

Evaluation the Impact of Environmental Conditions in New Valley Governorate on the Concentrations of Major, Trace, and Toxic Heavy Metals in Different Cow's Milk Samples

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Abstract

The El-Kharga City/Oasis has hyper arid climate condition and a limited supply of water. In this area, the fossil groundwater is the primary water source. This study aimed to measure the concentrations of major, trace elements, and toxic heavy metals in 50 samples of fresh cow's milk taken from three regions in El-Kharga city, New Valley Governorate (NVG), Egypt. Slight significant differences in the concentrations of major elements were observed among the different regions. The average contents of Sodium (Na), Potassium (K), Calcium (Ca), Magnesium (Mg), and Phosphorus (P) were 477.27, 1416.04, 1115.10, 119.91 and 848.49 mg/L, respectively. Ranges of trace elements were Fe (1.02- 10.30), Zn (1.82-7.11), and Mn (0.07- 3.98) and the average was 3.09, 4.00 and 0.612 mg/L, respectively. While, toxic heavy metals ranges were Cu (0.01 - 0.48), Pb (0.00 - 0.04)(0.11), and Cd (0.00 - 0.03) as well the average was (0.113, 0.054) and (0.005 mg/L). respectively. The highest average concentration of Fe, Zn, Mn and Cd (4.88, 4.25, 1.29 and 0.007 mg/L, respectively) was in El Mounira villages while those of Cu in Naser El Thowra villages and Pb in Al-Shula area. Furthermore, Zn, Mn and Pb contents were higher while Fe and Cd were lower, and Cu was within the permissible limits recommended by international dairy federation Standard (IDF) and Egyptian standards. As the concentrations of toxic heavy metals did not exceed the safety limits, it could not pose a serious danger to public health.

Key words: Major elements, trace elements, toxic heavy metals, maximum permissible limit, New Valley Governorate.

Introduction

Egypt's largest governorate by land area is the New Valley Governorate (NVG), which makes up 44 % of the country's total land area and approximately 66 % of the Western Desert area. It includes three oases/cities, El-Kharga, El-Dakhla, and El-Farafa. The Nubian-Sandstone-Aquifer (NSA) is the primary source of underground water in this area, serving as the main water source for the region (Soliman and Solimsn, 2017). El-Zeiny and Elbeih (2019) findings indicate that, the primary and potentially the only source of water for agricultural and domestic purposes in this area is subterranean water. Due to the governorate's desert nature and geographical location, this prevents access to water from the Nile River. Besides, the utilization of natural water infusion systems by planters in the New Valley region to dispose of excess water, coupled with the area's light-textured soils, suggests that human activities in the region could potentially have a considerable impact on the build-up of mineral contaminants in the subterranean water. Recently, increasing levels of salinity and water harvesting have begun to emerge in the NVG. Specifically, it is the cities of El-Kharga and El-Farafra that require particular attention (IFAD, 2017). The significance of water cannot be ignored in the exposure of humans to heavy metals. Mebrahtu and Zerabruk (2011) found that, heavy metals concentration in water exceeded the permissible limits compared to estimates provided by the WHO and US-EPA. A significant number of studies have pointed out the occurrence of potentially toxic elements (PTEs) in soil sediments at considerably great levels within the NVG (Mohamaden et al., 2017). Even though the groundwater in the NVG is located at a considerable depth, the extreme application of mineral nourishments, insecticides, and the discharge of dirt water into the land without proper dirty water drainage systems have emerged as significant issues of concern (Rahmati and Melesse, 2016; Abbas and Bassouny, 2018). There is a possibility that the quantities of these pollutants in the belowground water may continue to rise, ultimately exceeding permissible levels in several areas.

In milk and its byproducts, there are over twenty distinct trace elements present, with the majority being essential elements like zinc (Zn), copper (Cu), iron (Fe) and manganese (Mn) (Pennington et al., 1995). Environmental contamination by heavy metals has recently increased due to growing industrial and agricultural activity. The presence of these metals in milk can be attributed to various factors; animal diets, quality of water consumed by the animals, the production and processing of milk, or the materials used for storage and packaging (Khaniki, 2007). In dairy animals' tissue, heavy metals are accumulated and ultimately excreted into milk due to their pervasive and non-biodegradable or thermally biodegradable nature (Ismail et al., 2014). Furthermore, heavy metals accumulation in ruminants results in harmful effects in humans, who consume their contaminated meat and milk (Pilarczyk et al., 2013). Various physiological functions rely on heavy metals as co-factors for enzymes, highlighting their significance in biological processes, their absence results in disturbances in body functions and pathological diseases. Essential elements are necessary for good health in normal doses, but in higher doses, especially when it exceed 40 to 200-fold, it become "toxic" (Tchounwou et al., 2012). Heavy metal intoxication can impair the lungs, kidneys, blood, liver, brain and others essential for proper bodily function (Jaishankar et al., 2014). Heavy metals exposure might lead to metabolic disorders with severe consequences, including acute and chronic neurotoxic effects (Malhat et al., 2012). Lead (Pb) and Cadmium (Cd) are known hazard elements and have been linked to the development of several diseases affecting vital systems like the cardiovascular, nervous, renal, skeletal, and hematopoietic systems. Assessing the heavy metal content in milk can serve as a valuable "direct indicator"

of the milk's hygiene, and as an "indirect indicator" of environmental contamination, levels in the region where the milk was sourced (González-Montaña et al., 2012).

Therefore, the current study aimed to evaluate the impact of environmental conditions on the concentrations of major, trace, and poisonous heavy metals of different samples of cow's milk grazed in New Valley governorate and compare the obtained results with different international guidelines for food as well as with previous reports from the available literature.

Materials and methods

The collection and preparation of milk samples from different geographical locations:

Fifty cow milk samples (each approximately 100 mL of fresh milk) were randomly collected from different locations in three regions of El-Kharga city, NVG, Egypt, which included El Mounira villages, Al-Shula area and Naser El Thowra villages, (n = 16, each, except ElMounira villages n=18) during March to April 2022. To avoid contamination, sterilized screwcapped bottles were used to store the milk samples. The specimens were transported to the laboratory of Department of the Dairy Science, Faculty of Agriculture, New Valley University (NVU), Egypt, and the samples were promptly frozen at -20°C upon collection to preserve their integrity until analysis. The collected samples from different areas in NVG, Egypt are depicted in Fig. 1.

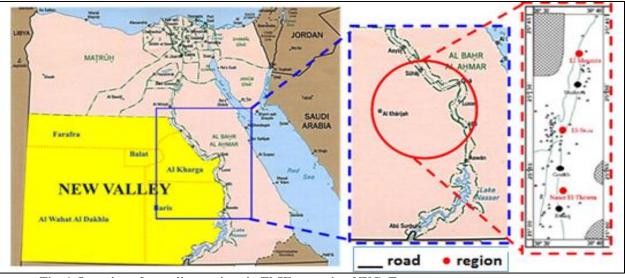


Fig. 1. Location of sampling regions in El-Kharga city, NVG, Egypt.

Sample digestion and determination

Milk samples (1 g) were digested with a solution consisting of concentrated nitric acid (HNO₃) and concentrated hydrogen peroxide (H₂O₂). Double deionized water was used to dilute the digested samples to a final volume of 50 mL. A blank digestion solution was used for comparison. Minerals including K, Na, and Ca were estimated using a Flame Photometer (Jenway, U.K.). Fe, Mn, Zn, Pb, and Cd were estimated using an Atomic Absorption Spectrophotometer (Thermo Electron Corp., China). The P element was estimated colorimetrically according to **AOAC (2005).**

This research was conducted in cooperation between the Dairy Science Department, Faculty of Agriculture, NVU, and the Food Technology Department, Faculty of Agriculture, Suez Canal University in Egypt.

Statistical analysis

Statistical analysis of the data was performed using one-way analysis of variance (ANOVA) with the aid of SPSS 16.0 software (SPSS Inc., Chicago, II., USA). The experimental procedures were replicated three times, and the results were reported as the mean \pm SD.

Results and discussion

The major element content of raw milk collected from the NVG. Major elements are essential factors in many biochemical and nutritional functions in the human body. Based on their quantities and reciprocal ratios, these elements could be acted as poisons or nutrients, and their deficiencies can cause some diseases (Brewer, 2010). The major elements found in milk samples obtained from different regions located in El-Kharga city, NVG, were Sodium, Potassium, Calcium, Magnesium, and Phosphorus and are presented in Fig. 2. The content of K was the most abundant mineral, followed by Ca, P, Na, and Mg. Furthermore, the Na, Ca and P contents of El Mounira villages. K and Mg contents of Naser El Thowra villages were the highest values when compared to milk samples obtained from the other regions. The average concentrations of the primary elements present in the milk samples gathered from various regions are also displayed in the Fig.5. The average of Na, K, Ca, Mg and P concentrations were 477.27, 1416.04, 1115.10, 119.91 and 848.49 mg/L, respectively. These differences in mineral content from the studied regions are related to the animals' dietary composition, mineral content in the soil, water sources, grains, forages and mineral supplements as well as the use of synthetic fertilizers (Yoo et al., 2013). The recommended dietary of elements intake were approximately Na,1500; K, 4700; Ca.1200; Mg was 310-320 for females and 400-420 for men and P was700 mg/day and about 1250 mg for pregnant women and teenagers, respectively (Zamberlin et al., 2012).

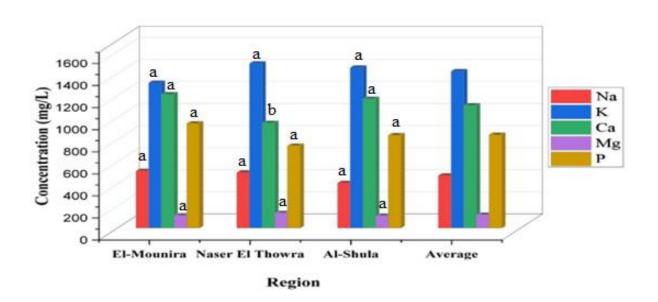


Fig. 2. Major minerals content (mg/L) in milk samples obtained from various regions of NVG.

However, in the current study, Ca content was higher and P content was lower than those reported by **Vahčić et al. (2010)**, which was 900 and 970 mg/L, respectively. In

humans and animals, either too little or extreme concentrations of macro and microelements have negative health consequences. Minerals are necessary to overall mental and physical health, as well as teeth and neurons growth. Furthermore, minerals serve as catalysts for numerous biological activities in the body such as muscular contraction and neuronal signaling (Anonymous, 2010). Therefore, daily mineral intake below the recommended levels causes osteoporosis, hypertension, colon and breast cancer, atherosclerosis and oxidative stress as in the case of Ca and Mg (Zamberlin et al., 2012).

Trace elements content of raw milk collected from the NVG

Essential elements are involved in several vital functions of living organisms when in minute concentrations but may become toxic at higher concentrations (Jaishankar et al., 2014). The content of trace elements such as Fe, Zn and Mn in the milk samples obtained from three distinct regions is shown in Fig. 3. Milk samples obtained from the villages of El Mounira had significantly higher of Fe, Zn and Mn concentrations in comparison to samples collected from the other regions. However, the Zn concentrations were not significantly different between milk samples, excluding El Mounira villages, which exhibited the highest levels. The high percentage of Fe, Zn and Mn in the El Mounira villages may be due to its near busy roads with heavy car traffic. Generally, the exceeded trace metal values in the investigated areas could have originated from the underground water and animal feed (Rajaganapathy et al., 2011).

As illustrated in the Fig.5, the average concentrations of Fe, Zn and Mn were 3.09, 4.00 and 0.612 mg/L, respectively. The findings are consistent with the levels reported by **Dawd et al., (2012)** for unpasteurized milk. The concentrations of Fe were greater than

those found by Enb et al., (2009) and Zamberlin et al., (2012). However, the concentrations were less than those estimated by Malhat et al., (2012) and Abdulkhaliq et Palestine. in Egypt and al., (2012)respectively. The obtained values were lower than Egyptian standards (EOSOC, 1993), recommending the maximum permissible limit (MPL) of Fe in milk to be 5 mg/Kg (Table 1). The daily consumption (mg/day) of Fe from milk and milk-based products has been reported to vary between 0.04 to 1799 mg/Kg/day (Meshref et al., 2014).

The Zn levels detected in the milk samples were less than those observed in milk samples obtained from the Cairo governorate (Abou-Arab, 1991) and Addis Ababa, Ethiopia (Dawd et al., 2012). Conversely, the results were higher than those of Enb et al., 2009). However, the contents of Zn were greater than that of IDF (1977), which mentioned that MPL for Zn in milk is 0.328 mg/Kg. Studies have reported a range of 0.036 to 1.258 mg/day for the daily intake (mg/day) of Zn from dairy products, including milk (Meshref et al., 2014).

In the current study, the Mn values were greater than those stated by Enb et al. (2009) and Vahčić et al., (2010). Besides, the obtained results in this study were closer to the reported of Belete et al., (2014) who found that the average was 0.427 mg/Kg. The MPL of Mn recommended by standard Tables of Food Composition in Japan was 0.2 ppm (Watanabe and Kawai, 2018). Thus, the mean concentration of Mn in the current study was over the permissible limit. The safe and appropriate daily intake of Mn for adults is 2-5 mg/day and 2.5-25 g/Kg for newborns (Perveen et al., 2005).

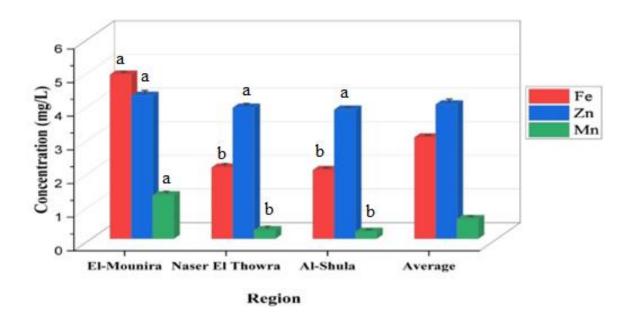


Fig. 3. Trace elements content (mg/L) in milk samples obtained from various regions of NVG.

Heavy metal toxicity in raw milk collected from the NVG

Heavy metals well-known are contaminants in the environment because of their toxic properties, bio-accumulative nature, and stability in the environment. Some heavy metals are required for appropriate metabolic processes in living organisms, while other minerals are not vital and do not play a biological role (Khan et al., 2014). Cd and Pb are hazardous metals found in the environment for animal and human health (Ali et al., 2019), because of its resistance to biodegradation, it accumulates in the environment causing several public health concerns for human and agriculture sector. Poisoning of Pb is characterized for human by anemia, muscle pain, changes in arterial elasticity resulting in hypertension, toxic effects on the reproductive system, and Pb nephropathy (Jaishankar et al., 2014). Fig. 4 depicts the levels of toxic heavy metals in milk samples obtained from different locations in El-Kharga city. The Cu and Pb concentrations in raw milk samples obtained from Naser El Thowra villages and

Al-Shula area exhibited significantly higher than that of other regions, respectively. Furthermore, Cd concentrations were almost empty for Naser El Thowra villages when compared with milk samples collected from the other regions as well as El Mounira villages had the lowest concentrations of Cu. Milk samples may become contaminated with Cu through various means, such as consumption of animal feed containing Cu, exposure to water with high Cu levels, and contact with milking equipment made of Cu metal or containing Cu bearings (Mitchell, 1981). Copper is known for its antimicrobial properties and has been used as a disinfectant. which can result in leached copper. This can serve as an alternative source of copper in situations where dietary intake is inadequate. However, concerns about acute and chronic copper toxicity in drinking water exist, as copper ions can accumulate from various sources, including industrialization, electronic waste treatment, urban waste treatment, and natural metal erosion and dissolution (Fitzgerald, **1998**). The average

concentrations of Cu, Pb and Cd were 0.113, 0.054 and 0.005 mg/L, respectively (Fig.5). The concentrations of Cu, Pb and Cd exceeded the Codex Alimentarius Commission's (2007) recommended values, where the maximum residue limits for those minerals in milk were 0.01, 0.02 and 0.0026 μ g/g, respectively. However, the mean concentration

of Cu observed in this study was comparable to the findings of earlier studies conducted by **Ogabiela et al., (2010)** and **Malhat et al.,** (2012). While it was considerably greater than the results reported by **Bilandžić et al. (2011)**. Moreover, **Enb et al. (2009)** and **Belete et al.,** (2014) reported much lower than that of our results.

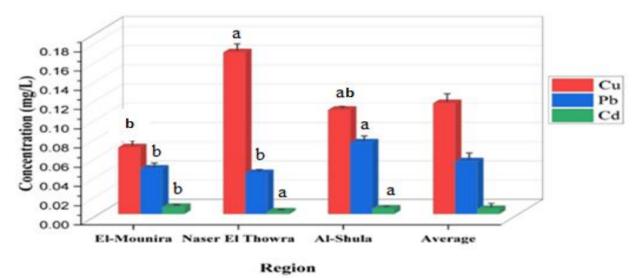


Fig. 4. Toxic heavy metals content (mg/L) in milk samples obtained from various regions of NVG.

(11)	I. F .L.).								
	- Group	Level of minerals (mg/L)						Numbers and % of	
Mineral		Positive samples		Min	Max	Mean \pm SD	M.P.L. (ppm)	samples exceed M.P.L. (ppm)	
		NO.	%					No.	%*
Fe	Trace elements	50	100	1.467	5.95	3.047±1.683	5 ^a	17	34
Zn		50	100	3.355	4.465	3.984 ± 0.444	0.328 ^b	50	100
Mn		50	100	0.165	2.563	0.626 ± 0.951	0.2°	28	56
Cu	Toxic	50	100	0.042	0.237	0.115±0.066	0.1ª,	13	26
Pb	heavy	42	83.0	0.000	0.085	0.052±0.033	0.02 ^{a,d}	20	47.6
Cd	metals	34	45.8	0.000	0.013	0.005 ± 0.005	0.05 ^a	12	35.3

Table 1: Mineral contents in the examined raw	milk samples and	percent above maximur	n permissible limits
(M.P.L.).			

a = Egyptian Organization for Standardization and Quality Control "EOSQC"

b = IDF (International dairy federation standard) (1977)

c = Standard Tables of Food Composition in Japan (Watanabe & Kawai, 2018)

- d = Codex Alimentarius Commission (2007)
- M.P.L.= Maximum permissible limits
- ppm = mg/kg

* % = Calculated as a percentage of examined samples

The heavy metal concentrations obtained from Mout-El-Dakhla city in Egypt's NVG by El-Bassiony et al. (2016) for Pb and Cd were higher, but the concentration of Fe was lower when compared to our study. This could be attributed to contamination of the animal feed, climate conditions such as wind, agrochemical and pesticide use, wastewater, and drinking water (Jan et al., 2009). Furthermore, these cows graze near busy roads with heavy car traffic, which also could contribute to the presence of Pb in milk samples (**Ogabiela et al., 2010**).

It is clears that some regions in the present study revealed no measurable levels of Cd (Naser El Thowra villages). Cadmium contamination may result from underground water sources that are polluted by sewage sludge and might be due to fertilizer use or pesticides, producing increased Cd levels in animal feed and milk. According to the Egyptian standard (EOSOC, 1993), the MPL for Cd in food is 0.05 ppm. The results of our study showed that, the concentration of Cd in all milk samples was below the limi. The mean levels of Cd reported for cow milk from Egypt by Enb et al. (2009) and Malhat et al. (2012); from Nigeria by Ogabiela et al. (2010) and from Ethiopia by Dawd et al. (2012) were much higher compared to the current findings. However, a very low Cd level was reported by Pilarczyk et al. (2013) in Poland; Bilandžić et al. (2011) in Croatia; Sola-Larrañaga and Navarro-Blasco (2009) in Spain and Khan et al. (2014) in South Korea, demonstrating improved Cd control in these countries. The tolerable Cd intake established by WHO (1980) is 60 μ g/day for women and 70 μ g/day for men.

Finally, Fig. 5 shows that displays of major, trace and toxic heavy metal concentrations present in milk samples obtained from various regions. The levels of different elements in the three subject regions can be clearly summarized such that El Mounira villages had the highest concentrations of Na, Ca, P, Fe, Zn, Mn and Cd; the lowest concentrations of K, Pb and Cu. Whilst, Naser El Thowra villages had the highest concentrations of Mg and Cu; the lowest concentrations of Ca, P and Cd. In addition, Al-Shula area had the uppermost concentrations of pb and the lowest concentrations of Na. On the other hand, El Mounira villages and Al-Shula area were similar and lowest in Mg. Naser El Thowra villages and Al-Shula area were similar and lowest in Mn, Zn and Fe. However, El Mounira villages were similar and lowest in Pb.

Conclusion

To the best of our knowledge, this is one of only few studies that evaluated the impact of environmental conditions on the concentrations of major, trace, and poisonous heavy metals in raw milk collected from several regions in Egypt's NVG. Data indicated that the mineral content exhibited large variability in their concentrations for the different regions showing that how environmental conditions impact on its concentrations in milk. Moreover, some regions showed nil levels of some heavy metals in the raw milk samples, and others were above the maximum residue levels (MRLs). This can be attributed to increased contamination of the environment (air, water and soil) in these regions. In addition, our results revealed that the NVG is one of the least polluted governorates of Egypt, especially considering toxic minerals, because it is less urban, agricultural activities and the least expansion. industrial We recommend conducting further studies on the level of toxic metals by taking a larger number of milk and its products samples from other locations and sub-cities in NVG.

Conflicts of Interest/ Competing interest

All authors declare that they have no conflicts of interest. **Data availability statement**

All data sets collected and analyzed during the current study are available from the corresponding author on reasonable request.

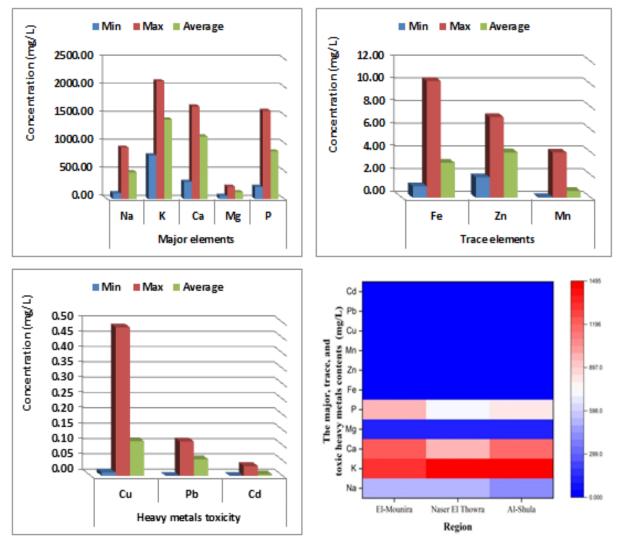


Fig. 5. Displays the concentrations of major, trace and toxic heavy metals present in milk samples obtained from diverse regions.

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