TOXICOLOGICAL ANALYSIS OF INDIAN BULLFROG SPECIES ORGANS AND WATER SAMPLES COLLECTED FROM SIALKOT AND GUJRANWALA DISTRICTS

A THESIS SUBMITTED TO

KINNAIRD COLLEGE FOR WOMEN

IN FULFILLMENT OF THE REQUIREMENTS

FOR THE DEGREE OF

BACHELORS (BSc. Hons.) IN ZOOLOGY

By

SHAZMA YAMIN

RABYIA AFZAL

DEPARTMENT OF ZOOLOGY

KINNAIRD COLLEGE FOR WOMEN, LAHORE

2018-2022

RESEARCH COMPLETION CERTIFICATE

It is certified that Ms. Rabyia Afzal and Ms. Shazma Yamin of BS Hons. (Session 2018-2022), Department of Zoology have carried out this research work entitled **"Toxicological Analysis of Indian Bullfrog Species and Water Samples Collected from Gujranwala and Sialkot Districts"** under my supervision.

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

"All changes suggested by the examiner during defense are incorporated in this final copy".

Ms. Mehak Ayub
Lecturer
Supervisor

Dated: 16/01/2023

Ms. Shamaila Nadeem

Head of the Zoology Department

ANTI-PLAGIARISM DECLARATION

We certify that this is our own research work. The work has not, in whole or in part, been presented anywhere else for assessment. Where material has been used from other sources, it has been properly acknowledged. The similarity index of the research report is 15%. If this statement is untrue and we are found guilty of plagiarism, the punitive actions against us should be taken as per Kinnaird Anti Plagiarism Policy.

Rabyia Afzal, Shazma Yamin

Registration No: F18BZOL013, W18BZOL011

Program: BS (Hons) Zoology

Ms. Mehak Ayub

Supervisor

Ms. Shamaila Nadeem

Head of the Zoology Department

ACKNOWLEDGMENTS

This research work has been done with the help and guidance of many people. First of all, we are thankful to Allah Almighty for all the strength and persistence he has bestowed upon us during this period. We would like to thank our Principal Dr. Rukhsana David and Vice Principal Dr. Nikhat Khan for providing us this opportunity to prove ourselves. We would like to express our gratitude towards our Head of the

Zoology Department Ma'am Shamaila Nadeem for her constant encouragement and supervision.

We would especially like to thank our Supervisor Ma'am Mehak Ayub for her constant support, guidance and supervision at every step.

Furthermore, we would like to specially thank our parents; Muhammad Afzal and Samia Yamin for supporting us emotionally and financially during all this period. Last but not the least, we would also like to thank Sir Haseeb, Sagheer, Sanwal and Shahzad for helping us throughout our work.

Rabyia Afzal

Shazma Yamin

ABSTRACT

Amphibians are a class of cold blooded animals, which include frogs, salamanders, and caecilians etc. The Indian bullfrog *(Hoplobatrachus tigerinus),* native to the Indian subcontinent is a vertebrate tetrapod which is primarily a freshwater aquatic species. The aim of following research was to collect Indian bullfrogs from Gujranwala and Sialkot Districts and study their morphological characteristics and analyze the presence of heavy metals in the liver and skin of the sample species. Furthermore, heavy metal analysis of the water samples of the respective study areas was carried out. Amphibian samples were collected from the research sites using methods such as sweep netting and bait. They were transported to Kinnaird College Lahore, they were excised and their skin and liver was preserved in 10% buffered formalin. Water samples were digested with HNO_3 and evaluated using Atomic Absorption Spectrometer for heavy metal identification, and the results were compared to standard stock solution values. The metals under consideration included Cadmium, Chromium, Copper, Cobalt, Nickel, Manganese and Zinc. The analyzed values were then matched with standard limits recommended by WHO. The morphological characteristics appeared normal in almost all frog species. Both the study areas are the agricultural areas, untreated water and dumping of solid wastes results in the formation of liquid from solid wastes leaches into the ground due to which sewage mixed with groundwater. Since the frogs were collected from ponds containing groundwater therefore heavy metals were detected in the bodies of the frogs which must have absorbed in their bodies from water. Heavy Metals are naturally present in ground water. The values of heavy metals were within the WHO permissible limits in the skin and liver samples. However, the values of Cadmium exceeded the permissible limit in the water samples. Thus, it was concluded that the quality of both study areas was considerably better. However, with regular monitoring, it can be improved further.

LIST OF ABBREVIATIONS

AAS	Atomic Absorption Spectroscopy
As	Arsenic
BAF	Bio-Accumulative –Factor
BAS	Bellawila-Adittiya Health Sanctuary
CDHB	6-chloro-2, 3dihydrobenzoxazol-2-one
Cd	Cadmium
Cr	Chromium
Cu	Copper
EL	Eye Length
FA	Fenoxaprop
Fe	Iron
FE	Fenoxaprop-ethyl
GSH	Glutathione
HgO	Mercury Vapor
HL	Head Length
HW	Head Width
Hg	Mercury
IARC	International Agency for Research on Cancer
IN	Internal Space
IOB	Inter-orbital Distance
MCL	Maximum Level of Contaminants

Mn	Manganese
MDA	Malondialdehyde
Pb	Lead
SOD	Superoxide Dismutase
ST	Snout Length
TD	Tympanum Diameter
Ti	Titanium
WHO	World Health Organization
Zn	Zinc

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1

INTRODUCTION

Vertebrates possess backbone which runs through the entire body. Mammals, birds, amphibians, reptiles and fishes are the main categories of vertebrates. The vertebrates are composed of about 45,000 self-existing species. Species from several classes are present all over the world. The size of vertebrates varies from tiny fish to elephants and whales. The presence of a vertebral column distinguishes vertebrates from other animals [1].

Toads, frogs, newts, caecilians and salamander (bearing poorly developed eyes) are all members of the amphibian family of cold-blooded vertebrates. Amphibians are the most endangered group of creatures on the planet. Amphibians, particularly frogs, are increasingly being exploited as bio indicators of contaminant accumulation. Since invertebrates are major source of food supply, they are major pathway for the contaminants to enter the body of amphibians and they play vital role in food chain. Amphibians take in water through their ventral skin, and since they spend most of their time in water, toxins may accumulate in their body. The population of amphibians is facing major downfall, and the causes for this remain unknown. Infectious diseases, ultraviolet radiation, habitat degradation and pollution have all been mentioned as possible causes of the frog population drop. The effect of fresh water quality on biota is fundamentally observed on the aquatic bodies that have been extensively polluted by specific sources, such as mines, industry and other anthropogenic pollutants. Low-level pollutants, on the other hand, are poorly understood since they are uncontrollable in natural settings [2].

The Indian bullfrog is primarily a freshwater aquatic species that is mostly solitary and nocturnal. In the Pakistani plains, *Holobatrachus tigerinus* is considered to be the largest frog. During the winter and during droughts, it hibernates by burrowing in the dirt. *Holobatrachus tigerinus* is a voracious feeder, bouncing on and swallowing anything that moves. It can use its anterior limbs to push larger food into its mouth if necessary. It eats a variety of things, including mice, shrews, baby frogs, earthworms, roundworms, juvenile snakes and tiny birds, in addition to a wide variety of insects.

Their ecological linkages with the area's biota reveal that amphibians are more susceptible to pollution because their skin is more permeable than other groups. This is why toxins can invade their critical organs and can be detected in species' tissues and blood. The presence of more amphibian species in an environment indicates that the pollution level of that area is low [3].

Frogs are often used as bio indicators of environmental quality and changes. Frogs are primarily influenced by changes in their freshwater and terrestrial environments. As a result, they are valuable bio indicators of environmental quality and change. Frogs have been utilized as bio indicators in the past, but their use isn't common. Frogs (and especially tadpoles) are known to be sensitive to a variety of water-borne chemicals, making them good candidates for bio-indicators. They are particularly sensitive to environmental changes and can provide scientists with significant information on how an ecosystem functions. The frog *Rana ridibunda* accumulates heavy metals and this frog can be used as a bio-indicator of heavy metal fluctuations in the water bodies in western [2].

Frogs are used as bio indicators of environment. The changes in freshwater and terrestrial environment mainly affect the frogs. As a result, they are valuable bio indicators of environmental quality and change. Frogs have been utilized as bio indicators in the past, but their use isn't common. Frogs (and especially tadpoles) are known to be sensitive to a variety of water-borne chemicals, making them good candidates for bio-indicators. They are specifically sensitive to the environmental changes which provide significant information on the functioning of ecosystem to the scientists. The frog *Rana ridibunda* assemble heavy metals and it can be used as a bioindicator [2].

Heavy metals are abundant in the Earth's crust. They cannot be broken down. They enter into our body in minute amount from breathing, drinking water and food. Some heavy metals like zinc, selenium and copper are used as trace elements essential for the metabolic activity. However, they cause toxicity in higher amounts. The contamination of drinking water from heavy metals cause poisoning and high ambient air concentrations near emission sources. Immunotoxic, genotoxic, cytotoxic, hepatotoxic, nephrotoxic and endocrine disruption are all impacts of heavy metal accumulation in vertebrates. Biochemical markers were found to be useful indicators of stress induced consequences of heavy metal toxicity because they were linked to the animal's oxidative stress systems.

Heavy metals are precarious because of their proclivity of bioaccumulation. Bioaccumulation means an increase in the concentration of a chemicals in a biological organism. When compounds enter into the body they stored quickly as compared to being expelled out. Therefore, they accumulate in the body. Unlike organic contaminants, heavy metals are non-biodegradation due to which they accumulate in living body. Most of them referred to as carcinogenic. Long-term exposure to heavy metals results in health problems [4].

It is noted that heavy metals have adverse effects on health and last for a long period of time, exposure of heavy metals is increasing throughout the world. Heavy metals are major pollutants of environment and the increase in their toxicity is the prominent problem for environmental, ecological, nutritional and evolutionary reasons. Heavy metals have been linked to a variety of illnesses. An allergic reaction is the most prevalent adverse health consequence of nickel in humans, followed by chronic bronchitis, lung and nasal sinus cancer, moderate pulmonary fibrosis and so on [5]. Manganese contamination causes psychomotor agitation and sudden death. It is also known as manganese toxicity, is a progressive illness that begins with minor symptoms and progresses to include dull affect, altered gait, fine tremor, and psychological disorders. Chromium poisoning has been linked to stomach upsets and ulcers, seizures, kidney and liver damage, and even death. Nickel, manganese, and chromium are metals that are released during combustion and spills and are linked to automotive pollution [5].

Cadmium (Cd) is a soft, ductile and silvery-white chemical metal. Its atomic number is 48. It belongs to the d block and period 5 of the periodic table's group. It is mined as a by-product of sulphide deposits, which are mostly made up of copper, zinc and lead. Cadmium is toxic to persons as well as the environment. When cadmium is present in low concentrations in the atmosphere, water, or food and is inhaled by humans, it may cause death. Cadmium is found in a variety of places in the environment. Mining and volcanic eruptions all expose individuals to cadmium as a poison through the earth's crust. Plants are the source of cadmium exposure in nonsmokers and persons who have never been exposed to it in the workplace. It is dangerous metals for human health. Cadmium acute exposures can cause inflammation, cough, nasal and throat dryness and irritation, headache, dizziness, chest pain, pneumonitis, and pulmonary edoema [6].

Chromium is present in large amount in environment in two valence states: trivalent chromium and hexavalent chromium. Chromium exposure can come from both natural and industrial sources. Chromium III is a significantly safer alternative to chromium. Chromium significantly effects the respiratory tract. The respiratory tract is primarily the target organ for both acute (short term) and chronic (long term) exposure. Humans require chromium to function properly. Acute exposure of chromium causes coughing, breath and wheezing while chronic exposure of chromium causes ulcerations, bronchitis, pneumonia, and impaired pulmonary functioning etc. Inhaled chromium is a human carcinogen, resulting in an elevated risk of lung cancer. Chromium has been demonstrated in animal tests to cause lung cancers when inhaled [7].

Nickel is a naturally present as silvery-white, shunt metallic element. It is fifth most important element on planet and is present in large amount in earth's crust and core. Nickel is present as trace element plants, animals, and oceans and is used in meteorites. Since nickel is a nutritionally important trace element for at least a few animal species, microorganisms, and plants, it can cause deficiency or toxicity symptoms. Wind-blown dust due to the weathering of rocks and soils, forest fires, volcanic emissions and vegetation are included in the natural sources of atmospheric nickel. Inexpensive jewellery, dental or orthopedics implants, stainless steel kitchen utensils and tobacco are all sources of lower levels of nickel in the environment. Despite the fact that nickel has a multitude of biological impacts, a deficient state in humans has yet to be described. Compounds of nickel have various commercial and industrial uses, and due to industrialization the pollution is gradually increasing to a drastic level. Although Nickel is ubiquitous and essential for the survival of many organisms, it is harmful to live organisms in particular locations due to anthropogenic emission and naturally changing quantities. Nickel poisoning is mostly caused by inhalation exposure in professional settings, immune system and respiratory tract is affected. Nickel is consumed with the intake of food and drinking water. It doesn’t affect individuals particularly those who deal with stainless steel and nickel plated products used in everyday life [8].

Cobalt is a bluish white d-block transition metal. Cobalt is present in first period of periodic table and found between iron and nickel. These two elements have several chemical and physical properties with cobalt. Ionic radii of cobalt ranges from 56 pm to 90 pm, depending on shape and surroundings. Cobalt is a compatible, transitional and siderophile element having a high melting point of 1495°C. The electronegativity of cobalt is 1.88. Vitamin B-12, commonly known as cobalamin, essential for animal and human health, growth and nutrition. The amount required is minuscule. In the typical population, dietary Cobalt intake is predicted to be between 5 and 40 mg per day. Cobalt exposure at high levels and on a regular basis can harm the neurological system and induce axonopathy. Cobalt dust inhalation can cause diffuse inflammatory reactions of the bronchial mucosa, as well as chronic respiratory tract illnesses. In large doses, some cobalt forms are carcinogenic [9] [10].

Manganese is fifth most abundant metal and twelfth most abundant element on earth. It is a silver gray, brittle, and glossy transition metal. All metals, including manganese, have natural and anthropogenic environmental sources. When harmful metals are released into the environment, even at low concentrations, they accumulate and have a long-term deleterious effect on living species. Despite the fact that the use of manganese-containing compounds is on the rise, nothing is known about the long term effects of manganese exposure from all sources in community settings [12]. Both a deficiency and an excess of manganese are harmful to one's health. Ingestion and inhalation can lead to severe central nervous system disease. Heart disease, skeletal abnormalities, hypertension, infertility and altered lipid and glucose metabolism are common symptoms of manganese deficiency [11].

Zinc is present in group IIb along with two other toxic elements of the periodic table cadmium and mercury. Zinc, unlike the other two metals, is a necessary trace element for all living things. It is one of the most extensively utilized metals and is necessary for life. Three major dietary groups which provide zinc consumption are meat, poultry, milk products, and cereals and cereal products, accounting for 68 percent of total zinc intake [13] [14]. Zinc has an important role in homeostasis, immunological function, oxidative stress, apoptosis, and ageing and substantial illnesses of public health concerns are linked to zinc deficiency. Concurrent zinc shortage can exacerbate the symptoms of a variety of chronic conditions, such as neurological disorders, atherosclerosis, cancer, ageing, autoimmune diseases, age-related degenerative diseases and Wilson's disease, by affecting immunological status, increasing oxidative stress and causing the production of inflammatory cytokines. It's found in roughly 300 enzymes and an even greater number of other proteins, indicating its importance to human health [15].

Copper is naturally found in metallic form as well as in the form of ores and minerals, and is regarded as one of the earliest metals used by humans. The ability to create bronze, which is made up of copper and tin in a 9:1 ratio, signaled the end of the Stone Age and the start of the Bronze Age. Copper along with its alloys are widely used in home and other plumbing systems, as well as in the production of cooking equipment. Copper is also used to make electrical wires and microelectronic devices, as well as electroplating and photography, roofing and act as a catalyst in the chemical industry. Exposed of copper is due to drinking water and ingestion of food. The proportion of copper consumed via food vs water varies by geography; in general, drinking water accounts for roughly 20–25 percent of copper consumption. Copper is both a necessary mineral for human health and a potentially harmful substance, depending on the amount consumed. Copper is associated with the health of bones, increased risk of infection and immunoglobin function [16].

Since not much work has been done to assess the pollution status of Sialkot and Gujranwala districts, by using frogs as pollutions indicators. Therefore, the research would be helpful in providing baseline data for future generations.

1.1 Study Area

The study areas were Sialkot and Gujranwala Districts Punjab, Pakistan.

Sialkot:

District Sialkot is one of Pakistan's districts. It is located in the Punjab province's northeast. Sialkot city is the district's capital. It has 32°29″ 34″ N, 074°31″ 53″ E geographical coordinates. District of Sialkot covers an area of 3,016 km².

During the summer and winter, Sialkot is hot and humid. The warmest months are June and July. In winter the greatest winter temperature might decrease to −2 °C. The earth is usually flat and fruitful. The yearly average precipitation is approximately 1000 mm. The district has more than 25.82% of its population [17].

Figure 1.1: Map of Sialkot District

Sialkot is referred as one of the largest producers of wheat, sugarcane, tobacco, millet, vegetables, fruits and rice in the world. Major crops grown are rice, wheat, cotton and sugarcane. Despite its remarkable and significant production, the country is still striving with high levels of food insecurity. Potatoes and sunflower are prominent among the minor crops in the district. Most of the land allocated for oilseeds and vegetables have unfortunately fallen prey to mushroom growth of housing societies emerging around urban centers [17].

Gujranwala:

Gujranwala District is located between 32.161N and 74.181E, it is bordered on the west by Sheikhupura, on the south by Gujarat and Lahore. Gujranwala District is 226 meters above sea level and covers 3198 m² with a population of 1,961,360 people. During winter temperature drops to zero while three months May, June and July are the hottest. Grasslands have been changed to bush lands and over grazing is the common cause of this transformation whereas rich diversity of herbs and shrubs are present which plays significant role in health care system. Canal irrigation is the major source of cultivation. The soil is rich in medicinal plants and the vegetation is in the form of grasslands and bush lands. Vegetation pattern has shown significant variation in last few years.

Woodlands have been replaced by crop farming areas. The crops grown include wheat, cotton, rice, barley and millet [18].

Figure 1.2: Map of Gujranwala District

AIMS AND OBJECTIVES

The aims of the research were:

- to study the morphological characteristics of Indian Bullfrog species collected from the selected study areas.
- to analyze heavy metals (Cadmium, Chromium, Manganese, Nickel, Zinc, Cobalt and Copper) in the water samples collected from the respective study areas.
- to analyze the above mentioned metals in the liver and skin of Indian Bullfrog species.

RATIONALE

Indian Bullfrog is considered to be the largest frog inhabiting the Pakistani plains. It is considered as a key stone species and an indicator of pollution of the respective area where it is found. The study aims to provide future researchers with baseline data on the status of heavy metal pollution occurring in Gujranwala and Sialkot districts and will further aid them in developing sustainable methods in order to reduce heavy metal pollution there in the respective study areas.

CHAPTER 2

LITERATURE REVIEW

Frogs are used as bio indicators of environment. The changes in freshwater and terrestrial environment mainly affect the frogs. As a result, they are valuable bio indicators of environmental quality and change. Frogs have been utilized as bio indicators in the past, but their use isn't common. Frogs (and especially tadpoles) are known to be sensitive to a variety of water-borne chemicals, making them good candidates for bio-indicators. Heavy metals are abundant in the Earth's crust. They cannot be broken down. They enter into our body in minute amount from breathing, drinking water and food.

In 2008, O.B. Stoylar *et al*., studied about seasonal and regional changes that were explored for the heavy metals in the liver of frog *Rana ridibunda*. Weight of the liver were seen to rise between spring and October. The metal concentration was detected in frog liver in the order >Cu>Zn>Mn>Cd. The highest concentration of metals in the frogs were discovered in urban areas. In the spring, Copper was shown to be greatest in the liver, the highest level in the agricultural location. During the summer, other metals were observed. It was likely that in the wetland there were pollutant discharge of Copper fungicides in the rural area. Iron high content reflects its water fluctuations. Copper bio-availability was almost 1000 times higher in comparison to other metals. Cadmium is discovered in the liver of frogs despite its very low quantity of water. This is an indicator that, despite the very low levels measured in water, tissues collect Cadmium. This can be a sign that frogs are exposed to Cadmium intermittently and other heavy materials [19]. Another research was done by Jayawardena UA, Angunawela P. in 2017 about the contamination of heavy metals, particularly in amphibians and their detrimental consequences on wetland biota. The study assessed the biochemistry and histopathology of the *E. hexadactylus* and laboratory exposure groups, i.e., 28 days of exposure to a mixture of zinc, lead, chromium, copper and cadmium in the metals contaminated. The loss of structural and functional integrity of hepatocyte membrane are added to considerably elevated alanine transaminase, transaminase aspartate, alkaline phosphates and glycemic glutamide transferase in frog liver homogeneity. Signification of hepatic and renal failure indicated dramatically reduced whole serum albumin and protein and high level of urea and creatinine in frogs. The findings confirm metabolic abnormalities associated to histopathology as potential indicators [20].

In 2010, Anyakora CA and *et al.* researched about contamination of heavy metals of the ground water in the district of Lagos. WHO states 0.2, 0.003 and 0.01 mg/L as the maximum levels of contaminant (MCL) for lead, aluminum and cadmium. However, 93.88 per cent of the tests indicated that the metals were present in the analysis. About forty-nine water wells and borehole samples were evaluated for lead, aluminum and cadmium through Atomic Absorption Spectrophotometer. The values were compared with the maximum contamination levels defined by the WHO. Cadmium was present in more than 38% and cadmium values were 32.65% more than MCL in the samples. Due to the harmful effect of these metals the manual dug well or borehole is the sole source of their water supply, Therefore the study highlights an important concern for the community [21].

In 2014, Taiwo IE, Henry AN, Imbufe AP, Adetoro OO studied that human activities gradually effect animal population in urban areas with heavy metal contamination. The aquatic amphibians bear permeable skin due to which they are exposed to heavy metal contamination. The activities of reduced level of lipid peroxidation product, glutathione (GSH) and superoxide dismutase (SOD) were examined in sampled frog's liver. Zinc was the accumulated metal, followed by nickel, copper and iron [22]. In 2013, Shaapera U, Nnamonu LA, performed a research and investigation showed statistically significant quantities of metals in frog's liver, skin and intestines. Generally, levels of lead, chromium, iron and manganese in all organs were higher than the maximum tolerance of sample sizes except for cadmium, copper and zinc [23]. In 2015, Cobbina, Duwiejuah *et al*. researched about heavy metal levels in drinking water sources that were measured in two small-scale mining settlements in northern Ghana (Nangodi and Tinga). In the two mining settlements, 72 samples of water were collected from hand dug wells, boreholes, dugouts, and a stream. An atomic absorption spectrophotometer was used to measure zinc (Zn), cadmium (Cd), arsenic (As), mercury (Hg) and lead (Pb) levels by atomic absorption spectroscopy (AAS). The value of heavy in water samples from the Nangodi and Tinga communities recorded were as follow: 0.038 and 0.064 Mercury (Hg), 0.031 and 0.002 Arsenic (As), 0.250

and 0.031 Lead (Pb), 0.034 and 0.002 Zinc (Zn), and 0.534 and 0.023 Cadmium (Cd). In general, levels of zinc (Zn), lead (Pb), cadmium (Cd), mercury (Hg) and arsenic (As) in water from Nangodi surpassed WHO drinking water standards of 0.010 for Mercury, Arsenic, and Lead, 3.0 for Zinc, and 0.003 for Cadmium, and levels of Mercury, Lead, and Cadmium observed in Tinga surpassed WHO standards. Residents in these mining areas may be at danger of serious health problems if they drink water that has been contaminated with high amounts of Mercury, Arsenic, and Cadmium. It is advised that the drinking water sources quality in these two localities must be monitored on a regular basis [24].

In 2021, Anyanwu and Adetunji, carried out a research to find out the presence and amounts of heavy metals in spring waters and whether they were safe to drink. There were eight heavy metals recorded (Nickel, Cadmium, Copper, Manganese, Zinc, Iron, lead).Nickel, lead, copper, iron, zinc, cadmium and manganese levels were found to be beyond permitted levels, which might be attributable to a geogenic source aggravated by seasonal and human factors. The concentrations of some of these metals were significant, posing a major health danger to spring water consumers. As a result, while spring waters are not acceptable for drinking, they can be used for other household purposes [25].

In 2016, Bibi, Khan *et al*. examined the presence of various elements in the drinking water of the district of Lakki Marwat. Iron, lead, zinc, chromium and cadmium concentrations in water samples collected from seventeen different regions in the Lakki Marwat district were studied. Iron concentrations were found to be over normal in all samples, with the highest concentrations in the Wanda and Machen Khel districts of Lakki Marwat. Zinc, on the other hand, was found to be well below the recommended levels in all places. The highest concentration of lead was identified in Kaka Khel, while the lowest concentration was observed in the Bego Khel and Mir Janan areas. Cadmium levels were found to be higher than the WHO threshold in eight locations, while the remaining locations had lower cadmium levels. Thirteen areas exhibited lower chromium concentrations, whereas the remaining locations had chromium concentrations above WHO guideline limits. The current investigation shows that iron levels in drinking water in the district of Lakki Marwat frequently surpass the recommended levels, while the remaining elements are found in different quantities in different regions [26].

In 2015, Priyadarshani S. and *et.al* studied about the effects of heavy metal poisoning of the Indian green frog's immune system, they were explored in Bellanwila Attidiya, an urban wetland polluted with high levels of heavy metals, and compared to a reference site in Bolgoda, Sri Lanka. According to Atomic Absorption Spectroscopy cadmium (Cd), lead (Pb), zinc (Zn) and copper (Cu) accumulation in frog's liver and gastrocnemius muscle was significantly higher in the polluted site than in the reference site. Standard immunotoxicity tests were performed, including total white blood cell, bone marrow cell counts, splenocyte and spleen weight/body weight ratio, neutrophil/lymphocyte ratio, and basal immunoglobulin levels. The polluted location's frogs had much lower test parameters than their counterparts in the reference site, indicating that the former site's frogs had a poorer immunological response. In vitro exposure of frog phagocytes to Copper, Zinc, Lead, and Cadmium at concentrations of 10-2– 10-10 M demonstrated immunomodulation, with low concentrations stimulating phagocytosis and increasing concentrations showing a trend toward immunosuppression. The decreasing order of phagocytosis inhibition potential was Cd>Zn>Cu>Pb based on IC_{50} values. In conclusion, heavy metalstimulated immunomodulation of *E. hexadactylus* was convincingly proven in the work. The potential of phagocytosis as a biomarker in Ecoimmunotoxicology to detect aquatic heavy metal pollution was plainly proposed by in-vitro investigations [27].

In 2008, Hasan M. and *et.al* collected samples from three populations in Khulna (southern part), Banglades, Char Nilokhia, and Netrokona (central portion) to determine the morphological and genetic variation of the Indian bullfrog *Hoplobatrachus tigerinus*. The Khulna population had significantly higher proportions of eye length and tympanum diameter (EL: TD), internarial space and interorbital distance (IN: IOD), head length and snout length (HL: SL), head length and head width (HL: HW) and internarial space and distance from nostril to tip of snout (IN: NS) than the other two populations (p 0.05). Four enzymes were examined for the allozyme investigation, and five presumptive scorable loci were discovered. The Khulna population had the highest mean proportion of polymorphic loci (0.95), mean number of alleles per locus, and mean number of heterozygous loci per individual (60 percent, 1.60 and 14.0 percent) respectively. Between the Khulna and Netrokona populations, the pairwise genetic divergence (FST and Neis D) was the highest (0.279 and 0.157) respectively. The Char Nilokhia and Netrokona populations formed one cluster, while the Khulna population alone formed another, according to the Unweighted Pair Group Method with Arithmetic Mean dendrogram. These findings suggested that Khulna population diverged genetically and physicaly from the other two populations. To fully understand all elements of difference in *H. tigerinus* from Bangladesh, more research would be required [28].

In 2011, Marques SM. And *et.al* studied about the human mining activities that result in severely damaged areas that remain poisoned for extended periods of time, resulting in extreme ecosystems. The formation of metal-rich effluents is a common feature of mining sites. The chemical and physical properties of water from a deactivated uranium mine pond and a reference site, as well as their metal concentration, were determined in this study. Furthermore, metal deposition in the liver and skin of *Pelophylax perezi* from reference site and *P. perezi* from deactivated uranium mine pond was evaluated and compared. The activities of catalase, glutathione reductase, glutathione peroxidase and glutathione-S-transferases in the liver, lungs, heart and kidney were also measured. In addition, the thiobarbituric acid reactive substances assay was used to quantify lipoperoxidation in the same tissues, and the activity of lactate dehydrogenase was measured in muscle. The majority of metals were present in higher amounts in the tissues of deactivated uranium mine pond species, according to findings. This tendency was particularly noticeable for whose content differed by 1350 times between reference site and deactivated uranium mine pond organisms. Despite the fact that none of the organs evaluated for antioxidant defenses exhibited Left Posterior Oblique, all organs from the deactivated uranium mine pond frogs showed enhanced total seleniumdependent Glutathione Peroxidase and Glutathione Peroxidase activity with the exception of the liver. However, only the lung showed a significant response, most likely as a result of the strong inhibition of Continuous Auto Transmission System upstream and the concomitant increase in H_2O_2. In frogs from the uranium mine area, the anti-oxidant stress system was found to be more sensitive in the lungs [29].

In 2017, Jing X. and *et.al* studied that pesticides have long been considered to be a contributing cause in the loss of amphibian populations. The major metabolites fenoxaprop (FA) and 6-chloro2, 3-dihydrobenzoxazol-2-one (CDHB) were examined. The enantiomers' acute toxicity and nontoxicity to tadpoles were also investigated. FE was not identified in frogs after oral administration or exposure to an aqueous solution, but FA generated was accumulated in the liver, kidney, eggs, brain, thigh muscle, blood and skin, with R-FA accumulating preferentially. The occurrence of FA in frog eggs show maternal transmission and potential effects on offspring in females. FA elimination in frog tissues was also enantioselective, with R-FA (kidney) or S-FA (liver) metabolism taking precedence (liver, eggs, skin, muscle and whole blood). After aqueous solution exposure, FE and FA were barely detectable in tadpoles, whereas CDHB was accumulated and removed as first-order kinetics with a half-life of 37.1 h. The enantioselective acute toxicity and nontoxicity were assessed using tadpole mortality and micronucleus rate in peripheral blood erythrocytes. CDHB was the only substance that caused significant acute toxicity in tadpoles [30]. In 2015, Qureshi IZ. And *et.al* investigated the concentration of Chromium, Manganese, Lead, Cadmium, Copper, Nickel, Iron and Zinc in two frog species, the *Rana tigrina* captured in industrial waste water in the town of Sialkot, in selective body tissue (liver, stomachs, lungs, heart, kidneys, skeleton muscles and liver). The darker color, distinct wet body weight and length of the frog was distinctly different in the two species. The results showed that in the samples taken from industrial sites, heavy metal concentrations were elevated relative to nonindustrial areas. There were few significant variations between two sites in the different tissues of *R. tigrina*. Heavy metal contents in *R. tigrina* tissues were higher than *E. cyanophlyctis*. *R. tigrina* showed relatively higher mean content of Manganese, Nickel, Iron, Copper, Chromium and Cadmium which were higher in both species as compare to other bodies in liver and kidney concentrations. In addition, there was a decrease in weight and body length as well as increased buildup of metal from frogs gathered from industry facilities. The results help the conservation authorities for these frog species that are affected by heavy metal pollution [31].

Akyengo O, Yakubu A, Adejube AA. Studied about the bioaccumulation of heavy metals in Bullfrog species. Samples of Indian bullfrogs from several river sample stations A (upstream) and B (downstream) located in Kumbotso Local Government Area, Nigeria have been examined for contaminants in the shape of heavy metals, such as chromium, cadmium, zinc and iron. For the testing of these heavy metals with an

Atomic Absorption Spectrophotometer, surface water and effluent discharg into the river from industry were analyzed. The outcomes got uncover that in a specific order Iron>Copper>Zinc>Chromium>Lead>Cadmium the centralization of those metals has developed for every species. Iron (Fe) in all samples studied had the highest documented concentration of heavy metals, and cadmium (Cd) had the lowest. In the following order Iron>Zinc>Chromium>Cadmium>Copper>Lead, the concentration of heavy metals measured by surface water was observed up and downstream (sample sites). Cadmium, copper, iron and chromium fixations from test point B of Indian frog over the W.H.O/F.A.O and F.E.P.A limitations, except for zinc, which was over the cutoff for Indian Bull Frog, when lead and zinc were inside those bodies. For both the samples in the sample point A, the concentration of heavy metals reaches the maximum permitted limits, with the exception of chromium and iron. The resulting bioaccumulative factor shows the copper factor of Indian Bull Frog in all sampling plants having the highest bio accumulative factor (B.A.F.) whereas cadmium had the lowest B.A.F. The detected heavy metal content demonstrate that Challawa is contaminated with heavy metals, which can clearly be recognized to the wastewater released from the industrial property of Challawa [32].

In 2021, Jayawardena UA and *et.al* studied the significant issue about the ecology and health concerns include heavy metal poisoning in the wetland habitats. A cytogenytoxicity study was performed in the Sri Lankan Bellanwila-Attidiya Health Sanctuary (BAS) employing a battery of cytogeny-toxicity tests to assess cytogenotoxical toxicity of the previously demonstrated pollution (Cadmium, Chromium, Copper, Lead and Zinc daily <5 ppm each). Field controls did not show significant effect on bioassay, however individual to heavy metals reported lower effects than their combined exposure under laboratory conditions; $Pb^{2+}$was regarded as most toxic metal, with the highest mitotic inhibition ($Pb^{2+}>Cd^{2+}>Zn^{2+}>Cr^{6+}>Cu^{2+}$), mutagenic potential as evaluated in the percentage incidence of chromosomal aberrations ($Pb^{2+}> Zn^{2+}> Cu^{2+}> Cr^{6+}> Cd^{2+}$) and pytotoxicity evaluated by the incidence of cell apoptosis and necrosis ($Pb^{2+}>Cr^{6+}>Cu^{2+}>Cd^{2+}>Zn^{2+}$). Thus, the test battery of micro-nucleus and comet assays that reveal differential aspects of cytogeny-toxicity may act as a valuable tool in monitoring of environment, primarily to screen for complex environmental mixtures of heavy metals that may affect ecological health [33].

In 2016, Sriwastav AK and *et.al* studied that the awareness of the significant reduction of the amphibian population has been rising worldwide. There have been numerous explanations for the amphibian population decrease. A heavy metal cadmium is introduced to the terrestrial and aquatic environment both from natural sources. The toxicity of heavy metal cadmium to Indian *Rana cyanophlyctis* was assessed in this study. Four-day static renewal of acute toxicity tests were employed to determine LC50 values for cadmium. The concentrations of cadmium chloride recorded was as follow (15, 20, 25, 30, 35, 40, 45 and 50 mg/L) to five replicates of every 10 frogs. During various lengths of exposure (24, 48, 72 and 96 h), frog mortality was tested for the LC50 and 95% confidence levels using Probit POLO-PC software. CC50 values of Cadmium Chloride were 32,586, 29,994, 27,219 and 23,048 mg/L for frog *R. cyanophlyctis* at 24, 48, 72, and 96 h [34].

In 2016, Intamat S, Phoonaploy U, Sriuttha M, Tengjaroenkul B, Neeratanaphan L. investigated about the quality of water and the pollution of heavy metals in fish, frogs, water, sediments and the Bio-Accumulative- Factors found on fish and frogs across the gold mining zone. The samples for water quality were evaluated for temperature, pH, and dissolved oxygen. Samples for heavy metals were evaluated using optical emission spectrometry combined with inductive plasma. The quality of the water was within the standard units. Five frog species were gathered; the *pulchra Kaloula*, the *heymonsi Microhyla*, the *limnochari Fejerarya*, the *rugulosus Hoplobatrachus* and the *pulchra Microhyla*. The concentrations of heavy metals in frog were observed. TheBioAccumulativeFactors were frogs. Heavy metal build-up in the sediment was greater than in the water. Many aquatic species use heavy metals just near the gold mine in the environment [35].

In 2010, Aktar MW and *et.al* examined the surface water quality of the Ganga River surrounding Kolkata from November 2005 to October 2006. Samples were analyzed in order to determine the heavy-metal (Nickel, Chromium, Cadmium, Manganese, Iron, Copper, Zinc and Lead) concentrations of surface water at four different river Ganga locations, around the river Kolkata from two points (center of river and the discharge point) at each location for a number of physical and chemical parameters.

Out of 96 samples tested, at concentrations ranging from 0.013 to 5.49, 0.022 to 1.78, 0.003, 0.303, 0.005 to 0.293 and 0.045 to 0.24 mg L−1, Iron, Manganese, Copper, Zinc and Nickel were detected in samples 71, 47, 38, 60, and 45 respectively. From the range of 0.005 to 0.006 and 0.05 to 0.53 mg L − 1, cadmium and b were found in six and 21 samples. In any of the samples analyzed, however, Chromium was not identified. There were no major variations in the metals with regard to both sample sites and disposal sites. However, these metals were concentrated differently with the season, its concentration were lowered during the winter months [36].

In 2019, Kujwa TS and *et.al* examined the content of heavy metals (Cadmium,Copper, Manganese, Lead, and Zinc) from irrigation project Kadawa, Kano State, Nigeria in water and *crowned bullfrog (Hoplobatrachus occipitalis)* species. Atomic Absorption spectroscopy was performed using Buck Scientific VGP-210 Atomic Absorption Spectrometer (AAS) model (2008). The mean concentration in water samples for heavy metals in Copper, Manganese, Lead and Zinc was 0.11 mg/L, 0.18 mg/L, 0.26 mg/L and 3.65 mg/L, respectively. The accumulating sequence of metal was Zinc > Lead > Manganese > Copper in all organs. The greatest concentration in the lung was observed in Zinc (77.38 mg per kg), Lead (1.81 mg/kg), and Manganese (0.68 mg/kg), and Copper (0.07 mg/kg). In all the analyzed samples, cadmium was not discovered. In the range of 77.38 mg/kg – 18.10 mg/kg and 1.81 mg/kg – 0.13 mg/kg zinc were most accumulated in every organ / portion of the body. The lung and liver contained the greatest concentration of metals. The pattern for accumulation was lung > hepatitis < trunk > muscle > leg for Zinc, Lead and Manganese and hepatitis > lung > trunk > muscle > leg for Copper. The accumulation pattern was the same. Heavy metals bioaccumulate highly in the lung and the liver [37].

In 2016, Mahmood T, Qadosi IQ, Fatima H, Akrim F, Rais M. studied about the concentrations of metals in common skittering frog. From October 2010 to March 2012, at four separate times Samples were taken from three chosen polluted locations and a reference site. All metals examined indicated high levels of heavy metals from all polluted locations in river water as well as in liver of frogs. In particular, in all the matrices analyzed from polluted locations Manganese exhibited considerably greater amounts as compared to the reference site. Abnormal hepatocytes were found in the history of frog liver subjected to high metals, but their kidney areas revealed discontinuous glomeruli with dead or broken cells with inadequate cytoplasmic deposition forms. The study shows that the frogs in the Korang River are potentially at danger of environmental metal toxicity [38].

In 2012, Singare PU and *et.al* examined the measurement of sediments of Mithi River in Mumbai for hazardous heavy metals accumulated. The study was conducted for two years from 2009 to 2012 in three different settings along the Mithi river flow. Aluminium, Lead, Nickel, Mercury, Cadmium, Chomiumr and Manganese were the various heavy metals. The study results show that these hazardous heavy metals have a concentration level of 1.2-5.8 µg/g over the two evaluation years. The results are a clear indicator of a growing day-to-day amount of pollution in the Mithi River that has a detrimental influence on river biology. The results emphasize the necessity for continuous scientific monitoring and revision of existing pollution management techniques of various contaminants that adversely affect the ecosystem [39].

In 2019 Thanomsangad P and *et.al* conducted a research in water and on frogs surrounding a waste disposal site with heavy metals (Arsenic, Cadmium, Chromium, and Lead). Bio-species of three frog species were evaluated for the bio-accumulation factor (BAFs). The quantities of Cadmium and Lead in water samples and of Arsenic and Lead in e-waste dumping site soil samples had surpassed limits. In the muscles of three frog species, the amounts of heavy metals recorded was: Chromium > Lead > Arsenic > Cadmium, and no major variations except in Lead were seen across the frog species ($p<0.05$). The food quality criteria were only surpassed by the Chromium concentration. Due to water and soil absorption, the relative order in frogs of the BioAccumulative Factors was Chromium > Arsenic > lead > Cadmium which showed a higher uptake of water than from the soil. The health risk index and carcinogenic risk (CR) evaluation showed the possible health impacts of Arsenic, Chromium and Lead on human health by frog intake [40].

Jayawardena UA and *et.al* studied about the histopathological quantification of heavy metal mediated tissue damages detected in *Euphlyctis hexadactylus*. In comparison to a reference place in the Labuagama reservoir, the study measured the histopathological damages of Indian green frog *Euphlyctis hexadactylus*, which was naturally exposed to heavy metals of an urban, contaminated wetland, Bellanwila Attidiya sanctuary. Contaminated with Copper>Lead>Zinc>Cadmium at considerably high levels (~5 ppm; p<0.05), the water and frog tissues of the contaminated environment, were compared to a location of reference. Double stained micro-tome segments of frogs' livers, kidneys, and skin sections were prepared for histopathological studies, in order to measure the tissue damage of frogs by accurately defining a range of grades from 0 to 8 for increasing degree of organic historical injury. Sections of tissue of *E. hexadactylus* revealed numerous damage in the contaminated region whereas no such disturbances were found in their counterparts. Ashcroft's histological damage was shown to result in substantial liver tissue damage (1.4: t=3.38, p=.004). Mildly injured skin epidermis glandular cells (0.394: t= 1.93, p=0.074) were also observed. The study created a reliable technique for measuring tissue damage in a sentinel amphibian species caused by aquatic heavy metals [41].

In 2020, Nasir M and *et.al* examined that copper metal was determined by means of the Atomic Absorption Spectrometry technique in organs of frogs, caught from industrial and from a nonindustrial location, 27.56±9.92 μg/g, of Copper was found in the samples. The results of the study showed that in some countries frogs are effective biomarkers to evaluate heavy metal contamination levels of different sources of water [42].

In 2021, Santana E and *et.al* studied that the amphibians are particularly vulnerable to contaminants discharged into the natural environment. They examined the connection of physiological and morphological changes with air, water and soil pollution in *Rhinella ornata*, an Atlantic forest endemical toad, a hotspot for world biodiversity [43].

CHAPTER 3

METHDOLOGY

Samples were collected from the agricultural fields of two study areas namely; Gujranwala and Sialkot Districts and the following methodology was adopted:

3.1: Materials and Methods

PLAN OF WORK

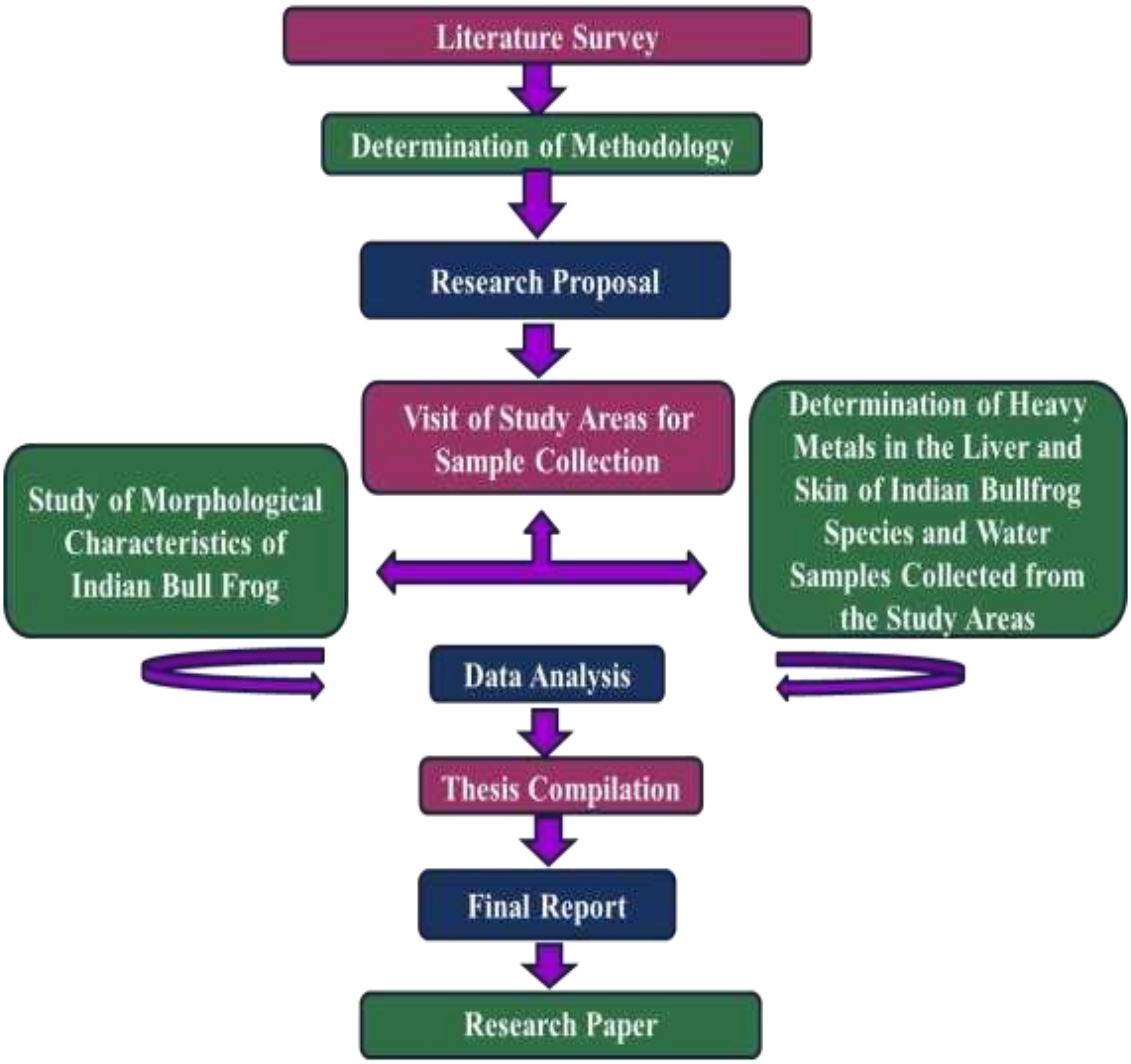


Figure 3.1: Flow Chart showing the Plan of Work

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.	Chloroform	500 ml
14.	Weighing Scale	Benchmark
15.	Measuring Tape	1
16.	Atomic Absorption Spectrophotometer	210 A BUCK Scientific
17.	Sweeping Nets	2
18.	Plastic Bucket Fitted with Nylon Mesh	2
19.	Muffle Furnace	***WiseTherm*** FHP Programmable Digital ***Muffle Furnace***
20.	Ceramic Bowls	10
21.	Nitric Acid (Concentrated)	200 ml
22.	Per chloric Acid	50 ml
23.	Sulphuric Acid	50 ml
24.	Pipettes	5

Table 3.2: Collected Samples and their Identification Codes

Sr. No	Sample	City	Identification Codes
1.	Sample 1	Sialkot	S1
2.	Sample 2	Sialkot	S2
3.	Sample 3	Sialkot	S3
4.	Sample 4	Sialkot	S4
5.	Sample 5	Sialkot	S5
6.	Sample 6	Sialkot	S6
7.	Sample 7	Sialkot	S7
8.	Sample 8	Sialkot	S8
9.	Sample 9	Sialkot	S9
10.	Sample 10	Sialkot	S10
11.	Sample 11	Gujranwala	G1
12.	Sample 12	Gujranwala	G2
13.	Sample 13	Gujranwala	G3
14.	Sample 14	Gujranwala	G4
15.	Sample 15	Gujranwala	G5
16.	Sample 16	Gujranwala	G6
17.	Sample 17	Gujranwala	G7
18.	Sample 18	Gujranwala	G8
19.	Sample 19	Gujranwala	G9
20.	Sample 20	Gujranwala	G10
21.	Sample 21	Gujranwala	G11

Table 3.3: Water Samples and their Identification Codes

Sr. No	Sample	City	Identification Codes
1.	Sample 1	Sialkot	SW1
2.	Sample 2	Sialkot	SW2
3.	Sample 3	Gujranwala	GW1
4.	Sample 4	Gujranwala	GW2

3.2: Sample Collection

Water samples were collected in thick plastic bottles from the selected sites, while adult Indian Bullfrogs, irrespective of gender were randomly captured from water ways using bait. Frogs were caught from agricultural fields of the study areas where small streams and water system (tube-well) containing ground water was present. The frogs were transported to the Kinnaird College Laboratory in plastic buckets covered with nylon mesh for aeration, half filled with water, for further analysis. [44].

3.3: Observation of the Morphological Characteristics of Frogs

The frogs collected from the respective study areas were weighed respectively using an electronic weighing scale and their body length and snout vent length was measured, using a measuring tape. Furthermore, the skin and the limbs of the frogs were carefully observed and analyzed for any signs of anomalies and the observations were recorded. The internal organs during dissection were carefully analyzed for any signs of abnormalities [46].

Figure 3.2: Steps for Observation of Morphological Characteristics of the Frog Samples for Signs of Anomalies

3.4: Sample Preparation

3.4.1: Water Sample Digestion:

In order to ensure the removal of organic impurities from the samples and thus prevent interference in analysis, the samples were digested with concentrated nitric acid. 10 ml of nitric acid was added to 50 ml of water sample in a 250 ml conical flask. The mixture was evaporated to half its volume on a hot plate after which it was allowed to cool and then filtered prior to heavy metal analysis [45].

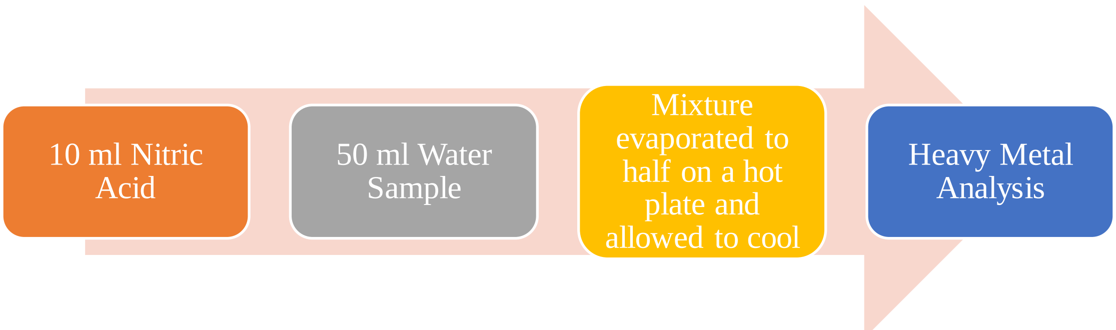


Figure 3.3: Steps for Water Sample Digestion

3.5: Organ Excision and Preservation

The frogs were carefully pinned down on a wooden board after being anesthetized using chloroform and were dissected from their ventral side. The skin was carefully excised from the body of the frog, by making first a horizontal, followed by ventral and lateral incisions. After that the tissues were cut in order to fully expose the internal organs of the frog and the liver was excised from the frog's body. Both the liver and skin samples were then being preserved in 10% buffered formalin till further analysis [47].

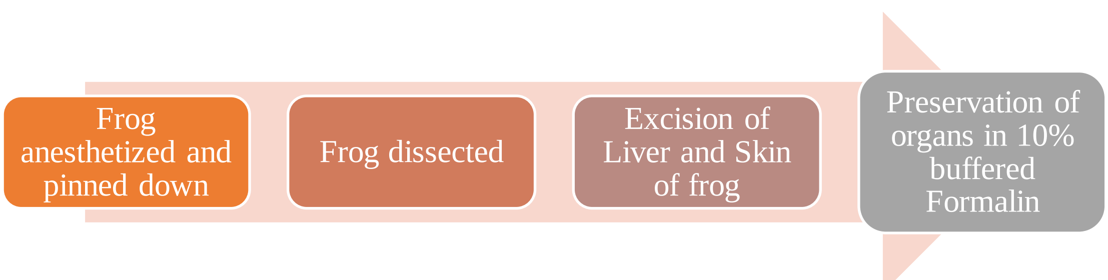


Figure 3.4: Steps for Organ Excision and Preservation of Frogs

3.6: Organ Digestion

Frog's liver and skin was burnt to ash at (600°C) for two hours in a muffle furnace and then acid digested ($1HNO_3$: $1H_2SO_4$). The digested samples were then filtered to remove any solid particles present and were later analyzed for the presence of heavy metals (Mercury, Zinc, Chromium, Nickel, Cobalt, Cadmium, Copper, Lead and Manganese) using an Atomic Absorption Spectrophotometer (AAS) [47].

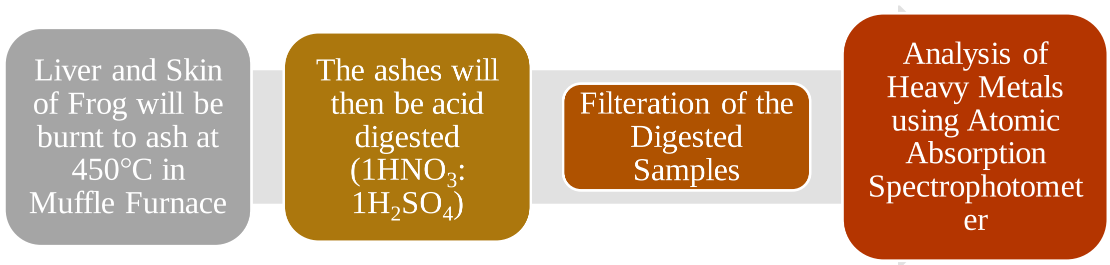


Figure 3.5: Steps Involved in the Organ Digestion of Frogs

3.7: Data Analysis

The obtained values of heavy metals present in the water samples and the above mentioned organs were then compared with the WHO Standards for Heavy Metals and the data was projected in the form of bar graphs, made with the help of Microsoft Excel. Wavelength of each of Heavy Metals is 228.8 nm for cadmium, 213.9 nm for zinc, 324.8 nm for copper, 279.5 nm for manganese, 240.7 nm for cobalt, 232 nm for nickel and 357.9 nm for chromium [48].

Table 3.4 Wavelength of Heavy Metals

Heavy Metals	Wavelengths(nm)
Cadmium	228.8
Chromium	357.9
Copper	324.8
Manganese	279.5
Cobalt	240.7
Nickel	232.0
Zinc	213.9

CHAPTER 4

RESULTS AND DISCUSSION

Samples of Frogs were collected from the agricultural fields of Gujranwala and Sialkot Districts and the following results were obtained.

4.1 Detection of Morphological Characteristics of Indian Bull Frog

Morphological characteristics of collected samples (11 from Gujranwala and 10 from Sialkot) were observed. The morphological result examined were frog skin color, snout vent length, body length, liver color, weight, heart condition, forelimbs and other observable characteristics on skin, prior to dissection, their snout vent length and body length were measured their photographs were taken. The results showed that all frogs had normal and standard body length, snout vent length, skin color and most had normal liver and heart appearance except for a few. The heart and skin appeared morphologically normal. No limb anomalies were observed in the samples.

Table 4.1: Identification of Morphological Characteristics of India Bullfrog Species

Sr No.	Specimen Number and Area	Skin colour	Weight	Total Length	Snout Vent Length	Other Observable Characters on Skin	Limb Anomalies Observed	Heart Condition
1.	Specimen 1 Gujranwala	Dark muddy green with dark green patches on dorsal side.	0.177 kg	11 inches	5.5 inches	Reddish colored webbed feet	No	Normal
2.	Specimen 2 Gujranwala	Dark muddy green skin colour with dark green patches on dorsal side	0.250 kg	12 inches	6.5 inches	No	No	Normal
3.	Specimen 3 Gujranwala	Dark green and muddy green colour with dark green patches on dorsal side	0.257 kg	12 inches	6.5 inches	No	No	Normal

4.	Specimen 4 Gujranwala	Dark and muddy green colour with brown and yellow patches on dorsal line	0.236 inches	12 inches	6.25 inches	No	No	Normal
5.	Specimen 5 Gujranwala	Dark muddy green colour with a tinge of light green and yellow patterns on dorsal side	0.165 inches	10.5 inches	5 inches	No	No	Normal
6.	Specimen 6 Gujranwala	Dark and muddy green with dark green patches and on dorsal and ventral side	0.123 kg	12 inches	5.25 inches	No	No	Normal
7.	Specimen 7 Gujranwala	Dark muddy green colour with yellow and green patches on dorsal side	0.255 kg	11.9 inches	6.25 inches	No	No	Normal
8.	Specimen 8 Gujranwala	Dull green with muddy green patches on dorsal side	0.261 kg	11.5 inches	5.8 inches	Vocal pouches prominent	No	Normal
9.	Specimen 9 Gujranwala	Dark and muddy green colour with dark green patches on dorsal side	0.162 kg	10.5 inches	4.75 inches	No	No	Normal
10.	Specimen 10 Gujranwala	Light muddy green in colour	0.150 kg	10.2 inches	5.2 inches	Vocal pouches are prominent	No	Normal
11.	Specimen 11 Gujranwala	Dark green with black patches on dorsal side	0.09 kg	9.1 inches	4.7 inches	Vocal pouches are prominent	No	Normal
12.	Specimen 1 Sialkot	Muddy green color	0.406 kg	13 inches	6.9 inches	No	No	Normal
13.	Specimen 2 Sialkot	Dark muddy green colour patches on dorsal side	0.16 kg	10.2 inches	5.5 inches	Vocal pouches prominent	No	Normal

14.	Specimen 3 Sialkot	Muddy to dark green colour, black patches on dorsal side	0.202 kg	11 inches	5.9 inches	Vocal pouches prominent	No	Heart enlarged
15.	Specimen 4 Sialkot	Dark muddy green patches on dorsal sides	0.173 kg	10.5 inches	5.5 inches	Vocal pouches prominent	No	No
16.	Specimen 5 Sialkot	Muddy light green in color dark green patches on dorsal side	0.163 kg	11.25 inches	5.5 inches	No	No	Normal
17.	Specimen 6 Sialkot	Dark muddy green colour yellow pattern on dorsal side	0.227 kg	11.5 inches	5.9 inches	Vocal pouches prominent	No	Normal
18.	Specimen 7 Sialkot	Dark muddy green colour on dorsal side	0.161 kg	10.25 inches	5.3 inches	Vocal pouches prominent	No	Normal
19.	Specimen 8 Sialkot	Dark muddy green colour with dark green patches on dorsal side	0.220 kg	11.75 inches	5.95 inches	Vocal pouches prominent	No	Normal
20.	Specimen 9 Sialkot	Dark muddy green with dark patches on dorsal side	0.157 kg	10.1 inches	5.25 inches	No	No	Normal
21.	Specimen 10 Sialkot	Dark muddy green with dark green patches.	0.175 kg	11.1 inches	5.5 inches	No	No	Normal

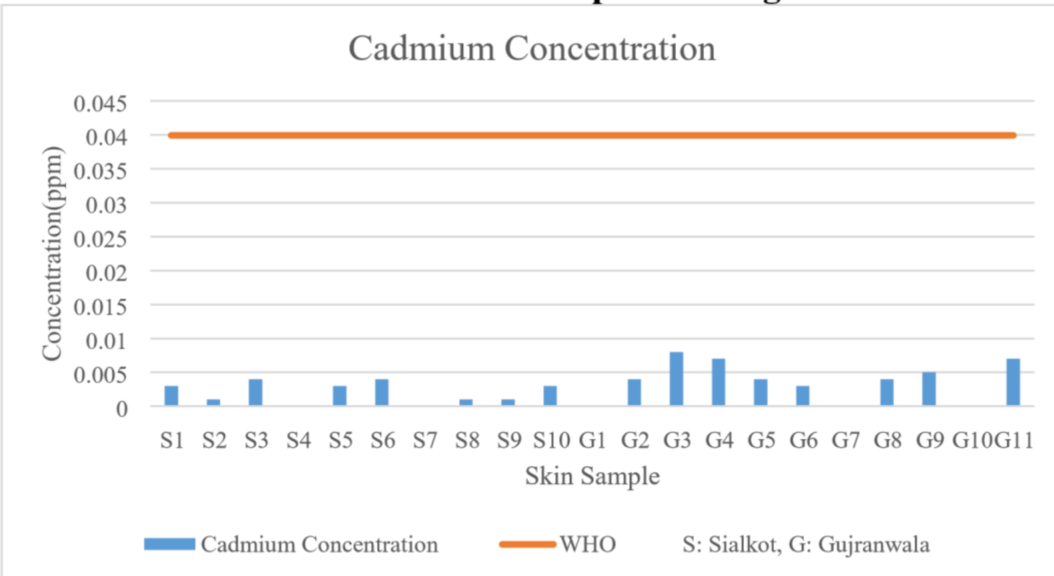
4.2: Concentration of Heavy Metals in the Skin and Liver Samples of Indian Bullfrog Species

Table 4.2.1: Concentration of Heavy Metals in Skin Samples of Indian Bullfrog Species:

Table 4.2: Concentration of Heavy Metals in Skin Samples of Indian Bullfrog Species from Gujranwala and Sialkot Districts

Sr. No	Sample	Cd	Cr	Ni	Co	Cu	Mn	Zn
	WHO	**0.04**	**0.07**	**0.2**	**0.05**	**2**	**0.5**	**5**
1.	S1	0.003	0.000	0.000	0.008	0.000	0.000	0.001
2.	S2	0.001	0.001	0.003	0.001	0.001	0.001	0.005
3.	S3	0.004	0.000	0.001	0.000	0.002	0.008	0.009
4.	S4	0.000	0.004	0.008	0.010	0.000	0.009	0.005
5.	S5	0.003	0.005	0.007	0.000	0.000	0.010	0.001
6.	S6	0.004	0.006	0.009	0.007	0.001	0.008	0.009
7.	S7	0.000	0.000	0.008	0.005	0.002	0.013	0.004
8.	S8	0.001	0.004	0.001	0.004	0.004	0.010	0.003
9.	S9	0.001	0.005	0.000	0.006	0.003	0.007	0.009
10.	S10	0.003	0.006	0.002	0.004	0.001	0.008	0.008
11.	G1	0.000	0.000	0.001	0.003	0.005	0.001	0.001
12.	G2	0.004	0.008	0.001	0.006	0.001	0.003	0.005
13.	G3	0.008	0.000	0.002	0.000	0.001	0.004	0.001
14.	G4	0.007	0.003	0.004	0.002	0.001	0.001	0.002
15.	G5	0.004	0.002	0.003	0.001	0.000	0.002	0.001
16.	G6	0.003	0.004	0.000	0.008	0.008	0.001	0.001
17.	G7	0.000	0.001	0.007	0.001	0.002	0.007	0.001
18.	G8	0.004	0.001	0.008	0.002	0.001	0.008	0.004
19.	G9	0.005	0.002	0.006	0.001	0.000	0.000	0.005
20.	G10	0.000	0.001	0.004	0.001	0.001	0.001	0.001
21.	G11	0.007	0.004	0.002	0.000	0.000	0.000	0.009

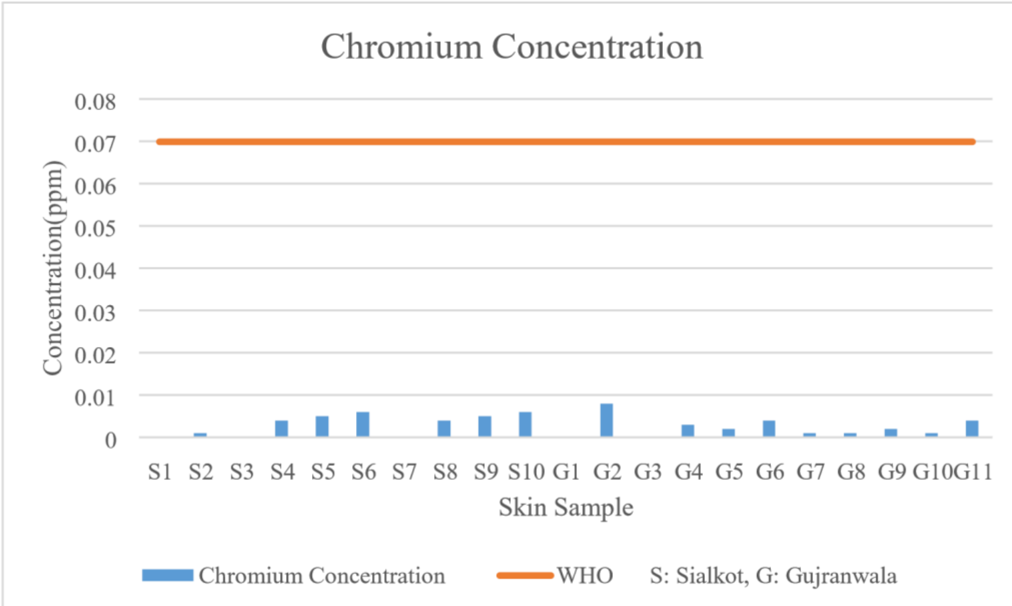
Cadmium concentration in Skin Samples of Frogs:



Graph 4.1: Cadmium concentration in Skin Samples of Indian Bullfrog

This graph shows the concentration of Cadmium in skin samples of Indian Bullfrogs of Gujranwala and Sialkot Districts. The concentration of cadmium did not exceed the WHO permissible limit which is 0.04 ppm. The highest value recorded in Gujranwala's sample 4 and 11 was 0.007 ppm. The main sources of cadmium are welding, burning of coal and municipal waste. Since the frogs were collected from ponds containing groundwater and both the study areas were industrial areas, therefore heavy metals were in fewer concentration in skin samples of frogs because they are slightly soluble in skin [49] [50].

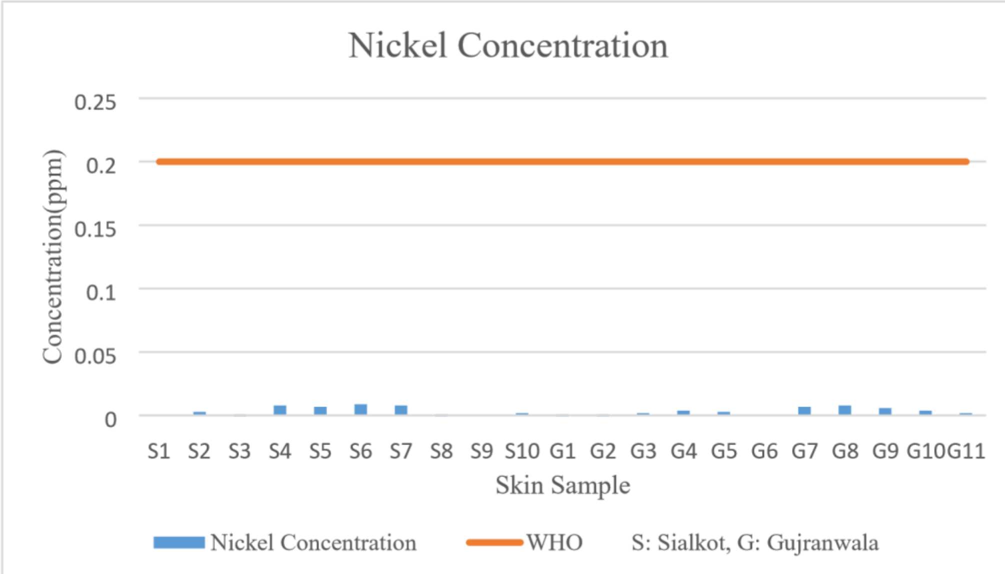
Chromium Concentration in Skin Samples of Frogs:



Graph No: 2 Chromium Concentration in Skin Samples of Indian Bullfrog

The graph shows the concentration of Chromium in skin samples of Indian Bullfrogs of Gujranwala and Sialkot Districts. The concentration of Chromium did not exceed the WHO permissible limit which is 0.07 ppm. Maximum value of chromium was observed as 0.008 ppm in Gujranwala's sample G2. This is maybe because when tannery wastewater passes through the agricultural fields of Gujranwala it increases its pollution level, particularly level of chromium [49] [50].

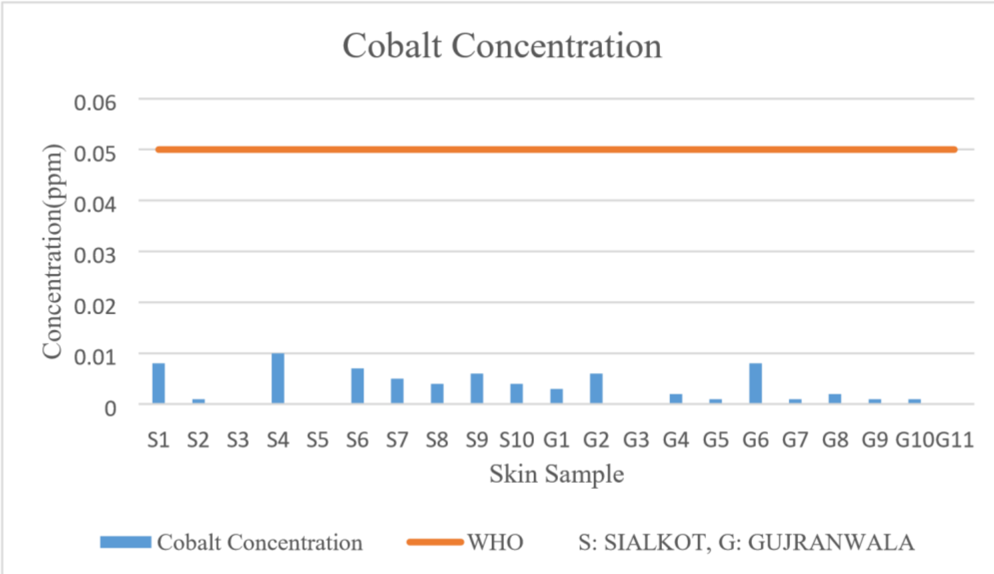
Nickel Concentration in Skin Samples of Frogs:



Graph 4.3: Nickel Concentration in Skin Samples of Indian Bullfrog

The graph shows the concentration of Nickel in skin samples of frogs of Gujranwala and Sialkot Districts. The highest value was 0.009 ppm in Gujranwala's sample 7. The concentration of nickel did not exceed WHO permissible limit which is 0.2 ppm. The sources of nickel are incineration of waste and sewage and combustion of coal, diesel oil and fuel oil. The heavy metals were in fewer concentration in skin samples because they are slightly soluble in skin [49] [50].

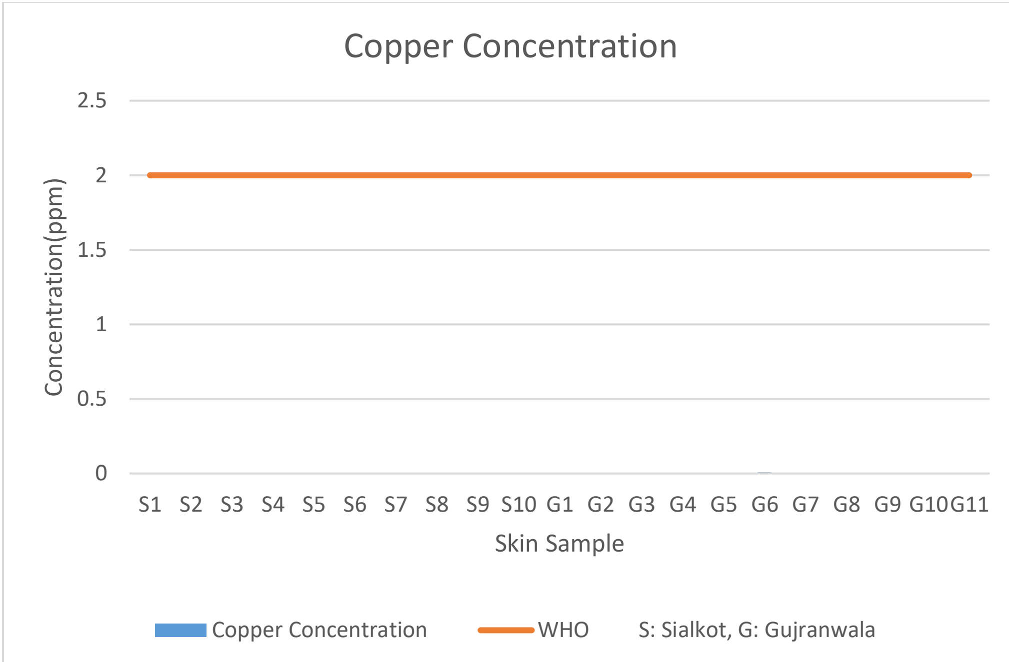
Cobalt Concentration in Skin Samples of Frogs:



Graph 4.4: Cobalt Concentration in Skin Samples of Indian Bullfrog

The graph shows the concentration of Cobalt in the skin samples of frogs of Gujranwala and Sialkot Districts. Maximum concentration of Cobalt was observed in S1 which was 0.011 ppm. The concentration of cobalt did not exceed the WHO permissible limit which is 0.05 ppm. Since the frogs were collected from ponds containing groundwater, therefore due to untreated water and solid waste being dumped into ground, heavy metals were present in frog's organs. The sources of cobalt are combustion of coal, industrial processes, incineration of waste and sewage etc [49] [51].

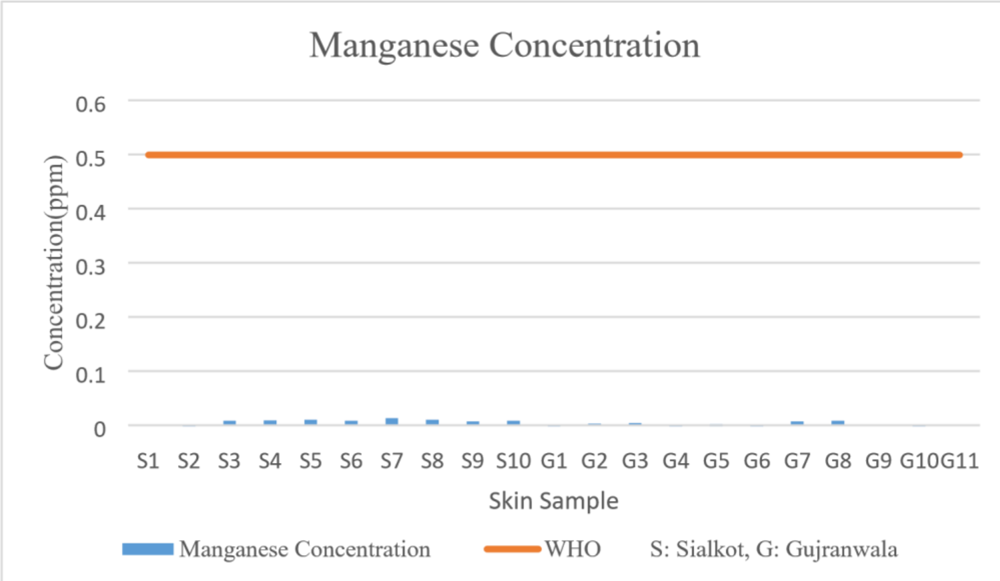
Copper Concentration in Skin Samples of Frogs:



Graph 4.5: Zinc Concentration in Skin Samples of Indian Bullfrog

The graph shows the concentration of Copper in the skin samples of frogs collected from Gujranwala and Sialkot Districts. The maximum concentration of Cobalt was observed in S1 which was 0.009 ppm. The Copper concentrations were well below the WHO permissible limit of 2 ppm in the skin samples of frogs. The concentration of copper was less in skin samples because copper is slightly soluble in skin [49] [51].

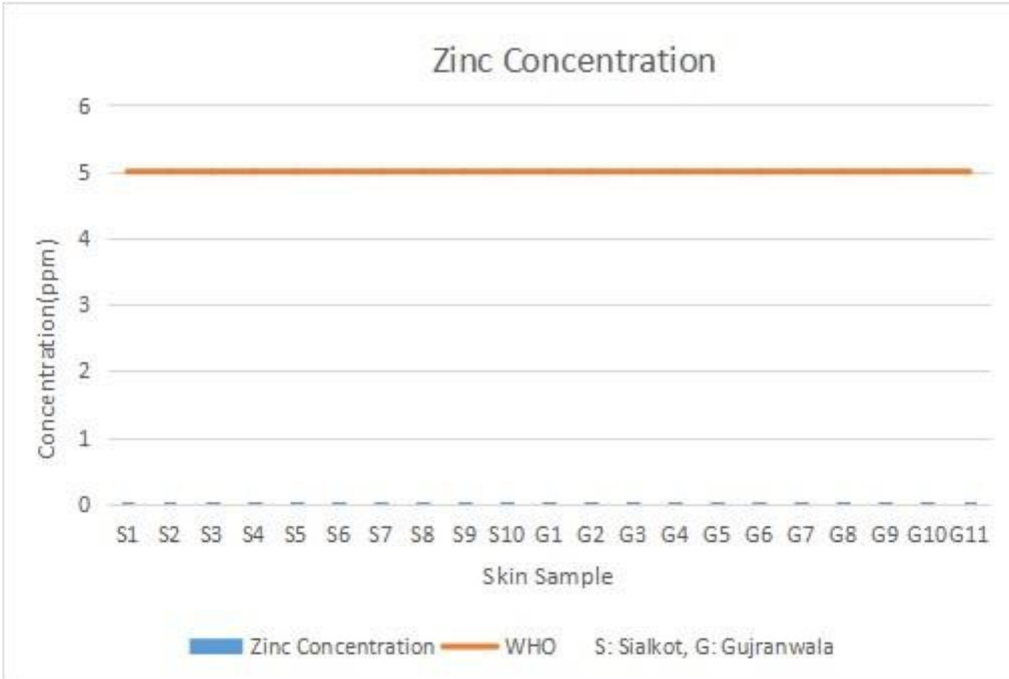
Manganese Concentration in Skin Samples of Frogs:



Graph 4.6: Manganese Concentration in Skin Samples of Indian Bullfrog

The graph shows the concentration of Manganese in the skin samples of frogs of Gujranwala and Sialkot Districts. Manganese was present in very small quantity in all the samples. Concentrations did not exceed the WHO permissible limit 0.5 ppm. The highest concentration was 0.013 ppm in Sialkot sample 7. The sources of magnesium include industrial production, mining, untreated sewage sludge and diffuse sources such as metal piping, traffic etc [49] [52].

Zinc Concentration in Skin Samples of Frogs:



Graph 4.7: Zinc Concentration in Skin Samples of Indian Bullfrog

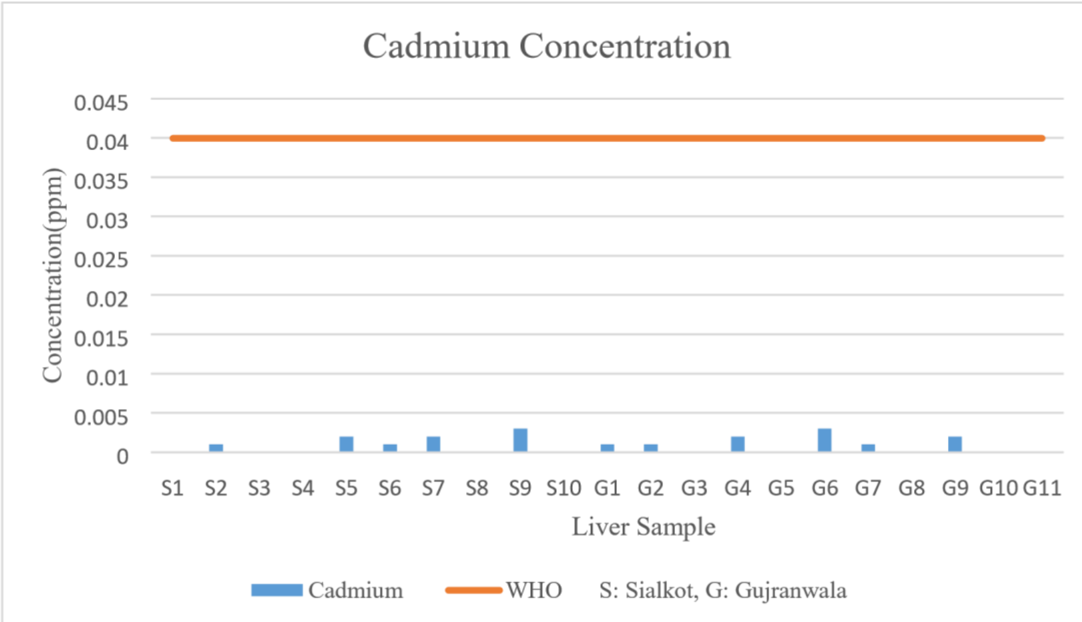
The graph shows the concentration of Zinc in the skin samples of frogs of Gujranwala and Sialkot Districts. Zinc was present in very small quantity and its concentrations did not exceed the WHO permissible limit 5 ppm. The highest concentration was 0.009 ppm. The potential sources of zinc include industrial wastes, agricultural runoff, aging water supply systems, mining etc [49] [52].

4.2.2: Concentration of Heavy Metals in Liver Samples of Indian Bullfrog Species

Table 4.3: Concentrations of Heavy Metal in Liver Samples of Indian Bullfrog Species Collected from Gujranwala and Sialkot Districts

Sr. No	Sample	Cd	Cr	Ni	Co	Cu	Mn	Zn
	WHO	**0.04**	**0.07**	**0.2**	**0.05**	**2**	**0.5**	**5**
1.	S1	0.000	0.001	0.001	0.005	0.005	0.003	0.009
2.	S2	0.001	0.000	0.004	0.004	0.001	0.004	0.013
3.	S3	0.000	0.002	0.000	0.001	0.004	0.000	0.008
4.	S4	0.000	0.000	0.003	0.003	0.003	0.001	0.003
5.	S5	0.002	0.003	0.004	0.001	0.002	0.003	0.011
6.	S6	0.001	0.001	0.005	0.000	0.005	0.005	0.015
7.	S7	0.002	0.004	0.006	0.003	0.000	0.001	0.023
8.	S8	0.000	0.000	0.001	0.004	0.008	0.004	0.001
9.	S9	0.003	0.002	0.002	0.001	0.001	0.003	0.030
10.	S10	0.000	0.001	0.001	0.000	0.002	0.000	0.019
11.	G1	0.001	0.003	0.000	0.002	0.003	0.001	0.022
12.	G2	0.001	0.004	0.003	0.003	0.004	0.003	0.009
13.	G3	0.000	0.001	0.004	0.001	0.001	0.002	0.007
14.	G4	0.002	0.002	0.001	0.003	0.005	0.001	0.051
15.	G5	0.000	0.004	0.001	0.0005	0.006	0.000	0.011
16.	G6	0.003	0.000	0.004	0.004	0.004	0.004	0.009
17.	G7	0.001	0.001	0.008	0.001	0.003	0.005	0.008
18.	G8	0.000	0.001	0.001	0.006	0.001	0.000	0.005
19.	G9	0.002	0.000	0.003	0.002	0.003	0.001	0.011
20.	G10	0.000	0.001	0.001	0.003	0.002	0.000	0.031
21.	G11	0.000	0.001	0.002	0.000	0.000	0.005	0.029

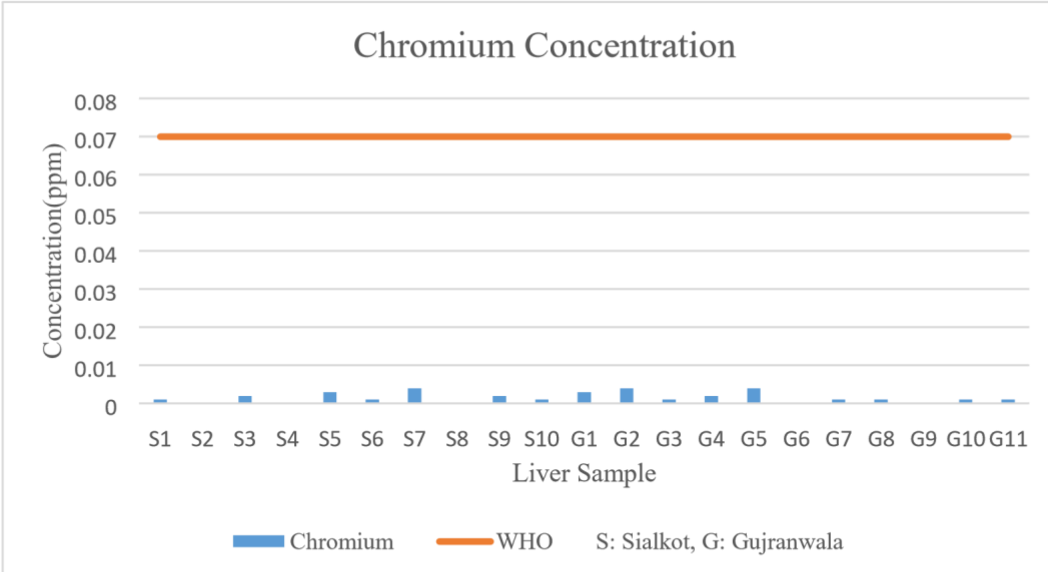
Cadmium Concentration in Liver Samples of Frogs:



Graph 4.8: Cadmium Concentration in Liver Samples of Frogs

The graph shows the concentration of Cadmium in the liver samples of frogs collected from Gujranwala and Sialkot Districts. The concentration of Cadmium in samples from both areas does not exceed the WHO permissible limit which is 0.04 ppm. The highest concentration was 0.003 ppm. The main sources are welding, burning of coal and untreated municipal waste. Since the frogs were collected from ponds containing groundwater, therefore due to untreated water and solid waste being dumped into ground, heavy metals were present in frog's liver [49] [50].

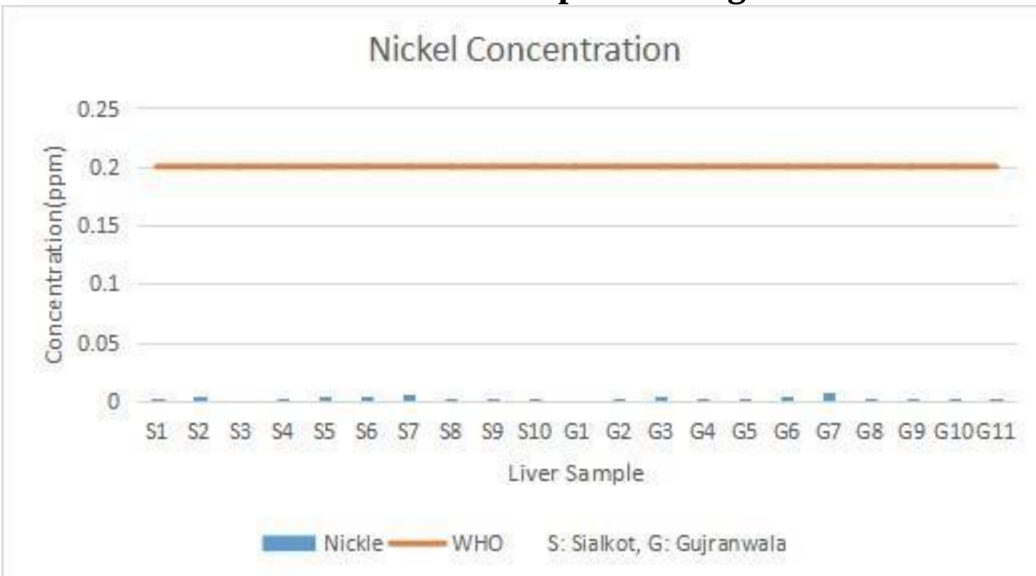
Chromium Concentration in Liver Samples of Frogs:



Graph 4.9: Chromium Concentration in Liver Samples of Indian Bullfrog

The graph shows the concentration of Chromium in liver samples of Indian Bullfrog of Gujranwala and Sialkot Districts. The concentration of Chromium did not exceed the WHO permissible limit that was 0.07 ppm. The highest concentration was 0.004 ppm. It is naturally occurring heavy metal in industrial processes. Chromium was present in less quantity in frog's liver as heavy metals are slightly soluble in frog's organs [49] [50].

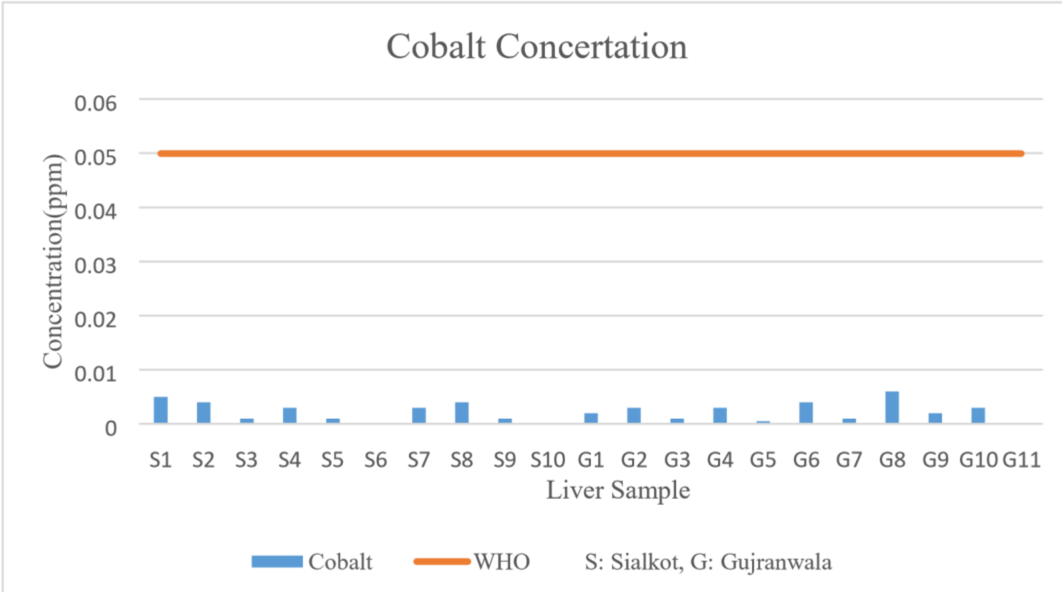
Nickel Concentration in Liver Samples of Frogs:



Graph 4.10: Nickel Concentration in Liver Samples of Indian Bullfrog

The graph shows the concentrations of Nickel in liver samples of frogs collected from Gujranwala and Sialkot Districts. The values of the nickel are within the permissible limit of WHO that was 0.2 ppm. The maximum concentration of nickel was observed in G7 that was 0.008 ppm. The sources of nickel are combustion of coal, incineration of waste and sewage etc [49] [50].

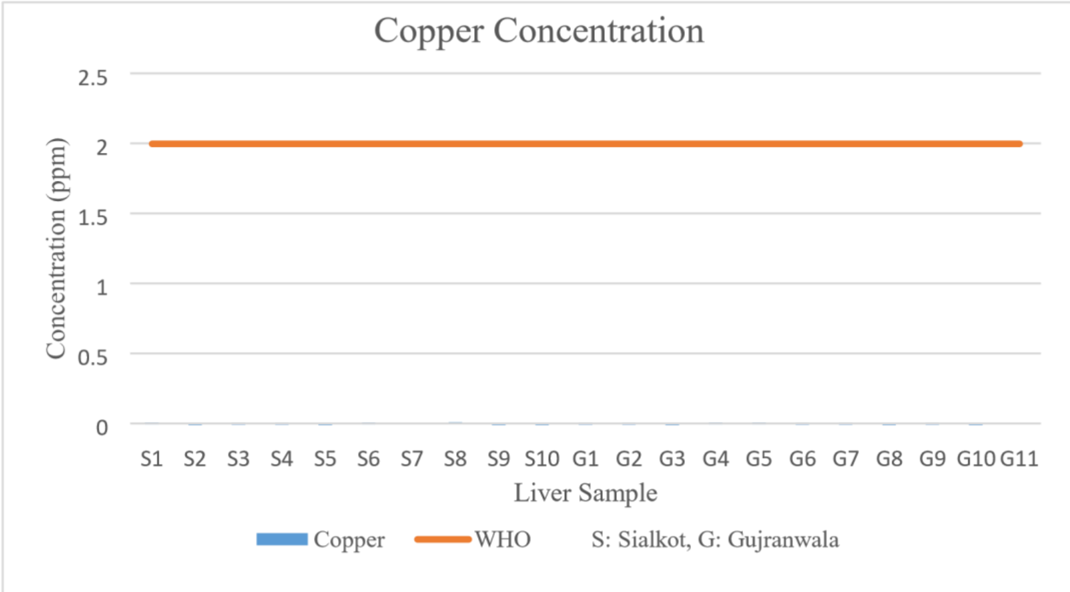
Cobalt Concentration on Liver Samples of Frogs:



Graph 4.11: Cobalt Concentration on Liver samples of Indian Bullfrog

The graph shows the concentration of Cobalt in the liver samples of frogs collected from Gujranwala and Sialkot Districts. The maximum concentration of cobalt was observed in G8 which was 0.006 ppm. The values of the Cobalt are within the permissible limit of WHO that was 0.05 ppm. The sources of cobalt are combustion of coal, industrial processes, incineration of waste and sewage etc [49] [51].

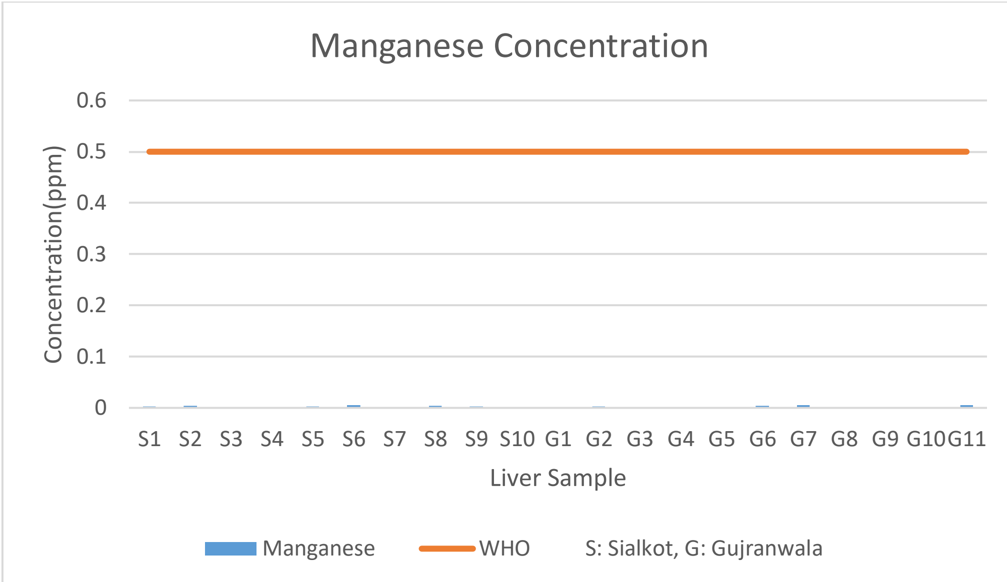
Copper concentration in Liver Samples of Frogs:



Graph 4.12: Copper concentration in Liver samples of Indian Bullfrog

The graph shows the concentrations of copper in the liver samples of frogs collected from Gujranwala and Sialkot Districts. The maximum concentration of cobalt was observed in S8 which was 0.008 ppm. The value of the copper is within the WHO permissible limit that was 2 ppm. Since the frogs were collected from ponds containing groundwater, therefore due to untreated water and solid waste being dumped into ground, heavy metals were present in frog's organs [49] [51].

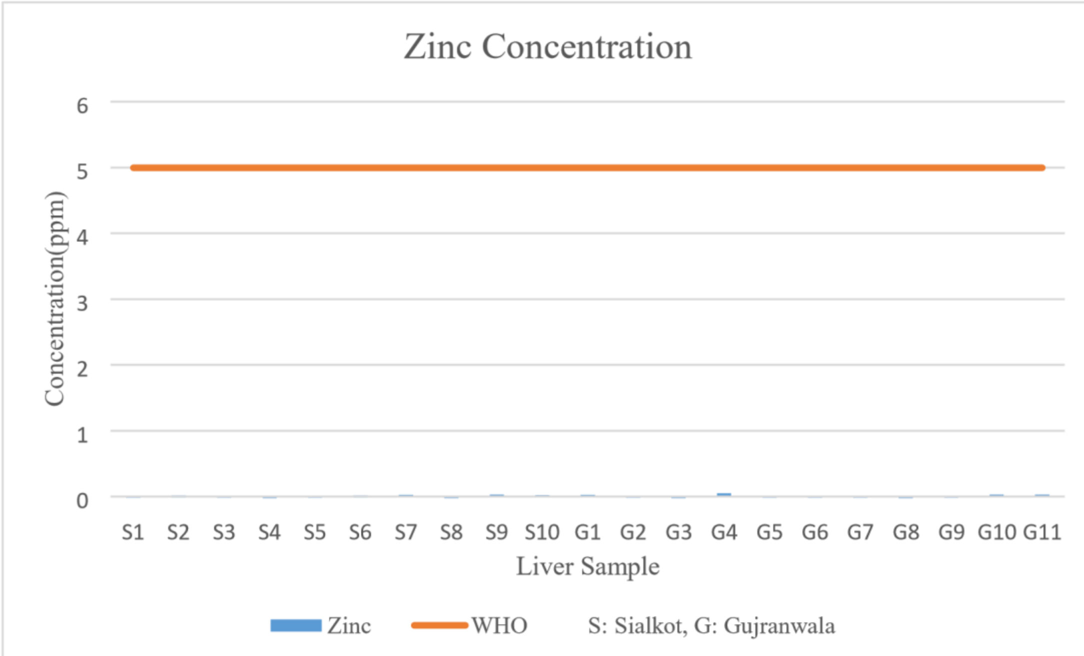
Manganese Concentration in Liver Samples of Frogs:



Graph 4.13: Manganese Concentration in Liver Samples of Frog

The graph shows the concentration of manganese in the liver samples of frogs collected from Gujranwala and Sialkot. The concentration of manganese did not exceed the WHO permissible limit which is 0.05 ppm. The maximum concentration of manganese was 0.005 ppm. The sources of magnesium include untreated sewage sludge, diffuse sources such as metal piping, traffic etc., mining and industrial production [49] [52].

Zinc Concentration in Liver Samples of Frogs:



Graph 4.14: Zinc Concentration in Liver Samples of Indian Bullfrog

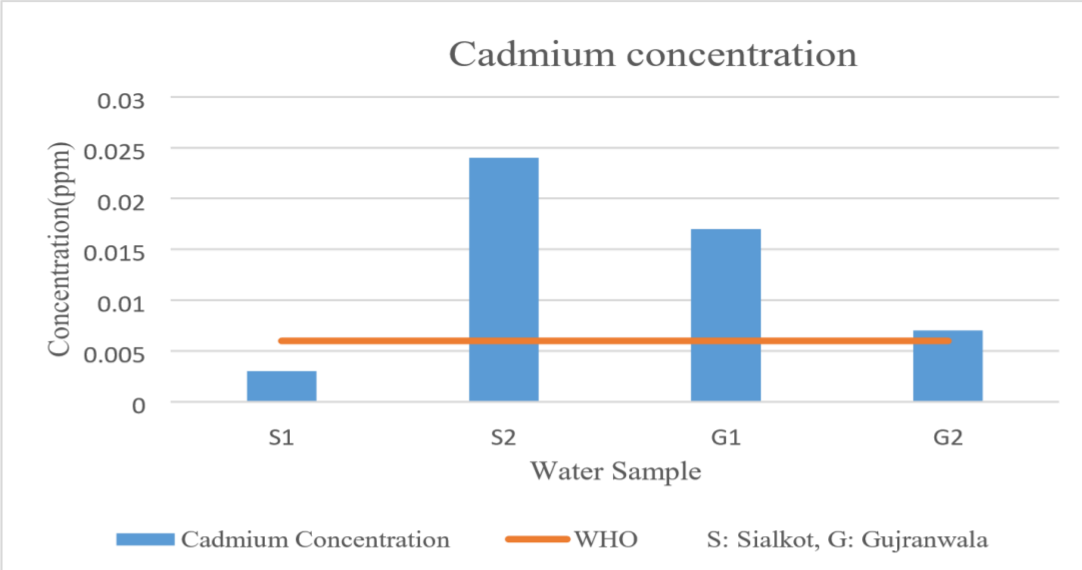
The graph shows the concentration of Zinc in the liver samples of Indian Bullfrog specie collected from Gujranwala and Sialkot Districts. Very minute concentrations of zinc in comparison to the WHO permissible level of 5 ppm. The maximum concentration of zinc was 0.031 ppm in Sialkot Sample 9. The sources of zinc include industrial wastes, agricultural runoff, aging water supply systems, mining etc [49]

4.2.3: Concentration of Heavy Metal in Water Samples

Table 4.4: Concentration of Heavy Metal in Water Samples of Gujranwala and Sialkot Districts

Sr. No	Sample	Cd	Cr	Ni	Co	Cu	Mn	Zn
	WHO	**0.006**	**0.1**	**0.07**	**0.05**	**0.1**	**0.26**	**15**
1.	SW1	0.003	0.001	0.005	0.017	0.009	0.000	0.009
2.	SW2	0.024	0.001	0.004	0.011	0.004	0.001	0.017
3.	GW1	0.017	0.005	0.000	0.025	0.007	0.002	0.008
4.	GW2	0.007	0.003	0.005	0.009	0.003	0.001	0.011

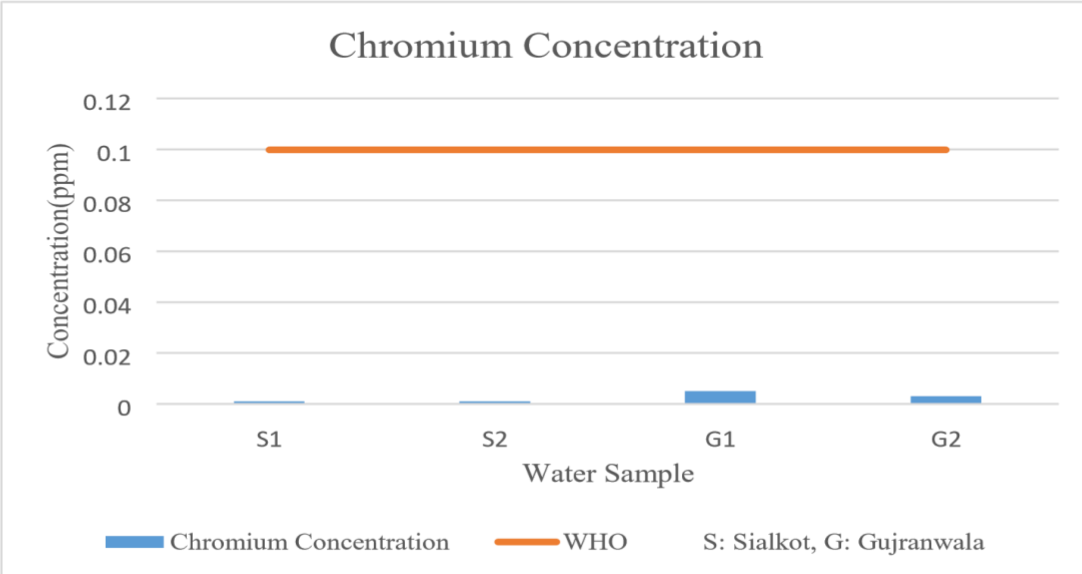
Cadmium Concentration in Water Samples:



Graph 4.15: Cadmium Concentration in Water Samples

The graph shows that the concentration of Cadmium in water samples of Gujranwala and Sialkot Districts exceeds the WHO permissible limit 0.006 ppm. The highest concentration was 0.024 ppm in Sialkot water sample 2 (SW2). Both the study areas were industrial areas, therefore heavy metals were present in water samples [49] [50].

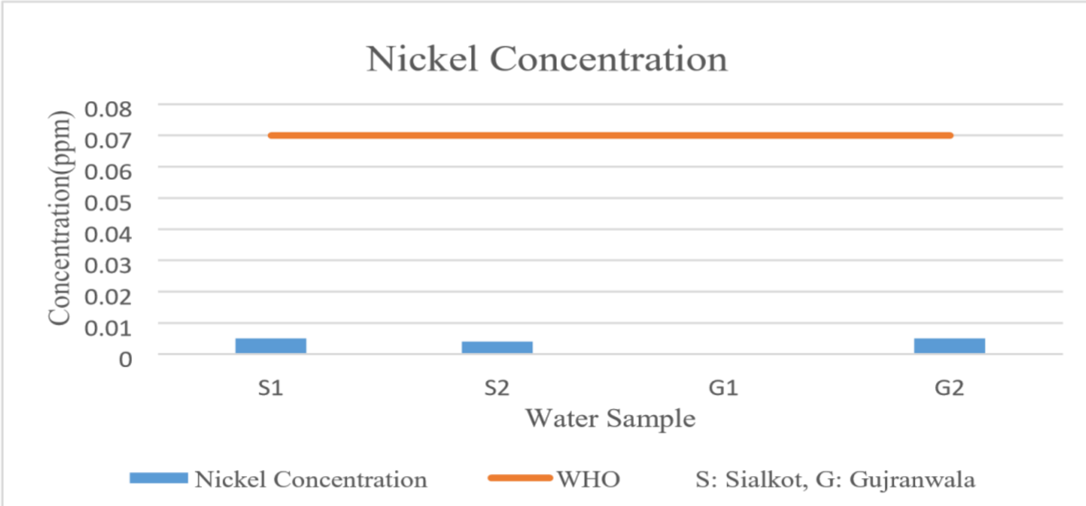
Chromium Concentration in Water Samples:



Graph 4.16: Chromium Concentration in Water Samples

The graph shows the concentration of Chromium in water samples of Gujranwala and Sialkot Districts. The concentrations of Chromium did not exceed the WHO permissible limit that was 0.1ppm. The highest concentration was 0.005 ppm in Gujranwala Water Sample 1 (GW1). The main sources of chromium are industrial processes [49] [50].

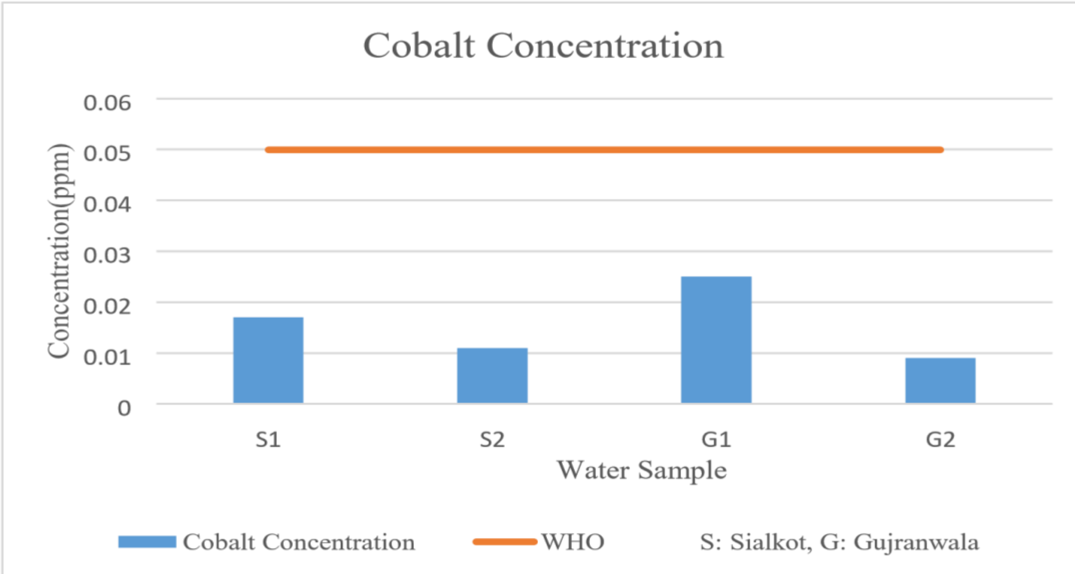
Nickel Concentration in Water:



Graph 4.17: Nickel Concentration in Water Samples

The graph shows the concentration of Nickel in water samples of Gujranwala and Sialkot Districts. The Concentration did not exceed the WHO permissible limit 0.07 ppm. The highest concentration was 0.005 ppm in Sialkot Water Sample1 (SW1) and Gujranwala Water Sample 2 (GW2). The sources of nickel are untreated sewage sludge and diffuse sources such as metal piping, traffic [49] [50].

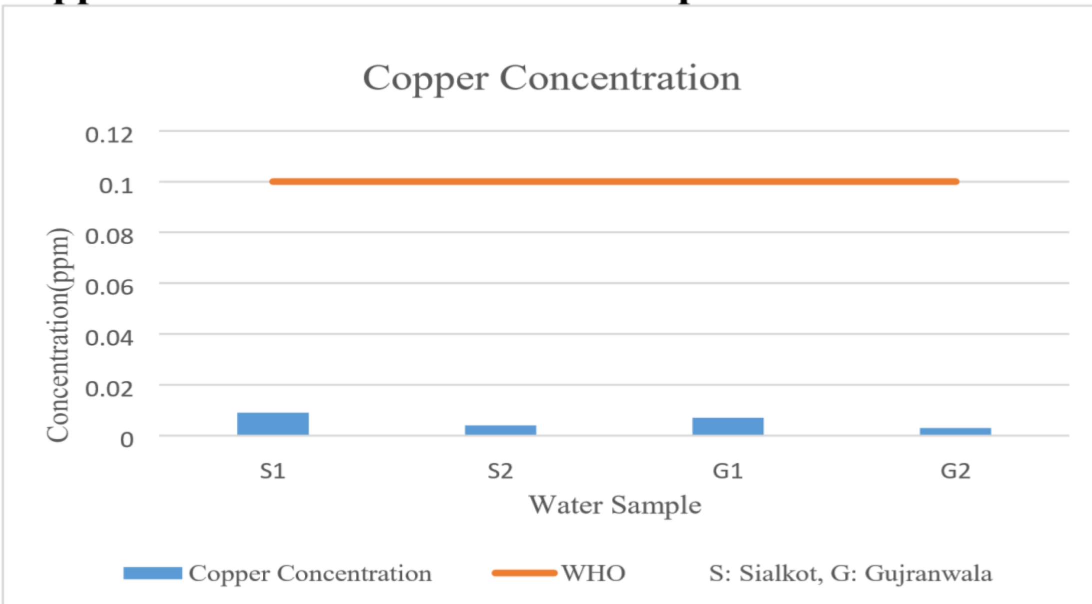
Cobalt Concentration in Water Samples:



Graph 4.18: Concentration of Cobalt in Water Samples

The graph shows the concentration of Cobalt in water samples of Gujranwala and Sialkot Districts. The concentration of cobalt did not exceed the WHO permissible limit 0.05 ppm. The highest concentration was 0.025 ppm in Gujranwala Water Sample 1 (GW1). Both the study areas were industrial areas and untreated water and solid wastes being dumped into the ground, therefore cobalt was present in water samples [49] [51].

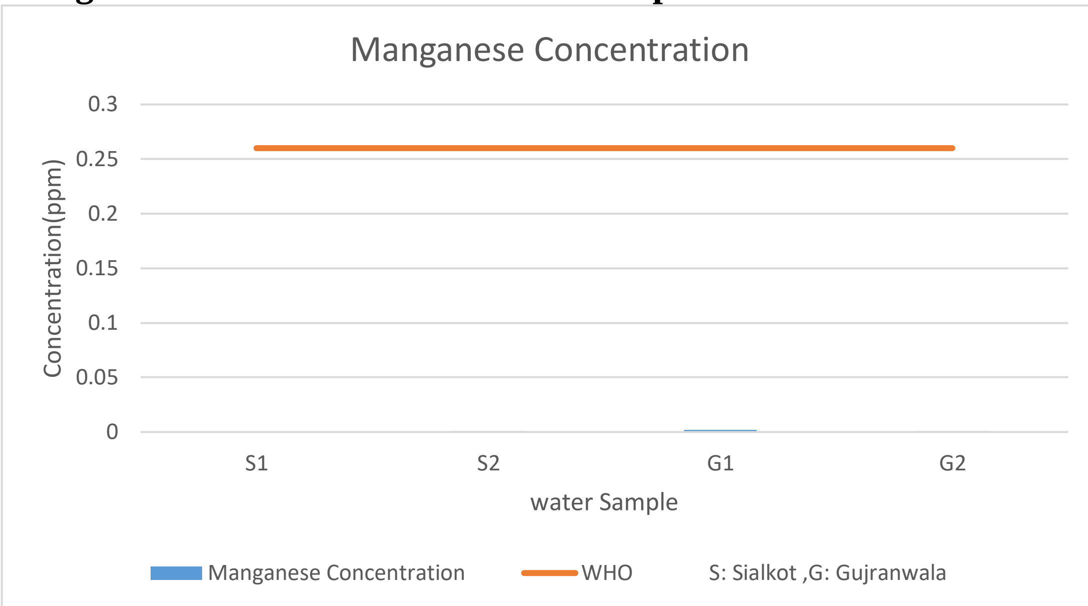
Copper Concentration in Water Samples:



Graph 4.19: Copper Concentration in Water Samples

The graph shows the concentration of Copper in water samples of Gujranwala and Sialkot Districts. The concentration of copper did not exceed the WHO permissible limit 0.1 ppm. The highest concentration was 0.009 ppm in Sialkot Water Sample 1 (SW1). Both the study areas were industrial areas, therefore heavy metals were present in water samples [49] [51].

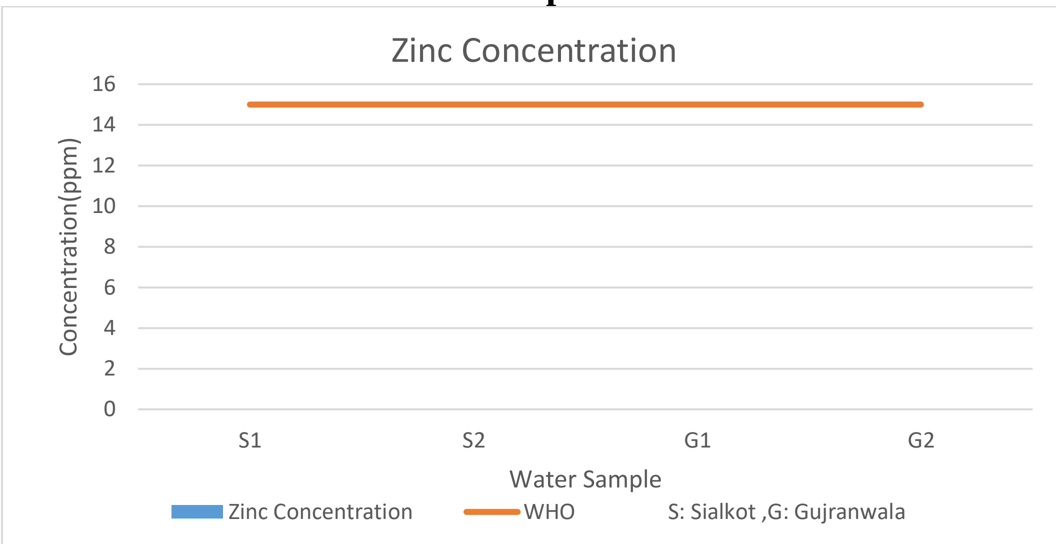
Manganese Concentration in Water Samples:



Graph 4.20: Concentration of Magnesium in Water Samples

The graph shows the concentration of Magnesium in water samples of Gujranwala and Sialkot Districts. The concentration of Magnesium does not exceed the WHO permissible limit 0.26 ppm. The highest concentration was 0.002 ppm in Gujranwala Water Sample 1 (GW1). The sources of magnesium include mining, industrial production, untreated sewage sludge and diffuse sources such as metal piping, traffic etc [49] [52].

Zinc Concentration in Water Samples:



Graph 4.21: Zinc Concentration in Water Samples

The graph shows the concentration of Zinc in water samples of Gujranwala and Sialkot Districts. The concentration of Zinc does not exceed the WHO permissible limit 15 ppm. The highest concentration was 0.017 ppm in Sialkot Water Sample 2 (SW2). The sources of zinc include industrial wastes, agricultural runoff, aging water supply systems, mining etc [49] [52].

Both the study areas are industrial areas, untreated water and dumping of solid wastes results in the formation of liquid from solid waste leaches into the ground due to which sewage mixes with groundwater. Since the frogs were collected from ponds containing groundwater therefore heavy metals were detected in the bodies of the frogs which must have absorbed in their bodies from the water. Heavy metals did not exceed the permissible limits in the skin and liver samples of frogs because heavy metals are slightly soluble in organs of frogs.

River and well water are primarily used for drinking and agriculture. It is studied that Heavy Metals are naturally present in ground water. This information exhibits rather a normal situation in terms of heavy metal quality in drinking water. Cu, Mn, and Zn concentrations were below WHO permissible values. The level of Chromium crossed the WHO standards by 21% [53].

CONCLUSION

Indian Bullfrog species were collected from Gujranwala and Sialkot Districts. Twenty-one samples were under observation; 11 from Gujranwala and 10 from Sialkot. Their morphological characteristics and the presence of heavy metals in the liver and skin of the sample species were observed. Furthermore, heavy metal analysis of the water samples of the respective study areas was carried out. Sialkot and Gujranwala both the cities are industrial areas with tanneries. Waste water pollution has influenced the biota of the area. In order to observe the pollution indicating capability of amphibians, standard procedures of dissection, organ preservation and organ digestion were performed. Atomic Absorption Spectrophotometer analysis were performed to obtain the results. The obtained values of heavy metals present in the water samples and the organs were then compared with the WHO Standards for heavy metals. Majority of frogs of both areas had normal and standard body length, snout vent length, skin color and liver color, weight and heart condition, with no appearance of any morphological abnormalities.

The values of heavy metals (cadmium, chromium, cobalt, nickel, zinc, manganese and copper) were well within the permissible limits of WHO, in the liver and skin samples. However, the concentrations of Cadmium, in water samples (SW2, GW1 and GW2) exceeded the WHO permissible limits while the remaining metals were well within the WHO limits in the water samples of the selected study areas. Since both the frogs and water samples were taken from the agricultural fields of the selected areas, where ground water was used as a source of irrigation therefore there may be possibility that the industrial effluent waste and sewage must have leached into the soil and contaminated the groundwater thus resulting in the occurrence of heavy metals in the water and in the body of amphibian species. Heavy metals are naturally present in ground water. Thus it was concluded that about the quality of respective study areas was considerably better. However, legislation and policies, regular monitoring, installation of the treatment plants in industries and awareness among people about the detrimental untreated consequences of dumped waste can further improve the condition of the areas.

REFERENCES

1. Gupta RS. Molecular signatures that are distinctive characteristics of the vertebrates and chordates and supporting a grouping of vertebrates with the tunicates. Molecular phylogenetics and evolution. 2016 Jan 1; 94:383-91.

2. Stolyar OB, Loumbourdis NS, Falfushinska HI, Romanchuk LD. Comparison of metal bioavailability in frogs from urban and rural sites of Western Ukraine. Archives of environmental contamination and toxicology. 2008 Jan; 54(1):10713.

3. Leontyeva oa, semenov dv, esenin av. Frog (Rana temporaria) in Moscow Province. Advances in Amphibian Research in the Former Soviet Union. 1997; 2:125.

4. Ibeto CN, Okoye CO. High levels of Heavy metals in Blood of Urban population in Nigeria. Research Journal of Environmental Sciences. 2010; 4(4):371-82.

5. Jaishankar M, Tseten T, Anbalagan N, Mathew BB, Beeregowda KN. Toxicity, mechanism and health effects of some heavy metals. Interdisciplinary toxicology. 2014 Jun; 7(2):60.

6. Bernhoft RA. Cadmium toxicity and treatment. The Scientific World Journal. 2013 Jun 3; 2013.

7. Barnhart J. Occurrences, uses, and properties of chromium. Regulatory toxicology and pharmacology. 1997 Aug 1; 26(1):S3-7.

8. Barceloux DG, Barceloux D. Nickel. Journal of toxicology: clinical Toxicology. 1999 Jan 1; 37(2):239-58.

9. Barceloux DG, Barceloux D. Cobalt. Journal of Toxicology: Clinical Toxicology. 1999 Jan 1; 37(2):201-16.

10. Lison D. Cobalt. InHandbook on the Toxicology of Metals 2015 Jan 1 (pp. 743763). Academic Press.

11. Schroeder HA, Balassa JJ, Tipton IH. Essential trace metals in man: Manganese:A study in homeostasis. Journal of chronic diseases. 1966 May 1; 19(5):545-71.

12. Das AP, Ghosh S, Mohanty S, Sukla LB. Advances in manganese pollution and its bioremediation. InEnvironmental microbial biotechnology 2015 (pp. 313-328). Springer, Cham.

13. Plum LM, Rink L, Haase H. The essential toxin: impact of zinc on human health. International journal of environmental research and public health. 2010 Apr; 7(4):1342-65.

14. Lim KH, Riddell LJ, Nowson CA, Booth AO, Szymlek-Gay EA. Iron and zinc nutrition in the economically-developed world: a review. Nutrients. 2013 Aug; 5(8):3184-211

15. Chasapis CT, Ntoupa PS, Spiliopoulou CA, Stefanidou ME. Recent aspects of the effects of zinc on human health. Archives of toxicology. 2020 May; 94(5):144360.

16. Linder MC. Biochemistry of copper. Springer Science & Business Media; 2013 Nov 21.

17. Ullah R, Malik RN, Qadir A. Assessment of groundwater contamination in an industrial city, Sialkot, Pakistan. African Journal of Environmental Science and Technology. 2009; 3(12).

18. Mahmood A, Mahmood A, Malik RN, Shinwari ZK. Indigenous knowledge of medicinal plants from Gujranwala district, Pakistan. Journal of Ethnopharmacology. 2013 Jul 9; 148(2):714-23.

19. Stolyar OB, Loumbourdis NS, Falfushinska HI, Romanchuk LD. Comparison of metal bioavailability in frogs from urban and rural sites of Western Ukraine. Archives of environmental contamination and toxicology. 2008 Jan; 54(1):107-13.

20. Jayawardena UA, Angunawela P, Wickramasinghe DD, Ratnasooriya WD, Udagama PV. Heavy metal–induced toxicity in the Indian green frog:

Biochemical and histopathological alterations. Environmental toxicology and chemistry. 2017 Oct; 36(10):2855-67.

21. Momodu MA, Anyakora CA. Heavy metal contamination of ground water: The Surulere case study. Res J Environ Earth Sci. 2010 Jan 10; 2(1):39-43.

22. Taiwo IE, Henry AN, Imbufe AP, Adetoro OO. Heavy metal bioaccumulation and biomarkers of oxidative stress in the wild African tiger frog, Hoplobatrachus occipitalis. African Journal of Environmental Science and Technology. 2014 Jan 14; 8(1):6-15.

23. Shaapera U, Nnamonu LA, Eneji IS. Assessment of heavy metals in Rana esculenta organs from River Guma, Benue State Nigeria. American Journal of Analytical Chemistry. 2013 Sep 4; 2013.

24. Cobbina SJ, Duwiejuah AB, Quansah R, Obiri S, Bakobie N. Comparative assessment of heavy metals in drinking water sources in two small-scale mining communities in northern Ghana. International journal of environmental research and public health. 2015 Sep; 12(9):10620-34.

25. Anyanwu ED, Adetunji OG. Assessment of the Occurrence and Concentration of Heavy Metals in Spring Waters. Modern Advances in Geography, Environment and Earth Sciences Vol. 4. 2021 May 10:74-81.

26. Bibi S, Khan RL, Nazir R, Khan P, Rehman HU, Shakir SK, Naz S, Waheed MA, Jan R. Heavy Metals Analysis in Drinking Water of Lakki Marwat District, KPK, Pakistan. World applied sciences journal. 2016; 34:15-9.

27. Priyadarshani S, Madhushani WA, Jayawardena UA, Wickramasinghe DD,

Udagama PV. Heavy metal mediated immunomodulation of the Indian green frog, Euphlyctis hexadactylus (Anura: Ranidae) in urban wetlands. Ecotoxicology and environmental safety. 2015 Jun 1; 116:40-9.

28. Hasan M, Khan MM, Sumida M. Morphological and genetic variation in three populations of Hoplobatrachus tigerinus from Bangladesh. Progressive Agriculture. 2008; 19(2):139-49.

29. Marques SM, Antunes SC, Nunes B, Gonçalves F, Pereira R. Antioxidant response and metal accumulation in tissues of Iberian green frogs (Pelophylax perezi) inhabiting a deactivated uranium mine. Ecotoxicology. 2011 Aug; 20(6):1315-27.

30. Jing X, Yao G, Liu D, Liu C, Wang F, Wang P, Zhou Z. Exposure of frogs and tadpoles to chiral herbicide fenoxaprop-ethyl. Chemosphere. 2017 Nov 1; 186:8328.

31. Qureshi IZ, Kashif Z, Hashmi MZ, Su X, Malik RN, Ullah K, Hu J, Dawood M. Assessment of heavy metals and metalloids in tissues of two frog species: Rana tigrina and Euphlyctis cyanophlyctis from industrial city Sialkot, Pakistan. Environmental Science and Pollution Research. 2015 Sep; 22(18):14157-68.

32. Akyengo O, Yakubu A, Adejube AA. Bioaccumulation of Heavy Metals in Fish (Tilapia zilli) and Bullfrog.

33. Jayawardena UA, Wickramasinghe DD, Udagama PV. Cytogenotoxicity evaluation of a heavy metal mixture, detected in a polluted urban wetland: Micronucleus and comet induction in the Indian green frog (Euphlyctis hexadactylus) erythrocytes and the Allium cepa bioassay. Chemosphere. 2021 Aug 1; 277:130278.

34. Srivastav AK, Srivastav S, Suzuki N. Acute toxicity of a heavy metal cadmium to an anuran, the Indian skipper frog Rana cyanophlyctis. Iranian Journal of Toxicology. 2016 Jun 10; 10(5):39-43.

35. Intamat S, Phoonaploy U, Sriuttha M, Tengjaroenkul B, Neeratanaphan L. Heavy metal accumulation in aquatic animals around the gold mine area of Loei province, Thailand. Human and Ecological Risk Assessment: An International Journal. 2016 Aug 17; 22(6):1418-32

36. Aktar MW, Paramasivam M, Ganguly M, Purkait S, Sengupta D. Assessment and occurrence of various heavy metals in surface water of Ganga River around Kolkata: a study for toxicity and ecological impact. Environmental monitoring and assessment. 2010 Jan; 160(1):207-13. 37.

37. Kuiwa TS, Mbah CE, Abolude DS, Lawal N, Aminu MA. Determination of Heavy Metals in Hoplobatrachus occipitalis (Crowned Bullfrogs) and Water from Some Reservoirs in Kadawa Irrigation Project Kano, Nigeria. Journal of Applied Sciences and Environmental Management. 2019; 23(12):2131-7.

38. Mahmood T, Qadosi IQ, Fatima H, Akrim F, Rais M. Metal concentrations in common skittering frog (Euphlyctis cyanophlyctis) inhabiting Korang River, Islamabad, Pakistan. Basic and Applied Herpetology. 2016 Oct 23; 30:25-38.

39. Singare PU, Mishra RM, Trivedi MP. Sediment contamination due to toxic heavy metals in Mithi River of Mumbai. Adv Anal Chem. 2012; 2(3):14-24.

40. Thanomsangad P, Tengjaroenkul B, Sriuttha M, Neeratanaphan L. Heavy metal accumulation in frogs surrounding an e-waste dump site and human health risk assessment. Human and Ecological Risk Assessment: An International Journal. 2019 Feb 22.

41. Jayawardena UA, Wickramasinghe DD, Ratnasooriya WD, Udagama PV.

Histopathological quantification of heavy metal mediated tissue damages detected in Euphlyctis hexadactylus (Anura: Ranidae) in an urban polluted wetland in Sri Lanka.

42. Nasir M, Ansari TM, Javed H, Yasin G, Khan AA, Shoaib M. Analytical quantification of copper in frogs (Rana tigrina) found from various aquatic habitats. African Journal of Biotechnology. 2020 Feb 29; 19(2):121-8.

43. Santana E, Schiesari L, Gomes F, Martins M. Morphophysiological traits of an amphibian exposed to historical industrial pollution in a Brazilian biodiversity hotspot. AmphibiaReptilia. 2021 Mar 5; 1(aop):1-3.

44. Jayawardena UA, Angunawela P, Wickramasinghe DD, Ratnasooriya WD, Udagama PV. Heavy metal–induced toxicity in the Indian green frog: Biochemical and histopathological alterations. Environmental toxicology and chemistry. 2017 Oct; 36(10):2855-67.

45. Momodu MA, Anyakora CA. Heavy metal contamination of ground water: The Surulere case study. Res J Environ Earth Sci. 2010 Jan 10; 2(1):39-43.

46. Qureshi IZ, Kashif Z, Hashmi MZ, Su X, Malik RN, Ullah K, Hu J, Dawood M. Assessment of heavy metals and metalloids in tissues of two frog species: Rana tigrina and Euphlyctis cyanophlyctis from industrial city Sialkot, Pakistan. Environmental Science and Pollution Research. 2015 Sep; 22(18):14157-68.

47. Jayawardena UA, Angunawela P, Wickramasinghe DD, Ratnasooriya WD, Udagama PV. Heavy metal–induced toxicity in the Indian green frog: Biochemical and histopathological alterations. Environmental toxicology and chemistry. 2017 Oct; 36(10):2855-67.

48. Renberg I. Concentration and annual accumulation values of heavy metals in lake sediments: their significance in studies of the history of heavy metal pollution. InPaleolimnology IV 1987 (pp. 379-385). Springer, Dordrecht.

49. Ibe FC, Opara AI, Ibe BO. Application of pollution risk evaluation models in groundwater systems in the vicinity of automobile scrap markets in Owerri municipal and environs, southeastern Nigeria. Scientific African. 2020 Jul 1; 8:e00450.

50. Bradl HB. Sources and origins of heavy metals. InInterface science and technology 2005 Jan 1 (Vol. 6, pp. 1-27). Elsevier.

51. Sörme L, Lagerkvist RJ. Sources of heavy metals in urban wastewater in Stockholm. Science of the total environment. 2002 Oct 21; 298(1-3):131-45.

52. Rehman W, Zeb A, Noor N, Nawaz M. Heavy metal pollution assessment in various industries of Pakistan. Environmental Geology. 2008 Jul; 55(2):353-8.

53. Mustafa S, Baloch N, Muhammad S, Malik Y, Khan T, Bibi M, Qadir A, Razaque G, Baloch IA. Determination of trace and heavy metals in drinking water of Jhal Magsi district of Balochistan, Pakistan. Pure and Applied Biology (PAB). 2017 Mar 10; 6 (1):9-17.

ANNEXURES I

Figure No.1: Observing the Morphological Characteristics

Figure No.2: Dissection of Frog

Figure No. 3: Observing Liver of Frog

Figure No. 4: Digestion of Water Samples

Figure No. 5: Filtered Samples for Organ Specimen

ANNEXURES II

Table 1: Morphological Characteristics of Specimen1 of Indian Bullfrog

Sr. No	Morphological Characteristics	Comments
1.	Specimen Number and Area	Specimen 1 Sialkot
2.	Weight	0.406 kg
3.	Total Length	13 inches
4.	Snout Vent Length	6.9 inches
5.	Color of Skin	Muddy green, dark green color, dark green patches, on dorsal side, ventral side yellow to pink.
6.	Other Observable Characters on Skin	No
7.	Limb Anomalies Observed	No
8.	Heart Condition	Normal
9.	Appearance of Cysts on the Body	No
10.	Color and Condition of Liver	Appearance normal
11.	Any other Morphological Abnormality Observed	No

Table 2: Morphological Characteristics of Specimen 2 of Indian Bullfrog

Sr. No	Morphological Characteristics	Comments
1.	Specimen Number and Area	Specimen 2 Sialkot
2.	Weight	0.16 kg
3.	Total Length	10.2 inches
4.	Snout Vent Length	5.5 inches
5.	Color of Skin	Dark muddy green colour patches on dorsal side
6.	Other Observable Characters on Skin	Vocal pouches prominent
7.	Limb Anomalies Observed	No
8.	Heart Condition	Normal
9.	Appearance of Cysts on the Body	No
10.	Color and Condition of Liver	Appearance normal
11.	Any other Morphological Abnormality Observed	No

Table 3: Morphological Characteristics of Specimen 3 of Indian Bullfrog

Sr. No	Morphological Characteristics	Comments
1.	Specimen Number and Area	Specimen 3 Sialkot
2.	Weight	0.202 kg
3.	Total Length	11 inches
4.	Snout Vent Length	5.9 inches
5.	Color of Skin	Muddy to dark green colour, black patches on dorsal side
6.	Other Observable Characters on Skin	Vocal pouches prominent
7.	Limb Anomalies Observed	No
8.	Heart Condition	Heart enlarged
9.	Appearance of Cysts on the Body	No
10.	Color and Condition of Liver	Yellowish reddish brown in colour
11.	Any other Morphological Abnormality Observed	No

Table 4: Morphological Characteristics of Specimen 4 of Indian Bullfrog

Sr. No	Morphological Characteristics	Comments
1.	Specimen Number and Area	Specimen 4 Sialkot
2.	Weight	0.173 kg
3.	Total Length	10.5 inches
4.	Snout Vent Length	5.5 inches
5.	Color of Skin	Dark muddy green patches on dorsal sides
6.	Other Observable Characters on Skin	Vocal pouches prominent
7.	Limb Anomalies Observed	No
8.	Heart Condition	Normal
9.	Appearance of Cysts on the Body	No
10.	Color and Condition of Liver	Appears normal
11.	Any other Morphological Abnormality Observed	No

Table 5: Morphological Characteristics of Specimen 5 of Indian Bullfrog

Sr. No	Morphological Characteristics	Comments
1.	Specimen Number and Area	Specimen 5 Sialkot
2.	Weight	0.163 kg
3.	Total Length	11.25 inches
4.	Snout Vent Length	5.5 inches
5.	Color of Skin	Muddy light green in color dark green patches on dorsal side
6.	Other Observable Characters on Skin	No
7.	Limb Anomalies Observed	No
8.	Heart Condition	Normal
9.	Appearance of Cysts on the Body	No
10.	Color and Condition of Liver	Appearance normal
11.	Any other Morphological Abnormality Observed	No

Table 6: Morphological Characteristics of Specimen of Indian Bullfrog

Sr. No	Morphological Characteristics	Comments
1.	Specimen Number and Area	Specimen 6 Sialkot
2.	Weight	0.227 kg
3.	Total Length	11.5 inches
4.	Snout Vent Length	5.9 inches
5.	Color of Skin	Dark muddy green colour yellow pattern on dorsal side
6.	Other Observable Characters on Skin	Vocal pouches prominent
7.	Limb Anomalies Observed	No
8.	Heart Condition	Normal healthy heart
9.	Appearance of Cysts on the Body	No
10.	Color and Condition of Liver	Slightly yellowish in colour
11.	Any other Morphological Abnormality Observed	No

Table 7: Morphological Characteristics of Specimen of Indian Bullfrog

Sr. No	Morphological Characteristics	Comments
1.	Specimen Number and Area	Specimen 7 Sialkot
2.	Weight	0.161 kg
3.	Total Length	10.25 inches
4.	Snout Vent Length	5.3 inches
5.	Color of Skin	Dark muddy green colour on dorsal side
6.	Other Observable Characters on Skin	Vocal pouches prominent
7.	Limb Anomalies Observed	No
8.	Heart Condition	Normal
9.	Appearance of Cysts on the Body	No
10.	Color and Condition of Liver	Appears normal
11.	Any other Morphological Abnormality Observed	No

Table 8: Morphological Characteristics of Specimen of Indian Bullfrog

Sr. No	Morphological Characteristics	Comments
1.	Specimen Number and Area	Specimen 8 Sialkot
2.	Weight	0.220 kg
3.	Total Length	11.75 inches
4.	Snout Vent Length	5.95 inches
5.	Color of Skin	Dark muddy green colour with dark green patches on dorsal side
6.	Other Observable Characters on Skin	Vocal pouches prominent
7.	Limb Anomalies Observed	No
8.	Heart Condition	Normal
9.	Appearance of Cysts on the Body	No
10.	Color and Condition of Liver	Appears normal
11.	Any other Morphological Abnormality Observed	No

Table 9: Morphological Characteristics of Specimen of Indian Bullfrog

Sr. No	Morphological Characteristics	Comments
1.	Specimen Number and Area	Specimen 9 Sialkot
2.	Weight	0.157 kg
3.	Total Length	10.1 inches
4.	Snout Vent Length	5.25 inches
5.	Color of Skin	Dark muddy green with dark patches on dorsal side
6.	Other Observable Characters on Skin	No
7.	Limb Anomalies Observed	No
8.	Heart Condition	Normal
9.	Appearance of Cysts on the Body	No
10.	Color and Condition of Liver	Appears normal
11.	Any other Morphological Abnormality Observed	No

Table 10: Morphological Characteristics of Specimen of Indian Bullfrog

Sr. No	Morphological Characteristics	Comments
1.	Specimen Number and Area	Specimen 10 Sialkot
2.	Weight	0.175 kg
3.	Total Length	11.1 inches
4.	Snout Vent Length	5.5 inches
5.	Color of Skin	Dark muddy green with dark green patches, yellow pattern lines on dorsal and ventral side
6.	Other Observable Characters on Skin	No
7.	Limb Anomalies Observed	No
8.	Heart Condition	Normal
9.	Appearance of Cysts on the Body	No
10.	Color and Condition of Liver	Appears normal
11.	Any other Morphological Abnormality Observed	No

Table 11: Morphological Characteristics of Specimen of Indian Bullfrog

Sr. No	Morphological Characteristics	Comments
1.	Specimen Number and Area	Specimen 1 Gujranwala
2.	Weight	0.177 kg
3.	Total Length	11 inches
4.	Snout Vent Length	5.5 inches
5.	Color of Skin	Dark muddy green with dark green patches yellowish pink ventral side
6.	Other Observable Characters on Skin	Reddish colored webbed feet
7.	Limb Anomalies Observed	No
8.	Heart Condition	Normal
9.	Appearance of Cysts on the Body	No
10.	Color and Condition of Liver	Appears normal
11.	Any other Morphological Abnormality Observed	No

Table 12: Morphological Characteristics of Specimen of Indian Bullfrog

Sr. No	Morphological Characteristics	Comments
1.	Specimen Number and Area	Specimen 2 Gujranwala
2.	Weight	0.250 kg
3.	Total Length	12 inches
4.	Snout Vent Length	6.5 inches
5.	Color of Skin	Dark muddy green skin colour with dark green patches on dorsal side, light yellow to pink on ventral side
6.	Other Observable Characters on Skin	No
7.	Limb Anomalies Observed	No
8.	Heart Condition	Normal
9.	Appearance of Cysts on the Body	No
10.	Color and Condition of Liver	Appears normal
11.	Any other Morphological Abnormality Observed	No

Table 13: Morphological Characteristics of Specimen of Indian Bullfrog

Sr. No	Morphological Characteristics	Comments
1.	Specimen Number and Area	Specimen 3 Gujranwala
2.	Weight	0.257 kg
3.	Total Length	12 inches
4.	Snout Vent Length	6.5 inches
5.	Color of Skin	Dark green and muddy green colour with dark green patches on dorsal side
6.	Other Observable Characters on Skin	No
7.	Limb Anomalies Observed	No
8.	Heart Condition	Normal
9.	Appearance of Cysts on the Body	No
10.	Color and Condition of Liver	Appears normal
11.	Any other Morphological Abnormality Observed	No

Table 14: Morphological Characteristics of Specimen 14 of Indian Bullfrog

Sr. No	Morphological Characteristics	Comments
1.	Specimen Number and Area	Specimen 4 Gujranwala
2.	Weight	0.236 inches
3.	Total Length	12 inches
4.	Snout Vent Length	6.25 inches
5.	Color of Skin	Dark and muddy green colour with brown and yellow patches and lines on dorsal line
6.	Other Observable Characters on Skin	No
7.	Limb Anomalies Observed	No
8.	Heart Condition	Normal
9.	Appearance of Cysts on the Body	No
10.	Color and Condition of Liver	Appears normal
11	Any other morphological abnormalities	No

Table 15: Morphological Characteristics of Specimen of Indian Bullfrog

Sr. No	Morphological Characteristics	Comments
1.	Specimen Number and Area	Specimen 5 Gujranwala
2.	Weight	0.165 inches
3.	Total Length	10.5 inches
4.	Snout Vent Length	5 inches
5.	Color of Skin	Dark muddy green colour with a tinge of light green and yellow patterns on dorsal side
6.	Other Observable Characters on Skin	No
7.	Limb Anomalies Observed	None
8.	Heart Condition	Normal
9.	Appearance of Cysts on the Body	No
10.	Color and Condition of Liver	Appears normal
11.	Any Other Morphological Abnormality Observed	N/A

Table 16: Morphological Characteristics of Specimen of Indian Bullfrog

Sr. No	Morphological Characteristics	Comments
1.	Specimen Number and Area	Specimen 6 Gujranwala
2.	Weight	0.123 kg
3.	Total Length	12 inches
4.	Snout Vent Length	5.25 inches
5.	Color of Skin	Dark and muddy green with dark green patches and on dorsal and ventral side
6.	Other Observable Characters on Skin	No
7.	Limb Anomalies	No
8.	Observed Heart Condition	Normal
9.	Appearance of Cysts on the Body	No
10.	Color and Condition of Liver	Darker in colour
11.	Any other Morphological Abnormality Observed	No

Table 17: Morphological Characteristics of Specimen of Indian Bullfrog

Sr. No	Morphological Characteristics	Comments
1.	Specimen Number and Area	Specimen 7 Gujranwala
2.	Weight	0.255 kg
3.	Total Length	11.9 inches
4.	Snout Vent Length	6.25 inches
5.	Color of Skin	Dark muddy green colour with yellow and green patches on dorsal side
6.	Other Observable Characters on Skin	No
7.	Limb Anomalies Observed	No
8.	Heart Condition	Normal
9.	Appearance of Cysts on the Body	No
10.	Color and Condition of Liver	Appears normal
11.	Any other Morphological Abnormality Observed	No

Table 18: Morphological Characteristics of Specimen of Indian Bullfrog

Sr. No	Morphological Characteristics	Comments
1.	Specimen Number and Area	Specimen 8 Gujranwala
2.	Weight	0.261 kg
3.	Total Length	11.5 inches
4.	Snout Vent Length	5.8 inches
5.	Color of Skin	Dull green with muddy green patches on dorsal side
6.	Other Observable Characters on Skin	Vocal pouches prominent
7.	Limb Anomalies Observed	No
8.	Heart Condition	Normal
9.	Appearance of Cysts on the Body	No
10.	Color and Condition of Liver	Yellowish brown in colour
11.	Any other Morphological Abnormality Observed	No

Table 19: Morphological Characteristics of Specimen of Indian Bullfrog

Sr. No	Morphological Characteristics	Comments
1.	Specimen Number and Area	Specimen 9 Gujranwala
2.	Weight	0.162 kg
3.	Total Length	10.5 inches
4.	Snout Vent Length	4.75 inches
5.	Color of Skin	Dark and muddy green colour with dark green patches on dorsal side
6.	Other Observable Characters on Skin	No
7.	Limb Anomalies Observed	No
8.	Heart Condition	Normal
9.	Appearance of Cysts on the Body	No
10.	Color and Condition of Liver	Appears normal
11.	Any other Morphological Abnormality Observed	No

Table 20: Morphological Characteristics of Specimen of Indian Bullfrog

Sr. No	Morphological Characteristics	Comments
1.	Specimen Number and Area	Specimen 10 Gujranwala
2.	Weight	0.150 kg
3.	Total Length	10.2 inches
4.	Snout Vent Length	5.2 inches
5.	Color of Skin	Light muddy green in colour on dorsal side, yellow and pink in colour on ventral side
6.	Other Observable Characters on Skin	Vocal pouches are prominent
7.	Limb Anomalies Observed	No
8.	Heart Condition	Normal
9.	Appearance of Cysts on the Body	No
10.	Color and Condition of Liver	Appears normal
11.	Any other Morphological Abnormality Observed	No

Table 21: Morphological Characteristics of Specimen of Indian Bullfrog

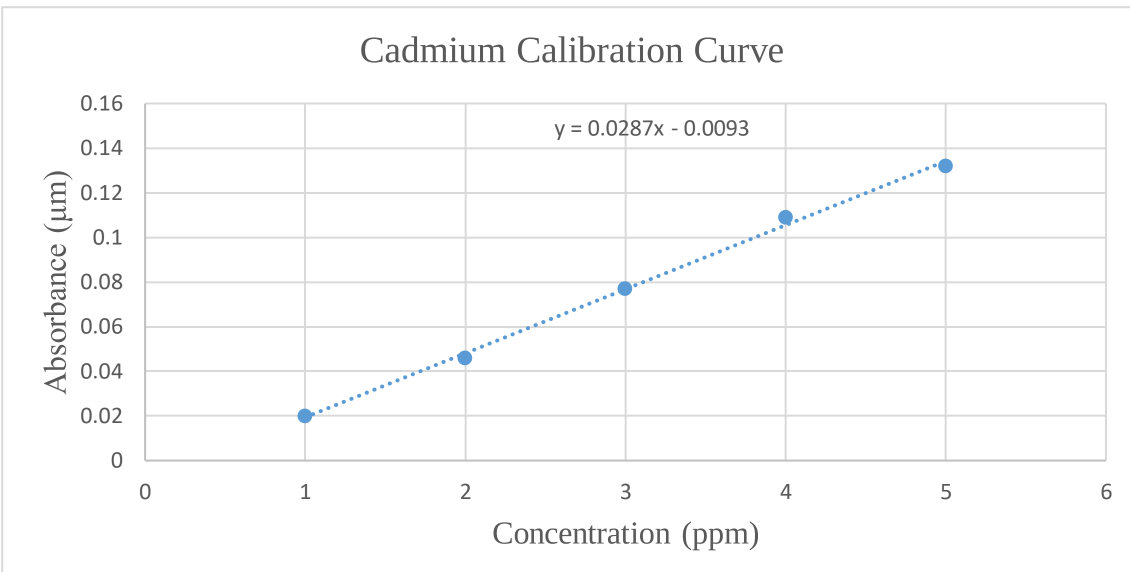
Sr. No	Morphological Characteristics	Comments
1.	Specimen Number and Area	Specimen 11 Gujranwala
2.	Weight	0.09 kg
3.	Total Length	9.1 inches
4.	Snout Vent Length	4.7 inches
5.	Color of Skin	Dark green with black patches on dorsal side
6.	Other Observable Characters on Skin	Vocal pouches are prominent
7.	Limb Anomalies Observed	No
8.	Heart Condition	Normal
9.	Appearance of Cysts on the Body	No
10.	Color and Condition of Liver	Greenish, yellowish in colour
11.	Any other Morphological Abnormality Observed	No

ANNEXURESIII

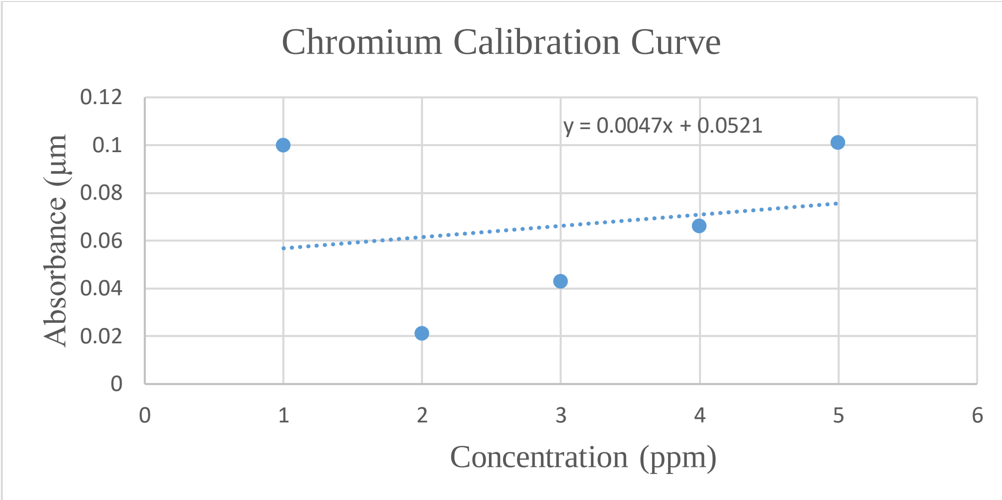
Table 1: Stock Solutions of Heavy Metals

Sr. No	Sample	1 ppm	2 ppm	3 ppm	4 ppm	5 ppm
Graph 1.	**Cadmium**	0.02	0.046	0.077	0.109	0.132
Graph 2.	**Chromium**	0.10	0.021	0.043	0.066	0.101
Graph 3.	**Nickel**	0.005	0.011	0.017	0.023	0.029
Graph 4.	**Cobalt**	0.009	0.020	0.028	0.037	0.046
Graph 5.	**Copper**	0.001	0.003	0.045	0.060	0.075
Graph 6.	**Manganese**	0.003	0.007	0.011	0.015	0.018
Graph 7.	**Zinc**	0.061	0.125	0.190	0.261	0.289

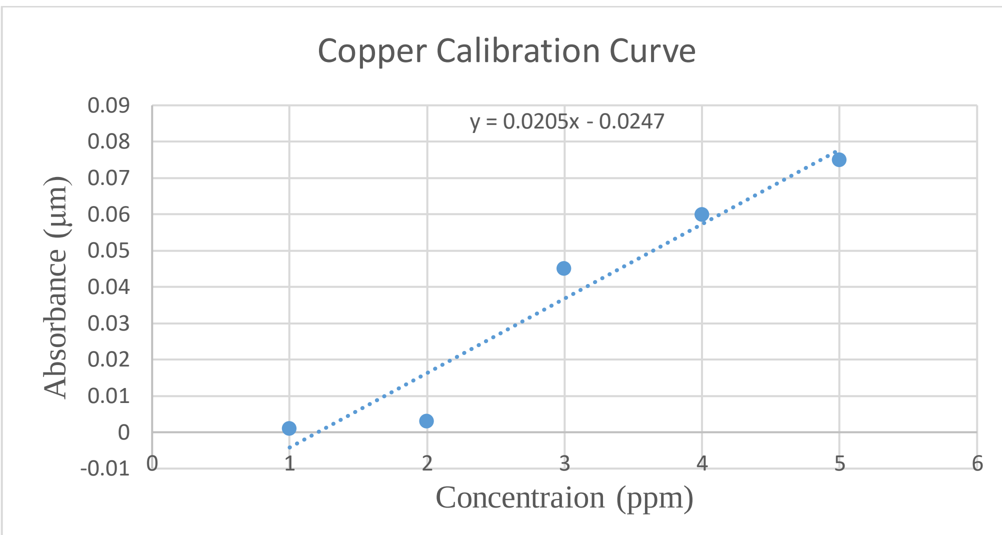
Calibration Curves of Stock Solutions:



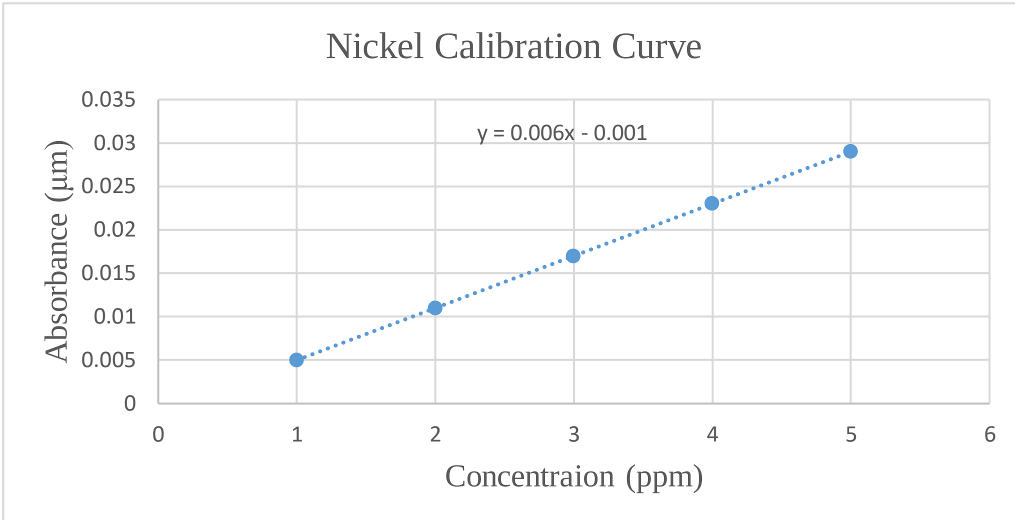
Graph 1: Calibration Curve of Stock Solutions of Cadmium



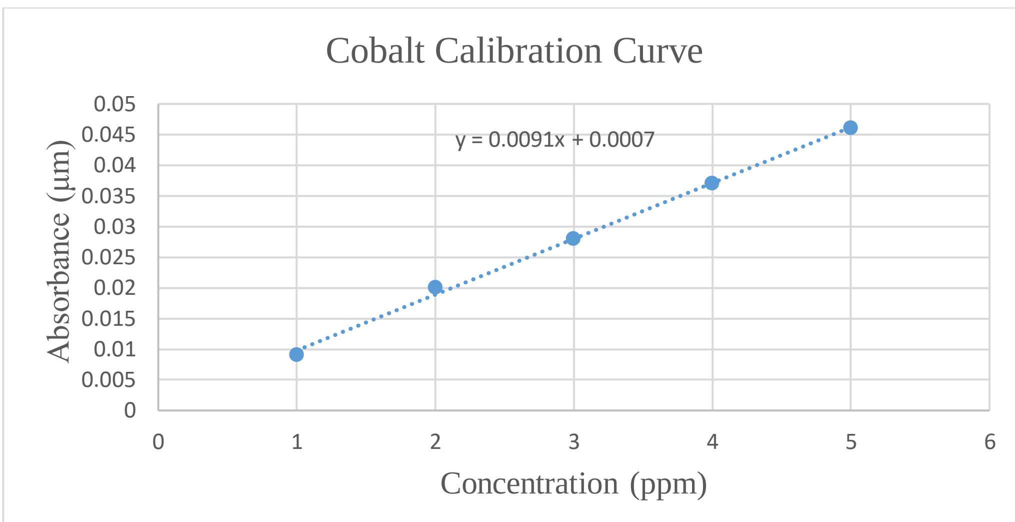
Graph 2: Calibration Curve of Stock Solutions of Chromium



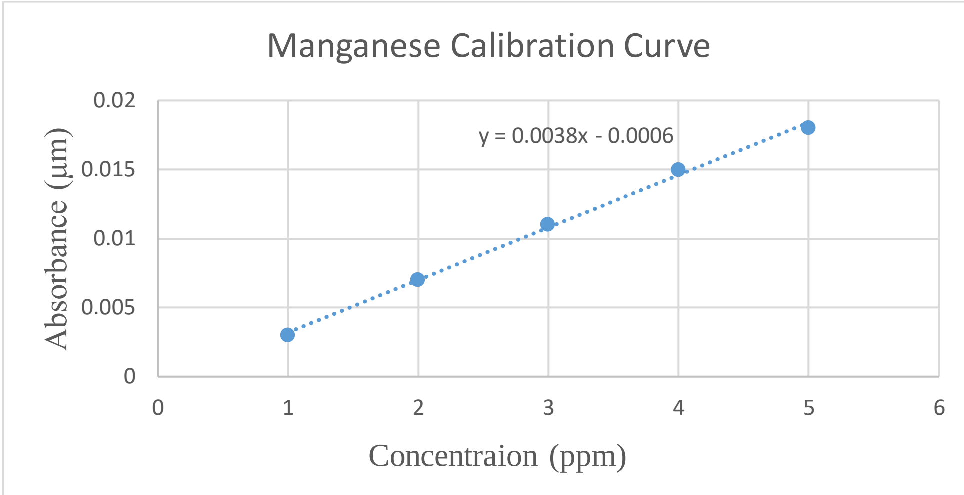
Graph 3: Calibration Curve of Stock Solutions of Copper



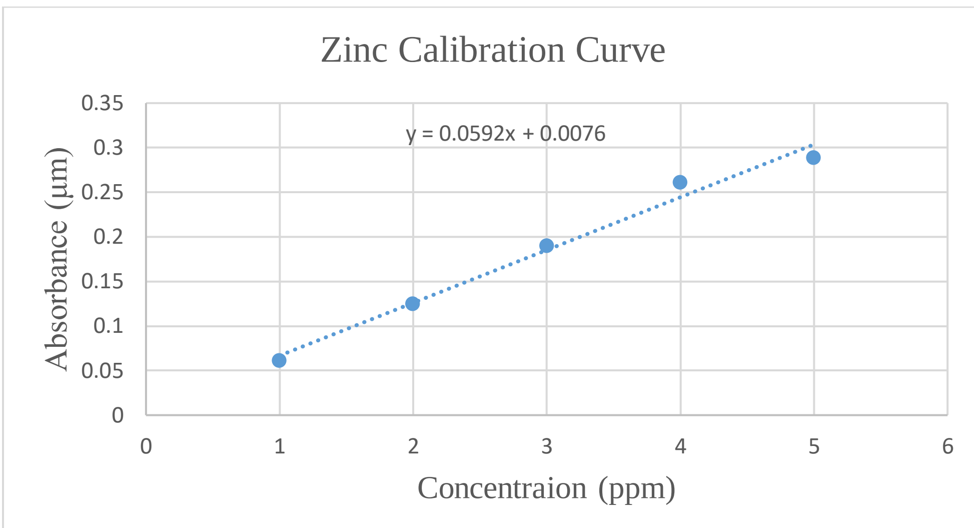
Graph 4: Calibration Curve of Stock Solutions of Nickel



Graph 5: Calibration Curve of Stock Solutions of Cobalt



Graph 6: Calibration Curve of Stock Solutions of Manganese



Graph 7: Calibration Curve of Stock Solutions of Zinc

Assignments
Students
Grade Book
Libraries
Calendar
Discussion
Preferences

Zoology

Eiuaza
29/11/22

About this page
This is your assignment inbox. To view a paper, select the paper's title. To view a Similarity Report, select the paper's Similarity Report icon in the similarity column. A ghosted icon indicates that the Similarity Report has not yet been generated.

BA/ BSc January Defense 2023

Inbox | Now Viewing: new papers ▼

Submit File Online Grading Report | Edit assignment settings | Email non-submitters

Delete | Download | move to...

Author	Title	Similarity	web	publication	student papers	Grade	response	File	Paper ID	Date
Nimra Tariq	Identification of Macroinvertebrates and...	5%	2%	2%	1%			download paper	1966157550	29-Nov-2022
Nadia Khan	Anticancer and Skin Repair Potential of ...	9%	7%	6%	2%			download paper	1966156600	29-Nov-2022
Sehar Munir	Evaluation of Skin Regeneration & Antiba...	9%	7%	7%	2%			download paper	1966157059	29-Nov-2022
Fajjar Azhar	Wound healing and Antifungal Properties ...	10%	6%	8%	1%			download paper	1966155397	29-Nov-2022
Laraib Fatima	Determination of Heavy Metal Toxicity in...	12%	5%	10%	3%			download paper	1966156240	29-Nov-2022
Bisma Noor	Determination of Heavy Metal Toxicity in...	12%	5%	10%	3%			download paper	1966154357	29-Nov-2022
Faria Zainab	Effect of hermal plant on cellular viabi...	12%	10%	8%	3%			download paper	1966155758	29-Nov-2022
Jamila Fatima	Efficacy of Pro-Biotic Supplementation o...	13%	11%	6%	3%			download paper	1966155960	29-Nov-2022
Shazma Shazma	Toxicological Analysis of Indian Bullfro...	15%	9%	11%	4%			download paper	1966157293	29-Nov-2022
Damna Tariq	Antifungal and injury regeneration poten...	16%	12%	9%	5%			download paper	1966154976	29-Nov-2022