International Journal of Environmental Science and Technology
https://doi.org/10.1007/s13762-022-04000-6

ORIGINAL PAPER

Insights of microorganisms role in rice and rapeseed wastes as potential sorbents for metal removal

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Received: 5 August 2021 / Revised: 20 January 2022 / Accepted: 25 January 2022

Abstract

Rice and rapeseed agricultural wastes, as nonliving biomass, are proposed for heavy metal remediation in polluted effluents (chromium, cadmium, copper and lead). The physicochemical characterization of these biomasses shows that the surface of both sorbents is negatively charged (zeta potential), the surface area of sorbents is 4.39 and 40.7 (Brunauer–Emmett–Teller), and the main functional groups are carboxylic and hydroxyl (attenuated total reflection Fourier-transform infrared spectroscopy). The main purpose of this work is to evaluate the insights of microorganisms associated with these nonliving biomasses in the removal of heavy metals from synthetic aqueous solutions, adjusted at pH 4.0 (as the best acidic condition for the sorption process). The isolates *(Bacillus* genus in rice and *Escherichia, Micrococcus* and *Staphylococcus* genus in rapeseed) remove heavy metals from mentioned solutions, mainly in consortia, with contribution percentage over than 80% of total metals. In addition, when they are present in biomass, they provide an additional metal removal effect, especially in rapeseed biomass system and with multiple heavy metals aqueous solutions: i.e. Cr(III) removal, at 4 mmol/L, increases from 70 to 100%. This knowledge makes possible the use of the nonliving biomasses with no need for any special pretreatment against the microorganisms, prior to their use as metal sorbents that implies their good feasibility for application from an economical point of view.

Keywords Biomass waste valorization · Sorption · Uptake heavy metals · Bacteria

Introduction

Heavy metal (HM) pollution has mainly become a serious threat to the environment due to anthropogenic activities (Bhattacharya et al. 2015). This increase in attention is due to the high toxicity, persistence in sediment and accumulation in biological tissues of HMs (Martín et al. 2015). HMs can be incorporated into the food chain, which can lead to gastrointestinal, neurological, haematic and renal diseases, among others (Kim and Kan 2016; Zhao et al. 2020). Consequently, the world is facing a critical water quality problem, which is threatening to animals and human beings (Azizullah et al. 2011). Relatively modest concentrations of Cr(III), Cd(II) and Pb(II) have toxic effects on the environment and humans. Cu(II) is also a potential toxicant at high doses (Tchounwou et al. 2012).

The main heavy metal pollutants and their main sources have included fertilizer leaching, sewage discharge, industrial wastewater and urban construction over the last decades (Nguyen et al. 2013; Addel-Ghani and El-Chaghaby 2014; Luo et al. 2017). In particular, the pollution of water

Editorial responsibility: Maryam Shabani.

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Published online: 19 February 2022

resources by HMs is an alarming issue in China, where large amounts of HMs have been found in groundwater and drinking water (Dai et al. 2018).

Conventional physicochemical techniques for treating effluents laden with HMs include ion exchange, solvent extraction, electrochemical treatment and membrane filtration technologies (Yin et al. 2019). These methods are usually too expensive and not eco-friendly due to the liberation of toxic chemical sludge that can cause secondary pollution (Carolin et al. 2017; Kumar and Khan 2021). Therefore, using low-cost biosorption approaches for the removal of harmful heavy metals from water resources and wastewaters is one of the most important goals in environmental science (Zhao et al. 2019; Zhang et al. 2020; Zhao et al. 2020). One of the types of biosorption HM removal technologies, which consists of the use of nonliving biological materials, has recently emerged as a cost-effective, eco-friendly and simple alternative to remove toxic HMs from water or to render these pollutants harmless (Senthil Kumar and Gunasundari 2018; Calderón et al. 2020). Therefore, nonliving and metabolically inactive biomaterials from plants have been increasingly studied (Jain et al. 2015; Zhao et al. 2020). In particular, rice (*Oryza sativa L.,* RO), which is the staple food for more than 50% of the world's population (Zhou et al. 2014), and rapeseed (*Brassica napus L.,* LO), which is an oilseed crop and the second largest source of vegetable oil (FAO 2014), are both widely planted in Hunan Province, China. RO and LO have been widely studied in environmental fields, in which RO and LO crop varieties have been planted in contaminated soils for heavy metal uptake and soil remediation (Cárdenas-Aguiar et al. 2020; El-Hassanin et al. 2020). After harvesting, nonliving RO and LO residues (biomass), such as their husks (Zhan et al. 2020) and straw compost (Yu et al. 2020), have been studied as potential adsorbents for the removal of heavy metals from contaminated water. Otherwise, it is largely known that living organisms (bacteria and others) are generally exposed to mixtures of metals in the environment (Vijver et al. 2011; Mebane et al. 2020). Hence, bacteria show resistance mechanisms such as extracellular sequestration, intracellular sequestration, active export and enzymatic detoxification, which help them interact with metals and tolerate their toxicity and deleterious effects (Yin et al. 2019; Villagrasa et al. 2021).

Despite the extensive information mentioned above, nothing is known about the role of RO- and LO-associated microorganisms. Consequently, the aims of this work are (i) to quantify the HM sorption potential of nonliving RO and LO biomass in an HM-contaminated aqueous solution, (ii) to determine the identity of RO and LO biomass-associated microorganisms and (iii) to unravel their role in the HM sorption process. Thus, to discuss the role of isolated microorganisms in the HM sorption process, a multiple analytical approach was followed by using different techniques: (i) attenuated total reflection Fourier-transform infrared (ATR-FTIR), zeta potential and Brunauer–Emmett–Teller (BET) techniques are employed to identify the properties of the assayed nonliving biomass (type of functional groups and electronic charge present on the surface of materials along with the area, volume and morphology of materials), and (ii) the adsorption capabilities of the nonliving assayed biomass are compared and studied with/without microorganisms via batch adsorption experiments. With this purpose, an economical and natural alternative/substitute for the decontamination of HM-containing effluents is developed by taking advantage of RO and LO straw residues. Moreover, to the best of our knowledge, this paper is the first to describe the role of microorganisms isolated from LO and RO wastes in regard to HM sorption capacity.

Materials and methods

Materials and solutions

Rice (*Oryza sativa L.,* RO) and rapeseed (*Brassica napus L.,* LO) were provided by the Oil Crops Research Institute of the Chinese Academy of Agriculture (Wuhan, China). Both biomasses were first immersed in a clean water solution, phases were separated by decantation, and then, biomasses were ground and dried for further use in corresponding metal sorption experiments. All chemicals were of analytical grade. Stock solutions (approximately 1000 mg/L) were prepared by dissolving the required amounts of $Cr(NO_3)_3 9H_2O$, $Cu(NO_3)_2 3H_2O$, $Cd(NO_3)_2 4H_2O$ and $Pb(NO_3)_2$ (all 99% from Panreac, Barcelona, Spain).

Characterization

Physicochemical characterization

RO and LO nonliving biomasses have been properly characterized. Therefore, the physicochemical properties of the biomass, including the pH, mineral composition, surface area and surface charge, along with the identification of functional groups, can help to explain the process of metal sorption. Attenuated total reflection Fourier-transform infrared (ATR-FTIR, Tensor 27, Bruker, USA) spectroscopy was performed to identify the chemical functional groups present on the biomass wastes used as sorbents. The FTIR data were obtained from 600 to 4000 cm^{-1} with an average of 64 scans at a 4.0 cm^{-1} resolution; this characterization was performed at the Chemistry Analysis Service of our university ("*Servei d'Anàlisi de Química*") (https://sct.uab.cat/saq/es) (UAB, Catalunya, Spain). The detailed procedures to determine the biomass pH, mineral composition, surface

area and surface charge are summarized in Supplementary Material (Text S1).

Characterization of microorganisms

Strain isolation Microbial strains were isolated from the RO and LO waste plants. For bacterial growth, 0.100 g of each plant was mixed with 10.0 mL of Luria–Bertani (LB) broth medium containing tryptone (10.0 g/L), yeast extract (5.00 g/L) and sodium chloride (10.0 g/L) at pH 7.0 and then placed in an orbital shaking incubator (Infors HT, Ecotron, Switzerland) at 150 rpm and 27 ± 1 °C for 24 h. Subsequently, 100 µL aliquots of each sample were spread onto LB agar plates consisting of LB broth medium along with bacteriological agar (15.0 g/L) and incubated again for 24 h at 27 ± 1 °C.

To determine the number of colony forming units per volume (cfu/mL) in each original biomass (RO and LO), 0.1 mL of the corresponding biomass/solution suspension, which was 0.1 g of biomass in 10 mL of sterile double deionized water, was spread in triplicate onto LB agar plates. Then, the samples were incubated at 27 ± 1 °C for 24 h.

Phenotypic characterization Phenotypic characterization of the different microorganisms associated with rice and rapeseed biomass samples was performed from single colonies collected from well-isolated cultures grown on LB plates. Morphological cell characteristics were examined with a conventional Olympus BH2 (Olympus, Japan) light microscope. Gram, Wirtz–Conklin spore and capsule staining were performed according to Doestch (1981), Schaeffer and Fulton (1933) and Anthony (1931), respectively. Oxidase activity was tested employing oxidase strips (Oxoid Ltd., Basingstoke, UK). Catalase enzymatic activity analysis was performed by applying a 3% (v/v) H_2O_2 solution on each isolate to evaluate enzyme-generated oxygen bubble formation (Bergey and Holt 1994). Moreover, DNase activity and the degradation of Tween 80 were examined according to the methodology reported by Høvik Hansen and Sørheim (1991). The detection of phosphate-solubilizing microorganisms was performed following the protocol reported by Nath et al. (2017). Biochemical characterization was accomplished through BioMérieux™ Clinical Diagnostic Kits (BioMérieux®sa, Marcy-l'Etoile, France).

Further characterization was developed by analysing microorganism growth in different selective solid and/or differential media after 24 h of incubation at 27 °C. These media included Vogel–Bonner E (VBE; Southern Group Laboratory, UK), minimal medium Vogel–Bonner (MMVB; Southern Group Laboratory, UK), mannitol salt and MacConkey (Scharlau, Spain). In addition, haemophilic activity was assessed through blood agar plates (Scharlau, Spain), and anaerobic growth was assayed on LB agar plates for 1 week at 27 °C using an Anaerocult system (Merck KGaA, Darmstadt, Germany). Antibiotic resistance profiles were recovered by applying an antibiotic susceptibility test based on the Kirby–Bauer disc diffusion method (Sayah et al. 2005). Here, the tested antibiotics and their respective concentrations were as follows: ampicillin—10 µg, tetracycline—30 µg, and nalidixic acid—30 µg.

Genotypic characterization For genotypic characterization, the total genomic DNA was extracted from each isolate using a bacterial genomic DNA isolation kit (Norgen Biotek Corp, Canada). The template DNA concentration was assessed by measuring the absorbance at 260 nm using an Ultrospec 1100 Pro UV/Vis spectrophotometer (Amersham Biosciences, USA). PCR cycling was executed with a Techne FTC 3G/02 thermal cycler (Techne, USA) and conducted for 5 min at 95 °C for initial denaturing, followed by an amplification cycle, 23 cycles of 1 min at 95 °C, 1 min at 55 °C, 2 min at 72 °C and a final extension of 10 min at 72 °C. Amplicons were visualized by electrophoresis on a 1.2% agarose gel in 1 × TAE buffer (Figure S1) and further purified with Monarch® nucleic acid purification kits (New England BioLabs, USA). DNA sequencing was performed at the UAB Genomics and Bioinformatics Service ("Servei de Genòmica i Bioinformàtica") (http://sct.uab.cat/genomica-bioinformatica) (UAB, Catalunya, Spain). Universal 16S rRNA forward 27F (AGAGTTTGGATCMTGGCTCAG) and reverse 1492R (GGTTACCTTGTTACGACTT) primers were used.

To analyse the sequences of partial gene amplicons of 16*S rRNA* and to exclude ambiguous data, SEQUENCHER software version 5.4.6 (http://www.genecodes.com/) was used. The obtained sequences were then compared to those existing in the NCBI database and matched with accessible references (www.ncbi.nlm.nih.gov) through the Ribosomal Database Project at Michigan State University (RDP-II) (http://rdp.cme.msu.edu/) and BLAST software (https://blast.ncbi.nlm.nih.gov/Blast.cgi) (Cole et al. 2014). Finally, they were deposited in the GenBank sequence database.

Experimental procedures

Batch sorption experiments

Sorption experiments were carried out at room temperature (25 ± 1 °C). A multimetal solution composed of 0.18 mmol/L solutions of Cr(III), Cd(II), Cu(II) and Pb(II) was prepared. When conducting the isotherm sorption experiments for single-metal systems, the initial heavy metal concentrations ranged from 0.05 to 6 mmol/L.

Batch sorption experiments were performed with the following steps: 25 mg of each sorbent was placed in 5 mL Falcon tubes, and then, 2.5 mL of a heavy metal aqueous

solution was added into the tube. Later, samples were placed in a rotary mixer (CE 2000 ABT-4, SBS Instruments SA, Barcelona, Spain) and shaken at 25 rpm for 24 h. The two phases were separated by decantation, and later, the aqueous phase was filtered through 0.22 µm Millipore filters (Millex-GS, Millipore). Finally, inductively coupled plasma-mass spectrometry (ICP-MS, XSERIES 2 ICP-MS, Thermo Scientific, USA) was used to analyse the final concentration of heavy metals in the supernatant phase.

The sorption/adsorption of the selected heavy metals by the sorbents was expressed as the sorption percentage, which was calculated according to Eq. (1). Furthermore, the capacity of the sorbent was calculated with Eq. (2) (Zhao et al. 2019):

$$\%\ \text{Sorption/Adsorption} = \frac{(C_0 - C_e)}{C_0} \times 100 \quad (1)$$

$$q_e = \frac{(C_0 - C_e)\ \times\ V}{m} \quad (2)$$

where q_e (mmol/g) is the sorption capacity of heavy metals, V (L) is the volume of the heavy metal solution, C_0 and C_e are the initial and equilibrium heavy metal concentrations in solution (both in mg/L), respectively, and m (g) is the dry weight of the sorbent. All the results are expressed as the mean value of minimum duplicate measurements, and the standard deviation (SD) was used to analyse data errors.

Sorption experiments were carried out to optimize the working conditions, including the influence of pH, initial metal concentration and contact time. Furthermore, kinetics and isotherm models were chosen to study the sorption process. The pseudo-first-order (PFO), pseudo-second-order (PSO) and Elovich models (Wang et al. 2015) are typically used to describe sorption processes, while the Langmuir and Freundlich isotherm models are typically used to describe the sorption mechanism for the interaction of heavy metal ions on a sorbent surface. The above-mentioned information details can be found in Supplementary Material (Text S2).

Additionally, experiments with sterilized biomasses were performed as described above. A complete autoclave cycle (Raypa, Terrassa, Spain) was performed for 1 to 1.5 h at 121 °C to eliminate live microorganisms, thereby allowing the uptake potential of sterile biomass to be tested. Therefore, sorption experiments were prepared with common biomasses and sterile biomasses, and the roles of the biomass-associated microorganisms in the metal uptake process were evaluated.

Exposure of isolated strains to a heavy metal solution

For each individual microbial strain, an aliquot of 2.00 of each strain culture (OD_{600} = 1.2–1.4) grown on LB liquid medium for 24 h at 27 °C was inoculated into 18.0 mL of LB broth medium containing 0.18 mM of a heavy metal mixture (Cr(III), Cd(II), Cu(II) and Pb(II)). For consortia, aliquots of 0.5 mL of individual strain were inoculated according to their isolation source into 18.0 (RO) or 18.5 (LO) mL of the above-mentioned LB mixed metal solution. Therefore, two microbial consortia (corresponding to each biomass) were formulated by mixing equal proportions of the pure cultures. Then, the cultures were placed in an orbital shaking incubator (150 rpm) for 24 h at 27 °C.

After 24 h, the samples were centrifuged (Centrifuge 5430, Eppendorf, Germany) at 8000 rpm for 15 min. Finally, the solution was analysed by using ICP-MS (XSERIES 2 ICP-MS, Thermo Scientific, USA) to analyse the concentrations of heavy metals in the supernatant phase.

Table 1 Comparison of the adsorption capacity of metal ions from multiple metal systems (initial concentration of 0.18 mmol/L of Cr(III), Cu(II), Cd(II) and Pb(II)) by different adsorbents under the same experimental conditions ($T = 25 \pm 1$℃, 25 mg of adsorbent, 2.5 mL of solution and a contact time of 24 h)

Adsorbents	Cr(III) $q \times 10^3$ (mmol/g)	Cu(II) $q \times 10^3$ (mmol/g)	Cd(II) $q \times 10^3$ (mmol/g)	Pb(II) $q \times 10^3$ (mmol/g)
Rice	9.81 ± 0.01	9.75 ± 0.01	10.5 ± 0.1	8.92 ± 0.01
Rapeseed	7.24 ± 0.01	3.78 ± 0.01	6.76 ± 0.01	8.92 ± 0.01
Pine	7.35 ± 0.01	4.02 ± 0.01	1.48 ± 0.01	9.09 ± 0.01
Poplar	9.41 ± 0.01	7.04 ± 0.01	1.93 ± 0.01	7.04 ± 0.01
Corn	11.8 ± 0.1	10.1 ± 0.1	4.87 ± 0.01	1.78 ± 0.01
Coffee shell	2.48 ± 0.01	4.61 ± 0.01	0.75 ± 0.01	20.1 ± 0.1
Sugar cane	2.46 ± 0.01	3.41 ± 0.01	0.52 ± 0.01	9.59 ± 0.01
Cork	7.98 ± 0.01	5.92 ± 0.01	2.53 ± 0.01	11.5 ± 0.1
Coffee grounds	3.48 ± 0.01	7.10 ± 0.01	0.14 ± 0.01	10.8 ± 0.1

Results and discussion

Taking into account previous research with other biomass materials (Dai et al. 2018), different agricultural wastes are compared in Table 1 in terms of their sorption capacities for the removal of HMs from multiple metal aqueous solutions containing Cr(III), Cu(II), Cd and Pb(II). Pine, poplar, coffee shell, sugar cane and coffee grounds all show high sorption capacities for Cr(III), Cu(II) and Pb(II) and low sorption capacities for Cd(II), as seen from the collected results (Table 1). In the case of corn wastes, it shows high sorption capacities for Cr(III), Cu(II) and Cd(II) and low sorption capacities for Pb(II). It is worth noting that the prepared RO and LO samples show good sorption capacities for all metal ions compared with the other sorbents listed here. In particular, the case of Cd(II) is highlighted because both

Table 2 Total concentrations of the minerals and heavy metals (mg/Kg) along with the zeta potential and BET results of rice (RO) and rapeseed (LO) biomass

	Fe	Mn	Ni	Cu	As	Pb	Mg	*P*	*K*	ZETA	BET
RO	6,291	2,348	70.5	747	15.2	1,691	2,051	25,108	28,700	−12.4	4.39
LO	6,410	208.1	40.5	229	15.5	568.7	554.0	24,280	37,900	−1.44	40.7

sorbents (RO and LO) show higher affinity for Cd than the majority of other biomass materials.

In this work, the authors wanted to distinguish between the nonliving biomass and the living microorganism systems that are present in them.

Nonliving biomass characterization

The concentrations of some minerals (P, K and Mg) and major metals (Fe and Mn) in the rice (RO) and rapeseed (LO) biomass materials are listed in Table 2. RO and LO have similar concentrations of some minerals. However, they differ in the contents of some others, such as Mn and Mg, with RO having higher concentrations of these metal ions than LO; thus, RO has different physicochemical properties (Wang et al. 2015; Zhao and Zhou 2019). These differences have not been related to differences in the corresponding treatment of crops but can have an understandable role in the sorption process of each biomass waste system.

Table 2 also shows the zeta potential and BET values of RO and LO. Both biomass materials show negative zeta potential values, so a negative charge on their surface probably facilitates the sorption of cation metal ions via electrostatic attraction (Plank and Christian 2007). In addition, the difference in surface area of sorbents obtained from BET results gives enhanced surface area and a higher number of possible sorption sites for heavy metals (Sčíban et al. 2007) for LO in comparison with RO.

ATR-FTIR analysis was carried out to identify the functional groups present in the RO and LO biomass wastes that might be involved in the biosorption process. In Fig. 1, the FTIR spectra display several absorption peaks, indicating the complex nature of the examined biomaterials. As mentioned previously, cellulose, hemicellulose and lignin are the main components of agricultural straw biomass (Ghaffar and Fan 2014), and their functional groups (such as carboxylic and hydroxyl groups) can be found in the present biomasses (RO and LO) used as sorbents, which may participate in the proposed metal sorption processes (Zhao et al. 2020).

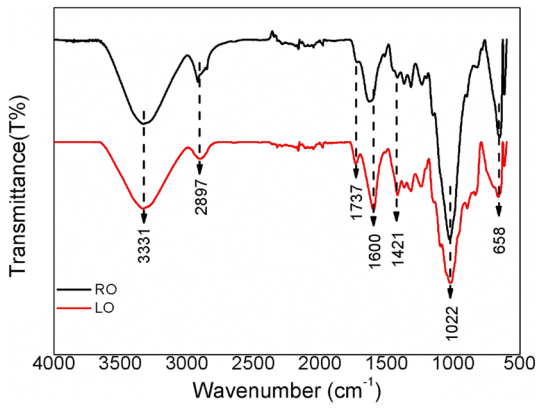

Fig. 1 ATR-FTIR spectra of RO and LO

Microorganism identification

Phenotypic and genotypic characterization

Through phenotypic analysis, a total of 8 microorganisms associated with the rice (R01, R02, R03 and R04) and rapeseed (L01, L02, L03 and L04) biomass samples were differentiated. A summary of the isolated strain characterization is shown in Table 3. After the colonies were grown on LB agar plates for 24 h at 27 °C, a preliminary phenotypic characterization of strains was performed, including macroscopic trait observations and cellular examination with optical microscopy. On the one hand, R01, R02, R02 and R04 present a similar macroscopic appearance, forming smooth, circular, nonpigmented and very mucoid colonies. On the other hand, L01 and L02 formed dry, nonpigmented, opaque and mucoid colonies, while L03 formed small, raised and white-coloured colonies, and L04 formed circular and convex colonies showing a bright yellowish pigment. Regarding microscopic cellular features, R01, R02, R03 and R04 are short, rod-shaped and gram-positive; additionally, all of them, except R03, produce spores. In contrast, L01 and L02 are rod-shaped, gram-negative and non-spore-forming bacteria. L03 and L04 are gram-positive and non-spore-forming cocci. In the first strain, cells are organized in grape-like clusters, while they are arranged in tetrads in the second strain. They all showed different patterns of motility and capsule formation. To further characterize the isolated strains, a physiologic and metabolic analysis was developed.

Table 3 Phenotypic and genotypic characterization of the microorganisms isolated from the rice and rapeseed biomass wastes

Phenotypic characterization							
Characters	RICE				CANOLA		
Cell morphology	Rod-shaped	Rod-shaped	Rod-shaped	Rod-shaped	Rod-shaped	Cocci in grape-like clusters	Cocci arranged in tetrads
Gram stain	+	+	+	+	−	+	+
Spore formation	+	+	−	+	−	−	−
Motility	+	−	−	+	+	−	−
Capsule formation	−	−	−	+	+	−	−
Enzymatic activity							
Catalase	+	+	+	+	+	+	+
Oxidase	+	+	−	−	−	−	+
H_2S production	−	−	−	−	−	−	−
Urease	−	−	−	−	−	−	−
Indole production	−	−	−	−	+	−	−
DNase	+	−	+	+	−	+	+
Tween-80	+	−	−	+	+	−	−
Phosphate solubilization (mm)	10	8	5	3	8	−	−
Antibiotic resistance							
Ampicilin	S	R	R	R	R	S	R
Tetracycline	R	R	R	S	R	R	R
Nalidixic Acid	R	R	R	R	R	R	R
Genotypic characterization							
Strain	*Bacillus sp.* R01	*Bacillus sp.* R02	*Bacillus sp.* R03	*Bacillus sp.* R04	*Escherichia coli* L01 *Escherichia coli* L02	*Staphylococcus sp.* L03	*Micrococcus sp.* L04
Accession number	MK94285	MK934695	MK934698	MK937732	MK940239 MK937745	MK942713	MK942714

All strains were positive for catalase, while only R01, R02 and L04 were positive for oxidase. R01, R02, R03, R04, L01 and L02 produce β-haemolysis, whereas L03 and L04 induce an α-haemolytic response. All isolates exhibited no H_2S production and were urease negative, and only L01 and L02 were positive for indole production. Moreover, all of them reveal distinct responses to DNA and Tween 80 (a non-ionic surfactant) hydrolysis (Table 3). R01, R02, R03 and R04 are facultative anaerobes that develop a chemoorgano-heterotrophic metabolism, and L01, L02, L03 and L04 are strictly aerobic bacteria. Regarding their growth on minimal, selective and differential media, R01, R02, R03 and R04 were able to grow on mannitol salt agar and MMVB medium but not on MacConkey agar or VBE medium. In the case of rapeseed-isolated microorganisms, L01 and L02 grew on MacConkey agar and MMVB, while L03 and L04 grew only on mannitol salt. The antibiogram results indicate that they are all resistant to nalidixic acid, but they show different susceptibility patterns in regard to tetracycline and ampicillin (Table 3). Last, rod-shaped strains from both the rice and rapeseed biomasses present the ability to solubilize inorganic phosphorus from insoluble compounds. This capacity, which is not detected with rapeseed coccoid strains, is considered one of the most relevant traits associated with plant phosphate nutrition by rhizosphere microorganisms (Alori et al. 2017).

For genotypic characterization, the DNA band of each strain obtained from PCR cycling was visualized in a 1.2% agarose gel before being further purified and sequenced (Supplementary Material, Figure S1). The processed sequence was then entered into the Ribosomal Database Project website at Michigan State University (RDP-II) (https://rdp.cme.msu.edu/classifier/classifier.jsp). The sequence analysis of the isolated strains shows their genus with a 100% probability. The sequence was then introduced in BLAST (Basic Local Alignment Search Tool) (https://blast.

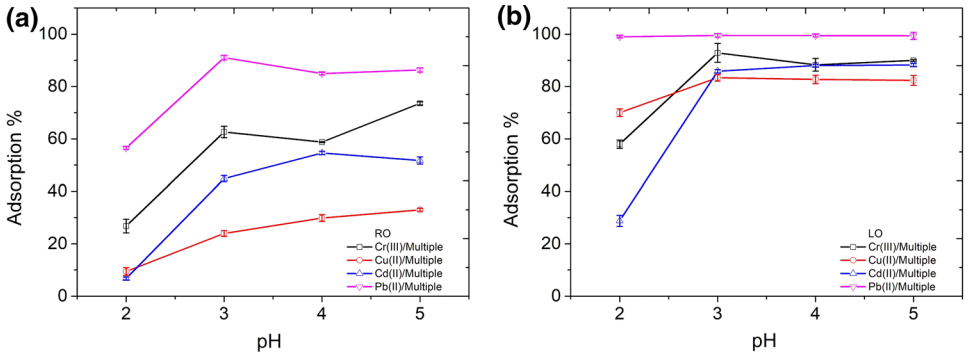

Fig. 2 Influence of the aqueous initial solution pH on the **a** RO and **b** LO sorption systems. Experimental conditions: $T = 25 \pm 1$℃, 25 mg of sorbent, 2.5 mL of aqueous solution with 0.18 mmol/L of each element and a contact time of 24 h. The lines are a guide for the eyes

ncbi.nlm.nih.gov/Blast.cgi), displaying the following high degrees of gene sequence similarity: R01 *Bacillus thuringiensis* (99.6%); R02 *Bacillus subtilis* (98.99%); R03 *Bacillus pumilus* (99.3%); R04 *Bacillus cereus* (99.8%); L01 and L02 *Escherichia coli* (99.71%); L03 *Staphylococcus epidermidis* (99.79%); and L04 *Micrococcus luteus* (99.42%). According to these results, the isolates were identified only at the genus level since further gene sequences are needed to certainly state that they belong to the specified species. At this point, the rapeseed-associated strain L01 was no longer considered for future experiments, as it was estimated to be the same as strain L02.

In general, terms, these findings are understandable, taking into account the origin of the samples, since the species of *Bacillus* and *Micrococcus* genera are widely distributed in the environment (Maldonado et al. 2010; Hashem et al. 2019). Moreover, isolated *Bacillus* strains have high phosphate solubilization potential, especially R01 and R02, which is related to their rhizosphere (roots) provenance and their ability to improve plant nutrient uptake. On the other hand, *Micrococcus* has been extensively shown to be both phyllospheric and rhizospheric genera that promote plant growth activities, specifically with rapeseed plants (Ghavami et al. 2017). The presence of *E. coli* suggests a faecal origin and can be explained by possible contamination during sample transport and handling and/or the erratic distribution of microorganisms in irrigation water (Luyt et al. 2012). In this way, the presence of *Staphylococcus* is presumably due to anthropogenic activities, since it is part of the human skin microbiome (Wood and Kelly 2010).

Optimization of sorption conditions

Influence of the solution pH

To check the influence of the pH of the aqueous metal solution, the initial pH was adjusted to 2, 3, 4, 5 and 6 with either HCl or NaOH. The following detailed experimental procedure is included in Text S2 (Supplementary Material).

As shown in Fig. 2, LO has a better sorption capacity than RO, especially at pH ≥ 3.0. As expected, the metal sorption percentage for both biomaterials increased with increasing pH. At pH 2.0, the lowest sorption percentages were found, probably because hydronium ions (H_3O^+) on the surface functional groups usually limit cation sorption due to their repulsive force (Feizi and Jalali 2015). Competitive sorption of heavy metal cations is also found for both biomaterials, as shown in Fig. 2. The sorption sequence of heavy metals by RO was ranked as Pb(II) > Cr(III) > Cd(II) > Cu(II) in all pH ranges, as shown in Fig. 2a. LO has a similar sorption trend. The higher adsorption percentage values found with LO for all heavy metal cations can be related to its enhanced surface area compared with RO (see Table 2), which leads to possibly higher sorption sites available, as mentioned previously. Considering that an aqueous solution pH above 5.0 can cause heavy metal precipitation (Shen et al. 2017), pH 4.0 was chosen in the following studies.

Kinetics modelling

The kinetics models used here to describe the sorption process include the PFO, PSO and Elovich models, which can be described in Text S2 (Supplementary Material). The experimental kinetic data for RO and LO from Figure S2, following the corresponding procedure described in Text S2, are adjusted to the models, as shown in Figure S3. The RO and LO constant values from the kinetic models, such as k_i, q_i, α and the calculated q_e (experimental sorption capacity), are listed in Table 4, together with the R^2 values of each model.

The results of RO and LO indicate that the PFO, PSO and Elovich equations all fit well with the experimental data for all metal ions with high correlation coefficients ($R^2 > 0.960$). In summary, the obtained results and model adjustments can explain why the biosorption processes of RO and LO are rate-controlled mainly by physisorption, and they can also follow a multilayer biosorption process, which is probably related to the aromatic structure of lignin, which

Table 4 Biosorption kinetic constant values for Cr(III), Cu(II), Cd(II) and Pb(II) by RO and LO for each model

	Experimental	PFO			PSO			Elovich		
	$q_{exp}\times10^3$ (mmol/g)	k_1 (min^{-1})	$q_1\times10^3$ (mmol/g)	R^2	k_2 (g/mmol*min)	$q_2\times10^3$ (mmol/g)	R^2	A	B	R^2
Cr(III)										
RO	8.70	160	8.10	0.972	67.6	3.80	0.972	6,063	$2.8*10^3$	0.965
LO	9.90	0.624	9.70	0.995	322	9.80	0.997	7,152	$3.1*10^{24}$	0.998
Cu(II)										
RO	3.80	0.107	3.70	0.961	67.6	3.80	0.971	6,063	$2.8*10^3$	0.968
LO	9.90	0.662	9.70	0.997	418	9.80	0.998	11,970	$7.0*10^{44}$	0.998
Cd(II)										
RO	6.70	0.194	6.60	0.997	168	6.60	0.997	12,110	$1.2*10^{28}$	0.997
LO	9.70	0.543	9.40	0.986	206	9.60	0.991	6,306	$1.4*10^{20}$	0.992
Pb(II)										
RO	8.90	0.153	8.60	0.996	60.2	8.80	0.999	4,870	$1.1*10^{12}$	0.998
LO	14.0	0.845	14.0	0.999	746	14.0	0.999	8,526	$5.6*10^{44}$	0.999

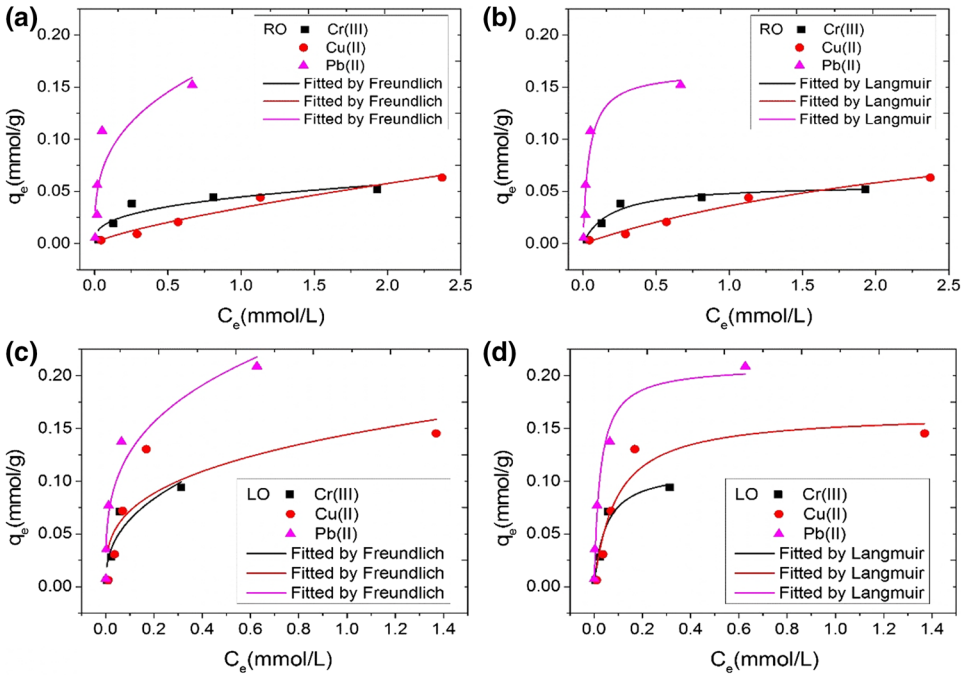

Fig. 3 Freundlich and Langmuir biosorption isotherms of heavy metals in individual-element systems by **a**, **b** RO and **c**, **d** LO. Biosorption conditions: $T=25\pm1$ °C, pH 4.0, 25 mg of sorbent, 2.5 mL of aqueous solution and a contact time of 24 h. The lines are a guide for the eyes

is predominant in the composition of both rice and rapeseed biomass wastes (Sharma et al. 2004; Hubbe 2021).

Isotherm modelling

To evaluate the maximum biosorption capacity of RO and LO, Langmuir and Freundlich isotherm models were used. Data results from the experiments of the initial metal concentration variation (Figure S4) are used in this case (following the corresponding procedure described in Text S2). The Langmuir model assumes that the uptake of metal ions occurs on a homogeneous surface, whereas the Freundlich model assumes it occurs on a heterogeneous surface, which can be described in Text S2 (in supplementary material).

The plots of q_e (sorption capacity) versus C_e (concentration at the equilibrium state) of each metal in a system with a single heavy metal are shown in Fig. 3. The corresponding constant values are shown in Table S1, including the R^2 values for each model (see Text S2 for further Langmuir constant explanation).

In the present study, the Langmuir equation fits the experimental data better than the Freundlich equation in a system with a single heavy metal content (either by RO or LO). The Freundlich model cannot properly fit all the experimental

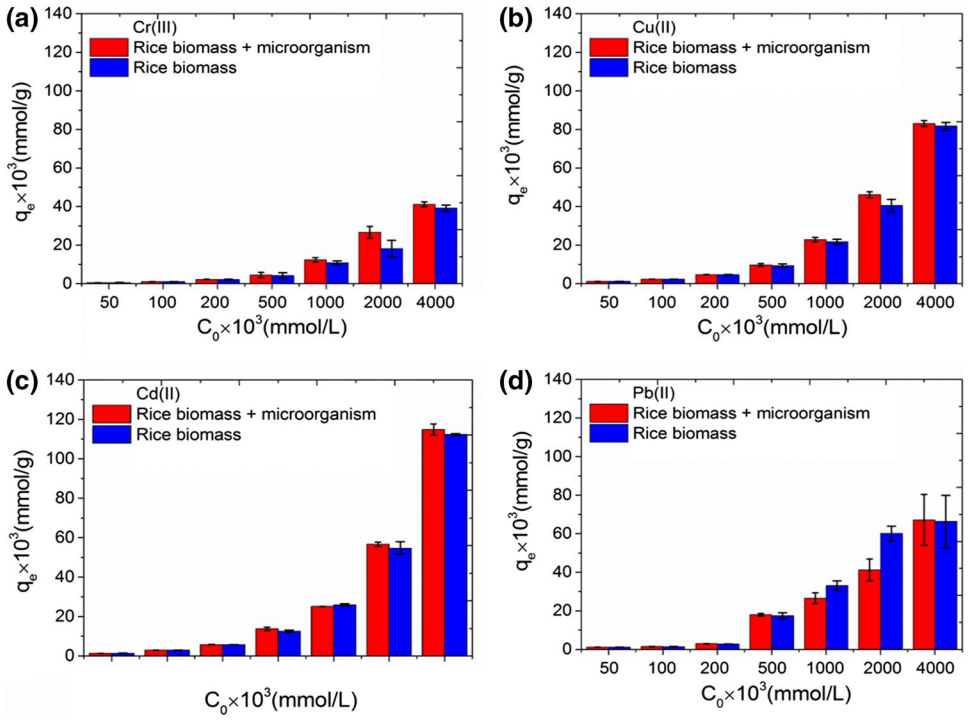

Fig. 4 Comparison of the sorption capacities of non-sterile and sterile rice (RO) at different concentration levels of multimetal aqueous solutions. Experimental conditions: $T = 25 \pm 1$ °C, pH 4.0, 25 mg of sorbent, 2.5 mL of metal solution and a contact time of 24 h

data, as the R^2 values are generally lower than the R^2 values with the Langmuir model (shown in Table S1 and represented in Fig. 3). In addition, the Langmuir R_L values are between 0 and 1 for all metal ions in all systems and indicate that the biosorption of all the metal ions on RO and LO is favourable. The good agreement achieved with the Langmuir model suggests that there is probably an interaction between the metal species and homogeneous sorbent surfaces.

Furthermore, the maximum sorption capacity values for Cr(III), Cu(II) and Pb(II) in systems with a single heavy metal are properly collected in Table S1. As expected from our previous results, RO shows lower maximum sorption capacities than LO for all heavy metals in this study, which can be related to the different BET values of RO and LO (Table 4). Compared with RO, LO has higher BET values, which corresponds to a higher surface; thus, higher possible sorption sites and subsequently higher sorption rates are possible with LO, as evidenced here.

Comparison of nonliving sterile and nonsterile biomass sorption capacity

To investigate the contribution of microorganisms to the HM sorption process, nonliving biomass with and without microorganisms was used as a sorbent for HM removal.

The RO and LO bacterial cfu counts in the original nonliving biomass were $4.3 \cdot 10^1$ and $3.7 \cdot 10^1$ cfu/mL, respectively. Thus, the original biomass naturally contains the associated microorganisms; consequently, rice and rapeseed biomass wastes were pretreated in an autoclave to eliminate live microorganisms to test the metal uptake of sterile biomass. In the same way, the role of biomass-associated microorganisms in the metal uptake process was quantified by using the original nonliving biomass. The sorption experiment results of both the original and sterile plant biomass wastes are plotted in Figs. 4 and 5 for rice (RO) and rapeseed (LO), respectively. Solutions containing multiple heavy metals in different amounts, from 0.05 to 4 mmol/L, were evaluated.

Regarding RO, comparable findings were observed (Fig. 4). A higher specific HM removal (q) with the original biomass is clear for all the tested concentrations of Cr(III), Cu(II) and Cd(II), highlighting the removal obtained at initial concentrations in solution of approximately 4 mmol/L for Cr(III) and Cu(II). However, no bacterial resistance to Pb(II) is observed here, indicating that the role of RO microorganisms is insignificant and that most HM sorption is achieved by rice biomass, which is most efficient at 4 mmol/L.

The results concerning LO original biomass show higher sorption capacity removal values compared with microorganism-free samples (sterile biomass), especially when exposed to Cr(III), Cu(II) and Pb(II) (Fig. 5). This behaviour can be explained by the complementary role of the associated microorganisms in the HM capture process. However, at 4 mmol/L Pb(II), a similar adsorption capacity (q) was detected between both systems (original biomass and sterile biomass, with and without microorganisms, respectively). Most likely, because microorganisms cannot survive at such high concentrations of Pb(II) (Yuan et al. 2015), they cannot

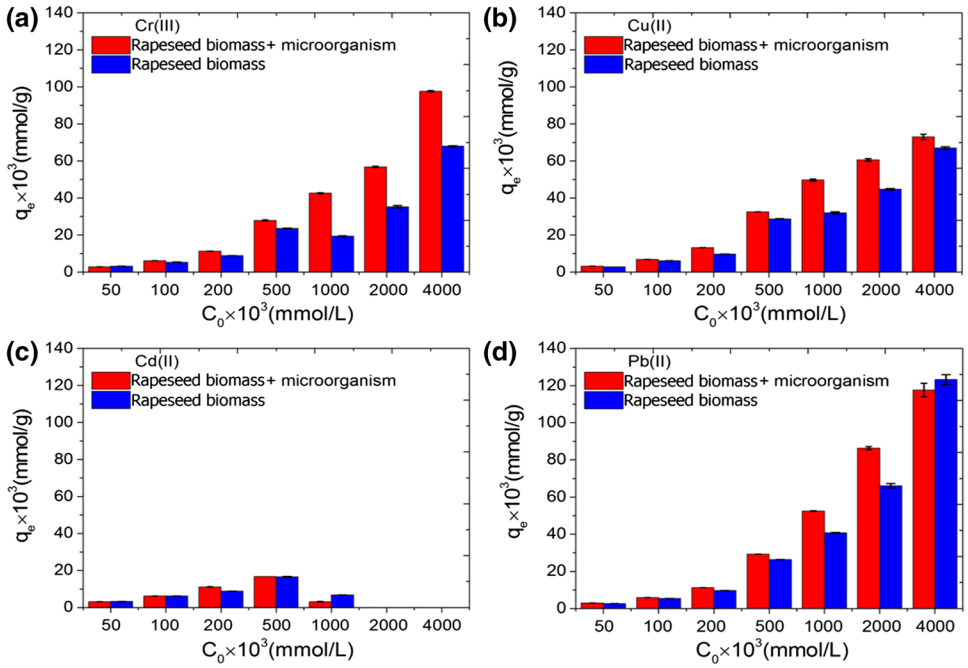

Fig. 5 Comparison of the sorption capacities of nonsterile and sterile rapeseed (LO) at different concentration levels of aqueous solutions with multiple HMs. Experimental conditions: $T = 25 \pm 1$ °C, pH 4.0, 25 mg of sorbent, 2.5 mL of metal solution and a contact time of 24 h

have any role in the rice and rapeseed sorption capacity. Interestingly, microorganisms show a lower capacity for Cd(II) removal than for the other metals under checked, presumably due to a low tolerance to this metal (Fig. 5c), as reported previously (Villagrasa et al. 2021).

These experiments demonstrated the likely role of biomass-associated microorganisms in enhancing heavy metal removal (Ayangbenro and Babalola 2017), especially in the case of rapeseed original nonliving biomass systems. Thus, to determine whether microorganisms exhibit resistance to HMs and their corresponding adsorption capacity to remove HMs (at the same concentration range), the isolated microorganisms were examined as sorbents in aqueous solutions with multiple HMs.

Removal capabilities of the isolated microorganisms

Metal exposure experiments with cell cultures were developed to address the following: whether the strains can indeed contribute to HM removal and the amount of HM captured by them. These experiments were tested in a rich culture medium (LB) to determine the role of microorganisms without being under nutrient starvation conditions.

The results referred to this section are provided in Table 5. First, pure isolated microorganism cultures were evaluated. Once they reached the exponential phase of growth, they were subjected to Cr(III), Cd(II), Cu(II) and Pb(II), each culture in separate aqueous solutions, at a final concentration of 0.18 mmol/L of each metal (in a multiple HM system). These experiments resulted in the active participation of all the isolated strains in the Pb(II) capture process, with

Table 5 Sorption of HMs by microorganisms (mg/L). The initial HM solution was 0.18 mmol/L for each element (Cr(III) 9.35, Cu(II) 11.4, Cd(II) 20.3 and Pb(II) 38.0 mg/L)

Strain	Cr(III)	Cu(II)	Cd(II)	Pb(II)
CONTROL*	0	0	0	0
R01	0	0.180 ± 0.020	0	9.72 ± 0.08
R02	0	0	0.700 ± 0.040	13.7 ± 0.1
R03	0	0	0	5.24 ± 0.08
R04	0	0	1.02 ± 0.04	14.8 ± 0.1
L01–L02	0	0	0.620 ± 0.060	11.8 ± 0.1
L03	0	0	0	4.64 ± 0.08
L04	0	0	0	7.66 ± 0.08
Mix rice strains	8.66 ± 0.02	11.9 ± 0.1	18.4 ± 0.1	36.8 ± 0.1
Mix canola strains	8.66 ± 0.02	11.9 ± 0.1	18.6 ± 0.2	37.1 ± 0.1

relatively high efficiencies, the most notable being 38.00% (strain R04), 35.30% (strain R02) and 30.44% (strains L01-L02). The values collected for R01, R03, L03 and L04 are 25.02%, 13.50%, 11.95% and 19.72%, respectively. Nonetheless, the involvement of the strains exposed to Cr(III), Cd(II) or Cu(II) can be dismissed. Although positive specific HM removal capacity (q) values are recovered for R01-Cu(II); L01-Cd(II); L02-Cd(II); R02-Cd(II); and R04-Cd(II), they are too low to consider the strains as active participants in the HM uptake process. These findings may be largely related to the metal toxicity susceptibility of these strains when they are single cultured (Nguyen et al. 2015; Aparicio

et al. 2018). It has been widely reported that certain concentrations of HM ions can affect the morphology, growth and metabolism of microorganisms by drastically altering their cell membranes, changing their nucleic acid structure, restricting enzyme activity, etc., which consequently reduces cell viability (Fashola et al. 2016; Igiri et al. 2018).

As RO and LO biomass microbiomes are composed of mixed populations, mixed cultures must also be explored. The importance of testing consortia cultures remains in the cooperative actions they perform in an ecosystem that are not contemplated in pure cultures. As previously described, consortia culture cells were grown to exponential phase and then added to a 0.18 mmol/L HM multiple solution system. The results acquired here are remarkable, with some removal capacity found in that case for Cr(III), Cd(II) and Cu(II), compared with previous results in single cultures. Particularly for Pb(II), as noted from the pure culture experiments (Table 5), both biomasses demonstrate the participation of these consortia in the HM uptake process. The contribution percentage is over 80% of the total HM. Interestingly, similar ranges of metal removal and uptake efficiency (Pb(II) > Cd(II) > Cu(II) > Cr(III)) were found with the consortia of both rice and rapeseed wastes.

Role of nonliving biomass-associated microorganisms in the HM biosorption process

From the above-mentioned results, it can be confirmed that after 24 h of exposure, the microorganisms associated with both biomasses play a role in the HM biosorption process. In addition, with longer exposure times, these microorganisms (with some resistance to HM) would develop exponentially, and therefore, they would participate in promoting the HM biosorption process. Several studies have previously reported the use of a microbial consortium for remediation, which is considered more effective than single strain pure culture (Dipak and Sankar 2015; Gupta and Kumar 2017). As expected, microorganisms are more resistant to HMs in mixed cultures, probably due to the physiological diversity between each strain and the different tolerance mechanisms towards HMs. Therefore, this resistance will develop a properly adapted and strong consortium through the exchange of genetic material between the present strains. Therefore, most strains can tolerate and remove these heavy metals and thus assist rapeseed and rice biomass in HM elimination.

Further studies are required to determine the mechanisms of the present strains on the HM removal capability and efficiency and to understand the metabolic pathways and genes responsible for these processes (Mosa et al. 2016). However, it can be initially predicted that the obtained results will be diverse since the genus *Bacillus* has been largely described to adopt both metal biosorption and bioaccumulation mechanisms (Çolak et al. 2011; Huang et al. 2018; Wu et al. 2017; Jin et al. 2018). Moreover, it is well known that gram-positive microorganisms have excellent biosorption capability due to their thick layer of peptidoglycan, which includes an abundant number of sorption sites (Song and Tao 2013; Yin et al. 2019).

Conclusion

In this work, rice (RO) and rapeseed (LO) residues are successfully used as nonliving biomass sorbents for the removal of Cr(III), Cu(II), Cd(II) and Pb(II) from multimetal aqueous systems, and the sorption processes are investigated and discussed in this paper. Furthermore, it is important to highlight that this is the first time that the presence of microorganisms in these nonliving biomasses has been taken into account and properly described, being different in each RO and LO case. Regarding the RO biomass, gram-positive bacteria are exclusively from the genus *Bacillus*, while the LO contains both gram-positive bacteria that belongs to the genera *Micrococcus* and *Staphylococcus* and gram-negative bacteria (genus *Escherichia*). The results show that both nonliving biomass residues can be used to remove heavy metals from wastewater systems, and the participation of isolated microorganisms in consortia during the metal sorption process has been confirmed here.

In this sense, it can be concluded that there is no need for any special pretreatment against the microorganisms in the nonliving biomass waste samples of RO and LO prior to their use as metal sorbents, which implies their good feasibility for application from an economic point of view.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s13762-022-04000-6.

Acknowledgements All the authors are grateful to L. López who helped on the performance of some experiments and the analysis of heavy metals by ICP-MS.

Authors' contributions X.J. Shen, J. Zhao and N. Bonet-Garcia contributed to conceptualization, methodology, formal analysis, investigation, data curation and writing—original draft preparation. E. Villagrasa was involved in conceptualization, methodology and writing—reviewing and editing. A. Solé contributed to conceptualization, methodology and reviewing. X. Liao and C. Palet were involved in conceptualization, methodology, writing—reviewing, editing, supervision and funding acquisition. All authors reviewed and approved the final manuscript for publication.

Funding Open Access Funding provided by Universitat Autonoma de Barcelona. This research was supported by grants from the National Key Research and Development Program of China (No. 2018YFD0200904) and the Agricultural Science and Technology Innovation Program of China (No. CAAS-ASTIP-2013-OCRI), by the Spanish research projects (Nos. CTM2015-65414-C2-1-R and AGL2015-70393-R) and by the China Scholarship Council (for J. Zhao—No. 201509110114).

Data availability All data generated or analysed during this study are included in this manuscript.

Declarations

Conflict of interset The authors declare no competing interests.

Consent to publish The authors declare their consent for the publication of this manuscript.

Ethical approval Not applicable.

Consent to participate Not applicable.

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