

**DETERMINATION OF ENVIRONMENTAL
CONTAMINATION OF BALLOKI HEADWORKS
USING FISH AS BIOINDICATORS**



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By

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RESEARCH COMPLETION CERTIFICATE

It is certified that Ms. Mahnoor Butt, Ms. Fizza Hafeez and Ms. Nade Ali of BS Hons. (Session 2019-2023), Department of Zoology have carried out this research work entitled “**Determination of Environmental Contamination of Balloki Headworks Using Fish as Bioindicators**” under my supervision.

It is assured that their research work is original and has not yet been published anywhere else.

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Dated: 16th June 2023



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ABSTRACT

Fishes are aquatic chordates, known to inhabit the freshwater and marine ecosystem for millions of years. They come in a wide variety of sizes, shapes, and colors and are an important source of food for humans and other animals. The aim of this research was to determine the concentration and distribution of heavy metals in gills, skin and muscles of fish. For this purpose fish samples were collect from Balloki Headworks. For heavy metal detection in the samples of skin, gills and muscle tissues of fishes were burn to ashes in the muffle furnace at 600°C for 2 hours respectively and then acid digested with Nitric and Sulphuric acid in (1:1 ratio). Atomic Absorption Spectrophotometer was used to analyze the digested samples after filtering them to remove any solid particles. The results obtained were compare with standard stock solution values. The analyzed values were then matched with the standard permissible limits recommended by World Health Organization. Experimental results further showed that all the studied metals Cadmium, Chromium, Nickel, Copper, Cobalt, and Manganese were present in excessive amount in gills, skin, muscle tissues and water samples of Balloki Headworks and value of Zinc is negligible in gills, skin, muscle tissues and water samples of Balloki Headworks. The reason for the remarkable low observed concentration of Zinc may be its least solubility in ground water and formation of an insoluble shielding layer. Fish are excellent pollution indicators as they accumulate toxins and pollutants from their environment in their tissues. Heavy metal analysis in fish is an important process to ensure food safety and protect public health. Balloki Headworks is a major source of drinking water and use for agricultural practices in nearby areas like Lahore and Kasur. This research will help to identify presence of heavy metals in water of Balloki Headworks and assess the risks to human health, aquatic organisms and the environment.

LIST OF ABBREVIATIONS

AAS	Atomic Absorption Spectroscopy
Cd	Cadmium
Co	Cobalt
Cr	Chromium
Cu	Copper
kg	Kilogram
Pb	Lead
ml	Milliliter
Mn	Manganese
Ni	Nickel
WHO	World Health Organization
Zn	Zinc

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CHAPTER 1

INTRODUCTION

Water is essential for all living things and is vital for maintaining life on Earth. It is used for drinking, irrigation, hygiene, industrial processes, and energy generation. Water also plays a crucial role in regulating the Earth's temperature and in preserving the natural environment through its role in the water cycle. It is an essential resource for agriculture, and its scarcity can lead to food insecurity and malnutrition. Access to clean water is a basic human right, yet millions of people lack access to this life-sustaining resource. Protecting and preserving water sources is crucial to maintaining a healthy and sustainable planet. Water is essential for the survival of aquatic life. It provides a habitat, serves as a means of transport, and supports all metabolic processes. Aquatic plants and animals depend on water for photosynthesis, respiration, and reproduction. The water's temperature, chemical composition, and oxygen levels all play critical roles in supporting aquatic ecosystems and determining the types of species that can thrive in a particular environment. Maintaining the quality and quantity of aquatic habitats is important to preserve biodiversity and sustain the delicate balance of these ecosystems.

Fishes are a diverse group of cold-blooded aquatic vertebrates that have been in existence for over 500 million years. They are found in almost every aquatic environment on the planet, from freshwater rivers and lakes to saltwater oceans and coral reefs. Fishes are known for their gills, fins, and scales, which are adaptations that help them thrive in their aquatic habitats. Fishes come in a variety of shapes and sizes, with the smallest being the Paedocypris fish that can fit in the eye of a needle, and the largest being the Whale Shark, which can grow up to 60 feet in length. They are also known for their colorful appearance, with many species having bright, vibrant patterns and hues. Fishes play an important role in the ecosystem, serving as both predators and prey. They are an important food source for many species of birds, marine mammals, and other fish. Additionally, some species of fish, such as the Sardine, play a crucial role in the ocean's food chain, serving as a source of food for larger predators like whales and seals. Humans have

been using fishes as a source of food for thousands of years, and many species of fish are commercially harvested for this purpose. Overfishing, habitat destruction, and pollution have led to a decline in fish populations, and many species are now considered threatened or endangered. Conservation efforts are underway to protect fish populations and restore their habitats (1).

Fisheries in Pakistan play an important role in the country's economy, providing livelihoods to thousands of people and contributing to food security. Pakistan has a long coastline along the Arabian Sea and the Bay of Bengal, which provides favorable conditions for fishing and aquaculture activities. The major fish species caught in Pakistan include sardines, mackerel, and catfish. However, overfishing, illegal fishing, and pollution are major challenges faced by the Pakistani fishing industry. Overfishing has depleted fish stocks, leading to reduced catches and impacting the livelihoods of fishermen. Illegal fishing practices, such as the use of banned fishing gear and fishing in protected areas, also pose a threat to fish stocks and marine ecosystems. To address these challenges, the government of Pakistan has taken steps to improve the management of the fishing industry. This includes implementing regulations to control overfishing, enforcing penalties for illegal fishing, and promoting sustainable aquaculture practices. The government has also introduced programs to support the development of the fishing sector, such as providing loans and training to fishermen, improving access to markets, and investing in infrastructure such as ice plants and fishing harbors. These efforts aim to improve the competitiveness of the Pakistani fishing industry and contribute to sustainable development in coastal communities. Fisheries in Pakistan are an important source of food and livelihoods, but face significant challenges that must be addressed to ensure the long-term sustainability of the industry. The government's efforts to improve the management of fisheries and support the development of the fishing sector are positive steps towards a more sustainable future for Pakistani fisheries (2).

Rahu fish is a species of predatory fish commonly found in freshwater rivers and lakes across South and Southeast Asia. This species is known for its unique

appearance, with a distinct elongated head, long dorsal and anal fins, and a long, slender body that is usually gray or brown in color. Rahu fish is also considered to be a highly prized food source in many cultures and is often consumed either as a fresh dish or dried and salted as a form of preservation. In traditional medicine, various parts of the Rahu fish are used to treat a wide range of health conditions, including headaches, digestive issues, and joint pain. Some people also believe that consuming Rahu fish can bring good luck and prosperity. In addition, this species is considered to be an important symbol in Hindu mythology, representing the head of the demon Rahu, who was beheaded by Lord Vishnu. In terms of fishing, Rahu is known for being a challenging species to catch, requiring specific techniques and gear. The fish are typically found in deep water, and anglers typically use lures or live bait to entice them. Due to overfishing and habitat loss, the populations of Rahu fish in some regions have declined in recent years, leading to conservation efforts and regulations aimed at protecting this species. Rahu fish is a fascinating species that holds both cultural and economic importance in many parts of the world. Despite challenges in fishing and conservation efforts, this species continues to be enjoyed as a delicious food source and treasured in traditional beliefs and practices (3).

Thalla fish, also known as Barramundi or Asian Seabass, is a species of fish found in freshwater and saltwater environments in Asia. It is a popular food fish due to its delicious flavor and firm texture. The fish is omnivorous and feeds on small fish, crustaceans, and plants. Thalla fish is a highly valued species in the aquaculture industry as it is fast growing and can be raised in both freshwater and saltwater ponds. The fish has a high market demand and is widely consumed in Southeast Asian countries, Australia, and India. Thalla fish has a high level of omega-3 fatty acids, making it a healthy addition to the diet. The fishing industry has had a positive impact on the economy of many countries, providing livelihoods for local communities and boosting export earnings. Thalla fish is a versatile and delicious species that is widely cultivated and consumed, making it an important part of the global seafood industry (4).

Barilius is a genus of freshwater fish belonging to the family Cyprinidae, commonly known as mountain stream fishes. They are native to the fast-flowing rivers and streams of South and Southeast Asia, including countries such as India, Pakistan, and Bangladesh. Barilius species are small in size, typically reaching a length of only 6-12 cm. They have an elongated, slender body shape with a slightly flattened head and a slightly upturned mouth. The dorsal and anal fins are long and angular, and the tail fin is forked. Barilius fish are hardy and adaptable to a range of water conditions, making them popular with aquarists. They are known for their vibrant, eye-catching colors, with different species exhibiting a range of patterns and hues, from silvery-blue to yellow and green. They are omnivores, feeding on a variety of small crustaceans, insects, and plant matter. In the wild, Barilius species are an important source of food for a variety of predators, including larger fish, birds, and mammals. They are also important for local commercial and subsistence fishing, providing a valuable source of protein for local communities. Despite their popularity and importance, some Barilius species are considered threatened or endangered, largely due to habitat destruction and degradation caused by human activities such as dam construction, deforestation, and pollution. Conservation efforts are underway to protect these species and their habitats, including the introduction of captive breeding programs and the creation of protected areas (5).

Fishes are often considered as bio indicators of water pollution. They are sensitive to changes in their environment and can be affected by pollutants such as heavy metals, oil spills, and chemicals. Fish can absorb these pollutants through their gills and skin, and their tissue can serve as a measure of the pollution levels in the water. Fish also play a key role in the food chain and the presence of contaminants in their tissues can have ripple effects throughout the ecosystem. By monitoring the health and populations of fish, scientists and environmental managers can gain insight into the overall health of a waterway and its ability to support aquatic life. Additionally, fish can help identify specific sources of pollution and inform cleanup and management efforts (6).

Heavy metals in water can come from natural sources or human activities such as mining, industrial processes and the use of pesticides. These metals can be toxic to humans and animals and can cause serious health problems if consumed in high concentrations. Some common heavy metals found in water include lead, mercury, cadmium, and arsenic. It is important to regularly test water sources for heavy metal contamination and take steps to remove them if necessary. This can include treatment with activated carbon, reverse osmosis, or ion exchange resin. It is also important to use caution when using products that contain heavy metals and to properly dispose them to prevent contamination of water sources (7).

Cadmium is a chemical element with the symbol Cd and atomic number 48. It is a silvery-white metal that is soft and ductile. Cadmium is primarily obtained as a by-product of zinc production and is widely used in batteries, pigments, coatings and as a stabilizer in plastics. It is highly toxic and exposure to cadmium can cause health problems, including kidney damage and osteoporosis. Cadmium is also a persistent environmental pollutant, as it does not break down in the environment and accumulates in food chains, leading to potential health risks to animals and humans. Despite its harmful effects, cadmium continues to be used due to its unique properties and versatility in various industrial applications (8).

Chromium is a chemical element with the symbol Cr and atomic number 24. It is a hard, brittle metal that is used for making steel alloys, plating metals, and producing pigments. The element has many industrial uses due to its corrosion-resistant properties and its ability to form thin, hard coatings on metal surfaces. Chromium is also an essential trace element in the human body, playing a role in glucose metabolism and insulin regulation. However, exposure to high levels of chromium can be toxic, leading to health problems such as skin irritations, respiratory issues, and cancer (9).

Zinc is a chemical element with the symbol Zn and atomic number 30. It is a metal that is essential for human health and is commonly found in foods such as red meat, poultry, and seafood. It plays a role in many biological processes such as DNA synthesis and protein metabolism. Zinc is also used in many industrial applications,

such as galvanization (protecting iron from corrosion) and as a component of alloys. Zinc oxide is widely used in sunscreens and other cosmetic products due to its ability to block ultraviolet (UV) light. Zinc deficiency can lead to various health problems, and excessive intake can cause side effects such as nausea and vomiting (10).

Manganese is a chemical element with the symbol Mn and atomic number 25. It is a silvery-grey metal that is widely distributed in nature. Manganese is an essential component of steel, being used as a purifying agent and strengthening component. It is also used in the production of aluminum alloys, batteries, and in the manufacturing of fertilizers and ceramics. Manganese is important for human health as it is involved in several enzymatic reactions and the metabolism of proteins, carbohydrates, and cholesterol. However, excessive manganese intake can lead to toxicity and adverse health effects. Manganese is primarily mined in countries such as South Africa, Australia, and China (11).

Copper is a reddish-brown metal that is highly conductive of heat and electricity. It is the third most abundant element in the Earth's crust, and is widely used in electrical wiring, plumbing, coins, and jewelry. Copper is also an essential nutrient for all living organisms, and is a key component in enzymes involved in energy production and oxygen transport. The metal can be extracted from sulfide and oxide minerals through a process called smelting. Copper is also widely used in alloys, such as brass and bronze, for its strength and durability (12).

Cobalt is a metallic element with the symbol Co and atomic number 27. It is a hard, lustrous, and silvery-blue metal that is highly valued for its magnetic, wear-resistant, and high-temperature properties. Cobalt is commonly used in the production of batteries, magnets, jet engines, and cutting tools. The largest producers of cobalt are the Democratic Republic of the Congo, followed by China, Russia, and Canada. Cobalt is also a critical component in the production of lithium-ion batteries, which are widely used in consumer electronics and electric vehicles. However, the mining of cobalt often involves unethical labor practices and

environmental degradation, leading to calls for increased regulation and greater transparency in the cobalt supply chain.

Nickel is a silvery-white metal with a shiny appearance and is a chemical element with the symbol Ni. It is a transition metal with a relatively high melting and boiling point, making it ideal for use in high-temperature applications. It is highly resistant to corrosion and is commonly used in alloys, such as stainless steel, to enhance their strength and resistance to corrosion. Nickel is widely used in coins, batteries, and electroplating. It is also used in the production of various other metal alloys, catalysts, and magnets. Despite its wide range of uses, nickel can be toxic in high concentrations and prolonged exposure to nickel compounds can cause skin irritation and lung problems (13).

Balloki Headworks is a dam located on the River Ravi in Pakistan, near the city of Kasur. It was completed in 2012 and serves as a source of irrigation and hydroelectric power generation. The dam has a capacity to store nearly 1.1 million acre-feet of water and generate 132 MW of electricity. It is a key part of the Indus Basin Project and helps to regulate the water supply for agriculture in the region. The Balloki Headworks diverts water from the River Sutlej to supply nearby communities with drinking water, hydropower generation, supporting the local economy and livelihoods. The dam has also helped to mitigate the risk of floods in downstream areas and provide a stable water supply for communities. Overall, Balloki Headworks is a critical infrastructure project that has brought significant benefits to the local area (14).

This research aims to investigate the presence and levels of heavy metals in fish and to evaluate the potential impacts of heavy metal contamination on fish health and reproduction.

1.1 STUDY AREA

The study areas was comprise of Balloki Headworks Districts Punjab, Pakistan.

Balloki Headwork's

Initially built on the River Ravi in 1913, the Balloki Headwork was subsequently applied to connect with the Sulemanki Headwork in 1956 in order to supply the River Sutlej's canal system at the time of year when water is scarce. The Balloki Headworks are about 65 kilometers from Lahore (district Kasur) in Punjab (Lat. 31029/ N, Long. 73085/ E). Lahore and the surrounding industrial districts pour their unprocessed urban and commercial garbage straight into the River Ravi. Numerous studies, including those on waterbodies, metal toxicity, and heavy metals, were carried out to determine the extent of water contamination at different Balloki Headworks locations. The trout population in the water habitats provides additional evidence of contamination levels. Include over 56 native and foreign fish species have been recorded in Balloki Headworks. Balloki Headworks is one of the primary industrial fishermen places of the River Ravi. Due to the rising toxicity in the River Ravi over the recent years, the number of such fish has severely decreased (15).



Figure 1.1: Map of Balloki Headworks

AIM AND OBJECTIVE

The aims of the research was:

- to assess the presence of heavy metals (Cadmium, Chromium, Nickel, Zinc, Cobalt, Copper, and Manganese) in the muscles, skin and gill of fish found in Balloki Headworks Punjab, Pakistan.

RATIONALE

Fish play significantly important role in the maintenance of our ecosystem. They have vital role in food chains as predators and prey. The determination of environmental contamination in muscles, gills and skin of fish species presented in selected study area of Balloki headworks would provide future researchers knowledge and the study would be helpful in providing the baseline information to the researchers regarding the toxicity of heavy metals in Balloki Headworks, using fish as pollution indicators.

CHAPTER 2

LITERATURE REVIEW

SA Mustafa *et.al* in this study detect the level of Lead and Cadmium in the water of fish tissue. The 100 sample of fish and water collected from three different site of the River Tigris. The atomic absorption spectrophotometry technique was used to detect the level of Pb and Cd in samples. The concentration of these metal highest in liver and gill and above the acceptable limit of WHO. The pollution in the River increasing day by day and effect the human health (16).

Ambedkar G *et al.* in this research study freshwater fish were collected from Kollidam Stream, Nagai. The fish organs dissected to analysis the level of metal .The degrees of heavy metals were detected by utilizing AAS. The outcome shows the copper had extreme levels followed by Zn<Cr<Pb<Cd. The most elevated level of copper was seen in liver tissue of *Saurida undosquamis* and the lowest degree of zinc was seen in muscle tissue of *Saurida undosquamis*. The level of heavy metals amassed by the five freshwater fish species may be because of the expansion agricultural and some other activity (17).

Olowu RA *et al.* in this research determined heavy metals in fish tissues, water and sediment. The samples were examined for the presence of zinc, nickel and iron utilizing atomic absorption spectrophotometer. The remains of sediments were found to have higher amount of iron for fishes and water. Concentrates on the various pieces of the fish uncovered higher convergences of 4.00 µg/g Nickle in head of tilapia fish followed by 2.40 µg/g Nickle in the digestive tract of feline fish. The most elevated centralization of Zinc was identified on the top of the feline fish while the least was kept in tilapia head. The convergence of Zinc in the water is inside the cutoff points allowed by the Lagos State Ecological Assurance Organization (LASEPA) of 1.0 mg/L Zn set for water (18).

Salam MA *et.al* in this study analyze the level of heavy metals in sediment, water and three fish species. The sample collected from the different area of jajmau which is

important fishery hatchery. The heavy metal, Nickel, Iron, Zinc, Lead, Cadmium and Chromium were evaluated in the organ of fish. The bottom feeder fish show the highest concentration of heavy metals than surface feeder. The result of this analysis is in order of $Co < Cu < Zn < Cd < Ni < Pb < Cr < Fe$. Sediment show high concentration of Iron and Chromium. It suggested that the river should be monitored properly for the protection of human (19).

Anjum *et al.* determine the amounts of heavy metals (zinc and lead) in the muscle tissues of *Labeo rohita* found in local streams and on farms. Lahore Siphon (upstream) and Balloki Headworks were the two places along the Ravi River where *Labeo rohita* was found (downstream). Furthermore, samples were collected from privately owned and operated fish farms in Pakistan's Lahore District. The fish muscles were digested, filtered, and their Zn and Pb concentrations were determined. The concentration of heavy metal ions differed significantly across two sites along the Ravi River, as well as between government-run and privately-owned fish farms. Balloki Headworks had significantly higher Zn and Pb concentrations, whereas muscle samples from private fish farms showed the lowest Zn and Pb concentration. Furthermore, heavy metal concentrations in *Labeo rohita* muscles from Balloki Headworks were found to be significantly higher than the WHO/FAO permissible values (20).

Zaghloul GY *et al.* detect the heavy metal in organ of fish is the serious problem. Due to the presences of heavy metals in fields and sewage wastewater which threaten aquatic life. Five commercial fish samples collected from the Hurgada to check the health risk with low and high consumption. To analysis heavy metal in organs of fish the technique of Atomic Absorption Spectrometer was used. The low value Cadmium, Iron, Zinc, Nickel and Copper were found in liver and gills. The study showed that the high intake of fish is harmful especially for children (21).

Zara *et al.* investigated the threats to human health posed by the Head Balloki fishery's heavy metal-polluted water. At six different places across Head Balloki, three water samples were collected for each Pre and Post Monsoon. At six separate

sites, fish were caught, and their gills and liver were removed for heavy metal analysis. This analysis discovered that the concentration of chromium Cr in water was higher than the content of nickel Ni. On the other hand, the concentration of Ni was higher than the concentration of chrome Cr in Liver $Ni > Cr$ whereas the concentration of Chrome Cr was higher in Labeo rohita fish organs Gills $Cr > Ni$ (22).

Mehar .S *et al.* discussed that marine life fish is the one who consume a large number of toxic and heavy metals in their body from which enter in human life and cause harmful effect. In this research the aim of study to analyze the heavy metal in the samples of fish tissue that collected from Karachi by using Atomic Absorption Spectrometer. The copper and chromium found high in stomach than muscles. The level of iron is low in skin than liver. The water and sediment detected to check the pollution and metal from the selected site. The result are shocking that pollution is greater in sediment, than in water and least in fish tissues. The selected site is highly polluted and disturb the food chain as well (23).

Stephen *et al.* in this study discussed that the River Ravi has been severely disrupted as a result of toxic industrial waste and household sewage discharges. The researchers discovered significant differences in six fish organs, including the gills, kidney, liver, skin, muscle, and scales at Balloki. Cu accumulation in each of the six fish organs varied substantially. The fish liver, kidney, and scales were the organs that gathered most of these metals. The metals dropped in the following order in the fish tissue: $Cu > Cr > Cd > Co$. Heavy metal concentrations were $Cu > Cr > Cd > Co$ in both river length and tributary waters. The metal toxicity of Catla's body organs and water was statistically significant in a favorable direction. The outcomes of this study indicated that Catla consumed metals present in the river system via water, sediments, and food, independent of their biological needs (24).

Ayanda *et al.* in this research study the level of a few heavy metals in four fish species *O.niloticus*, *M.electricus*, *P.obscura*, and *C.nigrodigitatus* in Ogun Stream, Nigeria. The fish species were collected and investigated for Manganese, Zinc, Lead,

Cadmium, Nickel and Zinc by using AAS. The outcomes showed different level of metals among the four fish species. Lead showed highest concentration in *O. niloticus* while Magnesium showed lowest concentration in *C. nigrodigitatus*. But in some cases Lead and Nickel showed highest concentration in muscles of *O. niloticus* and *P. obscura*. The concentration of heavy metal accumulated more liver and gills than the muscles. The difference in values were not critical among fish species. In this study the pollution status with heavy metals of studying site also depicted (25).

Canpolat *et al.* in this research study the degrees of cobalt, iron, manganese, cadmium, nickel, lead, copper, zinc, chrome, arsenic, mercury, calcium, and magnesium in the kidney, gill, liver, muscle, skin, and testicle of *C. umbla* got from Lake Hazar of Turkey were examined. The level of recognized heavy metals were the most elevated in the liver and the least in the muscle. Furthermore, it has been observed that the level of metals and minerals were viewed as changed in different tissues and organs. The measures of metals showed contrasts as per the age, length, weight, and sex gatherings of the fish. The outcomes matched with average value for weighty metals given by the WHO, Turkish Food Codex, PA and FAO to check if it effect humans (26).

Parvin sadeghi *et. al* in this research analyze the level of heavy metal like lead, copper and zinc in the tail fin, liver, gill and muscles tissues in fish sample collected from Oman sea. The heavy metal detected by using flame atomic, Absorption spectrophotometer. Liver show the significantly higher value than other tissues. The trend of concentration of metal in tuna is $Pb < Cu < Zn$. It Suggested that the metal intake for tuna is below tolerable range and it save for human health (27).

Tekin-Özan S *et al.* in this study analyze the concentration Pb, Cd, Zn, Mn, Cr, Fe and Cr in muscle, gill and liver of carp. During heavy metals analysis Chromium, Lead Cadmium are below the 0.03 ppm. The high concentration of metal observed in liver, gill and muscle. From summer to winter the concentration of heavy metal in carp increase and from autumn to spring it decreased. The study suggest that proper monitoring required for the wellbeing of human and aquatic environment. In order to prevent the pollution in the lake for future proper treatment of water needed (28).

Al-Ghanim KA *et al.* in this study analyzed the level of 10 heavy metals in the different organs of fish species. The samples were collected from four different sites. The greater significance value ($P < 0.01$) observed in fish species and accumulation of all metals in organs .The level of iron was generally significant than Zinc. The level of cadmium was observed lowest in all organs. The high concentration of metal observed in liver. Muscles had the concentration of all metals. It has been seen in this study the L. nebulosus species observed the highest concentration of all metal in each tissue or body (29).

Jabeen G *et al.* in this research study the level of toxic metals in fish. The samples collected from main fishing sites Ravi River, Baloki and barrage Sidhnai. The level of metals in the body organs of three fish species detected. The results showed that the concentration of heavy metal varies in fish of selected sites. The Aluminium and Zinc were observed high in samples that collected from three selected sites. Meanwhile the fish present at Sidhnai showed low concentration of heavy metals. Fish liver showed the high concentration of heavy metal during analysis, and less in other organs. Fish liver and kidney showed more accumulation of heavy metals and toxic pollutants (30).

Ebrahimpour M *et al.* in this study analyze the concentration of heavy metals in fresh water fish. They correlate the concentration of metal in different species. The highest concentration of heavy metal like Cadmium, Copper, Zinc, Lead, and chromium observed 1.96, 24.2, 49.6, 5.4 and 4.4 respectively in liver. While the lowest concentration heavy metals cadmium, copper, zinc, lead and chromium 0.21, 7.2,

19.4, 0.9 and 0.6 $\mu\text{g/g}$) found in the muscle. Experimental results obtained from the analysis of concentration of heavy metals in increasing order zinc < copper < lead < chromium < cadmium, similarly in the tissue liver < kidney < gill < intestine < muscle (31).

El-Moselhy KM *et al.* in this research measured the level of heavy metals in the liver, gills and muscles. The specimen collected from three regions in the Egyptian Red Ocean. The level of heavy metals change among fish species and organs. The muscles had the minimum level of all heavy metals. In this study the liver was the objective organ for Cu, Zn and Fe accumulation. Pb and Mn showed the high level heavy metals in the gills. Various types of fish showed variation for take-up, for age, geological conveyance and species. Concentration of metals in the current fish muscles were acknowledged by the global regulation and considered to be within permissible limits for human utilization (32).

Jenifer SA *et al.* in this research observed the concentration of heavy metals in Balloki Headwork's. The industrial disposed waste water in main supply system. Toxicity of metals such as iron, zinc, lead, nickel, and manganese to freshwater fish such as *Labeo rohita* is limited at Balloki. The toxicity of Acute *Labeo rohita* to fish has been studied. The substance's lethal toxicity and 96-hour LC50. Metals that are poisonous to fish the sensitivity of three fish species with different day's period to check the metal poisoning. Water temperature, pH, lead, dissolved oxygen, zinc ammonium, sodium, potassium, cobalt and carbon monoxide were all investigated in the experiment. This fish showed highest concentration for Nickel and low concentration for lead and zinc. When the metal level of the increased, the fish's ammonia excretion increased. However, with higher metal concentrations, the carbon dioxide level also increased (33).

CHAPTER 3 METHODOLOGY

Samples were collected from the Balloki Headworks and the following methodology was adopted:

3.1: MATERIALS AND METHODS

PLAN OF WORK

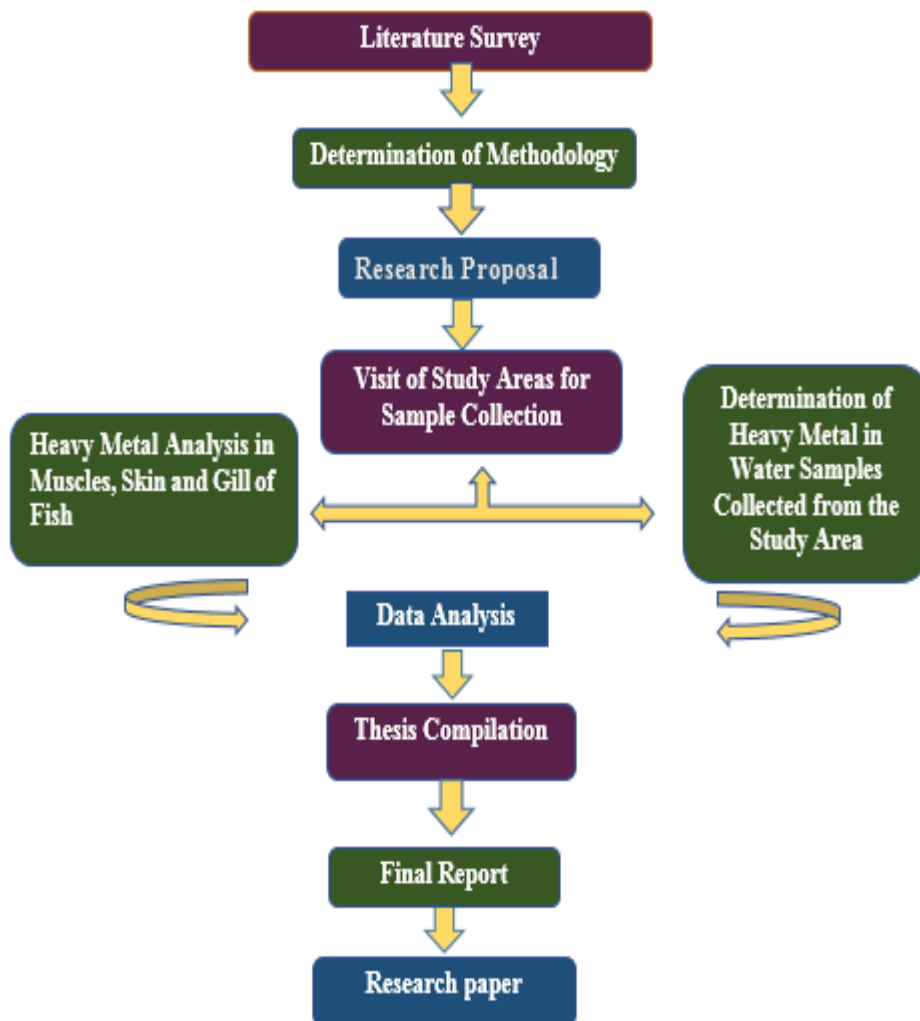


Figure 3.1: Flow sheet for the Plan of Work

3.2: MATERIALS REQUIRED

The following material were required for the research:

Table 3.1: Materials and Reagents that were Required for Sample Collection and Analysis

Sr. No	Materials/Reagents Required	Quantity/Specification
1.	Plastic Jars	50
2.	Glass Vials	100
3.	Test Tubes with Covers	100
4.	Test Tube Stands	6
5.	Dissection Kits	2
6.	Gloves	50
7.	Cotton	1 Big Roll
8.	Formalin (10%)	1000 ml
9.	Volumetric Flasks	20
10.	Beakers	20
11.	Measuring Cylinders	20
12.	Hot Plate	Scilogex
13.	Atomic Spectrophotometer	Absorption 210 A BUCK Scientific
14.	Plastic Bucket	2
15.	Muffle Furnace	<i>WiseTherm FHP Programmable Digital Muffle Furnace</i>
16.	Ceramic Bowls	10
17.	Nitric Acid (Concentrated)	200 ml
18.	Per chloric Acid	50 ml
19.	Pipettes	5
20.	Sulphuric Acid	50 ml

The following Identification were used for the samples:

Table 3.2: Collected Samples and their Identification Codes

Sr.no	Sample	Area	Identification Codes
1.	Sample 1	Balloki	B1
2.	Sample 2	Balloki	B2
3.	Sample 3	Balloki	B3
4.	Sample 4	Balloki	B4
5.	Sample 5	Balloki	B5
6.	Sample 6	Balloki	B6
7.	Sample 7	Balloki	B7
8.	Sample 8	Balloki	B8
9.	Sample 9	Balloki	B9
10.	Sample 10	Balloki	B10
11.	Sample 11	Balloki	B11
12.	Sample 12	Balloki	B12
13.	Sample 13	Balloki	B13
14.	Sample 14	Balloki	B14

Table 3.3: Water Samples and their Identification Codes

Sr.	Sample	Area	Identification Codes
1.	Balloki water 1	Balloki	BW1
2.	Balloki water 1	Balloki	BW2

3.2: SAMPLE COLLECTION:

Water samples was collected in thick plastic bottles from the selected site, the fish was randomly captured from water ways using bait irrespective of gender. The fish were transported to the Kinnaird College Laboratory in plastic buckets covered and half filled with water, for further analysis. Collected fish was first acclimatized to aerated glass tanks, containing dechlorinated tap water for two days prior to further analysis (34).

3.3: SAMPLE PREPARATION

3.3.1: Water Sample Digestion

In order to ensure the removal of organic impurities from the samples and thus prevent interference in analysis, the samples were digest with concentrated nitric acid. 10ml of nitric acid was added to 50ml of water sample in a 250ml conical flask. The mixture was then evaporated to half its volume on a hot plate after which it was allowed to cool and then filtered prior to heavy metal analysis (35).

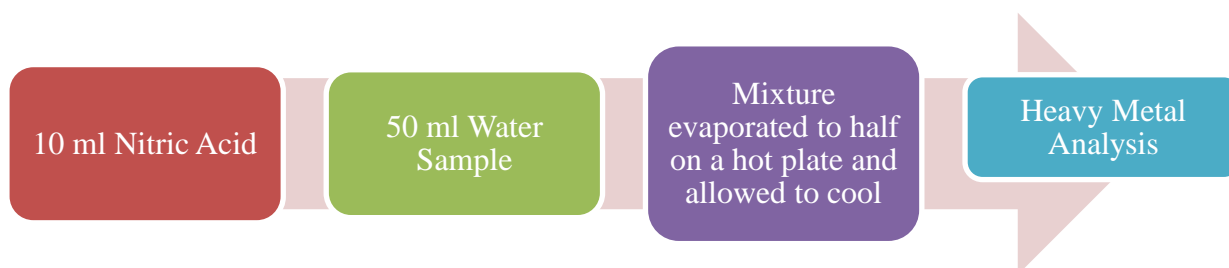


Figure 3.2: Steps for Water Sample Digestion

3.3.2: Organ Excision and Preservation

The fish was anesthetized before dissection. It was put on dissection tray and the scales were removed. The skin was cut from the midsection of the fish's anterior to 8 anal fin and muscles were cut from belly to operculum. The operculum and fin were removed to uncover gills. After remove skin and muscles the inside organs of fish's fully exposed. Muscles, skin and gill samples were then preserved in 10% buffered formalin till further analysis (36).



Figure 3.3: Steps for Organ Excision and Preservation of Fish

3.3.3: Organ Digestion

Fish muscles, gill and skin was burnt to ash at (600°C) for two hours in a muffle furnace and then acid digested (1HNO₃: 1H₂SO₄). The digested samples was then filtered to remove any solid particles present and then analyzed for the presence of heavy metals Zinc, Chromium, Nickel, Cobalt, Cadmium, Copper, and Manganese) using an Atomic Absorption Spectrophotometer (AAS) (34).

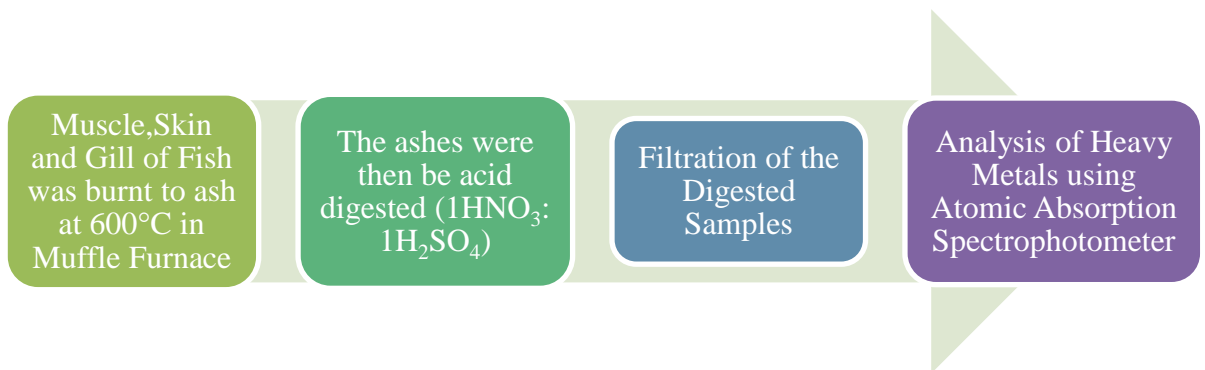


Figure 3.4: Steps Involved in the Organ Digestion of Fish

3.4: DATA ANALYSIS

The obtained values of heavy metals present in the water samples and the abovementioned organs were compared with the WHO Standards for Heavy Metals and the data was analyzed using graph pad prism.

CHAPTER 4

RESULTS AND DISCUSSION

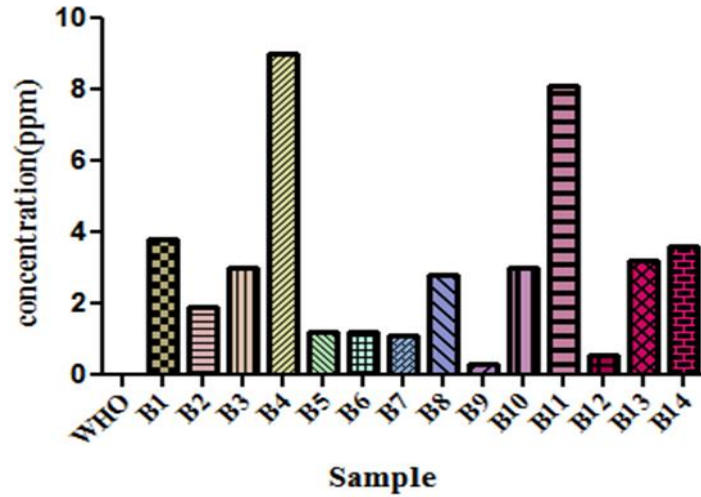
Samples were collected from the Balloki headworks and the following heavy metal concentration results were obtained.

4.1: Concentration of Heavy Metals in Muscle Tissues:

Table 4.1: Concentration of Heavy Metals in Muscle Tissue

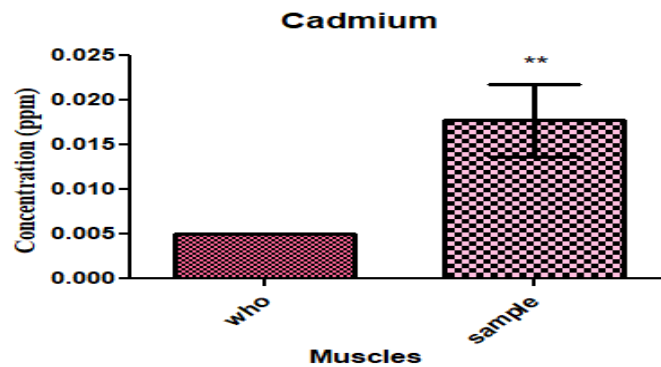
Sr. No	Sample	Cd (ppm)	Cr (ppm)	Ni (ppm)	Co (ppm)	Cu (ppm)	Mn (ppm)	Zn (ppm)
	WHO	0.005	0.1	0.070	0.01	1.0	0.400	5
1.	B1	0.010	0.018	0.008	0.018	0.043	0.014	0.098
2.	B2	0.019	0.011	0.009	0.027	0.052	0.009	0.070
3.	B3	0.041	0.029	0.008	0.023	0.062	0.011	0.060
4.	B4	-0.013	0.032	0.007	0.026	0.071	0.005	0.052
5.	B5	0.022	0.050	0.006	0.025	0.042	0.006	0.071
6.	B6	0.022	0.038	0.005	0.037	0.055	0.034	0.077
7.	B7	0.027	0.041	0.004	0.042	0.062	0.009	0.105
8.	B8	0.040	0.035	0.008	0.030	0.071	0.008	0.104
9.	B9	0.026	0.023	0.005	0.037	0.037	0.003	0.076
10.	B10	0.014	0.020	0.004	0.039	0.032	0.003	0.109
11.	B11	-0.009	0.025	0.003	0.042	0.029	0.004	0.067
12.	B12	0.025	0.018	0.004	0.059	0.040	0.005	0.104
13.	B13	0.013	0.017	0.009	0.053	0.049	0.006	0.076
14.	B14	0.011	0.029	0.003	0.040	0.052	0.009	0.077

4.1.1: Cadmium Concentrations in Muscle Tissue Samples of Fish



Graph 4.1.1.1: Concentration of Cadmium in Muscle Samples of Fish

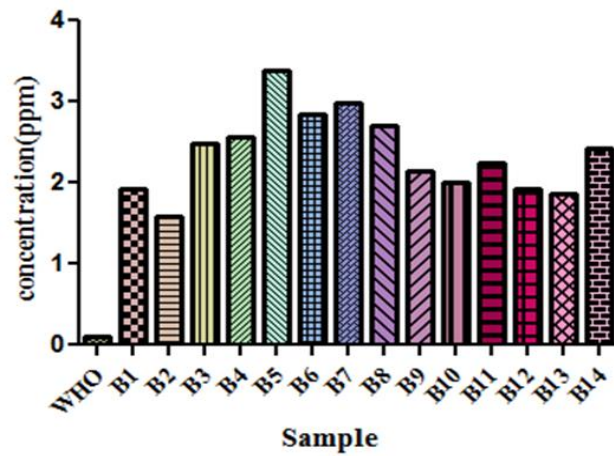
The graph showed that the concentration of Cadmium in muscles Samples of fish collected from Balloki Headworks which exceeded the WHO permissible limit which is 0.005 ppm. The sample B4 show the highest concentration of cadmium (37).



Graph 4.1.1.2: Values are expressed as Standard Error Mean (SEM)

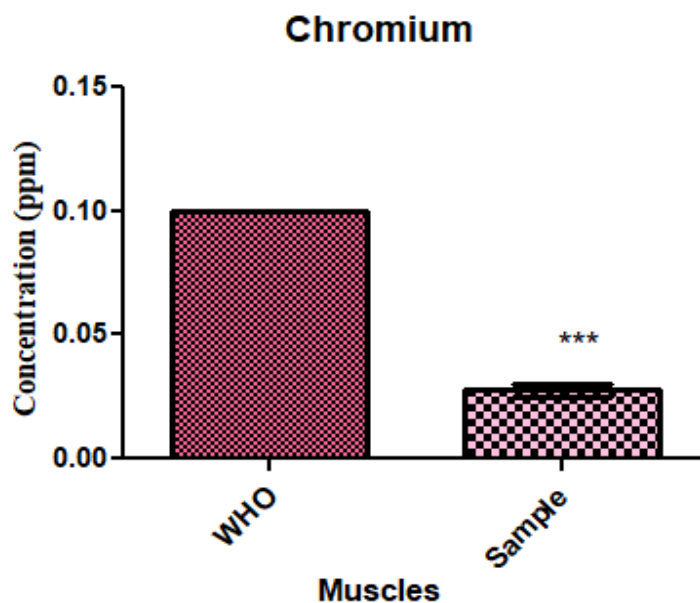
The graph showed the computative results of cadmium in all sample. The value expressed as Standard Error Mean.

4.1.2: Chromium Concentrations in Muscles Tissue Samples of Fish



Graph 4.1.2.1: Concentration of Chromium in Muscle Samples of Fish

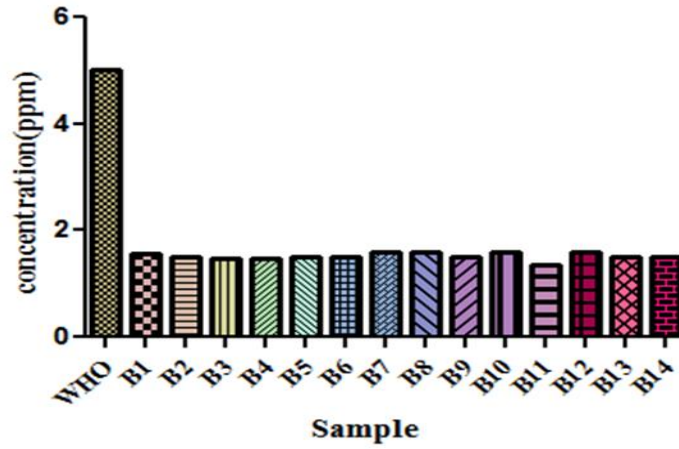
The graph showed that the concentration of Chromium in muscles Samples of fish collected from Balloki Headworks exceeded the WHO permissible limit which is 0.1 ppm (38).



Graph 4.1.2.2: Values are expressed as Standard Error Mean (SEM)

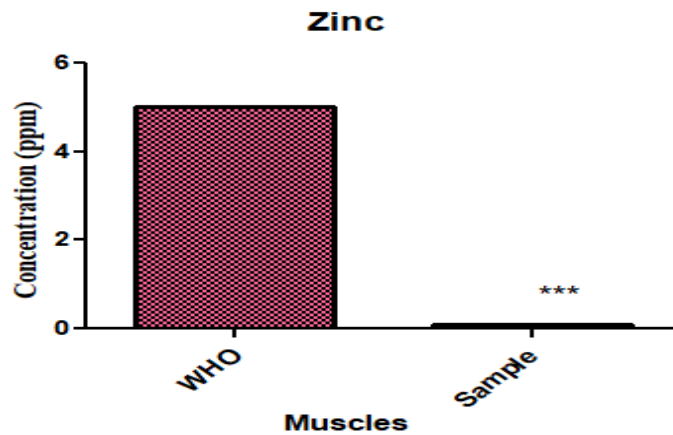
The graph showed the computative results of chromium in all sample. The value expressed as Standard Error Mean.

4.1.3: Zinc Concentrations in Muscle Tissue Samples of Fish



Graph 4.1.3.1: Concentration of Zinc in Muscle Samples of Fish

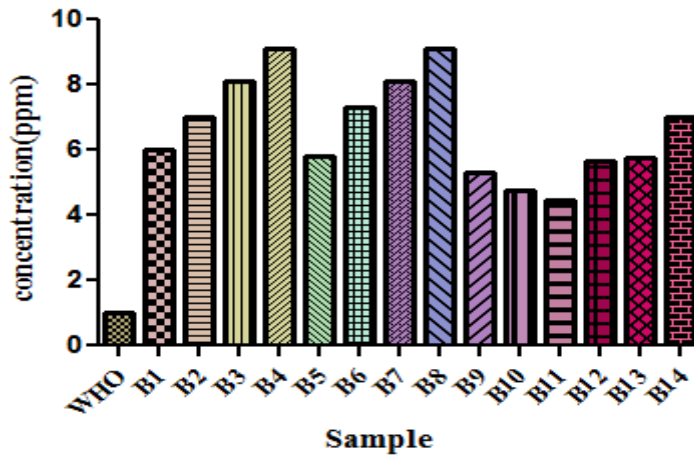
The graph showed the concentration of Zinc in the muscle samples of fish collected from Balloki Headworks. Zinc concentrations were observed to be negligible and did not exceed the WHO permissible limit of 5 ppm. (39).



Graph 4.1.3.2: Values are expressed as Standard Error Mean (SEM)

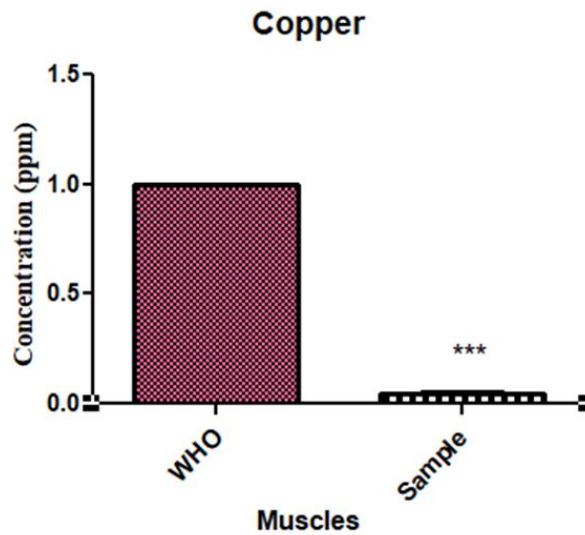
The graph showed the computative results of zinc in all sample. The value expressed as Standard Error Mean.

4.1.4: Copper Concentrations in Muscle Tissue Samples of Fish



Graph 4.1.4.1: Concentration of Copper in Muscle Samples of Fish

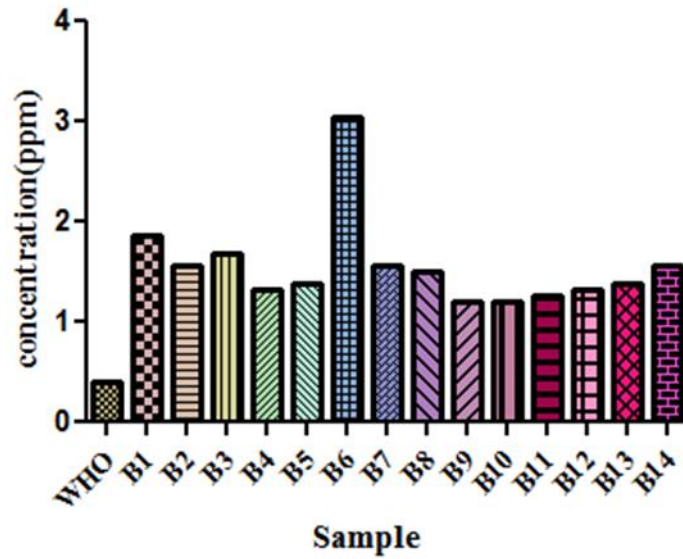
The graph showed the concentration of Copper in the muscle Samples of fish collected from Balloki Headworks exceeded the WHO permissible limit (37).



Graph 4.1.4.2: Values are expressed as Standard Error Mean (SEM)

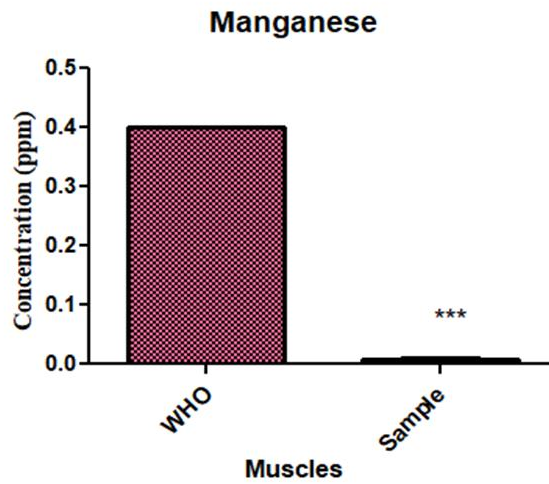
The graph showed the comparative results of copper in all sample. The value expressed as Standard Error Mean.

4.1.5: Manganese Concentrations in Muscle Tissue Samples of Fish



Graph 4.1.5.1: Concentration of Manganese in Muscle Samples of Fish

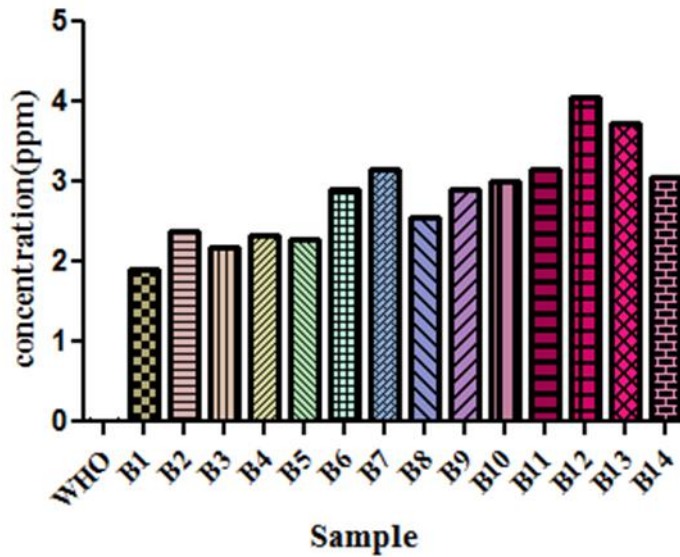
The graph showed the concentration of manganese in the muscle Samples of fish collected from Balloki Headworks exceeded the WHO permissible limit which is 0.4 ppm (38).



Graph 4.1.5.2: Values are expressed as Standard Error Mean (SEM)

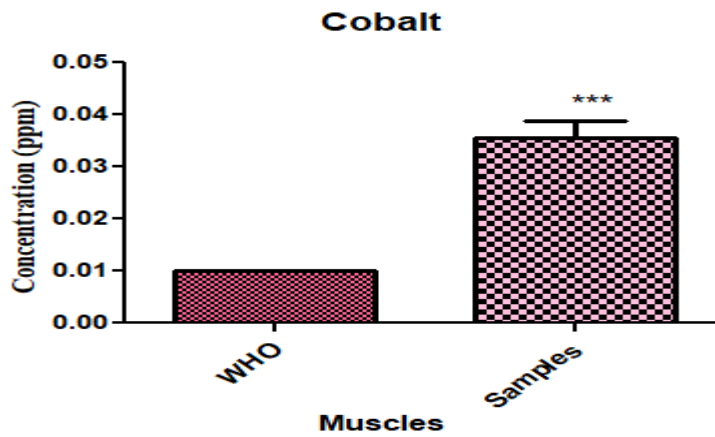
The graph showed the computative results of manganese in all sample. The value expressed as Standard Error Mean.

4.1.6: Cobalt Concentrations in Muscle Tissue Samples



Graph 4.1.6.1: Concentration of Cobalt in Muscle Samples of Fish

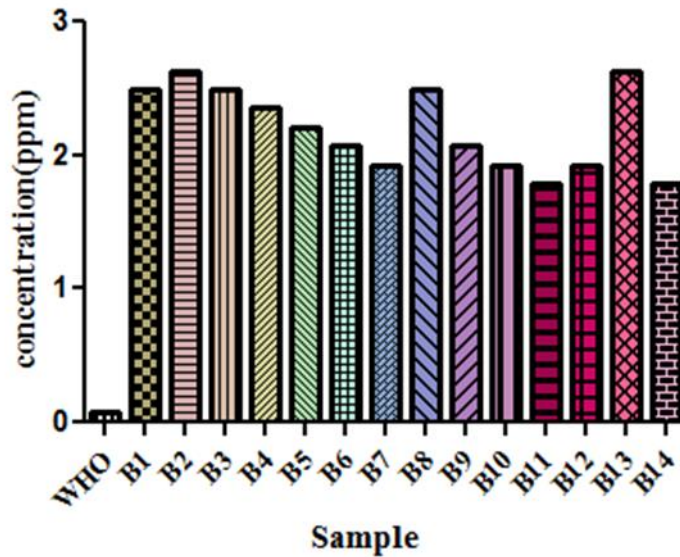
The graph showed the concentration of cobalt in the muscle Samples of fish collected from Balloki Headworks exceeded the WHO permissible limit which is. 0.01 ppm. The maximum values of Cobalt were observe in the samples B12 and B13 (37)



Graph 4.1.6.2: Values are expressed as Standard Error Mean (SEM)

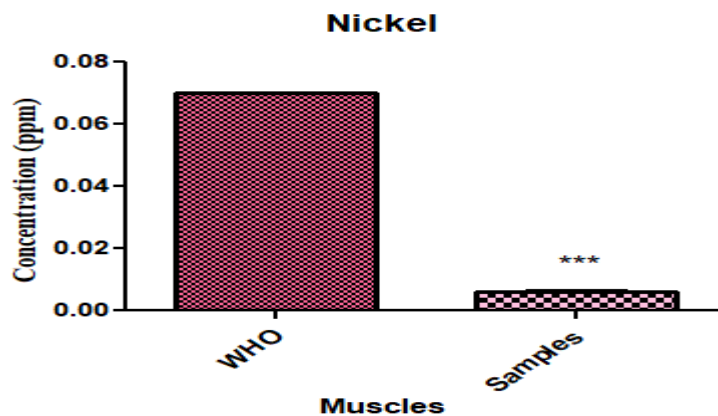
The graph showed the computative results of cobalt in all sample. The value expressed as Standard Error Mean.

4.1.7: Nickel Concentrations in Muscle Tissue Samples



Graph 4.1.7.1: Concentration of Nickel in Muscle Samples of Fish

The graph showed the concentration of Nickel in the muscle Samples of fish collected from Balloki Headworks exceeded the WHO permissible limit which is 0.070 (39).



Graph 4.1.7.2: Values are expressed as Standard Error Mean (SEM)

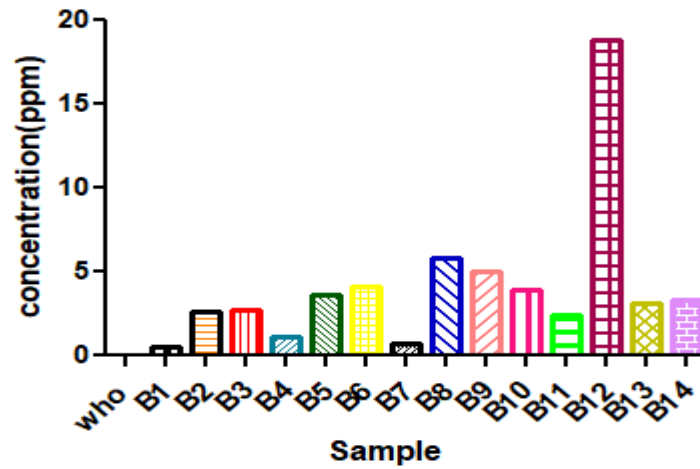
The graph showed the comparative results of nickel in all sample. The value expressed as Standard Error Mean.

4.2: Concentration of Heavy Metals in Gills Tissues

Table 4.2: Concentration of Heavy Metals in Gills Tissue

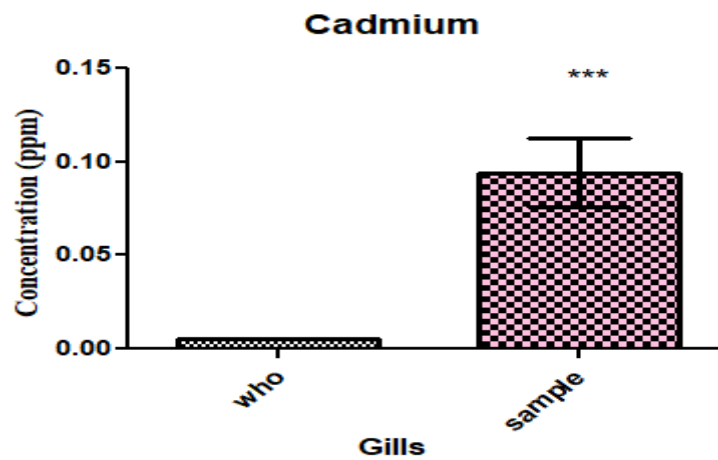
Sr. No	Sample	Cd (ppm)	Cr (ppm)	Ni (ppm)	Co (ppm)	Cu (ppm)	Mn (ppm)	Zn (ppm)
	WHO	0.005	0.1	0.070	0.01	1.0	0.400	5
1.	B1	0.025	0.058	0.108	0.004	0.043	0.016	0.008
2.	B2	0.147	0.062	0.085	0.006	0.025	0.097	0.007
3.	B3	0.148	0.038	0.089	0.009	0.040	0.083	0.004
4.	B4	0.080	0.047	0.075	0.004	0.052	0.047	0.005
5.	B5	0.044	0.083	0.080	0.005	0.040	0.031	0.006
6.	B6	0.009	0.040	0.095	0.009	0.035	0.029	0.007
7.	B7	0.024	0.035	0.072	0.008	0.042	0.039	0.008
8.	B8	0.001	0.030	0.066	0.004	0.047	0.041	0.004
9.	B9	0.050	0.043	0.056	0.005	0.052	0.038	0.003
10.	B10	0.203	0.041	0.087	0.006	0.063	0.041	0.009
11.	B11	0.137	0.038	0.079	0.008	0.048	0.037	0.008
12.	B12	0.111	0.042	0.083	0.009	0.035	0.042	0.009
13.	B13	0.163	0.043	0.072	0.007	0.046	0.038	0.003
14.	B14	0.177	0.035	0.060	0.008	0.052	0.040	0.004

4.2.1: Cadmium Concentrations in Gills Tissue Samples of Fish



Graph 4.2.1.1: Concentration of Cadmium in Gill Sample of Fish

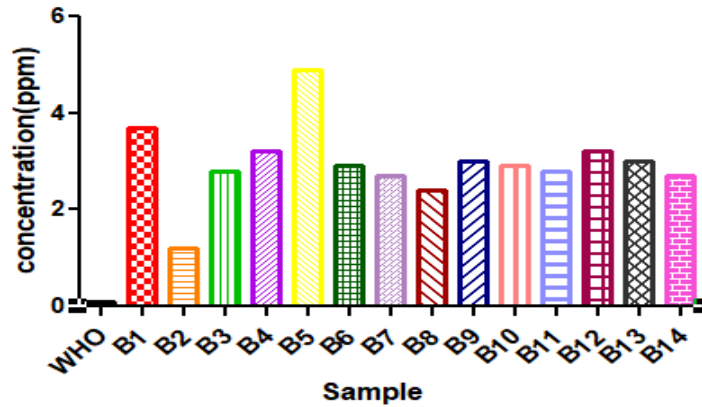
The graph showed that the concentration of Cadmium in gill samples of fish from Balloki Headworks exceeded the WHO permissible limit which is 0.005 ppm. Maximum value of cadmium was observed in B12 (37).



Graph 4.2.1.2: Values are expressed as Standard Error Mean (SEM)

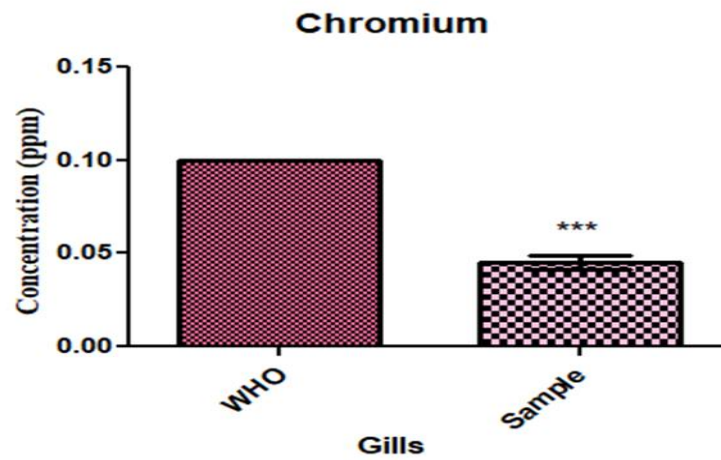
The graph showed the computative results of cadmium in all sample. The value expressed as Standard Error Mean.

4.2.2: Chromium Concentrations in Gills Tissue Samples of Fish



Graph 4.2.2.1: Concentration of chromium in Gill Sample of Fish

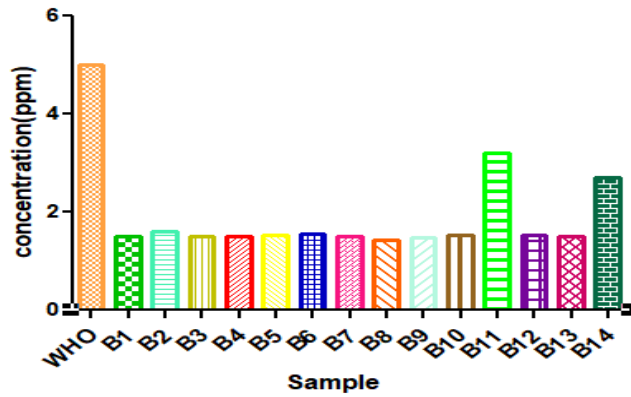
The graph showed that the concentration of Chromium in gill Samples of Balloki Headworks was above the WHO permissible limit which is 0.1 ppm. Maximum value of chromium was observed in B5 sample (40).



Graph 4.2.2.2: Values are expressed as Standard Error Mean (SEM)

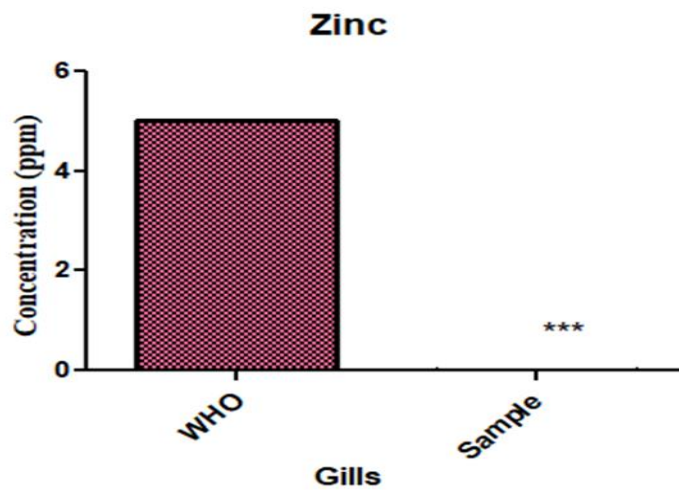
The graph showed the computative results of chromium in all sample. The value expressed as Standard Error Mean.

4.2.3: Zinc Concentrations in Gills Tissue Samples of Fish



Graph 4.2.3.1: Concentration of zinc in Gill Sample of Fish

Zinc concentrations were observed to be negligible and did not exceeded the WHO

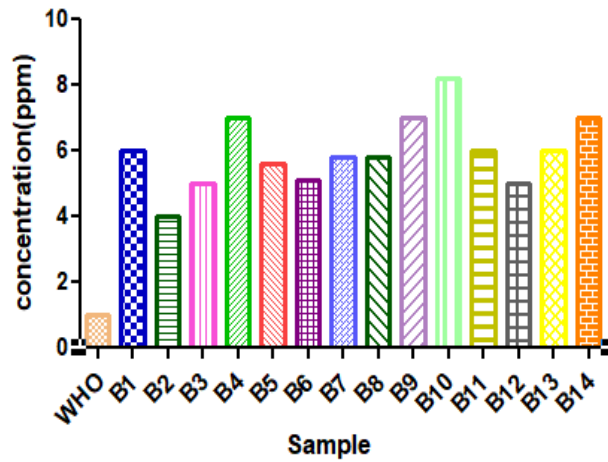


permissible limit of 5 ppm (41).

Graph 4.2.3.2: Values are expressed as Standard Error Mean (SEM)

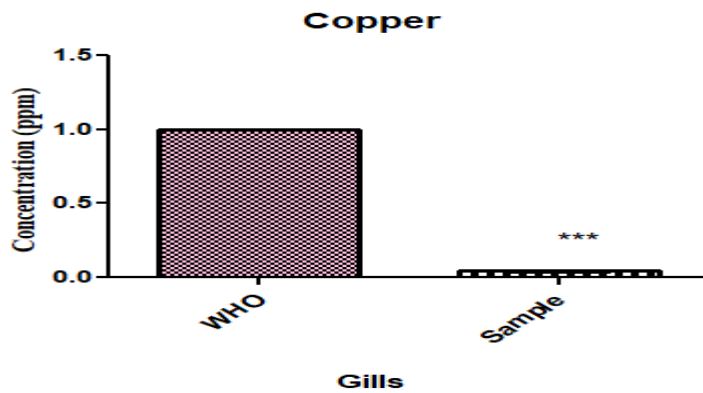
The graph showed the computative results of zinc in all sample. The value expressed as Standard Error Mean.

4.2.4: Copper Concentrations in Gills Tissue Samples of Fish



Graph 4.2.4.1: Concentration of copper in Gill sample of Fish

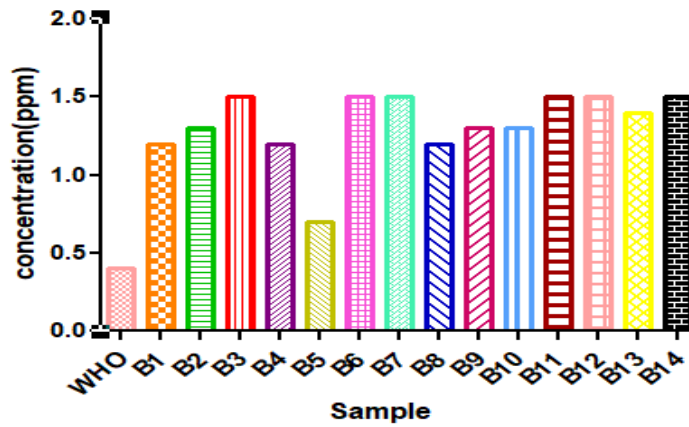
The graph showed the concentration of Copper in the Gill Samples of fishes. Concentration of copper was observed to be greater than the WHO permissible limit. Maximum value of copper was observed in B10 (38).



Graph 4.2.4.2: Values are expressed as Standard Error Mean (SEM)

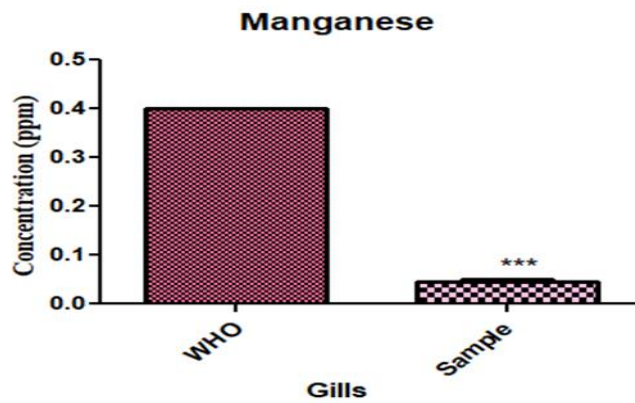
The graph showed the computative results of copper in all sample. The value expressed as Standard Error Mean.

4.2.5: Manganese Concentrations in Gills Tissue Samples of Fish



Graph 4.2.5.1: Concentration of manganese in Gill sample of Fish

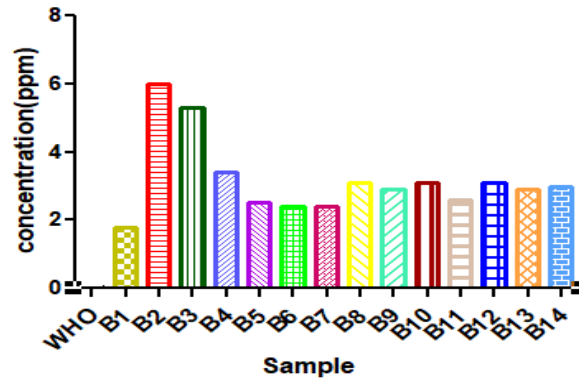
The graph showed the concentration of manganese in the Gill Samples of fishes. Concentration of manganese was observed to be greater than the WHO permissible limit which is 0.4 ppm(37).



Graph 4.2.5.2: Values are expressed as Standard Error Mean (SEM)

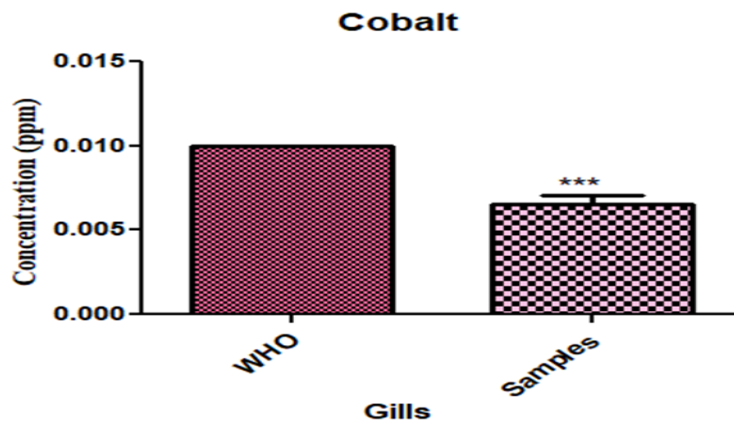
The graph showed the computative results of manganese in all sample. The value expressed as Standard Error Mean.

4.2.6: Cobalt Concentrations in Gills Tissue Samples of Fish



Graph 4.2.6.1: Concentration of Cobalt in Gill sample of Fish

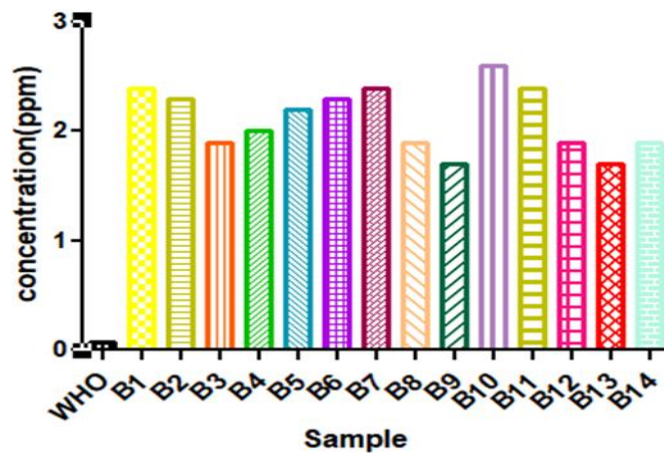
The graph showed the concentration of cobalt in the Gill Samples of fishes. Concentration of cobalt was observed to be greater than the WHO permissible limit. The maximum values were observe in the samples B2 and B3 (39).



Graph 4.2.6.2: Values are expressed as Standard Error Mean (SEM)

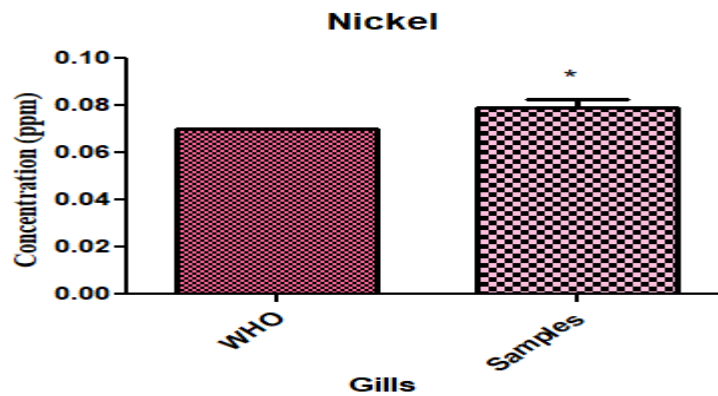
The graph showed the computative results of cobalt in all sample. The value expressed as Standard Error Mean.

4.2.7: Nickel Concentrations in Gills Tissue Samples of Fish



Graph 4.2.7.1: Concentration of Nickel in Gill sample of Fish

The graph showed the concentration of Nickel in the Gill Samples of fishes. Concentration of Nickel was observed to be highly greater than the WHO permissible limit. The maximum values was observe in the sample B10 (40).



Graph 4.2.7.2: Values are expressed as Standard Error Mean (SEM)

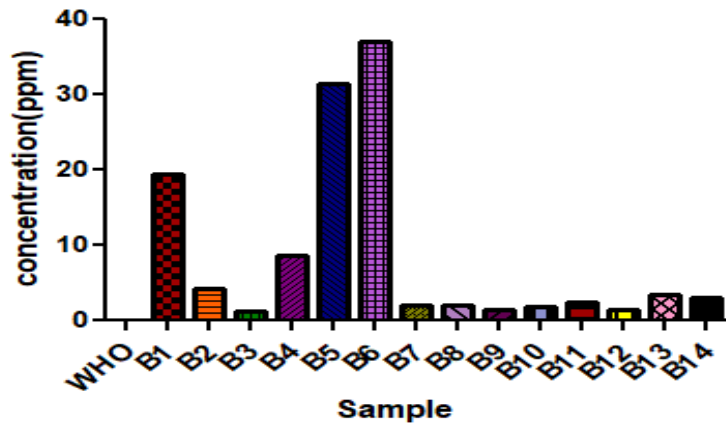
The graph showed the computative results of nickel in all sample. The value expressed as Standard Error Mean.

4.3: Concentration of Heavy Metals in Skin Tissues:

Table 4.3: Concentration of Heavy Metals in skin Tissue

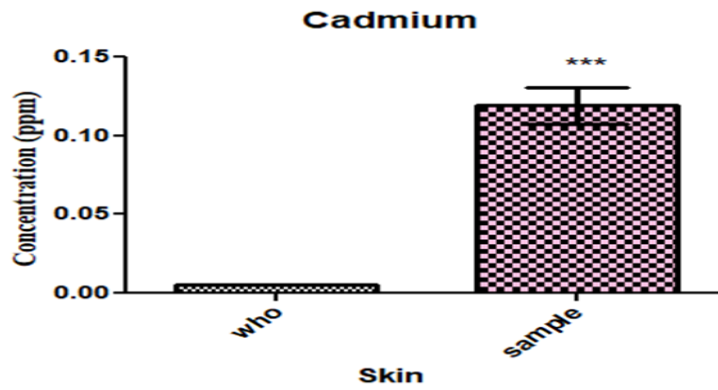
Sr. No	Sample	Cd (ppm)	Cr (ppm)	Ni (ppm)	Co (ppm)	Cu (ppm)	Mn (ppm)	Zn (ppm)
	WHO	0.005	0.1	0.070	0.01	1.0	0.400	5
1.	B1	0.115	0.042	0.072	0.009	0.062	0.080	0.009
2.	B2	0.147	0.051	0.065	0.004	0.071	0.065	0.008
3.	B3	0.033	0.060	0.050	0.003	0.048	0.072	0.002
4.	B4	0.067	0.032	0.062	0.005	0.052	0.071	0.003
5.	B5	0.169	0.050	0.076	0.005	0.043	0.016	0.005
6.	B6	0.194	0.047	0.058	0.004	0.062	0.023	0.006
7.	B7	0.133	0.063	0.064	0.009	0.040	0.040	0.007
8.	B8	0.133	0.035	0.067	0.006	0.048	0.032	0.009
9.	B9	0.092	0.042	0.056	0.007	0.052	0.040	0.004
10.	B10	0.109	0.038	0.072	0.008	0.068	0.062	0.008
11.	B11	0.099	0.042	0.080	0.009	0.022	0.070	0.009
12.	B12	0.094	0.040	0.063	0.008	0.082	0.083	0.002
13.	B13	0.112	0.052	0.075	0.002	0.043	0.060	0.003
14.	B14	0.171	0.065	0.078	0.006	0.041	0.043	0.008

4.3.1: Cadmium Concentrations in Skin Tissue Samples of Fish



Graph 4.3.1.1: Concentration of Cadmium in Skin Sample of Fish

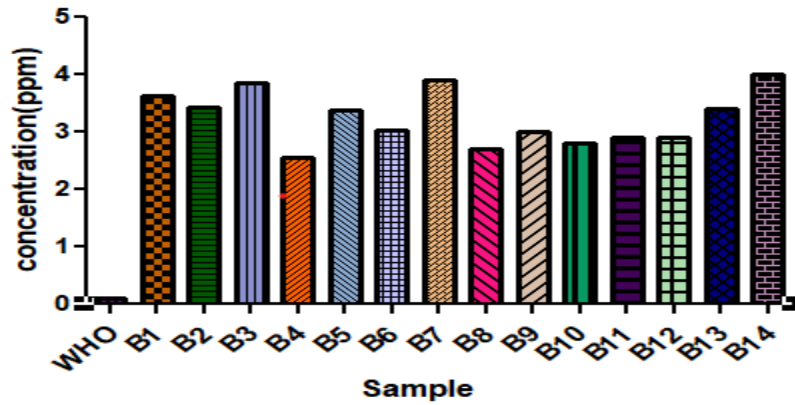
The graph showed that the concentration of Cadmium in water Samples of fish from Balloki Headworks exceeded the WHO permissible limit which is 0.005 ppm (38).



Graph 4.3.1.2: Values are expressed as Standard Error Mean (SEM)

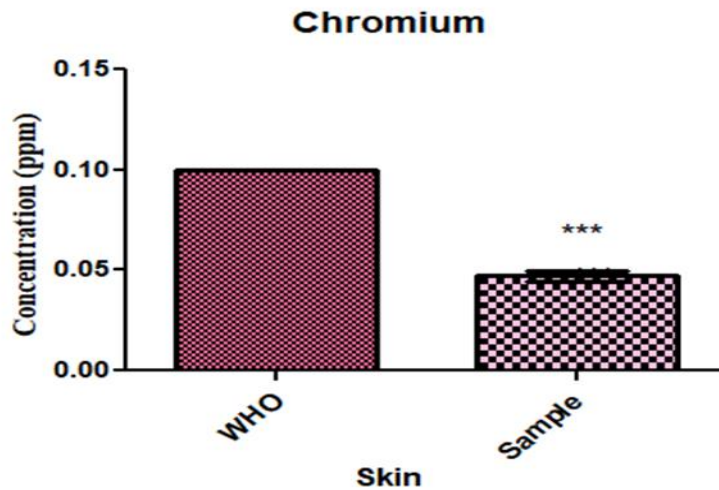
The graph showed the computative results of cadmium in all sample. The value expressed as Standard Error Mean.

4.3.2: Chromium Concentrations in Skin Tissue Samples of Fish



Graph 4.3.2.1: Concentration of Chromium in Skin Sample of Fish

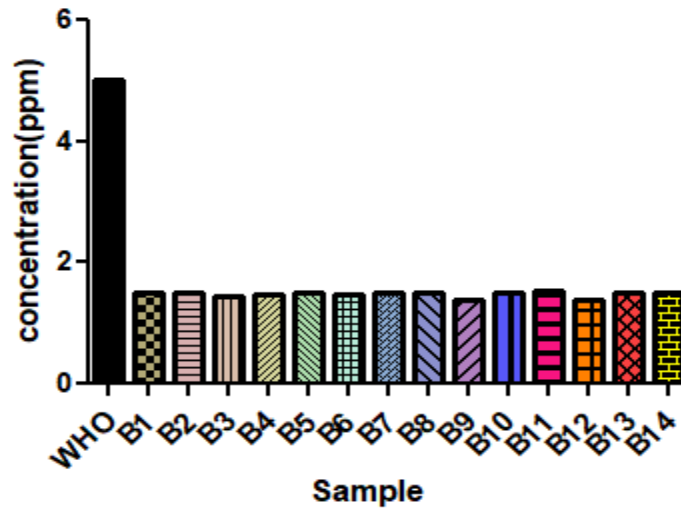
The graph showed that the concentration of Chromium in water Samples of fish from Balloki Headworks exceeded the WHO permissible limit which is 0.1ppm. The maximum values was observe in the sample B14 (40).



Graph 4.3.2.2: Values are expressed as Standard Error Mean (SEM)

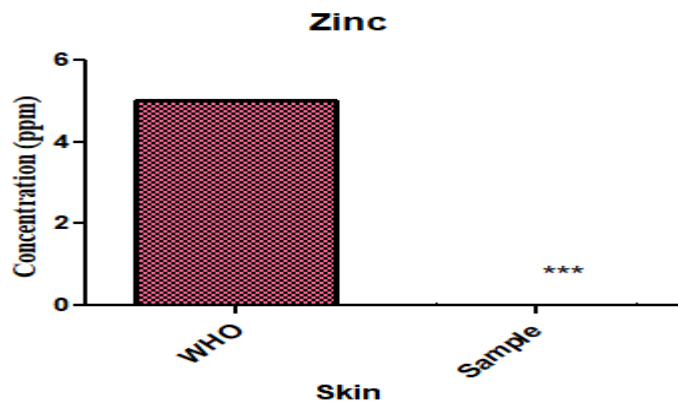
The graph showed the computative results of chromium in all sample. The value expressed as Standard Error Mean.

4.3.3: Zinc Concentrations in Skin Tissue Samples of Fish



Graph 4.3.3.1: Concentration of Zinc in Skin Sample of Fish

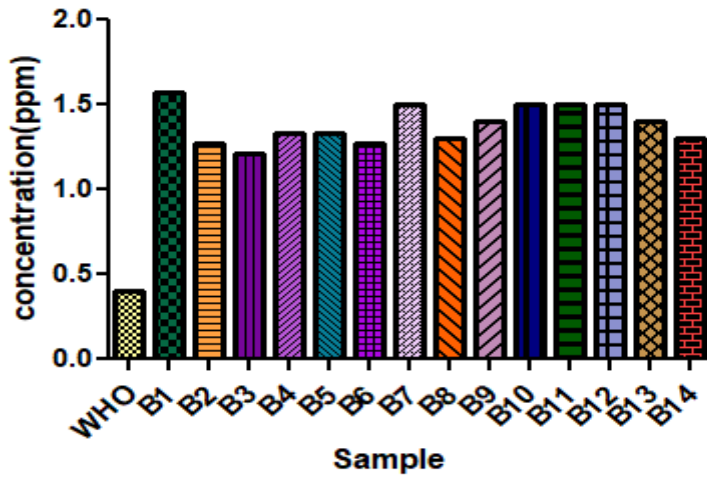
The graph showed that the concentration of Zinc in Skin Samples of fish from Balloki Headworks exceeded the WHO permissible limit which is 5.0ppm (39).



Graph 4.3.3.2: Values are expressed as Standard Error Mean (SEM)

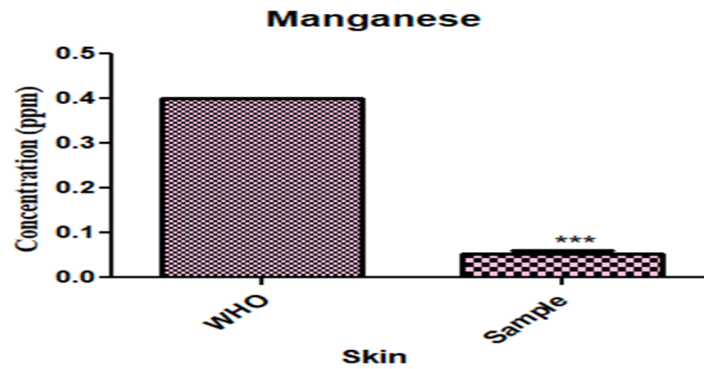
The graph showed the computative results of zinc in all sample. The value expressed as Standard Error Mean.

4.3.4: Manganese Concentrations in skin Tissue Samples of Fish



Graph 4.3.4.1: Concentration of Manganese in Skin Sample of Fish

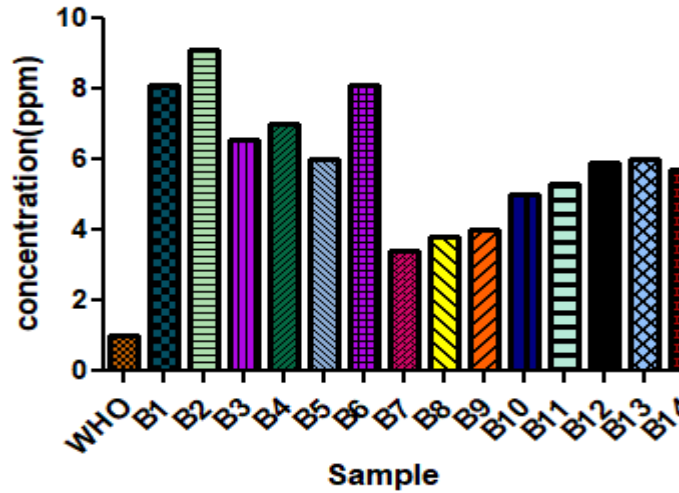
The graph showed that the concentration of Manganese in Skin Samples of fish from Balloki Headworks exceeded the WHO permissible limit which is 0.400ppm. The maximum values were observe in the samples B1 and B7 (37).



Graph 4.3.4.2: Values are expressed as Standard Error Mean (SEM)

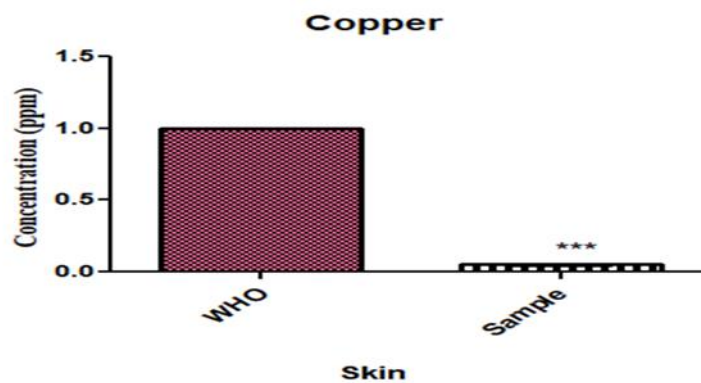
The graph showed the computative results of manganese in all sample. The value expressed as Standard Error Mean.

4.3.5: Copper Concentrations in skin Tissue Samples of Fish



Graph 4.3.5.1: Concentration of Copper in Skin Sample of Fish

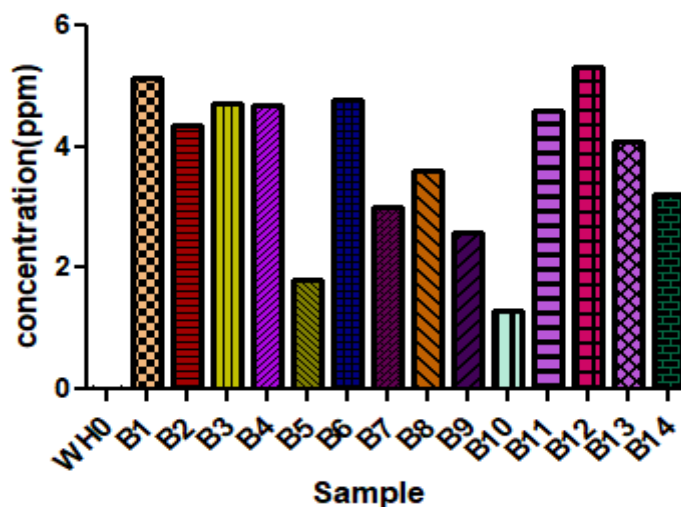
The graph showed that the concentration of Copper in Skin Samples of Fish from Balloki Headworks exceeded the WHO permissible limit which is 1.0ppm. The maximum values was observe in the sample B2 (39).



Graph 4.3.5.2: Values are expressed as Standard Error Mean (SEM)

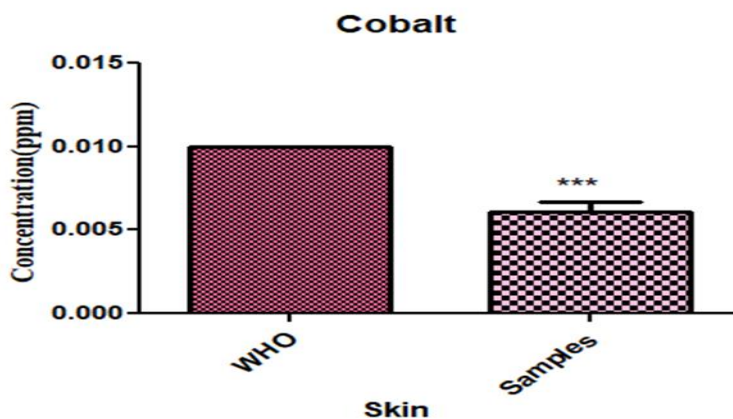
The graph showed the computative results of copper in all sample. The value expressed as Standard Error Mean.

4.3.6: Cobalt Concentrations in skin Tissue Samples of Fish



Graph 4.3.6.1: Concentration of Cobalt in Skin Sample of Fish

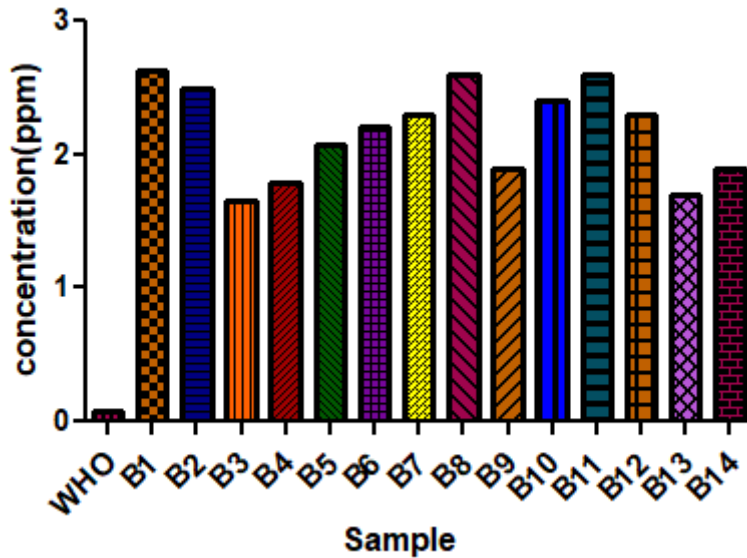
The graph showed that the concentration of Cobalt in Skin Samples of fish from Balloki Headworks exceeded the WHO permissible limit which is 0.01ppm. The maximum values were observe in the samples B1 and B12 (38).



Graph 4.3.6.2: Values are expressed as Standard Error Mean (SEM)

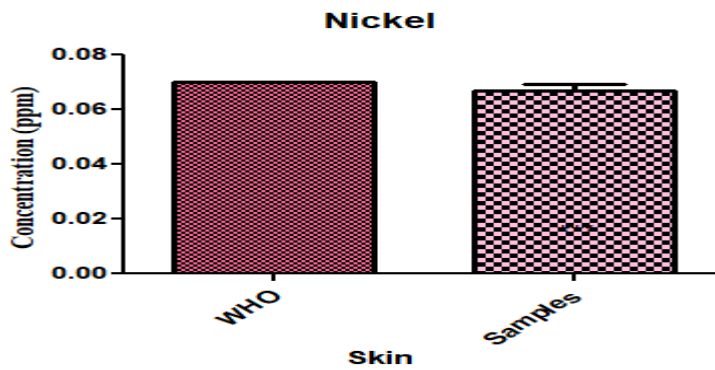
The graph showed the computative results of cobalt in all sample. The value expressed as Standard Error Mean.

4.3.7: Nickel Concentrations in skin Tissue Samples of Fish



Graph 4.3.7.1: Concentration of Nickel in Skin Sample of Fish

The graph showed that the concentration of Nickel in Skin Samples of Fish from Balloki Headworks exceeded the WHO permissible limit which is 0.070ppm. The maximum values were observed in the samples B1 and B8 (40).



Graph 4.3.7.2: Values are expressed as Standard Error Mean (SEM)

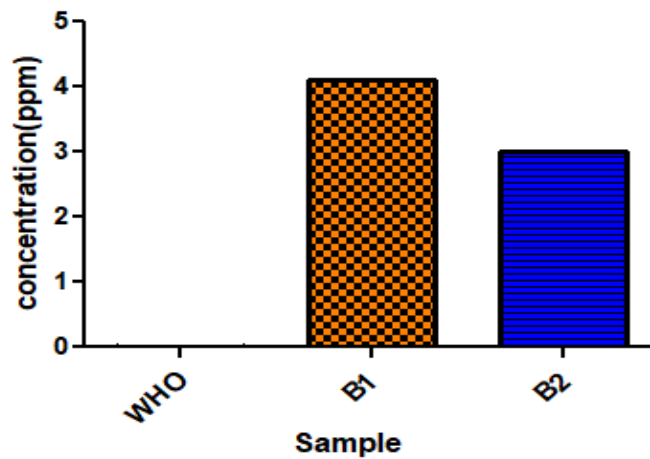
The graph showed the comparative results of nickel in all sample. The value expressed as Standard Error Mean.

4.4: Concentration of Heavy Metals in water samples of fish:

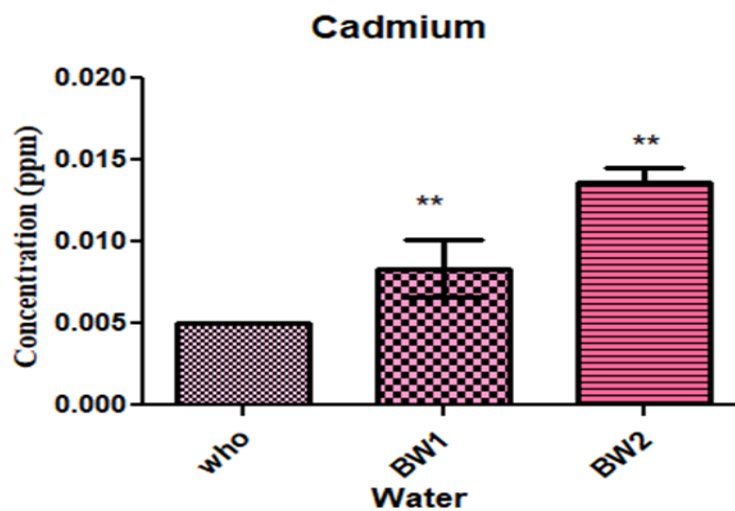
Table 4.4: Concentration of Heavy Metals in water sample of fish

Sampl e	Cadmiu m (ppm)	Chromiu m (ppm)	Zinc (ppm)	Manganes e (ppm)	Coppe r (ppm)	Cobal t (ppm)	Nicke l (ppm)
Balloki 1	0.009	0.043	0.019	0.009	0.017	0.016	0.005
Balloki 2	0.014	0.032	0.078	0.013	0.032	0.019	0.004

4.4.1: Cadmium Concentrations in water Samples of Balloki Headwork

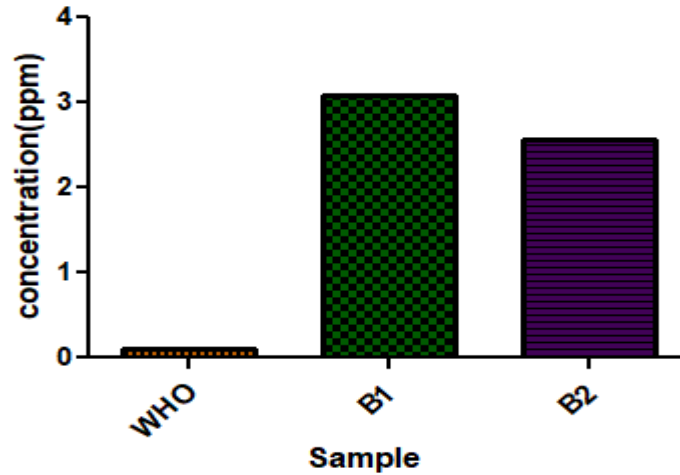


Graph 4.4.1.1: Concentration of Cadmium in water sample of Balloki Headworks
The graph showed that the concentration of Cadmium in water Samples from Balloki Headworks exceeded the WHO permissible limit which is 0.005 ppm. The maximum value was observed in the sample B1 (39).

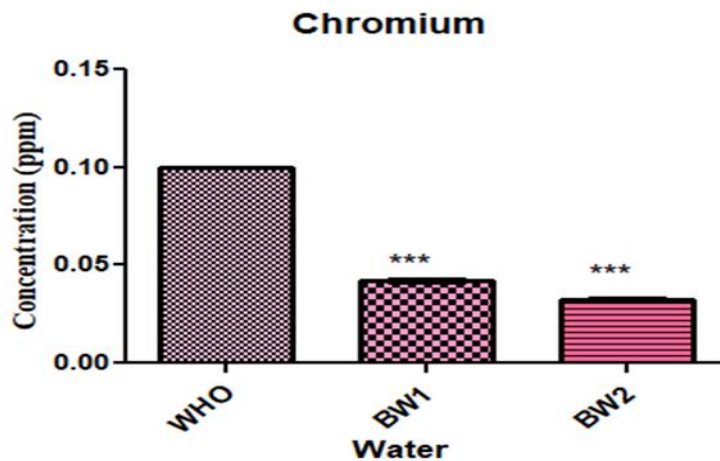


Graph 4.4.1.2: Values are expressed as Standard Error Mean (SEM)
The graph showed the computative results of cadmium in all sample. The value expressed as Standard Error Mean.

4.4.2: Chromium Concentrations in water Samples of Balloki Headworks

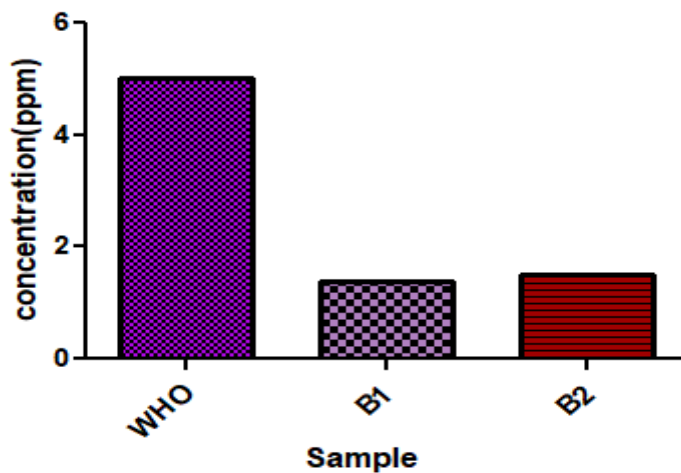


Graph 4.4.2.1: Concentration of Chromium in Water Sample of Balloki Headworks
The graph showed that the concentration of Chromium in Water Samples from Balloki Headworks exceeded the WHO permissible limit which is 0.1 ppm. The maximum value was observed in the sample B1 (37).



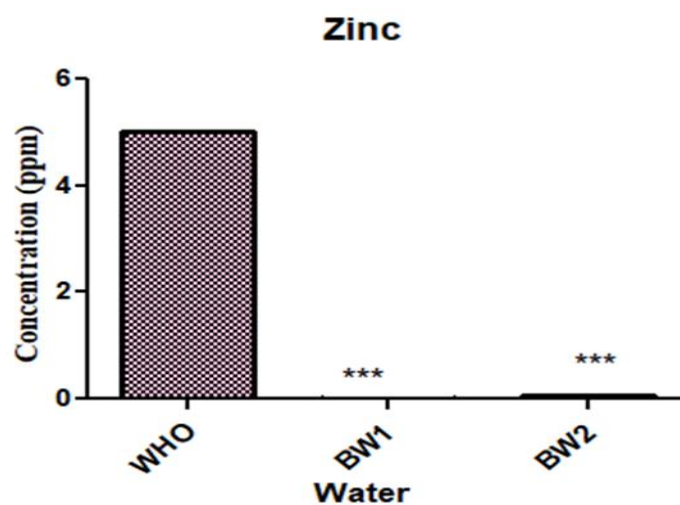
Graph 4.4.2.2: Values are expressed as Standard Error Mean (SEM)
The graph showed the computative results of chromium in all sample. The value expressed as Standard Error Mean.

4.4.3: Zinc Concentrations in Water Samples of Balloki Headworks



Graph 4.4.3.1: Concentration of Zinc in water sample of Balloki Headworks

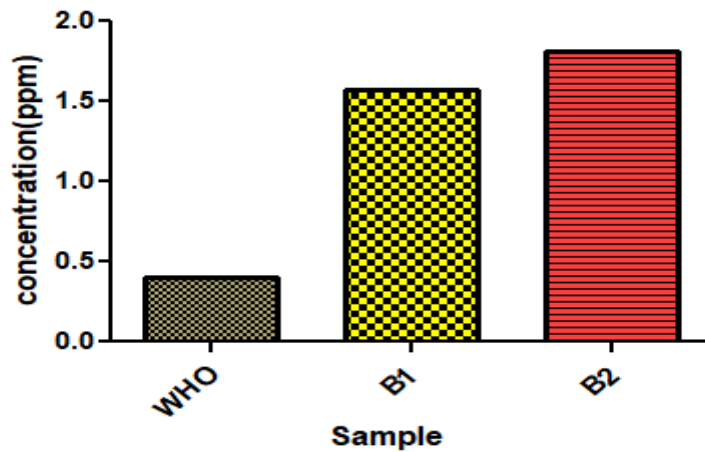
The graph showed that the concentration of zinc in water Samples from Balloki Headworks was less then WHO permissible limit which is 5.0 ppm (40).



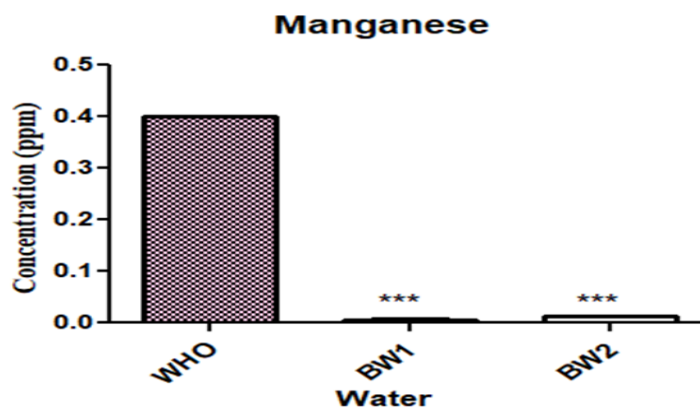
Graph 4.4.3.2: Values are expressed as Standard Error Mean (SEM)

The graph showed the computative results of zinc in all sample. The value expressed as Standard Error Mean.

4.4.4: Manganese Concentrations in water Samples of Balloki Headworks

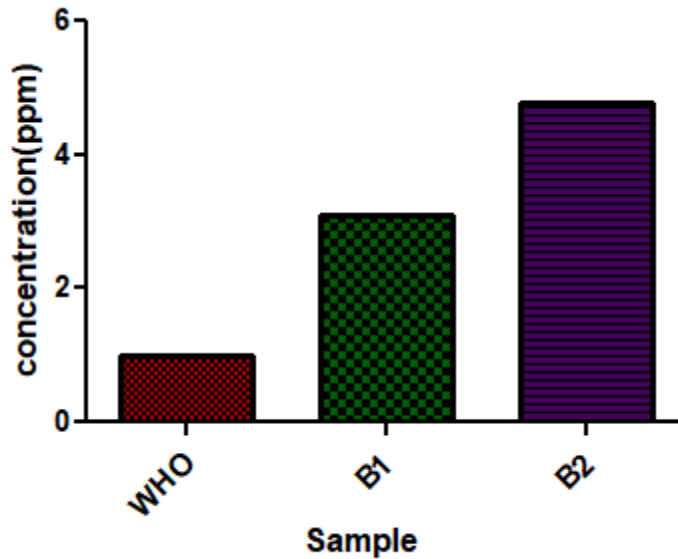


Graph 4.4.4.1: Concentration of Manganese in water samples of Balloki Headworks
The graph showed that the concentration of Manganese in Water Samples from Balloki Headworks exceeded the WHO permissible limit which is 0.400 ppm. The maximum value was observed in the sample B2 (41)

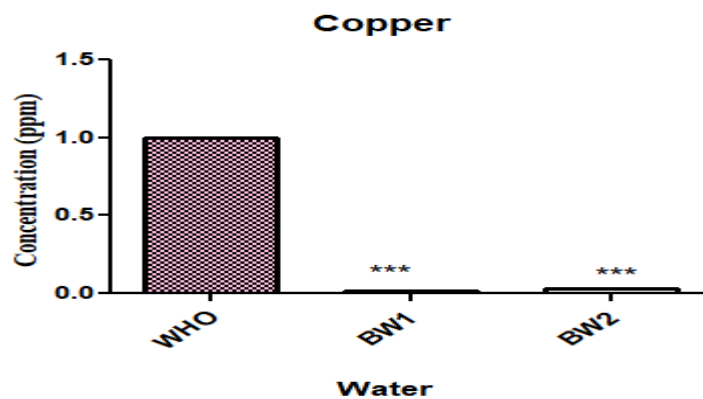


Graph 4.4.4.2: Values are expressed as Standard Error Mean (SEM)
The graph showed the computative results of manganese in all sample. The value expressed as Standard Error Mean.

4.4.5: Copper Concentrations in Water Samples of Balloki Headworks

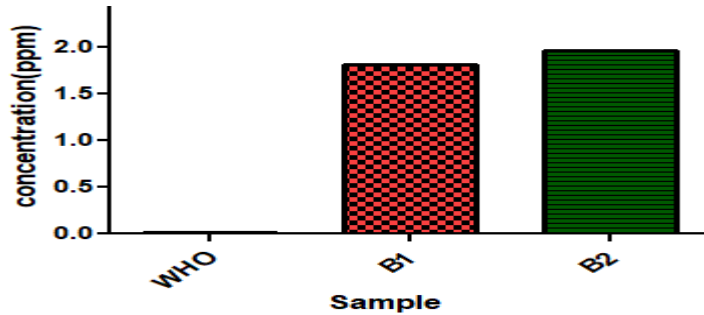


Graph 4.4.5.1: Concentration of Copper in water samples of Balloki Headworks
The graph showed that the concentration of Copper in water Samples from Balloki Headworks exceeded the WHO permissible limit which is 1.0 ppm. The maximum value was observed in the sample B2 (38).



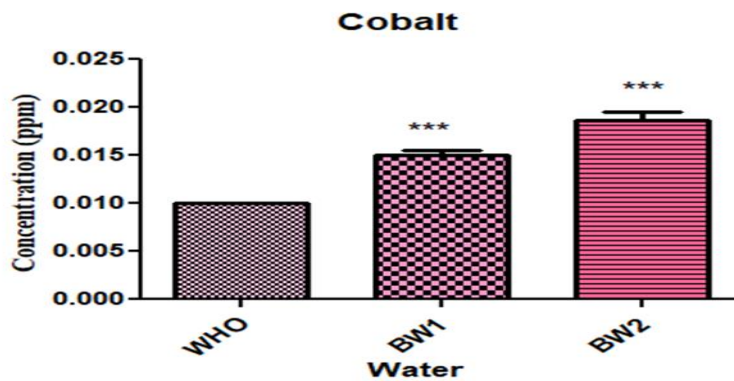
Graph 4.4.5.2: Values are expressed as Standard Error Mean (SEM)
The graph showed the computative results of copper in all sample. The value expressed as Standard Error Mean.

4.4.6: Cobalt Concentrations in water Samples of Balloki Headworks



Graph 4.4.6.1: Concentration of Cobalt in water sample of Balloki Headworks

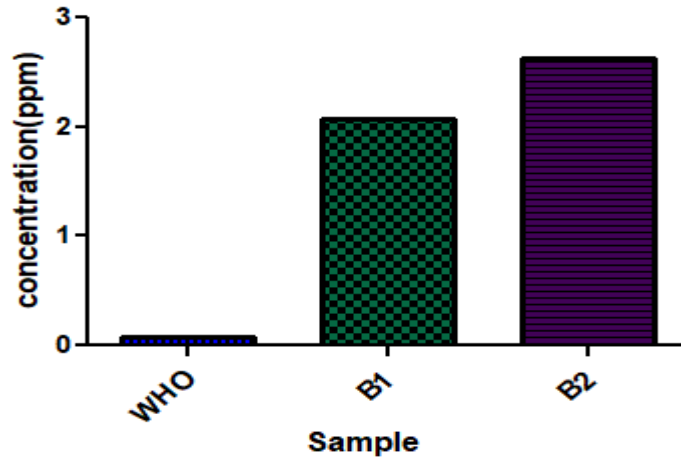
The graph showed that the concentration of Cobalt in water Samples from Balloki Headworks exceeded the WHO permissible limit which is 0.01 ppm. The maximum value was observed in the sample B2 (41).



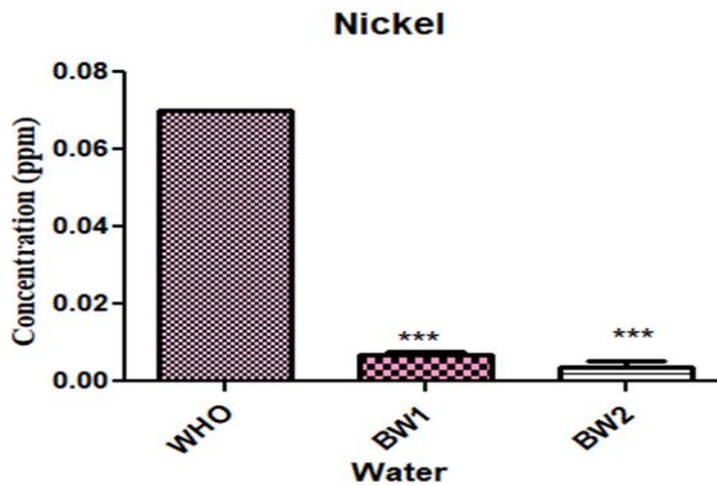
Graph 4.4.6.2: Values are expressed as Standard Error Mean (SEM)

The graph showed the computative results of cobalt in all sample. The value expressed as Standard Error Mean.

4.4.7: Nickel Concentrations in water Samples of Balloki Headworks



Graph 4.4.7.1: Concentration of Nickel in water samples of Balloki Headworks
 The graph showed that the concentration of Nickel in water Samples of fish from Balloki Headworks exceeded the WHO permissible limit which is 0.070 ppm. The maximum value was observed in the sample B2 (38).



Graph 4.4.7.2: Values are expressed as Standard Error Mean (SEM)
 The graph showed the comparative results of nickel in all sample. The value expressed as Standard Error Mean.

Heavy metal pollution resulted from industrial waste that contains heavy metals contaminating the water supply in Balloki Headworks. This endanger aquatic life and human health as well. Fish from Balloki Headworks contain heavy metals due to industrial and agricultural activities upstream. Consumption of such fish can lead to accumulation of heavy metals in bodies which can cause various health problems.

Balloki Headworks is a major source of drinking water and use for irrigation practices in nearby areas (37).

The results showed that all the studied metals Cadmium, Chromium, Nickel, Copper, Cobalt, and Manganese were present in excessive amount in gills, skin, muscle tissues and water samples of Balloki Headworks and exceeded the WHO permissible limit. The concentration of Zinc did not exceeded the WHO permissible limit 5.0 ppm. The value of Zinc was negligible in gills, skin, muscle tissues and water samples of Balloki Headworks. The reason for the remarkable low observed concentration of Zinc may be its least solubility in ground water and formation of an insoluble shielding layer. The undissolved heavy metal Zinc did not mixing up in the ground water which result in less absorbance of Zinc. The sources of zinc include industrial wastes, agricultural runoff, aging water supply systems, mining etc (38,40).

The proper monitoring of water is need because nearby communities use this water for drinking purpose. Installation of treatment plants should be done and the water should be treated before the release of industrial effluents in the waters of Balloki Headworks. We should aware community to not pollute the water bodies and protect the environment (39).

Conclusion

Fish samples were collected from Balloki Headworks Punjab, Pakistan. There were 14 samples analyzed in Balloki Headworks. Balloki Headworks is a barrage on the Ravi River in the Punjab, Pakistan. It is 70 km distance from Lahore. Pollutants found in wastewater can affect the growth, development, and survival of aquatic animals in Balloki Headworks. Excess amounts of heavy metals cause formation of algal blooms, which reduce oxygen level in water and affect the aquatic life. Accumulation of heavy metals cause delay in hatching of fish eggs. To analyze the presence of heavy metals fish organs were dissected and preserved. The Atomic Absorption Spectrophotometer technique were performed to obtain results. The obtained values of heavy metals present in the water samples and the organs were compared with the WHO standards for heavy metals. The values of heavy metals [Cadmium, Chromium, Cobalt, Nickel, Manganese and Copper] were above the permissible limits of WHO, in the muscles, skin and gills and water samples. The value of Zinc was negligible in gills, skin, muscle tissues and water samples of Balloki Headworks. The reason for the remarkable low observed concentration of Zinc may be its least solubility in ground water and formation of an insoluble shielding layer. Since, Fish samples were collected from the selected area. Heavy metal contamination in Balloki Headworks due to the untreated discharge of industrial and sewage water from city centers and industrial units. As a result, the ecosystem health of both of these rivers is degrading day by day. They have moderate metal contamination at a few sites. Contaminated fish from polluted rivers can badly impact human health. Heavy metal toxicity is responsible for the degradation of the population of aquaculture, causing physical deformities in organisms and polluting the aquatic environment. These toxic heavy metals cause various diseases in fishes.

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ANNEXURES I



Figure. 1: Sample collected



Figure. 2: Digestion of fish



Figure. 3: Preservation of Organs of Fish



Figure. 4: Specimen of Fish in Muffle Furnace for Incineration



Figure. 5: Ashes of Specimen



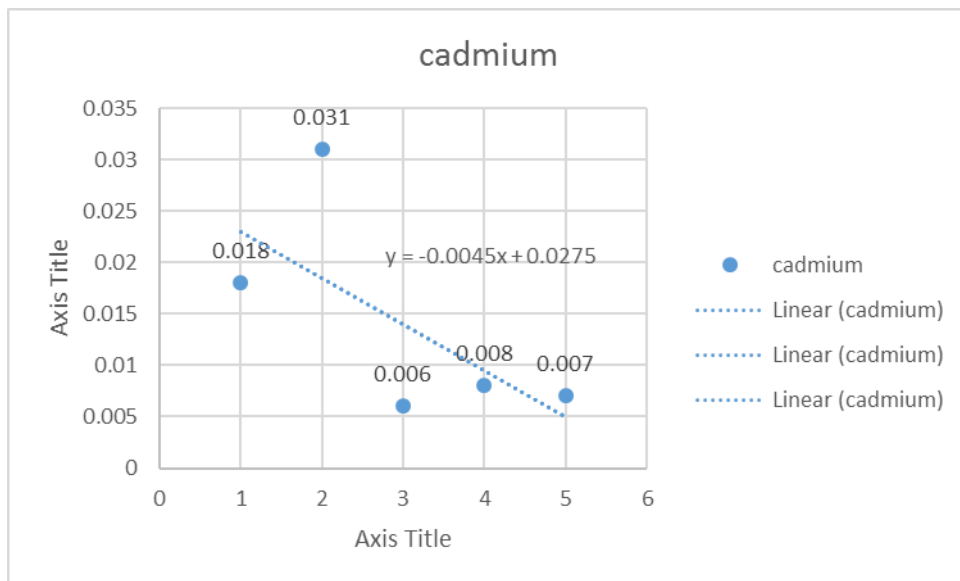
Figure. 6: Filtered Organ Samples of Fish

ANNEXURES II

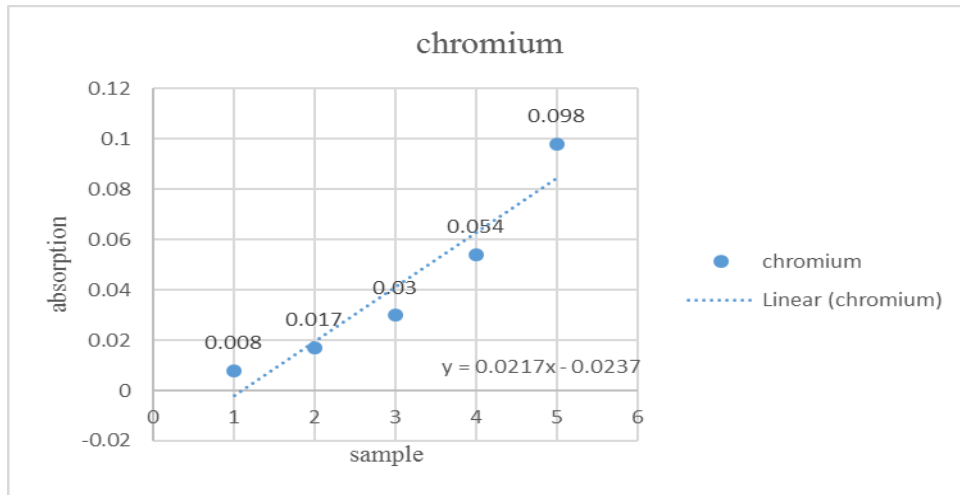
Table 1: Stock Solutions of Heavy Metals

Sample	Cadmium	Chromium	Zinc	Manganese	Copper	Cobalt	Nickel
WHO	0.005	0.1	5.0	0.400	1.0	0.01	0.070
1 ppm	0.018	0.008	0.000	0.007	0.002	0.006	0.001
2 ppm	0.031	0.017	0.203	0.011	0.005	0.019	0.003
3 ppm	0.006	0.030	0.613	0.029	0.013	0.021	0.008
4 ppm	0.008	0.054	0.900	0.043	0.021	0.057	0.016
5 ppm	-0.007	0.098	1.718	0.075	0.039	0.083	0.030

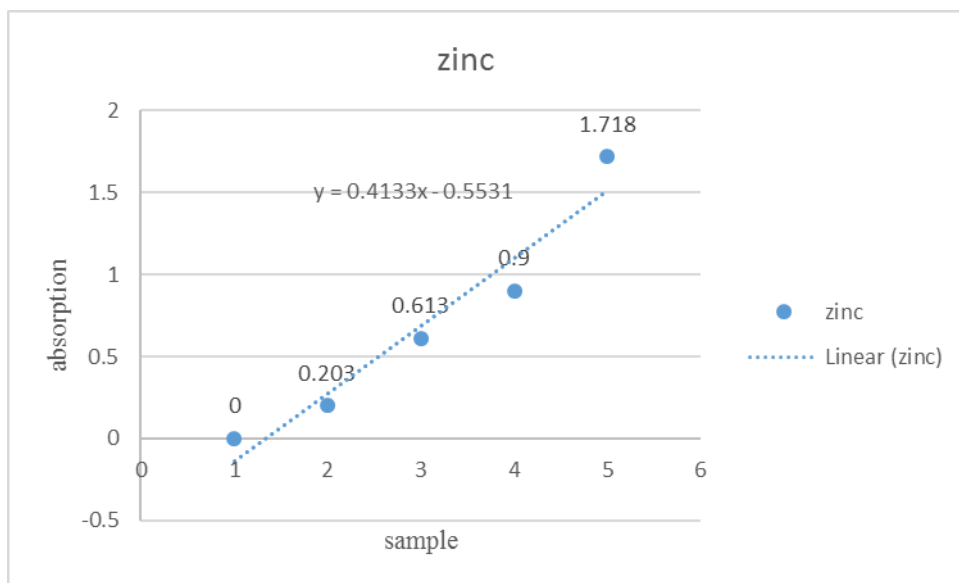
Calibration Curves of Stock Solutions:



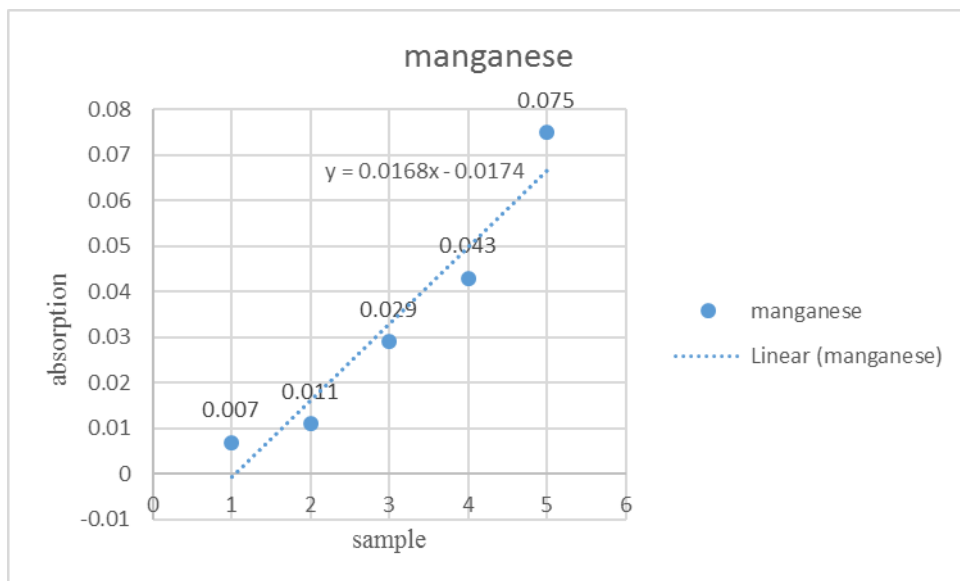
Graph 1: Calibration Curve of Stock Solution of Cadmium



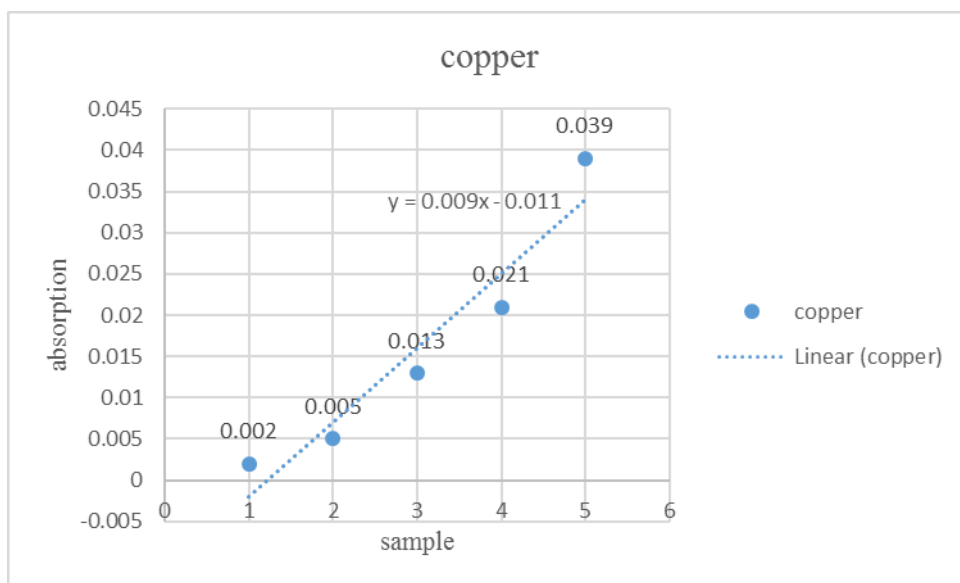
Graph 2: Calibration Curve of Stock Solution of Chromium



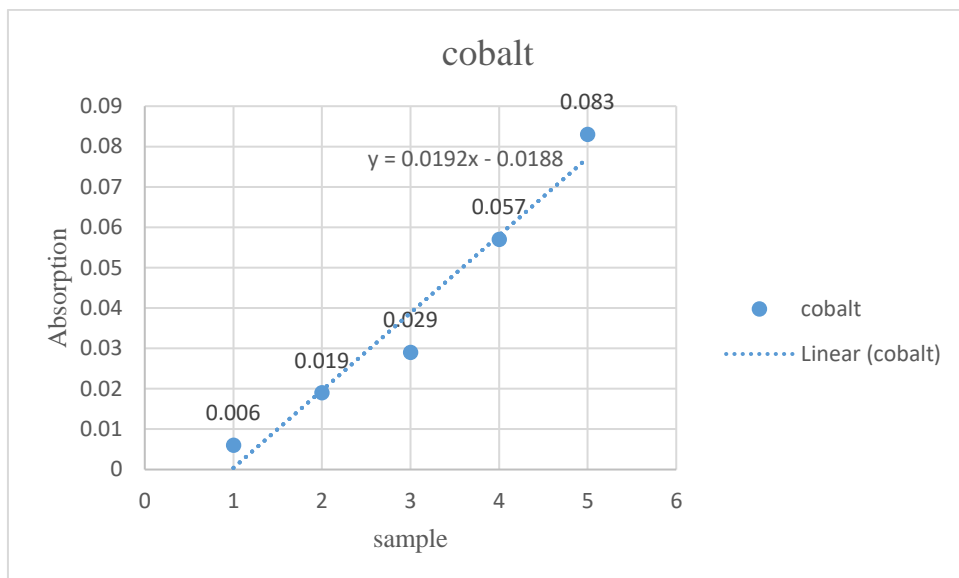
Graph 3: Calibration curve of Stock Solution of Zinc



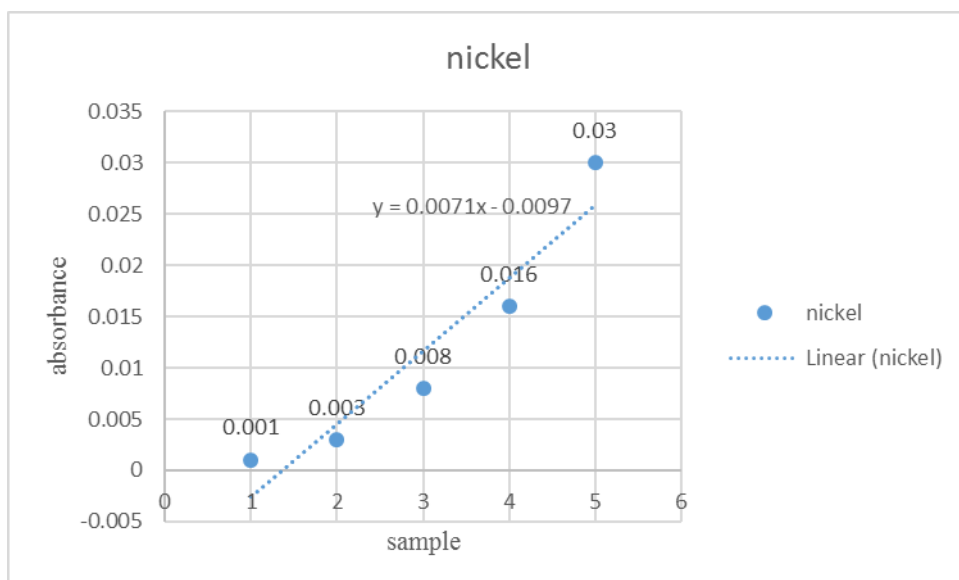
Graph 4: Calibration curve of Stock Solution of Manganese



Graph 5: Calibration Curve of Stock Solution of Copper



Graph 6: Calibration Curve of Stock Solution of Cobalt



Graph 7: Calibration Curve of Stock Solution of Nickel



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