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A paper-based electrochemical device for the detection of pesticides in aerosol phase inspired by nature: A flower-like origami biosensor for precision agriculture

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ABSTRACT

Pesticides are largely used at worldwide level to improve food production, fulfilling the needs of the global population which is increasing year by year. Although pesticides are beneficial for crop production, their extensive use has serious consequences for the pollution of the produced food as well as for soil and groundwaters. Indeed, it is reported that 50% of sprayed pesticides reach different destinations other than their target species, including soil, surface waters, and groundwaters. For this reason, we developed a flower-like origami paper-based device for pesticides detection in aerosol phase for precision agriculture. In detail, the paper-based electrochemical platform detects paraoxon, 2,4-dichlorophenoxyacetic acid, and glyphosate at ppb levels by measuring their inhibitory activity towards three different enzymes namely butyrylcholinesterase, alkaline phosphatase, and peroxidase enzyme, respectively. This integrated electrochemical device is composed of three office paper-based screen-printed electrodes and filter paper-based pads loaded with enzymes and enzymatic substrates. The pesticide detection is carried out by measuring through chronoamperometric technique the initial and residual enzymatic activity by using a smartphone-assisted potentiostat and evaluating the percentage of inhibition, proportional to the amount of aerosolized pesticides. This paper-based device was able to detect the three classes of pesticides in aerosol phase with limits of detection equal to 30 ppb, 10 ppb, and 2 ppb, respectively for 2,4-D, glyphosate, and paraoxon.

1. Introduction

Pesticides are used in agricultural practices to control pests, weeds, and diseases in plants with a remarkable contribution to food security in the last 50 years (Oerke, 2006). Despite the increased food availability, the presence of pesticides throughout the environment resulted in pollution, with a negative impact on the ecosystem and human health (Beketov et al., 2013; Nicolopoulou-Stamati et al., 2016). The problem of pesticides at European level has been highlighted by European Commission through policies with the overriding goal to control and reduce the use of pesticides. In this regard, several pesticides including chlorfenvinphos, chlorpyriphos, and atrazine have been reported in the priority list of hazardous substances within the Directive 2008/105/EC, in the frame of European Water Policy. In the case of the herbicide glyphosate, its use is currently approved in the agricultural practices until 15 December 2022 and after this date, it needs to be authorized by national authorities following a safety evaluation (https://www.efsa.europa.eu/en/topics/topic/glyphosate). Pesticide spraying is the most common way of dispensing them because of the low cost and efficient performance (Giles et al., 2008). However, this type of application sprayed on the target no more than 55% of the total volume sprayed, while the remaining 45% reaches the ground or it is lost as small airborne droplets (Panneton et al., 2001).

Furthermore, the sprayed droplets can contaminate unwanted areas and cause serious consequences, including damage to adjacent crops sensitive to environmental contamination. Indeed, pesticides can be transported to surface waters and groundwater through runoff and infiltration, causing pollution to water bodies with the reduction of usability of water resources. A recent study published in Nature Geoscience Journal reports that 74.8% of the global agricultural land (approximately 28.8 million km^2) is at risk of pesticide pollution by more than one active ingredient, and 31.4% falls within the high-risk

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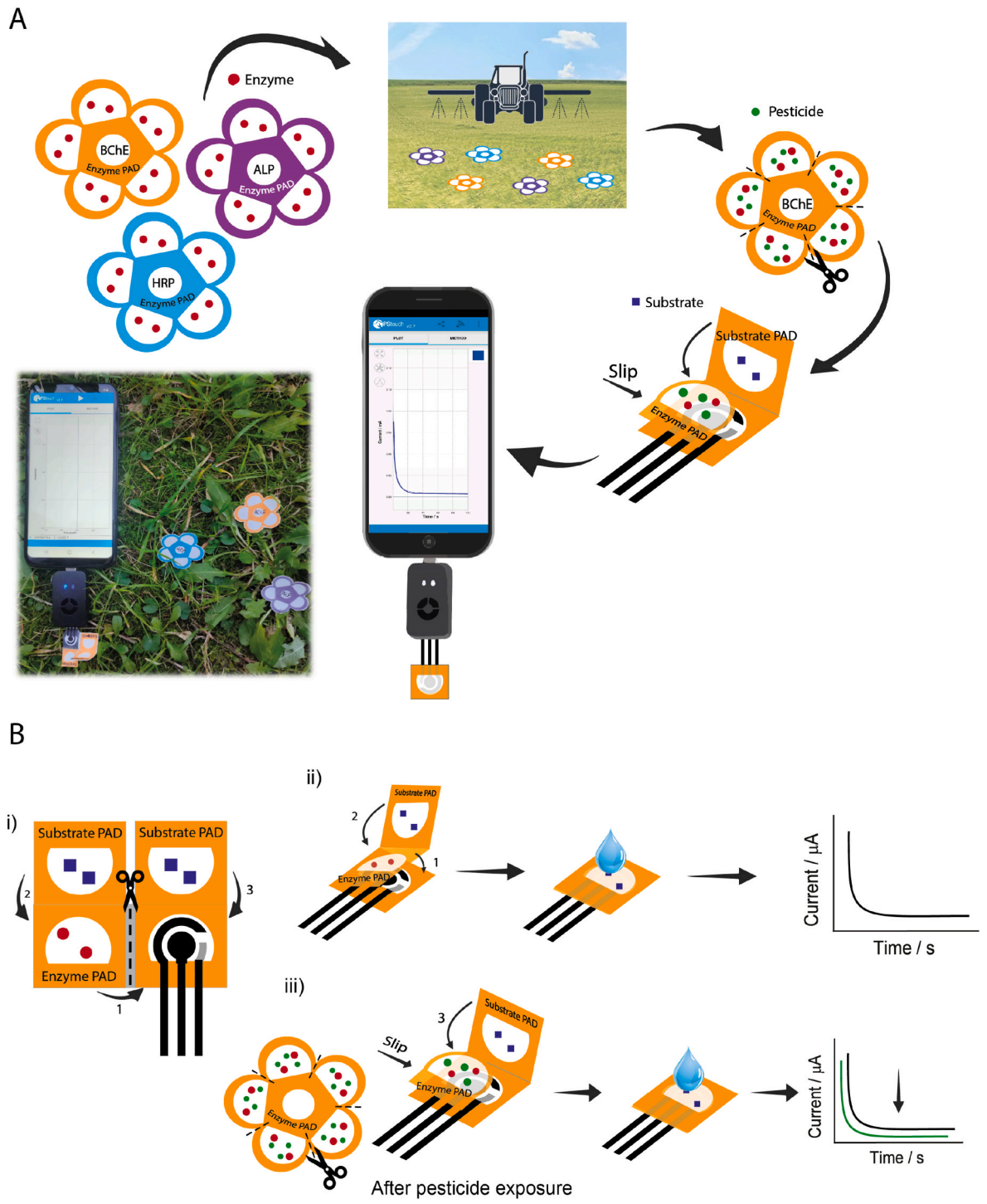

Fig. 1. A) The flower-like origami paper-based electrochemical device able to detect pesticides using a smartphone-assisted potentiostat. **B)** Schematic representation of the origami paper-based electrochemical biosensor composed by an office paper-based screen-printed electrode and three filter PADs (i), mechanism of measurement of the initial enzymatic activity in absence of pesticide (ii), and of the residual enzymatic activity after exposure to pesticide solution (iii).

class. Among all regions, Asia has the largest land area at high risk, with China ranked first, followed by Kazakhstan. In contrast, Europe has the 61.7% of the agricultural land at high risk of pesticide pollution (Tang et al., 2021).

Because the vision of precision agriculture has the overriding goal to increase the long-term farm production efficiency, productivity, and profitability as well as to minimize unintended impacts on wildlife and the environment, we designed a novel electrochemical biosensor to quantify the overuse of pesticides, by measuring the pesticides that reach the soil in the aerosol phase. To do this, electrochemical miniaturized devices turn out suitable analytical tools for pesticide detection also at ppb levels in different matrices, including aerosol. Using electrons for the chemical reactions, these platforms allow for the reduction of the amount of needed chemicals, also avoiding the use of organic solvents when compared with liquid chromatographic technique. In addition, being miniaturized, these electrochemical sensors can also be applied for *in situ* analysis, allowing real-time monitoring for pollution prevention, in agreement with the 11th principle of Green Chemistry

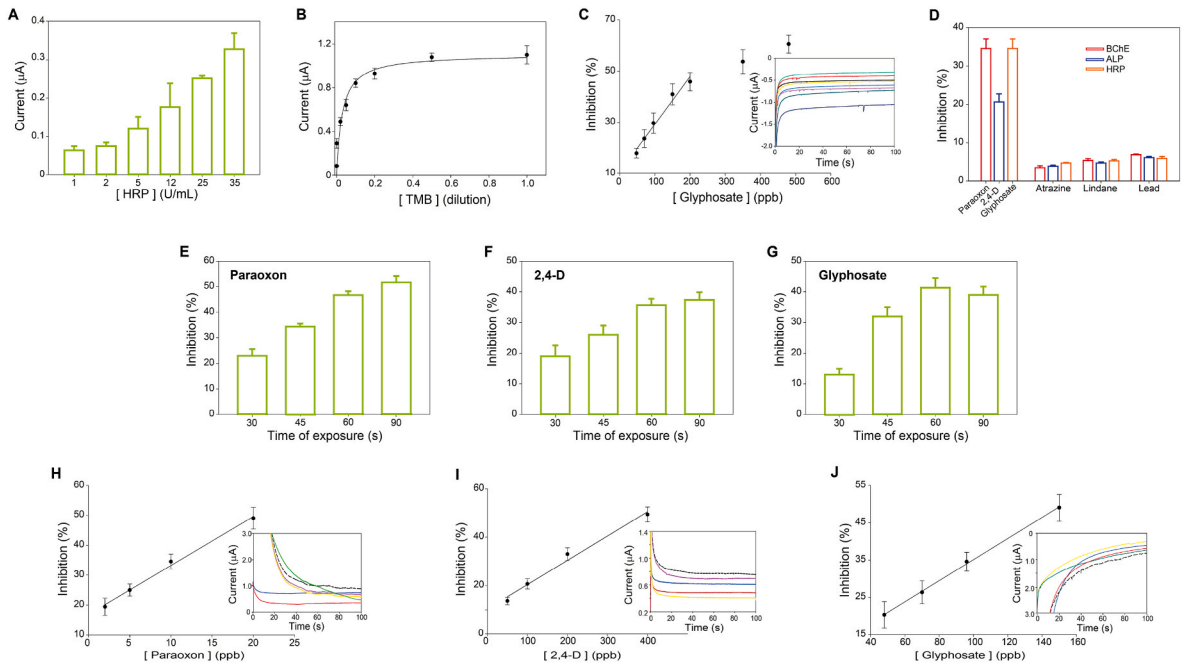

Fig. 2. A) Optimization of peroxidase concentration. Concentration tested: 1, 2, 5, 12, 25 and 35 U/mL. Reaction time: 2 min [TMB] diluted 1:5 (v/v) (n = 3). **B)** Calibration curve of TMB obtained testing different dilutions from 1:2 to 1:100 (v/v). [HRP] = 25 U/mL. **C)** Calibration curve of glyphosate obtained testing different concentrations in liquid phase: 50, 70, 100, 150, 200, 350, 480 ppb. Inhibition time = 5 min, [HRP] = 25 U/mL, [TMB] = 1:5 (v/v) (n = 3). Inset: chronoamperometric measurement, E = -0.2 V and t = 100 s. **D)** Selectivity study measuring the inhibition activity of paraoxon 10 ppb, 2,4-D 100 ppb, Glyphosate 96 ppb, atrazine 100 ppb, lindane 10 ppt, and lead 10 ppb against BChE 1 U/mL, ALP 50 U/mL, and HRP 25 U/mL. **E)** Time of exposure of PAD to a final concentration of 20 ppb of paraoxon. [BChE] = 1 U/mL, [BTCh] = 10 mM. Chronoamperometric measurement: E = 0.3 V and t = 100 s (n = 3). **F)** Time of exposure of PAD to a final concentration of 200 ppb of 2,4-D. [ALP] = 50 U/mL, [1-naphthyl phosphate] = 10 mM. Chronoamperometric measurement: E = +0.3 V and t = 100 s (n = 3). **G)** Time of exposure of PAD to a final concentration of 120 ppb of glyphosate. [HRP] = 25 U/mL, [TMB] diluted 1:5 (v/v). Inset: chronoamperometric measurement: E = -0.2 V and t = 100 s (n = 3). **H)** Calibration curve of paraoxon obtained testing different concentrations in aerosol phase: 2, 5, 10, 20 ppb. Time of exposure = 60 s, [BChE] = 1 U/mL, [BTCh] = 10 mM (n = 3). Inset: chronoamperometric measurements, E = +0.3 V and t = 100 s. **I)** Calibration curve of 2,4-D obtained testing different concentrations in aerosol phase: 50, 100, 200, 400 ppb. Time of exposure = 60 s, [ALP] = 50 U/mL, [1-naphthyl phosphate] = 10 mM (n = 3). Inset: chronoamperometric measurements, E = +0.3 V and t = 100 s. **J)** Calibration curve of glyphosate obtained testing different concentrations in aerosol phase: 50, 70, 100, 150 ppb. Time of exposure = 60 s, [HRP] = 25 U/mL, [TMB] = 1:5 (v/v) (n = 3). Inset: chronoamperometric measurement, E = -0.2 V and t = 100 s.

Table 1
Recovery studies.

Inhibitor	Concentration added (ppb)	Concentration Found (ppb)*	Recovery %
Paraoxon	2 ppb	2.0 ± 0.7	100 ± 35
	5 ppb	5.0 ± 0.5	100 ± 10
	10 ppb	8.9 ± 0.4	89 ± 4
	20 ppb	19.4 ± 0.7	97 ± 4
2,4-D	50 ppb	46 ± 4	92 ± 8
	100 ppb	103 ± 1	103 ± 1
	200 ppb	208 ± 4	104 ± 2
	400 ppb	396 ± 3	99 ± 1
Glyphosate	48 ppb	47 ± 1	98 ± 2
	70 ppb	72 ± 5	103 ± 7
	96 ppb	96 ± 1	100 ± 1
	150 ppb	157 ± 9	105 ± 6

*expanded uncertainty for a 95% confidence level.

(Anastas and Warner, 2000). Furthermore, the use of paper as substrate for the production of the electrochemical cells reduces the environmental impact of these devices and confers them sustainable features, due to the reduction of energy, by exploiting the capillarity force of the paper that avoids the use of an external pump, and the reduction of sample treatment, by exploiting the porosity of the paper to preload and treat the sample. Despite the ten-year use of paper as substrate to fabricate electrochemical biosensors (Lan et al., 2014; Nie et al., 2010; Noviana and Henry, 2020; Pollok et al., 2020), in the literature still few examples are reported related to paper-based devices for the detection of pesticides (Cinti et al., 2017; Arduini et al., 2019; Cioffi et al., 2021; Ruan et al., 2021). Thus, starting from our previous works focused on an origami paper-based enzymatic electrochemical device able to detect three different types of pesticide in river water samples (Arduini et al., 2019) and an origami wearable paper-based enzymatic device for the detection of mustard agents in the aerosol phase (Colozza et al., 2019), we propose here a flower-like origami paper-based electrochemical device able to detect paraoxon, 2,4-dichlorophenoxyacetic acid, and glyphosate in aerosol phase.

Herein, we demonstrated for the first time the exploitation of the multiple detection of analytes in the gas phase by using an origami paper-based device. To do it, we started by our previous work in which an origami paper-based platform was designed and developed for the detection of different pesticides in water samples. That paper-based device was constituted of two pairs of different pads, one pair containing the enzyme, the other two pads containing the substrate, separated by an empty piece of paper (Arduini et al., 2019). The so-developed paper-based origami was coupled with a portable potentiostat, EmStat3 Blue from PalmSens, which was connected to a laptop, and it was able to detect simultaneously paraoxon, 2,4-D, and atrazine in the liquid phase. The measurements were carried out just sampling few microliters of the solution to test and adding them on the enzyme pad.

In this manuscript, we present a new device able to detect simultaneously pesticides in the aerosol phase, composed of i) a paper strip where an office paper-based screen-printed electrode was located, coupled with one enzymatic PAD and two substrate PADs, one for the measurement of the blank (PAD sample not exposed to pesticides) and the other one for the pesticide detection; ii) a flower-like PAD, composed

Table 2
Electrochemical sensors for pesticides detection and for analyses in aerosol phase.

Paper-based electrochemical sensors for pesticides detection

Pesticide	Type of paper	Device structure	Electrochemical cell	Electroche-mical technique	Linear range /LOD	Environ-mental matrix	Ref.
Paraoxon	Filter paper	A single paper layer; lateral flow configuration	Screen-printed graphite electrodes (W.E. and C.E.) and Ag/AgCl (R.E.); W.E. modified with CB/PBNPs	Chronoam-perometry	5–25 ppb /3 ppb	River water Wastewater	Cinti et al. (2017)
1) Paraoxon 2) 2,4-D 3) Atrazine	Filter paper and office paper	Origami device based on the multiple folding of PADs on two different screen-printed electrodes	Two screen-printed graphite electrodes (W.E. and C.E.) and Ag/AgCl (R.E.); W.E. modified with CB for atrazine and 2,4-D; W.E. modified with CB/PBNPs for paraoxon	Chronoam-perometry	1) 2–20 ppb/2 ppb 2) 100–600 ppb/ 50 ppb 3) 10–100 ppb/2 ppb	River water	Arduini et al. (2019)
Paraoxon	Office paper	A single paper layer strip	Screen-printed graphite electrodes (W.E. and C.E.) and Ag/AgCl (R.E.); W.E. modified with CB/PBNPs	Chronoam-perometry	Up to 25 ng/mL /1.3 ng/mL	Soil-extracted Vegetables-extracted	Cioffi et al. (2021)
1) Atrazine 2) Acetochlor	Immuno-chromatographic strips on an adhesive polyvinyl chloride backing card	Two paper strips composed of a sample PAD, a conjugate PAD, a nitrocellulose membrane, and an absorbent PAD; lateral flow configuration; 3D holder	Commercial screen-printed electrodes	DPV	1) 0.1–500 ppb /0.24 ppb 2) 1–1000 ppb /3.2 ppb	River water	Ruan et al. (2021)
Methyl parathion	Not reported	Not reported	Carboxyl-group functionalized carbon nanotubes modified carbon paper electrode (W.E.) platinum wire (C. E.) saturated calomel electrode (R.E.)	DPV	0.06–30 μM /27 nM	Human urine	Wang et al. (2021)
p-nitrophenol	Whatman #1 chromatography paper	Origami device based on the multiple folding of PADs	Pencil graphite electrode (W.E.), C.E. and R.E. drawn on paper	DPV	10–200 μM /1.1 μM	Water sample	Santhiago et al. (2014)

Electrochemical sensors for analyte detection in aerosol phase

Analyte	Type of sensor	Electrochemical technique	Linear range /LOD	Ref.
H_2O_2	Paper-based sensor with PB-mediated carbon electrode (W.E.), carbon electrode (C.E.) and Ag/AgCl (R.E.)	Chronoamperometry	5–320 μM Sensitivity: 0.19 nA μM^{-1} mm^{-2}	Maier et al. (2019)
H_2O_2	Filter paper-based screen-printed graphite electrodes (W.E. and C.E.) and Ag/AgCl (R.E.); W.E. modified with CB/PBNPs	Chronoamperometry	1–7% w/w (11–78 × 10^3 ppm) Sensitivity: 64 μA/% (w/w) cm^2 (5.8 × 10^{-3} μA ppm^{-1} cm^{-2})	Fiore et al. (2021)
Sulfur mustard agent	Filter paper-based screen-printed graphite electrodes (W.E. and C.E.) and Ag/AgCl (R.E.); W.E. modified with CB/PBNPs	Chronoamperometry	Linear range not reported /0.019 g/m^3	Colozza et al. (2019)

W.E. = working electrode; C.E. = counter electrode; R.E. = reference electrode; CB/PBNPs = carbon black/Prussian blue nanoparticles; CB = carbon black.

of five petals preloaded with the enzyme, which will be exposed to the aerosol and then used for the pesticide measurements. Moreover, the integration of the origami sensor with the smartphone-assisted potentiostat (Sensit Smart, PalmSens) made the device more miniaturized and user-friendly in respect to the previous one. The dedicated PStouch app allowed for viewing in real-time the amperometric measurements on the smartphone display making easier the detection of pesticides *in situ*.

Thus, this smart device is able to detect multiple pesticide at ppb levels in aerosol phase thanks to:

i) the porous structure of the paper to load the reagents as well as to load the analyte in aerosol, allowing for the measure in the thin film layer of the solution within the cellulose network;
ii) the inhibition of these pesticides towards specific enzymes: paraoxon towards butyrylcholinesterase enzyme, 2,4-D towards alkaline phosphatase, and glyphosate towards peroxidase enzyme for the quantitative analysis;
iii) the use of cutting-edge technologies, namely wax printing to produce paper-based analytical devices (PADs) able to preload the reagents necessary for the enzymatic reaction; screen-printing to print the miniaturized electrochemical cell; and nanomaterials to enhance the electrochemical performances of the device;

With the final aim to produce a sustainable device for the customization of pesticide use, boosting the precision agriculture.

2. Experimental section

2.1. Reagents and equipment

Alkaline phosphatase from bovine intestinal mucosa (ALP), butyrylcholinesterase from horse serum (BChE), peroxidase from horseradish (HRP), 3,3′,5,5′-tetramethylbenzidine Liquid Substrate (TMB), 2,4 Dichlorophenoxyacetic acid (2,4-D), paraoxon, glyphosate, lindane (γ-hexachlorocyclohexane), lead, atrazine, potassium dihydrogen phosphate, dipotassium hydrogen phosphate, potassium chloride, 1-Naphthyl phosphate disodium salt were purchased from Sigma Aldrich. MO, USA. S-butyrylthiocholine chloride (BTCh) was purchased by Santa Cruz Biotechnology, Inc. TX, USA. Carbon black and carbon black/Prussian blue nanoparticles dispersions were prepared according to the procedure reported in our previous work (Arduini et al., 2019). The office paper-based electrodes (S4M-OP02) were supplied from SENSE4MED Company (Rome, Italy) and consisted in graphite working and counter electrodes and silver/silver chloride pseudo-reference electrode (geometric working electrode surface area equal to 12.56 mm^2). A portable potentiostat, Sensit Smart (PalmSens, Netherlands) connected to a smartphone was used to carry out the electrochemical measurements. The dedicated app PStouch allows for viewing in real-time the amperometric measurements on the smartphone display.

2.2. Origami paper-based device construction

Screen-printed office paper-based electrodes were used for the construction of the electrochemical device. More precisely, office screen-printed electrodes with working electrodes modified with 2 μL of carbon black dispersion were used for the detection of 2,4-D and glyphosate. For the detection of paraoxon, instead, the working electrode was modified with 2 μL of carbon black/Prussian blue nanoparticles dispersion. For the collection of pesticide samples, a flower-like PAD (composed of five petals, each consisting in a separate PAD) was designed using a drawing software and the pattern was printed on filter paper using wax printing technique to produce hydrophobic zones around hydrophilic regions. Each paper-based electrochemical biosensor consisted in a screen-printed electrode integrated with adhesive tape to three filter PADs used to load the enzyme and the substrate.

For paraoxon and 2,4-D, the biosensors were developed in agreement with our previous work (Arduini et al., 2019). For glyphosate measurement, the enzyme PAD was loaded with 2 μL of HRP 25 U/mL in phosphate buffer 0.05 M + KCl 0.1 M, pH = 7.4, while the substrate PAD was loaded with 20 μL of TMB diluted 1:5 (v/v) in phosphate buffer 0.05 M + KCl 0.1 M, pH = 7.4.

2.3. Pesticide measurement

For the measurement of the inhibition activity of pesticides, each flower-like waxed PAD was initially pre-loaded with the selected enzyme and exposed to the pesticide solutions. Then, the five enzymatic PADs were cut, and each PAD was separately used for pesticide monitoring. Indeed, the slipping of the single pesticide-enzyme PAD into the origami paper-based electrochemical biosensor allows for the measurement of the desired pesticide using a miniaturized potentiostat connected to a smartphone (Fig. 1A).

For the measurement of the initial enzymatic activity, the first couple of enzymatic and substrate PADs was put in contact and 20 μL of distilled water were added to these combined PADs. In 2 min, the reaction occurs producing the enzymatic by-product which was detected chronoamperometrically (Fig. 1B ii). For the measurement of aerosolized solution of pesticide, a system (volume 3 L) was used for the electrochemical measurements. The solutions were aerosolized using a nebulizer (SIMBR aerosol system) and the measurements were carried out by introducing the flower-like PAD into the closed box. In detail, 500 μL of solution were aerosolized for 1 min into the nebulizer with a flow rate of 500 μL/min. After the nebulization, the contaminated enzymatic PAD was integrated into the electrochemical biosensor and the residual enzymatic activity was measured as described above. In this case, a decrease in current occurs due to the inhibition activity of the pesticide toward the enzyme (Fig. 1B iii). The inhibition percentage was measured by using the following equation (Amine et al., 2016):

$$I\ \% = [(I_0 - I_i)/I_0)] \times 100 \quad (1)$$

where I_0 and I_i are the response of enzymatic activity in the absence and in the presence of pesticides.

3. Results and discussion

The development of this origami paper-based device exploited the inhibition activity of paraoxon, 2,4-dichlorophenoxyacetic acid, and glyphosate against butyrylcholinesterase, alkaline phosphatase, and peroxidase, respectively. (Arduini et al., 2010; Mazzei et al., 2004; Songa et al., 2009). To deliver a reagent-free device, we developed a 3D paper-based electrochemical device composed of an office paper-based screen-printed electrode coupled to two substrate PADs and one enzymatic PAD linked to each other thanks to adhesive tape (Fig. 1B i). For the measurement of the "blank" signal, in the absence of the pesticide, it is only necessary to fold a couple of PADs (enzymatic and substrate PADs) already linked to the electrode, delivering a 3D device composed of the electrode as the first layer and the two filter PADs as the upper layers (Fig. 1B ii). For the measurement of the sample, the enzymatic preloaded PAD exposed to the contaminated airborne solutions (slip PAD) was added to the 3D structure as the middle layer, above the electrode layer, and below the substrate PAD (Fig. 1B iii).

3.1. Pesticide detection in liquid phase

In our previous work (Arduini et al., 2019), we already evaluated the suitability of this type of 3D electrochemical platform to detect paraoxon and 2,4-D in liquid phase. Indeed, it was reported that this device was able to detect paraoxon and 2,4-D with a detection limit of 2 and 50 ppb, respectively. Herein, we developed the third part of this biosensing platform for the detection of glyphosate, due to its large use in agricultural practices. In this case, it was necessary to optimize the

experimental conditions of the electrochemical device before evaluating its suitability to detect the glyphosate in liquid and aerosol phase. The measurement of this pesticide was carried out by analyzing the inhibition degree of peroxidase enzyme, since it is reported in the literature that this enzyme is inhibited in a reversible way by glyphosate (Songa et al., 2009). We have chosen TMB (liquid solution commercially available) as the substrate of peroxidase because this solution already contains the hydrogen peroxide, the co-substrate of the enzymatic reaction, allowing for the development of a reagent-free device without the need for an external source of hydrogen peroxide for the reaction.

For the development of this biosensor, the concentration of the enzyme was optimized. In detail, five different enzyme concentrations were evaluated in a range comprised between 1 and 35 U/mL. As shown in Fig. 2A, the best compromise between sensitivity and repeatability was obtained with 25 U/mL (percent relative standard deviation, RSD %, = 3%), thus this value was selected for the further experiments. The response of the biosensor toward the enzymatic substrate was then evaluated. Not knowing the concentration of TMB, we evaluated different dilutions of the substrate, and Fig. 2B showed the calibration curve obtained, $K_M^{app} = 0.029 \pm 0.006$ (in the graph, "1" is referred to the solution not diluted, the other numbers to the subsequent dilutions). The dilution 1:5 (v/v) (0.2 value in the calibration curve) was selected as the amount of substrate to use in the further experiments, having RSD% equal to 5.5%. The results obtained demonstrated the suitability of this biosensor to detect different amounts of TMB.

The optimized paper-based platform was then applied to glyphosate detection in liquid phase (Fig. 2C). A linear range between 50 and 200 ppb described by the following equation, inhibition percentage = $(10.5 \pm 2.1) + (0.2 \pm 0.1) \times$ (glyphosate concentration/ppb), $R^2 = 0.907$.

3.2. Selectivity study

Successively, the selectivity of the electrochemical platform was investigated against different possible interfering species like lead and pesticides such as atrazine and lindane (γ-hexachlorocyclohexane). Lead accumulation in soils is of serious concern in agricultural practices due to the harmful effects on soil microflora, crop growth, and food safety. In soil, speciation of lead greatly affects its bioavailability and thus its toxicity on plants and microbes (Kushwaha et al., 2018). Atrazine and lindane are consistently found in sediment and soil in regions where they are applied during the agricultural activity being sprayed as pesticides, because they have a relatively long half-life of 261 days and 2292 days, respectively (de Souza et al., 2020; Fan and Song, 2014), depending on the soil and microbial environment (Atrazine, 2006; He et al., 2019).

Thus, the herbicide atrazine, the insecticide lindane, and the heavy metal lead were tested in liquid phase at levels of 100 ppb, 10 ppt, and 10 ppb, respectively (European Parliament, 2016; U.S. Environmental Protection Agency, 2020). The activity of the three enzymes, BChE 1 U/mL, ALP 50 U/mL, and HRP 25 U/mL, was measured before and after the exposure to these interfering species and the inhibition percentages were calculated. For each enzymatic biosensor, atrazine, lindane and lead gave low inhibition percentages in the range of 3–7% as showed in Fig. 2D, underlaying the selectivity of the proposed integrated paper-based to measure the selected pesticides.

3.3. Pesticide detection in aerosol phase

Once demonstrated the suitability of the developed origami biosensor for pesticide detection in liquid phase, the fabricated analysis system was used to evaluate the suitability of the electrochemical device to measure aerosolized solutions, simulating the spraying of pesticides in the crop field. Pesticide solutions were aerosolized thanks to a commercial nebulizer and let flow through the flower-like origami PAD preloaded with the enzyme and placed inside the closed box. After the reaction between enzyme and pesticide, the flower-like origami was cut into five different PADs and it was slipped into the paper-based device. Then the substrate layer (the upper layer) was wet with 20 μL of distilled water and the inhibition activity was measured.

Initially, different times of exposure were tested for each pesticide, namely 30, 45, 60, 180 s. As shown in Fig. 2E, F, G, only in the case of paraoxon there is a difference in the biosensor response increasing the time of exposure, because it is an irreversible inhibitor of the cholinesterase enzymes. For 2,4-D and glyphosate, there is a slight difference between the different times tested, confirming the reversible nature of the inhibition mechanism (Arduini et al., 2015). For further study, a time of exposure of 60 s was used for the selected three pesticides as a good compromise between repeatability, fast analysis, and high sensitivity (paraoxon RSD% = 3%, 2,4-D RSD% = 6%, glyphosate RSD% = 7%).

Fig. 2H, I, J, have shown the calibration curves obtained measuring the pesticides at different concentrations in aerosol phase. In detail, the origami platform resulted to detect paraoxon in a linear range between 2 and 20 ppb obtaining a calibration curve described by the following equation $y = (16 \pm 1) + (1.6 \pm 0.1)$ x ($R^2 = 0.955$) and a limit of detection equal to 2 ppb, calculated as the 20% of inhibition. For the calibration curve of 2,4-D described by the equation $y = (10 \pm 1) + (0.100 \pm 0.006)$ x ($R^2 = 0.965$), a linear range between 50 and 200 ppb is observed whit a LOD of 50 ppb, calculated as the 15% of inhibition. For what concern glyphosate, the calibration curve described by the following equation $y = (7 \pm 2) + (0.30 \pm 0.02)$ x ($R^2 = 0.943$) was observed linear until 150 ppb with a LOD equal to 10 ppb, calculated as the 10% of inhibition.

To evaluate the accuracy of the developed flower-like origami, we carried out the recovery studies for each inhibitor at four concentration levels as reported in Table 1, demonstrating satisfactory recovery values. The results obtained highlighted that the sensing platform developed can alert for the presence of airborne pesticides at ppb levels in only 60 s allowing for fast and *in situ* analyses without the need for external reagents.

4. Conclusions

In this work, we demonstrate the suitability of the 3D origami paper-based device to detect multiclass of pesticides in liquid and aerosol phase, allowing for environmental pollution monitoring at ppb levels, going beyond the state of art for both the detection of analytes in aerosol phase and the smart detection of pesticides by using paper-based devices (Table 2). This electrochemical platform was developed by exploiting cutting-edge technologies including screen-printing, wax-printing, and nanomaterials, resulting in an eco-friendly and sustainable device, thanks to the possibility to be safely disposed. The properties of paper were exploited, and thanks to the porosity of the filter paper all the reagents needed for the measurements were preloaded on the PADs delivering a reagent-free sensing platform. In conclusion, this platform results be a promising screening tool to detect pesticides in liquid samples and in aerosol phase.

CRediT authorship contribution statement

Veronica Caratelli: Experimental investigation, paper-based sensor fabrication, Methodology, Formal analysis, Writing – review & editing. **Greta Fegatelli:** Experimental investigation, Formal analysis. **Danila Moscone:** Writing – review & editing, Funding acquisition, Project administration. **Fabiana Arduini:** Conceptualisation of the biosensor, Supervision, of experiments, Funding acquisition, Project administration, Writing – review & editing.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bios.2022.114119.

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