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# PHYSICOCHEMICAL PROPERTIES AND MINERAL ELEMENT COMPOSITION OF POMEGRANATE (*PUNICA GRANATUM* L.) FROM BALOCHISTAN, PAKISTAN: AN ATOMIC ABSORPTION SPECTROSCOPIC STUDY

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**Abstract**

***Background:*** *The pomegranate (Punica granatum L.) is a significant fruit crop because of its nutritional and health-promoting characteristics. Nonetheless, variations in environmental conditions and the accumulation of heavy metals might have an impact on the quality of the fruit and on food safety. The present study evaluated pomegranate's selected physicochemical properties and heavy metal contents for samples obtained from various areas of Balochistan, Pakistan.*

***Methodology:*** *Khuzdar, Zehri, Baghbana, Zeedi, and Wadh are amongst the various regions of Balochistan where samples were collected. Cadmium (Cd), Copper (Cu), Nickle (Ni), Lead (Pb), and Zinc (Zn) were analyzed using Atomic Absorption Spectroscopy (AAS) after standard sample preparation and acid digestion while Physicochemical properties, including pH, ash content, and titratable acidity, were also determined.*

***Results:*** *The ash content and titratable acidity ranged from 0.3–0.7% and 0.5–1.5 respectively, while pH ranged from 2.2 to 3.8, confirming the acidic nature of pomegranate. All the metals analyzed were found with a significant level of regional variation. Zinc was the most abundant metal, followed by copper, nickel, lead and cadmium. Khuzdar generally exhibited higher metal concentrations than Wadh, which showed comparatively lower concentrations for most of the analyzed metals. These variations may be due to differences in soil, irrigation water, agricultural methods, and other environmental conditions.*

***Conclusion:*** *The results revealed the regional differences in the physicochemical and elemental composition of pomegranates from Balochistan. The physicochemical results in general could not be directly related to metal accumulation because region-specific values were not reported; however, the findings highlight the need for regular monitoring of heavy metals in fruits and their growing environments to support food safety and maintain fruit quality.*

**Keywords:** *Atomic absorption spectroscopy (AAS), Balochistan, Heavy metals, Pomegranate*

## INTRODUCTION

One of the most primitive fruit species to be cultivated, the pomegranate (*Punica granatum* L.) is a member of the *Punicaceae* family and is widely grown in the Mediterranean basin, the Middle East, Central Asia, and South Asia. Pomegranate has become an economically significant fruit and is becoming more widely acknowledged as a functional food due to its remarkable nutritional profile and medicinal potential (1). Rich in polyphenols (2), ellagitannins, anthocyanins, flavonoids, organic acids, vitamins, dietary fiber, and essential minerals (3), the fruit's various anatomical parts such as the peel, arils, juice, and seeds all contribute to its powerful antioxidant, antimicrobial (4), anti-inflammatory (5), cardioprotective, antidiabetic (6), and anticancer properties (7, 8). Consequently, pomegranate has attracted considerable interest from researchers and the food industry for its potential applications in pharmaceuticals, functional foods, and nutraceuticals (9-11). Physicochemical characteristics are important attributes of pomegranate fruit, as they influence its nutritional and commercial quality and serve as key indicators of fruit maturity, processing suitability, storage stability, and consumer acceptability. Parameters such as moisture content, ash content, pH, total soluble solids (TSS), titratable acidity, electrical conductivity and soluble sugar concentration are frequently used to assess fruit quality and authenticity. Cultivar, geographic origin, climate, soil composition, irrigation techniques, and postharvest processing all have a significant impact on these traits (12). Therefore, thorough physicochemical characterization is necessary for standardization of processed pomegranate products, cultivar classification, and quality control (11).

As mineral elements are essential for maintaining normal physiological and metabolic functions in humans, the mineral composition of fruits has become an important aspect of nutritional evaluation, complementing conventional quality attributes. Potassium (K), calcium (Ca), magnesium (Mg), sodium (Na), and phosphorus (P) are essential macrominerals that support cardiovascular health, bone growth, muscle contraction, and electrolyte balance (13, 14). Likewise, trace metals like iron (Fe), zinc (Zn), copper (Cu), and manganese (Mn) are essential cofactors in many antioxidant and enzymatic defense systems (15). On the other hand, hazardous heavy metals like lead (Pb), cadmium (Cd), chromium (Cr), and nickel (Ni at excessive concentrations) can accumulate in edible plant tissues through contaminated soils, irrigation water (16), fertilizers, industrial emissions, or atmospheric deposition. Those heavy metals can pose substantial risk to human health even at low concentrations (17, 18). Therefore, determining simultaneously both hazardous and essential constituents is crucial for evaluating food items' safety and nutritional worth. For the quantitative determination of metallic elements in biological and food matrices, Atomic Absorption Spectroscopy (AAS) remains a widely used and established elemental analysis technique. This technique offers good sensitivity, selectivity, analytical precision, and reproducibility following appropriate sample preparations. AAS has become a standard analytical tool in food quality assessment (19) environmental monitoring, nutritional studies and regulatory surveillance for identifying essential minerals and potentially hazardous heavy metals because it is relatively accessible and cost-effective as compared to other advanced multi-element techniques. Its use is especially beneficial for assessing fruit quality and ensuring compliance with international food safety standards (20, 21).

Though several studies have focused on the phytochemical composition and biological activities (22-25) of *P. granatum*, comparatively limited information is available on the combined physicochemical and elemental composition. Furthermore, significant diversity in the chemical composition of minerals among pomegranates cultivars has been observed due to the genotype, geographical location, soil, cultivation methods, and environmental factors (26, 27). These variations emphasize the need for region-specific studies on the composition of pomegranates to be able to develop accurate nutritional databases and quality standards for pomegranate fruits. This information is vital especially in the development food authentication, nutritional labeling, quality assurance, and development of value-added functional food products. To the best of our knowledge, this is the first study to report the heavy-metal profile (Cd, Cu, Ni, Pb, and Zn) along with key physicochemical characteristics (pH, ash content, and titratable acidity) of pomegranates from the selected regions of Balochistan. Hence, the current research aims to establish the physicochemical properties as well as the elemental composition of *Punica granatum* through standard analytical methods. The physicochemical properties were analyzed to establish the qualities of the fruit, while the elemental composition was carried out through Atomic Absorption Spectroscopy (AAS) to quantify essential mineral elements and selected heavy metals. The findings of this investigation are expected to provide comprehensive baseline information regarding the nutritional quality, mineral composition, and safety profile of pomegranate, thereby contributing to food quality assessment, nutritional research, and future industrial applications of this economically important fruit.

# MATERIALS AND METHODS

## PLANT COLLECTION

Fresh pomegranate (*Punica granatum* L.) fruits were collected from five major producing regions of Balochistan, Pakistan. These sampling sites are located across the District Khuzdar zone, encompassing Khuzdar, Baghbana, Zeedi (District Khuzdar), district Wadh, and Zehri (District Surab). Sampling was carried out during the harvesting season of (15-10-2025) (Figure 1). A pomegranate fruit samples (5 samples from each region) were collected. Each sample represented fruits obtained from an individual orchard. Samples were collected randomly from different locations within each orchard to minimize sampling bias. Samples were not pooled and analyzed individually.

## PLANT IDENTIFICATION

The plant samples were identified by a texanomist from the Department of Botany, University of Balochistan, Quetta.

## SAMPLE PREPARATION

The fruits were washed with tap water, followed by deionized water, to eliminate any dust or contaminants from their surfaces. The fruit parts (edible portion) to be analyzed were then separated. The fruit samples were air-dried in an oven at temperatures between 60-65°C till the constant weight of samples was obtained. The dried samples were ground using a stainless-steel mill and kept in polyethylene containers until further analysis (Fig. 1).

## ACID DIGESTION

Approximately 0.5 g of dried, powdered sample was digested with 10 mL concentrated $HNO_3$. The mixture was allowed to stand for 15 min at room temperature and then heated gradually on a hot plate until a clear digest was obtained. After cooling, the digest was filtered, transferred to a 50 mL volumetric flask, and diluted to the mark with deionized water following the procedure described by Khan et al. (2024) after slight modification (Figure 1; 28). The resulting solution was subsequently used for metal analysis by AAS.


**Fig. 1.** Schematic representation of pomegranate sample preparation and heavy metal determination in pomegranate using Atomic Absorption Spectroscopy (AAS)

## ELEMENTAL ANALYSIS TECHNIQUE

Atomic Absorption Spectrophotometer (FAAS, Model S4 AA, Thermo Scientific, USA) was used to analyze the levels of heavy metals (Cd, Cu, Ni, Pb and Zn). It employed special hollow cathode lamps to represent each metal, and an air-acetylene flame to change the samples into atoms. The settings of the instrument, which include the wavelength, slit width and lamp current, were set as per the instructions of the manufacturer regarding each metal. Metal concentrations were calculated and expressed as mg/Kg dry weight (ppm).

## QUALITY CONTROL AND QUALITY ASSURANCE

To minimize potential contamination, all glassware was soaked overnight in 10% nitric acid and subsequently rinsed thoroughly with deionized water before use.

## STATISTICAL ANALYSIS

All heavy-metal measurements were performed in triplicate. However, no statistical analysis was conducted in the present study, and the analytical results were reported as individual concentration values.

## PHYSICOCHEMICAL ANALYSIS

The physicochemical standard parameters were determined following standard AOAC (2019) procedures, with modifications from new studies on pomegranate fruit and drinks.

## PH MEASUREMENT

The pomegranate fruits were manually deseeded, and the extracted juice was thoroughly mixed. The pH was determined using a calibrated and standardized digital pH meter. Readings were noted at atmospheric temperature.

## TITRATABLE ACIDITY (TA)

The pomegranate juice total acidity was determined by titrating 10mL aliquot against 0.1 $M_{NaOH}$ using phenolphthalein as an indicator (29). The acid level was measured as percentage (%) citric acid according to the equation.

$$TA(\%\ citric\ acid) = \frac{V_{NaOH} \times M_{NaOH} \times E}{V_{sample}} \times 100$$

Where $V_{NaOH}$ is the volume of titrant (L), $M_{NaOH}$ its molarity, E the equivalent weight of citric acid (64 g/mole), and $V_{sample}$ the juice volume (mL). This procedure has been used to pomegranate juice and concentrate characterization.

## ASH CONTENT

The mineral remaining (ash) of pomegranate extract were find out by incinerating dehydrated samples in a chamber furnace at 550 °C until a light gray ash was gained. Crucibles were dried to a constant mass in a desiccator and weighed, and the ash content were calculated as:

$$Ash(\%) = \frac{\text{Weight of ash}}{\text{Initial dry sample weight}} \times 100$$

These methods are accordant to latest work appraising pomegranate harvest for structural variations (29).

# RESULTS

The elemental composition of pomegranate samples from five major pomegranate-producing regions of Balochistan (Baghbana, Khuzdar, Wadh, Zehri, and Zeedi) was evaluated by Atomic Absorption Spectroscopy (AAS) to determine the concentrations of cadmium (Cd), copper (Cu), nickel (Ni), lead (Pb), and zinc (Zn). The concentration of heavy metals was different at different sampling sites, which showed regional differences in heavy metal accumulation. The zinc was found to be the most found element in all samples, and its concentrations were between 2.3683 mg/kg to 4.5192 mg/kg dry weight, where the highest amount of zinc was found in Khuzdar (4.5192 mg/kg), followed by Zeedi (3.9348 mg/kg), Baghbana (3.1158 mg/kg), Wadh (2.6794 mg/kg), and Zehri (2.3683 mg/kg), whereas the FAO/WHO limit is 60 mg/kg. Copper was the second most abundant metal, varying between 0.8345 and 2.0329 mg/g, with the maximum concentration observed in Khuzdar and the minimum in Wadh, whereas the FAO/WHO limit is 40 mg/kg. The levels of Nickel concentrations were moderate ranged from 0.1082 to 0.4583 mg/kg, with the highest concentration recorded in Zehri and the lowest in Wadh, whereas the FAO/WHO limit is 10 mg/kg. Lead concentrations were relatively low, ranging from 0.0175 to 0.3087 mg/kg, where Khuzdar exhibited the highest concentration and Baghbana the lowest, whereas the FAO/WHO limit is 0.1 mg/kg. Cadmium was detected at trace levels in all samples, varying between 0.0053 and 0.0368 mg/kg, with the highest concentration observed in Baghbana.

The results of the heavy metal analysis of pomegranate samples using AAS, along with the area-wise comparison of their elemental composition, are presented in Table I and Fig. 2, respectively.

**Table I.** Detection of heavy metals in pomegranate samples using AAS

| S. No. | Collection area | Cadmium | Copper | Nickel | Lead | Zinc |
|---|---|---|---|---|---|---|
| 1 | Khuzdar | 0.0082 | 2.0329 | 0.2307 | 0.3087 | 4.5192 |
| 2 | Zehri | 0.0121 | 1.1575 | 0.4583 | 0.0963 | 2.3683 |
| 3 | Baghbana | 0.0368 | 1.1865 | 0.2376 | 0.0175 | 3.1158 |
| 4 | Zeedi | 0.0114 | 1.5789 | 0.1185 | 0.2517 | 3.9348 |
| 5 | Wadh | 0.0053 | 0.8345 | 0.1082 | 0.0245 | 2.6794 |
| | FAO/WHO permissible limit (mg/kg) | 0.05 to 0.1 | 40 | 10 | 0.1 | 60 |

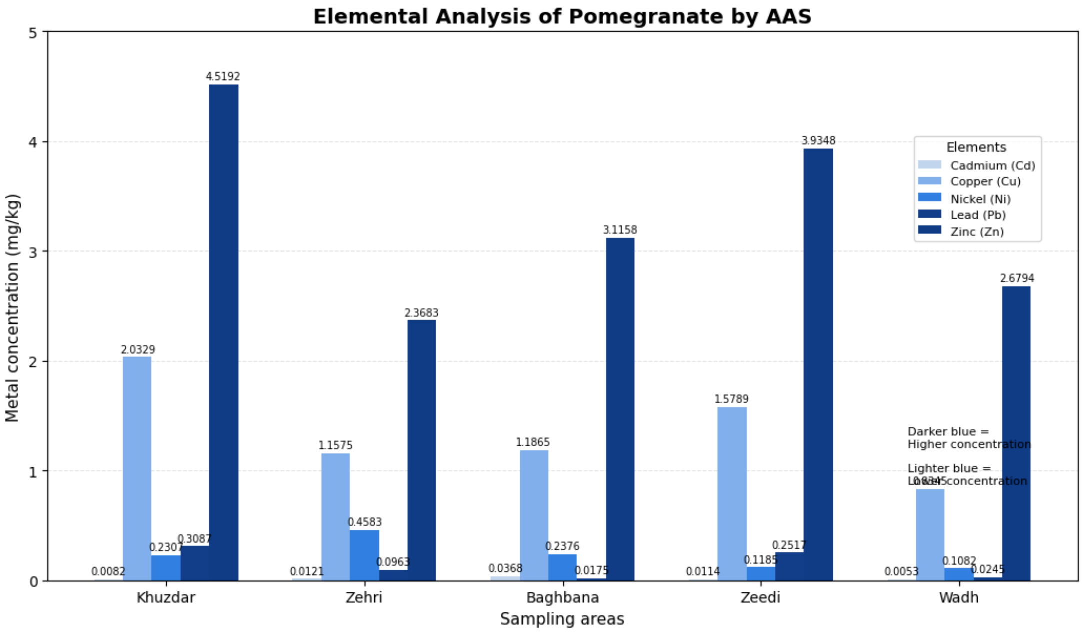


**Fig. 2.** Area wise comparison of elemental analysis of pomegranate by using AAS

## PHYSICOCHEMICAL ANALYSIS

The fundamental characteristics of a substance that comprise both the physical and chemical nature of a substance are known as physicochemical properties. These properties provide valuable insights into the appearance, behavior, and response of a material. Physical properties are characteristics that can be observed or measured without changing the chemical identity of a substance, such as color, odor, density, and temperature. In contrast, chemical properties describe how a substance interacts or reacts with other substances, such as its acidity, pH, and the presence or composition of specific elements or compounds.

The physicochemical properties of pomegranate samples are presented in Table II, while the comparative analysis of pH, ash content, and acidity is shown in Fig. 3.

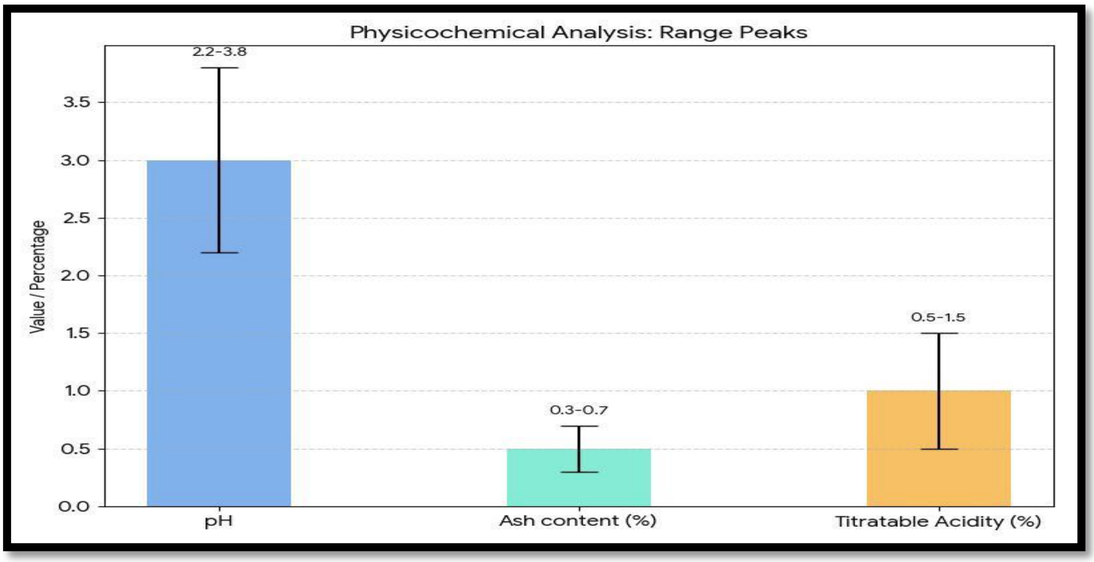


**Fig. 3.** Comparative analysis of pH, ash content, and acidity in pomegranate samples

**Table II.** Determination of physicochemical parameters of pomegranate

| Properties | Physicochemical analysis<br>Range | Factors for variations |
|---|---|---|
| pH | 2.2-3.8 | Increases with ripeness |
| Ash content | 0.3%-0.7% | High mineral salinity |
| Titratable Acidity | 0.5%-1.5% | Heavily dependent |

# DISCUSSION

In the present study, the concentrations of the selected heavy metals (cadmium, copper, nickel, lead and zinc) in pomegranate samples from five regions of Balochistan (Baghbana, Khuzdar, Wadh, Zehri, and Zeedi) were examined by Atomic Absorption Spectroscopy (AAS). Overall, zinc and copper were the predominant trace elements detected in the analyzed pomegranate samples collected from Khuzdar, Zeedi, Baghbana, Wadh, and Zehri, whereas cadmium and lead occurred at comparatively lower concentrations. The concentrations of zinc ranged from 2.3683 to 4.5192 mg/kg dry weight, with the highest concentration recorded in Khuzdar (4.5192 mg/kg), followed by Zeedi (3.9348 mg/kg), Baghbana (3.1158 mg/kg), Wadh (2.6794 mg/kg), and Zehri (2.3683 mg/kg). The elevated levels of zinc found in Khuzdar and Zeedi could be attributed to the differences in the mineral content of the soil, irrigation water, fertilizers, and other agricultural methods which impact the absorption of these metals by plants. Zinc is a trace element necessary for several physiological and biochemical activities such as enzyme function, protein synthesis, antioxidant protection, and immunity (30-31), hence its predominance in pomegranate fruit is not unexpected, but rather nutritionally advantageous at safe levels (60 mg/kg). Likewise, the presence of copper at considerable levels was evident in all the samples. This is another essential trace element that is necessary for plant functions like chlorophyll production, photosynthetic process, lignin production, and the activities of some oxidative enzymes, whereas in humans, it is essential for iron metabolism, production of connective tissues, and antioxidants (30). The levels of zinc and copper were both well below international safety standards of food, implying that these minerals will not cause any adverse effects on health through pomegranate fruits intake.

However, cadmium and lead levels were found to be comparatively low in all the samples. Cadmium was found in the range of 0.0082 mg/kg in Khuzdar to 0.0368 mg/kg in Baghbana, with the intermediate levels being recorded at the other sampling stations, suggesting only minor spatial variation in cadmium accumulation. The concentration levels of lead were found to be low in all samples, which indicated minimal contamination of the cultivation sites. Unlike zinc and copper, cadmium and lead do not play any biological role in the body and are considered even toxic elements that may accumulate in human tissues following prolonged dietary exposure. Chronic exposure to cadmium, on the other hand, has been linked to kidney malfunctioning, bone injury, and abnormalities in calcium homeostasis, while high levels of lead can cause nervous disorders, hematological problems, and mental retardation, especially in children. Consequently, the relatively low levels of both toxic metals in this study indicate minimal environmental contamination of the investigated cultivation areas (32).

The observed differences regarding heavy metal concentrations in Khuzdar, Zeedi, Baghbana, Wadh, and Zehri is probably linked to geochemical properties of soil in the regions, climatic conditions, water for irrigation, use of fertilizers and pesticides, proximity to road networks or mining operations, and other anthropogenic influences. All these factors directly contribute to mobility, bioavailability, and absorption of metals by plant roots and consequently affect the accumulation in edible fruits. Even though Khuzdar had higher levels of trace elements compared to Wadh and Zehri, there was no case of excessive accumulation of toxic metals in any of the samples tested.

First of all, it must be stated that WHO does not prescribe maximum permissible concentration of heavy metals in individual fruits like pomegranate. On the contrary, WHO, through Joint FAO/WHO Expert Committee on Food Additives (JECFA), sets health-based guidance values for dietary exposures, and maximum permissible concentrations of contaminants in foods are set by the Codex Alimentarius Commission and regional regulatory agencies (33) like European Commission. Comparison of the present results with internationally recognized food safety standards clearly demonstrates that concentrations of zinc, copper, cadmium, and lead in all tested samples of pomegranate fruit were within the recommended permissible range for edible fruits. Thus, it is safe to state that pomegranate fruit grown in Khuzdar, Zeedi, Baghbana, Wadh, and Zehri are safe for consumption with respect to their heavy metal contamination.

Physicochemical analysis is an important aspect in the assessment of fruit quality because it helps in the determination of nutritional profile, maturity status, storability, processibility, and acceptability of fruits. Some of the variables which can be used in assessing the quality of pomegranate varieties and determining their quality for commercial purposes include pH, ash content, and titratable acidity. These values depend on factors like genetics, weather, soil, irrigation methods, maturity level, and post-harvest handling. In recent studies, geographical location has been found to have a significant impact on the physicochemical makeup of pomegranates, causing a lot of variability among pomegranates from diverse environments (34-35). Additionally, these quality traits have been strongly related to consumer acceptance and processibility (11).

The mentioned pH range (2.2-3.8) proves that pomegranate is naturally an acidic fruit. The acidity of pomegranate is mostly because of citric acid and malic acid in it. With the maturation of fruits, the organic acids get metabolized by means of respiration, thus leading to an increase in pH and a decrease in acidity. Therefore, pH is considered a criterion for the maturity of fruits, their storage potential, and suitability for processing (34, 36).

The measured ash content (0.3-0.7%) accounts for the mineral residue left behind after burning and serves as an indirect indicator of mineral content of the fruit. The higher the ash content is, the more is the mineral content including potassium, calcium, magnesium, and phosphorus, and hence the better is the nutritional quality of the fruit. Variation in ash content is mainly linked to soil nutrition, fertilization, water quality for irrigation, salinity, and uptake of nutrients by different varieties. Some recent studies have pointed out that mineral content is one of the key quality parameters for nutritional assessment and authentication of pomegranate and its products (37, 11).

Titratable acidity (0.5-1.5%) indicates the total amount of organic acids and is one of the major parameters that influence fruit taste, maturity, and processability. The concentration of titratable acidity decreases during fruit ripening because of the use of organic acid in the process of respiration, whereas sugar contents increase and there will be an increased sugar to acid ratio. Titratable acidity is therefore commonly measured along with total soluble solids to evaluate the stage of harvest maturity. Differences among varieties result from genotypic, environmental conditions, irrigation management, elevation, and harvesting maturity (33, 35).

One of the limitations of the current study is that even though the physicochemical properties (pH, ash content, and titratable acidity) were determined using individual pomegranate samples from each region, these results were reported as overall ranges without region-wise analysis. As a result, no direct correlation could be established between physicochemical properties of different regions and metal accumulation. It is recommended that future researches consider region-wise data concerning the physicochemical properties of pomegranates. At the same time, continuous environmental monitoring of heavy metals is recommended in agricultural soils, irrigation water, and fruit samples to ensure quality food products as well as to detect any possible future metal contamination from such sources as agricultural activities, industrial discharges, mining, and other environmental sources.

## CONCLUSION

The present study assessed cadmium, copper, nickel, lead, and zinc in pomegranate samples from five regions of Balochistan using atomic absorption spectroscopy. Zinc was the most abundant metal, followed by copper, while cadmium showed the lowest concentrations. Khuzdar generally exhibited higher metal accumulation, whereas Wadh showed comparatively lower levels, suggesting that geographical, soil, environmental, and agricultural factors may influence metal accumulation in pomegranate fruits. The findings provide useful baseline data for future monitoring.

The physicochemical analysis showed that the pomegranate fruits were naturally acidic, with variations in pH, ash content, and titratable acidity. These parameters provide useful information regarding fruit quality, maturity, mineral composition, nutritional characteristics, and processing suitability. However, the physicochemical results were presented as overall ranges rather than region-wise values, limiting the assessment of regional differences and the relationship between physicochemical properties and heavy-metal accumulation.

## Limitations:

The study was limited by the absence of formal statistical comparisons and detailed analytical quality-control parameters. Future studies should include larger and regionally representative sample sizes, replicate sampling, statistical analyses such as ANOVA, and comprehensive analytical validation, including calibration and recovery studies and certified reference materials.

## Recommendations:

Region-wise assessment of physicochemical and heavy-metal parameters, along with continuous monitoring of soils, irrigation water, and fruits, is recommended to improve data reliability and support the assessment of pomegranate quality and safety.

## Conflict of interest:

The authors declare that there are no conflicts of interest associated with the publication of this paper.

## Author’s contributions:

HU Methodology, writing & original draft; AI Conceptualization, supervision, project administration, writing original draft; SSK Methodology, Validation, review and editing; IA Methodology & Formal analysis, NF Software & writing, original draft; ST Methodology, Formal analysis & Data curation. KI, SM, AR & RU Writing, review and editing.

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