WET COFFEE PROCESSING AGRO-INDUSTRY WASTEWATER TREATMENT POTENTIAL OF CONSTRUCTED AND NATURAL WETLANDS : THE CASE OF KEGE PROCESSING PLANT IN SIDAMA NATIONAL REGIONAL STATE, ETHIOPIA

PHD DISSERTATION

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A DISSERTATION SUBMITTED TO THE DEPARTMENT OF BIOLOGY,
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ADVISORS' APPROVAL SHEET

This is to certify that the PhD Thesis entitled "**WET COFFEE PROCESSING AGRO-INDUSTRY WASTEWATER TREATMENT POTENTIAL OF CONSTRUCTED AND NATURAL WETLANDS: THE CASE OF KEGE PROCESSING PLANT IN SIDAMA REGIONAL STATE, ETHIOPIA**" submitted in partial fulfillment of the requirements for the **Ph.D** in Environmental Toxicology, the Graduate Program of the **Department/School of Biology** and has been carried out by **Yohannes Seifu Berego** Id. No **PhDEnTo/0006/11**, under our supervision. Therefore we recommend that the student has fulfilled the requirements and hence hereby can submit the Ph.D Thesis to the department.

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DECLARATION

I hereby declare that this PhD dissertation is my original work and has not been presented for a degree in any other University , and all sources of materials used for this dissertation have been duly acknowledged

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BIOGRAPHICAL SKETCH

Yohannes Seifu is a PhD student in Environmental Toxicology at Hawassa University, Faculty of Biological Science, and Department of Biology. He received a bachelor’s degree in Medical Laboratory Technology in 2010 from Hawassa University and a master’s degree in Ecotoxicology and Environmental Health in 2017 from Hawassa University. And also he obtained a master’s degree in General masters of Public Health in 2019 from Hawassa University. He is interested in Environmental Toxicology, Ecotoxicology, Heavy Metals, Environmental Risk Assessment, Water Treatment, Wastewater Treatment, and Waste Management.

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LIST OF ABBREVIATIONS AND ACRONYMS

ANOVA	Analysis of Variance
BCF	Bio-Concentration Factor
C deg	Contamination Degree
CF	Contamination Factor
DWFEDO	Dale Woreda Finance and Economic Development Office
ERi	Potential Ecological Risk Factor
ICO	International Coffee Organization
ICS	Institute of Coffee Studies
Igeo	Geo Accumulation
EPA	Environmental Protection Authority
FAAS	Flame Atomic Absorption Spectroscopy
FAO	Food and Agricultural Organization
mCdeg	Modified Contamination Degree
PLI	Pollution Load Index
RCTs	Randomized Controlled Trials
RI	Potential Ecological Risk Index
SPSS	Statistical Package for Social Sciences
SRSEPA	Sidama Regional State Environmental Protection Authority
WHO	World Health Organization

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ABSTRACT

Constructed wetlands are engineered to use natural processes to remove pollutants from contaminated water in a more controlled environment, using phytoremediators for effective wastewater treatment.The aim of this study was to establish the coffee berries processing Agro-Industry Wastewater Treatment Potential of Constructed and Natural Wetlands.The finding indicates that calcium had the highest concentration (1355±18.02mg kg^{-1}) of macro elements in soil samples (from the farmland), followed by K (681.43±1.52mg kg^{-1}). Similarly, Na (111.63±0.35 mg kg^{-1}), Cu (49.96±0.99mg kg^{-1}), Co (5.43±0.31 mg kg^{-1}), Mn (0.62±0.238mg kg^{-1}), Ni (0.194±0.01mg kg^{-1}), and Zn (0.163±0.007mg kg^{-1}) were detected among the microelements in soil samples (from farmland). Pb and Cr were not detected in all soil samples (from farmland). Potassium (K) was found to have the highest concentration (99.93±0.037mg kg^{-1}) followed by Ca (17.23± 0.36 mg kg^{-1}) among the macro elements in coffee beans from farmers' farms. Like coffee beans from farmland, samples from washing plants also contained the highest K (77.93±0.115mg kg^{-1}), followed by Ca (4.33±0.035 mg kg^{-1}). Metal levels in coffee bean samples from farmland are in the following order: K>Na>Ca >Mn>Cu> Ni>Zn. Metal levels were found to be K>Na>Ca >Mn>Cu> Zn>Ni in coffee beans from the washing plants. Co, Cr, Pb and Cd were not detection in all coffee bean samples. Except for calcium, potassium and manganese, the levels of metals in coffee beans from farmland and washing plants were not significantly different at a 95% confidence level within a kebele. Findings indicated that a natural wetland had a mean influent and effluent of total suspended solids (TSS) of 2190.78±448.46 mg/L and 972.67±234.312mg/L, respectively. A Mann-Whitney U test revealed that TSS was significantly higher in the natural wetland (median =1551.50) compared to constructed wetland (median =922.5), U =676.5, z=-2.435, p=0.015, r=0.257. Natural wetlands had a mean influent of biological oxygen demand (BOD) was 4277.94±157.02mg/L, while in the effluent the BOD it was 326.83±112.24 mg/L. In constructed wetland it was 4192.4±191.3mg/L, 782.72±507.6mg/L and 88.28±20.08mg/L in influent, middle and effluent respectively. The average chemical oxygen demand (COD) value at influent in natural wetlands was 8085.61±536.99 mg/L and in the effluent it was 675.33±201.4mg/L. In the constructed wetland, it was found to be 8409.8±592.9, 1372.6± 387.94, and 249.0± 7.68 for influent, middle and effluent respectively. Comparatively, the purification efficiency of organic pollutants (TSS, BOD, and COD) of constructed wetlands was better than natural wetlands, whereas natural wetlands had better purification efficiency of nitrogen compounds such as ammonium, nitrite, and nitrate. On average, removal rates for nitrogen compounds were 39.53% and-24.41% for ammonium, 79.44% and 55.4 % for nitrite, and 68.90% and 60.6% for nitrate in natural and constructed wetlands respectively, while the phosphate removal rate was 43.17% and 58.7 in natural and constructed wetlands, respectively. A Mann-Whitney U test revealed that there is no significance difference in nitrite, nitrate, ammonium, and phosphate concentration between natural and constructed wetlands ($p > 0.05$). Findings indicated that Ca (460.0 ppm) had the highest mean concentration of heavy metals, whereas Ni (0.50 ppm) had the lowest in soil samples of constructed wetland. Metal absorption by Vetiver Grass is the highest concentrations found in plant tissues grown in the following order K > Ca > Na > Mn > Fe > Zn > Cu > Ni > Cr in shoots. The order of the heavy metal contents in the roots of vetiver grass was K > Ca > Na > Mn > Fe > Zn > Cu > Ni > Cr. Based on translocation and bioconcentration factors, the plant was found efficient in the translocation of Mn and Ni from roots to shoot, whereas It served as a potential phytostabilizer for Ca, Cu, Cr, Fe, K, Na, and Zn since the TF values are lower than 1, which show that vetiver grass prefers to accumulate heavy metals in the roots rather than the shoot and so supports its potential for phytostabilization. From the present study, it was evident that vetiver grass is an ideal candidate for wastewater treatment using constructed wetland technology. Based on these results, both systems of treatment were effective in treating the coffee effluent since most of the values obtained were below the permissible EEPA limits. Even though the constructed wetland treatment plant performed better overall, in comparison, the natural wetlands had better purification efficiency for nitrogen compounds like ammonium, nitrite, and nitrate and the constructed wetlands had better purification efficiency for organic pollutants (TSS, BOD, and COD). We observed permitted levels of macro and trace elements in coffee beans from farmlands and washing plants. Only in the soil samples, cadmium concentrations are higher than those permitted for agricultural soil recommended by WHO and FAO. Overall, there is no health danger linked with the use of coffee beans due to detrimental and trace heavy metals.

Keywords: Coffee Bean, Heavy Metals, phytoremediation, Removal Capacity, Wetlands, Wastewater Treatment, Vetiveria zizanoides.

CHAPTER 1: GENERAL INTRODUCTION

1.1 Background and Justification of the Study

According to an International Coffee Organization (ICO) (2021), coffee is the third most consumed beverage in the world after water and tea, and it is the world's second most traded commodity after oil. Gure *et a*l.(2017) noted that Arabica (*Coffea arabica*) and Robusta (*coffea Canephora*) are the two species of coffee which are internationally traded. Almost 99% of the coffee consumed worldwide comes from these two species, 70% of which is Arabica Coffee.

For developing countries like Ethiopia, Coffee is a product of significant economic, social, and environmental significance (Gure *et al*., 2017) which generates 60% of its total export earnings, 30% of government direct revenue, and subsistence earnings of about 25% of the population (Kufa *et al*., 2011, Amamo, 2014). According to ICO (2021), Ethiopia ranks fifth in the world as a coffee producer and exporter after Brazil, Vietnam, Colombia and Indonesia and is the third largest consumer, after Brazil and Indonesia (ICO, 2021). The Ethiopian output in 2020 reached 7.375 million bags (60 kg each) of processed coffee, nearly 4.3% of the global production (ICO, 2021). It, therefore, becomes vital to assess the quality of the coffee in Ethiopia.

Heavy metal contamination is a global hazard that began with the industrial revolution, resulting principally from performing farming practices through the application of organic fertilizers, minerals and pesticides to the agricultural soil (Carolin *et al*., 2017, Hejna *et al*., 2018). The stability of soil and water is impacted by the pollution of heavy metals (Kobielska et al., 2018), resulting in environmental (Bello *et al*., 2018) and public health problems (Reyes *et al*., 2016).

Recent research shows some of our favorite brews can be laced with contaminants and to assess the concentrations of toxic and essential elements in coffee beans, numerous investigations have been carried out worldwide (Gure *et al*., 2017, Feleke *et al*., 2018, Rubio *et al.*, 2019, Albals *et al*.,2021,Dubale,2021).These metals can be absorbed by coffee plants, where they can either stor e in the roots or move into the shoots and grains(Silva*etal*.,2007).When these metals get to the coffee beans, they become sources of contamination for people, causing harmful health effects s uch as significantly reduced neurological and hepatic functioning, mutagenesis, and carcinogenesis (Matés *et al*., 2010).

Sidama is recognized, at some point in the world, by way of its coffee production, which has extraordinary style and aroma. In the international market, Sidama coffee beans have a trademark identified with the rights owned with the aid of Ethiopia. As a result, fees and consumption of Sidama coffee beans have been expanded for the previous few years (Gelaw, 2019). However, to the knowledge of the author, no research report was found on the levels of essential and toxic metals in coffee beans (from farmer's farms and coffee washing industries) of Dale Woreda, Sidama regional state. Due to its widespread production, use, and popularity, a thorough investigation of the concentrations of toxic and essential elements in coffee beans from Ethiopia's main growing region, Woreda, seems to be warranted.

Constructed wetlands (CWs), also called treatment wetlands, are engineered systems built to use natural processes and remove pollutants from contaminated water in a more controlled environment (Vymazal, 2010). Constructed watelands are engineered systems that mimic natural processes by removing pollutants or by reducing the level of pollutants to a dischargeable limit (Kadlec and Wallace, 2008). According to Dhanya and Jaya (2014), natural wetlands act as a bio filter, removing sediments and pollutants such as heavy metals from the water, and constructed wetlands can be designed to emulate these features.

Although first time in the early 1950s, Kathe Seidel used CWs for wastewater treatment (Vymazal, 2010), these techniques were hardly used in the earlier decades for contamination removal (Langergraber, 2011). But the evolutionary period started in the 1990s, whene agricultural effluents, landfill leachates, storm water, contaminated river water, urban runoff, food wastes, abattoir effluent, acid mine drainage, industrial effluents, and petrochemicals are all cleaned up using this CW approach (Daniel,2009, Vymazal, 2011, Qasaimeh *et al*., 2015).

There are two types of constructed wetlands, surface flow and subsurface flow, and two methods of conveying water through a constructed wetland, vertical flow and horizontal flow (Poor *et al*., 2020). Secondary treatment or biological treatment methods play a pivotal role in removing biochemical oxygen demand (BOD), chemical oxygen demand (COD), total phosphorous, total nitrogen, suspended and dissolved solids, etc. (Tian *et al*., 2019, Jafarinejad and Jiang, 2019).

As an interim technological option for treating wastewater, It has been suggested that wetlands, both constructed and natural, can be used to remediate wastewater (McEldowney *et al.*, 1998). Particularly constructed wetland technology (CW) is one of the emerging and acceptable technologies because it can successfully remove almost all types of pollutants from wastewater without harming the environment. These systems are economically appealing and relatively energy-efficient for wastewater treatment (Dhanya and Jaya, 2014). Many nations, especially those in developing regions, are pushing for the recovery and reuse of wastewater (Almuktar *et al*., 2018) with particular attention paid to the use of CWs as a method for wastewater cleanup. However, there are also issues with local knowledge and understanding of the technology's use; for instance, Ethiopia still lacks a coordinated national policy on wetlands (Dixon *et al*., 2021).

In the case of wetlands, Ebrahimi *et al*. (2013) noted that the choice of plants is crucial, and they must be resilient to toxicity and changes in the nature of the incoming wastewater. Pongthornpruek (2017) also indicated that the the Vetiver system was developed with the intention of conserving soil and water, especially in the areas of wastewater treatment and solid waste dumps. According to Pongthornpruek (2017), and Ng *et al*. (2019), one of the most promising species is Vetiver Grass because of its quick growth, deep and wide roots, and high resistance to environmental stress such as dramatic temperature changes (22^0C - 60^0C), soil pH (3.0-10.5) and, most critically, excellent tolerance to heavy metal stress. Based on the above recent suggestion, therefore, the Coffee berries wastewater treatment with vetiver grass is the alternative way emphasized in this study.

Due to the necessity for a lot of water to wash wet coffee beans and remove the pulp and mucilage, more than 1,249 wet coffee processing plants were built near water in Ethiopia (Dadi *et al*., 2018). According to the Sidama Regional State Environmental Protection office (2021), more than 383 coffee Berries processing (CBP) wet mills are located in the Sidama Regional State, the majority of which have not been renovated to minimize water usage or treat waste. It is obvious that as the number of wet coffee refineries grows, so does the amount of waste generated, which is discharged carelessly into neighboring natural waterways that flow into rivers and/or penetrate ground water, posing a serious threat to surface and ground water quality.

Recently, Gururaj *et al*. (2021) indicated that because of its large volume and complex organic constituents such as caffeine, lignin, pectin, tannin, melanoidin, and sugar, the treatment of wastewater generated by coffee processing remains a significant environmental contamination issue. Daniel (2009), Fia *et al.*, (2013), and Dhanya and Jaya (2014) emphasize that coffee processing plants wastewater treatment is required to alter the properties of wastewater so that it can be used or finally disposed of in compliance with the laws and regulations established by the relevant legislative bodies without negatively affecting the receiving bodies of air, water, or soil.

Limited data are available about the potential of constructed wetlands-oriented wastewater treatment of Coffee Berries Processing Agro-industry (CBPA), especially using Vetiver grass in Africa in general, and particularly in Ethiopia (Samuel, 2021). The constructed wetland system does not require high construction and operation costs as it is required for the construction of a conventional wastewater treatment system (Pongthornpruek, 2017, Kamal *et al*., 2019). With this in mind, we designed, built, and operated constructed wetlands in the Kege processing plant to evaluate the treatment potential of coffee wastewater.

1.2 Objective of the Study

The general objective of this study was to establish the wet coffee processing Agro-Industry Wastewater Treatment Potential of Constructed and Natural Wetlands: The case of Kege Processing Plant in Sidama Regional State, Ethiopia

The specific objectives were to:

1. Determine contents of essential and toxic metals in Coffee beans and soils in Dale Woreda, Sidama Regional State, Ethiopia (Chapter III).
    - To investigate the levels of essential metals such as Sodium (Na), Potassium (K), Calcium (Ca), Zinc (Zn), Manganese (Mn), Copper (Cu), Cobalt (Co), Chromium (Cr), Nickel (Ni), and toxic metals like Lead (Pb) and Cadmium (Cd), in coffee beans from coffee growing farms and coffee washing plants/industries in the selected Kebeles.
    - To determine the concentration of metals in coffee growing soil of farm land.

    - To estimate the potential of ecological risks of the heavy metals in soil.

2. Treatment performance assessment of Natural and Constructed Wetlands on wastewater from Kege wet coffee processing plant in Dale Woreda, Sidama Regional State, Ethiopia (Chapter IV).
    - To determine the physicochemical characteristics of the water used, effluent from Kege coffee processing plants and effluent from wetlands (NW and CW);
    - To determine the concentration of selected metals (Pb, Ni, Zn, Co, Cu, Na, K, ,Mg, Fe, Mn, Cr, Cd,) in the water used, effluent from Kege coffee processing plants and effluent from wetlands (NW and CW), and
    - To evaluate the treatment efficiency of the natural and constructed wetland to remove several pollutant from wastewater of coffee processing plant;

3. Evaluate pollutant removal efficiency of the Vetiver Grass in Constructed Wetland (Chapter V).
    - To determine the concentration of heavy metals in soil and the different parts of the plant Vetiver grass.
    - To determine Phytoremediation Quotient such as bioconcentration factor (BCF) and translocation factor (TF) by Vetiver grass
    - To determine whether Vetiver Grass growing in Kege constructed wetland has phytoremediation potential for removing heavy metals

1.3 Dissertation Structure

This thesis paper begins by Determining the concentration of essential and toxic metals in coffee beans and coffee growing soils in Dale district/Woreda, Sidama Regional State, Ethiopia has been studied as well. Moreover, the determination of metals concentration in coffee growing soil of farm land and estimate the potential of ecological risks of the heavy metals in soil have been studied.

Overviews of better water treatment technologies like a wetlands system are reported. In this study, the assessment of treated wastewater for irrigation purposes has been investigated compared to the thresholds.Finally, the Phytoremediation of coffee effluent using Vetiver grass in Constructed wetlands has been studied. This report is divided into the following sections:

Chapter 1:- Introduction

This chapter of the dissertation introduces the background and motivation for the study, technologies for the treatment of coffee wastewater, justification, objectives of the study and specific objectives and dissertation structure.

Chapter 2:- Review of Related Literatures

This chapter contains a comprehensive literature review which includes the heavy metal source in the Environment, Potential sources of heavy metals in farmland andagro products including coffee beans,the history of coffee and its origin, Coffee crops in Ethiopia and Worldwide, coffee processing, water consumption and wastewater generation during coffee pulping, Treatment options effluent from coffee processing plants, Classification of Constructed Wetlands for Wastewater Treatment, and Heavy metals removal in wetland systems are presented.

Chapter 3:- The Contents of Essential and Toxic Metals in Coffee Beans and Soils in Dale Woreda, Sidama Regional State, Ethiopia

This chapter presents the contents of essential metals (Na, K, Ca, Zn, Mn, Cu, Co, Cr, Ni), and toxic metals (Pb and Cd) that were investigated in coffee beans (coffee growing farms and coffee washing plants/industries) and soil. A sample of coffee beans (coffee growing farms and coffee washing plants/industries) was analyzed for heavy metals concentration. The level of heavy metals found in coffee beans was compared with the maximum permissible limit for medicinal and edible plants according to FAO 1993. Soil samples were analyzed for the determination of heavy metals concentration. The level of heavy metals was compared with FAO/WHO standards. The level of contamination of the farmland soil samples with studied heavy metals (Ni, Zn, Co, Cu, Cr, Mn, Pb and Cd) was determined by using various indices, such as an index of geo accumulation (Igeo), contamination factor (CF), contamination degree (Cdeg), modified contamination degree (mCdeg), Pollution Load Index (PLI), potential ecological risk factor (ERi) and potential ecological risk index (RI). Results obtained from this study were used to generate baseline information on the level of heavy metals in coffee beans and soil pollution.

Part of this chapter has been published as ‘’**The contents of essential and toxic metals in coffee beans and soils in Dale Woreda, Sidama Regional State, Ethiopia**’’ in the journal of peer j. https://peerj.com/articles/14789

Chapter 4:- Treatment Performance Assessment of Natural and Constructed Wetlands on Wastewater from Kege Wet Coffee Processing Plant in Dale Woreda, Sidama Regional State, Ethiopia

Focus on physicochemical characteristics and heavy metals of the water used, effluent from Kege coffee processing plants and effluent from wetlands (NW and CW);. Samples were analyzed for physicochemical parameters and heavy metals to determine pollutant removal efficiency and potential of the natural and constructed wetlands. Results of studied physicochemical parameters and heavy metals in water used for coffee processing, influent and effluent from the processing plant are presented and discussed in this part. Furthermore, a comparison has been made between the pollutants removal efficiency of the natural and constructed wastelands. The concentration of heavy metals in wastewater was compared with the standard limit of FOA for wastewater to be used for irrigation. Likewise, another physicochemical parameter was compared with (EEPA, 2003, WHO, 2011) wastewater discharge. Results obtained from this study were used to generate baseline information on the physicochemical and heavy metals of the water used, effluent from Kege coffee processing plants and effluent from wetlands (NW and CW).

Part of this chapter has been published as ‘’**Treatment performance assessment of Natural and Constructed Wetlands on wastewater from Kege coffee processing plant in Dale Woreda, Sidama Regional State, Ethiopia**’’ in the journal of SAGE Environmental Health Insight. https://doi.org/10.1177/11786302221142749

Chapter 5:- Evaluation of pollutant removal efficiency of the Veteveria Grass in Constructed Wetland

Numerous coffee processing facilities are located along the river system and use the river for their water supply. Phytoremediation, or the use of plants, was investigated as a low-cost remediation method to lessen the pollution, in order to address this issue. Because of its distinct qualities and capacity to absorb heavy metals, vetiver grass was used. The coffee processing plant generates wastewater containing a variety of organic and inorganic chemicals which potentially contaminate water ecosystems. The available and well-known coffee wastewater treatments are considered as an expensive and less effective method, therefore phytoremediation was used as an alternative friendly solution. Vetiver was planted in a constructed wetland. Soil, vetiver roots, and vetiver shoots were collected to determine heavy metal contents using Flame Atomic Absorption Spectrometry (FAAS). Bioconcentration Factor (BCF), Biological

Accumulation factor (BAC), and Translocation Factor (TF) were calculated to evaluate the plant's effectiveness in metal remediation processes.

Part of this chapter has been Under review as '**evaluate pollutant removal efficiency of the Veteveria Grass in Constructed Wetland**'' in the journal of SAGE Environmental Health Insight.

Chapter 6: – Summary, Conclusion and Recommendation

Contain the conclusion and recommendations for future research.

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CHAPTER 2: LITERATURE REVIEW

By definition, heavy metals are metallic elements with a high relative atomic mass and a density of more than 4 g/cm^3 that have been linked to contamination and possible toxicity (Hawkes, 1997, Aprile and Bellis, 2020). Arsenic, cadmium, chromium, cobalt, lead, mercury, nickel, and vanadium (V) are non-essential hazardous heavy metals in Plants, whereas copper, iron, manganese, and zinc are essential (Aprile and Bellis, 2020). It is an issue that affects the stability of both soil and water (Kobielska *et al*., 2018), thereby generating environmental (Bello *et al*., 2018) and public health problems (Reyes *et al*., 2016). Long-term or acute exposure to heavy metals has adverse impacts on plants, animals, and humans (Aprile and Bellis, 2020).

2.1. Heavy Metal Source in the Environment

In different environmental compartments, heavy metals can come from both natural and anthropogenic processes (Masindi and Muedi, 2018). Heavy metal pollution includes some natural sources such as the breakdown of rocks possessing metals, the eruption of volcanoes and certain human activities like the increase in urbanization, establishment of industries, and smelting and extraction processes (Aprile and Bellis, 2020, Pujari and Kapoor, 2021). Due to human activity, which is the primary cause of pollution, heavy metal pollution has arisen. This is primarily because of metal mining, smelting, foundries, and other industries that use metals, as well as the leaching of metals from various sources, including excretion, waste dumps, landfills, livestock and chicken manure, runoffs, automobiles, and road construction (Briffa *et al*., 2020). According to several studies, essential metals like copper, zinc, chromium, and manganese as well as non-essential metals like cadmium and lead, which have been building up in agro-ecosystems around the world, are very hazardous to soil microbes, aquatic life, animals, and humans in huge quantities (Lugwisha, 2016, Mahugija and Sheikh, 2018, Guo *et al*., 2020).

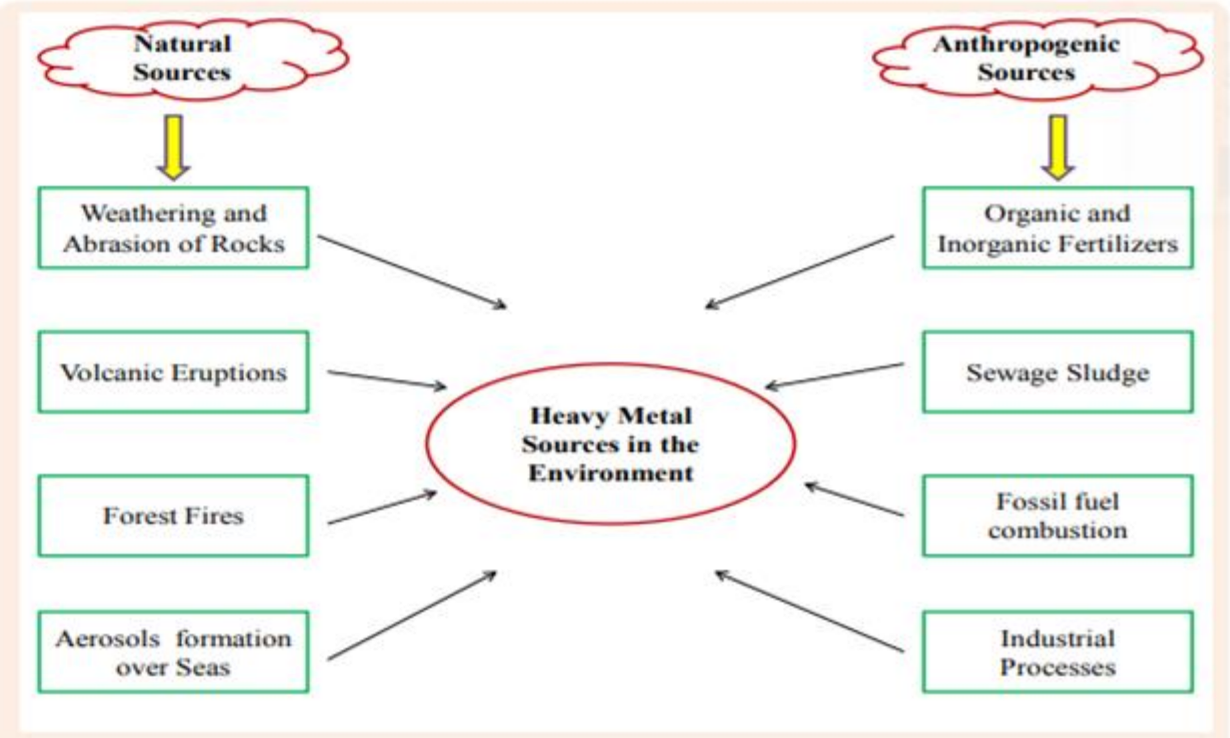

Figure 2.1 Source of heavy metals in the environment

2.1.1. Natural sources

All metals have been present on Earth since its formation (Briffa *et al*., 2020). Trace metals may occur by geographical phenomena like volcanic eruptions, weathering of rocks, and leaching into rivers (Walker *et al*., 2004). Natural weathering processes can lead to the release of metals from their endemic spheres to different environmental compartments. The most common heavy metals are lead, nickel, chromium, cadmium, arsenic, mercury, zinc and copper (Masindi and Muedi, 2018).

2.1.2. Anthropogenic sources of heavy metals

However, in most cases, metals become pollutants where human activity, mainly through mining and smelting, releases them from the rocks in which they were deposited during volcanic activity or subsequent erosion and relocates them into situations where they can cause environmental damage (Sankhla *et al*., 2016). Automobile exhaust releases lead, smelting emits arsenic, copper, and zinc, insecticides emit arsenic, and burning fossil fuels emits nickel, vanadium, mercury, selenium, and tin, among other important anthropogenic sources that significantly contribute to the contamination of the environment with heavy metals (Masindi and Muedi, 2018). The primary methods by which heavy metals enter the environment are Smelting or processing of metal ores; Mining; Burning of fossil fuels like coal, gasoline, and kerosene oil; discharging of industrial waste; discharging of domestic waste; and use of pesticides containing compounds (salts) of heavy metals (Armah *et al*., 2014).

2.1.2.1. Major anthropogenic sources of heavy metals

A. Agricultural practice

Due to poor waste disposal, the use of fertilizers and pesticides, the fast-expanding agriculture and metal industries, and other factors, inorganic pollutants are being dumped into our rivers, soils, and environment (Briffa *et al*., 2020). To grow and complete the life cycle, plants require not only macronutrients (N, P, K, S, Ca, and Mg), but also essential micronutrients. In some soils, heavy metals (such as Co, Cu, Fe, Mn, Mo, Ni, and Zn) are insufficient for optimal plant growth (Lasat, 2000). Fertilizers can be used as a soil additive or as a foliar spray on crops. Crops grown on Cu-deficient soils are sometimes fed Cu as a soil amendment, and cereal and root crops are sometimes provided Mn. Large volumes of fertilizer are administered regularly in intensive farming practices to provide adequate N, P, and K for crop growth (Raymond and Felix, 2011). Nowadays intensive farming practices use the application of organic fertilizers, minerals and pesticides to the agricultural soil to provide adequate N, P and K for crop growth (Carolin *et al*., 2017, Hejna *et al*., 2018). Organic fertilizer, mineral, and pesticide applications to agricultural soil result in significant amounts of these components in the resulting agricultural and animal products (Hejna *et al*., 2018). As impurities, the substances employed to supply these elements include trace levels of heavy metals (such as Cd and Pb), which, after repeated fertilizer application, may dramatically increase the soil's concentration of these impurities (Jones and Jarvis, 1981). The massive increase in the use of heavy metals has resulted in an impending influx of metallic compounds in both the terrestrial and aquatic environments (Gautam *et al*., 2016).

The potential for bio-solids to pollute soils with heavy metals has generated questions about their use in agricultural activities (Canet et al., 1998). Heavy metals like As, Cd, Cr, Cu, Pb, Hg, Ni, Se, Mo, Zn, Ti, Sb, and others build up in the soil as a result of the application of numerous bio-solids (such as livestock manures, composts, and municipal sewage sludge) to land (Basta *et al*.,2005). The manures produced by animals could have high levels of As, Cu, and Zn, which, if applied repeatedly to small areas of land, can lead to a significant buildup of these metals in the soil over time (Raymond and Felix, 2011).

B. Mining Activities and Mineral extraction

Heavy metals can infiltrate water resources naturally because they are present in the geological structures of the planet. For instance, persistent rain or flowing water can remove heavy metals from geological formations. When this geology is affected by commercial endeavors like mining, such processes are aggravated. These procedures subject the mined-out area to water and air exposure, which may have negative effects including acid mine drainage.The low pH conditions associated with acid mine drainage mobilize heavy metals (Sankhla *et al*., 2016). Extensive Pb and Zn ore mining and smelting have resulted in the contamination of soil that poses risk to human and ecological health (Raymond and Felix, 2011).

In the earth's crust, these heavy metals are present in ores which are recovered during mining activities as minerals. Significant amounts of heavy metal pollution can also be produced by mineral processing operations, both directly (which typically involves size reduction greatly increasing the surface area for mass transfer and generating effluents) and indirectly (through leaching from ore and tailings stockpiles).

C. Electronic waste

When ICT wastes are not disposed of properly, there is an alarming impact on the environment and human health due to the exponential development in the use of electrical and electronic equipment (EEE) (Mahipal *et al.*, 2016). Another industry that can produce lead pollution in the air and metal-rich effluents that can end up in surface water resources is the lead-acid battery manufacturing sector (and of course on land).

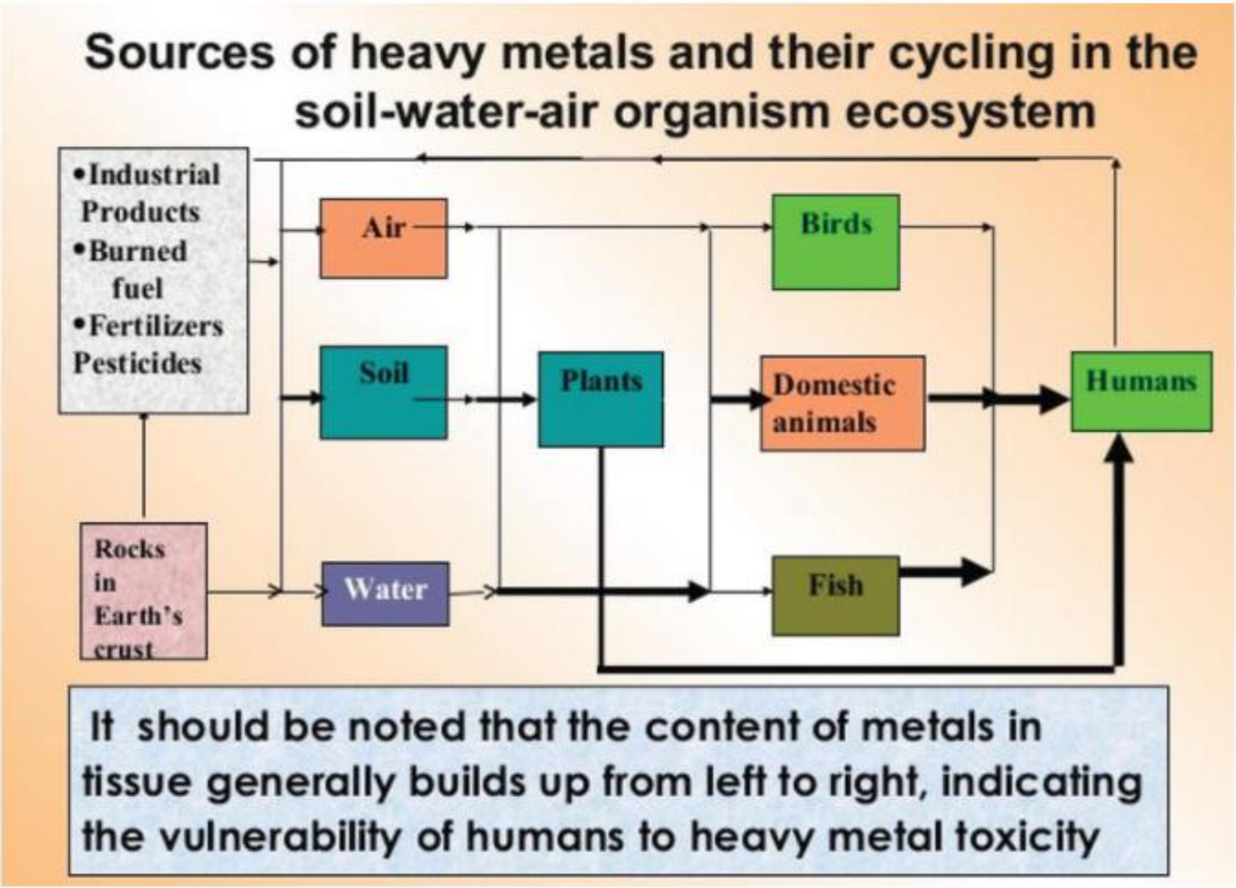

Figure 2.2 Sources of heavy metals and their cycle in the environment (Brady *et al*., 1994)

Table 2.1 Anthropogenic source of heavy metals in the environment

1. **Metalliferous mining and smelting**
    - a. Spoil heaps and tailings contamination through weathering, wind erosion (As, Cd, Hg, Pb)
    - b. Fluvially dispersed tailings deposited on the soil during flooding, river dredging, etc. (As, Cd, Hg, Pb)
    - c. Transported ore separates blown from conveyance onto the soil (As, Cd, Hg, Pb)
    - d. Smelting contamination due to wind-blown dust, aerosol, from stack (As,Cd, Hg, Pb, Se)
    - e. Iron and steel industry (Cu, Ni, Pb)
    - f. Metal finishing (Zn, Cu, Ni, Cr, Cd)
2. **Industry**
    - a. Plastics (Co, Cr, Cd, Hg)
    - b. Textiles (Zn)
    - c. Microelectronics (Cu, Ni, Cd, Zn)
    - d. Wood preserving (Cu, Cr, As)
    - e. Refineries (Pb, Ni, Cr)
3. **Atmospheric deposition**
    - a. Urban/industrial sources, including incineration plants, refuse disposal (Cd,Cu, Pb, Hg, V)
    - b. Pyrometallurgical industries (As, Cd, Cr, Cu, Ni, Pb, Zn)
    - c. Automobile exhausts (Pb, Cr)
    - d. Fossil fuel combustion including power stations (As, Pb, Se, V, Cd, Zn)
4. **Agriculture**
    - a. Fertilizers (e.g., As, Cd, V, and Zn in some phosphatic fertilizers)
    - b. Manures (e.g., As, and Cu in some pig and poultry manures, Zn in farmyard manure)
    - c. Lime (Pb, As)
    - d. Pesticides (Cu and Zn in fungicides, As and Pb used in orchards)
    - e. Irrigation waters (Cd, Pb, Se)
    - f. Corrosion of metals (e.g., galvanized and metal objects for fencing, troughs, etc. Pb, and Zn)
5. **Waste disposal on land**
    - a. Sewage sludge (Cd, Cr, Cu, Hg, Ni, Pb, V, Zn)
    - b. Leachate from landfill (As, Cd, Pb)
    - c. Scrapheaps (Cd, Cr, Cu, Pb, Zn)
    - d. Bonfires, coal ash, etc. (Cu, Pb)

Source: (Daniel, 2009)

2.2 Potential Sources of Heavy Metals in Farmlands and Agro-Products including Coffee Beans.

The potential sources of heavy metals in agro-ecosystem are both natural and anthropogenic; natural sources include emissions from dust, volcanoes and weathering product of rocks which are rich in metals, where their intensity vary along the landscape depending on other associated activities and climate changes (Shefali *et al.*, 2019, Briffa *et al.*, 2020). Anthropogenic sources include mineral mines, industrial and agrochemicals such as pesticides, fertilizers, herbicides, and growth regulators application in agricultural soils, and surface runoff from manufacturing and processing industries (Mng'ong'o *et al*., 2021). Studies in agro-ecosystems have identified industrial, agricultural, mining, domestic, and technological applications activities as the primary source of metal accumulation in agricultural soils (Kinuthia *et al*., 2020, Sayo *et al.*, 2020). Several studies have concluded that essential metals (Cu, Zn, Cr, and Mn) and non-essential metals (Cd and Pb) are highly poisonous to soil microbes, aquatic life, animals, and humans in large doses and have been accumulating in agro-ecosystem of in different parts of the world (Mahugija and Sheikh, 2018, Guo *et al*., 2020).

2.2.1 Fertilizers and pesticides

Agriculture was historically the first significant human impact on the soil (Scragg, 2006). Plants require important micronutrients in addition to macronutrients (N, P, K, S, Ca, and Mg) in order to grow and complete the life cycle. Some soils lack certain heavy metals (such as Co, Cu, Fe, Mn, Mo, Ni, and Zn), which are necessary for the healthy growth of plants (Lasat, 2000) and crops may be supplied with these as an addition to the soil or as a foliar spray (Raymond and Felix, 2011). In intensive agricultural systems, large amounts of fertilizers are routinely put into the soil to supply enough N, P, and K for crop growth (Raymond and Felix, 2011). Heavy metals (such as Cd and Pb) are contaminants present in trace amounts in the substances used to give these nutrients, and subsequent fertilizer applications may dramatically raise the soil's concentration of these impurities (Jones and Jarvis, 1981). There is no known physiological activity for metals like Cd and Pb. Certain phosphatic fertilizers might unintentionally contribute Cd and other potentially harmful metals to the soil, like F, Hg, and Pb (Raven *et al.*, 1998). Several common pesticides that were historically widely used in horticulture and agriculture included high levels of metals. Examples of such pesticides are copper-containing fungicidal sprays such as Bordeaux mixture (copper sulfate) and copper oxychloride (Jones and Jarvis, 1981).

2.2.2 Biosolids and manures.

Numerous biosolids, such as livestock manures, composts and municipal sewage sludge, are applied to land, which unintentionally causes the soil to become contaminated with heavy metals like As, Cd, Cr, Cu, Pb, Hg, Ni, Se, Mo, Zn, Tl, Sb, and others (Basta *et al.*, 2005). A popular practice in agriculture is to apply some animal wastes, such as chicken, cattle, and pig manures, to crops and pastures as solids or slurries (Sumner, 2000). In the pig and poultry industries, however, the Cu and Zn added to diets as growth promoters and the As present in poultry health products may also have the potential to lead to metal pollution of the soil (Chaney and Oliver, 1996, Sumner, 2000). The manures produced from animals on such diets contain high concentrations of As, Cu, and Zn and, if repeatedly applied to restricted areas of land, can cause a considerable buildup of these metals in the soil in the long run (Raymond and Felix, 2011).

In the course of treating wastewater, biosolids (also known as sewage sludge) are predominantly organic solid products that can be reused (USEPA, 1994). Many nations that permit the reuse of biosolids produced by urban populations regularly apply biosolids materials to the land (Weggler *et al.*, 2004). The potential for composting biosolids alongside other organic materials like sawdust, straw, or garden waste has also attracted a lot of interest. If this trend continues, there will be implications for metal contamination of soils (Raymond and Felix, 2011). The use of biosolids in agricultural activities has raised significant concerns due to the risk of heavy metal contamination of the soil (Canet *et al.*, 1998). Pb, Ni, Cd, Cr, Cu, and Zn are the heavy metals most frequently found in biosolids, and metal concentrations are affected by the type of industrial activity, its intensity, as well as the procedure used to treat the biosolids (Mattigod and Page, 1983).

Because heavy metals are getting into the food chain, the ecosystem is being ruined. Additionally, heavy metals reduce the biodegradability of organic contaminants, which has caused double the effect of polluting the environment. These metals are present in the soil and pose risks to the entire biosphere. They are ingested directly by organisms, and absorbed by plants, which can be dangerous for both the plant and the food chain that consumes it, and they change the soil's properties like pH, color, porosity, and natural chemistry, which lowers the soil's quality and contaminates water (Masindian and Muedi, 2018).

2.3. Coffee Crops in Ethiopia and Worldwide

The history of coffee begins with a legend, legend says the goat herder Kaldi first discovered the potential of these beloved beans (https://www.ncausa.org/About-Coffee/History-of-Coffee). The coffee plant was founded and cultivated by the Oromo people in the Kaffa province of Ethiopia which got its name around 1000 A.D and Arab people took the coffee seeds from this region and started the first coffee plantation. Then it spread to the whole of Europe (Adams, 1980, IAR, 1996, Haadis and Rani, 2008).

In 2020, Ethiopia produced 7.375 million bags (each weighing 60 kg) of processed coffee, accounting for approximately 4.3% of the world's total output (ICO, 2021). Ethiopia cultivates a variety of distinct regional kinds of *Coffee arabica* (Tessema *et al*., 2011, Gure *et al*., 2017). Depending on their geographical origin, Ethiopia produces numerous types of Arabica coffee with distinct characteristics and flavors (Amamo, 2014). Some of the coffee varietals produced in Ethiopia include Harar Coffee, Wollega Coffee (which includes Nekemt and Gimbi Coffee), Jima Coffee, Gedeo Coffee, Limu Coffee, Sidama Coffee, Kaffa Coffee, and others (IAR, 1996, Amamo, 2014). Each coffee-growing location and woreda has a wide variety of variations in the sorts of coffee grown (Gure *et al*., 2017). Even within a particular farmland, there might be differences in coffee types. Due to the fact that the regions in which coffee beans are cultivated have a significant impact on them, this variability has been noted. Coffee's body (or chemical composition), flavor, and the scent will all be greatly influenced by the soil, altitude, and climate of the regions where it is grown (Shalander *et al*., 2000). According to International Coffee Organization (2021), Ethiopia is the third-largest consumer of coffee in the world after Brazil and Indonesia, and ranks fifth in terms of production and exportation after Brazil, Vietnam, Colombia, and Indonesia (ICO, 2021).

Ethiopia is Africa’s largest producer of coffee and the world’s largest producer of organic Arabica coffee (ICO, 2021, FiBL, 2021). With coffee production forecasted at 7.62 million bags in 2021/2022, Ethiopia’s coffee sector is steadily expanding (USDA, 2021). Ninety percent of coffee is produced by smallholder farmers with an average farm size of 0.5-2 hectares (Solymosi and Techel, 2019). Production predominantly takes place in the Southwest, in the Oromia Region, and Southern Nations, Nationalities, and Peoples' Region (SNNPR). 15 million Ethiopian smallholders depend on coffee for their livelihoods, roughly one-fifth of the population (Reay, 2019).

Indonesia ranks fourth in global coffee production and third in total Robusta production (USDA, 2021). Robusta coffee is mainly produced in Southern Sumatra (Solymosi and Techel, 2019), and Arabica coffee is in Northern Sumatra. Other coffee-growing areas include Java, Sulawesi, Flores, and the Bali islands (Statista, 2021). There are an estimated 1.8 million smallholder coffee farmers in Indonesia (GCP, 2019), and coffee farms averaging between 1-2 hectares account for 98% of all coffee-growing areas (USDA, 2021).

2.3.1. Coffee species

The Coffea genus, which was created in 1737 by C. Linnaeus, is a member of the Rubiaceae family (Clifford and Willson, 1987). *Argocoffea*, *Paracoffea*, *Mascarocoffea*, and *Eucoffea* were the four sections, Chealier (1947) attempted to divide the species of the genus Coffea into (Clifford and Willson, 1987).

The two types that are traded globally are Robusta (*Coffea Canephora*) and Arabica (*Coffea Arabica*). Due to its more palatable flavor attributes, Arabica commands higher pricing (Deutsche, 2002). Robusta coffee is an important component of commercial coffee blends due to its characteristics of a rich "body" (Body is the viscosity, fullness and weight in the mouth of a beverage, ranging from "thin, watery" to "thick, heavy" (Viani, no date.). *Coffea liberica, Coffea abeakutyae, Coffea dewevrei, Coffea congensis,* and others are less commonly farmed coffea species. The appearances and behaviors of the various Coffea genus members are extremely varied (Clifford and Willson, 1987, Coste, 1992).

More than 80 coffee species have been identified and among them two are of economic importance. They are Coffea Arabica, popularly known as Arabica coffee, responsible for approximately 70% of the global coffee market and the other is *Coffea canephora* popularly known as Robusta coffee contributing to the remaining portion (Gururaj et al., 2021). The two types that are traded globally are Robusta (*Coffea Canephora*) and Arabica (*Coffea Arabica). Coffea liberica, Coffea abeakutyae, Coffea dewevrei, Coffea congensis*, and other species of coffee are less commonly grown. The appearances and behaviors of the various Coffea genus members are extremely varied (Clifford and Willson, 1987, Coste, 1992).

2.3.2. Uses of Coffee

Coffee is worth over 100 billion dollars (Global coffee industry facts, 2017). Coffee is a commodity of significant economic, social, and environmental relevance for developing countries like Ethiopia (Gure *et al*., 2017) which generates 60% of its total export earnings, 30% of government direct revenue, and subsistence earnings of about 25% of the population (Kufa *et al*., 2011, Amamo, 2014). The coffee sub-sector plays a significant role in Ethiopia's economy, as well as providing income for a large number of households: it is estimated that between 7.5 and 8 million households rely on coffee for a significant portion of their income, and coffee-related activities employ many more people (Dubale, 2021).

According to different studies, daily coffee use has been linked to a lower incidence of prostate cancer in men (Liu *et al*., 2015) and depression in women (Wang *et al*., 2016). The presence of antioxidants in coffee has been shown to reduce the risk of breast cancer, prostate cancer, and colorectal cancer, which is related to the presence of antioxidants in coffee (Nkondjock, 2009, Cao *et al*., 2014). High consumption (3–6 cups per day) promotes the release of the anti-diabetic hormone adiponectin, which reduces diabetes risk (Bidel and Tuomilehto, 2013). Another research revealed that coffee may protect against brain changes associated with Alzheimer's and Parkinson's disease (Wierzejska, 2017). Similarly, a review study showed that coffee drinking was linked to a lower risk of diseases like diabetes, obesity, and cardiovascular disease (Go¨kcen and Sxanlier, 2019). According to research, drinking coffee on a regular basis can boost glutathione levels and reinforce the body's protection against DNA damage (Martini *et al*., 2016).

2.3.3. Chemical composition of coffee beans

Coffee contains many classes of compounds such as carbohydrates, lipids, nitrogen compounds, vitamins, and minerals, as well as hazardous elements including Cadmium and Lead, which are all found in coffee (Go¨kcen and Sxanlier, 2019, Winiarska-Mieczan *et al*., 2021). The following ingredients of coffee beans are listed in order of abundance: 8% Phenolic Polymer (pulp), 6% Polysaccharides, 4% Chlorogenic acids, 3% Minerals, 2% Water, 1% Caffeine, 0.5% Organic Acids, 0.3 % Sugars, 0.2% Lipids, and 0.1 % Aromas (ICS, 2001).

The bioaccumulation of minerals in coffee beans varies depending on the trace elements, the variety, and the environment in which the coffee is grown (Gure *et al*., 2017). As reported by various researchers(Silva *et al*., 2017, Gure *et al*., 2017, Rubio *et al*., 2019), heavy metals such as Cd, Cr, Cu, Mn, Ni, Pb and Zn were found in the roasted and ground coffee beans and brew (Silva *et al*., 2017, Gure *et al*., 2017, Rubio *et al*., 2019).

A study done in Argentina revealed that all of the green coffee brands studied had Cd and As levels of less than 1.0 µg.g-1, and Pb levels of less than 3.0 µg.g-1(Rubio *et al*., 2019). Similarly, the mean concentration of metal in the three brands of coffee powder samples was (µg element/g): K (14488±467), Mg (1964±78), Ca (945±65), Na (484±12), Fe (52.0±4.0), Mn (23.0±0.9), Cu (14.0±0.6), Zn (15.0±0.8), Co (1.60±0.05) while that in their infusions (µg element/100 mL): K (37205±1501), Mg (2829±105), Ca (1619±102), Na (591±20), Fe (18.3±1.5), Mn (23.7±1.2), Cu (3.0±0.3), Zn (24.0±1.1), Co (1.8±0.1), respectively(Ashu and Chandravanshi, 2011).

2.3.4. Coffee Processing

There are two ways by which coffee can be processed. These are dry (natural) processing and wet (fermented and washed) processing (Genanaw *et al*., 2021, Bayata, 2021). During processing, the coffee berry is subjected to mechanical and biological operations to separate the bean or seed (Oliveira *et al*., 2000). The coffee berry's outer skin is called the pericarp; beneath it, there lies a thin layer of pulp (mesocarp) followed by a slimy layer called the pectin layer (parenchyma) (Gay, 2018). Approximately, half of the world's coffee harvest is processed by the wet method in which the coffee berry is subjected to mechanical and biological operation to separate the bean or seed from the exocarp (skin), mesocarp (mucilaginous pulp) and the endocarp (parchment) (Clark, 1985).

After picking coffee cherries, the fruit has to undergo several processing steps in order to remove the outer parts of the fruit, i.e. skin (exocarp), the pulp (mesocarp), the mucilage layer and the endocarpal \parchment (see **Fig. 2.3**).

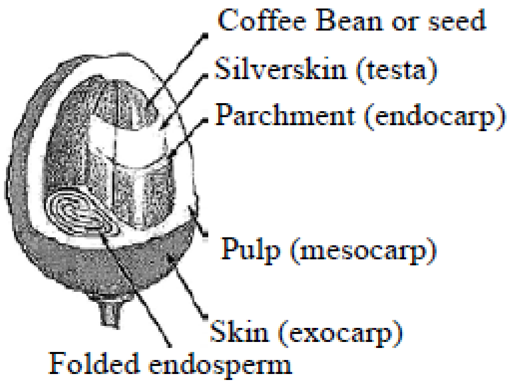

Figure 2.3 Morphology of coffee cherry (after Rothfos, 1979)

The final product's quality is determined by the processing method.The wet pulping method yields a superior quality product compared to the dry processing option (Gururaj *et al.*, 2021). Additionally, the possibility of pollution varies depending on the processing method. The dry method, which is used mostly for Brazilian Arabica but also a significant proportion of Robusta coffee, is the most straightforward and least polluting method of processing (Adams and Dougan, 1987). In this method, cherries are picked and left in the sun until the whole fruit reaches a moisture content of around 11%. The outer flesh and parchment are removed in one process after drying. In addition to requiring a big area for sun drying and rarely producing high-quality coffee, the dry technique can be readily affected by the rainy season in many regions of the world (Lucy, 2003).

Wet processing, in contrast to dry processing, requires a greater level of processing expertise and is typically used with Arabica coffee (Vincent, 1987). The wet method requires the mechanical removal of pulp using water with the simultaneous generation of wastewater (Gururaj *et al.*, 2021). Wet processing results in "mild coffees," which are of superior quality. The finer quality is due to a pre-sorting step of cherries which only allows ripe cherries in the process (**Fig. 2.4**). This is followed by de-pulping; during processing, exocarp and coffee pulp (mesocarp) are mechanically removed before the gelatinous and hygroscopic mucilage cover, which is coating the parchment, is removed. This is done during an approximate fermentation time of 36 hours depending on natural conditions like altitude and temperature (Rothfos, 1979). According to Por and Katzef (2001), the simplest test for full fermentation is to push one's palm into a pile of coffee beans until it creates a hole, at which point it is fully fermented (Por and Katzef, 2001). The coffee industry uses very large quantities of water at various stages of processing and production. About 50% of the total water used is consumed during the pulping process (Gururaj *et al.*, 2021). The clean parchment is only prepared for further processing, such as drying and hulling when the mucilage layer has been hydrolyzed and all leftovers have been removed (Vincent, 1987). The coffee's dryness is crucial because it boosts quality, which always raises the price, and because it prevents fungi from growing on the seed (Ijanu *et al*., 2020).

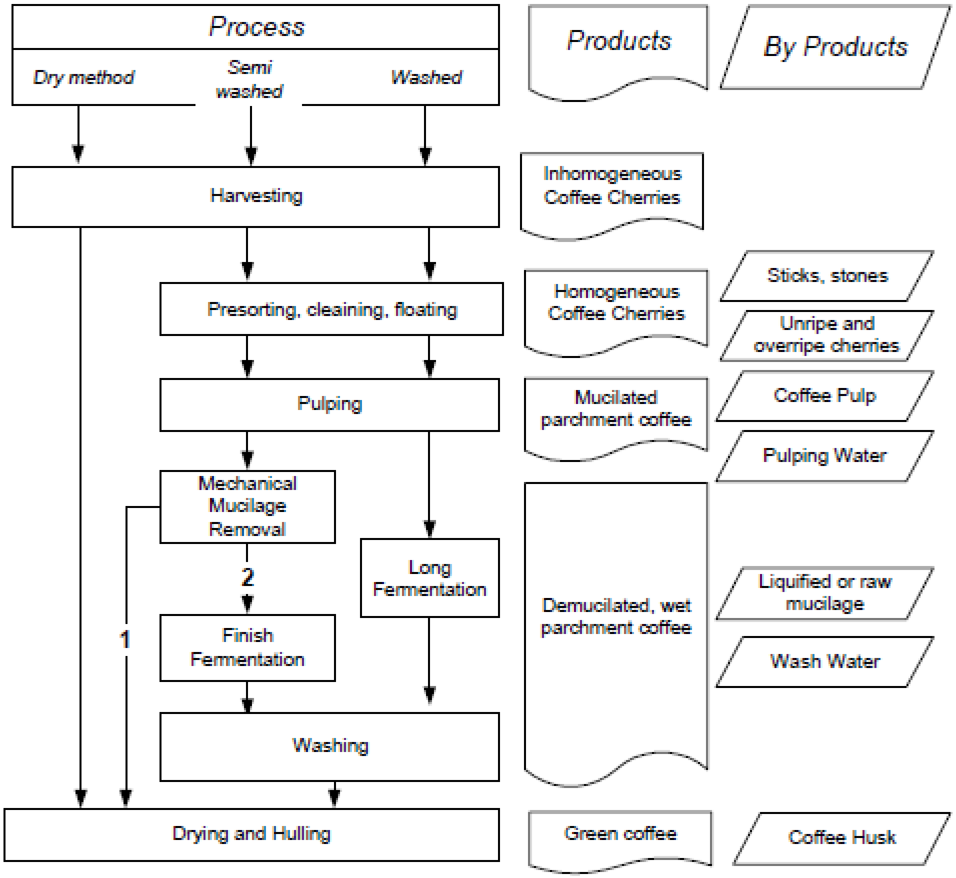

Figure 2.4 Coffee processing methods

2.4. Water Consumption and Wastewater Generation during Coffee Pulping

For pulping, fermenting, and washing the coffee cherry, the wet coffee processing industries need a lot of water (Chandrashekar *et al*., 2020, Genanaw *et al.*, 2021, Gururaj *et al.*, 2021,). Due to the seasonal nature of the coffee industry operating, the quantity of water used and the volume of wastewater generated in each coffee industry vary from one another depending upon the processing adopted (Gururaj *et al.*, 2021). In the processes of wet coffee production, about 5-15 liters of water are required to recover 1 kg of clean green coffee beans (the actual volume of water used depends on the pulping process, fermentation intensity and coffee bean transportation volume) (Haadis and Rani, 2008). Pulp makes up 43% of the by-products of coffee processing, along with mucilage (12%) and parchment (6.1%) *(Ijanu et al.,* 2020).

2.5 Treatment Options of Effluent from Coffee Processing Plants

Any treatment must consider operational costs, environmental friendliness, and the various demands for water quality in connection to water usage in order to maximize reuse based on the water-ft-for-use principle (Ijanu *et al*., 2019). Additionally, wastewater must be discharged within predetermined discharge limitations. Additionally, the stability and dependability of the management technique should be taken into account (Hubbe *et al*., 2016).

2.5.1Physicochemical treatment

Due to their capacity to break down complex compounds in wastewater in a controlled setting within a short period of time (e.g., a few hours), physicochemical treatment methods for industrial wastewater have lately gained popularity in comparison to biological treatment (Rodrigues *et al*., 2014, Takashina *et al*., 2018).

2.5.1.1 Zero-valent iron (ZVI) treatment

Zero-valent iron (ZVI) particulates have recently been employed to remediate both organic and inorganic contaminants in various wastewaters because they are non-toxic, abundant, and inexpensive (Tomizawa *et al*., 2016, Ijanu *et al*., 2019). Mechanisms of pollution removal are Reductive degradation, oxidative degradation, adsorption, and precipitation. Despite their effectiveness, spent ZVI particulates are difficult to regenerate, making the method uneconomical because materials must be replenished after treatment (Tomizawa *et al*., 2016).

2.5.1.2 Photo-Fenton method

This method uses the catalytic reaction between ferrous ions in solution and hydrogen peroxide producing hydroxyl (a specie with high oxidation potential) which is the key reagent in breaking down recalcitrant compound-related lignin (Hubbe *et al*., 2016). Although the photo-Fenton reaction is quite effective at decolorizing coffee eluent, there are several limitations, including the need for extra hydrogen peroxide supplies and the need to maintain an optimal pH of around 3.0 (Rodrigues *et al*., 2014).

2.5.1.3 Ultraviolet radiation catalysis (with ozone)

In comparison to photo-Fenton, which is a greater oxidant of many organic compounds, ozonation has been promoted as being a preferable choice (Satori and Kawase, 2014). According to studies, ozonation's greatest drawback is its low mineralization, but this is typically remedied by ultraviolet light or hydrogen peroxide (Hubbe *et al.*, 2016). Additionally, it was asserted that UV application alone is a less effective form of treatment unless it is paired with other treatment options that demonstrate the efficacious removal of significant amounts of organic compound loading, such as color and turbidity (Ashraf *et al.*, 2016, Takashina *et al.*, 2018).

2.5.1.4 Electro-oxidation

According to Hubbe *et al.* (2016), electro-oxidation could be defined as a process where voltages and other factors in an electrode are optimized for the in-situ generation of oxidizing and reactive species. In this method, organic compounds directly breakdown on the electrode through electrolysis, producing various oxidative radicals (such as hydroxyl and chlorine) and also organic compounds directly breakdown on the electrode through electrolysis, producing various oxidative radicals (such as hydroxyl and chlorine) (Ijanu *et al.*, 2019).

2.5.2 Biological methods

Various forms of filtration have been studied in connection with bioreactors to speed up biological decomposition (Hubbe *et al.*, 2016). The removal of BOD is one of the main goals of biological treatment, which is widely used. However, this method has been found to be ineffective at removing color or other acidic components of coffee wastewater, and its processes take a long time (a few months) to completely degrade organic materials (Ijanu *et al*., 2019). Aerobic and anaerobic treatment, spray irrigation, activated sludge, immobilization, enzyme treatment, and the utilization of bioreactors are all examples of biological treatment (Navitha and Kousar, 2018).

2.5.3 Wastewater treatment using constructed wetlands

The development of constructed wetlands was prompted by the presence of natural wetlands and the understanding of the function they play in the process of purifying water. Due to their performance and inexpensive construction and operating costs, constructed wetlands are being used more and more as an alternative method of treating wastewater (Kadlec *et al*., 2000). They are now recognized as the last stage in a conventional wastewater treatment system, "polishing" effluent before releasing it into the environment. The method is straightforward, economically and environmentally friendly, yet very effective and helps to achieve compliance with wastewater discharge rules (EU 2000/60). The concept of using constructed wetlands in Africa is relatively new, and the technique is quickly expanding throughout several nations.

Both vascular plants (the higher plants) and non-vascular plants (algae) are present in constructed wetlands, and both are significant in various ways. Algal photosynthesis raises the water's dissolved oxygen level, which has an impact on nutrient and metal interactions. Vascular plants aid in the treatment of wastewater by stabilizing contaminants in the substrates, which restricts channelized flow, slows speeds and allows suspended root systems to settle. These root systems then take up carbon, nutrients, and heavy metals and incorporate them into the tissues of the plants.

2.6. Classification of Constructed Wetlands for Wastewater Treatment

Depending on the flow path in the system there are two broad types of CWs: surface flow CWs (sometimes called free water surface CWs) and subsurface flow CWs. In surface flow CWs, the water slowly flows above a substrate medium, thus creating a free water surface and a water column depth usually of a few centimeters. On the contrary, in subsurface flow CWs, the water flows inside a porous substrate. Depending on the direction of the flow path, subsurface flow CWs can be subdivided into horizontal (HSSF) or vertical flow (VSSF) (Stefanakis *et al*., 2014, Vymazal, 2022). The layout, effectiveness at removing specific pollutants, space requirements, level of technological complexity, applications, and costs of various types of wetland systems all vary. Natural wetlands can be closely compared to free-water surface wetland technology. They are made up of sizable, shallow lagoons where various plant species can be found floating, submerged, or emerging. The biological treatment of the wastewater from the bio-films on the stems and leaves of the plants is carried out by the microorganisms(Mihret, 2014).

2.6.1. Surface flow constructed wetlands

Surface flow or free-water-surface wetland systems show, as the name suggests, a water flow primarily conducted above ground and exposed to the atmosphere (free water body). Re-aeration at the Surface is the major oxygen source in this wetland type. Below the free water body, the bed contains a soil layer which serves as a rooting media for the emergent vegetation. If rooted macrophytes are present, surface flow-constructed wetlands typically consist of shallow basins or channels with soil or other suitable mediums to support the establishment of macrophytes (Vymazal, 2022). Surface flow CWs can be divided into (a) free-floating macrophytes, (b) floating-leaved macrophytes, (c) submerged macrophytes, (d) emergent macrophytes, and (e) trees based on the type of macrophyte present (Vymazal and Kröpfelová, 2008). The majority of surface-flow constructed wetlands experience nitrification, although this process simply converts ammonia to nitrate and does not remove nitrogen from wastewater (Vymazal, 2022). At the bottom of the wetlands, amid a layer of decomposed plant matter, denitrification can remove nitrogen. Due to the minimal contact of wastewater with soil particles, phosphorus removal is typically very low, and as a result, there is little precipitation of Fe, Al, Mg, or Ca (Richardson, 1985). Figure 2.5 depicts a typical surface flow or free-water surface constructed wetland (FWS CW). A shallow sealed basin or series of basins, having 20-30 cm of rooting soil with a depth of 20–40 cm of the emerging macrophytes (typically more than 50%), depicts a free-water surface created wetland with emergent macrophytes (typically covering more than 50%)(Vynazal, 2007).

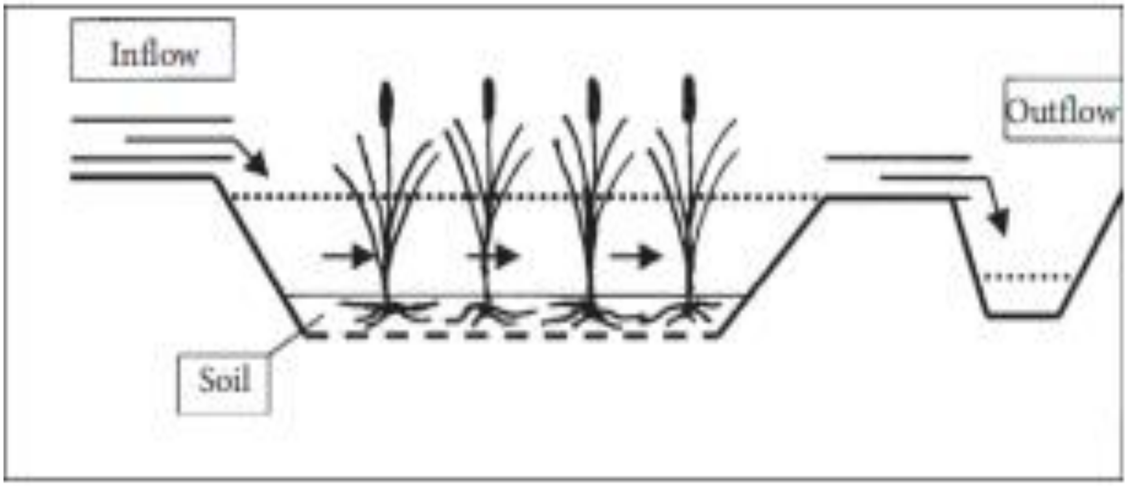

Figure 2.5 Schematic representation of the free-water surface constructed wetland with emergent macrophytes (source: Vynazal.J, 2007).

2.6.2. Subsurface flow (SSF) CWs

According to the flow direction, constructed wetlands with the subsurface flow can be divided into horizontal (HF CWs) and vertical categories (VF CWs). While the VF CWs are only occasionally fed, the HF CWs are fed continually (Vymazal, 2022).

2.6.2.1. Horizontal flow constructed wetlands

The main feature of horizontal flow systems is that the water level remains underneath the ground surface. The wastewater flows horizontally through a porous soil medium where the emergent plant vegetation is rooted and is purified during contact with the surface areas of the soil particles and the roots of the plants. During this transit, wastewater will come into touch with a network of aerobic, anoxic, and anaerobic zones. The aerobic zones contain roots and rhizomes that enter the substrate (Brix, 1994).

Capital expenses for horizontal flow constructed wetlands are higher than those for surface flow CWs because of the expense of sealing the bed and the expense of filtration material, including transportation (Vymazal, 2022). Because of their potential to limit the reproduction of disease-causing parasites, CWs are recommended. Nonetheless, a prevalent issue with horizontal flow systems continues to be a blockage, particularly in the input zone. Either faulty hydraulic design, unsuitable filtering media selection, or improperly controlled flow distribution inside the input zone are to blame for this kind of issue. This scenario slows down the system's operation, resulting in inadequately treated or subpar effluents (Worku *et al.*, 2018).

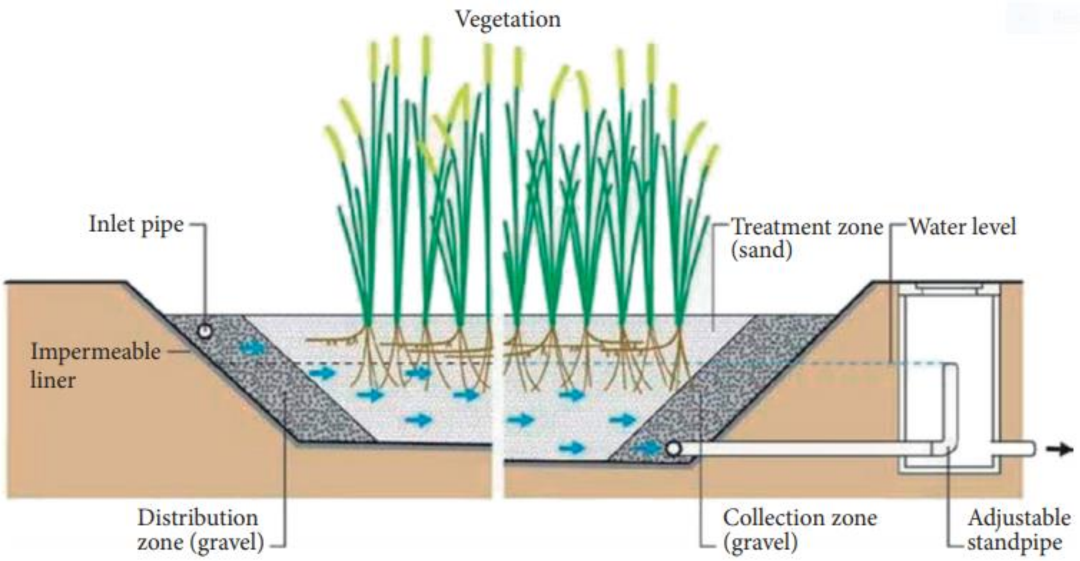

Figure 2.6 Schematic cross section of a horizontal-flow constructed wetland (HFCW) (Morel and Diener, 2006)

2.6.2.2. Vertical flow constructed wetlands

In vertical flow constructed wetlands, the water normally travels vertically through a bed of porous material (Vymazal, 2022). Although the removal of phosphorus is restricted, it can be increased by filtration material with a high sorption capacity, comparable to the horizontal flow CWS (Vymazal and Kröpfelová, 2008). Treatment efficiency is high for organics, suspended solids and ammonia, due to the aerobic conditions in the filter bed, and thus results in effective nitrification (Vymazal, 2022). The clogging of the filtration substrate, however, poses a serious danger to the VFS's ability to work well (Winter and Goetz, 2003, Chazarenc and Merlin, 2005). In order to prevent overloading certain areas of the surface, it is crucial to choose the filter material, hydraulic loading rate, and water distribution method effectively (Behrends *et al*., 2001).

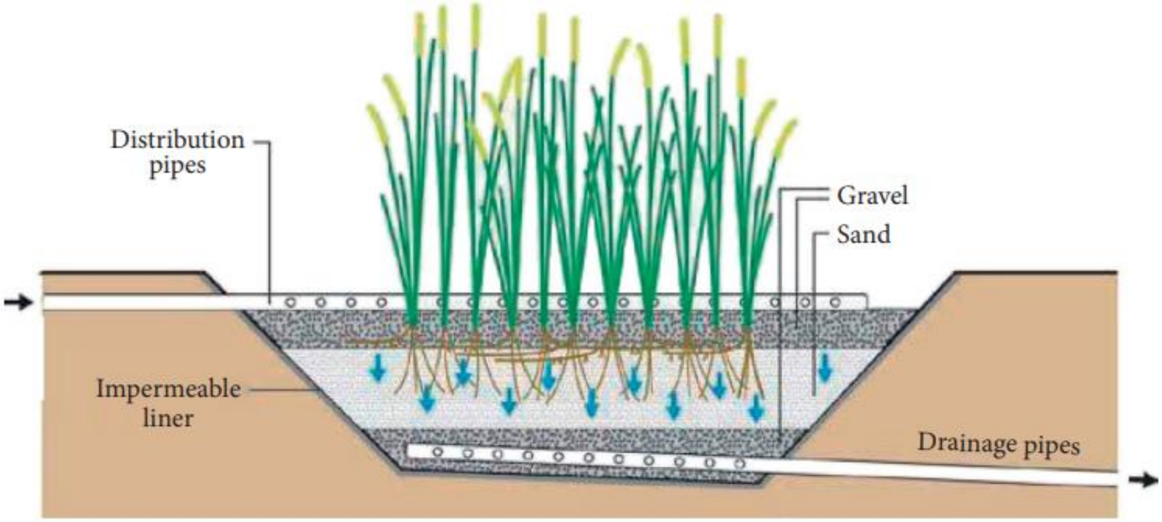

Figure 2.7 Schematic cross-section of a vertical-flow constructed wetland (VFCW) (Morel and Diener, 2006)

2.6.2.3. Hybrid constructed wetlands

It is possible to combine different CW types to increase the treatment efficacy, particularly for nitrogen. Due to the fact that these systems can combine the benefits of HSSF and VSSF systems to complement one another, such as in the case studies found in Egypt, there has been an increase in interest in hybrid systems (also sometimes called combined systems). Most typically, staged arrangements of VSSF and HSSF make up hybrid systems. **Figure 2.8** depicts the HF-VF hybrid system's schematic layout, however any kind of CW could be used in the system. An effluent with low BOD levels that is fully nitrified and partially denitrified, and therefore has significantly lower total-N concentrations, is conceivable (Cooper, 1999, Cooper, 2001). The design includes two stages of numerous parallel VF beds (filtration beds), followed by two or three HF beds (elimination beds), in series. The results show that the removal of organics (BOD and COD) and TSS is very good, while the removal of nitrogen is enhanced with no nitrate increase at the outflow (Cooper, 2001). Constructed wetland technology prevents diseases, safeguards the environment, and is a cheap, acceptable, and straightforward technology, all of which match the fundamental requirements of sustainable sanitation systems. Additionally, CWs produce highly-treated wastewater that encourages reuse, making them suitable for resource-focused sanitation systems (Langergraber, 2013). However, they are not recommended for the treatment of raw wastewater. On the contrary, they have gained acceptance worldwide (Tanner, 1996) due to their advantages as a wastewater treatment solution, which are both economically and environmentally sound, and because design, building, and operational experience have grown through time.

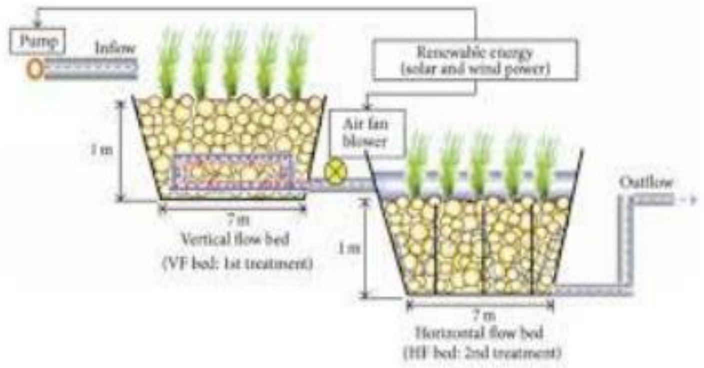

Figure 2.8 Schematic cross section of a Hybrid constructed wetlands (Morel and Diener, 2006)

2.7 Phytoremediation Potential

According to Dickinson *et al.* (2009), phytoremediation is a technology that uses plants and related microorganisms to remove, degrade, and isolate pollutants from the environment. Phytoremediation can be used to remove a wide variety of toxins from the environment. Heavy metals, metalloids, inorganic substances, radioactive chemical elements, petroleum hydrocarbons, pesticides and herbicides, explosives, and industrial organic wastes are some of them pollutants removed by Phytoremediation (Ensley, 2000).

2.7.1 Phytoremediation strategies

To clean up contaminated substrates, phytoremediation employs a variety of strategies. The contaminant's chemical make-up and other characteristics heavily influence the mechanism used. The features of the plant being employed in phytoremediation also have a role. So, there are five strategies that phytoremediation uses, and a plant might use more than one strategy at once. These methods include rhizodegradation, phytodegradation, phytostabilization, phytovolatization, and phytoextraction.

Phytodegradation is the process by which organic pollutants are broken down in plant cells. This process involves certain enzymes such nitroreductases, dehalogenases, and laccases (Rylott, 2008). Contaminants, whether organic or inorganic, are stabilized through phytostabilization when they are integrated into the humus or root cell walls. Finally, the pollutants are trapped in the substrate after being precipitated in insoluble forms by root exudates. The method limits the dispersion of pollutants in the substrate by preventing their mobility (Ali, 2013).

A process known as phytovolatilization allows some plants to absorb and volatilize metals or metalloids. These element ions can be taken up by the roots of the plants, changed into non-toxic forms, and then released into the atmosphere by the plants (Pilon-Smits and LeDuc, 2009). Heavy metals are transported and stored in the aerial parts of plants as a result of phytoextraction, which occurs when they are absorbed from the substrate into the roots of plants. The ability of plants known as hyperaccumulators to accumulate high amounts of particular heavy metals in their above-ground biomass is desired for the effectiveness of this process (Van der Ent *et al*., 2013).

Another method used by plants to treat wastewater is rhizodegradation. The best outcomes come from plants with a lot of root biomass and tolerance to heavy metals (Prasad, 2004). Growing roots encourage the development of rhizosphere bacteria, which then break down heavy metals in the process of rhizodegradation. Exudates and plant metabolites serve as a source of carbon and energy for the bacteria throughout this process. Additionally, plants release biodegrading enzymes into the environment (Prasad, 2004). This approach can only be used with organic pollutants, and the majority of the microorganisms found on roots belong to the genus Pseudomonas (Ali, 2013).

2.7.2 Phytoextraction and phytostabilization

The most crucial phytoremediation strategies for removing heavy metals and metalloids from polluted substrates are phytoextraction and phytostabilization. However, phytoextraction has drawn a lot of attention because of its generally high efficacy as a phytoremediation approach and the potential for the process to produce energy and metals with commercial value (Pedron *et al.*, 2009). Plants are recommended for use in phytoextraction because they possess several attractive qualities. The traits include the plant's capacity to endure high metal concentrations, its capacity to accumulate large amounts of metals in its above-ground biomass, and its capacity to grow quickly and create a lot of biomass (Shabani and Sayadi, 2012). Another desirable trait is the ability to be easily farmed and harvested, as well as having a thick root system (Shabani and Sayadi, 2012).

If the contamination can be removed through harvesting, phytoextraction as a phytoremediation approach will work more effectively. Only if the accumulated heavy metals are transferred to the plant's easily harvested aerial portions will this be accomplished. It is crucial to harvest at the right time since it should be done before the plants wither, die, and degrade (Blaylock and Huang, 2000). Phytomining can be used after harvest to recover metals from the plants absorbed during phytoremediation. The energy released when the plants are burned can be collected and used for other business purposes. The remaining ash can potentially be processed further to recover metals.

The goal of phytostabilization is to decrease pollutant mobility in the substrate. By covering the substrate with vegetation that can withstand harmful pollutants, soil erosion and contaminant leaching are constrained. Through both heavy metal adsorption and precipitation of the contaminants into the atmosphere, this phytoremediation technique lowers the mobility of heavy metals. It can be enhanced by oxidizing the root environment or by causing pH shifts. (Domingo *et al.* 2009) Utilizing plant species with the capacity to create large quantities of chelating compounds can also improve it. For the vegetation of mine tailings and contaminated areas, plants with the potential to stabilize the environment are extremely valuable (Antosiewicz *et al.*, 2008).

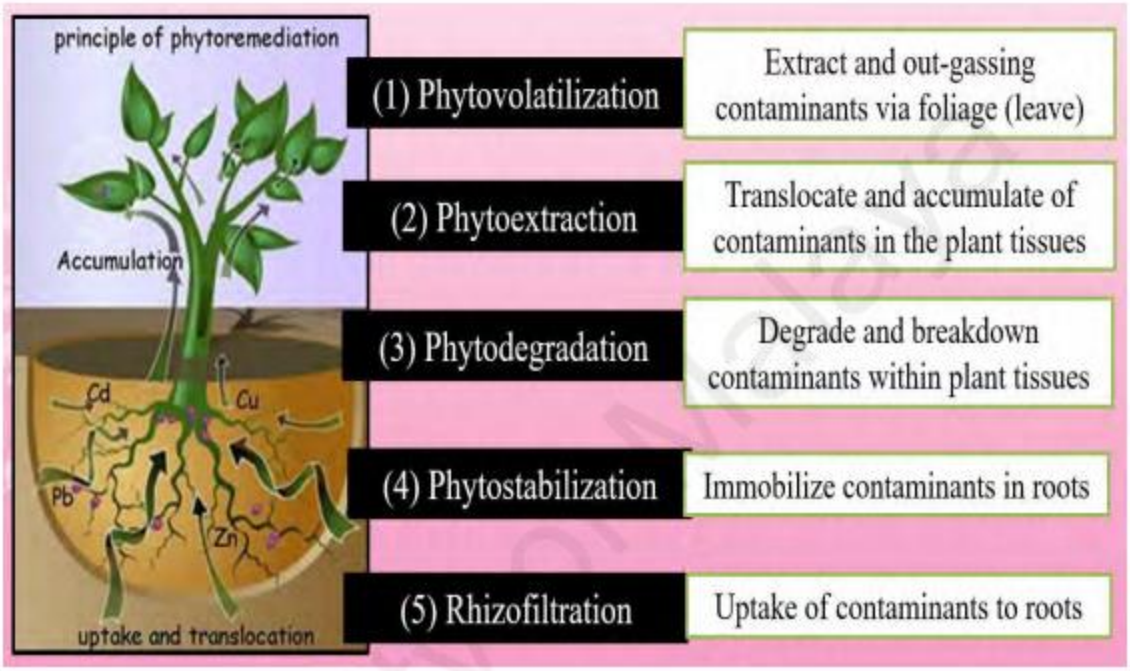

Figure 2.9 Major categories of phytoremediation.Adopted and modified fromPilon-Smits and LeDuc, (2009). Pedron *et al*., (2009). Ali, (2013) and Van der Ent *et al*., (2013).

2.7.3 Metal transfer coefficients

2.7.3.1 Bioconcentration factor (BCF)

A measure of a plant's capacity to accumulate a certain metal in its biomass relative to the metal concentration in the substrate is called the bioconcentration factor (Ghosh and Singh, 2005). It is employed to estimate the amount of heavy metals absorbed by the plant from the substrate. The bioconcentration factor (BCF), according to Zhuang *et al*. (2007), is the proportion of an element's total concentration in a plant's roots to its concentration in the substrate in which the plant is growing.

2.7.4 Translocation factor (TF)

The efficiency of phytoremediation can be quantified by calculating the translocation factor (TF). The TF describes a plant's ability to store a substance in its above-ground biomass. The ratio of the metal concentration in the upper section to that in the roots is what causes it (Chakroun *et al*., 2010). The plant's ability to move accumulated metal from its roots to its shoots is measured by the translocation factor (Padmavathiamma and Li,2007).

2.8 Pollutants Removal in Wetland Systems

Freshwater is an essential component of life. It deserves special attention because human activities have severely altered and threatened it. Lack of public awareness of environmental protection, population growth, rapid urbanization, industrialization, unsustainable use of pesticides and fertilizers, and rapid urbanization have all contributed to the pollution that threatens the physical, chemical, and biological integrity of the receiving aquatic environment (Bulton and Pitt, 2001). Untreated coffee-washed effluent discharge into the open environment and rivers can lead to several environmental and public health issues (Selvamurugan *et al*., 2010, Mussatto *et al*., 2011). Many hazardous substances, including tannins, alkaloids (caffeine), heavy metals, polyphenolic compounds, and nutrients including nitrate and phosphate, are present in coffee wastewater (Haadis and Rani, 2008, Gururaj *et al*., 2021).

In the western region of India, Yadav and Jadhav (2011) conducted a study using artificial wetland units paired with the surface flow and seeded with Eichhornia crassipes. The effectiveness of the treatment was assessed, and the results showed that BOD (95%), COD (97%), TSS (82%), NH_4^-N (43%), and PO_4-P (49%), had good mean removal efficiencies. In a similar vein, Atif Mustafa (2013) conducted a study using a pilot-scale built wetland (CW) that was ordered in Karachi. The study found that BOD (50%), COD (44%), TSS (78%), NH_4-N (49%), PO_4-P (52%), TC (93%) and FC (98%) all had good mean removal efficiencies.

Gitau and Kitur (2014) conducted a study on Tibia Wetland's effectiveness at treating wastewater. The study found that the wetland was important in the removal of contaminants, with TSS removal efficiency achieving the greatest results at 97.67%. Conductivity and Total Dissolved Solids were less than 50%, but BOD, Chromium, Nitrate, Total Solids, and Phosphate ranged from 50 to 96%. The effluent water was less acidic than the influent water due to a DO rise of 23.4% and a pH shift of -0.79%.

In a study conducted by Nyerere and Fouad (2018) on the analysis of the quality of wastewater produced by coffee processing facilities, a case study of coffee processing run by cooperatives in Burundi revealed that the effluent data from the existing coffee wastewater treatment design had not been able to guarantee accepted effluent levels regarding TSS, BOD5, COD, and pH allowable limits in accordance with Burundi standards. The research revealed the following values for the treated effluent's pH (4.82 and 5.29), TSS (432 mg/L and 176 mg/L), BOD5 (1050 mg/L and 950 mg/L), and COD (3960 mg/L and 1840 mg/L) characteristics.

Said *et al.* in 2019 using a continuous two-stage built wetland system. The coffee effluent employed in this investigation had a pH of 4.4, 399.3 mg/L of total suspended particles, 13,000 mg/L of COD, and 1720 mg/L of BOD. The removal rates of suspended particles, color, and COD by the treatment plant were 94%, 79%, and 95%, respectively.

A study by Haddis and Rani (2008): T ºC (upstream 25 and downstream 22), pH (upstream 3.57 and downstream 4.45), COD (upstream 25,600 mg/l and downstream 15,780 mg/l), BOD (upstream 14,200 mg/l and downstream 10,800 mg/l), phosphate (upstream 7.3 mg/l and downstream 4.6 mg/l), nitrate (upstream 23 mg/l and downstream 10.5 mg/l) and suspended solids (upstream 5870 mg/l and downstream 2080 mg/l).While the average values of the characteristics of nearby water bodies (River) before and after receiving coffee processing wastewater were the following: T ºC (before 15 and after 18), pH before 6.5 and after 5.15), BOD5 (before 120 mg/l and after 7800 mg/l), COD (before 176 mg/l and after 9780 mg/l), TSS (before 520 mg/l and after 2880 mg/l), phosphate (before 2.3 mg/l and after 4.1 mg/l) and nitrate (before 4.0 mg/l and after 7.5 mg/l).

In a wetland, heavy metals are dispersed among the substrate, water column, and flora (Sheoran and Sheoran, 2006). Heavy metal elimination occurs primarily through three wetland processes: adsorption by bacteria, algae, and plants; binding to soils, sediments, and particulate matter; and precipitation as insoluble salts (Kadlec and Knight, 1996). Important physical mechanisms that enable the removal of heavy metals linked to particulate matter include sedimentation and filtration. Long recognized as the primary method for removing heavy metals from wastewater, sedimentation (Sheoran and Sheoran, 2006). Adsorption and precipitation processes are two ways that heavy metals can bind to surfaces. Heavy metals can be adsorbed chemically or electrostatically, resulting in the creation of strong complexes (chemical adsorption) or comparatively weak complexes (physical adsorption) (chemisorption). Compared to physical adsorption, chemisorption more strongly binds metals (Evangelou, 1998). Cation exchange is a procedure that allows other cations to exchange metal ions that have been adsorbed on the substrate's surface. When solubility products are surpassed, metals can also precipitate with (oxy-) hydroxides, sulphides, carbonates, etc. in addition to the sorption and sedimentation reactions (Kadlec and Knight, 1996).

A study conducted in a constructed wetland in Sri Lanka found a significant reduction of 99.6% of Pb, 94.44% of Cd, and 94.84% of Zn (Jayaweera *et al*., 2006). Similarly, high removal efficiencies of 80.0%, 95.1% and 100% for Pb, Cu and Zn respectively have been reported at Nandi Hills tea estates wetland, Kenya (Gituku *et al*., 2015). Another study revealed that the removal efficiencies of the planted CWs for Iron, Copper, Manganese, Zinc, Nickel, and Cadmium were 74, 80, 60, 70, 71, and 70 %, respectively have been reported from pulp and paper industry wastewater (Arivoli *et al*., 2015).

Another study was done to investigate the efficiency of constructed wetlands in the removal of heavy metals and the result showed that the removal of heavy metals has been reported at 42% for manganese, 75–99% for cadmium, 26% for lead, 75.9% for silver and 66.7% for zinc (Odinga *et a*l., 2013). Similarly, a Previous study reported significant removal efficiencies of the CW for Pb, Cd, Fe, Ni, Cr, and Cu were 50%, 91.9%, 74.1%, 40.9%, 89%, and 48.3%, respectively, in Gadoon Amazai Industrial Estate (GAIE), Swabi, Pakistan (Khan *et at*.,2009).

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CHAPTER 3: THE CONTENTS OF ESSENTIAL AND TOXIC METALS IN COFFEE BEANS AND SOILS

ABSTRACT

For developing countries like Ethiopia, coffee is a commodity of great economic, social, and environmental importance. There are no detailed investigations performed on the contents of essential and toxic metals in coffee beans and soil in this study area. The levels of essential metals (Na, K, Ca, Zn, Mn, Cu, Co, Cr, Ni), and toxic elements (Pb and Cd) were investigated in coffee beans (coffee growing farmland and coffee washing plants) and soil samples (from farmland) using Flame Atomic Absorption Spectrometer (FAAS) and flame emission atomic spectroscopy. We selected 6 (20%) administrative units (Kebele) with a purposive sampling technique based on their coffee production capacity in Dale Woreda for the soil testing. After coffee sample preparation in a Microwave system with HNO_3 and H_2O_2 reagents, the accuracy of the optimized procedure was evaluated by analyzing the digest of the spiked samples. Soil samples were abridged accompanying a slight revision of the EPA 3050B acid digesting method. ANOVA was used to determine the significant differences in the mean concentration of metal within coffee beans from farmland at the various sampled sites at $p< 0.05$ significant level. To correlate the effect of one metal concentration on another metal in the coffee beans samples, the Pearson correlation matrices were used. Calcium had the highest concentration (1355±18.02mg kg-1) of macro elements in soil samples, followed by K (681.43±1.52mg kg-1). Similarly, Na (111.63±0.35 mg kg-1), Cu (49.96±0.99mg kg-1), Co (5.43±0.31 mg kg-1), Mn (0.62±0.238mg kg-1), Ni (0.194±0.01mg kg-1), and Zn (0.163±0.007mg kg-1) were detected among the microelements in soil samples. Pb and Cr were not detected in all soil samples. Potassium (K) was found to have the highest concentration (99.93±0.037mg kg-1) followed by Ca (17.23± 0.36

mg kg-1) among the macro elements in coffee beans from farmers' farms. Like coffee beans from farmland, samples from washing plants also contained the highest K (77.93±0.115mg kg-1), followed by Ca (4.33±0.035 mg kg-1). Metal levels in coffee bean samples from farmland are in the following order: K>Na>Ca >Mn>Cu> Ni>Zn. Metal levels were found to be K>Na>Ca >Mn>Cu> Zn>Ni in coffee beans from the washing plants. Co, Cr, Pb and Cd were not detection in all coffee bean samples. Except for calcium, potassium and manganese, the levels of metals in coffee beans from farmland and washing plants were not significantly different at a 95% confidence level within a kebele. The Pearson correlation coefficients also indicated strong to medium and positive or negative correlation among the metals in coffee beans.We observed permitted levels of macro and trace elements in coffee beans from farmlands and washing plants. Only in the soil samples, cadmium concentrations are higher than those permitted for agricultural soil recommended by WHO and FAO. Overall, there is no health danger linked with the use of coffee beans due to detrimental and trace heavy metals.

Key words: Coffee Bean,Dale Woreda,Heavy Metals.

3.1. Introduction

According to International Coffee Organization (ICO) (2021), coffee is the third most consumed beverage in the world after water and tea, and it is the world's second most traded commodity after oil. Numerous studies in the literature have noted the positive effects of moderate coffee consumption on human health, including the control of blood sugar levels, the prevention of cancers, circulatory and digestive diseases, Parkinson's and Alzheimer's diseases, and Parkinson's and Alzheimer's diseases (Tan *et at*., 2007, Kotyczka *et al*., 2011).

In Ethiopia, coffee is a valuable product for the country's economy, society, and environment (Gure *et al*., 2017) According to data from the Ethiopian Tea and Coffee Authority (2022), Ethiopia exported 300,000 metric tons of coffee in 2021, bringing in $1.4 billion and accounting for almost 30% of all export revenue and supporting the livelihoods of over 25% of its population (Tefera and Tefera, 2014). According to ICO (2021), Ethiopia ranks fifth in the world as a coffee producer and exporter after Brazil, Vietnam, Colombia and Indonesia and is the third largest consumer, after Brazil and Indonesia (ICO, 2021). The Ethiopian output in 2020 reached 7.375 million bags (60 kg each) of processed coffee, nearly 4.3% of the global production (ICO, 2021).

By definition, heavy metals are metallic elements with high atomic weights and densities that are at least five times greater than those of water. They are bio-accumulative, transferred up the food chain to humans, and stable, meaning they cannot be digested by the body. Heavy metals like lead and cadmium etc are extremely hazardous even at very low doses, and they can have negative effects (Mohiuddin *et al*., 2011). However, essential elements are those which even in small amounts play important roles as far as healthy animals or plant life is concerned. Essential elements are vital for life (Beasley *et al.*, 2000). Since the beginning of the industrial revolution, there has been an increased risk of heavy metal contamination. This risk is primarily due to farming practices that involve the use of organic fertilizers, minerals, and pesticides on agricultural soil (Carolin *et al.*, 2017, Hejna *et al.*, 2018) , With increased urbanization, industrialization, and the discharge of untreated or only partially treated industrial effluents, Ethiopia's pollution levels have reached alarming levels, with rising metal levels and declining agricultural soil quality (Ftsum and Abraha, 2018). The stability of soil and water is impacted by the pollution of heavy metals (Kobielska *et al.*, 2018), resulting in environmental (Bello *et al.*, 2018) and public health problems (Reyes *et al*., 2016).

Metal toxicity results when our body intake excess amounts through supplements, food and water (Ibrahim *et al*., 2006). Heavy metal exhibits specific toxic effects on human beings, damage to the kidney, liver, lungs, blood cells, and mental and central nervous systems (Dorne *et al.*, 2011, Winiarska-Mieczan *et al.*, 2021). Chronic exposure can lead to a gradual increase in neurodegenerative processes, which is related to diseases such as multiple sclerosis and Alzheimer’s disease (FAO, 2011, Butt and Sultan, 2011). The heavy metals toxic even in very small amounts such as arsenic, cadmium, chromium, nickel, and mercury are classified as category one (1) carcinogens, as they increase cancer risk in humans even with mild to moderate exposure (Jaishankar *et al.*, 2014, Kim *et al*., 2015).

Recent research shows some of our favorite coffee brews can be laced with contaminants and quite several studies have been conducted around the whole world to determine the levels of essential and toxic elements in coffee beans (Gure *et al*., 2017, Feleke *et al*., 2018, Rubio *et al.*, 2019, Albals *et al.*, 2021, Dubale, 2021). Another researcher has been conducted to determine heavy metal accumulation in beans of cacao (Takrama *et al.*, 2015, Bertoldi *et al*., 2016 , Aguirre-Forero *et al.,* 2020). Previous studies have also assessed the levels of hazardous components in grains from various countries (Meharg *et al*., 2013, TatahMentan *et al.,* 2020). Transfer of metals from soil to coffee beans or other crop products, such as cacao, grains can be absorbed by plants, where they can either store them in the roots or move them into the shoots and grains (Silva *et al*., 2007). When metals get to the coffee beans, they become the sources of contamination for people, causing harmful health effects such as significantly reduced neurological and hepatic functioning, mutagenesis, and carcinogenesis (Matés *et al*., 2010).

Sidama coffee beans are well-known on the global market for their superior quality. Due to this, Sidama coffee bean prices and consumption have increased over the past few years. (Gelaw, 2019). There have been studies before on the determination of metals in the coffee bean, but these had different in scope, such as coffee beans samples were taken from farmer's farms and coffee washing industries. To the best of our knowledge, there are no detailed investigations performed on the contents of essential and toxic metals in coffee beans and soil in Dale Woreda, Sidama Regional State. Moreover, the knowledge gap on the contents of essential and toxic metals in coffee bean and soil needs to be filled. Therefore, the aim of this study is to investigate the contents of essential and toxic metals in the coffee bean and soil in Dale Woreda, Sidama Regional State, Southern Ethiopia

3.1.1. Objective of the study

3.1.1.1 General objective

The general objective of this study was to investigate the contents of essential and toxic metals in coffee beans and coffee-growing soils in Dale district/Woreda, Sidama Regional State, Ethiopia.

3.1.1.2 Specific objectives

The specific objectives of the current research include:

1) To investigate the contents of essential metals such as Sodium (Na), Potassium(K), Calcium (Ca), Zinc (Zn), Manganese(Mn), Copper(Cu), Cobalt (Co), Chromium (Cr), Nickel (Ni), and toxic metals like Lead (Pb) and Cadmium(Cd) in coffee beans from coffee growing farmland and coffee washing plants/industries in the selected Kebeles.
2) To determine the concentration of metals in coffee growing soil of farm land.
3) To estimate the potential ecological risks of the heavy metals in soil.

3.1.2 Research questions

1. What are the contents of essential (Na , K, Ca, Mg, Fe, Zn, Mn, Cu, Co, Cr, Ni) and toxic metals (Pb, Cd) in coffee beans from farmer's farms and coffee washing industries in the selected Kebeles of Dale Woreda?
2. What is the concentration of metals in coffee growing soil of farm land?
3. Is there a potential of ecological risks of the heavy metals in soil?

3.2 Materials and Methods

3.2.1. Description of the study area

This study was carried out in Dale Woreda, Ethiopia. The geographic location of Woreda is between Latitude 6° 41' 35" north and Longitude 38° 21' 17" east. The capital town of Dale woreda is Yirgalem, which is located 45 km from Hawassa. Dale is bordered on the south by Aleta Wendo and Chuko, on the west by Loka Abaya, on the northwest by Boricha, on the north by Shebedino, and on the east by Wensho. The elevation of Dale Woreda varies from approximately 1200 meters above sea level along the Lake Abaya shoreline to approximately 3200 meters at its westernmost point. Rivers include the Gidabo. Coffee is an important cash crop in Dale, with 17.38 square kilometers planted with this crop, which produced a total of 12.3 million kilograms of beans in 2020/21. Industry in this Woreda includes 51 coffee pulpers (DWFEDO, 2021). Agricultural practices are using more and more farming chemicals, presumably to produce more yields. The mean annual temperature ranges between 9.6°C and 29.2°C. The area has a bimodal rainfall pattern with the first peak from April to May and the second peak from August and October. The lowest rainfall was recorded between November and February. The mean annual rainfall of the area is 1102 mm per year. Agroforestry practices appear to be the major features of the land use systems in the area.

Based on the 2007 Census conducted by the CSA(2007), this Woreda covered an area of 30,212 km^2 with a total population of 242,658, of whom 122,918 are men and 119,740 women; 30,348 or 12.51% of its population are urban dwellers. It has 30 rural and 2 urban Kebeles (the lowest administrative structures). There are large numbers of Coffee Arabica varieties that are grown in different parts of Ethiopia. The differentiation between the varieties is not clear and simple.

However, experts or researchers classify Ethiopian coffees based on the Farmland, Kebele, Woreda, or Zones in which they grow (Kufa *et al*., 2011, Amamo, 2014). The information from the Sidama Region's Environmental Protection Authourity indicates that 27,049 tones of the harvested Sidama coffee were exported in 2021/22 while the rest was used for domestic consumption (SRSEPA, 2021).

3.2.2 .Sampling sites selection and sample collection

For the collection of coffee beans and soil from farmland, 6 (20%) Kebeles (administrative units) of Dale Woreda were selected using a purposive sampling technique based on their coffee production capacity. The selected kebeles (sampling sites) were Kege named as sampling site 1 (SS1), Wenenata (SS2), Gane (SS3), Wondo (SS4), Bera (SS5), and Megara(SS6). The coffee beans from the coffee tree and the tree-supporting soil were sampled using the grab sampling method from the sites. In order to get quality coffee bean samples, eight farmers from each kebele were chosen based on the highest amount of coffee-producing farmers selected to sample (collect the beans). A minimum of five coffee trees/plants from each farmland were used for sampling. Finally, to establish a single sample of coffee beans that is representative of a single kebele, the full samples that were obtained from a single kebele were z homogenized. During the peak season of coffee production, ripe red cherries were carefully chosen and harvested for the study. The samples were sealed in polythene bags.

The coffee washing plant, on the other hand, has been chosen based totally on the following manner: To obtain coffee bean samples from coffee processing plants, the following procedure was used. Totally, there are 13 coffee washing/processing plants in the 6 selected sampling kebeles, and from each kebele one processing plant was selected purposively, making the selected plants 6. Therefore, a total of six coffee processing plants were selected for this study.

To obtain samples of coffee beans from washing plants, the washing company left the coffee beans to dry on beds after washing them, and we used those samples for sampling(we used washed and dried coffee beans for the study). All the samples were taken in triplicate.

Soil samples for this study were collected beneath each coffee tree selected from farmlands to sample coffee beans, as mentioned above. Soil samples were collected from the floor 15cm-25cm under each sampled coffee plant as described by Csuros and Csuros (2002) by using stainless steel soil sampling auger. Finally, the samples had been added into non-reacting polyethylene bags and carefully blended to make one composite pattern for each Kebele, which was once then delivered to the laboratory.

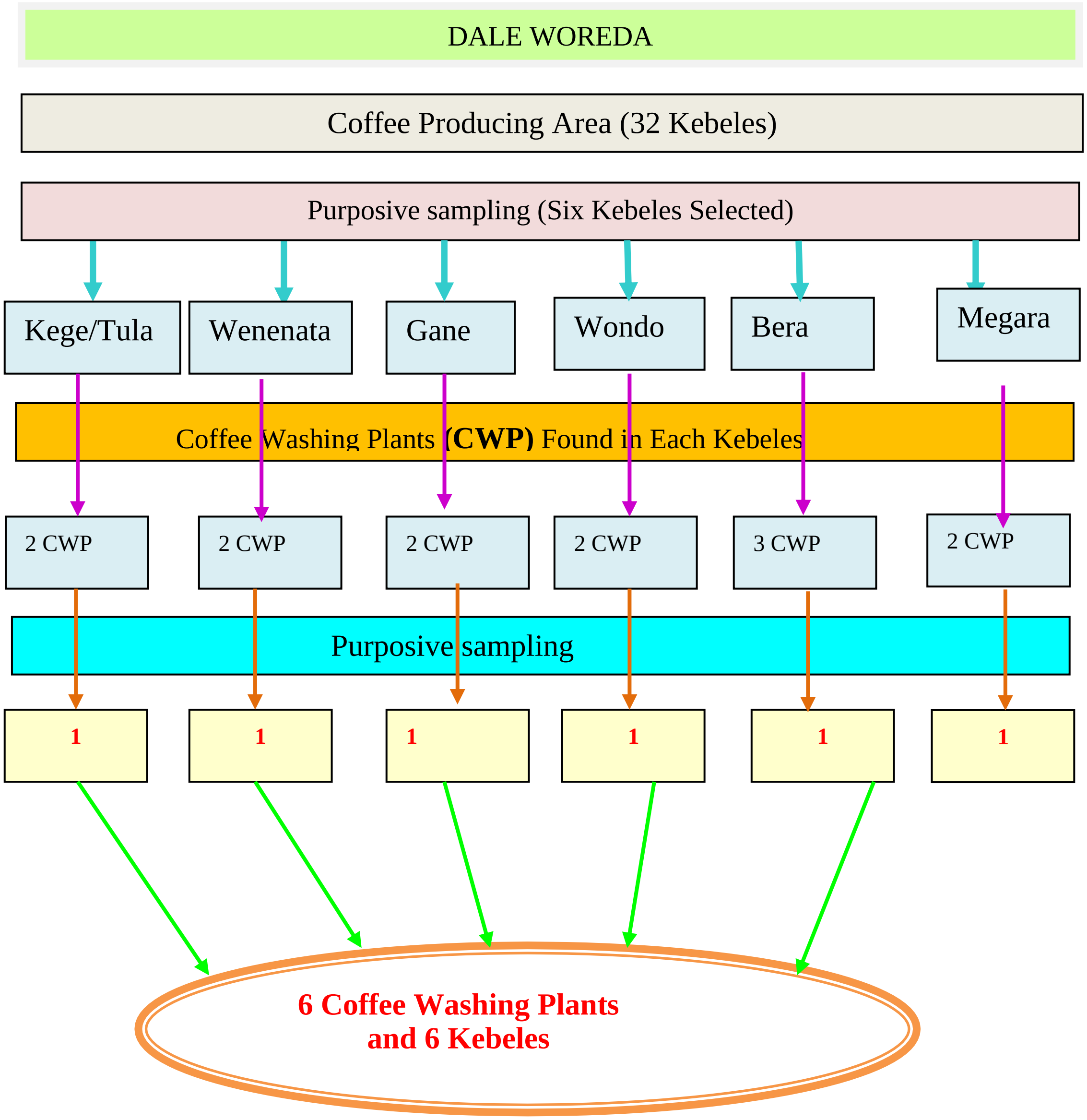

Figure 3.1 Schematic presentation of coffee beans and soil sampling procedure

3.2.3 Materials used in the study

A. Equipment and apparatus

Stainless steel soil sampling auger (Oakfield Apparatus Company, Oakfield, USA), ceramic mortar and pestle, electric motor grinder (Retsch, GmbH & Co. KG Type ZM 1, Hann 1, Germany), digital analytical balance (ADAM, Model AFP-110L, England), round bottom flask (100 mL) fitted with a reflux condenser, borosilicate volumetric flasks (25, 50, and 100 mL), Pipettes (Pyrex, USA), micropipettes (Dragonmed, 1-10 L, 100-1000 L, Shangai, China), a microwave digester (Buck Scientific Model BMS 1, USA), and flame atomic absorption spectroscopy (Buck Scientific Model 210VGP, USA) were utilized in this investigation.

B. Reagents and chemicals

All of the chemicals and reagents used in the experiment are analytical grade. HNO_3 (68 percent-70 percent), H_2O_2 (30 percent), HCl (37 percent) (UNI-CHEM® chemical reagent, China), and 0.1 percent $LaCl_3$ were utilized in this investigation. Stock standard solutions containing 1000 mg/L of the metals in H_2O (UNI-CHEM® chemical reagent, China)(Buck Scientific Puro-Graphic, USA).

C. Apparatus cleaning

Volumetric flasks, measuring cylinders, digestion flasks, and all other necessary apparatus were washed with detergents and tap water, rinsed with deionized water, soaked in 2 percent nitric acid for 24 hours, rinsed with deionized water five times, dried in an oven (Model N50C, England), and stored in a dust-free environment until analysis began.

3.2.4 . Preparation and digestion of samples

A. Preparation and digestion of coffee bean samples

On a firm, flat, clean surface, such as raised tables, the collected coffee cherries were dried in dir ect sunlight. Drying was used to remove moisture from the coffee bean in a slow continuous process as it takes up to 4 weeks before the cherries were dried to the optimum moisture content, depending on the weather conditions. The coffee beans are then removed from the dried husk. After grinding the dried coffee beans, 50 g were used for analysis. Finally, the powdered coffee bean samples were kept in polyethylene plastic bags and stored in desiccators containing calcium chloride to keep at constant dry weight till digestion (Mitra, 2003). Coffee bean samples from washing industries were also prepared in the same way as the beans from farmer's farmland.

For the evaluation of the concentration of elements using Flame Atomic Absorption Spectroscopy (FAAS) and Flame Emission Atomic Spectroscopy (FEAS) methods, samples of coffee require to be solubilized. According to Castro *et al.* (2009), this sample preparation step mostly aims at reducing matrix effects originating from organic compounds and releasing elements in the form of their simple ions. A procedure for the digestion of coffee powder was used as described by Suseela *et al*., (2001) with slight modification as follows. 0.3 g of coffee bean powder was directly weighed in a PTFE digestion vessel, 7 mL of HNO_3 conc. and 2 mL of H_2O_2 conc. were added and the vessels were placed in a fume hood for 10 min for pre-digestion before they were placed on the turntable of the microwave system. Finally, the samples were digested at the optimum conditions (as shown in Table 3.1) and for each bulk sample, the digestion was done three times. Then allowed to cool at room temperature. In parallel to the digestion of the samples, the same method was used to digest a reagent blank maintaining all other digestion settings. Six blanks were digested for bean samples. 0.1% $LaCl_3.7H_2O$ was added to the digested solution to eliminate the chemical interference of Ca and Mg ions and the solution was then filled to the mark (25 mL) with deionized water. The solutions were stored in the refrigerator until analysis.

B. Preparation and digestion of soil samples

Any visible plant remnants were removed, and the soil samples were air-dried. The dried soil samples were ground using pestel and mortar, and sieved by using 2 mm nylon sieves. The total amount of soil samples collected from a single Kebele provided over 500 g of sieved soil, of which 50 g was used for chemical analysis. The sieved soil samples were further dried in an oven at 50°C for one and a half hours to make their moisture content uniform. Finally, the samples were stored in sealed polythene and stored in desiccators containing calcium chloride to keep to constant dry weight till digestion.

For the digestion of soil samples, the EPA 3050B (Epa US, 1996) method was applied. The procedure used for the digestion of the soil sample was as follows: Initially, 500 mg of the dried and sieved soil sample was added into a digestion vessel. Then 10 mL of a solution prepared by mixing 1:1 ratio of HNO_3 and H_2O (deionized) was added into the vessel. Finally, after completion of digestion, the digest soil was allowed to cool, filtered through Whatman No. 42 filter paper and the resulting clear light yellow solution was made up to 50 mL with deionized water. Reagent blanks were also prepared and digested with the same procedure as that of the soil sample. All the solutions were stored in tightly capped polyethylene bottles and stored in a refrigerator until analysis.

3.2.5 Metals determination quality assurance methods

A. Optimization of digestion conditions

Different microwave digestion techniques were used in an effort to obtain a clear and colorless coffee digest solution that was adequate for FAAS analysis. HNO_3 and H_2O_2 volume, microwave digestion temperature, and digestion duration were the main factors. The three aforementioned factors were changed one at a time to create a total of 20 trails. The best digestion method was chosen based on the following factors: the clarity of the digests (solution free of residue and suspended materials), the smallest reagent volume, the shortest microwave digestion time, and the highest temperature. The developed optimum digesting conditions for coffee bean samples were presented in **Table 3.1** below.

Table 3.1 Optimum microwave digestion conditions of coffee bean samples.

step	1	2	3
Temperature ^{0}c	150	200	45
Time in minutes	5	10	10
Power-w	80	85	0

B. FAAS calibration procedure and determination of metals

To figure out the metal concentrations in soil and coffee bean sample solutions, calibration curves were prepared. The calibration curves for each metal were created using diluted stock standard solutions containing 1000 mg/L of each of the following metals: Ca, Cd, Co, Cu, Cr, K, Mn, Na, Ni, Pb and Zn in 2% HNO_3. Contents of the metals in the coffee bean and soil samples were made by using FAAS and flame emission atomic spectroscopy. To avoid loss through ionization, the concentration of Na and K was determined by the emission mode of the instrument. The same analytical approach was used to determine the elements in blank solutions and three replicate determinations for each metal was conducted.

C. Method validation

Method validation is the process used to confirmation by examination and provision of objective evidence that the particular requirements for a specific intended use are fulfilled. Results from method validation can be used to judge the quality, reliability and consistency of analytical results; it is an integral part of any good analytical practice (Ajay and Rohit, 2012, Magnusson B and Örnemark U, 2014). Since there were no approved standard reference materials available, the approach was validated using spiking. Four flasks containing samples of coffee beans were spiked. In four different flasks, 0.3 g of coffee bean powder sample was taken. 200 mL of 1000 mg/L Ca were spiked into the first flask. The second flask was spiked with 150m L of the same K concentration as the first flask. 25 L of 10 mg/L Cu, Mn, and Zn were spiked into the third flask. In the fourth flask, 15 µL of 10 mg/L of Ni, Co and Cr were spiked. All the spiked samples were digested in triplicate following the optimal digestion procedure developed for coffee bean samples.

3.2.6 Analysis of samples for metals

Determination of the metals in the coffee bean and soil samples was made by Flame Atomic Absorption Spectroscopy (FAAS) and Flame Emission Atomic Spectroscopy (FEAS) with an external calibration curve after the parameters such as burner and lamp alignment, slit width and wavelength adjustment were optimized for the maximum signal intensity of the instrument. For each metal, the respective hollow cathode lamp was inserted into the atomic absorption spectrophotometer, and therefore the solution was successively aspirated into the flame. To avoid loss through ionization, the concentration of Na and K was determined by FEAS. For other metals FAAS were used. Three replicate determinations were carried out for each metal and the same analytical procedure was employed for the determination of elements in blank solutions. Calibration curves were prepared to determine the concentration of the metals in coffee bean and soil sample solutions. Stock standard solutions (Buck Scientific Eurographics calibration standards, USA) containing 1000 mg/L of the metals Na , K, Ca, Zn, Mn, Cu, Co, Cr, Ni, Pb and Cd from which 10 mg/L of intermediate standard obtained were used for the preparation of calibration standards of each metal.

3.2.7 Calculation of bioaccumulation factors

Bio-concentration factor (BCF) has been defined that the ratio of heavy metal concentration in the edible part of the plant to heavy metal concentration in the soil sample (Rattan *et al*., 2005, Sharma *et al*., 2018). Accordingly, BCF was calculated by the formula used by (Kachenko and Singh, 2004) and given in Eq (3.1).

$$\mathrm{BCF} = \frac{\text{heavy metal content in plant}}{\text{heavy metal content in respective soil}} \quad \ldots\ldots\ldots\ldots\ldots\ \text{Eq (3.1).}$$

A value of BCF greater than 1 indicates that the plant is a potential accumulator for the metal being considered for analysis (Barman *et al*., 2000).

3.2.8. Soil pollution and Ecological Risk Assessment

Various pollution indices were calculated to investigate the level of pollution caused by heavy metals in the studied area, including the geo-accumulation index (Igeo), contamination factor (CF), Modified contamination degree (mCdeg), Pollution load index (PLI), potential ecological risk, and Potential ecological risk index which are widely used to evaluate heavy metal pollution in surface agricultural soil (Kumar *et al*., 2019, Barakat *et al*., 2019, Ennaji *et al*., 2020). These soil contamination indexes are summarized in the table below.

A. Geo Accumulation (Igeo)

The Geoaccumulation Index (*Igeo*) was calculated using the equation proposed by Muller (1969). $Igeo = \log_2(Cn/1.5Bn)$; where, Cn: the content of heavy metal in soil of the study area, *Bn* is the background value of the corresponding metal (*n*), and factor 1.5 is the background matrix correction due to lithogenic effects (Salomons and Forstner, 1984). $Igeo = log2\,\frac{Cn}{1.5Bn}$………………………………………….. Eq (3.2).

The *Igeo* classification proposed by Muller (1969) was used to determine the level of contamination. The seven classes in this classification are: uncontaminated ($Igeo \leq 0$; Class 0), uncontaminated to moderately contaminated (*Igeo*0–1; Class 1), moderately contaminated (*Igeo*1–2; Class 2), moderately to highly contaminated (*Igeo*2–3; Class 3), highly contaminated (*Igeo*3–4; Class 4), highly to very highly contaminated (*Igeo*4–5; Class 5), and very highly contaminated ($Igeo \geq 5$; Class 6) (Muller, 1969).

B. Contamination factor (CF)

CF provides the information to assess the pollution level of individual elements in the polluted soil as compared to the pre-industrial reference value for the same metals.Because there are no data on pre-industrial levels of heavy metal concentrations for the research site and no national heavy metal criteria in Ethiopia, Concentration of element in earth crust was used as a reference value (Loskaa *et al.*, 2004). Accordingly the reference value are Ni(75), Zn(70), Co(10), Cu(55), Cr(100), Mn(600).Pb(12.5) and Cd(0.15).

$CF = \frac{Ci}{Cn}$………………………………………………………………… Eq (3.3).

Where Ci : content of heavy metal in soil;

Cn: background value of heavy metal element i.

C. Pollution load index (PLI)

The pollution load index (PLI) provides simple but comparative means for assessing the level of heavy metal pollution (Tomlinson *et al*., 1980).Each sample site can be evaluated for the extent of the metal pollution by employing the method based on the pollution load index(PLI) developed by (Tomlinson *et al*., 1980) as below.

$\sum_{n=1}(\mathrm{CF1XCF2.CFn})\ 1/n$…………………………………………………… Eq (3.4).

Where CF is the contamination factors,n is the number of metals studied

CF^n is the contamination factors for the nth elements .According to Tomlinson *et al*., (1980) PLI <1 means that there is no indication of pollution , 1<PLI≤2 is indication of Moderate level of pollution , 2<PLI≤ 5 is high level ,and PLI>5 is extremely high level

D.Modified contamination degree (mCdeg)

It is the sum of all the contamination factors for a given set of samples divided by the number of analyzed metals.

mCdeg = ∑ CF÷n……………………………………………………… Eq (3.5).

i=1

Where CF: contamination factor of single heavy metal; n: number of heavy metals

E.Potential ecological risk factor (ERi.)

Potential ecological risk factor (ERi) is now widely used to assess the ecological risk of heavy metals in soil and it is the potential ecological risk factor of a single heavy metal.

$ERi = CFiXTRi$………………………………………………………………. Eq (3.6).

Where Tri : toxicity response coefficient of heavy metal; CFi : contamination factor of heavy metal.

The toxic response factors for Ni(6),Zn(1),Co(5),Cu(5),Cr(2),Mn(10).Pb(5) and Cd(30).

F. Potential ecological risk index(RI)

Potential ecological risk index (RI) initially is widely adopted to evaluate the potential ecological risk of the studied heavy metals in the soil

RI = $\sum$ ERi………………………………………………………………….. Eq (3.7).

i=1

Where ERi : potential ecological risk factor for heavy metal; n–number of analyzed heavy metals

Table 3.2 Summary of soil contamination indexes

Indices	Formula	Description	Limit Values	Classification	References
Index of geoaccumulation (Igeo)	$Igeo = log2\frac{Cn}{1.5Bn}$ Where Cn: the content of heavy metal in soil; Bn: background value; 1.5: constant	To assess the heavy metal contamination level in soils by comparing metal content in top soil with the geochemical background.	<0	Class 0: practically uncontaminated	(Muller, 1969)
			0-1	Class1:uncontaminated to moderately contaminated	
			1-2	Class 2: moderately contaminated	
			2-3	Class 3: moderately to heavily contaminated	
			2-4	Class 4: heavily contaminated	
			4-5	Class 5: heavily to extremely contaminated	
			>5	Class 6: extremely contaminated	
Contamination factor (CF)	$CF = \frac{Ci}{Cn}$ Where Ci : content of heavy metal in soil; Cn: background value of heavy metal element i. Accordingly the reference value are Ni(75),Zn(70),Co(10),Cu(55),Cr(100),Mn (600).Pb(12.5) and Cd(0.15)	CF provides the information to assess the pollution level of individual elements in the polluted soil as compared to the pre-industrial reference value for the same metals. Because there are no data on pre-industrial levels of heavy metal concentrations for the research site and no national heavy metal criteria in Ethiopia, Concentration of element in earth crust was used as a reference	<1	Class 1: low contamination	(Håkanson, 1980)
			1-3	Class 2: moderate contamination	
			3-6	Class 3: considerable contamination	
			>6	Class 4: very high contamination	

		value(Loskaa et al., 2004)			
Pollution load index (PLI)	$\sum_{n=1}(\mathrm{CF1XCF2.CFn})\,1/n$	PLI is often used to measure the average amount of soil pollution. This index provides a direct way to display the soil deterioration resulting from the accumulation of potential toxic elements	PLI≤1	Low level	(Tomlinson et al., 1980)
			1<PLI≤2	Moderate level	
			2<PLI≤ 5	High level	
			PLI>5	Extremely high level	
Modified contamination degree (mCdeg)	$\mathrm{mCdeg} = \sum_{i=1}^{n} \mathrm{CF} \div n$ Where CF: contamination factor of single heavy metal; n: number of heavy metals	It is the sum of all the contamination factors for a given set of samples divided by the number of analyzed metals	<1.5	Nil to very low degree of contamination	
			1.5-2	Low degree of contamination	(Håkanson, 1980)
			2-4	Moderate degree of contamination	
			4-8	High degree of contamination	
			8<16	Very high degree of contamination	
			16-32	Extremely high degree of contamination	
			>32	Ultra high degree of contamination	
Potential ecological risk factor (ERi.)	$ERi = CFiXTRi$ Where Tri : toxicity response coefficient of heavy metal; CFi : contamination factor of heavy metal. The toxic response factors for Ni(6),Zn(1),Co(5),Cu (5),Cr(2),Mn(10).Pb(	ERi is now widely used to assess the ecological risk of heavy metals in soil and it is the potential ecological risk factor of a single heavy metal.	<40	Low potential ecological risk	
			40-80	Moderate potential ecological risk	(Håkanson, 1980)
			80-160	Considerable potential ecological risk	
			160-320	High potential ecological risk	
			>320	Very high potential ecological risk	

	5) and Cd(30).				
Potential ecological risk index	$RI = \sum_{i=1}^{n} ERi$ Where Eri : potential ecological risk factor for heavy metal; n– number of analyzed heavy metals	RI initially is widely adopted to evaluate the potential ecological risk of the studied heavy metals in the soil	<150	Low ecological risk	(Håkanson, 1980)
			150-300	Moderate ecological risk	
			300-600	Considerate ecological risk	
			>600	Very high ecological risk	

3.2.9 Data analysis

An analysis was done using SPSS version 24 ANOVA was used to determine the significant differences in the mean concentration of metal within in coffee bean sample from the washing industries/Plants at $p < 0.05$ significant level. Additionally, t-tests had been performed in order to check whether there was a difference in metals concentration between coffee beans from farmland and washing industries. Finally, to correlate the effect of one metal concentration on the concentration of the other metal in the coffee beans samples, the Pearson correlation matrices using correlation coefficient (r) for the samples were used. Data were presented using tables, figures, etc. Mean and percentage removal efficiency calculations were done to describe the data.

3.3 Result and Discussion

3.3.1. Metal concentration in soil samples

The highest mean Ni concentration (0.194±0.009 ppm) was recorded in soil sampled from Kege Kebele (**SS1**) and the lowest (0.172±0.002ppm) was recorded in Bera Kebele (**SS5**). The concentration of Ni (0.194±0.009 ppm) soil sample in the present study is much lower (8.33 ± 0.55ppm) than that reported by the previous study (Dubale, 2021) in Ethiopian farms where coffee is grown, reported by (Ndungu *et al*., 2019) Ni (5.1 ± 3.8ppm) in agricultural land in Kenya, reported by (Tezottoa *et al*., 2012) Ni (133.1ppm) in Brazil farm where coffee is grown and reported by (Santos *et al*., 2009) Ni (1.92 ± 1ppm) from Brazilian coffees cultivated area. .However, the finding of the present study, was much higher (0.05 ppm) than that reported by (Raghavendra and Venkatesha, 2020) coffee plantations in the Western Ghats Region, Chikkamagaluru District, Karnataka, India. This is owing to the use of diverse farming practices, as well as geographical and meteorological differences between the study areas. Moreover, the application of livestock manure for the agricultural soil is a common practice in the study areas and it could be the possible reason for the high Ni concentration in the soil (Basta *et al*., 2005). The Ni levels analyzed was below the permissible limit set by FAO/WHO, 2001 (50.00 ppm) thereby these soil are free of its contamination.

The highest mean Zn concentration (0.163±0.007ppm) was recorded in soil sampled from SS1 and the lowest (0.141±0.001ppm) was recorded in SS3. The finding of the present study revealed that the concentration of zinc in the soil sampled was lower (52.5 ± 6 ppm) than that reported by (Santos *et al*., 2009) from the Brazilian coffee cultivated area, Tozottoa *et al*,. (2012) reported Zn (83.0 ± 33.5ppm) concentration, reported by (Ndungu *et al*., 2019) Zn (10.0 ± 3.1ppm) in agricultural land in Kenya and by (Dubale, 2021) Zn (47.14 ± 2.51ppm) from Gedeo Zone, Ethiopia. On the other hand, the present study finding much higher than reported by (Raghavendra and Venkatesha, 2020) Zn was (0.05 ppm). These differences in concentration of the heavy metals could be explained by the application of different methods of farming, geographical and climatic variation and human activity such as industrialization and urbanization in the study area. Moreover, the application of livestock manures for agricultural land is the common practice in the study area (Basta *et al*., 2005). The mean concentration of Zn in soils from all sample sites were below permissible limit set by FAO/WHO, 2001 (1000 ppm).this indicates that the soil from all sample sites is safe for agriculture with regard to the pollution of zinc.

The mean Co concentration was reported in two sites: 5.43±0.305ppm in soil samples from SS1 and 4.27±0.20 ppm at SS2. The highest mean Co concentration of the present study (5.43±0.305 ppm), when compared to Co concentration from other studies within Ethiopia, the concentration was lower than Co concentration obtained by (Dubale, 2021) that had high mean values of 11.66 ± 0.78ppm. However, the finding of the present study was higher than Co (3.57) concentration reported by (Oladeji and Saeed, 2015). This could be due to anthropogenic sources of contamination, such as the widespread use of manure, herbicides, fungicides, and fertilizers, which are high in Co as suggested by (Yasmeen *et al*., 2010).

The highest mean Cu concentration (49.96±0.99ppm) was recorded in soil sampled from SS3 and the lowest (22.34±0.25ppm) was recorded in SS4. The concentration of Cu (49.96±0.99ppm) in soil sample of the present study, is much lower (76.9 ± 1.34ppm) than that reported by (Dubale, 2021) in Ethiopian farms where coffee is grown. However, the concentration of Cu reported by the present study is much higher (7.10 ± 0.30ppm) than that reported by (Santos *et* *al*., 2009), reported by (Tezottoa *et al*., 2012) Cu (36.8 ± 5.9) in Brazil farm where coffee is grown, and by (Ndungu *et al*., 2019) Cu (0.26 ± 0.17) in agricultural land in Kenya. Numerous variables, such as air deposition from vehicle emissions or heavy traffic along the Hawassa-Moyale road, could be to cause of the heighest Cu concentration (Szwalec *et al.,* 2020). Furthermore, coffee species, geographic origin, coffee variation, usage of fertilizers with various chemical compositions, and other differentiating elements all have a substantial impact on the actual metal content of coffee beans (Kamal *et al.,* 2008). Copper concentration levels reported in this study were below the permissible limits for agricultural land use of 300ppm (FAO/WHO, 2001) implying that there was no Cu contamination in the soil.

Cadmium levels in the soil shown in Table 3.3, the mean concentration of Cd varied between 2.38 ± 0.044 and 3.36 ± 0.1ppm with the highest in the sample from SS1 and the lowest from SS4. In this study, the highest mean Cd concentration was (3.36 ± 0.1ppm), which is much lower (124.3 ppm) than reported by (Tezottoa *et al.*, 2012). However, higher than (0.1 ± 0.03 ppm) reported by (Ndungu *et al*., 2019) in agricultural land of Kenya; Cd (0.001 ± 0.0009ppm) reported by (Raghavendra and Venkatesha, 2020) coffee plantations in the Western Ghats Region, Chikkamagaluru District, Karnataka, India. On the other hand, values reported for Cd (3.49 ±0.26 ppm) by (Dubale, 2021) are comparable with the results obtained in the present study. These differences might be due to the difference in the anthropogenic sources like the application of fertilizers (Raymond and Felix, 2011). Cadmium levels reported in the soil are above the levels 3ppm, the permissible limit for agricultural soil (FAO/WHO, 2001). Therefore, there was Cd contamination in the soil.

Calcium had the highest concentration (1355 ± 18.02ppm) of macro elements in all soil samples, followed by K (681.43 ± 1.52ppm) and Na (111.63±0.35 ppm). Similarly, Cu (49.96 ± 0.99ppm) was detected in greater abundance among the microelements, followed by Mn (0.62 ± 0.238ppm), and Zn (0.163 ± 0.007ppm). Co>Cd>Ni was assigned to the remaining trace metals (**Table 3.3**). Ca^{2+} is present in three major forms in the soils: in solution, bound to exchangeable sites of clay minerals and organic matter and as minerals. Only a small fraction of the total Ca^{2+} is present in the soil solution. Plant roots can absorb only Ca^{2+} from soil solution (Ramírez-Builes et al., 2020). Several factors might influence the availability of Ca^{2+} in the soil solution, such as soil type, mineral fraction of the colloids, pH, organic carbon content, humid acids and cation exchangeable capacity (Ramírez-Builes *et al*., 2020).

In soil samples from six kebeles, metals such as Pb, and Cr were found to be not detected. Kege Kebele soil sample analysis shows that Ca (1355 ± 18.02 ppm) was the highest of all the detected metals. K and Na were found in the highest amount next to Ca with values of 673 ± 2.65ppm and 111.63 ± 0.35 ppm, respectively.

Out of the analyzed microelements, Cu (42.66 ± 2.52 ppm) was found to be in the highest amount followed by Co (5.33 ± 0.305 ppm) and Mn (0.62 ± 0.238 ppm). Similarly, the levels of Cd, Ni and Zn were 3.1 ±0.1 ppm, 0.191 ± 0.009 ppm and 0.161 ± 0.007 ppm, respectively. The average metal concentrations in the kege soil were determined to be in the following order: Ca>K>Na>Cu>Co>Cd> Mn>Ni>Zn (**Table 3.3**).

Table 3.3 Mean metal concentrations in soil samples

	Kege(SS1)	Wenenata(SS2)	Gane(SS3)	Wondo(SS4)	Bera(SS5)	Magara(SS6)	*MPL(FAO/WHO, 2001)
Ni	0.194±0.009	0.181±0.001	0.191±0.001	0.181±0.001	0.172±0.002	0.178±0.001	50.0
Zn	0.163±0.007	0.146±0.002	0.141±0.001	0.155±0.005	0.153±0.002	0.146±0.005	1000.0
Co	5.43±0.305	4.27±0.20	ND	ND	ND	ND	50.0
Cu	42.46±2.52	47.48±0.58	49.96±0.99	22.34±0.25	47.7±1.15	45.26±1.52	300.0
Cr	ND	ND	ND	ND	ND	ND	75.0
Ca	1355±18.02	1538.67±3.2	445±0.99	346.67±2.51	1352.67±2.5	1401±1.1	-
Mn	0.62±0.238	0.5±0.001	0.467±0.015	0.48±0.002	0.49±0.001	0.50±0.0005	-
Na	111.63±0.35	26.52±0.04	26.77±0.02	13.37±0.032	112±0.10	110.3±0.10	-
K	673±2.65	403.47±1.53	603±0.10	604.61±0.58	681.43±1.52	680.43±0.57	-
Pb	ND	ND	ND	ND	ND	ND	50.0
Cd	3.36±0.1	2.53±0.058	2.67±0.057	2.38 ±0.044	2.93±0.015	3.01±0.0058	3.0

***MPL**=Maximum Permissible Limit for Agricultural soils according to FAO 2001

Pearson's correlation coefficient was used to examine the correlations between the contents of different elements in the soil. According to Sharma and Raju (2013), a high correlation coefficient (near +1 or -1) indicates a good relationship between two variables, whereas a concentration around zero indicates no relationship at a significant level of 0.05 **%** level. If r > 0.7, it is strongly correlated, whereas r values between 0.5 and 0.7 indicate a moderate correlation between two different parameters.

The analysis revealed that there was a significant (p<0.01, p<0.05) positive correlation between heavy metals; Cd-Zn (r=0.511), Cd-Ca (r=0.507), Cd-Na (r=0.81), Cd-K (r=0.57), Ni-Co (r=0.882), Zn-Co (r=0.469), Cu-Ca (r=0.537), Ca-Na (r=0.652), Na-K (r=0.69); all have positive correlations (**Table 3.4**). Other's correlations were found to be non-significant, such as Cd with Ni, Co, Cu, Mn, and K, Ni found to be non-significant with Zn, Cu, Ca, Mn, and K , Zn also found to be non-significant with Cu, Ca, Mn, Na and K, Cu found to be non-significant with Mn, Na and Similarly Ca found to be non-significant with Mn and K. Likely Mn found to be non-significant with Na and K indicating that the concentration of one element may not have an impact on the concentrations of other elements in the study area. The positive correlation between Cd with Zn, Ca, Na and K indicate a correlation among the metals in farmland soil. The rise in levels of Cd increases the tendency of Zn, Ca, Na and K to increase. The rise in levels of Cd increases the tendency of Zn, Ca, Na and K to increase. This is in line with previously reported data reported by Dubale (2021) found that Mg, Ca, Cr, Mn, Zn and Co in farmland soils in Gedeo, Ethiopia may have a similar origin determined by a correlation study of soil heavy metals. Similarly,this is inline with the previousstudy reported by (Zhao *et al.*, 2019). They investigated the relationship between some selected heavy metals (Cd, Cu, Pb, Cr, Hg, As and

Zn) and found a correlation between Cd and Zn. Though there is a correlation between Cd and Zn in this study.

Table 3.4 Correlation Analysis of Heavy Metals of Soil in the selected Kebele of Dale Woreda

	Cd	Ni	Zn	Cu	Ca	Mn	Na	K
Cd	1							
Ni	0.337	1						
Zn	0.511*	0.241	1					
Co	0.359	0.482*	0.469*					
Cu	0.363	0.035	-0.421	1				
Ca	0.507*	-0.285	0.144	0.537*	1			
Mn	0.135	0.155	0.334	-0.014	0.266	1		
Na	0.81**	-0.161	0.396	0.369	0.65**	0.325	1	
K	0.57*	0.012	0.379	-0.075	-0.096	0.151	0.69**	1

* Correlation is significant at the 0.05 level (2-tailed). ** Correlation is significant at the 0.01 level

3.3.2. Metals in coffee bean sample from farmland

Potassium (K) was found to have the greatest concentration (99.93±0.037ppm) among the macro elements detected in coffee beans from all sampling farmland sites (Kege, Wenenata, Gane, Wondo, Bera, and Magara). As suggested by (Weis and Weis, 2004), the highest concentrations of K in coffee beans are likely to be attributable to the fact that nutrients like K,N, P, S, and Mg are highly mobile in plant tissues and can be translocated from old to young plant tissues.

According to (Çalişkan and Çalişkan, 2017), another reason for increased K concentrations is that this element is among the most important nutrients for plants and except for N, potassium is the element that plants absorb the most compared to other elements.

The concentration of K reported by the present study, which is much lower (99.93 ± 0.037ppm) than that reported by (Janda *et al*., 2020) K (18,634.66 ± 538.67) in Poland and by (Dubale, 2021) K (15042.8 ± 53.29ppm) from Gedeo Zone, Ethiopia. On the other hand, values reported for K(21.31-427.84ppm) by (Omer *et al.*, 2019) are comparable with the results obtained in the present study. The studied coffee beans were above the permissible limit set by FAO/WHO which is 32500ppm in plants. K is important for its diuretic nature and helps in the proper function of the brain as well as nerves, thereby preventing stroke. It plays part in acid-base and water regulation in the blood and tissues. K has been reported that a high potassium diet lowered blood pressure in individuals with raised blood pressure(He and MacGregor, 2008).

The finding of the present study revealed that Na and Ca were also detected in significant amounts in coffee bean samples from farmers' farms, with concentrations of 22.04 ± 0.042 ppm and 17.23 ± 0.36 ppm, respectively. This result is consistent with Na concentration (6.84-564.74 ppm) and Ca concentration (6.76-32.09 ppm) reported by (Omer *et al*., 2019) in Saudi Arabia and reported by (Adler *et al*., 2019) Na concentration (18.6 ± 11.31ppm) in the green coffee bean. However, the finding of Ca concentration (17.23 ± 0.36 ppm) in the present study was much lower (1252.93 ± 30.17 ppm) than that reported by (Dubale, 2021) from Gedeo Zone and reported by (Janda *et al*., 2020) Ca (1441.20 ± 49.49 ppm) in Poland. The finding of Na (22.04 ± 0.042 ppm) concentration in the present study, which was much higher (1.8–9.3 ppm) than that reported by (Martín *et al*., 1998) in the green coffee bean and Such great differences in Na concentration were also reported by (Grembecka *et al*., 2007) Na concentration (6.1 ± 2.3 ppm).

This variation could be attributed to the type of soil where the coffee was cultivated (Santos and Oliveira, 2001).

The highest concentrations observed for Manganese in this study was 0.927±0.004 ppm. When we compare the present experimental value with different countries earlier reported. The finding of the present study was much lower (23.60 ± 1.30 ppm) than reported by (Dubale, 2021). However, fairly agrees with the range (0.48-28.69 ppm) reported by (Omer *et al.*, 2019). The soil-plant system is highly specific for different, elements; plant species and environmental conditions (Gure *et al.*, 2017). As a result, coffee species, geographic origin, coffee kind, the application of fertilizers with varying chemical compositions, and other distinguishing characteristics all have a significant impact on the actual metal content of coffee beans (Kamal *et al.*, 2008). Mn has a high solubility at low pH, and its concentration in acidic soil is likely to be high (Wood, 1985) The permissible limit set by FAO (1993) for edible plants is 2 ppm.

Table 3.5 Concentration of non-toxic metal concentration in coffee bean sample from farmland in Dale Woreda, Ethiopia, 2021

		K	**Ca**	**Na**	**Mn**
Kege		99.93±0.037	15.15±0.614	22.04±0.042	0.927±0.004
wenenata		69.06±0.047	17.23±0.36	5.73±0.049	0.105±0.003
Gane		80.587±0.08	3.05±0.04	6.927±0.038	0.082±0.003
Wondo		78.10±1.081	3.96±0.063	3.10±0.10	0.088±0.003
Bera		69.36±0.55	16.11±0.11	5.73±0.049	0.105±0.003
Magara		99.27±0.714	15.33±0.189	21.077±0.99	0.093±0.004
*MPL	Medicinal plant	32500	-	-	-
	Edible plant	-	-	-	2

*MPL=Maximum Permissible Limit for medicinal and edible plants according to FAO 1993

The highest concentration of Ni (0.074 ± 0.003 ppm) reported by the present study, which is in good agreement with Ni (0.05–0.39 ppm) reported by (Grembecka *et al.*, 2007) and Ni (0.07 ± 0.11ppm) reported by (Omer *et al.*, 2019). However, the results presented in the present study are generally lower (0.415 ± 0.04 ppm) than those reported by (Adler *et al.*, 2019) in the green coffee bean, and reported by (Dubale, 2021) Ni (2.43 ± 0.14 ppm). The concentration of Ni elements is quite varied and this is associated with a vast influence of the origin (especially the type of soil where coffee plants are cultivated), the variety and the type of coffee, processes involved in the production of natural or soluble coffees and means of the confection and the storage of coffee (Santos and Oliveira, 2001, Grembecka *et al.*, 2007). The maximum permissible limit for Ni set by FAO (1993) for edible plants is 1.63 ppm.

Zinc concentration in the samples analyzed ranged between 0.054 - 0.076 ppm, which was much lower than Zinc (Zn) concentration ranged between (3.74 to 46.89 ppm) reported by (Nogaim *et al.*, 2014) in Yemeni green coffee beans, reported by (Silva *et al.*, 2017) Zn (5.53 to 55.83 ppm) in Brazil the roasted and ground coffee beans, reported by*(*Adler *et al.*, 2019) Zn (3.09 - 4.04 ppm) in the green coffee bean, and reported by (Dubale, 2021) Zn (8.74-12.75 ppm). However, this result is consistent with Zn concentration (0.0 - 4.59 ppm) reported by (Omer *et al.*, 2019) in Saudi Arabia. Zinc concentrations vary greatly depending on several factors, including the coffee's origin, variety, and type, the processes involved in producing natural or soluble coffees, and the methods of coffee confection and storage (Ashu and Chandravanshi, 2011).

In the present study, Co and Cr were not found. This finding is consistent with the previous study reported by Getachew and Worku (2014) for raw and roasted coffee beans and reported; by Santos and Oliveira (2001) in Brazilian soluble coffee. However, Abera Gure *et al.* (2017) have reported Co (2.6 - 8.4 ppm), and Cr (0.21 - 0.28 ppm) concentrations in Ethiopian green coffee beans. Similarly, Dubale (2021) have reported Co (2.47- 2.86 ppm), and Cr (1.04 - 1.92 ppm) concentrations in Ethiopian green coffee beans. Co and Cr have main sources of pollution from rock weathering, fertilizers, pesticides, mining, and manufacturing (Zhou *et al*., 2020). These sources may be responsible for the significant differences in the Co and Cr contents in the green beans. Due to the foolhardy use of fertilizers, wastewater discharge, coal and motor fuel combustion processes, and increased mining of the cobalt ore, the concentration of naturally occurring Co has increased (Saaltink *et al*., 2014). The maximum permissible limit for Chromium set by FAO (1993) for edible plants is 0.02 ppm. However; there was no maximum permissible limit for Cobalt set by FAO (1993) for edible plants.

Copper concentration the present study ranged between 0.14 - 0.28 ppm, Which is much lower (12-13 ppm) than the previous study reported by Getachew and Worku (2014) ; Abera Gure *et al.* (2017) Cu (11-23 ppm) for raw coffee bean; Adler *et al.* (2019) Cu (9.4-18.5 ppm) for green coffee; and Dubale (2021) have reported Cu (23.39-28.5ppm) coffee from farmland. However, the finding of the present study closely resembles the 0.13-10 ppm range reported by (Omer *et al*., 2019). The Cu was found low concentration in samples of coffee from farmland. The reason for this low concentration is that the soil where coffee plants growth and environmental conditions which affect the concentration (Ayele *et al*., 2015). There are different factors which affect the concentration such as fertilizer used with different chemical compositions, coffee species and fertilized land where crops were grown (Suseela *et al*., 2001). FAO/WHO (1993) defined an acceptable level of 3.00 ppm in edible plants. After comparing the metal limit in the investigated coffee beans to the FAO/WHO (1993) recommendations, it was discovered that all coffee beans collect Cu below this level. However, the WHO (2005) limits for Cu have not yet been determined for medicinal plants. Cu acceptable limits in medicinal plants were determined by China and Singapore at 20 ppm and 150 ppm, respectively (WHO, 2005).

In the coffee beans of the selected study site, Pb and Cd were not found. This finding is consistent with the previous study reported by Getachew and Worku (2014) for raw and roasted coffee beans; Abera Gure *et al.* (2017) for raw coffee beans and Dubale (2021) for coffee bean sample from farmland. However, Adler *et al.* (2019) have observed higher values for Pb (0.076 ± 0.0956) in Bosnia green coffee and reported by (Nogaim *et al.*, 2014) Pb (0.599–7.989 ppm) in Yemeni green coffee beans. This indicates that coffee beans' chemical composition differs depending on the region in which they are grown. These hazardous metals were not detected in the present study might be due to no environmental degradation due to industrial operations or chemical contamination from sources such as vehicles and pesticides in Dale Woreda. Furthermore, the absence of commercial fertilizers and pesticides for coffee plantations in Ethiopia may be evidenced by the low amounts of hazardous metals (Gure *et al.*, 2017). This may probably confirm that Ethiopian coffee is cultivated without the use of fertilizers. Furthermore, Pb and Cd have no nutritional value for humans, despite their very low concentration. As a result, consumers will not be exposed to any health risks as a result of these hazardous substances in dale Woreda coffee beans. The maximum permissible limit for lead and cadmium set by FAO (1993) for edible plants is 0.43 ppm and 0.21 ppm respectively.

In coffee beans from Kege farmer’s farms, Mn was determined to be the most abundant minor element, followed by Cu, Zn and Ni. Likewise, the levels of Cu (0.22 ± 0.0026 ppm) and Zn

(0.073 ± 0.003 ppm) were higher than that of Ni (0.055 ± 0.004 ppm). Except for Pb, Cd, Co and Cr, which had not been detected in the coffee bean, all kinds had equal minor element concentrations. In farmer's coffee beans from Wenenta, Wondo, Gane, Bera, and Magara Kebele, almost the same distribution pattern of metals was detected as in Kege Kebele.

Table 3.6 Concentration of toxic metal concentration in coffee bean samples from farmer's farms in Dale Woreda, Ethiopia, 2021

		Ni	Zn	Co	Cu	Cr	Pb	Cd
Kege		0.055±0.004	0.073±0.003	ND	0.22±0.0026	ND	ND	ND
Wenenata		0.053±0.003	0.065±0.004	ND	0.24±0.002	ND	ND	ND
Gane		0.074±0.003	0.055±0.002	ND	0.28±0.0037	ND	ND	ND
Wondo		0.052±0.005	0.07±0.0017	ND	0.14±0.0020	ND	ND	ND
Bera		0.057±0.003	0.067±0.002	ND	0.234±0.01	ND	ND	ND
Magara		0.057±0.008	0.07±0.001	ND	0.22±0.0005	ND	ND	ND
MPL	Medicinal plant	1.63	No MPL	No MPL	20	2	10	0.3
	Edible plant	No MPL	27.4	No MPL	3	0.02	0.43	0.21

3.3.3. Transfer factor of trace metals from soil to coffee bean/Bio-concentration factor

The amount of trace metals taken up by plants was calculated to determine the Bio-concentration factor (BCF) of trace metals from soil to plant. The principal pathway for potentially hazardous metals to enter the food chain is by the movement and deposition of heavy metals from soil to edible parts of plants (Sharma *et al.*, 2018). Bio-concentration factor is a significant measurement for the pollution assessment of soils with the highest level of trace metals. A value of BCF greater than 1 indicates that the plant is a potential accumulator for the metal being considered for analysis (Barman *et al*., 2000). The amount and types of heavy metals present, plant species, soil physicochemical properties, and other factors all affect how quickly heavy metals are transferred to and accumulated by plants at different rates (Sharma *et al*., 2018, Fonge *et al*., 2021). As a result, the transferability of heavy metals from soil to the plant species studied in this study was assessed. Table 3.7 shows the bio-concentration factor (BCF) data for the heavy metals studied.

Mn had the highest bio-concentration factor/transfer factor (1.495) among the elements examined. However, among the examined samples, Cu had the lowest transfer factor (0.0048), which is at the Magara locations (SS6). In Kege Kebele (SS1), the overall transfer pattern for trace metals was manganese > zinc > nickel > sodium> potassium > calcium > copper (**Table 3.7**). This demonstrates clearly that Mn bioaccumulation factors were higher in coffee samples than for other metals. This shows that the trace metal might originate from natural sources and is available for uptake and has a low retention rate of the metal in soil (EU, 2002). The higher transfer factor of Mn in the coffee bean can create a chance for higher human consumption. Mn influences plant function in addition to being harmful to human health. Suresh observed that excess soil Mn disrupted the stomata function in the plant(Suresh *et al*., 1987). Mn also caused human lung injuries like cough, bronchitis, and pneumonitis along with damage lungs (Boojar and Goodarzi, 2002).

In the analyzed samples, Cu had the lowest transfer factor (0.0048), which is at the Magara locations (SS6). It was apparent that the TF of some metals (Cu) decreased when the plants were grown in the soil with higher contamination (Mirecki *et al.,* 2017). It might become more tightly bound to the soil and alter the composition of the soil. This shows that the plant tissue absorbed most of the soil's trace metal concentration. The general pattern of trace metal transfer in Kege Kebele (SS1) was manganese > zinc > nickel > sodium> potassium > calcium > copper (**Table 3.7**). This clearly shows that Mn bioaccumulation factors in coffee samples were higher than for other metals.

Table 3.7 Bio-concentration factor (BFC) of heavy metals analyzed in coffee beans grown by Dale Woreda and its farmland

Sampling area	**Transfer of trace metals**						
	K	Ca	Na	Mn	Ni	Zn	Cu
Kege	0.149	0.011	0.197	**1.495**	0.284	0.448	0.0052
wenenata	0.171	0.011	0.216	0.21	0.293	0.445	0.0051
gane	0.134	0.0068	0.258	0.176	0.387	0.390	0.0056
wondo	0.129	0.0114	0.232	0.183	0.287	0.452	0.0063
Bera	0.102	0.0119	0.051	0.214	0.331	0.438	0.0049
Megara	0.146	0.0109	0.191	0.186	0.320	0.479	0.0048

3.3.4. Metals in coffee bean samples from the washing industry.

The beans from Megara coffee washing industries, like coffee beans from farmer's farms, have the maximum amount of K (77.93 ± 0.115 ppm), followed by Na (10.47 ± 0.058 ppm) and Ca (3.55 ± 0.114 ppm). In the washing industry's beans, Mn (0.92 ± 0.001ppm) was the highest accumulated trace metal, followed by Cu (0.277 ± 0.011 ppm) and Zn (0.094 ± 0.004 ppm). Other critical trace metal concentrations found in coffee beans were Ni (0.074 ± 0.003 ppm). The results show that the concentrations of Co, Cr, Pb and Cd were not detected. The distribution of metals in coffee beans from all six Kebele's washing industries follows the same trend, as illustrated in (**Table 3.8**). One-way ANOVA showed the results of metal concentration showed that no significant differences were found ($p > 0.05$) at 95% confidence levels for Ca, Cu, Mn and Ni in coffee samples collected from all washing plants.

The beans from Megara coffee washing industries, like coffee beans from farmer's farms, have the maximum amount of K (77.93 ± 0.115 ppm), followed by Na (10.47 ± 0.058 ppm) and Ca (3.55 ± 0.114 ppm). In the washing industry's beans, Mn (0.92 ± 0.001 ppm) was the highest accumulated trace metal, followed by Cu (0.277 ± 0.011 ppm) and Zn (0.094 ± 0.004 ppm). Other critical trace metal concentrations found in coffee beans were Ni (0.074 ± 0.003 ppm). The results show that the concentrations of Co, Cr, Pb and Cd were not detected.

Table 3.8 Mean concentration (mean ± SD, n = 3, ppm) of elements in coffee bean sample from the washing industry in Dale Woreda, Ethiopia, 2021

	Kege	**Wenenata**	**Gane**	**Wondo**	**Bera**	**Magara**
Ni	0.07±0.003^{a}	0.05±0.002^{a}	0.05±0.004^{a}	0.07±0.002^{a}	0.05±0.001^{a}	0.07±0.001^{a}
Zn	0.05±0.004^{d}	0.06±0.004^{c}	0.08±0.002^{b}	0.094±0.004^{a}	0.07±0.004^{c}	0.05±0.002^{d}
Co	ND	ND	ND	ND	ND	ND
Cu	0.24±0.003^{a}	0.19±0.001^{a}	0.25±0.004^{a}	0.15±0.004^{a}	0.19±0.001^{a}	0.28±0.011^{a}
Cr	ND	ND	ND	ND	ND	ND
Ca	3.73±0.03^{a}	2.82±0.021^{a}	3.71±0.015^{a}	4.33±0.035^{a}	2.8±0.017^{a}	3.55±0.114^{a}
Mn	0.92±0.001^{a}	0.08±0.003^{a}	0.09±0.004^{a}	0.09±0.003^{a}	0.08±0.005^{a}	0.09±0.001^{a}
Na	10.3±0.10^{b}	15.04±0.04^{a}	3.43±0.04^{c}	1.43±0.035^{d}	15.04±0.04^{a}	10.47±0.06^{b}
K	75.8±0.026^{a}	61.39±0.03^{c}	71.07±0.049^{a}	68.3±0.09^{b}	61.59±0.36^{c}	77.9±0.115^{a}
Pb	ND	ND	ND	ND	ND	ND
Cd	ND	ND	ND	ND	ND	ND

Maximum allowable limits of heavy metals in coffee beans were not found in the literature. As a result, standards for other foods and herbal plants were used to compare (**Table 3.9**). Trace heavy metal concentrations in plants were critical for the animal and human health, but the metals should be kept within allowed ranges, as established by FAO/WHO and other standard-setting authorities. Health is harmed by concentrations that are higher or lower than the prescribed limits (FAO/WHO, 1993, WHO, 2005).

Nickel concentrations in coffee beans from farmers' farms (CBFF) and coffee from the washing industry (CBWI) varied from 0.05 to 0.08 ppm. FAO/WHO (1993) defined a tolerable level of 1.63 ppm in edible plants. When the metal limits in the studied coffee beans were compared to those suggested by FAO/WHO (1993), it was revealed that all of the samples were Ni below this level. The WHO (2005) limits for Ni have not yet been defined for medicinal plants. Because Ni absorption by the body is quite low, Ni poisoning in humans is not very common (Onianwa *et al.*, 2000).

Zn levels in CBFF varied from 0.054-0.076 ppm and 0.051-0.09 ppm in CBWI. FAO/WHO (1993) defined an acceptable level of 27.4 ppm in edible plants. Following a comparison of the metal limit in the investigated coffee beans with those specified by FAO/WHO(1993), it was discovered that all samples fall within this range. The WHO (2005) limits for Zn have not yet been defined for medicinal plants.

Cu levels in CBFF and CBWI were 0.14 - 0.29 ppm and 0.14 - 0.28 ppm, respectively. FAO/WHO (1993) defined an acceptable level of 3.00 ppm in edible plants. After comparing the metal limit in the investigated coffee beans to the FAO/WHO (1993) recommendations, it was discovered that all coffee beans collect Cu below this level. However, the WHO (2005) limits for

Cu have not yet been determined for medicinal plants. Cu acceptable limits in medicinal plants were determined by China and Singapore at 20 ppm and 150 ppm, respectively (WHO, 2005). Cu levels in agricultural products should be between 4 and 15 parts per million, according to (Allaway, 1968).

Mn concentrations ranged from 0.08 to 0.11 ppm in CBFF and 0.07 to 0.10 ppm in CBWI. FAO/WHO (1993) defined an acceptable level of 2 ppm in edible plants. After comparing the metal limit in the investigated coffee bean to the FAO/WHO (1993) recommendations, it was discovered that all plants collect Mn below this level. The WHO (2005) limits for Mn have not yet been defined for medicinal plants.

Pb and Cd contents in the coffee beans tested were not detected. The FAO/WHO (1993) permitted limit for Cd and Pb in edible plants was 0.21 ppm and 0.43 ppm, respectively. However, the WHO (2005) set a permissible limit for Cd of 0.3ppm and a maximum for Pb of 10 ppm for medicinal plants

According to international heavy metal guidelines, the quantities of metals identified in coffee bean samples were below the maximum allowed limit. As a result, it may be concluded that there is no health risk linked to the use of Dale woreda coffee beans.

Table 3.9 Comparison of current results for coffee beans from farms and the washing industry with FAO/WHO, different organizations, and nations' maximum permissible values for metals.

Elements	Present study		MPL (ppm)	Type of plant	References
	CBFF	CBWI			
Ni	0.05-0.08	0.05-0.08	1.63	Edible plant	(FAO/WHO, 1993)
			No MPL	Medicinal plant	(WHO, 2005)
Zn	0.054-0.076	0.051-0.09	50	Grain	(USAD, 2000)
			No MPL	Medicinal plant	(WHO, 2005)
			100	Beans	(USAD, 2000)
			27.4	Edible plant	(FAO/WHO, 1993)
Co	ND	ND	No MPL	Medicinal plant	(WHO, 2005)
Cu	0.14-0.29	0.14-0.28	40	In food	(FAO/WHO, 1993)
			3	Edible plant	(FAO/WHO, 1993)
			20	Medicinal plant	(WHO, 2005) set by Singapore
			150	Medicinal plant	(WHO, 2005) set by china
Cr	ND	ND	2	Medicinal plant	(WHO, 2005)
			0.02	Edible plant	(FAO/WHO, 1993)
Ca	3.0-17.27	2.80-4.37	-	-	
Mn	0.08-0.11	0.07-0.10	No MPL	Medicinal plant	(WHO, 2005)
			2	Edible plant	(FAO/WHO, 1993)
Na	3.0-22.1	1.40-15.09	-	-	
K	69.0-99.97	61.4-78	32500	Medicinal plant	FAO/WHO, 1993)
Pb	ND	ND	0.43	Edible plant	(FAO/WHO, 1993)
			10	Medicinal plant	(WHO, 2005)
Cd	ND	ND	0.21	Edible plant	(FAO/WHO, 1993)
			0.3	Medicinal plant	(WHO, 2005)

T-tests were used to see if there was a significant difference in the mean concentration of metals in coffee beans from farmer's farms and washing industries. Except for Ca, Mn and K, all other quantified metals are not significantly different in coffee beans from farmer's farms and washing industries, according to the findings(**Table 3.10**).

Table 3.10 T-test among coffee beans from farmer's farms and washing industries

	t	Sig.	Mean Diff.	Std. Error Diff	95% CI	
					Lower	Upper
Ni	-1.91	0.064	-0.00594	0.003109	-0.01226	0.000373
Zn	-0.39	0.694	-0.00149	0.003757	-0.00912	0.006146
Cu	0.58	0.567	0.008444	0.014612	-0.02125	0.03814
Ca	5.77	**0.01**	8.310556	1.44024	5.383635	11.23748
Mn	2.28	**0.029**	0.006111	0.002682	0.00066	0.011562
Na	0.66	0.517	1.482222	2.264297	-3.11938	6.083828
K	3.89	**0.01**	13.36944	3.43744	6.383726	20.35516

The results in Table 3.11 show that the metal content found is more or less comparable to levels published in the literature. In this investigation, however, the concentration of K was found to be lower than previously reported values (Suseela *et al*., 2001, Adler *et al*., 2019). This may indicate that Ethiopian coffee is grown without the use of fertilizers. Pb and Cd concentrations were much below the technique detection limits, exactly as they had been in previous studies (Ashu and Chandravanshi, 2011, Gure *et al*., 2017, Dubale, 2021).

The findings of this study are more or less compatible with those reported by different researchers from various nations. Cu and Mn contents, on the other hand, are lower than the previous studies (Silva *et al*., 2017, Gure *et al*., 2017, Dubale, 2021). The current study's findings are in good agreement with the majority of published values. Furthermore, for macro-elements K > Na > Ca, the general trend of metal concentration is in good agreement. For the most part, the trace microelements Mn> Cu > Ni> Zn in coffee beans from farms' farms are followed.

Table 3.11 Metal Concentrations in Coffee Beans Compared to some of the Literature Values

	(Onianwa et al., 1999) Coffee beverage	(Suseela *et al.*, 2001) coffee powders	(Silva *et al.*, 2017) roasted and ground coffee	(Gure *et al.*, 2017) in green coffee	(Adler *et al.*, 2019) in green coffee	(Omer et al., 2019) in green coffee	(Dubale, 2021) in Ethiopia		**Present study**	
							CBFF	CBWI	CBFF	CBWI
Ni	0.04-2.58	NR	0.03-1.95	<0.04-2.5	0.42±0.04	0.0-0.25	1.66-2.43	1.56-2.32	0.05-0.08	0.05-0.08
Zn	4-14	2-9	5.53-55.8	4-21	3.6 ±0.67	0.0-4.59	8.74-12.7	9.41-13.0	0.054-0.076	0.051-0.09
Co	0.1-14	NR	NR	2.6-8.4	NR	NR	2.47-2.86	2.31-2.75	ND	ND
Cu	2-9	0.4-16	0.7-17.18	11-23	14 ±6.43	NR	23.4-28.5	23.1-28.2	0.14-0.29	0.14-0.28
Cr	0.89-6.98	0.4-1.00	0.03-0.10	0.21-0.28	NR	NR	1.04-1.92	0.94-1.90	ND	ND
Ca	NR	869-1171	NR	710-1250	789 ±132.23	6.76-32.1	1037-1253	1090-1270	3.0-17.27	2.80-4.37
Mn	NR	7-13	9.81-39.8	13-19	NR	0.48-28.7	17.3-23.6	17.2-22.6	0.08-0.11	0.07-0.10
Na	NR	NR	NR	NR	18.6±11.31	6.8-564.7	NR	NR	3.0-22.1	1.40-15.09
K	NR	14000-29000	NR	13010-17000	19898±445.48	21.31-427.84	14631-15043	14602-14980	69.0-99.97	61.4-78
Pb	0.09-0.91	NR	0.03-1.58	<0.05	0.076 ±0.0956	0.0-23.88	ND	ND	ND	ND
Cd	0.02-0.31	NR	0.03-0.10	ND	0.015 ±0.0005	0.00-8.01	ND	ND	ND	ND

3.3.5. Pollution of the soil and ecological risk assessment index for heavy metals

The level of contamination of the farmland soil samples with studied heavy metals (Ni, Zn, Co, Cu, Cr, Mn, Pb and Cd) was determined by using various indices, such as an index of geo accumulation (Igeo), contamination factor (CF), contamination degree (Cdeg), modified contamination degree (mCdeg), Pollution Load Index (PLI), potential ecological risk factor and potential ecological risk index (RI).

The Igeo index values for heavy metals in soils are listed in Table 3.12. The range of index of geo accumulation (Igeo) for heavy metals, Ni, Zn, Co, Cu, Mn, and Cd was −9.304 to 3.278, −9.540 to 2.927, −1.813 to 5.178, −1.885 to 10.60, -10.91 to -7.954, and −0.1573 to 3.742, respectively. This indicates that the agricultural soils in the research area ranged from no contamination to extremely high contamination. According to Muller's approach (Muller, 1969), the Igeo consists of seven classes. These criteria and results revealed that Geo-accumulation is highest for Cu (10.60), Mn (7.954) and Co (5.178) which can be concluded as extremely contaminated, whereas the remaining five metals were uncontaminated to heavily contaminated levels. The study conducted by Mamut *et al*. (2018) in Farmland soils concluded that the Igeo of the heavy metals were uncontaminated to moderately contaminated with Zn (1.69), Pb (0.14) , Cd (0.01) and practically uncontaminated by Cr (–0.15), Cu (–0.83) , Mn (–1.12) and Ni (–0.155). According to the findings of the current study, most of the sites were heavily contamination posed by Cd, which was consistent with a prior report (Chen *et al.,* 2015, Qi *et al.*, 2020). Based on geo accumulation values, the concentration of the studied metals are in the following order Cu > Mn> Co > Ni >Cd > Zn. According to Basta *et al*. (2005), this could be due to the continuous application of fertilizer and biosolids.

Table 3.12 Index of the geo accumulation (Igeo) of soil samples collected from Dale Woreda

	SS1	**SS2**	**SS3**	**SS4**	**SS5**	**SS6**
Ni	3.278	-9.279	-9.202	-9.279	-9.353	-9.304
Zn	2.927	-9.490	-9.540	-9.404	-9.423	-9.490
Co	**5.178**	-1.813	-	-	-	-
Cu	**10.60**	-0.797	-0.724	-1.885	-0.790	-0.866
Cr	-	-	-	-	-	-
Mn	**7.954**	-10.81	-10.91	-10.87	-10.84	-10.814
Pb	-	-	-	-	-	-
Cd	-1.573	3.491	3.569	3.403	3.703	3.742

The pollution level, depending on the values of contamination factor (CF), Håkanson (1980) defines four categories of CF which was low contamination (<1), moderate contamination (1–3), considerable contamination (3–6), very high contamination (>6). According to CF results, the soils had low pollution with Ni (0.0023 to 0.0026), Zn (0.002 to 0.0023), Cu (0.406 to 0.908), Mn (0.00078 to 0.001), and Co (0.427 to 0.543).Very high contamination with Cd (15.867 to 22.4). This finding was comparable with prior research findings, For example, very high contamination (CF>6) of soils with Cd metal was also reported by (Islam *et al*., 2017, Zhu *et al*., 2020). Indicating Cd may pose potential risks to the surrounding ecosystems (Rashed, 2010). Overall, the CF for the present study metals was in descending order: Cd > Cu > Co > Ni > Zn > Mn (**Table 3.13**).

Heavy metal pollution levels in farming soils were measured using the Pollution Load Index (PLI), which varied from 1.127 to 1.271, indicating a moderate level of pollution. Rahmania and Safari (2020) conducted research in South-West Iran on the pollution load index of selected heavy metals (Pb, Cd, Ni, and Mn). The Pollution Load Index ranged from 1 to 2.3, indicating that industrial activity and bedrock weather played a role in the research area's elevated PLI (Rahmanian and Safari, 2020).

Table 3.13 Contamination factor (CF) and Pollution Load Index (PLI) of the soil samples collected from Dale Woreda.

	Contamination factor (CF)					
	SS1	SS2	SS3	SS4	SS5	SS6
Ni	0.0026	0.0024	0.0025	0.0024	0.0023	0.0024
Zn	0.0023	0.0021	0.0020	0.002	0.0022	0.0021
Co	0.543	0.427	-	-	-	-
Cu	0.772	0.863	0.9084	0.4062	0.867	0.823
Cr	-	-	-	-	-	-
Mn	0.001	0.0008	0.00078	0.0008	0.00082	0.00083
Pb	-	-	-	-	-	-
Cd	**22.4**	**16.867**	**17.8**	**15.867**	**19.53**	**20.067**
	Pollution Load Index (PLI)					
	SS1	SS2	SS3	SS4	SS5	SS6
PLI	1.247	1.127	1.263	1.135	1.274	1.271

According to Loska *et al*., (2004) contamination degree (Cdeg) is the sum of all the contamination factors for a given set of soil samples. There are four categories of contamination degree (Cdeg) which low degree contamination (<8), a moderate degree of contamination (8–16), a considerable degree of contamination (16–32), and very high degree of contamination (>32). In the present study, the contamination degree (Cdeg) of the soil samples (18.16227 to 23.72095) suggested a Considerable degree of contamination (**Figure 3.2**). The contamination status of the study sites by heavy metals using contamination degree was in the order of SS1>SS6>SS5>SS3>SS2>SS4. The modified contamination degree (mCdeg) results showed that the soil quality in the study area ranged from (2.035 to 2.965) which was found in the category of a moderate degree of contamination (**Figure 3.3**).

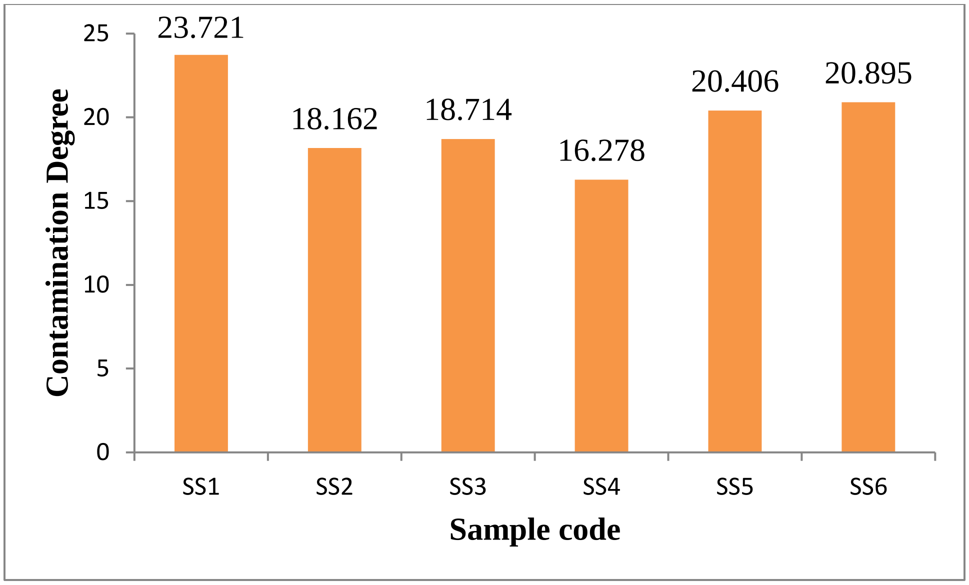

Figure 3.2 Contamination degree (Cdeg) of soil samples collected from in Dale Woreda, 2021

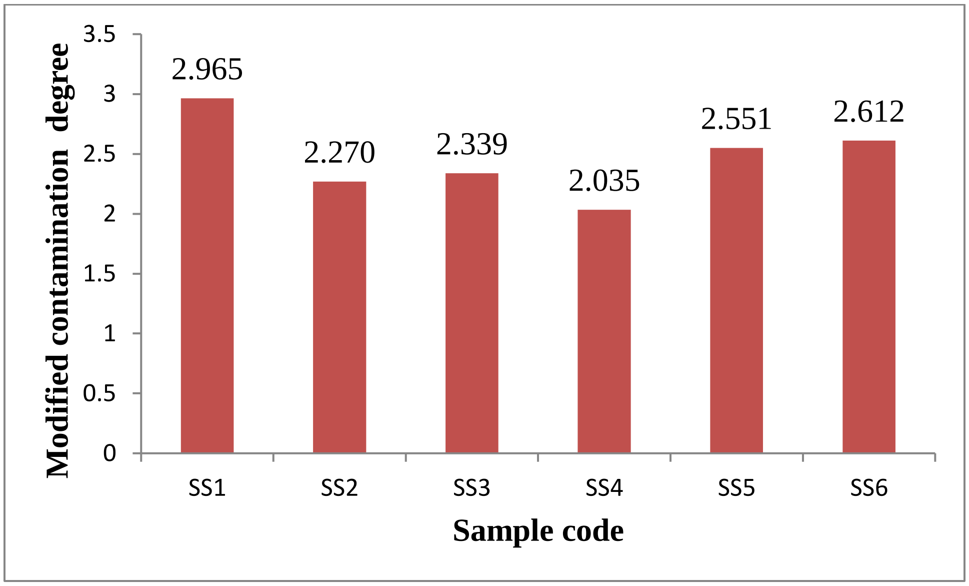

Figure 3.3 Modified contamination degree (mCdeg) of soil samples collected from in Dale Woreda, 2021

The results of ERi show Low potential ecological risk with Ni (0.0138 to 0.0155), Zn (0.0020 to 0.0023), Co (2.135 to 2.715), Cu (2.0309 to 4.5418), and Mn (0.0078to 0.010), while Cd (476 to 672) belonged to a very high potential ecological risk in soil samples from the research area (**Table 3.14**). This meant that Cd had a considerable impact on the heavy metal's environmental quality in the soil, and was the main controlling factor of heavy metal pollution in soils in Dale woreda. The finding of ERi of the current study was much higher than ERi value of Cu(0.25) and Cd (9.92) in agricultural soil from Kenya (Mungai *et al*., 2016), and reported by (Mamut *et al*., 2018) Cu (1.56) and Cd (0.93) in farmland Soils in Yanqi County, Xinjiang, Northwest China. However, the study conducted by Qi *et al*. (2020) in agricultural soil reported a high potential ecological risk (ERi ≥ 320) with Cd metal. This difference in ERi might be due to differences in pollution level between the study areas. The potential ecological risk was ranked as follows: Cd>Cu>Co>Ni>Mn>Zn.

The potential ecological risk index (RI) revealed that the soil samples have the maximum RI is 678.603, and the lowest ecological RI is 478.056, It was classified as being in the ecological risk categories of considerable ecological risk to very high ecological risk. Most sites had considerable ecological risk which was RI value (512.476) for SS1, (538.567) for SS3, (478.056) for SS4, and (590.360) for SS5. Barakat *et al*. (2019) found a considerable ecological risk in the peri-urban agricultural soil of Béni-Mellal city, Morocco, with RI values ranging from 184.49 to 292.46. The most important element determining RI was Cd, which poses a potential ecological risk to the soil of the study area. This was consistent with prior research findings. For example, metals Cd showed a higher ecological risk than the other metals in Shanxi (Pan *et al.*, 2016) and from agricultural soils in Kenya (Mungai *et al.*, 2016). Moreover, similar findings were also reported in recently published ecological risk assessment studies from the agricultural soil of

China (Qi *et al.*, 2020). Overall, most of the sampling sites in Dale Woreda suffered from the considerable, even very high ecological risk posed by heavy metals Cd. This could be because agricultural inputs such as pesticides, fungicides, contaminated fertilizers, and irrigation water are applied gradually, which could increase the burden of heavy metals and cause heavy metal pollution in agricultural soil (Mahmud *et al.*, 2021).

Table 3.14 Potential ecological risk factor and potential ecological risk index (RI) of soil samples collected from Dale Woreda

	Potential ecological risk factor					
	SS1	SS2	SS3	SS4	SS5	SS6
Ni	0.0155	0.0145	0.0153	0.0145	0.0138	0.0138
Zn	0.0023	0.0021	0.0020	0.0022	0.0022	0.0022
Co	2.715	2.135	-	-	-	-
Cu	3.86	4.3164	4.5418	2.0309	4.3364	4.336
Cr	-	-	-	-	-	-
Mn	0.010	0.0083	0.0078	0.008	0.0082	0.0082
Pb	-	-	-	-	-	-
Cd	672	506	534	476	586	602
	potential ecological risk index(RI)					
	SS1	SS2	SS3	SS4	SS5	SS6
RI $=\sum_{i=1}^{n} ERi$	678.60	512.476	538.567	478.056	590.360	606.139

3.4. Conclusion and Recommendations

Mean metal concentrations in the soil were determined to be in the following order: Ca>K>Na>Cu>Co>Cd>Mn>Ni>Zn. Except for Cd, all metals analyzed were below the permissible limit set by FAO/WHO. Cadmium levels reported in the soil are above the levels of 3ppm, the permissible limits for agricultural soil (FAO/WHO, 2001). Therefore, there was Cd contamination in the soil.

Metal levels in coffee bean samples from farmers' farms are in the following order: K>Na>Ca >Mn>Cu> Ni>Zn. Metal levels were found to be K>Na>Ca >Mn>Cu> Zn>Ni in coffee beans from the washing plants. In both coffee, the levels of toxic metals (Pb and Cd) were not determined, and trace heavy metal levels were below the FAO/WHO maximum permissible limits. As a result, there is no health risk linked with the use of Dale Woreda coffee beans due to harmful and trace heavy metals. Mn had the highest bio-concentration factor/transfer factor among the elements examined. However, Cu had the lowest transfer factor. The general pattern of trace metal transfer in Kege Kebele (SS1) was manganese > zinc > nickel > sodium> potassium > calcium > copper. According to the findings of this study, there are permitted levels of macro and trace elements in coffee beans from farmlands and washing plants. As a result, metal pollutants do not affect the coffee grown in Dale Woreda.

The various soil contamination indices such as the Igeo, CF, Cdeg, mCdeg, PLI, ERi, and RI were evaluated. The Igeo values of Ni, Zn and Cu indicate uncontaminated to heavily contamination, whereas Cu, Mn and Co show extremely contaminated in the studied area. The contamination factor values reveal that Cd show very high contamination in the study area. The results of the contamination degree and modified contamination degree show a considerable and moderate degree of contamination, respectively. The result of the Pollution load index revealed that the soil samples under the study area were found to show a moderate level of pollution. The results of ERi indicated a low potential Ecological risk of heavy metals in the case of Ni, Zn, Co, Cu and Mn. However, Cd shows a very high potential Ecological risk in the studied soil samples. Heavy metals pose a considerable to extremely high ecological risk in the examined area, according to the RI values.

Based on the above conclusion, the following are recommended:

- ✓ According to the findings of this study, there are permitted levels of macro and trace elements in coffee beans from farmer's farms and washing plants. As a result, metal pollutants do not affect the coffee grown in Dale Woreda. As a result, concerned bodies should make more marketing and raise awareness to increase and spread the recognition and consumption of Dale Woreda coffee beans in the national and international coffee market.
- ✓ It is very urgent to develop a soil remediation technique with consideration the type and degree of pollution, field characteristics, remediation targets, implementation schedule, cost-effectiveness and public acceptability (Liu *et al.*, 2018).

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CHAPTER 4: TREATMENT PERFORMANCE ASSESSMENT OF NATURAL AND CONSTRUCTED WETLANDS ON WASTEWATER FROM KEGE WET COFFEE PROCESSING

ABSTRACT Constructed wetlands are engineered systems built to use natural processes and remove pollutants from contaminated water in a more controlled environment. The research was Randomized controlled trials (RCTs), which is a type of comparative Randomized Experiment. In this RCTS experiment, the performance of a well-managed constructed wetland performance is tested against a well-managed natural wetland by providing a given amount of wastewater. The two vertical flow constructed wetland was built. The first wetland covered an area of 132 square meters. It has a 12m in width and 11m in length. Open space is constructed between two constructed wetlands with a dimension of 11m *3m *1m. The second wetland was constructed and its function is similar to the first one, from this wetland water is discharged to the Gidabo river. The construction of the wetland is accomplished by constructing 20cm wide furrows with a spacing of 30cm. Vetiver grasses have been planted with a spacing of 20cm intervals. The physicochemical data were recorded, organized, and analyzed using R software (version 4.1) and Microsoft Excel. Data were processed using parametric (one-way ANOVA) and nonparametric (Mann-Whitney's U-test) statistical tests of homogeneity. One-way analysis of Variance (ANOVA) was used to determine the significance of differences in variations in physicochemical variables within the constructed wetland sites. Tukey's multiple comparisons for differences between means were also assessed. Findings indicated that a natural wetland had a mean influent and effluent of total suspended solids (TSS) of 2190.78±448.46 mg/L and 972.67±234.312mg/L, respectively. A Mann–Whitney U test revealed that TSS was significantly higher in the natural wetland (median =1551.50) compared to constructed wetland (median =922.5), U =676.5, z=-2.435, p=0.015, r=0.257. Natural wetlands had a mean influent of biological oxygen demand (BOD) was 4277.94±157.02mg/L, while in the effluent of BOD, it was 326.83±112.24 mg/L. While in constructed wetland it was 4192.4±191.3mg/L, 782.72±507.6mg/L and 88.28±20.08mg/L in influent, middle and effluent respectively. The average chemical oxygen demand (COD) value at influent in natural wetlands was 8085.61±536.99 mg/l and in the effluent, it was 675.33±201.4mg/L. In the constructed wetland, it was found to be 8409.8±592.9, 1372.6±387.94, and 249.0±7.68 for influent, middle and effluent respectively. Comparatively, the purification efficiency of organic pollutants (TSS, BOD, and COD) of constructed wetlands was better than natural wetlands, whereas natural wetlands had better purification efficiency of nitrogen compounds such as ammonium, nitrite, and nitrate. On average, removal rates for nitrogen compounds were 39.53% and -24.41% for ammonium, 79.44% and 55.4 % for nitrite, and 68.90% and 60.6% for nitrate in natural and constructed wetlands respectively, while the phosphate removal rate was 43.17% and 58.7 in natural and constructed wetlands, respectively. A Mann-Whitney U test revealed that there is no significant difference in nitrite, nitrate, ammonium, and phosphate concentration between natural and constructed wetlands($p > 0.05$). In all sample sites, the mean concentration of heavy metals such as Ni, Zn, Cu, Na, Fe, and Mn was above the maximum permissible limit to be used as irrigation water. Based on these results, both systems of treatment were effective in treating the coffee effluent since most of the values obtained were below the permissible EEPA limits. Even though the constructed wetland treatment plant performed better overall, in comparison, the natural wetland had better purification efficiency for nitrogen compounds like ammonium, nitrite, and nitrate and constructed wetland had better purification efficiency for organic pollutants (TSS, BOD, and COD).

Key Words: Wetlands, Coffee Processing Wastewater, Removal Efficiency, Vetivar Grass

4.1 Introduction

Coffee wastewater discharged carelessly into neighboring natural waterways cause pollution to both the surface and groundwater (Dejen *et al.*, 2015). The effluent generated by agro-based industries has a high amount of pollutants, which can cause irreversible environmental harm if not properly disposed of (Gururaj *et al.*, 2021). According to Avellone *et al.*, (1999), Fia *et al.*, (2013), and Gururaj *et al.*, (2021), the effluent from coffee processing plants consists of different sugars, crude protein, crude fiber, different nutrients and chemicals which are generated from both pulping and mucilage fermentation processes. Haddis and Rani (2008) and Gururaj *et al.*, (2021) indicated that the effluent also consists of different toxic chemicals such as tannins, alkaloids (caffeine) and polyphenolic compounds and nutrients like nitrate and phosphate. Moreover, Selvamurugan *et al.*,(2010) and Mussatto *et al.*,(2011) noted that the discharge of such kinds of untreated coffee-washed effluent into the open environment and the river can bring various environmental and public health problems.

The use of natural and artificial (constructed) wetlands for wastewater treatment has been proposed as an intermediate technological solution for handling wastewater (McEldowney *et al.*, 1998). These systems are economically attractive and relatively energy-efficient for wastewater treatment, specially constructed wetland technology (CW) is one of the emerging and acceptable technologies because it can effectively remove almost all types of pollutants from wastewater without harming the environment (Dhanya and Jaya, 2014). Constructed wetlands are recommended as superior options for treating industrial or domestic wastewater because of their numerous benefits, including the provision of high wastewater treatment levels. Constructed wetlands are also recommended because they are environmentally friendly. It has been shown that this method can reduce wastewater contaminants to tolerable levels. The low cost of building is further aided by the fact that wetland systems require little to no energy and nothing in the way of equipment. Before considering using this method for complete or maximum contamination removal, it must first be fully established. Here, establishment denotes complete development/growth (Mthembu *et al.*, 2013). Several countries, particularly in the developing world, are promoting the reclamation and reuse of wastewater (Almuktar *et al.*, 2018) with an emphasis on the application of CWs as the technology for wastewater remediation. Nonetheless, local expertise and awareness in the application of the technology remain a challenge; for example, there has yet to be a national specific coordinated policy on wetlands in Ethiopia (Dixon *et al.*, 2021)

The choice of plants for wetlands is crucial, and they must be resistant to toxicity and changes in the character of the entering wastewater (Ebrahimi *et al.*, 2013). The vetiver system was created with the aim of soil and water conservation, particularly in the fields of wastewater treatment and solid waste dumps (Pongthornpruek, 2017). Vetiver grass is one of the most promising plants because of its rapid growth, deep and broad root system, and strong tolerance to environmental stress such as drastic temperature changes (22°C - 60°C), soil pH (3.0-10.5) and, most critically, excellent tolerance to heavy metal stress (Pongthornpruek, 2017, Ng *et al.*, 2019). Therefore, the coffee berry wastewater treatment with vetiver grass is the alternative way emphasized during this study.

For the past two decades, urbanization and expansion of industrial activities in the forest and wetland reserves have become an acute problem in Ethiopia. Not only does encroachment account for wetland and forest loss, but also biodiversity and aquatic life diversity depletion as well. Draining of wetlands and clearing of forests for urbanization and industrial development has had serious consequences on surface water hydrology and accelerated the process of water pollution (Dixon *et al.*, 2021).

Limited data are available about the potential of constructed wetland-oriented wastewater treatment of Coffee Berry Processing Agro-industry (CBPA), especially using Vetiver grass in Africa in general, and particularly in Ethiopia (Samuel,2021),. The constructed wetland system does not require high construction and operation costs as it is required for the construction of a conventional wastewater treatment system (Pongthornpruek, 2017, Kamal *et al.*, 2019). With this in mind, we designed, built, and operated the constructed wetlands in the Kege processing plant for the treatment of coffee wastewater. This study aims to assess the treatment performance of natural and constructed wetlands on wastewater from Kege wet coffee processing plant in Dale Woreda, Sidama Regional State, Ethiopia.

4.1.1Objectives of the study

4.1.1.1 General objectives

The general objective of this study was to assess the treatment performance of natural and constructed wetlands on wastewater from Kege wet coffee processing plant in Dale Woreda, Sidama Regional State, Ethiopia.

4.1.1.2 Specific objectives

The specific objectives of the current research were:

1). To determine the physicochemical characteristics of the water used, effluent from Kege coffee processing plants and effluent from wetlands (NW and CW);

2). To determine the concentration of selected metals (Pb, Ni, Zn, Co, Cu, Na, K, ,Mg, Fe, Mn, Cr, Cd,) in the water used, effluent from Kege coffee processing plants and effluent from wetlands (NW and CW), and

3). To evaluate the treatment efficiency of the natural and constructed wetland to remove several pollutant from wastewater of coffee processing plant;

4.1.2 Research questions

1) What are the physicochemical characteristics of the water used, effluent from Kege coffee processing plants and effluent from wetlands (NW and CW)?
2) What is the concentration of Pb, Ni, Zn, Co, Cu, Na, K, Ca, Mg, Fe, Mn, Cr, Cd in the water used, effluent from Kege coffee processing plants and effluent from wetlands (NW and CW)?
3) What is the performance of natural and constructed wetland to remove several pollutant parameters in wastewater of coffee processing plant?

4.2 Matterials and Methods

4.2.1. Study area

Kege wet coffee processing plant, one of the leading coffee-processingplants, is located in Dale Woreda of Sidama Regional State (SRS), near Aposto at the Gidabo River Bridge, at the side of the highway from Addis Ababa to Kenya (**Figure 4.1**). The information from the Sidama Region's Environmental Protection Authority indicates that 27,049 tones of the harvested Sidama coffee was exported in 2021/22 while the rest was used for domestic consumption. The range of the average yearly temperature of Dale Woreda is between 9.6°C and 29.2°C. There are two rainy seasons in the area, with the first peak being in April–May and the second from August–October. The least amount of rain falls between November and February. The area receives 1102 mm of rain on average yearly. Agroforestry practices appear to be the main features of the land use systems in the region (ILRI, 2013). During maximum coffee production, 64,000 litres or $64 m^3$ of wastewater is discharged from the Kege coffee processing plant.

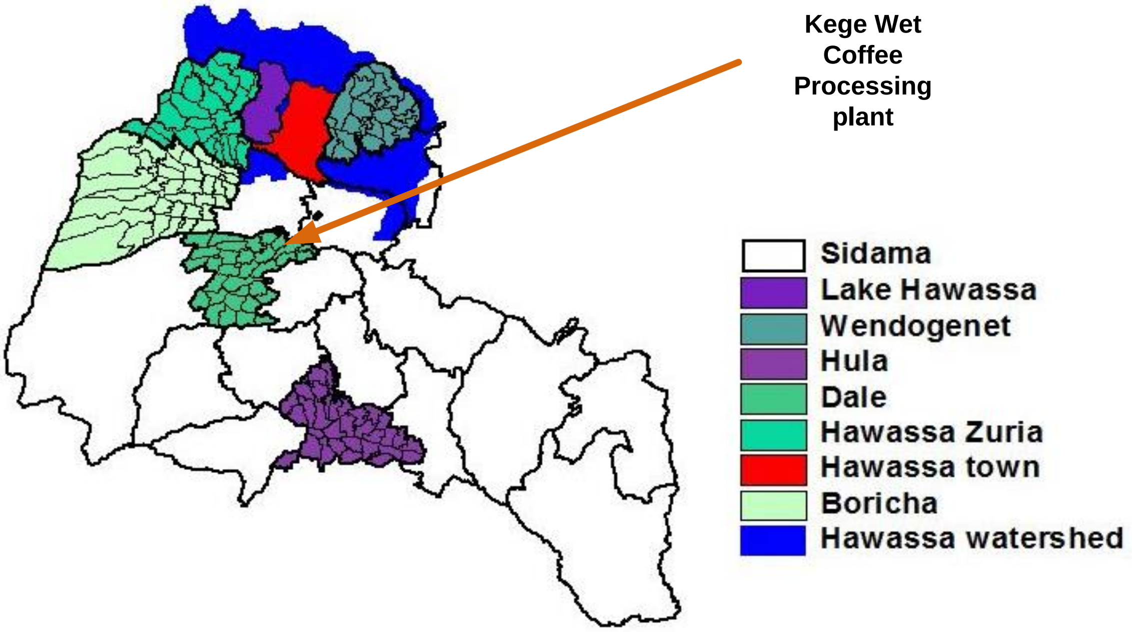

Figure 4.1 Kege Wet Coffee processing plant in SRS **(Source GIS)**

4.2.2 Constructed wetland unit preparation/ Field experimental setup

This study is a Randomized controlled trial (RCTs), which is a type of comparative Randomized Experiment. In this RCTs experiment, the performance of a well-managed constructed wetland performance is tested against a well-managed natural wetland by providing a given amount of wastewater. A variety of different wetland design and testing methods (either based on volume or area) are available. Each method carries its own set of assumptions, and different equation sets, and they have their strength and weaknesses. Volume-based methods use a hydraulic retention time (HRT) to assess pollutant reduction (Reed *et al.*, 1995) whereas area-based methods assess pollutant reduction using the overall wetland area (Kadlec and Knight, 1996).

Biodegradation of less-degradable pollutants generally requires a combination of anaerobic and aerobic processes. To treat such pollutants with constructed wetlands, therefore, anaerobic and aerobic processes should properly incorporate into wetland systems. Vertical flow constructed wetland systems in which anaerobic and aerobic processes take place sequentially are the most promising options for this purpose (Carballeira *et al.*, 2017).

The pond with 8m*8m*1m is constructed for storing wastewater discharged from the coffee processing plant. The pond is used to facilitate the sedimentation process in which heavy solid particles of wastewater are allowed to settle down in the pond. The dimensions of the pond are determined from the daily maximum discharge of wastewater. According to this, during maximum coffee production, 64,000 litres or $64m^3$ of wastewater is discharged from the coffee processing plant. Therefore, the sedimentation ponds need to have the capacity of storing this much wastewater per day. That is why the pond is constructed with 8m *8m*1m dimensions as it is shown in Figure **4.2**. This stabilization pond was used only for the constructed wetland.

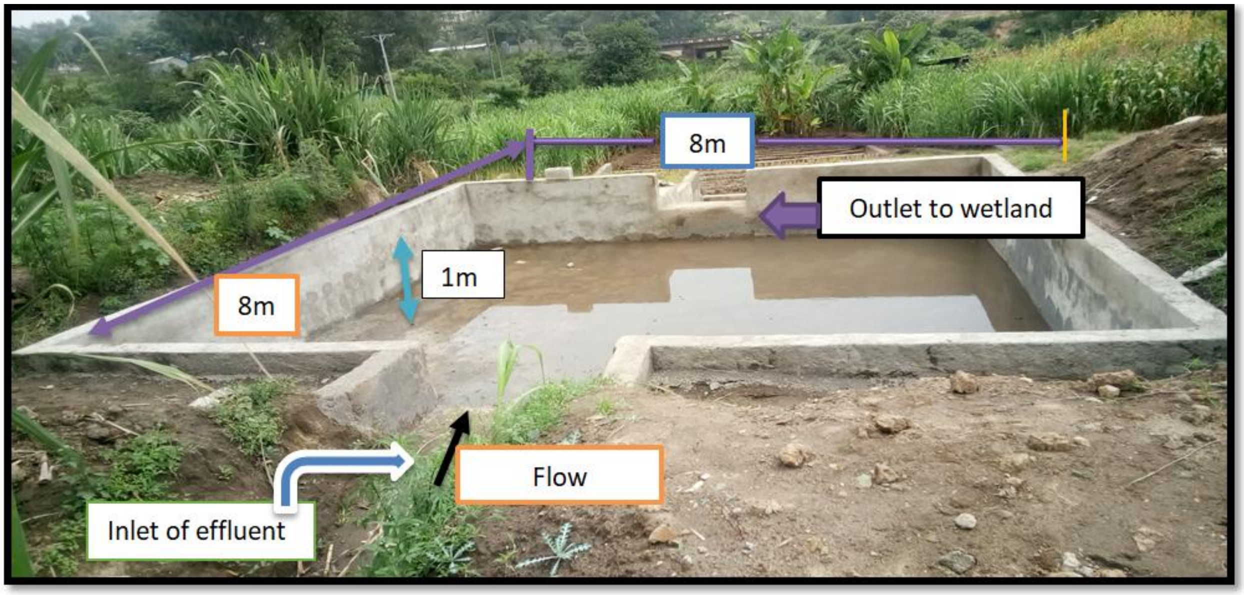

Figure 4.2 A Constructed sedimentation pond.

A drop structure was constructed to facilitate the mixing of wastewater with air (**Figure 4.3**) . This helps the wastewater for gaining adequate oxygen that required for the next aerobic reaction (especially the breakdown of acetic acid resulting from the fermentation of sugars and pectin). In sedimentation ponds, due to excess amount of organic pollution, there will be oxygen shortage, thus, there happens anaerobic reaction which leads to a bad smell through “rotting” and good growth conditions for health-threatening bacteria (USEPA,2000). Therefore, due to drop structures, the wastewater flows were highly disturbed and this helps to get enough oxygen from air so that there will be aerobic reaction in the next stage treatment unit.

a. During construction

b. After construction

Figure 4.3 Drop structure during construction and after construction

The filtration channel was constructed on an area of 15m^2, i.e. 1m*10 metre length with 1.5meter width. It was constructed between the drop structure and the first wetland (**Figure 4.4**). The depth of the channel is filled with different-sized gravels. The channel was used as filter media for filtration processes. Although heavy solid particles of wastewater are expected to be removed by sedimentation processes in the sedimentation pond, fine suspended particles remain in the wastewater so far and continue flowing with water in the succeeding steps. Since the channel was filled with different-sized gravels, which were used as filtering media, wastewater from drop structures immediately enter into the channel where it moves down and up through the gravels. Here the filtration processes have occurred. Through this filtration process,and the fine solid particles were removed from the wastewater. That is the reason why this particular structure is constructed.

Figure 4.4 Filtration Channel

The first wetland had a 12m width and 11m length that covered an area of 132 square meters. (**Figure 4.5A**). The design approach used for the Constructed Wetland design of Kege Wet Coffee Processing Plant in the current study is based on hydraulic and organic removal design criteria. In this work, the entire wetland design process mainly followed the criteria given by Kadlec and Knight (1996) and USEPA (2000) for vertical flow-constructed wetland systems.

The construction of the wetland was accomplished by constructing 20cm wide furrows with a spacing of 30cm (**Figure 4.5B**). Vetiver grasses have been planted with a spacing of 20cm intervals (Pongthornpruek, 2017). Each pilot unit was filled with soil and sand for plant cultivation to a depth of 60 cm and it was built with a slope of 1% from the inlet towards the outlet zones to prevent backflow (Pongthornpruek, 2017). Water is allowed to flow uniformly via the gravel zone overtopping the masonry wall on the surface of the first vertical flow constructed wetland and then drains down through the filter layer which consists of coarse sand and joins the open water pond downstream underground after passing through the first Vetiveria zizanioides plantation. All pilots were planted with vetiver grass (*Vetiveria zizanioides*) for wastewater treatment.

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Figure 4.6 First Wetland A) Area of the wetlans, B) Furrows constructed on the wetland

Open space is constructed between two constructed wetlands with a dimension of 11m *3m *1m. Thus, it covered an area of 33 square meters. Half of its depth is filled with different-sized gravels. The purpose of open space is to use it as filter medium next to the first wetland. Since it is open, water can get sunlight for a chemical reaction (**Figure 4.6**).

a. During excavation b. After construction

Figure 4.7 Construction of open space between two wetlands

The construction of the second wetland (**Figure 4.7**) was constructed in a similar manner to the first one and it has a similar function to the first one as it is discussed previously. From this, second wetland, the treated water is discharged into the Gidabo River .

Figure 4.8 Second wetland cell

Young plants of *Vetiveria zizanioides* were collected from natural wetlands and before planting, the tiller roots were carefully rinsed with tap water to get rid of any silt and soil that had adhered to them. The vetiver tillers' shoots and roots were cut back to 10 cm and 5 cm, respectively. Then the plants of Vetiveria zizanioides collected from natural wetlands were planted directly into the wetland cells (**Figure 4.8**). Vetiver grasses were planted in rows with a spacing of 20 cm so that wastewater flows in a meandering way to take a long time in the wetland for the natural processes to have enough hydraulic detention time for the service of natural processes. The experimental plants were acclimatized in the constructed wetlands by irrigating tap water for three weeks. After that, plants were given three months to grow in wastewater (before the real experimental treatments) as described by (Pongthornpruek, (2017) (before the real experimental treatments), by which time the majority of them had grown to a height of 1.0 to 1.5 meters.

Figure 4.9 Planting on wetlands

Construction of the footbridge and division boxes was constructed. The footbridge is used for traveling between the wetland and coffee processing units, whereas a division box is used to divide wastewater to the newly constructed wetland and the natural existing one during excess wastewater discharge (**Figure 3.9**). The overall composition of the wetland was presented in **Figure 3.10** below.

Figure 4.10 Foot Bridge

The area of the natural wetland was 4500 square meters. It has 150m length and 30m width. The natural wetland’s length-to-width ratio was 5 to 1. The water's depth ranged from 0.3 to 1.2 meters, and its maximum depth was 1.2 meters. The design inflow rate was 0.25 m^3/s, and the intended resident time was 3 days.

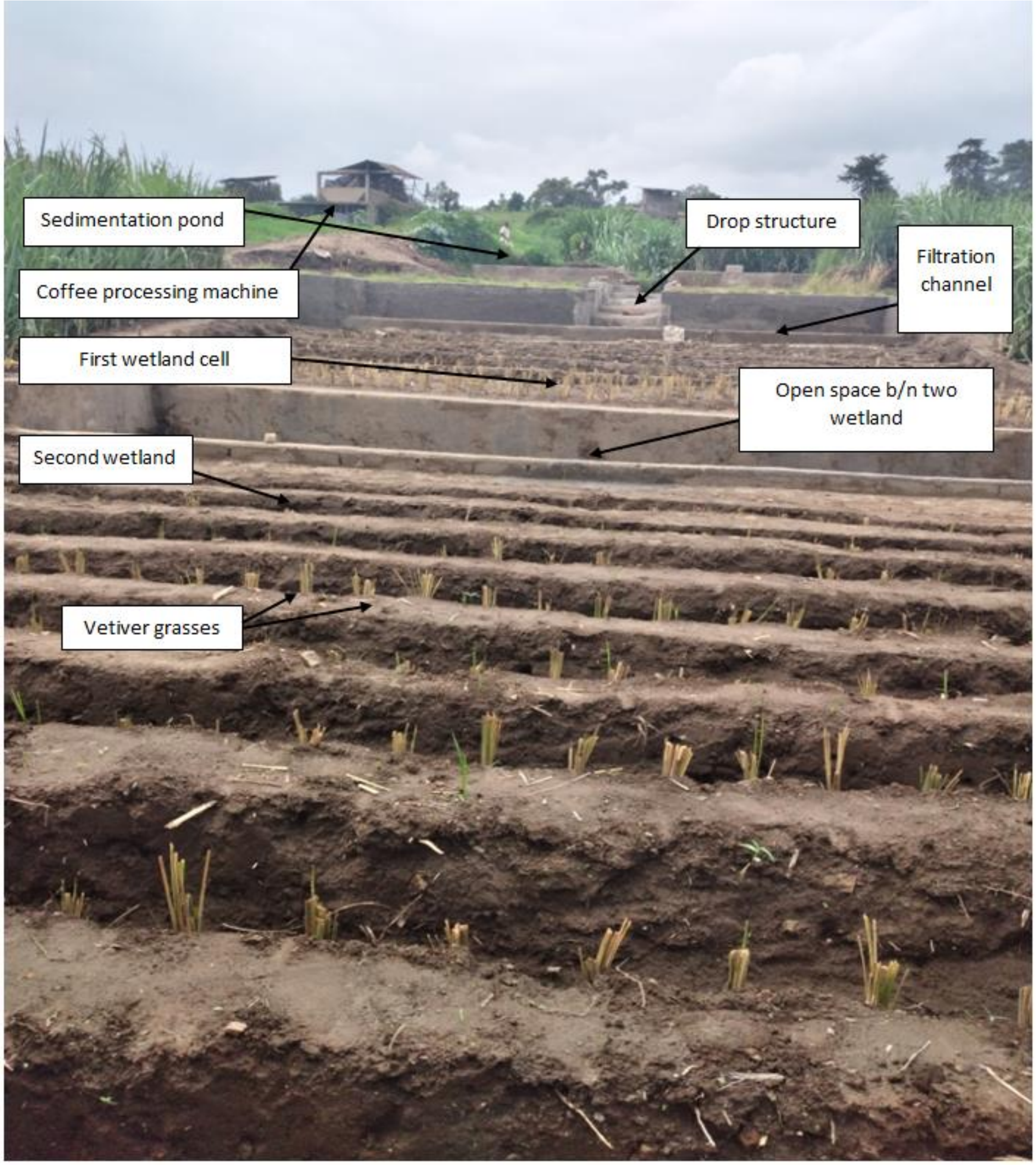

Figure 4.11 The whole system design of the constructed wetland

4.2.3. Samples collection and handling methods

A) Sampling time and selection of sampling sites

In SRS coffee growing areas, commonly coffee is harvesting time of the year is from October to December. Consequently, wet coffee processing usually begins at the end of September and proceeds until December. As a result, water and wastewater samples were collected to conduct the study from Kege coffee processing plants during October and December 2021.

Two kinds of samples, river water used for processing and wastewater from the plant were collected. A total of 7 sites were selected to collect samples, 1) inlet of river water into the processing plant,named as water sample 1 (WS1), 2) a point at pulping process stage (WS2), 3) inlet of wastewater (WW) into natural wetland (NW) (WS3); 4) outlet of WW from NW (WS6); 5) inlet of WW into CW (WS4); 6) WW in the middle of CW (WS5); and 7) outlet of CW (WS7). From each site samples were collected 3 times (triplicate) in 2 weeks interval from 10th October 2021 to 25th December 2021. Therefore, a total of 42 samples (14*3) were collected for analysis.

B) Sample collection procedure, transportation and storage

The wastewater samples were collected using sampling procedures described by American Public Health Association (APHA, 2017).Two liters of water (river water or wastewater) samples were collected in polyethylene sampling bottles. Before collection, these bottles were washed and rinsed thoroughly with distilled water and then re-rinsed three times with the respective water samples before sample collection. By employing the depth-integrated sampling technique, water samples were taken by inserting polyethylene sampling bottles in the opposite direction of the water flow and immediately capping them after filling them up to the tip of the mouth. Wastewater sampling and preservation techniques followed the standard preservation (APHA, 2017).

4.2.4 Sample preparation and analysis methods for metals

A) Sample preparation/digestion method

The wastewater samples from each sampling bottle were mixed thoroughly by shaking. In the process of digestion, an 8 mL wastewater sample was taken and 8 mL of HNO_3 was added and taken to the microwave digestion (CEM) adjusted according to APHA (2017). Blank digestion was also carried out in the same way as described in Birtukan and Gebregziabher, (2014). All reagents except for wastewater were present in the blank solution. All samples were digested in triplicates. The digests were analyzed for the heavy metals by using Flame Atomic Absorption Spectrophotometer (FAAS) (Buck Scientific, Model 210VGP AAS, USA) in Hawassa University, Chemistry Lab.

***B)* Flame Atomic Absorption Spectrophotometer (FAAS) operating conditions and calibration**

A total of twelve metals (Pb, Ni, Zn, Co, Cu, Na, K, Ca, Mg, Mn, Cr, and Cd) were analyzed. The digests were analyzed for the heavy metals by using FAAS (Buck Scientific, Model 210VGP AAS, USA) with an external calibration curve after adjusting the equipment to produce the strongest signal possible, factors like burner and lamp alignment, slit width, and wavelength adjustment were tuned. For each metal, the respective hollow cathode lamp was inserted into the Atomic Absorption Spectrophotometer, and the solution was successively aspirated into the flame. Metals were analyzed by the absorption mode of the instrument.

Table 4.1 Concentrations in the sample, Amount added, amount found in the spiked and Coefficient of determination of the calibration curve for analysis of wastewater samples.

Elements	Conc.in the sample	Amount added in ppm	Amount found in the spiked sample	% Recovery
Ni	0.137	0.10	0.160,0.167 and 0.166	
Zn	0.061	0.03	0.085,0.090 and 0.085	85.5
Cu	0.091	0.05	0.135,0.138 and 0.134	89.3
Ca	18.96	10	28.90,28.75 and 28.70	98.2
Mn	0.278	0.1	0.370,0.365 and 0.360	87
Na	33.98	10	43.78,43.88 and 43.78	98.3
K	16.93	10	26.81,26.85 and 26.67	98.5

C) Heavy metals analysis method.

Water and wastewater samples collected from each sampling site and digested were analyzed for metals concentration following the procedures outlined in APHA (2017) using FAAS. Iron and Magnesium were analyzed using a Spectrophotometer (Hach, DR6000, U.S.). Analysis was carried out in Hawassa University, Chemistry Department Lab. The concentration of each metal was calculated as described in Birtukan and Gebregziabher, (2014). using the formula below.

$$Final\ concentration\ (mg/L) = \frac{\text{CM xDF xNV}}{\text{SV}} \qquad \text{………………. Eqution (4.1)}$$

Where: **CM** = Concentration of metal, **DF** = Dilution factor, and **NV** = Nominal volume,

SV= Sample volume (mL)

4.2.5 Physico-chemical parameters analysis methods

A) In-situ analysis

Water quality parameters such as pH, water temperature, TDS, dissolved oxygen (DO) turbidity, and electrical conductivity (EC) were measured in-situ using a multi-parameter (Adwa AD8000, AD8000, Romania). For other remaining physicochemical water quality, the samples were properly and carefully labeled and transported to the laboratory of Sidama Regional State Water Bureau (SRSWB) and the Laboratory of the chemistry department, Hawassa University.

B) Lab analysis

The analytical method used for the determination of COD was the dichromate test method as described in APHA (20017). The dichromate chemical oxygen demand (COD) test measures the oxygen equivalent of the amount of organic matter oxidizable by potassium dichromate in a 50% sulfuric acid solution. Silver sulfate was used as a catalyst and mercuric sulfate was added to remove chloride interference. During the procedure, water samples were put in a cuvette with reagent and heated for two hours using a heater (Model HACH DRB 200). After the oxidation step was completed, the amount of dichromate consumed was determined calorimetrically. The intensity of color in the solution was directly related to the COD value in the sample and was measured with an ultraviolet spectrophotometer (model HACH DR 6000) at Sidama Regional State Water Bureau (SRSWB) lab. All the rest (Ammonium, Nitrite, Nitrate, Phosphate, Sulphate, BOD_5 and DO) parameter was done according to the standards of APHA (2017) (see Table 3.2 for methods used).

Table 4.2 Summary of water/wastewater quality analysis methods

Parameters	Instruments name and methods used for experimental analysis	**Units**
pH	AD8000 multipara meter	-
Temperature	AD8000 multipara meter	^{0}c
Conductivity	AD8000 multipara meter	µS/cm
TDS	AD8000 multipara meter	mg/L
Turbidity	AD8000 multipara meter	NTU
TSS	spectrophotometer	mg/L
NH_4^+	Spectrophotometer (Hach, DR6000, U.S.)	mg/L
Nitrite	Spectrophotometer (Hach, DR6000, U.S.)	mg/L
Nitrate	Spectrophotometer (Hach, DR6000, U.S.)	mg/L
Sulfate	Spectrophotometer (Hach, DR6000, U.S.)	mg/L
Phosphate	Spectrophotometer (Hach, DR6000, U.S.)	mg/L
BOD_5	Winkles methods	mg/L
COD	Spectrophotometer (Hach, DR6000, U.S.)	mg/L
DO	DO meter (Hach P/N HQ30d, Loveland. CO, USA)	mg/L
Heavy metals	FAAS (Buck Scientific, Model 210VGP AAS, USA)	

4.2.6 Wetlands pollutants removal performance calculation methods

During the monitoring period, the wetland's removal efficiency (E) was calculated as described by Dendup *et al*., (2021) using the formula shown below.

$E = \frac{\mathrm{Ci-Ce}}{\mathrm{Ci}} x100$ ………………………..Eq (4.2)

Where C_i = influent concentration of a pollutant

C_e = effluent concentration of a pollutant, and

E = wetland's removal efficiency (%).

4.2.7. Data quality assurance

Standard methods were used for all procedures in the set of experiments. The methodologies used in the series of experiments were all approved standard methods. The reagents were all analytical grade. A triplicate sample analysis was performed for each test to verify accuracy. On a prepared data registration form, all test results were recorded honestly and cautiously.

4.2.8. Data analysis

The physicochemical data were recorded, organized, and analyzed using R software (version 4.1) and Microsoft Excel. Data were processed using parametric (one-way ANOVA) and nonparametric (Mann-Whitney's U-test) statistical tests of homogeneity. The results of testing normality and homogeneity of variance for data from constructed wetlands were determined using Kolmogorov-Smirnov and Levene’s tests, respectively, and the data confirm that the constructed wetland data has not compromised the assumptions. One-way Analysis of Variance (ANOVA) was used to determine the significance of differences in variations in physicochemical variables and metal concentrations within the constructed wetland sites. Tukey’s multiple comparisons for differences between means were also assessed. Physico-chemical parameters were also correlated to see if statistical significance relations between them were employed. In addition, A Mann–Whitney test was performed to evaluate the presence of significant differences in physicochemical between the two wetlands at a 5% degree of error. The nonparametric statistical tests were chosen because the data from natural wetlands did not meet the assumption of normality. Statistical significance was set at $P< 0.05$ for all tests to identify differences. Data were presented using tables, figures, etc. Mean and percentage removal efficiency calculations were done to describe the data.

4.3 Results and Discussion

All the samples taken during the three-month period, which began on October 10th and ended on December 25th, 2021, were analyzed for physicochemical parameters and heavy metals to determine pollutant removal efficiency and potential of the natural and constructed wetlands. Results of studied physicochemical parameters and heavy metals in water used for coffee processing, influent and effluent from the processing plant are presented and discussed in the following parts. Furthermore, a comparison has been made between the pollutants removal efficiency of the natural and constructed wastelands.

4.3.1 Characteristics of phsico-chemical parameters and removal efficiency of the wetlands

A) Physico-chemical characteristics of water used and effluent of processing plant

A total of 14 Physicochemical parameters were analyzed in samples from water used, and the natural and constructed wetland such as pH, temperature, conductivity, Turbidity and Total Dissolved Solids (TDS) were analyzed immediately after collection (**Table 4.3**). The mean Ph value of water used (water entering the processing plant) and effluent of processing (Publing process stage) had 6.75±0.19 and, 4.52.30 ± 0.15, respectively. In this study, the concentration of total dissolved solids in the water used and at the effluent of processing plant had 110.57±26.14 mg/L and 184.5±2.74 mg/L, respectively. Turbidity was found to be 202.83 ± 8.45 NTU in the water used and 231.50 ± 17.93 NTU in the effluent of the processing plant(See more **Table 4.3**).

Table 4.3 Physico-chemical characteristics of water used and effluent of processing plant

	Water used/ entering the processing plant(WS1)	**Effluent of processing plant/ pulping process stage (WS2)**	**EEPA Discharge limit**
pH	6.75 ± 0.19	4.52 ± 0.15	6-9
T^0c	23.43 ± 0.45	24.18 ± 0.29	40
EC	170.67 ± 3.56	267.50 ± 5.24	<1000
TDS	110.5 ± 26.14	184.5 ± 2.74	3000
Turb	202.83 ± 8.45	231.50 ± 17.93	-
$NH4^+$	0.07 ± 0.02	0.53 ± 0.24	≤1
NO_2^-	0.06 ± 0.01	0.13 ± 0.29	-
NO_3^-	0.22 ± 0.24	13.63 ± 0.88	<10
SO_4^{2-}	4.32 ± 0.21	4.85 ± 0.56	200
PO_4^{3-}	2.04 ± 0.098	3.17 ± 0.34	10
DO	7.38 ± 0.20	2.22 ± 0.15	-
TSS	159± 28.6	2001.28±249.66	100
BOD	40.6±14.4	4117.45±253.06	80
COD	74.3±14	8010.24±646.59	250

B) Physico-chemicals contaminants removal efficiency of the wetlands (NW & CW)

The influent and effluent pH values in natural wetlands had the mean value of 4.72 ± 0.27 and 7.12 ± 0.215, respectively. The mean influent, middle and effluent pH in constructed wetland were 4.769 ± 0.247, 6.25 ± 0.723 and 6.9 ± 0.914 respectively. A one-way ANOVA revealed a statistically significant difference ($P<0.01$) in pH across the constructed wetland sites (WS4, WS5 and WS7). In both wetlands, there was a decrease in pH of the effluents. As indicated by Pidgeon and Cains, (1987), this decrease could be attributed to carbon dioxide released from the breaking down of organic wastes by bacteria and organic acids resulting from decaying vegetation. However, the mean effluent pH values from both the natural wetland (7.12 ± 0.215) and constructed wetland (6.9 ± 0.914) were within EEPA (6-9) limits of direct discharge to the river and WHO (6.5–9.2) limit for irrigation use.

The CW effluent pH (6.9) reported in current study is comparable to the 7.2 and 6.51 that reported by Rossmanna *et al.*, (2012) and more recently by Samuel (2021), respectively. However, much lower pH of 3.37 and 4.82 - 5.29 was reported by Genanaw *et.al*,(2021 and Nyerere and Fouad (2018), respectively. The low pH of effluent reported in previous researches could be attributed to a variety of issues, including poor construction design, plant and substrate type, hydraulic retention time and flow rate, and other factors.

A Mann-Whitney U test was performed to evaluate if any difference in effluent pH of CW and NW. The test revealed that the median pH value of the NW (median =5.95) and CW (median =6.4), with U = 954.50, z =-.145, p = 0.85, r = 0.001528, had no significant difference between the two wetland types (**Table 4.4**).

The mean influent and effluent values for temperature in the natural wetland had 24.29 ± 0.55 and, 23.30 ± 0.49, respectively, whereas, in constructed wetland the mean temperature at the influent wetland (WS4) was 24.26 ± 0.51, while at the middle wetland (WS5) and effluent wetland (WS7) it was 23.31 ± 0.35 and 21.36 ± 1.26 respectively. One-way ANOVA revealed that there was a significant difference in temperature along the constructed wetland sites. These values were below the permissible limit recommended by the national (Ethiopian) effluent direct discharge to rivers which is (40 ^{o}C). A decrease in temperature at the effluent of wetlands could be attributed to the shady effect of wetland vegetation and decreased organic matter concentration (Gitau and Kitur, 2014) and naturally, a local weather change could also be another reason. A Mann-Whitney U test revealed that temperatures were significantly lower in the constructed wetland (median =23.40) compared to the natural wetland (median =23.95), U =696.0, z=-2.295, p=0.022, r=0.242.

The mean influent and effluent conductivity values in natural wetlands were 363.78±66.81 mg/L and 245.83±105.4 mg/L, respectively. The mean influent (WS4), middle wetland (WS5) and effluent (WS7) conductivity values were 370.67 ± 67.32, 269.44 ± 117.93 and 224.83 ± 57.35 respectively. The data analysis revealed that the mean concentration of WS4 and WS5, and WS4 and WS7 were differed by a statistically significant ($P<0.01$). However, there was no statistically significant difference in WS5 and WS7 ($P>0.05$). Variation of EC in the wetland sites can be attributed to the physicochemical processes occurring there, which include the removal of ions through sedimentation, precipitation, adsorption and uptake by aquatic plants. These values are below the permissible limit endorsed by WHO 2006 (500 μs/cm) for irrigation and EEPA (2003) (1000 μs/cm) for direct discharge to rivers. The decrease in the conductivity level after the wetland could be attributed to a decrease in the concentration of TDS and TSS and the conversion of NO_3-N into diatomic molecular nitrogen (N_2) as the concentration of charged ions decreases (Gitau and Kitur, 2014). A Mann-Whitney U test revealed that there is an insignificance difference in conductivity between natural wetland (median =312) and constructed wetland (median =300), U =876.50, z=-.787, p=0.431, r=0.083.

The mean TDS levels in natural wetland influent and effluent were 189.78 ± 48.7 mg/L and 123.06 ± 52.36 mg/L, respectively. While in constructed wetland the mean TDS at the influent wetland was 187.61 ± 36.66 mg/L, while at the middle wetland (WS5) and effluent wetland (WS7) it was 128.72 ± 57.63 mg/L and 119.89 ± 43.09 mg/L, respectively. The decrease in TDS observed at the effluent of the two wetlands could be attributable to solid deposition caused by the slower water speed as it passes through the wetland, as well as uptake of some of the dissolved solids by wetland plants and also could be ascribed to the presence of organic matter due to decaying of plant and animal remains solids and ions deposition in the wetland (Gitau and

Kitur, 2014). The high values of TDS can be toxic to freshwater animals causing osmotic stress and can give increase obnoxious odors from the decay of organic matter and vulgar smell (Yemane-Tekle, 2015, Bisekwa *et al.*, 2020). The results show that the efficiency of TDS was noted with 35.16% and 36.1% removal efficiency in natural and constructed wetlands respectively. A Mann-Whitney U test revealed that there is an insignificance difference in TDS value between natural wetland (median =157.5) and constructed wetland (median =147.50), U =896.50, z=-.622, p=0.534, r=0.066 (**Table 4.4**).

During the present study, the inlet turbidity value in the natural wetland ranged between 200.0 - 812.0 NTU while that of the outlet ranged between 15.0-750.NTU with mean values of 386.83 ± 194.69 NTU and 226.19 ± 191.98 NTU at the influent and effluent, respectively. However, the influent ranged between 114-700 NTU in the constructed wetland, while the effluent ranged between 15-410 NTU, with mean values of 343.72 NTU and 138.94 NTU at the influent and effluent, respectively. Turbidity values in the effluent (138.94±148.25) of constructed wetland were found to be lower (378 ± 102.8) than the effluent reported by (Genanaw *et al.*, 2021) in Bokaso coffee processing plant effluent and turbidity in coffee wastewater might be attributable to a variety of solid by-products such as coffee pulp, skin, parchment, and bean. A mean reduction in turbidity was obtained with a removal efficiency of 41.53 % and 59.6% in natural and constructed wetlands respectively. A Mann-Whitney U test revealed that there is an insignificance difference in turbidity value between natural wetland (median =295) and constructed wetland (median =260), U=794, z=-1.468, p=0.142,r=0.155.

Table 4.4 mean value of physicochemical parameter and the removal efficiency of the wetlands in Dale Woreda,Sidama Region

	Natural wetland			Constructed wetland				EEPA
	Influent(WS3)	Effluent (WS6)	%	Influent(WS4)	Middle(WS5)	Effluent (WS7)	%	Discharge
pH	4.72 ± 0.27	7.12 ±0.215	-50.85	4.769±0.247^{a}	6.25±0.723^{b}	6.9±0.914^{c}	-44.68	6-9
T^{0}c	24.29 ± 0.55	23.30± 0.49	4.08	24.26±0.51^{a}	23.31±0.35^{b}	21.36±1.26^{c}	11.95	40
EC	363.78 ± 66.81	245.83±105.4	32.42	370.67±67.32^{a}	269.44±117.93^{b}	224.83±57.35^{b}	39.33	<1000
TDS	189.78 ± 48.7	123.06±52.36	35.16	187.61±36.66^{a}	128.72±57.63^{b}	119.89±43.09^{b}	36.1	3000
Turb	386.83 ±194.69	226.19±191.98	41.53	343.72±165.6^{a}	229.56±139.09^{ab}	138.94±148.25^{b}	59.6	-
NH_4^+	0.86± 0.71	0.52±0.59	39.53	0.88± 0.715^{a}	0.968± 1.095^{a}	1.13±1.578^{a}	-24.41	≤1
NO_2^-	0.18±0 .262	0.037± 0.014	79.44	0.287± 0.407^{a}	0.203± 0.243^{a}	0.128±0 .164^{a}	55.4	-
NO_3^-	33.05±24.21	10.28± 3.168	68.90	33.99±25.29^{a}	22.24±22.30^{ab}	13.38±20.58^{b}	60.6	<10
SO_4^{2-}	4.49±4.27	3.19± 3.69	28.95	4.47± 4.26^{a}	1.27± 1.324^{b}	0.42±0.61^{b}	90.6	200
PO_4^{3-}	3.66±0.75	2.08±0 .42	43.17	3.78± 0.87^{a}	2.45± 0.624^{b}	1.56± .621^{c}	58.7	10
DO	0.513±0.039	3.67±0.19	-	0.54±0 .056^{a}	3.263± 0.694^{b}	4.56± 1.011^{c}	-	-
TSS	2190.78±448.46	972.67 ±234.312	55.60	2253.2± 508.2^{a}	1048.61± 258.6^{b}	255.44±248.2^{c}	88.7	100
BOD	4277.94±157.02	326.83 ±112.24	92.36	4192.4±191.3^{a}	782.72±507.6^{b}	88.28± 20.08^{c}	97.9	80
COD	8085.61±536.99	675.33±201.4	91.65	8409.8±592.9^{a}	1372.6± 387.94^{b}	249.0± 7.68^{c}	97	250

For constructed wetland One Way ANOVA was done; numbers followed by the same letter superscripts in the same row do not vary significantly by **Tukey’s multiple comparisons** test at $p < 0.05$.

The mean Ammonium (NH_4^+) concentration in the natural wetland was 0.86 ± 0.71 mg/L and 0.52 ± 0.59 mg/L at the influent and effluent, respectively. While in constructed wetland the ammonium concentration was 0.88 ± 0.715 mg/L, 0.968 ± 1.095 mg/L and 1.13 ± 1.578 mg/L at the influent, middle and effluent, respectively. The mean effluent of Ammonium (NH4+) concentration from natural wetlands (0.52 ± 0.59 mg/L) was found within the national (Ethiopian) effluent discharge to rivers which is (≤1mg/l). However, the mean effluent in the constructed wetland was above the standard discharge limit for rivers. The results show that the mean effluent (0.52 ± 0.59 mg/L) of natural wetland was lower (4.99 ± 0.36ppm) than that reported by (Mosissa *et al*., 2016) and reported by (Bisekwa *et al.*, 2021) with mean of 5.55 ± 2.23 mg/L.This might be due to previous studies of wastewater effluents released without any treatment.

The mean concentrations of NH_4 were increased at the effluent (WS7), but the variation was non-significant. The enrichment of NH_4 in constructed wetland systems occurs for a variety of reasons, including the following: The process of ammonification produces ammonia from organic nitrogen in effluent. Both anaerobic and aerobic conditions can support this activity. In the wetland system, ammonification occurred due to anaerobic conditions. As opposed to this, an aerobic environment controls the nitrification process. In an oxic circumstance, the availability of inorganic carbon and NH_4, as well as temperature and pH ranges of 30-40^{0}C and 7.5-8.0, respectively, enhance nitrification rates in wetlands. It was warm enough for cultivated plants to develop quickly. As a result, nitrogen transformation from NH_4 to NO_3-N was completely inhibited due to the lack of a nitrification process (Xu *et al*., 2014). Ammonium is a critical parameter for fish in aquaculture due to its toxicity and it can eventually cause cell death in the central nervous system when it is in high concentration (Mburu *et al*., 2020). The removal efficiency of ammonium was 39.53% in the natural wetland whereas in constructed wetland it was -28.41%, Fig. 4.11 shows a little enlargement of ammonium during the flow through the constructed wetland. A Mann-Whitney U test revealed that there is a significance difference in ammonium concentration between natural wetland (median =0.29) and constructed wetland (median =0.4), U =954, z=-2.148, p=0.0442, r=0.226.

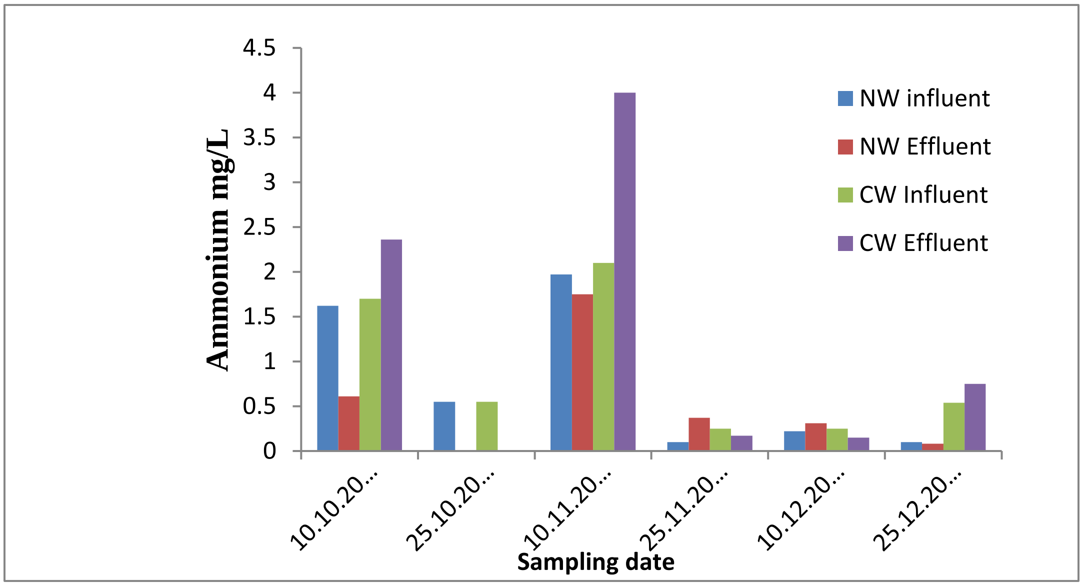

Figure 4.12 Influent and effluent of (NH_4^+) in these two wetlands (NW: Natural wetland; CW: Constructed wetland).

Nitrite (NO_2^-) concentrations were a mean of 0.18 ± 0.262 mg/l in influent of natural wetland whereas, at the effluent, nitrites concentrations were a mean of 0.037 ± 0.014 mg/L. The mean nitrite concentrations in the influent of a constructed wetland were 0.287 ± 0.407 mg/l, whereas nitrite concentrations in the middle constructed wetland was 0.203 ± 0.243 mg/l and the effluent was 0.128 ± 0.164 mg/L. One-way ANOVA revealed that there were no statistically significant differences in nitrite between WS4, WS5, and WS7 (P>0.05).

The results show that the mean effluent (0.037 ± 0.014 mg/L) of nitrite in the natural wetland of the current study was comparable (0.001 – 0.121 mg/L) with what was reported by (Xu *et al*., 2014) in the natural wetland. But lower (0.6 ± 0.1 mg/L) than reported by (Bisekwa *et al*., 2020) at Kayanza wet coffee processing plant. The mean nitrites concentrations of the effluent (0.128 ± 0.164 mg/L) found in a constructed wetland of this study were comparable with those reported by Hadad *et al*. (2006) who reported nitrite concentration (0.13 mg/L) and reported by (Xu *et al*., 2014) nitrite concentration (0.028–0.443 mg/L) in a constructed wetland. The removal efficiencies of nitrite were 79.44% and 55.4% in the natural and constructed wetland, respectively. The nitrites values of the samples from all the sites were below the recommended WHO (2006) standards. A Mann-Whitney U test revealed that there is an insignificance difference in nitrite between natural wetland (median =0.048) and constructed wetland (median =0.046), U =893.5, z=-0.648, p=0.517, r=0.0168.

Organic nitrogen in wastewater was transformed into ammonia by oxidative decomposition under the action of micro-organisms and then the ammonia was further removed through nitrification and denitrification processes (Xu *et al*., 2014). Despite this, the nitrate content increased. The mean nitrate concentrations in the influent of a natural wetland were 33.05 ± 24.21 mg/l, whereas nitrates concentrations in the effluent were 6.30 -15.0 mg/L. However, the mean nitrate concentration in the influent of a constructed wetland was 33.99 ± 25.29 mg/l, whereas nitrate concentrations in the middle constructed wetland were 22.24 ± 22.30 mg/l and the effluent was 13.38 ± 20.58 mg/L. The mean nitrate concentrations in wastewater WS4 and WS7 differed statistically significantly ($P<0.05$) according to a one-way ANOVA. However, there were no statistically significant differences between WS4 and WS5 ($P>0.05$), as well as

WS5 and WS6 (P>0.05). The mean effluent of nitrate concentration in each wetland was found to be higher than Ethiopia's (< 10 mg/l) national effluent discharge to rivers.

The mean effluent (10.28 ± 3.168 mg/L) of nitrate in the natural wetland in the present study was higher (3.39 ± 0.65 mg/L) than reported by (Tilahun *et al*., 2013) and reported by (Xu *et al*., 2014) nitrate concentration (0.091– 4.75 mg L), reported by (Sileshi *et al.*, 2020) nitrate concentration (0.26 - 1.11 mg/L), and reported by (Dendup *et al*., 2021) nitrate concentration (0.7 - 1.5 mg/L) in the natural wetland. The disparity might be explained by variations in the treatment's removal effectiveness of natural wetlands. The mean effluent nitrate values (13.38 ± 20.58 mg/L) found in a constructed wetland of the present study were comparable with those reported by Bisekwa *et al* (2020) nitrate (12.6 ± 2.9 to 27.4 ± 3.8 mg/L). However, lower (49.8 ± 12.4 mg/L) than reported by (Genanaw *et al*.,2021) in the constructed wetland at Bokaso coffee processing plant. But higher than those reported by Hadad *et al*. (2006), who reported a nitrate content of (1.45 mg/L), Reported by (Tilahun *et al.*, 2013) nitrate concentration (2.04 ± 0.34 mg/L) and reported by (Xu *et al*., 2014) nitrate concentration (0.54–2.13 mg/L) in the constructed wetland. The difference could be attributed to differences in the treatment's removal efficacy. The mean removal efficiencies of nitrate were 68.9% and 60.6% in the natural and constructed wetland, respectively. The decrease in nitrate concentration recorded at the wetland effluent could be attributed to denitrification where nitrate is converted to diatomic molecular nitrogen, deposition of nitrate in sediments at the wetland bottom and plant uptake (Gitau and Kitur, 2014). The nitrates concentrations level was above the national (Ethiopian) standards (EEPA, 2003) effluent discharge to rivers, which indicated that the wet coffee processing factories' effluents contribute to the pollution of the receiving water bodies. A Mann-Whitney U test revealed that there is an insignificance difference of nitrate between natural wetland (median =12.5) and constructed wetland (median =11.9), U =872.5, z=-0.82, p=0.412, r=0.08.

The mean Phosphate concentrations in the influent and effluent of natural wetlands were 3.66 ± 0.75 mg/L and 2.08±0.42 mg/L, respectively. The mean phosphate concentrations in the constructed wetland's influent, middle, and effluent were 3.78± 0.87 mg/L, 2.45± 0.624 mg/L and 1.56± .621 mg/L, respectively. The concentrations level of phosphate in the coffee wastewater of constructed wetland sites was found to be statistically significant ($P<0.05$). The mean effluent of phosphate concentration in the natural and constructed wetland was found to be below Ethiopia’s (10 mg/l) effluent discharge to rivers.

The current finding found in the discharge of wastewater (2.08±0.42 mg/L) in the natural wetland was comparable (3.32 ± 0.5 mg/L) those reported by (Tilahun *et al*., 2013). However, much higher (0.047–0.26 mg/L) than reported by (Xu *et al*., 2014), reported by (Sileshi *et al.*, 2020) phosphate concentration (0.67 ±0.52mg/L), reported by (Bisekwa et al., 2021) phosphate concentration (0.78 ± 0.34 mg/l) and reported by (Dendup *et al*., 2021) phosphate concentration (0.56 to 0.81±0.07 mg/L) in the natural wetland. The finding of the present study was lower (4.6 mg/l) than what was reported by (Haadis and Rani, 2008). This difference might be due to the complex combination of physical, chemical, and biological processes involving mainly adsorption, precipitation, sedimentation in pores of the substrate media, peat accretion and burial, and to a lesser extent biomass uptake (Dzakpasu *et al*., 2015).

The amount of wastewater discharged (1.56± .621mg/L) in the constructed wetland of the present study was lower (3.9 mg/L) than those reported by Saxena, (2016) in an effluent Treatment Plant of an Instant Coffee Production Unit in India, reported by Said *et al* (2019) phosphate (12.2 mg/L) from a coffee processing plant in Pulau Pinang, Malaysia and Genanaw *et al* (2021) phosphate (20±3.2 mg/L) coffee processing plant in Sidama region, Ethiopia. However, the effluent from constructed wetland was comparable with (2.26 ± 0.68 mg/L) reported by (Tilahun *et al*., 2013) and reported by (Xu *et al*., 2014) who reported phosphate concentration was (0.41–2.13 mg/L). The difference could be attributed to differences in the treatment's removal efficacy. The phosphorus removal mechanism in the wetlands includes fillers adsorption, plant uptake, and microbial assimilation. Bacteria and algae-containing wetlands are another factor for excess phosphorus removed from the effluent. Sedimentation of organic matter and incorporation into biomass by the macrophytes might cause this effect (Xu *et al*., 2014). In both wetlands phosphates concentrations of the effluent do not appear to pose any threat to the receiving water bodies according to EPA (2003), As a result, the receiving water bodies were not altered or polluted by this characteristic. Average removal efficiencies of phosphate were 43.17% and 58.7% in natural and constructed wetlands respectively. The plants' uptake of the phosphate in both wetland effluents or some of it is deposited in the wetland bottom with sediments and adsorption are two possible explanations for the lower concentration of phosphate (Gitau and Kitur, 2014).. Phosphate concentrations greater than 5mg/L, are attributed to human activities and contamination rise to excessive growth of algae (EEPA, 2003) and the presence of $PO4^{3-}$ in water increases eutrophication and similarly promotes the growth of algae (Bisekwa *et al.*, 2021). A Mann-Whitney U test revealed that there is no significant difference in phosphate concentration between natural wetland (median =2.5) and constructed wetland (median =2.4), U =827.0, z=-1.195, p=0.232, r=0.126.

In both wetlands, the finding of the present study shows an increasing range of dissolved oxygen in the effluent as compared to influent. The increase in the level of dissolved oxygen at the outlet could be attributed to the photosynthesis and biodegradation of compounds present in wastewater that previously used dissolved oxygen for various oxidation-reduction reactions and thus the release of oxygen through roots into the rhizosphere (Gitau and Kitur, 2014). In the present finding, the mean level of dissolved oxygen in the natural wetland was 0.513±0.039 mg/L in influent while that of the effluent was 3.67±0.19 mg/L. The mean DO (3.67±0.19 mg/L) effluent by natural wetland was comparable with (2.14 ± 0.72 mg/L) reported by (Tilahun *et al*., 2013) and reported by (Sileshi *et al.*, 2020) DO (3.12 ±1.24 mg/L) in Boye natural wetland. However, higher than the mean DO (1.9 mg/L) Tibia wetland in the treatment of wastewater (Gitau and Kitur, 2014). Lower DO concentrations in the effluent of the previous study might be due to relatively higher microbial activities in the water due to the presence of biodegradable organic compounds (Dendup *et al*., 2021). Also, increased nutrient loading can be one of the reasons for the depletion of dissolved oxygen in the wetland effluent (Aniyikaiye *et al*., 2019).

Dissolved oxygen concentrations at the influent of constructed wetland were 0.54±0.056 mg/L, at the middle wetland it was 3.263±0.694 mg/L and at the effluent 4.56± 1.011 mg/L. A one-way ANOVA revealed that the mean DO of wastewater at WS4, WS5, and WS7 was statistically significantly different from each other's ($P<0.01$). In the present study, the mean DO (4.56± 1.011 mg/L) of effluent in the constructed wetland was comparable (4.38 ± 0.63 mg/L) with reported by (Tilahun *et al*., 2013) in a constructed wetland. But, it is much higher than coffee effluents (0.9±0.46 mg/L) reported by (Genanaw *et al*., 2021). The difference might be due to poor construction design, plant and substrate type, hydraulic retention duration and rate, flow rate, and other variables (Genanaw *et al*., 2021). The photosynthetic activities in plants increase the DO in water, thus creating aerobic conditions in the system which also favors the aerobic bacterial activity to reduce BOD (Yadav *et al.,* 2011). An increase in DO facilitates the oxidation process within the wetland system (Shukla *et al.*, 2021). In the planted wetland, the effect of the root zone might have enhanced the concentration of DO. In addition to this increase DO level in the effluent of constructed wetlands might be related to the removal of organic substances through various means (Aniyikaiye *et al.*, 2019). The DO standard for sustaining aquatic life is set at 5 mg/L, and any concentration below this number has negative consequences for aquatic life (Chapman, 1996). All sample sites from coffee processing plants had mean DO concentrations of less than 5 mg/L, Discharging those effluents into rivers, as a result, would be harmful to aquatic life's survival. This study is in agreement with the study done previously (Tilahun *et al.*, 2013, Sileshi *et al.*, 2020) which reported DO concentrations of less than 5 mg/L. A Mann–Whitney U test revealed that dissolved oxygen was significantly lower in the natural wetland (median =1.98) compared to constructed wetland (median =3.30), U =737.0, z=-1.937, p=0.05, r=0.204.

Table 4.5 Mann-Whitney: U test statistics comparison of natural and constructed wastewater wetland

	Type of wetland	Median	U	z	r	p.value
pH	Natural	5.950	954.50	-0.145	0.00153	0.885
	Constructed	6.400				
T^0	Natural	23.95	696.0	-2.295	0.242	**0.022**
	Constructed	23.40				
EC	Natural	312.5	876.5	-0.787	0.083	0.431
	Constructed	300				
TDS	Natural	157.5	896.50	-0.622	0.066	0.534
	Constructed	147.50				
Turb	Natural	295	794	-1.468	0.155	0.142
	Constructed	260				
NH 4 +	Natural	0.29	954	-2.148	0.226	**0.0442**
	Constructed	0.4				
NO_2^-	Natural	0.048	893.5	0.648	0.0168	0.517
	Constructed	0.046				
NO_3^-	Natural	12.5	872.5	-0.82	0.08	0.412
	Constructed	11.9				
PO_4^{3-}	Natural	2.5	827.0	-1.195	0.126	0.232
	Constructed	2.4				
DO	Natural	1.98	737.0	1.937	0.204	**0.05**
	Constructed	3.30				
TSS	Natural	1551.50	676.5	-2.435	0.257	**0.015**
	Constructed	922.5				
BOD	Natural	2195	765.5	-1.776	0.187	**0.05**
	Constructed	630				
COD	Natural	4099	838	-1.104	0.116	0.27
	Constructed	1550				

Mann-Whitney U test statistics shown in bold are significant at $p<0.05$

As presented in table 4.4, the mean influent and effluent TSS values by natural wetland were 2190.78±448.46 mg/L and 972.67±234.312 mg/L, respectively which was above the permissible limits recommended by WHO (50 ppm), ISI (500 ppm) for irrigation and EEPA (100 ppm) for effluent discharge guideline. Removal of other pollutants like BOD, COD, and heavy metals from the water also leads to a decrease in TSS concentration (Gitau and Kitur, 2014). However, in the constructed wetland the mean TSS value in influent (WS4), middle (WS5), and effluent

(WS7) were 2253.2±508.2 mg/L, 1048.61± 258.6 mg/Land 255.44±248.2 mg/L respectively, which was above the permissible limits recommended by WHO (50 ppm) for irrigation and EEPA (100 ppm) for effluent discharge guideline. The data analysis revealed that the mean TSS values of WS4, WS5 and WS7 have statistically significant differences at (P<0.05). The decrease in TSS concentration noted at the effluent can be attributed to the luxuriant vegetation at the wetland which reduces the speed of the water flowing through the wetland hence causing most of the suspended solids to settle from within the water column and also removal of BOD, COD, and pollutants like heavy metals from the water also lead to decrease in TSS concentration (Gitau and Kitur, 2014).

The concentration of TSS in the natural wetland (972.67±234.312 mg/L) of the studied effluent was much lower (2880 mg/L) than in coffee effluents analyzed by (Haadis and Rani, 2008). However, higher than coffee effluents (259.5 ± 65.3 mg/L) reported by (Tilahun *et al.*, 2013) in the natural wetland. In the present study, the mean effluent value of TSS in the constructed wetland was 255.44±248.2 mg/L with a range of 80-701 mg/L which was much lower than coffee effluents of TSS (399.3 mg/L) reported by Said *et al.*, (2019), reported by Bisekwa *et al.*, (2020) TSS was in the range of 2481.3 ± 45.6 to 2640.9 ± 60.0 mg/l and reported by Genanaw *et al.*, (2021) TSS (1852.3±875.5 mg/L) in treating coffee wastewater at Bokaso coffee processing plant. Those differences are due to the different types and levels of coffee processing involved in each production plant, the chemicals or additional ingredients used and how the waste was handled individually in each plant. Because of the slow hydrolysis rate of the organic part of the material, solids discharge raises the turbidity of water and produces a long-term demand for oxygen. Sugar, proteins, and carbohydrates are all possible components of this biological substance. The natural biodegradation of proteins will eventually lead to the discharge of ammonium, which is ammonium oxidation into nitrite and nitrate by nitrifying bacteria, leading to extra consumption of oxygen on its oxidation by bacteria (Chapman, 1996). On comparing TSS values with EEPA permissible limits for discharging of treated effluent for irrigation purpose as given in Table 3.3, it was found that the concentration of TSS in both wetlands were very high. The mean removal efficiency of TSS in the natural wetland was 55.6%.whereas, in the constructed wetland, the average removal efficiencies of TSS were 88.7%. The finding of the present study of constructed wetland is in agreement with the removal efficiency of TSS (89%) in the study done previously (Terzakis *et al*., 2008) and TSS (94%) in coffee industry effluent(Said *et al*., 2019). A Mann –Whitney U test revealed that TSS was significantly higher in the natural wetland (median =1551.50) compared to constructed wetland (median =922.5), U =676.5, z=-2.435, p=0.015, r=0.257. In contrast to natural wetland wastewater treatment systems, the usage of constructed wetlands in wastewater treatment may offer solutions for reducing footprint and preserving the environment.

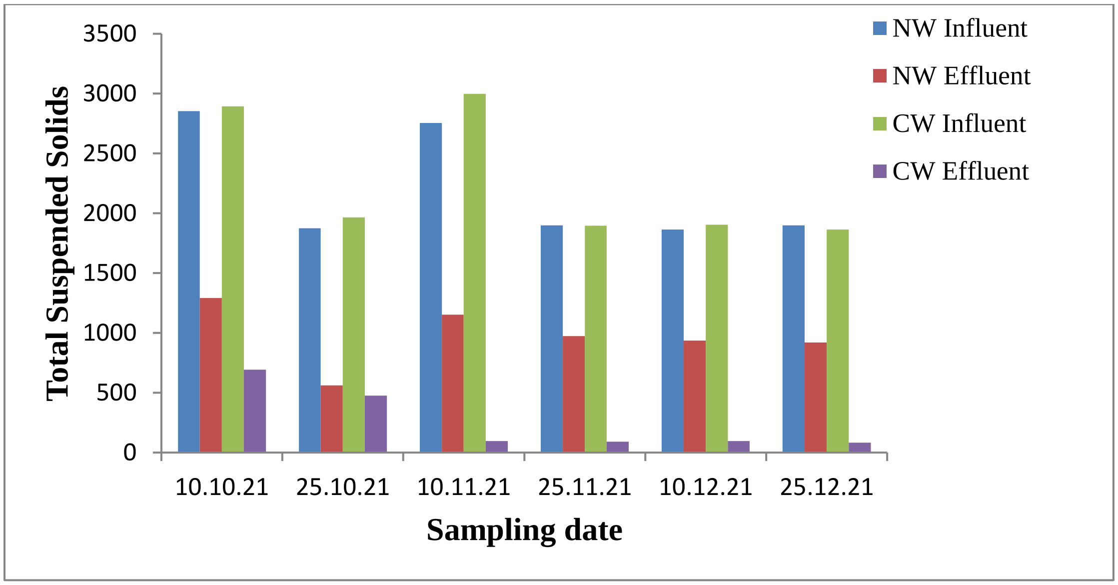

Figure 4.13 Influent and effluent of TSS in the two wetlands (NW: Natural wetland; CW: Constructed wetland).

COD and BOD were used to calculate the organic load. The BOD/COD ratio represents the biodegradability of an effluent (Skrzypiec and Gajewska, 2017). In the current study, the effluent of coffee processing obtains BOD/COD comparison as 0.48 and 0.36 in natural and constructed wetlands respectively which indicates that the ratio was between 0.36 to 0.54, showing that effluent from the production of coffee can be broken down and handled in a biodegradable manner (Abdelhakeem *et al.*, 2016). Biological oxygen demand at the influent natural wetland was (4277.94±157.02 mg/L) while the mean effluent of BOD (326.83±112.24 mg/L). The mean BOD value of the examined effluents (326.83±112.24 mg/L) in the natural wetland was much lower (1697 ± 390.67 mg/L) than reported by (Tilahun *et al.,* 2013). However, much higher (38.9 mg/L)   than reported by Gitau and Kitur, (2014) and reported by Xu *et al.*, (2014) BOD (8-15 mg/L) in the natural wetland. The reason for this difference might be due to the fact that the volume and strength of the effluent vary every day and primarily depends on the quantum of water used for coffee processing (i.e. lesser the water, higher the strength of effluent and vice-versa) (Gopinandhan *et al.*, 2022).

The mean BOD at the influent of constructed wetland was (4192.4±191.3 mg/L) while at the middle wetland (782.72±507.6 mg/L) and the mean effluent of BOD (88.28±20.08 mg/L) which is above the permissible limit set by WHO (2006) for irrigation and EEPA (2003) standard limits of 80mg/L for effluent discharge. The mean BOD of the studied effluent (88.28± 20.08 mg/L) in constructed wetland was much lower than the coffee effluent of BOD (3149±103.0 mg/L) in treating coffee wastewater at Bokaso coffee processing plant (Genanaw *et al.,* 2021) , BOD (171.5 mg/L) effluent of coffee processing plant in Pulau Pinang, Malaysia *(*Said *et al.*, 2019) and BOD (1946 mg/L) effluent of coffee reported by Saxena (2016). However, higher than the concentration of BOD (16 mg/L) in a pilot-scale constructed wetland for industrial wastewater treatment at Bahco Argentina (Hadad *et al*., 2006), reported by (Xu *et al*., 2014) BOD (3.61–27.67mg/L) in constructed wetland and BOD (25-49 mg/L) concentration of effluent in Hayatabad industrial Estate (Xie *et al*., 2022). Those differences might be due to the different types and levels of coffee processing involved in each production plant, the chemicals or additional ingredients used and how the waste was handled individually in each plant. In both, natural and constructed wetlands the mean effluent is above the permissible limit set by WHO (2006) for irrigation and EEPA (2003) standard limits of 80mg/L for discharge to river. The reduction in BOD_5 concentration at the effluent can be attributed to biodegradation of the organic matter by microbial bacteria's in the wetland and the trapping of particulate organic matter by wetland vegetation might have also contributed to the decrease in BOD_5 concentration at the effluent as the organic matter settle as sediment off the water column (Gitau and Kitur, 2014). The BOD_5 removal efficiency was 92.36% and 97.9% for natural and constructed wetlands, respectively. A Mann-Whitney U test revealed that there is a significant difference in BOD concentration between natural wetland (median =2195) and constructed wetland (median =630), U =756.5, z=-1.776, p=0.05, r=0.187. Because it is easier to administer and regulate a well-designed manufactured wetland, it can perform better than a natural wetland. This is why constructed wetlands outperformed natural wetlands.

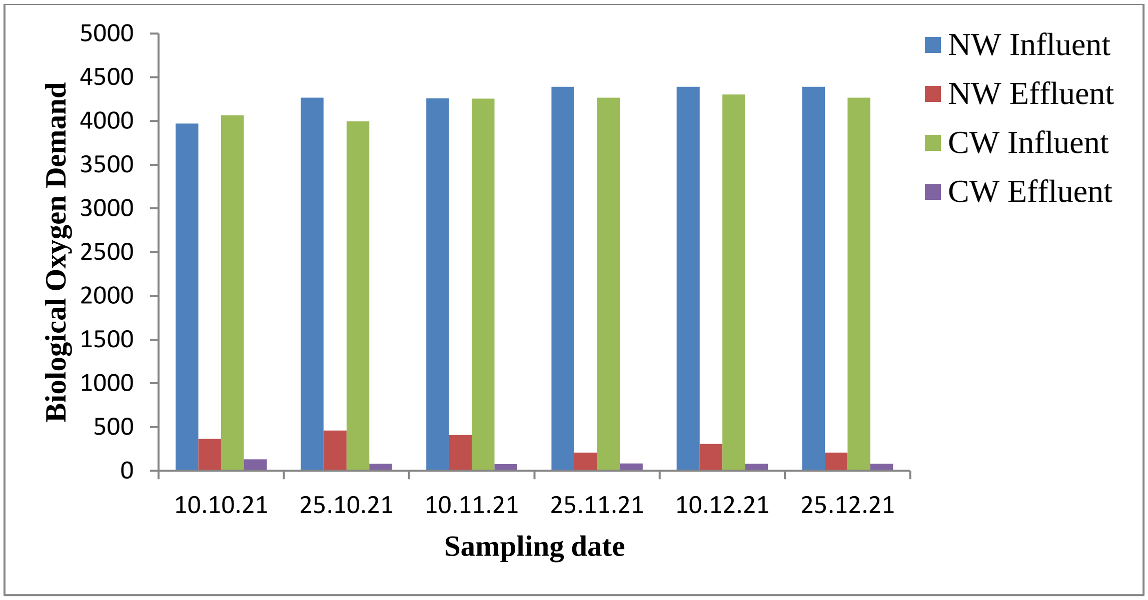

Figure 4.14 Influent and effluent of BOD in two these wetlands (NW: Natural wetland; CW: Constructed wetland).

COD shows the oxygen needed for the chemical oxidation process of organic substances. COD score indicates the number of dissolved organic substances which can be oxidized including all unraveled material content (Kalshetty *et al*., 2014).In the present study, COD of influent in natural wetlands fluctuated between 7198-9210 mg/L with an average of 8085.61±536.99 mg/l. The COD was reduced dramatically in the effluent of the system, which was found in the range of 400 -1000 mg/L with an average effluent of 675.33±201.4 mg/L which is above the national (Ethiopian) effluent direct discharge to rivers. The effluent of COD in natural wetland was lower (9780 mg/L) than reported by (Haadis and Rani,2008) and reported by (Tilahun *et al*., 2013) COD (5682.5 ± 304.45 mg/L). The possible justifications could be different flow velocities, surface area, and microbial activities in the wetland.

Whereas, the mean COD values in constructed wetlands were found to be 8409.8±592.9, 1372.6± 387.94 and 249.0± 7.68 at influent, middle and effluent respectively. COD content of coffee effluent in the constructed wetland (249.0 ± 7.68 mg/L) was noticed to be within the EEPA (2003) limit value for direct discharge to a river which is less than 250mg/L. A one-way ANOVA revealed that the mean COD of wastewater at WS4, WS5, and WS7 were statistically significantly different from each other's ($P<0.05$). As shown in the table 3.3, COD in the effluent wastewater (WS7) samples was significantly lower ($P < 0.05$) than the effluent (WS4) of the CW, indicating that the CW has effectively removed the COD from the wastewater.

In the present study, the effluent of COD (249.0±7.68 mg/L) values in the constructed wetlands was much higher than what was reported by the other researchers such as Haddad *et al*.,2006 who reported COD (40.0 mg/L); Terzaki *et al*.,2008 who reported COD (44-55.0 mg/L) and Xi *et al*., 2022 who reported COD (197-394 mg/L). But lower than the COD (3260±620 mg/L) reported by (Genanaw *et al*.,2021), reported by (Said *et al*., 2019) concentration of COD (13,000 mg/L) of coffee effluents in Malaysia, reported by Mosissa *et al.*, (2016) COD effluent ranged (1451-2735 mg/L), and COD (7785 mg/L) reported by Saxena (2016). This difference might be due to chemical composition which will vary from plant to plant from different geographic locations, also depending on their age, climate, and soil conditions (Murthy and Naidu, 2012). The COD removal was approximately 91.65% and 92% in a natural and constructed wetlands, respectively. High COD removal efficiency as obtained in this research was mostly caused by sedimentation, filtration, and absorption process. By using bacterial decomposition, sedimentation of particulate matter, and filtering by plant roots, COD was reduced (Selvamurugam *et al*., 2010). A Mann-Whitney U test revealed that there is an insignificance difference in COD concentration between natural wetland (median =4099) and constructed wetland (median =1550), U =838, z=-1.104, p=0.27, r=0.116.

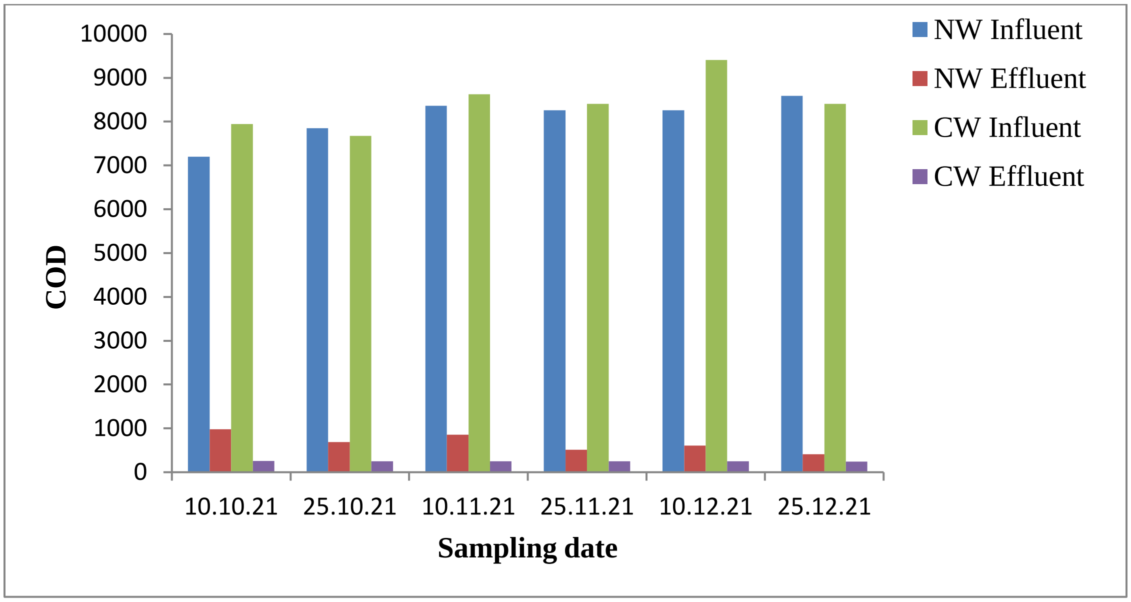

Figure 4.15 Influent and effluent of COD in two wetlands (NW: Natural wetland; CW: Constructed wetland).

4.3.2 Heavy metals concentrations in water used and effluent of the processing plant

A) Concentrations of heavy metal of water used and effLuent of processing plant

All of the identified heavy metals (Ni, Zn, Cu, Na, K, Ca, Mg, Fe, Mn, and Cr) were detected in the WS1, WS2, WS3, WS4, WS5, WS6 and WS7, except for Pb, Cd and Co.

The highest mean nickel concentration was detected in WS2 (0.167± 0.01098 ppm). The lowest mean nickel concentration was detected in WS7 (0.032± 0.0174 ppm). The rise in Ni seen in the WS2 in the processing plants investigated is most likely attributable to the type of machinery which is during the process and also could be owing to the most acidic pH values range (4.3-4.7) in this plant's pulping water, which could lead to increased machinery corrosion. The acidity of the pulping water, as well as friction between the coffee berries and the machinery, could have caused a change in the machinery enamel and paint (Siu *et al.*, 2007). Ni concentrations in discharge water from the two wetlands plants (WS6 and WS7) were lower than those found in pulping-process water, but WS6 was greater than those found in water entering the processing system. Fortunately, in all sample site, the mean concentration of nickel was above the FAO/WHO standard (WHO., 2006). A one-way ANOVA revealed a statistically significant difference ($P<0.05$) between the mean nickel concentrations in wastewater WS1 and WS2, WS3 and WS4. However, no statistically significant differences were between WS2, WS3, and WS4. Similarly, no statistically significant differences existed between WS1, WS5, WS6, and WS7.

The current study's findings revealed that the concentration of nickel in the effluent (0.032± 0.0174 ppm) of constructed wetland was substantially lower than earlier study which reported (23 ppm) Ni concentration in Soconusco, Chiapas, Mexico (Siu *et al.*, 2007) , reported by (Ayeni, 2014) which reported Ni (2.60 ppm) concentration from an industrial area in Ibadan, Nigeria and reported by (Mansourri and Madani, 2016) which reported Ni (0.487 ppm) concentration from wastewater of Bandar Abbas wastewater treatment plant. But higher than the concentration of Ni (0.00691 ppm) in wastewater from the Nairobi industrial area in Kenya (Kinuthia *et al.*, 2019). This discrepancy could be attributed to differences in natural and anthropogenic Ni concentrations in the study area.

The presence of Zn in coffee processing was found to be lowest in the WS1 (0.035±0.00807 ppm) and greatest in the WS3 (0.12±0.02625 ppm) and WS4 (0.118±0.02820 ppm), respectively. This could indicate that the Zn originated in fertilizers or chemical formulas used for pest control such as Zinc sulfate applied to coffee plants before the harvest (Rossi *et al.,* 2019). Zinc concentrations differ greatly between sampling sites. A one-way ANOVA showed a statistically significant difference ($P<0.05$) between the mean zinc concentrations in wastewater WS1 and WS3, WS1 and WS4, WS1 and WS5, WS1 and WS6. Similarly the difference between sampled from WS2 and WS3, WS2 and WS4, WS2 and WS6. However, no statistically significant differences were between WS3, WS4 and WS5.

The current investigation found that Zn (0.12± 0.02625 ppm) concentrations in wastewater were comparable with the previous study that reported Zn (0.269 ppm) in wastewater of the Bandar Abbas wastewater treatment plant (Mansourri and Madani, 2016) and by Bahiru (2020) which reported Zn (0.21 ppm) in wastewater around Eastern Industrial Zone, Central Ethiopia (Bahiru, 2020). However, the finding of the present study was lower than those reported by Siu *et al* (2007) Zn (13.7 ppm) in the coffee wastewater. This discrepancy could be attributed to differences in natural and anthropogenic Zn concentrations in the study area. In all the samples the mean concentration of Zn exceeded the recommended maximum concentration for crop irrigation (WHO, 2006). Zn is the least toxic and necessary component of the human diet since it is important for maintaining the healthy immunological function, normal brain function, and fetal growth and development. However, a very high concentration of zinc is exceedingly toxic and hence hazardous to the human body (Helen and Othman, 2014).

The highest mean concentrations of Cu were found in WS2 (0.3010±0.02517 ppm) and the lowest was found in WS7 (0.0283±0.06528 ppm). Higher values were found in the pulping stage water, which was WS2 (0.3010±0.02517 ppm), than in the entrance water, which was WS2 (0.189±0. 01592 ppm). This is probably due to the wearing of the machinery that is made mainly from copper. A one-way ANOVA revealed that there was not a statistically significant difference ($P>0.05$) among the mean copper concentrations in all sample sites. The threshold limit for the

WHO (2006) is 0.01ppm; by this recommendation, the water in all sites was described as unsafe for irrigation. This indicates that the coffee effluents from all locations contribute to water pollution in studied areas. Cu plays an important role in chemical and biological processes in the environment and that excessive exposure could lead to health hazards and it is acutely toxic to most forms of aquatic life at relatively low concentrations (Bisekwa *et al.*, 2021).

The present study found that means Cu concentrations in wastewater were lower than those reported in Coffee Processing Wastewater in Soconusco, Chiapas, Mexico which was (113 ppm) Copper concentration (Siu *et al.*, 2007) , Ayeni (2014) reported Cu concentration (6.7 ppm) in an industrial area in Ibadan, Nigeria and Birhanu (2020) report Copper (0.99±0.06 ppm) concentrations in around Eastern Industrial Zone, Central Ethiopia (Bahiru, 2020). This could be attributed to the reason of anthropogenic activities and industrial effluent released with treatment. The finding of the present study was comparable with the previous study which reported (0.4 ± 0.2 ppm) in Coffee growing ecological Zones in Burundi (Bisekwa *et al.*, 2020) and by Mansourri and Madani (2016) Cu (0.340 ppm) concentration in wastewater of Bandar Abbas wastewater treatment plant (Mansourri and Madani, 2016).

In the present study, the highest mean value of sodium concentration in the wastewater sample was in WS6 (46.97±2.03 ppm) and the lowest was found in WS1 (18.79±2.065 ppm). A one-way ANOVA showed a statistically significant difference ($P<0.05$) between the mean sodium concentrations in wastewater WS1 and WS2, WS1 and WS6, However, no statistically significant differences were between WS2, WS3, WS4, WS5, WS6 and WS7. The finding of the present study in all sample sites was above the permissible limit of the FAO's irrigation water quality standard FAO/WHO (2006) which is <3ppm. According to FAO, a concentration of sodium above 9 ppm is a severe restriction for irrigation.

In the present study, the highest mean value of potassium concentration in the wastewater sample was in WS2 (11.75±0.73 ppm) and the lowest was found in WS7 (3.98±0.165 ppm). Similarly, the highest mean value of calcium concentration in the wastewater sample was in WS4 (24.43±1.73 ppm) and the lowest was found in WS7 (10.67±1.165 ppm). Except for WS2, WS3, and WS4, all of the remaining sample sites were below the permissible limit of the FAO.

In the present study, the highest average concentration of Iron was found in WS4 (2.694±1.33 ppm) and WS3 (2.64±1.28332 ppm). The lowest average concentration of Iron was found in WS1 (0.985±0.07583 ppm). The discharge of wastewater (WS6 and WS7) has a higher Fe concentration than the water entering the plant (WS1) and the pulping process water (WS1). Iron concentrations in wastewater discharge increased as a result of corrosion and wear of the machinery, which is made of iron and copper, and as a result of naturally occurring minerals found in coffee. The mean copper concentrations in all sample sites did not differ statistically significantly (P>0.05) according to one-way ANOVA.

The concentration of Iron in the present study is much lower than the value reported by Siu et al (2007) Fe (1,872ppm) in coffee processing wastewater in Soconusco, Chiapas, Mexico and Birhanu (2020) Fe (5.13±0.06 ppm) in around eastern industrial Zone, Central Ethiopia. The difference might be due to the lower findings of the present study might be due to the differences in human activities and wastewater treated in the natural and constructed wetlands. The mean concentration of Iron in wastewater in all sites was above the recommended maximum concentration (RMC) for irrigation WHO (2006) is 0.3 ppm. Therefore, it may be claimed that the area's iron concentration is high for agricultural irrigation needs. These elements can oxidize easily, causing problems such as the staining of clothes, and blocking of pipes, pumps and other machinery related to water supply systems in communities downstream that are supplied by the river water (Siu *et al.*, 2007). High concentration of iron in wastewater contributes to soil acidification and loss of availability of phosphorus and molybdenum when applied to the soil (Ayers and Westcot, 1985). Iron-rich wastewater can make the soil more acidic and reduce the amount of phosphorus in the soil (Abagale *et al.*, 2013).

The highest mean Mn concentration (1.55±1.0 ppm) was recorded from WS2 and the lowest (0.209±0.29 ppm) in wastewater from WS4. The data analysis revealed that the mean concentration of WS2 and WS3 differed by a statistically significant ($P<0.05$). However, there was no statistically significant difference in WS2 between WS1, WS4, WS5, WS6, and WS7. Similarly WS3 did not differ statistically from WS1, WS4, WS5, WS6, and WS7.The highest mean concentration of Mn reported by the present study, is much lower (1.55±1.0 ppm) than that reported by Siu *et al* (2007) (420 ppm) in coffee processing wastewater in Soconusco, Chiapas, Mexico. The mean metal concentration in the samples surpassed the prescribed maximum concentration for irrigation at all sites (WHO., 2006). The sample concentration of Manganese can therefore be said to be high for crop irrigation purposes in the area.

The highest mean Cr concentration was found in WS6 (0.0097±0.023 ppm) and the lowest (0.0017±0.0038 ppm) in water from WS4. The results of the data analysis revealed that the mean concentration of all sample sites is not statistically significant ($P>0.05$). The highest mean concentration of Cr reported by the present study, which is much lower (0.0097±0.023 ppm) than that reported by Ayeni (2014) Cr (3.9 ppm) in wastewater obtained from an industrial area in Ibadan, Nigeria and reported by Sani *et al* (2019) Cr (2 ppm) concentration in industrial effluents, kano, Nigeria (Sani *et al.,* 2019). The entire site has a concentration below the maximum limit, according to the WHO (2006) standard of 0.1 mg/l. Therefore, by this standard, none of the sites is polluted because all are within the safe limit.

Overall, in WS1, the mean concentration of heavy metal was in decreasing order of Ca>Na>K>Mg>Fe>Mn>Cu>Ni>Zn>Cr, WS2 Na>Ca>Mg>K>Fe>Mn>Cu>Ni>Zn>Cr, WS3 Na>Ca>Mg>K>Fe>Mn>Cu>Ni>Zn>Cr. WS4 Na>Ca>Mg>K>Fe>Mn>Ni>Cu>Zn>Cr.WS5 Na>Ca>Mg>K>Fe>Mn>Cu>Zn>Ni>Cr. WS6 Na>Ca>Mg>K>Fe>Mn>Cu>Zn>Ni>Cr.

WS7 Na>Ca>Mg>K>Fe>Mn>Ni>Cu>Zn>Cr

Table 4.6 Mean heavy metal concentrations (ppm) detected in water used and effluent of the plant

	WS1	WS2	WS3	WS4	WS5	WS6	WS7	WHO,2006
Pb	ND	ND	ND	ND	ND	ND	ND	0.05
Ni	0.054^{a}	0.167^{b}	0.157^{b}	0.166^{b}	0.059^{a}	0.065^{a}	0.032^{a}	0.02
Zn	0.035^{a}	0.06^{ab}	0.12^{d}	0.118^{d}	0.082^{bc}	0.092^{cd}	0.0482^{a}	0.03
Co	ND	ND	ND	ND	ND	ND	ND	0.05
Cu	0.1893^{a}	0.3010^{a}	0.151^{a}	0.163^{a}	0.146^{a}	0.2278^{a}	0.028^{a}	0.01
Na	18.917^{a}	45.67^{b}	37.53^{ab}	38.88^{ab}	29.38^{ab}	46.972^{b}	23.883^{ab}	<3
K	7.617^{c}	11.75^{d}	8.59^{c}	8.95^{c}	4.97^{ab}	6.789^{bc}	3.983^{a}	
Ca	19.07^{ab}	23.133^{b}	23.83^{b}	24.43^{b}	19.63^{ab}	17.317^{ab}	10.678^{a}	0-20
Mg	1.317^{a}	20.12^{c}	15.28^{bc}	15.83^{bc}	11.45^{abc}	12.57^{abc}	6.21^{ab}	0-5
Fe	0.9850^{a}	2.325^{a}	2.64^{a}	2.694^{a}	2.24^{a}	1.58^{a}	1.662^{a}	0.30
Mn	0.983^{ab}	1.55^{b}	0.68^{ab}	0.209^{ab}	0.606^{ab}	0.324^{a}	0.646^{ab}	0.2
Cr	0.008^{a}	0.0085^{a}	0.0097^{a}	0.0017^{a}	0.009^{a}	0.0018^{a}	0.0075^{a}	0.10
Cd	ND	ND	ND	ND	ND	ND	ND	0.003

B) Heavy metals removal efficiency of the wetlands

In the present study, the removal level of metals in the natural wetland was 81.4% for Cr,58.6% for Ni, 55.6% for Mn 40.2% for Fe, 27.3% for Ca, 23.3% for Zn, 21% for K, -25.2% for Na and -50.86% for Cu. As can be observed, the least efficiency corresponds to the removal of Copper -50.86% and the highest level correspond to Chromium (81.4%). Removal of heavy metals is affected by the treatment system itself and this fact is confirmed by various researches including the present paper (Chipasa, 2003, Zadeh and Parvaresh, 2006, Mansourri and Madani, 2016). When calculating removal efficiencies, for example, only the heavy metal contents in the influent stream are compared to those in the effluent.

Whereas, in constructed wetlands the removal rates of Ni, Zn, Cu, Na, K, Ca, and Mg are higher than 50% ranging from 51.1% to 82.6%, while the removal rate of Fe was 38.3%.Heavy metal removal rates in constructed wetlands were comparable to those reported in other countries' research findings. The Ni removal efficiency of the constructed wetland was 80.72%. These findings are consistent with the removal of Ni (83.2%–88%) reported in Hayatabad Industrial Estate (Xie *et al*., 2022). These finding, on the other hand, was higher (69 %) than those reported (Hadad *et al.*, 2006) in a pilot-scale constructed wetland for industrial wastewater treatment**,** reported by (Khan *et al*., 2009) Ni removal efficiency of a constructed wetland was (40.9 %) in Gadoon Amazai Industrial Estate, Swabi, Pakistan and reported by (Zhou *et al*., 2018) Ni removal of a constructed wetland was (50.8%) in wastewater treatment plants (WWTPs) in China. This is due to the difference in wastewater type and its basic properties, which include high nutrient concentrations. The ANOVA analyses (Table 3.5) showed that the concentration of Ni in the effluent wastewater (WS7) samples were significantly lower ($P < 0.05$) than the influent (WS4) of the constructed wetland, indicating that the wetland has efficiently removed Ni from the wastewater.

In the present study, Zn removal efficiency of the constructed wetland was 59.2%, which was comparable (55%) to the previous study reported by (Hadad *et al*., 2006), reported by (Terzakis *et al.*, 2008) removal efficiency of Zn was (59%) from a constructed wetland in central Mediterranean and reported by (Zhou *et al.,* 2018) Zn removal efficiency was (65%) in wastewater treatment plants (WWTPs) in China. As shown in table 3.6, Zn concentrations in the effluent wastewater (WS7) samples were significantly lower ($P < 0.05$) than influent (WS4) of the CW, indicating that the CW has effectively removed the Zn from the wastewater.

This CW has shown 82.6% removal efficiency for Cu, which was significantly greater than (23%) of the reported data from a constructed wetland in the central Mediterranean (Terzakis *et al.*, 2008), reported (48.3%) removal efficiency in the constructed wetland in Gadoon Amazai Industrial Estate, Swabi, Pakistan (Khan *et al.*, 2009) and (53.1%) removal of copper (Zhou *et al.*, 2018). These findings, on the other hand, are consistent with the removal of copper (78.1%–93.2%) those obtained in Polish WWTP (Kulbat *et al*., 2003) and Cu removal efficiency (74%–93%) reported in Hayatabad Industrial Estate (Xie *et al*., 2022). The ANOVA analysis showed that the Cu concentrations in the effluent (WS7) wastewater samples were significantly lower ($P < 0.01$) than the influent (WS4) of the CW, showing that the Cu has been successfully extracted from the effluent by the CW.

In the present study, the efficiencies of potassium removal were 55.5% which was higher than those reported with the range of (21.3 - 30.7%) in the treatment of aerated coffee processing wastewater (Rossmanna *et al.,* 2012). The introduction of excessive rates of k nutrient to the systems may be responsible for the reduced efficiency of K removal. Because potassium removal efficiency in the CWs is entirely dependent on plant absorption, there was a significant difference ($p = 0.001$) between the average potassium removals of the influent and effluent.

The Fe removal efficiency of the CW was 38.3%, which was lower than those (74.1%) reported by khan *et al.* (2009) and reported Fe (47%) in the previous study (Jayaweera *et al*., 2008). This finding indicates that the CW was low in the removal of Fe from wastewater while the removal.

In the present study, the Cr removal performance of the CW was 77.3%, which is consistent with the findings (82%) of Cu removal constructed at Bahco Argentina (Hadad *et al*., 2006). But the finding of the present study was lower than reported (89%) removal efficiency in the constructed wetland in Gadoon Amazai Industrial Estate, Swabi, Pakistan (Khan *et al.,* 2009) and removal of Cu (88%–92%) those reported in Hayatabad Industrial Estate (Xie *et al*., 2022). The ANOVA analysis showed that the Cr concentrations in the effluent (WS7) wastewater samples were significantly lower ($P < 0.01$) than the influent (WS4) of the CW, indicating that the CW has effectively removed the Cr from the wastewater.

Table 4.7 Comparison of heavy metals removal efficiency between Natural and constricted wetland wastewater treatment system

	Constructed wetland			**Natural wetland**			**WHO (2006)**	**USEPA(2010)**	**EEPA(2003)**
Metals	Influent	Effluent	% Removal	Influent	Effluent	% Removal			
Pb	ND	ND	-	ND	ND	-	0.05	0.015	0.05
Ni	0.166	0.032	80.72	0.157	0.065	58.6	0.02	0.2	2
Zn	0.118	0.0482	59.2	0.12	0.092	23.33	0.03	2	5
Cu	0.163	0.0283	82.6	0.151	0.2278	-50.86	0.01	1.00	2
Na	38.88	23.883	38.6	37.53	46.972	-25.2	<3	<3	<3
K	8.95	3.983	55.5	8.59	6.789	21	-	-	-
Ca	24.43	10.678	56.3	23.83	17.317	27.3	0-20	0-20	0-20
Mg	15.83	6.21	51.1	15.28	12.57	17.74	0-5	0-5	0-5
Fe	2.694	1.662	38.3	2.64	1.58	40.2	0.30	0.30	10
Mn	0.646	0.206	68.1	0.68	0.324	55.6	0.2	0.1	5
Cr	0.0075	0.0017	77.3	0.0097	0.0018	81.4	0.1	0.1	1

4.3.3. Pearson’s correlation between heavy metals concentration in wastewater

Pearson's correlation coefficient was used to examine the correlations between the contents of different elements in wastewater. According to Sharma and Raju (2013), a high correlation coefficient (near +1 or -1) indicates a good relationship between two variables, whereas a concentration around zero indicates no relationship at a significant level of 0.05 **%** level. If r > 0.7, it is strongly correlated, whereas r values between 0.5 and 0.7 indicate a moderate correlation between two different parameters.

The analysis revealed that there was significance (p<0.01, p<0.05) positive correlation between heavy metals; Ni-Zn (r=0.708), Ni-Na (r=0.211), Ni-K (r=0.537), Ni-Ca (r=0.650), Ni-Mg (r=0.552), Zn-Cu (r=0.231), Zn-Na (r=0.292), Zn-K (r=0.332), Zn-Ca (r=0.301), Zn-Mg (r=0.251), Cu-K (r=0.396), Cu-Cr (r=0.339), Na-K (r=0.341), K-Ca (r=0.413), K-Mg (r=0.451), K-Cr (r=0.298), Ca-Mg (r=0.838), Ca-Fe (r=0.630), Ca-Mn (r=0.500), Mg-Fe (r=0.552), Mg-Mn (r=0.464) and Mg-Mn (r=0.464).whereas the result revealed that negative correlation between heavy metals; Zn-Mn (r=-0.26), Zn-Cr (r= -0.25), Cu-Fe (r= -0.31) and Na-Cr (r= -0.22) (**Table= 4.8**).

Table 4.8 Pearson correlation coefficient matrix for metal concentrations in the wastewater

	Ni	**Zn**	**Cu**	**Na**	**K**	**Ca**	**Mg**	**Fe**	**Mn**	**Cr**
Ni	1									
Zn	0.708**	1								
Cu	0.073	0.231*	1							
Na	0.211*	0.292**	0.065	1						
K	0.537**	0.332**	0.396**	0.341**	1					
Ca	0.650**	0.301**	-0.062	-0.129	0.413**	1				
Mg	0.552**	0.251*	-0.065	0.125	0.451**	0.838**	1			
Fe	0.341**	-0.038	-0.31**	-0.131	0.058	0.630**	0.552**	1		
Mn	0.074	-0.26**	-0.157	-0.07	-0.007	0.500**	0.464**	0.500**	1	
Cr	-0.154	-0.252*	0.339**	-0.220*	0.298**	-0.007	-0.099	-0.126	-0.074	1

4.4. Conclusions

Measurements of physicochemical sampling were taken from the coffee-processing wastewater samples using standard procedures. Findings indicate that the mean concentrations of TSS, BOD, and COD in water showed significantly and reduced dramatically in the effluent of natural and constructed wetland. However, the mean Nitrate, TSS and BOD values at the effluent of the two wetlands were above the EPA and Ethiopian effluent allowable discharge limits into inland surface waters. Parameters that meet the regulation in natural and constructed wetlands were Ph, temperature, EC, TDS, sulfate and phosphate. Comparatively, the purification efficiency of organic pollutants (TSS, BOD, and COD) of constructed wetlands was better than natural wetlands, because constructed wetland systems are designed specifically for wastewater treatment, they work more efficiently than natural wetlands. Whereas regarding nitrogen compounds such as ammonium, nitrite, and nitrate, natural wetlands had better purification efficiency.

Except for ammonium and nitrite, the mean concentrations of other parameters such as TSS, BOD, COD, nitrate, phosphate, sulfates, DO, Turbidity, TDS, and EC in the constructed wetland outlet (WS7) were significantly lower than in the inlet (WS4).

In all sample sites, the mean concentration of heavy metals such as Ni, Zn, Cu, Na, Fe, and Mn was above the maximum permissible limit to be used as irrigation water. Overall, the mean concentration of heavy metals in WS1 was Ca > Na > K > Mg > Fe > Mn > Cu > Ni > Zn > Cr, in WS2 Na > Ca > Mg > K > Fe > Mn > Cu > Ni > Zn > Cr, in WS3 Na > Ca > Mg > K > Fe > Mn > Cu > Ni > Zn > Cr. In WS4, the following elements are present: Na > Ca > Mg > K > Fe > Mn > Cu > Ni > Zn > Cr. Na > Ca > Mg > K > Fe > Mn > Cu > Zn > Ni > Cr in WS5, and Na >

Ca>Mg>K>Fe>Mn>Cu>Zn>Ni>Cr in WS6 and in WS7 the following order are present Na>Ca>Mg>K>Fe>Mn>Ni>Cu>Zn>Cr. Despite the fact that the constructed wetland treatment plant performed better overall, both systems were able to treat the effluent from the coffee processing process adequately.

4.5 Recommendation

- ✓ Therefore, Coffee processing plants should sufficiently treat effluent before discharging them to the environment; they regularly monitor treatment efficiency and abate the released pollutants;
- ✓ There should be strict regulations and law enforcement at all levels by government authority;
- ✓ Construction of wetlands should be required by law in all coffee processing facilities in order to treat wastewater

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CHAPTER 5: EVALUATION OF POLLUTANT REMOVAL EFFICIENCY OF THEVETEVERIA GRASS IN CONSTRUCTED WETLAND

Conventional methods for removing heavy metals from contaminated water are prohibitively expensive and, more significantly, ineffective, especially when the concentration of heavy metals is low. As a result, the use of alternative techniques, such as phytoremediation, is promoted. For effective wastewater treatment, phytoremediation uses plants known as phytoremediators, and careful selection of the phytoremediators is essential. This study, therefore, aimed to evaluate the pollutant removal efficiency of the Veteveria grass in a constructed wetland. A wetland with two vertical flows was constructed. The initial wetland was 132 square meters in size. 11 meters in length and 12 meters wide. The 11m * 3m * 1m open space between two constructed wetlands is developed. The second wetland was built, and it serves the same purpose as the previous one: it discharges water into the river. The construction of the wetland is performed by digging 20 cm wide, and 30 cm apart furrows. Vetiveria grasses have been planted at 20 cm intervals. Heavy metals (Ca, Cd, Co, Cu, Cr, Fe, K, Mn, Na, Pb, and Zn) were measured from soil and plant samples from the inlet to the outlet sampling sites using standard procedures from two compartments (soil, and macrophytes) of constructed wetland. Findings indicated that Ca (460.0 ppm) had the highest mean concentration of heavy metals, whereas Ni (0.50 ppm) had the lowest in the soil sample. metal absorption by Vetiver grass is the highest concentration found in plant tissues grown in the following order k > Ca > Na > Mn > Fe > Zn > Cu >Ni >Cr in shoots. The order of the heavy metal contents in the roots of vetiver grass was k > Ca > Na > Mn > Fe > Zn > Cu >Ni >Cr. The plant was found to be effective at transferring Mn and Ni from the roots to the shoots based on translocation and bioconcentration, whereas it served as a potential phytostabilizer for Ca, Cu, Cr, Fe, K, Na, and Zn since the TF values are lower than 1, which

show that vetiver grass prefers to accumulate heavy metals in the roots rather than the shoot and so supports its potential for phytostabilization. From the present study, it was evident that vetiver grass is an ideal candidate for wastewater treatment using constructed wetland technology.

Keywords: Constructed Wetland, Phytoremediation Quotient,Vetiver Grass

5.1. Introduction

Due to the inadequacies of conventional wastewater treatment facilities, wastewater treatment and disposal have been significant environmental challenges in developing countries (Josephat, 2018). Both organic and inorganic components can be attributed to anthropogenic soil and water pollution (Ali and Khan, 2017). The principal inorganic contaminants in wastewater include heavy metals such as chromium, manganese, nickel, copper, zinc, cadmium, lead, iron, and arsenic (Siu *et al*.,2007, Barakat, 2011, Khan *et al.*, 2011). Heavy metals by definition are metallic elements which have a high atomic weight and much high density at least 5 times that of water, often non-biodegradable and persistent in soils over a long duration (Ali and Khan, 2018). Urban sewage sludge disposal, industrial and agricultural practices, and other human activities release heavy metals into the environment (Khan, 2015). Some heavy metals, like copper, zinc, iron, and manganese, are essential soil micronutrients that living things need in very small amounts for biological metabolism (Pilbeam and Barker, 2007) and other heavy metals, such as Cd, Pb, Cr, Hg, and As, are not essential to the development of living organisms (Abaga *et al.*, 2021). Heavy metal contamination of soils and water has become a severe issue that affects soil biomass and causes bioaccumulation via the food chain as a result of metals being transferred from plants to soil (Khan, 2015, Ali *et al.*, 2019).

Phytoremediation is a technology that transfers pollutants from soils and sediments to the plant tissues without soil structure degradation and soil productivity decrease. The amount of heavy metals taken by plants is influenced by both plant physiology and the amount of metals in the soil (Danielson and Sutherland, 1986). One of the crucial factors in applying the phytoremediation method is choosing the right plant (Seroja *et al.*, 2018). Some plant species have a high capacity to accumulate metals in their roots and shoots (Neisi *et al.*, 2014, Gautam and Agrawal, 2017). Researchers have investigated and exploited vetiver in particular for a variety of environmental purposes, including improving water quality, reducing pollution, conserving soil and water, and restoring land (Abaga *et al.,* 2021, Mahmoudpour *et al.,* 2021). The huge biomass and extensive, 3 m-deep root system of vetiver are its most distinctive characteristics. The Vetiver system relies on the use of Vetiver grass, which was first identified as having "highly absorbent" characteristics suitable for the treatment of wastewater and leachate produced by landfills (Gupta *et al.*, 2012, Banerjee *et al.,* 2016).

Conventional systems, such as trickling filters and activated sludge, and non-conventional systems, like waste stabilization ponds (WSP) and constructed wetlands, are the two main categories of wastewater treatment methods (Aregu *et al.,* 2021). However, there are significant drawbacks to metal removal technology, including high application and maintenance costs, secondary pollution, and challenging operational procedures (Khalid *et al.*, 2017, Bolisetty *et al.*, 2019). To treat contaminated water, such as coffee wastewater, It is crucial to adopt remediation technology that is affordable, sustainable, eco-friendly, and successful. As a result, phytoremediation is regarded as an inventive, affordable, and ecologically friendly technique for eliminating toxins and hazardous substances from wastewater, such as organic or inorganic pollutants (Antiochia *et al.*, 2007, Suelee *et al.*, 2017). As, Cd, Cu, Cr, Pb, Hg, Ni, Se, and Zn are just a few of the heavy metals that Vetiver is highly tolerant to, demonstrating its distinctive physiological properties (Vargas *et al.,* 2016, Suelee *et al.,* 2017). Additionally, it is very capable of absorbing nutrients, particularly nitrogen (N) and phosphorus (P), as well as other organic components like biological oxygen demand (BOD) and chemical oxygen demand(COD) (Darajeh *et al.*, 2016).

In Africa generally, and in Ethiopia specifically, there is a lack of information about the pollutant removal efficiency of the Veteveria grass in a constructed wetland. However, Ethiopian researchers have explored the effective treatment of high-strength wastewater, specifically tannery effluent, utilizing Vetiver grass as a constructed wetland plant (Aregu *et al.,* 2021). However, the pollutant removal efficiency of the Veteveria grass for coffee wastewater quality treatment in this country has not been widely investigated. Previous studies have focused on the uptake of one element by the plant, but in this study, twelve elements were investigated. Also, transfer factors, bio-concentration factors and bioaccumulation factors have been studied. River pollution has become such a concern in Ethiopia as the number of wet coffee refineries grows, so does the amount of trash generated, which is discharged carelessly into neighboring natural waterways that flow into rivers and/or penetrate groundwater, posing a serious threat to surface and groundwater quality (Yemane-Tekle, 2015). The main objectives of the present work were to evaluate the pollutant removal efficiency of the Veteveria grass in the constructed wetland, and to determine the bioaccumulation of heavy metals in the different parts of the plant Vetiver grass.

5.1.1Objectives of the study

5.1.1.1 General objective

The general objective of this study was to evaluate the pollutant removal efficiency of the Veteveria grass in constructed wetland

5.1.1.2 Specific objective

1. To determine the concentration of heavy metals in soil and the different parts of the plant Vetiver grass.

2. To determine Phytoremediation Quotient such as bioconcentration factor (BCF) and translocation factor (TF) by Vetiver grass
3. To determine if vetiver grass growing in Kege constructed wetland have phytoremediation potential for removing heavy metals

5.1.2Research questions

1. What is the level of heavy metals in soil and the different parts of the plant Vetiver grass?
2. What are the bioconcentration factor (BCF) and translocation factor (TF) by Vetiver grass?
3. Vetiver plants growing in Kege constructed wetland have phytoremediation potential for removal of heavy metals?

5.2.Methods and Materials

5.2.1Study area

The research area's map was displayed in Picture 4.1, and the study setting was explained in Chapter 4 Section 4.2.1.

5.2.2 Constructed wetland unit preparation/ Field Experiment Design

The study wetland construction, set-up, planting and operation are defined in Chapter 4 (Section 4.2.2).

5.2.3 Selection of sampling sites, sample collection, transportation and storage

Experimental soils were taken from the soil surface (0 - 20 cm) of constructed wetland (**Figure 5.1**) as described by Kassa *et al.,* (2002) by using stainless steel soil sampling Auger. Plant samples were also collected in all the sampling sites: S1, S2, S3, S4,S5,S6,S7,S8,S9 and S10 (Figure 5.1) and rinsed in situ, blotted, pressed, and finally, the samples had been added to non-reacting polyethylene bags, which were then delivered to the laboratory.

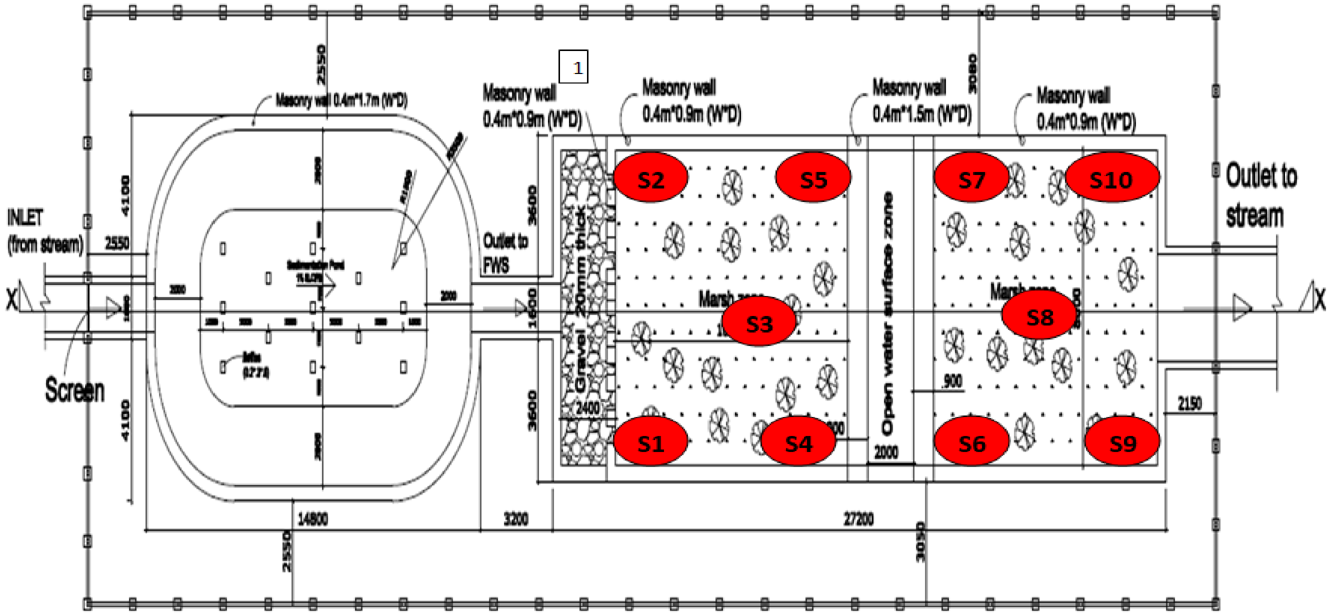

Figure 5.1 Schematics plan for constructed wetland and sampling sites for plants and soil sample

5.2.4 . Preparation and digestion of samples

A. Preparation and digestion of soil samples from constructed wetland

As described in Chapter 3 section 3.2.4(B), Any visible plant remnants were removed, and the soil samples were air-dried. The dried soil samples were ground using pestel and mortar, and sieved by using 2 mm nylon sieves. From the total amount of soil samples collected from constructed wetland sites, 500 g of sieved soil, 50 g of which were used for chemical analysis, were produced for each constructed Wetland site.The sieved soil samples were further dried in an oven at 50°C for one and a half hours to make their moisture content uniform. Finally, the samples were stored in sealed polythene and stored in desiccators containing calcium chloride to keep at constant dry weight till digestion.

For the digestion of soil samples, the EPA 3050B (Epa US, 1996) method was applied. The procedure used for the digestion of the soil sample was as described in in Chapter 3 section 3.2.4(B), as follows: Initially, 500 mg of the dried and sieved soil sample was added into a digestion vessel. Then 10 mL of a solution prepared by mixing 1:1 ratio of HNO_3 and H_2O (deionized) was added into the vessel and the digestion vessel was taken to the microwave digestion (CEM) adjusted according to the EPA standard for digestion process as described by (Kassa *et al.*, 2022) . After digestion is completed, 1 drop of perchloric acid was added to catch the acid and the digestion digestion vessel/tank was removed. Deionized water was added to the digested solution to a final volume of 50 mL

B.Preparation and plant digestion

Plant samples were collected from ten sampling sites (Figure 5.1) for plant tissue metal analysis. The plants were manually dug, washed properly with tap water, followed by distilled water to remove adsorbed soil particulates, trimmed carefully to separate root and shoot part of the plant, dry in a direct sunlight for more than 1 month first and finally an oven dry was done at 65°C until constant weight is obtained.From the dry weight of the biomass of each plant tissue, a representative sample was pooled and ground to pass a 100-mesh sieve.

For plant tissue digestion, as described by (Kassa *et al.,* 2022) 0.1 g plant tissue sample was pulverized using liquid nitrogen (100 mesh), 8 mL nitric acid was added to the sample and left overnight under Mars digestion (CEM recommended method), and in the next day (preferably after standing pure class), 2 mL 30% hydrogen peroxide (superior grade pure) was added to the plant samples.

5.2.5 Heavy metals analysis

Determination of the metals in the soil and plant samples was made by Flame Atomic Absorption Spectroscopy (FAAS) and Flame Emission Atomic Spectroscopy (FEAS) with an external calibration curve after the parameters such as burner and lamp alignment, slit width and wavelength adjustment was optimized for the maximum signal intensity of the instrument. For each metal, the respective hollow cathode lamp was inserted into the atomic absorption spectrophotometer, and therefore the solution was successively aspirated into the flame. To avoid loss through ionization, the concentration of Na and K was determined by FEAS. For other metals FAAS were used. Three replicate determinations were carried out for each metal and the same analytical procedure was employed for the determination of elements in blank solutions as described in chapter three section 3.6.2.

5.2.6 Determination of phytoremediation quotient

According to a method published by (Baker *et al.*, 2000, Shanker *et al.*, 2004, Ng *et al.*, 2020, Abaga *et al.*, 2021), the biological accumulation coefficient (BAC), biological concentration factor (BCF), and translocation factor (TF) were used to evaluate the capacity of vetiver grass for metal accumulation and translocation upwards.

BAC = Concentration of heavy metals in tillers ÷ Concentration of heavy metals in soil;

BCF = Concentration of heavy metals in roots ÷ Concentration of heavy metals in soil;

TF = Concentration of heavy metals in shoot ÷ Concentration of heavy metals in roots.

5.2.7 Data analysis

The statistical evaluations were carried out using SPSS 24. (SPSS Inc). The Shapiro-Wilk test was used to determine whether the data were normal. The Pearson correlation test was used to assess the association between the heavy metals. Based on confidence intervals of 95 and 99%, the statistical analyses' significance levels were 0.05 and 0.01, respectively.

5.3.Results and Discussion

5.3.1 Metal concentration in the sediment

The mean metal concentrations and associated information in the soil of the wetland are summarized in Table 5.1. Based on the mean concentrations, the target elements were arranged in the following descending order in the surface soil of the kege-constructed wetland: Ca> K > Na > Mg > Cu> Fe > Zn > Mn > Ni. Ca had the highest mean concentration of heavy metals (460.0 ppm), whereas Ni had the lowest mean concentrations of heavy metals in soil taken from a constructed wetland (0.50 ppm). The mean metal level found in the soil samples used in this investigation were below the US EPA Soil Quality Guideline (MacDonald and Ingersoll, 2002), and Mean Cd, Cu, Cr, Ni, and Zn concentrations were less than the probable effect concentration (PEC) , which is 3.53 mg/kg, 197 mg/kg, 90 mg/kg, 36 mg/kg, and 315 mg/kg, respectively.

Table 5.1 Concentration of metals in the soil of Kege constructed wetland (mg/kg).

	Ca	**Cd**	**Co**	**Cu**	**Cr**	**Fe**	**K**	**Mn**	**Na**	**Ni**	**Pb**	**Zn**
S1	445.0	-	-	33.0	0.024	10.0	500.0	7.80	150.0	0.70	-	8.90
S2	400.0	-	-	35.0	0.025	11.0	450.0	8.00	165.0	0.66	-	8.50
S3	455.0	-	-	30.0	0.025	9.4	440.0	7.60	145.0	0.80	-	8.00
S4	460.0	-	-	24.0	0.02	9.80	460.0	7.40	167.0	0.75	-	7.50
S5	440.0	-	-	26.0	0.02	11.0	400.0	7.70	180.0	0.65	-	8.90
S6	410.0	-	-	22.0	0.009	9.0	390.0	6.50	175.0	0.64	-	7.20
S7	380.0	-	-	25.0	0.016	10.0	389.0	6.20	160.0	0.61	-	6.70
S8	389.0	-	-	26.0	0.02	8.90	375.0	6.40	167.0	0.59	-	6.40
S9	375.0	-	-	24.0	0.02	9.20	350.0	6.00	172.0	0.55	-	6.20
S10	369.0	-	-	27.0	0.021	8.60	330.0	6.20	163.0	0.50	-	6.00
Mean	412.30	-	-	27.2	0.007	9.69	408.40	6.98	164.4	0.645	-	7.43
± SE	±34.87			±4.185		±0.83	±52.96	±0.78	±10.71	±0.089		±1.11

"-" indicates that the element was not detected.

According to the statistical analysis, there were significant correlations between the concentrations of Ca and K (p, 0.01), Ca and Mn (p, 0.01), Ca and Ni (p, 0.01), and Ca and Zn (p, 0.05). In addition, a correlation was observed for the Cu-Mn, Fe-Mn, and Fe-Zn heavy metal pair (p, 0.05).K-Mn, K-Ni, K-Zn were significanty correlated at (p , 0.05). Similary ,there were significant correlations between the concentration of Mn and Ni (p , 0.01), Mn and Zn (p , 0.01), and Ni and Zn (p , 0.05). Previous research found that the primary elements, including Cd, Hg, As, Co, Cu, Ni, Pb, and Cr, were correlated, suggesting that there was a human-made source for the heavy metals (Fu *et al.*, 2014, Maanan *et al.*, 2015). In this study, similarly, there were a number of paired elements strongly correlated with each other (P 0.01),

Table 5.2 Pearson correlations between the heavy metals in the soil sampled from the constructed wetland

	Ca	**Cu**	**Fe**	**K**	**Mn**	**Na**	**Ni**	**Zn**
Ca	1							
Cu	0.20	1						
Fe	0.38	0.48	1					
K	0.79**	0.59	0.54	1				
Mn	0.78**	0.69*	0.73*	0.84**	1			
Na	-0.27	-0.58	0.12	-0.48	-0.27	1		
Ni	0.91**	0.30	0.37	0.83**	0.74**	-0.46	1	
Zn	0.75*	0.59	0.79*	0.81**	0.94**	-0.17	0.68*	1

*Significant coefficient p, 0.05. **Significant coefficient p, 0.01

5.3.2 Heavy metal contents in shoots of vetiver

The study's findings for the average metal concentration in the vetiver grass shoots under study are shown in Table 5.3. The concentration of Ca, Cu, Cr, Fe, K and Mn ranged from 48.7 mg/kg to 110 mg/kg, 0.38 mg/kg to 0.913 mg/kg, 0.01 mg/kg to 0.04 mg/kg, 3.0 mg/kg to 6.07 mg/kg, 47.3 mg/kg to 118.3 mg/kg, 3.33 mg/kg to 8.17 mg/kg respectively. Na, Ni and Zn ranged from 37.3mg/kg to 69.7 mg/kg,0.33mg/kg to 0.88 mg/kg and 2.23mg/kg to 4.13 mg/kg respectively, Thus, for all metals, sample site one had the greatest concentration and sample site ten had the lowest concentration.

The mean metal concentrations in vetiver grass shoots along sampling sites from the inlet to the outlet did not exhibit a consistent trend (**Table 5.3**). The last sample site showed considerably (P 0:05) lower metal concentrations in the vetiver grass, showing that these macrophytes have the capacity to absorb metals and serve as biofilters for these substances, aiding in the retention of metals in the wetland. Pb and Cd concentrations in vetriver grass were not detected, indicating that there is only a very small amount of these metals in the environment. Metal concentrations in water and soil may have an impact on macrophytes' metal accumulations (Wang *et al.*, 2014).

Although vetiver grass absorbs metals, plant tissues cultivated in the following order had the highest quantities of metals: K > Ca > Na > Mn > Fe > Zn > Cu >Ni >Cr in shoots. Similar findings were made by Banerjee *et al.* (2016) who reported a high concentration of Mn, Fe, Zn, and Cu in the shoot of vetiver, and Gautam and Agrawal (2017) who also revealed a high concentration of Mn, Fe, Zn, and Cu in the shoot of vetiver.

Table 5.3 Heavy Metal Contents in Shoots of Vetiver grass

	Ca	**Cu**	**Cr**	**Fe**	**K**	**Mn**	**Na**	**Ni**	**Zn**
S1	110±1.0^{h}	0.913± 0.03^{g}	0.02±0.01^{b}	6.07±0.51^{f}	118.3±3.1^{h}	8.17±1.07^{g}	69.7±5.51^{f}	0.88±0.03h	4.13±0.31^{f}
S2	103.33±2.08^{g}	0.82±0.02^{f}	0.02±0.01^{b}	5.33±0.15^{e}	112.3±2.5^{g}	7.3±0.15^{f}	65.9±0.46^{ef}	0.81±0.02^{g}	3.77±0.25^{e}
S3	88.0±2.64^{f}	0.717±0.2^{e}	0.015±0.01^{a}	5.07±0.12^{e}	94.7±5.13^{f}	7.47±0.25^{f}	62.0±2.65^{de}	0.75±0.02^{f}	3.63±0.15^{de}
S4	81.7±6.65^{def}	0.65±0.01^{cd}	0.01±0.01^{a}	4.67±0.15^{d}	84.3±4.04^{e}	6.80±0.26^{def}	59.3±4.04^{cd}	0.72±0.02^{ef}	3.43±0.15^{d}
S5	87.33±2.52^{ef}	0.69±0.03^{de}	0.01±0.002^{a}	4.63±0.15^{d}	86.0±2.65^{e}	7.10±0.20^{ef}	66.0±2.65^{ef}	0.72±0.02^{f}	3.60±0.10^{de}
S6	81.0±6.56^{de}	0.68±0.02^{de}	0.008±0.002^{a}	4.37±0.15^{cd}	81.0±3.61^{de}	6.50±0.20^{de}	63.0±3.0^{de}	0.69±0.02^{de}	3.30±0.10^{cd}
S7	76.7±2.08^{d}	0.67±0.02^{cde}	0.014±0.002^{a}	4.50±0.10^{cd}	76.7±1.53^{d}	6.30±0.10^{d}	64.3±2.1^{def}	0.65±0.02^{d}	3.33±0.15^{cd}
S8	68.7±1.53^{c}	0.62±0.03^{c}	0.01±0.002^{a}	4.13±0.12^{c}	66.7±2.89^{c}	5.50±0.26^{c}	55.0±2.65^{c}	0.55±0.03^{c}	3.03±0.06^{bc}
S9	57.3±2.52^{b}	0.52±0.03^{b}	0.01±0.002^{a}.	3.50±0.30^{b}	57.3±2.52^{b}	4.23±0.25^{b}	44.3±2.52^{b}	0.45±0.02^{b}	2.73±0.21^{b}
S10	46.7±3.05^{a}	0.38±0.08^{a}	0.01±0.002^{a}	3.00±0.20^{a}	47.3±2.52^{a}	3.33±0.15^{a}	37.3±2.52^{a}	0.33±0.02^{a}	2.23±0.16^{a}

5.3.3 Heavy metal contents in roots of vetiver

The order of the heavy metal contents in the roots of vetiver grass was k > Ca > Na > Mn > Fe > Zn > Cu >Ni >Cr. The significant accumulation of K and Ca that were found in the root was possibly due to the translocation the metals ion from soils into the root because K and Ca are required macronutrients that are routinely taken by plant for life processes (Mengel and Kirkby, 2001). The observed variance in the amount of metals gathered by vetiver in its various portions suggests that vetiver's ability to absorb metals is mostly reliant on the soil's quality and the concentrations of metals in its natural soil environment (Chunilall *et al.*, 2005).

The roots accumulated a higher amount of K ,Ca ,Na , Mn , Fe , Zn ,and Cu than the shoots with the exception of Mn and Ni. These results are in agreement with the previous study reported a higher accumulation of metals Fe, Mn, Zn and Cu in roots of vetiver exposed to wastewater (Roongtanakiat *et al*., 2007, Banerjee *et al.*, 2019). This shows that vetiver grass can be used as a rhizofiltrator for potassium, calcium, sodium, iron, zinc, and copper due to the greater root absorption of the majority of heavy metals at various metal concentrations (Truong, 2000). Other researchers came to the conclusion that vetiver roots accumulate more heavy metals than the shoot does (Roongtanakiat *et al.*, 2007, Pleto *et al.,* 2019, Gravand and Hejazi, 2022). In general, vetiver accumulated more heavy metals in its roots than shoots; therefore it is suitable for phytostabilization as suggested by (Yoon *et al.*, 2006) and suggested by (Roongtanakiat *et al.*, 2008, Roongtanakiat *et al.*, 2009). Positive charges on metals allow them to be absorbed into negatively charged areas of root cell walls, leading to greater metal accumulation in roots than in shoots (Yang *et al.*, 2005).

Table 5.4 Heavy metal contents in roots of vetiver grass

		Ca	**Cu**	**Cr**	**Fe**	**K**	**Mn**	**Na**	**Ni**	**Zn**
S1	Root	562.7±14.2	40.7±5.92	0.04±0.001	13.0±0.50	567.3±12.5	3.77±0.25	241.3±2.3	0.48±0.08	4.6±0.2
S2	Root	546.7±15.3	33.8±0.15	0.04±0.002	12.0±0.50	558.3±12.6	3.47±0.15	238±7.5	0.38±0.03	4.33±0.15
S3	Root	470±17.3	33.7±0.21	0.03±0.001	11±0.2	447±21	3.33±0.15	200±10	0.31±0.01	3.73±0.15
S4	Root	433.3±15.3	33.5±0.15	0.029±0.00	11±0.2	408±10	3.41±0.15	196.7±5.8	0.32±0.00	3.53±0.06
S5	Root	420.7±10.1	30.7±1.15	0.028±0.00	9.6±0.2	338±8	3.2±0.2	178.3±2.98	0.30±0.01	3.43±0.15
S6	Root	373.3±20.8	9.3±2.52	0.02±0.002	8.43±0.2	361±14.9	3.23±0.15	155±5.0	0.26±0.04	2.9±0.24
S7	Root	374.3±4.01	29.0±3.61	0.02±0.03	8.0±0.0	291±50.9	2.83±0.25	142.7±20.5	0.26±0.02	2.0±0.00
S8	Root	305.7±5.13	22.3±2.52	0.02±0.00	6.3±0.23	216±12.2	2.25±0.05	118.3±7.6	0.21±0.01	1.91±0.04
S9	Root	213.3±15.3	17.7±3.05	0.014±0.00	4.4±0.1	168±10.4	1.90±0.1	112±2.64	0.17±0.01	1.6±0.2
S10	Root	161±6.6	13.7±1.53	0.01±0.002	3.3±0.15	139.7±4.5	1.50±0.05	102±2.66	0.13±0.02	1.45±0.05

5.3.4 Determination of phytoremediation quotient

Vetiver potential as a phytoremediation agent can be determined by some index including bio-concentration factor (BCF), bio-accumulation factor (BAC), and translocation factor (TF). The translocation factor and bioaccumulation factor are two indicators of how well plants can remove heavy metals from soil (Baker *et al.*, 1994, Dahmani-Muller *et al.*, 2000). The bioaccumulation factor calculates the capacity for plants to accumulate heavy metals in various areas of their bodies in relation to the levels of metals in the soil (Branquinho *et al*., 2007). A plant's ability to absorb more metal from the soil is indicated by a BAF value more than 1, while one with a BAF value less than 1 is a metal excluder (Yanqun *et al*., 2005). Vetiveria was determined to be a prospective metal excluder rather than a good candidate for the phytoextraction of metals (Ca, Cu, Cr, Fe, K, Na, and Zn) based on BAF values. Previous research corroborated the current study's conclusion that vetiver is a metal-excluder and tolerant plant (Banerjee *et al.*, 2016, Gautam and Agrawal, 2017).

Translocation factor measures the plant's potential to translocate heavy metals from roots to the aerial shoots (Baker *et al.*, 2000, Shanker *et al.*, 2004, Ng *et al.*, 2020, Abaga *et al.*, 2021). An accumulator has a translocation factor (TF) greater than 1 (Agunbiade *et al.*, 2009, Zhang *et al.*, 2014). A TF > 1 denotes more metal transfer from the plant's roots to its shoot portion. Vetiveria prefers to deposit heavy metal in the root more so than in the shoot, according to a TF value less than 1 (Aksorn and Chitsomboon, 2013). According to the results of the current study, significant amounts of Ca, Cu, Cr, Fe, K, Na, and Zn were absorbed by the roots but were not transported to the shoot system, as shown by TF values < 1. The results of this study are in line with those of Banerjee et al. (2016), who found that the Fe, Zn, and Cr contents of vetiver roots were greater than those of the shoots, and with Gautam and Agrawal (2017), who found that vetiver roots absorbed more Fe, Zn, Cu, and Cr than shoots. Long, narrow, waxy leaves and a fibrous root structure are specialized characteristics of vetiver grass that contribute to its ability to tolerate metals. Such specific properties of vetiver limit the transfer of metals via the xylem by reducing evapotranspiration rate (Boonyapookana *et al.*, 2005).

Based on the result, the present study revealed that the roots accumulated more heavy metals as the TF values are lower than 1, This confirms vetiver grass' capacity for phytostabilization by showing that it prefers to accumulate heavy metals in the roots rather than the shoot. These results are in agreement with the previous study reported by (Roongtanakiat *et al.*, 2007, Banerjee *et al.*, 2019, Pleto *et al.*, 2019). he fact that the shoots can be utilized for grazing or mulch because there is little heavy metal translocation into them is an important finding (Truong, 2000, Anjum *et al.*, 2013).

The manganese had the highest TF of **2.17** and cupper had the lowest with 0.02 at sample site one. The decreased bioavailable percentage of Cu in the soil may be the cause of the low TF for Cu observed in this investigation. For site two, nickel had the highest TF with 2.13 while cupper had the lowest with 0.02. for sample site three, nickel had a translocation factor of 2.42 which was the highest and cupper with only 0.02. The heavy metal nickel had the highest TF of 2.25 and nickel had the lowest with 0.02 for sample site four. For sample site five, nickel had the highest TF with 2.4 while cupper had the lowest with 0.02.

A plant is suitable for phytostabilization or root storage of heavy metals if its TF value is less than 1, and it is suitable for phytoextraction if its TF value is greater 1 (Nabaei and Amooaghaie, 2020). Two distinct types of phytoremediation—phytostabilization and phytoexreaction—involve the application of various functions and traits of plants to remove heavy metals from contaminated soils (Douchichea et al., 2012). The main mechanism of phytostabilization is the employment of species of plants that can withstand metals to immobilize heavy metal ions by storing them at the root level without attempting to remove the heavy metals from the upper plant and reduce their bioavailability, preventing their migration into the environment (Marques et al., 2009).On the other hand phytoextraction mainly refers to the use of plants to remove contaminants from the environment and concentrate them in above-ground plant tissue(Suman et al., 2018). ecause of this, phytoextraction entails removing above-ground biomass (shoots) in order to remove heavy metals from polluted soil (Lone et al., 2008).

A low TF (TF < 1) was observed for most of the heavy metals considered in this study. However Mn and Ni had high TF(TF > 1) which showed that vetiver grass can be utilized for Mn and Ni phytoextraction based on their remarkably high TF. The behavior of various metals, both antagonistic and synergistic, has a significant impact on the TF values, which in turn affects the uptake and distribution of those metals in plants (Eid and Shaltout, 2014).Mn and Ni more translocation to the shoots may be due to metal sequestration in leaf vacuole and apoplast(Gautam and Agrawal, 2017). On the contrary, Cr had a low TF (TF < 1) in all sample site. The results of this study were consistent with those of the earlier ones, which were TF < 1 (Tariq *et al*., 2016, Chintani *et al.,* 2021). The plant's low mobility of Cr from the roots to the shoots may be caused by Cr buildup and saturation in cell vacuoles and apoplast (Park *et al.*, 2011, Topcuoglu, 2012). Nickel plays an important role in plants. While it has no toxic effect on plants at low concentrations, nickel is poisonous for plants at high concentrations (Ziarati and Shad, 2017, Naeini and Rad, 2018). Excessive nickel may disturb electron transport chain during photosynthesis and prevent electron establishment and stomatal transactions (Chen *et al.*, 2004).

Most vetiver grass sites had BCF values for Cupper metal that were more than one (BCF>1) over the course of the investigation. The majority of BCF results were significantly higher than one, showing that the roots of vetiver plants may store a sizable quantity of Cupper metal. The TF values for calcium metal, on the other hand, were significantly below one (TF>1) throughout the study period. As a result, the research plant is a good phytostabilizer of Cupper metal. This indicates that in the studied plants, the transfer of copper metal from roots to shoots is restricted. This result is consistent with a related study by Pleto *et al*., 2019, which indicated that the roots had the highest concentrations of heavy metals and the shoots had the lowest concentrations.

Table 5.5 Determination of biological concentration factor (BCF), biological accumulation coefficient (BAC), translocation factor (TF)

		Ca	**Cu**	**Cr**	**Fe**	**K**	**Mn**	**Na**	**Ni**	**Zn**
S1	BAF	0.25	00.03	0.83	0.61	0.24	1.05	4.5	1.23	0.46
	BCF	1.26	1.23	1.67	1.3	1.13	0.48	1.61	0.69	0.52
	TF	0.20	0.02	0.5	0.47	0.21	**2.17**	0.29	**1.83**	0.90
S2	BAF	0.26	2.34	0.8	0.48	0.25	0.91	0.40	1.23	0.44
	BCF	1.37	0.97	1.6	1.2	1.24	0.43	1.44	0.58	0.51
	TF	0.19	0.02	0.5	0.44	0.2	**2.1**	0.28	**2.13**	0.87
S3	BAF	0.19	0.02	0.6	0.54	0.22	0.98	0.43	0.94	0.45
	BCF	1.03	1.12	1.2	1.20	1.02	0.44	1.38	0.39	0.47
	TF	0.19	0.02	0.5	0.46	0.21	2.24	0.31	2.42	0.97
S4	BAF	0.18	0.03	0.5	0.48	0.18	0.92	0.36	0.96	0.46
	BCF	1.04	1.40	1.45	1.12	0.89	0.46	1.18	0.43	0.47
	TF	0.19	0.02	0.35	0.42	0.21	2.0	0.30	**2.25**	0.97
S5	BAF	0.2	0.03	0.5	0.40	0.215	0.92	0.37	1.11	0.40
	BCF	1.05	1.2	1.4	0.87	0.845	0.42	0.98	0.46	0.39
	TF	0.21	0.02	0.36	0.48	0.25	**2.2**	0.37	**2.4**	1.01
S6	BAF	0.2	0.03	0.89	0.40	0.20	1	0.36	1.08	0.46
	BCF	0.91	1.33	2.2	0.77	0.90	0.5	0.89	0.41	0.40
	TF	0.22	0.02	0.4	0.52	0.22	**2.01**	0.41	**2.65**	1.14
S7	BAF	0.2	0.03	0.88	0.41	0.19	1.01	0.40	1.07	0.5
	BCF	1.0	1.2	1.25	0.73	0.73	0.46	0.89	0.43	0.30
	TF	0.21	0.02	0.7	0.56	0.26	2.23	0.45	**2.5**	1.65
S8	BAF	0.18	0.02	0.5	0.46	0.17	0.86	0.33	0.93	0.47
	BCF	0.79	1.00	1	0.71	0.54	0.35	0.71	0.36	0.30
	TF	0.22	0.03	0.5	0.66	0.31	**2.44**	0.47	**2.62**	1.57
S9	BAF	0.15	0.02	0.5	0.38	0.14	0.71	0.26	0.82	0.44
	BCF	0.57	0.75	0.7	0.48	0.42	0.31	0.65	0.31	0.26
	TF	0.27	0.03	0.71	0.79	0.34	2.23	0.39	**2.65**	1.71
S10	BAF	0.13	0.01	0.5	0.35	0.12	0.54	0.23	0.66	0.37
	BCF	0.44	0.5	0.5	0.38	0.35	0.24	0.63	0.26	0.24
	TF	0.29	0.03	0.99	1.1	0.34	2.22	0.36	2.54	1.54

5.4.Conclusion and Recommendation

The effluent from the coffee processing factory can potentially be cleaned up very well using the vetiver grass system. According to the findings, heavy metals had accumulated on roots and shoots. The vetiver grass absorbed harmful heavy metals like nickel, chromium, manganese, and copper. Based on the calculated translocation factor, the vetiver grass preferred to accumulate heavy metals in the roots. The mean concentrations of Ca, Cu, Cr, Fe, K, Mn, Na, Ni, and Zn in the aboveground tissue of vetiver grass growing in S1 and sampling S10 were substantially higher (P 0:05) based on statistical analysis and Tukey's multiple comparisons. The vetiver grass system is a relatively inexpensive technology with a significant potential benefit for reducing soil contamination.

Based on metal translocation and bio concentration factors, vetiver behaved as a phytostabilizer for all the heavy metals (Ca, Cu, Cr, Fe, K, Na and Zn) and efficient in translocation factor (TF > 1), of Mn and Ni from roots to shoot, serving as a good phytoextractor. We recommended that a follow-up investigation be carried out at a different season.

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CHAPTER 6: SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

6.1Summary and Conclusion

In most countries across the world, coffee wastewater discharges partially treated and occasionally untreated wastewater with nutrients into surface waters or through wetlands, causing pollution to both the surface and groundwater . Ethiopia is one country in Africa that has this issue. Although the effluent generated by agro-based industries has a high amount of pollutants, which can cause irreversible environmental harm if not properly disposed.The effluent from coffee processing plants consists of different sugars, crude protein, crude fiber, different nutrients and chemicals which are generated from both pulping and mucilage fermentation processes. The effluent also consists of different toxic chemicals such as tannins, alkaloids (caffeine) and polyphenolic compounds and nutrients like nitrate and phosphate. These wastes are no longer desirable to have in water bodies because they interfere with the species' ability to live sustainably. The discharge of such kinds of untreated coffee washed effluent into the open environment and the river can bring various environmental and public health problems.

The use of natural and artificial (constructed) wetlands for wastewater treatment has been proposed as an intermediate technological solution for handling wastewater. These systems are economically attractive and relatively energy-efficient for wastewater treatment, specially constructed wetland technology (CW) is one of the emerging and acceptable technologies because it can effectively remove all most all types of pollutants from wastewaters without harming the environment.

The Kege Natural Wetland, which is close to a coffee processing cooperative, is endangered by an increase in nutrient inflow, substantial encroachment for diverse land use activities, such as agriculture, high settlement densities, and being a site for the disposal of effluent. Kege natural wetland received untreated effluents from coffee processing continuously, particularly in the wet season. There is a possibility that pollutants such as (N and P) reach the Gidabo River and can cause pollution of the river. Wetlands should be created or intervened for their sustainable management. As a result, information on water quality in the wetland is needed for the planning of sustainable use of the wetland. It is currently assumed that the Kege wetland retains the organic matter and heavy metals carried by the wastewater, but there is no quantification of this function. Such information is necessary for the efficient planning of long-term sustainable use of the wetland and its biodiversity.

In the case of wetlands, plant selection is critical, and the plants chosen must be tolerant of toxicity as well as variations in the entering wastewater characte. Vetiver grass is one of the most promising plants because of its rapid growth, deep and broad root system, and strong tolerance to environmental stress such as drastic temperature changes (22°C - 60°C), soil pH(3.0-10.5) and, most critically, excellent tolerance to heavy metal stress. Therefore, the coffee berry wastewater treatment with vetiver grass is the alternative way to emphasize during this study.

For the past two decades, urbanization and expansion of industrial activities on forest and wetland reserves has become an acute problem in Ethiopia. Not only does encroachment account for wetland and forest loss, but also biodiversity and aquatic life diversity depletion as well. Draining of wetlands and clearing of forests for urbanization and industrial development has had serious consequences on surface water hydrology and accelerated the process of water pollution. Limited data are available about the potential of constructed wetland oriented wastewater treatment of Coffee Berry Processing Agro-industry (CBPA), especially using Vetiver grass in Africa in general, and particularly in Ethiopia. The constructed wetland system does not require high construction and operation costs as it is required for the construction of a conventional wastewater treatment system. With this in mind, we designed, built, and operated the constructed wetlands in the Kege processing plant for the treatment of coffee wastewater.The aim of this study was to establish coffee berries processing Agro-Industry wastewater treatment potential of constructed and natural wetlands: The case of Kege processing plant in Sidama Regional State, Ethiopia

Measurements of physicochemical sampling were taken from the coffee processing wastewater samples using standard procedures. Findings indicate that the mean concentrations of TSS, BOD, and COD in wastewater showed significant and reduced dramatically in the effluent of natural and constructed wetland .However, the mean nitrate, TSS, and BOD values of the effluent of the two wetlands were above the EPA and Ethiopian effluent allowable discharge limits into inland surface waters. Parameters which meet the regulation in natural and constructed wetlands were ph, temperature, EC, TDS, sulfate, and phosphate. Comparatively, the purification efficiency of organic pollutants (TSS, BOD, and COD) of constructed wetlands was better than natural wetlands, because constructed wetland systems are designed specifically for wastewater treatment, they work more efficiently than natural wetlands. Whereas regarding nitrogen compounds such as ammonium, nitrite, and nitrate, natural wetlands had better purification efficiency.Except for ammonium and nitrite, the mean concentrations of other parameters such as TSS, BOD, COD, nitrate, phosphate, sulfate, DO, Turbidity, TDS, and EC in the constructed wetland outlet (WS7) were significantly lower than in the inlet (WS4).

Findings indicate that the mean concentrations of all elements except Mn and Cr had showed significant and reduced dramatically in the effluent of constructed wetlands .However, the mean Ni ,Mn, Mg values in the effluent of the constructed wetlands were above the EPA and Ethiopian effluent allowable discharge limits into inland surface waters. Parameters which meet the regulation wetland were Zn , Cu, Na, K Ca, The removal rates of Ni, Zn, Cu, Na, K, Ca, and Mg are higher than 50% ranging from 51.1% to 82.6%, Overall, in water entering the processing plant(WS1), the mean concentration of heavy metal was in decreasing order of Ca>Na>K>Mg>Fe>Mn>Cu>Ni>Zn>Cr, at the pulping process stage (WS2) Na> Ca> Mg> K> Fe> Mn > Cu>Ni>Zn>Cr, Constructed wetland influent Na>Ca>Mg>K>Fe>Mn>Ni>Cu>Zn>Cr. In the middle of the constructed wetland Na>Ca>Mg>K>Fe>Mn>Cu>Zn>Ni>Cr. In the Constructed wetland Effluent Na>Ca>Mg>K>Fe>Mn>Ni>Cu>Zn>Cr.

In soil sampled from Kebele, mean metal concentrations were determined to be in the following order: Ca>K>Na>Cu>Co>Cd>Mn>Ni>Zn. except Cd, all metals analyzed were below permissible limit set by FAO/WHO. Cadmium levels reported in the soil are above the levels 3 mg kg^{-1}, the permissible limits for agricultural soil (FAO/WHO, 2001). Therefore, there was Cd contamination in the soil.

Metal levels in coffee bean samples from farmers' farms are in the following order: K>Na>Ca >Mn>Cu> Ni>Zn. Metal levels were found to be K>Na>Ca >Mn>Cu> Zn>Ni in coffee beans from the washing plants. In both coffee, the levels of toxic metals (Pb and Cd) were not determined, and trace heavy metal levels were below the FAO/WHO maximum permissible limits. As a result, there is no health risk linked with the use of Dale Woreda coffee beans due to harmful and trace heavy metals. Mn had the highest bio-concentration factor/transfer factor among the elements examined. However, Cu had the lowest transfer factor. The general pattern of trace metal transfer in Kege Kebele (SS1) was Mn > Zn > Ni > Na>K > Ca > Cu. According to the findings of this study, there are permitted levels of macro and trace elements in coffee beans from farmlands and washing plants.

We analyzed the different soil contamination indices, including the Igeo, CF, Cdeg, mCdeg, PLI, ERi, and RI. Cu, Mn, and Co show extremely contaminated in the examined area, but the Igeo values of Ni, Zn, and Cu indicate uncontaminated to heavily contaminated . The data for the contamination factor reveal that Cd contamination levels are very high in the research area. A considerable and a moderate degree of contamination are revealed by the findings of the contamination degree and modified contamination degree, respectively. The Pollution Load Index result showed that there was a moderate degree of pollution in the soil samples taken from within the studied area. In the case of Ni, Zn, Co, Cu, and Mn, the results of ERi suggested a low potential ecological risk of heavy metals. However, in the examined soil samples, Cd exhibits a very high potential ecological risk. According to the RI values, heavy metals constitute a considerable to extremely high ecological risk in the study area.

The effluent from the coffee processing factory can potentially be cleaned up very well using the vetiver grass system. The outcomes demonstrated that heavy metals had accumulated on roots and shoots. The vetiver grass absorbed harmful heavy metals like nickel, chromium, manganese, and copper. Based on the calculated translocation factor, the vetiver grass chose to accumulate heavy metals in the roots.Vetiver grass acted as a phytostabilizer for heavy metals (Ca, Cu, Cr, Fe, K, Na, and Zn) and was effective in the translocation of Mn and Ni from roots to shoots (TF > 1), acting as a good phytoextractor.

6.2 Recommendations

The researchers would like to make a few recommendations that, if implemented, could enhance the current environmental condition in Dale woreda in light of the study's specific findings as well as the overall overview and conclusion offered above.

- ✓ Coffee processing plants should sufficiently treat effluent before discharging them to the environment; they regularly monitor treatment efficiency and abate the released pollutants;
- ✓ There should be strict regulations and law enforcement at all levels by government authority;
- ✓ Construction of wetlands should be required by law in all coffee processing facilities in order to treat wastewater
- ✓ The results of this investigation show that coffee beans from farmer's fields and washing plants contain acceptable levels of macro and trace components. As a result, Dale Woreda's coffee is unaffected by metal contaminants. In order to increase and spread the consumption of Dale Woreda coffee beans in the domestic and worldwide coffee market, concerned bodies should conduct more marketing and awareness campaigns.

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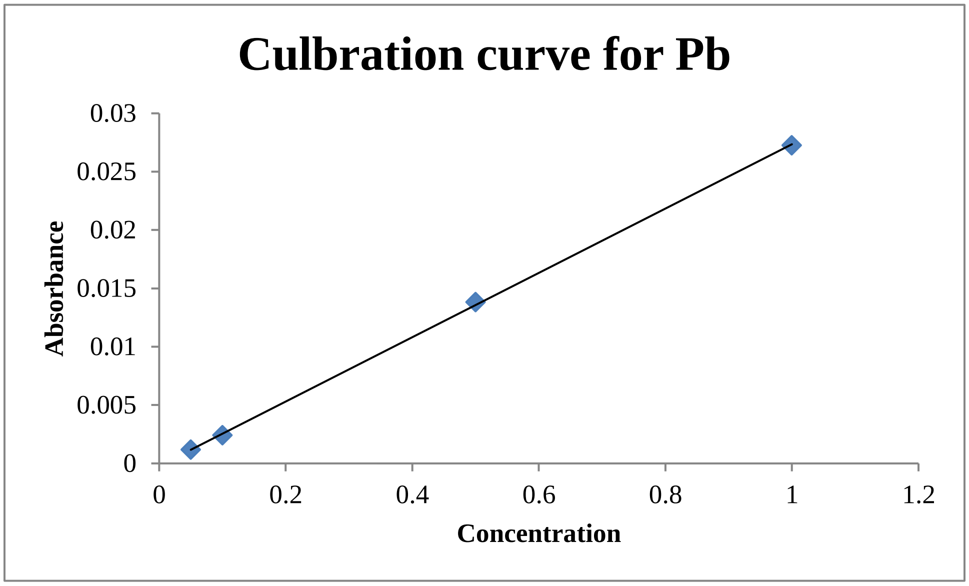
APPENDECES

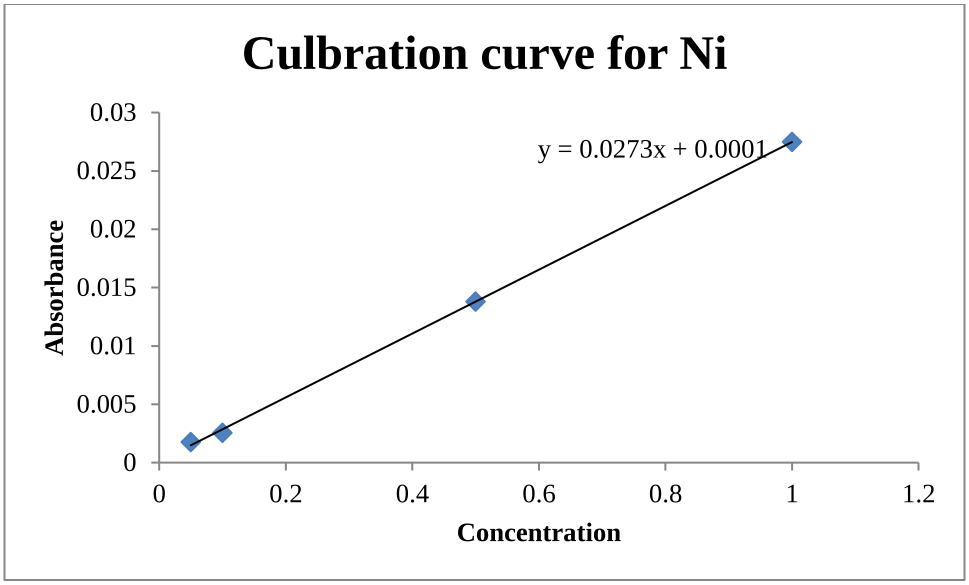
Appendece 1 Instrument operating conditions (slit width, lamp current, wavelength and others) for the determination of metals in coffee bean and soil samples by FAAS

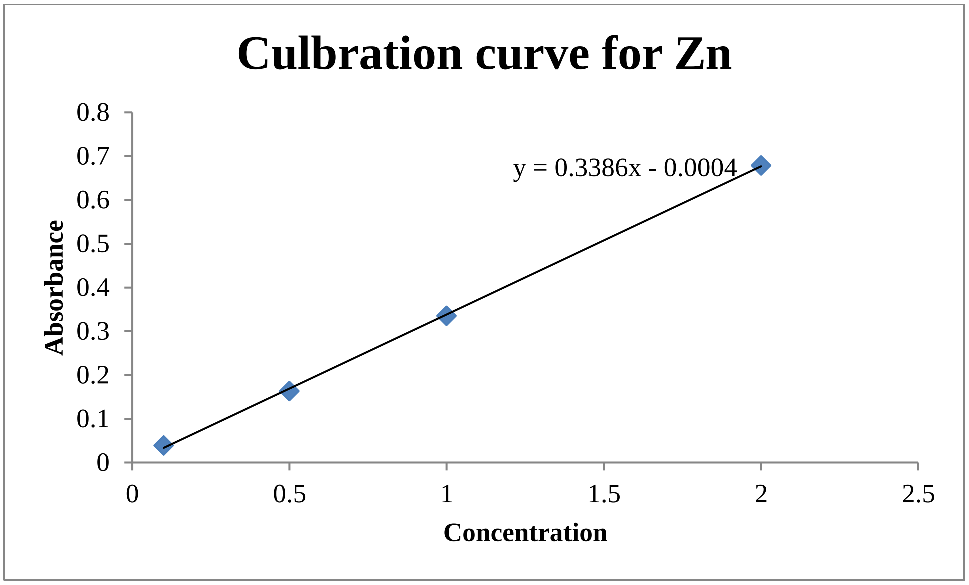
Metals	**Instrument operating conditions**				**MDL (mg/kg) for**		**Correlation Coefficients (r)**
	Wavelength	Slit width	Lamp current	IDL (mg/L)	Coffee bean sample	Soil sample	
Ni	232	0.2	7	0.04	0.2	0.1	0.99
Zn	213.9	0.7	2	0.005	0.16	0.17	0.99
Co	240.7	0.2	4.5	0.05	0.06	0.09	0.99
Cu	324.7	0.7	1.5	0.02	0.34	0.25	-0.90
Cr	357.9	0.7	2	0.05	0.09	0.1	0.99
Ca	422.7	0.7	2	0.01	0.4	0.35	0.99
Mn	279.5	0.7	3	0.01	0.3	0.32	0.99
Na		0.7	-	0.01			0.95
K	766.5	0.7	-	0.01	0.35	0.38	0.98
Pb	233.3	0.7	2	0.1	0.2	0.17	0.99
Cd	22.8	0.2	7	0.005	0.04	0.05	0.97

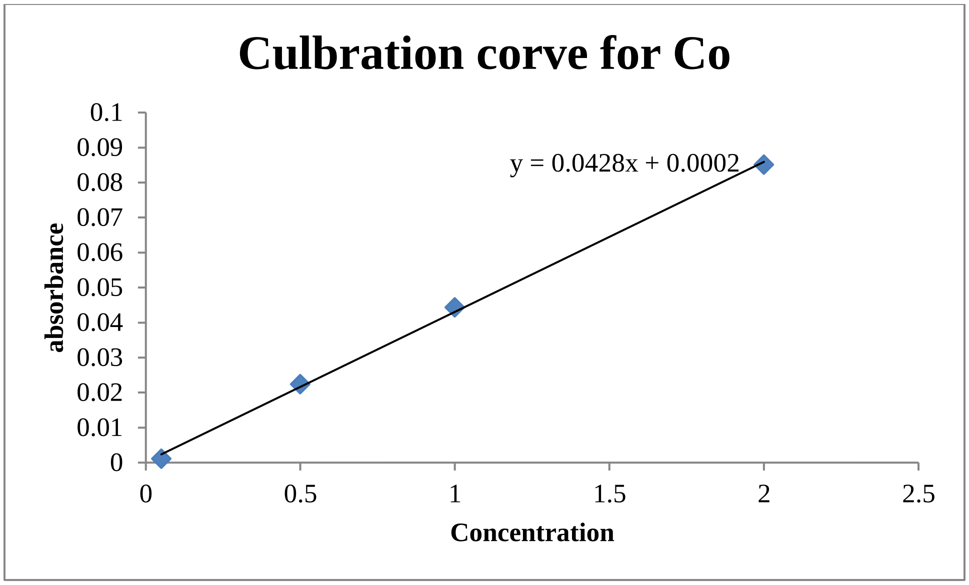
Annex 2 Analytical results obtained for validation of the optimized procedure

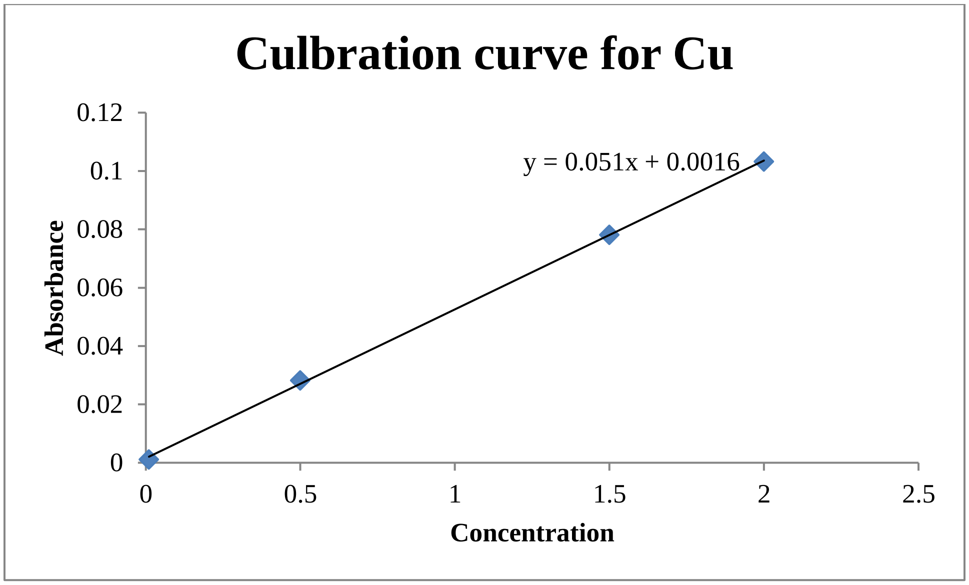
Metals	Concentration in sample(ppm)	Amount added	Amount found in spiked sample(ppm)	Recovery (%)
Ni	0.071	0.03	0.098±0.002	93.0±0.4
Zn	0.054	0.03	0.079±0.002	87±0.5
Cu	0.281	0.05	0.324±0.004	88±0.7
Ca	3.06	1	3.98±0.003	95±1.3
Mn	0.086	0.05	0.125±0.005	88±2.1
Na	6.97	5	11.75±0.005	97±3.2
K	80.66	25	105.42±0.9	99±5

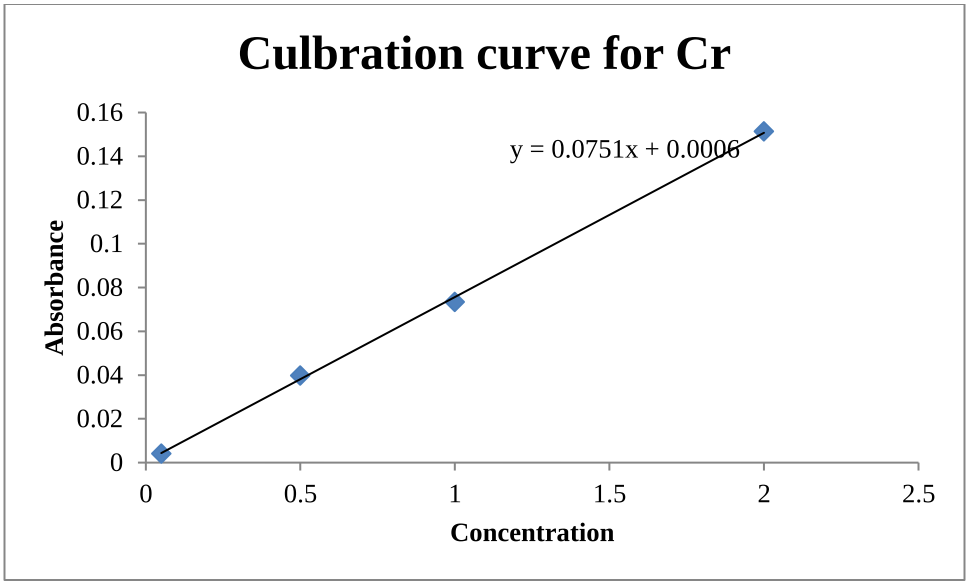



Culbration curve for Ni
Absorbance
0.03
0.025
0.02
0.015
0.01
0.005
0
y = 0.0273x + 0.0001
0
0.2
0.4
0.6
0.8
1
1.2
Concentration

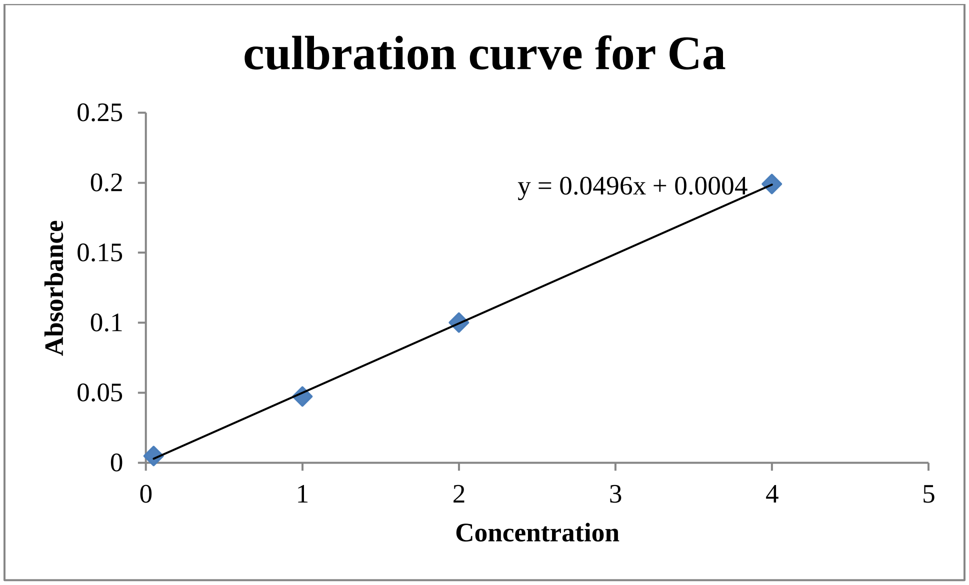

Culbration curve for Zn
Absorbance
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
y = 0.3386x - 0.0004
0
0.5
1
1.5
2
2.5
Concentration


Culbration corve for Co
absorbance
Concentration
y = 0.0428x + 0.0002
0.1
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
0
0.5
1
1.5
2
2.5

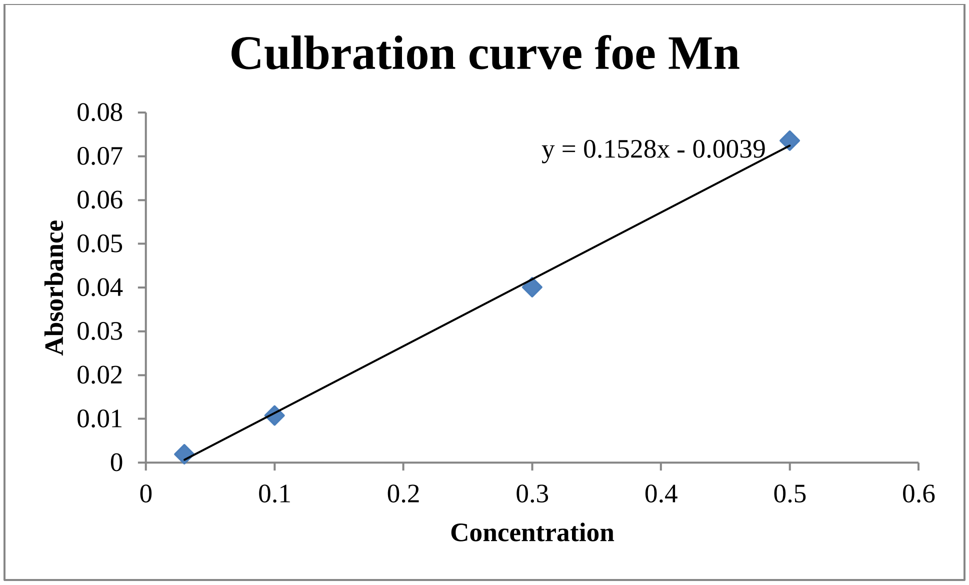

Culbration curve for Cu
Absorbance
Concentration
y = 0.051x + 0.0016
0.12
0.1
0.08
0.06
0.04
0.02
0
0
0.5
1
1.5
2
2.5



Culbration curve for Cr
y = 0.0751x + 0.0006
Absorbance
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
0
0.5
1
1.5
2
2.5
Concentration



culbration curve for Ca
y = 0.0496x + 0.0004
Absorbance
0.25
0.2
0.15
0.1
0.05
0
0
1
2
3
4
5
Concentration


Culbration curve foe Mn
y = 0.1528x - 0.0039
Absorbance
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
0
0.1
0.2
0.3
0.4
0.5
0.6
Concentration