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Supplementary Material

Recent Trends in Sustainable Remediation of Pb-Contaminated Shooting Range Soils: Rethinking Waste Management within a Circular Economy

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Supporting material consists of two parts. Part 1 ranges over three pages from 2-4 and involves a brief description of materials and methods used to conduct the practical experience presented in the main manuscript (Section 5). Part 2 contains a single table (Table S1, page 5) with the main chemical characteristics of studied amendments.

SM-Part 1. Materials and methods of Section 5 of the main manuscript entitled "Assessing the effectiveness of agro-industrial wastes as amendments for remediation Pb-contaminated shooting range soil. A practical experience in the Basque Country."

Microcosm experiment set-up

First, a microcosm experiment was conducted to investigate the effects of different soil amendments on the extractable fraction of Pb and to adjust the design of field experiment. Soil samples were collected from the surface layer (0-20 cm) of at an outdoor shooting range located in Oiartzun, Basque Country, Northern Spain (43º18′50.4′′N,1º51′53.6′′O). Subsequently, freshly collected samples of soil were air-dried, crushed to pass through a 4-mm sieve and stored in closed plastic bags until their use. Fragments of the bullets were manually removed. The field site exhibits a sandy loam (Gleic Luvisol) soil type (USDA-NRCS, 2019) [1]. The particle undersize 2 μm was determined by the sedimentation method (NEN 5753 procedure) [2] and found to be 20.4% (w/w). Soil pH was 5.2 (1:2.5 w/v soil:water ratio) and organic matter was around 5% (w/w) (using a gravimetric method according to NEN 5754 procedure [3]).The total concentration of Pb in tested soil was 2,058 ± 352 mg kg^{-1}, as determined by the ICP-AES following USEPA method 3051A [4]. The total Pb concentration in soil exceeded maximum allowable concentration for agricultural soils established by the legislation of the Basque Country (Law 4/2015 for the prevention and correction of soil contamination) [5], as well as those set out by Spanish (Law 22/2011 on waste and contaminated soils) [6] and European legislation (Directive 2004/35/EC) [7].

Amendments of spent mushroom substrate (SMS), biochar (BC) and bone meal (BM) were selected to assess their effectiveness on Pb immobilization in a shooting range soil. Spent mushroom substrate, BC and BM were kindly supplied by Mushroom Research Technological Centre (CTICH, Autol, La Rioja), Palaterra mbH (Hengstbacherhof, Germany), and Ecoforce S.L. (Oliva, Valencia), respectively. The SMS is a by-product of edible mushroom production, which annual output is over 830,000 tons in Spain (calculated on the basis of official production data for the year 2019 [8] and knowing that for every kilogram of mushrooms produced results in approximately 5 kg of by-product [9]). The SMS from *Pleurotus* sp. cultivation used in the present study was a composed of cereal (wheat) straw subjected to a pasteurisation process. Prior its application to soil SMS was manually ground to pass through a 4-mm sieve. Commercially available BC was produced from forest wood residues pyrolysed at 700 °C. The BM was prepared from cow bones after removing impurities by boiling water. Both BC and BM were grinded into homogenized powder of <1.0 mm particle size. The main characteristics of the amendments are shown in Table S1 (Part 2 of Supplementary Material).

The following four treatments were established in quadruplicate viz., untreated control soils (without amendment) (CTRL), and soils amended with SMS or BC or BM. Each amendment was applied at 5% weight basis of the contaminated soil. Specifically, 50 g of 4-mm sieved and air-dried soil was mixed thoroughly with 2.5 g of each amendment in a plastic pot. Then, pots were randomly placed in a growth chamber at 23 °C/18 °C (day/night) and 16/8 h (light/dark) photoperiod cycling using a fluorescent light for 7 days. Soil samples were collected from each pot just before the application of amendments and 7 day after, oven dried at 60 ºC and sieved to <0.125 mm.

The total Pb was extracted from the soil by acid digestion in a microwave oven (CEM Mars Corporation), as outlined in the USEPA 3051 method (2007) [4]. The 0.01 M $CaCl_2$-extractable of Pb, operationally defined as bioavailable fraction, was determined as described by Houba et al. (2000) [10]. Blank samples were included in all the analyses to verify any possible cross-contamination. Quantifications were performed by using ICP-MS (7700x, Agilent Technologies, Palo Alto, CA, USA), at the SCAB-SGIker facility of the University of the Basque Country (Spain). Soil pH was measured in mixture soil-deionized water (1:2.5 w/v soil:water ratio).

Field experiment set-up

A short-term field experiment was conducted in the shooting range in July 2019. Mean values of total and extractable Pb concentrations in soil were 1812 ± 90 mg kg^{-1} and 13.1 ± 1.4 mg kg^{-1}, respectively. The region has a temperate climate, with mean annual temperature of 12.5 °C and mean annual precipitation of 2,000 mm (climate data from Spanish Meteorological Agency –AEMet–). A preliminary vegetation analysis showed the predomination of monocots belonging to the genera Agrostis, Lolium or Festuca, and dicots as *Plantago lanceolata* and *Trifolium repens* L.. On a landscape scale, the field had low-intensity management regime and had not been cultivated before. Prior to the establishment of the experiment, field was mowed and, thereafter, the below-ground plant biomass was plowed back into the soil.

The experimental design included three treatments: (i) an unamended control; (ii) BC application and (iii) BM application, given that these two amendments resulted the most effective in immobilizing Pb in the soil of the microcosm experiment. Three plots (0.36 m^2) were produced for each treatment. Biochar and BM were applied into the soil at a rate of 5% (w/w), respectively, and later integrated to a depth of 20 cm by manual plowing. This resulted in a relatively uniform distribution of amendments to a depth of approximately 20 cm. A total of nine randomly taken soil cores (away from the edge of experimental plot) from a depth of 0 to 15 cm were collected and mixed at 0, 7 and 17 days after application of amendments. After removing plant debris and gravels, the soil from each sample was air-dried, crushed and passed through a 0.125 mm sieve. Soil pH was measured in mixture soil-deionized water (1:2.5 w/v soil:water ratio). Total and $CaCl_2$-extractable Pb concentrations in soils were determined following the procedures mentioned in the microcosm experiment set-up. Microbial activity was determined by basal respiration (BR) following ISO 16072 (2002) [11]. Soil basal respiration is frequently used as indicator of the negative impact of metals in soil as well as of the effectiveness of the remediation processes [12].

Statistical analysis

All statistical analyses were performed using the SPSS 20.0 package (IBM SPSS Statistics for Windows, Version 20; Armonk, NY: IBM Corp.). The normality of $CaCl_2$-extractable Pb and soil pH data was assessed using the Saphiro-Wilk test, which showed that the data were not normally distributed. To elucidate significant ($P<0.05$) differences in $CaCl_2$-extractable Pb concentrations and soil pH among different amendment treatments non-parametric tests were applied. The significance ($P<0.05$) of differences over time within the same treatment was treated statistically by repeated measurement test and assessed by the post hoc Games-Howell test (under a homogenous variance assumption) or DMS test (under a non-homogenous variance assumption). Finally, the Spearman's correlation was performed to establish relationships between variables.

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SM-Part 2.

Table S1. Physico-chemical characteristics of spend mushroom substrate (SMS), biochar (BC) and animal bone meal (BM) given on a dry weight basis.

	SMS	BC	BM*
Total Carbon (%)	39.2		
Organic Carbon (%)		21.0	
Total Nitrogen (%)	0.5	0.7	
Total Phosphorus (%)	0.006 (1)	0.2	12.0
Total Potassium (%)	0.4 (2)	1.3	0.1
pH in water (1:2.5, w/v)	7.6	10.0	6.6
Organic matter (%)	41.5	68.8	

(1) Available Phosphorus; (2) Available Potassium.

* Data acquired from the web page of the supplier.