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Research Article

Heavy Metal Bioremediation Potential of Autochthonous Lactic Acid Bacteria for Use in Edible Leafy Vegetables

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Heavy metals are well-known as destructive environmental pollutants that cause serious health problems. The use of bacterial biological biosorption has been proposed as a practical and environmentally friendly solution for the removal of heavy metals. The current study was conducted in in vitro and in situ conditions. Initially, seven strains of lactic acid bacteria with probiotic properties (*Lacticaseibacillus casei*, *Lacticaseibacillus rhamnosus*, *Lactiplantibacillus plantarum*, *Limosilactobacillus fermentum*, *Enterococcus faecium*, *Lactobacillus helveticus*, *and Lactobacillus acidophilus*) were screened for their ability to bind cadmium, lead, and nickel in an aqueous solution. Three of the potent probiotic strains that showed the highest biosorption efficiency at this stage were selected for further analysis. The effect of these bacteria mixed at a ratio of 1 : 1 : 1 on the removal of toxic metals in fresh leaves of edible vegetables including coriander, leek, and parsley was evaluated within 15 and 30 minutes. During in vitro analysis, the sorption percentage of Pb and Cd appeared higher than 99% during 15 minutes of initial contact, while increasing contact time (30 minutes) had no significant effect on the removal of these metals. While during in situ analysis, Ni sorption by the selected lactic acid bacteria (LAB) isolates was significantly enhanced with increasing contact time, such that the highest biosorption rate was recorded in coriander leaves at 30 minutes (91.15%). Overall, *E. faecium* showed the highest sorption of Pb, Cd, and Ni (79.75 ± 0.11, 75.28 ± 0.05, and 83.99 ± 0.10%), respectively. The combination of three bacterial strains had a synergistic effect on the toxic metal binding capacity compared to the single state of these bacteria, and the biosorption level increased to 99.94 ± 0.02, 99.91 ± 0.01, and 93.75 ± 0.04%, respectively. Scanning electron microscopic (SEM) observations and energy dispersive X-ray (EDX) analysis confirmed that the majority of Pb, Cd, and Ni were bound to the surface of the bacterial cell.

1. Introduction

With the expansion of human industrial activities, a significant increase in the number of heavy metals in soil, water, and air has been witnessed [1]. Heavy metals with a relative atomic density of more than 5 g.cm^{-3} are classified as essential and nonessential elements [2]. Cadmium (Cd), lead (Pb), mercury (Hg), copper (Cu), arsenic (As), zinc (Zn), nickel (Ni), and chromium (Cr) are the most prevalent hazardous heavy metals [3]. Although metals such as Zn, Mn, and Cu act as an essential cofactor in enzymatic reactions for normal cell growth in low concentrations, no

biological role is known for metals such as Pb, As, and Cd; hence, these metals are considered toxic in any concentration [4]. These elements are nondegradable inorganic pollutants that even at low quantity may cause various diseases such as osteoporosis, disorder in reproduction, mutagenicity, and carcinogenesis and are also known to damage different organs including the heart, nervous system, brain, liver, kidney, blood, lung, bone, and spleen [5–7].

While these metals enter the food chains through widespread use of sewage, pesticides, atmospheric deposition, coal combustion, petrol production, chemical fertilizers, and herbicides on farmland, mining processes can potentially have serious health consequences in man and animals [4, 7]. Methods of removing toxic metals from aquatic ecosystems can be divided into two categories: (i) biological process based on the sorption of toxic metals by plants or microorganisms and (ii) nonbiological process based on the removal of toxic metals using physicochemical processes such as deposition, ion exchange, and membrane filtration [8]. Absorption of toxic metals using microorganisms such as yeasts, fungi, algae, and bacteria in different food groups have been studied extensively [9–11]. There are two basic mechanisms by which microorganisms bind heavy metals: (1) bioaccumulation: the metabolism-associated process in which heavy metals penetrate the plasma membrane and accumulate inside the cell and (2) biosorption: the metabolism-independent binding of heavy metals to the cell surface [12]. The cell wall, which is mainly composed of polysaccharides, lipids, and proteins, has a variety of functional groups for binding to heavy metals, including carboxylate, hydroxyl, amino, and phosphate groups [13]. Electron microscopy observations and energy dispersive X-ray analysis confirmed that the majority of heavy metals bound to the surface of the bacterial cell [14], while many algae and bacteria can also produce secretions that absorb highly toxic elements [15]. Some of the advantages of biosorption methods include their low operating costs, use in foodstuff, selective removal for specific toxic metals, minimal use of chemicals (resulting in low sludge production), and high efficiencies at very low levels [16].

A number of probiotic species within the LAB group, including *Lactobacillus*, *Enterococcus*, *Pediococcus*, and *Bifidobacteria* classified as GRAS (generally recognized as safe), play a role in balancing the intestinal microbiota of mammals and prevent gastric and urinary infections, immune disorders, lactose intolerance, hypercholesterolemia, diarrhea, food allergy, etc [17]. Apart from possessing these properties, a number of LAB are shown to act as a biological sorbent owing to their high selectivity in eliminations of toxic metals being efficacious at a wide range of temperature and pH [18]. According to reports, the negative surface charge of the LAB helps them in binding to metal cations [15].

Potential bioremoval of heavy metals by a number of LAB has been reported, such as Cd by *L. plantarum* [19] and *L. plantarum* and *Bacillus coagulans* [20]; Pb and Cd removal by *L. acidophilus* [21], *L. rhamnosus* [22], and *L. acidophilus* [15]; and removal of Hg in potable water by *Saccharomyces cerevisiae* [23]. However, the uptake of heavy metals by microorganisms varies in different studies depending on different binding conditions [16, 24]. In this study, seven indigenous LAB strains were assessed for their ability to remove Pb, Ni, and Cd in the aqueous solution under constant pH and temperatures. The synergistic biosorption capacity of the selected LAB in edible vegetables including coriander, leek, and parsley was also evaluated at constant pH and after 15 and 30 min of contact time.

2. Materials and Methods

2.1. Bacterial Strains and Culture Conditions. Seven LAB isolates including *Lacticaseibacillus casei* (RTCC 1296-3), *Lacticaseibacillus rhamnosus* (RTCC 1293-2), *Lactiplantibacillus plantarum* (RTCC 1290), *Limosilactobacillus fermentum* (RTCC 1303), *Enterococcus faecium* (RTCC 2347), *Lactobacillus helveticus* (RTCC 1304), and *Lactobacillus acidophilus* (RTCC 1299) were obtained from Razi type culture collection (RTCC), located at Razi vaccine and Serum Research Institute, Iran. All isolates were cultured in MRS (deMan-Rogosa-Sharpe) (Scharlau, Spain) broth medium, at 37°C for 24 hours, under anaerobic conditions. Pure cultures were preserved for a long term by freezing at −70°C with 20% glycerol.

2.2. Biosorption in Aqueous Solution. Heavy metals including nitrate of Pb (II), Cd (II), and Ni (II) were purchased from Merck (Darmstadt, Germany). All standard solutions were prepared from the stock solutions containing 1000 mg.L^{-1} in distilled water. Other chemicals used in the study including nitric acid (65%) and hydrogen peroxide (37%) were also purchased from Merck, Germany. All the containers used were acid washed in 20% nitric acid for 48 hours.

The overnight grown LAB cultures of known cell concentrations (10^9 CFU·mL^{-1}) were centrifuged (10000g, 10 min), and cell pellets were mixed with sterile chilled ultrapure water. 1 mL of the bacterial suspensions was added to 9 mL of respective heavy metal concentrations. Heavy metal concentrations used were 5, 5, and 50 mg.L^{-1} for lead, cadmium, and nickel, respectively. After adjusting the pH to 6.5, the suspensions were incubated at 37°C for 1 hour and then centrifuged at 10,000g for 10 min. The residual Pb, Cd, and Ni concentrations were measured in the supernatant by using the inductively coupled plasma mass spectrometer (ICP-MS; ELAN DRC-e, PerkinElmer SCIEX, Canada) according to the previously described method [14, 18, 24]. Standard solutions of the heavy metal were prepared according to AOAC standard method [25], using five different concentrations (10, 50, 100, 500, and 1,000 μg.L^{-1}) of each heavy metals.

Percentage removal of the heavy metals by individual strains was estimated using the formula:

$$\text{Removal\%} = \frac{C_0 - C_1}{C_0} \times 100 \quad (1)$$

where C_0 and C_1 are the initial and residual concentrations of metals, respectively [18, 22, 26, 27].

2.3. Biosorption in Edible Leafy Vegetables. The leafy edible parts of the vegetables including coriander, leek, and parsley purchased from the local market in Tehran, Iran, were used in the study. The collected leaves of each vegetable were divided into three parts (100 g each), sealed in plastic bags, and stored at refrigerated temperatures before use. Before initiating the experiments, the amount of respective metals in the selected vegetables was estimated by the method described above in order to compare the concentrations of the metals before and after exposure to the tested LAB isolates.

Three most potent LAB isolates including *L. plantarum, L. fermentum,* and *E. faecium* showing highest percentage of heavy metal sorption in aqueous solutions were selected. Active cultures of the respective isolates were mixed in an equal proportion (1 : 1 : 1) before being added to the vegetable samples. Coriander, leek, and parsley samples were soaked in sterile distilled water for 30 min, and then, 10^8 CFU·mL^{-1} of the prepared bacterial suspensions was added. After 15 and 30 min, the samples were tested for the respective heavy metals by ICP-MS as described earlier [14].

2.4. Scanning Electron Microscopic (SEM) and Energy Dispersive X-Ray (EDX) Examinations. In order to investigate the effects of metal sorption on the cell structure and morphology of the tested LAB strains, metal treated, and nontreated (control), bacterial cell pellets were tested according to the method described by Ameen et al. [28] and Daisley et al. [22]. The prepared samples were scanned by using a scanning electron microscope (JEOL JSM 5400 LV, Japan). In addition, detection of metal elements in LAB cells was performed using energy dispersive X-ray (EDX) (JEOL JSM 6360 LA, Japan).

2.5. Statistical Analysis. All experiments were carried out based on complete randomized design, and the results represent the mean of at least three replicates. The data obtained were analyzed by the analysis of variance (ANOVA) method using SPSS 22.0 (SPSS Inc., IBM, Chicago, IL) statistical software. Significant differences between means were determined by Duncan's multiple range test at a probability level of $p < 0.05$.

3. Results

3.1. Action of Removal of Heavy Metals from Aqueous Solutions. According to the obtained results, a significant difference ($p < 0.05$) was recorded in heavy metal biosorption capacity of the tested LAB strains in the aqueous solution. *E. faecium* showed the highest biosorption percentage of Pb (79.75%), cadmium (75.28%), and nickel metals (83.99%). In contrast, *L. casei* showed lowest biosorption percentages that were 47.65% for Pb, 24.87% for Cd, and 67.72% for Ni (67.72%) It is worth noting that the percentage of Ni removal by all tested LAB isolates was significantly higher than the removal of other two metals, Pb and Cd ($p < 0.05$).

During initial screenings, considering that *E. faecium, L. plantarum,* and *L. fermentum* demonstrated the highest heavy metal biosorption percentages, the synergistic biosorption capacity of the mentioned three isolates in combination was also investigated. The results showed the synergistic effect of the isolates on the sorption of heavy metals, as the percentages of Pb, Cd, and Ni removal in aqueous solutions were significantly enhanced ($p < 0.05$) and estimated as $99.94 \pm 0.02\%$, $99.91 \pm 0.01\%$, and $93.75 \pm 0.04\%$, respectively (Table 1).

The bioremoval rates of heavy metals including nickel, cadmium, and lead in aqueous solutions by seven LAB isolates are shown in Table 1.

3.2. Concentrations of Pb, Cd, and Ni in Selected Leafy Vegetables. Prior to initiation of experiments, the purchased leafy vegetables used in the study were initially tested for the concentrations of Pb, Cd, and Ni by ICP-MS. As shown in Figures 1(a)–1(c), the amount of cadmium and lead in leek (2.14 mg.kg^{-1}), parsley (1.85 mg.kg^{-1}), and coriander (1.31 mg.kg^{-1}) was significantly higher than the allowed limits set by WHO/FAO, while as can been seen, the amount of Ni in these green leaves was lower than the set amounts.

3.3. Biosorption of Heavy Metals from Leafy Vegetables. The biosorption capacity of the bacterial suspension (mixture of the selected three LAB strains) on the removal of the heavy metals (Pb, Cd, and Ni) from the coriander, leek, and parsley leaves within 15 and 30 min of contact time is shown in Tables 2 and 3.

Based on the obtained results, the removal of Pb and Cd in all three vegetables during 15 min of contact time ranged from 99.50 to 99.91%, while in the case of Ni, the sorption rate was slightly lower and ranged from 68.59% in leek to 78.85% in coriander ($p < 0.05$). However, with increasing the contact time to 30 min, the rate of Ni biosorption in coriander, leek, and parsley leaves increased to 15.6%, 24.7%, and 22.5%, respectively. As evident, the sorption of Pb and Cd was highly significant within the first 15 min (>99%) of contact time and although with increasing exposure time (30 min), the process of biosorption was increased ($p < 0.05$), but numerically these changes were less than 0.5% (Tables 2 and 3), and the differences were nonsignificant. These results were indicative that soaking the leafy vegetables for 15 min in a solution containing 10^8 CFU·mL^{-1} of multiple LAB strains could decontaminate vegetables by removing approximately 99% of Pb and Cd and 68% of Ni.

3.4. Electron Microscopy Observation and EDX Analysis. As *E. faecium* showed highest biosorption of Pb, this isolate was selected for SEM and EDX analysis (Figure 2). Deposits of Pb were observed on the surface of the respective bacterial cells reflecting high-binding capacity (Figure 2(b)), in contrast to control samples (untreated cells), where no Pb deposits were visible (Figure 2(a)). In addition, SEM micrographs revealed, compared to the untreated control group, after exposure to Pb, the bacterial cells formed

Table 1: Heavy metals removal efficiency of indigenous LAB isolates in aqueous solutions [27].

Probiotic bacteria	Heavy metal removal (%)		
	Pb	Cd	Ni
L. casei	$47.65 \pm 0.20^{g,B}$	$24.87 \pm 0.17^{f,C}$	$67.72 \pm 0.12^{g,A}$
L. rhamnosus	$62.35 \pm 0.15^{d,B}$	$49.74 \pm 0.21^{d,C}$	$78.42 \pm 0.18^{e,A}$
L. plantarum	$66.60 \pm 0.12^{c,B}$	$53.06 \pm 0.10^{b,C}$	$81.82 \pm 0.22^{b,A}$
L. fermentum	$76.59 \pm 0.08^{b,B}$	$52.60 \pm 0.07^{c,C}$	$81.53 \pm 0.15^{c,A}$
E. faecium	$79.75 \pm 0.11^{a,B}$	$75.28 \pm 0.05^{a,C}$	$83.99 \pm 0.10^{a,A}$
L. helveticus	$50.70 \pm 0.12^{f,B}$	$22.27 \pm 0.09^{g,C}$	$78.99 \pm 0.08^{d,A}$
L. acidophilus	$55.62 \pm 0.18^{e,B}$	$48.34 \pm 0.14^{e,C}$	$76.21 \pm 0.21^{f,A}$

*Different small letters in each column and uppercase letters in each row indicate statistically significant differences between groups as determined by the Duncan test ($p < 0.05$).

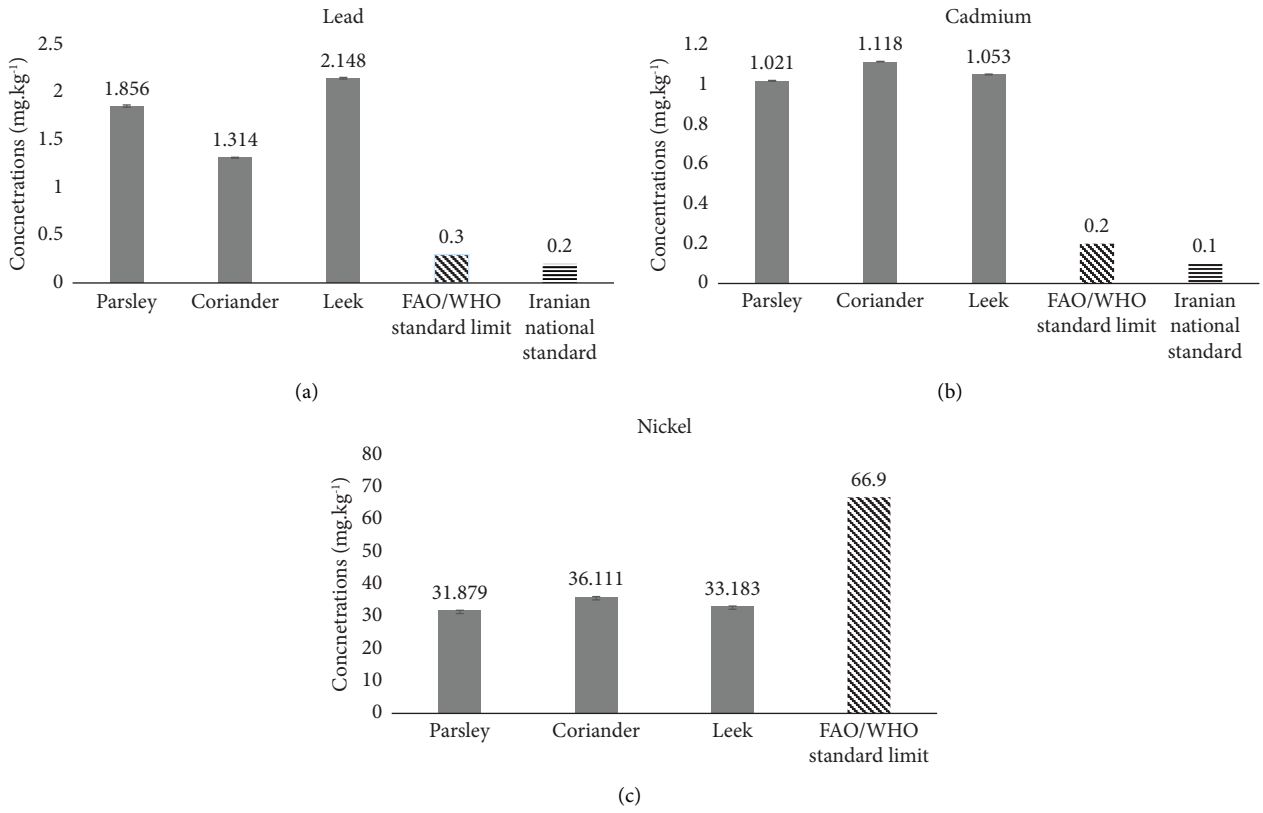

Figure 1: Concentration of (a) lead, (b) cadmium, and (c) nickel in parsley, coriander, and leek according to the FAO/WHO set standard limit and the Iranian national standard.

aggregates with no morphological changes. For the EDX analysis, no Pb signal could be detected in the untreated cells (Figure 2(c)), while a clear peak for Pb was observed in the treated cells (Figure 2(d)).

4. Discussion

Bulk green leafy vegetables and ready-to-eat packaged leafy vegetables such as leek, mint, basil, parsley, spinach, and dill are highly consumed in Iran and are a major part of the human platter. However, high health risks have been associated with the consumption of these leafy vegetables owing to the presence of high concentrations of heavy metals [29–35]. According to the FAO/WHO, the acceptable amount of lead, cadmium, and nickel in leafy vegetables is 0.3 and 0.2, and 66.9 $mg.kg^{-1}$, respectively [36]. While based on Iranian standards (Iranian National Standard Organization, INSO), maximum allowable concentration of Pb and Cd in fresh vegetables should not exceed 0.2 and 0.1 $mg.kg^{-1}$, respectively [37]. Unfortunately, no standard limits for nickel in leafy vegetables in the Iranian standard are yet available [2]. However, a number of studies have shown the presence of heavy metals in vegetables that exceed the standard limits and pose a threat to the health of man and animals. Long-term consumption of heavy metals in high concentrations through food such as vegetables may lead to chronic accumulation of these metals in the human body and affect human physiology and health and cause diseases

Table 2: Amounts of heavy metals in edible leafy vegetables before and after exposure to selected LAB isolates (*L. plantarum, L. fermentum,* and *E. faecium*) when used in combination for washing solution ($mg \cdot kg^{-1}$) [27].

Samples	Pb			Cd			Ni		
	Time (min)								
	Control	15 min	30 min	Control	15 min	30 min	Control	15 min	30 min
Coriander	1.314 ± 0.005	$0.0038 \pm 0.0003^{b,C}$	$0.0012 \pm 0.0001^{a,AB}$	1.118 ± 0.001	$0.0008 \pm 0.0001^{a,B}$	$0.0003 \pm 0.0001^{a,A}$	36.111 ± 0.009	$7.631 \pm 0.004^{a,E}$	$3.182 \pm 0.003^{a,D}$
Leek	2.148 ± 0.009	$0.0028 \pm 0.0002^{a,C}$	$0.0011 \pm 0.0001^{a,B}$	1.053 ± 0.002	$0.0009 \pm 0.0000^{a,B}$	$0.0003^{a,A} \pm 0.0001$	33.183 ± 0.008	$10.414 \pm 0.002^{c,E}$	$4.803 \pm 0.003^{c,D}$
Parsley	1.856 ± 0.012	$0.0086 \pm 0.0001^{c,D}$	$0.0039 \pm 0.0004^{b,C}$	1.021 ± 0.002	$0.0006 \pm 0.0001^{a,B}$	$0.0032^{a,A} \pm 0.0002$	31.879 ± 0.005	$9.111 \pm 0.005^{b,F}$	$4.002 \pm 0.004^{b,E}$

*Different small letters in each column and uppercase letters in each row indicate statistically significant differences between groups as determined by the Duncan test ($p < 0.05$).

Table 3: The heavy metal removal percentage by the LAB isolates (*L. plantarum*, *L. fermentum*, and *E. faecium*) when used in combination in edible leafy vegetable (%).

Samples	Pb removal %		Cd removal %		Ni removal %	
	Time (min)					
	15	30	15	30	15	30
Coriander	99.71	99.91	99.89	99.95	78.85	91.15
Leek	99.83	99.92	99.91	99.96	68.59	85.51
Parsley	99.50	99.77	99.91	99.95	71.41	87.44

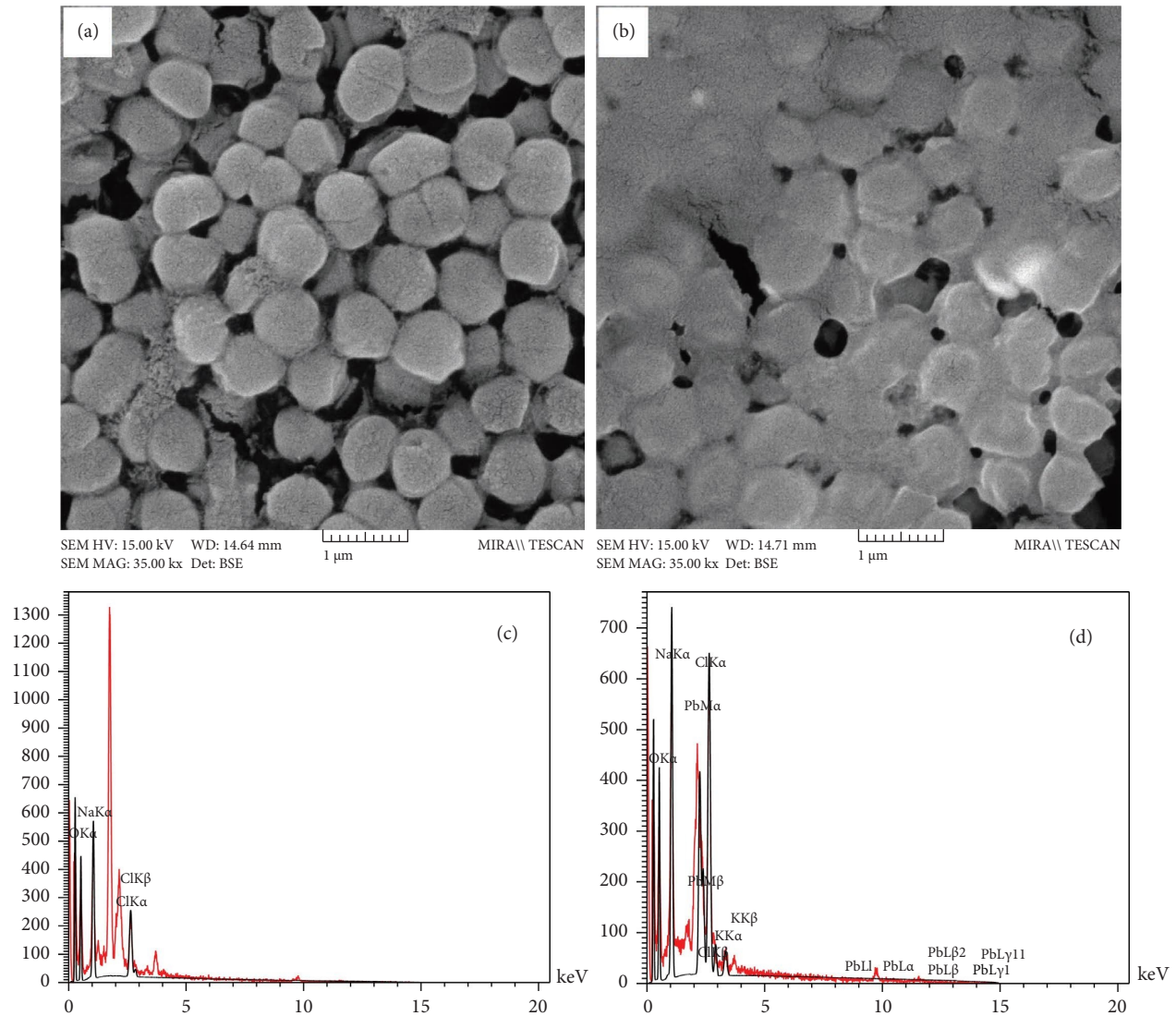

Figure 2: Scanning electron microscopy images of *E. faecium* before and after Pb binding: (a) untreated biomass, (b) biomass after Pb binding, (c) energy dispersive X-ray (EDX) spectra of untreated biomass, and (d) EDX spectra of biomass after Pb binding [27].

such as immunosuppression and mental retardation [38]. Some of the most important health risks factors related to ingestion of heavy metals may include kidney, cardiovascular, and hepatic diseases, while their involvement in congenital disabilities, premature births, neurobehavioral problems, bone injury, and cancers has also been reported earlier [31, 39, 40]. Although the main culprits responsible for heavy metal contaminations in plants are environmental pollution, the source of water and the nature of soil are also the other main agents responsible for such hazards [35, 41].

One of the natural safe strategies being studied for decontamination of heavy metals is the use of LAB species. A number of species belonging to this group of bacteria are claimed to be ideal for utilization as an effective biological tool for heavy metal removal from water and food sources, without posing any danger to our environment [12]. In this

study, we analyzed the metal-binding potential of several indigenous LAB strains that could allow uptake of heavy metals within min of exposure. Biosorption and bioaccumulation of heavy metals by LAB both are prosperous detoxification strategies as they prevent the exposure of heavy metals to body cells and tissues [14, 19, 42]. Initially, Pb, Cd, and Ni binding capability of seven food-grade bacteria, namely, *L. casei*, *L. rhamnosus*, *L. plantarum*, *L. fermentum*, *E. facium*, *L. helveticus*, and *L. acidophilus* in aqueous solutions, was evaluated at pH 6 and at 37°C. All tested strains showed variable levels of biosorption capacity in aqueous solutions and in leafy vegetables. The differences observed in the binding capacity of metals by different LAB species might be explained by the species and strain-specific nature of these bacteria. Correspondingly, Elsanhoty et al. [43] studied the ability of various LAB isolates (*L. acidophilus*, *L. rhamnosus*, *L. plantrum*, *Streptococcus thermophiles*, and *Bifidobacterium angulatum*) to remove heavy metals including Cd, Pb, and As from contaminated water. These researchers showed that the highest amount of heavy metal removal obtained at a pH close to neutral depended on the type of bacterial species. Similarly, Afraz et al. reported the strain-specific nature of LAB in biosorption of heavy metals. These researchers suggested that heavy metal bioremoval is a surface process due to the binding of metal cations to the anionic functional groups and depends on the capacity of the bacterial strains and metal electronegativity [44]. LAB have some polymers like peptidoglycan and lipoteichoic acid that can be responsible for the interactions between the heavy metals and the negative charge present on the surface of bacteria [15].

Besides, several other chemical and physical factors such as temperature, pH, incubation time, inoculum size, and heavy metal concentrations are known to play a considerable role in the bioremediation process. Among these parameters, temperature is one of the main physical factors that influence bioremoval efficiency [45]. A number of researchers have examined the bioremoval capability of probiotic bacteria using variable temperature ranging from 25 to 37°C [46, 47]. Another influencing factor in the biosorption of heavy metals is pH. While a number of studies have shown increasing pH values to be more effective and that metal sedimentations occur at alkaline situations, pH values ranging from 5 to 9.0 have been claimed to be the most suitable pH range [41]. In this study, constant pH (6.0) and temperature (37°C) were used, which appeared to be efficient in bioremoval of heavy metals. Similar results were reported by Elsanhoty et al. [43] who reported that heavy metal reduction by potential probiotics is pH-dependent and enhanced biosorption efficiency is observed at pH near neutral. Contact time is another main factor that influences the bioremoval activity [41]. During these studies, we observed significant heavy metal removal by LAB species during 15 min of exposure time that remained almost constant after 30 minutes. The binding of Pb and Cd by the tested LAB isolates showed no change during 15 or 30 min of exposure time. However, contrasting results have been reported by others, who showed that the efficiency of bacteria to remove heavy metals in a sample may be enhanced with increasing contact time. Elsanhoty et al. [43] showed that 85.5% of Pb and 86.8% of Cd were removed from contaminated water in 30 min. Similarly, *L. plantarum* was shown to remove more than 80% of Cd from rice in 24 h [48]. *L. acidophilus* bacteria removed lead and cadmium by 80% and 75%, respectively, within 4 days of incubation [15]. While findings similar to our results were stated earlier by Zhai et al. [14], who showed that with increasing the incubation time from 2 h to 36 h, no significant change in Cd reduction ($p \geq 0.05$) by *L. plantarum* CCFM8610 was evident. However, Ni absorption appeared to be time dependent as increasing contact time from 15 to 30 min significantly enhanced Ni absorbing ability of the selected LAB isolates.

In this study for the very first time, we show that multi-LAB cultures have enhanced biosorption ability compared to monostrain cultures. The synergistic effect of three LAB strains, namely, *E. faecium*, *L. plantarum*, and *L. fermentum*, became highly evident, and enhanced removal of the metals including Pb, Cd, and Ni was seen in aqueous solutions in the presence of the multistrain LAB cultures compared to monocultures. According to a vast number of research reports, multistrain probiotics (MSPs) broadly defined as a mixture, blend, or cocktail of two or more probiotic species or genera provide more benefits to their host, as they provide the synergistic effect in contrast to the use of a single or separate-strain probiotics [49–51]. Ibrahim et al. [52] showed that combination of *L. rhamnosus* and *Propionibacterium freudenreichii* could bind cadmium and lead efficiently at low concentrations. However, to our knowledge, this is the first report that shows the enhanced effectiveness of combination of multiple LAB cultures in removal of heavy metals in vegetable leaves.

SEM micrographs revealed that exposing *E. faecium* cells to Pb leads to enormous aggregation of the respective bacterial cells, compared to the untreated cells, with no morphological changes. This phenomenon might indicate self-protection of *E. faecium* cells caused by the change the surface charge and the degeneration of surface proteins enhanced by Pb exposure, leading to their high aggregation [23]. Morphological changes in *E. faecium* observed by SEM after Pb exposure were consistent with those in studies conducted by Teemu et al. [53], Zhai et al. [14], and Ameen et al. [28], who found major deposits of Pb on the surface of lyophilized *L. fermentum* ME3 and *Bifidobacterium longum* 46, Ni and Cr deposits on the surface of *L. plantarum* MF042018, and Cd deposits on the surface of *L. plantarum* CCFM8610 after binding.

5. Conclusion

During the current study, we observed that contamination of heavy metals in our local vegetables is much higher than the permissible limit. Although vegetables with such high levels of heavy metal should not enter the market, in most third-world countries where food safety monitoring is weak, the risks exist. The tested indigenous LAB strains were effective in eliminating heavy metals at pH 6 during 15 min of exposure time, and more interestingly, mixing multiple LAB

strains significantly enhanced the biosorption capability. As washing vegetables with water alone is not effective enough to decontaminate heavy metals, an effective biological liquid wash solution containing multistrain LAB could be used for decontaminating vegetables. The use of LAB strains is cost-effective, highly available, and biologically safe for use as a heavy metal decontaminating agent. Further studies are recommended for evaluating new LAB strains for their ability to bind toxic heavy metals in vegetables and other food products using varying concentrations of heavy metals at variable pH, temperatures, and incubation time.

Data Availability

The data used to support the findings of this study are included in the article.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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