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POLLUTION OF BUFFALO MILK BY LEAD AND CADMIUM

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ABSTRACT

This study was carried out at three different buffalo experimental stations belonging to animal production research institute, ministry of agriculture, Egypt. The stations were located in different areas. The first station was Seds station located in Bani Sweif governorate at middle Egypt. It was adjacent to a high density traffic way. The second station was Mehalet Mousa station located in Kafr El-Sheikh Governorate. It was 1.5 km away from a moderate density traffic way. The third station was Gemmeza station located in Gharbia governorate. It was 27 km far from a somewhat low density traffic way. The effect of environmental (traffic) pollution on components of milk and blood of buffalo was studied. Forty five adult lactating buffalo cows ranged from 4 to 10 years age, 380 to 770 kg weight and 1 to 6 parities. The highest level of lead in blood was found in Seds station (0.979ppm) followed by Mehallet Mousa (0.792ppm) and Gemmaza station (0.441ppm). Lead level in milk was significantly ($P<0.05$) higher in samples taken from Seds station than in Mehallet Mousa and Gemmaza stations (2.774 vs. 0.579 and 0.553ppm, respectively). Cadmium level in whole blood ranged from 0.006 to 0.08 ppm in the three stations. The highest level was in Seds station (0.034ppm) and the lowest one was in Gemmaza station (0.023ppm). Cadmium level in milk ranged from 0.01 to 1.56ppm.The highest level was in Seds station (0.543 ppm), and the lowest level was in Gemmaza station (0.237ppm). It could be concluded that the nearness of farm animals to traffic roads with high traffic density causes a rise in milk content of lead and cadmium.

Keywords: Buffalo, Milk, Blood, Traffic, Lead, Cadmium

INTRODUCTION

Environmental pollution is one of the most serious problems confronting human life. The most deleterious pollutants are heavy metals, among them lead (Pb) and cadmium (Cd) are of most concern. These metals intensively pollute the environment, because they are widely used in the industry, such as the manufacturer of batteries, electronics, jewelry, paint pigments, pottery glazes, inks, dyes, rubber, and plastics. Sources of air pollution are divers. It is often difficult to distinguish between them. The industrial sources, in general, contribute to a significant proportion of several types of environmental pollutants. Burning of coal and fossil fuel-based power generation contributes to several types of environmental pollutants. In addition, emissions of car exhaust greatly constitute the air pollution (Watkins, 1981).

The toxicity and metabolism of heavy metals may be influenced by a number of factors, such as age and the interaction between different metals. Diet may also influence the absorption and accumulation of the metals. The body burden of lead and cadmium is a balance between the amount being taken in, the amount distributed through the tissues, and the amount excreted. Milk and dairy products constitute a major source of food especially for infants and children, thus even relatively low contents of lead and cadmium in the food are considered hazardous for public health. Contamination of milk may occur during milk production, environmental contamination and/or milk processing. Regions having lead and cadmium residual levels are considered polluted, although these levels may considerably vary (Jeng *et al.*, 1994). The present study was designed to investigate the contamination of milk by lead and cadmium and the changes in milk composition due to such contamination.

MATERIAL AND METHODS

This study was carried out to investigate the presence of lead and cadmium in buffalo milk reared in three different buffalo breeding stations of animal production research institute, ministry of agriculture, Egypt.

Regions of the buffalo station*:* Three stations were namely Seds, Mehalet Mousa and Gemmiza, located at different regions and adjacent to different levels of traffic capacity were considered in this study. Seds station is located in Seds village in Bani Sweif governorate at mid-Egypt., and is adjacent to a high density traffic way. Mehalet Mousa station is located in Kafr El-Sheekh governorate, north of Nile Delta. It is adjacent to a low density traffic way and a train station and was 1.5 km far from a moderate density traffic way and 3 km far from a high density traffic way. Gemmeza station is located in Gharbia governorate at mid-Nile Delta. It is near from some low density traffic ways, and 27 km far from a high traffic way.

Animals*:* Forty five adult lactating buffalo cows were randomly and equally allotted to three groups according to age, weight and parity of animals,(4 to 10 years, weights, 380 to 770 kg and 1 to 6 parities, respectively).

Management: Animals were housed in semi-shaded open yard in all stations and fed according to the daily milk production (Ghoniem, 1966). Source of drinking water was the under-ground water in Mehallet Mousa and Gemmaza stations and the municipal water in Seds station.

Samples: Blood samples were individually collected from the animals via jugular vein. Ten and five ml of blood were collected in polypropylene tubes washed in nitric acid and coated with heparin as anticoagulant. The first one was immediately centrifuged and its plasma was stored at -20°C until use. The second one was immediately stored at -20°C until use. Twenty ml of milk were individually collected from lactating buffalo cows in polypropylene tubes washed in nitric acid, 10 ml were frozen at -20°C for the determination of lead and cadmium, and the other 10 ml were frozen at -20°C for the determination of milk constituents. Samples of fodder and water were collected from each farm at the same time of the collection of blood and milk samples upon use.

Chemical analysis*:* the frozen samples were left to thaw at room temperature. Determination of lead and cadmium levels in plasma and water samples was carried out at the Central Laboratory of Faculty of Agriculture, Cairo University, Giza, Egypt. Solar Series Atomic Absorption Spectrometers (UNICAM) was used for analysis. Principal lines for lead and cadmium were 217.0 and 228.8 nm, respectively. Samples of whole blood were digested using a mixture of nitric**,** perochloric and sulfuric acids at rates of 71:21:8 respectively, until a clear liquid appeared. The digested samples were increased in volume to 10 ml, using 0.1N HCl, (AOAC, 1984) for determining lead and cadmium proportion. Milk samples were dried, using a hot plate at 120°C. The dried samples were weighed in silica dishes and exposed overnight to 500°C in a muffle furnace to get the ash samples. The samples were left to cool, and 2-3 ml HCl were added to each sample. The total volume of each sample was diluted to 100 ml using de-ionized water, and analyzed using suitable standard solution for the determination of lead and cadmium (AOAC, 1984).

Clover samples were dried in an electric drier at 65°C for 72 hours. Each sample was ground and weighed in a silica dish. Ash of each sample was obtained and used to determine lead and cadmium, using the same procedures mentioned above. Rice straw and concentrate samples were individually ground and weighed. Ash was obtained and used to determine lead and cadmium, as mentioned previously.

Milk constituents and somatic cell count (SCC) were determined using MILKO-SCAN at Sakha laboratory of the Animal Production Research Institute, Ministry of Agriculture, Egypt.

Statistical analysis*:* The data set of the parameters was statistically analyzed for the effect of farm location using the Statistical Analysis System (SAS, 1999) including Duncan's multiple range tests which was used to separate means, whenever the model was significant.

RESULTS AND DISCUSSION

Lead level in blood

Table1 shows that lead level in whole blood and plasma of buffalo cows ranged from 0.08 to 1.53 and 0.01 to 0.91ppm, respectively. Seds station and Mehallet Mousa station significantly ($P<0.05$) had more blood lead level than Gemmeza station (0.979±0.096 and 0.792±0.116 vs. 0.441±0.053ppm, respectively).

Table (1) Effect of farm location on mean lead levels in whole blood, plasma and milk (ppm).

tissues	Range	Station		
		Seds	M.Mousa	Gemmeza
Blood	0.08-1.53	0.979±0.096^{a}	0.792±0.116^{a}	0.441±0.053^{b}
Plasm	0.01-0.91	0.369±0.096^{a}	0.329±0.070^{a}	0.162±0.029^{b}
Milk	0.042-5.1	2.774±0.408^{a}	0.579±0.136^{b}	0.553±0.096^{b}

Means with the same letter are statistically not significant ($P<0.05$) in each row.

This result indicated that animals lived in area adjacent to high traffic ways had more blood lead level than that away from the traffic. This result was in agreement with Stern, (1977), Chandra,(1980), Harrison, (1983), Dwivedi *et al.*, (1995), Dey *et al.*, (1996& 1997) and Swarup *et al.*, (1997). Plasma lead levels of buffalo cows in Seds and Mehallet Mousa stations were significantly higher than that in Gemmeza station (0.369±0.06, 0.329±0.070, vs.0.162±0.029ppm, respectively), similar conclusion was reported by Khalaf-Allah and Abdel-Aal (1999) who reported that the lead in serum samples of sheep grazing in polluted area was significantly higher than that of sheep grazing in unpolluted area. Buffalo had high tolerance to lead pollution (Dey *et al.*, 1996 and Dwivedi *et al.*, 2001). Moreover, lower values were reported, since Abdelhamid and El-Ayoty (1989) reported low blood pb- concentration (3.7±0.58ppb) in summer than in winter (29.9±4.76 ppb) with an overall mean of 21.0±3.59ppb for buffaloes.

Lead level in feed

Table2 shows that lead level in fodder "fed for animals" in Seds station was the highest one ($P<0.05$) followed by that in Mehallet Mousa and Gemmeza stations (7.88±0.006, 2.038±0.035 and 1.25±0.0119ppm, respectively). They significantly differed from each other. This result indicated that fodder samples taken from areas near the high traffic way had higher levels of lead than that at further areas. Dey *et al.* (1996& 1997) reported higher concentration of lead in the forage and leaf samples taken from polluted areas (319-1017ppm). Dwivedi *et al.,* (1995) found that the feed and fodder samples collected from urban area had higher concentrations of lead (54.15ppm) than that from rural area (31.73). Also, lower Pb-values were reported in Egyptian feed stuffs for buffaloes, being 8.05±0.45 ppb in summer and 32.0±3.45 in winter with an overall mean of 18±2.78ppb . The same trend was recorded for drinking water, being 8.91±0.98 and 23.5±6.21ppb in summer and winter, respectively and an overall mean of 15.2±3.85ppb (Abdellhamid and El- Ayoty, 1989).

Table (2) Lead level (µg/g) in feed and water consumed by the experimental animals/day

Feed Components	Seds	M. Mousa	Gemmeza
Clover	5.7	0.93	0.72
Grain mix	1.26	0.31	0.21
Rice straw	25.4	9.2	0.47
Water	2.0	2.0	1.5
Feed proportion%			
Clover	72.30	71.27	69.12
Grain mix	13.55	14.26	15.56
Rice straw	14.15	14.47	15.32
Contribution to feed (µg/g)			
Clover	4.1213 ^{a}	0.6628 ^{b}	0.4976 ^{c}
Grain mix	0.1707 ^{a}	0.0442 ^{b}	0.0327 ^{b}
Rice straw	3.5943 ^{a}	1.3314 ^{b}	0.7201 ^{c}
Total	7.8863 ^{a}	2.0384 ^{b}	1.2504 ^{c}

Means with the same letter are statistically not significant ($P<0.05$) in each row.

Lead level in milk

Table 1 shows that lead level in milk ranged from 0.042 to 5.1 ppm in the three stations. Lead level in milk was significantly ($P<0.05$) higher in Seds station than in Mehallet Mousa station and Gemmeza station (2.774±0.408 vs. 0.579±0.136 and 0.553±0.096 ppm, respectively). These results indicted that the higher concentrations were found in the areas adjacent to the main road. This finding was in agreement with Fotis *et al.*, (2001) who found that the highest heavy metal content was in milk samples taken from the industrial region followed by that from traffic intensive regions and rural regions (2.97-4.57ppm). Also Dey and Swarup (1996) found that, lead concentrations in milk samples obtained from the industrial areas and the areas adjacent to the high way were significantly higher ($P<0.05$) than that in the areas without any known source of lead pollution (0.01-0.117, 0.019-0.093 vs. 0.01-0.081ppm, respectively). Cattle and buffalo kept in urban areas and exposed to excessive environmental lead may carry high concentrations of the metal in milk >0.2ppm (Dwivedi *et al.* 1995 and Bhatia and Choudhi 1996). However, it was higher than that in normal cows (0.06-0.13ppm) and than that in cows from a lead-contaminated site (0.03-0.25ppm) (Dorn *et al.*1972), and than those found by Shehata and Saad (1992) who found that lead level in milk of cow and buffalo in Assiut governorate were 0.019±0.01, and 0.245±0.016ppm, respectively. The results somewhat coincided with El-Prince and Sharkawy (1999) who found that lead levels were 0.240±0.047ppm and 0.447±0.122ppm, respectively, Lead level ranges were 0.024-0.53, 0.003-2.4, and 0.001-4ppm of buffalo raw milk in Assiut, rifa, and Hawatica of Assiut governorate, respectively (Nasr *et al.*, 2001). Abdelhamid and El-Ayoty (1989) gave lower buffalo milk Pb-concentrations, being 6.4±0.37, 13.3±1.1 and 11.1±0.84ppb in summer, winter and as an over all mean, respectively.

Cadmium level in blood

Table 3 shows that cadmium level in blood ranged from 0.006 to 0.08 ppm in the three stations. Cadmium level was significantly ($P<0.05$) higher in Seds than that in Gemmeza (0.034±0.004 vs. 0.023±0.003ppm). But they did not differ from Mehallet Mousa (o.o26±0.003).

Table (3) Effect of farm location on mean cadmium levels in whole blood , plasma and milk (ppm)

tissues	Range	Station		
		Seds	M. Mousa	Gemmeza
Blood	0.006-0.08	0.034±0.004 ^{a}	0.026±0.003 ^{ab}	0.023±0.003 ^{b}
Plasma	0.003-0.3	0.014±0.002	0.012±0.002	0.011±0.002
Milk	0.08-1056	0.543±0.099 ^{a}	0.373±0.109 ^{ab}	0.237±0.073 ^{b}

Means with the same letter are statistically not significant ($P<0.05$) in each row.

This result indicated that areas adjacent to high traffic way and urban region had higher cadmium levels in blood of buffalo cows than that in the further areas. These results are in agreement with Swarup *et al.* (1997) who reported significantly higher concentration of cadmium in blood of cows in urban localities (0.006-0.024 ppm) than that of rural ones (0.006-0.016ppm). Also, Dwivedi *et al.*, (2001) reported that cattle and buffalo kept in urban areas were more exposed to high environmental cadmium levels than that in rural area. So, blood cadmium level was higher in urban area (0.11±0.01ppm) than that in rural area (0.09±0.01ppm). Cadmium levels in this study were higher than the normal level of about 0.0001ppm (WHO, 1992). Also, these levels were higher than those reported in rural and urban areas in India (0.013±0.009ppm) reported by Milhaud *and* Mehennaoui (1988). However, higher levels of Cadmium of 0.08±0.05ppm were obtained by Naresh *et al.* (1999&2003) in buffalo milk.

Cadmium level in feed

Table 4 shows that total cadmium levels in fodder ranged from 0.125 to 0.455ppm in the three stations. There were significant increases (P<0.05) in cadmium levels in fodder in Seds station than those in Mehallet Mousa and Gemmeza (0.44±0.002 vs.0.184±0.005, and 0.1290±0.0011 ppm, respectively). The results indicated that, fodder samples taken from areas near the high traffic way had higher cadmium levels than that further.

Table (4) Cadmium level (µg/g) in feed and water consumed by the experimental animals/day.

Feed components	Seds	M. Mousa	Gemmeza
Clover	0.47	0.16	0.16
Grain mix	0.05	0.02	0.02
Rice strow	0.66	0.47	0.10
Water	0.46	0.42	0.11
Feed proportion%			
Clover	72.30	71.27	69.12
Grain mix	13.55	14.26	15.56
Rice strow	14.15	14.47	15.32
	Contribution to feed(micro-g/g)		
Clover	0.3398[a]	0.1140[b]	0.1106[b]
Grain mix	0.0068[a]	0.0029[b]	0.0031[b]
Rice strow	0.0934[a]	0.0668[b]	0.0153[c]
Total	0.4400[a]	0.1849[b]	0.1290[c]

Means with the same letter are statistically not significant (P<0.05) in each row.

Cadmium level in milk

Table 3 shows that cadmium levels in milk ranged from 0.01 to 1.56 ppm in the three areas. Cadmium level was significantly (P<0.05) higher in Seds station than that in Gemmeza (0.543±0.099 ppm, vs. 0.237±0.073 ppm, respectively), but they were not differrent from Mehallet Mousa (0.373±0.109ppm). This could be due to the closeness of Seds station to the main road and urban region. Results were in agreement with Swarup *et al.* (1997) who reported that animal population kept in urban areas were exposed to toxic levels of heavy metals such as cadmium. It was higher in the urban area (0.004-0.014ppm) than in the rural area (0.002-0.009ppm) resulting in higher concentration in milk. Dwivedi *et al.* (1997) found that the mean cadmium concentration in different industrial localities was significantly higher (0.002-0.02ppm) than that of industry free rural locality (0.002-0.009ppm). Dwivedi *et al.* (2001) reported that levels of cadmium in milk of cows near the smelter were higher (0.03-0.1ppm) than that of cows from rural areas (0.002-0.009ppm). Naresh *et al.* (2003) found 0.12-0.14ppm of Cd in milk. Naresh *et al.*(1999) reported a cadmium level ranging from 0.05 to 0.17ppm as the acceptable level in milk. Levels of cadmium of 0.017 and 0.03ppm were reported in market milk by Murthy and Rhea (1968) and 0.02ppm in cow milk was given by Sharma *et al.* (1982). The maximum residual level of 50ppm was admitted for cadmium in milk for the Argentinean Alimentary Codex (Naresh *et al.*, 1999).

Milk composition

Figure 1 shows that there were no significant differences among the three areas in milk composition except lactose. Lactose percentages of milk collected from Seds and Gemmeza stations were significantly ($P<0.05$) different (5.55, and 4.88%, respectively). They did not significantly differ from Mehalet Mousa (5.053%). Fat percentages in the three stations Seds, Mehallet Mousa and Gemmeza were 6.45, 5.95 and 5.98, respectively. Protein percentage was 3.8, 3.82 and 4.01, respectively. Total solids percentage were 16.51, 15.53 and 15.58 in the three stations, respectively. Total solid not fat percentages were 10.06, 9.57 and 9.59 in the three stations, respectively.

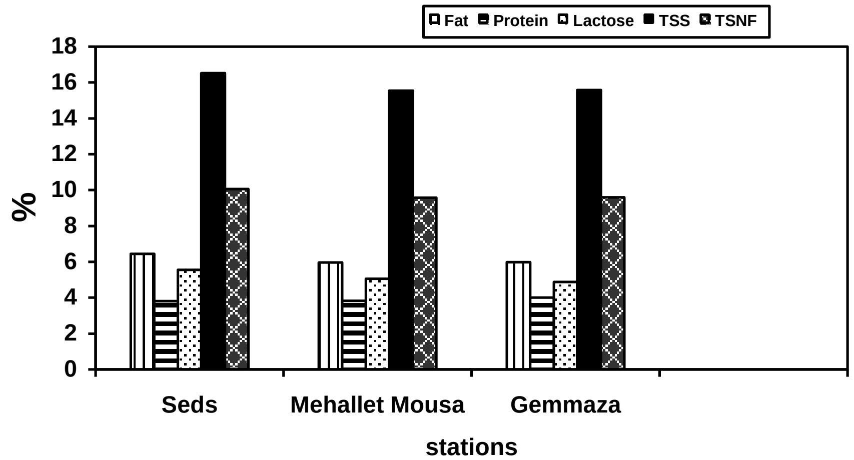

Figure (1). Mean milk coposition in expermintal stations

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تلوث ألبان الجاموس بالرصاص والكادميوم

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أجريت هذه الدراسة فى ثلاث مزارع بحثية مختلفة تابعة لمعهد بحوث الإنتاج الحيوانى- وزارة الزراعة- جمهورية مصر العربية. وكانت هذه المحطات فى ثلاث مناطق مختلفة الأولى هى محطة سدس وتقع فى محافظة بنى سويف فى منتصف مصر الوسطى (شمال الصعيد) وهى منطقة بها كثافة مرورية عالية,والثانية محطة محلة موسى والتى تقع فى محافظة كفر الشيخ والتى تبعد ١,٥ كم عن الكثافة المرورية المتوسطة, والمحطة الثالثة هى الجميزة وتقع فى محافظة الغربية واالتى تبعد ٢٧ كم عن الكثافة المرورية المتوسطة (منخفضة التلوث)، وقد تم دراسة التلوث البيئى على اللبن والدم فى الجاموس المصرى. وقد إستخدم فى هذه الدراسة ٤٥ جاموسة حلابة فى أعمار تتراوح بين ٤–١٠ سنوات, والتى قسمت عشوائيا إلى ثلاث مجموعات فى أوزان تتراوح من ٣٨٠ إلى ٧٧٠ كجم ومواسم تتراوح بين ١-٦ موسم.

وقد أظهرت النتائج أن أعلى مستوى من الرصاص كان موجودا فى عينات الجاموس الموجود فى محطة سدس (٠,٩٧٩ جزء فى المليون) وكان مستوى الرصاص فى الدم أعلى معنويا فى العينات المأخوذة من حيوانات محطة محلة موسى (٠,٧٩٢ جزء فى المليون) عن المأخوذة من محطة الجميزة (٠,٤٤١ جزء فى المليون)، وكان مستوى الرصاص فى اللبن أعلى بدرجة معنوية فى عينات محطة سدس عن محطة محلة موسى والجميزة (٢,٧٧٤ مقابل ٠,٥٧٩، ٠,٥٥٣ على التوالى)، وكان مستوى الكادميوم فى الدم يتراوح بين٠,٠٠٦ -٠,٠٨ جزء فى المليون فى الثلاث محطات، وكان أعلى مستوى فى سدس (٠,٠٣٤ جزء فى المليون) وأقل مستوى فى محطة الجميزة (٠,٠٢٣ جزء فى المليون)، وكان مستوى الكادميوم فى اللبن يتراوح بين ٠,٠١ – ١,٥٦ جزء فى المليون، و كان المستوى الاعلى معنويا فى محطة سدس (جزء فى المليون ٥٤٣.٠) وكان أقلها فى محطة الجميزة (٠,٢٣٧ جزء فى المليون). وتستخلص النتائج بأن قرب المزرعة من الطرق ذات الكثافة المرورية العاليه قد يسبب إرتفاعا فى نسبة تلوث الألبان بالرصاص والكادميوم.