

**HEALTH QUALITY ASSESSMENT OF A HIGHLAND
STREAM USING SELECTED PHYSICO-CHEMICAL
PARAMETERS, BIOLOGICAL FACTORS AND
MICROPLASTIC LEVEL**

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Degree of Master of Science

Department of Civil Engineering

University of Moratuwa

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May 2022

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Dissertation submitted in partial fulfillment of the requirements for the
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DECLARATION

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ABSTRACT

Ella is a popular tourism destination in Sri Lanka due to its salubrious climate and aesthetic quality of the surrounding environment. Beeralla stream (tributary of Kirindioya) flow through the Ella city by increasing recreational value of Ella City. Present study performs the health quality assesement of Beeralla stream with respect to the selected physico chemical parameters, biological factors and microplastic levels in suface water.

Ten physicochemical parameters (pH, Water temperature, Total Suspended Solids (TSS), Turbidity, Conductivity, Dissolved Oxygen (DO), Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Nitrate and Total Phosphorous (TP) concentration) of stream water were monitored at 05 sampling locations in January, March, July, September and November 2020 as representing the intermonsoonal and monsoonal periods. Sampling sites were selected for the study at Ella GN area after preliminary observations to cover the area close to different land use patterns. A microplastic level of surface water of the stream was investigated from March until December 2020 by analyzed by wet peroxidation method.

Results revealed that the measured water quality parameters significantly vary (<0.05) both spatially and temporally. Oneway Anova resulted that mean values of all parameters were significantly vary (<0.05) spatialy. Further, DO, TSS, Nitrate concentration, BOD, COD and TP concentration were significantly higher (<0.05) in January and March. Highest average TSS, BOD, COD and TP were recorded in the midstream (Site 3 and 4) indicating possible uncontrolled wastewater discharges and high urban waste runoff. Although average values of TSS, turbidity, DO, phosphate, COD and BOD levels are exceeded the Sri Lankan ambient water quality standards in sampling site 02, 03, 04 and 05.

Aquatic macroinvertebrates of each sampling sites were examined at the time sample collection for physicochemical parameters. A total of 15 individuals of macroinvertebrates representing 5 orders (Odonata, Hemiptera, Diptera, Coleoptera and Haplotaxida) were successfully collected from January until December 2020. Shannon Weiner Diversity Index was ranged 1.17 – 1.94 and Family Biotic Index ranged in between 5.56 to 8.26. The values of Family Biotic Index indicate that all sites of stream are subjecting to the organic pollution with poor water quality.

Samples collected from all sampling locations were contained microplastics with the average density of 5-13 Items/L. Mean micoplastc density levels of surface water were significantly varied among sampling periods (<0.05). Present study results reveal that water quality of the Beeralla stream is deteriorated with micoplastic pollution, vast nutient inputs with organic matter. Also, water quality is exceeded the

ambient water quality standards in Sri Lanka. Since Ella is a main tourism attracted area in Sri Lanka, it is vital to maintain its aesthetic quality and recreational value. Therefore, it is needed to identify point source and non- point sources of pollutants of Beeralla stream and control with an appropriate short-term and long-term technique.

Key Words

Aquatic ecosystems, physicochemical parameters, macroinvertebrates, microplastic pollution

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LIST OF ABBREVIATIONS

CEA	-	Central Environmental Authority
ANOVA	-	Analysis of Variance
BOD	-	Biochemical Oxygen Demand
COD	-	Chemical Oxygen Demand
DO	-	Dissolved Oxygen
TSS	-	Total Suspended Solids
WPO	-	Wet Peroxide Oxidation
FBI	-	Family Biotic Index
FTIR	-	Fourier-Transform Infrared

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1. INTRODUCTION

1.1 Aquatic eco-systems

Freshwater is an essential resource for human life, also it has no substitutes. It is crucial for many natural systems that support human well-being. Human activities are widely extended and changed the planet's freshwaters. Those alterations impact the physical, chemical, and biological features of aquatic systems (Stephen *et al*, 2011). Aquatic biomes are considered as the water ecosystems which are including marine ecosystems, brackish water ecosystems, estuarine ecosystems, and the freshwater ecosystems. Aquatic ecosystem services can be categorized into cultural, provisioning and regulating services. They act as a source of cultural services. Especially it provides aesthetic services, spiritual services and educational services. Also many of these ecosystems are tourism destinations with many recreational activities such as swimming, boating and bird-watching (Dodds, 2002).

As provisioning services, aquatic resources provide habitats for wide range of flora and fauna which provide valuable goods and cooperative services with potential useful application in the agricultural, pharmaceutical and innovative industries (Zabadal, 2005).

Also it is very important that aquatic environment provides supportive and regulatory services by supplying food and housing for crocodiles, crustaceans, fishes, whales, mollusks etc and nutrient supplies for economically important fish species (Zabadal, 2005). Other than that, aquatic environment performs other important environmental functions such as nutrient recycling, water purification etc. (Rodrigues and J. M. G, 2015).

Freshwater means that, water bodies with less than 0.05 parts per thousand of dissolved salts and comprise surface water bodies such as rivers, ponds, springs, lakes, wetlands, streams and artificial or man-made bodies such as reservoirs, ditches and reservoirs as well as underground water (Fransis, 2019). Two main types of freshwater bodies can be identified as – Lotic and Lentic water. Flowing water

bodies includes lotic systems, which are continuously fluctuate or are in unidirectional flow. They include streams, rivers, creeks, as well as seas and ocean which exhibit a tidal movement. Lentic water bodies are considered as ponds and lakes. There are many uses from lentic water bodies such as human recreation, tourism activities and transport industries especially in coastal regions of the world (Fransis, 2019).

1.2 Why are freshwaters changing?

Freshwaters are changing due to natural factors and antropagenic activities. Climate change as a natural factor, directly affects the ecology of freshwaters as a result of precipitation among different locations over time, sea level rise and increased water temperatures. Human alteration of water flows, land-use alterations, introduction of aquatic invasive species, chemical inputs and human harvesting activities including aquaculture are examples for the antropagenic activities. Direct discharges of wastewaters from mining activities, industries, or municipal sewage are main cause for the increase in chemical inputs to freshwaters. These anthropogenic chemicals added to freshwaters include organic compounds, heavy metals, acids, and alkalis which are toxic to aquatic organisms (Stephen *et al*, 2011). In order to protect the water quality, it is important to consider about the assimilative capacity of a water body in determining the safe release limits for industrial discharges (Brown and Rodgers, 2014).

Water pollutant can be defined as, any substance which is capable of causing or is likely to cause any adversarial effects in water. Any introduction of substances or energy into aquatic environment directly or indirectly which results in such deleterious effects as harm to living or non-living resources, hazardous to human health, interference to aquatic activities including fishing and other legitimate uses of the sea(River), diminishing of quality for use of water and reduction of features by man is refered as the aquatic pollution Generally water pollutants can be classified into several categories such as toxic, organic , petroleum, radiation, thermal, sediment runoff and pharmaceuticals pollutants (Wokoma, 2019). In addition to mentioned pollutants, microplastic pollution in water is a growing global issue.

Plastic production was increased all over the world rapidly for many uses due to its high durability, light weight, potential for diverse applications and low price (Woodall *et al.*, 2014 and Lee *et al.*, 2013). Earlier plastics were not considered as a threat to marine waters not much but now it is a emerging problem. During the past centuries, dumping of waste to ocean was practiced which contains organic and degradable materials. But unfortunately, synthetic materials, like plastics replace these degradable matters and emerge a great threat to the survival for aquatic environment of the world (Sheavly and Register, 2007). Recently past, many research studies reveal that microplastics accumulation in the marine environment. But microplastic pollution in freshwater and estuarine waters gets less attention. Several studies were proven the microplastic accumulation in freshwater via sediments and fish analysis (Ramasamy and Strthy, 2016). It has been found that, 5.25 trillion of floating plastic particles were spread in five ocean gyres. Therefore, plastics can be found in everywhere from North pole to South pole including water column, soil/sediment biota with wide size range from metres to micrometres (Ogunola *et al.*, 2018).

1.3 Health quality assessment and water resource management of streams

Health quality assessment of streams has a countless importance especially in developing countries as well as in semiarid countries. Now it is popular method of assessing and classifying the ecological water quality via indices-based techniques. It helps conserve and manage aquatic habitats. The most common method of measuring the physicochemical parameters is cost intensive, time-consuming, and specially depends on distinctive utensils. Usually physicochemical parameters show water quality at the moment of measurement, but these can alter over time. Nowadays, indicators-based water quality assessment programmes using absence or presence of aquatic organisms are developed rapidly and it is used to classify the ecological status of the aquatic environment (Azami *et al.*, 2015). Ecosystem health can be measured by evaluating the effects on biota which are exposed for environmental degradation at the end of the pollution chain in aquatic habitats and these biota act as an important indication (Norris and Thoms, 1999). Living organisms such as

invertebrates, small mammals, aquatic plants, algae, fish etc are considered to be biological indicators to assess water quality. Environmental pollution in aquatic habitats over a long period can be assessed by using fish species due to their long life cycle, high mobility, incorporate with different trophic levels. Also macroinvertebrates can be sampled relatively easy and they can inhabit clean water to highly polluted water. Therefore they are considered as a very biodiverse group. Macroinvertebrates play important role in organic matter cycling and high trophic level organisms procure food resources from them. The variation of macroinvertebrate richness causes for ecosystem function in aquatic environments. Moreover, many studies in Europe shows the importance of using biotic indices in water quality monitoring programmes (Azami *et al.*, 2015).

Major threat for water resources in Sri Lanka are the deterioration and degradation of the water quality caused by different anthropogenic activities. It is revealed that surface inland waters are polluted with domestic sewage and industrial effluents heavily specially in urban areas. Also, agricultural runoff is the reason for pollution of surface inland waters in rural areas (Silva, 2007). Fresh water is considered as a major part of tourism industry in Sri Lanka. Therefore, many water resources such as rivers, nature pools, lakes help to increase the foreign currency and revenue of the country. Also use of natural resources including freshwater and waste generation is high in tourism industry in Sri Lanka. Therefore, it is essential to increase awareness among Sri Lankan hotel sector regarding implementation of appropriate energy, waste management systems to adopt ecofriendly practices. Improper waste disposal is a major threat for aquatic ecosystems in tourism based areas in Sri Lanka (Sunlu, 2002).

1.4 Effects of tourism industry on water pollution in Beeralla stream

Tourism industry in Sri Lanka is growing rapid for centuries, Sri Lanka has been a popular place of attraction for foreign travelers. Also tourism is one of the leading foreign currencies earning industry in Sri Lanka which shows an increase trend in its development.

Ella is a picturesque little town 200 kilometres east of Colombo and at about 1,000 metres above sea level. The unique location of the town between the lowland rainforests and the highland tea plantations makes for a great rest stop while touring Sri Lanka (Ranaweera, 2019). Considering all over the country, proper wastewater management practices should be introduced to the hotel industries where discharge huge amount of wastewater. According to the rules and legislations, standard level of discharge limits for wastewater quality parameters were introduced by Central Environmental Authority (before discharge to the natural streams). But in practically, hotel owners in Ella do not comply with this legal status and discharge uncontrolled wastewater to natural waterways. Recent past, there was a rapid increase of tourism industry in Ella. With it's scenic beauty surrounded by rocks, many tourists were attracted this area. Unfortunately, with this rapid growth of tourism industry people have least concern on environmental protection of the area. Study done in Ella was identified that tourism negatively impact on environment of the area and local culture. Therefore, it is crucial to limit these negative impacts (Sharmini and Bandusena 2020). Villagers and hotel owners focused on tourism activities without cooperating ecotourism practices. Therefore unauthorized buildings, illegal constructions have been rapidly built in recent past in Ella. With this current status, many hotel and restaurant owners does not treat wastewater properly. The problem rise with this condition. Hotel and restaurant owners discharge wastewater from their industries without treated and make a risk of contamination of uncontrolled wastewater to natural ecosystems in Ella. Ella is enriched with natural ecosystems including forests, rivers, mountains etc. Popular Rawana Ella fall, Krindioya and tributary of Umaoya are major freshwater ecosystems that can be seen in Ella area. Beeralla stream (tributary of Kirindioya) flows through the Ella town area and many restuarants and hotels located at riverine habitat of this stream. Beeralla stream is a acommon water source for agricultural activities as well as washing and bathing purposes of villagers. Backward of many hotels and restaurants are directly face to Beeralla stream and their treated or untreated wastewater directly discharge to this tributary. Also, some common drains come from upper village area connect to this stream and it can be consisted with high pollution loads. Furthermore many

complaints regarding uncontrolled wastewater discharges to this stream was risen via Environmental Organizations. This is an unfortunate condition in Ella since it is recorded as a popular ecotourism destination in Sri Lanka. Due to these reasons, Beeralla stream becomes polluted and natural habitat of this area is degrading. Since this stream is adding value for aesthetic quality of the Ella, it should be assessed the health quality of the stream in order to get necessary actions to protect the stream by identifying its pollution level.

1.5 Aim of the study

To compare the selected physicochemical parameters, micoplastic level, and biological factors in terms of abundance of freshwater macroinvertebrates in shallow water in the selected sites of Beeralla Stream (tributary of Kirindioya) to identify the level of disturbance and asses the aquatic life health quality of the stream using Biotic Indices.

1.6 The specific objectives of the present study

- To compare the spatial and temporal variation of selected water quality parameters in the selected sites of Beeralla stream (tributary of Kirindioya).
- To compare the variation of aquatic macroinvertebrates diversity in the selected sites of Beeralla stream (tributary of Kirindioya).
- To assess the aquatic macroinvertebrates based rapid bio assessment indices to study the variation of shallow water in the selected sites of Beeralla stream (tributary of Kirindioya).
- To assess the variation of microplastic levels in surface water samples of Beeralla stream temporally and spatially

2. LITERATURE REVIEW

2.1 Freshwater pollution and effects of pollution on freshwater aquatic organisms

In recent years, freshwater ecosystem has experienced serious threats from human activities such as industrial effluents, waste management issues, agricultural activities and increase in urbanization. In addition, climate change impacts resulting changes in abiotic factors such as precipitation and temperature levels have affected the normal function of aquatic ecosystems. These pollution levels have also affected the habitats of aquatic flora and fauna (Patrick and Mahad, 2019). Virtually all known human activities have the potential to directly or indirectly impact on the quality of surface and underground water bodies. For example, fertilizers used by farmers in the field are gradually washed by rain into the ground water or nearby surface waters, thus polluting the water body. Pollution types are as varied as human activities and could arise from gaseous, liquid or solid matter, domestic, commercial or industrial activities as well as from natural or man-made sources (Fransis, 2019). The enrichment of a water body by the continuous addition of nutrients or organic matter stimulates growth of aquatic plants such as algal nuisances. It interferes the water uses, produce unpleasant odors and add to the Biochemical Oxygen Demand (BOD) of the water. This affects the rate of primary productivity and cause changes in the biota. The dominant biota changes and species diversity decreases, while plant and animal biomass increase. Anoxic conditions may develop and life span of the water body is shortened as a result of escalation in the rate of sedimentation. Runoff from the exposed soils such as highways, agricultural fields, construction sites etc. introduces excessive amounts of sediment into water bodies that can suffocate fish by clogging their gills. And also can block sunlight by inhibiting aquatic plants from photosynthesizing. Thus, reducing primary production, this ultimately will lead to death of aquatic biota (Zabadal, 2005). Thus, in order to protect global freshwater aquatic species and ensure the regular functions of ecosystems, it is crucial to identify the main pollution activities, their sources, and their fate in the aquatic

environment including their spatial distribution. Achieving these will provide more sustainable and lasting measures of protecting freshwater aquatic life from anthropogenic activities (Patrick and Mahad, 2019).

There are many industries such as gem mining, tea, rubber, fertilizer, chemical, textile etc in Sri Lanka which discharge large amount of environmental pollutants that contaminate freshwater bodies (Alagan, 2009). According to a study done in tributary of Baduluoya, water quality parameters such as BOD, phosphate and turbidity in surface waters exceed the ambient water quality standards of Sri Lanka. It is concluded that, domestic wastewater discharges and agricultural runoff can be the reasons for pollution level of water in Baduluoya tributary (Rajapaksha *et al.*, 2020). Kelani river below Ambathale is more polluted due to urban and industrial sources of pollution by threatening to aquatic life (Herath and Amaresekera, 2007).

2.2 Importance of river water quality monitoring

Good quality water in rivers is crucial for the sustainable socio-economic development of a country. Quality of water in a river depends on a number of physicochemical parameters and biological characteristics (Thirupathaiah *et al.*, 2012). Rivers which act as conduits of pollutants has their own ability to dilute the pollutants. But the partially treated or untreated wastewater which is discharged into water bodies can alter the physico-chemical and biological features of a water body. Therefore, vital need of assessing the trends in water quality has arisen in the present world (Bartram and Ballance, 1996). The discharge of uncontrolled wastewater could drain the assimilation capacities of the water bodies. The assimilative capacity determines the quantity of treated wastewater that can be discharged into the water bodies without harmful effects to the natural ecosystems while ensuring the compliance with the tolerance limits and standards for discharging treated wastewater. The basis of the assimilative capacity is the dilution available in the water body (Woodard, 2006). The ability of the water body to dilute the treated wastewater is based on several factors. Loads of organic and inorganic pollutants discharged, climatic and hydrologic factors, characteristics of the mixing zone where discharges and the receiving waters come together such as water depth, density,

water quality and flow rate (Carpenter *et al.*, 2011) In order to protect the water quality, it is important to consider about the assimilative capacity of a water body in determining the safe release limits for wastewater discharges (Brown and Rodgers, 2014). Permissible levels of wastewater that can be discharged into the rivers and surface water bodies are regulated by the governments of most countries in the world. In most developed countries they adopt a rigid strategy to overcome the consequences of river water pollution due to discharge of wastewater without any proper treatment. However, in developing countries like Nigeria, industrial waste water is discharged directly into the water bodies without any proper treatment causing number of adverse effects such as river pollution, loss of aquatic fauna, and uptake of polluted water by plants (Ado *et al.*, 2015). In Sri Lanka, the discharge of wastewater from industries is regulated by the Environmental Protection License (EPL) schemes implemented under the provisions of the National Environmental Act of 1980, section 23 (a). The physico chemical properties of the river water can be compared against the ambient water quality standards for National Environmental (Ambient water quality) Regulations No 1 of the 2019 Gazette notification No.2148/20 dated 05.11.2019 for category A (drinking water source for simple treatment) and category C (fish and aquatic life water).

However, these standards and permissible levels need to be coupled with a monitoring component. Monitoring of river water quality which is impacted by discharging industrial effluents would provide reliable data to ensure the compliance of permissible discharges from industries (Patil *et al.*, 2012). A monitoring should include testing of physicochemical and biological characteristics of water bodies. In most countries, industrial effluents and effluent receiving waters are assessed only by testing conventional physical and chemical characteristics. Testing of biological effects of industrial or any other wastewater is compulsory only for some countries (Gana *et al.*, 2008). Well-developed water quality monitoring programs involve the measurement of physical, chemical and biological parameters and provide valuable information on the impact on water quality. Biological monitoring, whereby living organisms are used to estimate the water quality or its chemical contents, is important in determining the health of an aquatic ecosystem. Physicochemical monitoring of a

water body is known to be insufficient to fully characterize its status or reliably detect adverse impacts (Carpenter *et al.*, 2011). However, it has been recognized as a vital component of an integrated assessment utilizing physicochemical and biological measure for assessing a waterway's condition. However, there are only a few studies using biotic indices in Asian countries such as Iran. The water quality monitoring programs in Iran are mainly based on the determination of some physicochemical parameters and water quality indices have generally not yet been use as a tool for the assessment and management of river ecosystems. Meanwhile, scientific efforts have often focused on improving freshwater resources that are of economic, cultural or spiritual importance. Unfortunately, most of these efforts have proceeded without documentation of the relative successes and failures of individual activities. Even though success is noted, there is often a lack of biotic data to identify specific results or endpoints for the river management activity (Aazami *et al.*, 2015). Physico-chemical parameters of surface water provide some details about water quality up to some extent. Therefore, testing of physico-chemical parameters of surface waters is also an important component in monitoring of water quality (Patil *et al.*, 2012). There are number of physico-chemical parameters that affect the quality of water and they act as indicators of water pollution (Kolawole *et al.*, 2011). Therefore, physico-chemical parameters of a water body are limiting factor of maintaining a healthy aquatic ecosystems. Temperature is a factor that controls the rate of chemical reactions occur in water and affect the wellbeing of the organisms live in water. Electrical conductivity of water depends on the presence of ions, total ion concentration and water temperature Dissolved Oxygen (DO), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) can be considered as important indicators of organic matter pollution in water bodies. DO is an important parameter which directly or indirectly correlates with bacterial activity, photosynthesis, availability of nutrients and stratification (Patil *et al.*, 2012). However Central Environmental Authority was published ambient water quality standards for all categories by National Environmental (Ambient Water Quality) Regulations, No 1 of the 2019 gazette No 2148/20 of 5th November.

The Categories referred to below-

Category A shall be water that requires simple treatment, for drinking

Category B shall be bathing and contact recreational water

Category C shall be water suitable for aquatic life

Category D shall be water source that require to undergo general treatment process, for drinking

Category E shall be water suitable for irrigation and agricultural activities

Category F shall be water with minimum quality but does not fall into categories A to E

Table 1: Ambient water quality standards in relation to the specified categories

	No	Parameter	Unit	Category					
				A	B	C	D	E	F
General	1	Colour	Pt mg/l, max	20	-	-	100	-	-
	2	Electrical Conductivity	µS/cm, max	-	-	-	-	700	-
	3	Turbidity	NTU, max	5	-	-	-	-	-
	4	TSS	mg/l, max	25	-	40	1500	2100	-
	5	Total Hardness (as CaCO ₃)	mg/l	250 des 600 max	-	-	-	-	-
	6	pH	-	6.0-8.5	6.0- 9.0	6.0- 8.5	6.0- 9.0	6.0- 8.5	5.5- 9.0
	7	DO at 25 ⁰ C	mg/l, min	6	5	5	4	3	3
	8	BOD ₅ at 20 ⁰ C	mg/l, max	3	4	4	5	12	15
	9	COD	mg/l, max	10	10	15	30	-	40
Nutrient	10	NO ₃ -N	mg/l, max	10	10	10	10	-	10
	11	NH ₃ -N pH<7.5	mg/l, max	-	-	0.94	-	-	9.1
		7.5≤pH<8.5		-	-	0.59	-	-	4.9
		8.5≤pH		-	-	0.22	-	-	1.6
	12	PO ₄ -P	mg/l, max	0.7	0.7	0.4	0.7	-	-
Micro Pollutant	35	Total Coliform	µg/l, max	1000	10000		1000 0	-	-
	36	Fecal Coliform	µg/l, max	500des 1000 Max	500des 1000 max	-	-	-	-

Source: (Central Environmental Authority, 2009)

2.3 Use of Biotic indices for assessment of stream water quality

It is needed to deploy significant effort to construct biological indices. However, consequent years of sampling is needed to gather sufficient data. Otherwise it is impossible to distinguish long term trends, which are impacted by anthropogenic causes and natural variations (Wallace *et al*, 1996). Indices are considered as a scale for showing the quality of an environment by indicating the types of organisms present in it. It is often used to assess the quality of water in rivers/ streams. Therefore many biotic indices will be used to assess the aquatic life health quality of the stream. A wide variety of biotic groups is used for biomonitoring. The biomonitoring is used to measure the response of aquatic communities to anthropogenic stressors like habitat quality, energy source, flow regimes, water quality and biotic interactions. A particular “indicator” organisms at a specific taxonomic level gets a numerical score through a well developed biotic index. The presence/absence, physiology, morphology, numbers (% abundance) or behavior of these specific indicator organisms can considerably predict the physico-chemical environments of a water body by defining the status of a given location. These indicator organisms have particular requirements regarding the chemical and physical conditions (Mandaville, 2002). When compared to physico chemical data, biological monitoring gives comparatively affordable means of environmental measurement for assess degradation of aquatic environment and loss of biological diversity due to anthropogenic disturbances. Biological indices, which integrate concepts such as integrity and biological diversity, offer significant measures of health of the ecosystem (Wallace *et al*, 1996).

In 1987, Hilsenhoff proposed Biotic Index for evaluate the water quality. But it is required to identify organisms into genus or species level. Then Hilsenhoff (1988) repropose a family-level biotic index (FBI). It provides a rapid evaluation of streams. Usually Biotic indices are specific to unique geographical area and they have to be adapted to ecosystem (Kazancı *et al.*, 2013).

2.3.1 Use of macroinvertebrates as bioindicators

Usually benthic macroinvertebrates are considered as sensitive organisms to the biotic and abiotic factors of their environment. Therefore, macroinvertebrate community commonly use as an indicator organisms of aquatic ecosystems (Mandaville, 2002). Aquatic insects, account for majority of macroinvertebrates and also an important component of aquatic ecosystems. They are very abundant and diverse group that inhabits a variety of aquatic environments. They play an important role in ecosystem functioning (Barman and Gupta, 2015). Also, they are one of the important components for food web in freshwater ecosystems. In the food web of lake ecosystems, aquatic insects are the main prey of nekton and have role as decomposer of organic matter. Therefore, aquatic insects can be used as a bioindicator of ecosystem stability and water quality. Meanwhile, biotic factors such as macro invertebrates by using aquatic insects as biotic index for water quality assessments can represent the prolonged period condition of aquatic ecosystems due to their prolonged exposure in aquatic ecosystem (Priawandiputra *et al.*, 2016). The concept of biological indicators using macroinvertebrates including aquatic insects is based on their diversity, abundance, and distribution in relation to the physical and chemical conditions of the habitats. Studies of freshwater aquatic insects as biological monitoring techniques have been widely reported and described. Many studies have done on the effects of anthropogenic activities, such as agriculture and recreational activities, silviculture, aquaculture, runoff from land clearing and urban development, river impoundment, organic and metal contamination by using freshwater aquatic insects (Ahmd *et al.*, 2011). The Biotic Index is one of the biotic indices which was originally developed to obtain a single ‘tolerance value’. This value can be considered as the average value of the tolerance values of all species who represents benthic arthropod community. This Biotic Index was modified with tolerance values which range from 0 to 10 (very intolerant – highly tolerant). This represents the family-level of macroinvertebrates and shows the sensitivity to organic pollution by constructing the Family Biotic Index (FBI) (Mandaville, 2002). Tolerance values are developed for each family by weighting species (Table 2) and this index is modified to include non-arthropod species.

Table 2: Evaluation of water quality using the family level biotic index (Hilsenhoff, 1998)

Family Biotic Index	Water Quality	Degree of Organic Pollution
0.00-3.75	Excellent	Organic pollution unlikely
3.76-4.25	Very Good	Possible slight organic pollution
4.26-5.00	Good	Some organic pollution probable
5.01-5.75	Fair	Fairly substantial Pollution likely
5.76-6.50	Fairly Poor	Substantial Pollution likely
6.56-7.25	Poor	Very substantial Pollution likely
7.26-10.00	Very Poor	Severe organic pollution likely

Table 3: Tolerance values for Macroinvertebrates for application in Family Biotic Index (Mandeville, 2002)

Plecoptera	Capniidae 1, Chloroperlidae 1, Leuctridae 0, Nemouridae 2, Perlidae 1, Perlodidae 2, Pteronarcyidae 0, Taeniopterygidae 2
Ephemeroptera	Baetidae 4, Baetiscidae 3, Caenidae 7, Ephemerellidae 1, Ephemeridae 4, Heptageniidae 4, Leptophlebiidae 2, Metretopodidae 2, Oligoneuriidae 2, Polymiatarcyidae 2, Potomanthidae 4, Siphonuridae 7, Tricorythidae 4,
Trichoptera	Brachycentridae 1, Calamoceratidae 3, Glossosomatidae 0, Helicopsychidae 3, Hydropsychidae 4, Hydroptilidae 4, Lepidostomatidae 1, Leptoceridae 4, Limnephilidae 4, Molannidae 6, Odontoceridae 0, Phipotamidae 3, Phryganeidae 4, Polycentropodidae 6, Psychomyiidae 2, Rhyacophilidae 0, Sericostomatidae 3, Uenoidae 3
Amphipoda	Gammaridae 4, Haylellidae 8, Talitridae 8
Isopoda	Asellidae 8
Decapoda	6
Acariformes	4
Mollusca	Lymnaeidae 6, Physidae 8, Sphaeriidae 8
Odonata	Aseshnidae 3, Calopterygidae 5, Coenagrionidae 9, Crodulengastridae 3, Corduliidae 5, Gomphidae 1, Lestidae 9, Libellulidae 9, Macromiidae 3
Megaloptera	Corydalidae 0, Sialidae 4
Lepidoptera	Pyralidae 5
Neuroptera	Sisridae 4, <i>Climacia</i> sp. 5
Dipter	Athericidae 2, Blepharariceridae 0, Ceratopogonidae 6, Blood-red Chironomidae (Chironomini) 8, Other Chironomidae (Including pink) 6, Dolichopodidae 4, Empididae 6, Ephydriidae 6, Muscidae 6, Psychodidae 10, Simuliidae 6, Syrphidae 10, Tabanidae 6, Tipulidae 3
Coleoptera	Dryopidae 5, Elmidae 4, Psephenidae 4
Collembola	<i>Istomurs</i> sp. 5
Oligochaeta	
Hirudinea	Bdellidae 10, <i>Helobdella</i> 10
Polychaeta	Sabellidae 6
Turbellaria	Platyhelminthidae 4
Coelenterata	<i>Hydra</i> sp. 5

Species including taxonomic orders of Odonata, Tricoptera, Plecoptera, Ephemeroptera and Odonata were found as highly abundant species in Dediya gála stream (Madhushanka *et al.*, 2014). During a study conducted in Badulu Oya catchment, a total of 38 benthic macroinvertebrate families were recorded. Among the macroinvertebrate indices which are calculated, catchment disturbance directly affects the total abundance and family richness. Total abundance of Macroinvertebrates and richness were decreased when catchment disturbances were decreasing (Gunawardhana *et al.*, 2018).

2.4 Microplastic pollution in aquatic ecosystems

Microplastics (MPs) are known as new type of pollutants, including very small (5-mm long) pieces of plastic (Krystian and Monika, 2020). Microplastics are plastic particles smaller than 5.0 mm in size. However the lower bound (size) of the microplastic is not defined. Microplastic debris in both marine and freshwater systems become an emerging issue over the past decade. It is important to understand the impacts of microplastics on aquatic wildlife, because the impacts on them still remain poorly understood. Municipal waste contains 10% plastic materials globally. Some plastics can be recycled, but most finally end up in land fill, breakdown and decompose. Also huge amount of plastic debris come to the ocean through several sources (Cole *et al.*, 2011). In 1970s, microplastics were first noted in North America as spherules in plankton tows along the coast of New England (Masura *et al.*, 2015). There are many examples for sources of microplastics in aquatic habitats such as plastic microbeads commonly used in personal care products, plastic fibers in laundry water of synthetic clothes. Microplastics accumulate waterborne contaminants such as heavy metals and Persistent, Bioaccumulative and Toxic (PBT) compounds due to its large surface to volume ratio and chemical composition (Sruthy and Ramasamy, 2016). Microplastics are accumulated in the environment as a result of anthropogenic activities both on land and in water bodies.

Microplastics are formed in two ways and enter to a water body which are named as primary and secondary microplastics. Primary microplastics from terrestrial areas are mainly derived from cleaning and cosmetic products, and secondary

microplastics including fibres or fragments come from larger plastic products (Szymanska and Obolewski, 2020). Primary microplastics contain manufactured raw plastic material, such as virgin plastic pellets, scrubbers, and microbeads. These plastics manufactured in microscopic size for various industrial or household applications. These plastics widely used in various purposes including cosmetics, air blasting media and medicine as vectors for drugs (Kershaw and Rochman, 2015). There were reported as polyethylene and polypropylene granules (less than 5 mm) and polystyrene spheres (less than 2 mm) which marketed as “micro- beads” or “micro-exfoliates” presence in the cosmetics. Composition, size, and shape of these microbeads can vary according to the product. After consumption, these products end up in the environment through wastewater and drains (Auta *et al.*, 2017). Finally these primary microplastics enter the ocean via runoff from land. Secondary microplastics occur when larger plastic items which are meso-and macro-plastics enter to beach or ocean and then undergo mechanical, photo (oxidative) and/ or biological degradation. This secondary microplastics breaks larger pieces into smaller plastic fragments gradually by biological, physical and chemical processes such as physical abrasion, weathering and ultraviolet radiation which can decrease the structural integrity of plastics. Ultimately it becomes to a undetectable level to the naked eye (Masura *et al.*, 2015). Several Environmental factors such as temperature and sunlight degrade the macroplastic debris in soil and water. This process varies with size and density like properties of the plastic polymer (Auta *et al.*, 2017). However microplastic in marine environment mainly origin from secondary sources by break down the larger plastics (Ogunola *et al.*, 2018).

2.4.3 Microplastic pollution in water

The constant increase of their concentration has recently become a foremost environmental issue. Global plastic production of recent times estimates ~8 million metric tons of mismanaged plastic waste entering the ocean each year (Hardesty *et al.*, 2017). Also it is a threat and potential toxicity in aquatic habitats, threatening biodiversity and human health because of their persistence, ubiquitous occurrence in the eco system. Scale of risks associated by the accumulation of microplastics in aquatic environments and the mechanisms underlying microplastics migration is poorly studied for decade. Although some studies on potential damage to the world's oceans has recently increased but the impacts of MP on freshwater ecosystems have received less consideration. However, the occurrence of plastics (including microplastics) in freshwater ecosystems such as lakes, rivers and river mouths acting as a plastic sink from multiple sources causing plastic pollution of marine environment (Krystian and Monika, 2020).

2.4.4 Impacts of microplastics pollution in water

It is recorded the severity of plastic pollution in many parts of the world such as North Central Pacific Gyres, North Pacific Subtropical Gyres and the Mediterranean Sea. Several fishes of these areas are affected by plastic pollution. In global studies which target fishes, 68% of the selected in the sea fish samples (highest percentage of plastic pollution) and freshwater fishes *L. macrochirus* and *L. megalotis* (45% of the selected samples) are affected by plastic pollution. The highest abundance of plastic reached 3.75 items/individual in Bogue (Boops boops). According to most field studies fibers are the dominant composition pattern recorded (Huahon *et al.*, 2016). A recent review on microplastics, (Eerkes-Medrano *et al.*, 2015) five studies were about freshwater sediments out of listed twelve studies on the occurrence of MPs in freshwater systems. In addition to these, few recent studies reveal microplastics contamination in estuaries: Naidoo *et al.*, (2015) reported the existence of microplasticss in five estuaries in South Africa. Also Zhao *et al.*, (2015) studied and reported microplastics contamination in three urban estuaries in China. Fok and Cheung (2015) have reported Pearl River as a potential source of micoplastics

contaminating the estuary in Hong Kong. Three Gorges reservoir Contamination, occurrence, and distribution of microplastics in Three Gorges reservoir in China has been reported by Zhang *et al.*, (2015). Most of these studies reveal the mismanagement of solid and liquid waste as prime cause of microplastics in water bodies. When considering the India, there are two major reports on microplastic contamination. But those particles movement, transportation and accumulation in the environment is poorly understood. Also microplastic particles get immovable when traveling through porous materials such as soil and sediment. But later break free and often continue to move substantially further (Bizmark *et al.*, 2020).

Impacts of microplastics on wildlife are not well understood. However, it was recorded that considerable numbers of organisms, both vertebrates and invertebrates have been found to ingest microplastics. These examples represent numerous organisms with differing feeding mechanisms, including detritivores, deposit feeders, and filter feeders. Examples include scleractinian corals, mussels (*Mytilus edulis*), as well as lugworms, amphipods, and barnacles (Thompson *et al.*, 2004). Microplastics can be considered as the bioavailable substance in marine food web. There is a potential to ingest low and high densities of microplastics when it floats in the surface water and availability of wide range of organisms may ingest passively or actively (Do Sul and Costa, 2014). Thus, major ecological risk of microplastic is ingestion by marine biota including filter, suspension and detritus feeders, invertebrates, fish, turtles, marine mammals, and marine birds (Zhang, 2017). Scientists are also concerned that organisms ingesting plastic debris may be exposed to contaminants absorbed to the plastic. Plastic debris acts as a sink and a source for chemical contaminants. Additives used in the manufacturing of plastics can leach into the marine environment. On the other hand, hydrophobic contaminants present in the water may absorb to the plastic particles. Thus, microplastics may provide a mechanism to transport concentrated contaminants to organisms (Browne *et al.*, 2007).

It is important to determine microplastic levels in freshwater and marine water in Sri Lanka. Because Sri Lanka is an island surrounded by the ocean and enriched with freshwater ecosystems which are highly threatened by plastic pollution due to anthropogenic activities. According to a study conducted in surface water, off

Colombo west Coast of Sri Lanka revealed that density of microplastics was 140.16 items per m³ (Athawuda, 2018). Also microplastic density was recorded as 0-29 items per m³ in Southern waters in Sri Lanka (Koongolla *et al.*, 2018). Past literature on microplastic contamination in Sri Lanka freshwater is very low. Appropriate numbers of studies were not conducted in freshwater as marine water in Sri Lanka. Also there are challenges in researches regarding microplastic pollution in water. Major challenge is the need of method harmonization and general definitions. It will help to compare results of studies done in different sites by different sampling methods, sample processing, and identification procedures all over the world.

3. MATERIALS AND METHODS

2.1 Study area

2.1.1 Study area and land use pattern

Ella is a quaint little town 200 kilometers east of Colombo and at about 1,000 meters above sea level.

Beeralla stream (tributary of Kirindioya) flows through Ella town which initiates from Ella rock and it connects to the Rawana falls at the downstream. This Beeralla stream flows parallel to the main road line (Ella to Wallawaya) and there are all sorts of accommodations and restaurants laid in that line of the main road. Study area is surrounded by urban landscapes, degraded natural habitats, and backyard of small scale and medium scale hotels, restaurant etc. This region of the stream receives urban runoff from common drains and wastewater from adjacent hotels. Therefore, this area is highly urbanized and threatened by anthropogenic activities. 05 sampling sites were selected for the study at Ella GN area after preliminary observations to cover the area close to different land use patterns.

Sampling Site 01 – This site is located at upper stream and used it as the reference site. It is surrounded by the natural vegetation. Also upper region for this sampling site belongs to the department of forest. No hotels and resorts located near to this site. Only 3 houses located around 300 meters away from the sampling location.

Sample site 02 – This site is located downstream to the first site and surrounded by cultivated lands. Vegetables were cultivated adjacent to this sampling point. Vilagers take water from this stream for cultivation purpose from this site. Also bathing place is located near to this site. Hotels/restaurants were located around 500 meters away from the location.

Sampling site 03 – This site of the stream is located at Ella town area. This site is fully surrounded by hotels/restaurants. Wastewater outlets from hotels/restaurants are directly open for the stream near to this location.

Sampling Site 04 – This site is located at Ella junction and closer to the bridge. Here also hotels located adjacent to the stream bank. Also drain comes from upper Ella area is directly open to the stream near this site area.

Sampling Site 05 – This site was located at lower stream and lower to the Ella junction. Some vegetation cover surrounds this sampling site. Hotels can be seen around 500 m closer to this sampling site.

2.1.2 Topography of the area

The elevation of the study area is 1041 m above the sea level. Temperature typically varies from 58°F to 82°F throughout the year. Usually wet season lasts from April to December and dry season lasts from December to April. Receives more rainfall during inter monsoonal period (September to mid November and March to mid April). The average annual rainfall in Ella is about 1652 mm (Sirisena *et al.*, 2016).

2.1.3 Land use pattern of the area

Majority of the study area included with hotels and restaurants. Also residential places are engaged with these hotels. In addition to those hotels vegetable cultivation can be seen in the study area. A visual survey was done in the surrounding area from the boundary of the study area and land use patterns were recorded. These were confirmed by Google Map (Appendix E).

Table 4: Coordinates of the sampling sites

Location No	Sampling Location	GPS Coordination
1	Origin of Kirindi Oya Tributary at Ambagolla, Ella	760128.74 N 505801.31 E
2	Origin of Kirindi Oya Tributary nearby Somasiri Restaurant/Shop at Ella	760045.00 N 505558.00 E
3	Origin of Kirindi Oya Tributary nearby 3600 Restaurant at Ella	759883.00 N 505372.00 E
4	Origin of Kirindi Oya Tributary nearby Ella Gap Hotel at Ella	759637.00 N 505462.00 E
5	Origin of Kirindi Oya Tributary nearby Silent Night Home Stay at Ella	758976.00 N 505752.00 E

Sampling Locations

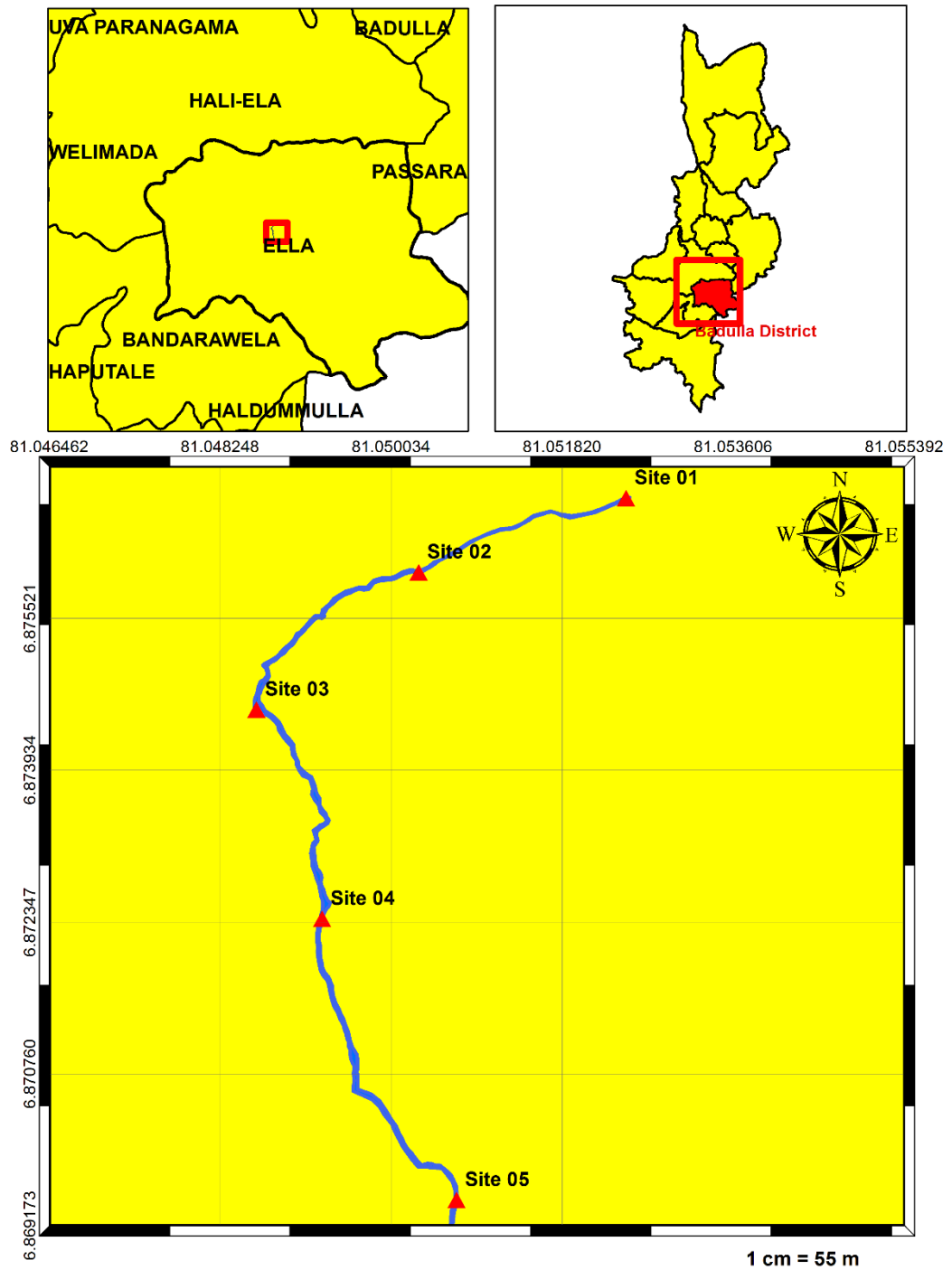


Figure 1: Map of the studied area of the Beeralla stream showing the sampling sites

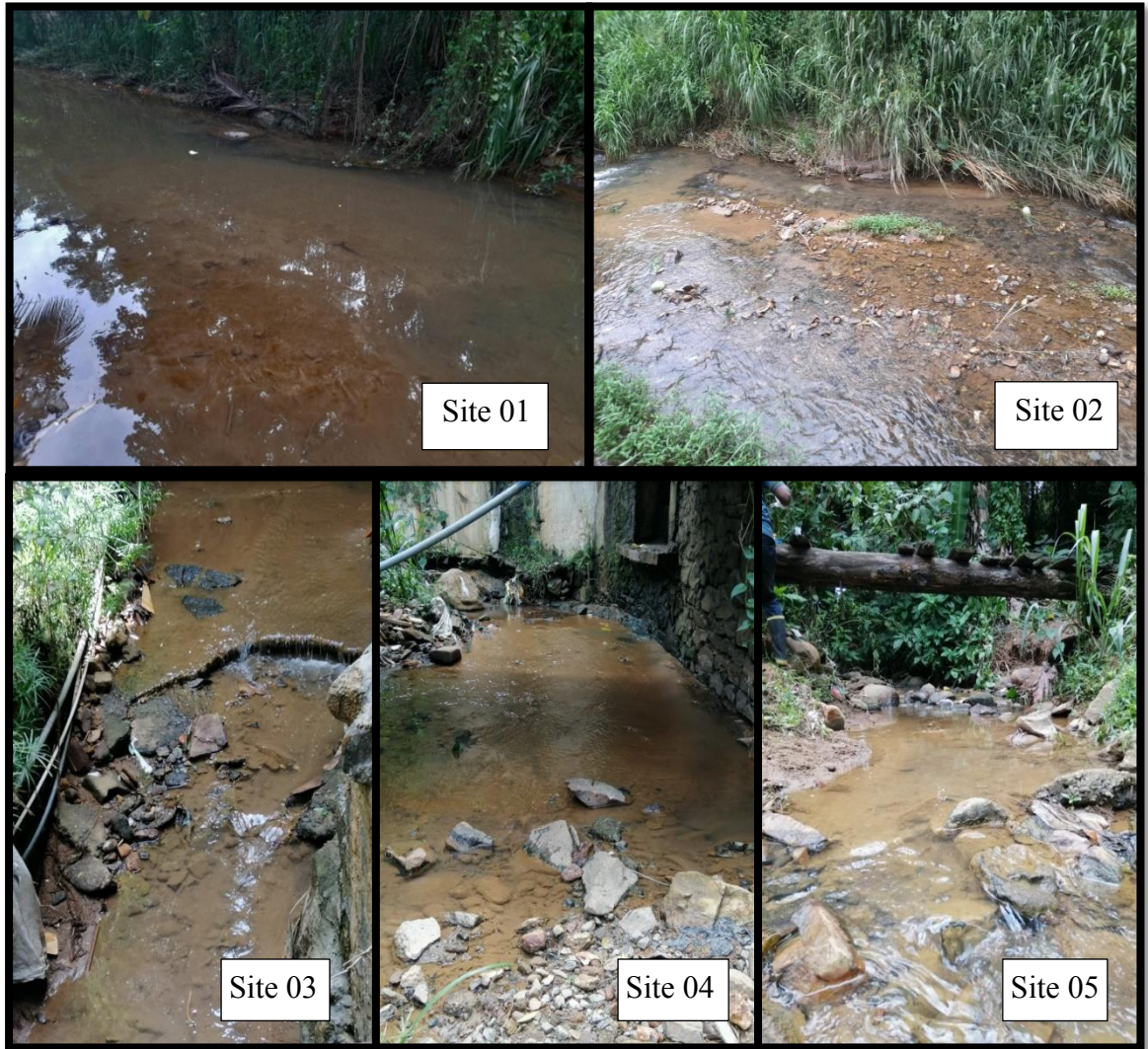


Figure 2: Photographs of sampling sites in Beeralla stream

2.2 Field sampling and in-situ analysis

2.2.1 Physico-chemical parameters

Field sampling was carried out in January, March, July, September, and November 2020 to cover the intermonsoonal and monsoonal periods. Samples were collected between 0930h and 1330h. The pH, Conductivity, Temperature, Total Suspended Solids (TSS), Turbidity, Dissolved Oxygen (DO), Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Nitrate and Dissolved Phosphate at each site were measured in triplicates. Coordinates of the selected sampling sites were recorded using a handheld GPS (Etrex/Model: Garmin summit). Water samples were collected from the same site on every subsequent day of sampling. Samplings were carried out in every three months period.

The pH was measured using a portable pH meter (WTW model No:315i/SET) by dipping the probe in to the water and keeping it until the reading was stabilized. Temperature, Turbidity and conductivity were measured using a pre-calibrated multimeter (YSI/Model: 556 MPS) by dipping the probe in to the water and keeping it until the reading was stabilized.

For the determination of five-day Biochemical Oxygen Demand (BOD₅), water samples were collected from each sampling site in triplicate in 250 ml glass stoppered dark bottles without trapping air bubbles. These bottles brought to the laboratory of the Central Environmental Authority, Uva Province for further analysis and incubated in 20 °C for five days as described by APHA (2017).

To measure the Nitrate and Total phosphorus contents, samples were collected in triplicate in 125 ml glass stoppered bottles as described by APHA (2017).

As described by APHA (2017), for Nitrate content analysis, in-situ filtration of each sample through a 0.45µm pore diameter membrane filter was carried out. As described by APHA (2017), concentrated sulfuric acid (0.25 mL) was added to each sample. Samples were brought to the laboratory of the Central Environmental Authority, Uva Province in ice at 4 °C and were stored in a refrigerator at 4°C for later analysis as described by APHA (2017).

2.2.2 Microplastic levels of surface water

Surface water samples were collected for 1 Litre glass bottles and collection was conducted during March 2020 to December 2020. Samples were transferred to the laboratory of the Aquaculture laboratory of Uva Wellassa University, Badulla and were stored in a refrigerator at 4°C for later analysis.

2.2.3 Aquatic macroinvertebrates

Aquatic macroinvertebrate samples were collected in triplicates at each sampling site by an aquatic D net (D-pond net) at the time of water sampling collection. Samples were placed in white trays for sorting and screening. The content of each sample (net) was transferred into properly labelled plastic containers, preserved in 80% ethanol and taken back to the laboratory for analysis.

2.2.4 Dragonflies

Count of dragonflies were recorded at the time of sampling of aquatic insects. Exact point of each location was selected. Dragonflies were visually observed in 2 meters radius from selected point and counted the number of dragonflies within the circle and identified using taxonomic key by Gehan de Silva Wijeyeratne (2015).

2.3 Laboratory Analysis

2.3.1 Determination of Biochemical Oxygen Demand (BOD₅)

5-day BOD test was carried out as described in APHA (2017). 250 mL dark bottles were filled with water completely without allowing the air to be trapped inside. Sample bottles were filled to determine the initial dissolved oxygen concentrations were fixed immediately using Manganese sulphate solution (1mL) and alkali-iodide

azide reagent (1mL) and shaken vigorously (Appendix 1). The bottles were kept stand and allowed the precipitate to be settled down.

Sample bottles were subjected to necessary dilutions with the oxygen saturated dechlorinated water for the samples. Diluted samples in the dark bottles (250 mL) were incubated in the dark for five days at 20°C. Another two dark bottles (250 mL) were filled with dilution water to determine initial DO and DO after 5 days as similar to the water samples.

The precipitate in the initial DO (DO_1) bottles were dissolved using concentrated sulfuric acid (1mL) at the time of processing. Portion (100 mL) of the sample was pipetted out and titrated immediately with Sodium thiosulphate titrant (0.025M) using starch as indicator. Few drops (0.20 mL) of starch were added into the titrand when the solution becomes pale yellow colour while titrating with sodium thiosulphate. Appeared dark blue solution was then titrated again until it turns into colourless. The burette reading was taken at this end point. Same procedure was carried out for the samples which were collected to determine the DO after 5 days and for the diluted samples. BOD was calculated using the following formula.

$$\text{BOD in water sample} = \frac{1000}{V} [(S_m - S_t) - (D_m - D_t)] \text{mg/L}$$

Where; V=Volume of the sample in 1L of the mixture,

S_m = DO of the mixture before incubation,

S_t = DO of the mixture after 5 days incubation,

D_m = DO of the dilution water before incubation,

D_t = DO of the dilution water after 5 days incubation

2.3.2 Determination of Nitrate concentration

Nitrate content was determined using the Ultraviolet Spectrophotometric Screening Method (4500-NO₃- B) as described by APHA (2017).

50 ml of the water samples fixed in-situ were taken and 1 ml of 1M HCl was added to each water sample. It was mixed thoroughly and the absorbance was read using 200-1000 nm UV-Visible Spectrophotometer (CECIL/Model: CE 1021) at 220nm and 275nm against distilled water set at zero absorbance. Sample absorbance due to Nitrate was calculated using the following equation as described by APHA (2017).

$$\text{Sample absorbance due to Nitrate} = E_{220} - (2 \times E_{275})$$

Where,

E_{220} = Absorbance of the filtrate at 220 nm

E_{275} = Absorbance of the filtrate at 275 nm

A standard curve was constructed by plotting absorbance due to NO₃⁻ against NO₃⁻ concentrations of the standards. Standards were prepared in the range of 0 to 1.4 mg/L using stock nitrate solution (Appendix 2). Standards were also treated in the same manner as samples. The nitrate concentration for each sample was then obtained using the standard curve.

2.3.3 Determination of Total Phosphorus concentration

Total Phosphorus concentration (TP) were measured using the Persulfate Digestion Method (4500- P B.5) followed by the Ascorbic Acid Method (4500-P E) as described by APHA (2017).

50 mL from each sample fixed in-situ was pipetted out to an Erlenmeyer flask after thoroughly mixing and Sulfuric acid (1 mL, Appendix 3) and solid Potassium persulfate (0.5 g) were added. Then the solution was boiled gently on a pre-heated hotplate until the final volume of 10 mL was reached. The sample was cooled to the room temperature, diluted to 30 mL with distilled water and Phenolphthalein

indicator (0.05 mL) was added. Then each sample was neutralized by adding Sodium hydroxide (1N) and the final volume was then made up to 100 mL with distilled water.

50 mL from each neutralized sample were pipetted out into a clean dry Erlenmeyer flask and Phenolphthalein indicator (0.05 mL) was added. Then Sulfuric acid (5N, Appendix 3) was added drop wise to until it gets a faint pink colour and then 8mL of the combined reagent (Appendix 3) were added. Sample was mixed thoroughly and kept for 10 minutes until the blue colour developed. The absorbance was measured using the 325-1000 nm visible Spectrophotometer (CECIL/Model: CE 1011) at 880 nm using the reagent blank as the reference solution.

Standards were prepared in the range of 0.01 to 0.13 mg/L by diluting the standard phosphate solution which was prepared using stock phosphate solution (Appendix 3). Standards were treated in the same manner as the samples. A standard curve was constructed by plotting absorbance against phosphate concentrations of the standards. The total dissolved phosphorous and total phosphorus concentrations were determined for each sample using the standard curves (APHA, 2017).

2.3.4 Determination of Chemical Oxygen Demand(COD)

Open reflux method (5220 B) was followed to determine the COD in water as described in APHA (2017) with some modifications. Aliquot of the well shaken sample (20.0 mL) was pipetted out to a 250mL refluxing flask. Mercuric sulfate (0.4g), several glass beads and standard potassium dichromate solution (0.0416M, 10.0 mL) were added (Appendix 4). Sulphuric acid reagent (30 mL) was added very slowly while mixing thoroughly by swirling (Appendix 4). The refluxing flask was attached to the condenser and the mixture was refluxed for 2 hours while the open end of the condenser was opened. Mixture was allowed to cool after 2 hours and reflux condenser was disconnected. Mixture was diluted to about 150 mL with distilled water and allowed to cool to the room temperature and titrated with dilute standard ferrous ammonium sulphate (FAS) (Appendix 4) using ferroin indicator (0.15 mL). Volume of FAS was recorded at the point of first sharp colour change

(blue green to reddish brown). A blank consisted of distilled water was treated as same as the samples. Molarity of the dilute standard FAS was determined as follows.

Dilute potassium dichromate solution (10.0 mL) was again diluted to 100 mL. Concentrated Sulphuric (30 mL) was added slowly and allowed to cool. This solution was titrated with dilute FAS using ferroin indicator (0.15 mL).

$$\text{Molarity of Dilute FAS solution} = \frac{\text{Volume of 0.04167 M K}_2\text{Cr}_2\text{O}_7}{\text{Volume of FAS used in titration} \times 0.250}$$

COD of the water samples was determined using the following formula,

$$\text{COD as mg O}_2\text{/L} = \frac{(A - B) \times M \times 8000}{\text{mL of the sample}}$$

Where; A = mL of ferrous ammonium sulphate used for blank

B = mL of ferrous ammonium sulphate used for sample

M = Molarity of dilute ferrous ammonium sulphate

8000 = milliequivalent weight of oxygen $\times$ 1000 mL/L

2.3.5 Determination of Total Suspended Solids (TSS)

100mL of sample was well mixed and filtered through a pre weighed standard glass-fiber filter (Gf/C Circle diameter 70mm). Washed the filter with at least three successive volumes of distilled water. Suction filtration method was allowed for this process. Suction was continued until all traces of water are removed. Filter was carefully removed using forceps from filtration apparatus and transferred to a weighing dish. Then filter was oven dried for 1 h in a 103-105 °C. Finally it was cooled in a desiccator to ambient temperature and weighed.

TSS of the water samples was determined using the following formula,

$$\text{TSS mg /L} = \frac{(A - B) \times 1000}{\text{Sample volume, mL}}$$

Where,

A = final weight of dried residue + dish, mg

B = Weight of dish, mg

2.3.6 Determination of microplastic levels of surface water

Determination of microplastic levels of surface water was followed as summarized in the flow chart .

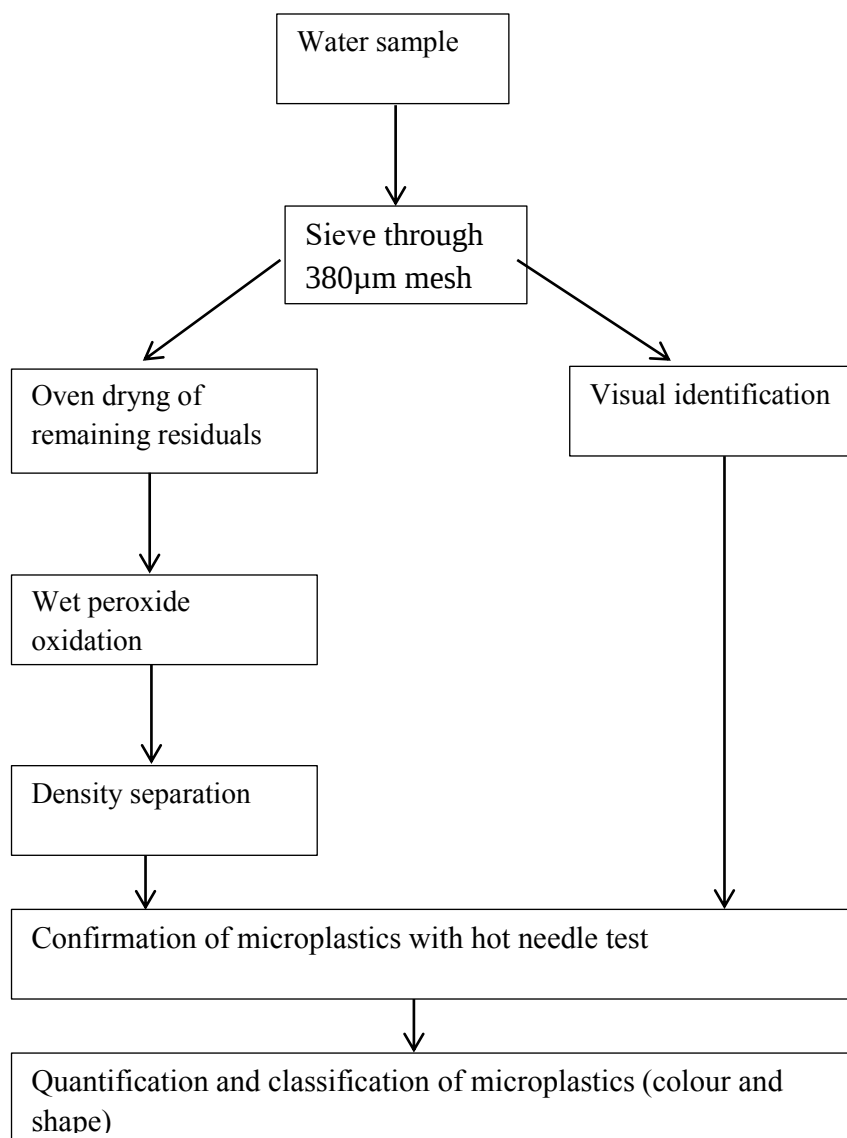


Figure:3 Flow diagram of the analysis of microplastics in water samples

2.3.6.1 Wet sieving

Sample was poured through a stacked arrangement 380 μm stainless steel mesh sieve. It was rinsed with squirt bottle filled with distilled water to transfer all residual solids to the sieve. Repeated the procedure again. Sieve was rinsed thoroughly using distilled water.

2.3.6.2 Transferring sieved solids

Clean, dry beaker (500 ml) was weighed to the nearest 0.1mg. All solids collected in the sieve were transferred to a tared beaker with minimal rinsing by using a distilled water squirt bottle. Beaker was placed in the drying oven at 90°C for 24 hours. Then the mass of total solid residuals were measured.

2.3.6.3 Wet Peroxide Oxidation (WPO)

Sample residuals were subjected to wet peroxide oxidation in order to digest the organic matter in the sample. Initially, Iron (Fe (II)) (0.05 M) (20.00ml) solution was added to the beaker which is containing residual solids and then 20.00 ml of 30% Hydrogen Peroxide solution was added. It was kept at room temperature for five minutes prior proceeding next step. Next stir bar was added to the beaker and covered with a watch glass. Then sample was heated upto 75 °C while stirring it. As soon as gas bubbles were observed at the surface, beaker was removed from the hotplate and placed it until boiling subsides. If reaction appears, distilled water was added to prevent overflowing the beaker. Then, it was reheated to 75 °C for an additional 30 minutes. Procedure was repeated until natural organic matter was invisible by adding 20.00 ml of 30% Hydrogen Peroxide. Then 3.5 g of Sodium chloride per 20 ml of sample was added to increase the density of the aqueous solution. Finally, mixture was heated 75 °C while stirring until the salt dissolves.

2.3.6.4 Density separation

When WPO solution was cooled, it was transferred to density separator by rinse with distilled water by ensuring all remaining solids were transferred to the beaker. Next it was allowed to settle overnight. Settled solids on the bottom layer were discarded, and upper supernatant layer was drained to the Whatman filter paper. Then density separator was rinsed with distilled water and drained into filter paper. Filter paper was placed in the Petri dish and allowed to air dry for 24 hours.

2.3.6.5 Microscopic examination for identification of microplastics

All the filters were examined visually using trinocular microscope (10×10 magnification) with 5 megapixels camera (model: B-500TPL, OPTIKA Microscopes, Italy). Particles present in the filter paper were sorted to identify microplastics visually. Each filter paper was read left to right, then move down and read from right to left. Finally particles were photographed.

2.3.6.6 Verification of microplastics

Microplastics were identified according to the Hidalgo-Ruz, 2012.

- Microplastics are not considered as a cellular/ organic structure, but organic material can be visible due to biofouling
- Fibers appear as equal thickness through the whole length
- Particles remaining as clear and consistent(homogenous) color through the variation
- If particles indicate transparent or white, must be detected under high magnification and a fluorescence microscope
- Usually biological particles may not break, salt crystals break apart and sand particles break with a sound like a breaking glass

Hot needle test was done for identified plastics to confirm whether those debris are plastic. According to the test, plastic particles melt or curl when hot needle is presence closer to particles (De Witte *et al.*, 2014).

2.3.6.7 Quantification and classification of plastics

Shape and colour of each plastic particle were recorded. Length of particles was measured by using image processing software (OptikaIsviiew software). Densities of the plastics were expressed in terms of numbers of items per liter. The mean density (Number of Plastic pieces /Filtered water volume) was calculated according to the locations and months. In terms of shape of the microplastics, plastic debris were classified as filaments, fragments and rod shape. Moreover, microplasticss were classified under 08 main colour categories (Blue, white, red, green, yellow, transparent, black, purple).

2.3.7 Diversity and Abundance aquatic macroinvertebrates

Diversity and the abundance of the macroinvertebrates were determined by the visual observing. In the laboratory, macroinvertebrates (including aquatic insects) were sorted and identified to the Family level using taxonomic key by C H Fernando (1990).

2.4 Calculation of Family Biotic Index (FBI)

Family Biotic Index for each site and each month was calculated using following equation described by Plafkin *et al.* (1989)

$$\text{Family Biotic Index} = FBI = \frac{\sum x_i t_i}{n}$$

where,

x_i = number of individuals within a taxon

t_i = tolerance value of a taxon n = total number of organisms in the sample

2.5 Calculation of Shannon-Wiener diversity index

Species richness, Shannon-Wiener diversity Index for each site and each month were calculated using following equations described by Heip *et al.* (1998).

$$\text{Shannon-Weiner Diversity index (H)} = - \sum_{i=1}^S P_i \ln P_i$$

where,

P_i = proportion of individuals of one particular species found divided by the total number of individuals found

2.6 Statistical Analysis

The pH, temperature, Dissolved Oxygen, TSS, Turbidity, conductivity, nitrate concentration, total phosphorus, COD, BOD₅ and microplastic density of each sampling site in each month also were subjected to one way ANOVA and two way ANOVA using the IBM SPSS statistic (version 23) software package and Microsoft office excel (version 2013) software after confining their conformity with normal distribution using the Anderson Darling test in order to test the significant variance at 0.05 significant level. When the null hypothesis in the ANOVA was rejected, Tukey's pairwise comparisons were carried out to multiple comparisons between sampling locations.

3. RESULTS AND DISCUSSION

3.1 Physico-chemical parameters of study sites

The pH values of five sampling sites varied in the range of 7.03-7.73 (Figure 4). Site 3, located in Ella town area showed the lowest mean pH value in March and Site 1 in upperstream area showed the highest mean pH value in December. Mean pH value of site 1 was significantly different from that of site 2, site 3, site 4 and 5 ($p < 0.05$) (Table 4). Also mean pH value of site 3 was significantly different from that of site 2 and site 4 ($p < 0.05$) (Table 5). Mean pH values of each sampling site were complied with the ambient water quality standards for all categories which are published by the Central Environmental Authority for National Environmental (Ambient Water Quality) Regulations, No 1 of the 2019 gazette No 2148/20 of 5th November. Temperature value varied from 18.57 °C in the site 1 in January to 23.1 °C in the site 5 in July (Figure 5). Mean temperature value of site 1 was significantly lower than that of other sites ($p < 0.05$) (Table 5). Temperature can directly affect the amount of oxygen dissolved in water and inversely related (Uttah *et al.*, 2008). Also site 1 is located at upperstream area and this site was covered with large trees. Probability of fallout sunlight directly to the stream is very low. This may be the reason for showing low temperature value at this site than that of other sites. Mean pH values and temperature values do not show a significant interaction between sampling sites and occasions ($p > 0.05$).

Total Suspended Solids (TSS) showed broad range of variations (Figure 6). Lowest TSS mean value of 0.17 mg/L was recorded in the site 1 in March and the highest value of 306.34 mg/L was recorded in site 4 in January (Figure 6). Mean TSS value of site 4 was significantly higher than that of other sites ($p < 0.05$) (Table 5). Mean TSS level of sampling site 1 is only site which comply with the ambient water quality standards. Other four sites does not comply with the ambient water quality standards for category A (water that requires simple treatment, for drinking) and category C (water suitable for aquatic life). According to the research done in Kirindi Oya, TSS levels of water was around 9.3 mg/L (Amarathunga *et al.*, 2006). This value shows huge variation with the present study values. High level of TSS in

present study may be due to soil erosion of stream bank. Also mean TSS level shows a significant interaction between sampling sites and occasions ($p < 0.05$).

Dissolved Oxygen (DO) values of the five sampling sites varied in the range of 3.97 mg/L – 7.20 mg/L (Figure 10). Lowest DO value was recorded in site 4 in September while highest value was recorded in site 1 in January (Figure 7). The mean DO contents in site 1 was significantly higher than that of other sites ($p < 0.05$) (Table 5). Oxidation process of the organic matter consumes the DO in water. Therefore available organic matter content reduce the DO levels in water (Rajapaksha *et al.*, 2020). According to the ambient water quality standards, mean DO level in site 1 is the only site which is suitable for drinking purpose after following simple treatment. Also sampling sites 3, 4 and 5 are not suitable for bathing, contact for recreational activities and survival for the aquatic life. Mean DO level does not significantly differ among sampling sites and occasions ($p > 0.05$).

The lowest turbidity of 3.33 NTU was recorded in site 1 in March while the highest value of 30.67 NTU was recorded in site 4 in January (Figure 8). The mean turbidity of site 1 was significantly different from that of site 2, site 3, site 4 and 5 ($p < 0.05$) (Table 5). Also mean turbidity value of site 4 was significantly different from that of site 2, site 3 and site 5 ($p < 0.05$) (Table 4). Also mean turbidity value of site 1 is the only site which comply with the ambient water quality standards for category A (water that requires simple treatment, for drinking). According to a previous study, turbidity showed 16.9 NTU at KirindiOya (Amarathunga *et al.*, 2006). Mean turbidity level shows a significant interaction between sampling sites and occasions ($p < 0.05$).

Conductivity varied from 0.023 mS/cm recorded in site 1 in March to 0.28 mS/cm recorded in site 4 in January (Figure 9). The mean conductivity of site 1 was significantly different from that of site 2, site 3, site 4 and 5 ($p < 0.05$) (Table 2). Also mean conductivity value of site 4 was significantly different from that of site 2, site 3 and site 5 ($p < 0.05$) (Table 5). Mean conductivity values of all sampling sites are complied with ambient water quality standards for all categories. Mean conductivity

level does not show a significant interaction between sampling sites and occasions ($p>0.05$).

Nitrate concentrations showed considerable temporal and spatial variation (Figure 10). Nitrate concentration varied from 0.057 mg/L recorded in site 1 in March to 3.56 mg/L recorded in January in site (Figure 10). The mean value for Nitrate concentration in site 1 was significantly lower than that of other sites ($p<0.05$) (Table 2). When considering the land use patterns of the study site, there are many pathways of incoming pollutants to the area where site 2, 3, 4 and 5 are located. Many hotels and restaurants are located closely to the site 3 and 4. Therefore many wastewater outlets from many hotels and restaurants are directly opened to the site 3 and 4. Agricultural area is located near for this site 2. Also mean Nitrate concentration value of site 4 were significantly different from that of site 2 and site 5 ($p<0.05$) (Table 5). This site 4 is located at the middle of the town area. Untreated wastewater discharge for this location is relatively high. However, mean nitrate concentration values of all sampling sites are complied with ambient water quality standards for all categories. Mean nitrate level significantly vary among sampling sites and occasions ($p<0.05$).

Total Phosphorus (TP) concentration varied from 0.017 mg/L recorded in site 1 in March to 2.11 mg/L recorded in site 4 in January (Figure 11). Mean TP contents in site 1 was significantly lower than that of other sites ($p<0.05$) (Table 5). Because pollution sources and disturbances are in low level in the area where site 1 is located. Also mean TP contents of site 3 and site 4 were significantly different from that of site 2 and site 5 ($p<0.05$) (Table 5). Site 3 and 4 show considerably higher values due to contamination of pollution loads. Untreated greywater from hotels and restaurants can be the reason for high nutrient contents of water. Also comparatively high concentration of nutrients site 2 and 5 may be due to drain of fertilizers/agrochemicals from near agricultural lands and urban runoff. However according to ambient water quality standards, mean total phosphorous levels in water presence in site 3 and 4 not suitable for aquatic life. Also mean TP level shows a significant interaction between sampling sites and occasions ($p<0.05$).

BOD is an important parameter to determine the amount of dissolved oxygen required for the biochemical decomposition the organic matter in water (Patil *et al.*, 2012). Biochemical Oxygen Demand (BOD₅) varied from 0.23 mg/L recorded in site 1 in March to 46.00 mg/L recorded in site 4 in March (Figure 12). Mean BOD value in site 1 was significantly lower than that of other sites ($p < 0.05$) (Table 5). Therefore excluding site 1, other sites receive high amount of organic matter. Also mean BOD value of site 4 was significantly different from that of site 2, 3 and 5 ($p < 0.05$) (Table 5). It means that comparatively site 4 receives high amount of organic matter. This may be due to kitchen wastewater discharge from hotels and restaurants. However mean BOD level in site 1 is the only one which comply with ambient water quality standards for all five categories. Mean BOD values of other four sites are does not comply with atleast with minimum quality of ambient water quality standards. Also mean BOD level significantly vary among sampling sites and occasions ($p < 0.05$).

COD is a measure of the required oxygen for the chemical oxidation of organic matter (Patil *et al.*, 2012). In present study, Chemical Oxygen Demand (COD) varied from 5.00 mg/L recorded in site 1 in March to 356.00 mg/L recorded in site 4 in January (Figure 13). Mean COD value in sites 1 and 2 were significantly lower than that of other sites ($p < 0.05$) (Table 5). It represents low organic matter content in site 1 and 2. High organic matter content in other 3 sites may be due to high pollution load intake. According to COD levels, sampling site 1 is the only one which comply with ambient water quality standards for all five categories. Mean COD values of other four sites are does not comply with atleast with minimum quality of ambient water quality standards. Mean COD level shows a significant interaction between sampling sites and occasions ($p < 0.05$).

According to the results of physico chemical parameters of the stream, midstream segment where site 3 and 4 located shows high nitrate, phosphate, BOD and COD levels than other sites. It reveals that this area influenced by point sources as well as non point sources which are associated with wastewater discharges and drains. Also this segment is accumulated by agricultural/cultivational discharges from upstream. Site 1 and 5 where considerably rich with riparian vegetation show low pollution loads due to support of phytoremediation and removal of nutrients. Flow rates and

turbulent flows due to stones and boulders in this segment of the stream is supporting continuous aerations and it helps to self-purification and natural deprivation of pollutants (Rajapaksha *et al.*, 2020). Riparian vegetation of mid stream area where site 3 and 4 are located is encroached by hotels and restaurants. This reduce the flow velocity of the stream and decrease the ability of water aeration and self purification.

Table 3 gives the mean $\pm$ standard error values for physico-chemical parameters for each sampling period. Mean values for pH, Turbidity and Conductivity in different sampling occasion were not significantly different throughout the study period ($p>0.05$) (Table 6). Mean water temperature in January was significantly lower than in other months ($p<0.05$). This may be due to annual temperature variation in the Sri Lankan reservoirs was not more than 5°C with a minimum in January (Silva, 2007). Also mean water temperature in March was significantly different from that of July, September and December ($p>0.05$) (Table 6).

Mean DO content in December was significantly different from January, March and September ($p<0.05$) (Table 6). This dissimilarity of measurements of temporal variation can be seen within considerable time period, because stream can be subjected to different kind of natural and anthropogenic activities.

Mean TP content was significantly higher in January than in other sampling occasions ($p<0.05$) (Table 6). Mean Nitrate concentration, TSS, BOD and COD content were significantly higher in January and March than in July, September and December ($p<0.05$) (Table 6). It is important to note that high nutrients loads shows at January and March. Usually tourism season starts from December. So Ella area is full crowded with tourists on January to March. But tourism industry was vastly decreased due to Covid 19 pandemic situation which is started at the end of March 2020 in Sri Lanka. It may be the reason for low pollution loads of Month July, September and December. Also monsoon season starts from October Sri Lanka. Low levels of nutrients, BOD and COD in water may be due to dilution of water with the rainfall.

Table 5: Mean values $\pm$ SEM of physico - chemical parameters in each sampling site

Parameter	Site1	Site2	Site3	Site4	Site5
pH	7.55 $\pm$ 0.04 ^a	7.23 $\pm$ 0.03 ^b	7.10 $\pm$ 0.07 ^c	7.33 $\pm$ 0.10 ^b	7.12 $\pm$ 0.02 ^{bc}
Water temperature (⁰ C)	19.81 $\pm$ 0.42 ^a	21.40 $\pm$ 0.78 ^b	21.43 $\pm$ 0.54 ^b	21.81 $\pm$ 0.41 ^b	21.96 $\pm$ 0.56 ^b
Total Suspended Solids (mg/L)	0.17 $\pm$ 0.05 ^a	45.02 $\pm$ 4.14 ^a	49.55 $\pm$ 3.77 ^a	127.82 $\pm$ 26.80 ^b	34.92 $\pm$ 3.56 ^a
Dissolved Oxygen (mg/L)	7.04 $\pm$ 0.47 ^a	5.09 $\pm$ 0.16 ^b	4.50 $\pm$ 0.20 ^b	4.51 $\pm$ 0.47 ^b	4.97 $\pm$ 0.32 ^b
Turbidity (NTU)	4.15 $\pm$ 0.43 ^a	13.67 $\pm$ 1.03 ^b	10.46 $\pm$ 1.54 ^b	19.56 $\pm$ 1.82 ^c	10.00 $\pm$ 1.01 ^b
Conductivity (mS/cm)	0.04 $\pm$ 0.02 ^a	0.17 $\pm$ 0.01 ^b	0.17 $\pm$ 0.02 ^b	0.25 $\pm$ 0.02 ^c	0.19 $\pm$ 0.01 ^b
Nitrate Concentration (mg/L)	0.09 $\pm$ 0.01 ^a	0.85 $\pm$ 0.07 ^{ab}	1.19 $\pm$ 0.20 ^{bc}	1.92 $\pm$ 0.31 ^c	0.80 $\pm$ 0.04 ^{ab}
Total Phosphorus (mg/L)	0.04 $\pm$ 0.09 ^a	0.33 $\pm$ 0.10 ^c	0.44 $\pm$ 0.13 ^{bc}	0.52 $\pm$ 0.12 ^b	0.19 $\pm$ 0.10 ^c
Chemical Oxygen Demand (mg/L)	6.20 $\pm$ 0.89 ^a	42.07 $\pm$ 3.40 ^a	130.47 $\pm$ 20.00 ^b	147.00 $\pm$ 21.10 ^b	102.40 $\pm$ 10.00 ^b
Biological Oxygen Demand (mg/L)	0.48 $\pm$ 0.07 ^a	11.00 $\pm$ 1.20 ^b	11.42 $\pm$ 1.40 ^b	20.29 $\pm$ 3.00 ^c	10.31 $\pm$ 1.23 ^b

For each parameter, mean values indicated by different superscript letters are significantly different from each other ($p < 0.05$).

Table 6: Mean values $\pm$ SEM of physico-chemical parameters in each sampling period

Parameter	January	March	July	September	December
pH	7.21 $\pm$ 0.22 ^a	7.21 $\pm$ 0.51 ^a	7.23 $\pm$ 0.20 ^a	7.25 $\pm$ 0.22 ^a	7.44 $\pm$ 0.17 ^a
Water temperature (⁰ C)	19.50 $\pm$ 0.52 ^a	20.49 $\pm$ 0.45 ^b	22.37 $\pm$ 0.24 ^c	22.07 $\pm$ 0.25 ^c	21.63 $\pm$ 0.21 ^c
Total Suspended Solids (mg/L)	122.31 $\pm$ 27.87 ^a	116.63 $\pm$ 12.72 ^a	7.18 $\pm$ 2.00 ^b	7.64 $\pm$ 2.86 ^b	3.72 $\pm$ 0.78 ^b
Dissolved Oxygen (mg/L)	4.82 $\pm$ 0.22 ^a	4.78 $\pm$ 0.34 ^a	5.40 $\pm$ 0.47 ^{ab}	5.23 $\pm$ 0.52 ^a	5.87 $\pm$ 0.50 ^b
Turbidity (NTU)	13.95 $\pm$ 3.74 ^a	13.96 $\pm$ 3.30 ^a	10.97 $\pm$ 1.33 ^a	9.82 $\pm$ 1.54 ^a	9.13 $\pm$ 1.58 ^a
Conductivity (mS/cm)	0.18 $\pm$ 0.06 ^a	0.16 $\pm$ 0.05 ^a	0.16 $\pm$ 0.05 ^a	0.15 $\pm$ 0.09 ^a	0.18 $\pm$ 0.06 ^a
Nitrate concentration (mg/L)	1.77 $\pm$ 0.31 ^a	1.65 $\pm$ 0.12 ^a	0.46 $\pm$ 0.110 ^b	0.46 $\pm$ 0.11 ^b	0.52 $\pm$ 0.10 ^b
Total Phosphorus (mg/L)	1.08 $\pm$ 0.02 ^a	0.26 $\pm$ 0.12 ^b	0.09 $\pm$ 0.05 ^b	0.05 $\pm$ 0.04 ^b	0.04 $\pm$ 0.08 ^b
Chemical Oxygen Demand (mg/L)	204.8 $\pm$ 40.31 ^a	180.4 $\pm$ 27.21 ^a	15.2 $\pm$ 4.22 ^b	15.8 $\pm$ 3.68 ^b	11.8 $\pm$ 1.23 ^b
Biological Oxygen Demand (mg/L)	24.52 $\pm$ 4.05 ^a	23.05 $\pm$ 3.32 ^a	2.92 $\pm$ 0.23 ^b	3.41 $\pm$ 0.31 ^b	2.60 $\pm$ 0.24 ^b

For each parameter, mean values indicated by different superscript letters are significantly different from each other ($p < 0.05$).

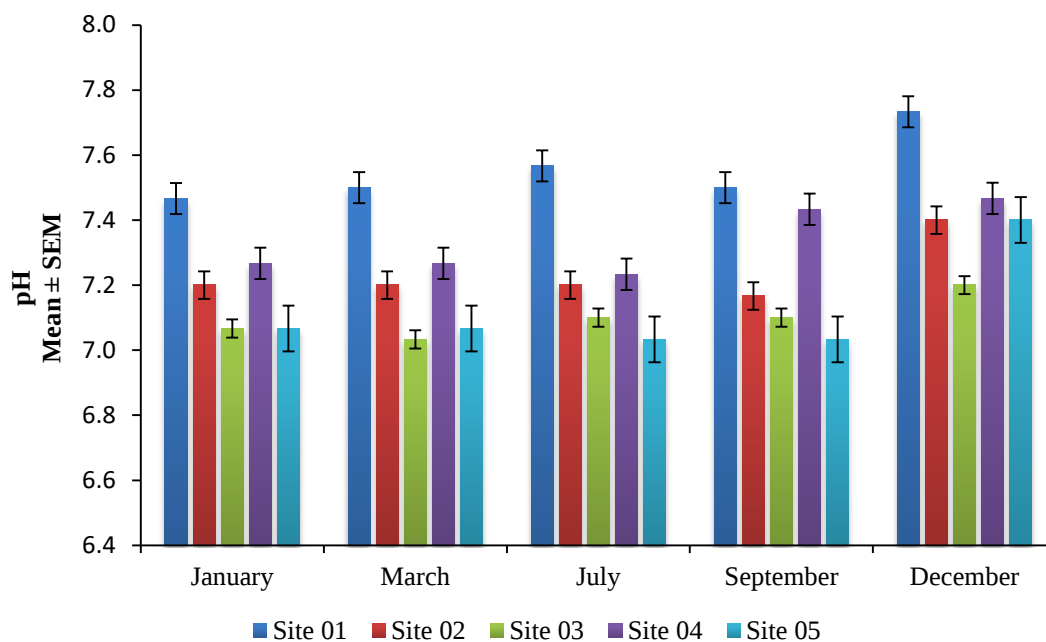


Figure 4: Variation of pH in different sampling sites during the study period. Error bars indicates the standard error of the mean.

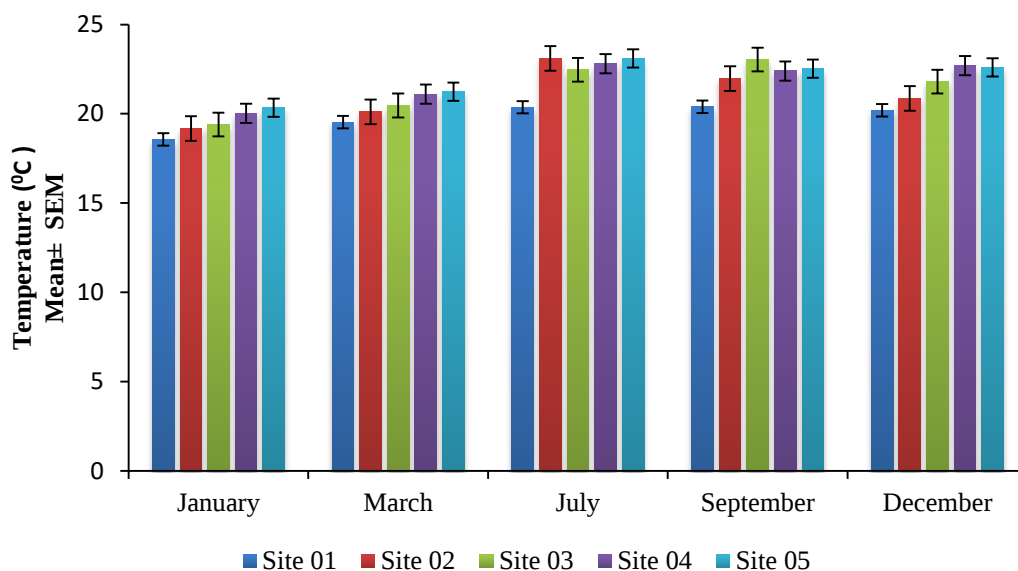


Figure 5: Variation of Temperature in different sampling sites during the study period. Error bars indicates the standard error of the mean.

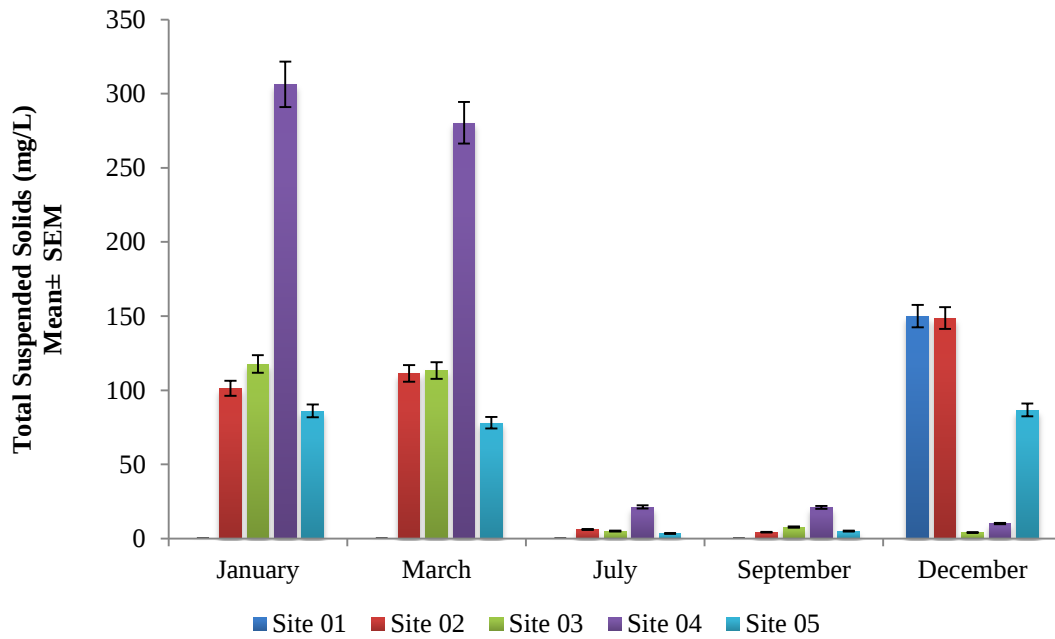


Figure 6: Variation of Total Suspended Solids in different sampling sites during the study period. Error bars indicates the standard error of the mean.

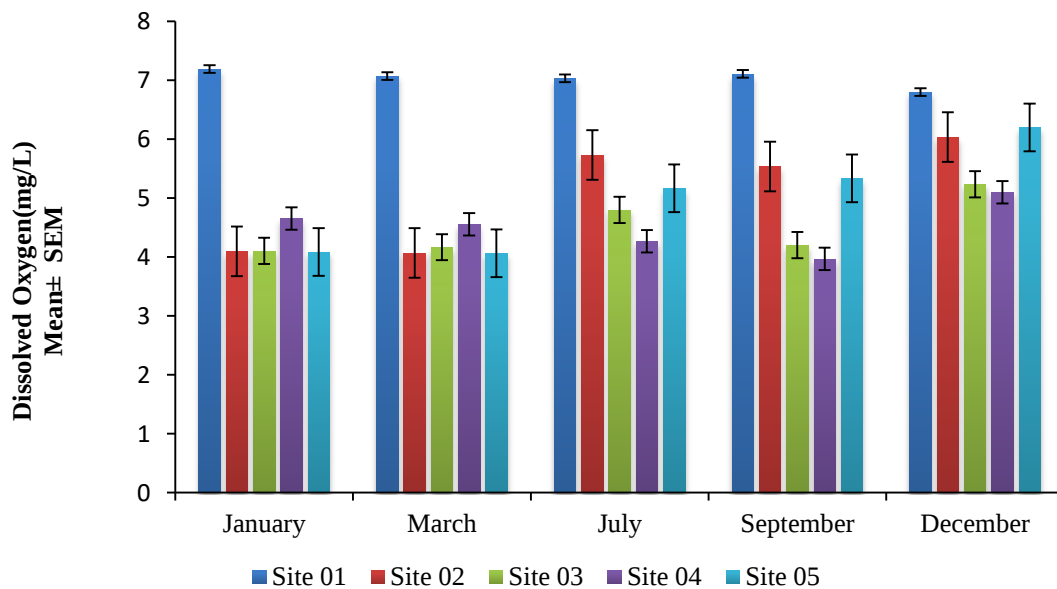


Figure 7: Variation of Total Dissolved Oxygen in different sampling sites during the study period. Error bars indicates the standard error of the mean.

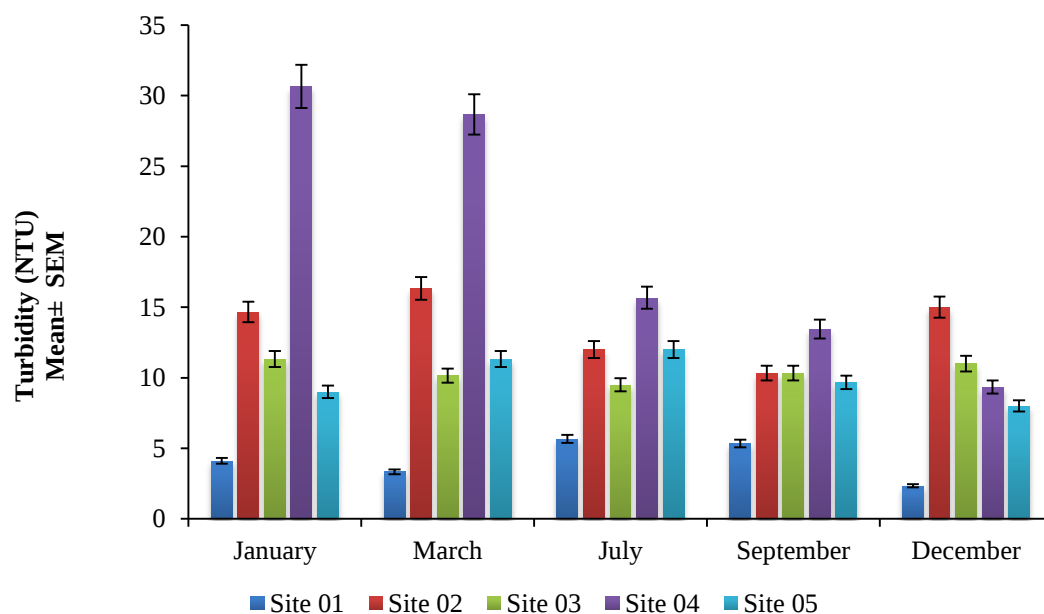


Figure 8: Variation of turbidity in different sampling sites during the study period. Error bars indicates the standard error of the mean.

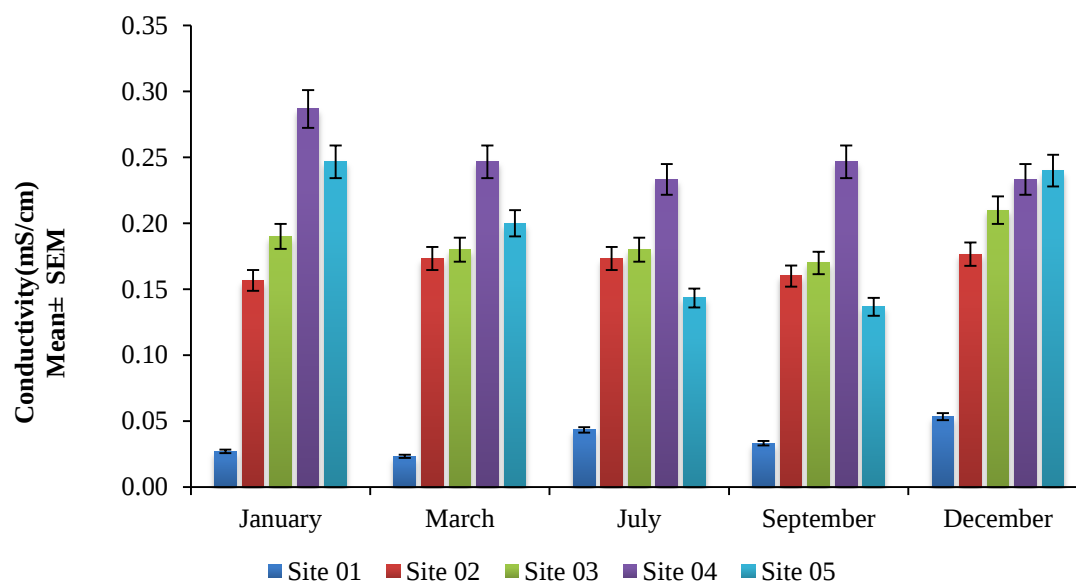


Figure 9 : Variation of Conductivity in different sampling sites during the study period. Error bars indicates the standard error of the mean.

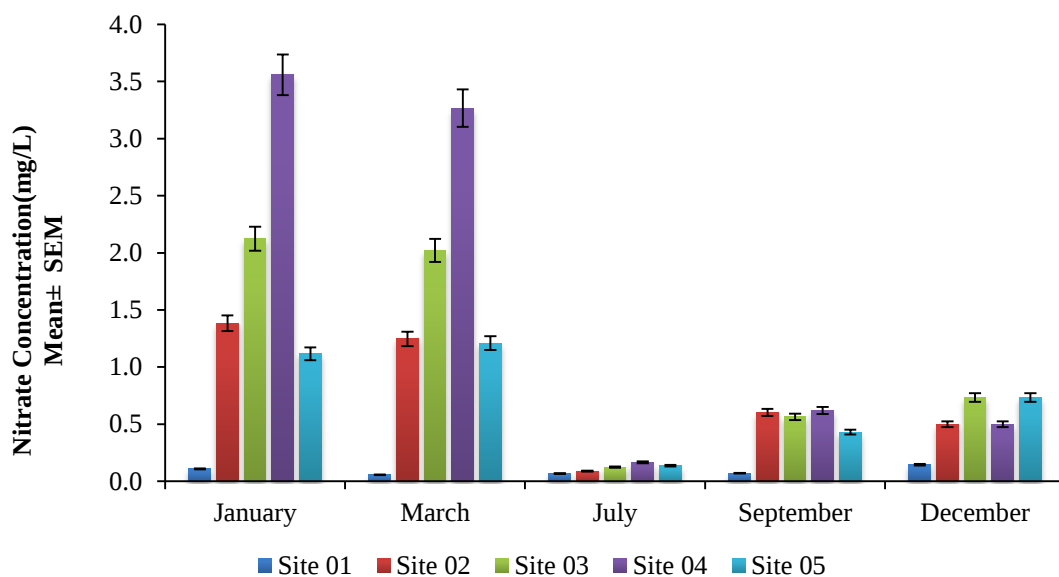


Figure 10: Variation of Nitrate concentration in different sampling sites during the study period. Error bars indicates the standard error of the mean

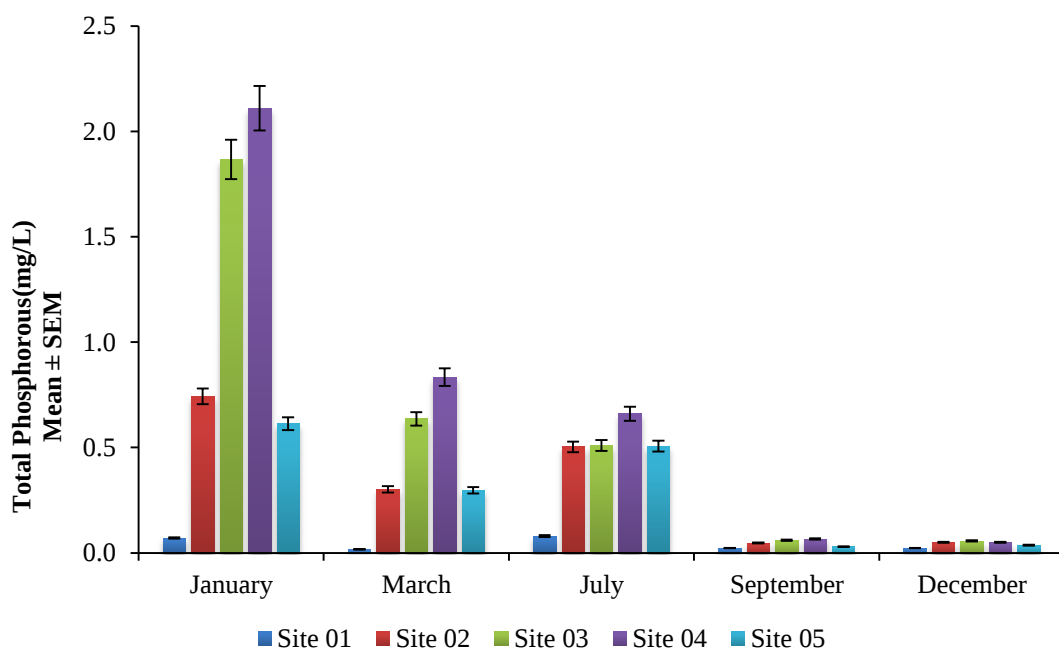


Figure 11: Variation of Total Phosphorous concentration in different sampling sites during the study period. Error bars indicates the standard error of the mean.

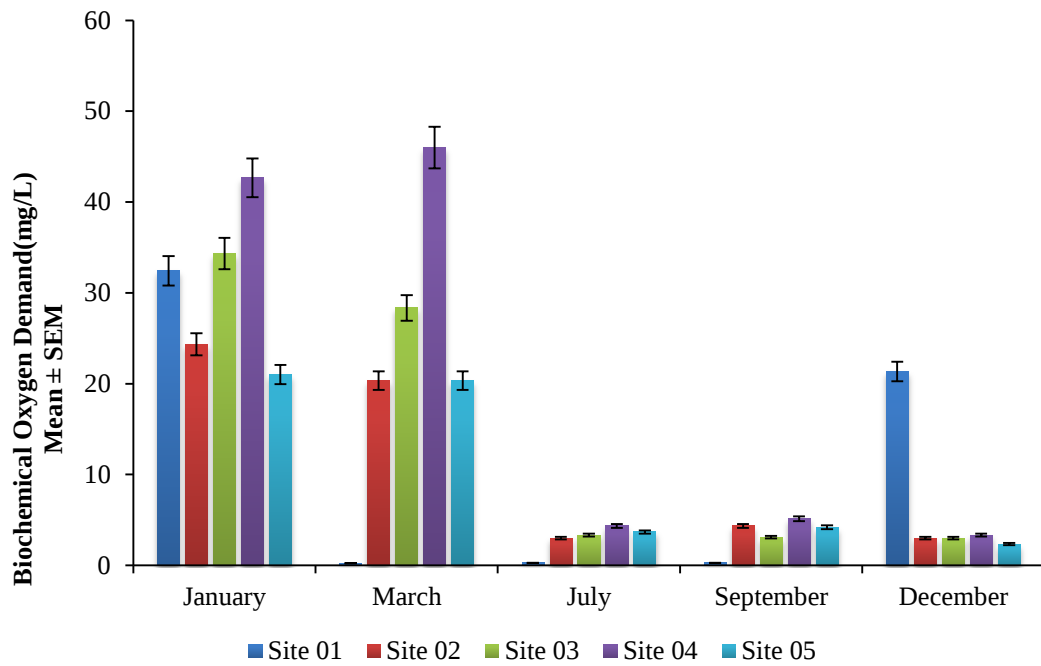


Figure 12: Variation of Biochemical Oxygen Demand in different sampling sites during the study period. Error bars indicates the standard error of the mean.

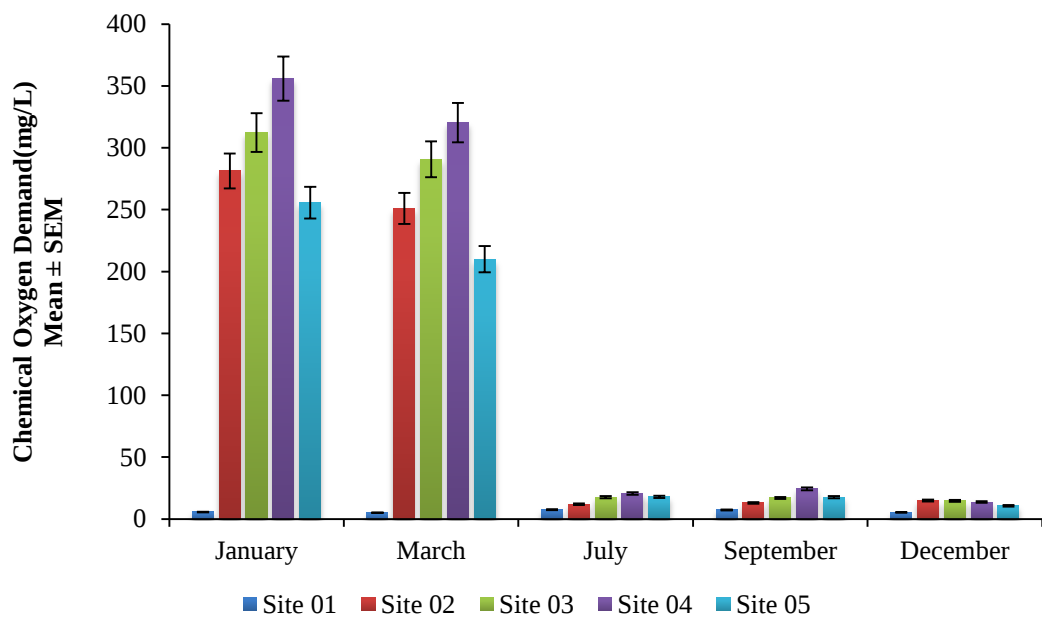


Figure 13: Variation of Chemical Oxygen Demand in different sampling sites during the study period. Error bars indicates the standard error of the mean.

3.2 Micoplastic levels of surface water samples of study sites

3.2.1 Microplastic density of study sites

Microplastics were identified in all the samples of Beeralla stream (tributary of KirindiOya). Microplastics density levels of surface water was varied from 5 Items/Litre recorded in site 1 in March to 12 Items/Litre recorded in site 2 in March (Figure 14). The mean micoplastic density levels are not significantly vary among each sampling sites ($p>0.05$) (Table 7). Majority plastic debris in marine environment is produced and introduced by rivers. But few studies shows that microplastics in freshwater ecosystems is severe as marine environment (Laforsch *et al.*, 2015). According to a study conducted in Greater London rivers, it is reported the microplastic concentration ranges between 3.3 to 9.9 items/L (Sofra *et al.*, 2010). Also micoplastic density was varied from 0.01 to 12.9 items/L in two rivers in California (San Gabriel and Los Angeles Rivers (USA)) (Moore *et al.*, 2011). After doing a two year survey in Danube River in Central Europe, it is reported the microplastic density as 0.00032 ± 0.00465 items/L (Lechner *et al.*, 2014).

Mean micoplastics density level of September was significantly lower than that of other months ($p<0.05$) (Table 8). Moore *et al.*, 2011 reports that microplastic density in rivers of San Gabriel and Los Angeles were higher after the rain event than dry season. Mean microplastic density level shows a significant interaction between sampling sites and occasions ($p<0.05$).

Study conducted in upstream and downstream of a wastewater treatment plant outlet in North Shore Channel in Chicago, microplastic density was varied from 0.00194 items/L to 0.01793 particles/L. It means that wastewater treatent plant discharges may play an vital source of microplastics in freshwater (McCormick *et al.*, 2014). It was observed at the field, treated wastewater released from hotels and restaurants to this Beeralla stream. Therefore it can be one of the sources for miroplastic pollution in this stream.

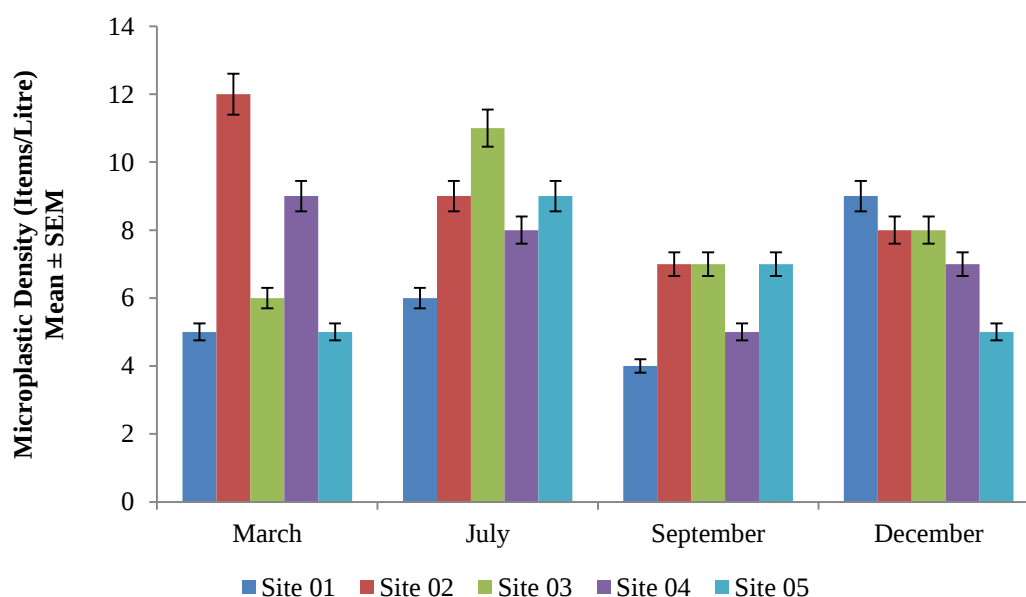


Figure 14: Variation of Microplastic Density in different sampling sites during the study period. Error bars indicates the standard error of the mean.

Table 7 : Mean values $\pm$ SEM of Micoplastic density in each sampling site

Sampling Site	Micoplastic density (Items/Litre)
Site 01	6 ± 0.90^a
Site 02	9 ± 1.08^a
Site 03	8 ± 1.02^a
Site 04	7 ± 1.09^a
Site 05	6 ± 0.88^a

Mean values indicated by different superscript letters are significantly different from each other ($p < 0.05$).

Table 8 : Mean values $\pm$ SEM of Micoplastic density in each sampling occasion

Sampling Period	Micoplastic density (Items/Litre)
March	7 ± 0.90^a
July	8 ± 0.98^a
September	5 ± 0.80^b
December	7 ± 1.05^a

Mean values indicated by different superscript letters are significantly different from each other ($p < 0.05$).

Table 9 : Mean microplastic density reported for surface waters around the world

Location	Density(Items/ L)	Reference
Tributaries of Great Lakes, USA	1900	Baldwin <i>et al.</i> , 2016
Manas River, China	21-49	Wang <i>et al.</i> , 2020
Ottawa, Canada	0.05–0.24	Vermaire <i>et al.</i> , 2016
Three Gorges Reservoir, China	1,597–12,611 x(10 ³)	Wang and wang, 2018
Seine River (France)	0.003 to 0.106	Dris <i>et al.</i> , 2015
Bohai Sea, China	10- 1230	Zhang <i>et al.</i> , 2017
Near the Southern California shore, Santa Monica Bay, California	3.92x10 ³	Lattin <i>et al.</i> , 2004
Surface water-Off Colombo west Coast of Sri Lanka	140.1x10 ³	Athawuda, 2018
Southern coastal water,Sri Lanka	17.45x10 ³	Athapaththu <i>et al.</i> , 2020

When considering the microplastic pollution in Sri Lanka freshwaters, there were no published records found in freshwater ecosystems.

3.2.2 Size variation of identified microplastics

Size of collected microplastics in surface water samples ranged from 0.43mm-4.7mm with an average length of 1.3mm. 10.27% of microplastics were in the size lower than 0.5 mm and 24.66% of the microplastics were in the size range between 0.5mm-1.0mm among the total number of micriplastics (Figure 18). In San Gabriel River, small micoplastics (1–4.75 mm) and larger plastic particles (> 4.75 mm) were identified (Moore *et al.*, 2011). 98% of plastics found in tributories of Great Lakes, USA were smaller than 4.75 mm in diameter (Baldwin *et al.*, 2016). According to a study done in the Laurentian Great Lakes (Lake Huron, Lake Superior and Lake Erie), plastic particles were categorized in to three groups (0.355 mm – 0.999 mm), (1.00 mm – 4.75 mm), (> 4.75 mm). Most of them were observed under the smallest category. It means that there is a similar risk for freshwater biota as well as in the marine habitat (Eriksen *et al.*, 2013). Majority of micoplastic particles identified in present study also lower than 1.5mm and have potential risk of accumution in biota and threat to biotic community of the stream.

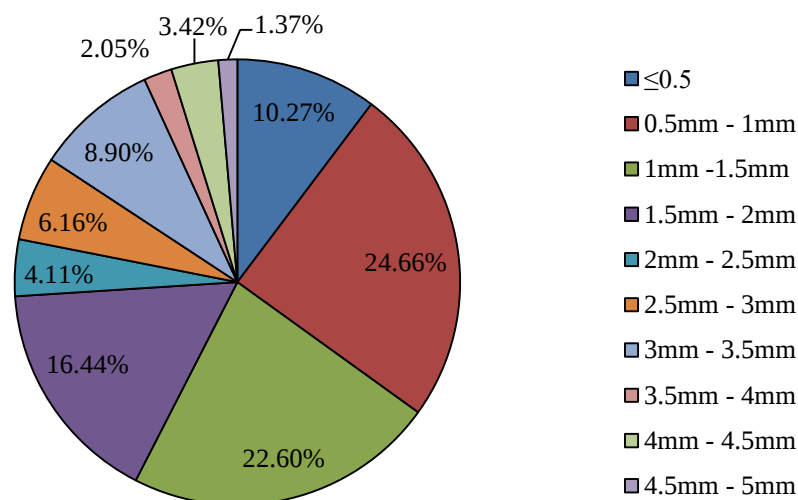


Figure 15: Size variation of identified microplastics

3.2.3 Shape variation of identified microplastics

Two shape categories (filament and rod shape) were identified from collected surface water samples. Filaments accounted for majority of microplastics in Beeralla stream (99.32%). Rest of microplastics were followed by rod shape (0.68%). These filament type microplastics can be a source of textile industry. Synthetic clothing fibres such as polyester, polyamide and polypropylene can be released during laundry washing machines and textile processes and their dimension and shape allow them to partially pass through wastewater treatment plants and reach directly to waterways. Moreover various types of materials or accessories used in clothes and textile products, such as prints, coatings, buttons and glitter (textile synthetics) are responsible for a global discharge of between 0.2 and 0.5 million tonnes of microplastics into the oceans each year (MacArthur, 2017). Mostly foamed plastics showed in San Gabriel River/USA and majority of plastic were fragments in Los Angeles River (Moore *et al.*, 2011). Common microplastics were fiber items, followed by films and granules, while plastic spherules occasionally reported from the Yangtze Estuarine ecosystem of China (Zhao *et al.*, 2014). Fibers are considered as the major component in the surface waters in

Stockholm Archipelago (Gewert *et al.*, 2017). Lusher *et al.*, 2014 revealed that four distinct types of microplastics (fibres, fragment, bead, foam) with the majority were fibers (95.9%).

Table 10 : Abundance of shape categories of microplastics

Shape	Percentage
Filament	99.32 %
Rod	0.68 %

3.2.4 Color variation of microplastics

Most common color categories of identified micoplastics were blue (30.26%), black (21.05%) and transparent (23.03%) followed by red (7.89%), green (3.29%), white (7.89%), yellow (5.26%) and purple (1.32%) from the total number of microplastics (Figure 19). Total mean microplastic density significantly vary with colour variations ($p < 0.05$). There had no significant effect ($p > 0.05$) on total microplastic density of colour categories among sampling sites and months. Blue colour plastic particles may be accumulated by discarded beverage bottles and packaging materials along the stream bank and stream body. There were pet bottles, empty plastic containers, plastic bags and plastic food wrappers along the stream bank. Also plastic debris were floating on the stream. In addition to uncontrolled wastewater discharges from restaurants and urban runoff, these kind of plastic pollutants (thrown litter) can be reason for the microplastic pollution of Beeralla stream.

Similarly, a range of colours were identified by Lusher *et al.*, 2014, with blue the most common 37.7% in Northeast Atlantic Ocean. Also it is reported that most common colours report in that study were blue, red, black and green for Filaments and white, black and blue for fragments. White and black were the most dominant colours which accounts 83% of microplastics found in the Manas River Basin China (Wang *et al.*, 2020). Another study has reported that most microplastics in coastal waters in Southern Sri Lanka were blue and green (Koongolla *et al.*, 2018).

Marine organisms as well as freshwater fish species and invertebrates can be ingested by microplastics. However, effects associated with microplastic ingestion is poorly examined and documented. Aquatic organisms such as fishes can be swallowed plastics by mistake as food. Also plastic associated additives and polymers can act as endocrine disruptors. These polymers can absorb nonparticles, toxic compounds and metal which can be adversely effected. Plastic debris can be a disease spread vector for alien species also (Dris *et al.*, 2015).

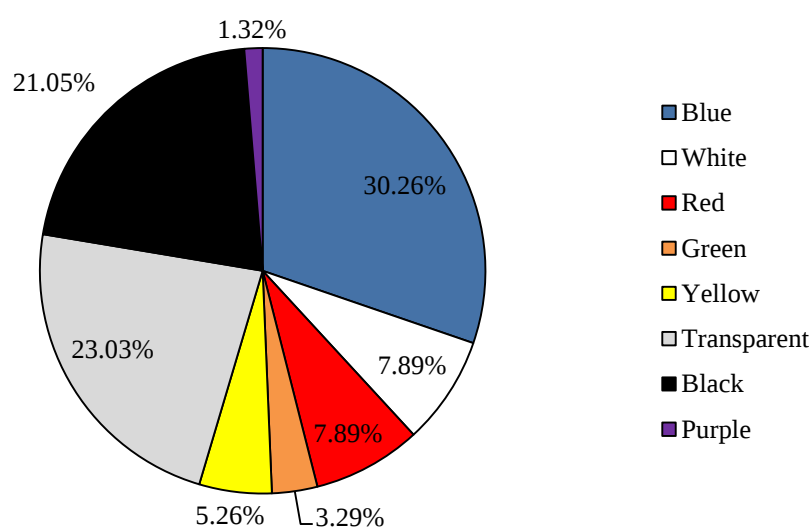


Figure 16 : Colour variation of identified microplastics

3.3 Diversity and abundance of macroinvertebrates

A total of 15 species of macroinvertebrates were identified in the samples of Beeralla stream, tributary of KirindiOya collected during the sampling period. The abundance of each of these species in each site is given in table 9. The highest mean abundance of macroinvertebrates was recorded in site 2 while the lowest mean abundance was recorded in site 3 (Table 11). The most abundant species was *Trithemis festiva* (Order Odonata) in site 2 (Table 11). The abundance of different macroinvertebrate species in each sampling period is given in Table 10. *Trithemis festiva* (Order Odonata) is the most abundant species in every sampling period. The highest mean abundance of macroinvertebrates was recorded in January while the lowest mean abundance was recorded in March (Table 12).

Highest species richness was recorded in site 5 while lowest value was recorded in site 1 (Table 11). Shannon-Wiener diversity index (H') is commonly and frequently used indicator to evaluate water quality. Highest Shannon-Wiener diversity index of 1.94 was recorded in site 4 and lowest value, 1.17 was recorded in site 2 (Table 11). When Shannon Wiener Diversity Index value is more than 3, it shows minor pollution or pollution free environment. If the value is in between 1 to 3, moderate pollution is taking place. Severe pollution in the environment implies by the value in between 0 to 1 (Chunje *et al.*, 2016). According to the results of Shannon Wiener Diversity Index, present study shows moderate pollution in Beeralla stream during the study period. Highest species richness and Shannon-Wiener diversity index of 2.347 were recorded in December while lowest values of species richness and Shannon-Wiener diversity index of 1.785 was recorded in January (Table 12). It means that this stream shows moderate pollution throughout the year 2020.

However, highest Family Biotic Index (8.26) was recorded in site 2 and the lowest value (5.56) was recorded in site 1 (Table 9). This Family Biotic Index (FBI) can be used to examine organic pollution. There are many advantages in using family level indices. Because it is need expert effort to identify macroinvertebrates in specific

geographical regions with high number of new species and highest endemism level. Also inadequate knowledge of various macroinvertebrate taxa in species and genus level in different regions is disadvantage of using indices based on species and genus level. Therefore it is easy to implement the family level index by persons than the expert (Kazanci *et al*, 2013). According to the FBI for each sampling sites, site 01 is fairly substantial pollution taken place. Site 4 and 5 are fairly poor water quality taken place. Site 2 and 5 have severe organic pollution with very poor water quality (Hilsenhoff, 1988). Study conducted in Dediyaagala stream in 2012, Family Biotic Index was varied 2.63 to 3.16 which shows good and suitable water quality for healthy aquatic life (Madhushanka *et al.*, 2014).

However, the highest Family Biotic Index of 7.24 was recorded in January and the lowest value of 5.80 was recorded in December (Table 12). According to the FBI values, comparatively stream shows fair water quality in December. Stream shows very substantial and severe organic pollution in other months. This may be due to dilution of pollutants in December with heavy rainfall. Also tourism season starts on January in Ella. Therefore stream shows high organic pollution due to uncontrolled wastewater discharges from hotels and restaurants.

When considering the land use patterns which are associated with land use activities such as tourism recreational activities, domestic activities (washing & bathing), agricultural activities (paddy cultivation, vegetable cultivation & tea cultivation) in study area directly affect on the ecological health of the stream. Forest department owned lands also located near to the study area. Some of them are natural forests and other lands are with timber plantations. But land under forest cover is low comparatively other land uses such as commercial, residential, recreational and transportation. Conversion of forest lands to tea and other commercial crops can be observed past years. Conversion of paddy cultivation lands and pasture lands in to commercial crop cultivations such as vegetables while other paddy lands seemed to be abandoned by farmers which became scrub jungles eventually. Also many homestays, hotels and restaurants are scattered in hilly area in Ella. Residential land uses area swapped for commercial uses. This process motivated by the growing

tourism industry and increasing the extent of commercial land use in the Ella town area. With the high demand of tourism based activities, extend of lands used for tea cultivation has been declining steadily and lands uses for road network is increasing. Especially improvements for Ella -Wallawaya road and Badulla – Colombo road were were done recent past years. It has been witnessed that the extent of lands used for commercial, residential and roads has been increased gradually while the lands kept under the channels and waterways have been declining gradually (Urban Development Authority, 2019)

Table 11: The mean abundance of dragonflies in each sampling site

Species	Family	Site 01	Site 02	Site 03	Site 04	Site 05
<i>Orthetrum chrysis</i>	Libellulidae	0	16	4	8	5
<i>Trithemis festiva</i>	Libellulidae	0	32	13	17	11
<i>Onychargia atrocyana</i>	Platycnemididae	0	0	0	0	1
<i>Brachythemis fuscopalliata</i>	Libellulidae	0	0	0	0	1
<i>Palpopleura sexmaculata</i>	Libellulidae	4	3	2	4	4
Abundance		4	51	19	29	22

Table 12: The mean abundance of dragonflies in each sampling period

Species	Family	January	March	July	September	December
<i>Orthetrum chrysis</i>	Libellulidae	9	7	9	6	2
<i>Trithemis festiva</i>	Libellulidae	21	16	21	10	3
<i>Onychargia atrocyana</i>	Platycnemididae	0	0	0	0	1
<i>Brachythemis fuscopalliata</i>	Libellulidae	0	0	0	0	1
<i>Palpopleura sexmaculata</i>	Libellulidae	4	3	4	2	0
Abundance		34	26	34	18	7

Table 13: The mean abundance of macroinvertebrates in each sampling site

Species	Family	Site 01	Site 02	Site 03	Site 04	Site 05
Order Odonata						
<i>Palpopleura sexmaculata</i> (Juvenile)	Libellulidae	0	0	0	0	1
<i>Anisoptera</i>	Platycnemididae	0	0	0	0	3
<i>Zygoptera</i>	Libellulidae	0	0	0	0	5
Abundance		0	0	0	0	9
Order Hemiptera						
<i>Enithares abbreviate</i>	Notonectidae	5	0	0	0	1
<i>Gerridae</i>	Gerridae	16	2	0	0	3
Abundance	(Water strider)	21	2	0	0	4
Order Diptera						
<i>Chironomidae</i>	Chironomidae	0	0	17	12	15
Abundance		0	0	17	12	15
Order Coleoptera						
<i>Laccophlas ceylonicus</i>	Cheiridiidae	8	1	0	0	2
<i>Hhphydrus indicus</i>	Dytiscidae	6	0	0	1	0
Abundance		14	1	0	1	2
Order Haplotaxida						
<i>Tubifex tubifex</i>	Tubificidae	0	13	0	5	0
Abundance		0	13	0	5	0
Total Abundance		35	16	17	18	30
Species Richness		5	6	4	6	12
Shannon weiner Diversity Index		1.31	1.17	1.81	1.94	1.53
Family Biotic Index		5.56	8.26	7.62	6.66	6.04

Table 14: The mean abundance of macroinvertebrates in each sampling period

Species	Family	January	March	July	September	December
Order Odonata						
<i>Palpopleura sexmaculata</i> (Juvenile)	Libellulidae	0	0	0	0	1
<i>Anisoptera</i>	Platycnemididae	4	3	3	5	6
<i>Zygoptera</i>	Libellulidae	0	1	1	2	1
Abundance		4	4	4	7	8
Order Hemiptera						
<i>Enithares abbreviate</i>	Notonectidae	0	2	1	1	2
<i>Gerridae</i>	Gerridae (Water strider)	3	4	2	3	9
Abundance		3	6	3	4	11
Order Diptera	Chironomidae	9	4	10	9	7
<i>Chironomidae</i>						
Abundance		9	4	10	9	7
Order Coleoptera	Cheiridiidae	2	1	2	4	4
<i>Laccophlas ceylonicus</i>						
<i>Hhphydrus indicus</i>	Dytiscidae	1	0	1	1	4
Abundance		3	1	3	5	8
Order Haplotaxida						
<i>Tubifex tubifex</i>	Tubificidae	3	3	2	5	8
Abundance		3	3	2	5	8
Total Abundance		22	18	22	30	42
Species Richness		9	10	10	11	13
Shannon Weiner Diversity Index		1.785	1.932	2.268	1.984	2.347
Family Biotic Index		7.24	6.75	7.22	6.65	5.80

4. CONCLUSION

This study reveals an overview about the health quality status of Beeralla stream (tributary of Kirindioya), Ella. Measured water quality parameters were significantly vary (<0.05) both spatially and temporally. According to the results, midstream segment of the stream shows comparatively high nitrate, phosphate, TSS, BOD and COD levels. This may be due to uncontrolled wastewater discharges from hotels and restaurants, nutrients inputs from urban runoff. It reveals that land use activities in study area directly affect on the ecological health of the stream. Although except site 01, TSS, turbidity, DO, phosphate, COD and BOD levels in other sites exceed the Sri Lankan ambient water quality standards. Mean nitrate, TSS, TP, BOD and COD levels in January and March were significantly higher than other months. With the start of tourism season on December/ January, nutrient concentration is high in the stream. Also tourism activities were vastly decreased with the Covid 19 pandemic situation which was started from the end of March. It can be the reason for decreased levels of nutrient concentration of the stream in other months.

It is important to note that, this study presents all samples collected from the study sites were contained microplastics. Mean micoplastic density levels vary significant in temporally. Data on contamination of microplastics in freshwater are lacking in Sri Lanka, while research on this evolving pollutant depicts more attention globally. Current study reports, the presence of microplastics in surface water of Beeralla stream. Occurrence of MPs in this stream and impacts on aquatic organisms of the whole food web are critical matters to be addressed. According to the morphology of micoplastics of this study, fragmented larger plastic debris and textile synthetics can be the sources for this microplastic pollution. It is recommended to do FTIR (Fourier-Transform Infrared spectroscopy) test in order to identify exact sources of microplastics. Also it should be controlled at the source of generation otherwise it is difficult to control or limit the distribution of microplastics after discharging into the environment. Almost there is no basic data on micoplastics contamination in freshwater ecosystems in Sri Lanka, this study would be a reference material for cognisance the microplastic contamination of freshwater Sri Lanka.

Generally, the water quality of Beeralla stream acts as an organic pollution spot with poor water quality based on the diversity and abundance of aquatic invertebrates and values of biological indices (Shannon Wiener Diversity Index and Family Biotic Index) used in this study. The Family Biotic Index indicates that all sites of stream are subjecting to the organic pollution and macroinvertebrates found in Beeralla stream can be used as bioindicators in biomonitoring programs in freshwater streams in Sri Lanka since they show responses to their surrounding environment. Therefore it is more productive to examine biological measure along with the physico chemical monitoring in order to assess the condition/health of a waterway since physico chemical parameters can be altered over the time and biological components last for long period of time.

Around 261 hotels and restaurants were located in Ella. Out of total hotel industries in Ella, 45% of industries represent homestays which is not considered as a prescribed activity. But these industries boom rapidly and scattered in hilly areas. Especially wastewater pollution arises due to limited space, urbanization and industrialists were not adapted to proper wastewater mitigatory measures.

5. RECOMMENDATIONS

Present study results reveal that water quality of the Beeralla stream is deteriorated with micoplastics, vast nutrient inputs with organic matter which cannot meet the ambient water quality standards in Sri Lanka. Since Ella is a main tourism attracted area in Sri Lanka, it is vital to maintain its aesthetic and recreational value. The Beeralla stream flows through the Ella town area and it is a water source for agricultural activities as well as bathing and washing purposes of people in the area. Therefore it should be identified point and non point sources of pollutants in the stream and control them.

As short term solutions, stream can be cleaned manually, stream bank enrichment by suitable vegetation cover and installation of manual barriers at stream bank to discontinue inflowing of macroplastics can be maintained. But water quality of the stream should be maintained up to good quality for long term period of time. Relevant government and non government institutes should be monitored and pay attention to protect stream from uncontrolled wastewater discharges from adjacent hotels, restaurants and residents. Awareness programs should be implemented to vulnerable groups (hotel owners and resident people) to aware about proper waste disposal procedures. Well maintained common/individual wastewater treatment system should be introduced to all hotels and restaurants which are located near and adjacent to the stream.

Even though the sampling sites of the stream located considerably close and are connected to each other, the physico-chemical and biological conditions vary significantly. Hence, even in a small water body in order to make conclusion on physico-chemical and biological parameters, it is necessary to take as many samples as possible and use FTIR test regarding identification of microplastic sources. Also some selected physicochemical parameters collectively can be affected to the degradation of water quality of the stream. Therefore it is recommended to carry out multiple linear regression model in order to estimate relationship in detail manner between quantitative dependent variable and two or more independent variables.

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APPENDICES

Appendix A

Preparation of solutions for the Biochemical Oxygen Demand (BOD) test

Manganese sulphate solution

MnSO₄.2H₂O (48 g) was dissolved in deionized water (80 mL).

The solution was filtered and diluted to 100ml.

Alkali-iodide-azide reagent

Sodium Hydroxide (NaOH, 50g) and Sodium Iodide (NaI, 13.5g) was dissolved in deionized water and diluted to 100mL. Sodium azide (NaN₃, 1 g) was dissolved in deionized water (4 mL) and added to the diluted solution.

Sodium Thiosulphate titrant (0.025M)

Sodium Thiosulphate (Na₂S₂O₃, 6.205 g) was dissolved in deionized water. Solid NaOH (0.4g) was added and mixes thoroughly to dissolve. The solution was diluted to 1000 mL.

Appendix B

Preparation of solutions for determination of Nitrate Concentration

Stock Nitrate solution (100mg/L)

Potassium nitrate (KNO_3) was dried in oven at 105°C for 24 hours and a portion of dried KNO_3 (0.7218g) was dissolved in distilled water and diluted to 1000mL. The solution was preserved with CHCl_3 (2mL).

Intermediate Nitrate solution (10mg/L)

Stock Nitrate solution (100 mL) was diluted to 1000mL with distilled water and preserved with CHCl_3 (2mL).

Standard solution series

Different volumes (0, 1.00, 2.00, 4.00, 7.00,... mL) etc. of intermediate nitrate solution was diluted to 50mL with distilled water.

Appendix C

Preparation of solutions for determination of Total phosphorous Concentration

Preparation of Sulfuric acid digestion reagent

Concentrated Sulfuric acid (300mL) was carefully added to distilled water (600mL) and diluted to 1L with distilled water.

Preparation of Combined reagent

- 5N Sulfuric acid

Concentrated Sulfuric acid (70mL) was diluted to 500mL with distilled water.

- Potassium antimony tartrate solution

Potassium antimony tartrate solid (1.3715g) was dissolved in distilled water (400mL) and diluted to 500mL with distilled water.

- Ammonium molybdate solution

Ammonium molybdate (20g) was dissolved in distilled water (100mL).

- Ascorbic acid solution (0.1M)

Ascorbic acid (1.76g) was dissolved in distilled water (100mL).

Combined reagent (100mL) was prepared by mixing the above reagents in the following order and amounts.

1. Sulfuric acid (50mL)
2. Potassium antimony tartrate solution (5mL)
3. Ammonium molybdate solution (15mL)
4. Ascorbic acid solution (30mL)

Preparation of Phosphate solutions for the construction of the standard curve

Stock Phosphate solution (50mg/L)

Anhydrous Monopotassium phosphate (KH_2PO_4 , 219.5mg) was dissolved in distilled water and diluted to 1000mL.

Standard Phosphate solution (2.5mg/L)

A portion of the stock phosphate solution was taken (50.0mL) and diluted to 1000mL with distilled water.

Appendix D

Preparation of solutions for Chemical Oxygen Demand (COD) test

Sulphuric acid reagent

Silver sulphate powder (Ag_2SO_4 , 2 g) was dissolved in concentrated Sulphuric acid (400 mL) in a dark glass bottle with glass stopper. The solution was allowed to stand for 2 days to dissolve.

Standard Potassium Dichromate Solution (0.04167M)

Potassium Dichromate, primary standard grade ($\text{K}_2\text{Cr}_2\text{O}_7$, 6.129 g) was previously dried at 1500 C for 2 hours. Dissolved in distilled water and diluted to 500 mL.

Standard Ferrous ammonium sulphate solution ($\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2$)-0.25M

Ferrous ammonium sulphate, analytical grade crystals ($\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$, 49 g) were dissolved in distilled water. Concentrated sulphuric acid (20 mL) was added. Allowed to cool and diluted to 1000 mL.

Dilute standard ferrous ammonium sulphate solution ($\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2$)-0.025M

Standard ferrous ammonium sulphate (100 mL) was diluted to 1000 mL.

Standardization of dilute ($\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2$)

Standard Potassium Dichromate Solution (10.0 mL) was diluted to 100 mL. Concentrated sulphuric acid (30 mL) was added and allowed to cool. Diluted solution was titrated with dilute ($\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2$) solution using Ferroin indicator. Endpoint colour change was identified as blue green to reddish brown.

Appendix E

Land use patterns around the study area

