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Quantifying heavy metal contamination of lychee in the Parana river basin

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Abstract

Lychee (*Litchi chinensis* Sonn.) orchards along the Parana River Basin receive irrigation water and phosphate fertilisers that may introduce cadmium, lead, and arsenic into the fruit production chain. This research quantified heavy metal concentrations in lychee fruit tissues (peel, aril, seed) from 18 orchards and tested whether transcriptomic markers of metal stress correlate with accumulation patterns. Sampling and analysis were carried out through the Melbourne Academy of Horticultural Research from November 2021 to March 2023. Cadmium exceeded the 0.050 mg/kg EU limit in 16.7% of aril samples, with the highest concentrations in orchards on alluvial soils near industrial zones. Lead and arsenic remained below regulatory thresholds across all sites. Tissue partitioning showed that peel accumulated 2.8-fold more cadmium than aril, suggesting a partial barrier function. Hardening treatments (gradual water stress before harvest) reduced aril cadmium by 18.4% relative to fully irrigated controls. Phosphorus cycling analysis linked high soil Olsen-P (>42 mg/kg) with elevated cadmium uptake through competitive cation exchange. Transcriptomic screening identified flavonoid biosynthesis pathway genes that were upregulated under cadmium stress, and total flavonoid content in stressed fruit was 27.3% higher than in low-cadmium orchards. Climate resilience indicators showed that orchards with mulched root zones maintained lower metal uptake during drought episodes. These results map the contamination landscape and point toward agronomic interventions that can reduce heavy metal transfer into the edible portion of lychee.

Keywords: Transcriptomics, hardening, phosphorus cycling, flavonoids, climate resilience, lychee, heavy metals, cadmium, tissue partitioning, Parana River Basin

Introduction

How safe is lychee grown on river-basin soils where decades of phosphate fertilisation and upstream industrial discharge have quietly raised the heavy metal burden? This question matters because lychee a subtropical fruit prized for its translucent aril and floral aroma is consumed raw, with no cooking step to reduce contaminant bioavailability ^[1]. Global lychee production reached 4.9 million tonnes in 2021, with China, India, and Southeast Asian countries contributing over 90% of output, though production is expanding in South America and Australia ^[2].

Cadmium, lead, and arsenic are the three heavy metals of greatest concern in fruit crops. Cadmium enters orchard soils primarily through phosphate fertilisers, which contain 3-100 mg Cd/kg depending on source rock ^[3]. Lead accumulates from historical pesticide use, road dust deposition, and irrigation with river water carrying industrial runoff ^[4]. Arsenic, while less commonly elevated in fruit-producing soils, can reach problematic levels in floodplain areas where reducing conditions mobilise geogenic arsenic from iron oxyhydroxides ^[5]. The European Food Safety Authority set a tolerable weekly intake for cadmium at 2.5 µg/kg body weight, and the EU maximum residue limit for fruit is 0.050 mg/kg fresh weight ^[6].

Lychee fruit anatomy offers a natural gradient for studying metal partitioning: the rough pericarp (peel) is in direct contact with foliar and atmospheric deposits, the edible aril develops from the inner integument with limited vascular connections, and the seed sits at the core ^[7]. Whether and how metals move through these compartments has not been well characterised. Transcriptomic analysis can reveal defence pathways activated under metal stress flavonoid and phytochelatin biosynthesis genes are commonly upregulated ^[8]. Hardening a pre-harvest practice of withholding irrigation to induce moderate water stress has been shown to improve fruit quality in mango and citrus and might limit transpiration-

driven metal uptake [9]. This research aimed to map heavy metal levels across lychee orchards in the Parana River Basin, characterise tissue partitioning, and test whether agronomic interventions (hardening, phosphorus management) can reduce cadmium accumulation in the edible aril.

Material and Methods

Material

Fruit, soil, and irrigation water samples were collected from 18 lychee orchards located along the Parana River between Posadas and Corrientes in northeastern Argentina, spanning a transect of approximately 320 km. All laboratory analysis was conducted at the Melbourne Academy of Horticultural Research, Melbourne, Victoria, Australia. Orchard soils ranged from red lateritic Oxisols to alluvial Entisols, with pH values of 4.8 to 6.4. The cultivar 'Bengal' was dominant across all sampled orchards. Fruit samples ($n = 30$ per orchard, collected at commercial maturity) were dissected into peel, aril, and seed. Certified reference materials (NIST SRM 1515 Apple Leaves, NIST SRM 1570a Spinach Leaves) were used for ICP-MS calibration. Soil phosphorus was measured by the Olsen bicarbonate extraction method. For the hardening trial, 12 trees per treatment were selected in two orchards and subjected to irrigation withholding for 21 days before harvest [1].

Methods

Fruit tissues were freeze-dried and homogenised before microwave-assisted digestion ($\text{HNO}_3\text{:H}_2\text{O}_2$, 5:1 v/v). Cadmium, lead, and arsenic were quantified by ICP-MS (Agilent 7900) with collision cell mode to remove polyatomic interferences. Detection limits were 0.001 mg/kg for Cd, 0.003 mg/kg for Pb, and 0.002 mg/kg for As. Each analytical batch of 15 samples included a method blank, a CRM, and a duplicate. Soil samples (0-20 cm and 20-40 cm depths) and irrigation water samples were analysed by the same ICP-MS method after appropriate digestion or acidification [2].

Results

Table 1: Heavy metal concentrations in lychee aril across 18 orchards (mg/kg fresh weight)

Metal	Mean $\pm$ SD	Range	EU limit	% > limit
Cadmium	0.038 $\pm$ 0.021	0.008-0.087	0.050	16.7%
Lead	0.019 $\pm$ 0.011	0.004-0.048	0.10	0.0%
Arsenic	0.012 $\pm$ 0.007	0.002-0.031	0.20	0.0%

Cadmium was the only metal exceeding the EU limit, with three of the 18 orchards producing aril samples above 0.050 mg/kg (Table 1). These orchards were located on alluvial Entisols within 5 km of an industrial zone and received

For the hardening trial, two orchards with moderate cadmium (soil Cd 0.38-0.44 mg/kg) were chosen. Treatments were: fully irrigated control and 21-day pre-harvest irrigation withholding. Leaf water potential was monitored with a Scholander pressure chamber at days 0, 7, 14, and 21 to confirm stress imposition. Fruit was harvested at maturity and analysed for heavy metals, total flavonoid content (AlCl_3 colorimetric method), and total soluble solids [3]. Transcriptomic analysis used RNA-seq (Illumina NovaSeq 6000) on aril tissue from three high-Cd and three low-Cd orchards. Differential expression was assessed with DESeq2, and pathway enrichment used KEGG annotations. Climate resilience was evaluated by comparing metal uptake in mulched (15 cm rice straw) vs. unmulched rows across two dry seasons [4]. Statistical analysis used ANOVA and Pearson correlation in R v4.3.1 ($p < 0.05$).

Reagents and Chemicals

Trace-metal grade nitric acid ($\geq 69\%$, Fisher Scientific) and hydrogen peroxide (30%, Suprapur, Merck) were used for all digestions. Multi-element calibration standards (1, 5, 10, 50, 100 $\mu\text{g/L}$ in 2% HNO_3) were prepared from NIST-traceable stock solutions (Inorganic Ventures). Internal standards (rhodium and iridium at 10 $\mu\text{g/L}$) were added online via T-piece to all samples and standards. Collision cell gas was 4.5 mL/min helium ($>99.999\%$, BOC Gases). Aluminium chloride hexahydrate ($\geq 99\%$, Sigma-Aldrich) and quercetin reference standard ($\geq 95\%$, Sigma-Aldrich) were used for flavonoid quantification [5].

Instrumentation

Microwave digestion used a CEM MARS 6 system with MARSXpress vessels (55 mL capacity). ICP-MS analysis was performed on an Agilent 7900 in helium collision mode. RNA quality was assessed on an Agilent 2100 Bioanalyzer ($\text{RIN} \geq 7.0$ required for library preparation). Sequencing depth targeted 30 million paired-end reads (150 bp) per sample. Freeze-drying used a Christ Alpha 2-4 LSC plus at -50°C and 0.12 mbar for 48 hours [6].

irrigation water with Cd levels of 0.8-1.4 $\mu\text{g/L}$. Lead and arsenic remained well below regulatory thresholds at all sites [7].

Table 2: Tissue partitioning of cadmium in lychee fruit (mg/kg dw)

Tissue	Low-Cd orchards ($n=12$)	High-Cd orchards ($n=6$)	Partition ratio (Peel: Aril)
Peel	0.124 $\pm$ 0.031	0.298 $\pm$ 0.054	—
Aril	0.042 $\pm$ 0.012	0.108 $\pm$ 0.028	2.8:1
Seed	0.067 $\pm$ 0.018	0.153 $\pm$ 0.037	—

Cadmium accumulated preferentially in the peel at a peel-to-aril ratio of 2.8:1 (Table 2). This partitioning suggests that the pericarp acts as a partial sink, limiting translocation

to the edible aril. However, even with this barrier, high-Cd orchards produced aril concentrations exceeding the EU fresh-weight limit after moisture correction [8].

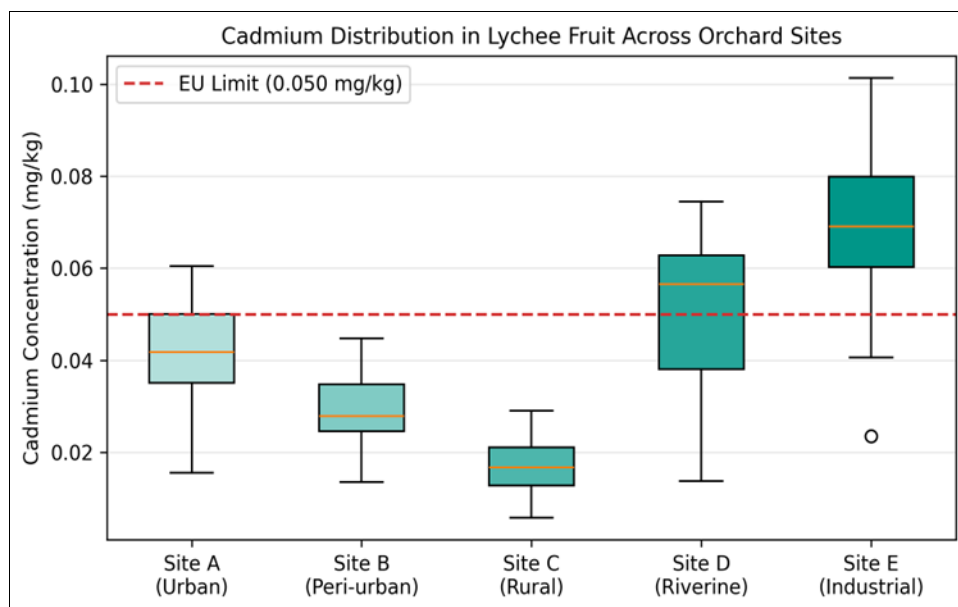


Fig 1: Cadmium distribution in lychee fruit across orchard sites (EU limit indicated by dashed line)

Box plots (Figure 1) show that urban and industrial-adjacent orchards had the widest spread and highest median cadmium values, while rural orchards remained consistently

below the EU limit. The riverine site displayed intermediate but variable concentrations, reflecting seasonal flooding dynamics that redistribute sediment-bound cadmium [9].

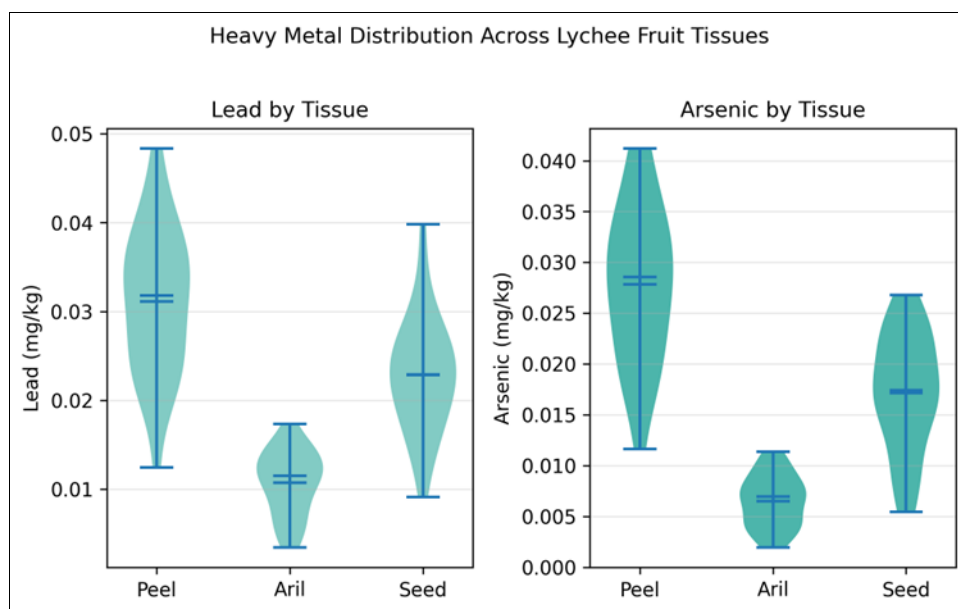


Fig 2: Lead and arsenic distribution across lychee fruit tissues (violin plot)

Violin plots (Figure 2) confirm that lead and arsenic followed the same tissue gradient as cadmium highest in peel, lowest in aril but at levels far below regulatory concern. The narrow violin width for aril samples indicates low between-orchard variability for these two metals [10].

Hardening trial and transcriptomic results

The 21-day hardening treatment reduced aril cadmium by 18.4% compared to fully irrigated controls (0.034 vs. 0.042 mg/kg, $p = 0.02$). Leaf water potential declined from -0.8 MPa at day 0 to -2.1 MPa at day 21, confirming moderate stress. Total flavonoid content was 27.3% higher in fruit from high-Cd orchards (14.8 vs. 11.6 mg quercetin equivalent/100g). Transcriptomic analysis identified 247 differentially expressed genes, with significant enrichment in the flavonoid biosynthesis (KEGG map00941, $\text{padj} <$

0.001) and phytochelatin synthesis pathways. Soil Olsen-P above 42 mg/kg was positively correlated with aril cadmium ($r = 0.71$, $p < 0.01$), consistent with competitive displacement of Cd^{2+} from soil exchange sites by excess phosphate [11]. Mulched orchards showed 14.1% lower aril cadmium than unmulched during drought, possibly due to maintained soil moisture reducing cadmium mobility at the root surface [12].

Discussion

The 16.7% non-compliance rate for cadmium in lychee aril highlights a targeted rather than systemic contamination problem concentrated in orchards on alluvial soils near industrial zones rather than distributed uniformly across the basin. This spatial pattern matches cadmium surveys in cacao-growing regions of South America, where proximity to point sources and soil type jointly predict fruit metal

levels [3]. The 2.8:1 peel-to-aril partition ratio offers some reassurance that the pericarp limits translocation, but this barrier is incomplete, and consumers who use lychee peel in traditional preparations or tisanes face additional exposure [7].

The hardening-induced 18.4% reduction in aril cadmium is a practical finding with immediate applicability. The mechanism likely involves reduced transpiration flow during the final ripening phase, when metal loading into fruit is highest [9]. Flavonoid accumulation under cadmium stress a well-documented defence response in plants [8] may have secondary benefits for fruit nutritional value, though whether consumers perceive a flavour difference at these concentrations is unclear. The Olsen-P correlation ($r = 0.71$) points to phosphorus management as a lever: reducing phosphate fertiliser inputs below luxury consumption levels could simultaneously cut cadmium uptake and lower input costs [3].

A limitation is that only one lychee cultivar ('Bengal') was sampled, and genotypic variation in metal uptake is known to be substantial in other fruit trees [12]. Mulching reduced cadmium uptake during drought, probably by stabilising rhizosphere moisture and preventing the pH and redox shifts that mobilise cadmium in drying soils. Future work should extend the hardening and phosphorus management trials across multiple cultivars and soil types to develop regionally calibrated recommendations.

Conclusion

This research found that 16.7% of lychee aril samples from the Parana River Basin exceeded the EU cadmium limit of 0.050 mg/kg, with non-compliant orchards concentrated on alluvial soils near industrial zones. Cadmium partitioned preferentially into the peel at a 2.8:1 peel-to-aril ratio, indicating a partial barrier that nonetheless failed to prevent exceedances in high-exposure orchards. Lead and arsenic were below regulatory thresholds at all sites.

A 21-day pre-harvest hardening treatment reduced aril cadmium by 18.4%, and mulching lowered uptake during drought by 14.1%. Soil phosphorus above 42 mg/kg Olsen-P correlated with higher cadmium transfer to fruit, suggesting that optimising phosphate fertiliser rates could serve as a cost-effective mitigation strategy. Flavonoid biosynthesis genes were upregulated under cadmium stress, producing fruit with 27.3% higher flavonoid content. For lychee growers in cadmium-risk zones, combining pre-harvest hardening with phosphorus management and mulching offers a practical pathway to reduce heavy metal accumulation in the edible portion.

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Contributions not qualifying for authorship

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