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The statistical evaluation of digestion methods for Myrtus Communis and Peganum Harmala

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Abstract:
Element concentrations in two selected medicinal plants Myrtus Communis and Peganum Harmala have been determined using four digestion procedures for samples preparation. These elements include Ca, Mg, Na, K, Fe, P, Si, Mn, Zn, Cu, Co, Ni, Cr, Al, Hg, Pb and Cd which were identified using ICP-ES instrument. Regardless of the digestion methods, Ca, Mg, K, Na, P and Fe as macronutrients were presented in maximum quantities in these plants. Meanwhile, the highest level of micronutrients was recorded for Si, Mn and Zn respectively. The toxic element Al represents the highest concentration according to other toxic elements in medicinal plants selected for this study which could be referred to environmental composition.
The statistical results exhibit a significant variation between water extraction procedure and other digestion procedures. This variation could be related to the ability of water to extract organic and inorganic salts only. In mean time, (HNO3/HClO4) digestion procedure has the superiority over there methods for sample preparations. The statistical data also exhibit significant variance according to the elemental composition. for instance, these herbs have significant differences in concentration of Mn, Cu, Cr, Zn, Sn and Si, meanwhile no significant variances were detected with the concentration of Co and Ni.

Introduction

Herbs defined as the plants which are utilized as remedy to treat and prevent diseases. The medicinal plants have been used in the traditional treatment for many diseases since ancient times [1-3]. Medical plants that form the basis of healthcare since long time are still widely utilized and have the importance in international treatment and continues to expand rapidly through the whole world because of the belief that herbs cold be more effective compared to synthetic pharmaceuticals for treatment of many disease [4-6]. In 2002, Food and Agriculture Organization (FAO) indicate that around 50000 herbs are unitized through the world [7].

Elements participate in the formation of the active compounds present in herbs and so there are responsible for the medicinal as well as toxic behaviours [8, 9]. Some elements are essential and trace nutrients and become toxic at high level meanwhile other some elements known as toxic even in relatively low concentration [8].

This research was conducted with the aims to investigate the elemental compassion-macronutrients, micronutrients and toxic elements in two Libyan medicinal plants, Myrtus Communis and Peganum Harmala grown in Libya. Moreover, to develop a suitable digestion method to prepare the samples for measurements.

Methodes

Chemicals

Deionized water was supplied by West Tripoli Power Station. The chemicals that used in this research were analytical grade and supplied by Fluka chemika , Farmitalia Cario Erba, Ventron Gmbh and Merck (Darmstadt, Germany).

Preparation of samples

Herbs were collected and washed with distilled and deionized water. After that, air dried for approximately three weeks. Herb species were chopped up finely and dried in an oven for approximately 24 hours at 90C°. These medicinal plants were grounded in a ceramic mortar and stored in glass jar. In this study, three digestion methods and water extraction were utilized to extract the elements from samples.

Wet digestion method 1

20 grams of each herb samples were introduced into 80-mL beakers. 20 ml of con. HNO_3 were poured to each container and covered with watch glasses after NO_2 bubble was released. The next step, the samples were refluxed gently until the samples volume became less. After that the samples were allowed to cool for approximately 5 minutes at room temperature, then 5 ml of $HClO_4$ were poured and boiled to almost dryness then the contents were cooled to room temperature. The samples were dissolved in 0.1N HNO_3, boiled, then cold to room temperature. After that, the samples filtered through a glass filter into 50-ml volumetric flasks and diluted with 0.1N HNO_3 to the marks.

Wet digestion method 2

Wet digestion method 2 is similar to the pervious method. The only difference is utilizing H_2O_2 instead of $HClO_4$ for prepare the sample mixture.

Dry ashing method

2.00 g of each herb samples were brought into crucibles and place in a muffle furnace. The samples were fired slowly to 500°C for 8 hrs. the crucibles were taken out from the furnace and cooled. Crucibles contents (ash) were dissolved in 10 ml of (1:1) nitric acid and filtered via a glass filter and poured into 50-mL volumetric flasks and diluted with 0.1N HNO3 to the marks.

Water infusion method

2.000 grams of herb samples were brought into 80-mL beakers and covered with watch glasses. A sufficient water was suppled to each beaker and refluxed for 1 hour. After that, the samples were filtered and transferred into 50-mL volumetric flasks and diluted with 0.1N HNO_3.

Measurement techniques

Plant Samples were measured for, Ca, Mg, K, Na, P, Fe, Si, Mn, Zn, Cu, Co, Cr, Ni, Sn, Al, Hg, Pb and Cd by Inductively Coupled Plasma Emission Spectroscopy (ICP-MS) technique, Model S (Industrial research center, Tripoli-Libya). Na and K were detected by flame photometer.

Result and discussion

The concentration of macronutrient, micronutrients and some toxic metals were detected in two Libyan herb plants. These plants were Myrtus Communis and Peganum Harmala. Three digestion procedure and water extract were used to prepare the sample for measurements. The measurements were conducted by Inductively Coupled Plasma Atomic Emission Spectroscopy technique (ICP-MS). The first part of discussion represents the elemental concentrations for the selected plants. The elemental concentrations of these plants were presented in the corresponding labeled graphs and tables.

Macronutrients

Regardless of digestion procedure, the concentration of calcium, magnesium, potassium, sodium, phosphorus and iron were detected at a higher concentration in Myrtus Communis and Peganum Harmala. The mean concentration of these elements depends on digestion procedures with $HNO_3/HClO_4$, HNO_3/H_2O_2, dry ashing and water infusion were found in Table 1 and Figure 1.

Table 1: The mean concentration (µg/g) of macronutrients in M. Communis and P. Harmala with different digestion methods*.

plant	method	Ca	Mg	Na	K	Fe	P
M. Communis	$HNO_3/HClO_4$	10175	6114	301	2136	259	697
	HNO_3/H_2O_2	9080	5585	295	1530	181	649
	ashing	7773	4120	309	1280	101	680
	water infusion	388	448	154	190	2.97	282
P. Harmala	$HNO_3/HClO_4$	9300	11170	910	8195	205	919
	HNO_3/H_2O_2	8646	9874	848	6195	195	606
	ashing	8677	10000	751	5777	195	482
	water infusion	403	2030	9.8	370	20	26.02

*Average of triplicate determinations, %R.S.D.= 0.03-0.10

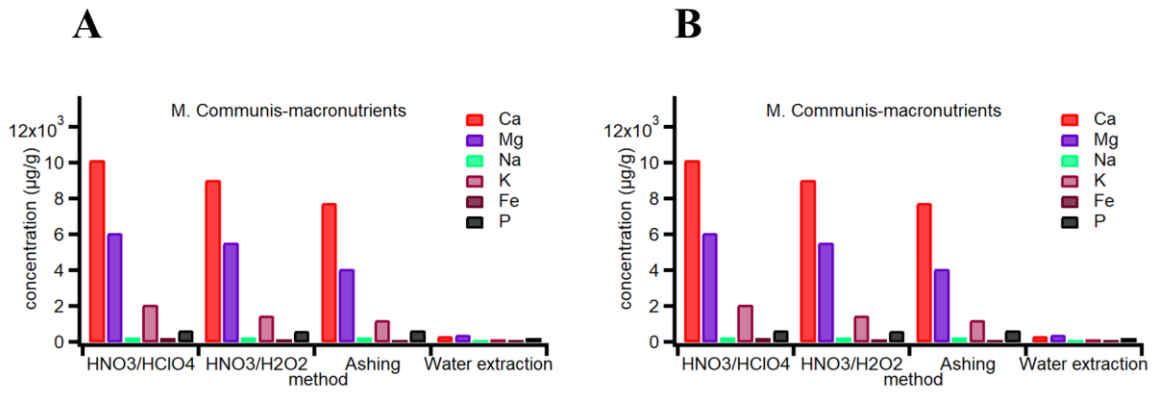

Figure 1: The concentration of macronutrients elements in M. Communis and P. Harmala with different digestion methods.

Both herb samples, calcium concentration is higher than magnesium and potassium concentration over sodium regardless any procedure followed for plant samples preparation. This is could be related to these herbs' selectivity towards these metals. The results also exhibit phosphorus and iron concentration are lower compared to calcium and magnesium concentrations but still the concentration of phosphorus and iron represent significant amounts in these herbs which could be a good supply for human body. From the data, its clear that the elemental concentration that extracted by water are lower compare to other digestion procedures. This may be explained by the lower solubility of the compounds of these elements in water. In spite of that, these elements exist in the water extract in sufficient concentration which may contribute to daily allowance recommended. It noticed that for the two plant samples, the concentrations of these elements are followed this order:

$$Ca > Mg > K > Na > P > Fe$$

The concentration order of these micronutrients was found in agreement with many previous studies [10-18].

The micronutrients

The mean concentration of these elements relays on digestion procedures with $HNO_3/HClO_4$, HNO_3/H_2O_2, dry ashing and water infusion were shown in Table 2 and Figure 2.

Table 2: The mean concentration (µg/g) of micronutrients in M. Communis and P. Harmala with different digestion methods*

.

plant	method	Zn	Mn	Cu	Cr	Co	Si	Sn	Ni
M. Communis	$HNO_3/HClO_4$	32.95	28.94	11.07	4.11	16.05	140	2.21	6.05
	HNO_3/H_2O_2	24.93	22.39	10.06	4.63	10.2	121	2.11	4.09
	ashing	13.6	15	6.93	3.63	8.65	619	2.13	4.65
	water infusion	1.98	2.22	1.33	0.32	0.85	3.02	0.0	0.51
P. Harmala	$HNO_3/HClO_4$	52.4	63.68	7.48	4.4	20.55	163	7.17	4.29
	HNO_3/H_2O_2	29.67	47.98	6.16	4.05	18.86	131	6.03	3.67
	ashing	19.65	38.32	6.21	3.74	8.8	579	6.67	3.32
	water infusion	0.96	8.43	0.85	0.24	2.18	13.08	0.0	0.54

*Average of triplicate determinations, %R.S.D.= 0.02-0.25

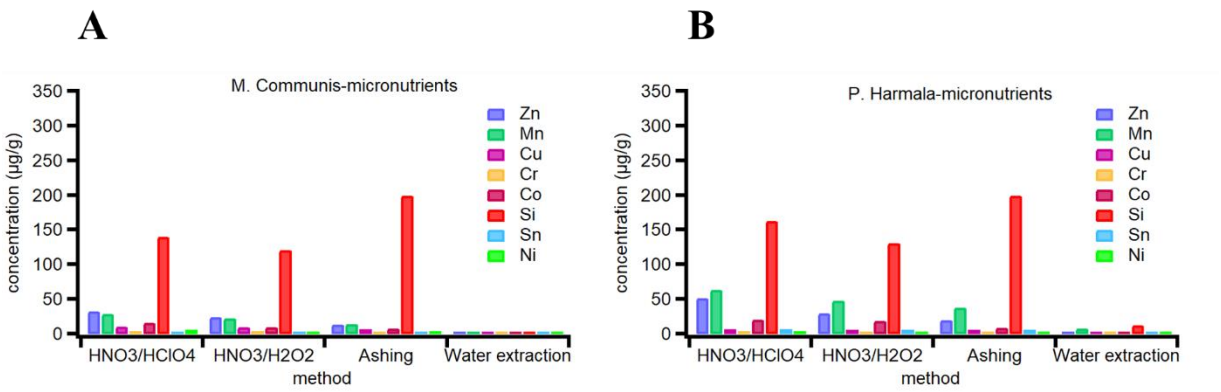

Figure 2: The concentration of micronutrients elements in M. Communis and P. Harmala with different digestion methods.

The results exhibit that silicon has highest value followed by manganese while, tin has the smallest concentration. For the most plants' samples, micronutrients concentrations followed this order:

Si >> Mn > Zn > Cu > Co > Ni > Cr >Sn

Micronutriments concentrations observed to be in a good agreement with many other researches on medicinal plants through the world [10, 13, 15, 16, 18, 19].

The toxic elements

The observed concentration of aluminum is relatively high compared to other toxic metals that may related to the environmental and soil contents, as seen in Table 3 and Figure 3.

Table 3: The mean concentration of toxic elements in M. Communis and P. Harmala with different digestion methods*.

plant	method	Al	Hg	Pb	Cd
M. Communis	$HNO_3/HClO_4$	141	3.72	7.85	5.99
	HNO_3/H_2O_2	158	3.31	4.85	6.01
	ashing	100	2.46	3.26	5.64
	water infusion	3.04	0.00	1.51	1.19
P. Harmala	$HNO_3/HClO_4$	195	7.64	4.87	5.61
	HNO_3/H_2O_2	174	3.4	3.76	5.22
	ashing	167	3.21	2.7	5.44
	water infusion	10.01	0.00	0.78	0.51

*Average of triplicate determinations, %R.S.D.= 0.03-0.24.

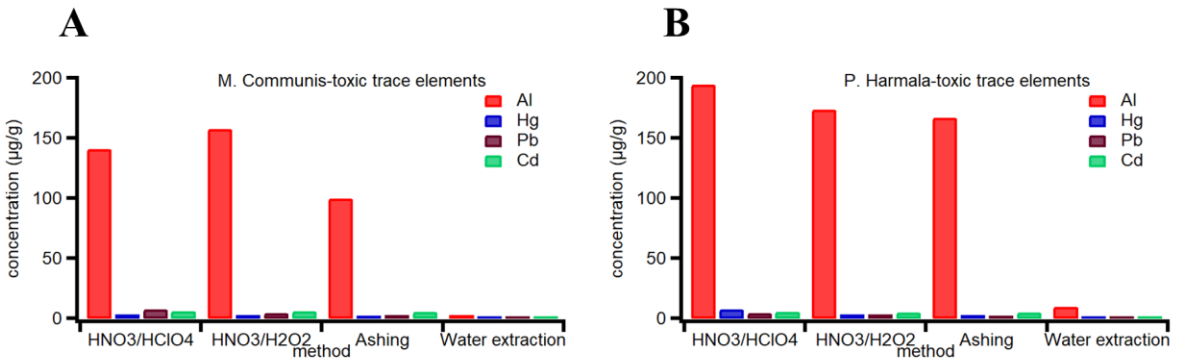

Figure 3: The concentration of *toxic Elements* in M. Communis and P. Harmala with different digestion methods.

Toxic elements concentrations were observed in agreement with many studies through the word [13, 17].

Statistical analysis

The statistical methods were performed to evaluate the significant differences between M. Communis and P. Harmala regarding to the elemental concentrations as well as to identify any significant variations arising from the different sample-preparation methods used for the selected herb plants. Statistical tools involve Principal Component Analysis (PCA), Partial Least Squares Discriminant Analysis (PLS-DA), Hierarchical Cluster Analysis (HCA) and boxplots were employed to assess the differences in elemental concentrations between M. Communis and P. Harmala and to identify variations associated with the different sample-preparation methods. The PCA data indicates that there is strong variation between the two plants results. As it can be a notable in Figure 4, there is a significant difference between the methods that used to prepare the samples, with the first two principal components showing 89% of the total variance. In addition, there are significant variance between water extract method and other procedures (wet and dry procedures). This is could be related to the lower solubility of elemental compounds in water. Moreover, a slight difference was notice between $HNO_3/HClO_4$ method and other two digestion methods.

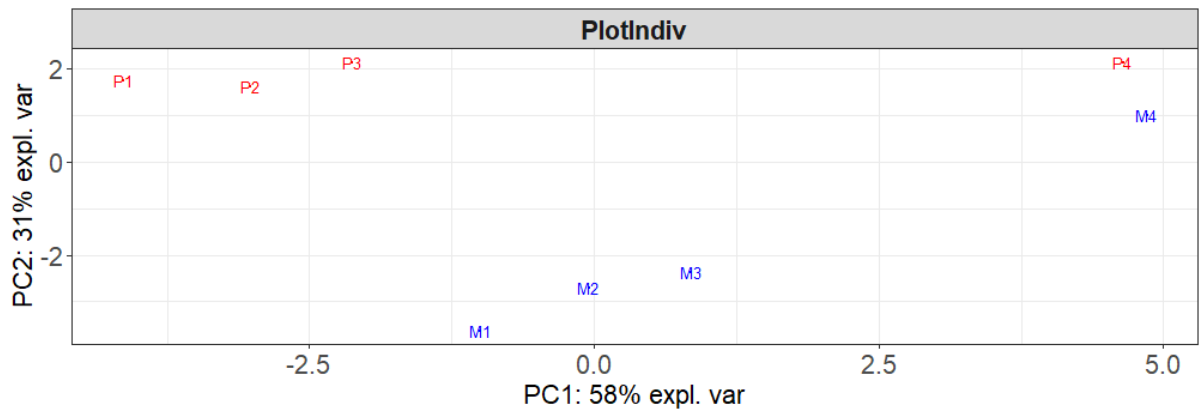

Figure 4: PCA analysis of elemental composition of M. Communis and P. Harmala; M1 ($HNO_3/HClO_4$ for M. Communis), M2 (HNO_3/H_2O_2 for M. Communis), M3 (dry ashing for M. Communis), M4 (water extraction for M. Communis, P1 ($HNO_3/HClO_4$ for P. Harmala), P2 (HNO_3/H_2O_2 for P. Harmala), P3 (dry ashing for P. Harmala), P4 (water extraction for P. Harmala).

PLS-DA result also supports PCA data and exhibits a clear separation between M. Communis and P. Harmala relaying on the method that used to prepare the samples. There is a clear variation between water extract method and other digestion methods due to the low solubility of metal salts in water as seen in Figure 5.

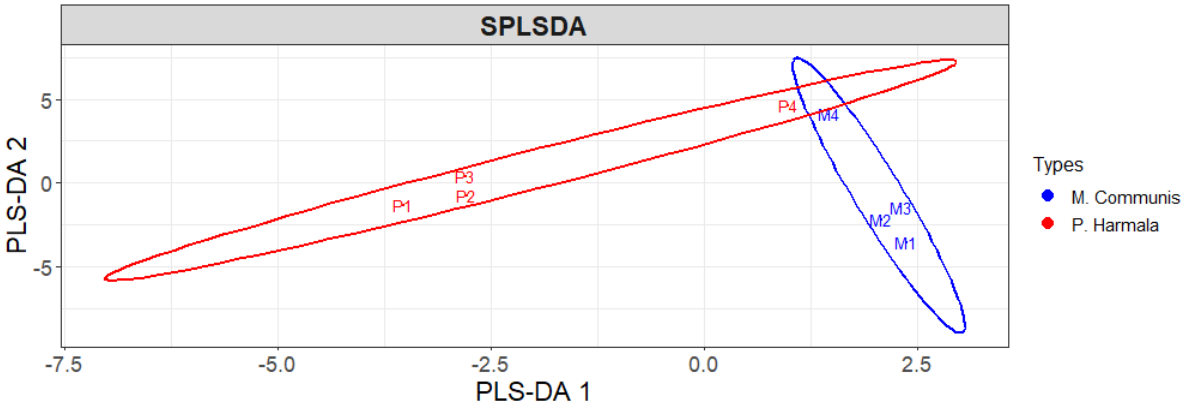

Figure 5: PLS-DA analysis of elemental composition of M. Communis and P. Harmala; M1 ($HNO_3/HClO_4$ for M. Communis), M2 (HNO_3/H_2O_2 for M. Communis), M3 (dry ashing for M. Communis), M4 (water extraction for M. Communis, P1 ($HNO_3/HClO_4$ for P. Harmala), P2 (HNO_3/H_2O_2 for P. Harmala), P3 (dry ashing for P. Harmala), P4 (water extraction for P. Harmala).

The cluster dendrogram plots exhibit clearly distinguish between water extraction method and other digestion methods. Similar groups are located together, reveal that these methods have a similar ability to extract the metals from the samples, Figure 6.

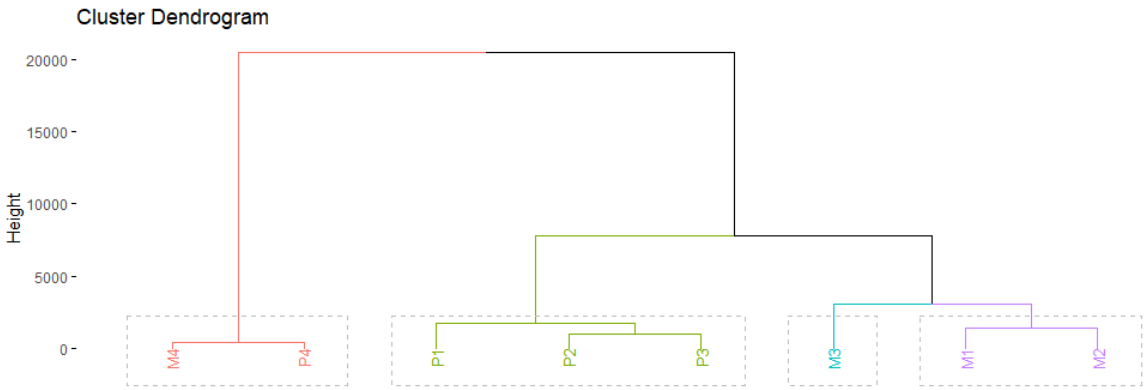

Figure 6: Dendrogram plot showing variation between digestion methods that used to extract elements from M. Communis and P. Harmala; M1 ($HNO_3/HClO_4$ for M. Communis), M2 (HNO_3/H_2O_2 for M. Communis), M3 (dry ashing for M. Communis), M4 (water extraction for M. Communis, P1 ($HNO_3/HClO_4$ for P. Harmala), P2 (HNO_3/H_2O_2 for P. Harmala), P3 (dry ashing for P. Harmala), P4 (water extraction for P. Harmala).

Boxplot graph shows there is a significant variation between M. Communis and P. Harmala according to elemental concentration. For example, these plants have significant differences according to the concentration of manganese, coper, chrome, zin, tin and silicon. This could be attributed to the different ability of these plants to absorb these elements. On the other hand, no significant variance was observed between M. Communis and P. Harmala in terms of cobalt and nickel concentrations, as seen in Figure 7.

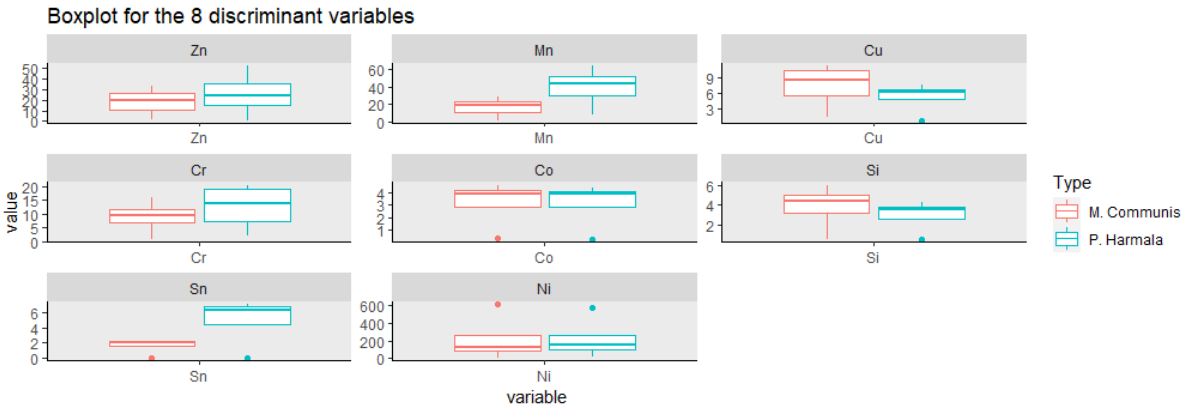

Figure 7: Grouped boxplot exhibit the possible elements which considered as marker to distinguish between M. Communis and P. Harmala.

Conclusion

The concentration of macronutrients, micronutrients and some toxic metals were determined in M. Communis and P. Harmala. From the result, it can be concluded that the concentration of these elements in the most samples followed this order: Ca >> Mg > K > Na > P > Fe > Al > Si > Mn > Zn > Cu > Co > Pb > Ni > Cd > Cr > Hg > Sn.

The macronutrient calcium concentration was observed in major level, while other macronutrients particularly, magnesium, potassium, sodium, phosphorus and iron found to be in lower level**.** In contract, the micronutrient silicon exists in major level compared to the other micronutrients, zinc, manganese, copper, chromium, tin, nickel and cobalt. The toxic elements were found in trace level.

The obtained results also demonstrated that, there are significant concentrations of macro and micronutrients in the water extract for these herbs which may be supported by the daily allowance recommended by WHO.

the statistical analysis indicated that there is significant variance in the selected plants according to their element concentrations. Furthermore, the obtained data demonstrated that (HNO_3/$HClO_4$) digestion method has superiority over other digestion procedure to prepare the samples for measurements.

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