



Porous microneedle-based electrochemical sensor for the *in vivo* detection of metals in *Alliaria petiolata* at an urban industrial wasteland

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ABSTRACT

The site under study, located in Vieux-Charmont, France, is a former industrial and household waste landfill contaminated with several toxic elements, notably zinc (Zn), lead (Pb), cadmium (Cd), arsenic (As), copper (Cu), nickel (Ni), chromium (Cr) and mercury (Hg). These metals are non-biodegradable and can pose a risk to the environment and human health. The plant *Alliaria petiolata* grows readily in contaminated areas of this site and is capable of accumulating certain heavy metals. It can therefore serve as a pollution bioindicator and potentially be used for phytoremediation.

To measure the metals present in *Alliaria petiolata* leaves, a low-cost system consisting of a porous polymeric microneedle array — fabricated *via* molding — attached to a commercial screen-printed carbon electrode was used. The microneedles penetrate the leaf slightly, allowing for the extraction of the apoplast-enriched leaf fluid, where metal ions can be detected.

In *Alliaria petiolata* leaves from contaminated areas, the differential pulse anodic stripping voltammetry (DPASV) electrochemical method enabled the detection and quantification of three metals: cadmium, lead, and copper. The concentrations measured in the apoplast-enriched leaf fluid were approximately: Cd: 0.60 mg/L; Pb: 2.54 mg/L; Cu: 1.71 mg/L. The same order of concentrations (lead > copper > cadmium) was obtained by analyzing dried plant material using ICP-AES.

1. Introduction

Marginal sites containing various organic and inorganic compounds in soil were formed due to human activities that released large quantities of potentially toxic elements into the environment. The cost of contamination management led to a lack of interest in these sites, rather than rehabilitation. In the European Union, in 2018, approximately 690,000 industrial sites were identified [1]. The most common contaminants in these sites are heavy metals; being non-biodegradable, they can impact the environment and human health, even at very low concentrations [2,3]. Zn, Pb, Cd, Cu, and Ni are considered to have the greatest impact on organisms; they cause oxidative stress, generate neurotoxic effects, and increase the risk of cancer [2]. Some plants can be used as bioindicators of the degree of contamination if they can accumulate contaminants as a function of soil content. An industrial wasteland located in Vieux-Charmont, Bourgogne-Franche-Comte

region, France, is contaminated with several heavy metals (Zn, Pb, Cd, As, Cu, Ni, Cr, Hg), due to its use as an uncontrolled landfill for industrial and household wastes. The total element content of the soil samples was measured on dry soil samples using inductively coupled plasma atomic emission spectrometry (ICP-AES) [4]; these elements are heterogeneously distributed throughout the investigated zone. The accumulation of these heavy metals by the vegetation in the wasteland was studied. The study demonstrated differences in the accumulation of heavy metals, depending on the plant genus. *Alliaria petiolata* grows easily in contaminated environments and was identified as a bioindicator of Zn. Moreover, the accumulation capacity of this species allow to consider the possibility of using it for managing the site by phytoremediation [5].

Microneedle-based sensors for plant characterization constitute a new direction of research in the field of sustainable agriculture. They allow the monitoring of plant growth status [6]. The detection technique is electrochemistry, with the microneedle array being metallized and

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modified if necessary. In this way, species of interest for plant health were electrochemically detected in the apoplast-enriched leaf fluid, a crucial component in plants. Salicylic acid [7] was detected by MIP-modified metallized microneedles, pH [8] was monitored under different conditions by a metallized microneedle array modified with polyaniline as a pH-responsive layer, and L-glutamate was detected by a metallized microneedle functionalized with glutamate oxidase [9]. The association of a microneedle array for sample extraction with commercialized screen-printed electrodes is a rising field [10,11], leading to low-cost devices. It can greatly simplify the fabrication technology. Hollow microneedle arrays were 3D-printed and associated with modified graphite screen-printed electrodes for the detection of H_2O_2 , glucose, and pH in the apoplast-enriched leaf fluid of different plants [11].

In this work, we consider, for the first time, the *in vivo* detection of heavy metals in the apoplast-enriched leaf fluid of *Alliaria petiolata* leaves, using porous polymethacrylate microneedle-based electrochemical sensors. Porous microneedles were obtained by molding through a two-step process, and then stuck onto commercial carbon screen-printed electrodes. The system was clamped on the leaf surface of *Alliaria petiolata* for ten minutes before the electrochemical detection of heavy metals using differential pulse anodic stripping voltammetry. The simultaneous detection of Cd^{2+} , Pb^{2+} , and Cu^{2+} was obtained. The detected concentrations were compared to those obtained through the analysis of the dry plant.

2. Materials and methods

2.1. Study site

The study site is a 2 ha industrial wasteland located in the city of Vieux-Charmont (47°31'15.8"N, 6°50'23.8"E) in eastern France. The site was used as an uncontrolled landfill for industrial and household wastes, found buried and on the surface. The wasteland was closed to the public in 2007 after soil analysis revealed contamination by organic (PAH, PCB) and inorganic (Zn, Pb, Cd, As, Cu, Ni, Cr, Hg) compounds [12].

2.2. Fabrication of the 3D-printed hollow microneedles

2.2.1. Fabrication of the mold of a microneedle array

The mold was designed using SOLIDWORKS software. The stainless steel (X153CrMoV12) master with 54 holes (diameter 300 μm) was machined in the KERN machine center using a CHARMILLES electrical discharge (EDM) machine (CNC Machines, Winter Springs Florida, USA). The stainless steel (X35 ST+) tips were machined using a STAR automatic lathe (Star, Shizuoka, Japan). A stainless steel master with 54 holes was first created (Fig. 1A), and 54 tips (Fig. 1B) were machined and inserted into the created holes (Fig. 1C). The dimensions of the tips were: length 700 μm , larger diameter 300 μm , smaller diameter 30 μm .

2.2.2. Fabrication of the hollow microneedle array

The fabrication procedure took place in two stages, following previous work [13]:

1. The fabrication of a female polydimethylsiloxane (PDMS) mold was obtained by pouring the 1/1 basis-catalyzer mixture (Silicone poly-addition FA5408, Prevent Silicones, Chateaufort-sur-Isere, France) into the stainless steel mold, vacuum degassing for 1 h at room temperature, and curing it at 70 °C for 20 mins. After unmolding, the female PDMS mold could be used for the successive preparations of hollow needle arrays.
2. Trimethylolpropane trimethacrylate (TRIM) and triethylene glycol dimethacrylate (TEGDMA) were used as cross-linkers for PGMA (monomer glycidyl methacrylate). Polyethylene glycol (PEG) solution in 2-methoxyethanol was used as a porogen. A porogen stock solution was prepared by dissolving PEG (2.0 mmol) in 2-

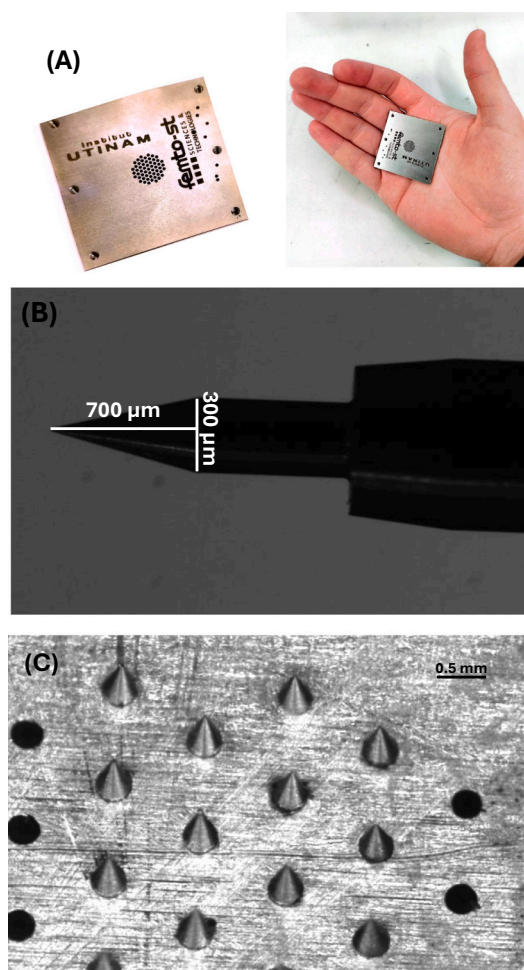


Fig. 1. (A) The stainless steel master with 54 holes (diameter 300 μm). Total diameter: 8 mm. (B) A stainless steel tip (length 700 μm , larger diameter 300 μm , smaller diameter 30 μm). (C) Fabrication of the master: Insertion of the tips into the holes of the master.

methoxyethanol (100 g) at 50 °C. A monomer stock solution was prepared by mixing the monomer glycidyl methacrylate (1 equiv.), cross-linker TRIM (0.26 equiv.), and cross-linker TEGDMA (0.79 equiv.), and this monomer solution was stored at -15 °C. Before use, this solution should be heated to room temperature. A concentration of porogen of 49% allowed a satisfactory penetration of water in the microneedle and a satisfactory penetration efficiency in pig skin [13]. Next, a photoinitiator Irgacure 184 (0.10 g, 1 wt% to the monomer) was put into the mixture of the monomer and porogen stock solution: PEG (6.0 g) and 2-methoxyethanol (30 g). After putting the mixture into the female PDMS mold, the mold with the mixture was put under vacuum for 1 h to make sure that bubbles were removed. The mold was put under 365 nm UV light for 1 h to polymerize the solution to make a microneedle array. After dissolving the porogen PEG in the microneedles with methanol/water (1,1 volume), microneedles with a porous structure were obtained (Fig. 2).

SEM images of the porous microneedle array were obtained using a high-resolution scanning electron microscope MIRAN3 TESCAN with an electron beam energy of 7 keV.

2.2.3. Electrochemical detection of heavy metal ions in birch sap and in the leaves of *Alliaria petiolata*

Differential pulse anodic stripping voltammetry (DPASV) using

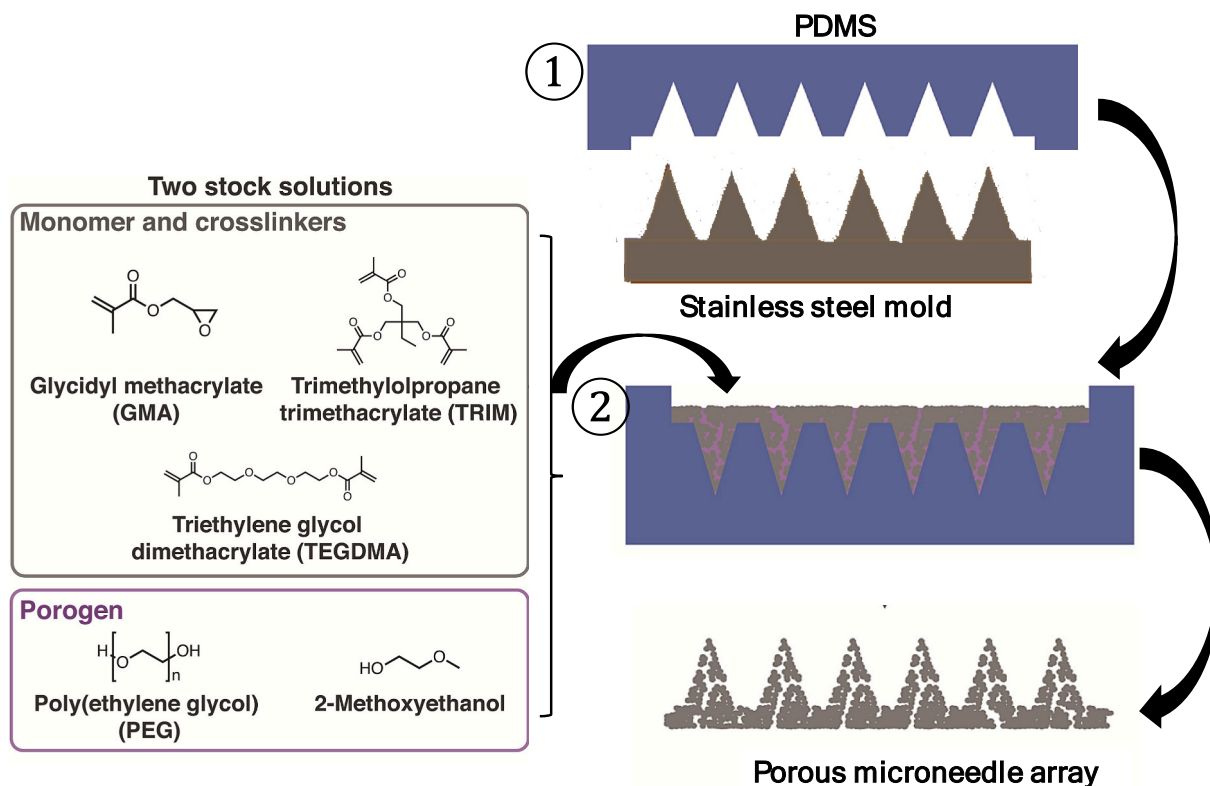


Fig. 2. A female polydimethylsiloxane (PDMS) mold was obtained by pouring the mixture into the stainless steel mold. A GMA, TRIM, TEGDMA mixture, mixed with a porogen mixture, was poured into the female PDMS mold and then exposed to 365 nm UV light for 1 h. After dissolving the porogen in the microneedles with 1/1 v/v methanol/water mixture, the hollow microneedles were obtained.

Palmsens 4 potentiostat (Palmsens, Houten, Netherlands) was used. A potential of -1.3 V was held for 10 min for ion reduction, and then differential pulse voltammetry was conducted at 50 mV/s in a large potential range from -1.8 V to -0.6 V using the following parameters: pulse potential 50 mV, pulse time 10 ms, equilibrium time 0 s, step potential 10 mV, and scan rate 50 mV.s $^{-1}$.

3. Results and discussion

3.1. Porous microneedle optimization

The obtained porous microneedle array was observed using a scanning electron microscope (Fig. 3), showing that all the microneedles are well molded. The MPA was then stuck onto a carbon screen-printed

electrode (Fig. 4).

3.2. Detection of heavy metals in the leaves of *Alliaria petiolata*

Alliaria petiolata plants (Fig. 5A), situated in zones 1 and 5 of the study site [4], present the highest Pb and Cd content. Electrochemical measurements were performed on their leaves through an active fluid extraction by keeping the thumb pressed on the SPE/PMA system on the leaf (Fig. 5B). In this way, a larger volume of apoplast-enriched leaf fluid is extracted [11]. After ten minutes, the process of DPASV begins. The DPASV signal registered is presented in Fig. 7, the black curve is obtained from plants grown in the contaminated zones. Five different plants were measured. The error on the intensity of the peak maximum for each heavy metal is 5%.

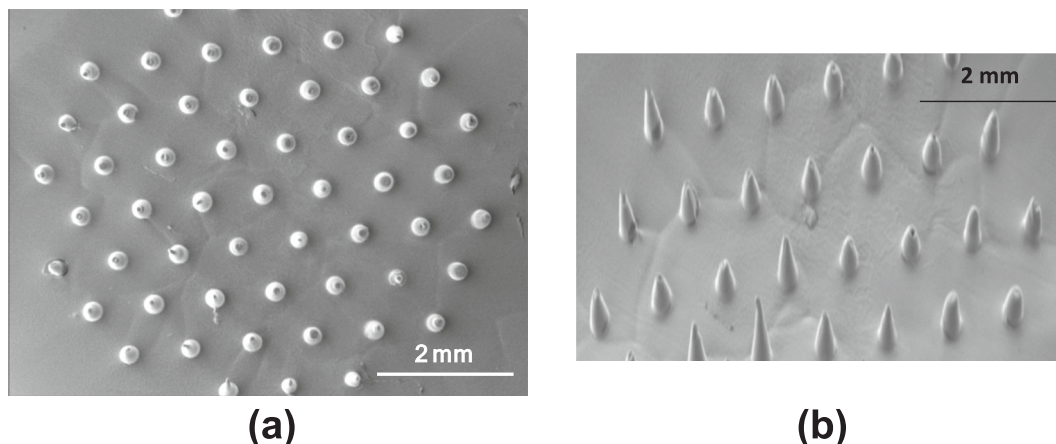


Fig. 3. SEM images of the porous microneedle array (a) top view (b) profile view.

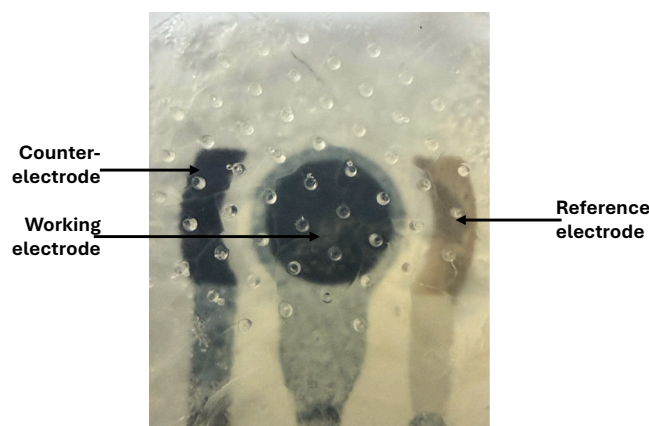


Fig. 4. The porous microneedle array stuck onto the carbon screen-printed electrode.

To identify the different peaks in the DPASV signal and estimate the concentration of heavy metal ions in the apoplast-enriched leaf fluid, the DPASV signal of the heavy metals was registered in birch sap (Carrefour, Ecully, France). From the potentials of the different peaks in Fig. 6, the identified heavy metal ions are: cadmium (-0.8 V), lead (-0.46 V), and copper (-0.16 V).

When *Alliaria petiolata* plants are grown in non-contaminated zones (Fig. 6, red curve), no DPASV signal is detected, showing that the metal concentration was not detectable under the applied conditions.

The DPASV signals of heavy metals in birch sap are presented in Fig. 7. The positions of the different oxidation peaks are: $\text{Cd}^{2+} - 0.88$ V; $\text{Pb}^{2+} - 0.7$ V; $\text{Cu}^{2+} - 0.25$ V. When comparing these potential values to those of the heavy metal oxidation peaks in *Alliaria petiolata* plant, we can observe that they are systematically cathodically shifted by -0.08 V for Cd^{2+} , -0.24 V for Pb^{2+} , and -0.09 V for Cu^{2+} . This point shows that in the plant, the heavy metal ions are oxidized more easily than in the birch sap due to different metal speciation (different pH values, different anionic concentrations). The influence of K^+ , Ca^{2+} , Mg^{2+} , organic acids in the apoplast are not directly detected using DPASV. Nevertheless they can influence the DPASV signal of heavy metals by modifying the ionic strength or the metal speciation, then influencing the stripping potential and/or the peak intensity.

The equations of the calibration curves of the three heavy metal ions in birch sap, in the 0 to 100 μM range, determined from three series of measurements, are the following:

$$\text{Cd}^{2+} : I (\mu\text{A}) = 49 \log_{10} C (\mu\text{M}) + 261 \quad (R^2 = 0.95) \quad (1)$$

$$\text{Pb}^{2+} : I (\mu\text{A}) = 16.5 \log_{10} C (\mu\text{M}) + 91 \quad (R^2 = 0.94) \quad (2)$$

$$\text{Cu}^{2+} : I (\mu\text{A}) = 18.6 \log_{10} C (\mu\text{M}) + 98 \quad (R^2 = 0.98) \quad (3)$$

I is the intensity of the peak maximum in μA , and C is the concentration of the metal in μM .

The detection limits, determined through the equation $3\sigma/S$ (σ being the error on the background, S being the slope of the calibration curve (sensitivity)) are 0.1 μM for Cd^{2+} , 0.4 μM for Pb^{2+} and 0.7 μM for Cu^{2+} , the quantification limit being respectively 0.3 μM , 1.2 μM , and 2.1 μM . The precision is 5% for each metal.

The concentrations of the different heavy metal ions in the apoplast-enriched leaf fluid of *Alliaria petiolata* second-year plants are calculated by reporting the intensities of the peak maximum in the DPASV signal (Fig. 6) in Eqs. (1)–(3) corresponding to the different metals. The results are:

Cadmium: $5.4 \pm 0.3 \mu\text{M}$ ($0.60 \pm 0.03 \text{ mg/L}$).

Lead: $12.3 \pm 0.6 \mu\text{M}$ ($2.54 \pm 0.13 \text{ mg/L}$).

Copper: $26.9 \pm 1.3 \mu\text{M}$ ($1.71 \pm 0.08 \text{ mg/L}$).

In the dry *Alliaria petiolata* second-year plants, the concentration of cadmium was found to be 1 mg/kg, that of copper 7 mg/kg, and that of lead 10.7 mg/kg [4]. The same order of concentrations is found in the

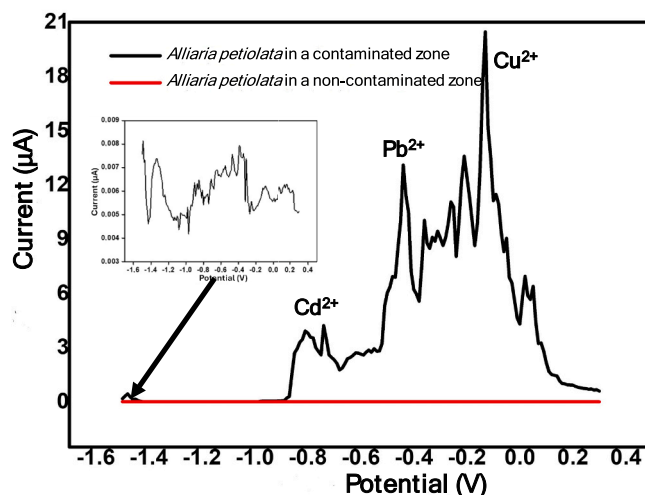


Fig. 6. DPASV signal registered in *Alliaria petiolata* in a contaminated zone (black curve) and in a non-contaminated zone (red curve). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)



(a)



(b)

Fig. 5. (a) *Alliaria petiolata* second-year plant. (b) Electrochemical measurements in a leaf of *Alliaria petiolata* through an active fluid extraction by keeping the thumb pressed on the leaf.

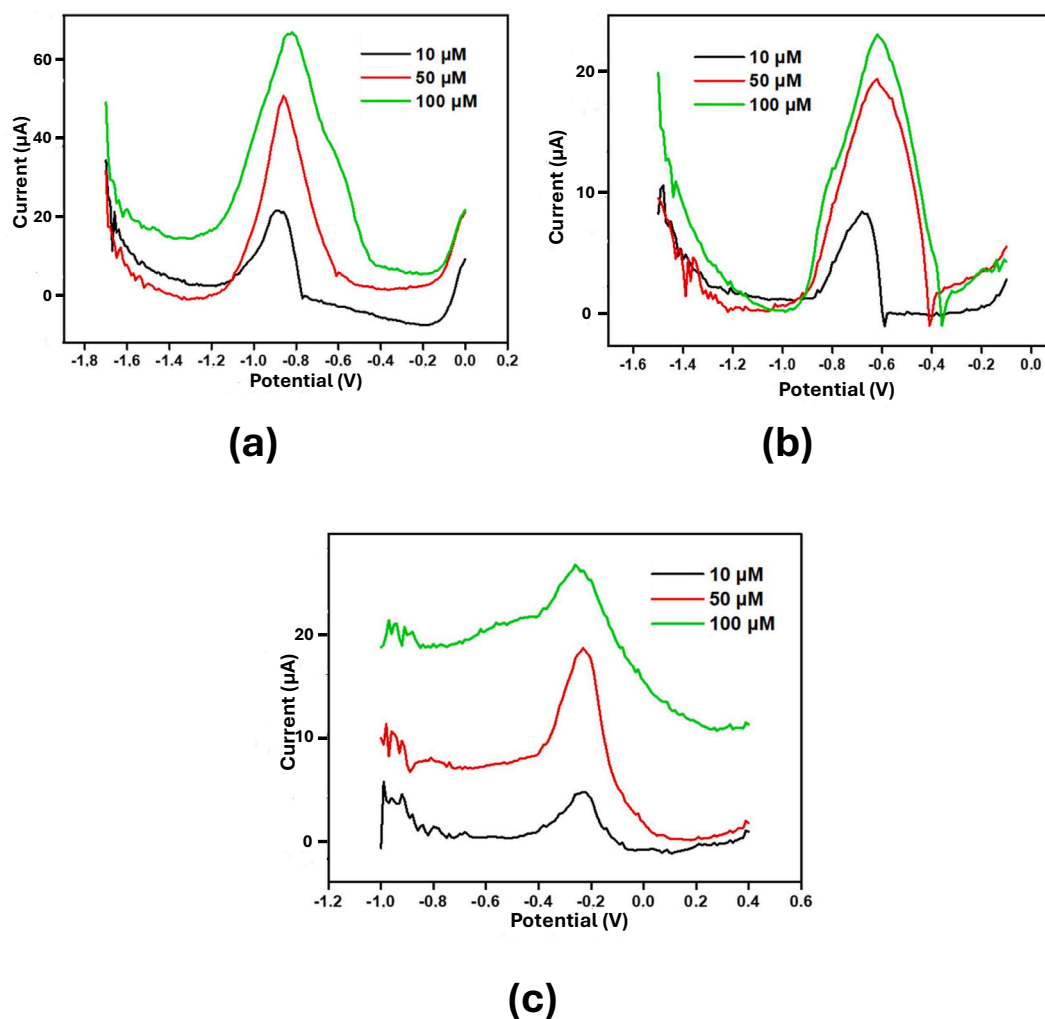


Fig. 7. DPASV signal of heavy metal ions in birch sap. (a) Cd²⁺, (b) Pb²⁺, (c) Cu²⁺.

apoplast-enriched leaf fluid of *Alliaria petiolata* second-year plants, using PMA/carbon SPE system. The two values refer to different matrices and cannot be directly compared quantitatively. The main point supported by the data is the consistent ranking Pb > Cu > Cd.

Another metal found to be highly concentrated in *Alliaria petiolata* second-year plants is zinc (1250 mg/kg), and nickel was also detected in dry plant (10.7 mg/kg). These metals were not detected using our PMA/carbon SPE system. The modification of the carbon surface of the SPE with a mercury thin film electrode would allow the detection of zinc and nickel [14,15].

The main strategies for soil decontamination are phytoremediation, the use of herbaceous and woody plants as well as associated microorganisms to extract HM from soil, which, however, face limitations to become successful at commercial scale and, then, often generate scepticism regarding their usefulness. Phytomanagement strategies often rely on the use of metal-accumulating plants and bioaugmented microbial consortia, which may improve plant uptake of the most abundant heavy metals, Ni, Cu, Zn and Cd. However, uptake and hence plant metal accumulation may vary according to seasons, soil metal availability, environmental conditions, plant density and developmental stages. Implementing technologies such as microneedle-based sensors to evaluate the plant potential for phytoremediation is therefore crucial. The use of a rapid and low-cost system to screen toxic elements in plant tissues represents a real advance over traditional approaches, which are time- and labour-consuming. Additionally, this approach may allow us to quickly assess the quality of the biomass produced on contaminated

soils, providing valuable sources of renewable biomass for the bio-based economy, e.g., bioenergy, biocatalysis and platform molecules, and eco-materials.

The present study thus addresses many of the most pressing issues of our time, including food security, biodiversity loss, and climate change. By proposing innovative and optimised solutions (phytomanagement) for the restoration of contaminated lands, it supports several Sustainable Development Goals (SDG), including SDG15 and the Zero pollution strategy; SDG3 contributing to reduce the exposure to pollutants and to contaminated groundwater and soil, bringing benefits in terms of human health and the sustainable development of regional water resources [16]; SDG13 and the New EU forest Strategy promoting the cultivation of trees in contaminated areas, facilitating the long-term locking-up of carbon; finally v) the Biodiversity Strategy by setting up vegetation covers on contaminated and often abandoned lands that will boost e.g. microbial, entomological and plant biodiversity.

4. Conclusion

In the industrial wasteland located in Vieux-Charmont, *Alliaria petiolata* second-year plants were identified to present accumulation capacities of heavy metals. A porous microneedle array, obtained by molding, was stuck on a carbon screen-printed electrode to obtain a low-cost system for the extraction and detection of heavy metals from the apoplast-enriched leaf fluid of these plants. Cadmium, lead, and copper were detected and quantified. The order of concentrations (lead >

copper > cadmium) was also found in the dry plants using ICP-AES. Other metals (zinc and nickel) were detected in the dry plant material but were not detected using the current setting. A modification of the carbon surface of the screen-printed electrode with a mercury film should allow the detection of all the heavy metals. The *in-situ* detection of heavy metals will allow the monitoring of heavy metals throughout the plant life, in different environmental conditions, helping to define the best conditions for managing the site through phytoremediation techniques.

CRedit authorship contribution statement

Hamdi Ben Halima: Writing – original draft, Methodology, Investigation. **Martial Personeni:** Methodology, Investigation. **Sébastien Thibaud:** Methodology, Investigation. **Piel Noble:** Methodology. **Betty Baudinot:** Methodology. **Michel Chalot:** Methodology, Investigation. **Nicole Jaffrezic-Renault:** Writing – review & editing, Conceptualization.

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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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Data availability

Data will be made available on request.

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