**ORIGINAL ARTICLE**

# Protective effects of *Lactobacillus casei* on Lead-induced biochemical deteriorations in Rats

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## ABSTRACT

**Aim:** To eliminate or reduce lead poisoning in experimental animals (rats) by the biological action of *Lactobcillus casei*.
**Methods**: The purity of the isolates of this bacterium was confirmed based on the culture and microscopic characteristics and the biochemical tests. A lead acetate solution was prepared at a concentration of 2 mg / mL. A biological experiment was designed in rats with 3 treatments, these are: the treatment of infection by dosing with lead acetate solution and the treatment by dosing with a liquid bacterium *Lactobcillus casei*. After the end of the trial period of (11 weeks), the animals were killed and blood samples were taken for which tests were conducted for the parameters of blood images, blood lipids, creatine and urea, and liver enzymes. The animals were also dissected and extracted from them the organs of the brain, liver and kidneys, ashes were estimated for these three members and the element lead was estimated in samples of the organs ashes.
**Results:** The results showed that treatment with the Lactobcillus casei probiotic booster had a clear significant effect on the parameters of blood images (WBC, PCV, RBC, HB), cholesterol, creatinine, and the liver enzyme (ALP), whereas, the treatment with this bacteria returned these values to the same values in a healthy control experiment.
**Conclusion:** The treatment gave significant positive results without treating or reducing the concentration of lead to normal concentration (healthy control) in liver, kidney and brain samples.
**Keywords**: *L.casei*, Lead Pb, blood and lipid profile, liver enzymes

## INTRODUCTION

Heavy metal pollution is one of the most important and dangerous problems that arose as a result of the industrial revolution and the human need for development, which led to many damages to the components of the environment (such as air, soil, and the tissue of the organism). A heavy metal is any chemical element with a relatively high density, toxic, highly toxic, or toxic in low concentrations[1]. It is one of the most dangerous heavy metals to humans, and the most prevalent in the animal environment and the most important of which is lead, as car road dust contains a high concentration of heavy metals, especially lead in general.

The concentration of lead increases with the increase in vehicle traffic and metal welding in industrial cities, which leads to the absorption of lead by the leaves of plants planted near the roads, or it may be inhaled by people present as a result of pollution from car exhaust[2]. Lead is widely used in industry, such as batteries, rubber, dyes, metal welding wires, and in coating pipes, tanks, and plates used to protect against X-rays and electrical cables, as well as in the manufacture of pesticides and phosphate fertilizers, as well as in the petroleum industries, where it is added to automobile fuel to reduce cracking, and in the manufacture of alloys, mining and smelting metals[3].

Recent studies have also shown that some strains of microscopic germs can reduce the absorption of pesticides and toxicity of the elements in humans and animals. Where researches indicated that different types of lactic acid bacteria enable them to absorb heavy metals on their cell wall surfaces (biological absorption) or accumulate inside the bacterial cell (bioaccumulation), which prompted the use of these organisms to detoxify heavy metals, where the bacterial binding to metal ions is produced by electrostatic interactions between the net negative surface charge of the bacterial cell and the positively charged metal ion[4].

Therefore, it was decided to conduct this research, which aims to eliminate or reduce poisoning with lead in experimental animals (rats) by the biological action of the bacterium enhancer (*Lactobcillus casei*).

## MATERIALS AND METHODS

**Preparation of probiotic bacteria isolates:** The isolates of the probiotic (*Lactobcillus casei*) were obtained, dried and prepared from the Italian company SaccoSri in the form of capsules and cultivated, and activated on the medium of MRS Proth and MRS agar, where their purity was confirmed by conducting culture, microscopic and biochemical tests on them designated for their diagnosis. The chemical tests included assays: catalase, oxidase, degradation of gelatin, fermentation of sugars, and the following culture tests were performed to ensure the purity of the bacterial isolate: Growth in MRS - CaCO3 medium, growth at different temperatures, growth at different pH levels, and growth test on aerobically fed agar.
**Preparation of the bacterial suspension:** After the purity of the isolate was confirmed, the bacterial suspension was prepared for the probiotic by growing the isolate in 100 ml of milk, liquid sorting in volumetric flasks and incubated anaerobically at 37 °C for 48 hours. Then, 1 ml of the bacterial suspension was taken and transferred to 9 ml of liquid MRS environment and anaerobically incubated at 37 ° C for 24 hours, compared with McFarland's solution at a concentration of 0.5 to obtain numbers of 1.5 x $10^8$ cells / mL. Then, at the start of the treatment, the experimental

animals were given an amount of 1 ml of bacterial suspension per day for each animal using a dosing machine [5].

**Preparation of lead acetate solution:** Lead was used in the form of lead acetate salt, where it was dissolved in distilled water, and to reach the appropriate concentrations, one gram of lead acetate was dissolved in 500 mL of distilled water, so the concentration was 2 mg / 1 mL.

**Preparation of laboratory animals:** Laboratory animals were obtained from the College of Veterinary Medicine / University of Tikrit, which are (white rats / females of the Albino type) with 9 rats with ages ranging between (3-4) weeks and weights ranging between (88-105 gm). It was confirmed that it was free of diseases through an examination by the veterinarian who presents in the animal house.

**Experiment design:** The 12 female white rats were divided into three groups. Each group contained 4 animals and they were as the following:

1. The first group: a healthy control group, where the rats were given distilled water and fed on a standard diet (NRC, 2002) for 11 weeks.
2. The second group: It is the affected group, where the members of this group dosed daily with 1 ml of lead acetate at a concentration of 2 mg / ml per rat by oral administration for a period of 11 weeks.
3. The third group: which is the treatment group, where the members of this group were dosed daily with 1 ml of lead acetate at a concentration of 2mg/ml for five weeks, and then they were dosing with 1 ml of the activated bacterial suspension of *Lactobacillus casei* and through oral feeding for the remain six weeks (of 11 weeks in total).

After the end of the experiment period (11 weeks), the total weights of the animals were recorded, then they were killed, and blood samples were taken for which the following tests were performed later: 1- blood picture, 2- blood lipids, 3- creatine and urea, 4- liver enzymes. Animals were also dissected; brain, liver and kidney organs were extracted.

**Practical steps**

**First: The tests that were made on the blood of the animals, as the following:**

**Measuring the blood parameters:** The total red blood cell count (RBC), total white blood cells (WBC), total white blood cells ($m^3 \times 10^3$) and platelets ($10^9$/ liter) were measured using the Hemocytometer. Hemoglobin concentration (g/dl) and Packed Cell Volume (PCV) (%) using open-ended glass capillary tubes that are filled with blood to two-thirds and then one end of which is closed with clay and then placed in its own Hematocrit Micro-Centrifuge for 15 minutes at a speed of 3000 rpm, and then the result was read from the capillary tube, which represents the percentage of the volume of compacted blood cells.(Turgeon, 2012)[6].

**Determination of the triglycerides (TG) concentration in serum:** A ready-made examination kit was used from a German company, Spectrum, which depends on the enzymatic method for analyzing triglycerides to glycerol as in (Tietz, 2005)[7]. It was calculated as shown in the equations below.

:

$$TG\ conc.\left(\frac{mg}{100mL}\right) = \frac{Sample\ absorption\ intensity}{Solution\ absorption\ intensity}\ X\ St.solution\ conc.\left(\frac{200mg}{100mL}\right)$$

**Estimation of the total cholesterol concentration in the blood serum:** The total cholesterol concentration in the blood serum was calculated using the German-origin Spectrum ready-made assay kit, which depends on the enzymatic method for converting cholesterol and cholesterol esters to the cochineal red quinoneimine, according to (Tietz, 2005) [(7)]. As in the equation below (same equation in point 2):

$$Cholesterol\ conc.\left(\frac{mg}{100mL}\right) = \frac{Sample\ absorption\ intensity}{Solution\ absorption\ intensity}\ X\ St.solution\ conc.\ \left(\frac{200mg}{100mL}\right)$$

**Determination of serum HDL-C cholesterol:** HDL-C cholesterol was determined using a test kit from a German company, Spectrum, based on what was mentioned in (Tietz, 2005) [(7)], and the HDL-C concentration was calculated as in the following equation:

$$HDL-C\ conc.\left(\frac{mg}{100mL}\right) = \frac{Sample\ absorption\ intensity}{St.\ solution\ absorption\ intensity}\ X\ 1.1\ X\ St.HDL\ conc.\left(\frac{50mg}{100ml}\right)$$

Where 1.1 is the dilution factor

**Calculation of the concentration of low-density lipoprotein (LDL-C) cholesterol in blood serum:** The concentration of low-density lipoprotein cholesterol in the blood serum was determined using a test kit from a German company, Spectrum, according to what was mentioned (Faas, 2002) [(8)], as shown in the following equation:

$$LDL-C\ level\ \left(\frac{mg}{100mL}\right) = conc.og\ cholesterol - \left(conc.of\ HDL + \frac{con.of\ TG}{5}\right)$$

**Determination of creatinine concentration in serum:** The concentration of creatinine in the blood serum was estimated using the ready-made analysis kit of the Spanish company Spinreact and as mentioned in (Tietz, 2005) [(7)] and the concentration was calculated as in the following equation:

$$Creatinine\ conc.\left(\frac{mg}{dl}\right) = \frac{Sample\ absorption\ intensity}{Standardsolution\ absorption}\ X\ St.sloution\ conc.\left(\frac{2g}{100mL}\right)$$

**Determination of serum urea concentration:** The level of urea was calculated according to the method (Kaplan et al., 1984) [(9)] in the blood serum using a ready-made test kit (Kit) from the Spanish company Spinreact according to what was mentioned in (Tietz, 2005) [(7)] and the urea concentration was calculated according to the formula:

$$Urea\ conc.\left(\frac{mg}{100mL}\right) = \frac{Sample\ absorbance}{Absorbance\ of\ St.solution}\ X\ St.solution\ conc.\left(50\frac{mg}{100}mL\right)$$

**Erythrocyte sedimentation rate (E.S.R):** The erythrocyte sedimentation rate was measured, and it is one of the tests used to evaluate inflammatory responses in the laboratory. An amount of blood is added to a special tube (Blood Collection Vacuum Tube) containing 3.8% sodium citrate and placed in a microsed-system for half an hour, then the results was read via the device screen.

**Estimation of the efficacy of the enzyme Aspartate transaminase (AST) in serum:** The enzymatic method was used to estimate the activity of the enzyme AST in blood serum, as the method included the use of the analysis kit equipped from the German company Spectrum, the method is based on the ability of the enzyme to work on the base material (aspartic acid and alpha-ketoclutaric acid). The conversion of aspartic amino acid to alpha-ketoclutaric acid (pyruvic) which can be converted into a reddish brown derivative compound by adding the reagent 2,4 Di-Nitrophenyl hydrazine, then the absorbance is read using a spectroscope at a wavelength of 505 nanometers (Tietz, 2005)[7] as in the following equation:

$$\boldsymbol{AST = L - Aspartate + Oxagultarate\ Oxalacetate + L - Gultamate}$$

**Estimation of the efficacy of the enzyme transaminase ALT in serum:** The enzymatic method was used, as it used the analysis kit prepared from the German company Spectrum, the ALT enzyme works on the base material (alanine and alpha-ketoglutaric acid) by converting the amino acid alanine into pyruvic acid directly, which is easily converted into a compound derived from the hydrazine with a reddish brown color by adding the reagent 2,4 Di-Nitrophenyl hydrazine, The absorbance is read on a spectrophotometer at a wavelength of 505 nm (Tietz, 2005) [(7)] as in the following equation:

$$\boldsymbol{L - Alanine\ +\ 2 - Oxaglutarate\ \rightarrow\ Pyruvate\ +\ L - glutamate}$$

**Estimation of the activity of alkaline phosphatase (ALP) in serum:** The effectiveness of the enzyme (ALP) was estimated using the German Spectrum kits as mentioned in (Tietz, 2005)[7].

**Second: The tests performed on organ samples:** Incineration (ash): Ash was estimated in the internal organs (liver, kidneys, and brain) of laboratory animals as reported in A.O.A.C, (2004)[10]. By taking 2 grams of organ samples in a ceramic jar of known weight, then transferring it to an oven for the purpose of removing the moisture, then to the incineration oven at a temperature of 600 ° C until the weight stabilizes and obtaining a white or gray powder and after the end of the incineration the lid is transferred to an isolated container (Discutor) and left, until the weight became stable.

**Estimating the element of lead in organ samples:** To detect the presence of lead in the internal organs (liver, brain, kidney) of the laboratory animals used in the experiment, the obtained ash is taken on it and 5 ml of nitric acid at a concentration of 5% is added to the ashes obtained and mixed well and then filtered using filter paper. The estimation was made by using the atomic absorption device for each mineral to be identified and measured by placing the sample in the form of a transparent liquid in the device and estimating its percentage directly as in (2004, A.O.A.C.)[10].

**Statistical analysis:** The results were analyzed statistically using the statistical program (SPSS) StatisticaL Package for the Social Sciences according to the ANOVA test, and the arithmetic averages were compared using the Duncan polynomial test to determine the significance of the differences if they existed between the different averages at a probability level (0.05 and 0. 01).

## RESULTS AND DISCUSSION

**Diagnostic results for *Lactobacillus case* isolation:** The purity of the isolate of the probiotic *L.casei* was confirmed by conducting culture, microscopic and biochemical tests on it for diagnosis, which included chemical tests such as; Catalase, oxidase, hydrolysis of gelatin, fermentation of sugars. The following culture tests were carried out to ensure the purity of the bacterial isolate: growth in MRS-$CaCO_3$ medium, growth with different temperature degrees, growth in different PH degrees, and growth test on aerobic feeding media as shown in table 1, these results were identical to what the researchers reported: (Al-Sheikhani, 2020)[11], (Holt et al., 1994)[12], (Al-Muhareb, 2019)[13], (Al-Nasiri, 2018)[12], Majeed and Ahmad, 2013)[15], (Frece et al., 2005)[16].

**The effect of injury and treatment factors on some results of the blood picture**

**Results of the treatment effects on the total and differential number of WBC:** The results in table 2 shows the increases in the total number of white blood cells WBC among the subjects of the infected control treatment with a significant increase at a probability level of 0.05 compared to the healthy control treatment, which recorded (13.800x109 and 9500109 x cells / $mm^3$), respectively. This result was in agreement with Al-Hamdani and Al-Rasheed (2011)[17]. As for the treatment with *L. casei* probiotic booster, the white blood cell count in this treatment came back to the same number in the healthy control experiment, as it recorded (9500 x 109 cells / $mm^3$). This result significantly confirms the efficacy of treatment with *L. casei* probiotic enhancer in removing the effect of the element lead on the content of white blood cells. This result is consistent with what the researchers found, Ghazanfarpour et al, (2019)[18].

The reason for the significant increase in the total number of white blood cells may be explained by the effect of lead on various inflammatory events in the body, such as smooth muscles, liver, kidneys, lungs and skin, which leads to an increase in the production of white blood cells from the bone marrow as a defensive response to infections occurring in the body. This result also agreed with the results obtained (Al-Muharib, 2019)[13], which showed that the probiotics work to repair body tissues and modify the

defect in the circulatory system, which leads to the restoration of the level of white cells within normal limits.

**The effect of treatments on RBS, hemoglobin and blood cells volume:** The results of table 3 showed a significant decrease with a probability level of 0.05 for the concentrations of Hb hemoglobin, erythrocyte and PCV among the subjects of the infected control treatment compared with the healthy control subjects. The effect of lead on multiple enzymes of the heme synthesis pathway is one of the important mechanisms of lead toxicity, as the hematopoietic system is one of the critical organs targeted by lead[19].

These results also agreed with Hadi (2014)[20] regarding the results of the group infected with lead acetate, which showed a decrease in the results of the total number of red blood cells and hemoglobin concentration. The cause is due to the oxidative stress caused by lead in the blood and other soft tissues, which is one of the possible mechanisms for the toxic effects of lead, and the poisoning lead will results to decrease the volume of red blood cells, hemoglobin and PCV, which leads to anemia due to the effect of lead on the lifespan of red blood cells[21]. As for the treatment of the treatment with the *L. casei* probiotic, the hemoglobin concentration and PCV in it were significantly closest to the healthy control values, as shown in table 3. This result is in agreement with the results obtained by Ghazanfarpour et al. (2019)[18] in their study of the protective effect of *Lactob acillus* fermentum on the toxicity of lead. The effect of poisoning with lead can be explained by the inhibitory effect of lead on the factors contributing to the synthesis of red blood cells in addition, as the biological promoters can bind heavy metal ions and expel them outside the body.

**The effect of treatments on lipid parameters:** The results of table 4 showed a clear significant change at the probability level of 0.05 with respect to the concentrations of blood lipid parameters. The level of LDL, Cholesterol, and Triglycerides values increased, while the HDL value decreased among subjects in the affected control treatment compared to the healthy control treatment.

This result was in agreement with the results of Junker and Al-Dabbagh 2008[22] in their study on the effect of bioaccumulation of lead on a number of biochemical variables of the human being, they explain that the mineral pollutants cause an increase in the cholesterol concentration through a decrease in the activity of the enzyme hydoxysteroid dehydrogenase, a change in the synthesis of steroids, and changes in lipid metabolism. As for the treatment with the l.casei probiotic, they lowered the cholesterol and LDL significantly to the level of its value when a healthy control was tried. As for the rest of the factors, the treatment improved it, but not significantly (Table 4). These results are in agreement with what happened (ALAjeeli et al., 2013), where their study concluded that nutritional supplements containing milk bacteria play a role in controlling hyperlipidemia.

**The effect of treatments on liver enzymes:** The results of table 5 showed that the values of all liver enzymes changed significantly at a probability level of 0.05 in the treatment of infection with an increase in the treatment of healthy control, and this result is in agreement with the results obtained by Al-Azzawi, (2015)[23]. This has been interpreted as lead to generate active oxygen varieties and to produce free radicals. *L. casei* probiotic booster treatment significantly reduced the ALP enzyme to a level close to its value when trying a healthy control. As for the other two enzymes (ALT, AST), the treatment improved it by decreasing the patient, but not significantly (Table 5). The result of the protective effect of the bio-promoter is in agreement with the results reported by Djurasevic et al (2017)[24], in their study of the protective effects of the bio-promoter bacteria on the toxicity of cadmium in mice. The study indicated that the probiotics reduce the concentration of minerals in the blood and increase the percentage of minerals in the stool due to their ability to bind minerals to the cell wall.

**The effect of the parameters on the indicators of kidney function:** The results in table 6 showed a significant increase in the indicators of renal function (urea and creatinine) at a probability level of 0.05 in the treatment of infection with an increase in the treatment of the correct control. While the treatment with the probiotic booster *L. casei*, the value of creatinine decreased significantly to the same value when trying the healthy control, while the value of urea, the action of the treatment improved it by decreasing it to a value close to a healthy control, but not significantly. This result is in agreement with the results of Al-Dulaimi (2016)[25], who indicated an increase in the values of urea and creatine in animals exposed to lead and cadmium infection without treatment, but the effect of treatment with nettle leaves and moringa seeds reduced the effects of cadmium and lead poisoning in male mice.

The results were also in agreement with Patra et al. (2018)[26], the fact that *Lactobacillus plantarum* was a probiotic promoter that had the ability to treat kidney function in rats suffering from chronic urinary and kidney infections, reach normal rates, and are resistant to oxidative stress. The reason for the decrease in the results of urea and creatine in the groups treated with the probiotic may be explained by the ability of the bio-booster to produce glucan, which may be the cause of the displacement of amino acids in the intestine, leading to a decrease in the composition of the main protein in the production of urea and an increase in creatinine, thus reducing their presence in the animals blood serum.

**The effect of different treatments on the presence of lead in organ samples:** The results in table 7 showed a significant increase in the accumulation of lead element significantly at a probability level of 0.05 in the treatment of injury in the organs of the brain, liver and kidneys, compared with the treatment of healthy control. Where the concentration of lead element after the injury treatment was 1.125, 1.915, and 1.898 ppm, respectively, while the lead concentration in the three members in the healthy controls treatment was 0.01 ppm.

The results agreed with what they found (AL-Najare et al., 2016)[27] regarding the ability of heavy metals to accumulate in the tissues of various organisms, as they indicated the presence of high concentrations in the liver, heart and brain in lead-hungry rats. The results of this experiment showed that the treatments of *L. casei* probiotic enhancer reduced the concentrations of lead element in all the members significantly to the same values as the sound control treatment (0.01) ppm, and this result is very encouraging to consume this vital reinforcer to avoid environmental pollution with lead.

The results of this study are in agreement with Monachese et al. (2012)[28] and Giri et al. (2018)[29] who indicated that the

probiotics work to bind heavy metals to their cells by the mechanisms of ion exchange reactions with peptidoclycones and with tequic acid in the cell walls of the bio-promoter.

Table 1: Results of culture, microscopic and biochemical examinations of *L. casei* bacteria

| **Item** | **The type and outcome of the tests** | | | | | **Temperatures rising** | | | |
|---|---|---|---|---|---|---|---|---|---|
| Culture and microscopic tests | Cell shape | Colonies shape | Cram dye | Formation of spores | The movement | 15 °C | 25 °C | 37 °C | 45 °C |
| Result | Bacillus | Regular white circles | + | - | - | - | - | + | + |
| Biochemical tests | Catalase | Oxidase | Fructose | Lactose | Galactose | Sucrose | Maltose | Anaerobic growth | |
| Result | - | - | + | + | + | + | + | + | |
| Growth below pH | pH 2.0 | pH 3.0 | pH 4.0 | pH 5.0 | pH 6.0 | pH 7.0 | pH 8.0 | pH 9.0 | |
| Result | - | + | + | + | + | + | - | - | |

Table 2: The effect of lead on white blood cells and treatment of that with *L. casei* bacteria

| **Number** | **Treatment** | **The total number of WBC (x 10 $^{9}$/mm$^{3}$)** |
|---|---|---|
| 1. | Healthy control | 9500 ± 1100C |
| 2. | Infected control | 13800± 200 A |
| 3. | Infected and treated with the vital booster *L. casei* | 9500± 1838 C |

Table 3: The effect of lead on red blood cells, hemoglobin, and PCV volume, and treatment of that with *L. casei* bacteria

| **Number** | **Treatment** | **HB (mg/dl)** | **RBC (10$^{6}$/mm$^{3}$)** | **PCV%** |
|---|---|---|---|---|
| 1. | Healthy control | 14.100 ± 0.500 A | 9.150 ± 0.500 B | 43.50 ± 1.500 A |
| 2. | Infected control | 9.600 ± 0.652 F | 3.700 ± 0.400 F | 31.80 ± 2.10 G |
| 3. | Infected and treated with the vital booster *L. casei* | 13.950 ± 0.495 Ab | 8.200 ± 0.424 D | 43.00 ± 1.41 A |

Table 4: The effect of lead on blood lipid parameters and treatment of that with *L. casei* bacteria

| **Number** | **Treatment** | **HDL mg\dl** | **LDL mg\dl** | **Cholesterol mg\dl** | **Triglycerides mg\dl** |
|---|---|---|---|---|---|
| 1. | Healthy control | 51.00 ± 2.00 Cd | 130.00 ± 5.00 C | 95.50 ± 3.50 B | 109.00 ± 13.00 C |
| 2. | Infected control | 48.00 ± 4.00 E | 158.00 ± 18.0 A | 101.00 ± 1.000 A | 145.50 ± 0.500 A |
| 3. | Infected and treated with the vital booster *L. casei* | 54.00 ± 1.000 B | 124.00 ± 0.0 De | 93.50 ± 9.50 b | 134.50 ± 0.500 A |

Table 5: The effect of lead on liver enzymes and treatment with *L. casei* bacteria

| **Number** | **Treatment** | **GOT (AST) U/L** | **GPT (ALT) U/L** | **ALP U/L** |
|---|---|---|---|---|
| 1. | Healthy control | 201.50 ± 9.50 B | 85.00 ± 7.00 B | 227.00 ± 14.00 C |
| 2. | Infected control | 355.00 ± 5.00 A | 101.50 ± 5.50 A | 389.50 ± 10.50 A |
| 3. | Infected and treated with the vital booster *L. casei* | 187.50 ± 4.50 C | 61.50 ± 9.50 D | 230.50 ± 1.500 C |

Table 6: The effect of lead on indicators of kidney function and treatment of that with *L. casei* bacteria

| **Number** | **Treatment** | **B. Urea (mg/dl )** | **S. Creatinine (mg/dl)** |
|---|---|---|---|
| 1. | Healthy control | 36.50 ± 0.500 Bc | 1.0500 ± 0.0500 B |
| 2. | Infected control | 55.99 ± 8.00 A | 1.6000 ± 0.1000 A |
| 3. | Infected and treated with the vital booster *L. casei* | 30.50 ± 2.50 De | 1.0500 ± 0.0500 B |

Table 7: The effect of the treatments on the accumulation of lead in the organs and the effect of *L. casei* bacteria on **it**

| **Number** | **Treatment** | **Brain (ppm)** | **Liver(ppm)** | **Kidney (ppm)** |
|---|---|---|---|---|
| 1. | Healthy control | 0.010 B | 0.010 b | 0.010 B |
| 2. | Infected control | 1.125 A | 1.915 a | 1.898 A |
| 3. | Infected and treated with the vital booster *L. casei* | 0.010 B | 0.010 b | 0.010 B |

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