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Faculté des Sciences de la Nature et de la Vie et Sciences de la Terre et de l'Univers
Département d'Ecologie et Génie de l'Environnement
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THÈSE

EN VUE DE L'OBTENTION DU DIPLOME DE DOCTORAT EN 3ème CYCLE

Domaine : Sciences de la Nature et de la Vie Filière : Ecologie-Environnement
Spécialité : Agro Ecologie

Présentée par

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Intitulée

Lait cru de vache: propriétés, niveaux de contaminants émergents et risques potentiels pour la santé humaine

Soutenue le : 14/03/2023 Devant le Jury composé de :

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Faculty of Natural and Life Sciences, Earth and the Universe
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THESIS IN VIEW OF OBTAINING THE 3rd CYCLE DOCTORATE DIPLOMA

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Submitted by

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Raw cow milk: properties, emerging contaminants levels, and potential risks to human health

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PUBLICATIONS

1. “Pesticide residues levels in raw cow’s milk and health risk assessment across the globe: a systematic review”. **<u>Ali BOUDEBBOUZ</u>**, Sofiane BOUDALIA, Meriem Imen BOUSSADIA, Yassine GUEROUI, Safia HABILA, Aissam BOUSBIA, George K. SYMEON. *Environmental Advances*. 2022/07/14/ 2022:100266. https://doi.org/10.1016/j.envadv.2022.100266.
2. “Determination of Heavy Metal Levels and Health Risk Assessment of Raw Cow Milk in Guelma Region, Algeria». **<u>Ali Boudebbouz</u>**, Sofiane Boudalia, Aissam Bousbia, Yassine Gueroui, Meriem Imen Boussadia, Mohamed Lyamine Chelaghmia, Rabah Zebsa, Abed Mohamed Affoune, George K Symeon. *Biological Trace Element Research.* 2022. https://doi.org/10.1007/s12011-022-03308-1.
3. “Challenges and Opportunities of the Mediterranean Indigenous Bovine Populations: Analysis of the Different Production Systems in Algeria, Greece, and Tunisia”. Aziza Mohamed-Brahmi, Dimitrios Tsiokos, Samia Ben Saïd, Sofiane Boudalia, Samir Smeti, Aissam Bousbia, Yassine Gueroui, **<u>Ali Boudebbouz</u>**, Maria Anastasiadou and George K. Symeon. *Sustainability*, 2022, vol. 14, no 6, p. 3356. https://doi.org/10.3390/su14063356
4. “Heavy metals levels in raw cow milk and health risk assessment across the globe: A systematic review”. **<u>Ali Boudebbouz</u>**, Sofiane Boudalia, Aissam Bousbia, Safia Habila, Meriem Imen Boussadia, Yassine Gueroui. *Science of The Total Environment*. 2020, 141830. https://doi.org/10.1016/j.scitotenv.2020.141830.
5. “Characterization of traditional Algerian cheese “Bouhezza” prepared with raw cow, goat and sheep milks”. Sofiane BOUDALIA, **<u>Ali BOUDEBBOUZ</u>**, Yassine GUEROUI, Aissam BOUSBIA, Mhamed BENADA, Choubaila LEKSIR, Zeki BOUKAABENE, Aya SAIHI, Hadjer TOUAIMIA, Abderrahmane AÏT-KADDOUR, Mabrouk CHEMMAM. *Food Sci. Technol.* 40(suppl.2): 528-537. 2020. Epub July 17, 2020. ISSN 1678-457X. https://doi.org/10.1590/fst.35919.
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ABBREVIATIONS

ADI	Acceptable Daily Intake
AI	Artificial insemination
AOPP	Advanced oxidation protein products
ARfD	Acute Reference Dose
As	Arsenic
ATSDR	Agency for Toxic Substances and Disease Registry
CB	Carbamates
Cd	Cadmium
Cu	Copper
Cr	Chromium
DALYs	Disability-Adjusted Life Years
DDT	Dichlorodiphenyltrichloroethanes
EFSA	European Food Safety Authority
EPA	Environmental Protection Agency
FAO	Food and Agriculture Organization
GAP	Good Agricultural Practices
GHG	Greenhouse gas
Hg	Mercury
HCH	Hexachlorocyclohexane
IARC	International Agency for Research on Cancer
IPM	Integrated Pest Management
JECFA	Joint FAO/WHO Expert Committee on Food Additives
JORA	Journal Officiel de la République Algérienne
MDEST	Metal dietary exposure screening tool
MRL	Maximum residue limit
OC	Organochlorine
OP	Organophosphates
Pb	Lead
PTDI	Provisional tolerated daily intake
PY	Pyrethroids
Ni	Nickel
RH	Relative humidity
RDAs	Recommended Daily Allowances
RfD	Reference doses
TDI	Tolerated daily intake
WHO	World Health Organization
Zn	Zinc

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Declaration

The work described in this thesis has not been previously submitted for a degree in Guelma University (Université 8 Mai 1945, Guelma) or any other university, and unless stated otherwise, it is the author’s own work.

Statement of copyright

Résumé

En raison de sa très large consommation et son importance sur le plan alimentation humaine, des efforts ont consenti pour améliorer sa qualité. Dans ce contexte, les travaux de cette thèse ont été réalisés à l'échelle régionale afin d'évaluer les qualités physicochimiques et bactériologiques. De plus, nous avons estimé la variabilité spatiale des concentrations de sept métaux lourds (Pb, Cd, Cr, Zn, Fe, Cu and Ni) dans le lait cru de race bovine locale collecté au niveau de plusieurs communes du nord-est algérien. Le potentiel risque de la consommation de lait pour la santé humaine via une approche théorique (quotient de danger - HQ) a été calculé. De plus, une enquête a été exécutée afin de déterminer le potentiel d'adaptation des races bovines locales à maintenir leur production laitière sous les effets du changement climatique. Au niveau international, l'analyse des taux et l'évaluation du potentiel risque de la consommation du lait pour la santé humaine ont été analysés via des approches mathématiques pour deux familles de contaminants émergents (les métaux lourds et les pesticides).

Au niveau local, l'analyse toxicologique des métaux lourds a révélé que dans l'ensemble des échantillons analysés (N= 88, 100 %), des concentrations supérieures aux limites maximales de résidus (LMR) pour le Pb, Cd et Cu. De plus, 82,95 %, 42,04 %, 15,90 % et 5,68 % des échantillons analysés contiennent des concentrations de Zn, Fe, Cr et Ni qui dépassent leurs LMR, respectivement. Les valeurs du quotient de danger (THQ) suggèrent que les niveaux de Ni, Zn, Cu et Fe ne causaient pas de risque pour la santé des consommateurs. Par ailleurs, les résultats indiquent, qu'il peut y avoir un risque, en particulier de Pb, pour les nourrissons.

L'analyse physico-chimique (N = 122) a montré une qualité du lait acceptable selon le Codex Alimentarius, mais sa qualité bactériologique est très mauvaise. L'analyse de l'historique du rendement laitier et la comparaison avec les données collectées a révélé un rendement laitier par vache très stable. Ce qui suggère que l'exploitation des races locales bovine soit une stratégie intéressante d'adaptation face aux effets du changement climatique. Les programmes de conservation de ces races peuvent favoriser la biodiversité et maintenir un écosystème équilibré. L'éleveur peut bénéficier d'un programme d'amélioration génétique qui peut augmenter la productivité et la rentabilité, cependant la lutte contre toute source de pollution doit être une priorité pour assurer un produit sain pour le consommateur.

Au niveau international, l'analyse des données extraites a révélé que le lait cru de vache présente des niveaux de contamination aux métaux lourds et aux pesticides, spécialement dans les pays en voie de développement (ex. Pakistan, Inde et la Turquie). L'évaluation du potentiel risque pour la santé humaine a montré un risque important pour la santé des consommateurs, en particulier dans les pays en voie développement : Pakistan, la Colombie, Soudan et l'Égypte.

En conclusion, la race bovine locale semble une bonne stratégie pour lutter contre les effets du changement climatique. Néanmoins, il est fortement nécessaire de surveiller les niveaux de contaminations avec les pesticides et les métaux lourds afin d'assurer un aliment sain exempt de tout risque pour les consommateurs.

Mots clés : Lait cru de vache, évaluation de risque pour la santé, changement climatique, analyses physico-chimiques et bactériologiques, race locale, contaminants émergeants.

Abstract

Due to the increasing consumption of milk and dairy products in the human diet, efforts have been made to improve its quality. This thesis was conducted to assess the physicochemical and bacteriological qualities and to determine the spatial variability of seven heavy metals (Pb, Cd, Cr, Zn, Fe, Cu and Ni) content in raw milk of local bovine breed collected at the level of various communes of the Northeast of Algeria. A theoretical hazard quotient (HQ) indicator was used to evaluate the possible risk of milk consumption to human health. Additionally, a survey was conducted to identify the potential of local cattle breeds to maintain their production ability under global warming. At the international level, the assessment of the potential risk of raw cow milk consumption for human health has been studied using mathematical approaches for two families of emerging contaminants (heavy metals and pesticides).

At the national level, the concentrations of Pb, Cd and Cu in all analysed samples (N=88, 100%) of the study area were higher than their corresponding Maximum Residue Levels (MRLs), while 82.95%, 42.04%, 15.90% and 5.68% of Zn, Fe, Cr and Ni samples exceeded their MRLs, respectively. The Task Hazard Quotient (THQ) values suggest that the levels of Ni, Zn, Cu and Fe in the raw cow milk samples were not causing a health risk for consumers. Moreover, the results indicated that there might be a potential risk of toxic metals, especially Pb, for infants via the consumption of raw cow milk.

Physicochemical analysis (N=122) revealed acceptable quality according to Codex Alimentarius but poorly bacteriological quality. The analysis of the milk yield history and the comparison with the collected data revealed a very stable milk yield per cow, suggesting that exploiting local cattle breeds is an interesting adaptation strategy for climate change effects. Conservation programs for these breeds can promote biodiversity and maintain a balanced ecosystem. The breeder can benefit from a genetic improvement program to increase productivity and profitability. However, eliminating any pollution source must be a priority to ensure a healthy product for the consumer.

At the international level, data extracted and then analysed revealed high levels of heavy metal and pesticide residues in raw cow milk, especially in developing countries (e.g. Pakistan, India and Turkey). Moreover, the evaluation of the potential risk for human health revealed a significant human risk, particularly in developing countries such as Pakistan, Colombia, Sudan and Egypt.

In conclusion, the local cattle breed seems to be an interesting strategy to fight against the effects of climate change; however, it is strongly recommended to monitor the contamination levels with pesticides and heavy metals to ensure healthy food free of any risk.

Keywords: Raw cow's milk, health risk assessment, climate change, physicochemical and bacteriological analyses, local breed, emerging contaminants.

ملخص

نظرًا لاستهلاكه الواسع جدًا وأهميته في التغذية البشرية، تم بذل مجهودات لتحسين جودة الحليب. في هذا السياق، أجرت أطروحتنا بحثًا على المستوى الإقليمي من أجل تقييم الخصائص الفيزيائية والكيميائية والبكتريولوجية وتقدير التباين المكاني لتركيزات سبعة معادن ثقيلة (Pb، Cd، Cr، Zn، Fe، Cu وNi) في الحليب الخام لسلالة الأبقار المحلية التي تم جمعها على مستوى عدة بلديات شمال شرق الجزائر. كما تم التعرف على المخاطر المحتملة على صحة الإنسان نتيجة إستهلاك الحليب من خلال حساب النهج النظري لحاصل المخاطر (HQ). بالإضافة إلى ذلك، تم إجراء تحقيق لتحديد إمكانية تكيف سلالات الماشية المحلية للحفاظ على إنتاج الحليب تحت تأثير تغير المناخ.

على المستوى الوطني، تم تحليل نسب وتقييم المخاطر المحتملة لاستهلاك الحليب على صحة الإنسان باستخدام مناهج رياضية لعائلتين من الملوثات الناشئة (المعادن الثقيلة والمبيدات). أما على المستوى المحلي، فقد أظهر التحليل السمي للمعادن الثقيلة أنه في جميع العينات التي تم تحليلها (88عينة، 100٪)، كانت التركيزات أعلى من الحدود القصوى للمخلفات (MRLs) للرصاص والكادميوم والنحاس. بالإضافة إلى ذلك، تحتوي 82.95٪ و 42.0٪ و 15.90٪ و 5.68٪ من العينات التي تم تحليلها على تركيزات من الزنك والحديد والكروم والنيكل والتي تتجاوز حدود مخلفاتها على التوالي. كما تشير قيم حاصل المخاطر (THQ) إلى أن مستويات Ni و Zn و Cu و Fe لا تشكل خطراً على صحة المستهلكين، و تشير النتائج كذلك إلى أنه قد يكون هناك خطر محتمل، خاصة من الرصاص للرضع.

أظهر التحليل الفيزيائي الكيميائي (122 عينة) جودة حليب مقبولة وفقًا لـ Codex Alimentarius ، لكنه ذو جودة بكتريولوجية رديئة جدًا. كما أظهر تحليل مردودية إنتاج الحليب والمقارنة مع البيانات التي تم جمعها أن مردود إنتاج الحليب لكل بقرة مستقر للغاية، هذا يشير إلى أن استغلال سلالات الماشية المحلية هو استراتيجية مثيرة للاهتمام نظرا لتكيفها مع آثار تغير المناخ. يمكن لبرامج المحافظة على هذه السلالات أن تعزز التنوع البيولوجي وتحافظ على نظام بيئي متوازن. يمكن أيضا أن يستفيد المربي من برنامج التحسين الوراثي الذي يمكن أن يزيد الإنتاجية والربحية، ولكن يجب أن تكون مكافحة أي مصدر للتلوث أولوية لضمان منتج صحي للمستهلك.

على الصعيد الدولي، كشفت بياناتنا أن حليب البقر الخام يحتوي على مستويات عالية من المعادن الثقيلة و جد ملوث بالمبيدات، خاصة في البلدان النامية (مثل باكستان، الهند وتركيا). كما كشف تقييم عامل المخاطر المحتملة على صحة الإنسان عن وجود مخاطر كبيرة على صحة المستهلكين، ولا سيما في البلدان النامية: باكستان، كولومبيا، السودان ومصر.

ختاماً، يبدو أن سلالة الماشية المحلية استراتيجية جيدة لمكافحة آثار تغير المناخ، ومع ذلك، فمن الضروري بشدة مراقبة مستويات التلوث بالمبيدات الحشرية والمعادن الثقيلة من أجل ضمان غذاء صحي خالٍ من أي خطر على المستهلكين.

الكلمات المفتاحية: حليب البقر الخام، تقييم المخاطر الصحية، تغير المناخ، التحليل الفيزيائي والكيميائي والبكتريولوجي، السلالات المحلية، الملوثات الناشئة.

Contents

GENERAL INTRODUCTION

Consuming milk and dairy products have been among the most traditional consumption habits throughout history. They are viewed as major sources of nutritious foods, especially for children and the elders, and are essential for growth, bone development, and immune functions in animals and humans because they contain macro- and micronutrients like vitamins and special fatty acids, conjugated linoleic acid with nutraceutical action (Boukria et al., 2020; Claeys et al., 2013; Hansen & Ferrão, 2018; Leksir, Boudalia, Moujahed, & Chemmam, 2019; Malbe, Otstavel, Kodis, & Viitak, 2010). In addition, several randomized controlled trials have shown that eating three or more servings of dairy products per day has positive effects on nutrients, energy intakes, and the need for calcium, magnesium, and vitamin D for adults compared to people who had one or fewer portions of dairy foods per day (Rice, Quann, & Miller, 2013).

In addition to milk's benefits for the human body, global milk consumption per person will increase from 10.6 kg to 13.5 kg in developing nations and from 22.2 kg to 23.1 kg in advanced countries by 2027 (OCDE/FAO, 2018). According to FAO (2013b), daily milk consumption In Algeria is approximately 0.276 kg/ per capita; however, this figure does not appear to be in line with the country's regional average for the consumption of milk and dairy products in rural and pre-urban areas. Based on a study of 750 consumers in Tebessa region (East of Algeria), Bentaleb, Sersar, Bendjama, and Bencharif (2020) found that the daily calcium intake (854.4 ± 364.5 mg/day) equated to daily consumption of 0.733 ± 0.312 kg of milk equivalent. Moreover, the pre-urban agglomeration purchases about 80% of local output for their self-consumption and to supplement breastfeeding. These non-negligible quantities of raw cow milk are obtained in an unregulated (black market) method (Belhadia, Yakhlef, Bourbouze, & Djermoun, 2014). Moreover, milk and its derivatives contain necessary minerals for human body health including copper (Cu), iron (Fe), and zinc (Zn). These elements can play an important role in metabolism and serve a range of biochemical purposes in living organisms and are co-factors in several enzymes. However, they may become harmful to human health if present in excess in animal and human bodies exceeding sanitary guidelines (Gall, Boyd, & Rajakaruna, 2015; Licata et al., 2012; Varol & Sünbül, 2020). Other substances, such as non-essential elements like cadmium (Cd), lead (Pb), and mercury (Hg), can be harmful even at very low doses and have no biological function (Varol & Sünbül, 2020). Additionally, previous studies have shown that antibiotics (such as sulfamethoxazole, chloramphenicol, and trimethoprim), pesticide residues, phthalates, and bisphenol A can contaminate milk and dairy products globally through animal consumption of contaminated water, feed, veterinary drugs, and grass or corn silage, or during transformation (Boudebbouz et al., 2022; Boudebbouz et al., 2021; Bousbia et al., 2019; Fierens, Van Holderbeke, Willems, De Henauw, & Sioen, 2012; Gill et al., 2020; Lu et al., 2021; Santonicola, Ferrante, Murru, Gallo, & Mercogliano, 2019).

Some of these contaminants can interact with the endocrine system and act as endocrine disruptors through non-monotonic dose-response relationships (Auxietre et al., 2014; S. Boudalia, Belloir, Miller, & Canivenc-Lavier, 2017; Sofiane Boudalia, Bousbia, Boumaaza, Oudir, & Canivenc Lavier, 2021). Humans are consequently exposed to various mixes of these pollutants depending on consumption levels, which amplify the consequences, particularly for vulnerable populations like infants and young children. This age category is especially vulnerable to many contaminants due to their high intake of milk and dairy products, their body weight and the immaturity of their defence mechanisms against chemical stressors (Nougadère et al., 2020).

Due to their ability to pass the placental barrier, heavy metals like lead (Pb) and cadmium (Cd) can have neurotoxic effects on developing fetal brains, including intelligence quotient IQ decline, memory loss, and language impairment (Khalil et al., 2009; Payton, Riggs, Spiro, Weiss, & Hu, 1998; Rehman, Fatima, Waheed, & Akash, 2018; Schwartz et al., 2000). Furthermore, the estrogenic activity of cadmium can be harmful to reproductive systems by upsetting the androgen-estrogen ratio and increasing the levels of steroidal hormones, both of which are associated with an increased risk of breast cancer (Johnson et al., 2003; Nagata, Nagao, Shibuya, Kashiki, & Shimizu, 2005). They may result in renal failure, an increase in blood pressure, and a decline in intelligence quotient (Malhat, Hagag, Saber, & Fayz, 2012); teratogenic, carcinogenic, and neurotoxic (Flora & Agrawal, 2017; Zhong et al., 2018); neurologic and immunologic (Ismail et al., 2017); cytotoxicity (Rahmani et al., 2018); Wilson's disease, cramps and nausea (Lawal, Mohammed, & Damisa, 2006).

In the same way, pesticide residues including organochlorine, organophosphorus, carbamate, and synthetic pyrethroid can build up as organic pollutants in fatty base foods like milk and dairy products, which when consumed by humans can lead to cancer and damage to the neurological, immunological, and endocrine systems (Ramezani et al., 2022). They can easily reach the food chain and build up in the fatty texture of both people and animals. Due to excessive toxicity, food quality may be compromised, resulting in a risk to human health, including genetic abnormalities, cancer, congenital impairments, and nervous system illnesses (Mir et al., 2022; Tajdar-oranj, Peivasteh-roudsari, Mahdavi, Keikavousi Behbahan, & Mousavi Khaneghah, 2021).

In addition to this, global warming and climate change can exacerbate these pollution effects on, agriculture, human and animal health. In a two-way process (interaction), climate change is one of the main drivers of ecosystem disruption, but this ecosystem disruption undermines nature's ability to regulate greenhouse gas (GHG) emissions and protect against extreme weather, thus accelerating climate change and increasing vulnerability to it. Regardless to forestry or changes in land use, the agriculture industry produced 12.3 million tons of CO2 equivalent (MtCO2e) GHG emissions in 2012 in Algeria. This amount corresponds to 5.63% of the total emissions (219 MtCO2e) (Climate Watch, 2021; FAO, 1997). More than 83% of all agricultural emissions come from livestock, with enteric fermentation accounting for the majority (approximately 5.5 MtCO2e) and manure left on pastures for the remainder (4.5 MtCO2e) (Climate Watch, 2021). Nearly 10% of the population in Algeria is affected by drought, which is ranked 18^{th} out of 184 nations by Prevention Web (3,763,800 people) (WBG, 2022).

Additionally, some studies have predicted a 5–30% decrease in annual total rainfall (Christensen JH, 2007). Climate change, whichis explained by both increasing temperatures and decreasing precipitation, is dislodging the northern temperate zone in favor of the desert (Zeroual et al., 2020; Zeroual, Assani, Meddi, & Alkama, 2019). Moreover, in the upcoming years, these effects are anticipated to be severe, extensive, and irreversible (IPCC, 2019; Mariotti, Pan, Zeng, & Alessandri, 2015; Zeroual, Assani, & Meddi, 2016) putting risk food and nutrition security, agricultural productivity, and animal production (FAO, 2013a). The level of heat stress that cows suffer depends on a variety of meteorological parameters, including ambient temperature, radiant energy, photoperiod, relative humidity (RH), and wind speed (Hammami, Bormann, M'hamdi, Montaldo, & Gengler, 2013). Milk production decreases as a result of dairy cows using behavioral and physiological strategies to deal with heat stress, such as cutting back on feed and drinking more water to reduce metabolic heat production (Herbut, Angrecka, & Walczak, 2018). As a result, the dairy cow has to be in its thermoneutral zone, a range of temperatures where it can maintain its body temperature without having to waste more energy (Marumo, Lusseau, Speakman, Mackie, & Hambly, 2022). As an illustration, the Holstein dairy cow prefers temperatures between 5°C and 25°C (the lower and higher critical temperatures, respectively) (Kadzere, Murphy, Silanikove, & Maltz, 2002; Marumo et al., 2022). Subsistence farmers in developing countries will feel the consequences of increased heat stress on cows more acutely as climate change and global warming progress(Ekine-Dzivenu et al., 2020; Hernández et al., 2011). Local dairy breeds are typically less susceptible to disease than imported bovine breeds because of their metabolism, which is linked to excessive heat production and the challenges of sustaining isotherm in hot regions(Bernabucci et al., 2014). These indigenous species can also endure challenging weather conditions like high heat, droughts, and a lack of nutrients and water (Sejian et al., 2015). Apart of the threat posed by climate change, the decline in the number of native cattle breeds, and the import of new breeds less resistant to local climatic conditions like high temperatures and humidity, drinking raw cow milk is growing in popularity and may be harmful to people's health. This is encouraged by the notion that heating milk renders it less nutritious and healthful, and may even have adverse effects (Claeys et al., 2013).

CHAPTER 1

SCIENTIFIC CONTEXT AND PROBLEMATIC

1. Emerging contaminants-Climate-driven milk changes

Milk and dairy products are a type of food that is seen as attractive and valued, and they are vital sources of nutrition for people of all ages, especially for children, adults and elderly people (Xin Li et al., 2019). Milk composition differs significantly among species because breast (or Udder) secretion is physiologically and physically tied to the nutritional requirements of new-borns from each species (Birhanu, Mohammed, Kedebe, & Tadesse, 2015; Saha, Malchiodi, Cipolat-Gotet, Bittante, & Gallo, 2017). Several factors have been reported to influence milk composition, such as seasons, lactation phases, diseases, and feedings (Renhe, Perrone, Tavares, Schuck, & de Carvalho, 2019), parity estrus, diurnal and environmental temperature (Park, Albenzio, Sevi, & Haenlein, 2013), and milk structure, which defined as the physical configuration of chemical elements(Lopez, 2011). Table 1 shows the average composition of the elements that appear in the highest concentrations for the three principal commercial species: cow's milk, buffalo's milk, and goat's milk, which underlines the variability among the three species (Renhe et al., 2019).

Table 1: Comparative composition of cow's milk, buffalo's milk, and goat's milk

Composition (g.100 g21)	Cow	Buffalo	Goat
Total dry matter	12.7	17.6	12.5
Lipids	3.7	7.0	3.8
Casein	2.6	3.5	4.7
Whey proteins	0.6	0.8	0.4
Lactose	4.8	5.2	4.1
Ash	0.7	0.8	0.8

Source: Renhe et al. (2019)

Milk is high in macro- and micronutrients such as lipids and proteins (polyunsaturated fatty acids), calcium, phosphorus, essential amino acids, carbohydrates, vitamins, and a variety of important bioactive substances for biochemical and physiological processes. Furthermore, enzymatic (superoxide dismutase, catalase, and glutathione peroxidase) as well as non-enzymatic (lactoferrin, casein, a-LA, b-LG, tryptophan, cysteine, tyrosine, lysine, carotenoids, uric acid, vitamins A, C, and E) antioxidants have also been discovered in the milk of many mammalian species. As a result, milk appears to have health-promoting and functional effects against the generation of reactive oxygen species and oxygen-free radicals, which would otherwise cause oxidative stress (Baniasadi, Azizkhani, Saris, & Tooryan, 2022).

Given its nutritional importance, milk consumption per capita has increased in developed countries from 22.2 kg in 2015 to 23.1 kg in 2017 and from 10.6 kg in 2015 to 13.5 kg in 2017 in developing countries (OCDE/FAO., (2018)). Furthermore, in the last three decades, world milk production has increased by more than 59%, from 580 million tons in 1988 to 843 million tons in 2018 (FAO, 2018). Also, by 2050, the global population is expected to reach 9.7 billion, an increase of around one-third from 2015 (Food and Agricultural Organization, 2018). It is a challenge that involves supplying more food for a growing population and considering health inequalities such as malnutrition and obesity (Henchion, Moloney, Hyland, Zimmermann, & McCarthy, 2021).

In Algeria, FAO (2013b) reported that the per capita milk consumption is about 0.276 kg/day; however, this value does not seem to correspond to the regional value of milk and dairy product consumption in the rural and pre-urban areas. Using a survey of 750 consumers in the Tebessa region (east of Algeria), Bentaleb, Sersar, Bendjama, and Bencharif (2020) reported that the daily calcium intake (854.4±364.5 mg/day) corresponded to daily consumption of 0.733±0.312 kg of milk equivalent. Moreover, Belhadia, Yakhlef, Bourbouze, and Djermoun (2014) reported that a non-negligible quantity of raw cow milk is used to supplement breastfeeding and family self-consumption and is also sold through uncontrolled (informal to the) pre-urban agglomeration (80% of local production). These important quantities were not included in official data on milk consumption in Algeria. Furthermore, it is estimated that informal market traders handle almost 80% of the milk market. Milk produced by dairy cattle farms, especially from extensive livestock, is sold directly to urban markets (Sraïri, Benyoucef, & Kraiem, 2013). Therefore, the safety of raw cow milk must be assured, even more so when 80% of this milk is consumed directly by rural and pre-urban populations (Belhadia et al., 2014).

Milk production in Algeria comes mainly from the intensive livestock system (breeding of imported breeds such as premium Holstein, Montbéliarde, Normande and Charolaise) and the extensive livestock system (local breeds) known as "*Brune de l'Atlas*" (Brown Atlas). They are little animals that have evolved to withstand extreme climate conditions, limited food resources, sickness, and parasites (Ben Jemaa et al., 2018). Other names for Maghreb indigenous cattle have been given to them based on their geographical origin and morphological traits. For example, “Guelmoise (grey)”, “Cheurfa (white)”, “Krouminiène”, “Chelifienne”, “Sétifienne”, Tlemcenienne (tawny), and “Djerba” populations originated in Algeria are highly suited to both hard climate conditions and limited food resources, as well as disease and parasites that are common in northern Algeria’s mountainous and forests environments (Boushaba et al., 2019; Rahal et al., 2021). Indigenous breeds have special features, resistant to severe environmental circumstances and diseases, and are well adapted to the location where they are raised.

The Algerian population has more than doubled from 13.7 in 1970 to 37.1 in 2021 (Mamine, Bourbouze, & Arbouche, 2011). However, their number is considered very low (1.6 million in 2012) compared to other countries, such as Morocco, with 2.8 million cattle (Sraïri et al., 2013). Official policies in the country favour an increase in average milk yield per cow rather than an increase in cattle numbers. One of the most effective strategies used to accomplish this rise in milk yield was a program of crossbreeding local strains with high genetic merit breeds, such as the Holstein, Montbéliarde, and Brown Swiss (Sraïri et al., 2013). As a result, pregnant heifer imports have increased to 387,000 since the early 1960s. In addition to cattle imports, Algerian authorities have initiated artificial insemination (AI) programs employing the semen of high genetic merit dairy cattle (Sra'I'ri & Farit, 2001). As a result of this politic, the genetic structure of the dairy herd in Algeria deteriorated, resulting in a sharp drop in the number of indigenous animals. Consequently, the percentage of indigenous cattle breeds in the population has decreased from 82 per cent in 1986 to around 48 per cent in 2016 (FAO, 2012; Mohamed-Brahmi et al., 2022).

As mentioned previously, Algerian local cattle breed are highly suited to both hard climate conditions and limited food resources, as well as disease and parasites, but the milk yield remains low (1175 litter/cow/year (Mamine et al., 2011)) compared to the imported cattle which vary from 1480 to 6703 litter/cow/year) depending on the temperature in each region (Bouzida, Ghozlane, Allane, Yakhlef, & Abdelguerfi, 2010).

However, it is important to note that the performance of imported breeds is lower in hot environments than in their native environments (Madani & Mouffok, 2008; Nigm, Sadek, Yassien, Ibrahim, & El-Wardani, 2015). It is well established in the literature that when dairy cattle are under heat stress, there is an increase in water intake and a decrease in dry matter, protein and fat content of milk as well as milk yield (Gorniak, Meyer, Südekum, & Dänicke, 2014). The microbiological qualities of milk are also affected because contamination and pathogen proliferation increase under excessive heat and humidity (Montcho et al., 2021), resulting thus in economic loss from dairy farms (Bohmanova, Misztal, & Cole, 2007; Martín-Sosa, Martín, García-Pardo, & Hueso, 2003). On the other hand, local breeds can perform well in adverse climatic conditions like high temperature, drought, feed and water scarcity (Sejian et al., 2015a) because they are more robust and genetically better adapted to their environment (Rodríguez-Bermúdez et al., 2019). Moreover, since the beginning of the 20th century, industrialization, urbanization, agriculture mechanization and intensification have led to increased environmental pollution (such as heavy metals and pesticides), negatively impacting livestock systems and milk quality.

1.1. Climate change effects on milk quality and production

For many decades, persistent challenges to the food system, such as rising greenhouse gas (GHG) emissions and temperature, decrease in precipitation, loss of natural ecosystems, and decreased biodiversity as a result of increased land and freshwater consumption to feed a growing population (IPCC, 2019), lead to the realization that is existing food production and consumption habits are unsustainable (Steenson & Buttriss, 2020). Global (COP 21: United Nations Framework on Climate Change Paris Agreement) and regional (European Union Farm to Fork strategy (European Commission, 2020)) promises to reduce Greenhouse gas (GHG) emissions, improve water quality and biodiversity, combat antibiotic resistance, and improve diets and health. The agriculture sector faces a huge challenge in becoming an important part of the solution (Henchion et al., 2021).

In Algeria, the agriculture sector contributed 12.3 million tons of CO_2 equivalent ($MtCO_2e$) GHG emissions in 2012, ignoring land-use change and forestry. This value represents 5.63 per cent of total emissions (219 $MtCO_2e$) (Climate Watch, 2021; FAO, 1997). Livestock emissions account for more than 83 per cent of overall agricultural emissions, with enteric fermentation accounting for the most (about 5.5 $MtCO_2e$) and manure left in pasture accounting for the rest (4.5 $MtCO_2e$) (Climate Watch, 2021). According to Prevention Web, Algeria is ranked 18th out of 184 countries most vulnerable to drought, affecting almost 10% of the population (3,763,800 people) (WBG, 2022).

Moreover, some studies have suggested a projected decline in total annual rainfall of 15-30% (Christensen JH, 2007), desert climatic expansion at the expense of the northern temperate zone, which is explained both by growing temperature and precipitation decreasing (Zeroual et al., 2020; Zeroual, Assani, Meddi, & Alkama, 2019). Furthermore, these consequences are expected to be "severe, extensive, and permanent" in the coming years (IPCC, 2019; Mariotti, Pan, Zeng, & Alessandri, 2015; Zeroual, Assani, & Meddi, 2016), posing a danger to animal production, agricultural yields, and food and nutrition security (FAO, 2013a).

Meteorological factors such as ambient temperature, radiant energy, photoperiod, relative humidity (RH), and wind speed all play a role in the degree of heat stress experienced by cows (Hammami, Bormann, M'hamdi, Montaldo, & Gengler, 2013). Dairy cows adopt behavioural and physiological methods to cope with heat stress, such as reducing feed intake and drinking more water to minimize metabolic heat production, decreasing milk supply (Herbut, Angrecka, & Walczak, 2018). Therefore, the dairy cow must be in its thermoneutral zone, a temperature range in which it does not have to increase its energy expenditure to maintain a constant internal body temperature (Marumo, Lusseau, Speakman, Mackie, & Hambly, 2022). For example, the Holstein dairy cow prefers a temperature range of 5°C (lower critical temperature) to 25°C (higher critical temperature) (Kadzere, Murphy, Silanikove, & Maltz, 2002; Marumo et al., 2022).

As climate change and global warming grow, the intensity of heat stress effects on cows will aggravate, and this impact will be felt more by subsistence farmers in developing countries (Ekine-Dzivenu et al., 2020; Hernández et al., 2011). Local bovine breeds are generally more resistant to disease than imported dairy breeds, whose metabolism is linked to excessive heat production and the difficulties of maintaining isothermic in hot environments (Bernabucci et al., 2014). These native varieties may also survive in harsh climatic conditions such as high temperatures, drought, and a lack of feed and water (Sejian et al., 2015b). Apart from the risk of climate change, the drop in the number of local cattle breeds and the importation of other breeds less resistant to climatic conditions in the country, such as high temperature and humidity, consumption of raw cow milk could pose a risk to human health. Consumption of raw milk is getting more popular. This is fuelled by the belief that boiling milk loses its nutritional and health benefits and can even have negative consequences (Claeys et al., 2013).

1.2. Emerging contaminants

Since the beginning of the 20th century, industrialization, urbanization, and agriculture mechanization and intensification have increased environmental pollution, negatively affecting livestock systems and milk production (yield and quality). Among these contaminants, we can find heavy metals and pesticides.

It has been discovered that the primary cause of food and feed contamination with emerging contaminants such as pesticides and heavy metals in milk resides in a transfer (external environment - internal environment). Cows can absorb contaminants through water and grass that have been polluted by a variety of sources, including industrial waste that is carelessly discharged into the environment, pesticide residues from agricultural use, water released from metropolitan areas, and in some areas, by natural processes like volcanic activity, where fine particles are released into the air, then transferred to the water and soil (Numa Pompilio, Francisco, Marco Tulio, Sergio Samuel, & Fernanda Elisa, 2021). Hence, the presence of heavy metals and pesticide residue compounds in milk is not only a direct indication of its hygienic condition but also an indirect indication of the environmental contamination where it is produced (González-Montaña, Senís, Gutiérrez, & Prieto, 2012).

1.2.1. Heavy metals

Heavy metals are a group of a term used for metals and semimetals (also known as metalloids) that have been linked to contamination and possible toxicity and ecotoxicity (Duffus, 2002). On the other hand, Heavy metal terminology has diverse definitions based on laws and scientific studies. Before 1936, there was no such thing as a definition (Duffus, 2002). Heavy metals such as cadmium (Cd), lead (Pb), mercury (Hg), nickel (Ni), chromium (Cr), arsenic (As), copper (Cu) and zinc (Zn) can be characterized as components having a particular gravity over 5 g/cm^3, or atomic weights within the range of 63.5–200.6 g/mol (Zhou, 2019). Heavy metals are toxic to humans even at lower concentrations (Gumpu, Sethuraman, Krishnan, & Rayappan, 2015; Tekaya et al., 2013). With the fast advancement of the economy and society, heavy metal contamination has become one of the most widespread environmental issues around the world, with unfavourable effects on the oceanic, terrestrial, and atmospheric environments (Mishra et al., 2019). Copper, nickel, and zinc are required for life in very low quantities (also known as microelements or trace elements) because they play vital roles in metabolic processes in living cells. However, high concentrations of these metals are toxic to most organisms, including animals, plants and humans (Gadd & White, 1993; Kaplan, 2013). Table 3 shows the reliable food sources, stability, associated body systems, functions, deficiency, and toxicity symptoms of these metals.

Other heavy metals, including cadmium, lead, and mercury, are non-essential and have been shown to harm species at extremely low doses. Metals can be found in the environment in various chemical forms (metal speciation), such as ions dissolved in water, vapours, salts or minerals in rocks, sand, and soils. Toxic metals, such as arsenic, cadmium, lead, and mercury, tend to bioaccumulate in critical organs and tissues. Excessive accumulation of these metals can be harmful or lethal to organisms (Gupta Mahendra, Kiran, Amita, & Shikha, 2014; Nagajyoti, Lee, & Sreekanth, 2010).

Heavy metal toxicity has a deleterious influence on humans and animals, as heavy metals are known to cause neurotoxicity, nephrotoxicity, fetotoxicity, and teratogenicity in humans (Mishra et al., 2019). They may induce blood and circulatory system disruptions, alterations in detoxification pathways (colon, kidney, liver, and skin), and gastrointestinal, reproductive, and mental system disorders (Abdulkhaliq, Swaileh, Hussein, & Matani, 2012; Iftikhar, Arif, Siddiqui, & Khattak, 2014). More precisely, it may cause changes in mental and neurological functions, as well as changes in neurotransmitter production and utilization, intellectual and behavioural deficits, hyperactivity, neuro disorders, decreased intelligence quotient, and endocrine disorders, which are all common in infants and young children who are directly exposed to heavy metal contamination (Bischoff, Higgins, Thompson, & Ebel, 2014; Jusko et al., 2008).

Figure 1 highlights the food chain routes through which the population is exposed to metal poisoning. Plant-animal-human and/or soil-plant-human and/or soil-water-animal could be potential metal accumulation food chain pathways in human populations (Purakayastha & Chhonkar, 2010). In developed countries, the concentration of heavy metals in the environment is decreasing because of the strict regulations on the production and usage of various chemicals, whereas, in developing countries, the release of these contaminants is not regulated (Birhanu et al., 2015). Heavy metal is a serious concern in developing countries due to unmanaged pollution in the environment, and it has been found in a variety of foods, including livestock, aquatic animals, processed foods, pistachio, milk and honey (Naseri, Salmani, Zeinali, & Zeinali, 2021; Sobhanardakani, Tayebi, & Hosseini, 2018).

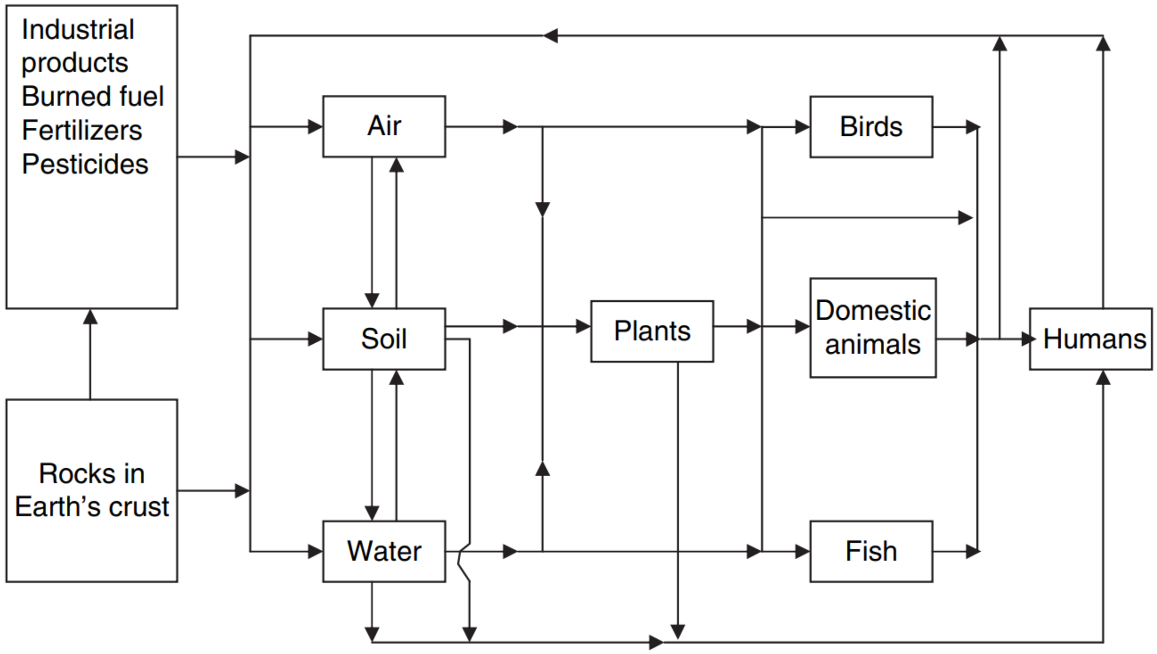

Figure 1: Possible pathways humans may be exposed to trace metals. (Adapted from (Purakayastha & Chhonkar, 2010)

Due to its intake by age groups most susceptible to heavy metal toxicity, milk is under severe regulatory scrutiny. Because of their high toxicity, several regulatory organizations have set permitted limits for heavy metals in different foods, including milk and dairy products. Values from the EPA, the Agency for Toxic Substances and Disease Registry (ATSDR) and the Joint FAO/WHO Expert Committee on Food Additives (JECFA) were used to determine daily maximum acceptable exposure levels for heavy metals. These exposure limits are typically referred to as "Oral Reference Dose," "Provisional Total Daily Intake," or "Minimal Risk Level" by these different agencies. Their goal is the same, regardless of the method used to derive them. All of the model's reference values come from dose-response data that compares exposures to observed effects in humans or laboratory animals (Wang, Su, Gu, Song, & Zhao, 2017).

European Commission (2002) laying down the general guidelines and requirements of food regulation and launching the European Food Safety Authority (EFSA) and procedures in food safety sets out the basis for this harmonization. In the pursuit of community policy, a high level of protection of human life and health should be ensured, and free movement of food and feed within the community can only be realized if food and feed safety criteria do not vary markedly from Member State to Member State.

In its appendix, European Commission (2006) (as modified) establishes maximum limits for various pollutants in foods, including the metals lead, cadmium, mercury/methyl-mercury, and inorganic tin in various foods. The European Commission's typical framework of Expert Working Groups and Committees routinely reviews and adds to the limits in this regulation, which are immediately applicable in all member states (unless temporary derogations are approved) and to all imports. Expert risk assessments are considered to determine the maximum limits in food. The European Food Safety Authority (EFSA) is in charge of this role, while the Joint FAO/WHO Expert Committee on Food Additives (JECFA) of the World Health Organization (WHO) and the Food and Agriculture Organization (FAO) of the United Nations is in charge of Codex Alimentarius Commission (Hargin & Shears, 2013; Udimal et al., 2022).

In addition, default exposure limits for five metals often found in food (e.g., arsenic, cadmium, chromium [III and VI], lead, and mercury) were calculated with a metal dietary exposure screening tool (MDEST) using publicly accessible chronic daily exposure limits such as the tolerated daily intake (TDI), provisional TDI (PTDI), or oral reference doses (RfDs). Background exposure from food and water sources was considered MDEST's default exposure limits, often known as the "MDEST portion." By deducting a high-end background exposure estimate from the relevant TDI or RfD, the MDEST fraction is calculated (Tran, Barraj, Scrafford, Bi, & Troxell, 2015). The values of MRL, TDI or Rfd of different heavy metals are shown in table 2.

Table 2: Maximum residues limit (MRL), Provisional Tolerable Daily Intake (PTDI)/ Recommended Daily Allowances (RDAs) of heavy metals in milk

Element	MRL mg/kg	PTDI/ (mg/kg BW/day)	RDAs mg/kg
Pb	0.02	0.0036	-
Cd	0.0026	0.00083	-
Cr	0.2	0.3	-
Ni	1	-	1
Zn	3.28	25	-
Fe	0.37	-	45
Cu	0.01	-	0.9

1.2.1.1. Lead

According to Agency for Toxic Substances and Disease Registry ATSRD (2015), lead is considered the most hazardous heavy metal pollutant after arsenic. Lead could be present in the environment from crustal sources, the background concentration of lead in seawater is about 0.003 ppb, and it is the 37^{th} most abundant element in the Earth's crust (Sarkar D, Datta R, & R, 2011). Moreover, lead could be found in the environment from natural activities, including volcanic activity, geochemical weathering, sea spray emissions, forest fires, the erosion of rocks and soils, volcanic eruption and remobilization of historic sources UNEP (2010). However, environmental lead comes mainly from anthropogenic activities such as mining, smelting and refining operations. Further, lead is used daily in numerous manufacturing products such as pesticides, glassware, paints and batteries Tangahu BV (2011). Lead is the top poisonous aggregate environmental contaminant due to its mobility and toxicity in soil, plant and human system because of its perseverance within the environment that influences different body organisms and is especially destructive to youthful children (Assi, Hezmee, Sabri, & Rajion, 2016). According to WHO (2013), about 1.43 million cases of death happen due to lead poisoning every year, along with 6.0 million new cases of children with intellectual disabilities. In addition, in 2017, the Institute for Health Metrics and Evaluation estimated 1.06 million deaths and 24.4 million Disability-Adjusted Life Years (DALYs), including years lost due to premature death and years lived with a proven disability due to lead exposure (IHME, 2015). However, Lead has not been known to have any role in biological systems, including humans, animals and plants (Giri, Mahato, Bhattacharjee, & Singh, 2020).

The concentration of lead in the human body higher than the MRLs may cause a serious effect on several organs like the brain, gastrointestinal tract, nervous system and kidney (Baldwin & Marshall, 1999), it can also severely affect cardiovascular, reproductive, and renal functions (Abdullahi, 2013; FAO/WHO, 2011), raise blood pressure and reduction in intelligence quotient (Malhat, Hagag, Saber, & Fayz, 2012). In addition, NSC (2009) reported that a high concentration of lead exacerbated headache, constipation, loss of appetite and anaemia.

1.2.1.2. Cadmium

According to the Agency for Toxic Substances and Disease Registry ATSRD (2015), Cd is the eighth most dangerous and toxic metal. Cadmium is classified as carcinogenic to humans by International Agency for Research on Cancer IARC (1991). Humans and animals are more likely to be affected by Cd contamination by inhalation or ingestion from various sources, including metal industries, rotten and wasted food, cigarettes, and Cd products associated with factories and workplaces (IARC, 1990; Mishra et al., 2019). Cadmium is not degraded metabolically; very toxic for humans (teratogenic, carcinogenic, hepatotoxic, nephrotoxic, skeletal, and reproductive effects) even at very low concentrations (0.35 µg/kg and 0.30 µg/kg) for men and women, respectively (Jarapala SR, 2014) with a long half-life (15–30 years) (Flora & Agrawal, 2017; Zhong et al., 2018). Furthermore, several organs could be affected by cadmium accumulation. It is effectively kept in the kidney for 10–30 years, causing bone injury due to kidney damage (Chirinos-Peinado & Castro-Bedriñana, 2020; Mazzocco et al., 2020). It could also cause several cancers type due to its bioaccumulation in the liver, the lungs, urinary, reproductive, and cardiovascular systems (Amegah, Sewor, & Jaakkola, 2021; Chirinos-Peinado & Castro-Bedriñana, 2020; Tinkov et al., 2018), it can also disrupt steroidogenesis, resulting in a testosterone imbalance that disrupts endocrine function (Bazid, Attia, Yousef, Fawal, & Mostafa, 2022; Ranganathan, Rao, Sudan, & Balasundaram, 2018).

Laboratory studies have shown that cadmium has a negative impact on adipose tissue physiopathology, contributing to increased insulin resistance and diabetes. However, uncertainties about the link between Cd exposure, diabetes, and obesity persist (Tinkov et al., 2017). Cd toxicity is linked to iron, zinc, selenium, magnesium, potassium, chromium, cobalt, and copper deficiency, and their toxicity is linked to pro-inflammatory properties, oxidative stress, genotoxicity, and the development of atherosclerosis, though the evidence is still limited (Mazzocco et al., 2020; Tinkov et al., 2017; Tinkov et al., 2018). The release of Cd from natural sources is 10 times lower than that of anthropogenic activities such as mining and smelting operations, waste disposal, metal smelting and electroplating and fertilization (DalCorso, Farinati, Maistri, & Furini, 2008; J. Liu, Zhang, Qu, & Wang, 2016). Cadmium in food can occur from polluted soil, which was contaminated by irrigation water, or from deposition caused by air pollution, phosphate fertilizer, or livestock manure used as fertilizer (Rebelo & Caldas, 2016). Furthermore, the highest mean concentrations were recorded in edible offal, legumes, grains, and potatoes (EFSA, 2009; Rebelo & Caldas, 2016).

1.2.1.3. Nickel

Nickel (Ni) is ubiquitous in nature; it is the 22nd most prevalent element in the earth’s crust (twice as Cu) and a vital trace metal. It accounts for about 0.008% of the earth’s crust in the forms of sulphide and silicate minerals (Hedfi, Mahmoudi, Boufahja, Beyrem, & Aïssa, 2007;

Shahzad et al., 2018). Ni is an indispensable mineral component for humans. It functions as a cofactor for various enzymes and hormones such as urease, [NiFe]-hydrogenase, carbon monoxide dehydrogenase and, acetyl-CoA synthase, coenzyme M reduction (Ismail et al., 2017; Zamble, 2017).

The human body requires a daily intake of approximately 0.3− 0.6 mg of Ni to produce red blood cells and is used as a catalyst for various metabolic reactions (Khodadoust, Reddy, & Maturi, 2004; Mishra et al., 2019; Yang & Ma, 2021). However, it may become toxic and lead to cell damage, alteration of enzyme and hormone activities, oxidative stress and neurotoxicity above this daily intake value (Ismail et al., 2017). Although nickel is not a cumulative toxin, greater concentrations and industrial exposure make it hazardous and even carcinogenic, causing occupational hazards (Mishra et al., 2019). Nickel is found in various foods, and the human body is expected to have 10 mg of nickel (Mislankar & Zirwas, 2013; Zirwas, 2018). Some vegetables have high nickel content, but the amount of nickel in any given food varies substantially depending on the nickel level of the soil where it was grown (Mislankar & Zirwas, 2013). Ambient air contains nickel due to industrial activity, fossil fuel burning, and waste incineration (Mansour, 2014). Inhalation, ingestion, and cutaneous contact can lead to human exposure. Furthermore, elemental nickel, nickel compounds, complexes, and alloys, as well as fumes from alloys used in welding and brazing, can induce occupational exposure (Yoon, Han, & Rana, 2007).

1.2.1.4. Zinc

Zinc is the 29th most abundant metal in the Earth's crust. In fact, with background values of 10–100 mg/kg, Zn is one of the most plentiful elements on Earth (Xingyuan Li, Zhou, & Zhang, 2021). Human activities like mining and metal smelting are mostly responsible for increasing Zn in the environment (F.-l. Li, Shi, Jin, Wu, & Sheng, 2017). More than 300 enzymes belonging to six major categories, namely oxidoreductases, transferases, hydrolases, lyases, isomerases, and ligases, require zinc as a cofactor (Mansour, 2014; McCall, Huang, & Fierke, 2000; Pajarillo, Lee, & Kang, 2021), this shows its importance, in a variety of biological functions and activities, including immunity and cell survival, growth, development, and health (Bakirdere, Kizilkan, & Yaman, 2011; J. Cao et al., 2016; Karaaslan Ayhan & Yaman, 2022; Yin et al., 2017). Zinc also plays essential functions in metabolic synthesis, degradation processes, and plasma membrane stabilization (Karaaslan Ayhan & Yaman, 2022; Shils, Olson, & Shike, 1994). Moreover, Zn plays a role in cellular growth protection and neoplastic cell formation (Chanihoon, Afridi, Talpur, Kazi, & Baig, 2022; Kolachi, Kazi, Afridi, Kazi, & Khan, 2012), and it is well known that Zn aids in the synthesis of glutathione peroxidase, a protective enzyme that protects humans from oxidative and free radical damage (Kurutas, 2016) (Torgovnick & Schumacher, 2016).

It is essential to evaluate the amount of these components absorbed through food because excessive or insufficient consumption can result in nutritional deficiency symptoms as well as could lead to a variety of diseases such as loss of appetite, growth retardation, skin changes and immunological abnormalities (Younas, Fatima, Ahmad, & Ayyaz, 2022). Other diseases like poor growth and development, skin rashes, decreased immune function, loss of taste and poor wound healing could also appear due to the lack of these components (Jarapala, Kandlakunta, & Thingnganing, 2014; Martínez-Ballesta et al., 2010). Zinc deficiency is a major problem that can induce development retardation, pathogenic infection, immunological dysfunction, and decreased cognition in humans. Nearly 2 billion people, mostly children and the elderly, are afflicted in underdeveloped nations (Pajarillo et al., 2021; Prasad, 2003). Also, mental function issues might be caused by zinc insufficiency (Roney et al., 2006). Excessive zinc exposure has been linked to toxic effects such as metabolic dysfunction and oxidative damage, implying that zinc levels in the body must be carefully monitored and maintained to avoid serious harm and health concerns (Becker & Skaar, 2014). Excess Zn can impede Fe species, causing haematopoiesis to be disrupted and resulting in iron deficiency anaemia in humans (Du, Yang, Peng, Liang, & Mao, 2019; Xingyuan Li et al., 2021).

1.2.1.5. Iron

Iron (Fe) is the most abundant trace mineral in the body and is an essential trace element that participates as a catalyst in several metabolic reactions. In mammals, haemoglobin contains approximately 70% iron, while myoglobin contains only about 5% to 10%. Iron is required to transport, store, and consume oxygen (Meshref, Moselhy, & Hassan, 2014). It is a cofactor for enzymes such as peroxidase, catalase, and cytochrome (Mansour, 2014), and a lack of it can cause anaemia and other diseases like pale red blood cells, low haemoglobin, weakness, pallor, headaches, reduced immunity, inability to concentrate and cold intolerance. Early infancy's iron needs are provided by the small amount of iron in human milk. The demand for iron increases exponentially 4–6 months after birth, peaking at around 0.7–0.9 mg/day for the rest of the first year. Between 1 and 6 years, the body's iron content doubles again (FAO/WHO, 2004). Adolescents' iron requirements are extremely high (0.7–0.9 mg/day), especially during growth spurts. Those portions of the population with insufficient access to foods rich in absorbable iron during periods of high iron demand have the largest risk of iron deficiency. Children, adolescents, and women of reproductive age, particularly during pregnancy, fall into these categories (FAO/WHO, 2004; Tako, 2022). However, excess iron can result in cell damage, organ failure and increasing carcinogenic risk (Eid, Arab, & Greenwood, 2017; Puliyel, Mainous, Berdoukas, & Coates, 2015). Iron poisoning is also a leading cause of unintentional death in children under the age of six years. Because there is no mechanism for excreting iron, toxicity is determined by the amount of iron already present in the body (Vilke et al., 2011).

1.2.1.6. Chromium

Chromium (Cr) is a highly hazardous heavy metal that occurs naturally and is widely applied in industrial processes. It can be released mainly from natural sources, principally in the earth's crust (Yang & Ma, 2021). Ultramafic rocks have an average chromium concentration of 2400 mg/kg, whereas basaltic and granite rocks have 200 mg/kg and 10 mg/kg, respectively (JORA, 1998). Industrial wastes from leather and tanning, petroleum and mineral refining, electroplating, and pulp industries are all anthropogenic sources of chromium in the environment in liquid and solid forms(Jarapala SR, 2014; JORA, 1998; Purakayastha & Chhonkar, 2010). Also, Cr could be released into the environment from chemical, mineral, steel, metal plating, textile dyeing, cement production, metallurgical, and other industries (Mamyrbaev, Dzharkenov, Imangazina, & Satybaldieva, 2015; Yang & Ma, 2021).

The toxicity of Cr to human health has been demonstrated in several investigations (Blades, Ayton, Hung, Bush, & La Fontaine, 2021; Sun, Brocato, & Costa, 2015). (Blades et al., 2021). Cr compounds can cause mutagenicity in human cells by interfering with the natural cell cycle (Mamyrbaev et al., 2015). Moreover, this hazardous substance in high quantities contributes to genotoxic and carcinogenic effects in human organs (Wang et al., 2017).

1.2.1.7. Copper

Cu is a major member of heavy metals. It is the third most abundant necessary trace element in organisms after zinc and iron (D. A. da Silva et al., 2022). Copper is the world's third most used metal and the 25th most plentiful component of the Earth's crust (Shabbir et al., 2020). It exists naturally in the soil, with a mean concentration of 30-35 mg/kg^2 in dry soil, although the average Cu content in the earth's crust is 60 mg/kg^2 (Kupiec et al., 2019).

Cu deficiencies in the diet can have long-term repercussions, including delayed cardiovascular development, bone deformity, and persistent neurologic and immunologic problems into childhood and beyond (Bost et al., 2016; Georgieff, 2007). Also, long-term marginal Cu deficiency in adults has been linked to changes in cholesterol metabolism (Blades et al., 2021) (Klevay et al., 1984).

However, Cu high concentration leads to various diseases, including capillary damage, gastrointestinal irritation, heart diseases, brain damage (de Moraes et al., 2020), liver and kidney disease due to necrotic changes in some tissues (Chowdhury & Saha, 2011), various hepatic and neurological disorders including Wilson's disease, Parkinson's disease, Alzheimer's disease, MENDIK syndrome, and Menkes disease (D. A. da Silva et al., 2022; Gaetke, Chow-Johnson, & Chow, 2014; V. Kumar et al., 2021). Excessive consumption of Cu in beverages and drinking water causes nausea, vomiting, and diarrhoea in people (WHO, 2017). Moreover, early-stage Cu poisoning causes weakness, lethargy, and anorexia, whereas later-stage Cu poisoning affects the gastrointestinal tract and causes renal necrosis (V. Kumar et al., 2021). Also, excess Cu could lead to mitochondrial damage, DNA breakage and brain injury (Desai & Kaler, 2008).

Copper contamination can also be of human activity origin. Refining, metallurgy, fertilizer, printed circuit board production, pesticides, chemical production, paints, mine drainage, agricultural wastes, stormwater runoff and traffic emissions are all anthropogenic sources of Cu (Ameh & Sayes, 2019; Leygraf, Chang, Herting, & Odnevall Wallinder, 2019; Shabbir et al., 2020), while, weathering of rocks and soils, volcanoes, forest fires and many disturbances in soil are the main natural sources of Cu in the environment. Cu levels in soil properties and sediments have increased due to rapid industrialization and urbanization (V. Kumar et al., 2021). Cu is widely utilized as a construction material and is a component of numerous alloys such as sterling silver, cupronickel, and constantan, which are used in jewellery, coinage, and instrument gauge. Due to its unique electricity conducting qualities, it has a wide range of applications, making it difficult to replace. Due to the likely rise of copper-intensive low-carbon energy and the electrification of transportation systems, it may become more significant for the general public (Schipper et al., 2018).

Table 3: Minerals: reliable food sources, stability, associated body systems, functions, deficiency, and toxicity symptoms

Minerals and adult requirements	Reliable food sources	Associated body systems, functions, deficiency/toxicity symptoms	Deficiency probability
Iron RDA: M: 8 mg/day F (19–50 years): 18 mg/day; (>50 years): 8 mg/day UL: 45 mg/day	Red meat, fish, poultry, shellfish, eggs, legumes, dried fruits, molasses, whole, enriched, or fortified grains	Systems: circulatory, endocrine, immune, muscular, nervous Functions: part of haemoglobin and myoglobin; electron carriers in electron transport chain; immune function Deficiency: iron deficiency anaemia − small, pale red blood cells, low haemoglobin, weakness, pallor, headaches, reduced immunity, inability to concentrate, cold intolerance Toxicity: gastrointestinal upset (GI upset), iron overload, infections, liver damage, acidosis, shock	Common in at-risk groups. Deficiency may be associated with unusual blood loss, parasites, or malabsorption At risk: infants and preschool children; adolescents; women of childbearing age; pregnant women; athletes; vegetarians
Zinc RDA: M: 11 mg/day F: 8 mg/day UL: 40 mg/day	Meat, seafood, poultry, whole grains, legumes, wheat bread, eggs	Systems: immune, integumentary, muscular, nervous, reproductive Functions: regulates protein synthesis; functions in growth, development, wound healing, immunity, antioxidant protection, vitamin A transport, fetal development Deficiency: poor growth and development, skin rashes, decreased immune function, loss of taste, poor wound healing Toxicity: decreased copper absorption, depressed immune function, kidney failure	The extent of inadequacy is unknown. Conditional deficiency can occur with systemic childhood illness and individuals who are nutritionally depleted or have experienced severe stress such as surgery At risk: vegetarians; low-income children; elderly
Copper RDA: 900 μg/day	Organ meat, seafood, nuts, seeds,	Systems: immune, muscular, nervous Functions: part of proteins needed for iron	No evidence At risk: those who over-

UL: 10 mg/day	whole grains, cocoa	absorption, lipid metabolism, collagen synthesis, nerve and immune function, and antioxidant protection Deficiency: anaemia, poor growth, bone abnormalities Toxicity: vomiting, diarrhoea	supplement with zinc; Menkes the disease is a genetic disorder resulting in copper deficiency
Chromium AI: M (19–50 years): 35 µg/day F (19–50 years): 25 µg/ day UL: no UL	Brewer's yeast, meat, nuts, whole grains, mushrooms	Systems: endocrine Functions: associated with glucose metabolism; enhances insulin action Deficiency: abnormal glucose metabolism; high blood glucose Toxicity: can occur with occupational exposure; causes damage to skin and kidneys	Found in those with severe malnutrition and may be a factor in diabetes development in older adults At risk: malnourished children

[a] Requirements are based on the Institute of Medicine Dietary Reference Intakes: Recommended Dietary Allowance (RDA), Adequate Intakes (AI), and Tolerable Upper Intake Level (UL) (Medicine, Food, Board, & Staff, 1998; Tako, 2022). F: female and M: male

1.2.2. Pesticide residues

Pesticides are compounds or mixtures of substances used to prevent, remove, or control pests such as insects, fungi, rodents, or undesired plant species that cause harm to crops during production and storage (Xin Li et al., 2019). The word "pesticide" is a broad term that includes insecticides, herbicides, fungicides, and rodenticides which can be used to eliminate specific pests. Pesticides are classified as chemical or bio-pesticides based on their sources of origin (Chen, Quandt, Grzywacz, & Arcury, 2011).

Chemical pesticides have performed an important and potentially beneficial role in increasing agricultural productivity by reducing pests and plant diseases (Chawla, Kaushik, Shiva Swaraj, & Kumar, 2018; Malarkodi, Rajeshkumar, & Annadurai, 2017; Moreno-González et al., 2017). Also, throughout the world, approximately 9000 kinds of insects and mites are the primary reason for the lack of desired agricultural produce products for human consumption; hence, controlling damaging insects is a critical responsibility for improving agriculture supply production (Fan, Zhao, Yu, Pan, & Li, 2014; X. Liu, Mitrevski, Li, Li, & Marriott, 2013).

However, despite their beneficial effects, their use has been linked to several negative consequences, including unfavourable effects on non-target species and severe environmental and human health consequences such as contaminated soil and water, the food chain's bioaccumulation and biomagnification and their potential link to effects on human health (Simeonov, Macaev, & Simeonova, 2014). Pesticides can be categorized in a variety of ways. According to Drum (1980), they could be classified depending on their source (Table 4), their function (Table 5), or the target pest species (Table 6).

Table 4: Classification of pesticides based on origin (Chen et al., 2011)

Origin	Sources and examples
Organic sources	Natural-plant phytochemical (essential oil, plant extracts, leftover oilseed cakes) synthetic-produced by chemical synthesis Pyrethroids, organophosphates, carbamates, organochlorine
Inorganic sources	Inorganic-mixture of inorganic salts Bordeaux mixture Cu $(OH)_2$. CaSO4 Malachite Cu $(HO)_2$. $CuCO_3$ and sulphur
Biological	Biological: microbial pesticide (bacteria, virus, and fungi)

Table 5: Classification of pesticides based on function (Chen et al., 2011).

Action	Functions	Examples
Feeding deterrents	Prevent an insect or other pest from feeding	Azadirachta Indica A. Juss
Ovipositor deterrent	Prevent egg laying by gravid female	Azadirachta indica
Repellents	Deters pests from approaching toward Crops	Plant essential oil
Attractants	A chemical that lures pests	Gossyplure
Fumigants	Kills the target pests by producing vapour	Phosphine
Insect growth regulator	A substance that works by disrupting the growth or development of an insect	Diflubenzuron
Synergist	A chemical that enhances the toxicity of a pesticide but is not by itself toxic to the pest	Piperonyl butoxide

Table 6: Classifications of Pesticides Based on Target Pest Species (Chen et al., 2011)

Pesticides class	Target pests	Examples
Acaricides	Mites	Bifonazole
Algaecides	Algae	Copper Sulfate
Avicides	Birds	Avitrol
Bactericides	Bacteria	Copper Complexes
Fungicides	Fungi	Azoxystrobin
Herbicides	Weeds	Atrazine
Insecticides	Insects	Aldicarb
Larvicides	Larvae	Methoprene
Molluscicides	Snail	Metaldehyde
Nematicides	Nematodes	Aldicarb
Ovicides	Egg- prevents hatching of eggs in insects and mites	Benzoxazine
Piscicides	Fishes	Rotenone
Repellents	Insects	Methiocarb
Rodenticides	Rodents	Warfarin
Termiticides	Kills termites	Fipronil
Viricides	Viruses	Scytovirin

In this thesis, we are interested in pesticide residues classified depending on synthetically produced by chemical synthesis, which are pyrethroids (PY), organophosphates (OP), carbamates (CB), and organochlorine (OC). After the Second World War, the use of commercial synthetic pesticides to control insects as disease vectors and pests in agriculture increased dramatically on a global scale. These "pioneer" compounds have demonstrated environmental stability and long-term efficacy. However, they accumulate in the biosphere due to their lipophilicity and resilience to biodegradation, and detectable levels can be observed in numerous foods, including milk and dairy products worldwide (Fischer, Schilter, Tritscher, & Stadler, 2016).

Regarding residual pesticides, bio-concentration is a well-known process for a food chain, and humans are always at the top of the food chain. As a result, there is a high likelihood of more concentrated pesticide residues accumulating in the human body, as illustrated in Figure 2.

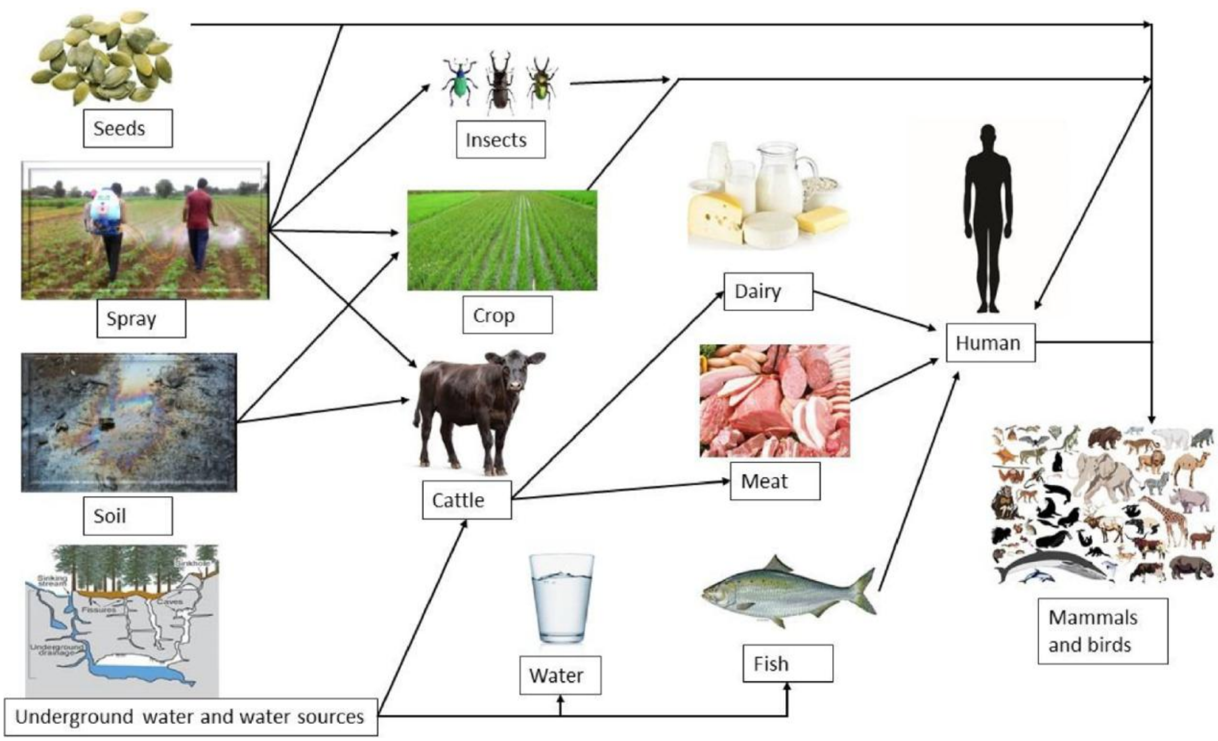

Figure 2: Bio-concentration of residual pesticides in the food chain (Chawla et al., 2018)

A rising number of pesticides are entering our environment, posing a risk to human and animal health and the ecosystem (Bedi, Gill, Aulakh, & Kaur, 2015; Gill & Garg, 2014). Long-term pesticide exposure can cause liver and kidney damage (Peres, Moreira, Rodrigues, & Claudio, 2006), endocrine system disruption, nervous and immune systems diseases and breast, lung, cervix, and prostate cancer risk (Bedi et al., 2015) (Figure 3).

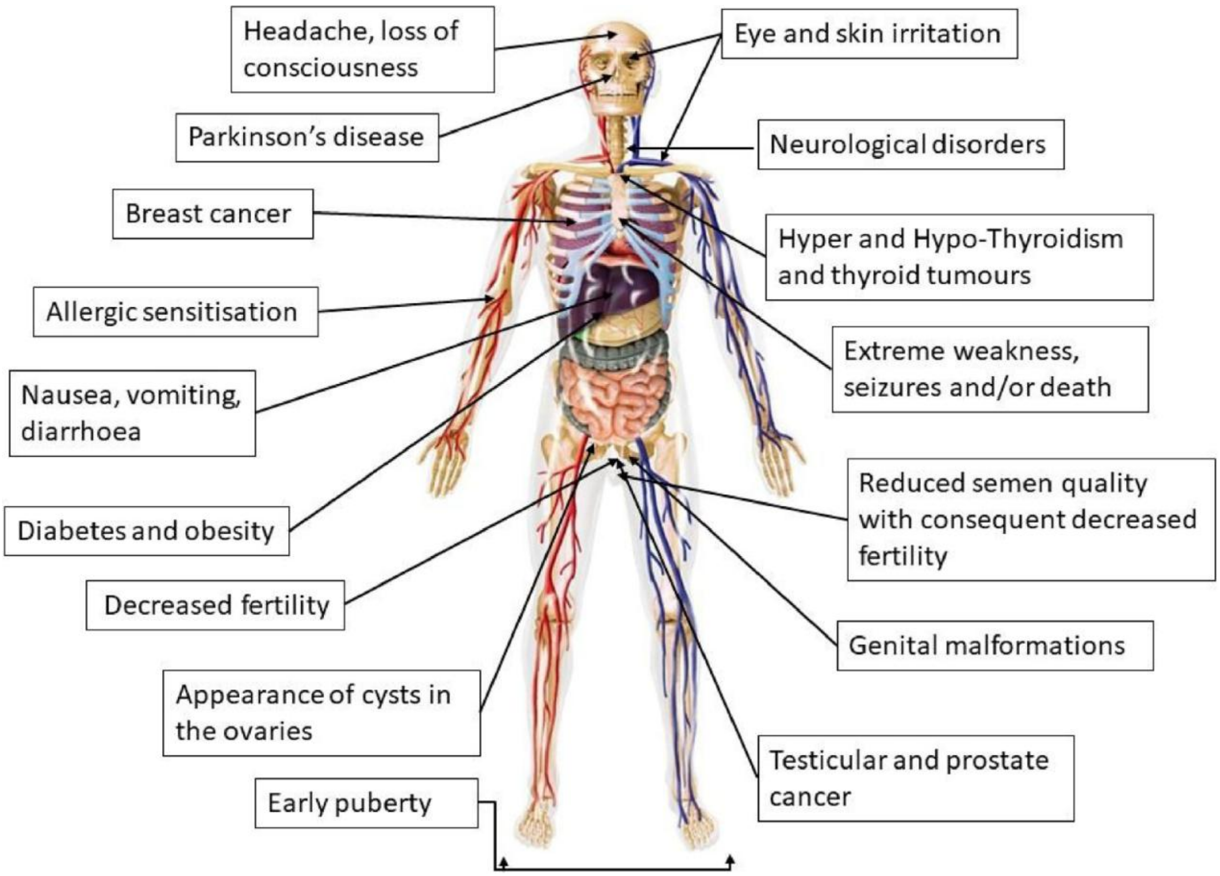

Figure 3: Effects of residual pesticides on the human body (Chawla et al., 2018)

Pesticides have been found in water bodies, soil, air, and biota due to their widespread use to increase agricultural productivity (JORA, 1998). As knowledge of the detrimental effects of many pesticides increased, many governments implemented pesticide restrictions to safeguard human and environmental health (Purakayastha & Chhonkar, 2010). The use of dichlorodiphenyltrichloroethane (DDT), for example, was forbidden globally in the Stockholm Convention of 2001 due to its negative impact on the environment and the health of living beings (Jarapala SR, 2014). More international conventions, such as the Rotterdam Convention of 2006 and the Montreal Protocol from 1993, have been adopted to limit the use of very toxic pesticides (Yang & Ma, 2021). There are two different definitions of residues under the law. The first is to assess the potential harm to human health and the environment.

On the other hand, the second is utilized for pesticide application monitoring and is highly dependent on the matrix. The term's use in European legislation is based on research into plant and animal metabolism and degradation while considering toxicology, ecotoxicology, agronomy, and exposure. The term "residue" can refer to both the pesticide's active ingredient and its transformation products (JORA, 1998).

European Commission (2005) establishes the MRL as the upper legal limit for a residual of an active pesticide ingredient in/on food or feed in Europe. They are calculated according to acceptable agricultural practices, minimizing consumer interaction with the pesticide to protect the most vulnerable. Good Agricultural Practices (GAP) standards and MRLs have been established by international regulatory organizations such as the EU and FAO/WHO to protect human and environmental health (Mamyrbaev et al., 2015). Pesticide MRLs for European Union countries are set down in European Commission (2005) and are continually monitored in the environment. MRLs are typically set at the lowest quantities detectable by standard analytical procedures, allowing the active ingredient to be detected at even lower concentrations. They generally range from 0.01 to 10 mg/kg, while items of plant or animal origin are restricted to not exceeding 0.01 mg/kg if no MRL has been established (Article 18, 1b). Pesticides are frequently banned as a protective measure if there is proof that they represent a health danger to organisms. Once adequate scientific information becomes available, the ban can be revaluated. After reviewing the literature available at the time, the European Food Safety Authority (EFSA) revaluated the 2006 endosulfan ban in 2011. They agreed that the pesticide should stay illegal in the European Union and be regulated as a substance that should not be used in animal feed.

Furthermore, the European Food Safety Authority (EFSA) noted that more research was needed to better understand pesticides' toxicity in diverse species (Sun et al., 2015). The situation is different in developing nations, where governments argue that they cannot readily ban specific chemicals due to several factors, including controllability, affordability, and efficacy. As a result, many developing nations have utilized or still use these substances in huge quantities (Boudebbouz, Boudalia, et al., 2022a; Ecobichon, 2001). Additionally, while developed countries utilize more than 80% of the pesticides produced globally, developing countries only use 20% of them. However, the fatality rate from improper or indiscriminate pesticide use is 13 times higher in developing nations (Ansari et al., 2021), there are no regulations for pesticide application, and farmers lack the necessary knowledge and training (Udimal et al., 2022).

Even though MRL is a good tool to evaluate the potential exposure assessment of pesticide residues, residue levels at or below the MRL would not give intakes that exceed the ADI/ARfD, but, despite this, there is public concern over such residues (Sun et al., 2015). The toxicological reference values used in dietary risk assessment are the Acceptable Daily Intake (ADI) and the Acute Reference Dose (ARfD).

Based on all available facts at the time of the evaluation, the Acceptable Daily Intake (ADI) is an estimate of the number of pesticides in food or drinking water that can be taken daily throughout a lifetime without posing a significant health risk to the consumer. However, the Acute Reference Dose (ARfD) estimates the number of pesticides in food or drinking water that can be taken over 24 hours without posing a significant health risk to the consumer. Both (ADI/ARfD) are measured in pesticide milligrams per kilogram of body weight. The pesticide’s dietary intake is calculated using a combination of national or regional food consumption statistics and predicted residues in food and/or drinking water. When the estimated long-term and short-term dietary consumption of pesticide residues do not exceed the recommended daily intake (ADI) and acute reference dose (ARfD), the consumer is considered effectively protected (Mamyrbaev et al., 2015).

Over the last decades, several pesticide residues have been detected worldwide in milk and dairy products. Table 7 shows all pesticide residues detected with their MRL in the milk and dairy products and the ADI/PTDI for each pesticide. The MRL and ADI/PTDI could be obtained from the pesticide database set by the Codex Alimantarius Commission (2020) for all pesticide residues in foods other than milk and dairy products.

Table 7: Maximum residues limit (MRL), Provisional Tolerable Daily Intake (PTDI)/ of Pesticide residues in milk, Data extracted from Codex Alimantarius Commission (2020)

Pesticide residues	Class	MRL (Year of Adoption) (mg/kg)	ADI/PTDI (Year of Adoption) (mg/kg BW)
o.p'-DDE	Organochlorine	-	-
o.p'-DDE	Organochlorine	-	-
o.p'-DDE	Organochlorine	-	-
o.p'-DDE	Organochlorine	-	-
Sum of DDT	Organochlorine	0.02 (1997)	0.01 (2000)
Alpha endosulfan	Organochlorine	-	-
Beta endosulfan	Organochlorine	-	-
Endosulfan sulphate	Organochlorine	-	-
Sum of alpha endosulfan, beta endosulfan and endosulfan sulphate	Organochlorine	0.01 (2007)	0.006 (1998)
α HCH	Organochlorine	-	-
α HCH	Organochlorine	-	-
α HCH	Organochlorine	-	-
α HCH	Organochlorine		
ΣHCH	Organochlorine	0.001 (2016)	0–0.005 (2002)
Aldrin	Organochlorine	-	-
Dieldrin	Organochlorine	-	-
Aldrin and Dieldrin	Organochlorine	0.006 (-)	0.0001 (1994)
Drins	Organochlorine	-	-
Endrin	Organochlorine	-	0.0002 (1994)
Heptachlor	Organochlorine	-	-
Heptachlor epoxide	Organochlorine	-	-
Sum of heptachlor and heptachlor epoxid	Organochlorine	0.006	0.0001 (1994)
Malathion	Organophosphorus	-	0-0.3 (1997)
Dimethoate	Organophosphorus	0.05 (2003)	0.002 (1996)
Chlorpyriphos	Organophosphorus	0.02 (2003)	0-0.01 (1999)
Profenofos	Organophosphorus	0.01 (2009)	0-0.03 (2007)
Dichlorvos	Organophosphorus	0.01 (2013)	0-0.004 (2011)
Methamedophos	Organophosphorus	0.02 (2005)	0-0.004 (2002)
Ethion	Organophosphorus	-	0.002 (1990)
Parathion methyl	Organophosphorus	-	0.003 (1995)
Cypermethrin	Pyrethroid	0.05 (2009)	0-0.02 (2006)
Permethrin	Pyrethroid	-	0.05 (1999)
Bifenthrin	Pyrethroid	0.2 (2011)	0-0.01 (2009)
Cyhalothrin	Pyrethroid	0.2 (2009)	0-0.02 (2007)
Deltamethrin	Pyrethroid	0.05 (2004)	0-0.01 (2000)
Carbaryl	carbamate	0.05 (2004)	0-0.008 (2001)
Aldicarb	carbamate	0.01 (-)	0.003 (1992)
Carbofuran	carbamate	-	0-0.001 (2008)

1.2.2.1. Organochlorine pesticides

Organochlorine pesticides (also called chlorinated hydrocarbons) are organic molecules linked to five or more chlorine atoms. They represent one of the oldest categories of pesticides ever synthesized and are widely employed in agriculture (Chen et al., 2011; Xin Li et al., 2019). Because of their chemical stability, long biological half-life (ranging from a few years to more than 10 years), and high biomagnification in the food chain, organochlorine pesticides (OC) (also known as chlorinated hydrocarbons) are considered the most harmful and persistent substances in the environment (Chen et al., 2011; Serrano, Blanes, & López, 2008). OC pesticides may be found in higher concentrations in human tissues such as the liver, kidney, thyroid, heart, mammary gland and testes (Nag, 2010).

Human researchers have revealed several negative health outcomes linked to OC pesticide exposure. They show that the presence of OC pesticides in human organs leads to endocrine-disrupting activity and can cause chronic toxicity after long-term exposure (Ansari et al., 2021; Martins, Amaya Chávez, Waliszewski, Colín Cruz, & García Fabila, 2013). In addition, Organochlorine pesticides can also cause non-Hodgkin's lymphoma, hepatotoxicity, immunotoxicity, developmental abnormalities, neurobehavioral disorders and population drops (Qu, Suri, Bi, Sheng, & Fu, 2010).

Sixteen (16) out of 30 chemicals targeted by the Stockholm Convention listed in the annexes of the convention text are Organochlorine pesticides (OC): aldrin, endrin, dieldrin, chlordane, chlordecone, dichlorodiphenyltrichloroethanes (DDTs), heptachlor, mirex, toxaphene, endosulfan and isomers, hexachlorobenzene (HCB), alpha-hexachlorocyclohexane (a-HCH), beta-hexachlorocyclohexane (b-HCH), lindane, Dicofol and penta-chlorobenzene (Stockholm Convention, 2009).

1.2.2.2. Organophosphorus pesticides

Organophosphorus pesticides are phosphoric acid-derived pesticides considered wide-spectrum pesticides because of their numerous functions. They manage a wide range of pests, weeds, and plant diseases. They are cholinergic cholinesterase inhibitors, which disrupt neurotransmitter transmission across a synapse (Saha et al., 2017). Organophosphate insecticides quickly replaced stable OC (which were banned in many countries in developed and developing countries in the 1970s) chemicals, resulting in a gradual decrease in OC residues such as DDT (and its metabolites) and hexachlorocyclohexane (HCH) isomers in the environment and, as a result, in different foods (Fischer et al., 2016).

Organophosphorus (OP) are phosphoric, phosphonic, phosphinic, and thiophosphoric acid-derived pesticides. They are a widely used class of pesticides that account for around 38% of all pesticides used worldwide (Vijayan P & Abdulhameed, 2020). According to several scientific reports, OP usage is the greatest of all pesticides, putting 3 million individuals in danger of OP poisoning each year (Cavaliere et al., 1998; Derbalah, Chidya, Jadoon, & Sakugawa, 2019; Obare et al., 2010; Sharma, Nagpal, Pakade, & Katnoria, 2010; Soltaninejad & Shadnia, 2014).

Even though OP residues such as malathion, chlorpyriphos, dichlorvos, profenofos, coumaphos, methamedophos, ethion, and dimethoate are less persistent in the environment than organochlorine pesticides, some authors describe the presence of OP residues in different foods such as vegetables, fruits and even in the milk and dairy products (Nag, 2010). In addition, OP and its metabolites have been found in various crops, water sources, and soils (Songa & Okonkwo, 2016). OP pose significant life-threatening diseases and genetic illnesses that directly impact billions of people's productivity and efficiency (N. Kumar, Kumar, Mann, & Seth, 2016).

1.2.2.3. Carbamates

Carbamate (CB) pesticides, including carbaryl, carbofuran, and aminocarb, are organic ester compounds derived from dimethyl N-methyl carbamic acid. They are similar in structure and purpose to OP pesticides (Hassaan & El Nemr, 2020; Xin Li et al., 2019). Carbamate pesticides are used as herbicides, insecticides, nematicides and fungicides for household, home, and agriculture purposes (Blodgett & Means, 2013). The acute poisoning symptoms arising from CB pesticides are similar to those of organophosphorus pesticides and are often severe. These poisoning symptoms can be found in several organs, including bronchial tree, cardiovascular effects, eye (Miosis and blurred vision), gastrointestinal manifestations and central nervous system effects (Roberts & Routt, 2013). In addition, numerous studies have shown that carbamate compounds have been linked with undesirable human health, such as cancer, reproduction toxicity (L. C. C. da Silva, Beloti, Tamanini, & Netto, 2014), and neurotoxic effects on the young human at excess intake (Herbert et al., 2021).

1.2.2.4. Pyrethroid pesticides

Pyrethroids are natural and synthetic insecticides derived from the pyrethrum extracts of chrysanthemum flowers known as pyrethrin found in Kenya (Xin Li et al., 2019). It can act on the central nervous system, which causes fluctuations in the dynamics of sodium cation channels in the nerve cell membrane, which leads to an increase in the time of opening of the sodium channels. The sodium cation stream extends across the membrane in vertebrates and insects (Kamita, Kang, Hammock, & Inceoglu, 2005; Perry, Yamamoto, Ishaaya, & Perry, 2013).

Due to the severe need for large quantities of these pesticides and the growing shortage of essential oils necessary for manufacturing natural organic pyrethrums, scientists have turned to the production of synthetic pyrethroids (Hassaan & El Nemr, 2020). Pyrethroid insecticides are mainly characterized by low toxicity to birds and mammals, high toxicity to arthropods since small amounts are needed to destroy insects, and high toxicity to fish when applied in water. They are ineffective at entering the soil to kill subsurface pests because they securely stick to soil and organic matter (Gupta & Crissman, 2013; Hassaan & El Nemr, 2020). They have been used since the 1980s worldwide due to their photodegradation, effectiveness against various insects, and low toxicity compared to other pesticides such as OC, OP, and CB (Yoo, Lim, Kim, Lee, & Hong, 2016). However, despite their low toxicity and strong efficacy against target organisms, pyrethroid insecticides can provoke major health problems by affecting organisms' neurological, circulatory, immunological, and genetic systems (Tang et al., 2018).

2. Objectives and outlines

Owing to the rising demand for milk and dairy products across the globe and in Algeria, as well as increased national milk production and the existence of different pathogens in raw cow milk and polluting sources that could cause direct or indirect contamination of raw cow milk, it is necessary to assess raw cow`s milk qualities (physicochemical, bacteriological and toxicological characteristics) produced in rural areas, particularly for emerging contaminants such as heavy metals and pesticides, which can be dangerous to human health.

Many researchers have already studied raw cow milk's physicochemical and bacteriological qualities in Algeria, but little information is available for milk from local cattle breeds in Northeast Algeria. Therefore, the innovative aspect of this thesis offers the first report on raw cow`s milk contamination by heavy metals and is considered an important guideline to the different stockholders (breeders, health authorities, and policymakers) for knowing the status of raw cow milk consumed by the population under climate change uncertainty context

At the international level, and despite the difficulty in understanding the multifaceted aspect of food security concerning cow's milk consumption, the potential risk to human health from raw cow milk consumption should be evaluated. This thesis aims to:

1. Assess the spatial variability of seven heavy metals contents in raw cow's milk produced in Northeast Algeria;

2. Summarize livestock production practices and milk quality and discuss the potential of local cattle breeds to maintain production ability under climate change context;

3. Assessing the potential risk to human health using a theoretical approach such as the hazard quotient (HQ) and the hazard index (HI) in Northeast Algeria;

4. Compare the levels of heavy metals (copper (Cu), iron (Fe) and nickel (Ni), aluminium (Al), cadmium (Cd), lead (Pb), and mercury (Hg)) and synthetic pesticide residues (organochlorine (OC), organophosphorus (OP), carbamate (CB), and pyrethroid (PY)) in raw cow's milk samples recorded in different countries, and discussing contamination sources and regulations;

5. Estimate daily intake (EDI), hazard quotient (HQ), and hazard index (HI) of pesticide residues and heavy metals from consuming raw cow's milk using data extracted for pesticide residues and heavy metals levels recorded from different areas across the globe.

CHAPTER 2

LOCAL CATTLE BREED IN ALGERIA: MILK QUALITIES AND PRODUCTION ABILITY UNDER CLIMATE CHANGE UNCERTAINTY CONTEXT

From Ali BOUDEBBOUZ, Sofiane BOUDALIA, Rassim KHELIFA, Meriem Imen BOUSSADIA, Aissam BOUSBIA, Asma BEN CHABANE, Lamiss BOUMENDJEL, Meryem SAHRI, Dounya Achwak CHEMMAM, Yassine GUEROUI, George SYMEON. Local cattle breed in Algeria: milk qualities and production ability under climate change uncertainty context.

Local cattle breed in Algeria: milk qualities and production ability under climate change uncertainty context

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Abstract

Algerian indigenous cattle breeds are well adapted to the local harsh arid and semi-arid environment. They are essential to enhance sustainable farming systems, which promote biodiversity and keep a balanced ecosystem. This study aimed to summarize livestock production practices, milk quality and to discuss the potential of local cattle breeds to maintain production ability under global warming. To collect data related to livestock farming practices, 175 smallholder farmers who practice the breeding of the Algerian local cattle breed were interviewed using a formal questionnaire. Then, 122 milk samples were collected to carry out the physicochemical and bacteriological analysis. Climate data variability from the study area was evaluated.

Results reveal that breeders have a low educational level (39.4% unlettered). They own small breeding essentially consisting of local cattle breeds (6.84 ± 8.66 cattle). The results also show that the average daily milk production was 4.13 ± 2.12 L/cow/day, with an acceptable physicochemical quality but poor bacteriological quality. Considering the vulnerability of the study area, we can consider that the exploitation of local breeds seems to be the best adaptation strategy to climate change effects. On the one hand, conservation programs of local breeds can promote biodiversity and keep a balanced ecosystem. On the other hand, it may benefit from a genetic improvement program that can increase productivity and profitability. This can be beneficial for smallholders; and can provide them with a fair and stable income and good working conditions and could contribute significantly to the social equity and local economies.

Keywords: sustainable livestock, global warming, local bovine breed, milk quality, milk yield.

1. Introduction

The most prominent cause for climate change is the increased greenhouse gas emissions (GHGs) in the atmosphere, such as nitrous oxide (N_2O), carbon dioxide (CO_2), and methane (CH_4), causing irregularity, variability, and unpredictability of rainfall, floods, and drought periods (IPCC, 2021). More than 83% of the total agricultural emissions are due to livestock emission sources. Enteric fermentation is considered the biggest contributor (about 5.5 $MtCO_2e$) of livestock emissions, followed by manure left in pasture (4.5 $MtCO_2e$) (Climate Watch, 2021).

Among the polluting sectors in Algeria, agriculture contributed 12.3 Million tons CO_2 equivalent ($MtCO_2e$) GHG emissions in 2012, which represented 5.63% of its total emissions excluding land-use change and forestry (219 $MtCO_2e$) (Climate Watch, 2021; FAO, 1997). According to Prevention Web, Algeria is ranked 18 of 184 of the most exposed countries to drought, and about 10% of its population (3,763,800 inhabitants) is exposed to droughts (WBG, 2022).

Several studies predict a further future decrease in total annual rainfall by 15-30% (Christensen et al., 2007) and desert climate expansion at the expense of the temperate northern zone, which is explained both by increasing temperature and decreasing precipitation (Zeroual et al., 2020; Zeroual et al., 2019). Moreover, these effects will likely be "severe, pervasive and irreversible" in the years to come (IPCC, 2021; Zeroual et al., 2016; Mariotti et al., 2015; IPCC, 2014; Sahnoune et al., 2013), which can negatively affect livestock production, crop yields, and threaten food and nutrition security (FAO, 2013).

In order to deal with these effects, it is urgently needed to transform the agriculture, livestock farming and food systems towards more sustainable production methods. The reduction of carbon footprint and greenhouse gas fluxes as well as the genetic conservation and preservation of local breeds which are well adapted to the local environment, are both strategies that can be profitable and safeguard natural resources for the future generations (Khelifa et al., 2021; Brini, 2021; Bousbia et al., 2021; Martin et al., 2020; Wainwright et al., 2019; IPCC, 2014).

The Algerian Brown Atlas breed is well adapted to the local harsh arid and semi-arid environment and is characterized by tolerance to heat stress and diseases resistance (Boushaba et al., 2019; Djaout et al., 2017; Derradji et al., 2017). Its population has been estimated by the "*Recensement National des Exploitations Agricoles et d'élevage RGA*" (MADR, 2001) at nearly 896,287 subjects. Nevertheless, the breed has low milk production, which accounts for 1175 litter/cow/year (Mamine et al., 2011). To remedy this low yield, foreign breeds were imported (Holstein and Montbéliarde breeds), which has led to a profound change in the genetic structure of the dairy herd in Algeria, resulting in a drastic fall on the numbers of local cattle. Thus, the share of local breeds has been reduced from 82% of the total in 1986 to about 48% of the total in 2016 (Wilson, 2018).

The performance of imported breeds is lower under hot environments than in their native environments (Nigm et al., 2015; Madani and Mouffok, 2008). It is well established in the literature that when dairy cattle are under heat stress there is an increase in water intake and a decrease in dry matter, protein and fat content of milk as well as milk yield (Gorniak et al., 2014). The microbiological qualities of milk are also affected, because contamination and pathogen proliferation increases under excessive heat and humidity (Montcho et al., 2021), resulting thus in economic loss from dairy farms (Bohmanova et al., 2007; Martín-Sosa et al., 2003). On the other hand, local breeds can perform well in adverse climatic conditions like high temperature, drought, feed and water scarcity (Sejian et al., 2015) because they are more robust and genetically better adapted to their environment (Rodríguez-Bermúdez et al., 2019).

Since the local bovine breeds farming sector is not well studied in Algeria, the objectives of this study were *a)* to summarize the farming practices of local bovine farms in the northeast of the country, *b)* to evaluate the physicochemical and microbiological properties of raw milk from the local bovine breed, and *c)* to highlight the climate variability in the study area, and discuss the potential of the local cattle breed to contribute to climate change mitigation and increasing resilience through adaptation.

2. Material and Methods

2.1. Ethical statement

This study was carried out as part of the BOVISOL project (Breeding and management practices of indigenous bovine breeds: Solutions towards a sustainable future www.rias.gr/bovisol). The BOVISOL project (2018-2022) is a cooperation of scientific teams from Greece, Tunisia, and Algeria and has been formed around the hypothesis that the local bovine breeds must be preserved since they possess a valuable genetic pool and they are a part of the landscape and the biodiversity of rural areas (Boudalia et al., 2020). The local Data Protection Board (DPB) and the local ethics committee have approved experimental protocols. The study involved data collection from different farms so the farmers were informed of the purpose of the project and have given their consent for their participation (complete the survey questionnaire, and/or provide a sample of the milk), and the use of data collected and generated for scientific publications.

2.2. Study area and environmental characterization

The present study was conducted in the northern-east of Algeria (Figure 1) from June 2018 to August 2021. The region is characterized by a subhumid climate in the center and in the North and semi-arid in the South. The climate is mild and rainy in winter, and hot and dry in summer (Figure 2A, B). To determine the historical (1980-2018) and projected future (2081-2100) climatic changes, the annual average temperature and annual precipitation from Worldclim2 was used (Fick and Hijmans, 2017). Moreover, land use data of Venter et al. (2016) and particularly cropland and pasture data were used to determine the temporal change of the area of these two land use types (cropland data are provided for 1992 and 2005 whereas pasture data are provided for 1993 and 2009). Croplands are coded as 0 (absence) or 7 (presence). Pastures are scored in four categories (None [0%] = 0, sparse [<12.5%] = 1, medium [>12.5%] = 2, dense [>50%] = 3). The pastures were categorized into presence and absence to estimate the historical change in percent cover in the study area.

2.3. Interviews and data collection

The study was conducted from June 2018 to August 2021. Detailed information was collected via a formal questionnaire developed in the BOVISOL project and used in Greece, Tunisia and Algeria (Boudalia et al., 2020). It included open-ended and closed questions and covered the following topics: demographic information on the farmers, gender dimension, and details on the farms, breeds, the animals' performance, production systems and market channels. 175 face-to-face interviews with farmers practicing the breeding of local bovine breed were conducted, 2 to 3 visits to each farm, in the local dialect, where the content of the questionnaire was read and interpreted to all the interviewed farmers. The data were coded, entered in a database, corrected and validated by the research group. In this research article, we present only preliminary results concerning farms and farmers' data in Algeria.

2.4. Samples collection

122 out of the 175 participating farmers agreed to provide milk samples for analysis. A total volume of about 0.5-1 L of milk was collected from each farm in sterile glass bottles and placed immediately in a cooler, then transported to the laboratory for analysis. All bottles were previously autoclaved at a temperature of 121 °C, under a pressure of 1 bar for 15 minutes. The vials were filled from a container of mixing milk, respecting the Good Laboratory Practices (GLP), and the rules of asepsis (disinfection of the hands). In order to take account of the real field conditions, no conservative was added.

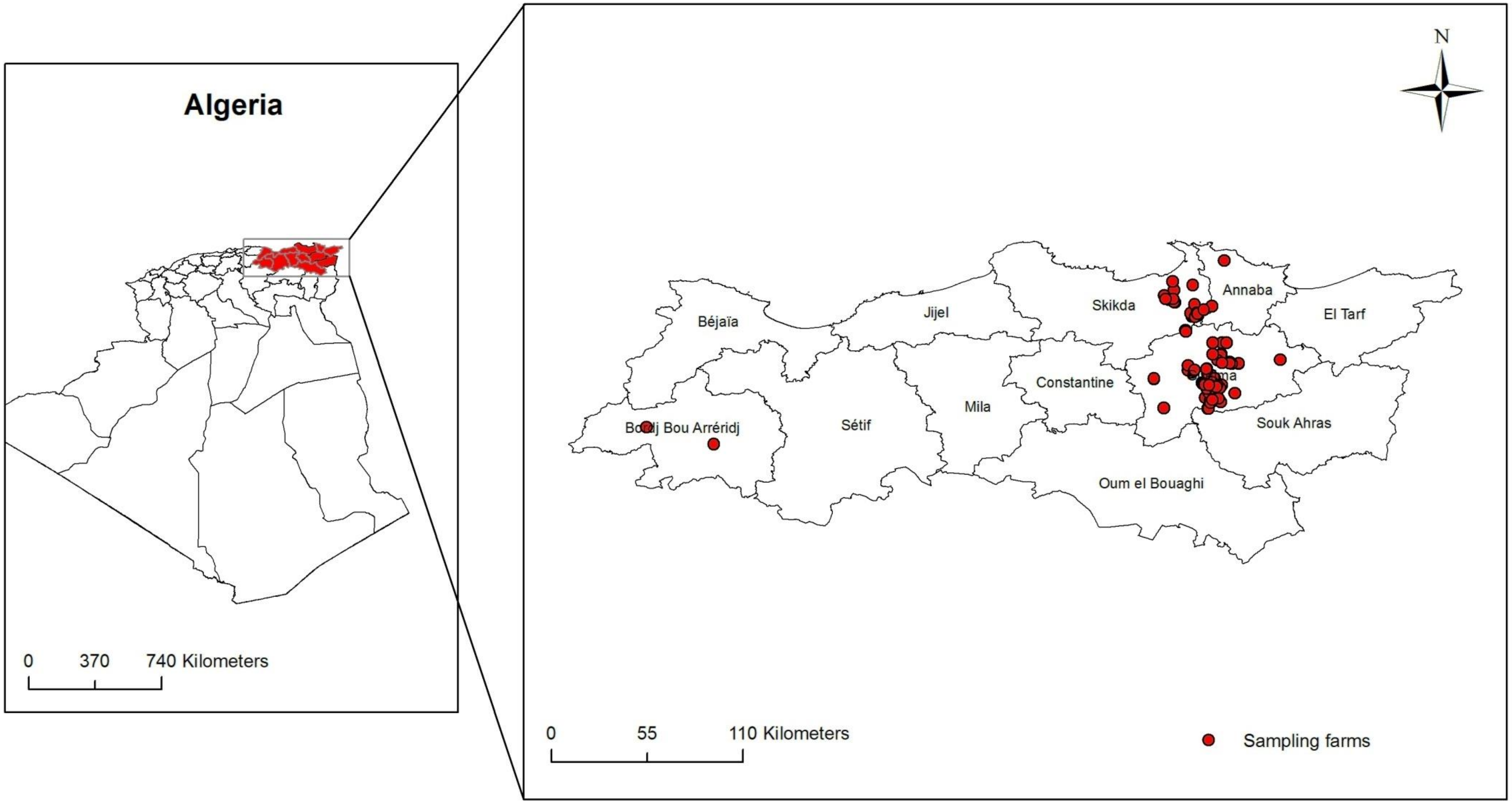

Figure 1. Study area. Map showing the locations of the municipalities investigated. Map created using the Free and Open Source QGIS.

2.5. Physicochemical properties

For physicochemical analysis, pH was measured using a pH meter Adwa, AD1000 and acidity (°D) was determined according to the method detailed in Tadjine et al. (2019). Freezing point (°C), conductivity (µS/cm), fat content (g/kg), protein content (g/kg), lactose content (g/kg), mineral content and vitamins (g/kg) of milk were measured with a Lactoscan milk analyzer (Milkotronic Ltd, Nova Zagora, Bulgaria) according to the manufacturer's instructions.

2.6. Microbiological analysis

For bacteriological analysis, samples preparation and dilutions were performed according to the recommendations of the International Dairy Federation (IDF, 1991): 1). The Total Mesophilic Aerobic Flora (TMAF) was enumerated using Plate Count Agar (PCA) and incubated at 30 °C for 72 h. 2). The Total Coliforms and Fecal Coliforms were determined using Violet Red Lactose Bile agar (VRBL) incubated at 37 °C for total coliforms, and 44 °C for fecal coliforms. 3). Sulphite Reducing *Clostridium* was determined using enrichment method in a liquid medium. 4). The enumeration of Staphylococci suspected pathogens was conducted using a selective medium (Chapman) and incubated at 37 °C for 24 to 48 hours. A positive culture of Staphylococci is indicated by the formation of a black precipitate surrounded by a white halo. 5). For *Salmonella*, two mediums were used to enumerate the colonies: Selenite-Cystine for enrichment at 37 °C for 12 h, and SS medium (*Salmonella-Shigella*) for isolation at 37 °C for 24 h. *Salmonella* appears like colorless and transparent colonies with or without a black center of small size (2 to 4 mm in diameter).

2.7. Data analysis

The results of the physicochemical analysis were expressed in the form of means ± SD (Standard Deviation). All the colonies were counted as Colony Forming Units per ml of milk (CFU/mL) (IDF, 1991). Average slopes of the historical change of temperature and precipitation across farms were carried out using linear regressions. The data was processed using IBM SPSS Statistics package version 25 (IBM SPSS, 2017). The minimum threshold of significance retained is $p < 0.05$.

3. Results and Discussion

3.1. Climate variability

Across the selected farms, annual average temperature increased by 0.3 ± 0.001 °C yr^{-1} between 1980 and 2018 (Figure 2C). Between 1980-2000 and 2081-2100, future scenarios of climate show that annual average temperature will increase in the region by 1.18°C for SSP1-2.6, 2.33°C for SSP2-4.5 and 4.59°C for SSP5-8.5. Annual precipitation declined by -0.99 ± 0.24 mm yr^{-1} between 1980 and 2018 (Figure 2D), and is projected to decline by 22.5 mm for SSP1-2.6, 44.4 mm for SSP2-4.5, and 95.2 mm for SSP5-8.5 between 1980-2000 and 2081-2100. These data are in accordance with those of Zeroual et al. (2020); (2019) who have shown that predicted increased temperatures may further exacerbate droughts and water shortages, which will lead to an expansion of desert climate zone at the expense of the temperate and steppe climate zones by the end of twenty-first century (2045-2100).

The decrease in precipitation and the increase in air temperature were accompanied by an increase in cropland and a decline in pasture areas. The cropland cover in our study area increased by 90.3% from 1992 to 2005 (Figure 2E, F). The pasture area declined by 53.7% from 1993 to 2009 (Figure 2G, H). This rapid change in land use has impacted the distribution of high-quality foraging lands for livestock, including natural vegetation.

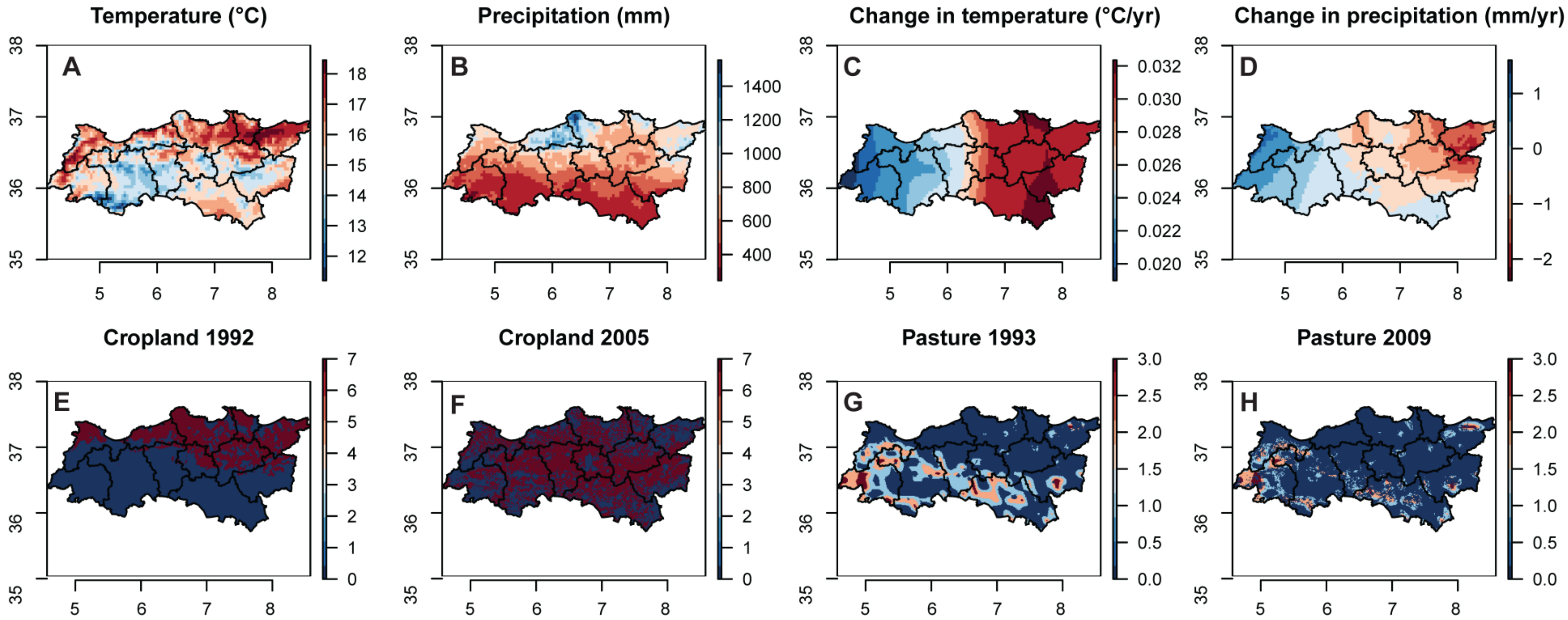

Figure 2. Study area. Environmental characteristics of study area. The plot shows annual average temperature (A), annual precipitation (B), change in annual average temperature during 1980-2018 (C), change in annual precipitation (D), cropland cover in 1992 (E), cropland cover in 2005 (F), pasture cover in 1992 (G), and pasture cover in 2009 (H). Cropland is coded as 0 (absent) or 7 (present). Pastures are scored in four categories (None [0%] = 0, sparse [<12.5%] = 1, medium [>12.5%] = 2, dense [>50%] = 3).

3.2. Socio-Demographic Characteristics

Table 1 summarizes farms data collected from June 2018 to August 2021. The average number of cattle is 14.41 ± 14.65 per farm, with 6.84 ± 8.66 representing the local cattle breed, which includes several traditional ecotypes such as the Guelmois, Sétifien, Cheurfa, and Fawn, meeting the standard of the local bovine breed (Bousbia et al., 2021). The level of education of breeders is often very low, where 39.4% of breeders are unlettered and 34.9% have a primary education level. Low literacy is a concept often observed in rural areas in Algeria, and it’s partly explained by the farms location in remote areas, without schools and cultural centers (Mouhous et al., 2020; Benidir et al., 2020).

All interviewees were men, without any women among 175 respondents. This gender inequality represented by complete men dominance is in agreement with that already found by Laouadi et al. (2018) with small-holders’ goat production systems in the area of Laghouat, located in southern Algeria (only one woman among 106 respondents), and Kadi et al. (2013) in the mountainous area of Kabylie in Algeria (86.2%). Although several studies show that global warming and climate change can amplify the effects of gender inequality in rural communities (Balehey et al., 2018), this question remains poorly documented in Algeria. This could be due to the traditional and cultural structure of society (customs) where men do not let women participate in interviews. Moreover, the majority of the surveyed farmers rely on family labor in their agricultural activities (65.1%). The man is considered the head of the family; he relies on family members to accomplish the various tasks on farm, where women and children play an important role in the functioning of farms.

In general, breeders with more than 20 years of experience are the most prominent, while new investors (≤ 5 years) represent only 0.57% of the total surveyed farmers. Moreover, the low percentage of breeders below 30 years old (1.7%) could indicate that young people are not interested in local cattle raising, and are moving towards the practice of intensive production system and/or other professions with better working conditions, fast and easy revenue such us fattening cattle, poultry farming and business. These results are in agreement to the reports of Laouadi et al. (2018) in Algeria and Yakubu et al. (2019) in Nigeria.

The daily average milk production was 4.13 ± 2.12 L/cow/day very close to those reported for the Algerian local breed (Brown Atlas) with 1400 L/cow/year (≈ 4 L/ cow/day) by Yakhlef (1989). However, they are lower compared to those recorded in the Kabylie region with 10.52 L/cow/day for crossbred cattle (indigenous × Holstein-Friesian of unknown percentage of genetic composition) (Mouhous et al., 2020), and higher than those recorded in the Central of Uganda (2.6 ± 0.19 L/cow/day) for indigenous cattle breed (Nalubwama et al., 2016). Concerning foreign imported breeds, like Holstein and Montbéliarde, which are more adapted to cold climate, milk yield recorded in semiarid and arid climates presents high variability from one farm to another with 1480 to 6703 L/cow/year (4.05 to 18.36 L/cow/day) in the mountainous region of northern Algeria (Bouzida et al., 2010), 9.15 L/cow/day in eastern region of Algeria (Yozmane et al., 2019). In Muscat city in Oman, Alqaisi et al. (2020) reported a yield equivalent to 17.08 and 11.35 L/cow/day for Holstein and Jersey breed, respectively.

It should be noted that milk yields of Holstein breeds remain significantly lower than those reported in cold-climate regions, where these breeds originated. Rémond and Bonnefoy (1997) reported an average milk yield of 29 L/cow/day for multiparous Holstein in France, while a value of 28.3 L/cow/day was reported in Southwest Quebec (Canada) for the same breed (Ouellet et al., 2019), 25.07 ± 5.61 L/cow/day for Holstein breeds in Oita prefecture (Japan) (Kino et al., 2019) and 24.36 ± 0.01 L/cow/day for Holstein breeds in Ukraine during summer (Mylostyvyi et al., 2021).

The reported changes in the climatic conditions negatively affect cattle milk yield which, in its turn, has adverse effects on the farm income. For the period from 1950 to 1999, yields in the United States showed a decrease of 0.55 L/day/cow, which causes an economic loss of 670 US Dollars million per year. Predictive analysis show a more important decrease of 1.35 L/day for the 2050s and 1.84 L/day for the 2080s, with economic losses as a whole of 1.7 billion US Dollars and 2.2 US billion per year in the 2050s and 2080s, respectively (Mauger et al., 2015). Same results were reported by other studies (Mylostyvyi et al., 2021; Gisbert-Queral et al., 2021; Ouellet et al., 2019).

Overall, considering the vulnerability of our study area towards the changing climate conditions, it seems obvious that the exploitation of foreign breeds such as the Holstein breed is not the best adaptation strategy to climate change effects.

Table 1. Socio-economic characteristics of cattle farmers in the study area

Variable		**Results**
Farms		
Total surveyed farm		175
Total animals (Number of cattle)		14.41 ± 14.65
Ecotypes	Number of local cattle	6.84 ± 8.66
	Number of improved-bred cattle	2.10 ± 4.60
	Number of ecotype Guelmois	2.51 ± 2.93
	Number of ecotype Cheurfa	0.60 ± 1.37
	Number of ecotype Sétifien	1.85 ± 2.88
	Number of ecotype Fawn	1.88 ± 3.69
	Crossbreed phenotype (local × local)	2.68 ± 4.78
	Crossbreed phenotype (local × exotic)	2.77 ± 3.98
Farmers		
Gender	Men	175
	Women	0
Age (years)	21-30	3 (1.7%)
	31-40	27 (15.4%)
	41-50	39 (22.3%)
	> 50	106 (60.6%)
Education level (%)	None	39.4
	Primary	34.9
	Medium	19.4
	High	6.3
Experience working on animal production (%)	< 5 years	0.57
	5 at 10 years	2.85
	10 at 20 years	29.75
	> 20 years	66.85
Number of family members		4.01 ± 2.261
Labor		
Family members working in farm (%)	0	61 (34.9%)
	1	66 (37.7%)
	2	29 (16.6%)
	3	13 (7.4%)
	> 4	4 (3.4%)
Economical aspects		
Milk production (L per Female per Day)		4.13 ± 2.12
Production products (%)	Milk	18 (10.28)
	Meat	16 (9.14)
	Mix Production products	141 (80.58)

3.3. Milk properties

3.3.1. Physicochemical properties of raw milk

Table 2 shows the physicochemical properties of raw milk collected from local bovine breeds in the northeastern Algeria. In general, lactose, protein and fat content, corresponds to the values of cow's milk standards. Matallah et al. (2017) showed similar results for raw cow milk from El Taref province with an average pH of 6.9 ± 0.37 vs. 6.5 ± 0.07, acidity of 18.7 ± 3.32 vs. 18.9 ± 1.11°D, density of 1031 ± 0.6 *vs.* 1030 ± 2.78, protein content of 32.8 ± 4.32 g/l *vs.* 32.51 ± 8.87 g/l, but a lower fat content of 33.3 ± 3.93 g/l vs. 33.99 ± 14.47 g/l. Moreover, when comparing the results of this study with those conducted in four provinces (Guelma, Souk Ahras, Annaba, and El Taref) by Mahieddine et al. (2017), the results were within the range of acidity values [16.83-20.71°D], fat [32.01-60.00 g/l], lower for pH [6.97-7.23], but greater for density [1025-1027 kg/m^3] and protein [28.7-31.23 g/l]. Furthermore, a study in the Kabylie region in highlands of central-North Algeria showed higher density (1032 ± 0.06 kg/m^3), fat (61.6 ± 2.64 g/l) and protein (69.8 ± 5.61 g/l). This could be due to the higher plant species richness and abundance in mountain areas (Manganelli et al., 2001). In the same way, milk qualities results were also close to ours obtained in recent study on raw cow milk heat treatments effects in northeastern Algeria (Tadjine et al., 2019), and in the study on the raw milk of central Algerian farms from Tissemsilt province conducted by Elhadj et al. (2015).

From the literature, and especially for extensive livestock farming system, where grazing is the main source of feed, the nutritional composition of milk is highly related to changes in feed quality and availability, which itself varies according to the climatic conditions (Rojas-Downing et al., 2018). Cattle grazing on poor quality pastures during periods of drought would lead to a decrease in dry matter intake and therefore lower milk, protein and casein yields (Pastorini et al., 2019).

Table 2. Physicochemical characteristics of the analyzed samples

Parameters	N	Min-Max	Mean ± SD	CV (%)	Standard
pH	122	5.68-7.76	6.95 ± 0.37	5.42	6.6 to 6.8
Density (mg/cm^3)	122	1.005-1.044	1.031 ± 0.006	0.58	1.028 to 1.033
Freezing point (°C)	122	-0.80 - -0.19	-0.56 ± 0.06	-11.54	-0.53 to -0.55
Conductivity (µS/cm)	122	4.20-8.03	5.03 ± 0.52	10.50	4 to 5.5
Titratable Acidity (°D)	122	10.33-31.33	18.78 ± 3.32	17.67	15 to 17
Fat content (g/kg)	122	10.83-86.70	33.99 ± 14.47	42.58	31 to 33
Protein content (g/kg)	122	11.10-51.03	32.51 ± 8.87	27.28	32 to 34
Lactose (g/kg)	122	40.10-66.03	49.49 ± 4.27	8.63	45 to 51
Minerals and Vitamins (g/kg)	122	5.23-8.79	7.25 ± 0.43	6.02	7 to 7.5
Dry Degreased Extract (g/kg)	122	22.63-108.93	87.55 ± 10.22	11.68	91

N: Number of the analyzed samples; SD: Standard Deviation; CV: Coefficient of Variation; Max: maximum; Min: minimum.

3.3.2. Bacteriological qualities of raw milk

The descriptive characteristics of the enumerated flora reported in Table 3 show high variations between the different raw milk samples studied for the seven microbial groups analyzed. The concentrations values of Total Mesophilic Aerobic Flora of raw milk varied between 1.49 and 1.81×10^7 CFU ml^{-1} with an average of 2.55×10^5 CFU ml^{-1}. Moreover, the results show that 9% of the total analyzed samples exceed the standard of 10^5 CFU ml^{-1} required by the JORA (1998), indicating a very poor quality of raw milk. These finding are consistent with those reported by Bousbia et al. (2018) and Bachtarzi et al. (2015) in the same traditional extensive livestock system, where high contaminations were found with an average values of 11.69×10^5 CFU ml^{-1} and 28.8×10^6 CFU ml^{-1}, respectively.

The results of total and fecal Coliforms showed significant contamination with an average of 3.02×10^4 and 1.09×10^3 CFU ml^{-1}, respectively. These values were extremely variable with standard deviations exceeding the average for each flora. 17.21% of all analyzed samples are not complying with national standards for fecal Coliforms (JORA, 1998), they are similar to the results obtained by (Bachtarzi et al., 2015) in the region of Constantine with an average of 3.67×10^5 CFU ml^{-1}, but they are much lower than the results reported by Sraïri et al. (2005) in Morocco with an average of 2.0×10^6 CFU ml^{-1}. The average enumerations were very variable from one farm to another; this can result from the lack of hygiene practices, which remains scarce (washing the udder before and after the milking). The presence of Coliforms indicates poor hygienic and sanitary conditions during the milking and the subsequent manipulations (Yucel and Ulusoy, 2006).

The sulphite reducing *Clostridium* have been detected in 31 (25.40%) analyzed samples with an average of 1.15×10^3 UFC ml^{-1}. A contamination of 16.30 CFU ml^{-1} was reported by Bousbia et al. (2018) in the same region of Algeria. To our knowledge, few studies were conducted to estimate the frequencies of pathogenic bacteria in cattle raw milk collected from

Algeria; Hamdi et al. (2007) found that among 153 samples of milk collected from farms in Algiers and Blida, 3.18% were contaminated. For all analyzed samples, only 16 (13.11%) samples do not contain *Staphylococcus aureus*. The presence of high content of *S. aureus* in raw cow milk samples could be explained by the presence of mastitis (Montcho et al., 2021), or poor hygienic conditions. In addition, the contamination spreads very quickly, under favourable conditions such high temperatures and humidity, causing risk to human health (Alghizzi and Shami, 2021).

Microbiological analysis has shown that one sample was contaminated by *Salmonella spp.* The origin of this contamination might be related to the unhygienic husbandry practices in traditional extensive livestock system (Montcho et al., 2021). In our study, several breeders have confirmed that they apply dung and urine on pasture to help fodder production during drought period, the urea is used to increase nutritive value of poor fodder, which is in agreement with the literature (Gunun et al., 2013). However, animal manure and urea might be sources of milk contamination, especially when hygienic conditions are absent (Montcho et al., 2021). Moreover, manure is considered as an important source of GHGs emissions (25% from total livestock GHGs emissions), mainly as methane and nitrous oxide, which can exacerbate global warming (Tubiello et al., 2015; Petersen et al., 2013). Consequently, microbial contamination increases under the effect of environmental temperatures elevation (Zweifel et al., 2005).

Table 3. Descriptive characteristics of studied flora and milk standards

Flora (CFU ml^{-1})	**N**	**Min-Max**	**Mean ± SD**	**CV (%)**	**Standard (CFU ml^{-1})**
TMAF	122	$1.81\text{-}1.49 \times 10^{7}$	$2.55 \times 10^{5} \pm 1.79 \times 10^{6}$	6.99×10^{2}	10^{5}
T. Col.	122	$0\text{-}2.36 \times 10^{6}$	$3.02 \times 10^{4} \pm 2.16 \times 10^{5}$	7.14×10^{2}	10^{3}
F. Col.	122	$0\text{-}1.81 \times 10^{4}$	$1.09 \times 10^{3} \pm 3.06 \times 10^{3}$	2.79×10^{2}	10^{3}
Sulphite reducing *Clostridium*	122	$0\text{-}1.00 \times 10^{5}$	$1.15 \times 10^{3} \pm 9.28 \times 10^{3}$	8.03×10^{2}	50
Staphylococcus	122	$0\text{-}8.00 \times 10^{6}$	$2.12 \times 10^{5} \pm 9.75 \times 10^{5}$	4.58×10^{2}	Absence /0.1 ml
Salmonella	122	$0\text{-}2.6 \times 10^{5}$	$2.13 \times 10^{3} \pm 2.35 \times 10^{4}$	1.10×10^{3}	Absence
Yeasts and molds	122	$0\text{-}1.6 \times 10^{3}$	$1.11 \times 10^{2} \pm 2.48 \times 10^{2}$	2.22×10^{2}	/

TMAF: Total Mesophilic Aerobic Flora; T. Col.: total Coliforms; F. Col.: fecal Coliforms; N : Number of the analyzed samples; SD: Standard Deviation; CV: Coefficient of Variation; Max: maximum; Min: minimum.

4. Conclusion

In this study, it is confirmed that climate change is influencing the temperature and precipitation level, cropland and pasture areas in the study areas. Moreover, taking into account the productive data, it could be concluded that the exploitation of the Algerian local breeds seems to be one of the best adaptation practices to climate change effects as it can promote biodiversity and keep a balanced ecosystem. Nevertheless, smallholder farmers have a low educational level and small farms characterized by low productivity, poor bacteriological quality of milk but an acceptable physicochemical quality.

The implementation of selection and genetic improvement programs can increase the productivity and profitability of local cattle breeds. This can be beneficial for smallholder farmers and can provide them with a fair and stable income and good working conditions. Other strategies can also contribute to the fight against climate change effects like women empowerment promotion, policy issues development, development of suitable capacity building programs for different stakeholders. These could contribute significantly to social equity and local economies.

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Disclosure statement

The authors declare no potential conflicts of interest.

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Data availability

The data collected and analyzed for this study can be shared upon request.

Declarations

All the participants to the study were informed about the purpose of the study and gave informed oral consent to participate in the study. All the interviewees participated on a voluntary basis and discussion was held (at the end the interviews) to share our understanding on what will be reported in the study.

Author Contributions

Ali BOUDEBBOUZ: Investigation (Data collection, and experiments performing), Formal analysis, Writing – original draft.

Meriem Imen BOUSSADIA, Asma BEN CHABANE, Lamiss BOUMENDJEL, Meryem SAHRI, Dounya Achwak CHEMMAM: Investigation (Data collection, and performing the experiments).

Rassim KHELIFA, Aissam BOUSBIA, Yassine GUEROUI: Conceptualization, Supervision, Data curation, Investigation, Writing – review & editing.

Sofiane BOUDALIA and George SYMEON: Conceptualization, Supervision, Data curation, Formal analysis, Funding acquisition, Project Administration, Methodology, Writing – original draft– Review & Editing.

All authors have read and agreed to the published version of the manuscript.

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CHAPTER 3

DETERMINATION OF HEAVY METAL LEVELS AND HEALTH RISK ASSESSMENT OF RAW COW MILK IN GUELMA REGION, ALGERIA

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Determination of Heavy Metal Levels and Health Risk Assessment of Raw Cow Milk in Guelma Region, Algeria

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Abstract

During the recent decades, adverse effects of unexpected contaminants, such as heavy metals on raw cow milk quality, have threatened human health. The objective of this study was to determine heavy metal levels in raw milk collected from autochthonous bovine breeds in the eastern region of Algeria. Eighty-eight pooled milk samples were analyzed using atomic absorption spectrometry for Pb, Cd, Cr, Cu, Ni, Fe, and Zn, and dietary risks were estimated for infants, children, and adults with minimum, average, and maximum milk consumption scenarios. Results revealed that Pb (0.94 ± 0.49 mg/kg), Cd (0.03 ± 0.01 mg/kg), and Cu (0.14 ± 0.08 mg/kg) levels in all analyzed samples were higher than their corresponding maximum residue levels (MRLs). The task hazard quotient (THQ) values suggest potential risk for infants in the three scenarios from Pb, Cd, and Cr; for children in the three scenarios from Pb and in the high scenario from Cr; and for adults in the medium and high scenarios from Pb. The hazard index (HI) values were higher than 1, and the contributions of each metal to the overall HI followed a descending order of Pb, Cr, Cd, Ni, Zn, Cu, and Fe with values of 68.19%, 15.39%, 6.91%, 4.94%, 3.42%, 0.88%, and 0.28%, respectively. Our results indicated that there may be a potential risk of heavy metals, especially Pb, for infants through raw cow milk consumption. Moreover, data actualization and continuous monitoring are necessary and recommended to evaluate heavy metal effects in future studies.

Keywords Risk assessment · Heavy metals · Raw cow milk · Hazard index · Maximum residue levels · Permissible limits

Introduction

The consumption of milk and its derivatives belongs to the most ancient eating practices [1] since these products are considered the most balanced food found in nature, containing major sources of nutrients, especially for children, adults, and elderly people [2, 3]. Milk provides a good source of macro- and micronutrients such as lipids and proteins (polyunsaturated fatty acids), calcium, phosphorus, essential amino acids, carbohydrates, vitamins, and several bioactive compounds that play a vital role in biochemical and physiological functions [4–6]. In addition to its importance, worldwide milk consumption per capita is projected to grow in developed countries from 22.2 in 2015 to 23.1 kg in 2027 and from 10.6 to 13.5 kg in developing countries [7].

In Algeria, FAO [8] reported that the per capita milk consumption is about 0.276 kg/day; however, this value does not seem to correspond to the regional value of milk and dairy product consumption in the rural and pre-urban areas. Using a survey of 750 consumers in the Tebessa region (east of Algeria), Bentaleb et al. [9] reported that the daily calcium intake (854.4 ± 364.5 mg/day) corresponded to daily consumption of 0.733 ± 0.312 kg of milk equivalent. Moreover, Belhadia et al. [10] reported that a non-negligible quantity of raw cow milk is used to supplement breastfeeding and

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family self-consumption and is also sold through uncontrolled (informal to the) pre-urban agglomeration (80% of local production). These important quantities were not included in official data on milk consumption in Algeria. Furthermore, it is estimated that almost 80% of the milk marketed is handled by informal market traders. Milk produced by dairy cattle farms, and especially from extensive livestock, is sold directly to urban markets [11]. Therefore, the safety of raw cow milk must be assured, even more so when 80% of this milk is consumed directly by rural and pre-urban populations [10].

The main source of heavy metals in the different foods including cow milk is the environmental contamination [12], which originates from the earth's crust and anthropogenic activities such as fuel combustion, the proximity of roads, mining and industrial areas, municipal and agricultural wastewater, and solid wastes [12–14]. Plants and fodder grown on soils irrigated with contaminated water permit heavy metals to pass into the land and water and then accumulate in the animal feed [15–17]. Heavy metals may enter the trophic chain through the consumption of animal feed and are finally ingested by animals and human bodies [18]. The estimated human health risk for human revealed that ingestion is the primary route of exposure to heavy metals [19]. Lead (Pb) and cadmium (Cd) have no known beneficial role for animals and plants [20, 21]. These metals are counted among the most potential toxic substances, causing carcinogenic, neurotoxic, nephrotoxic, and hematologic effects [22, 23], as well as reproductive disorders even at low concentrations [24]. Other metals, like nickel (Ni), zinc (Zn), chromium (Cr), copper (Cu), and iron (Fe), are essential nutritional elements for the human body; they are all crucial for the metabolism, and they play an important role in biochemical functions [25]. Nevertheless, their presence at high concentrations in animals and the human body may become harmful to human health [13, 26]. Consuming foods containing metals may cause harmful effects on human health, such as renal dysfunction, raise blood pressure, and reduction in intelligence quotient in case of Pb [27]; teratogenic, carcinogenic, and neurotoxic in case of Cd [28, 29]; neurologic and immunologic in case of Ni [30]; cytotoxicity in the case of Zn [31]; and Wilson's disease, cramps, and nausea in case of Cu [32]; Fe can cause tissue damage and organ failure and increases the risk of cancer [33]. It should also be noted that the non-carcinogenic and carcinogenic risk of heavy metals related to milk consumption is affected directly by milk consumption quantity.

To our knowledge, no health risk assessment has been provided to estimate the carcinogenic risks among infants, children, and adults following the consumption of raw cow milk in Algeria [11]. Therefore, this study aimed (i) to determine the levels of heavy metals (Pb, Cd, Cr, Cu, Ni, Fe, and Zn) in raw cow milk samples collected from eighty-eight extensive dairy farms located in different areas in Guelma Province, Algeria; (ii) to compare results of this study with data reported in published studies related to raw milk metals from different countries; and (iii) to estimate daily intake (EDI), hazard quotient (HQ), and hazard index (HI) of heavy metals from consuming raw cow's milk.

Materials and Methods

Ethical Statement

This study was carried out as part of the Bovisol project (Breeding and management practices of indigenous bovine breeds: Solutions towards a sustainable future, www.rias.gr/bovisol). The Bovisol project (2018–2022) was a cooperation of scientific teams from Greece, Tunisia, and Algeria [34]. The local Data Protection Board (DPB) and the local ethics committee have approved experimental protocols. The study involved data and/or milk sample collection from different farms, and participants were informed of the purpose of the project; they have given their consent for their participation (complete the survey questionnaire and/or provide milk samples) and the use of the collected data and the generated results from our analysis for scientific publications [35]. For this research article, we present only toxicological results concerning milk collected in Algeria.

Milk Sampling

From the 175 visited farms, only 122 farmers agreed to provide milk samples for analysis. Of the 122 samples, 88 were used to perform the toxicological analysis (heavy metals). Raw milk samples were collected during the spring of 2019 from different farms located in different regions of Northeast Algeria (Guelma province) as shown in Fig. 1. From each farm, about 0.5–1 L of milk was collected in sterile glass bottles, placed immediately in a cooler and then transported to the laboratory, where it was stored at − 20 °C until analysis. All bottles were previously autoclaved at a temperature of 121 °C, under a pressure of 1 bar for 15 min. The vials were filled from a container of mixing milk, respecting the Good Laboratory Practices (GLP) and the rules of asepsis (disinfection of the hands). In order to take account of the real field conditions, no conservative was added.

Heavy Metal Quantification

Raw milk samples (10 mL) were digested using 4 mL nitric acid (HNO_3) at 68% (CAS: 7697–37-2) (Sigma Aldrich, St. Quentin Fallavier, France) in ceramic capsules. The acid solution of milk was digested in a heating plate up to 370 °C for 1 h and then placed in a muffle furnace at 500 °C for

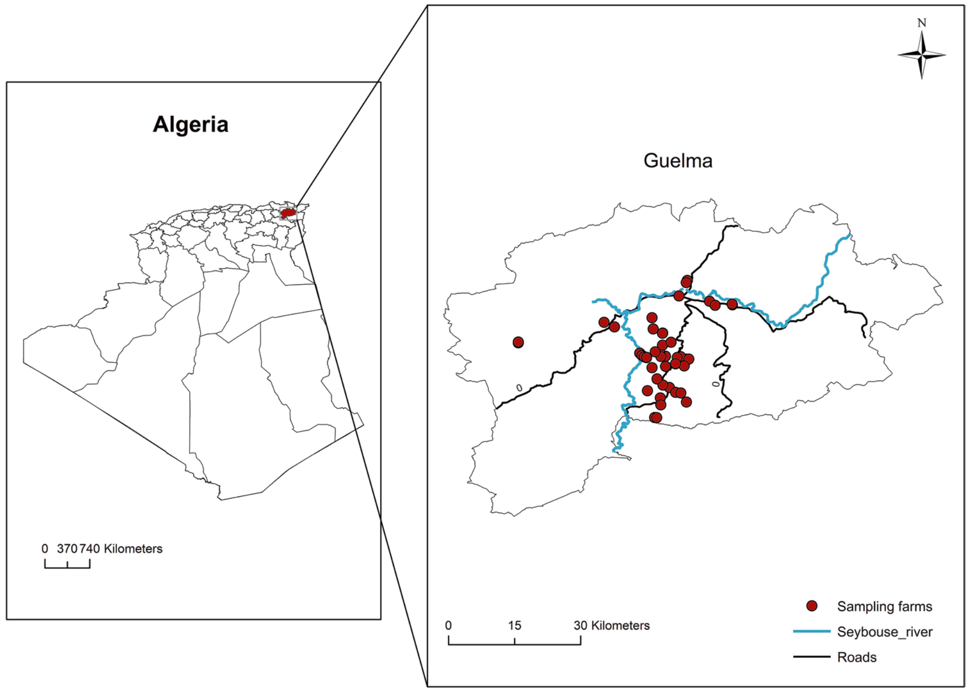

Fig. 1 Study area. Map showing the locations of the municipalities investigated. Map created using the Free and Open Source QGIS

4 h. The volume of digested samples was made up to 50 mL with distilled water containing 1% (v/v) of HNO_3. After, they cooled to the room's ambient temperature. Digested milk sample was filtered through a 0.45-mm membrane filter (Merck, Lyon, France) before analysis. Levels of Pb, Cd, Cr, Fe, Zn, Ni, and Cu were detected using a flame atomic absorption spectrometry (FAAS) (Aanalyst400, Perkin Elmer, USA).

Blanks, which were prepared with acid treatment, without samples, were subject to the same digestion procedures. Standard calibrations were developed to quantify the amounts of Pb, Fe, Cr, Ni, Zn, and Cd in raw milk samples. The standard solutions were prepared with multi-element standard solution (10 mg/L, SPEX, USA), and the calibration curves for Pb, Fe, Cr, Ni, Zn, and Cd were prepared based on six points. For all metals, the correlation coefficient of the regression line was calculated. The final results are the average of triplicates. The concentrations of each element are expressed in mg/kg.

Quality Control Procedure

All of the products complied with the quality standards of the European Pharmacopoeia (http://www.edqm.eu/en/Homepage-628.html). The developed method was validated for instrumental linearity and range, precision (expressed as relative standard deviation, RSD), accuracy, limit of detection (LOD), and limit of quantification (LOQ). Moreover, to check for possible contamination from the bottles used for storing the milk samples, three empty bottles were filled with 10 mL distilled water and placed in a – 20 °C freezer for the same duration of time as the milk samples. The thawed water aliquots were then analyzed for target element traces, and the results showed no evidence of target heavy metal contamination.

Linearity study was demonstrated by analyzing six different concentrations of heavy metals. For all metals, correlation coefficient of the regression line was greater than 0.99. In order to determine LOD and LOQ, ten blank samples were measured. For each element, LOD and LOQ were quantified as three times and ten times the standard deviation (SD) of the blank values, respectively [36, 37]. The LODs (LOQs) obtained for Ni, Zn, Cr, Cd, Cu, Fe, and Pb were 0.002 mg/kg (0.0055 mg/kg), 0.11 mg/kg (0.29 mg/kg), Cr 0.002 mg/kg (0.0056 mg/kg), 0.001 mg/kg (0.001 mg/kg), 0.009 mg/kg (0.026 mg/kg), 0.015 mg/kg (0.063 mg/kg), and 0.012 (0.036 mg/kg).

To ensure the accuracy of the analytical method, the recovery studies were carried out by adding a known quantity of analyte pre-analyzed by the proposed method. To check the accuracy of the analytical method, the recovery studies were performed to confirm the losses of heavy metals or contamination during sample preparation as well as matrix interferences during the measurement step. For the determination of the recovery, the spiking technique was used, i.e., the known concentration of Fe solution was added to the milk sample, and the resulting spiked samples were measured, calculated, and compared to the known value of Fe solution added. All analytical steps were performed in three replicates with three different levels of Fe concentration. All of the results of percentage recoveries for the studied metals ranged between 98.29 to 101.25%, which is within the expected SAA performance. The results of the recovery tests for samples were within the acceptable range for most metals [12]. Triplicate measurements of each sample ($n = 88$) were used for the analysis of trace metals in milk samples; values of relative standard deviations (% RSD) were less than 10% for all of the mean concentrations of metals; otherwise, the results were rejected according to the recommendations of Mitra and Brukh [38], and the measurement was repeated through a full reanalysis on new digestion.

Risk Assessment

Estimated Daily Intake (EDI)

Estimated daily intake (EDI) of Pb, Cd, Fe, Ni, Zn, and Cr by consumption of raw cow milk was calculated according to the following equation [39].

$$EDI = \frac{(CMetal * WMilk)}{BW}$$

where C_{Metal} (mg/kg, on wet weight basis) means the metal level of raw cow milk samples and W_{Milk} represents the daily average consumption of raw cow milk (kg/day). We assumed three consumption scenarios: low scenario (1 serving/day), average scenario (2 servings/day), and high scenario (3 servings/day) (consistent with the recommended serving size for the Food Dome Dietary Guidelines for Arab Countries) for infants, children, and adults, respectively [40, 41]. Moreover, to detect the most exposed groups, estimated daily intake was calculated for people of 3 various age groups: infant, children, and adult, taking into account the average body weight and milk intake.

BW (body weight) represents the average body weight of 10 kg for infants, 30 kg for children, and 70 kg for adults [42].

The EDI (mg/kg BW/day) of Pb, Cd, Cr, and Zn was compared with provisional tolerable daily intake (PTDI) set by the Joint FAO/WHO Expert Committee on Food Additives [43–45], while the daily intake (mg/day) of essential heavy metals (Cu, Ni, and Fe) was compared with recommended dietary allowances (RDAs) values established by the Food and Nutrition Board of the Institute of Medicine [46].

Target Hazard Quotient Determination

To assess the human health risk related to the consumption of raw cow milk with heavy metals, the target hazard quotient (THQ) was established by the US Environmental Protection Agency [47] for the estimation of non-carcinogenic risk associated with the reference dose and exposure. The THQ was evaluated based on the following equations [31, 48].

$$THQ = \frac{EDI}{RfDo}$$

In the above equation, EDI is already explained, and RfDo is the reference oral dose (mg/kg/day). The reference dose (RFDo) for Cd, Pb, Zn, Cu, Ni, Fe, and Cr are 0.001, 0.0035, 0.3, 0.04, 0.02, 0.7, and 0.003 mg/kg BW/day, respectively [49–51]. If the THQ value is greater than 1, potential non-carcinogenic effects could occur, whereas adverse health effects would be unlikely experienced when THQ < 1 [47, 52].

Hazard Index Determination

During our life, we are exposed to mixtures of contaminants and pollutants most often present in very low doses [53, 54]. Hazard index (HI) was performed to assess the cumulative risk of more than one metal contained in raw cow milk calculated by summing the THQ of each metal in this study according to the following equation [24].

$$HI = THQ_{Pb} + THQ_{Cd} + THQ_{Cr} + THQ_{Fe} + THQ_{Ni} + THQ_{Cu} + THQ_{Zn}$$

HI value below 1 means that the risk for human consumption was considered acceptable and safe, whereas an HI index higher than 1 indicates that its consumption should be considered a potential health concern [55–57].

Data Analysis

The results are expressed in the form of the mean ± SD (standard deviation). Data were analyzed using R 4.1.2 version [58]. Exposure to heavy metals from milk consumption in this region was calculated considering low (1 serving/day), medium (2 servings/day), and high (3 servings/day) daily milk consumption by infants, children, and adults.

Results and Discussions

Concentrations of Heavy Metals in Raw Cow Milk

Descriptive statistical data of the seven heavy metal concentrations in the raw cow milk collected from eighty-eight traditional farms with native bovine breeds in Guelma district in Algeria are presented in Table 1. The average concentrations (mg/kg) of the heavy metals were ranked as follows: Zn (4.02 ± 0.89) > Pb (0.94 ± 0.49) > Fe (0.76 ± 1.25) > Ni (0.39 ± 0.68) > Cu (0.14 ± 0.08) > Cr (0.18 ± 0.20) > Cd

Table 1 Heavy metal levels (Ni, Zn, Cr, Cd, Cu, Fe, and Pb) in raw cow's milk (mg/kg) in Guelma area, Algeria

Element	Ni	Zn	Cr	Cd	Cu	Fe	Pb
Mean ± SD	0.39 ± 0.68	4.02 ± 0.89	0.18 ± 0.20	0.03 ± 0.01	0.14 ± 0.08	0.76 ± 1.25	0.94 ± 0.49
% Samples exceeding MRLs	5.68	82.95	15.90	100	100	42.04	100
Min	0.007	0.33	0.007	0.01	0.032	0.063	0.041
Max	4.11	6.48	1.28	0.06	0.51	7.82	2.15
MRL	1	3.28	0.2	0.0026	0.01	0.37	0.02

SD standard deviation, *MRL* maximum residue limit values

(0.03 ± 0.01). The average concentrations of Cd, Zn, Cu, Fe, and Pb were above the maximum residue levels (MRLs) set by the International Dairy Federation (IDF) of 0.0026, 3.28, 0.01, 0.37, and 0.02 mg/kg, respectively [59], while average concentrations of Ni and Cr were below MRLs (Table 1). The results of our study revealed that the concentrations of Pb, Cd, and Cu in all the analyzed samples were more than their corresponding MRLs, while 82.95%, 42.04%, 15.90%, and 5.68% of Zn, Fe, Cr and Ni samples, respectively, exceeded their MRLs.

The high concentrations of Cd in raw cow's milk in the study area are related mainly to food production. Olsson [60] reported that the concentrations of Cd in raw milk vary depending on the Cd content in food production. A study conducted by Tu et al. [61] suggested that the main sources of Cd in animal feed are different minerals such as phosphate, fish meal, and trace element. Moreover, Caggiano et al. [62] indicated that Cd concentrations in milk coming from low environmental pollution areas were higher than those found in industrial and polluted areas. The highest levels of Pb in all analyzed milk samples might be mainly a consequence of Pb emission in the water and soil from road traffic as leaded gasoline is the major source of Pb in the atmosphere [63, 64]. Recently, and to counteract this Pb pollution, the Algerian government proceeded to the generalization of unleaded fuel throughout the country [65], a decision already taken in Europe since the 1970s, and it has already shown its effectiveness [66].

The highest Zn and Cr concentrations in raw cow milk were recorded in Bouchegouf and Djebala villages, where farmers of these villages use the Seybouse River for irrigation, farming, and watering animals. The Seybouse River would be the probably main contamination factor of heavy metals in water and animals feed, where high concentrations of Pb 1.75 ± 0.04 mg/L (1.72–1.80 mg/L), Cd 0.07 ± 0.00 mg/L (0.07–0.08 mg/L), and aluminum (Al) 0.37 ± 0.03 mg/L (0.36–0.39 mg/L) have been recorded [67]. Talbi and Kachi [68] reported high concentrations (58.8–235.2 mg/kg) of Ni in different locations of sediment in Seybouse River. The highest values of Fe in raw cow milk are related mainly to crustal sources. Moreover, Fe is associated with anthropogenic activities such as industrial processes and traffic sources [64]. The highest Fe concentration was observed in Maouna region close to a marble deposit area and brick factories, which is in agreement with data published previously in the same region [13].

Heavy metal concentrations in raw cow milk reported by previous studies across the globe are summarized in Table 2. The Cd level measured in this study was consistent with the levels reported in milk collected from cows reared close to highways in Turkey [69], as well as from milk collected in dairy farms in Bangladesh, [22], and from cows reared near the metallurgical complex in India [70], but were lower than the Cd concentrations reported in Slovak, from cows reared in agricultural sector [71], and in Ethiopia from high mineral enrichment and metal leaching [51]. However, the level was higher than the Cd level reported in Sudan from cows reared around sugar cane plants [72] and from cows reared in a rural region in Libya [73].

Fe concentrations detected in this study were in agreement with those reported by Ahmad et al. [86] in Pakistan but were lower than the Fe concentration reported in the trans-Himalayan high-altitude region in India [83] and Turkey, near highways. However, the concentration of Fe was higher than the concentration reported in China by Zhou et al. [75] and in Poland from milk collected in intensive production farms [84].

Lower concentrations of Cu than those recorded in this study were reported in industrial regions in Iran [81] and in Bangladesh [74] and higher than the level reported in Bangladesh from dairy farms [22], in Poland from intensive milk production farms [84], and in Romania from eastern Carpathians Rodnei mountains [87].

Concerning Ni, concentrations found in this study were higher than levels reported in Iran from traditional dairy farms [82], and in Pakistan from farms located near the cities with high urbanization and industrialization [30], but lower than levels reported in Slovak from the agricultural sector in Nitra region [71] and copper mining areas in India [80].

The level of Zn in raw cow milk collected in our study region was higher than the level reported in the trans-Himalayan high-altitude region in India [83] and from high mineral enrichment and metal leaching in Ethiopia [51]. However, Zn

Table 2 Heavy metal levels (Ni, Zn, Cr, Cd, Cu, Fe, Pb) in raw cow's milk published reported in research articles from other countries (mg/kg)

Country/specific location	Heavy metal levels (mg/kg), mean ± SD, and/or range (min–max)							Reference
	Ni	Zn	Cr	Cd	Cu	Fe	Pb	
Bangladesh/dairy farms	-	-	0.373 ± .008	0.02 ± 0.1	0.06 ± 0.01	0.33 ± 0.05	0.015 ± 0.002	Muhib et al. [22]
Bangladesh/industrial disposal areas	0.07–0.08	-	-	0.001–0.02	0.05–0.06	0.76–1.23	0.04 ± 0.04	Jolly et al. [74]
China	0.0057	-	-	0.00007	0.03	0.35	0.0014	Zhou et al. [75]
China/industrial areas	-	-	0.0018 ± 0.0012 0.00054–0.007	0.00011 ± 0.0006 0.0002–0.00031	-	-	0.002 ± 0.0036 ND–0.015	Su et al. [76]
Ethiopia/close to highways	BDL	6.21 ± 0.09	-	BDL	0.09–0.12		BDL	Belete et al. [77]
Ethiopia/high mineral enrichment and metal leaching	-	3.02 ± 0.10	0.64 ± 0.029	0.29 ± 0.01 (0.24–0.33)	1.12 ± 0.06 (0.84–1.53)	-	0.15 ± 0.01 (0.12–0.19)	Akele et al. [51]
Egypt/industrial air pollution areas	-	-	0.35 ± 0.10	0.02 ± 0.002	1.2 ± 0.4	5.1 ± 1.3	0.6 ± 0.2	El Sayed et al. [78]
Ethiopia/watering in textile treatment pound	1.6 ± 0.3	-	0.8 ± 0.2	0.2 ± 0.01	-	-	0.8 ± 0.1	Fenta [79]
India/copper mining areas	0.62	-	0.12	-	0.5	8.8	0.13	Giri, Singh [80]
India/iron mining area		-	0.12	-				Giri et al. [20]
Iran/industrial regions		0.57 ± 0.19	-	0.0011 ± 0.00051	0.42 ± 0.19		0.014 ± 0.0026	Shahbazi et al. [81]
India/metallurgical complex	-	-	-	0.02 ± 0.007	-	-	0.58 ± 0.018	Chirinos-Peinado, Castro-Bedriñana [70]
Iran/traditional dairy farm	0.04	-	-	-	-	-	-	Arianejad et al. [82]
India/Trans-Himalayan high-altitude	-	3.76 ± 0.33	-	0.0089 ± 0.0002	0.30 ± 0.01	4.91 ± 0.37	0.0056 ± 0.0003	Giri et al. [83]
Libya/rural region	-	-	-	0.0009	-	-	BDL	Elatrash, Atoweir [73]
Poland/intensive production farms	-	-	-	0.007 ± 0.004	0.04 ± 0.01	0.25 ± 0.06	0.01 ± 0.005	Król et al. [84]
Pakistan/near to the cities	0.01–0.08	-	-	0.001–0.004	0.04–0.09	-	0.014–0.033	Ismail et al. [30]

Table 2 (continued)

Country/specific location	Heavy metal levels (mg/kg), mean ± SD, and/or range (min–max)							Reference
	Ni	Zn	Cr	Cd	Cu	Fe	Pb	
Pakistan/urban area	0.8 ± 0.006	-	-	0.7 ± 0.07	1.4 ± 0.009	-	2.2 ± 0.2	Iftikhar et al. [85]
Pakistan	-	-	-	0.08 ± 0.006	0.14 ± 0.02	0.7 ± 0.3	BDL	Ahmad et al. [86]
Romania/Rodnei mountains	-	-	-	0.001	0.047	-	0.006	Cadar et al. [87]
Sudan/grazed around sugar cane plants	-	-	-		0.12	-	0.5	Abdalla et al. [72]
Slovak/agricultural sector	0.84 ± 0.13	13.09 ± 1.42	-	0.27 ± 0.06	2.12 ± 0.61	1.76 ± 0.36	3.8 ± 0.42	Capcarova et al. [71]
Turkey/close to highways	-	3.04 ± 0.12	0.13 ± 0.006	0.39 ± 0.02	0.62 ± 0.01	4.2 ± 0.16	1.85 ± 0.09	Bigucu et al. [69]

SD standard deviation, *BDL* below detection limit,—no data available

concentrations were lower than those reported by Capcarova et al. [71] in agricultural areas in Slovak and close to those reported near highways in Borena region in Ethiopia [77].

The level of Cr reported in our area was higher than the level reported near heavy industry plants in Turkey [69] and India from cows rearing near the vicinity of iron mining areas [55]. Cr levels were lower than levels reported in Egypt near industrial air pollution areas [78] and in Ethiopia from cows watering in textile treatment pounds [79].

Reported Pb levels in the present study were lower than levels measured close to highways in Turkey [69] and Pakistan from milk collected in urban areas [85]. However, they were higher compared to those recorded in raw cow milk from industrial areas in China [76] and a high mineral enrichment and metal leaching area in Ethiopia [51].

Health Risk Assessment

Estimated Daily Intake (EDI) and Tolerable Daily Intake (TDI)

Results of EDI, PTDI, and RDA of the metals in question are summarized in Table 3. The EDI values of Ni, Zn, Cu, Fe, and Cr for infants, children, and adults and in the three scenarios are lower than the relative legal limit (PTDI and RDA) values, and thus it can be suggested that raw cow milk consumption does not pose a health risk for consumers of all ages for Ni, Zn, Cu, Fe, and Cr. Most studies showed similar results to those reported in Guelma region. Boudebbouz et al. [39] reported that the EDI values of Cu and Fe across the globe were lower than their RDAs, while the EDI value of Ni was reported slightly higher than its RDA only in four out of 29 regions across the globe.

The mean EDI value of Cd was below 0.0083 mg/kg BW/day for an adult and children with the three scenarios and for an infant with the low scenario indicating that there is no health risk associated with the Cd intake from milk consumption for an adult and children consuming 1, 2, or 3 servings of cow milk per day and for infant consuming only 1 serving of cow milk per day (Table 3). However, the EDI of Cd was above 0.0083 mg/kg BW/day for an infant with medium and high scenarios indicating that there is a health risk associated with Cd intake from milk consumption for an infant consuming 2 or 3 servings of raw cow milk per day. In Peru, the same results were reported by Castro-Bedriñana et al. [88] in the extensive farming system near mining-metallurgical industries areas, where the EDI of Cd for adult consumers is below the PTDI value while in 2-year-old children with a high milk intake is exceeding the PTDI.

Results showed that the mean EDI value of Pb was far above 0.0036 mg/kg BW/day for an infant, children, and adult with the three scenarios indicating that there is a health risk associated with Pb intake from milk consumption for all ages consuming 3, 2, or even 1 serving by day. Several studies have reported values of Pb EDI higher than their PTDI values [71, 89].

Table 3 Daily intakes (mg/day) through milk consumption in comparison to PTDI values (for Pb and Cd) and to RDA values (for Ni, Fe, and Cu)

Element	Daily intakes (mg/day), mean ± SD, and range (min–max)								
	Adult			Children			Infant		
	1 S/day	2 S/day	3 S/day	1 S/day	2 S/day	3 S/day	1 S/day	2 S/day	3 S/day
Ni	0.0007 ± 0.0006 (0–0.0032)	0.0014 ± 0.0013 (0.0.0065)	0.0021 ± 0.0019 (0–0.0097)	0.0016 ± 0.0014 (0–0.0075)	0.0033 ± 0.0029 (0–0.015)	0.0049 ± 0.0044 (0–0.022)	0.0049 ± 0.0044 (0–0.022)	0.0099 ± 0.0088 (0–0.045)	0.015 ± 0.013 (0–0.067)
Zn	0.013 ± 0.0031 (0–0.22)	0.0272 ± 0.0061 (0–0.044)	0.0408 ± 0.0092 (0–0.0558)	0.031 ± 0.0071 (0–0.051)	0.0635 ± 0.014 (0–0.10)	0.0952 ± 0.021 (0–0.15)	0.0952 ± 0.021 (0–0.15)	0.1904 ± 0.042 (0–0.30)	0.286 ± 0.064 (0–0.46)
Cr	0.0006 ± 0.0007 (0–0.0043)	0.0012 ± 0.0013 (0–0.0086)	0.0018 ± 0.0020 (0.0001–0.013)	0.0014 ± 0.0015 (0.00006–0.010)	0.0029 ± 0.0031 (0.0001–0.020)	0.0043 ± 0.0047 (0.0002–0.030)	0.0043 ± 0.0047 (0.0002–0.03)	0.0086 ± 0.0093 (0.0003–0.060)	0.013 ± 0.014 (0–0.091)
Cd	0.0001 ± 0.0000 (0–0.0002)	0.0002 ± 0.0001 (0.0001–0.0004)	0.0003 ± 0.0001 (0.0001–0.0006)	0.0002 ± 0.00007 (0.00008–0.00047)	0.0004 ± 0.0001 (0.0002–0.0009)	0.0006 ± 0.0002 (0.0002–0.0014)	0.0006 ± 0.0002 (0.0002–0.0014)	0.0013 ± 0.0004 (0.0005–0.0028)	0.002 ± 0.001 (0.001–0.004)
Cu	0.0005 ± 0.0003 (0.0001–0.0017)	0.0009 ± 0.0005 (0.0002–0.0035)	0.0014 ± 0.0008 (0.0003–0.0052)	0.0010 ± 0.0006 (0.00025–0.0040)	0.0022 ± 0.0012 (0.0005–0.0081)	0.0033 ± 0.0018 (0.0008–0.012)	0.0033 ± 0.0018 (0.0008–0.012)	0.0065 ± 0.0036 (0.0015–0.024)	0.01 ± 0.005 (0.002–0.036)
Fe	0.0026 ± 0.0042 (0.0002–0.0256)	0.0052 ± 0.0084 (0.0004–0.053)	0.0078 ± 0.0126 (0.0006–0.079)	0.0060 ± 0.0098 (0.0005–0.061)	0.0121 ± 0.019 (0.001–0.12)	0.0181 ± 0.029 (0.0015–0.185)	0.0181 ± 0.029 (0.0015–0.185)	0.0362 ± 0.059 (0.003–0.37)	0.054 ± 0.089 (0.004–0.55)
Pb	0.003 ± 0.0017 (0–0.073)	0.006 ± 0.003 (0–0.015)	0.0095 ± 0.005 (0–0.022)	0.0074 ± 0.0039 (0–0.017)	0.015 ± 0.0078 (0–0.034)	0.022 ± 0.012 (0–0.050)	0.022 ± 0.012 (0–0.050)	0.044 ± 0.023 (0–0.10)	0.067 ± 0.035 (0–0.15)

SD standard deviation, *S/day* serving/day. PTDI values: Pb 0.0036 mg/kg BW/day; Cd 0.00083 mg/kg BW/day; Cr 0.3 mg/kg BW/day; Zn 25 mg/kg BW/day. RDA values: Ni 1 mg/day; Fe 45 mg/day; Cu 0.9 mg/day

PTDI tolerable daily intake, *RDA* recommended daily allowance

Non-carcinogenic Health Hazard (THQs)

The non-carcinogenic risks from consumption of raw cow milk by the adults, children, and infants were assessed for three scenarios (1, 2, and 3 servings of cow raw milk/day) based on the target hazard quotient (THQ). THQ values of Ni, Zn, Cu, and Fe, for adults, children, and infants, were < 1 in the three scenarios (Table 4). However, for Cd, the THQ for infants in the three scenarios (1, 2, and 3 servings of cow milk/day) was higher than 1. Also, Cr THQ values were recorded as higher than 1 for children in the high scenario (3 servings of cow milk/day) and for infants in the three

Table 4 Target hazard quotient (THQ) values and hazard index (HI) for heavy metals (Ni, Zn, Cr, Cd, Cu, Fe, and Pb) in raw cow's milk

Element	Target hazard quotient (THQ) values, mean ± SD, and range (min–max)								
	Adult			Children			Infant		
	1 S/day	2 S/day	3 S/day	1 S/day	2 S/day	3 S/day	1 S/day	2 S/day	3 S/day
Ni	0.065 ± 0.11 (0.0012–0.69)	0.13 ± 0.23 (0.002–1.39)	0.20 ± 0.35 (0.004–2.09)	0.15 ± 0.27 (0.003–1.63)	0.31 ± 0.54 (0.01–3.25)	0.46 ± 0.81 (0.01–4.88)	0.46 ± 0.81 (0.01–4.88)	0.92 ± 1.62 (0.02–9.75)	1.38 ± 2.42 (0.02–14.63)
Zn	0.045 ± 0.010 (0.003–0.073)	0.09 ± 0.02 (0.01–0.15)	0.14 ± 0.03 (0.01–0.22)	0.11 ± 0.02 (0.01–0.17)	0.21 ± 0.05 (0.02–0.34)	0.32 ± 0.07 (0.03–0.51)	0.32 ± 0.07 (0.03–0.51)	0.64 ± 0.14 (0.05–1.02)	0.95 ± 0.21 (0.08–1.54)
Cr	0.20 ± 0.22 (0.007–1.44)	0.41 ± 0.44 (0.02–2.88)	0.61 ± 0.67 (0.02–4.32)	0.48 ± 0.52 (0.02–3.63)	0.95 ± 1.03 (0.04–6.73)	1.43 ± 1.55 (0.06–10.09)	1.43 ± 1.55 (0.06–10.09)	2.86 ± 3.10 (0.11–20.18)	4.29 ± 4.66 (0.17–30.26)
Cd	0.09 ± 0.03 (0.03–0.2)	0.18 ± 0.06 (0.07–0.41)	0.28 ± 0.09 (0.10–0.61)	0.21 ± 0.07 (0.08–0.47)	0.43 ± 0.14 (0.16–0.95)	0.64 ± 0.21 (0.24–1.42)	0.64 ± 0.21 (0.24–1.42)	1.29 ± 0.42 (0.47–2.84)	1.93 ± 0.64 (0.71–4.27)
Cu	0.01 ± 0.006 (0.002–0.04)	0.02 ± 0.01 (0.01–0.09)	0.03 ± 0.02 (0.01–0.13)	0.03 ± 0.02 (0.01–0.10)	0.05 ± 0.03 (0.01–0.2)	0.08 ± 0.05 (0.02–0.30)	0.08 ± 0.05 (0.02–0.30)	0.16 ± 0.09 (0.04–0.61)	0.24 ± 0.14 (0.06–0.91)
Fe	0.003 ± 0.006 (0.0003–0.03)	0.01 ± 0.01 (0.001–0.08)	0.01 ± 0.02 (0.001–0.11)	0.01 ± 0.01 (0.001–0.09)	0.02 ± 0.03 (0.001–0.18)	0.03 ± 0.04 (0.002–0.26)	0.03 ± 0.04 (0.002–0.26)	0.05 ± 0.08 (0.004–0.53)	0.08 ± 0.13 (0.01–0.77)
Pb	0.90 ± 0.4 (0–2.07)	1.81 ± 0.95 (0.00–4.16)	2.72 ± 1.42 (0.00–6.24)	2.11 ± 1.11 (0.00–4.85)	4.23 ± 2.21 (0.00–9.70)	6.34 ± 3.32 (0–14.55)	6.34 ± 3.32 (0–14.55)	12.68 ± 6.64 (0–29.10)	19.02 ± 9.97 (0–43.66)
HI	**1.32 ± 0.57 (0.35–3.04)**	**2.61 ± 1.15 (0.70–6.08)**	**3.98 ± 1.73 (1.05–9.12)**	**3.10 ± 1.34 (0.82–7.09)**	**6.20 ± 2.69 (1.63–14.19)**	**9.30 ± 4.03 (2.45–21.28)**	**9.30 ± 4.03 (2.45–21.28)**	**18.59 ± 8.06 (4.89–42.57)**	**27.89 ± 12.10 (7.34–63.85)**

SD standard deviation, *S/day* serving/day

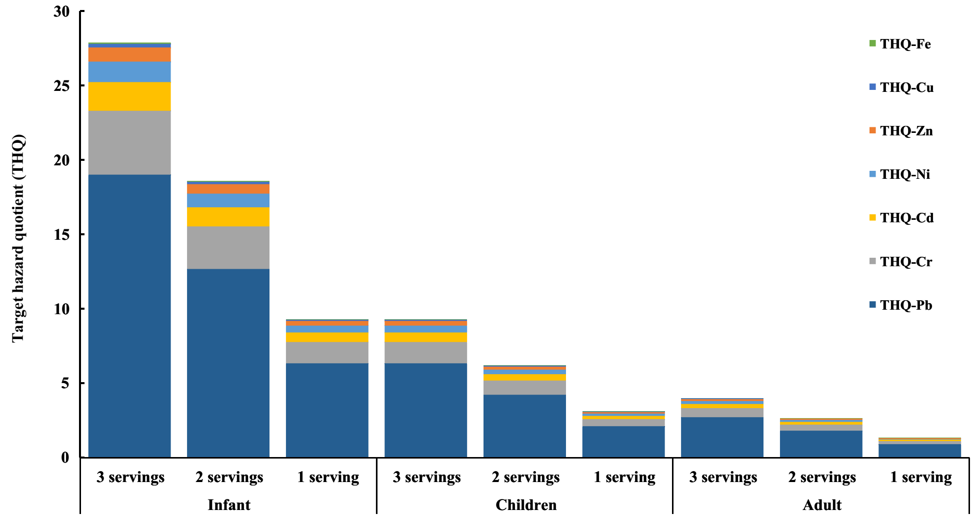

Fig. 2 Target hazard quotient (THQ) and hazard index (HI) for seven heavy metal exposure following raw co milk consumption for individuals by age and servings

scenarios (1, 2, and 3 servings of cow milk/day) (Table 4). Moreover, except for adults with a low scenario (1 serving of cow milk/day), all the THQ values of Pb were far higher than 1 indicating the greatest health risk for infants and children consuming 3, 2, or even 1 serving of cow milk by day and for an adult consuming 2 or 3 servings of cow milk by day.

In this study, the "infant" group seems to be the most exposed, which is due to their low body weight and higher milk intake [76], indicating that this exposure would be 2 to 3 times that of the general population on a bodyweight basis [15, 90].

Hazard Index (HI)

For the HI values, Pb made the largest contribution, followed by Cr, Cd, Ni, Zn, Cu, and Fe with values of 68.19%, 15.39%, 6.91%, 4.94%, 3.42%, 0.88%, and 0.28%, respectively (Fig. 2). The HI values for infants consuming 1, 2, and 3 servings were in the range of 2.44–21.28, 4.89–42.56, and 7.33–63.84, respectively, for children were in the range of 0.81–7.09, 1.63–14.18, and 2.44–21.28, while for adults, they were in the range of 0.34–3.04, 0.69–6.08, and 1.04–9.12 (Table 4). HI values were far higher than the threshold of 1. This indicates that the exposure level of the investigated heavy metals through milk consumption may cause adverse effects over a lifetime for all ages with the three scenarios (1, 2, and 3 serving cow milk by day). As mentioned above, HI for raw cow milk was largely driven by the Pb, Cr, and Cd THQs for all ages, while the highest HI values were recorded for infants and children, which is in agreement with data reported in Peru [88] and China [76].

Finally, it is important to keep in mind that this study only considers raw cow milk intake collected from extensive livestock, which is not the single food item consumed by the inhabitants of this region. Other studies have shown heavy metal contamination in fresh meat from cattle, sheep, chicken, and camel [91], in mollusk [92], as well as in sardine, swordfish, merlu, and Abramis [93–96]. Moreover, milk proportion of the total mass of food consumed per day varies significantly depending on age [76]. Consequently, the non-carcinogenic risk presented by heavy metals could be increased [97]. This also explains the EFSA [98, 99] recommendation, which advocates to investigate the risk of heavy metals in all products, including vegetables, grains, roots, fruits, and meat when the intake of children and adolescents considers milk and dairy products between 6 and 8%.

Conclusion and Recommendations

The average concentrations of heavy metals in the raw cow milk followed the order: Zn > Pb > Fe > Ni > Cu > Cr > Cd. The average concentrations of Cd, Zn, Cu, Fe, and Pb were above the maximum residue levels (MRLs), while the average concentrations of Ni and Cr were below MRLs. The estimation of the potentially harmful effects of heavy metals through raw cow milk consumption by comparing the estimated daily intake with toxicological limits (PTDIs or RDAs) showed that the consumption of milk does not pose a health risk for consumers of all ages with respect to Ni, Zn, Cu, Fe, and Cr. Whereas a health risk associated with Pb exposure for infants and children in the three scenarios and adults in high and medium scenarios were observed, moreover, a health risk associated with Cd concentration for infants in the medium and high scenarios was recorded.

THQ values of Ni, Zn, Cu, and Fe for infants, children, and adults were < 1 in the three scenarios. However, unacceptable potential risk levels of Pb, Cd, and Cr were found for infants in the three scenarios, for children in the three scenarios from Pb and in the high scenario from Cr, and for adults in the medium and high scenarios from Pb. HI values were far higher than 1, which means that the raw cow milk could cause adverse effects over a lifetime for all ages in the three scenarios. To our knowledge, there has been no study undertaken to assess the long-term effects of heavy metals in raw cow milk on infants, children, and adults in all regions of Algeria. Our results give a first clear picture of the impact of heavy metals in cow's milk consumed by the inhabitants of the region. Further studies in depth are needed to assess the risk of heavy metals in other regions in Algeria and to explore the correlation of metal levels in milk samples with feed, water, and soil. These will provide all the required information before the implementation of management measures and policies to make the right decisions regarding polluted areas. Also, particular attention should be paid to heavy metal residues, and a greater number of the main foodstuff should be measured in future studies to check the presence of toxicological risks.

It can be recommended that improvement and proper monitoring of cattle feed quality, as well as the techniques of milk processing, should be carefully considered for public health safety in Algeria. Moreover, as a preventive action and to reduce raw cow milk contamination by heavy metals, it is necessary to develop alternative eco-friendly tools and methods for conventional chemical inputs. Finally, to remediate heavy metal contamination of soil, water, and sediments, the use of necessary treatments such as chlorination, phytoremediation, thermal treatment, adsorption, chemical extraction, ion exchange, membrane separation, electrokinetics, and bioleaching is strongly recommended.

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Sofiane Boudalia: conceptualization, investigation, methodology, data curation, formal analysis, funding acquisition, project administration, methodology, and writing—original draft and review and editing.

Yassine Gueroui, Aissam Bousbia, and Rabah Zebsa: conceptualization, investigation, methodology, and writing—review and editing.

George Symeon: funding acquisition, project administration, and writing—review and editing.

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Data Availability The data that support the findings of this article can be found in the online version as supplementary.

Declarations

Ethical Statement The local Data Protection Board (DPB) and the local ethics committee have approved experimental protocols. The study involved data and/or milk sample collection from different farms, and participants were informed of the purpose of the project; they have given their consent for their participation (complete the survey questionnaire and/or provide milk samples) and the use of the collected data and the generated results from our analysis for scientific publications.

Competing Interests The authors declare no competing interests.

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CHAPTER 4

HEAVY METALS LEVELS IN RAW COW MILK AND HEALTH RISK ASSESSMENT ACROSS THE GLOBE: A SYSTEMATIC REVIEW

From Ali Boudebbouz, Sofiane Boudalia, Aissam Bousbia, Safia Habila, Meriem Imen Boussadia, Yassine Gueroui. Heavy metals levels in raw cow milk and health risk assessment across the globe: A systematic review. *Science of The Total Environment*. 2020, 141830. https://doi.org/10.1016/j.scitotenv.2020.141830.

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Review

Heavy metals levels in raw cow milk and health risk assessment across the globe: A systematic review

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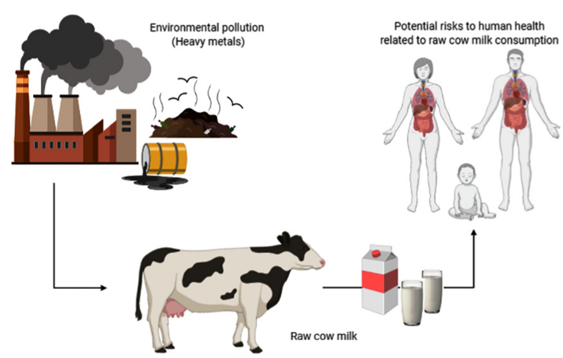
HIGHLIGHTS

- We analyzed 60 original published articles reporting raw cow's milk contamination.
- Heavy metals were detected in raw cow milk across the globe.
- Local environment and industrialization degree can affect heavy metals levels.
- Using Target Hazard Quotients, heavy metals exposure can affect human health.
- Data actualization is recommended to evaluate heavy metals effects.

GRAPHICAL ABSTRACT



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ABSTRACT

This systematic review presents the potential toxicity of heavy metals such as lead (Pb), mercury (Hg), cadmium (Cd), iron (Fe), nickel (Ni), aluminum (Al), and copper (Cu) in raw cow milk, focusing on their contamination sources and on the assessment of the related human health risk. Multiple keywords such as "raw cow milk, heavy metals, and human health" were used to search in related databases. A total of 60 original articles published since 2010 reporting the levels of these metals in raw cow's milk across the world were reviewed. Data showed that the highest levels of Ni (833 mg/L), Pb (60 mg/L), Cu (36 mg/L) were noticed in raw cow milk collected in area consists of granites and granite gneisses in India, while the highest level of Cd (12 mg/L) was reported in barite mining area in India. Fe values in raw cow milk samples were above the WHO maximum limit (0.37 mg/L) with highest values (37.02 mg/L) recorded in India. The highest Al level was (22.50 mg/L) reported for raw cow's milk collected close to food producing plants region in Turkey. The Target Hazard Quotients (THQ) values of Hg were below 1 suggesting that milk consumers are not at a non-carcinogenic risk except in Faisalabad province (Pakistan) where THQ values = 7.7. For the other heavy metals, the THQ values were >1 for Pb (10 regions out of 70), for Cd (6 regions out of 59), for Ni (3 out of 29), and for Cu (3 out of 54). Exposure to heavy metals is positively associated with diseases developments. Moreover, data actualization and continuous monitoring are necessary and recommended to evaluate heavy metals effects in future studies.

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Contents

1. Introduction

Over the centuries, milk and dairy products are considered as major sources of nutritious foods, especially for children because they contain macro- and micronutrients, such as vitamins and special fatty acids like conjugated linoleic acid with nutraceutical action, which are essential for growth, bone development, immune functions of the animal, and the human body (Leksir et al., 2019; Malbe et al., 2010). From human studies, several randomized clinical trials have demonstrated that consuming three or more servings of dairy foods per day has beneficial effects on nutrient and energy intakes in adults as well as of calcium, magnesium and vitamin D compared with intakes of individuals who consumed one or fewer serving of dairy foods per day (Rice et al., 2013). Several elements like iron (Fe), zinc (Zn) and copper (Cu) are essential for human body and play a crucial role in metabolism; they are considered as co-factors in many enzymes and have a variety of biochemical functions in the living organism. Nevertheless, their excess levels in animal and the human body above the sanitary recommendations may become toxic to human health (Gall et al., 2015; Licata et al., 2012; Varol and Sünbül, 2020). Other heavy metals such as cadmium (Cd), lead (Pb) and mercury (Hg) are non-essential elements and have no biological role and can cause toxic effects even at very low concentrations (Varol and Sünbül, 2020).

Therefore, the exposure to heavy metals present in food pose a threat to human health, it differs from the other types of pollutants with their long life exposure, their unsuitability for decomposition, for their non-degradability, and also for their high levels of accumulation along the food chain (Maas et al., 2011; Nkwunonwo et al., 2020).

Data from previous research studies show that the adverse human health effects are associated with exposure to environmental heavy metals, even at low concentrations, which considered as not harmful by health authorities. Heavy metals such as lead (Pb) and cadmium (Cd) can cross the placental barrier, and in utero exposure can affect fetal brain differentiation causing neurotoxic effect including a decrease in intelligence quotient, memory reduction and language disturbance (Khalil et al., 2009; Payton et al., 1998; Rehman et al., 2018; Schwartz et al., 2000). Moreover, the estrogenic activity of cadmium can affect reproductive systems including disturbance of androgen-estrogen balance and steroidal hormone levels which are correlated with high risk for development of breast cancer (Johnson et al., 2003; Nagata et al., 2005).

Data from previous studies indicate the presence of heavy metals in cow milk collected from different regions across the word (Elsaim and Ali, 2018; Licata et al., 2004; Malhat et al., 2012; Najarnezhad and Akbarabadi, 2013; Qin et al., 2009; Temiz and Soylu, 2012). However, heavy metals contamination degree is not constant and differs depending exposure routes, environmental condition, animal's nutrition, stage of lactation and animal breed (Bousbia et al., 2019; Fenta, 2014; Pilarczyk et al., 2013; Safaei et al., 2020). In lactating cows reared around industrial units, higher concentrations of lead and cadmium in milk were associated with higher concentrations of these toxic pollutants in forges and soil. Moreover, lead and cadmium can disturb the trace minerals profile of the milk and negatively affect its nutritional qualities (Patra et al., 2008).

Since the beginning of the 20th century, industrialization, urbanization, and agriculture mechanization lead to an increase in heavy metals pollution, which negatively impacts livestock systems and milk quality. The objectives of this systematic review were to compare the concentrations of selected heavy metals including copper (Cu), iron (Fe) and nickel (Ni) as well as toxic heavy metals involving aluminum (Al), cadmium (Cd), lead (Pb), and mercury (Hg) in the raw cow milk recorded across the world during the last decade (2010–2020) (Fig. 1). Contamination sources, regulations, and techniques used to detect heavy metals levels in milk were also discussed. Finally, the calculation of potential risks to human health related to milk daily consumption was performed using data extracted for heavy metals levels recorded from different areas across the world.

2. Materials and methods

2.1. Literature search

This systematic review was carried out based on published original articles in all publications (relevant research available 2010–2020). In

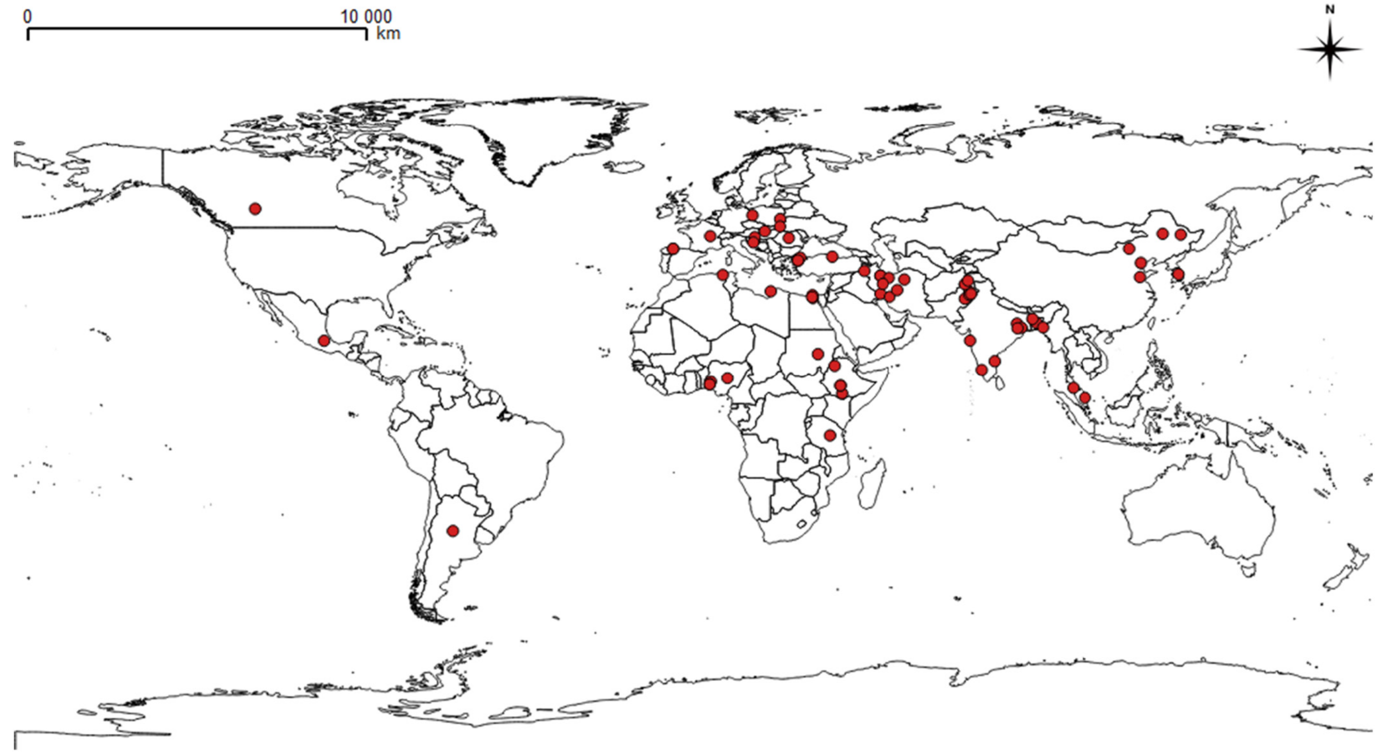

Fig. 1. Location map of raw cow milk samples collected from different countries across the world to measure heavy metals levels including copper (Cu), iron (Fe), aluminum (Al), cadmium (Cd), lead (Pb), mercury (Hg), and nickel (Ni) around the world during the last decade (2010–2020).

this review, international databases such as Google scholar, SCOPUS, Medline (using PubMed as the search engine), and Web of Sciences was searched for key words of: "heavy metals", "milk" in fields, title, abstract and keywords (A flowchart depicting the choice of studies is revealed in Fig. 2). After the first stage, the found articles were checked for eligibility for this review. Finally, the essential data was extracted through the selected articles and insert to spread sheet for further analysis.

2.2. Inclusion and exclusion criteria

The following inclusion criteria were adopted: (1) studies that assessed heavy metals levels in raw cow milk. The following exclusion criteria were adopted: (1) milk from other species such as goat, camel and sheep; (2) processed milk, which designates raw cow milk that has undergone several steps through various processes such as homogenization, sterilization or pasteurization, cream separation (whole milk, semi-skimmed milk or skimmed milk), packaging…etc.; (3) raw milk from cows who received daily oral metals administration; (4) scientific articles not published in English language and (5) review or conference abstracts or letters to the editor. For duplicate studies, the only article with further detailed information was included.

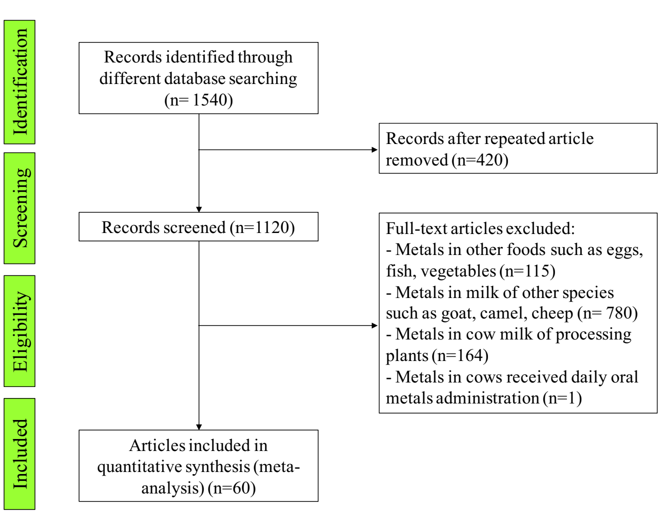

Fig. 2. Flow diagram of the studies selection process following the PRISMA (preferred reporting items for systematic reviews and meta-analyses).

2.3. Human risk assessment and exposure to toxic metals

2.3.1. Estimated Daily Intake

Metal Estimated Daily Intake (EDI) of Pb, Cd, Hg, Ni, Fe, Cu, and Al by consumption of raw cow milk was calculated using the following formula (Christophoridis et al., 2019).

$$EDI = \frac{(C_{Metal} \times W_{Milk})}{Body\ Weight\ (kg)}$$

where:

C_{Metal} (mg/kg, on wet weight basis) is mean metal level of raw cow milk samples.

W_{Milk} represents the daily average consumption of milk (kg).

BW (Body Weight): average body weight of an adult was considered as 70 kg.

The EDI (mg/kg BW/day) of non essential heavy metals such as Pb, Cd, Hg, and Al were compared with provisional tolerable daily intake (PTDI) set by the Joint FAO/WHO Expert Committee on Food Additives (Joint and World Health, 2012), while the daily intake of essential heavy metals (Cu, Ni, and Fe) were compared with recommended dietary allowances (RDAs) values established by the Food and Nutrition Board of the Institute of Medicine (USIMPM, 2001).

2.3.2. Target hazard quotients

To assess the human health risk from consuming raw cow milk with heavy metals, the target hazard quotients (THQ) was developed by the environmental protection agency (EPA) in the US for the estimation of potential human health risks (non-carcinogenic) associated with long term exposure to chemical pollutants (EPA, 1989). The THQ was evaluated based on the following THQ Equations (EPA, 2004; IRIS, 2010; Rahmani et al., 2018).

$$THQ = \frac{EF \times ED \times W_{Milk} \times C_{Metal}}{RFD \times Body\ Weight \times TA}$$

C_{Metal}, Body Weight, and W_{Milk} are already above explained.

The frequency of exposure (EF) and the exposure period equivalent to the mean longevity (ED) for an adult were considered to be 365 days in year and 70 years, respectively.

The TA (average time lifespan) was 25,550 days.

The reference dose (RFD0) for Cd, Pb, Hg, Cu, Ni, Fe, and Al are 0.001, 0.0035, 0.0003, 0.04, 0.02, 0.7, and 1 mg/kg BW/day respectively (USEPA, 2011; USEPA, 2012).

3. Results

3.1. Characteristics of eligible studies

Computerized literature search on multiple scientific databases resulted in a total number of 1540 documents. 420 were ruled out because they were duplicates, or they did not meet inclusion criteria and disqualified after review of title, abstract or manuscript [foods other than milk (115), milk from other species than cow (780), processed raw cow milk, which undergoes several steps through various processes (homogenization, sterilization or pasteurization, cream separation (whole milk, semi-skimmed milk or skimmed milk), packaging…etc.) (164), milk from cows who received daily oral metals administration ($n = 1$)]. In conclusion, 60 studies satisfied the inclusion criteria and were included in the systematic review.

3.2. Heavy metals contamination of raw cow's milk

3.2.1. Lead

Lead (Pb) is ubiquitous environmental metal, it is the most common industrial metal that can pollute air, water, soil and food chain (Raikwar et al., 2008). Widespread occurrence of lead in the environment is the result of anthropogenic activities, such as mining, smelting, and refining. Other sources of Pb in the environment include natural activities, such as volcanic activity, geochemical weathering and sea spray emissions, and remobilization of historic sources, such as lead in soil, sediment and water from mining areas (UNEP, 2010). In 2017, the Institute for Health Metrics and Evaluation has estimated that lead exposure accounted for 1.06 million deaths and 24.4 million Disability-Adjusted Life Years DALYs (IHME, 2015). Exposure to dietary Pb interrupts the nervous and circulatory systems as well as affecting several other organs of the body (Malhat et al., 2012). It can also cause renal dysfunction, raise blood pressure, spontaneous absorption, anemia, reduction in intelligence quotient, behavioral disturbance and neurodegenerative diseases (Eid and Zawia, 2016) and joint weaknesses (De Vasconcelos Neto et al., 2019; Gall et al., 2015).

There are a large number of published studies that have investigated the level of Pb in raw cow's milk. In the present review we were able to extract 55 studies that analyzed Pb in raw cow's milk samples collected in 70 regions around the world, 66% (36 studies) used Atomic Absorption Spectrometry (AAS) technique, followed by Inductively Coupled Plasma Mass Spectrophotometer (ICP-MS) (10 studies), other techniques used were Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES) (3 studies), Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) (3 studies), one study used Energy Dispersive X-Ray Fluorescence (EDXRF) technique, and one study used voltammetric analysis (VA) technique (Table 1).

The occurrence of Pb in raw cow's milk samples was presented in Table 1; the highest Pb level reported in the last ten years (60 mg/L) was obtained in raw cow's milk samples collected from matured grazing cow's reared in area consists of granites and granite gneisses of Archaean age with innumerable dyke swarms and isolated dykes situated in Tirupati area in Chittoor District, Andhra Pradesh (India) However, these concentrations are strongly influenced by seasonal variations, which are attributed to change in botanical composition of the herbage mainly in winter season (Raghu, 2015). In the same way, high Pb level was recorded (23.24 ± 0.30 mg/L) in cow's milk collected from animals during summer season and from cows drinking untreated main sewerage drainage water of Faisalabad city in Pakistan (Aslam et al., 2011), this value is far higher than the maximum residual limit (0.02 μg/mL) set by EC (2006). High Pb content (4.40 ± 1.60 mg/L) was also observed by Malhat et al. (2012) in raw cow milk collected in industrial air pollution area of Tokh city, El-Qaliubiya governance (Egypt). Also, high level of Pb (3.80 ± 0.42 mg/L) was obtained in raw cow milk samples collected from local producer of Nitra region (Slovak) compared to the permissible limit for milk according to Slovak law (0.02 mg/L) (Capcarova et al., 2019).

Zhou et al. (2019) show that at the farm level, Pb concentrations measured in milk produced from industrial polluted area in Tangshan province (China) were significantly higher than those found in milk from unpolluted areas in Qiqihar province (China) (1.43 μg/L vs. 0.16 μg/L, respectively). The mean concentration of Pb found in raw milk produced in major industrial area in Multan city, Pakistan was (0.03 ± 0.004 mg/kg), however, lower value was (0.01 ± 0.002 mg/kg) in samples collected in residential, and market zones (Ismail et al., 2015). Fenta (2014) show that the average concentration of Pb (0.8 ± 0.1 mg/L) in samples collected from cow's watered directly on a biological lagoon receiving the effluents of textile factory before discharged to the downstream, Tikur Wiha river in Hawassa, southern Ethiopia are significantly higher than those recorded in samples collected from animals watered on Tikur Wiha river before joining the waste water released from biological lagoon (0.4 ± 0.1 mg/L). In Turkey, Bigucu et al. (2016) have studied the impact of geographic livestock implantation on raw cow milk quality collected from three regions in Biga county of Çanakkale province: Yeniçiftlik, Gümüşçay and Şakirbey. The results show that the highest Pb concentration was found in milk from Şakirbey region which was close to highways (1.85 ± 0.09 mg/L), followed by those found in the milk collected from Yeniçiftlik region which was near to heavy industry plants (1.03 ± 0.05 mg/L) and the cow milk collected from Gümüşçay region which was close to food producing plants (1.01 ± 0.05 mg/L), respectively. In Ibadan, southern of Nigeria, Pb concentrations (0.34 ± 0.14 mg/L) obtained in milk collected from cow's reared in polluted area located some meters away from the dump site, which characterized with plants averagely growing in the vicinity of the slag and leachate were higher than those recorded in milk collected from animal reared in unpolluted area, where the lead content is undetectable (Ogundiran et al., 2012).

Derakhshesh and Rahimi (2012) reported a difference of Pb level from two regions in Iran, the concentration of Pb in cow's milk collected from area with industrial pollution of Tehran was high (0.02 ± 0.009 mg/L) compared to those found in the milk samples collected from unpolluted area located on Yasuj province (0.005 ± 0.002 mg/L). Pb concentrations above maximum residual limit (0.02 μg/mL) were also observed in raw cow milk samples collected in rural area without great industrial activities in different countries around the world. In Croatia (Bilandžić et al., 2011), in Slovak (Capcarova et al., 2019), Pakistan (Iftikhar et al., 2014), France (Maas et al., 2011), and in India (Pérez-Carrera et al., 2016). The high level of Pb content in milk collected from rural and unpolluted area could also be due to other factors or sources such as transhumance along roads and/or motorways, fodder contamination, climatic factors, such as winds, and the use of pesticide compounds (Derakhshesh and Rahimi, 2012).

Results from Table 1 indicate that raw cow milk samples analyzed in developing countries such as Pakistan, Ethiopia, Nigeria, Mexico, Argentina, Bangladesh, Egypt, Poland, and Sudan,have Pb concentrations beyond the standard limit; In contrast, developed countries like Spain, South Korea, Canada, and Croatia generally were found to have less Pb contamination in raw cow milk samples (Bilandžić et al., 2016; González-Montaña et al., 2012; Kim et al., 2016; Zwierzchowski and Ametaj, 2019).

From several studies, Pb levels in raw cow's milk samples were Below the Detectable Limit (BDL) of instrument. In Ethiopia, from cow's reared near to main high way in Moyale province (Belete et al., 2014), Sudan in grazing area (Elsaim and Ali, 2018), India from raw milk samples collected from uranium mining area (Giri et al., 2011), in Nigeria from cows reared in area considered free from contamination (Ogundiran et al., 2012), in Libya from rural region (Elatrash and Atoweir, 2014), and in China from small farms cooperatives, and in raw cow's milk samples collected in summer season from cows reared in granites and granite gneisses area (Qu et al., 2018; Raghu, 2015).

3.2.2. Cadmium

Cadmium (Cd) is one of the most toxic industrial and environmental heavy metals because of its long half-life (15–30 years) and multifaceted deleterious effects on human health, such as teratogenic, carcinogenic, hepatotoxic, nephrotoxic, skeletal and reproductive effects (Domingo, 1994; Flora and Agrawal, 2017; Zhong et al., 2018), it can be bio accumulated in various tissues especially liver and kidneys, which amplifies the deleterious effects on human health (WHO, 2005). Exposure to Cd is often mixed with industrial emission sources such as mining and smelting operations. Major industrial uses of Cd are in electroplating, pigments and, particularly, plastics, plastic stabilizers, and Ni-Cd rechargeable batteries (Flora and Agrawal, 2017).

In the present systematic review, we were able to retrieve 47 studies that analyzed Cd levels in raw cow milk samples collected in 59 regions across the world during the last ten years (Table 1). In most studies, Cd level was measured using AAS technique. Cd values were compared with the standard limit values (0.0026 μg/g) determined by the International Dairy Federation (IDF, 1979). This limit is outdated but still it is the only acceptable maximum limit of Cd level in milk (Ismail et al., 2019).

It should be noted that the Cd level in milk samples in thirty-six regions in the world (61%) were above standard limit of 0.0026 μg/g (IDF, 1979), in data extracted from 18 regions (31%), Cd levels are below standard limit (0.0026 μg/g), and in five studies (8%) Cd levels are below detectable limit of instrument (BDL) (Table 1). The highest Cd level (12 mg/L) was found in raw cow's milk samples collected from animal reared close to area consist of granites and granite gneisses situated in Tirupati area, in Chittoor District, Andhra Pradesh, in India (Raghu, 2015).

From literature, Cd levels in milk samples collected from cows watering directly on a biological lagoon that receives the effluents of textile factory before discharged to the downstream Tikur Wiha river in Hawassa, southern Ethiopia was (0.2 ± 0.01 mg/L) which is 2 times higher than the Cd levels in milk collected from cows watered on Tikur Wiha river before joining the waste water released from biological lagoon (0.1 ± 0.001 mg/L) (Fenta, 2014). In Turkey, Bigucu et al. (2016) obtained Cd level in raw cow milk are varied between 0.39 ± 0.02 mg/L for cows reared near to highways in the region of Şakirbey; and 0.19 ± 0.01 mg/L in the region of Gümüşçay which is near to food producing plants, and 0.19 ± 0.01 mg/L in the region of Yeniçiftlik near to heavy industry plants. Besides, in Pakistan, Iftikhar et al. (2014) studied Cd levels differences in cow's milk samples collected from two zones: the first one is an urban area, which is located in the centre of the city and hence was exposed to pollution, while the second one, is a rural area which is away from industrial and traffic pollution, results recorded were (0.7 ± 0.07 mg/L and 0.04 ± 0.01 mg/L) respectively. In the same way, Elatrash and Atoweir (2014) described a significant difference between Cd levels in milk samples collected from rural and heavy traffic intensity in Benghazi province, Libya (Table 1).

It could be observed that all studies reported a mean level of Cd in milk above (0.0026 mg/L) were collected from polluted area, except in three regions (5%) that showed a high Cd level in milk collected from unpolluted area (Bilandžić et al., 2011; Capcarova et al., 2019; Fenta, 2014; Iftikhar et al., 2014). Three studies conducted in polluted area reported a Cd level in milk samples below detectable limit of the instrument (BDL) (González-Montaña et al., 2012; Ogundiran et al., 2012; Raghu, 2015) (Table 1).

3.2.3. Nickel

Nickel (Ni) is an essential mineral element for humans, it acts as a cofactor for a number of enzymes as well as hormones but above certain levels Ni may become toxic, and lead to cell damage, alteration of enzyme and hormone activities, oxidative stress and neurotoxicity (Ismail et al., 2017). Data from twenty-two studies published from twenty-nine regions across the world since 2010 were extracted then analyzed. Fourteen out of twenty-two studies have analyzed Ni using AAS technique or by ICP-MS (five studies), then ICP-OES (two studies), and one study using EDXRF technique. The mean levels of Ni in raw cow milk are presented in Table 1. The upper level of Ni intake per day through dietary sources recommended by FNB (2001) is 0.3–1 mg/L.

The level of Ni in raw cow milk samples across the globe ranged between BDL to 833 mg/L. The maximum levels of Ni (833 and 63 mg/L) were recorded from milk samples collected from cows reared near to area consists of granites and genesis in Tirupati area, and cows reared near to Mangampeta barite mining area respectively in India (Raghu, 2015). Followed by Pakistan (23.5 ± 0.3 mg/L) from cows watering sewerage drainage of Faisalabad city (Aslam et al., 2011). Also, in Ethiopia, Fenta (2014) reported means level of Ni (1.6 ± 0.3 mg/L) in raw milk collected from cows watered directly on a biological lagoon of textile factory before discharged to the river downstream, was slightly higher than those recorded in raw milk from animals watered in river before joining the waste water (1.4 ± 0.2 mg/L). It should be noted that only five studies out of twenty-two (22%) found to exceed the maximum limit of Ni in raw milk (0.1–1 mg/L) recommended by the Food and Nutrition Board (FNB, 2001).

Ni levels in raw cow's milk collected from polluted area were usually higher than those collected from unpolluted area. For instance, in Pakistan, Iftikhar et al. (2014) found significant difference between samples collected from urban and rural area (0.8 mg/L vs.0.02 mg/L) respectively. In the same way, Ni level in raw cow's milk samples collected from cows reared in the vicinity of Iron mining areas (0.2 mg/L) was lower than those found in cows reared in the vicinity of copper mining areas in India (0.62 mg/L) (Giri and Singh, 2019).

3.2.4. Mercury

Mercury (Hg) is a naturally occurring chemical element which can be found in foodstuffs by natural causes. The most important anthropogenic causes of Hg pollution in the environment are mining and combustion, agricultural materials, and industrial and urban discharges (Bilandžić et al., 2011; Joint and FAO/WHO, 2011; Zhang and Wong, 2007). The European Union Regulation indicates 0.01 mg/kg as the maximum Hg content in any food including milk (EU, 2015). To our knowledge, there is not too much data on mercury residues in milk in comparison with other trace metals (Table 1).

Here, we extracted data from a total of twelve studies published since 2010 that analyzed Hg levels in raw cow's milk. The maximum Hg level (0.55 ± 0.01 mg/L) was reported in the raw cow's milk samples from cows drinking sewerage water in Pakistan (Aslam et al., 2011). Also; in Bangladesh, Kabir et al. (2017) found a high level of Hg in milk samples collected from cows fed grass of the forage near which industrial waste are discharged (0.4 mg/L). Other studies showed that Hg

Table 1
Heavy metals levels (Pb, Cd, Ni, Hg, Fe, Cu, Al) in raw cow's milk reported in research articles published since 2010.

Reference	Location	Characteristic of the collect area	N	Method	Heavy metals levels (mg/L). Mean ± SD/SEM and/or range (Min-Max)						
					Pb	Cd	Ni	Hg	Fe	Cu	Al
Abdalla et al. (2013)	Sudan	Grazed around sugar cane plants	51	AAS	0.5	0.0006	–	–	–	0.12	–
Ahmad et al. (2017)	Pakistan, Pakhtunkhwa	–	8	AAS	BDL	0.08 ± 0.006	–	–	0.7 ± 0.3	0.14 ± 0.02	–
Akele et al. (2017)	Ethiopia, North Gondar	High mineral enrichment and metal leaching	30	AAS	0.15 ± 0.01 (0.12–0.19)	0.29 ± 0.01 (0.24–0.33)	–	–	–	1.12 ± 0.06 (0.84–1.53)	–
Akhtar et al. (2015)	Pakistan, Multan	Market cities	24	AAS	0.2 ± 0.05	0.1 ± 0.02	0.18 ± 0.09	–	1.03 ± 0.28	0.15 ± 0.05	–
Arianejad et al. (2015)	Iran, Arak	Traditional dairy farm	16	AAS	–	–	0.04	0.0001	–	–	–
	Iran, Arak	Industrial dairy farm	16	AAS	–	–	0.13	0.00009	–	–	–
Aslam et al. (2011)	Pakistan, Faisalabad	Sewerage drainage of Faisalabad city	90	AAS	23.2 ± 0.3	0.17 ± 0.006	23.5 ± 0.3	0.55 ± 0.01	–	–	–
Bakircioglu et al. (2018)	Turkey, Edirne		2	ICP-OES	–	–	–	–	2–3.7	0.09–0.26	0.2–0.3
Belete et al. (2014)	Ethiopia, Borena Zone	Close to highways	–	AAS	BDL	BDL	BDL	–	–	0.09–0.12	–
Bigucu et al. (2016)	Turkey, Şakirbey	Close to highways	3	ICP-AES	1.85 ± 0.09	0.39 ± 0.02	–	–	4.2 ± 0.16	0.62 ± 0.01	17.32 ± 0.34
	Turkey, Yeniçiftlik	Close to heavy industry plants	3	ICP-AES	1.03 ± 0.05	0.19 ± 0.01	–	–	2.6 ± 0.08	0.65 ± 0.05	19.53 ± 0.18
	Turkey, Gümüşçay	Close to food producing plants	3	ICP-AES	1.01 ± 0.05	0.19 ± 0.01	–	–	4.05 ± 0.2	0.65 ± 0.005	22.5 ± 0.23
Bilandžić et al. (2011)	Croatia, Northern	Rural area	90	AAS	0.06 ± 0.08 (0.001–0.37)	0.001 ± 0.001 (0.001–0.01)	–	0.001 ± 0.001 (0.001–0.009)	–	0.93 ± 2.44 (0.002–0.017)	–
	Croatia, Southern	Rural area	67	AAS	0.03 ± 0.07 (0.001–0.48)	0.003 ± 0.003 (0.001–0.02)	–	0.007 ± 0.016 (0.001–0.09)	–	0.84 ± 1.19 (0.001–3.7)	–
Bilandžić et al. (2016)	Croatia	Rural areas	249	AAS	0.01 ± 0.008	–	–		–	–	–
Bousbia et al. (2019)	Algeria, Guelma	Polluted area	146	AAS	–	0.03 ± 0.013	–	–	1.43 ± 0.58	0.24 ± 0.17	–
Cadar et al. (2015)	Romania, Eastern Carpathians	Rodnei Mountains	–	AAS	0.006	0.001	–	0.001	–	0.047	–
Capcarova et al. (2019)	Slovak, Nitra region	Agricultural sector	10	AAS	3.8 ± 0.42	0.27 ± 0.06	0.84 ± 0.13	BDL	1.76 ± 0.36	2.12 ± 0.61	–
Castro-Gonzalez and Calderon-Sanchez (2018)	Mexico, Puebla	Waste water	60	ICP-OES	0.03 ± 0.01	–	0.01 ± 0.02	–	–	0.01 ± 0.01	–
Chirinos-Peinado and Castro-Bedriñana (2020)	India, Peru	Near to metallurgical complex	20	AAS	0.58 ± 0.018	0.02 ± 0.007	–	–	–	–	–
Derakhshesh and Rahimi (2012)	Iran, Tehran	Industrial air pollution in these regions.	10	AAS	0.02 ± 0.009	–	–	–	–	–	–
	Iran, Yasuj	Unpolluted area	5	AAS	0.005 ± 0.002	–	–	–	–	–	–
Dizaji et al. (2012)	Iran, East Azerbaijan	Close roads and/or motorways	100	AAS	0.01–0.18	0.0006–0.004	–	–	–	–	–
El Sayed et al. (2011)	Egypt, Menofia	Industrial air pollution in these regions	21	ICP-AES	0.6 ± 0.2	0.02 ± 0.002	–	–	5.1 ± 1.3	1.2 ± 0.4	–
Elatrash and Atoweir (2014)	Libya, Benghazi, Sidy Khalifa	Rural region	–	AAS	BDL	0.0009 ± 0.00002	–	–	–	–	–
	Libya, Benghazi, Garyounis	Heavy traffic intensity regions	–	AAS	0.005 ± 0.0003	0.003 ± 0.00002	–	–	–	–	–
Elsaim and Ali (2018)	Sudan, Merowe	Grazing areas	9	AAS	BDL	BDL	–	–	–	0.1–0.2	–
Fenta (2014)	Ethiopia, Chafe	Watering in textile treatment pound	15	AAS	0.8 ± 0.1	0.2 ± 0.01	1.6 ± 0.3	–	–	–	–
	Ethiopia, Dato	Textile treatment pound factory	15	AAS	0.4 ± 0.1	0.1 ± 0.001	1.4 ± 0.2	–	–	–	–
Giri and Singh (2019)	India, East of Singhbhum	Copper mining areas	–	ICP-MS	0.13	–	0.62	–	8.8	0.5	0.5
	India, West of Singhbhum	Iron mining areas	–	ICP-MS	0.09	–	0.20	–	11.4	0.31	0.26
Giri et al. (2011)	India, Jharkhand	Uranium mining area	60	AAS	BDL	–	0.4	–	4.9	0.6	–
González-Montaña et al. (2012)	Spain, Asturias	Close to iron and steel industries	36	ICP-MS	0.003 ± 0.0006	BDL	–	–	–	–	–
González-Montaña et al. (2019)	Spain, Asturias	Near high-density highway traffic	36	ICP-MS	–	–	–	BDL	–	–	0.14 ± 0.15
Iftikhar et al. (2014)	Pakistan, Peshawar	Urban area	30	AAS	2.2 ± 0.2	0.7 ± 0.07	0.8 ± 0.006	–	–	1.4 ± 0.009	–
	Pakistan, Peshawar	Rural area	20	AAS	2.08 ± 0.16	0.04 + 0.01	0.02 ± 0.005	–	–	0.09 ± 0.004	–
Islam et al. (2015)	Bangladesh, Bogra	Urban area	–	ICP-MS	0.15 ± 0.15 (0.006–0.7)	0.02 ± 0.03 (0.001–0.12)	1.3 ± 1.7 (0.07–8.3)	–	–	2.3 ± 1.5 (0.4–6.4)	–
Ismail et al. (2015)	Pakistan, Multan	Industrial zone	180	AAS	0.03 ± 0.004	0.003 ± 0.0001	0.03 ± 0.002	–	–	1.16 ± 0.04	–
	Pakistan, Multan	Residential and market zone		AAS	0.01 ± 0.002	0.0001 ± 0.0003	0.03 ± 0.003	–	–	0.83 ± 0.03	–
Ismail et al. (2017)	Pakistan, Punjab	Farms located near to the cities	240	AAS	0.014–0.033	0.001–0.004	0.01–0.08	–	–	0.04–0.09	–
Jigam et al. (2011)	Nigeria, Niger Dangana	Rural agrarian region	10	AAS	0.6 ± 0.2	–	–	–	–	0.6 ± 0.01	–

Jolly et al. (2017)	Bangladesh, Dhaka/Barishal	Industrial disposal areas	9	EDXRF	0.04 ± 0.04	0.001–0.02	0.07–0.08	BDL −0.03	0.76–1.23	0.05–0.06	–
Kabir et al. (2017)	Bangladesh, Karnafuli, Chittagong	Industrial waste	50	AAS	0.09 (BDL - 0.7)	0.03 (BDL - 0.3)	0.11 (0.1–0.7)	0.06 (BDL - 0.4)	7.64 (2.1–14.1)	0.12 (0.1–1.1)	–
Kim et al. (2016)	Korea, South part	–	143	ICP-MS	0.001 ± 0.001 (<0.0000–0.006)	0.0002 ± 0.0002 (<0.00005–0.002)	–	–	–	–	–
Król et al. (2012)	Poland, Lublin	Intensive milk production farms	72	AAS	0.01 ± 0.005 (0.003–0.02)	0.007 ± 0.004 (0.0003–0.008)	–	–	0.25 ± 0.06	0.04 ± 0.01	–
	Poland, Bieszczady	Extensive milk production farms	87	AAS	0.008 ± 0.003 (0.004–0.01)	0.002 ± 0.002 (0.0001–0.006)	–	–	0.37 ± 0.05	0.04 ± 0.01	–
	Poland, Biebrza	Extensive milk production farms	68	AAS	0.006 ± 0.004 (0.0005–0.01)	0.004 ± 0.003 (>0.00002–0.006)	–	–	0.33 ± 0.1	0.07 ± 0.02	–
Maas et al. (2011)	France, Besançon	Mountain area	61	AAS	0.009–0.12	0.0003–0.001	–	–	–	0.28–1.71	–
Malhat et al. (2012)	Egypt, El-Qaliubiya	Industrial air pollution	100	AAS	4.4 ± 1.6 (0.02–7.01)	0.28 ± 0.16 (0.004–0.67)	–	–	16.4 ± 8.4 (0.4–63.6)	2.9 ± 1.1 (0.01–5)	–
Meshref et al. (2014)	Egypt, Beni-Suef	–	22	AAS	0.2 ± 0.02	0.05 ± 0.005	–	–	8.9 ± 1.8	0.09 ± 0.04	–
Muhib et al. (2016)	Bangladesh, Dhaka	Dairy farms	30	AAS	0.015 ± 0.002	0.02 ± 0.1	–	–	0.33 ± 0.05	0.06 ± 0.01	–
	Bangladesh, Dhaka	Small household farmers	27	AAS	0.012 ± 0.001	0.05 ± 0.03	–	–	0.63 ± 0.1	0.12 ± 0.02	–
Najarnezhad and Akbarabadi (2013)	Iran, Khorasan	–	720	AAS	0.01 ± 0.0061	0.0003 ± 0.0003	–	0.003 ± 0.0003	–	–	–
Najarnezhad et al. (2015)	Iran, West Azerbaijan	–	100	AAS	0.007 ± 0.001	0.001 ± 0.001	–	–	–	–	–
Norouzirad et al. (2018)	Iran, Khuzestan	Close to oil fields	118	AAS	0.05 ± 0.004	0.004 ± 0.001	–	–	–	–	–
Ogundiran et al. (2012)	Nigeria, Ibadan	Uncontaminated area	20	AAS	BDL	BDL	–	–	–	29.2 ± 8.4	–
	Nigeria, Ibadan	Lead Slag contaminated sites	20	AAS	0.34 ± 0.14	BDL	–	–	–	0.18 ± 0.09	–
Pérez-Carrera et al. (2016)	Argentina, Southeast of Córdoba	Rural area		ICP-OES	0.02 ± 0.01 (>0.005–0.06)	–	0.04 ± 0.01 (0.02–0.14)	–	0.96 ± 0.73 (0.24–3.29)	0.03 ± 0.01 (0.009–0.09)	–
Pilarczyk et al. (2013)	Poland, Lubuskie	Organic farm Simmental breed	20	ICP-MS	0.03 ± 0.0008 (0.03–0.04)	0.003 ± 0.00007 (0.003–0.004)	–	–	0.25 ± 0.03 (0.12–0.7)	0.03 ± 0.005 (0.014–0.14)	–
		Organic farm Holstein-Friesian breed	20		0.04 ± 0.0008 (0.03–0.05)	0.004 ± 0.00007 (0.003–0.005)			0.2 ± 0.01 (0.14–0.49)	0.04 ± 0.003 (0.02–0.1)	
Qu et al. (2018)	Chine, Inner Mongolia	–	20	ICP-MS	BDL	–	BDL	0.002 ± 0.001 (0.001–0.004)	–	–	0.14 ± 0.13 (0.1–0.5)
	Chine, Heilongjiang	–	20	ICP-MS	0.01 ± 0.01 (0.003–0.03)	–	0.01 ± 0.01 (0.005–0.02)	0.002 ± 0.001 (0.001–0.004)	–	–	0.76 ± 0.95 (0.08–1.4)
Raghu (2015)	India, Tirupati	Granites and granite gneisses	6–8	AAS	60	12	833	–	–	36	–
	India, Mangampeta	Mining area	6–8	AAS	–	–	67	–	–	28	–
Rahimi (2013)	Iran (Isfahan, Yazd, Mashhad, Kerman, and Ahvaz cities)	Industrial cities	52	AAS	0.009 ± 0.004 (0.001–0.02)	0.0009 ± 0.0004 (0.0003–0.003)	–	–	–	–	–
Rao and Murthy (2017)	Tanzania, Dodoma, Ntyuka	Proximity to roads	10	AAS	–	–	BDL	–	–	0.25 ± 0.01	–
Safaei et al. (2020)	Iran, East Azerbaijan	–	5400	ICP-OES	0.010 ± 0.001	0.007 ± 0.001	–	–	–	–	–
Sarsembayeva et al. (2020)	Kazakhstan, Almaty	–	120	AAS	0.001–0.008	0.0027–0.0029	–	–	–	–	–
Shahbazi et al. (2016)	Iran (Ahvaz, Esfahan, Tehran, Tabriz and Mashhad cities)	Industrial regions	25	VM	0.014 ± 0.002	0.001 ± 0.0005	–	–	–	0.42 ± 0.17	–
Tahir et al. (2017)	Pakistan, Sargodha	Near traffic road	–	–	0.3–0.8	0.04–0.3	0.35–2.85	–	–	–	–
Temiz and Soylu (2012)	Turkey, south-east of Samsun	Industrial operations	144	ICP-MS	0.05 ± 0.04 (0.03–0.07)	0.01 ± 0.01 (0.001–0.01)	0.64 ± 1.04 (0.3–1.1)	–	0.47 ± 1.02 (0.12–0.64)	1.36 ± 1.16 (0.62–1.89)	–
Tona et al. (2013)	Nigeria, Ogbomoso		8	AAS	0.003 ± 0.0016	0.002 ± 0.0007	–	–	–	–	–
Zain et al. (2016)	Iran, South		9	ICP-MS	–	–	–	–	1.33 ± 0.03	0.93 ± 0.09	–
	Iran, North		12	ICP-MS	–	–	–	–	1.44 ± 0.05	0.83 ± 0.06	–
	Malaysia, Peninsula		63	ICP-MS	–	–	–	–	2.39 ± 0.09	1.29 ± 0.03	–
	Malaysia, Peninsula		93	ICP-MS	–	–	–	–	4.58 ± 0.06	1.30 ± 0.04	–
Zhou et al. (2017)	Chine, Shandong and Shaanxi cities	–	40	ICP-MS	0.0014	0.00007	0.0057	0.005	0.35	0.03	0.05
Zhou et al. (2019)	Chine, Tangshan	Industrial area	50	ICP-MS	0.0014	0.0001	–	–	–	–	–
	Chine, Qiqihar	Unpolluted agricultural area	10	ICP-MS	0.00016	0.00004	–	–	–	–	–
Zodape et al. (2012)	India, Mumbai		15	ICP-AES	0.14–5.9	–	–	0.01–0.02	–	0.03–37.29	–
Zwierzchowski and Ametaj (2019)	Canada, Alberta	Regions surrounding the cities	156	ICP-MS	>0.002	>0.001	–	–	0.08–0.5	0.03–0.1	0.007–0.07

AAS: Atomic Absorption Spectrometry, ICP-MS: Inductively Coupled Plasma Mass Spectrophotometer, ICP-AES: Inductively Coupled Plasma Atomic Emission Spectrometry, ICP-OES: Inductively Coupled Plasma Optical Emission Spectrometry, EDXRF: Energy Dispersive X-Ray Fluorescence, SV: Stripping Voltammetry, BDL: Below Detection Limit, VM: voltammetric analysis, **N**: sample size, Min: minimum values, Max: maximum values, SD: standard deviation, SEM: standard error mean, —: no data available.

level ranged from BDL to 0.06 mg/L (Arianejad et al., 2015; Bilandžić et al., 2011; Cadar et al., 2015; Jolly et al., 2017; Najarnezhad and Akbarabadi, 2013; Qu et al., 2018; Zhou et al., 2017; Zodape et al., 2012), while in Spain one study conducted by González-Montaña et al. (2019) analyzed milk samples collected from cows reared close to the steel industry, all milk samples analyzed had Hg values below the established detection limit. In Slovak, Capcarova et al. (2019) also reported Hg levels in raw milk samples below the detection limit. The present data indicate that the Hg levels in milk samples are generally in safe limits except in the study conducted in Faisalabad province, Pakistan (Aslam et al., 2011) (Table 1).

3.2.5. Iron

Iron (Fe) is an essential trace element that participates as catalyst in several metabolic reactions; and as a component of hemoglobin, myoglobin, cytochromes and other proteins, plays an essential role in the transport, storage and utilization of oxygen. It is a cofactor for a number of enzymes and its deficiency results in anemia and other pathologies (Meshref et al., 2014), however due to its ability to generate reactive oxygen species (ROS), the excess of iron can cause tissue damage and organ failure, and increases the risk of cancer (Eid et al., 2017; Puliyel et al., 2015). In milk and dairy products, a high Fe concentration can cause a problem in processing technology due to its catalytic effect on oxidation of lipids with development of unpleasant smell, bounding preferably proteins and membrane lipoproteins of milk fat globules (Lante et al., 2006).

In this systematic review, data from 21 studies published since 2010 that measure Fe concentrations in raw cow's milk in 30 regions across the world were extracted and analyzed.

The maximum limit for Fe recommended is 0.37 mg/L (IDF, 1979). The highest mean concentration of Fe in raw milk was found from cows reared close to industrial air pollution area in El-Qaliubiya district, Egypt (16.4 ± 8.4 mg/L) (Malhat et al., 2012), followed by samples of cows reared near to iron mining area in west of Singhbhum, India (11.4 mg/L) (Giri and Singh, 2019). Also, a high level was found in samples collected from local farms in Beni-Suef, Egypte (8.9 ± 1.8 mg/L) (Meshref et al., 2014). The mean level of Fe in raw cow's milk samples across the world ranged between 0.33 mg/L and 16.4 mg/L. Mean values of Fe in milk samples are presented in Table 1 They were generally above the maximum limit set by IDF (1979).

3.2.6. Copper

Copper (Cu) is an essential element for normal human growth but its excessive consumption lead to toxic effects on human health, primarily Wilson's disease which is characterized by deficiency of ceruloplasmin (Lawal et al., 2006). Here, 39 studies published since 2010 that measured the prevalence of Cu content in raw cow's milk in 54 regions around the world were used. Cu levels were recorded using AAS technique (24 studies), ICP-MS technique (7 studies), ICP-AES technique (3 studies), ICP-OES technique (3 studies), EXDRF technique (1 study), and SV technique (1 study). Cu levels in milk samples from different countries of world since 2010 were reported in Table 1. The mean level of Cu in raw cow milk samples across the globe ranged between 0.0136 mg/L and 36 mg/L, they are above the maximum limit (0.01 mg/L) (IDF, 1979).

The highest Cu levels were recorded in India from cows reared in area considered as one of the largest barite deposits of the world in Tirupati province (36 mg/L), and in area consists of granites and granite gneisses in Mangampeta in Kadapa District, Andhra Pradesh (28 mg/L) (Raghu, 2015). It should be noted that the concentrations of Cu in raw cow's milk in any area does not represent the concentration of the whole country. Cu concentrations were influenced by local environmental characteristic such as urban area, rural area, and industrial area. In Pakistan Ogundiran et al. (2012) reported that the milk collected from cows reared plants growing close to slag and leachate located some meters away from dump site of Lagelu government had mean Cu concentration (0.18 ± 0.09 mg/L) which was significantly lower than Cu concentration (29.2 ± 8.4 mg/L) obtained in the milk collected from region believed to be free from contamination located in northern parts of Pakistan. A comparative study conducted in Peshawar province, Pakistan showed that Cu concentration in milk from cows feed in rural area (0.09 ± 0.004 mg/L) was significantly lower than Cu concentration in milk (1.4 ± 0.009 mg/L) from cows reared in urban area in Peshawar province, Pakistan (Iftikhar et al., 2014). Also, a high Cu concentration (0.5 mg/L) was reported in the east of Singhbhum in milk collected from cows reared close to copper mining area compared to those reared in the west of Singhbhum close to iron mining area (0.31 mg/L) in India (Giri and Singh, 2019).

3.2.7. Aluminum

Aluminum (Al) has historically been considered to be relatively nontoxic in healthy individuals, without any apparent harmful effects. However, there is now abundant evidence that Al may cause adverse effects on the nervous system and high intakes of it through such sources as buffered analgesics and antacids may lead to pathological changes in the central nervous, skeletal and hematopoietic systems (Ayar et al., 2009).

Only seven studies investigating the content of Al in raw cow's milk samples collected in eleven regions in the world have been published from 2010 to date. Two conducted in China, two in Spain, one in Turkey, one in Canada, and one in India (Table 1). Two techniques were used to measure Al in raw cow's milk samples (ICP-MS and ICP-AES). Al levels in raw cow's milk samples across different countries ranged between 0.007 mg/L and 22.5 mg/L (Table 1).

Raw's milk samples from cows reared near to food producing plants in Gümüşçay district had higher Al levels (22.5 mg/L) compared to those obtained in samples collected from cows reared near to heavy industry plants (19.53 mg/L) in Yeniçiftlik district and those found in samples collected from cows reared near to highways (17.32 mg/L) in Şakirbey district, Turkey (Bigucu et al., 2016). Also, a low level of Al (0.05 mg/L) was recorded in Shaanxi district, China, this value was significantly correlated with Al level in feed (492.00 mg/L) (Zhou et al., 2017). The lowest level of Al (0.007 mg/L) was recorded in Alberta, Canada (Zwierzchowski and Ametaj, 2019).

4. Risk assessment of raw cow milk consumption

4.1. Estimated Daily Intake (EDI)

In Table 3, the daily intake via dairy products consumption (mg/day) were calculated for Ni, Fe and Cu and are compared to the Recommended Dietary Allowances (RDAs) that have been set for these metals.

The RDAs regarding Ni is set to 1 mg/day. The daily intake (mg/day) of Ni in raw cow milk across the globe ranged from 0 to 1.71 (mg/day), which represent 0–171.36% of the total RDA. The raw cow milk of Tirupati, India exhibited the highest EDI (1.71 mg/day) which represent 171.36% of RDA. It should be noted that the daily intake of Ni in 25 regions (86.2%) out of 29 through the globe were $<3.79 \times 10^{-3}$ which represent a maximum of 0.37% of RDA (Table 3).

As for Fe, the daily intake ranged from 1.18×10^{-4} to 2.49×10^{-2} mg/day which represent 0.0003% - 0.06% of RDA value of 45 mg/day (Table 3). In case of Cu, the RDAs is set to 0.9 mg/day. The daily intake of Cu through raw cow milk consumption ranged from 2.14×10^{-5} to 7.67×10^{-2} mg/day which represent 0.002% - 8.52% of RDA.

Dietary exposure to Pb, Cd, Hg, and Al through raw milk consuming was evaluated by calculating EDI of these metals based on the current analysis and compared with provisional tolerable daily intake (PTDI). The Joint FAO/WHO Expert Committee on Food Additives recommended the provisional tolerable weekly intakes (PTWI) of Pb as 25 μg/kg BW (equivalent to 3.6 μg/kg BW/day) (FAO/WHO, 1993). The calculation of the EDI_{milk} of collected data showed that the exposure of Pb through raw cow milk consumption around the world covers

Table 2
Estimated Daily Intake (EDI) in (mg/kg BW/day) of Pb, Cd, Hg, Al in comparison to PTDI values.

Reference	Location	Pb	Cd	Hg	Al
		EDI (% TDI)	EDI (% TDI)	EDI (% TDI)	EDI (% TDI)
Abdalla et al. (2013)	Sudan	2.60E-03 (72.31)	3.11E-06 (0.37)	–	–
Ahmad et al. (2017)	Pakistan, Pakhtunkhwa	–	3.37E-04 (40.6)	–	–
Akele et al. (2017)	Ethiopia, North Gondar	2.3E-04 (6.4)	4.52E-04 (54.4)	–	–
Akhtar et al. (2015)	Pakistan, Multan	8.43E-04 (23.41)	4.21E-04 (50.77)	–	–
Arianejad et al. (2015)	Iran, Arak	–	–	6.86E-08 (0.12)	–
	Iran, Arak	–	–	6.17E-08 (0.11)	–
Aslam et al. (2011)	Pakistan, Faisalabad	9.79E-02 (2715.8)	7.16E-04 (86.3)	2.32E-03 (4066.42)	–
Bakircioglu et al. (2018)	Turkey, Edirne	–	–	–	1.72E-03 (1.27)
Bigucu et al. (2016)	Turkey, Şakirbey	1.1E-02 (304.6)	2.31E-03 (278.5)	–	1.03E-01 (73.3)
	Turkey, Yeniçiftlik	6.1E-03 (169.6)	1.13E-03 (135.7)	–	1.16E-01 (82.7)
	Turkey, Gümüşçay	6.0E-03 (166.3)	1.13E-03 (135.7)	–	1.33E-01 (95.2)
Bilandžić et al. (2011)	Croatia, Northern	2.7E-04 (7.0)	4.44E-06 (0.54)	4.44E-06 (7.7)	–
	Croatia, Southern	1.3E-04 (3.7)	1.33E-05 (1.6)	3.11E-05 (54.5)-	–
Bilandžić et al. (2016)	Croatia	4.4E-05 (1.2)	–	–	–
Bousbia et al. (2019)	Algeria, Guelma	–	1.18E-04 (14.25)	–	–
Cadar et al. (2015)	Romania, Eastern Carpathians	4.8E-05 (1.3)	7.93E-06 (0.96)	7.93E-06 (13.9)	–
Capcarova et al. (2019)	Slovak, Nitra region	6.4E-03 (177.9)	4.55E-04 (54.8)	–	–
Castro-Gonzalez and Calderon-Sanchez (2018)	Mexico, Puebla	8.7E-05 (2.4)	–	–	–
Chirinos-Peinado and Castro-Bedriñana (2020)	India, Peru	1.29E-03 (33.1)	4.11E-05 (4.9)	–	–
Derakhshesh and Rahimi (2012)	Iran, Tehran	1.4E-05 (0.3)	–	–	–
	Iran, Yasuj	3.4E-06 (0.1)	–	–	–
Dizaji et al. (2012)	Iran, East Azerbaijan	6.9E-06 (0.2)	4.11E-07 (0.05)	–	–
El Sayed et al. (2011)	Egypt, Menofia	5.0E-04 (13.8)	1.66E-05 (2)	–	–
Elatrash and Atoweir (2014)	Libya, Benghazi, Sidy Khalifa		2.57E-06 (0.31)	–	–
	Libya, Benghazi, Garyounis	1.4E-05 (0.4)	8.57E-06 (1.03)	–	–
Fenta (2014)	Ethiopia, Chafe	1.2E-03 (34.60)	3.11E-04 (37.52)	–	–
	Ethiopia, Dato	6.2E-04 (17.30)	1.56E-04 (18.76)	–	–
Giri and Singh (2019)	India, East of Singhbhum	2.6E-04 (7.4)	–	–	1.04E-03 (0.7)
	India, West of Singhbhum	1.8E-04 (5.1)	–	–	5.3E-04 (0.4)
González-Montaña et al. (2012)	Spain, Asturias	9.9E-06 (0.2)	–	–	–
González-Montaña et al. (2019)	Spain, Asturias	–	–	–	4.62E-04 (0.33)
Iftikhar et al. (2014)	Pakistan, Peshawar	9.4E-03 (262.2)	2.91E-03 (350.34)	–	–
	Pakistan, Peshawar	8.8E-03 (243.5)	1.6E-04 (20.3)	–	–
Islam et al. (2015)	Bangladesh, Bogra	5.4E-05 (1.5)	7.14E-06 (0.86)	–	–
Ismail et al. (2015)	Pakistan, Multan	1.3E-04 (3.5)	1.26E-05 (1.5)	–	–
	Pakistan, Multan	4.2E-05 (1.1)	4.21E-07 (0.05)	–	–
Ismail et al. (2017)	Pakistan, Punjab	1.4E-04 (3.86)	1.69E-05 (2.03)	–	–
Jigam et al. (2011)	Nigeria, Niger Dangana	1.2E-04 (3.3)	–	–	–
Jolly et al. (2017)	Bangladesh, Dhaka/Barishal	1.4E-05 (0.4)	7.1E-06 (0.8)	1.07E-05 (18.8)	–
Kabir et al. (2017)	Bangladesh, Karnafuli, Chittagong	3.2E-05 (0.9)	1.07E-04 (12.91)	2.1E-05 (37.5)	–
Kim et al. (2016)	Korea, South part	1.4E-07 (0.004)	2.86E-08 (0.003)	–	–
Król et al. (2012)	Poland, Lublin	1.5E-05 (0.41)	1.03E-05 (1.24)	–	–
	Poland, Bieszczady	1.2E-05 (0.3)	2.9E-06 (0.3)	–	–
	Poland, Biebrza	8.8E-06 (0.2)	5.9E-06 (0.7)	–	–
Maas et al. (2011)	France, Besançon	1.9E-04 (5.2)	1.5E-06 (0.19)	–	–
Malhat et al. (2012)	Egypt, El-Qaliubiya	3.6E-03 (101.3)	2.3E-04 (27.9)	–	–
Meshref et al. (2014)	Egypt, Beni-Suef	1.7E-04 (4.6)	4.1E-05 (5.0)	–	–
Muhib et al. (2016)	Bangladesh, Dhaka	5.4E-06 (0.1)	7.14E-06 (0.86)	–	–
	Bangladesh, Dhaka	4.3E-06 (0.1)	1.79E-05 (2.15)	–	–
Najarnezhad and Akbarabadi (2013)	Iran, Khorasan	6.9E-06 (0.19)	2.06E-07 (0.02)	2.06E-06 (3.6)	–
Najarnezhad et al. (2015)	Iran, West Azerbaijan	4.80E-06 (0.1)	6.86E-07 (0.08)	–	–
Norouzirad et al. (2018)	Iran, Khuzestan	3.4E-05 (0.9)	2.74E-06 (0.33)	–	–
Ogundiran et al. (2012)	Nigeria, Ibadan	6.8E-05 (1.9)	–	–	–
Pérez-Carrera et al. (2016)	Argentina, Southeast of Córdoba	5.7E-04 (15.8)	–	–	–
Pilarczyk et al. (2013)	Poland, Lubuskie	4.4E-05 (1.2)	4.4E-06 (0.5)	–	–
	Poland, Lubuskie	5.9E-05 (1.6)	5.9E-06 (0.7)		
Qu et al. (2018)	Chine, Inner Mongolia		–	2.32E-06 (4.07)	1.62E-04 (0.1)
	Chine, Heilongjiang	1.2E-05 (0.3)	–	2.32E-06 (4.07)	8.81E-04 (0.6)
Raghu (2015)	India, Tirupati	1.2E-01 (3428.6)	2.47E-02 (2974.2)	–	–
Rahimi (2013)	Iran (Isfahan, Yazd, Mashhad, Kerman, and Ahvaz cities)	6.2E-06 (0.17)	6.17E-07 (0.07)	–	–
Safaei et al. (2020)	Iran, East Azerbaijan	6.9E-06 (0.2)	4.80E-06 (0.6)	–	–
Sarsembayeva et al. (2020)	Kazakhstan, Almaty	1.00E-05 (0.2)	2.7E-05 (3.2)	–	–
Shahbazi et al. (2016)	Iran (Ahvaz, Esfahan, Tehran, Tabriz and Mashhad cities)	9.60E-06 (0.2)	6.86E-07 (0.08)	–	–
Tahir et al. (2017)	Pakistan, Sargodha	3.4E-03 (93.6)	1.26E-03 (152.3)	–	–
Temiz and Soylu (2012)	Turkey, south-east of Samsun	3E-04 (8.2)	5.93E-05 (7.1)	–	–
Tona et al. (2013)	Nigeria, Ogbomoso	5.6E-07 (0.01)	3.71E-07 (0.04)	–	–
Zhou et al. (2017)	Chine, Shandong and Shaanxi cities	1.6E-06 (0.05)	8.11E-08 (0.01)	5.79E-06 (10.1)	5.79E-05 (0.04)
Zhou et al. (2019)	Chine, Tangshan	1.6E-06 (0.0)	1.16E-07 (0.01)	–	–
	Chine, Qiqihar	1.9E-07 (0.01)	4.63E-08 (0.01)	–	–

(continued on next page)

Table 2 (*continued*)

Reference	Location	Pb	Cd	Hg	Al
		EDI (% TDI)	EDI (% TDI)	EDI (% TDI)	EDI (% TDI)
Zodape et al. (2012)	India, Mumbai	1.2E-02 (337.1)	–	4.11E-05 (72.18)	–
Zwierzchowski and Ametaj (2019)	Canada, Alberta	1.7E-06 (0.05)	8.29E-07 (0.1)	–	5.8E-05 (0.04)

PTDI values: Pb 0.0036 mg/kg BW/day; Hg 0.000057 mg/kg BW/day (for inorganic mercury); Cd 0.00083 mg/kg BW/day; Al 0.14 mg/kg BW/day.
–: no data available.

Table 3
Trace elements daily intake (mg/day) for for Ni, Fe and Cu through consumption of milk in comparison to RDAs values.

Reference	Location	Ni	Cu	Fe
		DI (% RDA)	DI (% RDA)	DI (% RDA)
Abdalla et al. (2013)	Sudan	–	6.43E-04 (0.07)	–
Ahmad et al. (2017)	Pakistan, Pakhtunkhwa	–	5.90E-04 (0.07)	2.95E-03 (0.01)
Akele et al. (2017)	Ethiopia, North Gondar	–	1.74E-03 (0.19)	–
Akhtar et al. (2015)	Pakistan, Multan	7.59E-04 (0.08)	6.32E-04 (0.07)	4.34E-03 (0.01)
Arianejad et al. (2015)	Iran, Arak	2.74E-05 (0.003)	–	–
	Iran, Arak	8.9E-05 (0.01)	–	–
Aslam et al. (2011)	Pakistan, Faisalabad	9.9E-02 (9.9)	–	–
Bakircioglu et al. (2018)	Turkey, Edirne	–	1.54E-03 (0.17)	2.19E-02 (0.05)
Belete et al. (2014)	Ethiopia, Borena Zone	–	1.87E-04 (0.02)	–
Bigucu et al. (2016)	Turkey, Şakirbey	–	3.68E-03 (0.41)	2.49E-02 (0.06)
	Turkey, Yeniçiftlik	–	3.85E-03 (0.43)	1.54E-02 (0.03)
	Turkey, Gümüşçay	–	3.85E-03 (0.43)	2.40E-02 (0.05)
Bilandžić et al. (2011)	Croatia, Northern	–	4.13E-03 (0.46)	–
	Croatia, Southern	–	3.73E-03 (0.41)	–
Bousbia et al. (2019)	Algeria, Guelma	–	9.42E-04 (0.10)	5.64E-03 (0.01)
Cadar et al. (2015)	Romania, Eastern Carpathians	–	3.73E-04 (0.04)	–
Capcarova et al. (2019)	Slovak, Nitra region	1.42E-03 (0.14)	3.57E-03 (0.4)	2.97E-03 (0.01)
Castro-Gonzalez and Calderon-Sanchez (2018)	Mexico, Puebla	2.9E-05 (0.003)	2.91E-05 (0.003)	–
El Sayed et al. (2011)	Egypt, Menofia	–	9.94E-04 (0.11)	4.23E-03 (0.01)
Elsaim and Ali (2018)	Sudan, Merowe	–	1.04E-03 (0.12)	–
Fenta (2014)	Ethiopia, Chafe	2.49E-03 (0.2)	–	–
	Ethiopia, Dato	2.18E-03 (0.2)	–	–
Giri and Singh (2019)	India, East of Singhbhum	1.28E-03 (0.13)	1.03E-03 (0.11)	1.81E-02 (0.04)
	India, West of Singhbhum	4.11E-04 (0.04)	6.38E-04 (0.07)	2.35E-02 (0.05)
Giri et al. (2011)	India, Jharkhand	8.23E-04 (0.08)	1.23E-03 (0.14)	1.01E-02 (0.02)
Iftikhar et al. (2014)	Pakistan, Peshawar	3.37E-03 (0.34)	5.90E-03 (0.66)	–
	Pakistan, Peshawar	8.43E-05 (0.01)	3.79E-04 (0.04)	–
Islam et al. (2015)	Bangladesh, Bogra	4.64E-04 (0.05)	8.21E-04 (0.09)	–
Ismail et al. (2015)	Pakistan, Multan	1.26E-04 (0.01)	4.89E-03 (0.54)	–
	Pakistan, Multan	1.26E-04 (0.01)	3.50E-03 (0.39)	–
Ismail et al. (2017)	Pakistan, Punjab	3.50E-04 (0.03)	1.69E-04 (0.02)	–
Jigam et al. (2011)	Nigeria, Niger Dangana	–	1.19E-04 (0.01)	–
Jolly et al. (2017)	Bangladesh, Dhaka/Barishal	2.86E-05 (0.003)	2.14E-05 (0.002)	4.39E-04 (0.001)
Kabir et al. (2017)	Bangladesh, Karnafuli, Chittagong	3.93E-05 (0.004)	4.29E-05 (0.005)	2.73E-03 (0.01)
Król et al. (2012)	Poland, Lublin	–	5.89E-05 (0.01)	3.68E-04 (0.001)
	Poland, Bieszczady	–	5.89E-05 (0.01)	5.44E-04 (0.001)
	Poland, Biebrza	–	1.03E-04 (0.01)	4.86E-04 (0.001)
Maas et al. (2011)	France, Besançon	–	2.69E-03 (0.3)	–
Malhat et al. (2012)	Egypt, El-Qaliubiya	–	2.40E-03 (0.26)	1.63E-02 (0.03)
Meshref et al. (2014)	Egypt, Beni-Suef	–	7.46E-05 (0.01)	7.37E-03 (0.02)
Muhib et al. (2016)	Bangladesh, Dhaka	–	2.14E-05 (0.002)	1.18E-04 (0.0003)
	Bangladesh, Dhaka	–	4.29E-05 (0.005)	2.25E-04 (0.001)
Ogundiran et al. (2012)	Nigeria, Ibadan	–	5.80E-03 (0.64)	–
	Nigeria, Ibadan	–	3.57E-05 (0.004)	–
Pérez-Carrera et al. (2016)	Argentina, Southeast of Córdoba	1.14E-04 (0.01)	8.53E-05 (0.01)	2.73E-03 (0.01)
Pilarczyk et al. (2013)	Poland, Lubuskie	–	4.41E-05 (0.005)	3.68E-04 (0.001)
	Poland, Lubuskie		8.9E-05 (0.01)	2.9E-04 (0.001)
Qu et al. (2018)	Chine, Heilongjiang	1.16E-05 (0.001)	–	–
Raghu (2015)	India, Tirupati	1.71E+00 (171.3)	7.41E-02 (8.23)	–
	India, Mangampeta	1.38E-01 (13.8)	5.76E-02 (6.4)	–
Rao and Murthy (2017)	Tanzania, Dodoma, Ntyuka	–	2.29E-04 (0.03)	–
Shahbazi et al. (2016)	Iran, Ahvaz, Esfahan, Tehran, Tabriz and Mashhad cities	–	2.88E-04 (0.03)	–
Tahir et al. (2017)	Pakistan, Sargodha	1.2E-02 (1.2)	–	–
Temiz and Soylu (2012)	Turkey, south-east of Samsun	3.8E-03 (0.38)	9.66E-03 (1.07)	2.79E-03 (0.01)
Zain et al. (2016)	Iran, South	–	6.38E-04 (0.07)	9.12E-04 (0.002)
	Iran, North	–	5.69E-04 (0.06)	9.87E-04 (0.002)
	Malaysia, Peninsula	–	2.03E-04 (0.02)	3.76E-04 (0.001)
	Malaysia, Peninsula	–	2.04E-04 (0.02)	7.20E-04 (0.002)
Zhou et al. (2017)	Chine, Shandong and Shaanxi cities	6.67E-06 (8.52)	3.48E-05 (0.004)	4.06E-04 (0.001)
Zodape et al. (2012)	India, Mumbai	–	7.67E-02 (8.52)	–
Zwierzchowski and Ametaj (2019)	Canada, Alberta	–	8.29E-05 (0.01)	4.14E-04 (0.001)

RDA* (Recommended Dietary Allowances) Ni 1 mg/day; Fe 45 mg/day; Cu 0.9 mg/day. –: no data available.

Table 4
Target hazard quotient (THQ) values for heavy metals (Pb, Cd, Ni, Hg, Fe, Cu, Al) in raw cow's milk reported in research articles published since 2010.

Reference	Location	THQ						
		Pb	Cd	Ni	Hg	Fe	Cu	Al
Abdalla et al. (2013)	Sudan	7.4E-01	3.11E-03	–	–	–	1.61E-02	–
Ahmad et al. (2017)	Pakistan, Pakhtunkhwa	–	3.37E-01	–	–	4.21E-03	1.48E-02	–
Akele et al. (2017)	Ethiopia, North Gondar	6.7E-02	4.52E-01	–	–	–	4.36E-02	–
Akhtar et al. (2015)	Pakistan, Multan	2.4E-01	4.21E-01	3.79E-02	–	6.20E-03	1.58E-02	–
Arianejad et al. (2015)	Iran, Arak	–	–	1.37E-03	2.29E-04	–	–	–
	Iran, Arak	–	–	4.46E-03	2.06E-04	–	–	–
Aslam et al. (2011)	Pakistan, Faisalabad	2.80E+01	1.16E-01	4.95E+00	7.73E+00	–	–	–
Bakircioglu et al. (2018)	Turkey, Edirne	–	–	–	–	3.13E-02	3.85E-02	1.78E-03
Belete et al. (2014)	Ethiopia, Borena Zone	–	–	–	–	–	4.67E-03	–
Bigucu et al. (2016)	Turkey, Şakirbey	3.1E+00	2.31E+00	–	–	3.56E-02	9.19E-02	1.03E-01
	Turkey, Yeniçiftlik	1.7E+00	1.13E+00	–	–	2.20E-02	9.63E-02	1.16E-01
	Turkey, Gümüşçay	1.7E+00	1.13E+00	–	–	3.43E-02	9.63E-02	1.33E-01
Bilandžić et al. (2011)	Croatia, Northern	7.6E-02	4.44E-02	–	1.48E-02	–	1.03E-01	–
	Croatia, Southern	3.8E-02	1.33E-03	–	1.04E-01	–	9.33E-02	–
Bilandžić et al. (2016)	Croatia	1.3E-02	–	–	1.04E-01	–	–	–
Bousbia et al. (2019)	Algeria, Guelma	–	1.18E-01	–	–	8.05E-03	2.36E-02	–
Cadar et al. (2015)	Romania, Eastern Carpathians	1.4E-02	7.93E-03	–	2.64E-02	–	9.32E-03	–
Capcarova et al. (2019)	Slovak, Nitra region	1.8E+00	4.55E-01	7.08E-02	–	4.24E-03	8.93E-02	–
Castro-Gonzalez and Calderon-Sanchez (2018)	Mexico, Puebla	2.50E-02	–	1.46E-03	–	–	7.29E-04	–
Chirinos-Peinado and Castro-Bedriñana (2020)	India, Peru	3.41E-01	4.11E-02	–	–	–	–	–
Derakhshesh and Rahimi (2012)	Iran, Tehran	3.9E-03	–	–	–	–	–	–
	Iran, Yasuj	9.8E-04	–	–	–	–	–	–
Dizaji et al. (2012)	Iran, East Azerbaijan	2.0E-03	4.11E-04	–	–	–	–	–
El Sayed et al. (2011)	Egypt, Menofia	1.45E-01	1.66E-02	–	–	6.04E-03	2.49E-02	–
Elatrash and Atoweir (2014)	Libya, Benghazi, Sidy Khalifa	–	2.57E-03	–	–	–	–	–
	Libya, Benghazi, Garyounis	4.1E-03	5.71E-03	–	–	–	–	–
Elsaim and Ali (2018)	Sudan, Merowe	–	–	–	–	–	2.59E-02	–
Fenta (2014)	Ethiopia, Chafe	3.6E-01	3.11E-01	1.25E-01	–	–	–	–
	Ethiopia, Dato	1.8E-01	1.56E-01	1.09E-01	–	–	–	–
Giri and Singh (2019)	India, East of Singhbhum	7.6E-02	–	6.38E-02	–	2.59E-02	2.57E-02	1.03E-03
	India, West of Singhbhum	5.3E-02	–	2.06E-02	–	3.35E-02	1.60E-02	5.35E-04
Giri et al. (2011)	India, Jharkhand	–	–	4.11E-02	–	1.44E-02	3.09E-02	–
González-Montaña et al. (2012)	Spain, Asturias	2.8E-03	–	–	–	–	–	–
González-Montaña et al. (2019)	Spain, Asturias	–	–	–	–	–	–	4.62E-04
Iftikhar et al. (2014)	Pakistan, Peshawar	2.7E+00	2.91E+00	1.69E-01	–	–	1.48E-01	–
	Pakistan, Peshawar	2.5E+00	1.69E-01	4.21E-03	–	–	9.48E-03	–
Islam et al. (2015)	Bangladesh, Bogra	1.5E-02	7.14E-03	–	–	–	2.05E-02	–
Ismail et al. (2015)	Pakistan, Multan	3.6E-02	1.26E-02	6.32E-03	–	–	–	–
	Pakistan, Multan	1.2E-02	4.21E-03	6.32E-03	–	–	–	–
Ismail et al. (2017)	Pakistan, Punjab	4.0E-02	1.69E-02	1.75E-02	–	–	4.21E-03	–
Jigam et al. (2011)	Nigeria, Niger Dangana	3.4E-02	–	–	–	–	2.98E-03	–
Jolly et al. (2017)	Bangladesh, Dhaka/Barishal	4.1E-03	7.14E-03	1.43E-03	3.57E-02	6.28E-04	5.36E-04	–
Kabir et al. (2017)	Bangladesh, Karnafuli, Chittagong	9.2E-03	1.07E-01	1.96E-02	7.14E-02	3.9E-03	1.07E-03	–
Kim et al. (2016)	Korea, South part	4.1E-05	2.86E-05	–	–	–	–	–
Król et al. (2012)	Poland, Lublin	4.2E-03	1.03E-02	–	–	5.26E-04	1.47E-03	–
	Poland, Bieszczady	3.4E-03	2.94E-03	–	–	7.78E-04	1.47E-03	–
	Poland, Biebrza	2.5E-03	5.89E-03	–	–	6.94E-04	2.58E-03	–
Maas et al. (2011)	France, Besançon	5.4E-02	1.57E-03	–	–	–	6.72E-02	–
Malhat et al. (2012)	Egypt, El-Qaliubiya	1.E+00	3.32E-01	–	–	1.50E-02	6.01E-02	–
Meshref et al. (2014)	Egypt, Beni-Suef	4.7E-02	4.41E-02	–	–	1.05E-02	1.86E-03	–
Muhib et al. (2016)	Bangladesh, Dhaka	1.5E-03	7.14E-03	–	–	1.68E-04	5.36E-04	–
	Bangladesh, Dhaka	1.2E-03	1.79E-02	–	–	3.21E-04	1.07E-03	–
Najarnezhad and Akbarabadi (2013)	Iran, Khorasan	2.E-03	2.06E-04	–	6.86E-03	–	–	–
Najarnezhad et al. (2015)	Iran, West Azerbaijan	1.4E-03	6.86E-04	–	–	–	–	–
Norouzirad et al. (2018)	Iran, Khuzestan	9.8E-03	2.74E-03	–	–	–	–	–
Ogundiran et al. (2012)	Nigeria, Ibadan	–	–	–	–	–	1.45E-01	–
	Nigeria, Ibadan	2E-02	–	–	–	–	8.94E-04	–
Pérez-Carrera et al. (2016)	Argentina, Southeast of Córdoba	1.6E-01	–	5.69E-03	–	3.9E-03	2.13E-03	–
Pilarczyk et al. (2013)	Poland, Lubuskie	1.3E-02	4.41E-03	–	–	5.26E-04	1.10E-03	–
	Poland, Lubuskie	1.7E-02	5.9E-03	–	–	4.2E-04	1.4E-03	
Qu et al. (2018)	Chine, Inner Mongolia	–	–	–	7.72E-03	–	–	1.62E-04
	Chine, Heilongjiang	3.3E-03	–	5.79E-04	7.72E-03	–	–	8.81E-04
Raghu (2015)	India, Tirupati	3.53E+01	2.47E+01	8.57E+01	–	–	1.85E+00	–
	India, Mangampeta	–	–	6.89E+00	–	–	1.44E+00	–
Rahimi (2013)	Iran (Isfahan, Yazd, Mashhad, Kerman, and Ahvaz cities)	1.8E-03	6.17E-04	–	–	–	–	–
Rao and Murthy (2017)	Tanzania, Dodoma, Ntyuka	–	–	–	–	–	5.71E-03	–
Safaei et al. (2020)	Iran, East Azerbaijan	2.0E-03	4.80E-03	–	–	–	–	–
Sarsembayeva et al. (2020)	Kazakhstan, Almaty	2.9E-03	2.7E-02	–	–	–	–	–
Shahbazi et al. (2016)	Iran (Ahvaz, Esfahan, Tehran, Tabriz and Mashhad cities)	2.7E-03	6.86E-04	–	–	–	7.20E-03	–
Tahir et al. (2017)	Pakistan, Sargodha	9.6E-01	1.26E+00	6.01E-01	–	–	–	–
Temiz and Soylu (2012)	Turkey, south-east of Samsun	8.5E-02	5.93E-02	1.90E-01	–	3.98E-03	2.42E-01	–

(continued on next page)

Table 4 (*continued*)

Reference	Location	THQ						
		Pb	Cd	Ni	Hg	Fe	Cu	Al
Tona et al. (2013)	Nigeria, Ogbomoso	1.6E-04	3.71E-04	–	–	–	–	–
Zain et al. (2016)	Iran, South	–	–	–	–	1.30E-03	1.59E-02	–
	Iran, North	–	–	–	–	1.41E-03	1.42E-02	–
	Malaysia, Peninsula	–	–	–	–	5.37E-04	5.07E-03	–
	Malaysia, Peninsula	–	–	–	–	1.03E-03	5.11E-03	–
Zhou et al. (2017)	Chine, Shandong and Shaanxi cities	4.6E-04	8.11E-05	3.30E-04	1.93E-02	5.79E-04	8.69E-04	5.79E-05
Zhou et al. (2019)	Chine, Tangshan	4.6E-04	1.16E-04	–	–	–	–	–
	Chine, Qiqihar	5.30E-05	4.63E-05	–	–	–	–	–
Zodape et al. (2012)	India, Mumbai	–	–	–	1.37E-01	–	1.92E+00	–
Zwierzchowski and Ametaj (2019)	Canada, Alberta	4.7E-04	8.29E-04	–	–	5.92E-04	2.07E-03	5.8E-05

–: no data available.

values ranged from 0 to 0.123 (mg/kg BW/day) which represent 0–3428.57% of PTDI. It should be noted that mean Pb uptake by milk consumption in 10 regions (14.28%) out of 70 across the globe were extremely high, the EDI values were exceeding 100% of PTDI. In 27 (38.57%) regions, and the values were between 1% to 93% of PTDI. In 33 regions (47.14%) were representing values <1% of PTDI. The average consumption of Pb through milk reaches its maximum in milk collected in India, and Pakistan, the values were 3428.57%, and 2715.87% of PTDI respectively (Table 2).

For Cd, the provisional tolerable monthly intake (PTMI) given by Joint FAO/WHO Expert Committee on Food Additives is 25 μg/kg BW (equivalent to 0.83 μg/kg BW/day) (FAO/WHO, 2012). The exposure of Cd through raw cow milk consumption across the globe ranged between 0 and 0.024 (mg/kg BW/day) which represented the values ranged from 0 to 2974.18% of PTDI. The average consumption of Cd through milk collected in Mangampeta, China covers maximum 2974.18% of PTDI. The average Cd consumption exceed 100% of PTDI in 6 regions (10%) across the globe, while the values ranged between 1% and 86.32% of PTDI in 22 regions (37%), finally the EDI in milk collected in 31 regions which represent half of the analyzed milk across the globe exceeded 1% of PTDI (Table 2).

Regarding Hg, a recommended PTWI of 1.6 μg/kg have been set by the 67th JECFA in 2003 for methyl mercury (FAO/WHO, 2010), the average of Hg consumption through milk ranged between 0 and 0.0023 (mg/kg BW/day) which represent 0 to 4066.42% of PTDI. The average consumption of Hg in raw cow milk collected from Faisalabad, Pakistan covers maximum 4066.42% of PTDI, followed by the milk of Mumbai, India with represent 72.18% of PTDI (Table 2).

The 67 and JECFA has set a PTWI of 1 mg/kg BW/day (equivalent to 0.14 mg/kg BW/day) for Al (FAO/WHO, 2006). The EDI of Al through consumption of raw cow milk was ranged from 5.79E-05 to 1.33×10^{-1} (mg/kg BW/day) which represent 0.046% to 95.28% of PTDI. The highest EDI 1.33×10^{-1} (mg/kg BW/day) was recorded in milk collected in Turkey (Table 2).

4.2. Non-carcinogenic risk assessment

The non-carcinogenic risk of Pb, Cd, Hg, Ni, Fe, Cu, and Al for the milk consumers was determined by calculating THQ (target hazard quotient) value. THQ of these metals for adults was calculated based on the mean levels of these metals that obtained from the current review (Table 4). It must be noted that if THQ of metals is <1 non obvious risks are improbable to happen to the exposed population. Harmful impacts may happen to exposed population if THQ is >1 (Dadar et al., 2017; Rahmani et al., 2018).

The results of THQ values indicated that milk consumers in ten regions out of 70 in the globe during the last decade 2010–2020 were exposed to some potential health risk through the intake of Pb.

The highest value of THQ for Pb was estimated to be 3.53 E+01 in raw milk collected in Tirupati province, India. THQ values >1 (2.70E+00, 2.5E+00) in milk collected in Peshawar province, Pakistan. A health effect (THQ values >1) were observed in Turkey in raw cow milk collected in Şakirbey province, in Yeniçiftlik province, and in Gümüşçay province, the values were 3.1E+00, 1.7E+00 and 1.7E+00 respectively. Also THQ of Pb was more than one (2.8E+01) in milk collected from Faisalabad city, Pakistan, (1.8E+00) in milk collected in Nitra region, Slovak, (1.4E+00) in Industrial air pollution area of Tokh city, El-Qaliubiya governance, Egypt, and (3.5E+00) in milk collected in Mumbai region, India.

Regarding to Table 4, the THQ of Cd in six regions out of 59 that analyzed heavy metals in raw cow milk exceeded one indicating a greater risk for consumers. The highest THQ value of Cd was 2.47E+01 recorded in raw milk of Tirupati province (India). In turkey, THQ values were 2.31E+00, 1.13E+00, and 1.13E+00 recorded in milk collected in Sakirbey province, Yeniçiftlik province, and Gümüşçay province respectively. Also in Pakistan, THQ values (2.91E+00, 1.26E+00) were higher than 1 in raw milk collected in Peshawar province, and Sargodha province, respectively.

In the case of Cu, it was observed that THQ values were more than one only in three regions out of 54 regions around the world. The highest values (1.92E+00, 1.85E+00 and 1.44E+00) were recorded in India from Mumbai region, Turipati province, and Magampeta province respectively.

For Ni, THQ values exceeded 1 in three regions out of 29 regions in the world. The THQ values were 8.57E+01, 6.89E+00, and 4.95E+00 in raw milk of Tirupati province, and Mangampeta province, India, and in raw milk of Faisalabad region, Pakistan.

Hg THQ values are of concern (THQ values = 7.73E+00; Table 4) only for raw cow milk collected in Faisalabad province, Pakistan.

The results of non-carcinogenic risks from exposure to metals through milk consumption indicates that raw cow milk collected in all sites during the last decade was safe for human consumption in terms of the amounts of Al and Fe (THQ values <1; Table 4).

5. Conclusion

Milk is an important food source, it is rich in macro- and micronutrients which play an important role in health preservation; it impacts positively nutrient and energy intakes. However, heavy metals can counterbalance these benefits, and affect human health.

This systematic review covers 60 studies that assessed Pb, Cd, Hg, Ni, Fe, Cu, and Al levels in raw cow milk samples collected worldwide. The highest mean levels of Pb, Ni, Cu, Cd, and Fe in raw cow milk were reported in India while the highest values of Al, and Hg were recorded in Turkey and Pakistan respectively. The concentrations of Fe and Cu in raw cow milk collected worldwide were higher than the maximum limit recommended by the US Food and Nutrition Board. In the same way, according to our data, the overall concentration of Pb and Cd in cow milk was generally higher in developing countries and lower in developed countries, reflecting less strict regulation in developing countries.

The exposure assessment indicates that the exposure to Al and Fe through milk consumption were safe for human consumption. The THQ values of Hg were below 1 suggesting that milk consumers are not at non-carcinogenic risk except in Faisalabad province, Pakistan where THQ values = 7.7. However, the THQ values were >1 for Pb (10 regions out of 70), for Cd (6 regions out of 59), for Ni (3 out of 29), and for Cu (3 out of 54).

Data recorded in this systematic review show the difficulty to understand the multifaceted aspect of food security related to cow milk consumption. Moreover, data actualization and continuous monitoring are necessary and recommended to evaluate the potential adverse effects of heavy metals on human and animal health in future studies.

Ethical statement

None to be declared.

CRediT authorship contribution statement

Sofiane Boudalia, Ali Boudebbouz and Safia Habila has chosen the theme of the review, conceived the original idea and supervised the project.

Ali Boudebbouz, Sofiane Boudalia and Aissam Bousbia collected the data and contributed to the conceptualization of the manuscript and the overall writing and editing of the manuscript.

Meriem Imen Boussadia and Yassine Gueroui has contributed to manuscript revision and editing.

All authors discussed the review topic, contents and contributed to the final manuscript.

Declaration of competing interest

The author declares no conflict interests.

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CHAPTER 5

PESTICIDE RESIDUES LEVELS IN RAW COW'S MILK AND HEALTH RISK ASSESSMENT ACROSS THE GLOBE: A SYSTEMATIC REVIEW

From Ali BOUDEBBOUZ, Sofiane BOUDALIA, Meriem Imen BOUSSADIA, Yassine GUEROUI, Safia HABILA, Aissam BOUSBIA, George K. SYMEON. Pesticide residues levels in raw cow's milk and health risk assessment across the globe: a systematic review. *Environmental Advances*. 2022:100266. https://doi.org/10.1016/j.envadv.2022.100266.

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Pesticide residues levels in raw cow's milk and health risk assessment across the globe: A systematic review

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ABSTRACT

Milk is a widely consumed food rich in macro- and micronutrients that play an important role in health preservation. While it affects positively human nutrient and energy uptake, the presence of pesticide residues could, however, counterbalance these benefits and negatively affect human health. This systematic review provides an overview of studies on pesticide residues during the last decade and the related human health risk assessment. Thirty-five original articles published since 2010 reporting the levels of pesticide residues in raw cow's milk in 69 regions from 15 countries were reviewed. Data showed that pesticide residue levels were ranked as, DDTs> permethrin> bifenthrin> Drins> endrin> endosulfan> HCHs> cyhalothrin> cypermethrin> heptachlor> ethion> coumaphos> deltamethrin> dimethoate, chlorpyriphos> profenofos> malathion> dichlorvos> parathion methyl> carbaryl> aldicarb> carbofuran> methamidophos. High geographic variation was observed, and many regions appear as contaminated zones with high risks such as Punjab in Pakistan (× 3080 > MRL and × 113 > MRL for Cypermethrin and Drins, respectively), Sand Pedro in Columbia (× 1090 > MRL and × 200 > MRL for endrin and Drins, respectively), and Gezira State in Sudan (× 109 > MRL DDTs). The risk assessment for humans indicated that HQ Drins values were > 1 in Columbia (Sucre, Casa Azul, San Pedro, Costanera, Sabanas, Sinú Medio, and San Jorge regions), and in Pakistan (Punjab region). Moreover, the HQ values for endrin were > 1 in Sinú Medio (Colombia) and for heptachlor in Costanera region, Sinú Medio, and Sabanas (Colombia). Furthermore, HI values were > 1 in seven regions in Colombia, 1 region in Pakistan, 1 region in Egypt and 1 region in Turkey, suggesting a serious health risk. In conclusion, to avoid cow's milk contamination by pesticides, it is necessary to develop eco-friendly alternatives to chemical pesticides and promote integrated pest management (IPM) strategies.

1. Introduction

In order to deal with the effects of globalization, urbanization, mechanization, overpopulation, global warming, and climate change, it is of grave importance to improve the sustainability of agriculture and the food producing systems so that they can be profitable while at the same time they safeguard the natural resources for future generations (Boudalia et al., 2020; Bousbia et al., 2021; Martin et al., 2020; Wainwright et al., 2019). Commonly, conventional agriculture uses chemical inputs, such as pesticides, which are generalised across the globe, with several advantages including increasing crop yield and an effective fight against diseases and pests (Benada et al., 2021; Galani et al., 2020).

Abbreviations: ADI, acceptable daily intake; BDL, below detection limit; CAC, Codex Alimentarius Commission; CB, carbamate pesticides; DDD, dichlorodiphenyldichloroethane; DDE, dichlorodiphenyldichloroethylene; DDT, dichlorodiphenyltrichloroethane; EDI, estimated daily intake; EU, European Commission; FAO, food and agriculture organization; HCH, hexachlorocychlohexane; HI, hazard index; HQ, hazard quotient; MRL, maximum residue level; OC, organochlorine pesticides; OHPs, organohalogenated pollutants; OP, prganophosphorus pesticides; PTDI, provisional tolerable daily intake; PY, pyrethroid pesticides; WHO, World Health Organization.

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However, besides their benefits, several negative impacts of their use have been recorded, such as their undesirable adverse effects on non-target organisms and human health as well as negative environmental effects (Simeonov et al., 2014).

The consumption of milk and its derivatives belongs to the most ancient eating practices (Leksir et al., 2019) since these products are considered as the most balanced food ever found in nature, containing major sources of nutrients, especially for children, adults, and the elderly people (Nag, 2010). They are consumed by people worldwide due to their nutritive qualities (Boukria et al., 2020), mainly their contribution as the main source of calcium, but also for their macro- and micronutrients such as proteins, lipids (poly-unsaturated fatty acids), carbohydrates, essential amino acids, vitamins, and several bioactive compounds important for biochemical and physiological functions (Boudalia et al., 2016; Pereira, 2014).

Data from previous studies have shown that worldwide milk contamination with emerging contaminants such as heavy metals and pesticide residues originates mainly through animal consumption of contaminated water, feed, and grass or corn silage (Boudebbouz et al., 2022, 2021; Bousbia et al., 2019; Gill et al., 2020). Most pesticide residues are fat-soluble in milk and can affect human health at low doses (El-Saeid et al., 2021), even though they are considered as not harmful by health authorities (Aiassa et al., 2019). Some pesticides and their residues can interact with the endocrine system and can act as endocrine disruptors through non-monotonic dose-response relationships (Auxietre et al., 2014; Boudalia et al., 2017). Consequently, humans are exposed to diverse mixtures that amplify the effects, especially, for the vulnerable population such as infants and young children under 3 years of age. This specific age group is more sensitive to several pesticides due to their high intake of milk and dairy products in relation to their body weight and to the immaturity of their defence systems against chemical stressors (Nougadère et al., 2020; Simeonov et al., 2014).

Synthetic pesticides are classified into four major classes, namely organochlorine pesticides (OC), organophosphorus pesticides (OP), carbamate pesticides (CB), and pyrethroid pesticides (PY). Organochlorine pesticides were usually used as insecticides, herbicides and fungicides against a broad range of insects, and fungi in the agriculture sector (Abubakar et al., 2020). They have been detected in milk, and dairy products over the past three decades, particularly in the form of Dichlorodiphenyltrichloroethane (DDTs) and Hexachlorocychlohexane (HCHs) (Nag, 2010). Organophosphorus pesticides have been used increasingly in agriculture after banning or restricting organochlorine in use. Organophosphorus pesticides including malathion, dimethoate, chlorpyriphos, profenofos, coumaphos, dichlorvos, methamidophos, ethion, parathion methyl are highly toxic to mammals and are considered as mutagens, carcinogens, and teratogens substances (Needham et al., 2005; Sun et al., 2020; Wainwright et al., 2019). Carbamates are organic pesticides that include carbaryl, carbofuran, and aldicarb. They are similar to organophosphorus in terms of structure, however, their degradation is easy in the natural environment (Bhatt et al., 2021). Synthetic-pyrethroid including permethrin, cypermethrin, deltamethrin, cyhalothrin and bifenthrin can affect brain dopaminergic and serotonergic systems and provoke neurobehavioral changes in Wistar rats (Ansari et al., 2012). They can also affect learning, memory-related characteristics, and reduce the homing ability of *Apis mellifera* bee (Liao et al., 2018), or inhibit the acetylcholinesterase at the synaptic junction of the fish *Odontesthes bonariensis*, which provoke lethal effects (López Aca et al., 2018).

European Commission (2005) has established Maximum Residue Levels (MRL) as a measure to evaluate the maximum residue levels of pesticides in or on food and feed of plant and animal origin. The MRLs for milk and dairy products is ranged between 0.8 and 2000 μg/kg for the EU (European Commission, 2019), or range between 0.4 and 1000 μg/kg according to FAO and WHO (FAO/WHO, 2018). Moreover, a specific regulation for food consumed by children, and infants have been established by the European Commission (2013) to restrict the use of pesticides in infant formulas as much as possible.

In developing countries the situation is different, and governments claim that they cannot ban certain chemicals easily for several reasons such as difficulty to control, low cost and efficacy. Consequently, most of these chemicals have been or continue to be used in large quantities in many developing countries across the globe (Ecobichon, 2001; Ullah et al., 2010). Moreover, developing countries use 20% of total pesticides produced worldwide, while developed countries use more than 80%. Nonetheless, the fatality rate in developing countries is 13 times greater due to incorrect or indiscriminate pesticide application (Ansari et al., 2021), no standards in the application of pesticides and also farmers' lack of pesticide expertise and training (Udimal et al., 2022). Competent authorities of food safety and their related laboratory structures are poorly equipped, and despite the existing regulatory move, there is still a large gap between current international scientific knowledge and local laws and policies addressing pesticide use (Mahdavi, 2010). Furthermore, they do not have the required infrastructure to properly eliminate pesticides from the environment (Parra-Arroyo et al., 2022).

The objective of this systematic review is to compare the levels of synthetic pesticide residues including organochlorine (OC), organophosphorus (OP), carbamate (CB), and pyrethroid (PY) in raw cow's milk samples recorded in different countries in the last decade (2010-2021). Moreover, contamination sources and regulations are also discussed. Finally, estimated daily intake (EDI), hazard quotient (HQ), and hazard index (HI) analyses of pesticide residues from consuming raw cow's milk are performed using data extracted for pesticide residues levels recorded from different areas across the globe.

2. Materials and methods

2.1. Method of literature search

As shown in Fig. 2, publications search and selected articles were conducted according to Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines (Moher et al., 2015). A systematic search of the published research articles between 2012 and 2021 regarding pesticide residue identification and evaluation in raw cow's milk samples across the globe was performed using three different scientific databases i.e., Science Direct, Scopus and PubMed. During the identification phase, several keywords and their synonyms, related terms and variations were used. The keywords were "raw milk" "cow milk", "bovine milk", "pesticide", "pesticides", "Organochlorine", "Organophosphorus", "Pyrethroids", "Carbamate" combined with "OR" and/or "AND". The Endnote X software (version 5, Thomson ISI ResearchSoft, Philadelphia, USA) was used to import found references. Moreover, the references section of imported articles was checked to retrieve other related studies even in other databases such as Google Scholar. Duplicate articles were deleted, and the only articles with further details were kept.

2.2. Relevant screening, inclusion and exclusion criteria

The review was conducted independently by two researchers (AB and SB). With respect to the source of the publication, no limit was applied. An initial screening by reading the title and abstract of the articles was carried out, to ensure they were eligible for inclusion. After that, the full text of the retrieved articles was downloaded.

To be included in the analysis, studies had to meet these criteria:

(1) availability of full-text in the English language; (2) detection and monitoring of pesticide levels in raw cow milk samples across the globe; (3) preparation and instrumental analysis using analytical methods (GC-MS, GC-ECD, GC-MS/MS, HPLC UV and PAD and LC-MS...) should be detailed; (4) the process of quality control, insurance and validation should be included in the analysis.

In addition, the following categories of research articles were excluded: (1) articles published before 2010; (2) articles that developed and validated analytical methods to determine pesticide residues levels without random raw cow's milk sample testing; (3) articles that recorded the occurrence of pesticide residues in milk other than raw cow's milk e.g. breast milk, sheep milk, camel milk, goat milk and buffalo milk; (4) articles that included processed milk, which designates raw cow's milk that has undergone several steps through various processes such as homogenization, sterilization or pasteurization, cream separation (whole milk, semi-skimmed milk or skimmed milk), packaging, etc.; (5) studies concerning pesticide residues evaluation in other foods matrices such as meat, fruits, vegetables, nuts, cotton, seed products; (6) articles that were not peer-reviewed and not published in English language; (7) studies concerning contamination of cow's milk by other residues such as polybrominated diphenyl ethers (PBDEs), polychlorinated biphenyls (PCBs), hexabromocyclododecanes (HBCDs); and finally 8) included reviews, opinion pieces, conference proceedings, book chapters, conference abstracts and letters to the editor.

The selected articles were thoroughly reviewed, and the required information that was extracted was as follows: first author, year of study, provincial and the geographical location (longitude and latitude), sample size, pesticide residues level (recorded as mean concentration ± standard deviation (SD) and/or range (Min-Max) and positive occurrence frequencies (%). Moreover, all units of concentration of pesticide residues μg/L, ppb, and ng/g were converted to ng/L to obtain homogeneous data.

2.3. Bump graph analysis

To better visualize the subjects covered by the screened published research articles, keywords were extracted from each article using a software platform, with an online interface: CorTexT Manager (http://manager.cortext.net/). Moreover, according to Testoni et al. (2021) procedure, an Epic Epoch script was performed to follow the temporal evolution of these keywords over the years from 2010 to 2021.

2.4. Human risk assessment and exposure to pesticide residues

The average concentrations of pesticide residues are presented on the scale of mg/kg, ppm, μg/mL.μg/g was multiplied by 1000 to convert it to the scale of ng/g while the concentration presented on the scale of μg/L, μg/kg, and ppb was considered equal to the scale of ng/g.

In order to calculate the estimated daily intake (EDI), hazard quotient (HQ), and hazard index (HI), when articles presented data as a range, maximum values were used, and values reported as non-detected (ND) or below the limit of detection (LOD) were treated as zero (Martin et al., 2020).

2.4.1. Estimated daily intake

Estimated Daily Intake (EDI, in ng/kg BW per day) of pesticide residues by consumption of raw cow's milk was calculated to assess long-term dietary intake using Eq.01 (Boudebbouz et al., 2021).

$$\text{EDI} = \frac{(\text{C pesticide } \times \text{W milk})}{\text{Body Weight}} \quad (1)$$

Where:

C pesticide (ng/g, on a wet weight basis) is the mean pesticide level of raw cow's milk samples.

W Milk represents the daily average consumption of milk (g).

Body weight (BW): average body weight of an adult was considered as 60 kg (WHO, 1997).

The occurrence of each contaminant in raw cow's milk samples was compared with MRL obtained from the pesticide database set by the Codex Alimentarius Commission (2020). The average consumption of milk for each country was obtained from the FAO-data base (FAO, 2013).

In order to estimate the human risk from raw cow's milk consumption, the EDI was compared with Provisional Tolerable Daily Intake (PTDI), or Acceptable daily intake (ADI) obtained from the pesticide database set by the Codex Alimentarius Commission (2020). The database includes DDTs (op-DDE, pp-DDE, op-DDD, pp-DDD, op-DDT, pp-DDT), endosulfan (endosulfan sulfate, α endosulfan, β endosulfan), ΣHCH (α HCH, β HCH, γ HCH, δ HCH), heptachlor (heptachlor, heptachlor epoxide), Drins (aldrin, dieldrin), endrin, Organophosphorus pesticides, pyrethroid pesticides, and carbamate pesticides. Values exceeding the PTDI or ADI limit were considered not safe for consumers.

2.4.2. Hazard quotients

The long-term risk exposure of each pesticide residue was performed using the Hazard Quotient (HQ). According to Equation Eq. (02) (Galani et al., 2020; US ESPA, 2000), a hazard quotient value of HQ < 1 indicates that lifetime consumption of raw cow's milk samples containing the measured level of pesticide residues could not pose health threat in the long term or short term risks (Wu et al., 2021).

$$\text{HQ} = \text{EDI}/\text{ADI} \quad (2)$$

Where:

EDI: is the estimated daily intake of pesticide residues by consumption of raw cow's milk.

ADI: is the acceptable daily intake supposed to be a safe concentration for life exposure (US EPA, 1991).

2.4.3. Cumulative risk assessment

Because we are exposed during our life to mixtures of contaminants and pollutants most often present in very low doses (Gioiosa et al., 2015), the hazard index (HI) was evaluated to assess the residues of many pesticides in raw cow's milk samples and calculated by summing the HQs of the individual pesticide residue Eq. (3) (Galani et al., 2020).

HI values below 1 were considered acceptable and safe for human consumption, whereas an HI upper than 1 indicates that the consumption should be considered as a risk to the consumers (Alla et al., 2015; El Hawari et al., 2019; Galani et al., 2020).

$$\text{HI} = \text{HQ}_1 + \text{HQ}_2 + \text{HQ}_3 + \ldots\ldots\ldots\ldots + \text{HQ}_n \quad (3)$$

3. Results and discussion

3.1. Characteristics of eligible studies

A computerized literature search on the different scientific databases (Science Direct, Scopus and PubMed) resulted in a total number of 2742 documents. Four articles were retrieved after checking the references section on Google Scholar. A total of 150 articles were ruled out because they were duplicates, or they did not meet inclusion criteria and were disqualified after a review of the title, abstract or manuscripts. In conclusion, we screened 35 research articles that met our purpose (A flowchart depicting the choice of studies is shown in Fig. 1).

The used data covered more than 3612 raw cow's milk samples that were analysed for pesticide residues from 69 regions belonging to 15 countries around the world (Fig. 1). It appears that 35 pesticide residues were detected with different concentrations in 14 developing countries and one developed country (Spain) (Fig. 2). Recorded pesticide levels were mostly obtained by GC-ECD systems (21 studies); in 6 cases with confirmation by GC-MS. Two other works measured with GC-NPD approaches. Only one used other GLC, and in 4 cases HPLC UV and PDA systems were used. Only one study employed combined GC-MS/MS and LC-MS/MS approaches, which are a more updated wide scope multi-residue method (348 pesticides).

The majority of the pesticide residues (almost 18 residues) belonged to organochlorine pesticides (OC) (i.e., pp-DDT, pp-DDE, pp-DDD, op-

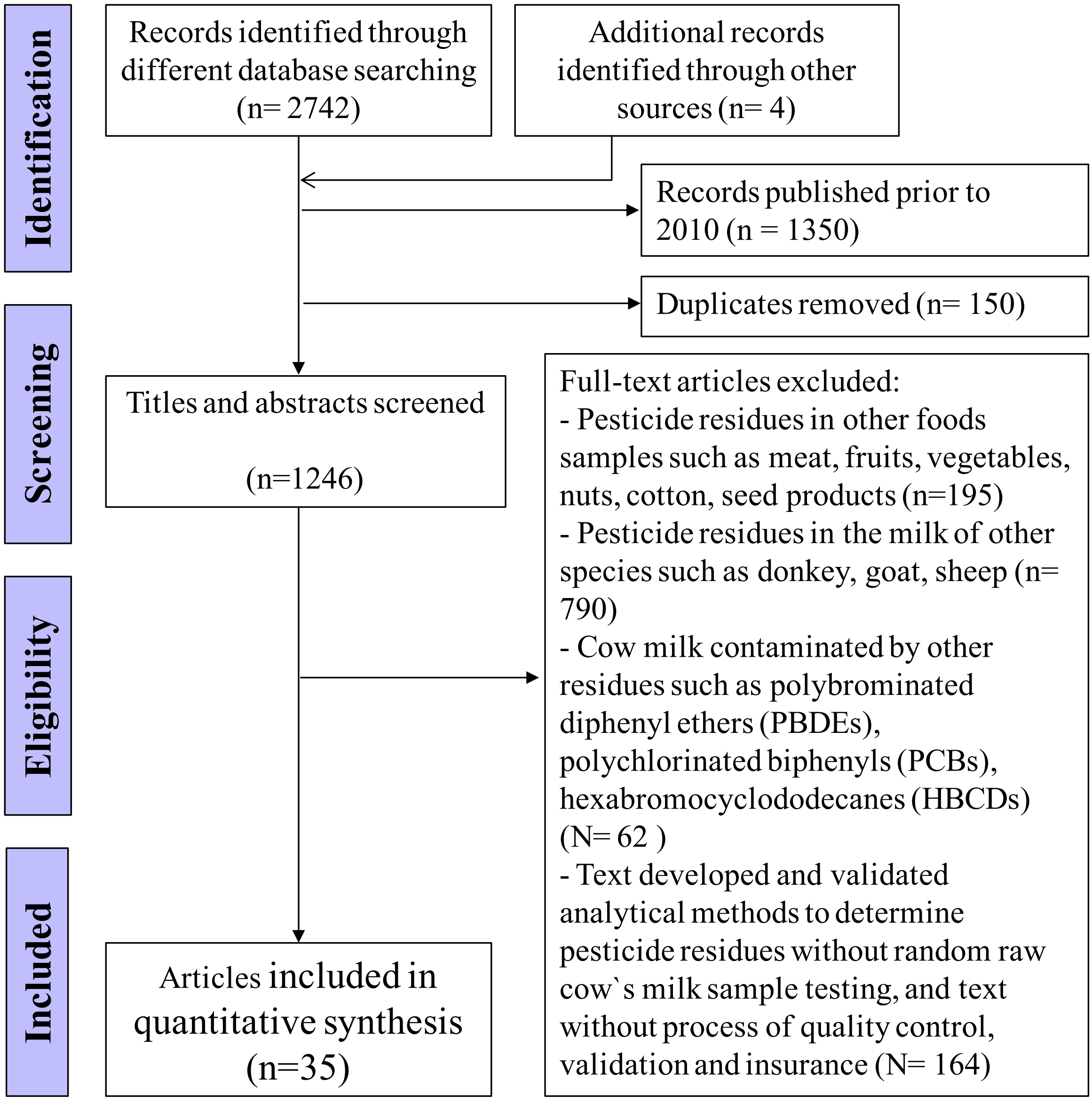

Fig. 1. Flow diagram of the studies selection process following the PRISMA (preferred reporting items for systematic reviews and meta-analyses).

DDT, op-DDE, op-DDD, α endosulfan, β endosulfan, endosulfan sulfate, α HCH, β HCH, γ HCH, δ HCH, heptachlor, heptachlor epoxide, aldrin, dieldrin, endrin), organophosphorus pesticides (OP) (almost 9 residues) (i.e., coumaphos, parathion-methyl, chlorpyriphos, dimethoate, dichlorvos, ethion, malathion, profenos, methamidophos), pyrethroid pesticides (PY) (5 residues) (cypermethrin, permethrin, bifenthrin, cyhalothrin, deltamethrin), and carbamate compounds (CB) (3 residues) (i.e., aldicarb, carbofuran and carbaryl).

3.2. Bump graph analysis

Fig. 3 shows a bump graph (Epic Epoch) visualizing the most used keywords and their evolution across different periods. It should be noted that the change of color hue during these periods showed how the ranks of the keywords changed. From 2010 to 2021, the subject areas of keywords used in the research field on “pesticides in raw cow’s milk” were “Agricultural and Biological Sciences”, “Environmental Science”, “Chemistry”, “Medicine and Human Health” and keywords recorded were in connection with the technical and/or the social aspects, such as “Gas chromatography”, “Mass spectrometry”, “risk assessment”, “food safety” and “Food contamination”.

Moreover, the keyword “Milk” was the most commonly occurring keyword from 2010 to 2016, but lost place in the later periods and started to drop from first to the third rank in the period between 2019 and 2020. The keyword “food contamination” was in the sixth and fifth rank in 2010 and 2011 respectively, but it appeared in the first rank in 2020.

3.3. Organochlorine pesticides

Organochlorine pesticides (OC) (also called chlorinated hydrocarbons) are considered the most dangerous and persistent compounds in the environment due to their chemical stability, long biological half-life, and high biomagnification in the food chain (Serrano et al., 2008). Most OC pesticides are used as insecticides for the control of malaria, typhus, and a wide variety of insects (Aktar et al., 2009). OC pesticides such as DDT, HCH, heptachlor, lindane, chlordane, endosulfan, aldrin and dieldrin may be found in higher concentrations in some human tissues such as liver, kidneys, thyroid, heart, mammary glands and testes (Nag, 2010). Several adverse health effects associated with exposure to OC pesticides have been reported in human studies. They show that the presence of OC pesticides in human organs leads to increased cancer

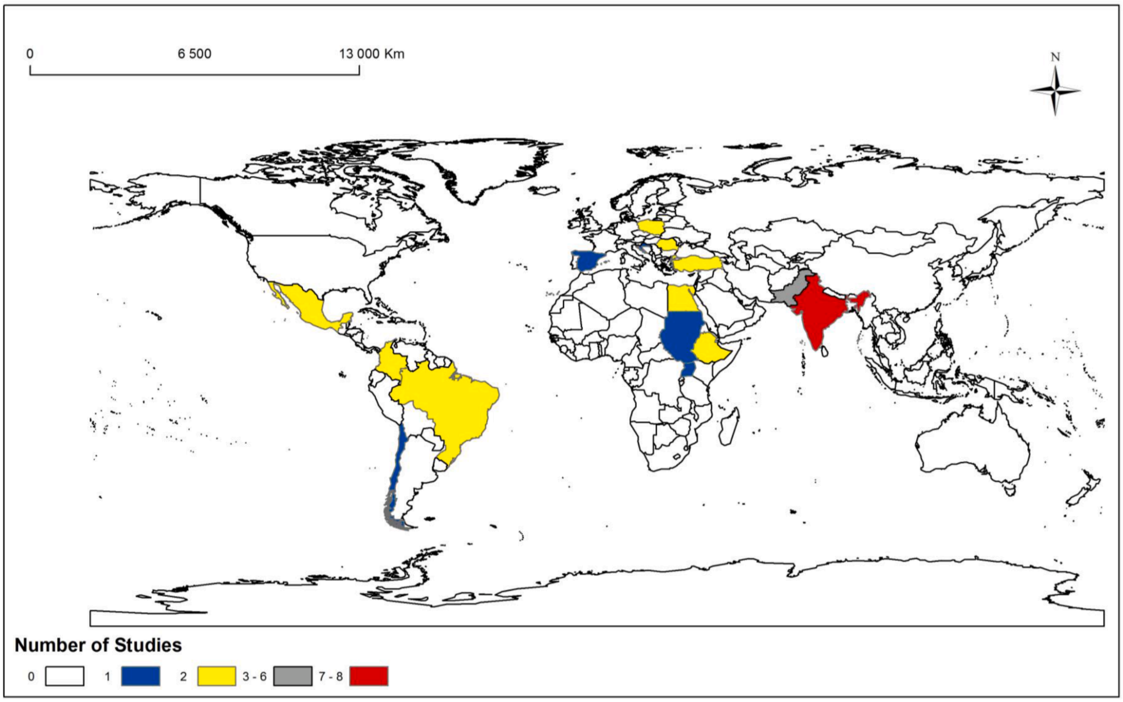

Fig. 2. Location map of raw cow milk samples collected from different countries across the world to measure pesticides levels including organochlorine, organophosphorus, pyrethroid, and carbamate pesticide residues around the world during the last decade (2010-2021).

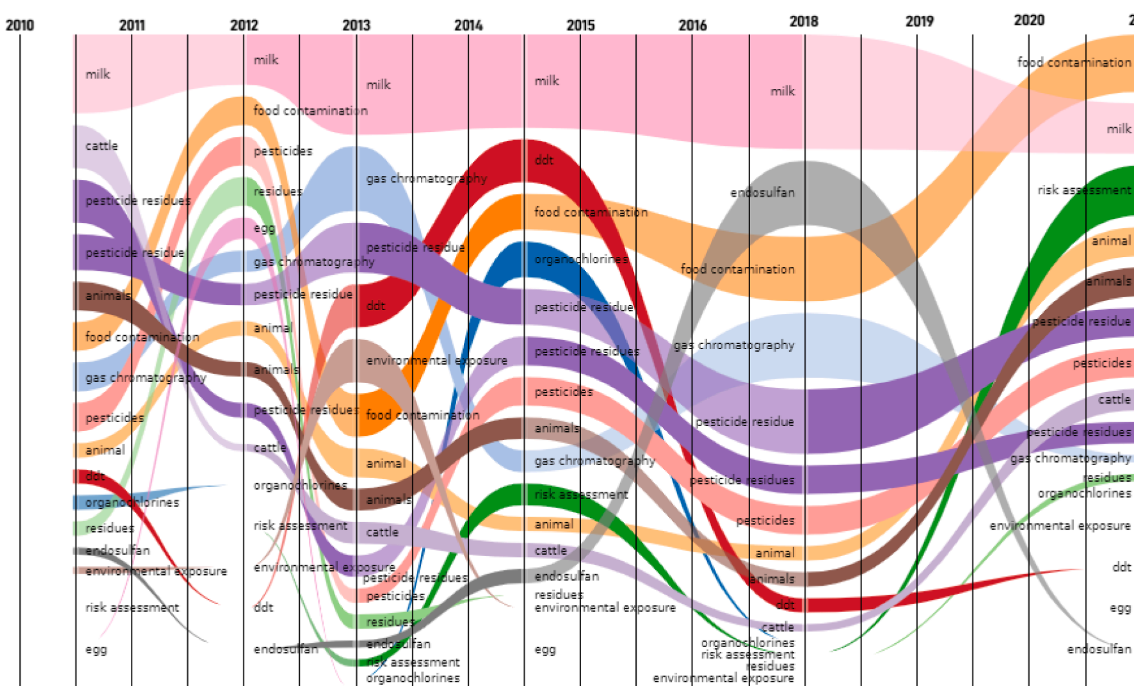

Fig. 3. "Epic epoch" graph allowing to visualize the most used keywords in each research article for each year, from 2010 to 2021.

risk, and many of them are known as endocrine disruptors, even at low concentrations (Ansari et al., 2021).

Among the organochlorine pesticides reported (32 studies) in raw cow's milk, DDTs have the highest monitoring frequency (27 publications), followed by endosulfan (20 publications), HCH (19 publications), Drins (16 publications), Heptachlor (11 publications), and endrin (8 publications).

3.3.1. DDTs levels in raw cow's milk

Dichlorodiphenyltrichloroethane (DDT) and its derivatives residues levels that were found in raw cow's milk samples in different countries are shown in Tables (1, S1). It can be seen that 3000 raw cow's milk samples collected from 59 regions in 12 countries namely Colombia, Egypt, Croatia, India, Ethiopia, Sudan, Pakistan, Uganda, Turkey, Mexico, Poland, and Romania were analysed for DDT and its derivatives. pp-DDT is the most reported DDT derivative, being monitored in twenty studies in forty-six regions across the globe, followed by pp-DDE which

Table 1
DDTs, ΣEndosulfan, ΣHCH, Drins, Endrin, Σ Heptachlor residues levels in raw cow's milk samples reported in research articles published since 2010.

Refs,	Location	N	Concentration (ng/g); Mean ± SD and/or range (Min-Max) and positive occurrence frequencies (%)					
			∑DDT	ΣEndosulfan	ΣHCH	Drins	Endrin	Σ Heptachlor
Abusalma et al. (2014)	Sudan, Gezira State	5	842 ± 810 110-2180	92.00	–	–	–	–
Arif et al. (2021)	Pakistan, Lahore	300	2.51 ± 0.55 (0.98-5.17)	73.46	0.92 ± 0.16	1.12 ± 0.17	–	–
	Pakistan, Lahore	60	4.64 ± 0.48	118.90	(0.72-1.89)	(0.89-3.06)	–	–
Ashok Kumar et al. (2013)	India, Palia Kalan	10	116 ± 2 80%	–	121 ± 4	–	–	–
Aydin et al. (2019)	Turkey, Konya	15	12.22 1.99-81.53 6%	16.31	63.6 (11.23- 127.19)	7.74 (1.96- 16.61)	17.32 (1.27-145)	11.71 (BDL-20.38)
Bedi et al. (2015)	India, Punjab	312	1.6 ± 3.9 10.3%	1.2	0.9 ± 3.5 7%	–	–	–
Bošnir et al. (2010)	Croatia, Karlovac county	40	0.37 (0.00-5.51) 87.5%	0.81	0.00 (0.00-2.67) 41.7%	–	–	(0-0.51) 13%
Bulut et al. (2011)	Turkey, Afyonkarahisar	50	–	9.70	91.32 (32.57- 172.63) 64%	1.52	4.57	0.34
Chandrakar et al. (2020)	India, Chhattisgarh	100	10	–	–	–	–	–
Deti et al. (2014)	Ethiopia, Lole	5	(BDL-256.4)	0.00	–	–	–	–
	Ethiopia, Gonde	5	(BDL-269.7)	0.00	–	–	–	–
	Ethiopia, Adami Tulu	5	(259.5- 1230.0)	95.60	–	–	–	–
	Ethiopia, Asendabo	5	(50.5-420.8)	155.20	–	–	–	–
Díaz Pongutá et al. (2012)	Colombia, San Jorge	18	47.2	–	260.2 ± 112.6	80.1	37.6 ± 12.2	61.7 ± 18.6
	Colombia, Sinú Medio	18	36.5	–	271	73.8	47.1 ± 19.3	77.3
	Colombia, Sabanas	18	34.3	–	469.6 ± 7.2	80.1	37.9 ± 10.7	60.1 ± 12.4
	Colombia, Costanera	18	37.1	–	157.2	30.7 ± 6.3	37.2 ± 4.5	BDL
Donia et al. (2010)	Egypt, Gizeh	60	223	–	36 ± 20 (12-46) 43.3%	50 ± 20 (30-72) 26.7%	12 ± 10 (10-16) 16.7%	98
Gebremichael et al. (2013)	Ethiopia, Asendabo	10	269	BDL	BDL	BDL	BDL	BDL
	Ethiopia, Jimma	10	477	BDL	BDL	BDL	BDL	BDL
	Ethiopia, Serbo	10	421	BDL	BDL	BDL	BDL	BDL
Gill et al. (2020)	India, Bangalore	216	0.53	10.70	0.76 ± 5.1	–	BDL	–
	India, Bhubaneswar	204	1.5	1.24	BDL	–	BDL	–
	India, Ludhiana	258	0.24	0.20	0.09 ± 1.5	–	0.24 ± 3.2 (–-50) 2%	–
	India, Guwahati	270	1.72	1.21 ± 10.7	0.79 ± 7.3	–	BDL	–
	India, Udaipur	235	0.65	0.00	0.17 ± 2.6	–	0.09 ± 1.3 (–- 20) 1%	–
Gutiérrez et al. (2012)	Mexico, Chiapas	36	1.53	5.15	18.7	0.77	0.66	0.67
Gutierrez et al. (2013)	Mexico, Hidalgo	12	0.27 ± 0.37	1.12	1.73	(BDL-7.03)	(BDL-5.95)	(BDL-5.84)
Hernández et al. (2010)	Colombia, San Pedro	16	630	650.00	–	620	820 ± 270	BDL
	Colombia, San Pedro	16	290	260.00	–	251 ± 100	530 ± 220	320 ± 130
	Colombia, San Pedro	16	810 ± 270	150.00	–	1200	510 ± 160	230 ± 70
Ishaq and Nawaz (2018)	Pakistan, Sahiwal	20	7.33 (0.05-15.61)	311.15	1.34	11.83 (2.08- 18.46)		
Jawaid et al. (2016)	Pakistan, Hyderabad	45	–	13.40	–	–		
Kampire et al. (2011)	Uganda, Kampala	54	50	2.00	26 ± 3 (1-86) 85%	16		
Kaushik et al. (2011)	India, Haryana	147	36.7 ± 38.5 (1.7-286.4)	–	29.3	–		
Kotinagu and Krishnaiah (2015)	India, Musi river belt	48	BDL	BDL	BDL	BDL		BDL
Kuba et al. (2015)	Poland, Slaskie	25	0.319 ± 0.444 (0.014-1.886)	–	–	–	–	–
	Poland, Zachodniopomorskie	25	0.387 ± 0.250 (0.074-1.140)	–	–	–	–	–
Muhammad et al. (2012)	Pakistan, Faisalabad	200	–	260 ± 20	–	–	–	–
Năstăsescu et al. (2020)	Romania, Campina	14	181	–	–	–	–	–
	Romania, Ploiesti	14	218	–	–	–	–	–
	Romania, Valea Doftanei	14	113.4	–	–	–	–	–
Nath et al. (2013)	India, Patna	23	9.26 ± 6.02 (BDL-22.2) 33.89%	30.53 ± 14.13 33.33%	–	–	–	–
Raslan et al. (2018)	Egypt, Zagazig	20	54.77 ± 11.14	–	83.09	–	–	–
Rusu et al. (2016)	Romania, Bacau	18	BDL	–	279.7	–	–	–

(*continued on next page*)

Table 1 (*continued*)

Refs,	Location	N	Concentration (ng/g); Mean ± SD and/or range (Min-Max) and positive occurrence frequencies (%) ∑DDT	ΣEndosulfan	ΣHCH	Drins	Endrin	Σ Heptachlor
Sajid et al. (2016)	Pakistan, Faisalabad	50	BDL	67.00	_	_	_	_
Singh et al. (2013)	West Bengal, Nadia	210	_	59.00	6 0.95%	_	_	_
ul Hassan et al. (2014)	Pakistan, Punjab	150	50 (45-1630) 10%	130.00	_	680	_	_
Witczak et al. (2013)	Poland, Chojna 1	5	1.72	_	0.72	0.534	0.116 ± 0.033	1.767
	Poland, Sobieradz	5	2.37	_	1.43	3.171	0.158 ± 0.029	1.634
	Poland, Chojna 2	5	2.37	_	1.21	3.21	0.024 ± 0.007	0.848
	Poland, Wielboki	5	2.48	_	1.65	1.255	0.082 ± 0.066	1.289
	Poland, Szczecin 1	5	2.58	_	0.917	2.501	0.10 ± 0.006	1.121
	Poland, Maszewo	5	2.63	_	1.521	2.156	0.114 ± 0.060	1.43
	Poland, Czaplinek 1	5	2.76	_	1.412	2.87	0.058 ± 0.033	1.049
	Poland, Rychlik	5	3.10	_	1.809	1.751	0.229 ± 0.021	1.976
	Poland, Czaplinek 2	5	3.20	_	2.371	1.451	0.106 ± 0.019	1.49 ± 0.150
	Poland, Kielce	5	3.63	_	1.254	0.204	0.309 ± 0.108	1.896
	Poland, Głowczyca	5	3.63	_	2.026	1.795	0.14 ± 0.069	1.117
	Poland, Łysinin	5	3.65	_	4.031	4.92	0.44 ± 0.010	2.307
	Poland, Szczecin 2	5	3.75	_	0.737	2.074	0.171 ± 0.121	0.984 ± 0.086
	Poland, Przesocin	5	3.90	_	1.289	0.888	0.162 ± 0.065	1.396
	Poland, Bierzwnik	5	7.08	_	1.762	1.74	0.207 ± 0.105	2.854

_: Data not available, BDL: Below Detection Limit, N: Samples sizes, %: occurrence frequencies.

is monitored in nineteen studies in 44 regions worldwide, then pp-DDD (12 studies in 29 regions), op-DDT (10 studies in 30 regions), op-DDD (8 studies in 29 regions), and op-DDE (5 studies in 8 regions).

It appears that pp-DDT was in the highest concentration among the DDTs levels (810 ± 270 ng/g), followed by op-DDT (482 ng/g), pp-DDE (213 ng/g), pp-DDD (174 ± 3 ng/g), and op-DDD was (170 ng/g) in the lowest concentration. DDTs total concentration (2180 ng/g) was found to be at the highest concentration of codex maximum residue levels (20 ng/g) (Codex Alimentarius Commission, 2020).

It should be noted that DDTs contaminated 18.88% (525 samples) of raw cow's milk samples across the globe with a concentration above the maximum residue level (20 ng/g). The highest value (2180 ng/g) was recorded in cow's milk samples collected from the Gezira state in Sudan where an unsafe use of pesticides was applied (Abusalma et al., 2014). Comparatively, a high concentration of total DDTs (1230 ng/g) was reported in the area treated recently with pesticides for malaria vector control in Ethiopia (Deti et al., 2014).

Total DDTs were detected in raw cow's milk samples from four countries (Colombia, Ethiopia, Romania and Egypt). In Colombia, DDTs concentrations were 810 ± 270, 630 ± 210 and 290 ± 120 ng/g and were detected in the milk of cows grazing on three traditional cotton farms (Hernández et al., 2010). In Ethiopia, DDTs concentrations were 477, 421 and 269 ng/g and were found in the milk of cows reared in three malarious and spraying DDT areas (Gebremichael et al., 2013), as well as 420.80, 253 and 243 ng/g in areas with pesticides use to control pests and malaria (Deti et al., 2014). In Romania, DDTs concentrations were 218, 181 and 113.40 ng/g and recorded in the milk of cows reared in three urban areas with intensive industrial and medium agriculture activities, urban areas with medium industrial and agriculture activities, and urban areas with agriculture activity, respectively (Năstăsescu et al., 2020). In Egypt, the concentration (223 ng/g) was reported in the milk of animal farms (Donia et al., 2010).

It should be noted that 1823 (60.76%) raw cow's milk samples out of 3000 that reported the DDTs level in raw cow's milk samples across the globe were collected from different regions in India. However, only 157 (8.61%) raw cow's milk samples had concentrations higher than the MRL (20 ng/g) (Codex Alimentarius Commission, 2020). Kaushik et al. (2011) reported a high mean DDTs value (36.7 ± 38.5 ng/g) compared to MRL in 147 raw cow's milk samples from Haryana, India. DDTs levels in raw cow's milk samples collected in Palia kalan region (India) were significantly higher than the MRL level (116 ± 2 ng/g) (Ashok Kumar et al., 2013).

Milk samples collected from different regions in Pakistan represent approximately 20% (580 samples) of milk analysed across the globe. Only 6 raw cow's milk samples (10%) out of 150 collected in Punjab, Pakistan were found to contain higher DDTs levels compared to MRL levels (ul Hassan et al., 2014).

DDTs levels in raw cow's milk samples collected in Colombia (7 regions), Ethiopia (7 regions), Romania (3 regions), Uganda (1 region), and Egypt (2 regions) were exceeding the MRL levels.

Additionally, Table S1 shows that DDTs levels in raw cow's milk samples vary significantly from one region to another in the same countries. In Ethiopia, the concentration of DDTs in raw cow's milk samples was reported very high in Adami Tulu region compared to Asendabo region, Gonde region, and Lole regions. These variations in concentration levels may be due to the presence of agrochemical companies in Adami Tulu region which are formulating DDT, and other pesticides for local consumption in that region (Deti et al., 2014).

Besides, it should be noted that the type of agriculture activities could affect the contamination level. In Romania, a recent study revealed that DDTs level in raw cow's milk samples collected in Ploiesti region, considered as an urban area with intensive industrial and medium agriculture activities was higher compared to those recorded in Campina, an urban area region with medium industrial and agriculture activities, and those recorded in Valea Doftanei, an urban area with agriculture activity (Năstăsescu et al., 2020).

Hernández et al. (2010) showed that the concentrations of DDTs residues in cow's milk samples collected from two traditional cotton Colombian farms using supplementation of seeds and soca to cattle feed were two times higher than the DDTs level in the raw milk collected from cows reared in a traditional cotton farm. In the same way, Arif et al. (2021) found that the total DDTs analysed in 60 raw cow's milk samples collected from dairy farms in Lahrore region, Pakistan was two times higher than the total DDTs analysed in 300 raw cow's milk samples collected from urban areas.

3.3.2. Endosulfan levels in raw cow's milk

Endosulfan was defined as persistent organic pollutants (POPs) in 2011 by the Stockholm Convention and its use has been restricted by several countries (UNEP-POP, 2011); however, endosulfan is being used in developing countries due to the absence of other organic choices and/or regulation lacking (Sathishkumar et al., 2021). Consequently, endosulfan residues have been found in farmed and cultivated food products, drinking water sources, and living organisms (Bertero et al., 2020; Souza et al., 2020).

Tables (1, S2) show the concentration of α endosulfan, β endosulfan, endosulfan sulfate and endosulfan in raw cow's milk samples from 9 different countries in 33 regions of the world, found in twenty-one studies. α endosulfan and β endosulfan were the most monitored contaminants with 13 studies in 18 regions, followed by endosulfan sulfate with 9 studies in 18 regions. β endosulfan represents the highest concentration of endosulfan in raw cow's milk samples (297.1 ng/g) followed by α endosulfan with 228.6 ng/g, then endosulfan sulfate with 42.02 ng/g.

Moreover, it should be noted that 62% of the analysed endosulfan samples (1813 out of 2923) have been reported in India, 27% in Pakistan, and 11% in Colombia, Croatia, Ethiopia, Mexico, Sudan, Turkey, and Uganda. It must also be noted that the mean concentration of endosulfan in 1418 raw cow's milk samples (48.36%) is much greater than their assigned MRL values (10 ng/g) (Codex Alimentarius Commission, 2020).

The highest value of endosulfan (650 ng/g) was reported in raw cow's milk samples collected in the north of Colombia from two traditional cotton cultivation farms that used soca as a nutritional supplement, and was from a farm which had a vocation of breeding and was traditionally supplemented by cotton seeds (Hernández et al., 2010). Furthermore, a high concentration of endosulfan (300 ng/g) was reported in raw cow's milk samples from different places in Gezira state (Sudan) after field spraying by endosulfan (Abusalma et al., 2014).

Deti et al. (2014) recorded pesticide concentrations equivalent to 155 ng/g and 95 ng/g in milk collected from cows reared in two areas that used pesticides to control pests and malaria in Ethiopia.

In addition, endosulfan concentration in raw cow's milk samples has been studied by several authors in different regions in Pakistan and the mean concentrations of endosulfan were above MRL (Arif et al., 2021; Ishaq and Nawaz, 2018; Jawaid et al., 2016; Muhammad et al., 2012; Sajid et al., 2016; ul Hassan et al., 2014). Endosulfan was detected above MRL (10 ng/g) in 1% (22) of raw cow's milk samples from 1831 samples collected in India. Singh et al. (2013) reported that 2 raw cow's milk samples out of 210 samples collected from west of Benga (India) were higher than MRL (10 ng/g). Also, Nath et al. (2013) found that 19 samples (33%) of raw cow's milk collected from Patna region (India) were slightly contaminated with endosulfan with a mean concentration of 30.53 ng/g.

In the same way, other studies revealed that the concentration of endosulfan in raw cow's milk samples from different regions in India was below MRL (Table S2) (Bedi et al., 2015; Gill et al., 2020; Kotinagu and Krishnaiah, 2015). The level of endosulfan was below the detectable limit in 235 raw cow's milk samples collected from Udaipur region, India (Gill et al., 2020), and in 48 raw cow's milk samples collected from Musi river belt, India (Kotinagu and Krishnaiah, 2015), and five regions in Ethiopia (Deti et al., 2014; Gebremichael et al., 2013).

3.3.3. *HCHs levels in raw cow's milk*

HCH is considered one of the most widely used pesticides and has been manufactured since the second world war (Vijgen et al., 2019). HCH and its isomers such as α-, β-, γ-, and δ HCH have been classified as POPs (persistent organic pollutants) by the Stockholm Convention and categorized as possible human carcinogens by the United States Environmental Protection Agency (EPA, 2003).

Tables (1, S3) show data extracted from 20 studies that analysed HCH in 44 regions around the world, 19 studies that analysed γ-HCH in 43 regions, 12 research articles that analysed α HCH in 31 regions, 10 studies that analysed β HCH in 26 regions and 11 studies that reported the level of δ HCH in 13 regions. Sixty-nine percent of the total samples were from India while the rest 31% were from Colombia, Croatia, Egypt, Ethiopia, Mexico, Pakistan, Poland, Romania, Turkey and Uganda.

The highest value of HCH isomers was recorded for α HCH with a concentration of 469 ± 7.2 ng/g, followed by γ-HCH with a concentration value of 212.4 ng/g, then β HCH and δ HCH with a concentration of 172.63, 48.65 ± 15.12 ng/g, respectively.

The ΣHCH residues in raw cow's milk samples ranged between the Below Detection Limit (BDL) to 469.6 ± 7.2 ng/g during the last ten years. One hundred (3.60%) samples out of 2772 collected in different areas across the globe exceeded MRL (100 ng/g) for ΣHCH (Kaushik et al., 2011; WHO, 1973).

Díaz Pongutá et al. (2012) reported that the level of ΣHCH in raw cow's milk samples collected from four different regions in Colombia was higher than MRL. Moreover, the level of ΣHCH in 10 raw cow's milk samples collected from Palia kalan region (India) was reported slightly higher than MRL (Ashok Kumar et al., 2013). Also, the level of ΣHCH was found more than two times higher than MRL in raw cow's milk samples collected from Bacau district in Romania (Rusu et al., 2016).

Tables (1, S3) show that 1910 raw cow's milk samples collected from ten regions in India were below MRL except in Palia Kalan region (Table S3). The level of ΣHCH residues was reported below MRL by several authors in different countries (Croatia, Egypt, Ethiopia, Mexico, Pakistan, Poland, Turkey and Uganda).

3.3.4. *Heptachlor levels in raw cow's milk*

Heptachlor, a chlorinated hydrocarbon insecticide, is one of the synthetic pesticides which has been used against termites and soil insects (Prado et al., 2009). Heptachlor pesticide can attack the central nervous system and causes neurological disorders such as Lewy pathology (Ross et al., 2019), and it has been also considered as carcinogenic to humans by WHO (1984). However, heptachlor insecticides are still used in agriculture and public health program in several developing countries due to their low cost in controlling different pests (Purnomo et al., 2013). Also, it is an environmentally ubiquitous contaminant found in food, human milk, soil, plants, marine animals and wildlife tissues (Chuang and Chuang, 1998; Prado et al., 2009).

Tables (1, S4) show that Σ Heptachlor (Heptachlor and Heptachlor epoxide) levels varied from BDL to 320 ± 130 ng/g in different countries. The highest values were recorded in San Pedro region, Colombia from two traditional cotton cultivation farms that used soca as a nutritional supplement, followed by milk samples collected from Egypt. Moreover, it should be noted that 129 (26%) of raw cow's milk samples collected from one region in Turkey, three regions in Colombia, and one region in Egypt were higher than MRL set by the Codex Alimentarius Commission (2020). Whereas, 357 (74%) of raw cow's milk samples collected from 30 regions in 7 countries (Colombia, Ethiopia, India, Macedonia, Turkey, Mexico and Croatia) were below MRL (Table S4).

Heptachlor levels varied from one region to another in the same country due to farming system models such as industrial farms or organic farms, and the degree of environmental contamination. In Colombia, Díaz Pongutá et al. (2012) reported that milk samples from the Sabanas, San Pedro and Sucre regions contained Heptachlor in values higher than MRL while in the Costanera region they were below the detection limit (Table S4).

In Mexico, Gutiérrez et al. (2013, 2012) reported that heptachlor in raw cow's milk samples was 0.4 ± 0.28 ng/g in industrial farms in Hidalgo region, and 0.670 ng/g in organic farms in Chiapas region. Moreover, in Turkey, Bulut et al. (2011) recorded a low level of heptachlor in raw cow's milk collected from Afyon karahisar region, while a high level was recorded from Konya district (Aydin et al., 2019). The urban air of Konya city was recorded in 2009 as contaminated by OHPs by the same authors (Ozcan and Aydin, 2009).

3.3.5. *Drins and endrin levels in raw cow's milk*

Fourteen studies reported the levels of Drins and endrin in 2213 raw cow's milk samples collected from 41 regions. Drins concentrations varied from BDL to 1200 ng/g. Table S4 shows that 60% (611 samples) of raw cow's milk samples analysed for Drins were below MRL (6 ng/g), while 40% (419 samples) were above MRL.

In Colombia, the level of Drins in raw cow's milk samples collected from different regions was exceeding the MRL and the highest level was recorded in San Pedro, Sucre region (Hernández et al., 2010). The level of Drins in raw cow's milk samples collected from 3 different areas in Ethiopia, and one area in India were below detectable limits (Gebremichael et al., 2013; Kotinagu and Krishnaiah, 2015). Moreover, the level of Drins in milk collected from two different regions in Mexico, and 15 different regions in Poland were below MRL (Gutiérrez et al., 2012; Witczak et al., 2013), while the level in milk collected from Egypt and Uganda were exceeded MRL levels (Donia et al., 2010; Kampire et al., 2011).

Other studies conducted in different regions in Turkey and Pakistan showed that the level of Drins in raw cow's milk samples varied from region to region depending on the spread of these environmental pollutants in the food chain and water. The level of Drins in raw cow's milk samples collected from Afyonkarahisar, Turkey was below MRL (Bulut et al., 2011) while the level was slightly higher than MRL in milk collected from Konya District, Turkey (Aydin et al., 2019). In the same way, in Pakistan, the level of Drins was below MRL in Lahore region, while the level was higher than MRL in Sahiwal region, and Punjab region, respectively (Ishaq and Nawaz, 2018; ul Hassan et al., 2014).

The level of endrin in raw cow's milk samples ranged from BDL to 820 ng/g. It should be noted that the level of endrin in 84.50% (1336 samples) of raw cow's milk samples collected from several countries (Mexico, Ethiopia, Poland, and India) was below MRL (1 ng/g). In the same way, 14.50% (245 samples) of raw cow's milk samples collected from three countries (Colombia, Turkey, and Egypt) were contaminated with endrin at levels higher than MRL (1 ng/g) (Codex Alimentarius Commission, 2020).

The highest levels of endrin in raw cow's milk samples were recorded in different regions in Colombia with the highest value (820 ng/g) in San Pedro, Sucre region (Hernández et al., 2010), and the lowest value was (37.2 ng/g) recorded in milk collected from Costanera region (Díaz Pongutá et al., 2012). The level of endrin in raw cow's milk samples collected from three regions in Ethiopia, and three regions in India were below detectable limits (Gebremichael et al., 2013; Gill et al., 2020).

Endrin levels in 15 different regions in Poland, one region in India, and one region in Mexico were not exceeding MRL (Bulut et al., 2011; Gill et al., 2020; Gutiérrez et al., 2012; Witczak et al., 2013), while the level of endrin was exceeding MRL in raw cow's milk samples collected from cows reared in Afyonkarahisar region, and in Konya district, in Turkey (Aydin et al., 2019; Bulut et al., 2011). In addition, Donia et al. (2010) reported that the level of endrin in raw cow's milk samples collected from different regions in Egypt was above MRL.

3.4. Organophosphorus pesticides

Organophosphorus (OP) are phosphoric, phosphonic, and thiophosphoric acids-derived pesticides. They are a commonly used group of pesticides and constitute roughly 38% of the total pesticide utilized universally (Vijayan and Abdulhameed, 2020). Regardless of being less persistent compounds in the environment compared to organochlorine pesticides, several authors reported the presence of OP residues including malathion, chlorpyriphos, dichlorvos, profenofos, coumaphos, methamidophos, ethion and dimethoate in milk. They can bioaccumulate in human organs (Chawla et al., 2018), and can cause harmful effects on human health, such as cancer (Sun et al., 2020), neurodegenerative diseases (Jokanović, 2018), and metabolic disorders like the risk of obesity and type 2 diabetes mellitus (Czajka et al., 2019).

Despite their wide use, a relatively low number of studies (12 publications) have reported organophosphorus pesticides analysis in raw cow's milk compared to other related pesticides such as organochlorine. A total of 9 organophosphorus pesticides were identified in raw cow's milk samples collected from different countries since 2010, including malathion, dimethoate, chlorpyriphos, profenofos, coumaphos, dichlorvos, ethion, and Parathion methyl. Of all organophosphorus pesticides reported here, chlorpyriphos had the highest monitoring frequency (7 publications), followed by malathion, and profenofos (5 publications), dimethoate (4 publications), coumaphos (3 publications) and dichlorvos, ethion, methamidophos, and parathion methyl (2 publications).

A total of 5106 (73.38%) samples analysed for organophosphorus were collected from India, and 1852 (26.62%) samples from Brazil, Egypt, Chile, Pakistan, and Spain. The level of each organophosphorus pesticide in raw cow's milk samples reported in available publications was summarized in Table 2.

Malathion has been found in 455 samples in 5 regions from 3 countries (Egypt, India and Brazil). Five out of 455 samples were exceeding MRLs while the highest reported mean value (20.20 ± 5.89 ng/g) was recorded in samples collected from Panta region (India) (Nath et al., 2013). Chlorpyriphos was reported in 1836 samples collected from 11 regions in 2 countries (India and Pakistan). Only 35 out of 200 samples (17%) collected in Faisalabad city in India were above MRL (12 ng/g) (Codex Alimentarius Commission, 2020). Dimethoate was reported in 168 samples from 4 regions in three countries (Brazil, India and Egypt) and only the mean concentration of 8 out of 48 samples collected from Musi river belt region, India was above the MRL (Kotinagu and Krishnaiah, 2015).

Profenofos was reported in 1625 samples collected from 9 regions in three countries (India, Pakistan and Egypt). The highest value was reported in the milk of Bangalore area, India (Gill et al., 2020). Coumaphos was reported in 302 samples collected from 3 regions in two countries (Brazil and Spain) and the highest value was reported in milk collected from the northwest region of Spain (Melgar et al., 2010). Methamidophos was reported in 34 samples collected in two countries (Pakistan and Chile) and all analysed samples were below the detection limit (Lapierre et al., 2019; Sajid et al., 2016).

Ethion was reported in 1495 samples collected in 6 regions in India. Ethion was detected in less than 14% of 209 samples (Bedi et al., 2015; Gill et al., 2020). Dichlorvos was reported in 242 raw cow's milk samples in Agreste region (Brazil) and was detected in 5.78% of milk samples at a level below MRL (Codex Alimentarius Commission, 2020; Fagnani et al., 2011). Finally, parathion methyl was reported in 272 samples collected from two countries (Spain and Brazil) and it was detected in less than 1% of samples with the highest value recorded in Spain (Melgar et al., 2010).

3.5. Pyrethroid pesticides

Pyrethroids are synthetic organic insecticides derived from the naturally occurring flowers of pyrethrums (Chrysanthemum Coccineum and Chrysanthemum cinerariae folium) (Zacharia, 2011). They have been used worldwide since 1980 and are considered as the safest compounds for use in food due to their photo degradation, effectiveness against various insects, and the low toxicity compared to other pesticides such as OC, OP, and CB (Yoo et al., 2016). However, despite low toxicity, and the high level of effectiveness against target organisms, prenatal exposure to pyrethroid pesticides and their metabolites may be associated with a variety of behavioural and executive functioning deficits (Furlong et al., 2017) and could affect the nervous, cardiovascular, immune, and genetic systems of organisms (Tang et al., 2018).

From literature, raw cow's milk contamination with synthetic pyrethroid is poorly documented. For the purposes of this review, 8 studies were reported synthetic pyrethroid pesticide content in raw cow's milk in 14 regions around the world. Of the 2024 raw cow's milk samples analysed, 1595 samples (78.80%) were collected from India, 420 (20.75%) from Pakistan, and 9 samples from Chile (Table 3).

Cypermethrin was reported in 1870 samples in 9 regions from two countries (Pakistan and India). In Pakistan, Cypermethrin was detected in 40 out of 200 samples collected from Faisalabad area, and 31 out of 150 samples collected from Punjab region at levels above MRL (5 ng/g) (Muhammad et al., 2012; ul Hassan et al., 2014). The highest value (15400 ng/g) was reported in milk collected from Punjab area. Permethrin was recorded in 1367 samples from ten regions in three

Table 2
OP residues levels in raw cow's milk samples reported in research articles published since 2010.

Pesticide	Refs.	Location	N	Concentration (ng/g); Mean ± SD and/or range (Min-Max) and positive occurrence frequencies (%)
Chlorpyriphos	Bedi et al. (2015)	India, Punjab	312	2.2 ± 8.5 (6.4%)
	Jawaid et al. (2016)	Pakistan, Hyderabad	45	0.6 (0.1-1.6)
	Gill et al. (2020)	India, Bangalore	216	(_-81) 1.71 ± 11.1 5%
		India, Bhubaneswar	204	(_-71) 1.30 ± 9.1 4%
		India, Ludhiana	258	(_-85) 1.57 ± 10.3 6%
		India, Guwahati	270	(_-65) 0.76 ± 6.5 4%
		India, Udaipur	235	(_-130) 1.62 ± 12.7 4%
	Kotinagu and Krishnaiah (2015)	India, Musi river belt	48	BDL
	Muhammad et al. (2012)	Faisalabad, Pakistan	200	72 ± 10 (17%)
	Nath et al. (2013)	India, Patna	23	1.6 (5.56%)
	Sajid et al. (2016)	Pakistan, Faisalabad	25	4 ± 0.05
Dichlorvos	Fagnani et al. (2011)	Brazil, Agreste	30	0.06 ± 0.13 (0.01-0.05)
	Melgar et al. (2010)	Spain, Northwest	242	9 (6 - 20) 5.78%
Dimethoate	da Silva et al. (2014)	Brazil, Parana	30	(BLD-0.15)
	Donia et al. (2010)	Egypt, Gizeh	60	BDL
	Fagnani et al. (2011)	Brazil, Agreste	30	0.01 ± 0.02 (0.01-0.11)
	Kotinagu and Krishnaiah (2015)	India, Musi river belt	48	(BDL-130)
Ethion	Bedi et al. (2015)	India, Punjab	312	0.3 ± 2.9 1.30%
	Gill et al. (2020)	India, Bangalore	216	1.05 ± 8.9 (_-86) 3%
		India, Bhubaneswar	204	0.68 ± 6.7 (_-73) 2%
		India, Ludhiana	258	0.80 ± 7.4 (_-74) 3%
		India, Guwahati	270	1.46 ± 20.0 (_-320) 2%
		India, Udaipur	235	1.02 ± 9.0 (_-83) 3%
Malathion	Bedi et al. (2015)	India, Punjab	312	0.4 ± 3.9 (0.9%)
	da Silva et al. (2014)	Brazil, Parana	30	(BDL-1.46)
	Donia et al. (2010)	Egypt, Gizeh	60	BDL
	Fagnani et al. (2011)	Brazil, Agreste	30	0.02 ± 0.03 (0.01-0.20)
	Nath et al. (2013)	India, Patna	23	20.20 ± 5.89 (22.22%)
Methamidophos	Lapierre et al. (2019)	Chile, Los Ríos	1	BDL
		Chile, La Araucanía	3	BDL
		Chile, Los Lagos	5	BDL
	Sajid et al. (2016)	Pakistan, Faisalabad	25	BDL
Parathion methyl	Fagnani et al. (2011)	Brazil, Agreste	30	BDL (0.01-12.89)
	Melgar et al. (2010)	Spain, Northwest	242	7 (5-9) 0.83%
Profenofos	Bedi et al. (2015)	India, Punjab	312	0.2 ± 1.6 (1.6%)
	da Silva et al. (2014)	Brazil, Parana	30	(BDL - 0.53
	Donia et al. (2010)	Egypt, Gizeh	60	BDL
	Fagnani et al. (2011)	Brazil, Agreste	30	0.04 ± 0.016 (0.01-0.87)
	Jawaid et al. (2016)	Pakistan, Hyderabad	45	2.1 (1-9)
	Gill et al. (2020)	India, Bangalore	216	0.94 ± 8.0 (_-74) 3%
		India, Bhubaneswar	204	0.32 ± 4.6 (_-66) 1%
		India, Ludhiana	258	0.78 ± 7.2 (_ - 71) 3%
		India, Guwahati	270	(_-68) 0.25 ± 4.1 1%
		India, Udaipur	235	0.31 ± 4.8 (_-73) 1%
	Melgar et al. (2010)	Spain, Northwest	242	93 (29-220) 2.06%
	Sajid et al. (2016)	Pakistan, Faisalabad	25	1.3 ± 1.7

_: Data not available, BDL: Below Detection Limit, N: Samples sizes.

countries (Pakistan, India and Chile). The highest value (1840 ng/g) was reported in milk collected from Udaipur (India) (Gill et al., 2020).

Bifenthrin was reported in 220 samples in three regions from Pakistan. The highest value (1768 ng/g) detected in milk samples collected from Punjab areas in Pakistan at levels above MRL (200 ng/g) (ul Hassan et al., 2014).

Cyhalothrin was identified in 1720 samples from eight regions in two countries (Pakistan and India). Thirty-eight out of 200 samples (19%) collected in Faisalabad region (Pakistan) were contaminated with Cyhalothrin at a level above MRL (200 ng/g) (Muhammad et al., 2012). Deltamethrin was reported in 587 samples in four regions from two countries (Pakistan and India). Ten out of 150 samples (7%) of milk collected in Punjab region (Pakistan) were contaminated with levels above MRL (50 ng/g) (Codex Alimentarius Commission, 2020). In this region, the range of Deltamethrin varied between 2450 and 5030 ng/g (ul Hassan et al., 2014).

3.6. Carbamate pesticides

Carbamate (CB) pesticides including carbaryl, carbofuran, and aminocarb are organic compounds similar in structure and purpose to OP pesticides (Zacharia, 2011). Carbamate insecticides are used abundantly by households and for agriculture purposes (Blodgett and Means, 2013). Milk contamination with CB pesticides could cause a serious risk to humans and animals since these compounds have been linked with cancer, reproduction toxicity (da Silva et al., 2014), and neurotoxic effects (Herbert et al., 2021; Vidair, 2004).

Only two studies were found, investigating the content of carbamates (carbaryl, aldicarb, and carbofuran) in raw cow's milk samples collected in two regions in Brazil (Table 3). Concentrations of carbaryl (0.02 ± 0.06 ng/g), aldicarb (0.02 ± 0.04 ng/g), and carbofuran (0.01 ± 0.01 ng/g) in 30 raw cow's milk samples collected in Agreste region (Brazil) were below MRL (Fagnani et al., 2011), while their concentrations were below detection limits in 30 samples collected in Parana State, Brazil (da Silva et al., 2014).

Table 3
Pyrethroid, and Carbamates residues levels in raw cow's milk samples reported in research articles published since 2010.

Refs.	Location	N	Pyrethroid Concentration (ng/g); Mean ± SD and/or range (Min-Max) and positive occurrence frequencies (%)					Carbamates		
			cypermethrin	permethrin	Bifenthrin	Cyhalothrin	deltamethrin	Carbaryl	Aldicarb	Carbofuran
Bedi et al. (2015)	India, Punjab	312	0.9 ± 5.0 4.10%	_	_	0.8 ± 4.3 4.50%	0.5 ± 3.4 2.20%	_	_	_
Chandrakar et al. (2020)	India, Chhattisgarh	100	_	_	_	_	7 ± 3 (BDL-196) 5%	_	_	_
da Silva et al. (2014)	Brazil, Parana	30	_	_	_	_	_	BDL	BDL	BDL
Fagnani et al. (2011)	Brazil, Agreste	30	_	_	_	_	_	0.02 ± 0.06 (0.01-0.33)	0.02 ± 0.04 (0.01-0.22)	0.01 ± 0.01 (0.01-0.04)
Gill et al. (2020)	India, Bangalore	216	1.20 ± 8.3 (_-76) 5%	0.31 ± 3.4 (_-45) 2%	_	0.11 ± 1.18 (_-15) 2%	_	_	_	_
	India, Bhubaneswar	204	BDL	11.1 ± 74.2 (_-650) 5%	_	0.39 ± 4.07 (_-50) 2%	_	_	_	_
	India, Ludhiana	258	1.74 ± 21.4 (_-340) 5%	0.30 ± 2.8 (_-32) 3%	_	0.62 ± 6.9 (_-102) 3%	_	_	_	_
	India, Guwahati	270	0.39 ± 3.7 (_-44) 3%	1.80 ± 22.9 (_-370) 4%	_	0.79 ± 8.3 (_-120) 3%	_	_	_	_
	India, Udaipur	235	BDL	28.17 ± 172.3 (_-1840) 10%	_	0.17 ± 2.6 (_-40) 1%	_	_	_	_
Jawaid et al. (2016)	Pakistan, Hyderabad	45	_	_	4.7 (3-9)	_	_	_	_	_
Lapierre et al. (2019)	Chile, Los Ríos	1	_	BDL	_	_	_	_	_	_
	Chile, La Araucanía	3	_	(BDL-13)	_	_	_	_	_	_
	Chile, Los Lagos	5	_	(BDL-14)	_	_	_	_	_	_
Muhammad et al. (2012)	Pakistan, Faisalabad	200	85 ± 20	_	_	380 ± 20	_	_	_	_
Sajid et al. (2016)	Pakistan, Faisalabad	25	29.9 ± 15	45 ± 15	19 ± 11	BDL	18 ± 3	_	_	_
ul Hassan et al. (2014)	Pakistan, Punjab	150	227 (_-15400)	1235.00	1768.00	_	210.00	_	_	_

_: Data not available, BDL: Below Detection Limit, N: Samples sizes.

3.7. Risk assessment of raw cow's milk consumption

3.7.1. Estimated daily intake (EDI)

3.7.1.1. Organochlorine. The EDI of DDTs, HCHs, endosulfan, Heptachlor, Drins, and endrin (expressed in ng/kg body weight (BW)/day) for raw cow's milk samples is shown in Table 4. Unlike other foods such as sugar and fruit juice, milk consumption in different countries across the globe is lower at older ages and higher at younger ages (Singh et al., 2015). The acceptable daily intake regarding DDTs is set to 10000 ng/kg BW. The EDI of DDTs in raw cow's milk samples across the globe ranged from 0 to 5090 ng/kg BW/day. It was notable that the estimated daily intakes of DDTs in all milk analysed across the globe were lower than guidelines values(Codex Alimentarius Commission, 2020).

As for HCHs, the Provisional Tolerable Daily Intake (PTDI) given by the Codex Alimentarius Commission (2020) is 5000 ng/kg BW. The exposure to HCHs through raw cow's milk across the globe ranged between 0 and 2590 ng/kg BW/day, while the highest EDI value for HCHs was recorded in milk collected from Bacau district in Romania. Furthermore, no analysed samples exceeded the acceptable daily intake (ADI) and/or PTDI (Table 4).

In the case of Heptachlor, the ADI/PTDI is set to 100 ng/kg BW/day and the estimated daily intake of Heptachlor through raw cow's milk consumption ranged from 0 to 349 ng/kg BW/day. The highest value of EDI was recorded in milk collected from Costanera region (Colombia). Except for three regions (9.37%) in Colombia, the EDIs of Heptachlor in analysed milk were lower than ADI/PTDI (Codex Alimentarius Commission, 2020) (Table 4).

The ADI value regarding Drins is set to 100 ng/kg BW/day. The EDI values of Drins ranged from 0 to 5420 ng/kg BW/day while the raw milk of Sucre region in Colombia revealed the highest EDI value (5420 ng/kg BW/day). Pesticides level recorded from 9 regions (23.68%) out of 38 were above ADI/PTDI (Table 4).

Concerning Endrin, the ADI is 200 ng/kg BW/day and the EDI for Endrin ranged between 0 and 2130 ng/kg BW/day. The highest EDI value was recorded in milk collected from Sinú Medio region in Colombia. Endrin value in one region out of 29 (3.44%) was exceeding the ADI/PTDI value (Table 4).

Regarding Endosulfan, the recommended maximum ADI/PTDI is 6000 ng/kg BW/day (Codex Alimentarius Commission, 2020). The average Endosulfan consumption through milk ranged between 0 and 2940 ng/kg BW/day. The highest EDI (2940 ng/kg BW/day) was recorded in milk collected in Sucre region in Colombia.

3.7.1.2. Organophosphorus. The EDI of organophosphorus pesticides including malathion, dimethoate, chlorpyriphos, profenofos, coumaphos, dichlorvos, methamidophos, ethion, parathion methyl (expressed in ng/kg BW/day) for raw cow's milk samples did not exceed the guideline values (Table 5).

3.7.1.3. Carbamates. None of the EDI-carbaryl, EDI-aldicarb, or EDI-carbofuran values exceeded the provisional tolerable daily intake (PTDI) or the acceptable daily intake (ADI) recorded from analysed data (Table 5).

3.7.1.4. Pyrethroid pesticides. EDI of PY pesticides including cypermethrin, permethrin, cyhalothrin, deltamethrin, and bifenthrin were within the standard limit values (Table 6).

Table 4
EDI mean (ng/kg BW/day) and HQ and HI of pesticides in raw cow's milk reported in research articles published since 2010.

Refs.	Location	EDI DDT	HCHs	Drins	Endrin	Endosulfan	Heptachlor	HQ DDT	HCH	Drins	Endrin	Endosulfan	Heptachlor	HI
Abusalma et al. (2014)	Sudan, Gezira	5.09E+03	_	_	_	5.52E+02	_	5.09E-01	_	_	_	3.03E-01	_	0.81
Arif et al. (2021)	Pakistan, Lahore	1.23E+01	4.52E+00	5.51E+00	_	3.61E+02	_	1.23E-03	9.05E-04	5.51E-02	_	6.02E-02	_	0.11
	Pakistan, Lahrore	2.28E+01	5.90E+00	9.49E+00	_	5.85E+02	_	2.28E-03	1.18E-03	9.49E-02	_	9.74E-02	_	0.19
Ashok Kumar et al. (2013)	India, Palia Kalan	2.78E+02	2.90E+02	0.00E+00	_	_	_	2.78E-02	5.81E-02	0.00E+00	_	_	_	0.085
Aydin et al. (2019)	Turkey, Konya District	8.45E+01	_	5.35E+01	_	1.13E+02	8.10E+01	8.45E-03	_	5.35E-01	_	1.88E-02	8.10E-01	1.37
Bedi et al. (2015)	India, Punjab	3.84E+00	2.16E+00	_	_	2.88E+00	_	3.84E-04	4.32E-04	_	_	4.80E-04	_	0.001
Bošnir et al. (2010)	Croatia, Karlovac County	1.92	1.92E+03	_	_	4.20	2.64	1.92E-04	3.85E-03	_	_	7.00E-04	2.64E-02	0.03
Bulut et al. (2011)	Turkey, Afyonkarahisar	_	6.32E+02	1.05E+01	3.16E+01	6.71E+01	2.35E+00	_	1.26E-01	1.05E-01	1.58E-01	1.12E-02	2.35E-02	0.42
Chandrakar et al. (2020)	India, Chhattisgarh	2.40E+01	_	_	_	_	_	2.40E-03	_	_	_	_	_	0.002
Deti et al. (2014)	Ethiopia, Lole	4.66E+02	_	_	_	0.00E+00	_	4.66E-02	_	_	_	0.00E+00	_	0.46
	Ethiopia, Gonde	4.90E+02	_	_	_	0.00E+00	_	4.90E-02	_	_	_	0.00E+00	_	0.049
	Ethiopia, Adami Tulu	2.23E+03	_	_	_	1.74E+02	_	2.23E-01	_	_	_	2.89E-02	_	0.25
	Ethiopia, Asendabo	7.64E+02	_	_	_	2.82E+02	_	7.64E-02	_	_	_	4.70E-02	_	0.12
Díaz Pongutá et al. (2012)	Colombia, San Jorge	2.13E+02	7.10E+02	1.39E+02	1.70E+02	_	0.00E+00	2.13E-02	1.42E-01	1.39E+00	8.49E-01	_	0.00E+00	2.39
	Colombia, Sinú Medio	1.65E+02	1.18E+03	3.33E+02	2.13E+02	_	2.71E+02	1.65E-02	2.35E-01	3.33E+00	1.06E+00	_	2.71E+00	7.36
	Colombia, Sabanas	1.55E+02	1.22E+03	3.62E+02	1.71E+02	_	2.79E+02	1.55E-02	2.45E-01	3.62E+00	8.56E-01	_	2.79E+00	7.52
	Colombia, Costanera	1.68E+02	2.12E+03	3.62E+02	1.68E+02	_	3.49E+02	1.68E-02	4.24E-01	3.62E+00	8.40E-01	_	3.49E+00	8.39
Donia et al. (2010)	Egypt, Gizeh	2.03E+02	3.48E+01	4.83E+01	1.16E-02	_	9.47E-01	2.03E-02	6.96E-03	4.83E-01	5.80E-05	_	9.47E-01	1.46
Fagnani et al. (2011)	Brazil, Agreste	_	_	_	_	_	0.00E+00	_	_	_	_	_	0.00	0.00
Gebremichael et al. (2013)	Ethiopia, Asendabo	4.89E+02	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	4.89E-02	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.05
	Ethiopia, Jimma	8.67E-01	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	8.67E-02	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.087
	Ethiopia, Serbo	7.65E+02	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	7.65E-02	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.076
Gill et al. (2020)	India, Bangalore	1.27E+00	0.00E+00	_	_	0.00E+00	_	1.27E-04	0.00E+00	_	_	0.00E+00	_	0.001
	India, Bhubaneswar	3.60E+00	2.16E-01	_	_	0.00E+00	_	3.60E-04	4.32E-05	_	_	0.00E+00	_	0.001
	India, Ludhiana	5.76E-01	4.08E-01	_	_	4.80E-01	_	5.76E-05	8.16E-05	_	_	8.00E-05	_	0.001
	India, Guwahati	4.13E+00	1.82E+00	_	_	2.98E+00	_	4.13E-04	3.65E-04	_	_	4.96E-04	_	0.003
	India, Udaipur	1.56E+00	1.90E+00	_	_	2.57E+01	_	1.56E-04	3.79E-04	_	_	4.28E-03	_	0.007
Gutiérrez et al. (2012)	Mexico, Chiapas	5.20E+00	6.36E+01	2.62E+00	2.24E+00	3.39E+01	2.28E+00	5.20E-04	1.27E-02	2.62E-02	1.12E-02	5.64E-03	2.28E-02	0.079
Gutierrez et al. (2013)	Mexico, Hidalgo	9.18E-04	5.88E-03	4.42E-04	2.38E-04	3.81E-03	1.36E-03	9.18E-05	1.18E-03	4.42E-03	1.19E-03	6.35E-04	1.36E-02	0.021
Hernández et al. (2010)	Colombia, San Pedro	2.85E+03	_	1.13E+03	3.70E+00	6.78E+02	0.00E+00	2.85E-01	_	1.13E+01	1.85E-02	1.13E-01	0.00E+00	11.75
	Colombia, Casa Azul	1.31E+03	_	2.80E+03	2.39E+03	1.17E+03	1.04E+00	1.31E-01	_	2.80E+01	1.20E-02	1.96E-01	1.04E-02	28.35
	Colombia, Sucre	3.66E+03	_	5.42E+03	2.30E+00	2.94E+03	1.45E+00	3.66E-01	_	5.42E+01	1.15E-02	4.89E-01	1.45E-02	55.08
Ishaq and Nawaz (2018)	Pakistan, Sahiwal	3.60E+01	6.59E+00	5.82E+01	_	1.53E+03	_	3.60E-03	1.32E-03	5.82E-01	_	2.55E-01	_	0.84
Jawaid et al. (2016)	Pakistan, Hyderabad	_	_	_	_	6.59E+02	_	_	_	_	_	1.10E-02	_	0.01
Kampire et al. (2011)	Uganda, Kampala	8.42E+01	4.38E+01	1.99E+01	_	3.37E+00	_	8.42E-03	8.75E-03	1.99E-01	_	5.61E-04	8.42E-03	0.22
Kaushik et al. (2011)	India, Haryana	8.81E+01	7.03E+01	0.00E+00	_	_	_	8.81E-03	1.41E-02	0.00E+00	_	_	8.81E-03	0.02
Kotinagu and Krishnaiah (2015)	India, Musi river belt	0.00E+00	0.00E+00	0.00E+00	_	0.00E+00	_	0.00E+00	0.00E+00	0.00E+00	_	0.00E+00	_	0.15
Kuba et al. (2015)	Poland, Slaskie voivodeship	5.48E-01	_	_	_	_	_	5.48E-05	_	_	_	_	_	0.00
	Poland, Zachodniopomorskie voivodeship	6.64E-01	_	_	_	_	_	6.64E-05	_	_	_	_	_	0.00
Lapierre et al. (2019)	Chile, Los Ríos	_	_	_	_	_	_	_	_	_	_	_	_	0.00
	Chile, La Araucanía	_	_	_	_	_	_	_	_	_	_	_	_	0.00
	Chile, Los Lagos	_	_	_	_	_	_	_	_	_	_	_	_	0.00
Melgar et al. (2010)	Spain, Northwest region	_	_	_	_	_	_	_	_	_	_	_	_	0.02
Muhammad et al. (2012)	Pakistan, Faisalabad	_	_	_	_	1.28E+03	_	_	_	_	_	2.13E-01	_	0.32
Năstăsescu et al. (2020)	Romania, Campina	1.67E+03	_	_	_	_	_	1.67E-01	_	_	_	_	_	0.16
	Romania, Ploiesti	2.02E+03	_	_	_	_	_	2.02E-01	_	_	_	_	_	0.20
	Romania, Valea Doftanei	1.05E+03	_	_	_	_	_	1.05E-01	_	_	_	_	_	0.10
Nath et al. (2013)	India, Patna	2.22E+01	_	_	_	_	_	2.22E-03	_	_	_	_	_	0.002
Raslan et al. (2018)	Egypt, Zagazig	5.29E+01	8.03E+01	_	_	_	_	5.29E-03	1.61E-02	_	_	_	_	0.02

(*continued on next page*)

Table 4 (*continued*)

Refs.	Location	EDI						HQ						HI
		DDT	HCHs	Drins	Endrin	Endosulfan	Heptachlor	DDT	HCH	Drins	Endrin	Endosulfan	Heptachlor	
Rusu et al. (2016)	Romania, Bacau	0.00E+00	2.59E+00	_	_	_	_	0.00E+00	5.17E-01	_	_	_	_	0.51
Sajid et al. (2016)	Pakistan, Faisalabad	0.00E+00	_	_	_	3.29E+02	_	0.00E+00	_	_	_	5.49E-02	_	0.07
Singh et al. (2013)	India, Nadia	_	1.44E+01	_	_	1.42E+02	_	_	2.88E-03	_	_	2.36E-02	_	0.002
ul Hassan et al. (2014)	Pakistan, Punjab	2.46E+02	_	3.34E+03	_	6.39E+02	_	2.46E-02	_	3.34E+01	_	1.07E-01	_	34.62
Witczak et al. (2013)	Poland, Chojna 1	2.95E+00	1.24E+00	3.50E+00	1.99E-01	_	1.46E+00	2.95E-04	2.47E-04	3.50E-03	9.96E-04	_	1.46E-02	0.002
	Poland, Sobieradz	4.07E+00	1.27E+00	9.17E-01	2.71E-01	_	1.69E+00	4.07E-04	2.53E-04	9.17E-03	1.36E-03	_	1.69E-02	0.002
	Poland, Chojna 2	4.07E+00	1.57E+00	1.52E+00	4.12E-02	_	1.80E+00	4.07E-04	3.15E-04	1.52E-02	2.06E-04	_	1.80E-02	0.034
	Poland, Wielboki	4.26E+00	2.08E+00	2.15E+00	1.41E-01	_	1.92E+00	4.26E-04	4.15E-04	2.15E-02	7.04E-04	_	1.92E-02	0.42
	Poland, Szczecin 1	4.43E+00	2.15E+00	2.49E+00	1.72E-01	_	1.92E+00	4.43E-04	4.31E-04	2.49E-02	8.58E-04	_	1.92E-02	0.045
	Poland, Maszewo	4.51E+00	2.21E+00	2.99E+00	1.96E-01	_	2.21E+00	4.51E-04	4.43E-04	2.99E-02	9.79E-04	_	2.21E-02	0.053
	Poland, Czaplinek 1	4.74E+00	2.42E+00	3.01E+00	9.96E-02	_	2.40E+00	4.74E-04	4.85E-04	3.01E-02	4.98E-04	_	2.40E-02	0.055
	Poland, Rychlik	5.32E+00	2.45E+00	3.08E+00	3.93E-01	_	2.45E+00	5.32E-04	4.91E-04	3.08E-02	1.97E-03	_	2.45E-02	0.058
	Poland, Czaplinek 2	5.49E+00	2.61E+00	3.56E+00	1.82E-01	_	2.56E+00	5.49E-04	5.22E-04	3.56E-02	9.10E-04	_	2.56E-02	0.063
	Poland, Kielce	6.23E+00	2.83E+00	3.70E+00	5.30E-01	_	2.81E+00	6.23E-04	5.67E-04	3.70E-02	2.65E-03	_	2.81E-02	0.069
	Poland, Głowczyca	6.23E+00	3.02E+00	4.29E+00	2.40E-01	_	3.03E+00	6.23E-04	6.05E-04	4.29E-02	1.20E-03	_	3.03E-02	0.075
	Poland, Łysinin	6.27E+00	3.11E+00	4.93E+00	7.55E-01	_	3.25E+00	6.27E-04	6.21E-04	4.93E-02	3.78E-03	_	3.25E-02	0.087
	Poland, Szczecin 2	6.44E+00	3.48E+00	5.44E+00	2.94E-01	_	3.39E+00	6.44E-04	6.96E-04	5.44E-02	1.47E-03	_	3.39E-02	0.091
	Poland, Przesocin	6.70E+00	4.07E+00	5.51E+00	2.78E-01	_	3.96E+00	6.70E-04	8.14E-04	5.51E-02	1.39E-03	_	3.96E-02	0.097
	Poland, Bierzwnik	1.22E+01	6.92E+00	8.45E+00	3.55E-01	_	4.90E+00	1.22E-03	1.38E-03	8.45E-02	1.78E-03	_	4.90E-02	0.14
*PTDI/ADI		**10000**	**5000**	**100**	**200**	**6000**	**100**							

* Data from Codex Alimentarius commission (Codex Alimentarius commission, 2020), _: Data not available

3.7.2. *Hazard quotient (HQ)*

As mentioned above, quantitative non-carcinogenic risks are reported as hazard quotients by comparing predicted pesticide intakes directly to toxicity values in the form of reference doses. The non-carcinogenic risk for raw cow's milk consumers was determined by calculating HQ of: 1) OC including DDTs, HCHs, Drins, endrin, endosulfan, and heptachlor, 2) OP including malathion, dimethoate, chlorpyriphos, profenofos, coumaphos, dichlorvos, methamidophos, ethion, parathion-methyl, 3) PY including cypermethrin, permethrin, bifenthrin, cyhalothrin, and deltamethrin, as well as 4) CB including carbaryl, aldicarb, and carbofuran (Tables 4, 6, 7).

HQ of pesticide residues for adults was calculated based on the mean levels of the concentration of these pesticide residues obtained from the current data. It must be noted that if HQ of milk is less than 1, non-obvious risks are improbable to happen to the exposed population, while harmful impacts may happen to the exposed population if HQ is above 1 (Dadar et al., 2017; Fakhri et al., 2019; Rahmani et al., 2018).

HQ values indicated that adult consumers were not exposed to any potential health risk through exposure to DDTs, HCHs, and endosulfan recorded in raw cow's milk in 69 regions across the globe. For Drins, the HQ values exceeded 1 in 8 out of 38 (21%) regions in the world. The highest value was recorded in milk collected from Sucre region, Colombia. Also in Colombia, the HQ values of Drins were exceeding 1 in Casa Azul region (22.8), San Pedro region (11.33), Costanera region (3.6), Sabanas region (3.6), Sinú Medio region (3.33), and San Jorge region (1.38). An HQ value was also higher than 1 in Punjab region, Pakistan (33.40). The HQ value of endrin in 1 out of 29 regions (3.44%) that analysed raw cow's milk samples was higher than 1 and that region was Sinú Medio in Colombia.

The results of non-carcinogenic risks from exposure to pesticide residues through milk consumption indicate that raw cow's milk collected in 3 out of 32 regions (9.37%) across the globe during the last decade was not safe for human consumption in terms of the amounts of heptachlor (HQ values >1; Table 4). The highest value was recorded in raw cow's milk samples collected in Costanera region (Colombia). Besides, it was observed that (HQ values <1) have no potential health risk to adults in the case of OP, PY, and CB pesticides.

3.7.3. *Hazard Index (HI)*

Table 4 summarizes the cumulative risk assessment (HI) values for the pesticides in raw cow's milk samples during the last decade, related to adult consumption. Results show that raw cow's milk intake represents a serious health risk for consumers in 10 out of 69 regions across the globe (14.50%). It can be seen that HI values ranged from 0 to 55.8. The highest HI value was recorded in Sucre region in Colombia.

HI values were higher than 10 in 3 regions in Colombia, and 1 region in Pakistan. Moreover, they were between 1 and 10 in 6 out of 69 regions that were studied across the globe (Colombia -4 regions, Egypt -1 region and Turkey -1 region). These results show a high potential risk for human health in terms of residue ingestion. HI values were lower than 1 in 59 out of 69 regions across the globe (85.50%), indicating that the risk of human exposure to pesticides via intake of cow's milk was minimum in these regions.

4. Concluding thoughts and recommendations

Milk is an important and widely consumed food which is rich in macro- and micronutrients that play an important role in health preservation. While it affects positively human nutrient and energy uptake, the presence of pesticide residues could, however, counterbalance these benefits and negatively affect human health.

After reviewing the relevant literature since 2010, it was concluded that pesticide residues were detected in raw cow's milk samples in 14 developing countries (Egypt, Colombia, Pakistan, Turkey, Uganda, Poland, Sudan, Ethiopia, India, Romania, Croatia, Mexico, Brazil and Chile) and one developed country (Spain). In addition, these countries

Table 5
EDI mean (ng/kg BW/day) of OP, CB pesticides in raw cow's milk reported in research articles published since 2010.

Refs.	Location	Malathion	Dimethoate	Chlorpyriphos	Profenofos	Coumaphos	Dichlorvos	Methamidophos	ethion	Parathion-methyl	Carbaryl	Aldicarb	Carbofuran
Bedi et al. (2015)	India, Punjab	9.60E-01	_	2.04E+01	4.80E-01	_	_	_	7.20E-01	_	_	_	_
da Silva et al. (2014)	Brazil, Parana	9.42E+00	9.68E-01	_	_	3.42E+00	_	_	_	_	0.00E+00	0.00E+00	0.00E+00
Donia et al. (2010)	Egypt, Gizeh	0.00E+00	0.00E+00	_	0.00E+00	_	_	_	_	_	_	_	_
Fagnani et al. (2011)	Brazil, Agreste	1.29E-01	6.45E-02	_	_	2.58E-01	3.87E-01	_	_	0.00E+00	1.29E-01	1.29E-01	6.45E-02
Gill et al. (2020)	India, Bangalore	_	_	4.10E+00	2.26E+00	_	_	_	2.52E+00	_	_	_	_
	India, Bhubaneswar	_	_	3.12E+00	7.68E+00	_	_	_	1.63E+00	_	_	_	_
	India, Ludhiana	_	_	2.47E+01	1.87E+00	_	_	_	1.92E+00	_	_	_	_
	India, Guwahati	_	_	1.56E+01	6.00E-01	_	_	_	3.50E+00	_	_	_	_
	India, Udaipur	_	_	3.05E+01	7.44E-01	_	_	_	2.45E+00	_	_	_	_
Jawaid et al. (2016)	Pakistan, Hyderabad	_	_	2.95E+00	1.03E+01	_	_	_	_	_	_	_	_
Kotinagu and Krishnaiah (2015)	India, Musi river belt	_	3.12E+02	0.00E+00	0.00E+00	_	_	_	_	_	_	_	_
Lapierre et al. (2019)	Chile, Los Ríos	_	_	_	_	_	_	0.00E+00	_	_	_	_	_
	Chile, La Araucanía	_	_	_	_	_	_	0.00E+00	_	_	_	_	_
	Chile, Los Lagos	_	_	_	_	_	_	0.00E+00	_	_	_	_	_
Melgar et al. (2010)	Spain, Northwest	_	_	_	_	3.30E+02	3.20E+01	_	_	2.49E+01	_	_	_
Muhammad et al. (2012)	Pakistan, Faisalabad	_	_	2.95E+00	_	_	_	_	_	_	_	_	_
Nath et al. (2013)	India, Patna	4.85E+01	_	3.84E+00	_	_	_	_	_	_	_	_	_
Sajid et al. (2016)	Pakistan, Faisalabad	_	_	2.46E-01	6.39E+00	_	_	0.00E+00	_	_	_	_	_
***ADI/PTDI (ng/g BW)**		**300000**	**2000**	**10000**	**30000**	_	**4000**	**4000**	**2**	**3**	**8**	**3**	**1**

* Data from Codex Alimantarius commission (Codex Alimantarius commission, 2020), _: Data not available

Table 6
EDI mean (ng/kg BW/day) and HQ of PY in raw cow's milk reported in research articles published since 2010.

Refs.	Location	EDI					HQ				
		cypermethrin	permethrin	bifenthrin	cyhalothrin	deltamethrin	cypermethrin	permethrin	bifenthrin	cyhalothrin	deltamethrin
Bedi et al. (2015)	India, Punjab	2.16E+00	_	_	1.92E+00	1.20E+00	1.08E-04	_	_	9.60E-05	1.20E-05
Chandrakar et al. (2020)	India, Chhattisgarh	_	_	_	_	1.68E+01	_	_	_	_	1.68E-04
Gill et al. (2020)	India, Bangalore	2.88E+00	7.44E-01	_	2.64E-01	_	1.44E-04	1.49E-05	_	1.32E-05	_
	India, Bhubaneswar	0.00E+00	2.66E+01	_	9.36E-01	_	0.00E+00	5.33E-04	_	4.68E-05	_
	India, Ludhiana	4.18E+00	7.20E-01	_	1.49E+00-03	_	2.09E-04	1.44E-05	_	7.44E-05	_
	India, Guwahati	9.36E-01	4.32E+00	_	1.90E+00	_	4.68E-05	8.64E-05	_	9.48E-05	_
	India, Udaipur	0.00E+00	6.76E+01	_	4.08E-01	_	0.00E+00	1.35E-03	_	2.04E-05	_
Jawaid et al. (2016)	Pakistan, Hyderabad	_	_	2.31E+01	_	_	_	_	2.31E-03	_	_
Lapierre et al. (2019)	Chile, Los Ríos	_	0.00E+00	_	_	_	_	0.00E+00	_	_	_
	Chile, La Araucanía	_	2.67E+01	_	_	_	_	5.33E-04	_	_	_
	Chile, Los Lagos	_	2.87E+01	_	_	_	_	5.74E-04	_	_	_
Muhammad et al. (2012)	Pakistan, Faisalabad	4.18E+02	_	_	1.87E+03	_	2.09E-02	_	_	9.34E-02	_
Sajid et al. (2016)	Pakistan, Faisalabad	1.47E+02	2.21E+02	9.34E+01	0.00E+00	8.85E+01	7.35E-03	4.43E-03	9.34E-03	0.00E+00	8.85E-04
ul Hassan et al. (2014)	Pakistan, Punjab	1.13E+03	6.07E+03	8.69E+03	_	1.03E+03	5.65E-02	1.21E-01	8.69E-01	_	1.03E-02
ADI/PTDI*		**20000**	**50000**	**10000**	**20000**	**100000**					

* Data from Codex Alimantarius commission (Codex Alimantarius commission, 2020), _: Data not available

total almost a third of the world's population (>2 450 000.00 Thousand inhabitants), which point to the existence still of considerable risks for the presence of recalcitrant contaminants such as OCPs banned all over the world several decades ago.

The most common found pesticide residues were from the OCs DDTs and Drins, from the OPs permethrin and bifenthrin, from the PYs ethion and coumaphos, and from the CBs carbaryl and aldicarb. While some pesticides such as DDT and HCH have been banned from use in developed countries, they are still used in many developing ones and therefore they are still detected at a high level in raw cow's milk. High geographic variation was observed, and many regions appear as contaminated zones with high risks such as Punjab in Pakistan (× 3080 > MRL and × 113 > MRL for Cypermethrin and Drins, respectively), Sand Pedro in Columbia (× 1090 > MRL and × 200 > MRL for endrin and Drins, respectively), and Gezira State in Sudan (× 109 > MRL DDTs). However, it is very difficult to assign this heterogeneity to a specific factor; it can depend on the intensity and/or the type of agriculture activities, and the presence of agrochemical companies.

The exposure assessment using the EDI, HQ, and HI revealed that EDI values were higher than PTDI/ADI for Drins in 9 regions out of 38, for heptachlor in 3 regions out of 32 and for endrin in 1 region out of 29. In addition, HI was far above 1 in 10 out of the 69 studied regions across the globe, which indicates a significant health risk for the consumers.

We recommend the adoption of more sustainable policies to reduce pesticide use and enhance collaboration between north-south countries to strengthen pesticide risk. Moreover, the development of eco-friendly alternatives for chemical pesticide use and the promotion of integrated pest management (IPM) strategies should be promoted. Dissemination and training programs for technical officers to inspect and monitor pesticide use should be developed. Finally, sanctions for non-compliance, unreasonable use or contamination of the environment must be firm for more effectiveness.

The studied data in this systematic review showed the difficulty to understand the multifaceted aspect of food security with respect to cow's milk consumption. Under this spectrum, the concepts of data actualization and continuous monitoring are necessary and recommended for the evaluation of the potential adverse effects of pesticide residues on human and animal health. The monitoring of this large list of compounds (herbicides, fungicides and insecticides) requires the use of updated analytical methodologies supported by chromatography coupled with mass spectrometry in a wide resolution and capability configurations. Moreover, the development of eco-friendly alternatives for the conventional chemical inputs in agriculture is necessary in order to ensure its sustainability, profitability and the preservation of natural resources for future generations.

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Data availability

All data are mentioned in the body of the manuscript, tables, and figures.

Table 7
HQ mean of OPs, and CBs residues in raw cow's milk reported in research articles published since 2010.

Refs.	Location	Organophosphorus								Carbamates		
		Malathion	Dimethoate	Chl	Pro	Dich	Meth	ethion	Parathion-methyl	Carbaryl	Aldicarb	Carbaryl
Bedi et al. (2015)	India	3.20E-06	_	2.04E-03	1.60E-05	_	_	3.60E-04	_	_	_	_
da Silva et al. (2014)	Brazil	3.14E-05	4.84E-04	_	_	_	_	_	_	0.00E+00	0.00E+00	0.00E+00
Donia et al. (2010)	Egypt	0.00E+00	0.00E+00	_	0.00E+00	_	_	_	_	_	_	_
Fagnani et al. (2011)	Brazil	4.30E-07	3.23E-05	_	_	9.68E-05	_	_	0.00E+00	1.61E-05	4.30E-05	6.45E-05
Gill et al. (2020)	India	_	_	4.10E-04	7.52E-05	_	_	1.26E-03	_	_	_	_
	India	_	_	3.12E-04	2.56E-05	_	_	8.16E-04	_	_	_	_
	India	_	_	2.47E-03	6.24E-05	_	_	9.60E-04	_	_	_	_
	India	_	_	1.56E-03	2.00E-05	_	_	1.75E-03	_	_	_	_
	India	_	_	3.05E-03	2.48E-05	_	_	1.22E-03	_	_	_	_
Jawaid et al. (2016)	Pakistan	_	_	2.95E-04	3.44E-04	_	_	_	_	_	_	_
Kotinagu and Krishnaiah (2015)	India	_	1.56E-01	0.00E+00	0.00E+00	_	_	_	_	_	_	_
Lapierre et al. (2019)	Chile	_	_	_	_	_	0.00E+00	_	_	_	_	_
	Chile	_	_	_	_	_	0.00E+00	_	_	_	_	_
	Chile	_	_	_	_	_	0.00E+00	_	_	_	_	_
Melgar et al. (2010)	Spain	_	_	_	_	7.99E-03	_	_	8.28E-03	_	_	_
Muhammad et al. (2012)	Pakistan	_	_	2.95E-04	_	_	_	_	_	_	_	_
Nath et al. (2013)	India	1.62E-04	_	3.84E-04	_	_	_	_	_	_	_	_
Sajid et al. (2016)	Pakistan	_	_	2.46E-05	2.13E-04	_	0.00E+00	_	_	_	_	_

_: Data not available.

Ethical statement

None to be declared.

CRediT authorship contribution statement

Ali Boudebbouz: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft. **Sofiane Boudalia:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft. **Meriem Imen Boussadia:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft. **Yassine Gueroui:** Conceptualization, Writing – review & editing. **Safia Habila:** Conceptualization, Writing – review & editing. **Aissam Bousbia:** Funding acquisition, Project administration, Writing – review & editing. **George K. Symeon:** Funding acquisition, Project administration, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

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GENERAL DISCUSSION

One of the most comprehensive definitions of "agro-ecology" to date is "food system ecology", and it aims to transform our food system by considering environmental issues (preserving and redeployment of biodiversity, developing climate-smart strategies (e.g. eco-friendly alternatives to chemical pesticides), fighting against global warming and climate change effects, reduction of carbon footprint and greenhouse gas fluxes via the decrease in the use of conventional energies); social and societal (work on hunger eradication, gender issues and women's empowerment) and health (ensure a healthy diet, to stop the development of chronic diseases such as metabolic disorders). Consequently, these strategies can be profitable and safeguard natural resources for future generations (Brini, 2021; IPCC, 2014; Martin et al., 2020; Wainwright, Glenk, Akaichi, & Moran, 2019).

In the same way, ensuring healthy food without any risk for the consumer means assessing the harmlessness of all contaminants, especially chemicals (such as heavy metals, pesticide and drug residues, and migrants from packaging). Some of these molecules can potentially be toxic to animals and humans; because they are involved in developing several pathologies such as cancers, obesity, and neurological and behavioural disorders (Arisekar, Shakila, Shalini, Jeyasekaran, & Padmavathy, 2022; S. Cao et al., 2022; Zhang et al., 2022).

In this current thesis, we tried to approach the "HEALTH" issue of the "food system transformation" through an assessment of milk safety. Identification and quantification of heavy metals in raw cow milk were made, and potential risks associated with milk consumption among different classes of consumers according to their age were assessed.

<u>At national level</u>

In northeast Algeria, we reported that the annual average temperature increased by 0.3 0.001 °C.yr^{-1} between 1980 and 2018, and we predicted that future annual average temperature will rise by 1.18°C, 2.33°C, and 4.59°C from several scenarios between 2081-2100. Also, we revealed that between 1980 and 2018, annual precipitation decreased by 0.99 ± 0.24 mm.yr^{-1} and is expected to fall by 22.5 mm, 44.4 mm, and 95.2 mm between 1980 and 2000 and 2081-2100 for different scenarios. The increase in air temperature and the decrease in precipitation were accompanied by an increase in cropland and a decline in pasture areas. From 1992 to 2005, agricultural cover grew by 90.3%. Between 1993 and 2009, the pasture area decreased by 53.7%. The distribution of high-quality foraging sites for livestock, particularly natural vegetation, has been influenced by this rapid change in land usage (*Publication in review*). These results are in accordance with those reported by Zeroual et al. (2020); (2019), who have shown that predicted increased temperatures may further exacerbate droughts and water shortages, which will lead to an expansion of desert climate zone at the expense of the temperate and steppe climate zones by the end of the twenty-first century (2045-2100).

In this thesis, to address the notion of "biodiversity redeployment and genetic resources protection", we collected samples only from local cattle breeds previously characterised by our laboratory (Aissam Bousbia et al., 2021). The results showed that the average daily milk production was 4.13 ± 2.12 L/cow/day, with an acceptable physicochemical quality but poor bacteriological quality. Milk yield was very close to data reported by Yakhlef (1989), with 1400 L/cow/year (≈ 4 L/ cow/day). However, they are higher than those recorded in Central Uganda (2.6 ± 0.19 L/cow/day) for indigenous cattle breeds (Nalubwama, Kabi, Vaarst, Smolders, & Kiggundu, 2016). Overall, considering the vulnerability of the study area to the changing climate conditions, it seems obvious that the exploitation of foreign breeds such as the Holstein breed is not the best adaptation strategy to climate change effects. Moreover, local cattle breeds seem to be the best adaptation practices. Nevertheless, are locally bred cattle spared from pollution and contamination?

To answer this question, we assessed heavy metals contamination in milk produced by local cattle breeds in northeast Algeria. The results of this study revealed that the concentrations of Pb, Cd and Cu in all analysed samples (100%) were more than their corresponding MRLs. In comparison, 82.95%, 42.04%, 15.90% and 5.68% of Zn, Fe, Cr and Ni samples exceeded their MRLs, respectively (Boudebbouz, Boudalia, Bousbia, et al., 2022), which in accordance with the data published previously in our laboratory in raw cow milk collected from foreign breeds (in intensive livestock system) in the same region (A Bousbia et al., 2019).

We reported values of target hazard quotients (THQs) higher than 1 for Cd for infants in three scenarios (1, 2, and 3 servings of cow milk/day). Moreover, Cr THQ values were recorded higher than 1 for children in the high scenario (3 servings cow milk/day) and infants in the three scenarios (1, 2, and 3 servings cow milk/day). Except for adults with a low scenario (1 serving cow milk/day), all the THQs values of Pb were far higher than 1, indicating the greatest health risk for infants and children consuming 3, 2 or even 1 serving cow milk by day and for an adult consuming 2 or 3 servings cow milk by day (Boudebbouz, Boudalia, Bousbia, et al., 2022). Hazard Index (HI) values were higher than 1, which indicates that the exposure level may cause adverse effects over a lifetime for all ages. HI for raw cow milk was largely driven by the Pb, Cr, and Cd THQs for all ages, while the highest HI values were recorded for infants and children, which is in agreement with data reported in Peru (Castro-Bedriñana, Chirinos-Peinado, Ríos-Ríos, Machuca-Campuzano, & Gómez-Ventura, 2021) and China (Su et al., 2021).

<u>At international level</u>

In this thesis, we extracted and then analysed published data concerning the emerging contaminants (Heavy metals and pesticide residues) in raw cow milk across the globe. Moreover, the potential health risks of these pollutants to human health were assessed. The research strategy was based on published articles between 2010 and 2020 (regarding heavy metals levels) and 2012-2021 (regarding pesticide residue) to identify and analyse the level of these contaminants in three different scientific databases (Science Direct, Scopus and PubMed) (Boudebbouz et al., 2021; Boudebbouz, Boudalia, et al., 2022b).

The reported results indicated that the levels of Cu and Fe in raw cow milk collected worldwide were higher than the maximum limit recommended by the US Food and Nutrition Board (Boudebbouz et al., 2021). However, Cd and Pb levels in cow milk were higher in developing countries and lower in developed countries, reflecting high strict regulations in developed countries. In addition, the exposure assessment indicates that the exposure to Fe and Al via milk consumption was safe for human consumption (Boudebbouz et al., 2021).

Results of chapter 4 show that pesticide residues were recorded in raw cow's milk samples in one developed country (Spain) and 14 developing countries (Egypt, Colombia, Pakistan, Turkey, Uganda, Poland, Sudan, Ethiopia, India, Romania, Croatia, Mexico, Brazil and Chile). In addition, results showed that some pesticides such as DDT and HCH had been banned from use in developed countries; they are still used and detected with a high concentration in many developing countries. The health risk assessment using the EDI, HQ, and HI indicates that EDI values were higher than PTDI/ADI for several pesticide residues in different areas in the world, especially in developing countries, which indicates a significant health risk for the consumers (Boudebbouz, Boudalia, et al., 2022b). Our reported results are in accordance with data published in several regions across the globe (Dong, Zhang, & Quan, 2020; Năstăsescu et [al., 2020](); [Parween, Ramanathan, & Raju, 2021](); [Taghizadeh et al., 2021](); [Zafeiraki, Kasiotis, Nisianakis, Manea-Karga, & Machera, 2022]()).

<u>Relationships between heavy metals and milk composition</u>

Here, using Spearman correlations tests, we looked for the relationship between heavy metal levels reported in northeast Algeria and raw cow milk components (lactose, protein, fat and Minerals) (Figure 4). Weak positive correlations were estimated for Pb-Lactose (0.31), Ni-lactose (0.12), Cr-Fat (0.15), and Cd-MM and Cd-Fat (0.17 and 0.19, respectively) (Figure 4).

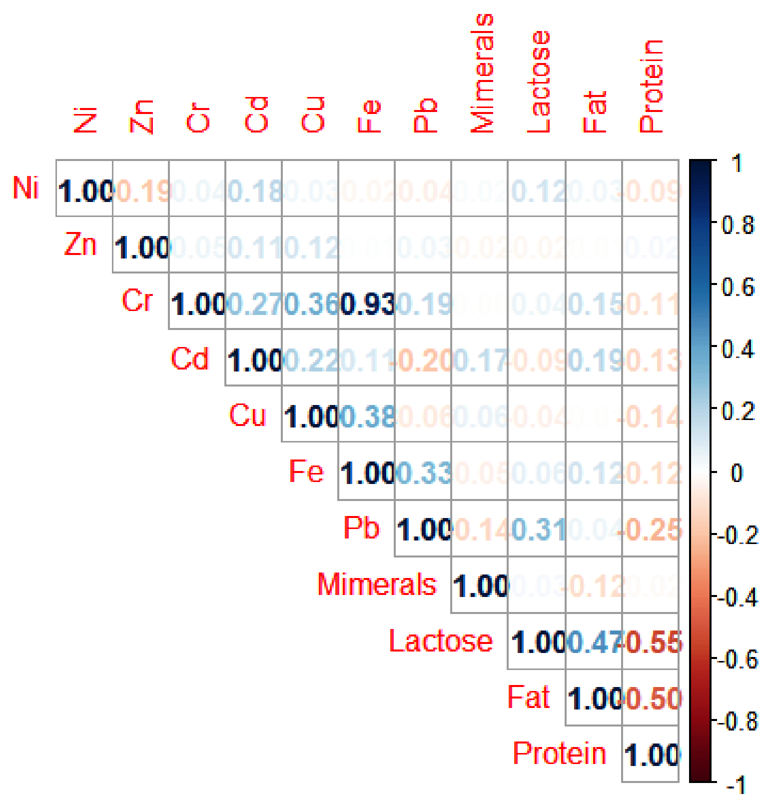

Figure 4 Spearman correlation coefficient number between heavy metals and milk composition

Contents of Pb and Ni in raw cow milk revealed low correlations with lactose. These findings are consistent with those of [Zhou, Zheng, Su, Wang, and Soyeurt (2019)]() and [Muhammad et al. (2009)](). The authors calculated regression coefficients of 4.09, 0.096, and 0.023 for Pb, Cr, and Cd residues, respectively, in cow milk with respect to the milk fat percentage. However, an estimated weak positive correlation has been reported for Pb-protein, Cr-protein, and Cd-lactose (Zhou et al., 2019). The presence of Cd and Pb in the milk could cause a significant rise in protein oxidation compounds such as dityrosine concentration and advanced oxidation protein products (AOPP). Moreover, contaminated milk with heavy metals, especially Pb and Cd, significantly dropped both antioxidant enzyme activities and lysozyme content (Grassi, Simonetti, Gambacorta, & Perna, 2022). Therefore, it is crucial to keep an eye out for these harmful substances in milk because they can harm consumers' health directly through ingestion and indirectly through the degradation of milk stability.

CONCLUSION

At the national level, the results of the study revealed a high concentration of heavy metals, especially Pb, Cd and Cu, in all farms in the northeastern region of Algeria, even in areas considered unpolluted or not exposed to pollution sources (far from roads, mines and industry). According to the task risk quotient (TRQ) values, the levels of Ni, Zn, Cu and Fe did not affect consumers' health. However, the results suggest that there may be a risk, particularly for infants exposed to Pb. Despite the risk of milk contamination with heavy metals, acceptable physicochemical quality was recorded. However, the poor bacteriological quality and low milk production are also considered problems to be solved to improve the local cattle breed livestock, which we think is a very interesting strategy to fight against global warming in this vulnerable area.

The preservation of Algerian cattle breeds resources and the fighting against the propagation of emerging contaminants (heavy metals and pesticides) may be crucial for agro-ecology development, and it can be provided in two phases:

- ✓ Implementing selection and genetic development activities to improve the indigenous cow breeds' productivity and profitability. Consequently, smallholder farmers may benefit from this since it can give them a fair and steady income and comfortable working conditions. Other activities, such as promoting women's empowerment, formulating pertinent policy concerns, and creating suitable capacity-building initiatives for various stakeholders, help fight against climate change's effects and can contribute significantly to local economies and social integrity.
- ✓ The development of more environmentally friendly products and regulations can decrease the use of synthetic pesticides and, consequently, protect human health.

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ANNEXES

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Characterization of traditional Algerian cheese "*Bouhezza*" prepared with raw cow, goat and sheep milks

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Abstract

"*Bouhezza*" is an Algerian traditional fermented soft-ripened cheese, found and consumed in the Northeast of Algeria. The objective of this study was to explore the preparation process (traditional making diagram) of "*Bouhezza*" cheese and to study the effect of the type of raw milk (cow, goat and sheep) on the yield and organoleptic characteristics of the fresh "*Bouhezza*" cheese. "*Bouhezza*" cheese was handmade in a traditional way using milk of three species (cow, goat and sheep). The milk used has been the subject of physicochemical and bacteriological analysis. Cheese yield for sheep's milk is higher ($p < 0.05$) than cheese yield for cow's and sheep's milk. For sensory analysis, score registered for cheese of cow's milk ≥ 5 suggested higher acceptability for this cheese. Here, we exposed the "*Bouhezza*" cheese, its history, origin and manufacturing processes. From the physicochemical and bacteriological analysis of milk, results show that all criteria analyzed respond almost to the required standard. The sensory qualities of the three types of cheese show that cow cheese was classified as the most satisfactory cheese for the majority of criteria (taste. color and texture). Finally, and for higher yield, results supported the use of sheep milk as a raw material.

Keywords: traditional cheese; "*Bouhezza*"; bacteriological criteria; physicochemical parameters; sensory evaluation.

Practical Application: Algerian "*Bouhezza*" cheese: his history, origin manufacturing processes and characteristics.

1 Introduction

Traditional products are considered as a very important way to keep the regional and national identity of peoples. We meet traditional recipes handed down from generation to generation, challenging time and space. Among these products, traditional cheeses are one of the food product that have become the image of different countries or region of origin, they differ from each other by their making process, ripening time (if applied), type of milk used, texture, color, flavor, coagulation type (enzymatic and/or acid)…etc. Among these traditional cheeses we can cite "*Klila*" chesses produced in Algeria (Leksir et al., 2019; Leksir & Chemmam, 2015); "*Roquefort*", "Cheddar", "*Emmental*", "*Camembert*", "*Parmesan*" and "*Picodon*" produced in France (Bertozzi & Panari, 1993; Leclercq-Perlat et al., 2019; Quetier et al., 2005); "*Maraj´ o*" *cheese*, "*Manteiga*", "*Coalho*", "*Caipira*", "*Canastra*" and "*Minas*" cheeses produced in Brazil (Moraes et al., 2018; Sant'Anna et al., 2017; Kamimura et al., 2019); "*Quesillo*" cheese produced in Argentine (Oliszewski et al., 2007); "*Vlasina*" cheese produced in Serbia (Terzic-Vidojevic et al., 2013); "*Anevato*" cheese produced in Greece (Hatzikamari et al., 1999); "*Chihuahua*" cheese produced in Mexico (Sánchez-Gamboa et al., 2018) and "*Babia-Laciana*" cheese produced in Spain (Franco et al., 2003).

Unlike other countries, in Algeria traditional cheeses are few in number but not fully enumerated and as little been studied (Dubeuf et al., 2010); about ten types of cheese are known in different regions of the country (Aissaoui Zitoun et al., 2011). Among these cheeses are "*klila*", "*bouhezza*", "*mechouna*" and "*madghissa*", in the region of Chaouia, "*takammérite*" and "*aoules*" in the south, "*igounanes*" in the region of Kabylie (Aissaoui Zitoun et al., 2011, 2012; Ben Danou, 1929; Benamara et al., 2016; Benkerroum, 2013; Khoualdi, 2017; Leksir & Chemmam, 2015; Licitra et al., 2019; McSweeney et al., 2017; Medjoudj et al., 2017a, b; Ramalho Ribeiro et al., 2006).

Unfortunately, several of these cheeses are endangered, for various reasons including the unavailability of fodder, rural exodus and changing dietary habits. We do not know the future of these products, but we must do everything possible to know them, maintain their existence and encourage their manufacture. The preparation processes of these cheeses come from earlier generations and have been passed down from generation to generation (Leksir et al., 2019). So, registration of different information about traditional cheeses is part of the preservation of a nation's culinary heritage and culture which must be well characterized and protected. Also, the certification

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of a geographical indication "Protected Designation of Origin PDO) for these artisanal cheeses may encompass an essential milestone for adding value and, an economic resource for farmers (Kamimura et al., 2019).

"*Bouhezza*" cheese has long been known in the Chaouia region of eastern Algeria. It is made from goat, sheep, cow milk or mixture (Marino et al., 2012; Medjoudj et al., 2017a, b) and considered not only as a food product but also as an integral part of "*Chaouias*" people life. The manufacture of "*Bouhezza*" has the particularity of involving coagulation, draining, salting and refining simultaneously. The cheese is obtained after transformation of the "*Lben*" in a "*skinbag*" or a "*Chekoua*" made of goatskin previously treated with salt and juniper (Aissaoui Zitoun et al., 2011). "*Chekoua*" serves, at first sight, container and media filtering for sewage (Aissaoui Zitoun et al., 2011, 2012; Chaker, 1986). Outsides, these elements, it is scientifically poorly studied. The current study deals with the traditional preparation of "*Bouhezza*" by the people of "*Chaouias*" (traditional making diagram) and at the same time explore the effect of the type of raw milk of three species (cow, goat and sheep) on the yield and organoleptic characteristics of the fresh "*Bouhezza*" cheese.

2 Materials and methods

2.1 Study area and sampling plan

The samples are collected from four areas located in the in North-East of Algeria (Guelma, Souk Ahras and Tébessa) and in the center of Algeria: (Djelfa) (Figure 1). A total of 27 samples of raw milk of three species (goat, cow, and sheep) were collected and used for cheese making. From each farm, about 1.5-2 L were taken in sterile glass bottles and placed immediately in a cooler, then transported to the laboratory, where they are stored at 4 °C until analysis and cheese making. All bottles are previously autoclaved at a temperature of 121 °C, under a pressure of 1 bar for 15 minutes.

The vials are filled from a container of mixing milk, respecting the Good Laboratory Practices (GLP), and the rules of asepsis (disinfection of the hands). In order to take account of the real field conditions, no conservative was added. Total volumes of 20-50 mL from each sample were collected for microbiological physicochemical analysis.

2.2 Raw milk analysis

Physicochemical and bacteriological analysis

For physicochemical analysis, pH was measured using a pH meter Adwa, AD1000 and acidity was determined according to the method described by Tadjine et al. (2019). Freezing point, conductivity, fat content, protein content, lactose content, mineral content and vitamins of milk were measured with a Lactoscan milk analyzer (Milkotronic LTD Europe) according to the manufacturer's instructions.

For bacteriological analysis, samples preparation and dilutions were performed according to the recommendations of the International Dairy Federation (1991): 1) The Total Mesophilic Aerobic Flora (TMAF) was enumerated using Plate Count Agar (PCA) and incubated at 30 °C for 72 h; 2) The Total Coliforms and Fecal Coliforms were determined using Violet Red Lactose Bile agar (VRBL) incubated at 37 °C for total coliforms, and 44 °C for fecal coliforms; 3) Sulphite Reducing *Clostridium* was determined using enrichment method in a liquid medium; 4) The enumeration of Staphylococci suspected pathogens was conducted using a selective medium (Chapman) and incubated at 37 °C for 24 to 48 hours. A positive culture of Staphylococci is indicated by the formation of a black precipitate surrounded by a white halo; 5) For *Salmonella*, two mediums were used to enumerate the colonies: Selenite-Cystine for enrichment at 37 °C for 12 h, and SS medium (*Salmonella-Shigella*) for isolation at 37 °C for 24 h. *Salmonella* appears like colorless and transparent colonies with or without a black center of small size (2 to 4 mm in diameter).

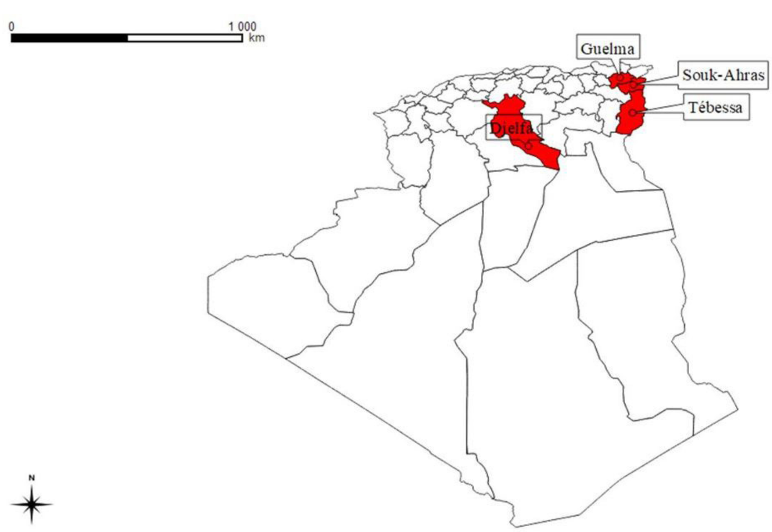

Figure 1. Location map of the study area. Milk samples are collected from four areas located in the North East of Algeria (Guelma, Souk Ahras and Tébessa) and in the center of Algeria: (Djelfa). Raw milk of three species (goat, cow, and sheep) were collected and used for "*Bouhezza*" cheese making.

2.3 Data collection about the traditional preparation of "Bouhezza"

A household survey focused on group discussion was performed in this study (Ghosh et al., 2014; Leksir et al., 2019). The present documentation of *"Bouhezza"* preparation is based on the questionnaires and insight observation from farmers. A total of 45 people including producers, sellers, and consumers were involved in the interviews and discussions, after taking their prior consent. The survey was conducted through face-to-face interaction to either heads or knowledgeable adults of households. During the data collection on *"Bouhezza"*, especially while conducting the interviews, observations were made and the comments of responders and other people were noted.

The research protocol for the sensory study and the household survey have been developed and validated by the ethics committee of the University of Guelma-Algeria.

2.4 Cheese making

The raw milk is left at room temperature, until its spontaneous coagulation that takes 24 to 72 hours depending on the seasonal temperature. This curdled milk by natural fermentation is called "*Rayeb*" (or *Raïb*). Then "*Rayeb*" must be churned for 30 to 40 minutes to make the "*Lben*" in the the "*Chekoua*". The addition of lukewarm water to the "*Raïb*" (about 10% (v/v)) makes it possible to reduce the temperature to the proper level to collect the grains of butter. After partial extraction of the traditional butter (*Zebda Beldia*), one obtains a thick liquid, the buttermilk named "*Lben*".

After thorough conversation and field observation, "*Bouhezza*" preparation stretches over a period of eight days, and it can be broadly divided into the following steps: salting is done in the "*Lben*". The added amount is an average of 1 tablespoon/L. The "*Chekoua*", in which the "*Lben*" is introduced (a quantity of 3.6 to 3.8 L) is suspended in a ventilated place and in the shade. Once the cheese is refined, the raw milk (100 mL/4 L) is added to adjust the acidity and salinity of the finished product. Cheese was stored in pottery jars/ glass or food containers for a few weeks at a temperature that varies between 4 °C and 8 °C.

2.5 Real yields in cheese, dairy whey and butter

After each series of production, the amount of whey, butter, and cheese is measured in order to calculate the yield using the Equation 1 (Tadjine et al., 2019).

$$\textit{Real yield of cheese } (\textit{butter or whey}) = \textit{The weight of cheese obtained } (kg) / \textit{volume of milk } (L) \times 100 \ (\textit{in } kg/100\,L) \quad (1)$$

2.6 Sensory analysis

In order to obtain basic information about the sensory characteristics of *"Bouhezza"* cheese, a consumer acceptability test was conducted using 9 point unstructured hedonic scale (Boudalia et al., 2016b; Oliveira et al., 2017; Ruvalcaba-Gómez et al., 2020); a randomized panel consisting of 20 students, and teachers-researchers of both sexes from University of 8 Mai 1945 Guelma: 91% of people are under the age of 30 years old, and 9% between 30 to 45 years old. The following five sensory characteristics were selected for evaluation, overall appreciation, taste, texture, smell and color. They were scored from 1 (dislike extremely) to 9 (like extremely). The overall assessment is requested at the beginning of the questionnaire to get as close as possible to the real conditions of purchase and to prevent the consumer from decomposing the sensations. Additional information on the sex, age and frequency of consumption of cheeses is also required to enable the characterization of the sample population interviewed.

Cheese is considered acceptable (from a hedonic point of view) if at least 50% of our participants give a score greater than or equal to 6 (likes slightly) (Conti-Silva et al., 2011; Volpini-Rapina et al., 2012).

Prior to the start of testing, all participants spread to questions about possible food allergies to cheese components (milk protein). Then, the cheese (40 g), which was freshly prepared, placed in plastic plates, then presented to the panelist for tasting. Participants answered questionnaires (Sęczyk et al., 2016). The questionnaires duly completed by the tasters were removed at the end of the evaluation and the data was organized and processed.

The research protocol for the sensory study and the household survey have been developed and validated by the ethics committee of the University of Guelma-Algeria.

2.7 Statistical analysis

The results of the physicochemical analysis, cheese yield, as well as the results of the sensory analysis, are expressed in the form of means ± SEM (Standard Error Mean). The differences between the different parameters are the subject of an analysis of variance (ANOVA) followed by a comparison of means (Dunnet test or Tukey test) when the conditions of normality and homogeneity of the variances are respected (test Kolmogorov-Smirnov), and possibly a non-parametric analysis of variance (Kruskal-Wallis). For bacteriological analyses, results were expressed by the presence or absence of germs. All the colonies were counted as Colony Forming Units per mL of milk (CFU/mL) (International Dairy Federation, 1991).

For sensory analysis, statistical analysis of the data was analyzed on the basis of a two-factor analysis of variance (ANOVA) "hedonic score *vs*. cheese, sex", considering "sex" and "cheese" as independent variables. "sex × cheese" interactions are also reported.

The data was processed using Minitab software (Minitab, Ltb., United Kingdom, Version 16). The minimum threshold of significance retained is $p < 0.05$.

3 Results and discussion

3.1 Physicochemical and bacteriological qualities of raw milk

Results from physicochemical and bacteriological analyses of raw milk for the three species presented respectively in Tables 1 and 2 satisfy the standard of analyzes criteria (Food and Agriculture Organization of the United Nations, 2002).

Table 1. Physicochemical qualities of the analyzed samples (N = 27 samples).

Parameters	Species	Mean	SEM	CV (%)	Min	Max
Fat content (%)	Cow	3.28^a	0.08	7.18	3.00	3.58
	Goat	3.23^a	0.09	8.36	2.81	3.80
	Sheep	1.82^b	0.08	13.44	1.57	2.30
Protein content (%)	Cow	3.13^b	0.20	18.69	2.44	4.15
	Goat	3.05^b	0.32	31.48	1.98	4.74
	Sheep	4.63^a	0.05	2.93	4.49	4.87
Lactose (%)	Cow	4.70^a	0.29	18.65	3.67	6.23
	Goat	2.97^b	0.29	29.33	1.89	4.50
	Sheep	4.30^a	0.07	4.63	3.90	4.62
Minerals and Vitamins (%)	Cow	0.70^a	0.04	18.69	0.55	0.93
	Goat	0.50^b	0.05	28.85	0.32	0.75
	Sheep	0.73^a	0.01	3.31	0.70	0.77
Dry Degreased Extract (%)	Cow	8.55^a	0.53	18.60	6.67	11.33
	Goat	6.61^b	0.65	29.28	4.21	10.01
	Sheep	9.77^a	0.10	3.13	9.40	10.28
Added Water (%)	Cow	3.62^b	2.11	174.46	0.00	18.44
	Goat	25.79^a	7.24	84.21	0.00	56.53
	Sheep	0.00^b	0.00	0.00	0.00	0.00
pH	Cow	6.48^a	0.06	2.93	6.00	6.63
	Goat	6.63^{ab}	0.03	1.18	6.52	6.78
	Sheep	6.71^b	0.08	3.65	6.26	7.05
Density (mg.cm^{-3})	Cow	1031.90^a	2.11	0.61	1024.90	1042.80
	Goat	1022.7^b	2.48	0.73	1013.00	1035.50
	Sheep	1033.10^a	1.36	0.39	1023.20	1035.90
Freezing point (°C)	Cow	-0.55^b	0.04	-20.57	-0.75	-0.42
	Goat	-0.38^a	0.04	-33.37	-0.58	-0.23
	Sheep	-0.56^b	0.01	-4.74	-0.60	-0.52
Conductivity (µS.cm^{-1})	Cow	4.94^a	0.25	15.15	4.03	5.82
	Goat	4.55^a	0.19	12.18	3.59	5.12
	Sheep	3.87^b	0.06	4.81	3.60	4.13

SEM: Standard Error Mean; CV: coefficient of variation; Max: maximum; Min: minimum. Means which are denoted by different letters (a, b) indicate significantly different mean values between milk from the here species and for the same parameter (Fat, protein and lactose, minerals and vitamins, Dry Degreased Extract, Added Water, pH, Density, Freezing point and Conductivity).

Table 2. Bacteriological qualities of the analyzed samples (N = 27 samples).

Flores (UFC/mL)	Species	Mean ± SEM	Standard (CFU/mL)
TMAF (10^5)	Cow	1.13 ± 1.26	10^5
	Goat	0.87 ± 1.05	10^5
	Sheep	1.37 ± 1.66	10^5
F. Col. (10^3)	Cow	1.03 ± 1.65	10^3
	Goat	0.56 ± 0.84	10^3
	Sheep	1.12 ± 1.33	10^3
T. Col. (10^3)	Cow	1.02 ± 1.45	10^3
	Goat	0.96 ± 1.01	10^3
	Sheep	1.15 ± 1.07	10^3
Sulphite reducing *Clostridium*	Cow	27 ± 15.60	50
	Goat	13 ± 18.35	50
	Sheep	51 ± 11.30	50
S. aureus	Cow	Absence	Absence
	Goat	Absence	Absence
	Sheep	Absence	Absence
Salmonella spp.	Cow	Absence	Absence
	Goat	Absence	Absence
	Sheep	Absence	Absence

TMAF: Total Mesophilic Aerobic Flora; T. Col.: total Coliforms; F. Col.: fecal Coliforms; SEM: Standard Error Mean.

Milk density is between 1.03 ± 6.33; 1.02 ± 7.44 and 1.03 ± 4.08 kg/m^3 for cow's milk, goat's milk, and sheep's milk, respectively. In addition, a significant difference is recorded between the milk of the three species, where the density of goat's milk is the lowest ($p < 0.05$).

Fat content recorded is 3.28% and 3.23% for cow's milk and goat milk respectively. These results are very close to those cited in the literature (3.7% and 4.1% for cow's milk and goat's milk, respectively) (Boudalia et al., 2016a; El Galiou et al., 2015). However, a very lean fat content was recorded for sheep's milk (1.82%). This significant difference ($p < 0.05$) is not consistent with data from the literature, where sheep's milk is considered as being a fatty milk (Fat content: 7.9%) (Park, 2006; Park et al., 2007). However, these results are probably due to the feed abundance, Hamidi et al. (2018) found lower fat content in a semiarid region of Algeria where plant abundant and richness is lower.

Results from dry degreased extract (TDE) shown that goat's milk contains less TDE (6.61%); this result is much below the standard (13.4%). In the same way, TDE results recorded in cow's milk (8.55%) and sheep's milk (9.77%)

remain relatively low compared to the standards (12.8% and 18.3% for cow's milk and sheep's milk respectively) (Food and Agriculture Organization of the United Nations, 2002; Renhe et al., 2019). The lactose content is (2.97%, 4.70% and 4.30%) in goat, cow and sheep respectively ($p < 0.05$, Table 1). The results obtained are slightly lower than data from the literature (Renhe et al., 2019).

Total protein (Table 1) indicates that the raw cow's milk is between 2.75 to 4.15% [27.5-41.5 g/L]. For goat and sheep respectively, the protein level of 3.05% and 4.63% was recorded. These rates are in line with the norms for goat's milk. In sheep, protein level remains higher than the protein content in milk from the other two species ($p < 0.05$).

Conductivity rate is 4.94 ± 0.75 mS/cm; 4.55 ± 0.55 mS/cm; 3.87 ± 0.19 mS/cm for cow, goat and sheep's milk respectively. These values were in good agreement with the data published by Park et al. (2007). The pH recorded is 6.48 ± 0.19, 6.63 ± 0.08, 6.71 ± 0.24 for cow, goat and sheep milk, respectively. These values are consistent with the standards (Park, 2006). Also, a significant difference is recorded between the pH of cow's milk and that of the sheep ($p < 0.05$), where cow's milk appears to be more acidic (Table 1).

Minerals and Vitamins (%) level observed were 0.70 ± 0.13%; 0.50 ± 0.14%; 0.73 ± 0.02% for cow's, goat's and sheep's milk, respectively. A significant difference was found between the level of minerals and vitamins (%) in cow's and sheep's milk *vs.* goat's milk where a goat's milk seems to be less rich (Table 1).

The freezing point recorded was -0.55 ± 0.11 °C; -0.38 ± 0.13 °C; -0.56 ± 0.03 °C for cow, goat and sheep milk respectively (Table 1). A significant difference was recorded, where goat's milk has a higher freezing point compared to the other two types of milk (cow and sheep). The values of cow's milk and sheep's milk are consistent with standards; however, the results of goat's milk are relatively lower compared to standards (Food and Agriculture Organization of the United Nations, 2002; Renhe et al., 2019). This difference in physicochemical qualities for goat milk may be due to a wetting of six samples of goat's milk (6/9).

For bacteriological analysis, counting of aerobic mesophilic flora for raw milk samples showed an average microbial load of 1.13×10^5; 0.87×10^5; 1.37×10^5 CFU/mL for cow, goat and sheep milk respectively. These values consistent with the results of raw cow milk gathered in Guelma region in the northeastern of Algeria (Boudalia et al., 2016a), and who have been a satisfactory quality of raw milk in light of standard (10^5 UFC/mL). The sulphite reducing *Clostridium* was less present with low concentrations in the samples analyzed for the three species. The averages of the enumerated bacteria for cow, goat and sheep's milk are <50 CFU/mL. Unlike studies of Ghazi & Niar (2011), Hamiroune et al. (2014) and Bachtarzi et al. (2015) in other regions in Algeria, no *Staphylococcus aureus* contamination was recorded. These results provide that the hygienic quality of the milk of the three species is very satisfactory and suitable for consumption or processing.

3.2 Data collection about the traditional preparation of "Bouhezza"

A survey was conducted among the local people of several provinces in the northeastern of Algeria to understand the traditional process and knowledge of "*Bouhezza*" preparation. This survey permitted to identify a common procedure for the "*Bouhezza*" cheese production. This procedure is schematically represented in Figure 2.

"*Bouhezza*" was traditionally the product of the processing of goat and sheep milk, but the current trend seems to be towards the use of cow milk (Aissaoui Zitoun et al., 2011, 2012; Licitra et al., 2019; Medjoudj et al., 2017a, b).

The cheese is obtained after transformation of the "*Lben*" in a "*Chekoua*" made of goatskin previously treated with salt and juniper (Aissaoui Zitoun et al., 2011). Draining, salting and refining "*Bouhezza*" are performed simultaneously in the "*Chekoua*". During the ripening period, "*Lben*" and milk are added to the contents of the "*Chekoua*".

In our study, nine manufacturing experiments were carried out via the traditional diagram and for ten weeks. During each experiment, "*Chekoua*" received each three-day an amount of 1.5 L of salted "*lben*" (25 g of salt per L). At the end of the manufacturing (for about 1 to 1.5 last weeks) and to adjust organoleptic characteristics of the "*Bouhezza*" cheese (salt and acidity), additions of whole raw milk were done. In this study, the additions of fresh whole milk were perused until the tenth week to observe eventual evolutions in this case. During manufacturing, the "*Chekoua*" was suspended in an aerated room and daily washed and scraped on the external face (Figure 3).

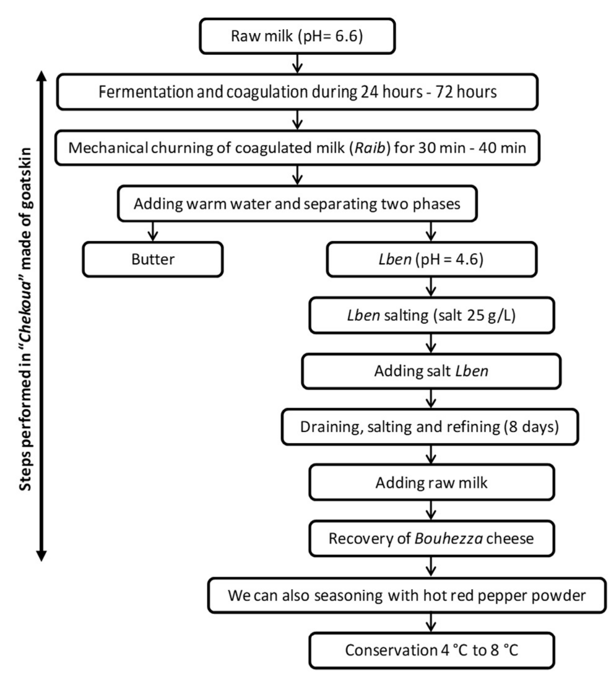

Figure 2. Illustrative global traditional diagram of the manufacturing processes of "*Bouhezza*" cheese-making. "*Bouhezza*" is obtained after transformation of the "*Lben*" in "*Chekoua*" made of goatskin previously treated with salt and juniper. Draining, salting and refining of cheese are performed simultaneously in the "*Chekoua*".

Figure 3. "*Bouhezza*" processing steps: After spontaneous coagulation of raw milk at room temperature which it takes 24 to 72 hours depending on the seasonal temperature, the curdled milk called "*Rayeb*" (or "*Raïb*" was obtained. A farmer woman use "*Chekoua*" or "*skinbag*" made of goatskin previously treated with salt and juniper to transform the "*Raïb*" (a quantity of 3.6 to 3.8 L) into "*Lben*" by churning for 30 to 40 minutes. During this step, the addition of lukewarm water to the "*Raïb*" (about 10% (v/v)) makes it possible to reduce the temperature to the proper level to collect the grains of butter. Extraction of traditional butter (*Zebda Beldia*) and salt adding (1 tablespoon/L) is realized at this stage (a) and (b). (c), (d) and (e) cheese draining; this step is carried out in "*Chekoua*". However, it can also be done in cloth bags to facilitate sewage. Raw milk (100 mL/4 L) can be added to adjust the acidity and salinity of the finished product. (f), (g) and (h) recovery of "*Bouhezza*" cheese is carried out after the draining step. (i), (j) and (k) "*Bouhezza*" Cheese is stored in pottery jars/ glass or food containers for a few weeks at a temperature that varies between 4 °C and 8 °C. People generally take it after lunch and dinner.

3.3 Cheese yields analysis

Figure 4 show the quantities of dairy products (whey, butter and cheese) following the transformation of milk from three species into "*Bouhezza*" cheese. Although the initial volume of milk used in cheese making is almost the same (no significant difference), a significant difference was recorded after processing in terms of *i)* volume of whey harvested after draining. This volume is greater than three liters for all the milks except for sheep's milk, which gave the lowest volume ($p < 0.05$); *ii)* butter quantity manufactured after churning which is around 0.24 kg for sheep's milk and 0.15 kg for cow's and goat's milk ($p < 0.05$); *iii)* "*Bouhezza*" quantity and cheese yield, indeed sheep's milk seems to be the most efficient in terms of cheese yield ($p < 0.05$) (Figure 4).

"*Bouhezza*" yield is an economically relevant variable which is influenced by different factors such as milk quality and cheese-making methods (Lucey & Kelly, 1994). Our results showed that there was an interspecific difference in cheese yield between cows, goats and sheep. While some studies show that cheese yield is higher in cows than goat (Rasheed et al., 2016), our study and others show the opposite (Hamidi et al., 2018; Mallatou & Pappa, 2005). Different factors might produce this interspecific difference, including those related to the milk composition and quality such as genetic variants of casein, fat and protein (Banks et al., 1981; Fenelon & Guinee, 1999; Verdier-Metz et al., 2001), seasonal variations (Sánchez-Gamboa et al., 2018), microbial counts and diversity (Vladimír et al., 2020) and cheese-processing methodology (Lawrence, 1993). In our study, "*Bouhezza*"-processing methodology and season are the same, however, we found higher fat and protein content in goat compared to cow milk, which probably contributed to the increase in cheese yield (Lucey & Kelly, 1994). In sheep milk, we found higher larger casein micelle size, which affect their renneting properties and coagulation time. Also, the higher casein content n, which functions as a chelator of divalent (or higher valence) ions, is associated with higher content of those mineral contents than in cow, and goat milk. The average fat globule size is smallest (<3.5 μm) in sheep milk followed by goat and cow milk. Therefore, cheese yield per volume of milk is the highest among ruminant milk (Silanikove et al., 2016).

3.4 Sensory analysis of "Bouhezza" cheese

The sensory evaluation scores are shown in Table 3 and Figure 5. From our panelist 50% are women, they are between 18 and 30 years old, and 95% of them consume cheese at least once a week. "*Bouhezza*" cheese from cow milk had a hedonic score greater than or equal to 5 for the 5 descriptors (overall appreciation, taste, texture, smell and color).

Panelists determined that "*Bouhezza*" cheese is accepted except "*Bouhezza*" from goat's milk, in which the hedonic note is less than 5. Furthermore, the sensory acceptance of the product tested in this study is very similar to that found by Dal Bello et al. (2017) for fresh cheese from raw cow milk. Also, from literature, goat and sheep cheese are not preferred by large proportion of people which are not appreciate a strong goaty or sheepy aroma, even though they are not very familiar with these aromas (Ryffel et al., 2008). In the same way, Costa et al. (2015) have evaluated the acceptance of fermented cow's and goat's milks. Results have shown that fermented cow's milk was well accepted compared to fermented goat's milk.

Table 3. Hedonic scoring test for "*Bouhezza*" cheese (with 5 descriptors).

Descriptors		Global appreciation	Taste	Texture	Odor	Color
Hedonic note	Cow	5.90 ± 0.81	5.25 ± 0.87	6.25 ± 0.82	7.75 ± 0.78	6.45 ± 0.86
	Goat	4.75 ± 0.90	4.00 ± 0.76	4.60 ± 0.71	7.60 ± 0.72	5.10 ± 0.92
	Sheep	5.50 ± 0.65	4.65 ± 0.76	4.55 ± 0.72	7.70 ± 0.64	5.10 ± 0.64

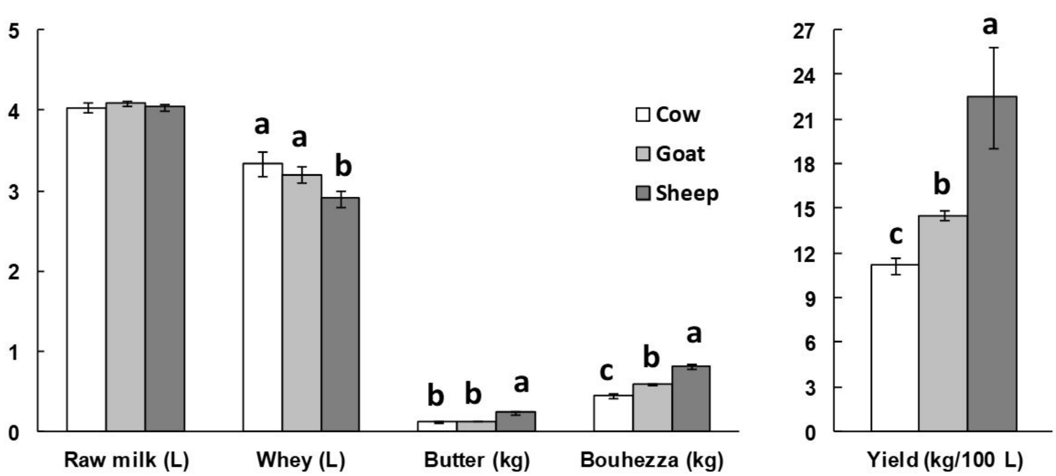

Figure 4. "*Bouhezza*" cheese yield analysis from cow's, goat's and sheep's milk collected in the regions of Guelma, Souk Ahras, Tebessa and Djelfa (n= 9/species). Results are expressed on average ± SEM. The letters on the diagrams show significant differences between each milk for the same parameter ($p < 0.05$) (One-way ANOVA, Tukey in post-hoc).

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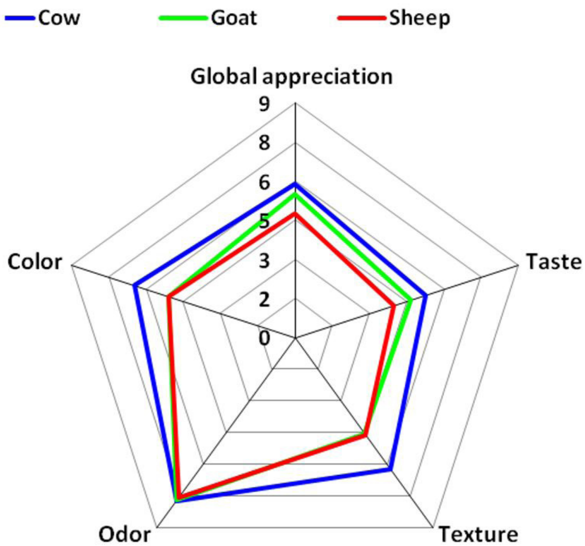

Figure 5. Mean scores of sensory acceptability test (with 5 descriptors) of different artisanal "*Bouhezza*" cheese produced from cow's, goat's and sheep's milk collected in the regions of Guelma, Souk Ahras, Tebessa and Djelfa.

4 Conclusions

In summary, *"Bouhezza"* cheese occupies a very important socio-economic place established in the rural and peri-urban environment. It is a fermented soft-ripened cheese produced empirically in several regions of Algeria. Originally, "*Bouhezza*" was traditionally the product of the processing of goat milk and sheep, but the current trend seems to be towards the use of cow milk (Aissaoui Zitoun et al., 2011, 2012; Licitra et al., 2019; Medjoudj et al., 2017a, b). In this study, we have elaborated the traditional fabrication diagram of this cheese from a field survey, and then we have produced the "*Bouhezza*" cheese from cow's milk, goat's milk and sheep's milk. Before cheese making, the raw milk from the tree species (cow, goat and sheep) was analyzed (physical, chemical, and microbiological properties). The cheese is obtained after transformation of the "*Lben*" in a "*Chekoua*" made of goatskin. Draining, salting and refining "*Bouhezza*" are performed simultaneously in the "*Chekoua*". During the ripening period, "*Lben*" and milk are added to the contents of the "*Chekoua*".

Results from the physicochemical and bacteriological analysis of milk show that all criteria analyzed respond almost to the required standard. The sensory qualities of the three types of cheese show that cow cheese was classified as the most satisfactory cheese for the majority of criteria (taste. color and texture). Based on a rate of return equivalent to that obtained in our milk production trials, the cheese processing seems very viable and cost-effective for the breeder better than their marketing as raw milk.

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Challenges and Opportunities of the Mediterranean Indigenous Bovine Populations: Analysis of the Different Production Systems in Algeria, Greece, and Tunisia

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Abstract: The indigenous cattle populations are threatened by extinction in many countries of the Mediterranean area. The objective of this study is the analysis of local cattle breeds' production systems in Algeria, Greece, and Tunisia and the identification of their future challenges and opportunities. A total of 385 surveys were conducted in these study areas: central and northern Greece (43); northern and northwestern Tunisia (167), and northeastern Algeria (175). Data collected concerned socio-economic parameters as well as the production system's functionality, constraints, and opportunities. Results revealed an average farmers' age of 52.6 years old. The illiteracy rate is high, especially in Algeria (39%) and Tunisia (44%), where the farm size is relatively small with an average of 14 and four animals per farm, respectively. In Greece, much higher numbers were recorded (89 animals/farm). The average cultivated feedstuffs' area is larger in Greece (12.07 ha) and smaller in Algeria and Tunisia (6.11 and 2.88 ha, respectively). Feeding resources are based on rangelands. Farming systems are traditional extensive and complemented when needed. Milk and meat marketing vary throughout countries and are not well valorized. The main constraints are high feeding costs, low milk and meat prices, and absence of labeling. Local and local-crossbred bovine populations could be valorized based on their good adaptation criteria when applying convenient genetic and development strategies.

Keywords: indigenous; cattle; Mediterranean; opportunities; challenges

1. Introduction

The indigenous cattle production systems contribute to milk and meat supply and represent an essential source for many communities in rural areas in the Mediterranean countries [1]. These populations are extremely valuable both at the local and regional level since they combine unique qualities: a valuable locally adapted genetic pool, substantial income to the local economies, and added-value animal products. Nevertheless, their numbers are declining due to the preference of farmers toward foreign, more productive breeds. Especially in the Mediterranean countries, the indigenous cattle breeds' populations face continuous challenges such as fear of extinction, anarchic breeding schemes, and harsh rearing conditions [2]. Generally, the global livestock sector is characterized by a growing contrast between livestock kept extensively by a large number of smallholders and pastoralists (600 million) in support of rural food security and livelihoods, and those kept in intensive commercial production systems [2,3]. In Southern Africa, over 90% of animal keepers are classified as smallholders and 75% of the farm animals, which largely consists of indigenous breeds, belong to the smallholder sector [4].

In Greece, the indigenous cattle populations have decreased to small numbers and are currently at risk of extinction, or already extinct, due to socio-economic reasons, geographic isolation, and crossbreeding with commercial breeds [5]; in 2020, four indigenous breeds were referred according to the Domestic Animal Diversity Information System of FAO (as provided by the Greek Ministry of Agriculture). They are used exclusively for meat production: the "Greek Red" (42,057 females), the "Vrahykeratiki" (9546 females), the "Katerinis" (728 females), and "Sykias" breed (2851 females) [6]. These animals are reared essentially in the mountainous grasslands. They are raised all year in the fields and housed only in extreme weather conditions. They are held in rough housing, and their dietary needs are covered mostly by grazing, while complementary feed is provided only in the winter. In past decades, the importation and use of foreign breeds and the disorganized breeding schemes have resulted in a great variety of phenotypes [1]. In the last 20 years, conservation programs have been set up to safeguard the indigenous populations.

In Tunisia, the indigenous cattle population with Iberian origin counts 191,920 females which are mainly (87%) localized in the north, especially in the mountainous area (120,000 heads). In this zone, indigenous cattle breeds contribute to 15–26% of the milk and meat production. This population has suffered from anarchic crossing, which affected its genetic structure [7]. Nevertheless, studies concerning this breed were mostly interested in genetic aspects. In fact, two breeds were identified: Atlas Brown and Blonde of Cap Bon. Moreover, the population of Atlas Brown has been declining over the years, and the population of the Blonde of Cap Bon is very limited, indicating that it is exposed to extinction [7]. The study of phenotypic variability based on a qualitative description of the characters showed that the differences between individuals are mainly manifested through the color and the general conformation of the animals.

Algerian indigenous cattle populations resemble the Atlas Brown, with pure bred animals still preserved in the mountainous regions. They are subdivided into several sub-populations, namely "Guelmoise", "Cheurfa", "Krouminiène", "Chelifienne", "Sétifienne", and "Djerba", which are clearly differentiated phenotypically [8]. These populations are characterized by their rusticity, and they constitute a very important socio-economic element, contributing to a large part to the feeding of the rural population [1,9]. Indeed, these populations have brought together qualities of adaptation to the harsh arid and semi-arid environment and to the food resources restriction [10–12]. Despite the perfect harmony between these indigenous cattle populations and their natural environment, productivity remains modest (1175 litter/cow/year [13]) both because of the often unfavorable rearing conditions and the low performance of the concerned breeds. Several trials for dairy intensification, based mainly on the importation of exotic breeds and the anarchic crossbreeding with the indigenous populations, led to a deterioration of the genetic structure of the dairy herd in Algeria, which resulted in a drastic fall in the numbers of local cattle. Thus, the percentage of indigenous cattle breeds' population is reduced from 82% of the total in 1986 to about 48% of the total in 2016 [14,15].

Therefore, the concept of the scientific cooperation project BOVISOL (Breeding and management practices of indigenous bovine breeds: Solutions toward a sustainable future) arose as a necessity between the partners (Algeria, Greece, and Tunisia) to preserve these populations by trying to find the best tools that will improve the production systems in terms of productivity and sustainability. The general aim of this project was to contribute to the sustainability of the indigenous bovine breeds' production systems by taking into account the adaptability of the animals to the local environment, the quality of the animal products, and the economic and cultural value of the systems. After all, as also very well documented by [16], it is not an easy task to balance between the values of biodiversity, cultural heritage, and productivity, as these values are differently perceived by the stakeholders in the sector.

There were three specific objectives of this work:

- socio-economic identification of the breeders of the indigenous cattle populations in the different study areas.
- analytical description of the existing farm and breeding practices.
- identification of constraints and proposition of solutions that will promote the sustainability of these production systems in the context of the climate change challenges.

2. Materials and Methods

2.1. Location of the Study Areas, Farmers Sampling, and Data Collection

In each country, the local Data Protection Board (DPB) and the local Ethics Committee have approved experimental protocols. The study involved data collection from different farms, and participants were informed of the purpose of the project; they have given their consent for their participation (complete the survey questionnaire and/or provide a sample of the milk) and the use of data collected and generated for scientific publications.

Study areas concerned regions in the three countries where indigenous cattle breeding and rearing is usually practiced. The collected data comprised a total of 385 questionnaires answered by owners or people who are responsible on the farms for the indigenous or crossbred cattle randomly selected from different villages in the study area. In Greece, the study was carried out in the central and northern Greece regions of Thessaly, Macedonia, and Thrace from March 2018 to May 2019. These study areas are known for their high density of cattle population. Cattle farms were selected taking into account the representation of all major indigenous cattle breeds, farm sizes, and typical Greek geographical conditions. In Tunisia, the study was carried out from January 2019 to June 2021. Surveys were conducted in two regions located in the north and northwestern Tunisia: Sejnan (Bizerte) and Tabarka-Ain Draham (Jendouba). These regions are plains and mountainous areas known for the predominance of indigenous and crossbred indigenous cattle breeding. In Algeria, surveys were conducted in the region of Guelma, Skikda, Annaba, and Bordj Bou Arréridj in northeast of the country from June 2018 to May 2019. The region is characterized by a subhumid climate in the center and in the north and semi-arid in the south. The climate is mild and rainy in winter and hot and dry in summer.

In all three countries, data were collected through direct interviews, using a quasi-structured questionnaire and personal observation at each visit during the study period. The interviewers followed a participatory way, where breeders had been asked to provide demographic information regarding the age, the education level, economic activities, as well as data regarding the livestock management as well as breeding and feeding practices.

2.2. Statistical Analyses

Details on the farms' structure, the breeds, the animals' performances, production systems, and market channels were digitized in spreadsheets (MS EXCEL 2016) separately for each country and coded, entered, corrected, and validated by the research team in accordance to the common format of the three countries before being imported in IBM SPSS Statistics package version 25 (IBM SPSS, 2017). From the 91 initial variables produced from the questionnaires, 17 variables were removed either due to missing data from one or two of the countries (more than 50% missing values in one country) or containing information irrelevant to the present study, mainly because the farmers found it difficult to understand the meaning of the questions. New variables were computed, where necessary, by combining variables from the questionnaires in order to reduce the data presented and to produce clearer results. Analysis was proceeded with quality control, corrections, and further validations. Farms with missing values were removed from the database. The custom tables function was used in IBM SPSS Statistics package version 25 (IBM SPSS, 2017) in order to create tables presenting all the results between the countries, and the created tables were imported in spreadsheets (MS EXCEL 2016) to produce the figures. Additionally, Pearson's chi-squared test was used to determine whether there was a statistically significant difference between the countries regarding the categorical variables and one-way analysis of variance (ANOVA) to determine whether there was a statistically significant difference between the countries regarding the continuous variables. A SWOT analysis was finally carried out relative to the sustainability of the indigenous cattle production systems in the study area, utilizing the data used in the previous parts of the work.

3. Results and Discussion

3.1. Farmers' Socio-Economic Identification

The main characteristics of farmers' identity are reported in Table 1. Results show that indigenous cattle farming is the main occupation for 82.3% of farmers, and 69% of them have a successor in the farm, which is a number that is greater in the North African countries (Table 1). Especially for Greece, the lower proportions of the existence of successors in the farms are a serious constraint factor, since particularly younger individuals reject traditional livestock farming because of the harsh working conditions and the low social status associated with this occupation [17]. The average age of the farmers is 52 years, and most of them are married (89%) with an average of three children.

Table 1. Socio-economic identification of farmers who participated in the survey.

Country	Farmers' Average Age	Married Farmers	Number of Children	Successor in the Farm	Farmers' with More than 20 Years' Experience	Farmers Working Full Time in Livestock
	One-way ANOVA $F(2, 380) = 4.582$, $p < 0.05$	Chi-Square = 14.577; df (2); $p < 0.01$; Cramer's V = 0.195	One-way ANOVA $F(2, 382) = 32.615$, $p < 0.01$	Chi-Square = 17.517; df (2); $p < 0.01$; Cramer's V = 0.213	Chi-Square = 34.770; df (4); $p < 0.01$; Cramer's V = 0.215	Chi-Square = 69.033; df (2); $p < 0.01$; Cramer's V = 0.423
Algeria	55.0	91.4%	4.0	74.3%	66.9%	56.6%
Greece	48.3	79.1%	1.6	51.2%	63.6%	100.0%
Tunisia	53.6	96.4%	2.8	82.0%	41.9%	90.4%
Overall Average	52.6	89.0%	3.2	69.2%	57.5%	82.3%

df: degree of freedom.

An attention-grabbing figure in the current study is the relatively high illiteracy rate, especially in Algeria (39%) and Tunisia (44%). Low literacy is a concept often observed in rural areas in Algeria and Tunisia, and it is partly explained by the farms' location in remote areas, without schools and cultural centers [18,19]. Even in Greece, where 80% of farmers have completed the basic education, only a very small percentage (5%) indicated that they have received some kind of training related to animal breeding. This is quite interesting, since it has been proven that there is a significant positive relationship between the level of farmers' education and the level of productivity [20], while value addition can also be promoted through training and capacity building [21].

In Tunisia and Algeria, more than 70% of the surveyed farmers opt for this profession due to heritage, while in Greece, the principal reason for this choice is the love for the profession (49%). Profit as a reason for practicing the profession varied throughout the three countries: 28% in Greece, 30% in Algeria, and only 5% in Tunisia, which was probably due to the limited productive performances of the indigenous cattle breeds (Chi-square = 275.687; df (18); $p < 0.01$; Cramer's V = 0.600) (Figure 1).

With respect to the reasons for choosing the indigenous versus the commercial breeds, the majority of farmers in Algeria and Tunisia (72.6% and 98.8%, respectively) chose the breeds' adaptation characteristics (Table 2). Moreover, more than 50% of the investigated farmers in the different countries indicated that they chose these breeds because of their productive performances, although there may be a misinterpretation between the performances (quantity of product produced) and robustness of the breeds. In Greece, where there are European and state funding conservation programs, 5% of the farmers mentioned that they are practicing this activity due to the subsidies, which was a relatively low proportion that was expected to be higher. Even though the farmers do not admit it openly, it has been reported that the conservation of indigenous breeds may not be viable without economic support [22].

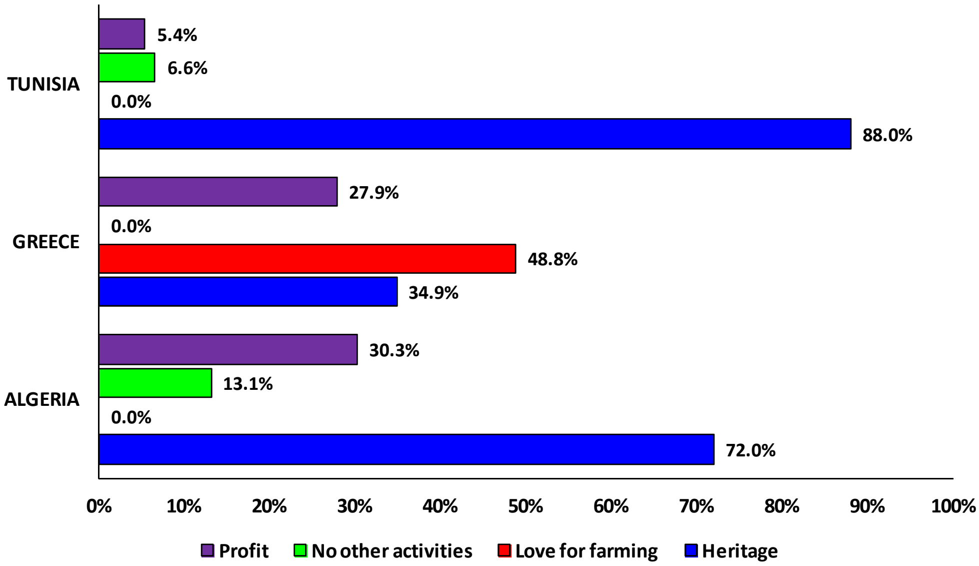

Figure 1. Reasons of practicing indigenous cattle farming in the study area.

Table 2. Reasons of choosing the indigenous cattle breeds (percentage of breeders).

	Adaptation	Performances	Subsidies
Algeria	72.6%	53.7%	0.0%
Greece	20.9%	51.2%	4.7%
Tunisia	98.8%	64.7%	0.0%

3.2. General Characterization of the Indigenous Cattle Production Systems

3.2.1. Farm Size and Type

Table 3 presents the main elements of farm sizing in the three studied countries. In terms of animal population, in Tunisia and Algeria, the farm size is relatively small with an average of four and 14 indigenous cows per farm, while in Greece, the respective figure is much higher (89 animals per farm). This is also depicted in the total farm area as well as the average number of cattle per hectare.

Table 3. Farm structure in the three countries.

		Algeria	Greece	Tunisia
Average cattle number per farm	One-way ANOVA F(2, 381) = 183.688, $p < 0.01$	13.97 (14.62) *	88.90 (72.6) *	3.76 (4.78) *
Total area of the farm (ha)	One-way ANOVA F(2, 336) = 19.801, $p < 0.01$	21.24 (16.17) *	8.85 (13.29) *	12.41 (11.87) *
Cattle per ha	One-way ANOVA F(2, 335) = 79.485, $p < 0.01$	0.72 (0.63) *	15.53 (19.40) *	0.91 (0.89) *
Area cultivated for feedstuffs (ha)	One-way ANOVA F(2, 351) = 14.883, $p < 0.01$	6.11 (5.82) *	12.07 (12.55) *	2.88 (3.26) *
Own cultivated area (%)	One-way ANOVA F(2,312) = 81.738, $p < 0.01$	62.95 (38.30) *	56.63 (37.52) *	100.00 (0.00) *

* Numbers in brackets represent standard deviations of the means.

In all three countries, the farmers cultivate feedstuffs in order to cover the feeding needs of the animals in a more efficient way than just purchasing the necessary feedstuffs from the market. The average cultivated area for feedstuffs is larger in Greece (12.07 ha) and smaller in Algeria and Tunisia (6.11 and 2.88 ha, respectively). In Tunisia, the cultivated area is totally owned by the farmer, while in Algeria and Greece, the farmers own approximately half of the cultivated area and rent the other half from other individuals (Table 3).

Results from the survey showed that relatively small proportions of the farmers reared other cattle breeds in the past, especially in Algeria (25%) and Greece (19%), indicating a close bond between the farmer and the breed (Chi-square = 224.157 ; df (2); $p < 0.01$; Cramer's V = 0.763). Nevertheless, in Tunisia, the interviewed farmers have chosen to crossbreed the indigenous cattle with commercial cattle breeds in an attempt to improve their productivity.

For the three countries (Algeria, Greece, and Tunisia), the indigenous cattle feeding resources are based on rangelands and pastures with concentrate feed complementation when needed during the critical climatic and physiological periods.

In Algeria, in more than 53% of farms, the cattle flocks are never stabled throughout the year (Figure 2). In Greece, the majority of farmers choose to stable the animals during the night (for protection from predators) and in the winter, while in Tunisia, the animals are stabled only at night. This is probably due to the different climate conditions in the two African countries, where there is no need to confine the animals, since the winter is relatively mild in contrast to Greece (Chi-square = 362.927; df (6); $p < 0.01$; Cramer's V = 0.687).

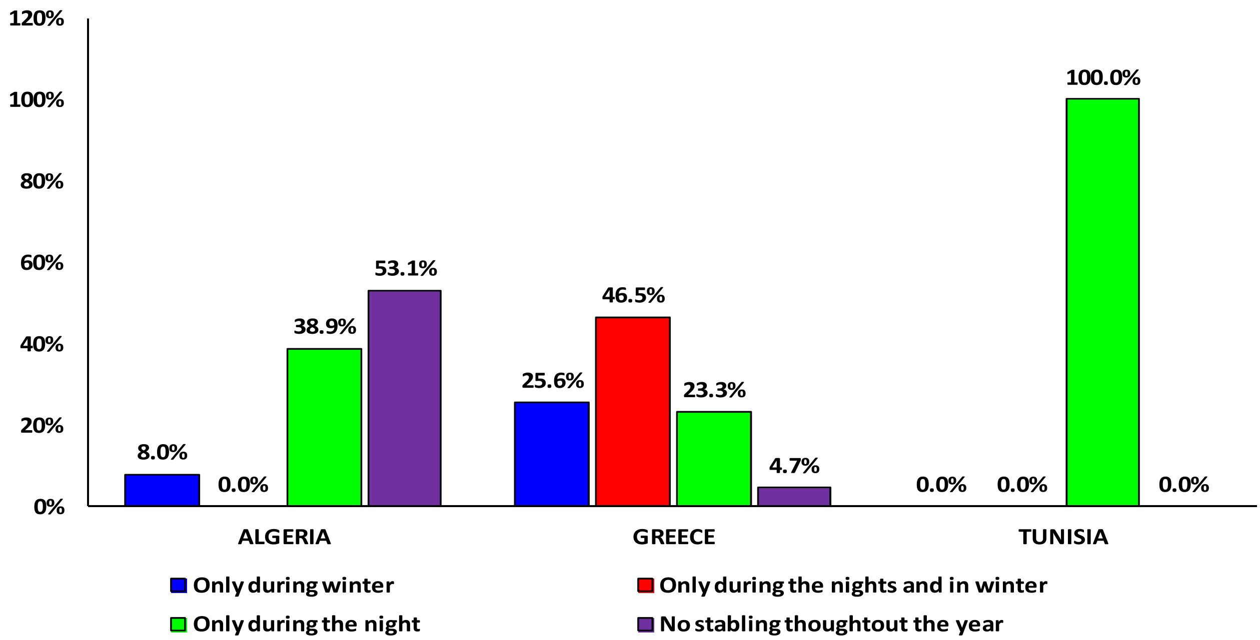

Figure 2. Periods of the indigenous cattle stabling in the different study countries (percentage of breeders).

Two types of cattle housings were found in the different study areas: concrete or built housing and loose housing that is more frequent in both Algeria and Greece (Figures 3 and 4). In Tunisia, two types of stables are used, but in 66.5% of the surveyed farms, local and crossbred cattle are housed in concrete stables, and 33.5% of them are housed in loose housing (Chi-square = 35.812; df (2); $p < 0.01$; Cramer's V = 0.306) (Figure 3).

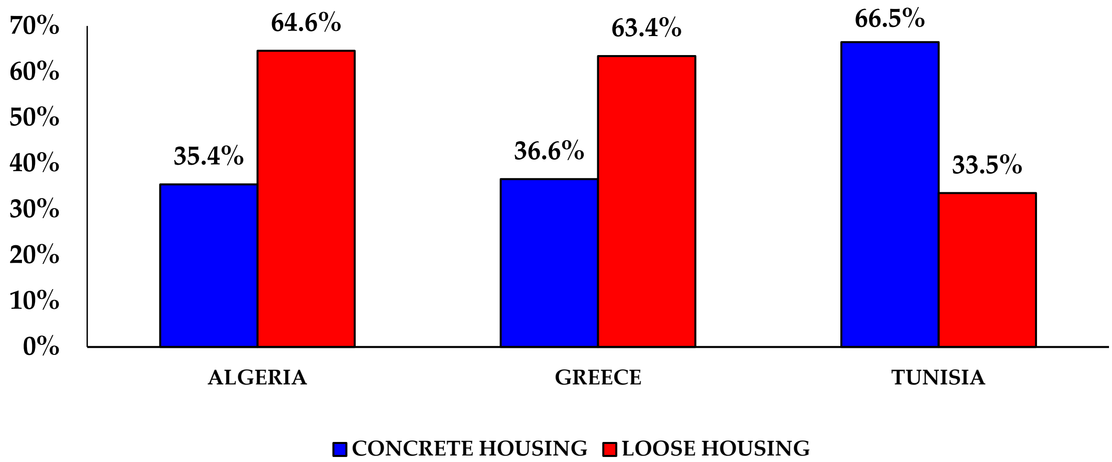

Figure 3. Indigenous cattle housing in the study area (percentage of farms).

Figure 4. Cattle housings: in Tunisia, cattle are housed in concrete stables or in loose housing (**A**–**C**); In Greece, cattle are generally kept in open yard housings (**D**–**F**); In Algeria, cattle housing are generally made of sheet metal and reused wooden planks (**G**–**I**).

3.2.2. Labor Force and Farming Practices

In the study area, farming is carried out almost exclusively by the family members with an average size of 1.1, 1.44, and 2.25, respectively, in Algeria, Greece, and Tunisia. External workers are rarely encountered in the interviewed farms. Daily and seasonal tasks differ from one country to another but some of them are practiced in the same way, such as the daily animals' watering and grazing, which is seasonal in 28% of the Greek interviewed farms and daily in Algeria and Tunisia (Chi-square = 32.914; df (2); $p < 0.01$; Cramer's

V = 0.293). Stables' cleaning is generally a daily activity in Tunisia (100%) and seasonal in Greece (81.3%) and in Algeria (87.4%) (Chi-square = 312.196; df (4); $p < 0.01$; Cramer's V = 0.637). Feed supplementation is seasonal in Tunisia (100%) and Algeria (98.3%) and is not applied in the majority of the Greek visited farms (72%) (Chi-square = 388.251; df (6); $p < 0.01$; Cramer's V = 0.710). Milking is also a seasonal task in both Algeria (72%) and Tunisia (100%); nevertheless, it is not practiced in Greece (94%), since the main direction of the production system is meat production (Chi-square = 272.750; df (4); $p < 0.01$; Cramer's V = 0.595). Dehorning and males castrating are never applied on the Tunisian cattle males, which is not the case in Algeria, where these two tasks are almost seasonal, and in Greece, where they are applied only by few farmers (11.6% and 2.3%, respectively, for dehorning and castrating practices).

As showed in Figure 5, animals' allocation in different groups is more practiced by the Algerian surveyed breeders (50%). In Greece, they just separate pregnant cows (14%) or animals that will be fattened (16%). Nevertheless, in Tunisia, no separation is practiced, which is generally related to the farms' small sizes and then to the limited area and feeding resources dedicated to these animals (Chi-square = 338.062; df (22); $p < 0.01$; Cramer's V = 0.765).

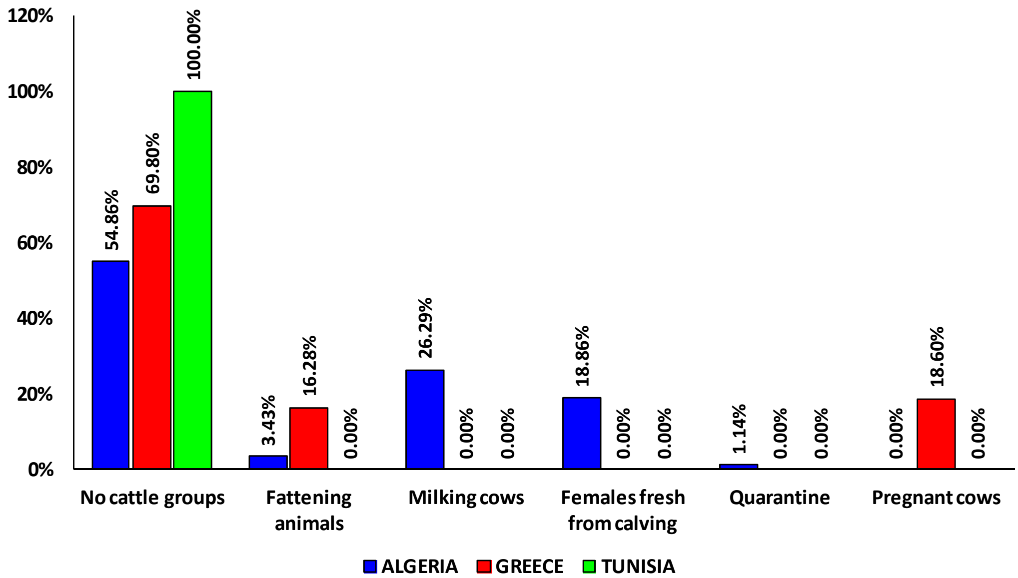

Figure 5. Animal's allotment in the surveyed farms.

Only 17% and 14% of the breeders in Algeria and Greece, respectively, declare that they do not purchase feedstuff to meet their animals' needs. In the other farms, feeding management is based on in-farm produced concentrate (32%) and straw (98%) and purchased by-products (44%), concentrate (38%), and straw (44%) in Algeria. Animals' feeding is based on purchased hay (100%), concentrate (100%), and straw (100%) in Tunisia where the main cultivated feedstuff for this kind of production system is the berseem (*Trifolium alexandrinum*) especially in the larger size farms where irrigation is possible. In Greece, the cultivated feedstuff is relatively diversified since alfalfa, corn, triticale, barley, and peas are cultivated respectively by 24%, 33%, 62%, and 28.6% of the interviewed farmers.

After birth, calves take colostrum directly in all the cases in the different study areas (100%). After that, they continue suckling their mothers in all the Greek and Tunisian farms but only in Algeria, they receive colostrum for 24 h after birth and generally continue to receive milk powder (95%) during a period of two months. The average weaning age is about four months and varies from 197.5 days in Greece to 213 days in Tunisia and to 221 days in Algeria (one-way ANOVA F(2, 377) = 1.572, $p = 0.209$). Weaning is natural in most cases and forced in respectively 9%, 21%, and 19% of the surveyed farms in Algeria, Greece, and Tunisia (Chi-square = 7.688; df (2); $p < 0.005$; Cramer's V = 0.141).

3.2.3. Reproduction and Breeding Management

Reproduction management is basic in the indigenous cattle farms and mainly refers to estrus and pregnancy detection as well as carvings grouping. In Greece, only a few farms use bulls to detect estrus, perform feeding supplementation, and perform calving grouping. In Algeria, almost all the breeders use bulls to detect estrus and also perform pregnancy detection. Nevertheless, in Tunisia, the indigenous and crossbred cattle breeders apply just the estrus detection (Figure 6).

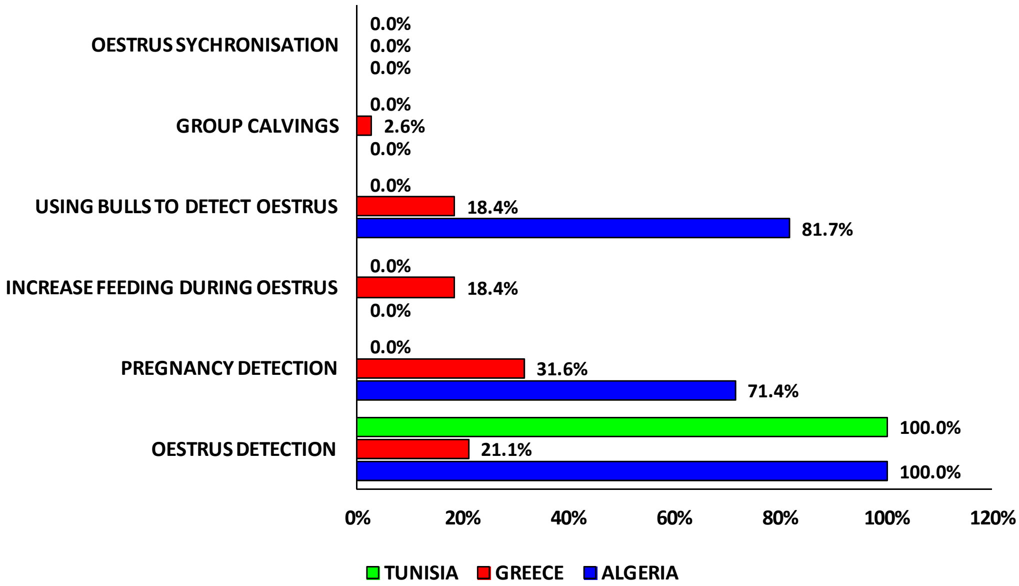

Figure 6. Reproduction practices in the surveyed farms.

As shown in Table 4, in the three countries, females enter into reproduction at an average age of 15 months ± 3.5. The average fertility and abortion rates are, respectively, 81.5% and 2%. Calving interval is relatively large (17.5 ± 5 months).

Table 4. Reproduction parameters in the surveyed farms.

	Age of Heifers' Entry into Breeding (Months)	Fertility Rate (%)	Abortion Rate (%)	Calving Interval (Months)
Mean	14.88 (3.41) *	81.47 (6.51) *	1.85 (3.77) *	17.34 (4.98) *
N	375	203	33	381

* Numbers in brackets represent standard deviations of the means.

The majority of the interviewed breeders in Algeria (86%) and Greece (98%) keep more than 18% of the owned cattle females and males for replacement, which was lower in Tunisia (65%), but with no significant differences between the countries (one-way ANOVA F(2, 364) = 0.920, p = 0.399). Replacement selection criteria are mainly animal phenotype in Tunisia (81%), animal growth performances in Algeria (66%), and parents' performances in Greece (81%), which confirm that the indigenous cattle breeding management and objectives are more or less the same in these three countries (Chi-square = 345.791; df (14); $p < 0.01$; Cramer's V = 0.671).

The basis for every well-designed breeding program is reliable data recording. Unfortunately, in Algeria and Tunisia, data recording in the farm was rarely performed (< 20%), which could be related to the general limited educational level of the indigenous cattle farmers. Nevertheless, in Greece, 78% of the farmers say that they record data on their farms. These data concerned the farm activities (16%), reproduction (84%), health (62.5%), and feedstuff use (91%) in the Greek farms, while in Algeria, only the financial data (100%)

were recorded, and in Tunisia, only information about the farm activities (100%) were recorded. About 62% of the Greek breeders are part of the national performances recording program, and more than 80% of them participate in a genetic resources' conservation program, which is a fact that is rarely seen in the African countries. Nevertheless, in all three countries, the animals' weights are usually estimated visually, and no means of animal identification is used, with the exception of Greece, where all farms use ear tags.

3.2.4. Production Systems Directions and Products' Commercialization

In Greece, the local cattle breeds are farmed exclusively for meat production, while in Algeria and Tunisia, the animals are farmed for both meat and milk production in the majority of farms (80.6% and 92.2%, respectively) (Table 5). This could be partly explained by the low meat and milk performances of these breeds, which oblige the farmers to profit from both the milk and meat performances of these flocks in order to increase their revenue.

Table 5. Production systems' objectives (percentage of breeders).

Objective(s)		Breeding Animals	Meat Production	Milk Production	Meat and Milk Production
Country	Algeria	5.7%	3.4%	10.3%	80.6%
	Greece	0.0%	100.0%	0.0%	0.0%
	Tunisia	0.0%	0.0%	7.8%	92.2%

The lack of a proper selection scheme in smallholder areas results in poor growth rates and possible inbreeding in cattle [23]. In Greece, the average slaughter age of the animals is 18.5 months at an average weight of 309 kg. In Tunisia and Algeria, the respective slaughter age is 17 months and 64 months, respectively, and the average weight is 250 kg and 172 kg, respectively (one-way ANOVA for slaughter age $F(2, 370) = 362.649$, $p < 0.01$; one-way ANOVA for slaughter weight $F(2, 337) = 133.883$, $p < 0.01$).

Periods of animals' commercialization differ from one country to another. In Algeria and Greece, most of the fattened indigenous cattle are commercialized occasionally throughout the year, while in Tunisia, all the interviewed breeders affirm that they sell these animals during the summer, especially for wedding occasions. In Algeria and Greece, 20% of the breeders have sales contracts for animals or products with butchers (94% of the Algerian breeders) or with the animal traders (85% of the Greek breeders) which is not the case in Tunisia, where selling practices are carried out privately between the breeders and their clients (consumers or animal traders) or in the local markets (Chi-square = 560.320; df (10); $p < 0.01$; Cramer's V = 0.916).

Products certification is only encountered in 11.6% of the Greek farms, even though all the interviewed breeders in the three countries believe that their product is of higher quality. In addition, only 17% of the Greek breeders affirmed that the products' prices are affected by their quality. Finally, in Algeria and Tunisia, breeders of the indigenous cattle do not belong to any organizational structure and do not practice any associative activity. Conversely, in Greece, 26.3% of the breeders are part of cooperatives that enable beneficiating from the technical consulting when needed from the cooperative, private, or public entities.

3.3. Production Systems Constraints and Improvement Ways

The limiting constraints to the indigenous cattle production systems encountered in Algeria, Greece, and Tunisia are presented in Figure 7, as perceived by the surveyed farmers. All the farmers agreed that a major constraint of the production system is the increased feeding cost (Chi-square = 73.749; df (2); $p < 0.01$; Cramer's V = 0.916). In Algeria and Tunisia, another major constraint is the low productivity of the animals (Chi-square = 298.615; df (2); $p < 0.01$; Cramer's V = 0.884), while both Greek and Tunisian farmers agree that the selling prices are low (Chi-square = 284.436; df (2); $p < 0.01$; Cramer's V = 0.863). The difficult management of the animals is moderately discussed by Greek and Algerian farmers

(Chi-square = 108.588; df (2); $p < 0.01$; Cramer's V = 0.533), while the Tunisian ones care more about the other costs of the production system (Chi-square = 152.661; df (2); $p < 0.01$; Cramer's V = 0.632) as well as for the lack of rules in the market (Chi-square = 251.980; df (2); $p < 0.01$; Cramer's V = 0.812).

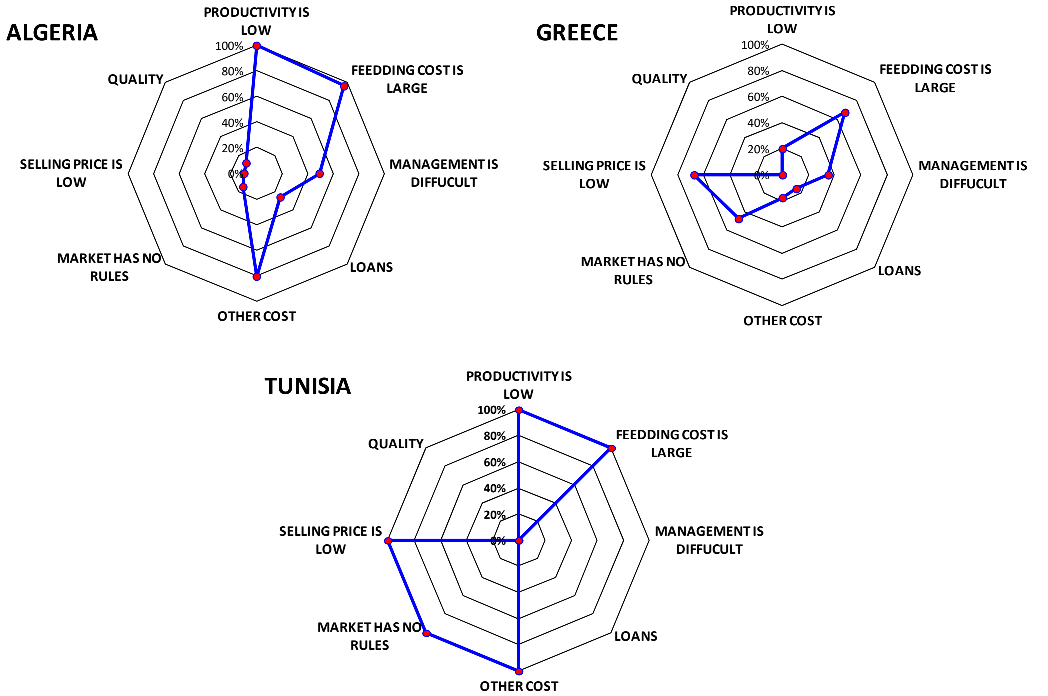

Figure 7. Limiting constraints in the studied production systems.

Figure 8 presents the farmers opinions regarding what actions could improve the outcome of the production systems. All farmers agree that higher selling prices (Chi-square = 45.103; df (2); $p < 0.01$; Cramer's V = 0.343) and state funding (Chi-square = 73.896; df (2); $p < 0.01$; Cramer's V = 0.439) would be two major steps in the right direction. In Greece, product certification (Chi-square = 101.108; df (2); $p < 0.01$; Cramer's V = 0.513) and advertisement (Chi-square = 68.196; df (2); $p < 0.01$; Cramer's V = 0.421) are also proposed as solutions while in all the countries, the genetic improvement of animals is moderately appreciated (Chi-square = 100.123; df (2); $p < 0.01$; Cramer's V = 0.511).

3.4. Opportunities toward Sustainable Indigenous Cattle Production Systems

Both in Europe and North Africa, indigenous cattle breeds are an essential supplier of food, agricultural power, agrarian culture and heritage, and genetic biodiversity [23]. Indeed, animal genetic diversity allows farmers to set up selection programs in collaboration with the specialized services or to develop new breeds in response to the continuously varying conditions associated to climate change, new or growing disease dangers, new knowledge of human nutritional requirements, and fluctuating market conditions or changing societal needs. It is important to develop concerted, coordinated, and comprehensive farmer training, research, and development programs to address these constraints for the breeders of the indigenous cattle populations that developed their own behavior to adapt with the unstable environmental and economic conditions. An integrated approach with due consideration to proper feeding, breeding, healthcare, and improved management practices are recommended to address the future challenges for sustainable conservation of these native breeds [24]. Subsequently, developing this sector needs both state interventions and adequate farmers' behaviors and activities to be implemented and applied.

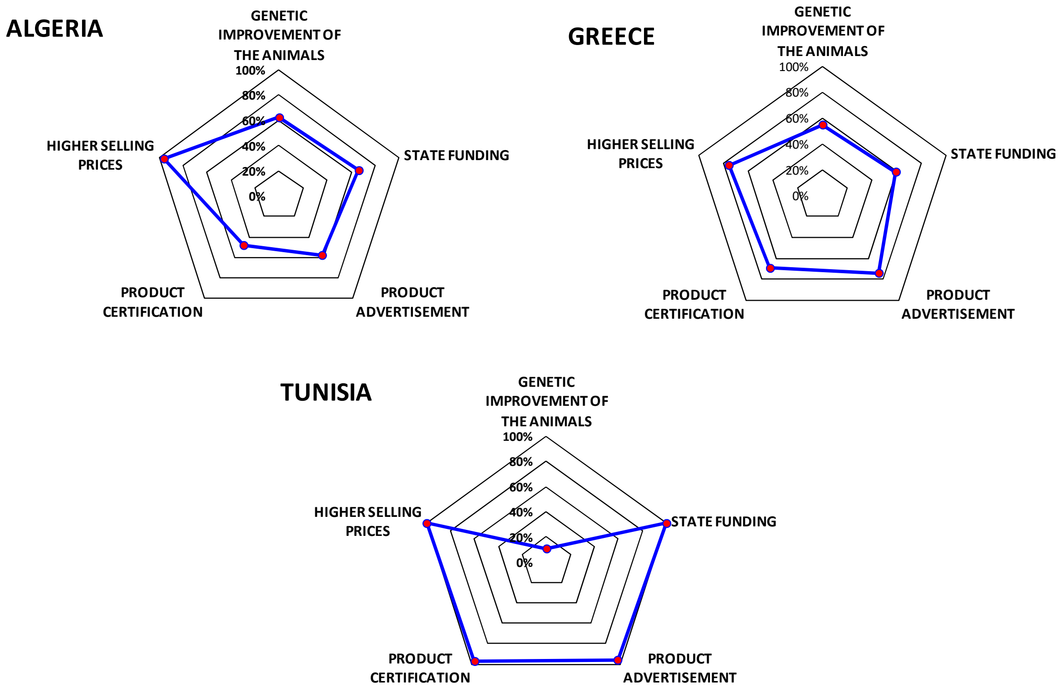

Figure 8. Farmers suggested improvement ways of the studied production systems.

3.4.1. State Funding

The agricultural policies in general lacked the necessary stakeholder support, both financial and moral, or commitment [25], which is also clear by the small involvement of the state especially in Tunisia and Algeria, where 100% and 66.3%, respectively, of the interviewed farmers stated the need of improvement in the form of state funding. Then, the state must have the leading role in the conservation of animal genetic resources; preserving their legacy for the future generations as a safety belt for the ever-changing environmental conditions is for the greater good. Under this context, it is necessary that the state funds such actions as well as supports financially the farmers that participate in such programs. Nevertheless, the safeguarding of the indigenous cattle breeds through programs and funding is mandatory to include all the relevant actors and foremost the farmers that must actively participate in all the steps. The tools provided should focus not only on conserving the breeds but also improving the management and productivity of the farms as well as preserving the characteristics of the local production systems while implementing current and future innovations. The key action that will ensure the survival of the production systems and the breeds is to find the proper balance between tradition and innovation. The indigenous breeds are highly connected to the local geographical, social, cultural, and economic conditions, and it is in the hands of the state and all the relevant actors to highlight and promote them to future generations.

3.4.2. Genetic Improvement of the Animals

The conservation of indigenous cattle breeds is critical for reversing the unprecedented loss of diversity and ensuring the security of cattle genetic resources for economic, ecological, and social benefits [26]. Genetic improvement programs must be organized and supervised by the state but implemented by farmers through collective organs. The genetic improvement of animals is a long process with additive results that usually takes years to be quantified and standardized and is important to be treated as such. In the case of indigenous breeds, the main goals would be the following:

- to clearly characterize the breeds and, in cases of crossbred populations,
- to stabilize the breeds,

- to take advantage of their close connection to the local environment and improve their adaptation characteristics, identified, especially in Algeria and Tunisia, as the main reason for choosing the indigenous breeds (72.6% and 98.8%, respectively), and
- to improve their productivity, which was reported by all farmers (100%) as a limiting factor in Algeria and Tunisia, without compromising their unique characteristics. In order to successfully implement genetic improvement programs, the active participation of all the famers of each breed is mandatory in order to have a complete register of the animals, along with accurate recording of their characteristics, performance, and environmental parameters. The responsible state bodies should supervise, coordinate, and control the peripheral actions taken by individual farmers and their collective organs.

An urgent need to establish a conservation plan that includes a well-designed genetic management program for the Tunisian indigenous cattle population was already underlined by [27,28]. This was not expressed by Tunisian breeders (10.8%) that are unaware of the effect of uncontrolled crossbreeding that could easily result in the extinction of the breeds and lack of programs, and when asked about the possible improvement ways, they do not mention the animals' genetic improvement in contrary to farmers in Algeria (62.3%) or in Greece (54.8%). It is important to mention that only farmers in Greece participate in a genetic resources conservation program (80% of the interviewed farmers) and performances recording program (62% of the interviewed farmers).

3.5. Farmers' Behaviors and Activities

3.5.1. Farmers Professional Organization

As presented in the results, few farmers in Greece (26.3%) and no farmers in Algeria and Tunisia are part of organizational or professional group. Motivation and sensitization of the breeders to join breeder's cooperatives or associations and farmers training to transform indigenous cattle milk into cheese or other milk derivatives are vital.

3.5.2. Animal Management Practices

As presented in the results, the management practices of indigenous breeds are far from being the optimum for the animals, which is partly due to the traditional character of the production systems. It is indicative that most farmers (96.6% in Algeria, 67.5% in Greece, and 100% in Tunisia) chose feeding costs as a limiting constraint, leaving room for improvement. Additionally, important reproduction practices are not performed in all three countries, with Tunisian farmers only performing estrus detection (100%), a small proportion of Greek farmers (less than 32%) performing increase feeding during estrus and estrus and pregnancy detection, and most of the Algerian farmers performing pregnancy detection (71.4%) and estrus detection (100%). Finally, most of the farmers in all three countries do not allocate animals in different groups (54.8% in Algeria, 69.8% in Greece, and 100% in Tunisia). Thus, small changes in the management practices, without altering the identity of the production systems, could have a great impact on the productivity and the overall welfare of the animals. The changes that are more easily applied, according to the information collected through the questionnaires, are the following:

(a) Improvement of the feeding of the animals with an overall goal to cover the needs of the animal at every production stage with a focus on young animals, females in gestation, and fattening cattle;

(b) Booster feeding before mating could improve the reproduction parameters of the animals;

(c) Control the females' insemination and natural mating;

(d) Better management of newly born calves in terms of feeding and hygiene in order to decrease infant mortality;

(e) Grouping of the animals in critical periods of their life such as gestation and calving.

3.5.3. Products Marketing, Certification, and Advertising

It is a common belief among farmers and consumers that the products of indigenous breeds are of high quality, but for most breeds, there is very little evidence that can support such a claim. However, the marketing of these products remains fragmented, as this sector is unsatisfactorily organized. Product certification and product advertisement were suggested as a way of improvement from farmers in all three countries (48.0% and 57.7%, respectively, in Algeria, 69% and 73.8% in Greece, 97% and 95.8% in Tunisia) Thus, it would be for the benefit of all the stakeholders to participate and, if possible, co-fund research projects that would evaluate the quality of the products and promote their unique characteristic and quality. Moreover, as the next step, the farmers could use the results of such projects in order to advertise their products and promote their advantages to the consumers. This would subsequently increase the demand for the products and create a brand name that would be recognizable and desirable by the consumer. As a result, this would also improve the selling prices for the products and the overall income of the farmer. The market promotion of these products will help to incorporate them into a profitable value chain. In this context, more milk and meat quality studies have to be carried out before studying the possibilities of certifying these local products (AOC, IG, AOP, IGP) and labeling, taking into account the consumers' expectations and preferences.

Table 6 presents a SWOT analysis related with the sustainability of the indigenous cattle production systems in the study area, utilizing the data used previously and results and discussion that preceded.

Table 6. SWOT analysis of the indigenous bovine production systems toward a sustainable development in Algeria, Greece, and Tunisia.

	Strengths	**Weaknesses**
✓ Economic axis	• Indigenous cattle activity is a source of income • Lower dependency on external inputs in marginalized areas	• Absence of financial support to small farmers • Low products' selling prices • Absence of products' certification • Management practices are not optimized
✓ Social axis	• Breeders attachment to continue practicing this activity • Inherited or/and own investment activity	• Low social value of this activity • Farmers aging • Absence of farmers' training in rearing and farm management techniques
✓ Environmental axis	• Valuable genetic pool adapted to local and harsh environment • Genetic resources conservation programs	• Problems related to the use of common lands • Over-use of grazing lands • The increase in the inbreeding level • Difficult to monitor animals that graze in far and difficult to access rangelands
	Opportunities	**Threats**
✓ Economic axis	• Distinction of specific products issued from these production systems (milk and meat) • Possibilities of breeders associations' creation and products' prices increase	• Large increase in animals feed • Higher external inputs' prices • Lower profitability of this activity
✓ Social axis	• Increasing human populations in the marginalized areas • Increasing the employment rate in these zones • Reducing the rural exodus • Consumers' demand for specific zones' products	• High rate of abandonment of this livestock activity • Guidance of farmers to other activities
✓ Environmental axis	• Considering the eco-systems particularities to advertise the products' quality	• Local cattle breeds are in danger of extinction • Higher level of inbreeding rate

4. Conclusions

The results of the current study show that although smallholder indigenous cattle populations in Algeria, Tunisia, and Greece still exist, their productivity is limited by several constraints that include low performances, limited feed availability, and poor marketing. In addition, the erosion of indigenous cattle populations genetic resources is becoming a serious problem especially in Tunisia. The conservation of these cattle genetic resources could be imperative, as these have been shown to be a useful integral part of agro ecosystems in smallholder areas. The reasons for conserving these flocks vary from their current utilization to the ability to meet future challenges in a dynamic environment. There is a big policy gap in the studied countries, especially Tunisia and Algeria, with regard to the genetic conservation programs. The costs of conservation activities can be met by increasing the market value of indigenous cattle products so that they eventually become self-sustaining. This requires the identification of the beef breeds, their characterization, and the development of marketable products from these breeds. There is a factual necessity to apply breed conservation strategies through securing long-term funding, revamping institutional activities, training technical personnel, and the co-ordination of management efforts, which will promote the conservation of the indigenous cattle populations and improve the sustainable development of these production systems.

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