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Screening of Some Lactic Acid Bacteria and Bifidobacteria for Their Capability for Removing of Heavy Metals and Aflatoxin B1 From Contaminated Water

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KEYWORDS

Biofilter, binding, Lactic acid bacteria probiotic bacteria, cadmium, lead, arsenic, aflatoxin B1.

ABSTRACT:

This study assessed the capacity of five microrganisms oflactic acid bacteria (LAB), Lactobacillus johnsonii ATCC 3320, Lactobacillus salivarius TISTR 390, Lactobacillus paracasei TISTR 453, and Lactobacillus helveticus LH-BO2L) and probiotic bacteria (Bifidobacterium lactis strain Bb12) and their combination to eliminate heavy metals (HM) like arsenic (As), lead (Pb), and cadmium (Cd) as well as mycotoxin aflatoxin B1 (AFB1) from contaminated water. Both single and mixed LAB and probiotic bacteria were used to test the biosorption parameters (pH, bacterial concentration, contact time, and temperature) of removal. The data showed that HM and AFB1 removal was influenced by the strain and that the process was very pH-dependent, with strong binding at a pH of about neutral. An increase in the bacterial content increased the binding of As, Pb, and Cd. Furthermore, increasing the contact time and treatment temperature improved the ability of LAB strains to eradicate HM. The effect of contact duration on Cd elimination was slightly different with freshly cultured cells. As the initial metal content increased, so did the perceptual elimination of Cd, Pb, and As. Bifidobacterium lactis strain Bb12, Lactobacillus johnsonii ATCC 3320, and the combination of the microbes were the most highly successful HM removers. It was determined that the system was sufficient for the HM concentrations being studied.By the end of the procedure, the HM concentration had reached the maximum permitted by WHO guidelines. These findings provide insights for selecting and optimizing the conditions to increase the capability of LAB for removing of heavy metals and aflatoxin B1 from contaminated water as primary basic biomethod for production of biofilter for purification of contaminated water.

1. Introduction

As the world's population grows and industrialization speeds up, environmental pollution has become a more serious issue. Unwanted changes brought on by the water contaminants have an impact on the environment's ecological equilibrium. Non-biodegradable substances known as heavy metals (HM) can take on various inorganic and biological forms. HM pose a major risk to human health when released into the water. Additionally, because of the effects on human health, the buildup of heavy metals (HM) in marine environments is of worldwide significance. Cd, Pb, Hg, and As are harmful even in minute amounts, while other HM like Fe, Cu, and Zn are necessary trace elements (**Kheradmand et al. 2006; Halttunen, 2007; Zoghi et**

al., 2014 Rogowska et al., 2019 Zhu et al., 2024; Ibrahim Fouad et al., 2025).

Heavy metals (HMs) are defined as metal elements with a density more than 5 g/cm3 (**Chen et al., 2020**). The HMs include harmful HMs like Pb, Hg, Cd, Cr, and As, as well as trace elements like Fe, Cu, and Zn that are essential to human health (**Adnan et al., 2024**).

According to WHO (2001), prolonged exposure to drinking water containing arsenic (As) can result in skin lesions as well as cancers of the kidney, bladder, and lungs. The two primary oxidation states of arsenic in groundwater are +5 (As(V)) and +3 (As(III)).

According to **Wang and Mulligan (2006),** oxidation of As(III) to As(V) is frequently necessary when using traditional water purification techniques, such as coagulation-precipitation, adsorption, ion exchange, and membrane filtering. A preliminary value of 10 µg/L for As in drinking water has been established by **WHO (2004)** due to its toxicity. A toxic heavy metal (HM), cadmium (Cd) can have a number of detrimental health impacts. The primary ways that people are exposed to cadmium are through food and tobacco use (**Satarug, et al., 2011; Zhai et al., 2015**, **Massoud and Zoghi 2022**).

A weekly acceptable consumption of Cd is 7 mg/kg body weight, according to the Joint FAO/WHO Expert Committee on Food Additives (**FAO/WHO, 2006; Zhai et al., 2015**). The United States Environmental Protection Agency's (EPA) Integrated Risk Information System (IRIS) established the reference Cd dose threshold at 0.5 mg/kg/day in water and 1 mg/kg/day in food based on human research involving chronic exposures (**IRIS, 2012; Zhai et al., 2015**, **Abdel-Megeed, 2021**).

Since there is currently no proven cure for cadmium poisoning (Nordberg et al., 2011), new approaches to preventing cadmium toxicity must be created. Lead (Pb) is a persistent environmental contaminant that causes a number of dysfunctions, impairments to the central and peripheral nervous systems, memory loss, and a decline in children's intellectual capacity (**Jarup 2003; Bhakta et al., 2012**). Adults (90%) and children (70%) have accumulated lead in their bones and teeth (**Halttunen, 2007**). However, a class of carcinogenic mycotoxins known as aflatoxins (AF) can cause liver cancer and a variety of acute or chronic intoxications (**Williams et al., 2004**). According to toxicological research, AFB1 is the most prevalent and harmful of the four naturally occurring AFs—AFB1, AFB2, AFG1, and AFG2 (**Zoghi et al., 2014**).

The main pathways for HM contamination in biological systems are food and water. The main sources of HM contamination in human bodies are aquatic animals, particularly fish (**Cheng and Gobas, 2007**). Therefore, the first step in addressing the issue of bioaccumulation should be the removal of heavy metals from water. Several techniques, such as flocculation, precipitation, ion exchange, and membrane filtration, were introduced to remove heavy metals (HM) from water. These techniques are said to have drawbacks, though, including the production of toxic sludge or other wastes that need to be disposed of, costly equipment and monitoring system requirements, incomplete HM removal, and high reagent or energy requirements (**Halttunen et al., 2007; Zoghi et al., 2014**).

Recently, removal of toxic HM using algal, fungal and bacterial biomass was introduced as an inexpensive and novel method. Mechanisms involved in HM biosorption include complex formation, ion exchange, adsorption, chelation and microprecipitation **(Halttunen, 2007; Zoghi et al., 2014, Wei et al. 2020).** Bifidobacteria and lactic acid bacteria (LAB), which are generally accepted to be innocuous, are the most well-known probiotic microorganisms for reducing biocontamination (**Mehta and Gaur, 2005, Perczak et al., 2018**, **Kotsiou et al., 2025**). Certain probiotics that can stick to intestinal cells will swiftly pass through the gastrointestinal tract when combining with toxins or heavy metals to generate a compound (**Zoghi, et al., 2021**). The general role of probiotics and potential probiotics in reducing and deactivating mycotoxins and heavy metals in food products is summed by **(Yao et al., 2024).**

Toxins and heavy metals can be effectively and economically removed from food items via bioremoval. To detoxify and deactivate harmful contaminants in the food matrix, several microorganisms are used (**Massoud et al., 2019**).

Aerococcus, Carnobacteriu, Enterococcus, Lactobacillus, Lactococcus, Leuconostoc, Oenococcus, Pediococcus, Sterptococcus, Tetragenococcus, Vagococcus, and Weissella are among the genera that are represented in LAB (**Axelsson, 2004**). Although they are phylogenetically unrelated and have distinct sugar fermentation processes, the lactic acid bacterium is also frequently associated with the genus Bifidobacteria. Because of their practical qualities or positive health effects, certain strains of LAB are employed to ferment food and drinks.

Heavy metals (HM) and mycotoxins (**Haskard et al. 2001; Turbic et al. 200**2) can be extracted from water in vitro by LAB (**Halttunen et al. 2007**). LAB has been observed to be strain dependent in its ability to eliminate mycotoxins and heavy metals from water (**Haskard et al. 2001; Halttunen et al. 2007; Nybom et al. 2007; Rachwał, and Gustaw, 2024**). Therefore, in order to efficiently remove several contaminants together, it could be required to use combinations of bacteria. It has been demonstrated that HM and AFB1 passively adhere to the bacterial surface through hydrophobic and electrostatic interactions (**Haskard et al. 2000; Lahtinen et al. 2004; Halttunen et al. 2007**: **Ye et al., 2020**). Cd and Pb were rapidly extracted from the bacterial surface by an ion exchange process (**Ibrahim et al., 2006; Elsanhoty et al., 2016, Amany et al., 2022**). LAB's negative surface charge makes them perfect for cation binding. However, species that are negatively charged could be more challenging to extract from water. A study by **Halttunen et al. (2007)** found that the concentration of As(V) and pH affected the amount of arsenic that Lactobacillus strains, both native and chemically modified, were able to remove. Additionally, it was discovered that while aminated L. casei did not remove As(III) from water, they did remove As(V). 33 LAB strains were examined by **Zhai et al. (2015)** for possible probiotic protection against Cd toxicity. Strong binding capacities and Cd tolerance were shown by both L. plantarum and L. rhamnosus. Additionally, it was found that AFB1 may be bound by LAB and probiotic bacteria (**Elsanhoty et al., 2013, 2016**).There wasn't much information in the literature on using biofiltration methods to clean contaminated water. The capacity of various LAB strains and their combinations to extract model toxins (Cd, Pb, As, and AFB1) from aqueous solution was assessed in this study.

2. Materials and methods

2.1.Materials

2.1.1 Chemicals

In this work, metal salts were used: sodium arsenate dibasic heptahydrate $Na_2HAsO_4.7H_2O$ (SIGMA-ALDRICH, Chemie GmbH-Germany); lead(II) nitrate $Pb(NO_3)_2$ (Fluka Chemie GmbH, Buchs, Schwitzerland); and cadmium nitrate $Cd(NO_3)_2$. To modify pH, SIGMA-ALDRICH (Chemie GmbH-Germany) provided sodium hydroxide (NaOH) and hydrochloric acid (HCl).

2.1.2 Cultured bacterial preparation, bacterial strains, and microbiological media

The Man Rogosa Sharp broth medium (MRS-broth) for subculturing and MRS Agar for slant culture were supplied by Oxoid (England).In this investigation, four LAB strains and one probiotic bacterial strain were used (Table 1). The strains were selected due to their widespread use in the food industry and the available data regarding their effects on mutagens. For the experiment, the MRS medium was used, and the appropriate amounts of each strain were maintained. Standard sterile techniques were used to inoculate the cells.To sanitize them, the microbe media and every piece of glassware were autoclaved for 20 minutes at 121˚C and 15 lb/in^2 of pressure.

2.2 Methods

2.2.1 HM solutions Preparation

2.2.1.1 Individual HM solution preparation

According to **Haskard et al. (2001),** metal concentrations of 5, 10, 15, 20, 30, and 40 mg/L were obtained by dissolving $Pb(NO_3)_2$ and $Cd(NO_3)_2$ in double-distilled water. A 1000 mg/L stock solution was made, from which various concentrations were made. Sigma (St. Louis, MO, USA) provided the AFB1, which was then dissolved in PBS using the procedures outlined by **Haskard et al. (2001).**

2.2.1.2 Binary metal solution preparation

Pb, Cd, and As binary metal solutions containing 10 mg/L were prepared and mixed in equal

parts. Before and after the removal trials, all glassware was treated with 0.1 M HCl to stop metals from sticking to it.

2.2. Preparation of biosorbent

In Duran bottles, one liter of MRS media was made in accordance with the guidelines in the Oxide Catalog for Microbiological Products. After adjusting the medium's pH appropriately, it was sterilized for 30 minutes at 121˚C and 15 lb/in2 of pressure before being allowed to cool. A loop full of bacterial culture was taken and streaked on the agar plate to produce more colonies in order to activate the stains. After that, they are moved to MRS broth and cultivated for subculturing. A 250 mL Erlenmeyer flask was filled with 100 mL of sterile culture medium. After letting the media cool, the microbial solution was added to the medium in a laminar air flow chamber. The aerobic strains were inoculated for two days at 250 rpm and 37˚C in an orbital shaker (Lab Line Orbit Environ-Shaker-Germany), and the anaerobic strains were incubated for 48 hours at 37ºC in a BBL anaerobic jar (Becton Dickinson Microbiology Systems, Sparks, MD) with a disposable BBL gas generating pack (CO2 system envelopes, Oxoid, Ltd., West Heidelberg, Victoria, Canada) in order to obtain biomass.Equal parts of each culture were added to create mixed civilizations. Centrifugation was used for 10 minutes at 9000 rpm to separate the cells from the media. The cells were re-suspended in phosphate buffered saline (PBS), which was made using purified water for washing, and centrifuged once more as previously indicated to ensure that no media remained on the cell surface. The supernatant was disposed of. To create lyophilized bacterial suspensions (2 g) for the experiment, the strains in lyophilized form were suspended. For 24 hours, the biomass was heat-killed at 60˚C in a traditional hot air oven. Experiments were conducted using this biomass. For mixed culture experiments, equal amounts of both biomasses (microbial cells) were added.

2.3. Experiment of biosorption

100 milliliters of metal solution were added to 250 milliliters of Erlenmeyer flask containing various quantities of freshly cultured and cleaned biomass (pure/mixed cultures). The flasks were allowed to equilibrate after being set on a shaker set to a steady 300 rpm. The amount of metal in the supernatant was measured after samples were taken at predetermined intervals and centrifuged as described above. The studies on biosorption were conducted in accordance with **Halttunen et al. (2007**). Centrifugation at 9000 rpm for 10 minutes was used to remove the biomass from the medium after the bacteria had grown in it. To ensure that no media remained on the cell surface, the cells were re-suspended in PBS made with filtered water for washing, and the supernatant was disposed of. They were then centrifuged once more as before. To achieve a final bacterial concentration of 1 g/L, 2 g/L, or 109 cells/mL, the bacterial suspension was spiked with the same solvent that contained either AFB1 or one of the metals. When required, diluted HNO3 or NaOH were used to bring the suspension's pH down to the desired level. Samples were incubated for five minutes to twenty-four hours at either 22°C or 37°C. Centrifugation was used to separate the bacteria from the supernatant following incubation, and a sample was extracted for either metal or AFB1 analysis. To preserve the samples for HM tests, a tiny amount of concentrated ultrapure HNO3 (Fluka Chemie GmbH, Buchs, Switzerland) was added. Every experiment was carried out at least twice, and both positive and negative controls—solvents that were used in place of bacteria and toxin, respectively—were included. For 24 hours, the biomass was heat-killed at 60˚C in a traditional hot air oven. The sorption studies were conducted using this biomass. Biomass as a function of temperature, contact time, pH, biomass concentration, and initial metal concentration was used in biosorption investigations.

2.4. Impact of temperature, pH, bacterial concentration, and duration of contact

The binding experiments were carried out as previously mentioned in order to investigate the effects of several parameters, including pH, biomass concentration, temperature, contact time, starting metal concentration, and metal binding. In every experiment, one condition was altered while the others remained unchanged. Before the experiment, the total number of lactic acid bacteria was 109 cells/g from fresh and lyophilized bacteria, after counting by flow cytometry (Virta et al., 1998), and the temperature was 25–50˚C. The experiments also varied the contact time (30–300 min), pH (1–7), and bacterial concentration (0.5-2 g/L), with the final bacterial concentrations being 1 g/L and 2 g/L. A theoretical model that allowed for the computation of particular descriptive parameters was fitted to the experimental findings of metal removal investigations with varying metal concentrations. This study used a Langmuir isotherm to assess the HM removal capacity of several LAB strains (**Davis et al., 2003**).

2.5. Bound HM desorption

After a binding experiment with ultra clean water containing 0.1 mM and 1.0 mM EDTA, the bacterial pellets were washed with 1.5 mM and 15 mM HNO3 and 1.5 mM NaOH to evaluate the desorption of bound metal. The binding experiment was conducted, as stated earlier. pH 5, 50 mg/L of metal, and 10 minutes of contact time were the settings for Cd and Pb, whereas pH 7, 0.5 mg/L of metal, and 5 minutes of contact time were the conditions for As. Following the initial binding test, the bacterial pellet was centrifuged to separate it from the supernatant. It was then re-suspended in an equivalent volume of one of the tested desorbents and allowed to sit at room temperature for ten minutes. The suspension was centrifuged for HM analysis, and a sample of the supernatant was extracted for metal analysis. The desorption process was carried out three times.

2.6. Resorption of HM

Using the techniques outlined by **Teemu et al. (2007),** the reusability of biomass used in HM binding was evaluated. The above-described binding experiment was carried out at pH 5 with a metal concentration of 100 mg/L and an incubation period of 10 minutes. To desorb the bound metal, the bacterial pellet was suspended in either 10 mM EDTA or 15 mM HNO3. Following desorption, the pellet underwent two ultra-pure water washes to eliminate any remaining EDTA/HNO3 before being utilized once more for metal binding. Three repetitions of this cycle were made. The method of **Rosenberg et al. (1980)** was used to characterize the elements that the microorganisms under inquiry used to remove HM.

2.7. LAB's surface characteristics

2.7.1.Hydrophobicity

Halttunen et al. (2008) state that the microbial adhesion to hydrocarbons (MATH) test was used to assess the hydrophobicity of *Lactobacillus salivarius TISTR 390*, *Lactobacillus paracasei TISTR 453*, *Lactobacillus helveticus* LH-BO2 *Lactobacillus johnsonii ATCC 3320*, *Bifidobacterium lactis* strain Bb12. To standardize the amount of bacteria (10^7 to 10^8 CFU/mL), the lyophilized cells were resuspended in the same buffer after being washed twice with 10 mmol/L PBS, pH 7.4, to an absorbance (λ = 600 nm) of roughly 0.25. P-xylene or n-hexadecane was added in an equal volume, and the two-phase solution was thoroughly mixed by vortexing for one minute. The absorbance at 600 nm was measured after the aqueous phase was removed after an hour of room temperature incubation. The formula [(A0-A)/A0] x 100 was used to calculate the adhesion percentage of affinity to hydrocarbons (hydrophobicity), where A0 and A0 represent the absorbance prior to and following extraction with organic solvents, respectively. The percentage decline in absorbance of the initial bacterial culture as a result of cell partitioning into the hydrocarbon layer was used to compute hydrophobicity from three replicates.

2.7.2 Auto/co-aggregation

According to the instructions for the MATH test, bacterial suspensions as well as their combination, were made. The absorbance (λ = 600 nm) of the bacterial suspensions was measured at 0, 1, 2, 3, and 4 hours after they had been incubated in aliquots at room temperature without stirring. This allowed for the assessment of auto/coaggregation. Aggregation index = [(A0) - (At)/(A0)] × 100 was used to express the auto/co-aggregation percentage, where A0 is the bacterial suspension's A600 at time 0 and at the A600 of the bacterial suspension during a 2-hour incubation period. In order to compare the results on auto- and coaggregation, a predicted value for co-aggregation was computed by multiplying the proportional bacterial concentration of each strain in the combination by the total of the auto-aggregation indexes of each strain.

2.8. Effect of biomass concentration, temperature, contact time and initial metal concentration

After the biomass was centrifuged at 9000 rpm, various biomass weights, ranging from 0.5 to 3 mg/mL, were distributed across solutions with a metal concentration of 10 mg/L. In order to maximize the metal ion's biosorption, the solutions were brought to the ideal pH. We let the flasks equilibrate. Using the

previously mentioned methods, the concentrations of metal ions were ascertained after centrifuging the solutions at 9000 rpm. To track the impact of temperature on biosorption, the ideal biomass content and pH were employed. Experiments were conducted on a rotary shaker set at 240 rpm and at various temperatures ranging from 10 to 50˚C for each culture. The samples were left to reach a state of balance. The sample was taken as previously mentioned at regular intervals and its metal content was examined. The cell pellet was dissolved in a metal solution with a concentration of 10 mg/L and a 100 mL working volume. At the ideal pH system, the experiment was conducted. On a rotary shaker set to 240 rpm, flasks were allowed to reach equilibrium before samples were taken on a regular basis. The supernatant's residual metal concentration was determined after centrifugation at 9000 rpm. In the biosorption studies, various starting metal concentrations were used while all other parameters, including biomass concentration, pH, temperature, and time, were fixed. Metal solutions were made as previously mentioned. A percentage increase in biosorption was noted when the metal content increased from 5 to 30 mg/L. At least two duplicates of each experiment were conducted.

2.9. Measurement of HM and AFB1

Using atomic absorption spectrometry (Atomic Absorption spectrophotometry-6800 Shimadzu-Japan), the concentrations of Cd and Pb were measured using either the flame method or the graphite furnace method, depending on the metal concentration. As quality control samples, Cd and Pb-spiked samples were employed in each assay. Using high performance liquid chromatography (HPLC), concentrations were examined using the techniques of **Meriluoto et al. (2005) and Lee et al. (2003)**. Removal % = 100 x [(C0-C1)/C0] was used to express the percentage of toxin/HM removed (bound by bacteria), where C0 and C1 represent the initial and residual concentrations of toxin, respectively, following removal. **Halttunen et al. (2007)** reported that the atomic absorption spectroscopic approach was used to determine arsenic. Filter syringes with a 25 mm diameter and 0.22 µm pore (Nalgene™ Syringe Filters) were used to isolate the bacterial cells that absorbed the HM and AFB1.

2.10. The impact of microorganisms and their combination on HM and AFB1 elimination

Following the procedures outlined in section 2.3, the bacteria and their combinations were generated in order to investigate the impact of LAB and their combination on the elimination of HM and AFB1. However, pH 7, bacterial concentration of 2 g/L, contact time of 300 min, and temperature of 40˚C were the conditions for biosorpation. Following the experiment, HM and AFB1 were identified. The microorganisms' combination (1:1) was prepared from the biomass as mentioned before using of the designed filter **(data not shown)**.

2.11. Statistics analysis

For statistical analysis, the analysis of variance (ANOVA) or paired t-test was computed using SPSS version 11.0. Depending on the homogeneity of variances, either the Tamhane test or Tukey's HSD was used to examine differences when ANOVA was employed.

3. Result and discussion

3.1. Effect of pH onHMremoval

Tables (2, 3, 4)present the effect of pHon the binding ofHM under study. Data showed that the binding is strongly pH-dependent with the highest binding at a pH close to neutral. There are similar effects on HMremoval with all studied strains. Increasing pH caused almost linear increase in removal processing wherein the highest binding of Cd(65.1%), Pb(70.1%)and As(74.8%)was achieved at pH 7. The effect of pH may be results from competition for negatively charged binding sites between HM cations and protons (H^+).The combinations between the microorganisms given the highly value for reduction of heavy metals.

The bacteria strain and pH determine whether LAB can bind to cationic HM. At pH values below 2-3, very little removal is usually seen; at pH values above 3, removal increases sharply, reaching its maximum at pH 4-6. Competition between protons and cationic metals for negatively charged binding sites is what causes the pH impact (**Halttunen, 2007; Zoghi et al., 2014**). According to **Hansen et al. (2006),** the ideal pH for Lessonia nigrescens to remove As(V) was acidic,

and As(V) was significantly removed across the pH range of 2.5–6.5. Rhizopus oryzae similarly showed lower elimination (**Mcafee et al., 2001**). The removal of As(V) from water by various microorganisms has only been examined in a small number of other papers. Low As(V) removal using a yeast biomass was noted by **Seki et al. (2005),** with amino and imidazol groups being the most likely binding sites. According to reports, the mechanism behind the elimination of arsenic (III) differs from that of As(V). As (III) was successfully extracted from water using immobilized biomass of the bluegreen algae Scytonema (**Prasad et al., 2006**). The mechanism of heavy metal removal was described by **Beveridge and Murray (1980)** and **Doyle et al. (1980)**, who also indicated that various mechanisms have been postulated to be involved in metal biosorption, including complex formation, ion exchange, adsorption, chelation, and microprecipitation. Ion exchange is most likely at least partially to blame for the observed cadmium and lead binding, according to the pH dependence**.** This finding is supported by the pH drop that was seen during the incubation, which is most likely the result of heavy metal ions replacing protons. The gram-positive bacterium *Bacillus subtilis* has been shown to involve anionic surface groups in metal binding. Cation absorption by isolated B. subtilis cell walls was decreased by removing the teichoic acid moieties (phosphodiester groups) and lowering the quantity of free carboxyl groups. While some cationic metals, like lead, create negatively charged complexes in water, such Pb(OH)3- and Pb(OH)42, which can interact electrostatically with positively charged amino groups, others, like copper, prefer to bond to neutral amino groups (**Beveridge and Murray, 1980, Elsahoty et al., 2016**).According to **Doyle et al. (1980**), amino groups typically obstruct the interactions between cationic metals and anionic surface groups on microorganisms. By chemically neutralizing the negative charge of carboxyl and phosphoryl groups, the binding of cadmium and lead to *L. fermentum* ME3 and *B. longum* 46 was decreased. This suggested that both of these groups are important for binding these metals and could be the locations of ion exchange.

3.2. Effect of bacterial concentration on HM removal

The impact of bacterial concentration on the removal of heavy metals from intentionally contaminated water samples is shown in **Tables (5, 6, and 7**). It is evident that the binding of Cd, Pb, and As was improved by an increase in the bacterial concentration. Particularly, *L. johnsonii* ATCC 3320 as a single strain and the combination of *Bifidobacterium lactis strain Bb12*, *Lactobacillus johnsonii* ATCC 3320, *Lactobacillus salivarius* TISTR 390, *Lactobacillus paracasei* TISTR 453, and *Lactobacillus helveticus* LH-BO2 all showed significant HM removal at pH 7. A greater quantity of accessible phosphate groups on the bacterial surface may be the cause of these outcomes. The results obtained were comparable to those of **Davis et al. (2003**). There have been reports of various microbes favoring some metals over others (**Davis et al., 2003; Teemu et al., 2007, 2008**). We observed from the obtained results that Pb was removed at a higher rate than Cd. This could be because Pb has a larger ionic radius than Cd (**Davis et al. 2003**). The data also showed that HM removal increased when the biomass concentration of both freshly cultivated and lyophilized cells increased. This may be explained by a greater biomass/metal ratio, which indicates that there are more binding sites available. Other research with comparable findings have noted the impact of biomass concentration (**Esposito et al., 2002; Ngwenya et al., 2003**). **Teemu et al. (2007, 2008) and Puranik and Paknikar (1999)** noted that at high biomass concentrations, there was a decrease in HM elimination.The creation of cell aggregates that decrease the surface area accessible for binding or the sorption of metals to dissolved organic acids that obstruct sorption to bacterial surface structures could be the cause of this. When using lyophilized cells, the binding of HM increased quickly.

The binding of both Cd and Pb was improved by increasing the bacterial concentration; this could be because metals sorb to dissolved organic acids, which prevents them from sorbing to bacterial surface structures, or because cell aggregates form, which decreases the surface area available for binding (**Harvey and Leckey, 1985; Zoghi et al., 2014, Elsanhoty et al., 2016**). According to reports, when the negative charge of carboxyl and phosphoryl groups is neutralized by chemical alteration, the binding of Cd and Pb by *L. fermentum* and *B. longum* is decreased. Both groups may be the locations where ion exchange takes place and play a major part in the binding of these metals. A larger biomass/metal ratio (i.e., more binding

sites available) may account for the rise in cadmium, lead, and arsenic removal with increasing biomass concentration of both lyophilized and newly cultivated lactic acid bacteria. Other research have found that biomass concentration has a similar effect (**Esposito et al., 2002; Ngwenya et al., 2003).** High biomass concentrations have been found to reduce metal removal (**Harvey and Leckey, 1985; Puranik and Paknikar, 1999**). The creation of cell aggregates that decrease the surface area accessible for binding or the sorption of metals to dissolved organic acids that obstruct sorption to bacterial surface structures could be the cause of this. This work did not observe such a phenomena, most likely as a result of not using high bacterial concentrations.

3.3. Impact of contact time on HM removal

The influence of contact time (min) on HM binding is shown in Tables (**8, 9 and 10**). According to the data, LAB strains' capacity to eliminate HM increases with longer contact times. All strains have comparable effects on the elimination of Cd, Pb, and As. The removal increased nearly linearly with increasing contact time, with the maximum binding of Cd (85.9%), Pb (85.6%), and As (86.8%) occurring at the end of contact period (300 min).Our findings showed that LAB and the investigated bifidobacteria could quickly and effectively bind HM and AFB1 in water. Electrostatic interactions between HM cations and anionic functional groups are thought to initiate the binding process. Based on the results, a commercial bio-filter that removes aflatoxins and heavy metals from contaminated water might be made using LAB and bifidobacteria.Similar results were obtained by **Elsanhoty et al., 2016 and Amany et al. 2022)**

3.4. Effect of temperature on HM removal

It was investigated how temperature (˚C) affected the removal of heavy metals (HM) from tainted water samples (Tables 11, 12 and 13). It was observed that HM's binding was improved at higher temperatures. The findings showed that as the temperature increased, so did the binding and removal of HM. The results were consistent with those of **Cho and Kim (2003),** who discovered that raising the incubation temperature considerably improved the amount of Cd removed by new biomass. This might have happened as a result of Cd actively entering the bacterial cells. It has been shown that *L. plantarum* accumulates Cd by an energy-dependent mechanism (**Hao et al., 1999**).According to our findings, the microbes that were being studied were capable of extracting As from tainted water. It has been investigated how various bacteria remove As(V) from water. The data showed that electrostatic interactions between the biomass's cationic surface groups and anionic As species were involved. While native, non-viable *Penicillium chrysogenum* biomass was shown to remove As in study-like conditions, the binding of anionic As species was most likely caused by the positively charged amino groups. Furthermore, there was minimal impact of temperature on *L. rhamnosus's* removal of Cd and Pb (**Halttunen, 2007, Elsanhoty et al., 2016, Ma 2024**). Most of the time, heat treatment improves the removal of Cd; this is because heat treatment increases the number of metal binding sites on the bacterial surface (**Göksungur et al., 2005; Zoghi et al., 2014 Amal Raj et al., 2024**).

The removal of HM and AFB1 by the LAB under investigation, as well as their combination, was detailed in Table **14**. Pb and Cd removal rates were high, ranging from 41.5 to 65.8% and 24.5 to 59.9%, respectively. As removal ranged from 31.9 to 48.9%, whereas AFB1 removal ranged from 14.8% to 22.9%. Depending on the strain, HM and AFB1 were removed in this investigation.The results showed that LAB and bifidobacteria quickly generated binding of HM and AFB1 in the aqueous solution. Similar findings have previously been documented for other bacteria, including *Bacillus subtilis* and *Pseudomonas putida* (**Fein et al., 1997; Pardo et al., 2003, George et al., 2021**), as well as for *Bacillus subtilis* and Pseudomonas aeruginosa (**Tarangini, 2009; Davis et al., 2003; Mehta and Gaur, 2005; Romera et al., 2006; Teemu et al., 2007, 2008**). In these instances, passive attachment to the bacterial surface—rather than accumulating within the cell and microbial biomass—was necessary for fast absorption and uptake. The LAB changed the adsorption capacity of HM and AFB1. The strains, their mixing, and the growth phase all affected the removal procedure. Variations in the number of functional groups involved in HM binding and the functional group in the strains' cell walls may be the cause of the highest HM binding capacities observed in this investigation. Previous studies have demonstrated that the various binding conditions cause variations in

the biosorption of Cd by microorganisms (**Bhakta, et al., 2012; Halttunen et al., 2007; Ibrahim, Halttunen, Tahvonen, & Salminen, 2006; Zhai et al., 2015**). According to **Halttunen et al. (2008),***Propionibacterium freudenreichii* shermanii that had been lyophilized had the highest clearance rate (49.1%). The elimination of Cd and Pb exhibits two phase kinetics: a fast binding phase that is followed by a gradual, almost continuous removal phase that lasts for hours. The first phase was Pb sorption to the lipopolysaccharide layer's outermost structures, and the second was the gradual diffusion of Pb deeper within the layer (**Zoghi et al., 2014**).

According to **Haskard et al. (2001),** physical adsorption on the bacterial surface seems to be the primary mechanism for the removal of heavy metals and AFB 1, but it is only partially in charge of the removal of MC-LR (**Meriluoto et al., 2005; Nybom et al., 2007; Surono et al., 2007**). Consequently, a reduced surface area or fewer accessible binding sites could account for the lower than anticipated clearance with combination. Bacterial auto- or co-aggregation would reduce the total surface area of the biomass. A negative linear association between AFB1 elimination and aggregation index was found, which lends credence to this theory. Nevertheless, coaggregation by ccobination did not exceed the level that might be inferred from the single strains' autoaggregation results. The observed and expected values of the coaggregation index were nearly equal. Since the elimination of model toxins was less than expected, the coaggregation of bacteria cannot account for this.

3.5. Adhesion to *n*-hexadecane and *p*-xylene as affected by bacteria and their combinations

Data in table **14** presented the percentages of adhesion to n-hexadecane and p-xylene for *Lactobacillus johnsonii* ATCC 3320, *Bifidobacterium lactis* strain Bb12, *Lactobacillus salivarius* TISTR 390, *Lactobacillus paracasei* TISTR 453, and *Lactobacillus helveticus* LH-BO2L are shown in Table 14. P-xylene generally had a greater adhesion percentage than n-hexadecane. *Lactobacillus johnsonii* ATCC 3320, *Bifidobacterium lactis* strain Bb12, *Lactobacillus salivarius* TISTR 390, *Lactobacillus paracasei* TISTR 453, *Lactobacillus helveticus* LH-BO2L, and combination all showed high adhesion percentages to p-xylene. *Lactobacillus johnsonii* ATCC 3320, *Lactobacillus salivarius* TISTR 390, and their combination showed a lower linkage to n-hexadecane, ranging from 44.0 to 47.7%. Strain Bb12 of *Bifidobacteriumlactis* had an adhesion of 44..8%.The results were comparable to those of **Tobin et al. (1990) and Halttunen et al. (2007)**, who discovered that *Aspergillus niger*, *Rhizopus arrhizus*, and LAB were less able to remove Cd, Pb, and As when carboxyl and phosphoryl groups were chemically modified. Previous findings suggested that HM and LAB were involved in electrostatic interactions (**Halttunen et al. 2007, Elsanhoty et al., 2016**). Bifidobacteria have also been shown to auto-aggregate due to hydrophobic interactions (**Canzi et al. 2005**).

According to **Haskard et al. (2000),** different binding-elimination processes and strain-specific traits most likely contributed to the lack of relationship between the removal of HM and AFB1. AFB1 seems to be mostly connected to surface proteins and carbohydrates by hydrophobic contacts, even while ion exchange to surface constituents seems to be significant in HM binding (**Lahtinen et al. 2004**), (**Halttunen et al. 2007**). Physical adsorption to the bacterial surface seems to be the main mechanism for the removal of HM (**Halttunen et al. 2007; Halttunen et al. 2008**) and AFB1 (**Haskard et al. 2001**).

Ngwenya et al. (2003) reported the surface chemistry characteristics of two gram-positive (*Bacillus subtilis and Bacillus licheniformis*) and two gram-negative (unknown and Schewanella putrefaciens) bacteria. They discovered that the carboxyl, phosphoryl, and hydroxyl/amine group surface densities were all marginally higher in the gram-positive strains. The amount of phosphoryl and hydroxyl/amine groups in the two gram-positive Bacillus species was also found to differ by a factor of two. It has been determined that the growth phase of B. subtilis affects the surface densities and deprotonation constants of its functional groups (**Daughney et al., 2001**).

According to **Daughney et al. (2001),** as biomass progressed from the exponential to the stationary and sporulated phases of growth, the surface densities and deprotonation constants of carboxyl, phosphoryl, and amine groups declined. **Chang et al. (1997)** showed a decrease in cadmium removal when using older

Pseudomonas aeruginosa cells, which supports our findings.However, the opposite was observed when lead removal was studied with the same biomass. (**Fein et al., 1997; Ngwenya et al., 2003**) reported that stability constants of a given metal vary with functional groups. Therefore, the observed inter-biomass differences in maximum metal binding capacities in this work may be attributed to differences in the quantity of functional groups involved in metal binding.

3.6.Effect of LAB and their combination on the removal of HM and AFB1

The dates in Table 15 detailed how certain LABs removed HM and AFB1 together. Pb and Cd were removed at higher rates, ranging from 28.3-59.9% and 72.6%, respectively. While the removal of AFB1 ranged from 14.8% to 22.7%, the removal of As was 49.8%. Depending on the strain, all poisons were eliminated in this investigation. The results showed that LAB and bifidobacteria quickly generated binding of HM and AFB1 in the aqueous solution. Similar findings have previously been documented for other bacteria, including *Bacillus subtilis* and *Pseudomonas putida***(Fein et al., 1997; Pardo et al., 2003),**as well as for *Bacillus subtilis* and *Pseudomonas aeruginosa* (**Tarangini, 2009; Davis et al., 2003; Mehta and Gaur, 2005; Romera et al., 2006; Teemu et al., 2007, 2008)**. In these cases, rapid absorption and uptake were dependent on passive binding to the bacterial surface.The LAB changed the adsorption capacity of HM and AFB1. The strains, their mixing, and the growth phase all affected the removal procedure. Variations in the number of functional groups involved in HM binding and the functional group in the strains' cell walls may be the cause of the highest HM binding capacities observed in this investigation.The biosorption of Cd by microorganisms varies from study to study because of the various binding conditions, according to previous reports (**Halttunen et al., 2007; Ibrahimet al., 2006; Zhai et al., 2015)**. The highest clearance rate (49.1%) was found in the lyophilized biomass of *Propionibacterium freudenreichii* shermanii (**Halttunen et al., 2008**). The elimination of Cd and Pb exhibits two phase kinetics: a fast binding phase that is followed by a gradual, almost continuous removal phase that lasts for hours. The first phase was Pb sorption to the lipopolysaccharide layer's outermost structures, and the second was the gradual diffusion of Pb deeper within the layer (**Harvey and Leckey, 1985; Zoghi et al., 2014**, **Elsanhoty et al., 2016, Amany et al., 2022, Rachwał, and; Gustaw, 2024)**.

4. Conclusion

Our results demonstrated that LAB and the bifidobacteria under study were capable of efficiently and rapidly binding HM and AFB1 in contaminated water.The strain *Lactobacillus johnsonii* ATCC 3320 (E) and the combination of lactic acid bacteria and bifidobacteria were given the highest capability for removing of heavy metals and aflatoxin B1 from contaminated water.Electrostatic interactions between HM cations and anionic functional groups in the strain's cell wall were thought to initiate the binding process. A commercial bio-filter that eliminates aflatoxins and heavy metals from contaminated water may be created utilizing LAB and bifidobacteria based on the results that were achieved.

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Table 1.Bacterial strains' origin and conditions of incubation

Strain	**Origin***	**Oxygen requirement**
Lactobacillus salivarius TISTR 390	**1**	Aerobic
Lactobacillus paracasei TISTR 453	**2**	Aerobic
Lactobacillus helveticus LH-BO2	**2**	Aerobic
Bifidobacterium lactis strain Bb12	**3**	Anaerobic
Lactobacillus johnsonii ATCC 3320	**1**	Aerobic

***(1)** Egyptian Microbial culture collection (EMCC) at Cairo Microbiological Resources Centre (Cairo MIRCEN), Faculty of Agriculture, Ain Shams University (Egypt).

(2) Thailand Institute of Scientific and Technological Research (Bangkok, Thailand).

(3) Germany Centre for culturecollection (DSMZ-Germany).

Table 2: Impact of pH on various LAB strains' cadmium binding. Mean of at least two replicates. Initial c(Cd2+)= 10 mg/l.

strains	**% removal of cadmium**						
	pH						
	1	2	3	4	5	6	7
Lactobacillus salivarius TISTR 390 **(A)**	3.4	5.3	13.4	27.7	31.2	44.9	56.9
*Lactobacillus paracasei TISTR 453**(B)***	2.9	5.2	12.8	29.1	32	49.3	59.9
Lactobacillus helveticus LH-BO2**(C)**	3.1	5.5	14.9	30.7	32.5	50.2	62.9
Bifidobacterium lactis strain Bb12***(D)***	2.1	5.4	15.2	29.9	32.8	51.9	61.7
*Lactobacillus johnsonii ATCC 3320**(E)***	4.0	5.5	16.3	30.9	34.4	53.3	63.5

Bifidobacterium lactis strain Bb12 + *Lactobacillus salivarius TISTR 390****(F)***	2.98	5.3	12.8	29.8	33.6	51.8	61.0
Bifidobacterium lactis strain Bb12 + *Lactobacillus paracasei TISTR 453****(G)***	3.2	5.5	13.2	30.9	34.5	52.7	62.2
Lactobacillus paracasei TISTR 453 + *Lactobacillus salivarius TISTR 390****(H)***	3.4	5.6	13.5	33.1	36.3	52.9	63.4
Bifidobacterium lactis strain Bb12 + *Lactobacillus Lactobacillus helveticus****(I)***	3.5	5.5	13.1	32.3	37.9	31	63.7
Bifidobacterium lactis strain Bb12 +*Lactobacillus johnsonii ATCC 3320H* ***(J)***	3.5	5.6	14.2	31.9	35.3	51.2	62.2
Lactobacillus johnsonii ATCC 3320 + *Bifidobacterium lactis* strain Bb12 + *Lactobacillus salivarius TISTR 390****(K)***	3.6	5.7	15.1	32.4	36.9	52.7	64.2
Lactobacillus johnsonii ATCC 3320 + *Bifidobacterium lactis* strain Bb12 + *Lactobacillus salivarius TISTR 390* + *Lactobacillus paracasei TISTR 453****(L)***	3.9	5.8	15.7	32.8	38.8	52.9	65.1
Lactobacillus johnsonii ATCC 3320 + *Bifidobacterium lactis* strain Bb12 + *Lactobacillus salivarius TISTR 390+ Lactobacillus paracasei TISTR 453+ Lactobacillus helveticus* LH-BO2 *(Estimation of Com****)(M)***	3.7	5.6	15.6	32.6	37.8	52.1	64.5

Table 3: Impact of pH on various LAB strains' lead by various LAB strains. Mean of at least two replicates.

strains	**% removal of lead**						
	Bacterial concentration (Biomass)						
	1	2	3	4	5	6	7
Lactobacillus salivarius TISTR 390 **(A)**	3.1	5.4	14.5	28.7	34.2	51.2	55.9
*Lactobacillus paracasei TISTR 453****(B)***	2.8	5.7	12.9	28.8	33	48.2	58.9
Lactobacillus helveticus LH-BO2**(C)**	3.3	5.8	17.9	31.6	38.5	53.2	69.9

Bifidobacterium lactis strain Bb12***(D)***	2.6	5.8	16.3	32.9	35.8	52.9	67.9
*Lactobacillus johnsonii ATCC 3320**(E)***	3.3	5.9	17.6	34.9	38.7	53..8	68.8
Bifidobacterium lactis strain Bb12 + *Lactobacillus salivarius TISTR 390**(F)***	3.2	5.8	13.9	28.9	34.6	53	69.9
Bifidobacterium lactis strain Bb12 + *Lactobacillus paracasei TISTR 453**(G)***	3.1	5.7	14.8	30.8	36.9	54.1	68.2
*Lactobacillus paracasei TISTR 453 + Lactobacillus salivarius TISTR 390**(H)***	3.4	5.8	14.7	32	37.4	55.1	70.7
Bifidobacterium lactis strain Bb12 + *Lactobacillus Lactobacillus helveticus**(I)***	3.4	6	15.5	32.8	38.2	55.3	71.1
Bifidobacterium lactis strain Bb12 *+Lactobacillus johnsonii ATCC 3320H **(J)***	3.0	5.7	14.4	31.2	32.8	51.8	66.2
Lactobacillus johnsonii ATCC 3320 + Bifidobacterium lactis strain Bb12 + *Lactobacillus salivarius TISTR 390**(K)***	3.2	5.9	15.4	33.2	32.7	52.1	68.1
Lactobacillus johnsonii ATCC 3320 + Bifidobacterium lactis strain Bb12 + *Lactobacillus salivarius TISTR 390 + Lactobacillus paracasei TISTR 453**(L)***	3.1	5.8	15.7	33.3	32.8	53.2	67.5
Lactobacillus johnsonii ATCC 3320 + Bifidobacterium lactis strain Bb12 + *Lactobacillus salivarius TISTR 390+ Lactobacillus paracasei TISTR 453+ Lactobacillus helveticus* LH-BO2 *(Estimation of Com)**(M)***	3.7	6.0	16.2	34.8	36.9	58.8	70.1

Table 4: Impact of pH on various LAB strains' arsenic binding by different LAB strains. Mean of at least two replicates are shown. Initial c(Cd2+)= 10 mg/l.

strains	**% removal of arsenic**						
	Bacterial concentration (Biomass)						
	1	2	3	4	5	6	7
Lactobacillus salivarius TISTR 390 **(A)**	2.6	5.1	15.2	27.6	32.1	50.2	58.9
*Lactobacillus paracasei TISTR 453**(B)***	2.9	5.5	13.8	26.8	34.6	49.2	58.9

Lactobacillus helveticus LH-BO2**(C)**	3.3	5.8	18.8	32.5	38.5	53.2	67.9
Bifidobacterium lactis strain Bb12***(D)***	3.4	6.4	19.5	33.9	39.8	55.9	74.7
*Lactobacillus johnsonii ATCC 3320**(E)***	2.7	5.5	14.8	26.9	33.4	52..8	56.8
Bifidobacterium lactis strain Bb12 + *Lactobacillus salivarius TISTR 390**(F)***	3.2	5.9	13.8	29.9	35.6	54.8	69.9
Bifidobacterium lactis strain Bb12 + *Lactobacillus paracasei TISTR 453**(G)***	3.4	5.9	15.7	31.7	37.8	55.6	68.4
*Lactobacillus paracasei TISTR 453 + Lactobacillus salivarius TISTR 390**(H)***	3.3	6.1	17.3	32.7	37.7	56.7	70.8
Bifidobacterium lactis strain Bb12 + *Lactobacillus Lactobacillus helveticus**(I)***	3.4	6.2	14.8	31.8	39.2	56.3	70.1
Bifidobacterium lactis strain Bb12 *+Lactobacillus johnsonii ATCC 3320H* ***(J)***	2.9	5.6	12.4	30.2	31.8	51.8	64.2
Lactobacillus johnsonii ATCC 3320 + Bifidobacterium lactis strain Bb12 + *Lactobacillus salivarius TISTR 390**(K)***	3.2	5.9	13.8	29.9	35.6	54.8	69.9
Lactobacillus johnsonii ATCC 3320 + Bifidobacterium lactis strain Bb12 + *Lactobacillus salivarius TISTR 390 + Lactobacillus paracasei TISTR 453**(L)***	3.3	6.1	17.3	32.7	37.7	56.7	70.8
Lactobacillus johnsonii ATCC 3320 + Bifidobacterium lactis strain Bb12 + *Lactobacillus salivarius TISTR 390+ Lactobacillus paracasei TISTR 453+ Lactobacillus helveticus* LH-BO2 *(Estimation of Com**)(M)***	3.5	6.5	19.8	34.5	39.9	56.9	74.8

Table 5. Impact of bacterial biomass (concentration) on the elimination of cadmium by various LAB strain. Mean of at least two replicates. Initial c(Cd2+)=10 mg/l.

strains	**Bacterial concentration (Biomass) g/L**					
	% removal of arsenic					
	0.5	0.75	1	1.25	1.5	2
Lactobacillus salivarius TISTR 390 **(A)**	32	54	63.4	74.7	84.9	90.3

*Lactobacillus paracasei TISTR 453**(B)***	33	54.2	64.3	71.2	82.2	89.9
Lactobacillus helveticus LH-BO2**(C)**	38	58.1	67.9	75.6	88..8	92.2
Bifidobacterium lactis strain Bb12***(D)***	39	58.9	67.3	76.9	88.8	92.2
*Lactobacillus johnsonii ATCC 3320**(E)***	31	56.5	62.6	71.9	82.4	92.2
Bifidobacterium lactis strain Bb12 + *Lactobacillus salivarius TISTR 390**(F)***	29.3	55.8	63.4	72.5	81.9	91.7
Bifidobacterium lactis strain Bb12 + *Lactobacillus paracasei TISTR 453**(G)***	30.2	54.2	66.9	74.3	83.4	92.6
*Lactobacillus paracasei TISTR 453 + Lactobacillus salivarius TISTR 390**(H)***	29.8	56.4	69.9	77.8	86.2	93.8
Bifidobacterium lactis strain Bb12 + *Lactobacillus Lactobacillus helveticus**(I)***	31.6	55.9	67.6	78.2	86.9	94.9
Bifidobacterium lactis strain Bb12 *+Lactobacillus johnsonii ATCC 3320H **(J)***	30.7	52.3	66.1	74.9	83.9	89.6
Lactobacillus johnsonii ATCC 3320 + Bifidobacterium lactis strain Bb12 + *Lactobacillus salivarius TISTR 390**(K)***	32.2	53.4	67.2	75.1	83.3	87.2
Lactobacillus johnsonii ATCC 3320 + Bifidobacterium lactis strain Bb12 + *Lactobacillus salivarius TISTR 390 + Lactobacillus paracasei TISTR 453**(L)***	32.1	53.8	67.2	75.2	83.7	88.6
Lactobacillus johnsonii ATCC 3320 + Bifidobacterium lactis strain Bb12 + *Lactobacillus salivarius TISTR 390+ Lactobacillus paracasei TISTR 453+ Lactobacillus helveticus* LH-BO2 *(Estimation of Com)**(M)***	39.1	59.9	67.4	76.9	88.9	92.2

Table 6. **Impact of bacterial biomass (concentration) on lead elimination by various LAB strains. Mean of at least two replicates. Initial c(Pb2+)=50 mg/l.**

strains	**Bacterial concentration (Biomass) g/L**
	% removal of cadmium

	0.5	0.75	1	1.25	1.5	2
Lactobacillus salivarius TISTR 390 **(A)**	29.5	44.2	51.4	61.7	72.9	80.1
*Lactobacillus paracasei TISTR 453**(B)***	31.3	45.2	54.3	61.2	71.2	82.9
Lactobacillus helveticus LH-BO2**(C)**	34.2	46.2	54.4	62.1	72.9	83.3
Bifidobacterium lactis strain Bb12***(D)***	38	48.1	57.9	65.6	75..8	85.6
*Lactobacillus johnsonii ATCC 3320**(E)***	36	46.2	53.3	63.9	72.4	83.2
Bifidobacterium lactis strain Bb12 + *Lactobacillus salivarius TISTR 390**(F)***	31	46.5	52.6	61.9	70.8	82.2
Bifidobacterium lactis strain Bb12 + *Lactobacillus paracasei TISTR 453**(G)***	32.1	46.2	51.8	60.9	71.2	82.1
*Lactobacillus paracasei TISTR 453 + Lactobacillus salivarius TISTR 390**(H)***	33.1	45.2	52.2	61.2	72.8	81.9
Bifidobacterium lactis strain Bb12 + *Lactobacillus Lactobacillus helveticus**(I)***	34.3	44.9	51.9	66.1	73.2	84.9
Bifidobacterium lactis strain Bb12 *+Lactobacillus johnsonii ATCC 3320H **(J)***	34.5	45.3	52.3	67.1	76.2	83.9
Lactobacillus johnsonii ATCC 3320 + Bifidobacterium lactis strain Bb12 + *Lactobacillus salivarius TISTR 390**(K)***	32.9	44.2	50.2	62.1	69.8	81.2
Lactobacillus johnsonii ATCC 3320 + Bifidobacterium lactis strain Bb12 + *Lactobacillus salivarius TISTR 390 + Lactobacillus paracasei TISTR 453**(L)***	32.4	45.1	51.2	62.3	69.5	81.7
Lactobacillus johnsonii ATCC 3320 + Bifidobacterium lactis strain Bb12 + *Lactobacillus salivarius TISTR 390+ Lactobacillus paracasei TISTR 453+ Lactobacillus helveticus* LH-BO2 *(Estimation of Com**)(M)***	37.1	48.1	56.8	63.1	71.9	83.9

Table 7.Impact of bacterial biomass (concentration) on the elimination of arsenic by various LAB strains. Mean of at least two replicates. Initial c(Pb2+)=50 mg/l.

strains	**Bacterial concentration (Biomass) g/L**					
	% removal of cadmium					
	0.5	0.75	1	1.25	1.5	2
Lactobacillus salivarius TISTR 390 **(A)**	22.5	39.2	48.4	60.7	71.9	80.2
*Lactobacillus paracasei TISTR 453**(B)***	24.3	40.2	51.3	61.3	70.2	81.9
Lactobacillus helveticus LH-BO2**(C)**	27.2	43.1	57.8	66.6	78..8	85.9
Bifidobacterium lactis strain Bb12***(D)***	30.2	44.2	58.3	68.9	78.9	86.2
*Lactobacillus johnsonii ATCC 3320**(E)***	26.0	40.5	51.6	61.9	70.7	80.2
Bifidobacterium lactis strain Bb12 + *Lactobacillus salivarius TISTR 390**(F)***	29.2	44.2	53.8	63.9	74.2	82.9
Bifidobacterium lactis strain Bb12 + *Lactobacillus paracasei TISTR 453**(G)***	31.1	45.2	53.2	65.2	71.8	84.8
*Lactobacillus paracasei TISTR 453 + Lactobacillus salivarius TISTR 390**(H)***	33.3	45.9	53.9	66.3	74.2	85.9
Bifidobacterium lactis strain Bb12 + *Lactobacillus Lactobacillus helveticus**(I)***	33.5	45.7	54.3	68.2	77.2	84.8
Bifidobacterium lactis strain Bb12 +*Lactobacillus johnsonii ATCC 3320H **(J)***	29.2	44.2	50.5	60.1	67.8	81.2
Lactobacillus johnsonii ATCC 3320 + Bifidobacterium lactis strain Bb12 + *Lactobacillus salivarius TISTR 390**(K)***	33.2	45.8	53.8	66.2	74.2	85.8
Lactobacillus johnsonii ATCC 3320 + Bifidobacterium lactis strain Bb12 + *Lactobacillus salivarius TISTR 390 + Lactobacillus paracasei TISTR 453**(L)***	31.9	44.9	57.8	66.7	74.3	85.9
Lactobacillus johnsonii ATCC 3320 + Bifidobacterium lactis strain Bb12 + *Lactobacillus salivarius TISTR 390+*	31.2	44.1	58.1	68.8	78.7	86.3

Lactobacillus paracasei TISTR 453+ Lactobacillus helveticus LH-BO2 *(Estimation of Com)**(M)***						

Table 8. **Effect of contact time on cadmium removal by different LAB strains. Mean of at least two replicates, Initial c(Pb2+)=50 mg/l.**

Strains	**% removal of cadmium**					
	Contact time (min)					
	30	60	120	180	240	300
Lactobacillus salivarius TISTR 390 **(A)**	21.5	38.1	48.3	61.7	70.9	81.1
*Lactobacillus paracasei TISTR 453**(B)***	24.6	40.2	51.2	61.6	71.2	81.7
Lactobacillus helveticus LH-BO2**(C)**	26.2	42.9	57.7	65.7	77..8	86.8
Bifidobacterium lactis strain Bb12***(D)***	29.4	41.2	55.4	64.9	76.4	85.3
*Lactobacillus johnsonii ATCC 3320**(E)***	26.0	40.5	51.6	61.9	70.7	80.2
Bifidobacterium lactis strain Bb12 + *Lactobacillus salivarius TISTR 390**(F)***	29.4	44.3	52.8	64.9	74.4	82.8
Bifidobacterium lactis strain Bb12 + *Lactobacillus paracasei TISTR 453**(G)***	30.3	45.7	53.6	65.7	71.9	84.7
*Lactobacillus paracasei TISTR 453 + Lactobacillus salivarius TISTR 390**(H)***	33.5	46.9	54.8	67.3	74.5	85.8
Bifidobacterium lactis strain Bb12 + *Lactobacillus Lactobacillus helveticus**(I)***	33.9	45.7	56.3	67.2	77.2	84.8
Bifidobacterium lactis strain Bb12 *+Lactobacillus johnsonii ATCC 3320H **(J)***	27.2	43.2	49.5	60.3	65.8	79.7
Lactobacillus johnsonii ATCC 3320 + Bifidobacterium lactis strain Bb12 + *Lactobacillus salivarius TISTR 390**(K)***	31.7	44.2	47.8	59.9	65.2	83.7
Lactobacillus johnsonii ATCC 3320 + Bifidobacterium lactis strain Bb12 + *Lactobacillus salivarius TISTR 390 +*	32.7	45.7	48.2	60.2	67.2	85.8

*Lactobacillus paracasei TISTR 453**(L)***						
Lactobacillus johnsonii ATCC 3320 + *Bifidobacterium lactis* strain Bb12 + *Lactobacillus salivarius TISTR 390*+ *Lactobacillus paracasei TISTR 453*+ *Lactobacillus helveticus* LH-BO2 *(Estimation of Com)**(M)***	34.9	45.9	49.1	61.6	68.2	85.9

Table 9. **Effect of contact time on lead removal by different LAB strains. Mean of at least two replicates are shown. Initial c(Pb2+)=50 mg/l.**

strains	**% removal of lead**					
	Contact time (min)					
	30	60	120	180	240	300
Lactobacillus salivarius TISTR 390 **(A)**	20.6	32.2	44.4	59.7	67.9	79.9
*Lactobacillus paracasei TISTR 453**(B)***	23.3	40.3	48.3	61.4	70.2	79.9
Lactobacillus helveticus LH-BO2**(C)**	26.2	42.1	52.8	65.6	76..8	82.5
Bifidobacterium lactis strain Bb12***(D)***	28.4	42.6	54.3	63.9	77.6	83.2
*Lactobacillus johnsonii ATCC 3320**(E)***	19.6	38.7	46.7	56.9	63.7	74.72
Bifidobacterium lactis strain Bb12 + *Lactobacillus salivarius TISTR 390**(F)***	29.7	44.5	54.9	62.9	75.9	83.1
Bifidobacterium lactis strain Bb12 + *Lactobacillus paracasei TISTR 453**(G)***	30.1	45.3	52.3	64.3	74.8	84.6
Lactobacillus paracasei TISTR 453 + *Lactobacillus salivarius TISTR 390**(H)***	33.6	45.8	54.8	65.6.	74.5	85.6
Bifidobacterium lactis strain Bb12 + *Lactobacillus Lactobacillus helveticus**(I)***	33.4	44.7	54.9	69.2	76.2	85.3
Bifidobacterium lactis strain Bb12 +*Lactobacillus johnsonii ATCC 3320H* ***(J)***	29.2	39.9	48.9	60.2	67.9	78.9
Lactobacillus johnsonii ATCC 3320 + *Bifidobacterium lactis* strain Bb12 +	33.7	39.8	52.9	63.6	70.2	78.9

*Lactobacillus salivarius TISTR 390**(K)***						
Lactobacillus johnsonii ATCC 3320 + *Bifidobacterium lactis* strain Bb12 + *Lactobacillus salivarius TISTR 390* + *Lactobacillus paracasei TISTR 453**(L)***	33.9	40.3	52.7	62.7	71.1	73.7
Lactobacillus johnsonii ATCC 3320 + *Bifidobacterium lactis* strain Bb12 + *Lactobacillus salivarius TISTR 390*+ *Lactobacillus paracasei TISTR 453*+ *Lactobacillus helveticus* LH-BO2 *(Estimation of Com)**(M)***	34.2	41.2	53.1	64.9	74.9	83.3

Table 10. **Effect of contact time on arsenic removal by different LAB strains. Mean of at least two replicates and standard deviation are shown. Initial c(Pb2+)=50 mg/l.**

strains	**% removal of arsenic**					
	Contact time (min)					
	30	60	120	180	240	300
Lactobacillus salivarius TISTR 390 **(A)**	20.6	32.2	44.4	59.7	67.9	79.9
*Lactobacillus paracasei TISTR 453**(B)***	23.3	40.3	48.3	61.4	70.2	79.9
Lactobacillus helveticus LH-BO2**(C)**	26.2	42.1	52.8	65.6	76..8	82.5
Bifidobacterium lactis strain Bb12***(D)***	28.4	42.6	54.3	63.9	77.6	83.2
*Lactobacillus johnsonii ATCC 3320**(E)***	19.6	38.7	46.7	56.9	63.7	74.72
Bifidobacterium lactis strain Bb12 + *Lactobacillus salivarius TISTR 390**(F)***	29.7	44.5	54.9	62.9	75.9	83.1
Bifidobacterium lactis strain Bb12 + *Lactobacillus paracasei TISTR 453**(G)***	30.1	45.3	52.3	64.3	74.8	84.6
Lactobacillus paracasei TISTR 453 + *Lactobacillus salivarius TISTR 390**(H)***	33.6	45.8	54.8	65.6.	74.5	85.6
Bifidobacterium lactis strain Bb12 + *Lactobacillus Lactobacillus helveticus**(I)***	33.4	44.7	54.9	69.2	76.2	85.3
Bifidobacterium lactis strain Bb12 +*Lactobacillus johnsonii ATCC 3320H* ***(J)***	29.2	39.9	48.9	60.2	67.9	78.9
Lactobacillus johnsonii ATCC 3320 +	32.7	42.8	51.9	63.2	69.9	82.1

Bifidobacterium lactis strain Bb12 + *Lactobacillus salivarius TISTR 390**(K)***						
Lactobacillus johnsonii ATCC 3320 + *Bifidobacterium lactis* strain Bb12 + *Lactobacillus salivarius TISTR 390* + *Lactobacillus paracasei TISTR 453**(L)***	33.2	43.7	52.9	64.2	74.4	85.8
Lactobacillus johnsonii ATCC 3320 + *Bifidobacterium lactis* strain Bb12 + *Lactobacillus salivarius TISTR 390*+ *Lactobacillus paracasei TISTR 453*+ *Lactobacillus helveticus* LH-BO2 *(Estimation of Com**)(M)***	33.8	44.9	53.7	66.9	74.9	86.8

Table 11: Effect of temperature on cadmium binding by different LAB strains and bifidobacteria. Mean of at least two replicates. Initial cadmium was 40 mg/l.

strains	**% removal of cadmium**						
	temperature						
	25	30	35	40	45	50	55
Lactobacillus salivarius TISTR 390 **(A)**	3.2	5.2	11.5	27.8	34.3	44.8	45.8
*Lactobacillus paracasei TISTR 453**(B)***	3.2	5.1	12.8	29.8	33	42.3	42.9
Lactobacillus helveticus LH-BO2**(C)**	3.9	5.8	15.4	34.5	39.6	54.3	54.5
Bifidobacterium lactis strain Bb12***(D)***	2.8	5.7	15.2	29.9	33.8	50.9	52.7
*Lactobacillus johnsonii ATCC 3320**(E)***	2.9	5.3	11.3	28.9	31.4	47.9	49.5
Bifidobacterium lactis strain Bb12 + *Lactobacillus salivarius TISTR 390**(F)***	2.8	5.7	12.8	28.8	33.9	52.8	54.0
Bifidobacterium lactis strain Bb12 + *Lactobacillus paracasei TISTR 453**(G)***	3.2	5.5	13.2	30.9	33.5	52.6	58.2
Lactobacillus paracasei TISTR 453 + *Lactobacillus salivarius TISTR 390**(H)***	3.3	5.8	14.3	33.1	36.3	52.9	62.6
Bifidobacterium lactis strain Bb12 + *Lactobacillus Lactobacillus helveticus**(I)***	3.8	5.7	13.6	32.9	37.4	54.1	62.7
Bifidobacterium lactis strain Bb12 +*Lactobacillus johnsonii ATCC 3320H **(J)***	3.7	5.8	13.9	33.3	34.8	52.3	57.8
Lactobacillus johnsonii ATCC 3320 +	3.6	5.6	13.9	33.7	36.7	53.4	58.9

Bifidobacterium lactis strain Bb12 + *Lactobacillus salivarius TISTR 390**(K)***							
Lactobacillus johnsonii ATCC 3320 + *Bifidobacterium lactis* strain Bb12 + *Lactobacillus salivarius TISTR 390* + *Lactobacillus paracasei TISTR 453**(L)***	3.8	5.8	14.2	32.8	39.2	54.0	59.5
Lactobacillus johnsonii ATCC 3320 + *Bifidobacterium lactis* strain Bb12 + *Lactobacillus salivarius TISTR 390*+ *Lactobacillus paracasei TISTR 453*+ *Lactobacillus helveticus* LH-BO2 *(Estimation of Com)**(M)***	3.9	6.0	14.9	34.8	39.0	54.2	62.0

Table 12: Effect of temperature on lead binding by different LAB strains. Mean of at least two replicates. Initial lead concentration was 40 mg/l.

strains	**% removal of lead**						
	temperature						
	25	30	35	40	45	50	55
Lactobacillus salivarius TISTR 390 **(A)**	2.3	3.4	10.5	26.7	31.2	47.2	51.6
*Lactobacillus paracasei TISTR 453**(B)***	2.7	3.7	11.8	27.8	32.1	47.2	53.9
Lactobacillus helveticus LH-BO2**(C)**	3.3	6.5	13.9	35.5	38.5	54.2	68.8
Bifidobacterium lactis strain Bb12***(D)***	2.7	4.7	14.3	31.9	36.8	51.9	67.9
*Lactobacillus johnsonii ATCC 3320**(E)***	2.8	5.3	14.2	31.2	37.4	53..8	56.7
Bifidobacterium lactis strain Bb12 + *Lactobacillus salivarius TISTR390**(F)***	3.3	5.9	13.9	32.9	37.6	53.9	69.8
Bifidobacterium lactis strain Bb12 + *Lactobacillus paracasei TISTR 453**(G)***	3.8	5.7	14.9	33.8	36.9	54.1	68.9
Lactobacillus paracasei TISTR 453 + *Lactobacillus salivarius TISTR 390**(H)***	3.6	5.9	15.7	32.9	37.6	55.4	70.8
Bifidobacterium lactis strain Bb12 + *Lactobacillus Lactobacillus helveticus**(I)***	3.9	6.1	16	33.8	38.2	55.3	71.9
Bifidobacterium lactis strain Bb12 +*Lactobacillus johnsonii ATCC 3320H* ***(J)***	3.8	4.9	15.7	33.9	33.1	52.3	59.8
Lactobacillus johnsonii ATCC 3320 + *Bifidobacterium lactis* strain Bb12 + *Lactobacillus salivarius TISTR 390**(K)***	3.9	5.8	15.9	34.8	35.9	53.2	57.9

Lactobacillus johnsonii ATCC 3320 + *Bifidobacterium lactis* strain Bb12 + *Lactobacillus salivarius TISTR 390* + *Lactobacillus paracasei TISTR 453**(L)***	3.7	5.7	14.9	34.9	36.8	54.1	59.7
Lactobacillus johnsonii ATCC 3320 + *Bifidobacterium lactis* strain Bb12 + *Lactobacillus salivarius TISTR 390*+ *Lactobacillus paracasei TISTR 453*+ *Lactobacillus helveticus* LH-BO2 *(Estimation of Com)**(M)***	3.9	5.9	15.9	35.2	37.6	55.0	69.0

Table 13: Effect of temperature on arsenic binding by different LAB strains. Mean of at least two replicates. Initial arsenic 40 mg/l.

strains	**% removal of arsenic**						
	temperature						
	25	30	35	40	45	50	55
Lactobacillus salivarius TISTR 390 **(A)**	2.4	3.2	11.4	27.8	32	46.7	57.1
*Lactobacillus paracasei TISTR 453**(B)***	2.6	4.6	12.8	28.8	46.8	46.2	57.9
Lactobacillus helveticus LH-BO2**(C)**	4.3	6.7	14.9	36.6	39.3	53.9	69.9
Bifidobacterium lactis strain Bb12***(D)***	4.2	6.0	16.4	33.8	36.9	52.9	69.2
*Lactobacillus johnsonii ATCC 3320**(E)***	2.7	5.4	14.1	26.2	30.4	49..8	53.8
Bifidobacterium lactis strain Bb12 + *Lactobacillus salivarius TISTR 390**(F)***	4.1	5.9	13.9	35	42.5.	53.8	72.9
Bifidobacterium lactis strain Bb12 + *Lactobacillus paracasei TISTR 453**(G)***	3.2	5.8	15.8	31.8	37.9	55.2	69.9
Lactobacillus paracasei TISTR 453 + *Lactobacillus salivarius TISTR 390**(H)***	4.9	6	16.7	37.6	41.2	57.8	74.9
Bifidobacterium lactis strain Bb12 + *Lactobacillus Lactobacillus helveticus**(I)***	3.4	6.2	17.8	36.8	39.9	56.8	73.1
Bifidobacterium lactis strain Bb12 +*Lactobacillus johnsonii ATCC 3320H* ***(J)***	3.4	5.8	14.8	35.3	39.7	50.5	63.8
Lactobacillus johnsonii ATCC 3320 + *Bifidobacterium lactis* strain Bb12 + *Lactobacillus salivarius TISTR 390**(K)***	3.9	6.2	15.2	34.9	41.7	51.4	68.6
Lactobacillus johnsonii ATCC 3320 + *Bifidobacterium lactis* strain Bb12 +	3.8	6.3	15.9	35	42.9	53.6	69.5

*Lactobacillus salivarius TISTR 390 + Lactobacillus paracasei TISTR 453**(L)***							
Lactobacillus johnsonii ATCC 3320 + Bifidobacterium lactis strain Bb12 + *Lactobacillus salivarius TISTR 390+ Lactobacillus paracasei TISTR 453+ Lactobacillus helveticus* LH-BO2 *(Estimation of Com)**(M)***	4.0	6.5	16.2	38.2	45.2	54.0	69.9

Table 14. Adhesion percentages of bacteria under study to to *n*-hexadecane and *p*-xylene

Strain(s)	Adhesion percentages	
	***n*-hexadecane**	***p*-xylene**
Lactobacillus salivarius TISTR 390 **(A)**	29.9	34.7
*Lactobacillus paracasei TISTR 453**(B)***	49.8	77.9
Lactobacillus helveticus LH-BO2**(C)**	45.8	75.6
Bifidobacterium lactis strain Bb12***(D)***	44.8	65.8
*Lactobacillus johnsonii ATCC 3320**(E)***	44.7	57.6
Bifidobacterium lactis strain Bb12 + *Lactobacillus salivarius TISTR 390**(F)***	40.5	68.6
Bifidobacterium lactis strain Bb12 + *Lactobacillus paracasei TISTR 453**(G)***	45.5	79.1
*Lactobacillus paracasei TISTR 453 + Lactobacillus salivarius TISTR 390**(H)***	46.4	74.1
Bifidobacterium lactis strain Bb12 + *Lactobacillus Lactobacillus helveticus**(I)***	44.9	75.6
Bifidobacterium lactis strain Bb12 +*Lactobacillus johnsonii ATCC 3320H **(J)***	45.4	75.7
Lactobacillus johnsonii ATCC 3320 + Bifidobacterium lactis strain Bb12 + *Lactobacillus salivarius TISTR 390**(K)***	46.3	76.3
Lactobacillus johnsonii ATCC 3320 + Bifidobacterium lactis strain Bb12 + *Lactobacillus salivarius TISTR 390 + Lactobacillus paracasei TISTR 453**(L)***	46.8	75.9
Lactobacillus johnsonii ATCC 3320 + Bifidobacterium lactis strain Bb12 + *Lactobacillus salivarius TISTR 390+ Lactobacillus paracasei TISTR 453+ Lactobacillus helveticus* LH-BO2 *(Estimation of Com)**(M)***	47.7	77.4

Table 15. Ability of bacteria under study to remove Cd, Pb, As and AFB1 from artificially contaminated aqueous solutions

Strain(s)	**% Removal**			
	Cd	**Pb**	**As**	**AFB1**
Lactobacillus salivarius TISTR 390 **(A)**	24.5	41.5	38.3	14.8
Lactobacillus paracasei TISTR 453 ***(B)***	29.5	43.3	42.6	19. 6
Lactobacillus helveticus LH-BO2 **(C)**	28.3	42.6	47.6	23.8
Bifidobacterium lactis strain Bb12 ***(D)***	59.8	45.8	44.7	19.5
*Lactobacillus johnsonii ATCC 3320**(E)***	65.5	72.6	49.8	20..9
Bifidobacterium lactis strain Bb12 + *Lactobacillus salivarius TISTR 390* ***(F)***	49.8	46.9	46.9	17.8
Bifidobacterium lactis strain Bb12 + *Lactobacillus paracasei TISTR 453* ***(G)***	52.6	45.6	38.2	19.4
*Lactobacillus paracasei TISTR 453 + Lactobacillus salivarius TISTR 390**(H)***	44.2	45.2	31.9	18.5
Bifidobacterium lactis strain Bb12 + *Lactobacillus Lactobacillus helveticus**(I)***	48.1	47.6	39.6	21.6
Bifidobacterium lactis strain Bb12 +*Lactobacillus johnsonii ATCC 3320H* ***(J)***	49.7	48.1	39.7	21.9
Lactobacillus johnsonii ATCC 3320 + Bifidobacterium lactis strain Bb12 + *Lactobacillus salivarius TISTR 390**(K)***	49..8	48.4	39.8	21.9
Lactobacillus johnsonii ATCC 3320 + Bifidobacterium lactis strain Bb12 + *Lactobacillus salivarius TISTR 390 + Lactobacillus paracasei TISTR 453**(L)***	49.9	48.7	40.8	21.8
Lactobacillus johnsonii ATCC 3320 + Bifidobacterium lactis strain Bb12 + *Lactobacillus salivarius TISTR 390+ Lactobacillus paracasei TISTR 453+ Lactobacillus helveticus* LH-BO2 *(Estimation of Com)* ***(M)***	59.9	65.8	48.9	22.7

*Average of three replicates at pH 7 and 37˚C. *Lactobacillus salivarius TISTR 390* **(A),** *Lactobacillus paracasei TISTR 453**(B,)*** *Lactobacillus helveticus* LH-BO2 **(C),** *Bifidobacterium lactis* strain Bb12***(D)*****,** *Lactobacillus johnsonii ATCC 3320**(E)*****,** *Bifidobacterium lactis* strain Bb12 + *Lactobacillus salivarius TISTR 390* ***(F)****,* *Bifidobacterium lactis* strain Bb12 + *Lactobacillus paracasei TISTR 453* ***(G)*****,** *Lactobacillus paracasei TISTR 453 + Lactobacillus salivarius TISTR 390**(H,)*** *Bifidobacterium lactis* strain Bb12 + *Lactobacillus Lactobacillus helveticus**(I)*****,** *Bifidobacterium lactis* strain Bb12 +*Lactobacillus johnsonii ATCC 3320H* ***(J)*****,** *Lactobacillus johnsonii ATCC 3320 + Bifidobacterium lactis* strain Bb12 + *Lactobacillus salivarius TISTR 390**(K)*****,** *Lactobacillus johnsonii ATCC 3320 + Bifidobacterium lactis* strain Bb12 + *Lactobacillus salivarius TISTR 390 + Lactobacillus paracasei TISTR 453**(L)*****,** *Lactobacillus johnsonii ATCC 3320 + Bifidobacterium lactis* strain Bb12 + *Lactobacillus salivarius TISTR 390+ Lactobacillus paracasei TISTR 453+ Lactobacillus helveticus* LH-BO2 *(Estimation of Com)* ***(M)***