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Water chestnut (*Trapa natans*): a food of ancient times or a bioindicator of the modern world

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Abstract

This study investigates the bioaccumulation of metals in Water chestnut seed samples collected from different locations of Lake Tisza, Hungary. Aquatic macrophytes can absorb and accumulate pollutants from water and sediments, making them useful indicators of environmental contamination. The aim of this research is to measure the concentrations of selected metals in the collected seeds. By analyzing the differences between sampling sites, the study also evaluates the distribution of metals within the lake ecosystem and the potential of aquatic plants as indicators of environmental pollution.

Keywords

Trapa natans, water chestnut, heavy metals, metals, bioaccumulation, Lake Tisza, ICP-MS.

Sulyom (*Trapa natans*): az ókor tápláléka vagy a modern világ bioindikátora

Kivonat

A tanulmány a nehézfémek bioakkumulációját vizsgálja a Tisza-tó különböző helyszíneiről gyűjtött sulyom (*Trapa natans*) magjainak mintáiban. A vízi makrofíták képesek abszorbeálni és felhalmozni a vízből és az üledékből származó szennyezőanyagokat, így hasznos indikátorai lehetnek a környezeti szennyezésnek. A kutatás célja a vizsgált fémek koncentrációjának mérése a gyűjtött sulyom magjában. A mintavételi helyek közötti különbségek elemzésével a tanulmány a nehézfémek eloszlását a tó ökoszisztémáján belül, valamint a vízinövények potenciálját a környezeti szennyezés indikátoraként is értékeli.

Kulcsszavak

Sulyom (*Trapa natans*), nehézfémek, fémek, bioakkumuláció, Tisza-tó, ICP-MS.

INTRODUCTION

Lake Tisza

Lake Tisza is the second-largest lake in Hungary and is located in the eastern part of the country along the Tisza River. The lake is an artificial reservoir created in 1973 after the construction of the Kisköre Dam and hydropower plant. Its primary purposes include water level regulation, flood control, irrigation support, and hydroelectric power generation. The lake covers an area of approximately 127 km². Lake Tisza consists of a highly diverse mosaic of habitats. In addition to open water surfaces, the area contains extensive reed beds, marshes, oxbow-like channels, and shallow wetlands, which provide suitable habitats for numerous plant and animal species. A large part of the lake belongs to the Hortobágy National Park, making it an internationally significant wetland area.

The lake is well known for its rich aquatic vegetation, including several macrophyte species such as water

chestnut (*Trapa natans*). These plants play an essential role in the functioning of the ecosystem, as they contribute to nutrient cycling, provide habitats for aquatic organisms, and support natural water purification processes. Besides its ecological importance, Lake Tisza is also a valuable research site. Studies focusing on water, sediment, and aquatic vegetation can provide important information about environmental conditions and the distribution of pollutants such as metals.

Trapa natans

Water chestnut (*Trapa natans*) is a floating-leaved aquatic plant widely distributed in freshwater habitats across Europe and Asia (Hummel and Kiviat 2004). In Hungary, it commonly occurs in slow-flowing rivers, lakes, oxbow lakes, and reservoirs. The plant forms a characteristic floating rosette of leaves on the water surface, while its roots are anchored in the sediment. In many water bodies, the species can grow very densely, forming large stands that may hinder water use and navi-

gation. For this reason, the plant is often mechanically removed or mowed, and the collected biomass is typically not utilized but treated as waste.

However, the seeds of *Trapa natans* have considerable nutritional value. The white seed inside the hard shell is rich in starch and is naturally gluten-free, which makes it suitable for producing flour. In several Asian countries, water chestnut flour has traditionally been used to prepare different foods such as bread, cakes, and noodles. Therefore, the plant could represent a potential raw material for producing gluten-free food products (Jain *et al.* 2025).

Bioaccumulation of Metals in Water Chestnut (*Trapa natans*)

Aquatic plants play an important role in indicating and reducing environmental pollution in aquatic ecosystems. Macrophytes are significant because they are capable of accumulating various metals in their tissues. Water chestnut (*Trapa natans*) is an aquatic plant that, according to numerous studies, can absorb and store considerable amounts of metal ions. Therefore, it may serve as a useful indicator of pollution in aquatic environments and can also be applied for phytoremediation purposes.

Metals such as cadmium, lead, copper, zinc, and manganese can enter aquatic ecosystems through both natural and anthropogenic sources. Agricultural activities, industrial discharges, and municipal wastewater significantly increase the concentration of these elements in water and sediments. Aquatic plants are able to absorb these elements through their root systems and tissues, where they may accumulate in different plant organs.

Several studies have investigated the metal accumulation capacity of *Trapa natans*. For example, an Indian study measured the concentrations of six heavy metals (Cd, Cr, Cu, Fe, Mn, and Zn) in pond water, sludge, and different parts of *Trapa natans*. The results showed that the highest concentrations of metals were found in the roots, followed by the shoots, while the lowest concentrations were detected in the edible seeds. The order of metal accumulation in the plant was reported as follows: Cd < Cr < Cu < Zn < Mn < Fe (Rai and Sinha 2001).

Another study examined the role of aquatic plants in the purification of contaminated water. During phytoremediation experiments using municipal wastewater, *Trapa natans* significantly reduced several pollution parameters of the water, including biochemical oxygen demand and dissolved salts, as well as the concentrations of several metals. The results indicated that cadmium, copper, iron, manganese, and zinc were mainly accumulated in the leaves, while chromium and lead were predominantly concentrated in the roots (Kumar and Chopra 2018).

The accumulation of metals in macrophytes is influenced by several factors, such as the chemical composition of the water, the properties of the sediment, and the level of pollution in the habitat. A study conducted in a wetland ecosystem demonstrated that the concentration of metals may also vary seasonally. The highest concentrations in water and sediment were observed during the

winter season, whereas the plants accumulated the largest amounts of metals during summer (Taher *et al.* 2023).

Water chestnut is therefore not only an ecologically important species but also plays a significant role in environmental research. The plant can accumulate different metals while often maintaining lower concentrations in its edible parts. This is particularly important in regions where the plant is consumed as food.

Based on the results of various studies, *Trapa natans* can be considered an effective indicator of metal pollution in aquatic environments (Elbagory *et al.* 2026). The examination of this plant provides valuable information about the condition of water and sediment, as well as the extent of environmental contamination. Consequently, the study of water chestnut may play an important role in the monitoring of aquatic ecosystems and environmental pollution.

Hypothesis

Our hypothesis is that in water bodies with higher environmental load or pollution, the concentration of metals in the seeds will be higher. This assumption is based on the idea that plants can absorb different metals from the water and the sediment, which may accumulate in their tissues, particularly in the seeds.

Objective

The aim of the research is to carry out an elemental analytical investigation of samples found in the different basins of Lake Tisza that have varying levels of environmental load. The study focuses on the Tiszavalk, Sarud, Poroszló, and Abádszalók basins, as these areas of the lake are characterized by different degrees of pollution and human impact. During the analysis, we aim to determine the concentration of various metals present in the samples, especially in the seeds. The results of this research may help determine whether the plant can be safely used for human consumption or whether it could rather play a role in phytoremediation, meaning the natural purification of polluted water bodies by plants.

MATERIAL AND METHODS

The sampling on which the study is based was carried out in August 2024 in different basins of Lake Tisza (Figure 1). The samples were collected by boat from areas where at least a 5 m³ continuous water chestnut (*Trapa natans*) population was present (Figure 2). Within each plant patch, samples were taken from three randomly selected points. In total, samples were collected from 12 sampling sites.

The collected seeds were first dried in the laboratory at 70 °C until constant weight was reached. After drying, the samples were ground into a fine powder. This grinding step is important because inclusions and different parts of the plant tissues may accumulate metals differently; therefore, homogenization ensures more accurate and reliable analytical results. The elemental analysis of the samples was performed using ICP-MS (Inductively Coupled Plasma Mass Spectrometry), which allows the detection and precise quantification of metals even at very low concentrations.

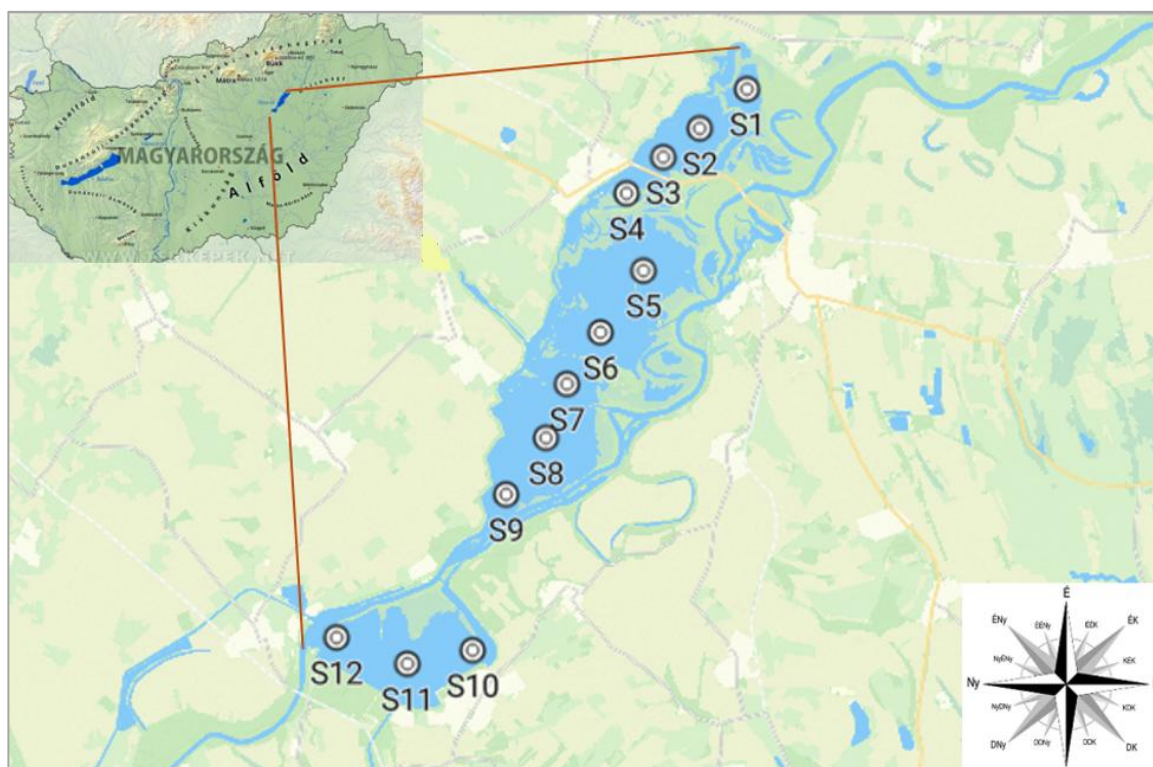


Figure 1. Sampling locations along Lake Tisza. S1-S12 represent the exact sampling points
 1. ábra: Mintavételi helyszínek a Tisza-tó mentén. Az S1–S12 jelöli a pontos mintavételi pontokat



Figure 2. *Trapa natans* fruit
 2. ábra. A *Trapa natans* termése

As part of the research, inorganic plant nutrient analysis from water samples is also carried out in order to determine the amount of dissolved nutrients in the water that are available for plant uptake.

In addition, the study aims to monitor abiotic and biotic changes in the environment, meaning both the physical–chemical environmental factors and the changes related to living organisms.

It is important to note that during the 2024 sampling, when the seeds were collected, sediment sampling was not carried out (3. Figure). However, in the following years of the research, sediment samples will also be collected. These samples will be taken using a core-type sampling method, and the collected sediment will be dried and ground before further analysis. For the sediment samples, the organic matter content and calcium carbonate content will be determined using the loss-on-ignition method. In this procedure, a measured amount of the sample is placed into a muffle furnace. In the first step, the sample is heated to 550 °C, during which volatile organic materials are removed. The resulting mass loss allows the determination of the organic matter content. In the second step, the sample is reheated to 950 °C, where carbonate compounds decompose. The additional mass loss observed at this stage is used to determine the calcium carbonate content.

RESULTS

Results of the one-way ANOVA analysis

The results indicate that there is a significant difference between the group means. Based on the results of

the one-way analysis of variance, the group effect was very strong: $F(30,341) = 291.7$, $p < 0.001$ indicating that the null hypothesis, that the group means are equal can be rejected. The effect size was also extremely large: ($\omega^2 = 0.959$), suggesting that most of the total variance is explained by differences between the groups (Table 1).



Figure 3. The seed of *Trapa natans* without the seed coat
3. ábra. A *Trapa natans* maghéj nélküli magja

Table 1. Test for equal means
1. táblázat. Az egyenlő átlagok vizsgálata

	Sum of sqrs	df	Mean square	F	p (sample)	Critical F (p=0,05)
Between groups	1.53072E06	30	51023.9	291.7	1,636E-223	1.143
Within groups	59645.5	341	174.914	Permutation p (n=99999)		
Total	1.59036E06	371	1,00E-05			

Since Levene's test yielded a significant result, the assumption of homogeneity of variances was violated; therefore, the results of the Welch test are also relevant. This procedure likewise showed a significant difference between the groups: $F(30, 117.8) = 827.4$, $p < 0.001$, confirming that the group means are not equal even when variances differ.

The Bayes factor is also extremely large, providing decisive evidence in favour of differences between the group means. Overall, the results indicate that there are statistically and practically significant differences among the examined groups.

The Kruskal–Wallis test also revealed a significant difference in the medians of the examined groups: $H(\chi^2) = 358.3$, $p < 0.001$. This suggests that at least one group's distribution differs from the others, and that the central tendencies of the groups are not identical.

Standardized Data Evaluation

The dataset was standardized prior to analysis, resulting in normalized values (z-scores) with a mean of zero

and a standard deviation of one. This transformation allows for direct comparison between elements with different original scales and highlights relative differences between samples.

Based on the standardized values, clear patterns can be observed across the samples. Positive values indicate above-average concentrations, while negative values represent below-average levels for a given element.

Sample TN_12 shows consistently high positive values across multiple elements, including Ni, Mn, Pb, Cr, Ba, Fe, and Al. This indicates that TN_12 is one of the most enriched samples in the dataset. Similarly, TN_6 also exhibits elevated values for several elements, particularly Ni, Mn, Cr, Ba, and Fe, suggesting a relatively high overall elemental concentration.

In contrast, sample TN_11 displays strongly negative values across nearly all elements, indicating consistently low concentrations compared to the dataset average. This makes TN_11 the most depleted sample.

Sample TN_7 also shows predominantly negative values, although less extreme than TN_11, suggesting generally lower-than-average concentrations.

Several samples, such as TN_5 and TN_9, show mixed behaviour, with both positive and negative deviations, indicating more balanced or moderate compositions.

The standardized dataset also reveals potential covariation patterns between elements. For instance, Ni, Mn, Cr, and Ba tend to increase together in samples such as TN_6 and TN_12, suggesting a possible common source or similar geochemical behaviour. Conversely, samples

with low values in these elements tend to show consistently negative deviations across multiple variables.

The Maucha diagram constructed from these standardized values allows for a clear visual comparison of the samples. Samples such as TN_12 and TN_6 would exhibit large, outward-extending shapes, reflecting high relative concentrations, while TN_11 would appear as a compressed shape near the centre, indicating low values across most elements.

Overall, the standardized data confirm strong heterogeneity among the samples and highlight distinct compositional groups, with enriched, depleted, and intermediate samples clearly distinguishable.

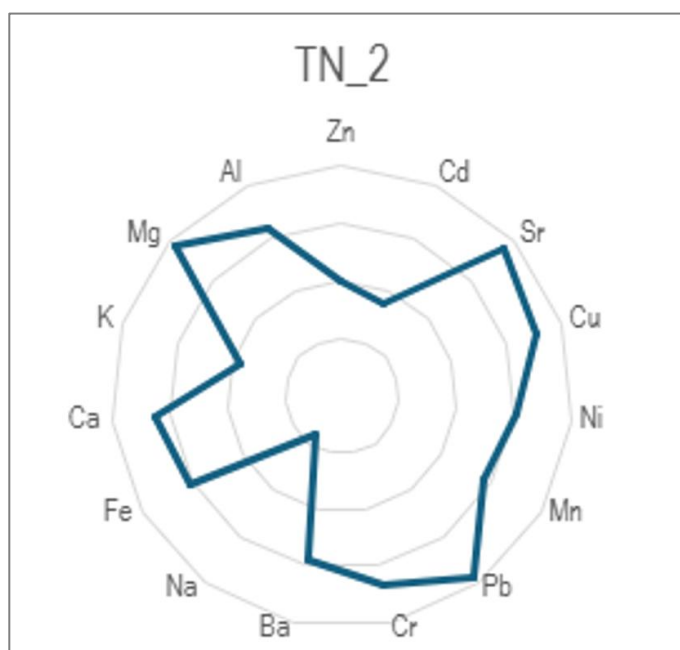


Figure 4. Maucha-diagramm of the samples-TN_2
4. ábra. A TN_2 minták Maucha diagramja

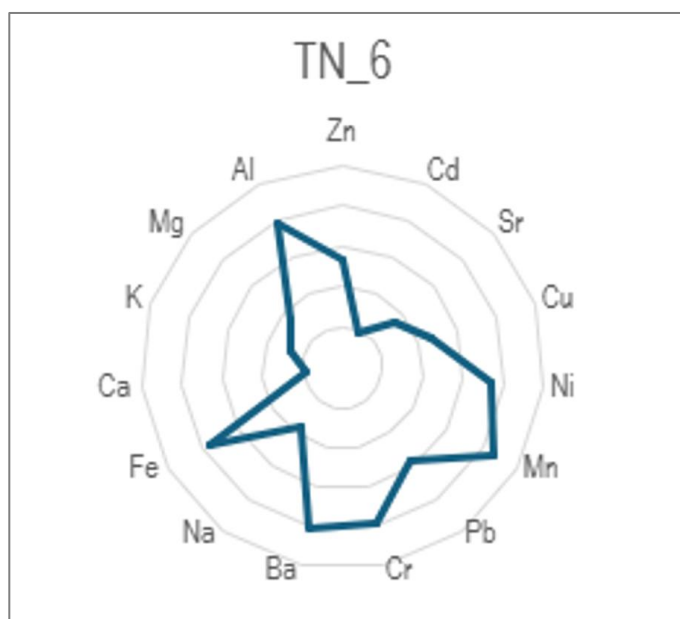


Figure 5. Maucha-diagramm of the samples-TN_6
5. ábra. A TN_6 minták Maucha diagramja

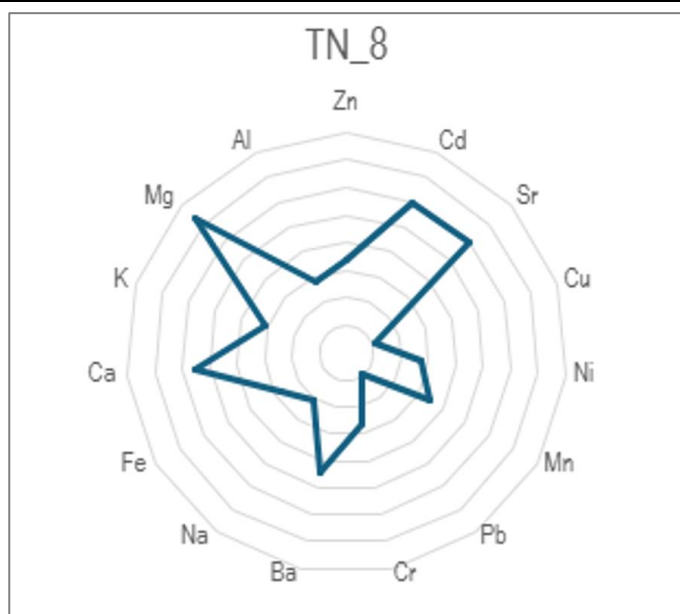


Figure 6. Maucha-diagramm of the samples-TN_8
6. ábra. A TN_8 minták Maucha diagramja

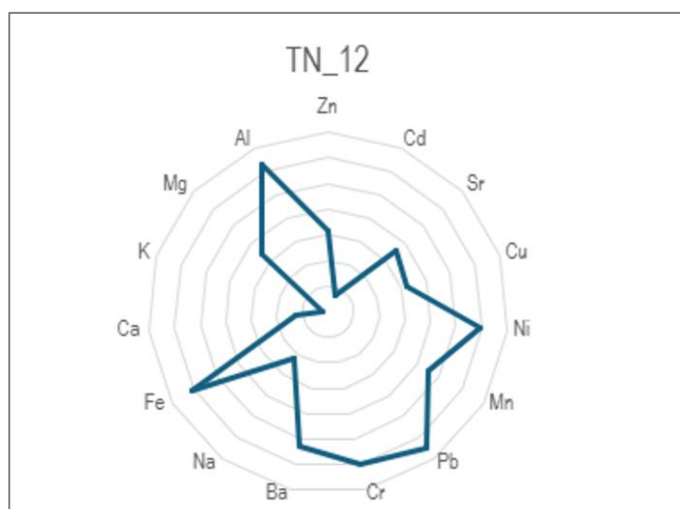


Figure 7. Maucha-diagramm of the samples-TN_12
7. ábra. A TN_11 minták Maucha diagramja

Metal and heavy metal content analysis

In this study, the elemental composition of 12 samples (TN_1–TN_12) was analysed based on the concentrations of 15 elements (Zn, Cd, Sr, Cu, Ni, Mn, Pb, Cr, Ba, Na, Fe, Ca, K, Mg, Al). The two provided tables contain the same data in different formats: one is element-oriented (elements in rows), while the other is sample-oriented (Figure 6, Figure 7).

Based on the data, substantial differences can be observed in the concentrations of the elements and their distribution across the samples. Some elements show relatively low variability; for example, cadmium (Cd) ranges between 0.006 and 0.011 across all samples, indicating minimal variation. In contrast, several elements exhibit considerable differences.

Sodium (Na) shows particularly high variability, with values ranging from 7.38 to 60.08. Sample TN_11 has an exceptionally high Na concentration (60.08), which clear-

ly stands out from the rest. Similarly, iron (Fe) displays a wide range (4.57–59.28), with TN_12 having the highest value and TN_7 the lowest.

Calcium (Ca) concentrations also vary significantly, ranging between 131.84 and 313.65. Sample TN_4 shows an outstandingly high Ca value (313.65), clearly distinguishing it from the other samples.

Manganese (Mn) exhibits moderate variability (0.772–2.375), while potassium (K), magnesium (Mg), and aluminum (Al) also show noticeable differences among the samples. Aluminum, in particular, spans a wide range (2.69–37.9), indicating substantial variation.

Among the heavy metals, lead (Pb) includes one negative value (TN_4: -0.011), which may indicate measurement uncertainty or an extremely low concentration. The remaining Pb values are positive and fall within a relatively narrow range.

Table 2. Measured concentration of metalloids and heavy metals from the seed of *Trapa natans*
2. táblázat. A *Trapa natans* magjában mért félfém- és nehézfém-koncentrációk

	TN 1	TN 2	TN 3	TN 4	TN 5	TN 6	TN 7	TN 8	TN 9	TN 10	TN 11	TN 12
	(mg/100 g seed dry matter)											
Zn	0.413	0.357	0.396	1.061	0.254	0.523	0.516	0.398	0.285	0.511	0.287	0.501
Cd	0.007	0.007	0.007	0.008	0.006	0.009	0.01	0.011	0.008	0.01	0.007	0.008
Sr	0.393	0.344	0.431	0.615	0.352	0.6	0.447	0.448	0.299	0.417	0.384	0.425
Cu	0.172	0.112	0.169	0.207	0.153	0.172	0.203	0.188	0.123	0.622	0.133	0.177
Ni	0.082	0.03	0.05	0.049	0.029	0.062	0.029	0.066	0.055	0.079	0.056	0.111
Mn	2.176	1.061	1.499	1.352	0.772	1.797	0.772	1.486	1.338	1.737	1.713	2.375
Pb	0.024	0.011	0.02	-0.011	0.02	0.015	0.01	0.022	0.027	0.028	0.027	0.041
Cr	0.049	0.012	0.031	0.025	0.014	0.038	0.006	0.037	0.031	0.045	0.026	0.078
Ba	0.279	0.172	0.203	0.228	0.132	0.251	0.16	0.253	0.188	0.267	0.205	0.333
Na	9.28	7.38	16.34	13.63	12.72	13.98	13.08	10.82	12.41	15.11	60.08	25.43
Fe	40.36	9.71	21.22	19.99	12.5	30.01	4.57	27.23	27.51	34.37	20.64	5.28
Ca	180.99	136.06	191.72	313.65	152.64	260.1	213.1	212.09	131.84	201.29	144.27	169.84
K	29.99	17.24	37.99	35.99	32.66	35.27	40.47	33.37	29.56	36.04	25.74	24.99
Mg	45.61	38.37	41.61	48.06	27.37	57.61	47.55	45.1	34.45	44.73	34.19	46.01
Al	25.71	5.69	13.38	12.54	7.42	19.82	2.69	17.71	15.7	22.67	11.88	37.9

By comparing the samples, it can be observed that certain samples consistently show higher concentrations across multiple elements. For instance, TN_4, TN_6, TN_10, and TN_12 belong to the higher-value group for several components (Figure 5). In contrast, TN_2, TN_5, and TN_7 tend to have lower concentrations across multiple elements (Figure 4).

Based on the observed patterns, potential covariations between elements can be identified. Calcium (Ca), magnesium (Mg), and potassium (K) often follow similar trends across samples, while iron (Fe) and aluminum (Al) also tend to be high or low together. In contrast, sodium (Na) often behaves differently from the other elements (Table 2).

Overall, the tables indicate that the elemental composition of the samples varies considerably, with concentrations spanning wide ranges. This suggests a heterogeneous system in which clear differences can be observed between the samples.

Results of the Principal component analysis (PCA)

Figure 8 presents a PCA biplot, which simultaneously displays the sampling sites and the measured variables. The arrows represent the variables, and their direction and length indicate their contribution to the principal components. Several elements, such as Fe, Mn, Cr, and Ni, point in similar directions, suggesting a common pattern or co-variation, while others (e.g., Na) show different orientations, indicating distinct behaviour. The sampling sites are clearly separated along the components, with TN_12 and TN_6 positioned on the positive side, indicating higher relative concentrations, whereas TN_11 is located on the opposite side, reflecting lower overall values.

Results of the non-metrical multidimensional scaling (NMDS)

The Figure 9 shows an ordination plot illustrating the relative similarity between the sampling sites. The distances between points represent differences in their multivariate composition: sites that are closer together are more similar, while those farther apart are more distinct. For example, TN_2, TN_5, and TN_9 appear relatively close to each other, suggesting similar characteristics, whereas TN_11 is clearly separated from the others, indicating a distinct composition. This visualization supports the presence of heterogeneity among the sampling sites.

DISCUSSION

The results of the present study are partly consistent with previous findings reported in the literature, while also revealing several notable differences.

According to earlier studies, *Trapa natans* is capable of accumulating significant amounts of metals, with the highest concentrations typically found in the roots, followed by the shoots, and the lowest levels detected in the edible parts of the plant (Rai and Sinha 2001). In the current research, only the seeds were analysed, and the results showed relatively low concentrations of heavy metals with limited variability, particularly in the case of cadmium (Cd: 0.006-0.011 mg/100 g seed dry matter). This observation supports the literature, suggesting that the edible parts of the plant tend to accumulate lower levels of heavy metals.

However, in contrast to some previous findings, the present study did not reveal consistently high accumulation levels across all examined elements in the seeds. This difference may be explained by the fact that heavy metals are primarily stored in other plant organs, such as roots and leaves, rather than in the seeds.

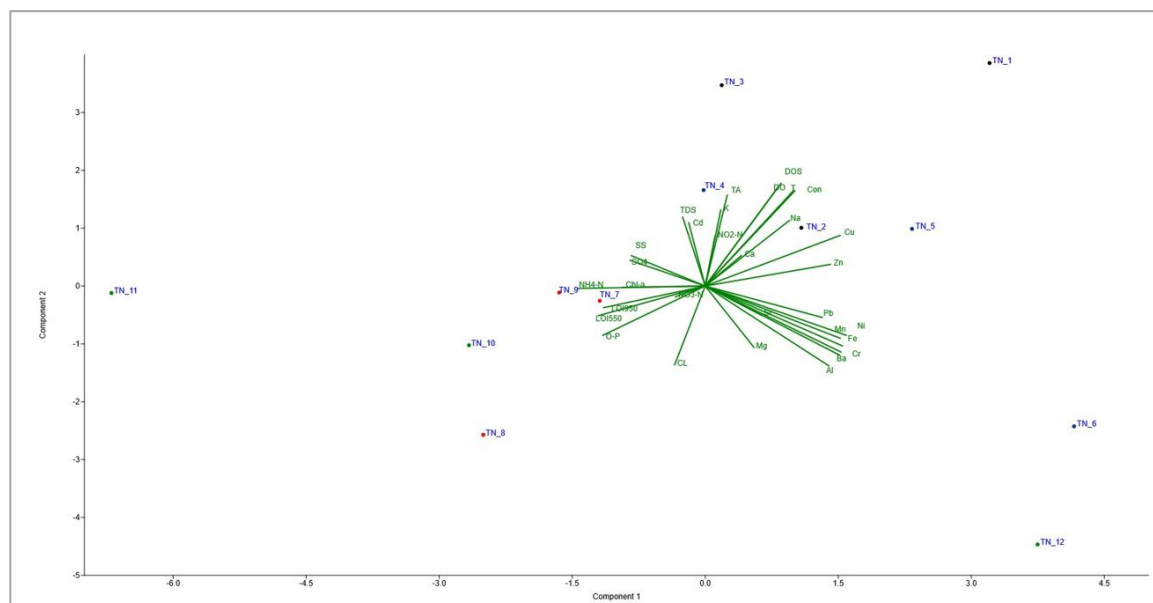


Figure 8. PCA biplot of the sampling sites and the measured variables
8. ábra. A mintavételi helyszínek és a mért változók PCA-biplotja

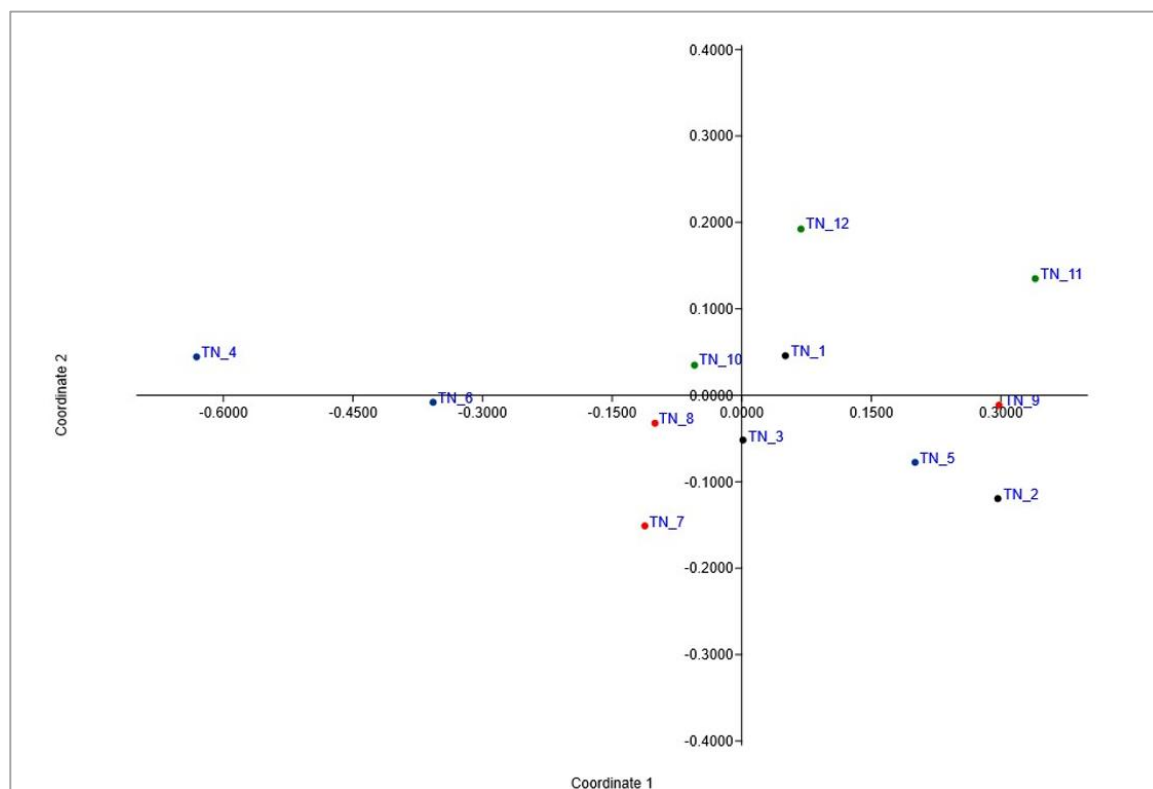


Figure 9. Similarity between sampling sites on an NMDS
9. ábra. A mintavételi helyszínek hasonlósága NMDS elemzés alapján

The results are also in agreement with the findings of *Kumar and Chopra (2018)*, who reported that certain elements (e.g., Cd, Cu, Fe, Mn, and Zn) are mainly accumulated in the leaves, while others (e.g., Cr and Pb) are predominantly concentrated in the roots. The relatively moderate concentrations measured in the seeds in this study indirectly support this distribution pattern within the plant.

Seasonal variation, as described by *Taher et al. (2023)*, may also play an important role in explaining the

observed results. Considerable differences were observed among the samples, indicating that local environmental conditions—such as water chemistry, sediment characteristics, and pollution sources—have a strong influence on metal accumulation.

One of the most important findings of this study is the pronounced heterogeneity among the samples. Statistical analyses, including one-way ANOVA and the Kruskal–Wallis test, confirmed highly significant differences between the sampling sites ($p < 0.001$). This result is con-

sistent with previous studies, which emphasize that the distribution of heavy metals in aquatic ecosystems is strongly influenced by spatial variability and environmental factors.

Certain samples (e.g., TN_12 and TN_6) exhibited elevated concentrations across multiple elements, while others (e.g., TN_11) showed consistently lower values. This pattern suggests that some areas of the lake may be subject to a higher environmental load, partially supporting the initial hypothesis.

However, the hypothesis was only partially confirmed. Although some samples showed higher metal concentrations, a clear and consistent relationship between the presumed pollution level and the metal content of the seeds could not be established. This indicates that metal accumulation is a complex process influenced not only by external environmental conditions but also by internal plant physiological mechanisms, including element transport and storage.

Overall, the findings of this study confirm that *Trapa natans* can serve as a useful bioindicator of heavy metal pollution in aquatic ecosystems. At the same time, the results highlight that analyzing only the seeds provides limited insight into the full extent of metal accumulation. Therefore, future studies should include the examination of roots, leaves, and sediments in order to obtain a more comprehensive understanding of the accumulation processes.

CONCLUSION

The present study aimed to investigate in detail the elemental composition and metal accumulation patterns of *Trapa natans* seeds collected from multiple basins of Lake Tisza. The analysis revealed a considerable degree of variability among the examined samples, clearly indicating that the distribution of elements within the lake ecosystem is not uniform but rather spatially heterogeneous. This heterogeneity suggests that localized environmental factors play a crucial role in shaping the chemical profile of aquatic plants.

The statistical evaluation of the data further supported these observations, as significant differences were detected between the various sampling sites. These differences were found to be highly significant, emphasizing that site-specific environmental conditions – such as water chemistry, sediment composition, and potential pollution sources – strongly influence the accumulation of elements in *Trapa natans*. Among the samples, certain ones (notably TN_12 and TN_6) consistently exhibited elevated concentrations across multiple elements, while others, such as TN_11, showed distinctly lower values. This pattern highlights the uneven exposure of different lake regions to environmental stressors.

The results partially confirm the initial hypothesis of the study. In some cases, samples originating from areas presumed to have higher environmental loads or pollution levels did indeed show increased concentrations of metals. However, this relationship was not consistent across all samples, indicating that the accumulation of metals in

Trapa natans seeds cannot be explained solely by external contamination levels. Instead, the uptake, translocation, and storage of these elements appear to be governed by a complex interplay of environmental conditions and intrinsic plant physiological mechanisms, including selective absorption and detoxification processes.

Consistent with findings reported in previous studies, the current results suggest that the edible parts of the plant (specifically the seeds) generally contain lower concentrations of metals compared to other plant organs, such as roots or leaves. This observation is particularly important from a food safety perspective, as it indicates a potentially lower risk associated with the consumption of *Trapa natans* seeds. Nevertheless, despite these encouraging findings, further comprehensive investigations are required to fully evaluate possible health risks, especially under conditions of prolonged exposure or in more heavily contaminated environments.

Overall, *Trapa natans* demonstrated considerable potential as a bioindicator species for assessing environmental conditions in aquatic ecosystems. Its ability to reflect variations in elemental composition makes it a valuable tool for monitoring pollution. At the same time, the study also highlights an important limitation: the analysis of seeds alone provides only a partial understanding of metal accumulation processes. For a more complete and accurate assessment, future research should incorporate the examination of additional components, including roots, leaves, surrounding water, and sediment samples. Such an integrated approach would enable a more comprehensive evaluation of pollutant dynamics and pathways within the ecosystem.

In conclusion, the findings of this study contribute to a deeper understanding of the role of aquatic macrophytes in environmental monitoring. They also emphasize the importance of adopting multidisciplinary and integrative methodologies when assessing ecosystem health and pollution levels, thereby supporting more effective environmental management and conservation strategies.

In the next phase of the research, the investigations will also include water and sediment pollution of Lake Tisza.

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