasing dissolved oxygen (DO);
ii. that the relative abundances and richness of the potentially vulnerable macroinvertebrate families would markedly decrease at the fine-sediment-influenced sites compared to the control sites (CLS).

5.2.3 Testing the predicted responses of macroinvertebrates in the Tsitsa River and its tributaries

To evaluate the predicted responses of macroinvertebrate taxa to sediment impact, the relative abundance and richness of the vulnerable and resilient families were regressed against DO, turbidity, total suspended solids (TSS) and embeddedness (EMB), using linear regression for both dry and wet seasons. These water quality variables were used as explanatory variables, whereas the biological metrics of the vulnerable taxa (VT) and resilient taxa (RT) were used as response variables (Libala *et al.,* 2019). The assumptions of normality and homogeneity of variance were investigated using the Shapiro-Wilk test and the Levene's test, respectively, and the data were normally distributed. Linear regression analyses were undertaken using STATISTICA software package version 13.3.

Box plots were used to graphically represent the distribution of the relative abundances and richness of the macroinvertebrate taxa: vulnerable or resilient in relation to the sites separately for the wet and dry seasons. The Kruskal-Wallis multiple comparison tests were used to test for significant differences ($p \leq .05$) between the sites in terms of the relative abundances of the VT and RT (Odume *et al.,* 2018; Libala *et al.,* 2019).

5.3 Results

5.3.1 Macroinvertebrate vulnerability and resilience to fine-sediment impact

Vulnerability scores were calculated for a total of 92 South African macroinvertebrate families. Of the 92 families, 29 were designated as potentially vulnerable and 63 as resilient to fine-sediment impacts (Appendix D).

5.3.2 Testing the prediction regarding relative abundances of the vulnerable and resilient taxa in relation to dissolved oxygen, total suspended solids, turbidity and embeddedness

The results indicated that, during the dry season (winter and spring), the relative abundance of the resilient taxa increased with increasing turbidity concentration (Figure 5.1). The relative abundance of the vulnerable taxa (VT) decreased with increasing turbidity. As expected, per the prediction, the relative abundance of the vulnerable and resilient taxa increased with the increasing concentration of dissolved oxygen (DO) (Figure 5.2). The relationship between the resilient taxa (RT), vulnerable taxa (VT) and DO was statistically significant ($P < 0.05$) as per the linear regression.

Regarding total suspended solids (TSS), as expected, the relative abundance of the VT decreased with the increasing concentration of TSS, and the relationship was statistically significant ($P < 0.05$) as shown in Figure 5.3. However, the relative abundance of the RT increased with increasing TSS concentration (Figure 5.3). In the case of embeddedness, the relative abundances for both resilient and vulnerable taxa during the dry season increased with increasing embeddedness (Figure 5.2), and both relationships were statistically significant ($P < 0.05$).

The results during the wet season (summer and autumn) indicated that increasing turbidity impacted negatively on the relative abundance of the vulnerable taxa. The resilient taxa increased in abundance with increasing turbidity (Figure 5.1). As with the dry season, the DO concentrations impacted positively on both the relative abundances of the vulnerable and resilient taxa, which also showed statistically significant relationships ($P < 0.05$). Increasing TSS impacted positively on the relative abundance of the VT and RT, but the regression analysis showed that the relationships were not statistically significant (Figure 5.3). The vulnerable taxa increased significantly with increasing embeddedness while the resilient taxa decreased with increasing embeddedness, as shown in Figure 5.4.

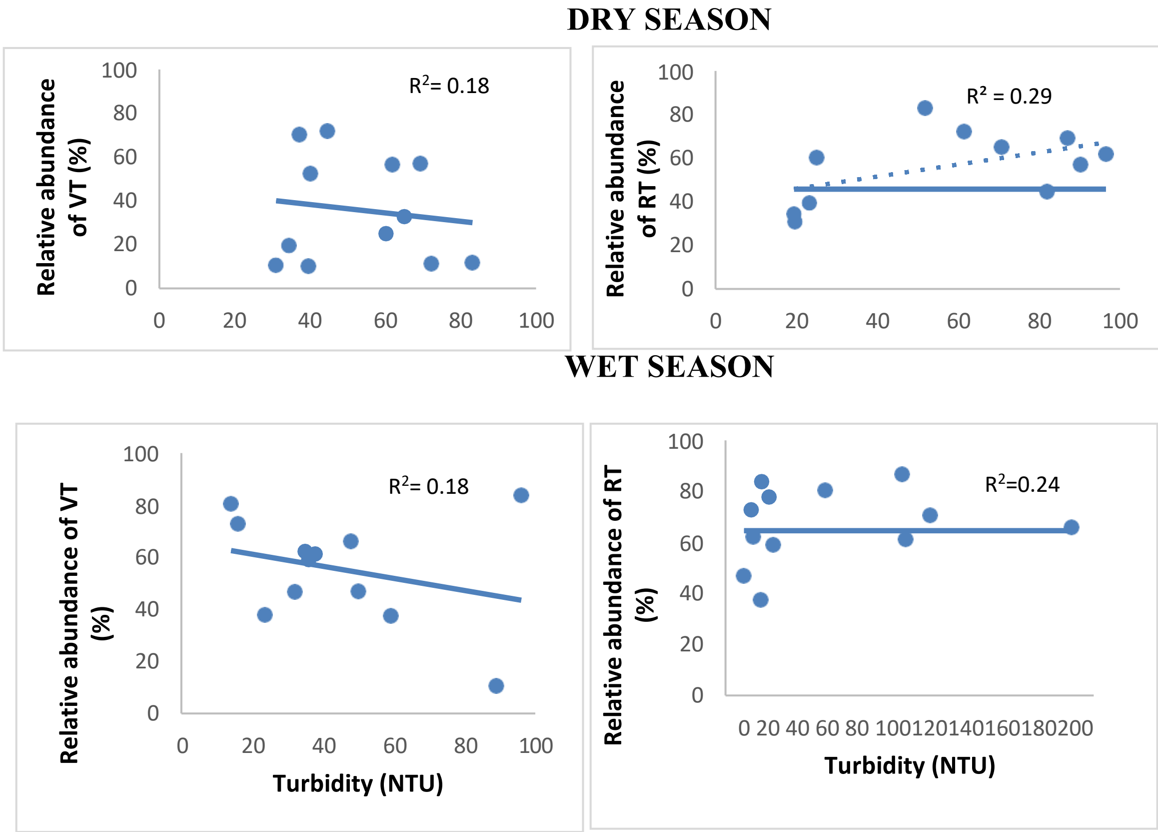


Figure 5. 1: Linear regression of the relative abundance of the vulnerable taxa (VT), resilient taxa (RT), and turbidity during the dry and wet seasons. *P* values are shown for relationships that are statistically significant ($P < 0.05$).

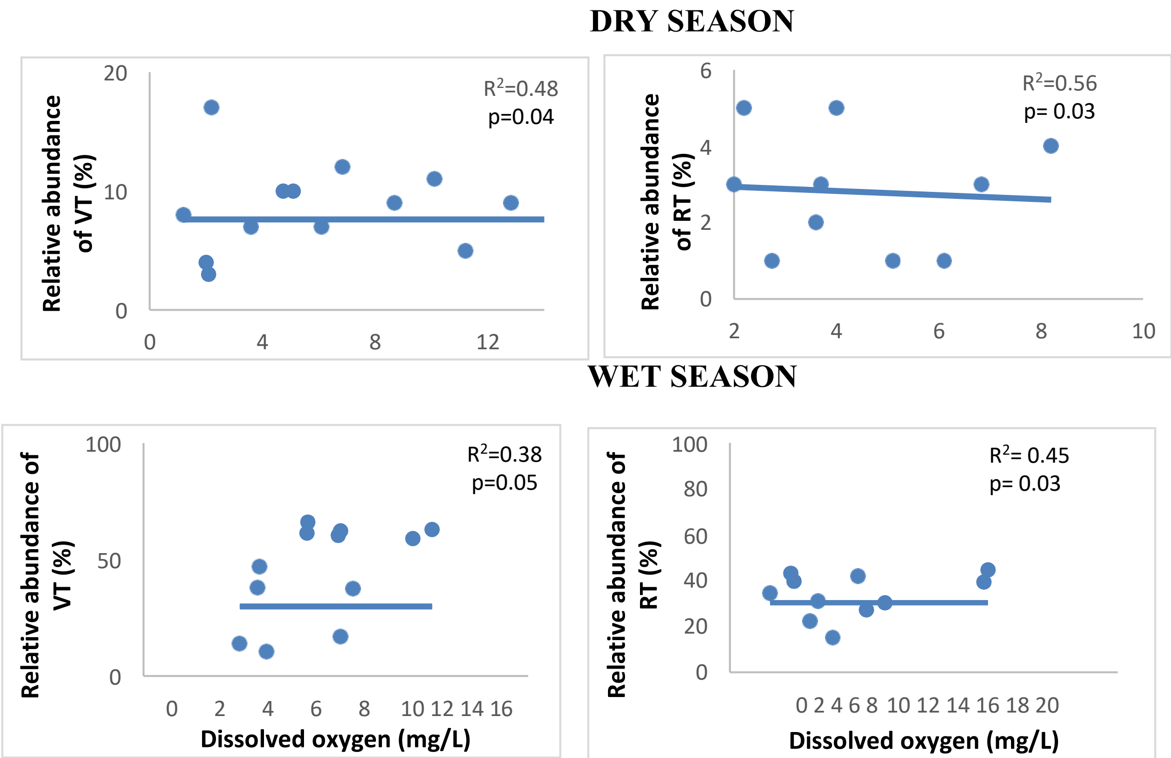
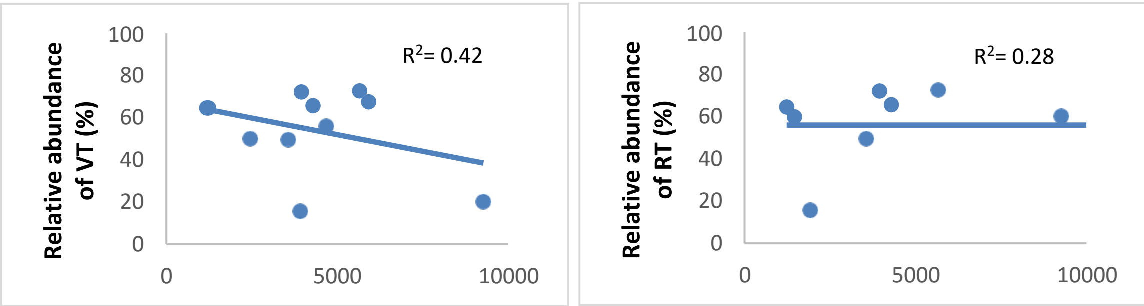


Figure 5. 2: Linear regression of the relative abundance of the vulnerable taxa (VT) and resilient taxa (RT) and dissolved oxygen during the dry and wet seasons. *P* values are shown for relationships that are statistically significant ($P < 0.05$).

DRY SEASON



WET SEASON

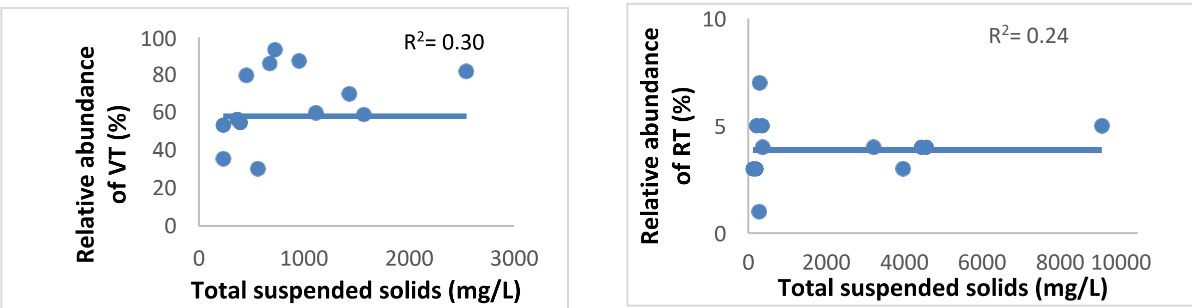
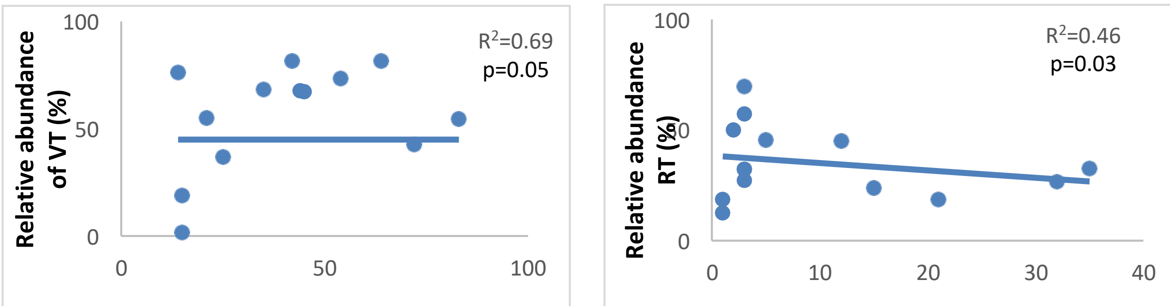


Figure 5. 3: Linear regression of the relative abundance of the vulnerable taxa (VT), resilient taxa (RT), and total suspended solids during the dry and wet seasons. *P* values are shown for relationships that are statistically significant ($P < 0.05$).

DRY SEASON



WET SEASON

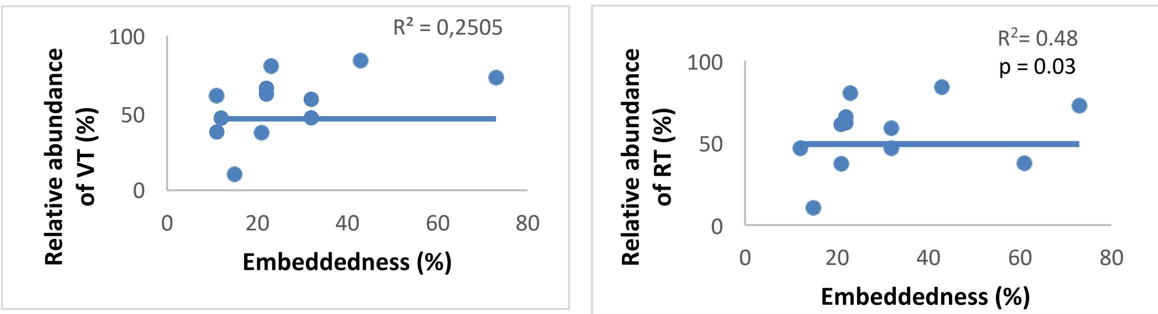


Figure 5. 4: Linear regression of the relative abundance of the vulnerable taxa (VT), resilient taxa (RT), and embeddedness during the dry and wet seasons. *P* values are shown for relationships that are statistically significant ($P < 0.05$). NB: higher percentage is lower embeddedness.

5.3.3 Testing the prediction regarding the richness of the vulnerable and resilient taxa in relation to dissolved oxygen, total suspended solids, turbidity and embeddedness

The results for taxa richness (vulnerable and resilient) during the dry season showed that the richness of the resilient taxa (VT) increased with increasing turbidity, but a marked decrease for richness of the VT in relation to increasing turbidity (Figure 5.5). The relationship between the richness of the VT and turbidity was statistically significant ($P < 0.05$). As expected, both the richness of the resilient and vulnerable taxa increased with increasing DO (Figure 5.6) concentration and showed statistically significant relationships ($P < 0.05$).

With regard to total suspended solids, as expected, RT richness increased with increasing TSS concentration (Figure 5.7). However, VT richness decreased with increasing TSS concentration and was statistically significant ($P <0.05$). As per embeddedness, the resilient taxa increased with increasing embeddedness and the richness of vulnerable taxa increased with increasing embeddedness; the relationships were statically significant ($P < 0.05$) for both VT and RT against embeddedness (Figure 5.8).

The results during the wet season (summer and autumn) indicated that an increase in turbidity positively impacted the richness of RT, while negatively impacted the richness of VT, with the relationship being statistically significant ($P<0.05$) for the RT as shown in Figure 5.5. As expected, the richness for both RT and VT increased with increasing DO. While the richness of RT increased with increasing total suspended solids with a statistically significant difference ($P < 0.05$), the richness of VT decreased with increasing total suspended solids.

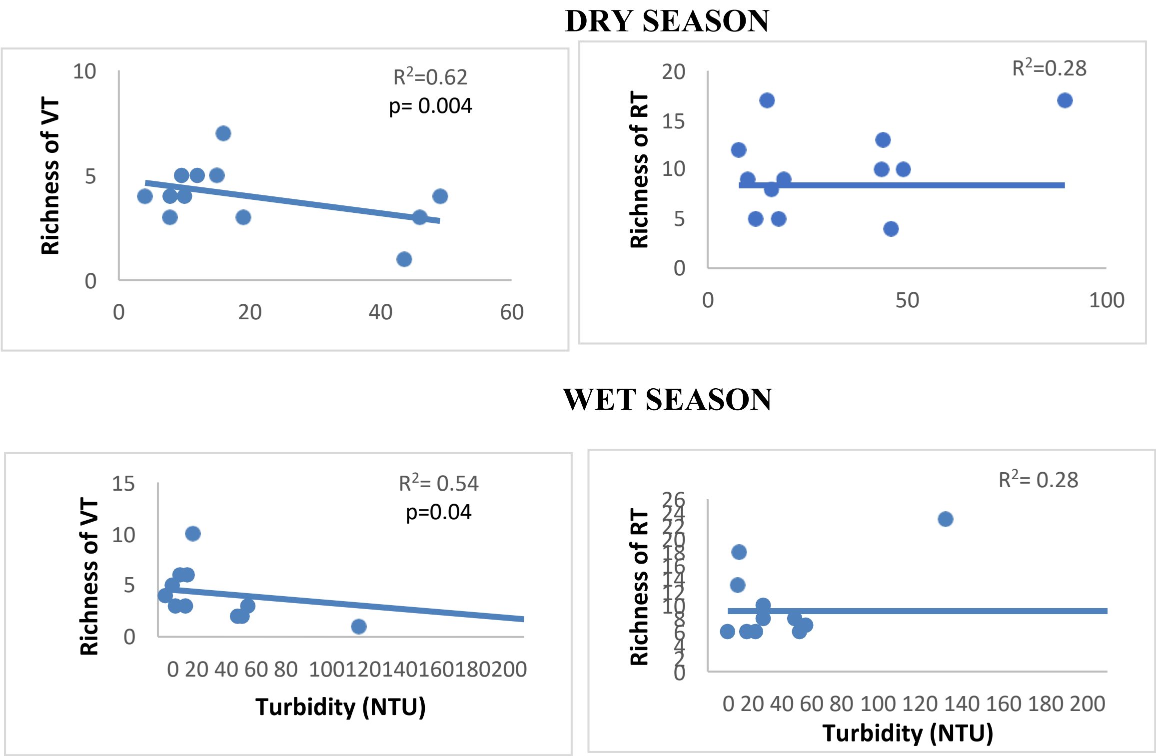


Figure 5. 5: Linear regression of the richness of the vulnerable taxa (VT), resilient taxa (RT), and turbidity during the dry and wet seasons. *P* values are shown for relationships that are statistically significant ($P < 0.05$).

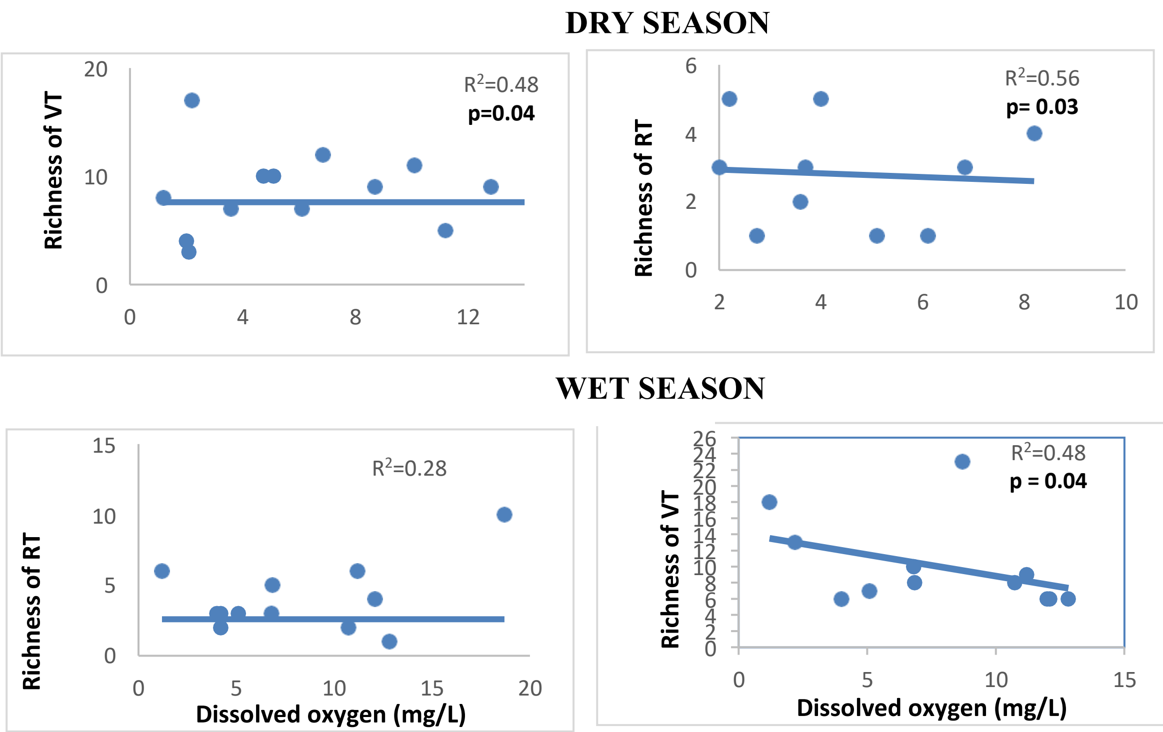


Figure 5. 6: Linear regression between the richness of the vulnerable taxa (VT), resilient taxa (RT), for dissolved oxygen during the dry and wet seasons. *P* values are shown for relationships that are statistically significant ($P < 0.05$).

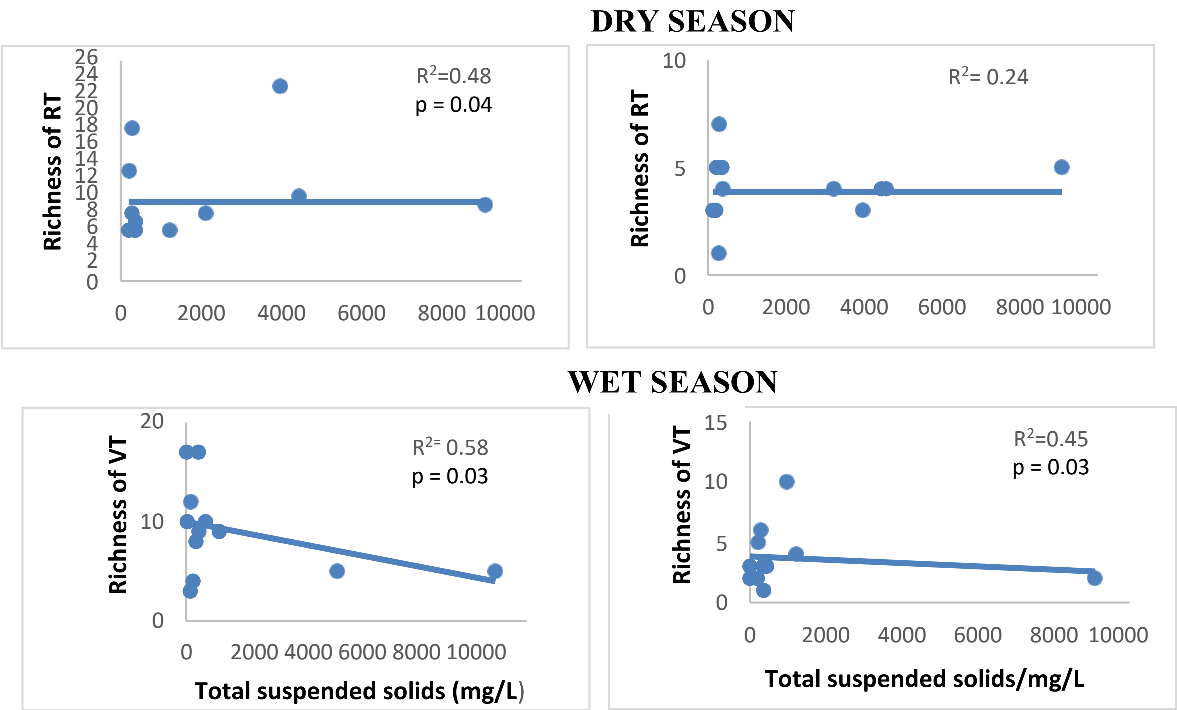


Figure 5. 7: Linear regression between the richness of the vulnerable taxa (VT), resilient taxa (RT), for total suspended solids during the dry and wet seasons. *P* values are shown for relationships that are statistically significant ($P < 0.05$).

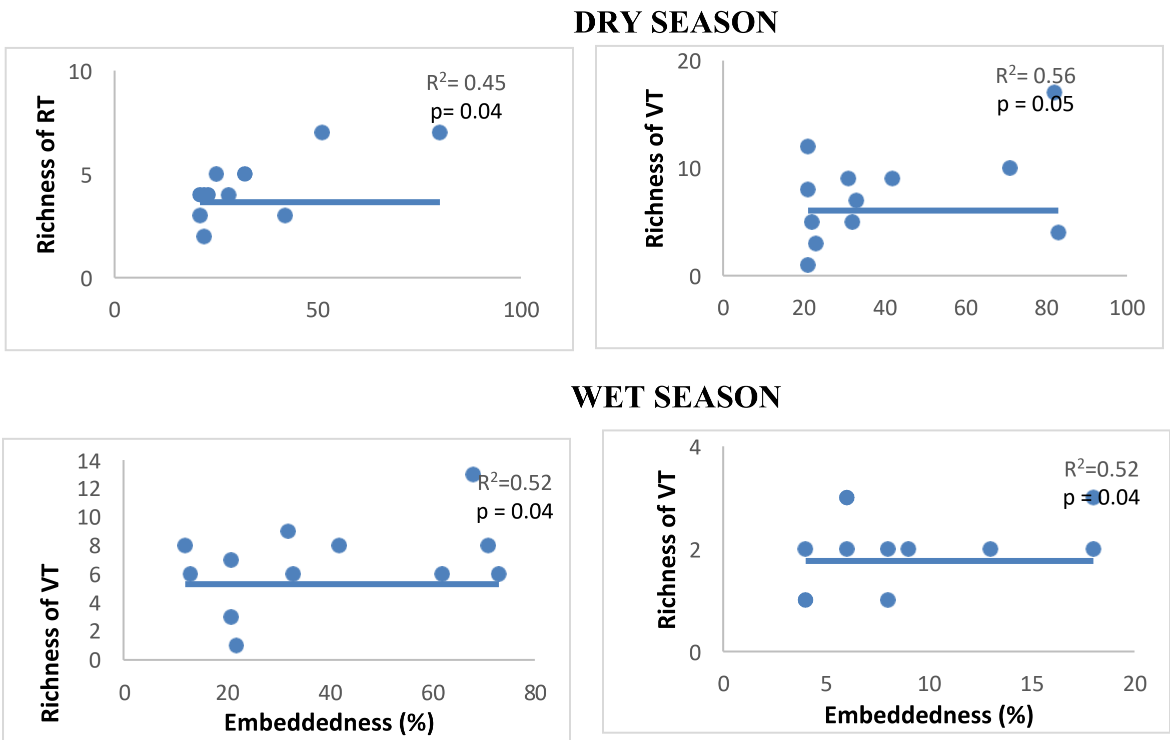


Figure 5. 8: Linear regression between the richness of the vulnerable taxa (VT), resilient taxa (RT), and embeddedness during the dry and wet seasons. *P* values are shown for relationships that are statistically significant ($P < 0.05$).

5.3.4 Spatial distribution of the abundance of vulnerable and resilient taxa

It was predicted that the relative abundance of the vulnerable taxa would decrease at the fine-sediment-impacted sites of Tsitsa upstream, Tsitsa downstream, Qurana tributary, Millstream upstream and Millstream downstream (i.e., TSU, TSD and QHR, MLU, MLD) compared with the control site (CLS). During the dry season, the relative abundance of taxa designated as potentially vulnerable decreased markedly at sites TSU, TSD and QHR, and the Kruskal-Wallis multiple comparison test indicated that the marked decrease was statistically significant ($P < 0.05$) (Figure 5.9). No prediction was made regarding the potentially resilient taxa as they were presumed to be able to occur across all sites, irrespective of sediment impact. The results during the dry season indicated that the relative abundance of the potentially resilient taxa were not statistically significantly different across all six sites, indicating that sediment impact had little or no effect on the distribution of these macroinvertebrate taxa (Figure 5.9).

The predicted results of the relative abundance for the vulnerable taxa during the wet season were similar to the results for the dry season. The relative abundance of the vulnerable taxa showed a marked decrease of macroinvertebrates at TSD and QHR compared with the rest of the highly impacted sites (Figure 5.9).

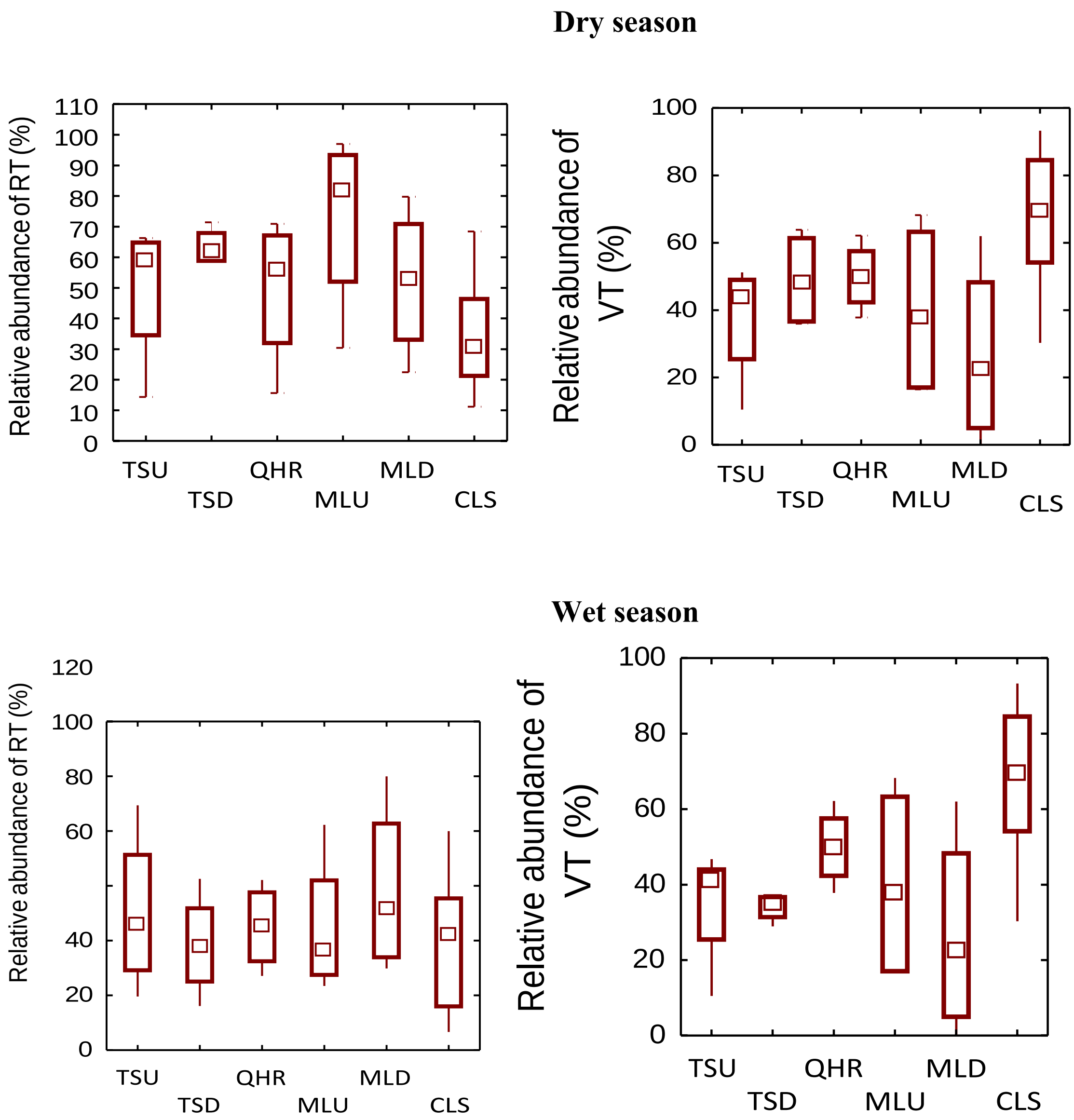
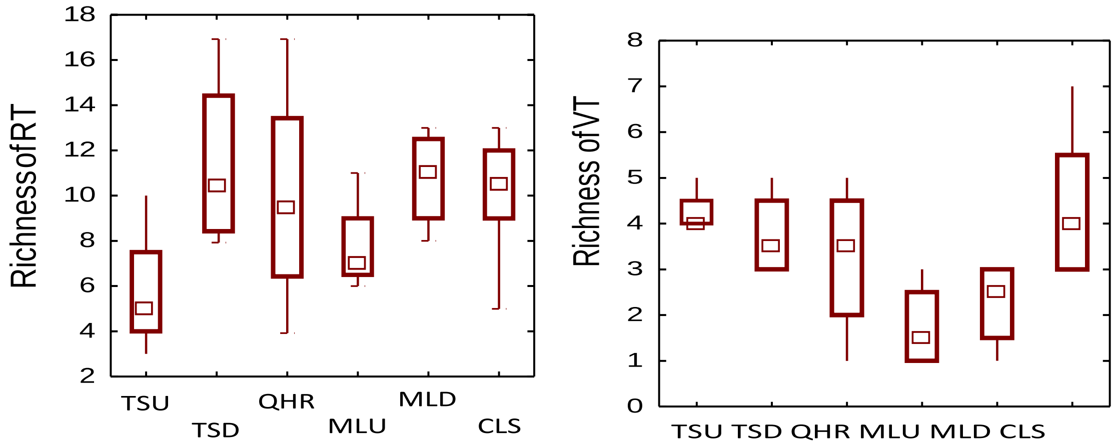


Figure 5. 9: The relative abundance of vulnerable and resilient taxa across the sites in the Tsitsa and its tributaries during the dry and wet seasons. Sites: TSU (Tsitsa upstream), TSD (Tsitsa downstream), QHR (Qurana tributary), MLU (Millstream upstream), MLD (Millstream downstream) and CLS (Control sites).

5.3.5 Prediction of VT and RT richness across sites

It was predicted that the richness of the designated vulnerable taxa would decrease at the highly sedimented sites (i.e., Sites TSU, TSD and QHR) compared with the CLS. During the dry season, the relative abundance of taxa categorised as potentially vulnerable decreased markedly at sites TSU, TSD and QHR and the Kruskal-Wallis multiple comparison test indicated that the marked decrease was statistically significant ($P < 0.05$) (Figure 5.10). No prediction was made regarding the potentially resilient macroinvertebrate taxa as they were recognised as having the potential to occur across all sites, irrespective of sediment impact. The results also indicated that the relative abundance of the potentially resilient taxa was not statistically significantly different across all six sites, indicating that sediment impact had little or no effect on the distribution of macroinvertebrates (Figure 5.10).

Dry season



Wet season

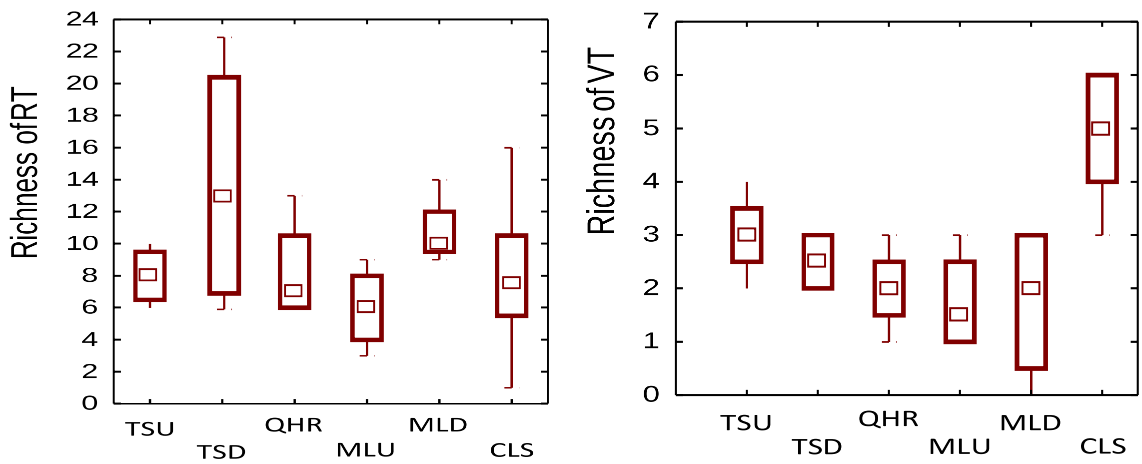


Figure 5. 10: The richness of vulnerable and resilient taxa across sites during the dry and wet seasons. Sites: TSU (Tsitsa upstream) TSD (Tsitsa downstream), QHR (Qurana tributary), MLU (Millstream upstream), MLD (Millstream downstream) and CLS (Control sites).

5.4 Discussion

In the present study, a trait-based approach was used to determine resilient and vulnerable macroinvertebrates. It was predicted that the relative abundance and richness of families designated as potentially vulnerable would increase with increasing DO and decrease with increasing TSS, turbidity and embeddedness. The decrease in the relative abundance of vulnerable taxa due to high turbidity and TSS concentration during both the dry and wet seasons (Figures 5.1–5.8) affirms the prediction made in this study and re-affirms the utility of TBA viz-a-viz predictive ecology. Taxa such as Caenidae, Gomphidae, Tabanidae (Appendix C) were deemed tolerant to sediment impact, while taxa like Oligonuridae, Perlidae were vulnerable. The majority of the taxa designated as vulnerable were those that obtain DO with external gills and were mostly filter feeders. These traits are features that increase an animal's susceptibility to the effects of elevated fine sediments through clogging (Bonada *et al.,* 2014).

The majority of the taxa designated as resilient had traits such as sclerotised body structure, a preference for slow-flowing water, use of both aerial and aquatic respiration, use of multiple feeding strategies (i.e., generalist feeders) and swimming as a mechanism for escape. These traits have been reported as having the potential to confer resilience on macroinvertebrates in relation to fine-sediment impacts (Doretto *et al.,* 2015). Therefore, these results suggest that possessing a combination of these traits is likely to provide an organism with adaptive potential to deal with the effects of sediment accretion.

The results also showed that relative abundance of vulnerable macroinvertebrate taxa during the dry and wet seasons was more strongly associated with the less sediment-impacted sites, MLU, MLD and CLS, than the highly sedimented sites, TSU, TSD and QHR groups. Furthermore, the results clearly indicated that the vulnerable taxa were more abundant at the control site during wet season, which may be attributed to the fact that sediment accretion was elevated during this season, and they were unable to cope with the increasing sediment impact at the impacted sites. Seasonality has been noted to play a mediating role in terms of sediment effects on macroinvertebrates (Akamagwuna *et al.,* 2019). During the wet season, fine-sediment influx is elevated, increasing the risk of gill clogging, filling up of interstitial spaces, and burial of less motile taxa. These factors may have led to the significant decrease of the vulnerable taxa at the impacted sites during the wet season compared with the dry season.

In the present study, the families of Chironomidae and Baetidae were excluded from the development of the predictive tool because species within these two families were found to have highly diverse autecological information regarding their potential vulnerability and tolerance to sediment impact. It is argued that these two families would be better described functionally at the species level rather than the family level because of their diverse autecology (Extence *et al.,* 2011; Odume *et al.,* 2018). However, as more trait information becomes available about the life history of Afrotropical macroinvertebrates, it may be possible to develop a similar approach at the species level for South African macroinvertebrates.

5.5 Conclusion

In this study, a novel trait-based approach was developed and was successfully used to classify South African macroinvertebrates into resilient and vulnerable taxa. The approach was tested using a case study from the Tsitsa River and its tributary, where it proved useful in enabling the prediction of macroinvertebrate assemblage response to sediment impact. While the approach shed light on the usefulness of traits in developing pressure-specific prediction through mechanistic understanding of the trait-environment relationship, important gaps, especially in the South African context, still need to be addressed. The major impediments to the adoption of the TBA in South Africa is the scarcity of life-history information, particularly that related to reproduction, necessitating the approach developed here at the family level. Nevertheless, the study makes an important contribution to the application and adoption of traits for freshwater biomonitoring in South Africa. As the outcome of the predictions were taxa rather than traits, the approach can be used to identify and protect potentially vulnerable taxa.

CHAPTER 6: GENERAL DISCUSSION, CONCLUSION, AND RECOMMENDATIONS

6.1 Introduction

Fine sediments are a natural and integral component of river systems (Owens *et al.,* 2005; Jones *et al.,* 2012; Vercruysse *et al.,* 2017). Sediment transport and deposition are important processes of riverine systems because they play a crucial role in structuring aquatic ecosystems (Owens *et al.,* 2005). In recent years, there have been high levels of fine sediment delivered into and transported by riverine ecosystems. Human-induced activities are the major contributors to fine-sediment delivery, not only in the Tsitsa River systems and its tributaries, where elevated fine sediment is the major water quality concern (Gordon *et al.,* 2013; Akamagwuna, 2018), but into riverine systems globally (Comte *et al.,* 2021). The present study used both taxonomic and trait-based approaches to better understand the effects of fine sediments on macroinvertebrates and, in the process, developed tools suitable for monitoring the impact of fine sediments as well as predicting biological responses. The objective of this chapter is to present a concise, integrative discussion of the results, summarising the key findings, making recommendations for further studies and stating important study limitations.

6.2 Elevated fine sediments as a water quality stressor

Elevated fine sediments have been implicated as a major driver (i.e., change in biodiversity) of water quality change and macroinvertebrate assemblage response (Gordon *et al.,* 2012). Several factors can affect how fine sediments impact macroinvertebrates: fine-sediment concentration, grain-size distribution, the sensitivity of the receiving environment (i.e., decrease in the abundance of sensitive taxa), sources of fine sediments and seasonality (Govenor *et al.,* 2019; Akamagwuna & Odume, 2020). In this study (Chapter 3) fine-sediment concentration and grain sizes were characterised in the Tsitsa River and its tributaries. The Tsitsa River is situated in a communally owned landscape with evidence of erosive degradation (Akamagwuna, 2018) compared to that of the Pot and Little Pot Rivers that are within a privately owned, well-maintained catchments. The results of the present study revealed that sites within the Tsitsa and Qurana Rivers were highly impacted by fine sediments, indicated by increased TSS and turbidity compared with sites in the Pot and Little Pot Rivers (control sites). This finding emphasised the importance of maintaining river catchments as a mechanism for reducing erosion and thus sediment influx into river systems.

The analysis of the grain sizes indicated that they were differentially distributed across the sites. The impacted sites within the Tsitsa River were dominated mainly by the smaller grain sizes, that is, clay and silts, whereas at the control sites the grain sizes were more evenly distributed. Several studies (e.g., Akamagwuna *et al.,* 2019; Mathers *et al.,* 2019; Giesiwen *et al.,* 2019) have indicated that smaller grain sizes are more deleterious to aquatic macroinvertebrates because they can easily clog fine structures of aquatic macroinvertebrates, and buried organisms often take longer to excavate and escape burial (Rabení *et al.,* 2005). The implication is that, in addition to fine sediments being elevated in the Tsitsa River, the dominance of smaller grain sizes at these sites presents an additional risk to aquatic macroinvertebrates.

The approach in Chapter 3 was used to investigate the structuring of the sites in terms of suspended and settled fine-sediment grain sizes for both the wet and dry seasons. During the dry season: the proportion of settled sediment grain size of very coarse silt and very fine sand (larger particles) influenced the structuring of the control site, the Qurana tributary and the Millstream, while clay (smaller particles) and embeddedness influenced the site structuring of Tsitsa River (Figure 3.7). Similar studies by Akamagwuna (2018) investigated the association of settled sediment grain sizes such as clay, very fine silt, medium silt, coarse silt, and indicated that smaller grain sizes were characteristic of the sites influenced by high sedimentation.

6.2.1 Developing sediment-specific multimetric index (SMMI)

The SMMI was developed following a five-step approach: i) selection of candidate metrics; ii) testing selected metrics for their potential to discriminate the control sites from the rest of the sampling sites; iii) testing metrics for redundancy; iv) integrating selected metrics into a unified multimetric index, and v) testing and validating the developed SMMI. The significance of developing an index specifically for assessing fine-sediment effects is based on the recognition that South Africa lacks a specific tool for assessing sediment impact on macroinvertebrates in freshwater systems. To assess the usefulness of the fine-sediment index, its performance was tested using different data sets (i.e., macroinvertebrates abundance) which were collected between 2017–2018. The SMMI proved useful in that it was able to indicate the degree of sedimentation between the sites, and to differentiate between the wet and dry seasons. This is the first time such an index has been developed in South Africa. The effectiveness of the index implies that it can be used for the routine assessment of effects of sedimentation in the Tsitsa River and its tributaries. At the time of this research, the National Department of Environmental Affairs was implementing a catchment rehabilitation programme aimed at improving the landscape and reducing erosion and thus the influx of sediments into the Tsitsa River. The developed SMMI can be used to monitor and evaluate the instream performance of the rehabilitation programme, thus contributing to national imperatives within the catchment.

6.3 Trait-based biomonitoring – the use of traits and ecological preferences in assessing sediment impact in riverine environments

Traits and ecological preferences play a major role in mediating organism-environment relationships (Odume, 2020; Hamilton *et al.,* 2020). Chapter 4 was devoted to reporting the development of a trait-based approach for assessing sediment impact in a freshwater system. It was hypothesised that the selected macroinvertebrates' traits and ecological preferences would be impacted differently by sediment impact. To determine the distribution pattern of traits and ecological preferences, Environmental variable: (R), macroinvertebrates taxa (L) and traits (Q) (RLQ) and fourth-corner analyses were conducted to determine traits sensitive to and tolerant of sediment impact in the Tsitsa River and its tributaries. The results revealed that the resilient/tolerant traits and ecological preferences were more strongly associated with the highly sedimented sites. These traits and ecological preferences included completely sclerotised, cased/tubed body, climbing, skating, and aerial breathing. The sensitive traits and ecological preferences were more strongly associated with the less sediment-influenced sites. These traits included exposed and soft body parts as well as collectors and filterers. The varied responses of the identified sediment-sensitive and -tolerant traits, and ecological preferences suggest that traits can be used as indicators of sediment impact in freshwater systems. This study adds to the growing body of knowledge on the usefulness of the trait-based approach in freshwater biomonitoring (Desrosiers *et al.,* 2019; Akamagwuna *et al.,* 2019, Odume, 2020; Edegbene, 2020)

6.4 Developing a trait-based approach (TBA) for predicting and assessing the potential vulnerability

6.5 of macroinvertebrates to fine-sediment impacts

Chapter 5 of this thesis developed a trait-based approach to assess and predict the potential vulnerability and resilience of macroinvertebrates to sediment impact in the Tsitsa River and its tributaries. Based on the developed trait-based approach (TBA), macroinvertebrates were classified into vulnerable and resilient taxa. The approach developed was based on ecological reasoning and it proved useful as a predictive-ecology tool which successfully predicted the responses of macroinvertebrate families to fine-sediment effects (i.e., fine sediments impacted the highly sensitive taxa). The richness and abundance of taxa designated as potentially vulnerable decreased with increasing turbidity, total suspended solids and embeddedness.

6.6 Conclusion

South African aquatic macroinvertebrates have been classified in terms of their potential vulnerability to sediment effect using the TBA. The TBA approach adopted in the study is rooted in ecological theory, taking cognisance of the fact that natural selection acts on the organisms through the interaction of the environment and traits. The classification can be regarded as tentative as it can be refined as our understanding of traits improves and more trait information become available. Through ecological reasoning and extensive review of the literature, a trait-based approach was developed, which proved useful and effective in predicting the potential vulnerability and resilience of aquatic macroinvertebrates to fine-sediment impact. The multimetric index developed for assessing sediments impact is the first of its kind in South Africa and thus serves as an important contribution towards a better understanding of sediment impact on riverine macroinvertebrates.

6.7 Limitations of the study

The concept of traits is relevant to all taxonomic groups and at family level only and not species level. Life-history research is not within the scope of the present study; however, in compiling the database, literature on life-history research and experts in the relevant disciplines were consulted and the research findings on life history information mainly based at family level. The main limitation of the work is that the compiled trait database is inadequate owing to the limited availability of trait information on South African aquatic macroinvertebrates in the literature. Further testing of the SMMI across different riverine ecosystems in the country is recommended. This would allow for identifications of areas requiring refinement and improvement. Existing SASS5 data collected from systems known to be stressed by sediments can serve this purpose.

6.8 Recommendations for future research.

- Testing the SMMI across different riverine ecosystems in the country is fundamental and would allow for identification of areas that need improvement.
- Development of modelling tools linking trait-mediated biotic response to specific water quality stressors, habitat characteristics, as well as stream hydrology.
- An understanding of the vulnerability and resilience of freshwater ecosystems to sediment stress, from a biological perspective, could help in planning and decision making.

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APPENDICES

Appendix A: Non- sensitive metrics not integrated into the SMMI for Tsitsa River and its tributaries.

CANDIDATE METRICS FOR COMPOSITION

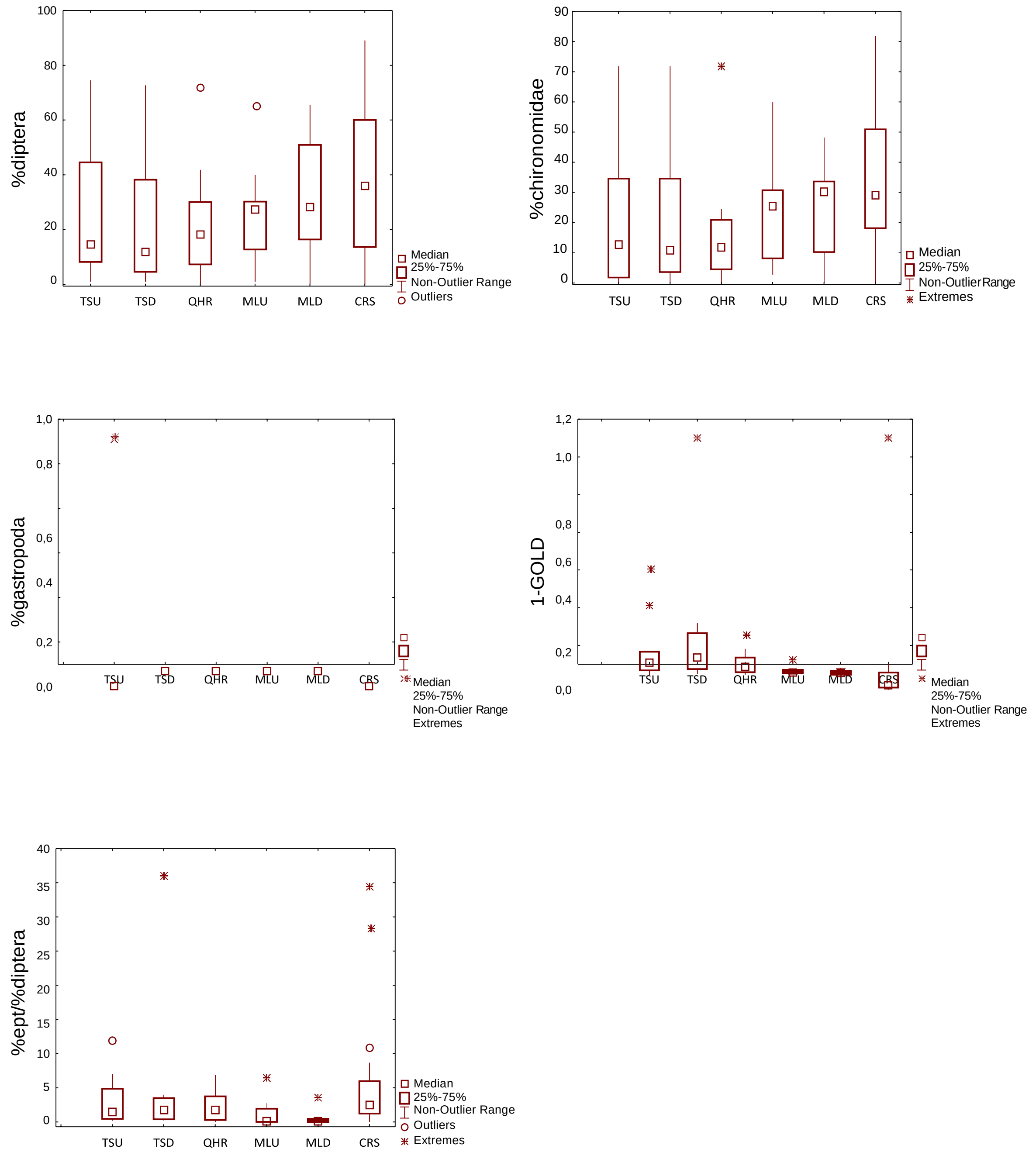
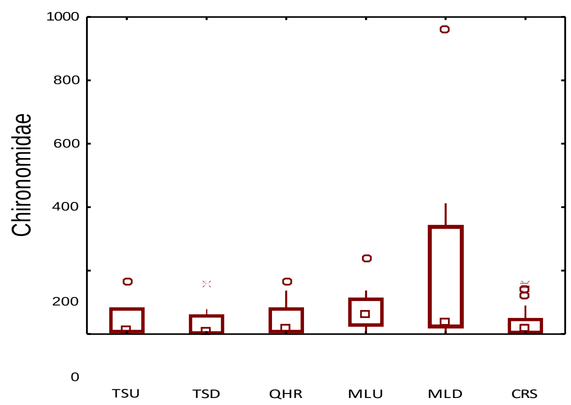
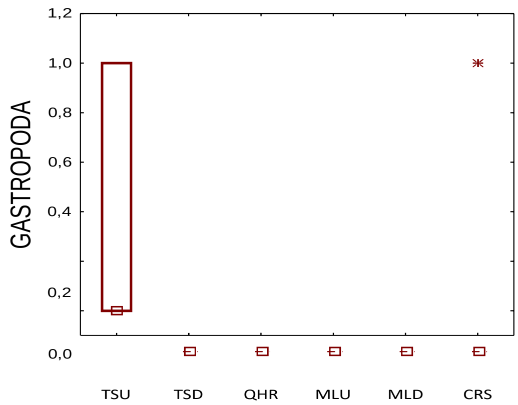


Figure A1: Six selected composition candidate metrics (%Diptera, %Chironomidae, %EPT/%Chiro, %Gastropoda, %1-GOLD and %EPT/%Diptera) selected in response to sediment in Tsitsa River and its tributaries during the two-year study period (August 2016–March 2018) across six sites groups: TSU (Tsitsa upstream), TSD (Tsitsa downstream), QHR (Qurana Tributary), MLU (Millstream upstream), MLD (Millstream downstream) and CRS (Control sites).

CANDIDATE METRICS FOR ABUNDANCE





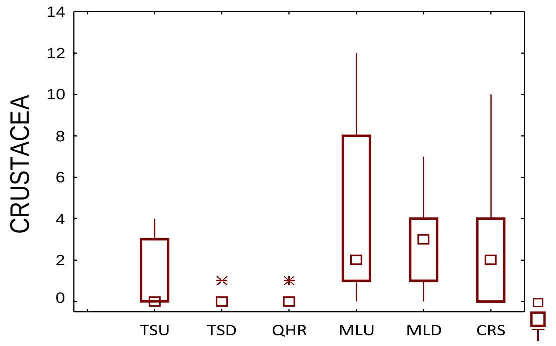
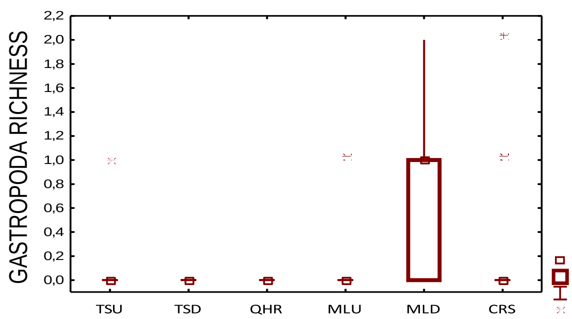
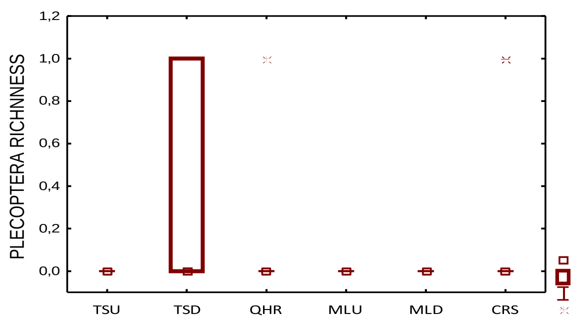


Figure A2: Four selected abundance candidate (Chironomidae, Chironomidae/Diptera, Gastropoda and Crustacea abundance) metrics selected in response to sediment in Tsitsa River and its tributaries during the two-year study period (August 2016–March 2018) across six sites groups: TSU (Tsitsa upstream), TSD (Tsitsa downstream), QHR (Qurana Tributary), MLU (Millstream upstream), MLD (Millstream downstream) and CRS (Control sites).

CANDIDATE METRICS FOR RICHNESS





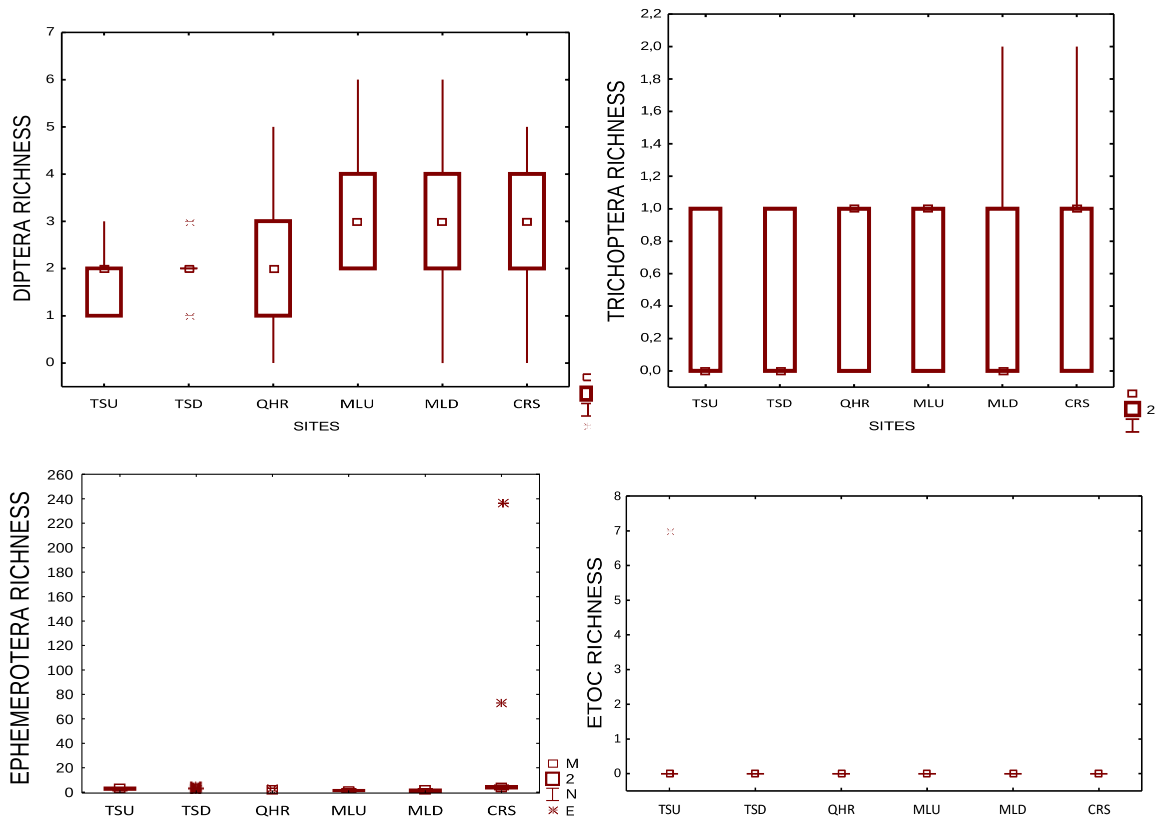


Figure A3: Seven selected richness candidate metrics selected (EPT, Gastropoda, Diptera, Plecoptera, Ephemeroptera, Trichoptera and ETOC richness) in response to sediment in Tsitsa River and its tributaries during the two-year study period (August 2016–March 2018) across six sites groups: TSU (Tsitsa upstream), TSD (Tsitsa downstream), QHR (Qurana Tributary), MLU (Millstream upstream), MLD (Millstream downstream) and CRS (Control sites).

Appendix B : Fuzzy coding of selected macroinvertebrate traits/ecological preferences for Tsitsa and its tributaries during the study period of (2016 –2018)

Code	A1	A2	A3	A4	A5	B1	B2	B3	B4	B5	C1	C2	C3	C4	C5	C6	D1	D2	D3	D4	E1	E2	E3	E4
TAXA																								
ANNELIDA																								
Oligochaeta	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0	5	0	0	0	5	5	1	0	0
Hirudinea	0	3	1	0	0	0	5	0	0	0	0	3	0	3	0	0	0	5	0	3	0	0	0	5
CRUSTACEA																								
Potamonautidae	0	1	1	3	1	5	0	0	0	3	0	5	0	1	0	5	0	1	3	0	0	1	3	3
EPHEMEROPTERA																								
Leptophlebiidae	0	5	3	0	0	5	1	0	0	0	0	3	0	5	0	0	1	1	0	6	5	1	1	0
Baetidae	3	5	0	0	0	5	1	0	0	0	0	3	0	5	0	0	1	0	0	5	5	1	3	0
Caenidae	3	5	0	0	0	5	1	0	0	0	0	3	1	3	0	1	0	3	0	5	5	0	5	0
Heptageniidae	0	1	5	0	0	5	1	0	0	0	0	3	3	1	0	0	0	5	0	0	5	0	5	0
ODONATA																								
Coenagrionidae	0	1	5	5	1	5	1	0	0	0	5	5	0	3	0	1	0	3	0	5	5	0	5	0
Lestidae	0	0	5	5	1	5	1	0	0	0	5	5	0	3	0	0	0	5	0	0	5	0	5	0
Aeshnidae	0	0	5	5	3	5	1	0	0	0	5	5	0	3	0	0	0	0	0	5	1	0	0	5
Corduliidae	0	1	5	3	1	5	1	0	0	0	5	5	0	5	0	0	0	0	0	5	1	0	1	5
Gomphidae	0	1	5	3	1	5	1	0	0	0	1	5	0	3	0	0	0	0	0	5	1	0	1	5
Libellulidae	0	3	5	1	1	5	1	0	0	0	1	5	0	3	0	0	0	0	5	0	1	0	1	5
Synlestidae	0	1	5	5	3	5	1	0	0	0	3	5	0	3	0	0	0	3	0	5	1	0	1	5
Protoneuridae	0	0	5	0	0	5	1	0	0	0	5	5	0	1	0	0	0	3	0	5	1	0	1	5
Platycnemidae	0	0	5	0	0	5	1	0	0	0	5	5	0	1	0	0	0	0	0	5	1	0	1	5
LEPIDOPTERA																								
Pyralidae	0	0	5	5	0	5	1	1	0	0	1	5	0	1	0	0	0	0	0	5	1	0	1	5
HEMIPTERA																								

Belostomatidae	0	1	3	5	1	0	0	5	5	0	3	3	0	5	0	1	0	3	0	5	3	1	3	0
Naucoridae	0	5	3	0	0	0	0	5	5	0	3	3	0	5	0	0	0	3	5	0	0	0	0	5
Nepidae	0	0	5	3	1	0	0	5	5	0	3	5	0	5	0	0	0	3	5	1	0	0	0	5
Pleidae	5	0	0	0	0	0	0	5	5	0	3	0	0	5	0	0	0	5	0	3	0	0	0	5
Veliidae	5	1	0	0	0	0	0	5	5	0	0	0	0	0	5	0	0	0	5	0	0	0	0	5
Gerridae	0	5	3	0	0	0	0	5	5	0	0	0	0	0	5	0	0	0	5	5	0	0	0	5
Corixidae	0	5	1	0	0	0	0	5	5	0	3	0	0	5	0	0	0	5	0	5	0	0	0	5
Notonectidae	1	5	1	0	0	0	0	5	5	0	3	0	0	5	0	0	0	0	0	5	1	0	5	0
TRICHOPTERA																								
Ecnomidae	1	5	3	0	0	1	5	0	0	0	0	5	0	1	0	0	0	0	0	5	3	3	1	3
Hydropsychidae	0	1	5	0	0	5	1	0	0	0	0	5	0	1	0	0	0	0	0	5	5	3	3	1
Leptoceridae	0	0	5	0	0	5	1	0	0	0	3	0	3	5	0	0	0	0	0	5	3	3	1	0
Hydrophilidae	5	0	0	0	0	1	5	0	0	0	3	0	3	0	0	0	1	3	5	5	1	0	5	0
Lepidostomatidae	0	5	3	0	0	1	5	0	0	0	1	0	5	0	0		0	0	0	5	1	5	3	0
COLEOPTERA																								
Dytiscidae	1	5	3	1	1	3	3	5	5	0	5	5	0	5	0	0	3	3	3	3	0	0	0	5
Gyrinidae	1	0	1	3	3	0	1	0	5	0	0	0	0	5	0	1	0	1	3	3	0	1	0	5
Hydraenidae	5	0	0	0	0	3	3	5	5	0	3	3	0	5	0	0	0	1	0	3	1	3	1	5
Hydrophilidae	1	5	3	1	1	3	1	5	5	0	5	5	0	5	0	0	0	0	5	5	0	3	3	5
Elmidae	5	1	0	0	0	5	1	5	0	0	1	5	0	0	0	0	0	3	3	3	5	3	1	0
Helodidae	1	5	0	0	0	5	3	1	0	0	5	5	0	1	0	0	0	0	0	5	1	3	0	3
DIPTERA																								
Ceratopogonidae	1	1	5	0	0	5	1	0	0	0	0	3	1	1	0	3	0	1	0	5	1	0	1	5
Chironomidae	3	5	1	1	0	3	5	0	0	0	0	3	3	1	0	5	0	0	0	5	5	1	3	3
Culicidae	1	5	0	0	0	0	0	3	3	0	0	0	0	5	0	0	0	0	0	5	5	1	1	0
Syrphidae	0	3	3	0	0	0	1	5	5	0	1	3	5	0	0	0	0	0	0	5	3	1	1	0
Muscidae	1	5	3	0	0	1	1	3	0	0	0	3	0	0	0	3	0	0	0	5	0	0	0	3
Simuliidae	3	5	0	0	0	3	5	1	0	0	0	3	0	1	0	0	0	0	0	5	5	1	1	0

Tabanidae	0	1	5	1	0	0	3	5	5	0	1	3	1	1	0	3	0	0	0	5	0	1	3	5
Tipulidae	0	3	1	1	0	3	3	5	0	0	0	1	1	0	0	5	0	0	0	5	5	1	1	3
Athericidae	0	3	3	0	0	3	3	0	0	0	1	5	3	0	0	5	0	0	0	5	1	3	1	3
Ephydridae	0	3	3	0	0	0	1	5	5	0	0	3	3	0	0	5	0	0	0	5	3	3	3	1
Psychodidae	3	3	0	0	0	0	0	5	0	0	0	5	0	1	0	0	0	3	0	3	5	3	1	0
GASTROPODA																								
Ancylidae	0	0	0	0	0	5	1	0	0	0	1	3	0	1	0	1	0	0	3	0	5	1	3	0
Lymnaeidae	0	0	0	0	0	0	5	0	0	5	1	3	0	1	0	0	0	0	3	0	3	1	3	0
Physidae	0	0	0	0	0	0	5	0	0	5	1	3	0	1	0	0	0	0	3	0	3	1	3	0
Planorbidae	0	0	0	0	0	0	5	0	0	5	1	3	0	1	0	0	0	0	3	0	3	1	3	0
HYDRACARINA																								
Hydrachnellae	5	0	0	0	0	0	5	0	0	0	1	5	0	1	0	0	0	0	5	0	1	0	0	3
Turbellaria	1	5	1	0	0	0	5	0	0	0	0	5	0	0	0	0	0	5	0	0	0	0	0	5
Code	F1	F2	F3	F4	G1	G2	G3	H1	H2	H3	I1	I2	J1	J2	J3	J4	K1	K2	K3	K4	L1	L2	L3	L4
ANNELIDA																								
Oligochaeta	3	3	1	0	5	5	0	0	0	0	0	0	0	3	0	1	5	3	1	0	5	5	3	3
Hirudinea	0	0	0	1	0	3	0	3	5	1	0	0	0	3	0	3	5	3	1	0	3	5	5	3
CRUSTACEA																								
Potamonautidae	1	0	3	0	0	3	3	5	3	1	5	0	5	5	5	3	0	3	0	5	5	5	5	5
EPHEMEROPTERA																								
Leptophlebiidae	3	1	3	0	0	3	0	0	0	0	5	0	3	5	3	3	0	0	0	0	1	3	5	3
Baetidae	5	1	3	0	0	0	3	5	3	1	5	0	3	3	5	3	0	0	0	0	3	3	5	5

Caenidae	5	0	3	0	0	3	3	5	3	1	5	0	3	5	5	3	0	0	0	0	5	3	3	3
Heptageniidae	5	0	3	0	0	0	0	3	5	1	5	0	5	5	5	3	0	0	0	0	1	5	5	3
ODONATA																								
Coenagrionidae	5	0	3	0	0	3	3	3	5	1	5	0	5	5	5	5	0	0	0	0	5	3	3	3
Lestidae	5	0	3	0	0	0	0	5	3	1	5	0	5	5	5	5	0	0	0	0	5	0	0	0
Aeshnidae	0	0	0	5	0	0	5	3	5	1	5	0	5	5	5	5	0	0	0	0	3	3	5	3
Corduliidae	0	0	0	5	0	0	0	0	0	0	5	0	5	5	5	5	0	0	0	0	5	1	0	0
Gomphidae	0	0	1	5	0	3	3	5	3	1	5	0	5	5	5	5	0	0	5	0	5	5	3	3
Libellulidae	0	1	1	5	0	3	3	5	3	1	5	0	5	5	5	5	0	0	0	0	3	3	5	3
Synlestidae	0	1	1	5	0	0	5	5	3	1	5	0	5	5	5	5	0	0	0	0	0	3	5	3
Protoneuridae	0	1	1	5	0	0	5	5	3	1	5	0	5	5	5	5	0	0	0	0	1	3	1	1
Platycnemidae	0	1	1	5	0	0	5	3	5	1	0	0	5	5	5	5	0	0	0	0	1	3	1	1
LEPIDOPTERA																								
Pyralidae	0	1	1	5	0	0	0	0	0	0	0	0	5	5	5	5	0	0	0	0	1	1	3	3
HEMIPTERA																								
Belostomatidae	0	0	3	0	0	0	0	5	3	1	0	5	5	5	5	3	0	0	0	0	5	1	0	0
Naucoridae	0	0	0	5	0	0	3	5	3	1	0	5	5	5	5	5	0	0	0	0	3	5	3	1

Nepidae	0	0	0	5	0	0	0	5	3	1	0	5	3	3	5	3	0	0	5	0	5	0	0	0
Pleidae	0	0	0	5	0	0	0	5	3	1	0	5	3	3	5	3	0	0	0	0	5	1	0	0
Veliidae	0	0	0	5	0	0	3	5	3	1	0	5	3	3	5	3	0	0	0	0	5	3	3	3
Gerridae	0	0	0	5	3	0	3	5	3	1	0	5	3	3	5	3	0	0	0	0	4	3	3	0
Corixidae	0	0	0	5	0	3	3	5	3	1	0	5	3	3	3	3	0	5	3	5	5	3	1	1
Notonectidae	0	1	0	0	3	0	0	5	3	1	0	5	3	3	3	3	0	0	0	0	5	3	0	0
TRICHOPTERA																								
Ecnomidae	0	3	0	3	0	0	3	1	3	5	1	0	3	5	3	3	0	0	5	3	1	3	3	1
Hydropsychidae	0	5	0	1	3	0	0	1	3	5	5	0	3	5	5	3	0	3	5	3	1	3	5	5
Leptoceridae	3	0	3	0	0	0	3	5	3	1	5	0	3	5	5	3	0	3	5	0	5	5	5	3
Hydrophilidae	0	0	5	0	0	3	0	5	3	1	1	0	3	5	5	3	0	3	5	5	3	5	5	3
Lepidostomatidae	0	0	1	0	0	3	0	5	3	1	1	0	3	5	3	3	0	3	5	5	0	5	3	1
COLEOPTERA																								
Dytiscidae	0	0	0	5	0	3	0	5	3	1	3	5	3	5	5	3	0	3	5	5	5	1	0	0
Gyrinidae	0	0	0	5	0	3	3	5	3	1	0	0	0	5	5	3	0	3	5	5	3	3	3	3
Hydraenidae	5	0	3	5	0	0	0	5	3	1	3	5	3	5	3	3	0	5	5	5	1	1	3	5
Hydrophilidae	5	0	0	5	3	0	3	5	3	1	3	5	3	5	5	3	3	5	3	5	3	3	3	3

Elmidae	5	1	0	0	0	0	3	5	3	1	5	5	3	5	5	3	3	5	3	5	1	3	5	5
Helodidae	0	0	0	5	0	0	0	3	5	3	5	1	5	3	5	3	0	0	0	0	5	3	3	5
DIPTERA																								
Ceratopogonidae	1	0	1	3	0	3	0	3	5	1	5	0	3	5	5	5	5	3	5	3	5	3	3	5
Chironomidae	5	0	3	3	0	3	3	5	3	1	3	0	3	5	5	3	5	3	3	3	5	5	5	5
Culicidae	0	5	1	0	0	3	0	3	5	1	0	3	1	3	5	1	0	0	0	0	5	1	0	0
Syrphidae	3	3	1	0	0	0	0	0	0	0	0	5	1	3	5	1	0	0	0	0	5	1	0	0
Muscidae	0	0	0	3	0	0	3	0	0	0	1	3	5	3	3	1	0	0	0	0	5	1	0	0
Simuliidae	0	5	1	0	0	0	3	3	5	1	3	1	1	3	5	1	0	0	0	0	1	3	3	5
Tabanidae	0	0	1	5	0	0	0	5	3	1	0	5	5	3	5	3	0	0	0	0	1	5	5	3
Tipulidae	1	0	1	5	0	0	0	5	3	1	3	5	1	3	5	3	0	0	0	0	3	5	5	5
Athericidae	0	0	3	3	0	0	0	0	0	0	3	0	3	5	5	3	0	0	0	0	3	5	5	3
Ephydridae	5	0	1	1	0	0	0	5	3	1	0	5	5	3	0	3	0	0	0	0	5	1	0	0
Psychodidae	5	0	3	0	0	0	0	0	0	0	0	5	5	3	5	1	0	0	5	0	0	5	5	0
GASTROPODA																								
Ancylidae	0	3	0	0	0	3	0	3	5	3	5	0	3	5	5	3	0	0	0	0	3	5	5	5
Lymnaeidae	0	0	3	0	0	0	5	3	5	1	0	0	0	5	5	3	0	0	0	5	5	3	1	0

Physidae	0	0	3	0	0	0	5	0	0	0	0	0	0	5	5	3	0	0	0	0	5	3	1	0
Planorbidae	0	0	3	0	0	0	0	3	5	1	0	0	0	5	5	3	0	0	0	5	5	3	3	3
HYDRACARINA																								
Hydrachnellae	0	0	0	0	0	0	0	0	0	0	0	0	0	1	5	3	0	0	0	0	0	0	0	0
Turbellaria	0	0	0	5	0	0	0	0	0	0	0	0	0	1	5	3	0	0	0	0	2	5	5	5

Appendix C : Macroinvertebrates taxa with vulnerability scores (VS) for Tsitsa river and its tributaries

TAXA	VULNERABILITY SCORE	
Notonemouridae	789709579,1	Vulnerable
Blepharoceridae	696129508,2	Vulnerable
Oligoneuridae	72074394,83	Vulnerable
Heptageniidae	52220428,73	Vulnerable
Barbarochthonidae	37970700,45	Vulnerable
Prosopistomatidae	21433470,51	Vulnerable
Turbellaria	16777216	Vulnerable
Pyralidae	4418690,634	Vulnerable
Polycentropodidae	1048576	Vulnerable
Sericostomatidae	964059,2358	Vulnerable
Petrothrincidae	759375	Vulnerable
Perlidae	619173,6422	Vulnerable
Perlidae	619173,6422	Vulnerable
Dipseudopsidae	421399,177	Vulnerable
Telagonodidae	132159,7159	Vulnerable
Trichorythidae	132159,7159	Vulnerable
Hydropsalpingidae	60242,97531	Vulnerable
Helodidae	56244,8656	Vulnerable
Sphaeridae	56244,8656	Vulnerable
Leptophlebiidae	51531,0178	Vulnerable
Haliplidae	42998,1696	Vulnerable
Oligochaeta	39152,97661	Vulnerable
Unionidae	39152,97661	Vulnerable
Lepidostomatidae	38416	Vulnerable

Psephenidae	34885,93827	Vulnerable
Simuliidae	34885,93827	Vulnerable
Limnichidae	30359,5776	Vulnerable
Empididae	28561	Vulnerable
Philopotamidae	28561	Vulnerable
Porifera	22153,3456	Resilient
Hirudinea	19829,64654	Resilient
Tabanidae	19829,64654	Resilient
Polymitarcyidae	10000	Resilient
Corbiculidae	5268,024	Resilient
Psychomyiidae	4357,37037	Resilient
Amphipoda	2823,149037	Resilient
Calamoceratidae	2370,37037	Resilient
Corydalidae	1876,037037	Resilient
Hydrobiidae	1404,928	Resilient
Hydropsychidae	1331	Resilient
Ephemeridae	1213,62963	Resilient
Atyidae	1213,62963	Resilient
Sialidae	512	Resilient
Athericidae	258,1377778	Resilient
Pisuliidae	227,0044444	Resilient
Hydraenidae	217,0711111	Resilient
Bulinae	183,1511111	Resilient
Ecnomidae	160,4444444	Resilient
Lymnaeidae	140,8177778	Resilient
Planorbinae	140,8177778	Resilient
Thiaridae	140,8177778	Resilient
Calopterygidae	100	Resilient

Coenagrionidae	94,73777778	Resilient
Hydrophilidae	85,87111111	Resilient
Culicidae	84,64	Resilient
Dixidae	78,02777778	Resilient
Protoneuridae	75,11111111	Resilient
Tipulidae	75,11111111	Resilient
Ephydridae	61,88444444	Resilient
Corduliidae	53,77777778	Resilient
Lestidae	53,77777778	Resilient
Belostomatidae	42,68444444	Resilient
Syrphidae	27,04	Resilient
Ancylidae	15	Resilient
Elmidae	14,2	Resilient
Ampullaridae	13,86666667	Resilient
Physidae	13,86666667	Resilient
Viviparidae	12,53333333	Resilient
Paleomonidae	12	Resilient
Chlorocyphidae	11,86666667	Resilient
Caenidae	11,46666667	Resilient
Libellulidae	11,46666667	Resilient
Psychodidae	10,93333333	Resilient
Ceratopogonidae	10,73333333	Resilient
Leptoceridae	10,46666667	Resilient
Aeshnidae	10,4	Resilient
Gomphidae	9,4	Resilient
Hydroptilidae	9,4	Resilient
Chlorolestidae	9,333333333	Resilient
Glossosomatidae	9,333333333	Resilient

Platycnemidae	9,333333333	Resilient
Gerridae	4,533333333	Resilient
Muscidae	4,533333333	Resilient
Nepidae	4,533333333	Resilient
Corixidae	2,533333333	Resilient
Hydrometridae	1,2	Resilient
Dytiscidae	1	Resilient
Gyrinidae	1	Resilient
Naucoridae	1	Resilient
Notonectidae	1	Resilient
Potamonautidae	1	Resilient
Veliidae	1	Resilient

TAXA	**TSU_W**	**TSU_Sp**	**TSD_W**	**TSD_Sp**	**QHR_W**	**QHR_Sp**	**MLU_W**	**M**
Hiru	0	0	0	0	0	0	0	0
Pota	6	5	10	4	9	1	3	4
Cae	14	68	17	103	168	6	91	66
Oli	6	0	20	7	21	1	1	12
Lepto	0	0	0	0	0	24	0	1
Hept	0	7	7	13	32	0	0	0
Total(Abundance)	26	80	54	127	230	32	95	83
V Ab	6	7	27	20	53	25	1	13
R Ab	20	73	27	107	177	7	94	70
Rel V	23,07692	8,75	50	15,74803	23,04348	78,125	1,052632	15
Rel R	76,92308	91,25	50	84,25197	76,95652	21,875	98,94737	84

TAXA	TSU_W	TSU_Sp	TSD_W	TSD_Sp	QHR_W	QHR_Sp	MLU_W	MLU_Sp	MLD_W
Hiru	0	0	0	0	0	0	0	0	0
Pota	6	5	10	4	9	1	3	4	3
Cae	14	68	17	103	168	6	91	66	71
Oli	6	0	20	7	21	1	1	12	5
Lepto	0	0	0	0	0	24	0	1	0
Hept	0	7	7	13	32	0	0	0	0
Baeti	17	52	41	53	142	28	0	19	6
Coen	0	25	34	8	17	6	82	7	81
Lest	4	8	8	9	18	0	2	0	1
Aesh	0	3	0	5	20	6	0	0	0
Cord	0	0	1	1	3	0	0	0	0
Gom	9	7	36	16	36	0	1	0	6
Libel	2	0	5	5	15	0	0	8	0
Chlo	0	0	0	0	0	0	0	0	0
Plat	0	0	0	0	0	0	0	0	1
Belo	0	0	0	0	0	0	0	0	0
Nau	0	0	9	0	3	2	8	6	5
Nep	0	0	0	0	0	0	0	0	0
Plei	0	0	0	0	0	0	0	0	0
Vel	0	1	2	5	9	0	0	0	0
Ger	0	0	0	0	0	0	0	1	0
Cori	0	0	0	0	0	1	0	1	1
Noton	0	0	1	1	3	2	0	1	2
Ecno	0	0	0	0	0	0	0	0	3
Hydro	3	1	5	2	3	1	0	0	0
Lepto	0	0	0	0	0	0	0	0	1
Hyph	0	2	1	3	7	0	0	0	5
Lepid	0	0	0	0	0	0	0	0	0
Dytis	0	4	1	8	11	4	0	0	0
Gyri	0	0	1	1	3	2	0	0	2
Hydrn	0	0	0	0	0	0	0	0	0
Elmd	4	6	4	13	12	0	4	5	5
Held	0	0	0	0	1	0	0	0	0
Cera	0	0	0	0	0	0	0	0	0
Chiro	20	8	35	37	96	21	93	26	340
Culi	0	0	0	0	5	0	0	4	2
Syphi	0	0	0	0	0	0	1	0	8
Musci	7	0	0	0	0	0	0	0	0
Simu	6	0	14	10	23	0	11	0	58
Taba	0	0	0	0	0	0	0	0	0
Tipu	0	0	0	0	0	2	9	0	0
Atheri	0	1	0	2	2	0	0	0	4
Psycho	0	0	0	0	0	0	12	0	8
Ancy	0	0	0	2	4	0	2	0	5
Lymn	0	0	0	1	2	0	0	14	0