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THE STUDY OF HEAVY METALS TRANSFER FACTORS ON DIFFERENT TYPES OF AQUATIC VEGETATION

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ABSTRACT. In this study, there were selected two sample types: sediments and three types of aquatic vegetation from Danube Delta Biosphere Reserve: *Scirpo- Phragmitetum*, *Typha angustofila* and *Stratiotetum aloides*. The samples were taken during 2008, from may to august. For the aquatic vegetations the humidity, the total carbon and the heavy metals (Cd, Mn, Ni, Pb, Zn) concentrations in the dry samples were measured. For the sediments the heavy metals concentrations were measured. Accumulation factor ratios of aquatic vegetations were calculated, and the three species were compared. The bioaccumulation ratios of *S. aloides* were higher comparing with *T. angustofila* and *S. phragmitetum for* nickel, lead and manganese. The study of the humidity and total organic carbon proves the fact that the *T. angustofila* is an important viable source of biocombustible (minimum humidity, maximum content of carbon), than *S. phragmitetum* (medium humidity, medium content of carbon) and *S. aloides* (maximum humidity and minimum content of carbon).

Keywords: *heavy metals, total carbon, humidity, Scirpo-Phragmitetum, S. aloides , T. angustofila, Danube Delta*

INTRODUCTION

Due to their different capacity to retain heavy metals, three types of aquatic vegetation were selected. Heavy metals like cadmium and lead were insolubilized as sulphides or hydroxides in large amounts pH-dependent in the sediment and finally deposited in the rhizomes and partially in the above aerial parts of the plants [4].

Plants can attenuate water flow, bind sediment, and directly accumulate metals. By these actions plants affect metal mobility.

Because of their acute toxic effects, heavy metals should be removed from the environment using cost-effective and appropriate methods, or should be converted into less-toxic forms. Phytoremediation means the use of green plants to reduce, remove, degrade or immobilize environmental toxins. [6] [8] [11] [12]

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In Danube Delta Biosphere Reserve, the dominant vegetation is the one specific for the swamps, as reed (*S. phragmitetum*) and rush (*T. angustofila*), which are helophila species. [1] The covering vegetation, under which have been developed peat deposits, is represented by *S. phragmitetum* association with tall reed (3–5m) in the areas characterized by an active circulation of fresh waters. The reed being located along the channels reduces the erosion of the river banks and helps in retaining of the sediments from flood water. The reed works as a self–driven system, optimizing the physical, chemical and microbiological processes which occur naturally in wet areas. Reed, like other plants which grow in the wet areas, conveys the atmospheric oxygen along the plant, through roots, in order to survive in the flooded soil.

The atmospheric oxygen transfer creates both aerobe and anaerobe conditions, which allows to an extraordinary variety of bacteria and fungi to grow. These are using organic pollutants as food source, reducing their concentration both in water and in soil [4]. The organic matter from water and soil are not only retained in reed but are also decayed there to harmless compounds. Other pollutants, heavy metals, are transformed from noxious ionic compounds and retained in soil by complexing chemical reactions [9]. In the last years, the aquatic vegetations (reed and rush) has been used in constructed wetlands for the treatment of industrial wastewaters containing metals. [6] [7]

S. aloides belongs to aquatic vegetation, it can be found in the shallow lakes, in the small pools located in rush-beds, in channels and in the banks of channels and brooks. Stratiotes is an underwater water plant species also known as “water soldiers”. A characteristic of the genus is the habit of the plants rising to the surface at flowering time. The plant appears to be associated with calcareous waters and there is a suggestion that changing levels of calcium carbonate on the leaves may explain the floating and submerging behavior.

The Dunavăț/Dranov region (19000ha) is one such peat area, lying as it does between two more or less parallel water courses: the Dunavăț Danube Branch in the north-west and the Dranov creek in the south-east. The Holbina/Dunavăț is situated in the Dunavăț/Dranov region and consists of three former fish-farm basins [5].

The aims of this study were to: (1) to compare the total humidity of reed, rush and stratiotes, (2) to compare the total organic carbon concentrations in the three types of aquatic vegetations, (3) to compare the the total heavy metals concentrations in the selected aquatic vegetations, (4) to evaluate the accumulations ratios oh heavy metals in reed, rush and stratiotes.

RESULTS AND DISCUSION

The pollutants in aquatic system are removed through a combination of biological, physical, and chemical processes including assimilation by plant tissue, microbiological transformations, sedimentation, precipitation, and adsorption to sediment particles. [2]

The study of heavy metals accumulation, proves that the inorganic pollutants have a different rate of accumulation in the aquatic vegetation

Tables 2 and 3 show the maximum, minimum, average, median and standard deviations values of the 25-th measurements of selected indicators for each sample type (sediment and aquatic vegetation from Dunavat and Holbina respectively), calculated using the excel interface.

In a totally ordered set, all elements are mutually comparable, so such a set can have at most one minimal element and at most one maximal element. Then, due to mutual comparability, the minimal element will also be the least element and the maximal element will also be the greatest element. Thus in a totally ordered set we can simply use the terms minimum and maximum. *T. angustofila* has the lowest minimum humidity value and the highest minimum total organic carbon value. In opposite, *S. aloides* has the highest minimum humidity value and the lowest total organic carbon value. The same trend was observed with the maximum calculated values, for humidity and the total carbon.

An average or central tendency of a data set refers to a measure of the "middle" or "expected" value of the data set. The humidity average has values between 40.671% and 93.076%. The *S. aloides* samples had the maximum value for humidity and the minimum values were found in the *T. angustofila* samples.

It was necessary to evaluate the content of organic carbon, because in the Danube Delta Biosphere Reserve it is a high quantity of aquatic vegetation, a possible source for the biocombustible. The maximum concentration of organic carbon it was determined in the *S. phragmitetum* and *T. angustofila* samples.

The average of heavy metals concentrations, expressed in mg/kg of dry substances, were represented in figure 1. The *S. aloides* samples accumulated the maximum heavy metals, with no exception. (Figure 1)

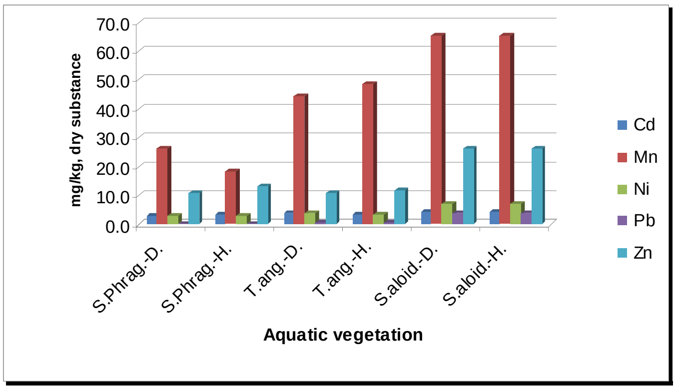

Figure 1. Total heavy metals medium concentrations in aquatic vegetation (D=Dunavat, H=Holbina)

A **median** is described as the number separating the higher half of a sample, a population, from the lower half. The *median* of a finite list of numbers can be found by arranging all the observations from lowest value to highest value and picking the middle one. If there is an even number of observations, the median is not unique, so one often takes the mean of the two middle values. At most half the population has values less than the *median* and at most half have values greater than the median. If both groups contain less than half the population, then some of the population is exactly equal to the median. The general distribution of the median is the same like the average, with the maximum values calculated in *S. aloides* .

In general, for all selected heavy metals, at Holbina and Dunavat, the calculated standard deviations have the lowest values (<1). A low **standard deviation** indicates that the data points tend to be very close to the average.

For the humidity and the total organic carbon, for the three type of aquatic vegetation, the standard deviations have high values. A high standard deviation indicates that the data are "spread out" over a large range of values.

As we can see, the both selected sampling points (Holbina and Dunavat) have the same general variation tendency of the statistical selected parameters: minimum, maximum, average, median, standard deviations. This can be explained by the fact the Danube Delta lakes are opened water, with channels and branches that assure the general input and output of the water.

Bioaccumulation factors which express the ratio of the metal concentration in the biological material to the metal concentration in soil or external solution were evaluated.[13], [3] It was found that the mobility within the chamomile plants decreased in the following order: Cd > Ni > Pb.

Tabel 1. Bioaccumulation factors in different aquatic vegetation

Sampling points	Sample type	BAF_{Cd}	BAF_{Mn}	BAF_{Ni}	BAF_{Pb}	BAF_{Zn}
Dunavat	*S.Phragmitetum*	0.823	0.074	0.082	0.010	0.283
	T. angustofila	1.779	0.254	0.125	0.018	0.281
	S. aloides	1.651	0.213	0.251	0.357	1.125
Holbina	*S.Phragmitetum*	1.039	0.050	0.085	0.009	0.361
	T. angustofila	1.150	0.221	0.102	0.017	0.320
	S. aloides	1.168	0.214	0.260	0.432	0.989

The bioaccumulation factor was calculated after the medium values of metals concentrations from aquatic vegetation and sediments, expressed in mg/kg of dry substance. By analyzing the values of bioaccumulation factors, we can observe the same general trend: $\mathbf{BAF_{Cd} > BAF_{Zn} > BAF_{Ni} > BAF_{Pb} > BAF_{Mn}}$. The calculated values of bioaccumulations factors are in interrelation with science literature. [13], [3]

Table 2. The minimum, maximum, average, standard deviation values of selected indicators from Dunavat area

Sample type	Parameter	Humidity	TOC	Cd		Mn		Ni		Pb		Zn	
		b	b	a	b	a	b	a	b	a	b	a	b
		%	mg/kg[1]	mg/kg[1]	mg/kg[1]	mg/kg[1]	mg/kg[1]	mg/kg[1]	mg/kg[1]	mg/kg[1]	mg/kg[1]	mg/kg[1]	mg/kg[1]
S.Phrag-mitetum	**Min. value**	63.652	65.995	2.542	2.028	350.796	24.194	30.116	1.998	14.575	0.062	32.501	4.324
	Max. value	86.635	84.625	4.843	3.893	356.854	27.451	32.204	3.658	14.923	0.318	44.730	15.701
	Average	74.231	77.254	3.691	2.859	353.725	26.128	31.358	2.565	14.770	0.147	38.036	10.539
	Median	73.654	77.625	3.783	2.887	354.195	26.225	31.489	2.461	14.781	0.110	37.766	10.369
	Std. dev	4.801	5.171	0.647	0.501	1.663	0.793	0.618	0.376	0.102	0.077	2.820	2.617
T. angustofila	**Min. value**	30.401	71.464	1.025	2.544	326.014	41.238	28.421	3.124	14.603	0.177	35.640	8.154
	Max. value	56.481	86.426	3.887	5.122	335.120	48.946	30.984	4.547	14.795	0.365	40.950	12.521
	Average	40.671	78.666	2.526	3.750	331.177	44.224	30.056	3.750	14.712	0.258	37.951	10.625
	Median	40.164	79.456	2.630	3.764	331.687	44.075	30.034	3.678	14.728	0.241	37.917	10.514
	Std. dev	6.276	3.988	0.786	0.661	2.635	2.018	0.667	0.375	0.052	0.052	1.291	1.105
S. aloides	**Min. value**	86.464	38.481	2.368	3.980	306.490	65.221	27.752	6.944	10.962	3.902	23.199	26.100
	Max. value	97.004	63.461	2.435	3.999	306.858	65.417	27.804	6.978	11.008	3.937	23.239	26.139
	Average	93.076	52.388	2.418	3.992	306.687	65.287	27.771	6.962	10.980	3.923	23.223	26.116
	Median	93.161	52.164	2.420	3.992	306.701	65.259	27.770	6.962	10.983	3.928	23.230	26.115
	Std. dev	2.701	5.709	0.012	0.005	0.107	0.059	0.012	0.009	0.013	0.011	0.013	0.011

a=sediment sample
b=aquatic vegetation sample
[1]=dry substance

Table 3. The minimum, maximum, average, standard deviation values of selected indicators from Holbina area

Sample type	Parameter	Humidity	TOC	Cd		Mn		Ni		Pb		Zn	
		b	b	a	b	a	b	a	b	a	b	a	b
		%	mg/kg[1]	mg/kg[1]	mg/kg[1]	mg/kg[1]	mg/kg[1]	mg/kg[1]	mg/kg[1]	mg/kg[1]	mg/kg[1]	mg/kg[1]	mg/kg[1]
S.Phrag-mitetum	**Min. value**	59.043	54.461	2.097	2.448	358.134	15.337	30.712	2.028	14.615	0.065	34.053	10.968
	Max. value	79.024	73.325	4.325	4.254	367.890	22.364	32.387	3.554	14.920	0.263	38.173	14.335
	Average	71.826	64.285	3.388	3.240	363.627	18.341	31.580	2.675	14.836	0.131	35.835	12.890
	Median	73.365	63.645	3.503	3.122	363.757	18.654	31.667	2.684	14.859	0.114	35.906	12.857
	Std. dev	5.171	5.250	0.680	0.553	2.118	1.671	0.370	0.324	0.081	0.056	0.992	0.876
T. angustofila	**Min. value**	52.164	60.134	0.902	2.014	321.353	42.532	29.448	2.294	14.516	0.119	34.788	8.964
	Max. value	82.655	80.716	4.861	5.222	334.005	52.686	31.895	4.311	14.864	0.421	40.151	14.298
	Average	71.202	70.612	3.499	3.150	326.055	48.496	30.743	3.125	14.681	0.254	36.953	11.758
	Median	72.467	70.964	3.612	3.114	325.792	48.822	30.763	3.245	14.676	0.255	36.833	11.642
	Std. dev	8.249	5.886	0.968	0.790	3.207	2.473	0.654	0.481	0.079	0.069	1.436	1.344
S. aloides	**Min. value**	68.264	39.630	3.314	3.900	305.589	65.231	26.765	6.942	9.044	3.907	26.391	26.101
	Max. value	89.474	52.326	3.533	4.018	305.822	65.338	26.793	6.966	9.096	3.928	26.420	26.129
	Average	80.144	46.532	3.416	3.988	305.671	65.282	26.776	6.958	9.070	3.919	26.404	26.116
	Median	80.461	47.552	3.412	3.992	305.654	65.286	26.774	6.958	9.078	3.920	26.404	26.114
	Std. dev	6.589	3.449	0.038	0.021	0.065	0.036	0.008	0.006	0.018	0.006	0.008	0.007

a=sediment sample
b=aquatic vegetation sample
[1]=dry substance

For all the heavy metals are made the flow charts and the calibration curves.

The coefficient R^2, for the calibration curves has the value 0.9994 (for cadmium), and 0.9997 (for lead) that represent a very good correlation between the intensity and the standards concentrations. (Figure 2 and Figure 3).

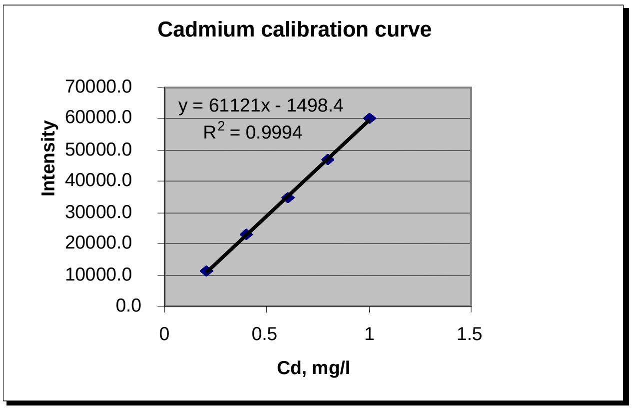

Figure 2. Calibration curve of cadmium

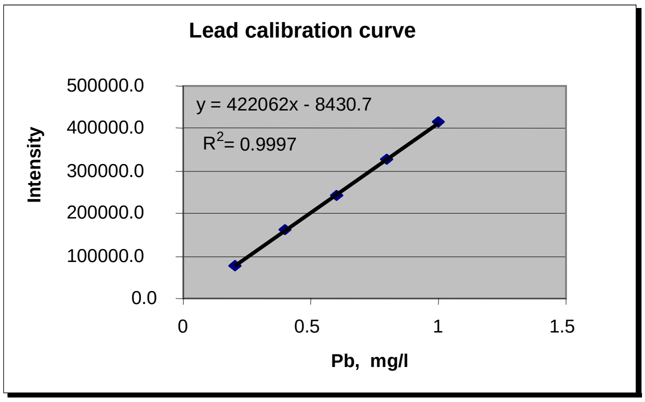

Figure 3. Calibration curve of lead

The quality control chart of cadmium was made in 2008, using a 0.6 ppm certified reference material PerkinElmer, according with a special procedure developed in our laboratory. The readings of 0.6 ppm standard at every cadmium samples measurements, assure a high quality control of analysis.

EXPERIMENTAL SECTION

In the field there were ten sampling points represented by aquatic vegetation and sediment samples. The samples are representative for Holbina Dunavat area. (Figure 4)

The aquatic vegetation samples (*S.phragmitetum*, *T. angustofila*, *S. aloides*) were taken with a reaper. Each sample is composed by the stems. The area where the samples were taken was 1 square meter. For each sample the humidity is determined by measurement and drying to the air oven, to 80 ^{0}C, until constant weight.

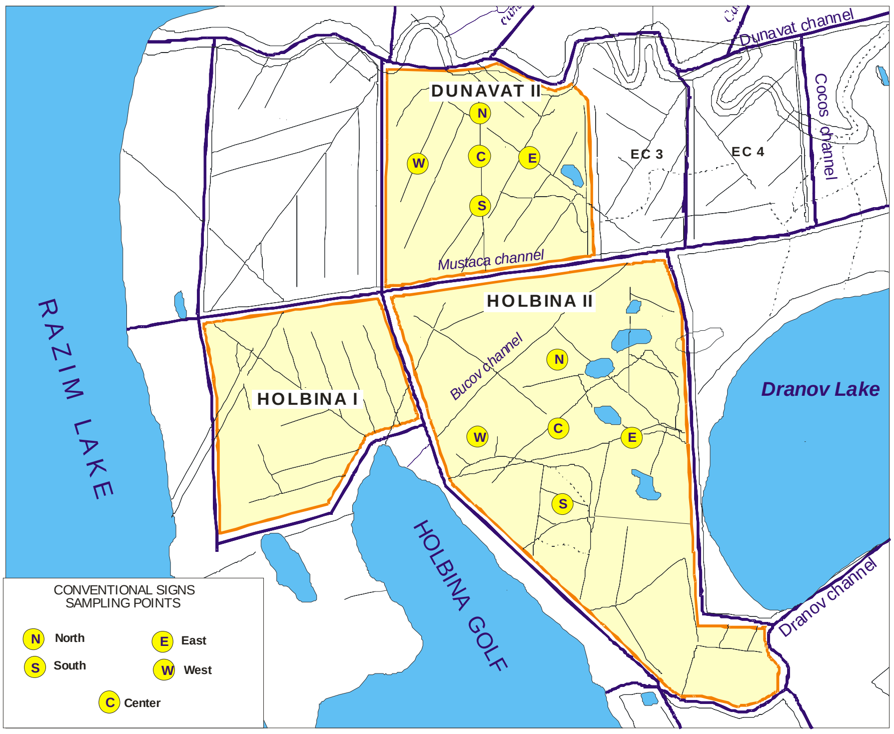

Figure 4. Sampling points of Holbina Dunavat area

The representative sample was obtained by the hashing of all the stems and the mixture until the homogenization.

The sediment samples are sampling according with SR ISO 5667 -12: September 2001- Water quality – Sampling, Part 12: Guidance on sampling of bottom sediments with dredge. The sediment samples were dried at room temperature, for avoid losing organic micro pollutant.

To obtain sub samples approaching 20g, it is made a pretreatment to a portion from the sample air-dried, grind fine and sprinkle through a sieve with meshes of 150 µm.

Reagents

All reagents used, have high chemical purity, and are Merck. The reagents used for organic carbon determination are: potassium bichromate, ($K_2Cr_2O_{7,}$ 1N), sulphuric acid (H_2SO_4 98%), Mohr salt (iron and ammonium double sulphate ($FeSO_4(NH_4)_2SO_4.6H_20$, 0.2N), ortho-phosphoric acid (H_3PO_4, 85%), biphenyl amine (colour indicator). For the heavy metals extraction we use nitric acid (HNO_3, 63%), and hydrogen peroxyde (H_2O_2), mainly used to increase the oxidizing effect of HNO_3 to destroy matrix residues.

Calibration solutions

The calibration curves were made using the Perkin Elmer Pure Plus Atomic Spectroscopy Standard, certified reference material 10 µg/ml, Multi-element ICP-MS calibration STD.3, matrix 5% HNO_3 The calibration curves are in five points and linear. Using the excell interface, it was calculated for each calibration curves, the equations and the coefficients R^2.

Instruments

Microwave digestion, necessary for heavy metals determination, according with EPA 3051/1994 - – Microwave assisted acid digestion of sediments, sludge, soil and plants for the determination of selected elements (Ag, Al, As, B, Ba, Be, Ca, Cd, Co, Cr, Cu, Fe, Hg, K, Mg, Mn, Mo, Na, Ni, Pb, Sb, Se, Sr, Tl, V, Zn); US EPA 3015/1994 – Microwave assisted acid digestion of aqueous samples and extracts.

Inductively coupled plasma – mass spectrometry (ICP-MS) is applicable to the determination of small concentrations of a large number of elements. When dissolved constituents are required, samples must be filtrated and acid-preserved before the analysis.

Acid digestion prior to filtration and analysis is required for groundwater, aqueous samples, industrial wastes, soils, sludge, sediments and other solid wastes [8].

The method measures ions produced by a radio-frequency inductively coupled plasma. Analyte species originating in a liquid are nebulized and the resulting aerosols are transported by argon gas into the plasma torch. The ions produced are entrained in the plasma gas and introduced, by means of an interface into a mass spectrometer. The ions produced in the plasma are sorted according to their mass-to-charge ratios and quantified with a channel electron multiplier. The analysis were made in the standard conditions of the instrument, without DRC.

Cell Gas A adjusts the flow rate of the first reaction cell gas. Cell Gas A (ml/min) specifies the flow rate in ml/min at which Cell Gas A flows into the reaction cell. The flow rate is controlled by a mass flow controller.

In the table bellow are represented the intrument experimental condition.

Table 4. Instrument experimental condition

Optimization Data		Summary		
Current value	**Description**	**Analyte**	**Mass**	**Meas. Intens. Mean**
0.91	Nebulizer Gas Flow [NEB]	Mg	24.0	37376.1
1.20	Auxiliary Gas Flow	In	114.9	30.9
15.00	Plasa Gas Flow	U	238.1	27.9
6.00	Lens Voltage	> Ce	139.9	88.5
1100.00	ICP RF Power	CeO	155.9	4.1
-1600.00	Analog Stage Voltage	> Ba	137.9	5264.9
750.00	Pulse Stage Voltage	Ba^{++}	69.0	175.6
0.00	Quadrupole Rod Offset Std [QRO]	220	220.0	0.8
-15.00	Cell Rod Offset Std [CRO]	8.5	8.5	1.1
15.00	Discriminator Threshold			
-13.00	Cell Path Voltage Std [CPV]			

The optimisation solution used, was ELAN 6100Setup /Stab./ Masscal. Solution. The optimisation solution contains the elements: Mg, Cu, Rh, Cd, In, Ba, Ce, Pb, U. The labeled concentrations are 10 ppb for each element. For one point, the measure time is about 3 minutes.

Sample preparation

The vegetation samples were dried until the constant mass, at 80^0C +/- 5^0 C. [10] The difference between the plant mass before and after drying process is a measure of drying substance and of water. In the humidity computing, the content of dry substance and water (humidity) are expressed in percentage, according with SR ISO 6496:2001: Fodders. The determination of humidity and other volatile substances.

A wet-oxidation technique is used for determining organic carbon in plant material. The oxidation is carried out by heating the plant sample with a mixture of potassium dichromate, sulphuric, and phosphoric acids for 10–15 min. The organic carbon is oxidation by chromic anhydride in excess, in the presence of sulphuric acid, at 100^0C temperature in 30 minutes. The excess of chromic anhydride are titrated with a Mohr salt 0.2N, in the presence of an oxido-reducing indicator. The content of organic carbon is computing after the chromic anhydride consuming for its oxidation. [14]

For the heavy metals determination, in quartz vessels are weighed to the analytical balance 0.25 -0.5 g of aquatic vegetation, in the 80 ml quartz digestion vessel, than added 5 ml nitric acids and 2 ml hydrogen peroxide.

After 15 minutes of pre-reaction time, the vessels are hermetic closed with the protective caps, are putting in the protective casings and put into the rotor of microwave oven.

The digest power program is presented in the table bellow.

Table 5. The digest program for aquatic vegetation

Phase	Power [W]	Ramp [min]	Hold [min]
1	1200	5.5	4.5
2	1400	0	5

After program and the cooling time end, the rotor is draw out, the digestion vessel are opened and the content is removed in graduated flask and dilute to 100 ml.

0.1 -0.2 g of well-mixed sediment samples are weighed to the analytical balance in the quartz digestion vessels and than added 10 ml concentrated nitric acid. If an intense reaction occurs, is allowed the reaction to stop before capping the vessel, after the pre-reaction, are putting in the protective casings and put into the rotor of microwave unit.
The digest power program is presented in the table bellow.

Table 6. The digest program for sediment

Phase	Power [W]	Ramp [min]	Hold [min]
1.	600	5.30	0
2.	1200	4.30	20

At the end of the microwave program, allow the vessels to cool for at least 10 minutes before removing them from the microwave unit. If the digested sample contains particulates which may clog nebulizer or interfere with injection of the sample into the instrument, the sample may be centrifuged, allowed to settle, or filtered, and then diluted in 100 ml graduated flask to 100 ml with distilled water and than analyzed to ICP-MS.

CONCLUSIONS

The study of the humidity and total organic carbon proves the fact that the *T. angustofila* is an important viable source of biocombustible (minimum humidity, maximum content of carbon).

The aquatic vegetation represents a real and a cheaper depolutting source for heavy metals and the nutrients, the major pollutants of the Danube River**.**

The *S. aloides* accumulated the maximum heavy metals, with no exception.

The bioaccumulation factor has the biggest values for cadmium and the minimum values for manganese.

The heavy metals calibration curves and flow charts assure high accuracy of measurements.

The future studies will be focused on the heavy metal accumulation in different morphological parts of aquatic vegetation, like roots, submerged stems, aerial stems, buds and rhizomes.

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