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Original Research Article

Assessing of Heavy Metals in Serum of Barki Sheep in Rainfed Area at Matrouh Governorate

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ABSTRACT

The present study was conducted to determine serum concentrations of lead (Pb), cadmium (Cd), copper (Cu), zinc (Zn), iron (Fe), and selenium (Se) of Barki sheep reared in the rainfed area at Matrouh Governorate, Egypt. Forty apparently healthy Barki sheep (2-4 years of age) were randomly selected from Marsa Matrouh and El Negaila farms (twenty each) for this study. In addition to twenty healthy Barki sheep aged 2-4 years, obtained from Borg El Arab farm, represented as control. Animals classified into 3 groups according to water source: Borg El Arab as control (Tap water); Matrouh (Rainfall water); El Negaila (Rainfall Water). Water and serum samples were prepared for the detection and estimation of selected metals by using Flame Atomic Absorption Spectrometer. Metal analysis revealed that Pb levels in different water samples were recorded above the EWQS of 0.01 ppm in following: El Negaila > Matrouh > control, Cd levels were recorded in El Negaila (RW) and Matrouh (RW) above recommended limit of 0.003, Se levels were recorded above maximum limit EWQS of 0.01 ppm in control (TW) followed by El Negaila and Matrouh however the levels of Cu, Zn, and Fe from the surveyed areas were below EWQS limits. Serum mineral analysis, Pb level was significantly increased only in examined sheep of El Negaila, Cd levels were higher in examined sheep of El Negaila and Matrouh however, Cu, Zn, and Fe levels were significantly lower compared to the control group. Conclusion: water analysis showed that Cu, Zn, and Fe concentrations were within the levels recommended by EWQS except for Pb, Cd and Se levels which need more attention. Serum analysis revealed high levels of Pb, Cd, and Se however deficient to marginal levels of Zn and Cu were detected in examined sheep. These results should provide a database for the mineral status of Barki sheep and their drinking water quality in Matrouh and El Negaila as well as evaluate environmental pollution status in this area.

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1. Introduction

Environmental pollution is a topic of great interest because it directly affects the quality of ecosystems and of all living organisms at different trophic and systematic levels (Ferrante et al., 2017). In recent years, water pollution by heavy metals such as lead (Pb) and cadmium (Cd) has accelerated dramatically due to natural and anthropogenic source (Masindi and Muedi, 2018) and subsequently, these metals are taken by plants and animals and take their way into food chain (Miedico et al., 2016). In Matrouh governorate, the native rangelands are deteriorating due to environmental and human reasons (Halmy, 2019). The risk associated with the exposure to Pb and Cd present in food products had serious health hazards to animals and may lead to accumulation of these metals in the body and cause metal toxicity (Milam et al., 2017).

Minerals are important to support animal health as well as productivity. Numerous studies have revealed that their deficiency or even imbalance can lead to various disorders, impaired growth and reproduction and depressed immunity of livestock (Gonul et al., 2009; Dar et al., 2014; Elayaraman et al., 2019). Copper (Cu), zinc (Zn), iron (Fe), and selenium (Se), among others trace minerals, are required for the normal functioning of basically all biochemical processes in the body. They are part of several enzymes, such as superoxide dismutase, glutathione peroxidize and glutathione reductase. They are essential components of the antioxidant defense against reactive oxygen species so protect tissues from oxidative damage (Evans and Halliwell, 2001; Čobanová et al., 2017).

Sheep, the most abundant ruminant livestock species in Egypt, is used mainly as a source of meat with wool as a secondary product (Elshazly and Youngs, 2019). The current number of sheep in Egypt is 5.69 million heads (FAO, 2017), one-third of which is held in the western desert especially in the northwestern coastal area. Barki sheep, the dominant coarse wool fat-tailed breed of this area, is known to be well adapted to harsh climates, poor feeding, and diseases (Tibbo et al., 2008). Sheep and goat production on range constitute a major part of the Bedouin's income. Over the past decades, sheep production in Matrouh governorate has declined sharply due to severe 15-year drought in the northwestern coastal zone from 1995 to 2010 with an annual rainfall of less than 150 mm over the period. Due to the duration of the drought, all the families have been led to reducing drastically their reproductive flock to cover the costs of feed and food (Osman et al., 2012; Alary et al., 2014).

The evaluation of the mineral status of livestock is an important tool in health management. Adequate provision of essential trace elements is necessary to avoid nutritional disturbance and to sustain animal production efficiency and animal welfare. Over the past few years, mineral deficiency has been often observed especially in cases of wool loss and lameness (Hill and Shannon, 2019). Hence, the study was conducted to investigate the serum mineral content of blood sheep and water sources besides monitoring the pollution status in the rainfed area to advise the supplementation strategy for ensuring optimum production performance and prevention of health disorders.

2. Materials and Methods

2.1. Study area

Matrouh Governorate, located on the northwestern coastal zone of Egypt at 30° 50′ 30.67″ N, 29° 23′ 38.56″ E (Figure 1). The climatic conditions of the study area are typically arid to semi-arid, characterized by long hot dry summer, mild winter with little rainfall, high evaporation with moderately to high relative humidity and elevation ranging from 0 to more than 40 meters above sea level and slopes towards the Mediterranean Sea (Bornkamm and Kehl, 1989). The study area consisted of the following regions i.e. Marsa Matrouh and El-Negaila. According to the global positioning system, Marsa Matrouh is neighboring district to El-Negaila positioned at 31° 20′ 0″ N, 27° 13′ 0″ E and 31° 24′ 25.6″ N, 26° 34′ 59.44″ E respectively. Distances between locations sampled are, Matrouh- El-Negaila: 90 km.

2.2. Animals and study design

Forty Barki sheep aged between 2 - 4 years of both sexes, selected from different flocks in Marsa Matrouh and El Negaila city at Matrouh governorate, during March 2018. The randomly chosen sheep were neither pregnant nor they recently have given birth. All selected sheep were apparently healthy, which was confirmed by physical and clinical examination to ensure that they are free from any apparent disorders (Morsy et al., 2019). In the study area, sheep were provided with rainfall water and fed on cultivated forage besides wheat straw and concentrates. These animals classified into 2 groups according to water source: Matrouh (RW); El Negaila (RW).

Twenty healthy Barki sheep aged 2-4 years, obtained from Animal Production Research Station at Borg El Arab, represented as control (Tap water). Animals were clinically healthy, nonpregnant and free from internal and external parasites. They were nourished by ordinary ration, consumed fresh tap water, and kept under complete veterinary supervision.

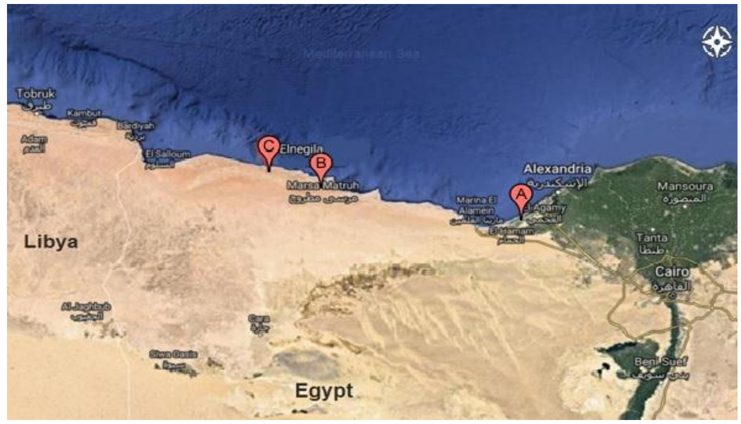

Fig. 1 Map of Matrouh governorate showing sampling locations, A. Control; B. Marsa Matrouh; C. El Negaila

2.3. Sampling

All glass tubes and plastic containers used in this study for mineral estimation were cleaned twice with diluted HNO_3, then rinsed with deionized distilled water twice and then air-dried before used.

2.3.1. Water samples collection

Water samples were collected to evaluate the mineral status of drinking water to monitoring the pollution status. Three water samples were collected from each ranch in acid leached polyethylene bottles. Before collecting each sample, the bottle was repeatedly washed with water of the sample examined. The only pretreatment was acidification to pH 2 with nitric acid, which was performed immediately after collection. The technique of water sampling was conducted according to the recommendation of American Public Health Association (APHA, 2012).

2.3.2. **Blood sample collection**.

Blood samples collected to investigate the mineral content of sheep blood to monitoring the effect of the pollution status. Five milliliters of blood were collected from a jugular vein of all sheep into a labeled centrifuge tube without anticoagulant and was allowed to clot at room temperature for one hour and then kept for the refrigerator for three hours to allow serum separation. Serum was obtained by centrifugation of blood samples at 3000 r.p.m for 20 minutes and the obtained serum was collected and frozen at -20°C until required for analysis.

2.4. Mineral analysis

Water samples were prepared according to the method described by AOAC (2016). Serum samples are prepared following Meret and Henkin (1971) protocol, Briefly, one portion of samples was diluted to 10 (1:10) with a 6% 2-butanol solution. Water and serum samples were analyzed by Flame Atomic Absorption Spectrometer (model SensAA, GBC, Australia) (Meret and Henkin, 1971; AOAC, 2016) in Animal Health Research Institute for detection Pb, Cd, Cu, Zn, Fe and Se concentrations, at suitable wavelengths of 217.0, 328.1, 324.7, 213.9, 248.3, and 196.0 nm, respectively. The other instrumental parameters (like bandwidth, lamp current) were set up separately for the estimation of specific metals according to the manufacturer recommendation. Highly purified deionized water was used to prepare calibrated standards. Appropriate quality assurance procedures and precautions have been carried out to ensure reliability of the results. The results of water samples were compared with the MPL of Egyptian drinking water quality standards (EWQS, 2005).

2.5. Statistical analysis

All data are expressed as mean ± Standard error and the variance was analyzed by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) by using IBM SPSS Version 25 (IBM, 2017) and

Differences are considered significant when $P < 0.05$.

3. Results

3.1. Mineral concentrations in water samples

Mineral concentrations of water samples as related to the sample collection (tap water, and rainfall water), in comparison with Egyptian drinking water standards limits (EWQS, 2005), were given in Table 1. It can be clearly observed that Pb, Cd and Se levels in different water samples were recorded above the maximum permissible limits. The difference between areas in Pb concentration values is not much but the maximum concentration in water samples was recorded in the rainfall water sample of El Negaila followed by Matrouh and control (TW) above the EWQS of 0.01 ppm. The concentrations of Cd were recorded only in the rainfall water sample of El Negaila and Matrouh above the recommended limit of 0.003. The concentrations of selenium were recorded above maximum limit EWQS of 0.01 ppm in control (TW) followed by El Negaila (RW) and Matrouh (RW). On the other hand, the levels of Cu, Zn, and Fe from surveyed areas were below the EWQS limits, 2 ppm, 3 ppm, and 0.3 ppm, respectively.

Table 1. Concentrations (ppm) of Pb, Cd, Cu, Zn, Fe, and Se in different sources of water from surveyed localities in Matrouh Governorate compared to Egyptian drinking water standards

Minerals (ppm)	Locality			EWQS
	Control (TW)	Matrouh (RW)	El Negaila (RW)	
Pb	0.07	0.112	0.25	0.01
Cd	ND	0.006	0.015	0.003
Cu	0.053	ND	ND	2
Zn	0.044	ND	ND	3
Fe	0.039	0.03	0.088	0.3
Se	0.83	0.28	0.68	0.01

ND= Not detectable. TW= tape water, RW=rainfall water.

EWQS (Egyptian drinking water quality standards, Decision number 1589/2005)**.**

3.2. Mineral concentrations in serum samples

Mean concentrations and ranges of selected metals in the serum of Barki sheep from different flocks in Matrouh and El Negaila are given in Table 2. The mean serum concentration of Pb was higher in examined sheep of El Negaila (RW) but not detected in other serum samples. The mean serum concentrations of Cd were increased in examined sheep of El Negaila and Matrouh. Similarly, the mean serum Se concentrations were significantly ($P < 0.05$) higher in examined sheep of Matrouh and El Negaila than the control group. Conversely, the mean serum concentrations of Cu and Zn were significantly ($P \leq 0.001$) decreased in examined sheep of surveyed areas compared to the control. Also, the mean serum Fe concentrations were slightly decreased in Matrouh and El Negaila groups compared to the control group.

Table 2. Statistical summary of concentrations (ppm) of Pb, Cd, Cu, Zn, Fe, and Se in the serum of Barki sheep in the rainfed area, along with reference values

Minerals (ppm)	Locality						*P*-Value	Ref. Value
	Control (TW)		Matrouh (RW)		El Negaila (RW)			
	Range	Mean ± SE	Range	Mean ± SE	Range	Mean ± SE		
Pb	ND	ND	ND	ND	ND – 1.2	0.48± 0.20	–	–
Cd	ND	ND	ND– 0.08	0.05± 0.01	0.01 – 0.1	0.06± 0.01	–	–
Cu	1.55–1.90	1.72^{b} ±0.06	0.55–0.69	0.62^{a}± 0.02	0.48 – 1.21	0.77^{a}± 0.13	<0.001	0.75–1.7
Zn	0.55–0.86	0.70^{b} ±0.05	0.43–0.54	0.48^{a}± 0.02	0.37 – 0.55	0.45^{a}± 0.03	0.001	0.55–1.2
Fe	1.99–2.32	2.15^{b} ±0.06	1.7 – 2.04	1.90^{ab}± 0.06	1.5 – 2.12	1.79^{a}± 0.11	0.025	0.90–2.7
Se	0.09 – 0.3	0.18^{a} ±0.04	1.8 – 8.21	5.16^{b}± 1.15	0.57 – 8.04	5.28^{b}± 1.33	0.006	0.06–0.2

Means of different variables within the same raw having different superscripts are significantly different ($P < 0.05$). ND= Not detectable. (–) not available. Reference values according to **Herdt and Hoff (2011).**

4. Discussion

Drinking water quality is a worldwide concern. It is important to monitor its quality and evaluate the risk of exposure to heavy metals (Priti and Paul, 2016). The obtained concentrations of analyzed 6 elements in different sources of water samples from Matrouh and El Negaila are summarized in Table 1. The results of Pb concentrations in all water samples from different areas are higher than the permissible limit value of 0.01 ppm according to Egyptian drinking water quality standards (EWQS, 2005). The difference between areas in Pb concentration values is not much but the maximum concentration of Pb in water samples was recorded in rainfall water samples from El Negaila, followed by Matrouh and Control (tap water), respectively. These findings are in line with the various studies conducted (El Gohary et al., 2017; El Baz and Khalil, 2018). El-Hamid et al. (2016), stated that the sediments from the Egyptian Mediterranean coast were mostly contaminated with Pb. The contamination of water sources with Pb may be attributed to the direct contamination with the exhaust of engines of vehicles that traffic the international road and the other paved roads. These results may be confirmed by what was observed in the field where most cisterns water is exposed. Moreover, Pb is an element, which is extensively used and is one of the most widespread metals in the environment largely due to human activities or anthropogenic origin (El Nemr et al., 2007). Other sources of Pb in the environment include smelting and refining of lead, burning of Pb based petroleum fuels containing lead additives (Soliman et al., 2015). Cadmium is considered one of the most toxic metals in the environment with a wide range of organ toxicity and long elimination half-life (Patrick, 2003). Cd concentrations were detected in rainfall water samples of El Negaila and Matrouh. These findings are in line with El-Sayed et al. (2010) who found that Pb and Cd are with high concentrations in some cisterns water in the northern part of the Egyptian Western Desert. In the study area, grazing sheep exposed to different sources of Cd like industrial activities or intensive agricultural practices contributing to environmental contamination. Moreover, the concentrations of Cu, Zn, and Fe from all surveyed areas were below Egyptian drinking water quality standards 2 ppm, 3 ppm, and 0.3 ppm, respectively (EWQS, 2005). Another interesting result of our study, the concentration of Se in the study area showed a wide variation in the contents. The highest concentration of Se in Control (tap water) followed by El Negaila and Matrouh (rainfall water). These recorded values were higher than the permissible limit value of 0.01 ppm according to EWQS (2005). These findings could be explained by the fact that selenium can be reached to soils or an aquatic environment through weathering of rocks, aeolian deposition, fluvial input, suspension load and biological effect (El-Badry and Khalifa, 2017). Recently, high levels of selenium are steadily found in groundwater and drinking water around the world (Golubkina et al., 2018).

Lead and Cadmium are toxic elements that present as an indicator of environmental pollution. In the present study, according to the obtained results that are listed in Table (2), the greatest Pb value was observed in serum samples of the sheep raised in El Negaila (RW). As compared to the values of the studies carried out at Pb-contaminated sites, The present value was greater than the values of Liu (2003) reported serum Pb value of the control group as 0.05 ppm, and Vıcıl et al. (2012) reported the same values as between 0.05 – 0.06 ppm. The present value corresponds to subclinical Pb poisoning (0.06 – 0.35 ppm; Ma (2011)). Similar with the present finding, Swarup et al. (2006) recorded high blood levels of 0.316 ± 0.040 µg/ml in goats reared around Pb-Zn smelter and Smith et al. (2010) detected blood Pb concentrations of 147 µg/l in sheep grazing metal-contaminated sites. The present finding revealed that sheep grazing in El Negaila is possibly exposing to extremely high levels of Pb in soils, plants, and water (Higueras et al., 2012). This was also substantiated by finding that lead concentration in a water sample collected from El Negaila (RW) was 0.25 ppm. This might have resulted in increased levels of toxic heavy metals in the animal body through the ingestion of contaminated water. This could explain the elevated blood Pb levels detected here. Meanwhile, no external signs of apparent toxicity were observed in all corresponding sheep. Indeed, clinical signs are not always correlated to blood Pb concentrations (Waldner et al., 2002). Furthermore, serum Cd values were significantly higher in examined sheep of El Negaila (RW) group as compared with M. Matrouh (RW). The present values were significantly greater than the value of Liu (2003) reported for healthy sheep (20 µg/l). A similar study was also conducted by Gowda et al.

(2003) showed comparatively higher Cd (0.065 ppm) levels in the blood plasma of dairy animals. Based on our data, elevation of serum Cd could be due to high concentration of Cd in water so blood concentration of Cd acts as an indicator for reflecting their environmental existence, this is in conformity with (Liu et al., 2001).

Serum mineral concentration Cu, Zn, Fe, and Se in the control group were within published reference ranges recommended by Herdt and Hoff (2011) for domestic sheep (Table 2). Copper is an essential trace element that presents a variety of functions in animal organisms. it plays a key role in enzyme function, maturation of red blood cell, collagen synthesis and immune response (Suttle, 2010). Regarding, serum Cu levels, presented in Table (2) highly significant ($P < 0.001$) decreased in the serum of Matrouh (RW) and El Negaila (RW) as compared with the control group. These findings suggest that serum Cu values in these groups ranged from deficient to marginal level as indicated by Herdt and Hoff (2011). Copper deficiency was found to be an important mineral limitation for grazing cattle in tropical regions (McDowell, 1997). Moreover, secondary deficiencies of Cu could be due to interference with other minerals provided in excess, such as Cd, Zn, Fe, S or Mo in the diet (Sefdeen, 2017; Hill and Shannon, 2019). Furthermore, Cu deficiency could be attributed to competitive or antagonistic inhibition by a high level of Cd through disturbs Cu metabolism by reduced plasma ceruloplasmin concentration (Blanco-Penedo et al., 2006). In the present study, similar mechanisms might be responsible for the lowered copper concentration in blood of examined sheep from Matrouh and El Negaila with high cadmium levels.

Zinc is an essential trace element for animals, being involved in protein synthesis and as a constituent of many metalloenzymes (Underwood and Suttle, 1999). Our findings demonstrated that a significant decrease ($P < 0.001$) in the serum Zn values of Matrouh (RW) and El Negaila (RW) versus the value observed in control ones. These results are consistent with the findings of (Bayoumi., 2013; Sellaoui et al., 2016). It is known that zinc absorption is affected by high concentration of Ca, P, Cu, Fe and Cd in the diet, among other things (Goff, 2018). Based on our results, the concentration of zinc could be affected by a high level of Pb and/or Cd. This is attributed to the interaction between Cd and Zn as they belong to the same chemical group, occur as divalent cations in biological environments and bind to the same proteins as albumin in blood stream and metallothionein and other proteins in tissues. Thus, Cd^{+2} interacts with Zn^{+2} ions at the stage of absorption, distribution, accumulation, and excretion and Cd may displace Zn at a number of biological processes (Brzóska and Moniuszko-Jakoniuk, 2001). Mahmoud (2016) found a negative correlation between blood cadmium and zinc levels.

Iron is the most essential element for both humans and animals as well. The main portion of Fe is bound to hemoglobin but Fe is also essential in the prosthetic groups of numerous enzymes and regulatory proteins (NRC, 2007; Kohgo et al., 2008; Zhang, 2014). The mean serum Fe concentration in examined sheep significantly lower than the control group but within reference range recommended by Herdt and Hoff (2011).

Selenium is a key component of several selenoproteins and selenoenzymes with vital biological functions such as antioxidant activity, anti-inflammatory, antagonistic roles and anticarcinogenic effects (Hosnedlova et al., 2017). In the current study, the mean serum selenium concentrations in examined sheep were higher ($P < 0.001$) than the control. The present results suggested that mean Se concentrations of examined sheep from surveyed areas were above the critical concentration of 0.5 ppm as suggested by Radostits et al. (2007). The higher Se concentrations in examined sheep most probably because of the elevated use of selenium supplementation. Naturally, the strongest factor influencing blood Se concentration in sheep was found to be Se supplementation (Ademi et al., 2017). This observation was in accordance with previous studies, Perry et al. (1976) suggested that tissue concentration of Se increased with increase in intake of Se by the ruminants. Moreover, Cristaldi et al. (2005) reported that the dietary intake of Se was reflected in the increasing concentrations of Se in serum ($P < 0.001$). Ademi et al. (2017) indicated that selenium-supplemented sheep had significantly higher Se concentration in blood than non-supplemented sheep ($P < 0.01$). Also, Morsy et al. (2019) reported a highly significant ($P < 0.001$) increase in Se level in Barki sheep reared in El Hammam city above the recommended level. Furthermore, there is individual variability to selenium tolerance in examined sheep which been reported by other authors under more control conditions (Pavlata et al., 2011; Hall et al., 2012; Faixová et al., 2016; Saeed et al., 2019). Ruminants are better adapted to a diet containing excessive selenium than are monogastric, as the ruminal microbiota metabolizes selenium into a less absorbable form which is excreted in the feces (Colegate and Dorling, 1994). The level of blood Se appeared to be a good indicator of dietary selenium status (Mudgal et al., 2018). Selenium toxicosis can either be acute or chronic, depending on the dosage and the period of exposure. Most chronic forms of the disease are due to high levels of selenium in the diet (Gupta and Gupta, 2000; Raisbeck, 2000; Tokar et al., 2012).

5. Conclusions

The present study provides crucial information on the level of metals in water used for drinking livestock, showing that Pb, Cd, and Se concentrations were above MPL recommended by EWQS, this may have a negative impact on animal health in these areas. Rainfall water cisterns must be improved as soon as possible with constant monitoring programs for water quality as the present data showed a degree of pollution. Furthermore, serum analysis revealed high levels of Pb, Cd and Se detected however deficient to marginal levels of Zn and Cu in serum of examined sheep from surveyed localities. The results could be used to balance and improve the animal diet and thus meliorate growth and production. Moreover, selenium should be used carefully as a dietary supplement.

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