Anomalous colourations in surface water bodies: causes and examples reported by the Regional Agency for Environmental Protection and Energy of Emilia Romagna (ARPAE)

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ABSTRACT

Surface water can exhibit a range of colours beyond the expected blue or green. These anomalous colourations can be triggered by different factors, both natural and anthropogenic, and not always are signs of pollution. This note explores the phenomenon of water discolouration, focusing on natural causes and analysing how factors such as algal and bacterial blooms influence water colour. The study provides examples of specific events that occurred in the Emilia-Romagna region and documented by ARPAE and highlights the importance of monitoring these colouration events in order to provide valuable insights into the health of water bodies and the surrounding environment.

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Introduction

Over the past decades, there has been a constant increase in reports from citizens of phenomena concerning the presence of anomalous colours in surface water bodies. Often, it is not easy to identify the cause of such events, as well as to explain how these colours are not always linked to specific water pollution phenomena. However, it is important to emphasize that changes in surface water colour are not necessarily indicative of pollution. Indeed, in many cases, they are associated with algal and bacterial blooms, which frequently are triggered by the natural degradation of organic matter under certain environmental conditions (Trüper and Genovese, 1968; Johnson *et al*., 2012; Belila *et al*., 2013; Van Vuuren and Levanets, 2020).

One of the tasks of the Regional Agency for Environmental Protection and Energy of Emilia Romagna (ARPAE) is to assess the causes of these phenomena and explain them to citizens or stakeholders, informing them against common false alarms that arise in response to these anomalous colouration events.

This short note will provide a brief overview of some specific cases of water discolouration handled by ARPAE, with a focus on the main causes of these phenomena, which are mainly attributable to natural events.

Usually, ARPAE manages these phenomena, performing both biological and chemical determinations on the collected water sample. Biological analysis involves the use of an Uthermol sedimentation chamber and an inverted optical microscope for the characterization and enumeration of bacteria and phytoplankton, as described in the ISTISAN 07/05 protocol. Chemical analysis generally includes the determination of pH, Biochemical Oxygen Demand (BOD5), total organic carbon, and dissolved organic carbon, following the methods APAT CNR IRSA 2060, APAT CNR IRSA 5120, and the European and International standards UNI EN 1484:1999 and ISO 15705:2002. Moreover, given the microorganisms observed in the samples and their role in causing the anomalous colourations, the quantities of iron and manganese, or alternatively sulphides and sulphates are analysed, following the European standards UNI EN

ISO 17294-2:2016 and the methods APAT CNR IRSA 4160 and 4020, respectively.

Results and Discussion

Anomalous colourations due to algal blooms: the case of *Euglena sanguinea*

As reported by Van Vuuren and Levanets (2020), freshwater ecosystems exhibit a limited diversity of microorganisms responsible of turning surface water from the expected blue or green colour into red colour.

Among these species, the euglenoid *Euglena sanguinea* Ehrenberg is widely considered the most prevalent phytoplankton species responsible for the red colouration of freshwater ecosystems (Van Vuuren and Levanets, 2020). Documented occurrences of blooms caused by this species have been reported extensively across the globe, resulting in the formation of red surface scums or a pronounced red discolouration of the water (Rahman and Khan, 2007; Van Vuuren and Levanets, 2020).

Blooms of this species mostly occur in the spring and summer months, when waters are affected by strong solar radiation, high temperatures, and a considerable supply of nutrients (Van Vuuren and Levanets, 2020). Indeed, most of the events concerning the presence of extensive red colouration of ponds or rivers reported by citizens to ARPAE during the summer months were attributable to blooms of *E. sanguinea*. Among these events of algal blooms, notable cases include those reported for the Migliarina canal (MO) in August 2009 (44°49’18.58”N, 10°49’43.93”E) and the Cento canal (FE) in August 2017 (44°44’48.41”N, 11°18’45.92”E). Other events reported to ARPAE in recent years include the red colouration of the Vallicella River (MO) in July 2020 (44°49’13.14”N, 11°11’5.19”E) and red scums in the river Canala dei Canali (Ra) between July and August 2020 (44°25’46.90”N, 12° 9’24.47”E). These phenomena were mainly characterized by the presence of red scum or red discolourations on the surface of the water column and by the absence of unpleasant odours (Figure 1a). The action plan typically outlined by ARPAE protocols in this scenario entails the collection of a water sample and its subsequent transfer to the laboratory units where chemical and biological analyses are performed. Biological analyses involve the search for certain species of algae capable of altering the water’s colour, while chemical analyses involve the measurement of pH, dissolved oxygen, and total organic carbon. Water samples collected for each of these cases were dominated by the presence of *Euglena sanguinea* in its characteristic red spherical shape (Figure 2a), while very low concentrations and diversity of other algal species were present; the chemical analyses revealed high levels of dissolved oxygen and a basic pH value (8.2-8.34).

Euglena sanguinea is a unicellular planktonic organism that possesses a complex of red pigments, the hematochrome, whose main function is to protect the cell from high light intensity (Van Vuuren and Levanets, 2020). In the presence of high solar radiation, the hematochrome pigments are distributed throughout the cell volume to protect the cell, which appears red and assumes a spherical shape (Figure 2a). In the evening and night hours, when low radiation is present, red pigments are concentrated in the centre of the cell, which resumes its classic green colour (Laza-Martinez *et al*., 2019).

Consequently, the main factors that can aid citizens in identifying *E. sanguinea* as the main responsible for a red discolouration phenomenon include the formation of a red layer on the water’s surface, sometimes accompanied by the presence of red scum, while below the surface layer the water usually appears clear and clean. Other factors can include the absence of unpleasant odours and the occurrence of this phenomenon during the summer months, when temperatures are extremely high and solar radiation is intense. Moreover, other conditions favourable for the development of *E. sanguinea* blooms are the presence of canalized waters with a high organic load and a considerable hydrodynamic stasis (Van Vuuren and Levanets, 2020). The alga is not harmful to humans, but it can produce a very potent ichthyotoxin, that is potentially harmful and lethal to fish (Zimba *et al*. 2004). In our cases, no detectable effects on the fish fauna were recorded.

Anomalous colourations due to bacterial blooms: purple sulphur bacteria and iron-oxidizing bacteria

In other cases, the phenomenon of freshwater discolouration is not due to algal blooms, but to typical ecological and geological conditions of the aquatic ecosystem that might trigger the proliferation of bacterial blooms, such as the presence of suspended minerals or high levels of organic load in the water column.

Among the events of anomalous colouration of surface freshwater reported by citizens to ARPAE and associated with bacterial blooms, we can list some records characterized by the development of extensive pinkish-purple colouration of the water, recurring periodically in small and shallow water bodies with hydrodynamic stasis (Figure 1b). These cases occurred in the province of Ravenna, specifically in a shallow private pond in August 2017 and March 2021 (44°22’53.23”N, 12°16’11.75”E) and in a eutrophic lake within the San Vitale Pine Forest in August 2020 (44°31’23.56”N, 12°14’2.24”E). More recently, purple scums were reported for the Zaniolo canal (RA) (44°29’48.22”N, 11°49’0.39”E) in July 2023, during the flood events that characterized Emilia-Romagna. During these blooms, citizens also reported a strong hydrogen sulphide smell and the presence of suspended solids. The microscopic analysis of the water samples revealed that these phenomena were associated with the presence of purple sulphur bacteria (Figure 2b, 2c), while physicochemical analysis showed a high presence of chlorides and sulphates, severe oxygen deficiency and a large amount of hydrogen sulphide responsible for the bad smell. Indeed, these organisms are classified as “anoxygenic phototrophic purple bacteria” as they typically grow under anoxic conditions, in presence of light and hydrogen sulphide (Pfenning and Trüper, 1992). They utilize sulphide as an electron donor for their photosynthetic processes, which is subsequently oxidized to sulphate. This oxidation process involves the intermediate formation of elemental sulphur globules that are accumulated inside or outside the cell depending on the bacterial species (Pfenning and Trüper, 1992).

As reported by other studies (Caumette, 1986; Belila *et al*., 2013), these organisms proliferate in anoxic zones and in situations characterized by an overload of organic matter. These environmental conditions are observed in wastewater stabilization ponds (Belila *et al*., 2013) or in shallow coastal lagoons that both receive freshwater inputs with organic material from the continent and marine waters with a high concentration of sulphates (Trüper and Genovese, 1968; Caumette, 1986).

The purple sulphur bacteria detected in the water samples analysed by ARPAE were identified as members of the family Chromatiaceae and likely belonging to the genus *Thiohalocapsa* and *Thiocapsa*, after a careful examination from taxonomic experts in this field (Figure 2b and 2c).

Visual features that can help citizens understand the natural water colouring phenomenon caused by purple sulphur-oxidizing bacteria include the presence of extensive pinkish-purple colouration of the water body and the occurrence of an unpleasant odour of hydrogen sulphide. Moreover, the proliferation of sulphur-oxidizing bacteria usually occurs in shallow water bodies with a small surface area, stagnant warm water, and an overload of organic matter.

In other cases, the action of other microorganisms is responsible for further events of red-rust and orange colourations of surface waters reported by citizens to ARPAE. These events were characterized by the presence of orange flocculent material deposited along the water body and colloidal material in suspension of the same colour (Figure 1c).

Figure 1. Anomalous colourations in surface water bodies were detected by the Regional Agency for Environmental Protection and Energy of Emilia-Romagna (ARPAE). **a)** Bloom of *Euglena sanguinea* in the canal Canala dei Canali (RA) let the water assume a red colour with red scum. Cyanobacteria of the genus *Oscillatoria* were responsible for the green scum visible in the picture. **b)** Purple discolouration of a private freshwater lake in the province of Ravenna caused by purple sulphur bacteria. **c)** Orange flocculent material deposited along the Samoggia stream (BO) due to the presence of iron-oxidizing bacteria.

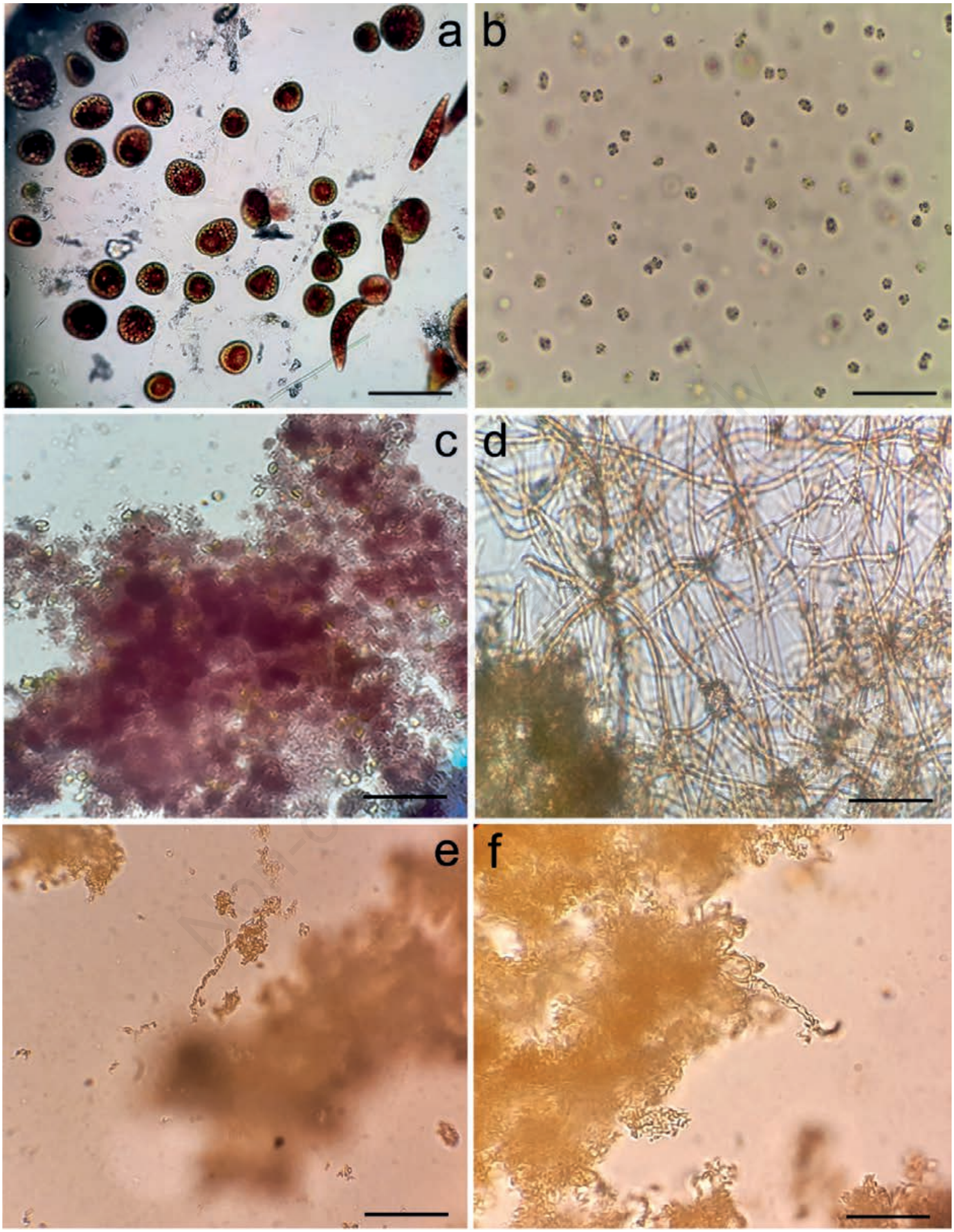

Figure 2. Microorganisms are responsible for anomalous colourations detected in surface water. **a)** *Euglena sanguinea* in its characteristic red spherical shape (Scale bar 100 µm). **b)** Purple sulphur bacteria of the genus *Thiohalocapsa* (Scale bar 50 µm). **c)** Purple sulphur bacteria of the genus *Thiocapsa* (Scale bar 50 µm). **d)** Iron oxidizing bacteria of the genus cf. *Leptothrix* enclosed in straight sheaths (Scale bar 50 µm). **e)** Iron oxidizing bacteria of the genus cf. *Gallionella* with twisted stalks (Scale bar 50 µm). **f)** Iron oxidizing bacteria of the genus cf. *Sphirophyllum* with threads twisted in a spiral form (Scale bar 50 µm).

These cases were observed along the Samoggia stream (BO) (44°37’19.14”N, 11°12’54.78”E) in the years 2016 and 2017, along the Savio River (FC) (43°50’26.99”N, 11°58’2.12”E) in January and February 2021, and finally in Borgo Maggiore (San Marino) (43°57’12.66”N, 12°26’56.70”E) in March 2021. The microscopic analysis performed by ARPAE allowed the observation of the structure of the colloidal material and revealed that it was mainly composed of filamentous iron-precipitating bacteria, likely belonging to the species *Leptothrix ochracea* Kützing and to the genera *Spirophyllum* and *Gallionella* (Figure 2d, 2e, and 2f). The analytical results of both water and sediment samples showed the absence of anthropogenic substances (hydrocarbons, heavy metals) and show a very high concentration of iron and manganese, supporting the hypothesis that these phenomena of reddish-orange colouration of water bodies were attributed to the presence of iron-oxidizing bacteria. The presence of ferrous and manganese ions in the water samples can be attributed to the environmental characteristics of the groundwater and likely linked to the minerals present in the soils and rocks of the area under investigation, such as the provinces of Bologna and Forlì-Cesena.

Thanks to their copious metallic depositions, these organisms can be identified by their morphological characters visible under an optical microscope. Members of the genus *Leptothrix* are single thread form composed of cylindrical cells placed end to end and generally enclosed in sheaths, usually straight or curved (Figure 2d), while species belonging to the *Gallionella* genus present long branching twisted stalks coated with ferric oxides (Figure 2e) (Harder, 1919; Johnson *et al*., 2012). *Spirophyllum* spp. are the most abundant of the iron thread bacteria, and they have the form of flat ribbon-like threads twisted in a spiral form (Figure 2f) (Harder, 1919).

These bacteria can thrive thanks to the energy obtained from the oxidation of ferrous and manganese ions present in the water (Harder, 1919; Emerson and Revsbech, 1994; Wang *et al*. 2011). This process leads to the deposition of ferric hydroxide that can be stored inside the cell or accumulate as extracellular matrices in the form of reddish-orange deposits (Harder, 1919).

Factors that can help citizens recognize the natural phenomenon of water colouration caused by iron-oxidizing bacteria include the presence of orange colouration in the water body with reddish-orange deposits and the absence of unpleasant odours. Moreover, unlike other algal and bacterial blooms that are more prevalent during warmer months, iron bacteria can persist and cause discolouration events throughout the years, including autumn and winter months. Iron-oxidizing bacteria thrive even in well-oxygenated water bodies with flowing, non-stagnant waters.

These microorganisms have been found in a range of iron-rich environments, such as freshwater wetlands, marine and freshwater hydrothermal vents, water distribution pipes, and salt and freshwater marshes (Karl *et al*., 1988; Johnson *et al*., 2012). All these cases suggest the ubiquitous presence of iron-oxidizing bacteria within wetland soils and sediments, supporting their pivotal role in metal cycling in aquatic and wetland environments.

This study aimed to highlight natural water discolouration phenomena that are poorly understood and documented in Italy, with the goal of sharing important information within the scientific community. To begin addressing these little-known phenomena, ARPAE’s technical approach was grounded in basic research, employing fundamental techniques such as optical microscopy for the taxonomic characterization of these microorganisms. As the Agency’s knowledge of these phenomena has expanded, future perspectives will involve the development of more advanced technical methodologies to refine analyses and obtain more accurate results. In this regard, the use of molecular techniques could be integrated with the taxonomic approach to better characterize the microbial communities responsible for water discolouration and to provide more accurate identification.

Conclusions

Reports of water discolourations recorded by ARPAE and associated with algal and bacterial blooms are constantly increasing, probably due to a growing public awareness of environmental issues as well as a rising frequency of changes in environmental conditions. Often it is not easy to immediately identify the cause of such events, as well as to explain to citizens that these phenomena are not always linked to anthropogenic causes or specific water pollution. Therefore, it is crucial to emphasize the importance of correctly contextualising these phenomena**,** providing comprehensive answers to citizens in order to avoid and limit their concerns.

The increased experience gained by ARPAE regarding these phenomena and collaboration among various professional figures within the Agency has allowed a clearer understanding of these events over the years. There are various results of this collaboration, including timely communication to the police enforcement engaged in environmental protection, as well as the effective response to citizen reports. Moreover, proper communication regarding the nature of these events through publication on local websites and institutional platforms promotes a greater reassurance among the population but can also contribute to creating in citizens the feeling of effective and professional environmental surveillance by the Agency. This, in turn, contributes to the development of a reciprocal relationship of collaborative trust between citizens and the public administration.

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