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Research article

Evaluation of an alternative method for wastewater treatment containing pesticides using solar photocatalytic oxidation and constructed wetlands

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The present study proposes an integrated system based on the synergetic action of solar photocatalytic oxidation with surface flow constructed wetlands for the purification of wastewater contaminated with pesticides. Experiments were conducted at pilot scale using simulated wastewater containing the herbicide clopyralid. Three photocatalytic methods under solar light were investigated: the photo-Fenton and the ferrioxalate reagent as well as the combination of photo-Fenton with TiO_2 P25, which all led to similar mineralization rates. The subsequent treatment in constructed wetlands resulted in further decrease of DOC and inorganic ions concentrations, especially of NO_3^-. Clopyralid was absent in the outlet of the wetlands, while the concentration of the detected intermediates was remarkably low. These findings are in good agreement with the results of phytotoxicity of the wastewater, after treatment with the ferrioxalate/wetlands process, which was significantly reduced. Thus, this integrated system based on solar photocatalysis and constructed wetlands has the potential to effectively detoxify wastewater containing pesticides, producing a purified effluent which could be exploited for reuse applications.

1. Introduction

The uncontrolled use of pesticides during the 20th century due to widespread intensive agriculture, is polluting water resources. Although their necessity in agriculture is undeniable, pesticides can cause significant problems upon their release to the environment (Zhang and Pagilla, 2010). United Nations estimate that less than 1% of all pesticides used in agriculture actually reaches the crops. The remaining contaminates the land, the air and particularly the water. These xenobiotics are in many cases toxic and non-biodegradable, with the potential to cause adverse acute or chronic toxic health effects to non-target organisms and to accumulate in the environment through the global trophic network with unpredictable consequences (NATO, 2012). Increasing concern involves the importance of small residues of pesticides, often suspected of causing acute neurologic toxicity, chronic neurodevelopmental impairment, cancer and endocrine dysfunction (Arias-Estevez et al., 2008; Readman et al., 1993; Sharma et al., 2008; Thurman and Meyer, 1996).

Thus, the need for applying alternative methods for wastewater purification, is progressively becoming a priority for government agencies, regulatory agencies and the general public. However, the wide range of pesticides in use makes research extremely difficult for producing a single method for the removal of pesticides that applies universally (Kock-Schulmeyer et al., 2013). In view of this, it is necessary to develop sustainable technologies that promote the degradation of such bio-recalcitrant organic compounds.

Advanced oxidation processes (AOPs) are characterized by the production of hydroxyl radicals (OH•), one of the most powerful oxidants, which can easily attack organic molecules leading to the production of organic peroxide-radicals and their final conversion to CO_2, H_2O and inorganic species (Malato et al., 2009). AOPs often take place under mild operating conditions and are considered to be promising technologies, allowing the contribution of renewable sources of energy (solar energy) to the process of environmental cleaning and restoration. Among these, heterogenous and

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homogenous solar photocatalytic detoxification methods (TiO_2/H_2O_2/UV-A, Fe^{3+}/H_2O_2/UV-A, Vis) have gained great interest for the treatment of wastewater containing pesticides (Fagan et al., 2016).

Constructed wetlands on the other hand, are engineered systems that have been designed and constructed to utilize natural processes involving wetland vegetation, soils and their associated microbial assemblages to assist in treating wastewater. They are designed to take advantage of many of the processes that occur in natural wetlands, but do so within a more controlled environment (Vymazal and Bfezinova, 2015). They have the ability to efficiently treat a variety of wastewaters, removing organics, suspended solids, pathogens, nutrients and heavy metals, while their construction and maintenance cost is relatively low, since they are practically self-sufficient (Antoniadis et al., 2007; Papadimitriou et al., 2010).

In this work, a low cost treatment system based on the combination and the synergetic action of solar photocatalytic oxidation with surface flow constructed wetlands is developed (Fig. 1), to investigate its ability to be used as an alternative process of treating toxic, non-biodegradable pollutants, such as pesticides (Antoniadis et al., 2007; Arana et al., 2008). The positive interactions between solar photocatalysis and constructed wetlands in a combined system result, among others, to a reduction of establishment and operational costs, enhancement of biodegradability in wetlands, low concentrations of nitrate and phosphate ions, etc (Antoniadis et al., 2007). The current paper demonstrates the results from the experimental evaluation of this innovative system in the detoxification of simulated wastewater containing the herbicide clopyralid. Our group has already published data concerning photocatalytic mineralization of clopyralid in laboratory scale (Berberidou et al.). Clopyralid (3,6-dichloro-2-pyridine-carboxylic acid) is a systemic herbicide from the chemical class of pyridine compounds, often detected in drinking water (Donald et al., 2007). It is used to control annual and perennial broadleaf weeds in certain crops and turf and provides control of some brush species on rangeland and pastures. It may be persistent in soil under anaerobic conditions and with low microorganism content, with half-life ranging from 15 to more than 280 days (Corredor et al., 2006). It presents high solubility in water and is particularly stable against hydrolysis and photolysis. Its chemical stability, along with its mobility, enables this herbicide to penetrate through soil, causing a long term contamination of ground and surface water supplies (Huang et al., 2004; Sakaliene et al., 2009). Clopyralid is hazardous to a number of endangered plant species, beneficial insects (Hassan et al., 1994) and toxic to certain mammals (Hayes et al., 1984).

Many studies indicate that a wide range of pesticides are readily degradable by means of heterogenous or homogenous photocatalysis. Often, the reduction or elimination of ecotoxicity under the investigated conditions is also reported, employing microorganisms, i.e. *Vibrio fischeri, Daphnia magna etc.* Nonetheless, the potential residual toxicity of the treated wastewater on higher plants has rarely been investigated (Arana et al., 2008). This is a very important point since, the generated effluent, if effectively purified, could be exploited in reuse applications, i.e. irrigation. It has been clearly demonstrated in several studies that even if the parent compound is completely eliminated, the generated intermediates can be more toxic. In this study, after purification employing the integrated system photocatalytic oxidation/constructed wetlands, the use of higher plants (*Sorghum saccharatum*, *Lepidium sativum* and *Sorghum alba)* is reported for the evaluation of residual toxicity of wastewater containing clopyralid using a simple, sensitive and inexpensive test (Phytotoxkit, MicroBioTests Inc). According to our knowledge, this is the first report in which data concerning the reduction of phytotoxicity of clopyralid subjected to photocatalytic treatment, employing eukaryotic plants, are presented.

2. Materials and methods

2.1. Materials

Lontrel 100AS®, a commercial herbicide, (active ingredient: clopyralid, 10% w/v) was a product of Dow Agrosciences. Clopyralid (3,6-dichloro-pyridine-2-carboxylic acid, CAS No. 1702-17-6, M_r: 192 g mol^{-1}, Product No: 36758, Pestanal, analytical standard) was a product of Fluka (Sigma-Aldrich Laborchemicalien GmbH).

TiO_2 P25 was a product of Evonik Industries (anatase/rutile = 3.6/1, BET: 50 m^2 g^{-1}, nonporous). All other reagent-grade chemicals were purchased from Merck and were used without further purification.

2.2. Pilot scale photocatalytic experiments

Experiments were conducted at pilot scale using simulated

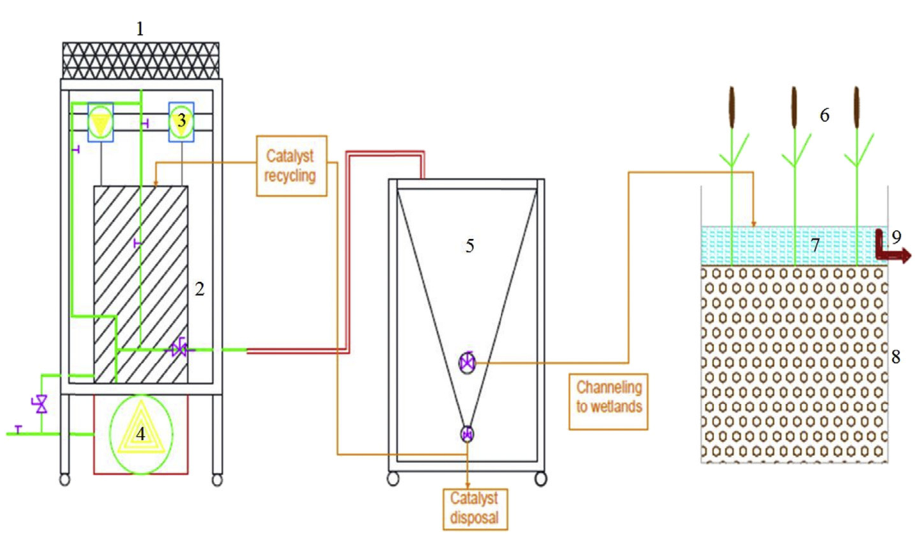

Fig. 1. Integration of solar photocatalysis with constructed wetlands for the purification of simulated wastewater containing pesticides: 1. photoreactor, 2. storage tank, 3. dosimetric pump, 4. pump, 5. Imhoff tank, 6. *Typha* spp. plants, 7. wastewater, 8. soil, 9. wetland outlet.

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wastewater containing clopyralid, prepared by appropriate dilutions of *Lontrel 100AS*® with tap water. Photocatalytic treatment was conducted under solar irradiation in a pilot-scale unit, able to treat 20 L of wastewater (Supplementary material, Fig. 1S). The unit constitutes of three parts: a) a photocatalytic, fountain type, reactor. The main idea is based on the design of six nozzles, through which the waste to be processed, enters the tank from the bottom of the unit, to the reactor. The nozzles create parallel, turbulent flow and vigorous stirring of the wastewater, which is exposed to solar light. Excess of the wastewater overflows and returns back to the tank and is then recirculated to the reactor by a pump. b) A reservoir located at the lower part of the reactor, for storing wastewater and c) an Imhoff type tank for separation of the treated effluent from the catalyst (Fig. 1). Solar light (UV-A) intensity was determined using a PMA 2100 pyranometer (Solar Light Company), equipped with a UV-A sensor (S/N 8773). Photocatalytic oxidation was conducted at a working volume of 15 L, at 3.1 ± 0.1 initial pH, after adjustment with H_2SO_4. The wastewater's temperature ranged from 25 to 32 °C depending on the month on which the experiment was performed. Experiments under solar irradiation took place in the region of Thessaloniki (latitude: 408620, longitude: 228950), Greece, from April to June 2015, where the UV-A intensity of solar light varied between 2.5 and 4.5 mW cm^{-2}.

2.3. Experiments in constructed wetlands

Three surface flow wetlands were constructed, according to the method suggested by EPA (Supplementary material, Fig. 2S) (EPA, 1998). The dimensions of each wetland were 60 cm × 30 cm × 50 cm. The water level was 10 cm above the soil surface, the hydraulic residence time of each wetland was 6 days and the hydraulic loading rate was 1.5 L d^{-1}. As wetland substrate a mixture of sandyloam soil and zeolite (5:1) was used. All wetlands were planted with *Typha spp*. macrophytes. The design characteristics for each wetland are presented in Table 1S of the Supplementary material. Constructed wetlands were supplied daily with 1.35 L of the photocatalytically treated simulated wastewater containing clopyralid, for 10 consecutive days, while samples were collected at the output of the wetlands on the 8th, 9th and 10th day. Experiments took place in the region of Thessaloniki (latitude: 408620, longitude: 228950), Greece, from April to June 2015.

2.4. Analytical procedures

Determination of dissolved organic carbon (DOC) was conducted according to standard methods by a total organic carbon (TOC) analyser (Shimandzu V_{CSH} 5000).

Inorganic ions were determined in a Shimadzu system consisting of a LC-10 AD pump, a CDD-6A conductometric detector (0.25 μL flow-cell) and a CTO-10A column oven. Cations were separated on an Alltech Universal column (100 mm × 4.6 mm) preceded by a guard column (7.5 mm × 4.6 mm) of the same material using 3 mM methanesulfonic acid at 1.5 mL min^{-1} constant flow. Anions were separated on an Alltech Allsep column (100 mm × 4.6 mm) preceded by a guard column (7.5 mm × 4.6 mm) of the same material using a phthalic acid and lithium hydroxide mixture of 4 mM (pH 4.00) at 1.5 mL min^{-1} constant flow. Column and conductivity cell temperatures were held constant at 35 °C and 38 °C, respectively. Mobile phases were degassed with helium stream before LC analysis. Calibration curves (0.01–10 mg L^{-1}) were constructed for each ion, while their detection limits (LOD) are presented in Table 2.

Some photocatalytic experiments were repeated three times to check the reproducibility of the experimental results. The accuracy of DOC values and of the inorganic ions analysis was ±10%.

Clopyralid and its transformation products (TPs) were determined by a Shimadzu liquid chromatograph with a photodiode array (PDA) detector coupled in series with a mass spectroscopy (MS) detector (LC-MS-2010 EV) equipped with an atmospheric pressure electrospray ionisation source (ESI). The high-performance liquid chromatography (HPLC) system consisted of a SIL 20A autosampler with the volume injection set to 20 μL and LC-20 AB pump both from Shimadzu (Kyoto, Japan). The column used was a CNW HPLC Athena C18 column, 4.6 mm × 250 mm and 5 μm pore size. Chromatographic conditions, MS parameters, quality assurance and quality control for LC-DAD-ESI/MS analysis have been described in our recent study (Berberidou et al.).

2.5. Phytotoxicity evaluation

Phytotoxicity measurements of clopyralid subjected to photocatalytic treatment were carried out using the standard Phytotoxkit microbiotest (MicroBioTests Inc., Belgium), a commercial toxicity bioassay based on three species of higher plants: the monocotyl sorgho *Sorghum saccharatum* and the dicotyls garden cress *Lepidium sativum* and mustard *Sorghum alba*. This microbiotest, measures both the decrease/absence of seed germination and the decrease of root growth after 3 days of exposure of the seeds to the sample.

For this purpose, 90 cm^3 of reference soil were added in the lower compartment of a test plate (delivered by manufacturer) and hydrated with 35 mL of the sample. A control test was performed using identical volumes of distilled water. The surface of the soil was flatten and covered with paper filter. The tests were carried out in three replicates for each sample and for each type of plant. Prepared Phytotoxkit plates were incubated at 25 °C for 3 days. Digital pictures of the plates were analyzed using ImageJ v1.49 (Wayne Rasband, National Institutes of Health, USA) software. For a comprehensive interpretation of the data, the length of the roots was measured. Average values were used for final calculation of the

Table 1
Concentrations of inorganic nitrogen species and phosphate ions of the simulated wastewater containing clopyralid during the treatment phases of the combined system in the presence of photo-Fenton, ferrioxalate and TiO_2/photo-Fenton (7 mg L^{-1} Fe^{3+}, 100 mg L^{-1} H_2O_2, 33 mg L^{-1} $C_2O_4^{-2}$, 0.5 g L^{-1} TiO_2 P25). Initial clopyralid concentration: 40 mg L^{-1}.

Photocatalytic method/treatment stage	NO_3^- (mg L^{-1})	NO_2^- (mg L^{-1})	NH_4^+ (mg L^{-1})	PO_4^{3-} (mg L^{-1})
Photo-Fenton/photocatalysis inflow	1.80	0.46	1.49	0.36
Photo-Fenton/photocatalysis outflow	23.47	Non detected	1.86	0.15
Photo-Fenton/wetlands outflow	2.58	0.43	Non detected	0.61
Ferrioxalate/photocatalysis inflow	1.91	0.38	1.72	0.41
Ferrioxalate/photocatalysis outflow	16.39	0.07	3.37	Non detected
Ferrioxalate/wetlands outflow	2.10	0.04	Non detected	0.74
TiO_2-photo-Fenton/photocatalysis inflow	1.73	0.48	1.66	0.32
TiO_2-photo-Fenton/photocatalysis outflow	20.37	0.03	1.95	1.32
TiO_2-photo-Fenton/wetlands outflow	6.93	0.34	Non detected	1.13

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Table 2
Chemical parameters determined at the outflow of the combined system in comparison to the limits determined by Greek legislation concerning the disposal of wastewater at Thermaikos gulf (prefectural decision 22374/91/94) and the European directive concerning drinking water quality.

Parameter	Unit	LOD (mg L^{-1})	Photo-Fenton/ wetlands outflow	TiO_2-photo-Fenton/ wetlands outflow	Ferrioxalate/ wetlands outflow	Limits	
						European directive (drinking water)	Thermaikos gulf (prefectural decision 22374/91/94)
pH	–	–	7.85	7.81	8.17	≥6.5 and ≤ 8.5	6–9
Specific conductivity	μS cm^{-1}	–	869.78	1221.56	1194.78	2500	
Total hardness	°F	–	23.73	21.67	88.68	500	
Sodium ions (Na^+)	mg L^{-1}	0.06	102.95	121.20	167.90	200	
Ammonium ions (NH_4+)	mg L^{-1}	0.03	0.18	Non detected	Non detected	0.5	
Potassium ions (K^+)	mg L^{-1}	0.13	6.56	5.12	25.41	Not affecting	
Calcium ions (Ca^{2+})	mg L^{-1}	0.38	82.58	75.55	199.94	Not affecting	
Magnesium ions (Mg^{2+})	mg L^{-1}	0.15	7.55	6.81	14.36	Not affecting	
Fluoride ions (F^-)	mg L^{-1}	0.09	0.20	0.26	0.48	1.5	30
Chloride ions (Cl^-)	mg L^{-1}	0.06	29.13	80.21	31.98	250	
Nitrite ions (NO_2^-)	mg L^{-1}	0.16	0.43	0.34	0.04	0.5	5
Nitrate ions (NO_3^-)	mg L^{-1}	0.03	2.58	6.93	2.10	50	30
Phosphate ions (PO_4^{3-})	mg L^{-1}	0.17	0.61	1.13	0.74	7	92
Sulphate ions (SO_4^{3-})	mg L^{-1}	0.07	126.73	179.17	184.18	250	1000
Total dissolved solids (TDS)	mg L^{-1}	–	460.67	645.67	640.67	1500	–

percentage inhibition of root growth, according to the following equation:

$$\frac{A-B}{A} \times 100 = \text{inhibition}(\%),$$

where.

A: mean root length in the control water and
B: mean root length in the test sample.

3. Results and discussion

3.1. Mineralization experiments

Initially, photocatalytic oxidation of the simulated wastewater containing clopyralid in the fountain-type photoreactor was performed, aiming to the degradation of the ingredients of the wastewater that contains, among others, 40 mg L^{-1} clopyralid and to the reduction of the total organic content, under solar irradiation. The initial dissolved organic carbon (DOC) of the simulated wastewater ranged between 25 and 28 mg L^{-1}. Three different photocatalytic systems were investigated: the photo-Fenton reagent (7 mg L^{-1} Fe^{3+}, 100 mg L^{-1} H_2O_2), the ferrioxalate reagent (7 mg L^{-1} Fe^{3+}, 100 mg L^{-1} H_2O_2, 33 mg L^{-1} $C_2O_4^{-2}$) and the combination of photo-Fenton with TiO_2 P25 (3.5 mg L^{-1} Fe^{3+}, 100 mg L^{-1} H_2O_2, 33 mg L^{-1} $C_2O_4^{-2}$, 0.5 g L^{-1} TiO_2 P25) (Fig. 2). Application of TiO_2 mediated photocatalysis alone or in the presence of H_2O_2 led to particularly low degradation and mineralization rates and was thus, not further investigated. The optimal conditions employed in the present study (irradiated volume, Fe^{3+} and H_2O_2 concentrations, etc) were determined in preliminary experiments (data not shown). The fundamentals of homogenous photocatalysis, mediated either by the photo-Fenton or the ferrioxalate reagent are thoroughly presented in several review articles (Jack et al., 2015; Malato et al., 2009).

The combination of TiO_2 and photo-Fenton is known to have beneficial effects, mainly attributed to interactions between iron species and TiO_2 (Nahar et al., 2009). There are three parallel processes of OH• generation in the system that include iron species, which may be described by the following reactions. The first is a photocleavage of Fe^{3+} hydroxoaqua complex (Eq. (1)), the second one is the dark Fenton reaction (Eq. (2)) and the third is the reaction of adsorbed Fe^{3+} with the photogenerated electrons (Eq. (3)) (Mestankova et al., 2009):

$$Fe(OH)^{2+} + h\nu \rightarrow Fe^{2+} + OH^{\bullet} \quad (1)$$

$$Fe^{2+} + H_2O_2 + H^+ \rightarrow Fe^{3+} + OH^{\bullet} + H_2O \quad (2)$$

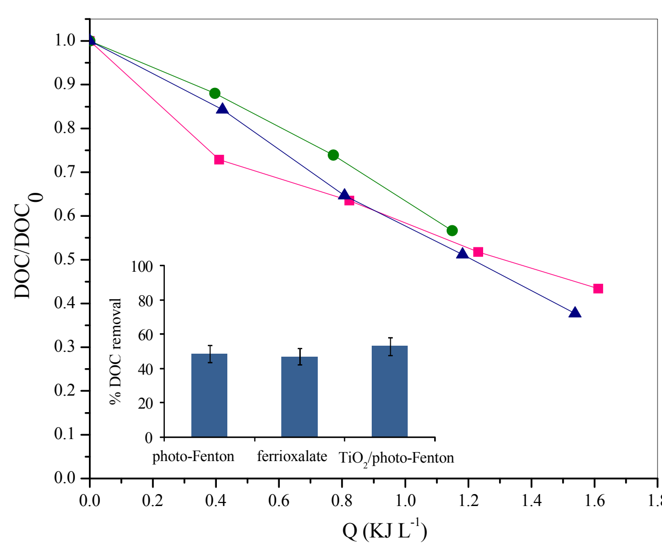

Fig. 2. Reduction of dissolved organic carbon (DOC) of the simulated wastewater containing clopyralid vs solar energy density, Q, for three photocatalytic systems: (■) photo-Fenton, (●) ferrioxalate and (▲) TiO_2/photo-Fenton (7 mg L^{-1} Fe^{3+}, 100 mg L^{-1} H_2O_2, 33 mg L^{-1} $C_2O_4^{-2}$, 0.5 g L^{-1} TiO_2 P25). Inset: % DOC reduction within 60 min of solar illumination employing the three photocatalytic systems. Initial clopyralid concentration: 40 mg L^{-1}.

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$$Fe^{3+} + TiO_2(e^-) \rightarrow Fe^{2+} + TiO_2 \quad (3)$$

This last reaction lowers the recombination rate of photogenerated holes and electrons resulting in an enhancement of the concentration of OH• radicals (Ohno et al., 1997). Electron trapping by soluble Fe^{3+} aggregates has a positive influence on this synergy because such species are easily reduced onto the TiO_2 surface. The synergetic effect of the $TiO_2/Fe^{3+}/H_2O_2$ system is also affected by the concentration of TiO_2, iron species, the organic substrate studied etc (Lam et al., 2005; Mrowetz and Selli, 2004). Here, the addition of 3.5 g L^{-1} TiO_2 P25 led to a small increase of the % DOC removal from 48.6 to 53% within 60 min of illumination, in comparison to the photo-Fenton system alone, while the concentration of Fe^{3+} used was lowered to half (Fig. 2). Meštankova et al. showed that it is possible to use more than a ten times lower concentration of TiO_2 in the presence of Fe^{3+} to reach similar efficiencies concerning the degradation of monuron in comparison to TiO_2 alone (Mestankova et al., 2005). It is well evidenced that in the $Fe^{3+}/TiO_2/H_2O_2$ process, the adsorption of Fe^{3+} onto titania is the key reaction leading to synergy (Cernigoj et al., 2010).

After 60 min of solar illumination, in all cases, the organic load of the wastewater was reduced to a value of ~12–15 mg L^{-1} DOC, which was the desirable inflow concentration for the constructed wetlands according to the design of the system. The wastewater was, then, channeled into the Imhoff tank, where pH was adjusted to 6.5–7.5, to aid separation of the catalyst (TiO_2 P25 and/or Fe^{3+}) from the liquid phase. After catalyst discharge, the wastewater was channeled to the constructed wetlands for further purification.

Fig. 3 shows the variation of dissolved organic carbon (DOC) at the different stages of the solar photocatalytic oxidation/constructed wetlands system. The decrease of organic load in total reaches 93, 92 and 87%, while photocatalytic oxidation itself leads to a 49, 47 and 53% DOC reduction in the case of photo-Fenton, ferrioxalate and TiO_2 P25-photo-Fenton, respectively, within 60 min of illumination. Subsequently, constructed wetlands lead to an additional DOC reduction in all three cases, proving its beneficial contribution to the removal of the organic content of the simulated wastewater. DOC reduction in constructed wetlands may be attributed to microbial degradation and chemical and physical processes in a network of aerobic, anoxic and anaerobic zones with aerobic zones being restricted to the areas adjacent to roots where oxygen leaks to the substrate, or to substrate adsorption (Vymazal, 2010). It must be noted that the DOC values of the effluent at the outflow of the constructed wetlands are considerably lower than the limits defined by Greek legislation concerning the disposal of wastewater at Thermaikos gulf (prefectural decision 22374/91/94).

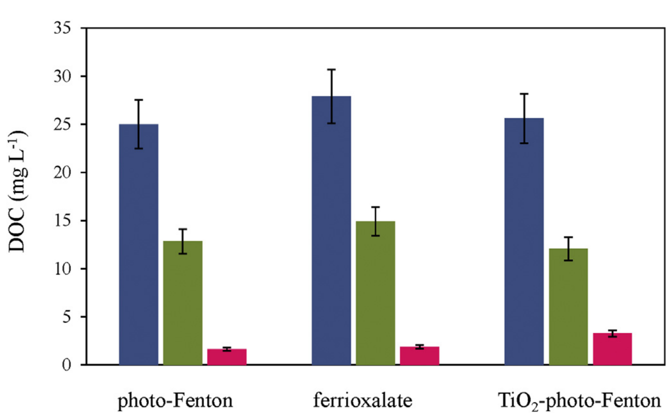


Fig. 3. Dissolved organic carbon of simulated wastewater containing clopyralid in the presence of solar irradiation and photo-Fenton, ferrioxalate or TiO_2-photo-Fenton (7 mg L^{-1} Fe^{3+}, 100 mg L^{-1} H_2O_2, 33 mg L^{-1} $C_2O_4^{-2}$, 0.5 g L^{-1} TiO_2 P25) during the treatment phases of the combined system (■): photocatalysis inflow (■), photocatalysis outflow and (■) wetlands outflow. Initial clopyralid concentration: 40 mg L^{-1}.

To investigate what happens to the simulated wastewater that contains 40 mg L^{-1} clopyralid as it is being treated by the combined system, besides the DOC reduction, samples were analyzed with respect to the release of nitrogen species and phosphate ions in the reaction mixture (Table 1). Concerning nitrate ions, their concentration was increased in all cases during photocatalytic oxidation, regardless of the method employed, due to the mineralization of the organic nitrogen. The followed treatment by constructed wetlands decreased, as expected, their concentration, leading to a 89, 87 and 66% reduction in the case of photo-Fenton, ferrioxalate and TiO_2 P25-photo-Fenton, respectively, compared to the outflow of photocatalytic oxidation. It is well known that inorganic nitrogen species may serve as nutrients in constructed wetlands during photosynthetic processes, however, these phenomena are limited in the period of root growth and development of the plants. Overall, inorganic nitrogen species removal is mainly attributed to nitrification/denitrification processes and to a lesser extend to plant intake (Reed et al., 1995).

As far as ammonium ions are concerned, no significant changes in their concentration is observed during the treatment of the simulated wastewater with the combined system. Their concentration in the outflow of the three photocatalytic systems is slightly increased to values which in all cases do not exceed 3.37 mg L^{-1} and is then decreased to values lower than the LOD of the method, resulting to 100% removal rates at the outflow of the combined system. The main process of ammonium removal in surface and subsurface wetlands is biological nitrification followed by denitrification. Although nitrification usually takes place under aerobial conditions, it may also occur in conditions with relatively low dissolved oxygen (Kadlec, 1996). Moreover, ammonium is exploited by plants and microorganisms for the development of new biomass. Reduction of NH_4^+ levels, to a lower extend, may be attributed to other phenomena like plant intake, conversion of NH_4^+ to NH_3 and adsorption in the pores of the substrate zeolite (Brix, 1993).

In the case of nitrite and phosphate ions, their concentrations were very low in all steps of the treatment, thus, any variation in their concentration is considered to be negligible. Furthermore, the values of other chemical parameters determined at the constructed wetlands outflow were in all cases lower than the limits defined by Greek legislation concerning the disposal of wastewater at Thermaikos gulf (prefectural decision 22374/91/94), as well as than the limits defined by the European directive concerning water for human consumption (European Council, 1998) (Table 2).

3.2. Transformation products

In order to further evaluate the efficiency of the integrated solar photocatalysis/constructed wetlands system in the purification of simulated wastewater containing clopyralid, the determination of potential transformation products (TPs) by liquid chromatography, with diode array detection and electrospray ionization mass spectrometry (LC-DAD-ESI-MS) was conducted. Clopyralid was already absent in the outlet of the pilot photocatalytic reactor regardless of the method employed, as well as in the outlet of the constructed wetlands, indicating that the final effluent is, indeed, free of the parent herbicide molecule (Table 3). Among the TPs, TP-207 which yielded a mass at m/z 208, was found in both the inflow and the outflow of the wetlands (Table 3). TP-207 originated from the addition of a hydroxyl group at the *para* position of the pyridine ring (TP-207-3,6-dichloro-4-hydroxypicolinic acid). TP-173, which yielded a mass at m/z 174, was detected in both the inflow and the outflow of the ferrioxalate/wetlands system, whereas in the case of photo-Fenton/wetlands and TiO_2-photo-Fenton/wetlands systems this TP was present only in the outlet of the wetlands. TP-173 can be formed by the elimination of one chlorine atom of the clopyralid molecule with subsequent addition of a OH group, to form a dechlorinated-hydroxylated product, namely 6-chloro-3-hydroxypicolinic acid. Detailed description for these TPs has been previously reported in our recent study (Berberidou et al.).

The amount of the hydroxylated TP-207 was further decreased at the outflow of the photo-Fenton/wetlands, ferrioxalate/wetlands and TiO_2-photo-Fenton/wetlands systems (45.93, 26.61 and 48.25%, respectively) in relation to the respective ones at the inflow of the wetlands, suggesting that this compound may be further transformed, forming second generation products (Table 3). The reduction in the concentration of TP-207 in constructed wetlands may be attributed to the indirect effect of *Typha* spp. plants. The formation of a particular macroclimate around the root area supports the growth of specific populations of microorganisms, which decompose xenobiotic organic matter, using it as a source of carbon and nitrogen, either they metabolize it via the co-metabolism phenomenon (Lv et al., 2016; Runes et al., 2003). The substrate consisting of sandy loam and zeolite possibly contributes to the reduction of the concentration of TP-207, due to its' absorption onto the active sites in these materials. The elimination of organic matter in wetland systems can, also, be attributed to plant-uptake or sorption processes (Vymazal and Bfezinova, 2015). The same decreasing pattern was also observed for the TP-173, in the case of the ferrioxalate/wetlands treatment, while in the cases of photo-Fenton/wetlands and TiO_2-photo-Fenton/wetlands processes, this product was found at trace levels only at the outflow of constructed wetlands (Table 3).

3.3. Phytotoxicity evaluation

The evaluation of potential phytotoxicity of the samples during the various stages of treatment with the integrated system solar photocatalysis (Ferrioxalate: 7 mg L^{-1} Fe^{3+}, 33 mg L^{-1} $C_2O_4^{2-}$, 100 mg L^{-1} H_2O_2)/constructed wetlands (*Typha* spp*)*, was conducted by employing three higher plant species (*Sinapis alba, Lepidium sativum* and *Sorghum saccharatum*). Growth inhibition was not observed in any of the three types of seeds, therefore the study was focused on the calculation of root inhibition (RI).

The RI profile of the simulated wastewater in the presence of ferrioxalate and solar irradiation, is shown in Fig. 4. The RI of the initial wastewater containing 40 mg L^{-1} clopyralid displayed a slight variation between the three types of plants (*Sorghum saccharatum:* 50.90%, *Sinapis alba:* 52.42 and *Lepidium sativum:* 57.59). Clearly, the untreated simulated wastewater exhibits a noteworthy degree of initial phytotoxicity, in comparison to the one of distilled water (0% RI). At photocatalysis outflow, the RI values demonstrated for all types of plants, a significant decrease in comparison to the initial clopyralid solution (63.7, 75.2 and 82.15% for *Sorghum saccharatum*, *Sinapis alba* and *Lepidium sativum*, respectively), resulting in all cases in RI values ranging from 10.34 to 18.47%. This reduction of the RI values may be owed, mainly, to the complete degradation of clopyralid by the ferrioxalate reagent. At the outflow of the wetlands, RI showed a subsequent reduction for all three types of plants, possibly due to further degradation of TP-208 and TP-173, that were generated during the photocatalytic process (Table 3). These findings demonstrate that phytotoxicity is significantly reduced after the use of solar photocatalysis/constructed wetlands, highlighting the efficiency of this integrated system in the reduction of phytotoxicity of wastewater containing

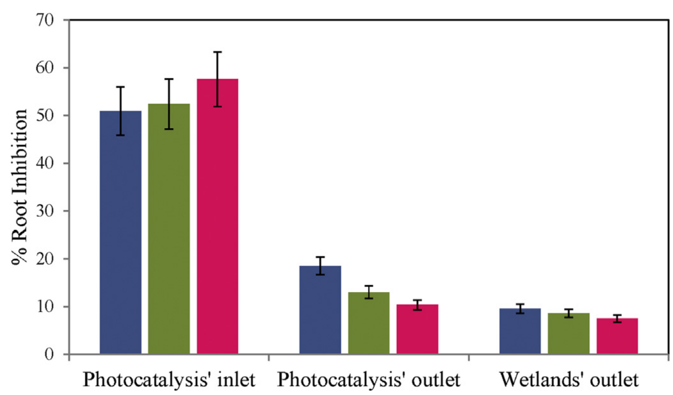
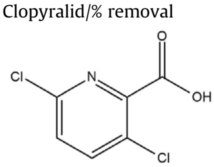
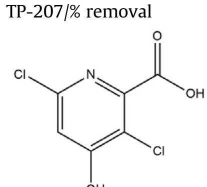
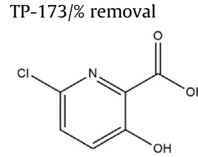


Fig. 4. Effect of simulated wastewater containing clopyralid to the length of the root system of the plants (■): *Sorghum saccharatum* (■), *Sinapis alba* and (■) *Lepidium sativum*, during treatment employing the integrated system solar photocatalysis (ferrioxalate: 7 mg L^{-1} Fe^{3+}, 33 mg L^{-1} $C_2O_4^{2-}$, 100 mg L^{-1} H_2O_2)/constructed wetlands (*Typha spp.).* Initial clopyralid concentration: 40 mg L^{-1}.

Table 3
Clopyralid and its' transformation products (TPs) identified during treatment in the combined solar photocatalysis/constructed wetlands system. (+) or (−) indicate the presence or absence, respectively, of the three compounds during the treatment phases of the combined system. Conditions: 7 mg L^{-1} Fe^{3+}, 100 mg L^{-1} H_2O_2, 33 mg L^{-1} $C_2O_4^{-2}$, 0.5 g L^{-1} TiO_2 P25, *Typha spp.* plants.

Process/treatment phase	Clopyralid/% removal	TP-207/% removal	TP-173/% removal
	 3,6-dichloro-pyridine-2-carboxylic acid	 3,6-dichloro-4-hydroxypicolinic acid	 6-chloro-3-hydroxypicolinic acid
Photo-Fenton/photocatalysis inflow	+	−	−
Photo-Fenton/photocatalysis outflow	−	+	−
Photo-Fenton/wetlands outflow	−	+/45.93[a]	+
Ferrioxalate/photocatalysis inflow	+	−	−
Ferrioxalate/photocatalysis outflow	−	+	+
Ferrioxalate/wetlands outflow	−	+/26.61[a]	+/46.91[a]
TiO_2-photo-Fenton/photocatalysis inflow	+	−	−
TiO_2-photo-Fenton/photocatalysis outflow	−	+	−
TiO_2-photo-Fenton/wetlands outflow	−	+/48.25[a]	+

[a] (%) removal in wetlands outflow was calculated in relation to photocatalysis outflow measurements.

clopyralid.

4. Conclusions

The efficiency of a system combining solar photocatalysis with horizontal flow constructed wetlands aiming to the detoxification of simulated wastewater contaminated with the herbicide clopyralid, was investigated. Three different photocatalytic systems were investigated: the photo-Fenton and the ferrioxalate reagent as well as the combination of photo-Fenton with TiO_2 P25, which all resulted to similar mineralization efficiences. Clopyralid was already absent in the outlet of the pilot reactor, regardless of the photocatalytic method employed. The detected intermediates TP-207 and TP-173 were found at trace levels at the outflow of the three systems. Phytotoxicity of the wastewater after treatment with the ferrioxalate/wetlands process, evaluated by the calculation of the RI of higher plant species, was significantly reduced. Our findings highlight the potential of this integrated system to effectively purify wastewater containing pesticides according to the principles of sustainable treatment technologies, able to be either safely discharged, according to Greek legislation, or to be reused (i.e. irrigation, firefighting, etc). The exploitation of solar energy is an important advantage of the proposed system, especially for countries with abundant sunlight. It must be noted, however, that the specific operational conditions proposed here must be redefined in real applications, based on the characteristics of the specific wastewater.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jenvman.2016.06.010.

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