Macroinvertebrate indices versus microbial fecal pollution characteristics for water quality monitoring reveals contrasting results for an Ethiopian river

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Abstract

Awash River is one of the major surface water sources used by millions of people in the central Highlands of Ethiopia. However, numerous pollution sources exert significant pressure on the river. Different approaches for assessing the status of water quality exist, but few studies compared the performance of distinct methods. Therefore, this study aims to evaluate the consistency of fecal indicator bacteria for environmental health assessment of rivers by comparing them to assessments of physicochemical tests as well as newly developed macroinvertebrate indices. Physicochemical, biological (macroinvertebrates) and microbiological (*Escherichia coli* and Enterococci) parameters were assessed at five sites along the upper Awash River. For *E. coli* and Enterococci moderate to strong fecal pollution levels, ranging from 7.9 × 102 to 7.6 × 103 cfu/100 ml and 7.6 × 102 to 1.1 × 104 cfu/100 ml, were observed, respectively. The concentrations of both fecal indicator bacteria exceeded the standards set by the European Union and the World Health Organization for safe recreational water. Hence, all sites were categorized as poor for swimming and recreation. In contrast, three African benthic macroinvertebrate indices (South African Scoring System 5, Tanzanian River Scoring System, Ethiopian Biotic Score) indicated a natural or good water quality with slight ecological degradation at the upstream sites, and a moderate to poor ecological status at the downstream sites. While macroinvertebrate communities were able to reflect anthropogenic disturbances, mainly caused by different land uses, fecal indicator bacteria, most likely driven by the high pressure of extensive livestock fecal emission and overgrazing in the whole catchment, did not. This study underpins the necessity of combining different indicator systems to analyze human pressures in Africa in a holistic way, which can serve as a basis for management and sustainable use of fundamental resources such as water from freshwater ecosystems.

1. Introduction

Rivers and streams are vital ecosystems that sustain the life of humans and animals (Rajiv et al., 2012). In many countries, such as Ethiopia, rivers are essential water sources for domestic, agricultural, industrial and recreational (e.g., open bathing and swimming) purposes (Rochelle-Newall et al., 2015). However, such human uses have inevitably reduced the ecological integrity of lotic ecosystems (Atique and An, 2018, Wang et al., 2013). Although humans depend on intact river systems, numerous anthropogenic activities severely degrade the water quality in many systems (Ebenstein, 2012, Hayzoun et al., 2014, Nhiwatiwa et al., 2017), as the input of pollutants, e.g., through industrial discharges and non-point source like agricultural surface runoff, are common stressors (Bo et al., 2017, Chang et al., 2017, Zhao et al., 2014). Another frequent problem, especially in developing countries, is the microbial fecal pollution of rivers that has wide-ranging impacts on various human activities that require appropriate river water quality (Byamukama et al., 2000, Djuikom et al., 2006, Goshu et al., 2010, Kirschner et al., 2017). For example, in such countries, the local communities often depend on river water for drinking, domestic purposes, crop irrigation and watering of animals (Chigor et al., 2013).

In order to protect people as well as the environment, the water quality must comply with several physicochemical and microbiological standards before water can be used for drinking, farming or recreational purposes (Fewtrell and Bartram, 2001, Jerves-Cobo et al., 2018). Hence, water quality is monitored regularly by assessing different physicochemical, microbiological and biological parameters that are important for ecological and environmental health evaluations (Atique and An, 2018, Popović et al., 2016). Also globally, there is an increasing interest in monitoring freshwater ecosystems, aiming to improve their value for ecological, recreational and economic purposes (WFD, 2000, Lear et al., 2009).

The water quality of rivers depends on their physical, chemical and biological properties, whereby the biological quality is defined by the types of living organisms present in the water, as well as their abundance and diversity. In contrast to traditional physicochemical assessment techniques, biological indicators provide a cumulative measure of ecosystem health resulting from the combined responses of the targeted communities to all stressor types they encounter in the aquatic habitat (Lear et al., 2011, Tanaka et al., 2016). Therefore, by assessing species composition and community structure of a subset of organisms, biological indicators are very useful in providing an overall index of ecosystem health (Lear et al., 2009). Many groups of organisms have been used in the assessment of aquatic ecosystems, including periphyton (McPherson et al., 2005), diatoms (Gonçalves et al., 2008), benthic invertebrates (Armitage et al., 1983, Böhmer et al., 2004, Ofenböck et al., 2004), and fish (Naigaga et al., 2011). In Europe, almost 300 different biological assessment methods, based on various organism groups, are in use (Birk et al., 2012). Successful monitoring requires the ability to accurately describe ecological changes through quantitative indicators (Ryder & Miller, 2005). Therefore, biological indicators, such as fish, macroinvertebrates or diatoms, have been commonly used to provide an integrated measurement of water quality (Bae et al., 2014, Beyene et al., 2009, Lainé et al., 2014, Lear et al., 2009). However, in developing countries, of which many are situated in tropical regions, biomonitoring approaches for assessing river pollution have not yet been studied extensively (Elias et al., 2014), although in some countries, such as Ethiopia, the use of biological indicators to assess water quality is substantially increasing (Ambelu et al., 2013, Lakew, 2015, Lakew and Moog, 2015a, Lakew and Moog, 2015b, Mekonen et al., 2016, Woldeab et al., 2018). Particularly biotic indices using benthic macroinvertebrates have recently been developed and applied for conservation and management of aquatic resources in Ethiopia (Lakew & Moog, 2015b). Nevertheless, though benthic invertebrate indices are widely used in Europe, North America, Australia and South Africa, little information is available on their use and applicability for water quality monitoring in Ethiopia.

Furthermore, indicator bacteria can be used to investigate pollution of aquatic environments (Gotkowska-Plachta et al., 2016, Jin et al., 2004). The most commonly used indicators worldwide are *Escherichia coli* and Enterococci (EU Bathing Water Directive, 2006, Liška et al., 2015, USEPA, 1986). Fecal indicator bacteria are usually counted to evaluate the level of microbial water contamination. The abundance of these fecal indicator bacteria is supposed to correlate with the level of microbial fecal pollution (Byamukama et al., 2000, Byamukama et al., 2005) and health risks associated with river water (Koffi et al., 2011, Teklehaimanot et al., 2014).

The expanding population coupled with the quest for improved livelihoods have resulted in anthropogenic campaigns that have led to the continuous release of pollutants (including sewage) into virtually all environmental matrices (Liyanage & Yamada, 2017). Globally, contaminated water is a serious threat to human health and ecosystem integrity (Sabater et al., 2018). Although different approaches are used worldwide for assessing the status of water quality, their performance has not been empirically compared. To the best of the authors' knowledge, this is the first study to examine the consistency of fecal indicator bacteria as an indicator for the environmental health and human water usage related issues of rivers and compare it with standard physicochemical tests as well as with newly developed macroinvertebrate indices for ecological and biological water quality assessment.

2. Materials and methods

2.1. Description of the study area and sampling sites

This study was conducted along the upper Awash River, located in the central Ethiopian Highlands (Fig. 1). The river's source is on a high plateau of the central highlands west of Addis Ababa, at an altitude of about 3000 m a.s.l. (Kinfe, 1999, WGC and AwBA, 2013). The upper part of the catchment is characterized by headwater streams which are surrounded by indigenous forest (Chilimo forest). Chilimo forest is part of the dry Afro-montane forest dominated by mixed broad-leaved and coniferous trees such as *Juniperus procera*, *Podocarpus falcatus*, *Prunus africanum*, *Olea europaea*, and *Hagenia abyssinica*, and has an overall coverage

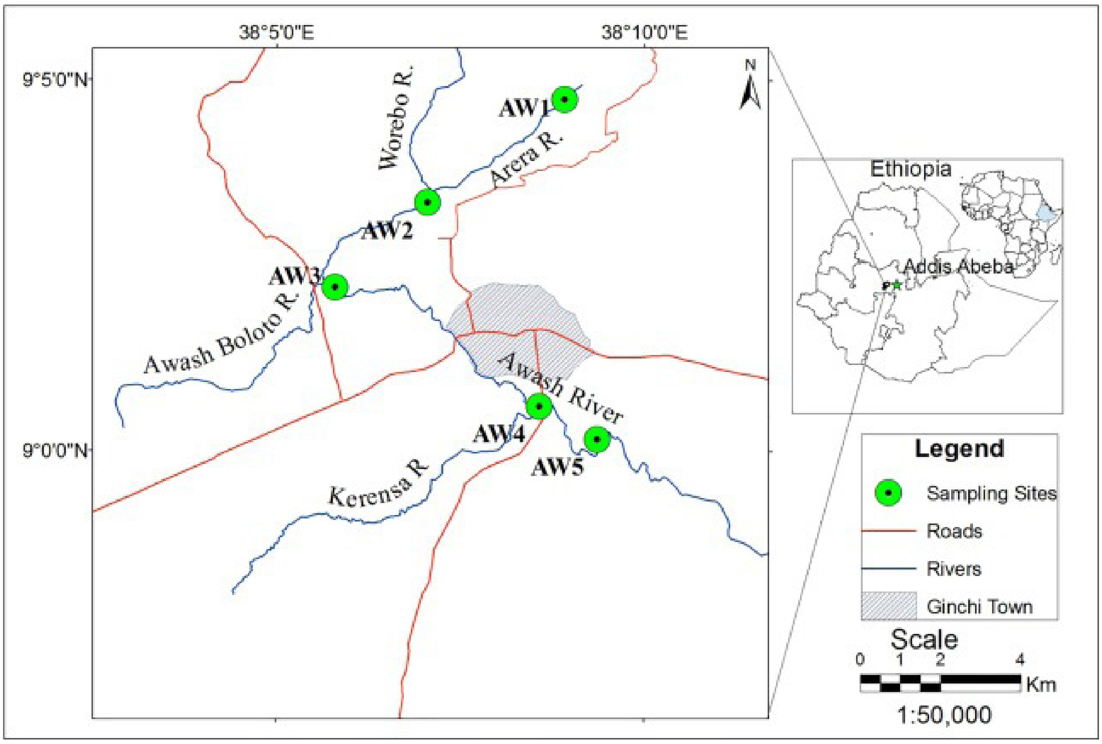

Fig. 1. Location of investigation sites along the upper section of Awash River, Ethiopia.

of about 5000 ha (Ameha et al., 2014, Getacher and Alemtsihay, 2012, Tesfaye, 2015). The lower part of the catchment is subject to a mixture of different anthropogenic activities. Farming, deforestation, urbanization, industrial uses, bathing and grazing are rather common. These anthropogenic activities, however, may lead to an enrichment of fecal bacteria (Paule-Mercado et al., 2016, Rochelle-Newall et al., 2015) and excessive nutrients into Awash River through, among others, open defecation around the riverbanks, in-stream washing of animals (i.e., cattle, donkeys, horses) and automobiles, wastewater releases from households as well as from one factory.

Sampling sites were selected along a pressure-gradient, ranging from river sections with high forest cover and fewer human interventions upstream to more degraded sites affected by multiple stressors downstream. In total, five representative sampling sites along an about 22 km long stretch were identified (Fig. 1; Table 1). The sites were not equidistant from each other because they were carefully chosen based on changes in anthropogenic pressure along the river section stretch. Sampling site AW1 lies within the Chilimo forest which is dominated by indigenous tree species. There are no human settlements in the area, and anthropogenic activities are minimal. Only some grazing livestock and wildlife animals can be occasionally observed. Site AW2 is located downstream of site AW1, at the confluence of two streams, the Arera and Worebo. The second site is mainly characterized by scattered human settlements in the forest and small-scale farming activities (vegetables and cereal crops) in the catchment. The forest around site AW2 is mainly dominated by indigenous (e.g., *J. procera*, *H. abyssinica*, *P. falcatus*) but also exotic (e.g., *Eucalyptus saligna*, *E. camaldulensis*, *Pinus patula*, *Cupressus lusitanica*) tree species. The communities living along AW2 use the river for domestic purposes. Site AW3 is located in the mid-section of the sampling reach, downstream from the inflow of Awash Boloto River, which joins the mainstream after passing through the grazing fields. Site AW4 is located directly downstream of Ginchi town, an urban area with a population of approximately 18,000. Various in-stream activities are present

Table 1. Sampling sites, geographic latitude and longitude, and land use characteristics around the sampling sites.

Sampling sites	Geographic latitude and longitude	Elevation (m a.s.l.)	Land use characteristics
AW1	N 9°05’19.00”, E 38°09’13.01”	2484	No human settlements; near-natural forest mainly dominated by indigenous tree species; wildlife such as monkeys and apes are present, as well as some grazing animals such as cattle and horses.
AW2	N 9°04’07.00”, E 38°08’23.02”	2459	Presence of few human settlements (approximately 75 households) in the mixed forest; small-scale farming; grazing of cattle, donkeys and horses.
AW3	N9°02’21.80”, E 38°05’52.05”	2254	River bank farming and grazing is common.
AW4	N 9°00’47.03”, E 38°08’53.33”	2208	Industrial waste released from a paper mill factory; rain-fed crop farming on the left bank of the river; regular washing of clothes, vehicles, and cattle in the river; animal watering; dumping of domestic wastes on the riverbanks.
AW5	N 9°00’08.94”, E 38°09’23.26”	2181	Rain-fed crop farming on both banks of the river; irrigation (for onions, cabbages, tomato and maize); sand excavation from the riverbed.

at site AW4, including washing of animals and vehicles, animal watering, open defecation, domestic waste dumping, waste disposal from a paper mill factory, and erosion/siltation from rain-fed crop farming. Site AW5 is mainly characterized by agricultural activities, such as rain-fed as well as irrigated crop farming, and sand excavation (Table 1).

2.2. Measuring of physicochemical parameters and fecal indicator bacteria

Measurement of physicochemical and microbiological parameters was conducted from March 2017 to February 2018. For each sampling site (AW1–AW5), twenty-four water samples were collected, respectively. Hence, in total, 120 samples were taken. Regarding physicochemical parameters, water temperature, Wtemp. (°C), dissolved oxygen, DO (mg/l), pH and electrical conductivity, EC (μS/cm) were measured in-situ with a multiparameter probe (Hach HQ 40d, USA). Water samples for the determination of total phosphorous (TP), orthophosphates (PO_4), nitrates (NO_3), and total suspended solids (TSS) were taken below the water surface in free-flowing river sections at a depth sufficient to exclude surface scum but without introducing bottom sediment. All samples were stored in ice-cooled boxes and then transported to the laboratory at Ambo University, Ambo, Ethiopia. Physicochemical parameters were determined following methods as described by EPA (1983).

Sterile glass bottles were used for water sampling to determine the status of fecal indicator bacteria (Goshu et al., 2010). After opening, the bottles were horizontally placed 30 cm below the water surface in a free-flowing river section, while the bottle mouth faced the water current. Bottles were moved against the river flow to capture 500 ml of water for fecal indicator bacteria analysis. All water samples were kept in an ice-cooled box and transported to the laboratory where they were analyzed not later than eight hours after collecting the first sample (Byamukama et al., 2005). Water samples were assessed for *E. coli* and Enterococci using a membrane filtration technique with Chromocult coliform agar for *E. coli*, and Slanetz Bartley medium for Enterococci (Merck, Darmstadt, Germany). To select against possible growth of background bacteria, Cefsulodin (5 mg dm−3 Sigma, Vienna, Austria) was added into Chromocult coliform agar. A range of volumes (0.001 to 100 ml) of water samples was prepared and filtered through 0.45 μm pore size and 47 mm diameter cellulose nitrate membrane filters (Sartorius, Vienna, Austria). For enumeration of *E. coli* and Enterococci, the membrane filters were placed onto the respective agar plates and incubated at 37 °C for 24 h for *E. coli* and 48 h for Enterococci. It should be noted that this study determined *Escherichia coli* and Enterococci concentrations in the investigated river system irrespective of where they come from as there is no guideline(s) currently existing for the techniques that track their source(s) in the environment.

The water quality was classified into one of four classes (Table 2) based on the 90th and 95th percentiles (see Eqs. (1), (2)) of *E. coli* and Enterococci in accordance with the EU Bathing Water Directive (2006). Also, the WHO guideline classification for microbial quality of recreational waters (WHO, 2003) was applied (the WHO guideline focuses only on Enterococcus, for which it requires the 95th percentile). For calculations of the percentiles (P), the arithmetic mean (μ) and standard deviations (σ) of all bacterial counts (log10 values) were obtained.

(1) $$P_{90} = antilog(\mu + 1.282 \times \sigma)$$

(2) $$P_{95} = antilog(\mu + 1.65 \times \sigma)$$

Table 2. Water quality category limits of the EU Bathing Water Directive (2006) for a colony forming unit (cfu/100 ml) of Enterococci and Escherichia coli in inland waters.

Parameter	Excellent quality	Good quality	Sufficient quality	Poor quality
Intestinal Enterococci (cfu/100 ml)	≤200[a]	201–400[a]	≤330[b]	>330[b]
Escherichia coli (cfu/100 ml)	≤500[a]	501–1000[a]	≤900[b]	>900[b]

a Based on 95^{th} percentile.
b Based on 90^{th} percentile.

2.3. Benthic macroinvertebrate sampling and analysis

Sampling of benthic macroinvertebrates was conducted from 19 to 20 February 2018. Macroinvertebrates were collected with a standard hand net with a frame size of 25 × 25 cm and a mesh size of 500 µs. For each sampling reach of 100 m river length, a multi-habitat sampling (MHS) scheme was implemented to sample all major habitat units according to their proportional representation within the investigation reach. A sample consists of 20 distinct sampling units which are collected from all microhabitat types which have at least a share of 5% of all habitats in the reach (Barbour et al., 1999, Moog, 2007). Sampling was conducted in upstream direction, starting from the most downstream sampling unit. After completion of one multi-habitat sample, the collected specimens of subsamples were combined in one homogenous sample and then preserved in 4% formaldehyde. In the laboratory, each multihabitat sample was passed through a set of sieves to separate different size classes of macroinvertebrates. Specimen of benthic macroinvertebrates were then sorted and counted to the family level, except Baetidae and Hydropsychidae which were identified to genus level. Three well-used African biotic indices were selected to assess the status of water quality and ecosystem health of Awash River, and to compare the results of the respective indices. Therefore, the South African Scoring System version 5 (SASS5) (Dickens & Graham, 2002), the Tanzanian River Scoring System (TARISS) (Kaaya et al., 2015), and the Ethiopian Biotic Score (ETHbios) (Lakew & Moog, 2015b) were used. The scoring systems (TARISS and ETHbios) are based on the SASS, underlining the necessity for index comparison. The score of these three biotic indices was calculated as the sum of sensitivity score of each taxon present in a sample (Eq. (3)).

$$(3)\quad \text{Biotic score} = \sum_{i=1}^{n} Score\ i$$

The Average Score Per Taxon (ASPT) was calculated as a total biotic score divided by the total number of taxa considered in the calculation, where score i is the score of taxon i and n is the number of taxa considered in the calculation (Eq. (4)).

$$(4)\quad \text{ASPT} = \frac{\sum_{i=1}^{n} Score\ i}{n}$$

2.4. Statistical analysis

The physicochemical data collected did not meet the assumptions of parametric tests, as confirmed by Shapiro-Wilk tests. Therefore, non-parametric Kruskal-Wallis H tests were used to determine the overall differences in physicochemical parameters among sites. The Mann-Whitney U test was used to conduct pair-wise post-hoc tests. Pearson correlations were carried out to determine the correlation between the fecal indicator bacteria variables (using the average value from each of the investigated sites) and the biotic scores, as well as between the different diversity indices of benthic macroinvertebrates. The data for the microbiological analysis was log-transformed (log x + 1) prior to analysis. To assess the status of the invertebrate community in relation to land use and environmental variables, a principal component analysis was conducted. All statistical tests were performed with IBM SPSS 20 and PC-ORD Version 5.33.

3. Results

3.1. Physicochemical parameters

Mean values of most physicochemical parameters, such as water temperature, pH, electrical conductivity, total phosphate, nitrate, orthophosphates and total suspended solids slightly increased from upstream to downstream sampling sites (Table 3). Five of eight measured physicochemical variables showed significant differences between the sampling sites ($p < 0.05$, Kruskal-Wallis H test). Only dissolved oxygen, total phosphorus and orthophosphates did not differ between the sites. Comparing AW1 and AW2, electrical conductivity was the only significantly different parameter between the two sites ($p < 0.05$, Mann-Whitney U test).

3.2. Concentrations of fecal indicator bacteria and assessment of bathing water quality

In general, the concentration of fecal indicator bacteria ranged from moderate to strong fecal pollution levels at the five sampling sites, according to the fecal classification scheme for river water (Kirschner et al., 2009), whereas concentration of indicator bacteria increased from upstream to downstream sampling sites (e.g., from 7.9 × 102 cfu/100 ml (AW1) to 7.6 × 103 cfu/100 ml (AW5) for *E. coli*, and from 7.6 × 102 cfu/100 ml (AW1) to 1.1 × 104 cfu/100 ml (AW5) for Enterococci.

The health-related water quality assessment for recreational environments showed that both, *E. coli* and Enterococci concentrations exceeded the threshold set by the EU Bathing Water Directive for the 90th and 95th percentile at all five sampling sites. Similarly, Enterococci concentrations exceeded the maximum WHO threshold at all sampling sites. Hence, based on both documents, all sampling points along the upper Awash River are not qualified for bathing as their water quality was classified as poor (Table 4).

Table 3. Summary statistics of environmental variables for the five sampling sites, showing the mean and the standard deviation (in brackets) for each physicochemical parameter (n = 5 sites × 24 measurements = 120 for all variables, except DO which was only measured 22 times).

Variable	AW1	AW2	AW3	AW4	AW5	Kruskal-Wallis H Test (p value)
Wtemp (°C)	13.05 (±2.49)	14.54 (±2.51)	21.17 (±2.86)	20.30 (±2.76)	19.74 (±2.11)	0.000**
pH	8.35 (±0.54)	8.52 (±0.57)	8.66 (±0.52)	8.48 (±0.61)	8.64 (±0.52)	0.024*
DO (mg l^{-1})	7.54 (±0.71)	7.47 (±0.63)	7.79 (±1.24)	6.91 (±0.86)	7.44 (±1.13)	0.058
EC (µs cm^{-1})	183.67 (±50.65)	247.92 (±94.45)	316.46 (±115.35)	366.13 (±175.28)	365.83 (±172.47)	0.000**
TP (mg·l^{-1})	0.11 (±0.06)	0.19 (±0.35)	0.29 (±0.44)	0.33 (±0.40)	0.37 (±0.47)	0.125
PO_4 (mg l^{-1})	0.037 (±0.026)	0.039 (±0.035)	0.041(±0.048)	0.048 (±0.058)	0.048 (±0.062)	0.942
NO_3 (mg l^{-1})	1.23 (±0.61)	1.37 (±0.81)	2.14 (±1.11)	2.19 (±1.24)	2.28 (±1.08)	0.000**
TSS (mg l^{-1})	62.84 (±104.25)	183.36 (±472.59)	681.99 (±1320.10)	690.20 (±1140.66)	673.96 (±1008.03)	0.000**

Wtemp = water temperature; DO = dissolved oxygen; EC = electrical conductivity; TP = total phosphorus; PO4 = orthophosphate, NO3 = nitrate; TSS = total suspended solids.

* = significant at the 0.05 level. ** = significant at the 0.01 level.

Table 4. Measured concentrations of E. coli and Enterococci in Awash River, and water quality classification based on the 90th and 95th percentiles according to the EU Bathing Water Directive (2006) and WHO guidelines for safe recreational water environments (2003).

Sampling site	Fecal indicator bacteria	Average[a]	±SD[a]	95th Percentile[b]	90th Percentile[b]	EU Bathing Water Directive (based on *E. coli* and Enterococci)	WHO guideline values (based on Enterococci)
AW1	*E. coli*	2.69	0.42	2400	1685	****	*****
	Entrococci	2.66	0.38	1917	1392	****	*****
AW2	*E. coli*	2.90	0.52	5701	3676	****	*****
	Entrococci	2.86	0.62	7549	4478	****	*****
AW3	*E. coli*	3.12	0.72	20,412	11,093	****	*****
	Entrococci	2.97	0.76	16,514	8,706	****	*****
AW4	*E. coli*	3.30	0.59	18,690	11,339	****	*****
	Entrococci	3.29	0.75	33,099	17,577	****	*****
AW5	*E. coli*	3.28	0.77	35,479	18,456	****	*****
	Entrococci	3.02	0.88	29,449	14,017	****	*****

SD = standard deviation.
a Values are log (x + 1 cfu/100 ml) transformed.
b Values are presented as cfu/100 ml.
**** Poor water quality class according to EU Bathing Water Directive.
***** Category D of WHO guideline values for microbial quality of recreational waters.

3.3. Status of benthic macroinvertebrate communities and comparison with microbial water quality classification

A wide range of physicochemical characteristics and land use types determines benthic invertebrate community structure in the Awash River. The principal component analysis showed that the presence of sensitive species (e.g., Leptoceridae, Heptageniidae, Lepidostomatidae, Ecnomidae, Simuliidae and Tipulidae) was associated with forest cover around the sampling site, while the presence of tolerant species (e.g., Oligochaeta, Chironomidae, Corbiculidae and Physidae) correlated with agricultural land (Fig. 2).

Two biotic indices, the South African SAAS5 and the Tanzanian TARISS, classified the five sampling sites exactly the same: AW1 and AW3 were categorized as "natural", AW2 as "good", AW4 as "fair", and AW5 as "poor". According to the Ethiopian ETHbios, the water quality of AW1–AW3 was classified as "good", whereas AW4 and AW5 were classified as "moderate" and "poor", respectively (Table 5). Based on a health-related assessment using the EU Bathing Water Directive and the WHO guideline values, however, all sites were classified as "poor" as the concentrations of fecal indicator bacteria exceeded the established thresholds (Table 5).

Pearson correlations were performed to assess the relation between the fecal indicator bacteria, the biotic indices for macroinvertebrates as well as selected metrics of benthic macroinvertebrates

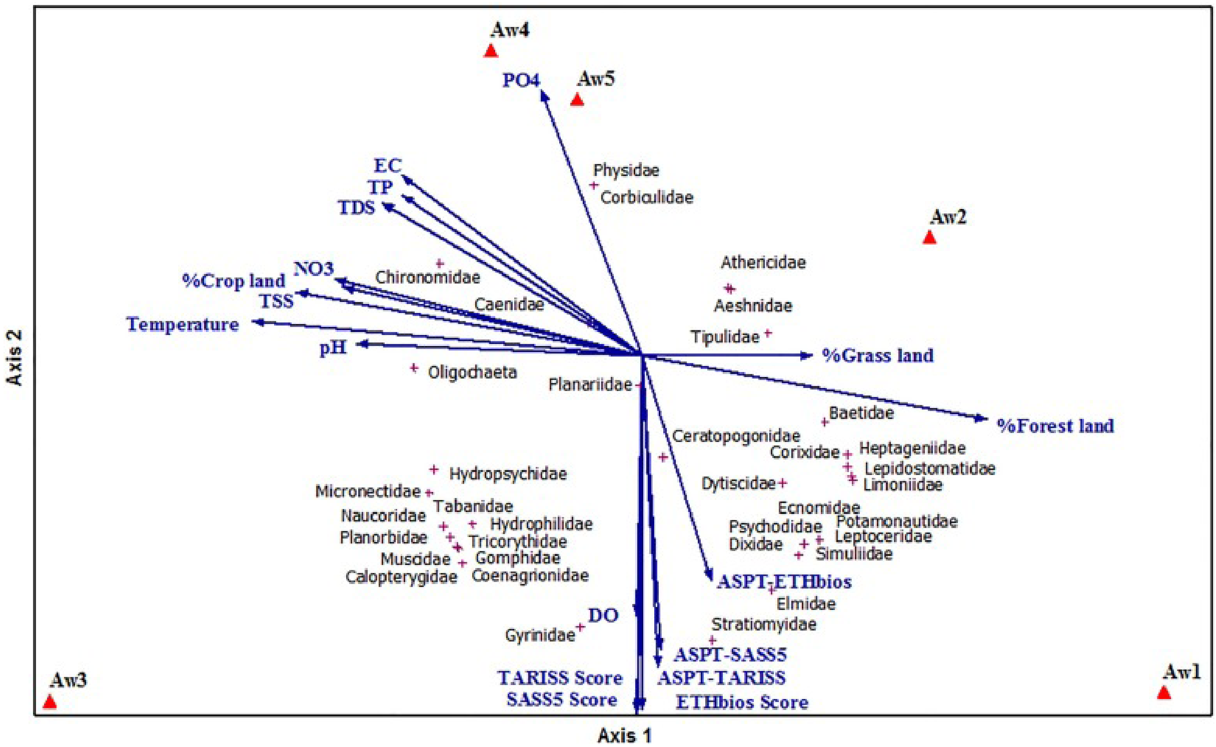

Fig. 2. Principal components analysis (PCA) biplot of macroinvertebrate community in relation to land use and environmental variables along sampling sites in the upper section of Awash River (the first two axes explain 72.15% of the total variance).

Table 5. Water quality classes obtained for each sampling site based on the biotic indices SASS5, TARISS, ETHbios, as well as the EU Bathing Water Directive (2006) and the WHO guideline values for microbial quality of recreational waters (2003).

Sampling Point	SASS5	TARISS	ETHbios	EU Bathing Water Directive (based on *E. coli* and intestinal Enterococci)	WHO recreational water quality guideline (based on intestinal Enterococci)
AW1	*	*	**	****	*****
AW2	**	**	**	****	*****
AW3	*	*	**	****	*****
AW4	***	***	***	****	*****
AW5	****	****	****	****	*****

Classification of ecological status according to the macroinvertebrate indices SASS5, TARISS, ETHbios: * = natural water quality, ** = good water quality with slight ecological degradation, *** = moderate water quality with significant ecological disturbance, **** = poor water quality with major degradation.

Classification of water quality class according to the EU Bathing Water Directive: **** = poor; according to WHO guideline values: ***** = category D.

(no. of taxa, percentage of Ephemeroptera and Trichoptera (ET) taxa). The correlation matrix (Table 6) shows that *E. coli* counts were strongly correlated with Enterococci abundance. However, concentration of both fecal indicator bacteria, *E. coli* and Enterococci, was negatively correlated with metrics of benthic invertebrates, such as number of taxa, percent of ET taxa, as well as the scores and ASPT values of the African biotic indices (SASS5, TARISS, ETHbios). The scores and ASPT values of the three indices were highly correlated with each other (>0.99 and >0.9, respectively), which was significant on the $p < 0.05$ level. The concentrations of fecal indicator bacteria rose as the impact of human pressure increased along the river in downstream direction. In contrast, however, the percentage of ET taxa, biotic scores and ASPT values decreased as the degree of anthropogenic pressures rose from AW1 to AW5.

4. Discussion

4.1. Physicochemical water quality parameters

In this study, we assessed and compared physicochemical water quality parameters, fecal indicator bacteria and benthic invertebrate communities along a pressure-gradient in the Awash River. We found that the levels of various physicochemical parameters increased from upstream to downstream sampling sites. In particular, the two sampling sites below the urban area of Ginchi town had significantly higher values for most physicochemical parameters (e.g., water temperature, electric conductivity, total phosphorus, nitrite and total suspended solids) when compared to upstream sites. Among others, some of these increases can be attributed to the presence of agriculture which often has great effects on water quality. For example, Goshu et al., 2010, Gotkowska-Plachta et al., 2016 reported high values of total suspended solids and electrical conductivity at sites which are mainly impacted by agricultural and urban activities. Furthermore, urbanization and deforestation in the lower sections of Awash River result in increased soil erosion and, subsequently, higher sediment loads and nutrient input into the river systems. The varying concentrations of orthophosphate and total phosphorus between the sampling sites might be caused by different land use types. Similarly, also Gotkowska-Plachta et al. (2016) reported that the lowest concentrations of both forms of phosphorus were measured in forested areas, whereas it was abundant in samples from agricultural and urban areas.

Regarding dissolved oxygen, the levels were the highest in the mid-section of Awash River (AW3), Nevertheless, these slightly higher values could be due to relatively abundant filamentous algae at this site. Similarly, Klose et al., 2012, Morgan et al., 2006 reported high DO concentrations during the day when filamentous algae were present.

4.2. Fecal indicator bacteria

In Awash River, concentrations of fecal indicator bacteria varied depending on the type of land use at immediate catchment area. Concentrations of *E. coli* and Enterococci were higher at sites close to urban and agricultural areas than at sites situated in forested areas. This pattern is similar to other studies which reported that densities of fecal indicator bacteria in the watershed exhibited a clear dependency on the land use type in the surrounding area (Gotkowska-Plachta et al., 2016). For example, Goto & Yan (2011) measured significantly higher concentrations of *E. coli* and Enterococci in the urban section of the stream than in the forested section. Similarly, in the Buffalo and Tyume River, South Africa, higher concentrations of fecal indicator bacteria were observed in lower river reaches as anthropogenic activities, such as

Table 6. Pearson correlation coefficients between pooled data of fecal indicator bacteria, selected macroinvertebrate metrics, and biotic indices for benthic invertebrates.

	E. coli	Enterococci	No. of taxa	% ET taxa	SASS5 score	TARISS score	ETHbios score	ASPT SASS5	ASPT TARISS	ASPT ETHbios
E. coli	1									
Enterococci	0.915*	1								
NO. taxa	−0.578	−0.408	1							
% ET taxa	−0.609	−0.739	−0.240	1						
SASS5 score	−0.628	−0.472	0.993	−0.149	1					
TARISS score	−0.612	−0.456	0.997	−0.176	0.999**	1				
ETHbios score	−0.676	−0.505	0.989	−0.105	0.997**	0.995**	1			
ASPT SASS5	−0.813	−0.699	0.900	0.189	0.941*	0.930*	0.951*	1		
ASPT TARISS	−0.817	−0.714	0.906	0.183	0.944*	0.935*	0.954**	0.998*	1	
ASPT ETHbios	−0.844	−0.697	0.869	0.239	0.914	0.900*	0.932*	0.992**	0.985**	1

* Correlation is significant at the 0.05 level.
** Correlation is significant at the 0.01 level.

urban influence, increased (Chigor et al., 2013, Sibanda et al., 2013). Also, studies conducted on the Belgian Zenne River and the Portuguese Ave River reported high levels of fecal indicator bacteria (*E. coli* and Enterococci) as anthropogenic factors increased along the river in downstream direction (Barbosa-Vasconcelos et al., 2018, Koffi et al., 2011).

According to several studies (e.g., Arnold et al., 2016, Benjamin-Chung et al., 2017), the majority of microorganisms harmful to health present in aquatic systems are of fecal origin. Contamination of bathing waters is, therefore, a serious environmental problem with potential negative effects on water users (Marion et al., 2010). However, in many parts of the world, such as in the Ethiopian Awash catchment, rivers and streams are used as open bathing and recreation areas. The standards of the U.S. Environmental Protection Agency (USEPA, 1986), the EU Bathing Water Directive (2006) and the World Health Organization guidelines for safe recreational water uses (WHO, 2003) are frequently applied for monitoring the bathing water quality criteria regarding bacteria. In Ethiopia, however, there is no guideline to monitor bathing water quality. Hence, in this study, we used the EU Bathing Water Directive and the WHO guidelines for classification of the environmental health status in terms of recreational activities of humans. Our results showed that concentrations of *E. coli* and Enterococci reflected critical to strong fecal pollution levels at the investigated sampling sites. According to EU and WHO water quality standards they had to be classified as poor water quality locations for bathing and recreational activities. According to Mayer et al., 2016, Reischer et al., 2013, this is a common outcome for rivers with a significant input of fecal pollution, such as upstream emissions from communal waste water treatment plants without further disinfection or non-point emissions from intensive livestock farming activities. Even though a study conducted on the Mur River, Austria, obtained concentrations less than those of our study, the numbers of fecal indicator bacteria were still too high at many sampling points, making the river not fit for recreational uses such as bathing (Kittinger et al., 2013). Similarly, high concentrations of fecal bacteria were also reported at some sampling points along the Danube River (Kirschner et al., 2017). Similar to our study, Gotkowska-Plachta et al. (2016) reported that all sampling sites, with the exception of a forested site, of Łyna River, Poland, were characterized by high levels of bacterial contamination and the water quality was, therefore, also classified as poor according to the EU Bathing Water Directive. Studying the Bufalo River, South Africa, Chigor et al. (2013) discovered that 71–79% of all water samples for fecal coliforms and 82–85% of all water samples for Enterococci exceeded the U.S. EPA and South African water quality guideline limits for recreational waters. As a violation of water quality guideline thresholds can pose serious health risk to people, it can be assumed that full contact activities, such as bathing, washing, or domestic uses, can endanger communities living along these rivers. In this regard, recent studies underlined the necessity to monitor the status of fecal indicator bacteria in rivers and streams to prevent unwanted health consequences in recreational areas (Abia et al., 2016, Arnold et al., 2016, Benjamin-Chung et al., 2017, Cordero et al., 2012, Marion et al., 2010). Communities in the upper Awash River basin, and especially the most upstream sampling points, however, not only use river water for outdoor bathing but also for drinking, domestic purposes and washing of clothes.

Recreational water with high fecal-associated bacterial content can lead to outbreaks of serious illnesses, causing hospitalization and even death (Centers for Disease Control and Prevention, 2015). Children, in particular, are among the highest risk group because they tend to play for longer periods in such water bodies and may swallow water during swimming. Moreover, in Eastern Africa, children commonly fetch water for household consumption which increases the frequency they are in contact with the river water. Indeed, Arnold et al. (2016) highlighted that, in the United States, the youngest children showed high gastroenteritis risk and associated burdens related to recreational water exposure. In addition to recreational and household activities, the community in the upper section of Awash River uses water for small-scale irrigation to grow vegetables, such as onions, cabbages and tomatoes, which are consumed fresh. Human pathogens present in irrigation water can, therefore, be transmitted to plants and subsequently passed on to humans through consumption of vegetables irrigated with surface water that may contain pathogens (Pachepsky et al., 2011). According to Herman et al. (2015), such disease outbreaks show a larger association to leafy vegetables than to other food types. Similarly, Chigor et al. (2013) showed that 89% of all water samples of unrestricted irrigation of crops likely to be eaten uncooked exceeded the U.S. EPA and South African water quality guidelines for fecal coliform bacteria. Therefore, the presence of higher fecal indicator bacteria concentrations in the irrigation water of farming communities along the Awash River suggests that fecal pollution levels and thus the potential presence of enteric pathogens are likely a public health threat and thus should be considered in the future.

4.3. Macroinvertebrates

We found that longitudinal diversity of macroinvertebrates was affected by land use and physicochemical parameters. The presence of tolerant species (e.g., Oligochaeta, Chironomidae) was strongly associated with surface area of agricultural land around the sampling site. In contrast, the presence of sensitive species was strongly associated with forested land. Hence, sensitive taxa mostly occurred in the two upstream sampling sites with high forest coverage.

In previously established taxa scores, e.g., ETHbios, sensitive taxa such as Leptoceridae, Heptageniidae, Lepidostomatidae, Ecnomidae and Simuliidae obtained relatively high taxa score values for the same study area in the upper Awash river basin (Lakew & Moog, 2015b). High values of PO_4, NO_3, EC, TP, temperature and TSS were strongly associated with downstream sampling sites where tolerant taxa, such as Oligochaeta and Chironomidae were relatively abundant. Also, Ghani et al. (2018) reported that Oligochaeta, which are very tolerant to pollution were found in high abundance in a polluted urban river. The high presence of sensitive macroinvertebrate taxa at upstream sites, as well as the high abundance of tolerant taxa at the downstream sampling sites, shows that upstream sampling sites are less exposed to human disturbances. A similar pattern was also found in previous studies (Barbosa-Vasconcelos et al., 2018, Herringshaw et al., 2011, Lakew, 2015), which indicates that the land use type within watersheds has considerable effects on biological communities in streams and rivers.

The principal component analysis also showed that the biotic indices were positively correlated with forestland and shrubland on the first axis. In this study, DO content significantly correlated with all biotic indices at the middle section of the river where the concentration of DO is highest, which reflected its desirable association with the macroinvertebrates. Furthermore, the intolerant species such as Ephemeroptera (Baetidae, Caenidae, Heptageniidae and Tricorythidae) and Trichoptera (Hydropsychidae, Lepidostomatidae, Ecnomidae and Leptoceridae) contributed to the high score of the three biotic indices (SAAS 5, TARISS and ETHbios). Hence, calculated biotic indices scores and principal component analysis showed their tendency to classify the upstream section of the rivers as having clean water quality.

The three African benthic macroinvertebrate-based biotic scores indicated a natural or good water quality with slight ecological degradation at the upstream sites, and a moderate to poor ecological status at the downstream sites. Comparing the biological indices, the results revealed that the South African SASS5 and the Tanzanian TARISS classified all sampling sites into the same ecological water quality class. In contrast, the Ethiopian index ETHbios classified two sampling sites different than the other two indices. Therefore, based on water quality classes of each of the sampling sites, ETHbios only had a 60% overlap with SASS5 and TARISS, which indicates that the results of the three biotic indices showed high similarities with each other.

4.4. Comparison of fecal indicator bacteria and benthic macroinvertebrates as indicators of water quality

We hypothesized that, in the Awash River, the population of fecal indicator bacteria and benthic macroinvertebrates would both be a reliable community indicator of ecological health of a freshwater system exposed to different land use types. The results indicate that the benthic invertebrate indices were effective tools for monitoring the biological quality and ecological status of the upper Awash River. All three macroinvertebrate indices were able to reflect the gradient of anthropogenic disturbances, presumably caused by changes in land use type from natural forests in the headwaters to highly modified agricultural landscapes in the lower reaches. In contrast, communities of fecal indicator bacteria were not able to indicate land use changes along the river. Although, in some studies, the fecal indicator bacteria analysis was sensitive to discriminate between the most impacted and the least impacted sites (Barbosa-Vasconcelos et al., 2018, Gotkowska-Plachta et al., 2016), this was not the case in our study, as the occurrence of fecal indicator bacterial, which was mainly driven by the high pressure of extensive livestock overgrazing in the entire catchment, could not distinguish heavily impacted sites by fecal pollution and other anthropogenic pressures from less impacted sites. Hence, this is the first study reporting the contrasting results of water quality status from fecal indicator bacteria and macroinvertebrates during water quality analysis. Consequently, there is a necessity of combining different indicator systems to analyze human pressures from a more holistic perspective, which can serve as a basis for integrated management and sustainable use of fundamental resources such as water from freshwater ecosystems.

5. Conclusion

The study compared physicochemical parameters, fecal indicator bacteria (*E. coli* and Enterococci) as well as benthic macroinvertebrate indices as indicators of water quality in the upper Awash River, Ethiopia. We found that river water quality decreased in downstream direction which reflected the effect of anthropogenic (land use) activities in the catchment which may be attributed to agricultural and urban runoff, waste from a paper mill factory, and various instream activities. Except for dissolved oxygen and water temperature, tested physicochemical parameters and fecal indicator bacteria levels increased from upstream to downstream sampling sites, while biotic indices values decreased. While the assessment of fecal indicator bacteria resulted in a poor classification of all sites if used for bathing and recreational activities, the macroinvertebrate indices generally resulted in better status classes and were able to reflect land use changes along the river. Nevertheless, as the overall bacteriological water quality in Awash River was classified as poor, its use for unrestricted irrigation of fresh produce, full contact recreation, and domestic purposes may cause significant public health hazards. In the interest of public health, future research should focus on the assessment of these surface waters for the presence of bacterial, viral and protozoan pathogens. Also, in order to identify fecal indicator bacteria sources, microbial source tracking should be performed. Provision of adequate sanitary infrastructure and public health education will help to prevent water source contamination from fecal pollution.

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