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# Critical evaluation of hazardous pollutants in edible insects: A simple review

## Diana Amorello<sup>a</sup>, Salvatore Barreca <sup>b,\*</sup>, Silvia Orecchio<sup>a</sup>

<sup>a</sup> *Department of Science and Technology Biological, Chemical and Pharmaceutical University of Palermo, Viale Delle Scienze, Ed. 17, Palermo 90100, Italy* <sup>b</sup> *Department of Chemical Sciences, University of Catania, Viale Andrea Doria 6, Catania 95125, Italy*



potential health risks associated with their consumption.

### **1. Introduction**

On average, one third of the world's population, especially those living in Africa, Asia and South America (Raheem et al.,2018; Raheem et al 2019; Hlongwane et al., 2020), have traditionally use many insect species in their diet. This practice is mainly driven by the lack of other readily available protein sources (Raheem et al., 2018). This food integration is possible because the nutritional characteristics of insects are similar to those of many traditional foods (meat, fish, etc.) (Barreca et al., 2023). Another advantage of using insects is that their production time is generally limited to a few weeks compared to several months needed to produce other types of protein food (Oonincx et al., 2019). For example, a few months are required for chickens, six months for pigs and two years for cattle (Huis et al., 2013; Orkusz et al., 2021). Consequently, the use of insects is advantageous from the perspectives of environmental protection, particularly regarding the production of ecological processes such as pollination, greenhouse gases and the consumption of water and soil. Recently, some species of edible insects have been classified as novel foods in the European Union. Until the end of 2022, the production of food containing insects or parts of them was subject to Regulation (EU) 2015/2283. Since the beginning of 2023, the European Commission (Gazzetta Ufficiale Repubblica Italiana 2023) has authorised the use of many insect species such as the house cricket (*Acheta domesticus*), the yellow mealworm (*Tenebrio molitor*), the migratory locust (*Locusta migatoria*) and the lesser buffalo mealworm (*Alphitobius diaperino*) (European Commission, 2023). However, it must be taken into account that the nutritional characteristics of the aforementioned species are highly variable, not only between the different species but also depending on the metamorphic development, diet, habitat and production techniques (Huis et al., 2013; Mlcek et al., 2021a; Mlcek et al., 2021b).

It is known that many toxicologically hazardous substances can accumulate in different environmental (Orecchio et al., 2022) and food matrices (Orecchio et al., 2009; Orecchio et al., 2019a; Orecchio et al., 2019b; Amorello et al., 2023; Barreca et al., 2023) and, in particular, in plant and animal tissues (Gianguzza et al., 2006; Culotta et al., 2008).

These substances can enter organisms through the diet, especially if they are fed with waste materials or from environmental matrices, among other sources. Generally, most of these substances can be classified as metals and persistent organic pollutants such as polybrominated diphenyl ethers, polybrominated diphenyl ethers (PBDEs) some pesticides and other pollutants (Amorello et al., 2022). In this context, it is necessary to pay attention to the assessment of the health risk posed by pollutants ingested through insects. In detail, similarly to

\* Corresponding author. *E-mail address:* salvatore.barreca@unict.it (S. Barreca).

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other animal products, insects can accumulate hazardous chemicals (Truzzi et al., 2019), such as heavy metals (Lindqvist et al., 1992; Zhuang et al., 2009; Bednarska et al., 2012), dioxins (Devkota and Schmidt., 2000) and flame retardants (Gaylor et al., 2012; Poma et al., 2017).

This review analyses the major contaminants in insects used in the food industry over the last twenty years using Google Scholar and Scopus database, resulting in the selection of 105 significant papers for consideration.

### **2. Chemical pollutants**

### *2.1. Metals*

Metals play a crucial role in the functioning of both plants and animals with micro and macro elements being essential for normal physiological processes (Malematja et al., 2023). Mineral nutrients can be classified into major, secondary and micro or trace minerals (Barreca et al., 2023; Orecchio et al., 2014). Macro elements such as calcium, chlorine, magnesium, potassium, phosphorus, sulphur and sodium are required in amounts greater than 100 mg day $^{-1}$ , while micro elements such as chromium, copper, fluorine, iron, iodine, manganese, silicon, and zinc are essential in smaller quantities, less than 100 mg day $^{-1}$ . Ultra trace elements like arsenic, boron, molybdenum, nickel, selenium, and vanadium are required in even smaller amounts, less than 50 ng day<sup>-1</sup>. Other metals (Ba, Br, Cd, Li, Pb, etc.) contribute to biological processes but are not been essential (Senila et al., 2023). Non-nutritive metals such as Al, Bi, Ga, Au, Hg and Ag have no known function, can be detected as contaminants in food matrices and cause toxic effects. Several trace metals are not metabolised into other intermediate compounds, which makes it difficult for them to be broken down in the body (EFSA Scientific Committee, 2015; Mlˇcek et al., 2017; Rehman et al., 2021). Furthermore, deficiencies or excesses of metals can lead to significant health issues (Bailey et al., 2010; Gao et al., 2019; Barreca et al., 2023). In this context, monitoring the intake of micro and macro elements is crucial for determining appropriate nutrient recommendations (Bailey et al., 2010) and, for this reason, element intake levels for several people are documented (Ysart et al., 1999; Radwan and Salama., 2006; Beccaloni et al., 2013; Orecchio et al., 2015).

Metals taken through diet or other ways by organisms are chemically complexed in various subcellular compartments. Indeed, some types of complexes are more stable than others, and invertebrates has sequestration mechanisms to detoxify metals and prevent their interaction with vital biomolecules. These mechanisms include binding the metal to heatstable and heat-denatured proteins, lysosomes, mitochondria and other ligands, and storing the metal in inorganic granules (Wallace et al., 2003a; Wallace and Luoma., 2003b, Rosabal et al., 2012; Ding et al., 2013; Bednarska Agnieszka and Świątek., 2016). Recently, the Italian Ministry of Agriculture, Food Sovereignty and Forestry (GURI 29/12/2023), regulated the production of foods and food preparations for human consumption, obtained by using the use of partially defatted house cricket (*Acheta domesticus)* powder, yellow mealworm (*Tenebrio molitor larva*), locust (*Locusta migratoria)* and buffalo mealworm (*Alphitobius diaperinus)* larvae (lesser mealworm) frozen, dried or in powder form.

#### *2.2. Polybrominated diphenyl ethers*

Polybrominated diphenyl ethers (PBDEs) are a class of persistent organic pollutants (POPs) that includes more than 200 congeners (Gaylor et al., 2012). In industrial applications, three types of commercial mixtures of PBDEs are generally used as penta, octa and deca-BDE. These compounds have generally been used as flame retardants in various production sectors including electronics, building materials, textiles, etc. (Abbasi et al., 2019). Penta-BDE is the most bio accumulate and toxic and has been listed as a persistent organic

pollutant under the Stockholm Convention. Penta-BDE has been used primarily as a flame retardant in polyurethane foam products, which are widely used in furniture upholstery and in the automotive sector for carpet underlay and in many other applications (Alcock et al., 2003; Polyurethane Foam Association, 2011). Considering that the aforementioned compounds do not form covalent bonds with the polymer matrices to which they are added, they can easily be released into the environment and/or fed into matrices not only with which they come into contact (Guo et al., 2009) but are also transported over long distances, resulting in almost ubiquitous environmental contaminants (Abbasi et al., 2019). Due to their lipophilic properties, PBDEs tend to accumulate in fatty matrices. High concentrations of PBDEs have been found in human samples, including breast milk, serum, placenta, adipose tissue and other tissues (Covaci et al., 2008a; Covaci et al.,2008b; Leung et al., 2010. Some studies (Zheng et al., 2017) have highlighted that PBDEs are endocrine disrupting molecules and their exposure in the body of animals and humans can cause health problems.

### *2.3. Benalaxyl*

Benalaxyl (Wang et al., 2017) is the common name of the unresolved isomeric mixture of methyl N-(phenylacetyl)-N-(2,6-xylyl)-D-alaninate (EFSA, 2013). Benalaxyl is a systemic pesticide product with a protective, curative and eradicative activity, which is commonly used in the production of many plant foods (grapes, tomatoes, potatoes, peppers, onions, tobacco and soya) to control of diseases caused by Oomycetes (Rosso et al., 2000). Benalaxyl molecules have an asymmetric centre and consist of two enantiomers. It is known that only the R enantiomer has physiological activity (Qiu et al., 2007). Differences in degradation between the two enantiomers were observed in several vegetable foods, soil, rabbit plasma, liver microsomes from rat and rabbit, and freshwater alga Scenedesmus obliquus.

### *2.4. 2,4,6-trinitrotoluene*

2,4,6-trinitrotoluene, also known as tritol, abbreviated to TNT, is an aromatic nitro derivative obtained from toluene by nitration. It is highly explosive and is used in the manufacture of explosive mixtures mixed with ammonium nitrate. The United States Environmental Protection Agency (EPA) considers TNT to be toxic to humans, mutagenic and possibly carcinogenic to humans (EPA). A recommended lifetime health advisory of 2 µg/L of TNT in drinking water has been established (Lee et al., 2007; Meyers et al., 2007).

TNT in soils, through chemical, biological and physical processes, gives rise to variety toxic by-products which can transfer into different environmental matrices. In surface waters, TNT is rapidly degraded by sunlight, whereas degradation groundwater and sediments is less in rapid. Biological degradation, both under aerobic and anaerobic conditions, gives rise to the formation of 2,4-dinitrotoluene (2,4-DNT), 2 amino-4,6-dinitrotoluene (2A-DNT) and 4-amino-2, 6-dinitrotoluene (4A-DNT) (Esteve-Núñez et al., 2001).

### *2.5. Pesticides*

The generic term pesticide refers to several different chemical compounds used for different purposes: herbicide, insecticide, fungicide, ovacide, acaricide, veterinary materials, nematicide, etc. to protect crops from damage caused by weeds, plant diseases and insects. It is known that pesticides can lead to an improvement in the quantity and quality of agricultural food production (Ahmadi et al., 2024), and could therefore improve the quality of human life by increasing food production and ensuring its availability for human consumption.

Considering the chemical composition, their classification is rather complex. Indeed, they are practically divided into four main groups; organochlorines, organophosphorus, carbamates, pyrethrins and pyrethroids. In general, modern pesticides are organic chemicals, including synthetic and plant origin compounds. However, some inorganic compound is also used as pesticides. Insecticides are an important class of pesticides, which can be further classified into several subclasses.

It has been estimated that almost one third of agricultural products are produced using pesticides (Ahmadi et al., 2024). Chronic exposure to pesticides can lead to several health problems such as an increased risk of cancer, neurological disorders, Parkinson's disease, etc. The World Health Organization (WHO) estimates that approximately one million people suffer from pesticide poisoning every year (Ahmadi et al., 2024). Exposure to pesticides may be responsible for 70 % of these deaths.

### *2.6. Polybrominated diphenyl ethers*

Polybrominated diphenyl ethers (PBDEs) are a class of organobromine compounds generally used as flame retardants. Structurally, they are similar to polychlorinated diphenyl ethers (PCDEs), Polychlorinated biphenyls (PCBs) and other polyhalogenated compounds consist of two halogenated aromatic rings. The life-saving benefits of flame retardants have resulted in their presence in all environmental and food matrices. Due to their chemical structure, PBDEs are very stable and persist in various environmental matrices for many years. Being very lipophilic and not easily degradable (metabolized), they tend to accumulate in the fatty part of organisms, giving rise to the phenomenon of bioaccumulation. The content of contaminants is transferred along the food chain (plant-herbivore-carnivore) so that the concentration of these compounds increases going from a lower to a higher level of the trophic chain, giving rise to the phenomenon of biomagnification. This phenomenon implies that an organism occupying a higher level in the food chain may have higher internal levels of contaminants.

### **3. Edible insects**

### *3.1. Tenebrio molitor (Linnaeus, 1758)*

Tenebrio molitor, commonly known as the yellow mealworm (Coleoptera: Tenebrionidae), is a beetle of the Tenebrionidae family. It is a native species of Europe, although it is now widespread throughout the world, with a preference for a temperate areas and the northern hemisphere, but is incapable of reproducing in the tropics. In recent years, it has become increasingly sought after as a favourable source of protein font for animal feed and food products (Li et al., 2013; Siemianowska et al., 2013). The species has a short life cycle and is very efficient method to bio-convert organic waste to animal proteins (Ramos-Elorduy et al., 2002; Van Broekhoven et al., 2015). The larvae and adults are typically nurtured on wheat bran, or flour, with supplementation of soybean flour, skimmed milk powder or yeast, fruits, etc. (Makkar et al., 2014). T*enebrio molitor L*., as it is a common and efficient species for the production of insect protein, and it is also used for both animal and human consumption (Vera et al., 2012; Li et al., 2013; Rumpold and Schluter., 2013; Houbraken et al., 2016).

### *3.2. Locusta migratoria (Linnaeus, 1758)*

It is the most widespread locust species and the only species in the Locusta genus. It natural habitat is in Africa, Asia, Australia and New Zealand. It was also found in Europe but is now become rare. There are numerous subspecies of the migratory locust, although not all researchers agree on the validity of some of these subspecies. The migratory locust is an insect that can be used as food. In Europe, such use was initially authorized in Switzerland (May 2017). In July 2021, the European Food Safety Agency (EFSA, 2021) published a scientific opinion stating that consumption of the aforementioned frozen, dried or ground insect is safe for humans (EFSA, 2021): Safety of frozen and dried formulations from migratory locusts (*Locusta migratoria*) as a novel food under to Regulation (EU) 2015/2283 (Regulation (EU) 2015/2283). On November 2021, the Member States of the European Union authorised

the EU Commission to allow the placing on the market of migratory locusts as food (European Commission, 2021).

### *3.3. Acheta domesticus (Linnaeus, 1758)*

*Acheta domesticus* (Linnaeus, 1758) or house cricket or hearth cricket is an orthopteran insect of the family Gryllidae, probably native to south-western Asia and imported to America in the 18th century. It is an omnivorous insect. Its diet consists mainly of vegetables, fruits, cereals and small nocturnal insects. The production efficiency of *Acheta domesticus* is very advantageous because 2.1 kg of dry feed are needed to produce 1 kg of product. In comparison, 4, 5, 9 and 25 kg of feed are required to produce 1 kg of edible product from poultry, pork and beef respectively (van Huis et al., 2013).

### *3.4. Alphitobius diaperinus*

*Alphitobius diaperinus* is a beetle in the Tenebrionidae family. It is better known as the lesser mealworm or litter beetle. It is found in almost all everywhere in the world. It is also known to be a harmful organism, because it is a vector of many types of pathogens, present in many cereal-based preserved food products and in chicken farms. The insect can feed on a variety of materials, including waste, bird droppings and bat guano, mold, feathers, eggs (including other insects) and carrion. It commonly feeds on sick or weakened live animals. The adult female usually lays 200–400 eggs, but she has been known to produce up to 2000.

On 4 July 2022, EFSA published an opinion confirming the safety of frozen and freeze-dried larvae of *Alphitobius diaperinus* for human consumption. (EFSA, 2022). Approval as novel food in the European Union followed on 6 January 2023 with the EU commission's publication of Implementing Regulation 2023/58 authorising the placing on the market of the frozen, paste, dried and powdered forms of *Alphitobius diaperinus* larvae. (European Commission, 2023).

#### **4. Pollutant levels in insects**

In 2003, Dutch researchers (Vijver et al., 2003) carried out some biological tests to determine the degree of absorption of Cd, Cu, Pb and Zn by *Tenebrio molitor* larvae using different soil types. The insect larvae (8–10 weeks old) used in this research were in their second stage of moulting. Specifically, the tests were performed using 13 natural soils, one soil spiked with analytes, four mixtures of soil and sediment and one artificial soil prepared according to Organization for Economic Co-operation and Development OECD., 1984) enriched with Cd or Zn. Body concentrations of Cu and Zn in the zinc-enriched OECD soils, field soils and soil-sediment mixtures persisted mostly constant. Significant variation was observed for all insects grown in natural soils and soil-sediment mixtures. For Cd-enriched and OECD soils, body concentrations increased almost linearly over time. In the case of cadmium and lead, non-essential elements, the concentrations in the larval bodies were mainly related to the total metal concentration in the soil. Cd sorption at similar total Cd concentrations was in the same range between metal-spiked soils, natural soils, and mixtures. Comparison of the results of the aforementioned authors with studies on other species highlighted that metal uptake patterns depend on the type of metal, the type of soil and the exposed species. The authors conclude that soil organisms can be classified based on the difference in ecophysiological characteristics, determined, for example, by the (non)permeability of the external integument (Table 1).

Concentrations of Hg, Cd and Pb in plants and *Migratory locust* from a Chinese province were quantified (Zhang et al., 2009; Zhong et al., 2009; Zhang et al., 2010; Zhang et al., 2012) and correlated with those of the soil to determine the biogeochemical processes of possible accumulation in the food chain. The concentration factors for the transfer soil-plant-herbivorous insect-carnivorous insect food chain were 0.18,

#### **Table 1**





6.57 and 7.88 for mercury, 6.82, 2.01 and 0.48 for cadmium, 1.47, 2.24 and 0.57 for lead. From the research results Hg was biomagnified to a greater extent, but Cd and Pb did not accumulate in carnivorous insects, as expected, since the food chain extends to secondary consumers. The results, as already seen previously, indicated that the concentration factors in the food chain were dependent on the type of element and the insect species. As an example, the average biomagnification factors (BMFs) of Hg, Pb and Cd for the *Migratory Locust* feed with A. chinensis–Mantis are 12.5, 4.7 and 0.7 respectively.

A study published in 2010, conducted by (Zhang et al., 2010) reported the findings on the distribution and bioaccumulation of total mercury (T Hg) in the soil-plant-grasshopper system relating to the city of Huludao (China), a highly polluted industrial area due to the presence of an industrial plant for the production of chlor-alkali and two smelters for the production of zinc. In *L. migratoria manilensis* the total mercury concentrations ranged from 0.015 to 0.346 mg  $kg^{-1}$  with an average of 0.119 mg kg<sup>-1</sup> (Table 2). Total mercury concentrations in soil, plant leaves, *Locusta migratoria manilensis*, *Acrida chinensis* and *spider* were 0.151, 0.119, 0.167 and 0.134 mg  $\text{kg}^{-1}$ , respectively (Table 2). Analysing the data according to the area involved in this research, the authors concluded that most of the element originates from the chlor-alkali plant and two zinc smelters. The highest concentration of Hg was found in the wings and organs of the insects studied. Although spiders are predators, the total amount of mercury in their bodies was not high and was similar to that quantified in grasshoppers, probably due to the particular lifestyle habits of spiders. Considering the soil-plant-grasshopper-spider food chain, the bioaccumulation factors were 0.03, 0.79–1.11 and 0.80–1.13, respectively. These results suggest that the biomagnification of mercury in terrestrial food chains is not as pronounced as in aquatic food chains (Hyun et al., 2012). From the results of experiments (Gaylor et al., 2012), concerning polybrominated

diphenyl ethers (PBDEs), some American researchers concluded that house crickets (*Acheta domesticus*) fed for 28 days with uncontaminated food and raised in containers containing small pieces of polyurethane foam (PUF), used in the packaging of fragile goods and containing Penta-BDE (8.7 % in dry weight), accumulate non-negligible amounts of this compound (13.4 mg kg<sup>-1</sup> of ΣPenta-BDE lipid) (Table 3). In detail, in the fat of insects able to purify their intestinal contents, the concentrations of BDE were three orders of magnitude higher than those generally present in humans, while in non-purified insects the content of ΣPenta-BDE was even higher (81 mg kg<sup>-1</sup> of lipids). The distribution of congeners in crickets and moults was very similar to that of the packaging material in contact with the organisms and the commercial formulation Penta-BDE, DE-71, indicating that no appreciable biotransformation occurred in the body of the aforementioned insects. The calculated accumulation factor (AF) value (104−103) was low and uncertain due to the difficulty in determining the quantity of PUF truly ingested.

Chinese researchers (Gao et al., 2013) conducted a study using HPLC-MS/MS analysis, to investigate the enantiomerisation and enantioselective bioaccumulation of benalaxyl in the larvae of *Tenebrio molitor* larvae. These larvae were fed a diet supplemented with the aforementioned compound (Gao et al., 2013) under laboratory conditions. Over a 21-day period of exposure to pure R-benalaxyl and S-benalaxyl enantiomers, significant enantiomerization occurred in *T. molitor* larvae, with the formation of the R-enantiomer from S-enantiomer and vice versa. However, this transformation was not observed in wheat bran. During bioaccumulation tests, the enantiomeric fraction in *T. molitor* larvae was maintained at approximately 0.6, while in wheat bran it was maintained at 0.5. The results show that the bioaccumulation of benalaxyl in *T. molitor* larvae was enantioselective.

In most cases, during the production of agricultural products, vegetable wastes are produced that cannot be used for human consumption (Houbraken et al., 2016). In the context of circular economy and respect for the environment, as well as, for an economic interest, wastes are used as substrate for the production of some insects intended for human consumption. Because pesticides are used in agricultural production, they could accumulate in the insects during their life cycle causing a health risk to humans who consume them. In one study, eight pesticide residues were quantified in the larvae of the yellow mealworm, *Tenebrio molitor* fed on fresh waste (Guo et al., 2014). The results show that insects preferentially accumulate pesticides (Table 4) with higher  $log(K_{ow})$ values, on the other hand, the excretion by insects was inversely proportional to the  $log(K_{ow})$  values of the pesticides (Houbraken et al., 2016).

In 2016, Polish researchers (Bednarska Agnieszka and Świątek., 2016) investigated the distribution of cadmium (a non-essential element) and zinc (an essential element) in different fractions (S1 or cytosolic fraction containing organelles, thermosensitive and thermostable proteins, S2 or cellular debris fraction and G or fraction of metal-rich granules) of larvae of *Tenebrio molitor* by feeding them by flour contaminated with Cd at 100, 300 and 600 mg  $\text{kg}^{-1}$ , or Zn at 1000 and 2000 mg  $\text{kg}^{-1}$ . The concentration of Cd and Zn in each fraction was measured 0, 7, 14 and 21 days after administration of the meal. Understanding the internal speciation and breakdown of a given metal in the organism, especially if it is in a metabolically available form, is more useful than knowledge of the total concentration in predicting possible toxic effects. The Cd concentration in the meal affected the relative amount measured in each larval subcellular fraction, whereas the Zn concentration in the meal affected only the concentration in the S2 and G fractions. The Cd and Zn concentrations in the mealworms have remained almost constant during the exposure time in all three fractions, but Cd concentrations were much higher than those found in the larvae before exposure (day 0). On the other hand, the concentration of cadmium in the flour had no effect on the percentage of this element in the S1 fraction. The contribution of Cd in the G fraction to the total amount of Cd was similar in all Cd treatments (30–40 %). The concentration of

### **Table 2**

Concentrations of various heavy metals for several edible insects (mg  $\text{Kg}^{-1}$ ).



## **Table 3**

Concentrations of contaminants in insects feed with enriched food or environment.



zinc in the three fractions was not influenced by that of the flour and by the relative contributions of each subcellular fraction with respect to the total load of Zn which generally remained constant for both control and for the treated larvae. In general, the larvae sequester approximately 30 % of Cd and Zn in the S1 fraction, which is important for the transport of metals to higher trophic levels in a food chain.

#### **Table 4**

Characteristics of pesticides used for the contamination of carrot.



A 2009 research (Karnjanapiboonwong et al., 2009) examined the effects of ingestion of 2,4,6- trinitrotoluene and its metabolites 2,4-dinitrotoluene (2,4-DNT), 2-amino-4,6- dinitrotoluene (2A-DNT) and 4-amino-2,6-dinitrotoluene (4A-DNT) on *Acheta domesticus*. The interest in TNT derives from the fact that this compound was used as an explosive during the First and Second World Wars (Robertson et al., 2007). Being chemically and biologically quite stable, TNT and their metabolites are still present in some environmental matrices and in particular in

### **Table 5**

Pollutants concentrations (µg  $\text{Kg}^{-1}$ ).

certain soils, where its concentrations and those of other nitro aromatic compounds and nitrosamines of up to 87,000 mg  $kg^{-1}$  have been quantified (Meyers et al.,2007). Considering that cricket embryos are sensitive to a wide range of pollutants that can cause negative effects on the morphology and development of young crickets (Walton et al.,1983; Zhang et al.,2006), the researchers concluded that TNT and its metabolites were not. Furthermore, mutagenic or teratogenic for crickets, they establish that concentrations in soil of aromatic nitro compounds including TNT at 10 µg  $g^{-1}$  can represent a risk to invertebrates such as crickets. The same conclusion is also valid for the molecules formed from the degradation of TNT. In particular, the presence of these compounds in soil with low organic carbon content (*<* 1.5 %) where cricket live leads to a decrease in hatching rates.

In a study conducted by Belgian researchers (Poma et al., 2017), the concentrations of various organic pollutants (including flame retardants, polychlorinated biphenyls, dioxins, pesticides, etc.) and nine heavy metals (Arsenic, Cadmium, Cobalt, Chromium, Copper, Nickel, Lead, Tin, Zinc) (Table 1) were quantified in samples of four different species of edible insects (greater wax moth, migratory locust, mealworm beetle and buffalo worm). The analytical results show that the concentrations (on dry weight) of organic pollutants (Table 5) were relatively low (polychlorinated biphenyls: 27–2065 pg  $g^{-1}$ ; organochlorine compounds:  $46-368$  pg  $g^{-1}$ ; flame retardants (BFRs, DPs, and PFRs): up to



NF= Not Found

flame retardants (BFRs, DPs, and PFRs),

organochlorine compounds (PCBs, OCPs: DDT, HCH, HCB) dioxins and dioxin-like PCBs, pesticides.

36 pg g<sup>-1</sup>; PFRs 783–23800 pg g<sup>-1</sup>; dioxin compounds: up to 0.25 pg WHO-TEQ  $\rm g^{-1}$ . Vinyl toluene and tributyl phosphate were found in 75 % of the samples, while 50 % contained a pesticide: pirimiphos-methyl. The concentrations of Cu and Zn in the analysed samples were similar to those found in meat and fish in other studies, while the concentrations of hazardous heavy metals (As, Co, Cr, Pb, Sn) concentrations were relatively low in all samples (<0.03 mg kg<sup>−1</sup>). The results of the aforementioned research confirm that the insect species studied can be used for food purposes without any additional risks compared to the consumption of traditional foods of animal origin. The results show that different chemical contaminants accumulate in the farmed insect, however, the concentrations were similar or even lower than those of meat, fish and eggs. Furthermore, the insect species studied can provide some micronutrients necessary for the human organism (Cu and Zn).

In a research of 2016 (Houbraken et al., 2016), the authors investigated the bioaccumulation of pesticides by a single exposure in edible insects: the yellow mealworm. In this study, 12 pesticides (Table 3) were added to carrots which were used to nourish *T. molitor* larvae under laboratory conditions. Quantification of the active principles was performed using a multiresidue method by Liquid chromatography-mass spectrometry (LC–MS/MS) on a triple quadrupole system. Pesticide residues in the *T. molitor* were increased to levels well above the LOQ for diflufenican (0.1 ng g<sup>-1</sup>), fenpropimorph (0.1 ng g<sup>-1</sup>), isoproturon (0.1 ng g<sup>−1</sup>), linuron (0.1 ng g<sup>−1</sup>), mefenoxam (1 ng g<sup>−1</sup>), pendimethalin (0.13 ng g<sup>−1</sup>), pyrimethanil (0.1 ng g<sup>−1</sup>) and tebuconazole (0.1 ng g<sup>−1</sup>). Residues up to 72 ng  $g^{-1}$  were quantified when the insects were exposed to these pesticides for 48 h to these pesticides. No quantifiable concentrations of 2,4-D, bentazone, bifenthrin and clopyralid were measured.

After a 24-hour of fasting period, pesticide residues reduced as a function of pesticide log( $K_{ow}$ ) values. Compounds having high log( $K_{ow}$ ) value were adsorbed more easily and excreted to a lesser extent, while, pesticides with a low  $log(K_{ow})$  values they were excreted rather quickly. The researchers conclude that although artificial contamination of food cannot be compared to real conditions of feeding with vegetable waste, it is necessary to prudently estimate the composition of products of unknown quality. Consequently, pesticide-contaminated plants having a low log ( $K<sub>ow</sub>$ ) can be used, provided that a sufficiently long period of food starvation is used before humans consume the insects. Foods contaminated with pesticides with a high log  $(K<sub>ow</sub>)$  are not recommended because they have been shown to be excreted to a lesser extent and accumulate easily.

In a study (Truzzi et al.,2019) the concentrations of Cd, Pb, Ni, As, Hg were quantified in *T. molitor* larvae fed with solid residues obtained from olive oil production. Furthermore, the bioaccumulation factor and the mercury-selenium balance were estimated. With the exception of mercury, the quantification of the other metals was carried out using atomic absorption spectrophotometry equipped with a graphite furnace and background correction using the Zeeman effect, while for mercury it was determined using the Direct Mercury Analyser. All metal concentrations found in feed substrates were below the legal limit for undesirable substances in animal feed (2002/32/EC). The concentration ranges of the analytes in the larvae, expressed in mg kg<sup>-1</sup> wet weight, were Cd 0.008–0.016, Pb 0.063–0.079, Ni 0.03–0.63, As 0.021–0.023, Hg 1.2  $\times$  $10^{-4}$  - 4.9 ×  $10^{-4}$  and Se 0.057–0.085. The authors conclude that, only for mercury, there is a good or statistically significant correlation between the concentrations of the metal in the food and in the larvae. From the results of the aforementioned study, in this case, it is clear that the risk of exposure to metals resulting from the consumption of mealworm larvae is relatively low and complies with European Union legislation. The development and validation of a semi-quantitative analytical multi-residual method by UHPLC-Q-Orbitrap-HRMS is described in a paper (De Paepe et al., 2019) for the simultaneous analysis of 77 compounds selected on the most common hazardous compounds (veterinary drugs, pesticides and mycotoxins) in environmental and food matrices and, in particular, for their possibility of bioaccumulation in edible

insects including larvae *Tenebrio molitor*, adults of *Locusta migratoria* and *Acheta domesticus*. In particular, the researchers estimated the concentrations of residues (semi-quantitatively) (Table 5) in the real Lesser mealworm, Black soldier fly, House cricket and Grasshopper. These results indicate limited toxicological risks associated with the consumption of insect tissues.

A paper (Köhler et al., 2019) reports the concentration of several metals (Table 1) in four insect species from Thailand: Bombay locust *Patanga succincta*, scarab beetle *Holotrichia sp*., house cricket *Acheta domesticus* and mulberry silkworm *Bombyx mori*, sampled from street vendor (SH) or at supermarket (SM). For mineral content analysis, samples were preliminarily defatted by use of petroleum ether and mineralized in a microwave oven using nitric acid. The quantification of Ca, Fe, K, Mg, Mn and Na was performed by ICP-OES while for Cd, Cu, Mo, Pb and Zn by ICP-MS. Arsenic and selenium were measured using of Cold Vapour Atomic Absorption Spectrometry (CV-AAS) for Hg atomization. As and Hg were highest in the scarab beetle, 0.576 mg and 0.08 mg  $\text{Kg}^{-1}$ , respectively. Lead was highest in the house cricket from the street vendor at 0.155 mg  $Kg^{-1}$ , while the cadmium level in all the insect samples was below 0.05 mg Kg<sup>-1</sup>. Table 4 shows the concentrations of the hazardous metals in the house cricket samples.

The absorption of aflatoxin B1 (AFB1), ochratoxin A (OTA) and fumonisin B1 (FB1) and their clearance rates in *T. molitor* larvae, fed on contaminated cereals, have been studied (Mancini et al., 2020) (Table 5). The paper describes a new analytical method to extract and quantify these hazardous compounds. The analytes were extracted with methanol and quantified by the LC-UV-MS method. The authors established that *T. molitor* larvae do not accumulate the three mycotoxins to detectable or hazardous concentrations. A 24-hour fasting time ensures a sufficient clearance rate of residual AFB1, OTA and FB1. In particular, for each mycotoxin, two concentrations of diet contamination were studied to obtain concentrations similar to the maximum residue limit (MRL) admitted in cereals for human consumption by EU legislation (Commission Regulation (EC) No 1881/2006).

Hydroxymethylfurfural (HMF), also known as 5-(hydroxymethyl) furfural, is an organic compound that is almost completely absent in fresh foods, but it is naturally produced in sugar-containing food during heat treatments such as drying or cooking, and is formed by the dehydration of reducing sugars (Gonzales et al., 2020). The toxicological and analytical interest in HMF is due to the fact that it can be transformed into other hazardous compounds such as 5-sulphoxymethylfurfural (Gokmen et al., 2006). The European Food Safety Authority (EFSA, 2005) has established that the aforementioned metabolite can generate genotoxic and mutagenic effects in vitro (Pastoriza de la Cueva et al., 2017). Recent studies have shown nephrotoxicity and hepatotoxicity both in vitro and in vivo studies. Although there are no maximum concentrations for this compound in most foods, the Codex Alimentarius and the European Union (European Commission, Directive 20 01/110/EC) have established a maximum concentration in honey and apple juice of 40 and 50 mg  $kg^{-1}$  respectively. To quantify this compound in food products containing insects, some researchers (Gonzales et al., 2020) developed and validated an analytical method based on solid-liquid extraction, followed by solid phase extraction, employing functionalized mesostructured silica as sorbent and liquid chromatography coupled to mass spectrometry (HPLC-MS/MS) analysis. This method was used to quantify hydroxymethylfurfural in samples of cereals and insect bars. Concentrations ranged from 336 to 962 mg  $\text{kg}^{-1}$  in all samples analysed.

Selenium is an essential micro element necessary to preserve the health of humans and animals (Kieliszek et al., 2019, Dong et al., 2021). Selenium deficiency is responsible for the development of several metabolic syndromes, chronic diseases and even cancer (Kieliszek et al., 2013). The amounts of selenium in most daily food is relatively low. Consequently, dietary supplementation of Se is necessary. This metal can exist in inorganic and organic forms. Organic selenium exhibits higher bioavailability than inorganic selenium. It is common to use

edible microorganisms, plants and livestock to produce organic bioavailable selenium. Some authors (Dong et al., 2021) have studied the accumulation of Se in the larvae of *Tenebrio molitor* (Table 1). The total Se content in the larvae increased by about 83 times, reaching concentrations of 54  $\mu$ g g<sup>-1</sup>, by nourishing the larvae by feed enriched with 20  $\mu$ g g<sup>-1</sup> of sodium selenite (Table 1). The authors established that 97 % of the Se in the *Tenebrio molitor* larvae was in organic form, in particular, the element was distributed in the protein fraction with the following order: alkali-soluble protein-bound selenium (36 %) *>* salt-soluble protein-bound selenium (19 %) *>* protein-bound soluble in water selenium (17 %) *>* alcohol-soluble protein -bound selenium (3 %).

In 2021, (Pastel et al., 2021) quantified some heavy metals in the *Acheta domesticus* fed with three different diets indicated as FE1, FE2, FE3. FE1 contained oats, wheat, barley, turnip rapeseed meal, potatoes and fava beans manufactured by the researchers. FE2 was a commercial chicken feed (control) containing wheat, barley, oat, calcium carbonate, textured soy, protein granules, vegetable oil, turnip-rapeseed pellets, calcium sodium phosphate, vitamin and trace element supplementation, sodium chloride and amino acids. Vitamin and trace element supplementation included vitamins A, D3, and E, iron, iodine, copper, manganese, zinc, and selenium. FE3 was manufactured by the researchers and contained wheat, turnip-rapeseed meal, potato, fava beans, peas, and barley. Heavy metals in feeds and cricket samples were quantified by inductively coupled plasma optical emission spectrometry (ICP-OES) and inductively coupled plasma mass spectrometry (ICP-MS). Concentrations of As, Cd, Cr, Ni and Pb are reported in Table 3. The authors' conclusion suggests that the use of three different feeds has no impact on the concentration of heavy metals in the crickets. Importantly, none of the observed concentrations of heavy metals exceed the maximum limits outlined by Commission Regulation (EC) No. 1881/2006 for insects.

In 2021, Spanish researchers (González-Gómez et al., 2021) optimized and validated an analytical method for the simultaneous quantification of toxicological substances (acrylamide, 5-hydroxymethylfurfural, 5-methylfurfural and furfural) in *Acheta domesticus* and insect-based products such as bars, crackers, and flours. Most of these substances are formed during the Maillard reaction or browning reaction (Table 6).

In particular, acrylamide is a toxic compound formed mainly by the reaction of asparagine with reducing sugars, very common in starch-rich foods subjected to high processing temperatures (generally above 120 ◦C) and low humidity. The International Agency for Research on Cancer IARC., 1994, classified acrylamide as probable carcinogen (group 2 A). The 5-Hydroxymethylfurfural, the 5-methylfurfural and furfural are intermediate products generated during the caramelization reaction. Eleven insect-based products were obtained from different local markets in Madrid (Spain). The insect-based food samples were a cricket flour (100 %, *Acheta domesticus*), a cricket flour with chocolate, six cricket bars and three cricket crackers. Three cereal bars were also considered for comparison. All trace analysis were performed by high performance liquid chromatography coupled to triple quadrupole mass spectrometry (HPLC-QqQ-MS/MS) (Table 5).

Considering the analytical results, the researchers conclude that foods containing insects can be a good alternative to traditional ones to reduce dietary exposure to furan compounds, mainly to hydroxymethyl-furfural.

During 2021(Kolakowski et al., 2021), 511 pesticides in 43 samples of crickets (whole insects, protein bars, powders, flour) and 4 samples of silkworm (whole insects) were quantified. The samples were obtained from online retailors or collected from retail establishments located in Ottawa (Canada). Thirty-nine samples contained up to four pesticides, 34 samples were compliant and 5 were noncompliant to the Canadian legislation. The pesticides were quantified by LC-MS/MS after extraction in acidic (2 % HCl) methanol. Only seven pesticides were detected, in particular, glyphosate and its metabolite (Table 7). Arsenic, cadmium, mercury and lead were quantified in only nineteen of the samples analysed for pesticides. As, Cd and Pb were quantified by inductively coupled plasma mass spectrometry, while Hg was quantified by cold vapour atomic fluorescence spectroscopy. All insect products analysed contained detectable levels of arsenic. The detected concentrations

### **Table 7**





**Table 6** 





Table= not detected; *<*LOQ: below the limit of quantification.

ranged from 0.030 mg kg<sup>-1</sup> (whole silkworm pupae) to 0.34 mg kg<sup>-1</sup> (cricket powder). The average concentration of As resulted 0.12 mg kg<sup>-1</sup> in cricket products and 0.049 mg  $kg^{-1}$  in silkworm pupae. These concentrations are not consistent with literature data, which report a maximum of 0.03 mg/kg for crickets (Poma et al., 2017; Hiun et al., 2021). None of the silkworm pupae–based products and all cricket-based samples contained detectable concentrations of cadmium that ranged from 0.031 to 0.23 mg  $\text{kg}^{-1}$ . Also in this case, these results are not consistent with previously reported Cd concentrations of no more than 0.03 mg kg<sup>-1</sup> (Poma et al., 2017). Lead was found in 58 % of all samples analysed, in 25 % of products containing silkworm pupae and in 67 % in the products made from crickets. Lead concentrations ranged from 0.019 to 0.059 mg kg  $^1$ . The concentration in silkworm pupae–based samples was 0.020 mg  $k^{-1}$ versus an average level of 0.033 mg  $\text{kg}^{-1}$ in cricket products. These values do not concur with the reported result of no more than 0.03 mg  $kg^{-1}$  for cricket samples. As with cadmium, none of the silkworm pupae–based products and all cricket-based samples contained quantifiable concentrations of mercury. The amount of mercury was in the range from 0.94 to 28  $\mu$ g kg<sup>-1</sup>. These concentrations in crickets are low compared to the 125 and 109 μg kg<sup>-1</sup> observed in one study (Ortiz et al., 2015). The resulting concentrations of heavy metals ranged from 0.030 to 0.34 mg kg<sup>-1</sup> for As, 0.031–0.23 mg kg<sup>-1</sup> for Cd, 0.019–0.059 mg kg<sup>-1</sup> for Pb, and from 0.94 to 28  $\mu$ g kg<sup>-1</sup> for Hg (Table 7). With the absence of microbiological contamination and considering the concentrations of pesticides and metals, the researchers affirm that all the analyzed insect products can be deemed safe for human consumption.

Some authors (Egonyu et al., 2021) use literature data to evaluate the possibility of using the desert locust for food purposes. They establish that this insect contains similar or even higher concentrations of iron and zinc than mutton, beef and pork, with 1–6 and 2.4–12.5 mg/100 g, respectively (Ahmad et al., 2018; Sun-Waterhouse et al.,2016). Concentrations of heavy metals in locust are within limits established for other foods (Ahmad et al.,2018; Poma et al.,2017).

#### **5. Conclusion**

The present review provides a comprehensive overview of the available data on the estimation of hazardous contaminants in edible insects for the purpose of risk assessment to ensure food safety. Therefore, it is strongly recommended to control the levels of in the production of insects for human consumption.

By systematically analysing the results of more than 150 studies, this review highlights the critical need for stringent monitoring and regulatory frameworks to minimise contamination levels in insect-based foods. The implementation of rigorous quality control measures and adherence to standardised guidelines will be essential to protect public health and promote consumer confidence in the emerging insect-based food market. In addition, ongoing research and collaboration between scientists, industry stakeholders and regulators will play a key role in advancing our understanding of contaminant dynamics and developing innovative solutions to mitigate potential risks. Through these concerted efforts, the sustainable integration of insects into the global food supply can be achieved, contributing to improved food security and environmental sustainability.

### **Ethics statement**

No animal or human experimentation is conducted in this review manuscript.

### **Author agreement statement**

We the undersigned declare that this manuscript is original, has not been published before and is not currently being considered for publication elsewhere.

We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed.

We further confirm that the order of authors listed in the manuscript has been approved by all of us.

We understand that the Corresponding Author is the sole contact for the Editorial process.

### **CRediT authorship contribution statement**

**Diana Amorello:** Writing – original draft, Data curation. **Silvia Orecchio:** Writing – original draft, Data curation. **Salvatore Barreca:**  Writing – review & editing, Data curation.

### **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.

### **Data Availability**

Data will be made available on request.

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