

Supplementary Materials

Following the occurrence and origin of titanium dioxide nanoparticles in the Sava River by single particle ICP-MS

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Table S1: Information about the Sava River sampling sites, summarized from the study of Milačić et. al. [1] and Sanchis et al. [2].

Sampling label	Sampling site	Country	Altitude (m)	Distance to the outfall (km)	Collected samples	Potential stressors identified
MOJ	Mojstrana	Slovenia	661	990	Water, sediment	none
RAD	Radovljica	Slovenia	409	830	Water, sediment	small urban area, metal industry
LIT1	Litija 1	Slovenia	225	810	Water, sediment	small urban area, potential impact from VRH dam
LIT2	Litija 2	Slovenia	225	810	Water	
VRH	Vrhovo	Slovenia	194	750	Water, sediment	hydropower plant, potential impact from different industries nearby
CAT	Čatež	Slovenia	137	736	Water, sediment	rural/agricultural area
ZAG	Zagreb	Croatia	110	644	Water, sediment	large urban area, industrial activities
JAS	Jasenovac	Croatia	87	500	Water, sediment	intense agricultural activities
SLB	Slavonski Brod	Croatia	82	360	Water, sediment	oil refinery, metal and other industries, large urban area
ZUP	Županja	Croatia	77	262	Water, sediment	intense agricultural activity, oil refinery, metal and other industries, mining
SRM1	Sremska Mitrovica 1	Serbia	72	118	Water, sediment	untreated wastewater, intense agricultural activity, oil refinery, urban area, industry
SRM2	Sremska Mitrovica 2	Serbia	72	118	Water	
SAB1	Šabac 1	Serbia	71	106	Water, sediment	untreated wastewater, urban area, metal and other industries
SAB2	Šabac 2	Serbia	71	106	Water	
BEO	Beograd	Serbia	69	0	Water, sediment	untreated wastewater, urban area, industrial activities

Figure S1: Particle size distribution of TiO₂NPs determined in non-treated and treated (3 min bath sonication) river water sample collected at BEO sampling site (bin size: 4 nm). 271 ± 17 and 1035 ± 6 particles/min (N=2) were detected in the non-treated and treated water sample, respectively. Prior to splCP-MS analysis, river water sample was 20-times diluted with water.

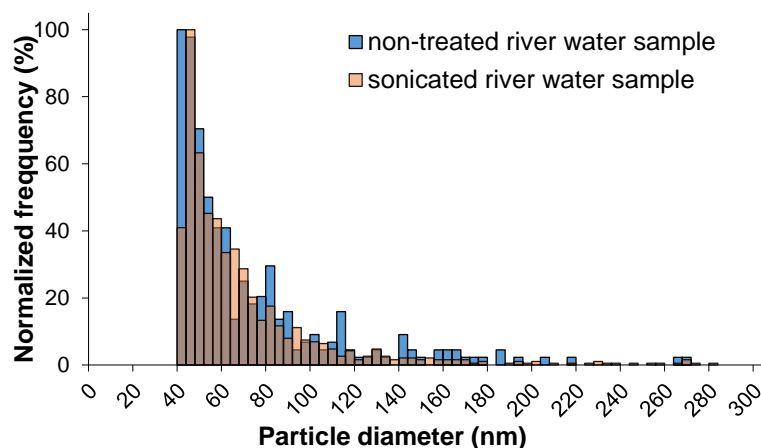


Table S2: Instrumental parameters for splCP-MS analysis of TiO₂ and Al-containing NPs.

Parameter/Isotope	Ti	Al
Sample introduction		
Nebuliser	MicroMist, concentric	
Spray chamber	Quartz, double-spray, Scott type	
Skimmer and sampler cone	Ni	
Sample depth	8 mm	
Sample uptake flow rate	0.300–0.330 mL min ⁻¹ ^a	
Plasma conditions		
Forward power	1550 W	
Plasma gas flow	15.0 L min ⁻¹	
Carrier gas flow (Ar)	1.05 L min ⁻¹	
Auxiliary gas flow	0.90 L min ⁻¹	
Cell parameters		
Cell gas flow rate	5.0 mL min ⁻¹ H ₂ , 5 % O ₂	4.3 mL min ⁻¹ He
Octopole bias (V)	-5.0	-18.0
Octopole RF (V)	190	200
KED (V)	-7.0	5.0
Data acquisition parameters		
Data acquisition mode	Time resolved analysis	
Integration time per isotope	3 ms	
Total acquisition time per isotope	60 s	
Scan type	MS/MS	SQ
Isotopes monitored	Q ₁ = ⁴⁸ Ti ⁺ , Q ₂ = ⁴⁸ Ti ¹⁶ O ⁺	Q ₁ = Q ₂ = ²⁷ Al ⁺

^a Determined on a daily basis at peristaltic pump speed of 0.1 rps

Preparation of SRM 1898 standard solution

SRM 1898 standard solution was prepared daily by weighing approximately 50 mg of SRM 1898 powder into 50 mL MilliQ water, resulting in 1 mg/ml TiO₂NPs suspension. Suspension was sonicated with the use of probe sonicator, operated at 80% amplitude for 15 min in pulse operation mode (corresponding to 17 kJ of delivered energy). During the sonication, vial was placed into an ice bath to prevent heating of the suspension. SRM 1898 suspension was diluted 20 million-times with MilliQ water to achieve TiO₂NPs concentration of 50 ng/L prior to spICP-MS analysis. This resulted in around 1000 particles detected per min. Blank sample, i.e. MilliQ water that was sonicated, diluted and analysed under the same conditions as SRM 1898, was also prepared to evaluate the release of Ti-bearing particles from the sonicator probe. Around 17-31 particles per min were detected in the blanks samples and subtracted from the values of SRM 1898 sample.

Figure S2: Particle size distribution of TiO₂NPs in 50 ng/L SRM 1898 suspension, determined by spICP-MS/MS method. Bin size: 2 nm.

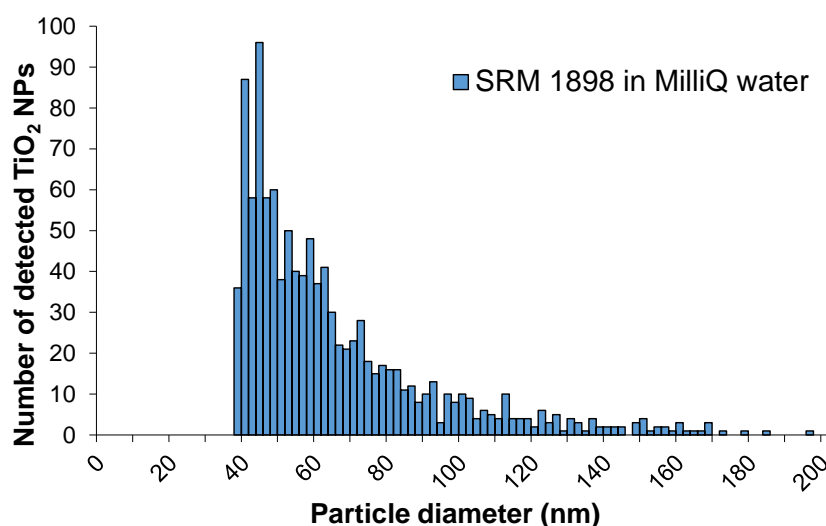


Table S3: Mass recovery, number concentration (expressed as particle number per gram of TiO₂ powder) and mean particle diameter of TiO₂NPs in SRM 1898 solution, determined by spICP-MS/MS on each day of the sample analysis. Result from each day represents an average \pm standard deviation of at least three replicate measurements (N=3).

Analysis day	NP mass recovery (%)	NP number concentration (10 ¹⁴ particles/g)	Mean particle diameter (nm)
#1	93 \pm 5	8.7 \pm 0.4	63.7 \pm 0.5
#2	75 \pm 3	8.0 \pm 0.6	65.0 \pm 1.5
#3	116 \pm 5	12.0 \pm 0.3	65.9 \pm 1.5
#4	121 \pm 9	11.6 \pm 0.8	64.3 \pm 1.2
#5	100 \pm 14	8.4 \pm 0.5	65.1 \pm 1.7
#6	94 \pm 15	10.2 \pm 1.6	60.1 \pm 1.4
#7	104 \pm 24	11.4 \pm 0.4	58.7 \pm 0.6
#8	110 \pm 14	10.0 \pm 1.3	64.8 \pm 1.4
#9	102 \pm 8	13.5 \pm 2.3	59.9 \pm 4.7
#10	66 \pm 7	8.0 \pm 0.4	60.7 \pm 2.1
Mean value	98	10.2	62.8
Standard deviation	16	1.79	2.5
Relative standard deviation	16.6 %	17.6 %	4.0 %

Table S4: Information on particle size distribution of TiO₂NPs detected in river water samples by spICP-MS. Data represent the mean \pm STD of four replicate samples (N=4)

Sampling site	Average particle diameter (nm)	Median particle diameter (nm)	Minimum particle diameter	Maximum particle
MOJ			not detected	
RAD	63 \pm 4	45 \pm 2	32	345 \pm 76
LIT1			not detected	
LIT2			not detected	
VRH	51 \pm 2	37 \pm 3	28	353 \pm 76
CAT	55 \pm 1	42 \pm 1	31	279 \pm 32
ZAG	51 \pm 1	40 \pm 2	31	277 \pm 67
JAS	62 \pm 1	48 \pm 1	34	713 \pm 100
SLB	60 \pm 2	48 \pm 1	36	409 \pm 67
ZUP	54 \pm 1	44 \pm 0	33	364 \pm 56
SRM1	54 \pm 2	43 \pm 1	33	376 \pm 131
SRM2	59 \pm 2	44 \pm 2	31	409 \pm 52
SAB1	61 \pm 2	50 \pm 1	37	444 \pm 68
SAB2	58 \pm 3	46 \pm 1	35	370 \pm 88
BEO	55 \pm 1	43 \pm 1	33	358 \pm 75

Table S5: Comparison of mass concentrations of Ti-containing NPs (Ti-NPs), ionic Ti and total Ti (calculated as sum of Ti-NPs and ionic Ti concentrations), determined in diluted river waters by spICP-MS, and total Ti mass concentration determined in the acid-digested river waters by conventional ICP-MS. Data represent the mean \pm STD of four replicate samples (N=4) for spICP-MS analysis and two replicate samples (N=2) for conventional ICP-MS analysis.

Analytical procedure	spICP-MS				Conventional ICP-MS	spICP-MS and conventional ICP-MS
Sampling site	Ti-NPs [$\mu\text{g/L}$]	Ti ⁺ + Ti-NPs <LOD _{size} [$\mu\text{g/L}$]	Total Ti [$\mu\text{g/L}$]	Ti-NPs/total Ti* [%]	Total Ti [$\mu\text{g/L}$]	Total Ti (spICP-MS/conventional ICP-MS)** [%]
MOJ	<LOQ ^a	<LOQ ^b	/	/	<LOD ^c	/
RAD	0.106 \pm 0.050	0.014 \pm 0.000	0.120 \pm 0.008	88.3	<LOD ^c	/
LIT1	<LOQ ^a	<LOQ ^b	/	/	3.51 \pm 0.10	/
LIT2	<LOQ ^a	<LOQ ^b	/	/	<LOD ^c	/
VRH	4.34 \pm 1.32	<LOQ ^b	4.34 \pm 1.32	100	54.2 \pm 3.7	8.01
CAT	0.129 \pm 0.023	0.017 \pm 0.004	0.144 \pm 0.025	89.4	7.78 \pm 0.06	1.86
ZAG	0.108 \pm 0.025	0.017 \pm 0.001	0.125 \pm 0.025	86.6	7.44 \pm 0.04	1.68
JAS	3.10 \pm 0.76	0.069 \pm 0.002	3.17 \pm 0.76	97.8	47.9 \pm 2.0	6.60
SLB	1.18 \pm 0.31	0.094 \pm 0.004	1.27 \pm 0.31	92.6	33.8 \pm 0.4	3.77
ZUP	0.601 \pm 0.127	0.074 \pm 0.006	0.675 \pm 0.133	89.0	41.1 \pm 1.8	1.64
SRM1	0.298 \pm 0.191	0.035 \pm 0.001	0.333 \pm 0.191	89.6	8.34 \pm 0.34	3.99
SRM2	0.226 \pm 0.030	<LOQ ^b	0.226 \pm 0.030	99.6	10.2 \pm 0.5	2.22
SAB1	0.426 \pm 0.046	0.057 \pm 0.004	0.483 \pm 0.044	88.2	12.3 \pm 1.7	3.92
SAB2	0.444 \pm 0.182	0.043 \pm 0.002	0.487 \pm 0.184	91.2	18.4 \pm 1.6	2.64
BEO	0.261 \pm 0.088	0.027 \pm 0.001	0.288 \pm 0.087	90.5	69.1 \pm 7.2	0.42

*Ti-NPs mass concentration was normalized to the total Ti mass concentration, both determined by spICP-MS.

**Total Ti mass concentration determined by spICP-MS was normalized to the total Ti mass concentration determined by conventional ICP-MS analysis.

LOQ^a: Limit of quantification for determination of Ti-NPs by spICP-MS was calculated to be 0.016 $\mu\text{g/L}$.

LOQ^b: Limit of quantification for determination of ionic Ti by spICP-MS was calculated to be 0.013 $\mu\text{g/L}$.

LOD^c: Limit of detection for determination of total Ti by conventional ICP-MS was calculated to be 2.50 $\mu\text{g/L}$ (3 x STD of 10 blank samples).

Figure S3: Spearman correlation between the TiO₂NPs mass concentration determined in water by splCP-MS and Ti mass concentration in water associated to SPM. Ti concentration in water associated to SPM was calculated by multiplying SPM concentration determined in river water (re-used from previously published data [1]) by the Ti mass concentration determined in the SPM as described in the work by Vidmar et al. [3]. Log-log plot.

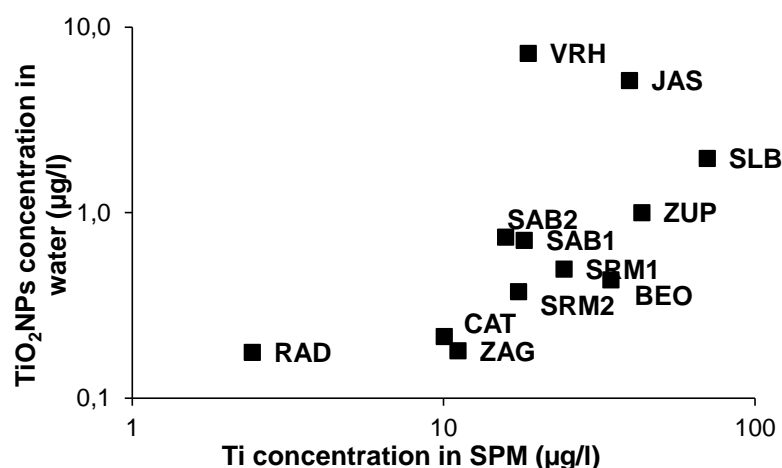


Table S6: Particle mass and number concentration as well as mean particle diameter for TiO₂NPs in non-spiked and spiked (with SRM 1898) sediment samples. Results were obtained after applying extraction procedure, followed by splCP-MS analysis. NP mass and number recovery were calculated based on the expected particle mass (1000 µg TiO₂NPs /g sediment) and number concentration (1.0×10^{12} TiO₂NPs /g sediment) in the spiked sediment samples. Results represent average \pm standard deviation of three replicates (N=3).

Sample	TiO ₂ NPs mass concentration (µg/g)	TiO ₂ NPs number concentration (#particles/g)	Mean particle diameter (nm)
BCR 320R	1148 \pm 133	(1.08 \pm 0.05) $\times 10^{12}$	66.7 \pm 1.9
SRM 1898 spiked to BCR 320R	2107 \pm 309	(2.10 \pm 0.07) $\times 10^{12}$	66.5 \pm 0.7
TiO₂NPs recovery (%)	100 \pm 14	102 \pm 4	/
Sediment MOJ	333 \pm 90	(0.46 \pm 0.09) $\times 10^{12}$	57.7 \pm 2.9
SRM 1898 spiked to Sediment MOJ	1257 \pm 158	(1.28 \pm 0.03) $\times 10^{12}$	68.0 \pm 0.6
TiO₂NPs recovery (%)	100 \pm 12	91 \pm 3	/

Figure S4: Particle size distributions of TiO₂NPs (SRM 1898) spiked to BCR-320R sediment reference material, determined by spICP-MS analysis after applying extraction procedure. Particle size distributions of a TiO₂NPs suspension (SRM 1898) in MilliQ water is shown for comparison. Bin size: 2 nm.

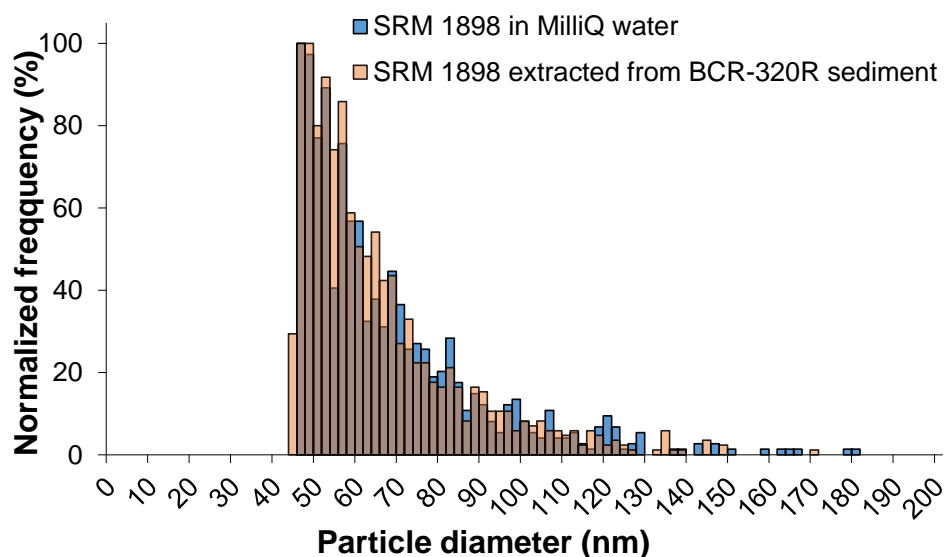


Table S7: Information on particle size distribution of TiO₂NPs detected in sediment extracts by spICP-MS. Data represent the mean \pm STD of six replicate samples (N=6)

Sampling site	Average particle diameter (nm)	Median particle diameter (nm)	Minimum particle diameter	Maximum particle
MOJ	59 \pm 3	50 \pm 3	38	247 \pm 61
RAD	60 \pm 2	50 \pm 1	37	274 \pm 70
LIT1	59 \pm 2	49 \pm 2	36	341 \pm 92
VRH	61 \pm 2	52 \pm 2	39	234 \pm 25
CAT	64 \pm 3	55 \pm 2	41	261 \pm 31
ZAG	63 \pm 2	54 \pm 2	42	224 \pm 30
JAS	62 \pm 4	53 \pm 3	41	241 \pm 35
SLB	65 \pm 2	56 \pm 2	44	232 \pm 64
ZUP	67 \pm 3	58 \pm 1	45	276 \pm 95
SRM1	64 \pm 2	55 \pm 2	42	250 \pm 35
SAB1	65 \pm 1	56 \pm 1	43	239 \pm 49
BEO	66 \pm 1	57 \pm 2	44	225 \pm 37

Table S8: Comparison of mass concentrations of Ti-containing NPs (Ti-NPs), ionic Ti and total Ti (calculated as sum of Ti-NPs and ionic Ti concentrations), determined in sediment extracts by spICP-MS, and total Ti mass concentration determined in the acid-digested bulk sediments by conventional ICP-MS. Data represent the mean \pm STD of six replicate samples (N=6) for spICP-MS analysis and two replicate samples (N=2) for conventional ICP-MS analysis.

Analytical procedure	spICP-MS				Conventional ICP-MS	spICP-MS and conventional ICP-MS
Sampling site	Ti-NPs [$\mu\text{g/g}$]	Ti ⁺ + Ti-NPs <LOD _{size} [$\mu\text{g/g}$]	Total Ti [$\mu\text{g/g}$]	Ti-NPs/total Ti* [%]	Total Ti [$\mu\text{g/g}$]	Total Ti (spICP-MS/conventional ICP-MS)** [%]
MOJ	219 \pm 56	30 \pm 11	249 \pm 66	88.0	367 \pm 28	68.0
RAD	431 \pm 104	56 \pm 7	486 \pm 102	88.6	1261 \pm 30	38.6
LIT1	513 \pm 113	33 \pm 10	547 \pm 115	93.9	957 \pm 44	57.1
VRH	400 \pm 61	85 \pm 11	485 \pm 61	82.5	1263 \pm 65	38.4
CAT	462 \pm 91	122 \pm 13	584 \pm 101	79.1	1150 \pm 34	50.8
ZAG	407 \pm 41	152 \pm 14	559 \pm 46	72.7	949 \pm 45	58.9
JAS	522 \pm 82	247 \pm 91	769 \pm 160	67.9	1319 \pm 233	58.3
SLB	418 \pm 64	237 \pm 13	656 \pm 65	63.8	1721 \pm 163	38.1
ZUP	490 \pm 127	331 \pm 45	821 \pm 165	59.6	3189 \pm 44	25.8
SRM1	413 \pm 84	199 \pm 14	613 \pm 94	67.5	1734 \pm 26	35.3
SAB1	446 \pm 52	234 \pm 7	681 \pm 53	65.5	1309 \pm 152	52.0
BEO	435 \pm 36	287 \pm 10	722 \pm 41	60.3	1241 \pm 148	58.2

*Ti-NPs mass concentration was normalized to the total Ti mass concentration, both determined by spICP-MS.

**Total Ti mass concentration in sediment extracts determined by spICP-MS was normalized to the total Ti mass concentration determined in acid-digested bulk sediments by conventional ICP-MS analysis.

Figure S5: Correlation between TiO_2NPs mass concentration in water and sediment samples determined by spICP-MS and calculated by A) including all sampling locations and B) excluding VRH and SLB sampling sites. Log-log plot.

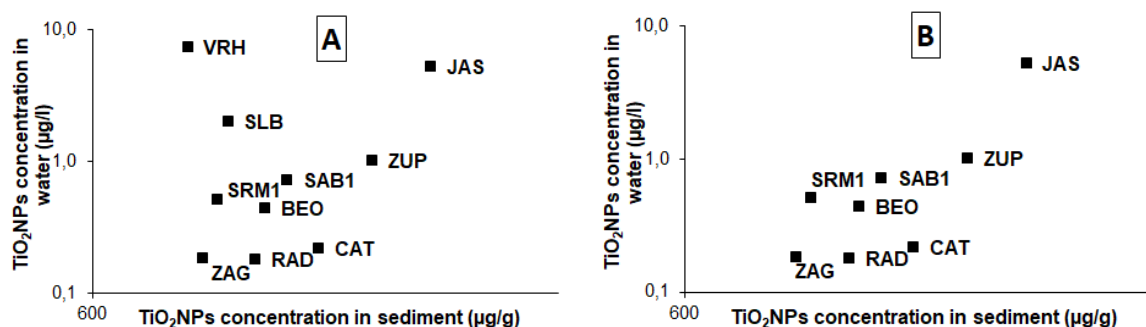


Figure S6: Correlation between total the Ti mass concentration in acid-digested water and sediment samples determined by conventional ICP-MS and calculated by A) including all sampling locations and B) excluding VRH and BEO sampling sites. Log-log plot.

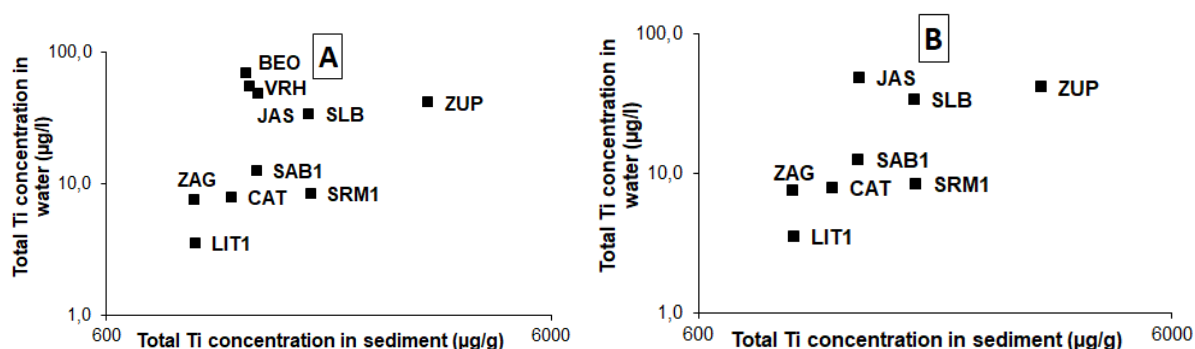


Figure S7: Correlation between TiO_2NPs mass concentration in water and sediment fraction $< 63 \mu\text{m}$. Log-log plot.

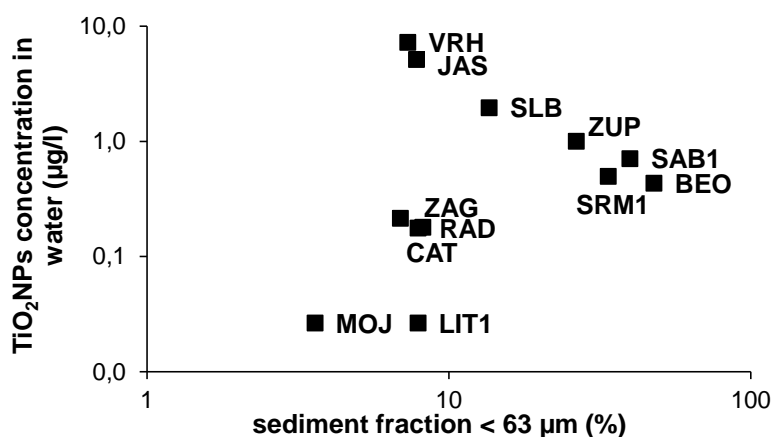
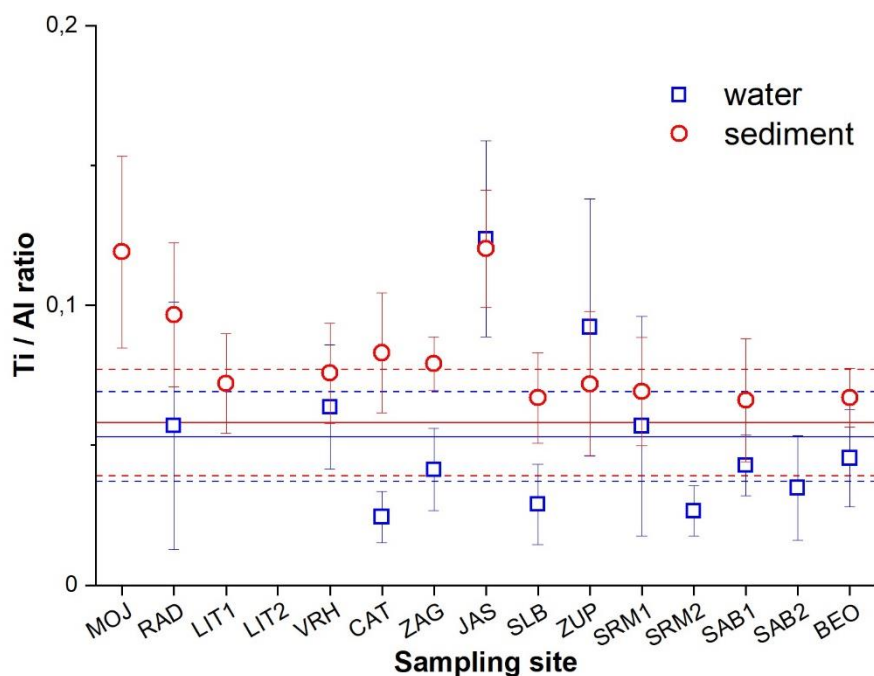


Figure S8: Ti/Al ratios determined in NPs in water (blue squares) and sediment extracts (red circles) at each sampling location. Each data represents the mean \pm STD of N replicates (N=4 for water and N=6 for sediment samples). As a reference, natural background Ti/Al ratio in SPM and natural background Ti/Al ratio in bulk sediment are presented as median values (solid line) \pm SD (dotted line).



References

- (1) Milačič, R.; Zuliani, T.; Vidmar, J.; Oprčkal, P.; Ščančar, J. Potentially Toxic Elements in Water and Sediments of the Sava River under Extreme Flow Events. *Sci. Total Environ.* **2017**, *605–606*, 894–905.
- (2) Sanchís, J.; Milačič, R.; Zuliani, T.; Vidmar, J.; Abad, E.; Farré, M.; Barceló, D. Occurrence of C60 and Related Fullerenes in the Sava River under Different Hydrologic Conditions. *Sci. Total Environ.* **2018**, *643*, 1108–1116.
- (3) Vidmar, J.; Zuliani, T.; Novak, P.; Drinčić, A.; Ščančar, J.; Milačič, R. Elements in Water, Suspended Particulate Matter and Sediments of the Sava River. *J. Soils Sediments* **2017**, *17*, 1917–1927.