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Analysis of Toxic Heavy Metal Content of the Most Widely Consumed Fruits

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ABSTRACT: *Fruits are a natural source of antioxidants which inhibit the production and accumulation of high reactive oxygen species (ROS), whereas heavy metals may initiate the production of ROS. Therefore, the most commonly used fruits such as apple, apricot, banana, cherry, grapes, guava, lemon, mango, orange, peach and pomegranate were analysed for the most toxic heavy metals (Cr, Co, Ni, Cd and Pb). Acid digestion method was used for metal extraction. The metal analysis was carried out using Flame Atomic Absorption Spectrophotometer (FAAS) (Perkin Elmer AAS-700). The results showed that the observed concentrations of all the five metals are much lower than that of the World Health Organization (WHO)'s maximum permissible limits. Although the obtained values are lower than the WHO standards, the effect of prolonged exposure to sub-limital levels of toxic metals are not known, therefore close monitoring of heavy metals in fruit and other food commodities remains important.*

Keywords: Cadmium, chromium, cobalt, fruits, lead, nickel, toxic metals

1. INTRODUCTION

The nutrients in fruits are vital for health and body maintenance.[1] Fruits are a good source of minerals and vitamins.[2] Fruits are the main source of many nutrients such as fibres, potassium, phosphorus, copper, calcium, sulphur, magnesium, thiamin, niacin, riboflavin, folic acid, pantothenic acid, vitamin B_6, vitamin C, minerals and flavonoids.[3–8] As such, citrus fruits are very rich in vitamin C.[9] Some of the animals such as fish and reptiles can produce vitamin C in their liver; however, unfortunately human beings does not have the ability to synthesise vitamin C. Therefore, an adequate amount of vitamin C is required to be taken from the food.[10] In addition, vitamin C has been considered as one of the best antioxidant. Antioxidants are the chemical compounds which inhibit oxidation.[7] Antioxidant also prevents the generation of high reactive oxygen species (ROS). The high ROS may destroy biomolecules and deoxyribonucleic acid (DNA).[11] Furthermore, the regular use of fruits in the diet reduces risk of cancer and cardiovascular and chronic diseases.[12] The regular use of fruits containing potassium reduces the risk of kidney stones, stroke and heart diseases and cancer.[13]

However, the use of industrial wastewater for the growth of fruit plants, and increased application of pesticides and fertilisers may elevate the heavy metal content of fruits.[14–17] As industrial wastewater and pesticides are the main sources of toxic heavy metals. Heavy metals may cause many diseases such as corrosion of mucosal layer, cancer, coagulopathy, hematemesis, hepatic injury, hematochezia, cardiotoxicity, nephrotoxicity, hepatotoxicity, immunotoxicity, shortness of breath, melena, cardiovascular collapse, kidney and bone diseases.[18–21] Alzheimer's and Parkinson's diseases are also linked with heavy metals.[22] In addition, heavy metals may initiate the production of high ROS which are the main cause of damaging biomolecules and DNA.[23]

Some of the heavy metals are essential for normal body growth and development at low concentration which is known as micronutrients. Therefore, presence of chromium (Cr) in blood reduces the demand for insulin. Cr also cooperates with insulin in protein synthesis while Cr deficiency disturbs glucose tolerance.[24] Similarly, cobalt (Co) is essential for haemoglobin synthesis and functioning of vitamins, hormones and enzymes.[25] Nickel (Ni) is an important trace element for bacteria, plants and eukaryotes. Ni is also a necessary component in the active site of several essential metallo-enzymes.[26] Among heavy metals, cadmium (Cd) and lead (Pb) are non-essential elements with well-known toxic effects on humans.[27]

Fruit water (juice) is a good source of antioxidants and inhibits the production of high ROS, while heavy metals initiate the production of such species. It could be concluded that high content of heavy metals may reduce the antioxidant property of fruits. Therefore, this study was designed to analyse the most widely used fruits in Khyber Pakhtunkhwa, Pakistan (apple, apricot, banana, cherry, grapes, guava, lemon, mango, orange, peach and pomegranate) for most toxic heavy metals such as Cr, Co, Ni, Cd and Pb.

2. EXPERIMENTAL

2.1 Collection and Digestion of Fruit Samples

The samples were collected from 28 fruit markets in Khyber Pakhtunkhwa, Pakistan. The identified fruits were selected for Cr, Co, Ni, Cd and Pb analysis. Each fruit item was purchased from 28 points in the capacity of 1 kg.

The samples were washed with distilled water and sliced into thin pieces with the help of iron knife and dried in oven at 100°C till constant weight. The sample was crushed gently with the help of mortar and pestle. The acid digestion method was used with slight modification. An amount of 2 g of dry sample was taken in an Eelenmeyer flask, and a mixture of 50 ml HNO_3 and $HClO_4$ (4:1) was added. The mixture was kept on electric hot plate at 250°C. The acid mixture was evaporated and the remaining pasty mass was dissolved in distilled water, filtered into volumetric flask (50 ml) and diluted with distilled water up to final volume of 50 ml. The solution was analysed for heavy metals using Atomic Absorption Spectrophotometer (Perkin Elmer AAS-700).

2.2 Analysis of Samples

The samples were analysed for toxic heavy metals (Cr, Co, Ni, Cd and Pb) using Flame Atomic Absorption Spectrophotometer (FAAS). The samples were analysed in triplicate while each solution was run in duplicate, mean values and standard deviation (SD) were calculated. The instrument conditions are listed in Table 1. The analysis of heavy metals was carried out in Centralized Resource Laboratory (CRL), University of Peshawar, Pakistan.

Table 1: Instrument analytical conditions for analysis.

Element	Fuel flow ($l\ min^{-1}$)	Air flow ($l\ min^{-1}$)	Wavelength (nm)	Slit width (nm)	LC* (mA)	Detection limit ($mg\ l^{-1}$)
Cr	2.0	17.0	357.9	0.7	25	0.0030
Ni	2.0	17.0	232.0	0.2	25	0.0060
Co	2.0	17.0	240.7	0.2	30	0.0090
Cd	2.0	17.0	228.8	0.7	4.0	0.0008
Pb	2.0	17.0	283.3	0.7	10	0.0150

**Lamp current*

3. RESULTS AND DISCUSSION

The study was designed to determine the concentration of toxic heavy metals in the most widely used fruits in Khyber Pakhtunkhwa, Pakistan. The domestic fruit samples were collected from the local markets. The average concentration of Cr, Co, Ni, Cd and Pb in fruit samples was compared with the maximum permissible limits as established by the World Health Organization (WHO). The WHO permissible limit of Cr, Co, Ni, Cd and Pb are 2.3 mg kg^{-1}, 50.0 mg kg^{-1}, 70.0 mg kg^{-1}, 0.1 mg $kg^{-1}$and 0.3 mg kg^{-1}, respectively.[11] The mean concentrations and SD of each metal along with the WHO's permissible limits have been summarised in Table 2. The observed concentration has been taken in mg kg^{-1} of the dry weight of fruits. It can be seen from the results that the observed mean concentrations of all the five heavy metals are found much lower than the permissible limits as established by the WHO. Khyber Pakhtunkhwa is a province of Pakistan, located between 34.9526°N and 72.3311°E, consisting of mountainous areas with almost negligible industrialisation. Furthermore, this province produces a large number of fruits which are distributed to the local markets. Second, the use of pesticides on fruit plants was negligible till 2011. In addition, due to the low number of industries and the limited use of pesticides, plants are not expected to accumulate high amounts of heavy metals. It can be summarised from the results that industrialisation and frequent application of pesticides may increase metal accumulation in plant tissues.

Table 2: Mean concentrations (mg kg^{-1}) of toxic heavy metals in fruits on dry weight bases.

Fruit	Cr		Co		Ni		Cd		Pb	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Apple	0.0417	0.0042	0.0215	0.0076	0.0106	0.0001	0.0065	0.0003	0.0052	0.0007
Apricot	0.0069	0.0004	0.0100	0.0006	0.0086	0.0009	0.0026	0.0015	0.0073	0.0008
Banana	0.0508	0.0169	0.0833	0.0009	0.1092	0.0047	0.0088	0.0006	0.0941	0.0062
Cherry	0.0281	0.0053	0.0094	0.0072	0.0170	0.0002	0.0920	0.0083	0.0934	0.0049
Grapes	0.0805	0.0018	0.0096	0.0005	0.0711	0.0040	0.0658	0.0003	0.1072	0.0095
Guava	0.1108	0.0642	0.0208	0.0004	0.0109	0.0083	0.0333	0.008	0.0117	0.0061
Lemon	0.1063	0.0049	0.0071	0.0001	0.0729	0.0036	0.0783	0.005	0.0692	0.0047
Mango	0.0805	0.0038	0.0484	0.0005	0.0609	0.0065	0.0099	0.0002	0.0905	0.0048
Orange	0.0069	0.0005	0.0073	0.0009	0.1608	0.0043	0.0106	0.0003	0.0392	0.0071
Peach	0.0641	0.0072	0.0180	0.0044	0.0399	0.0063	0.0507	0.0022	0.0738	0.0083
Pomegranate	0.0750	0.0041	0.0095	0.0007	0.0538	0.0072	0.0083	0.0006	0.0830	0.0005
WHO's limit	2.3		50		70		0.1		0.3	

The observed concentrations of Cr are shown in Figure 1. The results show the mean concentrations of Cr, 0.0417 ± 0.0042 mg kg^{-1}, 0.0069 ± 0.0004 mg kg^{-1}, 0.0508 ± 0.0169 mg kg^{-1}, 0.0281 ± 0.0053 mg kg^{-1}, 0.0805 ± 0.0018 mg kg^{-1}, 0.1108 ± 0.0642 mg kg^{-1}, 0.1063 ± 0.0049 mg kg^{-1}, 0.0805 ± 0.0038 mg kg^{-1}, 0.0069 ± 0.0005 mg kg^{-1}, 0.0641 ± 0.0072 mg kg^{-1} and 0.0750 ± 0.0041 mg kg^{-1}, respectively for apple, apricot, banana, cherry, grapes, guava, lemon, mango, orange, peach and pomegranate whereas, the WHO maximum permissible for food crops or fruits are 2.3 mg kg^{-1}. The highest concentration of Cd is observed in guava (0.1108 mg kg^{-1}) while the lowest concentration is noted in apricot and orange (0.0069 mg kg^{-1}). As such, the results show that the observed concentration of Cr has found within the permissible limits of the WHO. Therefore, the local population is safe and no such urgent measurements are needed.

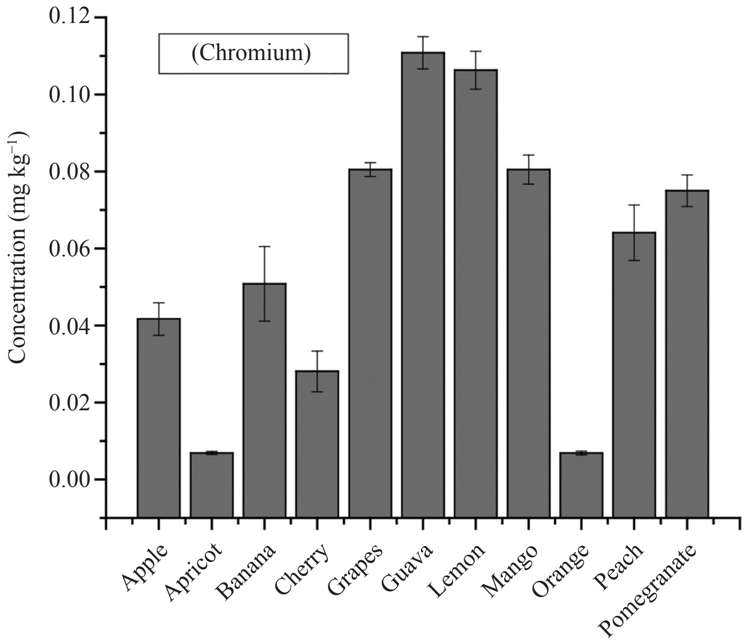

Figure 1: Mean concentration (mg kg^{-1}) of Cr in fruits on dry weight bases.

The average concentrations of Co in the selected fruits are shown in Figure 2. The observed mean concentrations in apple, apricot, banana, cherry, grapes, guava, lemon, mango, orange, peach and pomegranate are found to be 0.0215 ± 0.0076 mg kg^{-1}, 0.0100 ± 0.0006 mg kg^{-1}, 0.0833 ± 0.0009 mg kg^{-1}, 0.0094 ± 0.0072 mg kg^{-1}, 0.0096 ± 0.0005 mg kg^{-1}, 0.0208 ± 0.0004 mg kg^{-1}, 0.0071 ± 0.0001 mg kg^{-1}, 0.0484 ± 0.0005 mg kg^{-1}, 0.0073 ± 0.0009 mg kg^{-1}, 0.0180 ± 0.0044 mg kg^{-1} and 0.0095 ± 0.0007 mg kg^{-1} on dry weight bases, respectively.

The maximum concentration of Co is observed in banana (0.0833 mg kg^{-1}) while the minimum concentration is found in orange (0.0073 mg kg^{-1}). In addition, the observed concentrations have been found much lower than that of the WHO's maximum permissible limits (50 mg kg^{-1}). There is no risk of Co over exposure from the above fruits.

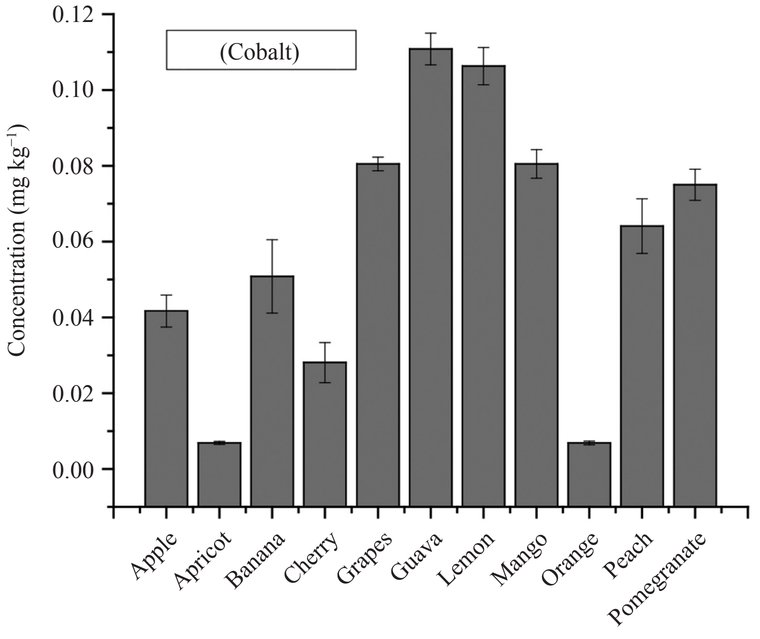

Figure 2: Mean concentration (mg kg^{-1}) of Co in fruits on dry weight bases.

The results of the observed concentrations of Ni are shown in Figure 3. The mean concentration of 0.0106 ± 0.0001 mg kg^{-1}, 0.0086 ± 0.0009 mg kg^{-1}, 0.1092 ± 0.0047 mg kg^{-1}, 0.0170 ± 0.0002 mg kg^{-1}, 0.0711 ± 0.0040 mg kg^{-1}, 0.0109 ± 0.0083 mg kg^{-1}, 0.0729 ± 0.0036 mg kg^{-1}, 0.0609 ± 0.0065 mg kg^{-1}, 0.1608 ± 0.0043 mg kg^{-1}, 0.0399 ± 0.0063 mg $kg^{-1}$and 0.0538 ± 0.0072 mg kg^{-1} is observed in apple, apricot, banana, cherry, grapes, guava, lemon, mango, orange, peach and pomegranate, respectively. The maximum concentration of Ni was found in orange (0.1608 mg kg^{-1}) while the minimum concentration was observed in apricot (0.0086 mg kg^{-1}). Similarly, the Ni concentration of all the selected fruits was found lower than that of the WHO's maximum permissible limit (70 mg kg^{-1}).

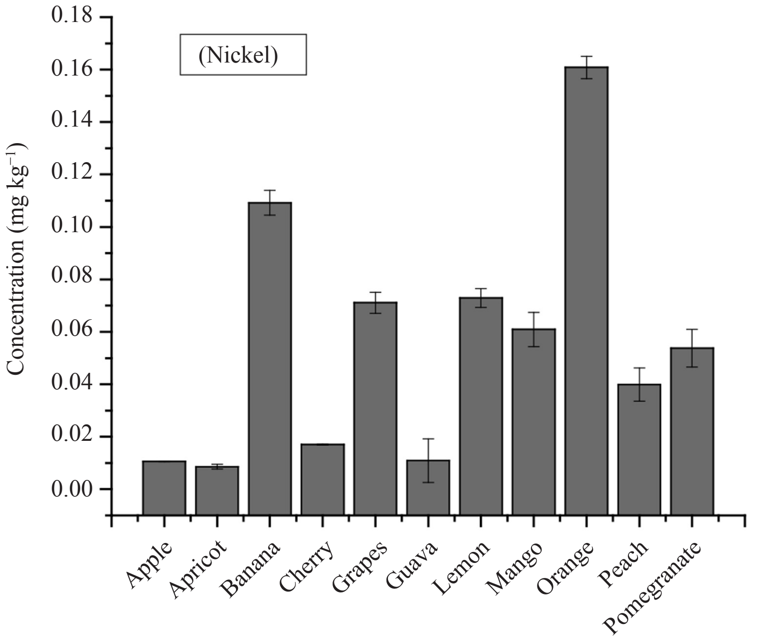

Figure 3: Mean concentration (mg kg^{-1}) of Ni in fruits on dry weight bases.

The mean concentrations of Cd and Pb are shown in Figures 4 and 5, respectively. Cd concentration of 0.0065 ± 0.0003 mg kg^{-1}, 0.0026 ± 0.0015 mg kg^{-1}, 0.0088 ± 0.0006 mg kg^{-1}, 0.0920 ± 0.0083 mg kg^{-1}, 0.0658 ± 0.0003 mg kg^{-1}, 0.0333 ± 0.008 mg kg^{-1}, 0.0783 ± 0.005 mg kg^{-1}, 0.0099 ± 0.0002 mg kg^{-1}, 0.0106 ± 0.0003 mg kg^{-1}, 0.0507 ± 0.0022 mg kg^{-1} and 0.0083 ± 0.0006 mg kg^{-1} while lead concentration of 0.0052 ± 0.0007 mg kg^{-1}, 0.0073 ± 0.0008 mg kg^{-1}, 0.0941 ± 0.0062 mg kg^{-1}, 0.0934 ± 0.0049 mg kg^{-1}, 0.1072 ± 0.0095 mg kg^{-1}, 0.0117 ± 0.0061 mg kg^{-1}, 0.0692 ± 0.0047 mg kg^{-1}, 0.0905 ± 0.0048 mg kg^{-1}, 0.0392 ± 0.0071 mg kg^{-1}, 0.0738 ± 0.0083 mg kg^{-1} and 0.0830 ± 0.0005 mg kg^{-1} of dry weight, respectively in apple, apricot, banana, cherry, grapes, guava, lemon, mango, orange, peach and pomegranate. Similarly, the concentration of both Cd and Pb was found lower than that of the WHO's suggested limits. The WHO's suggested limits for Cd and Pb are 0.1 mg kg^{-1} and 0.3 mg kg^{-1}, respectively. The lower concentration of Cr, Co, Ni, Cd and Pb in apple, apricot, banana, cherry, grapes, guava, lemon, mango, orange, peach and pomegranate might be due to limited industries in the area. In addition, almost negligible application of pesticides on food crops could result in lower accumulation of heavy metal in plants and animal tissues. Furthermore, the concentration of toxic heavy metals should be monitored in drinking water and food crops in order to ensure safety of the public health.

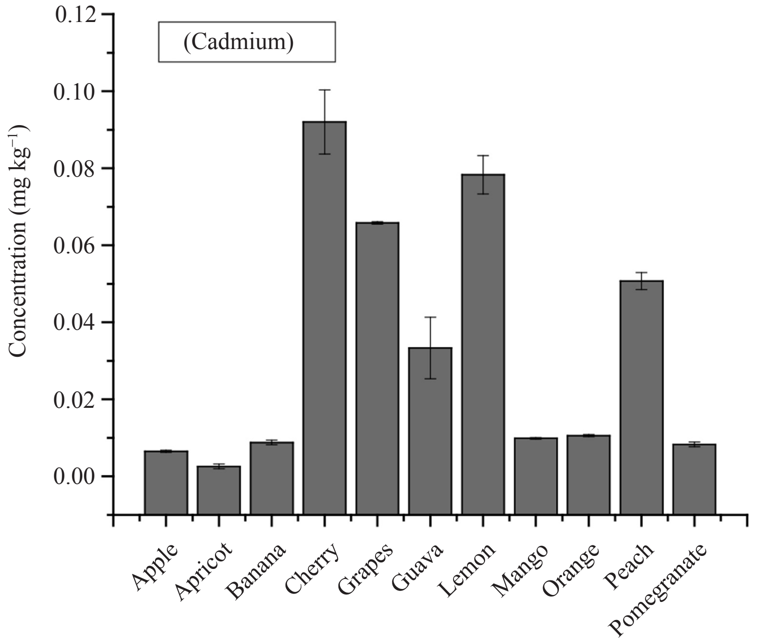

Figure 4: Mean concentration (mg kg^{-1}) of Cd in fruits on dry weight bases.

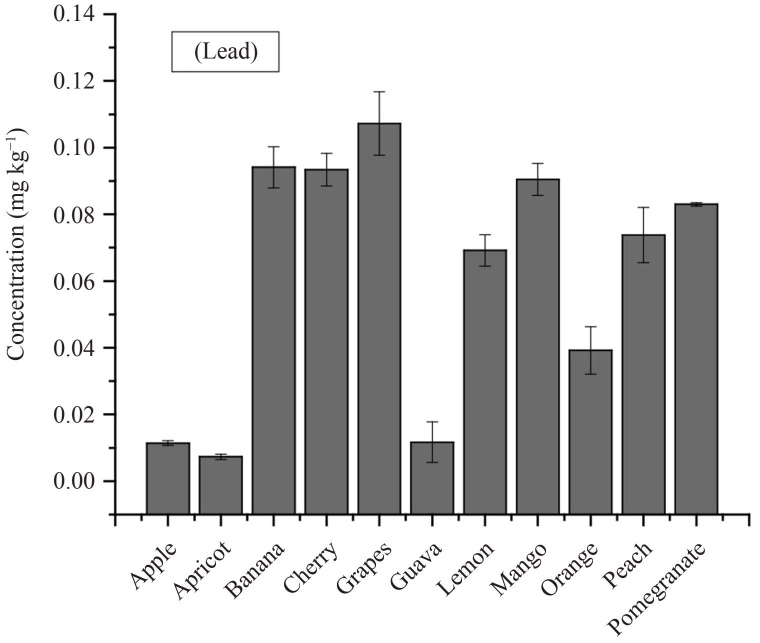

Figure 5: Mean concentration (mg kg^{-1}) of Pb in fruits on dry weight bases.

Heavy metals are the most dangerous environmental pollutants. Their toxicity is of great concern for environmental, ecological and nutritional point of view. Heavy metals are non-biodegradable and accumulate in living tissues. Therefore, analysis of various food items, crops and animal have been carried throughout the world. Ellen et al. analysed several fruits and higher concentration of Cd and Pb was observed in cherry (0.006 mg kg^{-1}) and lemon (0.039 mg kg^{-1}), respectively which are much lower than the WHO's limits.[28] Ellen et al. also observed lower concentrations of the metals because they analysed fresh fruit samples, and their observed concentrations (mg g^{-1}) are based on fresh weights. Meehye et al. studied Cd and Pb in apple, grapes and peach; the concentrations were found to be 0.004 mg kg^{-1}, 0.010 mg kg^{-1} and 0.009 mg kg^{-1} for Cd while 0.015 mg kg^{-1}, 0.025 mg kg^{-1} and 0.024 mg kg^{-1} for Pb in apple, grapes and peach, respectively.[29] Duran et al. analysed apple and apricot for several heavy metal in the Turkish fruits, as shown in Table 3.[30] His observed values indicate higher amount of Cd and Pb in apple, and Cr, Cd and Pb in apricot than suggested by WHO, whereas industrialisation was considered as the source of contamination. As such, Harmankaya et al.[12] analysed some fruits in Turkey, however he observed much lower concentration of Co, Ni and Cd in apricot as compared to study by Duran et al.[30]

Studies from other countries such as Russia, Libya, Romania, Germany, China, India, Bangladesh, Pakistan and Egypt are summarised in Table 3. The literature study indicates that our results are lower than most of the literature values.

Table 3: Literature comparison of the heavy metals in fruits from different countries.

Country	Fruit	Cr	Co	Ni	Cd	Pb	Unit	Reference
Netherlands	Apple	–	–	–	0.002	0.020	mg kg^{-1} (dry weight)	24
	Apricot	–	–	–	0.005	0.036		
	Banana	–	–	–	0.003	0.012		
	Cherry	–	–	–	0.006	0.014		
	Lemon	–	–	–	0.002	0.039		
	Orange	–	–	–	0.002	0.029		
Korea	Apple	–	–	–	0.004	0.015	mg kg^{-1} (dry weight)	25
	Grapes	–	–	–	0.010	0.025		
	Peach	–	–	–	0.009	0.024		
Turkey	Apple	0.80	0.20	0.60	0.28	5.50	mg kg^{-1} (dry weight)	26
	Apricot	4.02	1.15	5.78	0.81	12.4		

(*Continued on next page*)

Table 3 (*Continued*)

Country	Fruit	Cr	Co	Ni	Cd	Pb	Unit	Reference
Turkey	Apricot	–	0.0054	0.1338	0.0092	–	mg kg^{-1} (dry weight)	12
	Cherry	–	0.0058	0.0774	0.0075	–		
	Orange	–	0.0096	0.0582	0.0092	–		
	Peach	–	0.0119	0.1746	0.0113	–		
Russia	Apple				0.0093	0.0490	mg kg^{-1} (dry weight)	31
Libya	Apple	–	0.437	1.000	0.060	0.200	mg kg^{-1} (dry weight)	7
	Banana	–	1.168	1.316	0.050	0.100		
	Grapes	–	0.521	0.631	0.050	0.400		
	Mango	–	0.561	5.143	0.362	1.824		
	Orange	–	0.763	1.099	0.030	0.200		
	Peach	–	1.049	1.1190	0.020	0.250		
Romania	Apple	55.60	8.30	204.40	1.42	75.68	ug l^{-1} (juice)	32
	Apricot	55.45	7.00	146.60	0.78	5.36		
	Orange	28.21	2.76	145.00	0.64	10.03		
	Peach	39.64	7.60	53.12	1.38	18.58		
Germany	Apple				1.100	29.30	ug kg^{-1} (dry weight)	33
China	Apple	0.0250		0.0766	0.0021	0.0233	mg kg^{-1} (dry weight)	34
	Grapes	0.0153		0.0375	0.0013	0.0117		
	Peach	0.0322		0.1056	0.0037	0.0277		
India	Apple	–	–	–	20.37	224.4	ug l^{-1} (juice)	35
	Banana	–	–	–	12.24	132.9		
	Grapes	–	–	–	6.09	72.3		
	Orange	–	–	–	6.45	123.5		
	Pomegranate	–	–	–	9.18	200.7		
Bangladesh	Banana	0.010	0.004	0.030	0.030	0.030	mg kg^{-1} (dry weight)	36
	Guava	0.030	0.030	0.060	0.300	0.070		
Pakistan	Apple	2.18	–	12.23	7.62	0.06	mg kg^{-1} (dry weight)	37
	Banana	2.55	–	14.10	0.07	0.35		
	Guava	2.19	–	14.49	0.04	0.05		
Egypt	Apple	BDL	–	0.36	BDL	BDL	mg kg^{-1} (dry weight)	4
	Grapes	1.06	–	1.78	BDL	BDL		
	Orange	BDL	–	0.38	BDL	BDL		

BDL = Below detection level

4. CONCLUSION

Fruits are a rich source of antioxidant which prevents the production of high ROS, which could damage biomolecules and DNA. Whereas, heavy metals may initiate the production of high ROS. As such, heavy metals may decrease the antioxidant properties of fruits. Therefore, this study was conducted to analyse fruits for heavy metals.

The concentration of toxic heavy metals (Cr, Co, Ni, Cd and Pb) was determined in widely used fruits (apple, apricot, banana, cherry, grapes, guava, lemon, mango, orange, peach and pomegranate) in Khyber Pakhtunkhwa province of Pakistan. The local fruit samples were collected from 28 fruit markets. Acid digestion method was used for metal extraction. The metal analysis was carried out using FAAS (Perkin Elmer AAS-700). Interestingly, the results showed that the observed concentrations of all the five metals are much lower than that of the WHO's maximum permissible limits. This might be due to limited industries and less frequent application of pesticides. Although the obtained values are lower than WHO standards, the effect of prolonged exposure to sub-limital levels of toxic metals are not known, therefore close monitoring of heavy metals in fruit and other food commodities remains important.

5. ACKNOWLEDGEMENTS

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6. AUTHORS' CONTRIBUTIONS

All authors made significant contributions to the study. The idea was generated by Muhammad Rahim and Fazal Wahid. The collection of data was performed by Muhammad Rahim, Najm Us Saqib and Nadir Khan. Analysis of the data was performed by Muhammad Rahim. The first draft was completed by Muhammad Rahim, and all authors were involved in providing feedback and critical revision of the manuscript.

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