Supporting information

Sorption behaviour of psycho-active drugs onto sediment

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1 Sediment sampling

River sediment samples were taken at 9 different sites in Western Germany (Burgen, Dausenau, Lippe, Emscher, Wupper, Landgraben, Bieber, Rodau, Schwarzbach). Locations are shown in Figure S1. Except for the sites Burgen und Dausenau sediment samples were randomly taken from the sediment surface of the stream by a grabbing device (van Veen) which takes a sample at depths up to 15 cm, depending on the sediment thickness. Sediment samples from the Burgen and Dausenau sites were taken manually by a shovel at depth up to 10 cm. They were air-dried and sieved < 2mm prior to batch experiments.

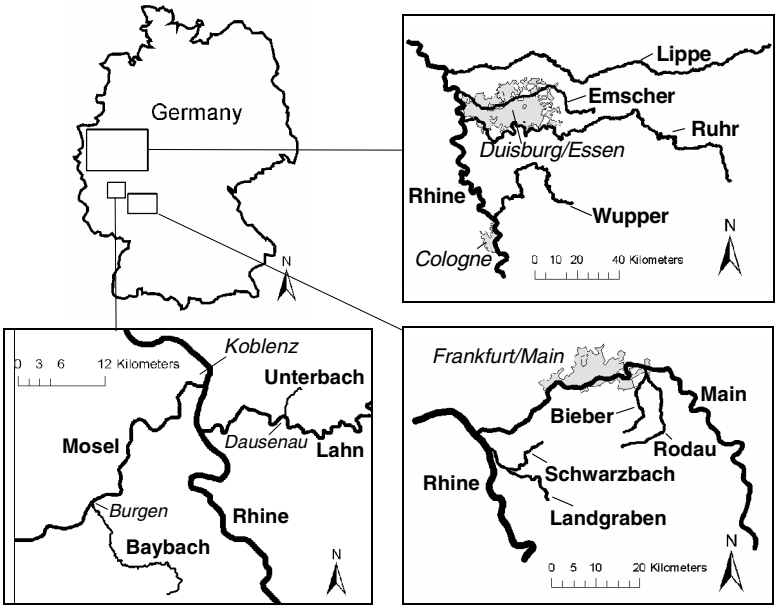

Figure S 1. Sediment sampling locations

2 Texture and organic carbon content of the examined sediments

Texture and organic carbon content of the solid matrices are summarized in Table S1.

Table S 1. Texture and organic carbon content of the examined sediments

Sediment	Burgen	Dause-nau	Lippe	Emscher	Wupper	Land-graben	Bieber	Rodau	Schwarz-bach	Schlau-engraben	Murn	reference sediment (Rhine)
Texture [mass% of dry weight] (DIN 19683-2)												
Sand	90	53	76	63	21	42	68	75	71	51	49	5
Clay + Silt	10[a]	47[b]	24	37	79	58	32	25	29	22	78	95
Total organic carbon (TOC) [mass% of dry weight]	0.74	4.36	1.47	1.89	7.6	3.97	3.56	1.72	4.03	1.3	3.2	---

[a]: clay: 4%; [b]: clay: 18%

3 Detailed description of the analytical procedures

3.1 Reference material and standards

Carbamazepine (Sigma, Deisenhofen, Germany), 10,11-dihydrocarbamazepine (Alltech, USA), 10,11-dihydro-10,11-dihydroxycarbamazepine (µ-Mol, Luckenwalde, Germany), diazepam, oxazepam, temazepam (Sigma, Deisenhofen, Germany), morphine as an ingredient of opium (Sigma-Aldrich, Steinheim, Germany), codeine, dihydrocodeine (Th. Geyer, Renningen, Germany), tramadol (Fluka, Buchs, Switzerland). We included sulfamethoxazole (Sigma, Deisenhofen, Germany) and its main metabolite N4-acetylsulfamethoxazole (EAWAG self-synthesis, Dübendorf, Switzerland) to allow referencing of the findings to previous work on the sorption of sulfonamides. Codeine-d_6, diazepam-d_5, morphine-d_6, nordiazepam-d_5 (Cambridge Isotopes Lab., Saarbrücken, Germany), oxazepam-d_6 (Sigma, Deisenhofen, Germany), carbamazepine-$^{13}C^{15}N$ (Campro Scientific, Berlin, Germany), sulfamethoxazole-d_4, and N4-acetylsulfamethoxazole-d_4 (Toronto Research Chemicals, North York, ON, Canada)

3.2 Detailed description of the solid phase extraction (SPE)

SPE was carried out at neutral pH using OASIS HLB 200 (200 mg, Waters, Milfort, USA). The OASIS HLB 200 cartridges (200 mg, Waters, Milfort, USA) were conditioned with 1 x 2 mL n-heptane, followed by 1 x 2 mL acetone, 3 x 2 mL methanol and 4 x 2 mL groundwater (pH 7). The water samples were then passed through the pre-conditioned SPE-cartridges with a flow rate of approx. 20 mL/min. Subsequently, the solid phase material was dried completely by a nitrogen stream for one hour and the analytes were eluted four times with 2 mL acetone. The combined extracts were evaporated to about 100 µL by a gentle nitrogen stream, then 100 µL methanol was added and the extract was again evaporated to 100 µL in order to remove most of the acetone. The remaining extract was filled up to 1 mL with 900 µL Milli-Q-water. Finally, detection was performed via LC-electrospray tandem MS (HPLC: Agilent 1100 from Agilent Technologies, Waldbronn, Germany; API 4000, Applied Biosystems, Foster City, CA, USA) using a 150 x 3 mm Synergi 4 µm Polar-RP 80 Å column (phenomenex®, Aschaffenburg, Germany).

3.3 Detailed description of the chromatographic LC and tandem MS conditions

The samples extracts (50 µL) were injected into the LC system (Agilent 1100 with degasser, quaternary pump and autosampler, Agilent Technologies, Waldbronn, Germany) using acetonitrile (A) and 10 mM ammonium formiate in water, adjusted to pH 4 with formic acid (B), as a mobile phase. The following gradient programme

was used: Start of the run with 10% A / 90% B, kept isocratic for 5 min, lineary increased to 80% A / 20% B within 13 min, returned to the initial conditions 10% A / 90% B within 2 min and kept isocratic for the last 10 min. During the analysis the flow rate was kept constant at 0.5 mL/min.

Table S 2. Composition and properties of groundwater used for the experiments

Parameter	**value**	**unit**
Visual appearance	clear	
Olfactory threshold	1	GSW
colouration (absorptance Hg 436 nm)	0.49	m^{-1}
turbidity	0.26	TE/F
Water temperature	13.6	°C
Air temperature	16.1	°C
conductivity	513	µS/cm
Redox potential (Pt, Ag, AgCl)	207	mV
pH	6.87	
pH after saturation with calcium carbonate	7.14	
Delta pH	-0.27	
Calcite capacity	53	mg/L
Dissolved oxygen	2.5	mg/L
Oxygen saturation index	25	%
Base capacity to pH 8.2	1.88	mmol/L
As dissolved carbon dioxide	82.7	mg/L
acid capacity to pH 8.2	n.d.	
acid capacity to pH 4.3	4.47	mmol/L
Carbonate hardness	12.5	°dH
Sum of alkaline earths	2.6	mmol/L
Total hardness	14.3	°dH
hardness (German Detergent Law)	3	
Sodium	14.1	mg/L
Potassium	4.95	mg/L
Calcium	77.9	mg/L
Magnesium	15.0	mg/L
Iron (total)	0.018	mg/L
Manganese	0.045	mg/L
Aluminium	<0.005	mg/L
Ammonium	0.09	mg/L
Nitrite	<0.005	mg/L
Nitrate	1.43	mg/L
Chloride	16.4	mg/L
Sulphate	28.5	mg/L
Phosphor (PO_4^{3-})	<0.04	mg/L
HCO_3^-	273	mg/L
Carbonate	n. d.	mg/L
Oxidation ability Mn VII>II calculated as O_2	0.13	mg/L
Oxidation ability Mn VII>II calculated as $KMnO_4$ consumption	0.51	mg/L
TOC	1.5	mg/L

3.4 Operating conditions of the LC tandem MS

The tandem MS was operated in positive electrospray ionization mode and was run in multiple reaction monitoring mode (MRM). Operational parameters were: collision gas, 41 kPa; curtain gas, 172 kPa; ion source gas 1 and ion source gas 2, both 275 kPa; source temperature, 450 °C; entrance potential, 10 V. The ionspray voltage was adjusted to 5.5 kV. Two MRM transitions for each substance were monitored for identification and quantification of the analytes. Parameters such as declustering potential, collision energy and cell exit potential were optimised in the auto-tuning program of the *Analyst* software. The retention times, the selected ion masses and the optimised parameters are listed in Table S3.

Table S 3. Precursor, product ions and retention times used in LC tandem MS detection

substances	retention time [min]	precursor ion [m/z]	product ion 1 (P1) [m/z]	product ion 2 (P2) [m/z]	DP [V]	CE (P1/P2) [eV]	CXP (P1/P2) [V]
Antiepileptics							
Carbamazepine	15.35	236.9	193.9	179.1	71	27/49	16/12
DHC	15.45	238.9	196.0	180.0	66	31/55	14/14
DHH	12.10	271.0	252.9	236.0	41	13/19	16/ 6
Tranquilizers							
Diazepam	17.80	284.9	221.9	193.0	76	45/34	14/20
Oxazepam	15.60	286.9	103.9	76.9	61	47/81	8/ 6
Temazepam	16.60	301.0	254.8	---	56	31	18
Opiates and opioids							
Morphine	5.20	285.9	201.0	152.0	86	35/77	6/14
Codeine	10.75	300.0	215.0	164.9	71	37/53	16/12
Dihydrocodeine	10.10	302.1	128.0	200.9	71	85/39	12/16
Tramadol	14.40	263.5	58.0	---	46	45	4
Antibiotics							
Sulfamethoxazole	13.75	253.9	155.8	188.0	66	23/21	12/14
N^4-Acetylsulfamethoxazole	13.90	296.1	134.0	197.9	81	35/25	12/14
Surrogate standards							
Carbamazepine ^{13}C ^{15}N	13.35	239.0	191.9	---	61	29	12
Diazepam d_5	17.75	290.0	198.0	261.9	91	45/31	14/ 8
Oxazepam d_6	15.60	291.9	235.9	109.0	81	31/49	20/ 8
Benzoylecgonine d_8	11.00	298.0	171.0	109.9	66	29/43	14/ 8
Morphine d_6	5.10	292.0	152.0	159.9	61	81/59	12/10
Codeine d_6	10.70	306.0	165.0	151.9	91	57/89	12/12
Sulfamethoxazole d_4	13.70	257.9	160.0	192.0	66	23/21	12/14
N^4-Acetylsulfamethoxazole d_4	13.90	301.0	202.7	139.0	81	27/37	18/16

DP = Declustering Potential CE = Collision Energy CXP = Cell Exit Potential

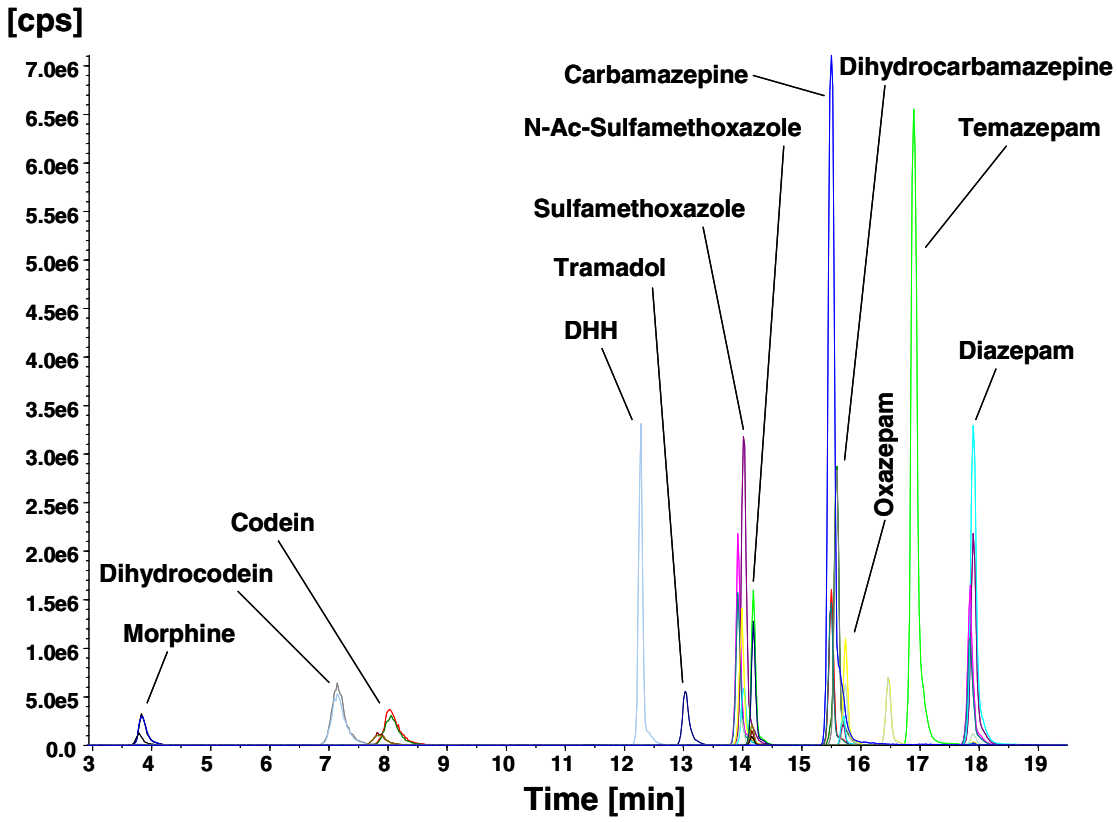

Figure S 2 Chromatogram of the analytes spiked with 200 ng to the reference sediment (rhine)

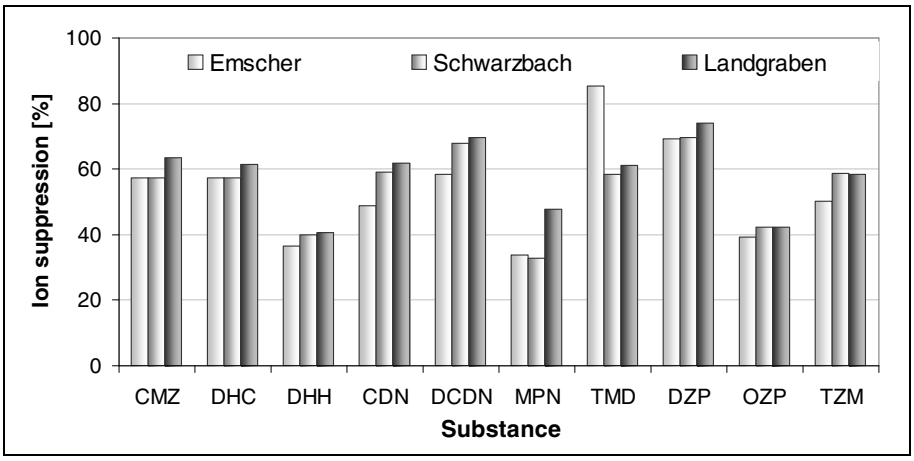

Figure S 3: Ion suppression in three different river sediments (CMZ – carbamazepine, DHC – dihydrocarbamazepine, DHH – dihydroxydihydrocarbamazepine, CDN – codeine, DCDN – dihydrocodeine, MPN – morphine, TMD – tramadol, DZP – diazepam, OZP – oxazepam, TZP – temazepam

3.5 Calibration

External calibration curves with 11 calibration points ranging from 0.5 to 2000 ng/mL were prepared by spiking MilliQ water. The linearity range was between 1 and 200 ng/mL. A quadratic fitting ($y = ax^2 + bx + c$) was used from 200 ng/mL to 2000 ng/mL (Table S4). In general, the correlation coefficients were higher than 0.993. Peak areas of the chromatograms were integrated and the ratios of the analyte/surrogate standards were calculated for each analyte. Resulting analyte concentrations were plotted versus the corresponding analyte peak areas or the respective ratios of analyte and surrogate standard peak areas.

Table S 4. Quadratic calibration parameters

substances	Calibration equation	r^2
Antiepileptics		
Carbamazepine	$y = -5.73x10^{-7} x^2 + 0.00466 x + 0.000737$	0.9993
DHC	$y = -1.45x10^{-6} x^2 + 0.0101x + 0.0019$	0.9993
DHH	$y = 1.6x10^{-6} x^2 + 0.0025 x + 0.000241$	0.9932
Tranquilizers		
Diazepam	$y = -3.8x10^{-7} x^2 + 0.00674 x + 0.00166$	0.9997
Oxazepam	$y = -2.16x10^{-6} x^2 + 0.0205 x + 0.00418$	0.9994
Temazepam	$y = -2.39 x10^{-4} x^2 + 0.907 x + 0.189$	0.9987
Opiates and opioids		
Morphine	$y = 1.76x10^{-6} x^2 + 0.0234 x + 0.0141$	0.9999
Codeine	$y = -9.52x10^{-7} x^2 + 0.0163 x + 1.49x10^{-7}$	0.9997
Dihydrocodeine	$y = -2.46x10^{-6} x^2 + 0.0276 x + 0.000189$	0.9992
Tramadol	$y = 7.46x10^{-7} x^2 + 0.00423 x + 0.000346$	0.9988
Antibiotics		
Sulfamethoxazole	$y = -1.91x10^{-7} x^2 + 0.00227 x – 5.48x10\text{-}9$	0.9994
N^4-Acetylsulfamethoxazole	$y = -1.11x10^{-5} x^2 + 0.0637 x + 0.0836$	0.9997

4 Environmental relevance

It was found that the sorbed fractions of carbamazepine and sulfamethoxazole are negligible compared to the dissolved fractions. However, the question arises whether these relative low sediment contamination levels are causing adverse effects for benthic organisms. Using the NOEC < 140 ng/g based on the reduced emerging rate found with *Chironomus riparius* (insect larvae) by Oetgen et al. (2) and the highest concentration in sediments of 49 ng/g found in this study, a MEC/PNEC of 17 is attained with an assessment factor of 50 (3). This ratio indicates a relatively high risk that adverse effects are caused by the presence of carbamazepine in sediments. For sulfamethoxazole effects at those low concentration levels are not described for sediment tests (3, 4). For most of the selected psycho-active drugs, even results of basic ecotoxicological test are currently not described in literature. The low sediment/water distribution coefficients (K_{OC} = 25-55.9 L/kg) of DHH and its elevated biodegradation (DT_{50}: 8d) in water-sediment-systems in comparison to carbamazepine (K_{OC} = 175,5-238,3 L/kg; DT_{50}: 328d (4)) might be the main reason that DHH was not found in sediments, although it was present at similar concentrations as carbamazepine in treated wastewater and river water (1,5). The K_d /K_{oc} values of sulfamethoxazole were comparable to DHH, but this antibiotic was detected in natural sediments at a few ng/g. The lack of biodegradation of sulfamethoxazole or better desorption of DHH may explain the presence of sulfamethoxazole and the absence of DHH in the natural sediments. Nevertheless, it is known that sulfamethoxazole can leach through soils without being significantly sorbed or degraded (6-8).

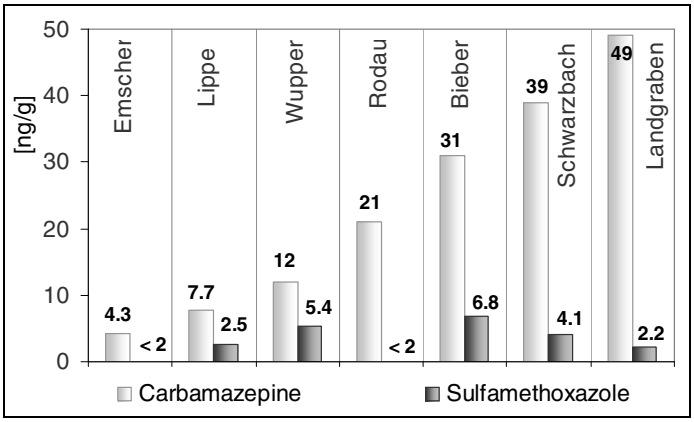

Figure S 4. Concentrations of carbamazepine and sulfamethoxazole in natural river sediments

5 Sorption kinetics

Since preliminary studies revealed that there might be losses of substances due to ageing or (bio)degradation (10,11), a total mass balance was calculated to assure that the data was not significantly influenced by those processes. For the sorption experiments, the sum of substance quantity detected in the sediment and the water phase was compared with the quantity spiked into the aqueous phase. For the desorption experiments, the sum of sorbed and dissolved quantities was compared with substance residues measured in the wet sediment after 24h of incubation. The solid-to-liquid ratios were 1/1 for CMZ, DHC, and DHH; 1/5 for DZP, OZP, TZP, CDN, DCDN, and TMD; and 1/3 for MPN on the Burgen sediment; and 1/25 for CMZ, DHC, DZP, OZP, TZP, and CDN; 1/10 for DHH, DCDN, and TMD; and 1/5 for MPN on the Dausenau sediment. Sediment masses between 1 and 10 g (± 0.01 g) and solution volumes (0.01 mol/L $CaCl_2$) between 10 to 50 mL (± 0.05 mL) were used.
Enclosed is the mass balance after 24h (Table S5) and the adsorption kinetics (Fig. S5-Fig. S14) of the target compounds in two different sediments with (right) and without (left) addition of sodium azide are shown.

Table S 5. Total mass balance in % (w/w) of the test substances for the sorption and desorption test period in both sediments after 24h [a]

Substance	Total mass balance in %			
	Burgen sediment		Dausenau sediment	
	Sorption	Desorption	Sorption	Desorption
Carbamazepine	93 ± 8	97 ± 8	105 ± 6	95 ± 22
DHC	93 ± 6	97 ± 6	103 ± 17	90 ± 21
DHH	106 ± 24	90 ± 21	133 ± 25	76 ± 19
Codeine	83 ± 4	126 ± 26	95 ± 11	84 ± 10
Dihydrocodeine	82 ± 8	144 ± 19	78 ± 10	112 ± 30
Tramadol	77 ± 11	108 ± 15	67 ± 10	129 ± 25
Diazepam	94 ± 9	139 ± 35	100 ± 8	102 ± 21
Oxazepam	93 ± 10	134 ± 22	106 ± 9	92 ± 27
Temazepam	86 ± 19	94 ± 44	96 ± 9	61 ± 12
Sulfamethoxazole	79 ± 10	55 ± 30	94 ± 11	58 ± 24
N^4-Acetylsulfamethoxazole	102 ± 8	104 ± 3	106 ± 4	84 ± 5

[a]: values reported as mean ± confidence interval (N=5, P=95%)

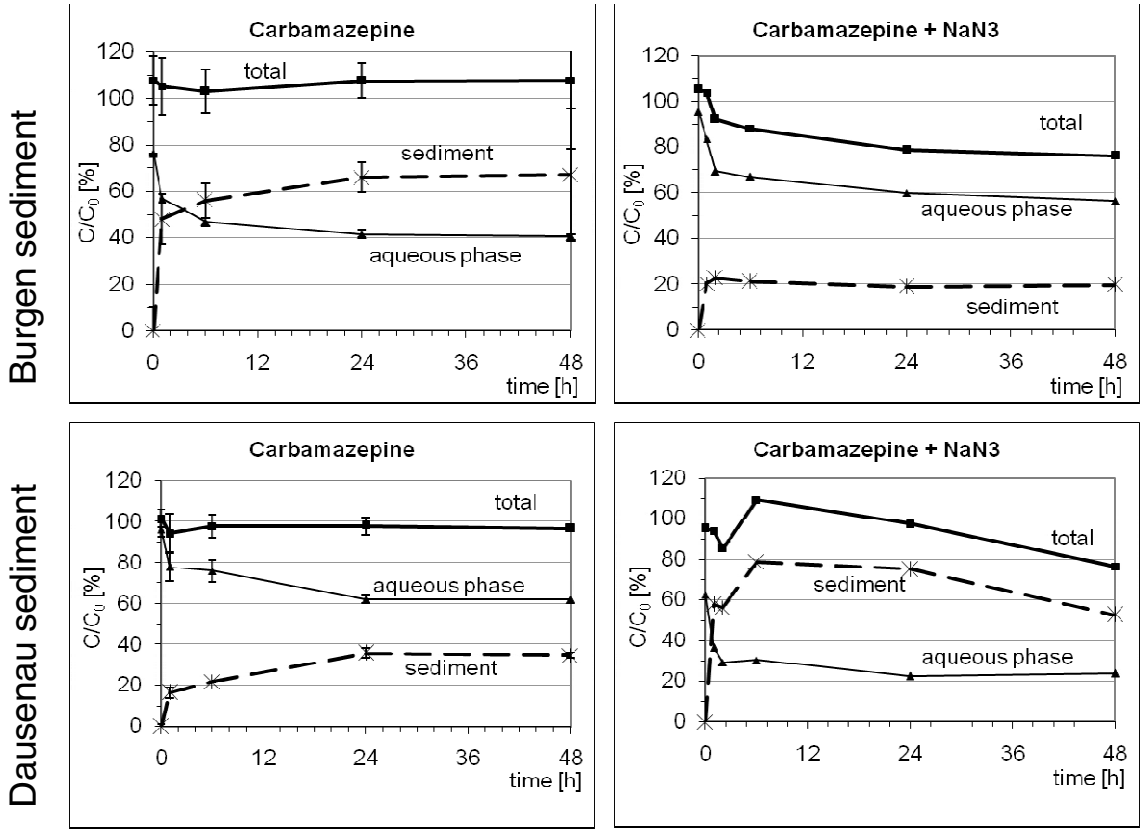

Figure S 5. Adsorption kinetics of carbamazepine in different sediments with (right) and without (left) addition of sodium azide

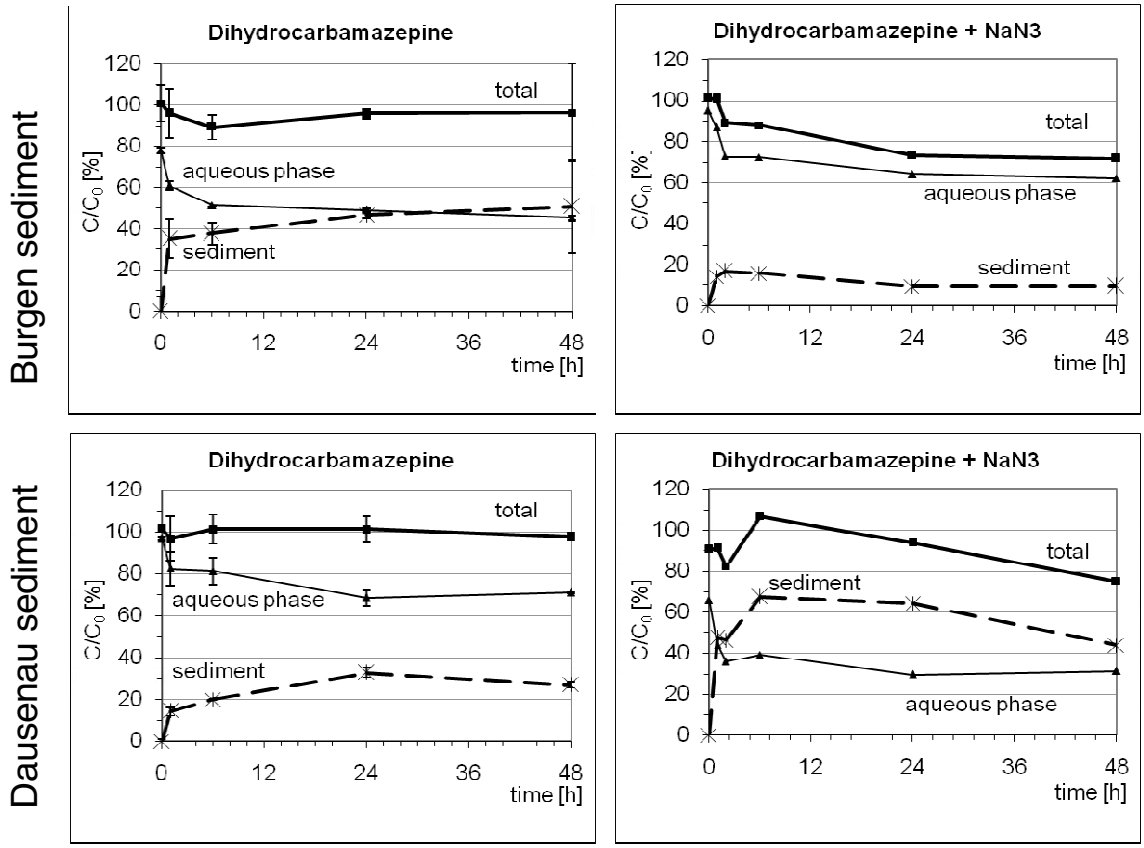

Figure S 6. Adsorption kinetics of dihydrocarbamazepine in different sediments with (right) and without (left) addition of sodium azide

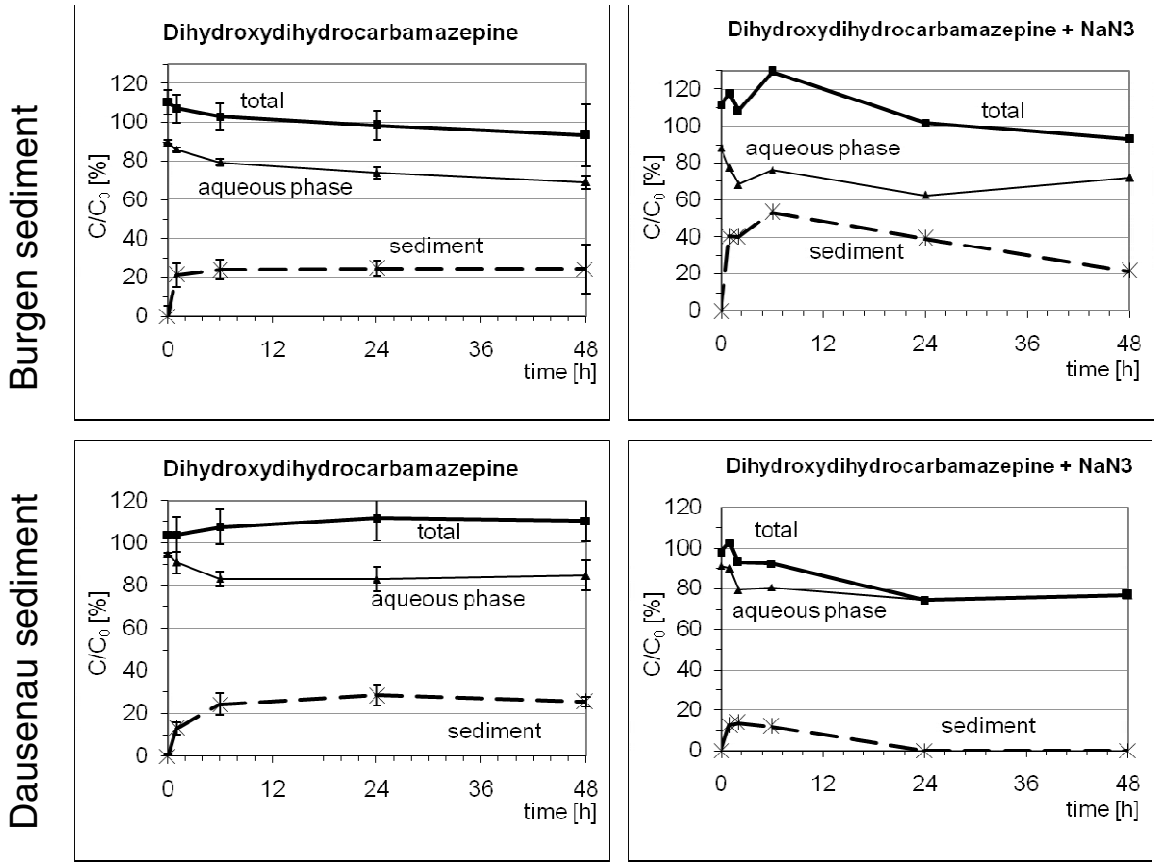

Figure S 7. Adsorption kinetics of dihydroxydihydrocarbamazepine in different sediments with (right) and without (left) addition of sodium azide

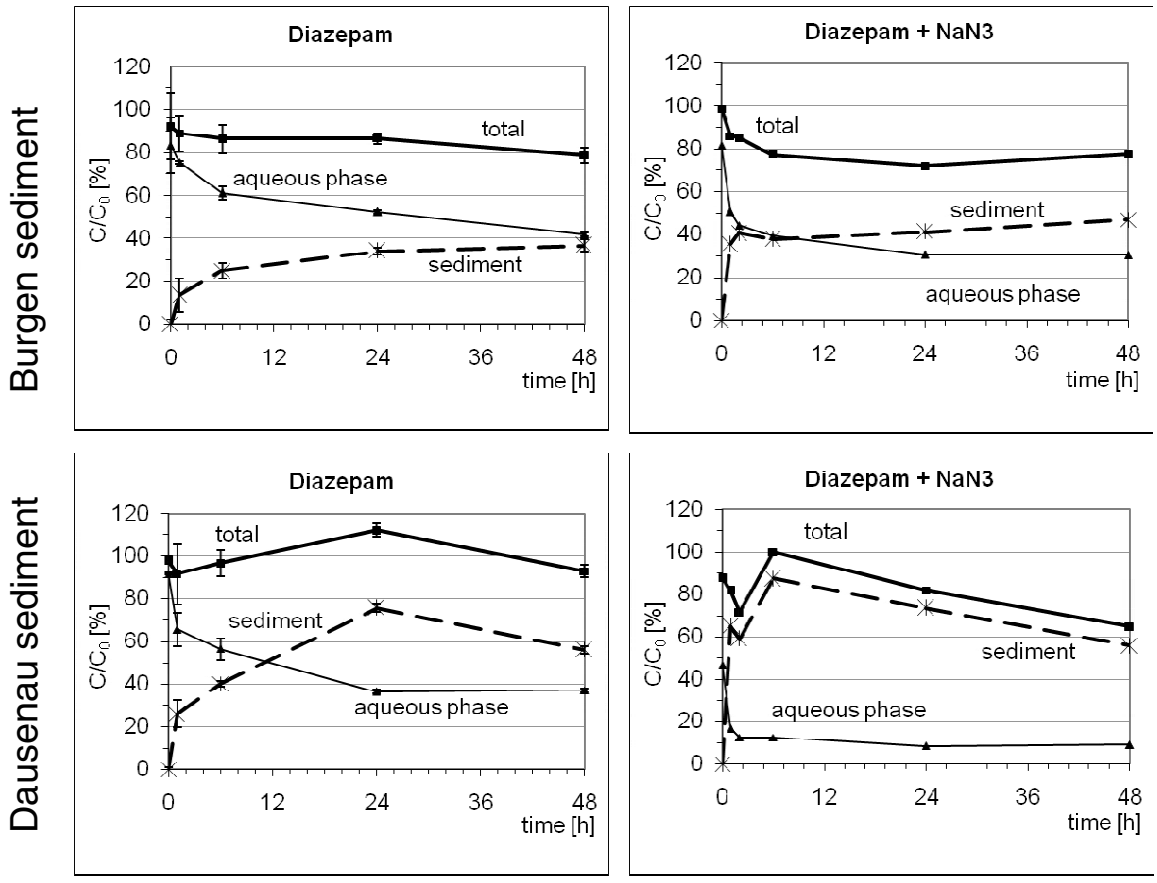

Figure S 8. Adsorption kinetics of diazepam in different sediments with (right) and without (left) addition of sodium azide

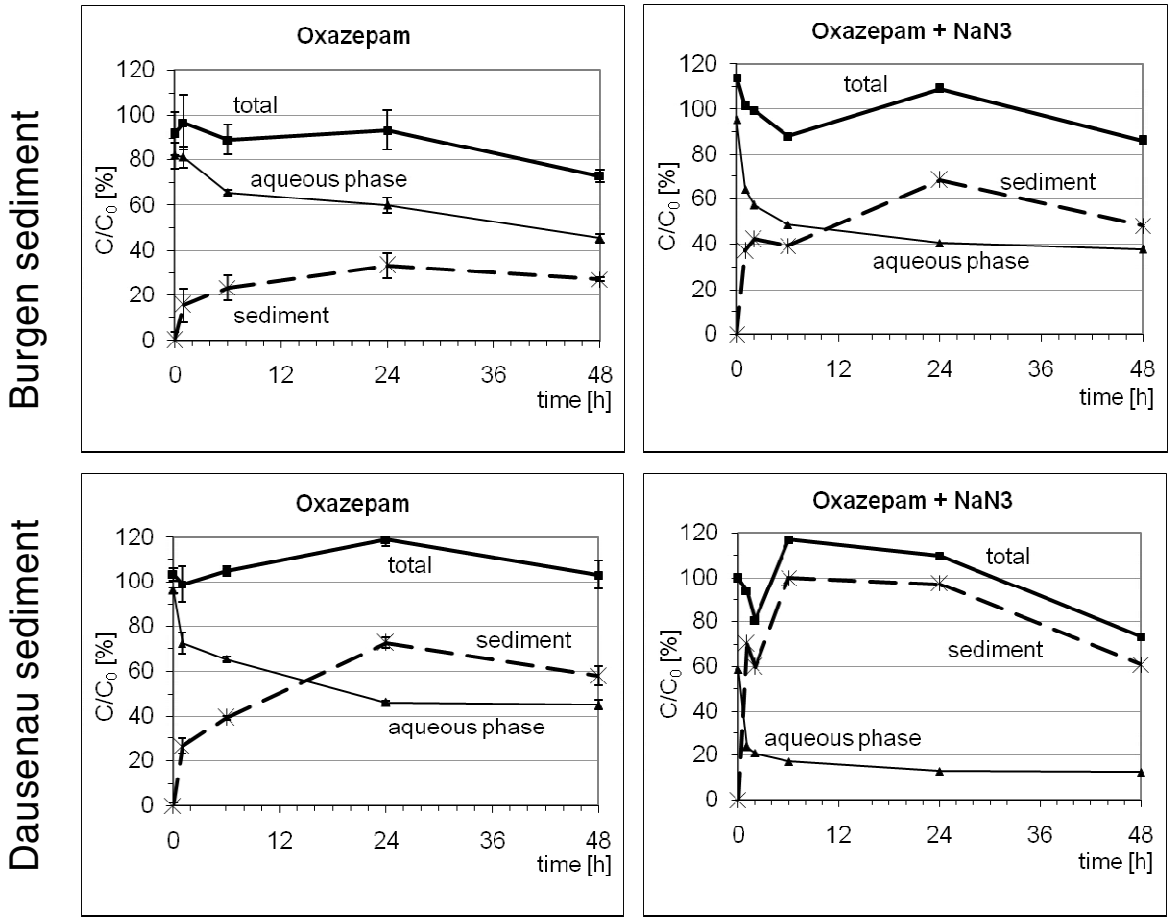

Figure S 9. Adsorption kinetics of oxazepam in different sediments with (right) and without (left) addition of sodium azide

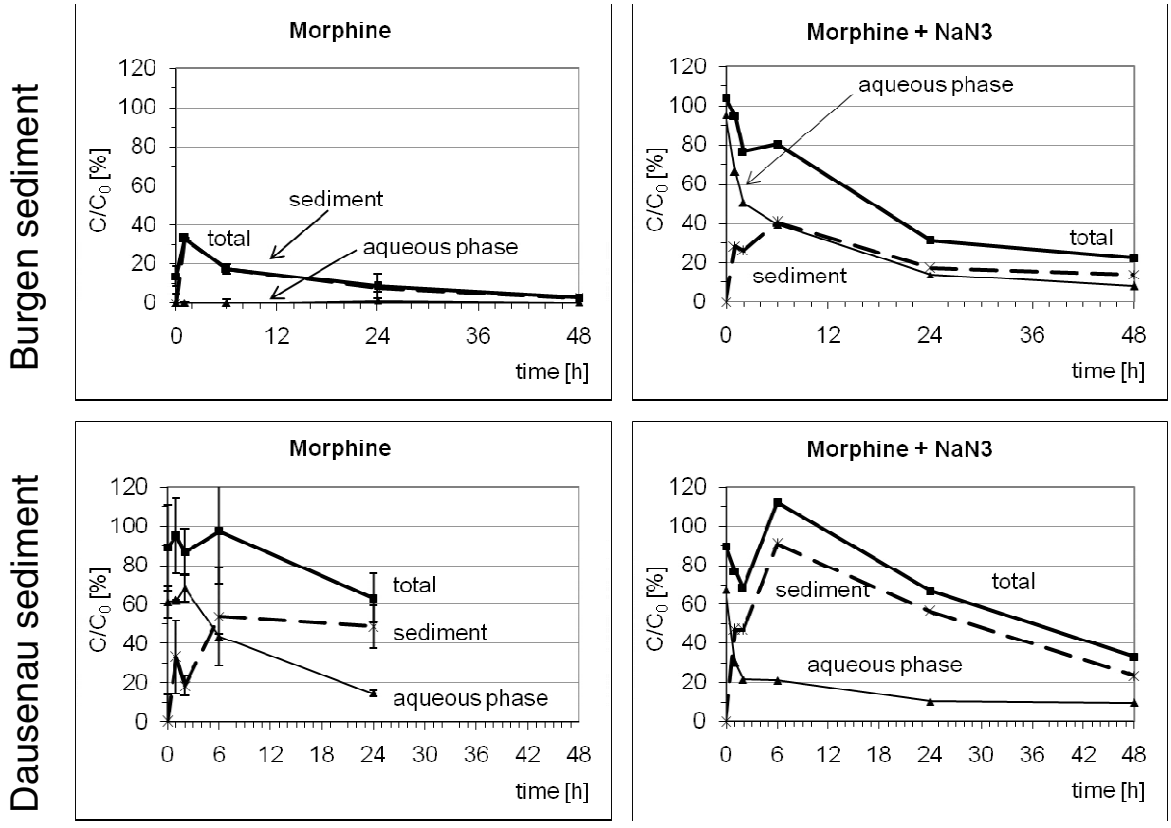

Figure S 10. Adsorption kinetics of morphine in different sediments with (right) and without (left) addition of sodium azide

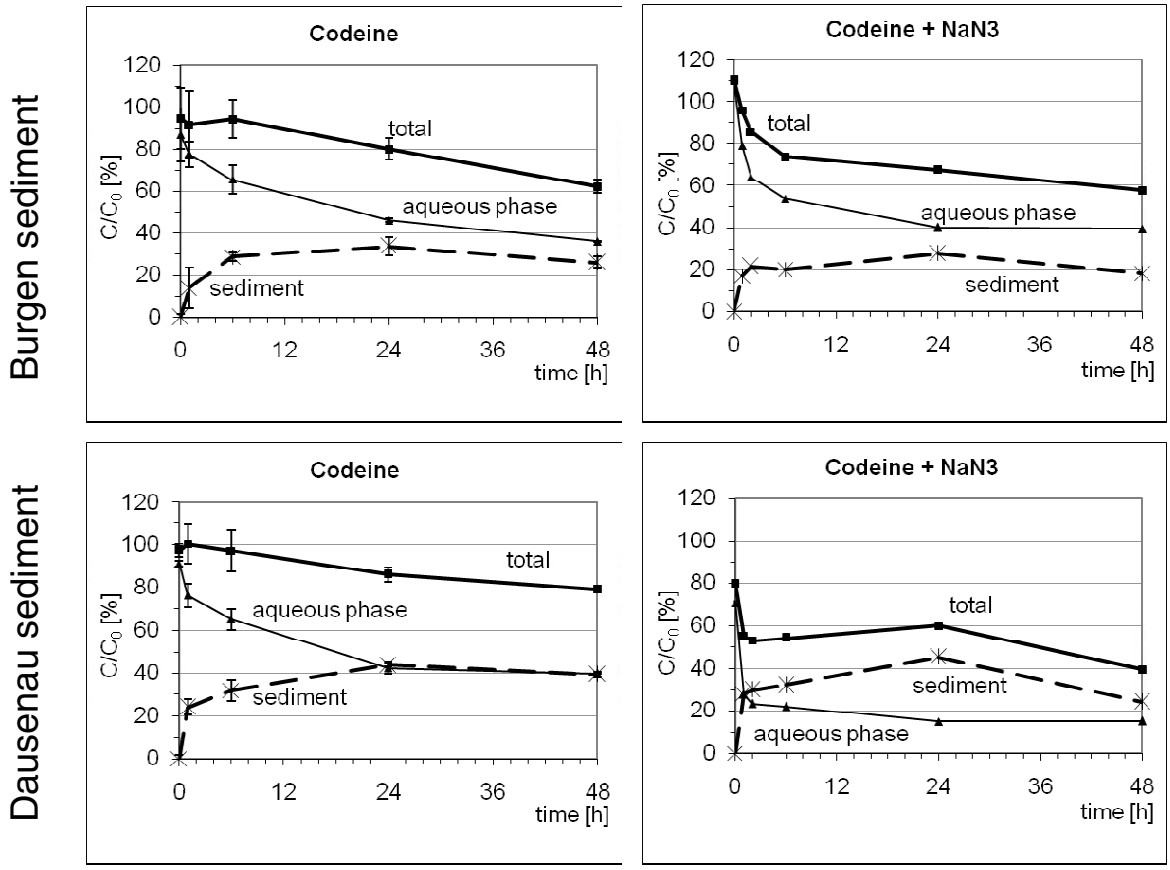

Figure S 11. Adsorption kinetics of codeine in different sediments with (right) and without (left) addition of sodium azide

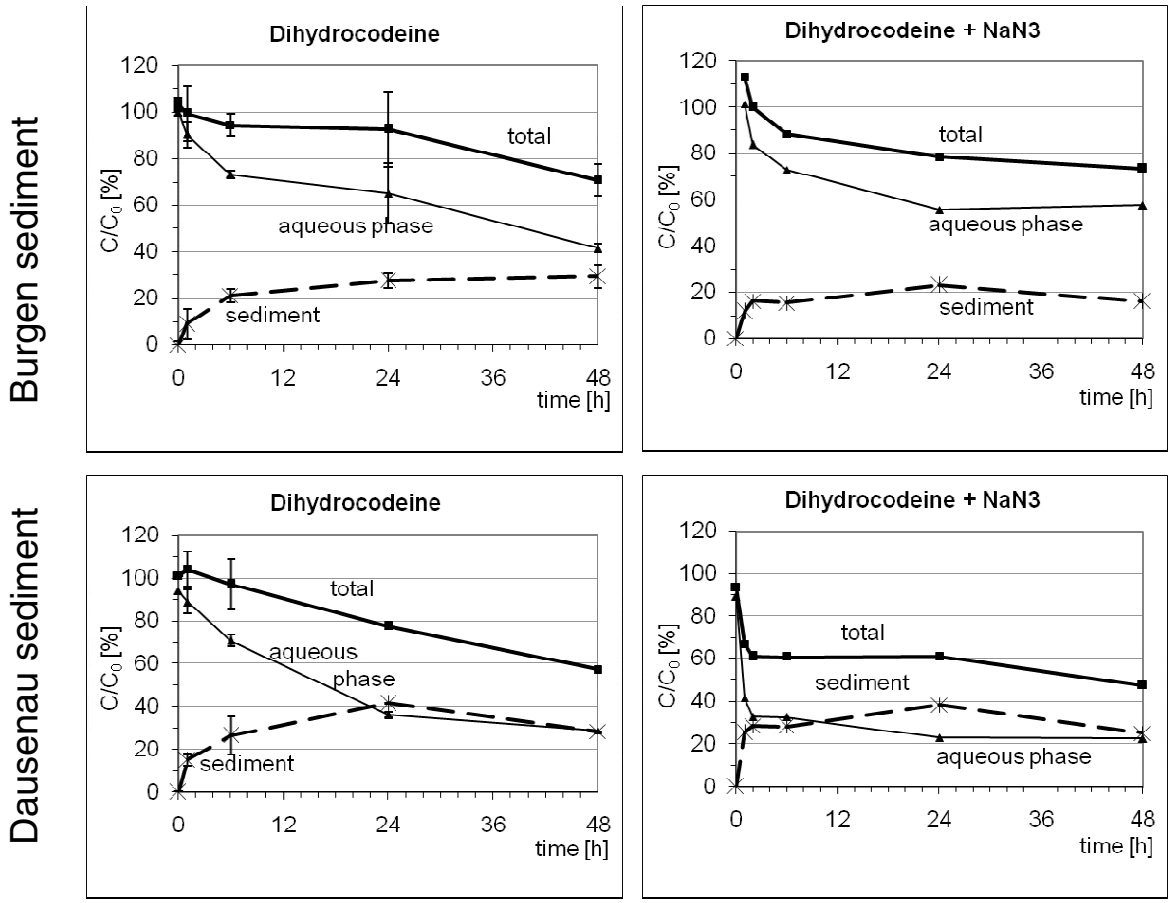

Figure S 12. Adsorption kinetics of codeine in different sediments with (right) and without (left) addition of sodium azide

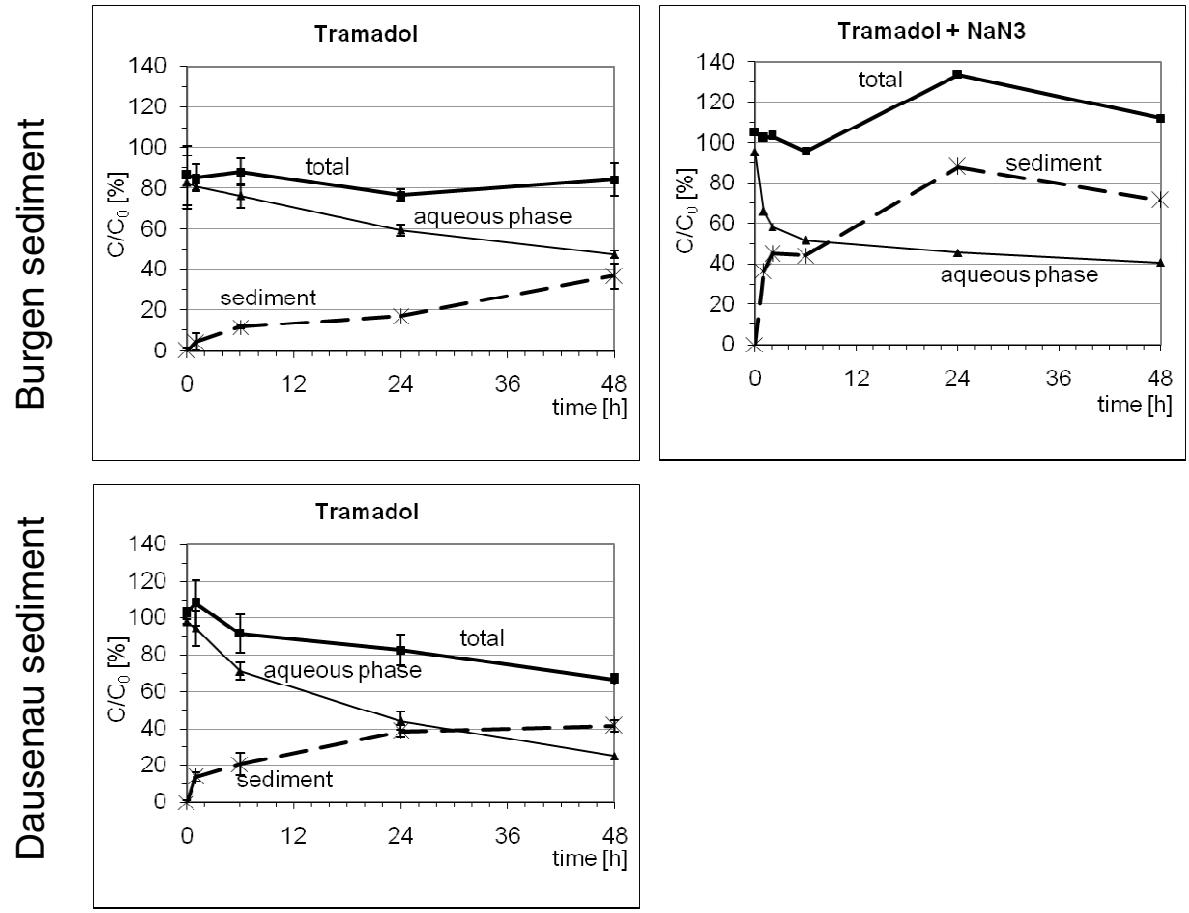

Figure S 13. Adsorption kinetics of tramadol in different sediments with (right) and without (left) addition of sodium azide

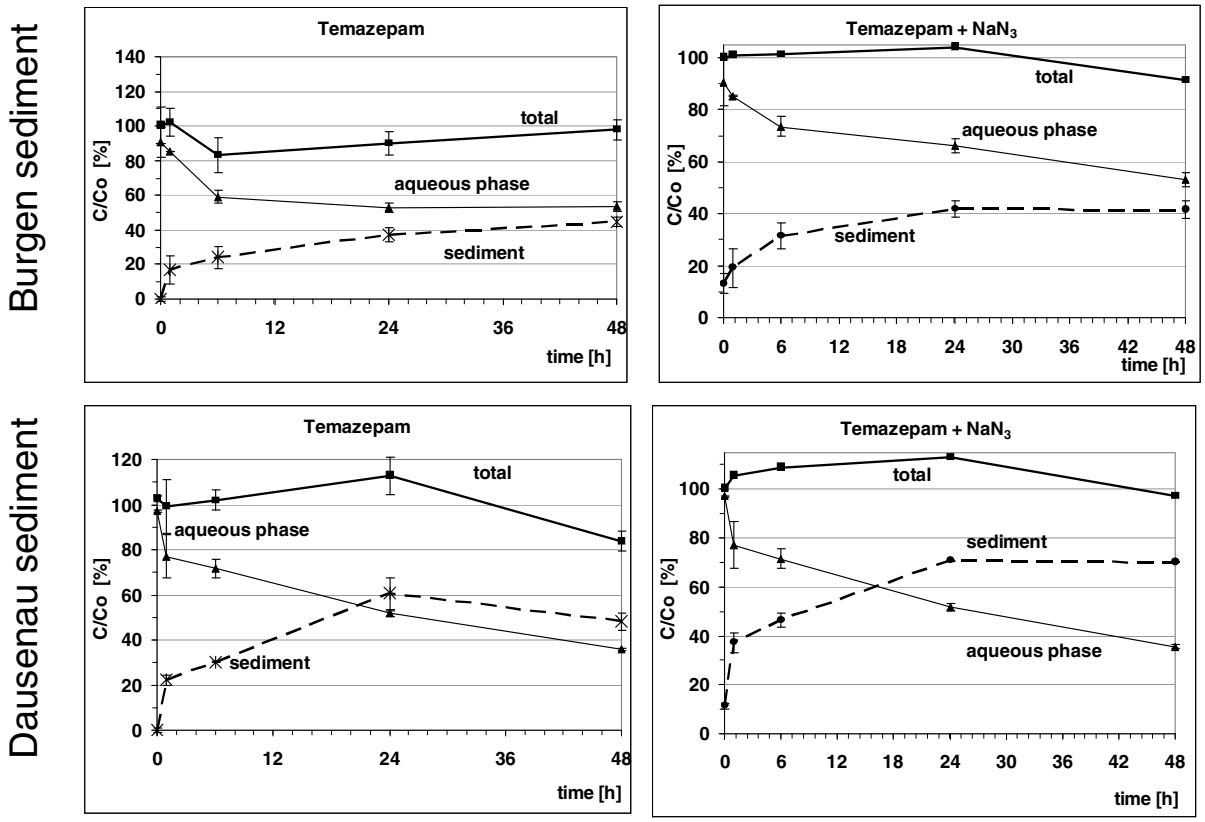

Figure S 14. Adsorption kinetics of temazepam in different sediments with (right) and without (left) addition of sodium azide

Table S 6. Calculated thermodynamic indices of irreversibility, TII for sorption-desorption data on Burgen and Dausenau sediments. The TII is zero for reversible sorption and approaches 1 for completely irreversible systems.

Compound	Concentration [ng/mL]	Tii	
		Burgen	Dausenau
Carbamazepine	25	-0.301	0.762
	50	0.253	0.841
	250	-0.137	0.902
	500	0.261	0.917
	2500	-0.286	0.678
10,11-Dihydrocarbamazepine	25	-0.211	0.243
	50	0.223	0.304
	250	-0.167	0.365
	500	0.285	0.341
	2500	-0.316	0.044
Diazepam	25	1.001	0.763
	50	0.809	0.826
	250	1.021	0.940
	500	0.917	0.938
	2500	0.518	0.662
Oxazepam	25	0.843	0.489
	50	0.667	0.626
	250	1.002	0.756
	500	0.983	0.890
	2500	0.480	0.658
Codeine	25	0.835	0.738
	50	0.828	0.720
	250	0.601	0.438
	500	0.757	0.652
	2500	0.626	0.631
Dihydrocodeine	25	0.932	0.894
	50	0.869	0.586
	250	1.003	0.551
	500	0.847	0.998
	2500	1.038	0.822
Temazepam	25	0.847	0.518
	50	0.272	0.614
	250	0.763	0.629
	500	0.812	0.447
	2500	0.173	-0.194
Tramadol	25	0.181	1.245
	50	-0.077	0.903
	250	0.492	1.193
	500	0.492	1.158
	2500	0.436	0.510

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