Water Practice & Technology

Water Practice & Technology Vol 17 No 7, 1523 doi: 10.2166/wpt.2022.081

# Assessing the influence of treated effluent on nutrient enrichment of surface waters using water quality indices and source apportionment

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## ABSTRACT

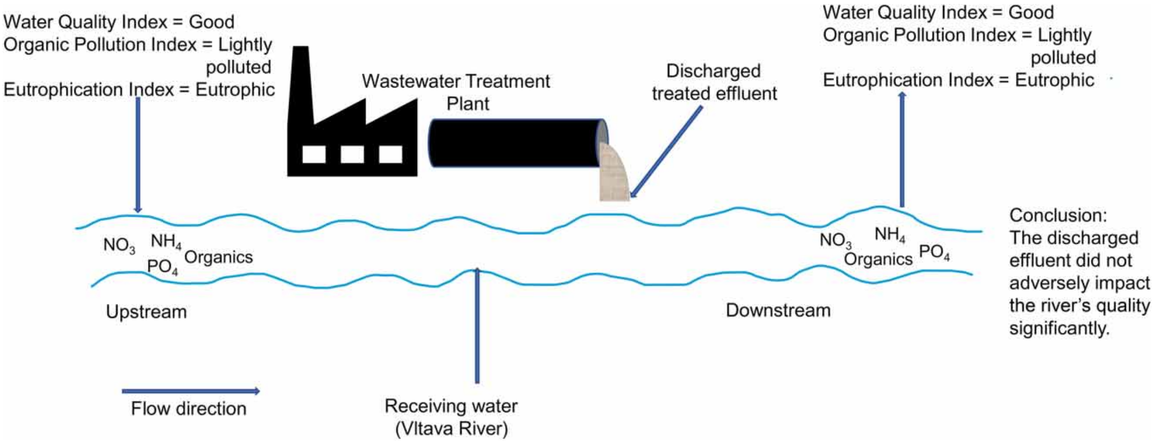
Discharges from wastewater treatment plants have been cited as one of the point sources contributing to surface water quality deterioration. However, does high-quality effluent affect water quality, and contribute significantly to nutrient enrichment or the eutrophication of receiving waters? The Vltava River and a wastewater treatment plant in the Czech Republic were used in a case study, to try to answer these questions through water quality indices and source identification. Samples were collected upstream and downstream of the effluent discharge point, and analyzed for temperature, pH, dissolved oxygen, chemical oxygen demand, nitrate, nitrite, ammonium, phosphate and sulfate. No statistically significant difference ($P > 0.05$) was observed between most of the upstream and downstream samples' physicochemical characteristics. The water quality, organic pollution and eutrophication indices of the river, upstream of the effluent discharge point were 83.48, 2.05 and 2.67, respectively, but increased to 99.06, 2.87 and 3.74 downstream. Nutrient source identification using principal component analysis suggests that the increase might be due to the effluent discharge. However, the river's comprehensive ecological (quality classification) status was the same upstream as downstream, indicating that the discharged effluent did not cause nutrient enrichment of the river.

**Key words:** eutrophication, effluent, pollution indices, principal component analysis, water pollution, water quality index

## HIGHLIGHTS

- The Vltava River's physicochemical characteristics were significantly unaffected by the effluent discharged.
- Water quality and pollution indices showed no adverse influence of effluent on the river's classification status.
- The discharged effluent did not cause nutrient enrichment.

## GRAPHICAL ABSTRACT



## ACRONYMS/ABBREVIATIONS

| | |
|---|---|
| AD | sampling point downstream of discharge point |
| BD | sampling point upstream of discharge point |
| CODcr | chemical oxygen demand (analyzed using dichromate) |
| ČSN | Česká Agentura pro Standardizaci (Czech Standardization Agency) |
| D | wastewater discharge point (to river) |
| DIN | dissolved inorganic nitrogen |
| DIP | dissolved inorganic phosphorus |
| DO | dissolved oxygen |
| EI | eutrophication index |
| EU | European Union |
| N | nitrogen |
| OPI | organic pollution index |
| P | phosphorus |
| PC | principal component |
| PCA | principal component analysis |
| WQI | water quality index |
| WWTP | wastewater treatment plant |

## 1. INTRODUCTION

Water scarcity is becoming a major issue not only in semi-arid and arid regions of the globe but also in regions of freshwater abundance (Becerra-Castro *et al.* 2015). The United Nations, through the Sustainable Development Goal 6, seeks to tackle this by increasing water-use efficiency and improving water quality through pollution reduction (United Nations 2015). The increasing scarcity arises not only from diminishing quantities but also deteriorating surface water quality. One such deterioration is nutrient enrichment or eutrophication of freshwater, which is a threat to surface water security (Jiang *et al.* 2019).

Eutrophication is the excessive nutrient enrichment of surface water, and is characterized by the overgrowth of autotrophs such as algae and cyanobacteria (Khan *et al.* 2014). The two nutrients considered responsible for eutrophication are phosphorus (P) and nitrogen (N) (Smith *et al.* 1999). Excessive nitrogen and phosphorus inflow into water bodies affects aquatic ecosystem structures, processes and functions negatively (Bhagowati & Ahamad 2019), and could trigger algal growth (Scannone 2016). The overgrowth of algae, non-vascular photosynthetic and chlorophyll-bearing aquatic organisms (Lopez-Gomez & Perez-Rivero 2019), may block light penetration and prevent re-oxygenation of the water (Ansari *et al.* 2011). Inorganic carbon depletion due to high rates of photosynthesis, as well as of dissolved oxygen, characterize the effects of eutrophication on aquatic ecosystems (Lehtiniemi *et al.* 2005; Tuner & Chislock 2010; Chislock *et al.* 2013). These effects could create hypoxic or anoxic conditions within the water environment, and deprive aquatic organisms (e.g. fish) of oxygen (Chislock *et al.* 2013).

Anthropogenic activities such as dam construction, agriculture run-off, and municipal and industrial effluent discharge, have increased the rate and magnitude of nutrient enrichment in surface waters greatly (Carpenter *et al.* 1998; Carpenter 2005; Zhang *et al.* 2010). Wastewater treatment plants (WWTPs) are considered major nutrient point sources in the enrichment of surface waters (USEPA 2021). However, this assertion is mostly based on discharges of untreated and relatively poorly treated effluent, not high-quality effluent. The question of whether high-quality effluent affects water quality negatively significantly or contributes significantly to nutrient enrichment needs answering. The study, therefore, seeks to provide insight by evaluating the impact of a high-quality effluent on nutrient concentration in a receiving water. Water quality, organic pollution, and eutrophication indices, and principal component analysis were used to demonstrate the impact of a WWTP's effluent on the Vltava River, in the Czech Republic, as a case study. In this study, a 'high quality effluent' means an effluent containing concentrations lower than the limits stipulated in the 'European Union Urban Wastewater Treatment Directive-1991," for 100,000 PE (European Commission 1991).

A water quality index (WQI) expresses the overall water quality of a given water body as an index number or single numeric value, reflecting the composite impact of the various water quality parameters (Mladenović-Ranisavljevic & Zerajic 2017; Ahn & Lyu 2020). A eutrophication index (EI) demonstrates the effect of an organic and nutrient pollutant load on water quality (Liu *et al.* 2011), and the organic pollution index (OPI) accounts for the multivariate effects of dissolved oxygen (DO), chemical oxygen demand (COD), dissolved inorganic nitrogen (DIN) and dissolved inorganic phosphorus (DIP) on water quality. Understanding the contribution and impact of high-quality effluent from WWTPs on surface water nutrient enrichment is vital to ensure water resource sustainability.

## 2. MATERIALS AND METHODS

### 2.1. Study area

The Vltava River flows through Prague and the Bohemian region of the Czech Republic, a landlocked country in central Europe covering 78,865 $km^2$ in total. It drains the country's entire southern region (Mahoney 2011) and is 430.2 km long, with an average flow rate of 151 $m^3 s^{-1}$ and a 28,090 $km^2$ catchment (Vltava 2020). The municipal WWTP studied is located within the river's catchment, uses activated sludge treatment process and has a population equivalent of over one million.

### 2.2. Sampling and analyses

Eleven sampling campaigns were carried out between November 2020 and January 2021 at both BD and AD (Figure 1). AD's location was chosen to avoid other point and diffuse pollution sources, thus ensuring that any nutrient flux observed could be evaluated with respect to the WWTP. Water samples were collected in one-liter, prewashed polyethylene bottles that were rinsed three times with river water at the time of sampling. Samples were taken below the water surface and against the current flow. 24-hour composite effluent samples were taken periodically from the WWTP outlet.

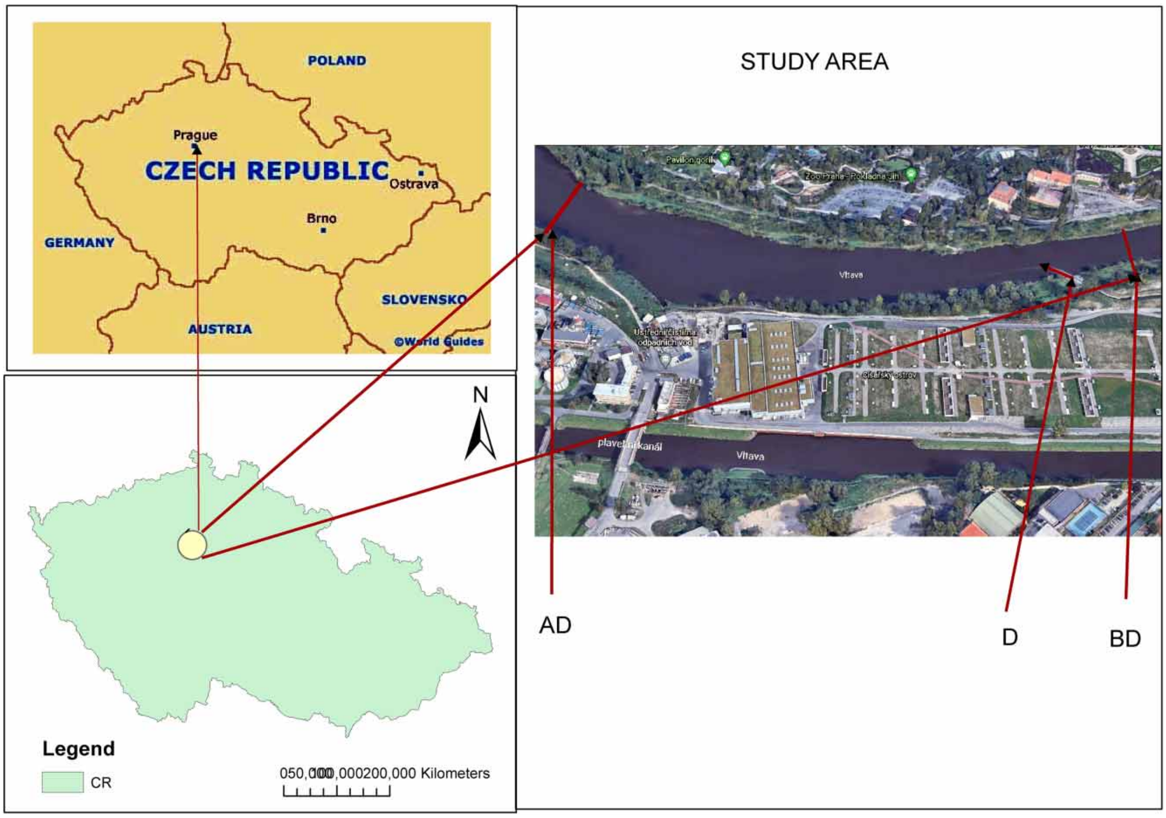


**Figure 1** | Study area showing the sampling and effluent discharge points. The river flows from right to left. D – effluent discharge point; BD – sampling point about 50 m upstream of D; AD – sampling point about 600 m downstream of D (Map with yellow background adopted from World Guides 2019).

All samples were analyzed for temperature, pH, DO, COD, nitrate, nitrite, ammonium, orthophosphate, sulfate, DIN and DIP. Temperature, DO and pH were measured with a portable multimeter, WTW Multi 3420 (WTW GmbH, Germany) immediately after sample collection. All other parameters were determined in triplicate in the laboratory using a photoLab 7100 Vis series spectrophotometer (WTW GmbH, Germany). Orthophosphate and COD were analyzed by colorimetry, and sulfate by turbidimetry (APHA 2012). Nitrate-N, nitrite-N and ammonium-N were determined according to the procedures outlined by Benáková *et al.* (2018) using the 2,6-dimethylphenol, SANED and Nessler's reagent methods, respectively. DIN was computed from the sum of the three forms of nitrogen measured, and DIP is equivalent to orthophosphate, as measured (National Water Quality Monitoring Council 2007).

### 2.3. Water quality and pollution indices

The WQI used in this study was adopted from Ravikumar *et al.* (2013) and is based on the sum of the water quality sub-indices of each parameter measured, as outlined in Equations (1)–(4).

$$WQI = \sum_{i=1}^{n} SI_i \tag{1}$$

where $SI$ represents the water quality sub-index for each parameter

$$SI = W_i q_i \tag{2}$$

$$q_i = \left(\frac{C_i}{S_i}\right) \times 100 \tag{3}$$

where $q_i$ is the quality rating, $C_i$ the water quality parameter concentration in each sample (mg $L^{-1}$) and $S_i$ the water quality standard set by the designated institution (Czech Water Quality Standards-ČSN 757221).

$$W_i = \frac{w_i}{\sum\limits_{n=1}^{n} w_i} \tag{4}$$

where $W_i$ denotes the relative weight, $w_i$ the weight of each parameter measured and $n$ the number of parameters measured. The water quality parameters' assigned and computed relative weightings for this study are reported in Table 1.

**Table 1** | Water quality parameters and assigned weightings

| **Parameter** | **Assigned Weight ($w_i$)** | **Computed Relative Weight ($W_i$)** |
|---|---|---|
| Temperature | 4 | 0.1111 |
| pH | 3 | 0.0833 |
| DO | 2 | 0.0556 |
| $COD_{cr}$ | 3 | 0.0833 |
| Nitrate-N | 5 | 0.1389 |
| Nitrite-N | 5 | 0.1389 |
| Ammonium-N | 5 | 0.1389 |
| Phosphate | 5 | 0.1389 |
| Sulfate | 4 | 0.1111 |
| | Sum = 36 | Sum = 1.0000 |

Except for tables, nitrate, nitrite and ammonium will be written without N for simplicity.

The assigned weights for sulfate and pH were obtained from Xiao *et al.* (2019) and Ravikumar *et al.* (2013), respectively. Nitrate, nitrite, ammonium, and phosphate were given the highest weight because they constitute the limiting parameters (N and P) in eutrophication; temperature and DO weights were based on their contribution to eutrophication (Yang *et al.* 2008).

OPI and EI were taken from the work of Liu *et al.* (2011).

$$OPI = \frac{COD}{COD_s} + \frac{DIN}{DIN_s} + \frac{DIP}{DIP_s} - \frac{DO}{DO_s} \tag{5}$$

$$EI = \frac{COD \times DIN \times DIP}{S_c} \tag{6}$$

where $COD$, $DIN$, $DIP$ and $DO$ are the measured concentrations, as defined above. $COD_s$, $DIN_s$, $DIP_s$ and $DO_s$ are their background/standard concentrations, respectively, as defined in water quality standards. $S_c$ is the mean of the product of $COD_s$, $DIN_s$ and $DIP_s$ (Xiao *et al.* 2007). The values of $COD_s$, $DIN_s$ and $DIP_s$ used were 26, 5.75 and 0.039 mg $L^{-1}$, respectively. The first two were computed from Czech surface water ecological background values (ČSN 757221 2015) and the latter from a European Union report (Phillips *et al.* 2018).

The water quality classification schemes for WQI, OPI and EI are reported in Table 2.

**Table 2** | WQI, OPI and EI classification scheme

| Index | Classification Range | Classification Status | Reference |
|---|---|---|---|
| WQI | $WQI < 50$ | Excellent | Ravikumar *et al.* (2013) & Wu *et al.* (2020) |
| | $50 < WQI < 100$ | Good | |
| | $100 < WQI < 200$ | Poor | |
| | $200 < WQI < 300$ | Very poor | |
| OPI | $WQI > 300$ | Unsuitable | Liu *et al.* (2011) |
| | $OPI < 0$ | Excellent | |
| | $0 \leq OPI < 1$ | Good | |
| | $1 < OPI < 2$ | Contamination begins | |
| | $2 < OPI < 3$ | Lightly polluted | |
| | $3 < OPI < 4$ | Moderately polluted | |
| | $OPI > 4$ | Heavily polluted | |
| EI | $EI > 1$ | Eutrophication | |

### 2.4. Data analyses

The data were analyzed using RStudio (1.3.1073 and 1.2.5042). Source identification analysis and evaluation were performed using principal component analysis (PCA), a statistical transformation technique that seeks to identify the fewest variables of a given dataset needed to explain most of the variance found and then arranges the variables from highest to lowest in terms of variance (Kuc *et al.* 2016; Shi *et al.* 2020). Student's t-test and Pearson correlation were used in establishing the significance of the difference between the datasets from BD and AD, and the correlations among the variables, respectively.

## 3. RESULTS AND DISCUSSION

### 3.1. The river's physicochemical parameters

#### 3.1.1. Temperature

The Vltava River's temperature downstream of D was higher (5.65 ± 1.16 °C) than that upstream (4.89 ± 1.38 °C). The temperatures at both BD and AD were within the Czech surface water ecological status background limit (Table 3). The effluent temperature was 19.69 ± 3.06 °C, relatively higher than the river, but the latter's temperature was not altered significantly by the effluent, perhaps because of the small discharge compared to the size and volume of the river. Student's t-test showed no significant difference in temperature between BD and AD ($P > 0.05$). A greater increase in temperature could stimulate the growth of organisms and promote the biological invasion of species (Hardenbicker *et al.* 2017).

#### 3.1.2. pH

The pH of the river (at both BD and AD) and the effluent were within the acceptable range of 5–9 (ČSN 757221 2015). The effluent – pH 7.54 ± 0.12 – was slightly alkaline, and the river more or less neutral – pH 7.11 ± 0.19 upstream of D and 6.95 ± 0.14 downstream. This indicates that the river is in good condition and can support aquatic life. The effluent did not impact the receiving water's acidity or alkalinity significantly ($P > 0.05$), and its influence could, therefore, be considered negligible.

#### 3.1.3. DO

DO is a measure of the amount of oxygen dissolved in water (Sari *et al.* 2020). The DO contents of the effluent, BD and AD were 8.93 ± 0.73, 11.85 ± 0.75 and 11.54 ± 0.54 mg $L^{-1}$, respectively, indicating that the river might be in good condition. No significant difference was observed between the DO contents at BD and AD, and this was confirmed statistically by Student's t-test, which gave $P > 0.05$. The river's ecological integrity was not affected adversely and, therefore, the river could support diverse aquatic life (Darius *et al.* 2019).

**Table 3** | Results of physicochemical water quality analysis of the Vltava River and WWTP effluent, and reference water quality

| Parameter | Unit | Vltava River at BD | Vltava River at AD | WWTP Effluent | Reference Water Quality (ČSN 757221 2015) |
|---|---|---|---|---|---|
| Temperature | °C | 4.89 ± 1.38 | 5.65 ± 1.16 | 19.69 ± 3.06 | 29 |
| pH | – | 7.11 ± 0.19 | 6.95 ± 0.14 | 7.54 ± 0.12 | 5.0–9.0 |
| DO | mg $L^{-1}$ | 11.85 ± 0.75 | 11.54 ± 0.54 | 8.93 ± 0.73 | >9 |
| $COD_{cr}$ | mg $L^{-1}$ | 16.94 ± 2.93 | 16.17 ± 4.04 | 19.71 ± 3.11 | 26 |
| Nitrate-N | mg $L^{-1}$ | 3.46 ± 0.16 | 3.64 ± 0.24 | 7.50 ± 0.84 | 5.4 |
| Nitrite-N | mg $L^{-1}$ | 0.014 ± 0.007 | 0.015 ± 0.008 | 0.016 ± 0.007 | 0.12 |
| Ammonium-N | mg $L^{-1}$ | 0.34 ± 0.08 | 0.41 ± 0.13 | 0.47 ± 0.27 | 0.23 |
| Phosphate | mg $L^{-1}$ | 0.08 ± 0.01 | 0.11 ± 0.02 | 0.78 ± 0.11 | NA |
| Sulfate | mg $L^{-1}$ | 15.44 ± 5.44 | 23.13 ± 5.49 | 103.87 ± 37.63 | 200 |

NA: limit not available.

### 3.1.4. COD

The concentration of organics in the river was lower than in the effluent discharged. The $COD_{cr}$ for BD, AD and D being 16.94 ± 2.93, 16.17 ± 4.04 and 19.71 ± 3.11 mg $L^{-1}$, respectively. The river's organic load is relatively low compared to others, such as the Biebrza River, which has COD of 36.51 mg $L^{-1}$ (Glinska-Lewczuk *et al.* 2016). A good connection exists between DO, pH and surface water organic content. Low pH and DO, for instance, could indicate high organic loads (Sari *et al.* 2020; Sukanya & Joseph 2020), which was not the case in this study. The DO was relatively high and remained unchanged. Considering the combination of the river's COD content, and the pH and high DO (at both BD and AD), it appears that the river's ecological status was not altered negatively by the effluent discharged.

### 3.1.5. Nutrients (nitrate-N, nitrite-N, ammonium-N and phosphate)

Nitrate, nitrite and ammonium increased from 3.46 ± 0.16 to 3.64 ± 0.24 mg $L^{-1}$, 0.014 ± 0.007 to 0.015 ± 0.008 mg $L^{-1}$, and 0.34 ± 0.08 to 0.41 ± 0.13 mg $L^{-1}$, respectively. Sulfate and phosphate, on the other hand, increased from 15.44 ± 5.44 to 23.13 ± 5.49 mg $L^{-1}$ and 0.08 ± 0.01 to 0.11 ± 0.02 mg $L^{-1}$ respectively (Table 3). Ammonium and phosphate both exceeded the respective Czech ecological limits (0.23 mg $L^{-1}$ and 0.039 mg $L^{-1}$) (ČSN 757221 2015; Phillips *et al.* 2018). The data showed no nutrient enrichment at AD relative to BD, however, and this was confirmed by the different water quality indices.

## 3.2. Water quality

The Czech ČSN 75 7221 surface water limits were used as the study's reference values and the majority of the parameters analyzed met the standard's requirements (ČSN 757221 2015; Mičaník *et al.* 2017). Ammonium and phosphate were the only two, out of nine, that exceeded the reference limits. The ammonium concentration at both BD and AD exceeded the 0.23 mg $L^{-1}$ limit. A similar observation was made for phosphate. At both AD and BD, the phosphate content was between twice and thrice the 0.039 mg $L^{-1}$ reference value. (ČSN 757221 does not provide a limit for phosphate, the value of 0.039 mg $L^{-1}$ was adopted from the EU report 'Best practice for establishing nutrient concentrations to support good ecological status' (Phillips *et al.* 2018)).

The WWTP effluent also met all stipulations of the EU Urban Wastewater Treatment Directive (European Commission 1991), although the Czech surface water standards were used in the study (Table 3), in the assessment of effluent quality. In other words, the approach taken was conservative and the effluent was regarded, hypothetically, as a 'tributary' of the Vltava. In this regard, five of the nine water quality requirements were met.

The nutrient level variations between BD and AD showed a pattern of marginal increase that was not statistically significant ($P > 0.05$) in most cases. The increase observed could be attributed to ammonium nitrification and organic matter remineralization (Santos *et al.* 2008), and the influence of the WWTP effluent. Santos *et al.* (2008) also made a similar observation in their studies on the Vieira Creek, Brazil, attributing the increase partly to the discharged effluent. As there are no other anthropogenic sources such as industrial discharges or agricultural activities between BD and AD, except the WWTP, it seems likely that the observed increase may be due to the effluent. However, the increase did not constitute enrichment as it was insignificant.

### 3.3. Correlation matrix and PCA

At BD (Table 4a), the temperature had a strong correlation (r = 0.84) with nitrite, which also had a moderate correlation with nitrate, suggesting they might share a common source. The source of nitrate and nitrite could be natural processes such as the nitrification of ammonium. Ammonium oxidizing microorganisms in the river might have oxidized ammonia, to produce nitrate and nitrite (Fan *et al.* 2015). Phosphate, pH and sulfate had no strong correlation with any other variables, indicating that they do not share their sources with the other nutrients. Phosphate may have been released into the river from its sediments (Sugiyama & Hama 2013).

**Table 4** | Correlation matrix of BD (a) and AD (b) showing the correlations among the different water quality parameters

| | *Temperature* | *pH* | *DO* | *COD$_{cr}$* | *Nitrate-N* | *Nitrite-N* | *Ammonium-N* | *Phosphate* | *Sulfate* |
|---|---|---|---|---|---|---|---|---|---|
| (a) | | | | | | | | | |
| Temperature | 1.00 | | | | | | | | |
| pH | −0.03 | 1.00 | | | | | | | |
| DO | −0.94 | 0.29 | 1.00 | | | | | | |
| COD$_{cr}$ | −0.21 | 0.16 | 0.43 | 1.00 | | | | | |
| Nitrate-N | 0.28 | −0.04 | −0.41 | −0.43 | 1.00 | | | | |
| Nitrite-N | 0.84 | 0.13 | −0.82 | −0.49 | 0.57 | 1.00 | | | |
| Ammonium-N | 0.02 | 0.47 | 0.20 | 0.52 | −0.54 | −0.12 | 1.00 | | |
| Phosphate | −0.08 | −0.35 | 0.01 | 0.26 | 0.19 | −0.01 | −0.06 | 1.00 | |
| Sulfate | −0.25 | −0.33 | 0.05 | −0.46 | −0.14 | −0.30 | −0.23 | −0.39 | 1.00 |
| (b) | | | | | | | | | |
| Temperature | 1.00 | | | | | | | | |
| pH | 0.02 | 1.00 | | | | | | | |
| DO | −0.90 | 0.31 | 1.00 | | | | | | |
| COD$_{cr}$ | 0.14 | 0.80 | 0.14 | 1.00 | | | | | |
| Nitrate-N | 0.05 | −0.39 | −0.03 | −0.34 | 1.00 | | | | |
| Nitrite-N | 0.74 | −0.17 | −0.66 | −0.13 | 0.53 | 1.00 | | | |
| Ammonium-N | 0.01 | −0.05 | −0.03 | −0.39 | 0.25 | 0.49 | 1.00 | | |
| Phosphate | 0.60 | −0.17 | −0.46 | −0.19 | 0.16 | 0.68 | 0.30 | 1.00 | |
| Sulfate | −0.52 | −0.33 | 0.19 | −0.67 | −0.07 | −0.32 | 0.41 | −0.39 | 1.00 |

At AD (Table 4b) nitrite had strong to moderate correlations with both nitrate (r = 0.53) and phosphate (r = 0.68), indicating a possible common origin, which could be anthropogenic. In a similar study on the Karamana River (India), Sukanya & Joseph (2020) found a moderate correlation between sulfate, nitrate, DIN and DIP. They suggested that these nutrients might have originated from effluent discharged to the river. It also seems likely that the marginal increments at AD might have originated from the WWTP effluent. Such increments were so small, however, that the river did not become enriched. COD$_{cr}$ had a strong correlation with pH (r = 0.80), indicating the influence of organics on pH. Phosphate and temperature had a moderate correlation (r = 0.60).

PCA is an important tool in pollution source identification. Borůvka *et al.* (2005) and Wu *et al.* (2020) applied PCA to identify pollutant sources in soil and water, respectively. Four principal components (PCs) with eigenvalues exceeding 1 were obtained for both BD and AD. BD's PC1, PC2, PC3 and PC4 had eigenvalues of 3.41, 2.07, 1.56 and 1.07, respectively, accounting for 90% of the total variation in the data (Table 5a). At BD, nitrite had the highest loading (0.90) in PC1, followed by temperature (0.81) and nitrate (0.68). The source of nitrate and nitrite at BD is likely to be natural. PC2 for BD had ammonium with the highest loading (0.68), with pH and COD$_{cr}$ showing moderate loadings, suggesting that the pH of the river water might have influenced its ammonium and organic content. Phosphate had a loading of 0.91 for PC3 at BD, and pH and nitrate 0.57 and 0.56, respectively. Phosphate may have been released into the river from its sediments. Cerozi & Fitzsimmons (2016) made similar findings concerning the release of orthophosphate into water in a study involving aquaponics.

**Table 5** | PCA showing the variables' loadings at the river sampling points

| | PC1 | PC2 | PC3 | PC4 |
|---|---|---|---|---|
| (a) BD | | | | |
| Temperature | **0.81** | 0.42 | −0.14 | −0.34 |
| pH | −0.2 | **0.57** | −0.51 | **0.57** |
| DO | −0.91 | −0.13 | 0.05 | 0.36 |
| $COD_{cr}$ | −0.64 | 0.54 | 0.31 | −0.19 |
| Nitrate-N | **0.68** | −0.11 | 0.28 | **0.56** |
| Nitrite-N | **0.9** | 0.35 | −0.11 | 0.09 |
| Ammonium-N | −0.43 | **0.68** | −0.3 | −0.27 |
| Phosphate | 0 | 0.14 | **0.91** | −0.05 |
| Sulfate | −0.06 | −0.8 | −0.42 | −0.24 |
| Eigenvalue | 3.41 | 2.07 | 1.56 | 1.07 |
| Proportion | 0.38 | 0.23 | 0.17 | 0.12 |
| Cumulative proportion (%) | 38.0 | 61.0 | 78.0 | 90.0 |
| (b) AD | | | | |
| Temperature | **0.85** | 0.42 | −0.18 | −0.18 |
| pH | −0.32 | **0.71** | **0.51** | −0.24 |
| DO | −0.81 | −0.11 | 0.43 | 0.27 |
| $COD_{cr}$ | −0.23 | **0.92** | 0.2 | 0.06 |
| Nitrate-N | 0.41 | −0.39 | 0.28 | **0.73** |
| Nitrite-N | **0.94** | 0 | 0.28 | 0.06 |
| Ammonium-N | 0.33 | −0.5 | **0.69** | −0.37 |
| Phosphate | **0.79** | 0.06 | 0.13 | −0.09 |
| Sulfate | −0.34 | −0.81 | −0.02 | −0.4 |
| Eigenvalue | 3.43 | 2.6 | 1.17 | 1.01 |
| Proportion | 0.38 | 0.29 | 0.13 | 0.11 |
| Cumulative proportion (%) | 38.0 | 67.0 | 80.0 | 91.0 |

Bold figures represent positive significant loadings under each principal component.

The four PCs for AD (PC1 to PC4) had eigenvalues of 3.43, 2.60, 1.17 and 1.0, respectively, and explained 91% of the data variability (Table 5b). PC1 had nitrite, temperature and phosphate loadings of 0.94, 0.85 and 0.79, respectively, accounting for 38% of total variability. The strong positive loadings of nitrite and phosphate suggest that these two parameters have the same source, and that temperature might have influenced their availability in the river. Under neutral pH conditions, almost no phosphorus is released from sediments (Li *et al.* 2013), so the phosphate increment at AD was not from the sediment as the pH there was almost neutral. As there is no potential source for these nutrients in the study area other than the WWTP, the increases in these nutrients must arise from the discharged effluent. Sukanya & Joseph (2020) found much the same in their similar study.

The variables were projected together with their loadings in a biplot (Figure 2). The biplot for BD captured 60.9% of the variance (Figure 2(a)). As can be seen, temperature and nitrite are highly correlated, suggesting that the nitrite concentration in the river might be affected by temperature. Since the source of nitrite is thought to be natural, the water temperature was crucial to its availability (Sukanya & Joseph 2020). Ammonium and $COD_{cr}$ exhibited moderate correlation, suggesting a similar origin. The ammonium and organic content at BD is thought to have been influenced by human activities such as agriculture and effluent discharge upstream (Nireti *et al.* 2017). The sources of phosphate and sulfate may be distinct from that of the other nutrient variables at BD as they show no significant correlation with the other parameters.

The biplot of AD explained 67% of the variability (Figure 2(b)). Phosphate and nitrite are moderately correlated, indicating that they might have originated from the same source, probably anthropogenic.

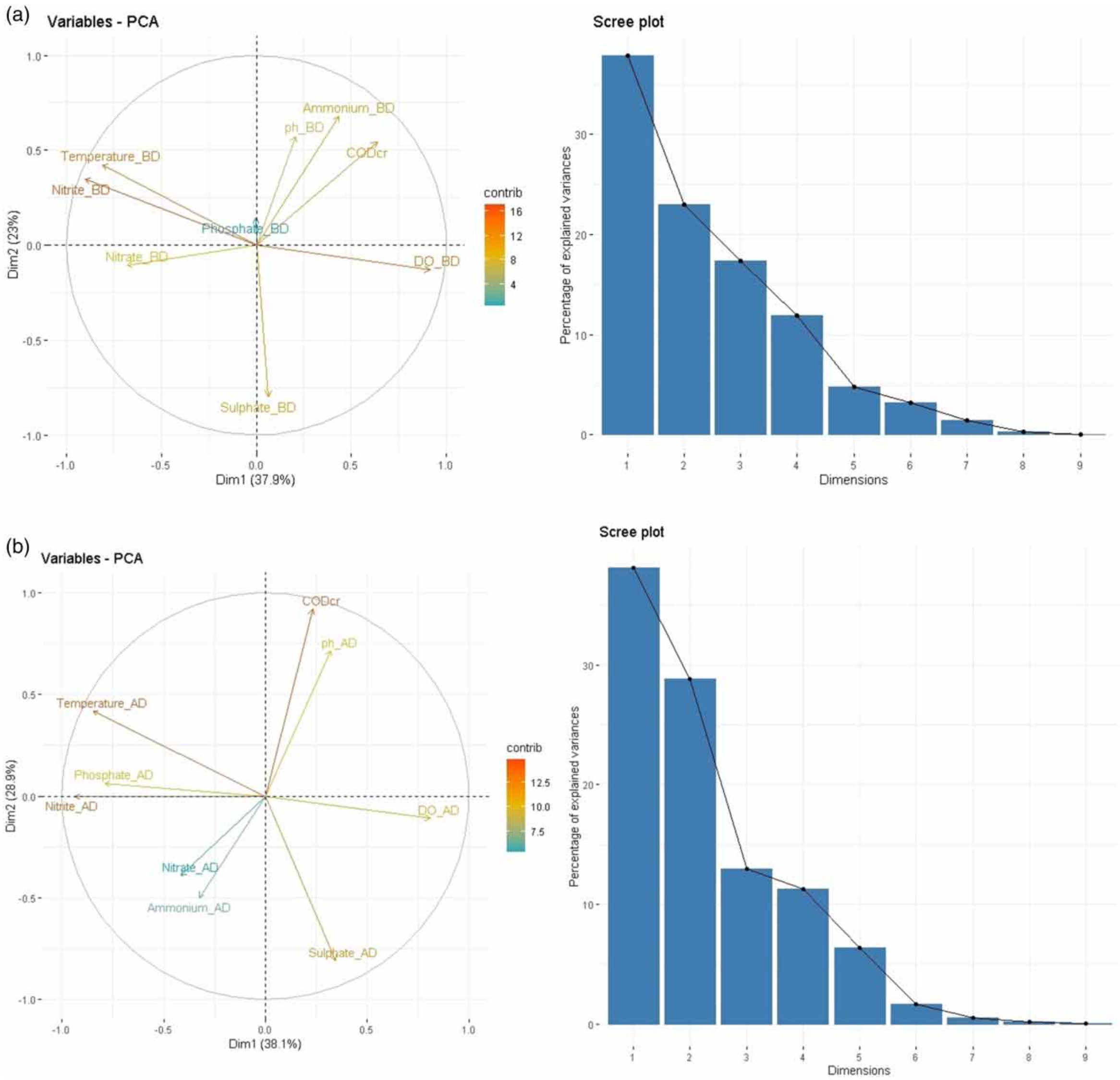


**Figure 2** | Projections of the variables, with their loadings, and scree plots (a) BD (b) AD.

The correlation matrix and PCAs suggest that the slight increments observed at AD are due to anthropogenic influence, and that the effluent from the WWTP might be the cause. This may not necessarily be the case, however. It might have arisen partly through biological or biochemical processes like nitrification and organic matter remineralization (Santos *et al.* 2008) in the river's ecosystem. A Student's t-test showed a statistically non-significant difference between nitrate concentrations at AD and BD. It is noted in this context that the effluent discharge is relatively small (less than $2\ m^3\ s^{-1}$) compared to the flow in the river ($151\ m^3\ s^{-1}$).

A Student's t-test also showed that the difference in phosphate between BD and AD was statistically significant ($P<0.05$). Since the only source of phosphate in the river is the WWTP, it is likely that the increase was caused by the effluent discharge. While the increase was statistically significant, no phosphate enrichment occurred. The increase in sulfate downstream of D arises from both natural (groundwater intrusion, and biological and chemical reactions in the water environment) and anthropogenic (effluent) causes.

### 3.4. WQI, OPI and EI

The WQI showed that BD (83.48) and AD (99.06) have the same classification status – 'good' (Table 6). The classifications imply that the effluent did not change the river's comprehensive quality status, and hence did not impact the river negatively. This is contrary to the common perception that WWTP discharges are a major source of nutrient input to surface waters (USEPA 2021). While this is true for poorly treated effluent, this study suggests otherwise for high quality effluents. In the case of poorly treated effluent, continuous nutrient (phosphate) input into a river over a long period could enrich it and trigger eutrophication, which could affect the river's ecosystem severely. This could cause the river to become hypoxic or anoxic, posing a significant risk to fish and other aquatic organisms that depend on DO to survive (Chislock *et al.* 2013). Considering the high quality of the effluent, its relatively low discharge rate and the river's cleansing ability, such water quality deterioration is not anticipated in this case.

**Table 6** | Water quality classification of BD and AD according to the different indices

| | OPI | Status | EI | Status | WQI | Status |
|---|---|---|---|---|---|---|
| BD | 2.05 | Lightly polluted | 2.67 | Eutrophication | 83.48 | Good |
| AD | 2.87 | Lightly polluted | 3.74 | Eutrophication | 99.06 | Good |
| Diff. | 0.82 | | 1.07 | | 15.58 | |

Diff.: index value differences between BD and AD; The unfiltered concentrations of nitrate, nitrite, ammonium and phosphate were used for the computation as ČSN 75 7221 does not provide limits for DIN and DIP.

The OPI at BD and AD was 2.05 and 2.87, respectively, characterizing both as lightly polluted. This index accounts for the multivariate effects of organics, nutrients and DO, and has also shown that the effluent did not change the river's ecological status. Even though water quality at AD was degraded compared to BD, indicating the possible impact of the effluent, that impact was insignificant. The river's ecological state was the same upstream and downstream of D. The lightly polluted status can arise from both point and diffuse source pollution, as well as wastewater discharges upstream of the study area.

Both BD and AD were eutrophic according to the EI, although no algal growth was observed at the time of sampling. This may be attributable to the river's relatively fast current and low retention time, which may have hindered biomass growth. The growth of algae and other aquatic plants does not depend solely on the availability of limiting nutrients (N and P). Other factors such as chlorophyll, temperature and sunlight are also relevant (Yang *et al.* 2008), but these factors may not have been conducive to support algal growth at the time. Also, the limiting nutrient concentrations may have been inadequate to support algal growth, considering the river's volume and flow. The EI, like the WQI and OPI, thus indicates the negligible influence of the effluent discharge on the river. The river's eutrophic status may have been induced by human activities including agriculture, and industrial and municipal wastewater discharges upstream of BD.

## 4. CONCLUSIONS

Applying water quality and pollution indices coupled with PCAs, enabled assessment of the influence of high-quality WWTP effluent on nutrient enrichment of surface waters, using the Vltava River as a case study. It was found that the river's water quality parameters downstream of the effluent discharge point were altered slightly, but the change was statistically insignificant, except for sulfate and phosphate. The river's general pollution status in terms of water quality, organic pollution, and nutrient enrichment remained the same (Table 6). This implies that the effluent discharged by the WWTP has no adverse effect on the river. This could be attributed to the relatively high quality of the effluent discharged.

## ACKNOWLEDGEMENTS

The support of the Biological Wastewater Treatment Research Group of the University of Chemistry and Technology, Prague, is duly acknowledged. Furthermore, we are grateful to the WWTP for providing the effluent for analysis. This work was supported partly by the European Commission within the Horizon 2020 project: Achieving wider uptake of water-smart solutions (H2020-SC5-2019-2), grant number: 869283.

## DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

## CONFLICT OF INTEREST

The authors declare there is no conflict.

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First received 26 May 2022; accepted in revised form 8 July 2022. Available online 13 July 2022