

Supplementary Information to
Biogeochemical causes of sixty-year trends and seasonal variations of river water
properties in a large European basin

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(Supporting Information includes 11 pages, 15 figures, and 2 tables)

Part SI-1: Details to section: Site description
Slapy catchment

The Slapy catchment (Fig. SI-1) is almost identical with the administrative South Bohemian Region prior to 2000, when it consisted of eight shires: České Budějovice, Český Krumlov, Jindřichův Hradec, Písek, Prachatice, Strakonice, Tábor, and Pelhřimov. Annual statistics on agricultural activities (livestock numbers, application of synthetic fertilizers, crop production and its composition), forest area, and population are available for this region from 1957 onwards in the Yearbooks of the Czech Statistical Office. For the whole Slapy catchment, statistical data from the original administrative South Bohemian Region were proportionately recalculated on the basis of their total areas. Climatic data for the South Bohemian Region (regional average monthly precipitation and monthly average air temperature) are available in the database of the Czech Hydrometeorological Institute (<http://portal.chmi.cz/historicka-data/pocasi/zakladni-informace>) from 1961. The largest artificial waterbodies in the Slapy catchment and dates of their constructions are given in Table SI-1.

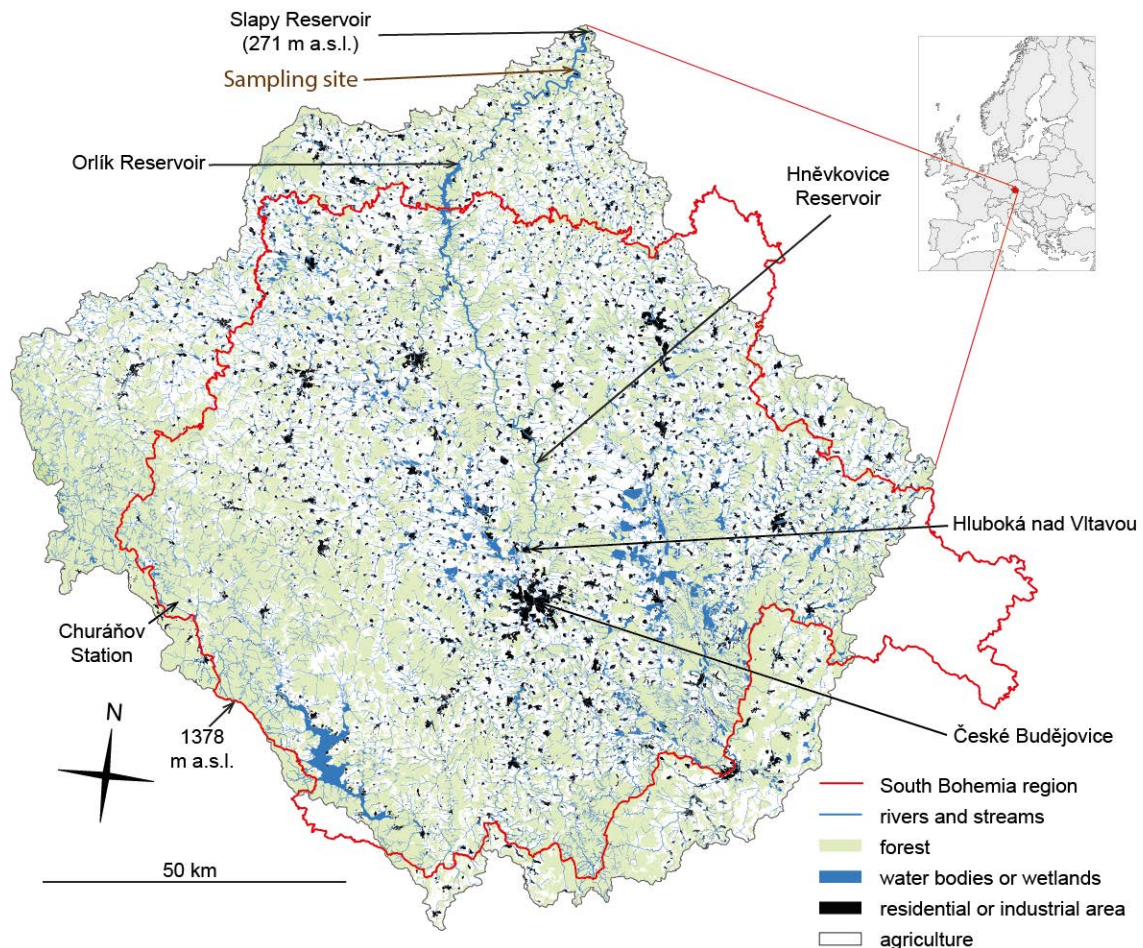


Fig. SI-1. Catchment of the Slapy reservoir (Vltava River) and its location in Europe. Map source: OpenStreetMap®. The catchment elevation ranges from 271 to 1378 m (average of 579 m) and its area is 12,968 km².

Table SI-1. Reservoirs in the Slapy catchment (Broža 2001).

| Reservoir | Stream | Dam construction | Year of filling | Volume (million m ³) | Area (km ²) | Mean depth (m) |
|------------|-----------|------------------|-----------------|----------------------------------|-------------------------|----------------|
| Husinec | Blanice | 1934-1939 | 1939 | 5.6 | 0.57 | 9.8 |
| Slapy | Vltava | 1947-1957 | 1957 | 269.3 | 13.92 | 19.3 |
| Lipno I | Vltava | 1952-1960 | 1960 | 306.0 | 48.70 | 6.3 |
| Lipno II | Vltava | 1952-1960 | 1960 | 1.7 | 0.45 | 3.8 |
| Kamýk | Vltava | 1957-1963 | 1963 | 12.8 | 1.95 | 6.6 |
| Orlík | Vltava | 1954-1963 | 1963 | 716.5 | 27.32 | 26.2 |
| Římov | Malše | 1974-1978 | 1978 | 33.6 | 2.11 | 15.9 |
| Humenice | Stropnice | 1985-1988 | 1988 | 0.8 | 0.15 | 5.4 |
| Hněvkovice | Vltava | 1986-1991 | 1991 | 22.2 | 3.12 | 7.1 |

Part SI-2: Details to section: Materials and methods*Water analysis*

Acid neutralizing capacity and bicarbonate: Acid neutralizing capacity (ANC) was determined by 0.1 M HCl titration with a bromphenol blue indicator and corrected for distilled water blanks in the 1959–1996 period. Since 1996, Gran titration (0.01M HCl, four end-points between pH of 4.3 and 3.7, calculation according to Mackereth et al. 1978) has been used. Besides water from the Slapy Reservoir (Table SI-2), a good comparability of both methods was proven for numerous water samples (paired two-sample student's *t*-test showed no significant differences between the methods; Kopáček 2018). Consequently, the ANC data based on Gran titration were used in the long-term trend since 1996. Because pH values varied between 6.3 and 10.2, with a median of 7.2 and only ~11% of all samples were outside the pH range of 6.7–9.0, we assumed for the purpose of this study that ANC was equal to the HCO₃⁻ concentration.

Ammonium: Concentrations of NH₄⁺ were determined by nesslerization of the distillate at pH ~7.8 from 1959–1963 (for details see Procházková 1977), then by a colorimetric, ammonium-specific (rubazoic acid) method (Procházková 1964) till 1992, and finally by a modified version, excluding the original extraction step (Kopáček and Procházková 1993) in the 1992–2019 period. The detection limit of this method is ~0.4 μmol L⁻¹ (i.e., ~6 μg L⁻¹ NH₄-N). The comparability of both modifications of the rubazoic acid method has been demonstrated (Kopáček and Procházková 1993). Since 1996, NH₄⁺ has also been regularly determined by ion chromatography (IC). Both (colorimetric and IC) methods provide almost identical results (Kopáček 2019). We use data based on colorimetric methods in this study, because they are also used for the determination of organic N.

Base cations: Concentrations of calcium (Ca²⁺) and magnesium (Mg²⁺) were determined by ethylenediaminetetraacetic acid (EDTA) titration (Hrbáček et al. 1974; APHA 1976) continuously since 1959, with detection limits of ~25 and 200 μmol L⁻¹ (i.e., 1 and 5 mg L⁻¹), respectively, and by the IC method since 1996, with detection limits of ~0.4 μmol L⁻¹ (i.e., ~0.02 mg L⁻¹) for both ions. After confirmation that both methods provide similar results (Table SI-2), the data based on the IC method were used in the long-term trend since 1996. Sodium (Na⁺) and potassium (K⁺) concentrations were determined by flame emission spectroscopy from 1962–1988, inductively coupled plasma mass spectrometry (PU 7450, Leemans Labs Inc., USA) from 1989–1996, and by IC from 1996–2019. Detection limits of the methods were ~0.4 μmol L⁻¹ (i.e., ~0.02 mg L⁻¹) for both elements. As in the case of divalent cations, analytical methods provided similar values for Na⁺ and K⁺ concentrations in 1996 (Table SI-2).

Chloride: Concentrations of Cl⁻ were measured by a mercurimetric method [0.015 M Hg(NO₃)₂ titration with diphenylcarbazone indicator solution, corrected for distilled water blanks] (Hrbáček et al. 1974; APHA 1976) during 1959–1996, and then by the IC method during 1996–2019. The methods did not differ at a significance level of 0.05 in the paired two-sample Student's *t*-test and provided similar values (Table SI-2). The detection limits of the mercurimetric and IC methods were ~100 and 0.5 μmol L⁻¹ Cl⁻ (i.e., ~4 and 0.02 mg L⁻¹), respectively, i.e., sufficiently below the measured values.

Chlorophyll a: During 1963–2017, chlorophyll *a* (Chl-*a*) was determined spectrophotometrically after extraction of seston samples on Whatman GF/C filters by 90% acetone in mixed samples from the upper reservoir layer (samples were taken using a 4-m long tube from ~10 different places). Pheophytins were subtracted after acidification of the extract according to Lorenzen (1967). The Chl-*a* determination was irregular prior to 1975 and then regular throughout 1975–2019. From 2014–2019, Chl-*a* was analysed spectrophotometrically in ethanol extracts according to Arvola (1981) using the same filters (Whatman GF/C). A four-year parallel use of both methods (2014–2017) showed a tight linear relationship between both methods (R²=0.97); however, the results based on the ethanol extraction method were 25% higher. To maintain consistency in the long-term data series based on the acetone extraction method till 2017, the results of the ethanol method used from 2018 in this study were divided by a factor of 1.25.

Fluoride: Concentrations of F^- were measured by the IC method from 1998–2019 with a detection limit of $\sim 0.5 \mu\text{mol L}^{-1}$ (i.e., $\sim 0.01 \text{ mg L}^{-1}$).

Nitrate: Concentrations of NO_3^- were determined colorimetrically after reduction to nitrite with alkaline hydrazine according to Procházková (1959) since 1959 and by the IC method since 1996. Because both methods provided almost identical values (Table SI-2; Procházková and Blažka 1999), the data based on the IC method were used in the long-term trend since 1996 even though data on both methods are available. The detection limit of both methods is $\sim 0.15 \mu\text{mol L}^{-1}$ (i.e., $\sim 2.0 \mu\text{g L}^{-1} \text{NO}_3\text{-N}$).

Nitrite: Concentrations of NO_2^- have been determined spectrophotometrically (Bendschneider and Robinson 1952) since 1961. The detection limit of this method is $\sim 0.03 \mu\text{mol L}^{-1}$ (i.e., $\sim 0.5 \mu\text{g L}^{-1} \text{NO}_2\text{-N}$).

Organic carbon: Chemical oxygen demand (COD) was determined using a standard dichromate technique (APHA 1976) as modified by Procházková and Blažka (1984) from 1959–1988, and by a dichromate semi-micro method (Hejzlar and Kopáček 1990) from 1987–2019. Comparability of both methods was demonstrated by Hejzlar and Kopáček (1990) and results of the latter method have been used in the database since 1988. Dissolved organic carbon (DOC) has been analyzed since 1995, with a detection limit of $\sim 5 \mu\text{mol L}^{-1}$ (i.e., $< 0.05 \text{ mg L}^{-1} \text{C}$). The previous trend in DOC was calculated from COD data as $\text{DOC} = \text{COD}/3.3$ (both values in mg L^{-1}). A coefficient of 3.3 was obtained as the arithmetical mean of all COD:DOC ratios from 1995–2010 ($n = 272$, standard deviation of 0.7). The ratios varied within 2.0 and 5.7, with 75% of all values within the 2.6–4.0 range (Kopáček et al. 2017). Particulate C (PC) has been determined since 1995 by combustion of glass-fibre filters (pore size of $0.4 \mu\text{m}$; GF-5, Macherey-Nagel, Düren, Germany) with the retained seston.

Organic nitrogen: Concentrations of total organic nitrogen (TON; the difference between the Kjeldahl N and $\text{NH}_4\text{-N}$) have been determined since 1959 by Kjeldahl digestion according to Procházková (1960), with 25–50 ml of samples previously evaporated with sulphuric acid to obtain a detection limit of $\sim 2 \mu\text{mol L}^{-1}$ (i.e., $\sim 25 \mu\text{g L}^{-1} \text{N}$). Since 1995, concentrations of dissolved organic N (DON) have been analysed analogously to TON after sample filtration with glass-fibre filters (pore size of $0.4 \mu\text{m}$; GF-5, Macherey-Nagel, Düren, Germany). Particulate N (PN) was the difference between TON and DON.

Oxygen: Concentrations of dissolved O_2 were determined using the Winkler titrimetric method (APHA 1976) from 1959–1994, then using different types of probes with sensors for temperature and dissolved oxygen.

pH: Values of pH were determined colorimetrically in the field immediately after sampling with comparators (Tintometer, Hellige, and Lovibond) from 1959–2010. Indicators used for different pH ranges were: Methyl red (pH of 4.4–6.0), bromothymol blue (pH of 6.0–7.6), cresol red (pH of 7.2–8.8), thymol blue (pH of 8.0–9.6), and thymolphthalein (pH of 9.3–10.5) (Hrbáček et al. 1974). Potentiometric determination of pH was performed in laboratory within < 12 hours after sampling using different types of combined glass pH electrodes (Radiometer). This method has been used since 1993. Potentiometric values were higher than the colorimetric pH, with greater differences at low than at high pH values (Fig. SI-2). The equation of linear regression between results of both methods during 1993–2010 ($\text{pH}_{\text{potentiometry}} = 0.891 \times \text{pH}_{\text{colorimetry}} + 1.064$; $R^2 = 0.93$, $n = 300$) was used to recalculate the potentiometric pH to colorimetric pH during 2011–2019, to obtain a consistent long-term pH trend for the whole period.

Phosphorus: Concentration of total phosphorus (TP) was determined by perchloric acid digestion followed by the molybdate method according to Popovský (1970) in 1963–1991, then by a semi-micro modification of this method (Kopáček and Hejzlar 1993). In both modifications, samples were concentrated by evaporation (with diluted perchloric acid at $\sim 100^\circ\text{C}$) prior to digestion to obtain a detection limit of $\sim 0.02 \mu\text{mol L}^{-1}$ (i.e., $\sim 0.5 \mu\text{g L}^{-1} \text{P}$). Both modifications of the method provided almost identical results (Kopáček and Hejzlar 1993). The TP concentrations were determined in water filtered through a $40\text{-}\mu\text{m}$ sieve from 1959–2019 and through a $200\text{-}\mu\text{m}$ from 1993–2019. Because the TP concentrations determined in the $200\text{-}\mu\text{m}$ filtrate were on average only $< 5\%$ higher than those in the $40\text{-}\mu\text{m}$ filtrate, no correction of TP concentrations prior to 1994 were applied in this study. Since 1995, concentrations of dissolved P (DP) have been analysed analogously to TP after sample filtration with glass-fibre filters (pore size of $0.4 \mu\text{m}$; GF-5, Macherey-Nagel, Düren, Germany). Particulate P (PP) was the difference between TP and DP.

Silica: Concentrations of dissolved reactive silica were measured colorimetrically using a molybdate method following Golterman and Clymo (1969) from 1997–2019. The detection limit of the method was $\sim 2 \mu\text{mol L}^{-1}$ (i.e., $\sim 0.05 \text{ mg L}^{-1}$).

Sulphate: Concentrations of SO_4^{2-} were determined by a colorimetric barium chloranilate method (Procházková 1961) irregularly (5–10 samples per year) from 1960–1969 and regularly in 1966 and 1970–1996. Since 1996, SO_4^{2-} has been determined by the IC method. The methods did not differ at a significance level of 0.05 in the paired two-sample student's t -test and provided similar average concentrations (Table SI-2). Nevertheless, this comparison showed that the colorimetric method overestimated SO_4^{2-} concentrations in some series in 1996, despite similar averages. Consequently, we also checked all results on SO_4^{2-} concentrations prior to 1996 by means of the charge balance (Kopáček et al. 2000), a comparison of the measured and calculated conductivities, and $\text{SO}_4^{2-}:\text{Cl}^-$, $\text{SO}_4^{2-}:\text{Na}^+$, $\text{SO}_4^{2-}:\text{K}^+$, $\text{SO}_4^{2-}:\text{Ca}^{2+}$, and $\text{SO}_4^{2-}:\text{Mg}^{2+}$ ratios. If the sum of anions was

higher than the sum of cations, the calculated conductivity higher than its measured value, and the sulphate to other component ratios higher than their neighbouring values, the actual SO_4^{2-} concentration was excluded and replaced with a new value calculated as a difference between the sum of cations and the sum of HCO_3^- , Cl^- , and NO_3^- concentrations, provided this change also improved the conductivity test and the sulphate to other component ratios. This correction was applied to ~20% of samples prior to 1996. The same method was also used to reconstruct the missing SO_4^{2-} concentrations from 1960–1970. This calculation provided an average $\text{SO}_4\text{-S}$ concentration of 8 mg L^{-1} in the 1960–1961 period, which was in reasonable agreement with the average value of 7 mg L^{-1} observed in the Slapy Reservoir by Fiala (1962) in 1958–1959. The detection limits of SO_4^{2-} determinations prior to and after 1996 were ~60 and $0.6 \mu\text{mol L}^{-1}$ (i.e., ~2 and $0.02 \text{ mg L}^{-1} \text{ SO}_4\text{-S}$), respectively, i.e., sufficiently below the measured values.

Transparency: Water transparency was measured as the Secchi depth with a standard Secchi disk.

Used instruments: We used the following ionic chromatographs with conductometric detection from 1996–2019: Thermo Separation Products AS 100, USA (1996–2000); Dionex IC25, USA (2001–2011); Dionex ICS-3000, USA (2012–2018); and Dionex (Thermo Fisher) ICS-5000+, USA (2018–2019).

Organic carbon (DOC and PC) was determined using the following analyzers: LiquiTOC (Foss/Heraeus, Germany) in 1991–2000; TOC 5000A (Shimadzu, Japan) from 2000–2015; Elementar vario TOC cube (Germany) 2010–2016, and TOC-L (Shimadzu, Japan) from 2016–2019.

For Gran titration, we used automatic titrators Tacussel EBX-3 (France) from 1996–2011, and TitraLab TIM865 Radiometer Analytical (France) from 2011–2019.

Concentrations of dissolved O_2 were determined using probes OXI-196 (WTW, Germany) from 1995–2010 and YSI ProODO (Xylem Inc.) from 2011–2019.

Conductivity at $25 \text{ }^\circ\text{C}$ was measured using various laboratory conductometers: Conductoscop Type4 (Czechoslovakia) and Radelkis (Hungary) prior to 1988, WTW LF2000 (Germany) from 1988–2010; and WTW Cond720 (Germany) from 2010–2019.

Table SI-2. Comparisons of old and new methods used for determining water composition in the Slapy Reservoir prior to 1997 and since 1996. Data represent average (\pm standard deviation) concentrations in $\mu\text{mol L}^{-1}$ in 1996, $n = 17$. The paired two-sample student's t -test (Strum and Kirk 1998) with a significance level of 0.05 was used to test for differences between the methods; the zero hypothesis was that the sample means were equal.

| | Old/new method | Old | New | p |
|--------------------|---|--------------|--------------|------|
| HCO_3^- | 0.1M HCl titration/Gran titratio | 863 \pm 98 | 843 \pm 96 | 0.12 |
| NO_3^- | Spectrophotometry/IC | 238 \pm 51 | 243 \pm 51 | 0.36 |
| Cl^- | $\text{Hg}(\text{NO}_3)_2$ titration/IC | 397 \pm 40 | 407 \pm 40 | 0.90 |
| SO_4^{2-} | Spectrophotometry/IC | 457 \pm 73 | 447 \pm 62 | 0.30 |
| Ca^{2+} | EDTA titration/IC | 665 \pm 20 | 640 \pm 60 | 0.15 |
| Mg^{2+} | EDTA titration/IC | 286 \pm 31 | 303 \pm 35 | 0.72 |
| K^+ | FES/IC | 112 \pm 6 | 114 \pm 18 | 0.52 |
| Na^+ | FES/IC | 417 \pm 41 | 416 \pm 72 | 0.35 |

Abbreviations: FES (flame emission spectroscopy), IC (ion chromatography), EDTA (ethylenediaminetetraacetic acid), p -value (significance of paired two-sample student's t -test).

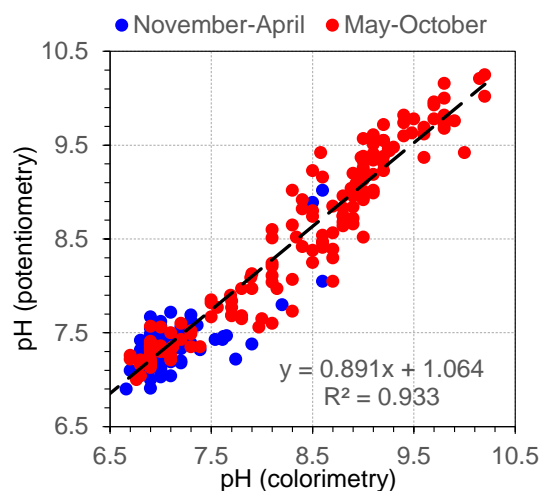


Fig. SI-2. Relationship between pH values of in the Slapy surface water layer (1996–2010) determined colorimetrically in the field immediately after sampling and potentiometrically in laboratory within <12 hours after sampling. Blue and red points denote samples from November-April and May–October periods, respectively. The dashed line represents the linear regression for all data; the related equation is given in the figure.

Part SI-3: Details to section: Results
Basic physical-chemical variables, pH, and chlorophyll

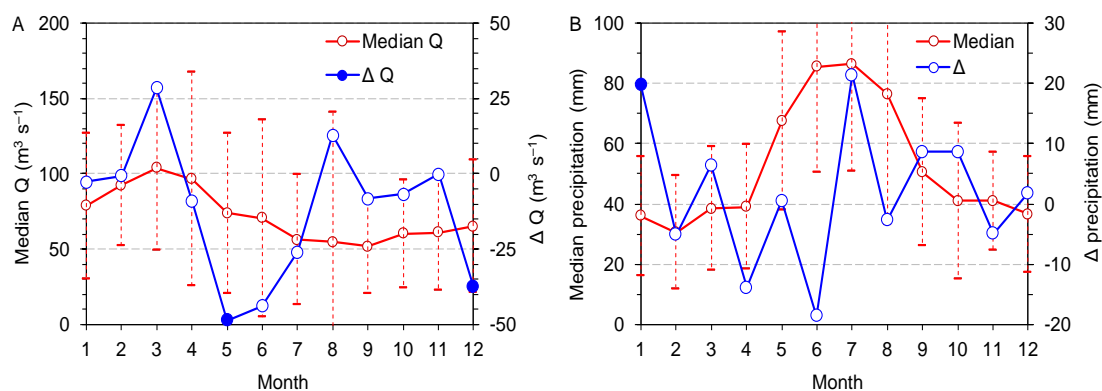


Fig. SI-3. Median values (\pm standard deviation, dashed lines) of (A) monthly average discharge of Vltava in the Slapy reservoir (Q, 1960–2019) and (B) monthly precipitation in the Slapy catchment (1961–2019). Blue lines show a net change (Δ) calculated from the monthly trend values from 1960/1961 to 2019. Significant changes ($p < 0.05$) are indicated by solid blue points.

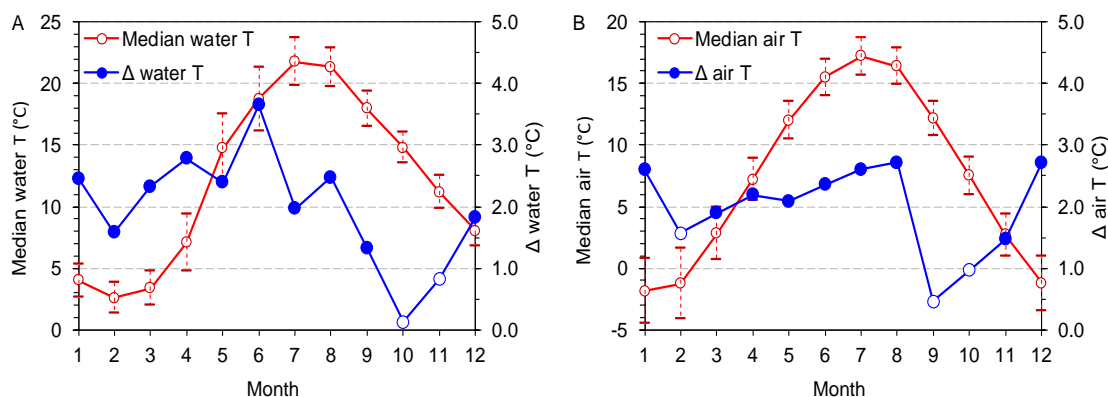


Fig. SI-4. Median values (\pm standard deviation, dashed lines) of (A) monthly average water temperature in the Slapy surface layer (1960–2019) and (B) monthly average air temperature in the Slapy catchment (1961–2019). Blue lines show a net increase (Δ) in the temperature calculated from monthly trends from 1960/1961 to 2019. Significant changes ($p < 0.05$) are indicated by solid blue points.

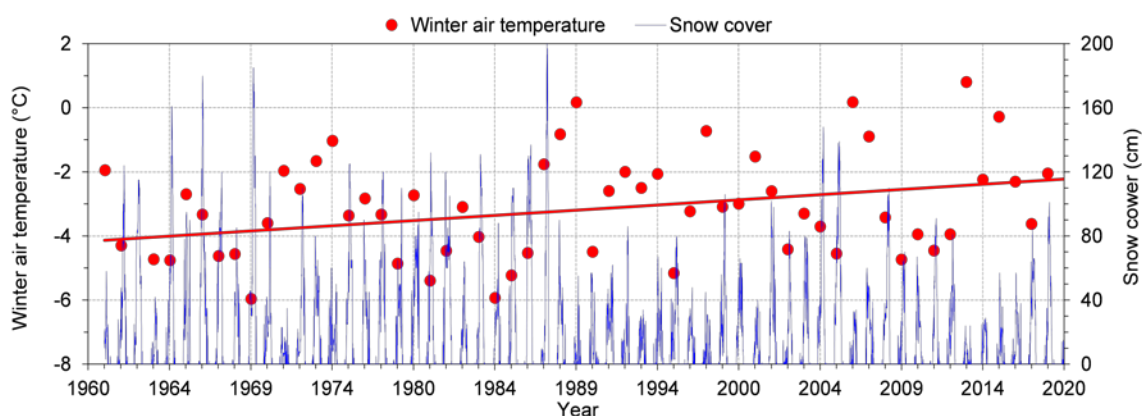


Fig. SI-5. Average winter (December–February) air temperature and snow cover at Churáňov station in the Bohemian Forest from 1961–2019 (data by the Czech Hydrometeorological Institute). The solid red line shows linear regression of temperature increase ($p < 0.05$). The average increase in winter air temperature was 2.1 °C from 1961–2019.

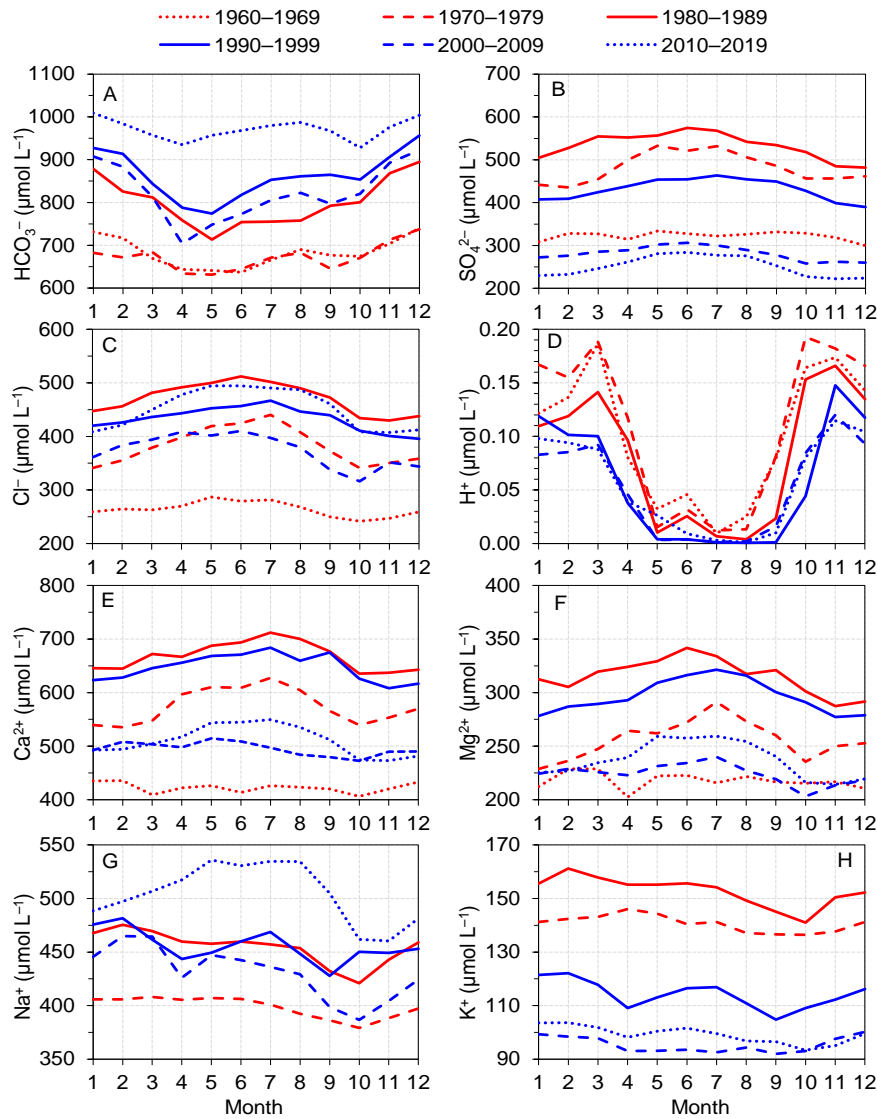


Fig. SI-6. Seasonal variations of mean concentrations of nutrients in the Slapy surface layer computed as geometrical means for individual months over six decades from 1960–2019: A, bicarbonate; B, sulphate; C, chloride; D, hydrogen (proton); E, calcium; F, magnesium; G, sodium; H, potassium.

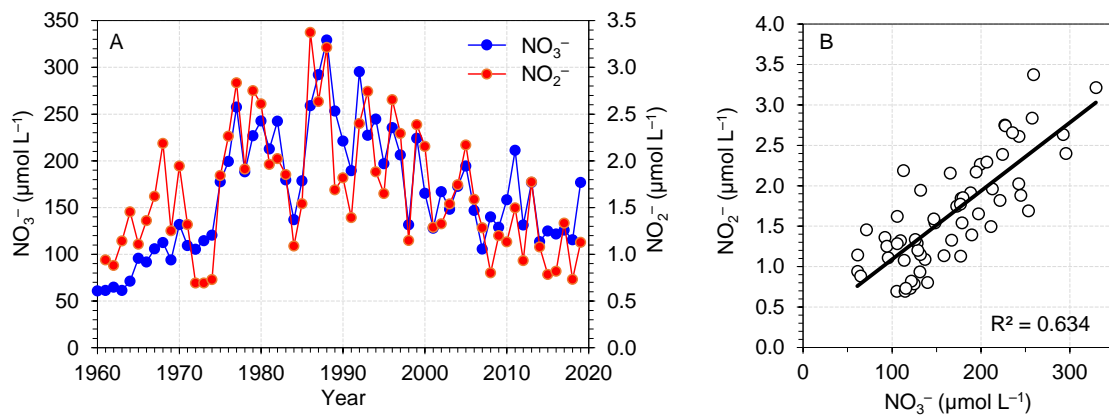


Fig. SI-7. Annual average concentrations of nitrate and nitrite in the Slapy surface layer from 1960 to 2019 (A) and (B) their relationship (the solid line, $p < 0.001$).

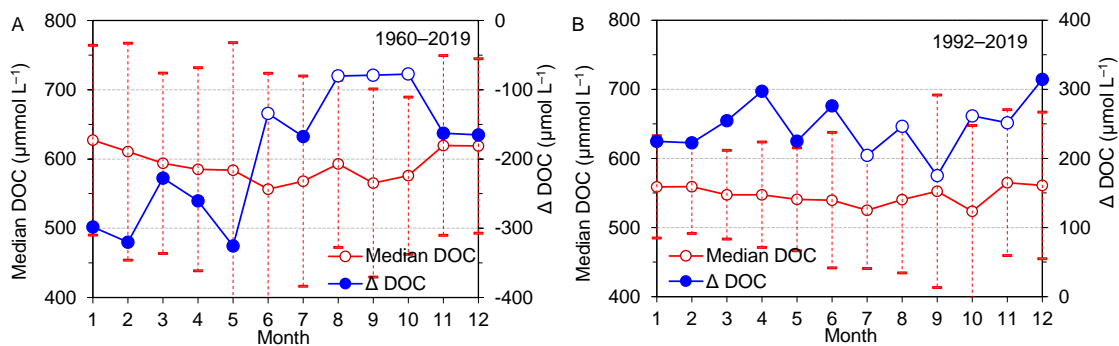


Fig. SI-8. Median DOC values (\pm standard deviation, dashed lines) in individual months during the 1960–2019 (A) and 1992–2019 (B) periods. Blue lines show a net change (Δ) in DOC calculated from monthly trends during individual periods. Significant changes ($p < 0.05$) are indicated by solid blue points.

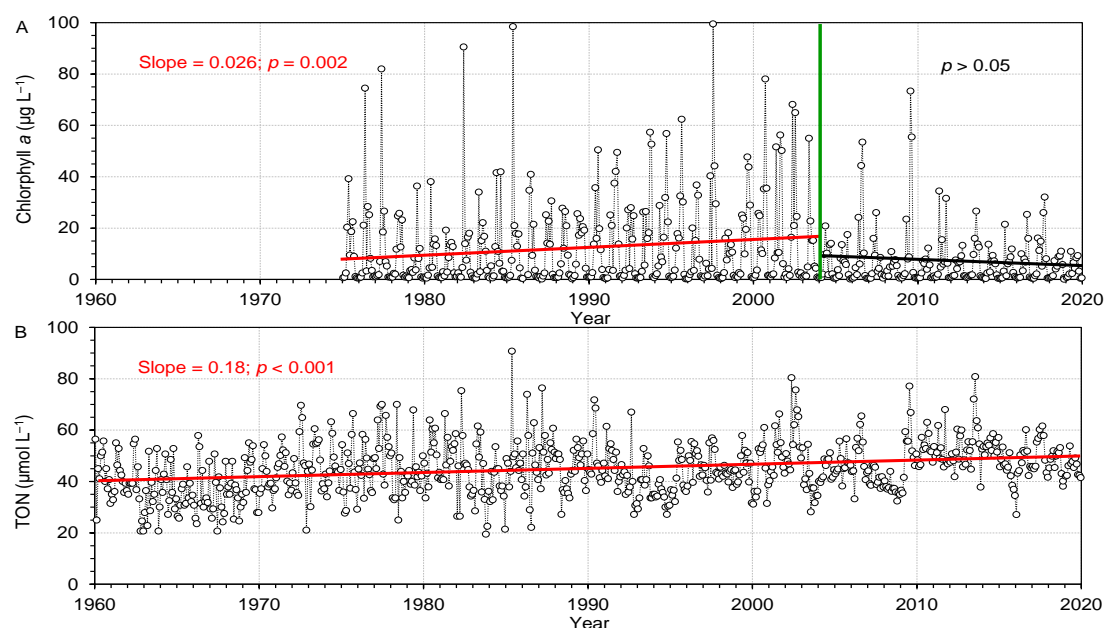


Fig. SI-9. Monthly mean concentrations of chlorophyll *a* (A), and TON (B) in the Slapy surface layer from January 1960 through December 2019. Red lines show significant trends of seasonal Mann-Kendall test (R Core Team 2019), the black line an insignificant trend, and green vertical line shows breakpoint. Data on chlorophyll *a* were not always complete prior to 1975, and thus not used in the statistic.

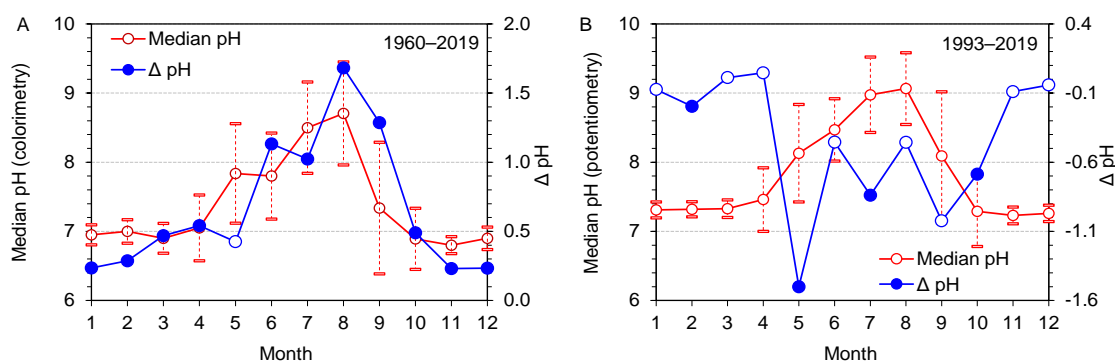


Fig. SI-10. Median pH values (\pm standard deviation, dashed lines) in individual months during the 1960–2019 (A) and 1993–2019 (B) periods. Blue lines show a net change (Δ) in the pH values calculated from monthly trends during individual periods. Significant changes ($p < 0.05$) are indicated by solid blue points.

Part SI-4: Details to section: Discussion

Element inputs to the Slapy catchment by synthetic fertilizers and atmospheric deposition

Synthetic fertilizers: Statistical data on the application of synthetic fertilizers in the Czech Republic and the South Bohemian Region came from the Yearbooks of the Czech Statistical Office. The [FAO database](http://www.fao.org/faostat/en/#data/RA) (<http://www.fao.org/faostat/en/#data/RA>) provided data on the annual application of nutrients (N, P, and K) in the Czech Republic from 1961–2008, and on the detailed composition of individual fertilizers used from 1982–2002. The Czech Statistical Office provided annual applications of total N, P, and K in the South Bohemian Region from 1959–2015. The major fertilizers used in the Czech Republic were: ammonium nitrate, ammonium phosphate, ammonium sulphate, calcium ammonium nitrate, calcium nitrate, potassium sulphate, urea, single superphosphate, concentrated superphosphate, muriate, and complex fertilizers with N, P, and K in different proportions. The input of Ca and Mg to agricultural land was calculated as the sum of their two major sources: (i) fertilizers and (ii) liming (with limestone and dolomite) of acidic soils. The total flux of SO_4^{2-} associated with synthetic fertilizers was calculated from the application of S-bearing P-fertilizers (in a single, concentrated superphosphate, or complex fertilizers), N-fertilizers (in ammonium sulphate), and K-fertilizers (in potassium sulphate). The Cl^- inputs with fertilizers were computed from the use of potassium (K) fertilizers (muriate and complex fertilizers) and impurities in the mineral kieserite (MgSO_4). The input of Na to agricultural land with synthetic fertilizers was estimated from the use of K fertilizers, assuming that NaCl formed ~3% of KCl (Jäger and Hegner 1987). For details see [Kopáček et al. \(2017\)](#). The inputs of major elements to agricultural soils in the Slapy catchment are given in Fig. SI-11.

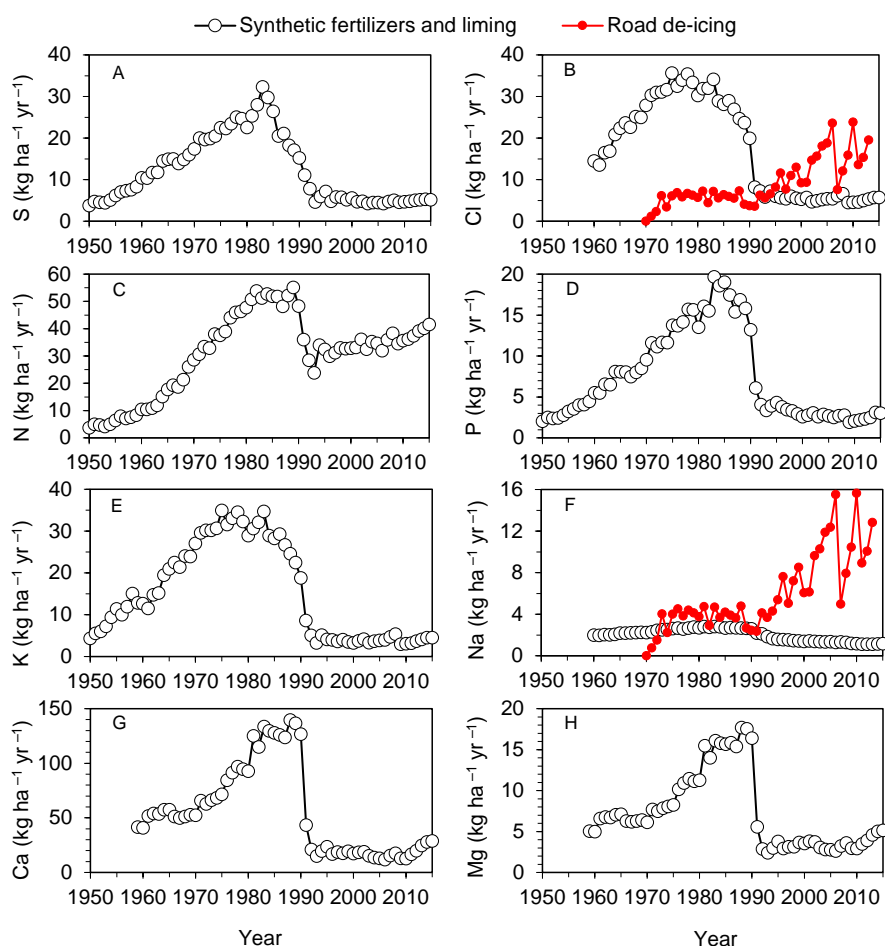


Fig. SI-11 Inputs of (A) sulphur, S; (B) chloride, Cl; (C) nitrogen, N; (D) phosphorus, P; (E) potassium, K; (F) sodium, Na; (G) calcium, Ca; and (H) magnesium, Mg, associated with (i) synthetic fertilizers, liming, and NaCl for animals, and (2) road de-icing in the Slapy catchment during 1950–2015 ([Kopáček et al. 2017](#)). Data are calculated as total annual inputs to the total catchment area, and normalized to ha^{-1} .

Atmospheric deposition: Element inputs to the Slapy catchment (Fig. SI-12) were calculated by [Kopáček et al. \(2012, 2017\)](#) as bulk deposition to treeless areas including water surfaces and throughfall deposition in forests.

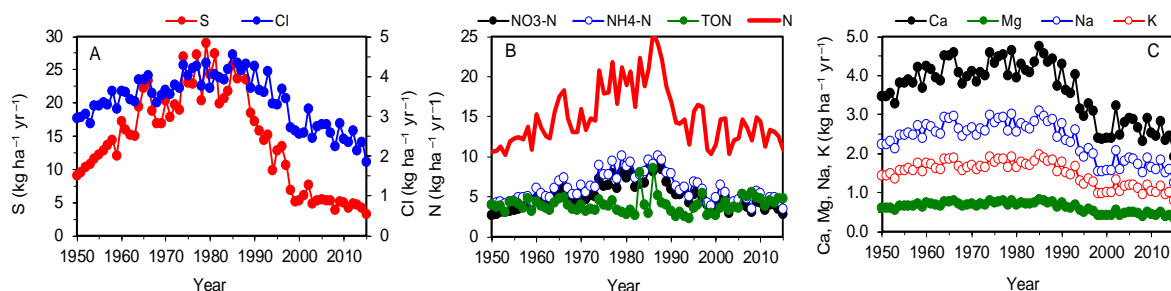


Fig. SI-12. Atmospheric inputs (bulk deposition in treeless area and throughfall in forests) of (A) sulphur (S) and chloride (Cl); (B) total nitrogen (N) and its individual forms; and (C) base cations (Ca, Mg, Na, and K; only bulk deposition) in the Słapy catchment (Kopáček et al. 2012, 2017). Data are calculated as total annual inputs to the total catchment area, and normalized to ha⁻¹.

Part SI-5: Details to section: Discussion

Organic and nutrient pollution of the upper Vltava River associated with wastewaters

Values of biochemical oxygen demand (BOD₅) and chemical oxygen demand (COD) determined either using oxidation with KMnO₄ (COD-Mn) or with K₂Cr₂O₇ (COD-Cr) have long been used as indicators of water quality. The BOD₅, COD-Mn, and COD-Cr are used as estimates of concentrations of readily biodegradable organic substances, chemically easily degradable organic substances, and total organic substances (Pitter 1990). All these indicators show significant water pollution of the Vltava water by municipal and industrial wastewaters from the 1960s to 1990 and a significant improvement since 1991 (Fig. SI-13). The annual flux of COD-Cr decreased by 45 Gg yr⁻¹ in 1991 compared to 1990, which is equivalent to approximately 18 Gg of total organic carbon (TOC), according to a conversion factor of 2.5 (Pitter 1990).

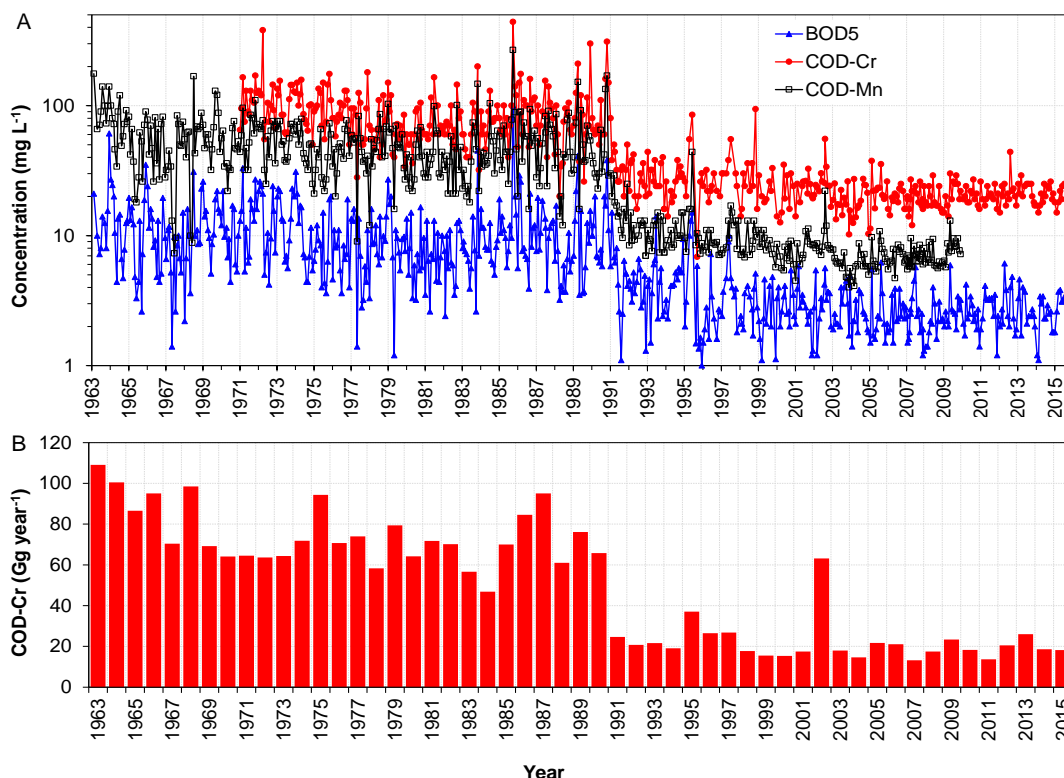


Fig. SI-13. Time series of measured indicators of organic pollution in the Vltava below České Budějovice (Hluboká nad Vltavou profile; Fig. SI-1): (A) Monthly concentrations (random point sampling) of biochemical oxygen demand (BOD₅), chemical oxygen demand by the permanganate method (COD-Mn), and chemical oxygen demand by the dichromate method (COD-Cr); (B) annual fluxes of COD-Cr calculated from the respective monthly average discharges and concentrations (data by the Vltava River Board; <http://hydro.chmi.cz/isarrow/>).

COD-Cr, phosphorus and nitrogen loads associated with wastewaters: Determination of COD-Cr, P, and N production by point wastewater sources in the upper Vltava catchment (Fig. SI-14) was based on development of population; its percentage attached to sewerage systems; percentage of waste waters treated in wastewater treatment plants (WWTPs); sewage treatment facilities and types of wastewater treatment technologies (mechanical, biological, chemical); and specific daily per capita production of COD, P, and N; and COD discharges from WWTPs in the catchment (Statistical Yearbooks of the Czech Republic; Kopáček et al. 2013a; Vystavna et al. 2017; Hejzlar, unpubl. data).

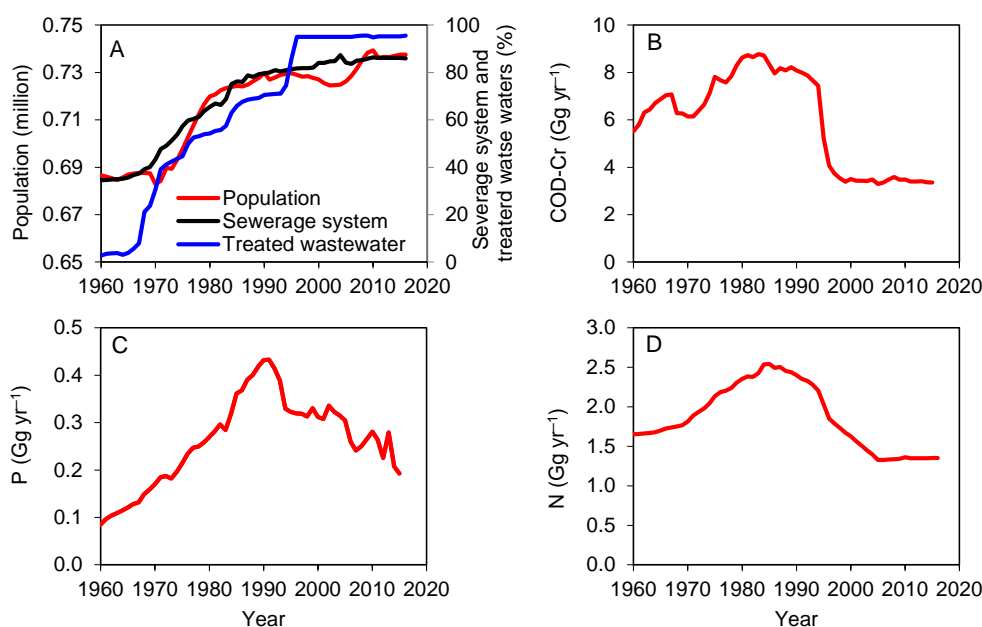


Fig. SI-14. Development of population, its percentage attached to sewerage systems, and percentage of waste waters treated in wastewater treatment plants in the upper Vltava catchment during 1900–2016 (Statistical Yearbooks of the Czech Republic) (A). Annual loads of organic pollution (chemical oxygen demand by the dichromate method, COD-Cr, Hejzlar, unpubl. data) (B), phosphorus (Vystavna et al. 2017) (C), and nitrogen (Kopáček et al. 2013a) (D) in waste waters.

Part SI-6: Details to section: Discussion

pH

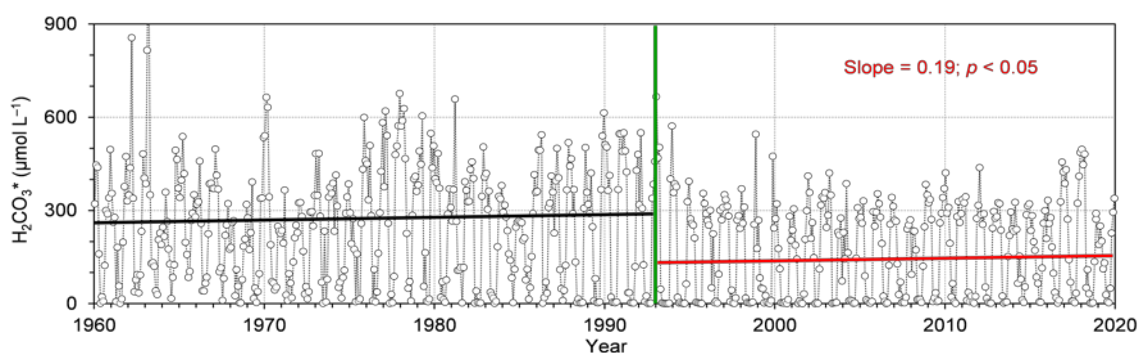


Fig. SI-15. Monthly average concentrations of the sum of dissolved carbon dioxide and carbonic acid ($H_2CO_3^*$) in the Slapy surface layer from January 1960 through December 2019. The red line shows a significant trend of the seasonal Mann-Kendall test (R Core Team 2019), the black line an insignificant trend, and the vertical green line shows the breakpoint. The $H_2CO_3^*$ concentrations were computed from ANC and in-situ pH and water temperature according to Stumm and Morgan (1981).

Part SI-7: Details to section: Acknowledgements

This study would not have been possible without the reprehensible behavior of the Communist Party of China that enabled the unprecedented outbreak of the Chinese flu (COVID-19) throughout the whole world. The following pandemic prevented our work on new projects and provided me abundant time in home office for evaluating existing data. J.K.

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