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Monitoring of Certain Pesticide Residues and Some Heavy Metals in Fresh Cow's Milk at Gharbia Governorate, Egypt

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Abstract: This monitoring study of 40 samples of cow's milk collected from different locations at Gharbia Governorate during the four different seasons of the years 2005-2006, was conducted to determine the contamination levels of seven pesticide residues including four organophosphorus (chlorpyrifos, diazinon, dimethoate and malathion) and three synthetic pyrethroids (cypermethrin, deltamethrin and fenvalerate), as well as five heavy metals (copper, iron, cadmium, zinc and lead). The results showed that there were no contamination with investigated pesticide residues found in all analyzed samples, except for malathion, which was detected in a single sample with a negligible existence (0.018 mg kg^{-1}), by ratio of (0.02%) of all samples. In relation to heavy metals detection, data revealed that all milk samples were contaminated with heavy metals all year around. The contamination with heavy metals was generally dominated at summer season. Level of lead was higher than other metals. The mean levels of Cu, Fe, Cd, Zn and Pb were 0.251, 0.607, 0.159, 0.371 and 2.462 mg kg^{-1} , respectively. Cu, Fe and Zn level were under the permissible limits, while the Cd and Pb were exceeded the permissible limits, indicating serious heavy metals pollution in the region.

Key words: Pesticides, heavy metals, contamination, cow's milk

INTRODUCTION

Liquid milk is an essential nutritional food for infant's as well as the aged. Contamination of this milk with residues of pesticides is a matter of serious concern. Milk is an ideal liquid to dissolve environmental chemicals. Most environmental contaminants are the fat-soluble type and milk has about four percent fat. The water-soluble chemicals dissolve easily in the predominantly aqueous part of milk. Therefore, all types of chemicals can be found in milk, fat-soluble and water-soluble, because milk offers both environments. Milk, therefore, is a true reflection of the pollution in our environment-not a very pretty picture.

A wide range of organic and inorganic compounds may occur in feedstuffs, including pesticides, industrial pollutants and heavy metals. Organic chemicals comprise the largest group and include plant toxins, mycotoxins, antibiotics and pesticides. Inorganic compounds include heavy metals and radionuclide. Pesticides that may contaminate feed originate from most of the major groups, including organochlorine, organophosphate and pyrethroid compounds (Van Barneveld, 1999).

Cow's milk makes an excellent medium to contract dangerous chemicals components found in the environment. Pesticides are so essential to a good crop that it is almost impossible to visualize any feed without some contamination.

The origin of these residues in milk is often attributed to two main factors. The first is the residues and their metabolites secreted in milk. The second is the direct contamination of milk after milking with pesticides used in spraying of the dairy barns, plants and milking animal itself. Milk producing animals, such as cow and buffalo's accumulate residues of pesticides through contaminated feed, grass/hay and inhaled air (Waliszewski *et al.*, 1977). Factors that determine the occurrence and concentration of pesticides in milk can be categorized into chemical and material characteristics (Gallenberg and Vodcnik, 1989). Chemical characteristics include the degree of ionization (plasma to milk pH gradient). Lipid solubility, molecular mass and extent and nature of association of pesticides with other blood constituents. A maternal characteristic includes the exposure of animals to chemicals, milk fat content, maternal age and parity and maternal adipose tissue mass. Pesticide residues in milk and dairy product

have been studied by Abd Rabo *et al.* (1989) Dogheim *et al.* (1990) Abd Alla *et al.* (1991) and Dabiza *et al.* (1999).

Milk is also contaminated with heavy metals such as Zinc, lead, cadmium, selenium, sulfur, iodine and possibly even more dangerous arsenic and cyanide (Richard Dorn *et al.*, 1975; Hura *et al.*, 1998; Licata *et al.*, 2004). Heavy metals are released into the environment by metal smelters and other industrial activities, unsafe disposal of industrial wastes and the use of lead in water pipes and petrol. Cow on farms near industrial areas have exceptionally higher levels of heavy metals in their milk. Still another source of feed contamination is recycled farmyard waste. The most dangerous metals, when concentrated above naturally occurring levels, include lead, mercury, cadmium, arsenic, copper, zinc and chromium. These have as diverse effects relating to cancers (arsenic and cadmium), genetic damage (mercury) and brain and bone damage (copper, lead and mercury). (Buck *et al.*, 1994; Fisher *et al.*, 1996). In a recent study of Egyptian Policemen, urinary excretion of NAG was positively correlated with duration of exposure to lead from automobile exhaust, blood lead and nail lead (Mortada *et al.*, 2001)

The purpose of the present study was to determine the residue concentrations of the seven pesticides commonly used in Egypt for pest control, i.e., four organophosphorus and three synthetic pyrethroides insecticides in milk samples collected from different locations through four different seasons of the year 2005-2006 at Gharbia Governorate. In addition to detection the levels of five heavy metals, i.e., Cu, Fe, Cd, Zn and Pb in the same samples were also determined.

MATERIALS AND METHODS

Sampling: Forty composite samples of fresh cow's milk were randomly collected from different location at Gharbia Governorate at various times during four seasons, namely Autumn (October and November), Winter (December, January and February) Spring (March, April and May) and Summer (June, July, August and September), (ten samples per season) at the year 2005-2006. Immediately after collection, milk samples were cooled to about 4°C and were quickly transferred in an ice box to the Central Agricultural Pesticide Laboratory, Agric. Res. Center, Dokki, Giza, Egypt where they were kept frozen at -20°C until the day analysis. Before analysis, the samples were thawed and homogenized by hand shaking. Each Milk sample was divided into three portions. The first portion was analyzed for milk composition, the second was analyzed for pesticide residues and the third was analyzed for heavy metals.

Analytical procedures

Milk composition analysis: The milk samples were analyzed for Total Solids (TS), fat, protein and lactose contents using the methods described in (Anonymous, 1990a). Solids No Fat (SNF) were calculated from the difference between TS and fat content. Ash content was determined using a furnace muffle.

Pesticide residues detection

Extraction: Samples were analyzed according to method of (Luke and Doose, 1984) for multiple residue determination, slightly and modified. For milk samples contain 4% fats or less, weight 50 g milk into a blender cup. Add 20 g aluminum oxide, 25 mL distilled water and 280 mL acetonitrile (If significant GC interferences are present in the aluminum oxide, then wash with 200 mL methanol 95% followed by 200 mL hexane and dry on a steam bath). Blend 2 min supernatant liquid with suction through sharkskin filter paper (previously washed with 100 mL acetonitrile). Measure 250 mL of the filtrate and transfer to 1 L separatory funnel. Add 100 mL petroleum ether and shake for 30 sec. Add 10 mL saturated NaCl solution and 500 mL distilled water. Shake for 1 min and allow the layers to separate. Transfers the lower aqueous layers to a second 1 L separatory funnel contain 100 mL petroleum ether and shake for 1 min. Once again, allow sufficient time for the layers to separate and then discard that lower aqueous layer. Then combine the remaining layer with the layer in the first separatory funnel and wash the combined layers with 2×100 mL portions of distilled water. Dry the petroleum ether extract through 1 layer of anhydrous, granular Na₂SO₄ (if GC interferences are present in the Na₂SO₄, then heat to 6000°C for 1 h), concentrate on a steam bath to 10 mL (Luke *et al.*, 1981).

Clean up: The clean up was carried out according to the methods reported by (Lappe *et al.*, 1983). The extract passing through a florisil (17 g) column, which had been activated at 130°C for 12 h and partially deactivated with 0.5% water. From this column, three fractions were collected: the first fraction with 70 mL of n-hexane; the second fraction with 50 mL of n-hexane/dichloromethane (70:30) and then a third fraction with 40 mL of dichloromethane and n-hexane. All of the fractions were concentrated to about 1 mL by rotary evaporator and kept under refrigeration until determination.

Quantitative determination: Organophosphorus pesticide residues were determined by gas chromatograph, (Hewlett Packard GC. Model 6890) equipped with a Flame Photometric Detector (FPD) with phosphorus filter. A fused silica capillary (PAS-1701), column containing 14% cyanopropylsyloxane as stationary phase (30 m length×

0.23 mm internal diameter (i.d.) × 0.25 µm film thickness), was used for the separation in the GC. While CP-CIL _ 13 CB 14% phenyl, 86% dimethylpolysiloxane phase (50 m × 0.53 mm i.d. × 1 µm film thickness) was used to confirm the detected pesticides.

The following are the operating conditions of GC instrument: Injector and detector temperature were 240 and 250°C, initial oven temperature, 170°C for 2 min, raised at 7°C min⁻¹ and then hold at 230°C for 2 min then raised at 10°C min⁻¹ to 240°C and then hold to 2 min. The carrier gas was nitrogen at 3 mL min⁻¹ and hydrogen and air were used for the combustion at 75 and 100 mL min⁻¹, respectively. All solvents and chemicals used were pesticide residue analytical grade reagents free of interfering residues as tested by gas chromatography.

Synthetic pyrethroides pesticide residues were determined by gas chromatograph (Hewlett Packard GC Model 6890) equipped with Ni 63 Electron Capture Detector (ECD). The gas chromatograph condition: DB-17 (J and scientific) capillary column (30 m length × 0.32 mm internal diameter (i.d.) × 0.25 µm film thickness). Operating temperatures were: column temperature was programmed 160 to 230°C, at a rate of 3°C min⁻¹ and to 260°C at a rate 10°C then hold 10 min. Injector temperatures were 300°C and detector temperature 320°C with nitrogen carrier gas flow at 4 mL min⁻¹. All compounds were identified by their retention times compared to known standards. Peak identical was confirmed by running the samples and standard on a second column. PAS-5 methyl silicon (30 × 0.23 mm i.d. × 0.25 µm film thickness). By using these conditions of separation and determination, obtained retention times and detection limits are shown in (Table 1) for tested pesticides.

The pesticide residues components was identified by comparing their retention times with those of the standards quantified by extrapolation of corresponding sample peak areas with those from standard curves prepared for each pesticide standard. A small variation is corrected by obtaining fresh chromatograms of the standard mixture after every nine injection. Slandered solutions were prepared for each pesticide of concentrations ranging from 0.01 to 0.04 ppm and then 1 µL was injected into the GC. Peak areas of standard solutions were plotted against their concentrations. A line of best fit was drawn through the points and the limits of detection were taken at five times the detector noise level.

Table 1 shows that, the limits of detection for organophosphorus ranged from 0.004 to 0.006 mg kg⁻¹ and was 0.01 mg kg⁻¹ for pyrethroides. The measurements of pesticide residues lower than limits of detection were set to be Not Detected (ND).

Table 1: Detection limits and retention times of individual investigated pesticides in mg kg⁻¹

Investigated pesticides	Detection limits (mg kg ⁻¹)	Retention time (Rt.) (min)
Organophosphorus		
Chlorpyrifos	0.005	7.87
Diazinon	0.004	5.32
Dimethoate	0.006	7.49
Malathion	0.004	8.58
Pyrethroides		
Cypermethrin	0.010	31.48
Deltamethrin	0.010	37.31
Fenvalerate	0.010	35.15

Heavy metals determination: Samples were analyzed according to method of (Anonymous, 1990b) was selected for determination of Copper, Iron, Cadmium, Zinc and Lead in milk samples. About 0.20 g samples was digested with concentrated nitric acid: concentrated perchloric acid at an ratio of 2:1 and heated till colorless then diluted to known volume deionized water. Then determination carried out using Thermo Jarrell Ash Atomic Absorption (model: AA- Scan 1) using a specific hollow cathode for each element.

Statistical analysis: The data were analyses using the general linear models procedure of (SAS, 1996). Comparison of means was done using Duncan's Multiple Range test at p<0.05.

RESULTS AND DISCUSSION

Milk composition: Milk samples of different seasons were varied in their composition. These variations are primarily due to milk changes in the fat, protein and occasionally the ash content of milk (Table 2).

During the summer season, more fat and protein were found in milk samples than in winter season. The differences in fat and protein contents among the season were highly significant (p<0.05). When comparing the particular seasons of the year, we may state that the increase of milk production during the winter season compensates its lower (at that time) participation of milk components: fat and protein. These observations are consistent with data from (Hemingway and Aitchison, 1988). The lactose content of milk samples of different seasons did not significantly vary. This was expected since lactose plays an important role in maintaining the osmotic pressure of milk. A large proportion of the unexplained variation in the milk composition may be accounted for by the dry matter intake and feed type offered to the animals. The effects of the amount of protein, fat, metabolisable energy and roughage: concentrate ratio in dairy rations on milk yield and composition are well established.

Table 2: Mean composition of cow's milk samples collected from different locations at Gharbia Governorate during the four seasons in years 2005-2006

Component (%)	Seasons			
	Autumn	Winter	Spring	Summer
Solids not fat	8.89 ^a	8.80 ^a	8.90 ^a	9.30 ^a
Fat	3.90 ^b	3.30 ^d	3.60 ^c	4.40 ^a
Protein	3.40 ^{ab}	3.00 ^b	3.20 ^{ab}	3.60 ^a
Lactose	4.81 ^a	5.08 ^a	4.96 ^a	5.00 ^a
Ash	0.68 ^b	0.72 ^{ab}	0.74 ^a	0.70 ^{ab}

Mean of 10 cow's milk samples per season, Mean values with the same superscript letter in the same row are not significantly different ($p < 0.05$)

Forage quality and quantity may affect milk SNF. Increasing the intake of roughage such as grass and sorghum silage usually reduces SNF and milk production. The decrease is largely due to reduced energy or dry matter intake. Increasing energy or dry matter intake usually restores the SNF to normal. Good quality hay tends to increase SNF, but poor quality hay may reduce both intake and SNF.

Nutrition of the cow has a marked effect on milk composition, particularly the fat content of milk. The solids nonfat component of milk, which consists of proteins, lactose and minerals, may also vary with changes in the diet but to a lesser degree than the fat content. Several factors have been reported as effect solids nonfat milk production includes: nutrition, genetics, disease, stage of lactation and season of year (Ekern and MacLeod, 1978; Sanz Sampelayo *et al.*, 2007).

Some research has shown an increase in SNF percent when cows go on spring pasture. Generally, it is believed that the increase is due to an increased energy intake. However, cows receiving adequate to excess energy prior to exposure to pasture will usually show a drop in SNF when shifted to pasture. In general, summer SNF values are lower than fall or winter SNF values. Periods of drought, high temperatures and high humidity tend to result in lower SNF values (Harris and Bachman, 1988).

Generally, a depressed SNF problem is caused by a lack of energy to the higher producing cows. This condition may result from the use of pasture and more hay with a reduction in grain consumption or the feeding of high-fiber low-energy rations.

Detection of organophosphorus and synthetic pyrethroid insecticide residues in milk samples: Detection of organophosphorus insecticides such as, chlorpyrifos; diazinon; dimethoate and malathion and synthetic pyrethroides i.e., cypermethrin; deltamethrin and fenvalerate in fresh milk collected from different locations at Gharbia Governorate (Table 3). Results showed that no contamination with investigated pesticide residues found in all analyzed samples, excepted one

Table 3: Pesticide residues (mg kg^{-1}) detected in milk samples collected from different locations at Gharbia Governorate during four seasons in years 2005-2006

Investigated pesticides	Autumn	Winter	Spring	Summer
Organophosphorus				
Chlorpyrifos	ND	ND	ND	ND
Diazinon	ND	ND	ND	ND
Dimethoate	ND	ND	ND	ND
Malathion	0.018	ND	ND	ND
Pyrethroides				
Cypermethrin	ND	ND	ND	ND
Deltamethrin	ND	ND	ND	ND
Fenvalerate	ND	ND	ND	ND

ND: Not detected under the limit of detection

sample in autumn season contaminated by malathion was the only negligible existence (0.018 mg kg^{-1}), by ratio of (0.02%) of all samples. There is no maximum residue limits for malathion in milk, which has not been included in the codex classification of feed and animal feeds (Anonymous, 2003).

Present findings are in accordance with those previously reported by many researchers. In Egypt, Abd Rabo *et al.* (1989) reported that levels of organophosphorus pesticide residues were under the limits of detection in milk samples. Abd Alla *et al.* (1991) found that milk samples randomly collected from Giza Governorate were contaminated with malathion with an average of 0.08 mg kg^{-1} whereas, dimethoate was not present in all milk samples. Dabiza *et al.* (1999) indicated that fresh milk collected from Gharbia Governorate was contaminated with organophosphorus including malathion and diazinon with an average of 0.40 and 0.87 mg kg^{-1} , respectively. In another study, Ahmed and Saleh (2002) recorded the presence of diazinon and deltamethrin by concentration of 0.053 and 0.060 mg kg^{-1} , respectively in milk samples collected from several markets at Giza governorate. Pesticide residues in some ready-to-eat baby foods were monitored by Salama *et al.* (2003). The results showed that, no pesticide residues were found in all samples. In a similar study, in India Battu *et al.* (2004) analysis of 92 samples of liquid milk and butter from Ludhiana, India during 1999-2001. Results revealed that, non of the 92 samples analyzed showed the presence of commonly used organophosphorus compound or synthetic pyrethroids

Generally, the contamination of milk with organophosphorus pesticide residues may be due to the feeding of dairy cattle on feeds contaminated with these pesticides and/or to the exposing the animals to direct atmosphere and to oral intake of organophosphorus pesticides (IDF, 1979).

Detection the levels of five heavy metals, i.e., Cu, Fe, Cd, Zn and Pb in milk samples: Table 4 contains the monitoring detailed data on heavy metals by individual

Table 4: Some heavy metals detected (mg kg⁻¹) in individual milk samples collected from different locations at Gharbia Governorate during four seasons in years 2005-2006

Season	Sample No.	Copper (Cu)	Iron (Fe)	Cadmium (Cd)	Zinc (Zn)	Lead (Pb)
Autumn	1	0.289	1.138	0.177	0.395	2.640
	2	0.280	1.016	0.165	0.452	2.536
	3	0.110	0.491	0.175	0.365	2.368
	4	0.197	0.043	0.179	0.346	2.227
	5	0.192	0.073	0.186	0.369	2.304
	6	0.178	0.044	0.177	0.341	2.341
	7	0.167	0.297	0.183	0.334	2.358
	8	0.159	0.251	0.185	0.357	2.364
	9	0.142	0.312	0.167	0.349	2.253
	10	0.230	0.398	0.177	0.347	2.259
	Mean		0.195	0.406	0.177	0.366
Winter	11	0.092	0.063	0.176	0.409	2.310
	12	0.082	0.062	0.177	0.431	2.300
	13	0.067	0.747	0.173	0.354	2.342
	14	0.063	0.751	0.173	0.324	2.384
	15	0.054	0.665	0.173	0.446	2.288
	16	0.041	0.799	0.177	0.358	2.353
	17	0.033	0.825	0.165	0.393	2.254
	18	0.290	0.184	0.117	0.142	2.513
	19	0.273	0.023	0.065	0.149	2.160
	20	0.624	0.231	0.133	0.182	3.314
	Mean		0.162	0.435	0.153	0.319
Spring	21	0.470	0.173	0.121	0.204	2.293
	22	0.573	0.171	0.123	0.243	3.724
	23	0.391	0.160	0.152	0.162	2.040
	24	0.214	0.182	0.101	0.176	2.250
	25	0.383	0.250	0.125	0.371	1.782
	26	0.180	0.184	0.112	0.304	3.022
	27	0.191	0.200	0.113	0.175	2.324
	28	0.420	0.073	0.141	0.228	3.053
	29	0.214	0.251	0.117	0.208	3.044
	30	0.463	0.184	0.123	0.409	3.020
	Mean		0.350	0.183	0.123	0.248
Summer	31	0.306	1.219	0.204	0.332	3.493
	32	0.300	0.606	0.189	0.410	2.516
	33	0.301	0.595	0.185	0.631	2.716
	34	0.300	1.243	0.185	0.631	2.511
	35	0.298	0.866	0.182	0.512	2.652
	36	0.291	1.364	0.182	0.643	2.604
	37	0.291	0.692	0.179	0.424	2.501
	38	0.295	1.570	0.171	0.761	2.274
	39	0.288	0.788	0.182	0.549	2.747
	40	0.280	1.803	0.171	0.617	2.450
	Mean		0.295	1.075	0.183	0.551
Total means		0.251	0.525	0.159	0.371	2.462
MRL'S		0.400	5.000	0.100	1.000	0.020

Total means: The overall mean values of the four sets of data for the entire period

Table 5: Summary of mean levels (mg kg⁻¹) of heavy metals detected in milk samples collected from different locations at Gharbia governorate during four seasons of years 2005/2006

Elements	Seasons			
	Summer	Autumn	Winter	Spring
Copper (Cu)	0.295 ^{ab}	0.194 ^{bc}	0.162 ^c	0.350 ^a
Iron (Fe)	1.075 ^a	0.406 ^b	0.435 ^b	0.183 ^b
Cadmium (Cd)	0.183 ^a	0.177 ^a	0.153 ^b	0.123 ^c
Zinc (Zn)	0.551 ^a	0.366 ^b	0.319 ^{bc}	0.248 ^c
Lead (Pb)	2.646 ^a	2.365 ^a	2.422 ^a	2.655 ^a

Mean of 10 cow's milk samples per season, Mean values with the same superscript letter in the same row are not significantly different (p<0.05)

milk samples from which the summary information was compiled (Table 5). Data revealed that, there are considerable variations in the levels of heavy metals were detected in cow's milk samples from different seasons. The overall mean concentrations over the whole four seasons for copper, iron, cadmium, zinc and lead were 0.251, 0.525, 0.159, 0.371 and 2.462 mg kg⁻¹, respectively. It is obvious that concentration of lead was higher than other elements. These variations in heavy metals content were due to many factors; character of tested metals, site, date of samples and feed contamination is the recycled farmyard waste.

The mean concentrations of copper, iron, cadmium, Zinc and lead in milk samples are presented in Table 4 and 5. It is obvious that mean concentrations for Cu, Fe, Cd, Zn and Pb were 0.251, 0.525, 0.159, 0.371 and 2.462 mg kg⁻¹, respectively. Concentration of lead is higher than another metal. The different levels of tested metals in milk samples due to many factors; character of tested metals, site, date of samples and feed contamination is the recycled farmyard waste. The main sources of Cu and Pb in Egyptian irrigation system are industrial wastes and algacides (Cu), while those for Cd are the phosphate fertilizers used in agriculture (Saad and Emam, 1998). In another study, Salama *et al.* (2003) monitoring of pesticides, heavy metals and nitrates residues in some ready- to- eat baby foods. The results showed that, no pesticide residues were found in all samples. The highest level of contaminate was nitrate followed by Cu, Pb and Cd. The mean concentrations of Cu, Pb and Cd were 0.538; 0.036; and 0.008 mg kg⁻¹, respectively.

The permissible limits for Cu, Fe, Cd, Zn and Pb are 0.4, 5.0, 0.1, 1.0 and 0.02 mg kg⁻¹, respectively according to (Anonymous, 1998). The figures presented in this study revealed that Cu, Fe and Zn concentrations were under the mentioned permissible limits, while Cd and Pb exceeded the permissible limits.

Data concerning the seasonal distribution of the studied metals, i.e., Cu, Fe, Cd, Zn and Pb in milk samples, collected from different locations at Gharbia Governorate during the year 2005-2006. Concentrations of Cu and Fe in four seasons, i.e., summer, autumn, winter and spring were 0.295, 0.194, 0.162, 0.350, 1.075, 0.406, 0.435 and 0.183 mg kg⁻¹, respectively. The corresponding figures with Cd and Zn were 0.183, 0.177, 0.153, 0.123, 0.551, 0.366, 0.319 and 0.248 mg kg⁻¹. While a level of lead is higher than another metals, the mean concentration were 2.646, 2.365, 2.183 and 2.655 mg kg⁻¹.

Data also, showed that the levels of iron, cadmium and zinc were highest in the summer whereas those of

copper and lead were maximum in the spring. The winter values were generally low for all the metals studied except iron. Lead was the most dominant metal in milk samples through the entire period. All heavy metals except copper had maximum concentration in the summer season. Concentration of copper was highest in the spring.

Generally, the seasonal variations in the concentration of heavy metals, in our study, attributed may be environmental conditions, type of feed for animals, agriculture activity to increase during the summer season which resulted in huge amounts of wastes that must be disposal by burning and industrial activity in the area surrounded by sampling location (El-mahalla El-Kubra city) and also to the presence of two big public dump in (El-Dawakhliya village and Tanta city) at Gharbia Governorate.

CONCLUSION

The residues of organophosphorus and pyrethroides pesticides in cow's milk are generally free, with very low levels of malathion. However, particularly with heavy metals, they were detected in a much high proportion of milk samples. The study has also reported presence of some heavy metals, especially nonessential metals like cadmium and lead, in cow milk raising serious concern. These indicated that the local people are facing considerable risk of heavy metals poisoning in the area.

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