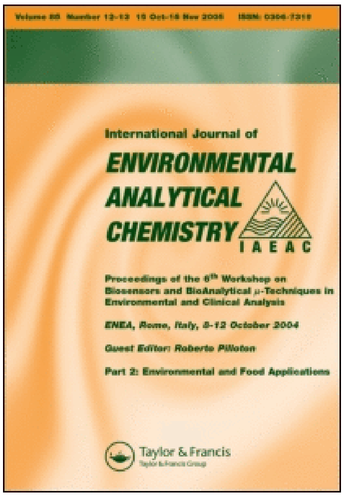

International Journal of Environmental Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

Neutron Activation Analysis of Trace Elements in Mixed Human Diet of Kashmir Region for Nutritional Adequacy and Safety Evaluation

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To cite this Article Waheed, S. , Zaidi, J. H. , Ahmad, S. and Saleem, M.(2001) 'Neutron Activation Analysis of Trace Elements in Mixed Human Diet of Kashmir Region for Nutritional Adequacy and Safety Evaluation', International Journal of Environmental Analytical Chemistry, 81: 3, 221 — 232
To link to this Article: DOI: 10.1080/03067310108044344
URL: http://dx.doi.org/10.1080/03067310108044344

Intern. J. Environ. Anal. Chem., Vol. 81, pp. 221–232
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NEUTRON ACTIVATION ANALYSIS OF TRACE ELEMENTS IN MIXED HUMAN DIET OF KASHMIR REGION FOR NUTRITIONAL ADEQUACY AND SAFETY EVALUATION

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(Received 11 May 2001; In final form 6 June 2001)

Instrumental Neutron Activation Analysis was applied to determine 34 major, minor and trace elements in mixed human diet representative of the Kashmir region. The purpose was to contrive a database for this region regarding adequacy and safety of trace elements through their food habits. The daily dietary intakes of essential and toxic elements were found to be within the RDA/ESADDI levels. Comparison of dietary patterns of the population of Kashmir has also been made with the representative diets of other countries.

Keywords: Trace elements; Diet; Intake; Kashmir; Safety

INTRODUCTION

In the past decades rapid urbanization, industrialization, development in agricultural practices through use of chemicals and sprays, combustion of fossil fuel and other anthropogenic activities for better living have led to a direction which is extremely alarming. Some of its ruinous effects can be seen as global warming and pollution of the biosphere with toxic metals. Air, water and soil around us are invariably being infected with these

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toxic elements which ultimately contaminate the food chains. Human exposure to the toxic trace elements is mainly through these food chains in the form of diet. Apart from the vulnerability of human beings to these undesired toxic elements, adequate intake of essential elements is also fundamental for the maintenance of good health and optimum human performance since many trace elements play a vital role in the biochemical processes through enzymatic reactions. On one hand high intake of toxic elements may lead to serious health problems and may cause impairment of the vital organs of the body such as brain, nervous system, kidney, liver, and lungs. On the other hand inadequacy of essential elements may lead to physiological deficiencies in the human body creating a vicious cycle of structural abnormalities by biochemical changes that takes more than one generation to rectify [1,2]. However some essential trace elements when taken in excess may create toxic effects [3]. This grave situation where diet intakes are not just about avoiding hunger, they are also about securing health, demands for systematically designed monitoring and data compilation of trace element contents in diets of different regions. Information on trace element contents in diets will help to assess the safety and adequacy of the regional food supplies.

The need for reliable analytical data on major, minor and trace element contents in diets of different regions was the basis of this study. A number of analytical techniques such as Atomic Absorption Spectrometry (AAS). Anodic Stripping Voltametry (ASV), Inductively Coupled Plasma–Mass Spectrometry (ICP–MS), Inductively Coupled Plasma–Atomic Emission Spectrometry (ICP–AES), Gas Chromatography–Mass Spectrometry (GC–MS), Direct Current Plasma–Emission Spectrometry (DCP–ES), and Particle Induced X-Ray Emission (PIXE) have been used to report such data for different countries [4–6]. In this study a sensitive, reliable and non-destructive technique of instrumental neutron activation analysis (INAA) has been employed to study integrated diet representative of the Kashmir region.

The territory of Kashmir is bordered on the north by Afghanistan and China, on the east by China, on the south by the state of Himachal Pradesh and the state of Punjab in India, and on the west by the North-West Frontier Province and the Punjab Province of Pakistan. The Indus River flows through Kashmir, and the Jhelum River rises in the northeastern portion of the territory. Kashmir possesses an equable climate. The majority of the people are engaged in diverse kinds of subsistence agriculture on terraced slopes, each crop adapted to local conditions. Rice, the staple crop, is planted in May and harvested in late September.

Corn (maize), millet, pulses (legumes such as peas, beans, and lentils), cotton, and tobacco, along with rice, are the main summer crops, while wheat and barley are the chief spring crops. Many temperate fruits and vegetables are grown in areas adjacent to urban markets or in well-watered areas with rich organic soils.

Apart from the concentrations of trace elements in the Kashmir diet, estimated daily dietary intakes of these elements have also been discussed. Emphasis has also been given to quality assurance through the use of IAEA and NIST certified reference materials (CRMs) to ensure the quality of the analytical data and reliability of the technique.

EXPERIMENTAL

Sampling and preparation of diet

Integrated mixed human diet of the Kashmir region was prepared in accordance with door-to-door survey regarding eating habits, appeal, and consumption of each family. All the basic food items needed for diet preparation according to their weekly consumption as listed in Table I, were collected and purchased in appropriate amounts from the local markets of the region. These individual food articles were washed with the local tap water and subsequently packed in clean polyethylene bags and

TABLE I Weekly intake plan of individual food articles as consumed by the adults of the Kashmir region

Serial No.	*Individual Food Articles*	*Moisture contents* (%)	*Wet Weight* (g)	*Dry Weight* (g)
1	Wheat	7.7	1550	1431
2	Rice	7.9	750	691
3	Pulses	7.7	130	120
4	Beef	72.8	250	68
5	Mutton	73.2	350	94
6	Chicken	74	350	91
7	Egg	73	300	81
8	Vegetables	83.6	820	135
9	Fruits	85	1075	161
10	Milk	85.5	2750	399
11	Edible fat	2.4	300	294
12	Sugar	9.5	240	218
13	Spices	2.5	40	39
Total	Weekly Intake		8905	3822
	Daily Intake		1272	546

transported back to our central laboratory for further processing. In the laboratory all the food samples were opened, cut into smaller pieces and further handled in laminar flow fume hood. Vegetables, fruits, meat, pulses, spices, food grains, eggs and milk samples were freeze dried, grinded, blended, homogenized, and mixed together into a diet as reported in our earlier works [7,8]. The prepared representative integrated Kashmir diet was stored in pre-cleaned polyethylene bottles with screw caps and placed in a freezer to avoid any decomposition. Six randomly selected aliquots from the diet sample were analyzed for their Mn contents in order to check the homogeneity of the sample. The results showed about 5% variation round mean value certifying the homogeneity of the prepared diet sample.

Preparation of multi-element standard

Optimum amounts of accurately weighed spec-pure elemental salts were mixed together, dissolved in aqua-regia, and brought to an aqueous medium to form a multi-element calibration and comparison base. 100 μl aliquots of this standard solution (checked with the CRMs) were transferred and dried on filter paper to be used as primary standard throughout this work.

Irradiation and counting

For short and medium irradiations aliquots of approximately 250 mg of the diet samples taken in triplicate, multi-element standard base, and IAEA-CRM mixed human diet (H-9), NIST-SRM bovine liver (SRM-1577a), NIST-SRM orchard leaves (SRM-1571) (for quality assurance of the analytical method) were separately sealed in precleaned polyethylene capsules. These targets were subjected to irradiation in a 27-kW Miniature Neutron Source Research Reactor (MNSR) with a thermal flux of $1 \times 10^{12}\,n\,cm^{-2}\,sec^{-1}$ at the sites near to the core. For longer irradiation similar set of target materials were sealed in pre-cleaned silica ampoules, placed in aluminum cans, cold welded and irradiated in the periphery of 10 MW swimming pool type research reactor with thermal flux of $5 \times 10^{13}\,n\,cm^{-2}\,sec^{-1}$. The adopted irradiation, cooling and counting protocols for this study have been previously optimized and reported in our earlier findings [9,10]. After appropriate irradiation and cooling the samples were transferred into pre-weighted polyethylene capsules for counting. The capsules were re-weighed to get the net weight of the counted sample.

NAA counting equipment

The neutron activation analysis (NAA) counting set up comprised of high purity germanium detector (Intertechnique), coupled the Personal computer-based multi channel analyzer (Intertechnique, Pro-286 e MCA). The resolution of the system is 1.9 keV for 1332.5 keV full energy peak of ^{60}Co. All the measurements were made using "Intergamma, Version 5.03" software. Calculations and statistical data treatment was done through a computer program developed in our laboratory.

RESULTS AND DISCUSSION

The integrated representative diet of the Kashmir region was analyzed using INAA. 5 major elements (concentration above 100 μg g^{-1} i.e. Ca, Cl, K, Mg, and Na), 9 minor elements (concentration from 1 to 100 μg g^{-1} i.e. Al, Ba, Br, Cu, Fe, Mn, Rb, Sr, and Zn), and 20 trace elements (concentration below μg g^{-1} i.e. Ag, As, Ce, Co, Cr, Cs, Eu, Gd, Hg, La, Mo, Nd, Sb, Sc, Se, Sn, Ta, Tb, Yb, and U) were characterized. Optimum adjustment in the irradiation, cooling and counting times prevented serious elemental interferences and all the peaks were well resolved except for Zn and Hg. The interference of the full energy peak of ^{65}Zn at 1115 keV and of ^{203}Hg at 279.2 keV were mathematically solved after subtracting the contribution of the overlapping peak of ^{46}Sc at 1120 keV and ^{75}Se at 279.5 keV respectively in accordance with our previous findings [11,12].

Employing the same set of irradiation and radio-assay protocols Certified Reference Materials, one from International Atomic Energy Agency (IAEA) and two from National Institute of Standard and Technology (NIST), were also analyzed for quality assurance of the technique and authenticity of the analytical data. Table II shows that our results for this work and the certified elemental concentrations for IAEA mixed human diet (CRM-H-9), NIST bovine liver (SRM-1577a), and NIST orchard leaves (SRM-1571) are reasonably comparable. Variation coefficient (νc) for most of the elements is below 10% showing fairly good accuracy and precision.

TABLE II Concentration of various elements in IAEA and NIST Certified reference materials (Concentrations in μg/g)

Element	IAEA (H-9) Mixed Human Diet		NIST SRM-1577a Bovine Liver		NIST SRM 1571 Orchard Leaves	
	Our Values	*Certified Values*	*Our Values*	*Certified Values*	*Our Values*	*Certified Values*
Al	19.6 ± 1.4	–	2.9 ± 0.3	(2)	329 ± 52	310
As	89.2 ± 7.3	88 ± 32	46.5 ± 3.9	47 ± 6	9.8 ± 0.9	10.0 ± 2.0
Ba	–	–	–	–	45.8 ± 4.2	(44)
Br	7.8 ± 0.6	7.5 ± 0.68	8.8 ± 0.9	(9)	10.5 ± 1.1	(10.0)
Ca	2324 ± 119	2310 ± 160	126 ± 9	120 ± 7	21022 ± 399	20900 ± 300
Cl	12488 ± 1097	12500 ± 1500	2791 ± 130	2800 ± 98	675 ± 89	(690)
Co	0.05 ± 0.006	0.043 ± 0.005	0.23 ± 0.02	0.21 ± 0.05	0.18 ± 0.02	(0.2)
Cr	0.14 ± 0.02	0.15 ± 0.04	0.68 ± 0.07	–	2.5 ± 0.2	2.6 ± 0.3
Cs	0.03 ± 0.003	(0.025)	0.021 ± 0.002	–	0.038 ± 0.004	(0.04)
Cu	–	–	163 ± 13	158 ± 6.9	12.1 ± 1.0	120.0 ± 1.0
Fe	33.7 ± 2.0	33.5 ± 2.2	196 ± 15	194 ± 20	301 ± 13	300 ± 20
Hg	0.006 ± 0.0006	0.005 ± 0.001	0.005 ± 0.001	0.004 ± 0.002	0.16 ± 0.01	0.155 ± 0.015
K*	0.86 ± 0.04	0.83 ± 0.066	1.1 ± 0.06	0.996 ± 0.007	1.47 ± 0.05	1.47 ± 0.03
Mg	778 ± 41	790 ± 50	605 ± 22	600 ± 15	–	–
Mn	11.7 ± 0.4	11.8 ± 0.8	10.0 ± 0.6	9.9 ± 0.8	92 ± 4	91 ± 4
Mo	–	–	3.2 ± 0.3	3.5 ± 0.49	0.37 ± 0.04	0.3 ± 0.1
Na	8121 ± 440	8100 ± 690	2419 ± 178	2430 ± 130	81 ± 5	82 ± 6
Rb	7.7 ± 0.5	8.0 ± 0.6	12.2 ± 1.0	12.5 ± 0.1	12.7 ± 0.8	12.0 ± 1.0
Sb	–	–	0.004 ± 0.001	(0.003)	3.1 ± 0.3	2.9 ± 0.3
Se	0.14 ± 0.02	0.11 ± 0.01	0.66 ± 0.06	0.71 ± 0.07	0.085 ± 0.009	0.08 ± 0.01
Sr	–	–	0.14 ± 0.013	0.138 ± 0.003	37.8 ± 2.3	37.0 ± 1.0
Yb	–	–	–	–	0.035 ± 0.004	(0.03)
Zn	26.0 ± 2.2	27.5 ± 1.8	120 ± 11	123 ± 8	25.3 ± 2.7	25.0 ± 3.0

*Concentrations in percent.
Values in parentheses are uncertified.

TABLE III Trace element concentration in mixed human diet of Kashmir

Elements	*Concentrations* μg g^{-1}	*Variation coefficient* (*vc*)%	*Elements*	*Concentrations* μg g^{-1}	*Variation coefficient* (*vc*)%
Ag	0.37 ± 0.03	8.1	La**	66 ± 7	10.6
Al	43.6 ± 4.0	9.2	Mg	601 ± 39	6.4
As**	121 ± 12.5	10.3	Mn	8.4 ± 0.3	4.6
Ba	2.0 ± 0.2	10.0	Mo	0.24 ± 0.02	8.4
Br	4.9 ± 0.6	12.2	Na	2976 ± 190	6.4
Ca*	1.5 ± 0.09	6.7	Nd	0.6 ± 0.05	8.4
Ce**	129 ± 13	10.0	Rb	2.6 ± 0.2	7.7
Cl	3186 ± 145	4.6	Sb**	3.7 ± 0.6	16.2
Co**	22.9 ± 2.5	10.9	Sc**	8.8 ± 1.2	13.6
Cr	0.26 ± 0.025	9.6	Se**	132 ± 15	11.4
Cs**	12 ± 1.6	6.5	Sn	3.2 ± 0.24	7.5
Cu	4.4 ± 0.3	6.8	Sr	2.7 ± 0.2	7.4
Gd	0.32 ± 0.03	9.4	Ta	0.14 ± 0.01	7.2
Eu**	10.6 ± 1.2	11.3	Tb	0.25 ± 0.02	8.0
Fe	26.0 ± 1.2	4.6	U**	44 ± 5	11.4
Hg**	5.3 ± 0.7	13.2	Yb	0.35 ± 0.03	8.6
K*	0.63 ± 0.04	6.3	Zn	24.5 ± 2.0	8.2

*Values in percentage.
**Values in ng g^{-1}.

The results presented in Table III represent concentrations of 34 elements in diet on dry weight basis and are the averages of at least 6 independent determinations. Most of the elements (Ag, Al, Ba, Ca, Ce, Cr, Cs, Cu, Gd, K, Mg, Mo, Na, Nd, Rb, Sn, Sr, Ta, Tb, Yb and Zn) have been determined with $vc \leq 10\%$ around the mean value. Cl and Mn show the highest precision since their $vc < 5$. However, As, Br, Co, Eu, Hg, La, Sc, Se, and U have $vc \geq 10\%$, with highest value of 16.2% for Sb showing relatively low precision. The elements present in ng g^{-1} levels show relatively low precision and low counting statistic could be illustrative for this high variation of coefficient.

The adequate intake values of essential, toxic and other elements were calculated from the average concentration of each element on the basis of daily consumption of 546 g of diet. Estimated daily dietary intake values for all the elements, along with recommended dietary allowances (RDA), and the estimated safe and adequate daily dietary intakes (ESADDI) [13,14] are presented in Table IV. The intake values of elements per day follow the pattern of K > Cl > Na > Ca > Fe > Zn > Mn > Cu > Sn > Mg > Cr > Mo > Co, where K, Cl, Na, and Ca being the major element constituents of the diet with intake values of 3440 mg, 1739 mg, 1625 mg, and 819 mg respectively. Nutritional adequacy has been interpreted by plotting percentage intake ratio of an element with the recommended dietary allowances against corresponding elements in Fig. 1. Figure shows the nutrient intake through diet follow the order Mn > Cr > Ca > Fe > Mg > Cu > K > Zn > Mo > Na > Sn > and Cl, designating reasonably ample supply of Mn, Cr, Fe, and Mg, through this diet however, it may not be a satisfactory source of Na, Sn, and Cl. For safe and adequate daily dietary intakes, estimated daily intakes of toxic elements namely As, Br, Hg, Sb, and Se,

TABLE IV Daily dietary intake values of trace elements in Kashmir diet (Consuming 546 g/day per person of diet)

Elements	*Daily intake* μg	*RDA/ESADDI*	*Elements*	*Daily intake* μg	*RDA/ESADDI*
Ag	202	–	La	36	–
Al*	24	60[b]	Mg*	328	0.35[m] 0.30[f]
As	66	120	Mn*	4.6	2.5–5.0
Ba*	1.0	–	Mo	131	75–250
Br*	2.7	–	Na*	1625	1100–3300
Ca*	819	800	Nd	327	–
Ce	70	–	Rb*	1.4	–
Cl*	1739	1700–5100	Sb	2	–
Co	12.5	3[a]	Sc	4.8	–
Cr	142	50–200	Se	72	50–200
Cs	6.5	–	Sn*	1.74	–
Cu*	2.4	1.5–3.0	Sr	1474	–
Gd	174	–	Ta	76	–
Eu	7	–	Tb	136	–
Fe*	14.2	10[m] 18[f]	U	24	–
Hg	2.9	42	Yb	191	–
K*	3440	1875–5625	Zn*	13.4	15

*Values in mg/person/day; [a]RDA in terms of vitamin B_{12}; [b]PTDI (Provisional Tolerable Daily Intake) Ref. [13]; [m]Male; [f]Female.

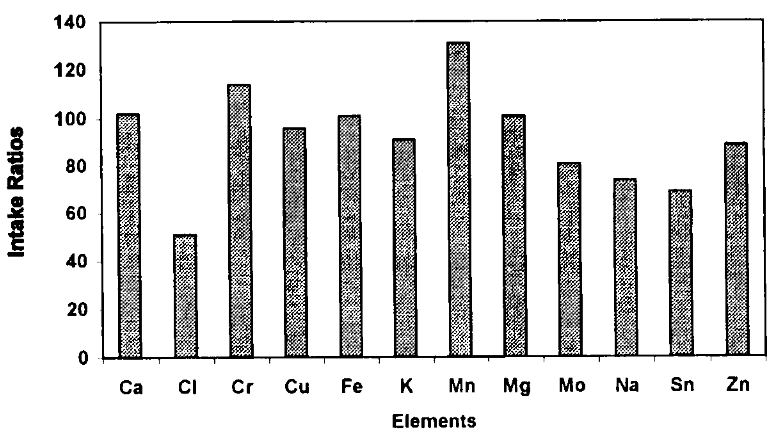

FIGURE 1 Intake ratio of trace elements in Kashmir diet.

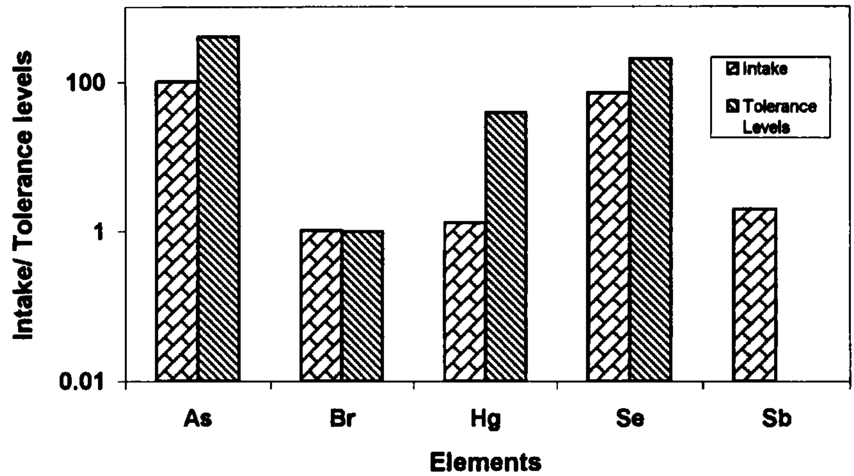

FIGURE 2 Intake levels and tolerance values of toxic elements in Kashmir diet.

have been presented in Fig. 2. Intake levels follow the pattern of Br > Se > As > Hg > Sb with corresponding values of these elements as 2.7 mg, 72 μg, 66 μg, 2.9 μg and 2 μg per day respectively. The toxic element intake values for Se, As, Hg, and Sb are well below the ESADDI safe limits.

Apart from essential and toxic trace elements, some other elements like Al, Ba, Rb, Cs, Sc, and rare earth elements (REEs) have also been quantified in the diet. Excess intake of Al above 3.8–51.6 mg [15] per day as given in literature can be toxic in nature. However, our value for Al intake is 24 mg per day, which is within the safe level of intake. Physiological importance if any has not been cited in the literature for Ba, Rb, Cs, and Sc, yet their presence in the body as 22 mg of Ba [15], 680 mg of Rb, 1.5 mg of Cs and ultra trace levels of Sc has long been established [16]. Our values of daily intake of these elements are 1.09 mg, 1.4 mg, 6.5 μg and 4.8 μg respectively which almost fall within the daily intake ranges of 0.27–1.29 mg of Ba [15], 1.2–6.3 mg of Rb [17,18], 4.4 to < 40 μg of Cs [19,20] and 0.11–2.2 μg of Sc [18–21] suggested in the literature.

With the extensive increase in demand for fine chemicals with REEs for industrial sector, they are likely to contaminate plants and animals food chains, thus effecting human health through diet [22]. REEs can penetrate and contaminate soil up to 1 m depth, and hence can be passable to crops [23]. A number of scientists have reported cyto-toxic effects of REEs for occupationally exposed workers causing REEs Pneumoconiosis [24–26]. 6 REEs (Ce, Eu, La, Nd, Tb, and Yb) have been determined in this work with relatively low concentrations and corresponding daily intake values of 70 μg, 7 μg, 36 μg, 327 μg, 136 μg, and 191 μg, respectively. These figures can be used to establish base line data of REEs in the Kashmir diet for estimation of future pollution levels and source identification.

Estimated dietary intake patterns of USA [4], Germany [27], Taiwan [28], India [29], and Brazil [30] along with the RDA and ESADDI values are compared to the Kashmir diet in Table V. Kashmir diet shows relatively high intake levels for Al, Ca, Mg, and Sc, while Ba, Cl, Na, Sb, and Sn levels are lower than other countries. USDIETS show high Rb, Sn, and Zn intake, low Cr intake and comparable intake of Ca, Co, Fe, Hg, Mg, Mo, and Sr with Kashmir diet. German representative diet has least intake values for Ca, and Mo and highest values for Na and Sb intakes. Daily intake of Sb is 4–5 folds higher than the Kashmir, USA, and Brazilian diets Taiwanese diet has lowest Al, Cs, Fe, K, Mg, Sc and Zn contents. K and Zn daily intakes in Taiwanese diet are extremely low and fall below the RDA values. Indian vegetarian diet shows highest daily intake of Cl, Cu, Fe, Mn, and Mo, where Cl, Fe, and Mn are almost two times

TABLE V Comparison of estimated intake values of trace elements of Kashmir diet with different countries (expressed in μg/person/day unless otherwise specified)

Elements	*Kashmir*	*USDIETS*	*Germany*	*Taiwan*	*India*	*Brazil*	*RDA/ESADDI*
Al*	24	11.8	–	3.6	–	–	60^b
As	66	47	83	–	–	139–159	120
Br*	2.7	4.68	2.5	3.53	–	–	1
Ca*	819	860	380	420	–	–	800
Cl*	1739	3950	6000	2070	7000	–	1700–5100
Co	12.5	14	17	11	17	–	3^a
Cr	142	< 39	62	41	250	397–398	50–200
Cs	13	9	13	8.3	–	–	–
Cu*	2.4	1.3	2.7	–	4	1.2–1.3	1.5–3.0
Fe*	14.2	14.35	13.9	10.4	28	–	10^m 18^f
Hg	2.9	3	12	–	–	11.0–14.4	42
K*	3440	2800	2400	1140	3500	–	1875–5625
Mg*	328	275	–	170	–	–	0.35^m 0.30^f
Mn*	4.6	2.49	2.7	2.13	8.0	–	2.5–5.0
Mo	131	125	70	–	200	–	75–250
Na*	1625	2820	4500	2130	3500	–	1100–3300
Rb*	1.4	2.44	1.9	1.43	–	–	–
Sb	2	4	23	–	–	5.7–6.7	–
Sc	4.8	0.4	1	0.35	–	–	–
Se	72	110	59	100	45	132.1–146.0	50–200
Sn*	1.74	3.2	–	–	–	–	–
Sr*	1.47	1.62	–	–	–	–	–
Zn*	13.4	16.0	11.8	6.71	12	–	15

*Values in mg/person/day; [a]RDA in terms of vitamin B^{12}; [b]PTDI (Provisional Tolerance Daily Intake) Ref. [13]; [m]Male; [f]Female.

higher than the other countries and are also above the RDA values. Highest As, Cr, and Se intake levels are present in the Brazilian diet, where As, and Cr contents are above the RDA/ESADDI levels.

CONCLUSION

Kashmir integrated human diet was analyzed for 34 major, minor and trace elements using INAA. The daily dietary intake values are well within the safe and adequate RDA and ESADDI limits. Kashmir diet represents higher intakes of Al, Ca, Mg, and Sc as compared to the reported dietary intake patterns of some countries, where as Ba, Cl, Na, Sb, and Sn levels are less than these countries. This data can be utilized by the national food and agricultural organization of the country to establish legislation for food and nutrition planning. Such studies can also provide a baseline for the region to aid in monitoring the degree of future contamination and help to identify the sources of pollution. This study is being extended to investigate trace elements in single food items for their dietary intakes so that proper protocols can be suggested for the modification and improvement of the dietary habits of the inhabitants of Kashmir region.

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