

BIOACCUMULATION AND SAFETY ASSESSMENT OF HEAVY METALS (PB, CR, CD) IN COMMERCIAL DATE PALM (*PHOENIX DACTYLIFERA* L.) CULTIVARS OF NORTHERN SINDH, PAKISTAN

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Abstract

The Heavy Metals Cadmium (Cd), Chromium (Cr) and Lead (Pb) analysed from five date palm varieties Aseel, Karbalain, Kupro, Dedhi and Fasli of five commercial date palm growers in Taluka Pano Akil, Sukkur, Pakistan. The results of ICP-OES analysis of harvested fruit samples revealed significant differences among the varieties in their uptake of Lead, Cadmium, Chromium and trace elements: Karbalain showed the highest Lead uptake (2.4 mg/kg), Aseel the highest Cadmium uptake (0.04mg/kg), Dedhi the highest Chromium uptake (2.3mg/kg), whereas Kupro showed the lowest Lead and Cadmium uptake, while Aseel showed the lowest uptake of Chromium and trace elements. The concentrations of metals were found to be below the Maximum Allowable Levels recommended by the international standard making bodies in the majority of cases in the safety evaluations. The concentrations of metals were found to be below the Maximum Allowable Levels recommended by the international standard making bodies in the majority of cases in the safety evaluations. In general, dates cultivated in Taluka Pano Akil are safe to be consumed by humans; however, environmental monitoring is suggested for future to control the pollution risk.

1.0 INTRODUCTION

Heavy metals (5 g/cm³) are persistent environmental pollutants because they are non-biodegradable, highly stable and accumulate in terrestrial food chains (Kazi, et al., 2018; Nizamani, et al., 2019). Natural background concentrations are set up by geogenic processes such as rock weathering and soil parent material erosion (Memon, et al., 2019). Nevertheless, the anthropogenic sources like industrial effluents, urban runoff, vehicle emissions, untreated wastewater irrigation, and intensive application of agrochemicals significantly increase toxic metal

levels in agricultural soils (Afridi, et al., 2017; Nizamani, et al., 2019). As a result of this, non-essential heavy metals (Lead (Pb), Cadmium (Cd), and Chromium (Cr)) cause severe toxicological effects upon chronic dietary ingestion such as nephrotoxicity, hepatotoxicity, neurodevelopmental deficits, cardiovascular diseases and carcinogenesis (US-EPA, 2002; Järup, 2003).

The date palm (*Phoenix dactylifera* L., family Arecaceae) is a socio-economically important horticultural crop in hot, dry regions of North Africa, the Middle East and South Asia (Abul-Soad, et al., 2017). In addition to high caloric

density (2,500-3,000 kcal/kg), dates provide a good source of dietary fiber, sugars (fructose and glucose), amino acids, vitamins and polyphenolic antioxidants that act as bioactive compounds (Baliga, *et al.*, 2010; Younas, *et al.*, 2020). Pakistan is a significant producer of date palm; Northern Sindh in particular is the Sukkur and Khairpur districts, which are the national commercial date producing areas (Jatoi, *et al.*, 2009). The area is mainly using Aseel cultivar for fresh and dried (tamar and chuhhara) processing while indigenous cultivars such as karbalain, fasli, dedhi, and kupro are also used. Fruit development is divided into five stages of phenology known as Hababouk (newly set fruits), Kimri (unripe green fruits), Khalal or Doka (physiologically mature fruits), Rutab (partially soft fruits) and Tamar (fully mature and shelf-stable fruits) (Abul-Soad, *et al.*, 2017).

1.1 Contamination Pathways and Research Justification

Date palms are physiologically tolerant to extreme climatic conditions such as heat and salinity, but they have large root systems and perennial leaves, which raise the exposure of date palms to environmental contaminants (Salama, *et al.*,

2019). The main routes of toxic metals entering the fruit matrix are through root uptake from polluted soil and irrigation water and by moving in the xylem to the pericarp. Moreover, heavy metals build-up occurs due to the direct deposition of airborne particulates from traffic emissions and industrial activities, as well as unhygienic post-harvest drying and storage in the roadsides (Salama, *et al.*, 2019; Al-Mazroui, *et al.*, 2020). Crop safety is vulnerable to the use of low quality irrigation water, unstandardized use of agrochemicals, and exposure to transport in development areas such as Taluka PanoAkil. Heavy metal assessment is essential to safeguard public health and meet international phytosanitary and export requirements in different production areas and cultivars

2.0 Methodology

Date fruit samples were collected from three different agricultural areas of Taluka PanoAkil of District Sukkur, Sindh, Pakistan namely from the urban/semi urban zone adjacent to commercial transport routes (PanoAkil City), peri-urban agricultural zone (Sanghi Village) and rural farming zone (Daffar Village).

2.1 Sample collection and procedures of analysis

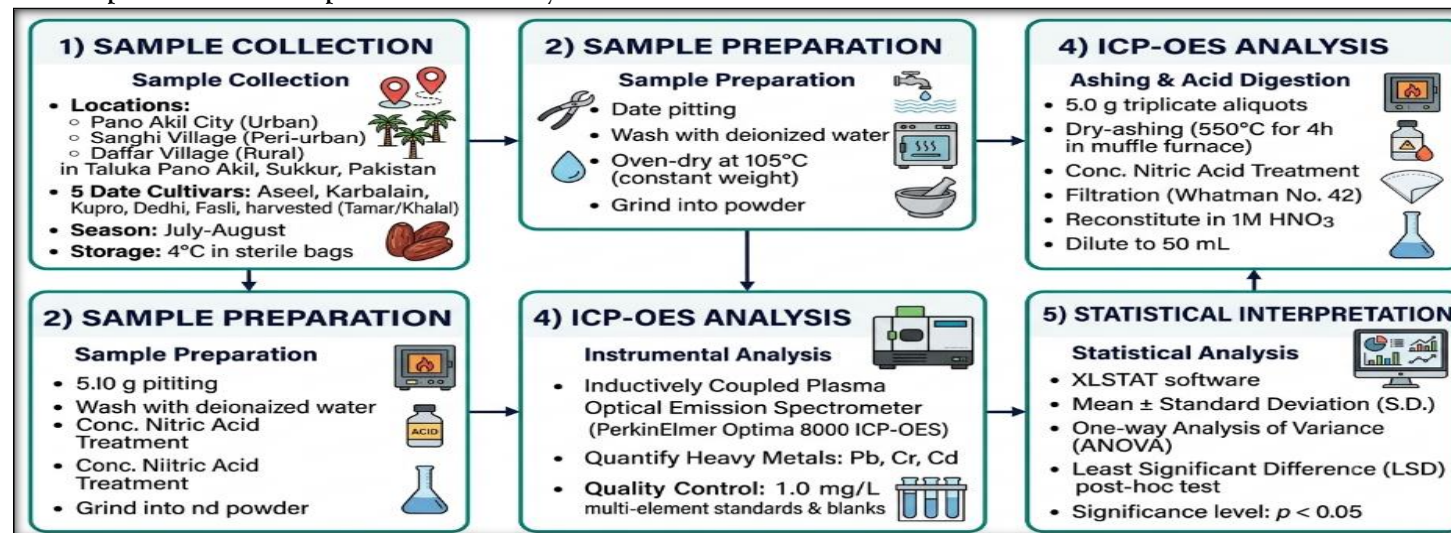


Figure: 01 shown the schematic representation of date fruit sample collection, sample preparation, ICP-OES analysis and statistical interpretation

Mature date fruits (Tamar or Khalal stage) of five cultivars (Aseel, Karbalain, Kupro, Dedhi and Fasli) were collected at the peak of the season (July–August) and put in sterile polyethylene bags in cold room (4°C).

2.2 Preparation and Digestion

Pitted fruit flesh was thoroughly washed with deionized water, dried at 105°C to constant weight, and ground to powder. Five (5) grams of each sample was dried ash at 550°C for four (4) hours in a muffle furnace. The ash produced was treated with very concentrated nitric acid, the ash was then reconverted into 1M nitric acid, filtered through Whatman No”42 paper and diluted to 50 mL with deionized water.

2.3 Instrumental Analysis

Heavy metal concentrations (Pb, Cr and Cd) were determined by Instrumental Analysis, Inductively Coupled Plasma Optical Emission Spectrometer (PerkinElmer Optima 8000 ICP-OES). A multi-element calibration standard (1.0 mg/L) and reagent blanks were used for analytical quality control.

2.4 Statistical Interpretation

Data were statistically analysed using XLSTAT software and expressed as Mean p values and standard deviation (S.D.) from triplicate determinations. Differences across date varieties and sampling plots were evaluated using one-way Analysis of Variance (ANOVA), with mean comparisons conducted via the Least Significant Difference (LSD) post-hoc test at a significance level of $p < 0.05$.

3.0 Results and discussion

To evaluate the safety of the examined dates, experimental results were compared against international Maximum Allowable Limits (MAL) established by the WHO/FAO, the European Union (EU), and Indian Standards (Awasthi, 2000; Khan, *et al.*, 2019). The detected elevated level of lead (Pb) in Karbalain (2.4193 mg/kg) and Fasli (1.7953 mg/kg) as compared to other cultivars corresponds with the results of the regional and international literature. Tunisia studies showed higher ranges of Pb (0.185 to 3.847 mg/kg; Ben Hassine, *et al.*, 2015). In Iran, mean Pb levels were found to range from 0.011 to 0.273 mg/kg, and in Tunisia were from 0.108 to 1.250 mg/kg (Akbari, *et al.*, 2019; El-Said, *et al.*, 2021, respectively).

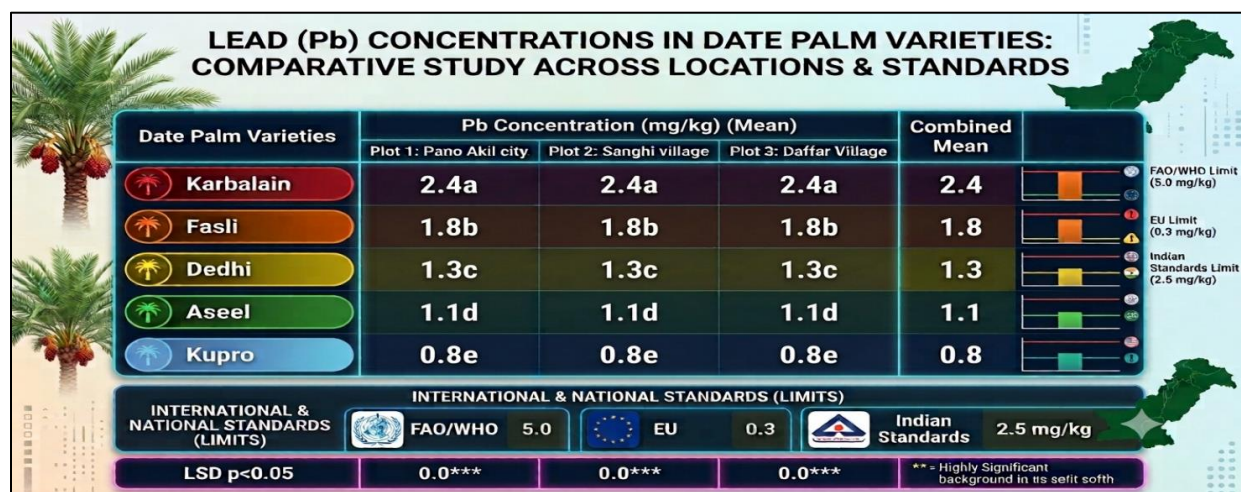


Figure: 02 Lead (Pb) concentrations in five date palm varieties across three sampling locations compared with international and national permissible limits.

The overall mean Pb concentrations followed the order: Karbalain (2.4 mg/kg) > Fasli (1.80 mg/kg)

>Dedhi (1.34mg/kg) > Aseel (1.1 mg/kg) >Kupro (0.78mg/kg). All varieties complied with the

WHO/FAO limit of 5.0 mg/kg and the Indian Standard of 2.5 mg/kg while all the varieties were found to have violated the EU limit of 0.3 mg/kg. The relatively high concentrations of Pb in Plot 1 (PanoAkil City) indicate that atmospheric deposition from vehicle exhausts and commercial transport along the urban roadways is a factor. During the soft ripening stage of dates (Rutab and Tamar), there is a high tendency to get sticky surfaces with high sugar content, which makes dates sticky and causes lead to accumulate on their surface (Salama, *et al.*, 2019). All cultivars met the WHO/FAO guideline (5.0 mg/kg) but not the strict EU

standard (0.3 mg/kg), which may cause problems with export markets to the EU.

The results of the statistical analysis showed significant difference ($P < 0.0001$) for the five date palm varieties in all the sampling locations in respect to the level of their Pb concentrations. The Dedhi variety had the highest mean level of Cr (2.307 mg/kg), which is close to the limits set by WHO/FAO and EU (2.3 mg/kg). The remaining cultivars showed much lower values, ranging from Kupro (0.9899 mg/kg) down to Aseel (0.0215 mg/kg). The Indian Standard MAL for all the samples was found to be below the limit of 20.0 mg/kg.

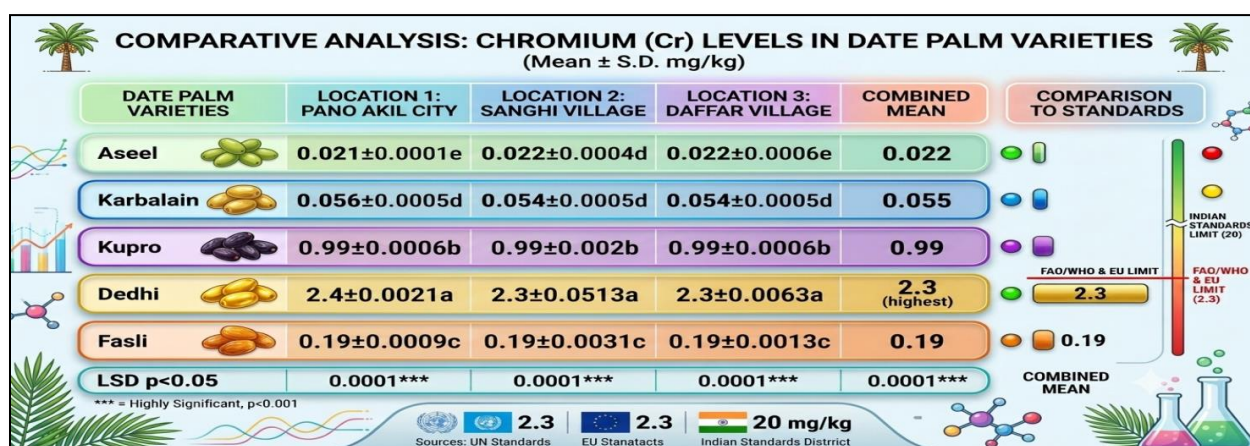


Figure: 03 Comparative analysis of chromium (Cr) concentration (mg/kg) in five date palm varieties collected from three locations in taluka Pano Aqil District Sukkur Sindh Pakistan.

The lowest level of chromium (Cr) was found in Aseel (0.02 mg/kg) and the highest level of chromium (Cr) was found in Dedhi (2.307 mg/kg). The results measured in Dedhi exceeded the threshold set by WHO/FAO (2.3 mg/kg). The concentrations of Cr in dates from Iran ranged from 0.10 to 1.30 mg/kg (Akbari, *et al.*,

2019) and 0.15 to 0.23 mg/kg in Omani dates (Al-Farsi, *et al.*, 2007). The reason for the higher Cr concentration in Dedhi could be the presence of certain physiological uptake mechanisms or soils with a parent material containing a higher concentration of trace chromium salts.

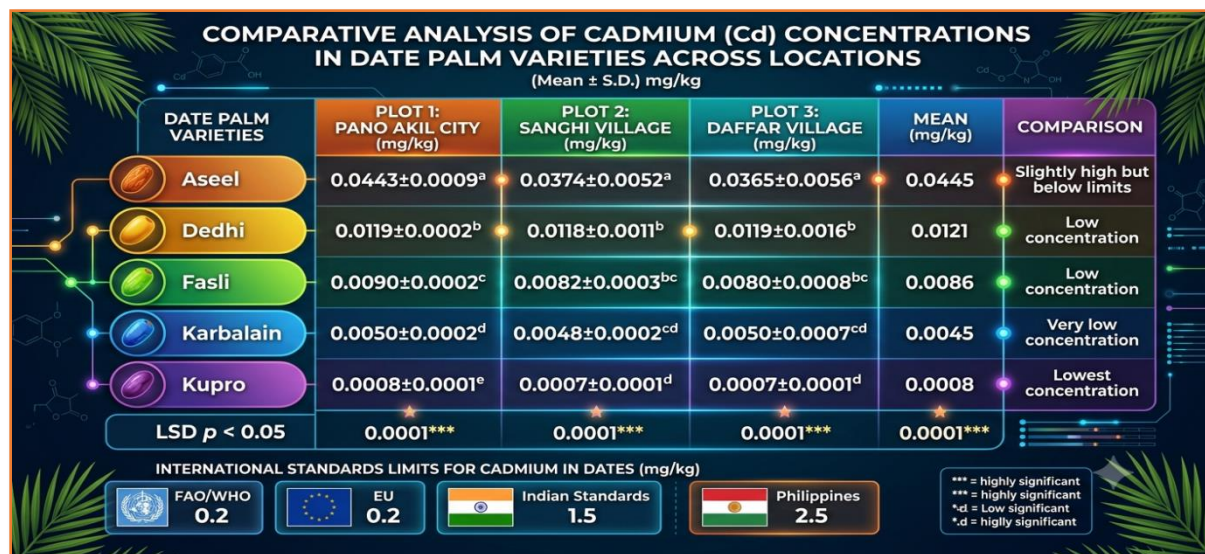


Figure: 04 Comparative analysis of Cadmium (Cd) concentrations (mg/kg) in five date palm varieties collected from three locations in taluka Pano Aqil, District Sukkur, Sindh Pakistan.

Cadmium, (Cd) levels varied somewhere between 0.0008 mg/kg (Kupro) and 0.04 mg/kg (Aseel). These results feel in step with Al-Farsi, *et al.*, (2007), where Cd was noted in the range of 0.002 to 0.024 mg/kg, and also with Al-Hooti, *et al.*, (2011), who reported Cd values from 0.005 to 0.013mg/kg among Kuwaiti dates. Now, the frequent use of phosphate fertilizer, basically acts like a routine agricultural route where cadmium can come into soils (Memon, *et al.*, 2019). Overall, Cd in every sampled date looked well

under the global benchmarks, WHO/FAO: 0.2 mg/kg, and Indian Standards: 1.5 mg/kg. The average values of Cd across all samples in descending order were Aseel (0.0445 mg/kg) >Dedhi (0.0121 mg/kg) >Fasli (0.0086 mg/kg) >Karbalain (0.0045 mg/kg) >Kupro (0.0008 mg/kg). All the samples were safe and within the range of recommended limits; below 0.2 mg/kg (WHO/FAO and EU) and under 1.5 mg/kg (Indian Standards).

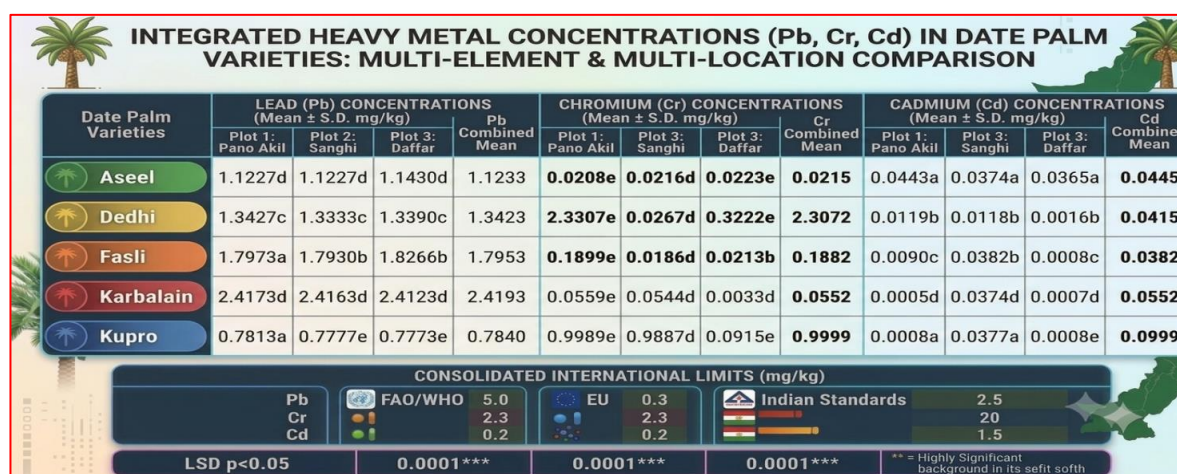


Figure: 05 integrated heavy metal concentration (Pb, Cr, Cd) in Date Palm varieties across three sampling locations.

This integrated dataset shows very significant statistical differences ($p < 0.0001$) in heavy metal accumulation (Pb, Cr, Cd) between the five date palm cultivation and three agriculture plots studied, thereby indicating synergistic combined effect of cultivar uptake mechanisms and localised environmental deposition. Karbalain, with 2.4193 mg/kg of lead, obtained the highest amount of lead, compared to Kupro (0.78 mg/kg). Although all the cultivars were below the WHO/FAO limits (5.0 mg/kg) and Indian Standards (2.5 mg/kg), every cultivar was higher than the European Union standard (0.3 mg/kg) largely due to high vehicle emissions in Plot 1 (PanoAkil City). Significantly high accumulation was observed in Dedhifor chromium (2.30 mg/kg), where the result

approached the WHO/FAO and EU limit (2.3 mg/kg) while Aseel showed minimum chromium accumulation (0.0215 mg/kg).

Cadmium accumulation was low in all the selected cultivars and the highest cadmium value was reported in Aseel 0.04 mg/kg which was found under the international standards (0.2 mg/kg). Thus, it can conclude that dates produced in Taluka PanoAkil are within WHO/FAO limit for human use and however, widespread pollution of European Union Lead standards is another factor which impedes the export in global market; thus, the cultivar with low accumulation of lead should be introduced in areas with high accumulation of lead, such as Kupro cultivar.

Table: 01 Shown the Pearson correlation analysis of Heavy metals (Pb, Cr, Cd).

PEARSON CORRELATION ANALYSIS OF HEAVY METALS (Pb, Cr, Cd)				
Symmetric Correlation Matrix (r)				
Heavy Metals	Lead (Pb)	Chromium (Cr)	Cadmium (Cd)	
Lead (Pb)	1.000	-0.383	-0.271	
Chromium (Cr)	-0.383	1.000	-0.274	
Cadmium (Cd)	-0.271	-0.274	1.000	
Heavy Metal Pair	Pearson Correlation (r)	p-value	Significance ($p < 0.05$)	Relationship
Lead (Pb) vs. Chromium (Cr)	-0.383	0.524	Non-Significant (ns)	Weak Negative
Lead (Pb) vs. Cadmium (Cd)	-0.271	0.659	Non-Significant (ns)	Weak Negative
Chromium (Cr) vs. Cadmium (Cd)	-0.274	0.656	Non-Significant (ns)	Weak Negative

Independent Accumulation: All three heavy metals exhibit weak, non-significant negative correlations ($p > 0.05$) with one another across the date palm varieties.
Biological Implication: The uptake, absorption, and translocation of Pb, Cr, and Cd operate independently driven by distinct physiological mechanisms and environmental contamination sources (e.g., vehicular emissions for Pb vs. agricultural inputs for Cd).

Pearson correlation showed non-significant and weak correlations between the examined pairs of the heavy metals ($p > 0.05$); Lead vs. Chromium showed $r = -0.383$, $P = 0.524$; Lead vs. Cadmium $r = -0.271$, $P = 0.659$ and Chromium vs. Cadmium $r = -0.274$, $P = 0.656$; this is evidence of the independent bio-accumulation of Pb, Cr and

Cd in date palm fruit that is attributed to their transport via independent plant nutrient uptake mechanisms and their availability through separate routes of exposure from the environmental environment such as auto emission for the Lead and agricultural practices for the Cadmium.

Table No: 02 Human-health risk assessment of EDI, THQ, HI and carcinogenic risk (CR) for Pb using different Date Varieties

Human Health Risk Assessment of Heavy Metals in Different Date Varieties						
Heavy Metal	Date Variety	Concentration (mg/kg)	EDI (mg/kg-day)	THQ	HI	CR
Lead (Pb)	Karbalain	2.4193	0.003456	0.987	0.987	2.94×10^{-5}
	Fasli	1.7953	0.002565	0.733	0.733	2.18×10^{-5}
	Dedhi	1.3423	0.001918	0.548	0.548	1.63×10^{-5}
	Aseel	1.1233	0.001605	0.458	0.458	1.36×10^{-5}
	Kupro	0.7840	0.001120	0.320	0.320	9.52×10^{-6}
Chromium (Cr)	Dedhi	2.3070	3.296×10^{-3}	1.0986	1.0986	1.65×10^{-3}
	Kupro	0.9899	1.414×10^{-3}	0.4714	0.4714	7.07×10^{-4}
	Fasli	0.1882	2.689×10^{-4}	0.0896	0.0896	1.34×10^{-4}
	Karbalain	0.0552	7.89×10^{-5}	0.0263	0.0263	3.94×10^{-5}
	Aseel	0.0215	3.07×10^{-5}	0.0102	0.0102	1.54×10^{-5}
Cadmium (Cd)	Aseel	0.0445	6.36×10^{-5}	0.0636	0.0636	3.88×10^{-4}
	Dedhi	0.0121	1.73×10^{-5}	0.0173	0.0173	1.05×10^{-4}
	Fasli	0.0086	1.23×10^{-5}	0.0123	0.0123	7.49×10^{-5}
	Karbalain	0.0045	6.43×10^{-6}	0.00643	0.00643	3.92×10^{-5}
	Kupro	0.0008	1.14×10^{-6}	0.00114	0.00114	6.97×10^{-6}
Exposure & Health Risk Hierarchies						
Pb Exposure & Risk: Karbalain > Fasli > Dedhi > Aseel > Kupro						
Cr Exposure & Risk: Dedhi > Kupro > Fasli > Karbalain > Aseel						
Cd Exposure & Risk: Aseel > Dedhi > Fasli > Karbalain > Kupro						

Exposure & Health Risk Hierarchies

Pb Exposure & Risk: Karbalain > Fasli > Dedhi > Aseel > Kupro

Cr Exposure & Risk: Dedhi > Kupro > Fasli > Karbalain > Aseel

Cd Exposure & Risk: Aseel > Dedhi > Fasli > Karbalain > Kupro

4.0 Conclusion

This study reaffirms a significant cultivar dependent build-up of metals in Taluka PanoAkil dates. Karbalain showed highest level of Lead (2.4 mg/kg) at this Taluka and Dedhi of Chromium (2.30 mg/kg) and Cadmium was highest in Aseel (0.04 mg/kg). In case of Kupro it showed the lowest uptake across all metals. Heavy metals were within WHO/FAO as well as Indian standards, thus indicating safe consumption of fruits. But lead crossed the limit of strict EU export standard (0.3 mg/kg) in all the cultivars due to road side atmospheric deposition and chromium crossed WHO/FAO standard in the variety Dedhi. Hence to avoid such problem, we should cultivate low accumulative varieties (Kupro) in high traffic areas, observe use of agro chemicals and improve processing after harvest.

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Conflict of interest

The authors declare that they have no conflict of interest regarding the research, authorship, or publication of this manuscript.

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