Trace Element Concentrations in Edible Wild Mushroom Samples From Turkey Determined by Atomic Absorption Methods Using Microwave Digestion vs. Wet Ashing

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INTRODUCTION

Mushrooms are a valuable, healthy food, low in calories and high in protein, vitamins, and minerals (1). Wild-growing mushrooms have been a popular delicacy in many countries, particularly in central and eastern Europe, and are a popular food source in Turkey (2–3). Mushroom picking is a “national hobby” in the Czech Republic, with a statistical mean of 5.6 kg per household per year. However, some individuals consume more than 10 kg per year (4–6). Mushrooms also have a long history of use in traditional Chinese medicine and are considered a therapeutic food in preventing diseases such as hypertension, hypercholesterolemia, and cancer. These functional characteristics are mainly due to the chemical composition of the mushrooms (2); thus, it is necessary to know the levels of their essential elements (7). Research regarding the trace element content of wild mushrooms has been ongoing since the 1970s (8).

Foods together with water provide the major portion of the total daily intake of trace elements. Knowledge about the trace element composition in foods is, therefore, important for both the consumer and the health professional. In recent years, food labeling legislation has highlighted this requirement (9). Heavy metal concentrations in mushrooms are consider-

ABSTRACT

In this study, the trace element concentrations of Fe, Cu, Mn, and Zn in mushroom species were determined by flame atomic absorption spectrometry (FAAS) and Cd, Cr, Ni, and Pb by graphite furnace (GFAAS) after wet ashing and microwave digestion. The results show that microwave digestion was best because it is simple, fast, and more accurate than wet ashing. The results were also compared to data reported in the literature. The mushrooms were collected in the middle region of Anatolia, Turkey, having metal concentrations of 21.4 µg/g (Cu), 1.10 µg/g (Cd), 2.81 µg/g (Pb), 74.5 µg/g (Zn), 78.6 µg/g (Mn), 389 µg/g (Fe), 1.30 µg/g (Cr), and 13.2 µg/g (Ni). These levels are allowable limits, except for lead. The concentration of the trace elements in the samples is dependent on the type of mushroom species since some accumulate trace elements at higher levels. The Pb levels in the mushroom samples (some edible) from this region were found to be higher than the legal limit. The accuracy of the method was verified by using certified reference material.

ably higher than in agricultural crop plants, vegetables, and fruit (2) since many wild-growing species accumulate elements at high concentrations (10) due to environmental conditions. Compared to green plants, mushrooms can build up high concentrations of the heavy metals such as Pb, Cd, and Hg. Therefore, great efforts have been made to evaluate their

possible danger to human health (11). The heavy metal concentrations in mushrooms are mainly due to the acidic and organic matter content in the ecosystem and soil. The uptake of metal ions in mushrooms is in many respects different from plants. For this reason, the variation in metal concentrations depends on the mushroom species and their ecosystems (11–13).

Turkey has a large edible wild mushroom potential and is becoming an important exporter. The purpose of this study relates to the determination of toxic and essential elements (Cu, Cd, Pb, Zn, Mn, Fe, Cr, and Ni) in some mushroom species collected from the middle region of Anatolia, Turkey.

EXPERIMENTAL

Instrumentation

For this study, a PerkinElmer® AAnalyst™ 700 flame atomic absorption spectrometer (FAAS), equipped with deuterium background corrector, was used for Fe, Cu, Mn, and Zn determination. The PerkinElmer® HGA® 700 graphite furnace AAS was used for the determination of Cd, Cr, Ni, and Pb (PerkinElmer Corporation, Shelton, CT, USA). An Elektro-mag m5040p oven (Elektro-mag A.S., Istanbul, Turkey) was used for drying the samples. The instrumental operating conditions are listed in Table I.

Microwave digestion was performed in an Ethos D microwave oven (Milestone, Italy). For wet ashing, a Model KS501D hot plate (IKA, Germany) was used.

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Samples

In this study, 20 mushroom samples of 18 different species were collected from the middle region of Anatolia (Sivas, Kayseri, Nevsehir, Nigde, and Yozgat). The samples were dried at 105 °C for 24 h in the Elektro-mag oven. The dried samples were homogenized using an agate mill and then stored in polyethylene bottles until analysis.

Reagents and Standard Solutions

All reagents were of analytical reagent grade unless otherwise stated. Double deionized water was obtained with a Milli-Q® system, MΩ-cm resistivity (Millipore Corporation, USA) and was used for all dilutions. Suprapur® HNO₃ and H₂O₂ were obtained from E. Merck, Darmstadt, Germany. All plastic and glassware was cleaned by soaking overnight in a 10% (w/v) nitric acid solution, then rinsed with deionized water. The standard solutions

of the investigated elements (1000 mg/L) were prepared by dissolution of pure metals or their salts (E. Merck) and were further diluted prior to use. The matrix modifiers of NH₄H₂PO₄, Pd, and Mg(NO₃)₂ were purchased from Sigma Chemical Co., St. Louis, MO, USA. The standard reference material NIST SRM 1515 Apple Leaves (National Institute of Standards and Technology, Bethesda, MD, USA) was used to establish the accuracy of the method.

Analytical Procedure

The elements Pb, Cr, Ni, and Cd in the samples were determined using the PerkinElmer® HGA® 700 graphite furnace AAS with argon as the inert gas. Pyrolytically coated graphite tubes with a platform were used for Cr, Ni, Cd, and Pb. The measurements for Fe, Cu, Mn, and Zn were carried out in an air/acetylene flame. The operating parameters for the working

elements were set as recommended by the manufacturer (Table I). All metal concentrations were determined on a dry weight basis.

The detection limit is defined as the concentration corresponding to three times the standard deviation of 10 blanks. The detection limits obtained by flame AAS were 0.10 µg/g for Cu, 0.15 µg/g for Zn, 0.20 µg/g for Mn, 0.25 µg/g for Fe. The detection limits for Pb, Cd, Cr, and Ni were below flame AAS detection and thus were determined by GFAAS. The values obtained were 0.01 µg/kg for Cd, 0.02 µg/kg for Pb, 0.03 µg/kg for Cr, and 0.04 µg/kg for Ni. The amount of matrix modifier added for Pb determination was 200 µg NH₄H₂PO₄, and for Cd, Cr, and for Ni it was 15 µg Pd+10 µg Mg(NO₃)₂.

Microwave Digestion Procedures

For digestion, a Milestone Ethos D microwave closed system (maximum pressure 1450 psi, maximum temperature 300 °C) (Milestone, Italy) was used. Samples of 0.5 g were digested with 6 mL of HNO₃ (65 %) and 1 mL of H₂O₂ (30%) in the microwave digestion system for 23 minutes, then diluted to 10-mL volume with deionized water. A blank digest was carried out in the same way. The digestion conditions for the microwave system were as follows: 2 minutes for 250 W, 2 minutes for 0 W, 6 minutes for 250 W, 5 minutes for the 400 W, 8 minutes for 550 W, and venting for 8 minutes.

Wet Ashing

A 0.5-g sample was digested with a mixture of HNO₃/H₂O₂ (6:2) on a hot plate (Model KS501D) for solubilization at 130 °C for 4 hours. This procedure was repeated two times. The residue was filtered through a Whatman filter paper (Whatman grade 589) and brought to 10-mL volume with distilled deionized water. The element con-

TABLE I
Instrumental Operating Conditions for FAAS and GFAAS

FAAS – PerkinElmer® AAnalyst™ 700 Flame AAS				
Element	Acetylene (L/min)	Air (L/min)	Wavelength (nm)	Slit Width (nm)
Fe	2.0	17.0	248.3	0.2
Cu	2.0	17.0	324.8	0.7
Mn	2.0	17.0	279.5	0.2
Zn	2.0	17.0	213.9	0.7
GFAAS – PerkinElmer® HGA® 700 Graphite Furnace AAS				
Elements	Pb	Cd	Ni	Cr
Argon flow (mL/min)	250	250	250	250
Sample Volume (µL)	20	20	20	20
Modifier (µL)	5	10	5	5
Heating Program Temperature °C, ramp time (s), hold time (s)				
Drying 1	100 (5, 20)	100 (5, 20)	100 (5, 20)	100 (5, 20)
Drying 2	140 (15, 15)	140 (15, 15)	140 (15, 15)	140 (15, 15)
Ashing	700 (10, 20)	850 (10, 20)	1300 (10, 20)	1600 (10, 20)
Atomization	1800 (0, 5)	1650 (0, 5)	2500 (0, 5)	2500 (0, 5)
Cleaning	2600 (1, 3)	2600 (1, 3)	2600 (1, 3)	2600 (1, 3)

centrations of the sample solution were determined by AAS. Blanks were prepared in the same way but without the samples.

Statistical Analysis

All of the data was subjected to a statistical analysis and correlation matrices were produced to examine the inter-relationships between the investigated trace element concentrations of the samples. A Student's *t*-test was employed to estimate the significance of the values.

RESULTS AND DISCUSSION

The recovery values were found to be nearly quantitative ($\geq 95\%$) for the microwave digestion and wet ashing methods. The relative standard deviations were less than 10% for all of the investigated elements. The Student's *t*-test was used in this study ($p < 0.05$). The accuracy of the method was evaluated by means of trace element determination in standard reference material NIST-SRM 1515 Apple Leaves (National Institute of Standards and Technology, Gaithersburg, MD, USA). The achieved results were found to be in good agreement with the certified values, and the results are listed in Table II.

A comparison of the results of the wet and microwave digestion methods for the standard reference material NIST SRM 1515 Apple Leaves shows that there are no statistical differences (Table II). Thus, we selected the microwave digestion method because it is simple, fast, and more accurate for the determination of trace elements by AAS.

In this study, 20 mushroom samples were analyzed for eight elements. The habitat and genetic families of the edible mushroom samples are listed in Table III. The trace element concentrations (mean $\pm$ standard deviation) in the mushroom species are listed Table IV.

The trace element levels for Cu, Cd, Pb, Zn, Mn, Fe, Cr, and Ni were 4.42–21.4, 0.11–1.10, 0.26–2.81, 14.5–74.5, 11.1–78.6, 101–389, 0.20–1.30, and 2.89–13.2 $\mu\text{g/g}$, respectively (see Figures 1–8).

Copper (Cu)

The minimum and maximum values of Cu were 4.42 $\mu\text{g/g}$ and 21.4 $\mu\text{g/g}$ (Figure 1). The highest and lowest levels of Cu were found in *Lepista nuda* and *Pleurotus*

TABLE II
Analysis of NIST SRM 1515 Apple Leaves After Microwave and Wet Digestion Methods ($\mu\text{g/g}$, Average $\pm$ S.D.)

Element	Certified Value	Microwave Digestion ^a	Recovery (%)	Wet-Ashing ^a	Recovery (%)
Cu	5.64	5.57 $\pm$ 0.30	99	5.48 $\pm$ 0.40	95
Cd	0.013	0.013 $\pm$ 0.001	100	0.012 $\pm$ 0.001	96
Pb	0.47	0.46 $\pm$ 0.04	98	0.45 $\pm$ 0.05	95
Zn	12.5	12.4 $\pm$ 0.5	99	11.9 $\pm$ 0.9	96
Mn	54	52.4 $\pm$ 3.7	97	51.6 $\pm$ 5.2	95
Fe	83	82.1 $\pm$ 5.2	99	81.3 $\pm$ 7.7	95
Cr	(0.3) ^b	0.29 $\pm$ 0.02	97	0.27 $\pm$ 0.03	95
Ni	0.91	0.88 $\pm$ 0.05	97	0.86 $\pm$ 0.08	95

^a Data presented as mean $\pm$ standard deviation.

^b The value in parenthesis is not certified.

Table III
Habitat of Edible Mushroom Species

Edible Mushroom Species	Habitat
<i>Suillus luteus</i> (L.) Roussel	Conifers, usually pine
<i>Coprinus atramentarius</i> (Bull.) Fr.	Near rotting or buried wood, or in grass
<i>Agaricus bitorquis</i> (Quél.) Sacc.	In gardens and at roadsides
<i>Pleurotus ostreatus</i> (Jacq.) P. Kumm.	On stumps, usually of deciduous trees
<i>Stropharia coronilla</i> (Bull.) Quél.	Lawns and pasture
<i>Agrocybe dura</i> (Bolton) Singer	At roadsides or in meadows
<i>Marasmius oreades</i> (Bolton) Fr.	In pasture or lawns
<i>Tricholoma argyraceum</i> (Bull.) Gillet	Singly or in groups in mixed woods
<i>Agaricus bisporus</i> (J.E. Lange) Imbach	Manure heaps, garden waste, and roadsides
<i>Hygrocybe nigrescens</i> (Quél.) Kühner	Among grass in fields or woods
<i>Stropharia coronilla</i> (Bull.) Quél.	On lawns and in pasture
<i>Coprinus comatus</i> (O.F. Müll.) Pers.	On roadsides, lawns, and other urban sites
<i>Lactarius piperatus</i> (L.) Pers.	Deciduous woods
<i>Pleurotus ostreatus</i> (Jacq.) P. Kumm.	Deciduous trees, especially beech
<i>Agaricus campestris</i> Schwein.	In pasture
<i>Lactarius piperatus</i> (L.) Pers.	Deciduous woods
<i>Lepista nuda</i> (Bull.) Cooke	In woodland, hedgerows, gardens
<i>Chroogomphus rutilus</i> (Schaeff.) O.K. Mill.	Conifers, usually pines

TABLE IV
Trace Elements Concentrations (µg/g) in Mushroom Species (mean ± standard deviation)

Sample Location	Mushroom Species	Cu	Cd	Pb	Zn	Mn	Fe	Cr	Ni
Sivas	<i>Suillus luteus</i>	11.5±1.8	0.32±0.03	0.26 ±0.02	36.7±3.4	12.2±1.1	161±14	0.32±0.03	3.91±0.34
	<i>Coprinus atramentarius</i>	10.7±1.0	0.21±0.02	0.36 ±0.03	65.3±6.2	21.3±2.0	193±18	0.32±0.03	4.60±0.43
	<i>Agaricus bitorquis</i>	17.2±1.4	0.18±0.02	0.67 ±0.07	46.0±3.9	35.5±3.2	176±18	0.85±0.07	3.17±0.30
	<i>Plevnotus ostreatus</i>	4.42±0.38	0.27 ±0.02	0.52 ±0.05	53.7±5.1	20.0±1.8	101±10	0.49±0.05	3.82±0.37
Kayseri	<i>Stropharia coronilla</i>	13.7±1.3	0.22±0.02	0.75±0.08	74.5±6.2	20.0±1.7	180±17	0.78±0.07	9.21±0.89
	<i>Agrocybe dura</i>	15.4±1.4	0.44±0.04	1.20±0.11	33.9±3.1	18.6±1.6	132±10	0.32±0.03	5.20±0.48
	<i>Marasmius oreades</i>	33.9±3.3	0.62±0.06	1.10±0.10	53.4±5.2	25.1±2.3	221±19	1.11±0.11	13.2±1.2
	<i>Tricholoma argyraceum</i>	12.3±1.2	0.80±0.08	1.90±0.11	69.5±6.0	15.5±1.4	107±10	0.42±0.04	5.75±0.49
Nevsehir	<i>Agaricus bisporus</i>	7.58±0.62	0.17±0.01	0.36±0.03	37.7±3.4	17.7±1.8	162±15	0.32±0.03	8.60±0.83
	<i>Hygrocybe nigrescens</i>	10.2±1.0	0.19±0.02	0.28±0.02	43.2±4.0	13.2±1.2	129±12	0.49±0.05	4.63±0.42
	<i>Stropharia coronilla</i>	7.13±0.70	0.18±0.01	0.52±0.05	20.6±1.9	22.3±2.0	295±26	1.08±0.10	6.81±0.67
	<i>Inocybe fastigiata</i>	5.46±0.48	0.23±0.02	0.23±0.02	27.7±2.8	16.3±1.6	166±12	0.38±0.04	3.95±0.38
	<i>Lepiota cristata</i>	10.2±1.0	0.12±0.01	0.52±0.05	17.8±1.6	15.4±1.3	101±10	0.38±0.04	5.60±0.54
Nigde	<i>Coprinus comatus</i>	20.4±1.9	1.10±0.10	2.81±0.24	16.4±1.4	29.4±2.5	336±29	0.23±0.02	4.73±0.43
	<i>Lactarius piperatus</i>	16.3±1.5	0.48±0.05	1.30±0.12	46.1±3.9	78.6±7.2	389±35	1.30±0.12	6.11±0.59
	<i>Pleurotus ostreatus</i>	13.1±1.3	0.52±0.04	2.31±0.20	73.5±6.9	23.2±1.9	267±24	0.34±0.03	2.89±0.27
	<i>Agaricus campestris</i>	15.9±1.5	0.63±0.05	1.40±0.14	48.3±4.5	11.1±1.1	360±29	1.11±0.10	4.83±0.44
Yozgat	<i>Agaricus campestris</i>	9.11±0.87	0.85±0.07	0.28±0.03	32.2±2.9	22.4±2.1	284±26	0.92±0.08	3.96±0.34
	<i>Lactarius piperatus</i>	6.82±0.64	0.54±0.05	0.52±0.05	68.6±6.6	21.1±2.1	102±10	0.26±0.02	3.63±0.35
	<i>Agaricus bisporus</i>	17.1±1.5	0.37±0.04	1.90±0.18	72.3±6.9	43.5±3.9	168±15	0.32±0.03	4.60±0.41
	<i>Lepista nuda</i>	21.4±3.1	0.18±0.02	0.52±0.04	61.4±6.0	16.8±1.3	154±15	0.21±0.02	3.88±0.38
	<i>Cbrogompus rutilus</i>	6.20±0.59	0.11±0.01	0.62±0.06	14.5±1.4	13.4±1.2	134±13	0.20±0.03	4.91±0.48
	<i>Fomes fomentarius</i>	8.23±0.79	0.23±0.02	0.67±0.06	34.2±3.4	17.0±1.6	162±15	0.20±0.02	5.64±0.43

TABLE V
Correlation Between Metal Concentrations

	Cu	Cd	Pb	Zn	Mn	Fe	Cr	Ni
Cu	1.000							
Cd	0.328	1.000						
Pb	0.410	0.649	1.000					
Zn	0.149	-0.064	0.127	1.000				
Mn	0.254	0.114	0.287	0.073	1.000			
Fe	0.287	0.440	0.389	-0.278	0.048	1.000		
Cr	0.275	0.114	-0.081	-0.087	0.427	0.630	1.000	
Ni	0.471	-0.003	-0.040	0.003	0.043	0.090	0.417	1.000

ostreatus. The Cu concentrations of mushroom samples reported in the literature are in the range of 4.71–51.0 µg/g (14), 12–181 µg/g (15), and 10.3–145 µg/g (16), respectively. These Cu values are higher than our values. The FAO/WHO provisional tolerable daily intake (PTDI) limits for heavy metal intake based on an average adult body weight of 60 kg are 214 µg for Pb, 48 mg for Fe, 3 mg for Cu, and 60 mg for Zn (17). The Cu levels in the analyzed samples were found to be in agreement with the FAO/WHO tolerable levels.

Figure 1. Distribution of Cu in mushroom species

Figure 2. Distribution of Cd in mushroom species

Figure 3. Distribution of Pb in mushroom species

Figure 4. Distribution of Zn in mushroom species

Figure 5. Distribution of Fe in mushroom species

Figure 6. Distribution of Mn in mushroom species

Figure 7. Distribution of Cr in mushroom species

Figure 8. Distribution of Ni in mushroom species

Cadmium (Cd) and Lead (Pb)

The mean Cd levels of the mushroom samples varied from 0.11 to 1.10 µg/g. The Pb concentrations were generally low, and the highest concentration was 2.81 µg/g in *Coprinus comatus*. The reported Pb values for mushrooms were 0.5–20 mg kg⁻¹ (16, 18) and for Cd were between 2.71–7.5 mg kg⁻¹ (7, 19, 16) (Figures 2–3). It can be seen that toxic metals can be present in high concentrations in mushrooms. According to FAO/WHO (1976) standards, the maximum permissible intake for an adult per week is 3 mg Pb and 0.5 mg Cd, while the recommended doses are only one-fifth of those quantities (20). The Cd and Pb levels found in the analyzed samples of this study are in agreement with the FAO/WHO tolerable levels.

Zinc (Zn)

Zinc is one of the most important trace metals necessary for normal growth and development of humans and animals. A deficiency in Zn can result from inadequate dietary intake, impaired absorption, excessive excretion or inherited defects in the Zn metabolism. Mushrooms are known as Zn accumulators and sporophores. The substrate ratio for Zn ranges from 1 to 10 mg kg⁻¹ (7, 21). In this study,

the highest Zn level observed was 74.5 µg/g in *Stropharia coronilla* and ranged from 14.5 to 73.5 µg/g in others (Figure 4). Reported Zn concentrations in mushrooms range from 9.9–118 µg/g (22), 30–150 mg kg⁻¹ (8), 21–100 mg kg⁻¹ (23), to 35–136 mg kg⁻¹ (24). The Zn levels in the analyzed samples of this study are in agreement with the FAO/WHO tolerable levels.

Iron (Fe), Manganese (Mn), Chromium (Cr)

The Fe levels in the mushroom samples studied were higher than the other metals. The highest concentrations of Mn, Fe, and Cr were 78.6 µg/g, 389 µg/g, and 1.30 µg/g in *Lactarius piperatus*, respectively (Figures 5–7). The lowest levels were for Mn at 11.1 µg/g in *Agaricus campestris*, Fe at 101 µg/g in *Pleurotus ostreatus*, and Cr at 0.20 µg/g in *Chroogomphus rutilu*. The Fe concentrations in mushroom samples have been reported in the range of 31.3–1190 mg kg⁻¹ (25), 50–842 mg kg⁻¹ (3), and 38.9–499.0 mg kg⁻¹ (26). The observed Fe values obtained in this study are lower than these reported data. For Fe, the concentrations were reported as 5.3–252 mg kg⁻¹ and for Mn between 5.42–41.3 mg kg⁻¹ (22). The Fe, Mn, and Cr levels in the analyzed samples of this study are

in agreement with the FAO/WHO tolerable levels.

Nickel (Ni)

The highest and lowest Ni levels were 13.2 µg/g in *Marasmius oreades* and 2.89 µg/g in *Pleurotus ostreatus*, respectively (Figure 8). Nickel values are reported as 1.18–5.14 µg/g (19) and 8.2–21.6 µg/g (28). Our nickel values are higher than the values obtained by Mendil et al. in 2004 (10) (4.2–8.2 mg/kg) and lower than the Mendil et al. values obtained in 2005 (28) (21.6 mg/kg). According to the Turkish Food Codex (29), the maximum Pb level permitted for wild edible mushrooms is 1 mg/kg. The Codex does not list any information about maximum permitted levels of other elements for wild edible mushrooms. The Ni levels in the analyzed samples are in agreement with the FAO/WHO tolerable levels.

Correlation Coefficients

The values of the correlation coefficients between the metal concentrations are listed in Table V. There is good correlation between cadmium-lead ($r=0.649$) and iron-chromium ($r=0.630$). There is positive correlation between copper-cadmium, copper-lead, copper-zinc, copper-manganese, copper-iron,

copper-chromium, cadmium-manganese, cadmium-iron, cadmium-chromium, lead-zinc, lead-manganese, lead-iron, zinc-manganese, zinc-nickel, manganese-iron, manganese-chromium, manganese-nickel, iron-chromium, iron-nickel and chromium-nickel. Negative correlations between copper-nickel, cadmium-zinc, cadmium-nickel, lead-chromium, lead-nickel, zinc-iron, and zinc-chromium were found as 0.471, 0.064, 0.003, 0.081, 0.040, 0.278 and 0.087, respectively.

CONCLUSION

Microwave digestion of samples in comparison to wet ashing was found to be more simple, fast, and accurate. The study showed that the concentration of trace elements in the samples is dependent on the type of mushroom species. Some species accumulate trace elements at high levels. The metal concentrations in the mushrooms collected in the middle region of Anatolia, Turkey, were determined by FAAS and the values found were: Cu at 21.4 µg/g, Fe at 389 µg/g, Mn at 78.6 µg/g, Zn at 74.5 µg/g, and by GFAAS for Cd at 1.10 µg/g, Cr at 1.30 µg/g, Ni at 13.2 µg/g, and Pb at 2.81 µg/g. These levels, except for Pb, are within the allowable limits. The lead levels in some edible mushroom samples were found to be higher than the legal limits. For public health reasons, wild edible mushrooms should, therefore, be sampled and analyzed more frequently to establish their toxic metal concentration status. The accuracy of the method was verified by using certified reference material and quantitative recovery values were obtained.

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