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Original Article

Risk Assessment of Arsenic, Cadmium, and Lead Concentrations and Microbial Contamination in Different Types of Children's Play Dough

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Abstract
Due to the widespread use of play doughs among children and the ability to absorb heavy metals and transfer contaminants through the skin, it is essential to determine the level of chemical and microbial contamination of these products to provide a clear picture of their quality. This descriptive cross-sectional study was conducted in the Reference Laboratory of Food and Drug Administration of the Ministry of Health and Medical Education of Iran. For this purpose, 12 samples of play dough were examined. A flame atomic absorption spectroscopy was used to evaluate the concentrations of heavy metals in different samples of play dough. Bacterial isolates were cultured in nutrient-rich microbial culture media and then used for phenotypic isolation and differential detection of bacteria. Based on the results, arsenic was observed in all samples and its mean concentration was 0.12 ppm. Cadmium and lead were present in all samples with mean concentrations of 0.06 and 0.12 ppm, respectively. According to the results, heavy metal concentrations did not exceed the threshold in any of the samples, and all concentrations were within the safe range. The results demonstrated that no microbial contamination related to *Escherichia coli*, coliforms, *Salmonella*, *Staphylococcus aureus*, molds, and yeasts was observed in the dough samples. Therefore, play dough products can be used as safe children's toys in Iran. However, due to the possibility of microbial contamination over time and repeated use, hygiene practices should be taught to children regarding the use of play toys such as frequent hand washing before and after use.
Keywords: Children, Heavy metals, Microbial contamination, Play dough, Risk assessment

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1. Introduction

In recent years, with increasing demand for food and industrial production, large amounts of hazardous chemicals and pesticides are used in various applications, which can lead to an increase in the accumulation of heavy metals in various products and pose a health risk to consumers (1). Arsenic, zinc, and cadmium rank among the priority metals due to their properties such as tissue accumulation, degradability, resistance to biological degradation, high toxicity, bioaccumulation in the food chain, and carcinogenicity. Arsenic, as one of the most important heavy metals, is widely used in various industries such as paint manufacture, pesticide production, and oil refining (2).

Acute exposure to arsenic is associated with a wide range of adverse health effects from gastrointestinal distress to death. Long-term exposure to arsenic is associated with skin cancer and serious damage to the bowel and liver (2,3). Cadmium is another heavy metal that is mainly produced as a by-product of zinc refining and most of its properties are similar to zinc. Cadmium and its compounds are highly toxic. Approximately 25 000 tons of cadmium enter the environment annually. About half of this cadmium enters the rivers through weathering of rocks (4,5). Forest fires, volcanoes, and human activities such as the production of industrial waste leachate and synthetic phosphate fertilizers are important sources of cadmium release into the environment. It mainly enters the human body through foods that contain high cadmium and eventually accumulates in the kidney. Cadmium is an element that does not perform any beneficial structural function in the body and can cause poisoning even in very small amounts.

Cadmium has several adverse effects on health including diarrhea, abdominal pain, vomiting, bone fracture, sterility, central nervous system disorders, immune system problems, psychological disorders, possible damage to DNA, and cancer (6). One of the most important issues regarding the entry of heavy metals into the body is that they are not metabolized by the liver. In fact, heavy metals are no longer excreted after entering the body and are accumulated in different tissues such as fat, muscles, bones, and joints, which can cause numerous diseases and complications in the body. Heavy metals can even replace some essential minerals in the body. For example, when there is a deficiency of zinc in the body, cadmium can replace zinc in certain dehydrogenating enzymes, leading to cadmium toxicity. Hence, the body is forced to use cadmium for repair. In general, exposure to heavy metals is associated with a wide range of adverse effects on human health including, neurological disorders (Parkinson's disease, Alzheimer's disease, depression, and schizophrenia), hormone imbalance, obesity, abortion, respiratory and cardiovascular disorders, liver, kidney, and brain damage, cancers, and death caused by acute exposure to high concentration of heavy (7,8). Pathogens can survive on different environmental surfaces and then enter the human body through hand, food, and so on. Survival of these pathogenic agents on different surfaces depends on several factors such as temperature and humidity (9). The survival of these pathogens is also higher on porous and humid surfaces (10). In this regard, kindergartens are often a place for the transmission of infectious agents among children. Playing with different toys like play dough in these places can cause the transmission of pathogenic germs such as bacteria (11).

Coliforms are bacteria that are mostly present in human and animal stool samples as well as soil and plant tissues. They include a variety of bacterial species such as *Citrobacter*, *Enterobacter*, *Escherichia*, *Klebsiella*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. These bacteria can cause infections in the urinary and respiratory tracts, bloody diarrhea, septicemia, soft tissue infection, and so on. Moreover, some molds and yeasts can also be transmitted indirectly through play dough (12).

Given that play dough is widely used among children and that it can absorb heavy metals and transfer contaminants through the skin, it is essential to determine the chemical and microbial contamination levels. This study can provide an overview of the contamination status of these products. Therefore, the present study aimed at evaluating the concentrations of arsenic, cadmium, and lead and microbial contamination in different types of play dough.

2. Materials and Methods

This descriptive cross-sectional study was performed on different types of play dough. For this purpose, 12 play dough samples from all brands available in the market were purchased and examined as new and untouched. Briefly, 300 g of each dough sample was prepared. The samples were kept at refrigerator temperature in the laboratory until they were analyzed. All laboratory dishes were washed with weak acid for further experiments, washed with distilled water, and then dried in an oven. After drying the samples, 2 g of each 300-g sample was removed and stored in the oven at 105 °C for 48 hours.

Afterward, 10 mL of perchlorate (70%), 5 mL of sulfuric acid, and 30 mL of nitric acid (70%) were added and kept at laboratory temperature for 30 minutes. Then, the resulting mixture was placed on a heater. The heated mixture was allowed to slowly boil to yield a 25-mL clear solution. Prepared samples were stored in the refrigerator to prepare all samples. A flame atomic absorption spectrometer (Shimudzu-AA-670, Japan) was used to determine the amount of heavy metals in different samples of dough. The obtained data were analyzed using Excel 2010 and Spectra AA software.

The values of the limit of detection (LOD) and limit of quantification (LOQ) for these three heavy metals are shown in Table 1.

The bacterial isolates were cultured in a nutrient-rich microbial growth medium (Merck, Germany). Then, specific differential media were used for the isolation and identification of bacterial phenotypes. Several media were also applied for the identification of the Enterobacteriaceae family including triple sugar iron agar (TSI), sulfide indole motility medium (SIM), Simmons citrate agar (SC), urea broth (U), and methyl red Voges-Proskauer broth (MR-VP). Moreover, the Coagulase and differential environment test (Mannitol salt Agar) were used for the growth of *S. aureus*. WL Nutrient Agar medium and microscopic detection were used to isolate yeast and mold (13).

At the time of analysis in the laboratory, 10% of all samples were evaluated as repeated with secret codes, and the test results were controlled and their accuracy was checked. A calculation formula was used to determine the accuracy of the test; this accuracy method is usually used to calculate the accuracy of the elements' concentrations;

Table 1. Values of Limit of Detection (LOD) and Limit of Quantification (LOQ) for the Investigated Metals

Heavy Metal	LOD (µg/L)	LOQ (µg/L)
Pb	0.0011	0.0035
Cd	0.002	0.0008
As	0.0012	0.004

the analysis error of repeated samples is calculated by equation 1 (14).

$$R = \frac{2}{n}\sum\frac{|x1 - x2|}{x1 + x2}\times 100 \quad (1)$$

3. Results and Discussion

3.1. Heavy Metals and Microbial Contamination

Based on the results, arsenic was found in all 12 different brands of play dough (100%) and its concentration was about 0.12 ppm. Additionally, various concentrations of cadmium were observed in all of the studied samples (100%). Table 2 presents the concentrations of the studied heavy metals in the play dough samples.

In this research, the microbial quality of the play doughsamples was investigated. As can be seen in Table 2, none of the samples were contaminated with E. coli, coliforms, Salmonella, S. aureus, molds, and yeasts. These results are presented in Table 3.

Since 2007, various measures have been undertaken to address toxic chemicals in toys in the United States. In this regard, the government established new regulations to increase corporate responsibility, limiting the use of hazardous chemicals and issuing environmental certificates for toys. These toys are evaluated by relevant institutes to confirm their quality in terms of dangerous chemicals, especially heavy metals (15). Despite these laws and regulations, there has been a growing concern regarding the chemical and microbial contamination of these products, especially in developing countries, which can pose a health risk to children.

According to previous studies on toys, polyvinyl chloride (PVC) is the most common material used in the manufacture of plastic toys. Lead compounds are incorporated in PVC materials in order to improve their stability, softness, brightness, and flexibility and make them more attractive to children. It has been reported that the presence of lead and cadmium in toys is the main cause of child poisoning worldwide (16-21). Lead compounds used in PVC materials include basic lead carbonate, lead stearate, basic lead stearate, tribasic lead stearate, dibasic lead stearate, and lead phthalate. Basic lead phthalate and lead are also used in the manufacture of PVC (18-21). The lead in PVC materials is released over time with repeated use of play dough; lead can enter the bloodstream through inhalation (21). Lead can significantly disrupt the balance of various ions, including calcium, iron, and zinc, and impair their vital function in the body (22,23). For this reason, in the present study, the concentrations of lead, cadmium, and arsenic as the main heavy metals existing in play dough were measured. The results showed that arsenic was observed in all play dough samples (100%) and its concentration was about 0.12 ppm. In the dough samples, the arsenic concentration was lower than the allowable limit for these elements. Moreover, the results demonstrated that various concentrations of cadmium were observed in all of the studied samples (100%), but the concentration of heavy metal was below the standard limit presented in Table 1. Table 1 presents the concentrations of the studied heavy metals in the play dough samples. The results implied that all samples had a high concentration of lead (0.06 ppm). However, the concentration of lead did not exceed the allowable limit in any of the samples; in other words, the lead content was within the safe range in all samples.

Increasing consumer concerns about the presence of heavy metals in play dough have led to the revision of Council Directive 88/378/EEC (European Economic Community). The recent guidelines of Directive 2009/48/EC and EN 71-3:2013 recommended the highest allowable concentrations of heavy metals in toys. Table 4 presents the maximum allowable concentration of arsenic, lead, and cadmium as the main heavy metals existing in toys according to the European Economic Community. In the present study, the concentrations of 3 toxic chemicals including arsenic, lead, and cadmium were in the ranges of 0.10-0.16, 0.04-0.10, and 0.05-0.07, respectively.

In the present research, the levels of arsenic, lead, and cadmium were examined in play dough samples, and it was found that their concentrations were lower than the maximum allowable limit recommended by Directive 2009/48/EC. These results are consistent with the findings of the study by Omolaoye et al on the measurement of lead and cadmium levels in PVC and non-PVC toys.

Table 2. Concentrations of Lead, Cadmium, and Arsenic in Play Dough Samples (ppm)

Code	Heavy Metals					
	As		Cd		Pb	
	ppm	SD	ppm	SD	SD	ppm
1	0.12	0.031	0.05	0.003	0.06	0.014
2	0.10	0.025	0.06	0.015	0.05	0.006
3	0.12	0.019	0.07	0.021	0.06	0.012
4	0.14	0.033	0.07	0.018	0.06	0.015
5	0.15	0.042	0.04	0.002	0.06	0.011
6	0.16	0.057	0.04	0.005	0.07	0.021
7	0.12	0.026	0.05	0.008	0.07	0.019
8	0.10	0.018	0.04	0.003	0.06	0.017
9	0.10	0.021	0.10	0.061	0.05	0.008
10	0.12	0.023	0.10	0.081	0.06	0.018
11	0.11	0.031	0.04	0.006	0.05	0.007
12	0.12	0.012	0.06	0.013	0.06	0.013

Table 3. Microbial Contamination in Play Dough Samples

Microorganism	Count
Total count of microorganisms	$5*10^2$
Coliforms	Negative
Escherichia coli	Negative
Salmonella	Negative
Staphylococcus aureus	Negative
Molds	Negative
Yeasts	Negative

In the mentioned study, the levels of heavy metals were lower than the standard level. They also reported that the lead and cadmium concentrations in non-PVC toys were lower compared to PVC toys. Moreover, among the metals studied in the study mentioned, the levels of lead in some toys were higher than the maximum allowable limit, which is inconsistent with our finding (21).

In another study conducted by Al-Qutob et al, the concentration of heavy metals (lead, cadmium, chromium, lead, lead, and zinc) in low-cost plastic toys imported into the Palestinian and Israeli markets was examined in 50 cases (25). These results showed that 40% of the Palestinian toy samples had a high concentration of lead (above the standard limit). Moreover, 20% of the samples were contaminated by different bacteria and a high concentration of cadmium and arsenic was observed in 30% and 42% of the toys, respectively. On the other hand, 95% of the Israeli toy samples were safe, and their concentration was below the maximum allowable limit. These results are not consistent with our findings, which may be attributed to poor production quality and lack of control over the production of these products. In the study conducted by Grynkiewicz-Bylina, it was reported that toys with polyester coating contained lead and several other heavy metals. Moreover, toys covered with liquid paint contained cadmium, lead, and other metals, and arsenic, lead, and cadmium were present in toys coated with enamel paints and impregnates. However, in 97% of the samples, the concentrations of the heavy metals were below the recommended standard, which is in agreement with the results of the present study (26). In line with our study, in the study conducted by Sindiku and Osibanjo in Nigeria, cadmium concentration was much lower than the standard threshold. Lead was also identified in almost all samples, but 76% of the samples had significantly higher lead concentrations than the recommended standard (27).

3.2. Risk Assessment

In the present study, in order to investigate the health-related risk of heavy metals present in play dough, chronic daily intake (CDI) of heavy metals through the skin was calculated using equation 2. For this purpose, the CDI value was calculated for each detected heavy metal in 12 brands of play dough. In order to calculate the non-carcinogenic risk of each metal, hazard quotient (HQ) was also calculated for each heavy metal. Table 4 presents the values of CDI and HQ for each studied heavy metal in the studied brands of play dough. According to the results, the values of the calculated indexes in all of the studied samples were less than 1, indicating that all of the play dough samples were in the safe range of heavy metal content and had a non-carcinogenic risk. The total CDI of arsenic, cadmium, and lead in each brand of play dough was also less than 1; in other words, their levels were within the safe range (28).

Table 4. The Maximum Allowable Concentration of Heavy Metals (mg/kg) According to the European Legislation on Toys (in Dry, Brittle, Powder-like Toy Material)

Directive	As	Pb	Cd	Reference
2009/48/EC	3.8	13.5	1.9	(24)
EN 71-3:2013	3.8	13.5	1.3	

$$D_{Dermal} = \frac{C \times SA \times SL \times ABS \times EF \times ED}{BW \times AT} \times 10^{-6} \quad (2)$$

Where D_{dermal} (mg/kg/d) is average daily intake of heavy metals through dermal exposure to children's play dough, C (mg/kg) is the concentration of the heavy metal in children's play dough, SA (cm^2) is the exposed skin area, SL (mg/cm^2) is skin adhesion factor, ABS (without unit) is the dermal absorption fraction for a given element, EF (day/year) is the exposure frequency, ED (year) is the exposure duration, BW (kg) is the body weight, and AT (day) is average exposure time (29). The AT was obtained using equation 3 (30).

$$AT = ED \times 365 \quad (3)$$

Where ED is the number of years that children's play dough has been used. Then, non-carcinogenic health risk was evaluated using equation 4 (31).

$$HQ = \frac{D_{Dermal}}{RfD} \quad (4)$$

3.3. Comparative Study

In this step, the results of the present study were compared with the findings of the previous studies. In this regard, the study of Njati, reviewed the lead concentration in both paints and PVC toys of children. In the mentioned study, high levels of lead in paints have been recorded in China (116 200 ppm), Cameroon (500 000 ppm), South Africa (189 000 ppm), Tanzania (120 862.1ppm), Uganda (150000 ppm), Thailand (505716 ppm), and Brazil (1705-258.4 ppm). In contrast to other studies, they reported a much higher level than the recommended limits in the paints and toys, which can cause serious health problems in children (17). As observed in Table 5, the mean concentration of all three types of studied heavy metals in the current study was lower compared to other studies conducted in different countries. Moreover, our findings are consistent with the findings of the study conducted by Charehsaz et al in Turkey (32).

The presence of toxic metals such as arsenic, cadmium, lead, and others in toys was also investigated in a study performed by Yazdanfar et al in Iran. They explained that all of the toxic elements in the examined children's toys were within the permissible limits, which confirms the results of our study (33).

Another study conducted in Nepal showed that 17% of tested toys had high concentrations of cadmium, lead, and other heavy metals (34). These metals have been highly varied in various toys in other studies, ranging from small amounts to very high and dangerous amounts

Table 5. Chronic Daily Intake and Non-carcinogenic Risk in Studied Brands of Play Dough through the Skin in Children

	As		Cd		Pb		Total	
	D_{Dermal}	HQ	D_{Dermal}	HQ	D_{Dermal}	HQ	D_{Dermal}	HQ
1	3.6E-14	4.5E-11	1.5E-14	6.0E-10	1.8E-14	3.4E-11	6.9E-14	6.79E-10
2	3.02E-14	3.7E-11	1.8E-14	7.0E-10	1.5E-14	2.9E-11	6.32E-14	3.71E-11
3	3.6E-14	4.5E-11	2.1E-14	8.0E-10	1.8E-14	3.4E-11	7.50E-14	4.51E-11
4	4.2E-14	5.2E-11	2.1E-14	8.0E-10	1.8E-14	3.4E-11	8.10E-14	5.21E-11
5	4.5E-14	5.6E-11	1.2E-14	5.0E-10	1.8E-14	3.4E-11	7.50E-14	5.61E-11
6	4.8E-14	6.0E-11	1.2E-14	5.0E-10	2.1E-14	4.0E-11	8.10E-14	6.01E-11
7	3.6E-14	4.5E-11	1.5E-14	6.0E-10	2.1E-14	4.0E-11	7.20E-14	4.51E-11
8	3.02E-14	3.7E-11	1.2E-14	5.0E-10	1.8E-14	3.4E-11	6.02E-14	3.71E-11
9	3.02E-14	3.7E-11	3.02E-14	12E-10	1.5E-14	2.9E-11	7.54E-14	3.71E-11
10	3.6E-14	4.5E-11	3.02E-14	12E-10	1.8E-14	3.4E-11	8.42E-14	4.51E-11
11	3.3E-14	4.1E-11	1.2E-14	5.0E-10	1.5E-14	2.9E-11	6.00E-14	4.11E-11
12	3.6E-14	4.5E-11	1.8E-14	7.0E-10	1.8E-14	3.4E-11	7.20E-14	4.51E-11

depending on the type of toy, the material, and the color. There are significant differences in the concentration of heavy metals, which can be attributed to the structure and color of the toys. However, the concentrations of these heavy metals in different toys in other studies were highly varied and ranged from low values (in the safe concentration range) to very high and dangerous values. These differences are related to the type of toys, material content, and type of colors used in their structure (35-39).

3.4. Microbial Contamination of Play Dough

In order to evaluate the microbial quality of play dough samples, the total number of colony-forming units (CFUs) was determined, which was equal to 2 × 102. However, no microbial contamination in relation to *E. coli*, coliforms, *Salmonella*, *S. aureus*, molds, and yeasts was observed in the play dough samples, as shown in Table 6.

According to the results of the present study, a low level of microbial contamination was detected in all types of the play dough samples, which indicated their safety as children's toys. However, due to the frequent use and contact with other objects and hands, these play doughs can become pathogenic over time, and their microbial load increases over time. In contrast with our findings, Stauber et al found that there was a high level of bacterial contamination in the studied toys and the level of *E. coli* was significantly lower than the total microbial population (42). Martínez-Bastidas et al reported that the contamination level of *E. coli* and *K. pneumoniae* was relatively high in the studied toys. In the mentioned study, *Salmonella* was identified in the studied samples, which is inconsistent with our study. The presence of microbial contamination could be related to the frequent use of these toys in the study conducted by Martínez-Bastidas et al (43).

4. Conclusion

This study aimed to evaluate the chemical and microbial concentrations in 12 different brands of play dough. For this purpose, the concentrations of lead, cadmium, and arsenic as the main heavy metals existing in play dough were measured in the present study. The results showed that arsenic was observed in all samples (100%) and its concentration was about 0.12 ppm. In the dough samples, the arsenic concentration was lower than the allowable limit for these elements. Moreover, the results demonstrated that various concentrations of cadmium were observed in all of the studied samples (100%), but

Table 6. Comparison of Previous Studies with the Present Study in Terms of Heavy Metal Concentrations

Author	Year	County	Item type	Heavy metal	Concentration
Ghaly et al (16)	2013	Egypt	Toys or dyes	Cd	<17 PPM
Njati et al (17)	2018	China Cameron South Africa Tanzania Uganda Thailand Brazil		Pb Pb Pb Pb Pb Pb Pb	116200 PPM 500000 PPM 505716 PPM 120862,1PPM 150000 PPM 505716 PPM 1702584 PPM
Omolaoye A (21)	2017	Nigeria		Pb Cd	2.5-1445 μg/g-1 0.5-373.33 μg/g-1
Gati et al (40)	2014	China		Pb Cd	<100 PPM <200 PPM
Shen et al (41)	2018	China		Pb	12-219 PPM
Turner A (35)	2018	UK		Pb Cd	5000 $\mu g/g^{-1}$ <16000 $\mu g/g^{-1}$
Garcia (36)	2010	Colombia		Pb	<1024 PPM
Greenway et al (37)	2010	Las Vegas		Pb	≥600 PPM
Sanders et al (23)	2012	USA		Pb	>100 PPM
Kawamura et al (38)	2006	Japanese		Pb Cd	1.3 - 1.5 mg/kg^{-1} 0.2-26 mg/kg^{-1}
Cui et al (39)	2015	China		As Cd Pb	0.22-19 mg/kg^{-1} 0.01-139 mg/kg^{-1} 0.08 – 860000 mg/kg^{-1}
Charehsaz et al (32)	2014	Turkey		As Cd Pb	0.18 mg/kg^{-1} 0.24 mg/kg^{-1} 17.84 mg/kg^{-1}
Current study	2020	Iran		As Cd Pb	0.12 PPM 0.06 PPM 0.06 PPM

these concentrations were below the standard limit (0.06 ppm). Moreover, lead was identified in all of the play dough samples with a mean concentration of 0.06 ppm, which was lower than the standard limit. The results demonstrated that no microbial contamination in relation to *E. coli*, *coliforms*, *Salmonella*, *S. aureus*, molds, and yeasts was detected in the dough samples. Therefore, play dough products can be used as safe children's toys in Iran. However, due to the possibility of microbial contamination over time and repeated use, hygiene practices should be taught to children regarding the use of play toys such as frequent hand washing before and after use.

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Competing Interests
The authors declare that they have no conflict of interests.

Ethical Approval
The study was approved by the Institutional Ethics Committee of Kermanshah University of Medical Sciences (Approval number: 980176, dated: 21/05/2019). Informed consent was waived because the study was observational.

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