Brazilian Journal of Analytical Chemistry
2025, Volume 12, Issue 48, pp 213-229
doi: 10.30744/brjac.2179-3425.AR-129-2024

BrJAC

ARTICLE

Diluted Acid and Microwave-Assisted Extraction for Trace Element Determination in Biochar by ICP OES and ICP-MS

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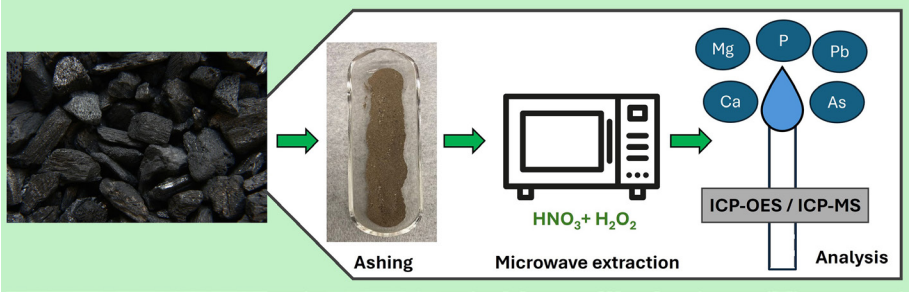

The determination of trace elements in biochar usually involves complex and inefficient sample preparation strategies due to the high carbon content and presence of silicates in the sample matrix, as well as to the variety of raw materials used in its production. Most methods are time-consuming, employ hazardous reagents (*e.g.* hydrofluoric acid), and are prone to analyte contamination and loss. Another issue is the lack of validation to ensure that these methods provide accurate results. In this study, we describe a sample preparation strategy to determine Al, As, Ba, Ca, Co, Cr, Cu, Fe, K, Mg, Mo, Mn, Na, Ni, P, Pb, S, Sr, Ti and Zn in biochar by inductively coupled plasma optical emission spectrometry (ICP OES) and inductively coupled plasma mass spectrometry (ICP-MS). The method includes a dry ashing step, followed by microwave-assisted extraction with diluted nitric acid, and hydrogen peroxide. Initially, it was evaluated for efficacy using a hog waste biochar and compared to a similar and commonly reported extraction with aqua regia. The method's accuracy was assessed by addition and recovery experiments, with analyte recoveries in the 89.6%-114% range. Limits of detection were in the 0.02-3000 and 0.006-0.02 mg kg^{-1} ranges for ICP OES and ICP-MS, respectively, with lower values for HNO_3 + H_2O_2 compared to aqua regia. Relative standard deviation (RSD) values using HNO_3 + H_2O_2 were below 10% for all analytes, except As (15%), Cr (12%), and Pb (11%), while aqua regia values were in the 15%-63% range. The method was then applied to the analysis of five commercially available biochar samples.

Cite: Fontoura, B. M.; Judd, N.; Schmittling, M.; Gross, M. D.; Jones, B. T.; Donati, G. L. Diluted Acid and Microwave-Assisted Extraction for Trace Element Determination in Biochar by ICP OES and ICP-MS. *Braz. J. Anal. Chem.* 2025, *12* (48), pp 213-229. http://dx.doi.org/10.30744/brjac.2179-3425.AR-129-2024

Submitted September 19, 2024, Resubmitted March 25, 2025, Accepted April 15, 2025, Available online May 8, 2025.

This article was submitted to the BrJAC special issue celebrating the achievement of Journal Impact Factor.

Keywords: sample preparation, high carbon content, recalcitrant material, biochar analysis accuracy, and atomic spectrometry

INTRODUCTION

Biochar is a material originating from the thermochemical conversion of plant biomass or animal waste (*e.g.*, hog waste, pine chips, stemwood, sugarcane bagasse, microalgae residue, etc.) through processes such as slow pyrolysis, torrefaction, fast pyrolysis, or gasification.[1] As expected, the type of feedstock and condition used in its production significantly impact the material's composition and physicochemical properties.[2]

Biochar has been used in several fields such as carbon sequestration,[3] water filtration,[4] soil remediation,[5] atmospheric greenhouse gas consumption,[6] and catalytic conversion.[7] When added to soil, it can increase cation exchange and fertility by the slow release of macro- and micronutrients (*e.g.* Ca, Cu, Fe, K, Mg, Mn, Na, P, S, Si, etc.) needed for plant growth.[8] On the other hand, due to its high surface area and porous structure, biochar is prone to the physical adsorption of organic and inorganic compounds, including toxic elements such as Al, As, Cd, Co, Hg, Pb, etc.[9] Thus, when directly added to the soil, it may cause environmental pollution and/or human health problems associated with food grown in contaminated soil.

Considering its many applications, it is important to accurately determine the elemental composition of biochar. However, converting biochar into a liquid solution, which is required for most quantitative analytical methods, may be a challenge due to its relatively high silica content.[10] Most procedures reported in the literature are time-consuming, and involve concentrated/dangerous acids and high temperatures. Wathudura *et al.*,[11] for example, evaluated the microwave-assisted digestion of nine carbonaceous sources using fuming HNO_3 and H_2SO_4, reagents known to be highly corrosive and toxic. Highly corrosive hydrofluoric acid is used in many standardized methods, such as those recommended by the United States Environmental Protection Agency (US EPA),[12] the International Organization for Standardization (ISO 15238 method),[13] and the European Biochar Certificate (EBC).[14]

The US EPA 3052 method proposes a closed-vessel microwave-assisted digestion using HNO_3 and HF to dissolve silicate and organic matrices.[12] It recommends decantation, filtration, or centrifugation of the final solution, as well as the addition of H_3BO_3 for fluoride complexion before analysis, in order to prevent damage to instrument parts made of glass or fused silica. The ISO 15238 method is recommended for Cd determination in coal but may be adapted for biochar analysis.[13] This strategy involves sample ashing at 450 °C for 2 hours, followed by water bath heating with a mixture of aqua regia and HF (3 mL/5mL). The EBC proposes a more specific method for biochar digestion and trace determination of As, Ag, B, Cd, Cr, Cu, Mn, Ni, Pb and Hg by atomic spectrometry.[14] The procedure requires several steps: sample drying at 40 °C, microwave digestion using HNO_3 + H_2O_2 + HF, fluoride masking, heating at 160 °C for 7 min, appropriate dilution, and final analysis by inductively coupled plasma mass spectrometry (ICP-MS) (method DIN EN ISO 17294-2). Mercury determination can be carried out by atomic absorption spectrometry (methods DIN EN ISO 12846 and DIN 22022-4), or by ICP-MS (methods DIN EN ISO17294-2 and DIN 22022-7).

A different approach is recommended by the EBC[14] for macro-elements determination such as Ca, Fe, K, Mg, Na, P, S and Si. Biochar is initially ashed in a muffle furnace at 550 °C under an O_2 atmosphere for 30-60 min. Then, the biochar ash is mixed with lithium metaborate in a platinum crucible and heated at 1050 °C. The melt is dissolved in HCl and appropriately diluted before analysis by inductively coupled plasma optical emission spectrometry (ICP OES) (method DIN EN ISO 11885) or ICP-MS (method DIN EN ISO17294-2). Ullah *et al.*[15] reported another, somewhat greener alternative, which uses the US EPA 3050B (1996) method for biochar analysis. The authors recommend a 3- to 4-hour sample digestion in an open-vessel conductive-heating system with concentrated HNO_3 and H_2O_2, which could cause analyte losses or contamination.

Most of these methods employ high concentrations of acids, which requires excessive dilution of the digested solutions to make them compatible with modern atomic spectrometry instrumentation based on

pneumatic nebulization. In this context, partial digestion/extraction strategies using diluted acids may be promising alternatives for achieving biochar digests with low acid concentrations.[16–20] De Mello *et al.*,[21] for example, described a partial digestion method for Al, Ca, Cu, Cr, Fe, K, Mg, Mn, Na, Ni, P, Pb, S, Si and Zn determination in complex matrices such as zinc oxide, kaolin, zinc residue, and zinc sulfide by ICP OES. Four mixtures containing 3 mL of H_2O_2 and 5 mL of nitric acid at different concentrations (2, 4, 7 or 14 mol L^{-1}) were investigated for closed-vessel microwave-assisted extraction and compared with a total digestion procedure using 2 mL of 40% v v^{-1} HF, 5 mL of concentrated HNO_3, and hot block conductive heating. In general, satisfactory results were observed using a HNO_3 concentration ≥ 7.0 mol L^{-1} in the partial digestion method.

Another important issue associated with biochar analysis is the lack of appropriated certified reference materials, and studies that include an evaluation of the method's accuracy. In one of the few works available in the literature that is concerned with this issue, Bachmann *et al.*[22] described an interlaboratory study involving 22 participating laboratories to determine macro- and microelements in biochar by ICP OES, ICP-MS, or graphite furnace atomic absorption spectrometry (GF AAS). However, inconsistent concentrations were reported for Ca, Fe, K, Na and P depending on the digestion method adopted. In general, higher analyte values were observed when fusion with $LiBO_2$ and subsequent dissolution in HCl was employed, which may be associated with greater analyte extraction. Nonetheless, the method is laborious and prone to contamination.

In the present study, we adopted diluted HNO_3 and H_2O_2 as reagents for closed-vessel microwave-assisted extraction of trace elements[21] from biochar. The main goals were to develop a sample preparation strategy that is (*i*) compatible with modern and sensitive analytical methods such as ICP OES and ICP-MS, (*ii*) a safer alternative to some of the methods currently available, which require high concentrations of acids, use of dangerous HF, or a laborious fusion procedure using $LiBO_2$, and (*iii*) accurate for trace element determination. The samples were first submitted to a drying/ashing procedure followed by microwave-assisted extraction and Al, Ba, Ca, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, P, S, Sr, Ti and Zn determination by ICP-OES, as well as As, Co, Mo and Pb determination by ICP-MS. Addition and recovery experiments were performed to evaluate the method's accuracy. The results were also compared with those from aqua regia extraction, which is commonly used for partial digestion of soil and sludge.[23]

MATERIAL AND METHODS

Instrumentation

A KSL-1100X muffle furnace (MTI Corporation, Richmond, CA, USA) and glass crucibles were used for sample drying and ashing. Microwave-assisted extraction was performed using an Ethos Up microwave-assisted digestion system (Milestone Inc., Sorisole, Italy) equipped with the SK-15 rotor and polytetrafluoroethylene (PTFE) vessels. An ICP OES instrument (Agilent Technologies, model 5100, Mulgrave, Australia) containing a SPS4 automatic sampler, a cyclonic spray chamber and a concentric nebulizer was used to determinate 16 elements in biochar samples. An ICP-MS instrument (Agilent Technologies, model 8800) was used for As, Co, Mo and Pb determination. Argon from a liquid Ar Dewar (99.999%, ARC3 Gases, South Dunn, NC, USA) was used for plasma generation in both ICP OES and ICP-MS. Instrument operating conditions are listed in Tables I and II for ICP OES and ICP-MS, respectively. An X-ray diffractometer (D2 phaser, A26-X1, Bruker, Karlsruhe, Germany) with a Cu Kα X-ray tube (30 kV, 10 mA, 1.5406 Å) was used for analyzing the solid remaining at the end of the biochar microwave-assisted extraction process.

Table I. ICP OES operating conditions used for biochar analysis

Instrument parameter	Operating condition
Radio frequency (RF) applied power (kW)	1.20
Plasma gas flow rate (L min^{-1})	12.0

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Table I. ICP OES operating conditions used for biochar analysis (continuation)

Instrument parameter	Operating condition
Auxiliary gas flow rate (L min^{-1})	1.00
Nebulizer gas flow rate (L min^{-1})	0.70
Stabilization time (s)	15
Peristaltic pump rate (rpm)	12
Uptake delay (s)	30
Viewing mode	SVDV
Number of replicates	3
Integration time (s)	5
Nebulizer	Concentric; glass
Spray chamber	Cyclonic; double pass; glass
Analytes (emission wavelength, nm)	Al I (396.153); Ba II (455.403); Ca II (396.847); Cr II (267.716); Cu I (327.395); Fe II (238.204); K I (766.491); Mg II (279.553); Mn II (257.610), Na I (589.592); Ni II (231.604); P I (213.618); S I (181.972); Sr II (407.771); Ti II (336.122) and Zn I (213.857).

I- atomic line; II- ionic line.

Table II. ICP-MS operating conditions used for biochar analysis

Instrument parameter	Operating condition
Radio frequency (RF) applied power (kW)	1.55
Sampling depth (mm)	8.0
Plasma gas flow rate (L min^{-1})	12.0
Carrier gas flow rate (L min^{-1})	1.01
Peristaltic pump rate (rps)	0.10
Number of replicates	3
Integration time (s)	0.1
Collision/reaction cell gas	He (As, Co and Mo); no gas (Pb)
He gas flow rate (mL min^{-1})	3.5
Octopole bias (V)	-18.0
Octopole RF (V)	190
Energy discrimination potential (V)	5.0
Analytes (mass-to-charge ratio - *m/z*)	As (75), Co (59), Mo (95), Pb (208)

Reagents and solutions

Distilled-deionized water (18 MΩ cm, Milli-Q® water, Millipore Sigma Merck KGaA, Darmstadt, Germany) was used to prepare all analytical solutions. Trace-metal-grade HNO_3 (Fisher Scientific, Pittsburgh, PA, USA), HCl (Fisher Scientific) and H_2O_2 (GFS Chemicals, Inc., Powell, OH, USA) were employed for microwave-assisted extraction. Single-element reference solutions of Al, Ba, Ca, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, P, S, Sr, Ti and Zn at 1000 mg L^{-1} (High-Purity Standards, North Charleston, SC, USA), and As, Co, Mo and Pb at 10 mg L^{-1} (High-Purity Standards) were appropriately diluted with distilled-deionized water to prepare the calibration solutions. Solutions used for addition and recovery experiments were prepared from adequate dilution of stock solutions of As, Co, Mo and Pb 100 mg L^{-1} (High-Purity Standards); Ba, Cr, Ni, Sr and Ti 1000 mg L^{-1} (High-Purity Standards); and Al, Cu, Mn, Na, S and Zn 20 000 mg L^{-1} prepared from their respective salts, *i.e.*, $Al_2(SO_4)_3 \cdot 18H_2O$ (99.8% purity, Fisher Scientific, Fair Lawn, NJ, USA), $CuSO_4 \cdot 5H_2O$ (99.7% purity, Fisher Scientific), $Mn(CH_3CO_2)_2 \cdot 4H_2O$ (99% purity, Acros Organics, Geel, Belgium), NaCl (99% purity, Fisher Scientific), $(NH_4)_2SO_4$ (99.8% purity, Fisher Scientific), and $Zn(CH_3CO_2)_2 \cdot 2H_2O$ (99.5%, Sigma-Aldrich, St. Louis, MO, USA). All glassware was decontaminated overnight in a 10% v v^{-1} HNO_3 bath and rinsed with distilled-deionized water prior to use.

The external standard calibration method (EC) was employed for analyte determination by ICP OES and ICP-MS. All calibration solutions were prepared in 4% v v^{-1} HNO_3, with analyte concentrations at 0.05, 0.1, 0.2, 0.5, 1.0, 2.0 and 5.0 mg L^{-1} for ICP OES, and at 5, 10, 20, 50 and 100 µg L^{-1} for ICP-MS analyses.

Sample and sample preparation

Six biochar samples were evaluated in this study: two produced from hog waste collected in the State of North Carolina (USA) by Montauk Renewables, Inc. (B1 and B2), and four commercially acquired and from different sources, *i.e.*, Yield Titan Premium (plants or animal waste), Char Bliss (from wood), Wakefield (plant-based), and Persist (pistachio shell).

Approximately 3 g of each sample was added to a crucible and the following muffle furnace heating program was applied: *(1)* ramp to 100 °C for 20 min, *(2)* hold at 100 °C for 15 min for sample drying, *(3)* ramp to 450 °C for 48 min, *(4)* hold at 450 °C for 180 min for sample ashing, and *(5)* cool down to room temperature for 60 min. The ashes were ground with a porcelain mortar and pestle for homogenization and, subsequently, 100 mg aliquots underwent microwave-assisted extraction in a closed vessel. Two extracting mixtures were evaluated: (P1) 5 mL of concentrated HNO_3 + 2 mL of 30% v v^{-1} H_2O_2 + 3 mL of distilled-deionized water,[21] and (P2) 2 mL of concentrated HNO_3 + 6 mL of concentrated HCl + 2 mL of distilled-deionized water. The microwave-assisted heating cycle had three simple steps: *(1)* ramp to 200 °C for 15 min, *(2)* hold at 200 °C for 15 min, and *(3)* cool down to room temperature for 15 min. The resulting solutions were filtered through a polyethersulfone membrane filter with 0.45 µm-dimeter pores (Whatman Inc., Clifton, NJ, USA), and their volumes were completed to 50 mL with distilled-deionized water.

Before trace element determination, P1 and P2 extracts were further diluted 2.5- and 4-fold, respectively, to reach a 4% v v^{-1} acid concentration. A 100-fold dilution was adopted for ICP OES determination of macro-elements such as Ca, Fe, K, Mg and P, which were found at high concentrations in the biochar samples. The remaining solids after extraction of one of the hog waste biochar samples (B1) were analyzed by X-ray diffraction to identify their composition and evaluate the efficiency of the sample preparation procedure.

Accuracy evaluation

Addition and recovery experiments were performed to evaluate the accuracy of the extraction method based on P1. The results were also compared with the aqua regia-based extraction procedure (P2). Approximately 3 g of hog waste biochar (B1) were spiked with 6 mg of Al, Cu, Mn, Na, S and Zn; 0.3 mg of Ba, Cr, Ni, Sr and Ti; 0.06 mg of Mo, and 0.03 mg of As, Co and Pb. The spike values were chosen based on the respective analyte concentrations found in the biochar samples.

It is important to note that the hog waste biochar sample was selected for accuracy evaluation due to its greater complexity compared to other commercial samples (plant-based, wood, or pistachio shell). Due

to the sample's hydrophobicity, the spiking solution was carefully placed in a well made by the analyst in the powdery sample. The main goal of this procedure was to prevent the spiking solution from touching the glass crucible to avoid any potential analyte loss. A preliminary study using ultrapure water was carried out to determine the maximum volume of spiking solution to be added to the sample without overflowing the well, which was found to be 0.9 mL. Considering the limitations of this maximum value, and the volume of spiking solution required to reach the desired analyte mass, the analytes were divided into five groups: (1) 0.6 mL of Mo 100 mg L^{-1} and 0.3 mL of a solution containing As, Co and Pb at 100 mg L^{-1} each; (2) 0.3 mL of single-analyte solutions of Ba, Cr and Ni at 1000 mg L^{-1}; (3) 0.3 mL of single-analyte solutions of Sr and Ti at 1000 mg L^{-1}; (4) 0.3 mL of single-analyte solutions of Al, Cu and Mn at 20 000 mg L^{-1}, and (5) 0.3 mL of single-analyte solutions of Na, S and Zn at 20 000 mg L^{-1}. Note that there was no instance were the total volume of spiking solution was greater than 0.9 mL.

Two crucibles were used for each drying/ashing run, *i.e.*, crucible 1 had a solid sample with the spiking solution, and crucible 2 had the sample with ultrapure water. The volumes of spiking sample and ultrapure water were paired for each run. At the end of the drying/ashing process, the sample was homogenized (thoroughly mixed using porcelain mortar and pestle) before microwave-assisted extraction. Figure S1 (Supplementary Material) shows pictures of the biochar sample at each step of the spiking experiment.

RESULTS AND DISCUSSION

Biochar's ash content

The ash content of the samples based on the drying/ashing program were 65.6 ± 1.5%, 68.0 ± 3.0%, 2.1 ± 0.1%, 4.0 ± 0.2%, 1.4 ± 0.1% and 3.6 ± 0.3% (n=3) for hog waste biochar 1, hog waste biochar 2, Yield Titan Premium, Char Bliss, Wakefield, and Persist, respectively. Analyte concentrations in the original biochar sample were calculated considering these values.

Limits of detection and quantification

Table III presents the limit of detection (LOD), and limit of quantification (LOQ) values calculated for each analyte following the sample preparation method based on microwave-assisted extraction with diluted HNO_3 + H_2O_2 (P1). These values were compared with those obtained for a similar extraction using aqua regia (P2). The LODs and LOQs were calculated according to IUPAC recommendations using the standard deviation of the instrument response for ten consecutive measurements (n = 10) of the blank solution (S_{blank}), and the EC calibration curve slope (m), where LOD = 3(S_{blank})/m, and LOQ = 10(S_{blank})/m.[24] The blank for each method corresponds to a solution containing the respective mixture of extractants without adding biochar, *i.e.*, 5 mL of concentrated HNO_3 + 2 mL of 30% v v^{-1} H_2O_2 + 3 mL of distilled-deionized water for P1, and 2 mL of concentrated HNO_3 + 6 mL of concentrated HCl + 2 mL of distilled-deionized water for P2. Blanks went through the same microwave-assisted extraction and filtration procedure as the samples.

The LODs (and LOQs) were in 0.006-3000 mg kg^{-1} (0.02-8600 mg kg^{-1}), and 0.02-2000 mg kg^{-1} (0.05-5500 mg kg^{-1}) ranges for P1 and P2, respectively. Except for K and P, the method based on diluted nitric acid provided generally lower values compared to aqua regia extraction.

Table III. Limit of detection (LOD) and limit of quantification (LOQ) for biochar analysis (n = 10). Values are reported in mg kg^{-1}.

Instrument	Analyte	HNO_3 + H_2O_2 (P1)		Aqua regia (P2)	
		LOD	LOQ	LOD	LOQ
ICP OES	Al I	2	6	2	8
	Ba II	0.1	0.4	0.2	0.6
	Ca II	3	9	6	20

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Table III. Limit of detection (LOD) and limit of quantification (LOQ) for biochar analysis (n = 10). Values are reported in mg kg^{-1}. (continuation)

Instrument	Analyte	$HNO_3 + H_2O_2$ (P1)		Aqua regia (P2)	
		LOD	LOQ	LOD	LOQ
ICP OES	Cr II	0.9	3	1	4
	Cu I	1	3	1	4
	Fe II	200	650	200	500
	K I	3000	8600	2000	5500
	Mg II	2	8	2	6
	Mn II	0.1	0.3	0.2	0.5
	Na I	10	30	40	120
	Ni II	4	10	7	20
	P I	400	1200	200	730
	S I	40	130	70	220
	Sr II	0.02	0.05	0.02	0.07
	Ti II	0.5	2	0.6	2
	Zn I	0.8	3	0.9	3
ICP-MS	As	0.007	0.02	0.04	0.1
	Co	0.01	0.03	0.02	0.05
	Mo	0.02	0.07	0.04	0.1
	Pb	0.006	0.02	0.02	0.06

I- atomic line; II- ionic line.

Accuracy

The accuracy of the microwave-assisted extraction method using diluted HNO_3 and H_2O_2 was evaluated by addition and recovery experiments using a hog waste biochar sample and the results are shown in Table IV. Arsenic, Co, Mo and Pb were determined by ICP-MS, while the other elements were determined by ICP OES. Except for Al (114%) and Sr (89.6%), analyte recoveries were within the 90%-110% range for all analytes evaluated.[25] These results demonstrate that the sample preparation method described in this study presents no significant analyte contamination or loss and is an effective alternative to previously described strategies for biochar trace element determination. The relative high RSD values may be associated with the heterogeneity of the sample and the manual nature of the spiking and post-ashing homogenization processes. Note that spiking solutions were manually added into wells that were also manually excavated into the solid biochar sample. The resulting ash was then manually homogenized before 0.1 g aliquots were submitted to microwave-assisted extraction. Although precision was relatively poor for some analytes, with relative standard deviations (RSD) higher than 10%, these values may be considered adequate for such a multiple-step and complex sample preparation method.[11,26,27]

Table IV. Analyte percent recoveries for biochar extraction using diluted HNO_3 + H_2O_2. Values are the mean ± 1 standard deviation (RSD, n = 3).

Analyte	Concentration added (mg kg^{-1})[a]	Recovery (%)
Al	2000	114 ± 8 (7)
Ba	100	98.7 ± 22.6 (23)
Cr	100	91.3 ± 5.0 (5)
Cu	2000	104 ± 6 (6)
Mn	2000	103 ± 4 (4)
Na	2000	104 ± 18 (17)
Ni	100	106 ± 11 (11)
S	2000	94.2 ± 14.3 (15)
Sr	100	89.6 ± 7.0 (8)
Ti	100	97.1 ± 9.2 (10)
Zn	2000	94.6 ± 14.6 (16)
As	10	106 ± 8 (7)
Co	10	105 ± 15 (14)
Mo	20	103 ± 22 (21)
Pb	10	107 ± 15 (14)

[a]Recoveries based on 3 g of biochar spiked with 6 mg of Al, Cu, Mn, Na, S and Zn; 0.3 mg of Ba, Cr, Ni, Sr and Ti; 0.06 mg of Mo; and 0.03 mg of As, Co and Pb.

Extraction residue

As expected with a sample usually presenting a high silica content such as biochar,[28,29] a solid residue (Figure S2) was observed at the end of the microwave-assisted extraction. No HF was present in either P1 or P2, so silica-based compounds were not expected to be dissolved during sample preparation.

To investigate the composition of the small solid grains remaining after the hog waste biochar 1 extraction procedure (Figure S2), they were filtrated out of the solution and analyzed by X-ray diffraction (XRD). As observed in Figure 1, the diffraction peaks match those reported for silicon dioxide (SiO_2), which confirms that all analytes were effectively extracted by the diluted HNO_3 + H_2O_2 solution.

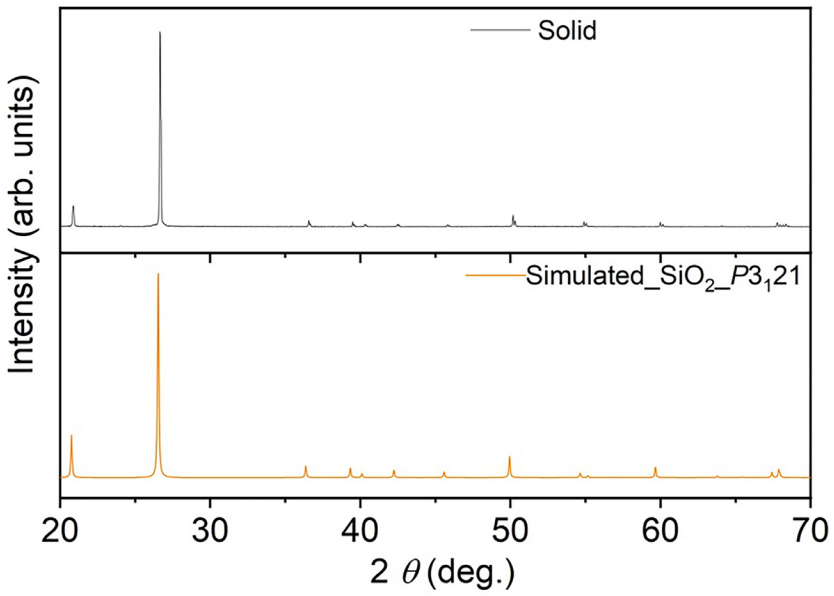

Figure 1. XRD spectra of the solid grains remaining after biochar microwave-assisted extraction.

Any analyte directly associated with silicate compounds may not be extracted using the method described here. However, if the biochar is used as fertilizer, these analytes would not be directly available to the soil's extractable fraction, and therefore, not available to plants.[23] Thus, the method based on biochar drying/ashing and microwave-assisted extraction with diluted HNO_3 + H_2O_2 is an effective strategy for evaluating the quality/toxicity of samples intended for agriculture applications.

Biochar elemental content

It is important to note that the analytes evaluated in this study were chosen based on the preliminary semi-quantitative analysis of the hog waste biochar 1 sample by ICP-MS. The semi-quantitative analysis routine, which is part of the ICP-MS instrument's controlling software, provides approximate analyte concentrations based on previously recorded and stored analytical signals. The semi-quantitative experiment helped us to focus on analytes that were in fact present in the sample evaluated. The analytes identified in the semi-quantitative analysis experiment were then quantitatively determined in the biochar sample by EC. Most elements were determined by ICP OES, with As, Co, Mo and Pb determined by ICP-MS.

Analyte concentrations found in hog waste biochar 1 using extraction methods based on diluted nitric acid or aqua regia are shown in Table V. As expected, macronutrients such as Ca, Fe, K, Mg and P, which are important for soil health and as plant nutrients,[22] were found in relatively high concentrations in this sample: 15500 - 94000 mg kg^{-1} and 11774 -136992 mg kg^{-1} for the diluted HNO_3 + H_2O_2 and the aqua regia methods, respectively. Other elements such as Al, Ba, Cr, Cu, Mn, Na, Ni, S, Sr, Ti and Zn ranged from 6.63 (Co) to 4600 (Na) mg kg^{-1} (6.1-3720 mg kg^{-1} for the aqua regia method). Toxic elements such as As and Pb were found at 10.4 and 8.42 mg kg^{-1}, respectively (8.9 and 6.2 mg kg^{-1} with the aqua regia extraction), which does not exceed the maximum limits recommended for biochar use in soil nutrition by the International Biochar Initiative, *i.e.* As (100 mg kg^{-1}) and Pb (300 mg kg^{-1}).[30]

A *t*-test at the 95% confidence level was used to compare the results from the microwave-assisted extraction method based on diluted HNO_3 + H_2O_2 with values obtained by extraction with aqua regia. Except for Ti, no statistically significant difference was observed between the two extraction methods. Boxplot representations of these results are shown in Figure S3.

Table V. Analyte concentrations found in the original hog waste biochar 1 sample following diluted HNO_3 + H_2O_2 or aqua regia extraction. Values are the mean ± 1 standard deviation in mg kg^{-1} (RSD, %, n = 3).

Analyte	HNO_3 + H_2O_2	Aqua regia
Al	2080 ± 130 (6.3)	2120 ± 520 (25)
Ba	62.1 ± 1.5 (2.4)	69.7 ± 14.5 (21)
Ca	85700 ± 3380 (3.9)	80600 ± 17400 (22)
Cr	89.8 ± 11.2 (12)	137 ± 86 (63)
Cu	548 ± 12 (2.2)	593 ± 95 (16)
Fe	94000 ± 7800 (8.3)	137000 ± 49000 (36)
K	15500 ± 500 (3.2)	11800 ± 2300 (19)
Mg	25700 ± 1600 (6.2)	21100 ± 4100 (19)
Mn	1480 ± 30 (2.0)	1820 ± 470 (26)
Na	4600 ± 200 (4.3)	3720 ± 670 (18)
Ni	78.0 ± 4.8 (6.2)	108 ± 45 (42)
P	34200 ± 1700 (5.0)	27900 ± 5100 (18)
S	3770 ± 180 (4.8)	3430 ± 570 (17)
Sr	139 ± 10 (7.2)	115 ± 28 (24)
Ti	103 ± 10 (9.7)	432 ± 65 (15)
Zn	2330 ± 170 (7.3)	2210 ± 400 (19)
As	10.4 ± 1.6 (15)	8.9 ± 3.2 (36)
Co	6.63 ± 0.32 (4.8)	6.1 ± 1.9 (32)
Mo	21.6 ± 1.8 (8.3)	23.2 ± 11.2 (48)
Pb	8.42 ± 0.93 (11)	6.2 ± 1.3 (20)

Also expected, commercial biochar samples produced from plant, wood, or pistachio shell presented lower nutrient contents compared to hog waste samples, as shown in Table VI. Macro-element concentrations ranging from 15.3 mg kg^{-1} (Na in Yield Premium) to 7900 mg kg^{-1} (K in Persist) were found. No sample exceeded the maximum limits allowed for As and Pb.[30]

Table VI. Analyte concentrations found in the original biochar samples using diluted nitric acid extraction. Values are the mean ± 1 standard deviation in mg kg^{-1} (RSD, %, n = 3).

Analyte	Hog waste 1	Hog waste 2	Yield Premium	Char Bliss	Wakefield	Persist
Al	2080 ± 130 (6.3)	1620 ± 90 (5.6)	337 ± 60 (18)	604 ± 22 (3.6)	91.8 ± 1.9 (2.1)	126 ± 5 (4.0)
Ba	62.1 ± 1.5 (2.4)	74.4 ± 13.9 (19)	9.0 ± 1.2 (13)	46.0 ± 3.3 (7.2)	25.9 ± 0.8 (3.1)	18.1 ± 0.3 (1.7)
Ca	85700 ± 3380 (3.9)	131000 ± 7000 (5.3)	1410 ± 320 (23)	5940 ± 310 (5.2)	2750 ± 130 (4.7)	3810 ± 26 (0.7)
Cr	89.8 ± 11.2 (12)	28.8 ± 5.1 (18)	0.70 ± 0.04 (5.7)	7.26 ± 0.56 (7.7)	0.40 ± 0.06 (15)	4.82 ± 0.40 (8.3)
Cu	548 ± 12 (2.2)	263 ± 33 (13)	1.8 ± 0.4 (22)	27.0 ± 5.5 (20)	3.65 ± 0.22 (6.0)	14.6 ± 0.8 (5.5)
Fe	94000 ± 7800 (8.3)	38200 ± 900 (2.4)	722 ± 44 (6.1)	748 ± 34 (4.5)	285 ± 14 (4.9)	291 ± 5 (1.7)
K	15500 ± 500 (3.2)	7730 ± 340 (4.4)	368 ± 43 (12)	3720 ± 230 (6.2)	1140 ± 40 (3.5)	7900 ± 70 (0.9)
Mg	25700 ± 1600 (6.2)	15600 ± 1500 (9.6)	288 ± 38 (13)	1050 ± 60 (5.7)	575 ± 37 (6.4)	995 ± 10 (1.0)
Mn	1480 ± 30 (2.0)	611 ± 83 (14)	60 ± 12 (20)	152 ± 10 (6.6)	126 ± 2 (1.6)	76.0 ± 2.5 (3.3)
Na	4600 ± 200 (4.3)	2500 ± 200 (8.0)	15.3 ± 1.7 (11)	1740 ± 110 (6.3)	35.9 ± 3.6 (10)	1210 ± 20 (1.7)
Ni	78.0 ± 4.8 (6.2)	27.0 ± 6.2 (23)	0.5 ± 0.1 (20)	3.0 ± 0.2 (6.7)	0.25 ± 0.03 (12)	2.77 ± 0.70 (25)
P	34200 ± 1700 (5.0)	22100 ± 2400 (11)	50 ± 12 (24)	410 ± 22 (5.4)	252 ± 16 (6.3)	1760 ± 50 (2.8)
S	3770 ± 180 (4.8)	2360 ± 200 (8.5)	31.5 ± 7.7 (24)	146 ± 10 (6.8)	66.7 ± 3.9 (5.8)	267 ± 5 (1.9)
Sr	139 ± 10 (7.2)	177 ± 9 (5.1)	6.2 ± 1.4 (23)	40.6 ± 3.0 (7.4)	12.5 ± 0.3 (2.4)	24.3 ± 0.1 (0.4)
Ti	103 ± 10 (9.7)	66.4 ± 5.0 (7.5)	7.8 ± 2.0 (26)	59.6 ± 2.0 (3.4)	3.10 ± 0.22 (7.1)	8.51 ± 0.34 (4.0)
Zn	2330 ± 170 (7.3)	1550 ± 140 (9.0)	8.4 ± 1.7 (20)	23.2 ± 3.9 (17)	4.02 ± 0.36 (9.0)	13.9 ± 0.7 (5.0)
As	10.4 ± 1.6 (15)	4.4 ± 0.8 (18)	0.083 ± 0.006 (7.2)	4.48 ± 0.52 (12)	0.017 ± 0.004 (24)	1.58 ± 0.08 (5.1)
Co	6.63 ± 0.32 (4.8)	2.2 ± 0.4 (18)	0.085 ± 0.008 (9.4)	0.49 ± 0.03 (6.1)	0.078 ± 0.001 (1.3)	0.175 ± 0.012 (6.9)
Mo	21.6 ± 1.8 (8.3)	6.2 ± 1.3 (21)	0.077 ± 0.018 (23)	0.102 ± 0.008 (7.8)	0.090 ± 0.003 (3.3)	0.130 ± 0.002 (1.5)
Pb	8.42 ± 0.93 (11)	7.4 ± 1.6 (22)	0.197 ± 0.030 (15)	2.74 ± 0.35 (13)	0.125 ± 0.008 (6.4)	0.453 ± 0.036 (7.9)

CONCLUSIONS

The increased use of biochar in several fields requires the development of reliable analytical methods that can ensure the efficient, safe, and environmentally friendly application of this material. The notorious diversity of raw materials used in biochar preparation, its high carbon content, and its intrinsic ability to absorb both nutritional and toxic elements, makes biochar sample preparation for trace element determination a challenging, but desirable task.

The method described in this study is a simpler, safer and effective alternative compared to strategies relying on concentrated and dangerous reagents (*e.g*. HF), or those based on fusion, which are prone to analyte loss and contamination. A simple drying/ashing step facilitates the decomposition of the sample's high carbon-content matrix, and the subsequent closed-vessel microwave-assisted extraction with diluted acid provides low blanks and is compliant with green chemistry precepts. It requires small volumes of reagents, which consequently generates less waste. The relatively low acidity of the digests also facilitates analyses by modern sensitive spectrochemical methods such as ICP OES and ICP-MS.

This study demonstrated the applicability of dry ashing and microwave-assisted extraction using diluted nitric acid and hydrogen peroxide for the analysis of biochar samples produced from a variety of sources. To the best of our knowledge, this is the first time a method with adequate accuracy evaluation is described for biochar sample preparation and subsequent determination of multiple trace elements. Analyte percent recoveries in 89.6-114% range were demonstrated for 15 elements, with 20 analytes successfully determined by ICP OES and ICP-MS.

Conflicts of interest

The authors declare they do not have financial or personal conflicts of interest.

Acknowledgements

The authors would like to thank the departments of Chemistry and Engineering at Wake Forest University for their support, Montauk Renewables Company for providing hog waste samples, Dr. Dundappa Mumbaraddi for his technical support on acquiring XRD spectra, and Dr. Jesse Ingham for his support on improving manuscript images.

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SUPPLEMENTARY MATERIAL

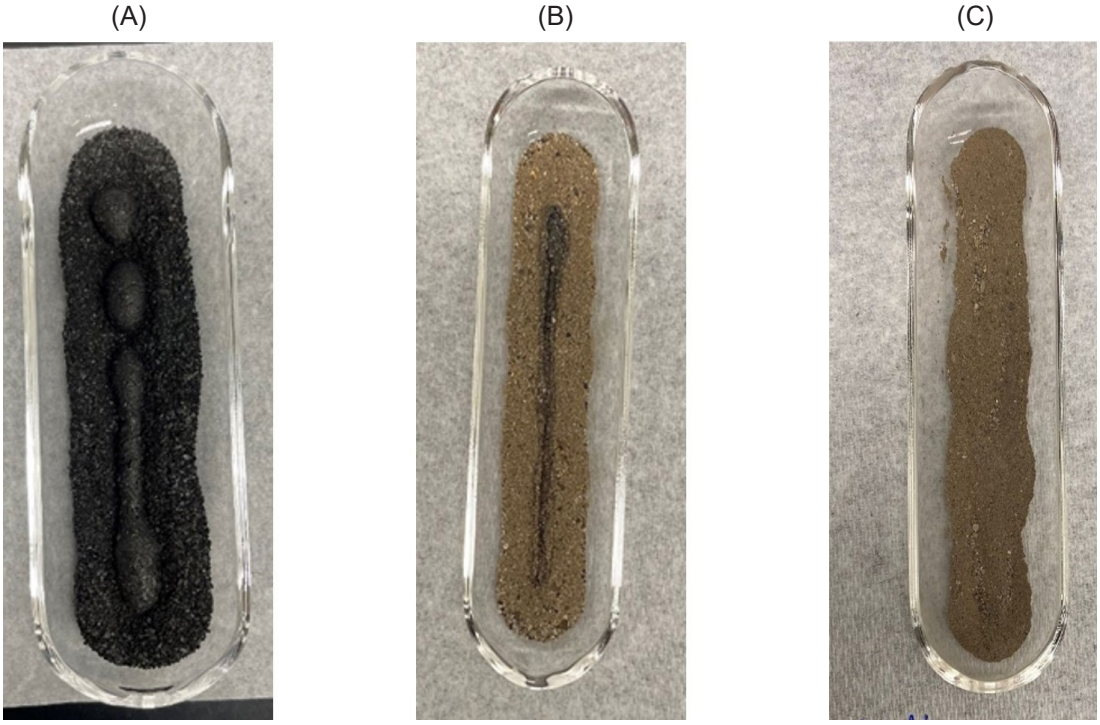


Figure S1. Drying/ashing process adopted for the addition and recovery experiment used to evaluate the accuracy of the biochar analysis method. (A) Spiking solution added to a well made into the biochar sample, (B) spiked biochar at the end of the drying/ashing process, and (C) ashed biochar after homogenization.

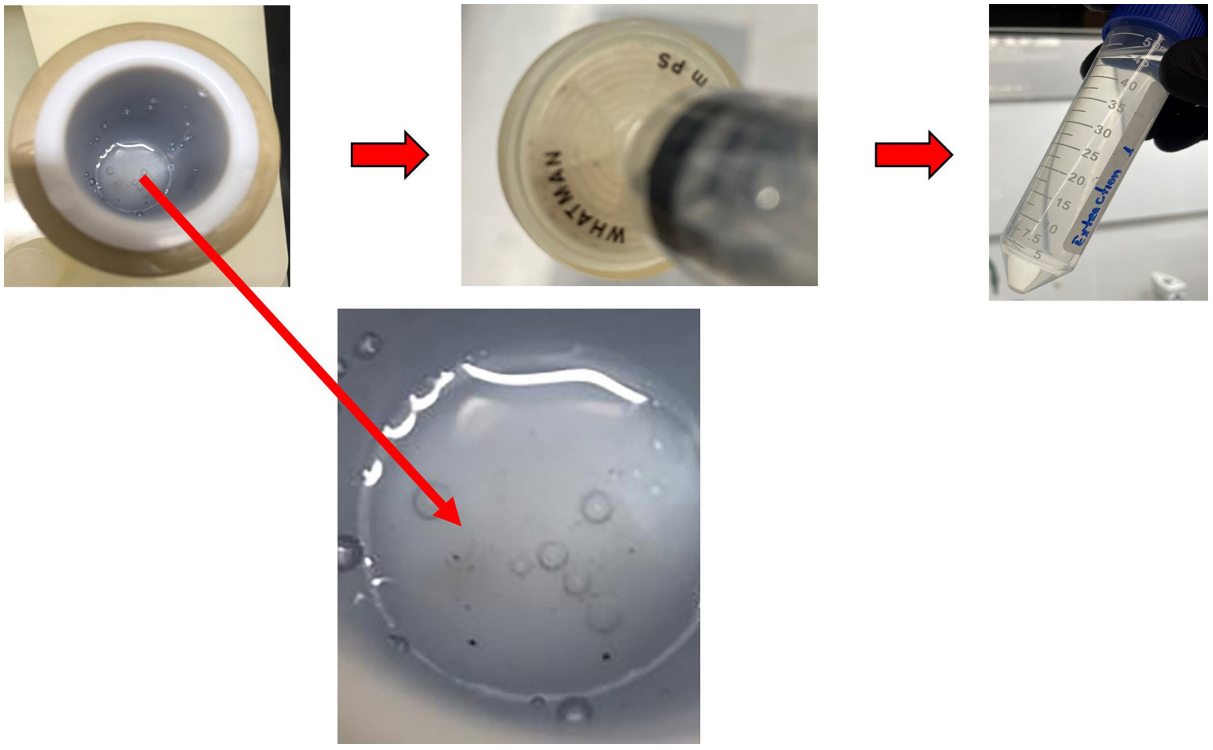


Figure S2. Filtration and dilution sequence after microwave-assisted extraction of the biochar sample. Solid residue (dark grains) remaining after the extraction procedure are shown in detail.

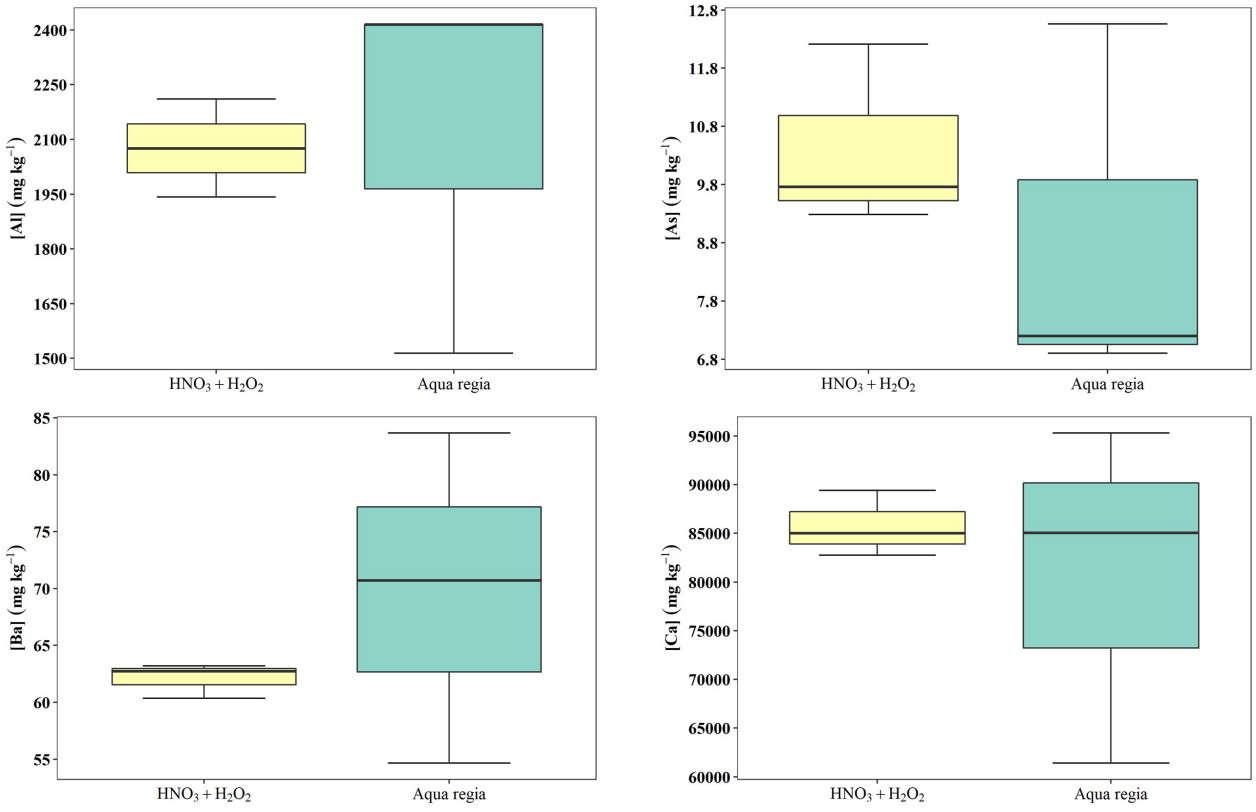
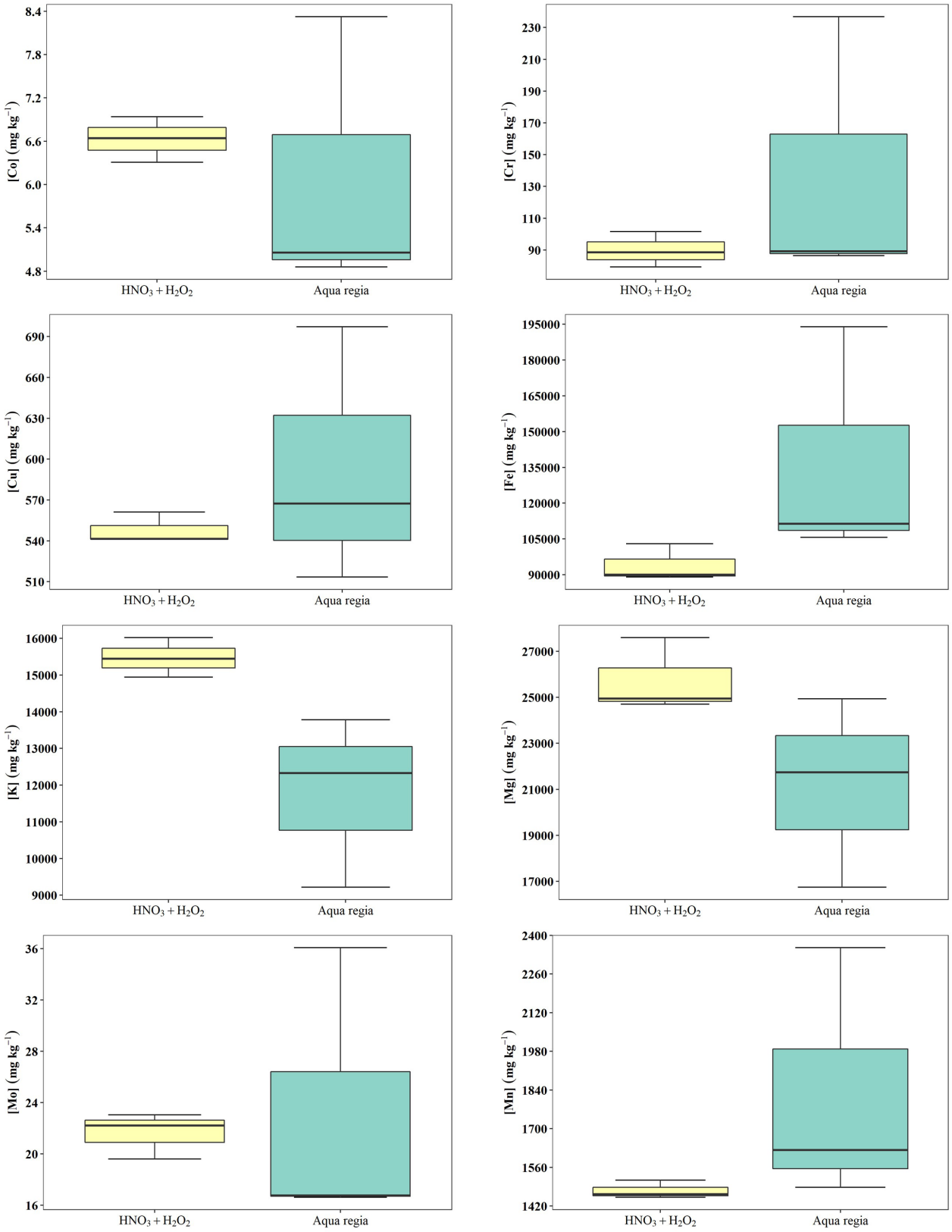


Figure S3. Boxplots showing analyte concentrations (n = 3) found in biochar following microwave-assisted extraction with diluted HNO_3 + H_2O_2 or aqua regia and determination by ICP OES and ICP-MS. (continues on next page)



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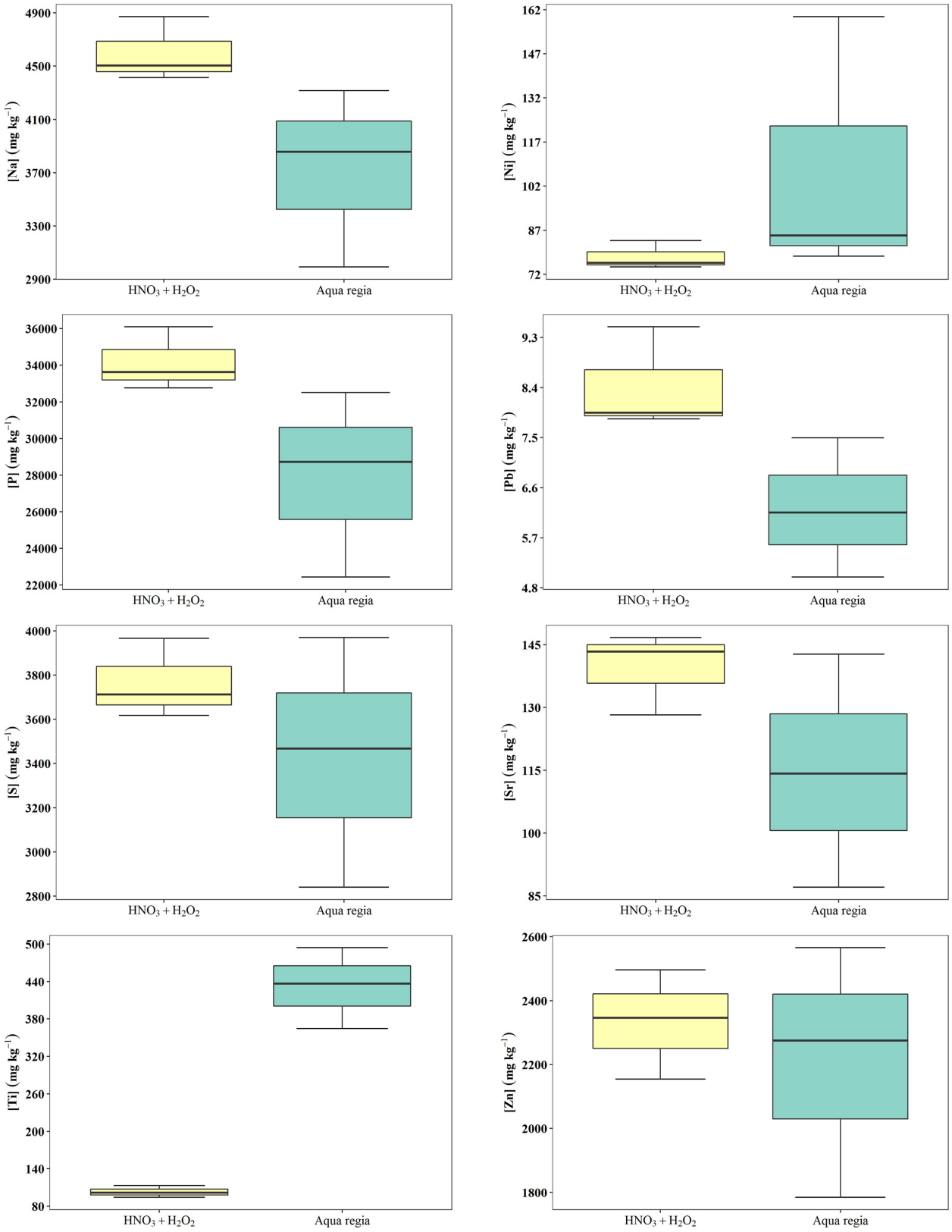

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