

Primary Research Paper

## The occurrence of a bloom-forming green alga *Pleodorina indica* (Volvocales) in the downstream reach of the River Malse (Czech Republic)

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### Abstract

In mid-August 2003 a massive bloom of the green alga *Pleodorina indica* (Iyengar) Nozaki (Volvocales) occurred in the downstream reach of the River Malše in the Czech Republic. An exceptionally long period of the hot dry weather resulted in low flow conditions and warm water temperatures. These environmental conditions combined with high nutrient concentrations led to the macroscopic appearance of *P. indica* in the form of an extensive surface scum formation with diurnal changes. The alga soon dominated the river phytoplankton, attaining concentrations of 2000 colonies per millilitre. The bloom persisted in the river from the 10–17 August when it was eventually flushed out. *P. indica* is an indigenous species of tropical climatic regions and prior to this its massive occurrence has not been reported in the temperate region of Central Europe.

### Introduction

Algae and cyanobacteria are commonly involved in a variety of biological processes that influence river water quality. When rivers host an increased growth of planktonic algae or cyanobacteria and such proliferation is dominated by a single (or a few) species, the phenomenon is referred to as an algal or cyanobacterial bloom (Chorus & Bartram, 1999). Mostly cyanobacterial blooms have been studied due to their potential toxin production (Sherman et al., 1998; Mitrovic et al., 2001), however algal blooms were also referred (Roos & Pieterse, 1996). Such an excessive growth of algae dramatically impairs water quality and, as the accumulated biomass is being decomposed by microorganisms, a serious oxygen depletion results that is often fatal to sensitive organisms.

Flagellated green algae are found in a variety of differing aquatic habitats. Some species tend to form blooms primarily in semi-permanent rain

pools (Al-Homaidan & Arif, 1998; Desnitski, 2000). In such environments their life cycle provides for rapid colonisation and further growth (Desnitski, 2000, 2002). Cultivated *Pleodorina* strains have been the subjects of numerous molecular, morphological and phylogenetic analyses (e.g., Nozaki, 1996; Nozaki et al., 1997, 1999; Angeler et al., 1999; Coleman, 2002; Nozaki, 2003a). Further, Desnitski (2003) reviewed the geographical distribution and morphology of *Pleodorina* in detail. Yet in all these studies of *Pleodorina*, the ecology of the different species was not addressed.

*P. indica* (Iyengar) Nozaki, which flourished in the River Malše is an indigenous species of tropical climates and prior to the Czech Republic bloom, it had not been reported in the temperate regions of Central Europe. The objective of this paper is: (1) to document the first known occurrence of this thermophilic green alga within the temperate regions of Central Europe and review its geographical distribution and (2) to describe the

particular ecological factors which resulted in the massive bloom of *P. indica*.

## Materials and methods

### Study site description

The River Malše is a shallow, eutrophic river originating in Austria (Malsch) and flowing through the southern part of the Czech Republic in Central Europe. Approximately 40% of the catchment area (961 km<sup>2</sup>) is covered by agricultural lands and forests. After traversing a distance of 89.5 km it converges from the east with the River Vltava in the city of České Budějovice at an altitude of 385 m a.s.l. At the confluence the river channel is 30 m wide and 3 m deep. It has been artificially confined by means of canals, which have sparse shoreline vegetation. The long-term mean discharge is 7 m<sup>3</sup> s<sup>-1</sup>. Downstream of the confluence is a manmade weir that impedes flows and impounds water for a distance of 1.5 km up the River Malše. This area, with its 'stagnant water' appearance, was the study site.

### Sampling and methods

In mid-August 2003, three sets of samples were collected from the surface at three separate sampling sites (A–C) along a longitudinal profile of the downstream reach of the River Malše (Fig. 1). Duplicated samples were taken from the shore. Diurnal changes were observed on two-hour intervals from 6 a.m. to 10 p.m. for three consecutive days (August 13–15). Water temperature and pH were measured *in situ* with a WTW 330i m. All chemical and biological parameters were laboratory analyzed immediately after sampling.

Samples of phytoplankton for biomass estimation were preserved with Lugol solution. Phytoplankton species were enumerated using the Utermohl sedimentation method on an inverted microscope (Olympus IMT-2, Lund et al., 1958). The mean cell dimensions were obtained for biovolume calculation using the approximation of cell to geometrical solids. Biovolume was converted into fresh mass (FM) assuming the specific density of phytoplankton to be equal 1 mg mm<sup>-3</sup> (Rott,

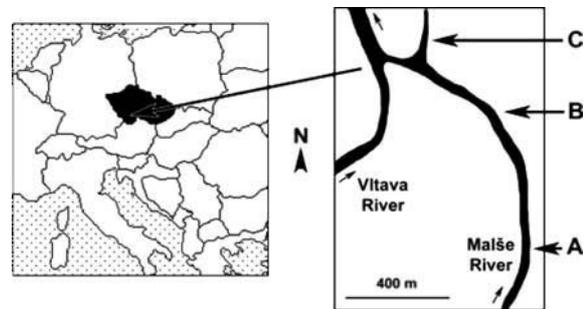


Figure 1. Map of the locality studied. Sampling sites are denoted by capital letters.

1981). Microphotographs were taken from the Olympus BX 51 microscope with an Olympus DP 70 digital camera using Nomarski differential interference contrast technique.

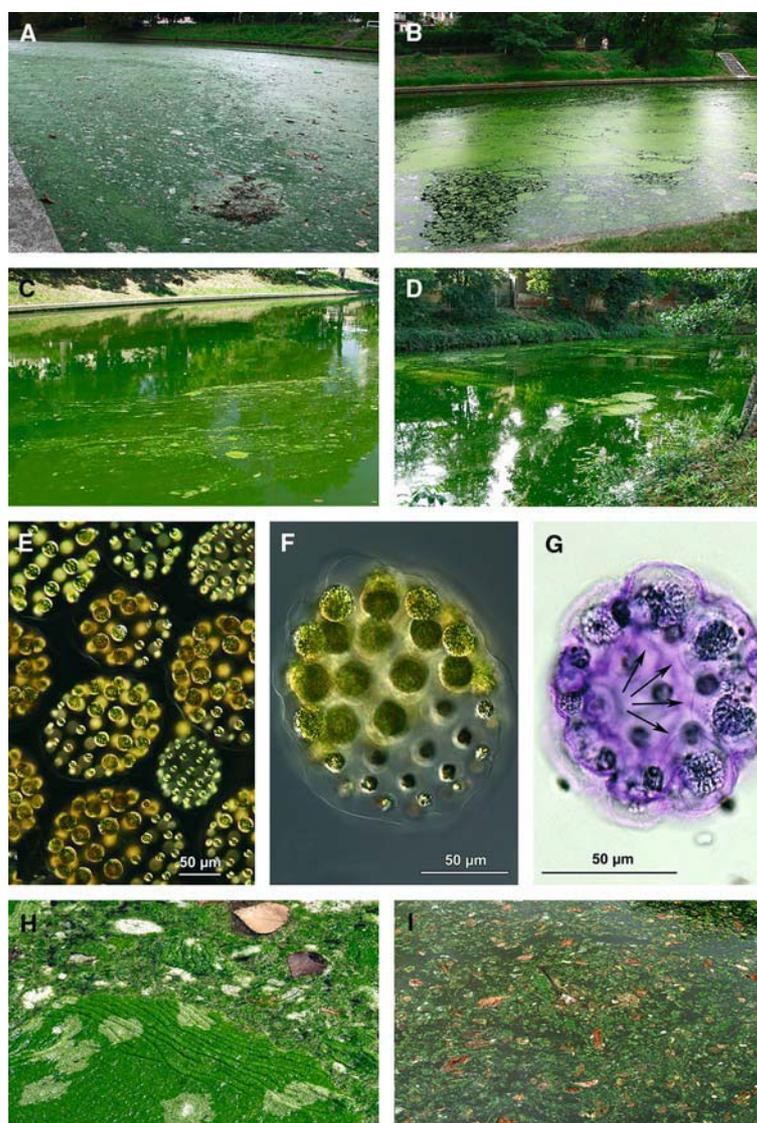
Total phosphorus (TP) and soluble reactive phosphorus (SRP) were analyzed spectrophotometrically according to Kopaček & Hejzlar (1993) and Murphy & Riley (1962), respectively. The concentration of NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> was determined following the procedure of Kopaček & Prochazkova (1993) and Prochazkova (1959), respectively. River discharge data were provided by the River Vltava authority, as measured at an upstream gauging station. Data on air temperature and rainfall were obtained from the Czech Institute of Hydrometeorology.

## Results

On August 11, 2003 we observed a massive bloom of green alga *P. indica* in the downstream reach of the River Malše that had already been reported the previous day. The formation of dense surface scums containing high cell density accumulated during the period that the phenomenon was being studied. At some locales scums of a foam-like consistency covered the surface of the entire river channel (Fig. 2(A, B)). The bloom persisted for 8 days until it was washed out by the flow.

### Weather conditions

The summer of 2003 was characterized by extremely high temperatures and low rainfall in the Czech Republic. During July and August, the



*Figure 2.* Accumulated colonies of *P. indica* formed scums in the River Malše (A, B). The dense scums redispersed into optically homogenous suspension (C, D). Microscopic view on the cenobial colonies of *P. indica* with differentiated somatic and reproductive cells (E, F). The colony is enclosed in gelatinous matrix, individual sheaths (indicated by arrows) are highlighted after methylene blue staining (G). Detail view on the mats of foam-like consistence, which were saturated by gas bubbles (H). Transition state before or after the aggregation into the macroscopic scum (I).

monthly average temperature was 2 and 4 °C higher, respectively than normal. Clear skies allowed for daily temperatures exceeding 30 °C, which prevailed over the period of algal bloom in the River Malše. On August 13, a record was set for that date of 36.8 °C, measured at a station located near the study locality. The heat, combined with low precipitation resulted in minimal

discharge in the River Malše, approximately four times lower than the long-term average (long-term August average flow  $6.2 \text{ m}^{-3} \text{ s}^{-1}$ ). This discharge of only  $1.3 \text{ m}^{-3} \text{ s}^{-1}$  was stable during the period studied. The only exception was measured on August 14, when the discharge temporarily rose to  $1.6 \text{ m}^{-3} \text{ s}^{-1}$ . This lasted only 18 h and then the flow rapidly dropped to its previous values.

### Physical and chemical parameters of the river

Water temperatures ranged between 23 and 26 °C (Table 1), reflecting the warmer than normal air temperatures. The highest temperatures were measured at sampling sites B and C on August 12 (Table 1) and a temperature of 23 °C was recorded at site A. On August 15 the temperatures decreased to 23 °C at all sampling sites. Particularly at the beginning of the period studied, a high pH was measured at all sampling sites following the same pattern as water temperature (Table 1). High concentrations of both TP and SRP were also found (Table 1). Ammonia ion concentration ranged from 0.3 to 0.6 mg l<sup>-1</sup>, however, nitrate nitrogen was occasionally depleted to undetectable levels (Table 1).

### The biomass of *P. indica*

On August 12 an overwhelming dominance of *P. indica* was recorded at the uppermost sampling sites, A and B (Fig. 3, Table 2). While the accumulated biomass formed dense surface scums at site A, the biomass was largely dispersed at site B (Fig. 2(C) and (D)). At site C colonial cyanobacteria (*Microcystis viridis*, *M. aeruginosa*) dominated the phytoplankton with *P. indica* accounting for only 3% of the total biomass (Fig. 3).

*P. indica* remained the dominant species at site A, but by August 15 there was an apparent decrease in the biomass. A reverse, upstream flow induced by the confluence of the Malse and Vltava

Rivers caused a translocation of phytoplankton biomass between sites B and C (Fig. 3).

By August 17 the situation on the river changed considerably. The most frequent algal species found in the phytoplankton at sites A and B were small centric diatoms (*Stephanodiscus* sp.), cryptophytes (*Cryptomonas reflexa* Skuja, *C. marssonii* Skuja), cyanobacteria (*M. viridis* (A. Br. in Rabenh.) Lemm., *M. aeruginosa* Kutz.) and a variety of green algae (*Scenedesmus* sp., *Tetraedron* sp., *Pediastrum duplex* Meyen). *P. indica* had vanished from both upstream sites. Dense surface scums were recorded only at site C, where the maximum value of *P. indica* biomass was measured (Fig. 3). The accumulated biomass subsequently washed out of the study locality. An increase of discharge measured on August 14 appeared to initiate the flushing of the algal biomass downstream. Downstream of the confluence the accumulated biomass of *P. indica* was likely dispersed by the combined flows of the two rivers and subsequently died out.

### Diurnal changes

Diurnal changes in the macroscopic appearance of the bloom were recorded on 2-h intervals. Daytime formation of dense scums having a foam-like consistency was observed. At certain river areas these scums or foams occasionally covered the entire river surface. Over the night the scums would disintegrate and in the morning the colonies were redispersed into an optically homogenous suspension. This cycle recurred for several consecutive days until the bloom washed downstream and dispersed.

### Morphology of *P. indica*

*P. indica* is a coenobitic colonial green alga assigned to the order Volvocales. Spherical, ovoid or ellipsoidal colonies (Fig. 2(E) and (F)) of 128 or 64 or 32 cells are arranged radially at the periphery of a gelatinous matrix (up to 300 µm long). Observed cells were spherical or ellipsoidal, each with two equal flagella, a stigma, many contractile vacuoles on the cell surface, and a massive cup-shaped chloroplast with single or multiple pyrenoid (Nozaki, 2003b). The anterior 1/5–1/3 of the cells in a colony were small, nearly spherical (up to 16 µm

Table 1. Physical and chemical parameters measured in longitudinal profile of the River Malse

	Site A		Site B		Site C	
	12-Aug	15-Aug	12-Aug	15-Aug	12-Aug	15-Aug
Water temperature (°C)	23	22.5	25.6	23.2	25.3	23.3
pH	8.1	7.6	9.5	8.2	8.9	8.3
NH <sub>4</sub> <sup>+</sup> (mg l <sup>-1</sup> )	0.6	0.4	0.3	0.4	0.4	0.3
NO <sub>3</sub> <sup>-</sup> (mg l <sup>-1</sup> )	b.d.l.*	0.6	0.6	0.8	0.3	0.3
TP (µg l <sup>-1</sup> )	233	146	91	103	53	117
SRP (µg l <sup>-1</sup> )	n.a. <sup>+</sup>	36	n.a. <sup>+</sup>	24	n.a. <sup>+</sup>	33

\* Beyond detection limit.

<sup>+</sup> Not available.

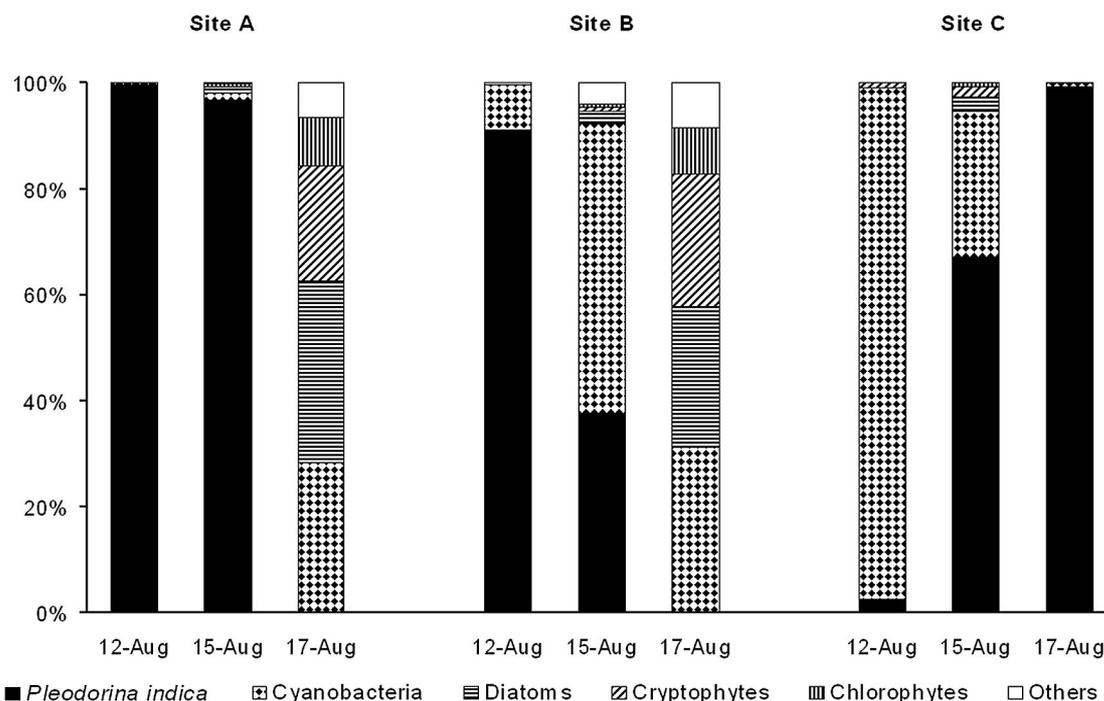


Figure 3. Algal and cyanobacterial composition of phytoplankton in longitudinal profile of the downstream reach of the River Malse.

in diameter) and obligately somatic. Remaining reproductive cells gradually increased in cell size from anterior to posterior pole reaching up to 25  $\mu\text{m}$  in diameter. Gelatinous matrix formed individual sheaths, which were highlighted after staining with diluted methylene blue (Nozaki et al., 1989, Fig. 2(G)). The presence of individual sheaths was the main taxonomic feature that dis-

tinguished *P. indica* from other species of the genus *Pleodorina*.

## Discussion

*Geographical distribution of the species of the genus Pleodorina*

*Pleodorina* is cosmopolitan in freshwaters and includes four species (Nozaki et al., 1989). *P. japonica* Nozaki was collected by Nozaki et al. (1989) from the immersed water near the bottom of rice plants in a paddy field (Japan). The authors reported only sparse presence of vegetative colonies of this taxon. Another species of *Pleodorina*, *P. californica* Shaw, appeared to occur abundantly but at a limited number of localities in the USA (Smith, 1916; Nozaki, 2003b; Coleman personal communication). According to Ettl (1983), *P. indica* (originally described as *Eudorina indica* Iyengar) is an endemic species, which is found only in

Table 2. Changes of biomass (FM,  $\text{mg l}^{-1}$ ) and abundance ( $10^3$  cells  $\text{ml}^{-1}$ ) of *P. indica* in longitudinal profile of the River Malse

Date of sampling	Site A		Site B		Site C	
	FM	Cell No	FM	Cell No	FM	Cell No
August 12	268	145.4	30	18.5	0.4	0.2
August 15	71	44.6	3.4	1.2	10.2	5.4
August 17	0	0	0	0	266	140.5

semi-permanent rainfall marshes in Mysore Province and also in the surroundings of Madras (both in India). However, this assumption was contradicted by later findings where *P. indica* was identified in Mexico and studied by light and electron microscopy (Nozaki et al., 1989; Nozaki & Kuroiwa, 1992). Also, according to Zalocar (1993), *P. indica* occurs in South America (Argentina). Moreover, Angeler et al. (1999) and Coleman (2002), in their studies of phylogenetic relationship among isolates of *Eudorina* and *Pleodorina* species, analyzed a *P. indica* strain (ASW05153), which originates from culture collection of algae at the University of Vienna (Austria, Central Europe). The strain was isolated from eutrophic backwater of the River Danube (locality Gießgang Greifenstein, Lower Austria) in July 1996. In contrast with massive occurrence of *P. indica* in the River Malše, it occurred sparsely in low abundance among a variety of other algal species and did not dominate the phytoplankton (Schagerl, personal communication). To date, *P. sphaerica* Iyengar appears to be the only endemic species that is found solely in India (Iyengar, 1933; Ettl, 1983; Desnitski, 2003). Unspecified *Pleodorina* was found near Cape Maclear in the phytoplankton of southern Lake Malawi (Haberyan & Mhone, 1991), however, it accounted for only 10% of total algal biomass. Al-Homaidan & Arif (1998), in their study of the ecology of algae in semi-permanent rain-fed pools in Saudi Arabia, observed that certain green flagellates (*Carteria multifilis* (Fresenius) Dill, *Chlamydomonas* sp.) do occasionally form water blooms.

Basically, there are two possible options clarifying the remarkable occurrence of *P. indica* in Central Europe. Besides tropical regions, *P. indica* may occur naturally also in temperate zone but only in very low abundance or at sparse number of localities. This would elucidate the lack of records regarding the occurrence of this green alga in the Czech Republic. The other options is an introduction, however, the method of introduction of *P. indica* in the River Malše is disputable. We hypothesize that *P. indica* might have been brought to the Czech Republic by waterfowl migrating from southern regions hospitable to the alga. Nonetheless, accidental human contamination of the waterway with this alga also can not be ruled out. There are a few documented incidents

where a rapid spread of a prominent species in a river system was attributable to accidental introduction or environmental changes (Talling & Lemoalle, 1998). In the August 2003 case in the River Malše, it was evident that *P. indica* was an indigenous species of warm climates and, as far we know, the bloom of this green alga has never been recorded in the temperate region of Central Europe. A parallel might be found in the geographical distribution of the invasive cyanobacterium *Cylindrospermopsis raciborskii* (Neilan et al., 2003). This species, which was originally described as a taxon of only tropical interests, has been now reported from temperate regions of Australia, Europe and America (e.g., Padisak, 1997). Presently, the massive occurrence of *P. indica* in the Czech Republic is considered a unique, exceptional event rather than a common feature of future phytoplankton development.

#### *Diurnal changes*

Diurnal changes in macroscopic appearance of the bloom were recorded. During the day the formation of a dense scum was observed, while over night the scums disintegrated and colonies redispersed into an optically homogenous suspension. Since *P. indica* is a flagellated green alga, active regulation of the vertical position in the water column is to be expected (Clegg et al., 2003). Therefore, the following explanation is proposed. The aggregated colonies formed foam-like scums saturated with oxygen bubbles evolved by the photosynthetic process. Once trapped on the surface, the colonies were not able to regulate their position in the water column and subsequent photodestruction resulted in partial scum discoloration (Fig. 2(H)). During the night the bubbles were either released into the atmosphere or the excessive oxygen diffused into the water and the colonies dispersed into the water (Fig. 2(I)). Each morning the photosynthetic production of oxygen prevailed over respiration and the whole cycle recurred. The proposed mechanism is based on repeated observation, however, the lack of detailed information impeded clarification of the mechanism involved in the aggregation process. Koblížek et al. (2002) concluded that the aggregation of unicellular cyanobacterium *Synechococcus* represented the light-adaptive mechanism affected by

irradiance. Light is assumed to be the driving force of algal vertical migrations (Clegg et al., 2003) and it might be also the prime impulse in the aggregation of *P. indica* colonies.

#### *Causes of the bloom*

It could be questioned as to why the occurrence of *P. indica* was restricted only to the downstream reach of the River Malše. The analyses of samples which were taken from an upstream reach of the river at the same time, only revealed the presence of low abundant common species of coenobial volvocalean algae (*Pandorina morum* (O. F. Muller) Bory, *Eudorina elegans* Ehrenb., *Gonium* sp.). *P. indica* was not found at all. The environmental conditions in the turbulent upstream reach of the River Malše appeared to preclude the growth and maintenance of this planktonic green alga. Nevertheless, the low-flowing artificially constrained river channel in the downstream reach exhibited a transitional habitat between the lotic and lentic systems. The lower flow permitted a greater retention time for planktonic algae, which in turn, enhanced biomass populations in a nutrient rich environment. Although not measured, the establishment of temporary thermal stratification at the sampling sites is assumed. According to Sherman et al. (1998), the establishment of persistent thermal stratification is a major factor in nurturing the phytoplankton species composition of a river. Additionally, high nutrient concentrations would also have been a factor contributing to the bloom of *P. indica*. High measured concentrations of both TP and SRP (Table 1) indicated that the growth of *P. indica* was not phosphorus limited. Exceptionally low concentrations of nitrate nitrogen (Site A, August 12, Table 1) were a probable result of the extensive nitrogen consumption by this alga.

The bloom of *P. indica* in the River Malše was likely triggered by the anomalous tropical weather conditions, resulting in minimal rainfall and low river discharge. It is not known whether the flourishing of this thermophilic species is an indication of the global warming phenomenon, however, this is the first record of the any massive occurrence of *P. indica* in the region of Central Europe.

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