EFFECT OF KOMBOLCHA TEXTILE FACTORY EFFLUENT ON SOIL, VEGETABLES AND LEYOLE RIVER, NORTH EAST ETHIOPIA

A Thesis Submitted to Department of Environmental Health Science and Technology, Faculty of Public Health, Institute of Health Science Jimma University, in Partial Fulfillment for the Requirements of Master of Science in Environmental Health.

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Jimma, Ethiopia

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APPROVAL SHEET

As thesis research advisors, we here by certify that we have read and evaluated this thesis prepared under our guidance by Gete Berihun: Effect of Kombolcha Textile Factory Effluent on Soil, Vegetables and Leyole river North East Ethiopia. We recommended that it is be submitted as fulfilling the thesis requirement.

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DECLARATION

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ABSTRACT

Background:-*Textile industries produce huge amount of effluents which is discharged to surface water bodies without proper treatment. Toxic heavy metals are released from textile industry effluents in to water, soil which are quite dangerous.*

Objective: - *To assess the effects of kombolcha textile factory effluent on soil, vegetables and "Leyole" river..*

Method:-*The study was conducted in Kombolcha textile factory in 2018 by using cross sectional study design. A total of 28 samples were collected from effluent, river, sediments, vegetables and soils for assessing physicochemical, heavy metals and macroinvertebrate as bio- indicators. Analysis was done by using standard guideline of American Public Health Association 2012.Heavy metals were analyzed by using Flame Atomic Absorption Spectrometric. Macroinvertebrate metrics were calculated to assess the level of pollution in the stream. SPSS version 20 and MS excel software were employed for statistical analysis. Mann Whitney statistics were carried out to assess the significance difference between sampling point.*

Result:-*The average concentration of Pb, Cd, Cr and Cu in soil and vegetable sample were 29.1, 1.63, 39.739.93 mg/l and 14.2, 1.09, 13.3 and 3.68 mg/kg respectively. The concentration of all investigated heavy metals except Pb in sediment was lower than international standard safe limits. The concentration of most physicochemical and heavy metal concentration were higher than the standards set by national and international agencies. A total of 1765 macro invertebrates belonging to 17 families and 7 orders were collected. The most abundant orders were Diptera with the value of 1079 (61.2%), Ephemeroptera 393(22.2%), Odonata 105(1.95%) and Hemiptera 99(5.6%) which were represented by13 families. The physicochemical and heavy metals in river showed great variation between sampling sites. Hilsenhoff family-level biotic index, percent Diptera, was higher at the most downstream site.*

Conclusion:-*Highest accumulations of heavy metals were present in soils while lowest concentration was found in the river***.** *Most physicochemical parameters of effluents and stream in the downstream were higher than the standards set by different authorized agencies. Most of tolerant macroinvertebrate were prevalent in the downstream while sensitive were more prevalent in the upstream. So the efficiency of the treatment plant should be improved.*

Keywords: Kombolcha, Textile, Effluents, Stream, Macroinvertebrate

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TABLE OF CONTENT

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

APHA:	American Public Health Association
ASPT:	Average Score per Taxa
BMI:	Benthic Macro Invertebrate
BMWP:	Biological Monitoring Working Party
BOD:	Biochemical Oxygen Demand
CF:	Contamination Factor
Cm:	Centimeter
COD:	Chemical Oxygen Demand
SDI:	Simpson Diversity Index
DC:	Degree of Contamination
DO:	Dissolved Oxygen
DW:	Distilled Water
EC:	Electrical Conductivity
EPT:	Ephemeroptera, Plecoptera Trichoptera
FAAS:	Flame Atomic Absorption Spectrometry
FAO:	Food Agricultural Organization
FBI:	Family Biotic Index
HM:	Heavy metal

HFBI:	Hilsenhoff Family Biotic Index
IFC:	International Finance Corporation
Igeo:	Geo- accumulation Index
Kg:	Kilogram
LOD:	Limit of Detection
LOQ:	Limit of Quantification
M:	meter
Mg/l:	Milligram per liter
Mm:	millimeter
ND:	Not Detected
NTU:	Nephelo Turbidity Unit
PLI:	Pollution Load Index
Ppm:	Parts Per Million
RA:	Relative Abundance
SD:	Standard Deviation
SPSS:	Statistical Package for Social Science
TDS:	Total Dissolved Solid
TS:	Total Solid
TSS:	Total Suspended Solid

USEPA:	United States Environmental Protection Agency
V/v:	Volume by Volume
WT:	Water temperature
W/v:	Weight by Volume
WHO:	World Health Organization

CHAPTER ONE

INTRODUCTION

1.1 Background

Environmental pollution is a terrible consequence of expanding populations and exponential developments in industrial field (Gupta *et al.*, 2014). The disposal Industrial effluents to water bodies are one of the main causes of environmental pollution and degradation. Many of these industries do not have proper waste treatment facility (Emigilati *et al.*, 2015). Industrial effluents which contains metals, and their accumulation in sediments and biota, present a persistent threat to health of ecosystem (Zinabu *et al.* , 2018).

Textile industries use large volumes of water in their operations which generates huge amounts of effluents proportionally. The effluent contains a variety of chemicals from the various stages of desizing, scouring, bleaching and dyeing and finishing (Sivakumar *et al*., 2011).Toxic heavy metals and trace metals are the major pollutants originating from such types of industries which are very dangerous to water and soil and vegetables (Poornima *et al*., 2011).

Effluents from the textile factory commonly contain high concentrations of organic and inorganic chemicals and are characterized by high Chemical Oxygen Demand (COD), Biological Oxygen Demand (BOD), Total Dissolved Solids (TDS), pH, Total Suspended Solids (TSS) values, and low dissolved oxygen (DO) value as well as strong color. The color of the effluent causes aesthetic problems with reduction of the visibility of receiving water bodies (Mehari *et al*., 2015). Heavy metals particularly, Pb, Cd, Cr, Cu and Ni are widely used for production color pigments of textile dyes. They are highly toxic which can be transferred to the environment and accumulate in human body, aquatic life, natural water bodies and soil (Bhardwaj *et al*., 2014).

Heavy /trace metals are among the most common environmental pollutants. The occurrence in waters and sediments indicates the presence of natural or anthropogenic sources. Heavy metal accumulation in sediment could affect quality of water, bio-assimilation and bio-accumulation of metals in aquatic organisms, resulting in potential long-term implications on human health and ecosystem (Olbasa, 2017).

Farmers use wastewater to get an opportunity for direct economic benefits due to lack of access to other source of water. Land application of effluent waste may cause excessive accumulation of heavy metals in soil and plants. Their accumulation in different environmental compartment is a potential risk for human health (Emigilati *et al.*, 2015).

Plants are an essential component of regular human diet. The food of plant origin is an irreplaceable source of vitamins, minerals and micro and macro elements. But they are amenable to absorb and accumulate heavy metal species from the soil, water and air. Intake those in too high doses can lead to pathological changes (Retka *et al*., 2009). Perishable vegetables are often grown around urban areas, which are usually prone to heavy metal contamination. Continuous use of waste water for irrigation leads to the accumulation of heavy metals in vegetables which can accumulate high content of heavy metals. Heavy metal transfer from soil to plant depends on transfer factor (Shirkhanloo *et al.*, 2015).

Sediments are the ultimate sinks for heavy metals discharge in to the environment. In the hydrological cycle, more than 99.9% of heavy metals are stored in sediments and soils while less than 0.1% are dissolved in the water. It frequently contains higher concentrations of pollutants which is used as carriers and possible source of pollution which can be released back to the water body. Contaminated sediments are known to be responsible for degradation of water quality in the natural waters (Abata *et al.*, 2013).

Benthic macroinvertebrate (BMI) are the most preferred group in bio-monitoring studies. They have limited habitat and less moving ability. As a result they cannot change their habitats quickly. Furthermore they respond to any pollutants by changing their community composition. The life cycles of BMI are long enough to understand what the differences are in their habitats before and after the pollution (Turkman and Kazanci, 2010).

BMI are useful in evaluating water quality and the overall health of flowing water systems which are affected by physical and chemical characteristics of the stream or river. They are sensitive in varying degrees to temperatures, dissolved oxygen, sedimentation and scouring; nutrient enrichment, chemical and organic pollution. This sensitivity allows them to be effective indicators of specific anthropogenic disturbances and climate changes. The most commonly used indices in biological evaluation of rivers include species richness, evenness, diversity and dominance indices, Biological Monitoring Working Party(BMWP), Average Score per Taxon (ASPT) and Ephemeroptera, Plecoptera and Trichoptera(EPT) (Yazdian et al.,2014).

1. 2 Statement of the problem

Textile factories use huge amount of water in different process which generates huge amounts of wastewater proportionally. Approximately 100.000 commercial dyes and dyestuff are used around the world of which 10-15% dye stuffs are directly lost to wastewater. Over 700,000 metric tons of dyestuff produced annually. These effluents are rich in dyes and chemicals, which are non-biodegradable which may cause a major threat to health and the environment (Manikandan *et al.*, 2015).The release of residual azo dye into industrial effluents deteriorates the water quality in terms of color and photosynthesis in aquatic plants. The dye is very toxic which produce a significant impact on human health due to their mutagenic and carcinogenic effects (Hmd, 2011).

The use of these large varieties of dyes and chemicals makes textile factories greatest challenge for environmental pollution both in the forms of liquid waste production and its chemical composition. Dyes could also have high level of BOD/COD, color, toxicity, surfactants, fibers and turbidity and may contain heavy metals. Dyeing process are usually contributing chromium, lead, zinc and copper to wastewater. Copper is toxic to aquatic plants and life at concentrations at 1.0 mg/l (Kaur and Sharma, 2015).

Heavy metals are one of the major components of textile wastewaters. The increased production can produce adverse effects on soil biological properties and toxic to plants. These microorganisms contribute to nutrient acquisition by plants which are important for reducing fertilizer inputs in sustainable plant production systems. Toxic metals significantly reduce soil fertility and also inhibit enzyme activity in the soil and alter soil acidity. Metals inactivate enzyme systems which leads to physiological changes and can cause tissue and cell necrosis (Tiruneh *et al* .,2014).

Chemical and physical assessments are widely utilized to evaluate the extent of pollution of water bodies from industrial and other sources. However, the combination of biological assessment with Physico-chemical assessment is the most appropriate means of detecting effects of pollution on the aquatic systems, because it can detect cumulative physical, chemical and biological impacts of adverse activities to an aquatic (Mehari *et al* .,2016).

In Ethiopia, macroinvertebrate composition has been used as stream and rivers water quality indicator by different researchers (Mehari *et al.*, 2015); (Mehari *et al* ,2016) ; (Derso *et al.*, 2017); (Beyene *et al.*, 2009); (Ambelu *et al.*, 2013); (Abraha ,2007); (Wosnie and Wondie, 2014); (Lakew,2015). However, the published article on using aquatic macroinvertebrate to assess the effects of textile wastewater in Ethiopia is very rare. In Ethiopia, Some researchers assessed the effects of textile factory effluents in river water and sediment quality. But it is hardly possible to find published research which was conducted on the effects of textile factory effluents on nearby soils and vegetables in Ethiopia.

There was no detailed and comprehensive national water risk assessment studies conducted on Ethiopia's textile and garment sector. Thus, knowledge of impacts from industrial effluent on humans and surrounding environment is very limited. Some studies reported that the wastewater from the textile and garment sector in Ethiopia is possibly the largest source of industrial soil and water pollution (Sima and Restiani, 2017). The present study was tried to fill the gap by assessing the effects of the effects of textile factory on soil, vegetable, stream water and stream sediment.

1.3 Significance of the study

The finding of the study showed the effects of Kombolcha textile factory effluents on the nearby environmental media. The finding indicated the concentration of different physico-chemical and heavy metal in effluent, river water sediment, soil and vegetables. The finding will give very important clue for factory owners to take an appropriate intervention based on the finding of the result. The finding will also be helpful to be used as input for other textile factories which have similar conventional wastewater treatment system. The result will be expected to be used as a baseline for other researchers who are interested in this area since much works needs to be done in the future. This information will be communicated to the concerned authorized body in order to mobilize the community on the health risks of through the consumption goods that are directly or indirectly related with use of improperly treated textile effluents.

CHAPTER TWO

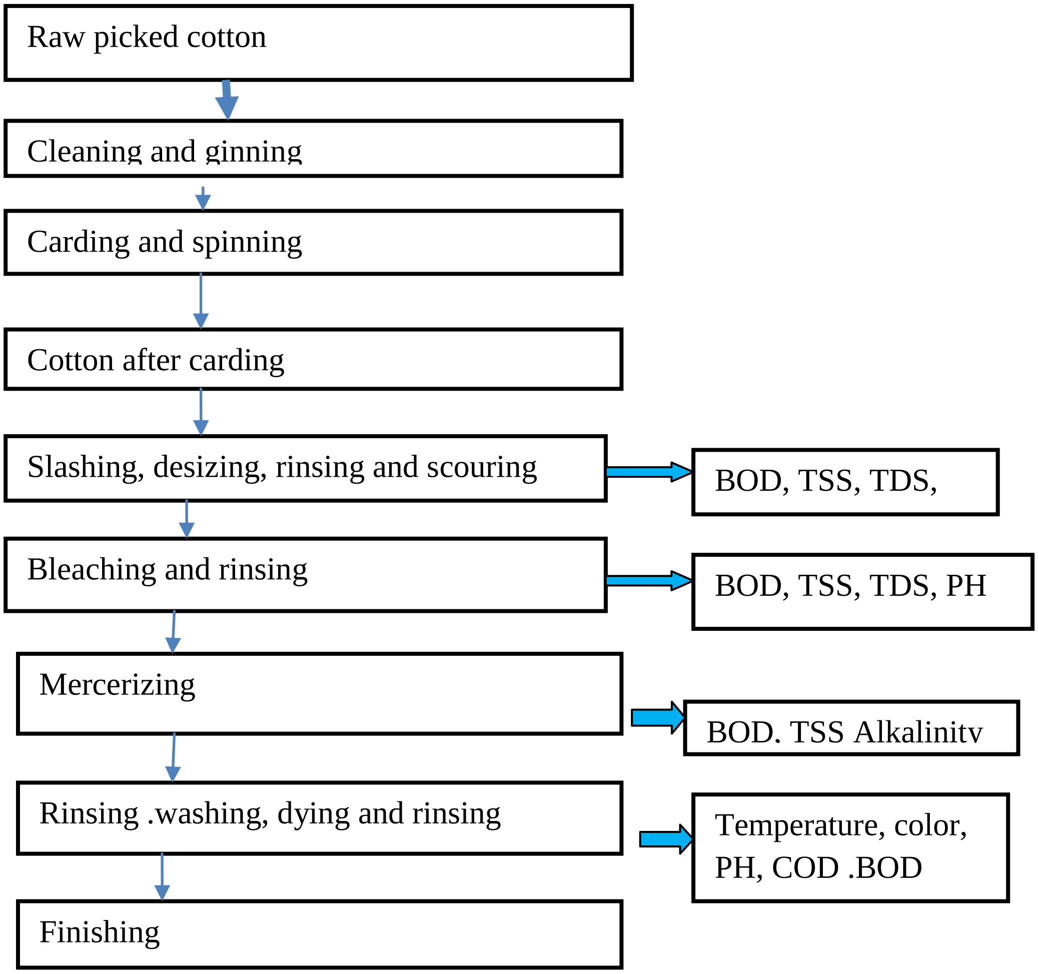
LITERATURE REVIEW

2.1 History of textile factory in Ethiopia.

The first textile factory in Ethiopia was established in 1939 in Dire-Dawa. Since 2010, the government of Ethiopia has given emphasis on potential of building a textile factory with governmental support, offering low-cost production and raw material and with a growing young population eager for jobs. The factory is one of the largest employers in Ethiopia, with 35,000 direct employees (cotton farming (10%) and textile/garment manufacturing (90%)), excluding the 500,000 engaged in the informal hand-loom weaving sector (Retka *et al*.,2009 ; (Abdella, 2008).

2.2 Textile printing and dying process

There are a number of processes that are conducted in textile factory from raw materials up to finishing. These are pretreatment, dyeing / printing, finishing. Pre-treatment includes desizing, scouring, washing, and other processes. In the process of dyeing, the dye is mainly dissolved in water. Printing is a special type of dyeing which is localized dyeing to a certain portion of the fabric that constitutes the design. In dyeing, color is applied in the form of solutions. The color is applied in the form of a thick paste of the dye in the process of printing. Both natural and synthetic textiles are subjected to a variety of finishing processes. In addition, in different circumstances, the singeing, mercerized, base reduction, and other processes should have been done before dyeing/printing. The most commonly used bleaching technologies are sodium hypochlorite bleaching; hydrogen peroxide bleaching and sodium chlorite bleaching. The first two techniques are the most common bleaching techniques. Normal concentration of chlorine dioxide in bleaching effluent is 10-200 mg/l. Chlorine dioxide is a strong oxidant which is very corrosive and toxic as well (Wang *et al.*, 2010).



(Islam and Guha, 2013).

Figure 1: Various steps involved in textile in cotton mill with their corresponding characteristics of waste

2.3 Characteristics of textile dyeing wastewater.

Continuous Discharge of industrial effluents is causing significant environmental problems. Hence the importance of the pollution control and treatment is the key factor for environmental sustainability and human future. The absence of proper wastewater treatment leads harm to the receiving water bodies and lands in the surrounding environments. High values of COD and BOD5, presence of particulate matter, sediments, oil and grease in the effluent causes depletion of DO. The reduction in DO has an adverse effect on the aquatic ecological and terrestrial system. Effluent from textile mills also contains chromium, which has a cumulative effect, and higher possibilities for entering into the food chain. The color of textile effluents is usually dark in color due the use of dyes and chemicals. This in turn affects the photosynthesis process, causing alteration in the habitat (Wang *et al.*, 2010).

Majority of wastewater generated from textile industry is in the process of dyeing and printing. On average, colored wastes contributes about 10-30% of the total BOD and in many cases reach 90%. Dyes also contribute about 2-5% of the (COD), while dye bath chemicals contribute about 25-35%. The accumulation of high in BOD and COD in dyes is very toxic to aquatic organisms including fishes (Hmd, 2011).

Textile wastewater contains high concentration of nitrogen in the forms of ammonia, nitrate, nitrite and organic nitrogen. Nitrogen can pose serious public health threat when present in drinking water above permissible concentrations. Nitrogen is commonly found in toxic water in the form of nitrate which is not dangerous but may cause methemoglobinemia in infants. But it becomes highly toxic when it is reduced to the form of nitrite through the activities of intestinal bacteria. Nitrates and phosphates are the main sources of nutrients to algae blooms in water bodies. The amounts necessary to trigger algae blooms in water bodies are not well established but concentrations as low as 0.01 mg/L for phosphorous and 0.1 mg/L for nitrate may be sufficient for eutrophication when other elements are optimal (Sivakumar *et al*., 2011).

Table 1 : Effluent composition and nature of pollutants in textile effluents (Assefa and Sahu, 2016)

Process	Effluent composition	Nature of pollutants
Sizing	Starch, waxes , carboxyl methyl cellulose (CMC) ,polyvinyl alcohol (PAV) ,wetting agents	High BOD, COD
Desizing	Starch ,CMC,PAV ,fats ,waxes pectin	High BOD ,COD, SS ,DS
Bleaching	Sodiumhypochlorite,Cl_2,phosphate,NaOH, H_2O_2 ,acids ,surfactants ,$NaSio_3$,short cotton fiber	High alkalinity, TSS
Mercerizing	NaOH ,cotton ,wax	High PH, low BOD , TDS

Dying	Dyestuff urea ,reducing agents ,oxidizing agents ,acetic acids ,detergents ,wetting agents	Strongly colored ,high , BOD , TDS ,low TSS heavy metal
Printing	Pastes ,urea ,starch ,gums , oils ,binders ,acids thickeners ,cross linkers ,reducing agents and alkalis	Highly color, high BOD , oily appearance ,TSS slightly alkaline

2.4 Physicochemical and heavy metal concentration in textile effluents

A study conducted by (Kumar *et al*.,2013) on the characteristics of textile effluents, TDS and EC were 4396.8±8.41 mg/l and 6.87±1.42ds/m respectively. The mean concentration of pH, DO and BOD were 6.87±0.31, 1.18±0.14mg/l and 2462.5±11.82 mg/l respectively. In the same way, the mean concentration of Chloride, NO_3 and SO_4 were 1298.00±10.95, 1139.5±7.72 and 954.5±5.97 mg/l respectively. Furthermore, the concentration of Cd, Cu and Cr were detected with the average value of 35.99±3.73, 29.50±2.60 and 47.54±4.37 mg/l respectively.

A study conducted by (Rajasthan, 2014) , the average concentration of heavy metals in textile effluents were 1.107±0.034, 4.794±0.17, 2.058±0.16 and 3.64±0.22 for Pb, Cu, Cd and Cr respectively. In addition, the results revealed that pH of the waste water ranges from 7.45 to 9.43 and EC between 0.97 to 1.25 mho/cm and total solids varies from 965.8 to 1562 mg/L. The total Hardness ranges between 568 to 1138 mg /l. The mean concentration of Chloride and DO values were in the range between 295 to 578.3 mg/L and 17.1 to 36.20 mg/L respectively.

A study conducted by (Das *et al.*, 2011) ,the concentrations of Cd, Pb, Cr and Cu in textile effluents were seasonally varied from 0.23-0.65, 0.96-3.89, 1.16-3.85 and 11.6-39.23 g/L respectively. Another study conducted by (Karim, 2015) reported that the pH and temperature of inlet effluent was 7.6 and 37℃ respectively while their corresponding outlet effluent was 8.38 and 35℃ respectively. Similarly, the mean concentration of TSS, TDS and BOD_5 were 900mg/l, 2933mg/l and 220mg/l respectively. In the case of outlet effluent, of the corresponding concentration of TSS, TDS and BOD_5 were 800mg/l, 1766mg/l and 120mg/l respectively.

A study conducted by (Hayyat *et al*., 2013) , textile effluent was brownish black in color. Its pH was 8.55 which were highly alkaline in nature while the mean value of turbidity was 75 NTU. The mean concentration of EC and DO in effluent was 5.72 dS/m and 0.12ppm respectively. Similarly the mean concentration of BOD and chloride was 216 mg/l and 7.1 mg/l respectively. The mean value of TDS and TSS was 1330 and 3900 mg/l, respectively. Another study reported by (Iram *et al.*,2013) , find out that the pH, temperature ,EC, TDS and turbidity was in the range of (7.16–8.29), (17.8–28.8 ℃), (1,005–3,347 μs/m), (754.3–2,519.5 mg/L), (272.8–487.05 NTU). Additionally, total hardness, nitrates, chloride, and sulphate were in the range of (300–452 mg/L), (10.11– 22.95 ppm), (127.72–396.16 ppm), (15.97–87.38 ppm) respectively. Furthermore Cd, Cr, Pb and Cu was in the range of (0.005–0.03 ppm), (0.2–7.4 ppm), (0.12–0.73 ppm), and (0.01– 0.06 ppm) respectively.

A study reported by (Adebayo *et al.,* 2007) on the effluents of textile, the average temperature, pH, EC ,turbidity was 27℃, 7.6 ,798μs/cm ,28 NTU respectively. In the same way, the mean value of total alkalinity, total hardness, TSS, BOD, DO were 1000mg/l, 1400mg/l .602mg/l, and 0mg/l respectively. Furthermore the average concentration of Pb, Cd, Cr, and Cu were ND, ND, 0.746mg/l and 0.178mg/l respectively.

The study conducted by (Mehari *et al*., 2015), the average DO and BOD of the effluent were 5.7 mg/l and 38.3mg/ respectively. In the same way, its average pH and effluent temperature were 8.1 and 23℃ respectively. Similarly, the mean concentrations of TDS, EC, total hardness and total alkalinity in the effluents were 501.65 mg/l, 942.9μs/cm, 95mg/l and 236mg/l respectively.

A study conducted by (Assefa and Sahu, 2016), on performance analysis of Bahirdar textile wastewaters, some physicochemical parameters showed great reduction of from inlet effluent to outlet effluent. Hence the pH reduced from 9.1 to 6.9 (24.17%) while BOD reduced from 476mg/l to 103mg/l (78.36%).In the same manner, TSS and TDS was reduced from 5996mg/l to 271mg/l (95.48%) and 5012mg/l to 2914mg/l (41.56%) respectively. Another study conducted by (Woldeamanuale, 2017) , the mean pH , EC, TDS, BOD , total alkalinity , chloride , and sulphate with the average value of 5.2 , 1205.3 ,1370 ,878.2 ,772.56 ,3405.2 ,and 500 mg/l respectively.

2.5 Physicochemical and heavy metal characteristics of adjacent river/stream

A study reported by (Parameswari, 2014) , the mean concentration of pH, EC and TDS of river water that receives textile effluents were 6.95, 0.12ds/m and 80 mg/l respectively. Furthermore, the mean concentration of sulphate and chloride was 15.2mg/l and 25.0 mg/l respectively.

A study conducted by (Poornima *et al*., 2011) , the mean concentration of Cr and Pb in textile effluents were 1.186± 0.654 and1.74 ± 0.546 respectively. The maximum values of Cr in soil contaminated by textile effluents were 5.950 mg kg-1 with average value of 3.488 ±1.275 mg/kg. In the contrary, the concentration of chromium water receiving the effluents samples mean average value of 0.456 ± 0.288 mgL-1.finally the researcher concluded that the order of metal contamination looks like the order: Soil > Effluent > adjacent pond water> Unpolluted control (reference site). This relationship indicates the role of effluents towards enhanced metal accumulation in the nearby soil system. The study conducted by (Das *et al.*, 2011), on the concentration of heavy metals in rivers adjacent to textile factory, the concentrations of Cd, Pb, Cr and Cu of Buriganga River water seasonally and spatially varied from 0.11-2.37, 1.18-8.59, 9.45-293.08 μg/l and BDL respectively. While the level of contamination in Karnatoli River, the concentrations of Cd, Pb, Cr and Cu were also seasonally and spatially varied from, 0.13-1.53, 0.53-6.8, 2.75-7.0 and 8.6-48.14μg/L respectively. Lowest concentrations of heavy metals were observed during rainy season which might be due to the dilution effect of rainfall whereas the highest concentration of these metals were found during dry season as industrial effluents are less diluted due to recede water in the river in this season. .

The study conducted by (Mehari *et al*., 2015) , on the effects of Bahirdar textile factory effluents on the head of Blue Nile River water quality was determined. The mean concentration of DO and BOD were 6.95mg/l and 20.6 mg/l respectively. The pH and water temperature was 7.4 and 22.35℃ respectively.The mean concentration of TDS, EC, total hardness and total alkalinity were 288mg/l, 555.85μs/cm,113.665 and 100.835 mg/l respectively. The mean concentration of DO was higher at the upstream site of the river, whereas BOD5, TDS, and total alkalinity were higher at wastewater outlet of the factory site.

2.6 Heavy metals in soil, vegetables and sediment

A study conducted by (Kumar *et al*.,2013) , the mean concentration of different heavy metals in textile effluent contaminated soil and vegetables were determined. The mean concentrations of pH, EC, Cd, Cu, and Cr in soil samples were 8.15±0.07, 3.04±0.14ds/m, 10.76±2.56 mg/l, 14.56±3.5mg/l and 7.85±1.32 mg/l respectively. While the mean concentration of Cd, Cu and Cr in vegetable samples were 1.95 mg/l, 3.16 mg/l and 1.30 mg/l respectively.

A study conducted by (Deepali and Gangwar, 2010) , on concentration of heavy metals in textile wastewater contaminated soils were 568.00±4.163, 308.40±3.02, 668.80±0.559, 109.54±0.315, 191.25±19.35, 83.62±0.119, ND µg/gm. respectively. Another study by (Poornima *et al.*, 2011) also reported that the mean concentration of Cr and Pb were (1.186mg/l, 1.74mg/l) and 3.488mg/l, 1.617mg/l) in effluents and soils respectively. Another study reported by (Karim, 2015), the study also assesses the pH of nearby soils and sediment. Were 7.7 and 7.92 respectively.

A study conducted by (Sorsa *et al.*, 2015), the concentrations of Heavy metals in sediment of the nearby stream to which the textile effluent is directed was measured. The result showed that some of the physico-chemical parameters such as pH, EC and TDS of the waste water from the two sampling sites were above provisional discharge limits set at national and/or international levels. The average concentrations in the sediment were 0.975 mg/Kg for Cd; (31.03 mg/Kg for Cr; 67.03 mg/Kg for Cu; 4.27 mg/Kg for Pb The concentration of almost all detected heavy metals in samples analyzed from both sites generally followed the order: sediment > flowing waste water. The heavy metals levels detected in sediment samples from both sites followed the order: Cu > Cr > Pb > Cd. Even though the concentrations of heavy metals in samples analyzed were below acceptable ranges of the provisional discharge limits, their accumulation over time and the potential threat on environment health and disruption of ecological integrity was overemphasized. This study suggests quick intervention and closes monitoring to arrest and solve the growing environmental pollution and associated problems in the area.

2.7 Macroinvertebrate monitoring

BMI are very large and taxonomically diverse array of organisms, encompassing any bottom-dwelling invertebrate large enough to be observed without magnification. The abundance and distribution of these BMI are highly affected by Hydro chemical characteristics like nutrient loading, dissolved oxygen, and organic and inorganic pollution, and hydro physical ones like temperature, turbidity, and Ultra Violet irradiation (Burgess, 2015).They are the most preferred group in bio-monitoring studies Because of their limited habitat and less moving ability. They cannot change their habitats quickly and respond to any pollutants by changing their community composition. In addition, the life cycles of this group are long enough to understand what the differences are in their habitats before and after the pollution (Turkman and Kazanci , 2010).

BMI have been used extensively in some countries as a biological indicator to assess the health status and ecological integrity of the waters, due to their crucial role in the food chain. They are also very sensitive to the environmental changes and characteristics of habitat caused by the presence of human activity, both natural and artificial. Most of the time, BMI are used to assess the deterioration of water bodies caused by organic pollution. But now a days they are used to assess the deterioration of water bodies caused by the accumulation of heavy metals ,sedimentation and climate change (Kartikasari *et al* .,2013).

Biological integrity is the ability of an aquatic ecosystem to support and maintain a balanced, adaptive community of organisms having species composition, diversity, and functional organization comparable to that of natural habitats within a region. River biological integrity reveals itself in the condition, abundance, and diversity of its biota. Protecting the biological integrity of water protect human uses of that water, whether for drinking, fishing, washing, flushing, shipping, irrigating, generating electricity, or making money in countless ways (Emana, 2016).

Healthy habitats of water bodies are characterized by high diversity and number of different species. The accumulation nutrient sources leads to the organic contamination of stream/river).As a result, the pollution status and number of tolerant species increases while sensitive species begin to disappear. The diversity and abundance of BMI in highly populated water bodies are decreasing. Important findings about the structure of a stream are gained by using the diversity indices (Mandaville, 2002).

BMI species are differentially sensitive to many biotic and a biotic factor in their environment. Consequently, macro invertebrate community structure has commonly been used as an indicator of the condition of an aquatic system (tolerance). In several countries, it has been developed many ecological indices for water quality monitoring in addition to the environmental index, such as (Biological Monitoring Working Party-BMWP, Average Score per Taxa-ASPT, Family Biotic Index-FBI, Extended Biotic Index-EBI, EPT% (Ephemeroptera, Plecoptera, Trichoptera) and EPT/Chironomus%. Order Ephemeroptera, Plecoptera and Trichoptera (EPT) taxa are very sensitive against pollutants such as metals and insecticides. But they usually occurred in clean water with the presence of high DO (Kartikasari *et al* .,2013).

2.8 Conceptual framework

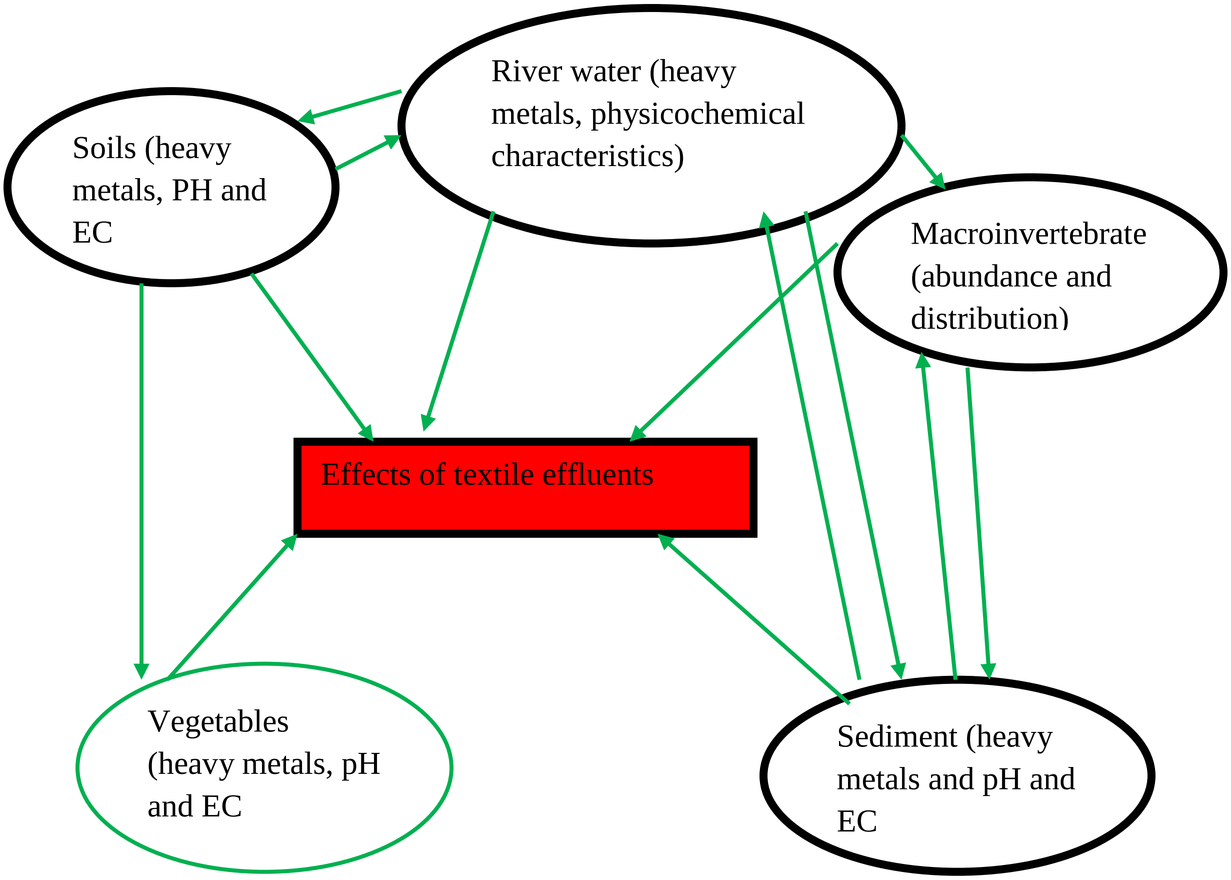


Figure 2: Conceptual framework of study adopted by reviewing different literature

CHAPTER THREE

OBJECTIVE OF THE STUDY

3.1 General objective

The objective of the study was to assess the effect of Kombolcha textile factory effluents on Leyole river, soil, and vegetables (Swiss chard) North East Ethiopia, 2018.

3.2 Specific objectives

- To determine the concentration of selected heavy metals (Pb, Cd, Cr and Cu) in soil, vegetable, river sediment, water and effluent.
- To determine the physicochemical characteristics of Kombolcha textile factory effluents and Leyole river
- To examine the effects of textile effluent on aquatic biota a using macroinvertebrate as bio indicators

CHAPTER FOUR

MATERIALS and METHODS

4.1 Study area

Kombolcha city is located in South Wollo Zone of the Amhara region, North East Ethiopia, which is located 380 km far from Addis Ababa. It has latitude of 11°5′N and longitude of 39°44′E with an elevation between 1842 and 1915 meter above sea level. The town covers 125 km^2 comprising rural upland landscapes in the north and populated lowlands in the south. Different land use types exist in the area, with extensive agriculture and forest land in the upland zone and peri-urban and heavily urbanized and industrial areas mainly in lowland plains. The area has annual bimodal rainfall seasons, usually from February to April, with heavier rainfall from July to September. Several tributary rivers rise from the surrounding escarpments and drain into two rivers, the Leyole and Worka rivers, which flow through an industrial zone of Kombolcha (Zinabu *et al* ., 2018) ; (Zinabu *et al* ., 2010).

Borkena River is the largest water body around Kombolcha which crosses the town emerging from the North West and running to the South East direction and finally entering to the Awash River. It receives effluents from industries directly and indirectly through its tributaries rivers mainly from locally named Worka and Leyole. The town is endowed with favorable conditions for developing urban agriculture through the application of irrigation using the available water sources. Hence, many residents in the city is cultivating vegetables like cabbage, lettuce, tomato, potato, onion, pepper and perennial crops including maize and coffee (Awole , 2015).

Kombolcha textile factory was established in 1986 in Kombolcha town. It is engaged in the production of towels, bed sheets and home fabrics using cotton. The factory consumes an average of 400,000 m^3 of water, 3,041,955kg (3042 ton) Lint cotton and 13,791,242.02 Kilo Watt Hour Electric power annually. The factory can produce 25 million square meter of fabric annually. The downstream part of a river is used for domestic activities including drinking and irrigation by using the wastewater (Serkalem *et al*., 2014).The factory uses lagoons as a treatment for textile effluent

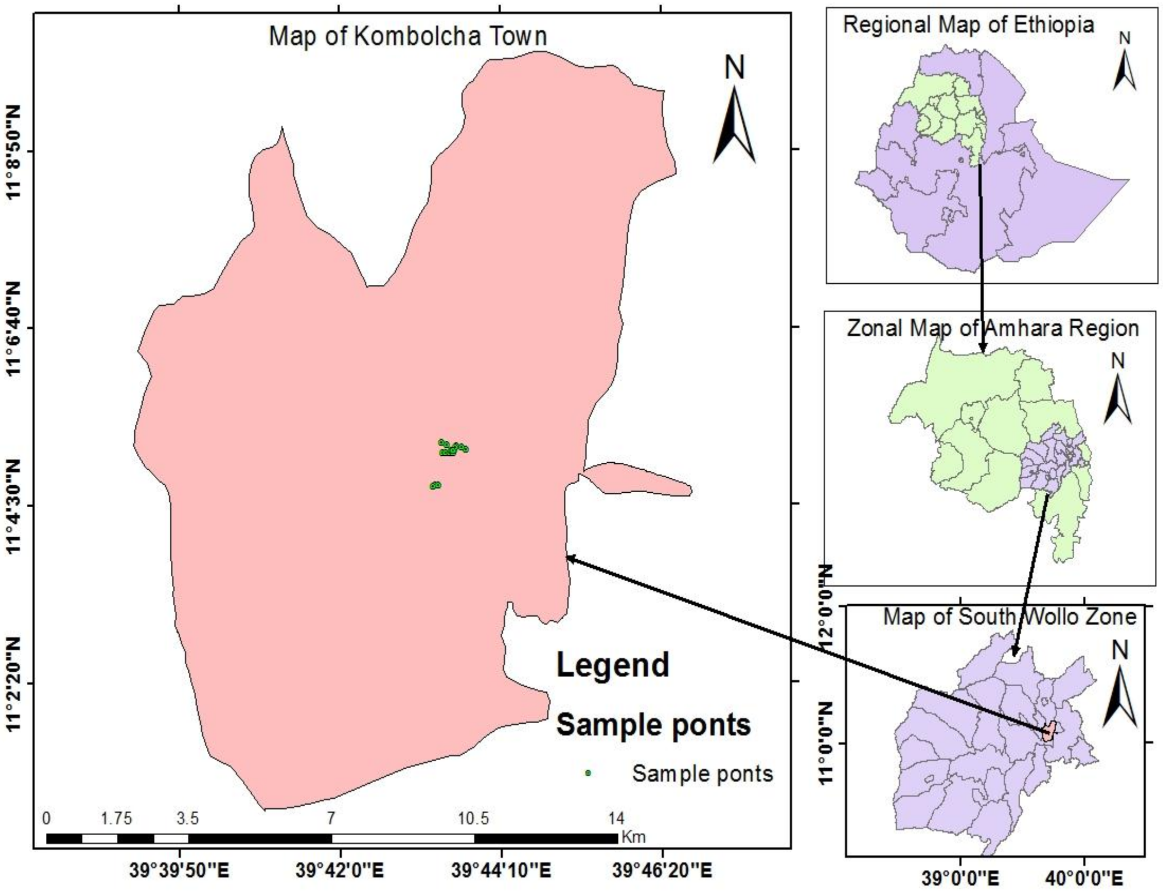


Figure 3: Map of kombolcha town of the study area.

Table 2: Summary of the sampling point along the Leyole river in kombolcha for sediment, river water and macroinvertebrate.

Site	Description
Ur2	The last upper stream point which is one reference point 200m above Ur1
Ur1	The lower upstream point before effluent joins stream (reference) 100m above the joining point of effluents.
Dr1	The immediate downstream point below after the joining point 5m below the joining point.
Dr2	The second downstream point below Dr1 200m below Dr1
Dr3	The last downstream sampling point below Dr2 200m below Dr1.

4.2 Study period

The study was conducted from May 1 to 30, 2018

4.3 Study design and variables

A cross sectional study of physical, chemical and biological components of Leyole stream, soil, vegetables and effluents was carried out.

Table 3: Physicochemical and heavy metal parameters with their corresponding Environmental compartments

No	Parameters	Influent	Effluent	River	Sediment	Soil	Vegetable
1	pH	yes	yes	yes	yes	yes	
2	EC	yes	yes	yes	yes	yes	
3	Ambient T^o	yes	yes	yes			
4	Water T^o	yes	yes	yes			
6	TS	yes	yes	yes			
7	TDS	yes	yes	yes			
8	TSS	yes	yes	yes			
9	Hardness	yes	yes	yes			
10	Alkalinity	yes	yes	yes			
12	BOD	yes	yes	yes			
14	DO	yes	yes	yes			
15	Nitrates	yes	yes	yes			
16	Sulphate	yes	yes	yes			
17	Chloride	yes	yes	yes			
18	Cd		yes	yes	yes	yes	Yes
19	Pb		yes	yes	yes	yes	Yes
20	Cr		yes	yes	yes	yes	Yes
21	Cu		yes	yes	yes	yes	Yes

4.4 sample collection and preparation

4.4.1 Sample Collection and preparation river water and effluent

Five composite samples were collected from the river water of which three of them were from downstream with immediate downstream point (Dr1), the second downstream below Dr1 (Dr2) and Dr3 was the final downstream sampling Point . The remaining two samples (Ur1 and Ur2) were collected from the upstream which was used as a reference site. The samples were taken in the effluents mixing zones within a 5 m long section immediately downstream of the effluent discharge points into the Leyole river. The mixing zones of Kombolcha's factories were not exactly determined. According to literature it is assumed that a 5 m long section was sufficient for complete mixing of the effluent, containing both the zone of initial dilution (ZID), near the effluent outfall, and the chronic mixing zone (impact zone) (Zinabu *et al* ., 2018). The samples were collected proportionally at 1/4, 1/2, and 3/4 of the width of the river and three times a day (morning, afternoon and before sunset) in a one liter polyethylene plastic container.

In the same way, six composite samples were collected from the effluents. The first three of them were collected from inlet effluents (immediately after its production, in midway journey and before entrance of preliminary treatment plant).The remaining three samples were collected from the outlet effluents (immediately after the release of treatment plant, in midway and before the joining of the river). In-situ measurements of DO, water T^o, ambient T^o, pH, and EC were measured using multiparameter probe (Hach-Model-HQ30d multiparameter digital meter). Other parameter of water and effluent determination was carried out by collecting and storing samples by clean polythene bottles previously cleaned by washing in non-ionic detergent. The containers were rinsed with tap water and thereafter soaked in 10% HNO3 for 24 h and finally rinsed with de ionized water. During sampling, sample bottles were first rinsed with the sampled water three times and then filled with sample. The time, date, location of the sample and other relevant information's of the sampling sites information was recorded properly for easily identification (Moges, 2014); (APHA, 2012).

After collection, the samples were stored in ice box containing well frozen ice cubes until they were taken to the laboratory for analysis. The bottles were filled and then sealed tightly to avoid head space that cause loss of samples because of oxidation. Samples for water quality were collected before sampling for macroinvertebrate to prevent contamination. The wastewater and stream samples were filtered before chemical analysis and heavy metal determination. For the assessments of heavy metals, Samples of stream water and effluent samples were preserved with 1 mL concentrated H2SO4 to keep the pH < 2 in order to prevent metal adsorption onto the sample container wall (Zinabu *et al.*, 2018) ; (APHA, 2012).

4.4.2 Soil Sample collection and preparations

Six composite soil samples were collected randomly from study and control sites. Three of the soil samples were collected from textile effluent contaminated soil which was used for cultivation of vegetables. The remaining three soil samples were collected from areas it was free from the textile effluent contamination and was used for vegetable cultivation at the time sample collection was taken as reference sample. From each sampling point, three samples were collected on the basis of the depth of the soil, 0 to5 cm, 5 to 10 cm and 10 to 15 cm depth of surface soil was dug from control and study site. An approximately of 50g soil resided beneath the dug surface soil was collected into a sterile container. Then it was put in clean plastic bags and transported to the laboratory. The samples were air-dried, crushed and passed through 2mm mesh sieve. After that it was put in clean plastic bags and sealed and ready for analysis (Herk, 2012).The pH soil was determined by adding (1:2.5) 10g of soil with 25 mL of doubly distilled water twice, stirred with a magnetic stirrer for 60 minutes at an ambient temperature of 22 ℃ ± 2 ℃. The suspension obtained was left to stand for 2 hours protected from the air.EC was measured using and electrical conductivity meter in 1:1 soil to water ratios (Mohad- Aizat *et al.* , 2014).

4.4.3 Vegetable Sample collection and preparation

In the same manner, six composite samples of vegetable were collected exactly from the point where soil sample was taken. Three of them were collected from contaminated soil through textile effluents and the remaining three were collected from control site. The samples were collected and placed in polyethylene plastic bags. Then, it was washed with tap water followed with distil water (DW).Then it was cut into small pieces and dried at 105°C for 18 hours. After drying, the samples were ground into powder form, which can easily pass through a 0.5 mm mesh and kept dry in a polyethylene bag in desiccators until analysis (Shirkhanloo *et al.*, 2015).

4.4.4 Sediment sample collection and preparation

Five sediment samples were collected from stream where water sample were collected. Sediment samples were collected from pool microhabitat at depths of 0 to 15 cm. The samples were wrapped in aluminum foils then stored in plastic bags and transported to the laboratory for heavy metal extraction and determination. Sediment sample was oven dried at 105°C for 24 h, followed by grinding and sieving using 0.18 mm sieve. 0.5 g of dry sediment samples were poured into a graduated test tube and mixed with 2 ml of aqua regia 1:3 (1 conc. HCl: 3 conc. HNO3). The mixture was digested on a hot plate at 95°C for 1 h and allowed to cool to room temperature. Then, the samples were diluted to 10 ml using distilled water and left to settle overnight. The supernatant was filtered prior to analysis using FAAS (Kihampa, 2013). The PH was determined by balancing of an ion mass of 10 g of sediment was added 25 mL of doubly distilled water twice, stirred with a magnetic stirrer for 60 minutes at an ambient temperature of 22 °C ± 2 °C. The suspension obtained was left to stand for 2 hours protected from the air. EC was measured using and electrical conductivity meter in 1:1 soil to water ratios (mohad-Aizat *et al.* , 2014).

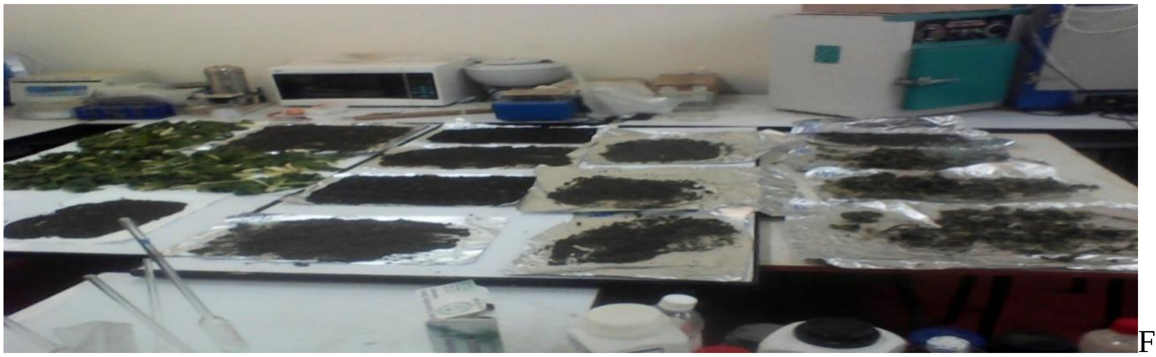


Figure 4: Soil and vegetable sample dried and prepared at room temperature

Figure 5: Crushing of dried soil, vegetable and sediment samples by using pestle and mortar

4.4.5 Macroinvertebrate sample collection

Samples of aquatic macroinvertebrate were taken by using D frame deep net with mesh size of 500µm from five selected points of Leyole stream. The bottom sediment was disturbed by foot during sampling in order to collect the BMI. In the field, visible organisms were removed by using forceps and put in to the specimen bottles. All samples were sorted live in a white plastic tray and then poured in to vials and preserved with 80% ethanol until laboratory analysis and counting. All the organisms in the sample were enumerated and identified to the family level using a dissecting microscope at Wollo University, Department of Environmental Health Science laboratory by using standard identification guideline (Bouchard ., 2004); (Galicia *et al*., 2011). A total of 20 sub samples were collected from each sampling point and merged to make one pooled sample per site from multi habitat using standard guideline. Sampling was done for a standard three minutes by disturbing a 1 m^2 area for each microhabitat (Mereta, 2013); (Nyakeya1 *et al*., 2009); (Abah *et al* ., 2018).

To obtain a visual record of sampling sites, digital photographs of the water body, upstream and downstream of the sampling locations were taken during sampling periods. Furthermore, for integration into a Geographic Information System (GIS) database longitude and latitude, and elevation of each sampling site were recorded using a global positioning system unit.

Figure 6: Sampling of benthic macroinvertebrate in Leyole river Kombolcha North East Ethiopia 2018

Figure 7: Identification of collected macroinvertebrates by using dissecting microscope

4.5 Habitat Quality Assessment and Human Disturbances Index

Habitat assessment was conducted at each sampling station using standard guideline of Rapid Bio-assessment Protocols for Use in Streams and Wadeable rivers (Barbour *et al*.,1999). Human disturbance score for each sampling site was assessed by using standard guideline of protocols for completing the biological monitoring wetland human disturbance assessment protocol (MEDP, 2013).

Physical parameters of the stream such as water depth, water velocity, and water width were measured. Canopy covers were measured on the left and right bank, and in four directions (upstream, downstream, left, and right) in the center of the channel cross section as partly open, partly shaded or shaded. Substrate composition were determined by size tallies, performed by placing a finger into the water and classifying the size of the particle first touched as bedrock (> 4000 mm), boulder (250–4000 mm), cobble (64–250mm), coarse gravel (16–64 mm), fine gravel (2–16 mm), sand (0.06–2.00 mm), fines (<0.06 mm),wood, hardpan (firm, consolidated fines). Embededness percentages were visually estimated from the area immediately surrounding each sampled particle (Derso *et al.*, 2015).

Figure 8: Habitat condition of Leyole river at different sampling point of the study area, Kombolcha North East Ethiopia 2018.

4.6 Digestion Method samples for heavy metal extraction

4.6.1 Wastewater and stream digestion for heavy metal analysis

For each water and wastewater sample, 100 mL was transferred to a conical flask. Then 5 mL of concentrated HNO_3 and a few boiling chips was added. The digestion was conducted under a slow boil and concentrated HNO_3 was added until digestion was completed. After that, the flask walls were washed with water and then filtered. The Filtrate was transferred into a 100 mL volumetric flask with two 5 mL portions of water. The filtrate was cooled and diluted to 100 mL in the volumetric flask 10mL of the solution was taken for metal determination (Syuhadah *et al.*, 2015).

4.6.2 Soil Digestion for heavy metal analysis

1.0 g of soil sample was measured. Then 5 mL of concentrated HNO3 was added to the measured soil sample and heated for 30 minutes which continues until the sample was completely digested. Then the mixture of the digestate and HNO_3 was evaporated to 5 mL and allowed to cool at ambient temperature. The digestion was continued by adding 2 ml of de-ionized water and 3 mL of 30% H_2O_2 then 1 mL aliquots of H_2O_2 was continued to be added until bubbling subsided. Then the volume was reduced to 5 mL then; 10 mL of concentrated HCl was added and heated for 15 minutes. Finally, the digestate was filtered and made up to 100mL with de-ionized (DI) water (Syuhadah *et al* .,2015).

4.6.3. Vegetables Digestion for heavy metal analysis

Portions of plant material was ground in a 30-50 ml porcelain crucible, and weighed to the nearest gram. After that, a porcelain crucible was placed in a furnace, and the temperature was increased gradually to 550℃. Ashing was continued for 5 hours after attaining 550℃. The furnace was shut off and the door was opened cautiously for rapid cooling. Then the product was dissolved in 5 mL portions of 1N hydrochloric acid (HCl), and was mixed. After 20 minutes, the volume was made up to 100 mL using distilled water (Syuhadah *et al.*, 2015).

4.7 Methods of assessment of contamination in soils and sediments

Contamination level of soils and sediments caused by heavy metals was assessed by conducting geo accumulation index (*I* geo), Concentration Factor (CF) Degree of Contamination (DC) and Metal Pollution Load Index (PLI)

4.7.1. Geo accumulation index (I geo)

The index of Geo accumulation (*I* geo) is widely used in the assessment of contamination by comparing the levels of heavy metals obtained to background levels originally used with bottom sediments. It was calculated using the following equation:

$$Igeo=\log_2 (Cn/1.5Bn) \text{ --- } (1)$$

Where, *C*n represents the measured concentration of the elements studied and *B*n is the geochemical background value of the element in fossil argillaceous sediment (average shale).The following classification is given for geo accumulation index: <0 = practically unpolluted, 0–1 = unpolluted to moderately polluted, 1–2 = moderately polluted, 2–3 = moderately to strongly polluted, 3–4 = strongly polluted, 4–5 = strongly to extremely polluted and > 5 = extremely polluted (Syuhadah *et al*., 2015).

4.7.2 Pollution Load Index

The pollution load index (PLI) is the ratio of element concentration in the study to the background content of the abundance of chemical elements in the continental crust. It was determined by the following equation.

$$PLI= (CF1 \times CF2 \times CF3 \times CF4 \times CF5 \times CFn)^{1/n} \text{ --- } (2)$$

PLI=pollution load index

CFn=concentration factor of heavy metals

The PI of each element is classified as either low ($PI \leq 1$), middle ($1 < PI \leq 3$) or high ($PI > 3$) (Naggar *et al* ., 2018).

4.7.3 Contamination Factors

Contamination factor (CF) is defined as the relative concentration of heavy metals caused by anthropogenic factors compared to the relative natural contamination level. Individual Contamination factors for heavy metals are calculated based on the following formula:

$CF=Mx/Mb$-- (3)

Where;

Mx is the concentration of the target metal

Mb is the concentration of the metal in the selected reference background,

Contamination factor is defined according to four categories as follows: $Cf < 1$ low contamination factor $1< Cf <3$ moderate contamination factor $3< Cf <6$ considerable contamination factor $Cf > 6$ very high contamination factor (Naggar *et al* ., 2018).

4.8 preparation of standard solution

Standard solutions were prepared to determine the concentration of the target parameters in the sample solutions. Intermediate standard solutions (10 mg/l) of each heavy metal was prepared from stock standard solutions containing 1,000 mg/l of Cu, Cd, Cr and Pb. Appropriate working standards was also be prepared for each of the metal solution by serial dilution of the intermediate solutions using de ionized water. Analytical wavelengths, color of the flame and slit width were adjusted according to the instrument operation manual for better sensitivity and working standards. Calibration curves were plotted with four points for each of the trace heavy metals standard using absorbance against concentrations (mg/l). Immediately after calibration using the standard solutions, the sample solutions were aspirated into the FAAS (Nov 400 p) instrument and direct reading of the metal concentrations was recorded. Triplicate determination was conducted out on each sample(Assefa and Sahu, 2016); (APHA, 2012); *(Deribachew et al.*, 2015).

4.9 Data processing and analysis

Physico-chemical and heavy metal analysis were conducted by using standard guidelines of (APHA, 2012) . Physicochemical characteristics of water, wastewater, soil and sediments were conducted in Wollo University, while heavy metals as well as nitrate and sulphate were determined in Amhara Design and Supervision Work Enterprise (ADSWE) in Bahirdar.

Figure 9: Laboratory analysis of selected physicochemical parameters in the laboratory.

Data generated in study was analyzed by using Microsoft excel spreadsheet and SPSS (version 20). Non parametric analysis of Mann Whitney test was applied to assess the significance difference in sampling station of the investigated parameters of heavy metals and physicochemical characteristics at 95% level of significance. The result of the finding is presented in tables and figures accordingly. Descriptive statistics were used to analyze the mean value and standard deviation of physicochemical and heavy metal concentration. Bivariate Spearman correlation test was also applied to test the relationship between various physicochemical, heavy metal concentration and macro invertebrate indices along the different sampling sites. The result was also compared with the available different national or international standard limits as well as other related studies which were conducted in different parts of the world.

MI metrics were determined at each sampling point for determining the quality of the streams. Shannon diversity and Simpson diversity index were examined to determine the diversity of BMI in five sampling points of Loyale stream sampling points. Furthermore Pielou evenness index (J) was determined in each sampling point to assess the evenness of their distribution among the different sampling point (Turkman and Kazanci,2010); (Smith and Wilson, 1996).

Shannon-Wiener Diversity Index (H') is a diversity index that incorporates evenness and richness. A high H indicates good water quality. It was calculated by using the following formula.

$$H' = -\Sigma [(ni / N) \times (\ln ni / N)] \text{ ———————————— } (7)$$

H' = Shannon Diversity Index

Ni: =Number of individuals belonging to" I" species

N=Total number of individuals

H is ranging from 0 for a community with a single family to over 7 for a very diverse community.

Simpson Diversity Index ***(***(SDI) It is a diversity indices derived by Simpson in 1949 which varies from 0 to 1.But while calculating, final result is subtracted from 1 to correct the inverse proportion.

$$SDI= [\Sigma ni (ni-1)]/N (N-1) \text{ ———————————— } (8)$$

SDI: Simpson Diversity Index

ni: Number of individuals belonging to i species

N: Total number of individuals

Pielou Evenness Index (Shannon evenness index) (J)

This index was derived from Shannon index by Pielou in 1966. The ratio of the observed value of Shannon index to the maximum value gives the Pielou Evenness Index result. The values are between 0 – 1. When the value is getting closer to 1, it means that the individuals are distributed equally (Turkman and Kazanci,2010);(Smith and Wilson, 1996).

J' = H' / H' max————————————————————————(9)

J': Pielou evenness index

H': The observed value of Shannon index

H' max: lnS

S: Total number of species

Family Biotic Index (FBI)

It is a biotic index which was calculated by multiplying the number of individuals of each family by an assigned tolerance values for the specified family. The assigned tolerance values ranges from 0 to 10 for families and increase as water quality decrease. It was calculated by using the following equation (Hilsenhoff, 1988).

HFBI =∑ [(TVi) (ni)]/N-- (10)

Where, TVi is the tolerance value of family i and ni is the total number of individuals of the family i and N is the total number of individuals in the sample collection. High HFBI community values are an indication of organic pollution, while low values indicate good water quality.

Biological Monitoring Working Party (BMWP)

The Biological Monitoring Working Party score (BMWP) provides single values, at the family level, representative of the organisms' tolerance to pollution. The greater tolerance towards pollution, the lower the values of BMWP score. BMWP is calculated by adding the individual scores of all families (Mandaville, 2002).

Average Score per Taxon (ASPT)

The Average Score per Taxon (ASPT) represents the average tolerance score of all taxa within the community, and was calculated by dividing the BMWP by the number of families represented in the sample (Mandaville, 2002).

Taxa Richness (TR)

TR indicates the health of the community through it's' diversity, and increases with increasing habitat diversity, suitability, and water quality.TR equals the total number of taxa represented within the sample. The healthier the communities, the greater the number of taxa found within that community. Furthermore different macro invertebrate metrics like % Ephemeroptera Plecoptera and Trichoptera(EP)T index , percent Diptera ,% dominant taxa and %chiromidae were determined at each sampling point as an indicator to assess the all over stream health condition (Mandaville, 2002);(Nyakeya1 *et al*., 2009).

4.10 Quality control strategy

The equipment and instruments used in this study were calibrated to check their status before and in the middle of the experiments. All sample containers were cleaned by 10% concentrated Nitric acid (HNO_3) in order to clear out any heavy metal on their surfaces. Apparatus such as volumetric flasks, measuring cylinder and digestion flasks were thoroughly washed with detergents and tap water and then rinsed with de ionized water. The digestion tubes were soaked with 1% (w/v) potassium dichromate in 98% (v/v) H_2SO_4 and the volumetric flasks in10% (v/v) HNO_3 for 24 hours followed by rinsing with de ionized water and then dried in oven and kept in dust free place until analysis began. Prior to each use the apparatus were soaked and rinsed in de ionized water. Reagents and chemicals used for the laboratory works were analytical grade: HNO3 (69- 72%), HClO4 (70%), Pb (NO3)2 (99.5%), Cd (NO3)2 (99.99%), Cr (NO3)2 (99.99%), H2SO4 (98%), A number of blank samples containing distilled- de-ionized water was processed through the same steps as those of the samples and metal determinations were similarly made (Moges 2014); (APHA, 2012).

4.11 Validation of experimental results for heavy metals

4.11.1 Accuracy

The accuracy of the method was determined by calculating percentage of recoveries. It was carried out by adding known quantity of analyte solution in to the sample by the proposed method. It is used to confirm the losses of analyte or contamination during sample preparation and matrix interferences during the measurement step for the determination of the recoveries of the analyte to be investigated. Spike recovery analysis of each metal was made to determine the recovery due to matrix effects. It was calculated using the equation given below (Moges 2014); (Ullah *et al.*, 2017).

$R= Cs-c/s *100$-- (4)

Where;

S= concentration equivalent of analyte added to the sample

Cs= metal content of the spiked sample

C = metal content of non-spiked sample

R = percent recover.

4.11.2 Method detection limit (LOD)

LOD is the amount of analyte that gives a signal equal to three times the standard deviation of a blank after digestion of three blank solutions containing .Three readings were taken for each blank samples and the standard deviation were calculated. The method detection limit of each element was obtained by multiplying the standard deviation of the reagent blank by three (Manikandan *et al.*, 2015).

$MDL=3x\delta blank$--- (5)

Where;

MDL= method detection limit

δ = standard deviation of blank solution reading

4.11.3 Limit of quantification (LOQ)

The lowest concentration level at which a measurement is quantitatively meaningful is called the limit of quantification (LOQ).The LOQ is 10xSD of the blank. The LOQ was calculated by multiplying pooled standard deviation of the reagent blank by using the following equation (Manikandan *et al.*, 2015).

$$LOQ = 10 \times SD_{blank} \text{ -- } (6)$$

Where;

LOQ=limit of quantification

SD blank =standard deviation of blank sample

4.12 Ethical clearance

Ethical clearance was obtained from Institutional Review Board (IRB), Institute of Health, and Jimma University. Formal letter of cooperation was written to Kombolcha textile factory higher officials including the objective the research clearly. Necessary permission for sample collection was taken from the authority of Kombolcha textile factory.

4.13 Plan for dissemination and ensuring utilization of finding

The findings will be presented to Jimma University scientific community as a defense and will be submitted to the Jimma University, Institution of Health Science Department of Environmental Health Science and Technology. The findings will also be communicated to kombolcha textile factory administrative officials to enable them to know the overall status of wastewater effluent discharged situations from their factories to take appropriate measures if needed. If possible, Publications in peer-reviewed, national, or international journals will also be done.

CHAPTER FIVE

RESULT

5.1 Evaluation of the analytical method for heavy metal analysis

Different method validation technique was carried out to assess the working situation of the laboratory experiments for determination of heavy metals in different environmental compartments of the study area. The Percentage of recovery was conducted by the method of spiking experiment in which known quantities of the metal standard solution were added to the samples to be analyzed. Percentage recovery values for individual heavy metal analysis for soil, effluent, and vegetable and sediments samples are presented in table 4 .The percentage recovery values of the metals for effluent, soil and sediment and vegetable samples were found to be within the range of 96.5–101.2%, 84.5-96.5 %, 80-88.5% and 83.5-94.5% respectively. The values were within the acceptable range (Ullah *et al.*, 2017) which confirmed the validity of the method utilized in the current study. Coefficient of correlation was determined by taking standard series of known solution which varies from 0.994 to 0.998.Additionlly, LOD and LOQ were also determined to assess quality of method determination that were used in the laboratory.

Table 4: Percentage recovery, limit of detection and limit of recovery of selected heavy metals

HM	Percent recovery				LOD	LOQ	Coefficient of correlation r'
	Effluent	Soil	Sediment	Vegetables			
Cr	98.6	84.5	86.2	87.5	0.008	0.027	0.998
Cd	101.2	88.6	80	94.5	0.007	0.023	0.996
Pb	101.2	96.5	88.5	90.2	0.003	0.01	0.994
Cu	96.5	91.2	81.6	83.5	0.005	0.017	0.997

5.2 Heavy metal concentration in soil

The mean concentrations of pH and EC in soil samples were 8.6±0.46 and 741±55.1µs/cm respectively. The corresponding value in control soil sample was 6.8±0.3,595±40.0µs/cm respectively. Similarly, the mean concentration of Pb, Cd, Cr and Cu in the in study soil samples were 29.1±6.0, 1.63±0.225, 39.17±19.1 and 39.93±6.4ppm respectively. The Mann Whitney's statistics showed that the mean concentration of PH, EC, Cr and Cu were statistically significant between study and control site with $p < 0.05$.

Table 5 : Average concentration of heavy metals (ppm), pH and EC (µs/cm) in soil sampling point of the study site, Kombolcha North East Ethiopia 2018

Point			Heavy metals			
	pH	EC	Pb	Cd	Cr	Cu
Study site 1	8.3±0.6	675±18.19	28.3±3.44	1.61±0.36	32.1±5.1	42.2±5.96
Study site 2	8.7±0.46	785±9.16	23.1±1.15	1.58±0.23	21.8±3.0	20.5±3.34
Study site 3	8.8±0.26	765±38.1	35.9±2.6	1.71±0.1	63.6±1.47	57.1±3.84
Mean of study	8.6±0.46	741±55.1	29.1±6.0	1.63±0.225	39.17±19.1	39.93±6.4
Mean of control	6.8±0.3	595±40.0	18.7±9.3	1.73±0.02	15.93±1.89	13.6±3.04
A			300	3	150	140
B			100	3	100	100

a (Kamunda *et al*., 2016)

b (Chiroma *et al*., 2014)

Table 6: Average concentration of heavy metals in study soil sample and control site in Kombolcha North East Ethiopia, 2018

Parameter	Sample soil	Control soil	P value
pH	8.6±0.46	6.8±0.3	0.009
EC	741±55.1	595±40	0.009
Pb	29.1±6.0	18.7±9.3	0.145
Cd	1.63±0.225	1.73±0.03	0.727
Cr	39.17±19.1	15.9±1.88	0.009
Cu	39.93±6.4	13.6±3.04	0.009

The table below shows, pH showed positive correlation with EC Pb, Cr, and Cu. In the contrary Cd had negative correlation with all of the assessed parameters of the soil samples. Highest strong correlation were observed between Cr and Cu with the correlation coefficient value of positive 0.944 while lowest correlation was observed between pH and Cd with the value of negative 0.081.

Table 7: Spearman correlation of pH, EC, Pb, Cd, Cr and Cu in soil sample of the study site 2018

Spearman correlation	pH	EC	Pb	Cd	Cr	Cu
pH	1.000					
EC	.642*	1.000				
Pb	.505	.371	1.000			
Cd	-.081	-.319	-.074	1.000		
Cr	.582*	.545	.832**	.084	1.000	
Cu	.674*	.552	.797**	-.091	.944**	1.000

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

5.3 Heavy metal contamination in vegetables

The mean concentration of Pb, Cd Cr and Cu in Swiss chard of the study sites were 12.4±2.06, 1.09±0.11, and 13.3±5.48 and 3.68±0.79 ppm respectively. The corresponding concentration in the reference vegetables were 14.95±1.1ppm, 1.09±0.005ppm, 6.05±0.85ppm and 2.04±0.28ppm respectively. The analysis of Mann Whitney statistics showed that Cu and Cr had significant difference between study and control site at $p < 0.05$.The average transfer factor of target heavy metal varies from 0.11 to 0.66. Cd had highest transfer factor with the value of 0.66 while Cu had lowest transfer factor with the average value of 0.11.

Table 8: Mean concentration of heavy metals, in each vegetable sampling point of the study site Kombolcha North East Ethiopia 2018.

Sampling point	Pb	Cd	Cr	Cu
Study site 1	15.3±2.86	1.06±0.087	19.7±4.3	3.91±0.89
Study site 2	12.4±0.95	1.08±0.176	8.5±1.05	2.99±0.78
Study site 3	14.9±1.47	1.12±0.069	11.7±0.89	4.13±0.157
Mean of study site	14.2±2.16	1.09±0.11	13.3±5.48	3.68±0.79
Mean of control site	14.95±1.1	1.09±0.005	6.05±0.85	2.04±0.28
(Chiroma *et al.*, 2014)	0.3	0.1		73
(FAO/WHO, 2015)	0.3	0.2		

Table 9: Average heavy metal concentration in vegetable sample in study and control site, Kombolcha north east Ethiopia 2018

HM	Sample Vegetable	Control Vegetable	p-value
Pb	14.2±2.16	14.9±1.15	0.282
Cd	1.09±0.11	1.09±0.000	0.727
Cr	13.3±5.48	6.05±0.85	0.009
Cu	3.68±0.79	2.04±0.28	0.018

Transfer of heavy metals in soil and vegetables

The correlation coefficient of examined heavy metals in soil and vegetables is presented in the table 10 below. Cd showed strong correlation coefficient with the value of 0.865. In the contrary, chromium had lowest value of correlation coefficient with the value of 0.045.

Table 10: Correlation coefficient of target heavy metals in soil and vegetables in Kombolcha North East Ethiopia, 2018

Heavy metals	R^2	r	Correlation strength
Pb	0.419	0.647	Moderate
Cd	0.748	0.865	Strong
Cr	0.002	0.045	Fair
Cu	0.695	0.833	Strong

5.5 Heavy metal concentration in stream

5.5.1 Heavy metal concentration in river sediment

The mean value of pH and EC in the downstream sediment of Leyole river was 8.77±0.75 and 865.7±101.4µs/cm respectively. The mean concentration of Pb and Cu in the downstream was .5±5.7 and 8.5±1.55 ppm respectively. Their corresponding concentrations in the upstream sites were 2.1±0.55 and 4.03±0.8ppm respectively. Mann Whitney test showed that pH, EC Pb and Cu showed significance difference between upstream and downstream sampling site with p value less than 0.05.

Table 11: Average concentrations of pH, EC and target heavy metals in sediment in downstream and upstream in Leyole river at study site Kombolcha North East Ethiopia 2018(in ppm unless specified)

Parameter	Down stream	Up stream	(Mortuza, 2017)	P value
pH	8.77±0.75	6.7±0.38		**0.000**
EC (µs/cm)	865.7±101.4	512.5±44.01		**0.000**
Pb	12.5±5.7	2.1±0.55	10	**0.000**
Cd	1.73±0.18	1.76±0.165	6	1.00
Cr	11.6±1.44	10.2±1.53	25	0.145
Cu	8.5±1.55	4.03±0.8	25	**0.000**

Spearman bivariate correlation showed that pH and EC showed positive correlation with examined heavy metals with highest coefficient of correlation value of 0.882. Copper had also strong correlation with pH, EC, and Pb with the value of 0.782, 0.768 and 0.09 respectively. Cd showed poor correlation with all examined parameters.

Table12: Spearman correlation of pH, EC and selected heavy metals in Leyole river sediments of the study site in Kombolcha, North East Ethiopia, 2018

correlation	pH	EC	Pb	Cd	Cr	Cu
pH	1.000					
EC	.882**	1.000				
Pb	.570*	.606*	1.000			
Cd	.108	.061	.054	1.000		
Cr	.465	.343	.424	.283	1.000	
Cu	.782**	.768**	.609*	-.039	.496	1.000

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

5.5.2 Heavy metal concentration in river water

The mean concentration of pH, EC in the downstream sampling station was 7.88±0.93 and 1602.47±322 µs/cm respectively. The mean value of Pb, Cd, Cr and Cu in the downstream of river water sample was 0.148±0.0134, 0.016±0.005, 0.91±0.66 and 1.08±.057 mg/l respectively. The analysis of Mann Whitney test showed that pH, EC, Pb and Cu showed significance difference between downstream and upstream of the sampling sites with the value of p less than 0.05

Table 13: Mean concentration of heavy metals in Leyole stream water sampling site in mg/l (ppm), Kombolcha, North East Ethiopia, 2018.

HM	Upstream	Downstream	Guideline value		p-value
			(Ayers, 1985)	(Chiroma *et al.*, 2014)	
pH	5.35±0.37	7.88±0.93	6.5-8.4		**0.04**
EC	219.5±40.1	1602.47±322	750		**0.00**
Pb	0.034±0.013	0.148±0.0134	5.0	0.065	**0.003**
Cd	0.012±0.002	0.016±0.005	0.01	0.01	0.113
Cr	0.545±0.38	0.91±0.66	0.1	0.55	0.529
Cu	0.44±0.17	1.08±.057	0.2	0.017	**0.012**

5.6 Metal Pollution Index of heavy metals in soil and sediment

The CF, DC and PLI and Igeo of examined heavy metals in soil sample are presented in table 14 below. The CF value in soil samples varied from 0.94 to 2.93. The highest and lowest value of CF was occurred in Cu and Cd respectively. In the same way, DC of in each sampling point varied from 5.04 to 11.11 with the average value of 7.9. Similarly, the PLI of the sampling point of soil samples were varied from 1.24 to 2.38 with the mean value of 1.8. Furthermore, Igeo of the sampling point varied from 0.19 to 0.59 with the highest and lowest values were occurred for Cu and Cd respectively.

Table 14: PLI, CF, DC and Igeo of target heavy metals in soil samples of the study site Kombolcha, North East, Ethiopia 2018

	HM	S1	S2	S 3	Average
Soil	Pb	1.51	1.24	1.92	1.56
	Cd	0.93	0.91	0.99	0.94
	Cr	2.01	1.39	4.0	2.46
	Cu	3.1	1.5	4.2	2.93
	Dc	7.55	5.04	11.11	7.9
	PLI	1.72	1.24	2.38	1.8
	Igeo				
	Pb	0.3	0.25	0.39	0.31
	Cd	0.19	0.18	0.2	0.19
	Cr	0.4	0.27	0.8	0.5
	Cu	0.62	0.3	0.84	0.59

The level of contamination in sediment sample is presented in the table 15 below. The CF value in stream sediment samples were varied from 0.98 to 6.38. The highest and lowest value of CF value was occurred in Cu and Cd respectively. In the same way, DC of in each sampling point varied from 8.16 to 13.71 with the mean value of 10.17. Similarly, the PLI of the sampling point of sediment sample varies from 1.68 to 2.19 with the average value of 1.9. Furthermore, Igeo of the sampling point varies from 0.13 to 0.64 with the highest and lowest values were occurred for Cu and Cd respectively.

Table 15 PLI, CF and Dc heavy metals in sediment and river water samples of the study site Kombolcha North East Ethiopia, 2018.

		Dr 1	Dr2	Dr3	Mean
Sediment	Pb	4.05	4.3	9.47	5.94
	Cd	0.94	1.04	0.99	0.98
	Cr	0.99	1.17	1.18	1.11
	Cu	2.18	2.13	2.07	6.38
	Dc	8.16	8.64	13.71	10.17
	PLI	1.68	1.83	2.19	1.9
	Igeo				
	Pb	0.25	0.27	0.59	0.37
	Cd	0.12	0.14	0.13	0.13
	Cr	0.2	0.23	0.24	0.22
	Cu	0.66	0.64	0.63	0.64

5.7 Physico-chemical and heavy metal characteristics effluents

The mean concentration of examined physicochemical and heavy metal charactesrtics of effluents with the average treatment efficiency of the treatment plant for each parameter is presented in table 16 below. The mean concentration of EC pH TDS and total alkalinity in the inlet effluent was 4754.24µs/cm, 11.25, 3663.43mg/l and 839mg/l respectively. The corresponding concentration in the outlet effluent of the treatment plant was 2875.47µs/cm,10.3,2601.33mg/l and 577.33mg/l respectively. In the same way, DO and BOD5 in the inlet effluents was 1.23mg/l and 680.83mg/l respectively while their average concentration in the outlet effluent of the treatment plant was 2.33mg/l and 531.3mg/l respectively. In addition to these parameters, the mean concentration of chloride, nitrate sulphate and total hardness in the inlet effluent was 384.25mg/l,74.3mg/l,439mg/l and 940mg/l respectively while the corresponding concentration in the outlet effluent was 294.04mg/l; 65.67mg/l 342.6mg/l and 703.67 mg/l respectively. Regarding to the mean concentration of Pb, Cd, Cr and Cu in the outlet effluent was 0.025mg/l,0.042mg/l,1.383mg/l and 1.02mg/l respectively. The table below also shows that the removal efficiency of the treatment plant varies from 4.02% to 39.52% .The highest and lowest treatment efficiency was achieved for EC and effluent temperature respectively. The treatment plant also achieved 8.44%, 29%, 21.9% reduction for PH, TDS, and BOD5 respectively. Similarly the treatment efficiencies of the treatment plant for total alkalinity, chloride, nitrate and sulphate was 31.66%, 23.46%, 11.6% and 21.96% of reduction respectively. From all parameters assessed DO showed increscent inlet effluent to outlet effluent with increasment efficiency 83.46%.

Table 16: Average concentrations of selected physicochemical and heavy metal characteristics in Kombolcha textile factory influent, effluent and its treatment efficiency Kombolcha North East Ethiopia, 2018. (All values are Mean ±SD expressed in mg/l, otherwise stated).

Parameter	Outlet Effluent(mean±sd	Inlet fluent(mean±sd	%removal efficiency	(EEPA , 2003)	(IFC, 2007)
Ambient T^{o}	27(±0.76)	27.4((±1.3)		-	
Water T^{o}	26.84(±1.25)	27.97(±1.04)	4.04	40	
DO	2.33(± 0.46)	1.27(±0.25)	-83.46	-	
EC(µs/cm)	2875.47(±242.2	4754.24(±669.9)	39.52	1000	
pH	10.3 (±0.8)	11.25 (±0.67)	8.44	6-9	6-9
Turbidity(NTU)	54.55 (± 4.92)	67.55(± 5.22)	19.24	-	
TS	3966.87(± 388.4)	5622.73(±442.8)	29.45		
TDS	2601.33(±240.83	3663.43(±172.53)	29	80	
TSS	1365.53(±208.32)	1955.97(±303.3)	30.18	30	50
BOD$_5$	531.3(±41.34)	680.83(±54.91)	21.96	50	30
Alkalinity	573.33 (±52.98)	839(±195.9)	31.66	-	
Chloride	294.04(±37.1)	384.25 (±36.38)	23.48	-	
Nitrate	6.567 (±0.92)	7.43 (±3.21)	11.6	50	
Sulphate	34.2.6(±5.01)	43.9(±7.93)	21.96	200	
Hardness	703.67(±65.13)	940.03(±104.5)	25.14	-	
Pb		0.025(±0.012)		0.5	
Cd		0.042(±0.039)		1	0.02
Cr		1.383(±0.631)		1	0.5
Cu		1.02(±0.276)		2	0.5

5.8 Physicochemical and heavy metal charactesrtics in river

The average concentration of selected physicochemical characteristics of the upstream and downstream of Leyole river that receives effluents from textile factory is presented in the table17 below. The average ambient T^{o} in the downstream and upstream was 26.47±1.11℃ and 23.85±0.66℃ respectively. The velocity of the river water in the upstream and downstream was almost similar with the average value of 0.31m/s and 0.28m/s respectively. The mean value of DO, pH, EC, TDS and BOD in the downstream was 3.97±0.69mg/l, 7.88±0.93, 1602.47±322.03μs/cm,1136.67±135.84mg/l and 103.93±41mg/l respectively. The concentration of total alkalinity, chloride, nitrate, sulphate and total hardness in the downstream was 421.33mg/l,128.05mg/l,6.32mg/l,32.9mg/l and 521.33mg/l respectively. The analysis of Mann Whitney statistics showed that most of the examined physico-chemical characteristics except ambient T^{o}, water velocity and depth of the water showed significance difference

Table 17: Mean concentration of selected physicochemical characteristics of upstream and downstream of Leyole river Kombolcha, North East Ethiopia, 2018 (in mg/l)

Parameter	Upstream (mean±sd	Downstream (mean±sd)	(Islam *et al*, [2018)	(Sarkar *et al*.,2016)	p-value
Ambient T(℃)	26.9±0.76	27.6±0.65	-		0.145
Water T(℃)	23.85±0.66	26.47±1.11	20-30	40	0.04
Velocity(m/s)	0.31±0.04	0.28±0.03			0.145
Depth (m)	0.33±0.02	0.28±0.06			0.181
Width (m)	2.1±0.23	1.76±0.14			0.012
DO	7.15±0.42	3.97±0.69	-	4.5-8	0.01
EC (μs/cm)	219.5±40.1	1602.47±322	1000	1200	0.00
pH	5.35±0.37	7.88±0.93	6.4-7.4	6-9	0.04
Turbidity(NTU)	11.35±2.03	31.07±7.48	-		0.03
TS	521±49.61	2025.12±422.			0.00
TDS	248.5±15.4	1136.67±136	1000	2100	0.00
TSS	267.5±52.5	888.57±290.5			0.00
BOD	15±2.34	103.93±41		50	0.00
Total alkalinity	88.55±16	421.33±33.49	-	-	0.00
Chloride	64.89±7.2	128.05±42.08	600	250 l	0.00
Nitrate	0.58±0.084	6.32±0.83			0.00
Sulphate	2.97±1.67	32.9±8.35			0.00
Total hardness	110.7±8.2	521.33±121.4	200-500	300	0.00

5.9 Human impact and habitat condition assessment

Human disturbance score in the habitats assessment condition is presented in the table 18 below. The result showed that the Habitant assessment three sampling stations were classified as suboptimal while the remaining points exhibited marginal level of quality. In the same way human impact score of each sampling site was low for the upstream sites and medium for the downstream sampling points.

Table 18 Human impact and habitat condition assessment scores and classes in ssampling sites of Leyole river, Kombolcha North East Ethiopia, 2018

Site no	Sampling site (habitat)	Human impact		Habitat condition	
		Score	Class	Score	Class
1	Ur2	21	Low	146	Suboptimal
2	Ur1	20	Low	138	Suboptimal
3	Dr1	39	Medium	106	Marginal
4	Dr2	35	Medium	108	Marginal
5	Dr3	37	Medium	132	Suboptimal

Human impact and habitat score categorization criteria; human impact score (<25 low, 25-75 medium,>75-125 sever (MEDP, 2013), habitat condition score (<60 poor, 60-109 marginal, 110-159 suboptimal, 160-200 optimal (Barbour *et al*., 1999).

5.10. Macroinvertebrate monitoring

A total of 1765 macroinvertebrate classified in to 7 orders and 17 families of were collected from five sampling sites of Leyole river. The most abundant orders were Diptera with the value of 1079 (61.2%), followed by Ephemeroptera 393(22.2%), Odonata 105(1.95%) and Hemiptera 99(5.6%) which were represented by13 families. The total number of families present at each site was varied from 7 in the immediate downstream (Dr1) to 14 (Ur1) in the immediate downstream site. The dominant families were Chironomidae, Culicidae, Caenidae and Ceratopogonidae with the value of 538(30.48%), 205(11.6%), 195(11.04%), 187(10.6%) respectively. In the contrary, the families with least occurred were Physidae, Gomphidae, Perlidae 2(0.1%), 3(0.17%), 17(0.96%), 6(0.34%)

respectively.

Among the order of Diptera, the family of Chironomidae was dominant and accounts 538 (50%). Order of mollusks (snails) were present in the least abundant and accounts only 0.1% which was present only in the downstream sampling point two (Dr2) of the sampling point. Regarding the number of individual macroinvertebrate at each sampling point, the highest number were collected from the downstream sampling point two (Dr2) which accounts 30% of the total macroinvertebrate collected from all sampling point of the study site. In the contrary, the least numbers of individuals' of macro invertebrates were collected from upstream sampling point two (Ur2) which accounts 13% of the all macroinvertebrate collected from all sampling point. Furthermore, the relative abundance of macroinvertebrate in different sampling points. EPT were dominated in the upstream sampling points (Ur1 and Ur2) compared to the downstream sampling points. In the contrary, the relative abundance of Diptera was relatively high in sampling sites of Dr1, Dr2 and Dr3 compared to the upstream sampling site. But the relative abundance of Odonata and Hemiptera show relatively even distribution among the downstream and upstream sites of the different sampling points of the study area.

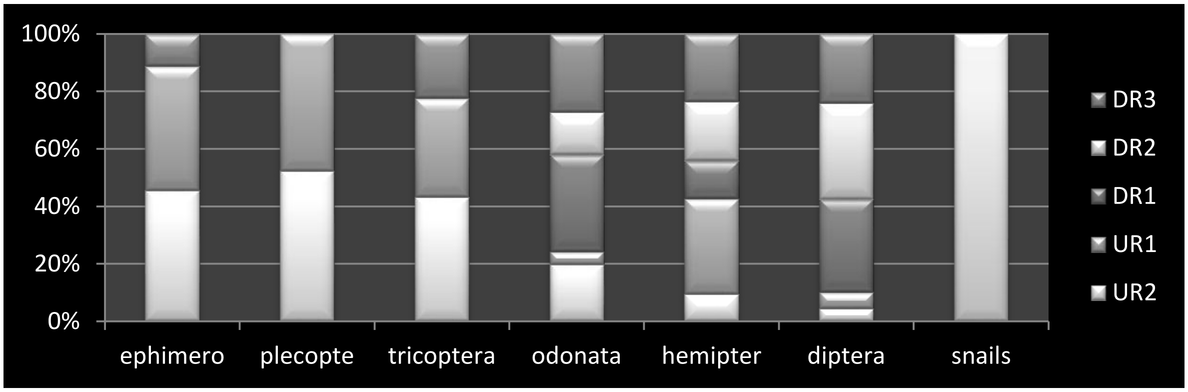


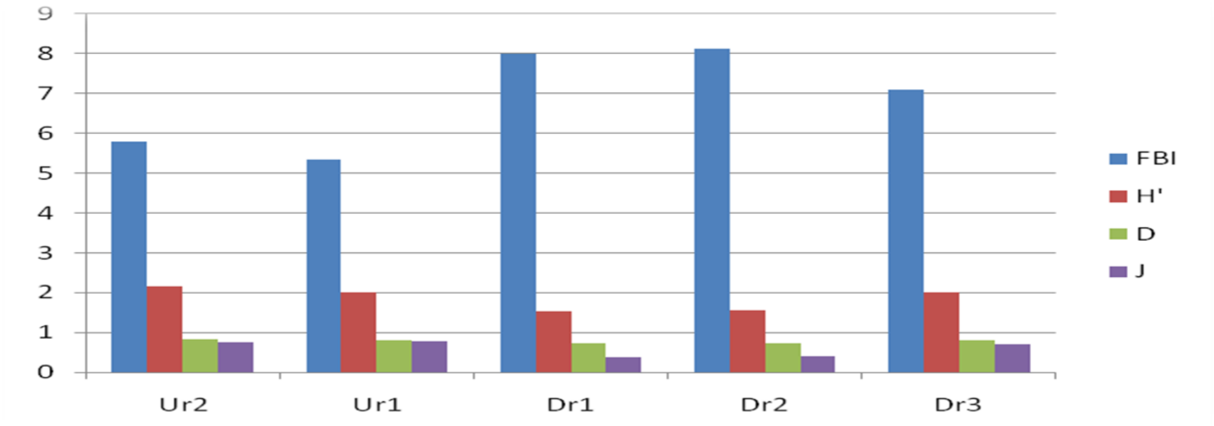
Figure 10: Relative abundance of macro invertebrates, at order level at five sampling points of Leyole river Kombolcha, north east Ethiopia 2018.

Table 19: Cumulative number of individuals for macro invertebrate taxa collected in five sampling sites along the Leyole river Kombolcha, North East Ethiopia 2018

s.no	**Order/ Family**	**TV**	**Sampling sites**					**Total**	**% covera**
			Ur2	Ur1	Dr1	Dr2	Dr3		
	EPHEMEROPTERA (**mayflies**)								
	Baetidae	4	74	39	0	1	29	143	8.1
	Heptageniidae	4	14	8	0	0	0	22	1.246
	Ephemerellidae	1	22	11	0	0	0	33	1.87
	Caenidae	7	97	86	0	0	12	195	11.1
	PLECOPTERA (**stoneflies**)								
	Perlidae	1	5	1	0	0	0	6	1.41
	TRICHOPTERA (**caddis flies**)								
	Hydropsychidae	4	19	14	0	0	8	41	2.3
	Hydroptilidae	4	12	4	0	0	5	21	1.19
	ODONATA (**dragon flies and damselflies**).								
	Gomphidae	1	0	3	0	0		3	0.17
	Aeshnidae	3	4	0	0	1	12	17	0.96
	Coenagrionidae	9	14	0	42	21	8	85	4.8
	HEMIPTERA (**water or true bugs**)								
	Belostomatidae	9	6	0	17	32	18	73	4.14
	Pleidae	8	3	23	0	0	0	26	1.47
	DIPTERA (**Two-winged** or “**true flies**”)								
	Culicidae (mosquitoes)	8	12	21	84	61	27	205	11.6
	Syrphidae	10	0	0	36	95	18	149	8.44
	Chironomidae	8	18	12	189	225	94	538	30.48
	Ceratopogonidae	6	8	2	67	84	26	187	10.6
	SNAILS								
	Physidae	7	0	0	0	2	0	2	0.1
	Total		318	233	435	522	257	1765	100
	Percent coverage per site		18.	13	25	30	14	100	

The figure below shows different indices and metrics of macroinvertebrate in five sampling point of the study area. The value of FBI varied from 5.33 to 8.12. The high value of FBI was occurred in the first two downstream of sampling point (Dr1 and Dr2) with the value of 8.0 and 8.12 respectively. In the contrary, the lowest FBI was registered in upstream site with the value of 5.55. Simpson diversity index varies from 0.73 to 0.83.The maximum and minimum Simpson diversity was presented in the upstream sampling point two (Ur2) and the immediate downstream sampling point of the study site respectively. In the same manner, Shannon diversity index also varied from 1.53 to 2.15 with maximum value was registered in the upstream sampling point two (Ur2) while its minimum value is presented in the immediate downstream sampling point of the study area.

Regarding to the dominance taxa, highest dominance tax was occurred in sampling point of downstream sampling point of Dr1, Dr2 followed by Dr3 with the average value 43.45%, 43.1% and 36.6% respectively. But the dominance taxa in the upstream sampling site were 30.5% and 36.9% for Ur2 and Ur1 respectively. The abundance of EPT was almost dominate in the upper stream sites while in the case of downstream it was almost zero especially for the first two immediate downstream sampling points. The distribution of % Chironominidae also follows the same distribution pattern with percent of dominant taxa.



FBI (Family biotic index), H’ (Shannon wiener diversity index), SDI (Simpson diversity index) and J (pioule evenness index)

Figure 11: HFBI, H’ SDI and J indices of the five sampling point of Leyole river in Kombolcha North East Ethiopia 2018

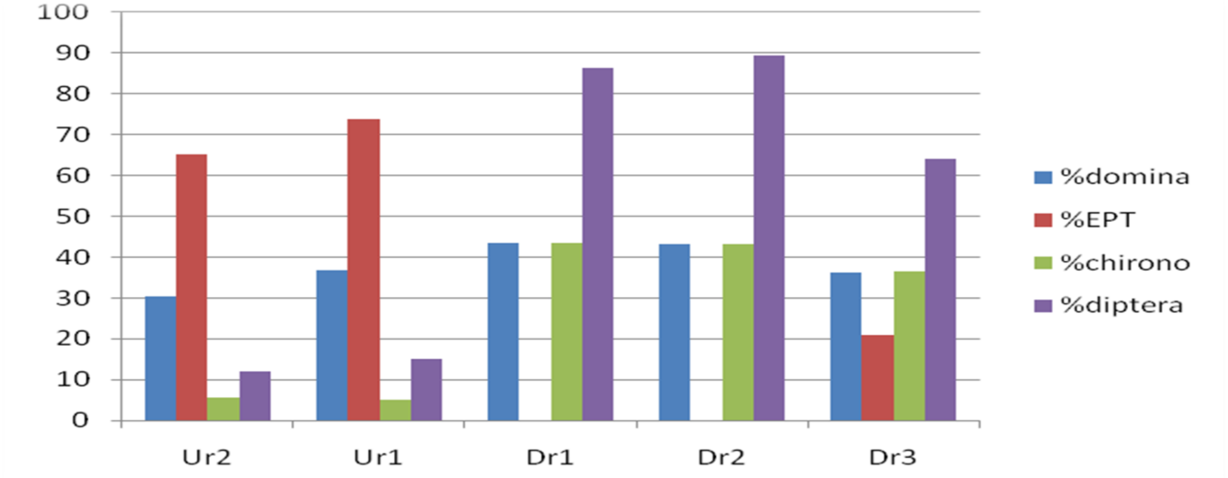
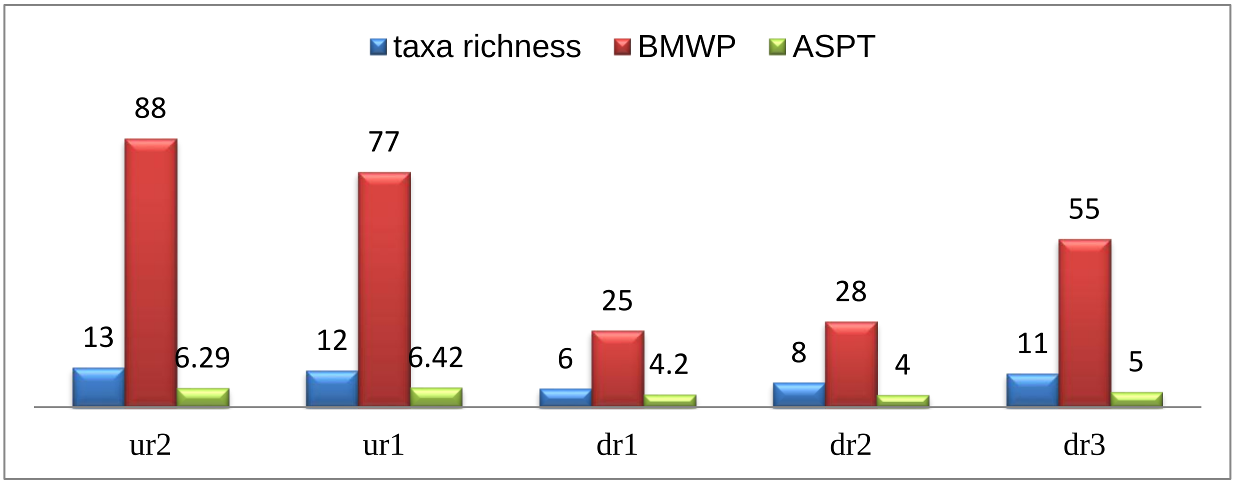


Figure 12: % Dominance taxa, %EPT, %Chironomidae and %Diptera indices of the five sampling point of Leyole river Kombolcha, North East Ethiopia, 2018**.**

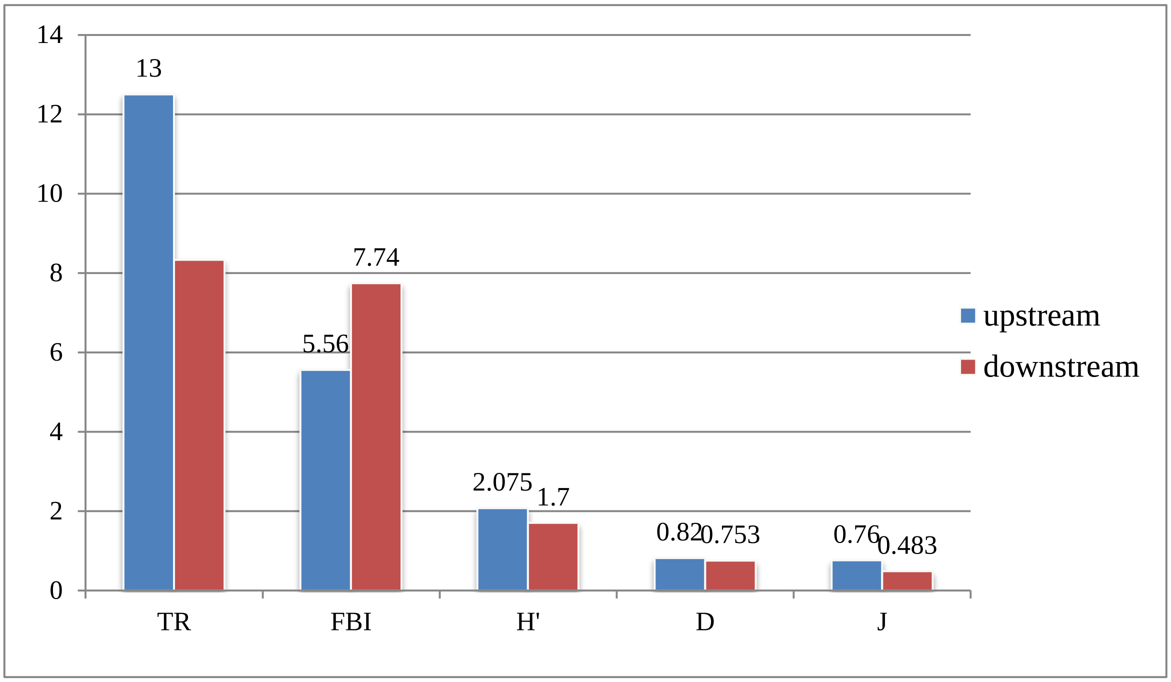


BMWP (Biological Monitoring Working Party) ASPT (Average Score per Taxa)

Figure 13: Taxa richness, BMWP and ASPT of five sampling points of Leyole river Kombolcha North East Ethiopia 2018

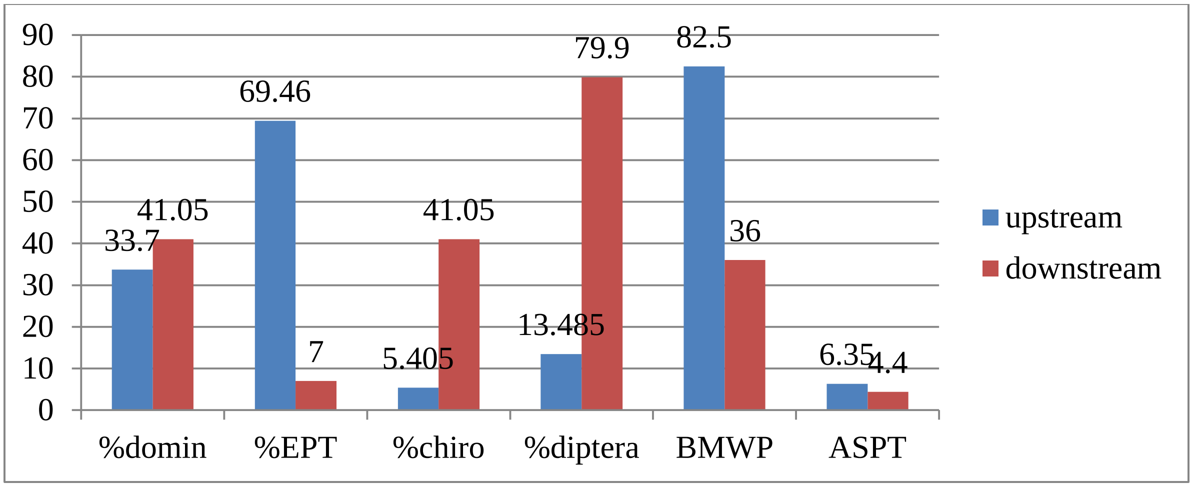
The average HFBI in the upstream and downstream was 5.56±0.32 and 7.74±0.56 respectively. Similarly, the average Shannon diversity index and Simpson diversity index in the downstream and upstream was (1.7±0.263, 2.075±0.11), and (0.753±0.04, 0.76±0.01) respectively. Furthermore, the percent of dominant taxa in the upstream and downstream was 33.7±4.53 and 41.05±3.86

respectively. In the contrary, percent of EPT was more prevalent in the upstream with the value of 69.46±6.17. BMWP and ASPT in the upstream and downstream of the stream was (82.5±7.78, 6.35±0.09) and (36±16.52, 4.4±0.53) respectively. Additionally, the percent of Chironomidae and Diptera in the downstream of the study site was 41.05±3.86 and 79.9±13.66 respectively.



FBI –family biotic index, H'-Shannon Weiner Diversity Index, SDI-Simpson diversity index, J-Evenness, TR-Taxa richness

Figure 14: Family biotic index, Shannon wiener diversity index, Simpson diversity index, and Shannon evenness index and taxa richness of the upstream and downstream of Leyole river Kombolcha, North East Ethiopia, 2018



Domin- Dominance taxa, Chiro-Chironomidae EPT-Ephemeroptera Plecoptera and Trichoptera, BMWP-Biological Monitoring Working Party, ASPT-average score per taxa

Figure 15: Percent of Ephemeroptera Plecoptera and Trichoptera, %Dominance taxa, % Chironomidae Diptera, Biological Monitoring Working Party, Average Score per Taxa

5.11 Correlation between physicochemical parameters and macro invertebrate metrics

Spearman bivariate correlation between physicochemical parameters and macroinvertebrates metrics is shown in table 20. Water temperature showed strong positive correlation with FBI, % Chironomidae and % Diptera with the value of r 0.9. In the contrary, it showed strong negative correlation with H’, SDI, J, % EPT, BMWP and ASPT with value of r 0.78, 0.872, 0.9, 0.975, 0.8 and 1.00 respectively. But the table showed that DO had strong positive correlation with H’, SDI, J, %EPT, BMWP and ASPT with the value of r 0.975, 0.975, 0.9, 0.872, 1.00 and 0.8 respectively. But it showed strong negative correlation with FBI, % of dominance taxa, % of Chironominidae and % of Diptera with value of r 0.9. Furthermore EC, pH, TDS, BOD, total alkalinity ,chloride, and total hardness showed strong positive correlation with FBI,% dominance taxa% of Chironomidae and % of Diptera with the value of r was greater than 0.75.But in the contrary, they showed strong negative correlation with H’, SDI, J, % of EPT, BMWP and ASPT with absolute value of r greater than 0.75 Furthermore the correlation strength of selected target heavy metals with different with different indices of macro invertebrate were varied from parameter to parameter. In general, Cu showed strong correlation either positive or negative with all selected indices of macroinvertebrate with absolute value of r was greater than 0.75 for most cases. In the contrary, Cd and Cr showed low correlation either in positive or negative with the absolute value of r was less than 0.25.

Table 20: Spearman correlation coefficients between selected environmental parameters and values of benthic macroinvertebrate metrics in Leyole river Kombolcha north East Ethiopia 2018

Variable	FBI	H’	SDI	J	%Domi	%EPT	%chi	%dip	Bmwp	ASPT
Ambient T^{o}	0.7	-0.359	-0.564	-0.2	0.3	-0.359	0.2	0.7	-0.4	-0.5
Water T^{o}	0.9^{*}	-0.718	-0.872	-0.9^{*}	0.6	-0.975^{**}	0.9^{*}	0.9*	-0.8	-1.00^{**}
Velocity	0.462	0.105	0.395	0.462	0.103	0.553	-0.462	-0.462	0.308	0.616
Depth	-0.4	-0.051	0.205	-0.1	0.1	0.103	0.1	-0.4	0.000	0.3
Width	0.564	0.553	0.658	0.82	0.359	0.763	-0.821	-0.564	0.718	0.667
DO	-0.9^{*}	0.975^{**}	0.975^{**}	0.9^{*}	-0.9	0.872	-0.9^{*}	-0.9^{*}	1.0^{**}	0.8
EC	0.8	-0.821	-0.872	-1.0^{**}	0.7	-0.975^{**}	1.00^{**}	0.8	-0.9^{*}	-0.9^{*}
pH	0.8	-0.821	-0.872	-1.0^{**}	0.7	-0.975^{**}	1.00^{**}	0.8	-0.9^{*}	-0.9^{*}
Turb	0.9^{*}	-0.975^{**}	-0.975^{**}	-0.9^{*}	0.9^{*}	-0.872	0.9^{*}	0.9^{*}	-1.0^{**}	-0.8
TDS	0.9^{*}	-0.975^{**}	-0.975^{**}	-0.9^{*}	0.9^{*}	-0.872	0.9^{*}	0.9^{*}	-1.0^{**}	-0.8
TSS	0.8	-0.821	-0.872	-1.0^{**}	0.7	-0.975^{**}	1.0^{**}	0.8	-0.9^{*}	-0.9^{*}
BOD	0.9^{*}	-0.975^{**}	-0.975^{**}	-0.9^{*}	0.9^{*}	-0.872	0.9^{*}	0.9^{*}	-1.00^{**}	-0.8
Alkalinity	0.9^{*}	-0.975^{**}	-0.975^{**}	-0.9^{*}	0.9^{*}	-0.872	0.9^{*}	0.9^{*}	-1.00^{**}	-0.8
Chloride	0.9^{*}	-0.718	-0.872	-0.9^{*}	0.6	-0.975^{**}	0.9^{*}	0.9^{*}	-0.8	-1.00^{**}
Nitrate	0.5	0.41	0.564	0.7	0.2	0.667	0.7	0.5	0.6	0.6
Sulphate	0.3	0.051	0.103	0.3	0.00	0.103	0.3	0.3	0.1	0.1
Hardne	1.00	-0.872	-	-0.8	0.8	-0.872	0.8	1.00^{**}	-0.9^{*}	-0.9^{*}

ss	**		0.975**							
Pb	0.6	-0.667	-0.667	-0.9*	0.6	-0.872	0.9*	0.6	-0.7	-0.8
Cd	-0.1	-0.103	-0.051	1.00	0.000	0.051	0.1	-0.1	-0.2	0.2
Cr	0.1	-0.154	-0.154	0.1	0.1	0.154	-0.1	1.00	-0.2	0.2
Cu	0.9*	-0.975**	-0.975**	-0.9*	0.9*	-0.872	0.9*	0.9*	-1.00**	-0.8

*Correlation is significant at the 0.05 level (2 tailed)

**correlation is significant at the 0.01level (2tailed)

FBI –family biotic index, H’-Shannon Weiner Diversity Index, D-Simpson diversity index, J-Evenness, EPT-Ephemeroptera Plecoptera and Trichoptera, BMWP-Biological Monitoring Working Party, ASPT-average score per taxa

CHAPTER SIX

DISCUSSION

According to (Hasnine *et al.*, 2017) , the application of wastewater causes changes in soil physico-chemical characteristics and heavy metal uptake by food crops, particularly vegetables. The pH of soil changes through the use of wastewater for irrigation purpose. The pH of soil has a great influence on the mobility and bioavailability of heavy metals. The mean concentrations of Cr, Pb, Cd and Cu in soil samples were 39.17 ppm, 29.1 ppm, 1.63 ppm and 39.93 ppm respectively. The average concentration of examined heavy metals follows the decreasing orders of soil, sediment, vegetables, effluent and stream water. The possible justification for this finding may be due to the low mobility of heavy metals in soil, the nature contribution of heavy metals to soil and sediment, dilution effect of stream water. The concentration of Cd in all examined compartment environment was low which may be due to low composition in textile dyes ingredient and minimum level of occurrence in the earth crust. In general the concentration of heavy metals in effluent was greater than stream water which may be due to effect of dilution. The finding showed that Cr and Cu showed significance difference between study and control soil samples which may be due the use of contaminated water for different agricultural purpose. The current finding of Cr and Pb was higher for than the study conducted by (Poornima *et al.*, 2011) with the corresponding concentration of 1.816±0.654 ppm and 1.74±0.546 ppm respectively. But in the contrary, all the current finding was lower than the study conducted by (Deepali and Gangwar,2010) with the average mean value of 568.±4.163 ppm 109.54±0.315 ppm, 191.25 ppm and 83.62 ppm for Cr, Cu, Pb and Cd respectively. The possible reason for this deviation may be due the difference physical characteristics of the soil, season of sampling, composition of the effluent discharged. But the overall finding of the investigated heavy metals in soils showed that there was low concentration of all examined heavy metals compared to international standards (Kamunda *et al.*, 2016); (Chiroma *et al*., 2014).

According (Oduma and Jimoh, 2013), pH is one of the factors which influence the bio-availability and the transport of heavy metal in the soil. Heavy metal mobility decreases with increasing soil pH due to precipitation of hydroxides, carbonates or formation of insoluble organic complexes. Heavy metals are generally more mobile at pH< 7 than pH>7. The amount of heavy metals mobilized in soil environment is a function of pH, properties of metals, reduction oxidation conditions, soil chemistry, organic matter content, clay content, cation exchange capacity and other soil properties. The current finding showed that all of the soil samples mean value of pH was greater than 7 which may lead to the reduction in the mobility of heavy metals. The pH of soil samples were in the range of 6.8±0.3 to 8.8±0.26. According (Bahiru, 2017) two of the soil points exhibited strongly alkaline property while the remaining soil sample was moderately alkaline. But in the contrary, reference soil samples were exhibited neutral or nearly neutral pH. The current finding was clearly supported by the fact that most of the chemicals that are used and released from textile factory have alkalinity property. So that most of the examined environmental compartments showed from slightly alkaline to highly alkaline nature.

According to (Sharma and Raju, 2013), bivariate Correlation Coefficient is one of the most important statistical tests to evaluate the strength and weakness of relationships among different variables including heavy metals. The high correlation co-efficient (near +1 or -1) shows a good relation between two variables, and its concentration around zero means no relationship between them at a significant level of 95% level, it can be strongly correlated, if $r>0.7$, whereas r values between 0.5 to 0.7 shows moderate correlation between two different parameters. Based on this fact, all of the selected heavy metals except Cd them showed significant correlations between the contaminants of Cr and Pb (0.832), Cr and Cu (0. 944), Cu and Pb (0.797) were highly significant in soil at $p < 0.01$ which indicate the same or similar source input or similar enrichment mechanism. This suggests that that the anthropogenic sources of these metals were closely related and was most likely expected to be from effluents of textile wastewater.

The average concentration of Pb, Cd, Cr and Cu in Swiss chard vegetable sample was 14.2 ppm, 1.09 ppm, 13.3 ppm and 3.68 ppm respectively. Likewise in soil, the concentration of Cr and Cu showed significance difference in sample vegetable compared control vegetables. The current finding showed that the concentration of Cr was higher than the study by (Kumar *et al*.,2013) with the average concentration of 1.30 ppm. But the mean concentration of Cd was lower than the above finding while the concentration of Cu was in line with the aforementioned finding. The deviation in the concentration of examined heavy metals may be due to the difference in the characteristics of vegetables species, time of sampling and the characteristics of soils in which the vegetable is grown. The current finding showed that the concentration all the examined heavy metals in vegetable samples were higher than the standards set by (Kumar *et al.*,2013); (Chiroma *et al.*, 2014).

The transfer factor of for Pb ,Cd ,Cr and Cu was 0.5 ,0.66 ,0.34 and 0.11 respectively which was slightly similar except Cd which was conducted by (Gebrekidan *et al.*, 2013) with mean value for Cu ,Cr ,Cd and Pb was 0.1,0.02,0.54 and 0.76 respectively. The order of TF was Cd>Pb>Cr>Cu while the sequence in the aforementioned study was in the order of Pb>Cd>Cu>Cr. The change in the sequence of transfer factor may be due difference in soil characteristics, vegetable type under investigation and season of sampling.

The current finding showed that the mean concentration of DO and BOD in textile effluent was 2.33mg/l and 531.3mg/l respectively. The mean value of DO was lower than the study conducted by (Mehari *et al.*, 2015) while the average concentration of BOD was higher than the aforementioned finding which may be due to high production organic matter in the production process. In the same way, the mean of pH and temperature of the effluent was 10.3 and 26.484℃ respectively which was higher than the study conducted by (Assefa and Sahu, 2016). The increment in pH of the effluent samples were above the normal background levels (7.00) which may be due to mercerization in textile processing requires NaOH. Alkaline pH could form insoluble hydroxides, oxides and carbonates which also precipitate examined heavy metals. Higher temperature in effluent was probably due to the increase load of suspended solids, soil particles and decomposed organic matter which can absorb more heat for certain period of time.

The mean concentration of EC, TDS, total alkalinity and total hardness was 2875.47µs/cm,2601.33mg/l,573.33mg/l and 703.67mg/l respectively which was higher than the study conducted by (Mehari *et al.*, 2015) for EC and TDS. The deviation of the current finding from the aforementioned finding may be due to change in the difference of the efficiency in the treatment plant of the factory, time of sampling, nature of raw materials as well as change in the environmental condition of the sampling areas. The current finding of pH, TDS, EC, BOD, nitrate and Sulphate concentration was higher than the recommended limit of (EEPA, 2003) But in the contrary, the average effluent temperature of the current finding was within the recommended of the aforementioned standard guideline. The pH and BOD of the current study finding were higher than the standards of (IFC, 2007).

Furthermore, the average concentration of examined heavy metals in the study of outlet effluents was 0.025mg/l, 0.042mg/l, 1.38mg/l and 1.02 mg/l for Pb Cd, Cr and Cu respectively. The current finding was higher than the study conducted by (Sorsa *et al.*, 2015) in Hawassa textile factory effluents which may be due to difference in raw materials, production process, treatment efficiency, environmental condition. The mean concentration of all examined heavy metals except Cr was within the recommended limit of (EEPA, 2003).But in the contrary, the average concentrations of all examined heavy metals were above the recommended standards(IFC, 2007).

The current finding showed that, there was significance difference in the mean value of Pb and Cu in sediment between downstream and upstream of the study site. The mean concentration of Pb and Cd in the downstream was 12.5 mg/l and 1.73 mg/l which was higher than the study conducted by (Sorsa *et al.*, 2015). But in the contrary, the concentration of Cr and Cu was lower than the previous study. The deviation in the difference of the concentration of examined heavy metals may be due to the difference in slop of the stream, flow velocity of the water, and time of sampling, environmental condition, discharge volume of the stream, sediment composition charactesrtics. All of the examined heavy metals except Pb in the sediment of Loyale stream were lower than the international standards set by (Mortuza, 2017).This may be due to the composition of sediment by itself and the prevalence of pool microhabitat which can easily store high amount of sediment and increase its composition proportionally.

The current finding showed that most of the physicochemical characteristics of Loyale stream of the study sites showed significant difference between downstream and upstream of the sampling site with the exception of Ambient T^o, velocity of water, depth of water, Cd and Cr. The current finding showed the concentration of EC, TDS sulphate and chloride was higher than the study conducted by (Parameswari, 2014). The mean concentration of BOD, Water T^o, TDS, EC, total hardness and total alkalinity was also higher than the study conducted by (Mehari *et al.*, 2015). But the mean value of DO was lower than the previous finding while pH was in line with it. The deviation of the current finding may be due to the change in time of sample collection, discharge volume of the stream, slop of the stream, dilution factor, and distance of the factory with receiving streams.

The mean value of examined heavy metals in the downstream of Loyale stream water was 0.148 ppm, 0.016 ppm, 0.91 ppm and 1.08 ppm for Pb, Cd Cr and Cu respectively. Although the mean concentration of all heavy metals in downstream of stream water was higher than the upstream site, only Pb and Cu showed significant difference. The current finding showed that all of the examined average heavy metals concentration was higher than the study conducted by (Das *et al.*, 2011).The mean value of Cd, Cr and Cu was higher than the standard (Chiroma *et al.*, 2014);(Ayers, 1985). The mean concentrations of Pb was higher than the standard set by (Ayers, 1985) but lower than the standard of (Chiroma *et al.*, 2014). All the examined heavy metals except Cd were higher than the standards set by Bangladesh (Islam *et al.*, 2018).Furthermore , the mean concentration of Cu and Cr were higher than the standard set for inland surface water *(*Sarkar *et al.*, 2016).

According to (Barbour *et al.*, 1999) family level richness is one of the metric which is used to assess the diversity number of different families found in a sub sample. It also reflects the health of the community as a measurement of the variety of families present. These metric increases with increasing water quality, habitat diversity, and habitat suitability. The current findings showed that family richness were in the range of 6 to 13 with highest in upstream and lowest in the downstream of sampling point. The reduction of taxa richness in downstream may indicates relatively decreased water quality, habitat diversity and habitat stability compared to the upper stream sites of the study area. The lower level of DO in textile waste receiving site provides lower number of taxa while the minimal impact of upper stream was able to support higher number of taxa. The decrease in the number of taxa at sites may be due to experiencing of depleted DO, nutrient enrichment and sedimentation.

The current finding showed that EPT was almost disappeared in the impacted sites than the reference (least impacted) which was in line with the finding of(Beyene *et al.*, 2009); (Abraha , 2007); (Akalu *et al.*,2011) . The possible justification may be due to the accumulation of different hazardous physicochemical chemicals and toxic heavy metal accumulation, nutrient enrichment, accumulation of organic matter and reduction of DO which is vital for the survival of living organisms.

The finding showed that most of sensitive macroinvertebrate were highly prevalent in the upper stream of the study site while highly tolerant macro invertebrates were highly occurred in the downstream of the study site. This may be due to their high tolerant capacity of disturbance within the downstream of the study site indicates. According to (Hilsenhoff, 1988) on the quality of river water based on FBI ranges from 0.00-10.00. The values which are less than 3.75 is considered as excellent while 3.76-4.25,4.26-5.00,5.01-5.75 are very good ,good and fair respectively. FBI which varies from 5.76 -6.5, 6.51-7.25 and 7.26-10.00 were fairly poor, poor and very poor respectively. Based on this fact, the current study showed reference sites were classified as fair level of water quality. The two immediate downstream sampling points were classified as very poor level of water quality Dr3 was classified as poor level of water quality. The reduction in the level of BOD may be due to the self-purification of a stream.

According to (Turkman and Kazanci, 2010) ,Shannon diversity index is the most preferred index and have a value of 0.0-5.0.Most values measured using this index range from 1.5 to 3.5, rarely exceeding 4.5. The Values of H' above 3.0 indicate that habitat structure is stable and balanced and values under 1.0 indicate the presence of pollution and degradation of habitat structure. Based on these criteria, none of the sites of Loyale stream sampling points were exceeded 3.0 level of the Shannon diversity index. H' less than 3 indicates the presence of elevated levels of pollution and degradation of habitat structure. Based on this criteria, two of the five sampling points which are situated immediately below downstream sampling point have a value of H' 1.53 and 1.56 while the upstream sampling and the remaining one downstream point have H' value ranges from 2.00 -2.15.

According to (Turkman and Kazanci, 2010), values measuring using the Simpson diversity index range between zero and one. Zero represents minimum evenness and one for the maximum. Based on this fact, all the sites fallen in the range from 0.73-0.83 in which higher values were presents in the upstream sampling site while lower values were documented in downstream of the stream which indicates the presence of severe pollution of Loyale stream which may be lead to reduction microbial diversity.

According to (Turkman and Kazanci, 2010) , J is the ratio of the observed value of Shannon index to the maximum value. The values are between 0 – 1. When the value is getting closer to 1, it means that the individuals are distributed equally. Based on this fact, the first two immediately downstream sampling points Shannon evenness index were 0.37 which is closer to 0 which implies the presence of un even distribution among different taxa in the sampling point. The possible justification for this finding may be due to the difference in the tolerance value for external environmental disturbance which may release from the textile factory.

Spearman bivariate Correlation tests showed, that most of environmental variables had strong correlation with macroinvertebrate biotic index with the value of r which is greater than 0.75.Water temperature, DO, TDS, BOD, and total alkalinity with FBI and H'with the value of r greater than or equal to the absolute value of 0.9. The correlation may be either in the form of positively or negatively depending on the variable under investigation. But the correlation between investigated heavy metals with FBI and H' showed very poor correlation except Cu which had very strong correlation with the value of correlation coefficient of 0.9. Family Biotic Index (FBI) indicates organic and nutrient pollution and provides an estimate of water quality for each site using established pollution tolerance values for each taxon. Based on (Hilsenhoff, 1988), the sampling sites were classified from fair quality to very poor.

The greatest abundance for macroinvertebrate belonging to the family Chironominidae which was recorded at the first two immediate downstream sampling points Dr1 and Dr2 which was in line with the study conducted by (Abraha ,2007) .The possible reason for this finding may be due to the release of very hazardous chemicals from textile factory in high amounts. The chemicals that may present in high amount may be the accumulation of chromium both in the form of (trivalent and hexavalent) and other related chemicals which are produced especially in the printing and finishing process. The hexavalent form of this metal is known for its high toxicity and thus might have contributed to the low faunal status of this sampling site..

According to the a study (Abraha , 2007) dominant taxa greater than 35% indicates poor water quality, between 25%-35% indicates fair water quality, and less than 25%indicates good water quality. Based on this criterion, four of the five sampling points were classified under poor water while the remaining sampling point was classified under fair water quality.

6.1 Limitation of the study

Environmental measurements are highly prone to variation in time and space change. But in this study the sample collection was conducted in May which is considered as dry season. The sample was not collected in wet season in which the effect may be variation due to the presence of runoff and rainfall which may lead to the occurrence of dilution. Furthermore the sample was collected only for short period of time.

CHAPTER SEVEN

CONCLUSIONS and RECOMMENDATION

7.1 Conclusion

The mean contribution of Cd caused by the effluent was low compared to other heavy metals. The average concentration of target heavy metals in soil and stream water followed the same order with Cu>Cr>Pb>Cd. Similarly, the order of heavy metals in stream sediment and vegetables followed the sequence of Pb > Cr> Cu > Cd. In general, the average concentration of examined heavy metals in different compartment of the study site followed the order of soil>sediment>vegetables>effluent> stream water. The value Igeo in soil and sediment was between 0 and 1 which is classified as unpolluted to moderately polluted contamination level. The mean concentration of examined heavy metals in soil was lower than the international standards but higher in vegetables.

The effect of textile factory effluents was also manifested on Loyale stream. The concentrations of most examined physicochemical characteristics of downstream sampling points were higher than the upstream sites. The downstream points of the stream exhibited alkaline nature.

A total of 7 orders which are classified in to 17 families of BMI were collected. Pollution tolerant macroinvertebrate were more prevalent in the downstream which indicates the presence of organic pollution caused by the discharge of effluents. Diptera were the most dominant order, of which Chironomidae was the most dominant one. But sensitive macro invertebrates were more prevalent in the upstream with Ephemeroptera was the most prevalent one compared to the downstream of the sampling point. In addition, aquatic macroinvertebrate indexes showed deteriorated water quality conditions.

7.2 Recommendation

- The factory owner's, administrators, and EPA local offices should work in collaboration on the way to improve to the efficiency of the treatment plant.
- The factory administrators should appoint a qualified health professional to monitor and follow up the working condition of the factory treatment plant.
- The health professionals should give continuous Awareness programs (sessions) to the local communities on not to use vegetables grown by textile contaminated effluents.
- Further research should be conducted on the chemical composition of dyes.
- The factory should try to use constructed wetland as supplementary of conventional wastewater treatment plant.

References

Abata, E. O., Aiyesanmi, A. F., Adebayo, A.O., Ajayi, O .O . 2013. Assessment of Heavy Metal Contamination and Sediment Quality in the Urban River : A Case Of Ala River in Southwestern – Nigeria. *J. Appl. Chem.* , 4(3): 56–63.

Abdella, A. 2008. Assessment of Challenges, Opportunities and Prospects of Textile Industry in Ethiopia: The Case of Yirgalem Addis Textile Factory, Unity University. 263–281.

Abraha, B.A. 2007. Assessment of Downstream pollution Profiles of Hawassa Textile Factory Effluent along Tikur Wuha River using Physico-chemical and Macroinvertebrate Indicators.

Adebayo, O.T., Balogun, A.M., Olubiyi, O.A. 2007. Chemical analysis of some Industrial Effluents that discharge into Lagos Lagoon, Nigeria. *Res. J. Environ. Sci,* 1(4): 196–199.

Akalu, S., Mengistu, S, Leta, S. 2011. Assessing human impacts on the Greater Akaki River, Ethiopia using macroinvertebrate. *Ethiop. J. Sci.,* 34(2): 89–98.

Ambelu, A., Lock, K., Goethals, P. 2013. Hydrological and anthropogenic influence in the Gilgel Gibe I reservoir (Ethiopia) on macroinvertebrate assemblages Lake and Reservoir Management Hydrological and anthropogenic influence in the Gilgel Gibe I reservoir (Ethiopia) on macroinvertebrate. *Lake and Reservoir Management*.

(APHA- AWWA- WEF). 2012. Standard Methods for examination of water and waste water. 22th edition.

Aschalew, L. 2015. Assessing anthropogenic impacts using Benthic Macroinvertebrate as bio-indicators in central highlands of Ethiopia. *Ethiopian Journal of Environmental Studies and Management,* 8(1): 45–56.

Assefa, T., Sahu, O. 2016. Performance Analysis of Textile Industry Wastewater Treatment Plant with Physicochemical Characterizations. *J. Environ. Treat Tech,* 4(1):22–30.

Awole, M. 2015. Assessment of Heavy Metal Contamination Levels of the Vegetables

Cultivated Along Two Tributaries of Borkena River at Kombolcha Town. A Thesis Submitted to the School of Graduate Studies Addis Ababa University in Partial Fulfillment of the Requirement for the Degree of Master of Science in Environmental Science.

Ayers, R.S., Westcot, D.W. 1985.Water quality for agriculture.FAO irrigation and drainage paper.

Bahiru, D.B. 2017. Levels of some toxic heavy metals in selected vegetables, soil and wastewater around eastern industry zone, central Ethiopia. A Thesis Submitted to the Department of Chemistry, Postgraduate Program Directorate, in Partial Fulfillment of the Requirements for the Degree of Master of Science in Analytical Chemistry.

Barbour, M.T., Gerritsen, J., Snyder, B.D., Stribling, J. B., 1999. Rapid Bio-assessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrate and Fish, Second Edition. U.S. Environmental Protection Agency, Office of Water, Washington, DC.

Beyene, A., Legesse, W., Triest, L., Kloose, H. 2009. Urban impact on ecological integrity of nearby rivers in developing countries : the Borkena River in highland Ethiopia, *Environ Monit Assess*, 153: 461–476.

Bhardwaj, V., Kumar, P., Singhal, G. 2014. Toxicity of Heavy Metals Pollutants in Textile Mills Effluents. *International Journal of Scientific and Engineering Research, 5*(7): 664–666.

Bouchard, R.W., Jr., 2004. Guide to aquatic macroinvertebrate of the Upper Midwest. Water Resources Center, University of Minnesota, St. Paul, MN. 208 pp.

Burgess, G.L. 2015. Effects of Heavy Metals on Benthic Macroinvertebrate in the Cordillera Blanca, Peru. Accepted in partial completion of the requirement for the Degree of Master of Science.

Chiroma, M., Ebewele, R.O., Hymore, F.K. 2014. Comparative Assessment Of Heavy Metal Levels In Soil, Vegetables And Urban Grey Waste Water Used For Irrigation In Yola And Kano.*(International Refereed Journal of Engineering and Science)*, 3(2): 1–9.

Das, M., Ahmed, K., Islam, S., Islam, M., Akter, M.S. 2011. Heavy Metals in Industrial Effluents (Tannery and Textile) and Adjacent Rivers of Dhaka City, Bangladesh. *Terrestrial and Aquatic Environmental Toxicology*, 5(1): 8–13.

Deepali, K.K., Gangawar, K.2010. Metals Concentration in Textile and Tannery Effluents , Associated Soils and Ground Water. *New York Science Journal*, 3(4):82-89

Deribachew, B., Made, M., Dechassa, N., Tadesse, A.M. 2015. Selected heavy metals in some vegetables produced through wastewater irrigation and their toxicological implications in eastern Ethiopia: *African Journal of Food Agriculture Nutrition and Development,* (15(3):10013–10032.

Derso, S., Abebe, B., Getachew, M., Ambelu, A. 2015.Assessment of ecological quality of hot springs in the Eastern Amhara Region. *Environ Syst Res , 4(19)*

Derso,S., Kidane, A.W., Teklu, K.T., Gizew, M., Abera, D., Mesaye, G.*et al*., 2017.Pollution status of Akaki River and its contamination effect on surrounding environment and agricultural products technical report.

Emana, A.N. 2016. Spatial and temporal trends in the water quality of Huluka River, Ethiopia: an assessment using selected physicochemical and biological methods A Thesis Submitted to Center for Environmental Science Presented in Fulfillment of the Requirements for the Degree of Doctor of Philosophy (Environmental Science).

Emigilati, M.A., Ishiaku, I., Usman, B.Y., Kuta, G.I, Dangana, K.2015 .Assessment of effluents discharged from textiles industries in selected villages in Kaduna State, Nigeria. *Afr.J. Environ.Sci.Technol, 9(5):* 385–389.

EEPA. 2003. Provisional standards for industrial pollution control in Ethiopia. pp. 1–35.

FAO/WHO. 2015. Codex Alimentarious International food standards.

Gebrekidan, A., Weldegebriel, Y., Hadera, A. 2013. Ecotoxicology and Environmental Safety Toxicological assessment of heavy metals accumulated in vegetables and fruits grown in Ginfel river near Sheba Tannery, Tigray. *Ecotoxicology and Environmental Safety*. Elsevier, 95: 171–

178.

Gupta, P., Asthana, M., Kumar, A., Barun, S. 2014.Physico chemical Analysis and Microbial Diversity of Yamuna Water and Industrial Effluents. *Int. J. Appl. Sci. Biotechnol*. 199–205.

Hasnine, T., Huda, E., Khatun, R., Saadat, A.M.H., Ahsan, M., Akter, S., *et al.*, 2017. Heavy Metal Contamination in Agricultural Soil at DEPZA , Bangladesh. *Environmental Ecology Research*, 5(7):510–516.

Hayyat, M.U., Mahmood R., Hassan S.W., Rizwan, S. 2013. Effects of textile effluent on growth performance of *Sorghum Vulgare* Pers CV. SSG-5000', *B*iologia, Pakistan, 59(1): 15–22.

Herk, A.V.2012. Physicochemical Parameters in Soil and Vegetable Samples from Gongulon.*International Journal of Chemistry,* 21–36.

Hilsenhoff, W.L. 1988 .Rapid field assessment of organic pollution with a family-level biotic index. *Journal of North American Benthological Society*, 7(1): 65–68.

Hmd, R.F.K. 2011. Degradation of Some Textile Dyes using Biological and Physical Treatments. Thesis Submitted for Partial Fulfillment of the Requirements for the Degree of Master of Science in Microbiology.

IFC. 2007. Environmental , Health , and Safety Guidelines for Textile Manufacturing. pp. 1–20.

Islam, A., Guha, A.K. 2013. Removal of pH , TDS and Color from Textile Effluent by Using Coagulants and Aquatic / Non Aquatic Plants as Adsorbents. *Resources and Environment,* 3(5): 101–114.

Islam, M.M, Mahmud, K., Faruk, O., Billah, M.S.2011. Textile Dyeing Industries in Bangladesh for Sustainable Development. *International Journal of Environmental Science and Development*, 2(6).

Islam, M.S., Mohante, SC., Siddique, M.A., Abdullah-Al-Mamun, M., Hossain, N., Bithi, U.H.2018. Physico-chemical assessment of water quality parameters in Rupsha River of Khulna region, Bangladesh. *International Journal of Engineering Science.* 7(1):57–62.

Iram,S.,Kanwal,S.,Ahmad,I.,Tabassam,T.,Suthar,V.,Mahmood-ul-Hassan,M.2013. Assessment of physicochemical parameters of wastewater samples. *Environ Monit Assess*. 2503–2515.

James, A. Evans, K. S., Dietlinde, N.N., 2018. Assessment of Zambezi river water quality using macroinvertebrate population diversity. *J. Fish. Aquat. Sci*., 13: 12-20.

Kamunda, C., Mathuthu, M., Madhuku, M. 2016. Health Risk Assessment of Heavy Metals in Soils from Witwatersrand Gold Mining Basin , South Africa. *Int. J. Environ. Res. Public Health*, 13(663).

Karim, E. 2015. Physico-Chemical and Microbiological Analysis of Textile Dyeing Effluents. *Journal of Environmental Science, Toxicology and Food Technology,* 9(7): 3–8.

Kartikasari, D., Retnaningdyah, C., Arisoesilaningsih, E. 2013. Application of Water Quality and Ecology Indices of Benthic Macroinvertebrate to Evaluate Water Quality of Tertiary Irrigation in Malang District. *J. Trop. Life Science,* 3(3): 193–201.

Kaur, H., Sharma, G. 2015. Removal of Dyes from Textile Industry Effluent . *International Journal of Humanities and Social Science*, 59–63.

Kihampa, C. 2013 .Heavy metal contamination in water and sediment downstream of municipal wastewater treatment plants, Dares Salaam, Tanzania. *International Journal of Environmental Science* 3(5):1407–1415.

Kumar, V., Chopra, A.K., Srivastava, S. 2013. Assessment of metals in vegetables irrigated with bio-methnated textile effluent at Haridwar (Uttarakhand), India. *Asian J. Plant Sci. Res*, 3(1):120–128.

Mandaville, S.M. 2002. Benthic Macroinvertebrate in Taxa Tolerance Values , Metrics , and Protocols.

Manikandan, P., Palanisamy, P.N, Baskar, R., Sivakumar, P., Sakthisharmila, P. 2015. Physico chemical analysis of textile industrial effluents from Tirupur city. *International Journal of Advance Research in Science and Engineering* .8354(4): 0–3.

(MEDP).2013. Protocols for Completing the Biological Monitoring Wetland Human Disturbance Assessment.

Mehari, A.K., Gebremedhin, S., Ayele, B. 2015. Effects of Bahir Dar Textile Factory Effluents on the Water Quality of the Head Waters of Blue Nile River, *Int. J. Analytic Chemistry.*

Mehari, A.K., Gebremedhin, S., Ayele, B. 2016 .Effects of Bahirdar municipal effluents on water quality of the head of Blue Nile River. *Ethiopian Journal of Environmental Studies and Management,* 9(2): 197–208.

Mereta, S.T. 2013. Water Quality and Ecological Assessment of Natural Wetlands in Southwest Ethiopia. Thesis submitted in fulfillment of the requirements for the degree of Doctor (PhD) in Applied Biological Sciences

Moges, A. 2014. Determination of levels of some heavy metals (Pb, Cr AND Cd) in three commercially available brands of milk powder found in Harar town, Eastern Hararge, Ethiopia. M.Sc. Graduate Project Research Submitted to the Collage of Natural and Computational Sciences In Partial Fulfillment of the Requirements for the Degree of Master of science in chemistry.

Mohad-Aizat, A., Mohamad-Roslan, M.K., Sulaiman, M.A., Karam, D.S.2014 .The relationship between soil pH and selected soil properties in 48 years logged -over forest. *International Journal of Environmental Science,* 4(6):1129–1140.

Mortuza, M.G., Minsad, F.A. 2017. Environmental Contamination and Assessment of Heavy Metals in Water , Sediments and Shrimp of Red Sea Coast of Jizan , Saudi Arabia. *Journal of Aquatic Pollution and Toxicology*, 1(5):1–8.

Najam, S., Nawaz, R., Ahmad, S., Ehsan, N., Khan, M.M., Nawaz, M.H. S. 2015. Heavy Metals Contamination of Soils and Vegetables Irrigated with Municipal Wastewater : A Case Study of Faisalabad. *Journal of Environmental and Agricultural Science,* 4:6-10.

Nyakeya, K.K., Okoth, R.P., Onderi, M.F., John, G. 2009. Assessment of pollution impacts on the ecological integrity of the Kisian and Kisat rivers in Lake Victoria drainage basin, Kenya. *Afr.J. Environ.Sci.Technol.*, 3(4): 97–107.

Olbasa, B.W. 2017. Characterization of Physicochemical Water Quality Parameters of River Gudar (Oromia region, West Shewa Zone, Ethiopia) for Drinking Purpose. *J. Appl. Chem.*, 10(5): 47–52.

Oscoz, J., Galicia, J.O, Miranda, R. 2011. Identification Guide of Freshwater Macroinvertebrate of Spain.

Parameswari, M. 2014. Effect of Textile and Dye Effluent Irrigation on Germination and its Growth Parameters of Green Gram , Black Gram and Red Gram. *IJESTR,* 2(1) : 6–10.

Poornima, K., Vasudevan, K., Venkateshwarlu, M. 2011. Evaluation of heavy metals in textile effluents in relation to soil and pond water. *Electronic J. Environ. Sci.,* 4: 79–84.

Rajasthan, J. 2014. Monitoring of Heavy Metal in Textile Waste Water of, *International Journal of Scientific and Research Publication,* 4(3): 3–6.

Retka, J, Maksymowicz, A., Karmasz, D. 2009. Determination of Cu, Ni, Zn, Pb, Cd by ICP-MS and Hg by AAS in Plant Samples. 15th ICHMET, 373–376.

Ringim, S.A. 2015. Determination of physicochemical parameters and some heavy metals in water samples from Challawa River and Wassai Dam in Kano state , Nigeria. in *Proceedings of The IRES 19th International Conference, Dubai, UAE, 5th December 2015*, 0–24.

Sarkar, M., Islam, B.J. 2016. Pollution and ecological risk assessment for the environmentally impacted. *J. Mater. Environ. Sci.*, 7(7): 2295–2304.

Yessuf, S.S., Moges, H.G., Ahmed, A.N.Determinants of occupational injury in Kombolcha textile factory, North-East Ethiopia. *Int J Occup Environ Med* 2014;**5**:84-93.

Sharma, S.M., Raju., N.S. 2013. Correlation of Heavy Metal contamination with Soil properties of Industrial areas of Mysore , Karnataka , India by Cluster analysis. *Int. Res. J. Environment Sci* , 2(10): 22–27.

Shirkhanloo, H., Mirzahosseini, S.A.H., Shirkhanloo, N., Moussavi-Najarkola, S.A, Farahani, H. 2015.The evaluation and determination of heavy metals pollution in edible vegetables, water and soil in the south of Tehran province by GIS. *Archives of Environmental Protection*.41 (2) :64-74.

Sima, S., Restiani, P. 2017. Water governance mapping repor*t :* Textile industry water use in Ethiopia. Accessed date1/30/2018.

Sivakumar, K.K., Balamurugan, C., Ramakrishnan, D., Bhai, L.H. 2011. Assessment studies on wastewater pollution by textile dyeing and bleaching industries at Kurur Tamil Nadu. *RASAYAN. J. Chem.*, 4(2): 264–269.

Smith, B., Wilson, J. B. and A, J. B. (1996). Consumer s Guide to Evenness Indices. 76(1): 70–82.

Sorsa, S., Chibssa, Y., Tilahun, G., Fitamo, D. 2015. Heavy Metal Concentrations and Physicochemical Characteristics of Effluent along the Discharge Route from Hawassa Textile Factory. *J. Environ. Anal. Toxicol*. 5(4): 1–7.

Syuhadah, S.N., MZ, Rohasliney, H. 2015. Determination of Heavy Metal Contamination from Batik Factory Effluents to the Surrounding Area. *International Journal of Chemical, Environmental and Biological Science,* 3(1): 3–5.

Tiruneh, A.T., Fadiran, A.O., Mtshali, J.S. 2014. Evaluation of the risk of heavy metals in sewage sludge intended for agricultural application in Swaziland. *International Journal of Environmental Science,* 5(1): 197–216.

Turkmen, G., Kazanci, A.N. 2010. Assemblages in streams in a national park in Turkey Applications of various biodiversity indices to benthic macroinvertebrate assemblages in streams of a national park in Turkey. *Review of Hydrobiology*, 3(2):111–125.

Uduma, A.U., Jimoh, W.L.O. 2013. Sequential extraction procedure for partitioning of lead , copper , cadmium , chromium and zinc in contaminated arable soils of Nigeria. *American Journal of Environment, Energy and Power Research*, 1(9): 186–208.

Ullah, AK., Maskud, M.A., Khan, S.R., Lutfa, F.N. 2017. Development and validation of a GF-AAS method and its application for the trace level determination of Pb, Cd , and Cr in fish feed samples commonly used in the hatcheries of Bangladesh. *Journal of Analytical Science and Technology*, 8(15).

Wang, Z., Xue, M., Huang, K., Liu, Z. 2010. Textile Dyeing Wastewater Treatment. Huazhong University of Science and Technology, China. Accessed date 1/10/2018.

Woldeamanuale, T.B. 2017. Study on Physico- Chemical Parameters of Waste Water Effluents from Kombolcha and Debreberhan Industrial Area , Ethiopia. *International Journal of Rural Development, Environment and Health Research*, 1(2): 10–17.

Wosnie, A., Wondie, A. 2014. Bahir Dar tannery effluent characterization and its impact on the head of Blue Nile River. *Afr. J. Environ. Sci. Technol*, 8(6): 312–318.

Yahya, Al N., Mohamed, S.K., Mohamed, A.G. Environmental Pollution by Heavy Metals in the Aquatic Ecosystems of Egypt. *Open Acc J of Toxicol.* 2018;3(1): 555603. DOI: 10.19080/OAJT.2018.03.555603

Yazdian, H., Jaafarzadeh, N., Zahraie, B. 2014.Environmental Health Relationship between benthicmacroinvertebrate bio-indices and physicochemical parameters of water : a tool for water resources managers. *Journal of Environmental Health Science and Engineering*, 12(30): 1–9.

Zinabu, E., Kelderman, P., Kuast, J.V., Irvine, K. 2018. Impacts and Policy Implications of Metals Effluent Discharge into Rivers within Industrial Zones : A Sub-Saharan Perspective from Ethiopia. *Environmental Management*. , 700–715.

Zinabu, E., Yazew, E., Haile, M. 2010. Assessment of the impact of industrial effluents on the quality of irrigation water and changes on soil characteristics (a case of Kombolcha town. *Fourteenth International Water Technology Conference, IWTC 14 2010, Cairo, Egypt,* 711–727.

Annex I

HABITAT ASSESSMENT FIELD DATA SHEET—HIGH GRADIENT STREAMS

		LOCATION	
SITE ID #	REACH ID	STREAM CLASS	
UTM N	UTM E	RIVER BASIN	
STORET #		AGENCY	
INVESTIGATORS			
FORM COMPLETED BY		DATE TIME AM	REASON FOR SURVEY

Habitat Parameter	Condition Category			
	Optimal	Suboptimal	Marginal	Poor
1. Epifaunal Substrate/ Available Cover	Greater than 70% of substrate favorable for epifaunal colonization and fish cover; mix of snags, submerged logs, undercut banks, cobble or other stable habitat and at stage to allow full colonization potential (i.e., logs/snags that are not new fall and not transient).	40-70% mix of stable habitat; well-suited for full colonization potential; adequate habitat for maintenance of populations; presence of additional substrate in the form of newfall, but not yet prepared for colonization (may rate at high end of scale).	20-40% mix of stable habitat; habitat availability less than desirable; substrate frequently disturbed or removed.	Less than 20% stable habitat; lack of habitat is obvious; substrate unstable or lacking.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
2. Embededness	Gravel, cobble, and boulder particles are 0-25% surrounded by fine sediment. Layering of cobble provides diversity of niche space.	Gravel, cobble, and boulder particles are 25-50% surrounded by fine sediment.	Gravel, cobble, and boulder particles are 50-75% surrounded by fine sediment.	Gravel, cobble, and boulder particles are more than 75% surrounded by fine sediment.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
3. Velocity/Depth Regime	All four velocity/depth regimes present (slow-deep, slow-shallow, fast-deep, fast-shallow). (Slow is < 0.3 m/s, deep is > 0.5 m.)	Only 3 of the 4 regimes present (if fast-shallow is missing, score lower than if missing other regimes).	Only 2 of the 4 habitat regimes present (if fast-shallow or slow-shallow are missing, score low).	Dominated by 1 velocity/ depth regime (usually slow-deep).
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
4. Sediment Deposition	Little or no enlargement of islands or point bars and less than 5% of the bottom affected by sediment deposition.	Some new increase in bar formation, mostly from gravel, sand or fine sediment; 5-30% of the bottom affected; slight deposition in pools.	Moderate deposition of new gravel, sand or fine sediment on old and new bars; 30-50% of the bottom affected; sediment deposits at obstructions, constrictions, and bends; moderate deposition of pools prevalent.	Heavy deposits of fine material, increased bar development; more than 50% of the bottom changing frequently; pools almost absent due to substantial sediment deposition.

SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	54 3 2 1 0
5. Channel Flow Status	Water reaches base of both lower banks, and minimal amount of channel substrate is exposed.	Water fills >75% of the available channel; or <25% of channel substrate is exposed.	Water fills 25-75% of the available channel, and/or riffle substrates are mostly exposed.	Very little water in channel and mostly present as standing pools.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	54 3 2 1 0

Habitat Parameter	Condition Category			
	Optimal	Suboptimal	Marginal	Poor
6. Channel Alteration	Channelization or dredging absent or minimal; stream with normal pattern.	Some channelization present, usually in areas of bridge abutments; evidence of past channelization, i.e., dredging, (greater than past 20 yr) may be present, but recent channelization is not present.	Channelization may be extensive; embankments or shoring structures present on both banks; and 40 to 80% of stream reach channelized and disrupted.	Banks shored with gabion or cement; over 80% of the stream reach channelized and disrupted. Instream habitat greatly altered or removed entirely.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
7. Frequency of Riffles (or bends)	Occurrence of riffles relatively frequent; ratio of distance between riffles divided by width of the stream <7:1 (generally 5 to 7); variety of habitat is key. In streams where riffles are continuous, placement of boulders or other large, natural obstruction is important.	Occurrence of riffles infrequent; distance between riffles divided by the width of the stream is between 7 to 15.	Occasional riffle or bend; bottom contours provide some habitat; distance between riffles divided by the width of the stream is between 15 to 25.	Generally all flat water or shallow riffles; poor habitat; distance between riffles divided by the width of the stream is a ratio of >25.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
8. Bank Stability (score each bank) Note: determine left or right side by facing downstream.	Banks stable; evidence of erosion or bank failure absent or minimal; little potential for future problems. <5% of bank affected.	Moderately stable; infrequent, small areas of erosion mostly healed over. 5-30% of bank in reach has areas of erosion.	Moderately unstable; 30-60% of bank in reach has areas of erosion; high erosion potential during floods.	Unstable; many eroded areas; "raw" areas frequent along straight sections and bends; obvious bank sloughing; 60-100% of bank has erosional scars.
SCORE ___ (LB)	Left Bank 10 9	8 7 6	5 4 3	2 1 0
SCORE RB)	Right Bank 10 9	8 7 6	5 4 3	2 1 0

9. Vegetative Protection (score each bank)	More than 90% of the streambank surfaces and immediate riparian zone covered by native vegetation, including trees, understory shrubs, or nonwoody macrophytes; vegetative disruption through grazing or mowing minimal or not evident; almost all plants allowed to grow naturally.	70-90% of the streambank surfaces covered by native vegetation, but one class of plants is not well-represented; disruption evident but not affecting full plant growth potential to any great extent; more than one-half of the potential plant stubble height remaining.	50-70% of the streambank surfaces covered by vegetation; disruption obvious; patches of bare soil or closely cropped vegetation common; less than one-half of the potential plant stubble height remaining.	Less than 50% of the streambank surfaces covered by vegetation; disruption of streambank vegetation is very high; vegetation has been removed to 5 centimeters or less in average stubble height.
SCORE ___ (LB)	Left Bank 10 9	8 7 6	5 4 3	2 1 0
SCORE ___ (RB)	Right Bank 10 9	8 7 6	5 4 3	2 1 0
10. Riparian Vegetative Zone Width (score each bank riparian zone)	Width of riparian zone >18 meters; human activities (i.e., parking lots, roadbeds, clear-cuts, lawns, or crops) have not impacted zone.	Width of riparian zone 12-18 meters; human activities have impacted zone only minimally.	Width of riparian zone 6-12 meters; human activities have impacted zone a great deal.	Width of riparian zone <6 meters: little or no riparian vegetation due to human activities.
SCORE ___ (LB)	Left Bank 10 9	8 7 6	5 4 3	2 1 0
SCORE ___ (RB)	Right Bank 10 9	8 7 6	5 4 3	2 1 0

Total Score __________

Annex II

Table results of qualitative habitat assessments of sampling points in Loyale streams

Parameter		Dr3	Dr2	Dr1	Ur1	Ur2
Epifaunal substrate		14	9	10	15	15
Embededness		11	8	9	12	13
Velocity/depth		6	**7**	8	10	9
Sediment deposition		10	5	5	9	11
Channel flow status		10	8	7	16	16
Channel alteration		19	16	17	18	19
Frequency of riffle		15	15	12	12	13
Bank stability	Left	8	8	8	7	8
	Right	8	8	7	8	8
Vegetation protection	Left	8	7	7	9	9
	Right	9	8	7	8	8
Riparian vegetation	Left	10	5	5	7	10
	Right	4	4	4	7	7
Score (200)		132	108	106	138	146
100%		66	54	60	69	73
Habitat score		Sub optimal	Marginal	Marginal	Sub optimal	Sub optimal

Habitat condition score: poor<60, marginal 60-109, sub-optimum 110-159 and optimum 160-200 (Barbour et al., 1999)

,

Annex III

Human Disturbance Ranking Form-Loyale stream (human disturbance of habitat)

Station #: ________ Date: ________ Time: ____________ Town: _________

Evaluator(s): ________ Name of river or water bodies: ________

The purpose of this ranking is to characterize the degree of human disturbance at given water bodies bio-monitoring station, including the portion of the watershed immediately surrounding the station, relative to other stations sampled. (Note that the human disturbance ranking is not intended to serve as an impact assessment in the absence of biological data.)For each wetland station assessed, score all potential factors in the five categories below using the following scale

Severity	Severity disturbance	Rank
Not observed or unknown	The stressor is not observed or has detrimental impacts on wetland condition	**0**
Observed minimal disturbance	The stressor is present and appears to have negligible impacts on wetland condition	**1**
Low disturbance	The stressor is present and appears to have minor impacts on wetland condition	**2**
Moderate disturbance	The stressor is present and appears to have moderately impacts on wetland condition	**3**
High disturbance	The stressor is present and appears to have significantly impacts on wetland condition	**4**
Sever disturbance	The stressor is present and appears to have major impacts on wetland condition	**5**

1 Hydrologic modification	**Observation /comment**	**Score**	**Section Subtotal:-**
Man made dikes or dams			
Cause ways roads or railroads bed crossing which impede water flow inadequate or obstructed culverts			
Ditching, draining or dewatering			
Filling or bulldozing			
Other hydrologic modification not included in this section (specify)			
2.Vegtative modification	**Observation /comment**	**Score**	**Section Subtotal**
Timber harvesting in wetlands			
Other clearing removal of vegetation of			

vegetation (roads utilities and lines)			
Plowing mowing or grazing in river bank			
Evidence of herbicide use in near to water bodies			
Other vegetative modification not included in this section (specify)			
3.Evidence chemical pollutants	**Observation /comment**	**Score**	**Section Subtotal 3**
Discharge pipes			
Oil ,petroleum ,chemicals observed or chemical odour present			
Soil staining and/or stressed or dying vegetation			
Trash ,chemical containers ,demolition debris ,drums e.t.c			
Other evidence of chemical pollutants not included in this section (specify)			
4.Impervious surface in water shade	**Observation /comment**	**Score**	**Section Subtotal 4**
Residential development			
Commercial or industrial development			
Recreational developments (campgrounds picnic or boat launch, areas ,trails boardwalks parking areas etc			
Additional roads ,highways bridges			
Other impervious surfaces not included in this section specify			
5.potential for NPS pollution	**Observation comment**	**Score**	**Section Subtotal 5**
Excess sediment accumulation or unstable or eroding soil from human activities (roads ,excavation sites ,agriculture ,forestry activities observed			
Alteration to wetland buffer (within 100 feet of wetland edge			
Livestock’s, feedlots or manure piles			
Evidence of fertilizers or pesticide use (lawns ,golf courses, agricultural crops etc			
Other NPS sources not included in this section			
Additional notes if needed			

Annex IV

Parameters and their corresponding method of determination

Parameter	Method of determination
PH	Ph meter
Temperature	Thermometer
Conductivity	Conductivity meter
TDS	TDS meter
TS	Gravimetric
TSS	Gravimetric
DO	Winkler
BOD	Winkler
Chloride	Argentometric Titrimetric
Total alkalinity	Titrimetric
Total hardness	Titrimetric
Sulphate	Spectrophotometer
Nitrate	Spectrophotometer
Heavy metals	FAAS

Annex V

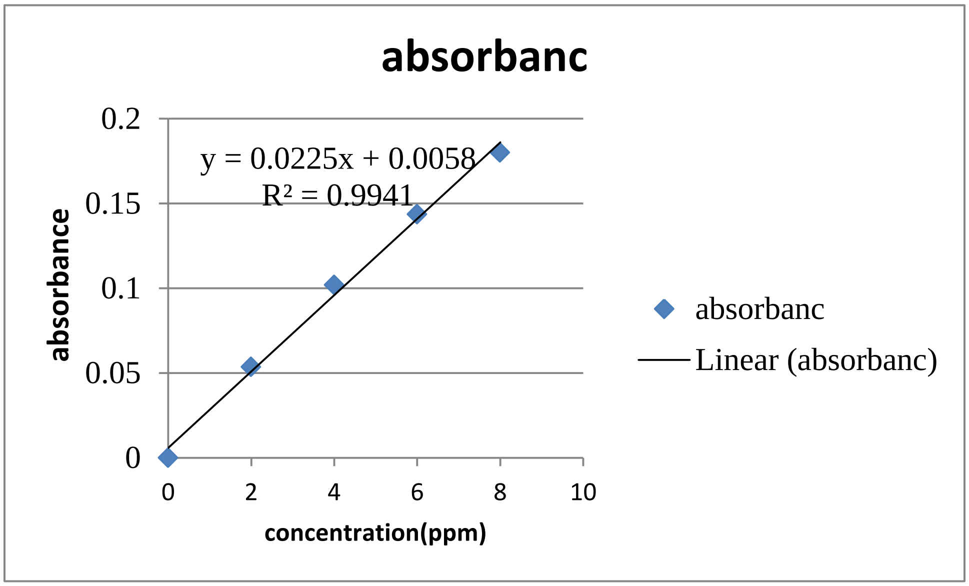
Textile wastewater limit Values for discharges to Water bodies Source (EEPA, 2003)

Parameters	Permissible Limit values (MPL)
pH	6-9
Temperature	40℃
Total dissolved solids	80mg/l
Conductivity	1000µs/cm
Sulphides	2mg/l
BOD	50mg/l(>90% removal)
COD	150mg/l(>80% removal)
Ammonia	30mg/l
Nitrate	50mg/l
Total phosphorous	10mg/l(>80% removal)
Total nitrogen	40mg/l(>80%removal)
Sulphate	200mg/l
Total suspended solids	30mg/l
Nickel	2mg/l
Chromium	1mg/l
Cadmium	1mg/l
Lead	0.5mg/l
Copper	2mg/l
Iron	1mg/l for dissolved iron
Zink	5mg/l

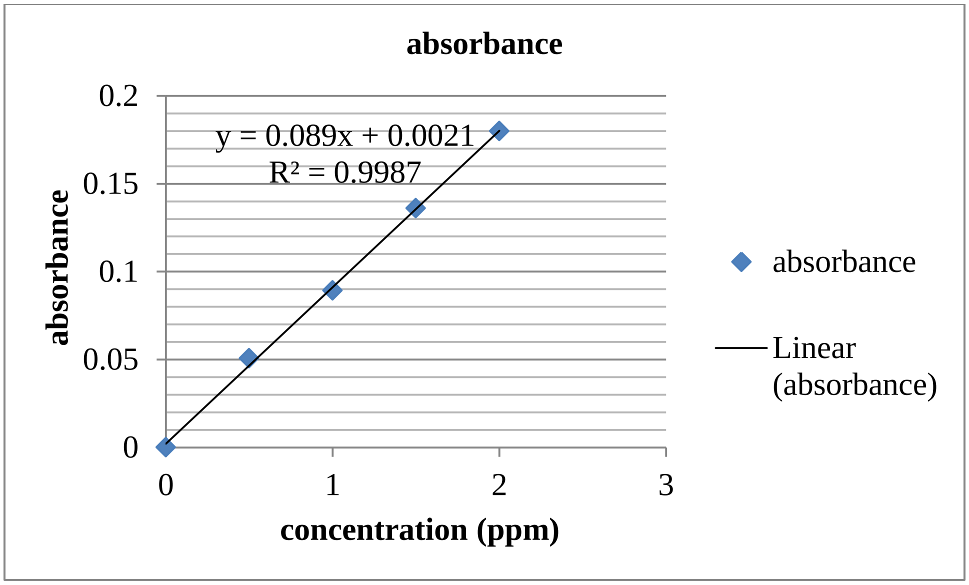
Annex VI

Determination of calibration curve for heavy metals

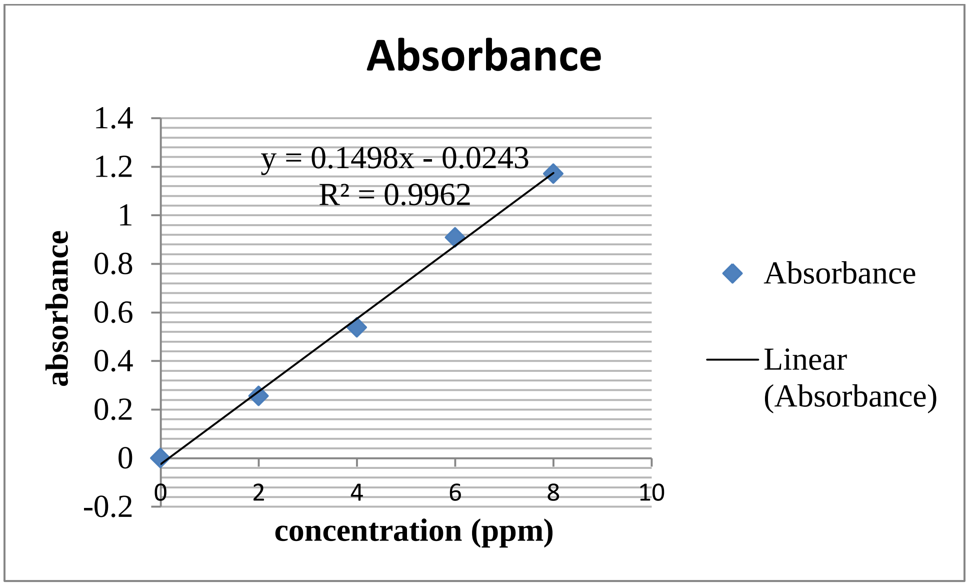
Calibration curve for lead



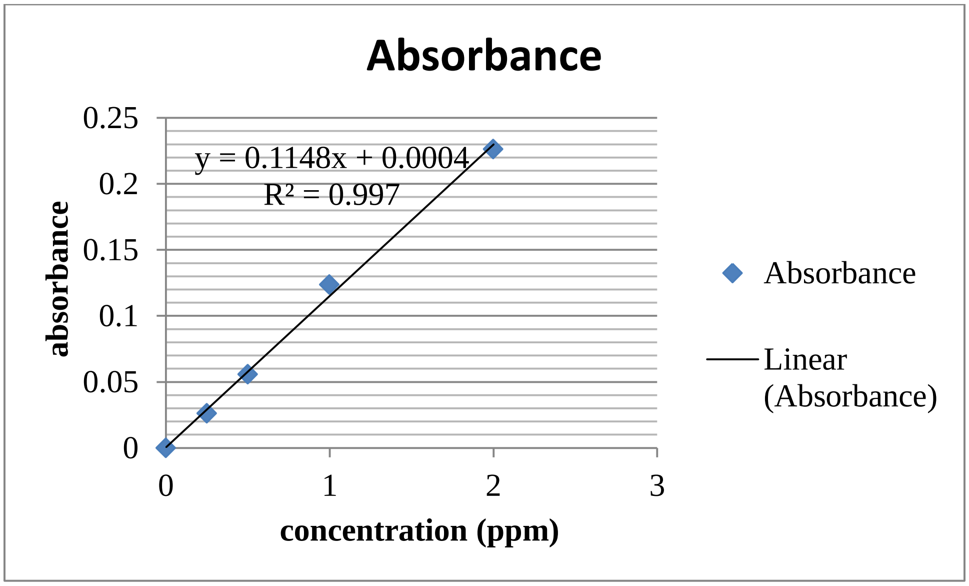
Calibration curve of chromium



Calibration curve of cadmium



Calibration curve of copper



Annex VII

Heavy metal transfer factor from of the study site kombolcha North East Ethiopia 2018

HM	Sampling point				
	S1	S2	S3	SC	Average
Pb	0.54	0.53	0.41	0.53	0.5
Cd	0.66	0.68	0.65	0.63	0.66
Cr	0.61	0.39	0.18	0.17	0.34
Cu	0.09	0.14	0.07	0.15	0.11

Average concentrations of PH, EC and selected heavy metals in Loyale stream sediment at each sampling site, Kombolcha, North East Ethiopia 2018

	Sd1	Sd2	Sd3	Ur1	Ur2
PH	8.7±0.44	9.5±0.61	8.1±0.46	6.9±0.35	6.5±0.36
EC	892±51.9	960±37.64	745±28.6	480±26.46	545±31.2
Pb	8.5±1.23	9.1±1.16	19.9±2.23	1.89±0.157	2.3±0.77
Cd	1.62±0.1	1.84±0.17	1.74±0.23	1.76±0.21	1.77±0.155
Cr	10.1±1.96	11.9±1.18	12±1.42	10.9±1.68	9.53±1.26
Cu	8.71±1.81	8.5±2.16	8.25±1.22	4.18±1.07	3.8±0.64

Average concentration of PH, EC and heavy metals in Loyale stream water of the study site Kombolcha North East Ethiopia 2018

	Ur2	Ur1	Dr1	Dr2	Dr3
EC	254±18.7	185±9.9	1996.7±26	1462±68.6	1348±63
pH	5.5±0.3	5.2±0.4	8.9±0.4	7.9±0.17	6.8±0.33
Pb	0.023±0.002	0.045±0.006	0.1±0.006	0.08±0.005	0.265±0.04
Cd	0.013±0.002	0.011±0.002	0.02±0.002	0.01±0.00	0.02±0.002
Cr	0.89±0.05	0.2±0.026	0.65±0.036	0.4±0.06	1.67±0.1
Cu	0.59±0.06	0.29±0.046	1.54±0.065	0.95±0.09	0.75±0.06

Average concentration of physicochemical and heavy metal charactesrtics of effluents in different sampling point in kombolcha textile factory effluents North East Ethiopia, 2018(in mg/l unless specified**)**

Parameter	Inlet effluent (mean±sd)(n=3)	Effluent1 (mean±sd)	Effluent2 (mean±sd)	Effluent3 (mean±sd)
Ambt T^{o} (℃)	27.4((±1.3)	27.5±0.608	26.5±0.98	27±0.46
Water T^{o}(℃)	27.97(±1.04)	26.5±0.53	27.2±0.624	26.9±2.3
DO	1.27(±0.25)	2.1±0.3	2.5±0.72	2.6±0.2
EC(μs/cm)	4754.24(±669.9	3105.4±116.2	2822±228.7	2698.9±196
pH	11.25 (±0.67)	10.2±0.34	10.5±0.95	9.4±0.72
Turbidity(NTU)	67.55(± 5.22)	58.2±2.8	54.57±5.48	50.58±3.48
TS	5622.73±442.8	4252.6±293.3	4098±241.55	3550±225.8
TDS	3663.43(±172.53)	2657±187.8	2789±108.69	2358±196.5
TSS	1956(±303.28)	1595.6±157.1	1309±136.36	1192±30.9
BOD	680.83±54.91)	535.1±43.91	564.8±29.32	494±16.18
Total Alkalinity	839(±195.9)	629±34.43	569±24.3	522±28.67
Chloride	384.25(±36.38	284.9±17.13	214.96±20.72	219.63±13.9
Nitrate	74.3 (±3.21)	52±4.0	78±	73±3.82
Sulphate	43.9(±87.93)	50.5±20.34	48.7±20.18	32.5±24.9
Total hardness	940.03(±104.5	780±39.94	685±16.7	646±29
Pb		0.04±0.026	0.019±0.0036	0.016±0.006
Cd		0.015±0.007	0.094±0.07	0.017±0.006
Cr		2.2±0.275	0.97±0.07	0.98±0.108
Cu		1.35±0.105	0.95±0.07	0.75±0.088

Average physicochemical and heavy metal characteristics of Loyale stream sampling point kombolcha north east Ethiopia 2018 (mg/l unless specified)

Parameter	Ur1	Ur2	Dr1	Dr2	Dr3
Ambient T^o(℃)	27.4±0.7	26.4±0.5	27±0.4	28±0.5	27.8±0.7
Water T^o(℃)	23.4±0.5	24.3±0.4	26.7±0.3	27.5±0.6	25.4±1.6
Velocity(m/s)	0.33±0.03	0.29±0.02	0.29±0.02	0.28±0.02	0.27±0.03
Depth(m)	0.33±0.02	0.34±0.02	0.36±0.01	0.22±0.03	0.26±0.01
Width (m)	2.0±0.26	2.2±0.17	1.75±0.13	1.8±0.1	1.75±0.2
DO	6.9±0.2	6.4±0.45	3.1±0.2	4.3±0.2	4.5±0.3
Ec(µs/cm)	185±9.9	254±18.7	1996.7±216	1462±68.6	1348±63
pH	5.2±0.4	5.5±0.3	8.9±0.4	7.9±0.17	6.8±0.33
Turb (NTU)	12.96±1.5	9.74±0.6	38.4±2.0	32.9±2.4	21.96±2.0
TS	479±2.2	563±29.3	2540.7±111	1945.4±76	1589.3±42
TDS	259±5.4	238±15.3	1285±61.6	1140±43.7	985±24
TSS	220±3.84	315±10.6	1255.7±49.3	805.4±35	604.6±18.7
BOD	16.9±1.5	13.1±0.9	154.3±13.3	95±6.2	62.5±3.3
Total alkalinity	102.5±5.3	74.6±5.2	459±15	425±24.8	380±8.3
Chloride	60±5.0	69.8±5.6	145±8.1	164.5±16.4	74.7±7.5
Nitrate	0.53±0.06	0.62±0.09	6.4±0.43	5.7±0.35	7.1±0.44
Sulphate	2.92±11.2	3.02±11.8	40.5±14	28.5±10.6	29.7±14.4
Total hardness	114.6±8.4	106.8±7.2	524±103.8	595±9.3	445±20.7

Annex VIII

Equipments and materials used

1. Different size beakers,
2. measuring cylinders,
3. micropipette,
4. volumetric flasks,
5. burettes,
6. funnel,
7. test tubes,
8. thermometer,
9. stopwatch,
10. ceramic mortar and pestle, 2 mm sieve,
11. Oven,
12. electronic-mill,
13. Plastic bags,
14. stirrer
15. Erlenmeyer flasks (different sizes),
16. refrigerator,
17. filter papers No. 42
18. . Hot plate
19. Conical (Erlenmeyer) flasks,
20. 125-mL, or Griffin beakers
21. 150-mL, acid-washed and rinsed with water,
22. volumetric flasks, 100-mL
23. Watch glasses ribbed and un ribbed
24. Safety shield, Safety goggles.
25. Standard D frame net 500µm mesh size
26. Sieve and bucket
27. Sieve and bucket
28. Ethanol 80%

29. Forceps
30. Vials
31. Labeling materials
32. Permanent markers
33. Sorting tray
34. Pencil
35. Clip board
36. Pipette
37. Bottle for water sample
38. Multi parameter probe
39. Distilled water
40. Cooled box
41. Field protocol
42. Portable Gps
43. Camera
44. Spectrophotometer
45. Bod digester
46. Dissecting microscope with magnification power of 10 times

Spearman correlation of physico chemical and heavy metals in effluents of textile factory in kombolcha town North East Ethiopia, 2018

	Wt	DO	EC	pH	Turb	Tds	Tss	Bod	TA	Chlor	No_3	SO_4	TH	Pb	Cd	Cr	Cu
WT	1																
DO	-0.77	1															
EC	0.79	0.98	1														
pH	0.78	0.97	0.96	1													
Turb	0.77	-1.00	0.98	0.97	1												
Tds	0.80	-0.99	0.97	0.98	0.99	1											
TSS	0.77	-0.98	0.98	0.97	0.98	0.97	1										
BOD	0.80	-0.99	0.97	0.98	0.99	1.00	0.97	1									
TA	0.78	-0.99	0.99	0.95	0.99	0.99	0.97	0.98	1								
Cl	0.76	-0.96	0.95	0.96	0.96	0.94	0.94	0.94	0.94	1							
NO_3	0.64	-0.73	0.75	0.77	0.73	0.78	0.69	0.78	0.74	0.7	1						
SO_4	0.26	-0.6	0.61	0.6	0.6	0.6	0.66	0.6	0.6	0.5	0.6	1					
TA	0.80	-0.99	0.97	0.96	0.99	0.98	0.96	0.98	0.98	0.97	0.7	0.5	1				
Pb	0.21	0.42	-0.41	-0.43	-0.42	-0.45	-0.3	-0.45	-0.45	-0.43	-0.6	-0.1	0.43	1			
Cd	0.31	-0.4	0.45	0.41	0.4	0.46	0.41	0.46	0.46	0.26	0.68	0.5	0.34	-0.4	1		
Cr	0.17	-0.62	0.55	0.57	0.62	0.58	0.55	0.48	0.58	0.63	0.52	0.43	0.61	0.4	.3	**1**	
Cu	0.5	-0.59	0.56	0.53	0.59	0.57	0.64	0.57	0.57	0.5	0.3	0.7	0.55	0.34	.2	**.3**	**1**