



# **The impact of Modjo Tannery on Modjo River: Diatom-based water quality assessment**

**By**

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## Acronyms

<b>BOD<sub>5</sub></b>	5-Day Biochemical Oxygen Demand
<b>CCA</b>	Canonical Correspondence Analysis
<b>CCME</b>	Canadian Council of Ministers of the Environment
<b>DO</b>	Dissolved Oxygen
<b>EEPA</b>	Ethiopian environmental impact Assessment
<b>GDI</b>	Generic diatom index
<b>PSI</b>	Pollution sensitivity index
<b>%(PTV)</b>	Pollution tolerant valve
<b>SRP</b>	Soluble Reactive Phosphorus
<b>RDA</b>	Redundancy analyses
<b>UNEP</b>	United Nations Environment Program
<b>USEPA</b>	United States Environmental Protection Agency
<b>WHO</b>	World health organization
<b>WMS</b>	Weighted Mean Sensitivity

## Definitions of Terms

A **metric** is a characteristic of the biota that changes in some predictable way with increased human influence (Barbour, *et al.*, 1999).

**Bi indicators** are species that can be used to as indicator the health of an environment or ecosystem (Chutter, 2003).

**Biological assessment** can be defined as the systematic use of biological responses to evaluate changes in the environment with the intent to use this information in a quality control program (Ward, 2001).

**Biological integrity** is defined as 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 (Moog and Chovanec, 2000).

**Diatoms** are a group of mostly unicellular and photosynthetic algae with siliceous cell wall (frustule) and whose size (length or diameter) is predominantly between 20 and 200 microns (De Zwart, 2003).

**Eigenvalues** are values that represent the amount of the original variance explained by each of new derived variables (Quinn and Keough, 2002).

**Water quality** is a term used to express the suitability of water to sustain various uses or processes (Chapman, 1996).

**Water quality monitoring** is the process needed to verify whether the observed water quality is suitable for intended uses, to determine trends in the quality of the aquatic environment and how the environment is affected by the release of contaminants, by other human activities, and/or by waste treatment operations. (Chapman, 1996)

## ABSTRACT

The present study aimed at assessing the impact of Modjo Tannery on Modjo River using diatoms as bioindicators. A total of 4 sampling sites were selected along the course of Modjo River for evaluation of physicochemical parameters and determination of the taxonomic composition and abundance of diatoms. Standard Method of sampling and analysis of samples were employed throughout the study period. Biotic indices and multivariate analytical methods, particularly Redundancy analyses were used for the analysis of the result. Most of the physico-chemical characteristics of the wastewater effluent were above the provisional discharge limit set out by the Ethiopian Environmental Protection Authority and world Health Organization. All physicochemical water quality parameters measured in samples from Modjo River except dissolved oxygen exhibited similar spatial variations with their levels declining consistently from their maxima at S2, the point of discharge of the tannery effluent, to those of the downstream sites. The lowest oxygen concentration of S2, which was far below the level capable of supporting aerobic heterotrophs, was significantly different from those of other sampling sites ( $p < 0.05$ ) and coincided with the present highest levels of BOD<sub>5</sub> and COD. Sites with relatively good water quality, S1 (reference site) and S4 (the most downstream site), were associated with such pollution-sensitive species of diatoms as *Cocconeis placentula*, *Fragilaria crotonensis*, *Gomphonema angustatum* and *G. gracile*, while sites with poor water quality, S2 and S3 (below S2), supported an assemblage of such pollution-tolerant species as *Fragilaria capucina*, *Gomphonema parvulum*, *Hantzschia amphioxys* and *Nitzschia palea*. Species richness, diversity and abundance of diatoms differed significantly among the sampling sites, with their highest and lowest levels occurring at S1 and S2, respectively. RDA of the association of sampling sites, diatom species and physico-chemical water quality clearly separated the reference site (S1) from S2 and S3 in terms of water quality, which was also suggested by the physico-chemical and biological data recorded in the present study. The present results clearly show the detrimental impact of the tannery effluent on the ecological integrity of Modjo River. The water quality of Modjo River represents a serious threat to public health and aquatic and terrestrial life. The concerned government bodies including EEPA should, therefore, do their utmost to put an end to the ever-increasing degradation of the water quality of this ecologically and economically important river.

**Key words:** bioindicators, diatoms, water quality, Modjo River, monitoring; RDA

# CHAPTER ONE: INTRODUCTION

## 1.1 Background and Justification

Freshwaters are finite resources, which are essential for agriculture, industry and hence human existence. Without freshwater of enough quantity and acceptable quality, sustainable development of a country is not possible. Nowadays aquatic ecosystems are threatened on a world-wide scale by a variety of pollutants as well as destructive land-use or water management practices. Some of these environmental problems have been present for a long time, but have only recently reached a critical level, while others are newly emerging (Bartram and Balance, 1996).

Deterioration of water quality has become a major environmental problem in Ethiopia due to rapid population growth, urbanization and various agricultural activities (Seyoum Leta *et al.*, 2003). The tannery industry in Ethiopia is among the country's largest external income earners. Consequently, during the past 20 years or so, the industry has expanded throughout the country due to significant government support. Modjo tannery is such an industry that disposes its liquid waste into Modjo River. Effluents, which are discharged from this and other tanneries and textile industries, contain high levels of metals, especially; chromium, copper and cadmium and other toxic compounds. These effluents are released onto the land or dumped into surface waters, ultimately leaching into ground water leading to its contamination.

Heavy metals are important for proper functioning of biological systems, but their shortage or excess could lead to a number of disorders or damage (Ward, 2001). In many parts of Ethiopia, wastewaters (industrial effluents), which are disposed into rivers, ponds, streams and non-functional treatment plants, are used as a source of irrigation and drinking water. Due to the high demand on irrigation water supply, farmers frequently faced the problem of utilization of poor quality water. Thus, the continued application of poor quality irrigation water can reduce the yield of different crops in farmlands (Eskindir Zinabu, 2002).

Industrial effluents cause a large number of environmental and health problems, which have become more complex and critical not only in developing countries but also in developed countries. The protection of public health and aquatic life necessitates assessment and monitoring of water quality. Therefore, the need for water quality assessment using well developed tools that show the past and present pollution status and demand less time for sample collection, preparation for observation and storage is unquestionable.

Water quality can be described in terms of physical, chemical and biological characteristics. Previously, water quality monitoring was based mainly on physicochemical parameters, which indicate the quality of water at the time of sampling only, and is still the commonest method in developing countries (Barbour, *et al.*, 1999). Anthropogenic (and in some cases natural) changes in these physical and chemical features of freshwaters can produce diverse biological effects. As each aquatic organism has particular requirements with respect to the physico-chemical and biological conditions of its habitat, changes in these conditions can result in reduction in species numbers, a change in species abundance or total loss of sensitive species by death or migration. Such changes indicate that the ecosystem and its organisms are under stress or that the ecosystem has become perturbed.

These changes in the aquatic environment can, therefore, be used to indicate the pollution level of the water body and even possible risks to human health and other organisms (Bartram and Balance, 1996). Thus, biological indicators are very important for assessing water quality because they show the cumulative effects of present and past conditions unlike chemical and physical data, which apply only to the moment of sampling. Therefore, biological indicators are extremely useful in evaluating anthropogenic impacts on aquatic environments.

Although the effects of anthropogenic activities on aquatic environment and their biota have been studied for decades, the findings have only recently been transformed into methods suitable for monitoring the quality of water bodies. Recently, biological indicators such as diatoms are getting more acceptance as routine monitoring tools especially in developed countries, while there is a need for the development of such tools in developing countries (Chapman, 1996).

Diatoms are a species-rich group of photosynthetic eukaryotes, with enormous ecological significance and great potential for environmental application. (Schmutz, 2007). Distribution, composition and abundance of diatoms are influenced by a variety of physical, chemical and biological factors. Diatoms have been used extensively for biomonitoring of aquatic ecosystems due to their wide geographical distribution, ease of sampling and preservation, high reproduction rate and rapid response to environmental changes and deterioration of water quality, especially from impacts such as eutrophication, acidification, and heavy metal contamination (Bunn, 2000).

Strong relationships between diatom species and water chemistry have been established and many diatom indices for water quality assessment of rivers and lakes have been developed (Kelly and Whitton, 1995) although some studies have shown that diatom-based indices vary in their capacity to predict trophic status, ionic composition, and organic pollution in rivers (Taylor et al., 2007). In Ethiopia, many rivers are heavily polluted owing to their flow through major cities and towns and their location near such industries as tanneries (Seyoum Leta *et al.*, 2003).

The unlimited use of toxic chemicals in modern tanneries and disposal of their effluents into water bodies and on land may have detrimental effects on human health and the aquatic environment mainly in developing countries (James, 2005). Modjo River, which is used as liquid waste disposal system by Modjo Tannery, contains chemical pollutants whose concentrations have surpassed the permissible levels set by the National Environmental Quality Standards (Seyoum Leta *et al.*, 2003).

Physical and chemical methods used to monitor water quality allow only instantaneous measurements that provide information on water quality at the time of measurement, while biological methods enable us to test long-term environmental impact on biota since these methods have the capacity of showing the conditions, which are not anymore present at the time of sampling and analysis. The most direct and effective measure of the integrity of a water body is, thus, the status of its biological system (Chvance *et al.*, 2000) as chemical, physical, or biological stressors impact the living characteristics of an aquatic environment (Bartram and Balance, 1996).

Therefore, physical and chemical criteria can be regarded as being complementary to biological criteria and together with biological criteria constitute the basis for a dependable assessment of the ecological integrity of running waters (Coste, 1991). In order to produce dependable information on the impact of Modjo tannery on the ecological integrity of Modjo River, the water quality of the river was, therefore, assessed using diatoms as bio-indicators.

## **CHAPTER TWO: LITERATURE REVIEW**

### **2.1. Biological Water Quality assessment**

Biological assessment can be defined as the systematic use of biological responses to evaluate changes in the environment with the intent to use this information in water quality management and development program (Eloranta and Soininen, 2002). This definition is often used in a restricted sense in which biological assessment refers to field studies on plankton, or any other form of biological community in a river to evaluate biological water quality. In this sense, biological assessment is a form of water body or ecosystem assessment and monitoring (De Zwart, 2003).

Biological community's parameters reflect water quality better than any physico-chemical measurements because communities respond to the entire range of biogeochemical factors in the environment. Preserving the biological integrity of a water body enables humans to use the water for drinking, fishing, washing, irrigation, generating electricity, etc. when water is no longer capable of supporting living things, it will no longer support human health. (Char and Chu, 2000).

Biological criteria represent a measurable and tangible goal against which the effectiveness of pollution control and other water quality monitoring efforts can be judged. However, biological assessments must be accompanied by appropriate chemical/physical measurements, and pollution source information necessary to establish linkages between stressors and the biological responses (Kwandrans *et al.*, 2001).

### **2.2. Advantages of biological assessment using diatoms**

Biological assessments and monitoring are important for the sustenance of the ecological integrity of aquatic ecosystems as it directly measures the result of pollution (Gadzala et al., 2005). Biological methods assess changes in population structure, productivity, and other ecological parameters and are hence better indicators of the overall consequence of pollution

than the physicochemical methods. Biotic communities integrate environmental conditions over a long period of time and require low-frequency sampling whereas chemical analyses show the condition of the environment at the time of sampling. Aesthetic, recreational and ecological dimensions of an aquatic environment can only be expressed in terms of biological or ecological features and be assessed by biological methods only (Metcalf, 2006).

Biological monitoring tools have been known as part of routine monitoring programmes due to certain shortcomings in standard physical and chemical methods. Because of the difficulty in and cost of chemically analyzing every potential pollutant in a sample of water, and of interpreting results in terms of impact severity, it makes sense to monitor aquatic biota. The main advantage of a biological approach is that it examines organisms whose exposure to pollutants is continuous. Thus, the biological community that is found in riverine ecosystems reflects both the present and past history of the water quality in the river, allowing detection of disturbances that might otherwise be missed (Ekhouste, 2006).

Biological communities reflect the overall ecological integrity by integrating various stressors, thus providing a broad measure of their synergistic impacts. Aquatic communities integrate and reflect the effects of chemical and physical disturbances that occur over extended periods of time. These communities can, therefore, provide a holistic and an integrated measure of the integrity or health of the river as a whole (Chutter, 2003). Biological communities reflect overall ecological integrity of the ecosystem (i.e. chemical, physical, and biological integrity). Biological communities integrate the effects of different stressors and thus provide a broad measure of their aggregate impact. Biological communities integrate the stresses over time and provide an ecological measure of fluctuating environmental conditions. Unlimited monitoring of biological communities can be relatively inexpensive, particularly when compared to the cost of assessing toxic pollutants, either chemically or with toxicity tests. Where criteria for specific ambient impacts do not exist (e.g., nonpoint-source impacts that degrade habitat), biological communities may be the only practical means of evaluating the water bodies (Barbour, *et al.*, 1999).

Biological monitoring should not be seen as an alternative to physical and chemical monitoring but as a useful complementary approach. Although physical and chemical analyses can indicate the presence and level of contaminants, biological methods can integrate responses to combinations of all contaminants and other sources of environmental stress thereby indicating overall effects on a water body. Physical and chemical analyses give a measurement, which is valid only for the short time when the sample was collected whereas biological monitoring shows the effects of the physical and chemical conditions to which the organisms were exposed over a period of time (Bartram and Balance, 1996).

Biological integrity is the main point in the biological water quality assessment (Karr, 1991). Therefore, reference condition, which represents the least impaired habitat type within a certain region along the gradient of human disturbance is important in the biotic integrity concept to provide an upper bound on the disturbance gradient against which biological attributes may be calibrated (Deberry and Perry, 2005).

### **2.3 Diatoms for river water quality Assessment**

Water quality assessment protocols based on the use of diatoms (*Bacillariophyceae*) are now well developed and their importance substantiated at an international level (Barbour, 1999). The method is applicable across a wide range of aquatic ecosystem types, namely freshwater, brackish, and estuarine, and is inclusive of lentic and lotic ecosystems, wetlands and their associated damp, marginal and littoral zones. They are also suited for monitoring severely impacted systems where other types of organisms are not present. The use of diatoms as a monitoring tool for water bodies was tested and found to be successful and promising in South Africa and Kenya, among African countries (Taylor *et al.*, 2007).

Diatoms are extremely important biological indicators for a variety of reasons. As living organisms, they reflect the overall health of the ecosystems they inhabit, as opposed to one-dimensional physical and chemical parameters that can change dramatically over a short period of time depending on the time of year, weather conditions, and other factors. Diatoms are better indicators usable for biological monitoring than many other organisms because of their ubiquity,

short generation time, sensitivity to changes in nutrient levels, and diverse species composition (De la Rey, *et al.*, 2004).

Diatoms also provide the following essential suite of diagnostic attributes (Barbour *et al.*, 1999; De la Rey *et al.*, 2004; Harding *et al.*, 2005):

- They are suited for monitoring very severely impacted systems where other types of organisms are not present. They indicate a broad range of tolerance along a gradient of aquatic pollution, with individual species having specific water chemistry requirements.
- Diatom assemblages are sensitive to some pollutants, which may not affect other aquatic assemblages, or may only affect other organisms at higher concentrations (e.g. herbicide).
- They have the shortest generation times of all other biological indicators. They reproduce and respond rapidly to environmental change and provide early measures of both pollution impacts and habitat restoration and recuperate quickly once the perturbation is removed.
- Diatom frustules have a lasting permanence in sediments, such that sediment cores provide details of changes in the quality of the overlying water for as far back as one is able to search.
- Diatoms can be found on substrata in streambeds even when dry and can be sampled at most times of the year and still accurately reflect recent or prevailing conditions.
- Their suitability for collection, preparation and storage (small sample volumes, no desiccation risk) for reference purposes.
- The existence of considerable amount of tested ecologically associative information world-wide mainly in developed countries.
- Their suitability for diversity analysis.
- The availability of interpretive software package.

Besides the afore-mentioned several advantages, diatom species occur in a wider variety of waters compared to invertebrates or fish, and are particularly useful as early warning indicators (CCME, 2006).

## **2.4 Diatom as indicators of different water quality status**

Diatoms are widely known and used as indicators of river and stream water quality, including trophic state conditions. They show cumulative impacts of nutrient concentration on water quality. Diatom species diversity, richness and abundance are strongly affected by the concentrations and ratio of nitrogen and phosphorus in the water (Lamaia, 2005). At different concentrations of nutrients, one can find different diatom species. If there is extremely high concentration of a nutrient, a small number of tolerant diatom species are found (Pan, 2001).

An increase in organic decay can change several biotic characteristics of river environments. Excessive concentrations of decay byproducts such as ammonia and hydrogen sulfide may influence the abundance and distribution of diatoms in the river (van Dam *et al.*, 1994). Heavy metal pollution in aquatic ecosystems has been a serious global environmental problem for a long time. Heavy metals are persistent in aquatic environments because of their resistance to decomposition under natural conditions. One of the greatest problems associated with the persistence of heavy metals is the potential for them to bioaccumulate and biomagnify, potentially resulting in long-term implications for human and aquatic environment problem (Soininen, 2002).

Changes in diatom composition have been shown to be good detectors of level of metal contamination of aquatic ecosystem and have, therefore, been subjected to extensive monitoring studies for several years (Prygiel, 1993). It has been long recognized that metal contamination can be harmful to diatoms. *In situ* studies conducted at sites exhibiting high levels of metals and microcosm experiments have demonstrated a decrease in productivity, abundance and changes in species richness of diatom community (Hill, 2001). Generally, diatoms are important indicators of trophic status, organic and metal pollution in the river.

## **2.5 Impacts of Tannery waste**

Tanning industry in Ethiopia is currently believed to be one of the sectors supporting economic development of the country. But, it is also pollutant intensive sector that threatens the environment at large. Ethiopian tanneries are now improving values to the products. This move

requires a series of additional processes/procedures and input of varieties of chemicals that can potentially increase the pollution load of the waste water effluent. The environment and development integration in a leather industry has to be strengthened to achieve the intended sustainable economic benefit (Haydar *et al.*, 2007).

Industrial waste is the most common source of water pollution nowadays (Ogedengbe and Akinbile, 2004) and it increases every year because almost all countries are getting industrialized. Worldwide, it is estimated that the industry is responsible for releasing 300-400 million tons of heavy metals, solvents, toxic compounds, and other solid and liquid wastes into water bodies each year (UNEP, 2010). Thus, the environment is under stress from wastes released from such industrial activities. As compared to other industries, leather tanning is one of the most polluting activities (Khan *et al.*, 1999) as it consumes huge amount of water in several stages, producing enormous amount of liquid wastes (Farenzena *et al.*, 2005), which are harmful to the environment into which they are discharged. Tannery wastewater is highly polluted in terms of biological oxygen demand (BOD), chemical oxygen demand (COD), suspended solids (SS), nitrogen, conductivity, sulphate, sulphide and chromium (Mondal *et al.*, 2005). In most developing countries tannery effluents are discharged directly into sewers or water bodies without treatment (Verheijen *et al.*, 1996).

High COD values indicate toxic state of the wastewater along with presence of biologically resistant organic substances. The high level of ammonia-N is harmful to aquatic life and nitrogen may cause eutrophic condition. The high salinity and total dissolved solids (TDS) of the effluent may result in physiologically stressful conditions for some species of aquatic organisms due to alterations in breathing conditions. Studies show that increase in salinity causes changes in biotic communities thereby limiting biodiversity, excluding less tolerant species and causing acute or chronic effects at specific life stages. Changes in the ionic composition of water can also eliminate some species while promoting population growth of others (Weber-Scannell and Duffy, 2007).

The pollutants result in food contamination and are hence toxic to human and aquatic life. In Ethiopia, although the use of leather and leather products goes back to prehistoric times, tanning

hides and skins of animals into leather has been practiced as industrial activity for the last few decades (EEPA, 2003). Presently, Ethiopia's leather industry is the leading of the leather sector development within the Eastern and Southern Africa region (UN, 2002). Currently, there are more than 20 operational tanneries that turnout wastes directly into the nearby water bodies like the other industrial activities. This makes industrial pollution one of the major environmental issues in the country (Zinabu Gebre-mariam and Zerihun Desta, 2002). This is becoming evident through the pollution of water bodies and human habitat in the major cities, rivers and lakes. Similarly, Modjo Tannery, the most important industry in the Modjo city, is discharging its effluent directly into Modjo River. The downstream part of the river is used for domestic activities including watering of livestock, drinking, irrigation and recreation (swimming and bathing). The use of the river in this way may lead to bioaccumulation of toxic pollutants like chromium, which is hazardous to human beings as well as livestock.

Industrial effluents from leather tanneries discharge higher amount of metals especially chromium. These effluents are released into a river or canal or dumped into ground waters leading to their contamination by chromium. Due to its accumulation, there is higher chance of exposure to chromium. The toxicity of chromium through drinking water is the major problem for human health (Javaid and Chaudhary, 2008). It has been reported that only about 20% of the large number of chemicals used in the tanning process is absorbed by leather and the rest is released as waste. The maximum concentration of these waste materials is absorbed by bioaccumulation process in cultivated crops irrigated by tannery effluent (Sahu *et al.*, 2008).

## **CHAPTER THREE: OBJECTIVES**

### **3.1 General objective**

- To assess polluted river using diatoms as bioindicators

### **3.2 Specific objectives**

- To assess the impact of Modjo Tannery on the physico-chemical parameters of Modjo River.
- To assess the impact of Modjo Tannery on the species composition, diversity, abundance of diatoms and other diatom metrics.
- To assess the association of sampling sites, diatom taxa and physico-chemical water quality parameters in Modjo River

## CHAPTER FOUR: MATERIALS AND METHODS

### 4.1 Description of the study area

Modjo River, the present study area (Fig. 1), is a perennial river that flows through Modjo, a town located some 73 km southeast of Addis Ababa. It is a tributary of Awash river receiving effluents of a number of industries including Modjo tannery and other tanning industries located within and in the proximity of Modjo town. In addition, extensive agricultural practices and human settlements have been altering the watershed and riparian zone of Modjo river.

The study area was reported to exhibit air temperature that averaged 20.40 °C varying between a minimum of 11.6 °C in August and a maximum of 29.20 °C in May, with average annual temperature decreasing with an increase in altitude. The study area had a maximum monthly rainfall of 79.3 mm in the month of August and a minimum of 56 mm in the month of February (National Meteorological Services Agency, 2012).

Open bushy woodland being the dominant, grass lands, shrubs and open shrub are the prominent types of vegetations that characterize the study area (Berehane Asfaw, 2007). In the study area, tuff, diatomite and ignimbrite, which are partially interblended in the lacustrine sediments forming an extensive layer at some places, are exposed in Modjo river area with thin but extensive spatial distribution of sands, silts, clays, tuffs, pumice, and ignimbrites (Berehane *et al.*, 1978). These sediments are mixed compositionally with quaternary volcanic products forming a specific mixed volcano-sedimentary rock type in most places (Tsegaye Abebe, 1999).

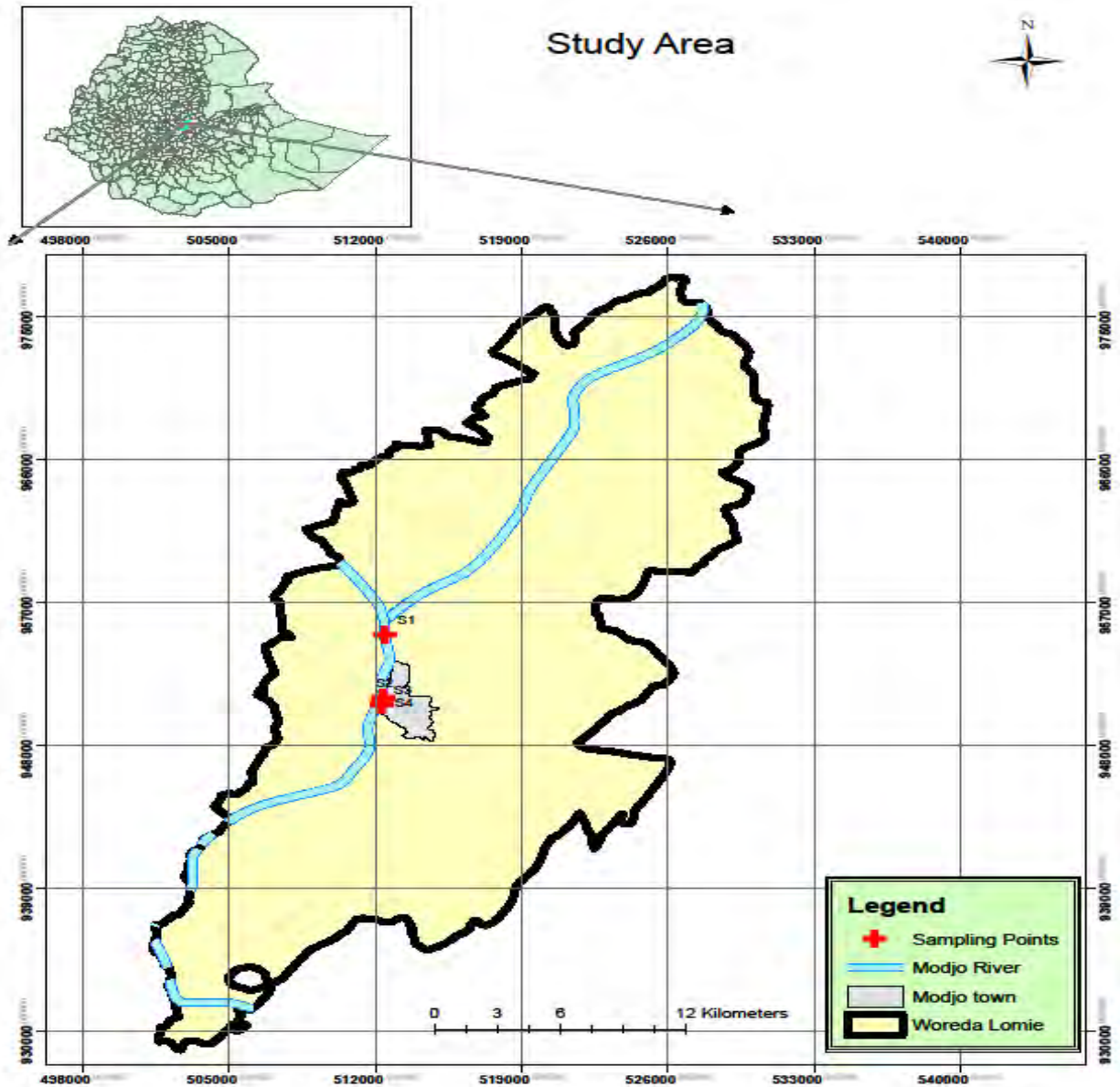


Figure1. Map showing Modjo River with sampling stations indicated with a "plus" sign “+”

## 4.2. Sampling Protocol

Four sampling sites, above and below the point of discharge of the liquid wastes of Modjo Tannery into Modjo river, were selected in line with the stated objectives and on the basis of their positions relative to the position of point of discharge of the liquid wastes. Water samples were collected in acid-washed plastic bottles (except. for phosphate, which requires glass bottle containers to avoid the effect of adsorption onto plastic bottles). The collected samples were

transported in ice-cooled boxes from sampling sites to the Limnology laboratory of Addis Ababa University for the analyses of physico-chemical parameters. Shortly before sample collection, *in-situ* measurements of water temperature, DO, electrical conductivity, salinity and pH were also made using a multiparameter device (HACH Multimeter HQ40d Model). The water samples collected from each site were used for the analyses of various chemical parameters.

Diatom samples were also collected from the same selected sampling locations simultaneously with those collected for physicochemical parameters and analyzed for such response (dependent) variables as species composition and abundance of diatoms. Three substrates (cobble stones) within 10 m distance were sampled randomly at each sampling reach (Kelly, 1998). A surface area of 25 cm<sup>2</sup> on each substrate was scrapped with tooth brush and samples obtained from the three substrates of each sampling site were pooled to form a single sample. The same type of substrate (cobbles) was sampled from each sampling site to make comparison among sites easier. Diatom samples were preserved with ethanol by adding 1-2 drops of ethanol into 10 ml of each sample (Taylor, 2007).

### 4.3 Analyses of samples in the laboratory

#### 4.3.1 Analysis of samples for chemical parameters

**Table 1.** Methods employed for the measurement of chemical parameters in the laboratory (APHA et al., 1999).

Parameters	Methods of Analysis
NH <sub>3</sub> + NH <sub>4</sub> <sup>+</sup> -N	Phenate method
NO <sub>3</sub> + NO <sub>2</sub> -N-	Sodium Salicylate method
SRP and TP	Ascorbic acid method
SiO <sub>2</sub>	Molybdosilicate method
TDS	Gravimetric method
Total alkalinity (TA)	Titration with strong acid using indicator solutions
BOD <sub>5</sub>	The Azide modification of the Winkler titrimetric method
Fe	APHA3111B,Air-Acetylene FAAS
COD	APHA 5220B. Open Reflux Methods
Cr	Atomic absorption spectrophotometry
SO <sub>4</sub>	Turbidimetric Method

## 4.4 Analysis of biological samples

### 4.4.1 Cleaning and Identification of diatom valves

Diatom frustules were cleaned with hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) according to Taylor (2007). Then cleaned samples were made up to a volume that provides adequate density of diatom valves and mounted on slides in Naphrax mounting medium. The slides were explored and diatom frustules found along transects were counted with a simple compound microscope at a magnification of 1000x. Diatoms were identified using standard taxonomic Keys (Krammer and Lang-Bertalot, 2000).

### 4.4.2 Estimation of biotic indices

The data generated from counting were converted to Percentage Abundance to calculate Weighted Mean Sensitivity (WMS) index (Kelly, 2001).

$$Index (WMS) = \frac{\sum_{j=1}^n a_j s_j v_j}{\sum_{j=1}^n a_j v_j}$$

Where, WMS= Weighted Mean Sensitivity,  $a_j$  = abundance or proportion of valves of species  $j$  in Sample,  $s_j$  = pollution sensitivity ('optimum') of species  $j$  and  $v_j$  = indicator value ('tolerance') This varies from 1 (for sites with very low nutrient concentration) to 5 (for sites with very high nutrient concentration). Trophic Diatom Index (TDI) is the WMS expressed on a scale of 0 to 100. It is calculated as follows:

$$TDI = (WMS \times 25) - 25$$

Other diatom indices were also calculated from WMS using the following equations.

$$\text{Generic diatom index (GDI)} = WMS \times 4$$

$$\text{Pollution sensitivity index (IPS)} = (4.75 \times WMS) - 3.75$$

$$\text{Pollution tolerant valve (\%PTV)} = \text{Percent of tolerant species}$$

The scale for both GDI and IPS runs from 4 to 20, with low values indicating heavy pollution. Values of GDI above 16 indicate no or minimal pollution. The IPS values were evaluated based on the following European standards especially those of France and Belgium, which are less stringent than that of Sweden (Siddaraju and Devi Prasad, 2011).

Table 2. Interpretation of IPS values (Source; Hungary Ministry of Environment and Water, 2005)

<b>Water Quality</b>	<b>France, Belgium</b>	<b>Sweden</b>
High	17 ≤ IPS ≤ 20	17.5 ≤ IPS ≤ 20
Good	13 ≤ IPS < 17	14 ≤ IPS < 17.5
Moderate	9 ≤ IPS < 13	10.5 ≤ IPS < 14
Poor	5 ≤ IPS < 9	7 ≤ IPS < 10.5
Bad	IPS < 5	IPS < 7

The influence of organic pollution was determined by considering percent pollution tolerant valves (% PTV) (Kelly, 200).

Table 3. Interpretations of proportions of counts of taxa tolerant to organic pollution in the sampling sites were based on Kelly and Whitton (1995).

<b>Proportion of count</b>	<b>Interpretation</b>
<20 % total valves belonging to tolerant taxa	Free of organic pollution
21-40% total valves belonging to tolerant taxa	Some evidence of organic pollution
41-60% total valves belonging to tolerant taxa	Organic pollution likely to contribute significantly to eutrophication of site
>61% total valves belonging to tolerant taxa	Site is heavily contaminated with organic pollution

Diatom diversity at all sampling sites was determined using Shannon's diversity Index. Shannon diversity index is probably the most widely used diversity index (Shaw, 2003). It is calculated from the proportional abundance of each Species (abundance of the species / total abundance)

$$H' = -H'' = -\sum_{i=1}^S (P_i \log_2 P_i)$$

Where,  $H'$  is the standard symbol for Shannon's diversity index, and  $\log (p_i)$  is the logarithm of  $P_i$ , the proportion of species  $i$ .

The minus sign at the start of the above equation is introduced to ensure that the calculated diversity is always positive value.

## 4.5 Data Analysis

RDA was used to relate diatom species (response variables) and chemical parameters (explanatory variables). Redundancy analyses (RDA) was used to estimate how much of the variance of the spatial distribution of diatom species was explained by the environmental variables using CANOCO for windows 4.5 version. RDA was chosen after confirming through a preliminary detrended analysis of correspondence (DCA) that the length of ordination axes in DCA was less than 3 (Jan and Petrš 2003). The statistical significance in RDA was assessed by Monte-Carlo permutation tests (499 permutations) and conclusions were made based on 5% level of significance ( $P < 0.05$ ). The results of RDA were visualized in the form of ordination diagrams in Canodraw for Windows. The relationship between physicochemical parameters on each site was examined using One-way ANOVA.

## CHAPTER FIVE: RESULTS AND DISCUSSION

### 5.1. Physico-chemical water quality parameters

Mean values of physico-chemical parameters determined in samples collected from the different sites along the Modjo River are given in Tables 4.

**Table 4.** Mean values ( $\pm$ SD) of physicochemical parameters of Modjo River. Aggregate chemical parameters, dissolved oxygen, iron and chromium determined for the different sites along the Modjo River (Units are  $\text{mg L}^{-1}$  unless otherwise indicated).

Physicochemical Parameter	Site1	Site2	Site3	Site4	Discharge limit
$\text{NH}_3+\text{NH}_4^+ \text{ N}$	0.055 $\pm$ 0.4	36.8 $\pm$ 4.1	12.9.1 $\pm$ 2.1	1.9. $\pm$ 0.1	30 mg/l
$\text{NO}_2\text{-N}$	0.9 $\pm$ 0.01	1.8 $\pm$ 0.2	1.8 $\pm$ 0.1	1.4 $\pm$ 0.1	-
$\text{NO}_3\text{-N}$	1.5 $\pm$ 0.1	16 $\pm$ 1.5	14 $\pm$ 1.1	1.5 $\pm$ 0.3	50 mg/l
SRP	1.4 $\pm$ 0.5	2.7 $\pm$ 0.4	2.3 $\pm$ 0.0	1.78 $\pm$ 0.0	-
TP	8.2 $\pm$ 2.7	32.2 $\pm$ 1.8	25.5 $\pm$ 3.3	11.7 $\pm$ 4.5	10)
Silica	0.7 $\pm$ 0.2	3.5 $\pm$ 0.15	0.85 $\pm$ 0.1	0.56 $\pm$ 0.04	-
$\text{SO}_4$	0.4 $\pm$ 0.1	255 $\pm$ 0.1	240 $\pm$ 0.7	23.6 $\pm$ 2.2	200 mg/l
TA	140 $\pm$ 13	384.7 $\pm$ 6.5	184 $\pm$ 3.1	186 $\pm$ 3	-
TDS	208 $\pm$ 21	3818 $\pm$ 22	2112 $\pm$ 19	1198 $\pm$ 17	80
Cr	ND	9.9 $\pm$ 1	ND	ND	1mg/l
Fe	16.4 $\pm$ 0.7	63.7 $\pm$ 4.3	50.5 $\pm$ 2.5	4.2 $\pm$ 1.9	1.0 mg/l
DO	7.8 $\pm$ 0.4	0.2 $\pm$ 0. 2	4.1 $\pm$ 0.1	5.5 $\pm$ 0. 3	-
Water Temp.( $^{\circ}\text{C}$ )	21.6 $\pm$ 2.1	23.3 $\pm$ 0.2	19.3 $\pm$ 4.1	17.2 $\pm$ 0.7	40 $^{\circ}\text{C}$
$\text{K}_{25}$ ( $\mu\text{S cm}^{-1}$ )	309.6 $\pm$ 15	2220 $\pm$ 20	396 $\pm$ 13	390 $\pm$ 9	1000 $\mu\text{S}/\text{Cm}$
pH	8.9 $\pm$ 0.2	9.6 $\pm$ 0.2	8.8 $\pm$ 0.9	8.2 $\pm$ 0.2	6-9
Salinity (ppt)Salinity (ppt)	0.23 $\pm$ 0.01	1.7 $\pm$ 0.03	0.29 $\pm$ 0. 1	0.29 $\pm$ 0. 1	-
$\text{BOD}_5$	5.4 $\pm$ 0.3	340.3 $\pm$ 2.9	27 $\pm$ 2.6	13.9 $\pm$ 2.1	50 mg/ l
COD	26.2 $\pm$ 2.3	628 $\pm$ 26	300.1 $\pm$ 1.9	127.9 $\pm$ 13	150 mg/l
Turbidity(NTU)	510 $\pm$ 20	659 $\pm$ 13.7	577 $\pm$ 6.5	503 $\pm$ 17.9	-

Source for discharge limit: EEPA (2003)

All physicochemical water quality parameters measured in the present study except dissolved oxygen peaked at S2, the point of discharge of the liquid waste of Modjo Tannery into Modjo River, making it warmest, turbid, polluted, saline and alkaline of the present study sites. This should be expected in light of the fact that this is the site that receives the unaltered raw liquid

waste of the tannery, which presumably contained high levels of organic load and inorganic substances including metals. All the afore-said physico-chemical parameters showed broadly similar spatial variations with their high levels at S2 and declining consistently to those of the downstream sites (Table 4). S2 was the only site where a high level of the potentially toxic heavy metal-Chromium (Cr) was also observed.

A similar study showed that the mean concentrations of pollutants along Blue Nile river exhibited a consistent decrease downstream from the point where the effluent joins the river to the last downstream site. This is attributed to the dilution capacity of the river, uptake of pollutants by the vegetation along the river gradient and some reactions that can change the identity of the pollutants as the water flows downstream from the point of pollution source (Assefa wosine and Ayalew Wondie, 2014).

The lowest oxygen concentration of S2 (Table 4), which was far below the level capable of supporting aerobic heterotrophs, was significantly different from those of other sampling sites ( $p < 0.05$ ) and coincided with the present highest levels of BOD<sub>5</sub> and COD. The critically low dissolved oxygen concentrations at S2 and S3, which were considerably below the level sufficient to sustain aquatic life (above 5 mg/L, WHO, 2005) are obviously the results of the discharge of untreated effluents of the tannery into the river water. COD mean values of S2 were above the discharge limit set by EEPA (2003) and significantly differed from those of other sites ( $p < 0.05$ ).

The high values of BOD<sub>5</sub> and COD at S2 (Table 4), resulted from the discharge of untreated tannery effluents, which contained higher organic matter load. The high levels of COD and BOD<sub>5</sub>, reflect the pollution strength of the wastewaters and seem to suggest the likely limiting role of oxygen available for aerobic living organisms that normally utilize the organic matter present in the wastewaters as a source of energy and carbon. High COD levels also imply toxic state and the presence of biologically resistant organic substances (Sawyer, 2006). Such low DO values (conditions) were also observed along the course of Borkena River in Dessie and Kombolcha town (Abebe Beyene and Worku Legesse, 2005).

BOD<sub>5</sub> and COD mean values of the present study are lower than those reported by Seyoum Leta *et al.* (2003) for the same segment of Modjo river. The present relatively low BOD<sub>5</sub> and COD values are attributable to presumably lower amount of organic matter and various chemicals used during the processing of hides and skins and/or to the set of samples collected during the rainy season, which might have been diluted by the resulting runoff. It has been reported that a significant part of the chemicals used in the tanning process is not actually absorbed in the process and hence discharged into the environment (UNIDO, 2001), thereby increasing the levels of BOD<sub>5</sub> in the effluent.

The nitrogen concentrations at S2 and S3 were higher than 0.3 mg/l. Concentration of inorganic nitrogen (NO<sub>2</sub> and NO<sub>3</sub>-N) greater than 0.3 mg/l can cause algae to grow in abundance (Nathanson, 2000). The standard provisional limits (EEPA, 2003) for NH<sub>3</sub>-N and TP of tannery wastewater are 30 and 10 mg/l, respectively (Table4). The levels of NH<sub>3</sub>-N at S2 were beyond the limit set by EEPA (2003). Similarly, the concentrations of phosphorus at S2, S3 and S4 were beyond the limit set by EEPA (2003). TP at such high levels normally cause eutrophication problem (USEPA, 2008). Eutrophication reduces the amount of oxygen dissolved in water and alters the river habitat available for aquatic life. This may disrupt the ecological integrity of the river so that certain biological communities experience severe mortality.

A similar study conducted by Behailu Berehanu (2007) has also shown the high degree of pollution in Modjo River, which was reflected in its very high BOD(2018mg/l), very high COD(8982), high turbidity, high dissolved solids, phosphate(8.5 mg/l), NH<sub>4</sub><sup>+</sup> (44.8 mg/l), and high conductivity value (Behailu Berehanu, 2007). The level of Cr at S2 was at a significantly high concentration although it was below the limit of detection of the method of analysis at all other sites. This seems to suggest that the only source of the metal is the tannery and that it is removed from the water immediately by biological uptake, sedimentation through adsorption or precipitation, etc. The high level of Cr at S2 is indicative of the potential toxicity of the river water at least at this same site and the high probability of its entering the aquatic food chain.

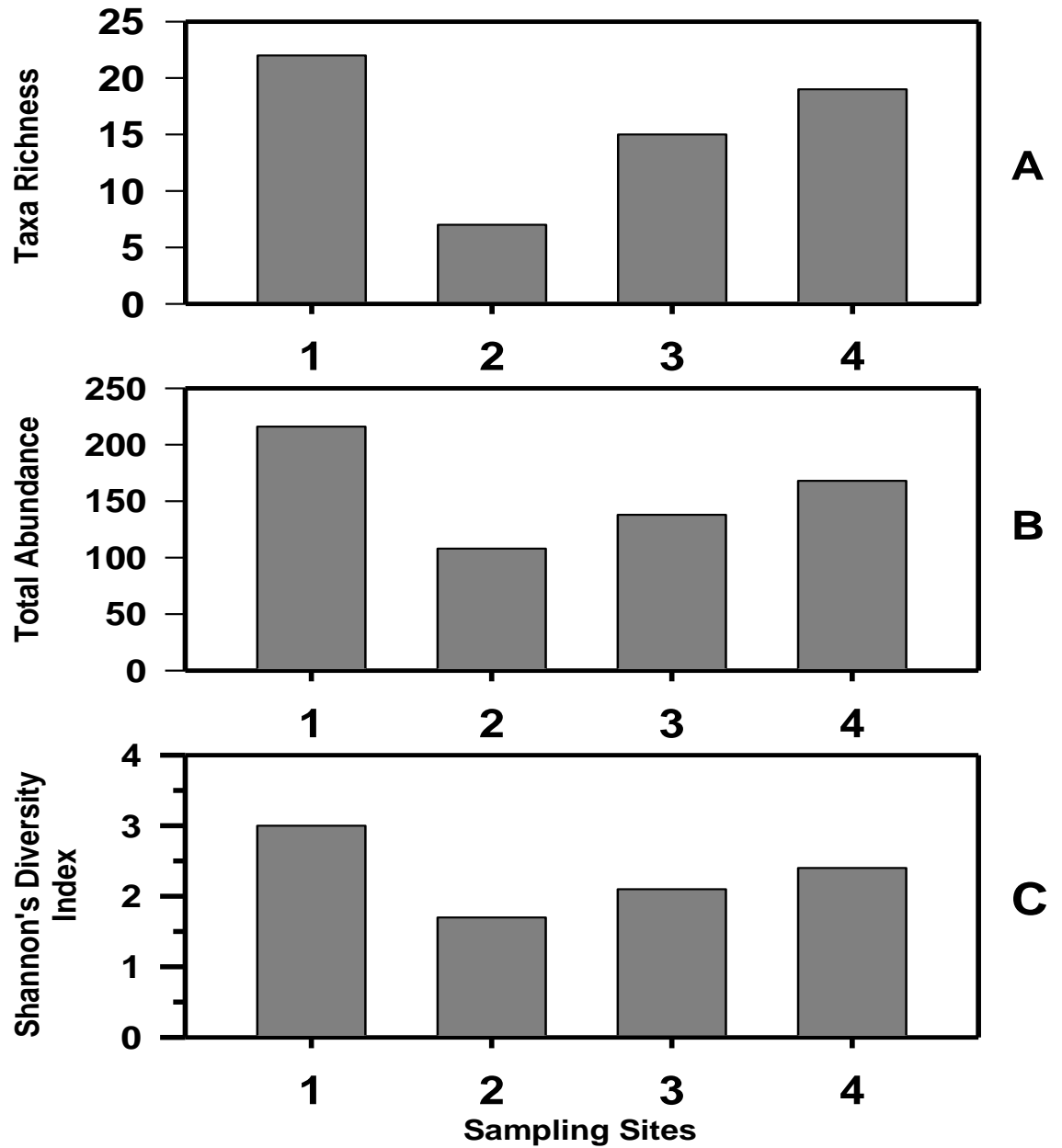
Although the variations in the levels of the various measured parameters at the reference site relative to other sites were not consistent, the fact that it is well-oxygenated, most dilute

chromium-free water and had considerably low level of organic and inorganic loads suggests that the major cause of the degradation of the water quality of Modjo River is Modjo Tannery, This argument is consistent with the reports of other studies made in the same and other rivers (Abebe Beyene and Worku Legesse, 2005).

## **5.2. Species composition, diversity and abundance of diatoms**

The diatom species identified during the present study totaled 44 belonging to 13 genera (Annex3). Different diatom species in a community respond differently to changes in water quality because of differences in the level of tolerance. Therefore, the composition of diatom communities at different locations, or at different points in time, provide useful information about the environmental conditions. Sites with relatively good water quality, S1 and S4, were associated with such species of diatoms as *Cocconeis placentula*, *Fragilaria crotonensis*, *Gomphonema angustatum* and *Gomphonema gracile*. At S2 and S3, which had very bad and bad water quality, respectively, the diatom assemblages consisted of such species as *Fragilaria capucina*, *Gomphonema parvulum*, , *Hantzschia amphioxys* and *Nitzschia palea*, .

The diatoms, which are representative of S2 and S3 are known for being tolerant of organic pollution and nutrient enrichment, growing well in eutrophic waters (Prygiel and Coste, 2000). Lobo *et al* (2002) described *Nitzschia palea* as a medium pollution tolerant species, which explains its high abundance (41), compared with other taxa observed in the present study, As pollution increased, the least pollution tolerant species such as *Fragilaria crotonensis*, *Fragilaria incognita* and *Gomphonema angustatum* were replaced by the highly pollution tolerant species such as *Gomphonema parvalum*, *Nitzschia palea*, and *Fragilaria capucina*. The tolerant species have been reported to be associated with waters of relatively high ionic strength and conductivity, and are known to be resistant to organic and heavy metal pollution (Duong *et al.*, 2006).



**Fig. 2. Variations in Taxa Richness (A), total abundance (B) and Shannon's diversity index (C) of diatoms: 1: Reference site; 2: site at the point of discharge of the liquid waste of ModjoTannery; 3 and 4: downstream sites.**

Species richness, diversity and abundance differed among sampling sites, tending to be higher at relatively unpolluted sites. Species richness of the diatom community was highest and lowest at S1 and S2, respectively, corresponding to the least and highly pollution-impacted segments of Modjo River (Fig. 2). Diatom species abundance, richness and diversity have been shown to change in response to salinity, pH, TP, water temperature and other environmental variables (Hinga, 2002). At different concentrations of nutrients, different assemblages of diatom species and abundance may be found with high concentrations of nutrients often resulting in the abundance of few tolerant diatom species (Pan and Lowe, 2001).

The increase in the level of the biological parameters from S2 to S4 seems to be associated with the consistently increasing dilution effect of the river water, while their observed maxima at S1 may be explained by the fact that this is a site, which is free from the impacts of the effluent of Modjo tannery. The high concentrations of silica, phosphorus and nitrogen observed at S2 (Table 4), would seem to favor better diatom growth. However, the lowest abundance, diversity and taxa richness of diatoms were observed at this site probably due to the high levels of potentially toxic substances including Cr, high levels of salinity, COD, BOD<sub>5</sub> nutrients and the extremely low level of dissolved oxygen, which may have adversely influenced the growth of diatoms. S2 is the only site where high levels of the potentially toxic heavy metal-chromium (Cr) - was observed (Table 4). It has been long recognized that high level of metal contamination can be toxic to diatoms. *In situ* studies and microcosm experiments conducted at sites exhibiting high levels of metals have demonstrated a decrease in productivity, diversity and changes in species composition of a diatom community (Hill, 2001).

The high level of Cr at S2 is indicative of the potential toxicity of the river water at least at this same site and the high probability of its entering the aquatic food chain. Heavy metals like chromium in wastewaters could negatively impact the aquatic environment through bioaccumulation over long periods (Sekhar, 2003). The levels of sulfur compounds are also known to affect the diversity and abundance of diatoms (Gomez and Licursi, 2001). Diatom species richness usually decreases as a result of river contamination by organics and metals (Khan, 2001). The unusually high pH (9.6) recorded at this waste disposal site of Modjo tannery (S2) may have been the reason for the disappearance of most taxa, which were found at the

upstream site-reference site (S1). At pH values above 9, the proportion of unionized ammonia (NH<sub>3</sub>) and hence toxicity of ammonia increase significantly due to deprotonization (Wetzel, 2001). The discharge of effluents with high salinity and TDS at S2 of the receiving system may have also resulted in physiologically stressful conditions for some species of aquatic organisms due to alterations in osmotic conditions. Studies show that increase in salinity causes shifts in biotic communities, limits biodiversity, excludes less tolerant species and causes acute or chronic effects at specific life stages of some organisms, while promoting population growth of others (Weber-Scannell and Duffy, 2007).

The extremely low level of oxygen concentration, which coincided with the highest levels of COD and BOD<sub>5</sub>, may have curtailed the growth of obligate and facultative heterotrophic diatoms. High levels of macronutrients do not always lead to enhanced growth of algae including diatoms. Poor liquid and solid waste management, discharge of untreated domestic, industrial and institutional liquid waste into rivers and mining activity are the major environmental stressors that affect the water quality of rivers (Taffere Addis, 2008).

### **5.3. The relationship between species composition and physicochemical Parameters.**

The results of RDA of the relationship between environmental parameters and the variance in Species of diatoms at the different sampling sites are summarized in Table 5 and Fig. 3.

Table 5. Summary of RDA of the relationship between environmental parameter and species of diatoms at different sites.

RDA Statics	Axis 1	Axis 2	Total variance
Eigenvalues :	0.631	0.279	1.000
Species-environment correlations:	1.000	1.000	
Cumulative percentage variance of species data :	63	91	
Species-environment relation:	63	91	
Sum of all eigenvalues			1.000
Sum of all canonical eigenvalues			1.000

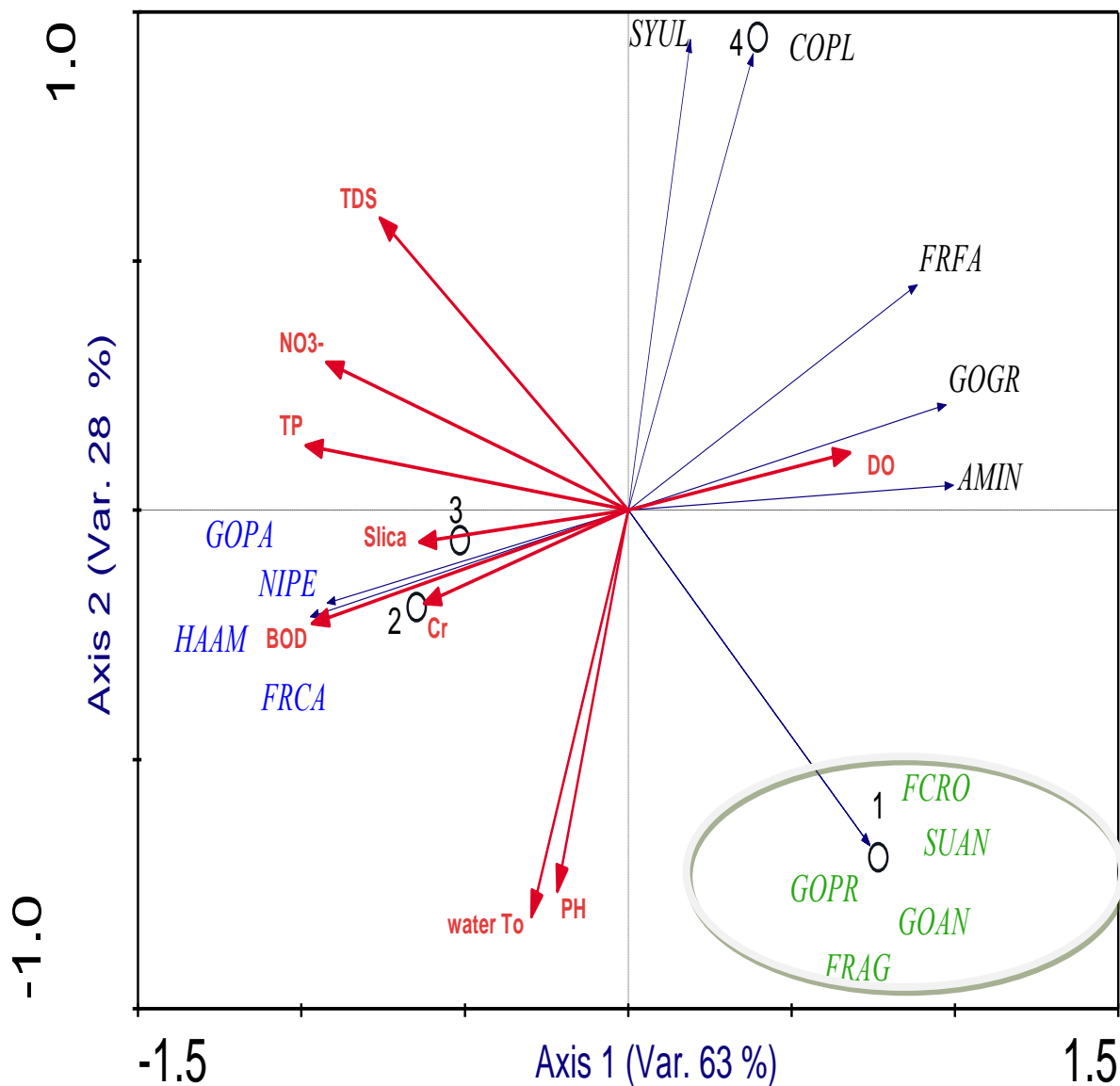


Figure 3. RDA of the association of diatom species, water chemistry and sampling sites of Modjo River. Full descriptions of abbreviations of names of diatom taxa and environmental variables are given in Annex 3. The arrows show the direction and proportional influence of the environmental variables.

Axis-1 (vertical) described more of the variance in species assemblage of diatoms (63%) than axis-2 (horizontal, 28%). In the above RDA biplot axis-1 clearly separated the reference site, S1,

from S2 and S3 in terms of water quality. The water quality of S2 was a function of NO<sub>3</sub>, TDS, COD, Cr, TP and silica (Fig. 3). S1 was dominated by pollution sensitive diatom species (e.g. DO sensitive species) with some of the species also occurring at site 4, while S2 and S3 were dominated by pollution tolerant diatom species (Fig. 3)

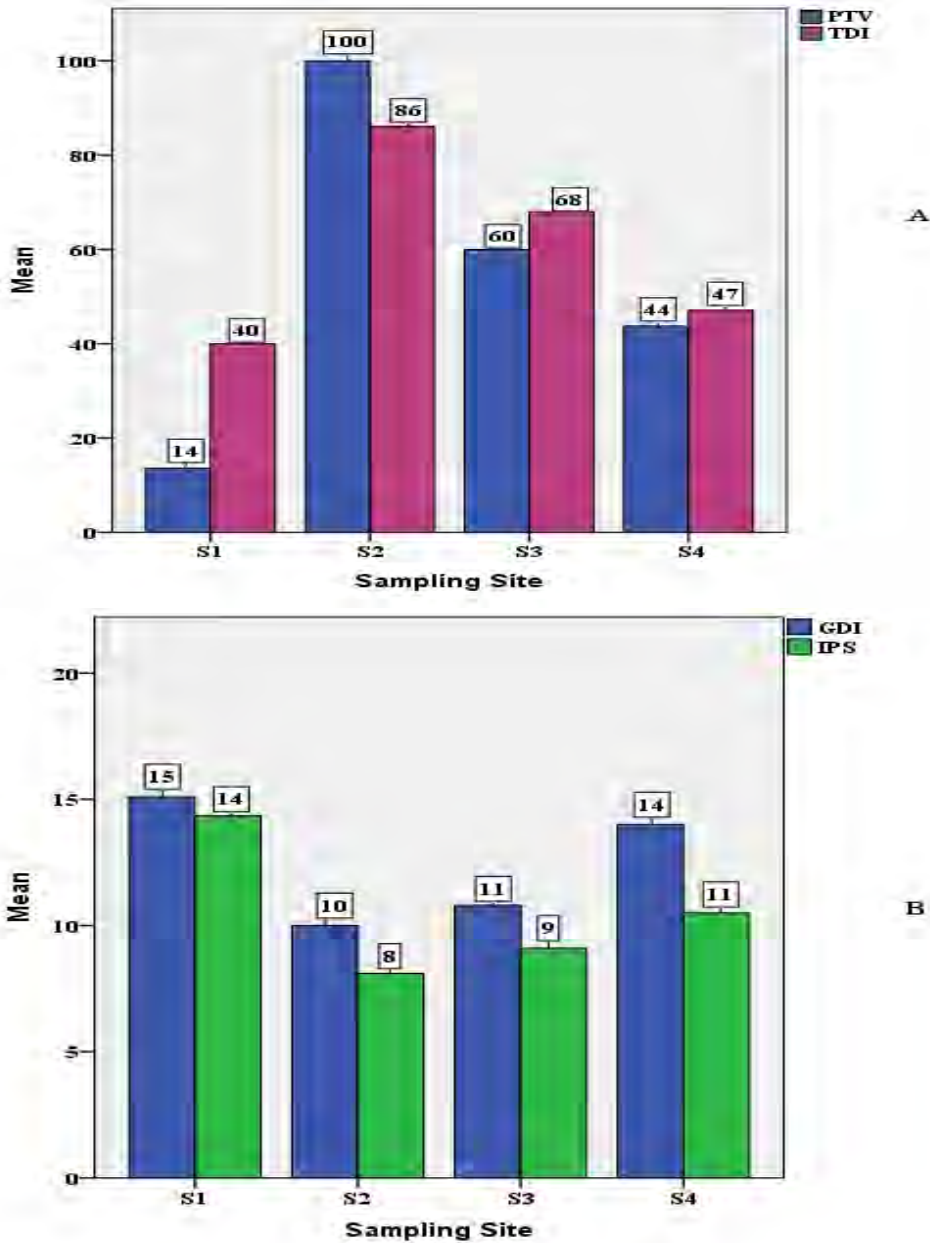
*Fragilaria capucina* was positively correlated with Cr, which is highly toxic to most diatoms and consequently adversely affecting their development and growth. Thus, the presence of high concentration of Cr at S2 did not adversely influence the growth and hence abundance of this diatom species, which is used as pollution indicator. This species is known to be resistant to high level of metal pollution owing to its unusual tolerance to potentially toxic metals (Duong et al., 2006). Thus, the presence of this species at S2 is indicative of the high level of metal pollution at this site. *Nitzschia palea*, which was among the most abundant diatom species at S2, was also positively correlated with the chemical parameters BOD<sub>5</sub>, TP, NO<sub>3</sub>. *Nitzschia palea* is typical of phosphate-enriched or organically polluted waters (Fore and Grafe, 2002).

Generally, significance of water quality difference among the sampling sites to the variations in diatom assemblage is reflected in the RDA results. The highly polluted sites S2 and S3 were grouped together and separated from other sites, which is consistent with their relatively high level of pollution and their association with the pollution-tolerant species like *Fragilaria capucina*, *Gomphonema parvulum*, *Hantzchia amphoxy* and *Nitzschia palea*, Axis1 also separated the diatoms associated with good quality environmental variables such as *Fragilaria crotonensis*, *Gomphonema gracile*, *Gomphonema angusta* and *Cocconies placentula*.

#### **5.4 Other diatom metrics (indices)**

As Figure 4 below shows, all the biotic indices calculated from diatom abundance data reflect the highest water quality at S1, which can be attributed to its position relative the point of discharge of the liquid waste of the tannery. The biotic indices determined for S2 and S3 also confirm the extent of pollution implied by the chemical data (Tables 4). According to the calculated %PTV values, S2, S3 and S4 had much higher level of pollution than S1 with the %PTV value for S2 of 100% suggesting the prevalence of a diatom assemblage composed entirely of pollution tolerant species at a site with heavy organic pollution (%PTV>61%). Fore and Grafe (2002) also found significant positive correlation between the number of eutrophic species (%PTV) and extent of

human disturbance. Based on their IPS values, the water quality of S1, S2, S3 and S4 were rated good, bad, bad and moderately good. The higher values of GDI indicated good water quality with low or total absence of pollution. The lowest values of GDI indicate deterioration in water quality of an aquatic system (Coste, 2000). The water quality of the segments of Modjo River ranged from bad to good.



**Figure 4.** Diatom indices determined for the present sampling sites. (TDI=Trophic diatom index, GDI= Generic diatom index, IPS= pollution sensitivity index and PTV= Pollution tolerant) valve).

The highest recorded GDI was 15 for S1, which is near the cut off point for no or minimal organic pollution (i.e. GDI equal to 16), while the GDI values for S2 and S3 (10 and 11, respectively) indicate their higher level of pollution. High concentration of phosphate accumulated at S2. The high concentration of this nutrient causes an overgrowth of plant and algae. As plant and algae die, they become decomposable organic matter in the water, which leads to the disappearance of organic pollution sensitive diatom species. The balance of existing sensitive species and tolerant species depends on alteration in water quality due to the disposal of different pollutants into an aquatic ecosystem, which affects physicochemical and biological features (Szabo and Acs, 2005). The present TDI mean values ranged from 40 at site1 to 86 at site 2 (Fig. 4). TDI is a measure of nutrient enrichment mainly phosphorus. The highest TDI (86.1) recorded for S2, coincided with the highest level of phosphorus measured for the same site ( $32.2 \text{ mg L}^{-1}$ ) indicating its incomparable eutrophication status.

## **CHAPTER SIX: CONCLUSION AND RECOMMENDATION**

Even though tanning industries are very important for the country's economy and livelihood of its citizens, their wastes are discharged directly into nearby aquatic ecosystems without treatment leading to their water quality deterioration. Most of the physico-chemical parameters investigated in this study were found to have surpassed the provisional discharge limit set by the Ethiopian Environmental Protection Authority (EEPA, 2003) indicating the ineffectiveness of the treatment plants owned by the tannery industry. The observed benthic diatom communities also reflected the water quality status of Modjo River. The presence of nutrient and organic pollution-tolerant taxa, which were found in the segment of Modjo River receiving the unaltered liquid waste of Modjo tannery, clearly shows the detrimental impact of the tannery effluent on the ecological integrity of Modjo River, while corroborating the usefulness of bioindicators especially diatoms as tools for water quality monitoring.

The water quality of Modjo River represents a serious threat to public health and aquatic and terrestrial life. The concerned government bodies including EEPA should do their utmost to put an end to the ever-increasing degradation of the water quality of this ecologically and economically important river.

## References

- Abebe Beyene and Worku Legesse (2005). Pollution status of river Borkena, Ethiopia. Sponsored Jimma University and Ethiopian Science and Technology Agency, unpublished
- American Public Health Association (APHA) (1999). Standard methods for the examination of water and waste water. Biopress Ltd, Washington, DC, pp. 5-112.
- American Water Works Association (AWWA), and Water Environment Federation (WEF) (1999). Standard methods for the examination of water and wastewater. 20th edn, APHA, Washington, DC
- Assefa Wosnie and Ayalew Wondie (2014). Bahir Dar tannery effluent characterization and its impact on the head of Blue Nile River. *Africa journal of Environmental science and Technology*. **8**(6), pp. 312-318.
- Bala, M. (2008). Determination of the level of some heavy metals in water collected from two pollution – prone irrigation areas around Kano metropolis. *Journal of Pure and Applied science*. **1**: 151-165.
- Bartram, J. and Balance, R. (1996). Large-scale regional variation in diatom–water chemistry relationship, rivers of the eastern United States. *Journal of Hydrobiologia*. **56**: 27– 57.
- Barbour, M. (1999). Use of epilithic diatoms to evaluate water quality in the Karasu River of Turkey. *Journal of Environmental Biology*. **23**(3): 239-46.
- Behailu Brehanu (2007). Geology of the Modjo area (Ethiopia). *Journal of Acta Vulcanologica*, **36**: 517-572.
- Berehane Asfaw (2007). Geology of the Debre Zeyt area (Ethiopia) (with a geological map at scale 1:100,000). *Journal of Acta Vulcanologica*. **36**:517-572–1999.
- Bunn, S. (2000). Biological processes in running waters and their implications for the assessment of ecological integrity. *Journal of Hydrobiology*. **422/423**: 61-70.
- CCME (2006). Developing biocriteria as a water quality assessment tool in Canada: scoping assessment. *Journal of Acta Vulcanologica*. **11**:517-572
- Chapman, D. (ed). (1996). *A Guide to the use of Biota, Sediment and Water in Environmental Monitoring*, Pergamon press, Oxford. 419 p.

- Char and Chu, (2000). Biological monitoring of aquatic ecosystems in Italy. *Journal of limnology*. **1**: 49-52.
- Chovanec, A., Jäger, P., Jungwirth, M., Koller-Kreimel, V., Moog, O., Muhar, S. and Schmutz, S. (2000). The Austrian way of assessing the ecological integrity of running waters: a contribution to the EU Water Framework Directive. *Journal of Hydrobiology*. **422/423**: 445-452.
- Chutter, D. (2003). Some considerations about bioindicators in environmental monitoring. *Journal of Environmental Studies*. **13** (5): 453-462.
- Coste M, (2000). Impact of urban pollution from the Hanoi area on benthic diatom communities collected From the Red, Nhue and Tolich rivers (Vietnam). *Journal of Hydrobiology*. **18**:216-562.
- Deberry, D.A. and Perry, J.E. (2005). The biotic integrity concept in freshwater wetlands. Virginia Institute of Marine Science. *Journal of Freshwater ecology*. **4**: 12-18.
- Dejene Hailu, (1997). Pollution status of Awetu stream as it crosses Jimma town. *Journal of Hydrobiology*. **7** (2):79-85.
- De la Rey, P.A., Taylor, J.C., Laas, A., Van Rensburg, L., and Vosloo, A. (2004) Determining the possible application value of diatoms as indicators of general water quality – A comparison with SASS 5. *Journal of Hydrobiology*. **30**(3): 325-332.
- De Zwart, E. (2003). Biological processes in running waters and their implications for the assessment of ecological integrity. *Journal of Hydrobiology*. 422/423: 61-70.
- Duong, O. Callegaro, V.L. & Lobo, E.A. (2006). Eplithic diatoms as indicators of water quality In Brazil. *Journal of Aquatic Science Technology*. **15**(1): 54-63.
- EEPA (2003). Provisional standards for industrial pollution control in Ethiopia. Prepared under the ecologically sustainable industrial development (ESID) project - US/ETH/99/068/Ethiopia, EPA/UNIDO, Addis Ababa.
- Ekhouste, H. (2006). Comparative application of indices of biotic integrity based on periphyton, macroinvertebrates, and fish to southern Rocky Mountain streams. *Journal of Applied Science*. **5**:117–136.
- Eloranta, P. and Soinen, J. 2002. Ecological Status of Some Finnish Rivers Evaluated Using Benthic Diatom Communities. *Journal of Applied Phycology*. **14**: 1-7.

- Eskindir Zinabu (2002). Heavy metals are important for proper functioning of biological systems but their deficiency or excess have determinant effect on their environment. Adama, *Journal of environmental science*. **1**(8):156-163.
- Fore, H. and Grafe, S. (2000). Most pollution tolerant diatoms of severely polluted rivers in the vicinity of Tokyo. *Journal of Phycology*. **30**: 188-196.
- Farenzena M, Ferreira L, Trierweiler JO, Aquim PM (2005). Tanneries: from waste to sustainability. Brazile. *Journal of Biotechnology*. **48**:281-289.
- Gadzala, R. Berecka, B. Bartoszewicz, J. (2005). Some considerations about bioindicators in environmental monitoring. *Journal of Environmental Studies*. **13** (5): 453-462.
- Giller, S. and Malmqvist, B. (1998). The biology of streams and rivers. Oxford University Press, New York. *Journal of Experimental Marine Biology*. **338**: 193-204.
- Gomez, N. and M. Licursi, (2001). The Pampean Diatom Index (IDP) for assessment of rivers and streams in Argentina. *Journal of Aquatic Ecology*. **35**: 173–181.
- Harding W.R., Archibald, C.G.M. and Taylor, J.C. (2005). The relevance of diatoms for water quality assessment in South Africa: *Journal of Environmental science*. **31**(1):41-46.
- Haydar S, Aziz JA, Ahmad MS (2007). Biological treatment of tannery wastewater using activated sludge process. Pakistan. *Journal of Applied Science*. **1**:61-66.
- Hill, J. (2001). Comparison of correlations between environmental characteristics and Stream diatom assemblages characterized at genus and species levels. *Journal of the North American Ethological Society*. **20**:299–310.
- Hinga, R. (2002). Effects of pH on coastal marine phytoplankton, England. *Journal of Marine Ecology*. **238**: 281-300.
- Hungary Ministry of Environment and Water (2005). ECOSURV: Biological quality environmental report on phytobenthos. Hungary, EuropeAid/114951/D/SV/2002-000-180-04-01-02-02.
- James R.K. (2005). The economic approach to environmental natural resources. Royal Irish Academy. *Journal of Environmental science*. **3**: 189-109
- Jan, K. and Petrš, L. (2003) Statistical inference in canonical correlation Analyses Exemplified the influence of North Atlantic on European climate. *Journal of metrological Society*. 1175/1520:20-42

- Jarvie, H. and Lycett, C. (2002). Patterns in nutrient concentrations and biological quality indices across the upper Thames river basin, UK. *Journal of Hydrobiology*. **3**: 282–283.
- Javaid, S. and Chaudhary, A. (2008). Assessment of Trace Metal Contamination of Drinking Water in the Pearl Valley, Azad Jammu and Kashmir. *Journal of Hydrobiology*. **36**: 216–221
- John, D. Whitton, B.A. and Brool, A. J. (eds) (2003). *The freshwater algal flora of the British Isles: An identification guide to freshwater and terrestrial algae*. Cambridge University Press, UK, pp. 1-702
- Khan SR, Khwaja MA, Khan AM, Ghani H, Kazmi S (2001). Environmental impacts and mitigation costs associated with cloth and leather exports from Pakistan. *Journal of Ecological Science*. **2**(1): 76-94.
- Krammer, K and Lang-Bertalot, H. (2000). Süßwasserflora von Mitteleuropa. Bacillariophyceae Part 5 (Vol. 2/5). (B. Büdel, G. Gärtner, L. Krienitz, and G. Lokhrost, Eds.) Heidelberg, Berlin: Spektrum Akademischer Verlag.
- Karr, J.R. (1991). Biological Integrity: A Long-Neglected Aspect of Water Resource Management. *Journal of Ecological Science*. **1**(1): 66-84.
- Karr, J.R. and Chu, E.W. (2000). Sustainable living rivers. *Journal of Hydrobiology*. **422/423**: 1-14
- Kelly, M. (1998). The conceptual basis of ecological status assessments using diatoms. Royal Irish Academy. *Journal of Hydrobiology* **3**:175-189.
- Kelly, M. (2000). Identification of common benthic diatoms in rivers of Canada. *Journal of Applied Biology*. **9**:583- 70
- Kelly, M. and Whitton, B.A. (1995). The Trophic Diatom Index: A New Index for Monitoring Eutrophication. *Journal of Applied Phycology*. **7**: 433–444.
- Kwandrans, J., Eloranta, P., Kawecka, B. and Wojtan, K.( 2001). Use of benthic diatoms to evaluate water quality in the Polish rivers (Southern Poland). *Journal of Applied Phycology*. **10**: 193-201.
- Lamaia, C. (2005). Toxicity and Accumulation of Lead and Cadmium in the Filamentous Green Alga *Ladophora fracta*: A laboratory study. *Journal Ecological Science*. **31**:121-127.

- Lobo, E. A., Katho, and Aruqa, A. (2002). Response of epilithic diatom assemblages to water pollution in rivers in the Tokyo metropolitan area, Japan. *Freshwater Biology* 34:191–204.
- Metcafe, F. (2006) Comparative environmental assessment in the studies of benthic diatom, macroinvertebrate, and fish communities. *Journal of Hydrobiology*. **89** (2): 121-138.
- Mondal, N. , Saxena,K. and Singh, S. (2005). Impact of pollution due to tanneries on groundwater regime in Italy. *Journal of Environmental science*. **88**(12):25.
- Moog, F. and Chovanec,K . ( 2000). Diatoms as ecological indicators in lakes of Mandya. *Journal of Applied Environmental Sciences*. **1**(2): 185-196
- Nathanson, S. (2000) Using diatoms to Assess the Quality of Washington Streams and to Describe Biological Expectations. Washington State department of Ecology. *Journal of Applied Ecology*. **3**: 97-332.
- National Meteorological Agency of Ethiopia, Adama Branch, March 2012,Adam Ethiopia.
- Ogedengbe K , Akinbile CO (2004). Impact of industrial pollutants on quality of ground and surface waters at Oluyole Industrial Estate, Ibadan, Nig. *Journal of Technology*. **4**(2):139-14
- Pan ,k. (2001). Spatial patterns and ecological determinants of benthic algal assemblages in mid Atlantic streams, united state of America. *Journal of Phycology*. **35**:460–468.
- Pan, k. and Lowe, J.(2001). Environmental requirements and pollution tolerance of freshwater diatoms. EPA-670/4–74–005. US Environmental Protection Agency, Cincinnati, Ohio.
- Porter, D. (2002). Biological and chemical indicators of eutrophication in the Yellowstone River and major tributaries during August 2000. Proceedings, 200 National Monitoring Conference, National Water Quality Monitoring Council URL: <http://www.nwqmc.org/>
- Prygiel, J. and Coste, M. (2000). Determination of the biological diatom index, France. *Journal of Applied Science*. 12: 90-354.
- Prygiel, J., and COSTE, M. (1993). The assessment of water quality in the Artois-Picardie water basin (France) by the use of diatom indices. *Hydrobiologia* 269/ 270:343–349.
- Quinn, G.P. and Keough, M.J. (2002). Experimental design and data analysis for biologists. Cambridge University Press, UK.

- Sahu, S. and Srivastava, J. (2008) Toxicity Assessment of Industrial Effluent by Bioassays. Clean – Soil, Air, and Water. *Journal of Applied science*. **36**: 517–520.
- Sawyer, G. (2006) The use of diatoms to assess past and present water quality. *Journal of Applied ecology*. **20** (1): 57–64.
- Schmitz, S. (2000). The Austrian way of assessing the ecological integrity of running waters: a contribution to the EU Water Framework Directive. *Journal of Hydrobiology*. 422/423: 445-452.
- Sekhar, A.(2003). Heavy metal polluted soil: effect on plants and Bioremediation methods. *Applied and Environmental Soil Science* . **14**: 1-12.
- Seyoum Leta, Fassil Assefa and Gunnel, D. (2003). Characterization of Tannery wastewater and assessment of downstream pollution profiles along Modjo River in Ethiopia. *Journal of Biological Science*. **2**(2): 157-168
- Shaw, P.J.A. (2003). Multivariate statistics for the environmental sciences. *Journal of Environmental Science*. **2**: 46-77
- Siddaraju, A. and Devi Prasad, G. (2011). Diatoms as ecological indicators in lakes of Mandya. *Journal of Applied Environmental Sciences*. **1**(2): 185-196
- Soininen, J. (2002). Response of epilithic diatom communities to environmental gradients in some Finnish rivers. *International Review of Hydrobiology*. **87**: 11–24.
- Stevenson, R. J., M. L. Bothwell and R. L. Lowe (eds), *Algal Ecology: Freshwater Benthic Ecosystems*. Academic Press, San Diego. *Journal of Applied Ecology*. **4**: 705–739.
- Szabo, K. and Acs E. (2005) Epiphytic diatoms of the Tisza River Kiskore Reservoir and some oxbows of the Tisza River after the cyanide and heavy metal pollution. *Acta Botanica Croatica, Journal of Phycology*. **64** (1): 1 – 46.
- Taffere Addis (2008). Comparative Study of Macroinvertebrates and Diatoms as bioindicators of River Water Quality in Addis Ababa. <https://www.researchgate.net/publication/223350111>.
- Tamiru Alemayehu (2001). The impact of uncontrolled waste disposal on surface water Quality in Addis Ababa. *SINET: Ethiopian Journal of Science*. **24** (1): 93-104.
- Taylor, J.C. (2007). Janse Van Vuuren MS, Pieterse AJH. The application and testing of diatom-based indices in the Vaal and Wilge Rivers, South Africa. *Journal of Applied sciences*. **33**: 51-59.

- Taylor, J.C., Prygiel, J., Vosloo, A., Pieter, A., Rey, D. and Ransburg, L.V. (2007). Diatom based pollution indices be used for biomonitoring in South Africa. A case study of the Crocodile West and Marico water management area. *Journal of Hydrobiology*. **592** (1): 455-464.
- Tsegaye Abebe (1999). Geology of the DebreZeyt area (Ethiopia) (with a geological map at scale 1:100,000). *Journal of Acta Vulcanologica*. **36** (4): 517-572
- UN (2002). Investment and innovation policy review Ethiopia. United Nations conference on trade and development investment. UNCTAD/ITE/IPC/Misc.4.
- UNEP (2010). Clearing the water: A focus on water quality solutions. Nairobi, Kenya.
- UNIDO (1991). Leather industry. Conference on ecologically sustainable industrial development. Case study No. 3. ID/WG.516/10. UNIDO Secretariat, Copenhagen, Denmark.
- USEPA (2008). Onsite wastewater treatment technology fact sheet 9, enhanced nitrogen removal - nitrogen. EPA/625/R/00/008. pp. 45-52.
- Van Dam, H., Mertens, (1994). A coded checklist and ecological indicator values of freshwater diatoms from the Netherlands. *Journal of Aquatic Ecology*. **28**: 117-133.
- Verheijen LAHM, Wiersema D, Hulshoff Pol LW, De Wit J (1996). Livestock and the environment finding a balance: Management of waste from animal product processing. International agriculture centre Wageningen, *The Netherlands. Journal of Applied Science*. **4**(9): 349-359.
- Ward, T. (2001). Response of epilithic diatom assemblages to water pollution in rivers in the Tokyo metropolitan area, Japan. *Journal of Freshwater Biology*. **34**:191–204.
- Weber-Scannell, P. and Duffy, K. (2007). Effects of Total Dissolved Solids on Aquatic Organisms: A Review of Literature and Recommendation for Salmonid Species. *Journal of Environmental Science*. **3**: 1-6.
- Wetzel, R.G. (2001). *Limnology: Lakes and Rivers ecosystems*, 3rd ed. Academic Press, San Diego San Francisco New York.
- WHO (2003). Scientific Assessment of Freshwater Harmful Algal Blooms. Volume 1, Coastal and fresh waters. World Health Organization. Geneva, Switzerland. 119pp.

Zinabu GebreMariam and Zerihun Desta (2002). The chemical composition of the effluent from Awassa Textile Factory and its effects on aquatic biota. *SINET:Ethiopian Journal of Science*. **25**(2):263-274.

## List of ANNEXES

### Annex 1 Description of sampling points

<b>Name of sampling site</b>	<b>Major sources of pollution</b>
S1( upstream of Modjo River)	Runoff from farm plots and pasture lands.
S2( discharging place of the liquid waste of Modjo Tannery)	Modjo Tannery
S3( downstream)	Modjo Tannery and also mining activity
S4(downstream)	Same as S3

### Annex2 Description of the codes for Diatom indices.

<b>Code</b>	<b>Name</b>
TDI	<b>Trophic diatom index</b>
GDI	<b>Generic diatom index</b>
IPS	<b>Pollution sensitivity index</b>
PTV (%)	<b>Percent pollution tolerant valve</b>

Annex 3 Diatom species found in the study area with their code, sensitivity and indicator Value (Kelly, 2000)

Code	Name of species	TDI		GDI/IPS	
		S	V	S	V
AMIN	<i>Achnanthes minutissima</i>	2	2	5	2
AMVE	<i>Amphora veneta</i>	2	1	3.6	1.7
COPL	<i>Cocconeis placentula</i>	3	2	2.6	1
COPE	<i>Cocconeis pediculus</i>	4	2	3.8	3.8
CYMI	<i>Cymbella minuta</i>	2	1	4.7	2.6
CYSI	<i>Cymbella silesiaca</i>	2	1	4.7	2.6
CYSP	<i>Cyclotella species</i>	0	0	2.9	1
CYPS	<i>Cyclotella pseudostelligra</i>	0	0	2.9	1
ENBE	<i>Encyonema bebridica</i>	2	1	4.7	2.6
ENMI	<i>Encyonema minuta</i>	2	1	4.7	2.6
EUSU	<i>Eunotia subarcuatoides</i>	1	3	4.8	2.3
FRBE	<i>Fragilaria berolinesis</i>	2	1	3.6	1.7
FRCA	<i>Fragilaria capucina</i>	2	2	3.6	1.7
FCRO	<i>Fragilaria crotonensis</i>	5	2	3.6	1.9
FRFA	<i>Fragilaria fasciculata</i>	2	1	3.6	1.7
FRGE	<i>Fragilaria germanin</i>	2	1	3.6	1.9
FRIN	<i>Fragilaria incognita</i>	2	1	3.6	1.7
FRNE	<i>Fragilaria neoproducta</i>	2	1	3.6	1.7
FRSU	<i>Fragilaria subsalina</i>	2	1	3.6	1.7
FRVA	<i>Fragilaria vaucheriae</i>	3	2	3.6	1.7
GOAF	<i>Gomphonema affine</i>	3	1	3.6	1.9
GOAN	<i>Gomphonema angustatum</i>	1	2	3.6	1.9
GAGR	<i>Gomphonema gracile</i>	3	1	3.6	1.9
GOPA	<i>Gomphonema paravlum</i>	5	3	3.6	1.9
GOPR	<i>Gomphonema productum</i>	3	1	3.6	1.9
HAAM	<i>Hantzschia amphioxys</i>	1	5	3.6	1.1
NAAC	<i>Navicula acomoda</i>	5	1	3.4	1.9
NAAT	<i>Navicula atomus</i>	1	4	3.4	1.9
NALE	<i>Navicula letostrata</i>	4	1	3.4	1.9
NAPR	<i>Navicula proteracta</i>	1	4	3.4	1.9
NASA	<i>Navicula salinicola</i>	4	1	3.4	1.9

NIAN	<i>Nitzschia angustata</i>	4	1	1	2.3
NIFI	<i>Nitzschia fibulafissa</i>	4	1	1	2.3
NIFL	<i>Nitzschia fliforms</i>	1	4	1	2.3
NIFL	<i>Nitzschia fleoxide</i>	4	1	2.3	1
NILI	<i>Nitzschia linearis</i>	2	4	1	2.3
NIPE	<i>Nitzschia pela</i>	5	1	1	2.3
NIUM	<i>Nitzschia umbonata</i>	1	4	1	2.3
PIBI	<i>Pinnularia biceps</i>	5	1	4.7	2.3
PUTR	<i>Pinnularia tropica</i>	5	1	4.7	2.3
SUAN	<i>Surirella angusta</i>	1	4	3.6	2.2
SYUL	<i>Synedra ulna</i>	5	1	3.1	1.8

Annex4. Spatial variation in diatom species composition and abundance along the Modjo River.

Species Code	S <sub>1</sub>	S <sub>2</sub>	S <sub>3</sub>	S <sub>4</sub>
AMIN	0	0	5	0
AMVE	0	14	0	0
COPE	4	0	0	13
COPL	17	0	0	46
CYMI	15	0	0	9
CYSI	11	0	11	13
CYPS	0	0	8	0
CYSP	3	0	0	7
ENBE	3	0	0	15
ENMI	4	0	0	4
FCRO	11	0	3	1
EUSU	9	0	4	0
FRAG	33	0	0	12
FRBE	10	0	0	0
FRCA	0	52	40	0
FRGE	9	0	0	4
FRIN	5	0	0	0
FRNE	3	0	0	4
FRSU	6	0	0	0
FRVA	3	0	0	4
GOAF	21	0	0	3
GOAN	27	0	0	25
GOCR	21	0	5	18

GOPA	0	10	38	0
GOPR	32	0	0	0
HAAM	0	15	0	0
NAAC	0	13	0	0
NAAT	0	0	5	5
NALE	12	0	0	0
NAPR	0	0	6	0
NASA	7	0	0	0
NIAN	7	0	4	0
NIFI	4	0	0	0
NILI	0	0	3	7
NIPE	0	41	28	0
NIUM	0	0	5	0
PIBI	7	0	0	5
SUAN	12	0	0	11
SYUL	0	7	5	5

Annex 5 ANOVA results

		F	P-value
NH <sub>3</sub>	Between Sites	242.248	.000
NO <sub>2</sub>	Between Sites	2.782	.110
NO <sub>3</sub>	Between Sites	2.737	.113
SRP	Between Sites	19.638	.000
TP	Between Sites	19.045	.001
Silica	Between Sites	895.755	.000

SO <sub>4</sub>	Between Sites	28977.150	.000
Fe	Between Sites	320.646	.000
BOD	Between Sites	100.316	.000
DO	Between Sites	560.555	.000
<u>Conductivity</u>	<u>Between Sites</u>	<u>11547.869</u>	<u>.000</u>