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Correcting a Major Error in Assessing Organic Carbon Pollution in Natural Waters

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Short title: Major Error in Assessing Organic Water Pollution

Teaser: A widely used method is found to overestimate organic pollution in natural waters and an alternative method is proposed

Abstract

Microbial degradation of dissolved organic carbon (DOC) in aquatic environments can cause oxygen depletion, water acidification and CO_2 emissions. These problems are caused by labile DOC (LDOC), and not refractory DOC (RDOC) that resists degradation and is thus a carbon sink. For nearly a century, chemical oxygen demand (COD) has been widely used for assessment of organic pollution in aquatic systems. Here we show, through a multi-country survey and experimental studies, that COD is not an appropriate proxy of microbial degradability of organic matter because it oxidizes both LDOC and RDOC, and the latter contributes up to 90% of DOC in high-latitude forested areas. Hence COD measurements do not provide appropriate scientific information on organic pollution in natural waters, and can mislead environmental policies. We propose the replacement of the COD method with an optode-based biological oxygen demand method to accurately and efficiently assess organic pollution in natural aquatic environments.

INTRODUCTION

About one quarter of the net carbon fixation on land enters the dissolved organic carbon (DOC) pool of natural waters (*1-3*). The nature and behavior of DOC in aquatic systems determine its fate and environmental effects. DOC is often classified based on how rapidly it turns over in the natural environment (*4*). Labile DOC (LDOC) is rapidly degraded and taken up by microbes, and when high amounts of LDOC are released into the environment, the resulting consumption of oxygen can cause oxygen depletion (*5, 6*), with detrimental effects on aquatic ecosystems. Also, respiration of LDOC to CO_2 can result in water acidification (*6, 7*) and release of excess CO_2 (supersaturation) to the atmosphere where it contributes to the greenhouse effect. In this way, a productive coastal region could become a source rather than a sink of atmospheric CO_2 (*7, 8*). It follows that the microbial degradation of LDOC can lead to a series of environmental problems. In contrast, the refractory components of DOC (RDOC) in the aquatic environment are not readily or rapidly metabolized by microbes and so can be preserved in natural waters where they act as a carbon sink (*4, 9*). While RDOC includes chelating agents (*10*) and therefore may act as an important transport pathway or sink for heavy metals, RDOC is not a pollutant but contributes instead to carbon sequestration. In fact, RDOC accounts for more than 95% of the total DOC in the deep ocean (*4, 11*) (man-made persistent organic pollutants are not discussed in this paper because they represent very small amounts of the DOC, *Supplementary Materials* 1.1). A purely chemical view of organic pollution could identify both LDOC and RDOC as organic pollutants, whereas the ecological view of organic pollution clearly restricts pollutants to LDOC. The negative environmental consequences of the microbial degradation of organic compounds mentioned above (i.e., oxygen depletion, acidification, and release of CO_2 to the atmosphere) depend only on the magnitude of the LDOC fraction.

The concern generated by organic pollution led governments to monitor microbial degradability of organic matter in water bodies, which became a fundamental tool in environmental policy and management. The biodegradability of organic matter can be evaluated via its oxygen demand by microorganisms, i.e., the amount of oxygen consumed by bacteria and other microorganisms while they decompose and metabolize the organic matter under aerobic conditions. The oxygen demand of LDOC in aquatic environments is best measured as the biological (or biochemical) oxygen demand (BOD) (*12*). However, the existing standard BOD method is time consuming as it requires 5-day incubations, and has low reproducibility as it involves initial and final measurements on different subsamples (bottles) of the water samples (*12*). Consequently, BOD estimates have largely been replaced by measurements of chemical oxygen demand (COD), i.e. the determination of the amount of oxygen consumed by chemical reactions in the solution. In COD, potassium dichromate or potassium permanganate is used to rapidly oxidize the organic matter (and also possibly existing reductive inorganic ions) by chemical reactions, thus shortening the measurement time to only a few hours. Given its convenience, relative simplicity, and repeatability, the COD method has been widely used by agencies and scientists for nearly a century for monitoring and managing waste effluents and natural waters (*13*) (*Supplementary Materials* 1.2 and 1.3). However, the COD method requires the addition of high amounts of strong chemical oxidants (i.e., potassium dichromate and permanganate), which do not exist in natural waters. Hence the COD method artificially oxidizes both LDOC and much or all of the RDOC, whereas oxygen consumption in natural waters is only caused by LDOC oxidation. Although the use of the COD method is justified when rapid measurements are required, such as in the management of wastewater treatment plants, we question its scientific basis and effectiveness for the assessment of organic pollution in natural water bodies.

Here, we hypothesize that the COD method substantially overestimates the DOC that can be degraded by microbes in natural waters, with the consequence that using this method could mislead the assessment of organic pollution in aquatic environments (Fig. 1).

To test our hypothesis, we analyzed COD and BOD data from experiments conducted for this study and measurements collated from the literature (see Materials and Methods). These data cover a wide variety of natural aquatic environments ranging from tropical to subarctic zones, farmlands to forest watersheds, high plateaus to coastal waters, and freshwaters to saline lakes and marine waters (Fig. S1 and Table S1). We thus examined how COD and BOD vary across gradients of salinity and DOC, with latitude and between ecosystems.

Two methods are currently used to determine COD, based on potassium dichromate ($\mathrm{COD_{Cr}}$) and potassium permanganate oxidation ($\mathrm{COD_{Mn}}$) (*14*) (see Materials and Methods). Comparison of $\mathrm{COD_{Cr}}$ and $\mathrm{COD_{Mn}}$ values determined on natural waters of different salinities and DOC concentrations ([DOC]), indicated that $\mathrm{COD_{Cr}}$ is not a valid measure of oxidized organic matter because salinity interferes with the determination of [DOC] (salinity effect already reported in previous publications including *14*), whereas $\mathrm{COD_{Mn}}$ may provide appropriate measurements (Figs. S2 and S3, Tables S2 and S3). Hence, we only use $\mathrm{COD_{Mn}}$ values in the following analysis. To simplify terminology, we use “COD” instead of “$\mathrm{COD_{Mn}}$” hereinafter, and “DOC” instead of total organic carbon (TOC) for the following discussion (*Supplementary*

Materials 1.4).

We also used a water-column macrocosm for a long-term incubation of natural water to better understand the problems involved in the current COD method, and the chemical nature of the organic matter oxidized by this method. We incubated in the Aquatron Tower Tank of Dalhousie University 100 m^3 of natural river water rich in humic material in darkness for 510 days (see Materials and Methods). The DOC in the incubated water was therefore subjected to microbial degradation for a much longer time than the 5 days required by the BOD method. Operationally speaking, any organic matter remaining after the 510-day incubation would have been refractory. We used, for the first time in the literature, ultra-high resolution Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) to analyze the water samples subjected to different treatments regarding the COD methodology (see Materials and Methods). A water sample from 1000-m depth in the South China Sea was used as a reference for natural-seawater RDOC.

RESULTS AND DISCUSSION

Investigating the COD Problems with Field Data

Data from our samples and the literature (Fig. S1 and Table S1) show that the relationships of COD and BOD with [DOC] vary in different natural environments (Fig. 2). The COD values increase linearly with increasing [DOC] in all the sampled natural environments, indicating that COD oxidizes both LDOC and RDOC, as expected. In contrast, the BOD values are much lower than their COD counterparts, as they reflect only LDOC. The relationships observed between BOD and [DOC] across different environments, and the variation therein can be explained by our current understanding of organic matter cycling as follows:

First, we explored latitudinal differences in COD and BOD as a function of [DOC] by grouping the data into high latitudes (subpolar areas), mid latitudes (temperate zone), and low latitudes (subtropical zone) (Fig. 2A-C). While the relationships of COD and BOD with [DOC] are totally decoupled at high latitudes (Fig. 2A), they are better aligned at lower latitudes (Fig. 2B and C). In the subpolar environment, the low BOD values and their independence from [DOC] (low correlation and regression coefficients, i.e. 0.16 and 0.39, respectively) indicate generally low LDOC concentration while the increase of (COD-BOD) with increasing [DOC] indicates accumulation of RDOC with increasing [DOC]. In the temperate zone, the values of COD and BOD both increase with [DOC], and the (COD-BOD) values are generally similar to the BOD values. This indicates similar concentrations of LDOC and RDOC at mid-latitudes. In the subtropical zone, the (COD-BOD) values are lower than BOD, indicating that COD is dominated by LDOC with less RDOC accumulating in the environment than at mid-latitudes (Fig. 2B and C). In terms of the absolute value of COD, the subpolar zone ranks the highest, followed by the temperate and subtropical zones, the latter being the lowest, whereas the BOD values remain at the same general level (Fig. 2). If these COD values were assessed according to conventions used in environmental management, the high COD values at high latitude would be interpreted as highly organic-polluted compared with low latitudes. Instead, the high COD values are due to high concentrations of non-polluting RDOC (see above).

Second, we grouped the same samples as in Fig. 2A-C by ecosystems (Fig. 2D-F). This led to the emergence of distinct patterns in LDOC and RDOC that reflect changes in organic matter as it moves through the hydrological cycle towards the sea. In waters of forested regions, BOD is almost independent of [DOC] and (COD-BOD) increased proportionally with [DOC]. The latter is consistent with DOC in forested watersheds consisting mostly of humic-like compounds, which are common components of RDOC (*15, 16*). In the other freshwater samples (Fig. 2E), the linear relations of (COD-BOD) and BOD with [DOC] were similar, indicating similar fractions of LDOC and RDOC in DOC. In coastal water samples, the values of BOD are higher than those of (COD-BOD), indicating that COD is dominated by LDOC. If the conventional COD standards were applied to the assessment of water quality in these different ecosystems, waters of forested regions would be considered as loaded with organic pollutants. Instead, the high COD values are due to high concentrations of non-polluting humic-like RDOC compounds (see above). This shows again the drawback of relying on COD as an indicator of organic pollution in natural aquatic systems.

Taken together, the above field observations are consistent with our hypothesis that COD reflects the oxidation of both LDOC, which can be naturally degraded by microbes, and RDOC, which resists biodegradation. In contrast, BOD reflects the oxidation of LDOC only. Hence, COD is not a valid indicator of the microbial degradability of organic compounds, and thus organic pollution in aquatic environments, whereas BOD provides realistic estimates of biodegradability.

Investigating the COD Problems Experimentally

To further investigate which fractions of naturally occurring organic matter are oxidized by the COD method, samples from the 510-day incubation in the Aquatron Tower Tank were treated with/out the COD method, and FT-ICR MS analyses were used to identify differences between treatments. Results are summarized in Fig. 3, where a water sample from 1000-m depth in the South China Sea provides a reference for natural-seawater RDOC (Fig. 3G) (*17*). The FT-ICR MS raw peak distributions (*m/z* 200~600) showed no major changes in components of the samples before and after the 510-day incubation (Figs. 3A and 3B, respectively), reflecting the natural richness of the river water in RDOC. In contrast, there was a large change in the FT-ICR MS peak distributions after the COD treatment of both the initial and the 510-day incubated samples (Figs. 3C and 3D). This shows that most DOC molecules in the *m/z* range of 200-600 persisted during the 510-day incubation (Figs. 3A and B), but were oxidized by the COD treatment of both the initial and incubated water (Figs. 3A vs. 3C, and 3B vs. 3D).

The FT-ICR MS analysis provides a proxy for the naturally occurring RDOC, i.e., the polygons in Figs. 3E to 3G delineate carboxyl-rich alicyclic-like (CRAM-like) molecules based on the literature (*10, 18*). These molecules dominate the DOC of the deep ocean, where they are identified as RDOC (*10*, *17*, *19*). Figures 3E and 3F show the molecules that were oxidized by the COD method (i.e., samples in Figs. 3A *minus* 3C, and 3B *minus* 3D, respectively). A large part of the oxidized molecules corresponds to the naturally occurring CRAM-like molecules in the deep seawater (Fig. 3G). These results demonstrate that the COD treatment rapidly oxidizes RDOC molecules that would otherwise persist for long periods as part of the carbon sink in natural waters.

Resolving Paradoxical Results Arising From Monitoring Pollution as COD

In the practical application of COD methods in natural waters, there is often a paradoxical decoupling between COD and BOD, including in situations where environmental protection measures have been carried out for decades. Representative examples are Lake Biwa in Japan (*20*), the Han River in Korea (*21*), and Finnish rivers (*22*) (Fig. 4). Lake Biwa was notorious for its organic pollution in the 1970s, but this pollution was reduced over the past three decades. It might therefore be expected that COD, as an indicator of organic pollution, would have decreased in response to the abatement of organic pollution. However, COD has increased since the 1980s, whereas BOD has decreased (Fig. 4A). This apparent paradox can be explained by a long-term accumulation of RDOC in the lake, which can be oxidized by the COD but not the BOD method. In cases such as Lake Biwa, environmental protection agencies should definitely use BOD instead of COD for assessing water quality. In the Han River (Fig. 4B), the COD values remained fairly constant over 20 years, whereas the BOD values continuously decreased. This means that the relative fraction of RDOC increased, while the LDOC fraction decreased over the past 20 years along with less eutrophication that would remobilize RDOC for decomposition (*23*). In Finnish rivers (Fig. 4C), COD and BOD both decreased over the past decades, and since BOD decreased faster than COD, the (COD-BOD)/COD ratio increased. This indicates that the relative fraction of RDOC increased and that of LDOC decreased.

Again, these long-term observations show that COD measurements on their own do not provide consistent estimates of organic pollution, whereas the BOD values yield such estimates in all circumstances. Furthermore, COD data used alone may fail to detect, or mask, the success of environmental protection countermeasures at curbing organic pollution.

Replacing COD by a Modern BOD Method

The above analyses clearly show that COD is not a valid indicator of the microbial degradability of organic compounds, and thus organic pollution in natural waters. This is because COD reflects the oxygen demand of both LDOC and RDOC, and the latter is not, or at least is very inefficiently, oxidizable by microbes and is thus part of the carbon sink. The fact that COD confounds RDOC with organic pollution implies that it can mislead environmental assessments or the application of environmental policies. However, COD is currently used widely for the monitoring and management of natural waters (*13*, *24-26*). As explained above, the present study has no bearing on the use of COD in wastewater treatment plants, but addresses its use with natural waters. Many governmental and international organizations presently rely on COD data to establish environmental standards and policies (*Supplementary materials* 1.2 and Table S4 and S5). Hence, there is a pressing need to prevent the misinterpretation created by the use of COD, especially considering that the International Organization for Standardization recommended COD as one of the parameters for assessing the quality of natural waters (*27*). Many countries use COD as the primary standard for water quality control and environmental assessment. For example, in China and Japan, the performance of environmental policies has been assessed based on the reduction of COD (Tables S4 and S5).

A substitute for the COD method is therefore required. TOC has been proposed for the monitoring of wastewater treatment and aquatic environments (*13*, *28*). However, TOC measurements include RDOC, which disqualifies it as an appropriate measurement to determine the microbial degradability of organic compounds. Similarly, total oxygen demand (TOD) (*29*) is not an appropriate alternative to COD as it incorporates the oxidation of RDOC. In contrast, BOD has clear scientific basis, and remains the first choice as an alternative to COD. However, the traditional BOD method has been abandoned in many instances because of its labor-intensive nature, and also the variability introduced by the heterogeneity among the bottles used for oxygen titrations before and after incubation. Here we recommend a modern BOD method that uses oxygen optodes (*30, 31*) instead of oxygen titration for the measurement of oxygen concentration, where the latter can be conducted in the same incubated bottles before and after incubation (or even continuously). This reduces the number of incubation bottles, reduces procedural errors, and eliminates the use of hazardous chemicals (see Materials and Methods). The optode-based BOD measurements provide repeatable values that are more indicative of water quality than COD (Figs. S4 and S5).

From Field Measurements to Environmental Policy

The above results of our multi-country field survey and experimental studies are consistent with our hypothesis that the COD method substantially overestimates the DOC that can be degraded by microbes in natural waters, showing that the application of the COD method often misleads the assessment of organic pollution in aquatic environments. We recommend the modern optode-based BOD method to replace the COD method for application in natural aquatic environments. Such measurements are required to inform policy makers of the risks of adverse environmental conditions that can be triggered by high concentrations of LDOC, and to design and implement measures to improve water quality. With the increasing need to monitor and manage water quality, it is imperative to refine and improve the scientific measurements on which policy depends.

MATERIALS AND METHODS

Sampling sites and data from the literature.

The field data used in this paper include new measurements made for this study and data collated from the literature. The field measurements were conducted in China (coastal water samples from the Xiamen Bay, Sanya Bay, Bohai Sea and Yellow Sea, and freshwater samples from Fujian, Sichuan and the Tibetan Plateau), Canada (Nova Scotia, New Brunswick, Quebec and British Columbia), and the USA (Florida). The sampling locations are shown in Fig. S1, and detailed information is provided in Table S1. In order to keep the data comparable, we only took from the literature COD values measured using the titration COD_{Mn} method and BOD data using the BOD_5 method (described below) (*20-22, 32-35*).

Potassium dichromate oxidation (COD_{Cr}) method

There are two methods for COD measurements: one with potassium dichromate as oxidant (COD_{Cr}) and the other with potassium permanganate (COD_{Mn}, which has two variants or protocols described next section). The measurement of COD_{Cr} was based on the International Standard ISO6060 "Determination of Chemical Oxygen Demand in Water" (https://www.iso.org/standard/12260.html), which was officially reviewed and confirmed in 2017. Briefly, 10 mL of a water sample was added into a 250 mL conical flask. Five mL $K_2Cr_2O_7$ solution was added into the water and thoroughly mixed. After connecting the conical flask into a condensing system, 15 mL $AgSO_4$-H_2SO_4 solution was slowly added into the mixture, which was then heated to boiling point for 2h. After that, about 45 mL of ultra-pure water was added. Cooling to room temperature, one or two drops of ferroin were added into the mixture to serve as a titration indicator, and titration was conducted with $(NH_4)_2Fe(SO_4)_2 \cdot 6H_2O$ solution and ended after the color of the mixture turned brown. The consumed volume of $(NH_4)_2Fe(SO_4)_2 \cdot 6H_2O$ solution was recorded as V_1 (mL). As a control, 10 mL ultra-pure water was used for the titration described above, and the consumed volume of the $(NH_4)_2Fe(SO_4)_2 \bullet 6H_2O$ solution was recorded as V_0 (mL). COD_{Cr} (mg O_2 L^{-1}) was calculated from the following equation: $COD_{Cr}=(c((NH_4)_2Fe(SO_4)_2 \cdot 6H_2O)\times(V_0-V_1) \times 8000)/10$. Triplicate water samples were analyzed at each station.

Potassium permanganate oxidation (COD_{Mn}) protocols

There are two COD_{Mn} protocols in the literature: alkaline COD_{Mn} and acidic COD_{Mn} protocols. The alkaline COD_{Mn} protocol is described in the Chinese national standard method of COD for seawater (GB1737.4-2007) and uses $KMnO_4$ as oxidant in alkaline conditions. Briefly, a 100 mL water sample was added into a 250 mL conical flask. One mL NaOH (250 g L^{-1}) and 10 mL $KMnO_4$ solution (0.01 mol L^{-1}) were added to the water and thoroughly mixed. The mixture was heated to boil for 10 min, after which it was quickly cooled to room temperature. After this, 5 mL H_2SO_4 solution (5 mol L^{-1}) and 0.5 g KI were added to the cooled mixture, which was then placed in the dark for 5 min. With the solution continuously shaken, titration was conducted with $Na_2S_2O_3 \cdot 5H_2O$ solution ($c \approx 0.01$ mol L^{-1}, calibrated by KIO_3 standard solution) until the color of the mixture turned light yellow. Then, 1 mL of starch solution (5 g L^{-1}) was added to the mixture and the titration was continued until the mixture turned transparent. The consumed volume of the $Na_2S_2O_3 \cdot 5H_2O$ solution was recorded as V_1 (mL). As a control, 100 mL ultra-pure water was used for the titration described above, and the consumed volume of the $Na_2S_2O_3{\cdot}5H_2O$ solution was recorded as V_0 (mL). Triplicate water samples were analyzed at each station. COD_{Mn} (mg O_2 L^{-1}) was calculated using the following equation:

Alkaline $COD_{Mn}=(c(Na_2S_2O_3{\cdot}5H_2O)\times(V_0-V_1)\times8000)/100$

The acidic COD_{Mn} protocol is described in the Chinese national standard method of COD for surface water (GB11892-89) and uses $KMnO_4$ as oxidant in acidic conditions. Briefly, a 100 mL water sample was added into a 250 mL conical flask. Five mL H_2SO_4 solution (5 mol L^{-1}) and 10 mL $KMnO_4$ solution (0.01 mol L^{-1}) were added to the water and evenly mixed. The mixture was heated for 30 min in a boiling water bath. Ten mL $Na_2C_2O_4$ solution (0.01 mol L^{-1}) was then added to the mixture, which turned transparent. With the solution continuously shaken, the titration was conducted with $KMnO_4$ solution (0.01 mol L^{-1}) until the color of the mixture turned pink, and remained pink for at least 30 sec. The consumed volume of the $KMnO_4$ solution was recorded as V_1 (mL). As a control, 100 mL ultra-pure water was used for the titration described above,10 mL $Na_2C_2O_4$ solution (0.01 mol L^{-1}) was added to the mixture, $KMnO_4$ solution was used as the titrant, and the consumed volume recorded as V_0 (mL). Triplicate water samples were analyzed at each station. Acidic COD_{Mn} (mg O_2 L^{-1}) was calculated using the following equation:

Acidic $COD_{Mn}=((10+V_1)\times10/V_0-10)\times c(KMnO_4)\times8000)/100$

The acidic COD_{Mn} protocol is recognized to be applicable for freshwater but not saline water, because of a salinity effect, i.e. an over-estimation due to interference of inorganic ions (*36-38*). We investigated this by comparing results from the acidic and alkaline protocols for samples with salinities ranging from 0 to 30 (Table S3). The data from the acidic COD_{Mn} protocol were systematically larger than those obtained with the alkaline COD_{Mn} protocol for salinities >0.3, and the difference increased with salinity (Fig. S3). In fresh waters (salinities ≤0.3), some data from the acidic COD_{Mn} protocol were smaller than those from the alkaline COD_{Mn} protocol. Since both methods use excessive amounts of oxidants, they are unlikely to produce underestimated values. Given that the data in this study came from freshwater, estuarine and marine environments, we decided to use only the values obtained with the alkaline COD_{Mn} protocol, in order to avoid overestimation of COD values in estuarine and saline waters.

Comparison of COD_{Cr} and alkaline COD_{Mn} measurements

The COD_{Cr} and alkaline COD_{Mn} measurements of natural water samples with different salinities showed that the COD values from the two methods were not influenced in the same way by the presence of inorganic reductants (Table S2). The values of COD_{Cr} were significantly higher than those of COD_{Mn} in all samples, the former being up to 80 times the latter in saline waters (Fig. S2A). In addition, the coefficients of correlation of COD_{Cr} and COD_{Mn} with salinity (r = 0.94, prob < 0.001, and r = 0.54, prob = 0.075, respectively) indicate a significant positive relationship between COD_{Cr} and salinity, and no relationship between COD_{Mn}, and salinity. As a consequence, the ratio COD_{Cr}/COD_{Mn} significantly increased with salinity (Fig. S2B). These results show that salinity had a systematic effect on COD_{Cr} measurements.

It follows that the COD_{Cr} does not provide a proper measure of oxidized organic matter especially in saline waters, whereas alkaline COD_{Mn} may provide realistic estimates of oxidized DOC. Hence in order to make all our data comparable, we only used COD_{Mn} measurements, either made for this study or collated from the literature (as listed in Table S1). To simplify terminology, we use "COD" instead of "alkaline COD_{Mn}" in this paper.

Protocol of Biochemical Oxygen Demand (BOD) by the BOD_5 Winkler method

Five-day BOD (BOD_5) measurement of oxygen consumption by Winkler titration is a standard method by the International Organization for Standardization. Water samples for BOD measurements were incubated in the dark at 20°C for 5 days. The measurement of dissolved oxygen ($[O_2]$) was based on the ISO 5813:1983 Determination of dissolved oxygen-Iodometric (Winkler) method (https://www.iso.org/obp/ui/#iso:std:iso:5813:ed-1:v1:en). Briefly, to determine the initial oxygen concentration, 1 mL of $MnCl_2$ solution (420 g L^{-1}) and 1 mL of alkaline KI solution (150 g L^{-1}) were sequentially added into triplicate water samples in 140 mL opaque BOD bottles, then mixed thoroughly and the samples allowed to sit in a water bath held at 20°C for at least 4 h. Thereafter, the precipitate was dissolved with, 2 mL of H_2SO_4 solution (5 mol L^{-1}). After transferring 100 mL of the solution into a 250 mL conical flask, the titration was conducted as described for the COD_{Mn} method, and the titrated volume of $Na_2S_2O_3{\cdot}5H_2O$ solution (c, mol L^{-1}) recorded as V_0 (mL). After the 5-day incubation, triplicate water samples were collected and treated by the same procedure as described above, and the titrated volume of $Na_2S_2O_3{\cdot}5H_2O$ solution was recorded as V_5 (mL). BOD_5 (mg O_2 L^{-1}) was calculated using the following equation:

$$BOD_5=c(Na_2S_2O_3\bullet 5H_2O) \times (V_0-V_5) \times 1000\times 8/100$$

Calibration for concentration of $Na_2S_2O_3{\cdot}5H_2O$ standard solution: 10 mL KIO_3 standard solution (0.0100 mol L^{-1}) were poured into a 250 mL conical flask, and 0.5 g KI and 1 mL H_2SO_4 solution (5 mol L^{-1}) were sequentially added to the flask, which was then sealed and gently shaken. After keeping the mixture in the dark for 2 min, 50 mL of ultra-pure water were added and gently mixed, in preparation for titration. The titration was conducted with a $Na_2S_2O_3{\cdot}5H_2O$ solution until the color of the mixture turned light yellow, after which 1 mL of starch solution (5 g L^{-1}) was added, and the titration was continued until the mixture became transparent. The consumed volume of the $Na_2S_2O_3{\cdot}5H_2O$ solution was recorded as V (mL). The concentration of the $Na_2S_2O_3{\cdot}5H_2O$ solution (mol L^{-1}) was calculated using the following equation:

$$c(Na_2S_2O_3\bullet 5H_2O)=10\times 0.0100/V$$

Determination of dissolved organic carbon (DOC)

Samples for DOC measurements were filtered through pre-combusted (450°C, 4 h) Whatman GF/F glass fiber filters. All the samples were then acidified to pH=2 with H_3PO_4 and stored at −20°C until analyzed. The carbon content of samples was measured using the high-temperature combustion method with a Shimadzu TOC-LCPH TOC analyzer and ultrapure water for system blank subtraction (*39*). Reference deep seawater (provided by the Hansell Organic Biogeochemistry

Laboratory at the University of Miami, USA) served as an additional control (*40*). The analytical methodology for DOC was the same for all studies reported in our work.

Aquatron Tower Tank and long-term incubation experiments

Located at Dalhousie University (Halifax, Canada), the Aquatron is the site of the Tower Tank, which is 10.64 m deep and 3.66 m in diameter, with an approximate water volume of 117 m^3, (https://www.dal.ca/faculty/science/biology/research/facilities/aquatron.html). It is well suited for biogeochemical research requiring depth and stratification. To conduct our long-term incubation experiment, we filled the Tower Tank with about 20 tons of humic-like water from the Ingramport River near Halifax. The incubation was run at room temperature (20°C) in the dark for 510 days. Water samples were taken before and after the incubation, and filtered through Whatman GF/F filters (pre-combusted as above) for solid-phase extraction of dissolved organic matter (see below).

Solid-phase extraction of natural DOM and COD treated DOM, and Fourier Transformation Cyclotron Resonance Mass Spectrometry (FT-ICR MS) analysis

DOM was solid-phase extracted following a standard procedure (*41*). For the initial (DOC concentration = 885.96±6.60 μmol C L^{-1}) and long-term incubation (510-day) (DOC concentration = 744.06±6.80 μmol C L^{-1}), DOM was extracted from water samples using 500 mg Bond Elut-PPL cartridges (Agilent) activated with HPLC grade methanol (Merck) and rinsed with acidified Milli-Q water (pH=2). An aliquot of 200 mL water sample was filtered through a pre-combusted (450°C) GF/F glass fiber filter and then passed by gravity through the cartridge, which was subsequently extensively rinsed with acidified Milli-Q water (pH=2) and completely dried before elution with HPLC grade methanol. Each cartridge was eluted with 3 mL HPLC grade methanol, and this DOM extract was adjusted to yield almost the same DOC concentration for each sample before injecting to the FT-ICR MS for analysis.

To investigate which fractions of naturally occurring organic matter were oxidized by the COD method, the initial and long-term incubation of river water samples were subjected to COD_{Mn} prior to DOM extraction. Two mL NaOH solution (250 g L^{-1}) and 20 mL $KMnO_4$ solution (0.01 mol L^{-1}) were added to a 200 mL aliquot of the water sample in a 500 mL conical flask. The mixture was then heated to boil for 10 min. After cooling down to room temperature and being neutralized by HCl (HPLC grade) to pH=7, 0.02 g of $NaHSO_3$ (Sigma-Aldrich) was added to the mixture to reduce the residue $KMnO_4$. Then DOM was extracted using the standard solid phase extraction procedure described above. As a control, 200 mL Milli-Q water was used for the COD treatment described above, and DOM molecules commonly existing in the control and the COD_{Mn} treated water samples were excluded from further analyses. All the glassware used for COD treatments was acid cleaned and combusted (480°C for 4 h).

The DOM extracts were adjusted to yield approximately 25 mM DOC and analyzed using a Bruker Apex Ultra FT-

ICR mass spectrometer equipped with a 9.4 T superconducting magnet. Sample solutions were infused via an Apollo II electrospray ion source (ESI) at 180 μL h^{-1} with a syringe pump. Typical operating conditions for negative ESI were as follows: spray shield voltage 3.5 kV, capillary column initial voltage 4 kV, and capillary column end voltage –320 V. The mass range was set to m/z 150–800. The 4M word size was selected for the time domain signal acquisition. A number of 128-time domain signals were co-added to enhance the signal-to-noise ratio and dynamic range. The magnitude threshold for the peak assignment was set to a signal-to-noise ratio of ≥4. The FT-ICR MS was calibrated using a known homologous series of the Suwannee River natural organic matter sample (obtained from the International Humic Substances Society, USA), which contained a relatively high abundance of oxygen-containing compounds. Molecular formulae were assigned with the calibrated mass data using in-house software (*40*). The elemental compositions were assigned from the m/z peaks using a mass calculator program limited to molecular formulae consisting of $^{12}C_{0-100}$, $^{13}C_{0-2}$, $^{1}H_{0-200}$, $^{14}N_{0-5}$, $^{16}O_{0-35}$, $^{32}S_{0-2}$ and $^{34}S_{0-1}$ (*40, 42*). All assigned formulae had to meet the following basic chemical criteria: (1) the number of H atoms should not exceed 2C + N + 2; (2) the sum of H and N atoms should be even (the "nitrogen rule"); and (3) the number of N or O atoms should not exceed the number of C atoms (*43, 44*).

Proposed new technique for BOD determination: The oxygen-optode BOD method

Oxygen optodes are optical sensors that are based on a chemical indicator (dynamic fluorescence quencher) (*45*). These robust and high-resolution sensors (*46, 47*) have been used to study a number of oxygen-related processes in freshwater and marine systems (*48-52*), yielding the same sensitivity as the chemical Winkler method (*53, 54*). For example, oxygen optodes have already been used in oceanography for measurements of community respiration rates in the mesopelagic and surface waters of the oligotrophic open ocean (*48-50*). They have also been used for measuring BOD in wastewater (*55, 56*). Here, we propose a novel, modern BOD method using oxygen optodes instead of the traditional Winkler titration for the measurement of dissolved oxygen concentration [O_2] in the BOD bottles. Oxygen optodes allow the recording of oxygen consumption in the incubated BOD bottles continuously in a non-invasive and non-destructive manner, so that the initial and final dissolved oxygen determinations can be conducted in the same BOD bottles (Fig. S4). This is a major advantage over the Winkler method, where the initial and final titrations must be done on different bottles. After proper calibration of the optodes (see below), the [O_2] and thus BOD values meet the quality standard of those obtained with the conventional Winkler titration method.

A detailed description of the BOD optode method will be provided in a separate specialized paper. Briefly, the steps are: (1) water sampling and filling of BOD bottles, which are incubated for 5 days; (2) oxygen optode measurements in triplicate incubated BOD bottles, and (3) calculation of BOD_5 (where the index 5 stands for 5 days) from oxygen-optode measurements.

Before using the OXSP5 optodes (PyroScience, Germany) for measuring O_2 in samples, we calibrated them with

Winkler titrations. We used a Metrohm auto-titrator (888 Titrando with a combined platinum ring electrode) for the Winkler O_2 determinations. The 5-day incubations were conducted at 20 ± 0.1°C. Winkler O_2 was measured at t=0, every second hour until t=8 hours, and every day until day 5 on triplicate bottles during the course of the incubation (total of 30 bottles). The optode signal was measured every 10 min in triplicate bottles during the course of the incubation, with one measurement every 3 s during 2.5 min to acquire 50 values at each Winkler sampling point (total of 3 bottles).

We tested the validity of the optode BOD method on a variety of natural freshwater and seawater samples. One example was from the DaRen River, Qingdao, China. The results showed good agreement between the optode and Winkler measurements (Fig. S5). The coefficient of variation (CV) of the measurements by the optode method (CV = 0.0824; n=145) was an order of magnitude lower than that of the Winkler method (CV =0.4472; n=42), indicating that the novel oxygen-optode approach can improve the reliability of the oxygen measurement in a BOD determination.

In summary, the novel oxygen-optode BOD method offers several advantages over the traditional Winkler method. Firstly, it provides a more precise measurement of BOD, i.e., replicates from a given sample measured by the optode method reveal a much smaller standard deviation than the respective Winkler replicates do. Secondly, it eliminates the chemical waste associated with the Winkler method as well as the need to take into the field chemicals that are toxic to aquatic life such as alkaline iodide azide and $MnCl_2$. Thirdly, it removes operator error associated with labor-intensive titrations.

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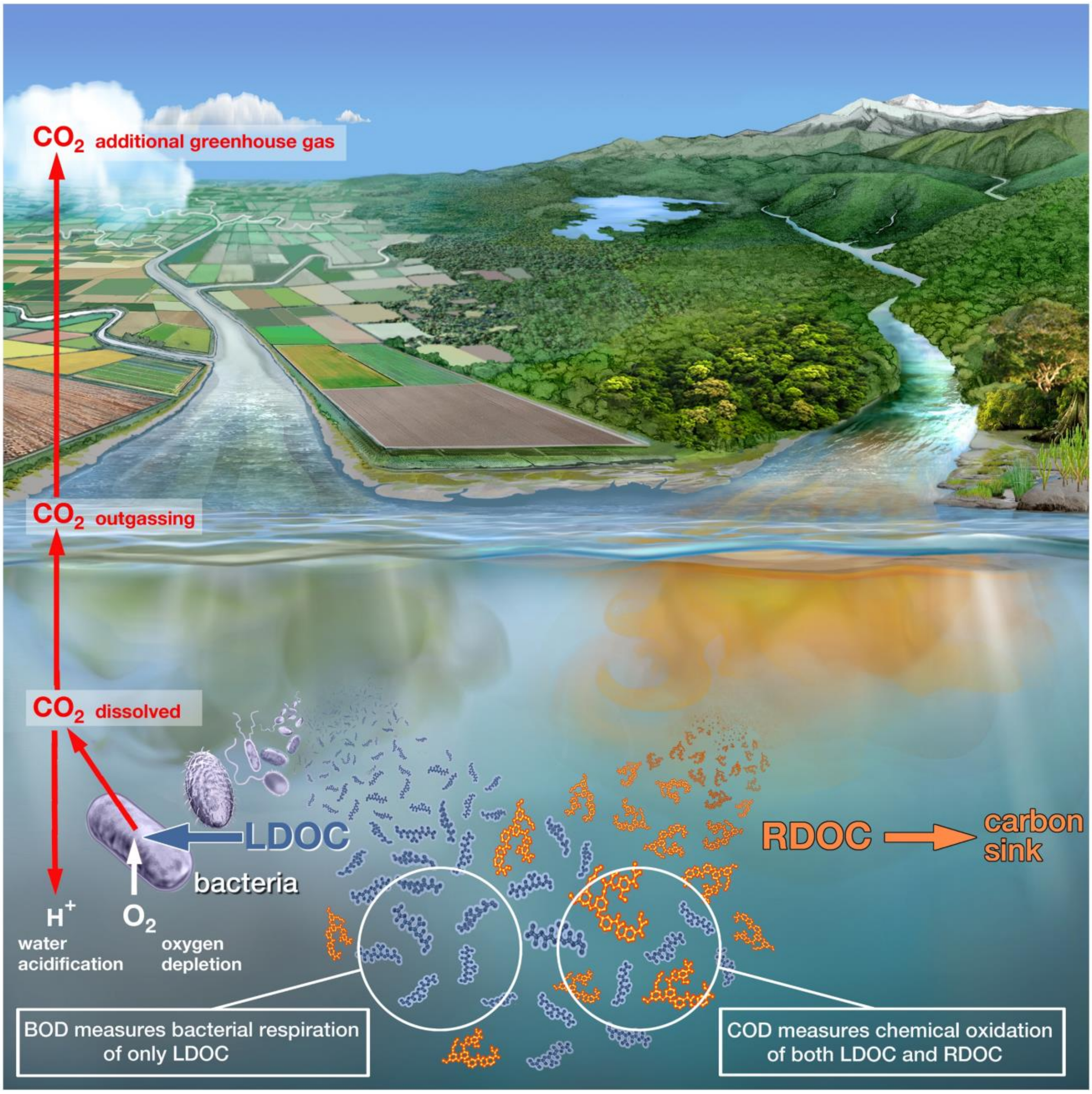

Fig. 1. Schematic representation of LDOC and RDOC in the environment, and their determination using the BOD and COD approaches. Degradation of LDOC can cause oxygen depletion, water acidification and CO_2 emission, and is best assessed by the BOD method. Only LDOC is readily degradable by microbes in natural waters; RDOC has no adverse environmental effects and is a carbon sink. The widely used COD method oxidizes both LDOC and RDOC, and so could result in a major error in the assessment of organic pollution.

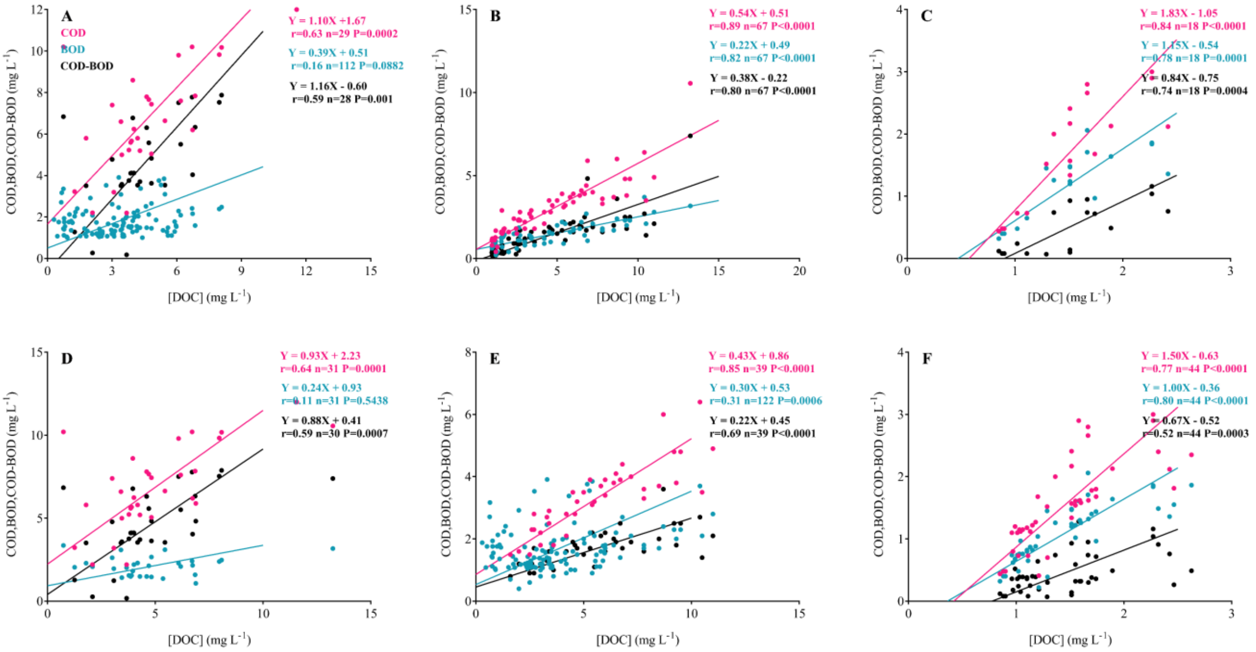

Fig. 2. COD and BOD as a function of [DOC] in different environments: (A) high latitudes/subpolar areas, (**B**) mid-latitudes/temperate zone, (**C**) low latitudes/subtropical zone, (**D**) forested watersheds, (**E**) fresh waters, (**F**) seawater. Note the different X and Y-axes scales of the different panels. The same samples were grouped by (**A-C**) latitudes and also (**D-F**) ecosystems. Regressions: Model 2 Standard Major Axis. n: number of samples.

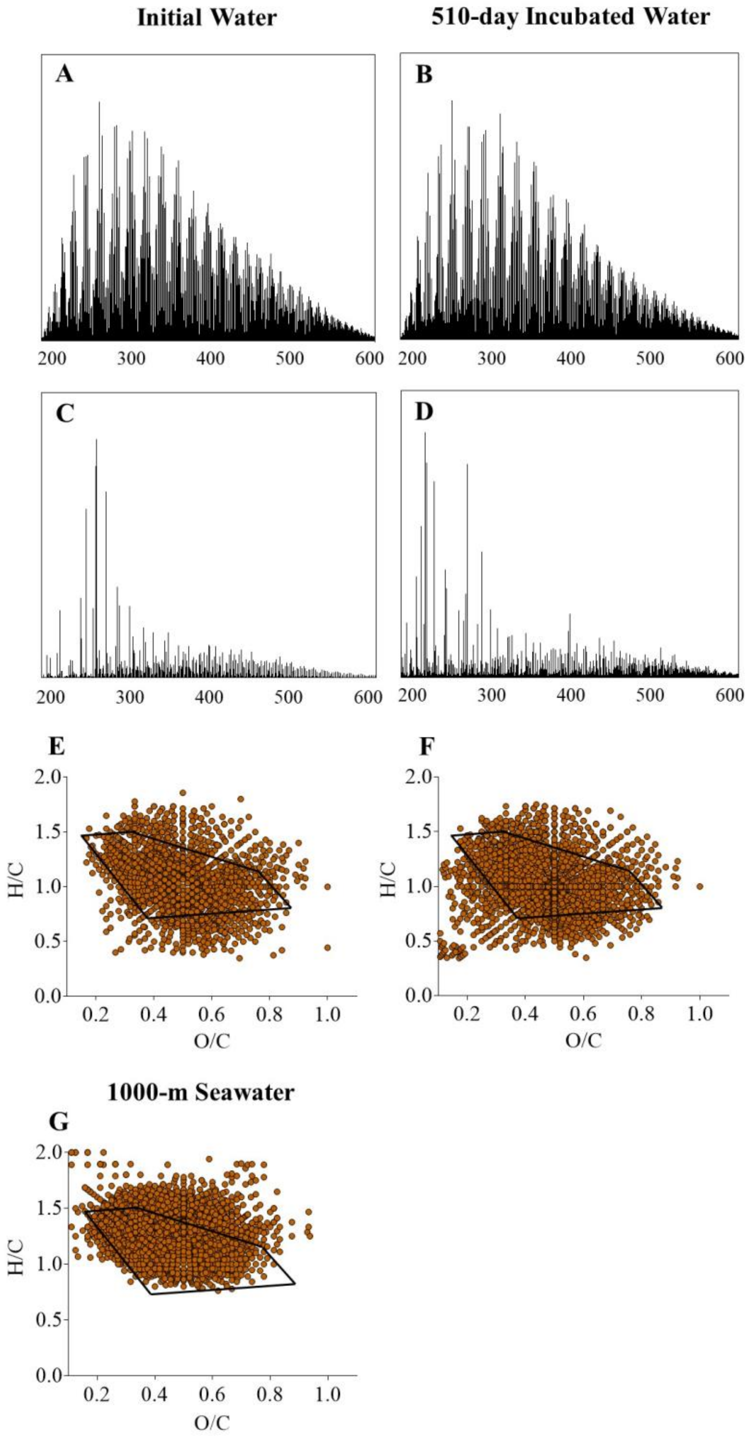

Fig. 3. Results of FT-ICR MS analyses showing changes in organic matter in the natural and incubated river water samples before and after COD treatment. Panels **A** and **B** are the raw peak distributions (m/z 200~600) of organic matter in the water samples before and after long-term (510 days) incubation, respectively, showing no major differences before and after long-term microbial degradation. Panels **C** and **D** show the raw peak distributions (m/z 200~600) of remaining organic matter after the COD treatment of the same samples as in panels **A** and **B**, respectively. Panels **E** and **F** show the van Krevelen diagrams of the RDOC-like components oxidized by the COD treatment, i.e., samples in panels **A** minus **C**, and **B** minus **D**, respectively. Panel **G** is a deep-water (1000-m) sample from the South China Sea, used as a RDOC reference. The black polygons in panels **E** to **G** delineate the CRAM-like molecules (RDOC proxy), based on the literature.

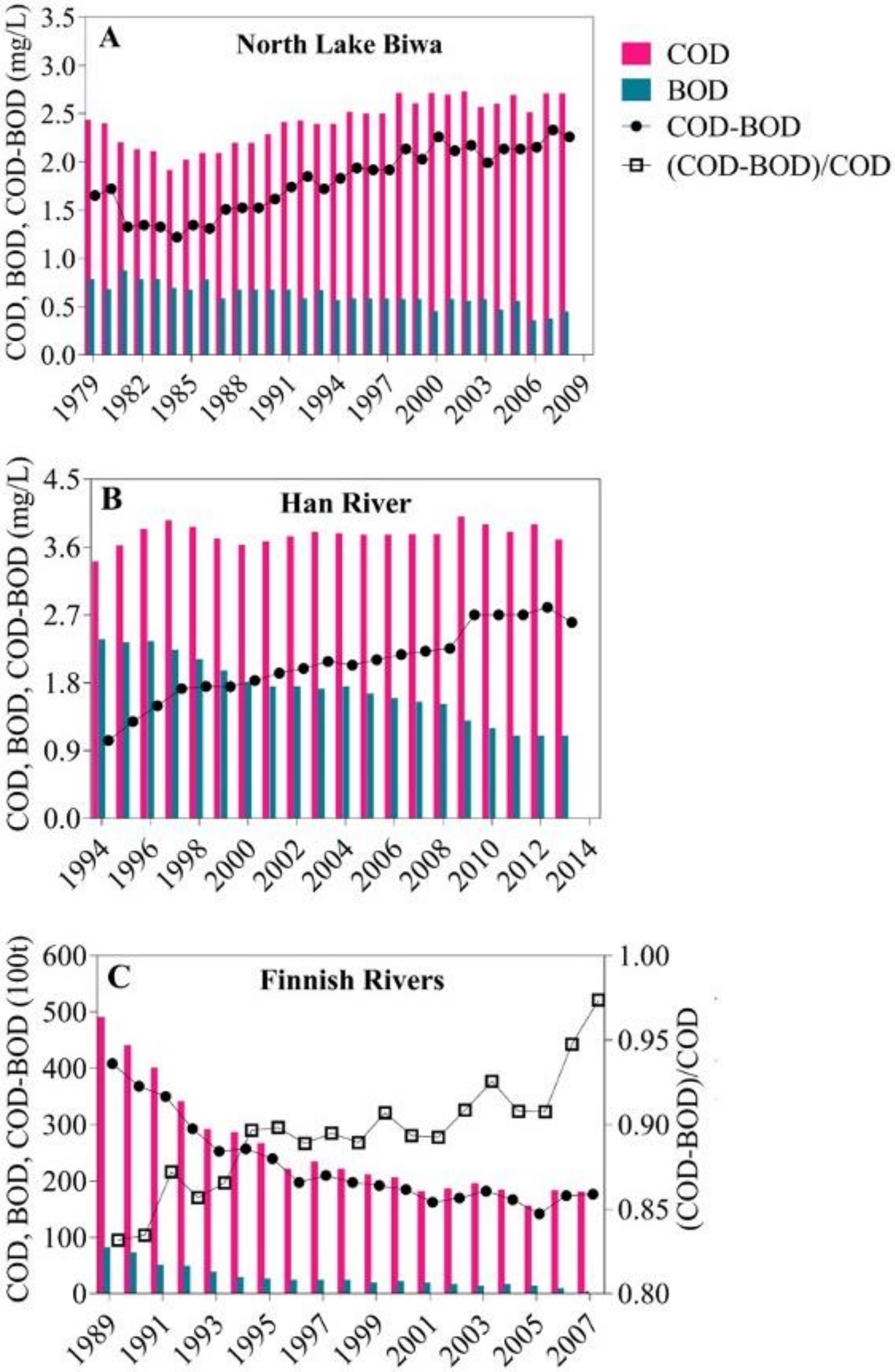

Fig. 4. Three representative field cases of long-term trends in COD and BOD changes: (A) increase in COD and decrease in BOD, and thus accumulation of RDOC (as indicated by [COD-BOD]) in Lake Biwa, Japan; **(B)** sharp decrease in BOD and relatively stable COD, and thus accumulation of RDOC in the Han River, Korea; and **(C)** decrease in both BOD and COD, but increase in the fraction of RDOC in Finnish rivers

Science Advances

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Supplementary Materials for

Correcting a Major Error in Assessing Organic Carbon Pollution in Natural Waters

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This file includes:

1. Supplementary statements
2. Supplementary Figs. S1 to S5
3. Supplementary Tables S1 to S5

1. Supplementary statements

1.1. Persistent organic pollutants (POPs)

Persistent organic pollutants (POPs) are organic compounds that persist in the environment for a long time, migrating in the air, water and soil and accumulating in sediments and food webs *(57, 58)*. The POPs are mainly man-made chemicals that can be divided into intentionally produced compounds (including industrial chemicals and organochlorine pesticides) and unintentional substances resulting from the combustion of organic compounds. The total concentration of recognized POPs in natural waters is usually very low, but can be high in some polluted areas, ranging from a few to thousands of nanograms per liter in natural waters *(59, 60)*. Although the COD method can oxidize many POPs *(61, 62)*, the total amount of carbon in POPs is trivial compared to natural DOC concentrations *(63, 64)*.

1.2. Applications of COD to water quality assessment, and related standards and policy

The COD methods have been used for nearly a century around the world for water quality assessment *(13)*. The International Organization for Standardization recommends COD as one of the parameters for assessing the quality of natural waters *(27)*. In many countries, such as China and Japan, reduction of COD in aquatic environments is used as a measure of environmental policy performance in the evaluation of governmental accomplishments (Table S4). Scientists from different countries also use COD as an indicator to monitor natural waters (examples are shown in the references of *(28, 36, 65-70)*. In addition, various countries (for example Japan, China, and Malaysia) categorize natural waters based on their COD values (Table S5).

1.3. Chemical oxygen demand, and dissolved organic carbon: A Google Scholar search

In a Google Scholar search conducted on January 26, 2020, there were 2,020,000 returns for "chemical oxygen demand" and 210,000 for a combination of “chemical oxygen demand” and “dissolved organic carbon". This shows the great interest of the community for the chemical oxygen demand, and the close connection of this topic with dissolved organic carbon.

1.4. Terminology of organic carbon

Total organic carbon (TOC) includes particulate organic carbon (POC) and dissolved organic carbon (DOC), the latter two being operationally differentiated based on filtration of water samples. Although the proportion of POC and DOC in a given water sample varies with the pore size of the filters used by the investigators, the term DOC is generally found in the scientific literature. Given the fact that DOC accounts for the majority of the TOC and is even equivalent to TOC in many waters, we only use DOC for the discussion in this study *(71, 72)*.

2. Supplementary figures

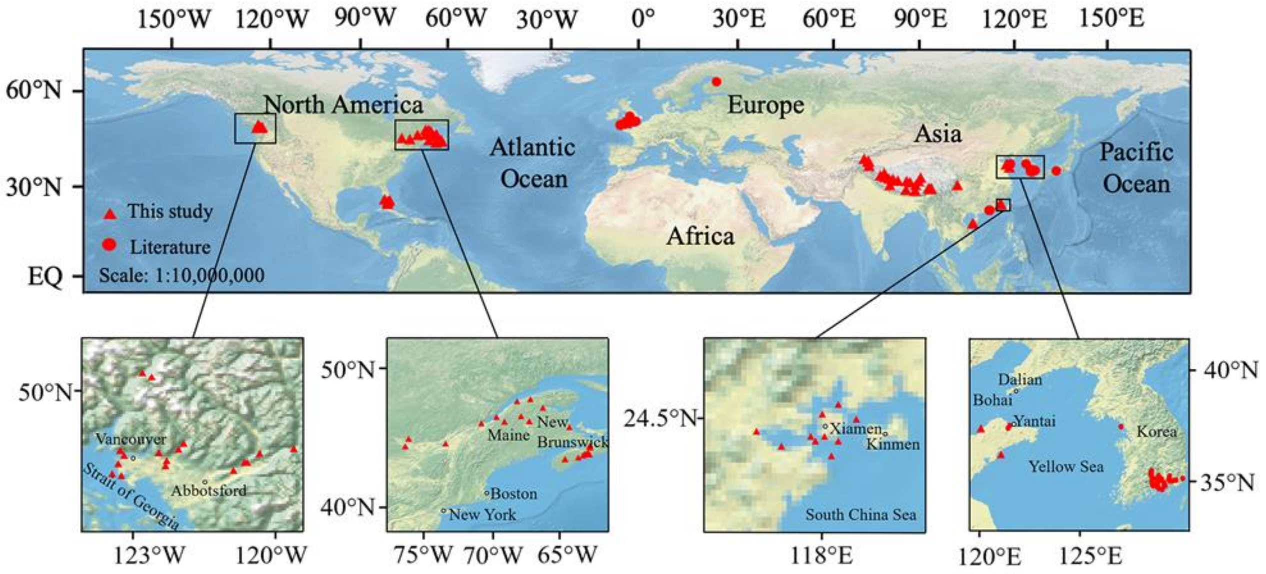

Fig. S1. Locations of the sampling sites (triangles) and data from the literature (circles).

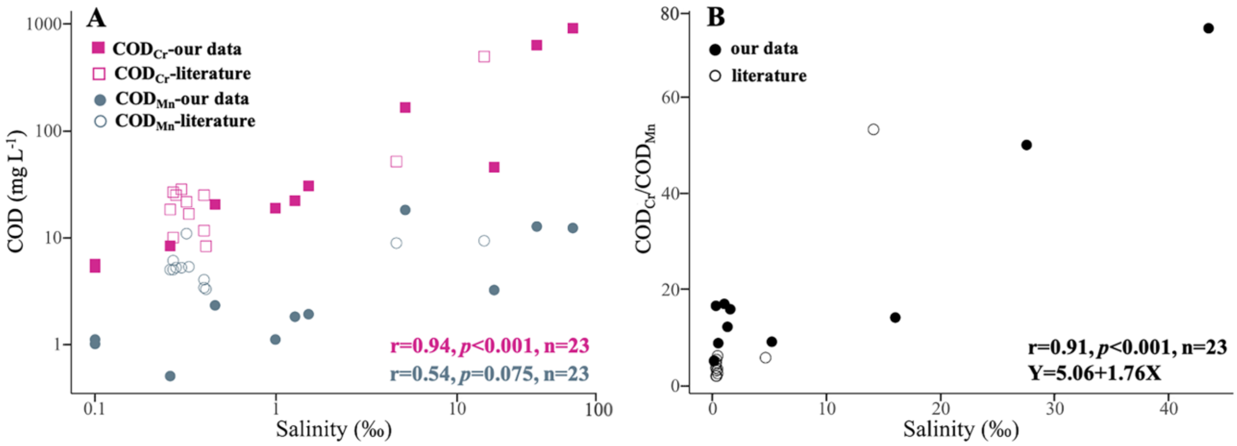

Fig. S2. (A) COD_{Cr} and alkaline COD_{Mn} as a function of salinity, and coefficients of linear correlations, and **(B)** ratio CODCr/CODMn as a function of salinity, coefficient of linear correlation and equation of Model 2 Standard Major Axis regression. n: number of samples.

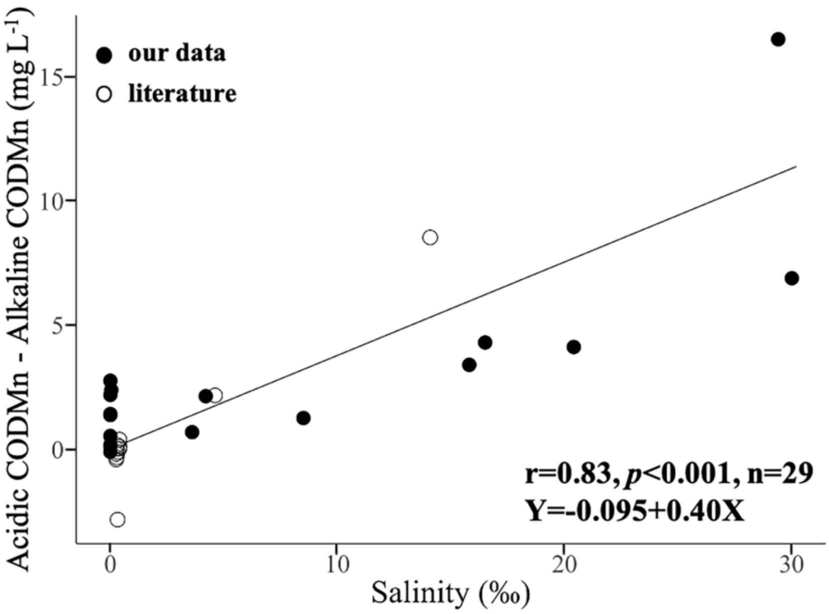

Fig. S3. **Acidic minus alkaline CODMn as a function of salinity.** Coefficient of linear correlation and equation of Model 2 Standard Major Axis regression. n: number of samples.

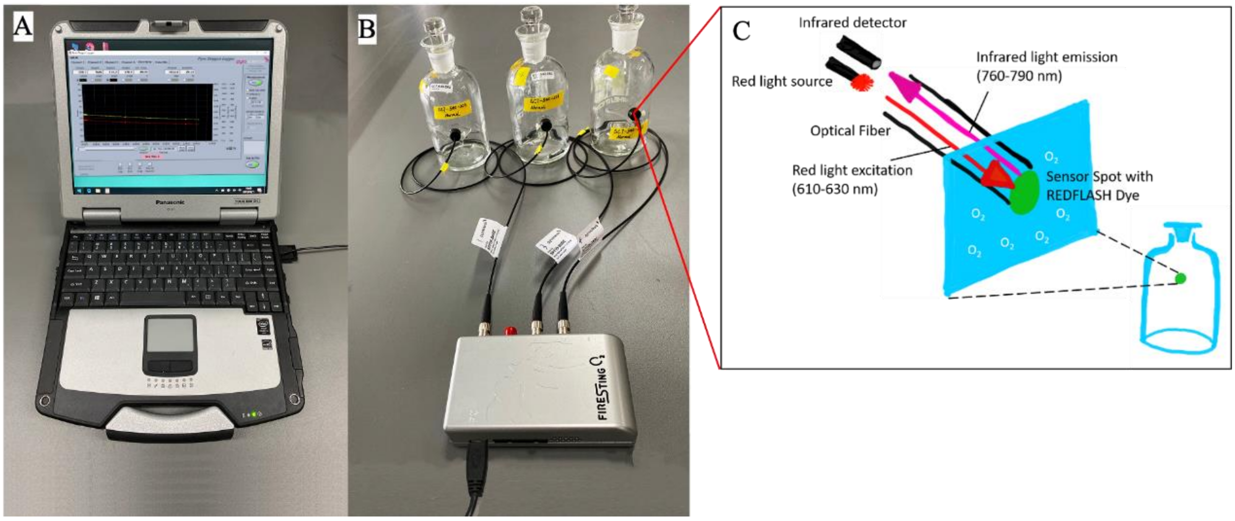

Fig. S4. System to measure dissolved oxygen concentrations with oxygen optodes in triplicate. (**A**) Computer with the data logging software (Pyro Oxygen logger) used for O_2 measurements. (**B**) Optical fiber sensors attached to the outside of BOD bottles with Pyroscience optodes installed internally, and connected to an optical oxygen meter (FireStingO_2) through which the signals are transmitted to the computer. (**C**) Diagram depicting how O_2 measurements are made using red light to excite the REDFLASH dye within the optode/sensor spot in contact with the water sample inside the BOD bottles. The dye emits infrared light that is channeled by the optical fiber into the optical oxygen meter, where it is converted into O_2 concentration (Modified from diagrams on the Pyroscience website: www.pyroscience.com). Photo Credit: Jihua Liu, Institute of Marine Science and Technology, Shandong University, Qingdao, China.

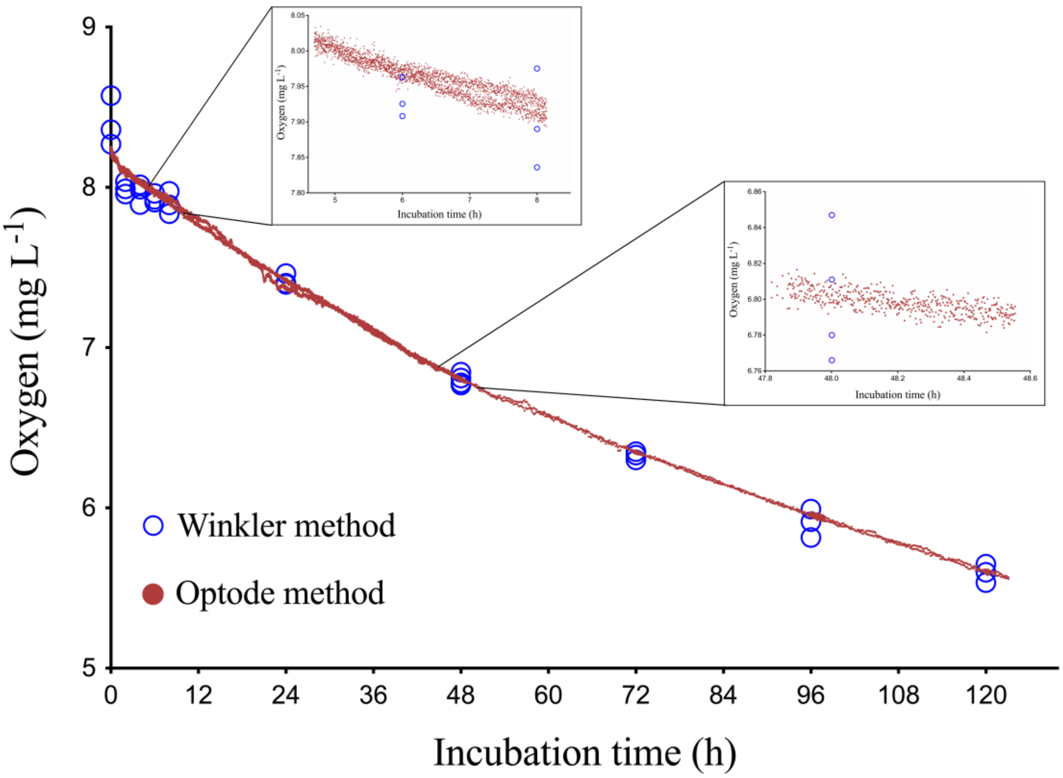

Fig. S5. Comparison of the new optode-based and usual Winkler BOD methods. Oxygen concentrations measured with the two methods during the course of the 5-day incubation. The incubated water was from the DaRen River, Qingdao, China**.** The two inset figures provide details of measurements with the two methods from 4.7 to 8.1 h, and 47.8 to 48.6 h.

3. Supplementary tables

Table S1. Sampling locations and data sources.

Locality	Latitude (°N)	Longitude (°E)	DOC (mg/L)	COD (mg/L)	BOD (mg/L)	COD-BOD (mg/L)	Source
Text Fig. 2							
(A) High latitudes / Subpolar region							
Richibucto River, Canada	46.65	-64.85	3.00	7.40	2.62	4.78	This study
Miramichi Bay, Canada	47.08	-65.21	3.41	6.60	3.09	3.51	This study
Chaleurs Bay, Canada	47.99	-66.74	3.08	3.20	1.96	1.24	This study
			3.78	5.23	1.55	3.76	This study
Lac Matapedia, Canada	48.54	-67.56	3.86	5.60	1.49	4.11	This study
			3.90	5.67	1.55	4.12	This study
Saint Lawrence River, Canada	48.45	-68.52	3.45	5.00	1.42	3.58	This study
			4.01	6.24	2.21	4.12	This study
Montmorency River, Canada	46.89	-71.15	1.79	5.80	2.29	3.51	This study
			4.18	5.80	2.30	3.54	This study
Grand Falls Dam, Canada	45.28	-67.48	4.60	7.80	1.49	6.31	This study
			4.70	7.66	2.13	5.58	This study
Saint John River, Canada	47.06	-67.78	3.67	2.20	2.02	0.18	This study
			4.82	7.44	2.66	4.83	This study
Bay of Fundy, Canada	45.81	-64.57	0.74	10.20	3.36	6.84	This study
			4.82	5.05	1.46	3.63	This study
Stewiacke River, Canada	45.14	-63.35	5.36		1.36		This study

Locality	Latitude (°N)	Longitude (°E)	DOC (mg/L)	COD (mg/L)	BOD (mg/L)	COD-BOD (mg/L)	Source
Shubenacadie River, Canada	44.93	-63.54	5.45	6.64	3.10	3.54	This study
			6.18	7.60	2.09	5.51	This study
			6.85	7.84	1.59	6.33	This study
Ingramport River, Canada	44.67	-63.97	11.56	12.00			This study
Mersey River, Canada	44.43	-65.21	7.97	9.83	2.39	7.53	This study
			6.71	10.20	2.42	7.78	This study
			8.08	10.18	2.48	7.88	This study
Kejimkujik Lake, Canada	44.38	-65.21	6.08	9.80	2.29	7.51	This study
Shubenacadie Grand Lake, Canada	44.89	-63.6	4.29	5.20	1.49	3.71	This study
Lake Major, Canada	44.72	-63.48	3.96	8.60	1.82	6.78	This study
Pockwock Lake, Canada	44.78	-63.84	6.73	6.20	2.16	4.04	This study
Yealm River D/S Yealmpton Stw, UK	50.38	-3.97	2.55		1.30		*(32)*
Yealm At Yealm Bridge, UK	50.31	-4.07	2.51		1.08		*(32)*
Yealm At Puslinch Bridge, UK	50.34	-4.01	2.77		1.08		*(32)*
Tort. Bk. Falfield, UK	51.64	-2.45	4.21		2.04		*(32)*
Tn Bwtr C N Newton, UK	50.31	-3.35	4.57		2.52		*(32)*
T R Avill Timberscom, UK	51.17	-3.5	3.55		1.07		*(32)*
T River Avill Timberscom, UK	NA	NA	5.90		1.30		*(32)*
Sydling Water, Downstream Shearplace, UK	50.22	-5.39	2.55		1.21		*(32)*
Sydling D/S Huish Fm, UK	51.18	-2.27	3.08		1.19		*(32)*

Locality	Latitude (°N)	Longitude (°E)	DOC (mg/L)	COD (mg/L)	BOD (mg/L)	COD-BOD (mg/L)	Source
St. Austell River Downstream Of, UK	50.33	-4.79	3.09		1.63		*(32)*
St Austell At Pentewan Bridge, UK	50.33	-4.79	2.35		1.11		*(32)*
Rodden D/S Langton Herring Stw, UK	50.64	-2.55	1.16		1.73		*(32)*
River Wey At Radipole, UK	50.61	-2.46	2.40		1.23		*(32)*
River Wey At Nottington, UK	50.61	-2.46	2.28		1.20		*(32)*
River Mole D/S South Molton Stw, UK	51.24	-0.31	1.80		1.08		*(32)*
River Cober D/S Helston Stw, UK	50.09	-5.28	2.99		1.19		*(32)*
River Char At Whitchurch Canonicorum, UK	50.75	-2.86	3.49		1.17		*(32)*
Ramsgate (Reen) Roseworthy, UK	50.20	-1.25	3.54		1.72		*(32)*
River Yeo (Lapford)-Bow Bridge, UK	50.86	-3.81	5.54		1.29		*(32)*
River Wey At Radipole, UK	50.63	-2.47	1.64		2.13		*(32)*
River Teign-Preston, UK	50.62	-3.64	3.30		2.46		*(32)*
River Teign-Chudleigh Bridge, UK	50.62	-3.64	3.50		1.59		*(32)*
River Teign D/S Heathfield Tip, UK	50.62	-3.64	3.50		1.85		*(32)*
River Char U/S Whitchurch Can., UK	50.75	-2.86	3.76		1.53		*(32)*
River Yeo Yeovil Bridge, UK	50.94	-2.61	2.44		1.10		*(32)*
River Yeo Yeovil Bridge, UK	50.94	-2.61	2.44		1.10		*(32)*
River Yeo Over Compton, UK	50.93	-2.60	3.31		3.91		*(32)*
River Yeo Goldings Lane, UK	51.00	-2.65	0.52		1.76		*(32)*
River Yeo Goathill, UK	50.95	-2.47	3.63		1.95		*(32)*

Locality	Latitude (°N)	Longitude (°E)	DOC (mg/L)	COD (mg/L)	BOD (mg/L)	COD-BOD (mg/L)	Source
R Wey At Nottington, UK	50.64	-2.48	4.32		3.38		*(32)*
River Wey At Broadwey, UK	51.15	-0.80	3.13		1.33		*(32)*
River Wey At Broadwey, UK	NA	NA	0.30		1.88		*(32)*
River Tone Coalharbor Br, UK	NA	NA	5.27		3.55		*(32)*
River Tone Clatworthy, UK	51.02	-3.14	1.72		1.44		*(32)*
River Tone Bishops Hull, UK	51.02	-3.14	2.41		1.43		*(32)*
River Tone Bathpool, UK	51.04	-3.05	4.15		1.56		*(32)*
River Brue Cow Bridge, UK	51.13	-2.71	1.10		1.81		*(32)*
River Banwell At M5 M'way, UK	51.36	-2.90	0.77		1.94		*(32)*
River Banwell @ M5 M'way, UK	51.36	-2.90	2.96		1.38		*(32)*
River Banwell @ Ebdon Br, UK	51.38	-2.92	5.04		1.86		*(32)*
River Axe Loxton, UK	51.29	-2.90	5.90		1.78		*(32)*
River Axe Henley Hill, UK	53.50	-1.40	3.49		1.07		*(32)*
River Axe Clewer, UK	51.26	-2.80	4.17		1.56		*(32)*
River Axe, UK	51.27	-2.83	2.40		1.05		*(32)*
River Avill Frackford Br, UK	51.16	-3.51	1.74		1.19		*(32)*
Point Above South Crofty R, UK	50.22	-5.28	3.54		1.13		*(32)*
Pill R Blue Anchor, UK	51.18	-3.39	0.74		1.44		*(32)*
Pill R Bilbrook Ford, UK	51.15	-3.40	0.64		2.71		*(32)*
Penponds Roseworthy Stream, UK	50.21	-5.32	3.22		1.58		*(32)*
Ozle. Bk Low. Barnes, UK	51.28	-0.13	3.95		1.28		*(32)*

Locality	Latitude (°N)	Longitude (°E)	DOC (mg/L)	COD (mg/L)	BOD (mg/L)	COD-BOD (mg/L)	Source
North Brook At Northbrook Park, UK	51.20	-0.85	1.52		1.09		*(32)*
Misc Lower Frome, UK	51.23	-2.32	5.98		1.54		*(32)*
Mill Stream D/S Horsepool, UK	51.16	-2.73	5.59		1.49		*(32)*
Medlyn Stream At Chy Bridge, UK	NA	NA	3.55		1.07		*(32)*
Lt. Dart R., D/S Chawleigh Stw, UK	50.91	-3.83	5.59		1.11		*(32)*
Lt. Dart R., D/S Chawleigh Stw, UK	NA	NA	3.09		1.07		*(32)*
Huish Trout Farm Easthill Inlet, UK	50.79	-2.52	1.10		1.36		*(32)*
Hancocks U/S St Ivel, UK	50.12	-5.29	5.17		3.76		*(32)*
Halse Water Tyler Br, UK	51.05	-3.25	0.99		1.76		*(32)*
Halse W Norton Br, UK	52.87	-2.19	1.38		2.41		*(32)*
Halse W Halse G Stn, UK	51.25	-3.13	0.78		1.76		*(32)*
Halse W Asylum Br, UK	51.25	-3.13	1.02		2.13		*(32)*
Gwithian Towans Red River, UK	50.22	-5.39	2.72		3.53		*(32)*
Frome U/S Louds Mill, UK	50.71	-2.41	5.76		1.11		*(32)*
Exeter Canal At A38 Bridge Countess, UK	50.69	-3.49	1.85		1.33		*(32)*
Ebble U/S Chalkvalley Inlet Rside, UK	51.04	-1.87	0.46		1.08		*(32)*
Ebble D/S Chalkevalley, UK	51.02	-1.99	4.14		1.34		*(32)*
Dawlish Water At Dawlish, UK	50.60	-3.51	4.14		1.34		*(32)*
Congresbury Yeo A370, UK	51.37	-2.81	0.77		1.41		*(32)*
Congresby Yeo Beam B, UK	NA	NA	2.08		1.18		*(32)*

Locality	Latitude (°N)	Longitude (°E)	DOC (mg/L)	COD (mg/L)	BOD (mg/L)	COD-BOD (mg/L)	Source
Congres Yeo Tutshill, UK	51.40	-2.40	3.40		1.37		*(32)*
Congres Yeo Iwood, UK	51.36	-2.79	5.96		1.59		*(32)*
Carminowe Creek Inflow To Loe Pool, UK	50.07	-5.28	5.41		3.85		*(32)*
By Brook Middlehill, UK	51.42	-2.27	0.89		1.05		*(32)*
By Brook Fosse, UK	51.50	-2.24	4.40		1.01		*(32)*
By Bk Long Dean Mill, UK	51.48	-2.22	0.92		1.01		*(32)*
Back Stream Fitzroy, UK	51.04	-3.15	0.66		3.17		*(32)*
Avon Scotland Road, UK	NA	NA	1.30		2.24		*(32)*
Avon Melksham Bypass, UK	51.37	-2.14	1.99		2.92		*(32)*
Avon Malford Church, UK	51.50	-2.06	0.81		1.55		*(32)*
Avon Lacock, UK	51.42	-2.12	2.27		1.74		*(32)*
At Austell At Molingey G Stat, UK	50.20	-4.48	2.81		1.67		*(32)*
Alphin Brook At Countess Wear Bridge, UK	50.70	-3.50	0.76		1.12		*(32)*
(B) Mid-latitudes/Temperate zone							
Edward B. Knight Pier, USA	24.55	-81.78	2.32	2.40	1.49	0.91	This study
South Pointe Pier, USA	25.76	-80.13	2.46	1.81	1.55	0.26	This study
Naples Pier, USA	26.13	-81.81	6.88	5.89	1.07	4.82	This study
Pine Glades Lake, USA	25.43	-80.72	13.24	10.56	3.17	7.39	This study
Nine Mine Pond, USA	25.25	-80.80	2.63	2.35	1.86	0.49	This study
Jiulong River, China	24.36	118.13	1.29	0.70	0.39	0.30	This study

Locality	Latitude (°N)	Longitude (°E)	DOC (mg/L)	COD (mg/L)	BOD (mg/L)	COD-BOD (mg/L)	Source
Jiulong River, China	24.42	118.06	1.21	0.41	0.22	0.19	This study
Jiulong River, China	24.4	117.91	1.55	1.61	1.02	0.59	This study
Jiulong River, China	24.46	117.8	1.58	2.90	1.61	1.28	This study
Xiamen Bay, China	24.44	118.04	0.99	1.20	0.68	0.52	This study
Xiamen Bay, China	24.42	118.16	0.97	0.80	0.62	0.18	This study
Xiamen Bay, China	24.51	118.24	1.04	1.12	0.97	0.15	This study
Xiamen Bay, China	24.57	118.16	1.13	1.12	0.87	0.25	This study
Xiamen Bay, China	24.53	118.09	1.20	1.68	1.04	0.64	This study
Bohai Sea, China	37.39	119.95	1.67	1.62	1.32	0.30	This study
Bohai Sea, China	37.39	119.95	1.59	1.62	1.30	0.32	This study
Bohai Sea, China	37.39	119.94	1.70	1.78	1.40	0.38	This study
Bohai Sea, China	37.39	119.93	1.60	1.58	1.26	0.32	This study
Bohai Sea, China	37.39	119.93	1.55	1.54	1.22	0.32	This study
Bohai Sea, China	37.39	119.94	1.74	1.80	1.44	0.36	This study
Yellow Sea, China	36.37	120.88	1.10	1.18	0.80	0.38	This study
Yellow Sea, China	36.37	120.88	1.15	1.22	0.82	0.40	This study
Yellow Sea, China	36.37	120.88	1.01	1.10	0.74	0.36	This study
Yellow Sea, China	36.37	120.88	1.18	1.27	0.88	0.39	This study
Yellow Sea, China	36.37	120.88	0.96	1.10	0.74	0.36	This study
Yellow Sea, China	36.37	120.88	1.03	1.15	0.76	0.39	This study
Yellow Sea, China	36.37	120.88	1.10	1.18	0.82	0.36	This study

Locality	Latitude (°N)	Longitude (°E)	DOC (mg/L)	COD (mg/L)	BOD (mg/L)	COD-BOD (mg/L)	Source
Yellow Sea, China	36.37	120.88	1.06	1.15	0.76	0.39	This study
Gilgok, South Korea	35.4	129.29	7.20	4.00	2.10	1.90	*(33)*
Shincheon, South Korea	35.36	128.64	6.40	3.80	1.80	2.00	*(33)*
Deokgok, South Korea	35.11	128.48	5.80	3.70	1.60	2.10	*(33)*
Imgok, South Korea	35.11	128.46	6.80	4.40	2.70	1.70	*(33)*
Jungchon, South Korea	35.13	128.43	3.00	1.80	0.80	1.00	*(33)*
Mari, South Korea	35.7	127.86	4.70	3.20	1.50	1.70	*(33)*
Anha, South Korea	35.31	128.83	9.20	4.80	2.30	2.50	*(33)*
Juwhang, South Korea	35.29	128.71	6.00	3.40	1.80	1.60	*(33)*
Wolpyeong, South Korea	34.95	128.35	2.70	2.80	1.10	1.70	*(33)*
Sangdong, South Korea	35	128.2	2.70	2.30	1.40	0.90	*(33)*
Deokam, South Korea	35.51	128.18	10.50	3.50	2.10	1.40	*(33)*
Uicho, South Korea	35.42	128.14	6.70	3.90	1.40	2.50	*(33)*
Daehyun, South Korea	35.45	128.16	7.80	3.30	1.70	1.60	*(33)*
Jikjun, South Korea	35.12	127.9	2.60	2.30	1.10	1.20	*(33)*
Hogye, South Korea	35.2	127.88	2.00	1.60	0.40	1.20	*(33)*
Sachon, South Korea	35.32	128.32	4.20	2.10	0.90	1.20	*(33)*
Ssanggye, South Korea	35.22	128.44	3.60	1.80	0.80	1.00	*(33)*
Gwigok, South Korea	35.64	127.84	5.30	3.90	2.20	1.70	*(33)*
Anui, South Korea	35.64	127.81	6.50	4.10	2.20	1.90	*(33)*
Jiwoo, South Korea	35.66	127.81	1.60	1.50	0.70	0.80	*(33)*

Locality	Latitude (°N)	Longitude (°E)	DOC (mg/L)	COD (mg/L)	BOD (mg/L)	COD-BOD (mg/L)	Source
Juksan, South Korea	35.52	127.81	6.00	3.90	1.70	2.20	*(33)*
Hachon, South Korea	35.23	128.12	10.40	6.40	3.70	2.70	*(33)*
Whagye, South Korea	35.11	128.1	11.00	4.90	2.80	2.10	*(33)*
Jinae, South Korea	35.23	128.14	8.70	6.00	2.40	3.60	*(33)*
Unjung, South Korea	35.49	128.68	4.20	2.80	1.20	1.60	*(33)*
Whansa, South Korea	35.14	127.99	5.00	3.50	2.00	1.50	*(33)*
Seongbang, South Korea	35.12	127.96	5.50	3.20	2.10	1.10	*(33)*
Daegok, South Korea	35.06	128.13	4.50	3.50	1.60	1.90	*(33)*
Gamgok, South Korea	35.05	128.15	3.40	2.50	1.20	1.30	*(33)*
Gobong, South Korea	35.04	128.17	3.30	2.70	1.40	1.30	*(33)*
Jeonggok, South Korea	35.41	127.91	3.00	2.20	1.00	1.20	*(33)*
Banggok, South Korea	35.36	127.87	9.30	3.80	2.00	1.80	*(33)*
Migok, South Korea	35.36	128.08	7.80	3.60	1.80	1.80	*(33)*
Wolgok, South Korea	35.49	127.86	9.50	4.80	2.30	2.50	*(33)*
Massang, South Korea	35.37	128.11	8.50	3.70	1.70	2.00	*(33)*
Garye, South Korea	35.33	127.86	3.40	2.90	1.30	1.60	*(33)*
Unam, South Korea	35.34	128.23	4.60	2.80	1.00	1.80	*(33)*
Jukjeon, South Korea	35.37	128.32	5.40	3.10	0.90	2.20	*(33)*
Whajung, South Korea	35.34	128.98	2.50	1.50	0.60	0.90	*(33)*
(C) Low latitudes/Tropical zone							
Sanya Bay, China	18.19	109.08	0.88	0.48	0.40	0.08	This study

Locality	Latitude (°N)	Longitude (°E)	DOC (mg/L)	COD (mg/L)	BOD (mg/L)	COD-BOD (mg/L)	Source
Sanya Bay, China	18.20	109.08	1.11	0.73	0.65	0.08	This study
Sanya Bay, China	18.19	109.07	1.02	0.73	0.48	0.24	This study
Sanya Bay, China	18.17	109.06	0.85	0.44	0.32	0.12	This study
Sanya Bay, China	18.15	109.18	0.90	0.48	0.40	0.08	This study
Mirs Bay, China	22.53	114.48	1.89	2.13	1.64	0.49	*(34)*
Mirs Bay, China	22.53	114.46	1.29	1.52	1.45	0.07	*(34)*
Mirs Bay, China	22.50	114.48	1.51	1.34	1.20	0.14	*(34)*
Mirs Bay, China	22.53	114.48	1.74	1.68	0.97	0.72	*(34)*
Mirs Bay, China	22.53	114.47	1.67	2.80	2.06	0.74	*(34)*
Mirs Bay, China	22.53	114.46	1.51	1.57	1.47	0.10	*(34)*
Mirs Bay, China	22.50	114.48	1.67	2.66	1.71	0.95	*(34)*
Mirs Bay, China	22.50	114.48	1.51	2.41	1.48	0.93	*(34)*
Mirs Bay, China	22.53	114.48	2.42	2.12	1.36	0.76	*(34)*
Mirs Bay, China	22.53	114.47	2.27	2.90	1.86	1.04	*(34)*
Mirs Bay, China	22.53	114.46	1.36	2.00	1.26	0.74	*(34)*
Mirs Bay, China	22.50	114.47	2.27	3.00	1.84	1.16	*(34)*
Mirs Bay, China	22.50	114.48	1.51	2.17	1.24	0.93	*(34)*
(D) Forest watershed							
Pine Glades Lake, USA	25.43	-80.72	6.88	5.89	1.07	4.82	This study
Nine Mine Pond, USA	25.25	-80.796	13.24	10.56	3.17	7.39	This study
Richibucto River, Canada	46.65	-64.85	3.00	7.40	2.62	4.78	This study

Locality	Latitude (°N)	Longitude (°E)	DOC (mg/L)	COD (mg/L)	BOD (mg/L)	COD-BOD (mg/L)	Source
			1.26	3.23	2.04	1.28	This study
Miramichi Bay, Canada	47.08	-65.21	3.41	6.60	3.09	3.51	This study
			2.09	2.21	2.08	0.27	This study
Chaleurs Bay, Canada	47.99	-66.74	3.08	3.20	1.96	1.24	This study
			3.78	5.23	1.55	3.76	This study
Lac Matapedia, Canada	48.54	-67.56	3.86	5.60	1.49	4.11	This study
			3.90	5.67	1.55	4.12	This study
Saint Lawrence River, Canada	48.45	-68.52	3.45	5.00	1.42	3.58	This study
			4.01	6.24	2.21	4.12	This study
Montmorency River, Canada	46.89	-71.15	1.79	5.80	2.29	3.51	This study
			4.18	5.80	2.30	3.54	This study
Grand Falls Dam, Canada	45.28	-67.48	4.60	7.80	1.49	6.31	This study
			4.70	7.66	2.13	5.58	This study
Saint John River, Canada	47.06	-67.78	3.67	2.20	2.02	0.18	This study
			4.82	7.44	2.66	4.83	This study
Bay of Fundy, Canada	45.81	-64.57	0.74	10.20	3.36	6.84	This study
			4.82	5.05	1.46	3.63	This study
Stewiacke River, Canada	45.14	-63.35	5.36		1.36		This study
			5.45	6.64	3.10	3.54	This study
Shubenacadie River, Canada	44.93	-63.54	6.18	7.60	2.09	5.51	This study
			6.85	7.84	1.59	6.33	This study

Locality	Latitude (°N)	Longitude (°E)	DOC (mg/L)	COD (mg/L)	BOD (mg/L)	COD-BOD (mg/L)	Source
Ingramport River, Canada	44.67	-63.97	11.56	12.00			This study
			7.97	9.83	2.39	7.53	This study
Mersey River, Canada	44.43	-65.21	6.71	10.20	2.42	7.78	This study
			8.08	10.18	2.48	7.88	
Kejimkujik Lake, Canada	44.38	-65.21	6.08	9.80	2.29	7.51	This study
Shubenacadie Grand Lake, Canada	44.89	-63.6	4.29	5.20	1.49	3.71	This study
Lake Major, Canada	44.72	-63.48	3.96	8.60	1.82	6.78	This study
Pockwock Lake, Canada	44.78	-63.84	6.73	6.20	2.16	4.04	This study
(E) Fresh waters							
Gilgok, South Korea	35.4	129.29	7.20	4.00	2.10	1.90	*(33)*
Shincheon, South Korea	35.36	128.64	6.40	3.80	1.80	2.00	*(33)*
Deokgok, South Korea	35.11	128.48	5.80	3.70	1.60	2.10	*(33)*
Imgok, South Korea	35.11	128.46	6.80	4.40	2.70	1.70	*(33)*
Jungchon, South Korea	35.13	128.43	3.00	1.80	0.80	1.00	*(33)*
Mari, South Korea	35.7	127.86	4.70	3.20	1.50	1.70	*(33)*
Anha, South Korea	35.31	128.83	9.20	4.80	2.30	2.50	*(33)*
Juwhang, South Korea	35.29	128.71	6.00	3.40	1.80	1.60	*(33)*
Wolpyeong, South Korea	34.95	128.35	2.70	2.80	1.10	1.70	*(33)*
Sangdong, South Korea	35.00	128.20	2.70	2.30	1.40	0.90	*(33)*
Deokam, South Korea	35.51	128.18	10.50	3.50	2.10	1.40	*(33)*
Uicho, South Korea	35.42	128.14	6.70	3.90	1.40	2.50	*(33)*

Locality	Latitude (°N)	Longitude (°E)	DOC (mg/L)	COD (mg/L)	BOD (mg/L)	COD-BOD (mg/L)	Source
Daehyun, South Korea	35.45	128.16	7.80	3.30	1.70	1.60	*(33)*
Jikjun, South Korea	35.12	127.9	2.60	2.30	1.10	1.20	*(33)*
Hogye, South Korea	35.20	127.88	2.00	1.60	0.40	1.20	*(33)*
Sachon, South Korea	35.32	128.32	4.20	2.10	0.90	1.20	*(33)*
Ssanggye, South Korea	35.22	128.44	3.60	1.80	0.80	1.00	*(33)*
Gwigok, South Korea	35.64	127.84	5.30	3.90	2.20	1.70	*(33)*
Anui, South Korea	35.64	127.81	6.50	4.10	2.20	1.90	*(33)*
Jiwoo, South Korea	35.66	127.81	1.60	1.50	0.70	0.80	*(33)*
Juksan, South Korea	35.52	127.81	6.00	3.90	1.70	2.20	*(33)*
Hachon, South Korea	35.23	128.12	10.40	6.40	3.70	2.70	*(33)*
Whagye, South Korea	35.11	128.1	11.00	4.90	2.80	2.10	*(33)*
Jinae, South Korea	35.23	128.14	8.70	6.00	2.40	3.60	*(33)*
Unjung, South Korea	35.49	128.68	4.20	2.80	1.20	1.60	*(33)*
Whansa, South Korea	35.14	127.99	5.00	3.50	2.00	1.50	*(33)*
Seongbang, South Korea	35.12	127.96	5.50	3.20	2.10	1.10	*(33)*
Daegok, South Korea	35.06	128.13	4.50	3.50	1.60	1.90	*(33)*
Gamgok, South Korea	35.05	128.15	3.40	2.50	1.20	1.30	*(33)*
Gobong, South Korea	35.04	128.17	3.30	2.70	1.40	1.30	*(33)*
Jeonggok, South Korea	35.41	127.91	3.00	2.20	1.00	1.20	*(33)*
Banggok, South Korea	35.36	127.87	9.30	3.80	2.00	1.80	*(33)*
Migok, South Korea	35.36	128.08	7.80	3.60	1.80	1.80	*(33)*

Locality	Latitude (°N)	Longitude (°E)	DOC (mg/L)	COD (mg/L)	BOD (mg/L)	COD-BOD (mg/L)	Source
Wolgok, South Korea	35.49	127.86	9.50	4.80	2.30	2.50	*(33)*
Massang, South Korea	35.37	128.11	8.50	3.70	1.70	2.00	*(33)*
Garye, South Korea	35.33	127.86	3.40	2.90	1.30	1.60	*(33)*
Unam, South Korea	35.34	128.23	4.60	2.80	1.00	1.80	*(33)*
Jukjeon, South Korea	35.37	128.32	5.40	3.10	0.90	2.20	*(33)*
Whajung, South Korea	35.34	128.98	2.50	1.50	0.60	0.90	*(33)*
Yealm River D/S Yealmpton Stw, UK	50.38	-3.97	2.55		1.30		*(32)*
Yealm At Yealm Bridge, UK	50.31	-4.07	2.51		1.08		*(32)*
Yealm At Puslinch Bridge, UK	50.34	-4.01	2.77		1.08		*(32)*
Tort. Bk. Falfield, UK	51.64	-2.45	4.21		2.04		*(32)*
Tn Bwtr C N Newton, UK	50.31	-3.35	4.57		2.52		*(32)*
T R Avill Timberscom, UK	51.17	-3.50	3.55		1.07		*(32)*
T River Avill Timberscom, UK	NA	NA	5.90		1.30		*(32)*
Sydling Water, UK Downstream Shearplace, UK	50.22	-5.39	2.55		1.21		*(32)*
Sydling D/S Huish Fm, UK	51.18	-2.27	3.08		1.19		*(32)*
St. Austell River Downstream Of, UK	50.33	-4.79	3.09		1.63		*(32)*
St Austell At Pentewan Bridge, UK	50.33	-4.79	2.35		1.11		*(32)*
Rodden D/S Langton Herring Stw, UK	50.64	-2.55	1.16		1.73		*(32)*
River Wey At Radipole, UK	50.61	-2.46	2.40		1.23		*(32)*
River Wey At Nottington, UK	50.61	-2.46	2.28		1.20		*(32)*

Locality	Latitude (°N)	Longitude (°E)	DOC (mg/L)	COD (mg/L)	BOD (mg/L)	COD-BOD (mg/L)	Source
River Mole D/S South Molton Stw, UK	51.24	-0.31	1.80		1.08		*(32)*
River Cober D/S Helston Stw, UK	50.09	-5.28	2.99		1.19		*(32)*
River Char At Whitchurch Canonicorum, UK	50.75	-2.86	3.49		1.17		*(32)*
Ramsgate (Reen) Roseworthy, UK	50.20	-1.25	3.54		1.72		*(32)*
River Yeo (Lapford)-Bow Bridge, UK	50.86	-3.81	5.54		1.29		*(32)*
River Wey At Radipole, UK	50.63	-2.47	1.64		2.13		*(32)*
River Teign-Preston, UK	50.62	-3.64	3.30		2.46		*(32)*
River Teign-Chudleigh Bridge, UK	50.62	-3.64	3.50		1.59		*(32)*
River Teign D/S Heathfield Tip, UK	50.62	-3.64	3.50		1.85		*(32)*
River Char U/S Whitchurch Can., UK	50.75	-2.86	3.76		1.53		*(32)*
River Yeo Yeovil Bridge, UK	50.94	-2.61	2.44		1.10		*(32)*
River Yeo Yeovil Bridge, UK	50.94	-2.61	2.44		1.10		*(32)*
River Yeo Over Compton, UK	50.93	-2.60	3.31		3.91		*(32)*
River Yeo Goldings Lane, UK	51.00	-2.65	0.52		1.76		*(32)*
River Yeo Goathill, UK	50.95	-2.47	3.63		1.95		*(32)*
R Wey At Nottington, UK	50.64	-2.48	4.32		3.38		*(32)*
River Wey At Broadwey, UK	51.15	-0.80	3.13		1.33		*(32)*
River Wey At Broadwey, UK	NA	NA	0.30		1.88		*(32)*
River Tone Coalharbor Br, UK	NA	NA	5.27		3.55		*(32)*
River Tone Clatworthy, UK	51.02	-3.14	1.72		1.44		*(32)*

Locality	Latitude (°N)	Longitude (°E)	DOC (mg/L)	COD (mg/L)	BOD (mg/L)	COD-BOD (mg/L)	Source
River Tone Bishops Hull, UK	51.02	-3.14	2.41		1.43		*(32)*
River Tone Bathpool, UK	51.04	-3.05	4.15		1.56		*(32)*
River Brue Cow Bridge, UK	51.13	-2.71	1.10		1.81		*(32)*
River Banwell At M5 M'way, UK	51.36	-2.90	0.77		1.94		*(32)*
River Banwell @ M5 M'way, UK	51.36	-2.90	2.96		1.38		*(32)*
River Banwell @ Ebdon Br, UK	51.38	-2.92	5.04		1.86		*(32)*
River Axe Loxton, UK	51.29	-2.90	5.90		1.78		*(32)*
River Axe Henley Hill, UK	53.50	-1.40	3.49		1.07		*(32)*
River Axe Clewer, UK	51.26	-2.80	4.17		1.56		*(32)*
River Axe, UK	51.27	-2.83	2.40		1.05		*(32)*
River Avill Frackford Br, UK	51.16	-3.51	1.74		1.19		*(32)*
Point Above South Crofty R, UK	50.22	-5.28	3.54		1.13		*(32)*
Pill R Blue Anchor, UK	51.18	-3.39	0.74		1.44		*(32)*
Pill R Bilbrook Ford, UK	51.15	-3.40	0.64		2.71		*(32)*
Penponds Roseworthy Stream, UK	50.21	-5.32	3.22		1.58		*(32)*
Ozle. Bk Low. Barnes, UK	51.28	-0.13	3.95		1.28		*(32)*
North Brook At Northbrook Park, UK	51.20	-0.85	1.52		1.09		*(32)*
Misc Lower Frome, UK	51.23	-2.32	5.98		1.54		*(32)*
Mill Stream D/S Horsepool, UK	51.16	-2.73	5.59		1.49		*(32)*
Medlyn Stream At Chy Bridge, UK	NA	NA	3.55		1.07		*(32)*
Lt. Dart R.,D/S Chawleigh Stw, UK	50.91	-3.83	5.59		1.11		*(32)*

Locality	Latitude (°N)	Longitude (°E)	DOC (mg/L)	COD (mg/L)	BOD (mg/L)	COD-BOD (mg/L)	Source
Lt. Dart R., D/S Chawleigh Stw, UK	NA	NA	3.09		1.07		*(32)*
Huish Trout Farm Easthill Inlet, UK	50.79	-2.52	1.10		1.36		*(32)*
Hancocks U/S St Ivel, UK	50.12	-5.29	5.17		3.76		*(32)*
Halse Water Tyler Br, UK	51.05	-3.25	0.99		1.76		*(32)*
Halse W Norton Br, UK	52.87	-2.19	1.38		2.41		*(32)*
Halse W Halse G Stn, UK	51.25	-3.13	0.78		1.76		*(32)*
Halse W Asylum Br, UK	51.25	-3.13	1.02		2.13		*(32)*
Gwithian Towans Red River, UK	50.22	-5.39	2.72		3.53		*(32)*
Frome U/S Louds Mill, UK	50.71	-2.41	5.76		1.11		*(32)*
Exeter Canal At A38 Bridge Countess, UK	50.69	-3.49	1.85		1.33		*(32)*
Ebble U/S Chalkvalley Inlet Rside, UK	51.04	-1.87	0.46		1.08		*(32)*
Ebble D/S Chalkevalley, UK	51.02	-1.99	4.14		1.34		*(32)*
Dawlish Water At Dawlish, UK	50.60	-3.51	4.14		1.34		*(32)*
Congresbury Yeo A370, UK	51.37	-2.81	0.77		1.41		*(32)*
Congresby Yeo Beam B, UK	NA	NA	2.08		1.18		*(32)*
Congres Yeo Tutshill, UK	51.40	-2.40	3.40		1.37		*(32)*
Congres Yeo Iwood, UK	51.36	-2.79	5.96		1.59		*(32)*
Carminowe Creek Inflow To Loe Pool, UK	50.07	-5.28	5.41		3.85		*(32)*
By Brook Middlehill, UK	51.42	-2.27	0.89		1.05		*(32)*
By Brook Fosse, UK	51.50	-2.24	4.40		1.01		*(32)*

Locality	Latitude (°N)	Longitude (°E)	DOC (mg/L)	COD (mg/L)	BOD (mg/L)	COD-BOD (mg/L)	Source
By Bk Long Dean Mill, UK	51.48	-2.22	0.92		1.01		*(32)*
Back Stream Fitzroy, UK	51.04	-3.15	0.66		3.17		*(32)*
Avon Scotland Road, UK	NA	NA	1.30		2.24		*(32)*
Avon Melksham Bypass, UK	51.37	-2.14	1.99		2.92		*(32)*
Avon Malford Church, UK	51.50	-2.06	0.81		1.55		*(32)*
Avon Lacock, UK	51.42	-2.12	2.27		1.74		*(32)*
At Austell At Molingey G Stat, UK	50.20	-4.48	2.81		1.67		*(32)*
Alphin Brook At Countess Wear Bridge, UK	50.70	-3.50	0.76		1.12		*(32)*
			(F) Seawater				
Edward B. Knight Pier, USA	24.55	-81.78	2.32	2.40	1.49	0.91	This study
South Pointe Pier, USA	25.76	-80.130	2.46	1.81	1.55	0.26	This study
Naples Pier, USA	26.13	-81.807	2.63	2.35	1.86	0.49	This study
Sanya Bay, China	18.19	109.08	0.88	0.48	0.40	0.08	This study
Sanya Bay, China	18.20	109.08	1.11	0.73	0.65	0.08	This study
Sanya Bay, China	18.19	109.07	1.02	0.73	0.48	0.24	This study
Sanya Bay, China	18.17	109.06	0.85	0.44	0.32	0.12	This study
Sanya Bay, China	18.15	109.18	0.90	0.48	0.40	0.08	This study
Jiulong River, China	24.36	118.13	1.29	0.70	0.39	0.30	This study
Jiulong River, China	24.42	118.06	1.21	0.41	0.22	0.19	This study
Jiulong River, China	24.40	117.91	1.55	1.61	1.02	0.59	This study

Locality	Latitude (°N)	Longitude (°E)	DOC (mg/L)	COD (mg/L)	BOD (mg/L)	COD-BOD (mg/L)	Source
Jiulong River, China	24.46	117.80	1.58	2.90	1.61	1.28	This study
Xiamen Bay, China	24.44	118.04	0.99	1.20	0.68	0.52	This study
Xiamen Bay, China	24.42	118.16	0.97	0.80	0.62	0.18	This study
Xiamen Bay, China	24.51	118.24	1.04	1.12	0.97	0.15	This study
Xiamen Bay, China	24.57	118.16	1.13	1.12	0.87	0.25	This study
Xiamen Bay, China	24.53	118.09	1.20	1.68	1.04	0.64	This study
Bohai Sea, China	37.39	119.95	1.67	1.62	1.32	0.30	This study
Bohai Sea, China	37.39	119.95	1.59	1.62	1.30	0.32	This study
Bohai Sea, China	37.39	119.94	1.70	1.78	1.40	0.38	This study
Bohai Sea, China	37.39	119.93	1.60	1.58	1.26	0.32	This study
Bohai Sea, China	37.39	119.93	1.55	1.54	1.22	0.32	This study
Bohai Sea, China	37.39	119.94	1.74	1.80	1.44	0.36	This study
Yellow Sea, China	36.37	120.88	1.10	1.18	0.80	0.38	This study
Yellow Sea, China	36.37	120.88	1.15	1.22	0.82	0.40	This study
Yellow Sea, China	36.37	120.88	1.01	1.10	0.74	0.36	This study
Yellow Sea, China	36.37	120.88	1.18	1.27	0.88	0.39	This study
Yellow Sea, China	36.37	120.88	0.96	1.10	0.74	0.36	This study
Yellow Sea, China	36.37	120.88	1.03	1.15	0.76	0.39	This study
Yellow Sea, China	36.37	120.88	1.10	1.18	0.82	0.36	This study
Yellow Sea, China	36.37	120.88	1.06	1.15	0.76	0.39	This study
Mirs Bay, China	22.53	114.48	1.89	2.13	1.64	0.49	*(34)*

Locality	Latitude (°N)	Longitude (°E)	DOC (mg/L)	COD (mg/L)	BOD (mg/L)	COD-BOD (mg/L)	Source
Mirs Bay, China	22.53	114.46	1.29	1.52	1.45	0.07	*(34)*
Mirs Bay, China	22.50	114.48	1.51	1.34	1.20	0.14	*(34)*
Mirs Bay, China	22.53	114.48	1.74	1.68	0.97	0.72	*(34)*
Mirs Bay, China	22.53	114.47	1.67	2.80	2.06	0.74	*(34)*
Mirs Bay, China	22.53	114.46	1.51	1.57	1.47	0.10	*(34)*
Mirs Bay, China	22.50	114.48	1.67	2.66	1.71	0.95	*(34)*
Mirs Bay, China	22.50	114.48	1.51	2.41	1.48	0.93	*(34)*
Mirs Bay, China	22.53	114.48	2.42	2.12	1.36	0.76	*(34)*
Mirs Bay, China	22.53	114.47	2.27	2.90	1.86	1.04	*(34)*
Mirs Bay, China	22.53	114.46	1.36	2.00	1.26	0.74	*(34)*
Mirs Bay, China	22.50	114.47	2.27	3.00	1.84	1.16	*(34)*
Mirs Bay, China	22.50	114.48	1.51	2.17	1.24	0.93	*(34)*
Text Fig. 3							
Ingramport River, Canada	44.67	-63.97					This study
Text Fig. 4							
Biwa Lake, Japan	35.33	136.17					*(20)*
Han River, South Korea	37.48	126.43					*(21, 73)*
Finnish rivers, Finland (1989)	64.00	26.00					*(22)*

Table S2. Sampling information and data corresponding to Fig. S2. The samples were from various lakes on the Qinghai Tibet Plateau, China (this study), and the Jiahe River valley (*35*, Table 2). The data cover a range of salinities from near zero to 43.

Locality	Salinity	COD_{Cr} (mg L^{-1})	COD_{Mn} (mg L^{-1})	COD_{Cr}/COD_{Mn}	Data source
Kala Kuli Lake, China	0.10	5.60	1.10	5.09	This study
Qiangyong Lake, China	0.10	5.20	1.00	5.20	This study
Songmuxi Co, China	0.26	8.30	0.50	16.60	This study
Bangong Co, China	0.46	20.30	2.30	8.83	This study
Namu Co, China	0.99	18.70	1.10	17.00	This study
Darebu Co, China	1.27	22.00	1.80	12.22	This study
Lang Co, China	1.51	30.20	1.90	15.89	This study
Gongzhu Co, China	5.16	164.10	18.00	9.12	This study
Daze Co, China	15.99	45.30	3.20	14.16	This study
Bieruoze Co, China	27.50	630.50	12.60	50.04	This study
Dong Co, China	43.46	906.70	12.20	74.32	This study
Jiahe River valley, China	0.40	24.74	4.00	6.19	*(35)*
Jiahe River valley, China	0.41	8.25	3.26	2.53	*(35)*
Jiahe River valley, China	0.40	11.55	3.37	3.43	*(35)*
Jiahe River valley, China	0.30	28.04	5.18	5.41	*(35)*
Jiahe River valley, China	0.26	18.15	4.97	3.65	*(35)*
Jiahe River valley, China	0.28	24.74	5.20	4.76	*(35)*
Jiahe River valley, China	0.27	9.90	4.97	1.99	*(35)*
Jiahe River valley, China	0.33	16.50	5.29	3.12	*(35)*
Jiahe River valley, China	0.27	26.39	6.04	4.37	*(35)*
Jiahe River valley, China	0.32	21.44	10.82	1.98	*(35)*
Jiahe River valley, China	4.61	51.14	8.82	5.80	*(35)*
Jiahe River valley, China	14.08	493.23	9.26	53.26	*(35)*

Table S3. Comparison of measurements with the alkaline $\mathrm{COD_{Mn}}$ and acidic $\mathrm{COD_{Mn}}$ protocols along a salinity gradient.

Sample Source	Salinity	Alkaline $\mathrm{COD_{Mn}}$ (mg L^{-1})	Acidic $\mathrm{COD_{Mn}}$ (mg L^{-1})	Acid. $\mathrm{COD_{Mn}}$ – Alk. $\mathrm{COD_{Mn}}$ (mg L^{-1})	Data source
Xiamen U. Reservoir, China	0	2.73	2.64	-0.09	This study
Furong Lake, China	0	10.19	11.62	1.43	This study
Xiang’an Mountain Lake, China	0	2.62	4.01	1.39	This study
Xiang‘an Artificial Lake, China	0	6.08	6.26	0.18	This study
Wenqing Lake, China	0	3.78	6.55	2.77	This study
Xiamen botanical Garden Lake, China	0	2.88	5.08	2.20	This study
Tibet Xironggou, China	0	1.61	1.74	0.13	This study
Tibet Lulang, China	0	11.15	11.70	0.55	This study
Jiulong R. Estuary, China	4.20	2.19	4.34	2.15	This study
Xiamen Bay, China	16.50	1.55	5.86	4.31	This study
Xiamen Bay, China	29.40	0.76	17.28	16.52	This study
Sackville River, Canada	0.04	4.82	7.22	2.40	This study
Sackville Estuary, Canada	3.60	4.82	5.52	0.70	This study
Sackville Estuary, Canada	8.50	5.08	6.35	1.27	This study
Sackville Estuary, Canada	15.80	2.78	6.19	3.41	This study
Sackville Estuary, Canada	20.40	2.15	6.28	4.13	This study
Northwest Arm, Canada	30.00	0.52	7.42	6.90	This study
Jiahe River valley, China	0.40	4.00	4.40	0.40	*(35)*
Jiahe River valley, China	0.41	3.26	3.38	0.12	*(35)*
Jiahe River valley, China	0.40	3.37	3.40	0.03	*(35)*
Jiahe River valley, China	0.30	5.18	5.36	0.18	*(35)*
Jiahe River valley, China	0.26	4.97	4.57	-0.40	*(35)*
Jiahe River valley, China	0.28	5.20	4.88	-0.32	*(35)*
Jiahe River valley, China	0.27	4.97	4.80	-0.17	*(35)*
Jiahe River valley, China	0.33	5.29	5.19	-0.10	*(35)*
Jiahe River valley, China	0.27	6.04	6.08	0.04	*(35)*
Jiahe River valley, China	0.32	10.82	8.00	-2.82	*(35)*
Jiahe River valley, China	4.61	8.82	11.00	2.18	*(35)*
Jiahe River valley, China	14.08	9.26	17.80	8.54	*(35)*

Table S4. Examples of the use of the COD index to natural water quality assessment by governmental organizations.

Country or organization	Application	Reference
United Nations Environment Program	Before Aquifer	*(74)*
USA	River	*(75)*
USA	Natural Water	*(76)*
USA-Geological Survey	Stream	*(77)*
USA-Geological Survey	Lake and River	*(78)*
USA-Geological Survey	Before Aquifer	*(79)*
USA	Natural Water	*(80)*
Europe Union	Lake and River	*(81)*
UK	Natural Water and Waste Water	*(82)*
UK	River and Running Surface Water	*(83)*
Netherlands	River	*(84)*
Romania	River and Drinking Water	*(85)*
Ireland	Surface Water	*(86)*
Sweden	Laker	*(87)*
South Africa	All water	*(88)*
China	Lake and River	*(89)*
China	Ocean	*(90)*
China	Reservoir	*(91)*
China	Natural Water	*(92)*
China	Drinking Water	*(93)*
Korea	River	*(73)*
India	Surface Water	*(94)*
Japan	River, Lake and Ocean	*(95)*
Tunisia	All Water	*(96)*
Uruguay	Estuary	*(97)*
Peru	All Water	*(98)*

Table S5. Classification of natural waters by COD standards and practical applications.

Nation	Document	Class	COD Standards	Applications	Practical Examples (reference)
Japan	Environmental Quality Standards for lakes	AA	0~1 mg/L	Drinkable after simple treatment	*(99, 100)*
		A	1~3 mg/L	Drinkable after normal treatment	
		B	3~5 mg/L	For industry after normal treatment	
		C	5~8 mg/L	For industry after specific treatment	
China	Environmental Quality Standard for Surface Water, GB 3838-2002	I	0~15 mg/L	Standard of headwaters	*(89)*
		II	0~15 mg/L	Standard of source of running water (first class), habitats of rare aquatic species	
		III	15~20 mg/L	Standard of source of running water (second class), aquaculture and swimming	
		IV	20~30 mg/L	Standard of industrial water	
		V	30~40 mg/L	For agricultural utilizations	
Malaysia	National Water Quality Standards of Malaysia	I	0~10 mg/L	Water Supply I – Practically no treatment necessary; fishery I – Very sensitive aquatic species	*(101)*
		II	10~25 mg/L	Water Supply II – Conventional treatment required; fishery II – Sensitive aquatic species; recreational use with body contact	
		III	25~50 mg/L	Water Supply III – Extensive treatment required; fishery III – Common, of economic value and tolerant species; livestock drinking water	
		IV	50~100 mg/L	Irrigation	

Nation	Document	Class	COD Standards	Applications	Practical Examples (reference)
		V	more than100 mg/L	None of the above	