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Heavy metal bioaccumulation and distribution in *Typha latifolia* and *Arundo donax*: implication for phytoremediation

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ABSTRACT
In this study we determined the concentration of metals (Cd, Ni, Zn and Cu) in sediment and aquatic plants (*Typha latifolia* and *Arundo donax*). The level of pollution in the sediment was assessed using contamination factor (CF), pollution load index (PLI) and geo-accumulation index (Igeo). Obtained results have exhibited that the distribution of trace elements in sediment follows: Zn (196.51 µg g^{-1}) > Ni (140.68 µg g^{-1}) > Cu (121.56 µg g^{-1}) > Cd (1.101 µg g^{-1}). However, comparison of sediment metal concentrations with several environmental contamination parameters, such as: probable effect level (PEC) and background levels, indicated that the concentrations of all investigated elements were less than PEC, except that of Ni, albeit higher than the background levels. The Igeo values revealed that Cd (1.28 µg g^{-1}) had been accumulated significantly in the Djendjen River. Contamination factor (CF) exhibited that the sedimentary samples were moderate in terms of all studied metal contaminations. The pollution load index (PLI) values were above one (>1), displaying an advanced decline of the sediment quality. In studied plants, results exhibited that the amount of concentrations in tissues is significantly dependent on the kind of organ and element. *A. donax* revealed a lesser capacity of bioaccumulation as well as a lesser efficiency of metal removal than *T. latifolia*. In contaminated aquatic ecosystems, the presence of *T. latifolia* may increase the removal of heavy metals, thus, their introduction contributed to a possible action of phytoremediation.

Key words: Trace elements, Macrophytes, Phytoremediation, Djendjen River, Algeria.

INTRODUCTION
In aquatic ecosystems water contamination by trace metals is one of the main types of pollution that may stress the biotic community (Baldantoni *et al.* 2004). Trace element contamination may originate from both natural geochemical processes (weathering of ultramafic rocks) and anthropogenic activities (such as mining and smelting, combustion of fossil fuels, utilization of fertilizers and pesticides, disposal of wastes) (Diez Lazaro *et al.* 2006). The exceeding of metal concentrations in surface water can pose a health problem to humans and the ecosystem. Restoration and conservation of surface water resources is thus necessary for the sake of their inevitable role in sustaining both aquatic and terrestrial life (Nazeer *et al.* 2014). The utilization of Macrophytes as biological indicators, particularly for biological monitoring of aquatic ecosystems, are widely studied (Demirezen & Aksoy 2006; Calzoni *et al.* 2007). These aquatic plants can accumulate trace elements 100,000 times higher than in the associated water (Mishra & Tripathi 2008). Thus, they have been used for trace elements retention from a various sources (Hassan et *al.* 2007). However, this process phenomena is highly influenced by seasonal variations in Macrophyte development, by plant tolerance for organic, metallic and nutrient loads, by the concentrations and mobility of elements in the surrounding environment, and also by bioavailability (Grisey *et al.* 2012).

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Aquatic plants differ in their ability to absorb metals in root tissues and in the proportions of metals transferred to the above ground parts (Jackson 1998; Manios *et al.* 2003). Some aquatic Macrophyte species are shoot accumulators. Others are named root accumulators, as they accumulate trace elements in their roots. Plant species with low accumulation are those that can reduce the retention when the substrate has high concentration of elements, or they have a high net efflux of certain elements. However, hyper accumulators can tolerate and absorb high levels of some trace elements which are toxic to most aquatic plants (Aksoy *et al.* 2005).
The Djendjen River, considered in this study, is a major riverine system in the alluvial plain of Djendjen, northeast of Algeria, which continues to be affected by direct human activities (urban effluents and fertilizers). The deteriorating quality of its surface water, becoming a major concern for managers and users of this precious resource (Krika & Krika 2018). The objectives of this study are to (i) assess the pollution status of the Djendjen river by estimating the levels of heavy metals in sediment; (ii) explore the degree of contamination and pollution impacts using the following pollution indicators, including contamination factor (CF), pollution load index (PLI), and geo-accumulation index (Igeo); (iii)) identifying the accumulation patterns in the root, stem, leaf and (iv) assess retention capacity of macrophyte plants for phytoremediation of aquatic ecosystem.

MATERIALS AND METHODS

Study area

The Djendjen River basin is located in north-eastern part of Algeria (Fig. 1) between 5°30' and 5°58'E longitude and 36°22' and 36°48' N latitude. It is characterized by humid Mediterranean climate. The average annual air temperature and precipitation is 18.4°C and 970.6 mm respectively, with the rainfall season between November and March. The total study area is 525 km^2. Agricultural wastes, fertilizers and raw sewage effluents constitute the predominant anthropogenic sources in the area (Krika & Krika 2018).

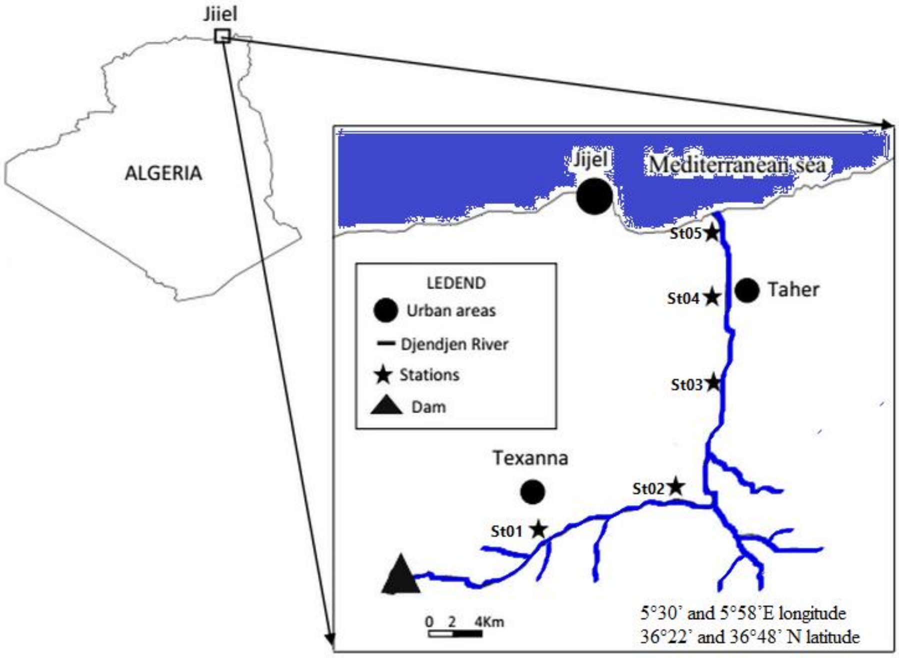

Fig. 1. Location of different sampling sites of Djendjen River.

Pollution evaluation indices

Practical tools namely pollution indices such as geoaccumulation index (I_{geo}), contamination factor (CF) and Pollution load index (PLI) were used to understand the pattern of trace element pollutions in the study area and to get information regarding to the origin of element pollutants and to evaluate pollution rank.

Geoaccumulation index (Igeo)

I_{geo} index was used to evaluate pollution by comparing the present and preindustrial trace element levels in sediments (Müller 1981).

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I_{geo} was determined using the following equation:

$$I_{geo} = Log_2 \frac{C_n}{1.5 \times B_n} \quad \textbf{(1)}$$

where C_n is the concentration of the studied metal (n) in the sediment and B_n is the geochemical background concentration of the metal (n). The constant 1.5 is the background matrix correction factor. The background values of Turekian & Wedepohl (1961) were utilised to determine I_{geo}. Muller (1981) has distinguished six grades of I_{geo} as shown in Table 1.

Table 1. Descriptive classes for Igeo values (Müller 1981).

Sediment quality	I_{geo}	I_{geo} Class
Unpolluted	$I_{geo} < 0$	0
Unpolluted to moderate polluted	$0 < I_{geo} \leq 1$	1
Moderately polluted	$1 < I_{geo} \leq 2$	2
Moderately to heavily polluted	$2 < I_{geo} \leq 3$	3
Heavily polluted	$3 < I_{geo} \leq 4$	4
Heavily to extremely polluted	$4 < I_{geo} \leq 5$	5
Extremely polluted	$I_{geo} > 5$	6

Contamination Factor (CF)

The contamination factor (CF) of a single metal was determined the same as recommended by Min *et al.* (2013) and Kerolli-Mustafa *et al.* (2015). It was employed to assess the contamination of trace element in our samples. CF is calculated as follow:

$$CF = \frac{C^i_{sample}}{C^i_{reference}} \quad \textbf{(2)}$$

where C^i_{sample} is the trace element level in sediment and $C^i_{reference}$ is a metal level of a control sample. The contamination levels were ranked according to their intensities on a scale of 1 to 6 (Table 2).

Table 2. Sediments contamination level based on contamination factor (CF) value (Hakanson 1980).

Contamination Factor level	CF value
Low contamination	CF < 1
Moderate contamination	1 < CF ≤ 3
Considerable contamination	3 < CF ≤ 6
Very high contamination	CF > 6

Pollution Load Index (PLI)

PLI was initially applied to establish sediment pollution load. It can furthermore provide a simple and relative ways for assessing the degree of pollution by metals (Tomlinson *et al.* 1980). This index is expressed as follow:

$$PLI = \sqrt[n]{Cf_1 \times Cf_2 \times Cf_3 \times \ldots Cf_n} \quad \textbf{(3)}$$

where n is the metal numbers and Cf is the contamination factor. The PLI value < 1 indicates no pollution while PLI>1 exhibits a heavy pollution (Tomlinson *et al.* 1980).

Sediment and plants analysis

Samples of sediment and plants were collected at five sites, namely St 01 to St 05, located at the Djendjen River and used by the local people for domestic and agricultural purposes. All samples were collected during the period from February to June 2018 in three replicates in each sampling sites.

To avoid metal contamination sedimentary samples were taken from each location using plastic sampling spatula and plastic gloves. After the collection, each sediment were placed in polyethylene bags and immediately refrigerated. Sediment samples were oven dried until constant dry weight at 60°C, and sieved with a 63-µm stainless steel sieve. Generally, fine fractions contain more trace elements than the coarser ones. This enrichment is mainly due to the high specific surface area of the smaller particles (Szefer *et al.* 1996). For trace element analysis, 0.1 g of dry sediment was digested using 10 mL $HCl-HNO_3-HF$ (Bai *et al.* 2011). Finally, the solution was diluted to a final volume of 25 mL with deionized distilled water.

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Two healthy aquatic plants of *Arundo donax* and *Typha latifolia* were collected by hand in each sampling sites and washed with river water to clear them from periphyton and detritus. The collected samples were preserved in plastic bags, labelled carefully for further analysis. The collected polythene tools were used in sampling.
Roots, stems and leaves were preliminarily dissected. Each set of roots, stems and leaves was thoroughly rinsed several times with deionized water and dried at 80 °C for 24 h (Demirezen & Aksoy 2006; Mishra & Tripathi 2008). Digestion process was carried out according to Karla (1998). The elemental state of trace elements was analysed by a flame atomic absorption spectrometer (AAS, PerkinElmer model 2380, USA) as prescribed in Standard Methods (APHA *et al.* 2005).
Two indicators were used to determine the potential of the two macrophyte species for phytoremediation including bioaccumulation factor (BAF) and translocation factor (TF)

$BAF = [\text{trace element}]_{root} / [\text{trace element}]_{sediment/water}$

$TF = [\text{trace element}]_{organ} / [\text{trace element}]_{root}$

Statistical analysis

To assess the contamination levels of heavy metals, the mean values were calculated for sediment and plant species. Data analysis was carried out using the packaged Statistica software version 8.0 for statistical analysis and variance analysis (One-Way ANOVA). The significance was reported as $p < 0.05$. The Newman-Keuls multiple mean comparison test was also used to complement the ANOVA (Zar 1999).

RESULTS AND DISCUSSION

Trace element concentrations in sediments

Table 3 illustrates the metal concentrations in the sediment. Generally, metal concentrations had the following order: Zn>Ni>Cu>Cd. For the assessment of the impacts of trace elements in sediments, the metal levels in Djendjen River were compared to metal background concentrations, also with other rivers sediments and the probable effect concentrations for sediments (PEC). The use of data of Turekian & Wedepohl (1961) was subjected as background data. The probable effect for sediment concentrations were developed by MacDonald *et al.* (2000). The results exhibited that average sediment metal levels from the Denjden River are higher than the background concentrations, but not exceeded the PEC, except for Ni, indicating low-moderate degrees of pollution (Table 3). In addition, metal concentrations in the sediments of the study area were lower than those reported by Varol & Şen (2012) and Mendez (2005). However, in contrast to our investigation, Ahmad *et al.* (2010), Bai *et al.* (2011) and Hongyi *et al.* (2009) have reported values less than our study.

Table 3. Mean concentrations of trace element (µg g^{-1}) in sediment compared to background world average and sediment quality guidelines (SQGs).

	Cd	Cu	Ni	Zn
Sediment (µg g^{-1}d.w.)	1.101 ± 0.07	121.56 ± 4.50	140.68 ± 2.52	196.51 ± 4.73
[a]Back.word. average	0.3	45	68	95
[b] SQGs				
PEC	4.98	149	36	459
[c]Bangshi River (Bangladesh)	0.61	31.01	25.67	117.5
[d]Yilong Lake (China)	0.76	31.40	1.20	2.13
[e]Pearl River (China)	1.72	348	-	391
[f]Tigris River (Turkey)	7.9	860	-	1061
[g]Rimac River(Peru)	31	796	-	8076

[a]Turekian & Wedepohl (1961)
[b]Sediment quality guideline (2000)
[c]Varol & Şen (2012)
[d]Mendez (2005)
[e]Ahmad *et al.* (2010)
[f]Bai *et al.* (2011)
[g]Hongyi *et al.* (2009)

Pollution evaluation indices

Based on the Igeo average values, metals in sediments varied with the following order: Cd> Cu> Ni≈ Zn. The values of the calculated index vary from 1.15 to 1.35 for Cd, 0.82 to 0.89 for Cu, 0.42 to 0.52 for Zn, and 0.43 to

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0.49 for Ni (Table 4). Furthermore, the Igeo values of Cu, Zn and Ni for all stations were less than 1, suggesting that sediments can be considered as not polluted to moderately pollute with these metals. In contrast, the average values of Igeo for Cd in all the stations is constantly greater than one (>1) indicating a moderately sediment pollutions with this metal.

CF and PLI are the most utilised indices to assess the level of heavy metal contamination in the sediments (Bhuiyan *et al.* 2010). The mean CF values of the metals studied are shown in Table 4.

The trends for CF values for the elements in the study zone followed the sequence: Cd> Cu> Ni ≈ Zn. The results indicated that for all metals, CF values were greater than 1 and a considerable CF for Cd was found in the study area. However, all the stations were found to be moderately contaminated with Cu, Zn and Ni.

PLI values were high in all sediment samples, ranging from 2.44 to 2.63, indicative of a heavy pollution, with respect to total of the metals studied. Sekabira *et al.* (2010) reported that a PLI greater than one mainly results from anthropogenic inputs.

Table 4. Geoaccumulation indexes (Igeo). Contamination factors (CF) and pollution load indices (PLI) of the studied metals in sediments of the study area.

Sites	I_{geo}				PLI	CF			
	Cd	Cu	Zn	Ni		Cd	Cu	Zn	Ni
S1	1.15	0.82	0.42	0.43	2.44	3.35	2.62	2.01	2.02
S2	1.29	0.84	0.44	0.44	2.52	3.68	2.68	2.03	2.04
S3	1.35	0.85	0.47	0.47	2.58	3.83	2.70	2.06	2.08
S4	1.30	0.86	0.47	0.47	2.56	3.70	2.71	2.08	2.08
S5	1.33	0.89	0.52	0.49	2.63	3.78	2.78	2.14	2.12
Mean	1.28	0.85	0.46	0.46	2.73	3.67	2.70	2.06	2.07

Trace element concentrations in different plant parts

The content of heavy metals in aquatic macrophyte has shown a strong anthropogenic influence in the natural environment. The studied species displayed a variety bioaccumulation of heavy metals (Table 5).

The average heavy metal concentrations in the *T. Latifolia* organs exhibited the following sequence: Zn > Cu > Ni > Cd. Compared with other investigated organs, the root of *T. Latifolia* enclosed the highest amount of Cd, Cu, Ni and Zn. Furthermore, the leaf parts of this species contained the main level of copper. In contrast, the mean metal concentrations in *A. donax* parts decreased following the sequence: Cu > Zn > Ni > Cd.

Table 5. Mean concentrations of trace metals in root, stem, and leaf of *T. latifolia* and *A. donax* (µg g^{-1} dry weight)

Elements	*Typha latifolia*			*Arundo donax*		
	root	stem	leaf	root	stem	leaf
Cd	1.10 ± 0.05^c	0.85 ± 0.07^a	0.93 ± 0.05^b	0.62 ± 0.07^c	0.35 ± 0.04^a	0.43 ± 0.04^b
Cu	30.20 ± 1.58^c	3.22 ± 0.41^a	6.55 ± 1.18^b	7.15 ± 0.60^c	1.94 ± 1.07^a	3.48 ± 0.56^b
Ni	26.90 ± 1.87^c	2.11 ± 0.18^b	1.80 ± 0.07^a	3.13 ± 0.39^c	0.88 ± 0.19^a	1.01 ± 1.10^b
Zn	98.10 ± 1.75^c	17.13 ± 2.35^b	2.19 ± 0.13^a	3.90 ± 0.57^c	1.85 ± 0.52^a	2.72 ± 0.53^b

Although *T. latifolia and A. donax* root enclosed the main concentrations of metals.

However, leaf and stem parts had higher Cu and Zn contents. Our results revealed, for the two species, that the greater amount of metals was reported in roots. To keep rhizomes and shoots from injuries caused by metals, the metal sequestration in roots and exclusion from above-ground, act as a metal ability strategy with filtering procedure

Bioaccumulation factor (BAF) and Translocation factor (TF)

Bioaccumulation factor (BAF), the quotient of heavy metal concentrations in plants and sediments, is shown in Table 6. BAF factors reveal the aptitude of plants to absorb and accumulate heavy metals from aquatic media. However, macrophytes communities respond differently to metal contaminated sediments depending on their capability to uptake and detoxify variety of trace elements.

According to Table 6, the roots of *T. Latifolia* were the major site of metal accumulations when compared to *A. donax* roots. In metal excluder macrophyte, translocation factors (TFs) were generally less than 1 (Zu *et al.* 2005).

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Table 6. Bioaccumulation and translocation factors (BAF and TF) in *T. latifolia* and *A. donax*.

	T. Latifolia			***A. donax***		
Element	**BAF**	**TF**		**BAF**	**TF**	
	Root/sediment	Stem/root	Leaf/root	Root/sediment	Stem/root	Leaf/root
Cd	0.99	0.77	0.84	0.56	0.56	0.69
Cu	0.25	0.11	0.22	0.06	0.27	0.48
Ni	0.19	0.10	0.04	0.02	0.29	0.57
Zn	0.50	0.17	0.02	0.02	0.47	0.70

Results exhibited a different bioaccumulation ability of heavy metals for both plants. BAF values for *A. donax* were lower than that of *T. Latifolia*. Due to high concentrations in the underground parts, the higher *T. latifolia* BAF values imply bioaccumulation as a dominant process, while *A. donax* seems to adopt a metal exclusion strategy, except for Cd (BAF > 0.5).

Moreover, translocation factor was used to assess the rate of trace element transfer between roots and shoots. In this study, the low values of translocation factor for stems and leave of both aquatic plants seems to be a characteristic of an excluder species, that decreased the absorption, transport and storage of heavy metals in above-ground parts (Karpiscak *et al.* 2001 ; Weis *et al.* 2004) due to ineffective metal transport systems (Zhao *et al.* 2002). Values for trace elements stem/root translocation factor were lower in *T. latifolia* compared to *A. donax* (except for Cd). This imply another trend of bioaccumulation in the different parts, i.e.; root>stem>leaf in *T. latifolia* (except for Cd and Cu) and root>leaf>stem in *A. donax* (for more elements).

Several investigations have shown that metal concentrations diminish in the following order root>leaf>stem in various aquatic vegetations such as *A. donax* (Clemens *et al.* 2002; Bonanno 2012). On the other hand, the pattern root/stem/leaf in *T. latifolia* appears relatively common in *Typha* species as establish by Giuseppe (2013).

CONCLUSION

The present study revealed that in sediments, abundance of trace elements was ranked as follows: Zn>Ni>Cu>Cd. However, all metal concentrations exceeded background values. The CFs exhibited that contamination level of Cd was higher than other heavy metals in sediments, such that the mean CF values for the metals in the study area followed the order of $CF_{Cd} > CF_{Cu} > CF_{Ni} \approx CF_{Zn}$. The PLI, which was within the range of 2.44-2.63, indicated heavy pollution of river sediments. The Igeo revealed that the pollution decreased in accordance with the following order: Cd> Cu> Ni≈ Zn, with Cd causing the most pollution and Cu, Ni and Zn, having nearly no pollution in river sediments. However, the present study confirmed that aquatic plants, growing in a metal contaminated environment, possess the ability to accumulate trace elements in their roots. Results exhibited that the concentration of elements in plant biomass is significantly depending on the type of element and organ. Concentrations of metals in *A. donax* and *T. latifolia* decreased as follows: root>leaf>stem and root>stem>leaf, respectively. This indicates that roots are the major sites of bioaccumulation. According to previous studies, roots accumulate higher metal amounts with regard to up tissues. Findings indicate that *T. latifolia* was performed than *A. donax* in terms of trace element bioaccumulation and the significant role of aquatic plants in aquatic environments, regarding of bioindication and bioremediation.

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تجمع زیستی فلزات سنگین و انتشار آنها در تیفا لاتی فولیا و آروندو دوناکس: کاربرد آن در تصفیه گیاهی

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چکیده

در این مطالعه ما غلظت فلزات (کادمیوم، نیکل، روی و مس) در رسوب و گیاهان آبی (تیفلا لاتی فولیا و آروندا دوناکس) را تعیین کردیم. میزان آلودگی در رسوب با استفاده از فاکتور آلودگی، شاخص بار آلودگی و شاخص تجمع زمین‌شناسی تعیین شد. نتایج به دست آمده نشان داد که انتشار عناصر کمیاب در رسوب به ترتیب زیر است: روی (۱۹۶/۵۱ میکروگرم در گرم) بیش از نیکل (۱۴۰/۶۸ میکروگرم در گرم) و بیش از مس (۱۲۱/۵۶ میکروگرم در گرم) و کادمیوم (۱/۱۰۱ میکروگرم در گرم). با وجود این، مقایسه غلظت فلزات موجود در رسوب با پارامترهای آلودگی محیطی مانند سطح موثره احتمالی، و سطوح زمینه‌ای، نشان داد که غلظت همه فلزات مورد بررسی به استثنای نیکل کمتر از غلظت مجاز عناصر می‌باشد، هرچند که از سطوح زمینه‌ای بیشتر بوده است. مقادیر شاخص تجمع زمین‌شناسی نشان داد که کادمیوم (۱/۲۸ میکروگرم در گرم) به طور معنی‌داری در رود جنجن تجمع یافته بود. فاکتور آلودگی نشان داد که نمونه‌های رسوب از نقطه نظر آلودگی به فلزات در حد متوسط قرار دارند. شاخص بار آلودگی بیش از ۱ بود که نشان دهنده افت شدید کیفیت رسوب است. در گیاهان مورد مطالعه نتایج نشان داد که مقدار غلظت در بافت‌ها بستگی به نوع اندام و نوع فلز دارد. آروندا دوناکس ظرفیت پایین‌تری از تجمع زیستی و همچنین ظرفیت پایین‌تری برای دفع کارآمد فلزات نسبت به تیفلا لاتی فولیا دارد. در بوم سازگان‌های آبی آلوده، حضور تیفلا لاتی فولیا باعث افزایش دفع فلزات سنگین می‌شود و بنابراین، معرفی آن می‌تواند در فعالیت‌های احتمالی تصفیه گیاهی نقش داشته باشد.

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