Caspian Journal of Environmental Sciences
Online ISSN: 1735-3866
Print ISSN: 1735-3033

Risk exposure of dairy cows to environmental contamination of brick kilns on milk content of antioxidants and heavy metals in Al-Nahrawan region, Iraq

Maha A. Razooqi

Department of Veterinary Public Health, College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq

** Corresponding author's E-mail: maha.a@covm.uobaghdad.edu.iq*

ABSTRACT

Long-term exposure to large quantities of toxic gases and heavy metals emitted by brick kilns can affect directly or indirectly on farm lactating animals, which influencing human health through consumption of their dairy products. Therefore, we investigated concentrations of heavy metals (HMs) including cadmium (Cd) and lead (Pb), as well as hydrogen sulfide (H_2S), antioxidants, catalase (CAT), glutathione peroxidase (GPx) and superoxide dismutase (SOD) in addition to malondialdehyde (MDA) in fresh milk. Totally, we selected 180 lactating cows; 140 reared / pastured near the active brick kilns as exposed group, and 40 cows existed at agricultural regions as control group during June-August 2021. The findings of exhibited a significant elevation in Cd, Pb, H_2S and MDA, while decrease in CAT, GPx and SOD levels in exposed group. Among different age groups, there were variable significant values for the examined parameters. In the cases of Cd, Pb and H_2S levels, significant higher values were observed in a group of >8 year-olds in comparison with a group of < 4, while in the cases of CAT, GPx and SOD, significant drops were detected in the over 8-year-old cows than in the 4-8 and < 4. Elevated values of MDA by age increasing were also observed. In conclusion, milk and its derivatives may likely exposed to HMs contamination. To date, available information is fragmentary; therefore, further studies of milky products are essential to monitor an existence of HMs regularly and to provide important information about their HM contents.

Keywords: Cadmium, Lead, Hydrogen sulfide, Catalase, Malondialdehyde, Iraq.
Article type: Research Article.

INTRODUCTION

Brick kilns are the fastest-growing industrial sector in several countries and among the top three sectors, along with vehicle exhaust and re-suspended road dust, contributing to the air pollution that consider as an important serious source of public health impacts as well as climatic changes (Skinder *et al.* 2014; Nasir *et al.* 2021). Different air pollutants (APs) were observed to be emitted as a result of inefficient fuel combustion involving carbon and nitrogen dioxides, and fine particulate matter that a mixture of sulphate, nitrate, black organic carbon (Rasheed *et al.* 2015; David *et al.* 2020), in addition to release large quantities of heavy metals such as lead and cadmium (Ravankhah *et al.* 2017), as well as toxic gases like hydrogen sulfide (H_2S; Shrestha & Thygerson, 2019). In the ecosystem, heavy metals (HMs) can accumulate geographically and biologically (Alizadeh & Mirarab- Razi 2016; Yabanli *et al.* 2016; Janbakhsh *et al.* 2018; Sattari *et al.* 2019a,b,c,d; Chirinos-Peinado & Castro-Bedriñana 2020; Khan *et al.* 2020; Sattari *et al.* 2020a,b,c,d; Forouhar Vajargah *et al.* 2020, 2021). Longer period of exposure for lowered amounts of Cd or Cd compounds can lead to lung impairment, fragile bones, and possible kidney diseases; whereas, the ingestion or inhalation of high levels cause severe damage to the lung, and severely irritates digestive system (Sinha *et al.* 2008; Martin & Griswold 2009). As it used in several products, exposure to high Pb levels could affect many body organs and systems resulting in many disorders such as anemia,

Caspian Journal of Environmental Sciences, Vol. 20 No. 3 pp. 491-502 Received: Nov. 23, 2021 Revised: Jan. 08, 2022 Accepted: March 03, 2022
DOI: 10.22124/CJES.2022.5642

Publisher: University of Guilan, Iran

weakness, decreased performance, damage to the brain and kidney, and probable cancer (Papanikolaou *et al.* 2005; Martin & Griswold 2009). In the case of hydrogen sulfide, a highly toxic colourless gas, though chronic exposure, causes permanent ocular and respiratory irritations and also large quantities can result in respiratory paralysis and death in a few minutes due to the direct respiratory centre dysfunction (Kilburn *et al*. 2010; Lewis & Copley 2015). The releasing of HMs has adverse effect, also, on agricultural soil that may be polluted with yielding of affected crops (Seyyednejad *et al.* 2011; Savci 2012; Sun *et al.* 2017). Biologically, interactions between APs and living tissues have demonstrated to cause disturbances in pro-oxidant and anti-oxidant balances, which result in induction of oxidative stress for maintaining level of reactive oxygen species (ROS) under the toxic thresholds (Jan *et al.* 2015; Fuertes *et al.* 2020). In cells, ROS accumulation can be neutralized through a defence mechanism involving antioxidant compounds such as catalase (CAT), glutathione peroxidase (GPx) and superoxide dismutase (SOD; Fuertes *et al.* 2020; Zhang *et al.* 2020). Oxygen radicals that are not neutralized by the antioxidant defences, react with polyunsaturated fatty acid residue in phospholipids, resulting in the production of reactive aldehydes. The most abundant of these is malondialdehyde (MDA; Romieu *et al.* 2008; Ayala *et al.* 2014). MDA is principally stable product of lipid peroxidation and is recognized to be relevant biomarker for oxidative stress (Gong *et al.* 2013). Worldwide, many studies used, experimentally, laboratory animals for studying the mechanism/s of air pollution-related health effects (Somers 2011; Huang *et al*. 2015; da Silveira *et al*. 2018). However, few studies were aimed to detect the negative impacts of APs on health of field animals in an epidemiologic context. In Iraq, these studies may be hindered by lacking detailed data about exposure and illness outcome. Hence, we performed this study to detect the levels of HMs (cadmium and lead), H_2S, antioxidants (CAT, GPx and SOD) and MDA in milk of cows exposed to APs released by the brick kilns.

MATERIAL AND METHODS

Ethical approval

This study approved by, and performed under the license of the Scientific Committee of the Department of Veterinary Public Health, College of Veterinary Medicine, University of Baghdad (Baghdad, Iraq).

Examined animals

Fresh milk samples were collected during June-August 2021 from a total of 180 adult cows. The examined animals included 140 cows reared or pastured close to the active brick kilns found in Al-Nahrawan region (Baghdad Province, Iraq) and considered as an exposed directly or indirectly group (Fig. 1). Additionally, overall 40 cows reared and pastured in agricultural regions away enough from APs were selected as non-exposed (directly or indirectly) healthy group as control group. The teats of each study animal, in both affected and control groups, were washed firstly with soap and water, dried well with a toilet paper and subjected then for direct milking to obtain an approximately 50 mL fresh milk into a labelled disposable plastic container. All samples were transferred cooled, and divided equally into two parts; one kept frozen and the other was centrifuged (8000 rpm / 5 min). The supernatant under the cream layer was pipetted into 10 mL free-anticoagulant glass tube that subjected to re-centrifugation (12000 rpm / 5 min), and clear supernatants (sera) were pipetted into the labelled 1.5-mL Eppendorf tubes, then kept frozen for further examinations. Age of all study cows was reported to estimate the concentration of each parameter among the different age groups.

Chemical analyses of HMs and H_2S

After preparation of the milk samples, reagents and chemicals, atomic absorption spectroscopy (AAS) method was used for detecting the Cd and Pb concentrations as described by Chirinos-Peinado & Castro-Bedriñana (2020). The wavelengths to quantify Cd and Pb were 228.8 nm and 283.3 nm respectively, with a respective 0.002 and 0.045 mg kg^{-1} detection limits. To generate the calibration curve, standards of 1000 mg kg^{-1} were used. The data from the standard solutions for Cd and Pb were 150 ± 0.05 and 155 ± 0.04 mg kg^{-1}, respectively. The concentration of each element in samples was expressed in mg kg^{-1}. In the case of H_2S, the concentration was measured using the gas-liquid chromatograph equipped with a flame photometric detector as described by Al-Attabi *et al.* (2014). The DMS calibration curve was applied to estimate the H_2S concentration using this equation: $Y = 1.1795x$ where Y represents the square root area and x, the concentration of H_2S. The data from the standard solution was 34.08 μg L^{-1}, and the H_2S concentration in examined milk samples was expressed in μg L^{-1}.

Biological analysis of antioxidants and MDA

Following the manufacturer instructions of Sandwich Enzyme-linked Immunosorbent Assay (ELISA) Kits (ABclonal Science, China) for antioxidants (CAT, SODs, and GPx) and MDA (AssayGenie, Ireland), the milk sera and standards were prepared, diluted, and processed using the provided reagents of each ELISA kit. Optical densities (ODs) of each plate were measured at 450 nm. Finally, the standard curve was plotted as the standard ODs in Y-axis and the respective concentration in X-axis with interpolating the ODs of milk sera to evaluate the concentration of the targeted parameters.

Statistical analysis

The study data was documented, tabled, figured, and analysed statistically using the Microsoft Office Excel (Version 2013) and GraphPad Prism (version 6.0.1). One-Way ANOVA test was applied to detect significant differences between values that expressed as mean ± standard errors (M ± SE) with range (R). Variation between groups was considered significant at $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***) and $p < 0.0001$ (****; Kim 2017).

RESULTS

Among 180 cows, our findings of HMs revealed significant elevations ($p < 0.05$) in the values of Cd, Pb and H_2S in milk obtained from exposed group, compared to that of control group (Tables 1-3).

Table 1. Concentration of Cd (mg kg^{-1}) in milk obtained from exposed (No: 140) and control (No: 40) cows.

Group	Mean	SE	Minimum	Maximum
Exposed	0.0378	0.0007	0.0159	0.0415
Control	0.0161	0.001	0.0069	0.0294
Significance			**	

Table 2. Concentration of Pb (mg kg^{-1}) in exposed (No: 140) and control (No: 40) cows.

Group	Mean	SE	Minimum	Maximum
Exposed	0.93	0.051	0.16	1.37
Control	0.27	0.042	0.09	0.74
Significance			***	

Table 3. Concentration of H_2S (μg L^{-1}) in exposed (No: 140) and control (No: 40) cows.

Group	Mean	SE	Minimum	Maximum
Exposed	67.81	2.71	43.96	95.5
Control	26.58	2.81	11.01	59.4
Significance			**	

In addition, the levels of Cd, Pb and H_2S in cows of exposed group was 234.78%, 344.44% and 257.69%, respectively, higher than those in cows of control group (Fig. 1). In comparison with cows of control group, the findings of exposed cows showed a significant drop ($p < 0.05$) in values of CAT, GPx and SOD, while significant rises ($p < 0.05$) in MDA (Tables 4 - 7).

Table 4. Concentration of CAT (ng mL^{-1}) in exposed (No: 140) and control (No: 40) cows.

Group	Mean	SE	Minimum	Maximum
Exposed	0.84	0.049	0.31	1.39
Control	3.76	0.15	1.49	5.07
Significance			****	

Table 5. Concentration of GPx (ng mL^{-1}) in exposed (No: 140) and control (No: 40) cows.

Group	Mean	SE	Minimum	Maximum
Exposed	3.65	0.19	1.81	6.69
Control	7.67	0.33	2.84	11.4
Significance			**	

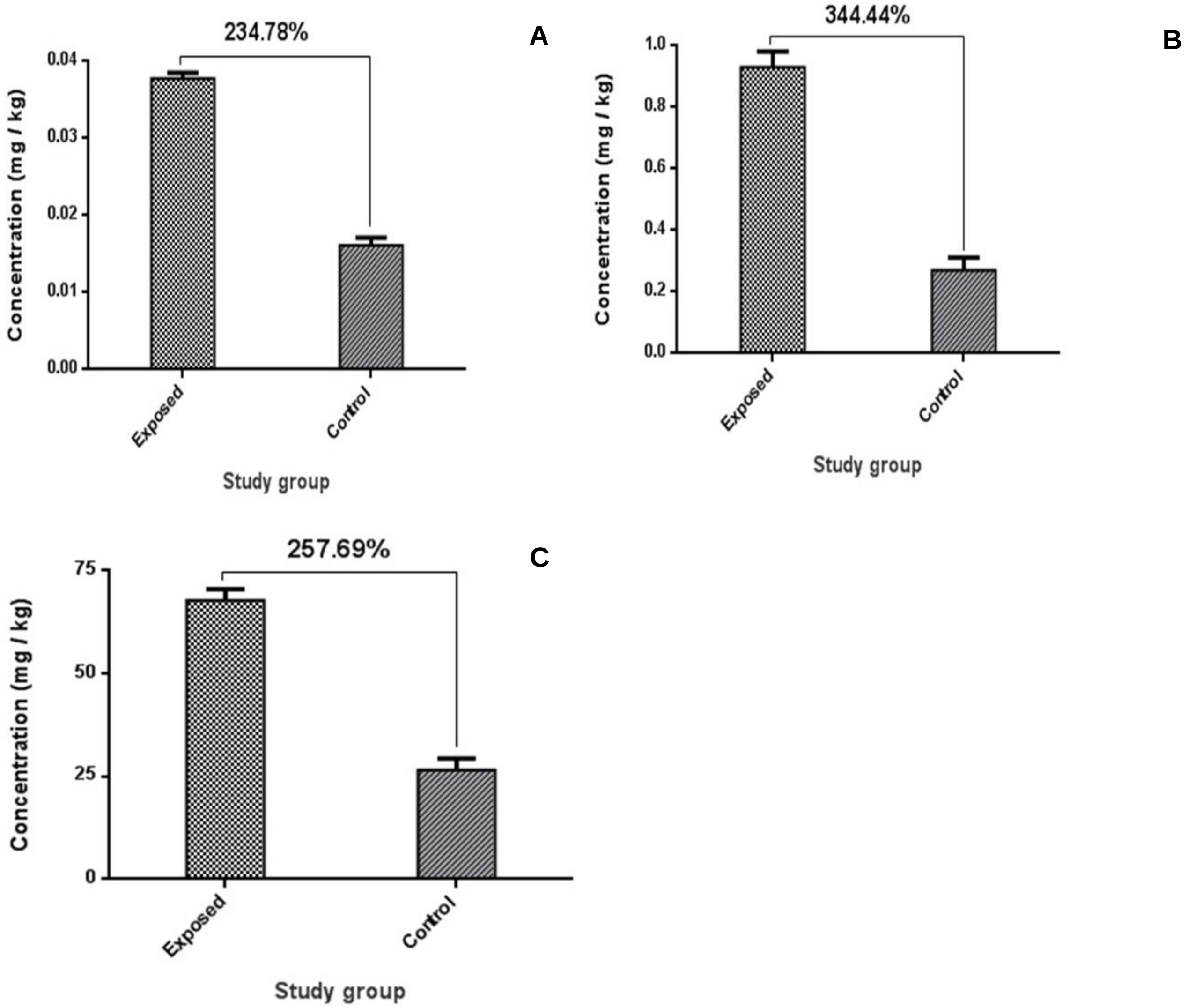


Fig. 1. Levels of Cd (A), Pb (B) and H_2S (C) among cows of exposed and control groups.

Table 6. Concentration of SOD (ng mL^{-1}) in exposed (No: 140) and control (No: 40) cows.

Group	Mean	SE	Minimum	Maximum
Exposed	0.71	0.034	0.25	1.16
Control	4.19	0.11	2.07	5.28
Significance			****	

Table 7. Concentration of MDA (nmoL mL^{-1}) in exposed (No: 140) and control (No: 40) cows.

Group	Mean	SE	Minimum	Maximum
Exposed	3.88	0.21	0.67	7.13
Control	1.16	0.13	0.36	3.14
Significance			***	

Additionally, lowered levels of CAT (447.62%), GPx (210.14%) and SOD (590.14%), and higher MDA (334.48%) were found in cows of exposed group, compared to control (Fig. 2). Relationships between HMs, H_2S, antioxidants and MDA in cows of exposed group and groups of age classes showed a significant variations ($p < 0.05$) in their values (Fig. 3). In the case of Cd, Pb and H_2S, significant higher values (R) were observed in the group of >8 year-olds [0.0328 ± 0.0009 (0.0159-0.0361) mg kg^{-1}, 0.67 ± 0.051 (0.16 -1.13) mg kg^{-1}, and 53.72 ± 2.44 (43.96-79.8)] µg L^{-1} compared to the group of ≤ 4 year-olds [0.0391 ± 0.001 (0.0228-0.0415) mg kg^{-1}, 1.07 ± 0.069 (0.32-1.37) mg kg^{-1}, and 80.29 ± 3.73 (43-95.5) µg L^{-1}] respectively. Additionally, though the findings of Cd and Pb showed insignificant differences ($p > 0.05$) in > 4-8 year-olds and > 8 year-olds, compared to those of 4-8 year-olds [0.0358 ± 0.001 (0.0205-0.0393) mg kg^{-1}, and 0.94 ± 0.051 (0.21-1.15) mg kg^{-1} respectively], however, the findings about H_2S [66.57 ± 3.04 (44.15-91.22) μg L^{-1}] were significantly higher than values of > 4 year-olds and lower than those of > 8 year-olds ($p < 0.05$; Fig. 3).

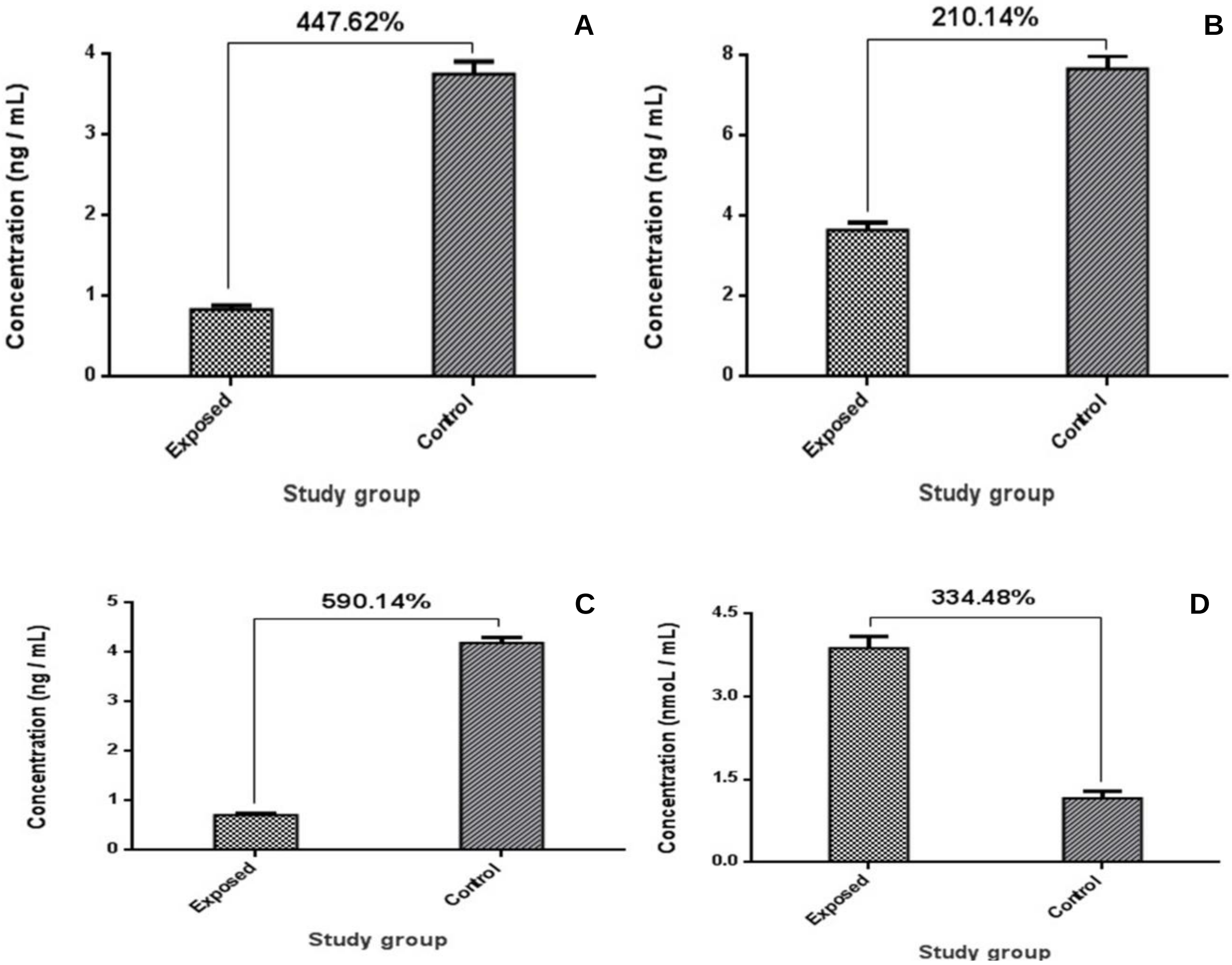
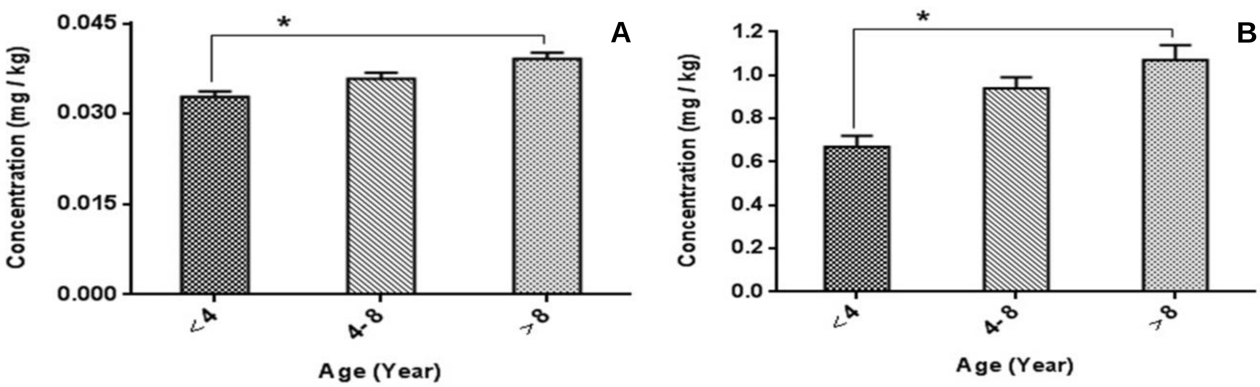


Fig. 2. Level of difference in CAT (A), GPx (B), SOD (C) and MDA (D) concentrations among cows of exposed and control groups.

Concerning the findings of CAT, GPx and SOD, significant decreases ($p < 0.05$) were detected in values (R) of > 8 year-olds [1.08 ± 0.038 (0.86-1.39) ng mL^{-1}, 4.73 ± 0.28 (1.91-6.69) ng mL^{-1}, and 0.9 ± 0.05 (0.29-1.16) ng mL^{-1}] in comparison with those of 4-8 year-olds [0.74 ± 0.037 (0.56 – 0.9), 3.34 ± 0.15 (1.87- 4.0) ng mL^{-1}, and 0.69 ± 0.038 (0.37-1.05) ng mL^{-1}] and < 4 year-olds [0.56 ± 0.05 (0.31-1.04) ng mL^{-1}, 2.48 ± 0.15 (1.81-3.13) ng mL^{-1}, and 0.53 ± 0.043 (0.25-0.96) ng mL^{-1}]. In the case of MDA, the values (R) were increased significantly by aging as 2.32 ± 0.44 (0.91-5.16) nmol mL^{-1}, 3.3 ± 0.31 (0.89-6.02) and 4.59 ± 0.24 (0.67- 7.13) nmol mL^{-1}, for age groups of < 4, 4-8, and > 8 year-olds, respectively (Fig. 4).



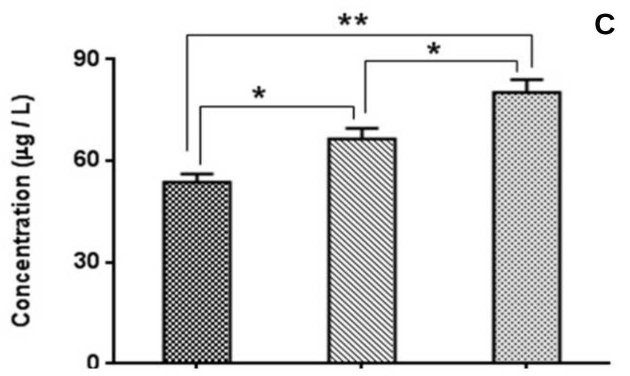


Fig. 3. Concentration of Cd (A), Pb (B), and H_2S (C) in exposed cows among different age groups.

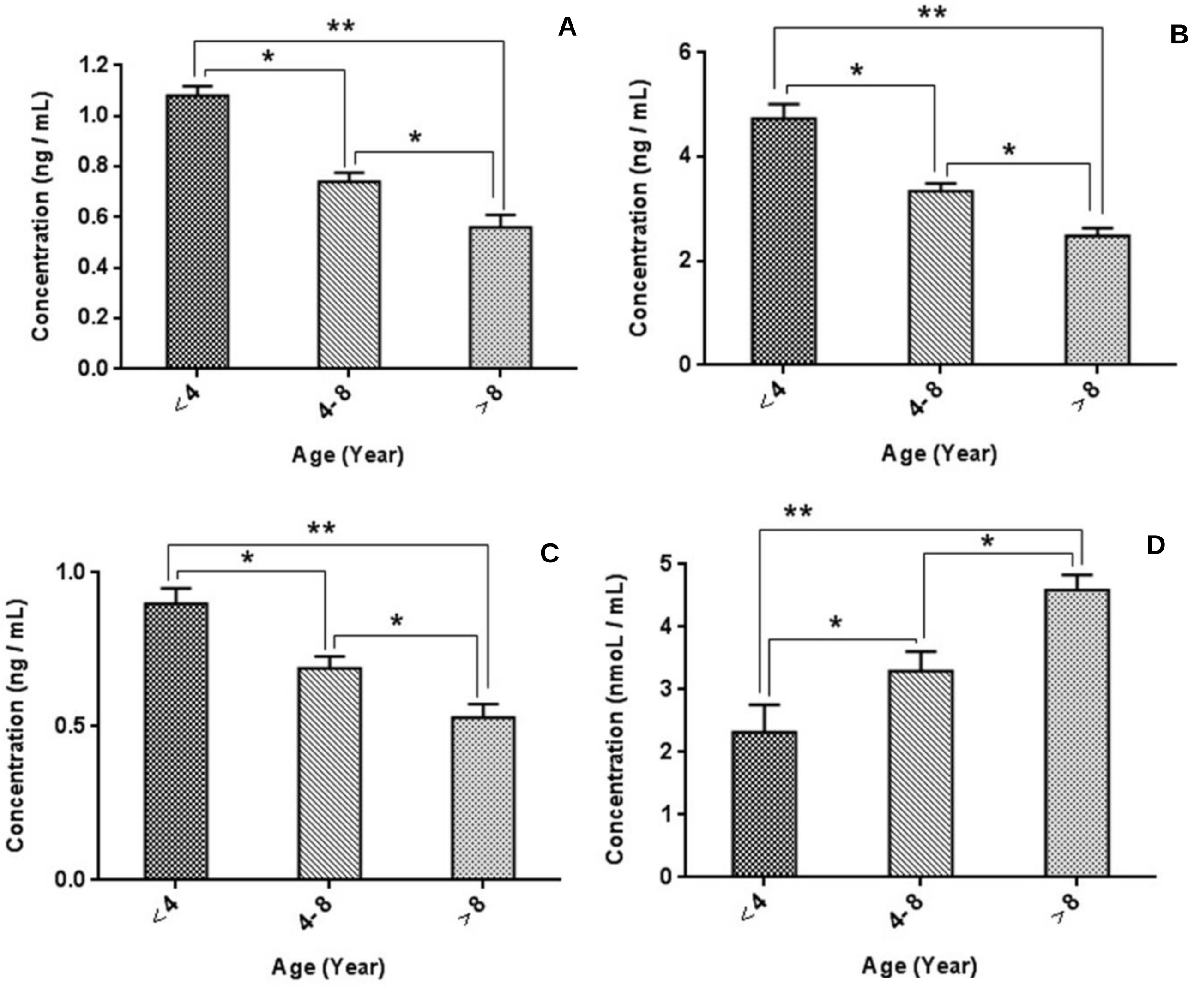


Fig. 4. Concentration of CAT (A), GPx (B), SOD (C), and MDA (D) for exposed cows distributed among different age groups.

DISCUSSION

Milk is considered as nearly as complete food since it is a good source of all basic nutrients required for mammals including human-like protein, fat and major minerals such as calcium, cupper, iron, magnesium, manganese, phosphorus and zinc (Hassan 2005; Enb *et al.* 2009). Increasing industrialization has been accompanied throughout the world by the new distribution of mineral substances from their natural deposit. Emissions from brick kilns comprised toxic fine dust particles and irritant gases, which have deleterious impacts on environment, animals and humans due to persistent nature, and their circulating from soil and water to plants (Achakzai *et al.* 2017; Hussan 2020). Our analysing of fresh raw milk in exposed cows was revealed a significant contamination by Cd, Pb and H_2S and exhibited hazardous effects on human health especially infants and children. However, HMs contents varied widely between cows of exposed group, which might be attributed to differences between breeds, animal exposure, amount of HMs transferred by the polluted soil to plants and grasses, and amount of ingestion from these plants. Globally, Cd and Pb are amongst the elements that have caused the most concern in terms of adverse effects on human health due to their readily transformation through food chains and are not known to serve any essential biological function (Duruibe *et al.* 2007; Mudgal *et al.* 2010). Many studies referred to that significant quantity of HMs can contaminate the plants and grasses causing accumulation of these potentially toxic metals in grazing ruminants, particularly cattle (Kloke *et al.* 1984; Nagajyoti *et al.* 2010; Bilal *et al.* 2018). This accumulation in ruminants causes toxic effects in cattle and also human consuming meat and milk contaminated with toxic metals (Miranda *et al.* 2003; Shen *et al.* 2019). In milk, HM contamination has been reported in different countries such as in Poland (Dobrzanski *et al.* 2005), Iran (Tajkarimi *et al.* 2008), Croatia (Bilandžić *et al.* 2011), France (Maas *et al.* 2011), Italy (Esposito *et al.* 2017), Turkey (Koyuncu & Alwazeer 2019), India (Singh *et al.* 2020), Peru (Chirinos-Peinado & Castro-Bedriñana 2020), and Slovakia (Anetta *et al.* 2021). The higher level of Cd (234.78%), Pb (344.44%) and H_2S (257.69%) in fresh raw milk might be due to high exposure of soil and water to sources of HMs near the hazardous waste sites (Tchounwou *et al.* 2012; Wani *et al.* 2015). In the case of H_2S, the high concentration and level in fresh raw samples might be attributed to emissions of brick kilns inhaled by the respiratory tract of examined cows, or due to its production in rumen as a result of ingestion excess quantities of sulphate (Von Essen & Auvermann 2005; Drewnoski *et al.* 2014). These large quantities can lead to decrease its implications in trace mineral absorption, dry matter intake, and overall cattle growth (DeWitt *et al.* 2021). Our findings showed that there were significant decreases in values and levels of antioxidants; catalase, GPx and SOD, by significant raises in values and levels of MDA, similar to several world studies (Swathy & Ayona 2017; Singh *et al.* 2019; Bhardwaj *et al.* 2021a). Dhaliwal & Chhabra (2016) reported that HMs directly interrupt the activity of enzymes and deactivated antioxidant sulfhydryl pools. Patrick (2006) pointed out that the levels of GPx are decreased due to multifactorial pathogenicity. In the case of SOD, Halliwell & Gutteridge (2015) stated that this enzyme needs to copper and zinc ions for its activity, and that both the metal ions are replaced by Pb which drops activity of SOD. Casalino *et al.* (2002) pointed out that Cd binds to imidazole group of SOD to result in significant reduction in effectiveness of SOD due to the substitution of Cd for manganese. According to a study, the average MDA level in the cattle upraised about three times more than the control, which might explain the effect of HMs on lipid peroxidation in body. However, the level of metabolites in free radical processes, which normally flow in all tissues, is an indicator of the activity of their metabolism. It is ensured by the rapid formation of oxygen active forms by the antioxidants protection system (Slivinska *et al.* 2020). Many studies showed the capability of HMs to accumulate in blood and to stimulate the formation processes of oxide active forms and lipid peroxidation (El-Beltagi & Mohamed 2013; Jaishankar *et al.* 2014; Forcina & Dixon 2019). In general, interaction between HMs usually results in a decrease in toxicity of these metals due to shorter half time or lower concentration in the target organ (Newairy *et al.* 2007; Tomza-Marciniak *et al.* 2011). Imed *et al.* (2008) suggest that some trace elements have a protective mechanism through their ability to form complexes with HMs. This might clarify the reason for different concentrations and levels of HMs and antioxidants among examined animals. In this study, the findings revealed a significant elevation in values of HMs and MDA, while a significant drop in antioxidant levels in cows > 8 year-olds in comparison with > 4-8 and ≤ 4 year-olds. Similarly, Rahimi (2013) reported that Cd and Pb concentrations in milk tend to elevate by age, and that higher levels were detected in cows > 3 year-olds than in ≤ 3 year-olds. A previous study showed that Cd and Pb concentrations were significantly higher in milk of cows ≥ 5 year-olds than in < 5 year-olds (Rubio *et al.* 1998). Anetta *et al.* (2021) suggested that toxicity of metals is closely related to age in addition to sex, route of exposure, level of intake, solubility, metal oxidation state, retention percentage, duration of exposure, frequency of intake, absorption rate and mechanisms / efficiency of excretion. Age-related variation in the HM impacts might be attributed to differences in binding of HMs to body organs, which raise by aging (Turkyilmaz *et al.* 2018), or due to more efficient metallothionein synthesis in particular in females as a result of the elevated HM retentions (Pilarczyk *et al.* 2013; Bhardwaj *et al.* 2021b). Different studies have reported that cows have significantly higher HM concentrations than calves, especially in blood, liver and kidney (Okareh & Oladipo 2015), which may reflex in an elevation in levels of these toxic metals in milk. Alonso *et al.* (2000) summarized that distribution of Cd and Pb in body organs is differed significantly, and that their concentrations in most tissues were significantly higher in cows than in calves.

CONCLUSION

In Iraq, the information about the presence of HMs in fresh raw milk of lactating cows is not available. We concluded that the direct and/or indirect exposure of lactating cows to emissions of brick kilns causes an elevated hazardous concentrations and levels of HMs in fresh milk samples, which can pose a high risk to human health because exposed cows will continue to deliver products legally not fit for human consumption during days or even weeks. Further investigation is required to identify all possible causes of elevated concentration in milk and milk products, and to obtain one identification and monitoring system to prevent inefficient sampling and analysis.

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Bibliographic information of this paper for citing:

Razooqi, M,A 2022, Risk exposure of dairy cows to environmental contamination of brick kilns on milk content of antioxidants and heavy metals in Al-Nahrawan region, Iraq. Caspian Journal of Environmental Sciences, 20: 491-502.