

DETECTION OF ENVIRONMENTAL POLLUTANTS BY MICROBIOLOGICAL EXAMINATION AND PESTICIDES RESIDUAL MEASUREMENT IN RAW MILK SAMPLES.

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ABSTRACT

Thirty raw milk samples including buffalo farm, bulk tank milk and cow's farm, 10 of each were randomly collected. Samples were collected from different dairy milk places in Mansoura city, Dakahlia governorate during summer and winter of 2006&2007. All samples were used for microbiological examination and to determine the residual of some pesticides. Obtained results represented the mean values of microbiological quality of raw milk on either general or specific cultivation media. Results exhibited high values of the microbial count in the tested raw milk samples appeared on tryptone glucose extract agar (TGEA), they were $82.03 \pm 79.19 \times 10^3$, $119.40 \pm 62.58 \times 10^3$ and $129.03 \pm 113.60 \times 10^3$ cfu/ml in examined buffalo, bulk tank, and cow's milk samples, respectively. Obtained results indicated that the pesticide residues concentrations in all samples collected during summer season were lower than that samples collected during winter season. Results showed that the mean values of aldicarb residue was the highest values in examined buffalo, bulk tank, and cow's milk samples, they were 0.0344, 0.0391 and 0.0129mg/kg, respectively. The residues levels of pesticides in different milk types were lower than the acceptable daily intake (ADI), in summer and winter seasons on contrast the residue concentration of aldicarb and carbofuran were higher than the ADI in winter season. Profenfos insecticide was found in all samples either times in summer or winter season.

INTRODUCTION

The presence of environmental pollution facilitate their entry into the food chain and thus increasing the possibility of them having toxic effects on human and animals. However, agriculture activities as the use of fertilizers and the irrigation of fields, can also be important sources of contamination of the environment, of the food chain and eventually of food products consumed by humans. Milk is a basic food in the human diet exposed to contamination with several types of microorganisms from different sources. Under suitable circumstances, these contaminant find their way to grow and multiply in the milk and its products leading to undesirable changes that rendering it of an inferior quality or even unfit for human consumption beside it may constitute a public health hazard. *Klebsiella pneumoniae* was isolated from 13.8% of

examined milk samples (Alonso *et al.*, 2002). *Staphylococcus aureus* was found in 38 out of 54 raw milk samples at concentration up to 8.9×10^5 cfu/ml (Rall *et al.*, 2008). Pesticides are being extensively used against livestock ectoparasites and agricultural pests. The usage of these highly persistent pesticides has resulted in trace contamination of air, water and soil with their residues. The residues of these pesticides were accumulated in milk-producing animals such as cow's and buffaloes if they fed on contaminated grass, hay and inhaled (Licata *et al.*, 2004). Eight organophosphorus pesticides residues were determined in raw milk samples, 30% of these samples were positive to acephate and chlorpyrifos contamination (Pagliuca *et al.*, 2004).

The microbiological examination and pesticides residues measurement of raw milk samples in this study were evaluated.

MATERIALS AND METHODS

Collection of samples:

Thirty samples of raw milk, buffalo farm, bulk tank milk and cow's farm, were randomly collected from different farms and markets in Mansoura city, Dakahlia governorate. Each sample was divided into two sub samples, the first was used for microbiological examination, while the second one was used for the determination of pesticides residues.

Microbiological examination:

Cultivation media:

The composition of general and specific cultivation media used in this investigation for different purposes were according to the standard methods reported in APHA (1992). These media were tryptone glucose extract agar (TGEA) for counting the total bacteria, *salmonella shigella* agar (SSA) to detect *Salmonella spp* and *Shigella spp*, mannitol salt phenol red agar (MSPRA) for detection of *Staphylococcus ssp.*, MacConkey agar (MaccA) for detecting coliform bacteria; Milk agar (MA) for isolation counting and screening of proteolytic bacteria, potato dextrose agar (PDA) for isolation of fungi and tryptose agar (TA) for detection of *Listeria spp.*, *Brucella spp.* and other pathogenic bacteria .

Chemical analysis:

Determination of pesticides residues in milk

Samples were taken at 2, 5, 8, 10 and 12 week over the course of a summer and winter seasons during 2006-2007. Milk (50ml) was blended with ethyl acetate for organophosphorus (OP) pesticides and with (10ml) acetone for carbamate pesticides and sodium sulphate (50g) was added and the mixture was shaken and then allowed to stand for 2-3 min. The upper layer was decanted, and 50 ml aliquot was evaporated to dryness in a rotating film evaporator (35°C). The residue was dissolved in hexane (10 ml) and then extracted with 2x25 ml acetonitrile saturated with hexane. Combined acetonitrile phase were evaporated to dryness, as described above. The residue was dissolved in 2 ml of hexane. The hexane extract after cleaning up was dried by passing through sodium sulphate anhydrous. The hexane

extract was rotary evaporated to few drops and quantitatively transferred to volumetric tube. The volume was adjusted to 1ml with hexane (Naik et al., 2000)

Liquid chromatographic analysis:

The residue of aldicarb, chlorpyrifos-methyl, carbofuran, methomyl and profenfos were analyzed using Agilent HPLC Series 1100 quartary pump, with ultra violet spectrophotometer detector and C₁₈ stainless column (2.4×250 mm). Duplicate injection (1μl) of calibration solution and each sample was injected and integrated area for each peak was recorded and standard curve. This was done at the Central Agriculture Pesticides Laboratory (CAPL), Agricultural Research Center (ARC), Dokki, Giza, Egypt.

Statistical analysis:

The experimental data were statistically analyzed using the analysis of variance by the factorial design method. This had been done in order to ascertain weather the experimental observations of the tested parameters were real and the differences between them are significant (SAS, 2001).

RESULTS AND DISSCUSION

Microbiological quality:

The mean values ±SD of the microbiological examination of the tested raw milk samples collected in summer and winter 2006-2007 seasons are listed in Table 1. Tabulated data exhibited high values ±SD on tryptone glucose extract agar (TGEA), they were 82.03± 79.19×10³, 119.40± 62.58×10³, and 129.03± 113.60×10³cfu/ml in examined buffalo milk, bulk tank milk and cow's milk samples, respectively. On specific cultivation media, the lowest mean values were found on Salmonella shigella agar (SSA), tryptose agar (TA) and potato dextrose agar (PDA), they were 2.10± 4.33×10³, 9.30± 18.00×10³ and 5.43± 10.5×10³ cfu/ml in examined buffalo milk, bulk tank milk and cow's milk samples, respectively. On the level of the sampling season, the high value was in winter season, being 99.05± 81.02×10³cfu/ml, but in summer season it was 95.61± 93.05×10³cfu/ml that exhibited on tryptone glucose extract agar (TGEA). Park et al., (2007) examined raw milk in Korea and they reported that *Staph. aureus*, *Streptococcus spp.*, *Enterococcus spp.*, *Escherichia coli*, *Pseudomonas spp.* and yeast could be isolated at different percentages. Muraoka et al., (2003) found *Listeria monocytogenes* in bulk milk samples, while Klossowska and Malinowski (2001) detected *Staphylococcus aureus*, *Escherichia coli* and *Enterococcus faecalis* in bulk tank milk.

Table(1): Mean values of the microbiological examination of the tested raw milk samples as affected by seasonal variations

Items	N	Specific cultivation media x 10 ⁴ cfu/ml						
		MA	MacCA	TGEA	PDA	SSA	TA	MSPA
		X ± SD	X ± SD	X ± SD	X ± SD	X ± SD	X ± SD	X ± SD
Sample:								
BTM	30	40.20±39.02	41.63±48.08	119.40±62.58	21.33±20.72	19.13±41.57	9.30±18.00	32.00±50.21
Buffalo	30	58.43±81.20	24.17±29.74	82.03±79.19	6.57±13.73	2.10±4.33	9.67±14.82	26.60±57.42
Cow	30	63.90±87.96	15.50±28.68	129.03±113.60	5.43±10.51	29.27±43.69	49.73±83.43	69.40±93.93
Season:								
Summer 1	75	65.32±78.56	39.67±63.07	95.61±93.05	22.90±28.64	31.91±48.09	36.49±56.59	54.69±75.49
Winter 2	75	49.85±61.58	20.32±28.01	99.05±81.02	28.67±40.11	1.31±3.81	1.09±4.71	7.65±13.85
Samplex Season Interaction:								
BTM X1	15	54.87±42.99	51.13±62.88	126.80±68.33	17.27±18.69	35.33±54.59	18.60±22.05	54.73±63.61
BTM X 2	15	25.53±29.05	32.13±25.33	112.00±57.68	25.40±22.46	2.93±6.02	0.00±0.00	9.27±8.36
Buffalo X 1	15	84.93±107.04	33.73±37.10	77.40±100.82	9.33±19.00	1.27±2.93	19.33±15.96	50.07±75.09
Buffalo X 2	15	31.93±26.42	14.60±16.12	86.67±52.71	3.80±3.63	2.93±5.35	0.00±0.00	3.13±3.23
Cow X 1	15	90.60±115.01	7.00±11.29	174.87±115.50	10.67±13.03	85.53±46.03	49.00±100.63	136.20±93.28
Cow X 2	15	37.20±35.64	24.00±37.71	38.20±94.29	0.20±0.56	0.00±0.00	5.47±9.57	2.60±3.29

BTM: Bulk tank milk

MA: Milk agar

SSA: Saimonella Shigella agar

TGEA: Tryptone glucose extract agar

PDA: Potato dextrose agar

MSPA: Mannitol salt phenol red agar

TA: Tryptose agar

MacCA: MacConkey agar

Values of pesticides residue:

The results of pesticides analysis represented the determination of aldicarb, carbofuran, chlorpyrifos- methyl, methomyl and profenfos residues in buffalo milk samples collected in summer and winter 2006-2007 seasons are listed in Table 2. The presence of negligible residues of pesticides in milk samples were taken at 5 times during summer season 2006-2007.

the average of detected pesticide residues in the buffalo milk after 2 weeks during summer season were (ND, 0.0025, ND, ND, and 0.0016 ppm) and were (0.0031, 0.0016, 0.0015, 0.0008, and 0.0026 ppm after 5 weeks during summer season and were (0.0010, 0.0003, 0.0014, 0.0002, and 0.0001 ppm) after 8 weeks during summer season and were (0.0039, 0.0004, 0.0024, 0.0009, and 0.0008 ppm) after 10 weeks during summer season and were (0.0015, 0.0008, 0.0006, 0.0003 and 0.0003 ppm) after 12 weeks during summer season for aldicarb, chlorpyrifos-methyl ,carbofuran, methomyl and profenfos, respectively. The average of the detected pesticides residues in buffalo milk samples after 2 weeks in winter season were (0.0344, 0.0005, 0.0018, 0.0008, and 0.0004 ppm) and were (0.0062, ND, 0.0031, 0.0016, and 0.0013 ppm) after 5 weeks and were (0.0099, 0.0003, 0.0017, 0.0024, and 0.0003 ppm) after 8 weeks and were (0.0045, 0.0008, 0.0023, 0.0011, and 0.0006 ppm) after 10 weeks and were (0.0152, 0.0087, 0.0048, 0.0038, and 0.0036 ppm) after 12 weeks at initial winter season for aldicarb, chlorpyrifos-methyl, carbofuran, methomyl and profenfos, respectively.

The pesticides residues in bulk tank milk samples were taken at 5 times during summer and winter 2006-2007 season are listed in Table 3. The pesticides residues in the bulk tank milk samples after 2 weeks in summer were (0.0021, 0.0011, 0.0001, 0.0003, and 0.0002 ppm) and were (0.0010, 0.0008, 0.0009, 0.0005, and 0.0002 ppm) after 5 weeks and were (0.0010, 0.0005, 0.0003, 0.0002, and 0.0004 ppm) after 8 weeks and were (0.0017, 0.0007, 0.0006, 0.0004, and 0.0004 ppm) after 10 weeks and were (0.0022, 0.0006, 0.0005, 0.0005, and 0.005 after 12 weeks at initial summer season for aldicarb ,chlorpyrifos-methyl ,carbofuran, methomyl and profenfos, respectively. But the pesticides residues in bulk tank milk after 2 weeks in initial winter season were (0.0391, 0.0080, ND, 0.0094 and 0.0009 ppm) and were (0.0224, 0.0132, 0.0056, 0.0058, and 0.0008 ppm) after 5 weeks and were (0.0207, 0.0188, 0.0045, 0.0051, and 0.0009 ppm) after 8 weeks and were (0.0164, 0.0097, 0.0041, 0.0041 and 0.0022 ppm) after 10 weeks and were (0.0323, 0.0120, ND, 0.0080 and 0.0011 ppm) after 12 weeks at initial winter season for aldicarb, chlorpyrifos-methyl, carbofuran, methomyl and profenfos, respectively.

The pesticide residues in cow's milk samples are listed in Table 4. The average of detected pesticide residues in cow's milk after 2 weeks during summer season were (0.0017, 0.0081, 0.0011, 0.0008, and 0.0006 ppm) and were (0.0012, 0.0004, 0.0004, 0.0003, and 0.0001 ppm) after 5 weeks and were (0.0039, 0.0004, 0.0029, 0.0009, and 0.0008 ppm) after 8 weeks and were (0.0015, 0.0008, 0.0006, 0.0003 and 0.0003 ppm) after 10 weeks and were (0.0081, 0.0017, 0.0011, 0.0008 and 0.0006 ppm) after 12 weeks in summer season for aldicarb, chlorpyrifos-methyl, carbofuran ,methomyl and profenfos, respectively. While the pesticides residues in cow's milk after 2 weeks during winter season were (0.0066, 0.0033, 0.0032, 0.0016, and 0.0010 ppm) and were (ND, 0.0017, 0.0021, ND, and 0.0006 ppm) after 5 weeks and were (0.0128, 0.0007, 0.0028, 0.0032, and 0.0008 ppm) after 8 weeks and were (0.0129, 0.0008, 0.0026, 0.0038 and 0.0007 ppm) after 10 weeks and were (ND, 0.0038, 0.0030, ND, and 0.0010 ppm) after 12 at initial winter seasons for aldicarb, chlorpyrifos-methyl, carbofuran, methomyl and profenfos, respectively.

Table (2): Pesticide residues (mg/Kg) in buffalo's milk during summer and winter 2007.

Pesticide tested	Season									
	summer					winter				
	Sampling period									
	2	5	8	10	12	2	5	8	10	12
Aldicarb	ND	0.0031 ± 0.013	0.0010 ± 0.0043	0.0039 ± 0.0170	0.0015 ± 0.0065	0.0344 ± 0.0007	0.0062 ± 0.0013	0.0099 ± 0.0021	0.0045 ± 0.0009	0.0152 ± 0.0032
Chlorpyrifos-methyl	0.0025 ± 0.0330	0.0016 ± 0.0213	0.0003 ± 0.0041	0.0004 ± 0.0064	0.0008 ± 0.0110	0.0005 ± 0.0003	ND	0.0003 ± 0.0074	0.0008 ± 0.0006	0.0087 ± 0.0057
Carbofuran	ND	0.0015 ± 0.0884	0.0014 ± 0.0816	0.0024 ± 0.1688	0.0006 ± 0.0343	0.0018 ± 0.0051	0.0031 ± 0.0087	0.0017 ± 0.0051	0.0023 ± 0.0067	0.0048 ± 0.0136
Methomyl	ND	0.0008 ± 0.0056	0.0002 ± 0.0017	0.0009 ± 0.0062	0.0003 ± 0.0024	0.0008 ± 0.0003	0.0016 ± 0.0005	0.0024 ± 0.0008	0.0011 ± 0.0004	0.0038 ± 0.0012
Profenfos	0.0016 ± 0.0049	0.0026 ± 0.0799	0.0001 ± 0.0041	0.0008 ± 0.0271	0.0003 ± 0.0096	0.0004 ± 0.0006	0.0013 ± 0.0020	0.0003 ± 0.0006	0.0006 ± 0.0010	0.0036 ± 0.0055

ND = Not Detected.

All values = means ± SE

Table (3): Pesticide residues (mg/Kg) in Bulk tank milk during summer and winter 2007.

Pesticide tested	Season									
	summer					winter				
	Sampling period									
	2	5	8	10	12	2	5	8	10	12
Aldicarb	0.0021 ± 0.0091	0.0010 ± 0.0077	0.0010 ± 0.0043	0.0017 ± 0.0073	0.0022 ± 0.0034	0.0391 ± 0.0080	0.0224 ± 0.0047	0.0207 ± 0.0044	0.0164 ± 0.0035	0.0323 ± 0.0068
Chlorpyrifos-methyl	0.0011 ± 0.1826	0.0008 ± 0.0102	0.0005 ± 0.0069	0.0007 ± 0.0091	0.0006 ± 0.0083	0.0080 ± 0.0053	0.0132 ± 0.0086	0.0188 ± 0.0123	0.0097 ± 0.0063	0.0120 ± 0.0283
Carbofuran	0.0001 ± 0.0606	0.0009 ± 0.0550	0.0003 ± 0.0151	0.0006 ± 0.0370	0.0005 ± 0.0292	ND	0.0056 ± 0.0161	0.0045 ± 0.0128	0.0041 ± 0.0139	ND
Methomyl	0.0003 ± 0.0020	0.0005 ± 0.038	0.0002 ±	0.0004 ± 0.0027	0.0005 ± 0.0034	0.0094 ± 0.0029	0.0058 ± 0.0016	0.0051 ± 0.0016	0.0041 ± 0.0013	0.0080 ± 0.0025
Profenfos	0.0002 ± 0.0057	0.0002 ± 0.0090	0.0004 ± 0.0023	0.0004 ± 0.0979	0.0005 ± 0.1204	0.0009 ± 0.0014	0.0008 ± 0.0012	0.0009 ± 0.0015	0.0022 ± 0.0047	0.0011 ± 0.0018

ND = Not Detected.

All values = means ± SE

Table (4): Pesticide residues (mg/Kg) in cow's milk during summer and winter 2007.

Pesticide tested	Season									
	summer					winter				
	Sampling period									
	2	5	8	10	12	2	5	8	10	12
Aldicarb	0.0017 ± 0.0074	0.0012 ± 0.0054	0.0039 ± 0.0097	0.0015 ± 0.0234	0.0081 ± 0.0044	0.0066 ± 0.0014	ND	0.0128 ± 0.0027	0.0129 ± 0.0027	ND
Chlorpyrifos-methyl	0.0081 ± 0.0053	0.0004 ± 0.0318	0.0004 ± 0.0064	0.0008 ± 0.0245	0.0017 ± 0.0113	0.0033 ± 0.0026	0.0017 ± 0.0011	0.0007 ± 0.0005	0.0008 ± 0.0005	0.0038 ± 0.0025
Carbofuran	0.0011 ± 0.0330	0.0004 ± 0.0239	0.0029 ± 0.0052	0.0006 ± 0.0091	0.0011 ± 0.0134	0.0032 ± 0.0021	0.0021 ± 0.0062	0.0028 ± 0.0080	0.0026 ± 0.0079	0.0030 ± 0.0092
Methomyl	0.0008 ± 0.0028	0.0003 ± 0.0020	0.0009 ± 0.0062	0.0003 ± 0.0024	0.0008 ± 0.0028	0.0016 ± 0.0005	ND	0.0032 ± 0.0010	0.0038 ± 0.0010	ND
Profenfos	0.0006 ± 0.0084	0.0001 ± 0.0059	0.0008 ± 0.0271	0.0003 ± 0.0096	0.0006 ± 0.0038	0.0010 ± 0.0016	0.0006 ± 0.0011	0.0008 ± 0.0013	0.0007 ± 0.0013	0.0010 ± 0.0016

ND = Not Detected.

All values = means ± SE

Pesticides can appear in milk due to several possible causes as use of insecticides directly on dairy cattle for ectoparasite control or animal feed manufactured from plant material that has been treated with insecticides and use of insecticides in stables or dairy factors. This study was carried out to determine the presence or absence of pesticide residues in milk samples (Buffalo, bulk tank and cow's) in summer and winter seasons during 2007. The pesticide residues concentration in all samples collected during summer season were lower than the pesticide residues concentration in all samples collected during winter season. The residues levels of pesticides in different milk types were lower than the acceptable Daily Intake (ADI), in summer and winter seasons on contrast the residue concentration of aldicarb and carbofuran were higher than the ADI in winter season. Profenfos insecticide was found in all samples at all times and in both summer and winter season, but its concentration within ADI value.

The results are in agreement with those obtained by Salas *et al.*, (2003) who studied residues of 13 organophosphorus pesticides in four different milk brands with high distribution were collected biweekly during a 12 month period .Approximately 39.67 of the samples contained detectable levels of OP pesticide residues. Average residues of 13 OP pesticides measured were below MRL's. Pagliuca *et al.*, (2004) who determined eight pesticide residues in milk, widely used in agriculture.

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الكشف عن الملوثات البيئية عن طريق الفحص الميكروبيولوجي وتقدير متبقيات المبيدات في عينات اللبن الخام

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تهدف هذه الدراسة إلى تقييم المحتوى الميكروبي وكذلك قياس متبقيات المبيدات في عينات اللبن الخام ، وهي ٣٠ عينة لبن خام تشتمل على لبن مزارع جاموسي، لبن بقري ولبن مجمع من محلات التجزئة (١٠ عينات لكل نوع) وجمعت هذه العينات من أماكن ومحلات مختلفة من مدينة المنصورة - محافظته الدقهلية أثناء موسم الصيف والشتاء (٢٠٠٦-٢٠٠٧). أظهرت النتائج قيم المتوسطات (Mean±SD) للفحص الميكروبي لعينات اللبن الخام سواء على اللبائن المتخصصة أو على البيئة العامة التي استخدمت في إجراء العد الكلي للميكروبات الموجودة بعينات اللبن المستخدم في الفحص.

وبينت النتائج ارتفاع قيمة الحمل الميكروبي لعينات اللبن تحت الدراسة على بيئة أجار مستخلص الجلوكوز والثرينتون وهي البيئة العامة المستخدمة للفحص حيث كانت $82.03 \pm 79.19 \times 10^3$ ، $119.40 \pm 62.58 \times 10^3$ ، $129.03 \pm 113.60 \times 10^3$

في عينات اللبن الجاموسي ، اللبن المجمع و اللبن البقري على الترتيب. وقد أوضحت النتائج وجود فروق عالية بين الثلاث أنواع من العينات في التحليل الميكروبي ، على مستوي اللبائن المستخدمة في الفحص وكذلك بين موسمي الصيف والشتاء.

وفي حالة تقدير المتبقي من المبيدات تم تقييم متبقيات لخمس مبيدات وهي الألديكارب، كلوربيرفوس ميثيل، كاربوفوران ، ميثوميل و بروفينفوس.

حيث أظهرت النتائج أن تركيز المتبقي من المبيدات في كل العينات كانت أقل في فصل الصيف عنه في فصل الشتاء. وعموماً وجد أن المستويات من المبيدات المتبقية في عينات اللبن المختلفة كانت أقل عن الحد المسموح به في موسمي الصيف والشتاء مع تباين في التركيز المتبقي من الألديكارب والكاربوفوران حيث كانت أعلى عن الحد المسموح به في فصل الشتاء. وأيضا تبين أن المتبقي من البروفينفوس وجدت في كل العينات في موسمي الصيف والشتاء .