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Article

Office Paper-Based Electrochemical Strips for Organophosphorus Pesticide Monitoring in Agricultural Soil

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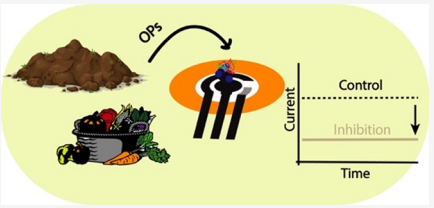
ABSTRACT: Although the use of pesticides has highlighted obvious advantages on agricultural yields, intensive and widespread pesticide use raises serious environmental and health concerns. In particular, organophosphate pesticides represent >40% of the totality used in the field of agriculture, and developing countries face the issue of agricultural poisoning, also due to scarce monitoring programs. In this work, a decentralized, miniaturized, sustainable, and portable paper-based electrochemical biosensor for the quantification of organophosphorus pesticides' level has been realized. The proposed approach highlights the use of a very common paper-based substrate, namely, office paper. Office paper offers several advantages due to its nature: it allows one to print conductive strips for electrochemical connection, loading bio-hybrid nanosized probes (Prussian blue, carbon black, and butyrylcholinesterase), evaluating pesticides and reducing waste disposal compared to plastic-based strips. The portable system has been characterized by a low detection limit of 1.3 ng/mL, and accordingly to total discovered pesticide contents in EU agricultural soils, up to ca. 3 μg/mL, it can offer a valuable tool for fast monitoring. To demonstrate its effectiveness, soil and fruit vegetables have been used to perform in situ quantification. Good recovery percentages between 90 and 110% have been achieved in different matrices, highlighting to be suitable for field measurements, and a good correlation has been obtained in comparison with LC−MS analysis.



KEYWORDS: *office paper, electroanalysis, wax printing, screen-printing, inhibition, paraoxon-ethyl, soil*

INTRODUCTION

Nowadays, phytomolecules, such as pesticides, herbicides, and insecticides, are largely used driven by the necessity in improving the agricultural production.[1,2] However, it should be noted that even if the use of these toxic compounds is capable of ensuring the quality and quantity of food products, a large amount of toxic species is dispersed in the environment.[3] The distance between the environment and humans is not far, and the risk associated with the use of contaminants is high. Although the one of the Goal 3 of UN Sustainable Development Goals is the following "by 2030, substantially reduce the number of deaths and illnesses from hazardous chemicals and air, water, and soil pollution and contamination,"[4] and the directive 2009/128/EC concentrates on the achievement of a sustainable use of pesticides in the EU through the reduction of risk and impact on human health and the environment,[5] the total amount of pesticides used in 2015 is ca. 3.5 M t/y world-wide of which 10% was in Europe.[6] A recent study on more than 300 European agricultural soils highlighted that more than 80% of them contained pesticide residues. In particular, the maximum individual pesticide content assessed in a soil sample is ca. 2 mg/kg, while the maximum total pesticide content was almost 3 mg/kg. High levels of pesticides in soils are the rule rather than the exception: it indicated the necessity to implement monitoring programs for better environmental risk assessment procedures.[7] In particular, organophosphate pesticides represent the 40% of the totality in the field of agriculture.[8] Presently, developing countries have been facing the issue of agricultural poisoning, and organophosphorus pesticide poisoning is the most common among global public health; e.g., worldwide, they are estimated to cause 300 k fatalities annually.[9] Several studies involving developing countries have demonstrated levels of organophosphorus pesticide residues in soils and drinking water sources exceeding regulations.[10,11] For instance, two separate studies that have been carried out in Thailand (where regulations exist but are scarcely enforced) demonstrated high levels of organophosphates in soil[12] (up to 90 mg/kg during winter) and fruits[13] (up to ca. 80 μg/kg). Other measurements in persimmon/jujube soils collected from China highlighted that chlorpyrifos was the most often found organophosphate in the soils, with levels up to ca. 25.5 μg/kg.[14] In addition, a recent US study on organophosphate pesticide residue monitoring, in agricultural settings, has demonstrated that >80% of the time, the pesticide residue level is higher than the maximum residue level.[15]

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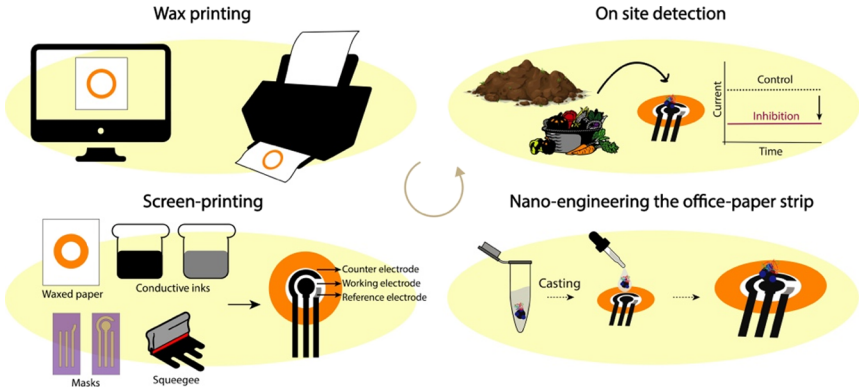
Scheme 1. Schematic Representation of the Manufacture of the Office Paper-Based Electrochemical Strip (Wax Printing and Screen-Printing), the Nanoengineering with the Electrocatalyst (Prussian Blue), Conductivity Enhancer (Carbon Black), and Sensing Bioelement (BChE), and the Typical Amperometric Inhibition-Based Response in the Presence of Organophosphate Pesticides



Good management practices and the implementation of point-source monitoring systems, accessible to farmers and easy to operate, can reduce the reducing pesticides' impact on the environment and health. Although several approaches, mainly including gas chromatography−mass spectrometry and liquid chromatography−mass spectrometry (LC−MS), are capable of quantifying pesticides with high sensitivity and specificity, the requirement for time-consuming workflows and expensive instruments represent major drawbacks for rapid and decentralized analysis.[16−18]

To overcome limitations due to laboratory-bound methods, other methods such as colorimetric, electrochemical, and fluorometric biosensors, nano/micromotors, and enzyme-linked immunosorbent as-say have been developed.[19−22] Among them, the electrochemical ones appear the most prone to in-situ application; in fact, compared to other architectures, the electro-chemical methods are not influenced from the turbidity and the matrix color, which is often the cause of interference.[23,24] The development of portable analytical methods is essential to ensure the quality of environmental sites and to protect human health from contaminant exposure and ingestion. Of course, novel solutions should be in agreement with the proposed sustainable development goals: the reduction of plastic and solvents, the use of low amounts of samples, and the easiness of application. To this regard, the use of smart materials for the sustainable manufacture of diagnostic tools is highly recommended, and the research on paper-based substrates for creating novel analytical solutions is providing satisfactory answers in many fields of action, from environmental to clinical, through the agri-food one.[25−29]

Although other paper-based platforms have been developed for electrochemical pesticide determination,[30,31] this work represents the first development uniquely based on the use of common office paper. With respect to the most commonly utilized Whatman No.1 chromatographic paper, office paper has the advantage of being available everywhere (not only in the laboratory settings) and cheaper (ca. 70-fold).[32]

Office paper merges together a biological sensing element (cholinesterase enzymes are inhibited by organophosphates), carbonaceous nanoaggregates (to enhance the electrical conductivity at the printed strip), and enzyme-like Prussian blue (to evaluate the enzymatic activity). These three components have been merged together in the same drop of hybrid-nanomodifiers, for the first time. The developed platform has been challenged to measure the presence of an organophosphate model directly in a small farm in the area of Naples (Italy). The adoption of a user-friendly equipment (laptop and portable reader) and convenient procedures has allowed us to quantify paraoxon in diverse matrices in the low ng/mL range, and the results have been positively compared with a validated LC−MS/MS method.

MATERIALS AND METHODS

Reagents and Equipment. Potassium ferricyanide, iron-(III) chloride, butyrylcholinesterase (BChE) from equine serum, butyrylthiocholine chloride, and paraoxon-ethyl have been bought from Merck, USA. Carbon black (CB) VXC72R powder has been provided by Cabot Corporation, Italy. All the species that have been tested for interference studies have been bought from Fluka (metal solutions for atomic adsorption experiments) and from Merck. Electro-chemical measurements have been carried out with the use of a portable potentiostat, EmStat[3] (PalmSens, Netherlands) connected to a laptop.

Strip Production. The manufacture of the printed strips is composed by wax printing and screen-printing (Scheme 1). An ad-hoc pattern is designed and printed onto common office paper with wax-based inks, using a solid ink printer, namely, ColorQube 8580 (Xerox, USA). This step is necessary to define an area of testing, avoiding aqueous solution diffusing toward the electrical connector and affecting the readout. Successively, electrodes are manually screen-printed using silver/silver chloride and graphite conductive inks, respectively, to obtain reference and working (and counter) electrodes.[33]

Strip Nanoengineering. The printed strips have been successively modified with the use of drop casting method-ology.[34] A mixture composed of CB, Prussian blue (PB), and BChE has been drop-cast on top of the working electrode. The detailed concentrations are reported in the Experimental Section.

Pesticide Quantification. The level of pesticides is obtained by measuring the change of the BChE activity in the presence of the inhibitor.[35,36] Briefly, the working area of the electrode, where the hybrid nanocomposite is adsorbed, is covered with 10 μL of a solution containing the inhibitor. After several minutes, the enzymatic activity is calculated by measuring the amount of the electroactive byproduct of the BChE substrate, namely, thiocholine. The current recorded in

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the presence and in the absence of the inhibitor is used to calculate the inhibition degree that is proportional to the inhibitor concentration.

Soil Spiking. Paraoxon-ethyl (liquid) has been added to 100 g of soil in a tube. The mixture of spiked soil has been shaken vigorously by hand for 10 min. Successively, 100 mL of aqueous solution (2% ethanol) has been added to the spiked samples. The tube has been shaken vigorously by hand and with the use of a vortex for 20 min. The test tube has been filtered with the use of a MF-Millipore Membrane Filter (0.45 μm), and the liquid phase has been directly drop-cast onto the paper-based biosensor that contained all the reagents necessary for the assay.

LC−MS/MS Analysis. To compare the effectiveness of the proposed method, paraoxon-ethyl has been measured in collaboration with Acea Elabori SPA (Rome, Italy) using the accredited method ISTISAN 19/7 CAC 015 utilizing the following instruments: ExionLC Series UHPLC (Sciex, USA) and mass spectrometer with a tiple quadrupole 5500 Q-Trap equipped with an ESI source (Sciex, USA).

RESULTS AND DISCUSSION

Electrochemical Optimization. Office paper offers a valuable substrate for producing electrochemical sensors and biosensors as reported in the literature.[37−40] However, before starting with the development of a sensing device, the quality of the printed strips needs to be evaluated to ensure a satisfactory starting point. The use of cyclic voltammetry, in the presence of a known electrochemical mediator, has allowed us to get a quick response. The electrochemical effectiveness of office paper-based strips has been evaluated performing cyclic voltammetry in the presence of 5 mM potassium ferricyanide. Experiments have been performed in solutions varying the scan rate in the range between 20 and 500 mV/s. As displayed in the Supporting Information (Figure S1), the presence of two similar peaks, namely, anodic and cathodic, is consistent with a typical reversible electrochemical behavior. As is clearly visible, the cathodic and anodic voltammetric peaks are linear to the square root of the scan rate as described in the Randles−Sevcik equation for quasi-reversible electron transfer processes: the calculated slope for both anodic and cathodic peaks is similar within the experimental errors, respectively, 0.18 ± 0.3 and 0.16 ± 0.2 mA/$v^{0.5}$. This behavior is consistent with a so-called semi-infinite diffusion of the electroactive species at the electrode surface: this means that the target is able to diffuse and to reach the electrode surface without entrapment due to the paper-based structure, which could alter the future quantification of the pesticide.[41−43] Since the good quality of strips printed onto office paper has been verified, the biosensor has been developed. The quantification method of paraoxon-ethyl is based on the irreversible inhibition of a cholinesterase enzyme, namely, BChE. BChE hydrolyzes its substrate, butyrylcholine, giving byproducts butyric acid and choline. Unfortunately, both the byproducts are not electroactive; then, the enzymatic activity cannot be observed at the printed electrochemical device. For this reason, butyrylthiocoline has been chosen as the enzymatic substrate: this allows us to byproduce thiocholine that is an electroactive compound that can be electrochemically revealed. A nanohybrid composite made with the combination of BChE, PB, and CB has been chosen because (i) BChE produces thiocholine, PB is sensitive to its oxidation at less interfering potential, and CB has the double function of hosting the composite and improving conductivity.[44−46] Thus, the biosensor built onto office paper has been optimized to obtain satisfactory sensitivity and repeatability. As shown in Figure 1, the amount of PB/CB and the working potential have been optimized.

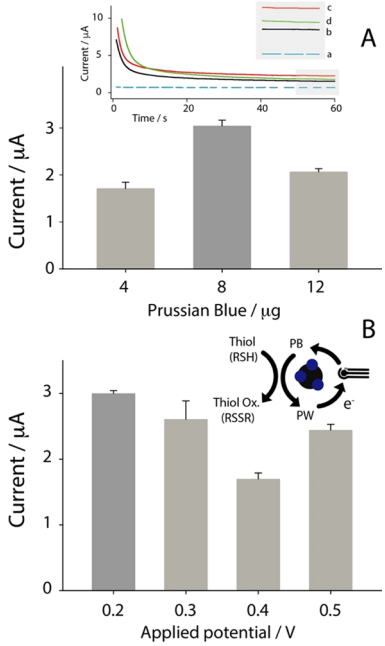

Figure 1. (A) Optimization of the electrocatalyst amount in the range between 4 and 12 μg Prussian blue. The inset shows chronoamperometric measurement results at 0.2 V (versus Ag/AgCl) in the absence (curve a) and in the presence of 5 mM of a model thiol, i.e., cysteamine with the strip modified with 4 (curve b), 8 (curve c), and 12 (curve d) μg of Prussian blue; (B) optimization of the applied potential for the oxidation of thiol, in the range between 0.2 and 0.5 V (versus Ag/AgCl) with an amount of Prussian blue equal to 8 μg.

Figure 1A highlights how the presence of 8 μg of the PB/CB composite resulted in the highest catalytic action toward the oxidation of model thiol, i.e., cysteamine, in comparison to 4 and 12 μg. This is due to the fact that the insulating nature of PB might provoke a decrease in the electrochemical performance, if a large amount of PB is present (12 μg). However, 4 μg of composite is not effective in producing a sensitive signal. Figure 1B shows the sensitivity reached by applying different potentials to oxidize thiols, and the maximum sensitivity has been achieved with 0.2 V (vs Ag/AgCl), and it has been characterized by a satisfactory repeatability with a relative standard deviation less than 7%.

Enzymatic Activity on the Strip. Successively, the concentrations of both BChE and the substrate have been taken into account. It is important to obtain the optimal condition to (1) have a good sensitivity, (2) evaluate future inhibition, and (3) reduce the costs. The effectiveness of the paper-based platform that has been engineered with the nanohybrid composite has been first evaluated in the presence of different levels of butyrylthiocholine, to decide about the optimal concentration to carry out the enzymatic reaction. As reported in Figure 2A, measurements have been performed using a volume of 100 μL in which the concentration of butyrylthiocholine has been varied up to 10 mM.

A typical Michaelis−Menten behavior has been observed. Considering that the difference between the responses due to 5 and 10 mM of enzymatic substrate is negligible, 5 mM has

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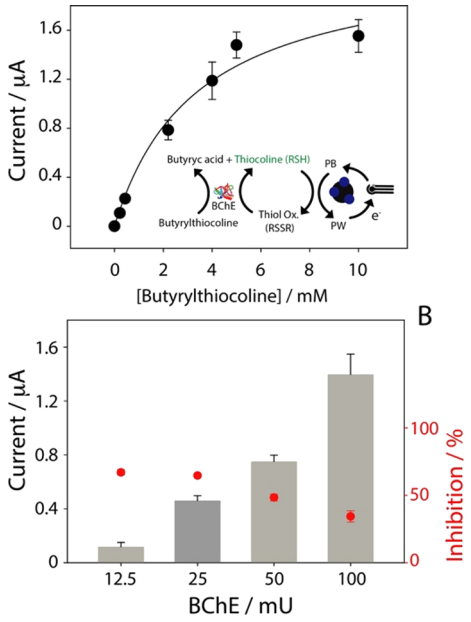

Figure 2. (A) Calibration curve in the presence of various levels of enzymatic substrates, i.e., butyrylthiocholine, in the 0−10 mM range, at an applied potential of 0.2 V (versus Ag/AgCl), reaction time of 2 min, using 100 mU of BChE onto the paper-based strip. The inset shows the mechanism of mediated oxidation at the Prussian blue-modified electrode; (B) evaluation of the enzymatic activity (histograms) between 12.5 and 100 mU BChE in the presence of 5 mM substrate, with the use of the optimized parameters. Red scatters are due to the evaluation of the inhibition degree in the presence of 100 ng/mL of paraoxon-ethyl.

been chosen as the minimum concentration of substrate to reach the Vmax and it has been selected for successive measurements in the presence of the inhibitor. Following the substrate optimization, the amount of BChE has been evaluated in order to have a good sensitivity and a high inhibition. After optimizing the amount of substrate to be used, we proceed with the measures to evaluate a high degree of inhibition but without losing the sensitivity (if the enzymatic activity is too low, the sensitivity of the method will not be enough for inhibitor quantification). As reported in Figure 2B, the enzyme amount increases as the sensitivity increases. However, performing experiments in the range between 12.5 and 100 mU of enzyme, the presence of a fixed amount of inhibitor (100 ng/mL) has displayed various degrees of inhibition. Although the use of 100 mU of BChE has yielded the highest substrate conversion, the inhibition associated has been low, i.e., less than 40%. The best compromise in terms of BChE amount and inhibition degree has been obtained by modifying the paper-based device with 25 mU of enzyme with a 60% inhibition in the presence of 100 ng/mL of paraoxon-ethyl.

Pesticide Quantification. After having optimized all the experimental parameters, including the inhibition time of 10 min (not shown), a calibration curve has been obtained in the range between 5 and 200 ng/mL of paraoxon-ethyl, as reported in Figure 3.

The intensity of the chronoamperograms, recorded in the presence of the inhibitor, highlights a decrease as the inhibitor concentration increases. A linear trend is observed up to 25 ng/mL, while a dynamic interval is obvious for higher levels of paraoxon-ethyl. The calibration curve is described by the

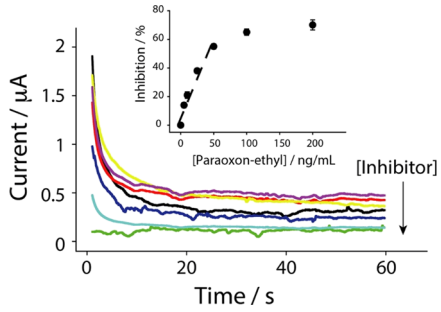

Figure 3. Chronoamperometric recordings obtained in the range between 0 and 200 ng/mL of paraoxon-ethyl. The inset shows the calibration curve obtained by analyzing paraoxon in the same range of the inhibitor. Experimental conditions are the same as described in previous optimization studies.

following equation: $y = 2.2 + 1.55x$, where y represents the % of inhibition and x is the concentration of paraoxon-ethyl in ng/mL, with $R^2 = 0.987$. The detection limit, calculated as a signal-to-noise ratio equal to 3, has been obtained to be equal to 1.3 ng/mL. The repeatability has been calculated on five measurements of 20 ng/mL standard solution, obtaining a value of 9%. It should be noted that all the measurements have been performed using one-shot platforms. The selectivity of the platform has been evaluated in the presence of common ions including toxic ones that could affect the enzymatic activity. The performance of the office paper-based device has been evaluated in the presence of nitrates, phosphates, potassium, chloride, ammonium, mercury, arsenic, copper, and cadmium. As reported in the Supporting Information (Figure S2), the signals that have been recorded in their presence have been of the same magnitude as the signal recorded for only paraoxon-ethyl (background signal), demonstrating the absence of the interference of these compounds at the level evaluated (1 ng/mL−1 µg/mL).

In order to evaluate the application of the novel platform, the applicability has been verified with the use of real samples of soil and vegetables, also considering that the established EFSA existing maximum levels for residues are in the 0.01−2 mg/kg range.[47] Additionally, since all members of the organophosphate pesticide family possess a similar mechanism of toxicological action toward cholinesterase enzymes, the office paper-based platform would be useful for evaluating the cumulative exposure. In particular, the presence of BChE inhibitors in fertilized and unfertilized soils, chili pepper, tomatoes, and potatoes has been evaluated with the portable strips. The samples have been obtained from a farm in the area of Naples (Italy). All the samples have been washed with potable water that is more common with respect to distilled water, and only in the case of soil samples, a 450 µm filter has been used to filter the gross impurities prior to the electrochemical detection. For all the samples tested (1 g of each sample has been immersed into 1 mL of water), the inhibition has not been observed at our detection limit and matrix effect has not been observed. However, in order to evaluate the accuracy of the paper-based platform, all the samples have been spiked with two levels of paraoxon-ethyl, namely, 10 and 25 ng/mL, and the obtained recoveries and relative standard solutions have been reported in Table 1.

As reported, the recoveries and the repeatability of the paper-based devices have been positively observed. In addition, thanks to the collaboration with Acea ElaboRI S.p.A. (Rome,

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Table 1. Percentage Recoveries of Two Levels of Paraoxon-Ethyl in Different Samples Obtained with the Office Paper-Based Electrochemical Biosensor (n = 3)

sample	[paraoxon]/ng/mL	inhibition/%	recovery/%	RSD/%
standard	10	19	/	11
	25	41	/	8
soil	10	16	84	11
	25	40	97	9
fertilized soil	10	18	95	10
	25	42	103	10
chili peppers	10	17	89	8
	25	40	97	11
tomatoes	10	18	95	7
	25	41	100	12
potatoes	10	19	100	9
	25	39	95	10

Italy), which provides one of the main analytical laboratory services at a national level, carrying out analyses of drinking water, wastewater, and environmental compartments, the effectiveness of the developed paper-based biosensor has obtained a 95% correlation with the LC−MS/MS accredited method (ISTISAN 19/7 CAC 015) for the analysis of paraoxon-ethyl in the ng/mL range in soil-extracted samples. These results demonstrated the effectiveness of the developed office paper-based biosensor to detect an organophosphate pesticide such as paraoxon-ethyl through easy and low-cost procedures. Taking into account the obtained results, the use of common materials such as the office paper has been demonstrated as a valuable platform for the realization of a portable device for organophosphate pesticide detection and might be considered in the risk assessment process for cumulative exposure of mixtures of organophosphate pesticides. Paraoxon-ethyl has been detected down to 1.3 ng/mL in few microliters of sample: the combination of office paper, wax-screen print-ed strips, biomimetic catalysts (PB), and BChE enzyme has allowed an accurate quantification of the target analyte. The final device is about 2 cm long, and it has been applied toward the detection of paraoxon-ethyl in a small farm setting. Soil and vegetables have been analyzed, and satisfactory recovery values have been obtained, i.e., 84−103%. The accuracy of the method has been evaluated by comparing the results in the ng/mL range with those obtained with the use of a validated LC−MS/MS method (in collaboration with Acea ElaboRi SpA.): a 95% correlation has been highlighted. It should be noted that the use of portable devices should be perceived as a substitute of traditional laboratory-bound approaches. The approaches are complementary, and the possibility in quickly evaluating abnormalities with portable methods could be effective in improving more accurate monitoring and remediations (in the case of positive samples) and to avoid unnecessary and expensive analysis (in the case of negative samples). The goal of a sustainable development can be reached with the combination of novel technologies and different analytical methodologies merged together. As reported in the literature, a step forward could be obtained in the near future with the inclusion of chemometrics, chemoinformatics, and artificial intelligence approaches,[48−50] both for designing accurate devices and to classify data from different matrices.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.1c01931.

Evaluation of electrochemical quality of office paper-based strips and selectivity study (PDF)

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Author Contributions

The manuscript was written through contributions of all authors. In particular, A.C. performed all the experiments as part of her Master's Degree thesis within the course of Environmental Toxicology Chemistry and revised the manuscript; M.M. and V.G. performed the LC−MS/MS measurements and revised the manuscript; S.C. designed and supervised the experiments and wrote and revised the manuscript.

Notes

The authors declare no competing financial interest.

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