ISSN (Online): 2091-2609
DOI Prefix: 10.3126/ijasbt
International Journal of Applied Sciences and Biotechnology (IJASBT)

Research Article

Analysis and Detection of Heavy Metals Content in Some Selected Packaged Fruit Juices of Kathmandu City by Flame Atomic Absorption Spectroscopy

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Article Information

Received: 15 July 2024
Revised version received: 5 September 2024
Accepted: 8 September 2024
Published: 30 September 2024

Cite this article as:
N. Paudel et al. (2024) Int. J. Appl. Sci. Biotechnol. Vol 12(3): 158-165. DOI: 10.3126/ijasbt.v12i3.70167

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Abstract

The determination of level of heavy metals in packaged fruit juices is crucial, as extended exposure to even small quantities of these toxic element can cause severe health issues and chronic poisoning. Hence, it is essential to gather comprehensive data on the presence of heavy metals in packaged fruit juice samples. The objective of this research work is to determine heavy metals (Fe, Cu, Zn and Pb) in four different types of packaged fruit juice samples viz Apple, Mango, Orange and Lychee of four different popular brands bought from retail market of Kathmandu city by flame atomic absorption spectroscopy and compare with acceptable limit set by WHO/FAO. Wet acid digestion method was used for digestion process of packaged fruit juice samples. The concentration of Iron were ranged from 0.572-0.762mg/L in Orange juices; 0.362-0.624mg/L in Mango juices; 0.277-3.649mg/L in Apple juices; 0.279-1.770mg/L in Lychee juices respectively. Copper concentration were ranged from not detected (ND)-0.574mg/L in Orange juices; ND for all selected samples in Mango juices; ND- 0.023mg/L in Apple juices; ND-0.041mg/L in Lyclee juices. Similarly Zinc concentration were ranged from 0.0511-0.2026mg/L in Orange juices; ND-0.0700mg/L in Mango juices; ND for all selected samples in Apple juices; ND-0.0287mg/L in Lychee juices. The concentration of hazardous element Lead was found below detection limit in all selected packaged fruit juice samples. The heavy metals concentration in selected brand of packaged fruit juice samples was in following order: Fe>Zn>Cu> Pb.

Keywords: Packaged fruit juice samples; Flame atomic absorption spectroscopy; heavy metals; Wet acid digestion; Hazardous.

Introduction

Easily available Packaged fruit juices are becoming important modern diet among Nepalese communities serving as nutritious beverages and offering flavourful taste (Tasnim *et al*., 2010). Packaged fruit Juice has become an ideal choice of today's busy way of life and its intake generally rises during summer season compared to other season providing all the essential nutrients of original product in convenient and concentrated forms (Farid and Enani, 2010; Anwar et.al., 2014). Fruit juice also provides some energy and glucose to the body due to their natural sugar content. Apart from this fruit juice also help to keep body stay hydrated and satiate thirst (Hassan *et al*., 2014). Fruit juice is rich in vitamins, minerals, antioxidants and phytonutrients that help to prevent different types of diseases and help promoting good health (Abbasi et.al., 2020; Jedah and Robinson, 2002). Packaged fruit juice often has minute amount of essential elements like Iron, Copper, Zinc vital for dietary balance in the human body and its very important to regulate the levels of these elements to ensure they are safe and beneficial (Ikem *et al*., 2002). Despite the necessity of these elements for normal bodily functions excessive level can result to serious health issues, diminished quality of life and potentially death (Senesse *et al*., 2009). Iron is crucial element for the formation of red blood cells and is vital element in the body (Tegegne, 2015). Copper and Zinc are vital for numerous Physiological functions but excessive dietary intake poses several health risks (Ihesinachi and Eresiya, 2014; Jalbani *et al*., 2010). Regarding Lead, even small amount can accumulate and cause serious health problems particularly harmful to nervous system, especially in the case of children causing development delays, attention disorders, learning difficulties (Balali-Mood *et al*., 2018). Considering potential harmful effects, the World Health Organization (WHO) estimated average daily intake of Iron to 17mg for males and 9-12 mg for females. Zinc intake approximated maximally to 20 mg per day for adults. Similarly, Copper intake estimated to be 0.5 - 0.7 mg for infants up to 6 months and 2-3mg for adults (FAO/WHO, 2024).

Verma and Rana, 2014 reported the Assessment of Cadmium, Chromium and Copper levels in Market fruit samples in Meerut, North India. Ofori *et al*., 2013 reported Heavy Metal analysis of Fruit Juice and Soft drinks bought from retail market in Accra, Ghana. Similarly, Kavikarage and Jayasundara, 2020 had determined heavy Metal Content in Several Commercially Available Fruit Juices in Sri Lanka. WHO/FAO established maximum Lead levels for fruit juices i.e, 0.03mg/kg (Maximum level does not apply to juices exclusively from berries and other small fruit), Grape juice 0.04 mg/kg, fruit juices exclusively from berries and other small fruits i.e, 0.05mg/L (FAO/WHO, 2024). However exact reference limit of Iron, Copper and Zinc in fruit juices were not set. Drinking water standards provide reference levels for many heavy metals including Iron, Copper and Zinc. Heavy Metals in beverages including soft drinks and Packaged fruit juices are typically compared to drinking water standards since these non-alcoholic beverages are highly dependent on water quality, which influence their purity (Magomya *et al*., 2015; Okeri *et al*., 2009; Dehelean and Magdas, 2013). The main objective of this study was to analyze Fe, Cu, Zn and Pb levels in some selected packaged fruit juice samples viz Apple, Mango, Orange and Lychee from four different most commonly consumed brands sold in retail market of Kathmandu city and comparing this findings with reference levels set by WHO/FAO for drinking water, i.e, 2mg/L (JECFA 1983), 2mg/L(WHO 2003), 3mg/L(WHO 2003) and 0.01mg/L for Iron, Copper, Zinc and Lead respectively (FAO/WHO, 2024).

Materials and Methods

Sample Collection

Sample collections was Carried out by choosing an area with a high percentage of fruit juice consumers. Sampling was conducted in Kathmandu, a city known for its wide variety of fruit juices, allowing consumers to select based on factors like price, quality, flavor and type. In this study, commercially available packaged fruit juice samples of four different flavors viz Orange, Apple, Mango, Lychee from four different most commonly consumed brands were purchased from the retail market within Kathmandu city.

Sample Preparation

50 mL of each fruit juice samples were taken in the small beaker then 5 mL of aqua regia was added to the sample. The solution was then mixed and placed on the hot plate to get the clear solution. When the solution started to boil then 5 mL of concentrated nitric acid was added to get the clear solution. After that samples were removed from hot plate and allowed to cool. Then the solution was filtered using Whatman No. 42 filter paper. The filtrate obtained was diluted up to 100 mL by adding double distilled water. All the samples were prepared three times to minimize the possibility of error.

Instrumentation

The concentration of heavy metals viz Iron, Copper, Zinc and Lead in the digested samples of packaged fruit juice were analyzed by Flame Atomic Absorption Spectrophotometer (Model AA240FS) with an air-acetylene flame. Prior to analysis, the instrument's fuel pressure and cathode lamp's light source were adjusted to meet the specific needs of each element and adhere to the instrument’s specifications. To ensure accuracy, the instrument was first calibrated using blank solution before being used to determine the metal content in the packaged fruit juice samples. Calibration curves were then constructed for each metal (Fe, Cu, Zn, Pb) under study by analyzing standard solutions prepared from stock solutions of known concentrations. Subsequently, the absorbance of the sample solution was measured, allowing for the calculation of metal concentrations based on their respective calibration curves. This method ensures precise determination of heavy metal levels in the packaged fruit juice samples.

Results and Discussion

Calibration Curves for Determination of Iron, Copper, Zinc and Lead in Packaged Fruit Juices

The calibration curves for the determination of Fe, Cu, Zn and Pb was obtained and shown in fig and the curve was plotted in absorbance as a function of concentration in mg/L.

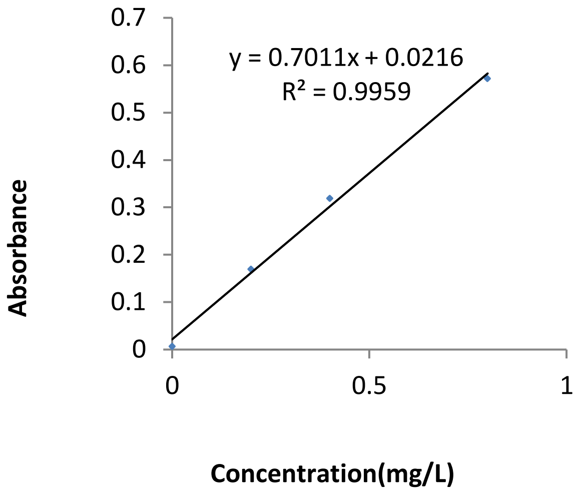

Fig. 1: Calibration curve for determination of Iron.

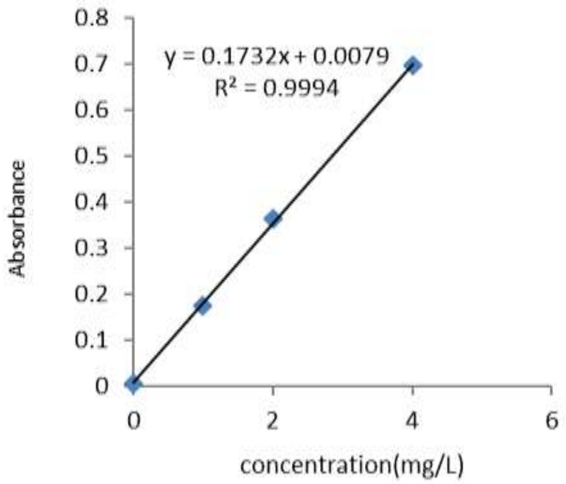

Fig. 2: Calibration curve for determination of Copper.

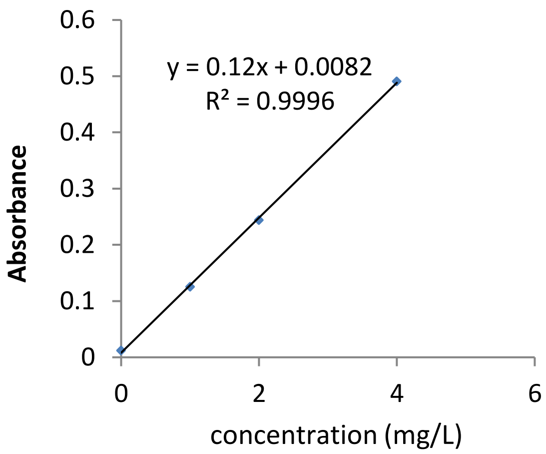

Fig. 3: Calibration curve for determination of Zinc.

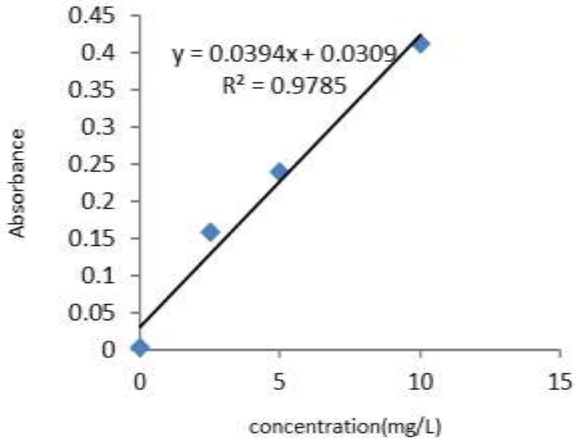

Fig. 4: Calibration curve for determination of Lead.

The adjusted linear equation and correlation coefficient are key components in quantifying the relationship between concentration and absorbance. The R^2 values obtained for the calibration curves indicate strong relationships between absorbance and concentration for all metals. Specifically, Iron exhibited an R^2 value of 0.995, while Copper, Zinc and Lead demonstrated R^2 values of 0.999, 0.999 and 0.978 respectively. These high R^2 values suggest excellent fit of the linear regression models to the reliability and accuracy of the calibration curves for metal analysis (Table 1).

Determination of Iron in Orange, Mango, Apple and Lychee Juice samples

Table 2 shows that the levels of Fe were found to be 0.572, 0.640, 0.655, 0.762mg/L in Orange juices; 0.362, 0.605, 0.624, 0.510mg/L in Mango juices; 0.347, 0.277, 3.649, 0.523 mg/L in Apple juices; 0.442, 0.336, 0.279, 1.770mg/L in Lychee juices for brand A, B, C and D respectively. The higher concentration of Iron detected was 3.649mg/L in Apple juice of brand C and lowest concentration detected was 0.279mg/L in Lychee juice of brand C. Iron is essential element necessary for various bodily function, including the formation of red blood cells and Oxygen transportation throughout the body. It's inadequacy can results in anemia. WHO did not establish a specific health-based guideline value for Iron in drinking water. However, it was noted that a value of around 2mg/L could be inferred from the Provisional Maximum Tolerable Daily intake (PMTDI) set by JECFA (Joint FAO/WHO Expert Committee on Food Additives) in 1983. The maximum concentration of Fe 3.649mg/L in Apple juice sample of brand C exceeds the acceptable limit recommended by FAO/WHO (i.e, 2mg/L) but this concentration falls within estimated average daily Iron intake of 17mg/day for males and 9-12mg/day for females.

Table 1: The adjusted linear equation and correlation coefficient

Metal	equation	R² value
Iron	y=0.701x+0.021	0.995
Copper	y=0.173x+0.007	0.999
Zinc	y=0.12x+0.008	0.999
Lead	y=0.039x+0.030	0.978

Table 2: Actual concentration of Iron in Orange, Mango, Apple and Lychee juices selected from most commonly consumed brands of Kathmandu city

S.N.	Juice type	Concentration of Iron(mg/L)			
		Brand A	**Brand B**	**Brand C**	**Brand D**
1	Orange	0.572	0.640	0.655	0.762
2	Mango	0.362	0.605	0.624	0.510
3	Apple	0.347	0.277	3.649	0.523
4	Lychee	0.442	0.336	0.279	1.770

Determination of Copper in Orange, Mango, Apple and Lychee Juice samples

Bar- diagrm (Fig 5) shows that the levels of Cu were 0.111, 0.574, 0.523 for brand A, C and D but below detection limit for brand B in Orange juices; below detection limit for all selected brands in Mango juice samples; 0.023mg/L for brand C and below detection limit for remaining brands in Apple juices; 0.041mg/L for brand C and below detection limit for other remaining brands in Lychee Juices respectively. The higher concentration of copper detected was 0.574mg/L for brand C in Orange juice and minimum detectable Copper concentration was 0.023mg/L for brand C in Apple juice sample. Copper is essential element crucial for human body, functioning as ligand in numerous protein and enzymes and plays a key role in cellular energy production (Tautua *et al*., 2013; Khair Un N *et al*., 2020). Copper deficiency impacts thyroid function, central nervous system disorders, hair abnormalities but excessive levels of copper cause abdominal pain, diarrhoea, nausea, electrolyte-imbalance, gastro-intestinal disorders, harmful impact on liver (Manne *et al*., 2022); Tautua *et al*., 2013). WHO (2003) established a guideline of 2mg/L for Copper in drinking water. The daily prescribed quantity of copper ranged from 0.5 to 0.7mg for infants up to 6 months to 2-3 mg for adults (WHO/FAO). The maximum concentration of Copper 0.574mg/L was within safest limit set by WHO/FAO (i.e, 2mg/L) respectively.

Determination of Zinc in Orange, Mango, Apple and Lychee Juice samples

Table 3 shows that the zinc levels were 0.0521, 0.1692, 0.2026, 0.0511mg/L for brand A, B, C and D in Orange juices; 0.0481, 0.0663, 0.0700mg/L in brands A, B, C and below detection limit for brand D in Mango juices; below detection limit for all selected brands in Apple juices; 0.0287 in brand D and below detection limit for all other remaining brands in Lychee juice samples respectively. The concentration of Zinc was found maximum 0.2026mg/L in brand C of Orange juice sample and minimum detectable concentration 0.0287mg/L in brand D of Lychee juice sample. Zinc is vital for numerous catalytic activities, DNA synthesis, wound healing, bone formation, brain development however an excess of Zinc is neurotoxin and could cause neuronal death, reduce cellular energy and disrupt mitochondrial membrane potential (Bahiru and Teju, 2019 ; Adlekan and Abegunde, 2011; Wang *et al*., 2021). Zinc intake recommended by WHO was maximally approximated to 20 mg per day for adults. WHO (2003) mentioned that health-based guideline value of Zinc in drinking water is not required. However, Zn levels above 3mg/L may not be accepted. The maximum concentration of Zinc detected 0.2026mg/L was within safest limit set by WHO/FAO (i.e, 3mg/L) respectively.

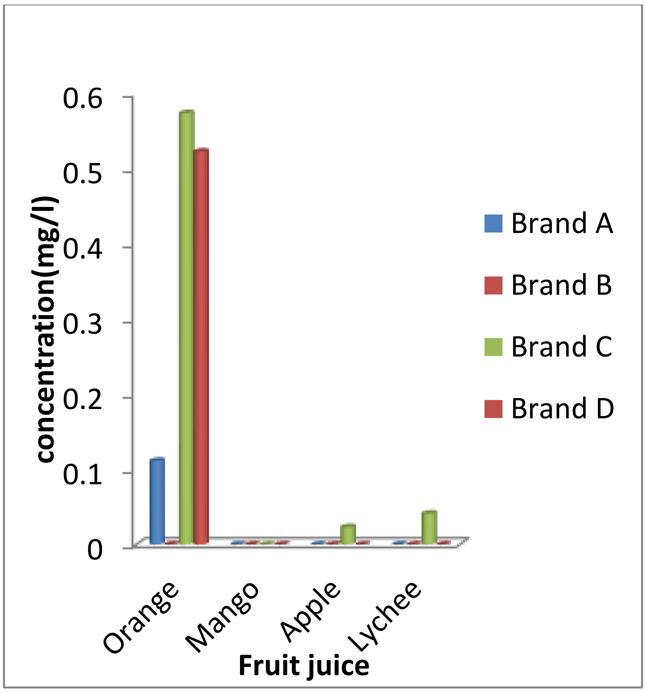

Fig 5: Bar-diagram representing concentration of Cu detected in mg/L in packaged fruit juice of four different brands of Kathmandu city

Table 3: Actual concentration of Zinc in Orange, Mango, Apple and Lychee juices selected from most commonly consumed brands of Kathmandu city.

S.N.	Juice type	Concentration of Zinc(mg/L)			
		Brand A	**Brand B**	**Brand C**	**Brand D**
1	Orange	0.0521	0.1692	0.2026	0.0511
2	Mango	0.0481	0.0663	0.0700	<0.01
3	Apple	<0.01	<0.01	<0.01	<0.01
4	Lychee	<0.01	<0.01	<0.01	0.0287

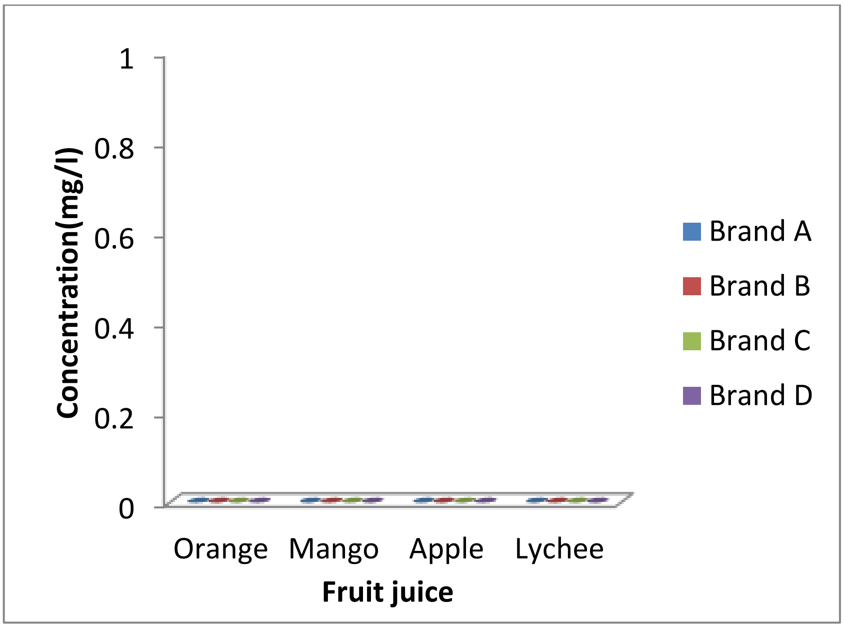

Fig 6: Bar- diagram representing concentration of Pb detected in mg/L in packaged fruit juice of four different brands of Kathmandu city

Determination of Lead in Orange, Mango, Apple and Lychee Juice samples

Bar-diagram (Fig 6) shows that the Lead levels were below detection limit in all selected Packaged fruit juices viz Orange, Mango, Apple and Lychee from four most commonly consumed brands purchased from retail market of Kathmandu City. Lead is hazardous in nature and has no biological function in human body. Exposure to Lead even in small amount can lead to several health issues including nervous system damage especially in the case of children causing development delays, Alzheimer's diseases, nephropathies, affect gastrointestinal tract leading to symptoms such as abdominal pain, constipation and nausea (Paz *et al*., 2019; Wani *et al*., 2015). The reference level set by WHO/FAO for Lead is 0.01mg/L. However, it was found detection limit in all selected samples shown in Fig. 6.

The data shown in the Table 4 shows the level of Fe in the fruit juice samples in Previous studies was reported in the range of 0.709-2.307mg/L (Zwaey-EI *et al*., 2022), 0.00-3.46mg/L (Maspalma *et al*., 2018), 0.015-0.097mg/L (Okeri *et al*., 2009). The concentration of Iron in present study ranged 0.5720-0.7620 in Orange juices; 0.362-0.624 in Mango juices; 0.277-3.649mg/L in Apple juices and 0.279-1.770 in Lychee juices respectively. Zwaey-EI *et al*., 2022 reported Copper content in the range of 0.099-0.513mg/L in canned fruit juices collected from some Benghazi city Markets while Maspalma *et al*., 2018 measured Cu ranging from 0.00-0.33mg/L in packaged fruit juices sold in Yola Metropolis, Adamawa State Nigeria. Similarly, Okeri *et al*., 2009 measured the level of Cu from 0.01-0.08mg/L in drinking water and fruit juice in Benin City, Nigeria. The concentration of copper in the present study ranged from ND-0.574mg/L in Orange juices; not detected (ND) in all selected samples of Mango juices; ND-0.023mg/L in Apple juices; ND-0.041mg/L in Lychee juices respectively. The level of Zinc in the present study ranged 0.0511- 0.2026mg/L in Orange juices; ND-0.0700 in Mango juices; ND in all samples of Apple juices; ND-0.0287mg/L in Lychee juices while in previous studies Zn ranged from 0.003-0.064mg/L, 0.00-0.30mg/L and 0.028-5.969mg/L reported by Zwaey-EI *et al*., 2022; Maspalma *et al*., 2018; Okeri *et al*., 2009. Lead in the tested samples were below detection limit in present study while measured in the range of 0.005-0.012mg/L in the studies reported by Zwaey-EI *et al*., 2022 and below detection limit in the studies reported by Maspalma *et al*., 2018 and Okeri *et al*., 2009.

Table 4: Comparison of concentration range of Fe, Cu, Zn and Pb reported in literature with the present study.

Heavy metal	Concentration range	Reference
Fe Cu Zn Pb	0.709-2.307mg/L 0.099-0.513mg/L 0.003-0.064mg/L 0.005-0.012mg/L	Zwaey-EI *et al*., 2022
Fe Cu Zn Pb	0.00-3.46mg/L 0.00-0.33mg/L 0.00-0.30mg/L ND	Maspalma *et al*., 2018
Fe Cu Zn Pb	0.015-0.097mg/L 0.01-0.08mg/L 0.028-5.969mg/L ND	Okeri *et al*., 2009
Fe Cu Zn Pb	0.5720-0.7620(O); 0.362-0.624(M); 0.277-3.649(A); 0.279-1.770(L) ND-0.574(O); ND(M); ND-0.023(A); ND-0.041(L) 0.0511-0.2026(O); ND-0.0700(M); ND(A); ND-0.0287(L) ND	Present study

(ND =Not detected; O= Orange juice; M= Mango juice; A=Apple juice; L=Lychee juice

Conclusion

The Fe, Cu, Zn and Pb from Packaged fruit juice samples was determined by Flame Atomic Absorption Spectroscopy method. Wet acid digestion method was used for the digestion process of packaged fruit juice samples. From, the research work it was found that the concentration of Fe was found maximum 3.649mg/L in brand C of Apple juice sample and minimum 0.277mg/L in brand B of Apple juice sample. The concentration of copper was found maximum 0.574mg/L in brand C of Orange juice sample and minimum detectable concentration 0.023mg/L in brand C of Apple juice sample; Copper was below detection limit in majority of other samples. Similarly, the concentration of Zinc was found maximum 0.2026mg/L in brand C of Orange juice sample and minimum detectable concentration 0.0287mg/L in brand D of Lychee juice sample. The Concentration of Fe in Apple juice sample of brand C 3.649mg/L exceeds the acceptable limit recommended by WHO i.e. 2mg/L but this concentration falls within estimated average daily Iron intake of 17mg/day for males and 9-12mg/day for females. Cu and Zn in selected packaged fruit juice samples were within accepted limit set by WHO/FAO (i.e. 2mg/L, 3mg/L) for human consumption and the Pb was found below reference limit (i.e. 0.01mg/L) in all samples. The concentration of Fe, Cu, Zn and Pb in all selected packaged juice samples may be due to manufacturing process, brand formulation and standards, packaging materials and storage conditions (Roberts and Orisakwe, 2011). The concentration of heavy metals Fe, Cu, Zn and Pb in analysed fruit juice samples was found within accepted limit set by WHO/FAO. Thus, they are safe for human consumption.

Conflict of Interest

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

Authors' Contribution

N. Paudel: Data acquisition, Analysis and interpretation of data, drafting of the manuscript, Critical revision of the manuscript as for important intellectual content, Final approval of the manuscript; *A. Dhakal*: Data acquisition, Drafting of the Manuscript; *S.S Pradhanang*: Conception and design, Analysis and interpretation of data, Critical revision of the Manuscript as for important intellectual content, Final approval of the manuscript.

Acknowledgement

We express our heartfelt appreciation to the Chemistry Department of Trichandra Multiple Campus for their generous provision of laboratory facilities. Additionally, we extend our sincere thanks to Nepal Academy of Science and Technology (NAST), for efficient analysis of packaged fruit

juice samples using flame atomic absorption spectroscopy (AAS).

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