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Research Article

Determination of Toxic Elements in Silver Leaf Coated Sugar Confectionaries By inductively Coupled Plasma-Mass Spectrometry and their Health Risk Assessment

Ligen Das*, Garima Dhakad, Poulami Basu, Arnab Kundu, Chezhiyan K, Debadutta Mishra and Dr. Geetanjali

National Food Laboratory, Food Safety and Standards Authority of India (FSSAI), India

Abstract

Exposure to heavy metal pollutants through air, soil, water and food is a growing concern due to its toxicity in living organisms. In this study, concentration of toxic metals like cadmium (Cd), nickel (Ni), arsenic (As), lead (Pb), mercury (Hg), and aluminium (Al) were analysed in silver leaf coated sugar confectionaries to evaluate their risk in humans. The elemental contamination can be due to various factors like industrialization, mining and over exploitation of natural resources, however this study focuses on adulteration of these toxic metals in food with emphasis on aluminium contamination in silver leaf along with other toxic metals. The identification of metals was accomplished by a validated technique employing inductively coupled plasma mass spectrometry (ICP-MS). The method was evaluated in terms of limit of detection (LOD), limit of quantification (LOQ), repeatability, recovery, accuracy, within-lab reproducibility, linearity and measurement of uncertainty. The concentrations of toxic metals were below the maximum residual limits for Pb, Hg, Cd, and As. To assess the toxicity of these metals, the Hazard Quotients were measured. The target hazard quotient (THQ) values for silver leaf-coated sugar confectionaries ranged from 9 to 10985 for aluminium in approximately 30% of samples, and were less than 0.01 for other metals. The highest levels of THQ were observed in aluminium > 1, suggesting a high health risk to humans. The correlation of samples with and without aluminium foil was investigated by a statistical evaluation of data employing the Karl Pearson's coefficient of correlation. In most cases, the food was found to be adulterated with aluminium and traces of nickel, whereas other toxic metals were detected well below the maximum detection limits (MRL).

INTRODUCTION

Heavy metal pollution is a growing concern across the world due to its extreme toxicity. The primary factors contributing to this phenomenon include elevated levels of pollution, urbanization, bioaccumulation, and industrialization. The presence of these contaminants in the earth, water and atmosphere can have a devastating impact on living organisms. The presence of these potentially harmful heavy metals is not essential for the human body and is gaining prominence as a significant health hazard Figure 1. This study aims to ascertain the concentrations of hazardous metals such as aluminium, arsenic, cadmium, lead, nickel and mercury in silver leaf coated sugar confectionaries. The major source of mercury contamination in the environment is power plants that use coal as a source of energy. The high concentrations of Pb, Ni, and Cd are the result of battery waste, sludge from the textile and chemical industries, etc. Arsenic is released into the environment through groundwater, mining processes and other sources like manufacturing of pesticides, glass manufacturing and the production of various alloys. Even essential metals can be poisonous if they are taken in large amounts. Heavy metals are exposed to the body in various ways. These metals can get into the body through food, water, pollution, industrial exposure, etc. and cause symptoms like nausea, vomiting, shortness of breath, and even kidney failure in extreme cases [1]. The ingestion of toxic levels of metals is commonly referred to as heavy metal poisoning. Aluminium is a common toxic metal present in the environment, which can be found in various foods and beverages. It enters the food chain through sources like food processing, packaging and using cooking vessels made of aluminium. Cooking vessels made of aluminium are at high risk of leaching. Acidic foods with low pH and high salt content significantly accelerate the rate at which aluminium enters the food. The contamination of aluminium can be caused by common food ingredients, such as baking powder and baking soda, which can absorb into baked goods during the cooking process. Dairy products, such as cheese, butter and processed or packed products are also found to contain aluminium due to leaching. Beverages packed in aluminium cans or aluminium packing material and additives used in food such as food colouring agents, anti-caking agents may also contain aluminium. Similarly, mercury and methyl mercury are present in the food chain due to the natural sources such as geological earthing and mining etc. Seafood is one of the major sources of mercury exposure in humans. Some of the common sources of

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***Corresponding author:** Ligen Das, National Food Laboratory, Food Safety and Standards Authority of India (FSSAI), Kyd Street, Kolkata 700016, India

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these toxic metals found in food are listed in Table 1. Silver has been used in the food industry since ancient times in the form of silverware, tableware, garnishing, and for serving food due to its antimicrobial properties. Silver is regarded as an antibacterial metal that is extremely beneficial to the body when consumed in moderate quantities. It has been discovered

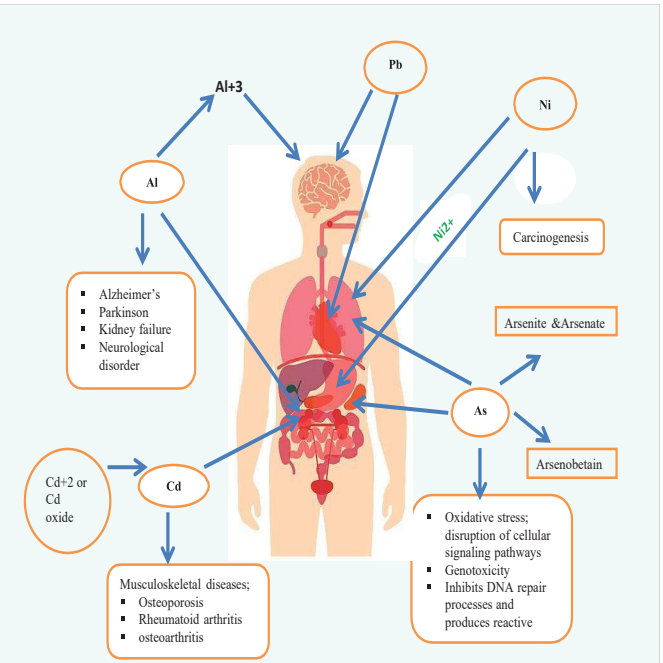


Figure 1 Effects of heavy metals in different vital organs of human health

Table 1: Food Sources of heavy metals [35-41]

Heavy metal	Food commodity	Range of Metals found in food commodity (mg/kg)
Mercury (Hg)	Fish (Tuna, king mackerel, marlin, orange roughly, and swordfish	0.128 mg/kg0.730 mg/kg 0.485 mg/kg More than 0.5 mg/kg
Arsenic (As)	Brown rice	0.170 mg/kg
Cadmium (Cd)	Leafy green vegetables Chocolate Nuts Cooked Meat Roasted meat Fried meat	0.05 mg/kgmg/kg 8.22-16.62 mg/kg 0.08 mg/kg 0.40 mg/kg 0.11 mg/kg
Lead (Pb)	Chocolate Cooked Meat Roasted meat Fried meat	0.048 mg/kg 0.16 mg/kg 0.46 mg/kg 0.28 mg/kg
Nickel (Ni)	Nuts	15.03-46.37 mg/kg
Aluminum (Al)	Tea	0.03mg/kg

that the silver foil utilized in the food industry primarily for garnishing sweets has been contaminated with aluminium. The fact that silver is a Nobel and highly priced metal can be the reason for adulteration with a cheaper look alike. The malleability, colour, and affordability of aluminium can be some of the reasons for their increasing use in the food industry [2]. The reason for the presence of aluminium in silver leaf requires investigation. The silver leaf coated confessionary were purchased from local markets across India from cities like Kolkata, Bhopal and Nagpur to study the presence of toxic metals using an ICP- MS.

Heavy metal and their toxicity mechanism.

Aluminium:

Aluminium (Al) is a ubiquitous element that is commonly found in the Earth's crust. It is a light, silvery-white metal, widely used in various industrial, medical, and household applications. Aluminium has a high affinity for bone tissue and can accumulate in bones over time. The accumulation of aluminium in the brain has been associated with various neurological disorders such as Alzheimer's disease [3]. It can exist in various forms such as free ions, complexes, and particles and its metabolism depends on its chemical form such as ionic (Al3+) or form complex with other molecules. The metabolism of aluminium also depends on the overall health status of an individual. Individuals with impaired kidney function are more prone to aluminium accumulation and toxicity. Its toxicity can cause various health problems such as bone disorders, neurological disorders, respiratory disorders, and reproductive disorders. The severity of aluminium toxicity depends on the dose and duration of exposure, the chemical form of aluminium, and the overall health status of an individual. The metabolism of aluminium involves absorption, distribution, metabolism and excretion. It has a strong affinity to DNA, RNA, and many mononucleotides, making them the cell structures most vulnerable to its effects. The amount of aluminium bound to DNA can vary widely, but it is thought that a complex with an Al/DNA-P ratio of about 1:3 is first formed.

However, excess amounts of Al3+ can increase this ratio to greater than 2:1, likely due to the binding of OH- ions as well. This increased ratio can lead to the complex acting as a crystallization nucleus for aluminium hydroxide [4].

Nickel:

Nickel helps in the absorption of iron from the intestine through the lungs, gastrointestinal tract and skin; they are stored in kidneys, lungs, and liver tissues in the body. The absorption of nickel depends on the chemical form and deposition site. It enters the body through food and water excretes most of the absorbed nickel through urine. Nickel is not broken down in the body but can undergo changes in its chemical form. Nickel's metabolism involves its binding to ligands and its transport throughout the body. Nickel can interfere with the physiological processes of other essential minerals such as manganese, zinc, calcium, and magnesium, which can lead to toxicity. In humans, most of the nickel ingested is not absorbed by the body and is eliminated mainly in the faeces. However, some of the nickel absorbed from the gastrointestinal tract is excreted in the urine, and it is associated primarily with low molecular weight complexes that contain amino acids. Additionally, nickel can also be eliminated through sweat and milk [5].

Nickel binds to biomolecules, such as proteins. At a physiological

pH, the strength of nickel's interactions with proteins depends on the type and position of amino acid residues in the protein molecule, as well as their accessibility. Ni2+ can coordinate with deprotonated peptide nitrogen, and it has the highest affinity for histidine imidazole nitrogen and cysteine sulfhydryl group. Thus, nickel is most strongly bound by histidyl and cysteinyl residues in peptides and proteins. Its toxicity and carcinogenesis involve the production of reactive oxygen species (ROS) [6]. Both soluble and insoluble nickel compounds produce ROS in cells. The oxidative effects of nickel depend on its abilityto form the Ni(III)/ Ni(II) redox couple at pH 7.4 when Ni(II) is complexed with certain natural ligands, including peptides and proteins. These complexes can react with endogenous O_2 or H_2O_2 to generate hydroxy radical (•OH) and other oxygen-, carbon-, and sulfur-centered radicals. Reactive intermediates can also be produced during the process of oxidative cellular solubilisation of nickel sulphides such as Ni_3S_2 and NiS. Gastrointestinal absorption of nickel is relatively quickly in blood, peaking around 2.5.

3 hours post-ingestion, and returning to normal levels in approximately 72 hours. The half-life of nickel ingested in a water-soluble form may vary widely, from 11 to over 30 hours, and that food taken with nickel greatly limits its absorption. Plasma proteins (mainly albumin) and poorly defined low-molecular-mass ligands carry nickelthroughout the body in the bloodstream [7].

Arsenic:

The contamination of arsenic in the environment can be caused by natural processes like erosion, burning fossil fuels and the presence of agricultural residues in soil and groundwater[8,9], as well as other sources like water and food contamination. Rice, rice- based products, and other grain-based processed products are the most common sources of inorganic arsenic exposure in the general population [10]. Inorganic compounds, such as arsenic trioxide, arsenic pentoxide, arsenious acid, and arsenic acid, exhibit higher levels of toxicity in comparison to organic compounds, such as monomethylarsonic acid(MMA), dimethylarsinic acid (DMA), and arsenobetaine. Arsenic forms in the third and fifth oxidation states are the most toxic [11-13].

Exposure to As is associated with numerous detrimental health consequences. Several studies have indicated that arsenic is a carcinogenic compound that has been linked to an increased risk of various types of cancers. In adults, prolonged exposure to As increases the risk of type 2 diabetes, peripheral vascular disease, skin lesions, and impaired lung function [14,15]. The arsenic metabolism is complex due to the diverse intermediates and products it yields, each with its own distinct behaviour and interactions in the human body. Various environmental exposure factors, as well as interand intraspecies variability, influence the formation of each metabolite. The primary cause of damage from exposure to arsenic is oxidative stress, which can lead to disruption of cellular signalling pathways and various diseases [16,17]. Studies on cell lines have shown that arsenical compounds can cause genotoxicity in both mice and humans [18,19]. The methylated form of arsenic inhibits DNA repair processes and produces reactive oxygen species in the spleen and liver as metabolic products. The accumulation of free radicals from reactive oxygen species (ROS) can lead to cell death through abnormal gene expression and lesions of cellular components such as DNA, lipids, and proteins.Additionally, chemical residues of As can bind to DNA-binding proteins, disrupting DNA repair processes and increasing the risk of carcinogenesis [20-23].

Cadmium

Cadmium is a naturally occurring metal found in the earth's crust. It is often producedas a by-product of zinc or lead smelting, it is used in various products, such as televisionscreens, batteries, and cosmetics. Human exposure to Cd is primarily mediated through inhalation or ingestion, with cigarette smoking being the primary mode of exposure. Individuals with iron, calcium, or zinc deficiency are more likely to absorb Cd and ingestion of contaminated food, water, drugs, or dietary supplements can have long- term health effects. Industrial exposure to Cd, such as through welding or soldering, hasthe potential to cause severe chemical pneumonitis. The consumption of rice that is highly contaminated with cadmium can result in the ltai-itai sickness, which can resultin bone damage. This disease can be caused by the accumulation of cadmium in the body over time, which can lead to metabolic issues due to enzyme inhibition. The risk is influenced by the overall diet composition and bio-availability of the cadmium compound. Cadmium is not linked to cancer however its exposure can lead to oxidative stress and damage to the brain, kidney and DNA [24]. Furthermore, it has been found to altercarbohydrate metabolism by limiting glycolysis and increasing amino acid decomposition. After cadmium exposure, increased lipid peroxidation has beenobserved in some brain regions, such as the cerebellum and cortex. The reactivity of oxygen produced by cadmium exposure leads to apoptosis and abnormal gene expression. Cadmium also exerts an impact on the activity of enzymes involved in energy metabolism and oxidation phosphorylation, resulting in damage and death of renal cells. After being absorbed, it is distributed throughout the body and typically binds to a protein called metallothionein. About 30% of Cd deposits are in the liver, 30% in the kidneys, and the rest is spread throughout the body. Cadmium is also associated with a decrease in the density of bones in postmenopausal women and an increased risk of musculoskeletal conditions like osteoporosis [25,26].

Mercury and Lead

The toxic effects of mercury and lead on human health are well-known. Mercury can specifically target the brain, but can also cause dysfunction in other organs such as nerves, kidneys, and muscles. Its action involves disrupting membrane potential and intracellular calcium homeostasis, as well as binding to freely available thiols because of its high stability constants. The Environmental Protection Agency (EPA) has classified lead as a carcinogen, and it has significant effects on numerous bodily systems. Lead is primarily deposited in skeletal bones, with more than 95% of the total body burden found in bones and teeth of adults. Children are especially vulnerable to lead toxicity due to their rapid growth rate and metabolism, which can have critical effects on their developing nervous system. Even low levels of exposure to lead in children aged between 0-5 years can result in developmental impacts and subsequent lowering of IQ. Lead poisoning can manifest as acute or chronic exposure, and is commonly observed in occupational settings or manufacturing industries that utilize lead. The symptoms may include a decrease in appetite, hypertension, abdominal pain, renal dysfunction, fatigue, sleeplessness, arthritis, hallucinations, and vertigo. Chronic exposure can cause serious health problems, such as mental retardation, birth defects, psychosis, autism, dyslexia, weight loss, hyperactivity, paralysis, brain damage, kidney damage, and even death. Despite the common method of

testing for lead exposure in humans, blood sampling may not accurately reflect an individual's level of intoxication. This is because lead can move from the vascular system and become deposited in bones,where it can remain for a prolonged period of time. Lead is majorly known for causing Oxidative Stress.

The metabolism of mercury in the body is complex and involves several pathways, including renal excretion, biliary excretion, and metabolism by enzymes such as glutathione S-transferase and the cytochrome P450 system. However, it is not easy to eliminate mercury from the body, and it may take several years for the body to eliminateall the accumulated mercury [27].

Regulatory limits of Metals in Food

The Food Safety and Standards Authority of India has established guidelines that regulate the permissible concentrations of various metals in food and food products in the India. These regulations are in place to ensure that food and food products are safe for consumption and do not contain harmful levels of metals that could pose a risk to human health. As per Food Safety and Standards regulation (FSSR) 2011 The maximum residual limit for nickel is 1,5 mg/kg for oil & fats, 1 mg/kg for vegetables whereas lead is limited to 2,5 mg/kg, cadmium to 1,5mg/kg, mercury to 1,0 mg/kg and arsenic to 1,1 mg/kg for food products under food not specified category. Aluminium is prohibited in silver leaf used in food. As per the regulation, silver leaf is grouped under sub regulation 2.11.4 under other food product and ingredients and the weight of the silver leaf should be up to 2,8g/sq m, and silver content should be of minimum 999/1,000 fineness [28].

The European Food Safety Authority (EFSA) has set regulations for the use of certain aluminium compounds in food commodities. For instance, E520 aluminium sulphate is permitted in egg white up to a maximum permissible limit of 30 mg/kg. Similarly, E521 aluminium sodium sulphate is allowed in candied, crystallized, and glacé fruit and vegetables up to a maximum limit of 200 mg/kg when used individually or in combination and expressed as aluminium. Other aluminium compounds, such as E522 aluminium potassium sulphate, E523 aluminium ammonium sulphate, E541 sodium aluminium phosphate (SALP, acidic form), sodium, potassium, and calcium aluminium silicate, and bentonite, are permitted as food additives under Directive 95/2 EC on food additives other than colours and sweeteners. However, the levels of these additives used in foodstuffs must not exceed the maximum limits set by EFSA. For instance, fine bakery wares such as scones and sponge wares are allowed to contain 1 g/kg of aluminium expressed as aluminium, sodium, potassium, and calcium aluminium silicate [29].

Aim of this study

Metals like Cd, As, Pb, Ni, Hg and Al are not necessary for human health and exposure to these metals through food can be very toxic. Silver leaf is now commonly used in the food industry, it can be found on sweets, paan (betel-leaf) and fruits etc. The reason why silver leaf is used is not just because it looks grand and opulent, but also because silver has anti-microbial properties and can increase the shelf life of food products. Edible silver or gold as vark is not considered harmful to the body, since the metal is in inert form and the quantities involved in normal use are minuscule. However, it has been observed that silver leaves are partially or fully containing Al. The consumption of such sweets and chocolates can cause severe stomach infections and can lead to food poisoning. The sole reason behind this practice is the cost of the two metals, as Al way cheaper than silver (Ag). The aim of this study is to estimate the concentration of toxic metals like Cd, As, Pb, Ni, Hg and Al in silver leaf-coated sugar confectionaries and to assess their health risk by measuring the target hazard coefficient (THQ). This study will also conduct a statistical evaluation of data employing the Karl Pearson's coefficient of correlation.

Material and Methods

Chemicals, reagents and standards

The certified reference standard solutions of (1000 mg L-1) of Cd, As, Pb, Ni, Hg and Al and internal standards of scandium (Sc), germanium (Ge), indium (In) bismuth (Bi) (Inorganic ventures, Christiansburg, Virginia) were used. Nitric acid (HNO3 65%) TracepurA grade, Hydrogen peroxide (H2O2 30%w/v) Tracepur grade, Hydrochloric acid (HCl 37%) TracepurA grade were used for sample pretreatment (Finar, Gujarat (India). Water (18.2 MOhm cm) (Elga, Herisau Switzerland).

Calibration standards

A calibration standard and blank were prepared by diluting the standards and internal standards in desired concentrations by the addition of hydrochloric acid. A linear range of each analyte was plotted using six calibration points prepared in class 'A' volumetric flask.

Sampling and study design.

For this study about 50 samples were collected from various retail stores across India from cities like Kolkata, Bhopal and Nagpur. The key ingredients of sweets are dry fruits, milk, cereals flour, sugar, and oil. Specifically silver leaf coated sweets were selected from different locations for this study. These samples were brought to the laboratory, homogenized, packed and stored at 4^0C until analysis.

For sample digestion the homogenized samples were thawed to room temperature and an aliquot of 0.5g (±0.01) of sample was weighed into a PTFE vessel using a calibrated weighing balance. A mixture of 1.5ml of double distilled water, 0.2ml of HCl, 1ml of H2O2 and 4 ml of 65% HNO3 is added to the sample. HCl (0.2 ml) is added to the sample to provide H+ and Cl- ions, which act as a basic salt, facilitating digestion. H2O2 (1 ml) is added to dissolve water with the organic matrix and digest low carbon residue. HNO3 (65%) is added to decompose strong organic matter. It is a strong acid that is highly soluble, forming nitrate salt with low chances of precipitate formation. The mixture was subjected to pre-digestion for about 30 minutes and digested using a multiwave 3000 microwave digester system (Anton Paar, Courtboeuf, France) (Table 2). The vessels prior to analysis were decontaminated using 10% of nitric acid 65% (v/v) and rinsed with double distilled water and dried in an oven at 40^0C prior to use. After the digestion the samples are transferred into 50ml polypropylene flask. The digested samples were made up to final volume by water after the addition of 50µl of internal standard (10mg/kg) to obtain a final concentration of 10µg/kg.

Instrumentation

Determination and Quantification of total metal content in the silver leaf coated sugar confessionary samples were performed using an ICP-MS (Thermo iCAP, Waltham, Massachusetts, U.S.). The equipment was optimized using isotopes of Li, Al, V, Mn, Co, Cu, Zn in the standard mode,

specific isotopes of Cd, As, Pb, Ni, Hg and Al were considered in the method to minimize the isobaric and polyatomic interference. Tuning solution (1µg/L) was used to optimize gases volume, torch position and Ion lenses to improve sensitivity of the instrument. The instrument parameters are listed in Table 3.

Table 2: Food Sources of heavy metals [35-41]

Stage	Temperature	Time (mmm:ss)	Fan level
Temperature ramp	100 degree C	20:00	1
Temperature hold		05:00	1
Temperature ramp	180 degree C	15:00	1
Temperature hold		30:00	1
Cooling	55 degree C	21:00	3

Table 3: Instrumental parameters of the ICP-MS system

RF Power	1548.6 W
Coolant argon flow(1/min)	14.018 l/min
Auxiliary argon flow(1/min)	0.7977 l/min
Nebulizer argon flow(1/min)	1.0381 l/min
Sample introduction system	Cross flow nebulizer with Scott spray chamber
Sample uptake rate(ml/min)	1
Detector mode	Dual mode
Sampler/skimmer cones	Nickel
Scanning mode	KED
Number of points per peak	1
Dwell time (s)	0.05
Sweeps per reading	30
Number of replicates	2

Quality Assurance and Control

The batch of sample were analysed along with a reagent blank. The reagent blank was subjected to digestion process along with other samples after addition of acids as per the procedure. Proven blanks were also analysed along with internal standards to accesses matrix interference. The samples were analysed in duplicate. The extraction efficiency of the analytical method was determined within the limits of variability using Control samples fortified with known volumes of elements.

Validation of method:

Method validation is an important step in ensuring the accuracy and reliability of the results. The method used for the determination of toxic metals in silver coated sweet confectionaries were verified using linearity, LOQ, repeatability, reproducibility, % recovery, and measurement uncertainty. Linearity refers to the ability of the ICP-MS method to produce results that are proportional to the concentration of the analyte in the sample. It was evaluated by analyzing samples with known concentrations of the analyte across a range of concentrations. The resulting data can be plotted as a calibration curve, and the linearity of the method can be assessed by examining the correlation coefficient and the residuals of the curve. The linearity of metals was plotted in the range of 0.001mg/kg to 0.04 mg/kg for Cd, As, Hg and Ni whereas 0.002mg/kg to 0.08mg/kg for Pb and 0.0025 mg/kg to 0.1 mg/kg for Al. The correlation coefficient were found to be 0.9996 for Hg , 0.9998 for Ni, 1.0000 for Pb, 1.0000 for Cd, 0.9998 for As,0.9996 for Al.

Repeatability

Repeatability, measures the intra-assay precision or precision under repeatability conditions, refers to the variation in results obtained by analyzing the same sample multiple times within a short period of time. Repeatability was evaluated by analyzing replicate samples fortified with different concentration at Limit of quantification (LOQ), Maximum residual limit (MRL) and two times of MRL (2MRL). Repeatability is calculated by the standard deviation (SD) or relative standard deviation (RSD) of the results.

The limit of quantification (LOQ)

It is a critical parameter in analytical chemistry, as it determines the lowest concentration of a substance that can be accurately and precisely measured. In the case of trace element analysis by inductively coupled plasma mass spectrometry (ICP-MS), the LOQ is typically defined as the lowest concentration that can be quantitatively determined with an acceptable level of repeatability and accuracy.

Recovery

Recovery can be assessed by analyzing a sample of spiked with a known concentration comparing the measured value to the spiked value and can be reported as a % of recovery. Recovery is a measure of the accuracy of an analytical method and refers to the ability of the method to accurately measure the amount of a substance in a sample. It was determined by analyzing sample that has been spiked with a known amount of the substance of interest, and comparing the measured value to the expected or “spiked” value. The spiked sample is then analyzed using the same analytical method that will be used for the actual samples. The measured value obtained for the spiked sample is compared to the expected value based on the known amount of substance added to the sample. The percent recovery is calculated by dividing the measured value by the expected value, and multiplying by 100%.

Reproducibility

It is a measure of closeness of values of single test results under reproducible test conditions (different operator, different apparatus, different laboratory, and different days). It is established by measuring HORRAT and its value shall be less than 2 to prove that the method reproducible. Reproducibility refers to the ability of a scientific experiment or measurement to be replicated by others, using different operators, apparatus, laboratories, and on different days. It is an important aspect of scientific research, as it ensures that the results obtained are reliable and can be trusted by the scientific community. To determine the reproducibility of a particular method, the HORRAT(r) “highest observed ratio of response to analytical target.” was determined. If the HORRAT value is less than 2, it indicates that the method is reproducible, meaning that the results obtained using different

test conditions are sufficiently similar to be considered equivalent. However, if the HORRAT (r) value is greater than 2, it suggests that the method may not be reproducible and that further investigation is required to identify the sources of variability and improve the method’s reliability. The validation parameters are summarized in Table 4.

Table 4: Method Validation Parameters

Metals	LOQ (mg/kg)	Repeatability(r)	Reproducibility(R)	Recovery %	Linearity(R^2)	Measurement ofuncertainty
Hg	0.2	0.10	0.01	95.36	0.9996	0.9566±0.03mg/kg
Ni	0.2	0.07	0.02	87.49	0.9998	0.8598±0.04mg/kg
Pb	0.4	0.18	0.03	86.02	1.0000	1.8862±0.08mg/kg
Cd	0.2	0.11	0.02	87.00	1.0000	0.8598±0.04mg/kg
As	0.2	0.10	0.02	92.49	0.9998	0.9010±0.04mg/kg
Al	0.1	0.71	0.12	118.86	0.9996	0.5326 ±0.03 mg/kg
Sn	1.0	0.25	0.07	84.45	0.9988	4.2912±0.43mg/kg

Health Risk Assessment

The health risk in humans associated with exposure to these toxic metals through food was estimated using the target hazard quotient (THQ). The risk assessment established by the

U.S. Environmental Protection Agency (USEPA) was used to determine the non- carcinogenic health effect of long-term metal exposure [30]. These critical tools that can help identify substances that pose potential health risks to humans. The potential hazards associated with a particular chemical or mixture of chemicals can be screened by comparing exposure levels with toxicity reference values. However, these tools have limitations and are not substitutes for a comprehensive risk assessment that considers all relevant exposure pathways and potential health effects. Therefore, a risk assessment that considers all the information available is needed to make informed decisions about the safety and regulation of chemicals. The non-carcinogenic risk of toxic metals such as Cadmium, Nickel, Arsenic, Lead, Mercury, and Aluminum was estimated by calculating the Target Hazard Quotient [31].

Estimated Daily Intake (EDI)

The daily intake of metals depends on the concentration of metals in silver leaf coated sugar confectionaries, their daily consumption, and body weight. The body weight of an Indian was taken to be 60 kg, calculated by averaging the male and female weights of 65 kg and 55 kg, respectively. Unfortunately, there is no information on daily intake of silver leaf coated sugar confectionaries in India. A survey was conducted in this regard to estimate the daily intake, and the average daily consumption of silver leaf coated sugar confectionaries was 0.001 kg/day.

The EDI is calculated using the formula (1)

$$EDI = \frac{Mc \times IR}{Rw \times 10^{-3}} \text{.....(1)}$$

Where:

Mc is the concentration (mg kg-1) of the heavy metals in silver leaf coated sugar confectionaries. IR stands for the daily average consumption of silver leaf coated sugar confectionaries. Bw represents the average body weight of Indians.

Target Hazard Quotient (THQ)

The target hazard quotient (THQ) relates the concentration of exposure of toxic element with the toxicological reference dose. EF is the exposure duration of the metal in days (365 days), ED is the exposure duration in a life time (67 years), RfD is the oral reference dose set by the USEPA Table 5, ATn is the average exposure time (365 days/67yrs) and 10−3 is the unit conversion factor [32-34].

The THQ is calculated using the formula (2)

$$THQ = \frac{(Mc \times IR) \times EF \times ED \times 10^{-3}}{(RfD \times Bw \times ATn)} \text{.....(2)}$$

THQ is used to express the level of concern and express the non-carcinogenic effects. The THQ ratio less one indicates no significant risk and the values greater than 1 indicates possible health risk.

Table 5: Reference dose of heavy metals

Metal	Oral RfD(mg/kg/day)	Source
Cadmium	1 x 10 $^{-3}$	Integrated risk information system, US.EPA
Arsenic	3 x 10 $^{-4}$	Integrated risk information system, US.EPA
Nickel	0.02	Integrated risk information system, US.EPA
Lead	3.6 x 10 $^{-3}$	World Health Organization (WHO)
Mercury	1.60 x 10 $^{-4}$	Integrated risk information system, US.EPA
Aluminium	7.00	World Health Organization (WHO)

Statistical analysis

Statistical analyses were performed using Karl Pearson's correlation coefficient to study the level of relation between the variables. A positive correlation is obtained when the direction of change between the two variables is the same and when the direction of the variables is opposite, relates to a negative correlation. A study was conducted to confirm that the silver foil used in sugar confectionaries is contaminated with aluminum. Samples with high concentrations of aluminum were retested after removing the silver leaf from the sample and correlation was estimated.

Result and discussion

The mean concentration of elements in silver leaf-coated sugar confectionaries is listed in Table 6. Compared to other toxic metals, the concentrations of aluminum were notably high, and traces of nickel were also observed in the sample. Toxic metals such as mercury, arsenic, lead, and cadmium were detected below the maximum residual limits as specified

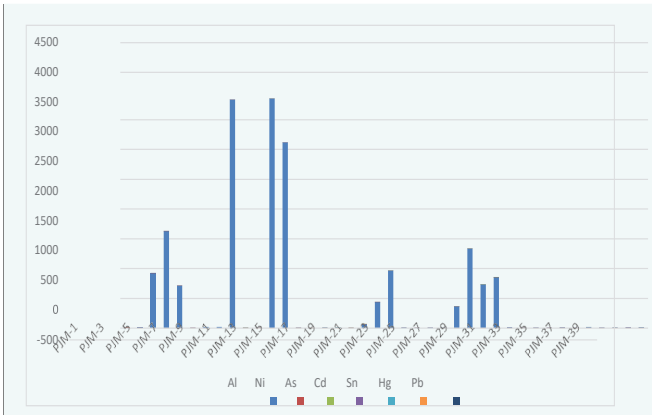
Table 6: Mean concentration of heavy metals

Sample	Al			Ni			As	Cd	Sn	Hg	Pb
	I	II	Mean	I	II	Mean					
PJM-1	2.78	2.40	2.59	1.73	1.73	1.73	0.00	0.00	0.00	0.01	0.03
PJM-2	9.37	8.61	8.99	0.47	0.45	0.46	0.00	0.00	-0.01	-0.01	0.08
PJM-3	965.61	882.33	923.97	0.99	0.98	0.99	0.00	0.00	-0.02	-0.01	0.01
PJM-4	1655.50	1603.53	1629.51	0.06	0.05	0.05	0.00	0.00	-0.03	-0.01	0.02
PJM-5	725.65	700.10	712.88	0.49	0.47	0.48	0.00	0.00	-0.02	-0.01	0.03
PJM-6	2.83	2.71	2.77	1.73	1.78	1.75	0.00	0.00	-0.02	-0.01	0.04
PJM-7	19.83	18.11	18.97	0.17	0.17	0.17	0.00	0.00	-0.01	-0.01	0.07
PJM-8	17.65	17.58	17.62	0.02	0.02	0.02	0.01	0.00	-0.01	-0.01	0.06
PJM-9	3887.37	3787.93	3837.65	1.70	1.73	1.71	0.00	0.00	-0.01	0.00	0.05
PJM-10	2.38	2.17	2.28	1.50	1.50	1.50	0.01	0.00	-0.01	-0.01	0.04
PJM-11	3.77	3.35	3.56	2.20	2.21	2.20	0.00	0.00	0.00	-0.01	0.04
PJM-12	3914.78	3787.36	3851.07	0.47	0.47	0.47	0.00	0.00	0.11	-0.01	0.05
PJM-13	3148.29	3093.96	3121.13	0.16	0.14	0.15	0.00	0.00	-0.01	-0.01	0.04
PJM-14	1.41	1.16	1.28	1.69	1.74	1.72	0.00	0.00	-0.02	-0.01	0.02
PJM-15	3.55	3.32	3.44	2.88	2.84	2.86	0.01	0.00	0.00	-0.01	0.01
PJM-16	1.58	1.58	1.58	1.75	1.84	1.80	0.00	0.00	0.02	-0.01	0.02
PJM-17	7.63	6.73	7.18	3.14	3.18	3.16	0.01	0.00	0.11	-0.01	0.11
PJM-18	2.14	1.91	2.03	2.89	2.92	2.90	0.01	0.00	-0.03	-0.01	0.01
PJM-19	67.32	62.53	64.93	0.22	0.23	0.22	0.01	0.02	0.00	0.02	0.06
PJM-20	465.92	409.32	437.62	0.02	0.02	0.02	0.00	0.00	-0.02	0.00	0.01
PJM-21	990.88	939.74	965.31	0.04	0.04	0.04	0.01	0.00	-0.02	-0.01	0.02
PJM-22	6.25	5.45	5.85	3.18	3.11	3.14	0.00	0.00	-0.03	0.00	0.04
PJM-23	3.69	3.53	3.61	1.92	1.95	1.93	0.00	0.00	-0.03	0.00	0.03
PJM-24	6.80	6.24	6.52	0.13	0.13	0.13	0.00	0.00	-0.03	0.00	0.03
PJM-25	4.86	4.11	4.49	0.28	0.27	0.28	0.01	0.02	-0.02	-0.01	0.02
PJM-26	376.79	351.32	364.06	0.29	0.29	0.29	0.04	0.01	-0.02	-0.01	0.02
PJM-27	1375.56	1299.54	1337.55	0.44	0.44	0.44	0.04	0.00	-0.01	-0.01	0.02
PJM-28	747.61	707.58	727.60	0.02	0.02	0.02	0.03	0.00	0.02	-0.01	0.06
PJM-29	886.55	821.44	854.00	0.36	0.37	0.37	0.01	0.00	0.00	-0.01	0.03
PJM-30	11.33	10.44	10.89	1.16	1.19	1.18	0.00	0.00	-0.02	-0.01	0.02
PJM-31	2.85	3.03	2.94	0.02	0.02	0.02	0.00	0.00	-0.02	-0.01	0.02
PJM-32	5.16	4.78	4.97	0.02	0.02	0.02	0.00	0.00	-0.02	-0.01	0.02
PJM-33	1.90	1.90	1.90	0.03	0.02	0.02	0.00	0.00	-0.01	-0.01	0.01
PJM-34	10.16	10.19	10.17	0.01	0.01	0.01	0.00	0.00	-0.01	-0.01	0.12
PJM-35	2.54	2.56	2.55	4.07	4.06	4.07	0.00	0.00	0.02	-0.01	0.03
PJM-36	13.67	13.52	13.60	0.96	0.92	0.94	0.00	0.00	0.02	-0.01	0.11
PJM-37	6.29	5.32	5.80	0.88	0.88	0.88	0.00	0.00	-0.03	-0.01	0.05
PJM-38	2.65	2.47	2.56	0.07	0.07	0.07	0.00	0.00	0.98	-0.01	0.03
PJM-39	10.02	9.14	9.58	0.26	0.25	0.25	0.00	0.01	-0.03	-0.01	0.02
PJM-40	8.57	8.41	8.49	0.17	0.17	0.17	0.00	0.00	0.08	-0.01	0.14

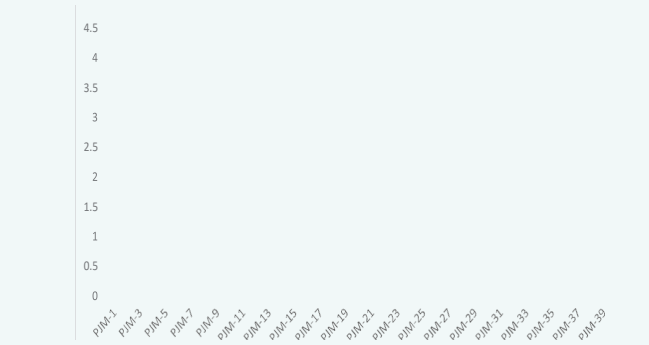
in FSSR. The analytical recovery of all the elements was within the acceptable range of 80-110%. Aluminum exhibited a recovery of 89%, Cadmium exhibited 85% recovery, Lead exhibited 91% recovery, Nickel exhibited 93% recovery, and Mercury exhibited 100% recovery. Higher levels of aluminum in silver leaf coated sugar confectioneries indicate that the silver leaf used in these foods was contaminated with aluminum Graph 1. The aluminum and nickel concentrations were in the range of 5-1000 mg/kg and 0.1- 10 mg/kg, respectively. A comparison of the obtained concentrations of Al and Ni with other metals is presented. Graph 2 and Graph 3. About 25% of the samples were detected with high levels of aluminum in the range of 500-3800 µg/kg, 7.5 % of samples were in the range of 50-500 µg/kg and 62.5% of samples were detected with aluminum in the range of 1-50 µg/kg. Nickel was detected in the range of 0.01 – 4.07mg/kg.

The concentration of aluminum was significantly reduced when the analysis was conducted without the silver leaf, in contrast to the samples tested with silver leaf. The presence of aluminum in silver leaf was attributed to this phenomenon. The statistical evaluation was conducted using a Karl Pearson's correlation coefficient using formula (3).

$$r = \frac{n(\sum xy) - (\sum x)(\sum y)}{\sqrt{[n\sum x^2 - (\sum x)^2][n\sum y^2 - (\sum y)^2]}} \quad \text{.....(3)}$$



Graph 1 A comparison of concentration (mean) of Al with Ni, As, Cd, Sn, Hg and Pb



Graph 2 A comparison of concentration (mean) of Ni with As, Cd, Sn, Hg and Pb

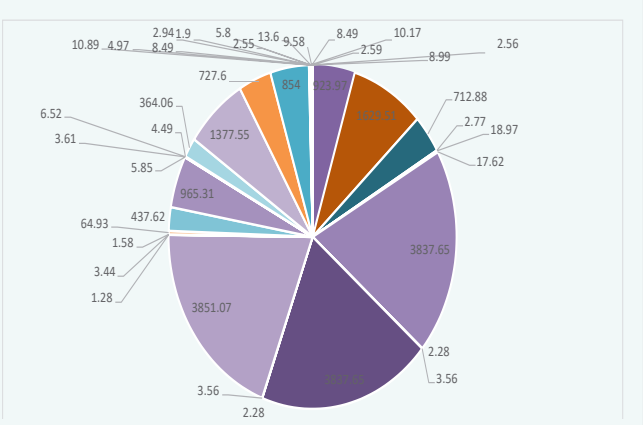
A perfect positive correlation between the two sets of data is indicated by the correlation coefficient between the aluminum samples with and without silver foil. A correlation coefficient of 1 indicates that there exists a robust and consistent correlation between the values of the two data sets. It is evident that aluminum (Al) found in silver leaf coated sugar confectionaries is primarily due to the adulteration of silver foil or VARK with aluminum. The results of the analysis support this conclusion, revealing a significant increase in the concentration of aluminum in samples with silver foil compared to those without.

The THQ of aluminium for about 12 samples were greater than 1, which indicates that the exposed adults are likely to experience adverse health effects. The THQ for other metals like arsenic, lead, mercury, nickel, cadmium was below 1 indicating no or minimal risk Table 7.

Table 6: Mean concentration of heavy metals

Sample id	THQ					
	Aluminium	Nickel	Arsenic	Cadmium	Lead	Mercury
PJM1	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
PJM2	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
PJM3	**153.32**	<0.01	<0.01	<0.01	<0.01	<0.01
PJM4	**840.98**	<0.01	<0.01	<0.01	<0.01	<0.01
PJM5	**70.41**	<0.01	<0.01	<0.01	<0.01	<0.01
PJM6	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
PJM7	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
PJM8	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
PJM9	**10985.27**	<0.01	<0.01	<0.01	<0.01	<0.01
PJM10	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
PJM11	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
PJM12	**11100.92**	<0.01	<0.01	<0.01	<0.01	<0.01
PJM13	**5909.49**	<0.01	<0.01	<0.01	<0.01	<0.01
PJM14	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
PJM15	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
PJM16	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
PJM17	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
PJM18	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
PJM19	0.05	<0.01	<0.01	<0.01	<0.01	<0.01
PJM20	**16.29**	<0.01	<0.01	<0.01	<0.01	<0.01
PJM21	**174.83**	<0.01	<0.01	<0.01	<0.01	<0.01
PJM22	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
PJM23	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
PJM24	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
PJM25	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
PJM26	**9.38**	<0.01	<0.01	<0.01	<0.01	<0.01
PJM27	**465.10**	<0.01	<0.01	<0.01	<0.01	<0.01

PJM28	**74.87**	<0.01	<0.01	<0.01	<0.01	<0.01
PJM29	**121.06**	<0.01	<0.01	<0.01	<0.01	<0.01
PJM30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
PJM31	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
PJM32	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
PJM33	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
PJM34	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
PJM35	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
PJM36	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
PJM37	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
PJM38	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
PJM39	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
PJM40	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01



Graph 3 Comparison of aluminium content in different samples

Conclusion:

Silver leaf coated sugar confectionaries were investigated for contamination of heavy metal such as lead, mercury, arsenic, cadmium, aluminium and nickel. The high levels of aluminium found in 27% of silver leaf-coated sugar confectionary samples are the result of the adulteration of aluminium with silver leaf used to embellish them. This assertion is substantiated by the observation that upon analysis of the sugar confectionary samples containing silver leaf without the inclusion of silver foil, the aluminum content was significantly diminished. As a result, it is apparent that the use of silver leaf in savory confections is the primary conduit for aluminum contamination. The outcomes of the research have implications for the wellbeing of individuals, as the consumption of high amounts of aluminum has been linked to health hazards. Therefore, it is important to implement measures to reduce the exposure to aluminum in sweet confectionery, such as using pure silver foil for coating or reducing the use of silver foil altogether. The non- carcinogenic risk of metals was assessed. The THQ values were within acceptable range for metals like nickel, arsenic, cadmium, lead, mercury, the only exception was aluminium with a higher contribution to toxicological limits.

The findings of this study can be utilized to formulate more effective guidelines to produce safe and healthy confectionery products. Further investigation is required to identify the potential sources of aluminum contamination in sweet confectionery and investigate alternative materials that can mitigate the exposure to aluminum.

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