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Zn, Cu, Mn and Fe Content in Commercial Organic and Non-Organic Vegetables and Meat Foodstuffs of Sikkim and Darjeeling of India

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Abstract: -

Sikkim and Darjeeling Himalayan Regions are famous for organic farming. Vegetables and meats food stuffs are an important source of metals like Cu, Zn, Mn and Fe which have very important biological roles as they participate in many proteins structure and functions. The concentration of these elements in vegetables and meat food samples of Gangtok and Darjeeling were studied using Atomic Absorption Spectrophotometer (AAS). Among the meat samples, concentration of Zn in the Mutton of both Gangtok (61.59 mg/kg) and Darjeeling (56.53 mg/kg) were above maximum permissible limit (MPL) of 50 mg/kg. Beef (58.63 mg/kg) from Darjeeling was also found to be above MPL. Cu in Chicken samples both from Gangtok and Darjeeling regions exceeded the MPL of 1mg/kg. Though there is no MPL set for Mn and Fe for meat samples, it can be referred from the present study that both the leafy vegetables and meat foodstuffs can be good source of Mn and Fe respectively. The results of this study supply valuable information about the metals contents in commercial organic vegetables and conventionally grown vegetables as well as meat foodstuffs commonly available in Gangtok and Darjeeling area. This is the first database of the chemical elements of the organic vegetables and conventionally grown vegetables as well as meat foodstuffs commonly available in Gangtok and Darjeeling Himalayan regions of India.

Keywords: Nutrition, human health, trace minerals, fertilizers, North Eastern India,

I. INTRODUCTION:

Metals have an important and significant role in maintaining proper health as they participate in important biological metabolisms that contributes to growth and healthy life. Governments health organization institute worldwide recommend daily consumption of meat and vegetables because they are major source of crucial elements which is one of the factors that govern one's physiology. The roles of metal ions in health and disease ranges from the requirement for intake of essential trace elements to toxicity associated with metal overload. The heavy metals enter the human body mainly by two pathways namely: inhalation and ingestion. The intake of heavy metals through ingestion pathway depends on the food habits. One of the major sources of metals is the diet we consumed. Some trace elements such as iron (Fe), zinc (Zn), manganese (Mn), chromium (Cr), cobalt (Co) and selenium(S) are essential since they are either inadequately synthesized or not synthesized in the body [1]. Therefore, it is important to ensure that the consumed diet contains the essential metals in adequate quantities. The first row of transition metals in the periodic table namely Fe, Mn, Ni, Zn, Cu, V, Co and Cr, together with Mo, Sn, Se and I are known to be important for health [2]. Each has its specific role in the metabolism and it cannot be wholly or partly replaced by any other element. Inadequate nutrition of these metal compromises with the immune functions, causes impaired physical and mental growth in children, affects reproductive performance, and reduces work productivity [3, 4]. Experimental data suggest that Cu, Mn and Zn are extremely important ions for enzymes of antioxidant system including superoxide dismutase (SOD) and so their deficiency as well as excess exposure results in increasing the oxidative stress [5]. Trace elements appear to enhance the biological effects of aflatoxin B1 (AFBl), a product of *Aspergillus flavus* and *A. parasiticus* is a mycotoxin in food, which is a hepatotoxin (toxic to the liver), a mutagen, an immunosuppressant and carcinogen [6]. There are also some reports of relation between malignancy and trace elements. Stocks et al., in 1964 found larger values for the ratio of Zn:Cu in the soil and malignancy in Wales (UK) [7]. Laker et al. in 1981 also noticed an uneven distribution in the prevalence of oesophageal cancer in rural parts of Transkei where the people eat mainly local produce [8].

Sikkim is a remote state in the North Eastern region of India and lies in the Eastern Himalaya biodiversity hotspots. The State has become the first organic state with adoption of 100% organic farming by preserving its rich natural resources with abundant flora and fauna, vibrant ecosystem and soil fertility with high organic matter content. It has become the major exporter for fruits, flowers, spices and vegetables in recent years. On the other hand, Darjeeling is a district in the State of West Bengal located in the Lesser Himalayas. The living populations of this region are consuming conventionally grown vegetables foodstuffs imported from neighboring states like Bihar and Uttar Pradesh. The aim of this study, therefore, was to provide a comparative database for the bioavailability of the metal levels in the commercial organic and conventionally grown vegetable as well as meat foodstuffs in Sikkim and Darjeeling.

II. MATERIALS AND METHODS

2.1. Study and Sample collection Area: Organic vegetables and meat foodstuffs were collected from the commercial organic markets of Gangtok, Sikkim while meat foodstuffs as well as non-organic vegetables were collected from the local markets of Kurseong, Darjeeling District. All the vegetables and meat foodstuffs were collected from two different commercial shops in two different seasons of the year during the month of October and January of 2019 respectively.

Fig. 1 Map of Sikkim Himalayan Region showing study sites i.e. Gangtok and Darjeeling

2.2. Selection and Collection of vegetables foodstuffs

For the present investigation, the commercial and conventionally grown vegetable foodstuffs were selected on the basis of frequency of consumption by the native of both Gangtok and Darjeeling areas and availability in the markets after primary field works and survey. After selection, twenty (20) vegetables food samples were purchased or collected from the commercial settings such as Markets or grocery stores of Gangtok and Kurseong town of Darjeeling, West Bengal. The sample collection was carried out in two phases to cover season-based food items as some vegetable's foodstuffs are seasonal. The first collections of vegetables food stuffs were carried out in the month of January while the second collection was conducted during October of the same year.

2.3. Preparation of vegetables and meat foodstuffs for analysis

Both commercial organic and conventionally grown vegetables were cut into small pieces and oven dried at a temperature <70^{o}C. The dried samples were then crushed into powder form. An acid mixture of nitric acid and perchloric acid in the proportion 9:4 was prepared. Then 0.5g of powdered vegetable samples was taken in a conical flask/beaker of 250 ml capacity. In this, adequate quantity (10 ml) of acid mixture was added. The meat food stuffs were also cut in to small pieces for easy and faster digestion. After that the conical flasks with the sample and acid mixture were kept in a hot plate for heating in an open and well-ventilated space. The red fumes of nitrous oxide were allowed to escape till white fumes started appearing. The white fumes were allowed to escape for a minute and heating was stopped (extra acid mixture was added whenever required). After this the extract was left to be cooled. In this, 20 ml of distilled water to the colourless extract was added and filtered through the filter paper. The colourless extract was taken in a 50 ml volumetric flask and the volume was made up full with distilled water. The sample was ready for analysis.

2.4. Analysis of Vegetables and meat foodstuffs by Atomic Absorption Spectrophotometer: Zn, Cu, Fe and Mn concentrations commercial organic and conventionally grown vegetables foodstuffs as well as meat food stuffs were estimated on Atomic Absorption Spectrophotometer (200 Series AA from Perkin Elmer) using hollow cathode lamps 213.9nm, 324.8nm, 248.3nm and 279.5nm for Zn, Cu, Fe and Mn respectively. Standard of Zn, Cu, Fe and Mn were prepared by dilution in triple distilled deionized water (TDW).

III. RESULTS AND DISCUSSION

The concentration of metals in vegetable as well as meat sample was in the order of Fe > Zn > Cu > Mn. The highest concentration of Cu was recorded in *Cucurbita pepo* L. (Pumpkin) of Darjeeling (30.97 ± 2.72 mg/kg) while the lowest concentration of Cu was recorded in *Raphanus sativus (L.) Domin* (Raddish) of Darjeeling (0.60 ± 0.006 mg/kg). In vegetables, most significant difference in vegetables of two regions was found in *Brassica juncea (L.)* Czern (Mustard Green, Raai) (p=0.010), the concentration of which were 1.60 ± 0.001 and 1.53 ± 0.002 mg/kg for Gangtok and Darjeeling respectively. The most significant difference in meat samples from two regions for Cu was found in Chicken (p=0.141), the concentration of which were 3.12 ± 0.02 mg/kg and 1.00 ± 0.004 mg/kg for Gangtok and Darjeeling respectively. Cu is an important metal which acts as a co-factor for many enzymes such as formyl glycine generating enzymes [9]. According to the World Health Organization (WHO), 1–3 milligrams per day of Cu are required to prevent any symptoms of deficiency. However, its overexposure leads to conditions such as diarrhea, nausea, jaundice and severe colic. Different organizations have set different maximum permissible limit (MPL) for heavy metals in vegetable and meat. The content of Cu in all the vegetable and meat samples were within the MPL that is 73 mg/kg for vegetables [10] and 20 mg/kg for meat (MPFO) [11]. Furthermore, it is important to mention that all the chicken samples exceed the MPL for Cu content in chicken samples set by FAO/WHO [12] and Codex Alimentarius Commission (1 mg/kg) [13]. The concentration of Cu in vegetables in our finding is higher than Nigeria [14] and lower in Saudi Arabia

[15]. However, the Cu content in meat samples in our study is in agreement with the study done in Bangladesh [16].

Table 1. Mean concentration of Cu, Zn, Mn and Fe (mg/kg) in organic vegetables and non-organic vegetable foodstuffs of Gangtok and Darjeeling. Values are ± SEM, n=10 each

Vegetable Items	Scientific Name	Area	Cu	Zn	Mn	Fe
Raddish	*Raphanus sativus (L.) Domin*	Gangtok	0.73 ± 0.001	16.10 ± 2.242	19.75 ± 3.16	ND
		Darjeeling	0.60 ± 0.006	19.62 ± 2.511	19.68 ± 3.21	32.25 ± 6.220
Common Beans	*Phaseolus vulgaris L.*	Gangtok	1.25 ± 0.001	14.48 ± 2.20	57.52 ±3 .12	11.62 ± 2.744
		Darjeeling	1.18 ±0.003	22.77 ± 2.68	10.73 ± 3.02	40.75 ± 4.58
Water Cress	*Nasturtium officinale*	Gangtok	1.93 ±0.001	29.10 ± 2 .59	41.93 ± 3.16	204.77± 4.58
		Darjeeling	1.00 ±0.011	35.25 ± 2.40	67.38 ± 3.25	75.18 ± 4.25
Carrot	*Daucus carota*	Gangtok	2.72 ±0.014	09.28 ± 2.42	8.37 ± 3.09	12.42 ± 2.12
		Darjeeling	0.95 ±0.000	11.68 ± 1.25	6.17 ± 3.06	19.57 ± 1.21
Potato	Solanum tubersum L	Gangtok	0.75 ±0.000	07.85 ± 2.016	3.13 ± 0.03	12.57 ± 2.26
		Darjeeling	0.90 ±0.001	12.88 ± 2.032	ND	49.68 ± 3.33
Chayote Root	*Sechium edule (Jacq.) Sw.*	Gangtok	0.65 ±0.000	06.75 ± 2.32	8.38 ± 1.42	0.63 ± 0.001
		Darjeeling	0.90 ±0.002	10.07 ± 2.47	ND	7.45 ± 1.20
Chayote	*Sechium edule (Jacq.) Sw.*	Gangtok	0.85 ±0.001	09.22 ± 2 .37	3.85 ± 1.02	21.35 ± 2.67
		Darjeeling	2.05 ±0.013	06.60 ± 2.53	ND	16.55 ± 1.81
Fenu Greek	*Trigonella foenum-graecum L.*	Gangtok	1.45 ±0.001	17.70 ± 2 .47	75.07 ± 3.23	168.07 ± 5.29
		Darjeeling	3.57 ±0.033	33.03 ± 3.16	22.85 ± 2.92	362.8 ± 2.12
Cauliflower	*Brassica oleracea var. botrytis L.*	Gangtok	0.95 ±0.004	14.47 ± 3.48	22.82 ± 2.14	4.01 ± 0.12
		Darjeeling	0.58 ±0.001	15.03 ± 2.34	8.80 ± 2.148	15.82 ± 2.39
Cabbage	*Brassica oleracea var.capitata L.*	Gangtok	0.70 ±0.002	10.73 ± 2 .54	44.08 ± 2.22	5.70 ± 1.21
		Darjeeling	0.85 ±0.001	16.53 ± 3.24	29.40 ± 2.38	52.42 ± 2.14
Bitter Gourd	*Momordica charantia L.*	Gangtok	1.10 ±0.001	14.40 ± 3.14	75.80 ± 2.45	5.00 ± 0.52
		Darjeeling	1.55 ±0.006	16.83 ± 3.20	15.57 ± 2.41	17.23 ± 2 .18
Bottle Gourd	*Lagenaria siceraria (Monila) Standl*	Gangtok	1.87 ±0.001	10.75 ± 3.06	13.95 ± 2.47	4.23 ± 0.110
		Darjeeling	1.62 ±0.005	14.57 ± 2.57	9.00 ± 2.69	28.63 ± 2.059
Cornona	*Solanum sessiliflorum L.*	Gangtok	18.30 ±0.190	8.38 ± 2.18	12.58 ±3.40	6.00 ± 1.20
		Darjeeling	1.25 ±0.002	11.57 ± 2.24	2.57 ± 0.02	5.00 ± 0.54
Brinjal	*Solanum melongena L.*	Gangtok	1.10 ± 0.001	09.98 ± 2.14	27.85 ± .25106	2.00 ± 0.001
		Darjeeling	5.55 ±0.050	11.30 ± 2.34	3.57 ± 1. 001	0.42 ± .001
Tomato	*Solanum lycopersicum L.*	Gangtok	10.40 ±0.162	07.95 ± 2.56	25.57 ± 4.40	ND
		Darjeeling	1.35 ±0.001	10.77 ± 2.28	7.98 ± 4 .488	25.40 ± 1.13
Tree Tomato	*Solanum betaceum Cav.*	Gangtok	1.17 ±0.001	08.83 ± 2.18	0.50 ± 0008	ND
		Darjeeling	1.55 ±0.006	08.65 ± 2.56	01.83 ± 0.78	0.95 ± 0.014
Mustard Green (Duku)	*Brassica juncea (L.) Czern*	Gangtok	1.28 ±0.003	21.12 ± 3.13	32.13 ± 3.171	3.27 ± 0.047
		Darjeeling	4.38 ±0.036	18.77 ± 3.58	23.60 ± 3.25	18.23 ± 1.20
Spinach	*Pinacia oleracea L.*	Gangtok	2.00 ±0.001	34.63 ± 3.57	156.53± 3.99	5.20 ± 4.70
		Darjeeling	3.37 ±0.025	41.70 ± 3.26	89.30 ± 3.358	249.97 ± 3.32
Mustard Green (Raai)	*Brassica juncea (L.) Czern*	Gangtok	1.60 ±0.001	14.37 ± 3.54	54.70 ± 5 .18	260.47 ± 5.47
		Darjeeling	1.53 ±0.002	23.78 ± 3.54	44.63 ± 5.314	96.38 ±4 .23
Pumpkin	*Cucurbita pepo L*	Gangtok	2.42 ±0.009	10.60 ± 3.27	0.18 ± 0. 0002	19.68 ± 2.21
		Darjeeling	30.97 ±0.007	13.60 ±3 .66	1.15 ± 0.001	5.93 ± 0.27

Table 2: Mean concentration of Cu, Zn, Mn and Fe (mg/kg) in meat foodstuffs of Gangtok and Darjeeling. Values are ± SEM, n=10 each

Meat Items	Scientific Name	Area	Cu	Zn	Mn	Fe
Pork	*Sus scrofa domestica*	Gangtok	4.00 ± 0.45	26.6 ± 1.621	1.40 ± 0.005	87.93 ± 2.05
		Darjeeling	13.81 ± 2.18	45.8 ± 1.46	0.10 ± 0.001	66.48 ± 2.09
Mutton	*Capra aegagrus hircus*	Gangtok	12.74 ± 2.15	61.5 ± 2.02	2.00 ± 0.007	111.17 ± 4.13
		Darjeeling	0.69 ± 0.001	56.5 ± 2.00	0.60 ± 0.010	91.32 ± 2.15
Chicken	*Gallus Gallus domesticus*	Gangtok	3.12 ± 0.02	18.3 ± 1.02	0.80 ± 0.007	63.71 ± 2.14
		Darjeeling	1.00 ± 0.004	26.2 ± 2.211	3.00 ± 0.006	62.02 ± 2.160
Beef	*Bos taurus*	Gangtok	1.17 ± 0.002	43.71 ± 2.108	1.50 ± 0.008	127.8 ± 0.204
		Darjeeling	1.27 ± 0.007	58.3 ± 2.058	ND	33.33 ± 5.52

Table 3 Correlation matrix for Mutton of Gangtok

	Mutton of Gangtok			
	Cu	Zn	Mn	Fe
Cu	1			
Zn	0.809	1		
Mn	0.080	0.038	1	
Fe	0.398	0.095	.715	1

Table 4: Correlation matrix for Mutton of Darjeeling

	Mutton of Darjeeling			
	Cu	Zn	Mn	Fe
Cu	1			
Zn	0	1		
Mn	0	-.120	1	
Fe	0	0.229	-.556	1

Table 5: Correlation matrix for Chicken of Gangtok

	Chicken of Gangtok			
	Cu	Zn	Mn	Fe
Cu	1			
Zn	0.862	1		
Mn	0.599	0.780	1	
Fe	0.952	0.754	0.365	1

Table 6: Correlation matrix for Chicken of Darjeeling

	Chicken of Darjeeling			
	Cu	Zn	Mn	Fe
Cu	1			
Zn	-.486	1		
Mn	-.432	0.852	1	
Fe	-.854	0.856	0.756	1

Zn is an important trace metal involved in numerous aspects of cellular metabolism and required for the catalytic activity of more than 200 enzymes [17]. Zn is also most important element required for the metabolism of several biochemical reactions and is found in every plant and animal tissue and plays a crucial role in maintaining healthy skin by controlling the enzymes that operate and renew the cells in our body [18]. The Zn containing metalloenzymes participates in the metabolism, growth and repair of the tissue and cell membrane stabilization and improves the immune response, especially T-cell mediated responses [19]. However, its long-term exposure can result in Cu deficiency, respiratory disorders as well as cancer. The results obtained from our study shows that the Zn concentration both in vegetables and meat foodstuffs of two regions ranges from 6.60 ± 0.0035 mg/kg to 61.59 ± 0.026 mg/kg. The concentration of Zn in all the vegetables were below the MPL[10] while *Capra aegagrus hircus (*Mutton) of both Gangtok (61.59 ± 2.26 mg/kg) and Darjeeling (56.53 ± 2.45 mg/kg) and *Bos taurus* (Beef) of Darjeeling (58.63 ± 2.58) exceeded the MPL (50 mg/kg) (MPFO). The concentrations of Zn in meat samples are comparably lower than the reported values from the state of Manipur, India [20].

Although, Mn is an essential nutrient involved in many chemical processes in the body, including processing of cholesterol, carbohydrates, and protein, exposures to high manganese levels can be toxic. Mn is also important in regulation of immune responses of the body by breakdown of amino acids, production of energy by regulating the metabolism of vitamin B_1, C, E and by activation of various enzymes important for proper digestion and utilization of foods [21]. Mn is also a component of the metalloenzyme manganese superoxide dismutase (also called the guardian of the power house) in the mitochondria, and is a constituent of the mitochondrial antioxidant defense system which protect from the free radical generated from the injured cells which is harmful to the skin [22] . The concentration of Mn in vegetables and meat samples were generally low compared to other metals studied. In this study the concentration of Mn ranged from 00.02 to156.53 mg/kg and 0.03 to 3.0 mg/kg in vegetable and meat respectively. The highest concentration of Mn was reported in Spinach of Gangtok (156.53 mg/kg) and Chicken of Darjeeling (3 mg/kg). Mn concentrations in all the vegetables were within the MPL [10]. Although there is no MPL for Mn in meat foodstuffs, Mn toxicity can result in a permanent neurological disorder with symptoms that include tremors, difficulty walking, and facial muscle spasms.

Fe other than having crucial role in oxygen transport also participates in metabolism as a component of proteins and enzymes. Fe deficiency is rarely found in non-vegetarians as their diet consists of meat through which they consume proteins like haemoglobin and myoglobin directly [23]. In contrast, vegetarians are more susceptible to the deficiency of Fe as they consume non-heme iron which needs to be altered before it can be absorbed by the body. Fe concentration was highest in *Trigonella foenum-graecum L.* (Fenu Greek) of Darjeeling (362.88 mg/kg) and Beef (127.86mg/kg) of Gangtok. Fe content in all the vegetables foodstuffs were found to be below the MPL [10] while no MPL for Fe in meat foodstuffs is reported in the literature. However, the recommended dietary allowances of Fe is 0-15 mg day^{-1} as per FAO/WHO. It is important to note that leafy vegetables and meat foodstuffs can be a good source of Fe and its deficiency may lead to anemia, myocardial infarction and increase susceptibility to gastro-intestinal infections [23].

CONCLUSION

The results obtained are favorably comparable with the findings of other researchers from other parts of the world and permissible limits stipulated by various agencies and organizations. The variation in the concentrations of Cu, Zn, Mn and Fe content in the vegetables may be mainly attributed to the differences in mineral composition of the soil in which the plants are cultivated and preferential absorbability of the plants, used of fertilizers, irrigation water and climatic conditions. Although, Sikkim is the first organic state of India, the metal contents of both Gangtok and Darjeeling were comparable to each other. The results of this study supply valuable information about the metals contents in commercial organic vegetables and conventionally grown vegetables as well as meat foodstuffs commonly available in Gangtok and Darjeeling. This is the first database of the chemical elements of the organic vegetables and conventionally grown vegetables as well as meat foodstuffs commonly available in Gangtok and Darjeeling. Moreover, these results can also be used to test the chemical quality of foodstuffs in order to evaluate the possible risk associated with their consumption.

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Conflict of Interest: The authors declare no conflict of interest.

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