Joint Danube Survey 3

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Quantity and Quality of Dissolved Organic Matter

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1 Introduction

Dissolved organic matter (DOM) is a heterogeneous C pool which provides microbes with major food source and therefore sustains the basis of riverine food web. Besides this ecological importance, DOM also provides other functions, such as light absorption, binding of heavy metals and organic contaminants, adsorption at surfaces and photochemical reactivity and also includes fingerprints of pollution, etc. (Baker et al. 2008). Dissolved organic carbon concentration is commonly measured in riverine monitoring programs as one of the parameters describing water quality as organic carbon delivery control microbial activity. However, DOM consists of a wide spectrum of different molecules and provides microbes with different food quality, which could result in different degree of microbial production based on the organic matter availability. In short, concentration of DOC can only give us insights on the C quantity, but not its quality aspect.

Increasingly, scientists are taking advantages of the optical properties of dissolved organic material (DOM) for describing the pools in natural aquatic systems.Various studies used optical properties of DOM to elucidate the anthropogenic influence on aquatic systems, including sewage effluents (Reynolds and Ahmad 1997), urbanization (Westerhoff and Anning 2000), and landfill leachates (Baker and Curry 2004). Forty to sixty percent of total DOM present in natural systems is fluorescent, primarily consisting of protein and organic acids derived from decayed organisms within the catchment. Those fluorescent substances are called fluorophores which can be measured by scanning excitation and emission wavelengths simultaneously through a set path length to create a 3-D contour plot which is called an Excitation-Emission matrix (EEM). The matrix is composed of peak intensities which are further related to the concentration of the fluorophore present in a water sample.

In large rivers such as the Danube which receive important inputs from human, terrestrial and *in situ* sources, the resulting DOM pools are a heterogeneous mixture of these carbon species (Massicotte and Frenette 2011). Especially in some sections of the Danube the sources are subject to anthropogenic environmental heterogeneity (e.g., disconnection of side-arms, drying of wetlands, constructing dams). By using this simple and time efficient method we can interpret the evolution and movement of DOM species both temporally and spatially in the Danube River. This approach is expected to differentiate the importance of these different factors and give some insights in the ecosystem functioning of a large river such as the Danube River.

2 Methods

2.1 Sampling strategy

Three samples, “Left bank (L)”, “Right bank (R)” and “Middle (M)” were collected along the transect for each JDS station. Exact location can be found in the general description of the JDS III. L and R sites were 10 to 15 meters away from the respective river banks; the sampling vessel was positioned in the middle of the river by GPS to collect “M” samples. Water was taken from 30 cm below the surface level and was filtered (pre-combusted Whatman GF/F, 2.5 h at 490°C) and stored in purged glass tubes (24 h in 10 % HCl, pre-combusted 4 h at 490°C) at 4°C until analysis.

2.2 Optical properties of DOM

Fluorescence values were measured by a Hitachi Fluorescence Spectrophotometer F-7000. The scanning method follows Baker (2001) with minor modification, with excitation wavelength 200–450 nm at 5 nm steps and emission wavelengths between 250–600 nm at 2 nm steps. Blanks of Milli-Q water were run before and after each run and were used to standardize to a mean Raman peak.

As the fluorospectrometer scanned a defined wavelength range, fluorophores which exist in samples result as peaks with intensity in their corresponding EEM. Peak B and T represent protein-like substances (Tyrosine-like and Tryptophan-like, respectively) (Baker, 2001), whereas peak A and C are related to humic-like substances (Table 1).

For data interpretation, we used the relative relationship between each peak intensity to obtain well-defined indices addressing different aspects of DOM sources according to previous references (e.g., Coble 1996; Welti et al. 2012). Table 2 shows the calculation of each index and the respective indication present in this report.

Table 1 Name and description of EEM peaks (fluorophores) after Hudson et al. 2007

EEM Peaks	Ex/Em wavelength (nm) at max fluorescence intensity	Description
A peak	237–260/400–500	Humic-like (Hydrophobic acid fraction)
C peak	300–370/400–500	Humic-like (Hydrophobic acid fraction)
B peak	225–237/309–321 and 275/310	Protein-like (Tyrosine-like)
T peak	225–237/340–381 and 275/340	Protein-like (Tryptophan-like)

Table 2 Name and representation of EEM indices

Peaks or indices	Peak intensity (int.) ratio	Representation	References
Fluorescence index (FI)	Intensity ratio between Em 450 nm and 500 nm (Ex 370)	Inversely related to the lignin content of DOM FI≒1.2: terrestrial/higher plant source FI≒1.8: dominant microbial source; from leachate of bacteria or algae	McKnight et al. 2001 Fellman et al. 2010
Freshness index (or β/α ratio,"BIX")	β(Em 380 nm) /α (max. Em at 420-435 nm), Ex 310	Relative contribution of recently produced DOM to highly degraded DOM BIX > 1: autochthonous microbial origin BIX < 0.6: allochthonous terrestrial origin	Parlanti et al. 2000
Humification index (HIX)	Peak area under Em spectra 435-480 nm/ 300-445 nm (Ex 255)	Directly proportional to the humic content of DOM HIX > 16: Strong humic character/dominant terrigeneous contribution (high fulvic acid content) HIX 6~10: Dominant humic character and weak recent autochthonous component HIX 4~6: Weak humic character and important recent autochthonous component HIX< 4: of direct aquatic microbial or biotic origin (not humified)	Kalbitz et al. 1999 Zsolnay et al. 1999 Huguet et al. 2009
T280/C ratio	Peak T (Ex 275/Em 350) to peak C (Ex 320-340 Em 410-430) ratio	Tryptophan/fulvic-like fluorescence intensity	Baker 2001

2.3 DOC measurement

An additional water sample at each site was prepared in the same manner and was analyzed for its DOC concentration by Croatian Waters.

2.4 Data analysis and visualization

Peaks picking of EEM dataset was done by using an Excel macro written according to description of Baker (2001). Each major peak was identified by picking out the highest fluorescence intensity within corresponding Ex/Em wavelengths in corrected EEMs. Peaks or indices from 3 samples, L, M, R, of each JDS station were illustrated in scatter plots, presented as spatial distribution along the main longitudinal Danube channel. Five Danube sections were marked with 5 different colors respectively, i.e., Upper Danube (●), Middle section (●), Lower Danube (●), Danube delta (●), and tributaries (○). Dataset was pooled and distribution probability range was determined using median (the line which divides dataset into 2 equal parts), lower control line (25% of the dataset lies beneath the line), and upper control line (75% of the dataset lies beneath the line).

3 Results

3.1 Different DOM related fluorescence peaks and rations

i. DOC

Among the 4 sections, the Upper Danube has the highest DOC concentration (MW=3.00 ± 1.34 mg L^{-1}, N=25) in which over half of its stations are above the upper control limit (Fig. 1). For the middle and lower sections and the delta, data points were scattered around the median line. However, most tributaries showed distinguishable higher DOC concentrations, indicating the DOC provided by the tributaries is different from that of the main channel. The Danube side-arm / tributary Moson Danube had the highest DOC concentration (18.03 mg L^{-1}) typical for side-arm systems (Welti et al. 2012).

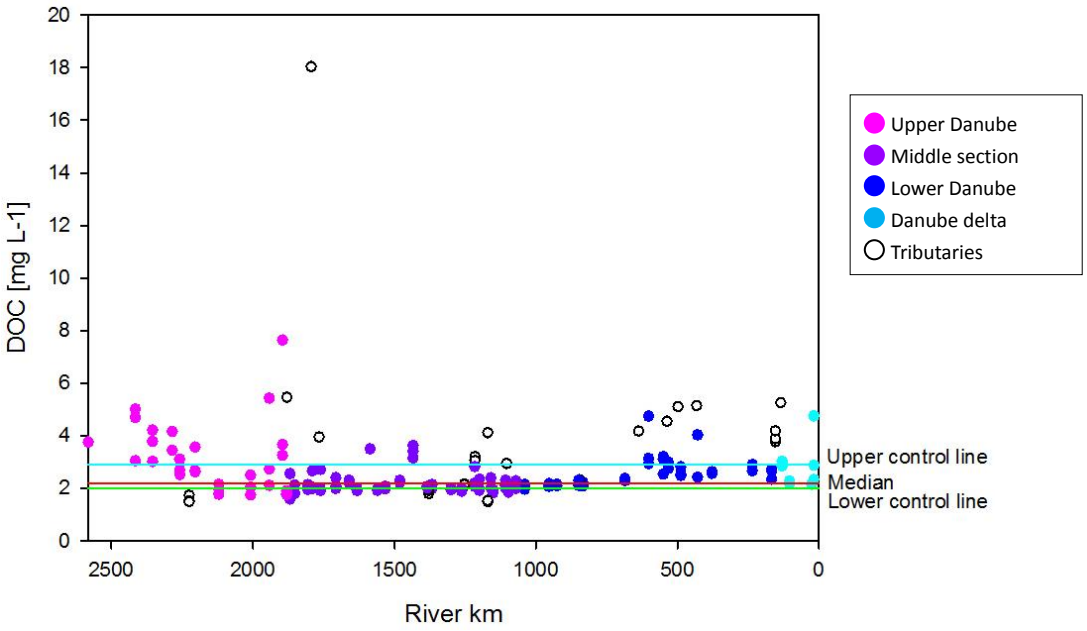

Fig. 1. DOC concentration plotted along the Danube River (river km).

ii. Peak A

Peak A shows a similar pattern as DOC values. Tributaries generally have signals either above the upper control limit or lower than the lower control limit. The high range of tributary values probably are owing to different characteristic of each tributary drainage basin.

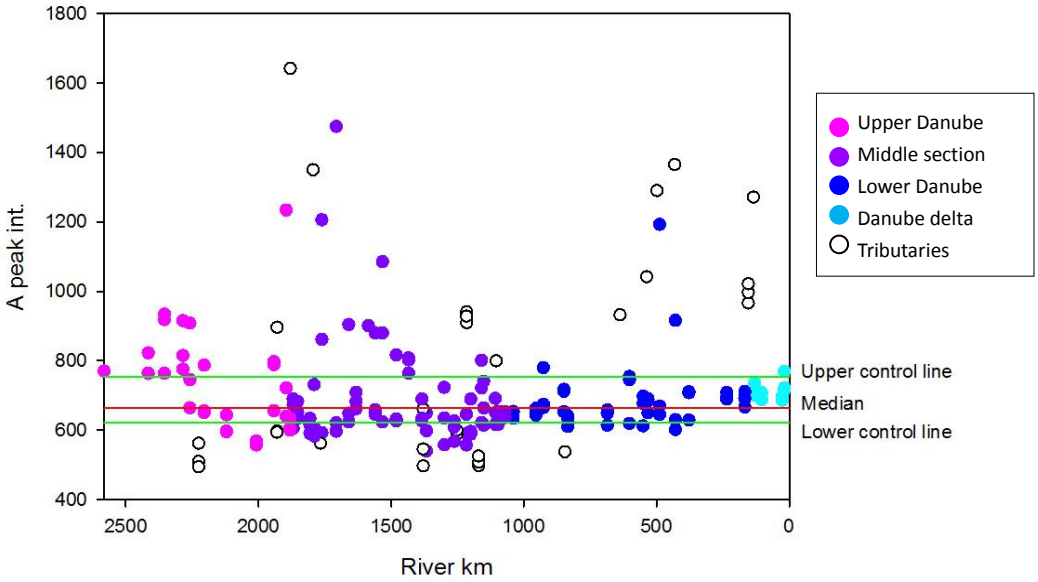

Fig. 2. Peak A plotted along the Danube River (river km).

iii. Peak C

Most of the dataset from middle and lower Danube were distributed within the control range of all samples (Fig. 3). The most upper river stretch of Upper Danube showed distinct higher C peaks which were above the upper control limit; whereas the lower parts of Upper Danube has lower C peaks below the lower control limit. Higher values were measured in the middle section were mostly downstream stations of tributaries or big cities, e.g., Vienna, Bratislava, and Budapest. Delta stations had slightly higher C peak values than the upper control line. Similar to peak A, tributary data was either higher or lower than the control range. Also, DOC concentrations from tributaries showed a positive relationship with C peak intensity (r^2= 0.33, p-value <0.0001), this could imply that tributary DOC is related primarily to fulvic-like (more labile) substances as other studies for other river systems (Hudson et al. 2007 and Baker et al. 2004) also showed.

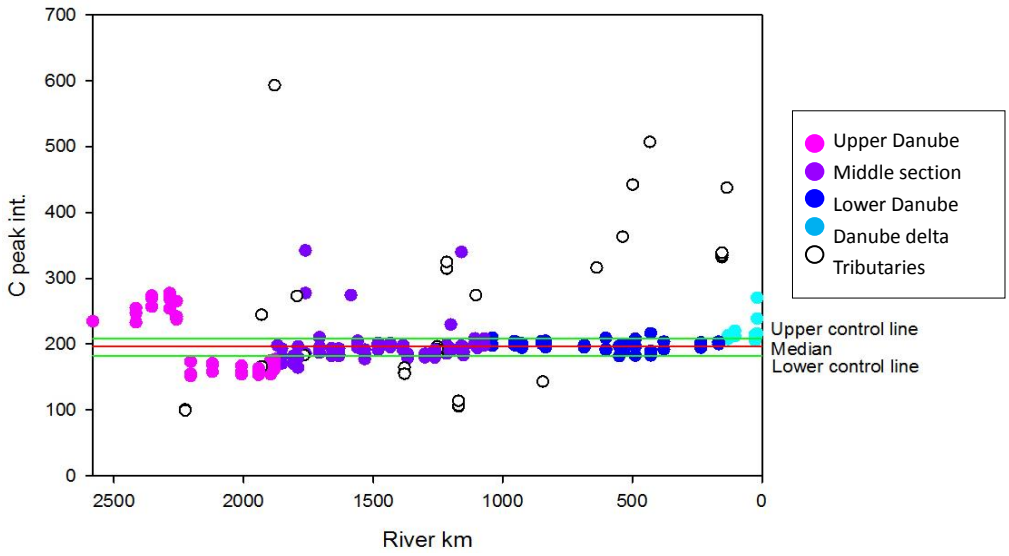

Fig. 3. Peak C plotted along the Danube River (river km).

iv. Peak B1 and T_{220}

The Upper Danube section had the highest standard deviation among L, M, R stations for both peak B1 and T220 (Fig. 4, 5). B1 and T_{220} are both indication of protein-related substances. Higher standard deviation was found in the upper and middle section, and decreased towards downstream sections. This might be due to the different hydromorphological conditions, or could be an indication for shifting of dominant primary producers and carbon sources, e.g., from (benthic) algae-dominant to vascular plants-dominant and the dominance of catchment derived organic material.

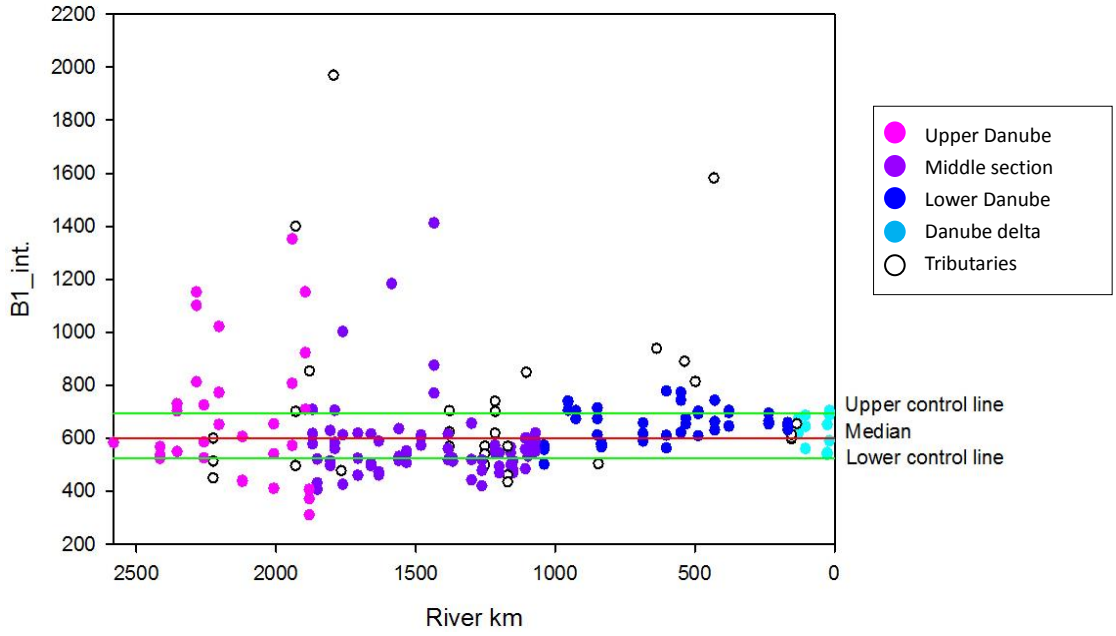

Fig. 4. Peak B1 plotted along the Danube River (river km).

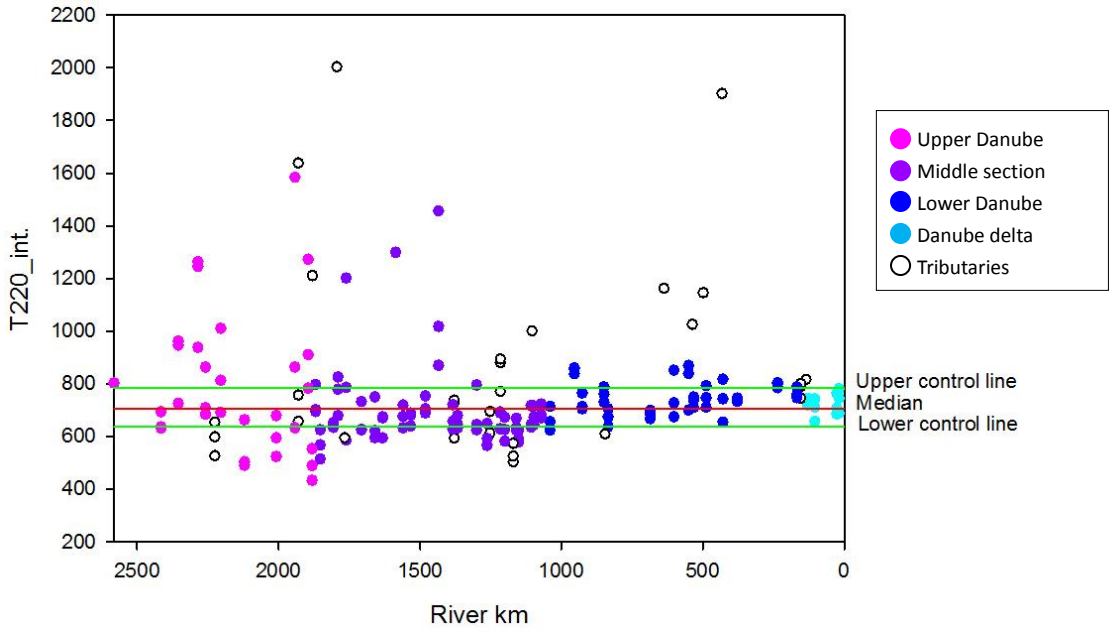

Fig. 5. Peak T_{220} plotted along the Danube River (river km).

v. Fluorescence index (FI)

The DOC concentration in large rivers is composed of both terrestrially derived DOM and microbially derived DOM, and these dual origins lead to FI values of bulk-water samples between the two end-member values (i.e. FI ≤1.4 for terrestrial DOM and ≥1.9 for aqueous microbially derived DOM). FI values of the water samples in the present study ranged between 1.25 and 1.65 (Fig. 6), in good agreement with literature values for other river water samples (McKnight et al. 2001, Chen et. al 2013).

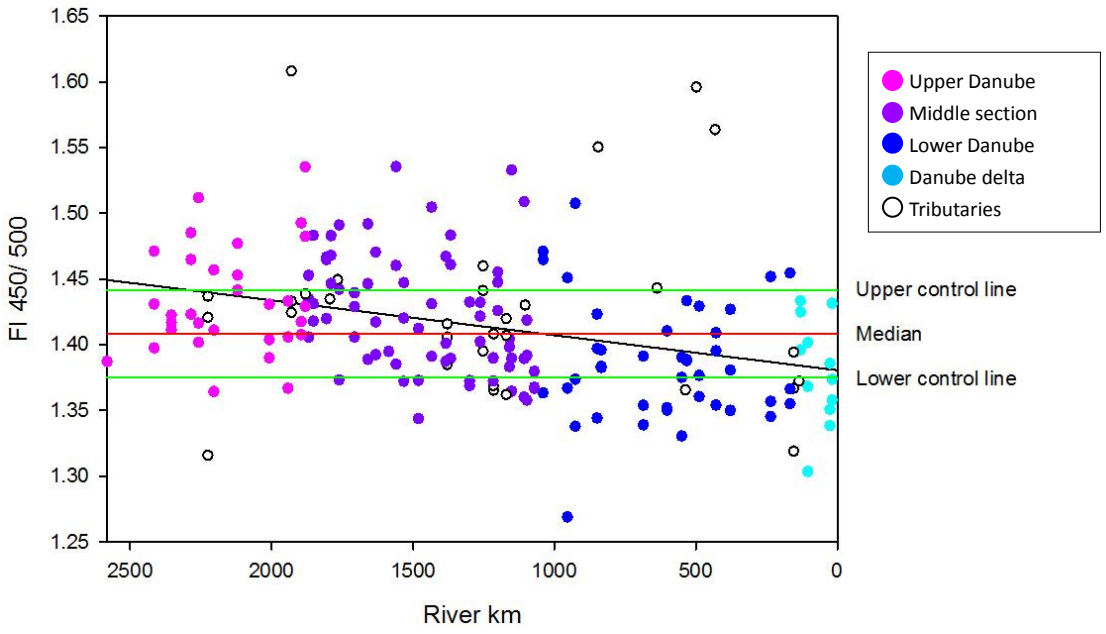

Fig. 6. FI plotted along river km. Black solid line indicates the trend line (r^2= 0.12, p-value< 0.0001)

vi. Humification index (HIX)

HIX is directly related to the degree of humification of DOM. In this study, tributaries had an increasing trend of HIX from alpine to the delta region. Tributaries from the alpine region were generally non-humified, which could contribute to the lower HIX signals for some Upper Danube stations (Fig. 7, red circle for sites in Upper Danube section). As for the delta, receiving more humified substances from the tributaries, the HIX showed a higher value. The lower HIX values found in the middle Danube corresponded to the phytoplankton peak (measured as chlorophyll-a).

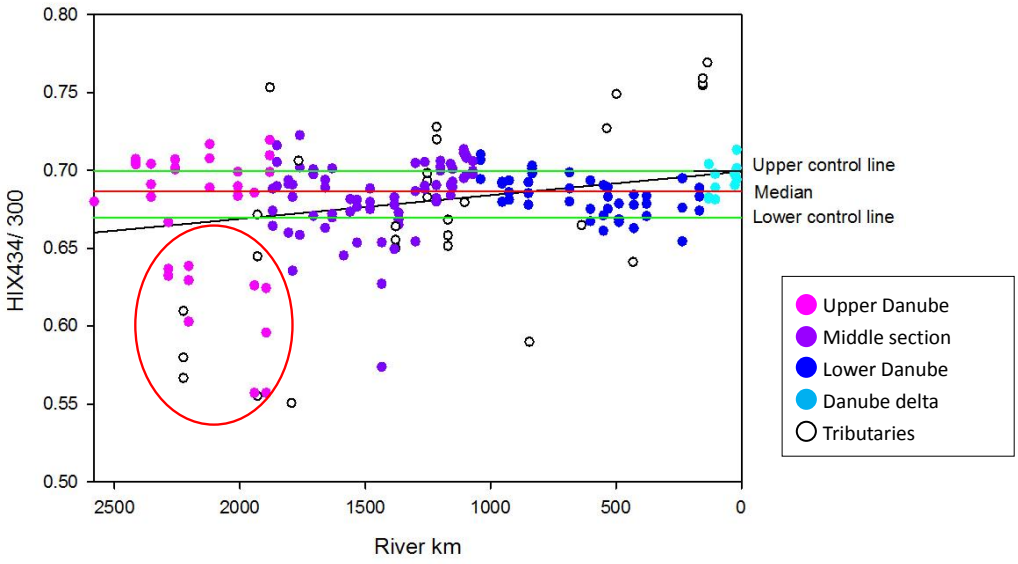

Fig. 7. Humification index (HIX) plotted along the Danube River (river km). Trend line r^2= 0.08, p-value< 0.0001.

vii. The β/α ratio

The β/α ratio represents decomposed material (α) versus recently-derived DOM (β), which can be used as an index for the degree of DOM decomposition. A spatial gradient of β/α ratio can be observed from up- to downstream (Fig. 8, r^2= 0.42). The β/α ratio was lower at most of the tributary stations, which could indicate a better physical mixing process that lead to evenly consumption of both young and old DOM material. The Upper Danube showed a lower β/α ratio due to higher α and lower β, whereas the higher ratio in the lower Danube was a result from both high α and β values.

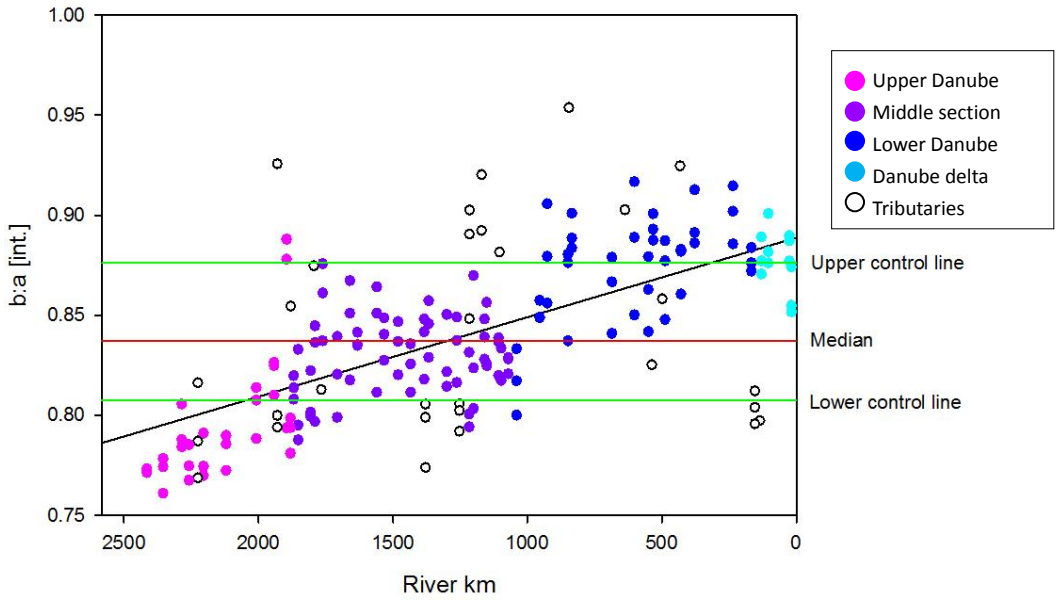

Fig. 8. The β/α ratio plotted along the Danube River (river km). Trend line r^2= 0.42, p-value <0.0001.

3.2 Differences in DOM parameters in the four Danube sections

As shown in the former chapters the mean values for DOC concentration were highest in the Upper Danube section (Tab. 3) and there significantly higher than the Middle Danube section (Tab. 4). Between the Middle and Lower Danube DOC concentration and 4 DOM parameters showed significant differences (Tab. 4). The Middle Danube section showed lower values for the peaks B1, T220 and the b:a ratio, while a higher FI was found compared to the Lower Danube section (Tab. 3). In the Lower Danube the protein-related compounds and the more autochthonous character led to these differences. The FI showed a decrease with the river course and significant differences between the Upper Danube and the Lower section and the delta confirmed these changes (Tab. 4). The DOM parameters analyzed here showed for many peaks and ratios, albeit small changes were observed, distinct changes between river sections indicating the differences in sources of DOM and changes in in-river processing.
The tributaries showed in all mean values clear differences to the Danube and a high standard deviation which can be attributed to the variety of different rivers combined in this group. In general high DOC concentrations and the highest mean peak values (A, C, B1, T220) were found (Tab. 3).

Table 3 Descriptive statistics of each parameter for 4 Danube sections and the tributaries

Parameters	Section group	Mean	Standard deviation	Range
DOC	Danube delta	2.66	0.75	2.61
[mg L^{-1}]	Lower Danube	2.55	0.55	2.78
	Middle Danube	2.16	0.38	2.03
	Upper Danube	3.19	1.31	5.89
	Tributaries	3.64	3.10	16.55
Chlorophyll-a	Danube delta	6.93	2.15	5.93
[µg L^{-1}]	Lower Danube	4.75	2.95	10.42
	Middle Danube	15.16	8.30	34.57
	Upper Danube	8.29	6.53	20.39
	Tributaries	14.75	13.75	50.61
Peak A	Danube delta	709.2	19.4	45.0
[Int.]	Lower Danube	687.7	50.5	207.9
	Middle Danube	695.5	99.7	305.5
	Upper Danube	735.4	101.4	308.9
	Tributaries	917.7	360.6	1131.9
Peak C	Danube delta	219.1	15.2	32.1
[Int.]	Lower Danube	198.1	3.2	11.0
	Middle Danube	199.9	25.6	97.4
	Upper Danube	202.3	45.6	108.4
	Tributaries	290.4	144.0	493.2
Peak B1	Danube delta	629.3	36.5	82.4
[Int.]	Lower Danube	656.3	49.0	183.0
	Middle Danube	598.2	163.3	711.2
	Upper Danube	694.0	206.9	659.2
	Tributaries	815.4	399.4	1493.1
Peak T220	Danube delta	723.2	16.0	35.0
[Int.]	Lower Danube	744.5	51.1	186.6
	Middle Danube	718.5	161.7	701.3
	Upper Danube	812.0	209.4	657.3
	Tributaries	974.2	428.5	1469.5
FI	Danube delta	1.38	0.03	0.06
	Lower Danube	1.39	0.02	0.07
	Middle Danube	1.42	0.03	0.09
	Upper Danube	1.44	0.04	0.12
	Tributaries	1.44	0.07	0.24
HIX	Danube delta	2.27	0.07	0.15
	Lower Danube	2.16	0.10	0.34
	Middle Danube	2.15	0.18	0.80
	Upper Danube	2.03	0.37	0.99
	Tributaries	2.22	0.65	2.11
β/α ratio	Danube delta	0.88	0.01	0.03
	Lower Danube	0.87	0.02	0.08
	Middle Danube	0.84	0.03	0.16
	Upper Danube	0.81	0.03	0.10
	Tributaries	0.85	0.05	0.16

Table 4 Kruskal Wallis Test with the four different Danube Sections and additional pairwise comparison with an oneway ANOVA. Level of significance: 0.05.

	Kruskal Wallis Test				One - way ANOVA after Kruskall Wallis (k sample)					
					Upper Danube - Middle Danube	Upper Danube - Lower Danube	Upper Danube - Danube Delta	Middle Danube - Lower Danube	Middle Danube - Danube Delta	Danube Delta - Lower Danube
	N	df	χ^2	p	adj. Sig.	adj. Sig.	adj. Sig.	adj. Sig.	adj. Sig.	adj. Sig.
DOC	141	3	36.35	**0.000**	**0.001**	1.000	1.000	**0.000**	**0.006**	1.000
peak A	146	3	9.03	**0.028**	1.000	1.000	0.516	0.417	**0.038**	0.893
peak C	146	3	33.26	**0.000**	0.135	**0.002**	**0.000**	0.340	**0.000**	**0.022**
peak B1	146	3	32.45	**0.000**	**0.008**	0.775	1.000	**0.000**	0.071	1.000
peak T220	146	3	21.01	**0.000**	0.064	1.000	1.000	**0.000**	0.151	1.000
FI	146	3	29.4	**0.000**	0.689	**0.000**	**0.004**	**0.000**	0.054	1.000
HIX	146	3	7.64	0.054						
β/α ratio	146	3	81.61	**0.000**	**0.019**	**0.000**	**0.000**	**0.000**	**0.000**	1.000

3.3 Fluorescence signals of organic pollution-impacted tributaries as compared to Danube sites

A review paper (Henderson et al., 2009) of recent literature demonstrated that by monitoring the fluorescence of dissolved organic matter (DOM), the ratios of humic-like (Peak C) and protein-like (Peak T) fluorescence peaks can be used to identify trace sewage contamination in river waters and estuaries. Additionally, strong correlations have been shown between Peak T and biochemical oxygen demand (BOD) in rivers, which is indicative of water impacted by microbial activity and therefore of sewage impacted systems.

We analyzed therefor the JDS 3 DOM data set whether we can find a distinct fluorescence signature to identify polluted tributaries or river sites in the Danube River Basin.

Figure 9 presents the tryptophan fluorescence intensity (T220) and the ratio of tryptophan/fulvic-like fluorescence ratio for all sampling locations.

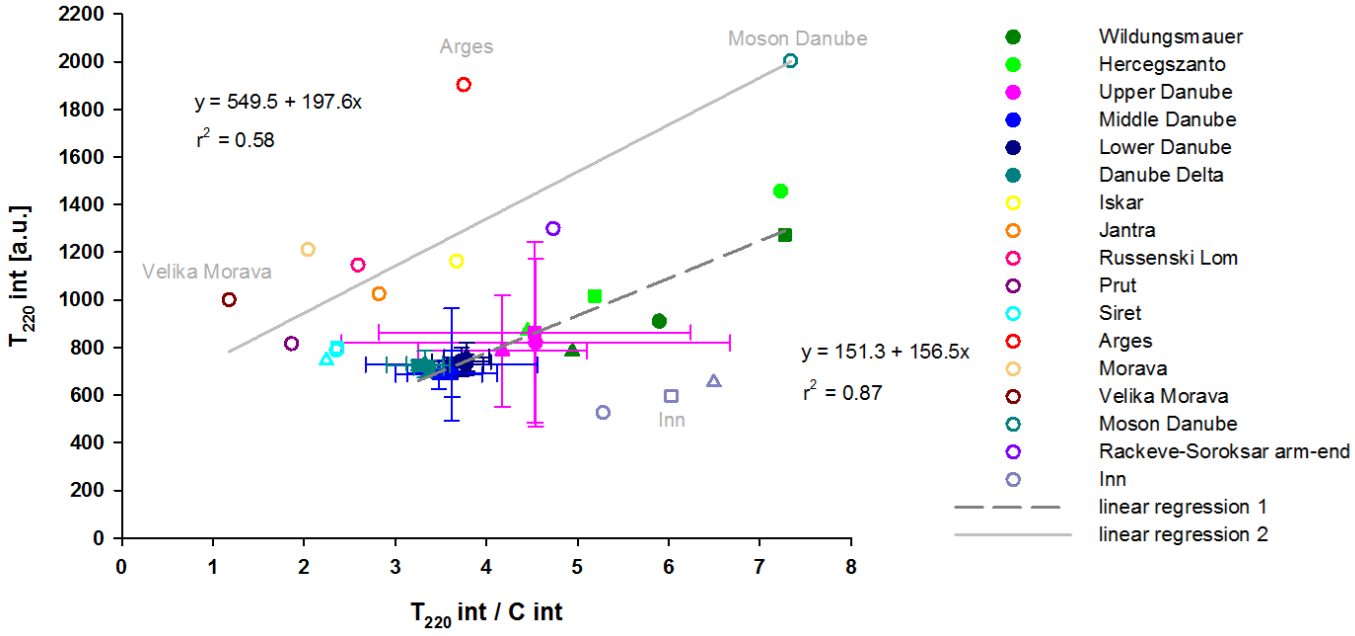

Fig. 9 Graph of tryptophan fluorescence intensity (T_{220} int) against tryptophan/fulvic-like fluorescence intensity (T_{220} int / C int). The two trend lines are linear regressions fitted to (i) pollution impacted tributaries and (ii) all other samples except tributary Inn. The dark green and light green symbols indicate two floodplain affected sampling sites (Wildungsmauer and Hercegszanto).

Pollution impacted tributaries were characterized by high tryptophan fluorescence intensity and a low to high ratio and can fit a linear trend line (r^2 0.58; p= 0.0024). The explanation of this linear trend is that on the one hand the tributaries show a different state of pollution (compare JDS 3 nutrient section) and also wastewater treatment plants situated next to the tributaries may use different treatment processes, which affect the residual fluorescence signal (Henderson et al, 2009). On the other hand these tributaries show different mean discharges (from 13.7 to 98.9 m s^{-1}), and thus, different levels of dilution.

The Danube main channel samples have a medium tryptophan/fulvic-like fluorescence ratio (median all sites: T220/C ratio 3.55) and also a low to medium tryptophan fluorescence intensity (median all sites: T220 703.7 int.). It is notable that floodplain impacted sites (e.g. Wildungsmauer and Hercegszanto) show also a high ratio and medium to high tryptophan fluorescence intensities, but still lower than from the pollution affected tributaries. This can be attributed to the fact that during connection of floodplains, aquatic bacteria can utilize reactive DOM from the river in these areas (Sieczko & Peduzzi, 2014). Therefor the microbial activity is elevated, which can be seen in the higher T220 signal in the inflow section of the floodplain area to the Danube River.

4 Conclusions

- Optical properties of DOM and DOC concentrations showed clear longitudinal patterns and differences for some sections and indicate the dominance of different sources such as waste water, terrestrial sources from the catchment and in-river sources
- The DOC concentrations were in the lower range typical for large, intensely used rivers
- DOM properties reflect the importance of in-river processes versus less input from the catchment for the JDS samples. An indication of severe organic pollution was not found for the Danube River, but in some tributaries DOM parameters point to higher pollution levels.
- The Danube sections showed significant differences for several DOM parameters, especially between the Middle and Lower Danube section.
- The Danube tributaries showed in several DOM parameters clearly different values compared to the respective Danube sections.
- There is evidence of all presented indices that besides terrestrial inputs, algal based sources significantly contribute to the overall carbon pool (as shown for the Upper Danube sections and this is in agreement with other findings as shown in the phytoplankton report).
- The results point to the low substrate availability and humic content in the upper section and more importance of terrestrial inputs in the lower parts. DOM of river water is influenced by site conditions and large scale patterns. Thus, these measurements are indicative for in-river processes (algal based sources) versus catchment effects and provide a link between water quality, catchment effects and biological components.

5 Acknowledgments

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