UPTAKE OF METALS AND METALLOIDS BY *CONYZA CANADENSIS* L. FROM A THERMOELECTRIC POWER PLANT LANDFILL

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Abstract: Fourteen metals and metalloids were determined in *Conyza canadensis* L. harvested from the fly ash landfill of the thermoelectric power plant "Kolubara" (Serbia). Fly ash samples were collected together with the plant samples and subjected to sequential extraction according to the three-step sequential extraction scheme proposed by the Community Bureau of Reference (BCR; now the Standards, Measurements and Testing Program). The contents of metals and metalloids were determined by inductively coupled plasma optical emission spectrometry (ICP-OES) in plant root and the aboveground part and correlated with their contents in the fly ash samples. The bioconcentration factor (BCF) and translocation factors (TF) were calculated to access uptake of metals from fly ash and their translocation to the aboveground part. Results regarding As revealed that fly ash samples in the proximity of the active cassette had higher amounts of the element. Principal component analysis (PCA) showed that As had no impact on the classification of plant parts. BCF for As ranged from 1.44 to 23.8 and varied, depending on the investigated area; TF for As ranged from 0.43 to 2.61, indicating that the plant translocated As from root to shoot. In addition to As, *Conyza canadensis* L. exhibited efficient uptake of other metals from fly ash. According to the calculated BCF and TF, the plant retained Al, Fe and Cr in the root and translocated Zn, Cd, Cu and As from root to shoot in the course of the detoxifying process.

Key words: arsenic; bioaccumulation; bioconcentration factor; *Conyza canadensis* L.; fly ash

INTRODUCTION

Soil is a major source of micronutrients for plant growth; however, it can also retain toxic quantities of heavy metals. Transformation of these elements in soil unfolds through many physicochemical processes such as precipitation-dissolution, adsorption-desorption and complexation. The mobility of metals in the soil depends on the total concentration of elements, soil properties, metal properties and environmental factors [1]. To evaluate metal uptake by plants it is very important to know the distribution of metals among soil components. For this purpose, various sequential extraction procedures for soil have been developed [2-4].

A three-step sequential extraction scheme proposed by the Standards, Measurements and Testing Program is commonly used to fractionate heavy metal chemical forms [4-6]. The metals are divided into acid-soluble/exchangeable, reducible and oxidizable fractions. Water soluble and exchangeable fractions are considered to be bioavailable, and reducible and oxidizable fractions may be potentially bioavailable. The relative mobility of metals is moderate and changes under redox conditions and can cause metal release. Metal mobility in the residual fraction is strongly dependent on environmental conditions and is unavailable to both plants and microorganisms.

The disposal of fly ash in the environment can be a serious problem due to the leaching of toxic heavy metals [7,8]. In our previous study, the leaching of elements from coal ash was investigated [9], and we showed that leaching of As, Cd and Pb can be a serious environmental problem. The relationship between soil metal fractions and plant uptake has been estimated by simple, partial and multiple correlation

procedures. The possibility of a metal to be absorbed by plants depends on the plant and the conditions of cultivation [10]. Some plants can tolerate and accumulate considerably higher concentrations of trace elements such as Cu, As and Cd [11]. Phytoremediation is a promising approach for treating contaminated soil [12]. It involves processes such as the removal, transfer, stabilization or degradation of contaminants from soil and sediment water [13]. The effectiveness and efficiency of phytoremediation depends mostly on plant characteristics, such as growth rate, quantity of biomass, suitability for harvesting, tolerance and accumulation of heavy metals in shoots [14,15].

Investigation of the potential use of the invasive species *Conyza canadensis* L. (formerly *Erigeron canadensis* L.) in phytoremediation has been undertaken previously [16]. The authors concluded that in view of its dominance in the vegetation cover and high biomass, this plant could be considered as a candidate for phytoextraction of Cd and Zn.

Contrary to our previous study, in this study the modified BCR (Commission of the European Communities Bureau of Reference) sequential extraction method was used to determine available concentrations of elements in fly ash. Fly ash samples were collected by different sampling patterns depending on their distance from the active cassette. Plant samples were collected in spring, prior to bloom, and divided into root and shoot (aboveground) parts. The aim of this research was to examine the correlation between metal fractions extracted from corresponding fly ash samples by the BCR method and the concentrations of metals in *Conyza canadensis* L with regard to the distance from the active cassette.

MATERIALS AND METHODS

Site characterization

The plant and fly ash samples used in this study were collected from the thermoelectric power plant "Kolubara" landfill [9,16]. The landfill was divided into four cassettes: A (active cassette), P (passive cassette) and two recultivated cassettes (R1 and R2) (Fig. 1). During sampling, one of the four cassettes was active. Samples of *Conyza canadensis* L., together with

the associated fly ash samples, were collected in April 2013 prior to bloom. In comparison to our previous study [16] when the fly ash samples were taken from the central area of the landfill where the vegetation cover was most abundant (Braun-Blanquet 5), this time sampling was performed diagonally from the borderlines with the active cassette to the center of the cassette that is subjected to recultivation.

Instruments and reagents

All chemicals were of analytical grade and obtained from Merck (Darmstadt, Germany). Multi-element stock solution containing 1000 mg/L of 22 elements was used to prepare intermediate multi-element standard solutions for ICP-OES measurements. All measurements were carried out in an inductively coupled atomic emission spectrometer, ICP-OES (Thermo Scientific, United Kingdom; model 6500 Duo). The operating RF power was 1150 W, argon plasma gas flow rate was 12 L/min; auxiliary 0.5 L/min; nebulizer 0.5 L/min. Selected wavelengths (nm) were: Ag (243.7), Cr (267.7), Al (396.1), Cu (327.3), As (193.7) Fe (238.2), Ba (493.4), Mg (279.5), Ca (393.3), Ni (231.6), Cd (214.4), Pb (220.3), Co (228.6), Zn (202.5). To check the accuracy, standard reference material was applied by BCR 701 (lake sediment). Microwave digestion was performed in a microwave oven (Ethos 1, Advanced Microwave Digestion System, Milestone, Italy).

Plant sample preparation

Plant samples (n=30) were washed, divided into belowground (root) and aboveground parts and oven-dried at 70°C to a constant weight. The samples were
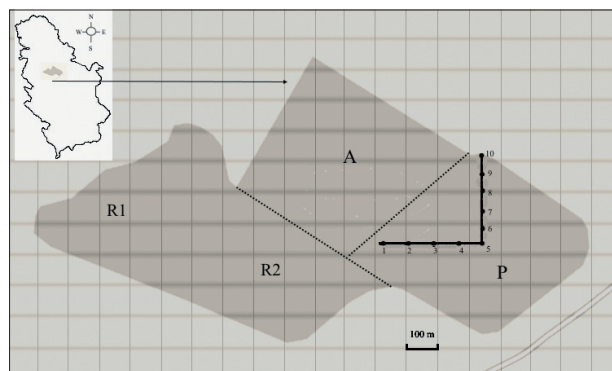
Fig. 1. Location map of the sampling area. A – active cassette; P – passive cassette; R1 and R2 – recultivated cassettes.

prepared for analysis by the microwave acid digestion procedure in 7 mL of 65% HNO_3, 1 mL 30% H_2O_2.

Fly ash sample preparation

Fly ash samples were taken in the root zone (0-30 cm) and dried at room temperature for 3 weeks. One g of each sample was subjected to the BCR sequential extraction procedure [5,6]. The following fractions were prepared: HOAc extractable (F1), reducible (F2), oxidizable (F3) and residual (R). For pseudo-total metal content determination, 1 g of each sample was digested in 15 mL of 12 mol /L HCl and 5 mL of 15.8 mol/L HNO_3 by heating for 5 h at 80°C.

Statistical analysis

Descriptive statistics and correlation analysis were performed by a demo version of NCSS statistical software (Hintze, 2001, Number Cruncher Statistical Systems, Kaysville, UT; www.ncss.com). Principal component analysis (PCA) was carried out by PLS ToolBox, v.6.2.1, for MATLAB 7.12.0 (R2011a). All data were autoscaled prior to any multivariate analysis to bring the values to compatible units. PCA was carried out as an exploratory data analysis using a singular value decomposition algorithm and a 0.95 confidence level for Q and T^2 Hotelling limits for outliers. The analysis was based on a correlation matrix and factors with eigenvalues greater than 1 retained.

RESULTS AND DISCUSSION

Fourteen elements were identified and quantified, including Ag, Al, As, Ba, Ca, Cd, Co, Cr, Cu, Fe, Mg, Ni, Pb, and Zn in the root and shoot sections of the *Conyza canadensis* as well as in the fly ash samples. The summarized parameters of descriptive statistics (mean, standard deviation, median, minimum and maximum values) obtained from metal content analysis in different parts of the plant *Conyza canadensis* are presented in Table 1. According to the results, higher amounts of Al, Cr, Fe, Pb and Co were found in the root, while the contents of Ca, Cd, Cu, Mg and Zn were higher in the aboveground part. The As, Ag and Ba contents were similar in both parts of the plant (root and shoot).

In order to select factors that could specify the particular part of the plant and evaluate the distribution of metals in the plant, a sophisticated chemometric technique, PCA, was applied. PCA was used for exploratory data analysis, not as a classification model but to intimate as to what could be expected from the current data and to check if there is a logical pattern in the data that could be explained. Input data consisted of the metal content.

PCA explained 86.32% of the total variance by a three-component model in which the first principal component, PC1, resulted in 54.20% of the overall data variance, PC2 for 21.41% and PC3 for 10.71%. The projections of factor scores and appropriate loadings for the first and third principal components (PCs) are presented in Fig. 2. Two distinctive groups of samples belonging to the shoot and root and separated alongside PC1 were obtained (Fig. 2a). The loading plot (Fig. 2b) showed that the highest positive influence on PC1 have the parameters of Co, Al, Fe and Cr, which determined the group of root samples, while the highest negative influence on PC1 was recorded by the metals Ca, Mg and Zn, which determined the

Table 1. Concentration of various elements (µg/g) in the root and shoot of plants.

	Parameter	Ag	Al	As	Ba	Ca	Cd	Co	Cr	Cu	Fe	Mg	Ni	Pb	Zn
Root	Mean	4.92	2310	2.24	17.83	1492	0.27	0.63	4.24	12.99	1325	769	6.01	2.61	19.47
	SD	1.27	990	0.90	6.78	270	0.15	0.23	2.16	4.46	558	194	1.75	3.58	3.69
	Median	4.45	2064	1.94	15.40	1364	0.23	0.58	3.53	11.69	1187	764	5.75	1.21	20.01
	Min	3.61	1166	1.56	10.46	1246	0.11	0.34	2.37	5.58	811	568	3.86	0.82	12.63
	Max	7.69	4638	4.40	35.82	2046	0.52	1.15	9.92	20.41	2722	1255	9.50	12.40	23.79
Shoot	Mean	4.33	594	2.83	14.73	5565	0.51	0.17	18.84	427	1520	3.71	0.49	68.75
	SD	1.00	405	2.25	3.17	1934	0.24	0.13	0.61	4.24	198	253	0.99	0.20	16.69
	Median	4.28	428	1.93	14.86	6252	0.40	0.12	1.10	18.72	380	1458	3.74	0.54	67.73
	Min	3.02	219	1.05	9.91	1010	0.30	0.04	0.46	12.05	197	1203	2.19	0.16	48.07
	Max	6.06	1393	8.37	19.62	7208	0.95	0.41	2.38	25.57	807	2016	5.73	0.78	102.16

n=30
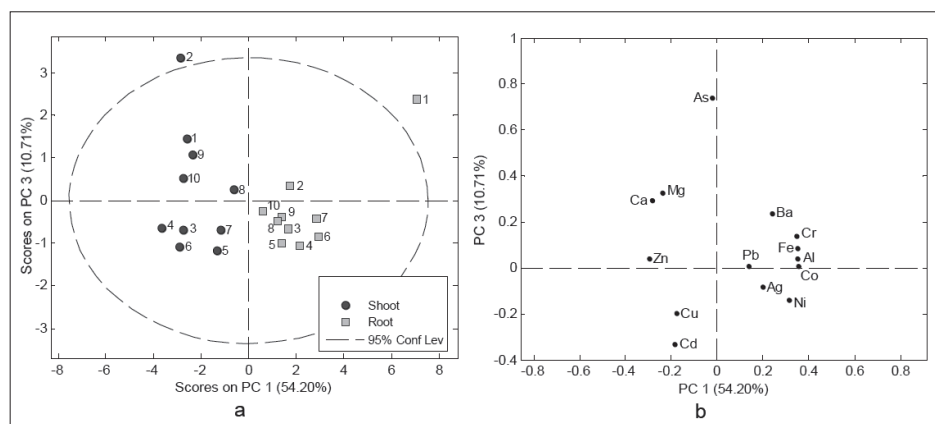
Fig. 2. Principal component analysis (PCA). A – score plot; b – loading plot.

group of shoot samples. Samples taken from the borderlines with the active cassette (samples 1, 2, 9 and 10) formed subclusters alongside PC3, and were more pronounced in the case of the shoot (Fig. 2a).

As does not significantly contribute to PC1 and has no impact on the classification of the plant part, but has a high positive influence on PC3, pointing to its higher content in borderline samples. The distribution of this metalloid is in accordance with previously published results on element leaching during the transport and storage of coal ash [9]. The filter fly ash and fly ash from the active cassette had a considerably higher content of As in comparison to the passive cassette. This could be the consequence of the continual leaching of As into the environment and is of toxicological importance. In contrast, samples collected closer to the center of the passive cassette, which was undergoing recultivation, had a higher content of Cu and Cd.

The sum of metal content obtained by the BCR sequential extraction procedure and the pseudo-total (PT) metal contents for fly ash samples (Table 2) were used to calculate recovery values. The recovery values of these metals ranged from 89 to 114%, indicating good agreement between the PT concentration and the sum of the BCR fractions. The metal contents in the fractions were evaluated in percentages relative to the total value (100%) and are presented in Fig. 3.

The distribution of various Cd fractions indicated that the highest amount was in the residual fraction (82.3% of the total), with the lowest content in the HOAc extractable fraction (F1) (3.9%). The reducible fraction (7.6%) was equivalent to the oxidizable frac-

Table 2. Concentration (μg/g) of elements obtained by sequential extraction and pseudo-total digestion.

(μg/g)	F1	F2	F3	R	BCR+R	PT	Recovery (%)
Al	64.1	665.0	245.8	17993.6	18968.5	20433.5	92.8
Ca	2025.8	1123.4	115.6	222.4	3487.1	3463.1	101.5
Fe	3.2	897.8	17.8	20553.6	21472.4	23091.3	93.2
Mg	476.1	233.9	257.8	3270.6	4238.3	4520.2	93.7
Ag	8.459	21.116	5.136	35.738	70.450	72.334	96.9
As	0.034	0.143	0.108	14.507	14.793	15.904	93.1
Ba	25.072	27.630	0.004	37.128	89.833	92.871	96.1
Cd	0.034	0.067	0.055	0.722	0.877	0.899	97.5
Co	0.836	4.164	0.434	5.623	11.056	11.526	95.3
Cr	0.193	0.873	1.322	88.190	90.577	95.826	94.9
Cu	0.799	3.085	13.873	86.968	104.726	104.532	99.3
Ni	4.786	8.635	4.381	40.148	57.950	59.800	96.8
Pb	0.503	2.056	0.367	18.689	21.615	23.374	92.6
Zn	0.532	1.725	12.461	111.156	125.874	122.001	105.1

n=10

tion (6.2%), indicating that this element has a mainly lithogenic origin [17]. As and Pb were primarily distributed in the residual fraction (98.1% and 86.5%, respectively), followed by the reducible (1.0% and 9.5%, respectively) and oxidizable (0.7% and 1.7%, respectively) fractions. The smallest amount of As and Pb were measured in the HOAc extractable fraction (0.2% and 1.7%, respectively). The dominant chemical forms of Cu and Zn were associated with the residual fraction, followed by the oxidizable fraction (27.1% and 10.5%, respectively). The Cu content in the sum of the first three phases varied in the samples, pointing to an anthropogenic input of these heavy metals [18]. The metals bound to the residual fraction (>90%) were Al, Fe, Cr and As. The greatest proportion of Ca noted during the study was associated with the HOAc
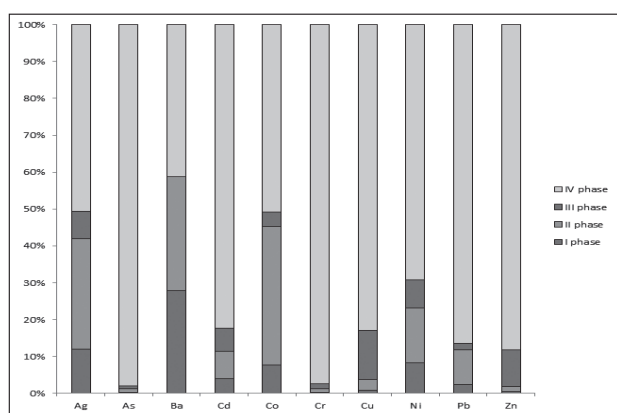
Fig. 3. Fraction of elements in fly ash samples (percent content) determined after BCR sequential extraction. BCR + R – the sum of the fractions F1 + F2 + F3 + residual; PT – pseudo total and Recovery (R%) = [(F1 + F2 + F3 + residual)/pseudo-total] × 100.

extractable fraction (58.1%), followed by the reducible (32.2%). A very small proportion of Ca was found in the residual fraction.

The risk assessment code (RAC), defined as the fraction of metal exchangeable and/or associated with carbonates (F1%), was determined for heavy metals, and the values were interpreted in accordance with the RAC classifications [19]. If the value of RAC is <1%, the soil is of no risk to the environment. Low risk, medium risk, high risk and very high risk are associated with RAC values of 1-10%, 11-30%, 31-50%, and >75%, respectively. The RAC value was determined by dividing the first fraction (F1) with the sum of three phases and the residual step of the BCR sequential extraction (F1+F2+F3+R). According to RAC analysis, the fly ash showed a low risk for all elements with RAC values <10% (Table 3). The RAC value of Ba in the present study was 28%, which is considered as dangerous, easily entering into the environment [19]. Co showed a low average value of RAC (7.6%); however, in some of the fly ash samples the RAC value exceeded 10%, indicating substantial risk of Co mobilization from fly ash across almost the entire study region. Also, the average value for Ni posed a low risk (RAC<10) but in some of the fly ash samples the RAC value exceeded 10%.

Correlation between metal concentrations in different fractions of fly ash and plant samples

The content of elements in different fractions of fly ash samples obtained by the modified BCR extrac-

tion procedure and the determined metal uptake of the root samples were compared using a correlation analyses (Table 4). Several statistically significant dependencies ($P<0.0001$ or better) were found, and the highest significant correlations ($r>0.6$, $P<0.000001$) were obtained between As ($r=0.740$) and the F3 phase of BCR extraction. There was a good negative correlation between Cd uptake and the HOAc extractable fraction (F1) ($r=-0.581$), and Pb uptake with the reducible fraction (R) ($r=-0.599$). Significant correlations were also found between Fe, Mg and Cr and the sums of the first three fractions (0.789, 0.738 and 0.640, respectively). A significant positive correlation was observed for Ba ($r=0.860$) with a residual fraction (R) and consequently with the PT concentration ($r=0.804$).

The sum of the concentrations of elements extracted with BCR+R (PT concentration) was an inadequate indicator for metal uptake by plants [5]. Therefore, the BCF was calculated as the ratio of the metal concentration in the root and the sum of the metal concentration in the first three extracted fractions. The calculated BCF and TF [16] are presented in Tables 5 and 6. The average BCF values lower than 1 were displayed by As, Zn, Al, Cd, Cr and Fe (in a de-

Table 3. The calculated RAC for fly ash samples.

	Ag	As	Ba	Cd	Co	Cr	Cu	Ni	Pb	Zn
RAC	12.00	0.23	27.91	3.86	7.56	0.21	0.76	8.26	2.33	0.42

Table 4. Correlation coefficients (*r*) between metal fractions [obtained by sequential extraction of fly ash i.e. F1, F2, F3, Residual (R), BCR+R (F1+F2+F3+R)] and the content of corresponding metals in root samples. Correlation is significant at 95% confidence level ($r_{critical}$ = 0.549, n = 10).

	F1	F2	F3	R	F1+F2+F3	BCR+R
Al	-0.131	0.146	-0.035	0.018	0.012	0.018
Ag	0.414	0.186	0.580	-0.029	0.286	0.287
As	0.432	-0.284	0.740	-0.243	-0.060	-0.237
Ba	0.191	0.640	0.000	0.860	0.551	0.804
Ca	0.094	0.535	-0.086	-0.304	0.336	0.160
Cd	-0.581	-0.493	0.546	-0.477	0.384	-0.088
Co	0.059	0.181	0.724	-0.729	0.250	-0.044
Cr	0.190	0.505	0.570	0.221	0.640	0.279
Cu	0.419	0.084	-0.179	-0.019	-0.157	-0.038
Fe	-0.076	0.785	0.553	-0.005	0.789	0.091
Mg	0.410	0.315	0.794	0.229	0.738	0.411
Ni	0.309	0.068	0.343	-0.072	0.184	0.142
Pb	0.052	-0.599	0.055	0.383	-0.499	0.218
Zn	0.371	0.023	0.453	-0.024	0.468	0.077

Table 5. Calculated bioconcentration factors.

BCF	Al	Ag	As	Ba	Ca	Cd	Co	Cr	Cu	Fe	Mg	Ni	Pb	Zn
Mean	2.70	0.18	11.50	0.35	0.51	2.21	0.15	1.96	0.96	1.51	0.81	0.43	0.19	6.82
Max	3.72	0.31	23.76	0.49	0.79	4.96	0.32	3.14	1.68	2.10	0.99	0.66	1.06	15.50
Min	0.71	0.05	1.44	0.19	0.32	0.65	0.06	0.66	0.22	1.07	0.64	0.14	0.00	1.20
SD	0.93	0.07	6.19	0.11	0.15	1.58	0.08	0.77	0.44	0.34	0.12	0.15	0.33	3.92
Median	2.80	0.19	11.69	0.35	0.45	1.31	0.14	2.07	1.04	1.47	0.86	0.47	0.08	6.08

Table 6. Calculated translocation factors.

	Ag	Al	As	Ba	Ca	Cd	Co	Cr	Cu	Fe	Mg	Ni	Pb	Zn
Mean	0.93	0.28	1.13	0.91	3.87	2.16	0.28	0.33	1.53	0.35	2.05	0.62	0.35	3.60
Max	1.40	0.81	2.61	1.49	5.77	3.05	0.70	0.82	2.33	0.82	2.86	0.97	0.90	4.96
Min	0.39	0.06	0.43	0.30	0.67	0.88	0.04	0.08	0.99	0.11	0.96	0.23	0.05	2.26
SD	0.28	0.24	0.68	0.34	1.47	0.75	0.25	0.23	0.40	0.23	0.52	0.20	0.25	0.86
Median	0.93	0.19	0.93	0.93	4.05	2.39	0.19	0.31	1.54	0.33	2.06	0.61	0.29	3.79

scending order). BCF showed considerable variation between samples, in particular the BCF values for As, which were in the range of 1.44 to 23.8 (Table 5). The content of As in the fly ash samples varied depending on the location from which it was taken. The samples in proximity to the active cassette had higher amounts of As. From the calculated BCF it was concluded that *Conyza canadensis* presented an efficient uptake of As, Zn, Al, Cd, Cr and Fe from the fly ash (the mean values in μg/g were: 11.50, 6.82, 2.70, 2.21, 1.96 and 1.51, respectively).

The elements Zn, Mg, Ca, Cd, Cu and As had average translocation factors higher than 1. Very high values of TF factors were found for Ca, Mg and Zn, while those of Cu and As were slightly higher than 1 (Table 6). The values of TF indicated that this plant in its aboveground parts accumulated considerable amounts of Ca, Zn, Mg, Cd, Cu and As (the mean values in μg/g were 3.87, 3.60, 2.05, 2.16, 1.53 and 1.13 respectively). In general, and according to the calculated BCF and TF, this plant exhibited a tendency to retain Al, Fe and Cr in the root and to translocate Zn, Cd, Cu and As from the root to the shoot. These findings support our data obtained in a previous study [16], except for the As. We thus conclude that *Conyza canadensis* displayed a potential for uptake and translocation of As from fly ash with a high content of this element, as was the case with samples closer to the active cassette.

Authors' contributions: VV and NRRA performed fly ash and plant analysis. JT and DM-O performed the statistical and chemometric analyses. RK performed plant and fly ash sampling. MM prepared the plant samples and prepared the manuscript. JM supervised the study, analyzed the data and prepared the manuscript.

Conflict of interest disclosure: The authors declare no conflicts of interest.

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