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HEAVY METALS CONTAMINATION OF SHEEP'S MILK: A REVIEW

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Abstract

Contamination with heavy metals and metalloids represents a danger to animal and human health. The most important sources of contamination are attributed to water and soil due to their potential for propagation in the trophic system. The process of contamination with heavy metals is based on a water-soil-plants-animals-human circuit, which is why it is particularly important to carefully monitor all influencing factors. Their presence even in small quantities affects the processes of cellular homeostasis of organisms with direct implications on renal and hepatic functions. The purpose of this article was to present the toxicity of heavy metals in sheep's milk focused on the sources of contamination, the possibilities of metabolism as well as the influence of age and lactation on the accumulation of heavy metals. In order to ensure an optimal level of security, it is recommended to perform periodic analyses of soil, water, feed and carefully monitor the management processes of dairy products.

Key words*: contamination, dairy products, heavy metals, milk quality, toxicity.*

INTRODUCTION

Heavy metals (cadmium, mercury, arsenic, lead, chromium) enter the soil cycle through various anthropogenic activities or natural processes and lead to toxicities of organisms and human beings (Li et al., 2019). Heavy metals have a density 5x higher than water (Kamran et al., 2013). In wastewater there are significant amounts of heavy metals that cannot be removed, subsequently penetrate the soil and interfere with plant roots being consumed by animals/humans and finally are incorporated into the food chain (Kamran et al., 2013). Borys et al. (2006) divide heavy metals into two categories: non-essential elements: cadmium and lead and essential elements: copper and zinc. Heavy metals are divided into two categories: toxic metals (Pb, Cd, As, etc.) and essential metals (Cu, Zn, Mn, Fe, Ni, Cr, etc.) and depending on their origin they are divided into natural and anthropogenic sources (Abdel-Rahman, 2021). High amounts of heavy metals have been observed in industrial areas compared to unpolluted areas (Zhou et al., 2019). The concentration of toxic elements in milk and dairy products is influenced by manufacturing processes and environmental conditions (Anastasio et al., 2006). The mineral composition of milk is correlated with the nutritional status of the animal, the stage of lactation and climate, genetic and environmental factors, reproduction as well as the handling, transport and processing stages after milking (Vahčić et al., 2010; Zamberlin et al., 2012). Even in small quantities, it causes health diseases in humans and animals, and exposure to heavy metals develops various types of cancer, affects the lungs and liver, and bones become more fragile (Vardhan et al., 2019; Singh et al., 2022). The assimilation of these toxic elements takes place in the liver and kidneys of animals. This result leads to the introduction of the risk of heavy metal transmission into the food chain (Wilkinson et al., 2003).

MATERIALS AND METHODS

To present a database for this study, we conducted a systematic search of scientific publications in the Google Scholar, ScienceDirect, Scopus and PubMed databases. The main keywords used were: heavy metals, influencing factors, sheep's milk, contamination, accumulation of heavy metals, presence of heavy metals in dairy products, possibilities of metabolism.

PRESENCE OF HEAVY METALS IN THE ORGANISM. PATHWAYS OF ENTRY INTO THE ORGANISM. ASSIMILATION, METABOLISM AND TOXICITY

The mechanism of entry of heavy metals into the human body is through inhalation, the gastrointestinal tract or through the skin. They disrupt cellular homeostasis by damaging lipids, enzymes, proteins, DNA membranes, oxidation of amino acid chains and oxidative stress due to the production of free radicals (Witkowska et al., 2021; Jan et al., 2015; Balali-Mood et al., 2021). Pb poisoning generates infertility and neurotoxicity, Hg has harmful effects on nursing mothers and As causes skin diseases and cancer (Kumar et al., 2018). The first target organ of heavy metal toxicity is the kidney, and their effect is dependent on the exposure time, dose and type of heavy metals (Lentini et al., 2017; Budi et al., 2022). The accumulation of heavy metals in the animal body in relatively small percentages (0.09% Cd, 0.06% Hg and 0.03% Pb) increases liver and kidney tissue concentrations by 5 to 20 times (Rehman et al., 2018). In the case of sheep, iron accumulates in the liver and cadmium in the kidneys and liver (Phillips & Tudoreanu, 2011). Another study highlighted the positive relationship between the level of cadmium analyzed in pastures and its presence in sheep milk, the presence of lead was identified in the kidneys, with higher amounts in autumn than in spring (Węglarzy K., 2010). Heavy metal toxicity can be combated by including the intake of minerals, antioxidants and phytochemicals (Kumar et al., 2018).

FACTORS THAT INFLUENCE THE PRESENCE OF HEAVY METALS IN WATER, SOIL AND FEED

Water is an important source of contamination with metalloids and heavy metals through irrigation with wastewater, application of fertilizer to the field or manure, as well as pesticides that disrupt the normal functioning of the human body (Sankhla et al., 2016). The highest values of the health coefficient were identified in agricultural farms irrigated with wastewater and the lowest values were found in water from tube wells (Iqbal et al., 2020). The main parameters that influence the chemical composition and digestibility of cultivated forages are: light, drought, temperature and nutrients in the soil, for example under the influence of low temperatures and low moisture stress are associated with a higher yield of qualitatively superior forages than high temperatures (Martiniello & Teixeira da Silva, 2011). In the case of pastures, the most significant changes in phosphorus, calcium, magnesium, nitrogen and soil organic matter were observed at depths of 0-15 cm and 15-30 cm, and with the decrease in water content from 40 to 10%, calcium, magnesium and potassium concentrations increased (Egeru et al., 2019). Grazing intensity (heavy, medium, light) influences changes in available water retention at intervals of 0-5 cm, soil nitrogen content at the interval of 30-45 cm, soil pH between 5-15 cm and electrical conductivity at all intervals except for the interval of 0-5 cm. Sheep grazing on vegetation contaminated with heavy metals favors the ingestion of Pb from the soil (Smith et al., 2010; Smith et al., 2009). The mineral composition of alfalfa is defined by 3 components (selenium, copper and zinc), the variability of grasses is formed by 4 elements (zinc, potassium, selenium and phosphorus) and silage corn is rich in iron, potassium and manganese concentrations (Marijanusic et al., 2017). The lowest levels of heavy metals were identified in alfalfa (*Medicago sativa* L.), hay, corn (*Zea mays* L.), cereals, silage, purchased mineral supplements and to a lesser extent in soy-based concentrates (Li et al., 2005). The inclusion of multiple plant species improves the qualitative and nutritional value of forage compared to forage monocultures (Bainard et al., 2020). The key to protecting soil health and nutrients is maintaining biodiversity because forage species have different nutrient concentrations and rooting patterns that influence soil biology and soil-plant-animal interactions (Crotty et al., 2015).

HEAVY METALS CONTAMINATION IN DAIRY PRODUCTS

In milk and dairy products, mineral elements are found in different forms: components of organic molecules (proteins, fats, carbohydrates and nucleic acids) or inorganic salts and ions, and the absorption and use of these elements in the body depends on their chemical form (Šimun et al., 2012). Heavy metal contamination can also occur from metals used in food processing or in the materials with which food products are packaged (Deshwal & Panjagari, 2020). Suhaj & Koreňovská (2008) demonstrated a positive correlation between elemental markers of grazing soil and milk, dairy products from sheep's milk.

The predominant element in the samples analyzed from raw milk, curd, whey, cheese, as well as other cheese varieties before and after salting was Al, followed by Cd and Pb (Toman et al., 2023).

The amounts of Pb, Cd, As and Se in dairy products presented the following values: 0.054 mg/kg milk powder, 0.009 mg/kg whey powder, 1,100 mg/kg and 1,051 mg/kg in cheese assortments and the highest values were identified for Pb.

Mansour et al. (2019) validates the same conclusion obtained in the study conducted, respectively, among the heavy metals monitored in milk and dairy products, lead was the most frequently detected element in a proportion of 66.7%.

It was found that among the species studied (goats, sheep, cows and buffaloes) higher values of Pb and Cd were found in sheep milk (Rahimi, 2013; Najarnezhad et al., 2015).

Regarding the presence of heavy metals in finished products, it has been observed that in curd, soft, brined, smoked and matured cheeses higher values of calcium, magnesium, phosphorus, chromium, zinc, iron and copper are identified (Borys et al., 2006). In cheeses, the elements that influence Pb and Cd concentrations are caused by the manufacturing process, maturation time, fat content and texture (Hashami et al., 2022).

The study conducted by Park (2006) highlights the highest percentage of zinc (415 mg/100 g) compared to the other species. The content of microminerals in sheep's milk is notable for Fe, Mg, Cu and in cow's milk for Zn and Ba. Potassium is the predominant element in cow's milk while Ca is higher in sheep's milk (Spiteri & Attard, 2017).

Higher amounts of Cu are found in cow's milk than in sheep's milk, while the proportion of Zn is higher in goat's milk than in sheep's milk (Gougoulias et al., 2014).

Table 1 shows the average values of heavy metals from various sources of contamination.

Table 1. Heavy metal content from different sources of contamination

Element	Soil (mg/kg)	Water (µg/L)	Feed (silage)(mg/kg)	Milk (µg/L)	Reference
Pb	17.57	0.07	1.38	0.03-10.46	Zhou et al. (2019)
As	7.91	3.58	0.36	0.004-1.53	Zhou et al. (2019)
Cr	39.93	1.80	1.57	0.02-5.01	Zhou et al. (2019)
Cd	0.16	2.04	0.05	0.01-0.27	Zhou et al. (2019)

According to the study conducted by Zhou et al. (2019), the elements with the greatest capacity to transmit heavy metals in soil and feed are lead and chromium, and in water analyses, arsenic illustrates the most significant values, and lead also highlights the highest share in milk.

INFLUENCE OF THE AREA OF ORIGIN OF THE ENVIRONMENT

Organic matter, soil pH and traffic emissions have a direct impact on the accumulation of heavy metals in urban soils (Wang & Zhang, 2018). Cd and Pb levels are correlated with the traffic area considered, so in the case of road sections with a high degree of traffic the concentration of these heavy metals increases predominantly and decreases with distance from high traffic areas (Yan et al., 2013). The following table shows the permissible limits of heavy metals in soil according to the relevant legislation (Table 2).

Table 2. Concentration [mg/kg d.w.] of metals in soil according to Romanian and European legislation

Elements		Mn	Fe	Ni	Cu	Zn	As	Cd	Pb
Ord. 756/1997	Normal value	900.00	3,000.00	20.00	20.00	100.00	5.0	1.00	20.00
	Maximum threshold	2,000.00	4,500.00	200.00	250.00	700.00	25.00	5.00	250.00
	Intervention threshold	4,000.00	7,000.00	500.00	500.00	1,500.00	50.00	10.00	1,000.00
European average value*		650.00	35,100.00	37.30	13.00	52.00	7.03	0.145	22.60

*Geochemical Atlas of Europe cited by Stirbescu et al. (2019)

The table below presents the average values of heavy metals from different locations (Table 3). The most representative values for Cu and Cd are in the study conducted by Levei et al., (2009) while the degree of lead toxicity was the lowest in Iași (Apostoae & Iancu, 2009). The Ni concentration obtained by Apostoae & Iancu

(2009) was lower than that identified by Chira et al. (2014).

Table 3. Heavy metal concentrations in different locations (mg kg^{-1})

Location	Cd	Cu	Cr	Ni	Pb	Zn	Reference
Baia Mare	1.9-25.4	78.3-962	-	-	87.7-9880	109-11500	Levei et al. (2009)
Zlatna	<330	41.0–12000	-	-	32.0–7860	89.0–15600	Levei et al. (2009)
Sibiu	-	29.6–150	-	-	28.6–761	-	Levei et al. (2009)
Baia Mare	2.7	289.5		65.3	847.9	516.6	Chira et al. (2014)
Iași	0.49	45.36	9.28	38.13	27.73	114.78	Apostoae & Iancu (2009)

HEAVY METALS CONTENT BASED ON PERIOD

Póti et al. (2012) investigated how the vegetation period (April-June) influences heavy metal concentrations. It was found that the Cd level increased with the vegetation period while the Cr concentration gradually decreased, the lowest Pb level was identified in April and the highest in May, followed by a decrease. According to the regulations (2005/87/EC) the maximum concentration of heavy metals in green fodder is 30 mg/kg, 1 mg/kg for Cd and 10 mg/kg for Pb at a content of 12%. A positive correlation of Pb was observed between milk and silage, and As in milk was positively correlated with As in water (Zhou et al., 2019). This indicates that heavy metals are transmitted from the source to the raw material, milk. A positive correlation was found between soil and feed in winter and summer seasons for As (Khan et al., 2021). Tahir et al. (2017) demonstrated that the level of heavy metals during a year varies depending on the season and factors such as soil, feed, milk. Thus, the following results were obtained:

Table 4. Heavy metal concentration over a year

Element	Maximum concentration	Minimum concentration
Ni	July-September	January-March
Co	July-September	October-December
Cr	July-September	October-December
Pb	July-September	January-March
Cd	July-September	October-December

Based on the results obtained by Tahir et al. (2017) it can be concluded that the highest values of heavy metals were recorded during the summer, this is due to soil erosion and the supply of contaminated water near the road that reaches the animals' bodies, and is subsequently transmitted to the milk for consumption. Khan et al. (2021) obtained similar results regarding the higher degree of contamination in the winter season than in the summer season for Cd. During the spring, sheep that grazed had high amounts of Pb, Cu, Hg and low levels of Cd and Zn (Kovacik et al., 2017). The absorption capacity of heavy metals from the soil in the feed is as follows: Zn>Cd>Cu>Pb (Miclean et al., 2019). According to ecotoxicological data, heavy metals or metalloids in soil are based on the following structure Se>Tl>Sb>Cd>V>Hg>Ni>Cu>Cr>As>Ba (Vodyanitskii, 2013).

THE INFLUENCE OF AGE ON THE ASSIMILATION OF HEAVY METALS

The assimilation of heavy metals in milk increases as animals age (Najarnezhad et al., 2015) and the toxicity of heavy metals is significantly higher in adult animals than in young animals (García et al., 2011; Rautio et al., 2010). Animals aged <3 years had lower concentrations of Cd (1.40 ± 1.05 mg/ml) and Pb (7.91 ± 3.60 mg/ml) than animals aged >3 years with values of 2.69 ± 1.67 mg/ml for Cd and 11.8 ± 5.71 mg/ml for Pb (Rahimi, 2013). Animals aged 9-10 years had the highest concentrations of cadmium compared to other age groups (Cui et al., 2013). Adult females have a greater capacity to accumulate heavy metals, especially Zn, while in males the predominant metals are Cd (kidneys) and Pb (liver, muscle and kidneys) (García et al., 2011).

THE INFLUENCE OF LACTATION ON THE ASSIMILATION OF HEAVY METALS

In the early and middle stages of lactation, a decrease in the elements Ca, Mg and Zn was observed, as well as an increase detected in the middle and end of the lactation period. At the same time, in the last stage of lactation, the concentration of Se and Fe increased significantly (Pšenková & Toman, 2020). In stage I of late lactation, the element with the most significant values is Cr, which almost doubled its level. The concentration of Pb shows the highest levels in early and late lactation, respectively average values in mid-lactation. Antunović et al. (2005) demonstrated that the elements Pb and Cd recorded the lowest values on the 2nd day of lactation, followed by a gradual decrease during the experimental period (10, 30 and 60 days) while As had a low concentration at the beginning and at the end of the 60 days it increased significantly. In Lacaune sheep, the content of heavy metals influences the milk profile in lactation as follows: in late lactation, the elements Co, Mn, Mo, Cr and Pb increase and the level of Zn, Cu decreases, respectively in mid-lactation, Cd increases and the concentration of Fe decreases slightly compared to the levels in early and late lactation (Antunović et al., 2023). Several authors have concluded that from the beginning of lactation to the last phase, the level of Zn is in a decreasing trend with values ranging between 8.9 mg/kg of milk at the beginning of lactation and 3.1 mg/kg of milk at the end of lactation (Antunović et al., 2020; Antunović et al., 2023, Pšenkova & Toman 2020). It has been observed that during lactation, the concentration of K decreases and the levels of Mg, Na, Se, Mn, Mo and Cd increase (Antunović, 2016).

CONCLUSIONS

To improve soil-plant-animal interaction, it is proposed to maintain the health of biodiversity, the use of more cultivated species to improve the nutritional characteristics of the feed consumed by animals. Heavy metal toxicity can be combated by including the intake of minerals, antioxidants and phytochemicals. Another way to prevent heavy metal contamination is to pay increased attention to the processing, transport and manufacturing processes. Periodic sampling by responsible organizations is recommended to ensure high security.

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