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Comparative Study of Heavy Metals Concentration in Watermelons (Citrullus lanatus) of different Districts of Balochistan

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Abstract

The current study was conducted to determine the concentration of heavy metals (Mn, Fe, Co, Ni, Cu, Cd, & Pb) in watermelon (Citrullus lanatus) collected from Hazarganji and Sabzi Street fruit market Quetta, Balochistan and to compare it with recommended limits set by WHO/FAO. Peels and fruity juicy portion of collected eleven watermelons samples were separated and was analysed by use of Flame Atomic Absorption Spectrophotometer (FAAS). Comparative study of fruits and peels samples shown that, concentration of Co was significantly high and concentration of Mn, Cu was slightly high in peels samples as compare to fruit samples, while concentration of Fe, Ni, Cd, Pb was slightly high in fruit samples in comparison of peel samples. Metals Mn, Co, Ni, Cd, Pb was within MPL and concentration of Fe, Cu was in very low concentration, while no metal was exceeded from permissible limit, which indicate that watermelons analysed samples was safe from any toxic metal however watermelon samples were not rich in essential metals specially iron. Findings of study shown that samples of watermelons obtained from Hazarganji fruit market Quetta, which were imported from various districts of Baluchistan had their metals limits less than MPL and consequently are free from any risk for consumers.

Keywords: Heavy Metals, Watermelon, Atomic Absorption Spectrometer, Balochistan.

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INTRODUCTION:

Metals are substances which are good conductors of heat and electricity and can be changed into different forms and wires. Heavy metals are the type of metals which possess high toxicity and density (>5g/cm^3). Suitable quantity of metals is required for both plants and animals for their survival. Intake of high quantity of heavy metals can lead to severe diseases or abnormalities [1]. The main task for researchers and nutritionists is to analyse toxic metals in various consumable products including fruits, vegetables, medicinal plants, meat and water. For healthy diet it is necessary to monitor concentration of toxic metals in fruits and vegetables. Minerals are needed in a suitable quantity for metabolism and growth of body and are crucial in production of vitamins, minerals and fibres[2]. Naturally metals are found in earth crust while contamination of food occurs due to human activities such as; use of chemical sprays, contaminated water, mining, fertilizers and industrial wastes [3]. Khan and his colleagues [4]reported the contamination of watermelons and vegetables due to heavy metals.

Ecologists are worried about the presence of minerals in the surroundings and their biological influence on human body. Food is the main cause of toxic metals which directly affect the human health. Several studies have been carried out for the purpose of the consumption of suitable levels of toxic metals through diet and their need for human body [5]. Some heavy metals like Cd, Pb and Hg are main toxins for humans through food consumption while others metals like Fe, Zn and Cu are crucial for biological processes in human organs [6]. The residues of toxic substances can cause serious nervous disorders, genetic problems, kidney failure, cardiac problem and different kinds of tumours [7].

Watermelons were initially cultivated in Africa, Sanskrit and prime Egyptian (over 4000 years ago) [8]. In Japan the seedless watermelons are cultivated by the crossing of tetraploid and diploids and such watermelons are still at low risk in United States [9]. Watermelon *(Citrullus lanatus)* is found in the tropical areas of Africa especially in Kalahari Desert which belongs to the family of *Cucurbitaceous*; is very famous due to its economical point of view and sweet taste. Watermelon of Kalahari Desert grows on the surface of the ground and has no specific stem. Its popularity was spread primarily in past and nowadays it is cultured across the globe. Melons belong to Cucurbitaceous family and are used for development of vine labelled as Catullus Lantus [8].There are two main types of watermelons on the basis of their colour (yellow and red); however red colour watermelons are more familiar [9]. More than 1200 species of watermelons are reported across the world on the basis of their shape, size, colour and taste. Naming of specific species of watermelon is done on the bases of their cultivated area, shape, size and colour [10].

Watermelon is important fruits for nutritional and clinical value, especially in the hot weather because it contains high levels of water and sugar which can help to reduce the risk of dehydration in the body [10].It has been reported that watermelon has ability to reduce the formation of kidney stones and bone loss due to aging. Watermelon contains amino acids and beta carotene which reduces the risk of heart disorders. Naturally watermelons contain large amount of coloring substance lycopene which protects from the infection of urinary and mouth tumors. The cotyledons of watermelons contain suitable quantities of metals like Na, Mg, P, K, Ca, Mn, Fe, Cu & Zn that is necessary for body development and metabolic process of living organisms [8].

Keeping in view beneficial effects of essential metals and hazardous effects of non-essential metals on human health the study was designed to monitor the levels of various seven metals (Mn, Fe, Co, Ni, Cu, Cd, & Pb) in eleven watermelon samples collected from Hazarganji and Sabzi Street fruit market Quetta, Balochistan.

MATERIAL AND METHODS

Chemicals and Reagents

All the standards and reagents were of analytical grade. The mixture of concentrated Nitric Acid (HNO_3), Sulphuric Acid (H_2SO_4) and Per Chloric Acid ($HClO_4$) was used for digestion purpose. The stock solution of each metal namely Manganese, Iron, Cobalt, Nickel, Copper, Cadmium, and Lead was provided by Merck (Darmstadt, Germany). The standards reagents were prepared from the 1000ppm stock solution (Merck) provided in 0.5 M solution of Nitric acid (HNO_3). A sequence of working standards solution (for calibration of FAAS) was prepared from stock solution and minimum three standards were used for particular metal analysis. Distilled water was utilized for dilution of samples and standards.

Sampling

Eleven samples of *Citrullus lanatus* (watermelon) were collected from Hazarganji and Sabzi Street fruit market Quetta, as these markets import watermelon from different districts of Baluchistan (Quetta, Noshki, Chaghi, Turbat, Qilla Abdullah, Pishin, Gawader, Khuzdar, Duki, Harnai and Mustang). Peels and fruity juicy portion of collected watermelons samples were used separately for analysis of heavy metals. Watermelon samples area, shape and colour are described in Table1.

Table 1: Watermelon samples area, shape and colour.

S.NO	Sample Codes	Districts	Shape and colors
1	Sample A	Nushki	Elongated white
2	Sample B	Chagai	Oval shiny rind
3	Sample C	Duki	Elongated white
4	Sample D	Turbat	Oval shiny rind
5	Sample E	Gwadar	Elongated white
6	Sample F	Khuzdar	Elongated watery
7	Sample G	Harnai	Elongated white
8	Sample H	Mastung	Oval shiny rind
9	Sample I	Quetta	Elongated white
10	Sample J	Qila Abdullah	Elongated white
11	Sample K	Pishin	Oval shiny peel

Washing and Drying

In research laboratory of Chemistry Department University of Balochistan the collected samples were washed with 5% Hydrochloric Acid (HCl) and then several times washed with deionized H_2O to remove contamination. Prior to analysis process samples were well sterilized at room temperature. Fresh samples of fruits were cut into small equal slices with clean cutter for separation of peels from fruits. The peels were chopped in to small parts and the portion of fruity samples was placed into the acidic condition. After washing the samples were dried at 150°C in furnace for 5 hrs till they develop stiff and crispy.

Grinding

The dried watermelon samples of pulp and peel were grinded with the help of mortar and piston for obtaining fine powder. Dried powdered samples were stored in polyethylene bags and each sample was labelled with specific code as shown in Table 1, for further processing.

Digestion

1g of each sample was digested by use of 10ml mixture of concentrated acids such Nitric Acid (HNO_3), Sulphuric Acid (H_2SO_4) and Per Chloric Acid ($HClO_4$) in ratio of 5:1:1 in 100mL Pyrex beaker on electric hotplate with constant stirring at 80 °C for 1 hr in the fuming cupboard until clear solution was obtained. Then the solution was cooled down and filtered with the help of Whitman filter paper No 42 into 100ml beaker and was diluted with deionized water to make it up to 50ml mark. Then the digested samples were stored for further analysis.

Analysis of Metals

The concentration of heavy metals was analysed by using Flame Atomic Absorption Spectrophotometer (FAAS), Thermos - Electron Corporation, S4 AA System, S. No, GE711544, China. Double beam and deuterium background standard hollow cathode lamps of Mn, Fe, Co, Ni, Cu, Cd, and Pb, used at specific wavelengths for analysis of these metals. For accurate results each sample of watermelons were analysed three times.

RESULTS AND DISCUSSION

In the present study concentration of seven various metals (Mn, Fe, Co, Ni, Cu, Cd, and Pb) were analyzed by use of AASP in fruits and peels of eleven watermelon samples (Nushki, Chagai, Duki, Turbat, Gwadar, Khuzdar, Harnai, Mastung, Quetta, Qila Abdullah, Pishin) collected from Hazarganji and Sabzi Street fruit market Quetta, Balochistan (Table 3 and Table 4 respectively). Consequently, the obtained data of concentration of heavy metals in watermelon samples was compared with MPL determined by World Health Organization (WHO)/ Food and Agriculture Organization (FAO), Codex General Standards for Contamination &Toxin in Foods (1996), Agency for Toxic Substance Disease Registry (ATSDR) (1994) and European Union (2006) (Table 5).

In fruit samples concentration of Fe>Mn>Pb>Ni>Cu>Cd>Co was 0.1641-0.7300>0.2746-0.6124>0.0215-0.1394>0.0053-0.1348>0.0189-0.0626>0.0220-0.0561>0.0069-0.0269 mg/kg and in peel samples concentration of Mn>Fe>Pb>Ni>Cu>Cd>Co was 0.4523-0.7818>0.2420-0.6768>0.0593-0.1212>0.0082-0.1148>0.0418-0.0706>0.0249-0.0512>0.0061-0.04394 mg/kg respectively. Fruit samples F, G H, J was rich in Pb, Cd, Ni, Fe and peel samples B, K was rich in Co and Mn, Cu respectively. Comparative study of fruits and peels samples shown that, concentration of Co was significantly high and concentration of Mn, Cu was slightly high in peels samples as compare to fruit samples, while concentration of Fe, Ni, Cd, Pb was slightly high in fruit samples in comparison of peel samples. Metals Mn, Co, Ni, Cd, Pb was within MPL and concentration of Fe, Cu was in very low concentration, while no metal was exceeded from permissible limit, which indicate that watermelons analysed samples was safe from any toxic metal however watermelon samples were not rich in essential metals specially iron.

Table 2: Standard operating conditions of Flame Atomic Absorption Spectrophotometer for analysis of heavy metals

Metals	*Wavelength(nm)*	*Lamp current(mA)*	*Slits width(mm)*
Mn	279.5	279.5	0.2
Fe	248.3	248.3	0.2
Co	240.7	240.7	0.2
Ni	232.0	232.0	0.2
Cu	324.8	324.8	0.7
Cd	228.8	228.8	0.7
Pb	283.3	283.3	0.7

Table 3: Concentration of Heavy metals (mg/kg) in Fruits samples of watermelons

Metals	**Mn**	**Fe**	**Co**	**Ni**	**Cu**	**Cd**	**Pb**
Permissible limits (mg/kg)*	**500.0**	**425.0**	**0.05-0.100**	**1.500**	**40.00**	**0.300**	**0.200-0.300**
Nushki	0.2746	0.1641	0.0140	0.0173	0.0189	0.0220	0.0262
Chagai	0.6443	0.5926	0.0072	0.0371	0.0382	0.0237	0.0910
Duki	0.3703	0.5835	0.0181	0.0053	0.0443	0.0426	0.0215
Turbat	0.5478	0.6951	0.0091	0.0366	0.0366	0.0460	0.0830
Gwadar	0.4062	0.2137	0.0269	0.0165	0.0444	0.0358	0.0873
Khuzdar	0.4301	0.3355	0.0069	0.0202	0.0471	0.0418	0.1394
Harnai	0.3920	0.1932	0.0171	0.0313	0.0625	0.0561	0.0876
Mastung	0.5429	0.4936	0.0189	0.1348	0.0357	0.0462	0.0728
Quetta	0.3781	0.2555	0.0179	0.0280	0.0353	0.0468	0.0841
Qila Abdullah	0.6124	0.7300	0.0193	0.0461	0.0626	0.0509	0.1097
Pishin	0.6045	0.5834	0.0097	0.0761	0.0434	0.0420	0.1252

FAO/WHO* stands for Food and Agriculture Organization/World Health organization (2007).

Table 4: Concentration of Heavy metals (mg/kg) in the peels of watermelons

Metals	**Mn**	**Fe**	**Co**	**Ni**	**Cu**	**Cd**	**Pb**
Nushki	0.6344	0.6768	0.0230	0.0348	0.0489	0.0249	0.0970
Chagai	0.7818	0.5000	0.0439	0.0379	0.0418	0.0290	0.0745
Duki	0.6945	0.6033	0.0065	0.0461	0.0636	0.0371	0.0912
Turbat	0.4523	0.2420	0.0172	0.1148	0.0488	0.0418	0.0593
Gwadar	0.5043	0.2840	0.0069	0.0310	0.0667	0.0467	0.0829
Khuzdar	0.5158	0.6272	0.0199	0.0280	0.0645	0.0443	0.0701
Harnai	0.4869	0.3346	0.0092	0.0472	0.0569	0.0489	0.0994
Mastung	0.5478	0.3549	0.0061	0.0196	0.0618	0.0508	0.0938
Quetta	0.6417	0.4037	0.0082	0.0318	0.0488	0.0512	0.1173
Qila Abdullah	0.7021	0.6168	0.0154	0.0082	0.0660	0.0488	0.1053
Pishin	0.4916	0.2926	0.0070	0.0132	0.0706	0.0476	0.1212

Manganese (Mn):

Manganese is natural occurring crucial elements found as a fifth most rich metal in the environments and all together the twelfth richest elements present in the Earth crust [11].It plays significant role in human body as anti-oxidant agent in biochemical process of carbohydrates, protein and lipids metabolism. Recommended dietary allowance (RDA) of Mn is 2.5 mg/day and it is stored in liver, skin and kidneys of the animals [12]. Approximately 12-20 mg of Mn is present in human body. Deficiency of Mn causes weakness of bones, weight loss, osteoarthritis and joints pain. Natural sources of Mn are fruits, vegetables, and whole grains [13].

The highest concentration of Mn was observed in peels Sample B (0.7818 mg/kg) and lowest in fruits Sample A (0.2746 mg/kg). Concentration range of Mn in fruits samples and peels samples was 0.2746 to 0.6124 mg/kg and 0.4523 to 0.7818 mg/kg respectively. However, no significant difference was observed in Mn content of fruits and peels samples of watermelons. It was concluded that concentration of Mn was within MPL (500 mg/kg) as recommended by WHO/FAO in all samples of watermelons.

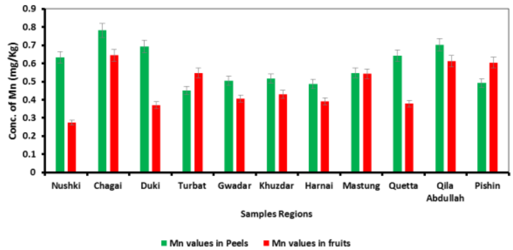

Figure 1: Comparison of conc. of Mn in fruits & peels Samples of watermelons

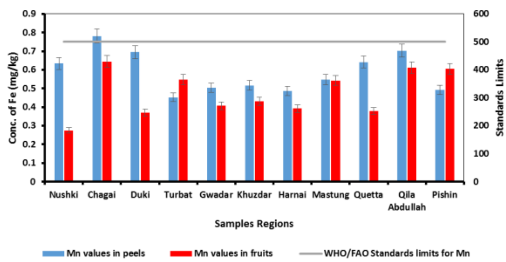

Figure 2: Comparison of Conc. of Mn in fruits & peels Samples of watermelons with WHO/FAO values (2001)

Iron (Fe):

Iron is an essential component for every single living life form and located in the first row of the d-block elements in the periodic table. It is the most fourth abundant metal in the earth crust. During the physiological process it is mostly exists in two freely interchangeable redox ferrous form and oxidized ferric form [14]. It is essential for maintaining the cell Homeostasis. It is the main component of hemoglobin in red blood cells (RBC) that carries Oxygen to the whole-body cells [15]. It is required for man and woman of all age groups in various amounts. Post-menopausal stage requirement of iron is 8 mg/day, while pre-menopausal stage requirement is 18mg/day for both man and women. The woman in pregnancy period needs larger amount of iron daily (127mg/day) and six years old child requires 11mg of iron per day [16]. High dosage of iron reduces the absorption of zinc and causes liver cancer and heart diseases; while deficiency of iron causes numerous disorders such as shortness of breathing, anima and spoon shaped of nails [17].

The highest concentration of Fe was observed in fruits Sample J (0.7300 mg/kg) and lowest in fruits Sample A (0.1641 mg/kg). Concentration range of Fe in fruits samples and peels samples was 0.1641 to 0.7300 mg/kg and 0.2420 to 0.6768 mg/kg respectively. However, no significant difference was observed in iron content of fruits and peels samples of watermelons. It was concluded that concentration of iron in the watermelons samples was much lower than MPL (425mg/kg) set by WHO&FAO (2001).

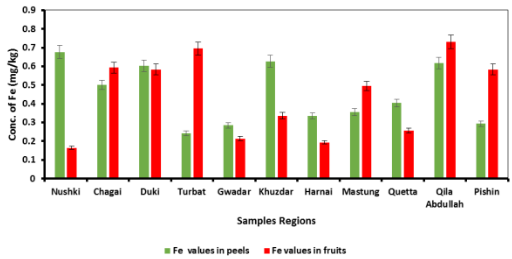

Figure 3: Comparison of Conc. of Fe in fruits & peel samples of watermelons

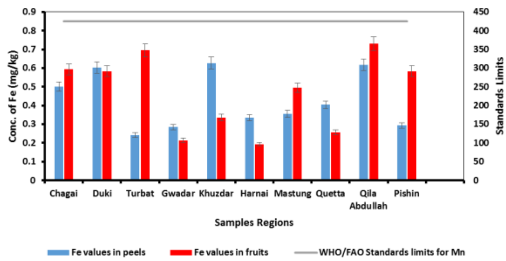

Figure 4: Comparison of Conc. of Fe in fruits & peel samples of watermelons with WHO/FAO Values (2001&1994)

Cobalt (Co):

Cobalt is an essential trace metal found in the earth crust in a chemically in combined state. It is the important constituent of vitamin B12 and helps in Production of RBC. It is also involved in reducing the risk of pernicious anemia and in proper functioning of Central Nervous System (CNS) [15]. More than 30 mg/day intake of Co can cause toxic effect on digestion, heart muscles, congestive heart failure, skin disorders; while its deficiency results in anemia[18]. Cobalt act like a manganese and zinc Cobalt so it can exchange Zinc in some biological processes[12].

Both highest and lowest concentration of Co was observed in peels Sample B (0.04394 mg/kg) and Sample H (0.0061 mg/kg) respectively. Concentration range of Co in fruits samples and peels samples was 0.0069 to 0.0269 mg/kg and 0.0061 to 0.04394 mg/kg respectively. However, no significant difference was observed in Co content of fruits and peels samples of watermelons. It was concluded that concentration of Co in the watermelons samples was found under the safe limits (0.05 to 0.1 mg/kg) of ATSDR (1994).

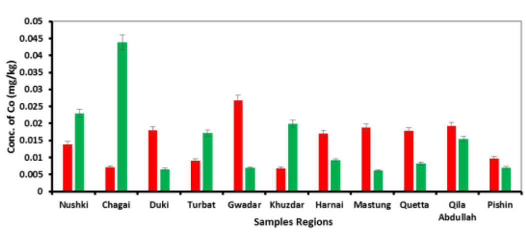

Figure 5: Comparison of Conc. of Co in fruits & peel samples of watermelons

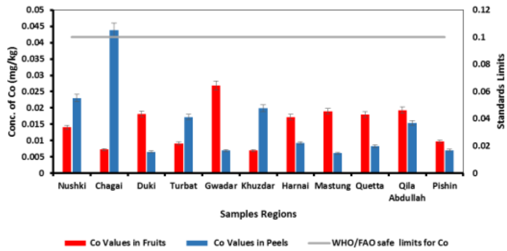

Figure 6: Comparison of Conc. of Co in fruits & peel samples of watermelons with WHO/FAO and ATSDR Values (1994)

Nickel (Ni):

Nickel is an important element that occurs naturally in higher amount in plants as compare to animals. It plays significant role in body functions including physiological processing, body's immune system, activates the enzymatic system and serves as a co-factor in Fe absorption from small intestine [19]. Excessive consumption of Ni causes weight loos, enlarged heart and reduced liver weight [20]. Ni causes skin allergies by physical interaction with the body because Ni ions are absorbed into the skin through the sweet capillaries [21]. Both highest and lowest concentration of Ni was observed in fruits Sample H (0.1348 mg/kg) and Sample C (0.0053 mg/kg) respectively. Concentration range of Ni in fruits samples and peels samples was 0.0053 to 0.1348 mg/kg and 0.0082 to 0.1148 mg/kg respectively. However, no significant difference was observed in Ni content of fruits and peels samples of watermelons. It was concluded that concentration of Ni in the watermelons samples was noticeably below the safe limits (1.5 mg/kg) proposed by WHO/FAO.

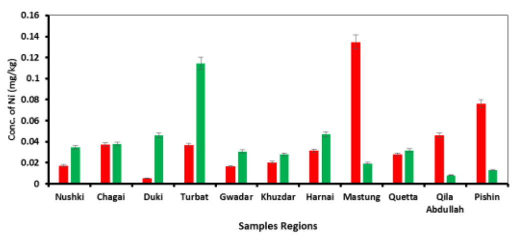

Figure 7: Comparison of Conc. of Ni in fruits & peel samples of watermelons

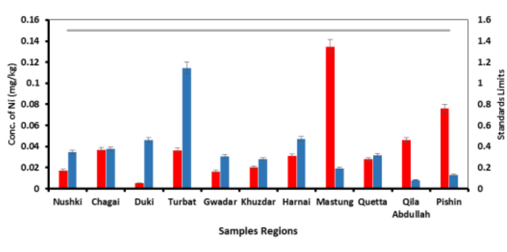

Figure 8: Comparison of Conc. of Ni in fruits & peel samples of watermelons with WHO/FAO Values (2007)

Copper (Cu):

Copper is a vital micronutrient which acts as a biocatalyst required for production of cellular energy in the living creatures [22]. About 9 gram of Cu is present in human body. It is present in the enzyme involved in the oxidation reaction and metabolism of estrogen which is crucial for maintaining women's fertility during pregnancy [23]. Higher intake of Cu decreases the level of hemoglobin in blood and increases the risk of cancer & death; while its deficiency affects the functions of thyroids glands, causes CNS disorders and abnormalities of hair [24]. Greater intake of Cu Cause Nausea, vomiting, headache, dizziness, abnormal pain, softness, and Metallic taste of mouth[25].

The highest concentration of Cu was observed in peels Sample K (0.0706 mg/kg) and lowest in fruits Sample A (0.0189 mg/kg). Concentration range of Cu in fruits samples and peels samples was between 0.0189 to 0.0626 mg/kg and 0.0418 to 0.0706 mg/kg respectively. However, concentration of Cu in peels samples of watermelons was higher in comparison of fruits samples. It was concluded that concentration of Cu in the watermelons samples was lower than MPL (40 mg/kg) as determined by WHO/FAO (2001).

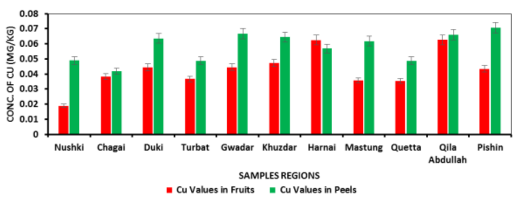

Figure 9: Comparison of Conc. of Cu in fruits & peel samples of watermelons

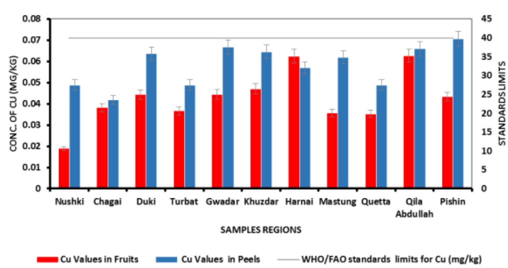

Figure 10: Comparison of Conc. of Cu in fruits & peel samples of watermelons with WHO/FAO Values (2001)

Cadmium (Cd):

Cadmium is naturally existing in combined state with metals copper, lead and zinc [26]. It is non-essential toxic element found in food, fresh water, phosphate fertilizers and petroleum products. Its impurities found in some products like petroleum's products, phosphate fertilizers, and washing detergents. The unclean Food is the main causes of Cd exposure [27]. Industrial wastes and acid rain increase the concentration of Cd in soil. Consumption of Cd contaminated fruits and vegetables cause diarrhea, stomach pain, vomiting, bone fracture and CNS failure [28].

Both highest and lowest concentration of Cd was observed in fruits Sample G (0.0561 mg/kg) and Sample A (0.0220 mg/kg) respectively. Concentration range of Cd in fruits samples and peels samples was 0.0220 to 0.0561 mg/kg and 0.0249 to 0.0512 mg/kg respectively. However, no significant difference was observed in Cd content of fruits and peels samples of watermelons. It was concluded that concentration of Cd in the watermelons samples was within MPL (0.1 to 0.3 mg/kg) recommended by WHO/FAO (2001) and European Union (2006).

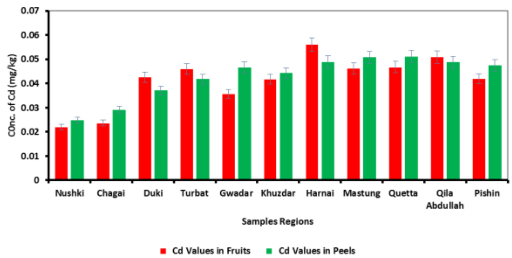

Figure 11: Comparison of Conc. of Cd in fruits & peel samples of watermelons

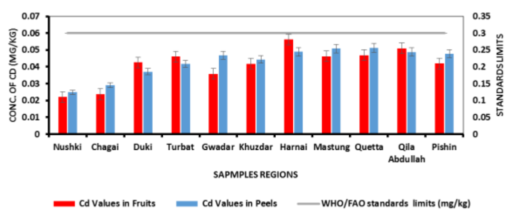

Figure 12: Comparison of Conc. of Cd in fruits & peel samples of watermelons with WHO/FAO Values (2001) & European Union (2006)

Lead (Pb):

Lead is a toxic heavy metal found worldwide and is extremely toxic for plants, animals, and microorganisms. Even minor quantity of lead can cause environmental contamination and health problems [29]. The environment has been contaminated with Pb for the past few eras with more than 50% of productions come from fuel. It has been estimated that 50% of inorganic Lead is absorbed into the lungs through breathing. Adults take in 10 to 15% of lead from foods, Although the children absorb 50% of lead through the gastrointestinal tract [30]. Human interaction to Pb occurs due to contaminated air, water, food and causes toxicity to RBC, kidney, reproductive system, affects CNS mainly intelligence quotient (I.Q) & behavers of children's [3].

Both highest and lowest concentration of Pb was observed in fruits Sample F (0.1394 mg/kg) and Sample C (0.0215 mg/kg) respectively. Concentration range of Pb in fruits samples and peels samples was 0.0215 to 0.1394 mg/kg and 0.0593 to 0.1212 mg/kg respectively. However, concentration of Pb in fruits samples was higher as compare to peels samples of watermelon. It was concluded that concentration of Pb in the watermelons samples was within MPL (0.2 to 0.3 mg/kg) set by WHO/FAO (2001&1994).

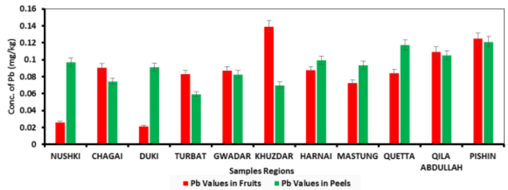

Figure 13: Comparison of Conc. of Pb in fruits & peel samples of watermelons

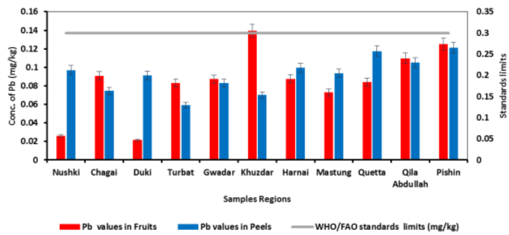

Figure 14: Comparison of Conc. of Pb in fruits & peel samples of watermelons with WHO/FAO Values (2001 & 1994)

Table 5: Permissible limit of Heavy metal in (mg/kg).

Metals	Permissible limits (ppm)	Organization's
Mn	500	WHO/FAO (2001& 1994)
Fe	425	WHO/FAO (2001& 1994)
Co	0.05-1.0	ATSDR (1994)
Ni	1.50	WHO/FAO (2007)
Cu	40.0	WHO/FAO (Codex General Standards for Contamination &Toxin in Foods (1996))
Cd	0.1 0.3	WHO/FAO (2001) & European Union (2006)
Pb	0.2	WHO/FAO (2001 & 1994)

(*Javed, M., & Usmani, N. (2012) & (Nisa, K.U., & Khan, N (2020).*

CONCLUSION:

From the findings of current study, it is concluded that all the analyzed watermelon samples were free from any hazardous metals. Comparative study of fruits and peels samples shown that, concentration of Co was significantly high and concentration of Mn, Cu was slightly high in peels samples as compare to fruit samples, while concentration of Fe, Ni, Cd, Pb was slightly high in fruit samples in comparison of peel samples. Metals Mn, Co, Ni, Cd, Pb was within MPL and concentration of Fe, Cu was in very low concentration, while no metal was exceeded from permissible limit, which indicate that watermelons analysed samples was safe from any toxic metal however watermelon samples were not rich in essential metals specially iron. Here it is concluded that samples of watermelons obtained from Hazarganji fruit market Quetta, which were imported from various districts of Baluchistan had their metals limits less than MPL and consequently are free from any risk for consumers.

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

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