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DETERMINATION OF FE, CU AND ZN CONTENT IN SOME SPICES SOLD WITHOUT PACKAGING IN VAN

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ABSTRACT
The content of Fe, Cu, and Zn present in black pepper, cumin and red pepper available at herb stores and local markets in Van were determined using ICP-MS. The Fe content was 557.5 mg/kg, 467 mg/kg, 122.5 mg/kg, the Cu content was 19.4 mg/kg, 12.3 mg/kg, 11.9 mg/kg, and the Zn content was 17.6 mg/kg, 36.5 mg/kg, 11.9 mg/kg in black pepper, cumin, and red pepper, respectively. The highest Fe, Cu and Zn content were found in black pepper and the lowest Fe, Cu and Zn content in red pepper. The results showed that the Cu and Zn content determined in black pepper, cumin, and red pepper was below the WHO standards and the Fe content determined in black pepper and cumin was found to exceed the WHO standards. Therefore, continuous heavy metal analysis in terms of Fe, especially in black pepper and cumin, is essential for human health.
Keywords: Spice, iron, zinc, copper

VAN İLİNDE AMBALAJSIZ OLARAK SATIŞA SUNULAN BAZI BAHARAT ÇEŞİTLERİNDE FE, CU VE ZN DÜZEYLERİNİN BELİRLENMESİ

ÖZ
Van ilinde baharatçı ve yerel marketlerden temin edilen karabiber, kimyon ve kırmızıbiberdeki Fe, Cu ve Zn düzeyleri ICP-MS cihazında tespit edildi. Karabiber, kimyon ve kırmızıbiberde Fe düzeyi sırasıyla 557.5 mg/kg, 467 mg/kg, 122.5 mg/kg, Cu düzeyi sırasıyla 19.4 mg/kg, 12.3mg/kg,11.9 mg/kg, Zn düzeyi sırasıyla 17.6 mg/kg, 36.5mg/kg, 11.9 mg/kg'dı. Analiz sonucunda en yüksek Fe, Cu ve Zn düzeyinin karabiberde, en düşük Fe, Cu ve Zn düzeyinin ise kırmızıbiberde olduğu belirlendi. Karabiber, kimyon ve kırmızıbiberde tespit edilen Cu ve Zn düzeyleri WHO'nun bildirdiği limitlerin altında, karabiber ve kimyonda tespit edilen Fe düzeyi ise WHO'nun bildirdiği limitlerin üstündeydi. Bu nedenle, özellikle karabiber ve kimyonda Fe yönünden ağır metal analizlerinin devamlı yapılması insan sağlığı açısından gereklidir.
Anahtar kelimeler: Baharat, demir, çinko, bakır

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INTRODUCTION

Spices are the dried bits of plants and are added to meals in small amounts. They are widely used for both home and industrial purposes to enhance the color, flavour, and palatability of foods. Black pepper is derived from fruits of the trees in the Piperaceae family (*Piper nigrum*), which are collected before they are not fully ripe. Black pepper is added in meat dishes, salads and sauces to increase pungency of the food. Red pepper (*Capsicum annuum*) is used in a variety of dishes, such as salads, soups, meat dishes, and some sauces. Cumin (*Cuminum cyminum*) is indigenous to Egypt and India. It is also grown in Konya and Ankara in our country. It is used to add a unique flavor to dishes, such as soups, meat dishes, sauces and fish (İzer, 1997).

The Codex Alimentarius Commission (CAC) identified food contaminants as chemicals that are contaminated as a result of production, processing, preparation, storage, packaging, transportation or environmental pollution, although they are not intentionally added to food (Codex Alimentarius Commission, 2011). In recent years, heavy metals are among the most dangerous food contaminants that threaten human health (Jarup, 2003). In the list of heavy metals, there are more than 60 metals including Pb, Cd, Fe, Co, Cu, Ni, Hg, and Zn. Some of these metals (Fe, Mg, Zn, Cu, Mn) are also essential for the proper functioning of biochemical processes in the human body. These trace metals are taken up the body from potable water, food, and inspiration. However, high levels of these essential heavy metals accumulate in the body and may cause acute or chronic toxicity (Kahvecioglu et al., 2003).

Heavy metals are one of the global contaminating factors that pose danger and risk to humans and all living organisms. Environmental pollution problems have started to arise due to factors such as rapid industrialization, transition to modern agriculture, urbanization, domestic and industrial wastes, close proximity of the cultivation areas to the city, heavy metals emitted from the chimney smoke or the exhausts of motor vehicles and distorted urbanization due to the rapidly increasing population in the world (Stresty and Madhava, 1999). Increasing environmental contamination has also caused soil contamination to increase to a level where it poses risks for living organisms. The major soil pollutants heavy metals are Cd, Cr, Hg, Pb, Cu, and Zn. These heavy metals accumulate in plant tissues and enter foods by the food chain (Çağlarırmak and Hepçimeni, 2010). Nutrition sources are contaminated through food-chain as a result of environmental and soil contamination, which may cause serious health conditions (Vural, 1993). In particular, heavy metal accumulation changes the physiological and genetic structure in the organisms (Öktüren and Sönmez, 2006; Okçu et al., 2009).

The aim of this study is to determine the levels of Fe, Cu, and Zn in the spices widely used in Van for flavoring such as red pepper, black pepper and cumin, and to evaluate the potential risks to public health. Spices are added in foods and consumed daily, so to be aware of the heavy mebal content of spices is essential for human health.

MATERIAL AND METHOD

Reagents

Analytical grade nitric acid, perhydrochloric acid (Merck, Germany) and deionised water (Milli-Q System, Millipore, Billerica, USA) were used for the preparation of all solutions. All plastic and glassware were soaked in 10% (v/v) HNO_3 overnight and rinsed with deionised water prior to use. Multielement ICP-MS calibration standard (İnorganic Ventures, USA) was used as standard solution for Fe, Cu, and Zn.

Sample collection

A total of 75 samples of red pepper, black pepper and cumin were obtained from herb stores and local markets in Van. These samples were taken from each sack with a probe to correspond to 1 sample per 300 kg. The samples were put into sterile bags and delivered to the laboratory in a short time in order to prevent air and high temperature.

The samples were preserved in the packages at room temperature until they were analyzed.

Sample preparation
For determination of heavy metal concentrations, a wet digestion of the dried samples was done according to the method described by Allen (1986) using % 65 HNO_3 and $HClO_4$ mixture. Each sample was weighed about 0.5 g and then combined with a 10 ml of 3:1 acid mixture (HNO_3 and $HClO_4$). After waiting for a while, the mixture was heated up to 70 ^{0}C until a transparent solution was obtained. The clear solution was transferred into 25 ml volumetric flask, and completed to the mark with double distilled deionized water. Blanks were also prepared according to the same digestion procedure for comparison.

Measurement of heavy metals
The metal content analysis of the samples was performed with an ICP-MS device (Thermo Scientific UK, X Series 2). For each of 75 spice samples, three paralel samples were analyzed and the final results were expressed by taking the average of the results of three measurements. Calibrations were carried out using a multi element ICP-MS Standard provided from Inorganic Ventures (Christiansburg, VA, USA) with Fe, Cu and Zn concentrations of 98 μg/L, 23 μg/L and 79 μg/L, respectively. The limits of detection (LODs), defined as the concentration of each element corresponding to three times the Standard deviation of 10 reagent blanks, were 0.021, 0.003 and 1,77 μg/g for Fe, Cu, and Zn respectively. The limits of quantification (LOQs), defined as the concentration of each element corresponding to 10 times the standard deviation of 10 reagent blanks, were 0.069, 0.010, and 5.84μg g^{-1} for Fe, Cu, and Zn respectively. The accuracy and precision of the digestion procedure was carried out in cumin samples by spiking the analyzed samples with aliquots of metal standard and then reanalyzing the samples. All the spiked samples were digested following the procedure described earlier in triplicates. Percentage of recoveries obtained was 95.4, 93.8, 91.5 % for Fe, Cu, and Zn respectively and the relative Standard deviations (RSDs) of replicates were less than 10%.

Statistical analysis
Data was analyzed with IBM SPSS V23. The Fe, Cu and Zn parameter was assessed by one-way analysis of variance. Tukey HSD, a multiple comparison test, was used. The results were shown as average ± standard deviation. $P<0.001$ was chosen as the significance level.

Human Health Risk Assessment:
Human health risk assessment was carried out with the estimation of the daily consumption of an individual, in order to determine the "safe" daily intake, which is the amount considered tolerable. The exposure rate to heavy metals was calculated by the below formula according to the average metal concentration detected in the samples, assumed 20 g daily intake consumed by 50 kg body weight (ATSDR, 2016).
Calculation of the daily heavy metal intake:
EDI = HMC X TDI/1000/ABW
EDI: Estimated daily intake (mg/kg/day)
HMC: Heavy metal concentration,
TDI: The daily intake (20 g)
ABW: Average body weight (50 kg)

RESULTS
The contents of Fe, Cu and Zn in black pepper, cumin, and red pepper were presented in Table 1. The values of metal concentrations were compared with the maximum permissible concentration as recommended by WHO (1984).

Table 1.The Fe, Cu and Zn content found in black pepper, cumin and red pepper

Spices	n	Fe (mg/kg)	Cu (mg/kg)	Zn (mg/kg)	*p*
Black pepper	25	557.5±315.83^a	19.4±7.2^a	17.6±7.56^a	<0.001
Cumin	25	467±225.5^a	12.3±3.9^b	36.5±14.1^b	<0.001
Red pepper	25	122.5±48.1^b	11.9±5.2^b	11.9±2.02^c	<0.001
WHO limit (mg/kg) (1984)		300	50	100	

a, b, c: There is no difference between the groups with the same letter on the same line for each parameter.

Fe contents of spice samples are given in Table 1 and Figure 1. The mean values of Fe analysis showed differences among the groups ($P<0.001$). The mean value was found 557.5 mg/kg in black pepper, 467 mg/kg in cumin and 122.5 mg/kg in red pepper. While there was no difference statistically between the mean values of black pepper and cumin, red pepper apeared to be significantly lower than the others (Figure 1). As comparing with standard limit, the blackpepper and cumin sample had the highest content of Fe that far exceeds the permissible level recommended by WHO (1984) (Table 1).

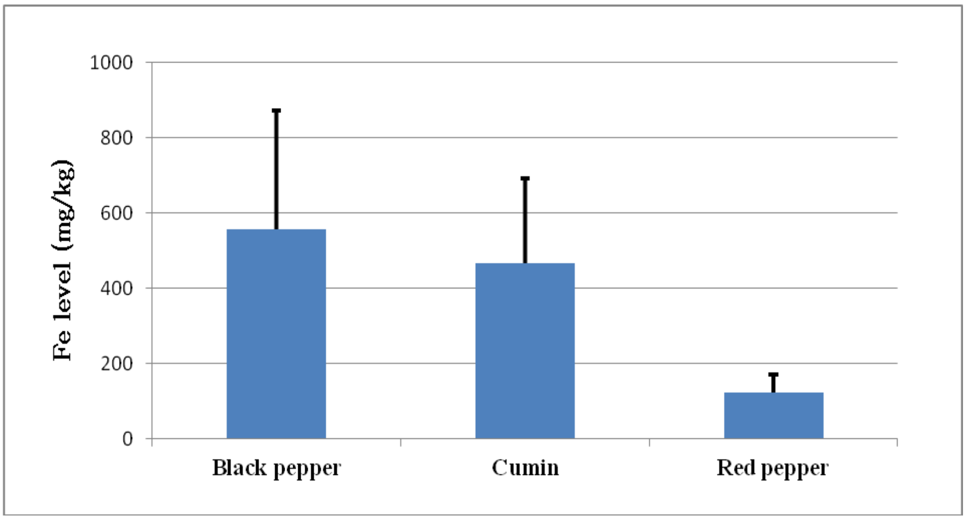

Figure 1. Levels of Fe in spice samples

As shown in Table 1, the mean values of Cu analysis showed differences among the groups ($P<0.001$). The mean value was 19.4 mg/kg in black pepper, 12.3 mg/kg in cumin and 11.9 mg/kg in red pepper. The mean value in cumin and red pepper showed no statistical difference ($P> 0.001$) while the mean value in black pepper appeared significantly higher than the others (Figure 2). The concentrations of Cu of all the samples were lower than the maximum permissible concentration (50mg/kg) of Cu (WHO, 1984).

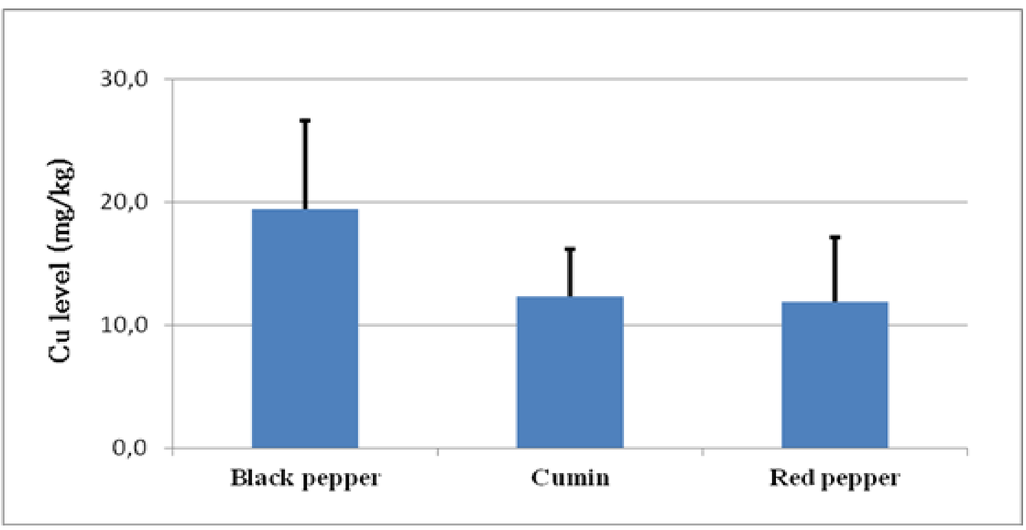

Figure 2. Levels of Cu in spice samples

The mean values of the Zn analysis differ among the groups ($P < 0.001$) in Table 1. The mean value was found 17.6 mg/kg in black pepper, 36.5 mg/kg in cumin and 11.9 mg/kg in red pepper. There were differences between the mean values of black pepper, cumin, and red pepper. The lowest mean value was found in red pepper, while the highest value was found in cumin (Figure 3). The concentrations of Zn of all the samples were lower than the maximum permissible concentration (100mg/kg) of Zn (WHO, 1984).

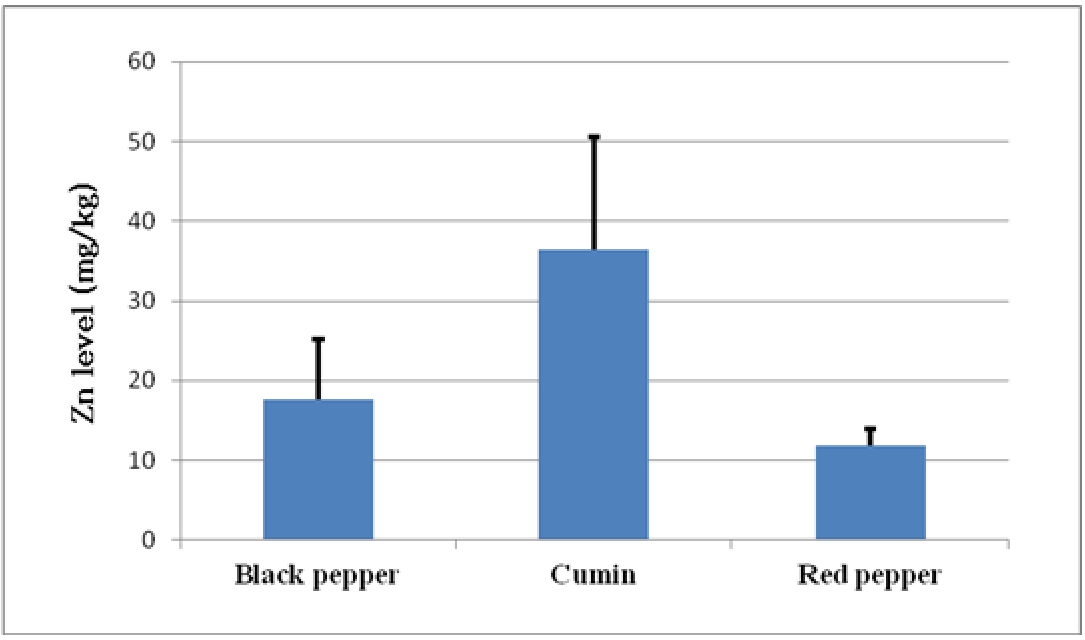

Figure 3. Levels of Zn in spice samples

The results of comparison of the daily intake (1mg/kg/day) calculated according to the average amount of heavy metals detected in spices with the minimum risk level (MRL) determined for heavy metals are given in Table 2.

Table 2. Comparison of the daily intake (1mg/kg/day) calculated according to the average amount of heavy metals detected in spices with the minimum risk level (MRL) determined for heavy metals.

Spices	Fe (mg/kg/day)	Cu (mg/kg/day)	Zn (mg/kg/day)
Black pepper	223×10^{-2}	7.8×10^{-3}	7×10^{-3}
Cumin	187×10^{-2}	4.9×10^{-3}	14.6×10^{-3}
Red pepper	49×10^{-2}	4.8×10^{-3}	4.8×10^{-3}
WHO MRL (1993)	70×10^{-2}	10×10^{-3}	300×10^{-3}

It was calculated assuming that a 50 kg weighing person consumed 20 g of spices daily.

DISCUSSION

Metals are not present in the natural structure of food, but can be transmitted to food in various ways. Due to the wear and tear of the machine parts during the grinding of spices in mills, contamination with heavy metals may occur. In addition, heavy metals can be transported from these machines, tools and materials, packaging materials, which are in contact with foodstuffs such as spices. Spices are continuously consumed in small amounts together with foods, so metals can accumulate in the human body and cause various health problems by consuming spices contaminated with heavy metals (Janitha et al., 1988). Therefore, routine checks and analysis of spices should be performed regularly. There are many studies analyzing heavy metal content in spices. Comparison of Fe, Cu and Zn values in this study with other studies presented in Table 3.

Table3. Comparison heavy metal concentration in this study with previous studies.

Literature and sample location	Spices	Fe (mg/kg)	Cu (mg/kg)	Zn (mg/kg)
This study, Turkey (Van)	Black pepper	557.5	19.4	17.6
	Cumin	467	12.3	36.5
	Red pepper	122.5	11.9	11.9
Ansari et al. (2004), Pakistan (Multan)	Black pepper	155	14.3	5
	Cumin	482	17	43
	Red pepper	3708	141	22.8
Krejpcio et al. (2007), Poland	Black pepper	-	5.44	7.15
	Cumin	-	5.11	17.88
	Red pepper	-	7.07	14.20
Ibrahim et al.(2012), Iraq (Erbil)	Black pepper	390	19.2	29.4
	Cumin	222.3	9.3	44
	Red pepper	-	-	-
Umar and Zubair (2014), Nigeria (Abuja)	Black pepper	275	8.9	40
	Cumin	2401	32.6	5
	Red pepper	191	10.4	42.5
Seddigi et al. (2016), Saudi Arabia	Black pepper	144	10.5	7.5
	Cumin	84.8	6	19.4
	Red pepper	192	8.7	10.2
Bazargani-Gilani and Pajohi-Alamoti (2017), Iranian (Hamedan)	Black pepper	222.33	9.92	11.40
	Cumin	98.3	3.31	5.46
	Red pepper	56.79	8.38	9.53

Fe is an essential element for vital functions. But excess iron accumulation causes damage to the organisms. The balance of Fe-blood levels in the body is principally controlled by modulation of the absorption of dietary Fe in the proximal intestine (Özsürekci, 2009). On average, 2-6 grams of Fe are stored in the body. 65% of the body Fe is found in hemoglobin, 25% contained in an Fe store, 10% in the myoglobin and the remaining in a variety of enzymes (Diri, 2007). Fe causes toxicity in the body as the human body has no mechanisms for the excretion of Fe. Therefore, the balance of Fe is mediated through a balancing mechanism that regulates the Fe absorption and excretion (Özsürekci, 2009). In this study, the level of Fe found in black pepper and cumin exceeds the maximum permissible limit recommended by WHO (Table 1). The high levels of Fe might be the result of the contamination during the grinding process. In previous studies, it was pointed out that spices are 3 to 5 times more contaminated with heavy metals in commercial grinders due to wear and tear of the machine parts of the mills (Janitha et al., 1988). Ibrahim et al. (2012) found higher Fe values in black pepper; Ansari et al. (2004), Singh et al. (2006) and Nkansah and Amoako (2010) found higher Fe values in cumin which were exceeding the maximum permissible limit. These results are in correlation with our study. In addition, Mubeen et al. (2009) found that the level of Fe (144.5-1260 mg/kg) in the spices of two of the most popular Pakistani brands were higher compared to the other metals. The daily intake of Fe determined in this study exceeds the MRL standards recommended by WHO for black pepper and cumin (Table 2). Therefore, consuming these spices may pose a risk to health.

Cu is both an essential and toxic element for many living creatures. Cu has the greatest catalytic activity among all other elements. It reacts with proteins and accelerates the chemical reactions. It is also necessary for carbohydrate/lipid metabolisms. Though only a trace amount is sufficient for functioning: Cu intake in excess of the nutrient needs can be toxic (Aksoy, 2000; Şahan, 2003). Cu is found in various foods such as oilseeds, whole grains, liver and other organs, seafood (Temurci and Güner, 2006). However, the Cu levels in foods are gradually increasing as a result of the contamination due to various factors such as industrial operations or toxic pesticides (Vincevica-Gaile and Klavins, 2012). Intake of Cu at excess level is toxic and inhibits the functions of some enzymes in the body. This inhibition of vital activities of enzymes by Cu may cause liver dysfunction. It can cause liver necrosis and eventually result in death. In addition, symptoms such as epigastric pain, nausea, vomiting, and diarrhea are observed due to an excessive Cu intake. Excessive Cu levels may damage the brain, kidney, and cornea and also causes dementia by affecting the nervous system (Aksoy, 2000; Şahan, 2003; Temurci and Güner, 2006). Our study reveals that all values of Cu found in the spices fall short of the MRL standards recommended by WHO (Table 2). In other studies (Ansari et al., 2004; Krejpciove et al., 2007; Ibrahim et al., 2012; Umar and Zubair, 2014; Bazargani-Gilani and Pajohi-Alamoti, 2017); the Cu content in black pepper, cumin, and red pepper are similar to the values obtained in this study. However, the level of Cu reported by Ansari et al. (2004) is much higher than the value in this study and all other Cu values found in other studies (Table 3). It also exceeds the maximum permissible limit recommended by WHO.

Zn exists in every cell of the body and plays an important role in growth and development. Zn is an essential cofactor for more than 300 enzymes and has a regulatory role for their activities. However, high levels of Zn in the body affect the metabolism and interfere with activities of other metals (Nwoko and Mgbeahuruike, 2011). In this study, the Zn content found in the spices is below the maximum permissible limit recommended by WHO (Table 1). In earlier studies (Ansari et al., 2004; Krejpciove et al., 2007; Ibrahim et al., 2012; Umar and Zubair, 2014; Seddigi et al., 2016; Bazargani-Gilani and Pajohi-Alamoti, 2017), the levels of Zn found in black pepper, cumin and red pepper show correlation with the levels found in this study. Zn level in the spices may vary depending on the soil conditions, geographical location and cultural methods (Khan et al., 2014). Zn levels of spices determined in this study was below the MRL standards recommended by WHO (Table 2). So, it does not pose a risk to health.

When a comparison is generally made according to the location of the samples in Table 3, Fe, Cu, and Zn levels in the samples sold without packaging in herb stores and local shops in Van appear to be higher than the Fe, Cu and Zn levels in the samples obtained from Saudi Arabia and Iran.

The results in Table 2 showed that no risk from daily intake of this spices for Cu, and Zn if the human intake is about 20 g of spices per day.

CONCLUSION

As a result, while the levels of Cu and Zn determined in black pepper, cumin and red pepper are below the limits recommended by the WHO, they do not pose a health risk, whereas Fe levels in pepper and cumin that are determined above the WHO recommended limits may constitute a health risk. Therefore, routine analyzes of Fe, especially in pepper and cumin, are important for human health. On the other hand, since the levels of Cu and Zn determined in black pepper, cumin and red pepper are below the limits recommended by WHO, these spices can be considered as an alternative source of Cu and Zn.

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