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ORIGINAL PAPER

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ANALYSIS OF TOXIC ELEMENTS IN LEAVES AND FRUITS OF LOQUAT BY INDUCTIVELY COUPLED PLASMA-MASS SPECTROMETRY (ICP-MS)

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ABSTRACT

This study aimed at analyzing the content of 10 toxic elements (Be, Al, Ti, As, Cd, Sn, Sb, Hg, Pb and Ag) in different tissues (leaf blade, seed, fruit peel and pulp) of loquat fruits, at different maturity stages (immature green, mature green and full ripe) from Fujian (Yun Xiao/Zhangzhou) in China, using inductively coupled plasma-mass spectrometry (ICP-MS), with microwave digestion. Results revealed that the concentrations of Be, Al and Hg in all observed tissues were significantly reduced with the change in maturity of fruits from immature green to full ripe. Whereas the toxicity of Ti, As, Cd, Sb and Pb were observed to be shifted from seed and pulp to peel and leaves. Tin concentration was increased in all observed tissues except seeds, while silver concentration was only increased in fruit peel of loquat. In sum up, toxic elements concentration detected in the fruit pulp of loquat, at full ripe stage, was found safe for human consumption.

Key words: loquat, toxic elements, ICP-MS, microwave digestion

INTRODUCTION

Loquat (Eriobotrya japonica Lindl.) is an evergreen fruit plant originated from the People's Repub-lic of China. It belongs to the family Rosacea, subfamily Maloideae. It is most widely grown in Japan, Korea, India, Pakistan and the south-central region of China. It is also grown as an ornamental shrub in California [LaRue 2020]. China is the leading producer and exporter of loquat, grows on more than 100 000 ha. The annual production reaches up to 380 000 t. More than 30 species of loquat are grown in temper-ate and subtropical regions of Asia [Lu et al. 2007]. It is a rich source of vitamin A, vitamin B6, potassium, magnesium and dietary fiber. It is a very beautiful orange-colored fruit with a mild sweet and sour taste. Due to soft and juicy pulp and thin skin, the fruit cannot be stored for a long period of time. It is the most favorite fruit of the people belonging to various parts of the world [Tian et al. 2011].

Although fruits and vegetables have low energy content but the nutritive value of the fruits has gained the interest nowadays. They are rich source of vitamins, fibers and minerals which are much essential for human body [Slavin and Lloyd 2012]. The excessive intake of fruits and vegetables can replace the food containing harmful saturated fats and sugars and enhance the consumption of healthy nutrients and dietary



fibre [Pem and Jeewon 2015]. To avoid certain cardiovascular diseases, it is recommended to consume 4400 g fresh fruits and vegetables per day [WHO 2015]. But the intake of some fruits and vegetables containing toxic elements can cause their entrance in human body and deposition in bones and fat tissues. The slow release of those toxic elements in the body can cause some severe diseases [Guerra et al. 2012]. Toxic elements e.g. Hg, As, Cd, and Pb, can influence the function of nervous system, and cause mental illness by affecting blood circulatory system [Hajeb et al. 2014]. Hence, it is very important to update the mineral contents of fruits and vegetables.

The mineral contents of the plants are influenced by many factors e.g. plant cultivar, soil and weather conditions, fertilizer use during fruiting and maturity stage of the fruits [Corelli-Grappadelli and Lakso 2004]. The quadrupole inductively coupled plasma mass spectrometer (ICP-MS) is the most suitable method for the determination of trace elements in fruits and vegetables and is prevailed as the most appropriate practice for clinical quantification [Bressy et al. 2013]. The element detection through ICP-MS has become a predominant methodology, because of its rapidity, determination limits, and the sample quantity needed for analysis [De Blas Bravo et al. 2007].

In present experiment, the contents of 10 toxic elements were analysed; beryllium (Be), aluminum (Al), titanium (Ti), arsenic (As), cadmium (Cd), tin (Sn), antimony (Sb), mercury (Hg), lead (Pb) and silver (Ag); from different tissues of loquat fruits on different maturity stages i.e. immature green (IMG), mature green (MG) and full ripe (FR) from Fujian province in China. Inductively coupled plasma-mass spectrometry (ICP-MS) was used to determine the contents of above-mentioned toxic elements.

MATERIALS AND METHODS

The loquat fruit samples were collected at three different maturity stages i.e. immature green (IMG), mature green (MG) and full ripe (FR) from an orchard located at Yun Xiao, Fujian (23°57'13.5"N 117°20'36.0"E). Stratified sampling in accordance with acceptable sunlight degree of plant parts was adopted to collect the fruits. Plant materials were washed (for 10 s approximately) with a solution of phosphate-free detergent, then with a 0.1 N HCl solution and finally with distilled water. Then, dried at 70°C, ground and passed through a 500 µm stainless-steel sieve [Madejon et al. 2006]. Soil samples were taken from the root zone of each tree, about 2 m from the trunk, and at two depths (0–25 cm and 25–40 cm), by using a spiral auger of 2.5 cm diameter. Three sub-samples around the trunk were taken to make a composite soil sample per tree. They were transported to the laboratory, oven-dried at 40°C and crushed to pass through a 2 mm sieve, and then ground to $<60 \,\mu m$ for total trace element determinations [Madejon et al. 2006]. The analysis of toxic elements was performed using inductively coupled plasma mass spectrometer (ICP-MS) at institute of Subtropical Fruits, Fujian Agriculture and Forestry University.

Instrumentation and reagents. The quadrupole inductively coupled plasma mass spectrometer (ICP--MS) used in this work was Agilent7700X (Agilent, USA). It was combined with a high-efficiency sample introduction desolvating system equipped with a quartz cyclonic spray chamber. It was also having an additional mixing chamber to further homogenize and stabilize the sample aerosol stream, resulting in a more stable signal from the ICP-MS and a PFA-400 nebulizer operating with peristaltic pump (APEX-IR, Omaha, NE, USA). The argon gas utilized was of spectral purity (99.9998%). Before each experiment, the instrument was tuned for daily performance, using Agilent 7700X Sensitivity Detection Limit Solution, Agilent, USA. It is an aqueous multi-element standard solution of Li, Y, Co, Ce and Tl for consistent sensitivity (7Li, 59Co, 89Y, 140Ce, 205Tl) and minimum doubly charged and oxide species levels (140Ce). The concentration of elements in the solution was 10 µg L⁻¹. The internal standard solution (226Re, 115In, 73Ge, 45Sc) had the concentration of 0.01 μ g mL⁻¹ for each element, was also purchased from Agilent, USA.

Standard solution (1000 μ g L⁻¹) for each element i.e. Be, Al, Ti, As, Cd, Sn, Sb, Hg, Pb, Ag was purchased from National Standard Material Research Center. Nitric acid (HNO₃) used in the experiment was analytically pure, obtained from CNW Technologies GmbH, Germany. The water was 18.3 M Ω deionized, made by our laboratory (Institute of Subtropical Fruits, FAFU).

In this experiment, the working parameters of the inductively coupled plasma mass spectrometer were

optimized before the test, and the parameter settings are shown in Table 1.

Sample preparation. After the cleaning of glassware and PTFE digestion tank used in the test, they were soaked in 20% nitric acid (1 + 4, V + V) for more than 12 h, and then rinsed with deionized water three times before use. After lyophilizing the loquat pulp with a freeze dryer, 0.5 g of the dry sample were added into a poly-tetra-fluoroethylene digestion tank. Then, 5 mL of concentrated nitric acid (analytical grade, CNW Technologies GmbH, Germany) was added. Then, the digestion tank was gently shook to make the sample completely immerse; microwave digestion (Mars5, HY-20-164, CEM, USA) under the set conditions (Tab. 2). After microwave digestion completed, it was let be cool naturally. After opening the cap, the digestion solution was transferred to a 25 mL volumetric flask, and washed the inner wall of the digestion tank with a small amount of deionized water

several times. The washing solution was combined in a 25 mL volumetric flask to make a constant volume, at the same time as a blank control.

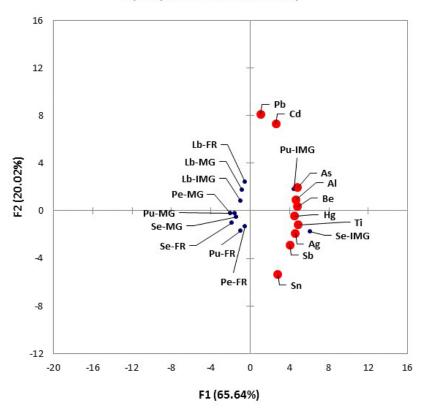
Determination. A single standard series solution was prepared. The mixed standard series solution and sample solution were measured by ICP-MS, and the standard curve method was used for quantification. The mixed internal standard was added to correct for matrix interference and instrument signal drift.

Statistical analysis. The experiment was conducted under the complete randomized design (CRD) with four replications. Collected data was analyzed for analysis of variance (ANOVA) and Fisher's least significance difference (LSD) method for pair-wise comparison of mean values at 5% significance level using analytical software package 'Statistix 8.1'. Principal component analysis (PCA) – Figure S1 – and correlation coefficient values were determined with Pearson (n) method using XLSTAT Version 2014.4.06.

| Working parameters | Set value | |
|--|-----------|--|
| RF power (W) | 1550 | |
| Plasma gas flow (L min ⁻¹) | 15 | |
| Carrier gas flow (L min ⁻¹) | 1.07 | |
| Compensation air flow (L min ⁻¹) | 0.00 | |
| Spray chamber temperature (°C) | 2 | |
| Octopole reaction cell mode | helium | |
| Oxide (%) | <3 | |
| Double charge (%) | <1.5 | |
| Sampling cone and intercepting cone | nickel | |
| Sampling depth (mm) | 10.0 | |

Table 1. The main working parameters of ICP-MS

| | Step | Power (W) | Heating rate (°C/min) Temperature (°C) Hold time (| | Hold time (min) |
|---|------|-----------|--|-----|-----------------|
| | 1 | 1200 | 12 | 120 | 5 |
| | 2 | 1200 | 30 | 150 | 5 |
| _ | 3 | 1200 | 19 | 190 | 35 |



Biplot (axes F1 and F2: 85.66%)

Fig. S1. Principal component analysis among 10 toxic elements and different plant tissues of loquat on different maturity stages

Abbreviations: IMG – immature green, MG – mature green, FR – full ripe, Lb – leaf blade, Se – seed, Pu – fruit pulp, Pe – fruit peel, Be – beryllium, Al – aluminum, Ti – titanium, As – arsenic, Cd – cadmium, Sn – tin, Sb – antimony, Hg – mercury, Pb – lead, Ag – silver.

RESULTS AND DISCUSSION

Soil

Soil is the major receiver of minerals and nutrients from the environment. The elements which are adsorbed by the clay particles of the soil and don't go through decomposition are become toxic elements [Wuana and Okieimen 2011]. Soil texture and structure can influence the nutrients availability from soil to plants [Huang et al. 2006]. Soil solution containing the ions of nutrients is the main reservoir of nutrients, available to plants [He et al. 2005]. There are many factors influencing the toxic elements concentration in the soil. Naturally, they can be deposited by the rock weathering. Humans can also be the source of toxic elements by adding industrial waste in the soil. The toxic elements present in the soil can affect the quality of fruits and vegetables, and can enter into the human body. These elements are very harmful for human health [Nieder et al. 2018]. In present study, the status of toxic elements (Be, Al, Ti, As, Cd, Sn, Sb, Hg, Pb, Ag) in the soil as well as in the loquat fruits grown there, was determined. Among all other toxic elements, aluminum (Al) was found

| Toxic elements | Concentration | |
|----------------|--|--|
| Beryllium | 511.92 ±72.68 μg kg ⁻¹ | |
| Aluminum | $54774.10 \pm 1137.5 \text{ mg kg}^{-1}$ | |
| Titanium | $1215.94 \pm 24.5 \text{ mg kg}^{-1}$ | |
| Arsenic | $58.88 \pm 1.5 \text{ mg kg}^{-1}$ | |
| Cadmium | $80.8 \pm 4.01 \ \mu g \ kg^{-1}$ | |
| Tin | $7745.31 \pm 241.01 \ \mu g \ kg^{-1}$ | |
| Antimony | $701.66 \pm 20.88 \ \mu g \ kg^{-1}$ | |
| Mercury | $34.1 \pm 4.08 \ \mu g \ kg^{-1}$ | |
| Lead | $47.97 \pm 1.13 \text{ mg kg}^{-1}$ | |
| Silver | $59.03 \pm 3.42 \ \mu g \ kg^{-1}$ | |

Table 3. Toxic elements concentration in the soil of loquat orchard

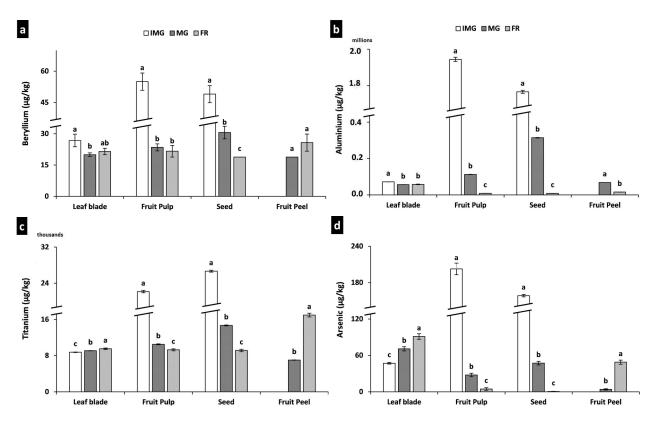


Fig. 1. Toxic elements [beryllium (a), aluminum (b), titanium (c) and $\operatorname{arsenic}(d)$] concentration in different plant tissues of loquat on different maturity stages. Vertical bars indicate average \pm standard error (4 replicates)

IMG - immature green, MG - mature green, FR - full ripe

in maximum quantity $(54774.10 \pm 1137.5 \text{ mg kg}^{-1})$ as shown in table 3. Aluminum is 3^{rd} abundant element present in soil crust after oxygen and silicon. Soil is the main source of Al for plants. Soil pH can also influence its uptake [Silva 2012].

Most of toxic elements i.e. Be (511.92 \pm 72.68 µg kg⁻¹), Ti (1215.94 \pm 24.5 mg kg⁻¹), As (58.88 \pm 1.5 mg kg⁻¹), Sn (7745.31 \pm 241.01 µg kg⁻¹), Sb (701.66 \pm 20.88 µg kg⁻¹), Hg (34.1 \pm 4.08 µg kg⁻¹), Pb (47.97 \pm 1.13 mg kg⁻¹), Ag (59.03 \pm 3.42 µg kg⁻¹) concentrations level was higher in the soil (Tab. 3) than plant tissues (Figs 1–3), except Cd (80.8 \pm 4.01 µg kg⁻¹). Toxic trace elements are present in the soil but deposition of industrial waste, sewage and sludge can increase their concentration [ATSDR 2002].

Loquat leaves and fruits

Toxic elements are categorized into two groups with respect to their environmental significance. Aluminum (Al), arsenic (As), cadmium (Cd), lead (Pb) and mercury (Hg) have the greater environmental significance than beryllium (Be), titanium (Ti), tin (Sn), antimony (Sb) and silver (Ag) [Nieder et al. 2018]. Micronutrients including toxic elements are called trace elements when they are present in quantity <1000 mg kg⁻¹, being toxic [Kabata-Pendias 2010]. The analysis results about the beryllium concentration in different plant tissues i.e. leaf blade, fruit pulp, seed and peel of loquat indicate a significant difference in beryllium level throughout maturity as shown in figure 1(a). Results were indicative that the toxicity of

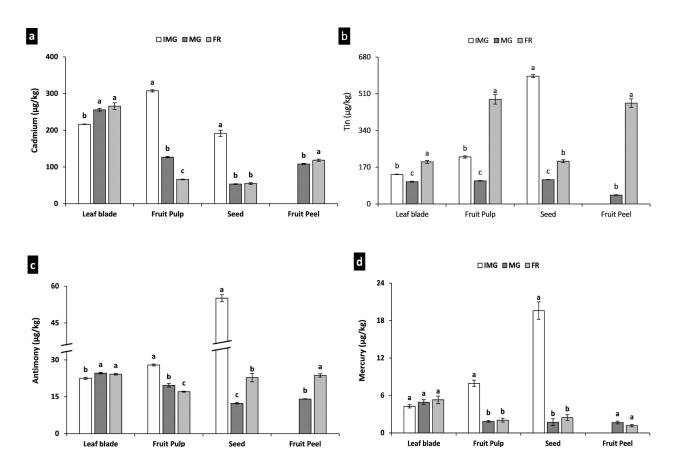


Fig. 2. Toxic elements [cadmium (a), tin (b), antimony (c) and mercury (d)] concentration in different plant tissues of loquat on different maturity stages. Vertical bars indicate average \pm standard error (4 replicates).

IMG - immature green, MG - mature green, FR - full ripe

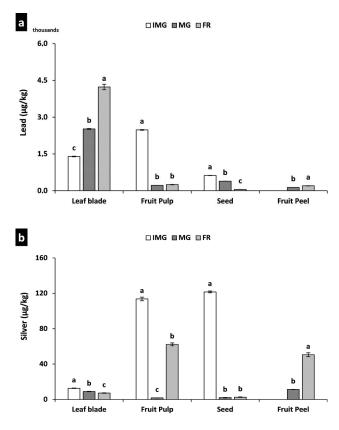


Fig. 3. Toxic elements [lead (a) and silver (b)] concentration in different plant tissues of loquat on different maturity stages. Vertical bars indicate average \pm standard error (4 replicates).

IMG - immature green, MG - mature green, FR - full ripe

beryllium in leaves, fruit seeds and pulp was reduced with the proceeding from immature green stage to full ripe stage. Whereas fruit peel didn't show any significant difference. At early maturity stage, toxic elements concentration in the peel of loquat fruits was not determined because the peel was negligible at that level. Fruit pulp having maximum beryllium level (110.11 $\pm 4.11 \ \mu g \ kg^{-1}$) at immature green stage showed significant variation among all other plant tissues, while the lowest beryllium concentration (18.82 $\pm 0.00 \ \mu g \ kg^{-1}$) was observed in loquat seeds at fruit ripening stage.

The results shown in Figure 1b indicate that the concentration of aluminum in all observed plant tissues i.e. leaves, seeds, fruit pulp and peel was reduced with the increase in fruit maturity. A great difference in aluminum concentration with fruit maturity was observed in fruit pulp and seed when

the concentration was decreased up to 1/16 and 1/5, respectively. At full ripe stage, loquat fruits depicted the aluminum concentration as 8.8 mg kg⁻¹. The analysis results about the titanium concentration in different plant tissues of loquat were indicative that the toxicity of titanium in leaves, seeds and fruit pulp was reduced with the proceeding from immature green stage to fruit ripening stage. Whereas fruit peel showed a significant increase in titanium concentration as shown in Figure 1c. Maximum titanium level $(4.27 \times 10^4 \pm 212.39 \ \mu g \ kg^{-1})$ was indicated by loquat seeds at early maturity stage, while the lowest concentration (8.75 \times 10³ ±53.62 µg kg⁻¹) was observed in loquat leaves at same maturity stage. A great difference in titanium concentration with fruit maturity was observed in fruit pulp and seed when the concentration was decreased up to 1/3, each.

Loquat fruit is one of those fruit crops which are highly sensitive to show the effect of irrigation water. Arsenic (As) is a toxic element which is present in the groundwater of many areas of the world and can contaminate fruits through irrigation water [Heitkemper et al. 2009]. There are many studies reported about the high-level toxicity of arsenic in those fruits, vegetables and cereal crops which were irrigated by arsenic contaminated water [Farid et al. 2003]. Our findings about the arsenic concentration in different plant tissues of loquat indicate that the toxicity of arsenic in seeds and fruit pulp was reduced with the proceeding from immature green stage to full ripe stage. Whereas fruit peel and leaves showed a significant increase in arsenic level. Maximum arsenic contents (404.25 $\pm 9.59 \ \mu g \ kg^{-1}$) were observed in the fruit pulp at immature green stage (Fig. 1d), which were reduced up to $4.4 \pm 2.1 \ \mu g \ kg^{-1}$ at full ripe stage, which was in much greater quantity as reported earlier [European Commission 2002]. While the lowest arsenic concentration $(0.46 \pm 0.21 \ \mu g \ kg^{-1})$ was observed in loquat seeds at fruit ripening stage. Arsenic is highly poisonous for humans. Its intake, greater than 10 mg kg⁻¹ day⁻¹ can cause hepatotoxicity and anemia [Singh et al. 2014].

More than 70% cadmium intake by humans are sourced from fruits and vegetables [Chen et al. 2012]. According to the findings of various scientists, cadmium is a mobile element in the soil, while it is immobile in plants, that's why it is found abundantly in plant's body [Rahman and Hasegawa 2011]. The results shown in Figure 2a indicate a significant decrease in cadmium concentration present in fruit pulp and seed of loquat, whereas a remarkable increase was observed in cadmium toxicity in fruit peel and leaf blade from early maturity to fruit ripening stage. It was might be a shift of toxic element from internal parts of fruit i.e. pulp and seed toward external peel and leaves. A great difference in cadmium concentration with fruit maturity was observed in fruit pulp and seed when the concentration was decreased up to 1/5 and 1/3, respectively. Cadmium intake by humans can cause cardiovascular-toxicity, nephrotoxicity and can affect on reproductive and renal excretory system [ATSDR 2012, Satarug 2018].

Tin concentration in different plant tissues of loquat were indicative that the toxicity of tin in leaves, fruit pulp and peel was increased with the proceeding from immature green stage to full ripe stage. Whereas seeds showed a significant decrease in tin concentration. While looking at Figure 2b, it can be said that the tin toxicity made a curve with the proceeding from early maturity (IMG) to fruit ripening (FR) stage, as fruit pulp and leaves showed that the tin content were decreased at fruit physical maturity but increased at ripening stage. Maximum tin level $(591.47 \pm 7.16 \,\mu g \, kg^{-1})$ was indicated by loquat seeds at early maturity stage, while the lowest concentration (41.07 \pm 1.39 µg kg⁻¹) was observed in fruit peel at mature green (MG) stage. At full ripe stage, tin concentration (484.20 \pm 22.09 µg kg⁻¹) in fruit pulp of loquat was observed under the safe limit (250 mg kg⁻¹) determined by USDA [USDA (FAS) 2018]. The results shown in Figure 2c indicate a significant decrease in antimony concentration present in fruit pulp and seed of loquat, whereas a remarkable increase was observed in antimony toxicity in fruit peel and leaf blade from early maturity (IMG) to fruit ripening (FR) stage. A great difference in antimony concentration with fruit maturity was observed in loquat seeds when the concentration was decreased up to 1/6 of initial value.

Various scientists have reported in their reports about toxic elements concentration in foodstuff that fruits contain mercury (Hg) as a trace element; in a minute quantity [Hajeb et al. 2014]. The reason of its presence in trace amount is might be its affinity to humic substances [Tangahu et al. 2011]. Our results are also in correspondence with the above-mentioned findings indicate trace availability of Hg in different plant tissues of loquat. Loquat seeds having maximum mercury level (19.6 $\pm 1.4 \ \mu g \ kg^{-1}$) at early maturity stage showed significant variation among all other plant tissues, while the lowest mercury concentration $(1.16 \pm 0.18 \,\mu g \,kg^{-1})$ was observed in fruit peel of loquat as shown in Figure 2d. Results were indicative that the toxicity of mercury in seeds and fruit pulp was reduced with the proceeding from early to late fruit maturity stage. Whereas fruit peel and leaves showed a non-significant difference in mercury level.

A lot of research has been done in last decade to eradicate its toxicity from foodstuff. In a report of USDA (Foreign Agricultural Service, China), the maximum limit of mercury in fruits and vegetables is reported as 0.01 mg kg⁻¹ [USDA (FAS) 2018]. Mercury is a highly toxic element much studied among toxic elements. Its exposure can cause severe damage to brain, kidneys and fetus [Castro-González and Méndez-Armenta 2008].

Lead (Pb) concentration (µg kg⁻¹) observed in different plant tissues of loquat indicated a significant decrease in its concentration present in fruit pulp and seed, whereas a remarkable increase was observed in lead toxicity in fruit peel and leaf blade from early maturity to fruit ripening stage. A great difference in lead concentration with fruit maturity was observed in fruit pulp when the concentration was decreased up to 1/11 of the initial value as shown in Figure 3a. Maximum lead level (4248.95 $\pm 111.98 \ \mu g \ kg^{-1}$) was indicated by loquat leaves at fruit ripening stage, while the lowest concentration (53.11 $\pm 1.3 \ \mu g \ kg^{-1}$) was observed in seeds of loquat fruit at mature green stage. Recent studies show that the lead has the opposite behavior of cadmium [Sridhara Chary et al. 2008]. Lead contents in the plants depend upon its availability in the soil (Tab. 3). The analysis results were indicative that the toxicity of silver in leaves, fruit seeds and pulp was reduced with the proceeding from immature green stage to full ripe stage. Whereas fruit peel showed a significant increase in silver concentration. Loquat seeds having maximum silver level (121.55 \pm 1.07 µg kg⁻¹) at early maturity stage showed significant variation among all other plant tissues, while the lowest silver concentration $(1.79 \pm 0.05 \,\mu g \, kg^{-1})$ was observed in fruit pulp of loquat at fruit maturity stage as show in Figure 3b.

CONCLUSION

The results presented in this paper suggest that the aluminum and lead concentrations in the soil, leaves and fruits of loquat are found maximum (>1 mg kg⁻¹) among all other toxic elements ($\leq 600 \ \mu g \ kg^{-1}$). The concentrations of Be, Al and Hg in all observed tissues were significantly reduced with the change in maturity of fruits from immature green to full ripe. Whereas the toxicity of Ti, As, Cd, Sb and Pb were observed to be shifted from seed & pulp to peel & leaves. The concentrations were below than recommended intake level, so the daily intake of toxic elements through fresh fruits may not constitute a health hazard for consumers. However, these amounts can be hazardous if the fruits are taken in large quantities. Although these results described the concentrations of toxic elements in loquat fruits, there is still lack of information about the toxic elements' concentrations in other fruits. Ultimately more research is needed to find out the toxicity level of fruits.

CONFLICT OF INTEREST

None

AUTHORS CONTRIBUTION

Authors of manuscript equally contributed in planning, execution, data collection, statistical analysis, draft development, commenting, revising and approving the manuscript for submission.

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