Evaluation of Heavy Metal Levels in Chicken Using Indeterministic Analysis: A Study Conducted in Localities of Lahore, Pakistan

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Abstract

The poultry industry is a significant source of animal protein, vitamins, and minerals, particularly through the consumption of chicken meat. A study conducted in Lahore collected 100 samples each of chicken liver, chicken feed and water to investigate the presence of Arsenic and selected heavy metals. The analysis revealed contamination above safe limits, with Copper (Cu), Zinc (Zn), and Cadmium (Cd) being the most prominent metals detected. Neutrosophic statistics proved to be a more informative approach for data analysis. These findings emphasize the importance of monitoring and mitigating heavy metal contamination in the poultry industry to ensure the safety and quality of poultry products.

1. Introduction

Items made from poultry, including eggs, meat, and meat-based items, are widely recognized as significant sources of vitamins, minerals, protein and energy worldwide due to their nutritional value, palatability, and affordability. These products contribute substantially to meet the daily suggested allowances for proteins, energy, and trace minerals set compiled by the World Health Organization (WHO) (WHO, 2007). However, it is important to acknowledge that poultry products can potentially carry risks associated with contamination by heavy metals. Environmental sources and feed additives are common pathways for heavy metal contamination in poultry. Broiler feed often contains supplemented microelements like Iodine (I), Manganese (Mn), Zinc (Zn), Copper (Cu), and Arsenic (As) to support bird growth and health (Ahmad, Muhammad, Zafar, Afzal, & Aslam, 2023; X. Li, Chen, Cheng, Wen, & Zhou, 2017; Sim & Yu, 1988). While minerals are essential for meticulous biological process, immunity, and metabolic activity, excessive levels of trace minerals can surpass the animals' requirements. Consequently, these excess trace minerals are excreted in manure, leading to potential negative environmental impacts (Koyunoğlu & Karaca, 2022). In addition to the aforementioned heavy metals, like Lead (Pb), Cadmium (Cd), and Mercury (Hg), which are non-essential for the body's authenticity and efficiency, the increased consuming these hazardous elements significant risks to both humans and animals. In terms of minerals animal products is essential for the development of a healthy human embryo and has been suggested as a potential bio-sindicator for environmental element pollution. But it’s crucial to understand that animal products can potentially exhibit elevated levels of hazardous elements, primarily originating by way of food, water, waste, and the environment(Satarug & Moore, 2004; Vardhan, Kumar, & Panda, 2019).

Cadmium (Cd), Arsenic (As), and Lead (Pb) are particularly toxic and may spread across the food chain, exerting harmful effects on each animal and human health. Lead (Pb), known as a neurotoxin, can cause metabolic harm and negatively impact renal, hemopoiesis, neurological, and gastrointestinal functions. Food plays a significant role in Cd contamination, as it comes from several food sources and the environment, ultimately animals and humans are exposed to it through the food chain, leading to pulmonary and hepatocellular damage, hypertension, kidney dysfunction, and kidney damage(Järup & Åkesson, 2009; Satarug, Vesey, & Gobe, 2017). Existing literature indicates that the presence of animal feed mineral contamination is a problem that cannot be avoided, as they are susceptible to contamination by heavy metals. Environmental pollution, the use of supplements and concentrates, as well as the machinery and equipment employed during the manufacturing process, can all contribute to heavy metal contamination in animal feeds (Kabata-Pendias & Szteke, 2015). Both humans and animals rely on the liver and kidneys for the crucial processes of detoxification and excretion of harmful elements. Consequently, these organs are particularly vulnerable to damage when there is an abundance of harmful elements present in the food or a diet (Haque, Hossain, Jolly, & Tareq, 2021; Soumaoro, Pitala, Gnandi, & Kokou, 2021; Valko, Morris, & Cronin, 2005).The liver plays a vital role in metabolizing and eliminating toxins from the body, while the kidneys are responsible for filtering and excreting waste products. However, when heavy metals contaminate animal feeds or food, the liver and kidneys can suffer significant harm. The excess toxic elements impair the detoxification processes of the liver and compromise the kidneys' ability to eliminate these harmful substances (Zheng et al., 2020).

F. Smarandache introduced the neutrosophic statistical approach(AboElHamd, Shamma, Saleh, & El-Khodary, 2021), this is a more thorough and expended version of the fuzzy statistical approach. This technique has acquired increasing attention in various fields for analyzing interval data. In applied sciences, neutrosophic statistics has been utilized for exploring and analyzing interval data, while in the field of medicine, it has been applied to diagnose data measurements. Additionally, it is used in humanities research as well as astrophysics to measure Earth speed data(Afzal & Aslam, 2023a; Riaz, Sherwani, Abbas, & Aslam, 2023). Different research articles have explored the definition and handling of indeterminacy in neutrosophic statistics. For instance, the work of Smarandache et al. introduced the concept of neutrosophic statistics and discussed its application in dealing with imprecise, uncertain, and contradictory data (Afzal & Aslam, 2023b; Smarandache, 2014). Furthermore, Neutrosophic statistics provides a framework to handle such situations by introducing the concept of indeterminacy in statistical analysis (Afzal & Aslam, 2023d; Smarandache, 2013).

1.1. Research Aim

The purpose of this research is to determine the concentration of Heavy Metals that is found in Liver, Feed and drinking Water of Chicken from the most populated province Punjab i.e. Lahore, and nearby area in province Punjab, Pakistan. We are intrigued to use Atomic Absorption Spectroscopy (AAS) to investigate the concentration of heavy metals in the water samples. Then, we compared our measured concentration of heavy metal with the basic Standards of the World Health Organization (WHO). For a better achievement of our result, we were motivated to compare our experimentally measured data with the currently published data. To deal with this situation, novel treatments will be proposed and applied with the idea that poultry products can potentially reduce the risks associated with contamination by heavy metals. To achieve our aim, we evaluate our data by using two methods i.e. Classical and Neutrosophic methods. Firstly, our goal is to analyze the data of heavy metals through the classical method by applying the classical formula. With the help of the table, we will convert all intervals into fix-point intervals from minimum to maximum value. It simplifies complex systems to single values or points. Then, we applied our second approach to analyze the data of heavy metals through the neutrosophic method by applying the neutrosophic formula. The neutrosophic analysis is a relatively new mathematical framework that extends the classical analysis by incorporating the concept of indeterminacy. The indeterminacy will be checked by using a neutrosophic approach. Then we lead to compare both of these methods. The purpose of the comparison between the two methods is to find a divine method to calculate the concentration of heavy metal in the chicken's feed, drinking water, and liver. Our expected result is that the neutrosophic approach will be a remarkable approach for the analysis of data.

2. Materials and Data Collection

2.1. Sample Collection and Analyses

- **Sample collection**

We collect most fresh samples of chicken from the Lahore and nearby area in Lahore, province Punjab, Pakistan. Province Punjab located in central eastern of Pakistan and Punjab is the largest province of Pakistan by population. Its climate is blazed and humid in summer. Main parts of body like as liver, breast muscles were collected to observe that these samples were collected in polyethylene bags then comprehensible and shifted in ice-box onward to the analysis laboratory.

- **Preparation of chicken's Liver sample**

The defrosted chicken's samples were cut into small pieces in deionized water. In 20ml of Nitric acid (HNO_3) was taken one gram sample of chicken and placed on a hot tube at 70–80°C temperature when sample placed on fireplace to start boiling. 10m amount of Per-chloric acid $(HCIO_4)$ was added into it and kept on same temperature until its volume reduces to 6ml. After cooling at room temperature the excerpt was filtered and followed by 49ml deionized water was added in almost 1ml excerpt. At the final the samples were stored in flask and with the required prior labeling to test heavy metal Atomic Absorption Spectroscopy.

- **Preparation of chicken's feed sample**

The samples of chicken have feed grinded in the form of powder. Sample of 1g powder mix with 18.6ml Nitric acid (HNO_3) 6.4ml of hydrochloric acid (HCL) and 5ml of hydrogen peroxide (H_2O_2). After this mixture was boiled for almost 30 minutes and followed by deionized water was added to make the final solution or 50ml. prior to filtration samples were cooled down at the room temperature. At last, the sample was test via Atomic absorption spectroscopy to assess the concentration of heavy metals such as Lead (Pb), Cupper (Cu), Cadmium (Cd), Arsenic.

- **Preparation of chicken's drinking water sample**

First one of these collected water samples PH almost ranges from 7.8–8.1. Addition of Nitric acid HNO_3 in 20ml water sample. The area of heavy metals concentration in the water's sample is determined covered by Atomic Absorption Spectroscopy (*A.O.A.C*, 1995).

Figure 1 is explaining the whole process of experiment.

Figure 1: Sample Analysis Atomic Absorption Spectrophotometer (AAS)

Now let we move see the algorithm for using the Atomic Absorption Spectrophotometer (AAS) for this experiment as follows:

- **Start of Program**:

Initialize variables and instruments required for Atomic Absorption Spectrophotometer analysis.

Set up the AAS instrument and calibrate it using standard solutions of known concentrations.

- **Interval**:

Define the concentration range or interval of the analytic you want to measure.

Determine the number of samples or data points to be analyzed within the interval

- **Uncertainty**:

Establish the desired level of uncertainty or precision for the analysis.

Determine the appropriate number of replicates or measurements to achieve the desired uncertainty.

- **Indeterminacy**:

Check for potential interferences or matrix effects that may affect the accuracy of the analysis.

Employ appropriate sample preparation techniques to mitigate interferences, if necessary.

- **Values**:

Prepare the samples to be analyzed, ensuring they are representative of the analyte.

Apply appropriate dilutions or adjustments to bring the sample concentration within the instrument's working range.

Determine the new concentration interval or range for each diluted or adjusted sample.

- **Execute**:

Perform the AAS analysis on each sample using the calibrated instrument.

Measure the absorbance or emission intensity of the analyte at the appropriate wavelength.

Record the instrumental parameters, such as slit width, lamp current, and integration time, for each measurement.

- **End of Program**:

Calculate the concentration of the analyte in each sample using the calibration curve obtained from the standard solutions. And record the values in interval form.

2.2. Data Collection

We have collected measured data of heavy metals from the experiment is expressed in Table 1:

Table 1
The concentrations of heavy metals in Chicken's drinking water, Feed, and Liver

Elements	**Chicken's Drinking Water (mg/L)**	**Chicken's Feed (mg/Kg)**	**Chicken's Liver (mg/Kg)**
Zn	[9.42–36.76]	[1.99–3.42]	[3.27–4.59]
Cu	[0.80–6.88]	[1.32–2.38]	[6.8–1.72]
Cd	[0.16–1.45]	[1.88–4.25]	[0.02–0.12]
Pb	[0.18–0.37]	[0.04–0.39]	[0.03–1.58]
AS	[0.04–1.96]	[0.46–0.90]	[0.25–1.85]

2.3 Analysis Methodology

We have two types of methodology as shown below:

- Classical Statistical
- Neutrosophic Statistical

2.3.1. Classical Statistical

In the classical method, a fixed value is computed by taking average of each interval. In classical analysis, one approach to converting interval data into single-point values is to use the midpoint of the interval as the representative value. This limitation can potentially mislead policy-makers, especially when high and low values are present within the intervals.

$$P\left(S\right)_i = \frac{\left(Q_i + R_i\right)}{2}$$

1

Here, $P\left(S\right)_i$ are the intervals and Q_i is Lower value and R_i is Upper value.

2.3.2. Neutrosophic Statistical

Let Z_{jN} is the neutrosophic numbers with Z_{jU} high values and Z_{jL} low values resulting in the jth intervals of neutrosophic formula(Afzal & Aslam, 2023c):

$$Z_{jN} = Z_{jL} + Z_{jU} I_N \in (j = 1,2,3,\ldots nN)$$

2

Here $Z_N \in [Z_L, Z_U]$ is a neutrosophic variable with size $m_N \in [m_L, m_U]$. The variable $Z_{jN} \in [Z_{jL}, Z_{jU}]$ having two parts; the lower values Z_{jL} a part of classical and high values $Z_{jU} J_N$ an part that is indeterminate and has an indeterminacy interval$J_N \in [J_L, J_U]$.

Now let we move to see the algorithm for analyzing the data through Neutrosophic approach as follows:

- **Step 1**: Get the interval of the data
- **Step 2**: Calculate the indeterminacy for the interval
- **Step 3**: Execute the Neutrosophic formula: $Z_{jN} = Z_{jL} + Z_{jU} j_N; j_N \in [j_L, j_U]$ and calculate the values
- **Step 4**: Move to step 1 for getting new interval. If all interval are analyzed move step 5
- **Step 5**: Plot the graph

3. Results and Discussion

From the experiment it is observed that, the chicken's feed is based on essential nutrients as compared to additives that concentrate the growth rate and weight of the chicken. In feed samples the detected heavy metals are (Pb, Zn, Cd, Cu, and As) values of these heavy metals are given in Table 2. The concentration ranges of heavy metals for (Pb, Zn, Cd, Cu and As) in the chicken's feed samples are 0.04–0.39, 1.99–3.42, 1.88–4.25, 1.32–2.38, 0.46-0.90mg/kg. Similarly, the chicken's drinking water is contaminated by human anthropogenic activities. In drinking water, the detected heavy metals are Zn, Cu, Cd, Pb, Hg and As. The values of these heavy metals are mentioned in table 1.2. In drinking water samples, the range of heavy metals are 9.42–36.76, 0.80–6.88, 0.16–1.45, 0.18–0.37, and 0.04-1.96mg/l. Moreover, in chicken's liver the detected heavy metals are Pb, Cd, Zn, Cu and As. Values are mentioned of the detected heavy metals in table 1.3. The concentration of detected heavy metals (Pb, Cd, Zn, Cu and As) are (0.03–1.58, 0.02–0.12, 3.27–4.59, 6.8–1.72 and 0.26–1.85) mg/kg. Let see the comparison of concentrations of heavy metals with WHO Standard:

Table 2
Comparison of measured concentration of heavy metal with WHO Standard

Element	Chicken's Drinking Water (mg/L)		Chicken's Feed (mg/Kg)		Chicken's Liver (mg/Kg)	
	This Work	WHO Standard	This Work	WHO Standard	This Work	WHO Standard
Zn	[9.42–36.76]	5.0(Paul, 2017)	[1.99–3.42]	2.0(P. Zhuang, B. Zou, H. Lu, & Z. Li, 2014a)	[3.27–4.59]	2.0(Zhuang et al., 2014a)
Cu	[0.80–6.88]	1.0(Paul, 2017)	[1.32–2.38]	1.0(Zhuang et al., 2014a)	[6.8–1.72]	1.0(Zhuang et al., 2014a)
Cd	[0.16–1.45]	0.005(Paul, 2017)	[1.88–4.25]	0.5(Mottalib et al., 2018)	[0.02–0.12]	0.5(Mottalib et al., 2018)
Pb	[0.18–0.37]	0.05(Paul, 2017)	[0.04–0.39]	0.1(Zhuang et al., 2014a)	[0.03–1.58]	0.1(Zhuang et al., 2014a)
As	[0.04–1.96]	0.05(Paul, 2017)	[0.46–0.90]	0.1(Mottalib et al., 2018)	[0.25–1.85]	0.1(Mottalib et al., 2018)

The detected heavy metals (Cd, Cu, Pb, Zn, As) in drinking water samples have higher levels except the mercury described by WHO(Paul, 2017). The concentration of Heavy metals like Cd, Cu, As and Pb in the chicken's liver and feed is observed to be higher than safe limits as defined by WHO(2002) (Zhuang et al., 2014a). In chicken's liver and feed amount of Zn is within safe limits according to WHO/FAO, Chinese and Australian Standard (Okoye, Ibeto, & Ihedioha, 2011; P. Zhuang, B. Zou, H. Lu, & Z. J. P. J. E. S. Li, 2014b). The concentration of Cadmium crosses the permissible limits of WHO, Pak, EC and Chinese in chicken liver and feed (E. Kurnaz & A. Filazi, 2011; Zhuang et al., 2014a; Zhuang et al., 2014b). The concentration of the Lead is within safe limits according to Pak Standard in chicken's liver and feed (E. Kurnaz & A. J. F. E. B. Filazi, 2011). According to WHO/FAO (2002), EC (2006), Canada's standard, the Lead range is higher than safe limits in chicken's liver and feed (E. Kurnaz & A. Filazi, 2011; Mahmoud & Abdel-Mohsein, 2015; Zhuang et al., 2014a). The detected Arsenic in chicken's liver crosses the permissible limit of WHO/FAO as detected values exceed 0.1 mg/kg (Mottalib et al., 2018). The permissible limit of Arsenic in water is 0.05mg/L according to WHO standards and water samples have an Arsenic levels above this (Paul, 2017). Similarly, chicken feed samples exceed the permissible limit of WHO. Lead metals have a higher levels than provided safe limits by WHO/FAO (2000), Australia, EC (2001) and Chinese (Mahmoud & Abdel-Mohsein, 2015; Okoye, Aneke, Ibeto, & Ihedioha, 2011; Zhuang et al., 2014a). Copper in chicken liver and feed exceeds from the allowed limit of WHO/FAO, Canada, Pak and EC (E. Kurnaz & A. Filazi, 2011; Mahmoud & Abdel-Mohsein, 2015; Zhuang et al., 2014a). The comparison (for liver concentration) of the current study with recently published data is given in Table 3:

Table 3
Comparison of heavy metals concentration with others

Area	Concentration of Metals (mg/kg)					Ref.
	Zn	Cu	Cd	Pb	As	
Bangladesh	1.02–19.4	-	0.01–0.015	0.03–2.73	0.04–0.06	(Ullah et al., 2022)
Iran	-	2.73–6.31	0.016–0.027	0.008 –0.051	-	(Naseri, Salmani, Zeinali, & Zeinali, 2021)
India	-	-	0.013 –0.022	0.647 –1.779	0.001 –1.511	(M, A, Rajarathinam, & S, 2021)
China	424.11	61.66	3.09	-	27.22	(H. Li et al., 2021)
Pakistan	3.27–4.59	6.8–17.2	0.02–0.12	0.03–1.58	0.26–1.85	Present Study

3.1. Data Analysis of Heavy Metals

Now, we next move on to the data analysis. By using both the classical and neutrosophic method, we examine the data related to heavy metals as shown in Tables 4 & 5.

Firstly, the results of analysis of the heavy metals data using the classical method are expressing in Table 4 by applying the classical formula. In table, it is clear that the classical analysis transformed all intervals into a fix-point without defining the variance of the interval's range of values from minimum to maximum. Classical analysis often simplifies complex systems by reducing them to single values or points, which may not capture the full range of variability within a given interval.

Table 4
Classical analysis of heavy metals

Elements	Chicken's Drinking Water	Chicken's Feed	Chicken's Liver
Zn	23.09 ± 13.67	2.705 ± 0.715	3.93 ± 0.66
Cu	3.84 ± 3.04	1.85 ± 0.53	1.2 ± 0.52
Cd	0.805 ± 0.645	3.065 ± 1.185	0.07 ± 0.05
Pb	0.275 ± 0.095	0.215 ± 0.175	0.805 ± 0.775
As	0.982 ± 0.978	0.68 ± 0.22	1.05 ± 0.8

Similarly, the results of analysis of the heavy metals data using the neutrosophic method are expressing in Table 5 by applying neutrosophic formula. It seems from these tables that neutrosophic analysis is a relatively new mathematical framework that extends classical analysis by incorporating the concept of indeterminacy. Neutrosophic analysis can capture the entire spectrum of information about the variance of heavy metals by allowing for degree of indeterminacy. For example, neutrosophic analysis gives an equation of a heavy metal like Zn = 9.42 + $3676J_N$ with the indeterminacy interval $J_N \in [0, 0.744]$. According to neutrosophic analysis the values of heavy metals lies between 9.42 and 36.76 by put the indeterminacy values.

Table 5
Neutrosophic analysis of heavy metals

Elements	Chicken's Drinking Water	Chicken's Feed	Chicken's Liver
Zn	$9.42 + 36.76J_N$; $J_N \in [0, 0.744]$	$1.99 + 3.42J_N$; $J_N \in [0, 0.418]$	$3.27 + 4.59J_N$; $J_N \in [0, 0.288]$
Cu	$0.8 + 6.88J_N$; $J_N \in [0, 0.884]$	$1.32 + 2.38J_N$; $J_N \in [0, 0.445]$	$0.68 + 1.72J_N$; $J_N \in [0, 0.605]$
Cd	$0.16 + 1.45J_N$; $J_N \in [0, 0.890]$	$1.88 + 4.25J_N$; $J_N \in [0, 0.558]$	$0.02 + 0.12J_N$; $J_N \in [0, 0.833]$
Pb	$0.18 + 0.37J_N$; $J_N \in [0, 0.513]$	$0.04 + 0.39J_N$; $J_N \in [0, 0.897]$	$0.03 + 1.58J_N$; $J_N \in [0, 0.981]$
As	$0.004 + 1.96J_N$; $J_N \in [0, 0.998]$	$0.46 + 0.9J_N$; $J_N \in [0, 0.889]$	$0.25 + 1.85J_N$; $J_N \in [0, 0.865]$

3.2. Heavy Metals: A Graphical Comparison of Neutrosophic and Classical Analysis

Graphical comparison of heavy metals analysis including neutrosophic and classical methods are shown in Figs. 2 & 3.

Classical analysis simplifies complex systems by reducing them to single values, supposing that the variability is negligible or not significant for the given context. Neutrosophic analysis allows for an extensive representation of uncertainty and variability, enabling a more comprehensive analysis. The variation between the classical and neutrosophic graphs for heavy metals is small, it suggests that the variability and indeterminacy associated with heavy metals are not significant, and classical analysis can provide a reliable approximation. One thing seems clear that the explanation of heavy metals using classical graph is less adaptable and helpful as they are depicted using fix-point values. However, neutrosophic graphs are versatile and instructive enough to describe and terminate the issue. Classical graphs, such as line plots or error bar graphs, are commonly used by researchers to represent the data variation. These plots are not analytically competent the error bar displays the error in the data rather than variance. The neutrosophic approach can be a noticeable method for data analysis in certain situations. As the result, the neutrosophic strategy is a remarkable approach to analysis the data analysis. For the analysis of the heavy metals we have opted to apply the neutrosophic method.

4. Conclusion

The present study focuses on the assessment of heavy metal concentrations in chicken liver. A comprehensive collection of one hundred samples each of chicken feed, water, and liver was undertaken from diverse areas in Lahore. Atomic Absorption Spectroscopy (AAS) was employed for the determination of heavy metal concentrations. The collected data was subjected to analysis using classical and neutrosophic approaches. The findings revealed that the concentrations of Cadmium (Cd), Copper (Cu), and Lead (Pb) in the collected samples of drinking water, feed, and liver exceeded the established safe limits set by WHO/FAO, Pak, EC, and Chinese standards. However, the concentration of Zinc (Zn) in chicken feed and liver remained within safe limits according to WHO/FAO and Australian standards, while it exceeded the safe limits in drinking water. The concentration of Arsenic (As) was found to be higher than the safe limits in drinking water, feed, and chicken liver, posing a significant threat to the food chain due to the associated risks of cancerogenic and non-cancerogenic diseases. Prolonged exposure to arsenic can adversely affect pregnant women, infants, and children, impacting various bodily systems such as the renal, dermal, nervous, hepatic, endocrine, cardiovascular, and hematological systems. Moreover, the analysis of data indicated that neutrosophic statistics provided a more insightful and flexible analysis of heavy metals, underscoring the urgent need for monitoring and mitigation strategies within the poultry industry to ensure the safety and quality of its products.

Declarations

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All authors have equally worked in this paper. **All authors have read and approved the manuscript.**

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Figures

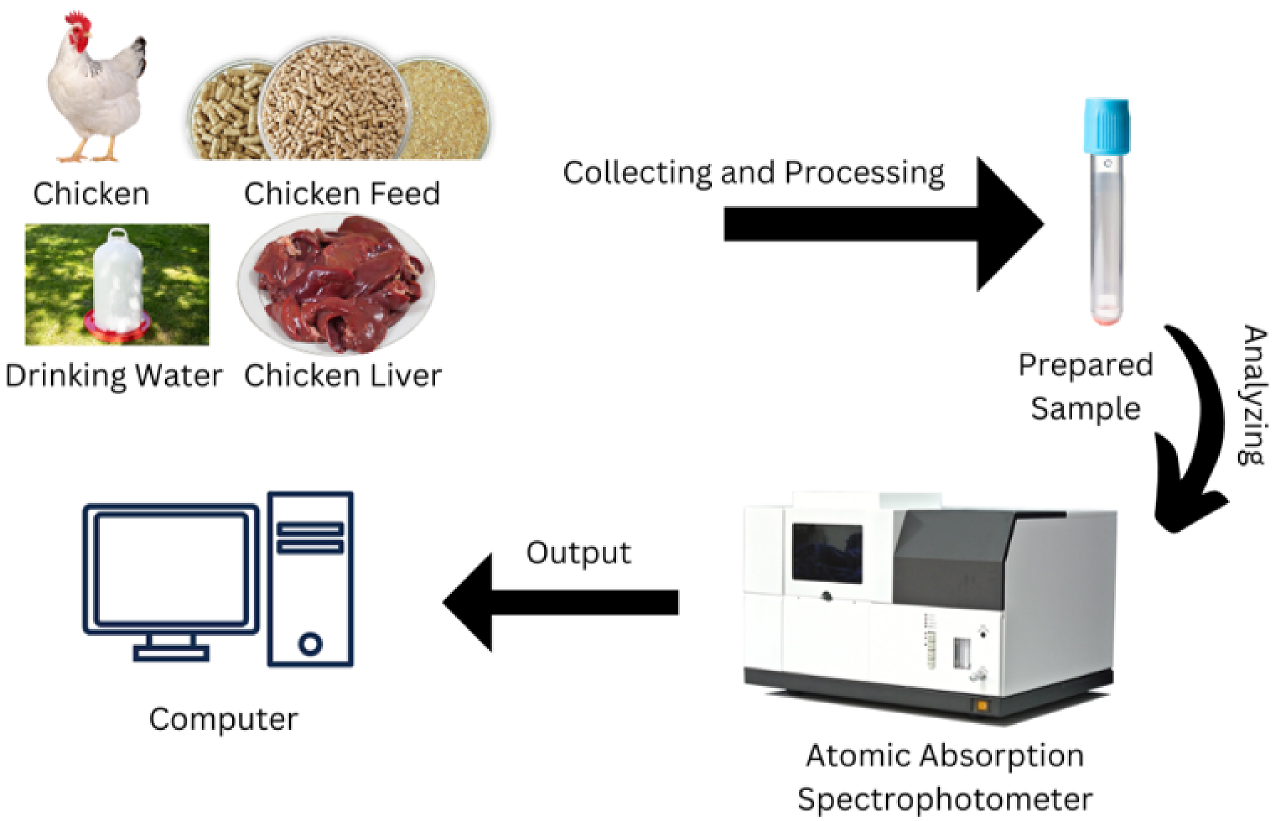

Figure 1

Sample Analysis Atomic Absorption Spectrophotometer (AAS)

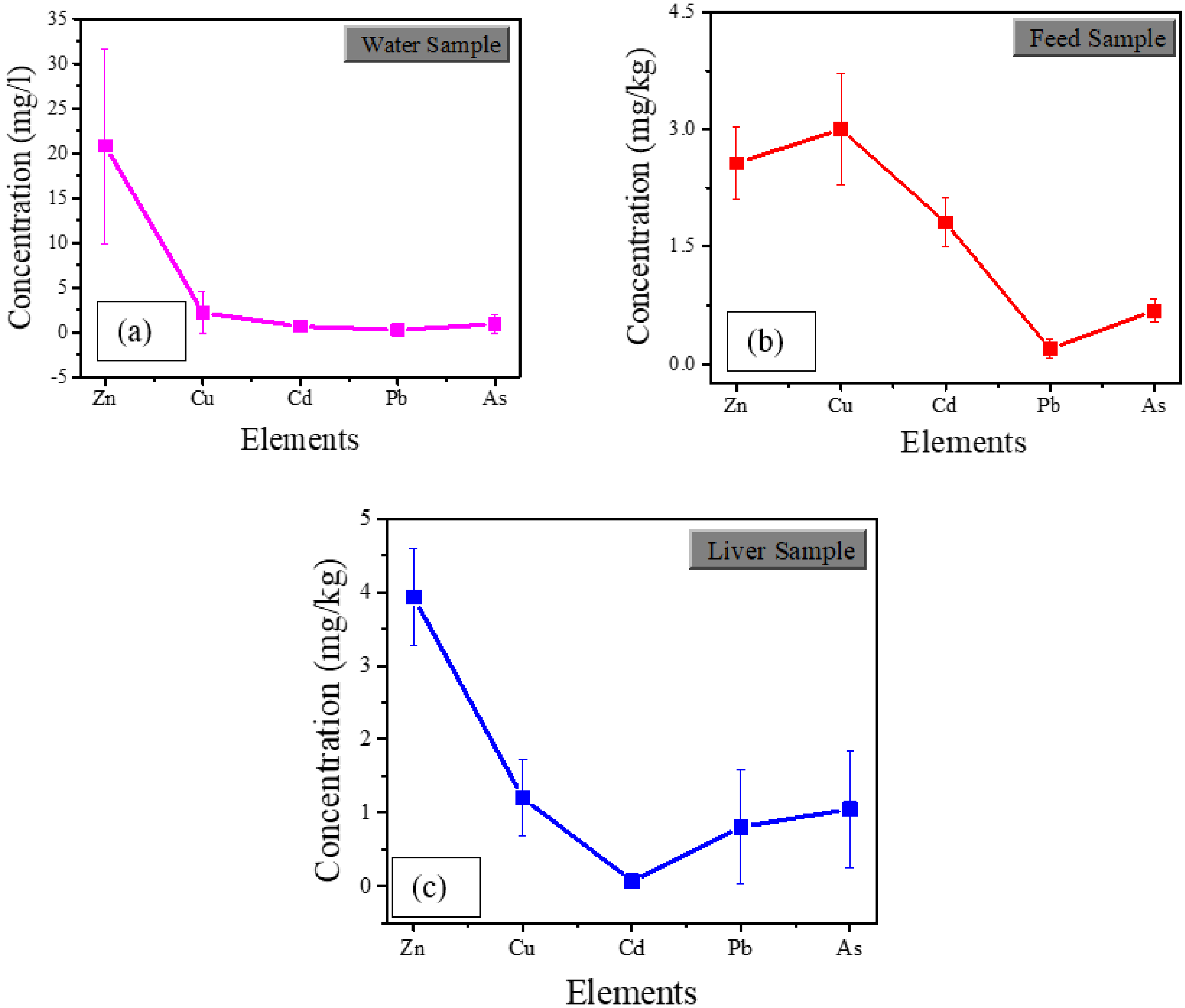

Figure 2

(a) is expressing the classical graph for water sample, (b) is expressing the classical graph for feed sample and (c) is expressing the classical graph for liver sample

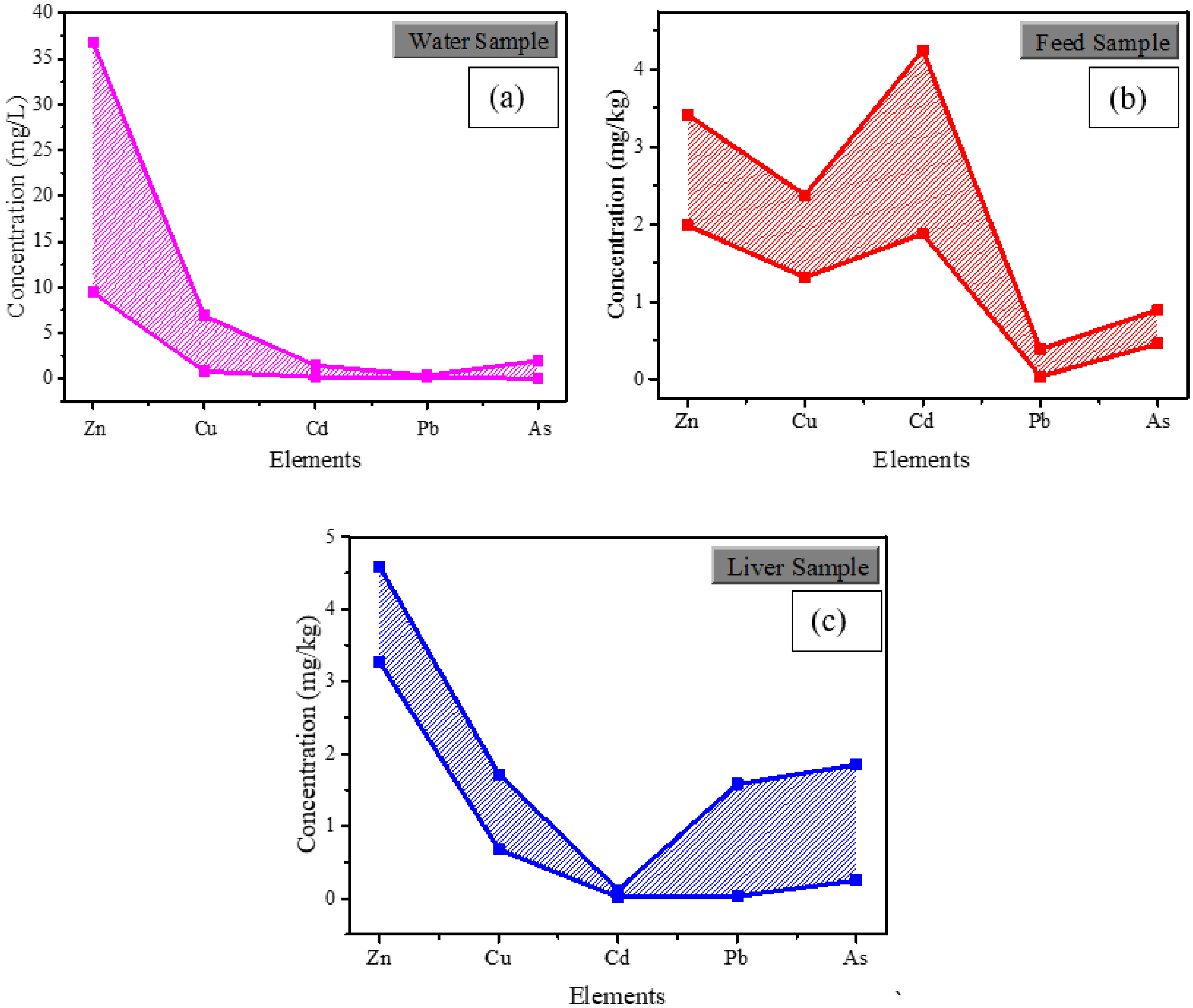

Figure 3

(a) is expressing the neutrosophic graph for water sample, (b) is expressing the neutrosophic graph for feed sample and (c) is expressing the neutrosophic graph for liver sample