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An assessment of metals content in *Phragmites australis* (cav.) Trin. Ex steudel grown in natural water reservoirs according to climate zone and salinity

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ABSTRACT

Studies on the abundance of trace metals in submerged reed were performed in various aquatic ecosystems of Greece and Poland in the period from 2005 to 2006. The analyzed metals were Cd, Pb, Zn, Cr, Ni, Cu, Co, Fe, Mn, K, Na, Mg, and Ca. The relation between the concentrations of metals and water characteristics (salinity, temperature, etc.) was explored by the use of Principal Component Analysis. The results showed that the temperature facilitates accumulation of Cd, Pb, and Co. Common reed grown in salty reservoirs contained more Na and Mg, which are present in water in the form of NaCl and $MgCl_2$. Water temperature plays no important role in accumulation of Ni and Cr in reed. Differences in the content of Cr and Ni in underwater parts of reed were caused by individual characteristics of the sampling point (i.e. intensity of recreation) and increased uptake in growing season. An independence of Cr and Ni concentration in reed on integrated water salinity was confirmed. Pools of Fe and Mn in *Phragmites australis* were similar in both countries, but reed collected in several locations in Vistula Lagoon allocated more Fe and Mn. Large accumulation of Cu in stems of reed was discovered in highly anthropopressed melioration ditches.

Keywords: *Phragmites australis*; Underwater parts; Heavy metals; Integrated salinity; Poland; Greece

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1. Introduction

Phragmites australis (Cav.) Trin. Ex Steudel is one of the most widely distributed species in aquatic and land ecosystems. Its natural distribution range is difficult to define since it easily adopts and exists almost everywhere around the world. It occurs in Europe, Asia, Africa, both American continents and even in Australia. The one exception is the Amazon basin [1]. It also commonly occurs in Poland, from the Baltic Sea to lower mountains and in mediterranean climate zones as Greece.

P. australis (Cav.) Trin. Ex Steudel, known as common reed, is a plant typically occurring in marsh or aquatic ecosystems, but due to its ecological tolerance, it can grow also on dry land. It tolerates wavy motions and permanent drawn, even up to 2 m. It grows in slowly flowing waters as well as in stagnating waters of various base. This species being extraordinary expansive creates mono phytocoenosis. Despite that the environmental salt gradient diversifies plant communities in spatial scales [2,3], it can withstand extreme environmental conditions. Common reed tolerates moderate salinity [3,4] and survives in ecosystems contaminated with heavy metals, such as Cr, Ni, Zn, Pb, and Cd [5]; however, it is still questionable whether the distribution of *P. australis* is related to its innate metal tolerance when it grows in contaminated ecosystems.

With respect to *P. australis*, the majority of investigations studied single or at least several metals accumulation by plants grown in glasshouse [6] or hydroponic conditions [7,8] or constructed wetlands [9,10]. In several studies, the authors analyzed metal concentration in a root system [5,8,11–14], rhizome [13,14], leaves [5,14], and above water shoots [8,11,13], while there are only a few studies devoted to underwater part of reed stem [15]. To our best knowledge, there is only one study addressed to the effect of climate and salinity on growth, nitrogen, and ion relations of *P. australis* [16]. However, the study was conducted in controlled, experimental plots. Therefore, the aim of presented investigation was to assess an abundance of metals in *P. australis* according to salinity of natural water reservoirs of various anthropopressure located in two different climatic zones, mediterranean and moderate ones.

2. Materials and methods

2.1. Sampling area

Submerged shoots of reed *P. australis* (Cav.) Trin. Ex Steudel were collected in 2005 and 2006 in selected water reservoirs of mediterranean (37–42˚N, Greece = Greek samples) and moderate (49–54.5˚N, Poland = Polish samples) climate zones which differed according to salinity. In Greece, sampling sites were located in the prefecture of Aetolia-Acarnania and in the area of Athens. The following aquatic ecosystems were investigated: Trichonis ($n = 2$) and Lyssimachia freshwater lakes ($n = 3$), saline Messolonghi Lagoon ($n = 3$), Kastrakiou ($n = 5$) and Marathon ($n = 1$) dam reservoirs, drainage canals in Athens ($n = 4$), the Evinos River ($n = 1$), and the Porto Rafti Bay ($n = 1$). Detailed characteristics of the studied aquatic ecosystems in Greece had been presented before [17]. In Poland, sampling sites were located in the Pomeranian Region. Samples were taken from Gardno Lake ($n = 22$), Żarnowieckie Lake ($n = 2$), Puck Bay ($n = 7$), and Vistula Lagoon ($n = 49$). Both Gardno Lake and Żarnowieckie Lake are separated from the sea with a belt of land. It extends for around 1 km and 4 km in the case of Gardno and Żarnowieckie Lakes, respectively, and this is why Gardno Lake could be classified as a brackish water lake, while Żarnowieckie Lake as freshwater lake. About 100 reed samples in total were collected at eight different locations in Greece and four different zones in Poland (Fig. 1). In all water reservoirs, no algae blooms were observed during sampling.

2.2. Samples preparation and analysis

The reed was transported to the laboratory, washed by the use of tap water in order to remove sediments and periphyton, and then it was rinsed with distilled water. The representativeness of the analytical sample of reed was achieved by joining at least 10 individual plant samples [18]. Shoots of reed were dried in 80˚C [19,20] and homogenized. Consecutively, an analytical sample of 1.000 g was weighted into 100 Pyrex beakers [21]. A mixture of H_2O_2 and HNO_3 was applied for mineralization under high temperature and pressure [9] by the use of microwave device MARS-5 (CEM Corp., USA). According to [1], microwave destruction, using a combination of HNO_3 and H_2O_2, is the most suitable method of determining heavy metals in *Phragmites*. Mass contents of Cd, Pb, Zn, Cr, Ni, Cu, Co, Fe, Mn, K, Na, Mg, and Ca in reed samples were determined by the use of flame adsorption spectrometer Varian 100A (Varian, Australia). The quality of analytical results was controlled by means of analysis of Certified Reference materials INCT-MPH-2 (mixed polish herbs) and INCT-TL-1 (tea leaves). Fresh herbs used to create INCT-MPH-2 were collected in a clean rural area, mixed, and processed for production of medicines, while tea leaves collected and processed in Argentina were used during

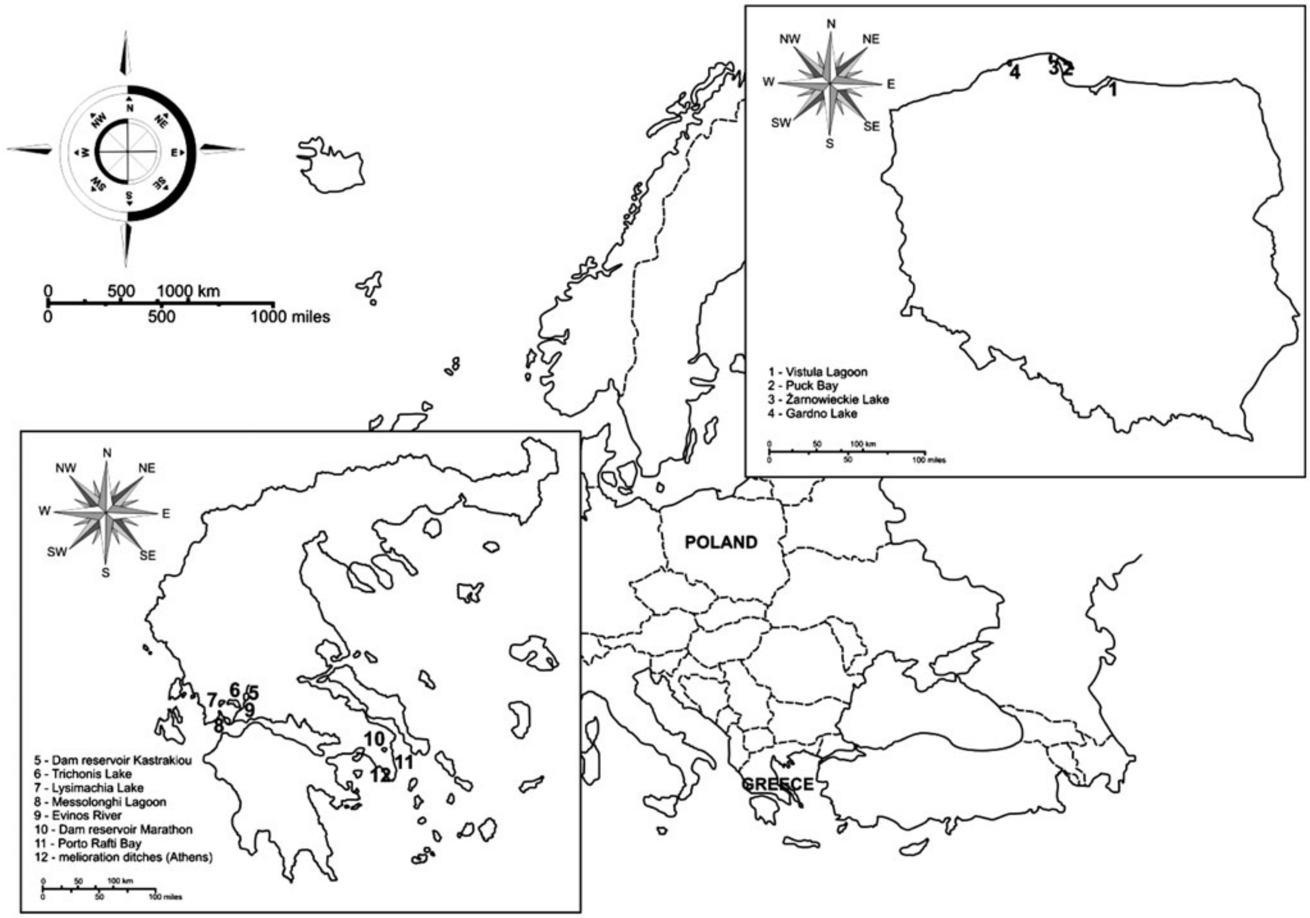

Fig. 1. Location of four sampling sites in Poland and eight sampling sites in Greece.

INCT-TL-1 production. Results obtained from the analysis of the certified reference mixture of herbs were as follows: Cd (199 ± 11 ng kg^{-1}), Co (210 ± 18 ng kg^{-1}), Zn (33.5 ± 1.8 mg kg^{-1}), Ni (1.57 ± 0.13 mg kg^{-1}), Cu (7.77 ± 0.45 mg kg^{-1}) and Mn (191 ± 10 mg kg^{-1}), while tea leaves as follows: Cd (30.2 ± 2.0 ng kg^{-1}), Co (387 ± 35 ng kg^{-1}), Zn (34.7 ± 1.9 mg kg^{-1}), Ni (6.12 ± 0.45 mg kg^{-1}) and Cu (20.4 ± 1.0 mg kg^{-1}). Calculated error did not exceed 5% of certified values.

Water temperature and salinity were analyzed on site by the use of Elmetron CC-401 (Elmetron, Poland).

2.3. Data analysis method

Differences in concentration of metals in submerged reed samples collected in water reservoirs in two different climatic zones were analyzed by nonparametric U Mann–Whitney test, while Principal Component Analysis (PCA) was used for overall exploration of the analytical data [22,23]. It enables the reduction of the dimensionality of the space of the variables in the direction of the highest variance of the system. New variables, called principal components being linear combinations of the previous variables, replace the old coordinates of the factor space. PCA results are indicated by two sets—factor scores giving the new coordinates of the factor space with the location of the objects and factor loadings which provide necessary data concerning the relationship between the variables. In the presented case study, factor loadings higher than 0.5 were taken into consideration.

Usually, the first principal component explains the maximal part of the system variation and each additional PC has a respective contribution to the variance explanation, however, less significant one when compared to the previous one. A reliable model in environmental studies usually requires such a number of PCs, so that over 70–75% of the total variation can be explained. Moreover, in the case of presented exploration, varimax-rotated PCA (as indubitably the most popular rotation method by far [24]) solution was interpreted. Rotation strategy simplifies the structure of factors and therefore makes its interpretation easier and more reliable since it strengthens the role of the latent factors with a higher impact on the variation explanation and diminishes the role of PCs with a lower impact [25,26]. The sequence of rotated factors might not be longer arranged in an order of decreasing percentage of variance explained, although the total variance explained is equal before and after rotation. All calculations were performed by the use of the software package STATISTICA 10.0 (Statsoft Inc., USA).

3. Results and discussion

In order to calculate basic statistics, an analytical data-set was divided for subsets concerning shoots of reed collected in Greece and Poland, respectively. The assessment of the differences in concentrations of metals in the sampled reed as water characteristics are summarized in Table 1.

The mean concentration of Cd, Cu, Co, K, and Ca in reed samples collected in Greece was significantly higher than in the case of Polish samples. In general, salinity of salty water reservoirs located in Poland is higher than in Greece, while in Greece, water temperature is higher. It was found that metal concentration in submerged common reed fell in the order: Ca > Na > Mg > K > Fe > Mn > Zn > Pb > Cr > Ni > Co > Cu > Cd for Polish samples, while the only change in the order for Zn and Pb was found in Greek samples. The order of macroions (Ca, Mg, K and Na) in Polish and Greek reed was similar to those collected in Balaton Lake [15]. Higher concentration of Fe compared to Co was reported by [14]. To compare metal abundance in shoots of common reed collected in this study and described in other references, respective data are summarized in Table 2.

PCA analysis gives the opportunity for specific interpretation of the data-set structure. Thus, the next step in the data exploration was performing PCA on normalized input data. The number of components for interpretation was determined according to eigenvalue ≥1 criterion (Kaiser criterion). Consecutively, the first five eigenvalues, which explained about 72% of the total variances, were selected and the sorted rotated factors are extracted thereafter (Table 3).

Such a distribution of the total variance means that it is possible to compress the information provided in the data-set onto the first five principal components without losing any substantial information. Factor 1 is highly correlated with temperature, Cd, Pb, Co, and Ca. It demonstrated that temperature facilitates accumulation of heavy metals in submerged reed. Factor 2 has a strong positive loadings on Salinity, Na, and Mg which represent the integrated salt characteristic of reed. Factor 3 has a strong negative loadings on Cr and Ni. Both Cd, Pb, Co, Ni and Cr belong to the group of heavy metals, but in the case of Ni and Cr, water temperature is irrelevant as a factor facilitating their accumulation in reed. Factor 4 positively correlates with Fe and Mn, while Factor 5 only with Cu.

The relation between submerged shoots of reed and water reservoirs according to their salinity and

Table 1
Differences in concentration of metals (mg kg^{-1}) in *P. australis* as well as water salinity (g L^{-1}) and temperature (℃)

	Poland ($n = 80$)			Greece ($n = 20$)			U Mann–Whitney test
	Mean	Min	Max	Mean	Min	Max	p level
Salinity	3.0	0.2	6.9	2.1	0.1	18.2	0.000003
Temperature	14.9	4.5	25.9	23.4	15.2	32.0	<0.005
Cd	1.4	0.6	2.7	1.8	1.4	2.0	0.000524
Pb	16.7	7.5	31.5	19.9	17.0	26.0	0.050244
Zn	16.9	7.3	77.4	18.5	8.6	44.3	0.657192
Cr	8.7	1.2	16.3	8.3	3.1	11.6	0.776099
Ni	4.4	2.0	8.4	4.5	2.9	6.5	0.520742
Cu	1.7	0.5	3.7	3.1	0.9	11.1	0.000012
Co	2.7	1.1	4.9	3.7	2.5	4.5	0.000241
Fe	235.0	76.9	1475.1	171.0	78.5	340.4	0.733567
Mn	66.5	13.4	198.5	57.1	20.1	130.3	0.590175
K	1595.7	133.0	7777.0	2272.7	513.0	5655.5	0.001541
Na	3826.0	111.5	9437.0	3543.0	499.5	12547.5	0.016593
Ca	6211.5	2050.0	21005.0	8007.3	5465.0	12280.0	0.000281
Mg	1634.9	89.0	7947.0	2605.3	394.0	14149.0	0.951900

Table 2
Metals concentration (mg kg^{-1}) determined in shoots of common reed distributed in wetlands and rivers

Location	Czech Republic[a]	India[a]	Italy[b]	Poland[b]	Poland[b]	Serbia[b]
References	[9]	[27]	[28]	[29]	[30]	[31]
Cd	0.013–0.15	0.34–1,500	0.63–1.13	0.3–5.3	0.7–4.9	0.56–9.17
Pb	0.13–7.4	10–1,800	9.8–16.54	0.6–30	1.0–28.1	–
Zn	11.6–61.3	10–1,400	10.04–104.10	49–446	5.6–463.6	–
Cr	0.18–14.7	1.7–1,758	0.4–6.97	1.1–44	1.2–76.9	–
Ni	0.91–14.4	0.9–1,758	0.48–9.12	2.3–38	1.1–167.3	–
Cu	10.1–33.0	5.2–560	2.31–14.98	4.4–97	2.2–8.2	6.61–68.28
Co	–	–	–	0.1–28.5	0.3–19	–
Fe	–	10–1,651	–	1,262–17,845	–	793–10,822
Mn	–	18–1,014	–	442–7,624	–	95–4,997
K	–	–	–	1,172–43,887	–	–
Na	–	–	–	1834–13,319	–	–
Ca	–	–	–	1,639–3,625	–	–
Mg	–	–	–	314–2,998	–	–

[a]Wetlands.
[b]Rivers.

Table 3
Varimax-rotated factor loadings

	PC1 Temp-Cd–Pb–Co–Ca	PC2 Salinity-Na–Mg	PC3 Cr–Ni	PC4 Fe–Mn	PC5 Cu
Salinity	−0.19	**0.82**	0.07	−0.12	−0.08
Temperature	**0.50**	0.15	0.12	−0.06	0.41
Cd	**0.94**	−0.10	0.11	−0.02	0.00
Pb	**0.86**	−0.09	0.02	−0.01	−0.03
Zn	0.41	−0.34	0.40	0.22	0.37
Cr	0.06	−0.20	**−0.88**	−0.03	0.06
Ni	−0.41	0.22	**−0.83**	0.04	0.05
Cu	0.04	0.08	−0.10	−0.02	**0.91**
Co	**0.91**	−0.11	0.16	0.01	0.08
Fe	−0.21	0.19	−0.36	**0.62**	0.27
Mn	0.10	−0.23	0.13	**0.83**	−0.13
K	0.37	0.33	0.09	−0.36	−0.04
Na	−0.19	**0.83**	0.01	−0.14	0.21
Ca	**0.55**	0.17	−0.02	0.33	0.25
Mg	0.07	**0.85**	−0.10	0.08	0.03
Eigenvalue	3.63	2.56	1.85	1.41	1.36
Explained variance	24%	17%	12%	9%	9%

Extraction method: PCA; rotation method: raw Varimax; bold values represent loadings higher than 0.5.

water temperature, and hence climate characteristics can be identified by the representation of the factor scored vs. monitoring sites. High factor scores correspond to the high impact of the given factor on a sampling site. The plot of sample scores of the PC1 vs. PC2 visualized according to country (A) and the water characteristics in a given water reservoir (B) are presented in Fig. 2.

It could be easily observed that, except for four samples (Messolonghi Lagoon and Porto Rafti Bay) located in the top of score plot, Greek samples create a homogeneous group, while Polish ones are spread. The location of samples along PC1 axis proves that common reed samples collected in Polish Vistula Lagoon are not as rich in the Cd, Pb, Co, and Ca compared to the ones collected in other water reservoirs located both on Polish and Greek territory. Reed samples collected from Gardno and Żarnowieckie lakes as well as Puck Bay accumulate Cd, Pb, Co, and Ca similarly as the samples collected in Greece. Comparison

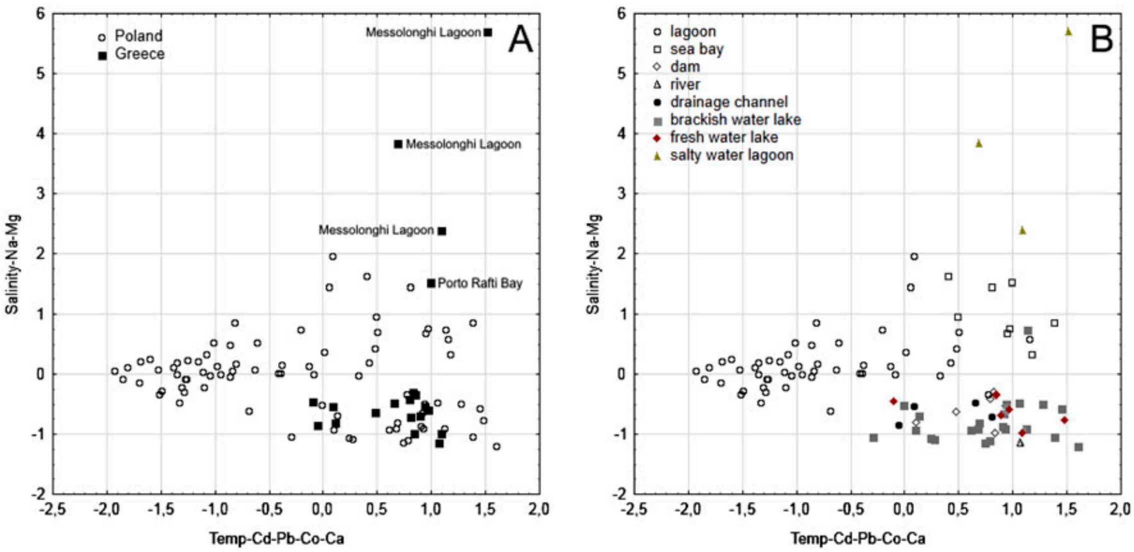

Fig. 2. PC1 (Temp-Cd–Pb–Co–Ca) and PC2 (Salinity-Na–Mg) scores according to geographic location (A) and the type of water reservoirs (B).

of mean water temperature of water reservoirs (Vistula Lagoon—13.7˚C; Gardno Lake—16.3˚C; Żarnowieckie Lake—15.9˚C, Greek reservoirs—23.4˚C) demonstrates clearly that reed's ability for heavy metal accumulation increases with water temperature. Although that phenomenon could be interpreted as the effect of climate zone on heavy metals accumulation by the *P. australis*, in this specific case, the impact of climate zone is not evident. Gardno and Żarnowieckie lakes being relatively small (24.8 km^2 [32] and 14.3 km^2, respectively) as well as Puck Bay which is shielded by the Hel Peninsula from the open Baltic Sea are able to become heated and hence similar to Mediterranean water reservoirs, which are generally warmer.

PC2 clearly distinguishes between reed collected in salty and brackish or sweet water reservoirs. The location of samples along PC2 axis demonstrated that reeds collected in Messolonghi Lagoon and Porto Rafti Bay in Greece as well as Puck Bay in Poland (all salty water reservoirs) are characterized by factor scores above 0.5. *P. australis* distributed in aquatic ecosystems impacted by salty water easily accumulates salt. However, as presented before [16], higher salt tolerance is observed in reed population growth in a moderate climate. Additionally, the decreasing depth of the water reservoir facilities salt accumulation, as observed for Messolonghi Lagoon of depth range between 0.45 and 1.65 m [17]. Salt accumulation ability of reed grown in shallow waters can be linked with their morphological differences comparing to reed grown in deeper waters as the number of internodes, the length of the lower internodes, and leaf sheaths [33]. The spread of factor scores (from −0.6 to 0.9) for common reed samples collected in Vistula Lagoon along PC2 indicated the effect of location of the sampling place on integrated salt characteristic of reed. Vistula Lagoon is isolated from the Puck Bay by the thin belt of land (around 1 km) with Krynica, Piaski, and Przebrno where *P. australis* samples were collected. However, reed stands in those locations are temporarily flooded by sea water or exposed to different flooding duration, especially during autumn storms. Similar flood-dependent salinity tolerance of *P. australis* was discussed before [3]. It enables diversified integrated salt characteristic of reed due to impact of salty, brackish, or freshwater in those locations. All reeds collected in freshwater lakes, rivers, melioration channels as well as dam reservoirs were characterized by a factor score values around −1 which is as expected. It could be concluded that common reed accumulates Na and Mg proportionally to their natural abundance in water. Moreover, it seems that salinity does not facilitate Cd, Pb, Co, and Ca accumulation in common reed since samples collected in fresh and salty water reservoirs were distributed along PC1 with positive factor scores.

Interesting pattern was revealed when PC1 and PC3 scores were displayed according to the country (A) and the water characteristics in a given water reservoir (B) (Fig. 3).

The location of samples along Cr–Ni axis demonstrated that Polish and Greek samples do not create separate groups but overlap. The findings suggested that Cr and Ni abundance in reed cannot be directly

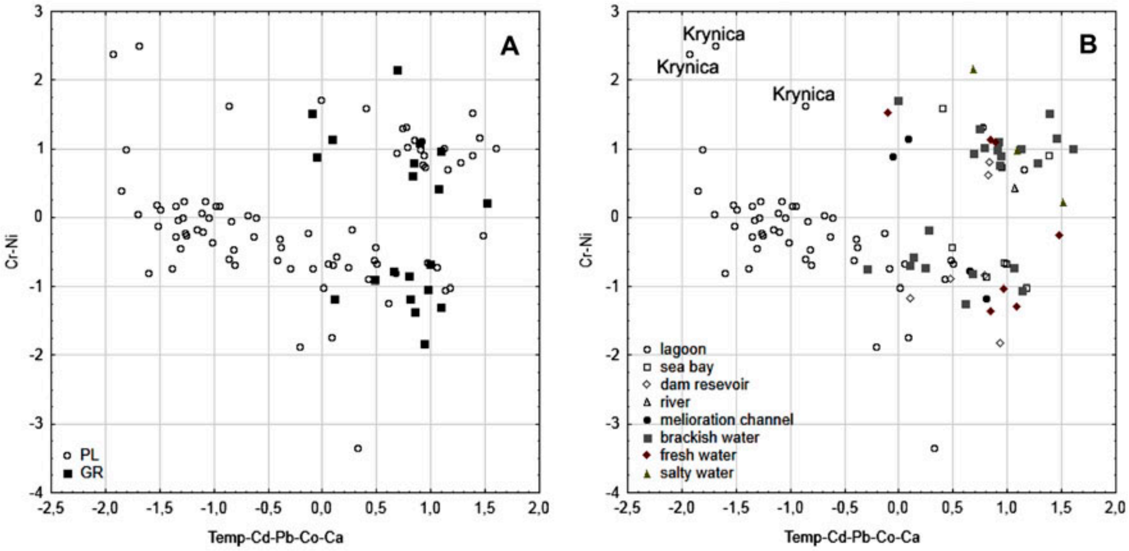

Fig. 3. PC1 (Temp-Cd–Pb–Co–Ca) and PC3 (Cr–Ni) scores according to geographic location (A) and the type of water reservoirs (B).

linked with the difference in the climate zone and is dependent rather on individual characteristic of the sampling point. In general, samples collected in freshwater Vistula Lagoon in Poland created homogeneous group of points characterized by a factor score values close or lower than 0. As mentioned above, the lower factor score value the lower PC's impact, and hence, it could be concluded that these samples are less contaminated with Ni and Cr than samples settled in the upper right quarter of the score plot. The only exception was three samples collected in Krynica. However, in that case, unexpected value of the factor score could be linked with an accidental sample contamination, since each of them was collected in different seasons: spring, summer, and autumn. In spite of occurrence of two well-separated clouds of points on a PC3 vs. PC1 score plot, the clue suggesting that Cr and Ni concentration in reed is dependent on an individual characteristic of the sampling point while independent on water salinity is distinct. Nine samples collected in Gardno Lake with brackish water were characterized by the Cr–Ni factor score value lower than 0 (low content), while 13 by a value higher than 0.7 (high content). In this case, clear seasonal variation appears since samples characterized by negative scores were collected in spring, while these characterized by positive scores in summer. On the one hand, this phenomenon could be connected with increasing touristic activity during the summer in the vicinity of this attractive lake area, and on the other hand, increased uptake during growing season. An increased mean concentration of Cr in shoots of *P. australis* grown in freshwater Lake Sapanca in Turkey exposed to heavy urbanization because of its natural beauty and proximity to the metropolitan Istanbul was also found [11]. Almost ninefold increase in Cr concentration in stem collected in April (0.3) and June (2.6) was reported by others [13]. An increased Cr concentration due to the recreation usage of the lake was also reported for

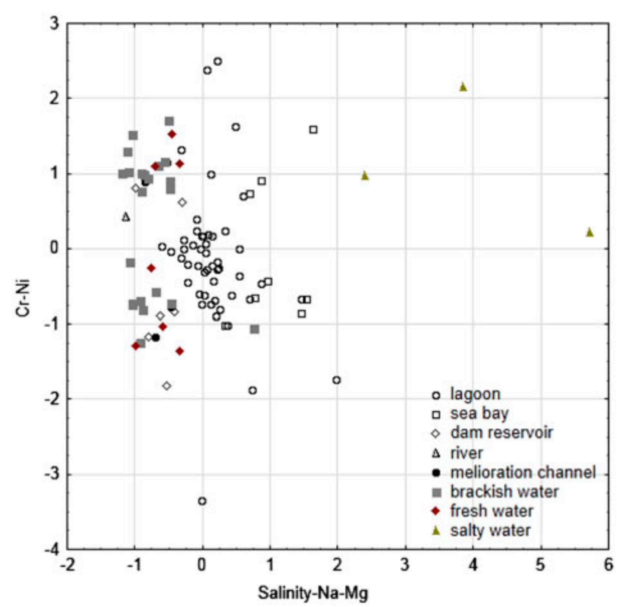

Fig. 4. PC2 (Salinity-Na–Mg) and PC3 (Cr–Ni) stores according to the type of water reservoirs.

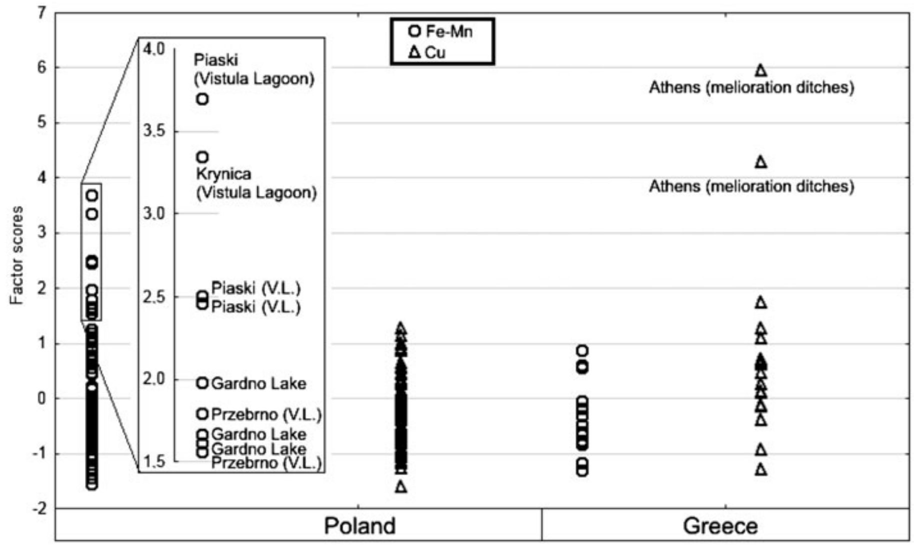

Fig. 5. The variation of PC4 (Fe–Mn) and PC5 (Cu) factor scores according to geographic location (V.L.—Vistula Lagoon).

Hungarian lakes [15]. The rest of the samples were apparently casually distributed among both clouds, however, some clear partition can be observed. Generally, reed samples collected in Kastrakiou dam reservoir and Lyssimachia freshwater lake were less contaminated with Cr and Ni since majority of samples collected in these water reservoirs were settled in the bottom cloud signifying lower contamination. Contrarily, in the upper cloud signifying higher contamination, all samples collected in freshwater Żarnowieckie Lake, Marathon dam reservoir, Messolonghi Lagoon, and Evinos river were settled. An independence of Cr and Ni concentration in reed on integrated salinity is confirmed by the presence of samples collected in brackish, fresh and salty water reservoirs in both clouds. Additional prove could be obtained by visualization of PC2 vs. PC3 scores (Fig. 4). As ensues from Fig. 4, any ordination which could suggest a salt-related gradient cannot be observed.

The lowest part of variation was explained by PC4 and PC5. Four samples from Piaski, two from Przebrno, one from Krynica (all Vistula Lagoon), and three from Gardno Lake had factor scores higher than 1.5 in PC4, while two samples from Athens had factor scores higher than 4 in PC5 (Fig. 5).

Excluding samples mentioned above the rest of samples were characterized by comparable factor scores according to PC4 (Fe–Mn) and PC5 (Cu). This demonstrates that Fe, Mn, and Cu variation in submerged common reed is independent of climate zones, while high concentration of metals in reed is caused by relatively minor reasons having little impact. The hypothesis concerning accidental allocation of metals in reed collected in one location becomes more convincing since two other samples collected in Athens, 17 in Gardno Lake, 16 in Krynica, 17 in Piaski, and nine in Przebrno were characterized by a factor scores in the range between −1.5 and 1.5 signifying low or at least moderate allocation of metals. One sample, which was collected in autumn in melioration ditches in Athens, was characterized by a large factor score value (>4). The other one was collected in summer. Two more samples collected in autumn in Athens were characterized by a factor score value close to 0. This proves that even in the case of relatively small geographic location, high variation in metal allocation could take place, however, the causes of this phenomenon could remain undiscovered.

4. Conclusions

The descending order of metals concentration in common reed collected in Poland and Greece is similar, however, Cd, Cu, Co, K, and Ca concentration in Greek samples was higher than in Polish ones. In lukewarm water reservoirs, an accumulation of Cd, Pb, and Co is facilitated, and hence *P. australis* distributed in mediterranean water reservoirs allocated heavier metals in submerged shoots. Shallow water reservoirs, as was in the case of Messolonghi Lagoon, favor an accumulation of salt components in reed. Cr and Ni accumulation in underwater parts of reed depends on individual characteristics of water reservoir or intensity of anthropogenic impacts with regard to its recreational usage. Generally, reed samples collected in both climatic zones were similarly abundant with Cu, while the accidental increase observed in melioration ditches could be caused by periodic reasons having little impact.

The large variability in metal concentrations found in common reed collected in different climate zones and water reservoirs of different salinity must be taken into account when designing monitoring programs, so that no incoherent conclusions would be reached when using slightly different sampling dates or sampling sites, as it was in the case of Cr and Ni abundance analysis in reed collected in Vistula Lagoon.

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