Supporting information

17 pages, 14 Figures and 1 table

Siderophores in cloud waters and potential impact on atmospheric chemistry: Photoreactivity of iron complexes under sun-simulated conditions

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Text S1 Nitrobenzene detection

The degradation of nitrobenzene was monitored by an HPLC system (Waters Alliance) equipped with a diode array detector (set at 210 nm) and a C18 Zorbax column (Agilent, 15 cm x 4.6 mm, 5 µm). A mixture of 50% water and 50% acetonitrile was used as the mobile phase at a constant flow rate of 1.0 mL min^{-1}.

The pseudo-first order decay of NB in the presence of 100 µM of Fe(III)-Pyo at pH 4.0 and 6.0 is showed in the following figure S1

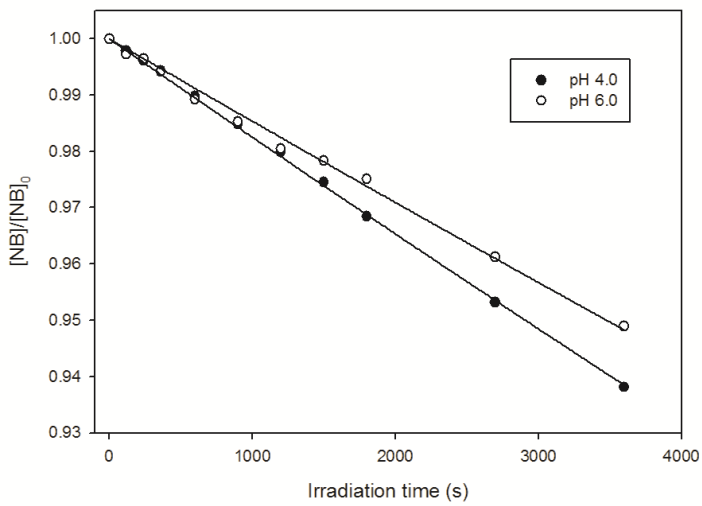

Figure S1: degradation of NB vs irradiation time in the presence of Fe(III)-Pyo 100 µM under polychromatic irradiations

The formation rate of hydroxyl radical ($R^{f}_{HO^{\bullet}}$) can be estimated using a kinetic in which competition between NB and Fe(III)-Pyo to trap hydroxyl radical is considered:

R1) $Fe(III)-Pyo \xrightarrow{h\nu} \rightarrow\rightarrow HO^{\bullet}$

R2) $HO^{\bullet} + NB \rightarrow NB_{ox}$

R3) $HO^{\bullet} + Fe(III)-Pyo \rightarrow products$

R1 summarise the complex mechanisms discussed in the text leading to the formation of $HO^{\bullet}$, R2) shows the reaction between $HO^{\bullet}$ and NB considering the second order rate constant ($k_{HO^{\bullet},NB}$) of 3.9×10^{9} M^{-1} s^{-1} and [NB] = 300 $\times10^{-6}$ M; R3) the reactivity of $HO^{\bullet}$ with Fe(III)-Pyo where for $k_{HO^{\bullet},Fe(III)-Pyo}$ it is reasonable to consider the value of 1.0 × 10^{10} M^{-1} s^{-1} and [Fe(III)-Pyo] = 100 $\times10^{-6}$ M.

It is then possible to calculate the percentage of HO• trapped by NB ($T_{HO,\,NB}$) as:

$$T_{HO,\,NB} = \frac{k'_{NB}}{k'_{NB} + k'_{Fe(III)-Pyo}} \times 100 = 54\%$$

The formation rate of HO ($R^{f}_{HO\bullet}$) can be then estimated considering that 54 % of hydroxyl radical reacts with NB:

$$R^{f}_{HO\bullet} = \frac{R^{d}_{NB}}{0.54}$$

$R^{f}_{HO\bullet}$ vs irradiation time for pH 4.0 and 6.0 is then plotted in the Figure S2

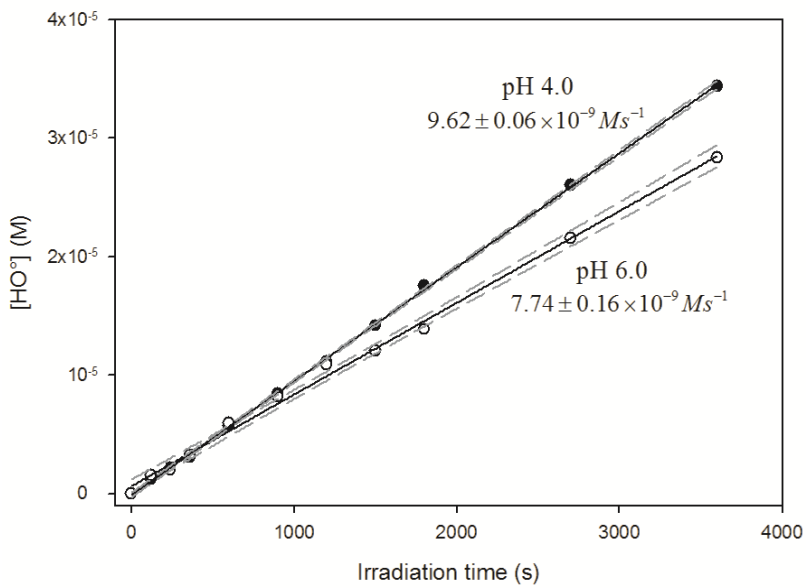

Figure S2: Concentration of photogenerated hydroxyl radical *vs* irradiation time in the presence of Fe(III)-Pyo 100 µM at pH 4.0 and 6.0.

Text S2 Formation rate and Quantum yield calculation

The time evolution of Fe(III)-Pyo data were fitted with a pseudo-first order equation $C_t = C_0 \times e^{-kt}$ where C_0 and C_t are the initial concentration of Fe(III)-Pyo and concentration at time *t* respectively and *k* the firt-order rate constant of Fe(III)-Pyo transformation. In this case the Fe(III)-Pyo degradation rate ($R^{d}_{Fe(III)\text{-}Pyo}$) is equal to $k \times C_0$.

The initial rates of Fe(II) formations were determined by fitting the experimental data with a linear equation $[Fe(II)] = R^{f}_{Fe(II)} \times t_{irr}$ considering the first 20 min of irradiation.

The quantum yield of photochemical reaction is defined as the ratio between molecules transformation and number of absorbed photons in the same period. This value gives an evaluation of the photochemical process efficiency independent on the experimental photochemical conditions.

In our experiments we assumed that Fe(III)-Pyo is the only absorbing specie present in water and the polychromatic quantum yield formation of Fe(II) ($\phi_{Fe(II)}$) can be estimated in the overlap range 290-600 (λ_1 and λ_2) by (eq S1).

$$\phi_{Fe(II)} = \frac{R^{f}_{Fe(II)}}{I_a} \qquad \text{eq S1}$$

where $R^{f}_{Fe(II)}$ is the Fe(II) formation rate (M s^{-1}) and I_a is the absorbed photon flux per unit of surface and unit of time in the overlap range 290-600 (λ_1 and λ_2) by Fe(III)-Pyo. The latter was calculated from eq S2:

$$I_a = \int_{\lambda_1}^{\lambda_2} I_0(\lambda)\,(1-10^{-\varepsilon(\lambda) l [Fe(III)-Pyo]})\,d\lambda \quad \text{eq S2}$$

Where I_0 is the incident photon flux, ε the molar absorption coefficient of Fe(III)-Pyo, l the optical path length inside the cells and [Fe(III)-Pyo] the initial Fe(III)-Pyo concentration.

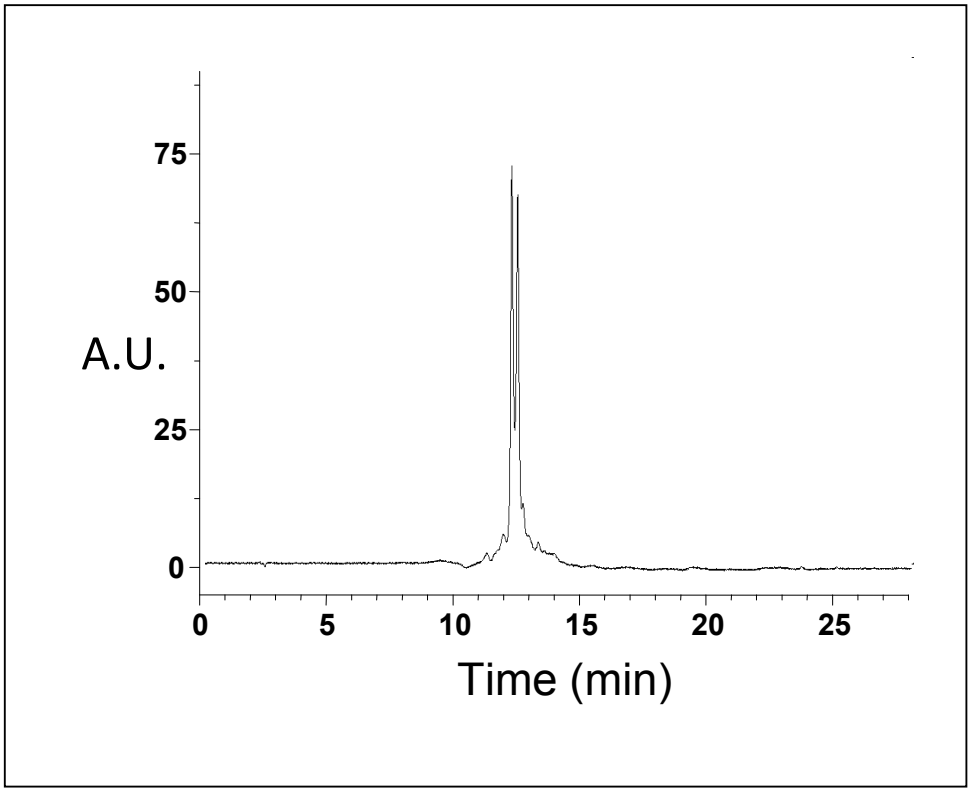

Figure S3: HPLC chromatogram of the purified pyoverdins obtained from *Pseudomonas fluorescens*36b5 culture.

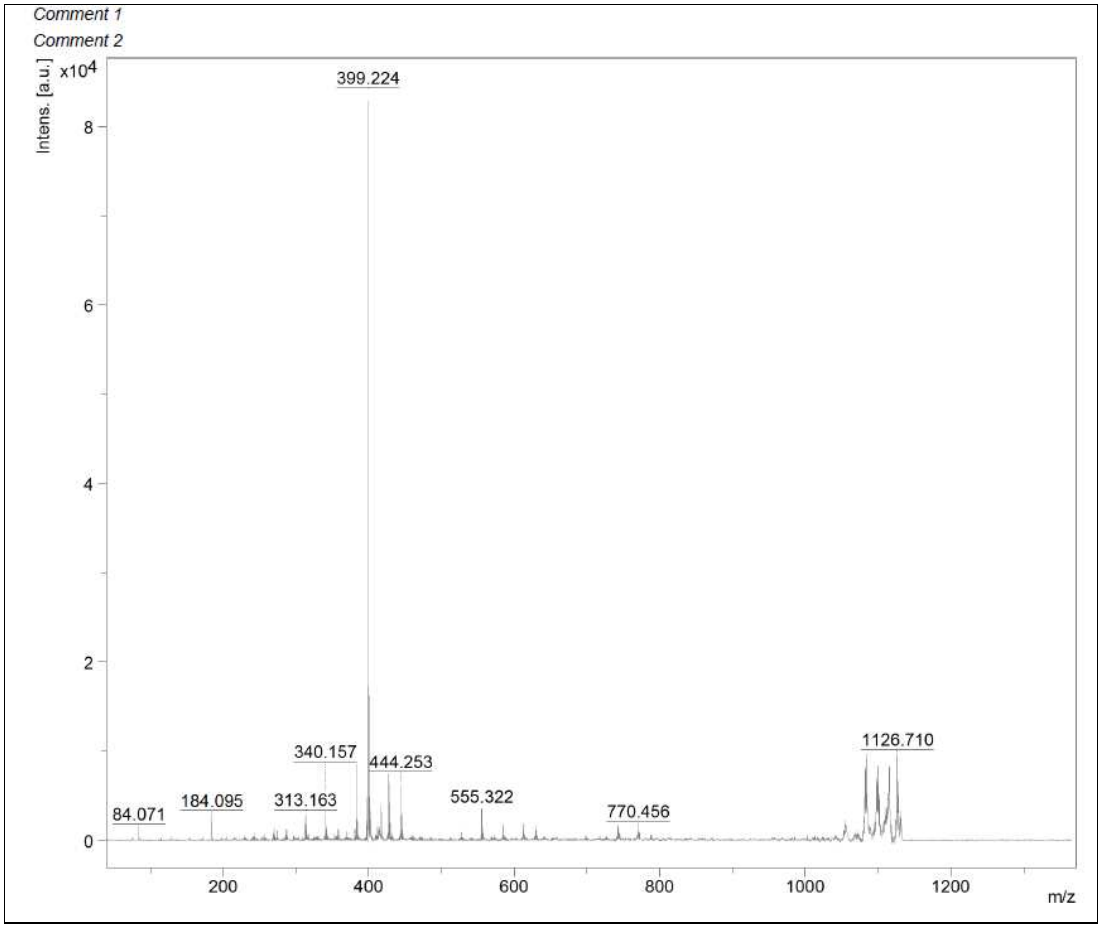

Figure S4: Lift (MS/MS) spectrum of the major form of pyoverdin (m/z 1143.607).

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m/z	Intensity	Attribution
84.071	1578	Lys1
129.113	480	Lys 1
184.095	3187	Y_2-H_2O-NH_3
204.104	567	Chromophore
256.147	753	Y_3 -H_2O -McLafferty
274.128	1130	Y_3-McLafferty
286.143	1441	Chromophore
313.163	3212	Y_3-NH_3-H_2O
399.224	84122	A_1-H_2O
417.223	3942	A_1
555.322	3776	B_2-H_2O-CH_3COOH
770.456	1985	Y_7-NH_3-H_2O
1069.62	1247	$[M+H]^+$-Mc lafferty
1082.743	9722	$[M+H]^+$-NH_3-CO_2
1088.811	1625	$[M+H]^+$-$NOHCOCH_3$+H_2O
1100.902	5324	$[M+H]^+$-NH_3-CO_2+H_2O
1126.71	9873	$[M+H]^+$-NH_3

95 **Table S1:** Structure of the relevant ions obtained after fragmentation of the major form of pyoverdin
96 (m/z 1143.607).

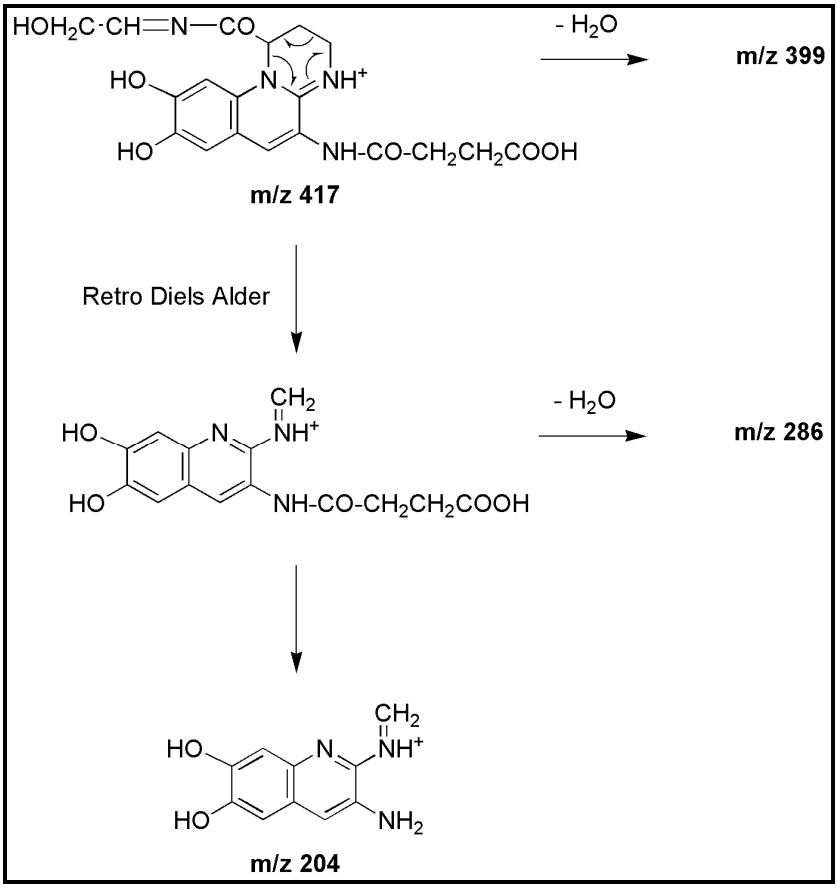


Figure S5: MS ions obtained after fragmentation of the chromophore.

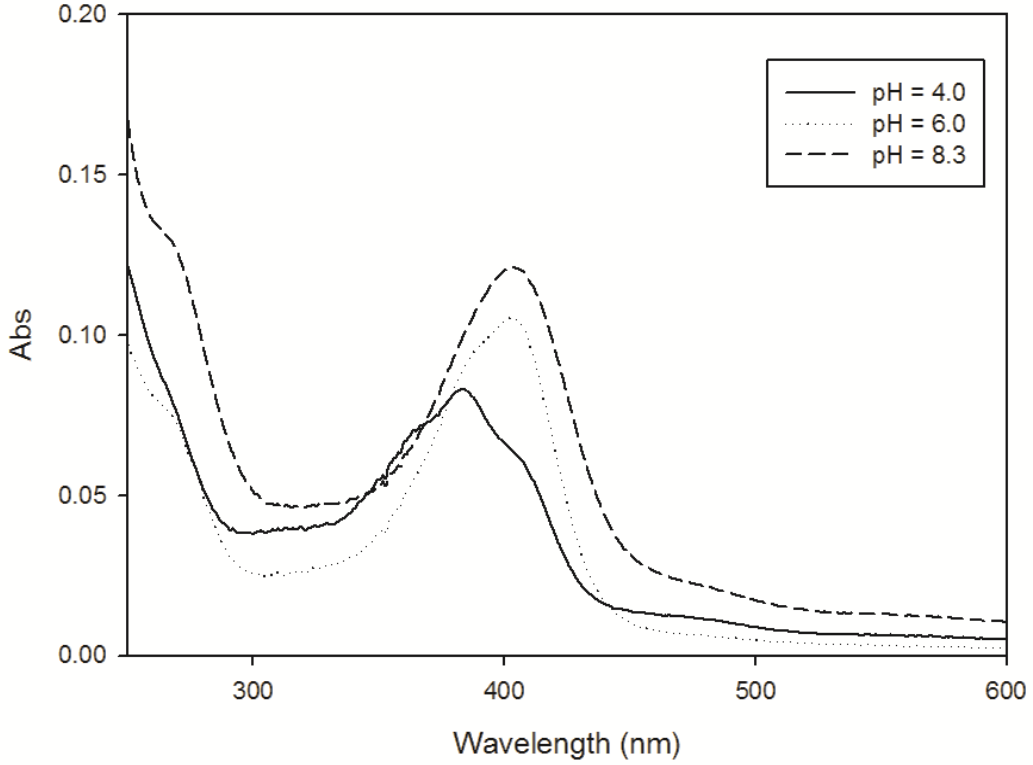


Figure S6. UV-vis absorption spectra of 100 µM pyoverdin solution in water at pH 4.3, 6.0 and 8.3 (natural pH).

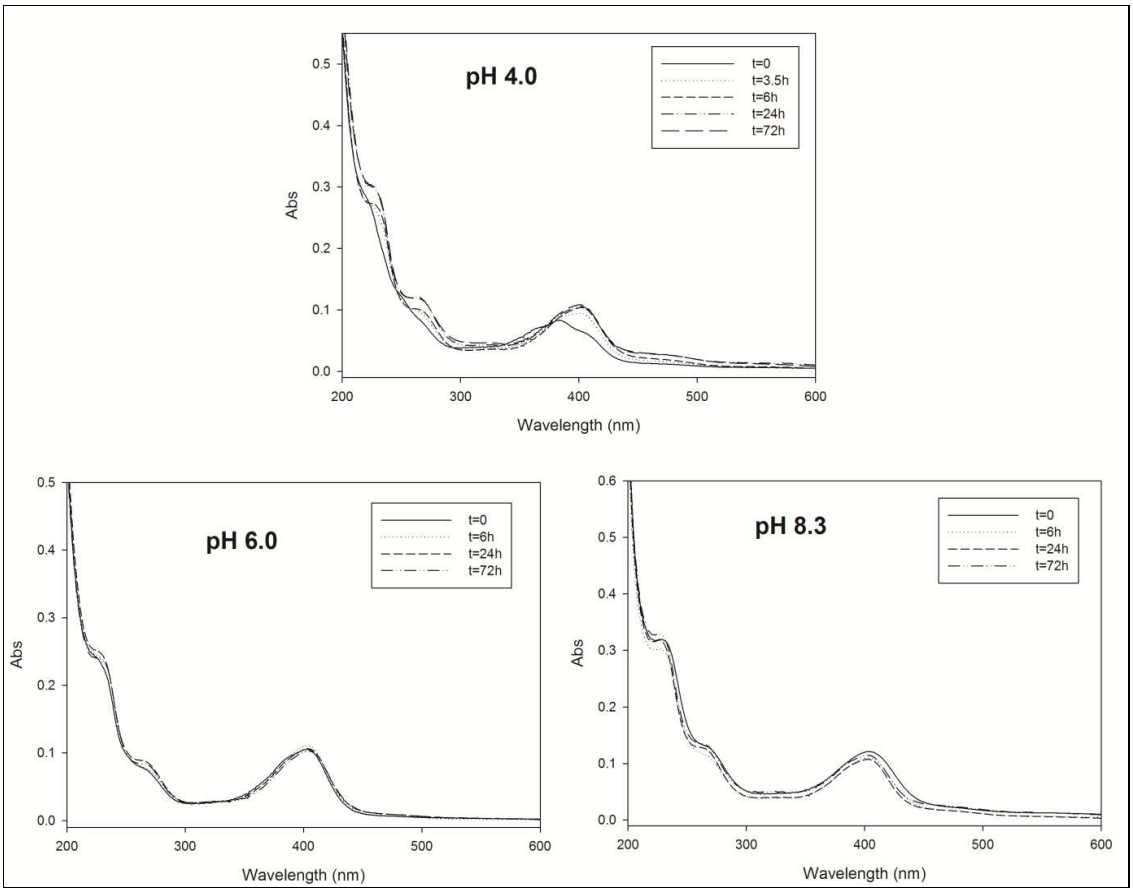
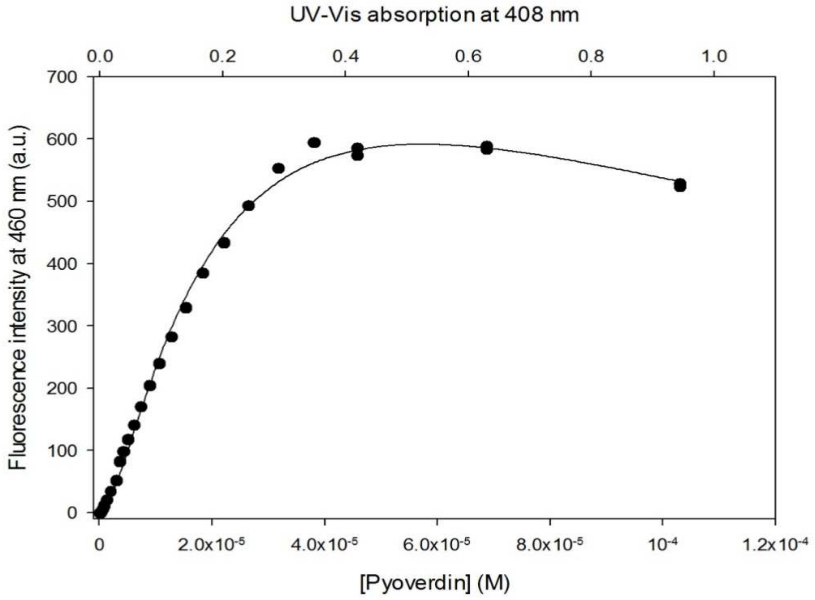


Figure S7. UV-vis evolution profile of Pyo100 μM at pH 4.0, 6.0 and 8.3 in dark condition and 283K.



108 **Figure S8.** Correlation between pyoverdin concentration, UV-vis absorption at 408 nm (excitation

109 wavelength) and fluorescence emission (λ_{em}) intensity at 460 nm for an excitation λ_{ex} = 408 nm.

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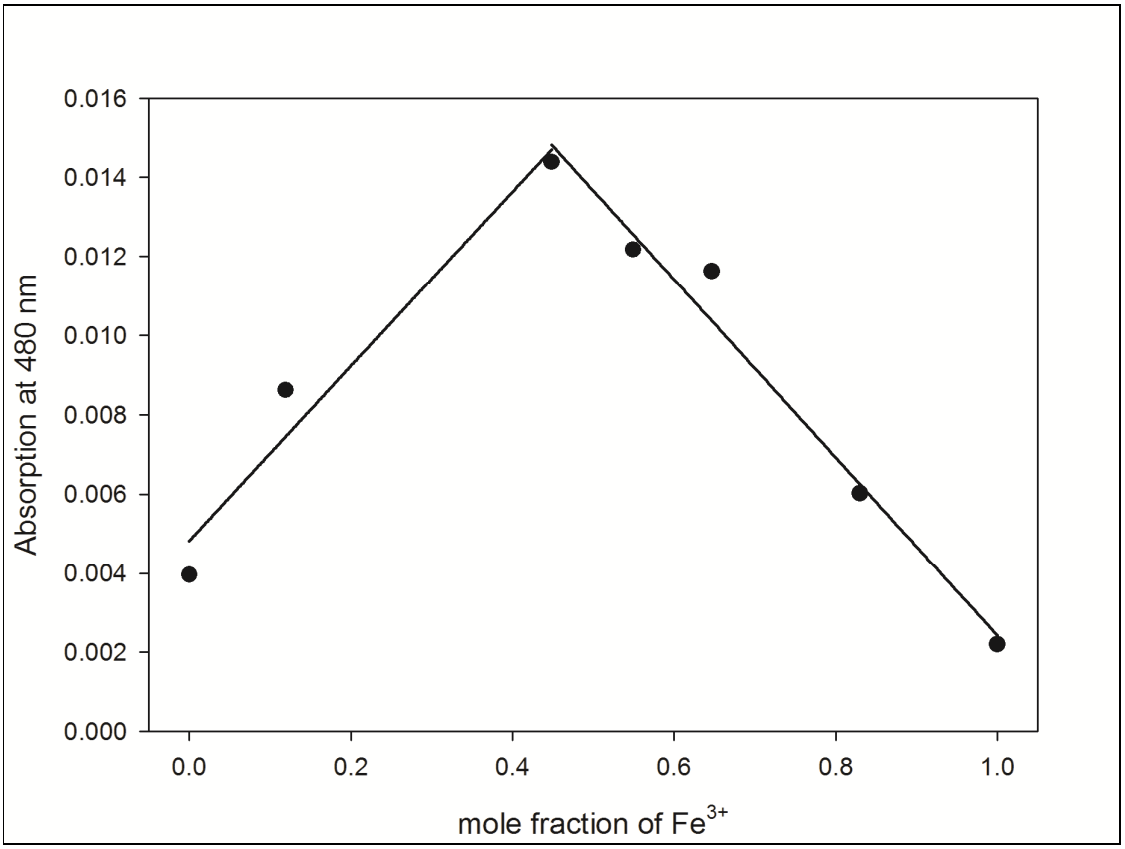

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112 **Figure S9.** Assessment of Fe(III)-Pyo complex. Figure shows the standard Job's method at pH 4.0.

113 Absorbance read at λ = 480 nm.

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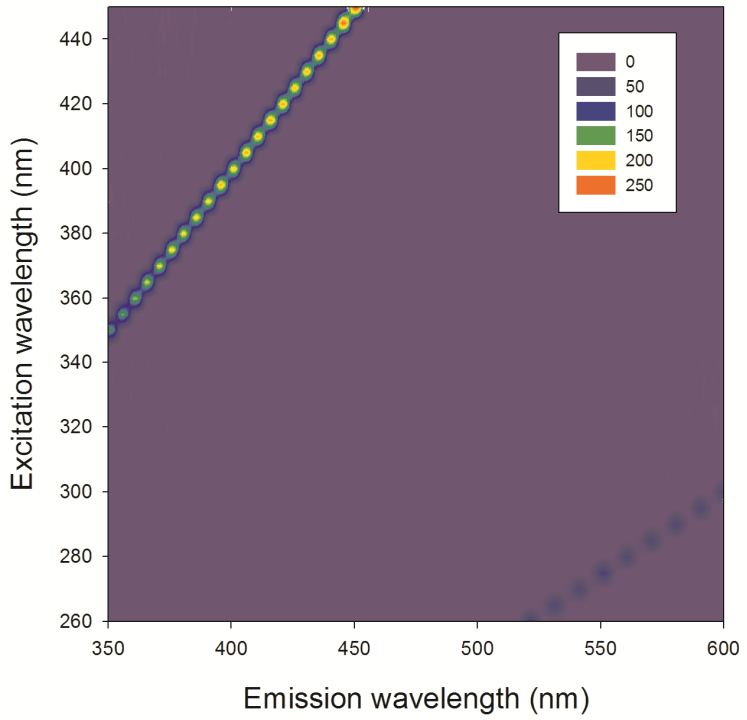

Figure S10: Fluorescence excitation-emission matrix (EEM) as contour plot of 10 μM Fe(III)-pyoverdin complex solution in water at pH 4.0.

Text S3 Fe-oxalate complexes

The Fe(III)-oxalate complex $Fe(C_2O_4)_2^-$ was prepared by mixing Fe(III) perchlorate (100 μM) with potassium oxalate (200 μM) and fixing the pH at 2.5 (Figure S11). A mixture of Fe(III)-oxalate complexes $Fe(C_2O_4)_2^-/Fe(C_2O_4)_3^{3-}$ 50/50% was prepared by mixing Fe(III) perchlorate (100 μM) with potassium oxalate (250 μM) and fixing the pH at 3.8 (Figure S12).

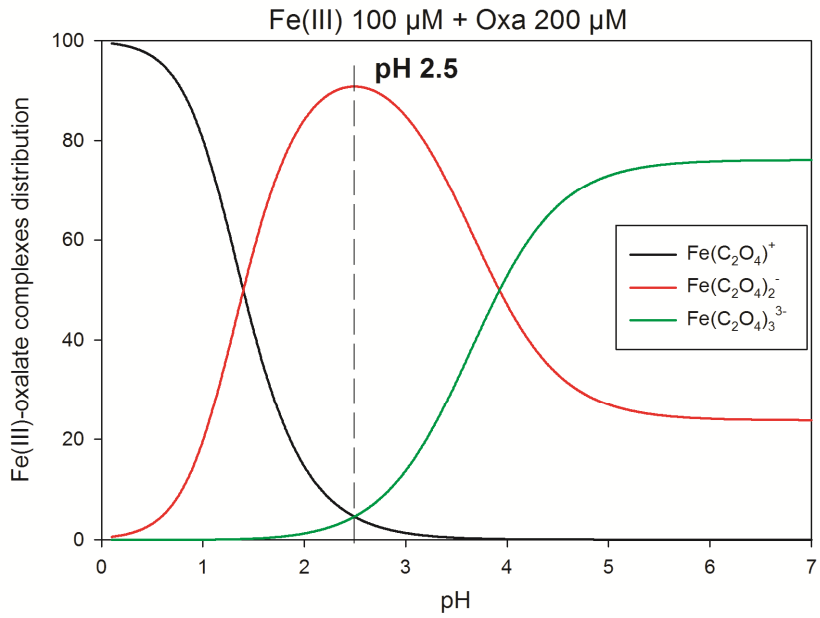

Figure S11: Fe(III) oxalic acid complex ([Fe(III)] = 100 µM, [oxalic acid] = 200 µM) speciation as a function of the pH.

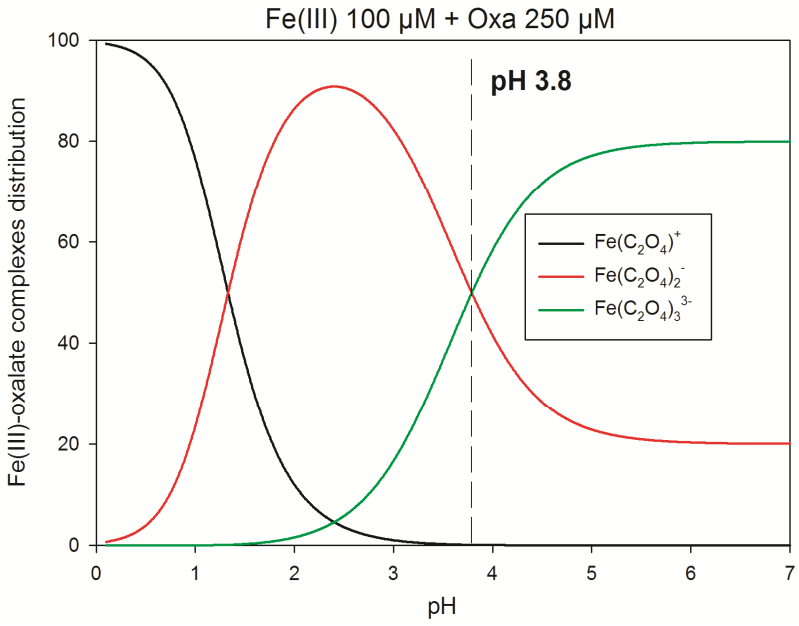

Figure S12: Fe(III) oxalic acid complex ([Fe(III)] = 100 µM, [oxalic acid] = 250 µM) speciation as a function of the pH.

Following parameters are used to determine iron-oxalato complexes speciation:

For oxalate Ka are 5.6×10^{-2} and 5.42×10^{-5} (corresponding to the first and second protonation) while stability constant of Fe(III)-oxalato complexes where 2.53×10^{9}, 6.3×10^{6}, 1.6×10^{4} respectively for $Fe(C_2O_4)^{+}$, $Fe(C_2O_4)_2^{-}$ and $Fe(C_2O_4)_3^{3-}$

Text S4 Competition between Fe(III)-Pyo and Fe(III)-Ox_2 complex formation

At pH 4, in the presence of oxalate (Ox) and pyoverdin (Pyo), Fe(III) can form complexes with both compounds following equations S3 and S42:

$Fe^{3+} + 2Ox^{-} \rightarrow (Fe(III)\text{-}Ox_2)^{-}$ (eq. S3)

$Fe^{3+} + Pyo^{2-} \rightarrow (Fe(III)\text{-}Pyo)^{+}$ (reported in this paper as Fe(III)-Pyo) (eq. S4)

The corresponding stability constants are described as follow:

$$K_{Fe(III)-Ox_2^-} = \frac{[Fe(III)-Ox_2^-]}{[Fe(III)]\times[Ox]^2} = 10^{16.2} \qquad \text{(eq. S5)}$$

$$K_{Fe(III)-Pyo} = \frac{[Fe(III)-Pyo]}{[Fe(III)]\times[Pyo]} = 10^{20} \qquad \text{(eq. S6)}$$

where $[Fe^{III}]$ is the free Fe(III) concentration, $[Ox]$ is the oxalate concentration, $[Pyo]$ is the pyoverdin concentration, $[Fe(III)-Ox_2^-]$ is the Fe(III)-oxalate complex concentration and $[Fe(III)-Pyo]$ is the Fe(III)-Pyo complex concentration, all of these being concentrations at equilibrium.

The free Fe(III) concentration at equilibrium can be obtained by the following equation:

$$[Fe(III)]_{eq} = \frac{[Fe(III)-Ox_2^-]_{eq}}{K_{Fe(III)-Ox_2^-}\times[Ox]_{eq}^2} = \frac{[Fe(III)-Pyo)]_{eq}}{K_{Fe(III)-Pyo}\times[Pyo]_{eq}}$$

In cloud water, the oxalate concentration is generally higher than the Fe(III) concentration. Therefore, the equilibrium concentration of free iron, $[Fe(III)]_{eq}$ is expected to be zero. To fulfil the condition of mass balance, the sum of $[Fe(III)-Pyo)]_{eq}$ and $[Fe(III)-Ox_2^-]_{eq}$ will be equal to the initial free Fe(III) concentration ($[Fe(III)]_i$):

$$[Fe(III)]_i = [Fe(III)-Ox_2^-]_{eq} + [Fe(III)-Pyo)]_{eq}$$

Considering the previous approximation, we can obtain the following system:

$$\begin{cases} [Fe(III)]_i = [Fe(III)-Ox_2^-]_{eq} + [Fe(III)-Pyo)]_{eq} \\ \dfrac{[Fe(III)-Ox_2^-]_{eq}}{K_{Fe(III)-Ox_2} \times [Ox]_{eq}^2} = \dfrac{[Fe(III)-Pyo)]_{eq}}{K_{Fe(III)-Pyo} \times [Pyo]_{eq}} \end{cases}$$

The only unknown values are $[Fe(III)-Pyo)]_{eq}$ and $[Fe(III)-Ox_2^-]_{eq}$. For better, the concentrations and the equilibrium constants are expressed as follows:

$Fe(III)]_i$ =m

$[Ox]_i$ =q

$[Pyo]_i$ =n

$K_{Fe(III)-Ox_2}$ =a

$K_{Fe(III)-Pyo}$ =b

$[Fe(III)-Ox_2^-]_{eq}$ =x

$[Fe(III)-Pyo)]_{eq}$ =y

where $[Ox]_i$ and $[Pyo]_i$ are the initial concentration of oxalate and pyoverdin respectively.

The simplified systems will be:

$$\begin{cases} m = x + y \\ \dfrac{x}{a(q-2x)^2} = \dfrac{y}{b(n-y)} \end{cases}$$

The solution of these simultaneous equations yields the concentrations of Fe(III)-Pyo and Fe(III)-Ox_2 complexes in different conditions (different Pyo, Ox and Fe concentrations).

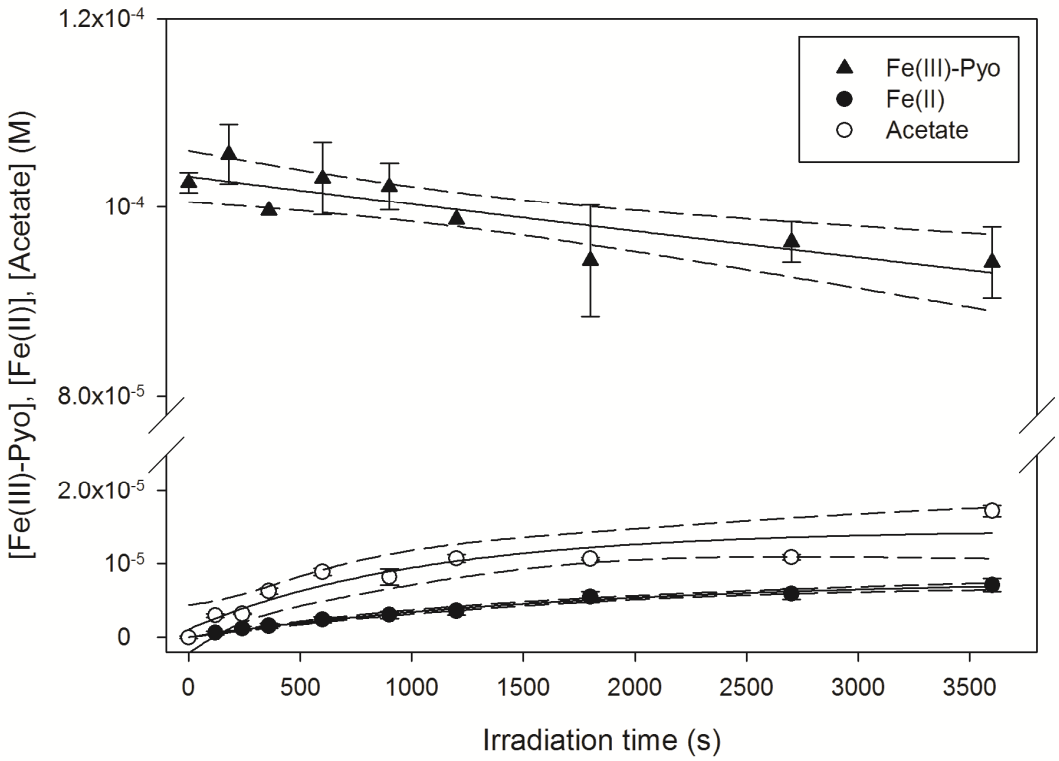

Figure S13. Fe(III)-Pyo degradation, Fe(II) and acetate formation profiles during polychromatic irradiation at pH 6.0. Experiments were performed at 278 ± 2 K. The solid line is the fit of experimental data using an exponential decay equation for Fe(III)-Pyo and an exponential rise to a maximum value equation for Fe(II) and acetate, dashed lines denote the 95% confidence of the fit.

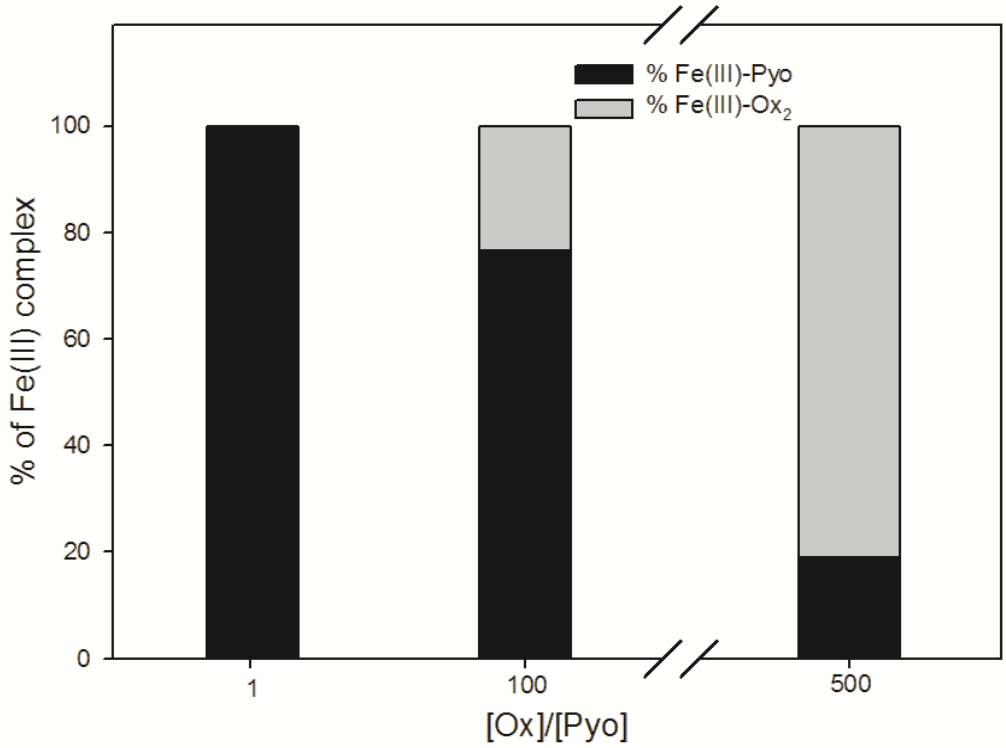

Figure S14. Fe(III) complex at varied Oxalate/Pyoverdin ratios ([Ox]/[Pyo]) considering an initial concentration of Fe(III) of 2μM and Pyo of 2 μM at pH 4.0.