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Research Article

QUANTITATIVE ICP-MS ASSESSMENT OF TRACE METAL DIETARY RISK AND MARGIN OF EXPOSURE IN PROTEIN POWDERS FROM DIVERSE SOURCES

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ABSTRACT

Objective: This study aimed to determine the concentration levels of harmful heavy metals – arsenic (As), cadmium (Cd), lead (Pb), and mercury (Hg) – in commercially available whey and vegan protein powders sourced from India, China, and Japan, and to assess the potential non-carcinogenic health risks associated with their consumption based on target hazard quotient (THQ) and hazard index (HI) values. The reliability and efficiency of the analytical method employed for trace metal determination were also evaluated.

Methods: Quantitative analysis of As, Cd, Pb, and Hg was performed on whey and vegan protein powder samples using microwave-aided acid digestion, followed by an analytical technique using ICP-MS, daily intake estimations, THQ, and HI were calculated to assess potential non-carcinogenic health risks.

Results: Heavy metals were detected in protein powder samples, posing potential health risks. On-carcinogenic risk, measured by hazard quotient (HQ), is concerning if > 1, based on estimated daily intake (EDI) and compared to provisional tolerable daily intake (PTDI). Non-carcinogenic metal transfer percentages are 14% (copper, lead, cadmium), 24% (mercury), and 35% (arsenic). A hazard index (HI) > 1 indicates cumulative risk. Carcinogenic risk is unacceptable if > 10^{-6}, calculated using slope factors (SF_0) such as 1.5 (arsenic) and 0.0085 (lead) (mg kg^{-1} day^{-1} $bw)^{-1}$. The analytical method used was reliable for trace metal detection in these samples.

Conclusion: The presence of heavy metals in commercially available protein powders and the associated potential non-carcinogenic health risks, as indicated by THQ and HI values, underscores the need for stringent quality control measures within the protein supplement industry. The validated analytical method provides a useful tool for future monitoring and risk assessment in this domain.

Keywords: Food contamination, ICP-MS, Risk assessment, Target hazard quotient, Hazard index, Margin of exposure, Health risk.

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INTRODUCTION

A balanced diet is key to fitness and athletic performance, and many believe high nutrient intake, especially protein, boosts results. Protein, from animal or plant sources, provides essential amino acids for muscle, tendon, and bone, and regulates metabolism and hormones. Athletes need varying protein amounts based on their sport and training. To meet these needs, protein supplements—available as drinks or powders—are widely used to support muscle growth, endurance, strength, and overall physical health [1]. The presence of protein powders made from varied sources such as animal-derived whey protein, a dairy industry byproduct that is famous for its full complement of amino acids and fast digestion rate [2], and an increasingly diverse set of plant-based options such as soy, pea, rice, hemp, and combinations thereof addresses the varied preferences and limitations in diet [3].

Even though their perceived health effects and popularity, dietary supplements such as protein powders have been repeatedly questioned in terms of safety and quality control [4]. These questions usually revolve around adulteration of ingredients, improper labeling, and the occurrence of unwanted contaminants, e.g., heavy metals. Heavy metals are of special interest because they are non-biodegradable and have the potential to bioaccumulate in the human body, causing a range of negative health effects even at low levels of chronic exposure [5].

The main causes of heavy metal contamination of food items are generally classified into environmental and anthropogenic [6]. Environmental sources comprise the natural presence of these elements in water and soil, which can be accumulated by plants that are used for making vegan proteins or enter indirectly into the dairy chain through feeds. Anthropogenic sources include industrial effluents, agricultural activities (e.g., the application of contaminated fertilizers and pesticides), and processing techniques during supplement production [7]. The geographical location of the raw materials and the production sites can thus have a major impact on the likelihood of heavy metal contamination.

Earlier studies have found heavy metals in different dietary supplements sold in different parts of the world. Interestingly, some studies have indicated that plant foods and supplements can have higher concentrations of some heavy metals than animal products because they take up heavy metals directly from soil and irrigation water [8]. This is highly applicable considering the growing popularity of vegan protein powder as a sustainable and ethical option [9].

The Indian, Chinese, and Japanese markets are large and increasing consumer pools for protein supplements. These countries also have unique patterns of agricultural cultivation, industry, and ecosystems that may affect the levels and forms of food product heavy metal contamination. Being aware of the individual contamination patterns of protein powders on sale in these markets is thus imperative for determining possible regional health hazards [10-14].

Based on previous studies, this work intends to present a comparative study of heavy metal pollution (As, Cd, Cr, Pb, Hg) in whey and vegan protein powders obtained from India, China, and Japan. Using a

validated analysis technique involving microwave-assisted acid digestion and Q-ICP-MS, endeavor to determine the trace metal levels in a set of commercially sold products accurately. Microwave digestion allows for efficient and complete organic matrix breakdown with ease, guaranteeing effective release of the analytes of interest for subsequent determination by Q-ICP-MS, an analytical technique of high sensitivity, selectivity, and precision for trace element quantitation. Validation of the method, as implied in the preliminary conclusion, is a necessary process to guarantee the robustness and reliability of the resulting data [15,16].

In addition, to better assess the potential health effects to consumers an integral risk assessment methodology, including the estimations of daily intake, target hazard quotients (THQ), hazard index (HI), and margin of exposure (MOE) calculations, enables complete estimation of possible health hazards of heavy metal intake via protein powder consumption [17-19].

Daily intake estimation takes into account the per capita intake of protein powder by various groups. The THQ is a quotient of the estimated daily intake of a contaminant and its reference dose and gives an estimation of non-carcinogenic risk for a single metal. The HI, which is the total of THQs for several metals, determines the likelihood of cumulative non-carcinogenic effects. The MOE, mainly applied to carcinogenic substances but also to non-carcinogens with a threshold of toxicity, is compared between the estimated level of exposure and a benchmark dose, which gives an estimate of the risk of adverse effects. With these integrated risk assessment criteria, this research seeks to have a more detailed understanding of the possible health effects on consumers in India, China, and Japan who intake both whey and vegan protein powder. The results will provide useful information for regulatory bodies and help consumer choices about protein supplementation [20-25].

METHODS

Materials and solutions

Eighteen dietary protein supplements [whey protein and Vegan protein powders], sourced from India, China, and Japan nutraceutical export exhibition, were utilized in this study. These supplements, available in powder sachet form, contained a daily recommended dose of 500 mg essential amino acidsalong with additional nutrients like vitamins and minerals. For microwave digestion, HNO3 and HCL (both trace metal grade, Fisher Chemical) and ultrapure water were sourced from a Direct Q (Millipore) water purification system (Millipore, UK) was required.

Sample preparation by microwave-assisted acid digestion

About 0.2 g of every whey and plant-based protein powder sample, precisely weighed in triplicate into acid-washed Teflon PFA vessels, was subjected to microwave digestion. 7 mL trace metal grade nitric acid (HNO_3) and 2.0 mL trace metal grade hydrochloric acid (Fisher Chemical) were added to each vessel. The vessels were sealed and digested in a milestone ethos microwave digestion system. The digestion procedure had a 10-min ramp-up to a final temperature of 160°C that was held for a further 20 min. This process successfully released metals from the organic protein matrix and left very little residual material. After digestion, every sample and standard was spiked with 10 µg of an internal standard, yielding an approximate concentration of 1 ng g^{-1} Rhodium (Rh) in a solution of 2% nitric acid (HNO_3). Two blank controls were run as controls. After digestion program completion and cooling, the digested samples were filled into 50 mL polyethylene vials and diluted to a final volume of 50 mL using 18.2 MΩ deionized water for subsequent analysis.

Standard stock solutions for calibration

High-purity standard solutions of lead, mercury, cadmium, and arsenic, were purchased from Sigma Aldrich. Analytical grade nitric acid (Merck) and guaranteed reagent grade hydrochloric Fisher's chemical were used as well. Single-element standard solutions were first diluted with 5% nitric acid to a concentration of 1 µg mL^{-1}. The above single-element standards were then mixed in specific volumes to prepare a multi-element reference stock solution by diluting with 5% nitric acid to obtain 5 µg mL^{-1} for Pb and As, 50 µg mL^{-1} for Cd, and 0.5 µg mL^{-1} for Hg. To make calibration curves for the formation of calibration curves, this standard stock solution was successively diluted using 5% nitric acid to form a series of standard mixtures of different concentrations (ng mL^{-1}) for all the target heavy metals.

ICP-MS parameters (Agilent 7800 ICP-MS)

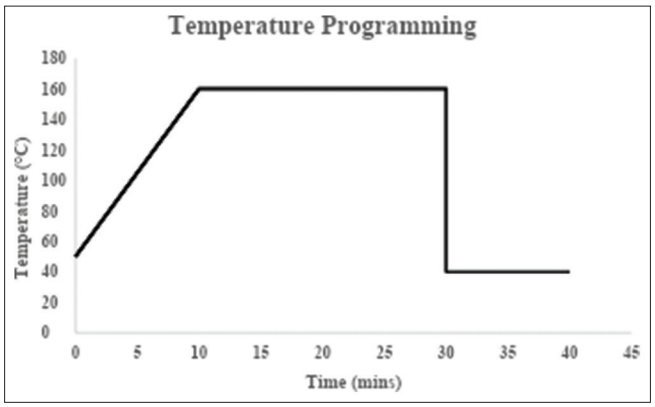
The analysis was carried out under certain operating conditions of the ICP-MS instrument. A Scott double-pass spray chamber and a concentric nebulizer were used for the introduction of samples. The radiofrequency (RF) power was adjusted to 1550 W, and the plasma gas (argon) flow rate was held constant at 1.05 liters per minute. Measurements were conducted in helium mode, consisting of 100 sweeps per replicate, and the total number of replicates used for each sample was three. The microwave digestion procedure, used for sample decomposition before ICP-MS measurement, consisted of a temperature profile with a 10-min ramp to 160°C (shown in Graph 1) followed by a hold period of 20 min at this temperature, shown in Table 1.

Determination of metals by ICP-MS

A calibration curve for each target element was constructed by averaging the signal intensity from the calibration blank and a group of standards at concentrations of 0.05, 0.1, 0.2, 0.5, 1, 2, and 5 parts per billion (ppb). Calibration curves were used directly to analyze the elemental content in the digested samples. Lead (Pb) and Cadmium (Cd) were determined in low-resolution mode, whereas Arsenic (As), and Mercury (Hg) were determined in medium-resolution mode. The signal intensities of the two procedural blank runs were then averaged for each element, and these average blank intensities were subtracted from the raw intensities obtained for the calibration standards and the protein powder samples. In the resulting calibration curve equation, the y-value was the blank-corrected intensity, and the corresponding X-value was the ICP-MS-determined concentration. This calibration process and the linearity were determined by establishing regression analysis and co-relation coefficient using standards and every digested

Table 1: ICP-MS parameters (Agilent 7800 ICP-MS)

Operating conditions	
Spray chamber	Scott double-pass
Nebulizer	Concentric
RF power (W)	1550
Plasma gas flow rate (l/min)	1.05
Measurement parameters	
Scanning mode	Helium mode
Sweeps/replicate	100
No. of replicates	3
Ramp (Mins)	10:00
Temperature (°C)	160
Hold time (Mins)	20:00



Graph 1: Temperature programming of Agilent 7800ICP-MS parameters

whey and vegan protein powder sample Table 2 and Table 3. Finally, to get the true concentration of every element in the original protein powder sample, the measured concentration in the ICP-MS was then multiplied by the correct dilution factor, considering the sample preparation procedure.

Method validation

Method validation was rigorously performed according to an internal procedure. Key validation parameters included recovery, selectivity, accuracy, repeatability, reproducibility, and linearity. Recovery studies involved fortifying powder samples at 10, 50, and 100 µg/kg in four replicates, with an acceptance range of 90.0%–120% and an RSD of <20.0%. Selectivity was verified by the absence of analyte response in blank samples for As, Cd, Hg, and Pb. Accuracy was further confirmed if recovery rates fell between 90.0% and 120%. Repeatability and reproducibility were established by ensuring %RSD did not exceed ±20.0%. Linearity was confirmed with a correlation coefficient (R2) ≥1.00 (Table 4). Comprehensive quality control measures, such as the use of solvent blanks, fresh calibration standards, spiked samples, and certified reference materials analyzed with each batch, guaranteed the reliability and accuracy of the results.

RESULTS AND DISCUSSION

Concentrations of Arsenic (Ar), Cadmium (Cd), Lead (Pd), and Mercury (Hg) were measured with ICP-MS, and the respective calibration curve data are given in Table 1 and Figs. 1-4. The linearity of these calibration curves and method accuracy were rigorously evaluated. Recovery

Table 2: Linearity of standards concentration (ppb) vs CPS representing Regression analysis Correlation Coefficient

Metal	As		Cd		Hg		Pb	
	Con (ppb)	CPS	Con (ppb)	CPS	Con (ppb)	CPS	Con (ppb)	CPS
Concentration	0.35	8682.49	0.35	21711.59	0.35	3938.78	0.35	215019.7
	0.5	13054.04	0.5	30488.39	0.5	5001.03	0.5	308556.5
	0.75	19762.81	0.75	46166.43	0.75	5301.32	0.75	459214.9
	1	25973.19	1	62070.82	1	6593.8	1	607331.1
	5	131895.7	5	306642.8	5	35974.1	5	2900452
	10	260934.7	10	609227.2	10	313401.3	10	5710839
Regression analysis	y=26145x+24.051		y=60921x+683.02		y=569230x+30493		y=32860x - 15959	
Correlation Coefficient	$R^2 = 1$		$R^2 = 1$		$R^2 = 1$		$R^2 = 0.9972$	

Table 3: Concentration of trace elements in whey and vegan powders from various sources

Element	As		Cd		Hg		Pb	
CPS/CONC	CPS	Conc	CPS	Conc	CPS	Conc	CPS	Conc
		(ppb)		(ppb)		(ppb)		(ppb)
S_1 WPC	427.15	0.02	1902.27	1.02	56795.42	1.21	51612.41	0.04
S_2 WPC	794.24	1.03	1364.94	0.01	3637.91	0.6	74752.6	0.08
S_3 WPC	767.54	1.03	5106.72	1.07	3340.88	0.59	88488.82	1.1
S_4 VPC	1398.29	10.05	3634.53	31.05	2125.89	0.55	85195.17	9.1
S_5 VPC	1037.87	12.04	4485.73	4.06	2850.19	2.57	279931.6	10.44
S_6 VPC	1161.33	10.04	2059.12	15.02	2392.91	1.56	122135.67	8.16
S_7 WPJ	1031.18	3.04	747.52	4	2135.89	0.55	84302.71	0.09
S_8 WPJ	573.98	0.02	400.46	ND	2539.76	0.56	45270.34	1.03
S_9 WPJ	1655.27	0.06	2653.22	2.03	1264.81	0.52	71598.43	1.07
S_10 VPJ	2509.69	6.1	2035.77	30.02	1188.06	3.52	75075.25	10.08
S_11 VPJ	3310.79	20.13	2312.77	12.03	1468.4	0.53	87618.95	6.1
S_12 VPJ	447.15	15.02	1302.27	10.01	56795.42	12.21	51612.41	7.04
S_13 WPI	694.24	0.03	1234.94	0.01	3637.91	ND	74752.6	0.08
S_14 WPI	667.54	ND	5206.72	0.07	3340.88	0.59	88488.82	0.1
S_15 WPI	1292.29	10.05	3624.53	1.05	2125.89	0.55	85195.17	1.1
S_16 VPI	1027.87	15.04	3485.73	10.05	2850.19	31.57	279931.6	10.44
S_17 VPI	1121.33	10.04	2159.12	12.02	2392.91	1.56	122135.67	8.16
S_18 VPI	1131.18	7.04	2647.52	10.03	2135.89	0.55	84302.71	8.09
Σ METAL		120.7		143.55		59.79		82.3
Averg. concentration		120.7		143.55		59.79		82.3
SD		27.49		9.7135		19.191		4.3124
RSD		22.76296		6.766674632		32.10035946		5.23996538

WPC: Whey protein sample from china, VPC: Vegan protein sample from china, WPJ- Whey protein sample from Japan;*VPJ-Vegan protein sample from Japan, WPI: Whey protein sample from India, VPI: Vegan protein sample from India, Σ METAL: Sum of total metals, SD: Standard deviation, RSD: Relative standard deviation

Table 4: Method validation data and The estimated dietary intake (EDI) of As, Cd, Hg and Pb with different level bound

Analyte	LOD (µg/L or ppb)	LOQ (µg/L or ppb)	LB (mg kg^{-1})	MB (mg kg^{-1})	UB (mg kg^{-1})	R^2	Recovery %	RSDd %
As	0.7	2.4	0.4	0.1	0.16	1	110	6.32
Cd	0.9	3	0.09	0.1	0.12	1	106	3.56
Hg	0.8	4	0.41	0.45	0.5	1	104	6.11
Pb	6.9	23	0.21	0.36	0.38	0.9972	115	3.02

LOD: Limit of detection (µg/L), LOQ: Limit of quantification (µg/L), R^2: Corelation coefficient, RSD: Relative standard deviation (n=8), UB: Upper bound, MB: Middle bound, LB: Lower bound

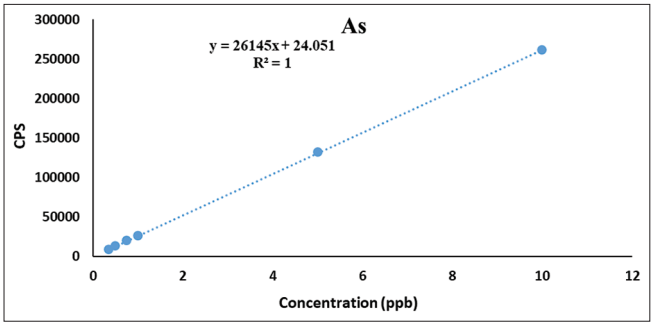


Fig. 1: Arsenic calibration curve

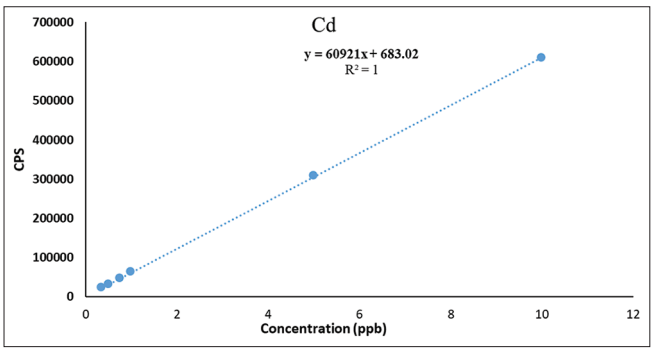


Fig. 2: Cadmium calibration curve

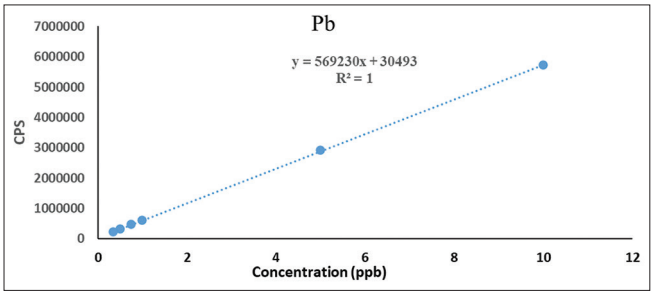


Fig. 3: Lead calibration curve

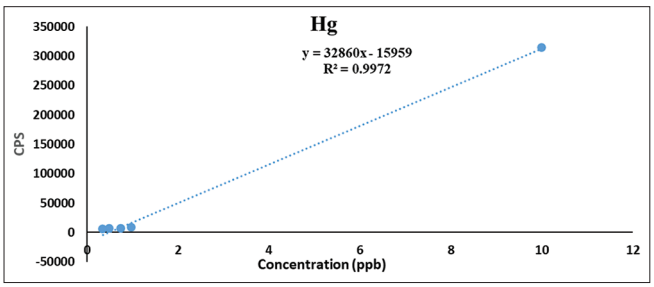


Fig. 4: Mercury calibration curve

tests, conducted on a reference material and spiked samples, showed satisfactory accuracy, with recovery percentages between 90% and 120%. Out of the 18 whey and vegan protein samples from China, India, and Japan, the Vegan protein samples contained the highest levels of the studied heavy metals. Table 2 and Fig. 5 summarize the concentration values of the elements measured in both the whey and vegan protein powder samples from the various geographical locations listed in the study.

The health risk assessments of heavy metal contamination

Exposure estimation, in the form of estimation of the daily intake (EDI) of the heavy metals, was carried out to assess the health effects

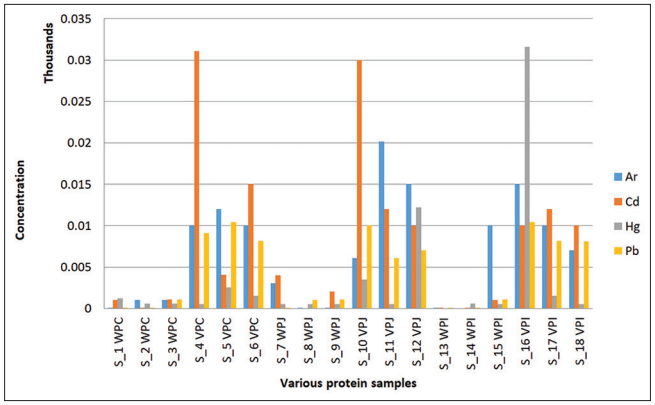


Fig. 5: Concentration of detected heavy metals in various protein supplements

potential linked with the intake of the protein powders. This was followed by non-carcinogenic and carcinogenic risk estimations. Non-carcinogenic risks were described using the hazard quotient (HQ) for a single metal and the hazard index (HI) for multiple metals' effect. To this end, the U.S. Reference dose (RfD) of the Environmental Protection Agency (EPA) was used. The RfD, which has superseded the tolerable daily intake (TDI), is, according to the EPA. Carcinogenic risks (CR) were also estimated for those metals with proven carcinogenic potential to give a complete picture of the possible long-term health effects.

Exposure assessment

The estimated daily intake (EDI) of every heavy metal among consumers of vegan protein and whey proteins was calculated using [17],

$$EDI = (C \times IRD) / BW$$

where C is the concentration of the heavy metal in mg kg^{-1}, and both the maximum, mean, and minimum concentrations were used. IRD is the ingestion rate per day of vegan protein in kg day^{-1}, and BW is the mean body weight in kg. The derived EDI values (mg kg^{-1} day^{-1} bw) for every metal (Table 4), were then contrasted with their corresponding PTDI values (mg kg^{-1} day^{-1}) as 0.00214 for As,0.00057 for Hg, 0.00357 for Pb, and 0.00083 for Cd. This comparison allowed for an initial estimation of the possible health hazards related to the daily consumption of vegan&Whey protein powders with the measured heavy metal content.

Non-carcinogenic risk assessment

Non-cancerous hazards posed by heavy metal exposure due to consumption of vegan proteins were estimated using the hazard quotient (HQ), which was given by [18]

$$HQ = (C \times IR \times Ef \times Ed \times t) / (AT \times BW \times RfD)$$

Here, C is concentration of metal (mg kg^{-1}), IR is ingestion rate per day (0.5 kg day^{-1}), Ef is frequency of exposure (90 days $year^{-1}$), Ed is duration of exposure (20 years), t is transfer percentage (14% for Cd, Cu, Pb; 35% for As; 24% for Hg), AT is average lifetime (365 days $year^{-1}$ × 70 years), and BW is average body weight (kg). An HQ < 1 signifies minimal likelihood of non-carcinogenic effects for one metal alone, whereas an HQ > 1 implies a possible risk. The overall non-carcinogenic risk from a combination of metals was evaluated by the hazard index (HI), a measure of the addition of individual HQs.

$$HI = \sum HQ$$

An HI > 1 signifies a possibility of cumulative non-carcinogenic effects on health.

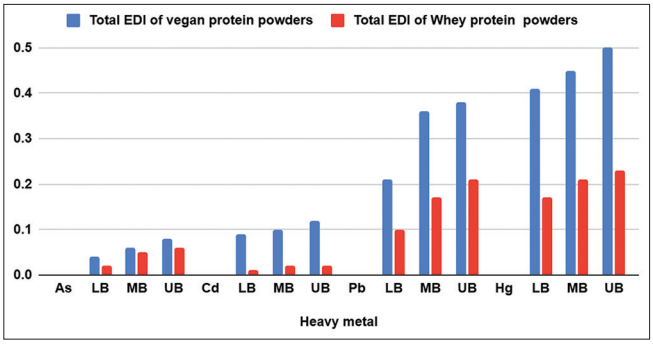


Fig. 6: Total EDI of vegan protein & Whey protein powders

Carcinogenic risk assessment

Carcinogenic risk (CR) due to heavy metal exposure through consumption of vegan protein was estimated using the formula [19],

$$CR = (C \times IR \times Ef \times Ed \times t \times SFo) / (AT \times BW)$$

In this, C is the metal concentration (mg kg^{-1}), IR is the rate of ingestion per day (0.5 kg day^{-1}), Ef is the frequency of exposure (90 days $year^{-1}$), Ed is the duration of exposure (20 years), and t is the rate of transfer (14% for Cd and Pb; 35% for As). The SF0 values for use were 6.1 for Cd, 1.5 for As, and 0.0085 for Pb (in (mg kg^{-1} day^{-1} bw) $^{-1}$). The carcinogenic risks of these three metals in the same vegan protein samples were added together to acquire the sum CR for one supplement. A CR value above 10^{-6} is taken to represent an unacceptable amount of carcinogenic risk.

Statistical analysis

The statistical analysis commenced with calculating the mean and standard deviation of heavy metal content in the samples, utilizing Microsoft Excel 2021. To accurately assess chemical contaminants, especially when concentrations fell below the limit of quantification (LOQ) or detection (LOD), data were treated according to methodologies established. This involved three distinct approaches: assigning zero for lower bound (LB) estimations, half the LOQ/LOD for Middle Bound, and the full LOQ/LOD for Upper Bound (Table 4). Levene's test was employed to investigate the homogeneity of variance in mean heavy metal content across the samples. Furthermore, data are presented using t t-test, and the p≤ .05 was considered statistically significant.

Risk assessment: daily intake estimation, target hazard quotient, hazard index, and margin of exposure

This study estimated dietary exposure to the investigated toxic elements. Total heavy metal intakes were quantified employing LB, Middle bound (MB), and upper bound (UB) methodologies (Fig. 6). Non-carcinogenic chronic risks from protein powder consumption, specifically for Arsenic (As), Mercury(Hg), Lead (Pb) and Cadmium (Cd), were evaluated using the THQ and hazard index (HI) calculated across LB, MB, and UB scenarios along with the MOE in the selected protein powders.

CONCLUSION

The present study offers an extensive evaluation of heavy metal contamination in commercially used whey and vegan protein powders from India, China, and Japan. Our investigation, using a confirmed microwave digestion and Q-ICP-MS technique, found detectable levels of Arsenic, Cadmium, Lead, and Mercury in the tested samples. Importantly, as opposed to some predictions, vegan protein samples had higher levels of the studied heavy metals than whey protein powders in the regions under study. The follow-up exposure and risk evaluation revealed possible health issues related to the intake of these protein supplements. Calculation of estimated daily intake (EDI) compared to provisional tolerable daily intake (PTDI) values implied possible exceedances for some heavy metals. Non-carcinogenic risk assessment, based on hazard quotients (HQ) and the hazard index (HI), non-carcinogenic metal transfer percentages are 14% (Copper, Lead, Cadmium), 24% (Mercury), and 35% (Arsenic), disclosed possible cumulative risks, especially based on consumption patterns and heavy metal levels. In addition, the carcinogenic risk assessment of Arsenic, Cadmium, and Lead showed possibilities of unacceptable carcinogenic risks in certain samples, according to the CR values calculated. These results highlight the importance of high-quality control procedures and analysis of heavy metal contents in whey and vegan protein powders to protect consumer health. Additional studies are needed to determine the points of contamination throughout the supply chains in India, China, and Japan and to have more precise regional consumption data for more refined risk estimations. Finally, this research adds worthwhile information to the body of knowledge regarding heavy metal contamination within a commonly used food supplement category and emphasizes the significance of regulatory control to guarantee the safety of protein powder nutraceutical products in the world market.

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Nil

AUTHORS CONTRIBUTIONS

All the researchers participated in the research and production ofthe text in an equal capacity throughout all phases.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

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