International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 6 Nov-Dec 2023, pp: 1323-1338 www.ijprajournal.com ISSN: 2249-7781

Analysis of heavy metal content of spices using inductively coupled plasma mass spectrophotometry and their effect ongrowth of Escherichia coli as a representative of gut microflora.

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Submitted: 25-11-2023 Accepted: 05-12-2023

ABSTRACT
This study investigates the presence of heavy metals in commonly used spices, employing Inductively Coupled Plasma Mass Spectrometry (ICP-MS) to analyze cadmium, lead, mercury, and arsenic concentrations. Samples were collected from three districts in Madhya Pradesh, assessing heavy metal accumulation in these globally consumed culinary additives. The research explores the connection between spice-related heavy metal exposure and changes in gut microflora composition. Findings reveal significant variations in heavy metal levels among different spice types, raising concerns about their contribution to overall dietary heavy metal intake. The study suggests a potential link between elevated heavy metal exposure from spice consumption and health effects, with certain metals showing heightened levels in specific districts. Additionally, a toxicity assay confirms the antimicrobial properties of heavy metals. The research emphasizes the importance of understanding spice heavy metal content for its potential impact on human health, particularly on gut microflora. It underscores the necessity for ongoing monitoring of heavy metal concentrations in food and their potential health effects, contributing valuable insights to the understanding of dietary heavy metal exposure and advocating for safe food practices to maintain a healthy gut ecosystem.

Keywords: Heavy metal toxicity, Contamination, Food contamination, Toxicity in humans, ICP-MS.

I. INTRODUCTION

Metals and their compounds, naturally occurring in the Earth's crust, can be brought to the surface through human and geological activities, posing a risk of human exposure to metallic pollutants.[1] Heavy metal contamination has adverse effects on aquatic, terrestrial, and atmospheric environments, originating from both natural sources and human activities, and resisting easy degradation.[2] The term "heavy metal" refers to metallic elements with high density (>5g/cm3) and toxicity at low concentrations, including Mercury (Hg), Cadmium (Cd), Arsenic (As), Chromium (Cr), Thallium (Ti), and Lead (Pb).[3]

Anthropogenic actions have inadvertently introduced these heavy metals into the food chain and interconnected food webs, primarily through irrigation with wastewater effluent from industries.[4] The scarcity of freshwater for irrigation, along with the use of fertilizers, insecticides, and other human activities, exacerbates this issue.[5]

Heavy metals not only pose toxicity to humans and animals but also impact microflora. Introduced into the environment through natural processes and human activities, these metals tend to accumulate in living organisms, including the human body, leading to detrimental effects.[6] Within the body, heavy metals are transported and sequestered in cells and tissues, binding to proteins and nucleic acids, causing degradation and interference with cellular functions.[7] This can result in disruptions in the central nervous system, damage to blood components, and harm to vital organs, contributing to various diseases. Prolonged exposure to heavy metals can lead to physical, muscular, and neurological degenerative processes resembling conditions like Parkinson's and Alzheimer's.[8] Additionally, repeated exposure to specific heavy metals or their compounds can induce damage to nucleic acids, trigger mutations, mimic hormonal activities, disrupt the endocrine system, impair reproductive functions, and contribute to cancer development.[9] The study underscores the critical need to understand and address heavy metal contamination for environmental and human health protection.[10]

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1. Sources of Heavy metal exposure to humans

Heavy metals, often found as ores in the Earth's crust, are extracted during mining operations. Examples include arsenic, iron, lead, zinc, gold, nickel, silver, cobalt (in sulfide forms), and manganese, aluminum, selenium, gold, and antimony (in oxide forms).[11] Some metals, like copper, iron, and cobalt, exist in both sulfide and oxide ores, and certain ores may contain multiple heavy metals, as seen in chalcopyrite ($CuFeS_2$) containing copper and iron. Mining activities release heavy metals from ores, leading to their dispersion into the environment and deposition in soil, transport through air and water, and potential environmental contamination. [12,13] Industrial use further contributes to heavy metal release during processes, combustion, and effluent discharge into soil or water bodies. Products like paints, cosmetics, pesticides, and herbicides can also serve as sources of heavy metals, transported to different areas via erosion, runoff, or the effects of acid rain.[14]

2. Heavy metal toxicity in humans

1. Arsenic

Exposure to arsenic can lead to acute or chronic toxicity, each presenting distinct health implications. Acute poisoning can damage blood vessels, gastrointestinal tissues, and affect the heart and brain.[15] Chronic arsenic toxicity, known as arsenicosis, is characterized by skin-related symptoms like pigmentation changes and keratosis.[16] Prolonged exposure to lower arsenic levels may cause symptoms such as nausea, vomiting, reduced blood cell production, blood vessel damage, irregular heartbeats, and tingling in extremities.[17] Over the long term, chronic exposure can result in skin lesions, pulmonary diseases, neurological issues, peripheral vascular diseases, diabetes mellitus, hypertension, and cardiovascular diseases.[18]

2. Lead

Lead exposure causes toxic effects known as lead poisoning, affecting the gastrointestinal tract and central nervous system in both children and adults.[19] The poisoning can manifest as either acute or chronic. Acute exposure induces symptoms like headaches, diminished appetite, abdominal discomfort, fatigue, insomnia, hallucinations, dizziness, renal dysfunction, hypertension, and arthritis.[20] In contrast, chronic exposure results in severe consequences such as birth defects, mental retardation, autism, psychosis, allergic reactions, paralysis, weight loss, dyslexia, hyperactivity, muscle weakness, kidney impairment, brain damage, coma, and even fatality.[21] Elevated lead levels can displace the blood-brain barrier's plasma membrane, potentially causing edema, and disrupt intracellular second messenger systems, impairing the central nervous system.[22]Developing fetuses and children, especially vulnerable to lead's neurotoxic effects, can experience intellectual development impairment even with low-level exposure (5–25 μg/dL in blood), leading to a loss of intelligence quotient points according to epidemiological studies.[23]

3. Mercury

Chronic exposure to mercury can lead to erethism, characterized by excitability, hand tremors, memory loss, timidity, and insomnia.[24] Occupational exposure is associated with declines in performance on neurobehavioral tests, impacting motor speed, visual scanning, visuomotor coordination, verbal and visual memory.[25] The compound dimethylmercury, highly toxic and able to penetrate the skin even with latex gloves, can cause central nervous system degeneration and death with minimal exposure.[26] Pregnant women exposed to mercury risk affecting their fetus, leading to mental retardation, cerebellar symptoms, retention of primitive reflexes, malformations, and other abnormalities.[27] Recent studies support these concerns, showing that pregnant women exposed to mercury through whale meat and fish consumption exhibit reduced motor neuron function, memory loss, impaired speech, and compromised neural transmission in their offspring.[28]

4. Cadmium

Long-term exposure to cadmium leads to its accumulation in bones and lungs, causing damage, particularly disrupting bone mineralization and resulting in issues like osteoporosis.[29] The "Itai-Itai" disease epidemic in Japan was linked to cadmium contamination, showing increased toxicity associated with higher risks of bone fractures, reduced bone density, and height loss in both males and females.[30] Cadmium's high toxicity to the kidneys can lead to renal dysfunction and disease, disturb calcium metabolism, contribute to renal stone formation, and result in hypercalciuria.[31] Classified as a Group 1 carcinogen for humans by the International Agency for Research on Cancer, cadmium intake is primarily through smoking, making smokers more

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 6 Nov-Dec 2023, pp: 1323-1338 www.ijprajournal.com ISSN: 2249-7781

susceptible to intoxication. Cadmium exposure also poses risks such as testicular degeneration and a potential factor for prostate cancer.[32]

5. Chromium

Hexavalent chromium (Chromium VI) is the most toxic form of chromium, while Chromium III compounds pose minimal health risks. Chromium VI, known for its corrosive properties, has the potential to trigger allergic reactions.[33] Exposure to higher concentrations of chromium compounds can inhibit erythrocyte glutathione reductase, impacting the conversion of methemoglobin into hemoglobin. In vivo and in vitro experiments show that chromate compounds can induce DNA damage, leading to DNA adducts, chromosomal abnormalities, alterations in DNA replication, sister chromatid exchanges, and changes in DNA transcription.[34] Substantial evidence suggests that chromium promotes carcinogenicity in humans, as elevated rates of stomach tumors have been observed in animals and humans exposed to Chromium VI in drinking water.[35]

3.**Gut Microbiome**

The term "microbiome" refers to the combined genetic material of all microorganisms in the human body, while "microbiota" and "microflora" denote the actual community of microorganisms within or on specific body niches.[36] The understanding that microbiota and microflora impact human health dates back to the early 1900s, initially explored in relation to nutrition and periodontal health. The widespread use of theterm "microbiome" emergedduring the "omics" era, driven by advancements in DNA sequencing technologies that allowed a more comprehensive assessment of microbiota composition.[37]

In the human gut microbiota, there are approximately 10 to 100 trillion microbial cells, potentially outnumbering the estimated 10 trillion cells constituting the human body.[38] Within the human gut, researchers have identified 1,000 to 1,150 distinct bacterial species, collectively hosting 3.3 million unique microbial genes, a count nearly 150 times greater than the total number of genes found in the human genome.[39]

4.**Dysbiosis and Eubiosis in gut microbiome**

In the realm of microbiome studies, two frequently used terms are "dysbiosis" and "eubiosis." Dysbiosis characterizes an unhealthy microbiome, while eubiosis signifies a healthy one.[40] A fundamental trait of a balanced and healthy microbiome is its adaptability to withstand various external influences, including abiotic factors like diet and environmental conditions, as well as biotic factors such as interactions with other microorganisms.[41] A well-balanced microbiome is crucial for supporting overall health, and any disruption in its equilibrium can lead to the onset of various disease states.

Numerous factors, both derived from the microbiome itself and the host, work tirelessly to maintain the microbiome's healthy status.[42] The intestinal immune system, for instance, plays a pivotal role in upholding eubiosis. Furthermore, microbiome-derived products have the capacity to stimulate the host immune system, as exemplified by short-chain fatty acids (SCFA) triggering the production of the host antimicrobial peptide cathelicidin-3.[43] Additionally, microbiome-produced bacteriocins selectively target and eliminate specific bacterial strains.

5. **Major functions of gut microbiome**

The gut microbiome benefits the host by fermenting non-digestible carbohydrates, producing short-chain fatty acids (SCFAs) like acetic acid, propionic acid, and butyric acid. SCFAs serve as an energy source for colon epithelial cells, associated with the type-1 enterotype, and play a role in stimulating the differentiation and proliferation of intestinal epithelial cells, indicative of a healthy microbiome.[44]Excessive protein and fat consumption can lead to a dysbiotic microbiome, especially in overweight individuals, characterized by overproduced branched-chain amino acids (BCAAs) like valine, leucine, and isoleucine.[45] Elevated BCAA levels are linked to insulin resistance and the development of type-2 diabetes. The increased presence of proteolytic bacterial populations can enhance gut mucin degradation, leading to heightened gut permeability.[46] This increased permeability can trigger inflammation due to the translocation of lipopolysaccharide (LPS) and other bacterial products.[47]

II. LITERATURE REVIEW

The toxicity of metals primarily stems from their ability to generate reactive oxygen species (ROS) and oxidize proteins, as well as disrupt the composition of cell membranes, particularly those containing fats. This process leads to oxidative stress. [48,49] Research conducted

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with human Caco-2 cells has indicated that the intestines can play a role in the pre-systemic metabolism of inorganic arsenic (As).[50] Exposure to As triggers an inflammatory and oxidative response in the body, potentially causing structural and functional alterations in the mucosal layer.[51] These changes can result in the breakdown of the epithelial barrier function. Moreover, sub-chronic exposure to As impacts the structure of the epithelium, resulting in the loss of microvilli, which are vital for intestinal absorption and digestion processes. This may also disrupt intestinal homeostasis while affecting the integrity of the intestinal mucosa.[52]

Exposure to cadmium (Cd) and lead (Pb) led to a notable reduction in the thickness of the intestinal mucosal and submucosal layers, as well as a decrease in crypt depth.[46] Conversely, exposure to mercury (Hg) resulted in degenerative damage to various segments of the gastrointestinal tract, primarily characterized by inflammation and infiltrations.[53]

1. **Heavy metal analysis techniques**

The assessment of trace elements in biological samples serves a valuable purpose across various clinical contexts. For nutritional monitoring, essential elements like iodine, manganese, copper, selenium, and zinc are examined.[54] These elements play crucial roles in a diverse array of biological processes, including electron and oxygen transport, hormone synthesis, and the facilitation of biological reactions.[55] Disruptions in the normal balance of these elements may contribute to, or indicate the presence of, one or more pathological conditions.[55]

Conversely, other elements such as arsenic, cadmium, mercury, and lead are recognized for their toxic effects, often stemming from various mechanisms. Consequently, these elements are measured to gauge exposure levels.[28] Throughout history, a wide array of analytical techniques has been employed for the analysis of trace elements. [43,46]

While ICP-MS has been in existence for over three decades, some laboratories continue to employ older techniques. From a laboratory standpoint, one of the most noteworthy benefits of ICP-MS lies in its ability to analyse multiple elements simultaneously within a single analysis.[87] This stands in contrast to methods like flame and graphite furnace atomic absorption, where the lamp is tailored for a limited set of elements, permitting the measurement of only one or a few elements at a time.[57] Paired with its brief analysis duration and straightforward sample preparation, ICP-MS provides the potential for exceptionally high sample throughput in laboratory settings.[58]

III. MATERIAL AND METHODOLOGY

The experimental study done at Instrument section of Microbiology laboratory at Food and Drug Administration (FDA), Bhopal in a duration of six months from March to August of2023.

Variety of different food samples were collected from three different districts of Madhya Pradesh. These samples were analysed by ICPMS- MS for detailed metal contamination analysis of spice samples and antimicrobial susceptibility assay was performed using standard agar well diffusion method for E. coli, using their aqueous infusion method in distilled water as solvent system.

Amoxicillin was used as positive control.

1. Experimental chemicals and reagents

Nitric acid (65 – 69%), Trace Metal TM Grade, Fisher Chemical TM (P/N A618-S212): 4mL; Hydrogen peroxide (30 – 32%), Trace Metal TM Grade, Fisher Chemical TM (P/N N/1929/23): 1 ml; Hydrochloric acid (35 – 37%), Trace Metal TM Grade, Fisher Chemical TM (P/N A617-S500): 0.5 mL; Deionized water (18.20 MΩ.cm) water purification system; Heavy metal salts; Nutrient agar plates.

2. Experiment procedure to analyse heavy metal

During the course of experimental procedures following standard steps are taken for the determination of metal analytes. It involves:
Sample preparation, Sample digestion (Microwave digestion), Sample analysis, Toxicity assay using standard agar well diffusion method.

2.1 Sample Preparation

Sample preparation is recognized to be the largest source of errors and one of the most critical points of each analysis. Food sample (solid or liquid) is weighed and mix with chemical reagents in appropriate concentration.
Nitric acid (HNO_3) – 2% (w/v); Hydrochloric acid (HCl) – 0.2 mL; Hydrogen peroxide (H_2O_2) – 1 mL; Sample weight – as per the sample.
The mixture is then subjected to microwave digestion.

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Types of samples: Two types of food samples can be processed in instrument that is liquid and solid sample.
Liquid sample: All types of water, effluents, oils, petroleum products, pharma, honey, blood, urine, fruits juice, etc. Solid sample: Metals, metal alloys, food, pharma textiles, rubber, ayurvedic medicines, etc.

2.2 Sample Digestion

Digested in Microwave Assisted Digestion through Wet ashing or Dry ashing depending upon the sample type (solid or liquid) under proper temperature (approx. 200 degrees Celsius) and pressure (between 20-80 bar) within 2 hours.

2.3 Sample Analysis

Following digestion, transparent solution in each vile is transferred to precleaned 50ml centrifuge tubes, 5ml at a time using DI water to make up the volume to 50ml.
50µl internal standard is added to each of the vile.

Significance of Internal Standard

Internal standardization is typically utilized to compensate for variations in instrument operating conditions and sample-specific matrix effects that can either amplify or diminish the signal of the analyte. A consistent quantity of internal standard is introduced into every sample, standard, and blank. The results are then computed by comparing the signal of the analyte to that of the internal standard. Ideally, the chosen internal standard will share similar physical and chemical properties with the analyte, ensuring that it behaves comparably. Consequently, the ratio between the analyte and internal standard should remain unaffected by changes in the sample matrix or fluctuations in instrument operating conditions.
Sample Name: Turmeric, red chili powder, coriander powder, black pepper, fenugreek seed powder.

3. Bacterial isolates and culture conditions

Procurement of ATCC cultures of bacteria from Himedia which is Escherichia coli (ATCC-25922). Proper aseptic techniques and standard laboratory procedures were followed during the study of these microorganisms.
3.1 Antimicrobial Susceptibility assay using standard agar well diffusion method

Extraction of spices (samples from district C) was done by aqueous infusion method in distilled water as solvent system. The obtained infusion was passed through 0.22 mm dissociable syringe filter aseptically for sterilised or contamination-free extract. For antimicrobial activity, Muller-Hinton agar plates were prepared for working with bacteria respectively. The bacterial inoculum was taken from broth of revived cultures using sterile swab and seeded onto the MH agar media followed by punching 5 wells of 6 mm diameter. 20 mm of each extract was poured into each different wells of pre-inoculated culture plates separately with different microbial species . Amoxicillin was taken as positive control and distilled water as -ve control. The culture plates were then incubated at 37°C for 24 hours respectively. Observations were taken in the form of zone of inhibition after incubation.

IV. RESULT AND DISCUSSION

The table 5.1, 5.2 and 5.3 show the contamination level of spices from the three districts namely, A, B and C (the name of the districts cannot be revealed as conditioned by the FDA). Four heavy metals: As, Cd, Hg and Pd analysed for 6 spice samples under study in different batches. Followed by graph of interpretation of the contamination levels of each spice against all four heavy metals and concluding which has the most and least level of contamination.

The three districts under study have shown elevated levels of lead and arsenic followed by cadmium and mercury. Samples of coriander powder from district A and C and chilli powder samples from district B have shown the highest level of contamination. Lead contamination varies from 150 to 400 ppb, arsenic from 30 to 120 ppb, mercury from 20 to 32 ppb and cadmium from 20 to 220 ppb in 0.25 gms of samples. The possible reasons for such high levels of contamination observed in spice samples could be the pesticides and poor quality of water being used for irrigation contaminated with heavy metals due to industry effluents and waste disposal in water bodies.

To test the growth inhibition, antimicrobial susceptibility assay was performed using standard agar well diffusion method. The results obtained are confirmatory of the antimicrobial activity of spices with taken from samples with highest contamination levels. Turmeric powder 11mm, fenugreek seeds 9mm, fennel 7mm, coriander powder 10mm, black pepper 10mm, chilli powder 12mm. Highest inhibition shown by chilli powder and turmeric powder, the same can be confirmed by the graph of

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 6 Nov-Dec 2023, pp: 1323-1338 www.ijprajournal.com ISSN: 2249-7781

samples from district C, where Pb and As are found in maximum content in chilli and turmeric powder. Lowest zones shown in fennel and fenugreek powder, in the graph as well the contamination levels of fennel and fenugreek are in minimum range across the heavy metals. Confirming the antimicrobial effects of spices contaminated with heavy metals altering the gut microbiota.

Amoxicillin used as a positive control as it helps confirm the experimental conditions, such as growth medium, temperature, and bacterial strain used. If amoxicillin inhibits bacterial growth in the positive control, it indicates that the experiment setup is functioning as expected and help analyse the antimicrobial nature. To test the antimicrobial properties of heavy metals, one gram-negative and one gram-positive bacteria were used. The results have confirmed the antimicrobial properties of heavy metals as they hinder the growth of microorganisms.

S no.	Label	75 As (KED) [ppb]	111 Cd (KED) [ppb]	202 Hg (KED) [ppb]	208 Pb (KED) [ppb]
1	QC CHECK	0.403 (80.6%)	0.515 (102.9%)	6.571 (131.4%)	53.130 (106.3%)
2	CHILLI POWDER	8.488	95.08	0.855	199.756
3	CORIANDER POWDER	23.227	49.595	5.794	91.661
4	TURMERIC POWDER	26.495	34.763	0.504	180.139
5	FENUGREEK POWDER	39.373	37.385	11.637	74.296
6	BLACK PEPPER	18.9497	16.2998	15.363	89.759
7	FENNEL	33.877	26.287	8.259	144.342
8	QC CHECK	0.407 (81.3%)	0.538 (1077.6%)	5.753 (115.1%)	52.487 (105.0%)
9	CHILLI POWDER	14.45	102.807	15.658	370.446
10	CORIANDER POWDER	110.024	78.218	2.362	350.543
11	TURMERIC POWDER	66.23	29.947	0.715	201.707
12	FENUGREEK POWDER	23.195	33.257	6.724	108.735
13	BLACK PEPPER	15.993	15.376	14.897	88.879
14	FENNEL	23.195	33.257	6.724	108.735
15	QC CHECK	0.405 (81.0%)	0.550 (102.9%)	5.458 (109.2%)	51.390 (102.8%)
16	CHILLI POWDER	7.479	88.173	46.854	289.055
17	CORIANDER POWDER	12.397	69.629	0.029	459.922
18	TURMERIC POWDER	44.65	58.868	1.231	346.338
19	FENUGREEK POWDER	3.731	26.914	0.042	13.429
20	BLACK	17.9499	9.786	0.089	10.897

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	PEPPER				
21	FENNEL	35.897	21.908	5.908	145.908
22	QC CHECK	0.470 (81.0%)	0.559 (102.9%)	5.897 (109.2%)	51.098 (102.8%)

S no.	Label	75 As (KED) [ppb]	111 Cd (KED) [ppb]	202 Hg (KED) [ppb]	208 Pb (KED) [ppb]
1	QC CHECK	0.399 (79%)	0.476(95.3%)	4.803 (96..1%)	50.584 (101%)
2	CHILLI POWDER	32.182	77.583	18.095	311.058
3	CORIANDER POWDER	23.195	33.257	6.724	108.735
4	TURMERIC POWDER	15.268	15.645	8.035	245.54
5	FENUGREEK POWDER	38.987	36.789	10.987	75.789
6	BLACK PEPPER	11.098	15.098	12.768	90.789
7	FENNEL	35.789	25.564	3.145	142.908
8	QC CHECK	0.432 (86.3%)	0.519 (103.7%)	5.351 (107.0%)	54.529 (109.0%)
9	CHILLI POWDER	45.344	79.359	9.972	446.111
10	CORIANDER POWDER	13.962	48.623	9.759	78.172
11	TURMERIC POWDER	3.325	14.997	0.406	65.09
12	FENUGREEK POWDER	30.789	29.897	1.89	70.786
13	BLACK PEPPER	3.908	13.445	0.098	89.067
14	FENNEL	0.57	10.098	4.648	39.099
15	QC CHECK	0.540 (108.0%)	0.515 (103.0%)	5.119 (102.4%)	53.609 (107.2%)
16	CHILLI POWDER	23.159	87.325	2.318	137.3
17	CORIANDER POWDER	30.079	54.786	7.924	429.253
18	TURMERIC POWDER	142.668	61.799	12.02	302. 674
19	FENUGREEK POWDER	10.731	10.075	5.19	19.626
20	BLACK PEPPER	10.87	13.759	0.0513	89.798
21	FENNEL	4.087	10.041	4.281	39.951

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22	QC CHECK	0.405 (81.0%)	0.550 (102.9%)	5.458 (109.2%)	51.390 (102.8%)
23	CHILLI POWDER	0.699	42.35	1.388	74.818
24	CORIANDER POWDER	2.888	24.68	4.987	64.883
25	TURMERIC POWDER	10.764	7.284	4.132	774.67
26	FENUGREEK POWDER	10.503	11.671	5.769	12.626
27	BLACK PEPPER	4.97	10.041	4.281	39.951
28	FENNEL	0.34	9.098	3.29	67.89

Table 5.1: Samples from district A

S no.	Label	75 As (KED) [ppb]	111 Cd (KED) [ppb]	202 Hg (KED) [ppb]	208 Pb (KED) [ppb]
1	QC CHECK	0.446 (89.2%)	0.549 (109.7%)	5.715 (114.3%)	51.390 (102.8%)
2	CHILLI POWDER	23.159	87.325	0.023	137.33
3	CORIANDER POWDER	166.343	54.785	0.078	135.789
4	TURMERIC POWDER	49.833	38.292	0.012	112.152
5	FENUGREEK POWDER	0.897	23.761	0.014	239.78
6	BLACK PEPPER	26.344	18.791	0.06	236.229
7	FENNEL	0.04	10.997	0.034	38.808
8	QC CHECK	0.476 (95.2%)	0.480 (96.0%)	4.896 (97.9%)	49.151 (98.3%)
9	CHILLI POWDER	3.456	19.567	0.019	74.897
10	CORIANDER POWDER	84.908	38.98	2.204	411.768
11	TURMERIC POWDER	64.908	27.347	0.08	218.085
12	FENUGREEK POWDER	1.289	24.89	0.078	89.09
13	BLACK PEPPER	0.0367	26.914	0.045	45.9
14	FENNEL	15.38	24.776	2.508	54.732
15	QC CHECK	0.540 (108.0%)	0.515 (103..0%)	5.119 (102.4%)	53.609 (107.2%)
16	CHILLI POWDER	14.45	102.722	15.657	370.446
17	CORIANDER POWDER	110.024	78.218	2.362	351.52

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18	TURMERIC POWDER	20.362	16.434	0.076	121.278
19	FENUGREEK POWDER	2.297	34.072	0.166	43.125
20	BLACK PEPPER	0.897	13.754	0.045	67.09
21	FENNEL	15.38	24.776	2.508	54.732
22	QC CHECK	0.569 (113.8%)	0.514 (102.9%)	5.200 (104.0%)	52.123 (104.2%)
23	CHILLI POWDER	14.546	109.809	5.334	145.809
24	CORIANDER POWDER	29.089	85.218	1.25	175.754
25	TURMERIC POWDER	31.134	28.897	0.342	108.89
26	FENUGREEK POWDER	85.978	23.78	0.089	78.09
27	BLACK PEPPER	0.078	36.98	0.089	213.89
28	FENNEL	18.89	9.89	0.0674	216.98

Table 5.2: Samples from district B

S no.	Label	75 As (KED) [ppb]	111 Cd (KED) [ppb]	202 Hg (KED) [ppb]	208 Pb (KED) [ppb]
1	QC CHECK	0.482 (97.2%)	0.523 (109.7%)	5.125 (112.3%)	51.395 (102.9%)
2	CHILLI POWDER	33.789	104.785	15.675	370.987
3	CORIANDER POWDER	30.789	24.989	0.098	79.897
4	TURMERIC POWDER	17.583	47.868	3.677	223.748
5	FENUGREEK POWDER	5.879	28.564	0.146	92.819
6	BLACK PEPPER	3.256	23.78	0.013	94.987
7	FENNEL	16.998	24.776	2.509	54.987
8	QC CHECK	0.415 (93.2%)	0.482 (93.0%)	4.878 (98.9%)	50.151 (99.3%)
9	CHILLI POWDER	7.689	88.173	46.876	289.055
10	CORIANDER POWDER	110.035	78.218	2.362	351.52
11	TURMERIC POWDER	44.65	58.868	0.192	370.869
12	FENUGREEK POWDER	5.98	287.585	0.146	92.819
13	BLACK PEPPER	67.98	8.789	0.098	87.795

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14	FENNEL	17.098	25.768	2.807	78.098
15	QC CHECK	0.567 (107.3%)	0.545 (99.067%)	5.190 (112.4%)	55.209 (107.2%)
16	CHILLI POWDER	38.467	108.89	15.89	97.897
17	CORIANDER POWDER	39.5	72.636	1.764	173.109
18	TURMERIC POWDER	31.577	15.625	0.49	576.778
19	FENUGREEK POWDER	2.298	34.901	0.164	43.768
20	BLACK PEPPER	65.875	8.768	0.087	34.67
21	FENNEL	16.784	24.98	0.0678	67.98
22	QC CHECK	0.599 (103.8%)	0.524 (99.89%)	5.209 (104.0%)	52.823 (109.2%)
23	CHILLI POWDER	36.098	108.098	15.675	38.09
24	CORIANDER POWDER	38.789	28.98	0.087	80.098
25	TURMERIC POWDER	18.883	46.98	3.786	223.89
26	FENUGREEK POWDER	5.879	14.89	0.0987	100.908
27	BLACK PEPPER	37.908	13.94	0.098	78.986
28	FENNEL	15.098	23.678	0.0356	78.907

Table 5.3: Samples from district C

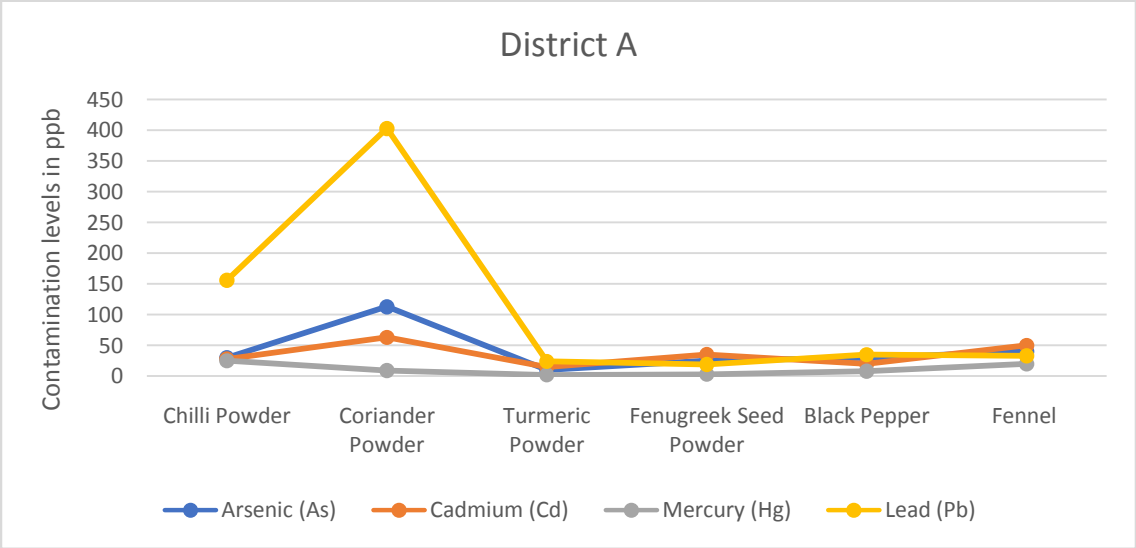

Figure 5.1: The graph shows contamination levels (in PPB) of spice samples from District A, taking the mean of triplicate experiments. The name of the districts cannot be revealed as conditioned by the FDA. This district particularly shows alleviated levels of lead (325.34

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ppb) in the samples, followed by arsenic (48.65 ppb) being the second highest. After arsenic, cadmium and mercury being the least in concentration. The higher levels might be indicating higher pesticides usage or due to poor irrigation water conditions. The coriander powder samples show the highest contamination levels in all the four heavy metals, followed by chilli powder and black pepper.

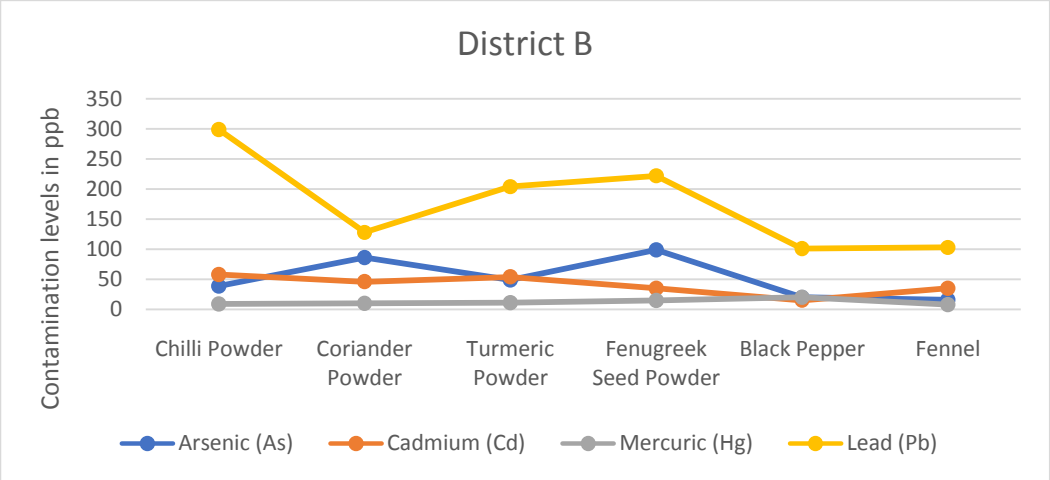

Figure 5.2: The contamination levels from the spice samples of district B is consistent with the trends shown in district A, here as well lead shows the highest concentration followed by arsenic, cadmium and mercury. Chilli powder has the highest contamination levels from this district, followed by fenugreek seed powder, turmeric powder and black pepper.

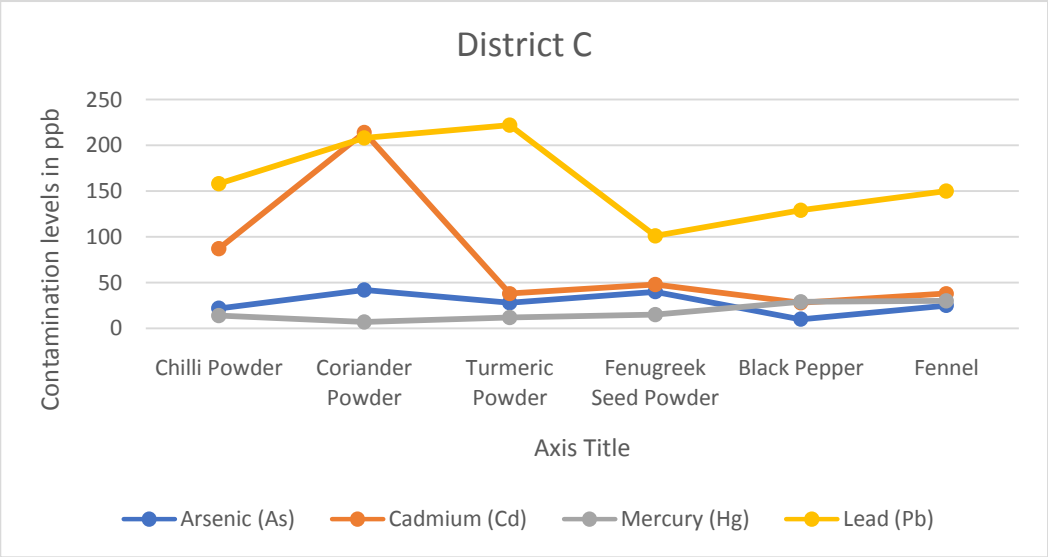

Figure 5.3: Contamination levels of district C shows highest contamination in coriander powder as shown by coriander powder samples from district A. The highest contamination here as well shown by lead followed by cadmium. Turmeric samples from this district show maximum levels of lead followed by coriander powder and black pepper samples.

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Observation and Results of antimicrobial susceptibility assay:

Fig: 5.4: Results of Antimicrobial Susceptibility Assay of turmeric, black pepper and red chilli powder.

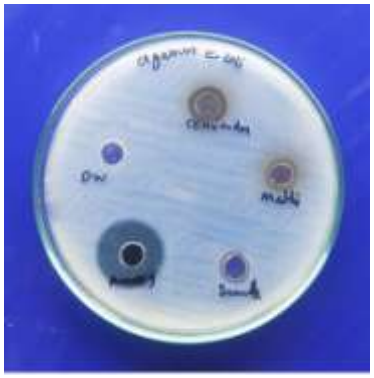

Fig: 5.5: Results of Antimicrobial Susceptibility Assay of coriander powder, fenugreek seed powder and fennel powders.

S.no.	Stock Extract (100%)	Zone of inhibition (in mm) against E. coli (ATCC-25922)
1	Amoxicillin (antibiotic)	19 mm
2	Turmeric powder	11 mm
3	Fenugreek seeds	9 mm
4	Fennel	7 mm
5	Coriander powder	10 mm
6	Black pepper	10 mm
7	Chilli powder	12 mm

Table 5.5: Experiment was carried out in triplicate manner and diameters of the zones of inhibition were measured by millimeter.

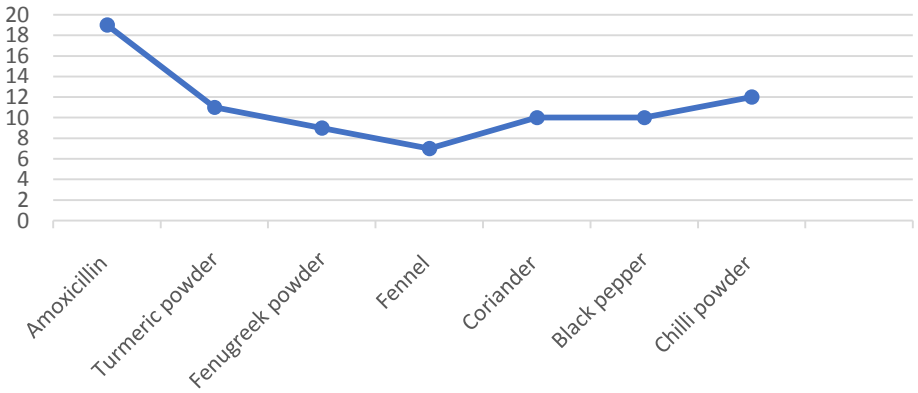

Fig 5.6: The graph shows the zone of inhibition (in mm) obtained in 6 spice samples.

V. CONCLUSION

After analysing the results on heavy metal content analysis and performing subsequent assay, it is inevitable to note that contamination levels are high in spice samples and upon long exposure can lead to various health problems. The reason behind such elevated levels can be soil pollution, excessive

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use of pesticides and contamination of water used for irrigation due to industrial effluents and dumping of sewage and garbage in water bodies.

In conclusion, this thesis has provided an analysis of heavy metals in spice samples, shedding light on an important aspect of food safety and quality control. Through the systematic collection of data, rigorous laboratory testing, and meticulous data analysis, gained valuable insights into the presence and levels of heavy metals in commonly consumed spices.

The findings of this study have highlighted the significance of monitoring heavy metal contamination in spice samples, as these contaminants can pose serious health risks to consumers. This underscores the need for stringent quality control measures in the spice industry to ensure the safety of these essential culinary ingredients.

The analysis of heavy metals in spice samples is not only a matter of consumer health but also contributes to the overall quality and safety of our food supply. The contamination seen could be from contaminated irrigation water carrying effluents from industries and chemical based pesticides used beyond permissible limits to cope up with the demand and poor management practices. As we move forward, it is imperative that we continue to prioritize research in this area to ensure the well-being of consumers and the sustainability of the spice industry.

VI. ACKNOWLEDGEMENT

Gratitude is intangible, a sensation that resides within, unfelt by the eyes but deeply experienced by the heart. The sentiments of the heart are often challenging to articulate. Behind every accomplished task stand numerous hands that contribute to its fulfilment. The journey to task completion remains incomplete without extending gratitude to those who made it attainable. A mere mention falls short in doing justice to their significant contributions. Thus, my acknowledgment extends far beyond the words expressed here.

Research is a lofty concept that tests our patience, energy, and commitment. I extend my gratitude to the divine for providing the strength to navigate this path. Each achieved result marks the commencement of a higher pursuit, necessitating the expertise of those well-versed in the field.

I express my sincere thanks to Dr. Richa Jain, Dr. Abin Mani, Dr. Shikha Jain and Dr. Prabhat K. Mishra for their unwavering support and guidance. Mr. Sandeep Victor of the Food & Drug Administration, Bhopal, for his authorization and the valuable opportunity to conduct my project in the well-equipped High End Equipment laboratory. A profound debt of gratitude is owed to my mentor, Ms. Farheen Ansari, ICP-MS, FDA Bhopal, for her encouragement, guidance, and continuous motivation throughout my endeavors. Without support and guidance during my study period, the fruition of my work would not have been possible.

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