Case Studies in Chemical and Environmental Engineering 4 (2021) 100167

Contents lists available at ScienceDirect

Case Studies in Chemical and Environmental Engineering

journal homepage: www.sciencedirect.com/journal/case-studies-in-chemical-and-environmental-engineering

Paper-based devices as new smart analytical tools for sustainable detection of environmental pollutants

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ARTICLE INFO

Keywords:
Electrochemical sensor
Eco sustainable device
Emerging pollutant
Environmental monitoring

ABSTRACT

The use of paper as a multifunctional material in electrochemical sensing has been intensively explored over the last decade. The combination among different kinds of paper as well as their coupling with different electrochemical cell configurations have been demonstrated, disclosing innovative sensing performances and features that are still to be fully investigated. This ongoing research has found applications in a variety of fields, including the biomedical, agri-food, security, and environmental ones, thanks to the high versatility and adaptability of the paper material. In this review, we report a critical and comparative analysis of electrochemical devices based on paper published within 2010–2021 and applied for the detection of pollutants of environmental interest in fresh water, seawater, and other real environmental matrices. Several paper types, from common office paper to Whatman filter paper with different filtering grades, were proved to be useful in this field. In detail, the multifarious roles played by the paper are discussed, highlighting how the paper can be a suitable material for electrochemical sensing while being capable of simplifying the measurement of complex real matrices or realizing programmable origami-like structures. Among the most important pollutants, a special focus is dedicated to the emerging pollutants. Furthermore, the unique advantages achieved by the paper have been analyzed and highlighted, reporting the future perspectives regarding the use of this surprising material.

1. Introduction

Sustainability is a hot keyword in this decade, because this approach is changing each sector starting from environment through economy until to society, with the overriding goal to create a new mode of thinking for a better life. As reported in the European Commission website [1] "*The European Commission aims to ensure coherence between industrial, environmental, climate and energy policy to create an optimal business environment for sustainable growth, job creation, and innovation. To* support *this, the Commission has established an ambitious agenda to transform EU economy into a circular one, where the value of products and materials is maintained for as long as possible, bringing major economic benefits. The Commission also* supports *European industry in the move to a climate-neutral economy and improves the energy efficiency of products through eco-design legislation*".

In detail, since 2015 United Nations Member States adopted the 2030 Agenda for Sustainable Development [2], which encompasses 17 Sustainable Development Goals (SDGs) to draw the attention of modern society to the most urgent and important concerns regarding people, prosperity, and the planet. These principles include ending poverty and hunger in all their forms, to ensure human rights, dignity, and equality, as well as harmony in technological and economic progress with respect the nature. Particularly, the protection of the planet's equilibria is one of the most concerning aspects, requiring switching toward a more far-sighted lifestyle in terms of production and consumption.

In this scenario, chemists, as well as scientists of other disciplines, are called to put their efforts into turning their research and activities into a sustainable manner [3]. Analytical chemistry and in particular the sensor field have the role to develop eco-design tools to boost the achievement of several SDGs, particularly: SDG3 by developing smart point-of-care devices for rapid diagnostic and for the management of precision medicine; SDG6 by integrating on-site sensors for the control of water quality and the efficiency of sanitation treatment; SDG16 by creating devices suitable for the monitoring of pollution concerning the

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https://doi.org/10.1016/j.cscee.2021.100167
Received 15 October 2021; Received in revised form 24 November 2021; Accepted 26 November 2021

oceans and marine resources.

Working in the analytical environmental field, we are aware that if we select analytical tools able to control the pollutant level but, at the same time, we produce waste generated by chemical analysis, we do not help our Earth. In this vision, electrochemical sensors are able to reduce the amount of needed chemicals by exploiting the reactions and exchanges of electrons to obtain a measurable quantity. This approach avoids the use of organic solvents, often used in conventional analytical methods such as liquid chromatography. In addition, the use of electrochemical sensors matches the requirement of the 11th principle of green chemistry [4] which allows for real-time analysis for pollution prevention.

With the aim to further reduce the environmental impact, several groups [5–11] developed electrochemical paper-based sensors over the last years. If the first attempt has been devoted to replacing the plastic substrate with the paper for delivering a plastic-free device, scientists found out that the paper confers to electrochemical devices unprecedented sustainable features, including the **reduction** of:

i. **Energy** by exploiting the capillarity force of the paper and avoiding the energy requirement of external pump;
ii. **Chemicals** because the analysis happens in the few μL layer entrapped in cellulose network as well as the reagents can be entrapped in cellulose networks to further reduce their amount;
iii. **Sample treatment** by using the porosity of the paper to treat the sample as well as by exploiting the capability of paper of loading and pre-concentrating the sample.

In the context of environmental pollutants, the European project that had the more impact in the last years on the environmental field in our opinion is the Norman project, which delivered the NORMAN network [12]. This network has the aim to exchange information on emerging environmental substances, encouraging the validation and harmonization of common measurement methods and monitoring tools, and promoting the synergies between research teams from different countries in the field of emerging substances. Within his project, the surface waters of several European Countries have been monitored, discovering several pollutants never found nor legislated before and thus leading the European Commission to update the water legislation with the Directive 2013/39/EU**.**

In this review, we have reported the most relevant examples of paper-based devices for the monitoring of pollutants, highlighting the emerging ones in agreement with 2013/39/EU, with the aim to show the advantages of using these eco-designed analytical tools in terms of analytical performances as well as of sustainable measurements for pollutant control. In detail, we report the studies under examination divided by inorganic and organic pollutants, then go through details among different types of pollutants within these two groups, namely heavy metals, pesticides, pharmaceuticals, and other inorganic and organic pollutants. The analytical features and the multiple advantages offered using paper are summarized in Table S1 and Table S2 (Supplementary Material).

2. Inorganic pollutants

2.1. Heavy metals

The release of metals and metal-containing compounds into the environment is a warning and ubiquitous concern in the modern world, involving all the environmental compartments (i.e., waters, soils, air) [13]. The intensive industrial, metallurgic, and mining activity carried out to supply the necessity of the current lifestyle and technology inevitably involves the processing of a variety of elemental metals and metal compounds with consequences on the environment and living beings that need to be monitored [14].

Transition metals such as iron (Fe^{2+}), nickel (Ni^{2+}), copper (Cu^{2+}), zinc (Zn^{2+}), silver (Ag^{+}), cadmium (Cd^{2+}), lead (Pb^{2+}), and mercury (Hg^{2+}) are indisputably identified as serious hazards for the environment as well as for living beings, particularly because of their persistence in common environmental conditions. More precisely, these elements are currently recognized as “heavy metals” [15], a class of metallic species characterized by high toxicity, persistence in the environment, (bio)accumulation (i.e., including the accumulation in tissues/organs of plants and animals), and characteristic physico-chemical properties [14].

The pollution of heavy metal ions in all environmental matrices hence represents a global issue that outreaches all ecosystems [14]. Therefore, novel analytical tools are required for outperforming the conventional spectrometry-based detection methods (e.g., atomic absorbance spectroscopy, mass spectrometry) and providing a rapid, sensitive, and simple approach for the determination of heavy metal ions at the trace level (i.e., ppb and below) according to national and international legislations.

To answer this need, electrochemical sensors have been developed for the detection of a heavy metals basing on the possibility of discriminating each metal by its characteristic discharge potential. In this regard, anodic stripping voltammetry (ASV) is among the most employed voltametric technique for the measurement of trace heavy metals because it provides sensitivity and low detection limit for many cationic metals thanks to the accumulation of the analytes by electrodeposition (in which the cationic metals are reduced on the working electrode surface to their metallic form) and the subsequent stripping phase, consisting of their re-dissolution by an oxidative potential scan [16]. Deposition potential and deposition time are the dominant parameters for this technique, determining the analytical performance. Further sensitivity can be achieved by applying a square wave potential scan (square wave anodic stripping voltammetry, SWASV). Thus, SWASV is commonly chosen for trace heavy metal detection taking advantage also of the reduced background noise that is ensured by the poor contribution from capacitive current to the voltametric response [16].

Furthermore, bismuth is typically used to improve the detection of some heavy metals, especially cadmium, lead, and zinc, which are capable of forming metal alloys with bismuth during the electrodeposition phase of SWASV [17]. This represents a more sustainable alternative in comparison with the classical mercury-based electrodes to detect simultaneously cadmium, lead, and zinc. Moreover, bismuth allows for performing the electrochemical measurement without deoxygenation, resulting to be more suitable for realizing in-field applicable sensors. The preferred medium as the working solution is usually acetate buffer since it ensures the stability of the involved metals in a relatively acid environment (e.g., pH = 4.6).

The first example of a paper-based sensor developed for the detection of heavy metal was reported by the group of Whitesides in 2010 [5], a pioneering group in the use of paper microfluidic for electrochemical sensing devices. They employed screen-printed electrodes obtained on paper (chromatography paper or a polyester–cellulose blend), on which microfluidic channels were patterned by photolithography or wax printing, to drive the sample throughout the device. A classical configuration of the three-electrode printed cell, based on conductive carbon ink or Ag/AgCl ink, was used in combination with the paper microfluidics to achieve a sensing device capable of measuring Pb^{2+} and glucose. For pursuing the topic of this review, only the detection of Pb^{2+} will be discussed in detail.

In this study, a pad of cellulose blotting paper was placed at the outlet of the microfluidic channel to act as a sink, realizing a lateral flow configuration. The presence of the sink pad served to obtain continuous adsorption of the sample by capillarity, allowing it to flow throughout the electrodes. This strategy facilitated the plating of metals and the cleaning of electrodes at the same time and was revealed to be advantageous with respect to the direct drop-casting of the sample on the electrodes or in the absence of the blotting paper.

In this work, the authors electrodeposited bismuth (i.e., Bi^{3+}) in situ simultaneously with Pb^{2+}, forming metal alloys on the working electrode and boosting the sensitivity of the analytical method. The detection of Pb^{2+} was successfully carried out by means of SWASV in an acetate buffer solution also in the presence of Zn^{2+}, without suffering from interferences and resulting in a limit of detection of 1.0 ppb. Considering that the WHO's guidelines established a threshold concentration in fresh water for Pb^{2+} equal to 10 ppb, the paper-based sensor here developed provided a competitive alternative to other electrochemical devices. To reach these satisfactory results, the device was characterized by electrochemical studies and the detection was optimized by studying the parameters of SWASV, demonstrating the promising features of paper-based electrochemical devices for the detection of heavy metals in environmental samples. Being a pioneering study on the use of paper for heavy metal detection (coupled with glucose detection), the authors did not test the sensor in real samples and did not compare its analytical performance with conventional methods.

A few years later, Mensah 2014 et al. have chosen potentiometry as the detection technique to measure Cd^{2+}, Ag^{+}, and K^{+}, realizing solid-contact ion-selective electrodes (SC-ISEs) based on a paper platform [18]. As common SC-ISEs, this device was provided with an ion-selective membrane based on copolymer methyl methacrylate-decyl methacrylate and containing ionic sites, lipophilic salts, and conventional ionophores for Cd^{2+}, Ag^{+}, and K^{+}, to achieve the selectivity toward the target ions. The innovative aspect was to use conductive paper strips as the working electrode, testing different modification approaches to prepare the strips for the potentiometric measurement. In detail, the chosen filter paper was coated with a suspension of single-wall carbon nanotubes by using a conventional paintbrush and left to dry firstly at room temperature and then by a thermal treatment. The authors observed that the porous structure of the filter paper allowed it to be deeply impregnated with the liquid suspension by means of the capillary forces. This modification process was applied six times for each strip, verifying that the electrical resistance of the resulting modified paper decreased exponentially. The conductive paper strips were tested before and after further modifications with the aim to improve their electrochemical performance, involving the use of poly(3-octylthiophene) (POT) and gold. More precisely, in one case five layers of POT were drop-cast on a 0.8 cm-diameter circle on one end of the strip, and in another case, a layer of gold was sputtered before the drop-casting of five layers of POT. It is noteworthy to describe how the authors dealt with the usual problem encountered for SC-ISEs, namely the formation of an undesired water layer between the interface of the solid support and the ion-selective membrane, which can entrap ions and cause interferences with the potentiometric detection. Indeed, the hydrophilicity of paper might enhance this phenomenon, affecting the limit of detection. However, the use of POT allowed the authors to obtain a hydrophobic surface on the modified paper strips, as verified by contact angle measurements. The best results in terms of hydrophobicity and of stability of the measured potential was achieved with the paper strips modified with both gold and POT, for which the authors stated that the water layer was not significantly present at the membrane/solid substrate interface also after soaking in a water bath for 65 hours. Interestingly, the paper strips modified with gold and POT resulted not to suffer from oxygen half-cell formation, which commonly affects the potential stability of SC-ISEs. Indeed, no drifting was observed in the presence of both gold and POT on the conductive paper strips, indicating high stability also in a solution saturated with oxygen.

The conductive paper strips modified with gold and POT were thus employed for the target ions. Satisfactory limits of detection of 1.2, 25.1, and 11.0 nM were obtained for Cd^{2+}, Ag^{+}, and K^{+}, respectively, with close-to-Nernstian slopes and high selectivity and reproducibility. These results represented an outstanding achievement for paper-based potentiometric devices, demonstrating for the first time the excellent performance of paper in SC-ISE development.

An alternative approach to manufacture paper-based sensors for lead detection was developed by Yu et al., in 2017, based on the lamination technique [19]. This approach allowed the authors for designing the electrode without the need for masks or screens typically used for the screen-printing technique, thus realizing a faster and simpler procedure for electrode production consisting of a two-step lamination. The conductive electrodes were applied on the paper, patterned by a CO_2 laser cutter, by means of a spatula and letting the ink penetrate the paper. The paper-based electrode layer was then placed in the middle of two other paper layers, both coated with a biodegradable polyester, polycaprolactone (PCL), in a sandwich-like overlapped configuration. This polymer was used to outline the electrode geometry instead of the more commonly used dielectric inks, photoresists, or wax, as well as to insulate the conductive traces. The PCL-coated layer placed on the top of the device presented a 4-mm circular hole in correspondence with the circular silver-modified paper electrode. When the assembly was carried out, the layers were placed between two aluminum foils, and the lamination was achieved by a thermal treatment. During this procedure, the melting of PCL allowed for the sealing of the layers. The resulting laminated electrodes were permanently assembled, thus creating a robust and handy device.

This interesting device was capable of measuring Pb^{2+} in the range from 50 nM to 500 nM (~from 10 ppb to 100 ppb), with a limit of detection equal to 16 nM by applying SWASV. A convenient deposition time (i.e., 30 s) was sufficient to sensitively detect Pb^{2+} and the detection resulted not significantly affected by possibly interfering ions, namely Fe^{3+}, K^{+}, Na^{+}, Ca^{2+}, Mg^{2+}. It is worthy of note that these satisfactory results were achieved without the use of any electrode modifier and in absence of bismuth. Finally, the device was tested in real water samples, obtaining good recoveries and proving its reliability for environmental applications.

Nitrocellulose paper (0.45 μm pore size) was used by Wang et al., in 2018 to realize a paper-based device suitable for multianalyte detection [20]. The paper-based electrodes consisted of a three-electrode cell, obtained through magnetron sputtering technology, thus sputtering a thin layer of gold (750 Å) on the paper. The reference electrode was then coated with an Ag/AgCl slurry, followed by thermal curing. A hydrophobic pattern was realized on the paper by applying wax to insulate the electrode traces. Moreover, a printed circuit board was obtained by 3D printing to contain the nitrocellulose layer and put it into contact with the wire electrodes. After designing this device, the authors verified the electrochemical performance of the sputtered electrodes on the nitrocellulose. The electrochemical resistance and morphological features were compared with commercial electrodes (e.g., platinum, Ag/AgCl), confirming the good performance of the developed device in terms of good conductivity and stability as well as a high electrode surface area.

The analytical capabilities of the device were firstly attempted toward the detection of Cu^{2+}, used to optimize the voltametric parameters. The resulting analytical features allowed for the detection of Cu^{2+} with two linear ranges, the first between 5 ppb and 200 ppb, and the second between 200 ppb and 1000 ppb, with a detection limit of 2 ppb. By studying the interference from Pb^{2+}, Cd^{2+}, Zn^{2+}, Bi^{3+}, Cl^{-}, ${SO_4}^{2-}$, ${CO_3}^{2-}$, ${PO_4}^{3-}$, Na^{+}, and K^{+}, a drop of the Cu^{2+} signal up to 10% was observed, reaching the value of 15% in the presence of Pb^{2+}, still proving a satisfactory performance of the device. Thus, the authors applied the developed devices to lake waters, spiked with known amounts of Cu^{2+}, demonstrating recoveries within 95–110%. The promising behavior of the device led the authors to test its analytical performance also for the detection of other metals, namely Zn^{2+}, Cd^{2+}, Pb^{2+}, and Bi^{3+}. The preliminary tests showed an increased response upon increasing the concentration of these ions, analyzed separately. Although the interesting possibility of multianalyte detection was disclosed, the authors postponed further investigations to future studies, necessary to clearly prove the device reliability toward the detection of multiple metal ions.

In 2019 Zheng et al. reported the first case of using g-C_3N_4, an emerging carbon-like polymer as an alternative material Cd^{2+} and Pb^{2+} detection, employing photo paper as the support material for the electrode screen-printing [21]. The sensor was designed with a three-electrode cell, composed of Ag/AgCl underlying traces and reference electrodes, while carbon-based ink was used as working and counter electrodes. A protective cover made of adhesive vinyl was placed on the top of the printed sensor. The authors observed that photo paper offer a dual advantage: on one hand, good adhesion of the printed electrodes on the photo paper surface is achievable, and on the other hand, the hydrophobicity of this paper material, together with the presence of the adhesive cover, allow for avoiding the undesired diffusion of the liquid sample throughout the sensor. After printing, the sensor was placed on a rigid support and the working electrode was coated by drop-casting with 1:1 w/w of bismuth nanoparticles and g-C_3N_4, both prepared in the laboratory.

The resulting sensor was investigated by morphological and electrochemical studies. A graphitic stacking of g-C_3N_4 layer and a crystalline structure of the bismuth nanoparticles were observed on the working electrode, while good electrochemical performance resulted from the cyclic voltammetry with ferro/ferricyanide probe.

The authors evaluated the use of g-C_3N_4 material in comparison with bismuth nanoparticles, highlighting the advantages of the former as a cheaper alternative with comparable or higher performance. Cd^{2+} and Pb^{2+} were separately detected, obtaining a linear range within 30 ppb and 120 ppb, with detection limits of 17.5 ppb and 8.1 ppb, respectively, in acetate buffer solution at $pH = 5$. In comparison, the bismuth nanoparticles-coated electrode gave detection limits equal to 21.8 ppb and 10.4 ppb. The benefit given by the combination of bismuth nanoparticles and g-C_3N_4 was confirmed also by analyzing real samples of tap water, spiked with known amounts of Cd^{2+} and Pb^{2+}, without the need for pH adjustment.

It can be observed that the various paper types exploited within these works can provide the developed devices with the capability of overcoming any eventual matrix effect coming from the measurement in real samples. This aspect was well highlighted by Medina-Sànches et al., who devised a paper layer as a lateral flow system to treat the sample and facilitate the detection of Cd^{2+} and Pb^{2+} in turbid water samples [22]. In detail, the diffusion of the sample through the channel by lateral flow was proved to filter the mud-containing sample and allowed for the electrochemical response without a significant matrix effect. The lateral flow system was realized by printing wax on the paper and thus outlining a 3 mm-wide and 25 mm-long hydrophilic channel. The flow of the sample allowed it to reach the electrochemical cell area, composed of three screen-printed electrodes with a width of 500 μm and a thickness of 4 μm, separated by 500 μm each other. These electrodes were obtained with a graphite-based ink for the working and counter electrodes, and an Ag/AgCl-based for the reference electrode. An underlying paper layer, printed with wax with the same pattern as the paper channel, was used to avoid leakages from the channel layer. Moreover, an adsorbent pad was placed at the end of the channel to withdraw the liquid and continuously renew the sample at the electrochemical cell. The layers were sealed together with an adhesive glue (Fig. 1A).

In this work, the authors carried out the measurement in SWASV mode by drop-casting just 25 μL of sample on the hydrophilic channel, opposite to the adsorbent pad and the electrochemical cell. Acetate buffer was chosen as a medium suitable for the metallic analytes, added with a small amount of surfactant (Triton X-100 0.1% v/v). The authors investigated separately the detection of Cd^{2+} and Pb^{2+} in the range 10–100 ppb, obtaining a limit of detection equal to 11 ppb and 7 ppb, respectively. Afterward, the simultaneous detection of both metals was also tested: while no significant difference was observed for the detection of Cd^{2+} in the separate and simultaneous modes, a slight shift of the Pb^{2+} current peak occurred toward more negative potentials, ascribed to the effect of the simultaneous reduction of the metals. The filtering properties of paper were thus demonstrated by analyzing real water samples with the characteristic of turbidity, namely muddy water and seawater, resulting in the satisfactory detection of the two heavy metals although the turbidity of the sample.

An additional aspect of the paper is its suitability for combining the electrochemical measurement with a colorimetric assay. This can be obtained by pre-loading specific reagents on the paper capable of reacting with a certain analyte to provide a coloring having a proportional intensity with the analyte concentration. This strategy has been employed also in the case of paper-based sensors for environmental applications, as reported by three examples in literature. However, it has to be noted that in this context, we will focus mainly on the description of the electrochemical detection modem to be consistent with the topic of this Review.

A first example of colorimetric/electrochemical dual detection on paper was provided by Chaiyo et al., in 2016 [23]. The authors developed a paper-based device capable of measuring Cu^{2+} by a colorimetric approach and Cd^{2+} and Pb^{2+} by electrochemical detection. To achieve this, the sensor was devised by combining a wax-patterned paper layer and a working electrode layer (Fig. 1B). The former presented hydrophilic areas, defined by wax, for both electrochemical (a circular 1 cm-area where a graphite-based counter electrode and an Ag/AgCl-based reference electrode were screen-printed) and colorimetric detection (two circular 3 mm-areas, one connected to the electrochemical area by a hydrophilic microfluidic channel, and the second separated and used as a control). The working electrode layer consisted of a bismuth-modified boron-doped diamond. The two layers were sealed together by means of a double-sided adhesive tape having an 8 mm-hole in correspondence with the electrochemical area, to allow for the sample deposition. The sensing approach relied on the drop-casting of 50 μL of liquid sample on the electrochemical area to measure Cd^{2+} and Pb^{2+}, then the liquid diffusion toward the colorimetric area enabled the colorimetric detection of Cu^{2+}. In this way, the eventual interfering effects toward Cd^{2+} and Pb^{2+} electrochemical detection coming from the presence of Cu^{2+} were overcome.

In this study, the authors used a working solution composed of NaCl at pH 6.0 used to optimize the simultaneous electrochemical and colorimetric detection, since it provided the best results among other buffers or acidic media commonly used for the detection of the selected metals. The colorimetric detection of Cu^{2+}, based on the catalytic etching of silver nanoplates with thiosulfate, will not be discussed in this context; regarding the measurement of Cd^{2+} and Pb^{2+}, bismuth was in situ electrodeposited during ASV technique application. After pointing out the most advantageous conditions for the ASV application, a detection limit of 0.1 ppb was obtained for both Cd^{2+} and Pb^{2+}. Moreover, the addition of possibly interfering ions 1000-fold (K^+, Mg^{2+}, Mn^{2+}, and Al^{3+}), 500-fold (As^{3+}, Fe^{3+}, and Cr^{3+}), 400-fold (Zn^{2+}, Hg^{2+}, Co^{2+}, and Ni^{2+}), and 200-fold (Cu^{2+}) did not significantly affect Cd^{2+} and Pb^{2+} signals. Thus, the authors choose stream water and groundwater to apply the developed device. Moreover, the devices were tested also in samples from commercial food products, namely rice and fish, which were appropriately digested. The recoveries obtained, within 80–103% for both Cd^{2+} and Pb^{2+} as well as the successful comparison with a reference spectrometry technique (coupled plasma optical emission spectrometry) allowed the authors to conclude that their device was a potential candidate for the monitoring of the selected metals in environmental samples.

Henry and colleagues have contributed to this topic by reporting a paper-based device for the detection of heavy metals. In detail, Rattanarat et al., in 2014 have developed a dual-mode detection, combining a colorimetric response with electrochemical measurements [24]. Such devices were conceived to have a three-dimensional configuration, composed of two layers on which the colorimetric and the electrochemical detection were carried out separately. In this way, the possibility of assembling the paper layers allowed for applying different sample preparation methods for each detection technique, according to the different needs.

The focus of this work was on metals present in the air particulate

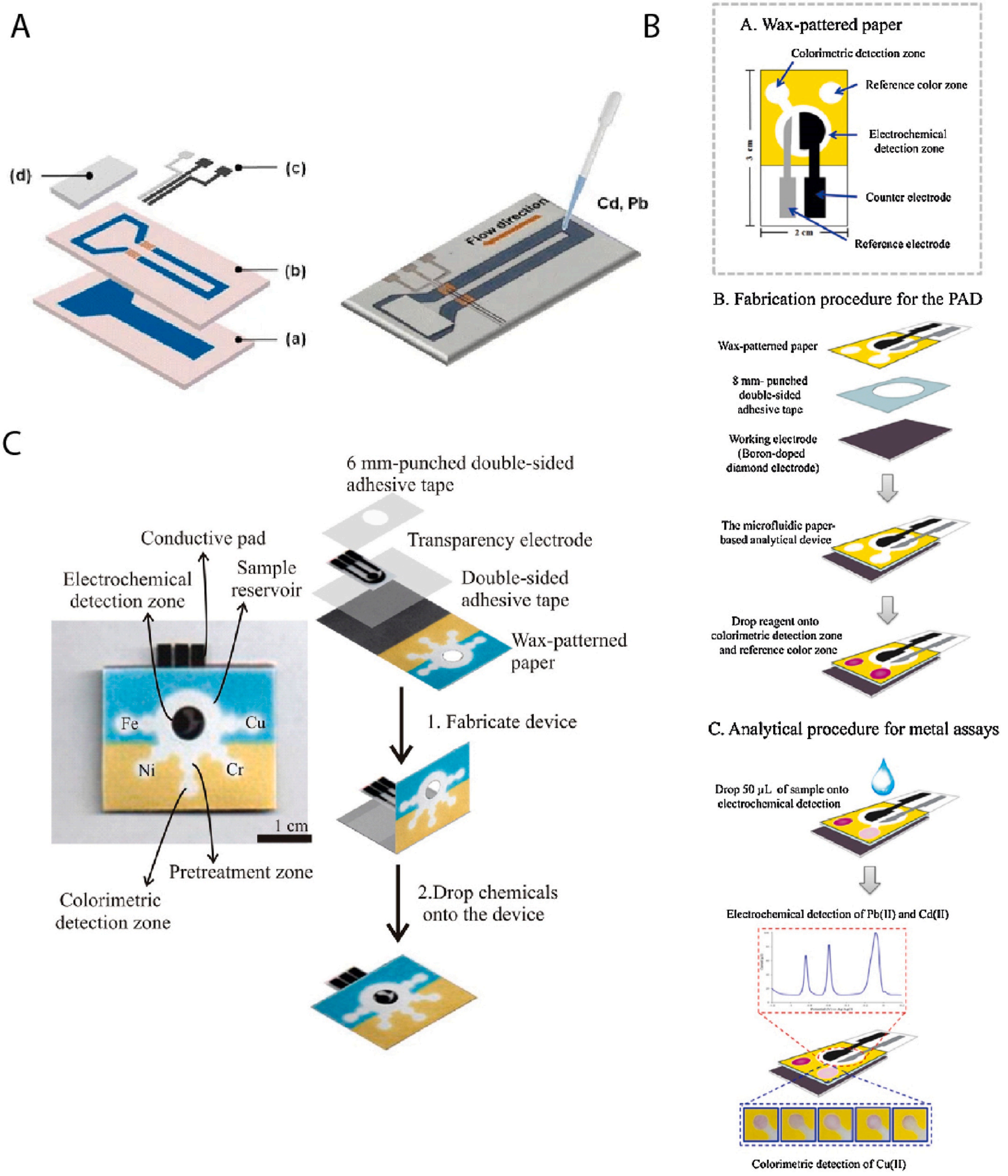

Fig. 1. **A)** Eco-friendly electrochemical lab-on-paper for heavy metal detection. Reprinted with permission from Ref. [22], 2015 Springer-Verlag Berlin Heidelberg. **B)** High sensitivity and specificity simultaneous determination of lead, cadmium and copper using µPAD with dual electrochemical and colorimetric detection. Reprinted with permission from Ref. [23], 2016 Elsevier. **C)** Multilayer paper-based device for colorimetric and electrochemical quantification of metals. Reprinted with permission from Ref. [24], 2014 American Chemical Society.

matter, in detail nickel, iron, copper, and chromium, analyzed by colorimetric approach, and lead and cadmium, detected by SWASV. These metals were collected on air sampling filters (37 mm Pallflex or mixed cellulose ester filters) as a particulate. The particulate collected was digested by microwave-assisted acid digestion, then the filters were placed on the center of the paper-based device and a drop of buffer was added to elute the metal content in two directions: outward, toward the colorimetric detection zones, and downward, to reach the electrodes. The device was designed using Whatman grade 1 filter paper, with a printed wax pattern to drive the sample toward the hydrophilic reaction areas, namely the sample reservoir, the pretreatment zone, and the detection zone (diameters of 1.2, 0.3, and 0.3 cm, respectively). Interestingly, the colors of the wax were chosen in order to be complementary to the shades generated by the colorimetric reactions, thus obtaining a more efficient reading of the response. These features represent some of the characteristic properties that make the use of paper advantageous with respect to other materials.

To accomplish the electrochemical detection, a 6 mm-diameter hole was created in the middle of the sample reservoir of this device, to allow the sample solution to reach the electrode, placed under the paper pad. A three-electrode cell was screen-printed on a polyester transparent film, which was placed and sealed under the paper pad by using an adhesive (Fig. 1C). For the measurement, acetate buffer containing bismuth and ferrocyanide was used to modify the working electrode surface, the latter composed of multiwalled carbon nanotubes-modified carbon ink. Also in this case, bismuth was chosen to improve the detection of Cd^{2+} and Pb^{2+}, while ferrocyanide was employed to remove the interference commonly given by copper ions. With this approach, Cd^{2+} and Pb^{2+} were simultaneously detected in the range of 5–150 μg/L, obtaining LOD of 25 ng (1 μg/L) for both the metals.

The selectivity of this sensor for the target analytes was demonstrated for possibly interfering metal species. In detail, Mg^{2+}, Mn^{2+}, Al^{3+}, Fe^{3+}, Zn^{2+}, Ba^{2+}, and V^{3+} did not significantly affect Cd^{2+} and Pb^{2+} detection, while Ni^{2+}, Co^{2+}, Cr^{6+}, and Cu^{2+} showed interfering effects on Cd^{2+} and Pb^{2+} detection. However, the authors successfully pointed out the use of ferricyanide to reduce the interference of copper, thanks to the complex formation between copper ions and cyanide ions. Indeed, the complete recovery of Cd^{2+} and Pb^{2+} was achieved in the presence of 30 μg (500 μg/L) of Cu^{2+} when 0.25 mM of ferricyanide was pre-deposited on the working electrode surface. To finally validate the device, baghouse dust samples with a known content of cadmium, lead, and chromium, and unknown amounts of other metals, were nebulized on the filters, to simulate the particulate sampling that usually occurs from the air. The resulting concentrations matched with the known quantities of each metal, confirming the applicability of the device.

A sophisticated and very interesting origami-like paper structure was recently reported by Henry's group. Indeed, Silva-Neto et al. presented a "plug-and-play" assembly approach designed to combine the colorimetric detection of iron, nickel, and copper ions with the electrochemical detection of zinc, cadmium, and lead ions [25]. The sensing principle was based on a vertical and reversible foldable mechanism that was configured to enable dual detection with a single drop of sample. In detail, an electrochemical screen-printed three-electrode cell and an *ad hoc* designed pad were obtained on Whatman grade 1 chromatography paper. The printed cell consisted of a working and counter electrode made of graphite ink containing multiwalled carbon nanotubes, while the pseudo-reference electrode was obtained by painting with silver-based ink. The colorimetric pad was composed of three channels (1.5 mm in width and 12 mm in length) originated from the same piece of paper, each one presenting three circular areas (3 mm in diameter), to be used as the pretreatment zone (PZ), colorimetric detection or reaction zone (RZ), and waste zone (WZ). After the printing step and the shape designing, the paper-based devices were laminated by thermal treatment on a flexible thermosensitive polyester film, which in this way fixed the paper-based printed electrochemical cell and the pad for the colorimetric detection together and at the same time defined the hydrophilic areas.

For carrying out the dual measurement, a 100 μL-drop of the sample was placed on the printed cell, then the device was folded twice, with a 45-degree angle, to put in contact the colorimetric pad with the sample drop. Thus, the sample was allowed to flow through the paper pad for 3 s for providing the colorimetric reactions. Immediately after, the device was unfolded and 1 μL of ferricyanide solution was added to remove the interference of copper ions while accomplishing the electrochemical detection. An additional external holder was produced by 3D printing to enable simple and reproducible assembly of the device as well as to ensure the robustness of the device and to provide a stable interface between the printed cell and the potentiostat. Particularly, the electrical contact was obtained using a USB cable.

The colorimetric assays for iron, nickel, and copper ions were performed exploiting well-known reactions with bathophenanthroline, dimethylglyoxime, and bathocuproine, respectively, but this will not be described in detail in this review. For the electrochemical measurement of Zn^{2+}, Cd^{2+}, and Pb^{2+} ions, bismuth was previously electrodeposited on the working electrode surface and SWASV war run in the presence of the target ions. The device showed a linear range of 100–1400 ppb for Zn^{2+} (detection limit = 10.5 ppb) and 10–1400 ppb for Cd^{2+} and Pb^{2+} (detection limit = 1.3 ppb and 0.9 ppb, respectively). In addition, the measurement carried out in the presence of potential interferences at different tolerance ratios, namely Mn^{2+}, Al^{3+}, Sb^{2+}, Ba^{2+}, Ni^{2+}, and Fe^{2+}, did not result in significant interference toward Zn^{2+}, Cd^{2+}, and Pb^{2+} detection. Real samples from river waters were analyzed by spiking in the range from 16 to 786 ppb, giving recovery values within 76–121%. These achievements together with the good comparison with a conventional method (i.e., atomic absorption spectrometry) demonstrated the promising features of such paper-based sensing architectures having the advantage of enabling rapid multianalyte measurement in a dual mode.

One of the most notorious and toxic heavy metals, namely mercury, was chosen by Bui et al., in 2016 as the target analyte for a paper-based sensor [26]. Interestingly, the authors developed a device capable of measuring both mercury and nitrate ions, the latter being an inorganic compound of crucial importance for aquatic environment equilibria. Other inorganic species that affect the environment safe conditions are discussed in the next paragraph.

In the work of Bui et al. the detection of mercury and nitrate was achieved by combining gold nanoparticles (12 ± 2 nm of diameter) with selenium particles (0.2–1 μm of diameter) to promote the nucleation of mercury at the working electrode surface as well as to catalyze the detection of nitrate. The sensing platform consisted of a carbon printed sensor realized on paper and coated with poly (ethyleneglycol) methylether thiol by drop-casting. Thiol groups ensured the good deposition of gold nanoparticles thanks to the high binding affinity toward gold. The adsorption of mercury ions on selenium particles served to improve its detectability. Interestingly, a morphological study highlighted how the use of poly(ethylene glycol) methylether thiol improved the surface area available for the adsorption of both gold and selenium particles on the carbon electrode, especially thanks to the higher number of interacting thiol groups. This allowed the authors to exploit the porosity of paper as an advantage for enhancing the sensitivity of the sensor.

Electrochemical investigations based on cyclic voltammetry were applied to characterize the electrode behavior toward Hg^{2+} and nitrate detection. The former was achieved at an oxidative potential of about 0.5 V, while nitrate could be measured at a reductive potential of about −0.4 V. The measurement was accomplished by using differential pulse ASV for Hg^{2+} and differential pulse voltammetry for nitrate. The ASV parameters were step by step studied, resulting in satisfactory analytical features by using an accumulation time of 600 s, namely a linear range of 14–3500 ppb and a detection limit of 1.0 ppb. On the side of nitrate detection, a linear range within 0.016–5 mM and a detection limit of 8.6 μM were obtained. Finally, to test the applicability of the device to real matrices, lake water samples were spiked with known amounts of Hg^{2+}, with recoveries of about 80%.

2.2. Other inorganic pollutants

Inorganic compounds including nitrates, ammonium, and phosphate are aquatic descriptors of water quality and good ecosystem equilibria, in terms of nutrient content [27]. Indeed, they are essential compounds for living beings in water ecosystems, ensuring the necessary supply of elemental phosphorus and nitrogen. However, an excessive amount of these compounds, for example as a consequence of intensive use of fertilizers in the agriculture field, can alter the optimal equilibria conditions and cause eutrophication [28]. On the other hand, reduced forms of these compounds, particularly nitrites and ammonia, turn to be harmful to the aquatic environment [29]. This claims the need for the monitoring of such compounds in waters, as demanded by European and international entities and legislations.

Besides the more conventional colorimetric assay, spectrophotometric methods, and chromatography, such compounds can be detected by electrochemical sensors. Several efforts have been spent to find out successful strategies for enabling electrochemical detection in this field, with the aim to provide the end-users with portable, rapid, easy-to-use, and cost-effective analytical tools for the monitoring of the target analytes. Here, the literature examples of paper-based electrochemical sensors for the detection of inorganic compounds relevant to the safety of the environment and living beings are analyzed and discussed. To our knowledge, such kinds of devices have been developed to date for the measurement of nitrates (as discussed in the previous paragraph [26]) and phosphates.

A first example of paper-assisted detection of phosphates is reported in a work of ours in 2016 [11]. A wax-patterned filter paper strip was designed to integrate the chemical modification of the paper itself, by pre-loading the reagents needed for the measurement (i.e., “reagentless” sensor), with screen-printed electrodes. Such devices needed a very small volume of the sample solution, namely 5 μL, thanks to the high porosity and surface area provided by filter paper, patterned by wax to outline the hydrophilic reaction areas. In detail, the reaction area was pre-loaded with 10 μL of a solution containing the reagents needed to carry out an on-paper adaptation of Murphy and Riley method for phosphate detection. This method is based on the use of a mixture of ammonium molybdate, ascorbic acid, and antimony potassium tartrate that, together with an acidic environment, lead to the formation of a P–Mo complex (i.e., $[PMo_{12}O_{40}]^{3-}$, also known as the Keggin anions), the latter being reduced by ascorbic acid and antimony tartrate to a molybdenum species with a mixed oxidation state. The amount of phosphate is then proportional to the intensity of the blue coloring given by the reduced product of this reaction. Alternatively, the reduction process of the P–Mo complex can be performed by electrochemical mode, thus enabling the development of electrochemical sensors for phosphate detection in liquid samples. This aim was achieved in our work by using the matrix of filter paper as a chemical reactor for the above-described reactions. After reagents pre-loading, namely molybdate ions, potassium chloride, and sulfuric acid, the paper was let to dry, and the electrodes were screen-printed to realize a three-electrode cell consisting of a graphite-based working and counter electrodes and an Ag/AgCl-based reference electrode (Fig. 2A). The working electrode was modified with carbon black to enhance the electrochemical sensing performance, for example in terms of electron transfer kinetics.

Despite the paper substrate’s advantageous properties, we explored possible drawbacks due to the cellulose matrix. Indeed, this could hinder the diffusion of the analyte, including the dissolution of the reagents pre-loaded within the cellulose itself, thus affecting the measurement. Actually, the diffusion coefficient, calculated using ferricyanide as the electrochemical probe and the Cottrell equation in chronoamperometry mode, resulted to be three-fold lower ($2.03 \pm 0.11 \cdot 10^{-6}$ cm^2/s) in the paper matrix with respect to the liquid solution ($6.08 \pm 0.20 \cdot 10^{-6}$ cm^2/s). However, the analytical performance of the sensor resulted

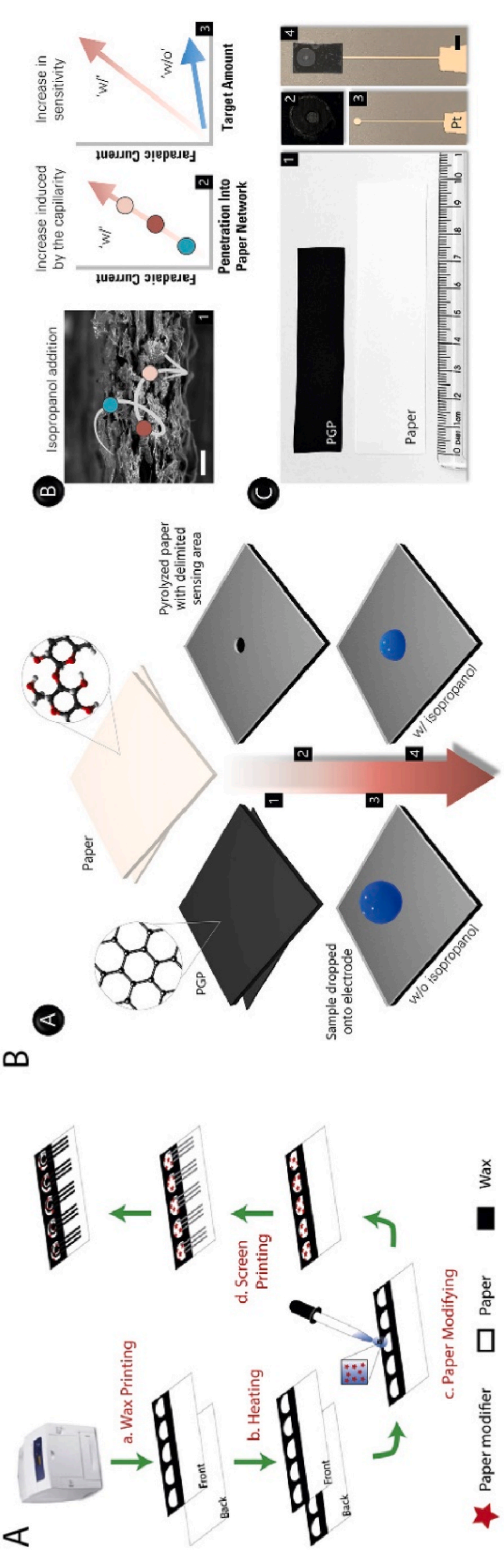

Fig. 2. **A)** Novel reagentless paper-based screen-printed electrochemical sensor to detect phosphate. Reprinted with permission of [11], 2016 Elsevier. **B)** Alcohol-Triggered Capillarity through Porous Pyrolyzed Paper-Based Electrodes Enables Ultrasensitive Electrochemical Detection of Phosphate. Reprinted with permission of [30], 2021 American Chemical Society.

satisfactory, allowing for the phosphate detection in a linear range up to 300 μM and with a detection limit of 4 μM by using cyclic voltammetry. These sensors were characterized by good reproducibility (relative standard deviation of nine sensors equal to 6%) and could be stored at RT dark condition for 30 days without undergoing any alterations of the response, indicating that the pre-loaded reagents were stable in the cellulose matrix. Lastly, river waters were analyzed as the real sample obtaining recoveries of 96 ± 10% after spiking with known concentrations of phosphate ions.

Later on, Shimizu et al. have studied the on-paper measurement of phosphate with a different strategy [30]. Similar to the previous example, the authors exploited the Murphy and Riley method and the formation of the P–Mo complex, which was obtained in a solution prior to adding the analyte and the reagents on the paper-electrode (Fig. 2B). In this work, a pyrolyzed graphitic paper was obtained by a thermal treatment at 1000 °C, then followed by the partial melting of a flexible film within the cellulose matrix. The resulting treated paper has acquired robustness, flexibility, and a defined area, but becoming hydrophobic at the same time. This substrate was used as the working electrode by conceiving an isopropanol-triggered strategy to enable the absorbance of aqueous solutions by capillarity in the pyrolyzed paper.

After pyrolysis, the paper presented a mixture of carbon units with sp^2 (~30%) and sp^3 (~58%) hybridization and oxygen-containing functional groups (~12%, including C–O, C=O, C–OOH). Since this substrate resulted to be hydrophobic, a 0.5 μL-drop of isopropanol was added on a pyrolyzed paper-based electrode to favor the good penetration of the sample through the pores. This approach was shown to allow for hindrance-free redox reactions and an increment of the active areas at the same time. Moreover, an advantage was found in the pyrolysis process due to the absence of needed reagents, thus avoiding waste during the paper modification.

The measurement was carried out by drop-casting 150 μL of a solution already containing the P–Mo complex in SWASV mode. For the reference and counter electrode, Ag/AgCl and platinum wires were used, respectively. Importantly, the current recorded in the presence of isopropanol resulted to be 10 times higher than the measurement carried out without the alcohol, confirming its role in improving the analytical performances of the pyrolyzed-paper electrode. This achievement was attributed by the authors to the enhanced wettability given by the alcohol adding on the pyrolyzed paper, as shown by contact angle studies. As suggested, the capability of isopropanol of penetrating the hydrophobic surface as well as interacting with the aqueous solutions by hydrogen bonding could decrease the interface tension of pyrolyzed paper. Further proof of this was found by applying energy-dispersive X-ray spectroscopy, which revealed the presence of the reagents (i.e., sulfur atoms from the electrolyte) within the pores of the pyrolyzed paper only when isopropanol was previously added. Also, the increased active surface area was confirmed by double-layer capacitance experiments, resulting in an area about 27-fold higher with respect to pyrolyzed paper not treated with isopropanol. The authors also tested other commonly used alcohols, namely methanol and ethanol, providing indications on how the sensing principle can be adapted also to other alcoholic solvents.

This platform provided a linear range up to 300 ppm for phosphate detection with the isopropanol-assisted measurement, and a limit of detection of 1.1 ppb, which is among the lowest values according to the literature. Real samples from lake water and tap water were analyzed with a satisfactory accuracy calculated by comparing the electrochemical responses with ion chromatography as a reference method.

3. Organic pollutants

3.1. Pesticides

Pesticides are defined as chemical substances used to prevent, destroy, or mitigate pests such as insects (i.e., insecticides), rodents (i.e., rodenticides), and weeds (herbicides) [31]. These chemicals include organochlorine pesticides, pyrethroids, organophosphorus, chloronitrophenols, and heavy metal ions, and are capable of producing harmful effects on the environment and living beings also when present at the trace level [32].

Despite these concerning aspects, the use of pesticides covers a central role in agricultural productivity thanks to the enabled agricultural intensification since the Second World War. The large use of pesticides in agri-food practices allows for unintentional pesticide poisonings of agricultural workers and pollution of the aquifer due to the percolation of pesticides through the soil.

Currently, the most exploited analytical methods for pesticides detection rely on laboratory-based techniques, mainly based on chromatography and mass spectrometry [32]. As well known, these techniques require an intensive sample treatment, including solvent extraction and purification processes to avoid interferences from the real matrices analyzed. Considering the sustainable development criteria and the ubiquitous concern represented by pesticides, there is an urgent need for on-site, organic solvent-free, and cost-effective analytical tools for the smart detection of pesticides.

In this overall scenario, electrochemical paper-based biosensors have covered a recent and interesting research field due to the capability of detecting classes of pesticides in a fast way, working as a “family doctor”. Using this approach for a fast screening of natural waters or plant samples, only in the case of the positive sample, the laboratory-based conventional analysis would be required, reducing the use of organic solvent and waste production and conferring a more sustainable vision to pesticide detection [33]. Among biosensors, typically based on aptamers, antibodies, and enzymes, inhibitive enzymatic biosensors are the most used devices for pesticides detection. The sensing principle is based on the capability of pesticides to inhibit specific enzymes [34]. As an example, organophosphorus insecticides are able to inhibit in an irreversible way a key enzyme of nervous transmission, namely acetylcholinesterase, thus allowing for the detection of organophosphorus insecticides by selecting an enzyme belonging to the cholinesterase class to design an enzyme inhibition biosensor [35]. With this strategy, it is possible to use the high specificity of enzymes to improve the analytical performance of the devices and reduce the occurrence of interference in the measurement. This advantage, coupled with the properties of the paper, has inspired the researchers in designing electrochemical devices based on paper and enzymes capable of revealing pesticides with high sensitivity and selectivity also in complex matrices, as described in the following paragraph.

In 2017, we developed the first example of an integrated paper-based screen-printed electrochemical biosensor able to quantify organophosphates [36]. The measurement using an inhibitive biosensor encompasses the measure of enzymatic activity in absence of pesticides followed by the measure in presence of pesticides, and in the case of the decrease of enzymatic activity in presence of the sample, the degree of inhibition is proportional to the quantity of pesticides present in the sample. Taking into account the need for two measurements, while paper-based devices usually allow for carrying out only one measurement per device, we designed the lateral flow microfluidic paper-based device by combining two parallel paper-based biosensors close to each other (Fig. 3A). In each case, a strip of a nitrocellulose membrane was loaded with the substrate butyrylthiocholine and integrated with a paper-based test area that included a screen-printed electrode and the pre-loaded enzyme. The addition of a drop of distilled water pushed the flow of the substrate towards the electrochemical cell zone, which contained the enzyme butyrylcholinesterase able to catalyze the hydrolysis of butyrylthiocholine to thiocholine and butyric acid. The electroactive thiocholine was then detected by chronoamperometry at +300 mV by using the carbon black/Prussian Blue nanocomposite present on the working electrode as the modifier. Paraoxon was chosen as an organophosphorus pesticide. For the sample analysis, a few microliters were added to the electrochemical cell zone, where the enzyme

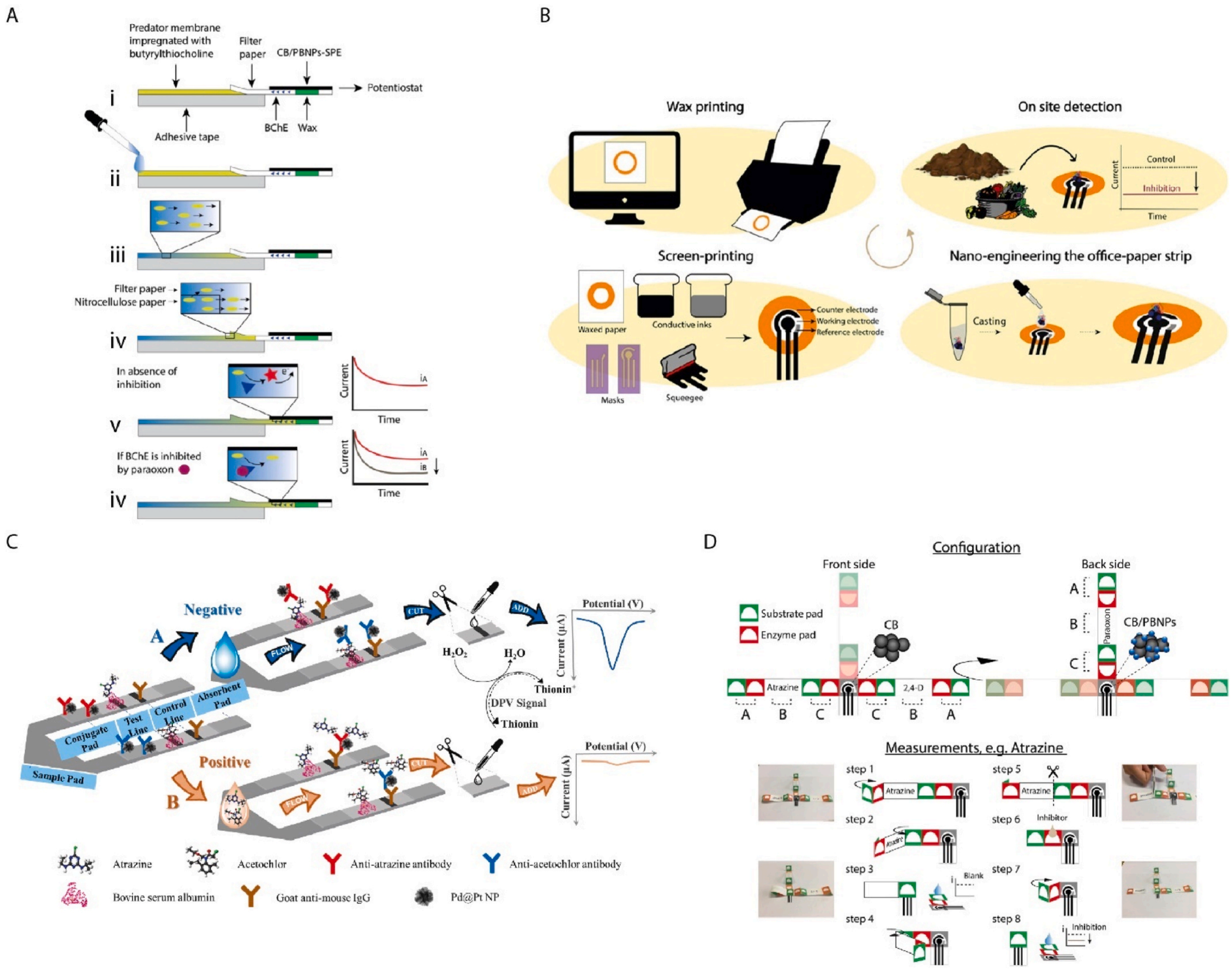

Fig. 3. **A)** Fully integrated ready-to-use paper-based electrochemical biosensor to detect nerve agents. Reprinted with permission of [36], 2016 Elsevier. **B) Office Paper-Based Electrochemical Strips for Organophosphorus Pesticide Monitoring in Agricultural Soil.** Reprinted with permission of [37], **2021 American Chemical Society. C)** Nanomaterial-enhanced 3D-printed sensor platform for simultaneous detection of atrazine and acetochlor. Reprinted with permission of [38], 2021 Elsevier. D) Origami multiple paper-based electrochemical biosensors for pesticide detection. Reprinted with permission of [9], 2018 Elsevier.

reacts with the pesticides during the incubation time. In standard solutions, this biosensor achieved a detection limit equal to 3 μg/L, calculated as the amount of paraoxon that gave 10% of inhibition. To demonstrate the suitability for the environmental samples, the biosensor was tested with a recovery study using a river water sample spiked with 25 ppb of paraoxon, obtaining values of percentage recovery for untreated river water equal to 89 ± 8. The sample was added on the back side of the working electrode, to allow the paper to filtrate the sample before reaching the working electrode surface, aiming to avoid the presence of particulate of the sample close to working electrode surface.

The sensing principle explored in this study was then further developed by our group for the multiplex analysis of pesticides, by realizing an inhibitive enzyme biosensor based on butyrylcholinesterase, alkaline phosphatase, and tyrosinase enzymes for the detection of organophosphorus insecticides, phenoxy-acid herbicides, and triazine herbicide, respectively. This device combined the vertical microfluidic and a programmable paper folding to obtain an origami configuration that presented three strips (Fig. 3D) [9]. Each strip was designed with two pads containing the enzyme (red pads) and two pads containing the substrate (green pads) and conceived to detect specifically one class of pesticide. For the measuring of the enzymatic activity without inhibitors, the distal couple of pads was folded to get into intimate contact with the electrochemical cell printed on office paper, followed by the addition of distilled water to carry out the electrochemical measurement. The adding of distilled water was suitable for dissolving the enzymes, substrate, and buffer salts, previously pre-loaded on the filter paper pads. For the sample measurement, some μL of water sample was loaded only onto the red pad containing the enzyme for having incubation time (5 min), then the pads were folded to obtain the measure in presence of inhibitors. The paper-based device demonstrated the capability to quantify paraoxon, 2,4-dichlorophenoxyacetic acid, and atrazine at ppb level in river water samples with good accuracy, without sample treatment and the need for adding further reagents, delivering a reagent-free device.

By following a similar approach, Cinti's group developed an office paper-based electrochemical biosensor for paraoxon-ethyl in several samples including soil and fertilized soil samples (Fig. 3B). In this case, the sample needed for the treatment adding 100 μL of aqueous solution (2% ethanol), followed by a vortex for 20 min and filtration with an MF-Millipore Membrane Filter (0.45 μm). The biosensor achieved the detection limit of 1.3 ng/mL, calculated as a signal-to-noise ratio equal to 3 in standard solutions, and recoveries values in soil equal to 84% and 97% for levels of 10 and 25 ng/mL [37].

A different strategy was reported by Du's group, developing a two-ways immunosensor for simultaneous detection of atrazine and acetochlor with a 3D-printed holder (Fig. 3C). The measurement was carried out using the mesoporous core-shell palladium@platium nanoparticle as the label of immunological chain, having peroxidase-like property on the redox reaction between thionin acetate and hydrogen peroxide. This multiple immunosensor demonstrated the capability to detect atrazine and acetochlor with a detection limit of 0.24 ppb and 3.2 ppb, respectively. The applicability in environmental samples was evaluated by testing water from the Snake River (Clarkston, WA) and a local well (Pullman, WA) with recoveries for the spiked atrazine samples between 97.0% and 109% for the river water matrix, and between 90.8% and 101% for the well water matrix, while for spiked acetochlor samples the recoveries resulted to be between 92.4% and 112% for the river water matrix and between 102% and 117% for the well water [38].

3.2. Pharmaceuticals

The population growth and the consequent strong increase in the consumption of pharmaceuticals (personal care products, hormones, antibiotics, and drugs) all over the world have led to concern about the high concentration of these products in the environment. Indeed, the United Nations has estimated that the pharmaceutical industry's annual growth rate is 6.5% and that the 10% of manufactured pharmaceutical products are an environmental hazard [39].

These pharmaceutical products and their residues can reach the environment in different ways. For example, they may result from i) the wastewater discharged from pharmaceutical manufacturing plants; (ii) domestic and hospital effluents containing pharmaceutical compounds excreted in the urine and feces of humans; (iii) the use of veterinary drugs and feed additives in livestock breeding; and (iv) improper disposal of unused or expired pharmaceutical products through the sink, toilet or in a general waste bin Other pathways have been reported, such as the applications of pharmaceutical substances directly to water for aquaculture and wastewater irrigation of agriculture [40].

Several studies indicate the presence of various pharmaceuticals in the effluents of wastewater treatment plants, surface water, and groundwater. For example, it is reported that in wastewater effluents, a range of around 16 to 54 types of pharmaceuticals is found in concentrations relatively higher than 1.0 μg/L or even extremely higher than 10.0 μg/L. Such existence or occurrence of pharmaceuticals at higher levels can be the inability of conventional wastewater treatment plants (WWTPs) or other natural elimination processes to remove them completely, and thus they can percolate through WWTPs and be incorporated into the aquatic systems (streams and rivers) [41]. From a consumers-based source view, partially or incompletely metabolized pharmaceutical excretion into the domestic sewage stream is the main cause of pharmaceuticals to the aquatic environment [42]. Thus, the presence of pharmaceutical residues as pollutants in the environment has been recognized as an emerging issue in environmental chemistry. The difficulty of removing these compounds is especially due to their molecular complexity based on variations in their stability, solubility, polarity, and ionization, which also depend on environmental properties [43].

Indeed, different types of pharmaceutical products have been introduced in the NORMAN list of emerging substances and the need to detect them rapidly and easily has been increased day by day. In this overall scenario, electrochemical paper analytical devices result in excellent instruments for detecting pharmaceutical residues in water samples due to their wide availability, straightforward fabrication, self-pumping abilities via capillary action, portability for field analysis, and easy disposal.

Among the pharmaceutical pollutants, antibiotics are not completely metabolized or eliminated, and a percentage ranging from 30% to 90% is excreted unchanged into the wastewater system [44]. The derived degradation products can sometimes be as toxic as their precursors or having even a higher ecotoxicity, which may affect human health and increase bacterial resistance, especially with their long persistence in the environment [45]. Reports have demonstrated that the incorporation of antibiotics, metabolites, and antibiotic resistance genes into the natural environment can influence microbial populations by bacteriostatic and bactericidal effects, leading to disappearing key microbial groups associated with ecological activities or affecting their physiological functions [46]. At worldwide level, 45 types of antibiotics have been found in effluents of wastewater treatment plants while the collected surface water data contain about 43 types of antibiotics from 24 countries. Sulfamethoxazole and trimethoprim antibiotics, which are used to treat bacterial and fungal infections, and pneumocystis pneumonia, are among the three most found antibiotics in wastewater treatment plants from 13 countries all over the world, according to the European Commission's Joint Research Center technical report.

Being essential to monitor their presence in the environment, Martins et al. developed a paper-based screen-printed sensor capable of detecting sulfamethoxazole and trimethoprim antibiotics simultaneously in real water samples (Fig. 4A) [47]. The electrochemical cell was characterized by a carbon working electrode with an area of 7 mm^2 modified with 6 μL of reduced graphene nanoribbons (0.1 mg/ml), a carbon counter electrode, and a silver pseudo-reference electrode. The use of reduced graphene nanoribbons as a working electrode modifier

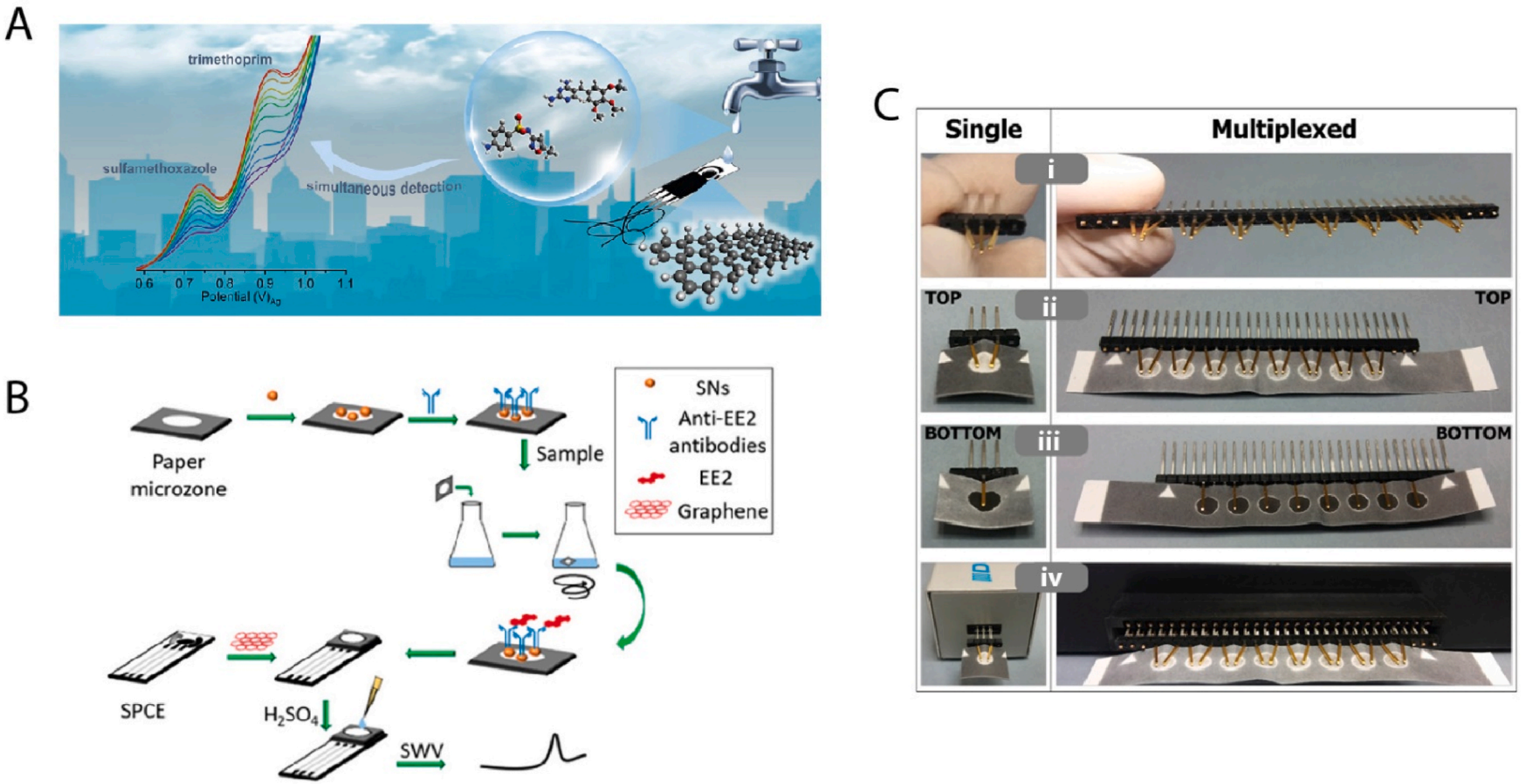

Fig. 4. **A)** Paper-based electrochemical sensors with reduced graphene nanoribbons for simultaneous detection of sulfamethoxazole and trimethoprim in water samples. Reprinted with permission from Ref. [47], 2021 Elsevier. **B)** Novel Electrochemical Paper-Based Immunocapture Assay for the Quantitative Determination of Ethinylestradiol in Water Samples. Reprinted with permission from Ref. [50], 2018 American Chemical Society. **C)** Preconcentration and sensitive determination of the anti-inflammatory drug diclofenac on a paper-based electroanalytical platform. Reprinted with permission from Ref. [53], 2019 Elsevier.

considerably increased the sensitivity for the detection, thanks to its two-dimensional structure which allowed a strong interaction with the analytes. Moreover, the electrodes were printed on parchment paper, which was an excellent candidate as substrate being biocompatible and non-toxic, flexible, and resistant to fluids, with moderate self-absorption. It also facilitated sample interaction and has high structural stability while offering printing capacity. Indeed, the parchment paper has a hydrophilic character which is a desired characteristic since hydrophilic substrates have high ink transfer rates with good reproducibility. Furthermore, the parchment paper used in the construction of the electrodes exhibited an excellent chemical and structural stability for applications in aqueous medium at different pHs. The calibration curves of the antibiotics were performed with differential pulse voltammetry measurements between 0.4 and 1.1 V in phosphate buffer (pH 6.0) and the paper-based sensor displayed a linear response from 1 μM to 10 μM for both antibiotics with detection limits of 0.09 μM and 0.04 μM for sulfamethoxazole and trimethoprim, respectively. For the recovery studies, the voltametric measurements were carried out in a phosphate buffer (pH 6.0) solution made with tap water without further dilution or pre-treatment, obtaining values for the simultaneous detection between 97.8 and 102.0% with a relative standard deviation below 4%.

Matos' research group proposed another paper-based device developed for antibiotic residues detection and more precisely for sulfanilamide antibiotic monitoring in environmental, pharmaceutical, and biological fluids samples by differential pulse voltammetry [48]. The electrochemical device employed a conductive ink based on graphite and nail polish in a ratio 1:1 (w/w), with cardboard paper (240 g/m^2) as a support material with 1 × 2 cm dimensions. The paper support was used for the construction of the working electrode (diameter of 0.4 cm) while Ag/AgCl, and platinum wire electrodes were used as reference and auxiliary electrodes, respectively. The electrochemical method showed an excellent analytical performance with a detection limit of 4.1 μM, wide linear range from 10 to 100 μM, adequate precision (relative standard deviation, RSD, $<2.0\%$), and recovery values ranging from 80 to 102% for the spiked samples, samples of lake and seawater, and minimum sample preparation step (simple dilution). Thus, the disposable proposed device was a promising analytical tool for routine analyses of clinical, environmental, and food samples.

Besides antibiotics, different hormones are considered emerging pharmaceutical pollutants. The most widely used oral contraceptive formulations contain ethinyl estradiol (EE2), a synthetic hormone derived from the natural estrogen estradiol. In contrast to the latter, EE2 is resistant to degradation by the liver. Therefore, a great amount of EE2 and its derivatives are introduced into the environment leading to be listed in the NORMAN emerging substances. EE2 is considered an endocrine-disrupting compound, having a negative impact on the reproductive system of wild animals [49]. For this reason, the quantitative determination of EE2 in natural water samples has become an important challenge. Scala-Benuzzi et al. developed a novel paper-based immunoassay for the quantitative determination of EE2 in water samples [50]. The method was based on the use of paper hydrophilic microzones, with a circular shape of 7 mm in diameter, modified with silica nanoparticles and *anti*-EE2 specific antibodies for the capture, preconcentration, and determination of EE2. The silica nanoparticles were used to increase the surface for immobilization of biomolecules, allowing for the enhancement of their incorporation into the device, leading to signal enhancement. Fig. 4B showed the schematic representation of the immunoassay. River water samples were added to the modified paper hydrophilic microzones for the capture and preconcentration of EE2. Then, the paper microzones were placed on the surface of reduced graphene on a screen-printed carbon electrode. The bound EE2 was desorbed adding onto the paper microzones a diluted solution of sulfuric acid 10 mM. The recovered EE2 was electrochemically detected by square-wave voltammetry where the obtained oxidation current was proportional to the EE2 concentration in the sample. In this case, the paper was not only used as substrate to sample and preconcentrate the analyte of interest, but also to treat the matrix. Indeed, water samples were passed by gravity through filter paper (Whatman) and by vacuum through glass microfiber filters (Whatman). Finally, the samples were adjusted to pH 7 using 0.1 M phosphate buffer. The proposed paper-based biosensor was able to detect different concentrations of EE2 obtaining a linear calibration curve up to 120 ng/L in standard solutions with a LOD equal to 0.1 ng/L. Five natural and spiked river water samples were analyzed to validate the reliability of the method. The recovery values obtained from spiked samples ranged from 97.5% to 103.7%, with a relative standard deviation of less than 4.9%, which indicated a good accuracy of this assay for quantitative detection of EE2 in water samples.

Another important environmental pharmaceutical pollutant is diclofenac (DFC), a common non-steroidal anti-inflammatory drug with analgesic and antipyretic properties [51], sold under the brand name Voltaren® among others. It is reported that the contents of diclofenac reached 0.99 μg/L in WWTP effluents, and a detectable level has been identified in drinking, surface, and groundwaters in the range of ng/L to μg/L in Sweden, Spain, Switzerland, and the Baltic region. Thus, DCF is considered an emerging pollutant as a consequence of its occurrence in surface waters and its potential toxicity towards aquatic organisms [52]. Taking the above-mentioned aspects into account, the quantification of DCF in environmental waters was of great importance, not only to improve the current state of knowledge regarding its pathways, fate, and effects in the environment but also to determine the efficiency of wastewater treatment plants. For this purpose, Costa-Rama et al. developed a paper-based device for the determination of DCF in spiked tap water samples [53]. The platform consisted of a paper-based carbon-ink working electrode, which did not need any stencil or screen for fabrication, combined with counter and pseudo-reference electrodes based on metallic wires. As shown in Fig. 4C, to define the area of the working electrode, first a circular pattern with a diameter of 4 mm was wax printed on Whatman chromatographic paper grade 1 and then it was cured at 100 °C for 1 min. The reference and counter electrodes were gold-plated pins of a commercial connector (previously cut to take a three-pin element) that crossed a black plastic piece (Fig. 4C, i). The pins were employed as electrodes and on the other hand as an easy connection that fitted the commercial interface that was connected to the potentiostat. The reference and counter electrodes were shaped to form a clip, in which the paper-based working electrode could be easily inserted without the need for adhesives, cables, or similar elements. Thus, the authors proposed an easy-to-use paper-based electroanalytical platform in which the porous paper matrix was exploited not only to detect DCF but also to preconcentrate the samples, an important step since emerging contaminants are often present at trace levels in environmental waters. For the preconcentrating step, a drop of DCF solution (in water) was deposited onto the working electrode surface (on the opposite side of the ink) and left to dry at room temperature or in an oven. The importance of this step was demonstrated by cyclic voltametric measurements which registered a 3-fold signal increase after preconcentration, due to the solvent evaporation, which approached DCF molecules to the electrode surface, making the mass transport more efficient. Overall, the paper played multiple roles, being the platform for fabricating the carbon ink-based working electrode (by deposition of the ink), the substrate for sample preconcentration (by solvent evaporation), and the container to pre-load the supporting electrolyte. Moreover, the spatial separation of complete electrochemical cells in the same platform allowed for performing simultaneous measurements when a multi-potentiostat is employed. Indeed, here the versatility of the design was demonstrated by constructing an 8 electrochemical-cell platform for multiplexed measurements (Fig. 4C, iv). The analysis time did not increase because the wax printing and diffusion, employed for delimiting working areas, as well as the deposition and curing of the ink, were carried out simultaneously for all the cells. Due to the predominance of adsorption at the lowest concentrations, the proposed lab-on-paper device was characterized by two linear concentration ranges: one comprised between 0.10 and 5.0 μM (with a slope of 0.85 mA/μM) and the other between 5.0 and 100 μM (with a slope of 0.48 mA/μM). A limit of detection of 70 nM was achieved with this simple device that provided accurate results with an RSD of ca. 5%.

3.3. Other organic pollutants

Among the aforementioned endocrine-disrupting compounds, such as pharmaceuticals and pesticides, listed as emerging pollutants because able to affect human hormonal activities [54], Bisphenol A (BPA) has attracted relevant attention by the scientific community for its toxicity and presence in the environment, being used in the industrial production of epoxy resins, polycarbonate plastics, and lacquer coating [55]. Moreover, several studies demonstrated its estrogenic-like properties correlating the BPA exposure with diabetes, heart diseases, obesity, breast, and prostate cancer, lowered sperm quality, neurotoxicity problem, and polycystic ovarian syndrome [56]. Furthermore, BPA is released in the fresh water when plastic-based bottles are incorrectly transported under sunlight, delivering contaminated water. The detection of BPA by using rapid and sensitive sensors is thus a key issue in analytical chemistry for easy assessment of safety of water and food samples, as well as the pollution of surface waters. Currently, electrochemical sensors are excellent candidates having intrinsic advantages favorable portability, short analysis time, and inexpensive equipment. In literature, two different paper-based electrochemical sensors are reported for the detection of PBA in environmental samples.

The first one is the paper-based potentiometric sensor developed by Kamel et al. [57] characterized by uniform-sized molecularly imprinted nanobeads as recognition receptors to provide a higher affinity for BPA since they have the larger binding capability and can be well dispersed in the polymeric membrane potentiometric sensor. Compared to natural receptors such as antibodies and aptamers, MIPs are cheaper, more robust, and easier to produce. Commercially available chromatography paper (Whatman #1) was used as the electrode substrate to build up the sensor. For the construction of the paper-based device, the printed Whatman chromatography paper was firstly kept in an oven at 150 °C for 2 min till the wax was penetrated completely into the paper to form hydrophobic wax barriers and then cooled to room temperature. On a 6.5 cm × 5.5 cm piece of paper, the carbon ink was then coated using a conventional paintbrush. The paper was then dried at room temperature for 5 min and then in an oven at 70 °C for 10 min. The conductive paper was cut into small strips (0.5 cm × 2 cm) and coated with an adhesive plastic film mask as a further insulating layer. Then, a 3 mm-circular orifice that can limit the sensing area of the membrane was punched from the plastic mask. A volume of 20 μL of the corresponding membrane cocktail was drop-casted onto this orifice to obtain the desired ion-selective membrane. These procedures were found to give optimum results (mechanical stability, reproducibility, and good calibration parameters, etc.). Other uncovered parts on the other side of the conductive paper were used to connect with the potentiometer. The proposed paper-based exhibits similar analytical performance with the classical glassy carbon-based sensor and showed a linear response over the concentration range from 0.5 to 13 μM with a detection limit of 0.15 μM and exhibits good selectivity over other phenols. To evaluate the feasibility of the sensor in real sample analysis, its performances were investigated against BPA released from commercial polycarbonate baby bottles collected from the local market. The samples were washed with deionized water and cut into small pieces and then mixed with 50 mL deionized water. The samples were heated in the oven at 90 °C for 6 h and left overnight in contact with its container to cool. After cooling, the samples were analyzed by the proposed sensors and HPLC method as a reference method. The recoveries results varied from 93% to 102.5%, and the data obtained by the polymeric membrane sensor agree well with those obtained by the HPLC method.

The second electrochemical paper-based sensor for bisphenol A reported in the literature was developed by our group. The proposed sensor was composed of a three electrode-based cell screen-printed on a paper substrate with the working and counter electrodes in graphite and reference electrode in silver/silver chloride. The paper-based device allowed detection of BPA directly on river water samples without any extra-task of end-users, exploiting the porosity of filter paper to load the reagents as well as to treat the sample, thanks to the presence of pores with dimension comprised between 1 and 3 μm which are able to block the particulate [58]. To achieve this task, the sample was loaded on the backside of the printed sensor to exploit the filtration property of the paper (Fig. 5A). Previously, some μL of buffer were loaded on paper waiting for solvent evaporation. In this way, the buffer salts remained in the cellulose network and the added sample allowed their dissolution, delivering a working solution at the pH selected for sensitive and reproducible BPA detection. Square wave voltammetry was used as electrochemical technique to detect BPA. Previously the sensor was tested in standard solutions at concentrations starting from 0.1 μM until to 50 μM, observing two linear ranges in the range comprised between 0.1 and 0.9 μM and in the range comprised between 1 and 50 μM, with $R^2 = 0.961$. The detection limit obtained was 0.03 μM (calculated as 3 times the standard deviation of the blank solution divided by the slope of the calibration curve). The high sensitivity was achieved by exploiting the low-cost carbon black as nanomaterial for direct BPA detection. For real samples measurements, before water analysis, 10 μL of phosphate buffer 0.1 M + KCl 0.1 M at pH 8.0 were loaded onto the paper-based sensor, waiting until it got dry, to deliver a reagent-free sensor. Then, the real water sample was loaded without any pre-treatment, on the backside of the printed sensor, in order to filter the sample. The device gave satisfactory recovery values demonstrating the effectiveness of this cost-effective and easy-to-use paper-based sensor to detect BPA in water samples.

The properties of paper as substrate for the development of electrochemical devices were also exploited to detect biological molecules such as bacteria, which can be a threat to the aquatic and the terrestrial ecosystem. In this context, our group developed a paper-based screen-printed electrochemical device for one-site detection of botulinum neurotoxins A and C. Botulinum neurotoxins (BoNTs) are metalloendoproteases and encompass seven toxin types or serotypes (A to G) and several toxin subtypes and are produced by soil neurotoxigenic anaerobic bacterium Clostridium botulinum. They are responsible for the foodborne intoxication named botulism and are listed as biohazard agents and potential biological weapons [59].

In this regard, we developed a smartphone-assisted paper-based antibody-free electrochemical device was developed exploiting the cleavage capability of these neurotoxins towards SNAP-25 protein whose inhibition causes muscular paralysis [60]. Indeed, a synthetic short peptide that mimics the protein SNAP-25 was labeled with the electrochemical molecule methylene blue and immobilized on the surface of a gold nanoparticles-modified graphite working electrode for the detection of the neurotoxins. The presence of BoNT/A and BoNT/C was evaluated by monitoring their proteolytic activity against the proteins through the square wave voltametric measurement of the methylene blue electrochemical response. In detail, BoNT/A and BoNT/C were able to cleave the portion of the peptide bound to methylene blue, leading to a decrease of the signal due to the removal of the electrochemical probe from the working electrode surface (Fig. 5B). The efficacy of the paper-based sensor to detect botulinum neurotoxin A in real matrix was evaluated. Real samples were spiked with different BoNT/A concentrations in the range between 0.01 and 10 nM and the calibration curve obtained was characterized by the following equation $y = (33.8 \pm 1.5) + (21.7 \pm 2.8)\,x$, $R2 = 0.860$. The accuracy of the sensor was estimated by recovery studies, spiking orange juice samples with 1 nM and 0.5 nM BoNT/A. Percentage recoveries of 104 ± 6% and 98 ± 9% were obtained, respectively demonstrating the suitability of the paper-based platform to measure botulinum neurotoxin also in real matrix. Moreover, the paper-based device showed high selectivity since was not able

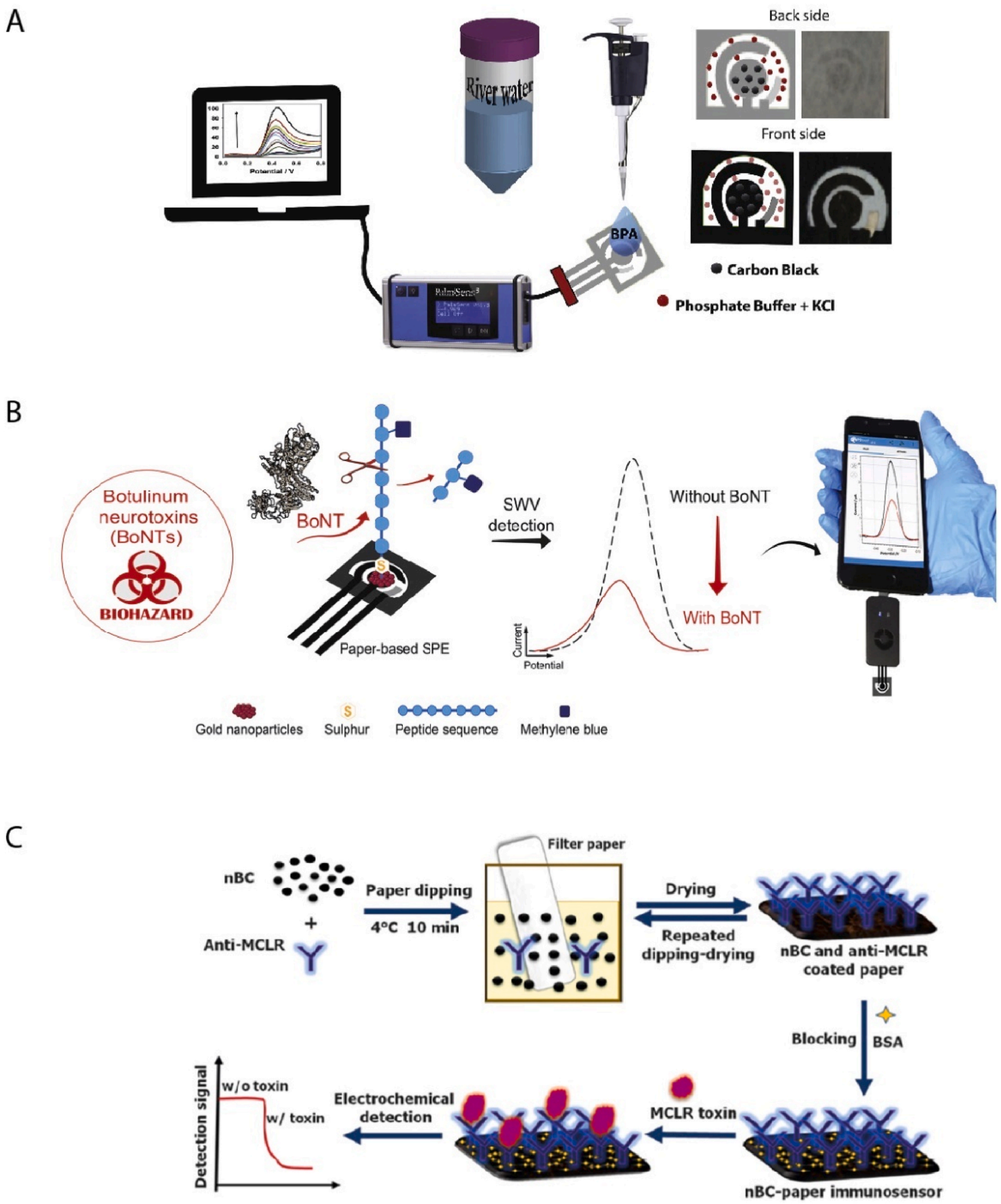

Fig. 5. **A)** Highly sensitive paper-based electrochemical sensor for reagent free detection of bisphenol A. Reprinted with permission from Ref. [58], 2020 Elsevier. **B)** Paper-based electrochemical peptide sensor for on-site detection of botulinum neurotoxin serotype A and C. Reprinted with permission from Ref. [60], 2021 Elsevier. **C)** Nanobiochar paper based electrochemical immunosensor for fast and ultrasensitive detection of microcystin-LR. Reprinted with permission from Ref. [61], 2020 Elsevier.

to detect BoNT/E, another neurotoxin subtype that cleaves the SNAP-25 protein in another site does not present in the immobilized synthetic sequence.

Yao et al. [61] developed a portable, cheap, and sensitive paper type electrochemical immunosensor for the algal toxin microcystin-LR in water which is recognized as the most common and tremendous threat to aquatic ecosystems and human health because of its high toxicity and it can seriously damage the lungs, kidneys, and brain of mammals [62]. Regarding the potential health risk of these toxins, a simple, rapid, specific, and cost-effective analytical method was developed for detecting the microcystin-LR in environmental water at trace level. The paper immunosensor was constructed by coating highly conductive and dispersible nanobiochar particles (nBC) and a monoclonal antibody on the filter paper via the dipping-drying method. The nBC-paper immunosensor was fabricated by the following steps. As shown Fig. 5C, firstly, a solution containing nBC (10 mg/mL) and anti-toxin monoclonal antibody (1 μg/mL) was prepared. Then, the filter paper was dipped in the solution containing the nBC and the MCLR antibody for 10 min and followed by drying at 4 °C. The dipping-drying processes were repeated for several cycles until a rational electrochemical property of the nBC paper was achieved. Finally, the obtained nBC-paper was blocked by using 20% of BSA solution for 30 min, and thoroughly washed with 0.1 M PBS (pH = 7.4). Then the prepared nBC-paper immunosensor was stored at 4 °C for further use. The presence of MCLR could be specifically amperometrically quantified by the nBC-paper immunosensor with a response time of less than 5 min, a very low detection limit of 17 pM (0.017 μg/L), and a wide linear response range of 0.1–100 nM. Moreover, the proposed immunosensor exhibited high selectivity, reproducibility, and storage stability. The sensor was also used for lake water samples with satisfactory recoveries of 95.00%, 100.80%, and 99.50% with RSDs of 7.8%, 5.1%, and 5.9% (n = 3). The successful fabrication of a low-cost and ubiquitous biochar-based paper-type electrochemical immunosensing system would have significant value for the development of a highly cost-effective electrochemical device.

4. Conclusions

In this review, we have critically discussed a variety of paper-based analytical devices reported in the literature from 2010 to 2021 for environmental applications. The roles of paper have been highlighted throughout these examples, and several unique properties have emerged from our analysis. The use of paper has disclosed novel sensing strategies and integrated sample treatment into simpler and faster detection approaches, going beyond the analytical features of the classical electrochemical sensors. The benefits offered by paper and the most relevant considerations about its features can be summarized as follows and are reported in detail in Table S2 (Supplementary Material).

- Many kinds of paper have been explored as the substrate on which realizing an electrochemical cell, such as Whatman paper, filter paper, chromatography paper, and photo paper, including functionalized or modified papers. This demonstrated the high versatility of paper and allowed for a functional choice of the most suitable type of paper to suites with the detection requirements and create novel sensing platforms.
- Multiple configurations have been conceived taking advantage of the high adaptability of the paper. Particularly, the paper enables the designing of vertical flow or lateral flow configurations, based on the diffusion of the liquid solutions through the cellulose matrix on a single layer or multiple paper layers, respectively. This is possible thanks to the porosity and capillarity of paper, which allows the solutions to flow through different layers after their overlapping or after the paper folding.
- Strategies for overcoming the fragility of paper, which can be ripped or degraded when wet, have been reported. In several examples, the paper-based device was combined with 3D printed structures, used as rigid holders to improve the robustness of the device. Alternatively, the lamination process has been proposed, resulting in paper-based devices with improved mechanical resistance and facilitated handling.
- Microfluidic channels can be obtained on the paper thanks to hydrophobic patterns, which can be realized by several techniques, mostly by wax printing. This serves also to define the hydrophilic areas where the chemical and electrochemical reactions take place, enabling analyte detection.
- Paper has shown to be capable of combining the use of different nanomaterials as well as electrode production. For instance, a variety of electrodes can be obtained by printing techniques or sputtering. Moreover, a wide range of nanomaterials and electrode modifiers are compatible with the paper, also allowing for easy modification procedures. Indeed, the porosity of the paper allows for easy modification of the cellulose matrix, realizing conductive paper devices.
- The adsorbent property of paper can be exploited to pre-load the reagents needed for the analyte detection into the cellulose matrix, where they are retained after solvent evaporation until the application. Such kinds of devices are “reagentless”, namely do not need the addition of reagents when the operator is carrying out the measurement, thus representing user-friendly analytical tools. In addition, the need for reduced volumes of reagents and samples, usually lower than 100 μL, makes the paper-based devices environmental-friendly by decreasing the use of reagents and cutting down the production of waste.
- The paper capability of promoting the analysis of complex real aqueous matrices has been reported as a promising feature for paper-based sensors. Indeed, the filtering properties can be used to prevent interfering substances to reach the detection area, reducing the negative effects from complex matrices and thus the steps for the analytical sample treatment.

The aspects above-described, together with its eco-sustainability and inexpensiveness, have turned to be successful features for the application of paper-based devices to the detection of a variety of substances of environmental interest, ranging from inorganic pollutants, such as heavy metals and other inorganic compounds, to organic species, including pharmaceuticals and pesticides. In detail, the properties of paper suit finely with the requirements for the analysis of several environmental matrices, particularly enabling detection in the aqueous phase, in the case of contaminants present in natural waters, as well as in the vapor phase, in the case of airborne pollutants. In this regard, paper-based sensors have been developed by our group for the detection of chemical warfare agents in the aerosol phase, exploiting the adsorbent properties of filter paper to capture these chemicals in the vapor phase [63]. Recently, the versatile applicability of paper has been proven also in the case of solid surfaces by our group [10]. Indeed, we have developed paper-assisted potentiometric sensors capable of monitoring the degradation of reinforced concrete buildings by exploiting the paper properties to interface the sensor with the solid surface of the concrete. Importantly, the versatility of paper allowed also in this case to measure several key parameters (e.g., pH, corrosion), again demonstrating the wide range of applications offered by this material by a proper design of the sensing principle. As a last but significant consideration, the adaptability and wide compatibility of the paper open the way for its integration with other platforms and electronics, with the perspective to develop wearable sensors as well as embedded sensors for the monitoring of multifarious analytes in all the natural matrices.

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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cscee.2021.100167.

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