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Evaluation of the Capacity of Probiotic in Detoxification of Zinc Metal in Contaminated Cow Milk

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A B S T R A C T

Food and water are increasingly contaminated with heavy metals due to environmental pollution. Zinc is a heavy metal that is harmful to humans and is present in soil, water, air, and food, especially milk. Ninety 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 ; viz. industrial facilities, high traffic and intensive agricultural activities. The current study aimed at investigating the contamination of cow's milk samples collected from the previously mentioned study areas with some types of heavy metals such as zinc by measuring their concentrations and comparing them with the permissible limits, which were determined by the Food Organization and the World Health Organization. Then, the study evaluated the ability of some genus lactic of acid bacteria to reduce the concentrations of these minerals in the milk samples. For this purpose, a commercial and local isolation was used that contained various types such as (*Lactobacillus plantarum*, *Lactobacillus acidophilus*).

Introduction

Heavy metals (minerals with a density of more than 5 g / cm^3) have a special accumulation. Bioaccumulation is stated by the World Health Organization (WHO) and has significant health risks. Zinc is a major microelement involved in many functions in the animal body. It enhances metabolic function, the synthesis of essential enzymes and the formation of hormones related to growth, reproduction and immunity. Cow's milk contains ~3-4 mg/L of zinc, 95% of which is bound to casein micelles, and the rest is bound to molecules of citric acid salts[1] and food safety is the basic condition for human health and public safety. It has become necessary to search for modern methods to ensure this; including bioremediation, which is a modern technique used to remove heavy metals and/or recover them from polluted environments[2]. This technology relies on biological mechanisms to eliminate dangerous pollutants using microorganisms[3]. It is an environmentally, friendly and cost-effective technique, when compared with traditional, chemical and physical techniques, which are often more expensive and inefficient especially for low metal concentrations[4].

The current research aims at showing the problems that cities suffering from, especially the city of Mosul including environmental pollution which has become a threat to societies. The research then clarifies special strategies to solve this problem through the science of the dairy industry and the formulation of a future vision for the production of healthy milk in the city and in the light of achieving sustainable health standards according to the current conditions and data, as well as future scientific developments and achieving the following goals:

1. Studying the levels of some heavy metals such as zinc in cow's milk and some of its products in a number of different regions of Nineveh Governorate and estimating zinc contamination extent to identify the most contaminated areas using milk as a biological indicator.
2. Evaluating the ability of probiotics (strains of Lactobacillus bacteria) to absorb zinc from contaminated milk in order to adopt them later on as one of the useful methods for the biological removal of heavy metals.

Methods and Materials

Collection of Milk Samples

Three replicates for each sample were used to collect 90 milk cows' milk samples from native breeds samples from different regions in Mosul city and its environs during the months of June 2022 and September 2022 for this study, taking into account the regional environmental conditions. Clean 1 liter polyethylene containers were used which were thoroughly cleaned before being filled and placed in a cool box and subjected to the necessary environmental conditions before being brought to the laboratory for the current research experiments.

Determination of Zinc in Milk Samples

Ten millimeter of milk were added to dry, known-weight bowel. Incineration and cremation processes were done by putting the bowl in the muffle (Electro. Mag., Germany) for one hour at 105 °C, then increasing the temperature to 550 °C for three hours until the white ash was obtained. 0.1 N Hcl ash was added to each sample at a rate of 10 ml Hcl to convert the precipitate into a liquid containing minerals in the form of dissolved electrolytes. By the atomic absorption apparatus, the concentration of the studied element zinc was estimated by the atomic absorption apparatus (Atomic Absorption) [5].

Isolation and Identification of *Lactobacillus Acidophilus*

The new 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 C^0 in anaerobic conditions . Later on, the gram positive rod shaped microscopic observed cells were purified and stored in $4C^{0.}$. Biochemical testes were proved *Lactobacillus acidophilus* to confirm these results 16srRNA gene PCR assay was carried out[6] . Total DNA was extracted by a Bioneer genomic extraction kit , amplified via the primers (F-5' AGAGTTTGATCCTGGCTCAG 3', R-5' AAGGTTACCTCAC-CGACTTC 3') primers (F-5' AGAGTTTGATCCTGGCTCAG 3', R-5' AAGGTTACCTCAC-CGACTTC 3') , conditions of reaction illustrated in Table (1-1) below :

Table (1-1) The Thermo polymer program

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 in the United States of America

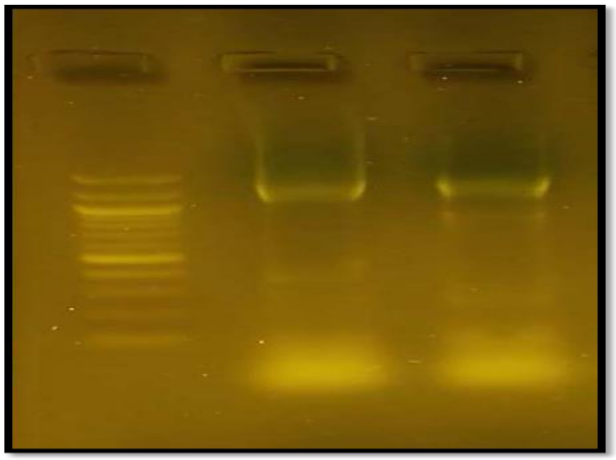

Figure 1 Shows the PCR reaction product of bacteria samples *Lactobacillus acidophilus* for 16SrRNA region (bp1200) migrated with 2% agarose gel.

Manufacturing of Yogurt

The yogurt has been made from raw cow's milk exposed to contamination. The concentrations of (zinc) were previously determined. Milk was pasteurized at a temperature of 84 c^o and then cooled at a temperature of 40c^0, impregnated with the starter at a rate of 3% per 100 ml of pasteurized milk, incubated for 4 hours and then kept cold at a temperature of 4 for 24 hours until the procedures for estimating the heavy metals in it were taken again[7].

Statistical Analysis

The information was analyzed using a system of simple experiments by means of a complete random design (CRD) to identify the nature of the difference among the levels of the treatments using the statistical program (SPSS) (Social Program Statistics System) to compare the arithmetic means at the level ($p < 0.05$) as mentioned by[8].

Results & Discussion

1- Effect of Adding Commercial Isolates to Milk Contaminated with Zinc

Table (1-2) shows the effect of the commercial isolate (*L. plantarum*) on raw milk contaminated with zinc as the different letters indicate that there was no statistically significant difference at the confidence level ($a \geq 0.05$) in the value of zinc concentration rates in contaminated raw milk samples collected from areas close to agricultural fields in which chemical fertilizers and pesticides were used and their concentration after the addition of the commercial isolate. The values of zinc before the addition in the areas shown in the table were (0.007, 0.006 and 0.004) ppm, while the values after the addition were (0.005, 0.004 and 0.003) ppm, respectively. The highest percentage of decline was recorded in areas close to agricultural fields in which chemical fertilizers and pesticides were used. Bioremoval ability varied with different incubation times, bacteria strains, temperature, pH, metal concentration or mycotoxins. Further evaluations were needed to discover the biological removal mechanism as well as the optimal cases to increase the removal yield. Indeed, more prospective studies on the combination of probiotics were needed to develop efficient bioremediation [9] [10][11].

Table (1-2) represents a comparison among zinc concentrations in contaminated milk before and after adding the commercial and local isolate

Region type	Zinc concentration ppm before adding (raw milk)	Zinc concentration ppm after adding commercial isolate
Areas close to industrial facilities	0.007±0.007 A	0.005±0.005 A
Areas close to high-traffic streets	0.007±0.006 A	0.006±0.004 A
Areas close to agricultural fields in which chemical fertilizers and pesticides are used	0.004±0.004 A	0.002±0.003 A

* The different letters on the means indicate a statistically significant difference at the confidence level ($a \geq 0.05$).

2- Effect of Adding Local Isolates to Milk Contaminated with Zinc

The results of Table (1-3) show the effect of the local isolate on raw milk contaminated with zinc. The different letters indicate that there was no statistically significant difference at the level of confidence ($a \geq 0.05$) in the value of zinc concentration rates in contaminated raw milk samples collected from the three regions and its concentration after adding the local isolate where the zinc values before addition in the regions shown in the table were (0.007, 0.006 and 0.004) ppm while the values after addition were 0.004 and 0.001 ppm respectively. The highest percentage of decrease was recorded in areas close to agricultural fields in which chemical fertilizers and pesticides were used. Although studies on the bio-absorption of zinc are few, the study conducted by[12] whose results are consistent with the current study confirmed that the initial concentration of heavy metals is an important factor affecting the process of bio absorption. The active binding sites and functional groups available on the surface of the bio sorbent were affected by the initial concentration of the metal ions [13].

Table (1-3) represents a comparison among zinc concentrations in contaminated milk before and after adding the commercial and local isolate

Region type	Zinc concentration ppm before adding (raw milk)	Zinc concentration ppm after adding local isolate
Areas close to industrial facilities	0.007±0.007 A	0.004±0.004 A
Areas close to high-traffic streets	0.007±0.006 A	0.004±0.004 A
Areas close to agricultural fields in which chemical fertilizers and pesticides are used	0.004±0.004 A	0.002±0.001 A

* The different letters on the means indicate a statistically significant difference at the confidence level ($a \geq 0.05$).

Conclusion

The results confirmed the absence of any contamination with the element (zinc) in the milk samples of cows that were grazing close to the three study areas, as its concentrations did not exceed the permissible limits in milk within the Iraqi and international standard specifications which were (0.328) ppm, and no significant differences were recorded in zinc concentrations at a probability level ($a < 0.05$) among samples from all regions. In this study, the absorption efficiency of zinc was evaluated. These results explained that when the amount of metal ions in the bacterial medium did not exceed its toxicity threshold. It cannot change the bacterial state or metabolism which led to a decrease in the efficiency of bioabsorption.

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