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PROGRAM: MSc. ENVIRONMENTAL CHEMISTRY

"Comparative study of the level of heavy metals in wastewater, sediment and aquatic organisms: a perspective of the tackling environmental threats for the food security, Environmental pollution and their removal using agricultural wastes»

A dissertation submitted to the Department of Chemistry, College of Science and Technology, University of Rwanda, in partial fulfilment of the requirements for the award of a Master's degree with honors in Chemistry (Environmental Chemistry).

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ABSTRACT

The ecological and environmental pollution is due to the deposition or poor disposal of wastes in the ecosystem. The evaluation of pollution may concentrate on various quality indicators like CF (Contamination Factor), Igeo (Geoaccumulation Index), pollution load index (PLI), and bioaccumulation factor (BAF). This study investigated the level of selected heavy metals such as cadmium (Cd), nickel (Ni), Zinc (Zn), copper (Cu), lead (Pb), and chromium (Cr) in wetland ecosystems (case study in Nyabarongo and Nyabugogo). The collected samples, including soil sediments, benthic species, and aquatic organisms, were analyzed for the concentrations of metals were analysed using an Atomic Absorption Spectrophotometer (AAS) according to standard protocols. The results revealed the average concentrations of the metals for waste water, soil sediment vegetables and aquatic organism , for waste water mean Pb (0.79 ± 0.04 ppm), Fe (2.16 ± 0.08 ppm), Mn (0.938 ± 0.11 ppm), Ni (1.034 ±0.02 ppm), Zn (1.33 ± 0.01 ppm), Cu (1.31 ± 0.005 ppm) and Cd (0.272 ± 0.001 ppm) , for the soil sediments Pb (0.52 ± 0.01 ppm), Fe (1.11 ± 0.02 ppm), Mn (1.58 ± 0.01 ppm), Ni (0.69 ±0.02 ppm), Zn (1.75 ± 0.03 ppm), Cu (1.81 ± 0.005 ppm) and Cd (0.172 ± 0.001 ppm) and for fish Pb (1.68 ± 0.04 ppm), Fe (2.26 ± 0.04 ppm), Mn (0.80 ± 0.03 ppm), Ni (1.34 ±0.006 ppm), Zn (1.65 ± 0.06 ppm), Cu (1.41 ± 0.004 ppm) and Cd (0.12 ± 0.007 ppm) and vegetables Pb (0.13 ± 0.01 ppm), Fe (0.36 ± 0.02 ppm), Mn (0.28 ± 0.01 ppm), Ni (0.33 ±0.002 ppm), Zn (0.65 ± 0.001 ppm), Cu (0.71 ± 0.003 ppm) and Cd (0.07 ± 0.001 ppm). Intriguingly, the mean concentration of all measured metals is under the limits, and also is within the permissible limits, comparatively lower than the national and international contexts. Additionally, the assessment gathered on the health risk signified no immediate effect on the group ages taken; meanwhile, all metals are below the threshold impact. These results provide important insights for environmental monitoring and management. The report further emphasizes the critical need for ongoing surveillance and regulation of materials containing heavy metals to protect ecosystems, public health, and food security. Bio-adsorption using rice husk biochar (RHB), which is an environmentally friendly, economical, efficient, and simple technique, was used to treat wastewater, considering various factors such as bio-sorbent dosage, pH, and contact time.The IR spectrum of the adsorbent after treatment exhibited the disappearance or shift of certain peaks, indicating successful adsorption of heavy metals and reflecting the highest percentage of heavy metal removalusing RHB is for 87.56% for Pb^{2+} *and* Mn^{2+} *is for 81.40%.*

Keywords: Heavy metal; AAS; Pollutant; Benthic, rice husks biochar, pyrolysis

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CERTIFICATION

The undersigned hereby certifies that he has thoroughly reviewed this final year project and officially recommends it for examination by the College of Science and Technology, University of Rwanda. "*Comparative study of the level of heavy metals in wastewater, sediment and aquatic organisms: a perspective of the tackling environmental threats for the food security, Environmental pollution and their removal using agriculture wastes».* In fulfilment of the requirements for the award of the Master's degree of Science in Chemistry (Environmental Chemistry) at the University of Rwanda.

Supervisors: Dr. Gratien HABARUREMA (PhD)
Co-supervisor: Dr Anaclet NSABIMANA (PhD)

DECLARATION AND COPYRIGHT

I, Jean de Dieu UTABARUTSE hereby declare that the present work "*Comparative study of the level of heavy metals in wastewater, sediment and aquatic organisms: a perspective of the tackling environmental threats for the food security, Environmental pollution and their removal using agriculture wastes*" is my original work and that it has not been presented or submitted and will not be presented anywhere for a similar or any other degree award.

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DEDICATION

- The Almighty God
- Our parents
- Our brothers and sisters
- Our classmates, friends, and colleagues;
- Our supervisors
- Our lecturers
- Staff of the Department of Chemistry
- University of Rwanda community

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List of abbreviation and acronyms

AAS: Atomic Absorption Spectrophotometer

Igeo: Geoaccumulation index

PLI: Pollution Load Index

CF: Contamination Factor

PERF: Potential Ecological risk factor

BCF: Bioconcentration Factor

BAF: Bioaccumulation factor

Zn: zinc

Cu: Copper

Fe: Iron

Mn: Manganese

Cd: Cadmium

Ni: Nickel

Pb: Lead

HMs: Heavy metals

RH: Rice Husk

RHB: Rice Husk Biochar

Ppm: parts per million

HCl: Hydrochloric acid

HNO_3 : Nitric acid

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1.INTRODUCTION

Environments, which leads to the owning to their toxicity and persistence to be accumulated with the living organism [1], it poses a significant risk to the ecosystem which the several repercussions. The most of the heavy metals (HMs) are geogenic and man –made sources for the domestic and mining waste disposal, transportation of the industrial wastes into the rivers, lakes and ocean which affect the water bodies and by increasing the concentration of HMs in related activities such as agricultural product and aquatic living organism, uncommon activities leading to the increase of bio-magnification in food chain [2]. HMs at lower concentrations are essential for living organisms, while higher concentrations are dangerous and hurt them.[1].

Heavy metal pollution represents a major risk to coastal sediments and aquatic life in rivers, lakes, and wetlands. Within aquatic systems, these metals can be either dissolved or attached to particles. Generally, Heavy metals are more likely to accumulate in sediments than in the water column because they tend to settle at the bottom of aquatic environments [3]. The distribution and behavior of these metals in sediments are affected by various factors, such as the metals' inherent properties, sediment composition, pH, organic matter content, and redox conditions.[2].There are several methods used to the assessment of the degree of the environmental contamination, analysis and monitoring hazardous components in water, sediment and aquatic organism [2], by assessing the root causes of the pollutant, evaluation of the ecological risks which are related to the heavy metal accumulation, monitoring the sediment range and yield of the contamination and measure for the essential methods used to the control the pollution [3].

The discharged water from the refinery oil industry in district near the swamps which affect the ecosystem, those discharged water contain toxins contaminates coastal waterway. Which will lead to the effect on the aquatic life like the marine fishes, the crabs, turtles and benthic organisms, this poses the health risks derived from the consumption the marine fishes, vegetables and using the water from the discharged water industry [6].

Wetland habitats, which are crucial ecosystems supporting diverse flora and fauna, face severe threats due to pollution. One of the primary sources of this pollution is the presence of heavy metals such as cadmium (Cd), nickel (Ni), cobalt (Co), copper (Cu), lead (Pb), zinc (Zn), and chromium (Cr)[4]. These pollutants contaminate the wetland environment, impacting both aquatic life and the surrounding ecosystem. Sediment, water, and benthic species, including crustaceans, mollusks, and polychaetes, are significantly affected by these heavy metals. To assess the extent of heavy metal contamination, advanced analytical techniques such as the double-beam Atomic Absorption Spectrophotometer (AAS) are employed[5], analyse the presence of heavy metals in wetland ecosystems [6]. The assessment reveals alarming concentrations of Cr and Co in sediment, Ni and Co in polychaetes, and Pb and Cu in crabs. Additionally, zinc contamination is noted in fish, highlighting the widespread nature of heavy metal pollution across different species. This study provides a comprehensive analysis of heavy metal pollution in wetland habitats, focusing on sediment, water, and various benthic species[7]. By intensively assessing multiple sources within the sanctuary, this research sheds light on the diverse aspects of pollution in wetlands [8]. By thoroughly analyzing the occurrence and distribution of heavy metals, this study seeks to provide meaningful insights that support the preservation and rehabilitation of these essential ecosystems.

1.2. General objective

Comparative study on the level of the heavy metal pollution in the water, sediment, and aquatic organism: a perspective of the tackling environmental threats for the food security and environmental pollution, removal of heavy metal efficiency using rice husk.

1.2.1: Research objectives

I. Assess Heavy Metal Pollution: Evaluate heavy metal levels in water, sediment, and aquatic species to understand threats to food security and the environment.

II. Compare Contamination Levels: Conduct a comparative analysis of heavy metal pollutants in different mediums, identifying patterns and risks to food security and environmental stability.

III. Identify Implications: Investigate how pollutants impact aquatic species, food chains, and biodiversity, assessing both food security and broader environmental concerns.

IV. Removal efficiency using the rice husks biochar (RHB) as bio-adsorbent

1.3: Significance of study

This research is crucial for thoroughly evaluating the potential risks that heavy metal contamination poses to food security and environmental sustainability. By measuring heavy metal concentrations in a range of samples collected from different locations, the study provides valuable insights into the complex relationship between environmental pollution and food safety. It highlights a pressing issue that affects both human health and biodiversity [7]. The comparative analysis of heavy metal pollutants across different mediums, such as soil, industrial water, and natural water sources like Nyabarongo water, offers valuable insights into the extent and variation of contamination. Through rigorous analysis, this study not only identifies the presence of pollutants but also discerns their impact on aquatic species and biodiversity. Understanding these pollutant effects is pivotal, as it directly correlates with the safety of our food sources and the overall health of our ecosystems. This research provides a nuanced understanding of the intricate relationship between heavy metal contamination, food security, and environmental concerns [8]. By quantifying and analysing samples from diverse geographical areas, this study contributes significantly to the scientific knowledge base. Moreover, it equips policymakers, environmentalists, and agricultural stakeholders with vital information necessary for devising targeted interventions aimed at mitigating pollution, safeguarding food sources, and preserving our ecosystems for future generations [9].Ultimately, this research lays the foundation for evidence-based decision-making, supporting initiatives aimed at fostering a safer and more sustainable environment for both humanity and the ecosystem.

1.4: Problem statement

In our daily lives, food security has become an increasing concern due to the widespread contamination of food sources. Several factors contribute to this problem, heightening the risks of food toxicity, particularly from heavy metals like Cu, Ni, Cr, Cd, Co, Pb, and Zn. This contamination affects not only the food itself but also the soil, further compromising the safety of agricultural products. The environmental contamination by heavy metals harms marine organisms, disrupting the ecosystem and damaging cellular structures. Various sources of pollution, including industrial waste, hospital waste, and e-waste, contribute to this issue [10]. Heavy metal pollution leads to the degeneration of aquatic species, causing physical abnormalities in marine life. These toxic metals also harm fish, resulting in reduced hatching rates, teratogenic effects, and bioaccumulation in tissues. As more people consume contaminated fish, this problem intensifies, impacting the environment over time. With high bio-accumulation potential, heavy metal contamination disrupts both fish and human health, impairing reproductive abilities and affecting physiology [11].

Industrial wastewater, hospital waste, and domestic waste are discharged into Nyabugogo and Nyabarongo. In recent years, this contaminated water has been increasingly used for irrigation of crops. As a result, heavy metals from these waste sources accumulate in agricultural vegetables consumed by people, posing serious health risks. The accumulation of heavy metals in edible vegetables and marine fish from the Nyabugogo and Nyabarongo areas results in food contamination, posing direct risks to human health. Prolonged exposure to these toxic elements can lead to severe health problems, adversely affecting the well-being and quality of life of local communities as well as the wider population[9].

Food analysis plays a crucial role, involving microbial, chemical, and physical assessments to accurately measure contamination levels. Contaminated food leads to spoilage and presents a significant threat to human health. Microorganisms like bacteria and parasites, along with toxic substances, make food unsafe for consumption[10]. Contamination enters the supply chain through various biological and chemical routes, from farm to fork. Bacterial hazards and chemical contaminants, including pesticides, heavy metals, and foreign chemicals, worsen the situation, making it a global issue[11]. Consuming contaminated food causes illnesses, while the intentional addition of harmful substances or poisons to food products constitutes a criminal act, creating a worldwide challenge. Food security suffers due to multiple contamination pathways, which render food unsafe and lead to both acute and chronic health issues. Heavy metals are specifically associated with a range of serious health conditions, including gastrointestinal and renal impairments, neurological disorders, skin abnormalities, vascular damage, immune system suppression, congenital defects, and various forms of cancer. These risks highlight the critical need for comprehensive measures to tackle this issue effectively. [12]

2.0. BACKGROUND OF STUDY

2.1 Introduction

Wetland habitats stand as some of the most diverse and vital ecosystems on our planet. These areas not only enrich biodiversity but also provide a suitable home for a wide variety of aquatic organisms. The nature of wetlands offers essential elements such as shelter, feeding grounds, and breeding spaces, supporting the growth of numerous species [12]. However, the delicate balance of these ecosystems faces threats, primarily from heavy metal pollutants contaminating the aquatic environments. Heavy metals, as significant pollutants in aquatic regions, disrupt the food chain within these ecosystems. These metals enter the water through a complex process involving soil degradation, agricultural activities, industrial operations, and urbanization [13]. The accumulation of heavy metals in aquatic ecosystems results from the interaction of both chemical and biological processes, including precipitation, diffusion, and adsorption, all of which contribute to the creation of toxic conditions for aquatic organisms. The presence of heavy metals in aquatic organisms especially within sediments, benthic habitats, and fish, poses serious environmental challenges. Although heavy metals naturally present in rocks and minerals are typically inert, they become hazardous once they dissolve in water. Aquatic organisms accumulate these metals mainly through consuming contaminated food and exposure to dissolved metals in the water [14]. Monitoring heavy metal concentrations in benthic species and fish is essential, as these organisms serve as reliable ecological indicators in pollution assessments. Benthic organisms, along with prawns and fish, are vital food sources for numerous migratory and native shorebirds. However, these birds are at high risk of harm due to heavy metal contamination in their diet, which threatens their health and the broader ecosystem [15. In light of these concerns, the present study aims to assess the concentrations of heavy metals in water, sediments, benthic organisms, prawns, and fish within the PCWL Ramsar site. This evaluation is intended to provide insight into the ecological condition of the sanctuary, which serves as a critical habitat for numerous migratory and endangered shorebird species annually. By comprehensively studying the heavy metal levels in these crucial components of the ecosystem, we aim to shed light on the extent of pollution and support the conservation efforts of this vital wetland habitat [16].

2.1: Removal of heavy metals using rice husks biochar as bio-adsorbent

2.1.1. Introduction

Introduction: bio adsorption as the independent methods for treating heavy metals using physio-chemical passive metabolite with deployed bio sorbent non-living biological materials, thus this process of bio adsorption is environmentally friendly, economical, efficient and simple techniques for treatment process of the heavy metal [17]. This technique is regarded as an economical and effective solution for eliminating heavy metals from dilute or low-concentration solutions. The current study investigates the potential of agricultural by-products, specifically rice husks, to remediate heavy metal-contaminated environments". It aims to evaluate and optimize the biosorption efficiency by analyzing key factors such as biosorbent dosage, pH, contact time, and the initial concentration of heavy metals. Additionally, the research assesses the adsorption isotherms for the removal of Lead (Pb^{2+}) and Manganese (Mn^{2+}) ions using rice husks [18].

2.1.2: Chemical composition of the rice husks (RHs)

Rice husks, the by-products of edible rice processing, contain a variety of components, which encompasses moisture, crude fat, crude protein, ash, crude fiber, as well as cellulose and hemicellulose [14]. Their chemical makeup consists of both organic and inorganic elements, which makes them well-suited for multiple uses, such as biochar production. Typically, the organic fraction of rice husks is composed of cellulose (30–50%), hemicellulose (20–25%), and lignin (15–20%) [15], inorganic compound such as silica (15-20%) Found in the outer layer of rice husks, silica is a major component that gives rice husks their abrasive and thermal-resistant properties, minerals (trace metals) such as K, Ca, Mg and phosphorus , moisture content, ash content which varies from 15–25%, with a significant portion being silica and carbon content for the rice husks (RHs) are rich in carbon, making them ideal for conversion into biochar or other carbon-based materials [19].

2.1.3: Properties of the rice husk biochar (RHB)

Rice husks undergo a series of chemical transformations under varying thermal conditions during pyrolysis, a key method for biochar production. The efficiency and quality of rice husk biochar (RHB) are largely affected by key parameters such as pyrolysis temperature, heating rate, and the flow rate of the carrier gas, all of which play a crucial role in determining the final yield, composition, structural characteristics, and physicochemical properties of the resulting biochar.

As pyrolysis temperature rises, the aromatic nature of rice husks intensifies, mainly as a result of the development of new functional groups. The degradation of cellulose and hemicellulose begins at moderate pyrolysis temperatures, leading to the generation of functional groups such as carbonyl, lactone, lactone alcohol, quinone, and chromene [3]. At temperatures exceeding 500°C, significant structural changes occur, particularly with the decomposition of lignin and other thermally resistant organic compounds. This results in the removal of oxygenated acidic functional groups, especially carbonyl groups, and a corresponding increase in basic functional groups [4].

RHB produced at lower pyrolysis temperatures (300–400°C) retains more organic features, including a higher content of C–O and –OH functional groups, and is typically associated with greater bioactivity. However, Figure 1 indicates that the higher pyrolysis temperatures (600–700°C) This process produces biochar with highly aromatic carbon structures, increased surface area, and improved porosity. These characteristics greatly enhance the adsorption capacity of rice husk biochar (RHB), making it more efficient for removing contaminants from wastewater [5].

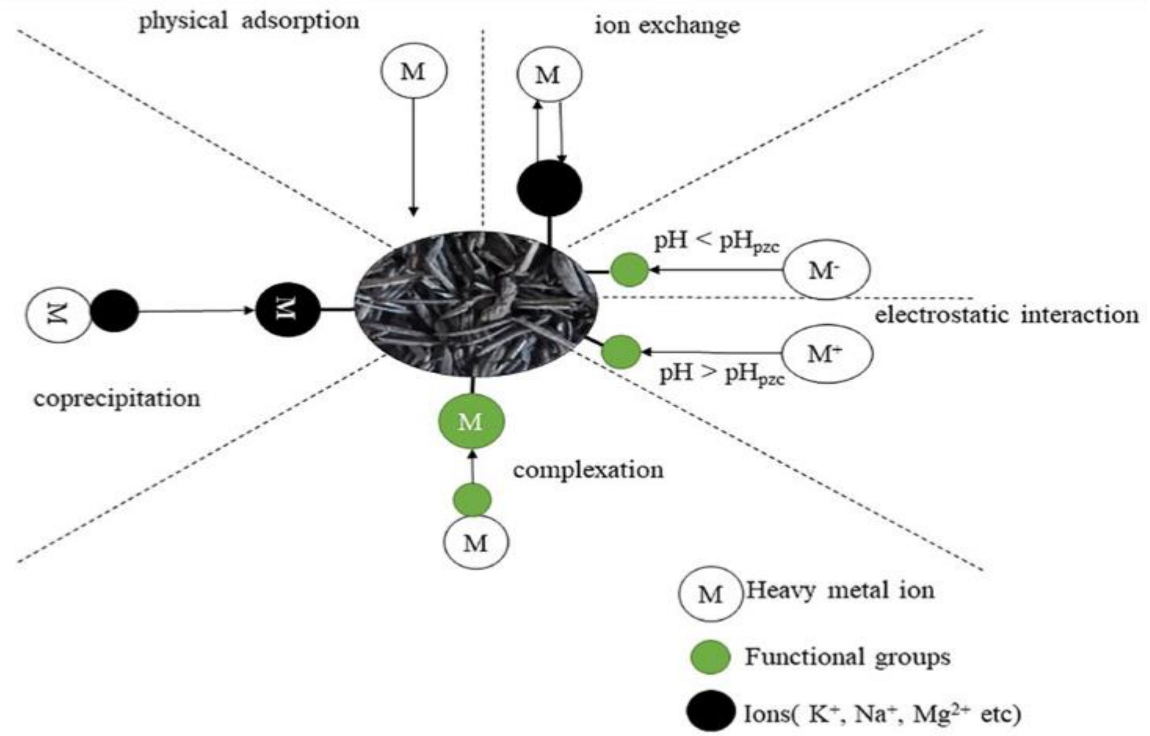

Figure 1: Rice husk characterisation and chemical properties for the production of good Rice husk biochar

3.0: MATERIAL AND METHODS

3.1: Sample Collection, Preparation of Samples, Sample digestion, and Sample filtration

3.1.1: Sampling location

Figure 2 illustrates the map of sampling locations within the wetlands of the Nyabugogo and Nyabarongo rivers. It highlights all collection points for wastewater, fish, vegetables, and soil sediments, covering the full extent of the study areas. Each sampling site is specified to ensure comprehensive representation of the environmental conditions. The detailed mapping supports the accuracy of data collection, facilitates precise analysis, and strengthens the interpretation of results obtained through instrumental methods. Additionally, the map aids in forming targeted observations and recommendations based on the findings [13].

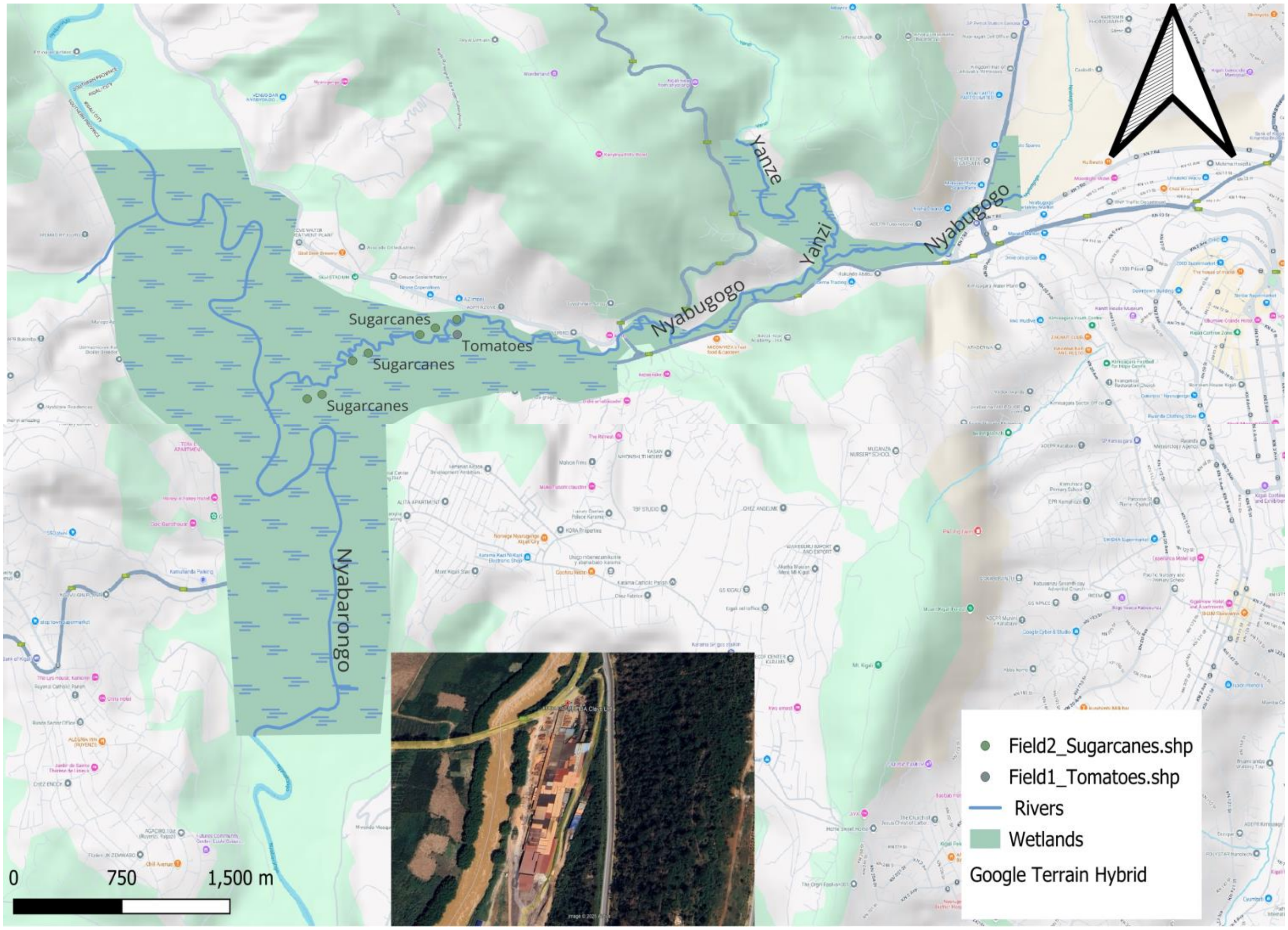

Figure 2: Map representing the sampling location (Nyabarongo and Nyabugogo).

3.1.2: Sample collection in wastewater, soil sediment, and aquatic species

3.1.2.1: Wastewater Sample Collection

Sampling of wastewater was conducted considering the distinct features and conditions of each source. This study focused on four sources: Mpazi, which receives significant domestic waste discharge, and multiple points along the Nyabugogo River. At each sampling site, surface water was collected from various locations to ensure representative sampling. 1000 mL of water was collected per site using clean polypropylene containers. To avoid contamination, samples were collected in clean containers. Immediately after collection, they were stored in an ice box to preserve their integrity during transportation to the analytical laboratory. Upon arrival, the samples were acidified using 1% nitric acid (HNO_3) and kept at 4°C to inhibit metal precipitation and microbial activity prior to heavy metal analysis. [21].

3.1.2.2: Collection of soil sediment samples

To assess heavy metal contamination in sediment, 20 composite sediment samples were randomly collected from multiple locations spanning the Nyabarongo Valley to Giticyinyoni near Gatsata. At each site, 500 grams of sediment were collected and sealed in airtight polyethylene bags. The collected samples were transported to the laboratory for detailed heavy metal analysis. Upon arrival, the sediment samples were oven-dried at 45 °C for 48 hours, then finely ground using a mortar and pestle (see Figure 3). The powdered samples were subsequently sieved through a 106 µm mesh and stored in polycarbonate containers. Core samplers, rinsed with 10% nitric acid (HNO_3), were used for sample collection and subsequently sent to the analytical lab for further examination [22].

Figure 3: The fine powder from the soil sediment.

3.1.2: Sample preparation

The sample preparation depends on the nature of sample and the purpose of the research study, in our study deals with the soil sample, aquatic samples, vegetables samples and also water samples, there are various methods of the sample preparation as listed below sample collection, drying of the sample, seizing and filtration the digestion followed after preparation [24]. For the vegetables, soils and fishes' samples must be dried at room temperature with 72hrs and the samples must be kept in good condition, a after drying the samples must mantled by reducing their size into the small size for passing into the seize materials, after preparation of the samples are kept in the laboratory for further digestion process [15].

3.2: Sample digestion in wastewater, soil sediment, and fish

3.2.1: Wastewater Sample Digestion

For the digestion process, 2 grams of sediment from each sampling site were measured and transferred into a 100 mL glass beaker. The samples were digested with 10 mL of concentrated nitric acid (HNO_3) and 5 mL of concentrated perchloric acid ($HClO_4$). The solution was then heated at 130 °C for about 5 hours until near complete evaporation... Complete digestion is confirmed when the solution becomes clear. The digested solution is then filtered using Whatman No. 41 filter paper and washed with a 1/10 M prepared acid solution[16].

For the water samples, 100 mL of the collected water is passed through a Whatman 0.45 µm filter paper to eliminate suspended particles. The resulting filtrate is combined with 5 mL of concentrated sulfuric acid (H_2SO_4) in a conical flask and heated at 105 °C for 2 hours until the volume is reduced. The digested sample is subsequently transferred into a 100 mL volumetric flask, and deionized water is added to reach the final volume. The resulting solution is then stored in a clean, properly labelled analytical bottle for later heavy metal analysis[17].

3.2.2: Soil Sediment Sample Digestion

Soil sediment samples are air-dried for three days to minimize moisture content. After drying, the samples are ground into a fine powder and sieved through a 2.0 mm mesh to achieve uniform particle size in preparation for metal analysis, A 100 mL aliquot of the powdered sample is placed into a volumetric flask and digested with a HNO_3/HCl mixture (1:3 v/v), prepared by combining 10 mL of concentrated nitric acid (HNO_3) with 30 mL of concentrated hydrochloric acid (HCl), and then diluting with 100 mL of deionized water. Following digestion, the solution is filtered through Whatman filter paper (42.00 mm pore size) to eliminate any remaining solid particles. The clear filtrate is then analysed for heavy metals using an Atomic Absorption Spectrophotometer(AAS)[10].

3.2.3: Acid Digestion of Benthic Organisms

The benthic organisms were rinsed thoroughly with double-distilled water to eliminate any contamination from external sources. Their tissues and organs are then used for metal analysis. For each benthic organism, 25 g of collected tissues are cut into smaller pieces or pooled together for analysis. The study includes mollusks and crabs[10]. Sample preparation involves treating 0.1 mg of tissue with 1 mL of concentrated nitric acid (HNO_3) at 50°C for 24 hours at room temperature. Additionally, 100 mL of hydrogen peroxide (H_2O_2) is used for preservation in separate sample tubes. After preparation and incubation, the samples undergo metal analysis [25].

3.2.4: Digestion of the fish sample

To release heavy metals into a detectable form, fish and prawn samples undergo acid digestion, which breaks down organic matter and dissolves the tissue. For each species, three tissue samples are collected. The samples were homogenized and pooled, with three replicates prepared for metal analysis[18]. A known weight of fish tissue (1–5 g) is carefully measured and placed in a digestion vessel (such as a Teflon beaker or microwave digestion vessel). Concentrated nitric acid (HNO_3) is added to break down the tissue, along with a small volume of hydrochloric acid (HCl) or perchloric acid ($HClO_4$) to assist in metal release and organic material decomposition[16]. After digestion, the sample is permitted to cool before being diluted to a predetermined volume using deionized water, typically in a volumetric flask. In this study, the digestion process for fish and prawn tissue used a HNO_3/HCl mixture in a 1:3 (V/V) ratio [26].

3.3: Sample Filtration

After digestion, the samples may contain particulate matter or undissolved residues, which must be removed to prevent interference with Atomic Absorption Spectroscopy (AAS) readings. To ensure a clear final solution, all digested samples are passed through Whatman filter paper (42 mm) to remove any remaining solid residues. The filtration process varies based on sample type: Soil samples: 2 g of soil is mixed with 50 mL of distilled water before filtration[19]. For wastewater samples, 25 mL of the digested solution is diluted with 50 mL of distilled water before filtration. In the case of vegetable samples, The resulting digest was subsequently diluted with 50 mL of distilled water and then filtered prior to analysis. This step ensures that the final solutions are free of solid residues, making them suitable for accurate metal analysis using AAS [20].

3.4: Quality Assessment and Instrumental Analysis

The quality assessment of all collected samples depends on the instrumental stability and quality control (QC) measures applied during the analysis of 30 samples. To ensure accuracy, the analysis follows systematic procedures that consider the relative standard deviation (RSD) for each metal. The RSD values range between 5-10% for the metals analyzed. Each metal (Cd, Hg, Ni, Pb, Co, Cr, Zn) has its calibration curve [10]. Prepared using standard solutions at different concentrations: 0.5, 1.0, 1.5, 2.0, 5.0, and 10.0 ppm. These calibration curves help determine the standard deviation, mean, and correlation of results [5]. The stock solution is prepared using the following ratios: 65% (v/v) HNO_3,30% (v/v) H_2O_2, H_2O (v/v = 1:1:3). Before use, all materials are rinsed with distilled water to prevent contamination. The instrument is calibrated using a blank solution to ensure precision. Finally, all air-dried samples are analyzed using Atomic Absorption Spectrophotometry (AAS), and the results are expressed in ppm [27].

3.4.0: Environmental Risk Assessment Factor (ERAF)

Environmental Risk Assessment (ERA) is the process of evaluating the potential adverse effects of human activities (e.g., pollution, industrial discharge, habitat destruction) on the environment. In the context of fish sampling in a river like the Nyabarongo, an Environmental Risk Assessment Factor (ERAF) helps quantify the risk posed by contaminants, especially heavy metals, to the aquatic ecosystem and human consumers[12].

3.4.1: Evaluation of Contamination in Sediment Samples

3.4.1.1. Determination of Geoaccumulation Index (Igeo) for Sediment Quality

The Geoaccumulation Index (Igeo) serves as a crucial environmental indicator for differentiating between metals derived from natural geological processes and those resulting from anthropogenic sources. Additionally, it is instrumental in assessing the extent of contamination in sediment samples [24]. The calculation of Igeo is outlined in Equation 1:

$$Igeo = log_2 \left(\frac{Cn}{1.5\ BAn}\right) \qquad \textit{Equation 1}$$

In the formula, Cn represents the concentration of the metal in the sediment sample, while Bn denotes the background geochemical concentration of the same metal (n). The constant factor of 1.5 is introduced to correct for natural variations in the background levels, such as those caused by lithogenic influences. This correction factor was established by Müller, who also developed the Geoaccumulation Index (Igeo) to classify the degree of metal pollution in sediments. Table 2 presents the classification categories defined by Müller for interpreting Igeo values.

Table 2: Classification of Geoaccumulation Index (Igeo) Levels

Igeo Value	Class	Pollution intensity
$Igeo \leq 0$	0	Unpolluted
$0 < Igeo \leq 1$	1	Unpolluted to moderately polluted
$1 < Igeo \leq 2$	2	Moderately polluted
$2 < Igeo \leq 3$	3	Moderately to strongly polluted
$3 < Igeo \leq 4$	4	Strongly polluted
$4 < Igeo \leq 5$	5	Strongly to extremely polluted
$Igeo > 5$	6	Extremely polluted

3.4.1.2: Evaluating Contamination Factor (FC) and Pollution Load Index (PLI)

The Contamination Factor (CF) serves as a useful tool for assessing the level of contamination by individual heavy metals in a specific area. It provides insight into how much a particular metal has accumulated compared to its natural background levels. In contrast, the Pollution Load Index (PLI) offers an overall evaluation of sediment quality in the study area. It combines multiple contamination factors to present a comprehensive view of pollution status, helping to determine the extent of environmental degradation [24].

$$CF = \frac{Cn\ (sample)}{Bn\ (shale)} \qquad \textit{Equation 2}$$

$$PLI = (CF1 * CF2 * CF3 * CF4 * \ldots\ldots\ldots * CFn)^{\frac{1}{n}} \qquad \textit{Equation 3}$$

In the evaluation of the Contamination Factor (CF), Cn represents the average concentration of a specific heavy metal in the sediment sample, while Bn corresponds to its natural background concentration. For the Pollution Load Index (PLI), the variable n refers to the total number of elements analyzed in the sediment sample.

3.4.2: Assessment of Potential Ecological Risk"

To assess the ecological risks posed by heavy metal contamination, the method introduced by Hakanson in 1980 is commonly applied. This technique evaluates ecological productivity and the potential for metal accumulation in aquatic systems, with an emphasis on the bioaccumulation risk in aquatic organisms within a specific region[13]. The Potential Ecological Risk Index (PERI) offers an integrated assessment of pollution levels in sediment samples. It facilitates a more detailed analysis of the Potential Ecological Risk Factor (PERF) for individual metals by accounting for both environmental and ecological implications. The method incorporates the toxicological profiles of metals to evaluate contamination severity and its potential impact on aquatic ecosystems.[28].

$$E_r = T * CF \qquad \textit{Equation 4}$$

$$C_f = \frac{Cn}{Co} \qquad \textit{Equation 5}$$

$$RF = \sum Er \qquad \textit{Equation 6}$$

The Risk Index (RI) indicates the overall ecological risk resulting from the combined presence of all detected heavy metals in sediments, water, and aquatic organisms. In comparison, the Potential Ecological Risk Factor (PERF) quantifies the ecological threat posed by each individual metal. These indices are calculated using the Toxic Response Factor (TRF), which reflects the harmful potential of each metal based on its toxicity

3.5.0: Bio- adsorption experimental procedures

Biosorption experiments were conducted under batch conditions by placing a measured quantity of biosorbent material (rice husks) into conical flasks. Each flask was then filled with 50 ml of a prepared heavy metal solution. After combining the biosorbent with the metal solution, the flasks were positioned on an orbital shaker set at 125 rpm and agitated for a predetermined period to facilitate adsorption [29]. Upon completion of the biosorption process, the mixtures were filtered using 125 mm filter paper. The filtered solutions were analysed with an Atomic Absorption Spectrometer (AAS) to measure the remaining heavy metal concentrations. Biosorption conditions were optimized by adjusting critical factors, including biosorbent dosage, contact time, and initial metal concentration. The targeted heavy metals for treatment using rice husks in this study included Pb^{2+} (lead), Fe^{3+} (ferric iron), and Mn^{2+} (manganese). It is important to note that each metal required specific standard procedures for batch biosorption experiments [26].

3.5.1: Sampling and preparation of adsorbents

The rice husk is sampled from Kabuye field in Gasabo District; samples are washed carefully using Samples were rinsed with tap water followed by deionized water to eliminate particulate matter from their surfaces prior to collection, then after the cleaned rice husks are dried in an oven at 100^0C for 24 hours. Followed by heavy metal concentrations prepared to be adsorbed by RH, and the filtrates are determined after the adsorption process, next for Atomic Absorption Spectrophotometer (AAS) analysis.

3.5.2 Adsorption mechanism and % adsorption calculation

Batch adsorption experiments were conducted using 25 ml aliquots of lead (Pb^{2+}) and iron (Fe^{3+}) solutions. For other heavy metals such as nickel and manganese, solutions of known concentrations were measured into 100 ml flasks containing accurately weighed quantities of the rice husk adsorbent [30]. The prepared rice husks were used at varying dosages, typically around 2.0 g per 100 ml of solution. Each mixture was placed in a flask and agitated at 120 rpm using an electric shaker for a predetermined duration to ensure equilibrium was achieved. After reaching equilibrium, the mixtures were filtered using filter paper with a 2 mm mesh size. The residual concentrations of lead (Pb^{2+}) and iron (Fe^{3+}) in the filtrates were analyzed using Atomic Absorption Spectroscopy (AAS). The adsorption capacity was then determined using Equation 7 as referenced in the study [27].

% Adsorption = $\frac{Co \quad - \quad Ce}{Co} x\ 100$ *Equation 7*

Where Co is the initial concentration and Ce is the equilibrium concentration of the adsorbate

3.5.3: Adsorption model

This model was used to investigate the group evaluated to the different adsorption of heavy metal pollutants, and this was also analysed using the Langmuir model (Equation 8) as well as the Freundlich model. The Langmuir isotherm model describes how the amount of adsorbate bound to the surface of an adsorbent at equilibrium relates to the concentration of the adsorbate remaining in the solution. It is based on the assumption of monolayer adsorption onto a surface containing a limited number of uniform sites, with each site accommodating only one molecule and no interactions occurring between adsorbed molecules.

$$\frac{qe}{qm} = \frac{Qmax^0 * K * Ce}{1+K*Ce}$$ *Equation 8*

Where:

- qe: Amount of adsorbate adsorbed at equilibrium (mg/g)
- qm: Maximum adsorption capacity of the adsorbent (mg/g), representing the monolayer adsorption capacity
- Ce: Equilibrium concentration of the adsorbate in solution (mg/L or mol/L)
- KL: Langmuir adsorption constant (L/mg or 1/mg), which reflects the adsorption energy

Additionally, the Langmuir equation can be rearranged into a linear form as follows:

$$\frac{1}{qe} = \frac{1}{KL\ Qm\ Ce} + \frac{1}{Qm}$$ *Equation 9*

This is the linear form of the Langmuir equation, which can be used to determine the constants KL and qm by plotting $\frac{1}{qe}$versus $\frac{1}{Ce}$

Freundlich equation model description

The Freundlich isotherm is an empirical model that describes heterogeneous surface adsorption. It is often used when the adsorption sites are not equivalent and can have different adsorption affinities

$$q_e = k_f c_e^{\frac{1}{n}}$$ *Equation 10*

Equation 7: Freundlich model equation

- ✓ qe = amount of adsorbate adsorbed at equilibrium (mg/g)
- ✓ Ce = equilibrium concentration of the adsorbate in solution (mg/L)
- ✓ KF = Freundlich constant, indicative of adsorption capacity (mg/g)

- ✓ $\frac{1}{n}$ = exponent related to the adsorption intensity or surface heterogeneity.

The Langmuir equation describing the adsorption process at equilibrium and this model is linear form, the biochar content enhancing the adsorption process and equilibrium is reached on the contact time ranged from 15 -20 minutes, then next is the model formula describing the adsorption process.

$$\frac{Ce}{X/m} = \frac{1}{KXm} + \left(\frac{1}{Xm}\right) Ce$$ *Equation 11*

Where X/m represents the metal ions adsorbed at equilibrium, Ce represents the metal concentration at equilibrium; Xm and K is Langmuir constant related to the maximum adsorption capacity (monolayer capacity) and adsorption energy.

3.4.4: Preparation of biochar as adsorbent

The biochar preparation from the rice husks involves utilizing rice husks and proper preparation of the biochar are effective adsorbent of the heavy metals such as Pb^{2+}, Mn^{2+} and Cu^{2+} due to the rice husks are rich in carbon and silica [15], there are numerous steps for the preparation of the biochar such as the selection and preparation of the rice husks this involves the milling of the rice husks into the fine powder for the pyrolysis using the temperate furnace, the rice husks should be cleaned to remove any foreign (dust, stones and other organic matter) by drying it to reduce the moisture less than 10%, followed by the pyrolysis, the fine powder from the rice husks undergo the pyrolysis by the thermal decomposition process in the absence of the oxygen which converts the organic materials in the husks into the biochar this done at the pyrolysis typical temperature condition between 400 °C and 700 °C [15].

The lower temperature around the 300 °C produce the biochar with more oxygen containing functional groups and for the higher temperature around (500 – 700 °C) yield biochar with higher carbon content specified for the removal of the heavy metals and greater surface area, and also happened on the heating rate (10-20 °C/min) to ensure the biochar quality, upon increasing the heating time from 30 min to 2hrs due to the desired biochar characteristics required. The pyrolysis reactor, RHs are heated in absence of the O_2 to decompose the organic matter by producing carbon rich biochar material and followed by physical activation (steam or CO_2 activation) the biochar can be activated by passing steam through the temperature between 700^0C and 900 °C, the steam reacts with carbon in biochar to form carbon monoxide (CO) and carbon dioxide (CO_2) and creating the more porous structure and increasing the surface [15].

To further enhance the adsorption capacity of rice husk biochar for specific pollutants (like heavy metals, dyes, or organic compounds), biochar can be functionalized by adding or modifying certain chemical groups on its surface. Treating the biochar with acids (e.g., HCl, HNO_3) to introduce carboxyl or hydroxyl groups, Acid washing: Treating the biochar with acids (e.g., HCl, HNO_3) to introduce carboxyl or hydroxyl groups, Acid washing: Treating the biochar with acids (e.g., HCl, HNO_3) to introduce carboxyl or hydroxyl groups, amine functionalization: Treating biochar with amine compounds to add amine groups, which increase the biochar's ability to adsorb metals or anionic pollutants. The preparation of rice husk biochar as an adsorbent involves several key steps: starting with the collection and cleaning of rice husks, followed by pyrolysis, activation, and functionalization processes[21]. The activated rice husk biochar has a high surface area and a range of functional groups, making it a versatile adsorbent for a variety of contaminants. The biochar can be tailored for specific applications by adjusting the pyrolysis and activation conditions, as well as by adding functional groups to enhance its adsorption capacity [15].

4.0: RESULT AND DISCUSION

4.1: Calibration curve for AAS

The analysis of heavy metals using Atomic Absorption Spectroscopy (AAS) began with preparing Standard solutions were prepared at concentrations of 0.01 M, 0.05 M, 0.1 M, 0.2 M, and 0.5 M, derived from a stock solution. The filtered samples were prepared using Whatman filter paper. A calibration curve was generated based on the absorbance values of these standard solutions. This curve was essential for determining the correlation between the sample concentration and the machine's limit of quantification. Additionally, it helped assess potential errors in standard preparation and the instrument's efficiency. The plotted calibration curve, obtained from reference standards containing all targeted heavy metals, was used to ensure accurate quantification of metal concentrations in the analyzed sample [31]. The calibration curve was obtained using a reference standard solution prepared from the stock solution containing all targeted heavy metals. The correlation results were reliable, as indicated by an R^2 value of 0.97438(see Figure 7), and the limit of quantification (LOQ) of the atomic absorption spectrophotometer (AAS) fell within an acceptable range. The calibration curve was utilized to determine the concentrations of heavy metals in various field samples, including wastewater, sediment, soils, aquatic organisms (fish), and vegetables such as carrots, garden eggs, sugarcane, cabbage, tomatoes, and *Amaranth viridis*. The curve demonstrated linearity. The relationship between absorbance and metal concentration is represented by the linear equation: y=0.7687x+0.2778 and (R2=0.9744). This equation enabled the accurate determination of heavy metal concentrations during the analysis with AAS. Figure 3 indicates the absorbance values (in ppm) for the standard solution, where each heavy metal was analyzed at its specific wavelength, ensuring precise quantification.

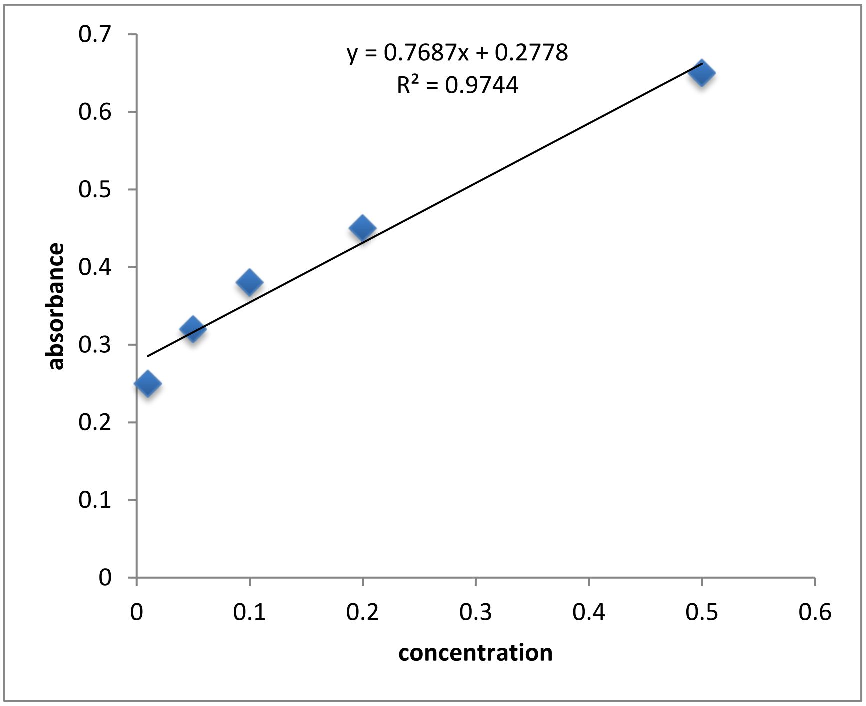

Figure 3: The Calibration curve used in AAS analysis.

4.2: Concentration of the samples analysed using AAS

The results obtained from the analysis using Atomic Absorption Spectrophotometer for all assessed measured data values from all samples, under the calibration curve prepared using standard material. Table 2 summarizes all measured values of all collected data to all targeted heavy metals (Cd, Fe, Mn, Pb, Cu, Ni, and Zn. All samples collected from the different sites are measured using AAS.

Table 1: The Results of all targeted heavy metals obtained from all measured samples

Name of samples	Cd(mg/kg)	Ni(mg/kg)	Pb(mg/kg)	Fe(mg/kg)	Mn(mg/kg)	Cu(mg/kg)	Zn(mg/kg)
S1 (Nyabarongo soil)	NA	9.467±0.04	0.732±0.01	0.349±0.014	0.456±0.015	1.629±0.01	0.693±0.001
S2 (Nyabarongo)	NA	0.339±0.01	0.262±0.05	0.597±0.016	0.382±0.011	1.867±0.01	0.153±0.011
S3 (Nyabugogo giticyinyoni)	NA	0.278±0.024	0.621±0.02	0.735±0.013	0.486±0.012	1.491±0.01	0.417±0.004
S3 (Nyabugogo giticyinyoni)	0.124±0.004	0.029±0.001	0.431±0.03	0.198±0.015	0.371±0.016	0.928±0.025	0.224±0.015
W2 (Nyabarongo)	0.342±0.01	0.195±0.006	0.234±0.01	0.217±0.011	0.205±0.024	2.514±0.02	0.161±0.011
W3 (Nyabarongo)	0.453±0.03	0.308±0.01	0.366±0.02	0.091±0.012	0.118±0.015	1.691±0.01	0.351±0.002
W4 (Giticyinyoni)	0.345±0.01	0.129±0.005	0.675±0.01	0.381±0.021	0.099±0.008	1.196±0.01	1.569±0.01
W5 (Garage Gatsata)	0.895±0.03	0.491±0.02	0.872±0.04	0.492±0.005	0.634±0.004	3.529±0.025	1.931±0.01
W6 (Mpazi)	0.784±0.02	0.207±0.015	0.926±0.01	0.892±0.024	0.829±0.006	NA	0.9231±0.007
W7 (Nyabugogo clay)	0.342±0.01	0.105±0.008	0.549±0.04	0.215±0.012	0.196±0.022	1.729±0.011	0.4111±0.003
W8 (inlet Mpazi)	0.246±0.02	0.2091±0.005	0.854±0.01	0.181±0.007	0.285±0.001	1.472±0.01	2.672±0.01
W9 (Nyabugogo tie fixing)	0.109±0.008	0.013±0.007	0.478±0.006	0.274±0.003	0.193±0.016	1.461±0.015	NA
Tomato	NA	0.108±0.001	0.182±0.014	0.184±0.014	NA	NA	NA
Cabbage	NA	NA	0.161±0.026	0.091±0.017	NA	0.184±0.025	0.295±0.025
Fish(100g)	0.145±0.004	0.307±0.002	0.285±0.011	0.211±0.001	0.398±0.002	1.145±0.01	1.048±0.005
Fish(500g)	0.211±0.004	0.618±0.004	0.191±0.006	0.544±0.071	0.112±0.021	0.731±0.003	NA
Fish(1kg)	0.256±0.004	0.996±0.004	0.091±0.026	0.629±0.021	0.198±0.001	NA	NA
Garden egg	NA	NA	0.137±0.014	0.014±0.031	0.182±0.003	0.157±0.012	0.176±0.012
Amaranth virdis	NA	0.117±0.004	0.119±0.007	0.097±0.041	0.122±0.035	NA	0.588±0.011
Carrot	NA	0.098±0.004	0.233±0.003	0.087±0.021	0.284±0.001	0.271±0.012	0.436±0.001

Hint: (NA: not detected, S1: soil sample from Nyabarongo, S2: soil sample from Nyabugogo near Nzove ,S3;soil sample from giticyinyoni, W1: Nyabarongo waste from clay manufacturing ,W2;waste water from Nyabarongo near Nzove ,W3: waste water from Gatsata garage to Nyabugogo, W4;waste water from Mpazi,W5; waste water from Kabuye *industry,W6;waste water from Mpazi inlet ,W7;waste water from Nyabugogo near Giticyinyoni , Cd;Cadmium,Fe; Iron , Mn ;Manganese; Lead, Zn; Zinc ,Cu ; copper ,Ni; Nickel).*

The World Health Organization (WHO) does not have official global standards for heavy metals in soil. Nevertheless, various entities like the Food and Agriculture Organization (FAO) and the United States Environmental Protection Agency (USEPA)[22], European Union (EU), and national environmental agencies have developed guidelines and permissible limits for heavy metals in soil wastewater water and vegetables. Table 3 shows some general reference limits for heavy metals in agricultural soils from different sources.

Table 3: The standard of the concentration of heavy metals in soil sediment, waste water and Vegetables from WHO/FAO

Heavy Metal	FAO/WHO (mg/kg)	USEPA (mg/kg)	EU (mg/kg)
Lead (Pb)	50–300	400	50–300
Cadmium (Cd)	1–3	70	1–3
Arsenic (As)	10–50	0.39 (residential)	10–50
Mercury (Hg)	0.3–5	0.3	1–1.5
Chromium (Cr)	50–150	100	50–150
Copper (Cu)	50–140	750	50–140
Zinc (Zn)	150–300	2,800	150–300

https://www.researchgate.net/profile/Kehinde-Adewuyi/publication/324088079/figure/tbl1/AS:667649331691528@1536191413389/FAO-WHO-guideline-for-metals-in-food-and-vegetables.png

4.3: Heavy metal description and results discussion

4.3. 1. Results of Cadmium

Cadmium (Cd) is introduced into the environment via natural processes as well as human activities. Natural sources mainly involve volcanic eruptions and the weathering of rocks, which contribute cadmium to soils and water bodies.. A considerable proportion of cadmium exposure in humans originates from agricultural products, as plants tend to absorb cadmium from contaminated soils. Moreover, cadmium is frequently present in phosphate fertilizers and other soil amendments, further contributing to environmental contamination [32]. In this study, cadmium concentrations were evaluated in various environmental samples from different locations. The measured concentrations ranged from a maximum of 0.9411 ppm to a minimum of 0.137 ppm, as outlined below: Gatsata Car Garage: 0.9411 ppm, Mpazi Inlet Water: 0.618 ppm, Nyabarongo River (near Brix Manufacturing): 0.4841 ppm, Fish Samples: 0.137 ppm. The accumulation of cadmium in sediments and aquatic organisms is a key indicator of environmental contamination and contributes to bioaccumulation and biomagnification within aquatic ecosystems. To assess the level of contamination, the Contamination Factor (CF) was calculated. The mean CF value for cadmium was 0.111, which is below 1 (CF < 1), classifying the environment as uncontaminated. This suggests a minimal environmental impact and a low risk to aquatic life in the studied areas [33].

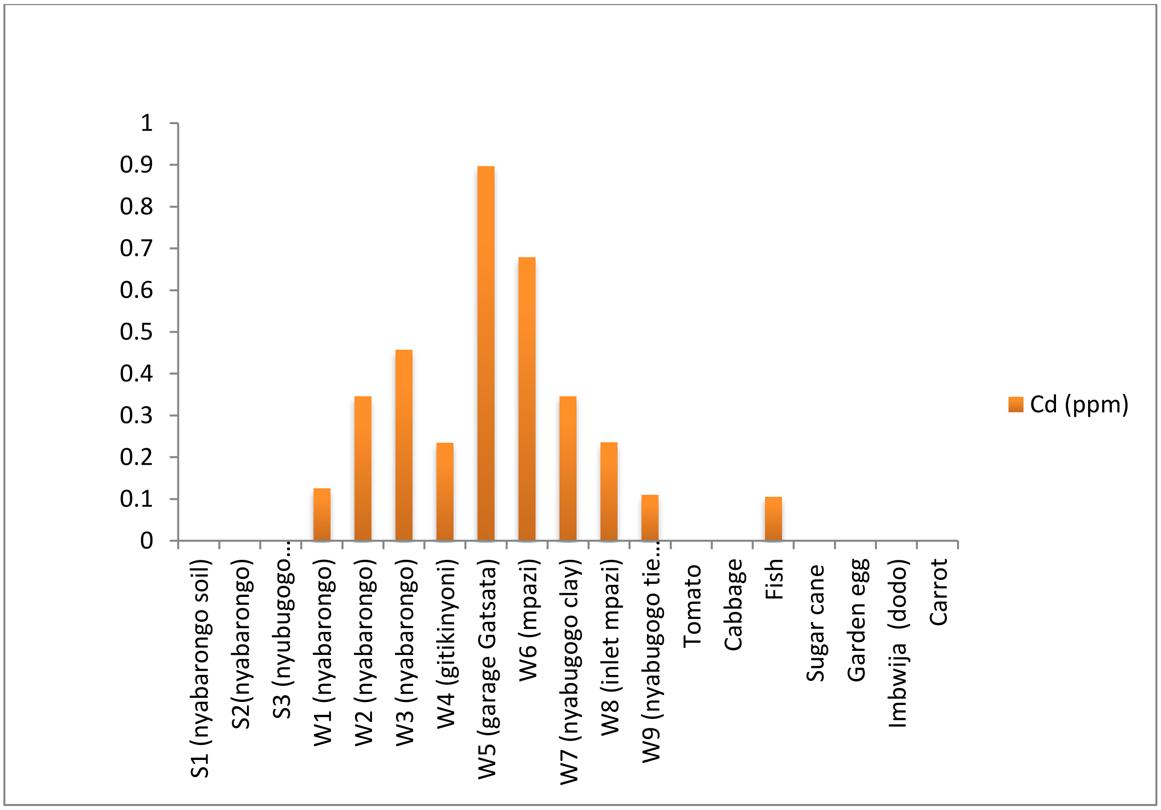

Figure 4: Cadmium description and level of the contamination in aquatic species, soil sediments, and vegetable plants from wastewater deposition.

(S1: soil sample from Nyabarongo, S2: soil sample from Nyabugogo near Nzove ,S3;soil sample from giticyinyoni, W1: Nyabarongo waste from clay manufacturing ,W2;waste water from Nyabarongo near Nzove ,W3: waste water from Gatsata garage to Nyabugogo, W4;waste water from Mpazi,W5; waste water from Kabuye industry,W6;waste water from Mpazi inlet ,W7;waste water from Nyabugogo near Giticyinyoni , Cd; Cadmium, Fe; Iron , Mn ;Manganese; Lead, Zn; Zinc ,Cu ; Copper, Ni; Nickel

4. 3.2. Results of Lead (Pb)

It is a persistent heavy metal known for its toxic effects on both human and animal health, especially targeting the kidneys and nervous system. In aquatic environments, lead contamination can arise from multiple sources, including atmospheric deposition, residues from coal combustion, and the discharge of industrial effluents. Marine and freshwater species are especially vulnerable to lead exposure, which can accumulate in their bodies over time. [32], In this study, among the heavy metals detected in wastewater from a site that collects domestic sewage and hospital effluents, lead was identified as the most prevalent.. Lead concentrations in fish samples ranged from 0.987 ppm to 0.3876 ppm, as observed in sediments and wastewater samples [33]. The AAS was used to determine lead concentrations, with Pb absorption occurring at 248.3 nm. Among the analyzed samples, fish collected from Nyabugogo contained the highest lead concentration, indicating bioaccumulation in aquatic organisms (see Figure 9). Additionally, lead concentrations were measured in various vegetables and fish species, as follows:

- Tomatoes: 0.2 ppm
- Cabbages: 0.1589 ppm
- Fishes: 0.2940 ppm
- *Amaranthus Virdis* (local vegetable): 0.1584 ppm

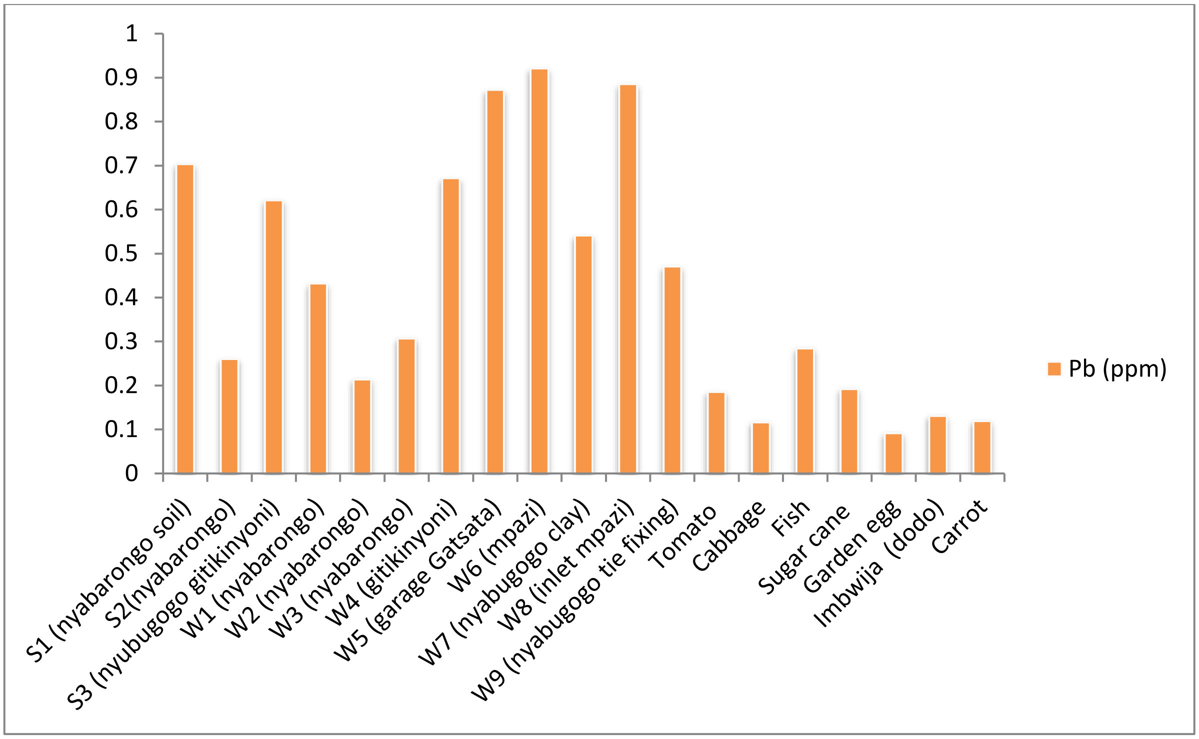

Figure 5: Lead description and the level of contamination in the soil sediment, aquatic species, and vegetables from heavy metal deposition.

(S1: soil sample from Nyabarongo, S2: soil sample from Nyabugogo near Nzove, S3; soil sample from giticyinyoni, W1: Nyabarongo waste from clay manufacturing, W2; waste water from Nyabarongo near Nzove, W3: waste water from Gatsata garage to Nyabugogo, W4; waste water from Mpazi, W5; waste water from Kabuye industry, W6; waste water from Mpazi inlet, W7; waste water from Nyabugogo near Giticyinyoni, P; lead).

4 3.3. Results of Iron (Fe)

Iron (Fe) is a naturally occurring element in marine environments, primarily introduced through crustal weathering and subsequently forming hydroxyl compounds that precipitate into sediments. Iron oxides and hydroxides are highly efficient at scavenging trace metals, playing a crucial role in regulating their concentrations in sediments, wastewater, aquatic species, and vegetables. The positive correlation observed between iron and mud suggests that mud significantly influences the distribution of iron.[34]. In this study, Figure 6 below describes all iron concentrations, ranging from 0.9826 to 0.5096 ppm in sediment samples collected. Nyabarongo Valley (near a brick manufacturing site): 12.9845 ppm, Nyabugogo Valley (near Giticyinyoni): 0.5096 ppm, Mpazi

Wastewater: 1.3275 ppm, Fish samples: 2.0395 ppm (highest accumulation) (see Figure10), Conversely, some wastewater and vegetable samples showed lower Fe concentrations, particularly in sites along Nyabarongo River (S1, S2, S3) based on the analyzed data. Contamination and Bioaccumulation Assessment, Geoaccumulation Index (Igeo): Fe is classified as moderately accumulated (Igeo = 1.075), since Igeo > 1 indicates moderate contamination. Contamination Factor (CF): CF > 1 signifies moderate contamination. The calculated CF for Fe is 1.0043, classifying Fe as moderately contaminating. Pollution Load Index (PLI): PLI > 1 also indicates moderate contamination. The PLI for Fe is 1.005, supporting this classification. Bioconcentration Factor (BCF): BCF > 1 suggests high accumulation in organisms. The BCF for Fe (1.001) confirms significant bioaccumulation, making Fe a highly accumulated and potentially harmful contaminant to surrounding organisms and ecosystems. The findings highlight iron's widespread presence in sediments, wastewater, and aquatic species, with notable contamination and accumulation levels. Given Fe's high bioaccumulation potential, it poses a contamination risk to marine life and surrounding species in the studied areas[23].

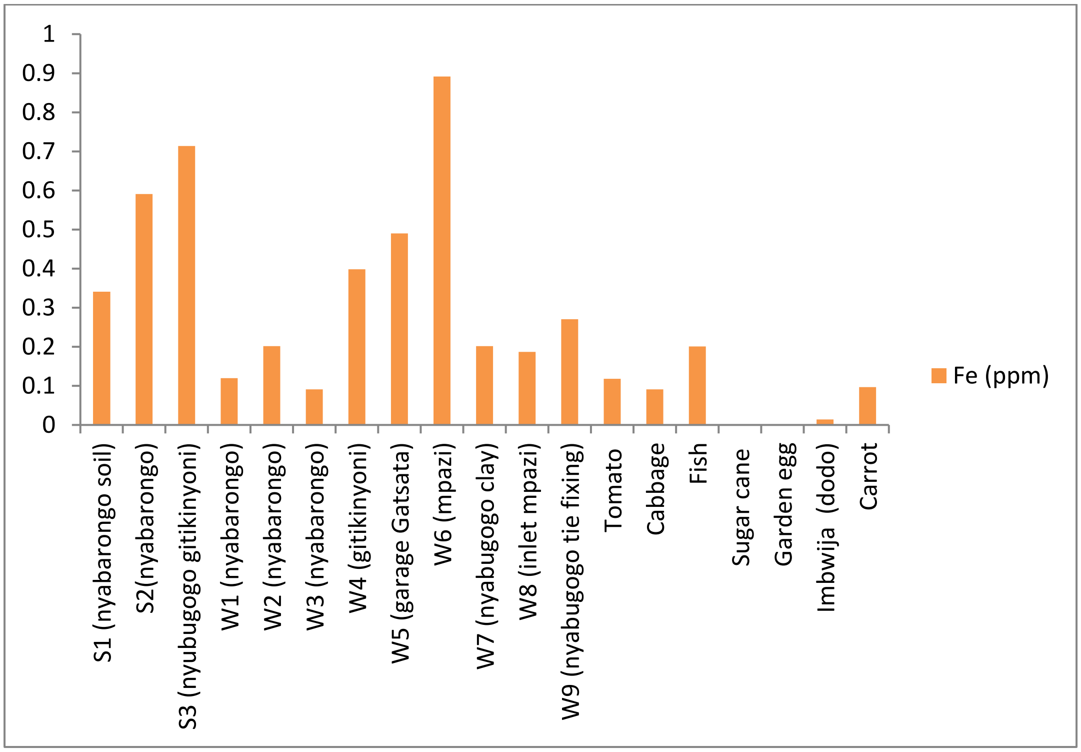

Figure 6;The General description of the Iron in aquatic species, soil sediment and vegetable plant with the same region as the results of the heavy metal deposition

(S1: soil sample from Nyabarongo, S2: soil sample from Nyabugogo near Nzove, S3; Soil sample from giticyinyoni, W1: Nyabarongo waste from clay manufacturing, W2; waste water from Nyabarongo near Nzove, W3: waste water from Gatsata garage to Nyabugogo, W4; waste water from Mpazi, W5; waste water from Kabuye industry, W6; waste water from Mpazi inlet, W7; waste water from Nyabugogo near Giticyinyoni, Iron; Fe)

4.3.4. Result of Nickel (Ni)

Nickel (Ni) is a non-biodegradable heavy metal recognized for its harmful properties, primarily because it can break down into toxic ions. It enters wastewater systems from both natural and human-related activities. Natural sources include volcanic eruptions, wind-transported dust, rock weathering, and biomass combustion. On the other hand, anthropogenic sources involve industries such as stainless-steel manufacturing, gas turbine production, battery and alloy fabrication, and electroplating processes [35]. Exposure to nickel has been linked to several adverse health effects, including persistent dry cough, cyanosis, respiratory complications, and even cancer. According to sampling data collected from various locations, nickel concentrations in Gatsata, particularly around the garage zone, were found to range from 0.098 ppm to 0.45 ppm, with noticeable accumulation in cabbage samples. The contamination factor (CF) had a mean value of 0.071 (CF < 1), indicating a very low risk and no significant nickel contamination in the analyzed sediment, The Geoaccumulation index (Igeo) for nickel was found to be 0.6255, suggesting that the sampled environment is "uncontaminated to moderately contaminated," as Igeo values between 0 and 1 indicate minimal contamination. Figure 7 illustrates the concentration of all selected samples in Nyabugogo and Nyabarongo, Additionally, fish samples collected from the Nyabugogo River contained 0.346 ppm of nickel, while samples from Nyabarongo, near a clay manufacturing site, had a higher concentration of 0.455 ppm (see Figure7). [36].

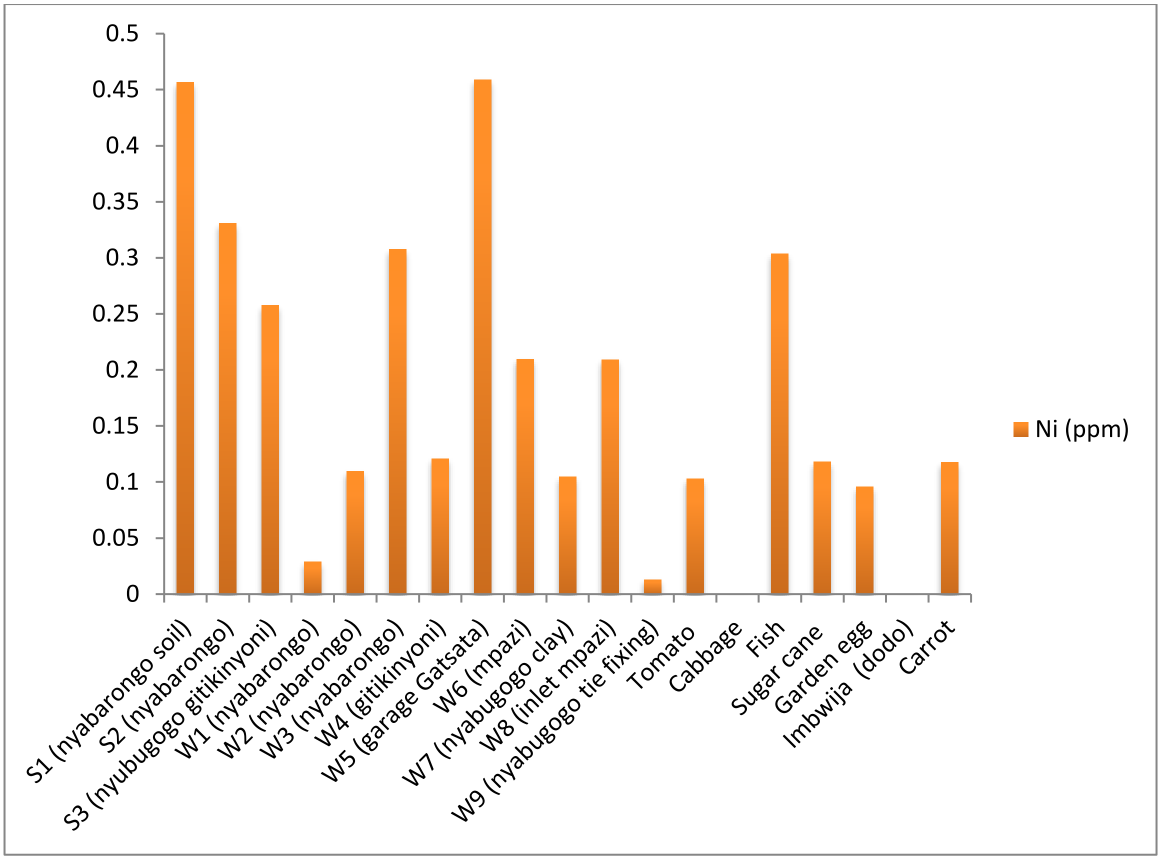

Figure 7: The Nickel content in aquatic species, soil sediment, and vegetable plants in the wetland as a result of the heavy metals deposition from domestic and industrial wastewater.

(S$_1$: soil sample from Nyabarongo, S$_2$: soil sample from Nyabugogo near Nzove, S$_2$; soil sample from giticyinyoni, W$_1$: Nyabarongo waste from clay manufacturing, W$_2$; waste water from Nyabarongo near Nzove, W$_3$: waste water from Gatsata garage to Nyabugogo, W$_2$; waste water from Mpazi, W5; waste water from Kabuye industry, W$_6$; waste water from Mpazi inlet, W$_7$; waste water from Nyabugogo near Giticyinyoni, Ni; Nickel)

4.3.5. Result of Manganese (Mn)

Manganese (Mn) is a metal that originates from the weathering of the Earth's crust in terrestrial environments, undergoing transformation into complex manganese hydroxyl compounds before settling into sediments. In this study, its concentration in sediments ranges from 0.8164 to 0.099 ppm in the region of Gatsata near the garage, the highest concentration of the Mn 0.8164 ppm and lowest concentration 0.099 ppm found in water from nyabugogo (Giticyinyoni), then averaged value of Igeo in the sediment 0.6481 which classified as particularly moderated contaminate due to the Igeo is greater than 0, Igeo > 0 (moderated contamination). The concentration factor (CF) in sediment is

1.1327(see Figure 8). This means that Mn has moderate contamination in the sediment sampled region; in fact, of some crops cultivated are affected by Mn in the bioaccumulation process, CF >1 = Moderate contamination [37]

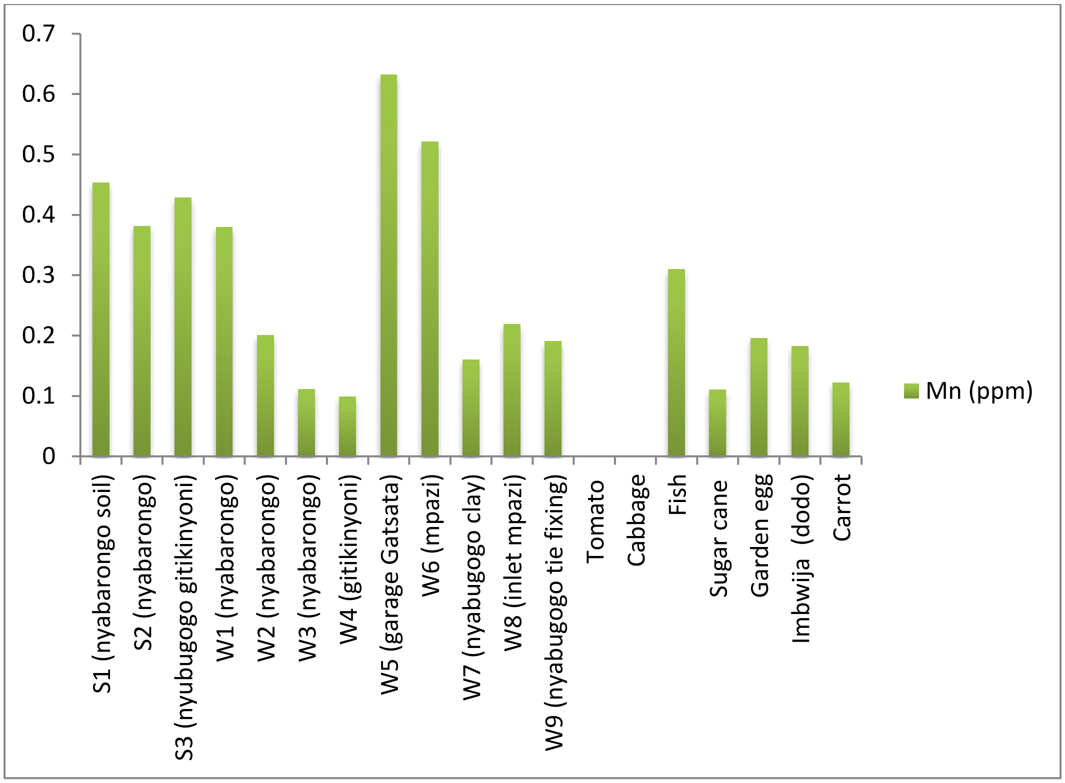

Figure 8:The Manganese description in the selected samples of the aquatic species, soil sediment, and vegetable plants as a result of the heavy metals deposition from the domestic and industrial wastewater.

(S1: soil sample from Nyabarongo, S2: soil sample from Nyabugogo near Nzove, S3: soil sample from Giticyinyoni, W1: Nyabarongo waste from clay manufacturing, W2: wastewater from Nyabarongo near Nzove, W3: wastewater from Gatsata garage to Nyabugogo, W4: wastewater from Mpazi, W5: wastewater from Kabuye industry, W6: wastewater from Mpazi inlet, W7: wastewater from Nyabugogo near Giticyinyoni, Mn: Manganese)

4.3.6. Result of Copper (Cu)

classified as a transition metal, is released into the environment from several sources, including mining operations, metal production processes, and runoff from agricultural lands, and activities of the chemical industry. Although it is an essential trace element required for haemoglobin synthesis and several enzymatic functions, elevated levels can pose serious risks to human health [40]. In this study, copper concentrations were found to vary across different sampling locations, with measurable levels detected in sediments, aquatic organisms, and wastewater samples. The observed concentration range is as follows: Highest concentration: Gatsata (near a garage) 3.6209 ppm, Nyabarongo River (near brick manufacturing) 2.5899 ppm, Nyabugogo River (near Nzove Water Treatment Plant) 1.8791 ppm Fish samples from Nyabugogo River 1.5509 ppm, Lowest concentration: Vegetables (cabbage and *Amaranthus viridis*) 0.9711 ppm [41] (see Figure). To evaluate the environmental impact, the Contamination Factor (CF) was calculated. The average CF value for copper was 1.5207, which is greater than 1 (CF > 1). This indicates moderate contamination in the sediment samples. While the local Cu concentrations suggest moderate contamination, they remain within safe limits when compared to national and international standards. These findings highlight the importance of continuous environmental monitoring, especially in regions affected by industrial activities and urban runoff, to prevent further copper accumulation and to safeguard both ecosystem and human health.

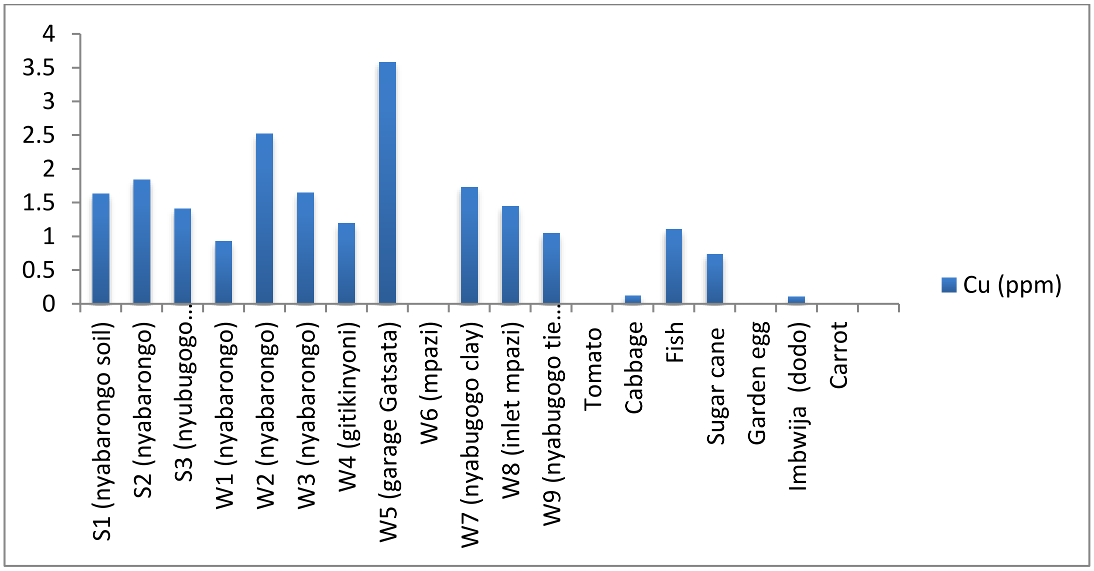

Figure 9: The Copper heavy metal concentration in the measured samples using AAS.

(S1: soil sample from Nyabarongo, S2: soil sample from Nyabugogo near Nzove, S3; soil sample from giticyinyoni, W1: Nyabarongo waste from clay manufacturing, W2; waste water from Nyabarongo near Nzove, W3: waste water from Gatsata garage to Nyabugogo, W4; waste water from Mpazi, W5; waste water from Kabuye industry, W6; waste water from Mpazi inlet, W7; waste water from Nyabugogo near Giticyinyoni, Cu; copper, AAS, Atomic absorption spectrophotometer)

4.3.7. Result of Zinc (Zn)

Zinc (Zn) is a naturally occurring element commonly present in the Earth's crust. It is frequently found alongside mud and various types of organic materials, such as green waste, food scraps, food-contaminated paper, non-hazardous wood residues, and landscaping or pruning debris. It enters the environment primarily through industrial and domestic waste disposal, as well as from the use of anti-corrosive paints containing zinc sulfate, which may be discharged into rivers and lakes. Once introduced into aquatic systems, zinc accumulates in aquatic organisms, and elevated concentrations can disrupt vital physiological functions, leading to health problems [42]. In this study, zinc concentrations ranged from 0.45632 to 2.3496 ppm, with specific values as follows: Highest concentration: Water inlet at Mpazi - 2.3496 ppm, Gatsata car garage water: 1.9801 ppm, Fish samples: 1.3075 ppm, Lowest concentration: *Amaranthus viridis* vegetable - 0.45632 ppm (see Figure 14). To assess the environmental risk of zinc contamination, three standard indices were applied: Contamination Factor (CF). The average CF value for Zn was 0.1009, which is less than 1 ($CF < 1$), indicating that the environment is not contaminated by zinc. Geoaccumulation Index (Igeo): The calculated Igeo value for Zn was 0.4570. Since $Igeo < 1$, this confirms that zinc is not causing contamination in the environment [43]. Pollution Load Index (PLI): The average PLI value for Zn was 0.620, which is less than 1 ($PLI < 1$), again classifying the environment as uncontaminated concerning zinc [44]. Overall, the zinc concentrations across water, fish, and vegetable samples fall within safe and uncontaminated ranges, suggesting minimal environmental impact from Zn in the study areas. While Zn plays a critical role in biological systems, excessive levels can be harmful, and these findings support the need for continued environmental monitoring to ensure concentrations remain below harmful thresholds.

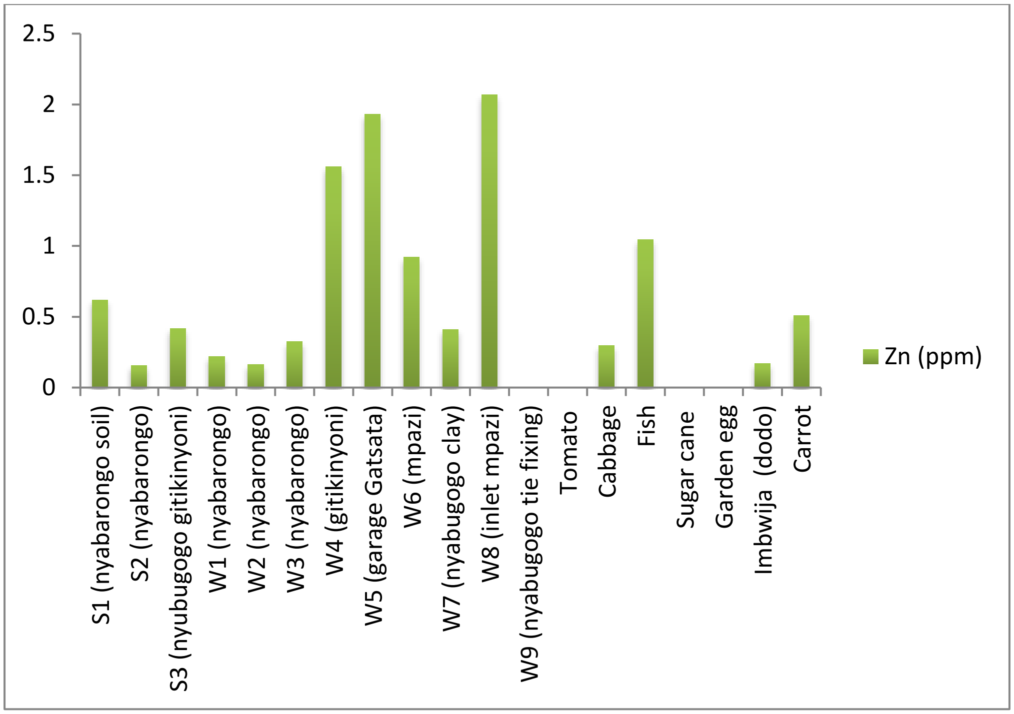

Figure 10: The Zinc description concentration in all samples collected in soil sediment, aquatic species, and vegetable plants in the Nyabugogo River and the Nyabarongo Wetland as a result of the heavy metal deposition from domestic, hospital, and industrial wastewater.

S1: soil sample from Nyabarongo, S2: soil sample from Nyabugogo near Nzove, S3; soil sample from giticyinyoni, W1: Nyabarongo waste from clay manufacturing, W2; waste water from Nyabarongo near Nzove, W3: wastewater from Gatsata garage to Nyabugogo, W4; wastewater from Mpazi, W5: wastewater from Kabuye industry, W6: wastewater from Mpazi inlet, W7: wastewater from Nyabugogo near Giticyinyoni, Zn: Zinc.

4.4: Evaluation of Contaminant Levels in Sediments

4.4.1. Geoaccumulation Index (Igeo)

The Geoaccumulation Index (Igeo) is an important ecological tool for distinguishing between metals originating from natural processes and those introduced through anthropogenic activities. It is also used to evaluate the contamination degree in sediment samples. The index is calculated using the formula Geoaccumulation index (Igeo): Igeo = $\log_2 \left(\frac{Cn}{1.5\,BAn}\right)$ where Cn is the concentration in sediment samples and Ban is the background geochemical metal concentration (n)

Table 2:The Geoaccumulation factor in all measured samples for all targeted heavy metals as the environmental assessed parameter for the pollution and environmental contamination

HMs resulted from AAS	*Average Igeo value*
Pb	*0.457±0.001*
Mn	*0.641±0.011*
Zn	*0.470±0.026*
Ni	*0.655±0.032*
Cu	*1.081±0.007*
Fe	*1.075±0.002*
Cd	*0.185±0.026*

(HMs, Heavy metals, Igeo; Geoaccumulation Index, AAS; Atomic absorption spectrophotometer, Cd, Cadmium, Mn; Manganese, Zn; Zinc, Ni; Nickel, Cu; Copper, Fe; Iron, Pb; Lead).

Based on the average Geoaccumulation Index (Igeo) values calculated in this study, the contamination level of heavy metals (HMs) in sediment samples follows the descending order: Mn>Zn>Pb>Cu>Ni>Fe. This ranking indicates that manganese (Mn) exhibits the highest Igeo value, suggesting a comparatively greater level of accumulation, whereas iron (Fe) shows the lowest Igeo value, implying minimal or no contamination [45]. According to the Igeo classification standards (see Figure 4), all metals fall within the "unpolluted" to "moderately polluted" range, thereby suggesting that the sediment across most study sites remains largely uncontaminated. However, notable deviations were observed at specific locations, particularly Nyabugogo near the Mpazi inlet, where higher concentrations of several metals were recorded (see Figure15). This localized increase in metal concentration led to elevated Igeo Pb, Mn, Ni, Cu, Cd, and Fe. Among these, Cu and Ni remained within the unpolluted category, indicating a lesser environmental threat. Nonetheless, the Igeo values for Pb, Mn, and Cd in this region were significantly higher compared to other sampling sites, highlighting potential contamination hotspots in the vicinity of urban and industrial discharge zones such as Mpazi and parts of Nyabugogo [46]. These findings underscore the importance of site-specific monitoring and targeted management strategies to mitigate heavy metal pollution in critical zones of the Nyabugogo and Nyabarongo river valleys.

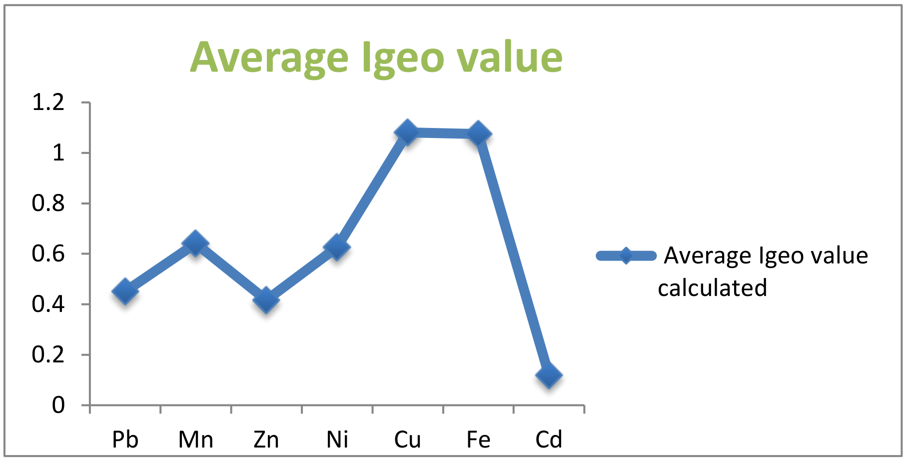

Figure 11:The average value of the Geoaccumulation index in all targeted heavy metals.

4.4.2. Evaluation of Contamination Factor (CF) and Pollution Load Index (PLI)

4.4.2.1: Contamination factor (CF)

The contamination factor (CF) and Pollution Load Index (PLI) are crucial factors indicating the contamination and pollution of heavy metals in the sediments. The contamination factor is calculated by the ratios of the mean concentration of the samples to the background of geochemical metal concentration (see Table 3).

Table 3: Concentration of the samples used in ERAF

Samples	**Pb(mg/kg)**	**Fe(mg/kg)**	**Cu(mg/kg)**	**Ni(mg/kg)**	**Mn(mg/kg)**	**Zn(mg/kg)**	**Cd(mg/kg)**
Nyabarongo (S1)	1.036±0.01	22.138±0.11	1.045±0.01	9.673±0.01	0.457±0.002	0.861±0.02	0.903±0.002
Nyabarongo (S2)	0.984±0.001	46.198±0.23	0.826±0.001	0.152±0.001	0.488±0.005	0.436±0.012	NA
Nyabarongo (S3)	0.732±0.003	23.161±0.11	0.772±0.016	0.422±0.015	0.468±0.001	0.529±0.015	0.115±0.001
Nyabugogo (S1)	0.624±0.004	20.867±0.17	0.469±0.014	0.562±0.01	0.528±0.001	0.441±0.001	0.451±0.006
Mpazi inlet 1	2.034±0.02	45.292±0.18	1.472±0.01	0.425±0.01	0.622±0.001	0.302±0.004	12.601±0.2
Gatsata garage	1.778±0.01	118.46±0.42	1.217±0.01	0.621±0.013	0.725±0.001	0.214±0.005	0.862±0.02
Mpazi inlet 2	1.031±0.01	40.264±0.27	1.139±0.01	0.725±0.011	0.674±0.001	0.406±0.001	8.546±0.11
Cn	1.172	45.191	0.991	1.797	0.566	0.452	3.355
CF	1.302±0.01	1.0043±0.01	1.515±0.01	0.071±0.001	1.132±0.002	0.109±0.003	0.111±0.003
PLI	1.038±0.01	1.005±0.01	0.783±0.02	0.686±0.003	1.017±0.01	0.514±0.017	0.731±0.001

$CF<1$=Low, $1<CF<3$=Moderate risk, $3<CF<6$=Considerable risk, $CF<6$=Very high risk

(Cn: mean concentration, CF: Concentration factor, PLI: pollution load index)

CF $=\frac{Cn}{bn}$ where the Cn is concentration mean and Bn is background of geochemical metal concentration. The background concentration standards for Cd, Zn, Cu, Ni, Mn, Fe, and Pb are provided as follows in mg/kg: 0.30, 95, 45, 65, 850, 47,200, and 20, respectively. The Pollution Load Index (PLI) reflects the cumulative environmental burden of heavy metals in the sediment and is calculated by multiplying the contamination factors [38].

PLI $=(CF1\ X\ CF2\ X\ CF3\ X\ CF4\ X\ \ldots X\ CFn)^{\frac{1}{n}}$ *Equation12*

Table 4: Mean values of CF calculated in sediment samples analysed.

HMS Analyzed	*CF mean value*	*Risk category*	*Decision*
Pb	*1.392±0.01*	*Moderate risk*	*Contaminated*
Cu	*0.015±0.01*	*Very low risk*	*Uncontaminated*
Mn	*1.132±0.02*	*Moderate risk*	*Contaminated*
Zn	*0.090±0.01*	*Very low risk*	*Uncontaminated*
Ni	*0.071±0.01*	*Very low risk*	*Uncontaminated*
Cd	*0.111±0.01*	*Low risk*	*Very low contamination*
Fe	*1.043±0.02*	*Moderate risk*	*Contaminated*

(*HMs; Heavy metals, CF; Contamination Factor, Pb; Lead, Cu; Copper, Mn; Manganese, Zn; Zinc, Ni; Nickel, Cd; Cadmium, Fe; Iron)*

The Contamination Factor (CF) serves as an essential metric for assessing the extent of heavy metal contamination in various environmental media, including sediments, water, vegetables, and aquatic life. A CF value below 1 (CF < 1) generally indicates a low risk of contamination, while values above 1 (CF > 1) suggest moderate to high contamination levels, potentially posing ecological or health risks. In this study, the CF values for cadmium (Cd), zinc (Zn), nickel (Ni), and copper (Cu) ranged from 0.099 to 0.111, identifying them as low-risk metals with negligible environmental effects. Conversely, lead (Pb) exhibited a CF value of 1.3027, placing it in the moderate contamination category. This level of contamination is particularly concerning in the Nyabarongo and Nyabugogo valleys, where elevated lead levels have been detected in vegetables grown in contaminated soils and in fish, raising public health concerns due to lead's carcinogenic and neurotoxic potential. Similarly, iron (Fe), with a CF value of 1.0043, also represents a moderate contamination risk, necessitating ongoing monitoring. The heavy metals, based on descending order of contamination severity (CF values), are as follows: Pb: 1.3027, Mn: 1.1324, Fe: 1.0043, Cd: 0.111, Zn: 0.0719, Cu: 0.00095 (see Figure 12). These findings underscore the need for targeted environmental surveillance and mitigation efforts, especially concerning lead and manganese, to minimize ecological degradation and protect both aquatic ecosystems and public health [48].

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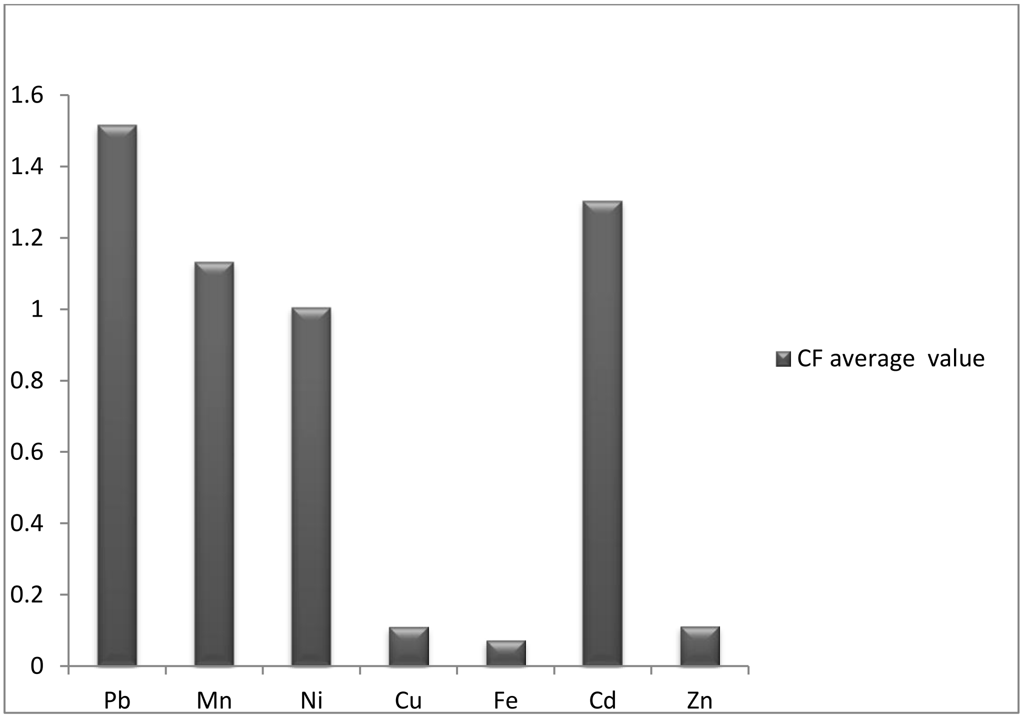

Figure 12: The Contamination factor value charts indicate the level of contamination in the selected heavy metals.

(CF; Contamination Factor, Pb; Lead, Mn; Manganese, Ni; Nickel, Copper, Fe; Iron, Cd; Cadmium, Zinc)

4.4.2.2: The pollution load index (PLI)

The Pollution Load Index (PLI) quantifies the overall burden of heavy metal contamination in sediment samples. It is derived by calculating the product of the contamination factors for each heavy metal present in the samples. PLI serves as a key metric to evaluate the relationship between heavy metal accumulation in the environment and its potential effects on aquatic organisms and plants. By combining the contamination factors of all assessed metals, PLI produces a single value that simplifies the evaluation of pollution levels in sediments and helps gauge ecological risks. The calculation of PLI follows the formula below [38].

PLI =$(CF1\ X\ CF2\ X\ CF3\ X\ CF4\ X\ \ldots X\ CFn)^{\frac{1}{n}}$ Then PLI (pollution load index), CF (contamination factor), n (number of samples taken), where: CF = Contamination Factor for each metal. n = Number of metals analyzed in the sample.

The Pollution Load Index (PLI) was calculated to assess the cumulative level of heavy metal contamination across various sample types, including water, aquatic species, and sediment. The PLI offers a comparative overview of pollution by summarizing the collective influence of multiple heavy metals in a single metric. Based on the results, the ranking of PLI values from highest to lowest is as follows: Zn > Ni > Cd > Cu > Fe > Mn > Patchogue zinc (Zn) topped the ranking in terms of individual PLI values, lead (Pb) exhibited the highest mean PLI across all sample types, suggesting areas of concern where Pb concentrations may be consistently higher (see Table 7).
Despite these variations, most PLI values were below 1, indicating uncontaminated or low-risk conditions for most of the study area. Only a limited number of samples exceeded the threshold of PLI > 1, pointing to minor contamination in localized zones. Most sites are within acceptable pollution limits. Localized PLI > 1 values suggest minor contamination hotspots. Continued monitoring is recommended, particularly for Pb and Zn, to prevent potential ecological and health impacts. These findings support the conclusion that, while the overall environment remains relatively uncontaminated, targeted management strategies should be developed for areas showing signs of increasing heavy metal accumulation [49].

Table 5: The Pollution Load Index (PLI) value from value selected heavy metals in the specific region

HMs	*PLI mean value*	*Risk assessment*	*Decision*
Fe	*1.05±0.01*	*Moderate risk*	*Less contamination*
Zn	*0.514±0.02*	*Very low risk*	*Uncontaminated*
Cu	*0.788±0.03*	*Very low risk*	*Uncontaminated*
Cd	*0.736±0.02*	*Very low risk*	*Uncontaminated*
Mn	*1.017±0.01*	*Moderate risk*	*Less contamination*
Ni	*0.686±0.02*	*Very low risk*	*Uncontaminated*
Pb	*1.038±0.02*	*Moderate risk*	*Less contamination*

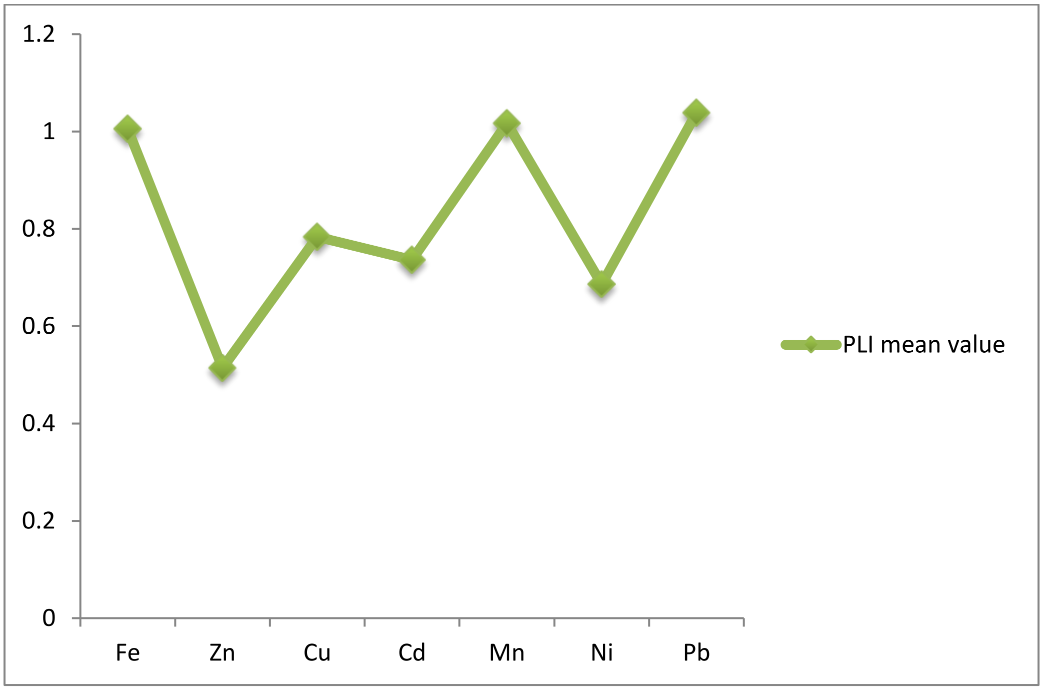

Figure:13. Pollution load index

4.4. 3: Bioconcentration, bioaccumulation, and biomagnification in the fish

The Bioconcentration Factor (BCF) was calculated using data from Atomic Absorption Spectroscopy (AAS) in a study focused on heavy metal pollution in the environment. BCF is an important metric that indicates how much heavy metals accumulate within organisms living in the same habitat and helps assess their potential effects on these organisms. The table below presents the average BCF values for various heavy metals found in fish. Bioconcentration describes the process by which organisms absorb chemicals from their surroundings mainly through respiration, skin surfaces, or sometimes diet, under natural environmental conditions [39] (See Table 8). The

BCF is calculated as the ratio of metal concentration in the organism's tissue to the concentration in the surrounding water. This measure enables quantification of metal accumulation levels in specific regions. BCF represents the balance between the rates of chemical uptake (such as through fish gills) and other absorption pathways. It provides insight into the extent of bioconcentration and underscores the environmental consequences of heavy metal contamination [23].

The bioconcentration factor is calculated as shown by the equation 12

$$\mathrm{BCF} = \frac{Concentration\ in\ biota}{concentration\ in\ ambient\ condition\ (water)} = \frac{CB}{CW} \qquad \textit{Equation 12}$$

Where the CB is the concentration of biota, as well as CW is for concentration of water in ambient conditions.

The BCF calculated, mentioning the degree of the accumulation or uptake of the chemical substances on the organism in the environment, BCF >1 indicating an uptake of the chemical substance that poses some effect on the living organism. As the bio concentration factor is greater than one (BCF is greater than 1) indicates that the concentration of heavy metal is greater than the medium where the samples are taken (water).

Table 6: The bioconcentration factor in fish for all targeted values of the heavy metals

Heavy metal	*Mean concentration (ppm)*	*BCF calculated*	*Decision*
Mn	*0.1596*	*0.515±0.002*	*BCF<1, i.e., less accumulation, the concentration in fish is less than in water, no further effect on the organism*
Pb	*0.5409*	*1.901±0.01*	*BCF>1 concentration of Pb in fish is greater than in water (moderate accumulation)*
Ni	*0.1045*	*0.344±0.002*	*BCF<1 i.e less accumulation, the concentration in fish is less than in water, no further effect on the food security*
Cu	*1.7299*	*1.566±0.026*	*BCF>1, i.e, the concentration of Cu in fish is greater than in water, which will affect the*

			surrounding organisms and lead to food insecurity
Zn	*0.1596*	*0.393±0.01*	*BCF <1, i.e, the concentration of Zn is below the medium of fish life, no effect on the aquatic organism (no great accumulation)*
Cd	*1.7299*	*3.303±0.12*	*BCF>1, i.e, the Cd concentration in a sample taken from Nyabugogo water is greater than the concentration of Cd in water, which will affect the next organism, including the aquatic species and human beings, and lead to the food insecurity*
Fe	*0.2019*	*100.001±0.2*	*BCF>1 i.e the concentration of Fe*

(*BCF; Bioconcentration Factor*)

Table 7: The bioconcentration factor value in the fish for all selected heavy metals and their decision

Heavy metal (HMs)	*Conc in Biota*	*Conc in Water*	*BCF in fish*	*Decision*
Cd	*0.145*	*0.352*	*0.302±0.022*	*Moderate accumulation*
Cu	*1.145*	*1.729*	*0.638±0.052*	*Moderate accumulation*
Mn	*0.398*	*0.156*	*1.941±0.016*	*Highly accumulation*
Fe	*0.211*	*0.215*	*0.998±0.023*	*Highly accumulation*
Ni	*0.337*	*0.145*	*2.906±0.025*	*Highly accumulation*
Pb	*0.285*	*0.549*	*0.525±0.012*	*Moderate accumulation*
Zn	*1.448*	*0.411*	*2.541±0.042*	*Highly accumulation*

(HMs; Heavy metals, BCF; bioconcentration factor)

4.4.5. Bio-concentration factor on vegetables (BCF$_{\text{Veg}}$) in *Solanum lycopersicum* (Tomato)

For this case, fish and mammals accumulate the organic and heavy metal pollutants, the plant tissues also accumulate those heavy metal pollutants. For the vegetables, the bio-concentration factors are expressed by the equation 11.

$$\text{BCF}_{\text{Veg}} = \frac{concentration\ in\ plant\ tissue}{concentration\ in\ soil} \qquad \textit{Equation 12}$$

Table 8: Bio-concentration factor on vegetables (BCFVeg) in Tomato

Heavy metal (HMs)	*Conc in soil*	*Conc in plant*	*BCF (Veg)*	*Decision*
Cd	*NA*	*NA*	*NA*	*No accumulation*
Cu	*1.491*	*NA*	*NA*	*No accumulation*
Mn	*0.486*	*NA*	*NA*	*No accumulation*
Fe	*0.735*	*0.184*	*0.165±0.006*	*Moderate accumulation*
Ni	*0.278*	*0.128*	*0.398±0.002*	*Moderate accumulation*
Pb	*0.621*	*0.182*	*0.298±0.004*	*Moderate accumulation*
Zn	*0.417*	*NA*	*NA*	*No accumulation*

4.4.6. Bio concentration factor on vegetables (BCF$_{\text{Veg}}$) in Cabbages

In analysis carried out to some vegetable and plant accumulate the heavy metal pollutant concentration in found in soil can be also accumulated by the plant or vegetables like cabbage in the same cases like the fishes and mammals accumulate the organic and heavy metals pollutant, even the plant tissues also accumulate those heavy metals pollutant, for the vegetables the bio concentration factors are expressed by the equation 13

$$\text{BCF}_{\text{Veg}} = \frac{concentration\ in\ plant\ tissue}{concentration\ in\ soil} \qquad \textit{Equation 13}$$

The bio-concentration results for cabbage cultivated in the soils of Nyabugogo near Giticyinyoni reveal a varying degree of heavy metal accumulation. Based on the calculated Bioconcentration Factor (BCF) values, the level of contamination follows a descending order from high to low BCF, indicating the extent of uptake by the cabbage from the surrounding soil. Among the analyzed heavy metals, lead (Pb) and iron (Fe) exhibited moderate levels of accumulation, suggesting a potential risk of contamination in cabbage grown in this area. These findings indicate that cabbage can bio-accumulate certain metals to levels that may pose concern for human consumption and public health if exposure persists over time. In contrast, other heavy metals analyzed in this study showed BCF values below 1, implying no significant accumulation or no contamination risk in the cabbage samples.

Table 9: Bio-concentration Factor in vegetable (BCFVeg) in Cabbage

Heavy metal (HMs)	*Conc in soil*	*Conc in plant*	*BCF (Veg)*	*Decision*
Cd	*NA*	*NA*	*NA*	*No accumulation*
Cu	*1.491*	*NA*	*NA*	*No accumulation*
Mn	*0.486*	*NA*	*NA*	*No accumulation*
Fe	*0.735*	*0.184*	*0.165±0.005*	*Moderate accumulation*
Ni	*NA*	*NA*	*NA*	*No accumulation*
Pb	*0.621*	*0.161*	*0.186±0.002*	*Moderate accumulation*
Zn	*0.417*	*NA*	*NA*	*No accumulation*

(NA; not detected, HMs; Heavy metals, conc; concentration, veg; vegetables)

4.4.7. Bio-concentration factor on vegetables ($\mathbf{BCF_{Veg}}$) in Sugar cane

The sugar cane cultivated in the Nyabarongo valley their soil containing some heavy metal concentrations, and the cultivated sugar cane in that soil also contains some concentrations of the heavy metals as a result of bioaccumulation. The above table summarizes the values of the BCF in sugar cane analyzed [15]. In analysis carried out to some vegetable and plant accumulate the heavy metal pollutant concentration in found in soil can be also accumulated by the plant or vegetables like sugar cane in the same cases like the fishes and mammals accumulate the organic and heavy metals pollutant, even the plant tissues also accumulate those heavy metals pollutant, for the vegetables the bio concentration factors are expressed by the equation 14.

$$\mathrm{BCF_{Veg}} = \frac{concentration\ in\ plant\ tissue}{concentration\ in\ soil} \qquad \textit{Equation 14}$$

Table 10 summarizes the Bio-concentration Factor (BCF) values for sugarcane, highlighting the extent of heavy metal accumulation and its potential environmental and biological impacts. The BCF values are presented in descending order, with higher values indicating a greater degree of metal uptake by sugarcane plants. Based on the analysis, all BCF values for the metals studied in sugarcane are below 1, indicating no significant accumulation. According to established classification criteria, BCF values less than 1 suggest minimal to moderate accumulation, with no immediate adverse effects on sugarcane or the surrounding ecological systems. Among the analyzed heavy metals, iron (Fe), lead (Pb), copper (Cu), and manganese (Mn) exhibit moderate accumulation, with their BCF values ordered as follows: Fe > Pb > Cu > Mn. This implies that these metals are more readily absorbed by sugarcane compared to others, but still within safe accumulation thresholds. Other metals, including zinc (Zn), nickel (Ni), and cadmium (Cd), show negligible or no accumulation, as their BCF values were significantly below 1. These findings collectively indicate that while some level of metal uptake occurs, the overall contamination risk is low, and sugarcane cultivation in the Nyabarongo Valley remains environmentally safe under current conditions.

Table 10 Bio-concentration Factor in Vegetable (BCFVeg) in Sugar cane

Heavy metal (HMs)	*Conc in Plant*	*Conc in Soil*	*BCF (Veg)*	*Decision*
Cd	*NA*	*NA*	*NA*	*No accumulation*
Cu	*0.118*	*0.457*	*0.258±0.001*	*Slightly accumulation*
Mn	*0.112*	*0.436*	*0.242±0.002*	*Slightly accumulation*
Fe	*0.781*	*1.629*	*0.453±0.005*	*Slightly accumulation*
Ni	*0.693*	*NA*	*NA*	*No accumulation*
Pb	*0.121*	*0.732*	*0.273±0.004*	*Slightly accumulation*
Zn	*NA*	*0.693*	*NA*	*No accumulation*

(HMs; Heavy metals, BCF; bioconcentration factor, NA; not detected)

4.4.8. Bio concentration factor on vegetables (BCF_{Veg}) in *Solanum melongena,* (Garden egg)

Based on the measured heavy metal concentrations in the soil of Nyabugogo Valley, especially near the Nzove Water Treatment Plant, and the corresponding metal levels in garden egg samples grown in that soil, Bioconcentration Factors (BCF) were calculated to assess the extent of contamination and metal uptake [19]. The findings suggest that certain plants and vegetables have the ability to accumulate heavy metals from polluted soils, much like aquatic species such as fish and mammals absorb contaminants from their environments. Edible plant parts, including those of garden eggs and cabbage, can bioaccumulate these harmful substances, which may then enter the food chain and pose health risks. In this study, the BCF values calculated for garden eggs reflect the extent to which these heavy metals are absorbed from the soil. The BCF provides critical insight into the mobility and transfer of metals from soil to plant tissues, indicating that bioaccumulation is not limited to animal species but also affects crops, with implications for human health and food safety (see equation 15).

$$\mathrm{BCF}_{\mathrm{Veg}} = \frac{concentration\ in\ plant\ tissue}{concentration\ in\ soil} \qquad \textit{Equation 15}$$

Table 13 shows a summary of the bio-concentration Factor (BCF) values for heavy metals found in garden eggs cultivated in the Nyabugogo Valley. The BCF values are presented in descending order to reflect the extent of metal accumulation within the plant tissues. Among the heavy metals analyzed, manganese (Mn) exhibited the highest BCF value at 0.4568, which is classified as moderate contamination (BCF < 1 and > 0.1). This is followed by nickel (Ni), with a BCF value of 0.3723, also falling within the moderate contamination range. All other heavy metals showed BCF values below 0.1(see Table 13), indicating low or negligible accumulation. These results suggest that only Mn and Ni present moderate uptake levels, while the remaining metals pose minimal bio-concentration risk. Overall, the bio-magnification potential of the assessed heavy metals in garden eggs is considered low, with no significant ecological or health impacts on surrounding organisms or food chains under current environmental conditions [23].

Table 13: Bio concentration factor on vegetables (BCFVeg) in Garden egg (Intoryi)

Heavy metal (HMs)	*Conc in Plant*	*Conc in Soil*	*BCF (Veg)*	*Decision*
Cd	*NA*	*NA*	*NA*	*No accumulation*
Cu	*NA*	*1.491*	*NA*	*No accumulation*
Mn	*0.198*	*0.426*	*0.456±0.002*	*Moderate accumulation*
Fe	*NA*	*0.735*	*NA*	*No accumulation*
Ni	*0.096*	*0.258*	*0.372±0.003*	*Moderate accumulation*
Pb	*0.091*	*0.621*	*0.146±0.001*	*Slightly accumulation*
Zn	*NA*	*NA*	*NA*	*No accumulation*

4.4.9. Bio-concentration factor in vegetable (BCF$_{\text{Veg}}$) in *amaranth virdis*

Based on the assessed data of the bioconcentration factor (BCF) from *Amaranthus virdis* sampled from Nyabugogo Valley soil, the BCF is determined using atomic absorption spectrophotometer (AAS) concentration values for each heavy metal across different samples[23]. These values are used to indicate the degree of soil contamination and its impact on corresponding plants, nearby species, and organisms. Additionally, they highlight the bioconcentration factor for each heavy metal and its associated risks or effects within the environment. The bioconcentration factor for plants or vegetables is calculated as shown in equation 16.

$$\text{BCF}_{\text{Veg}} = \frac{concentration\ in\ plant\ tissue}{concentration\ in\ soil} \qquad \textit{Equation 16}$$

Based on the Bioconcentration Factor (BCF) values determined for *Amaranthus viridis* using data obtained from Atomic Absorption Spectroscopy (AAS) and calculated through the standard BCF formula, the metals were ranked from highest to lowest in terms of accumulation potential. Manganese (Mn) exhibited the highest BCF value at 0.4246, indicating moderate accumulation, followed by zinc (Zn) with a BCF of 0.4091, which is also classified under moderate accumulation [50]. The remaining heavy metals analyzed showed very low or negligible BCF values, suggesting minimal or no accumulation in garden amaranth. According to recognized BCF classification standards, values between 0.1 and 1.0 indicate moderate accumulation, while values below 0.1 reflect low or no accumulation. Therefore, the BCF values assessed across different soils and vegetable samples, specifically for garden amaranth, indicate that all heavy metals fall within the low to moderate contamination range, with no significant environmental or ecological risks posed. These findings suggest that garden amaranth grown in the study area is relatively safe for consumption, and its cultivation contributes minimally to heavy metal transfer in the food chain [53].

Table 11 The table below illustrates the bioconcentration value with its associated risks or decision

Heavy metal (HMs)	*Conc in Plant*	*Conc in Soil*	*BCF (Veg)*	*Decision*
Cd	*NA*	*NA*	*NA*	*No accumulation*
Cu	*NA*	*1.821*	*NA*	*No accumulation*
Mn	*0.198*	*0.551*	*0.756±0.026*	*Moderate accumulation*
Fe	*NA*	*0.821*	*NA*	*No accumulation*
Ni	*0.096*	*0.278*	*0.683±0.003*	*Moderate accumulation*
Pb	*0.091*	*0.421*	*0.446±0.001*	*Slightly accumulation*
Zn	*NA*	*NA*	*NA*	*No accumulation*

4.4.10.Bio-concentration factor in Vegetable (BCF_{Veg}) in Carrot

The Bio-concentration Factor (BCF) serves as a critical metric in evaluating the degree to which heavy metals (HMs) accumulate in plants or vegetables grown in contaminated soils. This factor is calculated based on the ratio of the heavy metal concentration in the plant to that found in the corresponding soil. Elevated BCF values may indicate potential for bio-accumulation and, over time, bio-magnification through the food chain, posing risks to human and ecological health, especially with crops intended for large-scale consumption. In this analysis, the BCF in carrots was determined to evaluate the accumulation of heavy metals, offering insight into the eco-toxicological impact and potential health implications at various trophic levels (see equation 17).

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$$BCF_{Veg} = \frac{concentration\ in\ plant\ tissue}{concentration\ in\ soil} \qquad \textit{Equation 17}$$

The bio-concentration factor (BCF) values for carrots cultivated in the Nyabugogo Valley are summarized in Table 15 below and ranked from highest to lowest. Zinc (Zn) recorded the highest BCF value at 1.22, which classifies it as having a high degree of accumulation (BCF > 1). This indicates a potential health concern associated with the prolonged consumption of carrots grown in this area, due to elevated zinc uptake. Nickel (Ni) exhibited a BCF value close to 0.5, which falls within the range of moderate accumulation ($0.1 < BCF < 1$), suggesting a slight degree of contamination. The remaining analyzed heavy metals displayed BCF values below 0.1, signifying no significant accumulation in carrot tissues.

Overall, while zinc shows notable bio-accumulation, and nickel suggests minor contamination, the remaining heavy metals pose minimal risk. When assessed on a broader ecological scale, these values indicate limited contamination impact at higher trophic levels, with no significant biomagnification effects detected.

Table 12: Bio-concentration factor in Vegetable (BCFVeg) in Carrot

Heavy metal (HMs)	*Conc in Plant*	*Conc in Soil*	*BCF (Veg)*	*Decision*
Cd	*NA*	*NA*	*NA*	*No accumulation*
Cu	*NA*	*1.491*	*NA*	*No accumulation*
Mn	*0.122*	*0.486*	*0.284±0.032*	*Slightly accumulation*
Fe	*0.097*	*0.735*	*0.135±0.012*	*Slightly accumulation*
Ni	*0.176*	*0.578*	*0.456±0.024*	*Slightly accumulation*
Pb	*0.119*	*0.621*	*0.191±0.021*	*Slightly accumulation*
Zn	*0.588*	*0.417*	*1.225±0.025*	*Highly accumulation*

(HMs; Heavy metals, BCF; biococentration factor, conc; concentration, NA; not detected, CD; Cadmium, Cu; Copper, Mn; Manganese, Fe; Iron, Pb; Lead).

4.5. Potential ecological risk factor (PERF)

The heavy metal pollution has more impact to the endanger for health, environment and aquatic organism, this study presented to investigate the potential ecological risk (PER) of the heavy metals in the soils and aquatic organism, the range of the potential ecological risk for the risk assessments for the metal standards, presents the Potential Ecological Risk Factor (PERF) values for various heavy metals, highlighting their associated environmental and ecological risks. Among the evaluated sites, the highest PERF value was recorded for zinc (Zn) in fish and soil near Nyabugogo, close to Giticyinyoni, with a value of 40.752 and a cumulative ecological risk index (RI) of 204.21. According to established ecological risk assessment criteria: 100≤Eri<1501→ *Considerable risk*200≤RI<350→ *Very high risk.*

This places zinc contamination in this region within the high-risk category, posing a significant threat to aquatic life and the surrounding environment. The second highest risk was found in soil near the Nyabarongo brick manufacturing area, specifically for iron (Fe), with a PERF value of 33.4502 and a cumulative RI of 162.38. This falls under the classification of: 50≤Eri<100 → *Moderate to considerable risk,* 150≤RI<250→ *Significant cumulative risk*

These findings underscore the need for ongoing monitoring and potential remediation in the identified hotspots to mitigate the risks to ecosystems and public health [40].

Table13: Potential ecological risk factor (PERF) for the organism

Heavy metal	*Co*	*Cn*	*Cn/co*	*ER*	*RI*	*Decision*
Cu	*1.867*	*0.846*	*0.458*	*27.115*	*101.45*	*Low risk*
Cd	*0.352*	*0.141*	*0.408*	*26.95*	*100.06*	*Low risk*
Ni	*0.536*	*0.138*	*0.199*	*26.041*	*99.45*	*Low risk*
Pb	*0.675*	*0.321*	*0.478*	*32.28*	*135.66*	*Moderate risk*
Mn	*0.732*	*0.197*	*0.283*	*22.982*	*86.57*	*Low risk*
Fe	*0.467*	*0.207*	*0.439*	*33.452*	*162.38*	*Significant risk*
Zn	*0.735*	*0.434*	*0.606*	*40.752*	*204.21*	*High risk*

(PERF; Potential ecological risk factor, Co; initial concentration, Cn; mean concentration, ER; ecological risk, RI; risk index)

4.6. **Bioaccumulation in fish**

Bio-accumulation factor (BAF) is defined as the increased level of metal concentration in the organism that is present on the environment surface, some pollutant like HMs is taken up to the survival, more concentration of heavy metal accumulated by the effect on it. In that case most of the heavy metals are hazardous to the organism through the binding site on the organism's tissues which enhancing the metal binding and lead to the improper function of the organism (see equation 18) [41].

$$\text{BAF} = \frac{Concentration\ of\ substance\ in\ fish}{Concentration\ of\ substance\ in\ water} \qquad \textit{Equation 18}$$

The bio-accumulation factor (BAF) was calculated from the various data obtained after using Atomic Absorption Spectroscopy (AAS); therefore, (see Table 16) summarizes the bio-accumulation factor for the target sample analyzed. The bio-accumulation factor calculated from the data analysed from the sample of fish sampled from Nyabarongo and Nyabugogo with the concentration of that water analysed using AAS in ppm values, the concentration of biota as the fish is also in ppm values and as the BAF is calculated by the ration of the concentration of the biota or the accumulator species to the concentration of the media (water) then the range of the results are from the 10 to the 0.4 values of the BAF from different heavy metals concentration. BAF <1 = slightly accumulated, BAF>1 or 2 = moderate accumulated and BAF >5 = highly accumulated[24], for the BAF data values obtained, the Nickel(Ni) is mostly accumulated with the value of the 9.8 as the highest accumulated heavy metal found in the fish analysed, the zinc is also highly accumulated heavy metal with the value data of 4.9 and the slightly accumulated heavy metal found in cadmium and the bio-accumulation factor lead to the bio-magnification. These findings suggest a potential for bio-magnification, especially for Nickel and Zinc, as they move through the food web. This poses a significant ecological and human health risk, particularly due to fish consumption [19].

Table 14: Bioaccumulation for all selected heavy metals

HMs	*CA*	*CW*	*BAF*	*Decision*
Cd	*0.105*	*0.125*	*0.839*	*Slightly accumulation*
Cu	*1.145*	*0.928*	*1.191*	*Moderate accumulation*
Mn	*0.398*	*0.391*	*1.255*	*Moderate accumulation*
Pb	*0.211*	*0.421*	*2.148*	*Moderate accumulation*
Fe	*0.337*	*0.198*	*0.394*	*Slightly accumulation*
Ni	*0.283*	*0.029*	*9.803*	*Highly accumulation*
Zn	*0.048*	*0.224*	*4.919*	*Highly accumulation*

(HMs; Heavy metals, CA; concentration in fish, CW; concentration in water, BAF; bio-accumulation factor)

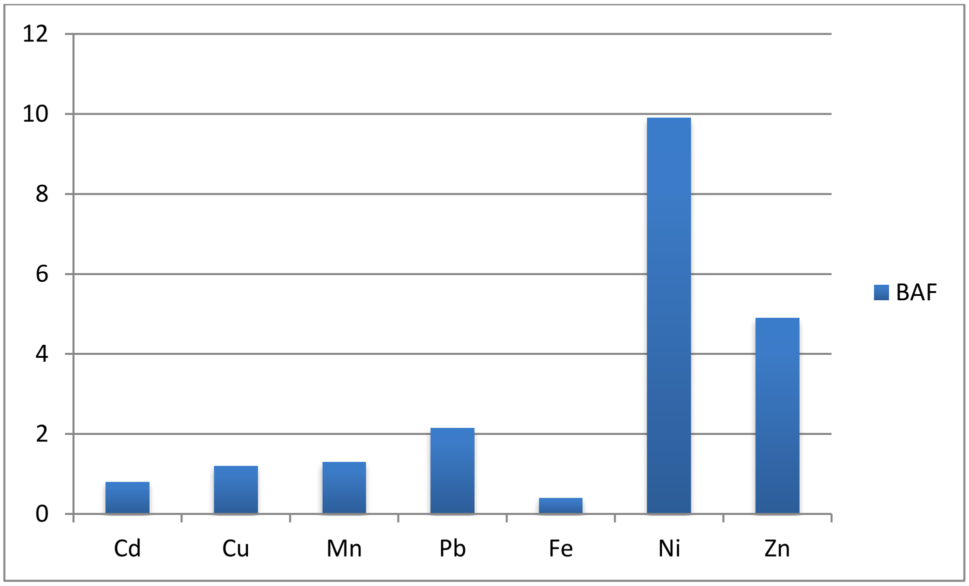


Figure:14. Bio-accumulation factor

7.0: Fourier transform infrared spectroscopy analysis (FTIR analysis)

Fourier Transform Infrared (FTIR) spectroscopy was utilized to identify and analyze the main functional groups present in rice husk-derived biochar, which play a vital role in the adsorption and precipitation of heavy metal ions from aqueous solutions. The FTIR spectra were recorded over the range of 4000 to 600 cm^{-1} using a Bruker Alpha-E spectrometer equipped with a ZnSe Attenuated Total Reflection (ATR) crystal. Rice husk biochar contains a variety of molecular components such as carbohydrates, proteins, fibers, and lipids, along with functional groups including N–H, O–H, Si–O bonds, aliphatic chains, and aromatic rings, all of which are key contributors to heavy metal adsorption. Significant spectral bands were observed for N–H, O–H, Si–O groups, as well as aliphatic and aromatic structures. In particular, C–H vibrations in aromatic and heteroaromatic compounds appeared in the 826–980 cm^{-1} region, indicating interactions with alkaline earth metals and oxidizing heavy metals. The peak at 1250 cm^{-1} indicates the presence of hydrogen-bonded hydroxyl groups, which enhance the binding affinity toward metal ions. A peak at 1400 cm^{-1} is attributed to CO_2 groups, while the 1600 cm^{-1} peak signifies the presence of carbonyl groups, typically formed from the thermal decomposition of cellulose. The 1613 cm^{1} peak corresponds to the carboxylate ($-COO^{-}$) group, and in some cases, overlapping signals from conjugated alkenes ($-C=C-C$) may also be present, contributing to metal ion coordination. These functional groups in the rice husk-derived biochar are instrumental in enhancing its adsorptive capacity, making it effective in the removal of heavy metals from contaminated water systems (see Figure15).

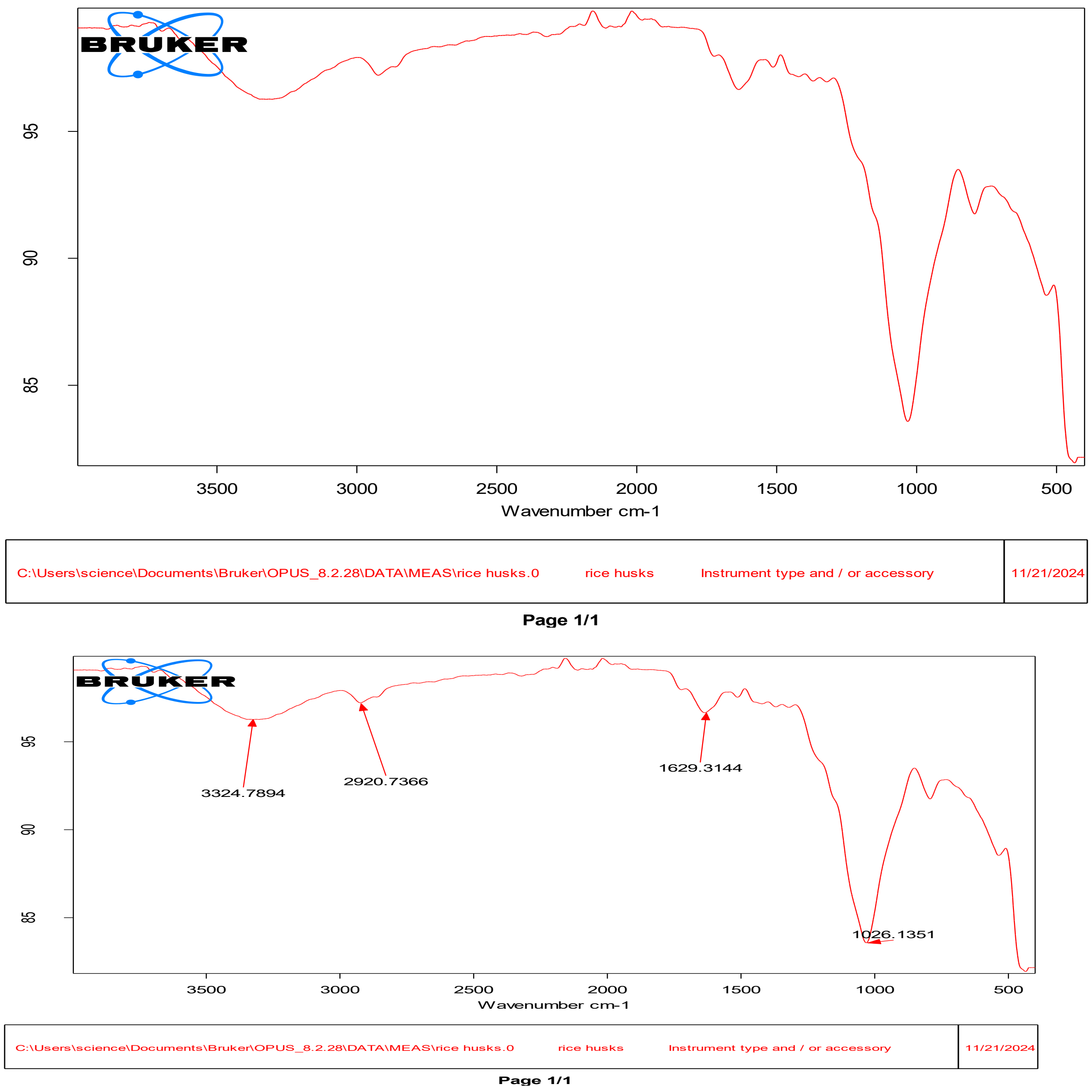


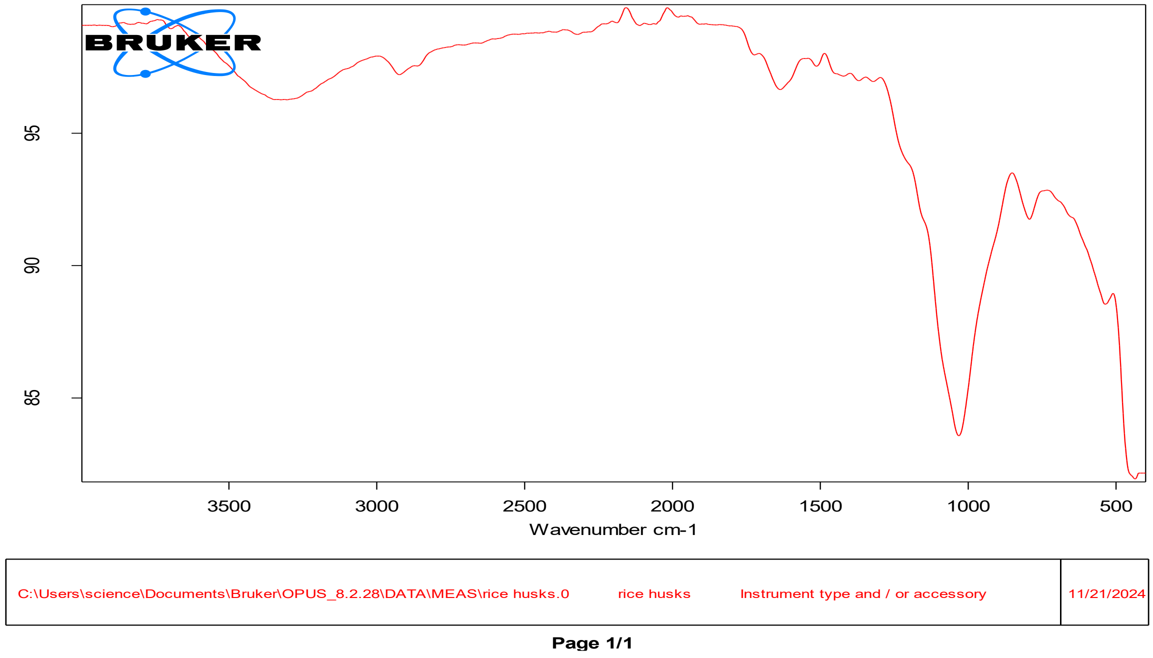
Figure 15: FTIR Characterization of Biochar Functional Groups for Heavy Metal Adsorption

The results, from the FTIR (Fourier transform infrared) analysis of the rice husks which are used as biochar for the adsorption of the heavy metals from the waste water, therefore the FTIIR showed the different compounds present in the rice husks according to their specific wave number and the transmittance. Then the cellulose and hemicellulose are present in rice husks due to the O-H stretching with range value from 3200-3600 cm^{-1}, this stretched of the OH functional group indicates the presence of the cellulose and hemicellulose compounds in the rice husks, the C=O,

C-O-H, C-H stretching also found in the rice husks also indicating the presence of the cellulose, hemicellulose and lignin compounds with the stretched valuorganism e arranged respectively 1020-

1100 cm^{-1},1200-1600 cm^{-1},2800-3000cm^{-1} all wavenumber their corresponding transmittance. Hence by the FTIR analysis results of the rice husks shows all target, compounds used for the adsorption process of the heavy metals which are cellulose with (OH-stretching) have high efficiency removal for Pb^{2+} and Mn^{2+}, the lignin and hemicellulose with (C=0, C-H, C-O-H) have the removal efficiency for the Fe^{2+} and Cd^{+} and for other heavy metals also are removed in the adsorption process.in analysis of the rice husks using the Fourier transform infrared also indicating the carbon compounds present which are used to make the biochar for the adsorption mechanism for the removing the heavy metals from the waste water sampled , hence for overall compounds analyzed by FTIR are responsible for the production of the good biochar, which are used in the adsorption process and as for the rice husks containing the cellulose ,hemicellulose and lignin produce the good biochar due to their contains numerous number of the carbon and good biochar leads to the high removal efficiency of the heavy metal ions like Pb^{2+},Fe^{2+},Mn^{2+} and other heavy metal ions remaining [29].

Figure C: The spectrum of Rice Husks Biochar from FTIR



7.1: Atomic Absorption Spectrophotometer (AAS) results after adsorption process

The filtered mixture of the rice husk biochar and waste water samples from the Mpazi, Gatsata garages, and Mpazi hospital waste water inlet those mixtures are filtered using the Whatman filter paper with 42.0mm. The standard solution is prepared from the stock solution containing all heavy metals to be analysed, such as Pb, Mn, Cd, Cu, Ni and Fe (0.05M ,0.01M ,0.1M, 0.2M and 0.5M) The calibration curve is derived from that standard with corresponding absorbance with R^2=0.8478+0.2368

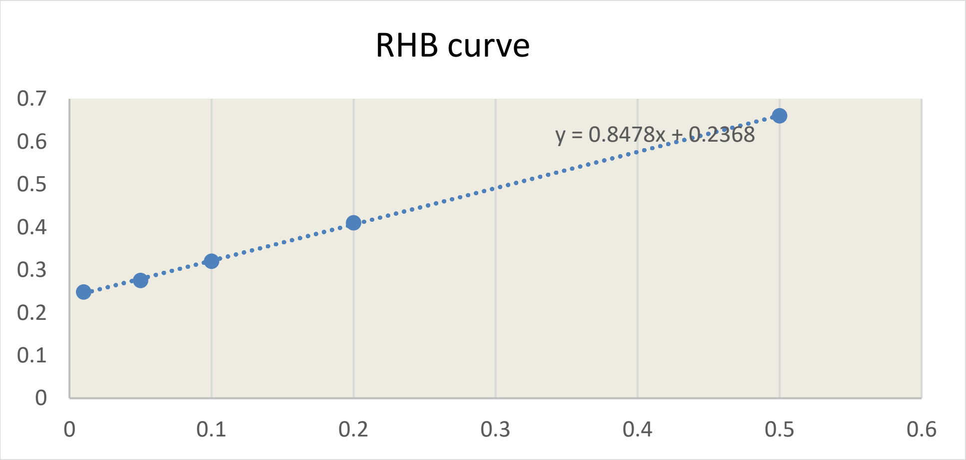


Figure 13:Calibration curve for the HMs analysis after adsorption

The adsorption process of heavy metals from wastewater samples was conducted using rice husk biochar (RHB) as an effective adsorbent. The process involved the adjustment of pH, variation in adsorbent dosage, and contact time, which are critical parameters influencing adsorption efficiency[25]. Following the adsorption process, the samples were filtered to remove suspended solids and then analyzed using Atomic Absorption Spectrophotometry (AAS) to determine the residual heavy metal concentrations. Baseline measurements were also conducted on the untreated wastewater and the rice husk biochar beforehand to evaluate the initial levels of heavy metals and the biochar's adsorption capacity [18] (see Table 15) below provides a summary of important parameters and the concentrations of heavy metals prior to adsorption, including factors such as pH, initial concentrations of Pb, Mn, Fe, Cu, Cd, Zn, Ni

Table15: Concentration of the wastewater before adsorption using RHB

Samples	Cd (mg/kg)	Pb (mg/kg)	Mn (mg/kg)	Ni (mg/kg)	Fe (mg/kg)	Cu (mg/kg)
Mpazi H1	0.06±0.004	2.36±0.11	1.87±0.03	1.35±0.024	4.47±0.024	2.04±0.024
Mpazi G1	0.09±0.005	2.05±0.14	2.21±0.024	1.49±0.024	3.72±0.024	1.79±0.024
Gatsata Gg	0.12±0.014	3.88±0.04	1.93±0.024	2.23±0.024	1.71±0.024	1.88±0.024
Mpazi I	0.02±0.023	3.03±0.02	2.18±0.024	1.62±0.024	2.25±0.024	1.42±0.024
RHB	Ntd	0.07±0.004	0.01±0.024	0.04±0.024	0.02±0.024	0.07±0.024

(RHB: Rice Husk Biochar as adsorbent, Gg: Garage inlet, G1: waste water inlet to Nyabugogo river, H1: Wastewater from CHUK, I: wastewater from Kabuye sugar work industry ltd)

Table 16: Concentration of waste water samples after adsorption using RHB

Samples	Cd (mg/kg)	Pb (mg/kg)	Mn (mg/kg)	Ni (mg/kg)	Fe (mg/kg)	Cu (mg/kg)
Mpazi H1	0.01±0.004	1.86±0.11	1.14±0.03	0.88±0.024	1.66±0.014	1.76±0.011
Mpazi G1	0.02±0.005	1.63±0.14	1.71±0.024	0.46±0.001	1.08±0.006	1.52±0.021
Gatsata Gg	0.04±0.014	2.68±0.04	1.02±0.024	1.28±0.015	1.35±0.015	1.61±0.017
Mpazi I	Ntd	1.93±0.02	1.09±0.024	0.77±0.024	0.94±0.004	1.31±0.024

(Gg: Garage inlet, G1: waste water inlet to Nyabugogo river, H1: Wastewater from CHUK, I: wastewater from Kabuye sugar work industry ltd)

7.2: The removal efficiency determination

The efficiency of heavy metal removal was assessed using the Langmuir adsorption model, which is commonly employed to evaluate adsorption processes involving activated carbon for eliminating both organic and inorganic contaminants. In this study, the Langmuir model was utilized to determine the percentage of heavy metal adsorption [42].

Our research focused on assessing the efficiency of rice husk biochar (RHB) in the removal of Pb^{2+} and Mn^{2+} as targeted heavy metals. The following formula was used to calculate the percentage removal of heavy metals across all samples, as well as to validate the Langmuir model. The data, obtained from Atomic Absorption Spectrophotometry (AAS) and analyzed in Excel, showed the following results:

- Highest removal efficiency: 85.56% for Pb^{2+} and 70.96% for Fe^{2+}
- Lowest removal efficiency: 7.74% for Cu^{2+}

These findings indicate that the adsorption of heavy metals using rice husk biochar is highly effective, particularly for Pb^{2+} and Fe^{2+} (see Table17). Additionally, RHB proves to be an efficient biochar material with strong adsorption properties for heavy metal removal [43].

$$\% \text{ Adsorption} = \frac{Co \quad - \quad Ce}{Co} x\ 100$$

Table 7:Measured concentration of the targeted heavy metals after absorption using RHB

samples	*Cd*	*Pb*	*Mn*	*Ni*	*Fe*	*Cu*
M1	*16.66*	*85.56*	*39.04*	*34.81*	*62.86*	*13.72*
M2	*22.22*	*62.44*	*22.64*	*69.12*	*70.96*	*15.08*
G1	*16.67*	*64.76*	*47.15*	*42.6*	*21.05*	*14.36*
G2	*10*	*57.33*	*50*	*52.46*	*58.22*	*7.74*

(M1: Mpazi inlet from CHUK, M2: Wastewater from Kabuye Sugar Work industry, G1: Wastewater from Gatsata garage inlet, G2: Mpazi wastewater inlet to Nyabugogo river)

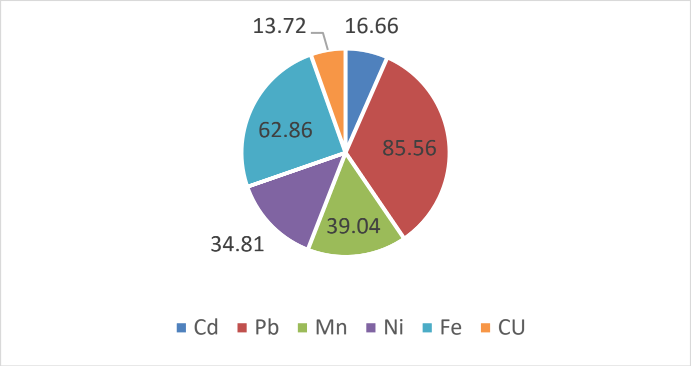


Figure14:Absorption percentages for all targeted heavy metals

7.3.1. Effect of pH on the % removal of heavy metal

The adsorption capacity of the adsorbent depends on several factors, such as adsorbent dosage, the pH of the medium, and contact time. After preparation, the biochar is used as a bio-adsorbent. The initial pH of the absorbent ranges from 4.65 to 5.01. To examine the adsorption capacity of the adsorbent, the pH is optimized from 1 to 6 [44]. The adsorption process is then carried out for targeted heavy metals such as Pb^{2+} and Mn^{2+}, along with other heavy metals, within a specific time interval. The percentage adsorption of these heavy metals is represented in the chart below. At a pH of 5, the highest adsorption percentages for Pb^{2+} and Mn^{2+} are observed, at 86% and 34%, respectively. Conversely, at a pH of 1, the lowest adsorption percentages for Pb^{2+} and Mn^{2+} are recorded, at 25% and 20%, respectively (see figure 16). Hence, as the adsorbent becomes weaker, an increase in acidity enhances the adsorption capacity for heavy metal removal [45].

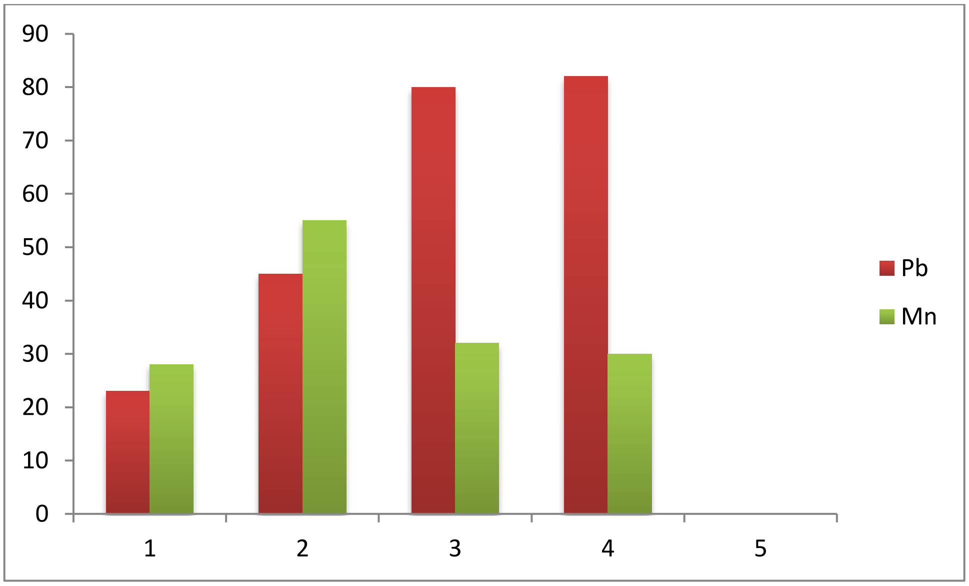


Figure 15:Effect of pH on the % removal of heavy metal

7.3.2. Effect of Adsorbent Dosage on the Efficiency of Heavy Metal Removal

By examining the effect of adsorbent dosage on determining the adsorption capacity of the bio-adsorbent, the dosage of rice husk biochar ranged from 1g to 5g. The adsorption process was conducted for all analysed heavy metals, with a focus on Pb^{2+} and Mn^{2+}. Increasing the adsorbent concentration enhances the availability of surface pores for metal adsorption onto the activated carbon, thereby improving the percentage removal of heavy metals [46]. According to the results presented below, Pb^{2+} and Mn^{2+} achieved maximum removal efficiencies of 85% and 80%, respectively, at the lowest adsorbent dosage of 1g. In contrast, at the highest adsorbent dosage of 5g, the removal efficiencies dropped to 25% and 20%, respectively (see figure17). Hence, as the concentration of biochar increases, the number of adsorption surface pores also increases, leading to an improvement in the percentage removal of heavy metals [47].

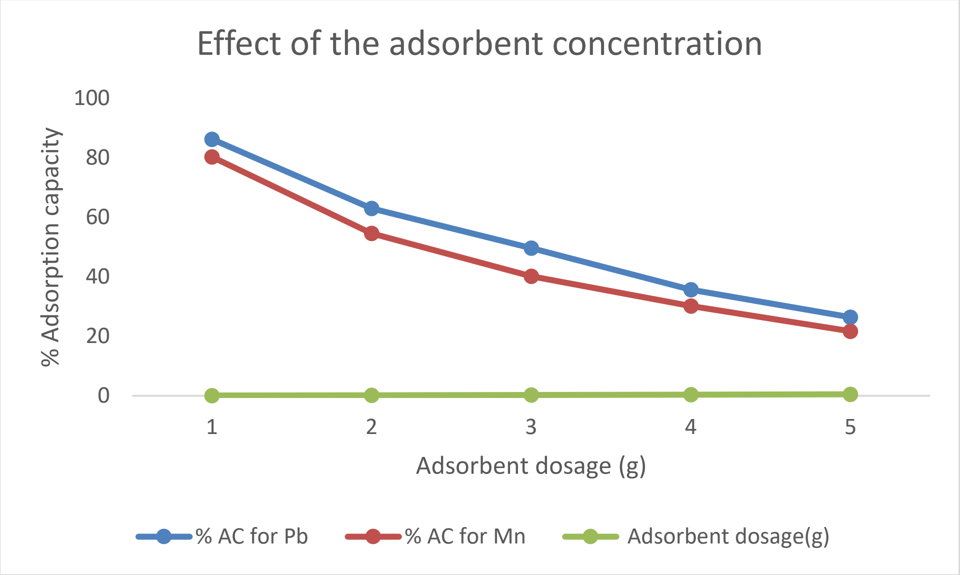


Figure 16:Effect of Adsorbent Dosage on the Efficiency of Heavy Metal Removal

7.3.4. Effect of the contact time on the % removal of the heavy metal

The contact time helps determine the optimal duration for achieving the highest percentage removal of heavy metals. In this study, the effect of contact time on the adsorption process was examined, with a duration ranging from 20 to 60 minutes [48]. By analysing the impact of varying contact times, the highest percentage removal was observed at 50 and 60 minutes, with Pb^{2+} and Mn^{2+} achieving 87% and 85% removal and also lowest % removal achieved at 20 min, respectively[26](see figure18). The study showed that fluctuations in adsorption efficiency occurred with changes in contact time, as illustrated in the chart below. Therefore, to achieve higher removal efficiency of heavy metals, a longer contact time is required to enhance the availability of adsorbent surface pores, allowing more heavy metals to be effectively adsorbed [49].

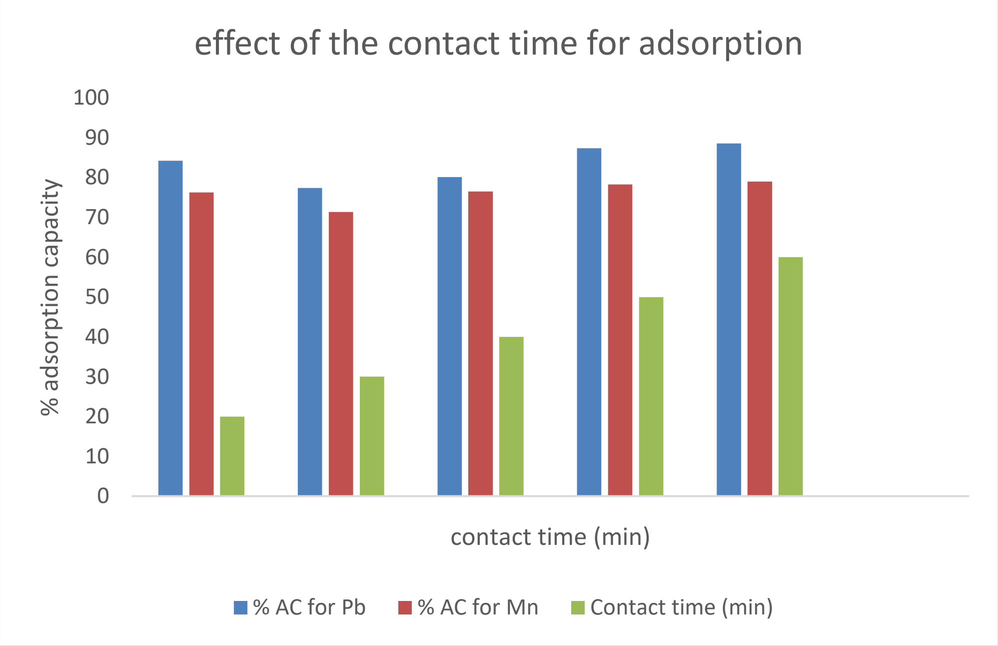


Figure 17:Effect of the contact time on the % removal of the heavy metal

8.0: Data Analysis and Interpretation

T-Table: Mean and Standard Deviation of Heavy Metals (ppm)

Heavy Metal	Mean (ppm)	Standard Deviation (ppm)	Min	Max	Detected in (samples)
Cadmium (Cd)	0.364	0.248	0.1045	0.8965	11
Nickel (Ni)	0.211	0.133	0.0130	0.4591	18
Lead (Pb)	0.472	0.244	0.0911	0.9206	20
Iron (Fe)	0.297	0.221	0.0140	0.8912	17
Manganese (Mn)	0.299	0.186	0.0990	0.8209	17
Copper (Cu)	1.419	0.849	0.1057	3.5829	17
Zinc (Zn)	0.768	0.607	0.1563	2.0672	16

Cadmium (Cd): Moderate presence with high variation, especially from garage and industrial zones Nickel (Ni): Relatively low mean but consistently found in almost all samples Lead (Pb): High variation and concern due to toxicity; detected in all types of samples Iron (Fe): Detected consistently but mostly in moderate to low amounts. Manganese (Mn): Similar trend to Iron, but with notable highs in Mpazi and Gatsata. Copper (Cu): Very high average and widest range due to industrial waste.

The wastewater is highly contaminated, particularly from industrial and garage areas. These samples exceed safe limits for drinking or irrigation. Cadmium (Cd), Lead (Pb), and Copper (Cu) levels in some samples exceed recommended safe levels. Consumption of contaminated vegetables or fish may lead to long-term health issues, including kidney damage, neurological disorders, and other toxic effects. The source of high pollution appears to be industrial discharge and improper waste management, several environmental and food samples contain notable levels of heavy metals, especially Cu, Pb, and Zn. Industrial activities are a major contributor [13]. There is an urgent need for monitoring and pollution control, especially in wastewater discharge zones. Public health awareness and treatment of water sources are highly recommended.

Table: Geoaccumulation Factor (Igeo) Analysis

Heavy Metal (HM)	Igeo Value	Mean	Std. Deviation (SD)	T-value
Pb (Lead)	0.4512	0.6295	0.2923	1.544
Mn (Manganese)	0.6411			
Zn (Zinc)	0.4170			
Ni (Nickel)	0.6255			
Cu (Copper)	1.0810			
Fe (Iron)	1.0750			
Cd (Cadmium)	0.1185			

The mean Igeo = 0.63, which indicates moderate pollution based on Müller's classification. The t-value = 5.70 suggests a statistically significant deviation from zero (i.e., from unpolluted condition), at $p < 0.01$, meaning there is clear evidence of contamination. Copper (Cu) and Iron (Fe) have the highest Igeo values, indicating they are the most accumulated and potentially the most concerning. There is moderate to significant pollution from heavy metals, particularly Cu, Fe, and Ni. The statistical significance of the Igeo values confirms that environmental contamination is real and measurable in the studied area[20]. The mean PLI is below 1, suggesting low overall pollution.

The negative t-value shows that the pollution levels are significantly less than the pollution threshold (PLI = 1) at ~95% confidence level. Therefore, most metals pose low environmental risk, with Pb, Mn, and Fe needing closer monitoring due to their PLI being near or slightly above 1.

Table 15:PLI data analysis

Metal	n	Mean (ppm)	SD	t-value	Significance
Cd	4	0.0725	0.0418	2.69	*Moderate*
Pb	5	2.278	1.430	3.47	Significant
Mn	5	1.64	0.954	8.37	Highly Significant
Ni	5	1.346	0.834	7.76	Highly Significant
Fe	5	2.434	1.614	5.86	Highly Significant
Cu	5	1.44	0.768	8.63	Highly Significant

T-value calculated with reference mean = 0.05 ppm and appropriate degrees of freedom (n-1). Cd has a moderate t-value — slightly elevated, but the sample size is small. All other metals (Pb, Mn, Ni, Fe, Cu) have statistically significant or highly significant values above background/reference levels (0.05 ppm). The RHB adsorbent has extremely low values, suggesting effective heavy metal reduction, suggesting a general tendency toward moderate bio-accumulation [27]. However, the t-test is not statistically significant (t = 1.34), meaning the average BAF is not significantly higher than 1 based on this small sample. Notably, Ni and Zn show very high individual BAF values, indicating potential ecological and food chain risks, even though the overall mean is not significant. Mean ER = 29.37, significantly below the threshold of 40, which supports the conclusion of overall low ecological risk. The t-value is large and negative, meaning the difference from 40 is statistically significant. Most metals fall under low or moderate risk, with only Zn contributing to high ecological pressure. The mean BCF (1.41) suggests moderate to high bio-accumulation overall. The standard deviation is high, showing large variability between metals. The t-value = 1.114 is not statistically significant at 95% confidence, meaning that while some metals (Ni, Zn, Mn) show high BCFs, the overall bio-accumulation is not conclusively above the danger threshold (BCF = 1) for all metals. The average BCF > 1, indicating moderate bio-accumulation risk overall[19]. However, the t-value = 0.74 suggests the result is not statistically significant at $p < 0.05$, meaning some metals bio-accumulate, but the group overall is mixed. Cadmium (Cd) shows the highest BCF (3.30), which is concerning for food safety. Pb, Cu, and Fe also exceed 1, meaning they accumulate in fish tissue, posing ecological and human health risks [27].

5. CONCLUSION AND RECOMMENDATION

5.1. CONCLUSION

This comprehensive study assessed the concentration of heavy metals across key environmental matrices wastewater, sediments, vegetable crops, and aquatic organisms within the Nyabarongo and Nyabugogo valleys. The primary objective was to inform ecological decision-making and lay a foundation for future environmental management strategies. The results indicated that the levels of lead (Pb), manganese (Mn), and iron (Fe) mostly remained within acceptable limits, suggesting that the environment is relatively unpolluted and supportive of both aquatic life and human health. When compared with findings from regional, national, and international studies, the heavy metal concentrations in the study area were notably lower. These results provide meaningful insights; however, the study acknowledges several limitations, including its restricted geographic scope, the absence of seasonal variation analysis, and the lack of investigation into the chemical speciation of metals, an important factor in determining bioavailability and toxicity. Despite moderate ecological risks, particularly to aquatic life, no substantial negative impacts were detected in sediment quality or agricultural produce such as vegetables. The health risk assessment further affirmed that all evaluated metal concentrations remain below harmful thresholds for all age groups analysed, indicating no immediate concern for public health. Overall, the findings highlight the current status of heavy metal contamination in the Nyabarongo and Nyabugogo river systems, including Mpazi a major wastewater discharge site. This study highlights the critical importance of ongoing environmental monitoring and strict enforcement of regulations to mitigate heavy metal pollution. Furthermore, it advocates for proactive and adaptive policy development to ensure the sustainable use and protection of aquatic ecosystems, ultimately safeguarding ecological balance, public health, and food security

5.2.RECOMMENDATION

To ensure the long-term environmental and human health protection, through this study underscores the urgent need for the sustained monitoring of the heavy metal’s contamination in aquatic and terrestrial ecosystem; by implementing the stricter regulations to control heavy metal deposition from the industrial and domestic sources, Adaptive and proactive policies are essential to promote the sustainable use and conservation of aquatic species. This study offers important insights for environmental monitoring and management, emphasizing the need for forward-looking actions to protect ecological integrity, human health, and food security. It is recommended that the Rwanda Environmental Management Authority (REMA) and the Ministry of Infrastructure (MoI) establish robust control mechanisms for the discharge of industrial wastewater, garage effluents, and domestic wastewater. Special attention should be given to waste streams containing heavy metals. Furthermore, Effective treatment methods must be applied to eliminate heavy metals and other contaminants before releasing effluents into the environment. This initiative will help prevent environmental contamination, protect aquatic ecosystems, and safeguard public health.

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