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Short Communication

Determination of Lead and Cadmium in cow's Milk and Elimination by Using Titanium Dioxide Nanoparticles

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ABSTRACT

Background and Objectives: Heavy metals such as cadmium and lead are the most important toxins spreading through various ways like water, soil, and air in nature and easily enter human food chain. It is essential to determine the cumulative and harmful effects of these metals in nutrients, especially in cow milk because it is a unique source of food for all ages and it contains both essential and nonessential trace elements.

Materials and Methods: A total of 100 milk samples were directly collected from healthy cows in Zabol located on east of Iran. The samples were tested to determine lead and cadmium residues. The rates of the heavy metals were determined using a Rayleigh atomic absorption spectrum equipped with hollow cathode lamps (HCL) at 283.3 nm for lead (Pb) and at 228.8 nm for cadmium (Cd). By using the photo-catalytic titanium dioxide nanoparticles, these toxic metals were removed.

Results: The mean ± SD of the concentration of lead and cadmium in raw milk were 9.175± 2.5 and 4.557 ± 1.081 ppb, respectively. Also, the P-values of Kalmogorov– Smiranov test for lead and cadmium were respectively 0.057 ppb ($P>0.05$) and 0.435 ppb ($P>0.05$). TiO_2 nanoparticles were used to eliminate and remove lead and cadmium in milk samples. The results showed that there was a significant difference between lead and cadmium contents before and after adding TiO_2 nanoparticles ($P<0.05$).

Conclusions: According to results of this study, there was a very low amount of toxic metals. So, it seems that it is not necessary to use TiO_2 in milk samples but these days it used frequently as an additive to some samples like milk to remove these pollutants.

Keywords: lead, cadmium, milk, atomic absorption spectroscopy, TiO_2 nanoparticles

Introduction

The entry of pollutants like heavy metals in food such as milk and dairy products is one of the issues that are very important. By far, various strategies and measures have been taken to improve milk quality and to lower the amount of pollutants in it (1).

Heavy metals have been considered as one of the deadly toxins that have accumulated in plant and animal tissues through the biological cycle and have dispersed across a wide geographical area. Some heavy metals such as lead and cadmium that are among the oldest historical background in creating this pollution are transported via the air. These particles enter air through the chimneys of factories and industrial units causing environmental contamination. Breathing this contaminated air transfers these particles to lungs and bronchi, stomach, blood, and other tissues leaving adverse effects. In addition, the heavy metals in the air are absorbed by plants and move indirectly into human body.

Because of the nature and properties of metals and their unwillingness to react with organic ligands in

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biological cycles, their toxicity is more than other toxic substances so that based on the codex 2000 (Committee monitoring Food Additives in Geneva), the acceptable levels of lead and cadmium in raw milk were 1000 ppb and 10 ppb, respectively (2).

Heavy metals accumulate in the tissues and are difficult to dispose, so they make severe side- effects the accumulated tissues. They also easily enter tissues such as blood vessels, muscles, bones, kidneys. Likewise, cadmium can replace some enzymes and lead to change in their spatial conformation result in loss of enzymatic catalytic activity. Furthermore, deformed bones, short stature, and bone fracture are other complications of cadmium accumulation in body. Some cadmium poisoning symptoms are asthma and kidney complications. It is carcinogenic, especially in lungs and prostate tumors. The complications of cadmium pollution among pregnant women are birth defects, decreased fetal weight, abnormalities in DNA and fetal proteins. Its high infection usually leads to abortion (3,4).

The negative effects of chronic exposure to high lead concentrations have long been known and its toxic effects were first introduced by Hippocrates. Various factors such as motor vehicles, factories, chemical and metal industries are involved in this regard. Lead and its organic and inorganic compounds are readily absorbed through skin, respiratory and gastrointestinal systems (5,6).

The kidneys are the major targets of cadmium toxicity following oral exposure. A specific indicator of cadmium-induced renal effects is tubular proteinuria. The most important digestive effect of lead is its gray pellet on gums known as the margin Burton (7). Lead toxicity in human includes adverse effects on circulatory system, muscles, endocrine glands, renal system, nervous system, reproductive system, deformation of gums. (8, 9, 10). Therefore, it is necessary to perform studies on the rate of milk contamination with these metals and to determine the rates of these heavy metals in different areas. Nano-sized metal oxides are known as available absorbents of heavy metals. Some of them are iron oxide, manganese oxide, aluminum oxide, titanium oxide, and cerium oxide, which have a particularly high level with a strong tendency to absorb heavy metals. Oxidation by sunlight or photo-catalysts can eliminate and destroy numerous kinds of environmental contamination at room temperature (11). As photo catalysts, oxidized metals play various roles in different conditions; however, scientific studies have proved that titanium dioxide (TiO_2) is mostly used as a suitable material for removing pollutants. The sol-gel method, including hydrolysis reactions and condensation of alkoxide precursors, is a safe method for the synthesis of very tiny metal oxides (12).At ultraviolet light, titanium dioxide creates a strong oxidizing effect, and can easily oxidize these heavy metals on its own surface (13).

In the east of Iran, Zabol is known as one of the poles of the livestock with an important source of animal products. Currently, there is a little information regarding the contamination of heavy metals in the Zabol milky cows. The objective of this study was to determine the concentration of lead and cadmium in the cows' milk of Zabol and also determine using of nanoparticles to remove these contaminations.

Materials and Methods

Cows' milk samples: From February to April 2014, a total of 100 milk samples were collected from healthy milky cows in Zabol, Iran. The samples were dumped in the bolted- door PE pipes washed with 10% nitric acid and were rinsed with distilled water. All the milk samples stored at -20°C in clean polyethylene containers, and away from light exposure.

Determination and elimination of lead and cadmium in milk samples: In this study, the furnace atomic absorption spectrometry as a sensitive method for measuring heavy metals (14, 15) was used to determine the levels of lead and cadmium in samples.

A volume of 2 ml raw milk was mixed with 4 ml concentrated nitric acid (65%, Merck, Germany) and was incubated for 24 hours at 85° C. Then, 4 ml of 30% hydrogen peroxide (Merck, Germany) was added and incubated for 1 h at 120° C a semi-closed glass digestion apparatus. Finally, it was diluted by 1% nitric acid (10 ml), and the amount of each metal was measured by spectrophotometer [16]. The concentrations of cadmium and lead were determined by graphite furnace atomic absorption spectrophotometry (Rayleigh, China) apparatus. Details of the analytical methods have been previously published (Ursinyova and Masanova, 2005). The detection limits calculated as three times the standard deviation were 0.038 μg/L and 0.041 μg/L of milk for cadmium and lead, respectively. The accuracy of the analysis was checked by various methods including the use of reference material (BCR No 150). Because of special properties of titanium dioxide like chemical stability, low cost and non-toxicity, this nano-catalyst is widely used in photo catalysis treatment of environmental pollutants. Photo-catalyzed oxidation processes are advanced oxidation methods in which heavy metals are oxidized as a result of exposure to UV light and in presence of Photo-catalysts such as titanium dioxide (UV/TiO_2 process).To determine metal removal by nanoparticles, 2 ml of TiO_2 nanoparticles (size :10-25nm ,purity:99% and prepared by Iranian Nanomaterials Pioneers Company) was added to 20 ml milk at a concentration of 0.1 ppm and was put at ultraviolet light for 2 hours to complete oxidation reaction. Then, it was filtered and like the previous stage, digestion was done. Finally, the remaining amount of lead and cadmium was calculated with atomic absorption spectrometry.

Statistical analysis: Using SPSS 18 statistical software (SPSS Inc,. Chicago, IL, USA), a Kalmogorov–Smiranov test analysis was performed and differences were considered significant at $P<0.05$. For the statistical analysis, values below the detection limit were set to half that level.

Results

The average concentration and the limit range levels of lead and cadmium available in cows' milk of five different regions of Zabol are presented in Table 1. Results showed that the average concentration of lead was 9.175 ± 2.581 ppb (range 2.87-14.67 ppb) and the average concentration of cadmium was 4.557 ± 1.081 ppb (range 1.5- 6.98 ppb)), respectively.

Table 2 showed the lead contents after adding TiO_2 nanoparticles to same samples mentioned above in five different regions of Zabol. By comparing the results of lead and cadmium contents before and after adding of TiO2 nanoparticles, we could observed a significant differences between them ($P<0.05$).

Table 1. Lead and cadmium concentration (ppb) in milk from healthy cows in five different regions of Zabol, Iran

Heavy metals	Number of samples	Mean concentration ± SD	Standard range
Lead	100	9.175 ± 2.581	2.87-14.67
Cadmium	100	4.557 ± 1.081	1.50-6.98

	% RSD n=5	uncertain	loq	lod	recovery	accre
lead	0.281	0.3	60	1.2	101.47	Y
cadmium	0.237	0.4	10	0.3	102.12	Y

Table 2. Lead and cadmium concentration (ppb) after adding TiO_2 nanoparticles in milk samples from healthy cows in five different regions of Zabol, Iran

Heavy metals	Number of samples	Mean concentration ± SD	Standard range
Lead	100	5.173 ± 2.517	0.17-11.46
Cadmium	100	1.905 ± 1.068	0.17-2.76

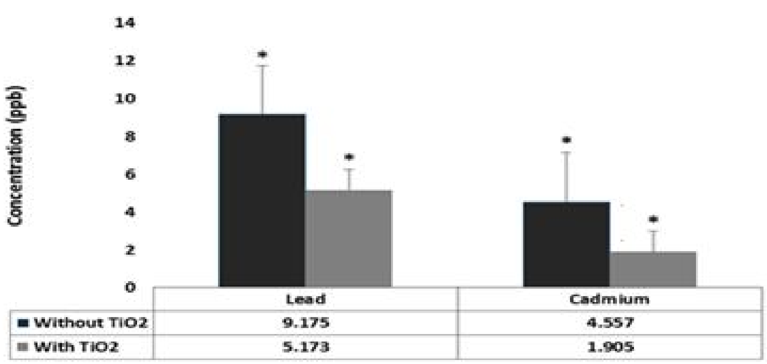

Figure 1. Difference between Lead and Cadmium concentration (ppb) in milk with and without adding TiO_2 Nanoparticles.

* $P<0.05$

Discussion

In this study, after adding nanoparticles and oxidizing lead under UV light, according to the average results, 46.5% of this heavy metal was oxidized and removed from the surface of titanium dioxide nanoparticle. After adding the nanoparticle and oxidizing cadmium under UV light, according to the average results, 59.4% of this heavy metal was oxidized and removed from the surface of titanium dioxide nanoparticles. Many studies have been conducted on milk poisoning with lead and cadmium in Iran. It is important to find and remove contaminated milk because it is not only beneficial for human public health, but also a useful way to inform farmers about the ways to eliminate the source of contamination and toxin among their cattle. It also warns them to pay more attention to analysis of materials, especially the amount of pollutants, while feeding the cattle so that they would be able to minimize gastrointestinal and breathing absorption of metals such as lead and cadmium as much as possible. One study in Khorasan-e-Razavi province, north east of Iran, Najar nezhad et al. reported that lead and cadmium concentrations in milk was 0.3 ± 0.3 ng/g and 0.6 ± 12.9 ng/g respectively (17). Using the atomic absorption method, Shakerian et al. stated the average concentration of lead and cadmium in the raw milk gathered in Isfahan as 0.25 ppm and 0.03 ppm (21). Javadi et al. tested the content of mercury, lead, cadmium and chromium in cow's milk and realized that the cow's milk contamination levels of lead and chromium was above the standard limit, whereas in case of cadmium, its contamination was lower than the limit (22). Shirzad Siboni et al. at 2013 study on Photo catalytic Removal of Hexavalet Chromium and Divalent Nickel from Aqueous Solution by UV Irradiation in the Presence of Titanium Dioxide Nanoparticles and The results showed that UV/TiO_2 was an effective method in removal of hexavalent chromium and divalent nickel from aqueous solutions (23).Another study by Sung Ho Kim et al. at 2003, designed the hybrid thin-film-composite (TFC) membrane consisted of self-assembly of TiO2 nanoparticles with photocatalytic destructive capability on microorganisms was devised as a novel means to reduce membrane bio fouling. Then, the anti-fouling and fouling mitigation on the actual commercialized TFC was verified (24, 25). Presence of various industrial centers and high production rate of vehicles could be a reason for the higher rate of lead and cadmium metals in some special areas. Likewise, one of the reasons for the differences in allowable values of lead and cadmium in different studies was the standard rates of lead and cadmium in studied Codex (Codex international food standards). According to Codex 2000, the standard limit of lead in milk was 1000 ppb but at 2007, this limit reduced to 20 ppb, respectively (17, 21-23, 26). According to results of this study, the rates of these heavy metals in the milk samples in Zabol city were much less than the global standard limits. This fact indicated that cow's milk in this region of Iran seems to be safe for human health. Reports have indicated that ingestion of TiO_2 nanoparticles as a photo catalyst in chemical reactions is harmless. In these experiments, due to a very low amount of toxic metals, it is not necessary to use this photo catalyst in milk samples and according to studies , TiO_2 nanoparticles is toxic at the highest dose (≥50ppm) but has no effect on body at lower levels (27-29).

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None of the authors have any conflict of interest to declare.

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