Journal of Environmental Management 398 (2026) 128569

Contents lists available at ScienceDirect

Journal of Environmental Management

journal homepage: www.elsevier.com/locate/jenvman

Research article

Ecotoxicological assessment of wastewater treated by the novel solar chlor-photo-Fenton process for sustainable crop irrigation

S. Belachqer-El Attar [a,b], P. Taborelli [c], P. Soriano-Molina [a,b], P. Roslev [c,*], J.A. Sánchez Pérez [a,b,**]

[a] Solar Energy Research Centre (CIESOL), Joint Centre University of Almería-CIEMAT, Almería, Spain
[b] Chemical Engineering Department, University of Almería, Almería, Spain
[c] Department of Chemistry and Bioscience, Section of Bioresources and Process Engineering, Fredrik Bajers Vej 7H, Aalborg University, Aalborg, Denmark

ARTICLE INFO

Keywords:
Quaternary wastewater treatment
Photo-Fenton
Photooxidation
Ecotoxicity
AOPs

ABSTRACT

Potential ecotoxicity of the solar chlor-photo-Fenton (SCPF) process as a novel approach for wastewater reclamation has been investigated in relation to reuse for crop irrigation. Several secondary effluents from wastewater treatment plants across different geolocations were treated by applying the process with low reagent concentrations of 0.1 mM of ferric nitrilotriacetate (Fe^{3+}-NTA), 0.73 mM of hydrogen peroxide, at different chlorine doses ranging from 0.13 to 0.40 mM. Chlorination, using comparable chlorine concentrations, and the solar photo-Fenton process were evaluated in parallel to compare with SCPF. A UVB-LED system was also used to perform the SCPF treatment, demonstrating its adaptability to both solar and artificial light-driven applications. Potential environmental toxicity was assessed using a battery of bioassays, including bacterial toxicity and genotoxicity, phytotoxicity in algae and plants, and toxicity toward mixed microbial communities (microrespirometry). Water treated with SCPF exhibited minimal bacterial and algal toxicity (<13 %), low phytotoxicity as assessed by plant germination (35 % at high chlorine concentration) and shoot/root elongation (22 % and –30 %), low toxicity toward mixed microbial communities (<30 %), and low apparent levels of genotoxicity. In comparison, chlorination yielded higher ecotoxic responses across the bioassays, suggesting that SCPF may mitigate these adverse effects. Although Fe^{3+}-NTA dissolution showed toxicity its ecotoxic impact was markedly reduced at the operational concentration of 0.1 mM used in SCPF. These results indicate that chlor-photo-Fenton can produce treated water with low apparent ecotoxicity, supporting its potential as an environmentally friendly and scalable solution for wastewater treatment and reuse.

1. Introduction

The increasing need for alternative water sources to address water scarcity highlights the importance of wastewater reclamation, particularly in the Mediterranean region of Europe. To meet this demand, it is essential to adhere to applicable policies, including the EU Regulation on water reuse (2020/741) and the Urban Wastewater Treatment Directive (2024/3019) (EU 2020/741, 25 May; EU, 2024/3019, 27 November), which both impose strict requirements regarding disinfection and outline the management of microcontaminants. As a result, there is a need to investigate treatment technologies that can satisfy these obligations by effectively removing microcontaminants, while also minimizing the formation of disinfection by-products (DBPs). To address these stringent requirements, some researchers have focused on advanced oxidation processes (AOPs), in particular the solar photo-Fenton process, which has been identified as one of the most effective. Research on this oxidative process has progressed significantly, with studies reaching demonstration scale (Gualda-Alonso et al., 2022; Rizzo, 2022). Chelating agents such as ethylenediamine-N, N-disuccinic acid (EDDS), ethylenediaminetetraacetic acid (EDTA), and nitrilotriacetic acid (NTA) have been proposed as potential candidates for such application (Ahile et al., 2021; De Luca et al., 2014). However, NTA has emerged as the preferred choice due to practical and environmental considerations. EDDS is significantly more expensive than the other agents, while EDTA raises concerns due to its potential toxicity, contribution to eutrophication in aquatic systems, and

* Corresponding author. Aalborg University, Denmark.
** Corresponding author. Solar Energy Research Centre (CIESOL), Joint Centre University of Almería-CIEMAT, Almería, Spain.
E-mail addresses: pr@bio.aau.dk (P. Roslev), jsanchez@ual.es (J.A. Sánchez Pérez).

https://doi.org/10.1016/j.jenvman.2026.128569
Received 8 November 2025; Received in revised form 24 December 2025; Accepted 4 January 2026
Available online 10 January 2026

environmental persistence stemming from its recalcitrant and poorly biodegradable nature (De Luca et al., 2014; Prete et al., 2021). Importantly, the evaluation of toxicity associated with iron chelates is critical when selecting agents for environmental applications. In this regard, the solar photo-Fenton process, mediated by the ferric nitrilotriacetate complex (Fe^{3+}–NTA), has demonstrated high efficiency as an AOP. Nevertheless, there is still a need to enhance disinfection efficiency, as long treatment duration poses bottlenecks for industrial-scale adoption. Scaling up the process is technically challenging due to prolonged reaction times, which reduce treatment capacity and conflict with the rapid throughput required for industrial/municipal wastewater systems (thousands of liters/hours), compared to mature technologies like ozonation or UV disinfection. Hence, an upgraded enhanced version based on the combination of solar photo-Fenton and chlorination, called solar chlor-photo-Fenton (SCPF), was introduced, successfully achieving its targets (Belachqer-El Attar et al., 2022), and subsequently registered as an utility model (N° 1,310,873) (Sánchez Pérez et al., 2024).

SCPF involves the concurrent use of hydrogen peroxide (H_2O_2) and sodium hypochlorite (NaOCl), as oxidants, with Fe^{3+}-NTA serving as the iron source (Fig. 1). Briefly, Fe^{3+}-NTA, in the presence of H_2O_2, forms Fe^{2+}-NTA and superoxide radicals ($O_2^{\bullet-}$) (Reaction 1). Fe^{2+}-NTA then reacts with H_2O_2 (Fenton reaction, Reaction 2) to regenerate hydroxyl radicals ($HO^{\bullet}$). Under sunlight, Fe^{3+}-NTA is photoactivated (Reaction 3) resulting in a fraction that photoreduces to Fe^{2+}, producing $HO^{\bullet}$ (Reaction 4), while the other fraction returns to its initial state, releasing heat (Q) (Reaction 5). The photo-reduced iron may remain as Fe^{2+}-NTA before yielding Fe^{2+} and NTA (Reaction 6), with the Fe^{2+} produced reacting with H_2O_2 via Fenton reaction (Reaction 7), forming $HO^{\bullet}$ and Fe^{3+}, which precipitates as ferric hydroxide ($Fe(OH)_3$) and $HO^{\bullet}$. Finally, free chlorine ($HOCl/OCl^-$) reacts with Fe^{2+} to yield $HO^{\bullet}$ and chlorine radicals ($Cl^{\bullet}$) are generated, enhancing disinfection (Belachqer-El Attar et al., 2023).

The SCPF treatment enables improved disinfection efficiency and the removal of microcontaminants while minimizing DBP generation, producing Class B treated water with up to 80 % of organic microcontaminants removed under mild oxidation conditions (Belachqer-El Attar et al., 2023; Pichel et al., 2023). Producing Class A reclaimed water is also possible by adjusting the oxidant concentrations, particularly the chlorine dose, although this also depends on the secondary effluent quality. It has the potential to mitigate the formation of trihalomethanes (THMs) and haloacetic acids (HAAs) (Belachqer-El Attar et al., 2025a), achieving concentrations well below the maximum levels of 30 μg/L and 60 μg/L for THMs and HAAs, based on the guidelines of the water reuse in Italy and drinking water in Spain, respectively (Italian Decree Article 99 n.152 and April 3, 2006; Royal Decree 3/23 and January 10). This is primarily due to the synergistic action of sunlight-driven photolysis and $HO^{\bullet}$-oxidation (Belachqer-El Attar et al., 2025a). In comparison with conventional treatments, ozonation and chlorination are effective, but they produce toxic DBPs like THMs and bromate, raising environmental concerns. UV radiation is eco-friendly but is limited to water with adequate transmittance and lacks residual protection, whereas peracetic acid (PAA) is biodegradable, but costlier and less effective against certain pollutants (Fatta-Kassinos et al., 2016). The results obtained at both laboratory and pilot scale encouraged implementing the SCPF process in a demonstration-scale raceway pond reactor plant. Accordingly, a SCPF plant was installed in a rural wastewater treatment plant (WWTP), demonstrating its resilience and robustness in treating rural sewage under real-world operational conditions (Belachqer-El Attar et al., 2025b). The system achieved over 60 % removal of organic microcontaminants while producing Class B reclaimed water under mild oxidation conditions with negligible DBP levels, in line with regulatory standards for non-potable water reuse applications. Additionally, there is room to increase the chlorine dose to produce the highest water quality (Class A) without compromising DBP levels (Belachqer-El Attar et al., 2025b). However, chemical and biological safety of water treated by the SCPF process must be thoroughly assessed before its application in agricultural irrigation. Some studies also suggest that the chelating agent, NTA, might be genotoxic and its resulting complex, Fe^{3+}-NTA, could also pose similar risks (European Commission, 2010; Nixon, 1971).

NTA raised concerns about toxicity under Annex I of the Cosmetics Directive 67/548/EEC. Notably, this classification is based on findings from animal studies, with limited evidence of genotoxic effects in human cells and no observed mutagenicity in bacterial assays (Regulation (EC)). Additionally, chlorine-based disinfection processes, widely used in wastewater reclamation, remain understudied in terms of DBP formation but also their toxicological impacts (Sánchez-Montes et al., 2023). Numerous studies have investigated the toxicity of traditional solar photo-Fenton processes conducted at acidic pH and using as an iron source the ethylenediamine-N,N′-disuccinic acid (EDDS), consistently reporting no significant toxicity. Moreover, these processes have demonstrated the capacity to reduce untreated effluent toxicity (Rivas et al., 2017; Maniakova et al., 2022; Freitas et al., 2017; Rueda-Márquez et al., 2020; Starling et al., 2017). However, to the best of our knowledge, no studies have yet assessed the potential toxicity of Fe^{3+}-NTA in the context of water reclamation using solar-driven processes such as SCPF or even conventional solar photo-Fenton. This gap in literature underscores the urgent need for further research to address this critical aspect and ensure the safety and sustainability of these advanced treatments and highlights the critical need for ecotoxicological evaluation of emerging treatment technologies to assess potential biological effects and minimize any harmful environmental and public health impacts.

The current study evaluated the environmental toxicity of wastewater treated by the SCPF process to assess its suitability for reuse in agriculture with a particular focus on the role of Fe^{3+}-NTA as the iron source. Such assessments are critical for advancing the large-scale application of SCPF in field of wastewater treatment. To this end, a series of bioassays were conducted to assess the toxicological potential of the wastewater treated from several municipal and rural WWTPs. These

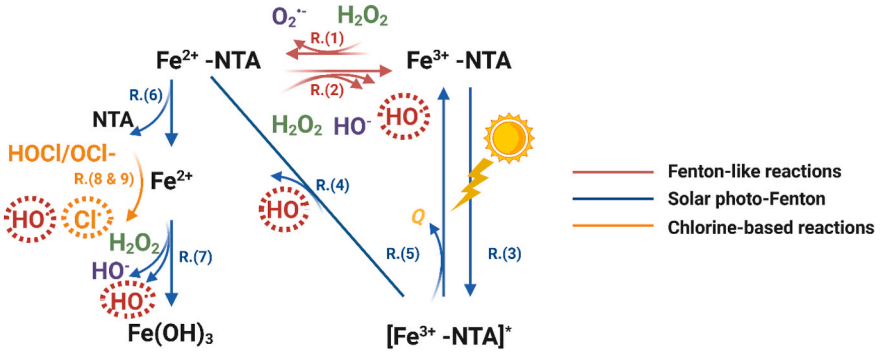

Fig. 1. Scheme of the main reactions involved in the SCPF process. Adapted from (Pichel et al., 2023).

bioassays included evaluations of microbial toxicity, phytotoxicity, and genotoxicity, providing a comprehensive framework for assessing potential environmental impacts.

2. Materials and methods

2.1. Chemical compounds

H_2O_2 (33 %, w/v) and ferric sulfate hydrate ($Fe_2(SO_4)_3{\cdot}H_2O$, 75 %, w/w) were supplied by Panreac (Barcelona, Spain). NTA (>99 %, w/w) and NaOCl (10 %, w/v) were provided by Sigma Aldrich (Steinheim, Germany). Sodium hydroxide (NaOH) was purchased from J.T Baker (Deventer, Holland). The Fe^{3+}-NTA preparation procedure is reported in detail elsewhere (Belachqer-El Attar et al., 2025b). Titanium (IV) oxysulfate solution ($TiOSO_4$,1.9 %, w/v), ortho-phenanthroline ($C_{12}H_8N_2$, 99 %, w/w), ascorbic acid ($C_6H_8O_6$,99 %, w/w) and sulfuric acid (H_2SO_4, 96 %, w/v), ammonium acetate (CH_3COONH_4, 96 %, w/w), and acetic acid glacial (CH_3COOH,> 99 %, w/v) were obtained from Panreac. DPD N° 1 and DPD N° 3 tablets for the determination of chlorine concentration were purchased from Lovibond® Water testing. Milli-Q ultrapure water was obtained from a Direct-Q Ultrapure Water System from Millipore® (Burlington, USA) (18.2-MΩ/cm resistance and 3-μg/L dissolved organic carbon (DOC). UHPLC grade acetonitrile and methanol (99 %, w/v), acetic acid glacial (CH_3CO_2H, 99 % w/v), ortho-phenanthroline (99 %, w/w), formic acid (98 %, w/v) and bovine liver catalase enzyme (0.1 g/L), were supplied by Panreac and Sigma Aldrich (Steinheim, Germany).

As for the toxicity bioassays, zinc sulfate monohydrate ($Z_nSO_4 \cdot H_2O$, 99 %, w/w), sodium sulphite (Na_2SO_3, 99 % w/w), L-histidine (99 %, w/w), D-biotin (99 %, w/w), ampicillin (>99 %, w/w), aminoantracene (2-AA, 96 %, w/w)), 4-nitroquinoline-N-oxide (4-NQO, ≥98 %, w/w), and boric acid (98 %, w/v) were acquired from Sigma Aldrich (Copenhagen, Denmark).

2.2. Analysis of wastewater composition

Actual secondary effluents were collected from urban and rural WWTPs, El Toyo and Uleila del Campo (Almería, Spain), respectively, and from Aalborg West WWTP (Aalborg, Denmark) for the validation experiment. Detailed information on Spanish WWTPs has been reported elsewhere (Belachqer-El Attar et al., 2023; Belachqer-El Attar et al., 2025c). Both Spanish plants employ extended aeration, while the Danish WTTP also incorporates a similar aerobic biological treatment.

Table 1
WWTP secondary effluent characterization.

		WWTPs		
		El Toyo (SE-A)	Uleila del Campo (SE-B)	Aalborg West (SE-C)
pH		7.3	8.1	7.1
Conductivity (mS/cm)		3.9	1.4	1.1
Turbidity (NTU)		2.1	0.70	2.0
IC (mg/L)		57	14	52
DOC (mg/L)		7.1	16	6.7
Anions (mg/L)	F^-	0.40	0.40	0.30
	Cl^-	930	190	230
	NO_2^-	1.6	1.8	n.d.
	Br^-	4.4	n.d.	0.48
	NO_3^-	5.1	4.4	12
	PO_4^{3-}	17	9.2	4.0
	SO_4^{2-}	410	410	71
Cations (mg/L)	Na^+	570	270	150
	NH_4^+	n.d.	n.d.	n.d.
	K^+	29	13	24
	Ca^{2+}	120	55	24
	Mg^{2+}	87	18	43

*n.d.: not detected.

Effluent compositions for the three WWTPs are shown in Table 1. Inorganic carbon (IC) and DOC were quantified using a Shimadzu TOC-VCSN analyzer (Kyoto, Japan). Cations and Anions were quantified by ion chromatography (Metrohm 850 Extension module 1 and 2 ion chromatography system configured for gradient analysis). Turbidity and conductivity were determined using a HI 93703 turbidity meter (HANNA, Gipuzkoa, Spain), and a EC110 handheld conductivity meter (HORIBA Scientific, Kyoto, Japan).

To prevent bicarbonate-induced hydroxyl radical scavenging, wastewater samples from El Toyo and Uleila del Campo WWTP effluents were pretreated overnight with sulfuric acid in stoichiometric amounts to neutralize bicarbonate ions and minimize their scavenging effect.

2.3. Wastewater reclamation treatments

Secondary effluents from rural and urban WWTPs located in Uleila del Campo and El Toyo (Almería, Spain), respectively, were treated using the SCPF process with low reagent concentrations representative of practical operating conditions (Belachqer-El Attar et al., 2023): 0.1 mM of Fe^{3+}-NTA (molar ratio 1:1, 5.5 mg/L Fe^{3+}), 0.73 mM of H_2O_2 (25 mg/L), at different chlorine dosages ranging from 0.13 mM (10 mg/L) to 0.39 mM (30 mg/L). The chlorine concentration of 30 mg/L is commonly used in wastewater chlorination processes (Belachqer-El Attar et al., 2023). In addition, conventional chlorination at comparable chlorine concentrations (0.13–0.40 mM) and solar photo-Fenton processes were evaluated in parallel to compare with the novel strategy. All treatments were carried out in batch mode for 60 min at lab scale in a 1-L beaker made of polyvinyl chloride (PVC) to prevent incoming radiation through walls, thus allowing us to assume that all the radiation entered through the liquid surface. Same batch of secondary effluent was used to avoid interferences due to the variability of matrix composition, taking samples after 30 min and 60 min of the reaction to evaluate the toxicity throughout the treatments. Control experiments to study the effect of the corresponding reagents were also conducted to provide a better understanding of the process phenomenology in terms of toxicity. Sunlight assays were carried out under an average solar irradiance of 28 ± 3.6 W/m^2. Additionally, the SCPF treatment was performed using a UVB-LED system (315 nm) to treat secondary effluent from the Aalborg West WWTP with an irradiance of ~19 W/m^2. Mild oxidation conditions were used (0.1 mM of Fe^{3+}-NTA. 0.74 mM of H_2O_2, and 0.13 mM of NaOCl).

2.4. Analysis of reagent concentrations

H_2O_2, total dissolved iron (Fe), along with free and total chlorine concentrations, were measured during each corresponding process using standard spectrometric techniques (DIN 38 402 H15, 2015; ISO 6332, 1988; ISO 7393-2, 2017). Free and total chlorine concentrations were determined using the DPD tablet method. To prevent H_2O_2 interference, catalase was added to the SCPF-treated water samples.

Samples were filtered using 0.2-μm nylon filters (Merck Millipore, Burlington, USA). Filtered samples of 2-mL were used to measure H_2O_2 and total dissolved Fe, while 10 mL was employed for chlorine measurements.

Further information regarding the analytical techniques is detailed in the literature (Belachqer-El Attar et al., 2023).

2.5. Toxicity analyses

2.5.1. Luminescent bacteria test using Aliivibrio fischeri

Bacterial toxicity was evaluated using a luminescent marine bacterium test, determining the inhibitory effect of water samples on the light emission of *Aliivibrio fischeri* (*A. fischeri*) in accordance with a modified version of ISO 11348-1 (ISO 11348-1, 2007). White 96-well microplates (CulturPlate™, PerkinElmer, Shelton, USA) were used to perform the incubation. Luminescence measurements were recorded using a

VICTOR™ X Multilabel Reader (PerkinElmer) after incubation for 30 min in the dark at 20 °C in a microplate shaker. Samples were subjected to ten serial two-fold dilutions, each in quadruplicate. Control tests included a blank (culture medium without bacteria), negative control (medium with bacteria but no toxicant), and positive control (medium with bacteria and the reference toxicant Z_nSO_4). Inhibition exceeding 10 % was considered indicative of detectable toxicity.

2.5.2. Freshwater algae test

Phytotoxicity was evaluated using a freshwater algal growth inhibition test with the unicellular green algae *Raphidocelis subcapitata* in accordance with ISO 8692:2012 (ISO 8692, 2012). Algal cultures (Micro BioTests Inc., Gent, Belgium) were maintained in a freshwater growth medium at 23 ± 2 °C under continuous illumination of 6000–10000 lux.

Ten serial two-fold dilutions of test samples were prepared in transparent 96-well microplates (ThermoFisher Scientific, United Kingdom (UK)), each in quadruplicate. Afterward a diluted culture of *Raphidocelis subcapitata* (1:50) was added to all the wells. Growth was measured after 24 h, 48 h, and 72 h at 450 nm using a Thermo Multiskan Plate Reader (ThermoFisher Scientific). The experiment included a negative control, which consisted of algae without any toxicant, a positive control where the algae were exposed to the toxicant $ZnSO_4$, and a blank that contained only the growth medium without algae to monitor for background interference or contamination.

The average specific growth rate (μi-j) was calculated for each replicate as follows:

$$\mu_{i-j} = \frac{\ln(X_j) - \ln(X_i)}{t_j - t_i} \quad \text{Equation 1}$$

Where X_i and X_j are the biomasses at time t_i and t_j, respectively, t_i is the time of the first measurement (start of exposure), and t_j is the time of the second measurement (end of exposure, 72 h). Inhibition exceeding 10 % was considered indicative of detectable phytotoxicity.

2.5.3. Seed germination and growth test

Phytotoxicity was also evaluated in duplicate using seedling emergence of *Lactuca sativa L.* in accordance with ISO 17126:2024 (ISO 8692, 2012). In brief, 100 g of sand in Petri dishes was moistened with test samples in series, with 2-fold dilutions or controls, to achieve a 70 % water-holding capacity. 40 lettuce seeds were individually placed on the surface to ensure even distribution. Each Petri dish was covered with 90 g of dry cover sand before being randomly placed inside sealed bags and incubated at 20–24 °C for 5–7 days. During the first 48 h, the plates were kept in the dark. After this period, a diurnal cycle with 16 h of light and 8 h of darkness, using fluorescent light at an intensity of 4300 lux was established for the remainder of the testing period. Water was used as a negative control, and boric acid as a positive control. The emerging seedlings were counted and recorded after 5, 6, and 7 days. At the end of the test, shoot and root elongations were measured using a digital caliper. Inhibition exceeding 20 % was considered indicative of detectable toxicity.

A germination index (GI) was calculated as follows (equation (2)) (López-Vinent et al., 2020):

$$germination\ index\ (GI) = \frac{seed\ germination \cdot root\ growth}{100} \quad \text{Equation 2}$$

Seed germination and root growth were determined as detailed in the *Supplementary Materials*.

A GI exceeding 80–85 % suggests the absence of phytotoxicity, as seed germination and root elongation proceed near control levels. A GI between 50 and 60 % indicates minimal impact on plant growth, with negligible injury to seedlings (ISO 11350, 2012). However, a GI below 20 % reflects severe inhibition of both germination and root development, signaling strong phytotoxic effects. Intermediate values (20–50 % GI) imply moderate phytotoxicity, where toxicants partially impair physiological processes (López-Vinent et al., 2020).

2.5.4. Reverse mutation (regulatory Ames) test

Mutagenicity was analyzed using the reverse mutation or regulatory Ames test without metabolic activation (S9) (ISO 11350, 2012). Strains of auxotrophic *Salmonella typhimurium*, TA 98 (detects frameshift mutations) and TA 100 (detects base-pair substitutions) were used. To facilitate selection, each strain was cultivated in nutrient broth growth medium supplemented with 1 mg/mL of L-histidine, 0.12 mg/mL of D-biotin, and 50 mg/mL of ampicillin solution at 37 °C on a shaker at 120 rpm. Pyrex tubes were prepared with 5 mL of melted minimum glucose agar, keeping them at 45–50 °C in a water bath to avoid solidification. 200 μL of 0.5-mM L-histidine and biotin solution, 500 μL of phosphate buffer, 200 μL of the sample or positive and negative controls, and 100 μL of cultured strain were added to the tubes. Each tube containing the corresponding compounds and strain was mixed and distributed in Petri dishes previously prepared with minimum glucose agar. The plates were covered and incubated for 48 h at 37 °C in the dark. The positive controls were 2-AA and 4-NQO. The negative control was water. For each test condition, the assay was performed in duplicate. Positive mutagenic response was detected as an increase in revertant colonies with respect to the negative control. Further information is detailed elsewhere (ISO 11350, 2012; Vijay et al., 2018).

2.5.5. Microrespirometry toxicity tests

Toxicity toward mixed microbial communities was evaluated using a microrespirometry-based test applied to a lake microplankton community and a soil microbial community. A quartz glass 24-well microplate system from Loligo Systems (Viborg, Denmark) was used to measure community oxygen consumption. The microrespirometry system was equipped with optical oxygen sensors in each of the 500 μL wells allowing non-invasive online monitoring of oxygen consumption in each sample. To calibrate the sensors, a two-point method was applied, using oxygen-saturated water and oxygen-depleted water. Oxygen-depleted water is obtained by dissolving 10 g of Na_2SO_3 into 500-mL distilled water.

For lake plankton communities, a sample from a Danish oligotrophic lake (Nors Sø) was collected at 30 cm depth and then filtered through a 45-μm sieve to ensure that only microorganisms ≤63 μm remained, including different bacteria, fungi, phytoplankton, and zooplankton. Under sterile conditions, 750 μL of lake water was spiked with 750 μL of the test sample in 1.5-mL Eppendorf tubes (1:1 dilution) and mixed. 500 μL of this mixture was added to the 24-channel plates, avoiding air trapping in the wells.

For soil microbial communities, a soil sample from an agricultural soil of a greenhouse at the University of Almería, (Spain) was collected at a depth of 15–20 cm and then sieved (1.5 mm mesh) to remove large objects such as pebbles and large roots. After that, 10 g were weighed and mixed with 50-mL of sterile demineralized water before being incubated on a shaker at 100 rpm for 3 h. Following this incubation, the samples were cooled to 5 °C to allow for sedimentation and subsamples from the supernatant were collected after 1 h.

For both lake and soil communities, samples were analyzed in quadruplicate using two-fold serial dilutions and were prepared using sterile distilled water. Oxygen consumption was monitored for 3 days in the microrespirometry system to assess microbial activity after exposure to relevant chemicals and treatments. Controls consisted of samples without the addition of chemicals or wastewater.

2.5.6. Data and statistical analyses

The inhibition percentage for the toxicity tests was calculated as follows (equation (3)):

$$q = 1 - \left(\frac{R_X}{R_0}\right) \cdot 100 = 100 - 100 \cdot \left(\frac{R_X}{R_0}\right) \quad \text{Equation 3}$$

Where q is the relative inhibition percentage between 0 and 100 %, R_x is the response measured at the different toxicant concentrations minus the blank, and R_0 is the response measured for the control samples without toxicant.

EC50 value (half maximum effective concentration) was calculated by fitting the dose-response or concentration-dose curve using a Hill-type model with four parameters (equation (4)). EC20 values were also determined (20 % effective concentration).

The dose-response curve was obtained by plotting q as a function of the log value for concentration.

$$q = \frac{100}{\left[1 + \left(\frac{EC_{50}}{X}\right)^{d}\right]} \quad \text{Equation 4}$$

Where q is the relative inhibition, a and b are the lowest and the highest values for inhibition, respectively, d is a Hill coefficient (Hill slope factor), and X is the toxicant concentration (log_{10} value).

Toxicity data were also expressed as the lowest observed effect concentration (LOEC) to identify the lowest concentration of a substance (or the highest dilution of a sample) at which an adverse (or stimulatory) response was observed relative to controls.

3. Results and discussion

3.1. Assessment of the environmental toxicity

The ecotoxicological potential of wastewater treated by the novel SCPF process was assessed with respect to its suitability for reuse in crop irrigation and effects if discharged into receiving freshwater bodies. To this end, SCPF at different chlorine dosages ranging from 0.13 to 0.40 mM was evaluated. In addition, conventional chlorination at comparable concentrations (0.13–0.40 mM), iron complex decomposition, and solar photo-Fenton were evaluated in parallel to enable comparison with the novel strategy. The iron source Fe^{3+}-NTA, which plays a pivotal role in the process, and its chelating agent (NTA), were also analyzed due to toxicity concerns. Both chemical stock solutions at concentrations of 100 mM and 300 mM, respectively, are commonly prepared for the treatment (EU, 2024/3019, 27 November; Sánchez Pérez et al., 2024).

3.1.1. Microbial toxicity

The pure chemicals, Fe^{3+}-NTA and NTA, exhibited significant toxicity toward *A. fischeri* at concentrations >1.78 mM and >39.8 mM, respectively (Fig. 2a). Dose-response analysis yielded EC20 values of 3.65 mM and 217 mM, respectively. These results indicate that the iron complex is more toxic than the chelating agent (Fig. 2a), likely due to its ability to generate reactive oxygen species and alter metal bioavailability (Fukuzawa et al., 2001). A review of the available literature suggests that specific EC20 values for either theFe^{3+}-NTA complex or free NTA in *A. fischeri* assays are scarce, thus making direct comparisons challenging.

The toxicity of effluents and treated samples from the SE-A and SE-B WWTPs was evaluated using the *A. fischeri* bioluminescence inhibition test after 30 and 60 min of exposure**.** No apparent toxicity was observed for either secondary effluents or SCPF-treated samples, regardless of wastewater origin (Fig. 2b).

Table 2 presents the LOEC values for samples treated by all processes from both WWTPs. These data reveal that effluent from SE-A WWTP generates toxicants under certain treatments, particularly chlorination, the Fe^{3+}-NTA photodecomposition, and in the dark, as well as the H_2O_2 (dark) process underscoring the impact of wastewater composition. Overall, chlorination exhibited apparent toxicity regardless of the effluent origin. Chlorination treatments showed clear time- and dose-dependent effects, with marked differences between the two WWTPs. For SE-A samples, chlorination with 0.13-mM NaOCl resulted in apparent toxicity at a 2-fold dilution (D2) and increased to a 4-fold

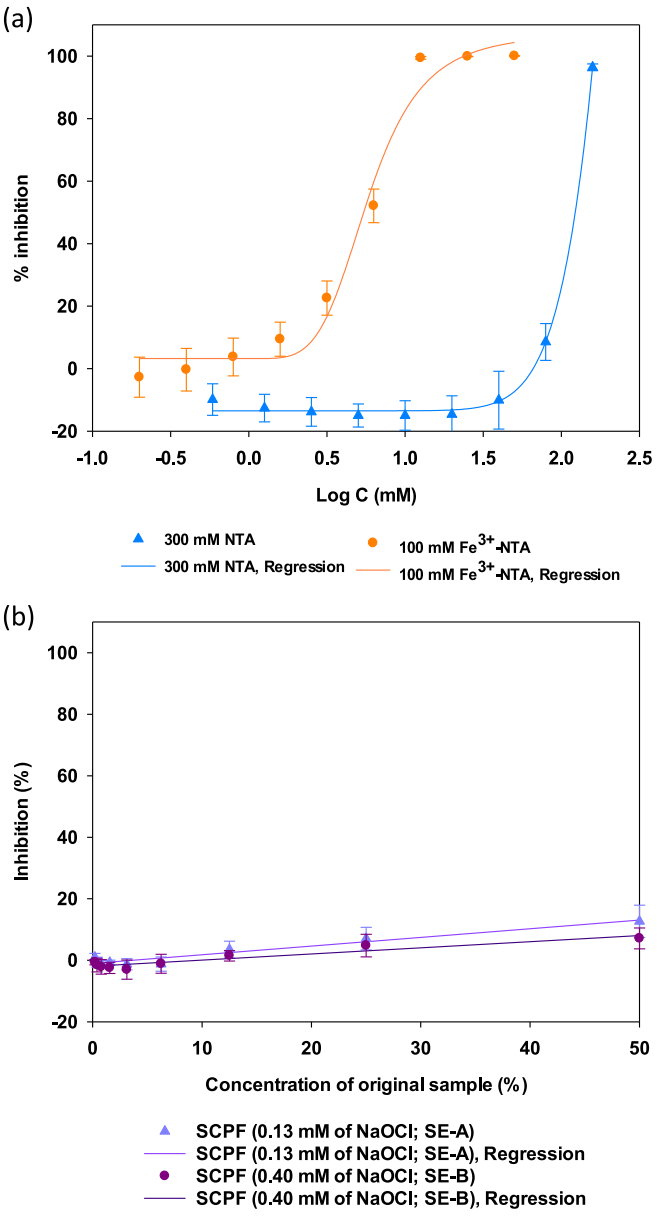

Fig. 2. Dose-response curves for *A. fischeri* exposed to Fe^{3+}-NTA and NTA solutions (a) and secondary effluent treated by the SCPF process (b). Data points represent the means of 4 replicates ± standard deviation.

dilution (D4) when the chlorine dose was increased to 0.26 and 0.40 mM, indicating the formation of toxic DBPs after a 60-min treatment. In contrast, SE-B samples overall demonstrated no apparent toxicity across all processes and time points, confirming their low ecotoxicological risk. However, chlorinated samples displayed toxic effects at D2 but only at 30 min, with inhibition values of up to 21 % for 0.13 mM and 0.26 mM of NaOCl, and no inhibition was detected at 60 min. Interestingly, contrary to what was expected, no apparent toxicity was observed at the higher chlorine concentration (0.40 mM), regardless of the treatment time. This is likely to have resulted from assay interferences, causing inhibition to be masked by signal quenching. This phenomenon could be due to excess chloride ions or residual oxidants, which may interfere with the bioluminescent signal without reflecting a real biological response (Side et al., 2017).

Fe^{3+}-NTA also exhibited apparent toxicity at D4 when exposed to wastewater treated for 60 min, whereas it decreased under sunlight to D2 after 60 min, suggesting the formation of toxic intermediates during oxidation, which was reduced through photodecomposition (Reaction

Table 2
LOEC values for bacterial toxicity based on inhibition of luminescence in *A. fischeri* after exposure to treated samples from SE-A and SE-B WWTPs. 30 min and 60 min refer to the treatment time for wastewater. A green color indicates no apparent toxicity at D2, whereas a red color indicates toxicity >10 % at D2. The dilution level where toxicity first appears is indicated for samples.

		SE-A		SE-B	
		time (min)		*time (min)*	
Processes		30	60	30	60
Chlorination	0.13 mM NaOCl				
	0.26 mM NaOCl		D4		
	0.40 mM NaOCl		D4		
0.74 mM H_2O_2 in the dark		D4			
0.1 mM Fe^{3+}-NTA	Dark		D4		
	Sunlight				
Solar photo-Fenton					
SCPF	0.13 mM NaOCl				
	0.26 mM NaOCl				
	0.40 mM NaOCl				

3). Apparent toxicity was also observed at D4 after 30 min, which decreased to D2 at 60 min for the 0.74-mM H_2O_2 process in the dark.

As for the photo-driven processes, solar photo-Fenton exhibited no apparent toxicity, as did SCPF, across most conditions, except for the lowest concentration of NaOCl (0.13 mM) after 30 min at D2 when treating effluent from SE-A WWTP. This response may be attributed to matrix effects influencing the bacteria biological activity, demonstrating that solar photo-Fenton with Fe^{3+}-NTA and SCPF are generally non-toxic and environmentally safe when exposed to *A. fischeri*. These findings are consistent with previous studies reporting similar behavior for solar photo-Fenton processes at acidic and neutral pH using other chelating agents such as Fe^{3+}-EDDS (Rivas et al., 2017; Maniakova et al., 2022; Freitas et al., 2017; Starling et al., 2017).

3.1.2. Phytotoxicity using algae and plants

Phytotoxicity was evaluated using two bioassays: freshwater algae inhibition and plant-based tests.

The pure chemicals were assessed with an algae inhibition test (Fig. 3a). The chelating agent exhibited significant phytotoxicity with inhibition percentages exceeding 60 % even at a concentration of 0.07 mM, but only 21.3 % at 25 mM for the iron complex, displaying no algal toxicity below a concentration of 3.13 mM. Notably, the concentration used in the SCPF (0.1 mM) process is well below this threshold, suggesting no or low apparent algal toxicity (Fig. 3a). This is also evident from tests with SCPF treated secondary effluents exhibiting negligible apparent toxicity (Fig. 3b).

Chlorination (Table 3), regardless of WWTP origin, induced phytotoxicity toward *R. subcapitata* at D4 for 0.13 mM of NaOCl and D8 when the concentration was increased to 0.26 mM for SE-A samples, while D4 was observed with the highest chlorine concentrations, 0.26 and 0.40 mM for SE-B samples, underlining the importance of wastewater composition. This is due to the fact that chlorination generates harmful DBPs, which have toxic effects on aquatic organisms, including algae (Li et al., 2022). Residual chlorine in the effluent may also directly affect algal growth and metabolism, by oxidizing essential cellular components, such as proteins and lipids, and causing cellular damage to algae (Cao et al., 2021). Delving into chlorine dose dependency, 0.4-mM chlorinated samples from SE-A exhibited no apparent toxicity, contrary to expectations, which likely results from the presence of interfering substances or wastewater matrix effects which act like quenching agents.

In contrast, all other tested processes, including solar photo-Fenton and SCPF (0.13–0.40 mM), showed no detectable algal toxicity and even promoted stimulation, probably due to the nutrient content of the wastewater. These findings are consistent with previous research that demonstrates the exceptional performance of SCPF in mitigating DBP concentrations, achieving low levels of THMs and HAAs compared to chlorination (Belachqer-El Attar et al., 2025a).

Phytotoxicity was also evaluated using the *Lactuca sativa* (garden lettuce) seed germination and growth bioassay.

NTA and Fe^{3+}-NTA solutions were highly phytotoxic at elevated concentrations (Fig. 4a), causing 100 % inhibition. NTA exhibited the highest inhibition, reaching 63 % even at a concentration of 18.75 mM. However, Fe^{3+}-NTA displayed EC20 and EC50 values of 1.20 mM and 2.39 mM, respectively, and no phytotoxic effects were observed below a concentration of 6.25 mM, which is lower than the concentration used in the novel photo-Fenton strategy (0.1 mM). This is also evident from tests with SCPF treated secondary effluents exhibiting negligible apparent toxicity (Fig. 4b).

Overall, the germination results (Table 4) indicated phytotoxicity, particularly in SE-A samples treated with chlorination, mainly after 30 min (the highest inhibition was 75 % at a concentration of 0.13-mM NaOCl), 0.1-mM Fe^{3+}-NTA (in the dark and under sunlight; 25 % for both), and solar photo-Fenton (35 % inhibition) after 60 min. In contrast, no phytotoxicity was observed in other cases. For the samples from SE-B, chlorinated water showed apparent toxicity after a 30-min treatment (25 % inhibition at 0.26 mM), while for SCPF-treated water (0.26-mM NaOCl) and solar photo-Fenton (with 30 % and 35 % inhibition, respectively), this took place after 60 min.

A previous study investigating the phytotoxicity of solar photo-Fenton using chelating agents, EDTA, diethylenetriaminepentaacetic acid (DTPA) and EDDS, found moderate phytotoxicity for EDTA and DTPA, and high phytotoxicity corresponding to EDDS (López-Vinent et al., 2020). However, solar photo-Fenton with EDDS has also been studied previously, reporting toxicity reduction when applying the algae test (Rivas et al., 2017; Freitas et al., 2017). As such, these significant variations are likely due not only to the concentrations and operational conditions applied during the treatments, but also the wastewater matrix used.

Fig. 5 shows seedling growth measurements exposed to pure chemicals, untreated secondary effluent, treated by chlorination processes (0.13 and 0.40 mM of NaOCl), and SCPF (0.13 mM of NaOCl). The SE-A

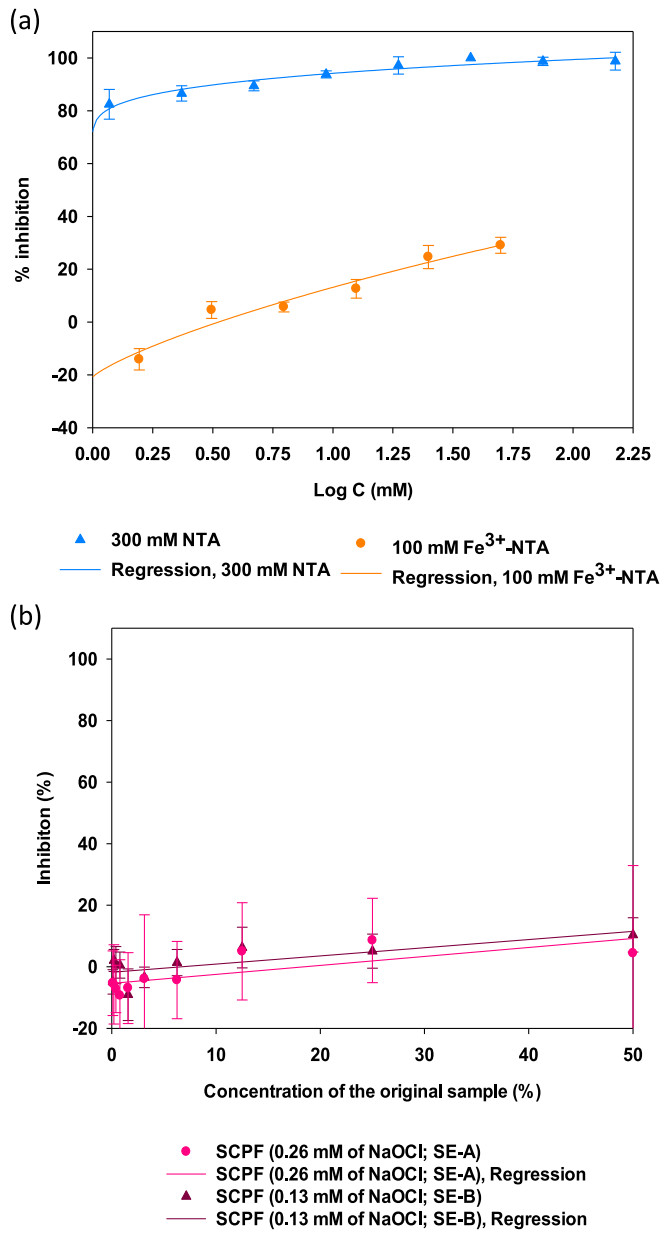

Fig. 3. Dose-response curve for freshwater algae (*R. subcapitata*) exposed to NTA and Fe^{3+}-NTA (a) and secondary effluent treated by the SCPF process (b). Data points represent the means of 4 replicates ± standard deviation.

WWTP, because most samples with apparent toxicity are from this plant. As expected, no growth was observed for NTA due to its phytotoxic effects. In contrast, seedlings exposed to Fe^{3+}-NTA showed average root and stem elongations of 5 and 3 mm, respectively.

The water samples treated by chlorination and SCPF (0.13 mM), respectively, resulted in comparable growth measurements, with root elongations ranging from 22 to 28 mm and stem elongations between 10 and 14 mm, corresponding to the highest values observed with SCPF. Notably, untreated secondary effluent resulted in the greatest growth, with root and stem elongations reaching 33 and 17 mm, respectively.

Accordingly, the complete inhibition of germination (GI = 0) for NTA even at 18.75 mM highlights the phytotoxicity of this chelating agent. Lower GI was determined for the iron complex at the highest concentration,100 mM (22 %), which is higher than the concentration applied in the treatment (Belachqer-El Attar et al., 2023). However, the samples treated by chlorination and SCPF (0.13 mM of NaOCl), and the untreated effluent showed GI >100 %, indicating the beneficial role of wastewater-derived nutrients in stimulating growth, specifically in the effluent (GI = 140 %). This suggests that the positive effects of nutrient availability compensate for any inhibitory factors in the raw effluent. Samples treated with chlorination and SCPF indicate that these wastewater treatments can preserve growth-promoting components while reducing risks. Chlorination likely reduces pathogens without stripping essential nutrients, while SCPF removes microcontaminants and inactivates microorganisms (Belachqer-El Attar et al., 2023; Pichel et al., 2023), while retaining dissolved nutrients.

3.2. Microbial community toxicity assessed using microrespirometry

The toxicity of treated wastewater toward mixed microbial communities was assessed using microrespirometry, with test biota consisting of microplankton from a freshwater lake and microbial communities from an agricultural greenhouse. Analyses revealed no apparent microbial toxicity toward plankton communities in treated wastewater samples irrespective of the treatment. These observed low levels of respiratory inhibition are consistent with previous studies that have evaluated the toxicity of treated wastewater on aquatic microorganisms. For instance, Maniakova et al. (2022) (Maniakova et al., 2022) reported similar findings when investigating solar photo-Fenton with Fe^{3+}-EDDS, where the advanced wastewater treatment process effectively reduced the toxicological impacts of effluents on microbial communities. By comparison, both pure chemicals, NTA and Fe^{3+}-NTA, demonstrated low toxicity, with respiratory inhibition percentages below 23 %. At lower concentrations, respiratory inhibition decreased slightly to ~20 % and 14 % for Fe^{3+}-NTA and NTA, i.e. up to 6.25 mM and 18.75 mM, respectively (Table S1).

As for soil microbial communities, Fig. 6 shows that Fe^{3+}-NTA and NTA caused severe inhibition (~80 % even at an 8-fold dilution (D8), indicating high toxicity to soil microorganisms due to metal chelation, increased bioavailability of heavy metals, and oxidative stress. Chlorination also induced strong inhibition (~70–80 % even at D8), reflecting the persistence of toxic DBPs that impair microbial metabolism. In contrast, SCPF treatment resulted in much lower inhibition (~30 % at D2 decreasing to 14 % at D8), demonstrating its effectiveness in reducing ecotoxicity and preserving microbial activity. This suggests that SCPF may not only degrade microcontaminants and disinfect, but also contribute to minimizing harmful residues, resulting in less residual toxicity from process reagents. Note that inhibition of SCPF was apparently mitigated by dilution, whereas chlorination and both chemicals remained toxic to soil microorganisms regardless of an 8-fold dilution (D8).

The differences between the two analyses may be attributed to variations in exposure conditions, community composition, organism interactions and dynamics, as well as ecosystem health and integrity(Edo et al., 2024). Furthermore, soil microbes were found to be highly sensitive to Fe^{3+}-NTA, but not in SCPF-treated water. While Fe3+-NTA alone caused ~80 % soil respiration inhibition, its toxicity was markedly reduced in SCPF-treated effluents. This is probably caused by photodegradation of the iron complex under natural sunlight, which reduces bioavailable iron and prevents reactive species bursting into the soil. Additionally, natural organic matter in wastewater may chelate residual iron, further mitigating toxicity (Oliveira Silva et al., 2025).

The findings highlight that while conventional treatments, such as chlorination, pose high risks to soil health, SCPF offers a safer alternative for wastewater reuse in agricultural or land application scenarios, where maintaining functional microbial communities is essential for ecosystem sustainability.

3.3. Genotoxicity assessment

Wastewater treated via the SCPF process also requires genotoxicity assessment, as the iron source, Fe^{3+}-NTA, employed in the process is suspected to be genotoxic due to its chelating agent, which has been

Table 3
LOEC values for phytotoxicity based on growth inhibition of the freshwater alga *R. subcapitata* after exposure to treated samples from SE-A and SE-B WWTPs. 30 min and 60 min refer to the treatment time for wastewater. A green color indicates no apparent toxicity at D2, whereas a red color indicates toxicity >10 % at D2. The dilution level where toxicity first appears is indicated.

		SE-A		SE-B	
		time (min)		*time (min)*	
Processes		30	60	30	60
Chlorination	0.13 mM NaOCl		D4		
	0.26 mM NaOCl		D8		D4
	0.40 mM NaOCl				D4
0.74 mM H_2O_2 in the dark					
0.1 mM Fe^{3+}-NTA	dark				
	sunlight				
Solar photo-Fenton					
SCPF	0.13 mM NaOCl				
	0.26 mM NaOCl				
	0.40 mM NaOCl				

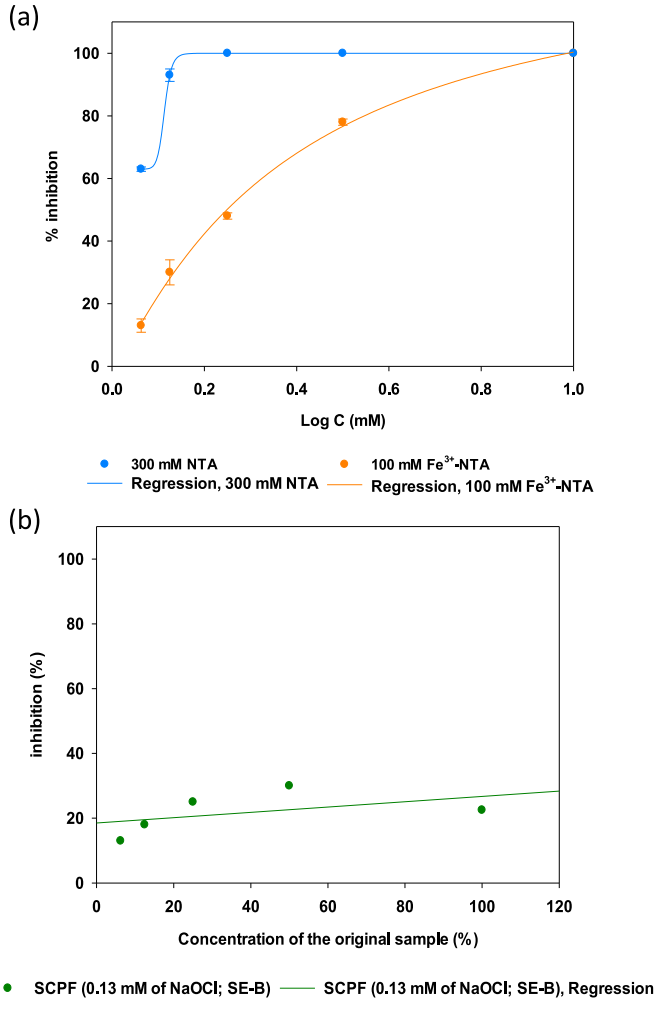

Fig. 4. Curve dose-response for seed germination test using garden lettuce exposed to NTA and Fe^{3+}-NTA (a) and secondary effluent treated by the SCPF process (b). Data points represent the means of 4 replicates ± standard deviation.

identified as a genotoxic compound (European Commission, 2010). Consequently, further evaluation is necessary. To this end, mutagenicity tests using *Salmonella* strains were carried out to analyze treated water applying the novel strategy with the lowest chlorine concentration (0.13 mM), and chlorination at the maximum concentration (0.40 mM of NaOCl) for both SE-A and SE-B. Additionally, the chlor-photo-Fenton process was performed using the UVC-LED system to treat SE-C.

The results demonstrate that neither NTA nor its iron complex exhibited apparent mutagenic activity at the highest tested concentrations, implying that lower concentrations of these compounds are also non-mutagenic (Table S2). In contrast to SE-A and SE-B treated effluents, only SE-C showed no detectable mutagenicity. Specifically, chlorination (0.40-mM NaOCl) and SCPF with 0.13 mM of NaOCl induced positive responses in both *S. typhimurium* strains (with revertant number up to 100 of TA98 and from 49 to >100 of TA100). The highest number of revertants was observed in the TA100 strain, which is sensitive to base-pair substitutions. This agrees with previous research that reported that this strain is more responsive for certain chemicals, indicating better mutagenicity screening (Schulze et al., 2024). Interestingly, this discrepancy between the treated samples by chlor-photo-Fenton could be due to variations in nutrient content could enhance the growth of test bacteria on agar plates, potentially leading to false-positive revertant counts but also surviving bacteria might metabolize compounds or produce secondary mutagens during the test (Pérez-Albaladejo et al., 2023). Additionally, differences in the organic matter load, DBP precursors, or residual pollutants might influence mutagen formation during treatments. For instance, the chlorination with higher chlorine concentration (0.40 mM) promotes the formation of DBPs, such as HAAs or THMs (Belachqer-El Attar et al., 2025a), which are known to induce DNA damage [43]. Conversely, water treated by SCPF with a lower NaOCl dose (0.13 mM) still resulted in mutagenicity, indicating that factors beyond chlorine dosage, such as wastewater composition, may be critical.

While these findings suggest that SCPF can produce water with low mutagenic risk, the observed variability across sources highlights the need to consider both treatment conditions and wastewater characteristics in ecotoxicological assessments.

The results of the tests demonstrated a significant advantage of SCPF over conventional chlorination (Table 5). Chlorination induced toxicity, with inhibitory effects at low dilutions in *A. fischeri* and algae tests, severe phytotoxicity (75 % germination inhibition in lettuce), strong

Table 4
Phytotoxicity of undiluted samples based on inhibition of seed germination in the lettuce *Lactuca sativa sp* after 6 days of incubation when exposed to treated samples from SE-A and SE-B WWTPs. 30 min and 60 min refer to the treatment time for wastewater. A green color indicates no apparent toxicity, whereas a red color indicates toxicity >20 %.

		SE-A		SE-B	
		time (min)		*time (min)*	
Processes		30	60	30	60
Chlorination	0.13 mM NaOCl				
	0.26 mM NaOCl				
	0.40 mM NaOCl				
0.74 mM H_2O_2 in the dark					
0.1 mM Fe^{3+}-NTA	dark				
	sunlight				
Solar photo-Fenton					
SCPF	0.13 mM NaOCl				
	0.26 mM NaOCl				
	0.40 mM NaOCl				

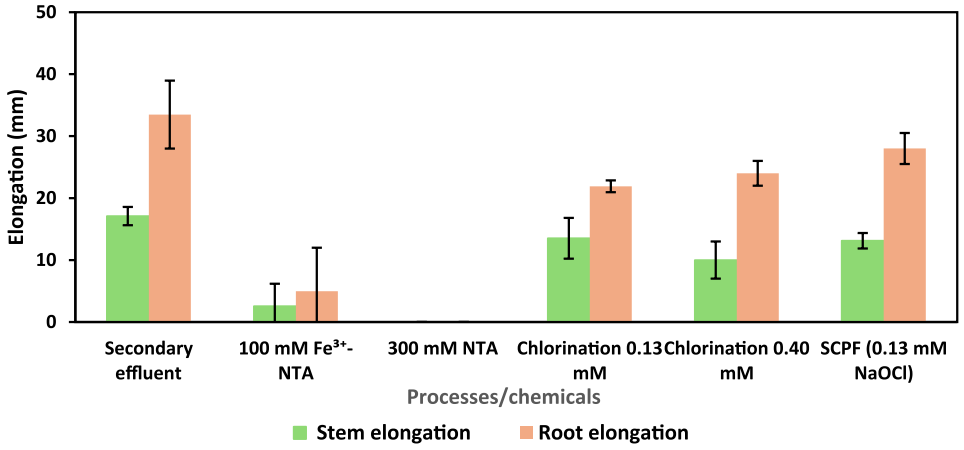

Fig. 5. Root and stem elongations of garden lettuce seedlings for the pure chemicals, secondary effluent, treated water by chlorination processes (0.13 and 0.40 mM of NaOCl), and SCPF (0.13 mM of NaOCl) from SE-A. Data points represent the means of 2 replicates ± standard deviation.

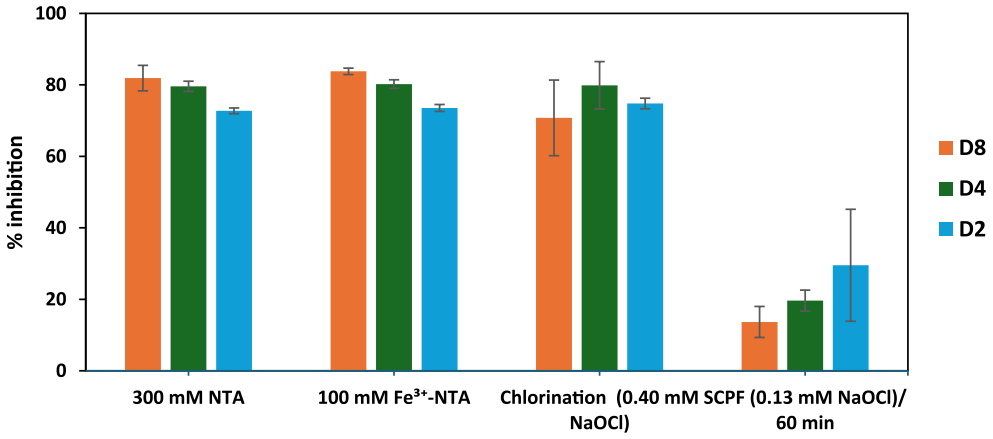

Fig. 6. Inhibition of soil microbial respiration after exposure to chemicals, 100-mM Fe^{3+}-NTA and 300-mM NTA, and treated wastewater samples from SE-A WWTP, incorporating: chlorination (0.40 mM NaOCl, 60 min), and SCPF (0.13 mM NaOCl, 60 min)0.2-fold dilution levels: D2, D4, and D8.

Table 5
Comparison of ecotoxicity profile of secondary effluents treated by conventional chlorination (0.4 mM NaOCl) and solar chlor-photo-Fenton (SCPF, 0.13 mM NaOCl).

Treatment	*A. fischeri*	Algae	Lettuce (germination)	Soil microbes	Genotoxicity
Chlorination (0.4 mM)	D2	D4	75 % inhibition	80 % inhibition	Positive
SCPF (0.13 mM)	Non-toxicity	Non-toxicity	25 % inhibition	30 % inhibition	Negative[a]

[a] See Section 3.3.

suppression of microbial soil respiration (80 % inhibition), and a positive mutagenic response. In contrast, SCPF at a much lower chlorine dose (0.13 mM) demonstrated negligible ecotoxicity. Thus, effluents treated with the novel strategy have no ecotoxic effects, making them suitable for crop irrigation. This effectiveness is attributed to the mechanistic processes involved, which combine HO· oxidation with a low chlorine concentration. This approach minimizes the formation of DBP precursors and, consequently, toxic DBPs, compared to traditional chlorination methods. Additionally, the influence of solar radiation on the degradation of these by-products must be considered. These findings demonstrate that SCPF not only achieves high treatment efficiency, but also reduces ecotoxicity, offering a sustainable solution that complies with the EU Water Reuse Regulation (2020/741). Soil microbial analyses were the most sensitive for detecting toxicity among the tested assays. The Ames test and *A. fischeri* assays, in turn, demonstrated high specificity for direct-acting toxicants. Algal growth and plant-based tests were comparatively less sensitive but still distinguished differences between treatments. Critically, treatment conditions and wastewater composition must be considered for mutagenicity test due to possible interferences.

4. Conclusions

For the first time, the SCPF process has been comprehensively evaluated for the ecotoxicological impact of treated secondary effluents. Compared to traditional chlorination, SCPF-treated water caused low to undetectable bacterial and algal toxicity, minimal phytotoxicity in terms of seed growth and germination, and limited inhibition of mixed microbial communities. Genotoxicity levels were also low, as indicated by preliminary tests. While pure Fe^{3+}-NTA exhibited inherent ecotoxicity at elevated concentrations, its impact was significantly reduced at the low concentration (0.1 mM) typically used in the SCPF process, confirming its suitability for practical water reuse applications. This research sheds light on the application potential of this novel process and may pave the way for its large-scale implementation. Ecotoxicological evaluations of this kind can help in establishing safe limits and water quality standards, ensuring environmental sustainability and the protection of aquatic ecosystems, as well providing support for regulatory decision-making.

CRediT authorship contribution statement

S. Belachqer-El Attar: Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **P. Taborelli:** Writing – review & editing, Investigation. **P. Soriano-Molina:** Writing – review & editing, Supervision, Conceptualization. **P. Roslev:** Writing – review & editing, Funding acquisition, Data curation, Conceptualization. **J.A. Sánchez Pérez:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

S. Belachqer-El Attar gratefully acknowledges the PPIT-University of Almería for her predoctoral research grant (CPRE2023-007), Junta de Andalucía- ERDF 2021–2027 (Program: 54. A), and her research stay grant (EST2024_25/038) at Aalborg University in Denmark. The authors also acknowledge the ANDROMEDA project (PID2022-140875OB-C31) funded by MCIN/AEI/10.13039/501100011033/FEDER, UE, and the IN2AQUAS project which is funded by the European Union under the Marie-Sklodowska-Curie Research and Innovation Staff Exchange' "(HORIZON – MSCA –2022-DN-JD-101119555-IN2AQUAS). Project N. 101119555.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jenvman.2026.128569.

Data availability

Data will be made available on request.

References

Ahile, U.J., Wuana, R.A., Itodo, A.U., Sha'Ato, R., Malvestiti, J.A., Dantas, R.F., 2021. Are iron chelates suitable to perform photo-Fenton at neutral pH for secondary effluent treatment? J. Environ. Manag. 278, 111566. https://doi.org/10.1016/j.jenvman.2020.111566.

Belachqer-El Attar, S., Soriano-Molina, P., De La Obra, I., Sánchez Pérez, J.A., 2022. A new solar photo-Fenton strategy for wastewater reclamation based on simultaneous supply of H2O2 and NaOCl. Sci. Total Environ. 834, 155273. https://doi.org/10.1016/j.scitotenv.2022.155273.

Belachqer-El Attar, S., Soriano-Molina, P., Pichel, N., París-Reche, A., Plaza-Bolaños, P., Agüera, A., Pérez, J.A.S., 2023. Continuous flow operation of solar photo-Fenton fused with NaOCl as a novel tertiary treatment. J. Hazard Mater. 460, 132354. https://doi.org/10.1016/j.jhazmat.2023.132354.

Belachqer-El Attar, S., Soriano-Molina, P., París-Reche, A., Jambrina-Hernández, E., Plaza-Bolaños, P., Agüera, A., Sánchez Pérez, J.A., 2025a. Phenomenological insights into the occurrence and abatement of disinfection by-products in the novel solar chlor-photo-fenton process. J. Hazard Mater. 491, 138019. https://doi.org/10.1016/j.jhazmat.2025.138019.

Belachqer-El Attar, S., Soriano-Molina, P., Casas López, J.L., Jambrina-Hernández, E., Agüera, A., Sánchez Pérez, J.A., 2025b. Addressing wastewater reuse challenges in rural decentralized areas: implementation of a solar chlor-photo-fenton demonstration plant. J. Water Process Eng. 77, 108616. https://doi.org/10.1016/j.jwpe.2025.108616.

Belachqer-El Attar, S., Rodríguez-García, D., Soriano-Molina, P., García Sánchez, J.L., Casas López, J.L., Sánchez Pérez, J.A., 2025c. Model-based scenario analysis to support the operation of solar photo-Fenton plants. J. Environ. Manag. 373, 123886. https://doi.org/10.1016/j.jenvman.2024.123886.

Cao, S., Zhang, D., Teng, F., Liao, R., Cai, Z., Tao, Y., Hu, H., 2021. Inhibitory effects of ultralow-dose sodium hypochlorite on microcystis aeruginosa and chlorella vulgaris: differences in sensitivity and physiology. Sci. Total Environ. 774, 145638. https://doi.org/10.1016/j.scitotenv.2021.145638.

De Luca, A., Dantas, R.F., Esplugas, S., 2014. Assessment of iron chelates efficiency for photo-Fenton at neutral pH. Water Res. 61, 232–242. https://doi.org/10.1016/j.watres.2014.05.033.

DIN 38 402 H15, 2015. Determination of Hydrogen Peroxide Spectrometric Method Using Titanium (IV) Oxysulphate.

Edo, G.I., Samuel, P.O., Jikah, A.N., Yousif, E., Onyibe, P.N., Opiti, A.R., Ikpekoro, V.O., Ezekiel, G.O., Thomas, B.E., Eboh, S.O., Ekokotu, H.A., Ugbune, U., Oghroro, E.E.A., Ainyanbhor, I.E., Akpoghelie, P.O., Owheruo, J.O., Essaghah, A.E.A., Ekpekpo, L.D., Isoje, E.F., Agbo, J.J., 2024. Sensitivity ecology and evolution, toxicology organism assessment model in the use of chemical applications for the management of toxic substances. Ecol Front 44, 890–908. https://doi.org/10.1016/j.ecofro.2024.06.001.

EU 2020/741, 25 May. Regulation (EU) 2020/741 of the European parliament and of the council of 25 May 2020 on minimum requirements for water reuse. Off. J. Eur. Union, L 177/32. .

EU 2024/3019, 27 November. Directive (EU) 2024/3019 of the European Parliament and of the Council of 27 November 2024 Concerning Urban Wastewater Treatment (Recast) .

European Commission, 2010. Directorate General for Health and Consumers. Opinion on Trisodium nitrilotriacetate (NTA), Publications Office, LU.

Fatta-Kassinos, D., Dionysiou, D.D., Kümmerer, K., 2016. Advanced Treatment Technologies for Urban Wastewater Reuse. Springer International Publishing, Cham.

Freitas, A.M., Rivas, G., Campos-Mañas, M.C., Casas López, J.L., Agüera, A., Sánchez Pérez, J.A., 2017. Ecotoxicity evaluation of a WWTP effluent treated by solar photo-Fenton at neutral pH in a raceway pond reactor. Environ. Sci. Pollut. Res. 24, 1093–1104. https://doi.org/10.1007/s11356-016-7101-7.

Fukuzawa, K., Tokumura, A., Kogure, K., Iemura, M., Gondoh, N., Fujii, M., Ueno, S., Shibata, A., 2001. A comparative study of the ability of ferric nitriloacetate and other iron chelators to assist membrane lipid peroxidation by superoxide radicals. Chem. Phys. Lipids 110, 69–84. https://doi.org/10.1016/S0009-3084(01)00127-X.

Gualda-Alonso, E., Soriano-Molina, P., Casas López, J.L., García Sánchez, J.L., Plaza-Bolaños, P., Agüera, A., Sánchez Pérez, J.A., 2022. Large-scale raceway pond reactor for CEC removal from municipal WWTP effluents by solar photo-Fenton. Appl. Catal. B Environ. 319, 121908. https://doi.org/10.1016/j.apcatb.2022.121908.

ISO 11348-1, 2007. Water Quality - Determination of the Inhibitory Effect of Water Samples on the Light Emission of Vibrio fischeri (Luminescent Bacteria Test): Part 1: Method Using Freshly Prepared Bacteria.

ISO 11350, 2012. Water Quality - Determination of the Genotoxicity of Water and wastewater-Salmonella/microsome Fluctuation Test (Ames Fluctuation Test).

ISO 6332, 1988. Water Quality - Determination of Iron: Spectrometric Method Using 1, 10-Phenanthroline.

ISO 7393-2, 2017. Water Quality - Determination of Free Chlorine and Total Chlorine Part 2: Colorimetric Method Using N, Ndialkyl-1,4-phenylenediamine, for Routine Control Purposes. International Organization for Standardization (ISO), Geneva, Switzerland.

ISO 8692, 2012. Water Quality - Fresh Water Algal Growth Inhibition Test with Unicellular Green Algae.

Italian Decree Article 99 n.152, April 3, 2006. Technical Standards for the Reuse of Waste Water, Pursuant to Article 99, Paragraph 1, of Legislative Decree, p. 152.

Li, N., Liu, Z., Wang, P., Suman, K., Zhang, J., Song, Y., 2022. Effects of sodium hypochlorite treatment on the chlorophyll fluorescence in photosystem II of microalgae. Sci. Total Environ. 833, 155192. https://doi.org/10.1016/j.scitotenv.2022.155192.

López-Vinent, N., Cruz-Alcalde, A., Malvestiti, J.A., Marco, P., Giménez, J., Esplugas, S., 2020. Organic fertilizer as a chelating agent in photo-Fenton at neutral pH with LEDs for agricultural wastewater reuse: micropollutant abatement and bacterial inactivation. Chem. Eng. J. 388, 124246. https://doi.org/10.1016/j.cej.2020.124246.

Maniakova, G., Salmerón, I., Aliste, M., Inmaculada Polo-López, M., Oller, I., Malato, S., Rizzo, L., 2022. Solar photo-Fenton at circumneutral pH using Fe(III)-EDDS compared to ozonation for tertiary treatment of urban wastewater: contaminants of emerging concern removal and toxicity assessment. Chem. Eng. J. 431, 133474. https://doi.org/10.1016/j.cej.2021.133474.

Nixon, G.A., 1971. Toxicity evaluation of trisodium nitrilotriacetate. Toxicol. Appl. Pharmacol. 18, 398–406. https://doi.org/10.1016/0041-008X(71)90132-3.

Oliveira Silva, M., Bernardes Marques, M., De Souza, D.R., 2025. Potential of organic complexing agents in enhancing fenton and photo-fenton reactions: a sustainable approach using solar irradiation for emerging contaminants treatment. Orbital - Electron. J. Chem. 70–79. https://doi.org/10.17807/orbital.v17i1.22735.

Pérez-Albaladejo, E., Pinteño, R., Aznar-Luque, M.D.C., Casado, M., Postigo, C., Porte, C., 2023. Genotoxicity and endocrine disruption potential of haloacetic acids in human placental and lung cells. Sci. Total Environ. 879, 162981. https://doi.org/10.1016/j.scitotenv.2023.162981.

Pichel, N., Belachqer-El Attar, S., Soriano-Molina, P., Sánchez Pérez, J.A., 2023. Demonstrating the feasibility of a novel solar photo-Fenton strategy for full-scale operationalization according to EU 2020/741 disinfection targets for water reuse. Chem. Eng. J. 472, 144935. https://doi.org/10.1016/j.cej.2023.144935.

Prete, P., Fiorentino, A., Rizzo, L., Proto, A., Cucciniello, R., 2021. Review of aminopolycarboxylic acids–based metal complexes application to water and wastewater treatment by (photo-)Fenton process at neutral pH. Curr. Opin. Green Sustainable Chem. 28, 100451. https://doi.org/10.1016/j.cogsc.2021.100451.

Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on Cosmetic Products.

Rivas, Ibáñez G., Bittner, M., Toušová, Z., Campos-Mañas, M.C., Agüera, A., Casas López, J.L., Sánchez Pérez, J.A., Hilscherová, K., 2017. Does micropollutant removal by solar photo-Fenton reduce ecotoxicity in municipal wastewater? A comprehensive study at pilot scale open reactors. J. Chem. Technol. Biotechnol. 92, 2114–2122. https://doi.org/10.1002/jctb.5212.

Rizzo, L., 2022. Addressing main challenges in the tertiary treatment of urban wastewater: are homogeneous photodriven AOPs the answer? Environ Sci Water Res Technol 8, 2145–2169. https://doi.org/10.1039/D2EW00146B.

Royal Decree 3/23, January 10. Royal Decree 3/23, 20 of January 10, Establishing the technical-health Criteria for the Quality of Drinking Water, Its Control and Supply.

Rueda-Márquez, J.J., Levchuk, I., Manzano, M., Sillanpää, M., 2020. Toxicity reduction of industrial and municipal wastewater by advanced oxidation processes (Photo-Fenton, UVC/H2O2, electro-Fenton and Galvanic Fenton): a review. Catalysts 10, 612. https://doi.org/10.3390/catal10060612.

Sánchez Pérez, J.A., Belachqer-El Attar, S., Soriano-Molina, P., Casas López, J.L., García Sánchez, J.L., Agüera, A., Plaza-Bolaños, P., 2024. Composición Para la Purificación De Aguas. Fecha de solicitud: 23/03/2023. Fecha de publicación: 03/10/2024. 23.

Sánchez-Montes, I., Santos, G.O.S., Dos Santos, A.J., Fernandes, C.H.M., Souto, R.S., Chelme-Ayala, P., El-Din, M.G., Lanza, M.R.V., 2023. Toxicological aspect of water treated by chlorine-based advanced oxidation processes: a review. Sci. Total Environ. 878, 163047. https://doi.org/10.1016/j.scitotenv.2023.163047.

Schulze, T., Neale, P.A., Ahlheim, J., Beckers, L.-M., König, M., Krüger, J., Petre, M., Piotrowska, A., Schlichting, R., Schmidt, S., Krauss, M., Escher, B.I., 2024. A guidance for the enrichment of micropollutants from wastewater by solid-phase extraction before bioanalytical assessment. Environ. Sci. Eur. 36, 165. https://doi.org/10.1186/s12302-024-00990-x.

Side, D.D., Nassisi, V., Pennetta, C., Alifano, P., Di Salvo, M., Talà, A., Chechkin, A., Seno, F., Trovato, A., 2017. Bacterial bioluminescence onset and quenching: a dynamical model for a *quorum sensing* -mediated property. R. Soc. Open Sci. 4, 171586. https://doi.org/10.1098/rsos.171586.

Starling, M.C.V.M., Dos Santos, P.H.R., De Souza, F.A.R., Oliveira, S.C., Leão, M.M.D., Amorim, C.C., 2017. Application of solar photo-Fenton toward toxicity removal and textile wastewater reuse. Environ. Sci. Pollut. Res. 24, 12515–12528. https://doi.org/10.1007/s11356-016-7395-5.

Vijay, U., Gupta, S., Mathur, P., Suravajhala, P., Bhatnagar, P., 2018. Microbial mutagenicity assay: Ames test. BIO-Protoc 8. https://doi.org/10.21769/BioProtoc.2763.