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ORIGINAL PAPER

Determination of concentration of essential and toxic metals in different varieties of fruits by atomic absorption spectroscopy

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ABSTRACT This research was intended to study the concentration of essential and toxic metals in different varieties of fruits available in local shops of Islamabad city and how these metals affect human health. A total of eleven samples of a varieties of fruits were examined in order to check trace levels of essential heavy metals comprising Fe, Cu, Co, Ca, Mg, K, Na and Zn and three toxic metals Pb, Ni and Cr. Acid digestion of dried samples was done with concentrated nitric acid and hydrogen peroxide and then analyzed by the Atomic Absorption Spectroscopy (AAS) for the determination of metal ion concentrations. Results showed that Ni, 0.059-1.487; Zn, 0.055-1.783; Cu, 0.150-3.960; Co, 1.474-2.896; Cr, 0-4.095; Fe, 5.735-27.345; Ca, 30.514-150.426; Mg, 17.422-29.499; Na, 7.744-23.539; K, 260.899-319.817 and Pb was analyzed as 0-1.571 mg/kg. These values were found to be comparable with the reported values.

Keywords: Essential and heavy metals, Fruits, Atomic Absorption spectroscopy, Wet acid digestion

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INTRODUCTION

It is generally known that fruits are vital sources of vitamin C, carotenoids, minerals (mainly Mg, K) and numerous types of antioxidants and dietary fiber (i.e. pectin) that protect against degenerative and chronic diseases i.e. cancer and heart diseases (Krejpcio et al., 2005). Fresh fruit contains phenolics, vitamins, minerals, sugars, amino acids, volatile compounds, organic acids, fatty acids, large amount of water and concentration of these components responsible for organoleptic properties (Aljuhaimi et al., 2016).

Industrial uses of metals and other domestic processes have introduced substantial amounts of potentially toxic heavy metals into the atmosphere and into the aquatic and terrestrial environments (Özcan & Hacıseferoğullari, 2007; Ozcan et al., 2012). Pakistani population, particularly that belongs to low and medium economic groups consume substantial amount of fruits and vegetables. The basic amount of fruits and vegetables in the diet of an individual should consist of 300-350 gm despite the fact per individual only utilize 80 to 90 gm of fruit and vegetables in their routine diet. Vegetables and fruits are known as protecting supplementary food. Minerals, vitamins, carbohydrates, dietary fibers and essential amino acid are necessary part of fruits that are important for the working of human metabolic reactions. They also neutralize the acid produced in stomach in the course of digestion (Bukhari et al., 2013). Various health issues such as kidney disorders are caused by the accumulation of heavy metals by ingestion. The poorest urban farmers are prone to toxic metal load as they are most likely to use high risk sites and undeveloped land (Zahir et al., 2009). The consistency of trace metals in fruits depends on different factors e.g. soil, weather, genetic, their supply to markets and the harvesting stage of maturity. Trace metals sustain the osmotic regularity, body pH and they are used as coenzyme which maintains the body biological reactions (Ismail et al., 2011).

Consumption of large dose of copper can cause various health issues such as green hair coloration, urinary problem, vomiting, jaundice and diarrhea. Inhaling copper dust is more hazardous to human health (Moses et al., 2011). Nickel plays an important role in the enzymes activity for either bacterial or vegetable, despite it is not critical for human beings and absorption of Nickel is 3% from the gastrointestinal tract. Depressed growth in mammals is reported due to the deficiency of Ni (Biego et al., 1998).

For animals and plants, zinc is a vital element. Sufficient amount of Zn is necessary to neutralize the poisonous effects of Cd. Zinc plays important function in the maintenance of a healthy central nervous system and prevention of anemia (Radwan et al., 2006). Iron plays a crucial role in the transportation of oxygen, vascular functions and cellular and glucose metabolism, as it is part of haemoglobin, heme-proteins, and myoglobin. Iron deficiency leads to insomnia, anemia and other health issues because its deficiency weakened the immunity system and inhibits the synthesis of haemoglobin (Alzahrani et al., 2016). When used in trace amount Co act as micronutrient for the growth of animals and human beings, Co accumulates in the liver and kidney of humans causing nervous, cardiovascular, bone and kidney diseases when used for prolonged time (Ali et al., 2012).

Ca is known for the growth and skeletal development. The deficiency of calcium causes loss of Ca deposit in bone. The acidity of soil causes the insufficiency of Mg in fruits. Mg plays a vital role in stability of nervous system and synthesis of protein. The daily recommended permissible limit for Mg is 320-420 gm/day. The risk of osteoporosis is increased, if the intake of Mg is less than 60% in skeletal muscles.

Na is an essential element required to regulate the blood pressure and transmit signals in nervous system. The deficiency of sodium causes seizures and confusion etc. The high dose of sodium has direct relation with the blood pressure. The concentration of sodium is responsible for osmotic regulation (Krejpcio et al., 2005). According to nutrition board 2004-2006, the daily suggested amount of potassium for adult is 4700 mg/day (Viarengo et al., 1996). Lead has toxic effects on nervous system, red blood cells, kidney and reproductive system. In Japan, lead poisoning has also been reported in which many people developed bodily abnormalities and many lost their lives (Ogunkunle et al., 2014).

Herein we have reported the determination of essential and toxic metals in different varieties of fruits collected from local market of Islamabad, Pakistan by AAS and to assess the impact of these metals on human health. With best of our knowledge such type of study in Islamabad by employing AAS has not yet been reported elsewhere.

MATERIALS AND METHODS

Apparatus and glassware

Digital Analytical Balance, hot plate, oven and Flame Atomic Absorption Spectrophotometer (Analyst-200 by Perkin Elmer) instruments were used. Volumetric flasks (25, 50 and 100 mL sizes), glasswares and 5-50 mL measuring cylinders, 1-10 mL pipettes (Pyrex, USA), micropipettes measuring devices were used.

Collection of samples

Total of 14 samples of different fruits were collected from the three markets of Islamabad city, Pakistan. A total of 14 varieties of fruits, produced locally, including lemon, apple, banana, papaya, mosammi, red mulberry, green mulberry, guava, grapefruit (chakotra), pear, kino, chikoo, melon and strawberry.

Sample preservation and handling

Samples of fruits were washed. Tap water was used to remove dust particles. De-ionized water was used to rinse the fruit samples three times and dried in oven at 60-80 ^{o}C. Porcelain piston and mortar was used to grind the sample and stored in plastic containers for AAS analysis. All samples were analyzed in triplicate and mean values reported.

Table 1 The conditions of Atomic Absorption Spectrometry employed for each analyte

Element	Wave length (nm)	Slit width (nm)	Fuel flow (L/min)	Oxidant (L/min)	Flame
Ca	422.67	2.7	2.70	10.00	
Mg	285.21	2.7	2.50	10.00	
Mn	279.48	1.8	2.50	10.00	
Fe	248.33	1.8	2.50	10.00	
Zn	213.86	2.7	2.50	10.00	
Cu	324.75	2.7	2.50	10.00	Air-
Cr	357.87	2.7	3.00	10.00	Acetylene
Co	240.73	1.8	2.50	10.00	
Ni	232.0	1.8	2.50	10.00	
Pb	283.31	2.7	2.50	10.00	
Na	589.00	1.8	2.50	10.00	
K	766.49	2.7	2.50	10.00	

Table 2 The working standards used to establish Atomic Absorption Spectrometry calibration curves

Elements	Working standards (mg/kg)	Correlation coefficient of calibration curve
Ca	10.0, 20.0, 30.0	0.998583
Mg	1.0, 2.0, 3.0	0.999407
Mn	5.0, 10.0, 15.0	0.999847
Fe	2.0, 4.0, 6.0	0.999667
Zn	0.5, 1.0, 1.5	0.995787
Cu	5.0, 10.0, 15.0	0.999816
Cr	50.0, 100.0, 150.0	0.995018
Co	5.0, 10.0, 15.0	0.997520
Ni	1.0, 2.0, 3.0	0.997744
Pb	5.0, 10.0, 15.0	0.995018
Na	0.5, 1.0, 1.5	0.991538
K	1.0, 2.0, 3.0, 4.0	0.998690

Table 3 Concentration of metals (mg/kg) ±SD in fruits samples [a]

Sample	Cu(mg/kg)±SD	Zn(mg/kg)±SD	Co(mg/kg)±SD	Ca(mg/kg)±SD
Lemon	0.317±0.0151	0.609±0.0304	1.474±0.0373	81.382±1.3447
Apple	0.15±0.0065	0.161±0.0080	1.782±0.0772	30.514±1.75153
Banana	3.96±0.0353	0.339±0.0129	2.132±0.1022	36.987±1.3487
Papaya	0.22±0.011	0.315±0.0105	1.841±0.0907	66.656±1.9559
Mosammi	0.807±0.0211	0.818±0.0400	1.946±0.0966	105.769±1.1591
Red Mulberry	0.291±0.0128	0.94±0.0339	1.844±0.0901	105.633±1.0556
Green Mulberry	0.259±0.0114	0.633±0.0146	2.04±0.0957	114.862±1.2390
Guava	1.89±0.00610	1.504±0.0363	2.023±0.1011	35.494±1.3983
Chakotra	0.187±0.0091	0.055±0.0021	2.525±0.1260	150.426±1.8155
Pear	0.846±0.0085	0.314±0.0125	2.635±0.1312	40.671±1.0967
Kino	0.184±0.0085	1.047±0.0500	2.323±0.1161	40.954±1.1644
Chiko	0.166±0.0065	0.262±0.0130	2.68±0.1119	50.564±1.1582
Melon	0.192±0.0071	1.738±0.0524	2.558±0.1249	42.794±1.4743
Strawberry	0.317±0.0110	1.783±0.3363	2.896±0.1209	46.59±1.4530
Permissible limit	0.5	1	2	NA

[a] SD = Standard Deviation

Table 4 Concentration of metals (mg/kg) ±SD in fruits samples [a]

Sample	Cr (mg/kg)±SD	Pb (mg/kg)±SD	Ni (mg/kg)±SD
Lemon	ND	0.001±0.0000	0.313±0.0141
Apple	ND	ND	0.579±0.02703
Banana	ND	0.415±0.0206	0.059±0.00245
Papaya	ND	0.152±0.0063	ND
Mosammi	ND	0.668±0.0331	1.26±0.0341
Red Mulberry	0.23±0.0094	0.603±0.0301	0.959±0.0301
GreenMulberry	3.975±0.1398	0.021±0.0017	1.469±0.0503
Guava	4.343±0.2086	0.103±0.0048	1.18±0.03015
Chakotra	4.304±0.2115	0.168±0.0064	0.804±0.0401
Pear	0.18±0.0098	0.222±0.0110	1.487±0.0681
Kino	3.931±0.0818	0.224±0.0110	1.099±0.0539
Chiko	ND	1.571±0.0311	1.279±0.0631
Melon	4.095±0.0447	0.138±0.0066	0.794±0.0247
Strawberry	2.864±0.1359	0.081±0.0026	1.31±0.0101
Permissible limit	0.24	0.3	2

[a] ND = Not Detected

Table 5 Concentration of toxic metals mg/kg in fruit samples [a]

Samples	Fe(mg/kg) ±SD	Na(mg/kg) ±SD	Mg(mg/kg) ±SD	K(mg/kg) ±SD
Lemon	8.057±0.71	7.744±0.1945	23.242±0.1945	265.312±5.0860
Apple	7.919±0.31	23.047±0.2398	22.163±0.2398	260.899±3.4742
Banana	8.717±0.41	16.912±0.4086	23.242±0.4086	289.616±2.1247
Papaya	6.588±0.27	10.617±0.2215	21.801±1.0215	319.817±8.4360
Mosammi	5.984±0.08	22.606±0.1698	22.036±1.1008	293.536±2.6717
Red Mulberry	22.632±0.60	16.907±0.0818	23.242±0.0000	289.462±3.4957
Green Mulberry	27.345±0.24	15.935±0.2438	23.643±0.2438	279.103±2.2342
Guava	7.334±0.04	17.122±0.0447	29.499±0.0447	291.525±1.7094
Chakotra	16.051±0.19	18.034±0.1959	23.95±0.1959	274.023±2.6966
Pear	5.735±0.19	13.418±0.1945	28.968±0.1945	268.137±1.9791
Kino	6.536±0.23	23.137±0.2398	17.422±0.2398	277.991±3.0869
Chiko	10.302±0.40	21.962±0.4086	21.482±0.9086	263.131±2.3764
Melon	6.486±0.22	23.539±0.2215	23.242±0.2215	270.094±2.1473
Strawberry	6.541±0.16	21.281±0.1698	19.968±0.1698	268.12±4.7687
Permissible limit	35.6	NA	NA	NA

[a] NA = Not Applicable

Apparatus clean-up

All glassware, plastic containers and polyethylene bags were filled with aqueous detergent solution; inner and outer walls wiped briskly with foam and brushes; sufficient amount of water was used to remove the detergent followed by de-ionized water rinsing. All the glassware were immersed overnight in 8N HNO_3 and washed with deionized water. Then the glassware was dried in oven and stored in clean dry places free of contamination.

Digestion of samples

Standard methods reported by AOAC, 1990 was used for the sample wet acid digestion. 4gm of dried fruit samples were added to 40ml concentrated HNO_3 in 100 mL beaker and then it was placed on the hot plate for 1 hour to get semi-dried sample of each fruit. Again 40 mL of conc. Nitric acid and 16ml of hydrogen peroxide were added to the sample and again vigorously heated on hot plate. Addition of H_2O_2 and HNO_3 was continued till the solution became colorless. The volume of fruit sample was reduced up to 2-3 mL. And then it was cooled and Whattmann filter was used to filter the sample. 10 mL sample bottles or vials were used to store the filtrate. De-ionized water was used to dilute up the sample to 100 mL before taking to Atomic Absorption Spectrometer. All the fruit samples were digested in the same way and diluted up to 100 mL with de-ionized water.

Instrument operating conditions

Commercially available stock solutions in the form of an aqueous solution (1000 ppm) were used to prepare Atomic absorption spectroscopic standard solutions. Working standards were prepared from purified de-ionized water. Three or four points of calibration curve were established by running the prepared working standard solutions in AAS.

The sample solutions were aspirated into the Atomic Absorption Spectrometry instrument immediately after calibration and direct readings of the metal concentrations was recorded. Three replicate determinations were carried out on each sample. The same analytical procedure was employed in the determination of elements in the blank. The operating conditions of AAS employed for each analyte are given in Table 1.

Instrument calibrations standards

Standard solutions prepared from stock solutions were use to set up AAS calibration curves and in turn used to determine the concentrations of selected essential elements in the specified plant samples. Concentration range of AAS standards are therefore presented in Table 2.

It can be noted from the values presented in Table 2 that, the concentration ranges of the working standards are wide enough to bracket the elemental concentrations of plant food sources analyzed in this research. Dilution was used to bring elements that were naturally abundant in fruits into working range. Therefore, the working standards used to establish AAS calibration curves were expected to have given precise and accurate levels of essential elements in the analyzed fruit samples.

RESULTS AND DISCUSSION

The fruits samples were analyzed for major trace essential and trace non-essential metals with FAAS. The instrument was operated as per the instrument's manual. Each of the sets of successive samples were aspirated one after another in to the FAAS instrument set in concentration mode using the hallow cathode lamp of the respective metal. Calcium uptake values in fruits were within the permissible limit set by WHO/FAO. The mean level of Ca in mulberry samples in the present study was higher than those found in Turkey (Hamurcu et al., 2010). In developing countries the daily permissible limit of Na is between 2400-5175 mg/day. As shown in Table 3 sodium uptake in fruits was higher in melon 23.539 mg/kg while apple showed lower Na contents, in lemon 7.744 mg/kg. Where all the values are within the permissible limit set by WHO/FAO. The daily recommended allowance for magnesium is 320-420 gm/day. Magnesium uptake in fruits was higher in guava 29.499 mg/kg while apple showed lower Mg contents, in kino 17.422 mg/kg. Where all the values are within the permissible limit set by WHO/FAO. The mean level of Mg in mulberry samples in the present study was higher than those found by Akbulut and Ozcan (2009) (Akbulut et al., 2009). Fe was found highest in green mulberry that is 27.345 mg/kg. The maximum permitted level for iron ion for food is 15 mg/kg, according to (Turkish Food Codex Anonymous Regulation, 2002). In other research, the concentration of Fe was reported as 35.6 mg/kg for raw foodstuff. The permissible limit for Zn is 20 mg/kg. According to WHO, the tolerable limit of Zn in different fruits is 100 mg/kg. The mean level of K in mulberry samples in the present study was lower than those found in Minsk, Belarus (Samus et al., 2016).

The daily allowable ingestion of zinc is 0.3–1 mg/kg (FAO/WHO, 1982). The concentration of Zn is maximum in strawberry and minimum in apple, the value for Zn in fruits were above the FAO/WHO's permissible values. The concentration of Cu was found minimum in apple and maximum in banana. Whereas the tolerable range is up to 1.0 mg/100g (Queirolo et al., 2000). And the suggested range for daily use is 2-3 mg/day. Sample of fruits contain the level of Cu within the permitted limit except banana, mosammi, guava and pear. Co contents were ranged from 1.474-2.896 mg/kg. The concentration of Co in some fruits is slightly higher than the permissible limit, cobalt variation in fruits is 0.006-0.009 mg/kg as shown in Table 3 & 4.

Most of the plants contained Cr in levels less than 1 ppm, while in others it ranges from 4 to 6 ppm, as shown in Table 5. The maximum concentration of Cr is 4.343 and minimum was 0 mg/kg, where concentration of Cr in certain fruits is high then the permissible limit set by WHO. The comparison of these metals in fruits is shown in Fig. 1 and Fig. 2.

The Food and Nutrition Board of the NAS/NRC states that a safe, sufficient ingestion of chromium for an adult is 50–200ug/day. The highest concentration of Cr is in Guava that was 4.343 mg/kg. The level of Pb was found greater in Chikoo and banana whereas the concentration of lead in other fruits is within the permissible limit set by WHO/FAO. The acceptable limit for Ni is 0.05-5 mg/dl. Whereas the concentration of Ni in all fruit samples are within the permissible limit set by WHO. The comparison of toxic metals in fruits is shown in Fig. 3.

CONCLUSION

The present study was conducted to check the concentration of metals in different varieties of fruits that were collected from local shops of Islamabad city Pakistan. The concentration of lead and chromium was slightly above the permissible limit due to the environmental contamination. It is concluded that the levels of trace metals are within permissible limits or slightly above for safe human utilization. Hence regular use of the different varieties of investigated fruits will provide the sufficient amounts of the macro and essential trace elements needed for human health.

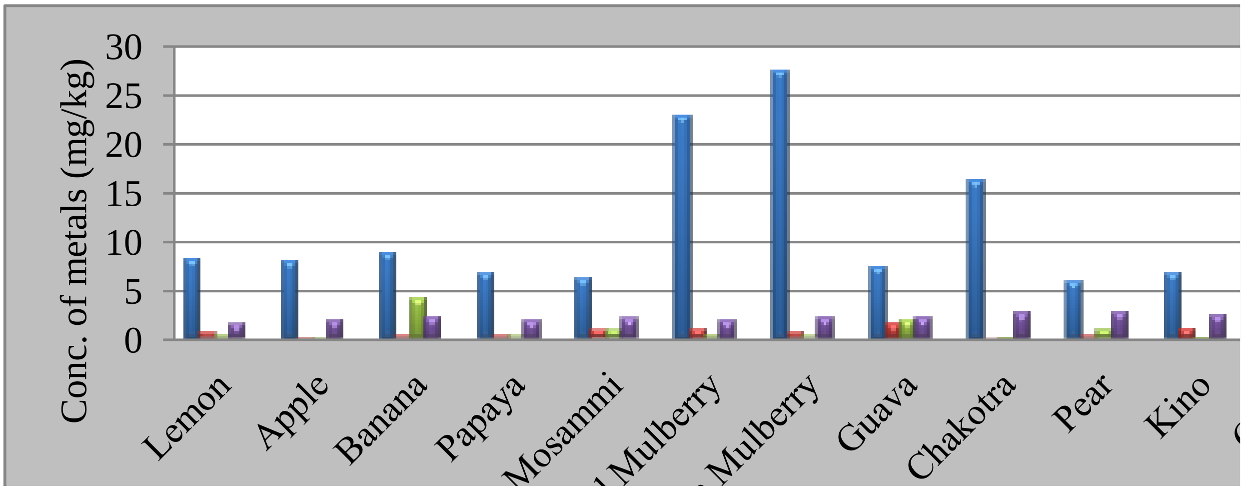

Fig. 1 Comparison of Micro elements level in fruits

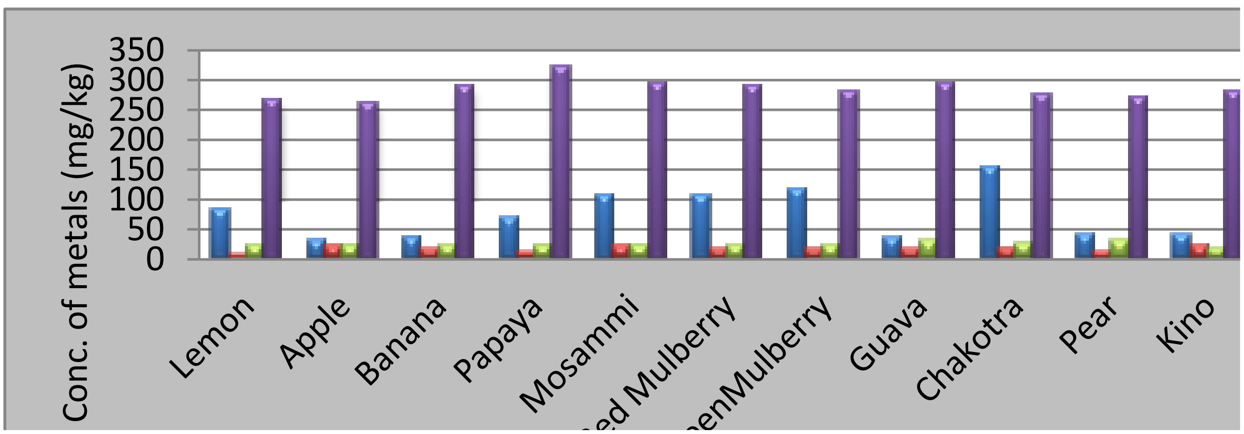

Fig. 2 Comparison of Macro elements level in fruits

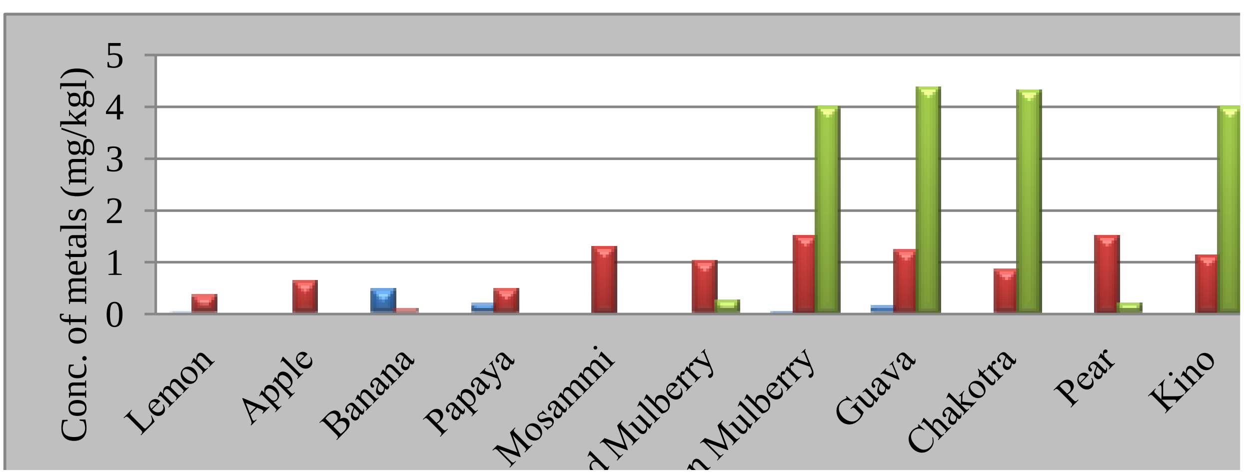

Fig. 3 Comparison of toxic elements level in fruits

Author Contribution Statement Moazzam Hussain Bhatti participated in the planning, coordination, arrangements of the study and helped to draft the document. Mehreen Gull conducted the research study and wrote the manuscript. The authors have approved the final document.

Conflict of Interest The authors have accepted the final version of the document being submitted. The article is the original work of the authors. Our submitted manuscript is not under consideration for publication in another journal. There is no conflict of interest between the authors.

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