

**EXAMINATION OF LEAD AND CADMIUM
CONTAMINATION IN MARKET- SOURCED
FILOPALUDINA BENGALENSIS FROM JHAPA,
MORANG AND SUNSARI DISTRICTS, EASTERN
NEPAL.**



**A PROJECT WORK SUBMITTED TO THE
DEPARTMENT OF ZOOLOGY
CENTRAL CAMPUS OF TECHNOLOGY INSTITUTE OF
SCIENCE AND TECHNOLOGY TRIBHUVAN UNIVERSITY
NEPAL**

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BACHELOR OF SCIENCE (Bsc.) IN BIOLOGY**

BY

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FILOPALUDINA BENGALENSIS FROM JHAPA, MORANG AND SUNSARI DISTRICTS,
EASTERN NEPAL.**

SMARIKA KAFLE

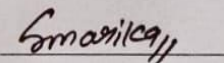
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DECLARATION

I hereby declare that the thesis report entitled “**EXAMINATION OF LEAD AND CADMIUM CONTAMINATION IN MARKET SOURCED FILOPALUDINA BENGALENSIS FROM JHAPA, MORANG AND SUNSARI DISTRICTS, EASTERN NEPAL**” is carried out under the supervision of Asst. Prof. Dr. Ganesh Tamang.

This project has been carried out in partial fulfillment of the requirement for the award of Bachelor of Science (B.Sc.) degree in Zoology at the Institute of science and Technology (IoST), Tribhuvan University (T.U.), Nepal. All activities described in this report have been conducted diligently, following the rules of academic writing, with all the sources duly acknowledged.

I hereby declare that this report has not been submitted to any other institution for any degree or benefit.



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[3, AUGUST, 2026]

RECOMMENDATION

The thesis entitled “EXAMINATION OF LEAD AND CADMIUM CONTAMINATION IN MARKET SOURCED FILOPALUDINA BENGALENSIS FROM JHAPA, MORANG AND SUNSARI DISTRICTS, EASTERN NEPAL”, submitted by **Ms. Smarika Kafle**, Symbol No: 500080051, T.U. Registration No: 5-2-8-87-2021 is prepared under my supervision as per the procedure and format requirement laid by the Department of Zoology, Central Campus of Technology, Institute of Science and Technology (IoST), Tribhuvan University(T.U), Nepal, as partial fulfillment of the requirements for the degree of Bachelor of Science (B.Sc) in Zoology.

To the best of my knowledge, this project represents Ms Smarika Kafles's original work and has not been submitted to any other institution for fulfillment of any degree requirement.



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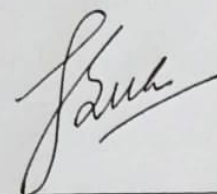
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LETTER OF FORWARD

On the recommendation of Asst. Prof. Dr. Ganesh Tamang, this project work was submitted by Ms. Smarika Kafle, Symbol No: 500080051, T.U. Registration No: 5-2-8-87-2021 entitled "EXAMINATION OF LEAD AND CADMIUM IN MARKET SOURCE FILOPALUDINA BENGALENSIS FROM JHAPA, MORANG, AND SUNSARI DISTRICT EASTERN NEPAL" is forwarded by Department of Zoology, Central Campus of Technology, Institute of science and technology (IoST), Tribhuvan University (T.U), Nepal.

She has fulfilled all the requirements laid down by the institute of science and technology (IoST), Tribhuvan University (T.U), Nepal, for the project work.

Date: August 3, 2026

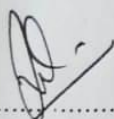


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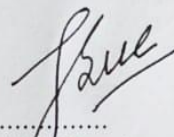
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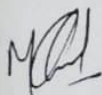
This project work (Pro-406) entitled "Examination of Lead and Cadmium in Market Source *Filopaludina bengalensis* from Jhapa, Morang, and Sunsari District Eastern Nepal" by Ms Smarika Kafle, Symbol No: 500080051 and T.U. Registration No: 5-2-8-87-2021 under the supervision of Dr. Ganesh Tamang in the Department of Biology, Central Campus of Technology, Institute of Science and Technology (IoST), Tribhuvan University (T.U.), is hereby submitted for the partial fulfillment of the Bachelor of Science (B.Sc.) degree in Zoology. This report has been accepted and forwarded to the controller of Examination, Institute of Science and Technology, Tribhuvan University, Nepal for the legal procedure.



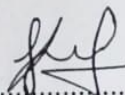
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LIST OF ABBREVIATIONS

Abbreviated form	Details of abbreviations
AAS	Atomic Absorption Spectrophotometry
ANOVA	Analysis of Variance
FAO	Food and Agriculture Organization
HI	Hazard Index
TR	Target Cancer Risk
US EPA / USEPA	United States Environmental Protection Agency
WHO	World Health Organization

ABSTRACT

Heavy metal pollution from contaminated water sources threatens aquatic life and human health. In eastern Nepal where Freshwater Snails (*Filopaludina bengalensis*) are commonly consumed as an edible mollusc, these pollutants may be absorbed into the snails through polluted waters which could cause harm to those who consume them and to assess the level of lead (Pb) and cadmium (Cd) present in snails sold at various markets in Jhapa, Morang and Sunsari districts in eastern Nepal, Atomic Absorption Spectrophotometry (AAS) was used to measure the levels of these metals and statistical analysis was carried out to compare the levels of each metal within each of the sampled locations and also to find the association between Pb and Cd. A comparison of the average amount of Pb and Cd measured indicated that Pb was significantly greater than Cd at all the sampling locations. At Site 3, the highest overall concentration of heavy metals was observed while Site 5 demonstrated the lowest concentrations of heavy metals of both Pb and Cd. One-way Anova and Kruskal Wallis Tests revealed significant variation in Pb and Cd levels among sample sites ($p < 0.05$). Analysis of paired comparisons confirmed that Pb levels were always greater than Cd levels at each site. However although there was a moderate positive association between Pb and Cd levels over sites this association was not statistically significant. These observations provide evidence for regional variations in the degree of contamination by heavy metals in *Filopaludina bengalensis* obtained from markets in eastern Nepal, also with lead being more problematic than cadmium in terms of contamination. Therefore, the results represent important baseline data on the levels of heavy metal contamination in commercially harvested freshwater snails and highlight the need for continued monitoring of freshwater foods for public health protection and improved food safety management in Nepal.

Keywords: Heavy metals, Lead(pb), Cadmium(Cd), *Filopaludina bengalensis*, Atomic Absorption Spectrophotometry(AAS)

शोध सार

यस अध्ययनको विषय “पूर्वी नेपालको झापा, मोरङ र सुनसरी जिल्लाका बजार स्रोतका *Filopaludina bengalensis* (घोंगी) मा लेड (Pb) र क्याडमियम (Cd) को प्रदूषणको परीक्षण” रहेको छ। अध्ययनको नमुना बजार स्रोतबाट पाँच स्थानहरूमा संकलन गरिएको थियो। ती स्थानहरूमा सुनसरी जिल्लाका इटहरी र झुम्का, मोरङ जिल्लाको मिरगौलिया र झापा जिल्लाका दमक र सुरुङ्गा रहेका थिए। क्षेत्रीय कार्य (fieldwork) नोभेम्बर २०२५ देखि मार्च २०२६ सम्म गरिएको थियो। संकलित नमुनाका नरम तन्तुहरू (soft tissues) अलग गरी ८०°C मा ओभन ड्राइ गरिएका, पिसाइ गरी लेड (Pb) र क्याडमियम (Cd) को मात्रा निर्धारणका लागि Atomic Absorption Spectrophotometry (AAS) विधि प्रयोग गरिएको थियो।

परिणाम अनुसार, Pb को एकाग्रता साइट १ (इटहरी) मा 0.370 ± 0.009 , साइट २ (झुम्का) मा 0.379 ± 0.006 , साइट ३ (मिरगौलिया) मा 0.465 ± 0.012 , साइट ४ (दमक) मा 0.399 ± 0.007 र साइट ५ (सुरुङ्गा) मा 0.232 ± 0.011 mg/g रहेको थियो। Cd को एकाग्रता क्रमशः साइट १ मा 0.091 ± 0.005 , साइट २ मा 0.118 ± 0.010 , साइट ३ मा 0.107 ± 0.003 , साइट ४ मा 0.049 ± 0.014 र साइट ५ मा 0.038 ± 0.016 mg/g रहेको थियो।

साइट ३ मा Pb को मात्रा सबैभन्दा बढी तथा कुल भारी धातु (Pb + Cd) को मात्रा 0.492 mg/g रहेको थियो भने साइट ५ मा कुल भारी धातुको मात्रा सबैभन्दा कम (0.270 mg/g) रहेको थियो। Cd को मात्रा सबैभन्दा बढी साइट २ मा रहेको थियो।

एकतर्फी ANOVA परीक्षणमा Pb ($F = 288.56$, $p < 0.001$) र Cd ($F = 27.34$, $p < 0.001$) का लागि साइटहरूबीच महत्वपूर्ण भिन्नता देखियो। युग्म तुलना (paired comparison) मा सबै साइटहरूमा Pb को मात्रा Cd भन्दा उल्लेखनीय रूपमा बढी रहेको पत्ता लाग्यो।

Pb र Cd बीचको Pearson सहसंबन्ध सकारात्मक तर सांख्यिकीय रूपमा असन्तुष्ट ($r = 0.710$, $p = 0.179$) रहेको थियो भने Spearman सहसंबन्ध पनि सकारात्मक तर असन्तुष्ट ($\rho = 0.500$, $p = 0.391$) रहेको थियो।

त्यसैले, बजार स्रोतहरूबीच Pb र Cd को प्रदूषणमा भिन्नता रहेको र Pb सधैं Cd भन्दा बढी रहेको निष्कर्ष प्राप्त भयो। अध्ययनले बजार स्रोतमा रहेको भारी धातु प्रदूषणको अवस्था उजागर गरेको छ। यसका साथै, पानी र अवसाद (sediment) को विश्लेषणसहित विशिष्ट स्रोत-केन्द्रित अध्ययन आवश्यक रहेको निष्कर्ष निकालिएको छ।

1.INTRODUCTION

1.1 Background

Molluscs, which are soft-bodied invertebrates found in terrestrial, freshwater, and marine settings, play an important part in the ecosystem. Molluscs are increasingly regarded as bioresources, benefiting both local people and the economy. Traditional knowledge on the usage of molluscs in food and medicine has had a significant impact on scientific research.

Molluscs have long been used as a food source and an important dietary component. They are also an excellent source of nutrients (Jadhav et al., 2023). *Filopaludina bengalensis*, also known as fresh water snails or Ghoongi is commonly found in various types of lowland aquatic habitats, particularly in stagnant and low saline water bodies. It inhabits rivers, streams, lakes, ponds, wetlands, marshes, ditches, and paddy fields. *F.bengalensis* are taken from rivers, canals, ponds, and marshes, and sold in local fish markets.(Rout et al., 2023). *Filopaludina bengalensis* is a freshwater These are edible freshwater gastropods that are widely consumed in Nepal and neighbouring countries and are regarded as an economical source of protein and minerals (Chakraborty et al., 2025). Its body is covered in shell. Overall, the shell is oval in shape. The upper portion of the shell is somewhat conoidal rather than conical. The aperture is sub-circular, with a narrow black edge. The colouration varies greatly, but it is never particularly bright. The foundation tint is greenish and opaque. The operculum is somewhat slender and dark brownish in hue. The viviparid (mystery-snail) females have a uterus in which they gestate the eggs until they become young snails, hence the term viviparous.(Bar, 2020). *F. bengalensis* is a crucial part of the fresh water ecology. It serves as a secondary consumer, digesting plankton and producing for carnivorous aquatic animals. This animal performs biofiltration while grazing on microorganisms and planktons. This species serves as a bioindicator for various ecological characteristics.(Bar, 2020).

Cadmium (Cd) and *lead* (Pb) bioaccumulate in freshwater ecosystems, posing significant risks to environmental health. The purpose of this work was to assess the level of heavy metals *cadmium* (Cd) and *lead* (Pb) in tissues of the edible fresh water snail *Filopaludina bengalensis*(Chatchakit & Nantararat, 2025). Heavy metals are inorganic metals found in minute concentrations in water and sediments. It has a high specific density, greater than 5g/cm³. Heavy metals are very poisonous even at low

concentrations, with devastating consequences for all forms of life. Heavy metals, like organic contaminants, are difficult to eliminate through natural processes due to their buildup in the environment and inability to decompose (Al-Taher et al., 2020). Metals like lead (Pb) and cadmium (Cd) are not often necessary for metabolic functions. Furthermore, even trace amounts of these heavy metals can be hazardous to animals. Aside from their carcinogenic effects, heavy metals can cause major complications such as liver illnesses, cardiovascular irregularities, kidney failure, and even death in extreme circumstances. Heavy metal poisoning, which exceeds the maximum tolerated limits, not only poses major human health hazards but also has a number of severe consequences on the ecosystem's natural equilibrium. Given these negative consequences and accompanying health hazards, heavy metal contamination is regarded as the most hazardous problem in aquatic environments (Biswas et al., 2021). In the current study, heavy metal levels (mg/g) in *F. bengalensis* from eastern Nepal were determined using the Atomic Absorption Spectrometry (AAS) method and compared to the maximum limits recommended by the FAO and WHO to determine whether these snails from the study areas are safe for human consumption. (Biswas et al., 2021).

1.2. Objective of the study

1.2.1 General Objective

To determine the concentrations of lead (Pb) and cadmium (Cd) in market-sourced *Filopaludina bengalensis* collected from Jhapa, Morang, and Sunsari districts of eastern Nepal.

1.2.2 Specific Objective

- I. To quantify the concentrations of lead (Pb) and cadmium (Cd) in soft tissue of *Filopaludina Bengalensis* collected from local markets in Jhapa, Morang and Sunsari districts.
- II. To evaluate public health risk associated with consumption of these mollusks by comparing metal levels against international food safety standards, such as WHO/ FAO limits.

1.3. Rationale of the study

1.3.1 Background

Although *Filopaludina bengalensis* is frequently consumed in eastern Nepal, data on heavy metal contamination in market-sourced specimens is scarce. Rapid industrialization, agricultural operations, and inappropriate waste management can all contribute to heavy metal pollution in aquatic ecosystems. Because these snails are consumed by local communities, determining their contamination levels is critical for food safety and public health. As a result, the motive of this study is to provide baseline data on lead and cadmium concentrations in *F. bengalensis* obtained from eastern Nepal's local markets.

1.3.2 Justification

This project will collect baseline data on lead and cadmium levels in *Filopaludina bengalensis* sold in eastern Nepal. The findings will aid in determining the safety of these edible snails for human consumption, as well as providing useful information to consumers, researchers, and public health officials. Furthermore, the research could help with future environmental monitoring and the creation of food safety policies.

1.3.3 Limitation

The study will analyze samples collected only from markets in Jhapa, Morang, and Sunsari districts. Only two heavy metals (Pb and Cd) have been analyzed and will focus only on soft tissue and not on shells or sediments. This also doesnot clarify that the ghoongi bought from itahari and Jhumka could be from same place or different place.

2.LITERATURE REVIEW

Molluscs are considered the second largest group of invertebrates after insects. The number of legitimate molluscan species is estimated to range from 50,000 to 55,000 marine species, 25,000 to 30,000 terrestrial species, and 6,000 to 7,000 freshwater species. Freshwater Mollusca occur in wide variety of aquatic habitats, including rivers, lakes, streams, swamps, springs, temporary ponds, drainage ditches, and other freshwater bodies. Nepal is home to approximately 80 freshwater mollusc species, including 30 bivalves and 50 gastropods . Freshwater gastropods, which make up the majority of the littoral fauna in tanks, ponds, beels, reservoirs, and rivers, are critical to aquatic ecosystem dynamics. The viviparids are mainly found in stagnant waterbodies, they are also seen in irrigated rice fields and occasionally in rushing water *Filopaludina bengalensis* (ghoṅgī) is a common edible river snail in South Asian populations, including Nepal (Filoparu et al., n.d.) Because it lives and feeds in freshwater habitats, it may accumulate hazardous heavy metals including lead (Pb) and cadmium (Cd) from contaminated water, sediment, and food. These metals may accumulate in the snail, causing physiological, tissue, and genetic problems, as well as food safety concerns if infected snails are consumed. Studies on allied *Filopaludina* species have shown seasonal fluctuation and considerable accumulation of Pb and Cd, whereas more recent research has specifically revealed Pb bioaccumulation in *F. bengalensis*. Monitoring Pb and Cd in ghoṅgī can help assess freshwater contamination and protect those that rely on it as a traditional food source (Saha et al., 2017)

Filopaludina bengalensis is a freshwater mollusc. Its body is covered in shell. Overall, the shell is oval in shape. The upper portion of the shell is somewhat conoidal rather than conical. The aperture is sub-circular, with a narrow black edge. The colouration varies greatly, but it is never particularly bright. The foundation tint is greenish and opaque. The operculum is somewhat slender and dark brownish in colour.(Bar, 2020). The front section houses the head, which has two tentacles, eyes, and a mouth. A large retractor muscle permanently attaches a snail's soft section to the columella of its shell. The visceral mass contains most of the internal organs and is enclosed within the mantle, a large fold of tissue that surrounds the body. Important organs, including the heart, kidney, and respiratory organs(lung), are situated within the mantle cavity. The margin of the mantle is involved in shell formation and growth. The digestive and

reproductive systems extend through the body, from the anterior region towards the foot. The visceral mass contains major parts of the digestive tract, reproductive organs, heart and nephridium. The intestine and rectum are located close to the major blood vessels and other internal organs. Ctenidium gills are responsible for the majority of respiration. The circulatory system is largely open, with blood moving through interconnected body sinuses. Before returning to the heart, the blood passes through the gills and nephridia, where gas exchange and excretory processes take place. In *Filopaludina bengalensis*, males and females can be distinguished by differences in the size and form of the right tentacles. Variation in the shell shape between the sexes has also been associated with differences in the diameter of the mantle region containing the reproductive organs. In males the reproductive system includes a well developed testis situated within the mantle cavity. The vast deferens extends toward the right tentacle and opens near its tip. In females, the reproductive system comprises a small ovary, a slender oviduct, a u-shaped glandular structure, a comparatively thick oviduct and an albumen gland. (Filoparu et al., n.d.)

The population status of *Filopaludina bengalensis* in Nepal has not been studied extensively. However, the species is frequently reported from freshwater habitats, particularly ponds, rivers, streams, and paddy fields in the Terai region and some middle districts of the country (Chakraborty et al., 2025b). As an edible freshwater snail, *F. Bengalensis* is consumed by people in different parts of Asia and Africa. It is also associated with traditional food practices and medicinal uses among various ethnic communities. "According to (Filoparu et al., n.d.)" freshwater snails constitute an important food resource for households belonging to several caste and ethnic groups.

In addition to its importance as food resource, *F. bengalensis* contributes to the functioning of fresh water ecosystems. Its feeding activities involve the consumption of detrital material, microorganisms, and other food particles, thereby contributing to the nutrient cycling and energy transfer within aquatic food webs. The species may also provide information about local environmental conditions because its occurrence and accumulation of contaminants can reflect changes in water quality (Filoparu et al., n.d.). Its role in the aquatic community includes participation as a consumer as well as serving as prey for other organisms. The species feeding and filtration activities may further contribute to the movement of organic material within freshwater ecosystems. *F. bengalensis* has a stronger ability for zinc, copper, and lead accumulation, and the

accumulated metal concentration in the snail's soft body tissues is higher than that of surface water and soil sediments. This specimen can serve as a candidate for standardising the freshwater sediment toxicity testing technique (Bar, 2020)

(Al-Taher et al., 2020) Heavy metals are inorganic metals found in extremely small amounts in water and sediment. It has a high specific density, greater than 5 g/cm³. Heavy metals are very poisonous even at low concentrations, resulting in devastating effects on all forms of life. Heavy metals, like organic contaminants, are difficult to eliminate through natural processes because they accumulate in the environment and do not decompose. Heavy metals enter aquatic ecosystems, especially fresh water, due to their use in civil and rural life, as well as increased population growth. Pollution from these metals can be natural or anthropogenic, with human events often being the primary source. While some metals are necessary for life, they can also be harmful in large concentrations. Heavy metals such as lead (Pb), cadmium (Cd), chromium (Cr), nickel (Ni), and tin (Sn) generally have no essential role in the normal metabolic processes of animals. Exposure to these metals, even at low concentrations, may adversely affect animal health. Prolonged or excessive exposure can result in toxic effects, including damage to the liver and kidneys, cardiovascular disturbances, and in severe cases, death. When heavy metals concentration exceed permissible limits, they may pose significant risk to human health and can also disrupt the natural balance of ecosystems. Therefore, heavy metal contamination is considered an important environmental concern, particularly in aquatic environments where these pollutants can accumulate and enter food chains (Biswas et al., 2021). To protect human health from the adverse effects of heavy metal exposure, international organizations such as the FAO and WHO have established permissible limits for various heavy metals in food and environmental matrices. However, these permissible limits alone do not provide an estimation of the potential carcinogenic and non-carcinogenic health risks associated with exposure. Therefore, the U.S. environmental protection agency (USEPA) has developed quantitative approaches for assessing health risks, including the hazard index (HI) for non-carcinogenic effects and target cancer risks (TCR) for carcinogenic effects. In the present study, atomic absorption spectroscopy (AAS) was employed to determine the concentrations of selected heavy metals, including lead and cadmium, in the analyzed samples (Biswas et al., 2021).

(Bar, 2020) *F. bengalensis* has the highest protein content (% dry weight) of any freshwater edible mollusca, as well as strong ethnomedicinal use. Previous in vivo and in vitro studies have reported the potential of *F. bengalensis* and its bioactive components to exhibit anti-inflammatory, anticancer, antioxidant, and antimicrobial properties. In addition, increasing global warming and industrial pollution have contributed to the deterioration of aquatic ecosystems, highlighting the importance of aquatic organisms as indicators of environmental quality. Due to its association with freshwater habitats *Filopaludina bengalensis* may have potential as a bioindicator for assessing water quality and may also contribute to biofiltration processes. The species is widely available and relatively inexpensive in local markets, making it a potentially useful organism for ecological studies and future investigations into its biological and medicinal properties. Freshwater snails are taken from rivers, canals, ponds, and other wetlands and distributed to consumers through local fish markets. However, there is little information available in Nepal about harvest patterns and customer preferences. In Nepal, its consumption is closely linked to the food traditions of various Terai and Madhesh people, including the Tharu and other local indigenous groups. However, it should not be seen as a meal restricted to a single ethnic group. The use and preparation procedures differ between communities and localities (Chakraborty et al., 2025a).

Heavy metal buildup can result in, Injury to tissues and organs, disturbance of normal metabolism, Oxidative Stress, DNA/genetic harm, Reduced growth and reproduction, In extreme contamination, survival is lowered in snail. *F. bengalensis* is consumed as food, hence contamination is a food-safety problem. If snails acquire Pb or Cd and are eaten regularly, these metals can reach the human body. Lead (Pb) can harm the neurological system, blood formation, kidneys, and other organs. Children are especially sensitive to lead exposure. Cadmium (Cd) can accumulate in the body, mostly harming the kidneys and bones after prolonged exposure.

Heavy metals are naturally occurring elements that have a large atomic weight and are poisonous. Many heavy metals produce toxicity through oxidative processes that endure and bioaccumulate in mitochondrial environmental systems, stress, and enzyme inhibition. Even at low carcinogenic disorders, certain metals—such as iron, zinc, and copper—dysfunction, genotoxicity, and endocrine disruption are necessary for and imbalance of essential metal homeostasis, biological functions, while others—such as lead, which causes neurological, renal, hepatic, mercury, cadmium, and arsenic—pose

serious risks to cardiovascular, reproductive, immune, and environmental health. Heavy metal concentrations in soil, water, and air have dramatically increased as a result of rapid industrialization, mining, an integrated evidence base to support intensive agriculture, recycling of electronic waste, and urbanization. This has altered natural biogeochemical cycles. Heavy metals are not biodegradable, in contrast to many organic pollutants, and they build up in food chains through contaminated water supplies, fish, and crops. The kind of metal, the method of exposure, the length of time, and the health of those exposed all affect how severe the toxicity is. Therefore, knowledge of the origins of contamination, exposure routes, and health consequences is still crucial for managing public health and protecting the environment.(Ajeboriogbon et al., 2026)

3. MATERIALS AND METHODS

3.1 Study area

This study was conducted on the Eastern Side of Nepal from Sunsari, Morang to Jhapa fish market. The field research was carried out from November 2025 AD to March 2026 AD. These districts lie in Eastern Terai Nepal and contain extensive wetlands, agricultural lands, and urban settlements. In sunsari, Jhumka and Itahari were the market for collection of sample likewise Mirgauliya in Morang and also Damak and Surunga from Jhapa district.

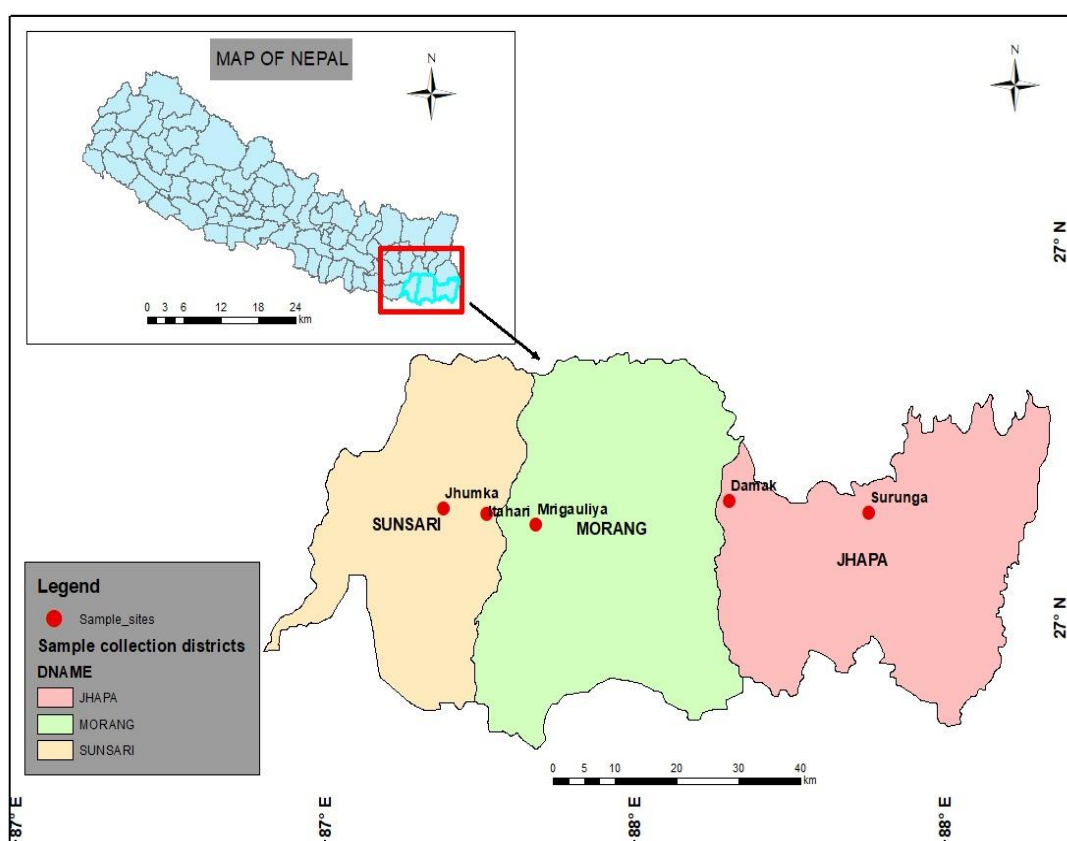


Figure 1 : Map of Study Area

3.2 Materials required

- Dissecting box
- Petriplates
- Zipper bags
- Mortar and pestle
- Refrigerator
- Hot air oven
- Scissors
- Gloves
- Weighing balance
- Almunium foil

3.3 Collection of samples

The sample were collected from five different fish market (Itahari, Jhumka, Mirgauliya, Damak, Surunga). After collecting the samples they were brought in plastic bags to the labrotary at the Department of Zoology on the Central Campus of the Dharan.

3.4 Preparation of samples for analysis

The samples from each market were individually cleaned and had their soft tissue removed from the shell and placed in the seprate petriplates for individual market,.After that they were dried in an oven at 80 degree celcius until it completely dry. Then, a mortar was used to make powder (sample wise). The sample were put in zipper bags and each bags with sample were weighed individually and sent to the lab for AAS test.

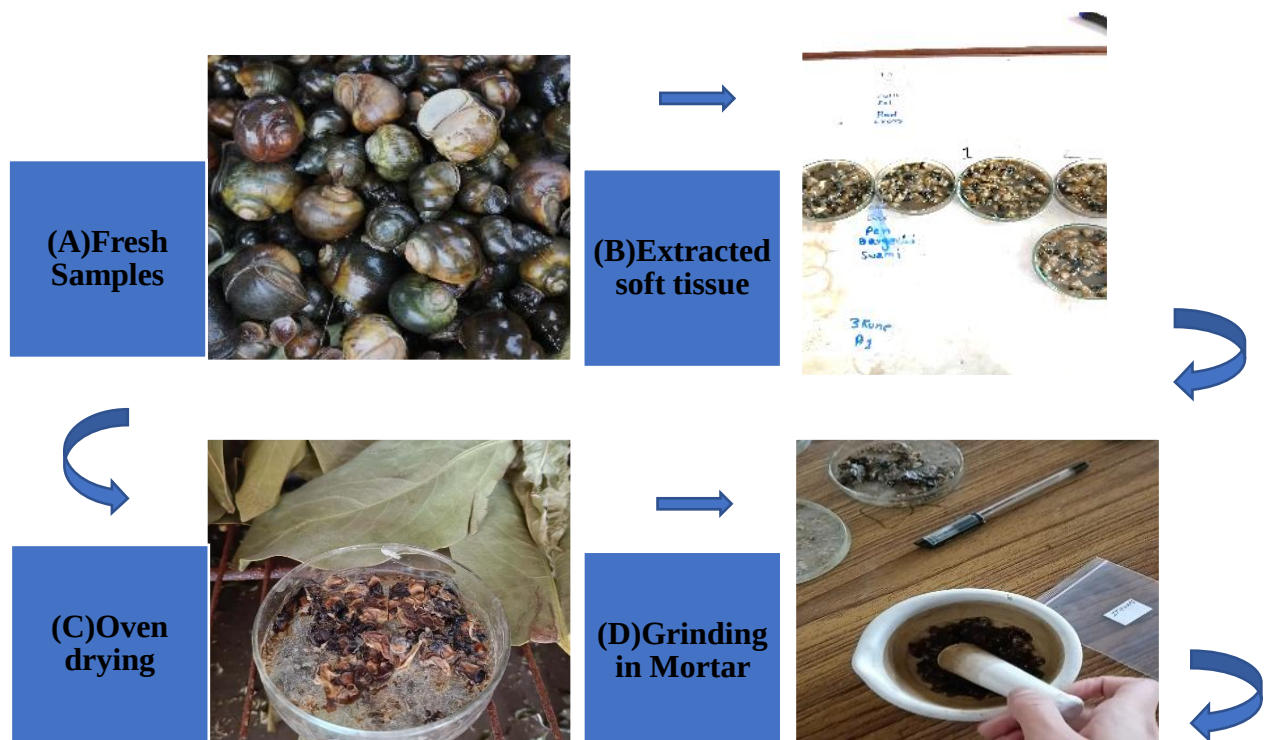




Figure 2: Process of sample preparation for Heavy meatal analysis

Table 1 : Sample name, Weight (in gram)

S.N	Sample name	Weight (in gram)
1	Damak	5.422
2	Surunga	7.451
3	Mirgauliya	6.513
4	Itahari	7.789
5	Jhumka	6.029
	Empty bag	1.5

3.5 Heavy metal analysis

The composition of Heavy metal in *F.Bengalensis* was determined using AAS(Atomic Absorbtion Spectrometry).This test was conducted to find the concentration of Lead(Pb) and Cadmiuim(Cd) in the sample collected.This analysis was carried out at the Nepal Batawaraniya Sewa Kendra, Biratnagar, Morang.

The concentrations obtained from the AAS analysis were recorded for each sampling location and subsequently used for statistical analysis and comparison among the study sites.

4. RESULTS

4.1 Site-wise concentrations of lead and cadmium

The concentrations of lead (Pb) and cadmium (Cd) varied among the five sampling sites. Overall, Pb concentrations were consistently higher than Cd concentrations at all sites. The mean Pb concentration ranged from 0.232 ± 0.011 mg/g at Site 5 to 0.485 ± 0.012 mg/g at Site 3. Similarly, Cd concentration ranged from 0.038 ± 0.016 mg/g at Site 5 to 0.118 ± 0.010 mg/g at Site 2.

Among the studied sites, Site 3 showed the highest Pb concentration, followed by Site 4, Site 2, Site 1 and Site 5. In contrast, Site 2 showed the highest Cd concentration, followed by Site 3, Site 1, Site 4 and Site 5. Site 5 showed the lowest concentration for both metals (Table 2).

Table 2. Mean concentration of Pb and Cd across five sampling sites. Values are presented as mean $\pm$ SD (mg/g).

Site	Pb (mg/g)	Cd (mg/g)	Pb/Cd ratio	Total Pb + Cd (mg/g)
Site 1	0.370 ± 0.009	0.091 ± 0.005	4.083	0.461
Site 2	0.379 ± 0.006	0.118 ± 0.010	3.206	0.498
Site 3	0.485 ± 0.012	0.107 ± 0.003	4.520	0.592
Site 4	0.399 ± 0.007	0.059 ± 0.015	6.758	0.458
Site 5	0.232 ± 0.011	0.038 ± 0.016	6.162	0.270

The total heavy metal concentration (Pb + Cd) was highest at Site 3, with a combined value of 0.592 mg/g, while the lowest combined concentration was recorded at Site 5 (0.270 mg/g), indicating that Site 3 had the greatest overall accumulation of the studied heavy metals.

4.2 Variation in metal concentration among sites

The variability of metal concentrations differed between Pb and Cd. Pb showed relatively low variation within triplicate measurements (Figure 3), with coefficient of variation (CV) values ranging from 1.52% to 4.64%. The lowest variation for Pb was observed at Site 2, while the highest was observed at Site 5.

Cd showed greater relative variability than Pb, particularly at Site 4 and Site 5. The CV for Cd ranged from 2.85% at Site 3 to 42.59% at Site 5, suggesting that Cd concentration was less uniform among triplicate measurements, especially at sites where its mean concentration was low (Table 3).

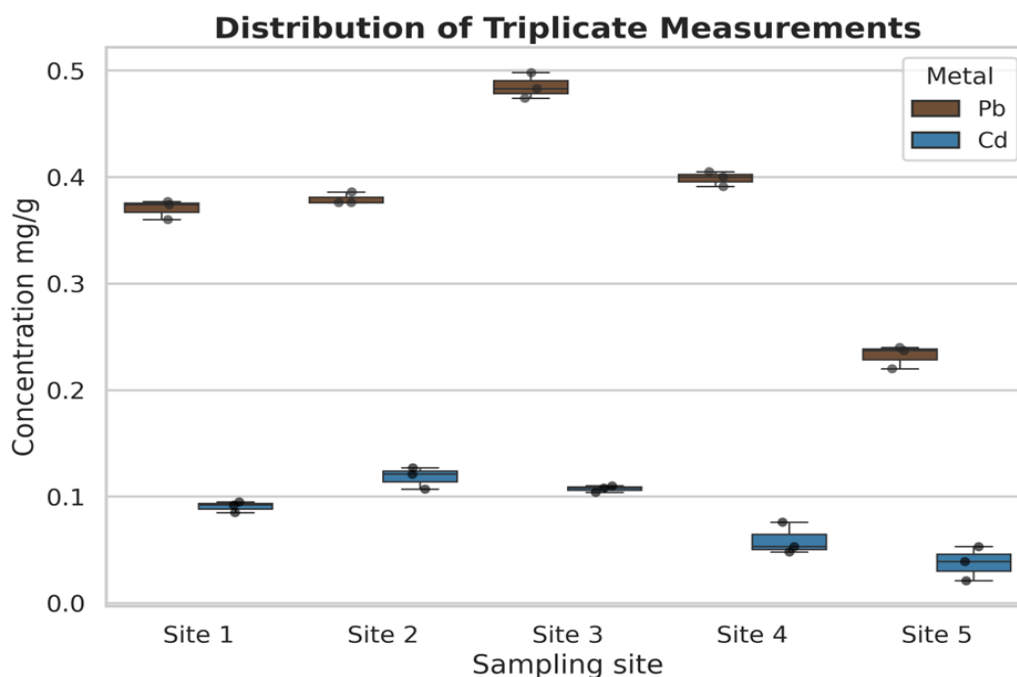


Figure 3. Distribution of triplicate measurements

Table 3. Descriptive statistics of Pb and Cd concentrations at each site.

Metal	Site	n	Mean (mg/g)	SD	SE	CV (%)
Pb	Site 1	3	0.370	0.009	0.005	2.45
Pb	Site 2	3	0.379	0.006	0.003	1.52
Pb	Site 3	3	0.485	0.012	0.007	2.50
Pb	Site 4	3	0.399	0.007	0.004	1.78
Pb	Site 5	3	0.232	0.011	0.006	4.64
Cd	Site 1	3	0.091	0.005	0.003	5.66
Cd	Site 2	3	0.118	0.010	0.006	8.67
Cd	Site 3	3	0.107	0.003	0.002	2.85
Cd	Site 4	3	0.059	0.015	0.009	25.31
Cd	Site 5	3	0.038	0.016	0.009	42.59

4.3 Statistical comparison of heavy metal concentrations among sites

One-way ANOVA showed that the concentration of Pb differed significantly among the five sites ($F = 288.56$, $p < 0.001$). The Kruskal-Wallis test also confirmed a significant site-wise difference for Pb ($H = 13.06$, $p = 0.011$), supporting the ANOVA result.

For Cd, one-way ANOVA similarly showed a significant difference among sites ($F = 27.34$, $p < 0.001$), and the non-parametric Kruskal-Wallis test confirmed significant variation among sites ($H = 12.68$, $p = 0.013$) (Table 4).

Table 4. Statistical comparison of Pb and Cd concentrations among sites.

Metal	Test	Test statistic	p-value	Interpretation
Pb	One-way ANOVA	$F = 288.56$	< 0.001	Significant difference among sites
Pb	Kruskal-Wallis	$H = 13.06$	0.011	Significant difference among sites
Cd	One-way ANOVA	$F = 27.34$	< 0.001	Significant difference among sites
Cd	Kruskal-Wallis	$H = 12.68$	0.013	Significant difference among sites

Post-hoc pairwise comparison indicated that most Pb concentrations differed significantly among sites. Site 3 differed significantly from Sites 1, 2, 4 and 5, while Site 5 also differed significantly from Sites 1, 2, 3 and 4. However, the difference between Site 1 and Site 2 was not statistically significant. For Cd, fewer pairwise differences remained significant after multiple-comparison correction; the strongest difference was observed between Site 2 and Site 5.

4.4 Comparison between Pb and Cd at each site

Paired comparison between Pb and Cd concentrations showed that Pb was significantly higher than Cd at all five sites. The mean difference between Pb and Cd ranged from 0.195 mg/g at Site 5 to 0.378 mg/g at Site 3 (Table 5). The largest Pb-Cd difference was observed at Site 3, largely attributable to the high Pb concentration at this site.

Although Site 5 had the lowest concentrations of both metals, Pb remained significantly higher than Cd.

Table 5. Paired comparison between Pb and Cd concentrations at each site.

Site	Mean difference Pb – Cd (mg/g)	t-value	p-value	Interpretation
Site 1	0.280	40.32	0.00061	Pb significantly higher than Cd
Site 2	0.261	62.69	0.00025	Pb significantly higher than Cd
Site 3	0.378	60.65	0.00027	Pb significantly higher than Cd
Site 4	0.340	27.54	0.00132	Pb significantly higher than Cd
Site 5	0.195	18.01	0.00307	Pb significantly higher than Cd

4.5 Relationship between Pb and Cd across sites

The relationship between site-wise mean Pb and Cd concentrations was positive but not statistically significant. Pearson correlation analysis showed a moderate positive relationship ($r = 0.710$, $p = 0.179$), and Spearman rank correlation also indicated a positive but non-significant association ($\rho = 0.500$, $p = 0.391$) (Table 6). Although sites with higher Pb tended to show higher Cd to some extent, the trend was not strong enough to attain statistical significance based on the five sampling sites.

Table 6. Correlation between site-wise mean Pb and Cd concentrations.

Comparison	Test	Correlation coefficient	p-value
Site mean Pb vs Cd	Pearson correlation	0.710	0.179
Site mean Pb vs Cd	Spearman correlation	0.500	0.391

5. DISCUSSION

This study assessed the level of lead (Pb) and cadmium (Cd) in market-sourced *Filopaludina bengalensis* from five fish markets of eastern Nepal. While Pb and Cd were found in the soft tissues of snail, concentration differed among sampling sites as revealed by the study. The concentration of metals varied, which shows that the level of contamination was not equal in all studied markets.

Lead values were higher than cadmium amounts in all five testing sites. The average Pb level varied between 0.232 ± 0.011 mg/g at Site 5 and 0.485 ± 0.012 mg/g at Site 3. Cd values ranged from 0.038 ± 0.016 mg/g at Site 5 to 0.118 ± 0.010 mg/g at Site 2. The steadily increasing Pb readings show that lead was most common contaminant in the snail samples analysed. Although previous study have shown that freshwater molluscs can absorb Pb and Cd from their aquatic environment (Al-Taher et al., 2020; Chatchakit & Nantararat, 2025).

Of the five sites, Site 3 showed the highest Pb concentration (0.485 ± 0.012 mg/g), while Site 5 recorded the lowest (0.232 ± 0.011 mg/g). Site 2 recorded the highest Cd concentration (0.118 ± 0.010 mg/g), whereas Site 5 had the lowest (0.038 ± 0.016 mg/g). The highest combined levels of Pb and Cd were found at Site 3 (0.592 mg/g). In contrast, Site 5 exhibited the lowest total concentration (0.270 mg/g). Such differences may indicate variations in the sources and extent of environmental contamination associated with the areas from which the market-sourced snails originated.

Statistical analysis also confirmed the variations in Pb and Cd concentrations. The obtained Pb concentration varied significantly among the five sites (one-way ANOVA: $F = 288.56$, $p < 0.001$). Kruskal-wallis also demonstrated a statistically significant difference ($H = 13.06$, $p = 0.011$). Likewise, Cd concentration also varied significantly among the sites (ANOVA: $F = 27.34$, $p < 0.001$; Kruskal-Wallis test: $H = 12.68$, $p = 0.013$). Consequently, it was statistically significant and not simply due to variation between very small measurement units.

At most sites, the triplicate measurements of Pb revealed comparatively little variation. Pb readings were relatively consistent, with a coefficient of variation ranging from 1.52% to 4.64%. The variance was higher for Cd, especially at Sites 4 and 5. At Site 5, the coefficient of variation for Cd was 42.59%. The comparatively low Cd concentration at

this location, where slight variations in measurements might result in a higher percentage of fluctuation, may be partially responsible for this increased variation.

Pb concentrations were substantially greater than Cd concentrations at all sampling sites, according to the paired comparison of Pb and Cd. Site 3, which likewise had the highest Pb concentration, had the most difference between Pb and Cd (0.378 mg/g). Site 5 showed the least amount of variation (0.195 mg/g). The conclusion that lead was the primary heavy metal found in the examined samples is further supported by this result.

The mean amounts of Pb and Cd at each site showed a positive correlation. Spearman correlation yielded $\rho = 0.500$, whilst Pearson correlation yielded $r = 0.710$. Nevertheless, neither association was statistically significant ($p > 0.05$). Although the current data were insufficient to show a statistically significant association between the two metals, this suggests that places with greater Pb concentrations tended to have higher Cd concentrations to some extent. The correlation result might have been impacted by the minimal number of sampling sites.

Because *F. bengalensis* is eaten in eastern Nepal, the presence of Pb and Cd in its soft tissues is significant. In addition to describing *F. bengalensis* as an edible freshwater mollusc, earlier research has emphasised its ecological significance as a bioindicator of environmental conditions (Bar, 2020; Rout et al., 2023). The tissue concentration of the snail can reveal important details about the quality of the surroundings connected to the collected specimens since the snail can store pollutants from aquatic settings.

The current study's results offer baseline data about Pb and Cd contamination in market-sourced *F. bengalensis* from eastern Nepal. Nevertheless, only two heavy metals were examined, and the study was restricted to five market sites. Additionally, the study did not look at the related water, sediment, or shell samples and instead concentrated on soft tissues. Therefore, the current investigation cannot identify the precise origins of Pb and Cd contamination.

Overall, the findings demonstrate that the *F. bengalensis* samples under investigation were contaminated with heavy metals, with Pb consistently exhibiting higher quantities than Cd. The notable variations across sampling locations imply that contamination levels differ geographically. Therefore, more research on edible freshwater molluscs and their aquatic habitats will be helpful in determining the origins and degree of heavy metal pollution in eastern Nepal.

6. CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The current study measured the levels of lead (Pb) and cadmium (Cd) in market-sourced *Filopaludina bengalensis* that were gathered from five fish markets in the eastern Nepalese districts of Jhapa, Morang, and Sunsari. The soft tissues of the snails were found to contain both Pb and Cd, with varying quantities across the sampling locations.

At each of the five locations, lead was consistently detected at higher amounts than cadmium. Site 3 had the greatest Pb concentration (0.485 ± 0.012 mg/g), whereas Site 5 had the lowest (0.232 ± 0.011 mg/g). Site 2 had the highest concentration of Cd (0.118 ± 0.010 mg/g), while Site 5 had the lowest concentration (0.038 ± 0.016 mg/g). Pb and Cd concentrations were highest at Site 3, whereas Site 5 displayed the lowest combined concentration

Pb and Cd concentrations varied significantly between the sampling locations, according to

statistical analysis. At every location, the concentration of Pb was likewise noticeably higher than that of Cd. The link between Pb and Cd was not statistically significant, despite the fact that it was positive across all sampling locations.

The results show that market-sourced *F. bengalensis* from the examined areas includes detectable levels of Pb and Cd, with lead being the most prevalent pollutant. Because the species is used as food, regular monitoring of heavy metals in edible freshwater creatures is critical for food safety and public health. More studies with a larger number of sampling sites, additional heavy metals, and water and sediment analysis are needed to identify potential sources of contamination and offer a more comprehensive assessment of heavy metal pollution in eastern Nepal.

6.2 Recommendation

The following key recommendation are proposed based on the findings of this study:

- Edible freshwater snails sold in eastern Nepal's local markets should be regularly monitored for Pb, Cd, and other potentially dangerous heavy metals.
- Future studies should include a greater number of sampling locations and samples from various seasons in order to obtain a more representative picture of heavy metal pollution.
- Additional research should be conducted to discover potential sources of Pb and Cd pollution in water and sediment, as well as snail tissues.
- Future studies should include other heavy metals such as mercury (Hg), chromium (Cr), arsenic (As), and copper (Cu) to provide a more comprehensive assessment of contamination.
- The concentration of heavy metals in edible *F. bengalensis* should be compared on a regular basis to appropriate food-safety standards in order to identify potential consumption concerns.
- Consumers and local communities should be informed about the potential impacts of heavy metal pollution in aquatic food resources, as well as the need of getting aquatic species from safer sources.
- Proper management of agricultural runoff, household trash, industrial waste, and other potential sources of heavy metal contamination should be promoted in locations near aquatic environments.
- Long-term monitoring programs should be implemented to generate baseline data and detect changes in heavy metal pollution in edible freshwater species in eastern Nepal.
- Seasonal variation in Pb and Cd accumulation should be addressed because environmental circumstances and pollution levels can alter across seasons.

PHOTO PLATE





नेपाल वातावरणीय सेवा केन्द्र NEPAL BATAWARANIYA SEWA KENDRA

Morang Merchants' Association Complex
Pdt. Meghraj Marga (Goshwara Road), Biratnagar-7 Nepal Tel : +977-21-576887

Date: 24th Chaitra 2082

Test Report

Name of the Client	Smarika Kafle	Location	Itahari, Sunsari
Type of Sample	Ghoongi	Sample Receiving Date	16-12-2082
Condition of Sample	Ok	Test Performance Date	22-12-2082
Lab Code	SN- 36 (F), 2082/83	Test Completion Date	22-12-2082

SN	Parameters	Unit	Results					Method
			A	B	C	D	E	
1	Lead	mg/100gm	0.38	0.37	0.48	0.39	0.23	AAS
2	Cadmium	mg/100gm	0.10	0.12	0.10	0.06	0.04	AAS

The reported results comply to the sample tested only.

A- Itahari, B-Jhumka, C-Mirgaula, D-Damak, E-Jhapa Road

Prepared by

Analyst

Authorized signature
Lab In-charge



P.O. Box 104, Email: nenscbt@gmail.com/seamnmmaesc@gmail.com

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