Prevalence of Some Trace and Toxic Elements in Raw and Sterilized Cow's Milk

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Abstract: A total of 80 random samples (40 each of raw and sterilized cow's milk) were collected from different outlets in El Dakahlia Governorate, Egypt and analyzed by Atomic Absorption Spectrophotometer to determine trace and toxic elements (lead, cadmium, aluminum, iron, selenium and manganese). Results revealed that the mean values in examined raw and sterilized cow's milk samples for lead, cadmium, aluminium, iron, manganese and selenium were (0.615 & 0.910); (0.416 & 0.355); (0.501 & 1.324); (5.303 & 5.681); (0.555 & 0.330) and (0.016 & 0.018) ppm, respectively. Analyzed data indicated that (90 & 100%); (100 & 92.5%); (60 & 100%); (75 & 50%); (90 & 80%) and (45 & 70%) of examined raw and sterilized cow's milk samples had residues of Pb, Cd, Al, Fe, Mn and Se above the permissible limit, respectively. The calculated daily intake of Pb, Cd, Al, Fe, Mn and Se from consumption of 200 ml raw and sterilized cow's milk per day were (123 & 182); (83.2 & 71); (100.2 & 264.8); (1060.6 & 1136.2); (111 & 66) and (3.2 & 3.6) µg, respectively, which contributed about (24.6 & 36.4 %); (118.9 & 101.43%); (8.35 & 22.07%); (22.1 & 23.67%); (2.22 & 1.32%) and (4 & 4.5%) from the Acceptable Daily intake of Pb, Cd, Al, Fe, Mn, and Se. Also, it is evident that the mean values of Pb; Al and iron were higher in sterilized milk than raw milk, but the mean values of Cd and Mn were higher in raw milk than in sterilized milk. However, the Se level in both was nearly equal. The public health significances of existing metals as well as the suggested measures to minimize the hazardous effect of these pollutants were discussed.

[Salah F. A. Abd- El Aal; Esmat I. Awad and Rania M. K. M. Kamal. **Prevalence of some trace and toxic elements in raw and sterilized cow's milk.** *J Am Sci* 2012; 8(9):753-761]. (ISSN: 1545-1003). http://www.jofamericanscience.org. 103

Key Words: Raw cow's milk, sterilized cow's milk, lead, cadmium, aluminum, iron, selenium, manganese.

1. Introduction

Milk contains protein and minerals essential to promoting the growth and maintenance of human life during the three periods of life: child hood; providing protein, minerals and fat to support the body's development, during the adolescence; offering conditions for a rapid growth building consistent muscles, bones and endocrine and for elderly people it represents a source of calcium essentially to maintain the integrity of bones **(IEA, 2007).** So, it is necessary that milk should be obtained from healthy animals as well as collected and stored in satisfactory healthy conditions free from environmental contamination. That is to be all containers applied for packaging milk at milking, at the collection or to store must be made of stainless steel, aluminum or iron foliated in perfect finishing and seamless.

Milk and milk products are the most diversified of the natural foodstuffs in terms of composition contain more than twenty different trace elements most of them are essential and very important such as copper, zinc, manganese and iron **(Pennington *et al.*, 1995).** These metals are co-factors in many enzymes and play an important role in many physiological functions and lack of these metals cause disturbances and pathological conditions **(Schuhmacher *et al.*, 1991)**.

Ingestion of contaminated feeding stuffs and water was considered the main sources of metal residues in secreted milk where they pass into the mammary gland and subsequently into the milk **(Antoniou *et al.*, 1989; Rossipal *et al.*, 2000** and **Frodello *et al.*, 2002**). The amount of metals in uncontaminated milk is admittedly minute, but their contents may be significantly altered through manufacturing and packaging process where metal contamination may occur at several stages during dairy processing e.g. from factory door, plant equipments, catering operations, ceramic, enameled utensils, metal containers and water used in dairy production **(Reilly, 1991).**

Toxicity of metal is closely related to age, sex, route of exposure, daily intake, solubility, metal oxidation state, retention percentage, duration of exposure, frequency of intake, absorption rate and mechanisms/efficiency of excretion **(Mertz, 1986).** The presence of heavy metals as arsenic, cadmium, lead and mercury even in low concentrations, leads to metabolic disorders with extremely serious consequences and causing serious problems as it causes many health problems such as weakness, heart failure, cancer and also affects the kidneys **(McCally, 2002** and **Licata *et al.*, 2004)**.

The aim of this investigation was to evaluate raw and sterilized cow's milk based on the concentration of metals as well as the influence of the heat treatment on their level.

2. Material and Methods

1- Collection of samples:

A total of 80 random samples (40 each of raw and sterilized cow's milk) were collected from different outlets in El Dakahlia Governorate, Egypt. The raw milk samples were collected in polyethylene bags while sterilized milk samples were collected in their original package. Collected samples were taken to the laboratory without delay. Each sample was labeled to identify the source, site and date of sampling. Delayed samples were stored in ice bag.

2-Preparation of samples:

A measured volume (2cc) of thoroughly homogenized raw and sterilized milk was transferred in clean and acid washed screw capped digestion tubes. The prepared two tubes from each sample were identified for analysis.

3- Analysis of the prepared samples:

For determination of Pb, Cd, Fe and Se the first tube of each prepared sample was digested according to **Tsoumbarisand Papadop (1994)**. While, for determination aluminium and manganese the procedure was carried out on the second tube according to **Dabeka and Mckenzie (1992).** All filtered samples were analyzed for their metal contents according to methods of **Medina *et al.* (1986).** All the filtered samples were analyzed for their metal contents by using "perkia-Elmer Atomic Absorption Spectrophotometer model d 2380, USA, 1998" at the microanalytical laboratory, Department of Chemistry, Faculty of Science, El-Mansoura University, Egypt. Instrumental analysis of Pb, Cd, Fe and Se and were conducted by air lacetylene Flame Atomic Absorption Spectrophotometer (FAAS). While for determination of Al and Mn use Graphite Furnace Atomic Absorption Spectrophotometer (GFAAS). The analytical detection limits of lead, cadmium, iron, selenium, aluminium and manganese for the used instrumentation were 0.02 ppm, 0.0006 ppm, 0.05 mg/kg, 0.002 ppm, 0.02 ppm and 0.01 ppm, respectively.

4- Calculation of the daily metal intake:

The daily metal intake was estimated from the consumption of raw and sterilized cow's milk and the mean data of the present study were combined with the consumption data obtained from **Nutrition Institute, Cairo, A.R.E., 1996**. Comparison of the calculated daily metal intake from raw and sterilized cow's milk with the Acceptable Daily Intake (ADI) values recommended by **FAO\WHO (1972, 1974c, 1982, 1987, and 1989), FAO\WHO (1999), Food and Nutrition Board (1980), Dreosti (1986) and Pennington (1987).**

5- Statistical analysis:

All the data analyzed using SPSS/PCT **(Foster, 2001).** Independent T-test was performed to evaluate differences.

3. Results

Table (1): Statistical analytical results of heavy metals and trace elements residues (ppm) in examined raw cow's milk samples (N=40).

Metals	positive samples		Minimum	Maximum	Mean ±S.E
	No	%			
Lead	40	100.0	0.10	1.25	0.615±0.049
Cadmium	40	100.0	0.10	1.20	0.416±0.059
Aluminium	24	60.0	0.00	2.20	0.501±0.107
Iron	40	100.0	2.60	10.05	5.303±0.272
Manganese	40	100.0	0.05	1.05	0.555±0.051
Selenium	40	100.0	0.005	0.090	0.016±0.003

Table (2): Frequency distribution of heavy metals and trace elements residues in examined raw cow's milk samples (N=40).

Metals	Permissible limit mg/kg (ppm)	Within permissible limit		Over permissible limit	
		No. of samples	%	No. of samples	%
Lead	0.2[a]	4	10.0	36	90.0
Cadmium	0.05[b]	0	0.0	40	100.0
Aluminium	0.06[c]	16	40.0	24	60.0
Iron	5[d]	10	25.0	30	75.0
Manganese	0.05[e]	4	10.0	36	90.0
Selenium	0.011[f]	22	55.0	18	45.0

a: Carl (1991) b: Egyptian standard (2001) c: Pennington (1987)
d: Egyptian standard (1993) e: Wenlock *et al.*(1979) f: Thorn *et al.* (1976)

Table (3): Comparison of Acceptable Daily Intake (ADI) value of heavy metals and trace elements with the calculated daily intake from raw cow's milk

Metals	ADI µg /70 kg person	Mean concentration of metals (µg/L) in examined samples	Calculated daily intake of metals from consumption of 200 ml raw cow's milk per day [(f)]	
			µg /day / person	%
Lead	500[a]	615	123	24.6
Cadmium	70[a]	416	83.2	118.9
Aluminium	1200[b]	501	100.2	8.35
Iron	4800[c]	5303	1060.6	22.1
Manganese	5000[d]	555	111	2.22
Selenium	80[e]	16	3.2	4

a: FAO/ WHO, Joint Expert Committee on Food Additives WHO Technical Report Series, 1972, 1974c, 1982, 1987 and 1989.
b: Pennington (1987). c: FAO/ WHO, Joint Expert Committee on Food Additives, 1999.
d: Food and Nutrition Board (1980). e: Dreosti (1986).
F: Daily consumption of milk for adult person according to Nutrition Institute, Cairo, A.R.E (1996).

Table (4): Statistical analytical results of heavy metals and trace elements residues (ppm) in examined sterilized cow's milk samples (N=40).

Metals	**% of positive samples**	**Minimum**	**Maximum**	**Mean ±S.E**
Lead	100.0	0.45	1.25	0.910±0.051
Cadmium	100.0	0.05	1.20	0.355±0.039
Aluminium	100.0	0.33	2.50	1.324±0.112
Iron	100.0	3.85	7.86	5.681±0.209
Manganese	100.0	0.04	0.75	0.330±0.034
Selenium	100.0	0.010	0.080	0.018±0.002

Table (5): Frequency distribution of heavy metals and trace elements residues in examined sterilized cow's milk samples (N=40).

Metals	**Permissible limit mg/kg (ppm)**	**Within permissible limit**		**Over permissible limit**	
		No. of samples	**%**	**No. of samples**	**%**
Lead	0.2[a]	0	0.0	40	100.0
Cadmium	0.05[b]	3	7.5	37	92.5
Aluminium	0.06[c]	0	0.0	40	100.0
Iron	5[d]	20	50.0	20	50.0
Manganese	0.10[e]	8	20.0	32	80.0
Selenium	0.011[f]	12	30.0	28	70.0

a: Carl (1991) b: Egyptian standard (2001) c: Pennington (1987)
d: Egyptian standard (1993) e: Wenlock *et al.*(1979) f: Thorn *et al.* (1976)

Table (6): Comparison of Acceptable Daily Intake (ADI) value of heavy metals and trace elements with the calculated daily intake from sterilized cow's milk.

Metals	ADI µg /70 kg person	Mean concentration of metals (µg/L) in Examined samples	Calculated daily intake of metals from consumption of 200 ml sterilized cow's milk per day [(f)]	
			µg /day / person	%
Lead	500[a]	910	182	36.4
Cadmium	70[a]	355	71	101.43
Aluminium	1200[b]	1324	264.8	22.07
Iron	4800[c]	5681	1136.2	23.67
Manganese	5000[d]	330	66	1.32
Selenium	80[e]	18	3.6	4.5

a: FAO/ WHO, Joint Expert Committee on Food Additives WHO Technical Report Series, 1972, 1974c, 1982, 1987 and 1989.
b: Pennington (1987). c:FAO/ WHO, Joint Expert Committee on Food Additives, 1999.
d: Food and Nutrition Board (1980). e: Dreosti (1986).
F: Daily consumption of milk for adult person according to Nutrition Institute, Cairo, A.R.E (1996).

Table (7): Multiple Comparison between heavy metals and trace elements in raw and sterilized cow's milk.

Metals	Raw milk X±S.E.	Sterilized milk X±S.E.	*P*
Lead	0.615±0.049	0.910±0.051	0.000**
Cadmium	0.416±0.059	0.355±0.039	0.396
Aluminium	0.501±0.107	1.324±0.112	0.000**
Iron	5.303±0.272	5.681±0.209	0.273
Manganese	0.555±0.051	0.330±0.034	0.000**
Selenium	0.016±0.003	0.018±0.002	0.613

P= Raw milk versus sterilized milk *P<0.05 = Significant. **P<0.001 = Highly significant.

4. Discussion

Raw cow's milk:

Results listed in table (1) declared that the average concentration of Pb, Cd, Al, Fe, Mn and Se in examined raw cow's milk samples were 0.615, 0.416, 0.501, 5.303, 0.555 and 0.016 ppm, respectively.

Concerning lead concentration, it was found that these results are nearly similar to those reported by **Ali et al. (2011)** and **Samuel et al. (2011).** While, lower levels could be revealed by **Ashraf et al. (2000)** and **Enb et al. (2009)** and higher concentrations were recorded by **Ijaz et al. (2009)** and **Nina et al. (2011).**

About cadmium, nearly similar levels were reported by **El-Wakeel and El-Mowelhi (1993)** and **Nasef (2002).** Higher levels were detected by **Amer et al. (2005)** and **Nina et al. (2011)** while lower concentrations were reported by **Enb et al. (2009), Ijaz et al. (2009)** and **Samuel et al. (2011).**

For aluminium, these results are nearly similar to those reported by **McBean and Speckmann (1980)** and **Jenness (1988).** Higher levels were detected by **Lo´pez et al. (2002)** and **Cristina and Inigo (2009).** While, lower concentrations were reported by **Muller et al. (1998)** and **Anna et al. (2010).**

At the meanwhile, iron result was agreed with that obtained by **Simek et al. (2000),** while lower concentrations were recorded by **Enb et al. (2009)** and **Herwig et al. (2011).** On the other hand, higher concentration was recorded by **Cristina and Inigo (2009)** and **Sandrine et al. (2012)**

Regarding manganese level, nearly similar result was reported by **Angel et al. (1995),** while lower levels were detected by **Enb et al. (2009)** and **Herwig et al. (2011).** However, **Erdogan et al. (2004)** and **Cristina and Inigo (2009)** detected higher levels.

Selenium results are nearly similar to those reported by **Cava –Montesions (2004).** However, **Ashraf et al. (2000), Prapaisri et al. (2005)** and **Herwig et al. (2011)** could detect selenium in lower levels. On the other hand, higher concentrations were recorded by **Cristina and Inigo (2009)** and **Miguel et al. (2011).**

From the achieved results (Table 2) it could be concluded that 36 (90%), 40 (100%), 24 (60%), 30 (75%), 36 (90%) and 18 (45%) of examined samples had Pb, Cd, Al, Fe, Mn and Se above the permissible limit, respectively.

Many conclusions could be gained from the above mentioned data. Lead level in raw cow's milk may have been increased by the oral intake of large quantities as a result of licking metallic objects, painted surfaces and burnt storage batteries or feeding on feed and water contaminated from industrial wastes, mills, organic and inorganic chemical, petrochemicals, alkalis, chlorines, fertilizers, petroleum refining and steel foundries **(Okada et al., 1997).** Moreover, high lead levels were found in raw milk samples collected from cows reared near major roads with heavy traffic, which considered as a principal source of lead (leaded petrol), as well as coal combustion, iron and steel production **(Juretric et al., 1997).** Also, mobilization of lead from its stores in the skeleton of lactating animals and may be also due to migrated contamination of raw milk from metallic utensils.

The presence of cadmium with high concentration in cow's milk may be due to consumption of contaminated feeding stuffs and water **(El-Wakel and El-Mowelhi, 1993)** where it comes from industrial emissions and fertilizers (phosphate rocks, which form the basis of commercial fertilizers and sludge) which can contaminate soil and crops. Also, inhalation of fumes and dusts from the industrial activities **(Dwivedi et al., 1997)** and cadmium-lined metal equipment used in commercial food processing, kitchenware enamel, pottery glazes and plastics containing cadmium **(Harrison, 1993).**

Routinely, milk usually contains very low concentration of aluminium except when dairy animals have consumed and/or drinking contaminated water, fodder and feeds **(Cabrera et al., 1995).** Environment represents the main source of aluminium burden in human populations with food being of much greater importance than exposure via air **(Jones and Bennett, 1986).** General possibilities of oral aluminium exposure of human via extensive use of aluminium-containing food additives, migration of aluminium from saucepan or food packing into food stuffs and drinking water. Also, electrochemical erosion when foods are left in contact with aluminium vessels for long periods, acidic and salty food are increasing concentration of complex ions **(Mei and Yao, 1994).**

Milk of all animal species is notoriously low in trace elements. Selenium concentration in milk is lower than the concentrations of other essentials trace elements (Fe, Cu or Zn) (**Hatano et al. 1985).** The low level of selenium may be attributed to selenium deficiency in our geographical area. There are other factors that can produce some variations in the selenium content of cow's milk. The rich sources of selenium were meats, cereals and cereals products **(Al-Ahmary, 2009)**.

Results given in table (3) showed that the average concentration of Pb, Cd, Al, Fe, Mn and Se in raw cow's milk samples were 615, 416, 501, 5303, 555 and 16 µg/L, respectively which gave daily intake of about 123; 83.2; 100.2; 1060.6; 111 and 3.2 µg, respectively for adult person from consumption of 200 ml milk per day and this contributed about 24.6; 118.9; 8.35; 22.1; 2.22 and 4 % of acceptable daily intake recommended by **FAO/WHO (1987)** and **Pennington (1987).** Lower results of lead and cadmium were reported by **Sharma et al. (1982).** However, lower results of aluminium are recorded by **Baxter et al. (1990)** and **Muller et al. (1998). Koutnik et al. (1996)** and **Hussein and Bruggemann (1999)** recorded lower results of trace elements.

These results declared that the intake of lead and cadmium from consumption of raw milk was relatively high, but aluminium was relatively low.

Sterilized cow's milk:

Regarding Pb, Cd, Al, Fe, Mn and Se in examined sterilized cow's milk samples results (Table 4) declared that the average concentration were 0.910, 0.355, 1.324, 5.681, 0.330 and 0.018 ppm, respectively.

Lead results were nearly similar to those reported by **Rodriguez et al. (1999a),** lower findings was recorded by **Vanessa et al. (2010),** while the highest levels were recorded by **Madeha et al. (1994).**

Cadmium results were similar to those reported by **Madeha et al. (1994)** and **Rodriguez et al. (1999a).** However, **Tsoumbaris and Tsoukail (1994)** detected lower level.

Manganese concentration nearly simulates result reported by **Tsoumbaris and Tsoukail (1994).** However, a lower concentration was recorded by **Anthony Lopez et al. (1985). Madeha (2002)** recorded nearly similar concentration of selenium.

Statistical analysis of different heavy metals in sterilized cow's milk samples (Table 5) revealed that all examined samples having lead and aluminium residues above the permissible limits**.** 37 out of 40 examined samples having cadmium levels above the recommended permissible limit. Otherwise, 50% of the examined samples having iron levels above the recommended permissible limit. While 32 out of 40 samples having manganese levels above the permissible limit and 28 (70%) out of 40 examined samples having selenium above the permissible limit**.**

From the above mentioned data, it can be concluded that the examined sterilized cow's milk samples were contaminated with heavy metals especially lead, cadmium and aluminium which may be attributed to manufacturing processes that play a key role in distribution of minor and trace elements **(Tsaneem et al., 2009).**

Aluminium foil, cans, pots and pans in contact with food and aluminium in drugs are considered sustainable sources for the cumulative load of aluminium in the human body eventually reaches critical levels **(Nage and Jobest, 1994).**

Sterilized milk may be contaminated with selenium from processing, equipment and accidental contamination during storage and leaching from containers **(Ukhun et al., 1990).**

The result recorded in table (6) indicated that the average concentration of Pb, Cd, Al, Fe, Mn and Se in sterilized cow's milk were 910, 355, 1324, 5681, 330 and 18 µg/L, respectively which gave a daily intake of about 182, 71 and 264.6, 1136.2, 66 and 3.6 µg /person, respectively from consumption of about 200 ml milk/ day. This contributed of about 36. 4, 101.43, 22.07, 23.67, 1.32 and 4.5 % of acceptable daily intake (ADI) recommended by **Food and Nutrition Board (1980), Dreosti (1986), Pennington (1987), FAO/WHO (1989) and FAO/WHO (1999).**

It could be concluded that the daily intake of lead cadmium from consumption of sterilized milk were relatively high and represented high proportion in-compared to ADI recommended by **FAO/WHO (1989).**

Concerning daily intake of trace elements it could be concluded that the daily intake of iron, manganese and selenium from consumption of the sterilized milk were relatively low. Also, our results showed that milk cannot contribute a considerable proportion of the supply of trace elements in the human diet.

Multiple comparisons between metals in different samples:

Result recorded in table (7) show the comparison between metals cadmium, lead, aluminium, iron, manganese and selenium in raw and sterilized cow's milk. The mean value of lead in sterilized milk was higher than in raw milk (0.910 and 0.615 ppm, respectively). Lead was mainly associated with caseins in cow's milk, freezing or heating did not cause significant changes in distribution of lead in cow's milk and milk products **(Mata et al., 1996)** but the desiccation process produces significant increases in lead concentration **(Moreno Rojas et al., 1999).**

However, the mean value of cadmium in raw milk was higher than in sterilized milk (0.416 and 0.355 ppm, respectively). The distribution change of cadmium after heat treatment of milk due to the formation of complexes between the whey proteins and the metal or to the desegregation of the cadmium bound to casein micelles.

Also, the mean level of aluminium in sterilized milk was higher than that in raw milk (1.324 and 0.501 ppm, respectively) this result from migration of aluminium from packaging materials into the milk products **(Poonam et al., 1977).**

Meanwhile, it is evident that the mean value of iron was higher in sterilized milk than raw milk, but the mean value of Mn was higher in raw milk than in sterilized milk.

However, the Se level in both was nearly equal. Selenium concentration in raw milk and sterilized milk were lower than the levels of other essential trace elements such Cu or Zn because it depends largely on animals feed and its geographic distribution **(Klassen et al., 1986).**

Public health hazardous:

From public health point, the lead has large affinity to thiol and phosphate containing ligands, inhibits the biosynthesis of heme and thereby affects the membrane permeability of kidney, liver and brain cells which reduce the function or completely breakdown of these tissues (**Forstner and Wittmann, 1983**). Therefore, C.N.S, kidney, liver and hematopiotic system are important targets of lead toxicity which manifested as peripheral neuropathy, nephritis, rheumatic finding and anaemia **(Goldfrank et al., 1990).**

While, cadmium acts on sulphydryl groups of essential enzymes, it binds to phospholipids and interferes with oxidative phosphrylation as well as it can replace zinc in metallo-enzymes which change its activity**,** also lowering blood iron, iron binding capacity, iron saturation, Hb and packed cell volume **(Jensen, 1995)**

The chronic cadmium toxicity included kidney damage with proteinuria, impaired regulation of calcium and phosphate manifesting by bone demineralization, osteomalacia and pathological fractures**.** Moreover, cadmium is a possible cause of hypertension, insomnia and testicular atrophy **(Friberg et al., 1986b).**

Aluminium toxicity is well recognized as an important factor in many clinical disorders, the most being Alzheimer's disease **(Cowburn et al., 1990).** It is also associated with the mobilization of bone phosphate and complication in dialysis dementia and some forms of sclerosis**.** The well recognized manifestations of systemic aluminium toxicity include fracturing, osteomalacia, dialysis, encephalopathy and microcytic hypochromic anemia **(Sahin et al., 1995)** where it inhibits fluoride absorption and may decrease the absorption of calcium and iron**.**

The major nutritional importance of iron lies in it being a component of the pigments of hemoglobin, myoglobin and cytochromes involved in human body, so the common consequence of incorrect nutrition was anemia **(Ziemlamski, 1994).**

Manganese is important in human nutrition and metabolism, it can be found in dairy products in different concentration. Milk is a poor source of manganese as its mean value in cow's milk from different breeds ranged from 10-50 µg/L **(Koops and Westerbeek, 1993).**

Selenium is now recognized to be a trace mineral of great importance for human health where it has several tasks in the body; important antioxidants, prevent blood clots by inhibiting platelets aggregation, increase the effectiveness of immune system and strengthens resistance to viral and bacterial infections and protects the heart muscle from disease **(Hanpaa, 1990).**

Conclusion

As a general conclusion, heavy metals and trace elements residues were contaminated samples of raw and sterilized milk in various levels. These are mainly due to greater pollution of the environment which have resulted in an increased concentration of heavy metals in air, water and soil subsequently, these metals are taken by plants and animals to take their way into milk.

Also, heavy metals may be added to milk during production, processing and storage as well as by contamination from containers.

On the other hand, continual increase in the number of industries and agricultural processes producing these pollutants and their participation in increasing the incidence of some chronic diseases, throw some light on the necessity of scientific substantiation and urgent solution of this problem.

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8/15/2012