



3D-printed extraction chamber and paper-based screen-printed sensors for zinc analysis in soil and Antarctic sediments

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ABSTRACT

Studying chemical substances in Antarctic soils, such as zinc ions, provides crucial insight into ecosystem changes. Conventional analyses typically require laboratory-based instrumentation (e.g., atomic absorption spectroscopy), with high energy consumption and use of high volumes of solvents, making them unsuitable for remote environments. Herein, we developed an analytical approach that combines Zn²⁺ extraction from soil with its analysis utilizing a simplified and green analytical methodology. We customized a 3D-printed chamber to extract water-soluble and exchangeable Zn²⁺ fraction, which is the most relevant for bioavailability in ecosystem exchanges. The analysis of extracted Zn²⁺ is carried out by square wave anodic stripping voltammetry with a miniaturized paper-based screen-printed sensor, based on ex-situ bismuth modification. Our results showed a linear range of 0.05–1.25 ppm, a limit of detection of 0.015 ppm, and good agreement with a reference analytical method. Our approach provides green innovation for a methodology applicable in remote areas, such as Antarctica, as proven by our analysis using RGBfast metrics, which is essential for observing soil ecosystem responses to global warming.

1. Introduction

Over the last 60 years, research on Antarctica has been focused on understanding the environmental transformations occurring due to climate change [1]. Insights into ecosystem changes in Antarctic geochemistry and the role of nutrients can reveal long-term implications for global biodiversity and climate resilience [2,3]. For instance, heavy metals, such as zinc, copper, nickel, and iron, are essential for plant and animal metabolism, acting as cofactors for enzymes and participating in redox reactions [4,5]. However, excess levels typically arising from intensive industrial/urban activities can lead to toxicity [4,5]. Overall, studying metal micronutrient dynamics in deglaciated Antarctic environments reveals unique insights into how trace metal geochemistry is influenced by climate-induced transformations [6,7].

Heavy metal content in Antarctica has been growing, ascribable to industrial emissions of heavy metals and consequent long-range atmospheric transport [8–10]. For instance, the total zinc increased from about 100 µg/g to almost 400 µg/g in Nelson Island (West Antarctica) during the 20th Century [1]; zinc accumulation in soils was observed particularly in nearby research stations with intensive fuel contamination and waste disposal, with value comprised between 5.2 and 38 mg/kg in Larsemann Hills and 22–256 mg/kg in Mirny station and Fulmar Island [9]. The accumulation of metals in Antarctic snow and ice represents a threat to Antarctic ecosystems, especially where ice melting can release large amounts of these substances in the newly uncovered soils [11,12].

However, monitoring zinc levels in remote areas such as Antarctica is a significant challenge. Importantly, the total amount of zinc in the soil

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can be divided into different fractions, distinguishable by their different chemical reactivity [13–15]. The total zinc content in target soils or sediments can be obtained through mineralization of solid samples via acid digestion, which allows for the complete transformation of different chemical forms into Zn^{2+} . Nevertheless, not all zinc fractions are equally significant to the ecosystem [13,15,16], especially in areas such as deglaciated soils in Antarctica, where limited types of vegetation can grow (e.g., mosses, lichens, algae). The aqueous soluble and exchangeable fraction of zinc is the most ecologically relevant and readily available for plants [17], determining zinc supply in the food chain [13, 18].

Here, we address these challenges with a new analytical methodology, combining low-cost, low-energy, and portable devices to improve the sustainability of zinc extraction and analysis from soils and Antarctic sediments (Fig. 1). Specifically, we designed an extraction chamber through 3D stereolithography printing, where the soil sample (e.g., 0.1 g) is placed. Following this, Zn^{2+} containing solution is extracted by flowing an extracting solution (e.g., 4 mL of 0.11 M acetic acid) using a syringe, driven by a peristaltic pump to obtain a semi-automatic system (Fig. S1). For the analysis of the extracted Zn^{2+} , we produced miniaturized paper-based screen-printed sensors as sustainable and low-cost devices. The detection principle relies on the ex-situ electrodeposition of a bismuth film and the detection using square wave anodic stripping voltammetry (SWASV) to achieve high sensitivity, even compared to atomic absorption spectroscopy (AAS), which is used as the reference method for metal analysis. This methodology was first optimized using common soil samples and then tested on Antarctic sediment samples. Thanks to the use of non-toxic reagents and eco-friendly materials (i.e., paper), our approach represents a green analytical method that combines the extraction and the analysis of water-soluble and exchangeable zinc fractions in soils for fast and cost-effective monitoring.

2. Experimental section

2.1. Materials and methods

All the reagents used were of analytical grade, including HNO_3 69 %, Bi^{3+} Standard Solution 1 g/L (in HNO_3), Zn^{2+} Standard Solution 1 g/L (in HNO_3), Acetic Acid 99 %, Sodium Chloride (Sigma Aldrich, USA). All solutions were prepared using double-distilled water using a Milli-Q system (Millipore). Teflon or polyethylene containers (beakers, test tubes, pipettes, round-bottom flasks) were preferred and previously rinsed with 3 % v/v HNO_3 to ensure thorough cleaning from metal

traces.

2.2. Sensors production

Paper-based screen-printed electrodes (SPEs) were home-produced, following a well-established procedure [19]. Firstly, a wax pattern was printed onto common office paper sheets through a ColorQube 8580 Xerox printer and then treated at 100 °C for 2 min to permeate the paper homogeneously. This hydrophobic pattern defines hydrophilic spots on the paper material where the electrochemical cell is printed using a 245 DEK (Weymouth, UK) serigraphic printer. The working and counter-electrodes were printed using graphite-based ink (Electrodag 423 SS) while the pseudo-reference electrode was printed using Ag/AgCl-based ink (Electrodag 6033 SS). Both conductive inks were purchased from Henkel. The resulting working electrode surface area is 12.6 mm². The obtained sensors are based on a green material (i.e., paper) and can be disposed of with low-impact waste (e.g., paper incineration).

2.3. Soil and Antarctic sediment sampling

Soil samples were collected from different areas surrounding Tor Vergata University and sieved with a plastic sieve having pores of 0.5–1 mm in size to obtain fine soil powder. The powder obtained is spread on a sheet of paper and placed to dry in an oven at 100 °C for 6 h. Finally, the dried powder is collected in a polyethylene test tube and used for Zn^{2+} extraction. These soils were used to point out the protocols for zinc extraction and analysis.

Antarctic sediments were sampled from seasonal coastal lakes near Terra Nova Bay, which are frozen in the winter and deglaciated during the austral summer [20]. In these lakes, the water supply comes only from snow and ice melting. The Antarctic sediments analyzed in this study were sampled during Italian Antarctic expeditions from 1990 to 2014 and then stored at the Sample Bank of the University of Genoa at –80 °C [21]. The samples analyzed in this work were provided by the Antarctic Environmental Specimen Bank (BCAA) of the University of Genoa and temporarily stored at –20 °C at Ca' Foscari University of Venice. Then, these sediments were freeze-dried for 3 days, sieved, homogenized using a Retsch RM 200 mortar mill, and finally stored in glass containers previously sterilized. In detail, the sediments here analyzed came from 5 lakes: two lakes at Edmonson Point (14 and 15A), which are close to the active volcano Mount Melbourne; lake Gondwana, which is near the South Korean Jang-Bogo base; lake 10B on Inexpressible Island,

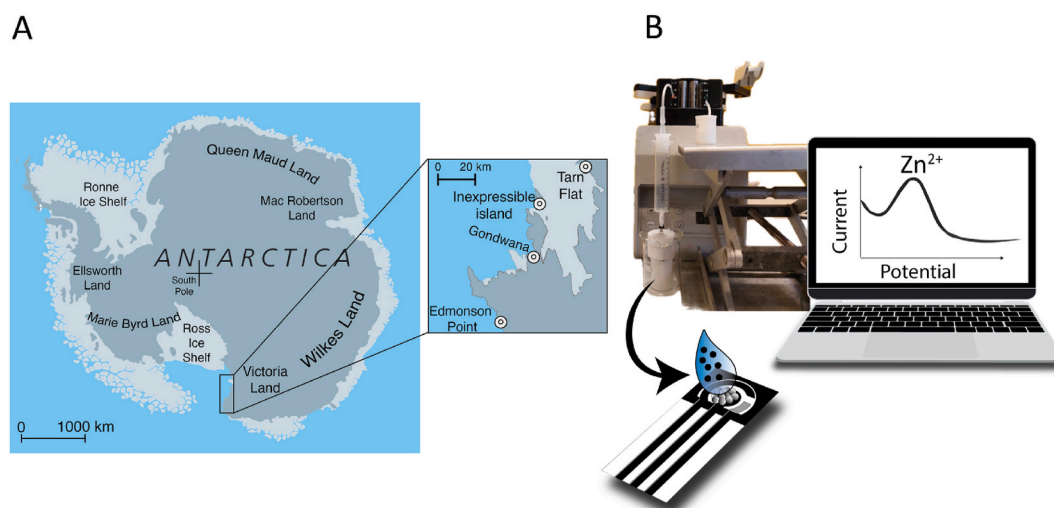


Fig. 1. (A) Sampling sites of the analyzed sediments in Antarctica. (B) The proposed analytical tool is based on the 3D-chamber extraction and office paper-based electrochemical sensor.

which is set on a moraine relief; lake 20 in Tarn Flat, which lies in an erosion depression 70 m below sea level. The samples analyzed are listed in Table 1. The geochemical features of these sediments were characterized in previous works [22]. The protocols for zinc extraction and analysis were applied to the Antarctic sediment samples without any other treatment.

2.4. BCR extraction method

The BCR procedure for sequential extraction of water-soluble and exchangeable zinc fraction was applied according to the literature [23]. In detail, 15 mL of 0.11 M acetic acid is added to 0.37 g of sieved and dried soil (or sediment) in plastic tubes, and the mixtures are kept under constant mixing for 16h in a rotary shaker (Digital tube revolver, Thermo Fisher Scientific); then, the mixtures are centrifuged for 15 min at 4000 rpm at a temperature of 23 °C; the resulting supernatant solution, containing the exchangeable Zn²⁺ fraction, is removed with a syringe and filtered using a polytetrafluoroethylene filter (Diameter 33 mm, pore size 0.22 μM, Cobetter Filtration). The extracted solution was immediately analyzed using AAS (see paragraph “2.6. AAS analysis”).

2.5. 3D-printed chamber extraction method

Our innovative extraction method is based on an ad hoc-designed and printed extraction chamber using a 3D stereolithography printer (FormLabs, Italy). The chamber is composed of two cylindrical and complementary units with a key-lock design, which are held together by six screws (Fig. S1A). The underlying unit has a concavity of 1-cm diameter in which 0.1 g of the soil powder is placed on a polyvinylidene fluoride membrane, to avoid leakage of soil powder. The upper unit is then overlapped to interlock with the underlying unit. The extraction is carried out by injecting 4 mL of 0.11 M acetic acid through the cell with a syringe connected to a peristaltic pump system (Fig. S1B), equipped with polyvinyl chloride tubes and set at optimal speed (see paragraph “3.6. 3D-printed extraction chamber” in the “Results and discussions” section). The extracted solution is then collected in a Teflon

beaker.

2.6. AAS analysis

AAS was used as the reference method for trace metal analysis. A Shimadzu AA6300 equipped with a hollow-cathode lamp and an air/acetylene flame (the gases were set at 4 atm and 1 atm, respectively). Standard parameters were used: Zn adsorption wavelength set at 213 nm, pre-spray time 7 s, integration time 5 s, response time 1 s, atomizer position 7 mm, burner angle 0°.

Before the analysis of extracted sample solutions, a calibration curve is run using standard solutions of Zn²⁺ (0.1 ppm, 0.2 ppm, 0.35 ppm, 0.5 ppm, 1 ppm) in double-distilled water and HNO₃ 1 % v/v. After the analysis of the extracted solutions, standard Zn²⁺ solutions at known concentrations are analyzed to check the stability of the AAS analysis, since the chemical composition of complex samples can affect the efficiency of the flame.

2.7. SWASV analysis

All the electrochemical measurements were carried out using a potentiostat, MultiEmStat4, equipped with 8 channels (PalmSens®, The Netherlands) and connected to a laptop.

The paper sensors are first modified by electrodeposition of a bismuth film. A 70 μL-drop of Bi³⁺ 50 ppm standard solution is placed on the printed electrochemical cell, and the electrodeposition is run by applying two SWASV scans with optimized parameters (Table S1). In detail, the first SWASV scan is run to deposit and strip the bismuth film, obtaining the electrode surface conditioning [24], while the second scan is run to deposit the bismuth film permanently. The so-prepared sensors are ready to analyze Zn²⁺ in extracted solutions.

For the calibration process, Zn²⁺ standard solutions (0.05 ppm, 0.1 ppm, 0.2 ppm, 0.35 ppm, 0.5 ppm, 1 ppm, 1.25 ppm) were prepared in 100 mM acetate buffer (pH 6.5).

For the analysis of Zn²⁺ extracted, known concentrations (i.e., 0.5 ppm and 0.75 ppm) of Zn²⁺ standard solution were added to the

Table 1

Concentrations of Zn²⁺ (ppm, mg/L) measured in Antarctic samples from deglaciated lake sediments, analyzed by spectrophotometric reference technique (AAS) after BCR extraction and our innovative system, based on ex-situ bismuth-modified paper-based sensors and optimized SWASV analysis after extraction with the pump-driven 3D-printed extraction chamber.

Sampling Site	Sediment sample	Date of sampling	Zn ²⁺ (ppm) BCR - AAS	Zn ²⁺ (ppm) Peristaltic pump-3D chamber - SWASV	BIAS %
Edmonson Point Sites 14 and 15A	6S 8B	25/12/1990	0.089 ± 0.001	0.093 ± 0.001	4
	9S 8B1	24/12/1993	0.047 ± 0.001	Not visible	
	11S 8B1	19/01/1996	0.061 ± 0.002	Not visible	
	21S 8B	31/01/2006	0.0702 ± 0.0002	0.0707 ± 0.0004	0.7
	25S 8B	14/12/2009	0.032 ± 0.001	Not visible	
	8b a2a	07/01/2012	0.115 ± 0.001	0.13 ± 0.03	13
	8b a3u1	14/01/2012	0.016 ± 0.001	Not visible	
	8b a3u3	14/01/2012	0.022 ± 0.001	Not visible	
	8c a1d	20/12/2011	0.114 ± 0.002	0.12 ± 0.02	5
	8c a1d	20/12/2011	0.044 ± 0.003	Not visible	
29S 8B	11/01/2014	0.052 ± 0.002	Not visible		
Inexpressible Island Site 10B	6S 18B	30/12/1990	0.063 ± 0.002	Not visible	10
	19S 18D	16/01/2004	0.028 ± 0.004	Not visible	
	21S 18D	22/01/2006	0.299 ± 0.003	0.27 ± 0.03	
	18d a2b2	14/01/2012	0.043 ± 0.003	Not visible	
	29S 18D	05/01/2014	0.016 ± 0.001	Not visible	
Tarn Flat Site 20	6S 12A	23/01/1991	0.009 ± 0.002	Not visible	1
	9S 12A	08/01/1994	0.041 ± 0.003	Not visible	
	19S 12A	24/01/2004	0.027 ± 0.003	Not visible	
	21S 12A	25/01/2006	0.084 ± 0.001	0.083 ± 0.002	
	25S 12A	30/12/2009	0.023 ± 0.004	Not visible	
	12A A1B	22/12/2011	0.016 ± 0.002	Not visible	
	12A B1BE	14/01/2012	0.0021 ± 0.0006	Not visible	
	29S 12A	11/01/2014	0.061 ± 0.001	Not visible	
Gondwana	24 a1d	31/01/2012	0.204 ± 0.001	0.167 ± 0.006	18

extracted samples, and analyzed in SWASV. Small volumes of concentrated buffer were used to reconstitute the 100 mM acetate buffer (pH 6.5) condition while avoiding significant dilution of the samples. The concentrations corresponding to the amounts of Zn^{2+} extracted were obtained by the extrapolation method.

The extracted solutions from target samples were analyzed under optimal conditions (100 mM acetate buffer, pH 6.5) and compared with the reference method (BCR coupled with AAS).

2.8. Electrode characterization

The morphology and chemical composition investigations of the working electrode before and after bismuth electrodeposition were carried out using an ultra-high resolution field emission scanning electron microscope (UHR FEG-SEM) CLARA 2 by Tescan, equipped with an Energy-dispersive X-ray spectroscopy (EDS) SDD detector AZtec Ultim® Max ("Infinity") 170 mm2 by Oxford Instruments.

The electrochemical properties of the Bi-modified paper-based sensor were investigated using Electrochemical Impedance Spectroscopy (EIS), in a solution of 5 mM ferro/ferricyanide (1:1 v/v) + KCl 0.1 M. A frequency range between 1 Hz and 10 KHz was set, with 10 frequencies per decade, at an open circuit potential, with a voltage amplitude of 0.01 V. Data fitting was carried out using Zview software, applying the Randles equivalent circuit.

3. Results and discussions

3.1. Paper-based sensor for zinc analysis

In this work, we combine the advantages of bismuth-based electrodes with ecodesigned screen-printed sensors based on office paper into a highly sustainable zinc detection method. Bismuth-based electrodes in metal stripping analysis are a sustainable alternative to the classical electrochemical mercury electrode owing to bismuth relatively low toxicity, a large negative potential range (e.g., down to -1.4 V), and insensitivity to dissolved oxygen [25,26].

In detail, we have implemented the expertise achieved in previous works, in which we thoroughly explored the use of bismuth film for trace metal analysis [19,24,27]. As a first determinant aspect, in-situ and ex-situ approaches for Bi^{3+} electrodeposition were evaluated. The first approach is carried out simultaneously with the deposition of the metal analyte in a single step, while the ex-situ deposition is carried out before the metal analysis [27,28]. The in-situ approach usually results in higher sensitivity and reduced time of sensor preparation but requires the addition of Bi^{3+} during the sample analysis [27,28]. This procedure can introduce analytical complexity when the analysis is carried out in the field, due to reagent transport and management, and accurate volume addition. The ex-situ approach requires two steps because the bismuth deposition is carried out separately from target metal analysis, but can be associated with long-term functional stability, delivering bismuth-modified sensors ready for in-field applications [26].

Here we used the following ex-situ protocol: i) in the first scan, the bismuth film is reduced on the paper-based graphite electrode surface during the deposition step and re-oxidated during the stripping step by setting the potential range to overcome bismuth oxidation potential (see Table S1), showing a bismuth peak at about -0.2 V; ii) in the second scan, the bismuth film is formed permanently on the electrode surface by ending the potential range before the bismuth oxidation potential. The ex-situ bismuth-modified paper sensor is then ready for in-field analysis without the need for reagent transport and addition.

To enhance the analytical performance in terms of electrochemical signal intensity, the sensor was further optimized by investigating the parameters of the SW-ASV electrochemical technique. As the Bi^{3+} deposition was carried out by the ex-situ approach, we were able to individually study the parameters that principally affect Bi^{3+} electrodeposition. Fig. S2A depicts the results obtained by varying the

deposition time of Bi^{3+} . The sensor response for Zn^{2+} detection is enhanced by increasing Bi^{3+} deposition time up to 300 s, while the current signal does not show any further increases beyond this time. Given the better sensitivity results for the 300 s and 450 s deposition times, a deposition time of 300 s was selected, taking advantage of a shorter analysis time.

The concentration of the buffer solution containing the Zn^{2+} was also tested. Acetate buffer provides an acidic pH value of 4.6, which helps metal cation stability [27] while different concentrations can affect the electrochemical measurement due to different ionic strength conditions. In this study, we investigated the ionic strength of the buffer solution in a concentration range of 50–500 mM. The concentration of 100 mM revealed better performance in terms of the current peak and repeatability of the measurements (Fig. S2B).

Then, SW-ASV parameters were optimized. First, the deposition time of Zn^{2+} was evaluated. Fig. 2A depicts an increasing trend up to 300 s, followed by a current decrease, suggesting a saturation effect of the electrode surface as a result of more Zn^{2+} ions being electrodeposited over time. Therefore, the time of 300 s was chosen as the optimal time for the deposition of the analyte.

Therefore, the pulse frequency was evaluated as an important parameter affecting the current peak intensity and resolution. In detail, the frequencies of 25, 50, 75, and 100 Hz were investigated. As depicted in Fig. 2B, the increase in pulse frequency from 25 Hz to 50 Hz resulted in an enhanced current signal, with no significant variation at higher frequencies. These findings led to the selection of the frequency value of 50 Hz for subsequent studies.

Next, the effect of the step potential, namely the step between a pulse and the next one, was studied. Fig. 2C shows an increase in the current signal corresponding to the step potential increase up to 7.5 mV. Given these results, 7.5 mV was chosen as the best compromise between sensitivity and repeatability.

Finally, the effect of the potential amplitude was evaluated by investigating potential values in the range of 10–100 mV (Fig. 2D). An increment of the current signal was recorded up to 50 mV, and no further increase in the current signal was observed beyond this value, but a decrease in repeatability. To obtain enhanced repeatability of the measurement, the value 50 mV was selected.

As the final aim of the developed analytical tool is to be applied for the detection of Zn^{2+} in Antarctica sediment samples, we investigated the possibility of using different concentrations of bismuth for the film electrodeposition in order to increase the sensitivity and repeatability, using a low concentration of Zn^{2+} equal to 0.1 ppm. Fig. 2E shows that using a concentration of bismuth equal to 50 ppm, higher current intensity, and repeatability were recorded, while increasing the bismuth up to 100 ppm, low repeatability was obtained, probably ascribed to a poor homogeneity of the electrodeposited layer.

The response of the Bi-modified paper sensor, under optimized SWASV conditions, was compared to the bare electrode in Fig. S3, for the analysis of Zn 0.5 ppm. As expected, the presence of bismuth as an ex-situ deposited film allows for a huge improvement in the detection capability toward zinc.

3.2. Electrochemical and morphological characterization

To gain further insight into the bismuth modification, EIS analysis was carried out (Fig. S4), comparing the Bi-modified paper-based sensor with the bare sensor. A decrease in the charge transfer resistance (R_{ct}) was observed with the Bi-modified paper-based sensor, showing a value of (302 ± 40) Ohm, compared to the non-modified sensor R_{ct} of (2400 ± 170) Ohm. This indicates faster charge transfer kinetics [29], which results in an improved electron transfer following the bismuth modification [30].

Moreover, SEM micrographs were recorded to deeply investigate the surface morphologies of the bare and Bi-modified working electrodes. The results are shown at different magnifications in Fig. S5 (A-F), while

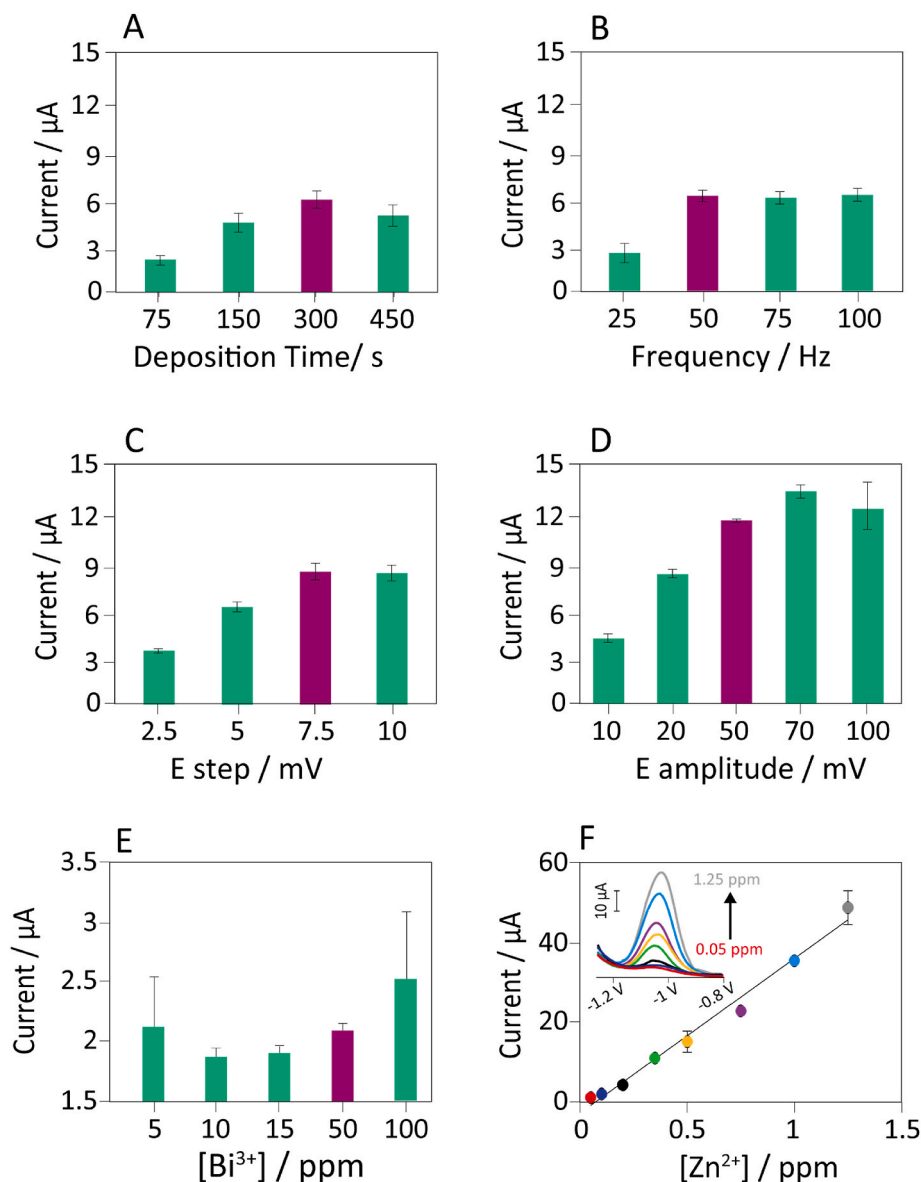


Fig. 2. Histogram bars obtained for the optimization of the parameters of the SW-ASV technique using office paper-based sensors, and a Zn²⁺ concentration of 0.5 ppm: (A) Comparison of the responses using deposition times of 75 s, 150 s, 300 s, and 450 s; (B) Comparison of the responses obtained in SW-ASV using pulse frequencies of 25 Hz, 50 Hz, 75 Hz, 100 Hz; (C) Comparison of the responses obtained investigating step potentials of 2.5 mV, 5.0 mV, 7.5 mV, 10 mV; (D) Comparison of the responses obtained using potential amplitudes of 10 mV, 20 mV, 50 mV, 70 mV, 100 mV; (E) Histogram bars obtained for the optimization of the Bismuth concentration of the SW-ASV technique using office paper-based sensors, and a Zn²⁺ concentration of 0.1 ppm. (F) Calibration curve obtained for the detection of Zn²⁺ at different concentrations using office paper-based sensors. Inset: Voltammograms obtained for the detection of Zn²⁺ at different concentrations using office paper-based sensors.

the chemical composition obtained via EDS mapping analysis is reported in Fig. S6 (A-F). Specifically, the bare sensor (Fig. S5 A-C) shows the typical morphology already observed in Refs. [19,31], with randomly oriented micrometric graphite flakes. As shown in Fig. S5 B-C, the flakes are uniformly covered with clusters of round nanometric particles, likely consisting of the cross-linking agents present in the original ink. The graphite flakes are still observed on the Bi-modified electrode surface, in the low-magnification micrograph of Fig. S5D. On the other hand, the aspect of the Bi-electrodeposited surface changes at a smaller scale: Fig. S5E and S5F show the presence of a coating layer, mostly visible on the cross-linking agent nanoparticles, whose round shape is not as clear as that observed in the bare sensor at the same magnification (Fig. S5B and S5C).

The compositional analysis reported in Fig. S6 shows the C signal matching the morphology of the graphite flakes in both electrodes. The

bismuth signal is absent in the bare sample (the observed uniformly distributed counts in Fig. S6C arise from background noise), while it is clearly present on the Bi-modified working electrode (Fig. S6F), tracing the surface morphology and exhibiting higher intensity on the nanoparticle clusters.

3.3. Analytical features

The analytical features of the developed electrochemical paper-based sensor were assessed by analyzing different concentrations of Zinc standard solution. A linear correlation was obtained in the range of 0.05–1.25 ppm, described by the following equation: y (μA) = $(38.8 \pm 1.9) x$ (ppm) - (2.6 ± 1.3) , ($R^2 = 0.986$) (Fig. 2F), observing a negative intercept in agreement with the literature [32]. The limit of detection (LOD) was calculated as the S/N ratio = 3 and found equal to 0.015 ppm,

while the limit of quantification (LOQ) was obtained as the $LOD \times 3.3$, resulting in 0.05 ppm.

The precision was assessed by measuring RSD% at different concentration levels, for instance, RSD% was found equal to 4 % when the measurement of 0.1 ppm was carried out in triplicate.

Batch-to-batch repeatability was finally assessed by measuring RSD % at a Zn concentration equal to 0.5 ppm. Using Bi-modified sensors fabricated on three different days, an RSD% equal to 3.9 % was obtained.

3.4. Zinc extraction

Heavy metal analysis in soils is typically carried out using methods based on acid digestion, which leads to the total conversion of different metal forms into the most acid-soluble cation form. This approach is useful for measuring the total amount of target metals, but does not enable the differentiation among different metal fractions. However, information about metal fractions is needed to study the role of metals in ecosystem dynamics. The mobility and availability of metal fractions typically decrease from water-soluble forms to reducible forms, oxidizable forms, and, finally, residual forms [14]. In detail, the water-soluble and exchangeable fraction is immediately available for plant uptake [13, 15,16].

Herein, our methodology is designed to reveal the zinc water-soluble and exchangeable fraction in soils and sediments, namely the fraction available for immediate uptake and transformation by plants, thus closely related to modifications that occur in the ecosystem. To develop our method, we translated the BCR protocol used for the extraction of zinc water-soluble and exchangeable fraction into a customized semi-

automatic extraction system based on a 3D-printed chamber and a peristaltic pump.

3.5. BCR method

The BCR extraction method is based on the sequential extraction of zinc fractions from soils or sediments, [22,23] in which the extraction of the water-soluble and exchangeable fraction is the first step. Starting from the original BCR protocol [23], several variations have been reported in the literature.

As shown in Table S2, a few variations have been proposed compared to the original method, related to i) the possibility of replacing the end-over-end shaking extraction with sonication, thus reducing the time required for the process to 10–15 min, and ii) the filtration of the supernatant extract after the extraction procedure. In detail, the long extraction time of the original BCR (i.e., 16 h) is a significant constraint, especially for analysis to be carried out in the field. For this reason, the replacement of end-over-end shaking with sonication has been attempted to reduce the extraction time [33,34].

In detail, we investigated the efficiency of key mechanisms in the extraction protocol: i) the shaking time, ii) the mixing procedure (rotating shaking vs sonication), iii) supernatant filtration, and iv) soil oven-drying. For these studies, soil sampled from gardens at the Department of Chemical Science and Technologies, University of Rome “Tor Vergata” was used. The amount of Zn^{2+} extracted was analyzed by flame AAS, as a suitable analytical reference method for trace metal analysis.

First, different extraction times were evaluated using a rotating shaker, from 16 h down to 5 min, at a constant temperature (about

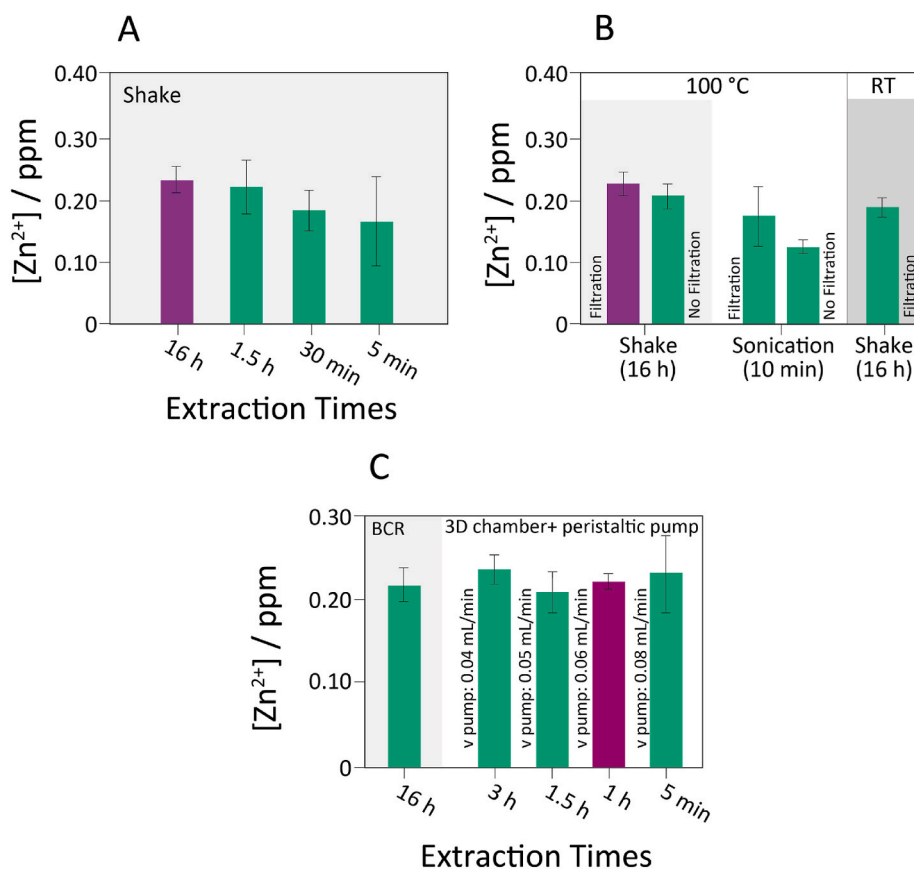


Fig. 3. (A) Comparison of the extraction times of the BCR extraction method as a function of the concentration of Zn^{2+} . (B) Histogram bars obtained for the comparative analysis of three steps of the BCR extraction method, including drying the sample in a furnace or air, shaking or sonicating the solution overnight, and filtering or not of the solution. (C) Comparison of the extraction times of the peristaltic pump as a function of the concentration of Zn^{2+} . AAS Measurements were carried out using soil samples from the University of Rome “Tor Vergata”.

15 °C) (Fig. 3A). Compared to 16 h, a loss of repeatability and amount of Zn^{2+} extracted was observed. This behavior can be ascribed to the kinetics that rule the adsorption-desorption of water-soluble and exchangeable Zn^{2+} from soil particles, which can require up to 16 h to reach the equilibrium and, thus, repeatability in the extraction [35].

The efficiency in the extraction carried out by shaking and sonication was also evaluated. The results shown in Fig. 3B reveal that a higher concentration of Zn^{2+} was obtained after 16 h of rotating shaking compared to 10 min of sonication (20 kHz, 20 % amplitude) [33]. For both shaking and sonication, the filtration of the supernatant solution (after centrifugation) led to a higher amount of Zn^{2+} extracted, suggesting the presence of suspended particles. Indeed, the filtration step allows for removing suspended particles, such as organic matter, which can adsorb Zn^{2+} and prevent its correct detection at the flame AAS.

Finally, the effect of oven drying was tested on the soil sample, upstream of the extraction process. Although most of the studies report an air-drying process (Table S2), we tested a drying treatment at 100 °C for 6 h to obtain a standard drying process. This approach can ensure controlled and repeatable drying conditions compared to air drying, which is subject to seasonal variations. In addition, heat treatment ensures the total evaporation of the aqueous content and the inactivation of microbial activity, which can alter the Zn^{2+} content in the samples [36,37]. The comparison between soil samples dried at 100 °C for 6 h and samples dried at room temperature conditions (both extracted by 16 h-shaking, centrifugation, and filtration as described above) showed that the 100 °C-treatment allows for higher Zn^{2+} extraction.

The most effective BCR protocol resulted in the sequence given by i) soil oven treatment at 100 °C for 6 h, ii) rotating shaking in 0.11 M acetic acid for 16 h, and iii) filtration of the supernatant solution after centrifugation using PTFE filters (0.45 µm). This procedure was therefore used as a reference to develop our innovative approach based on the 3D-printed extraction chamber.

3.6. 3D-printed extraction chamber

As described in the previous paragraphs, we designed a pump-driven approach combined with a 3D-printed extraction chamber to deliver an innovative, portable, and semi-automatic system for water-soluble and exchangeable zinc fraction from soil and sediment samples. We used a syringe to inject the solution into the 3D-printed extraction chamber (Fig. 1 and Fig. S1B). The solution is driven by the peristaltic pump into the upper part of the syringe, according to the speed of the pump. When the solution enters the syringe, it is dropped inside the syringe, reaching the bottom part. In detail, the pump flows the extracting solution (4 mL of 0.11 M acetic acid, as used in the BCR method, see Table S2) into a syringe connected to the 3D-printed chamber, in which the soil or sediment sample (0.1 g) is previously placed (Fig. S1A). In this way, the ratio (soil):(extracting volume) established by the BCR method is respected and translated into our innovative, portable, and user-friendly extraction system. The constant flow speed set on the pump allows for a controlled contact time between the solution and the soil in the extraction chamber. The system did not show any leaching of the solution, thanks to the controlled process, which avoids high pressure inside the syringe and the extraction chamber. This system has been thoroughly studied in our work, as described in this paragraph. This system resulted in a simple but efficient method to carry out zinc extraction from the solution.

The cell, fabricated by a stereolithographic 3D printer, is formed by a concave unit and a convex unit (Fig. S1A). A polyvinylidene fluoride (PVDF) membrane is placed at the inner base of the concave unit, preventing soil particles from leaking. Additionally, the membrane retains suspended matter contained in the matrix but does not adsorb zinc, owing to the inert nature of PVDF. Once the cell is closed with the aid of screws, Zn^{2+} extraction from the soil is carried out by flowing a volume of extracting solution through the cell, using a peristaltic pump, equipped with flexible polyvinyl chloride tubes and a syringe (Fig. S2B). A

Teflon tape was used to prevent solution leakage. The extracted solutions were filtered with 0.45 µm PTFE filters.

First, a study of the flow speed was carried out, testing pump flow rates from 0.04 to 0.08 mL/min were examined using soil sampled at the University of Rome "Tor Vergata". Taking inspiration from the BCR approach, in which an efficient extraction is obtained by continuously mixing soil for 16 h, we started investigating the pump flow with a slow rate, namely 0.04 mL/min, corresponding to an extraction time of 3h. Fig. 3C shows the amount (ppm) of Zn^{2+} extracted using our peristaltic pump-3D chamber system at different flow rates and compares it to the results previously obtained using the BCR method. Comparable results can be observed in terms of extracted Zn^{2+} (ppm) up to a flow rate of 0.06 mL/min, while 0.08 mL/min led to a higher variability of the concentrations of the extracted Zn^{2+} . Therefore, a pump rate of 0.06 mL/min was selected, corresponding to an extraction time of 1h. Noteworthy, this extraction time is extremely advantageous compared to the time required for the BCR method, i.e., 16 h. Finally, our peristaltic pump-3D chamber system does not require other equipment, such as shakers and centrifugation.

3.7. Antarctic samples analysis

The efficiency of our innovative extraction system was tested on Antarctic sediments and compared to the optimized BCR extraction method. The sediments were collected from lakes that are deglaciated during the short Antarctic summertime in the area of Terra Nova Bay (Fig. 1A).

The first one is the Edmonson Point site, located close to Mount Melbourne, one of the rare active volcanoes of Antarctica, and characterized by dark sediments. Lake 14 at Edmonson Point, which has experienced the largest size reduction among the lakes considered here, is eutrophic and sediments are covered in algal mats where numerous microalgal and cyanobacterial taxa were identified, and which were also observed at Gondwana, the second site. The third site is located on the mountainous island of Victoria Land, in a depression called Tarn Flat. The fourth site is the Inexpressible Island, which is set on a moraine relief. The latter two sites are characterized by the intensive stress from katabatic winds, which makes the sites even more inhospitable for life [22,38–40]. All lakes are characterized by a high salt content, increasing with proximity to the sea. Table 1 presents a comparison of the Zn^{2+} amount (ppm, mg/L) measured in these sediments using i) the BCR method coupled with flame AAS and ii) our innovative extraction method, based on the peristaltic pump-3D chamber extraction system coupled with paper-based printed electrodes and SWASV electrochemical measurements (Fig. 1B). Our innovative extraction systems resulted in accuracy and repeatability comparable with the reference method, with a bias between the range of 0.7–20 %.

As shown in Table 1, our system did not reveal Zn^{2+} amounts lower than 0.06 ppm (mg/L); however, these amounts are poorly significant in terms of ecosystem availability as well as pollution. Indeed, Table 2 summarizes the water-soluble and exchangeable zinc levels in Antarctic sediments that were reported in the literature and compares them to our results. For this comparison, the water-soluble and exchangeable zinc fraction was translated from mg/L to mg/kg, using the mass of the sample (0.1 g) and the volume of extracting solution used according to the formula (1):

$$Zn^{2+} \text{ (mg / kg)} = [Zn^{2+}] \text{ (mg / L)} \cdot 0.004 \text{ (L)} / 0.0001 \text{ (kg)} \quad (1)$$

It can be observed that zinc amounts lower than 0.06 ppm (mg/L), corresponding to 2.4 mg/kg, are close to the lowest values reported in other studies (see Table 2), thus, suggesting that our innovative 3D-printed extraction cell system, coupled with the paper-based sensors, is a promising method for the extraction and analysis of water-soluble and exchangeable zinc fraction in soils and sediments, even in remote areas such as Antarctica, taking advantage of in-field transportable

Table 2

Reference amounts of zinc water-soluble, acid-soluble, and exchangeable fractions in soils and sediments reported in the literature for several Antarctic sites. The lowest-highest value range is reported when possible, according to the data published in the reference articles. The values related to this work are calculated using the formula Zn^{2+} (mg/kg) = $[Zn^{2+}]$ mg/L • 0.004 L/0.0001 kg) for both the BCR + AAS method (a) and our innovative method (b). Where the symbol "≈" is reported, the values are approximated from the evidence reported in the corresponding articles. For simplicity, only data related to sediments are reported, excluding data related to soils, in the case of reference [22].

Antarctic site; Date of sampling	Zn ²⁺ water- soluble and exchangeable fraction (mg/ kg)	Extraction method	Analysis method	Ref.
Scott base; Date of sampling not available	<0.025–8.9	2 g of dry soil was shaken with 25 ml of deionized water for 1 h and centrifuged; the procedure was then repeated with 25 ml of 0.01 M HNO ₃	Inductively Coupled Plasma-Atomic Emission Spectroscopy or Mass Spectroscopy	[41]
Old Casey Station; 2017–2018	2.4–60	0.3 g of dry soil was shaken for 1 h with 30 mL of 1 M HCl, then	Inductively Coupled Plasma-Atomic Emission Spectroscopy	[17]
Wilkes Station; 2017–2018	1.7–36	centrifuged, and filtered by 0.45 μm filter		
Edmonson; Point, site 14 2003	≈5.5 (≈5 % of total zinc = 110)	BCR method: 0.5 g of dry sediment was shaken with 20 ml of 0.11 M acetic acid for 16 h and centrifuged	Inductively Coupled Plasma-Atomic Emission Spectrometer or Graphite	[22]
Inexpressible Island, site 10b; 2004	≈2.6 (≈30 % of total zinc = 8.75)		Furnace Atomic Absorption Spectrometer	
Tarn Flat, site 20; 2004	≈1.7 (≈2.5 % of total zinc = 69.7)			
Edmonson Point, sites 14–15A; 1993–2014	(a) 0.64–4.6 (b) 2.83–5.2	(a) BCR (as above) (b) method 0.1 g of dry sediment was placed in the 3D-printed extraction chamber and	(a) AAS (b) SWASV and paper-based sensor modified ex-situ with bismuth	This work
Inexpressible Island, site 10B; 1990–2014	(a) 0.64–11.96 (b) 10.8	extracted by flowing 4 mL of 0.11 M acetic acid for about 1 h		
Tarn Flat, site 20; 1991–2014	(a) 0.008–3.36 (b) 3.32			
Gondwana; 2012	(a) 8.16 (b) 6.68			

setup.

A great variability of zinc content in Antarctic soils and sediments can be observed in Table 2. While the average total contents of metals are defined by mineralization, threshold values of zinc fractions in soils are difficult to establish because they are sensitive to many environmental variables [42]. Particularly, the expected content of the water-soluble and exchangeable zinc fraction in Antarctica has shown significant heterogeneity depending on geographical distribution and date of sampling [43], low organic matter, proximity to biological inputs, extreme temperatures, and varying salt concentrations [9]. On one side, cold and wet soils are associated with low zinc content owing to low biological activity [42], which is the case of Antarctic lakes that are deglaciated only in summertime. On the contrary, high levels of zinc can be found in specific Antarctic sites owing to bird activity (i.e., guano) as well as intensive fuel contamination and activities in human Antarctic

stations [9]. Such variability is well reflected in the results that we obtained with the reference BCR-AAS method (Table 1). Interestingly, our innovative system revealed values in good accordance with zinc levels reported in the literature for the water-soluble and exchangeable fraction, extracted using BCR (Table 2) [22], highlighting again the promising applicability of our proposed approach.

3.8. Interfering agents

Potential interferences were analyzed according to expected values for unpolluted sediments, which are also reported in the literature. In detail, sediments are expected to contain high amounts of iron (up to 30000 ppm) while nickel and copper are usually found in total concentrations <50 ppm [22,44,45] in Antarctic sediments. However, the amount of these metals that can be extracted under the conditions of the first BCR step (water soluble and exchangeable fractions) results in much smaller quantities. For example, Malandrino et al. [22] reported that less than 1 % of the total amount of iron can be extracted. Burgay et al. [44] found that nickel can be extracted up to about 15 %, and copper between 3 and 4 % (with respect to the total amount of metal). According to this evidence, we chose to analyze 10 ppm, 50 ppb, and 20 ppb for iron, nickel, and copper, respectively, with the Bi-modified electrode. The results are reported in Fig. S7, where no peaks appear in the potential window, highlighting that these metals are not detected under these conditions.

Moreover, we analyzed cadmium and lead cations, which are known to form alloys with bismuth [27].

According to data from the literature, we analyzed concentrations of 0.5 ppm for lead and 0.1 ppb for cadmium. Fig. S7 shows that only lead is detected at about −0.55 V, which is far from the potential of zinc oxidation, avoiding peak overlapping.

We highlight that the metal concentrations discussed here refer to unpolluted sediments. Polluted sediments can contain much higher amounts of metals, and the fractions that can be extracted can increase as well. Moreover, each sediment should be analyzed thoroughly to disclose the full metal composition and metal fractions, which requires the use of multiple analytical techniques and a sampling plan. In this work, we present a disposable, low-cost, and sustainable method to analyze the water-soluble and exchangeable zinc fraction from soil and sediments, which can be seamlessly applied in remote areas owing to portable and low-energy-consumption equipment. This method is not meant to replace classical analytical methods for metal analysis, which ensure accuracy and comprehensive analysis (e.g., mass spectrometry), but represents a valid support for in-field analysis as well as an example of how to design sustainable analytical methods as novel complementary approaches.

3.9. Greenness and Whiteness assessment by the RGBFast tool

A final quantitative assessment was conducted on the proposed method and the BCR-AAS reference method, in accordance with the principles of Green Analytical Chemistry and White Analytical Chemistry. To evaluate the Greenness/Whiteness of the methods, a novel tool called “RGBfast” was used [46], which enables a straightforward comparison of methods applied for detecting a specific analyte.

An Excel spreadsheet was used to carry out the calculation and obtain the scores for each of the six criteria [46], providing a final table that summarizes the RGB and White color saturation (Fig. 4).

In detail, the “Trueness” value for the peristaltic pump-3D chamber - SWASV method was calculated by the % relative ratio between the results obtained using the proposed and the BCR-AAS method.

For the “Trueness” calculation of the BCR-AAS method, we used the recovery values reported by Žemberyová as reference values [47]. Precision values were calculated as the RSD% from a set of measurements, while LOD was calculated as $(3 \times \sigma_{10 \text{ blanks}})/S$ (where “ $\sigma_{10 \text{ blanks}}$ ” is the standard deviation of 10 blank measurements, “S” is the slope of the

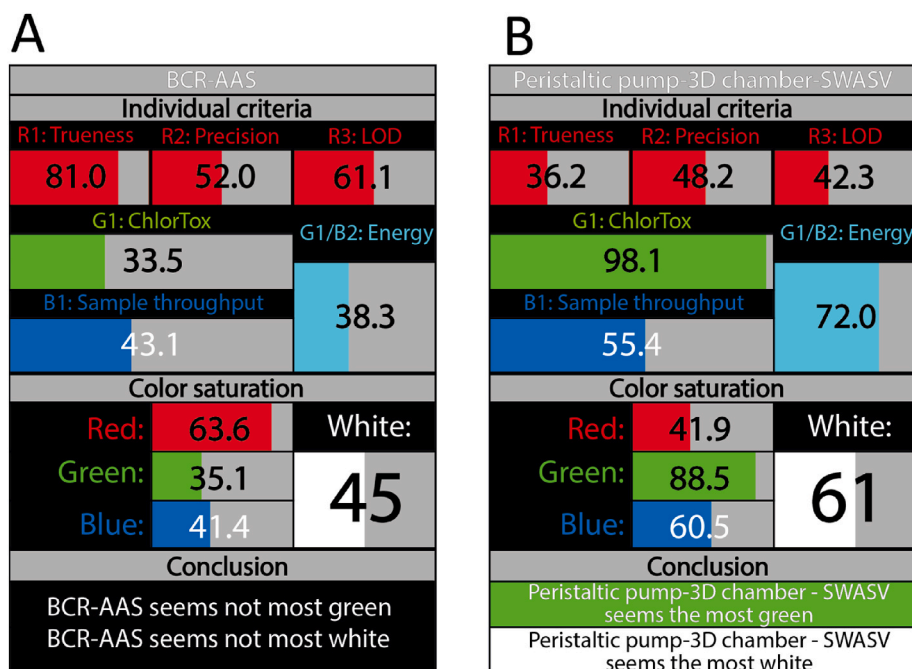


Fig. 4. Comparison of the RGBfast evaluation carried out for (A) the BCR-AAS method and (B) the proposed 3D-chamber-SW-ASV method.

calibration curve), and $S/N = 3$ (Signal to Noise ratio), for the BCR-AAS and proposed method, respectively.

The remaining values for “ChlorTox index”, “Sample throughput”, and “Energy results” were carefully estimated considering the suggestions provided by the RGBfast method [46], taking into account the amount of reagents used, the maximum number of analyses that can be done within 24 h, and the instrumentation used, respectively.

The results of the RGBfast assessment highlight that our proposed method features a higher Greenness and Whiteness (Fig. 4 and Fig. S8). It obtained the highest evaluation scores for Green and Blue color saturation. Unlike the BCR-AAS, our proposed method uses low-energy-consuming instruments based on a portable potentiostat and a peristaltic pump. In detail, several potentiostats can be supplied directly by a laptop or by a smartphone (e.g., Palmsens® portable laptop or smartphone-assisted potentiostats) while miniaturized pumps can be supplied by batteries. Moreover, our approach minimizes the use of toxic reagents. As a result, the levels of Green and Blue color saturation reach 88.5 and 60.5, respectively. Conversely, the BCR-AAS method is characterized by increased analytical performances, highlighted by the Red color saturation value of 63.6, resulting from the higher scores obtained for the Trueness, Precision, and LOD scores.

In summary, our proposed method has emerged as the greenest and whitest compared to the BCR-AAS reference method.

4. Conclusions

In this study, we presented an innovative analytical approach that combines the extraction and analysis of zinc in soils with an integrated analytical system. The strengths of our approach rely on the capability to distinguish the water-soluble and exchangeable fraction of zinc in soil, which is the most important fraction for plant uptake and the following introduction into the food chain. The analysis of the different zinc fractions in soil can be done using the BCR protocol, which stands as a reference method. Here, we translated its principles into a simplified procedure, utilizing an easy-to-use and semi-automatic system, namely a peristaltic pump and a 3D-printed extraction chamber, ad hoc customized for this purpose. Moreover, we coupled this innovative extraction method with a paper-based sensor to accomplish the analysis of the extracted zinc, replacing the need for expensive and bulky instruments

such as AAS.

Antarctic deglaciated lakes samples, collected during summertime expeditions from 1993 to 2014, were analyzed to prove the capability of our method to extract and detect the water-soluble and exchangeable zinc fraction in real sediments. The comparison of our results with the reference method, based on an optimized BCR extraction protocol and AAS analysis, confirmed the excellent analytical performance of our approach.

Moreover, the application of RGBfast assessment disclosed the superior features of our approach over the BCR-AAS method in terms of Blue and Green parameters, which stands for greater sustainability owing to lower energy consumption, lower use of toxic reagents, and the use of green materials (i.e., paper-based electrodes), paving the way for its wide use, even in remote areas of the world.

CRediT authorship contribution statement

Noemi Colozza: Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Vincenzo Mazzaracchio:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Formal analysis, Conceptualization. **Chiara Di Gregorio:** Investigation, Formal analysis, Data curation. **Narjiss Seddaoui:** Investigation, Formal analysis. **Daniel Aquilani:** Investigation, Formal analysis. **Alessio Pizziconi:** Investigation, Formal analysis. **Ludovica Gullo:** Investigation, Formal analysis. **Elena Argiriadis:** Writing – review & editing, Funding acquisition. **Fabiana Arduini:** Writing – review & editing, Methodology, Funding acquisition, Conceptualization.

Notes

The authors have obtained permission for the use of Antarctic Lake sediment samples for research purposes from the Italian National Antarctic Museum (MNA) and from the Bank of Antarctic Environmental Samples (BCAA) of Genoa.

Declaration of competing interest

The authors declare that they have no known competing financial

interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.talanta.2025.128718>.

Data availability

Data will be made available on request.

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