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DETERMINATION OF HEAVY METAL ACCUMULATION IN MILK AND THEIR PACKAGING MATERIALS AND STATISTICAL ANALYSIS*

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Abstract

Heavy metal accumulation occurs when foods and food contact materials contain excessive amounts of heavy metals. Heavy metal accumulation in foods can risk public health and cause diseases. Therefore, the concentration of heavy metals in food and packaging materials is an important parameter that needs to be analyzed. This study aimed to detect heavy metal accumulation in food and packaging materials. For this purpose, milk, which has an essential place among foods, was chosen. Products of 10 different milk brands used commercially today were supplied. Within the scope of heavy metal analyses, Lead (Pb), Arsenic (As), Cadmium (Cd), Iron (Fe), Manganese (Mn), Zinc (Zn), Copper (Cu), Aluminum (Al), Cobalt (Co) and Nickel (Ni) elements were determined. Pre-treatment of the samples was carried out using the efficient microwave method to ensure minimal sample loss through rapid thawing. Heavy metal analysis was then carried out using the widely used ICP-OES (Inductively Coupled Plasma-Optical Emission Spectrometer) device, a widespread technique in current practice. The range of heavy metal concentrations in all packaging samples were: Al (1.219–2.578), As (1.078–1.522), Co (0.219–0.239), Fe (0.862–81.440), Pb (1.784–2.170), Mn (0.042–1.884), Ni (0.782–9.758, Zn (1.814–2.522) mg/kg. Heavy metal concentrations in all packaging samples were found Al (51267.00–71601.25), As (31.626–42.371), Cd (1.035–1.209), Co (0.775–1.167), Cu (4.921–44.839), Fe (259.615–463.182), Pb (24.386–26.668), Mn (4.301–59.599), Ni (6.065–7.943), Zn (5.324–8.763) mg/kg. The heavy metals with the highest concentration among packaging materials are Al and Fe, followed by As and Pb. Additionally, the correlation between milk and its packaging samples were presented using SPSS.

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Keywords: Milk, food packaging, microwave, heavy metal accumulation, inductively coupled plasma–optical emission spectrometry (ICP-OES)

1. Introduction

Foods must be physically, chemically, and microbiologically suitable for consumption and not lose their nutritional value [1,2]. Food poses a risk to our health due to exposure to chemical contamination [3]. Nowadays, many parameters affect the quality of food. Heavy metals appear to be the most important [4]. Heavy metals threaten our health by contaminating foods from many different sources
[5]. Heavy metals cause various diseases depending on factors such as the person's immune resistance, genetics, age, and nutrition [6].

Milk and dairy products constitute the majority of foods widely consumed worldwide [7]. Minerals and proteins contained in milk and dairy products are beneficial components for human life [8]. Dairy products contain many macro-micro elements and vitamins in their structure. These elements are very important in nutrition [9]. Heavy metals in milk and dairy products have particular importance, and the amount of heavy metals must be determined, controlled, and compared [10]. In recent years, increased consumption of foods contaminated by heavy metals has accelerated studies in this field [11–13]. Especially from a toxicology perspective, the amount of heavy metals such as Cd, Pb, and Ni in milk must be defined [14]. In addition to foodstuffs, heavy metal accumulation resulting from packaging materials is also a significant issue [15,16].

The selection of packaging material is very important during the packaging stage of milk. Because the transfer of chemicals or heavy metals from packaging into milk is an important problem [17,18], many different materials can be used to package dairy products. Glass bottles with aluminum caps, LDPE bags, LDPE or LLDPE coated cardboard boxes, and PP bottles are used [19]. The most commonly used aseptic packaging material combines polyethylene film, laminated cardboard, and aluminum foil [19,20]. Aseptic cardboard packaging is a material designed with six layers to fulfill the task of protecting liquid food and preventing the leakage of microorganisms, gas or steam [17,21]. Despite these features, migration between the packaging and the product may occur due to adverse conditions (temperature, exposure to UV light, storage time) during heat treatment and storage [17,22]. For these reasons, the contents of milk packaging need to be determined.

This study aimed to determine the heavy metal accumulation of milks and packaging materials, which are a good source of macro and micronutrients, the production and use of which have been increasing daily in our country in recent years. In this context, Lead (Pb), Arsenic (As), Cadmium (Cd), Iron (Fe), Manganese (Mn), Zinc (Zn), Copper (Cu), Aluminum (Al), Cobalt (Co), Nickel (Ni) heavy metal contents were analyzed at ppm and ppb level. Then, the relationship between milk and milk packaging samples was examined by correlation analysis in SPSS.

2. Materials and method

The study conducted heavy metal analyses of milks and their packaging materials with ICP-OES. In this context, Lead (Pb), Arsenic (As), Cadmium (Cd), Iron (Fe), Manganese (Mn), Zinc (Zn), Copper (Cu), Aluminum (Al), Cobalt (Co), Nickel (Ni), which cause toxic effects, were determined at ppm and ppb levels. All standards and chemicals were used in analytical purity and ultrapure water obtained from the water purification system (Human, Power 2).

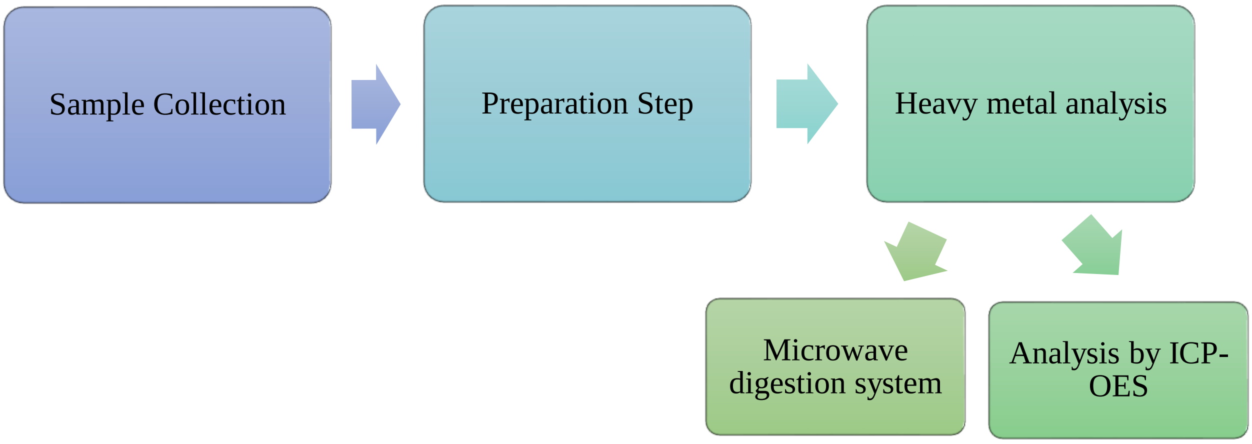


Figure 1. Methodology of study

2.1 Milks and packaging materials

A total of ten commercial milk samples (liquid) and packaging materials belonging to different milk brands sold in the market were provided. The brands of milk provided are **T1, T2**... from 1 to 10; their packaging is **T1P, T2P,** etc. labeled with tags. Milk samples were taken fresh and storage conditions were followed until analysis.

Microwave digestion methods for samples

The microwave digestion method, which is much faster than other methods in the literature, was used to liquefy the samples [23]. Thanks to the pressure system in the Teflon containers used in the Microwave digestion system, evaporation is prevented and sample loss is not experienced. Additionally, the time spent in sample preparation for analysis is reduced [24–26].

In this study, the CEM MARS6 brand microwave digestion device was used. The preliminary step procedure used for milk samples and packaging samples is different in microwave digestion system. Data for the preliminary phase are presented in **Table 1**. After the end of the period, the Teflon containers were cooled to room temperature and carefully opened. Then, the samples in solution were made up to 25 mL with ultra-pure (UHQ) water.

Table 1. Sample preparation data for microwave digestion system

	Milk samples	**Packaging samples**
Sample quantity	0.25 mL	0.1 g
Acid	10 mL Nitric acid (%97)	10 mL Nitric acid (%97)
Microwave temperature program	200 °C	210 °C

The temperature was increased to	15 min	20 min
Standby time	15 min	15 min
Maximum power	1400 W	1400 W

2.2 Heavy Metal Analysis

i. Elements determination by ICP-OES

The inductively coupled plasma-optical emission spectrometer (ICP-OES) is one of the most popular devices used for the analysis of macro and micro (trace) elements in various samples [27]. It plays an important role especially in the detection of elements that cause chemical toxicity. It is also among the leading techniques for measuring the quantitative amounts of elements [27–29]. ICP-OES is known for its simultaneous analysis of many elements, accuracy, precision, and sensitivity in measurements and its ability to reach low detection limits [30,31] .

For calibration, multielement standard stock solution IV (Merck, Germany) was used in the preparation of calibration standards. Standard calibration solutions were prepared from the mix standard stock solution IV of 1000 ppm by dilution. The ranges of the calibration curves (5-6 points) were selected and prepared calibration solutions will be analyzed on the SpectroBlue brand ICP-OES. Linearity was checked and detection limits as LOD (Limit of Dedection) and LOQ (Limit of Quantitation) were calculated. ICP-OES operating parameters, elements, and calibration data are summarized in **Tables 2 and 3**, respectively.

Table 2. The operating parameters of ICP-OES

Instrument	**ICP-OES**
Brand/Model	SPECTRO BLUE II
Wavelenght	Nm
Replicates	3
Spray Chamber	Cyclonic
Plasma Segment	Axial and radial
TochBox Temperature	Max 58°C
Nebulizer Flow (L/min)	0.8
Nebulizer Pressure	2.0-4.0 bar
Plasma Torch	Quartz
Gas	Argon (purity 99.9%)
Main Argon Pressure	6-8 bar

Coolant Flow (L/min)	13
Auxiliary Gas Flow (L/min)	0.8
Sample Pump Speed (rpm)	30
Plasma Power (W)	1400

2.3 Evaluation of results

i. Statistical evaluation (SPSS)

In this study, statistical evaluations of the results were carried out using the SPSS package program. The relationship between milk and packaging samples was examined. In this context, correlation analysis, a statistical analysis showing the degree of association between two variables (weak-moderate-high degree), was performed.

3. Results and discussion

A microwave digestion system was used to determine heavy metals in milk and their packaging materials by inductively coupled plasma–optical emission spectrometry (ICP-OES). Al, As, Cd, Co, Cu, Fe, Pb, Mn, Ni, and Zn were determined in milk and their packaging materials. Analytical parameters of analysis are an important. The emission wavelength of elements, LOD, LOQ, and correlation coefficient for the calibration graph are presented in **Table 3**.

Table 3. The analytical parameters of analysis of elements

Elements	**Wavelengths (nm)**	**LOD (mg/kg)**	**LOQ(mg/kg)**	**r^2 (correlation coefficient)**
Al	167.078	0.139	120.000	0.99972
As	189.042	3.358	120.000	0.99953
Cd	214.438	0.211	120.000	0.99994
Co	228.616	0.732	120.000	0.99969
Cu	324.754	0.689	120.000	0.99945
Fe	259.941	0.972	120.000	0.99991
Pb	220.353	1.771	120.000	0.99966
Mn	257.611	0.356	120.000	0.99994
Ni	231.604	1.074	120.000	0.99979
Zn	334.502	0.261	90.000	0.99964

Relative standard deviation is one of the ways to indicate the accuracy of the analysis results for the reliability of the values [32]. Relative standard deviation provides a more precise result than absolute standard deviation for assessing the data quality. Relative standard deviation is expressed as a percentage and is desired to be low. In this study, the datas of %RSD (Relative Standard Deviation) for results were below 6.56% (**Table 4**). According to the survey conducted with the analytical device, RSD values were quite good.

Table 4. %RSD of elements by ICP-OES

	Al	**As**	**Cd**	**Co**	**Cu**	**Fe**	**Pb**	**Mn**	**Ni**	**Zn**
T1	3.655	6.565	1.823	2.222	3.392	2.930	5.303	3.242	3.019	3.266
T2	2.322	4.666	1.362	3.034	2.247	0.195	1.401	2.564	1.329	0.463
T3	1.246	3.029	0.898	4.035	3.575	1.267	1.275	3.344	1.514	0.127
T4	0.974	1.677	1.091	3.395	4.408	0.347	0.929	0.413	1.768	0.277
T5	0.584	2.525	0.828	5.395	0.371	0.885	0.423	1.537	0.318	0.210
T6	0.488	4.114	0.708	2.088	3.867	1.352	1.079	3.696	1.208	0.600
T7	0.916	1.560	1.520	2.174	6.098	0.691	0.513	1.037	0.218	0.099
T8	0.392	0.551	0.189	4.962	2.687	0.324	0.322	2.412	0.991	0.407
T9	0.722	0.619	0.340	4.276	2.662	0.675	0.259	1.086	1.189	0.155
T10	0.509	3.525	1.238	3.421	2.011	0.578	0.645	0.580	1.152	0.815
T1P	0.691	0.675	3.596	1.044	2.299	0.615	0.585	0.637	0.141	0.436
T2P	0.732	0.920	3.489	1.694	0.289	0.353	1.166	0.268	0.549	0.074
T3P	0.562	0.949	4.932	2.756	0.272	0.199	0.543	0.435	0.525	0.267
T4P	0.188	0.909	5.064	1.176	0.905	0.719	0.488	1.056	0.405	0.930
T5P	0.385	1.227	3.689	3.037	0.450	0.061	0.413	1.013	0.629	0.132
T6P	0.694	0.897	3.589	1.625	2.586	0.064	0.624	0.055	1.295	0.308
T7P	0.594	0.517	1.673	2.381	0.761	0.040	0.281	0.158	0.726	0.158
T8P	0.209	0.536	4.392	1.380	0.945	0.394	0.825	0.305	0.563	0.460
T9P	0.196	0.082	3.229	1.812	2.399	0.890	0.331	0.641	0.512	0.954
T10P	0.847	0.373	2.837	3.219	0.877	0.394	0.396	0.560	0.334	0.606

The samples were measured in triplicate and calculated as mean values. The results are expressed as mean concentration ± standard deviation. The results (as mg/kg) are presented in **Table 5**. Heavy metal concentrations in all milk samples were found: Al (1.219–2.578), As (1.078–1.522), Co (0.219–0.239), Fe (0.862–81.440), Pb (1.784–2.170), Mn (0.042–1.884), Ni (0.782–9.758, Zn (1.814–2.522) mg/kg. In milk samples, it was observed that the highest concentration belonged to Fe in the T1 sample, and the lowest concentration belonged to the Mn element in the T3 sample. Cu was found below the lowest detection limit in all milk samples (**Table 5** and **Figure 2**).

Heavy metal concentrations in all packaging samples were found Al (51267.00–71601.25), As (31.626–42.371), Cd (1.035–1.209), Co (0.775–1.167), Cu (4.921–44.839), Fe (259.615–463.182), Pb (24.386–26.668), Mn (4.301–59.599), Ni (6.065–7.943), Zn (5.324–8.763) mg/kg. The heavy metals in the highest concentration among packaging materials are Al and Fe, followed by As and Pb. The concentrations of these metals range from 10.2 to 96.5 mg/kg for Al and 17.5 to 104.7 mg/kg for Fe. The highest concentration of Al (71601.25 ± 2.097) and Fe (463.182 ± 6.531) was found in the T2P sample. The lowest concentrations belong to the elements Cd and Co. The lowest limit for Cd is in T1P; It was determined that Co was T5P. While the amount of Cu could not be detected in milk samples, it was detected in the range of 4.92–43.066 mg/kg in its packaging (**Table 5** and **Figure 3**). As seen in Table 5, the heavy metal contents in the packaging for all elements were found to be higher than those of milks. It has been determined that Al is the element with the highest content, especially in packaging materials. The reason for this is that the majority of the layers in the packages consist of aluminum foil and polyethylene.

Table 5. The results for heavy metal in milks and their packaging materials

	Al	As	Cd	Co	Cu	Fe	Pb	Mn	Ni	Zn
T1	1.829 ± 1.338	1.522 ± 1.999	0.219 ± 0.080	0.393 ± 0.175	N.D.	81.440 ± 4.772	1.784 ± 1.893	1.884 ± 1.222	9.758 ± 5.892	2.005 ± 1.310
T2	1.219 ± 0.566	1.231 ± 1.149	0.226 ± 0.062	0.088 ± 0.054	N.D.	5.653 ± 0.221	1.920 ± 0.538	0.078 ± 0.040	0.941 ± 0.250	1.888 ± 0.175
T3	1.342 ± 0.335	1.435 ± 0.869	0.237 ± 0.043	0.081 ± 0.066	N.D.	0.862 ± 0.219	2.024 ± 0.516	0.040 ± 0.027	0.782 ± 0.237	1.814 ± 0.046
T4	1.789 ± 0.349	1.345 ± 0.451	0.235 ± 0.051	0.095 ± 0.065	N.D.	2.183 ± 0.152	2.022 ± 0.376	0.070 ± 0.006	0.800 ± 0.283	2.335 ± 0.130
T5	2.138 ± 0.250	1.385 ± 0.700	0.237 ± 0.039	0.1003 ± 0.108	N.D.	1.332 ± 0.236	2.063 ± 0.174	0.047 ± 0.015	0.843 ± 0.054	2.522 ± 0.106
T6	2.475 ± 0.242	1.078 ± 0.887	0.227 ± 0.032	0.125 ± 0.052	N.D.	1.815 ± 0.491	2.025 ± 0.437	0.060 ± 0.045	0.893 ± 0.216	2.098 ± 0.252
T7	2.055 ± 0.377	1.123 ± 0.350	0.228 ± 0.070	0.125 ± 0.055	N.D.	1.831 ± 0.253	2.036 ± 0.209	0.042 ± 0.009	0.852 ± 0.037	1.844 ± 0.037
T8	2.578 ± 0.202	1.224 ± 0.135	0.225 ± 0.009	0.129 ± 0.129	N.D.	2.785 ± 0.180	2.066 ± 0.133	0.070 ± 0.034	0.859 ± 0.170	2.265 ± 0.185
T9	1.322 ± 0.191	1.337 ± 0.166	0.236 ± 0.016	0.1179 ± 0.101	N.D.	6.294 ± 0.850	2.148 ± 0.111	0.056 ± 0.012	0.870 ± 0.207	2.321 ± 0.072
T10	1.755 ± 0.179	1.315 ± 0.927	0.239 ± 0.059	0.1114 ± 0.076	N.D.	2.806 ± 0.324	2.170 ± 0.280	0.059 ± 0.007	0.929 ± 0.214	2.303 ± 0.376
T1P	65314.00 ± 1.807	39.759 ± 1.074	1.035 ± 0.149	0.914 ± 0.038	4.921 ± 0.453	372.5 ± 9.167	25.464 ± 0.596	30.306 ± 0.773	7.569 ± 0.043	6.960 ± 0.121
T2P	71601.25 ± 2.097	39.818 ± 1.465	1.088 ± 0.152	1.167 ± 0.079	43.066 ± 0.499	463.182 ± 6.531	26.668 ± 1.243	4.329 ± 0.046	7.823 ± 0.172	7.754 ± 0.023
T3P	68640.00± 1.543	42.371 ± 1.609	1.047 ± 0.207	0.921 ± 0.102	23.076 ± 0.251	394.277 ± 3.135	26.499 ± 0.575	59.599 ± 1.036	7.265 ± 0.153	6.798 ± 0.073
T4P	63892.50 ± 0.481	39.687 ± 1.444	1.097 ± 0.222	0.885 ± 0.042	42.902 ± 1.552	385.985 ± 11.096	25.142 ± 0.491	5.818 ± 0.246	7.943 ± 0.129	8.763 ± 0.326
T5P	63306.25 ± 0.976	35.914 ± 1.762	1.067 ± 0.157	0.775 ± 0.094	37.919 ± 0.683	394.822 ± 0.967	24.744 ± 0.408	4.301 ± 0.174	6.065 ± 0.153	8.256 ± 0.044
T6P	55791.00 ± 1.548	34.685 ± 1.245	1.073 ± 0.154	1.006 ± 0.065	4.289 ± 0.444	327.405 ± 0.844	24.386 ± 0.609	22.907 ± 0.051	7.052 ± 0.365	5.890 ± 0.073
T7P	55745.75 ± 1.325	37.059 ± 0.767	1.209 ± 0.081	0.941 ± 0.090	17.064 ± 0.519	277.915 ± 0.442	24.559 ± 0.276	29.221 ± 0.185	7.289 ± 0.212	5.324 ± 0.034
T8P	51267.00 ± 0.429	31.626 ± 0.678	1.048 ± 0.184	0.769 ± 0.042	19.708 ± 0.745	262.13 ± 4.129	22.925 ± 0.756	12.336 ± 0.151	6.460 ± 0.145	6.268 ± 0.115
T9P	58058.25 ± 0.455	37.307 ± 0.122	1.078 ± 0.139	0.982 ± 0.071	13.144 ± 1.261	259.615 ± 9.240	25.359 ± 0.335	32.356 ± 0.830	6.847 ± 0.140	6.427 ± 0.245
T10P	59704.50 ± 2.024	37.216 ± 0.555	1.160 ± 0.132	0.932 ± 0.120	44.839 ± 1.573	278.467 ± 4.390	25.331 ± 0.401	5.683 ± 0.127	7.374 ± 0.099	6.379 ± 0.155

N.D. (Not Detected)

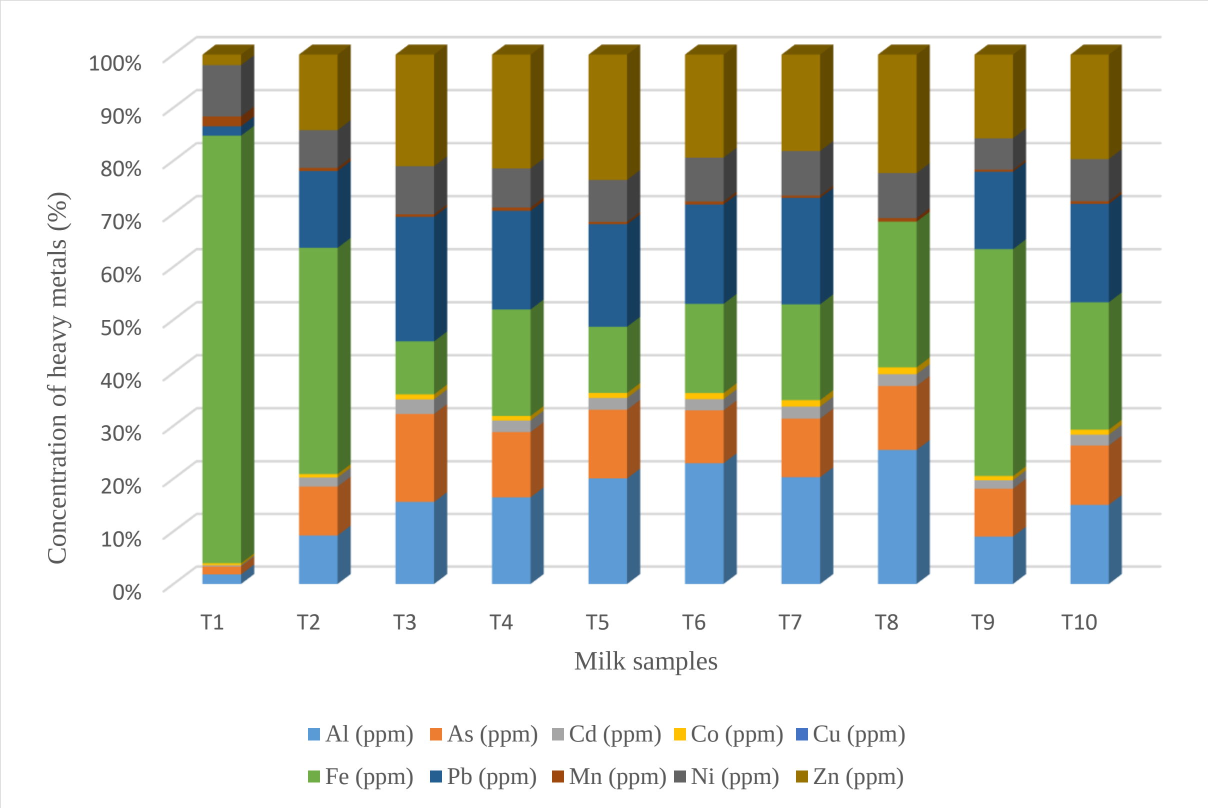


Figure 2. % Concentration of heavy metals in milk samples

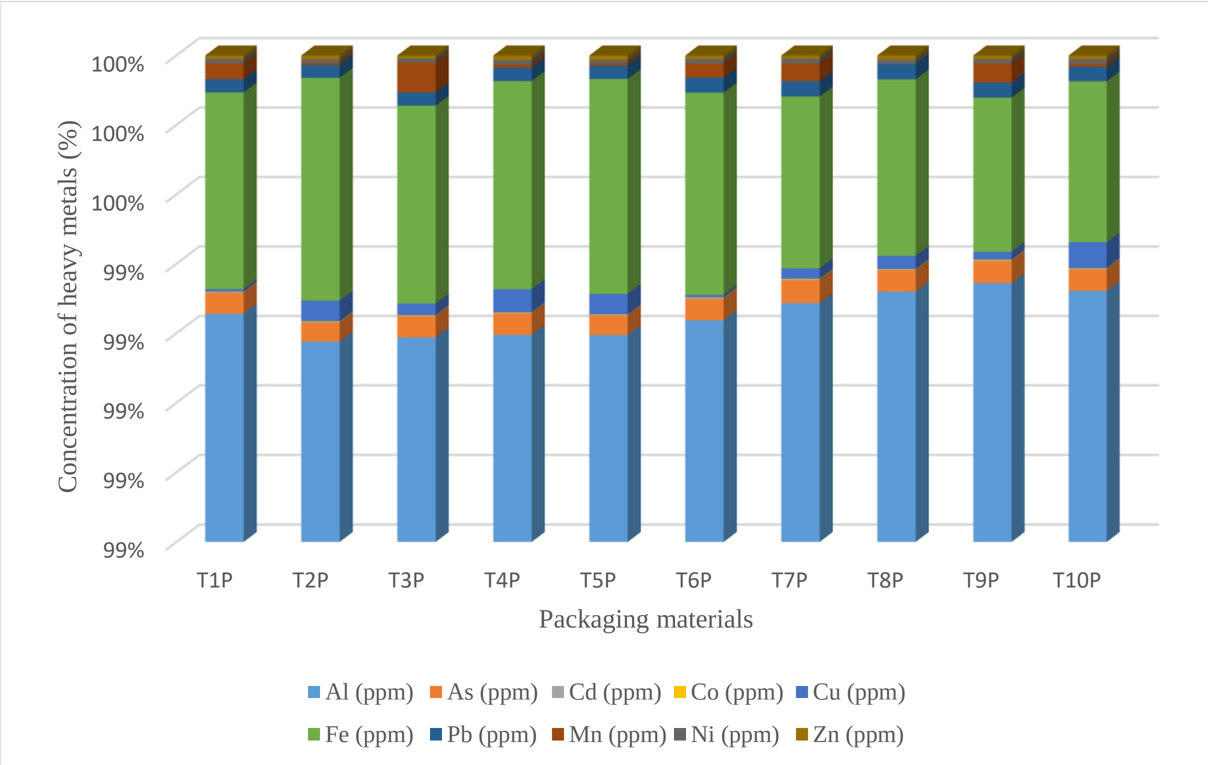


Figure 3. % Concentration of heavy metals in their packaging materials

Aluminum is necessary for some physiological processes in the body, but it is a toxic metal that can cause various neurological diseases due to excessive accumulation [33]. The main areas of aluminum use are packaging technology, electrical-electronics, drugs, and material technologies [34,35]. The allowable amount of aluminum in adults is announced as 1 mg/kg body weight/week by the BFAO/WHO Expert Committee on Food Additives [36]. In a study conducted on flavored milk samples, 0.957-0.984 mg/L Al was detected [13]. The aluminum concentration of Al in the raw bovine milk was found to be 2.93 μg/L [37]. In this study, the aluminum amounts of milk samples were 1.219-2.578 mg/kg. The aluminum value in milk packaging is significantly higher than in milk samples (**Table 5**). This is due to the aseptic aluminum foil layer in the structure of the aseptic packaging, which blocks the light.

Arsenic is one of the risky and toxic elements. It can be transmitted by humans and animals, mostly through drinking water [38]. In the literature, average arsenic contents of raw milk samples taken from different regions varied between 0.0002 and 0.05 mg/kg depending on the area [39]. The amount of As in flavored milk samples was found to be between 0.0038 -0.0057 mg/kg [13]. The amounts in our milk samples were found to be between 1.123 and 1.522 mg/kg.

Cadmium is a toxic element and poses a risk to human health [40]. Excessive accumulation may cause damage to the kidneys and reproductive system [41]. In this study, cadmium levels in milk and packaging samples were determined as 0.219 - 1.209 mg/kg. It was determined that the cadmium content of the milk samples was lower than that of the packages, but the values were close to each other. Cadmium contents in some reported studies it have been reported as 0.0032 mg/kg in fresh milk, and 0.0029 mg/kg in pasteurized milk [42]. In another study, Cd concentration in the studied milk samples (fermented, sterilized and modified milk) was found to be 0.0034, 0.0019, and 0.0041 mg/kg, respectively [43].

The recommended daily intake of **copper** for adults is 3 mg/day [44]. Although Cu is an important element in both humans and animals, excessive intake is highly toxic and damages the liver and kidneys [45]. In this study, average copper was not detected (below the detection limits). In the packages, it was detected between 4.921 and 44.839 mg/kg (**Table 5**). Bakırcıoğlu et al. Cu concentration in cow's milk was 0.138 mg/kg; They emphasized that it varies between 0.181 mg/kg and 0.111 mg/kg in milk-based products [46]. In another study conducted on vegan milks, the amount of Cu was in the range of 0.020-1.06 mg/kg [47]; In the study conducted on cow milk produced in Aydın province, it was found that it was below the detection limits [48].

Iron is an important mineral for life and is frequently encountered in various industries [33]. In foods, iron is found mostly in meats and is not abundant in milk, dairy products, and most non-green vegetables [49,50]. In our study, although the iron value of milk samples was low, the highest was detected in T1 brand milk (81 mg/kg). It was detected in the range of 0.862-6.294 mg/kg in the other nine milk samples. In the packaging, the amount of Fe is visibly higher than in milk (259.615-463.182). Another noteworthy point is that the highest Fe content is in T1 brand milk and in the packaging of T1 brand milk. Iron concentration has been emphasized to be not detected in vegan milks in the literature [12]. Ahmed et al. the average Fe value in fresh and pasteurized milk samples was found to be 1.915 and 1.274 μg/mL [42].

The element known as **lead** consists of a greyish-white, soft, and malleable metal. Since lead is not biodegradable, it can accumulate in the body, especially in bones and teeth. It is a very harmful metal, it can be found everywhere and if it progresses, it can cause serious environmental pollution [51]. According to the Joint FAO/WHO Expert Committee on Food

Additives, the maximum exposure to Pb is 1.2 μg/kg per day [52]. According to the Turkish Food Codex, the limit for Pb is 0.02 mg/kg in milk [53]. Lead values in milk samples were detected above the maximum limit (1.784-2.170 mg/kg) in this study. Lead levels in packaging materials were found to be higher than in dairy products. Cıddıroglu et al. in their study, lead levels in 59 milk samples were above the maximum detection limit; in 7 examples, they stated that it was in the range. They emphasized that the highest lead limit is 1.51 mg/kg [7]. In another study, it was reported that the amount of Pb in flavored milk samples was higher than the limits [13].

Manganese occurs naturally in living organisms such as plants and animals and is found in various food sources. Manganese is of great importance due to its vital role as one of the essential elements [54]. According to the World Health Organization (WHO), it is recommended that adults consume some manganese, between 2 and 9 mg per day [55]. In this study, the amount of manganese in milk samples was found to be between 0.040-1.884 mg/g. Karasakal et al. found the average Mn concentration in vegan milk samples to be in the range of 0.09-0.42 mg/kg [12]. In a different study conducted on coconut milk samples, the amount of Mn was found to be in the range of <0.11–3.88 mg/kg [56].

Nickel is believed to be a crucial element for humans due to its numerous potential roles in supporting and producing body cells. However, as the toxicity level of this metal increases, the level of allergic reactions in humans may also increase [57]. Nickel in foods can potentially come from manufacturing or storage processes, including drying, cooking, and packaging materials, as well as environmental contamination [58]. In this study, the nickel content in milk was reported to be in the range of 0.782-9.758 mg/kg. When the literature is examined, nickel metal is not very common among the elements detected in milk samples [12,39,42]. Özturan et al. While Ni was not found in goat, sheep, and human milk, they detected 0.27 mg/kg in cow's milk [10].

Zinc is the second mineral seen in cells after iron and is an important element for protecting immune cells. An imbalance in Zn intake causes the immune function to deteriorate, increasing the risk of developing diseases [59]. Based on clinical studies by FAO/WHO in 1966 and 1982, the recommended daily tolerable amount was determined as 0.3-1 mg/kg body weight [60]. According to the Turkish Food Codex, the maximum zinc concentration in foods is stated as 5 mg/kg [61]. In our study, Zn in milk was found in the range of 1.814-2.522 mg/kg. It was found to be lower than the maximum allowable Zn level.

According to the Turkish Food Codex Regulation, the limit value of Pb is given among the elements that may be present in milk. In all milk samples, milk samples with Pb levels above the LOD were above the limit reported by the Turkish Food Codex Regulation. Since there is no legal limit in the Turkish Food Codex Regulation for other elements in milk, they are evaluated based on acceptable daily intakes according to FAO/WHO.

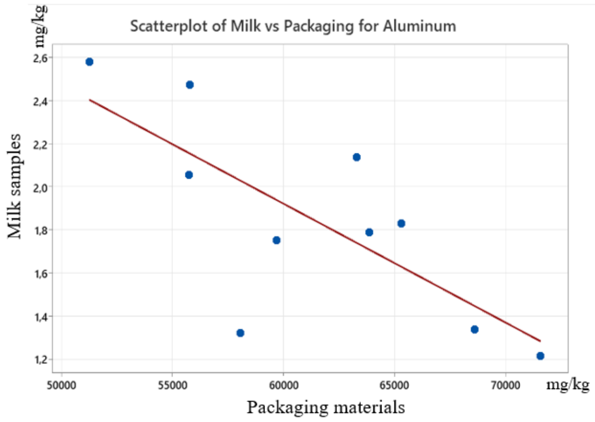
i. Statistical evaluation of the results

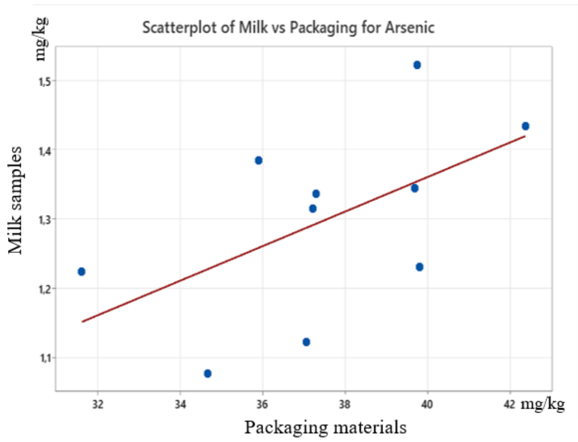
In this section, the relationship between milk and milk package samples was examined. Correlation analysis was performed between milk samples and their packaging for each heavy metal. Negative and positive correlations were detected. '*r*' values indicating the degree of relationship were noted for each relationship and are given in **Table 6**. Scatter graphs of milk and their packaging were created (**Figure 4**). The relationship levels between packaging and milk were stated. The correlation level is high for the element Al; As for moderate; and very weak for Cd, Co, Fe, Mn, and Ni; It was found to be weak for Pb and Zn. Since the Cu element remained below the detection limits in all milk samples, correlation analysis could not be performed. A scatterplot is a scatterplot that visually shows the pattern of relationships between two variables. A scatter plot is helpful to see outliers and those that are far apart.

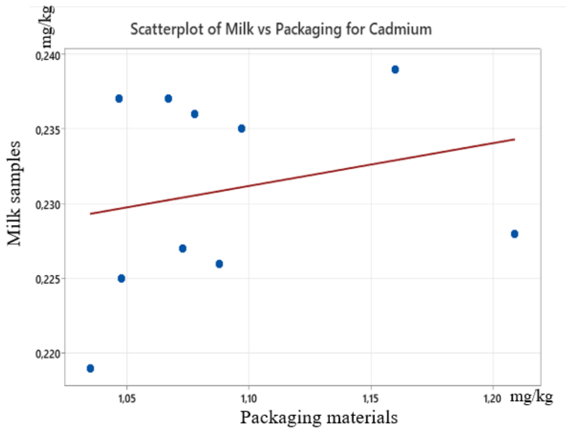
Table 6. Correlation values and degrees between milk and their packaging according to all elements

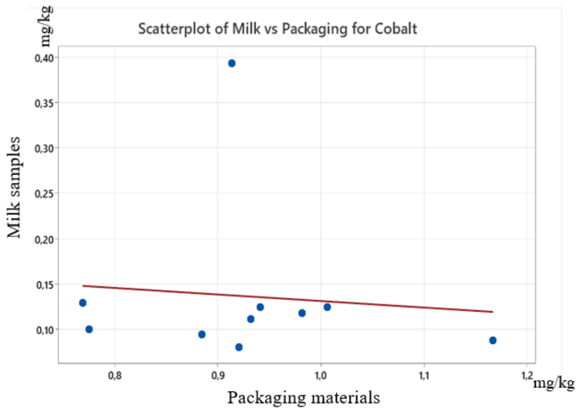
	Milks - Packaging									
	Al	As	Cd	Co	Cu	Fe	Pb	Mn	Ni	Zn
r	-0.742	0.556	0.232	-0.089	-	0.149	-0.281	0.175	0.242	0.401
Degree	H	M	VW	VW	-	VW	W	VW	VW	W

H: High; M: Moderate; VW: Very Weak; W: Weak;









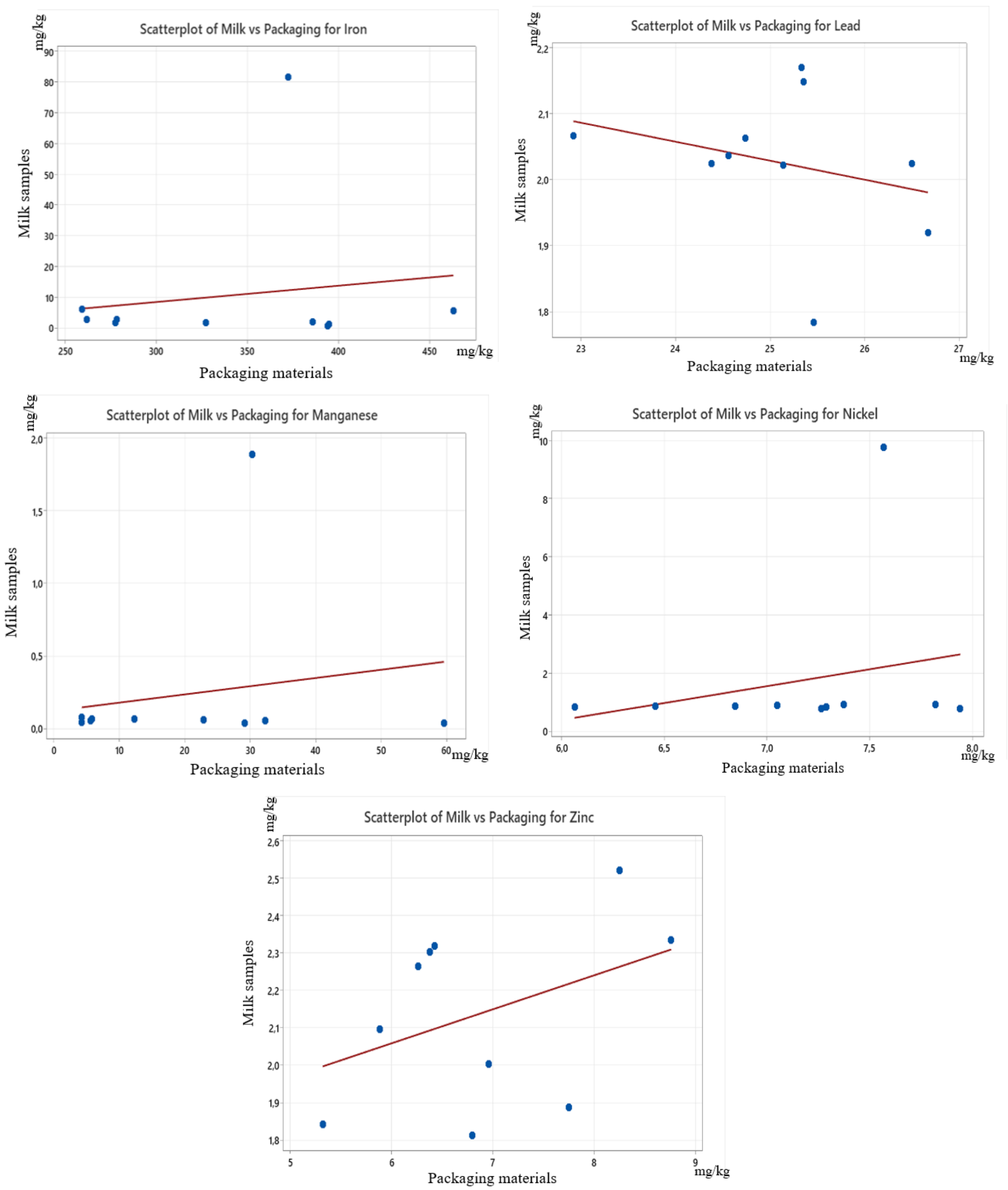


Figure 4. Scatterplots of milk vs packaging for concentrations of all heavy metals

3. Conclusion

In this study, heavy metals in the milk and packaging of ten different milk brands used commercially today were detected. Within the scope of heavy metal analyses, the elements Lead, Arsenic, Cadmium, Iron, Manganese, Zinc, Copper, Aluminum, Cobalt, and Nickel were determined using ICP-OES. The heavy metal concentrations obtained are comparable to similar studies. These concentrations were compared to those announced by the Expert Committee on

Food Additives. The relationship between milk and packaging was examined for each element. It was observed that the element Al had a negative correlation and a high level of relationship depending on the correlation value. It was determined that the relationship levels of the other elements, except Cu, were weak and very weak. It is thought that there may be an interaction between packaging and milk due to the high level of correlation in aluminum. This study paved the way for future toxicology studies by leading to the determination of heavy metals in food products. It also contributed to ensuring quality food and food safety by determining the heavy metal content of milk and milk packaging materials.

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