Evaluation the Ability of some Probiotic in the Detoxification of Some Heavy Metals in Cow Milk in Mosul city

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ABSTRACT

Food and water are increasingly contaminated with heavy metals due to environmental pollution. Lead and cadmium are heavy metals toxic to humans that can be found in air, soil, water, and even food. especially milk , to evaluate the ability of some specious of Lactic acid bacteria to remove and reduce the level of these heavy metals in milk this study was conducted . (90) samples of cow's milk from different areas exposed to pollution within the borders of Nineveh Governorate were collected . The areas were divided into three groups industrial facilities, high traffic and intensive agricultural activities .The current study aimed to investigate the contamination of cow's milk samples collected from the mentioned study areas with some types of heavy metals such as (lead, cadmium) by measuring their concentrations and comparing them with the permissible limits, which were determined by the Food Organization and the World Health Organization. Then evaluate the ability of some genus of lactic acid bacteria to reduce the concentrations of these minerals in the milk samples .For this purpose, a commercial isolation was used that contains various types such as (Lactobacillus plantarum, Lactobacillus acidophilus, Streptococcus thermophiles) ,The results showed that the (lead) in the milk samples collected from all study areas exceeded the permissible limits of milk within the Iraqi and international standard specifications, which are (0.02) parts in million (PPM) , and the areas near industrial facilities were the most contaminated with the element of lead, as its concentration reached (0.045) PPM, followed by the agricultural fields

The results also indicated that the (cadmium) in the milk samples of all study areas exceeded the permissible limits in milk within the Iraqi and international standards, which are (0.002) ppm, where the highest calculation value recorded in areas near industrial facilities and areas near streets with high traffic, both (0.008) ppm, and no significant differences were recorded . In order to solve the problem of milk contamination with heavy metals, commercial and local isolations of probiotic were used and added to contaminated milk. The results of the study showed that commercial isolation had a notable role in reducing the toxicity of heavy elements (lead, cadmium) in milk. The values of lead concentrations before addition were in cow's milk samples that are grazing soon from industrial facilities, streets with intensive irrigated movement, agricultural fields in which pesticides and fertilizers are used (0.064, 0.053 and 0.056) ppm were, respectively, while their values after addition (0.045, 0.030 and 0.043 0.007) part by million while their values after addition (0.004, 0.006 and 0.015) were part in million and respectively and all results recorded significant differences at probability level (a < 0.05). for local isolation, Lactobacillus acidophilus bacteria were isolated from cow's milk and microscopic tests were carried out on it, such as bacterial pigmentation and movement test, then the tests were conducted, chemical tests such as (catalyze test, oxidase test, gelatinase test, jacket consumption, and indole test, sugar with

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regard to local isolation, high efficiency was shown in the bioremoval of the toxicity of heavy elements (lead, cadmium), as the values of lead before addition were in the milk samples of the three regions mentioned, (0.064, 0.053 and 0.056) ppm , while their values after addition (0.031, 0.015,0.026) ppm respectively, and significant differences were recorded at a probability level ($P \leq 0.05$) for both lead and cadmium concentrations before and after addition.

Keywords : Milk , Probiotic, Detoxification, Heavy Metals.

.1INTRODUCTION

Food contamination is one of serious problems of pollution particularly in developing countries , it categorized as physical, chemical and biological(1), the chemical pollutants whether international or accidental are one of the main causes of food contamination , they have mentioned historically before eight thousands years ago, their origins vary from field to table, involves , air , water, soil environmental ,packing processes , they brings in its wake numerous health implications . Fortunately appropriate food management when applied correctly can prevent chemical contamination effectively.(2(

Milk, is an important part of the human diet for ages , it is a super beneficial product contain almost all of the essential nutrients such as carbohydrates , fats calcium , magnesium , zinc , vitamins in addition to being resource of protein. Milk gathering and processing exposed it to various contaminants , virtually 38 micro element have been registered in raw milk all over the world , including lead, copper, cadmium ,zinc , nickel, arsenic and mercury which consider dangerous for animal and individuals health ,their presences in milk and it is products vary depending on different factors like , cow health , period of lactation, climatic variation and environmental pollution (3). heavy metals (any metal its density more than 5g/ cm3) are one of the most frequent causes of milk contamination , , they are toxic cumulative elements , harmful to health even in low levels , heavy metals metabolic function is not well known but they have a toxic effect lead to cellular disruption. The major sources of milk metal contamination are industrial effluents, combustion forest fire ,chemical fertilizer and pesticides (4) , Long term of exposure to levels of heavy metals can induce oxidative stress and increase the risk of many disease especially cancer in the body, hence the world health organization (WHO) have established the metal maximum residual limit (MRL) value in the products of food in order to avoid the heavy metals hazards (5). Decontamination of heavy metals encompass chemical and physical and biological methods , In terms of economy or safety chemical and physical methods are not so appropriate , while the using of microorganisms in biodegradation of chemical contaminants was specific and eco-friendly to eliminate the levels of these pollutants(6 . (

Probiotics are defined as live microorganisms can provide a health advantage when they presence in sufficient amounts (7) , recently , there was a considerable interest in probiotic with high xenobiotic binding capacity which could be used to detoxifying food contamination , the vivo and vitro trials have been demonstrated that ability of probiotic microorganisms to bind and metabolize different pollutants such as heavy metals (8). Lactic acid bacteria (LAB) is a class of probiotics , they are gram positive ,in general nonspourulating, aero tolerant , capable of sugar fermentation , found in different food included milk and it is products(9). The strains of this probiotic have many valuable properties make it vtital in xenobiotics bio removal (10) , therefor utilization of these probiotic strains consider as promising tool for detoxification of heavy metals, and in this work we focused on the ability of new stains of (LAB) in detoxification of some metals in bovine milk.

.2Methods and Materials

2.1Collection of Milk Samples

During this study, 90 milk samples were collected From June to September 2022, from different areas of Mosul city

and its suburbs by three repeaters per sample, taking into account the environmental conditions specific to each region. using clean bottles of (1) liter polyethylene, which were washed several times before filling them, and then placed in cold box and in appropriate conditions to avoid changes in a number of properties of milk until reaching the laboratory for research tests.

kit , amplified via the primers primers (F-5' AGAGTTTGATCCTGGCTCAG3', R-5' AAGGTTACCTCAC-CGACTTC 3') primers (F-5' AGAGTTTGATCCTGGCTCAG 3', R-

2.2Determination of Heavy Metals in Milk Samples

(10)ml of milk were add to dry , known weight bowl.Incineration and cremation processes were done by putting the bowl in the muffle(Electro.mag-Germany) for one hour at 105 °C then increase the temperature to 550 °C for three hours until converted to ashes.,Lead, Cadmium and Zinc concentrations in the samples were measured by Atomic Absorption Spectrometry (burkert, Gasbox – Germany) and A hollow element cathode lamp operating at a wavelength of 283.3 nm was used for Pb , 228.8 nm for Cd and 213.9 nm for Zn.

2.3Isolation and idintification of LAB

The noval local strain bacteria LC741319.1 was isolated by taking one ml of the sample , centrifuged for 6 min at 1000 rpm , nine ml of normal saline were add to the solid phase . the diluted samples were grown on MRS agar (Merck , Germany), finally incubated for 48 hours at 45 C0 in anaerobic conditions . Later the gram positive rods shaped microscopic observed cells were purified and stored in 4C0.. Biochemical testes were proved L acidophilus to confirm these results 16srRNA gene PCR assay was carried out . Total DNA extracted by a Bioneer genomic extraction 5' AAGGTTACCTCAC-CGACTTC 3') , conditions of reaction illustrated in the table:(1-1(

Table (1-1) Showing the Conditions of the Reaction

No.	Stage	Temperature	Time	Cycle number
1	Initial denaturation	95	6 min.	1
2	Denaturation	95	45 sec.	35
3	Annealing	56	1 min.	
4	Extension	72	1 min.	
5	Final extension	72	5 min.	1

Products then migrated by electrophoresis on agarose gel (2%) , sequenced and submitted to NCBI gene bank database .

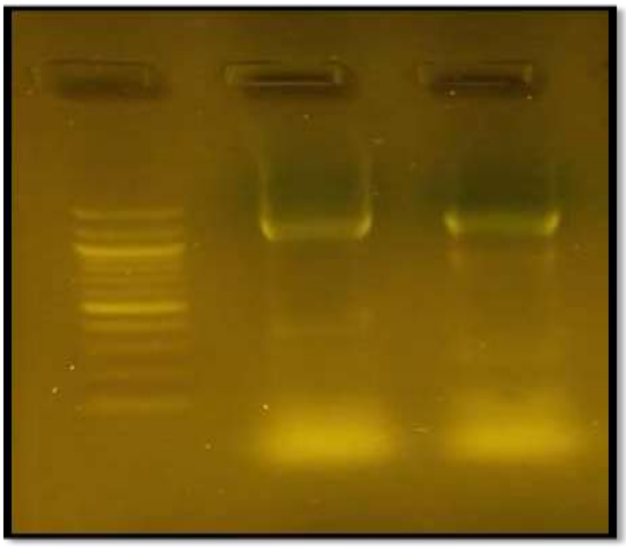

Figure 1: Shows the PCR reaction product of bacteria samples Lactobacillus acidophilus for 16SrRNA region (bp1200(migrated with 2% acarrose gel.

2.4Manufactured of Yogurt

The yogurt has been made from raw cow's milk According to (Rasmussen and Reinemann, (2010)) exposed to contamination, the concentrations of heavy metals in it (lead, cadmium,)was determined previously . Milk was pasteurization at a temperature of 84 co and then cooled at a temperature of 40c0 . impregnated with the starter at a rate of 3% per 100 ml of pasteurized milk at temperature 42-45c0 ,incubated for 4 hours and then kept it cold at a temperature of 4 for 24 hours until the procedures for estimating the heavy metals in it again.

.3Results and Discussion

3.1Estimation the concentration of lead in milk

Table (1-2) showed that the concentration of lead in milk samples collected from the three study areas was higher than the maximum limits allowed by WHO and FAO which were (0.02) ppm (11). The highest arithmetic value recorded in the areas close to the industrial facilities was (0.045) ppm The results in the table also showed that there were no

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significant differences ($P < 0.05$) in the concentration of lead in milk between the three regions and the reason for this may be due to the fact that all areas of sample collection are exposed to pollution of water, air and soil through what is released by car exhaust from fuel waste containing tetraethyl lead, in addition to factory nozzles, fertilizers and pesticides and studies that recorded results similar to the current study is a study by (12. (

Table (1-2) Average Concentration of Lead (ppm) in Milk Collected from Different Regions

Region type	Lead concentration in raw milk
Areas close to industrial facilities	$0.045\underline{+}0.03$ A
Areas close to high-traffic streets	$0.032\underline{+}0.02$ a
Areas close to agricultural fields in which chemical fertilizers and pesticides are used	$0.044\underline{+}0.02$ A

3.1.1Effect of adding probiotics to milk contaminated with lead

3.1.1.1Effect of adding commercial isolates to milk contaminated with lead

Table (1-3) shows the effect of adding the commercial isolate (a mixture of B)L. plantarum, L. acidophilus, Streptococcus thermophiles L.bulgaricus) on raw milk contaminated with lead, where the different letters indicate a statistically significant difference at the confidence level ($P \leq 0.05$) in the value of lead concentration rates in contaminated raw milk samples collected from The three regions showed its concentration after adding the commercial isolation, where the lead values before the addition were (0.064, 0.053, 0.056) ppm, respectively, while its values after the addition were (0.045, 0.030, 0.043) ppm, respectively. The highest percentage of decrease was recorded in areas close to streets with traffic high pass perhaps the reason behind this decrease is the effect of the commercial isolate containing four types of probiotics, whose ability to reduce metals has been proven by recent research because it has more than one mechanism for the absorption of heavy metals, including the absorption of minerals by various toxic substances on the cellular surface of microbes, in addition to the exchange of metal ions and formations complex with metal ions on the interactive chemical sites of the cell surface, and precipitation of excess metal ions occurs through reactions that occur on the cell surface as all microorganisms have aggregates with a negative charge on the surface of their cell, enabling them to bind to metal ions with a positive charge (metal ions heavy). The most important negatively charged groups involved in metal adsorption are alcohol, amine, carboxylate, ester, hydroxyl, sulfhydryl, phosphoryl, sulfonate and thiol groups (13). This mechanism has been studied on a large scale and among the studies, whose results are consistent with the current study is the study of (14)(15). Also, the published study of (16) reported that Enterococcus L. plantarum and L. bulgaricus has adsorption potential against toxic heavy metals, and the results of the study were consistent with the results of a study (17) on the effectiveness of commercial isolates in removing heavy metals in vivo and in vitro.

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Table (1-3) Showing the Effect of Adding Commercial Isolation on Lead-contaminated Milk Collected from Different Regions

Region type	Lead concentration before addingcommercial isolate (raw milk)	Lead concentration after adding commercial isolate
Areas close to industrial facilities	0.064±0.027 a	0.045±0.020 a-d
Areas close to high-traffic streets	0.053±0.031 a-c	0.030±0.017 de
Areas close to agricultural fields in which chemical fertilizers and pesticides are used	0.056±0.016 ab	0.043±0.018 a-d

3.1.1.2Effect of adding local isolates to milk contaminated with lead

The results of Table (1-4) showed the addition of the local isolate of L. acidophilus, which was isolated and diagnosed biochemically, microscopically and genetically and registered in the global gene bank for the first time through the study of raw milk contaminated with lead, where the different letters indicate the presence of significant differences at the level of probability ($P \leq 0.05$) in the value of lead concentration rates in contaminated raw milk samples collected from the three regions and its concentration after adding the local isolate, where the lead values before addition were (0.064, 0.053, 0.056) ppm, while the values after addition were (0.026, 0.015, 0.031) ppm, respectively, and the highest percentage of decrease was recorded in areas close to streets with high traffic, and the reason for the decrease is attributed to the ability of the local isolation of LAB bacteria, especially L. acidophilus, as it is more efficient in lead and cadmium ions and has specific membrane structures to passively bind metal ions and remove them from the body according to What was mentioned by (18), in addition to what was mentioned by the study (19) in evaluating the biological treatment of L. acidophilus KLDS strains to reduce lead toxicity, as the study stated that taking L. acidophilus KLDS encapsulated in capsules increases protection against lead toxicity and reduces levels lead in the blood Another reason for the reduction is attributed to what was mentioned in a study (20) about its ability to reduce intestinal pH, the production of bacteriocins and adhesion resources, the production of antitoxins and prevent its interference with the host to respond to toxins.

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Table (1-4) Effect of Adding local Isolation on Lead-Contaminated Milk Collected from Different Regions

Region type	Lead concentration before adding local isolate (raw milk)	Lead concentration after adding local isolate
Areas close to industrial facilities	0.064±0.027 A	0.026±0.015 de-
Areas close to high-traffic streets	0.053±0.031 a-c	0.015±0.010 e
Areas close to agricultural fields in which chemical fertilizers and pesticides are used	0.056±0.016 Ab	0.031±0.022 b-d

3.2Estimation the concentration of cadmium in milk

Table(1-5) shows the cadmium concentration of milk samples collected from the three regions was higher than the maximum limits allowed by WHO and FAO which were (0.002) ppm(11). The highest arithmetic value recorded in areas near industrial facilities and areas near streets with high traffic both was (0.008), and the table also showed that there were no significant differences ($P \leq 0.05$) in the concentration of cadmium in milk between the three areas, and it is likely that the reason lies in the fact that cadmium cadmium (cadmium) is used in the brake manufacturing process, and the friction resulting during braking leads to corrosion of the cadmium layer and the release of cadmium particles into the environment. Cadmium particles can enter water bodies and drinking water supplies through rainwater runoff (12) and also because these areas are exposed to environmental pollution produced mainly by humans in industrial activities and their presence in feed or water consumed by animals and fertilizers and their effect on soil properties .

Table (1-5) Average Concentration of Cadmium (ppm) in Milk Collected from Different Regions

Region type	Cadmium concentration in raw milk
Areas close to industrial facilities	0.008±0.006 a
Areas close to high-traffic streets	0.008±0.005 a
Areas close to agricultural fields in which chemical fertilizers and pesticides are used	0.007±0.003 a

3.2.1

Effect of adding probiotics to milk contaminated with cadmium:

3.2.1.1Effect of adding commercial isolates to milk contaminated with cadmium

Table (1-6) shows the effect of the commercial isolate on raw milk contaminated with cadmium. The different letters indicate that there are significant differences at the probability level ($P \leq 0.05$) in the average value of cadmium concentrations in milk samples collected from the three regions and its concentration after adding the commercial isolate. Cadmium values before addition and as shown in the table (0.007, 0.006 and 0.003)

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ppm, while the values after addition were (0.004, 0.003 and 0.001) ppm. Respectively, the highest percentage of decline was recorded in the areas close to an agricultural field using chemical fertilizers and pesticides. The reason for the reduction is attributed to the possibility of commercial isolates containing different types of probiotics to remove cadmium significantly, and this is consistent with what was mentioned by the study (21) as L. plantarum bacteria reduce cadmium, as these strains have the ability to survive in a stress environment in the digestive system, including the intestine, and later the intestinal mucosal cells are committed to the exercise of vital activity and the removal of heavy metals, and these results were also consistent with the results from (22) in their study of the in vitro digestion model of cadmium by different types of probiotics.

Table (1-6) Effect of Adding Commercial Isolation on Cadmium-contaminated Milk Collected from Different Regions

Region type	Cadmium concentration before adding commercial isolate (raw milk)	Cadmium concentration after adding commercial isolate
Areas close to industrial facilities	0.007±0.012 a	0.004±0.008 b-d
Areas close to high-traffic streets	0.006±0.011 Ab	0.003±0.007 b-d
Areas close to agricultural fields in which chemical fertilizers and pesticides are used	0.003±0.01 a-c	0.001±0.006 cd

3.2.1.2 Effect of adding local isolates to milk contaminated with cadmium

The results of Table (1-7) indicated the effect of the local isolate added to raw milk contaminated with cadmium, as the different letters indicate that there is a statistically significant difference at the level of confidence ($P \leq 0.05$) in the value of cadmium concentration rates in samples of contaminated raw milk collected from the three regions and their concentration after adding the local isolate where the values of cadmium before addition, shown in the table, were (0.007, 0.006, and 0.003), while its values after addition were (0.003, 0.002, and 0.001)ppm. respectively, the highest percentage of decline was recorded in areas close to an agricultural field using chemical fertilizers and pesticides. The reason for the decrease is attributed to the ability of the local isolate of LAB bacteria, especially L. acidophilus, as it is more efficient in lead and cadmium ions and has specific membrane structures to passively bind metal ions and remove them from the body, according to what was mentioned (18) as well as what was mentioned by the study (21). Five LAB probiotic isolates were investigated for their high resistance to lead and cadmium toxicity with outstanding metal binding and removal efficiencies and potential to reduce bioavailability of heavy metals

Table (1-7) Effect of Adding Local Isolate to Cadmium-contaminated Milk Collected from Different Regions

Region type	Cadmium concentration before adding local isolate (raw milk)	Cadmium concentration after adding local isolate
Areas close to industrial facilities	0.007±0.012 A	0.003±0.004 D
Areas close to high-traffic streets	0.006±0.011 Ab	0.002±0.005 Cd
Areas close to agricultural fields in which chemical fertilizers and pesticides are used	0.003±0.01 a-c	0.001±0.004 D

.4Conclusion

The presence of heavy metals in food and water is a serious health concern worldwide. Pb and CD are toxic heavy metals for people. Milk is the most commonly used dairy product that should be safe to consume. In this project, Pb and CD were successfully removed from contaminated milk by using commercial and local isolates of lactobacillus. . This study revealed that both of isolates are effective natural biosorbent for removing Pb and CD at very low concentration levels (ppm) of milk. Our findings are the first step to investigating the ability of heavy metals to bind by LABs in milk. Further studies on other LAB strains in milk and other foods are still needed to eliminate the harmful effects of toxic heavy metals.

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