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Assessment of chemical contaminants in fresh and packaged tender coconut (*Cocos nucifera*) water

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Abstract: Pesticide residues and heavy metals were analysed in both fresh tender coconut water (FTCW) (n = 161) and packaged tender coconut water (PTCW) (n = 126) samples collected from three southern states of India [Andhra Pradesh (AP), Kerala (KL), and Tamil Nadu (TN)]. A method validated in the laboratory using liquid chromatography-tandem mass spectrometry (LC-MS/MS) was used for pesticide residues, while heavy metals were analysed using a validated method of inductively coupled plasma-optical emission spectrometry (ICP-OES). Significant differences in heavy metal concentrations were assessed using analysis of variance (ANOVA) and post hoc test (between different varieties collected 'within' and 'among' states). FTCW samples [n = 9 (6%)] collected from TN showed Monocrotophos and Malathion residues in the range of 1.0 μg L^{-1} to 51.6 μg L^{-1} and 0.5 μg L^{-1} to 0.6 μg L^{-1}, respectively, while they were detected in n = 5 (4%) of the PTCW samples at a range of 0.90 μg L^{-1} and 0.82 μg L^{-1} to 1.56 μg L^{-1}. Heavy metals such as cadmium (Cd), chromium (Cr), lead (Pb), and stannum (Sn) were detected in different varieties collected from all three states. Some of the PTCW samples also contained traces of Cd, Cr, cobalt (Co), and Pb. Arsenic (As) was found in one sample from KL, while none of the samples was contaminated with mercury (Hg). The present study accentuates the need for fixing standards for the pesticide residues in coconut water.

Keywords: natural isotonic beverage; pesticide residues; heavy metals; standards

Coconut plant products contribute more than USD 19 677 810 000 (15 000 crore rupees) to the country's gross domestic product and 72% of the world's total production is from India alone (Raghavi et al. 2019). The total value of exports was 42% higher than that of the export earnings for the previous year (Jayasekhar

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et al. 2019). Tender coconut water is popular and widely consumed, as it is a refreshing, highly nourishing, wholesome non-alcoholic isotonic beverage rich in natural sugars, salts, vitamins, amino acids and phytohormones with a calorific value of 19 calories (100 mL)$^{-1}$, while it is 39 calories (100 mL)$^{-1}$ for bottled coconut water (Jnanadevan 2016). It is also useful as an intravenous solution, as a blood plasma substitute and has a positive effect on cholesterol (Priya and Ramaswamy 2014; Thomas et al. 2017).

The coconut palms are frequently attacked by various insects such as *Macroplectranararia*, *Leucopholisconeophora* Burm., *Rhynchophorusferrugineus*, *Opisinaarenosella*, *Oryctesrhinoceros* and *Eriophyesguerreronis* (TNAU 2014) and hence they are susceptible to various diseases. The major insect pests of the coconut are rhinoceros beetle, red palm weevil, black headed caterpillar, mealy bugs, etc., while major diseases are root wilt, Thanjavur wilt, tatipaka, bud rot, white fly, stem bleeding, and crown choking (KAU 2021). Adopting economically viable farming systems by scientific management is an effective measure to control insect infestation. The coconut board has recommended Carbaryl, Dichlorvos, Fenthion, Monocrotophos, Dimethoate, Chlorpyriphos, Phorate and other neem-based pesticides (Azadirachtin) (Ramesh et al. 2013). Further, the Central Insecticides Board of India has recommended Monocrotophos and Bromadiolone. In addition, pesticides such as Malathion and Phosalone are also frequently used to spray over the leaves and injected through the stem and root systems. Although spraying pesticides over the leaves may not directly contaminate the kernels, it may occur through leaching into the soil through rainwater and thereby entering the stem and root system. Contamination of coconut water with pesticide residues and kernel through root and stem administration was reported (Reddy et al. 1998).

The application of pesticides on a long-term basis may result in the accumulation of heavy metals in the topsoil which may be due to the tendency of a plant to take them up and subsequently get biomagnified in the food chain (Parth et al. 2011). Earlier studies revealed that toxic heavy metals get bioaccumulated in soil, edible portions of plants and coconut water (Roberts and Orisakwe 2011; Islam et al. 2018).

Studies on chemical contamination in the fresh/packaged tender coconut water (FTCW/PTCW) samples are scarce. Therefore, the present study aims to assess the extent of contamination with pesticide residues and heavy metals in both FTCW and PTCW collected from three major coconut growing states from southern India.

MATERIAL AND METHODS

A survey was conducted in the identified districts of three southern states of India [Andhra Pradesh (AP), Kerala (KL), and Tamil Nadu (TN)] wherein coconut crop cultivation is predominant. The information on demographic particulars, extent of the land holding, farming experience, plantation, duration of cultivation, details on cropping/harvesting, varieties of coconuts cultivated, pest management methods adopted, frequency of application, and time interval given between the last spray and harvest were recorded from the local coconut farmers. A random sampling procedure was adopted for sample collection. The FTCW samples (n = 161) of the most popular varieties for consumption were collected from three different locations in each district of the three states. The PTCW samples available at the selected places (n = 126) of the three states from the local markets and the processing units were also collected. Both were labelled carefully, transported to the laboratory, and stored at –20 °C until analysed (deep-freezer HF 500 CHP; Carrier, US).

The certified reference materials of the predominantly used pesticides for coconut crops and the internal standard (triethyl phosphate) were supplied by Sigma Aldrich (Germany). The ultra-high-performance liquid chromatography-tandem mass spectrometry (UHPLC-MS) grade solvents such as methanol, acetonitrile and formic acid were procured from Biosolv (France). The standard solution mixture containing 1 000 µg mL^{-1} of each metal was procured from Merck Millipore (Germany). The ultrapure water was obtained from the Direct Q water purification system (Merck Millipore, Germany).

Preparation of standard solutions. The standard stock solutions of all the pesticides and the internal standard were prepared at 100 µg mL^{-1} and were stored at –20 °C (deep-freezer HF 500 CHP; Carrier, US) until analysed. The primary standard solution was prepared every week from the standard stock solution.

The linearity check was done using the stock solution of the standard metals mixture diluted from 0.01 µg mL^{-1} to 50 µg mL^{-1} by directly injecting the standard solutions into the instrument inductively coupled plasma-optical emission spectrometry (ICP-OES) (iCAP 6500 Duo series; Thermo Scientific, China).

Sample preparation. For the assessment of pesticide residues, 50 mL of the sample was filtered through a 0.22 µm cellulose filter (Nupore Filtration Systems, India), of which 20 µL of the sample was directly injected into liquid chromatography-tandem mass spec-

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trometry (LC-MS/MS) (triple stage quadrupole TSQ Altis with RSLC 3000 ultimate; Thermo Fisher Scientific, US) for detection and quantification of the analytes in the selected reaction monitoring (SRM) ion mode. For heavy metals analysis, 25 mL of the sample was extracted using 2 mL of 65% pure nitric acid [high-performance liquid chromatography (HPLC) grade; Merck, India] and digested on a hot plate (VWR International, US) at 100 °C for 10 min. The digested solution was filtered and transferred for analysis using ICP-OES (iCAP 6500 Duo system; Thermo Scientific, China) for quantification.

Instrumental conditions. LC-MS/MS with TSQ Altis triple stage quadrupole mass spectrometer connected to RSLC 3000 ultimate model quaternary gradient pump and auto-sampler (Thermo Fisher Scientific, US) was used for analysis. The ionization was carried out in the atmospheric pressure chemical ionization mode with source temperature at 350 °C and transfer line temperature at 320 °C, respectively. Nitrogen was used as the sheath, auxiliary and collision gas at 60, 20, and 2.5 units of pressure, respectively. The separation of analytes was carried out on the C18 column (length 150 mm, 5 µm particle size; Chromatopak, India). Water with 0.1% formic acid and acetonitrile with 0.1% formic acid were used as mobile phases A and B, respectively. The analytes were separated in a gradient elution mode with 30% mobile phase B maintained between 18.1 min and 20 min (Table 1).

Heavy metals analysis was carried out on Thermo Fisher Model ICAP6500 Duo system (Thermo Scientific, China) with argon as a nebulizer, plasma, and auxiliary gas at 0.7, 12, and 0.5 mL min^{-1}, respectively. The radio frequency power was maintained at 1 150 W by maintaining the pump speed at 50 rpm to monitor the target analytes such as arsenic (As) 193.75 nm, cadmium (Cd) 228.80 nm, cobalt (Co) 228.61 nm, chromium (Cr) 283.56 nm, copper (Cu) 324.75 nm, mercury (Hg) 184.95 nm, stannum (Sn) 189.98 nm, and lead (Pb) 220.35 nm.

Validation. The analytical method validation was performed as per EC (2017) guidelines. The lower limit of detection for each pesticide was measured at a signal to noise (S/N) ratio 3 : 1, which varied from 0.1 µg L^{-1} to 1.5 µg L^{-1}. The lower limit of quantification measured at the S/N ratio of 10 : 1 was in the range of 0.5 µg L^{-1} to 2 µg L^{-1}. The method was linear with a correlation coefficient $R^2 > 0.99$ and in the range of 0.1 µg L^{-1} to 1 000 µg L^{-1}. Inter and intraday precisions determined at 0.5, 50, and 500 µg L^{-1} were < 15%. Further, it is not necessary to conduct any recovery studies as the method used in the present

Table 1. Selected reaction monitoring (SRM) parameters

Compound	Precursor (*m*/*z*)	Qualifier ion	Quantifier ion	Collision energy (V)
Aldicarb	116.00	70.09	88.93	10.23
Carbaryl	145.07	117.00	126.99	16.44
Oxamyl	163.00	118.93	128.93	19.89
Triethyl phosphate	182.98	98.99	155.04	19.06
Acephate	183.96	124.86	142.99	18.30
Propoxur	210.01	111.00	168.07	14.81
Dichlorvos	220.95	108.99	144.99	17.54
Carbofuran	222.07	122.90	165.05	21.60
Monocrotophos	224.06	193.00	126.92	15.69
Methiocarb	225.99	120.97	169.05	19.14
Dimethoate	229.95	124.92	198.96	21.49
Phorate	261.08	74.93	144.99	10.23
Parathion methyl	263.91	108.97	222.84	22.93
Fenitrothion	278.02	124.82	245.92	20.16
Quinalphos	299.07	147.00	163.04	22.13
Diazinon	305.11	153.16	169.00	20.35
Malathion	331.12	127.06	99.00	20.10
Malaoxon	314.97	99.04	127.04	23.38
Imidan	318.00	132.99	160.06	36.20
Phosalone	367.99	181.97	321.99	15.80

m/*z* – mass-to-charge ratio

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study was developed and validated previously; the values obtained represent true levels of residues in the samples. Hence, the samples were analysed directly after filtration without subjecting them to extraction (Deme et al. 2013).

The heavy metals were estimated using a validated method of ICP-OES. The limit of detection and limit of quantification for each analyte measured varied from 0.0001 μg mL^{-1} to 0.0056 μg mL^{-1} and from 0.0003 μg mL^{-1} to 0.0168 μg mL^{-1}, respectively. Further, the method was also found to be linear as the correlation coefficient (R^2) value was falling in the range of acceptance criteria (0.9994 to 1.0000) and the precision of the method determined for each target element studied was in the range of 0.08–0.69% relative standard deviation (RSD) at all levels of the study.

Statistical analysis. The mean and standard deviation (SD) values for pesticide residues and heavy metals were calculated for FTCW and PTCW samples. Analysis of variance (ANOVA) test was conducted to assess significant differences between the concentration levels of heavy metals in both types of samples. Post hoc least significant difference (LSD) test was also done to determine the significant difference between the different varieties of both types of samples collected 'within' and 'among' the states.

RESULTS AND DISCUSSION

The information collected from 156 farmers was tabulated (Table 2). Details pertaining to cultivation are given in Table 3. The details on different coconut varieties cultivated in each village revealed that a total of nine varieties of coconuts are being cultivated in AP, of which Malayan Yellow Dwarf (MYD), Chowghat Orange Dwarf (COD), Ganga Bondam (GB), Godavari Ganga (GG), East Coast Tall (ECT), and Orange Dwarf (OD) are predominant cultivated varieties. Similarly, in KL out of fourteen cultivated varieties West Coast Tall (WCT), MYD, COD, Tall × Dwarf (T×D), GG, and Gouri Gathram (GGt) are predominantly cultivated, while in TN out of seven varieties cultivated, COD, MYD, Chowghat Green Dwarf (CGD), T×D, and ECT are major cultivated and consumed varieties.

LC-MS/MS determination of pesticide residues. All the samples of FTCW and PTCW collected from all three states were analysed for pesticide residues. No pesticide residues were detected in the FTCW collected from AP and KL. While among 34 samples from TN, four samples (one sample each of T×D and COD and two samples of MYD) were detected to contain Monocrotophos residues in the range of 1 μg L^{-1} to 51.5 μg L^{-1} and five samples (three COD and two T×D samples) contained Malathion residues in the range of 0.5 μg L^{-1} to 0.6 μg L^{-1} (Figure 1). It was observed from the present study that the use of pesticides is negligible in AP and KL as compared to TN. Further, in TN, a majority of the farmers are applying systemic pesticides like Monocrotophos, Phorate, and Chlorpyrifos through root feeding and spraying.

Only five PTCW were detected with pesticide residues, one with Monocrotophos (0.9 μg L^{-1}), four with Malathion (0.8 μg L^{-1} to 1.56 μg L^{-1}) (Figure 2). Studies are available on different validation methods to determine pesticide residues in commercial coconut water, but studies on the contamination with pesticide residues in FTCW and PTCW are scarce (Ferreira et al. 2016).

ICP-OES determination of heavy metals. The levels of heavy metals such as Cd, Cu, Cr, Pb, Sn were detected in all the popularly cultivated/consumed FTCW varieties collected from all three states varied. The results of ANOVA among different varieties of FTCW collected from AP have shown a significant difference in Cd, Co, Cr, Pb, and Sn levels. Further, the post hoc test has also shown a significant difference between the states for Cd, Co, Cr, Pb, and Sn. A significant difference was found for heavy metals like Cd and Cu between the different varieties of FTCW collected from TN. However, no such significant difference was observed between the different varieties of samples collected from KL except for Sn. While, in AP, there was found a significant difference between COD *vs.* GB and OD with respect to levels of Cu. Studies are available on the heavy metal contamination in the lake and mine waters (Begum and Krishna 2010).

Table 2. Demographic profile

State	Number of farmers (*n*)	Extent of land holdings (acres)			Average experience (years)
		1–4	5–10	> 10	
AP	60	31 (51.6%)	19 (31.6%)	10 (16.6%)	30.0
KL	49	12 (24.5%)	18 (36.7%)	19 (38.8%)	28.3
TN	47	14 (29.79%)	22 (46.81%)	11 (23.4%)	26.3

AP – Andhra Pradesh; KL – Kerala; TN – Tamil Nadu

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Table 3. Farming particulars

State	Average cultivation period (years)	Average harvesting period (days) / frequency (times per year)	Time interval between spray and harvest (days)	Average number of plants per acre
AP	36.5	60 / 6	40	59.6
KL	35.3	45–50 / 7–8	N/A	68.0
TN	31.3	40–50 / 7–12	30–35	70.0

AP – Andhra Pradesh; KL – Kerala; TN – Tamil Nadu; N/A – not applicable

Contamination with heavy metals in fresh coconut kernels, coconut cream, and coconut milk powder was also reported elsewhere (Warsakoon 2010). It is interesting to note that Co was detected in 61.9, 11.9, and 30.9% of FTCW collected from AP, KL, and TN, respectively (Table 4). This may be due to the discharges from paper, plastic, oil, gas, and brick industries within the 10-km radius around the collection sites, which might have contained metals in a loosely bound state that leached into the environment, leading to contamination (Butylina et al. 2018). Further, metals from airborne sources may also release as particulates and thereby contaminate

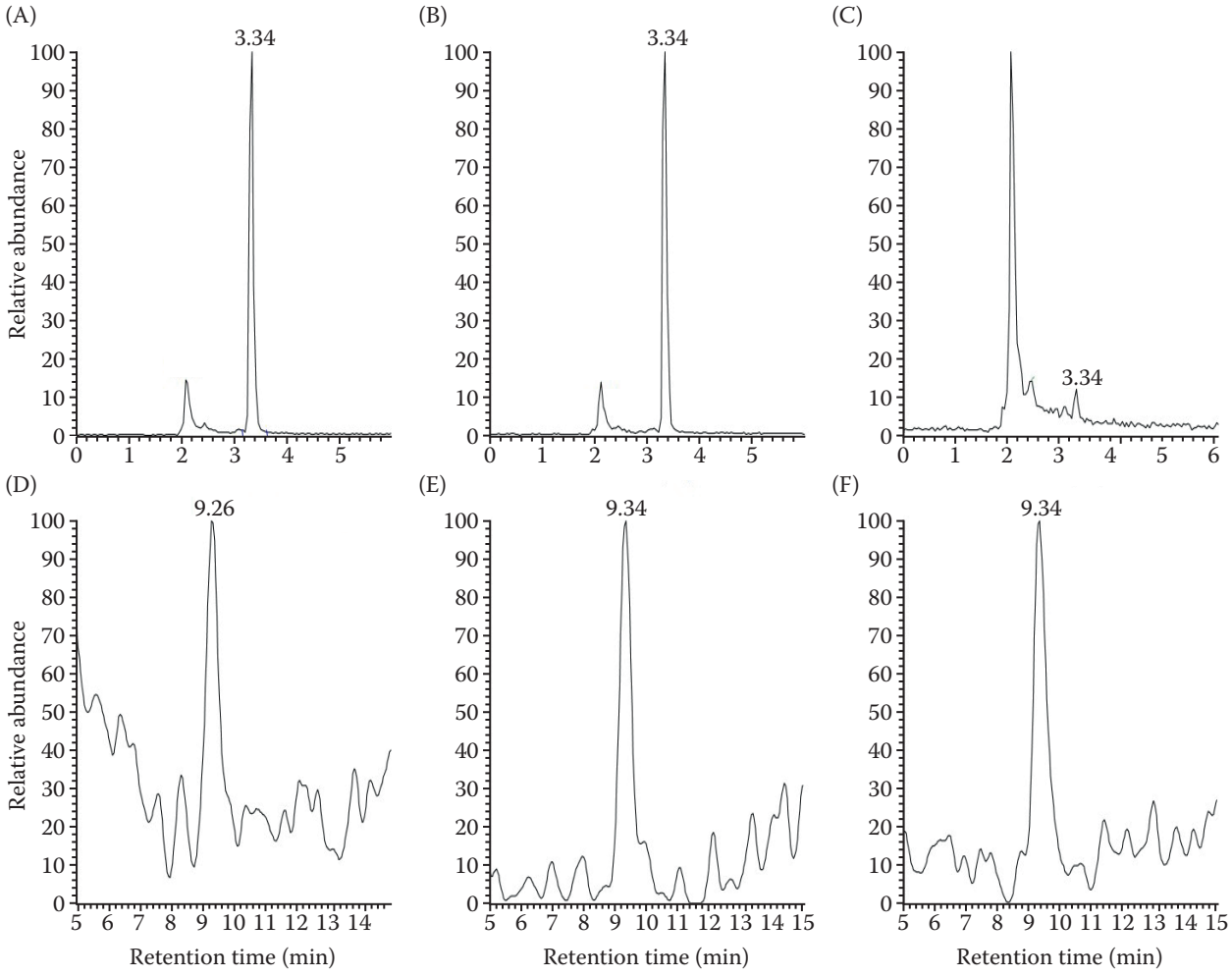

Figure 1. Spectra showing pesticide residues in FTCW from TN: Detection of (A) Monocrotophos in MYD1, (B) Monocrotophos in MYD2, (C) Monocrotophos in COD4, (D) Malathion in COD3, (E) Malathion in COD52, and (F) Malathion in T×D44

FTCW – fresh tender coconut water; TN – Tamil Nadu; MYD – Malayan Yellow Dwarf; COD – Chowghat Orange Dwarf; T×D– Tall × Dwarf; retention time of +0.1 min is acceptable as per the EC (2017) guidelines

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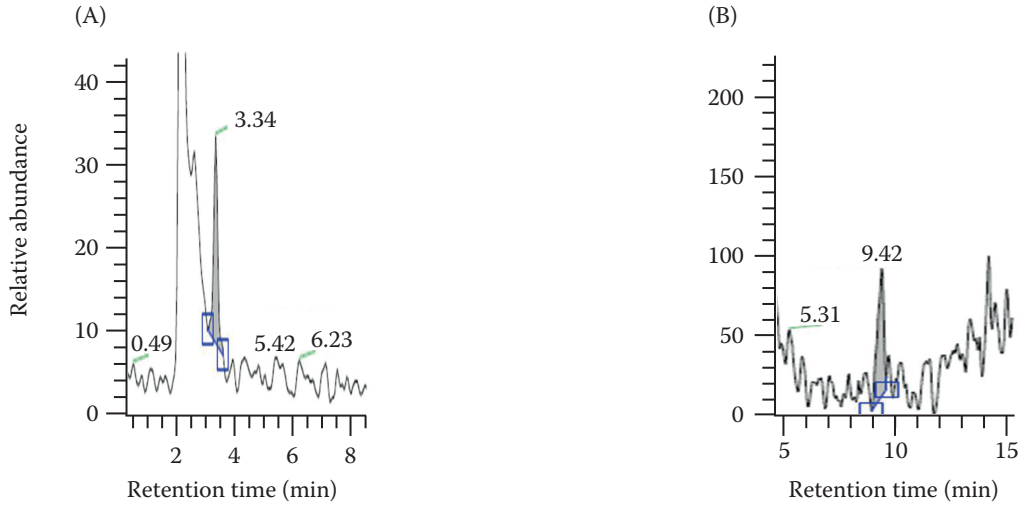

Figure 2. Spectra showing pesticide residues in PTCW: (A) Monocrotophos and (B) Malathion

PTCW – packaged tender coconut water

the agricultural lands located adjacent to roads (Wuana and Okieimen 2011; USEPA 2014).

It was observed that the concentration of Cd, Cr, and Pb in FTCW was exceeding the permissible limits set by WHO (2011), Bureau of Indian Standards (BIS; IS 10500:2012), and Food Safety and Standards Authority of India (FSSAI; 2011) for drinking water. Similar results were found in the studies conducted elsewhere (Islam et al. 2018; Pantipkayee et al. 2018). In contrast, the levels of Pb, Cd detected in natural/industrialised coconut water samples were found to be below the Maximum Tolerable Limits recommended by Brazilian Health Surveillance Agency (Paixao et al. 2019).

All the twelve brands of PTCW collected from all three states were contaminated with Sn, while heavy metals such as Cd, Cr, Co, Pb, and Cu were also detected in traces. The results of ANOVA were significant for Cd, Cr, Sn, and Cu. The average levels of Cu were higher in AP and TN as compared to KL (Table 5). None of the FTCW and PTCW was found to have been contaminated with Hg, while one FTCW collected from KL was detected to contain As (0.0002 μg mL^{-1}).

Table 4. Heavy metals detected in FTCW (μg mL^{-1})

Heavy metals	State						ANOVA (*F*-value)	Comparison between states (LSD)
	AP ($^{\#}n$ = 42) (6 varieties)		KL ($^{\#}n$ = 42) (6 varieties)		TN ($^{\#}n$ = 42) (5 varieties)			
	n	mean ± SD	*n*	mean ± SD	*n*	mean ± SD		
Cd	42	0.0027 ± 0.0009	42	0.0024 ± 0.0006	42	0.0034 ± 0.0009	15.35**	AP *vs.* TN* KL *vs.* TN*
Co	26	0.0007 ± 0.0004	5	0.0002 ± 0.0001	13	0.0002 ± 0.0001	9.43**	AP *vs.* KL* AP *vs.* TN*
Cr	42	0.0306 ± 0.0196	41	0.0253 ± 0.0066	42	0.0356 ± 0.0129	5.47**	KL *vs.* TN*
Pb	40	0.0093 ± 0.0052	42	0.0114 ± 0.0044	42	0.0148 ± 0.0081	8.16**	AP *vs.* TN* KL *vs.* TN*
Sn	42	0.0161 ± 0.0077	41	0.0173 ± 0.0085	42	0.0504 ± 0.0269	55.08**	AP *vs.* TN* KL *vs.* TN*
Cu	40	0.0778 ± 0.0560	42	0.0733 ± 0.0230	42	0.0778 ± 0.0447	0.149	–

*Significance $P < 0.05$; **F-value > 2.5 significance; FTCW – fresh tender coconut water; ANOVA – analysis of variance; AP – Andhra Pradesh; KL – Kerala; TN – Tamil Nadu; SD – standard deviation; LSD – least significant difference; $^{\#}n$ – total number of samples analysed; n – number of samples detected with heavy metals

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Table 5. Heavy metals detected in PTCW (μg mL^{-1})

Heavy metals	Brand											
	A ($^{\#}n$ = 15)		B ($^{\#}n$ =12)		C ($^{\#}n$ =10)		D ($^{\#}n$ =21)		E ($^{\#}n$ =15)		F ($^{\#}n$=9)	
	n	mean ± SD	*n*	mean ± SD	*n*	mean ± SD	*n*	mean ± SD	*n*	mean ± SD	*n*	mean ± SD
Cd	15	0.0018 ± 0.0003	12	0.0013 ± 0.0003	10	0.0011 ± 0.0002	20	0.0014 ± 0.0003	14	0.0012 ± 0.0004	9	0.0023 ± 0.0003
Co	0	nd	3	0.0001 ± 0.0001	0	nd	3	0.0001 ± 0.0011	1	0.0002	0	nd
Cr	15	0.0120 ± 0.0033	12	0.0041 ± 0.0019	9	0.0080 ± 0.0014	21	0.0076 ± 0.0033	12	0.0168 ± 0.0030	8	0.0143 ± 0.0009
Pb	6	0.0083 ± 0.0044	0	nd	1	0.0060	6	0.0024 ± 0.0018	1	0.0073	3	0.0056 ± 0.0027
Sn	15	0.0087 ± 0.0030	12	0.0040 ± 0.0018	10	0.0500 ± 0.0188	21	0.0286 ± 0.0205	15	0.0073 ± 0.0024	9	0.0275 ± 0.0252
Cu	13	0.0324 ± 0.0183	0	nd	5	0.0242 ± 0.0047	17	0.0094 ± 0.0043	10	0.0247 ± 0.0198	7	0.0138 ± 0.0021

Table 5. To be continued

Heavy metals	Brand												ANOVA (*F*-value)
	G ($^{\#}n$ =11)		H ($^{\#}n$ =11)		I ($^{\#}n$ =3)		J ($^{\#}n$ =3)		K ($^{\#}n$ =10)		L ($^{\#}n$ =6)		
	n	mean ± SD	*n*	mean ± SD	*n*	mean ± SD	*n*	mean ± SD	*n*	mean ± SD	*n*	mean ± SD	
Cd	10	0.0018 ± 0.0001	11	0.0016 ± 0.0002	3	0.0024 ± 0.0003	3	0.0009 ± 0.00005	7	0.0025 ± 0.0001	5	0.0016 ± 0.0001	21.17**
Co	0	nd	0	nd	0	nd	0	nd	1	0.0005	0	nd	2.38
Cr	10	0.0057 ± 0.0017	8	0.0171 ± 0.0008	3	0.0168 ± 0.0021	3	0.0030 ± 0.0007	9	0.0123 ± 0.0018	6	0.0131 ± 0.0023	33.51**
Pb	3	0.0055 ± 0.0019	3	0.0048 ± 0.0009	2	0.0079 ± 0.0003	0	nd	1	0.0082	1	0.0072	1.72
Sn	11	0.0041 ± 0.0004	11	0.0205 ± 0.0041	3	0.0292 ± 0.0342	3	0.0037 ± 0.0002	10	0.0194 ± 0.0040	6	0.0330 ± 0.0154	11.51**
Cu	11	0.0290 ± 0.0016	10	0.0174 ± 0.0009	3	0.0091 ± 0.0003	0	nd	9	0.0213 ± 0.0054	5	0.0549 ± 0.0204	10.13**

**F-value > 2.5 significance; PTCW – packaged tender coconut water; nd – not detected; $^{\#}n$ – total number of samples analysed; n – number of samples detected with heavy metals; SD – standard deviation; ANOVA – analysis of variance

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It was observed from the present investigation that all the samples of PTCW were contaminated with Sn, while 94.5, 92.06, 21.43, and 6.34% were detected to contain Cd, Cr, Pb, and Co, respectively. This could be due to the continuation of contamination from FTCW to PTCW during processing, as the PTCW samples were collected from similar locations and regions as those of FTCW. Further, the metal ion load coming through processing and packaging might also be an additional factor for contamination which was reported previously (Magomya et al. 2015). In addition, the authorised use of stannous chloride (E512) as 'antioxidant' could also have been another source of contamination (Hague et al. 2008).

CONCLUSION

To conclude pesticide residues were detected in very few samples of both FTCW and PTCW, while heavy metals were detected in all samples collected from all three states. In India, there are no limits yet set for pesticide residues and heavy metals in FTCW and PTCW by regulatory bodies like FSSAI. The findings from this study would provide the necessary insights for the Indian regulatory bodies for setting standards for this natural isotonic beverage both from food safety and public health points of view. However, a systematic integrated approach with a larger sample size from other geographies can provide further information on the chemical contamination of coconut water.

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