

# Akaki River | Ethiopia



**POLLUTION STATUS OF AKAKI RIVER AND ITS CONTAMINATION EFFECT ON SURROUNDING ENVIRONMENT AND AGRICULTURAL PRODUCTS: TECHNICAL REPORT 2017**



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## SUMMARY

**Background:** Urbanization is one of the main causes for environmental problems due to the introduction of undesirable materials into soils, water and air. Such changes in the characteristics of soil, water and air, may have a direct effect on the health of people or other living things. In addition, problems associated with human settlements can carry risks for rivers, streams and other water reservoirs if insufficient care is taken and human habitation are sited near to the water bodies. Furthermore, industrialization is among the major cause of surface water pollution in Ethiopia. Industrialization is expansion in Ethiopia particularly in urban areas. Addis Ababa is well known by its numerous industries and most of the industries are located a long side of Akaki Rivers. Accelerated pollution and eutrophication of rivers, streams, springs and other water reservoirs because of anthropogenic activity which results from aforementioned factors are a concern throughout the world including Africa particularly Ethiopia is a case since as developing countries lack and have not stringent regulations that have been implemented to restrict the discharge of untreated wastewater into rivers, streams and other water bodies. As we know the existing pollution legislation of Ethiopia is weak and generally not adequately enforced into action to protect the water bodies and other environmental entities. Due to severely pollution of Akaki River, there is a very high risk to human health, and the surrounding environment (air, soil, and water). Exposure to these wastes, which contain toxic components such as chemicals, pathogens, is of great concern, as it poses not only health risks to humans but also potentially unacceptable ecological risks to plants, animals and macroinvertebrates, which are abundant to water bodies

**Objective:** To understand the Pollution status of Akaki River and the extent of microbial, and trace metals transportation in food and environmental matrices adjoining the river and its tributaries

**Method:** River water samples were collected from 27 different sampling sites of the Akaki River applying the procedure in APHA. The 500-ml water sample, which was intended for anion analyses, was left unfiltered and unacidified. The other unfiltered 250-ml water sample was acidified with 2 ml of concentrated nitric acid; this acidified sample was used later for cations analysis. 1-L Sterilized bottles were used for bacteriological analysis and samples were stored in a refrigerator at 4°C and subjected to analysis within 24 hours. The water quality parameters were analyzed in accordance with the standard method for the examination of water and waste water (APHA, 1996). The parameters such as pH, Conductivity and Turbidity were determined at

sampling site using in-situ instruments. All other water quality variables were analyzed in the laboratory.

The procedure described by Weldegebriel et.al, 2011 was followed for the collection of the vegetable samples. During the dry season of 2017, 38 samples were collected and 15 in the wet season of 2016. In the sample preparation, a microwave digestion system with the aid of acid mixture (6ml of 65% HNO<sub>3</sub> and 2ml of 30% H<sub>2</sub>O<sub>2</sub>) was used for the dissolution of the vegetable samples. Finally, Atomic Absorption Spectroscopy (novAA400P) was used to determine the metal concentrations.

In the same periods of the vegetable samples collected, 13 samples in the dry (February, 2017) and 12 in the wet season (August, 2016) were taken, using plastic bags, applying a rectangular sampling strategy in the study farms. The samples air dried, grinded, sieved with 2mm mesh, then subjected to acid digestion and analyzed for trace metal concentration by Atomic Absorption Spectrophotometer.

**Result:** The result of physicochemical, nutrients and trace metal of the Akaki River collected in dry and wet seasons from a total of 27 sampling sites are summarized in this report. The median value of Nitrate, and Nitrite, in Akaki river water in mg/L was 32.21, and 0.34 with minimum and maximum value of 6.36-192.59, and 0.1-4.84, respectively. Approximately 30% of study sites' nitrate concentration was found above the standard. Relatively, high proportion of sampling sites (55.6 %) Nitrite concentration did not comply with any of the standards mentioned. Phosphate median concentration in Akaki River was 0.19 mg/L with minimum and maximum range of 0.0-1.4mg/L and approximately, 32% of river water samples phosphate concentration surpass any of the standard or guideline values. The Akaki River water BOD ranged from 0-319.2 mg/L with a median value of 15.54 mg/L while COD values were ranged between 0-738.67 mg/L. Overall, 95.9 % and 61.1% of river water samples' COD and BOD respectively, did not comply with standards and guideline values. The overall concentration of iron, manganese, zinc, chromium, lead, and cadmium in the Akaki River water samples ranged from 0 -38.55 mg/L, 0.01 - 777.0 mg/L, 0 - 0.42 µg/L 0 - 858.4, 0-26.22 µg/L, and 0-7.49 µg/L with median value of 3.36, 1.06, 0.12, 5.33 mg/L, 5.33 µg/L, and 6.23 µg/L, respectively. A total of 23 soil samples were collected and analyzed for selected trace metals. The values of Zn, Co, Ni and Cd are within the standard limit of the European directives for soil contaminants. However, Cu, Cr and Pb have surpassed this limit. In this study, a total of 51, widely consumed vegetables samples, of which, 22 are

Ethiopian Kale, 10 Lettuce and 14 Swiss chard, were collected and subsequently analyzed for selected heavy metals, Fe, Mn, Zn, Pb, Cr and Cd. Zn was detected in all vegetable types, where around 51% of the samples have exceeded amount of Zn when compared to standard limit of 99mg/kg. The minimum value is 58mg/kg and maximum 157mg/kg. The order of accumulation is in order of decreasing concentration, Lettuce>Swiss chard> E.Kale.

The present study attempted to determine the percentage of vegetable contamination with Total aerobic plate count (TAC), coliform bacteria (TCC) and faecal coliforms (FC) as well as their microbial loads through total aerobic count (TAC), total coliform counts (TCC) and fecal coliforms (FC). The overall mean count of E. coli and Non-E. coli from water samples in the present study were 2.09 and  $>3.48 \log_{10} \text{CFU } 10 \text{ mL}^{-1}$  which is higher than the WHO recommended standard. The mean count of TC, FC, and TAC on collected vegetables irrigated with Akaki River were 3.22, 1.37, and 4.72 in dry season, and 3.87, 2.57, and 5.09  $\log_{10}\text{CFU}$  per gram in wet season, respectively. All fresh vegetables were contaminated with total coliform, fecal coliform and total aerobic in dry season.

The daily exposure of farmers to selected heavy metals through ingestion and inhalation pathways is analyzed and the total chronic daily intake of heavy metals is higher in female farmers ( $4.80e-4$ ) than male farmers ( $6.10e-4$ ). The intake of lead and chromium through ingestion of vegetable is higher than intake through inhalation. Even though, it is lower than ingestion, intake through inhalation proves that farmers in the urban and peri-urban areas of Addis Ababa are at risk of occupational exposure to heavy metals. The intake of heavy metal via inhalation is in the order of Ni>Co>Cr>Pb.

**Conclusion:** It has been evident from our findings that, the water quality of the Akaki River shows pattern of behavior linked to anthropogenic sources with the intensity of human pressure associated with industrial effluent, domestic wastes and agricultural activities. The assessment of macroinvertebrate taxa provides a clue what happens in Akaki River and the water quality effect on species diversity. Therefore, all macroinvertebrate indices along with human disturbance and poor habitat quality suggests that both little and Great Akaki streams are severely modified by human influences and it needs immediate restoration and rehabilitation tasks.

The Akaki River was shown to be heavily contaminated with non-E. coli & E. coli coliforms and did not meet the WHO guideline criteria for safe irrigation. Target microorganisms commonly used as indicators for the hygiene status of foods frequently exceeded the HACCP/QM and

ICMSF limit values for safe consumption. This indicates that the presence of these organisms on produce might be due to a transfer from fecally contaminated irrigation water, which might place consumers at risk. Consequently, we showed that fresh produced vegetables (especially Ethiopian Kale, lettuce, Cabbage, and swish chard) might contain pathogenic microorganisms and represent a risk for consumers regarding foodborne disease.

Vegetable farms in and around Addis Ababa, which were irrigated with contaminated waters exhibited increased concentrations of metals both in the soils and in the vegetables grown on them. Nevertheless, it was noticed that different vegetables accumulate and translocate variable amounts of metals from the soil into their tissues. Without regard to bioavailability, the vegetables lettuce, Ethiopian kale, and Swiss chard grown in these farms showed Cd at levels that could raise health risk concerns to consumers. However, zinc has the lowest translocation factors in all vegetables analyzed. The findings dictate immediate need for measures to protect the safety of consumers and the general environment.

**Keywords:** Akaki River, Pollution, Trace metal, Soil, Vegetable, Macroinvertebrate, Season

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## **1. Background and Justification:**

Population growth and industrialization are among the cause of surface water pollution. On the other hand, urbanization is alarmingly increasing in Ethiopian particularly in the capital of Addis Ababa. Recent findings of UN-HABITA indicating that the current population of Addis Ababa is about 4 million and hosting 30 percent of the urban population with predicting population of 12 million by 2034 (Tigabu and Semu, 2008). Urbanization is one of the main causes for environmental problems due to the introduction of undesirable materials into soils, water and air. Such changes in the characteristics of soil, water and air, may have a direct effect on the health of people or other living things. In addition, problems associated with human settlements can carry risks for rivers, streams and other water reservoirs if insufficient care is taken and human habitation are sited near to the water bodies. The chemical risks to Akaki River hugely associated with human settlements and related activities. On-site sanitation and sewerage systems, waste disposal, urban runoff, fuel storage, handling and disposal of chlorinated solvents, and pesticide application for public health and vector control and Spills of many chemicals found in urban areas (including petroleum and fuel oils) are also a source of contamination of both ground waters and surface waters in Addis Ababa.

Furthermore, industrialization is expansion in Ethiopia particularly in urban areas. Addis Ababa is well known by its numerous industries and most of the industries are located a long side of Akaki Rivers. There are 15 major industries in Addis Ababa. The most important of all are food and beverage, furniture, leather, paper and printing, non-metallic mineral products, metallic products and textiles. Except for some very old enterprises, most of the large and medium industries in Addis Ababa are located in industrial zones of Akaki (CSA, 2010) where Akaki Rivery is crossing down.

Accelerated pollution and eutrophication of rivers, streams, springs and other water reservoirs because of anthropogenic activity are a concern throughout the world including Africa particularly Ethiopia is a case since as developing counties lack and have not stringent regulations that have been implemented to restrict the discharge of untreated wastewater into rivers, streams and other water bodies. As we know the existing pollution legislation of Ethiopia is weak and generally not adequately enforced into action to protect the water bodies and other environmental entities (Kumie and Kloos, 2006).

Most Ethiopian cities lack waste treatment systems, including Addis Ababa, the capital city of Ethiopia (pop. More than 4 million). About 90% of the industrial firms in Addis Ababa discharge their effluents directly into the nearby streams without any form of treatment. In addition, oil pollution to rivers from waste discharge from car wash and garages are very common situations of Akaki River.

Akaki River water use for domestic, recreational purposes, as irrigation water sources and as a disposal sites for all domestic, institution and industries wastes (Alemayehu, 2001). The study conducted on Akaki River revealed that physicochemical parameters like dissolved oxygen sharply depleted and biochemical oxygen demand is sharply increase downstream of Akaki. Similarly, other nutrients, like phosphate, nitrate and ammonia plus ammonium were also elevated in downstream sites.(Beyene et al., 2009).

Besides to wastes discharged from aforementioned sources, the Akaki Rivers suffers from Diversion of its tributaries, pumping of water for irrigation, deforestation, erosion, and town settlement around the river side. This has made life difficult to the surrounding fringe dwellers as they depend on the Akaki to irrigate their vegetable patches and give its water to their cattle to drink. This highly polluted river is a tributary to the Awash River, which pretty much dilutes these pollutants because of its large volume, but one should ask for how long? The Awash River irrigates most of the large-scale farms that are exporters of sugar, fruits and many more agricultural products. We should think of the loss of healthy vegetables due to the pollution at national scale as we throw our garbage into the rivers and streams passing our backyard. As evidence the concentrations of trace metals like cadmium, copper and lead in the vegetables irrigated with Akaki river water were found above the maximum recommended limits (Weldegebriel et al., 2012).

Due to severely pollution of Akaki River, there is a very high risk to human health, and the surrounding environment (air, soil, and water). Exposure to these wastes, which contain toxic components such as chemicals, pathogens, is of great concern, as it poses not only health risks to humans but also potentially unacceptable ecological risks to plants, animals and macroinvertebrates, which are abundant to water bodies (Melaku et al., 2004). One study conducted in Akaki River showed that the Prevalence of perceived illness among farmers working on irrigation farms within and around Addis Ababa were including the intestinal nematodes, diarrhea and skin disease, and the result varied significantly between the wastewater and

freshwater areas. The prevalence was also higher for farmers working in downstream than upstream wastewater farm areas (Weldesilassie et al., 2011).

Previously FMOH has initiated the need for a comprehensive study on the status of exposure of Akaki population to hazardous toxic chemicals, solely heavy metals. The short coming of the previous study was that, the sampling technique did not include population groups that are believed to be receivers of the maximum effect of the pollution. These individuals are farmers and their families, who cultivate different kinds of fruit and vegetables on the urban agricultural field adjoining the river Akaki and its catchment. Farmers and their families are believed to have three times higher consumption rate of vegetables than the general population of Addis Ababa. For this reason, risk assessment focusing on this part of the community is required to provide preliminary information on the health risk that farmers are facing with the consumption of vegetables that they are growing and exposure to heavy metals through occupational inhalation of heavy metals while working on the field. To characterize the risk, both primary and secondary data were extracted and used for the risk estimation. The risk assessment has followed four distinct standardized steps; the general process encompasses, hazard identification, exposure assessment, dose-response assessment and risk characterization

## **2. OBJECTIVE OF THE RESEARCH PROJECT**

### **General Objectives**

To understand the Pollution status of Akaki River and the extent of microbial, and trace metals transportation in food and environmental matrices adjoining the river and its tributaries

### **Specific Objectives**

To evaluate the quality of Akaki River based on physicochemical, trace metal and biomonitoring indices (Macroinvertebrates)

To appraise the level of trace metals in selected vegetables irrigated by Akaki River and its tributaries;

To investigate the major point and non-point pollution sources of the Akaki River

To assess the degree of microbial contamination of river water and selected vegetables irrigated by the river and its tributaries;

To determine metal content of Akaki River water

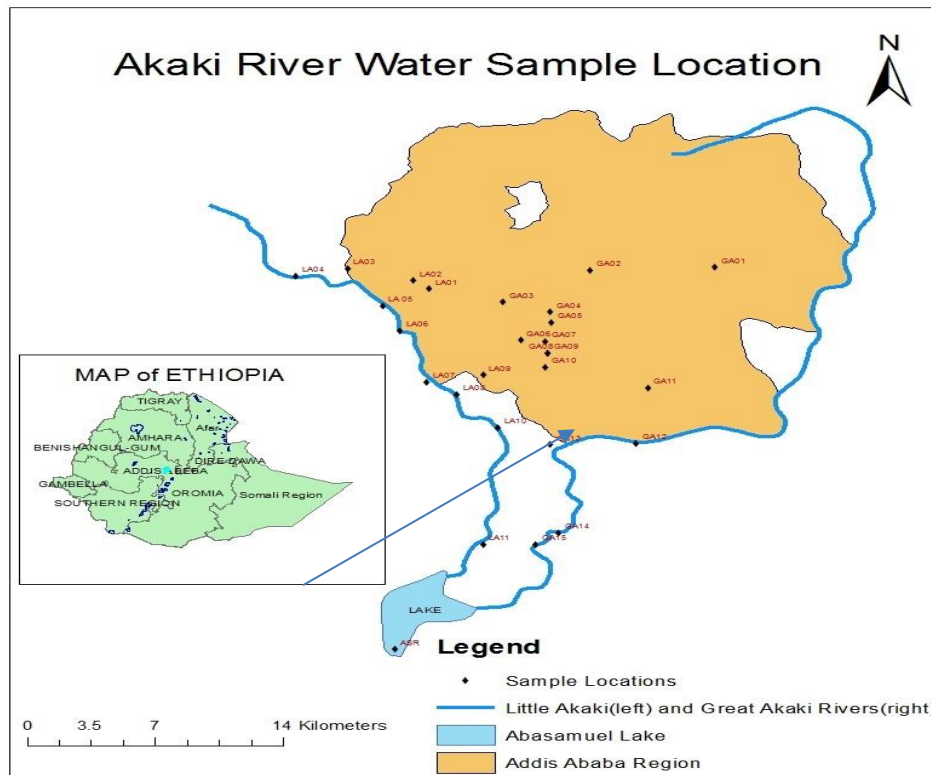
To identify concentration of heavy metals on irrigated farmlands.

To determine both cancerous and non-cancerous health risks, amongst farmers living and working around agricultural fields that extends along the Akaki river catchment.

## 2. Materials and methods

### Study Area

The study was conducted on Akaki River and its tributaries from upstream to down Akaki. The Akaki catchment is located in central Ethiopia along the western margin of the Main Ethiopian Rift. The catchment is geographically bounded between 8°46′–9°14′N and 38°34′–39°04′E, covering an area of about 1500 km<sup>2</sup>. The entire catchment is bounded to the north by the Intoto ridge system, to the west by mount Menagesha and the Wechecha volcanic range, to the southwest by mount Furi, to the south by mount Bilbilo and Guji, to the southeast by the Gara Bushu hills and to the east by the mount Yerer volcanic center (Demlie and Wohnlich, 2006).



**Figure 1: Map of sampling point, Akaki River Ethiopia, 2017**

Despite its proximity to the equator, the study area experiences a temperate Afro-Alpine climate. Daily average temperatures range from 9.9 to 24.6 °C and annual mean rainfall is 1254 mm, as measured at Addis Ababa Observatory (Demlie, 2015). The climate of the Akaki catchment is characterized by two distinct seasonal weather patterns. The main wet season, locally known as Kiremt extends from June to September, contributing about 70% of the total annual rainfall. A

minor rainy season, locally known as Belg, contributes moisture to the region from mid-February to mid-April (Demlie et al., 2007). The remaining five months are dry season.

For better description of the Akaki River and the associated land use along the river course, the catchment was segmented into three parts. These are the upper, middle and lower segmentations. The upper catchment comprises small streams which drain from different parts of mount Entoto, Geferes, and Legedadid dams to join together to form both little Akaki flowing from west to south and Great Akaki flowing down from north to south. The upper catchment of both Little and Great Akaki is mostly dominated by residential settings. However, the upper catchment of Little Akaki is occupied by few medium and large industries (Addis Ababa and Dire Tanneries, Addis Ababa glass factory, Ethio-Marble Factory, Tikur Ababy shoe Factory, Dil oil and Gulele soap factories) than Great Akaki streams (Tegegn, 2012). In addition, commercial activities are very common in this catchment, especially around Kolfe sub city. In this part of the river section, we identified 12 sampling sites for general monitoring and 5 vegetable farms. Soil samples were collected in all vegetable farms.

The middle catchment includes the full course of the river inside the city before leaving the suburbs of Akaki- Kality. This part of the river contains more tributaries than the upper catchment. Little Akaki drain the Addis Ababa cement factory and join the main river at a point near Bihere Tsige Park. While the Great Akaki and Bulbula are joined together below Arsema Church around Worku sefer to form the final Great Akaki. The river also traverses through a highly populated and big commercial section of the city. In this part of the river, we identified 11 general monitoring grids and 5 vegetable sampling sites.

Down below the middle catchment there exist many large and medium size industries, workshops and big garages. Tanneries, abattoirs, oil and beverage factories are among the industries found at the riverbanks of both Little Akaki and Great Akaki streams. Other factories are also found in a short distance from the river. In this catchment area, horticultural crops are also grown by irrigation. The irrigation system also extends toward the farming field of Akaki in the lower catchment (Tegegn, 2012). The lower catchment passes through the rural parts of the city and finally enters into the Aba-Samuel reservoir. In this part of the catchment, there is a point source where wastes are discharged from Kaliti sewage treatment plant. After treatment of the collected sewage from the city, the final waste is disposed into Little Akaki. This catchment is also used for agricultural and domestic purposes. In this catchment, we identified 5 general sampling points and 5 vegetable sampling sites.



## Study Design

A cross-sectional study design was used to study physicochemical, trace metal and biological parameters of the Akaki River and its tributaries, irrigated soil, and vegetables in dry and wet seasons.

## Reconnaissance survey

Before the actual survey was commenced, a reconnaissance survey was carried out. During this survey, we assessed preliminary information, which are necessary for sampling, preservation, and transportation of sample before the actual engagement. In addition, monitoring grids, site description, pollution source profiling, types of fruits and vegetables produced identification of irrigated fields and estimates of amount of fruits and vegetables produced in each area, and seasonal variation on the production type were documented.

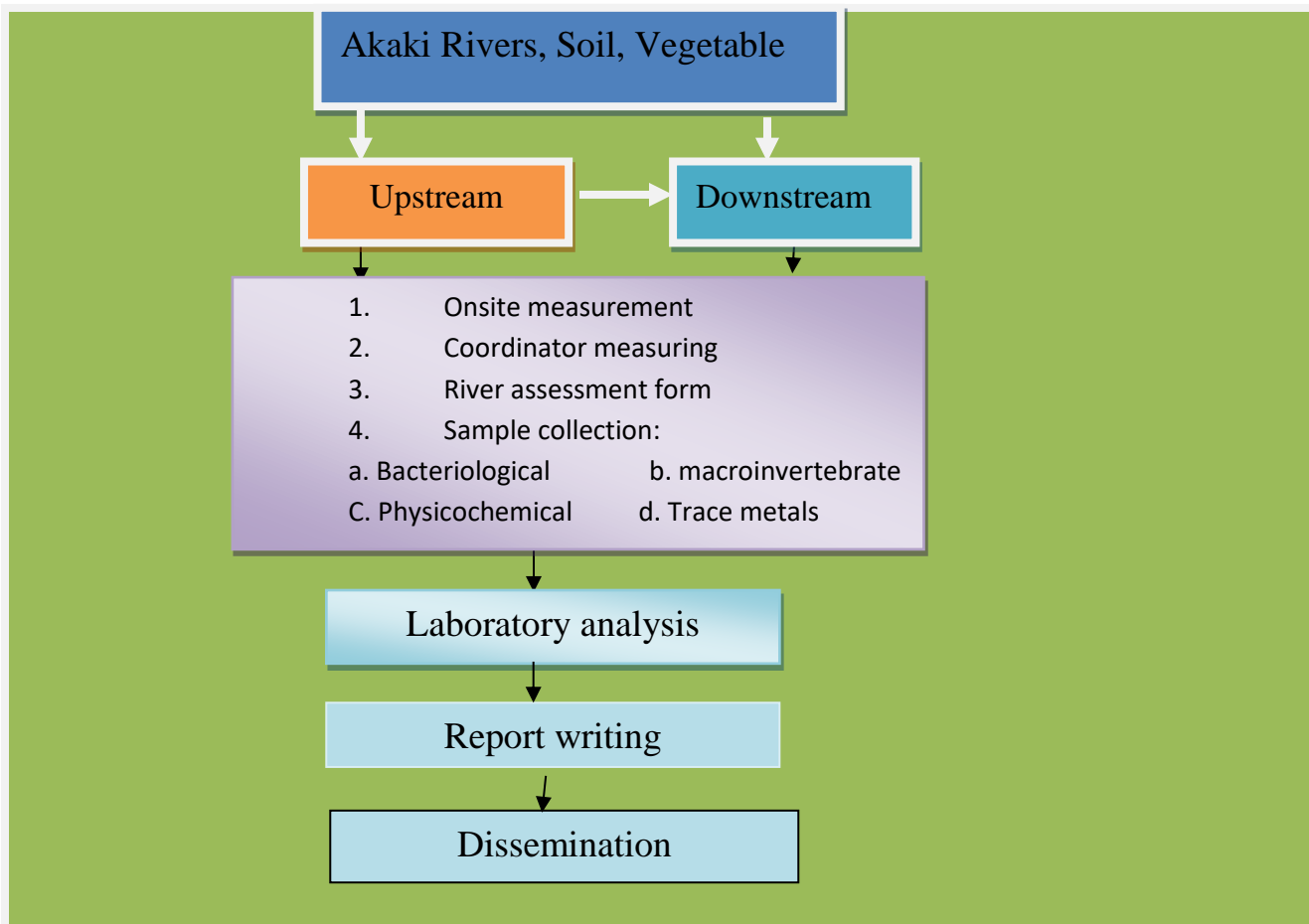


Figure 2: data flow diagram, Akaki River Ethiopia, 2017

During the site assessment, the following issues were outlined in a questionnaire:

- Hectare of land irrigated
- Production rate
- Proportion of vegetables and fruits produced
- Frequency of production and variation on fruits and vegetables produced seasonally
- Market distribution
- Point source and non-point sources of pollutions
- Habitat quality assessment
- Human disturbance score

## **Sampling and sample pretreatment**

### ***I. River water***

Representative river water samples were collected from 27 sampling sites from Little and Great Akaki Rivers, including diversion points for irrigation. The sites were chosen based on locations of industries, agricultural activities, population density, and other possible sources of pollutants. At each sampling stations 2 liters and 250ml of river water samples were collected by pre-cleaned polyethylene bottles for physicochemical and trace metal analysis. Sterilized bottles were used for bacteriological analysis and samples were stored in a refrigerator at 4°C and subjected to analysis within 24 hours. Samples for trace metal analysis were centrifuged and filtered through 0.45 mm micropore filters and stored in a refrigerator at 2°C by adding a preservative (1 ml of 70% HNO<sub>3</sub>).

### ***II. Vegetable***

Three types of fresh vegetables (Ethiopian Kale, Lettuce and spinach) were collected from farms irrigated by Akaki River in the dry and wet seasons. During the dry month of February 2017, 38 samples were collected and 15 in the wet season of August 2016. Vegetables were taken from every corner of plots to make sampling representative. Samples were collected in plastic bags, cleaned with deionized water to remove dust and extraneous matter. Edible parts were sliced and air-dried to remove excess moisture and oven-dried at 70 °C for 24 hrs. Samples were then grinded using mortar and pistil, homogenized and passed through a 2-mm-mesh sieve and stored in tightly closed sample bottles at ambient temperature before analysis.

### ***III. Soil***

Soil samples were collected by applying a rectangular sampling strategy. Five sampling points were selected with in the designated rectangular frame of the typical farm. 13 samples in the dry season (February, 2017) and 12 in the wet (August, 2016) were taken. Samples from each point

were mixed in a polyethylene bag amounting 1.5Kg. During the sampling process, the top soil, which is composed of litters and vegetation, were cleared out and samples at a depth of about 25 cm were taken. Samples were then transported to EPHI laboratory and air dried on a plastic tray. After drying, 500gm sample was dried and sieved by 2 mm sieve.

## ***VI. Biomonitoring assessment***

### **Sampling and identification of Macroinvertebrates**

Macroinvertebrates were collected using a D-shaped sweep-net to provide a qualitative description of the community composition of the stream. Sweeping was done (first on surface column and then on stream bed) in a vigorous action for 3 min for a distance of 10 m with multi-habitat approach to dislodge macroinvertebrates attached to any substrates or vegetation present at each sampling point (Gabriels et al., 2010). Collected organisms have been removed and sieved. Identification of macroinvertebrate in species level has been performed in the laboratory using identification key and a microscope.

### **Habitat Quality Assessment and Human Disturbances Index**

Physical habitat information was collected at each site with visual estimate measurement technique. Bank angles were estimated at each of six evenly spaced channel cross sections, wetted width, bankfull width, bankfull and incised heights. Canopy cover 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 (64–250 mm), 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). Embeddedness percentage were visually estimated from the area immediately surrounding each sampled particle. Immediately following cross section surveys, large wood (>six in diameter) were tallied and organic layer accumulation in depositional zones were measured.

Human Disturbance index was used to characterize the degree of human disturbance at a given waterbodies' biomonitoring station, including the portion of the watershed immediately surrounding the station, relative to other stations sampled. The index was determined by considering hydrological and vegetative modification, evidence of chemical pollutions, impervious surface and potential of non-point sources to each sampling sites.

### **Sample Digestion**

#### ***I. River water***

100 ml portion of the sample was acidified with 5ml concentrated HNO<sub>3</sub> and evaporated on hot plate to the lowest volume possible (10 ml to 15 ml).

## ***II. Vegetables***

For sample dissolution, a microwave digestion system (ETHOS One, Milestone, Italy) with Microwave Digestion Rotors (MDR) technology was used with Tetrafluormethaxil (TFM) vessels. 0.5gm powdered vegetable sample was weighed and placed into a TFM vessel and an acid mixture (6ml of 65% HNO<sub>3</sub> and 2ml of 30% H<sub>2</sub>O<sub>2</sub>) was added. The vessels were gently swirled to homogenize samples.

We performed a complete digestion of samples by two heating steps at different temperatures (110 °C, 200 °C). The digested solution was passed through a filter paper (Whatman No. 42). Following this, the filtrate was made up to 100 ml using deionized water. The final diluted sample was stored in a refrigerator at 4 °C in acid-washed polyethylene bottles.

## ***III. Soil***

Soil samples were digested in a microwave digestion system with the mixture of acid solutions (nitric acid: hydrochloric acid and hydrofluoric acid: boric acid). 0.5g of the dried soil sample was weighed in to the TFM vessel, an acid mixture (8ml of 65% HNO<sub>3</sub>: 5ml of 37% HCl and 1ml of 40% HF: 5ml of 5% H<sub>3</sub>BO<sub>3</sub>) were added into the sample containing TFM vessel. The TFM vessels were gently swirled to homogenize the sample with the acids. We performed a complete digestion of samples by two heating steps at different temperatures (110 °C, 200 °C). The digested solution was passed through a pre-washed filter paper (Whatman No. 42). Following this, the filtrate volume was made up to 100 ml using deionized water. The final diluted sample was stored in the refrigerator at 4 °C in acid-washed polyethylene bottles before final analysis.

## **Sample analysis**

### ***water***

#### ***Bacteriological analysis***

Isolation and enumeration of *E. coli* were carried out using membrane filtration method (WHO, 1997). After incubation, the number of colonies per 100 mL of a sample were evaluated from plate count. Blanks were run for each analysis.

#### ***Trace Metal Analysis***

Samples were analyzed according to Standard Methods of Water and Wastewater Analysis (APHA 1996). The level of Cr, Pb, Fe, Mn and Zn were determined by using atomic absorption spectrophotometer (Analytikjena novAA400P) equipped with a graphite furnace, deuterium background correction system, MP-60 auto-sampler, and graphite tubes with integrated L'vov platform in Ethiopian Public Health Institute, department of environmental public health.

### ***Physico-chemical analysis***

Electrical conductivity, pH, TDS and turbidity were measured on situ, whereas fluoride, chloride, ammonia, total alkalinity, total hardness, Ortho-phosphate, nitrate and nitrite concentrations were analyzed by using Uv-Vis Spectrophotometer (Shimadzu UV-1800) at EPHI laboratory according to APHA, 1996.

### ***Vegetables***

#### ***Trace Metal***

Atomic Absorption Spectroscopy (novAA400P) was used to determine the concentrations of Cu, Co, Fe, Cd, Ni, Cr, Mn, Pb and Zn. All results were reported in mg/kg dry weight (DW) of vegetables.

### ***Soil***

#### ***Trace Metal***

The same instrument analyzed the levels of Cr, Mn, Pb and Zn in soil similarly and all results were reported in mg/kg dry weight (DW) of soil.

## **Methods of health risk assessment**

The health risk assessment is aimed at assessing the health risk that could emanate from the consumption of carcinogenic and non-carcinogenic chemicals. The process has four distinct steps of hazard identification, exposure assessment, toxicity (dose-response) assessment, and risk characterization.

The hazard identification focuses on the identification of potential pollutants that are health hazards; in this case selected heavy metals are identified and their concentration in water, vegetable and soil samples of the perturbed environment were characterize. Lead, Chromium, Nickel, Cobalt, Cadmium, Manganese, Zinc and iron were identified as possible hazards for the community.

The exposure assessment is vital in estimating the quantity, frequency and duration of the heavy metals, which the population has exposed. In the current study, chronic daily intake of farmers for selected heavy metals through ingestion and inhalation of vegetable and soil particulates were estimated respectively.

The dose response assessment estimates the toxicity of the heavy metals to the farmers at risk. For this Endeavour, two indices were taken, this are cancer slope factor (CSF, Carcinogen potency factor) and reference dose (RFD, a non-carcinogenic threshold).

The last step, risk characterization, uses all this data, in order to estimate the potential cancerous and non-cancerous health risk posed to farmers living and working adjoining the river Akaki and its catchments.

Ingestion of heavy metals via vegetable consumption:

$$CDI_{ing} = \frac{Cv \times IR \times EF \times ED}{BW \times AT}$$

$CDI_{ing}$  = Chronic daily ingestion of vegetable (mg/kg/day)

$Cv$  = concentration of heavy metal in vegetable samples (mg/kg)

$IR$  = ingestion rate (Kg per day)

$EF$  = Exposure frequency (days/year)

$ED$  = exposure duration in years

$BW$  = body weight of the exposed individual

$AT$  = the time period over which the dose is averaged in days, which is different for carcinogenic and non-carcinogenic risk, that is 365 days for the non-cancer risk and 365x70d for cancer risk.

Inhalation of heavy metals via soil particulates:

$$CDI_{inh} = \frac{Cs \times IR_{air} \times EF \times ED}{BW \times AT \times PEF}$$

$CDI_{inh}$  = chronic daily intake of heavy metals through inhalation, mg/kg/day

$Cs$  = concentration of heavy metals in soil (mg/kg)

$IR_{air}$  = inhalation rate (m<sup>3</sup>/day)

$PEF$  = particulate emission factor (m<sup>3</sup>/kg)

$EF$ ,  $ED$ ,  $BW$  and  $AT$  are identified earlier in equation 1.

Non-carcinogenic risk estimation

The non-carcinogenic hazard is termed as hazard quotient (HQ), which is a unit less number that expresses the probability of an individual suffering an adverse effect. It is a quotient of CDI divided by RfD (chronic reference dose). For n numbers of heavy metals, the total non-carcinogenic risk is termed as hazard index, which is the summation of hazard quotient of each heavy metal.

$$HQ = \frac{CDI}{RfD} \quad HI = \sum_{k=1}^n \frac{CDI}{RfDs}$$

If the HI is less than one, the exposed population is unlikely to experience adverse health effects; if the HI is greater than one, then there may be concern of non-carcinogenic effects ()

Carcinogenic risk estimation

The risks are estimated as the incremental probability of an individual developing cancer over a life time as a result of exposure to the potential carcinogen.

$$\text{Cancer risk (each of ingestion and inhalation)} = \sum_{k=1}^n \text{CDI} \times \text{CSF}$$

The total cancer risk is estimated based on the summation of cancer risks estimated for exposure pathways, ingestion and inhalation.

$$\text{Total cancer risk} = \sum \text{cancer risk ing} + \text{cancer risk inh}$$

### ***Quality Control and Quality Assurance***

Blanks were run for every analysis to correct measurements. Calibration of the AAS was made using standard solutions (analytikjena, 1000 mg/l stock solutions, Germany). For sets of ten samples, a procedure blank and a spiked sample containing all reagents were read to check contamination and triplicate measurements were taken for each sample.

For the sake of quality assurance data were assessed carefully using standard operating procedures and Double entry of data were performed to assure the quality of data.

### **Data Analysis**

Data analysis were carried out using spreadsheet and IBM SPSS Statistic 20 software. Median, Mean, standard error of mean and standard deviation of each heavy metal were calculated. ANOVA and correlation tests were calculated for comparing mean concentration of heavy metals between the vegetables and determining their correlation level, respectively. In addition, Family biotic indices, and Simpson and Shannon diversity were carried out to assess the pollution status of the Akaki River.

### **Ethical Clearance**

The study was conducted after getting permission from Scientific and ethical review committee of Ethiopian Public Health Institute.

## 4. Result and Discussion

### 4.1 River Water

#### 4.1.1 Physicochemical distribution

The result of physicochemical, nutrients and trace metal of the Akaki River collected in dry and wet seasons are summarized in Table 1 and Table 2 respectively. During the sampling of two round campaign, a total of 27 sampling sites were covered.

#### Selected Physicochemical parameters

The median hydrogen concentration of Akaki River in both dry and wet season was near to neutral pH 7.87 with minimum (5.96) and Maximum (9.01) ranges, which is in agreement with the limit of the Canadian Council of Ministers of the Environment (CCME) guidelines for livestock watering and irrigation water (CCME, 2001).

**Table 1: Distribution of Physico-chemical parameter results in river water, Akaki River Ethiopia, 2017**

Parameter	Unit	Min.	Max.	Mean	Median	Standard	MAC (%)
pH		5.96	9.01	7.80	7.87		3.8
Turbidity	NTU	10.30	2000	285.00	131		83.3
Conductivity	μS/cm	70.20	3330	663.23	590		11.3
TDS	Mg/L	45.9	2540	463.86	436		5.7
Nitrate	mg/l	6.36	192.59	44.74	33.21		27.8
Nitrite	mg/l	0.1	4.84	0.69	0.34		55.6
Ammonia	mg/l	0	51.16	9.49	5.66		59.3
Phosphate	mg/l	0	1.4	0.28	0.19		31.5
Chloride	mg/l	0	799.75	60.85	37.98		1.9
BOD	mg/l	0	319.20	55.76	15.54		61.1
COD	mg/l	0	738.67	200.36	169.53		95.9
DO	mg/l	5.20	8.45	6.98	6.90		47.2



In addition, the finding is in agreement with previously conducted on Little Akaki River (Melaku et al., 2007). This is may be due to calcium carbonate bedrock weathering or may reflect the importance of dissolution of limestone and dolomites in the watershed.

Akaki river turbidity ranged from 10.3 to 2000 NTU for all the water samples in both dry and wet seasons with median value of 131.0 NTU.

The electrical conductivity (EC) indicates the amount of material dissolved in water and the Akaki River water EC value was ranged from 70.2 at sampling site LA05 (Lomimeda) to 3330.0 at LA02 near winget area (Bedada River) with median value of 590  $\mu\text{S}/\text{cm}$ . The median EC value of Akaki River water in both wet and dry season was within the limit of the Ethiopian Environmental protection authority standards (1000  $\mu\text{S}/\text{cm}$ ) (Admasu, 2007). On the other hands, the maximum value of EC was recording at sampling point where tannery industry and other bleaching and gas factories join to Bedada stream, which is one of Little Akaki tributary. Generally, high value of EC possibly related to phenomena of mineralization or weathering of sediments, and probably largely due to discharge of industrial and domestic wastes (Melaku et al., 2007, Alemayehu, 2001) as well noted across the course of Akaki River up to downstream.

#### **Nitrate, Nitrite, Ammonium, Ortho phosphate**

The median value of Nitrate, and Nitrite, in Akaki river water in mg/L was 32.21, and 0.34 with minimum and maximum value of 6.36-192.59, and 0.1-4.84, respectively. Overall, about 70.2 % sampling sites of the Akaki River Nitrate concentration was found in agreement with the limit of 50 mg/L of Canadian surface water standard and European Union drinking water maximum permissible limit (CCME, 2001, Reeve, 2002). On the other hand, approximately 30% of study sites' nitrate concentration was found above the standard. Relatively, high proportion of sampling sites (55.6 %) Nitrite concentration did not comply with any of the standards mentioned. The presence of nutrients in the Akaki River is may be originated from overland runoff from riverine agricultural fields where irrigated cash crops are grown and the use of inorganic fertilizers is rather frequent. The high concentration of nitrites may also originate from decomposition of nitrogen containing organic compounds such as proteins and urea occurring in industrial and municipal wastewater discharges or open defecation practiced along the course of the river. Moreover, study (Vega et al., 1998) indicated that in the presence of high levels of organic matter, nitrate can be reduced in some extent to nitrite, what could explain the high concentration of this

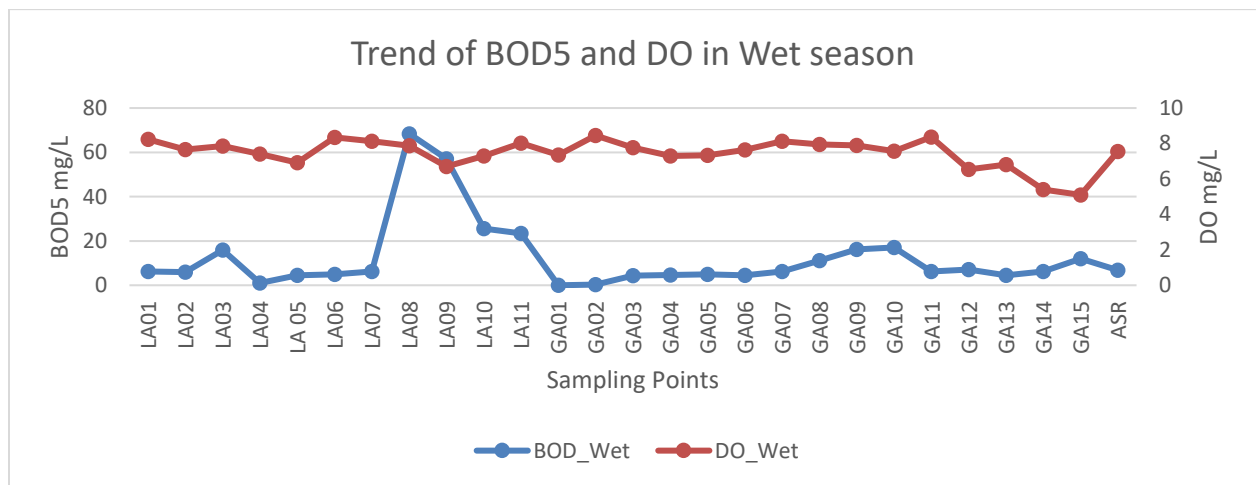
pollutant in Akaki River. This is also my related to high concentration of Ammonia in the Akaki River with maximum value of 51.16 mg/L.

Phosphate median concentration in Akaki River was 0.19 mg/L with minimum and maximum range of 0.0-1.4mg/L and approximately, 32% of river water samples phosphate concentration surpass any of the standard or guideline values. This may be due to the consequences of municipal wastewater discharge and urban-agricultural practices since these are an important component of detergent and inorganic fertilizers.

**Pollution loading parameters (COD and BOD)**

The Akaki River water BOD ranged from 0-319.2 mg/L with a median value of 15.54 mg/L while COD values were ranged between 0–738.67 mg/L. The COD medial value of Akaki River in both dry and wet season was 169.53 mg/L. The median value of both BOD and COD are exceeds any of standard in the world. Overall, 95.9 % and 61.1% of river water samples’ COD and BOD respectively, did not comply with standards and guideline values. This could be related with the disposal of industrial and municipal wastes directly to the river. This was notified during site visit overall the river courses.

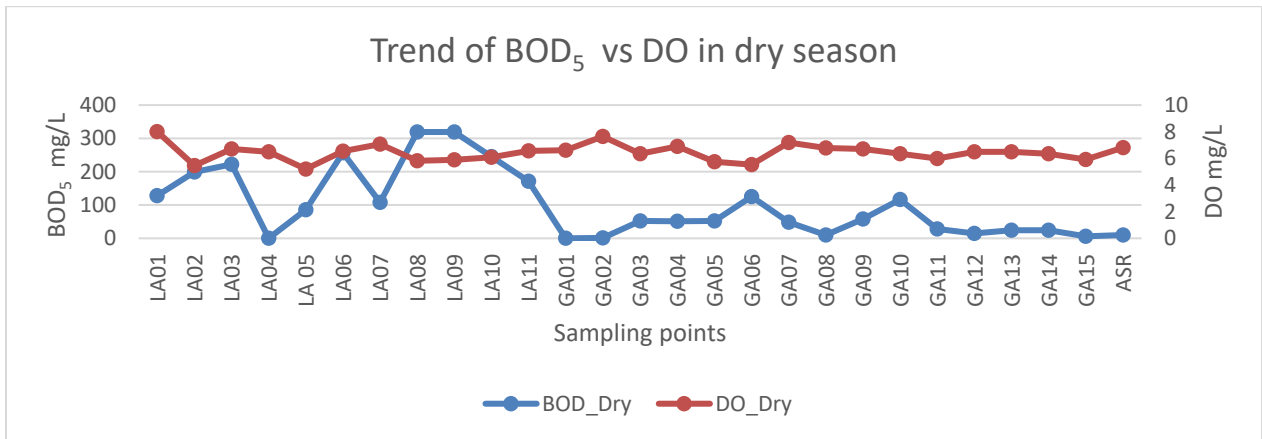
Since Biochemical Oxygen Demand (BOD) is a measurement of organic pollution and depletion of oxygen, it is presented on Figure3 and 4 to determine the trend in both dry and wet season.



**Figure 3: shows the Akaki River water BOD<sub>5</sub> and DO Trend in Wet Season, Akaki River Ethiopia, 2017**

The higher BOD values along with the lower DO (in both dry and wet seasons) accompanied by the continuous input of all kinds of wastes into the Akaki River overestimated the assimilative (the natural self-purification) capacity of the river. This in turn greatly impairs the water quality

of the river and harms aquatic life. However, few improvements is noticed at the final downstream site (Abasamuel); this may be due to the current rehabilitation of the dam.



**Figure 4: shows Akaki River water BOD<sub>5</sub> and DO Trend in Dry Season, Akaki River Ethiopia, 2017**

#### 4.1.2 Distribution of heavy metal in River water

The concentration of Fe, Zn, Mn, Pb, Cd, and Cr in river water were quantified; the results are presented in Table 2. The overall concentration of iron, manganese, zinc, chromium, lead, and cadmium in the Akaki River water samples ranged from 0 –38.55 mg/L, 0.01 – 777.0 mg/L, 0 – 0.42 µg/L 0 – 858.4, 0-26.22 µg/L, and 0-7.49 µg/L with median value of 3.36, 1.06, 0.12, 5.33 mg/L, 5.33 µg/L, and 6.23 µg/L, respectively.

**Table 2: the overall distribution of selected heavy metals in water sample, Akaki River Ethiopia, 2017**

Parameter	Unit	Min.	Max.	Mean	Median	Standard	MAC (%)
Iron	mg/l	0	38.55	5.89	3.36		56.6
Manganese	mg/l	0.1	4.3	1.15	1.06		54.7
Zinc	mg/l	0	0.42	0.14	0.12		0
Chromium	µg/L	0	858.4	56.48	5.33		17
Lead	µg/L	0	26.22	6.92	6.23		28.3
Cadmium	µg/L	0	7.49	1.02	0.43		20.4

The concentration of iron and manganese was found exceeding in more than half of the water sample collected from Akaki River in both seasons. Among toxic metals, lead takes the highest proportion, of which 28.3% of the sampling sites have surpassed the limit, followed by Cadmium

(20.4%) and Cr (17%). Contrarily, Zink was the only heavy metal, which was found 100% within or below the standard.

**Table 3: Distribution of trace metals in water samples, Akaki River Ethiopia, 2017**

Catchments	Concentration (Mean $\pm$ SE)					
	Fe(ppm)	Mn(ppm)	Zn(ppm)	Cr(ppb)	Cd(PPb)	Pb(PPb)
Lower	12.00 $\pm$ 4.76	98.87 $\pm$ 96.88	0.15 $\pm$ 0.03	16.72 $\pm$ 8.77	0.41 $\pm$ 0.10	5.56 $\pm$ 1.35
Middle	3.83 $\pm$ 0.92	0.99 $\pm$ 0.09	0.12 $\pm$ 0.02	16.50 $\pm$ 10.13	0.51 $\pm$ 0.11	6.45 $\pm$ 1.35
Upper	5.82 $\pm$ 1.39	1.04 $\pm$ 0.15	0.16 $\pm$ 0.02	112.74 $\pm$ 48.50	1.81 $\pm$ 0.48	7.92 $\pm$ 1.32
Akaki river Contamination	5.89 $\pm$ 1.04	1.15 $\pm$ 0.1	0.14 $\pm$ 0.01	56.48 $\pm$ 21.40	1.01 $\pm$ 0.22	6.92 $\pm$ 0.83

The minimum level of lead was detected in sampling sites that are found in the upper catchment, sites such as Burayu Gefersa, Tinziz wonze around France embassy and Yeka Abado. This was ascribed, relatively to the absence of human and industrial disturbance. Hailu, (2007), has reported that the level of Pb, Cd and Cr around Burayu area were below the detection limit, whereas the current investigation has shown 1-3-fold higher concentration of lead, however the value is within the standard limit. An assessment made by Itanna, (1998), in a river crossing Kera and Peacock reported an exceeding amount of lead when compared to the current study. Ejigu (1997) reported similar trend in a river that is crossing Biheretsige garden. This shows that the level of Lead is decreasing when compared to studies carried out before 10-20 years.

**Table 4: Calculated heavy metal pollution index in terms of the most toxic metals, lead, cadmium and chromium, Akaki River Ethiopia, 2017**

River catchment	Heavy metal pollution index (HPI)	Interpretation (Mohan et al., 1996)	
General Akaki river	91.6	0-25	Excellent
Upper catchment	310	26-50	Good
Middle Catchment	278	51-75	Moderately polluted
Lower catchment	24100	76-100	Very poor
		Above 100	Unsuitable

The main source of chromium in the river catchment is the presence of leather processing industries. In the current study, areas that are situated along tanning industries have exhibited higher concentrations. For instance, Bedada River, River behind Bihere Tsige garden and Beyene Hordofa Bridge have chromium as high as 673 $\mu$ g/L, 32.37 $\mu$ g/L and 233 $\mu$ g/L respectively. When compared to previous studies such as Ejigu, 1997, a level of chromium in Biheretsige site was around 0.1 $\mu$ g/L; it explains, after 20 years, the level of chromium in this

specific place has increased more than 300 folds. A comparable result is, however reported by Mersha, (2008) and Itanna, (1998). In general, the level of chromium is within a predictable range; however, there are places with peculiar results and these areas are highly impacted by the presence of tanning industries.

According to Mohan et al., (1996), a river’s pollution status in terms of heavy metal pollution can be best represented applying heavy metal pollution index. The index is bounded between 0 and 100; any value beyond 100 explains that the river is not suitable for irrigation purpose. In the current study, the heavy metal pollution index is calculated and presented in table 4. The result presents that Akaki River has a heavy metal pollution index of 91.6, which explains the river has a poor water quality and somewhat unsuitable for agriculture purposes. The worst case can be observed in the lower catchment, which has 264 times higher pollution index, compared to the overall Akaki River index; this was ascribed to the cumulative effect of the pollution load. In general, the Akaki River is unsuitable for irrigation.

## Macroinvertebrate Result

### 4.2.1 Macroinvertebrate Order

A total of 2,593 and 1,603 of macroinvertebrates in which belongs to nine orders were collected from 27 sampling sites from Akaki Rivers in wet (August 2016), and Dry (February 2017) seasons, respectively.

The most abundant orders during the wet season were Diptera 1,021 (39.37%) and Ephemeropetra 534 (20.59 %) while Diptera 851 (53.98%) and Odonata 434 (27.07%) were the most dominant order in dry season.

**Table 5: percentage of macroinvertebrate orders, Akaki River Ethiopia, 2017**

SN	Order	Wet Season		Dry Season	
		Number	%	Number	%
1	Coleopteran	1	0.04	12	0.75
2	Dipteral	1,021	39.37	851	53.98
3	Ephemeroptera	534	20.59	107	6.68
4	Gastropoda	60	2.32	1	0.06
5	Hemiptera	15	0.58	52	3.24
6	Nematoda	476	18.36	136	8.48
7	Odonata	290	11.18	434	27.07
8	Oligochaeta	181	6.98	9	0.56
9	Tricoptera	15	0.58	1	0.06
Total		2,593	100.00	1,603	100.00

### 4.2.2 Macroinvertebrate Indices

## Shannon Index

The Shannon diversity index of macroinvertebrate communities was significantly lower at all sampling sites in both seasons where macroinvertebrate was found with range from 0.105-0.816 during the wet season and from 0.024 to 0.892 in the Dry season.

**Table 6: macroinvertebrate indexes, Akaki River Ethiopia, 2017**

Sn	Sampling Sites	Wet Season		Dry Season		Remark
		Shannon Diversity (H')	Simpson Diversity (D)	Shannon Diversity (H')	Simpson Diversity(D)	
1	LA01	0.396	0.551			
2	LA02					
3	LA03	0.596	0.367			
4	LA04	0.695	0.207	0.892	0.13	
5	LA 05	0.526	0.413	0.22	0.764	
6	LA06					
7	LA07	0.603	0.309	0.059	0.939	
8	LA08	0.614	0.253	0.024	0.98	
9	LA09	0.531	0.305			
10	LA10					
11	LA11	0.569	0.238	0.276	0.467	
12	GA01	0.816	0.162	0.972	0.109	
13	GA02	0.736	0.245	0.024	0.98	
14	GA03	0.64	0.228	0.057	0.943	
15	GA04	0.752	0.221	Only 1 family	1	
16	GA05	0.482	0.441	0.292	0.4	
17	GA06	0.589	0.276	0.373	0.436	
18	GA07	0.682	0.238	Only 1 family	1	
19	GA08	0.662	0.237	Only 1 family	1	
20	GA09					
21	GA10	0.291	0.509	0.024	0.98	
22	GA11	0.513	0.345	0.196	0.667	
23	GA12	0.817	0.192	Only 1 family	1	
24	GA13	0.541	0.308	0.241	0.63	
25	GA14	0.435	0.4	0.115	0.862	
26	GA15	0.463	0.38	0.148	0.807	
27	ASR	0.105	0.877	0.298	0.499	

The Shannon diversity index value is relatively higher in upstream sites namely Gefersa (LA04), Yekabado (GA01), and Tinziz wonz (GA02) above Farnese embassy in both wet and dry seasons. Most values measured using the Shannon diversity index (Turkmen and Kazanci, 2010) range from 1.5 to 3.5, rarely exceeding 4.5. Values 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

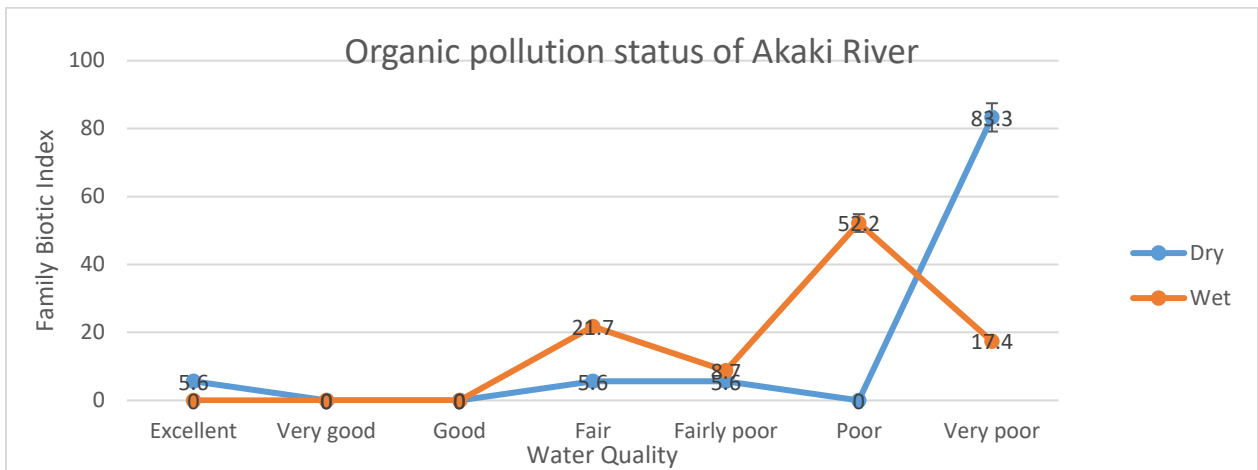
structure. Based on these criteria, all of sampling sites of Akaki river fallen below one level of the Shannon diversity index in both dry and wet seasons (Table 6). It further indicating that the presence of elevated levels of pollution and degradation of habitat structure in the studied area.

### **Simpson diversity index**

The Simpson diversity index of macroinvertebrates communities were also significantly lower at all sampling sites where the diversity was found ranging from 0.162 - 0.877 in Wet season and 0.13 –1 in dry season. According to (Smith and Wilson, 1996), values measuring using 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 nearly zero and indicated the presence of severe pollution in all sites of the Akaki river from upstream to down to Akaki in both dry and wet seasons.

### **Family level biotic index**

The family level biotic index showed significant variation among the studied sites in both wet and dry seasons. About 52 % of the sampling sites are classified as poor water quality, which are substantially pollution likely to organic pollution sources during dry season and approximately 83% of study sites in both Little Akaki and Great Akaki were fallen under very poor water quality that are severely organic pollution likely. The family biotic index showed a strong organic pollution level in all sites of the Akaki River. Although this biotic index was originally formulated to provide a single ‘tolerance value’, which is the average of the tolerance, values of all species within the benthic arthropod community(Hilsenhoff, 1988), these results showed that the index responded well to loading of organic pollutants. These results occurred because the more intolerant genera and species in each family predominate in clean streams, whereas the more tolerant genera and species predominate in polluted streams (Mandaville, 2002). Based on these criteria, all sites’ macroinvertebrate family scored high family biotic index value (Figure 5) and all the sites were severely deteriorated by anthropogenic activities, including open defecation, linkage of toilets from nearby dwellers, washing and other industrial influents.



**Figure 5: family biotic index and water quality, Akaki River Ethiopia, 2017**

The habitat classes of Akaki River and its tributary could be categorized into three (marginal, sub-optimal, and optimal) as shown from Table 7. Only 15 percent of the study sites are classified with optimum habitat quality index whereas the majority sampling points are fallen under marginal (45 %), and sub-optimum (40 %). On the other hands, Human disturbance score in the study area is varied considerably among sites. None of the sites is classified as low disturbance. Twenty-five sites out of 27 categorized under severely disturbed class and the remaining two sampling sites are grouped under moderately disturbance class. Although the river supports a diverse and abundant invertebrate community consisting of aquatic, semi-aquatic species as depending on the human disturbance and habitants score level. The most abundant orders were Diptera in both wet (39%) and dry (54%) seasons (Table 5). Most of families found in Akaki River belonging to families called generalists. This group uses a variety of food resources, including detritus, plants, epiphytic algae and other organisms (Barbour et al., 1999) and is able to resist disturbance when food resources change. In addition, Invertebrate assemblages were relatively poor taxon and had low densities in those locations with high fine sediment, detritus, and mud content.



**Table 7: Habitat and Human disturbance scores in River water, Akaki River Ethiopia, 2017**

Sampling Site	Human disturbance Score		Habitat Quality Index	
	Score	Class	Score	Class
LA01	110	Sever	105	Marginal
LA02	120	Sever	64	Marginal
LA03	115	Sever	107	Marginal
LA04	60	Moderate	171	Optimum
LA 05	105	Sever	80	Marginal
LA06	100	Sever	73	Marginal
LA07	105	Sever	135	Sub-Optimum
LA08	110	Sever	87	Marginal
LA09	105	Sever	80	Marginal
LA10	115	Sever	77	Marginal
LA11	100	Sever	90	Marginal
GA01	50	Moderate	171	Optimum
GA02	50	Moderate	171	Optimum
GA03	90	Sever	122	Sub-Optimum
GA04	100	Sever	150	Sub-Optimum
GA05	85	Sever	154	Sub-Optimum
GA06	90	Sever	150	Sub-Optimum
GA07	100	Sever	157	Sub-Optimum
GA08	100	Sever	152	Sub-Optimum
GA09	115	Sever	93	Marginal
GA10	95	Sever	127	Marginal
GA11	85	Sever	148	Sub-Optimum
GA12	85	Sever	156	Sub-Optimum
GA13	105	Sever	145	Sub-Optimum
GA14	105	Sever	120	Sub-Optimum
GA15	100	Sever	104	Marginal
ASR	100	Sever	116	Sub-Optimum

Habitat condition score: poor<60, marginal 60-109, sub-optimum 110-159 and optimum 160-200(Barbour et al., 1999), and Human Disturbance Score: low disturbance<25, moderate disturbance >25-75 and sever disturbance >75-125(MDEP, 2009).

### **Trace metal in soil amended with polluted Akaki River**

A total of 23 soil samples were collected and analyzed for selected trace metals, Fe, Mn, Zn, Co, Cu, Ni, Cr, Pb, and Cd. The results are presented in table 7. The values of Zn, Co, Ni and Cd are

within the standard limit of the European directives for soil contaminants. However, Cu, Cr and Pb have surpassed this limit. In addition, the results have a different phenomenon when aggregated across the river catchment, upper, middle and lower catchments.

**Table 8: Trace metals in Soils amended with River water, Akaki River Ethiopia, 2017**

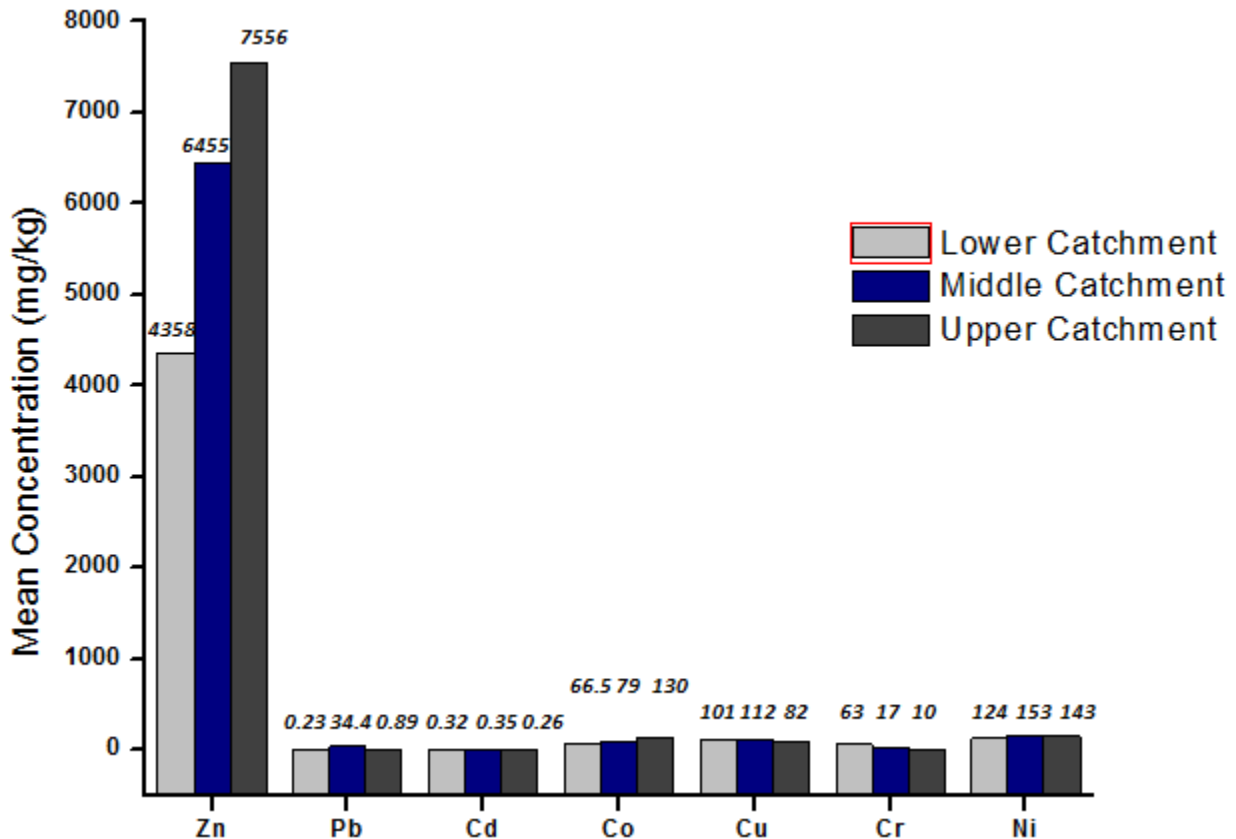
Trace Metal (mk/kg)	Mean ±SE	Min value	Max value	Guideline value (China, mg/kg)
Iron	104550.87±4915.38	72210.00	150500.00	
Manganese	3317.3261±201.00	1828.00	5562.00	
Zinc	5856.74±642.61	2435.00	162110.00	250
Cobalt	82.25±9.72	29.84	223.90	50
Copper	103.16±7.22	54.08	186.00	40
Nickel	139.91±9.57	59.72	232.30	250
Chromium	40.52±12.97	0.00	228.00	0
Lead	13.91±10.96	0.00	249.70	
Cadmium	0.31±0.04	0.04	0.71	

It was found that Zn in the lower catchment is the highest with a concentration of 7556.25 mg/kg. Surprisingly, this concentration is about 38 times higher than the recommended maximum value set by both the European and the Chinese standards for Soil (200 mg/kg). In addition, the concentration of Zn is significantly different ( $p < 0.05$ ) among the catchments.

The quantitative chemical analysis of chromium in the three catchments revealed that its concentration is somewhat higher in the upper catchment (63.42 mg/kg), but with statistically no significant difference ( $p > 0.05$ ) to the other two. The European standard for soil sets a maximum value for chromium to be 100 mg/Kg, whereas the limit value recommended by China is 250 mg/Kg. Previous studies of Tamiru and Alemahyehu, (2006) and Amare Hailu, (2007), have reported a comparable result. A higher concentration of chromium as observed in both periods could be attributed to the presence of three industries, namely Addis Ababa tannery Share Company, Bedada leather processing industry and Ethio marble industry.

At the lower catchment of the Akaki River, higher concentration of cobalt was found to be 130.50 mg/Kg, which is higher than the upper and middle, 66.50 mg/Kg, 79.00 mg/Kg respectively, and there is no statistically significant ( $p > 0.05$ ) concentration difference among them. However, these concentrations are above the maximum limit value set by the European Standards (20 mg/Kg). Compared to the result by Itanna, (2004), the concentration of Co in peacock and kera farms are 2-3 times lower than results exhibited in the middle catchment of the present investigation.

Although there is no significant difference ( $p>0.05$ ) in the concentration of copper among the catchments, the middle and upper catchments contain higher concentration, 112.67mg/Kg, 101.75 mg/Kg respectively, which are above the concentration limit set by the European standards (100 mg/Kg); the Chinese set 50 mg/Kg as the maximum acceptable concentration. The level of copper in the lower catchment is 80.00 mg/Kg. Tamiru and Alemahyehu, (2006), reported a 29mg/kg of Cu, in one of the sites of the upper catchment, Burayu farm, which is around 4 times lower than the current study.



**Figure 6. Mean concentration of heavy metals in upper, middle and lower catchments of the soil irrigated with Akaki River, Akaki River Ethiopia, 2017.**

The level of nickel in the middle catchment is 153.56 mg/Kg, which is higher than the upper and lower catchments and at the same time higher than the recommended maximum value set by the European (50 mg/Kg) and Chinese standards (40 mg/Kg). It was found that there is no statistically significant ( $p>0.05$ ) concentration difference among the three catchments. Previous study on two of the large farms that found in the middle catchment reported a lower concentration of nickel when compared to the current mean value of 153.56mg/kg.

In general, the deposition of heavy metals in farms irrigated with Akaki River seems to increase, especially on the upper area, which is usually taken as a less perturbed environment. However,

the lower catchment farms are highly impacted due to the inflow of all the rivers towards this part of the river.

#### 4.4 Trace metal in Vegetable samples irrigated with Akaki River

In this study, a total of 51, widely consumed vegetables samples, of which, 22 are Ethiopian Kale, 10 Lettuce and 14 Swiss chard, were collected and subsequently analyzed for selected heavy metals, Fe, Mn, Zn, Pb, Cr and Cd. The results are presented in Table 9.

**Table 9: Trace metal contamination in vegetable samples amended with River water, Akaki River Ethiopia, 2017**

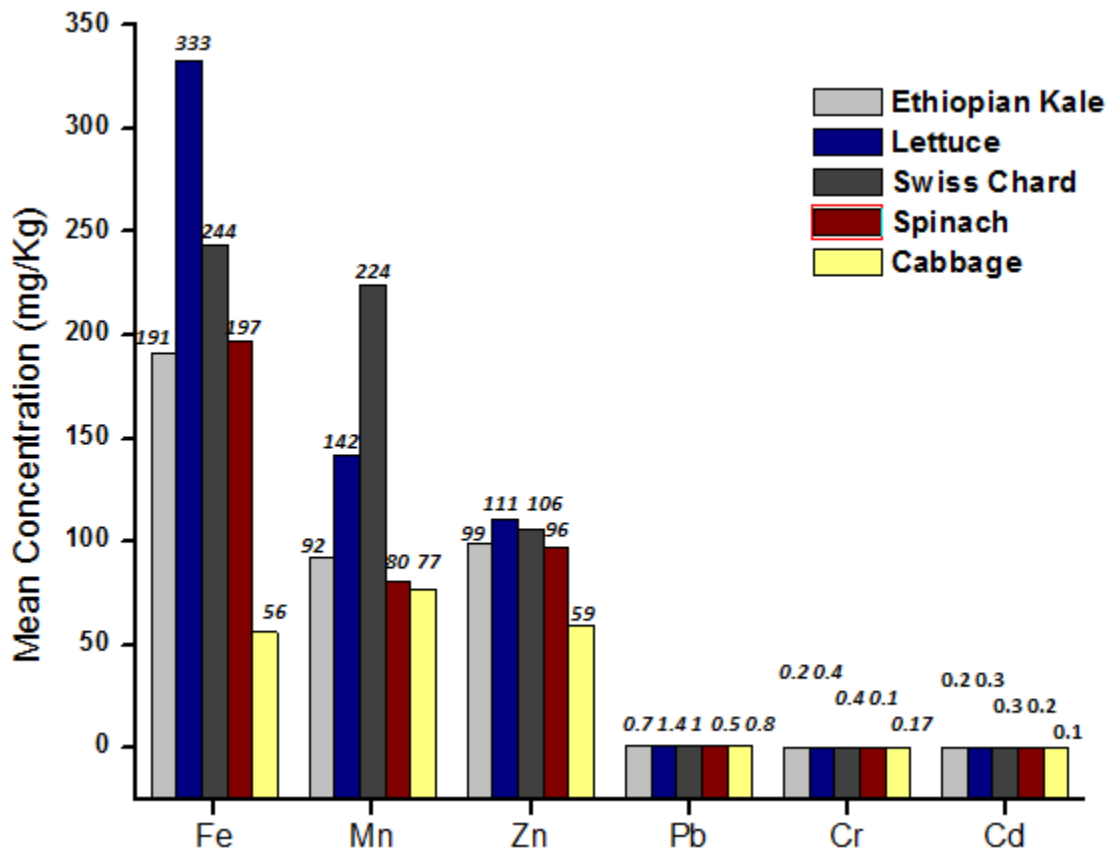
Trace metal	Statistics	Vegetable Type			Compliance %	FAO/WHO standard limit
		Ethiopian Kale	Lettuce	Swiss Chard		Mg/kg
<b>Fe</b>	Min	28.5	85.83	133.5	90.10	426
	Max	483.7	671.4	401.75		
	Mean± SD	191±26.4	333.7±55.7	244±19.61		
<b>Mn</b>	Min	11.74	13.82	71.35	100	500
	Max	466	411.5	481.1		
	Mean± SD	91.88±20.9	142.7±36.5	224.09±31.8		
<b>Zn</b>	Min	58.35	84.89	49.07	49	99
	Max	150.1	150.4	167.0		
	Mean± SD	99.87±5.93	111.2±6.45	106.11±11.1		
<b>Pb</b>	Min	0.00	0.00	0.00	17.60	0.3
	Max	2.43	3.0	2.26		
	Mean± SD	0.72±0.11	1.43±0.37	1.06±0.19		
<b>Cr</b>	Min	0.13	0.08	0.07	100	2.3
	Max	0.61	1.4	0.71		
	Mean± SD	0.28±0.03	0.45±0.14	0.45±0.06		
<b>Cd</b>	Min	0.12	0.28	0.30	100	0.2
	Max	0.41	0.57	0.50		
	Mean± SD	0.25±0.02	0.39±0.04	0.37±0.02		

FAO/WHO limits Fe in vegetables to be at a concentration of 426mg/Kg. In the current study, 90% of the vegetable samples are in compliance with this standard. The minimum accumulation was 28.5 mg/kg (near to Aba Samuel reservoir) and the maximum quantity is around 484 mg/kg (Behind Biheretsige garden). Of the accumulation, lettuce (334mg/Kg) is the highest accumulator of all, followed by Swiss chard (244mg/Kg) and E. Kale (191mg/Kg). Itanna, (2002), reported similar accumulation potential of lettuce and Swiss chard. Unlike Fe, all the vegetable samples have Mn within the standard limit. Zn was detected in all vegetable types,

where around 51% of the samples have exceeded amount of Zn when compared to standard limit of 99mg/kg. The minimum value is 58mg/kg and maximum 157mg/kg. The order of accumulation is in order of decreasing concentration, Lettuce>Swiss chard> E.Kale.

In the current study, pH of the soil was between 6.5 and 7.4(Average 6.97) at 20 cm soil depth. Soil scientists have reported that the pH was an essential factor that influenced cation mobility and regulated the solubility of the heavy metals in the soil as most of the metals tend to be available to plants in acidic pH. (Rodriguez et al., 2008). Moreover, the soil in the study area has moderate to high cation ex-changing capacity (CEC) in the range of 31.44 to 51.12 Meq/100mg. Hence, the low soil organic carbon (2.22g/kg), High CEC and a relatively neutral soil pH could lead to poor heavy metal availability to plants.

In terms of toxicity, the values of manganese and chromium found in the vegetable samples were lower than the critical values set by international guidelines and other researches. However, some of these values were high for the case of iron, lead, zinc and cadmium. For the case of lead and cadmium this will mean eating such vegetables would pose health risk. (Codex Alimentarius Commission, 2001)



**Figure 7: Comparison of Trace Metals Concentration in vegetable samples, Akaki River Ethiopia, 2017**

Heavy metals in smallholder agriculture were mostly related to the addition of organic and inorganic nutrients to the soil. The result of cluster analysis by (Nigatu et al., 2016) showed that the three major factors that contributed for heavy metal stock in soils (Contributed for more than 60% variation) were anthropogenic factors such as the flow of factory effluents to farms, addition of organic products and natural factors such as soil sediments.

In the current investigation, no significant difference was observed between the levels of iron in Ethiopian Kale irrigated across the three catchments (Kruskal-Wallis,  $n=51$ ,  $p=0.81$ ). Likewise, the spatial variability of manganese and lead was found to be not significant (Kruskal-Wallis,  $n=51$ ,  $p=0.48$ ). On the other hand, the distribution of Zinc and Chromium in Ethiopian Kale was found to be statistically significant ( $p<0.05$ ). A further statistical analysis using Mann-Whitney Test confirmed the manifestation of such difference in the concentration of zinc and chromium primarily between the upper and middle catchments. Such variation in heavy metal content of vegetable samples were attributed due to the land use types.

The distribution of heavy metals in lettuce exhibited no spatial variability across the three catchments. Table 10 shows the significance level associated with the distribution of trace metals in lettuce samples across the three catchments. In Swiss chard samples, except for chromium (Kruskal-Wallis,  $n=51$ ,  $p=0.02$ ) the other trace metals were indifferent across the catchments. A further statistical analysis using Mann-Whitney Test confirmed that the observed difference in chromium distribution was manifested between the upper and middle catchments (Mann-Whitney,  $n=51$ ,  $p=0.00$ ). As to cadmium, a significant difference ( $P<0.05$ ) was recorded between upper and middle catchment.

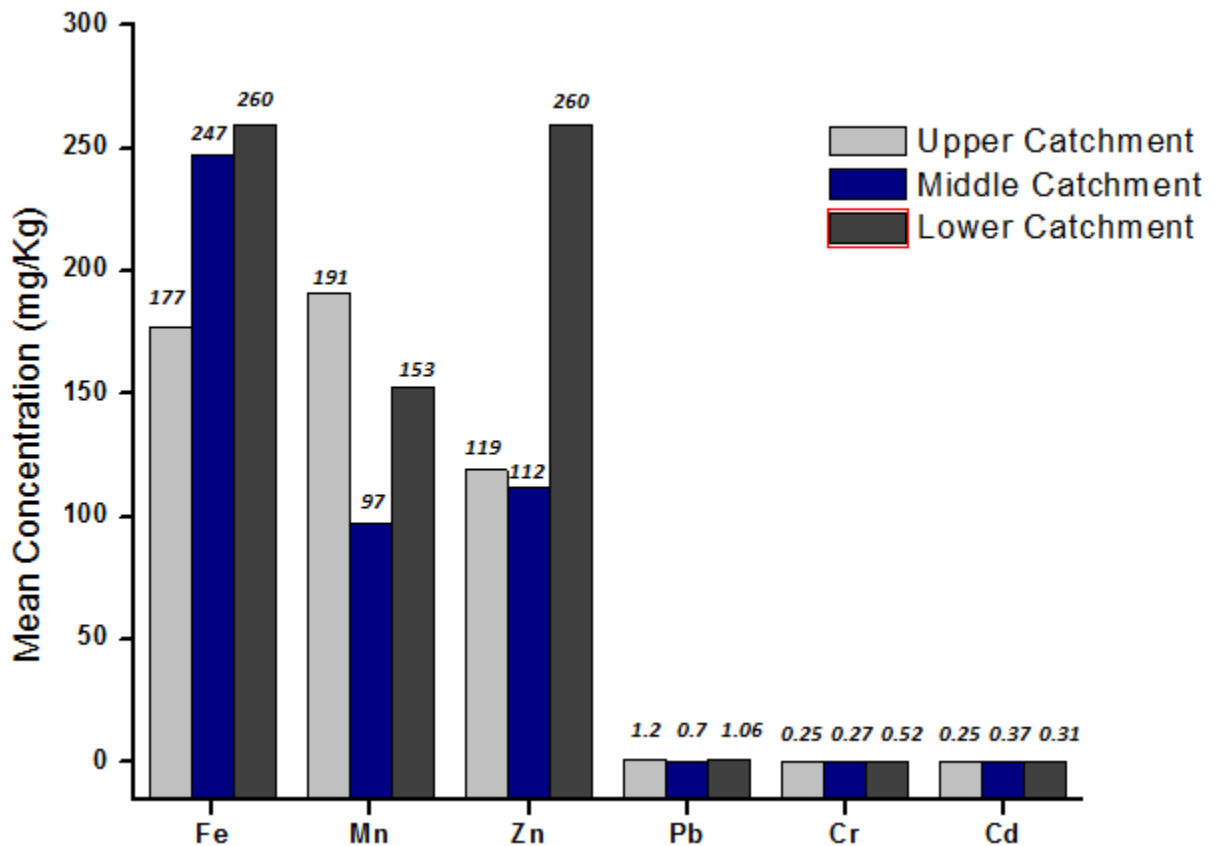
**Table 10: Distribution of trace metals in selected vegetables by catchment, Akaki River Ethiopia, 2017**

Trace Metals (mg/kg)	Catchments (Mean ± SE)			Significance level ( <i>p</i> )		
	Upper	Middle	Lower	E. Kale	Lettuce	S. Chard
Fe	260.11±30.32	247.28±34.3	177.4±30.87	0.815	0.286	0.51
Mn	153.78±23.84	97.56±18.2	191.1±48.52	0.176	0.117	0.69
Zn	260.11±30.32	112.11±5.25	119.7±9.38	0.034	0.556	0.06
Cr	0.52±0.6	0.27±0.4	0.25±0.05	0.007	0.266	0.02
Pb	1.06±0.15	0.74±0.22	1.19±0.25	0.485	0.317	0.09

Cd	0.25±0.08	0.37±0.11	0.32±0.08	0.300	0.156	0.04
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A slight decreasing pattern in mean Iron concentration was observed from upper catchment towards the lower. The distribution of manganese showed no consistent pattern across the catchments. However, the highest mean concentration of manganese was detected in lower catchment (191.1±48.52 mg/Kg) followed by upper and middle catchment respectively.

The mean zinc concentration detected at the Lower catchment was approximately twice the concentration detected at the upper and middle catchment (Figure 8). Similar pattern was observed for chromium across catchments. Lead concentration was relatively similar across the catchments.



**Figure 8: Comparison of Trace Metals Concentration of vegetable samples by Catchment, Akaki River Ethiopia, 2017**

WHO/FAO limit for Pb in vegetables is 0.3mg/kg; in the present investigation, around 83% of the samples have surpassed this limit. The highest value is 3mg/kg in samples taken from mekanisa area, which is 10 time higher than FAO’s standard. Amongst the vegetables, Lettuce was the highest accumulator (1.43mg/kg), succeeded by Swiss chard (1.06mg/kg) and the least accumulator is E.kale (0.72 mg/kg). The results are comparable to Rahlenbeck and

Zimmermann, (1999) and Itanna, (2002), Amare Hailu, (2007). The value of translocation factor as indicated in Table 11 provides that the transfer rate of the heavy metals from the soil to the plant. In general, Lettuce has the highest translocation factor, where a considerable amount of the heavy metals was transferred to the plant, followed by E.Kale and Swiss chard. Even though the concentration of cadmium is within the allowable limit, of all cadmium has the highest translocation value, which explains that cadmium that presents in the soil are uptake into the plant material considerably. On the contrary, Itanna, (1994) have reported a lower accumulation of Cadmium, which was enunciated due to the highest concentration of copper in the vegetable. In the current study, Zn has the lowest translocation factor, which could be attributed to the amendment of the soil with phosphate fertilizer, which most farmers apply.

**Table 11: Soil to plant transfer of heavy metals in three vegetable types growing with River water, Akaki River, Ethiopia 2017**

Vegetable type	Trace metal	Translocation factor
E.kale	Fe	1.82E-3
	Mn	2.8E-2
	Zn	1.7E-2
	Pb	5.1E-2
	Cr	6.9E-3
	Cd	80.6E-2
Lettuce	Fe	3.19E-3
	Mn	4.3E-2
	Zn	1.9E-2
	Pb	10.2E-2
	Cr	1.1E-2
	Cd	1.26
Swiss chard	Fe	2.3E-3
	Mn	6.8E-2
	Zn	1.81E-2
	Pb	7.6E-2
	Cr	1E-2
	Cd	1.19

#### 4.5 Microbial Contamination of Water and fresh vegetables

The present study attempted to determine the percentage of vegetable contamination with Total aerobic plate count (TAC), coliform bacteria (TCC) and faecal coliforms (FC) as well as their microbial loads through total aerobic count (TAC), total coliform counts (TCC) and fecal coliforms (FC). This study also showed the percentage of Akaki River water contamination with E. coli and non- E- E. coli.



**Table 12: Microbial quality of river water during dry season, Akaki River Ethiopia, 2017.**

Test	N	Count/10ml	Log
E-coli	26	148.85(0-320)	2.09(1-2.51)
Non-E-coli coliforms	26	>3000/ml	>3.48

Table 12 presents the microbial quality of river water in dry season campaign. The overall mean count of E.coli and Non-E.coli from water samples in the present study were 2.09 and >3.48  $\log_{10}$  CFU 10 mL<sup>-1</sup> which is higher than the WHO recommended standard (WHO, 2006). Non-E. coli level were always higher than E. coli counts, which is not surpassing since non-E. coli originate from non-fecal source such as plants and soil. According to the standard, the fecal coliform level must not exceed 1000 counts 100 mL<sup>-1</sup> for the safe use of wastewater for irrigation of vegetables.

**Table 13: Average microbial load of vegetables in Dry Season, Akaki River Ethiopia, 2017**

Test	N	Mean(min-max)	Log (min-max)
<b>Total coliform</b>	35	11088.86(10-68800)	3.22(1-5)
<b>Fecal coliform count</b>	35	132.57(10-2800)	1.37(1-3.45)
<b>Total Aerobic count</b>	35	318640(2100-6700000)	4.72(3-7)

The mean count of TC, FC, and TAC on collected vegetables irrigated with Akaki River were 3.22, 1.37, and 4.72 in dry season, and 3.87, 2.57, and 5.09  $\log_{10}$ CFU per gram in wet season, respectively. All fresh vegetables were contaminated with total coliform, fecal coliform and total aerobic in dry season.

**Table 14: Microbial load of vegetable during wet season, Akaki River Ethiopia, 2017**

Test	n	Mean colony forming unit ml (min-max)	Log (min-max)
<b>Total coliform count</b>	18	70592.78	3.87(1.9-5.71)
<b>Fecal coliform count</b>	13	<10	
<b>Fecal coliform count</b>	5	368(20-1400)	2.57(1.3-3.15)
<b>Total Aerobic count</b>	18	347066.7 (5600-2016000)	5.09(3.75-6.3)

The overall mean aerobic mesophilic count observed in this study ranged from 3-7 in dry season, and 3.75-6.3  $\log_{10}$  CFU g<sup>-1</sup> in wet season, which are in agreement with similar studies conducted in Awash River and Awetu stream in Jimma (Benti et al., 2014, Weldezigina and Muleta, 2016).

Generally, there is no specification set for the permissible level of microbes for raw food being served in Ethiopia. However, Hazard Analysis and Critical Control Points-Total Quality Management (HACCP/TQM) Technical Guidelines lay down the microbial quality for raw foods, where the food containing less than 4.4– 6.69, 6.69–7.69 and greater than 7.69 log CFU g<sup>-1</sup> (aerobic plate count) is rated as good, average, poor, and spoiled food, respectively (Aycicek et al., 2006). Based on these criteria, most of vegetables irrigated with Akaki River particularly in dry seasons fall under poor categories. This may be due to lack of dilution in dry period and farmers are not in use river water during wet season.

The mean count of total coliform and fecal coliform ranged from 1-5 and 1-3.45 log<sub>10</sub> CFU g<sup>-1</sup> in dry period, and 1.9- 5.71 and 1.3- 3.15 log<sub>10</sub> CFU g<sup>-1</sup> in wet season. However, more than 72% of vegetables collected during wet season (n=13) were not contaminated with fecal coliform. This may be related to river water dilution and as we noticed during sample collection most of irrigation, diversions were closed and the river water was not used. The high percentage of vegetables contaminated with coliform bacteria and fecal coliforms may suggest high risk of acquiring infectious diseases through the consumption of these vegetables. The occurrence of such indicator microorganisms is an indication of the contamination of the vegetables with faecal matter derived from humans and other animals (Cornish et al., 1999).

**Table 15: Microbial load in different type of vegetables in dry season, Akaki River Ethiopia, 2017.**

type of sample	N	Total coliform count (min-max)		Fecal coliform count(min-Max)		Total Aerobic plat count(min-max)	
		Count	Log	Count	Log	Count	Log
Ethiopian Kale	13	11088.86 (10-67000)	3.22 (1-4.83)	132.57 (10-2800)	1.39 (1-3.45)	318640.00 (2100-6700000)	4.72 (3.32-6.83)
Lettuce	9	9961.61 (1800-68800)	3.15 (3.28-4.84)	57.74 (10-400)	1.32 (1-2.72)	352745.16 (4800-689000)	4.75 (3.68-5.84)
Swiss Chard	11	10110.00 (100-7000)	3.17 (2-3.85)	57.10 (10-110)	1.31 (1-2.04)	330780.65 (7200-294000)	4.69 (3.86-5.49)
Cabbage & spinach	2	1100.00(500-1700)	2.96(2.7-3.23)	20 (10-30)	1.24(1-1.48)	62400.00 (30800-94000)	4.73 (4.9-4.97)

Table 15 and 16 present the mean microbial count of fresh vegetable irrigated with Akaki River in wet and dry seasons. The mean of Total coliform count, fecal coliform and total aerobic count of Ethiopian Kale were 3.22, 1.39, and 4.72 log<sub>10</sub> CFU g<sup>-1</sup> in dry; and 3.49, 2.86 and 4.97 log<sub>10</sub> CFU g<sup>-1</sup> in wet seasons respectively. On Lettuce, the mean of total coliform, fecal coliform and

total aerobic plate count were 3.15, 1.32, and 4.75 log<sub>10</sub> CFU g<sup>-1</sup> in dry and 4.01, 1.02 and 5.49- log<sub>10</sub> CFU g<sup>-1</sup> in wet seasons. While the mean of total coliform, fecal coliform and total aerobic plate count of Swish chard were 3.17, 1.31, and 4.69 log<sub>10</sub> CFU g<sup>-1</sup> in dry period, and in wet season only total coliform (4.72 log<sub>10</sub> CFU g<sup>-1</sup>), and total aerobic plate count (5.49 log<sub>10</sub> CFU g<sup>-1</sup>) were identified.

**Table 16: Microbial load in different type of vegetables during wet season, Akaki River Ethiopia, 2017**

type of sample	N	Mean Total coliform/gm (min-max)		Mean Fecal coliform/gm (min-Max)		Mean Total Aerobic count/gm (min-max)	
		Count	Log	Count	Log	Count	Log
Ethiopian Kale	9	41952.22 (80-333600)	3.49 (1.9-5.52)	885 (370-1400)	2.86 (2.57-3.15)	263622.2 (9800-928000)	4.97 (3.99-5.97)
Lettuce	4	19625 (1000-44000)	4.01 (3-4.64)	20 (10-30)	1.02 (<1-1.48)	648250 (7400-2016000)	5.49 (4.87-6.3)
Swiss Chard	2	256700 (5400-508000)	4.72 (3.73-5.71)	<10	-	323000 (238000-408000)	5.49 (5.38-5.61)
Cabbage & spinach	3	100400 (4400-291200)	4.29 (3.64-5.46)	<10	-	211866.7 (5600-604000)	4.56 (3.75-5.78)

The total coliform level recorded in this study was higher all type vegetables analyzed in both wet and dry seasons. The mean value of TC of Ethiopian kale, Lettuce, and Swish chard ranged from 1-4.83, 3.28- 4.84 and 2-3.85 in dry period and 1.9-5.52, 3-4.64, and 3.64-5.46 log<sub>10</sub> CFU g<sup>-1</sup> in wet seasons, respectively. Similarly, the mean of fecal coliform count of Ethiopian Kale, lettuce, and Swish Chard ranged from 1-3.45, 1-2.72, and 1-2.04 log<sub>10</sub> CFU g<sup>-1</sup> in dry season respectively and in wet season fecal coliform identified in Ethiopian Kale (2.57-3.15 log<sub>10</sub> CFU g<sup>-1</sup>), and Lettuce ((<1-1.48 log<sub>10</sub> CFU g<sup>-1</sup>). However, fecal coliform was <10 log<sub>10</sub> CFU g<sup>-1</sup> in Swish charge, cabbage and spinach during wet season campaign. Except swish Chard, cabbage and spinach in wet season, the mean fecal coliform values of all the vegetable samples exceed the World Health Organization (WHO) and International Commission on Microbiological Specifications for Food (ICMSF) recommended level of 10<sup>3</sup> fecal coliform g<sup>-1</sup> fresh weights in both dry and wet season campaigns (ICMSF, 1998, Blumenthal, 2000). This may be due to the Akaki River water, which is higher than the WHO recommended standard used for irrigation of vegetables particularly in dry season that flows from upstream through town's major industrial, commercial, institutional and residential, taking in a whole burden of all types of raw effluent. In addition to this, application of organic manures is common practices of farmer for production of crops in that area.

## 4.6 Risk of Contamination and farmers health

The daily exposure of farmers to selected heavy metals through ingestion and inhalation pathways is presented in table 16. The total chronic daily intake of heavy metals is higher in female farmers ( $4.80e-4$ ) than male farmers ( $6.10e-4$ ). The intake of lead and chromium through ingestion of vegetable is higher than intake through inhalation. Even though, it is lower than ingestion, intake through inhalation proves that farmers in the urban and peri-urban areas of Addis Ababa are at risk of occupational exposure to heavy metals. The intake of heavy metal via inhalation is in the order of  $Ni > Co > Cr > Pb$ .

**Table 17: Chronic daily intake of heavy metals through ingestion of vegetables and inhalation of soil particulates, Akaki River Ethiopia, 2017**

Receptor	Ingestion mg/kg/day		Inhalation				Total chronic daily intake
	Pb	Cr	Pb	Cr	Co	Ni	
<b>Male Farmer</b>	$3.5e-4$	$1.3e-4$	$8.6e-10$	$3.7e-8$	$1.13e-7$	$1.71e-7$	$4.80e-4$
<b>Female Farmer</b>	$4.4e-4$	$1.7e-4$	$1.08e-9$	$4.66e-8$	$1.4e-7$	$2.12e-7$	$6.10e-4$

When the non-cancer risk value is less than 1, there is no health risk to the farmers, but if these values exceed 1, there may be concerns of potential non-carcinogenic effects. When analyzed, the risk quotient for each metal in each exposure pathways are less than one, which is an indication that the heavy metals do not impart non-carcinogenic effect independently. However, in real life scenario farmers are exposed to many chemicals at the same time; thus, the action of the chemicals could be additive, non-additive or synergistic. In many cases, two or more substances may act at the same target organ and that can be additive or non-additive or it may even be very complex interaction of two or more chemicals that act at different, but related targets. In extreme cases there may be synergistic effects, in that case the effects of two substances together are greater than the sum of individual effects. However, our analysis assumes only an additive effect of chemicals. According to US EPA, the hazard index can be accurate when used with mixture of components that have similar toxic action (US EPA, 1986). The sum of hazard quotient, which is aggregated as a total non-cancerous health risk, is greater than one for both male and female farmers (table 18). This result explains that the non-cancerous health risks associated with the consumption and inhalation of Pb, Cr, Co and Ni would be perhaps those outlined in table 18.

**Table 18: Non-cancerous risk of heavy metals through ingestion and inhalation of soil particulates, Akaki River Ethiopia, 2017**

Receptor	Ingestion		Inhalation				Total non-cancerous risk
	Pb	Cr	Pb	Cr	Co	Ni	
Male Farmer	0.82	0.045	5.79e-7	0.19	1.88e-2	8.56e-3	1.08
Female Farmer	1.02	0.06	2.57e-8	1.14e-9	1.42e-8	1.25e-7	1.08

**Table 19: Selected heavy metals and their potential health concern identified in polluted water and soil, Akaki River Ethiopia, 2017**

Heavy metals	Potential health effects	References
Chromium	Lung and skin damage, Cancer	National research council, 1998; WHO,1999; WHO,1992; WHO, 1991; WHO,1989; WHO, 2006; ATSDR,2000,
Lead	Nervous and immune system and kidney damage, memory deterioration	
Nickel	Brain and kidney damage, embryo/fetotoxic, lung, brain, kidney, liver, spleen and skin damage, cancer	
Cobalt	Allergy skin, bronchial asthma	

The tolerable standard for cancer risk is in between  $10^{-6}$  and  $10^{-4}$ ; beyond this range, the exposed group is expected to be at risk of cancer. According to New York State Department of Health (NYSDOH, 2007) the total cancer risk ranges are described as, follows: if total cancer risk  $\leq 10^{-6}$  = Low;  $10^{-4}$  to  $10^{-3}$  = moderate;  $10^{-3}$  to  $10^{-1}$  = high;  $\geq 10^{-1}$  = very high. In the current investigation both male and female farmers, have exhibited low cancer risk, which is  $6.53 \times 10^{-5}$  and  $3.9 \times 10^{-6}$  respectively. Thus, farmers working on the river Akaki catchment are not at risk of any kind of cancer risk when exposed to Pb, Cr, Co, and Ni through ingestion and inhalation exposure pathways combined. However, this does not diminish the future risk that is posed, as the pollution index is increasing exponentially, with the relentless disposal of liquid waste from domestic and industrial establishment, with inadequate or no treatment of their disposal. In addition, the awareness of farmers on the possible health risk associated with working on polluted river and soil seems lower as they are not following any precautionary measures to minimize the risk of health problem. Almost all farmers have inadequate or no personal protective equipment (PPE), where most of them do their daily activities with bare foot or without glove and mouth

cover, that chronic intoxication through dermal exposure of the lower and upper extremities to polluted water and soil is high.

**Table 20: cancerous risk of heavy metals through ingestion and inhalation of soil particulates, Akaki River Ethiopia, 2017**

Receptor	Ingestion		Inhalation				Total cancerous risk
	Pb	Cr	Pb	Cr	Co	Ni	
Male Farmer	2.56e-6	6.01e-5	3.28e-11	1.39e-6	9.92e-7	2.62e-7	6.53e-5
Female Farmer	1.69e-7	4.47e-7	4.07e-11	1.72e-6	1.23e-6	3.25e-7	3.89e-6

Moreover, there is one important exposure pathway, which is not considered in the present risk estimation protocol, that is, dermal exposure of farmers to pollutants carried out through the soil and water compartment used for the irrigation. During working hours, it is evident, that different body parts of farmers, forearms, hands, lower legs and feet of the farmers are continuously immersed into the polluted soil and water matrices. Since this report is a preliminary assessment, the dermal exposure was not included; however, had it been included, the cancer risk would have been increased.

## 5. Conclusion

It has been evident from our findings that, the water quality of the Akaki River shows pattern of behavior linked to anthropogenic sources with the intensity of human pressure associated with industrial effluent, domestic wastes and agricultural activities.

The assessment of macroinvertebrate taxa provides a clue what happens in Akaki River and the water quality effect on species diversity. Therefore, all macroinvertebrate indices along with human disturbance and poor habitat quality suggests that both little and Great Akaki streams are severely modified by human influences and it needs immediate restoration and rehabilitation tasks.

The Akaki River was shown to be heavily contaminated with non-E. coli & E. coli coliforms and did not meet the WHO guideline criteria for safe irrigation. Target microorganisms commonly used as indicators for the hygiene status of foods frequently exceeded the HACCP TQM and ICMSF limit values for safe consumption. This indicates that the presence of these organisms on produce might be due to a transfer from fecally contaminated irrigation water, which might place consumers at risk. Consequently, we showed that fresh produced vegetables (especially Ethiopian Kale, lettuce, Cabbage, and swish chard) might contain pathogenic microorganisms and represent a risk for consumers regarding foodborne disease.

Vegetable farms in and around Addis Ababa, which were irrigated with contaminated waters exhibited increased concentrations of metals both in the soils and in the vegetables grown on them. Nevertheless, it was noticed that different vegetables accumulate and translocate variable amounts of metals from the soil into their tissues. Without regard to bioavailability, the vegetables lettuce, Ethiopian kale, and Swiss chard grown in these farms showed Cd at levels that could raise health risk concerns to consumers. However, zinc has the lowest translocation factors in all vegetables analyzed. The findings dictate immediate need for measures to protect the safety of consumers and the general environment.

## 6. Recommendations

Akaki River is found to be under high impact and is impaired. On the other hand, the river water is used for a variety of purposes such as irrigation, cattle drinking and domestic purposes without prior treatment. For sustainable management of this water resource, environmental protection agencies at different levels and other concerned administrative and/or nongovernmental bodies should take strict as well as technical measures. Enforcement of law and propagating environmental education to the community with special target to those contributors of the present degradation could be one solution. Providing different advantages such as taxation, cooperativeness and market value for those industrial firms with treatment plant and good environmental management could be another option. It also necessitates avoiding establishment of additional industries near the river. Continuous monitoring using parameters such as those used in this study should be employed to assess timely status of the system.

This study in the Addis Ababa urban area revealed two biologically highly stressed rivers impacted primarily by physical habitat degradation and both point and nonpoint pollution. The low macroinvertebrate composition was liable to physical habitat destruction and poor chemical water quality. This calls the responsible authorities need to take urgent ameliorative and preventive measures to improve the ecological integrity of these rivers as part of efforts to restore their ecology and reduce public health risks within the urban area and downstream river stretches. The untreated and inadequately treated effluents from industries have a considerable effect on the water quality of the receiving water bodies. The levels of most parameters monitored were generally higher in the discharge point of the rivers. For instance, high EC value was recorded around Winget where effluent of industrial wastes joins the stream. Thus, cause many fold increase at downstream of both Little and Great Akaki rivers. This study suggests that there is a need of remediation of the rivers. There should also be an intervention of appropriate regulatory bodies to ensure production of high quality treated final effluents by all industries and protect the natural surface waters quality.

The mean fecal coliform values of Ethiopian kale and Lettuce vegetables exceed the World Health Organization (WHO) and International Commission on Microbiological Specifications for Food (ICMSF) recommended level of  $10^3$  fecal coliform g<sup>-1</sup> fresh weights in both dry and wet season campaigns. Such findings warn the importance of adequate measures throughout the farm-to-table food chain should be emphasized. Therefore, this calls for farmer's and consumer's



awareness on the dangers of contacting with Akaki River water and consuming pathogen contaminated vegetables and the need to insist on properly processed/stored sliced produce needs to be re-awakened. In addition, to minimize potential risks associated with river water irrigation, the proper use of river water as well as cheap and efficient methods to reduce microbial loads in microbially contaminated water used for irrigation needs to be implemented.

In general, to reduce water pollution in Addis Ababa, the following measures should be taken:

Construction of low cost sewerage lines and proper management of wastewater,

Properly control industrial effluents discharges and implementing industrial park principles,

Should be developed a robust solid waste collection and management systems,

Establishment of proper landfilling facilities

Constructing adequate public latrines that help to attain open defecation free areas in the city

Enhancing the environmental awareness of the population,

Empowering the local authorities to take an active participation in waste disposal regulations and its implementations

The systematic application of the aforementioned controlling mechanisms could cut the surface water pollution of the Akaki River and its tributaries.

## Reference

- ADMASU, T. 2007. ASSESSMENT OF BIOLOGICAL INTEGRITY USING PHYSICO-CHEMICAL PARAMETERS AND MACROINVERTEBRATE COMMUNITY INDEX ALONG SEBETA RIVER, ETHIOPIA.
- ALEMAYEHU, T. 2001. The impact of uncontrolled waste disposal on surface water quality in Addis Ababa, Ethiopia. *SINET: Ethiopian Journal of Science*, 24, 93-104.
- AYCICEK, H., OGUZ, U. & KARCI, K. 2006. Determination of total aerobic and indicator bacteria on some raw eaten vegetables from wholesalers in Ankara, Turkey. *International Journal of Hygiene and Environmental Health*, 209, 197-201.
- BARBOUR, M. T., GERRITSEN, J., SNYDER, B. D. & STRIBLING, J. B. 1999. *Rapid Bioassessment Protocols For Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates, and Fish*, Washington, DC 20460, EPA 841-B-99-002.
- BENTI, G., KEBEDE, A. & MENKIR, S. 2014. Assessment of bacteriological contaminants of some vegetables irrigated with Awash River water in selected farms around Adama town, Ethiopia. *Journal of Microbiology and Antimicrobials*, 6, 37-42.
- BEYENE, A., ADDIS, T., KIFLE, D., LEGESSE, W., KLOOS, H. & TRIEST, L. 2009. Comparative study of diatoms and macroinvertebrates as indicators of severe water pollution: Case study of the Kebena and Akaki rivers in Addis Ababa, Ethiopia. *Ecological Indicators*, 9, 381-392.
- BLUMENTHAL, U. J., MARA, D.D., PEASEY, A., RUIZ-PALACIOS, G. AND STOTT, R 2000. Guidelines for the microbiological quality of treated wastewater used in agriculture: recommendations for revising WHO guidelines. *Bulletin of the World Health Organization*, 78, 1104-1116.
- CCME 2001. Canadian Environmental Quality Guidelines. In: ENVIRONMENT, C. C. O. M. O. T. (ed.). Canada: CEQG.
- CORNISH, G., MENSAH, E. & GHESQUIRE, P. 1999. Water quality and peri-urban irrigation: An assessment of surface water quality for irrigation and its implications for human health in the peri-urban zone of Kumasi, Ghana.
- CSA 2010. REPORT ON LARGE AND MEDIUM SCALE MANUFACTURING AND ELECTRICITY INDUSTRY SURVEY. *Annual Report*. Addis Ababa, Ethiopia: FDRE-CSA.
- DEMLIE, M. 2015. Assessment and estimation of groundwater recharge for a catchment located in highland tropical climate in central Ethiopia using catchment soil–water balance (SWB) and chloride mass balance (CMB) techniques. *Environmental Earth Sciences*, 74, 1137-1150.
- DEMLIE, M. & WOHNLICH, S. 2006. Soil and groundwater pollution of an urban catchment by trace metals: case study of the Addis Ababa region, central Ethiopia. *Environmental geology*, 51, 421-431.
- DEMLIE, M., WOHNLICH, S., GIZAW, B. & STICHLER, W. 2007. Groundwater recharge in the Akaki catchment, central Ethiopia: evidence from environmental isotopes ( $\delta^{18}\text{O}$ ,  $\delta^2\text{H}$  and  $^3\text{H}$ ) and chloride mass balance. *Hydrological processes*, 21, 807-818.
- GABRIELS, W., LOCK, K., DE PAUW, N. & GOETHALS, P. L. 2010. Multimetric Macroinvertebrate Index Flanders (MMIF) for biological assessment of rivers and lakes in Flanders (Belgium). *Limnological-Ecology and Management of Inland Waters*, 40, 199-207.
- HILSENHOFF, W. L. 1988. Rapid field assessment of organic pollution with a family level Biotic Index. *J. N.Am.Benthol.Sco.*, 65-68.
- ICMSF 1998. *Microbial Ecology of Food Commodities*, Blackie Academic & Professional.
- KUMIE, A. & KLOOS, H. 2006. Occupational health and industrial pollution. *The epidemiology and ecology of health and disease in Ethiopia*, 171-195.
- MANDAVILLE, S. M. 2002. Benthic Macroinvertebrates in Freshwaters Taxa Tolerance Values, Metrics, and Protocols. New York: Soil & Water Conservation Society of Metro Halifax.
- MDEP, M. D. O. E. P. 2009. Quality assurance project plan for biological monitoring of Maine's rivers, streams, and freshwater wetlands. Augusta Maine: Bio-Monitoring Program, QAPP Bureau of Land and Water Quality.

- MELAKU, S., WONDIMU, T., DAMS, R. & MOENS, L. 2004. Simultaneous determination of trace elements in Tinishu Akaki River water sample, Ethiopia, by ICP-MS. *Canadian journal of analytical sciences and spectroscopy*, 49, 374-384.
- MELAKU, S., WONDIMU, T., DAMS, R. & MOENS, L. 2007. Pollution status of Tinishu Akaki River and its tributaries (Ethiopia) evaluated using physico-chemical parameters, major ions, and nutrients. *Bulletin of the chemical society of Ethiopia*, 21.
- REEVE, R. N. 2002. *Introduction to environmental analysis*, John Wiley & Sons.
- SMITH, B. & WILSON, J. B. 1996. A consumer's guide to evenness indice. *OIKOS* 70-82.
- TIGABU, T. & SEMU, G. 2008. Ethiopia; Addis Ababa urban profile. UN HABITAT, regional and technical cooperation division. UNON publishing services section, Nairobi. <http://www.unhabitat.org>.
- TURKMEN, G. & KAZANCI, N. 2010. Applications of various diversity indices to benthic macroinvertebrate assemblages in streams of a natural park in Turkey. *BALWOIS*.
- VEGA, M., PARDO, R., BARRADO, E. & DEBÁN, L. 1998. Assessment of seasonal and polluting effects on the quality of river water by exploratory data analysis. *Water Research*, 32, 3581-3592.
- WELDEGEBRIEL, Y., CHANDRAVANSI, B. S. & WONDIMU, T. 2012. Concentration levels of metals in vegetables grown in soils irrigated with river water in Addis Ababa, Ethiopia. *Ecotoxicology and Environmental Safety*, 77, 57-63.
- WELDESILASSIE, A. B., BOELEEE, E., DRECHSEL, P. & DABBERT, S. 2011. Wastewater use in crop production in peri-urban areas of Addis Ababa: impacts on health in farm households. *Environment and Development Economics*, 16, 25-49.
- WELDEZGINA, D. & MULETA, D. 2016. Bacteriological contaminants of some fresh vegetables irrigated with Awetu River in Jimma Town, Southwestern Ethiopia. *Advances in Biology*, 2016.
- WHO 2006. *Guidelines for the safe use of wastewater, excreta and greywater*, Geneva, Switzerland, World Health Organization.

## Annex

### Annex 1: Sampling Site code with coordinator

SN	Sampling site code	Literal name	GPS Coordinator		Status
			Easting	Northing	
1	LA01	Around Fenance,	0469057	1000164	Upper
2	LA02	Winget	0468233	1000816	Upper
3	LA03	Burayu Kera	0464616	1001769	Upper
4	LA04	Gefersa, head site	0461823.7	0999517.2	Upper
5	LA 05	Lomi Meda, on bridge road to	0466537	0998805	Upper
6	LA06	Back of Coca cola factory	04669563	0996814	Upper
7	LA07	Mekanissa-Teklehaymanot	0468928	0992721	Upper
8	LA08	Mekanisa area below national Liquor	0470579	0991730	Middle
9	LA09	Kera A.A abattoir	0472025	0993284	Middle
10	LA10	Behind Beheretsige	0472845	0989325	Middle
11	LA11	Bellow Shitu (waste water treatment) under main bridge	0472024.9	0984218.5	Lower
12	GA01	Yeka Abado	0484714	1001894	Upper
13	GA02	Tinziz wonze	0477876	1001634	Upper
14	GA03	Ras mekonen dildiye	0473129	0999106	Upper
15	GA04	Kebena	0475716	0998311	Middle
16	GA05	Meles Foundation	0475770.7	0997469.3	Middle
17	GA06	St Estifanos church	0474123	0996041	Middle
18	GA07	St Urael church	0475425	0995959	Middle
19	GA08	Atlas down to St. Ureal Church	0475661	0995304	Middle
20	GA09	Atlas bellow former A.A Health Bureau	0475296	0995505	Middle
21	GA10	Bole road under the bridge"- Civil service	0475443	0993912	Middle
22	GA11	Woro Gono	0481029	0992253	Upper
23	GA12	Ketime	0480397	0987814	Upper
24	GA13	St Arsema church	0475725	09998724	Middle
25	GA14	Turneshi Bejing Hospital	0476188	0980678	Lower
26	GA15	Akaki Beseka	0474920	0979731	Lower
27	ASR	Aba Samuel, downstream	0532393.7	0971378.4	Lower

## Annex 2: HABITAT ASSESSMENT FIELD DATA SHEET—Akaki River research Project

STREAMNAME		LOCATION	
STATION # _____ RIVERMILE _____		STREAMCLASS	
LAT _____ LONG _____		RIVER BASIN	
STORET #		AGENCY	
INVESTIGATORS			
FORM COMPLETED BY		DATE _____ TIME _____ AM PM	REASON FOR SURVEY

	Habitat Parameter	Condition Category			
		Optimal	Suboptimal	Marginal	Poor
Parameters to be evaluated in sampling reach	<b>1. Epifaunal Substrate/ Available Cover</b>	Greater than 50% 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 <u>not</u> new fall and not transient).	30-50% 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).	10-30% mix of stable habitat; habitat availability less than desirable; substrate frequently disturbed or removed.	Less than 10% stable habitat; lack of habitat is obvious; substrate unstable or lacking.
	<b>SCORE</b>	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
	<b>2. Pool Substrate Characterization</b>	Mixture of substrate materials, with gravel and firm sand prevalent; root mats and submerged vegetation common.	Mixture of soft sand, mud, or clay; mud may be dominant; some root mats and submerged vegetation present.	All mud or clay or sand bottom; little or no root mat; no submerged vegetation.	Hard-pan clay or bedrock; no root mat or vegetation.
	<b>SCORE</b>	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
	<b>3. Pool Variability</b>	Even mix of large-shallow, large-deep, small-shallow, small-deep pools present.	Majority of pools large-deep; very few shallow.	Shallow pools much more prevalent than deep pools.	Majority of pools small-shallow or pools absent.
	<b>SCORE</b>	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
	<b>4. Sediment Deposition</b>	Little or no enlargement of islands or point bars and less than <20% of the bottom affected by sediment deposition.	Some new increase in bar formation, mostly from gravel, sand or fine sediment; 20-50% of the bottom affected; slight deposition in pools.	Moderate deposition of new gravel, sand or fine sediment on old and new bars; 50-80% 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 80% of the bottom changing frequently; pools almost absent due to substantial sediment deposition.
	<b>SCORE</b>	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
	<b>5. Channel Flow Status</b>	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.
	<b>SCORE</b>	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0

**HABITAT ASSESSMENT FIELD DATA SHEET—LOW GRADIENT STREAMS (BACK)**

Habitat Parameter	Condition Category																				
	Optimal					Suboptimal					Marginal					Poor					
<b>6. Channel Alteration</b>	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.					
<b>SCORE</b>	20	19	18	17	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1	0
<b>7. Channel Sinuosity</b>	The bends in the stream increase the stream length 3 to 4 times longer than if it was in a straight line. (Note - channel braiding is considered normal in coastal plains and other low-lying areas. This parameter is not easily rated in these areas.)					The bends in the stream increase the stream length 1 to 2 times longer than if it was in a straight line.					The bends in the stream increase the stream length 1 to 2 times longer than if it was in a straight line.					Channel straight; waterway has been channelized for a long distance.					
<b>SCORE</b>	20	19	18	17	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1	0
<b>8. Bank Stability (score each bank)</b>	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						
<b>9. Vegetative Protection (score each bank)</b>	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.					
Note: determine left or right side by facing downstream.																					
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						
<b>10. Riparian Vegetative Zone Width (score each bank riparian zone)</b>	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						

Parameters to be evaluated broader than sampling reach

**Total Score** \_\_\_\_\_

### Annex 3: Human Disturbance Ranking Form-Akaki River Project

Station #: \_\_\_\_\_ Date: \_\_\_\_\_ Time: \_\_\_\_\_ Town: \_\_\_\_\_

Evaluator(s): \_\_\_\_\_ Name of river or waterbodies: \_\_\_\_\_

For each wetland station assessed, score all potential factors in the five categories below using the following scale:

Not Observed	Minimal Disturbance	2	Moderate Disturbance	4	Severe Disturbance
0	1		3		5

The purpose of this ranking is to characterize the degree of human disturbance at a given waterbodies biomonitoring 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.)

<b>1. Hydrologic Modifications to Wetland</b>	<b>Observations/Comments</b>	<b>Score</b>	<b>Section 1 Subtotal:</b>
man-made dikes or dams			
causeways, roads or railroad bed crossings which impede water flow; inadequate or obstructed culverts			
ditching, draining or dewatering			
filling or bulldozing			
Other hydrologic modifications not included in this section (specify).			
<b>2. Vegetative Modifications to Wetland</b>	<b>Observations/Comments</b>	<b>Score</b>	<b>Section 2 Subtotal:</b>
timber harvesting in wetland			
Other clearing/removal of vegetation (roads, utility lines etc.)			
plowing, mowing or grazing in riverbank			
evidence of herbicide use in near to waterbodies			
Other vegetative modifications not included in this section (specify).			
<b>3. Evidence of Chemical Pollutants</b>	<b>Observations/Comments</b>	<b>Score</b>	<b>Section 3 Subtotal:</b>
discharge pipes			
oil, petroleum, chemicals observed, or chemical odor present			
soil staining and/or stressed or dying vegetation			
trash, chemical containers, demolition debris, drums, etc.			
Other evidence of chemical pollutants not included in this section (specify).			
<b>4. Impervious Surface in Watershed</b>	<b>Observations/Comments</b>	<b>Score</b>	<b>Section 4 Subtotal:</b>
residential development			
commercial/industrial development			
recreational development (campgrounds, picnic or boat launch areas, trails, docks, boardwalks, parking areas, etc.)			
additional roads, highways bridges			

Other impervious surfaces not included in this section (specify).			
<b>5. Potential for NPS Pollution</b>	<b>Observations/Comments</b>	<b>Score</b>	<b>Section 5 Subtotal:</b>
excess sediment accumulation or unstable/eroding soil from human activities (roads, construction or excavation sites, agriculture, forestry activities, etc.) observed			
alterations to wetland buffer (within 100 feet of wetland edge)			
livestock, feedlots or manure piles			
evidence of fertilizer or pesticide use (lawns, golf courses, agricultural crops, etc.)			
Other NPS sources not included in this section (specify).			
<b>Additional notes</b> (if needed):			<b>Total:</b>

#### Annex 4: Moldova standard for Surface water

Parameter (group)	Acronym	Unit	Use Class I	Use Class II	Use Class III	Use Class IV	Use Class V
Dissolved oxygen	O2	[mg O2/l]	≥7 (or BG)	≥7	≥5	≥4	<4
Biochemical oxygen demand (5 days)	BOD5	[mg O2/l]	3 (or BG)	5	6	7	>7
Chemical oxygen demand,	COD	[mg O2/l]	<7 (or BG)	7	15	20	>20
Nitrate	NO3	[mg /l]	4.43 (or BG)	13.3	24.8	50	>50
Nitrite	NO2	[mg /l]	0.033 (or BG)	0.2	0.4	1	>1
Ammonium	NH4	[mg /l]	0.3 (or BG)	0.5	1	4	>4
Ortho-phosphates	PO4	[mg /l]	0.2 (or BG)	0.3	0.6	1.5	>1.5
Chloride	Cl-	[mg/l]	200 (or BG)	200	350	500	>500
Sulphates	SO4	[mg/l]	<250 (or BG)	250	350	500	>500
pH	pH	[-]	6.5-9.0	6.5-9.0	6.5-9.0	6.5-9.0	<6.5 or >9.0
Total iron	Fetot	[mg/l]	<1 (or BG)	1	3	5	>5
Manganese	Mn	[mg/l]	<0.1 (or BG)	0.1	1	2	>2
Cadmium total (SS= 30 mg/l)	Cdtot	[µg/l]	<1 (or BG)	1	5	5	>5



Lead total (SS= 30 mg/l)	Pbtot	[µg/l]	<50 (or BG)	50	50	50	>50
Nickel total (SS= 30 mg/l)	Nitot	[µg/l]	10 (or BG)	25	50	100	>100
Copper total (SS= 30 mg/l)	Cutot	[µg/l]	<50 (or BG)	50	100	1000	>1000
Zinc total (SS= 30 mg/l)	Zntot	[µg/l]	<300 (or BG)	300	1000	5000	>5000
Coliforms total		[№/100 ml]	500	5,000	10,000	50,000	>50,000
Coliforms faecal		[№/100 ml]	100	2,000	10,000	20,000	>20,000
Escherichia coli		[cfu/100 ml]	<500	500	1,000	>1,000	>1,000

### Annex 5: Quality elements and physico-chemical quality standards for assessment of ecological status of surface water in Romania, 2006 (GD 161)

Parameter	Acronym	Unit	I	II	III	IV	V
Water Temp.		°C					
pH			6.5 – 8.5				
1	Dissolved oxygen (DO)	mg O2/l	9	7	5	4	<4
3	BOD5	mg O2/l	3	5	7	20	>20
4	COD – Mn	mg O2/l	5	10	20	50	>50
5	COD-Cr	mg O2/l	10	25	50	125	>125
<b>C.3. Nutrients</b>							
1	Ammonia (NH4+)	mg /l	0.52	1	1.55	4.13	>4.13
2	Nitrites (N-NO2-)	mg /l	0.03	0.1	0.2	1	>1
3	Nitrates (N-NO3-)	mg /l	4.43	13.29	24.8	50	>50
4	Total Nitrogen (TN)	mg N/l	1.5	7	12	16	>16
5	Orthophosphates (P-PO43-)	mg /l	0.31	0.61	1.23	0.58	>0.58
<b>C.4. Salinity</b>							
1	Conductivity	µS/cm					
2	Total residue at 105 °C	mg/l	500	750	1000	1300	>1300

3	Chlorides (Cl <sup>-</sup> )	mg/l	25	50	250	300	>300
4	Sulphates (SO <sub>4</sub> <sup>2+</sup> )	mg/l	60	120	250	300	>300
5	Calcium (Ca <sup>2+</sup> )	mg/l	50	100	200	300	>300
6	Magnesium (Mg <sup>2+</sup> )	mg/l	12	50	100	200	>200
7	Sodium (Na <sup>+</sup> )	mg/l	25	50	100	200	>200
<b>Specific toxic pollutants</b>							
1	Chromium total (Cr <sup>3+</sup> + Cr <sup>6+</sup> )	µg/l	25	50	100	250	>250
2	Copper (Cu <sup>2+</sup> ) <sup>5</sup>	µg/l	20	30	50	100	>100
3	Zinc (Zn <sup>2+</sup> )	µg/l	100	200	500	1000	>1000
4	Arsenic (As <sup>3+</sup> )	µg/l	10	20	50	100	>100
10	Barium (Ba <sup>2+</sup> )	mg/l	0.05	0.1	0.5	1	>1
5	Selenium (Se <sup>4+</sup> )	µg/l	1	2	5	10	>10
6	Cobalt (Co <sup>3+</sup> )	µg/l	10	20	50	100	>100
7	Lead (Pb) <sup>6</sup>	µg/l	5	10	25	50	>50
8	Cadmium (Cd)	µg/l	0.5	1	2	5	>5
8	Total iron (Fe <sup>2+</sup> + Fe <sup>3+</sup> )	mg/l	0.3	0.5	1.0	2	>2
9	Mercury (Hg) <sup>6</sup>	µg/l	0.1	0.3	0.5	1	>1
9	Manganese total (Mn <sup>2+</sup> + Mn <sup>7+</sup> )	mg/l	0.05	0.1	0.3	1	>1
10	Nickel (Ni) <sup>5</sup>	µg/l	10	25	50	100	>100

**Annex 6: Threshold and guideline values for metals in soils** (extract; MEF, 2007).

<b>Metal</b>	<b>Mg/KG</b>
Cadmium(Cd)	1
Cobalt(Co)	20
Chrome(Cr)	100
Copper(Cu)	100
Lead(Pb)	60
Nickel(Ni)	50
Zinc(Zn)	200