Elemental bioaccessibility and endogenic nanoparticles in farmed insects: In search of quality sustainable food

Ignacio Machado,[1*] Andrés Suárez Priede,[2] Mario Corte Rodríguez,[2] David Heath,[3] Ester Heath[3], Lenka Kouřimská,[4] Martin Kulma,[5] Jörg Bettmer,[2] María Montes-Bayón[2*]

[1]Área Química Analítica, Facultad de Química, Universidad de la República, Av. Gral. Flores 2124, Montevideo, Uruguay.

[2] Department of Physical and Analytical Chemistry, Faculty of Chemistry, University of Oviedo, Julián Clavería 8, 33006 Oviedo, Spain and Instituto de Investigación Sanitaria del Principado de Asturias (ISPA), Oviedo, Spain.

[3] Jožef Stefan Institute, Jamova cesta 39, 1000 Ljubljana, Slovenia.

[4] Department of Microbiology, Nutrition and Dietetics, Czech University of Life Sciences, Praha-Suchdol, Czech Republic.

[5] Department of Zoology and Fisheries, Czech University of Life Sciences, Praha-Suchdol, Czech Republic.

Abstract

Despite the many advantages on the use of insects as a sustainable food source and their approval by the European Food Safety Authority, insect farming is still susceptible to several hazards, making the need for further in-depth chemical studies. This work investigated the *in vitro* bioaccessibility of aluminum, copper, iron, manganese, lead, selenium, and zinc in three farmed insects: *Tenebrio molitor*, *Locusta migratoria* and *Acheta domesticus*. The high zinc bioaccessibility observed for all species (~92%) was one of the highlights, demonstrating the high nutritional value of this element. In addition, a higher accumulation of Se was observed upon increasing exposure concentration in *Acheta domesticus*, showing the possibility of insect food as a food supplement for this element. In addition, the presence of nanoparticulated Al and Fe species could also be proved using highly sensitive mass spectrometric techniques and transmission electron microscopy in some of the analyzed samples.

Keywords: insects, elemental composition, bioaccessibility, nanoparticles

Introduction

Recent years have seen insects receive significant attention in Europe due to their potential to address ongoing food, feed, and nutritional challenges (Van Huis., 2020. The reason is that farmed insects demand fewer natural resources such as water, feed, and land when compared to conventional livestock (Van Huis *et al*., 2013), and their production results in lower overall greenhouse gas emissions (Oonincx, 2017). As cold-blooded organisms, insects have a high feed conversion efficiency and the potential to transform organic matter into insect biomass (Gamborg *et al*., 2018; Oonincx, 2017). Insects also have good nutritional value, providing bioactive compounds such as vitamins, minerals, long-chain polyunsaturated fatty acids and a protein-rich food source (Roos, 2018). Furthermore, up to 80% of insect biomass is consumed and digested, compared to the average percentages of 55 % reported for chickens and pigs and 40 % for cattle (Heckmann *et al*., 2018; Van Huis *et al*., 2013). They are especially suitable for industrial production due to their rapid growth, high reproductive rate, short lifespan with quick turnover, efficiency in nutrient conversion, and ability to thrive in high density, which makes animal welfare less of an issue (Jensen *et al*., 2017; van Hui, 2021). In addition, the byproducts of insect production, including frass and exoskeletons, are high-quality crop amendments, which could reduce the need for nitrogenous fertilizers (Michel & Begho, 2023). Currently, several insects are awaiting the safety evaluation of the European Food Safety Authority (EFSA) and the marketing authorization as novel foods (Regulation (EU) No. 2015/2283), while preparations containing the species yellow mealworm (*Tenebrio molitor*), house cricket (*Acheta domesticus*), migratory locust (*Locusta migratoria*), and lesser mealworm (*Alphitobius diaperinus*) have already been approved by the European Commission (Delgado Calvo-Flores *et al*., 2022).

Despite all these advantages and the approval by EFSA of four species, insect farming, like all intensive livestock production, is susceptible to possible food safety hazards, including biological (bacteria, viruses, fungi, parasites), chemical (mycotoxins, pesticides, heavy metals, organic contaminants), and physical hazards (Belluco *et al*., 2018). Although regulatory frameworks are being developed, it is clear that further studies of chemical risks related to the rearing, handling, harvesting, processing, storing (shelf-life), and transporting of insects and insect-based products are needed (FAO, 2021). Bioaccumulation of chemical hazards generates a toxicological risk regarding insects' safety as food and feed sources (Belluco *et al*., 2018; Marone, 2016). Insect feed substrates may contain relatively high levels of environmental contaminants capable of bioaccumulating, such as some heavy metals. Their accumulation depends on the type of insect, the stage of growth, the element, the environmental contamination levels, and their bioaccessibility (Belluco *et al*., 2018). In this regard, *in vitro* bioaccessibility, defined as the amount of an element that is released from a food matrix and has the potential to be absorbed by the intestine after digestion, constitutes a better approximation to evaluate potential positive or adverse effects on human health (Iaquinta *et al*., 2021).

Moreover, insects may accumulate different types of exogenous nanoparticles from the environment, threatening animal and human health (Sezer Tunçsoy, 2018). Also, recent studies have revealed that various metal-containing endogenous nanoparticles occur naturally in insects, for example, in insects' wings and thoracic regions, aiding aerodynamics during flight (Bhattacharyya *et al*., 2010). Due to their small size, nanoparticles may cross barriers such as the human intestinal epithelium and enter the bloodstream, reaching secondary organs and bioaccumulating (Usman *et al*., 2022). Therefore, nanoparticles should be considered during any food safety assessment.

This study investigated the *in vitro* bioaccessibility of aluminum (Al), copper (Cu), iron (Fe), manganese (Mn), lead (Pb), selenium (Se), and zinc (Zn) in three farmed insects: *Tenebrio molitor*, *Locusta migratoria*, *Acheta domesticus*, and *Acheta domesticus* fed on a diet enriched in selenium. Selenium fortification was performed with the aim of evaluating the bioaccumulation capacity of the species for its use to promote animal and human nutrition. The work also characterizes and quantifies the presence of endogenous nanoparticles in these farmed insects. The information obtained will be valuable for the subsequent nutritional and toxicological characterization and assessment of farmed insects.

Materials and methods

Reagents

All chemicals were of analytical reagent grade or higher quality. Ultrapure water (18.2 MΩcm) was obtained using the Milli-Q system (Millipore, Bedford, MA, USA). Daily, working standard solutions were prepared by serial dilution of commercial 1000 mg L^{-1} stock solutions of each element (Merck Millipore, Darmstadt, Germany) in dilute nitric acid (HNO_3) at concentrations of 2% for elemental determinations and 0.1% for nanoparticle determinations. Concentrated HNO_3 (65%, Suprapur), hydrochloric acid (36%, Suprapur) and hydrogen peroxide (30%) were all purchased from Merck Millipore (Darmstadt, Germany). Digestive enzymes (pepsin, α-amylase, gastric lipase, trypsin) and bile salts were purchased from Sigma–Aldrich (Saint Louis, MO, USA). Sodium hydroxide (NaOH), calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$), ammonium acetate ($CH_3CO_2NH_4$), potassium chloride (KCl), potassium phosphate monobasic (KH_2PO_4), sodium bicarbonate ($NaHCO_3$), sodium chloride (NaCl), magnesium chloride hexahydrate ($MgCl_2 \cdot 6H_2O$) and sodium dodecyl sulphate (SDS, $NaC_{12}H_{25}SO_4$) were also

purchased from Sigma–Aldrich (Saint Louis, MO, USA). Glass beads for mini-beadbeater (diam. 0.5 mm) were likewise procured from Sigma–Aldrich (Saint Louis, MO, USA).

Samples

Four pooled samples (5 g each) from each insect species *Acheta domesticus*1, *Acheta domesticus*2, *Locusta migratoria*, and *Tenebrio molitor* (larvae) were studied. Insect samples were obtained from the rearing facility at the Department of Zoology and Fisheries, Czech University of Life Sciences Prague (CZU) using a rack-system (conditions for room with house crickets and mealworms: t = 27±1 °C, humidity 40-50 % RH; for room with locusts: t= 27±1 ºC, humidity 30-35% RH). The experimental crickets were kept in plastic rearing boxes (560 × 390 × 280 mm, SAMLA, IKEA, Prague, Czech Republic) until harvest at 50±5 days when most crickets were adult. The boxes were equipped with egg trays, two Petri dishes with feed, and two dishes containing water gel (Oslavan, Náměšť nad Oslavou, Czech Republic). The mealworms were kept in plastic containers (280×140×390 mm, SAMLA, IKEA, Prague, Czech Republic) in a feeding substrate. Fresh sliced carrots and apples were supplied as the only water source. Mealworms were harvested using sieving when the first pupae occurred (the approximate age of harvested larvae was 90±7 days). *Locusta migratoria* was kept in two flexariums 450×400×800 mm, each equipped with one 40 W bulb used as a local source of heating (12:12 hrs) and one Petri dish with water gel. The locusts for analysis were collected as adults.

Regarding insect diets, all species were provided with dry feed *ad libitum*. House crickets were fed chicken feed (77.9% wheat, 17.6% soybean meal, 1.8% rapeseed oil, 2.7% a premix of minerals, macronutrients, and micronutrients; particle size <1 mm) produced in collaboration with the experimental farm of Demonstrational and Experimental Centre,

CZU. Locusts were fed on wheat bran, hay and fresh Poaceae grasses, while Mealworms were fed wheat bran: chicken feed (4:1) diet. Prior to harvest, the experimental insects were starved for 24 hours. After that, they were freeze-killed at -18 °C, lyophilized and homogenized using laboratory mill A10 (IKA Werke GmbH & Co. KG, Staufen).

Acheta domesticus 2 belonged to a group of house crickets enriched with Se obtained from the National Research Council Canada (NRCC), subdivided into four groups: control, enriched with 5 ppm, enriched with 10 ppm, and enriched with 50 ppm.

In vitro bioaccessibility experiments

Bioaccessibility experiments were performed using a simulated *in vitro* gastrointestinal digestion system adapted from the INFOGEST protocol (Brodkorb *et al*., 2019). The procedure was miniaturized to be carried out in 2.0 mL microcentrifuge tubes. The simulated salivary fluid (SSF) comprised 15.1 mM KCl + 3.7 mM KH_2PO_4 + 13.6 mM $NaHCO_3$ + 0.15 mM $MgCl_2 \cdot 6H_20$ + 1.5 mM $CaCl_2$-$2H_20$ + 1.1 mM HCl + 75 U mL^{-1} α-amylase in ultrapure water. The simulated gastric fluid (SGF) was 6.9 mM KCl + 0.9 mM KH_2PO_4 + 25 mM $NaHCO_3$ + 0.12 mM $MgCl_2 \cdot 6H_20$ + 0.15 mM $CaCl_2 \cdot 2H_20$ + 15.6 mM HCl + 2,000 U mL^{-1} pepsin + 60 U mL^{-1} gastric lipase in ultrapure water (5 M HCl was used for pH adjustment). The simulated intestinal fluid (SIF) was 6.8 mM KCl + 0.8 mM KH_2PO_4 + 85 mM $NaHCO_3$ + 0.33 mM $MgCl_2 \cdot 6H_20$ + 0.6 mM $CaCl_2 \cdot 2H_20$ + 8.4 mM HCl + 100 U mL^{-1} trypsin + 10 mM bile salts in ultrapure water (5 M NaOH was used for pH adjustment).

A portion of 0.15 g of freeze-dried sample was placed in a microcentrifuge tube with 0.30 mL of SSF, and the mixture vortexed for 30 s and incubated for 2 min at 37 °C (pH = 7.0) in a digital dry block heater (Thermo Scientific, Bremen, Germany). Then, 0.60 mL of SGF was added, and the mixture was vortexed for 1 min and incubated at 37 °C for 2 h (pH = 3.0). Afterwards, 0.60 mL of SIF was added and the mixture was vortexed for 1

min and incubated for 2 h at 37 °C (pH = 7.0). Finally, the mixture was centrifuged at 5,000 *g* for 30 min, and the supernatant was used to determine the bioaccessible fraction (%BF). The %BF was calculated as %BF = (RF/TC) × 100, where RF is the released fraction of the element and TC is the total concentration of the element expressed in mg kg^{-1} (Iaquinta *et al*., 2023).

The accuracy of the assay was determined from the corresponding mass balance (%MB), where %MB was calculated as %MB = [(RF + RFR)/ TC] × 100, where RFR is the remaining fraction of the element in the residue and expressed in mg kg^{-1}.

Microwave-assisted total digestion method

Total elements in the samples and residues obtained from bioaccessibility studies were extracted using microwave-assisted acid digestion with an Ethos 1 microwave system (Milestone Srl., Sorisole, Italy). Ground insect biomass (or whole residue) was accurately weighted (0.1 g) into each microsampling insert, and 1.0 mL of concentrated HNO_3 and 1.0 mL of H_2O_2 were added. The inserts were then transferred into 100 mL Teflon vessels. The program consisted of a 30 min ramp time until 150°C, holding for 1 h, and then cooling to room temperature in 45 min. The output power was 1,500 W and controlled via a microprocessor. The elemental concentrations were then determined using inductively coupled plasma–mass spectrometry (ICP-MS) after appropriate dilution with 2 % HNO_3. All samples and reagent blanks were run in triplicate.

Ultrasound-assisted nanoparticles isolation

Nanoparticle extraction was achieved through mechanical lysis (R. Álvarez-Fernández García *et al.*, 2020, Taskova *et al*., 2006). For this task, 0.010 g of freeze-dried sample was placed in a microcentrifuge tube with 1.0 mL of 50 mM ammonium acetate

175 ($CH_3CO_2NH_4$) solution and 0.5 mL of glass beads and vortexed for 30 s. The mixture was
176 then sonicated for 10 min at 40 kHz in an Ultrasons-H ultrasonic bath (Selecta, Barcelona,
177 Spain) and subsequently vortexed for 5 min. The whole procedure was repeated two
178 times. The obtained mixture was centrifuged at 3,000 *g,* and the supernatant was
179 transferred to a clean tube. The procedure efficiently isolated Al nanoparticles (Al NPs)
180 and Fe nanoparticles (Fe NPs). Extracts were appropriately diluted with ultrapure water
181 prior to single particle–inductively coupled plasma–mass spectrometry using (sp-ICP-
182 MS) analysis.

183 ***Total element determinations***

184 Analytical determinations of Al, Cu, Fe, Mn, Pb, Se, and Zn were performed in an
185 iCAP^{TM} TQ ICP-MS (Thermo Fisher Scientific, Bremen, Germany) employing the single
186 quadrupole (SQ) or the triple quadrupole (TQ) mode, depending on the element. The ICP-
187 MS instrument was fitted with a concentric nebulizer and a cyclonic spray chamber.
188 Hydrogen was employed as a reaction gas to eliminate polyatomic interferences affecting
189 ^{63}Cu, ^{56}Fe and ^{64}Zn, respectively. The ^{80}Se isotope was monitored in TQ mode using O_2
190 as the reaction gas to form $^{80}Se^{16}O^+$, which allowed large interferences to be resolved.
191 ICP-MS operating parameters are shown in Table 1.

192 ***Single particle ICP-MS measurements***

193 The same instrumentation was used in the single particle mode (sp-ICP-MS) to detect the
194 presence of nanoparticles. In this case, a SC-SI-73 single-cell sample introduction kit
195 purchased from Elemental Scientific (Omaha, NE, USA) was employed. The sample
196 introduction flow rate was controlled via a Chemyx Fusion 100-X syringe pump
197 (Chemyx, Stafford, TX, USA) and set to 0.01 mL min^{-1}. Transport efficiency (%TE) was
198 assessed by analyzing an LGCQC5050 colloidal gold nanoparticle reference material (Au
199 NPs, 30 nm, 1.47×10^{11} NPs g^{-1}) and using the particle number method (Pace *et al.*,

2011). The same instrumental conditions as those for Al NPs and Fe NPs were used, but monitoring the m/z 197 for Au. Calibration standards of ionic Al and Fe were prepared in 0.1% HNO_3. NPs concentration and size distribution were calculated based on the %TE experimental value and the developed equations for data processing for counting and sizing NPs using sp-ICP-MS (Pace *et al.*, 2011). Extracts were diluted 1:5 and 1:50 with ultrapure water to detect Fe NPs and Al NPs, respectively. All dilutions were vortexed before their analyses by sp-ICP-MS to avoid sedimentation. The nebulization gas flow rate (Ar) was set to 0.34 L min^{-1}, the nebulizer sheath flow rate (Ar) to 0.825 L min^{-1}, the dwell time to 0.005 s, and the acquisition time to 120 s otherwise the instrument and operative conditions were the same as those summarized in Table 1.

Table 1. ICP-MS operating parameters

Parameter	Al	Cu	Fe	Mn	Pb	Se	Zn
Isotope monitored	^{27}Al	^{63}Cu	^{56}Fe	^{55}Mn	^{208}Pb	^{80}Se|$^{80}Se^{16}O$	^{64}Zn
Mode	SQ	SQ-H_2	SQ-H_2	SQ	SQ	TQ-O_2	SQ-H_2
Plasma RF power	1550 W						
Nebulization gas flow rate (Ar)	1.0 L min^{-1}						
Sample introduction flow rate	0.40 mL min^{-1}						
Dwell time	0.1 s						

Analysis of nanoparticles using HPLC-ICP-MS

To determine the presence of small NPs, i.e., below the size detection limit of SP-ICP-MS, high-performance liquid chromatography (1260 Infinity Series, Agilent Technologies, Tokyo, Japan) coupled to the ICP-MS (HPLC-ICP-MS) was employed as described by Helfrich *et al*. (2006). Separation was achieved on a Nucleosil C18 reversed-phase column (7 µm particle size, 1000 Å pore size, 250 x 4.6 mm, Macherey-Nagel GmbH & Co. KG, Düren, Germany). The mobile phase consisted of 10 mmol L^{-1} sodium dodecyl sulphate and 10 mmol L^{-1} ammonium acetate buffer (pH = 6.8). The flow rate

was 0.5 mL min^{-1}. The column was coupled to the iCAP TQ ICP-MS through a concentric nebulizer and a cyclonic spray chamber. The operating conditions were the same as in Table 1. Analysis time was 8 minutes. Column calibration is shown in Fig S1.

Transmission electron microscopy

Transmission electron microscopy (TEM) images were obtained with a MET-JEOL-JEM-1011 (Tokyo, Japan) operated at 100 kV. HR-TEM measurements were done in a JEOL-JEM 2100F transmission electron microscope with TEM operation voltage at 200 kV to image iron NPs suspensions deposited on copper grids. The instrument also allowed EDX analysis to evaluate the elemental composition of the nanostructures.

Statistical analysis

Differences in analyte concentrations were tested using the Student's *t*-test. Distinctions in mean concentrations, at a 5% significance level ($p < 0.05$), were regarded as statistically significant (Miller & Miller, 2010). All calculations were performed using Excel software (Microsoft Office 365), while analysis and graphing of NP data were performed using OriginPro 8 software.

Results and discussion

Determination of elemental composition

For the characterization of the analytical method based on ICP-MS, some figures of merit were herein evaluated. Six-point calibration curves with standards ranging from 1.0 to 50.0 μg L^{-1} were constructed. Linear ranges were verified by determination coefficients ($R^2 > 0.999$) and the random distribution of individual residuals. Limits of quantification estimated as 10 × (standard deviation of background noise / analytical sensitivity) were considered adequate for analyzing insects since they were far below the obtained levels in the analyzed samples. Precision (repeatability) expressed as percentage standard

deviation (%RSD) for three independent samples was ≤ 8% for all the studied elements. In addition, samples were spiked by adding a known amount of analyte to prove that the microwave-assisted digestion was adequate and that no matrix effects remained. Recoveries were calculated by comparing the mean spiked value and the mean value of the sample with the added concentration, which ranged from 95% to 102%, confirming the method's accuracy. In sum, the obtained figures of merit demonstrated the suitability for insect analysis. The use of microsampling inserts in the microwave digestion system allowed for a reduction in sample mass to 0.1 g and digestion volume to 3 mL, respectively. It also increased sample throughput by allowing the placement of three inserts in a 100 mL digestion vessel.

The total elemental concentrations are summarized in Table 2. The results agree with those previously reported for *T. molitor* samples (Sikora *et al*., 2023), *L. migratoria* samples (Turck *et al*., 2021) and *A. domesticus* samples (Ververis *et al*., 2022).

Table 2. Summary of elemental concentrations in analyzed insects

Sample	Mean concentration ± standard deviation (mg kg^{-1})						
	Al	Cu	Fe	Mn	Pb	Se	Zn
T. molitor	15.4 ± 0.1	13.2 ± 0.1	62.9 ± 1.2	13.24 ± 0.12	0.0105 ± 0.0002	0.130 ± 0.001	150.9 ± 2.7
L. migratoria	39.2 ± 2.9	23.7 ± 1.9	65.3 ± 5.8	5.86 ± 0.17	0.0586 ± 0.0027	0.121 ± 0.018	157.4 ± 18.1
A. domesticus 1	35.6 ± 3.5	14.2 ± 1.3	65.8 ± 7.8	38.6 ± 1.9	0.0779 ± 0.0077	0.223 ± 0.026	174.9 ± 4.6
A. domesticus 2 (Control)	33.5 ± 1.0	12.1 ± 0.2	68.9 ± 1.2	85.6 ± 0.3	0.1031 ± 0.0076	0.143 ± 0.003	188.2 ± 3.5
A. domesticus 2 (5 ppm)	38.3 ± 2.0	11.8 ± 0.3	74.1 ± 9.0	91.7 ± 0.6	0.0931 ± 0.0021	0.249 ± 0.004	186.5 ± 5.0
A. domesticus 2 (10 ppm)	32.0 ± 0.8	10.5 ± 0.1	95.4 ± 9.3	90.9 ± 1.1	0.0907 ± 0.0030	0.532 ± 0.014	182.1 ± 5.8
A. domesticus 2 (50 ppm)	95.7 ± 5.7	11.6 ± 0.1	205.4 ± 7.3	93.4 ± 0.1	0.0950 ± 0.0031	1.382 ± 0.045	182.9 ± 2.1

As previously stated, *A. domesticus* 2 comprised a pool of farmed *A. domesticus* enriched with Se in different concentrations. Statistically significant differences ($p < 0.05$) were observed between the control and the enriched samples, ranging from 0.143 ± 0.003 mg kg^{-1} (control) to 1.382 ± 0.045 mg kg^{-1} (50 ppm), revealing the Se bioaccumulation

capacity of *A. domesticus*. Selenium fortification was also performed to evaluate the formation of NPs since endogenous selenium NPs have been reported to have higher bioactivities and lower toxicity than bulk Se in nutritional, antimicrobial and anticancer applications (Alghuthaymi, 2022). In addition, Se levels were in the same order in all the analyzed insects when no Se was added.

Similar elemental concentrations between species were also observed for essential elements like Cu and Zn, with values ranging from 12.1 to 23.7 and 150.9 to 188.2, respectively. A low Zn bioaccumulation rate has been previously reported in the literature for *A. domesticus*, indicating their ability to regulate dietary exposure to this element, with accumulation only occurring at Zn concentrations above 40 mM/kg of dry feed (Fernandez-Cassi *et al*., 2019). Interestingly, *A. domesticus* 2 shows a statistically significant increase ($p < 0.05$) in Al and Fe in the sample enriched with 50 ppm of Se (Figure 1), indicating a possible correlation between these three elements. While Se enrichment may not directly impact Al and Fe levels, it is worth noting that excessive Se intake can adversely affect an organism's health (Genchi *et al*., 2023). Similar to other organisms, insects have mechanisms to regulate the uptake and utilization of various elements, including Se. However, it is known that high levels of Se can disrupt these mechanisms, potentially affecting the overall health of the insect and altering the uptake rate of other elements (So *et al*., 2023). In the case of Al, a toxic element for humans, the EFSA has recommended lowering the tolerable weekly intake to 1 mg kg^{-1} body weight/week.

In the case of heavy metals like Pb, concentrations were compared with the maximum limits established for other foods as set in Regulation (EC) No. 1881/2006, since in the current EU legislation, there are no maximum levels set for insects as food (Turck *et al*., 2021). Results were lower than those set for other related foods. For instance, the

maximum Pb limit for crustaceans is 0.50 mg kg^{-1}. The distribution of the mean Pb concentrations in the analyzed species reveals a significant difference ($p < 0.05$) between *T. molitor* (0.0105 ± 0.0002 mg kg^{-1}) and *A. domesticus* (0.1231 ± 0.0076 mg kg^{-1}). This finding may indicate interesting toxicological differences in the bioaccumulation capacity of toxic elements between species. Only a few studies have assessed Pb concentrations in crickets. Devkota & Schmidt (2000) found that Pb bioaccumulation was low compared to Cd or Hg due to its lower chemical activity, while Zhang *et al*. (2012) found a concentration factor of 0.43 – 0.85 in grasshoppers. This finding highlights the importance of controlling the feed used for rearing different types of insects. Differences in the metabolism and physiology between species may lead to differences in bioaccumulation rates, which are also influenced by seasonal variations and an insect's developmental stage (Fernandez-Cassi *et al*., 2019).

Determination of bioaccessible fractions

Table 3 shows the %BF for the three species. In all cases, the obtained %MB ranged from 90 to 110%, demonstrating the accuracy of the assay.

Table 3. *In vitro* bioaccessible fractions in farmed insects.

Sample	Bioaccesible fraction ± standard deviation (%)					
	Al	Cu	Fe	Mn	Se	Zn
T. molitor	1.2 ± 0.4	64.2 ± 5.1	92.6 ± 3.6	3.3 ± 0.2	45.0 ± 4.3	97.6 ± 5.7
L. migratoria	8.7 ± 0.9	47.2 ± 2.3	93.2 ± 0.8	5.4 ± 0.2	66.7 ± 1.1	86.0 ± 2.4
A. domesticus 1	7.7 ± 0.4	72.2 ± 3.9	46.2 ± 5.4	9.7 ± 0.3	55.8 ± 0.3	95.9 ± 3.6
A. domesticus 2 (control)	9.4 ± 0.2	68.4 ± 3.3	32.9 ± 0.5	10.3 ± 0.3	57.5 ± 2.9	97.6 ± 2.6

The mean %BF obtained for Al (1.2 to 9.4 %) for all three species is relatively low, < 10%, which constitutes an interesting finding from the toxicological point of view since this element is involved in the development of several diseases in humans (Klotz *et al*., 2017). For Cu, %BF values ranged from 47.2 to 72.2 %, the mean %BF for *L. migratoria* being statistically lower ($p < 0.05$) than *T. molitor* and *A. domesticus*. Iron showed the

highest %BF variation (32.9 to 93.2 %), being above 90% for *T. molitor* and *L. migratoria* values and almost twice that for *A. domesticus*, which is highly relevant from the nutritional point of view, considering the usual low bioaccessibility observed for this element and the fact that Fe deficiency is a worldwide health issue (WHO, 2020). It is important to highlight that Fe in insects is predominantly present as ferritin, a storage protein for Fe, thus each molecule is capable of containing thousands of Fe atoms in the ferrous state, which contributes to a higher bioaccessibility for this element when compared to other food sources (Mwangi *et al*. 2018). The results for Fe bioaccessibility for crickets in this work (32.9 to 46.2 %) agree with those previously reported by Latunde-Dada *et al.* (2016).

Results obtained for Mn showed a low bioaccessibility for this element, similar to that obtained for Al. The low %BF obtained for Mn (~10%) in crickets also agree with those previously reported by Latunde-Dada *et al.* (2016). Although Mn is an essential trace element, it poses recognized toxicity in humans with excessive intake. Thus, a relatively low bioaccessibility can be considered a safe condition, especially when total Mn levels are high.

The %BF of Se ranged between 45.0 and 66.7 %, which is interesting considering Se enrichment is a strategy employed in insect farming to benefit the nutrition of both animals and humans (Chen *et al*., 2023). Finally, %BF values obtained for Zn did not vary considerably between species, ranging from 86.0 to 97.6 %, which is outstanding from the nutritional point of view. All this information is highly relevant to providing new insights into insects' nutritional properties, considering the high consumption worldwide, especially in Eastern cultures. The differences in %BF observed between different elements may be due to the formation of more soluble species in some cases (Tissot & Machado, 2020). For instance, there is a long-known antagonism between Cu

and Zn and their interaction with proteins since food proteins are digested in gastrointestinal solutions and degraded into peptides or amino acids, forming complexes with Zn, increasing its bioaccessibility by enhancing its solubility (Iaquinta *et al*., 2021). Also, it has been reported that the bioaccessibility of minerals depends on the presence of both enhancer (ascorbic acid, organic acids, dietary proteins, polyphenols) and inhibitor (phytic acid, oxalates, polyphenols, dietary fiber) compounds (Jaiswal, Pathania, & Lakshmi, 2021). Food matrix inhibitors can form insoluble species with certain elements. For example, phytic acid can form insoluble complexes with Fe and Zn. Consequently, these elements may be less bioaccessible after digestion (Sulibursk a & Krejpcio, 2014).

It has been reported that chitin from insects can be partially digested in the human stomach in Eastern cultures by mammalian chitinase (Muzzarelli *et al*., 2012). However, other authors suggested that the low chitin intake in Western cultures may have reduced the expression of chitinase genes, thus resulting in loss of catalytic activity (Paoletti *et al*., 2009). Experts consider chitin an insoluble fiber that is not digested in the small intestine of humans to any significant degree, being excreted mainly unchanged. The fact that chitin can bind some minerals could be why low bioaccessibility is observed for some elements or even explain the differences between species, considering that different species may have different chitin amounts in their structure (Anastopoulos *et al*., 2017).

An appropriate intake of Cu, Fe, Mn, Se, and Zn is required for a healthy diet, according to its essentiality, based on the human body's requirements. Thus, the contribution of the studied insects to the recommended dietary allowance (RDA) of these elements was assessed. The RDA constitutes the average daily dietary intake level to meet the nutrient requirements of healthy individuals of a given group (NIH, 2023). The population sector selected for data interpretation comprised adult women over 19 years. The obtained results regarding the average contribution of the studied minerals (expressed in mg) to

362 the corresponding RDA are shown in Table 4. The average contribution to diet was
363 calculated as C (mg) = [Released Fraction (mg kg^{-1}) × 0.050 kg], while the coverage of
364 the RDA was calculated as CRDA (%) = [C (mg) / RDA] × 100.

365

366 **Table 4.** Nutritional data for daily consumption of insects per 50 g of dried material

Analyte	RDA (mg/day)	Average contribution to diet (mg) / Coverage of RDA (%)			
		T. molitor	*L. migratoria*	*A. domesticus* 1	*A. domesticus* 2
Cu	0.9	0.4 / 44.4	0.6 / 66.7	0.5 / 55.6	0.4 / 44.4
Fe	18	2,9 / 16.1	3.0 / 16.7	1.5 / 8.3	1.6 / 8.9
Mn	1.8	0.02 / 1.1	0.01 / 0.6	0.19 / 10.6	0.44 / 24.4
Se	0.055	0.003 / 5.45	0.004 / 7.3	0.006 / 10.9	0.004 / 7.3
Zn	8	7.4 / 92.5	6.8 / 85.0	9.3 / 116.3	9.2 / 115.0

367

368 From the data (Table 4), the studied insects constitute excellent sources of Cu and Zn to
369 the human diet, contributing significantly to the RDA, i.e., the consumption of 50 g of
370 insect (dry weight) per day would cover between 44.4 and 66.7 % of the RDA for Cu and
371 >85% for Zn. The consumption of 50 g of crickets would fully cover the RDA for Zn and
372 constitute a significant source of Zn in the human diet. The coverage of the RDA of the
373 other elements is more modest than in the case of Zn.

374 ***Nanoparticle characterization and determination***

375 Recent studies have revealed that several types of metal-containing NPs occur naturally
376 in the wings and thoracic regions, helping with aerodynamics during flight. Likewise, the
377 presence of magnetic iron nanoparticles in social insects acting as geomagnetic sensors
378 has been described (Bhattacharyya *et al*., 2010; Zhang & Liu, 2006). Mechanic lysis
379 assisted with ultrasound was successfully applied in this work to extract existing NPs in
380 insect samples. The extract obtained from *A. domesticus* 2 (Figure 1) exhibits spikes
381 corresponding to Fe NPs following the analysis by sp-ICP-MS. In this example, 209
382 events could be detected. However, after conducting a mass scan to identify NPs

consisting of other elements, only Al and Fe NPs were reliably detected. The transport efficiency, assessed by analyzing the colloidal Au NPs reference material, was 26.5 ± 1.8%, which allowed us to perform all the necessary calculations to characterize Al and Fe NPs following the method described by Corte-Rodríguez *et al*. (2020). The minimum intensity of an event to be distinguished from the background was set as the average of all data points plus three times their standard deviation in the iterative procedure previously described. Event intensities were then transformed into the mass of Al or Fe per event employing an external calibration using ionic elemental standards.

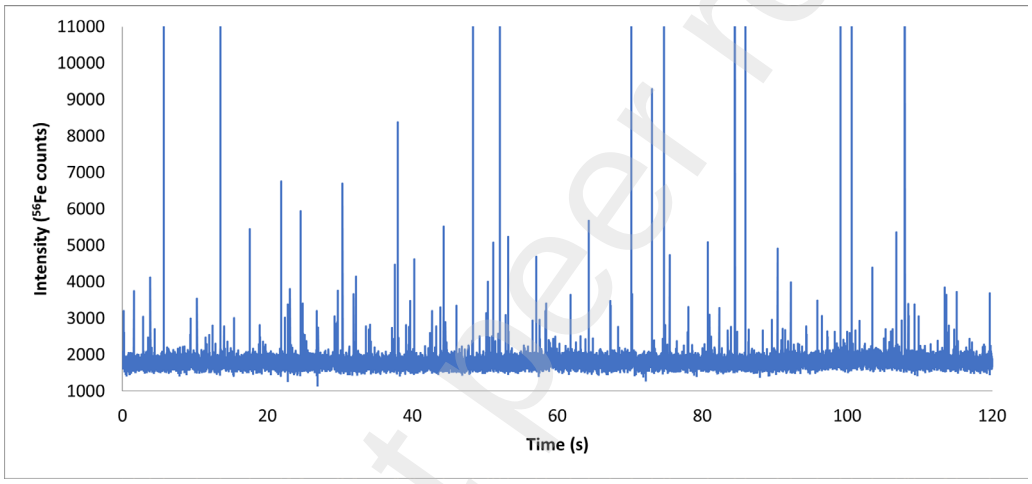


Figure 1. Iron events observed for *A. domesticus*-2 extracts after analysis by sp-ICP-MS.

Table 5 summarizes the number of detected events or spikes encountered in each insect extract and the mean NPs concentration per mg of insect. The number of events, and thus the concentration of NPs, increases in the order *T. molitor* < *L. migratoria* < *A. domesticus* 1 < *A. domesticus* 2 for both elements. After obtaining the corresponding mass of Fe and Al contained in the NPs detected for each element, the data sets were plotted as box and whisker plots (Figure 2). It is worth mentioning that the size of the nanoparticles could not be calculated from their mass because their exact composition and the chemical form of the Fe present in them are unknown, although we assume that higher mass derives from larger nanoparticles for the discussion.

403

404 **Table 5.** Summary of detected events and NPs concentration in the analyzed samples

Sample	Al		Fe	
	Detected events	Concentration (NPs mg^{-1})	Detected events	Concentration (NPs mg^{-1})
T. molitor	70	0.7×10^5	83	0.6×10^4
L. migratoria	193	2.1×10^5	116	0.9×10^4
A. domesticus 1	273	3.0×10^5	188	1.2×10^4
A. domesticus 2 (control)	300	3.2×10^5	209	1.5×10^4

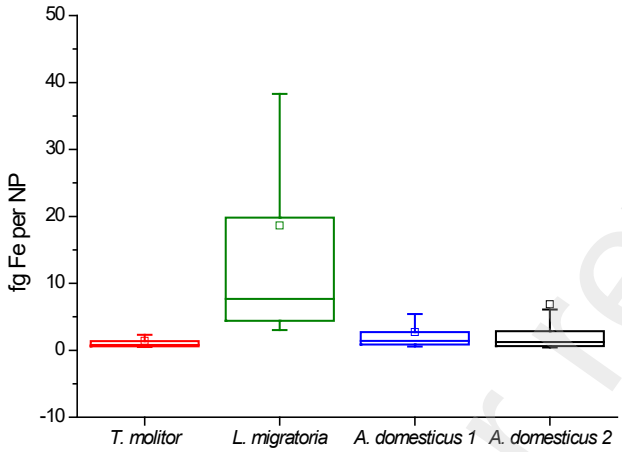
405

406 The mass of the FeNPs was relatively constant for *T. molitor* and *A. domesticus*, but the
407 mass, and therefore size, of the FeNPs, was significantly increased in the *L. migratoria*,
408 although the total Fe content in this sample was practically the same as the content of Fe
409 in *T. molitor*. In the case of Al, only the *A. domesticus 1* sample contained nanoparticles
410 with slightly higher Al content. In all cases, the obtained masses were higher than the
411 limit of detection, which was calculated as the minimum mass of particle that would be
412 possible to differentiate from the background. Therefore, the LODs were sample-
413 dependent, resulting in 0.5 fg of Fe and 48 ag of Al for *T. molitor*, 2.9 fg of Fe and 75 ag
414 of Al for *L. migratoria,* and 0.5 fg of Fe and 114 ag of Al for *A. domesticus*. Figure S2
415 depicts the mass of elements per NP frequencies for Fe and Al ones in the *A. domesticus*
416 *1* sample. As observed, there is a higher proportion of lower Fe containing NPs, with the
417 peak between 0.77 – 1.61 fg for Fe NPs and between 0.2 – 0.9 fg of Al for AlNPs.

418 To further prove the presence of Fe and Al nanoparticles in the extracts, particularly those
419 of small sizes that cannot be detected using single particle ICP-MS, the samples were
420 simultaneously analyzed employing reversed-phase HPLC-ICP-MS, following the
421 method of Helfrich *et al.* This method distinguishes small nanoparticles (< 50 nm) and
422 other low molecular mass species containing the element of interest. It operates on a size
423 exclusion-like mechanism, with the larger particles eluting first and the ionic species at

the end of the chromatogram (García Fernández *et al*., 2018, Rodríguez Pescador *et al*., 2022).

A)



B)

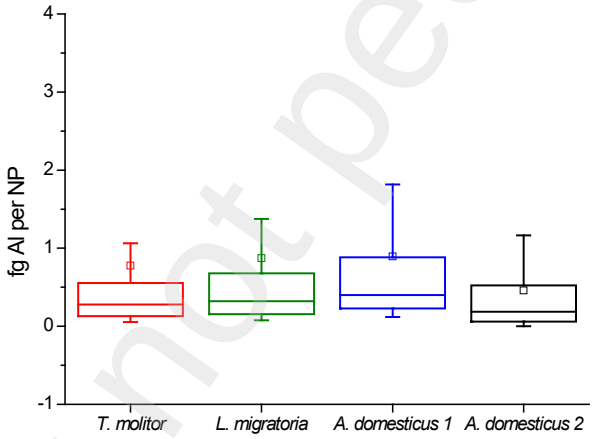


Figure 2. Boxplot graphs showing the mass distribution of the Fe (a) and Al (b) in NPs found in the analyzed insect samples.

As previously stated, the system was calibrated using commercial gold nanoparticles and ionic gold. Broad chromatographic peaks were obtained in all the samples for Al and Fe, as exemplified in the case of *A. domesticus 1* (see Figure 3) in the elution time region where retention times correspond to NPs < 30 nm, as deduced from the calibration with AuNPs. By comparing the Fe results with those previously obtained in cell models, the chromatographic peak between 250 and 300 seconds could correspond to Fe-ferritin

(about 12 nm of diameter), in agreement with previous studies, while the peak above 300 s could be assigned to smaller structures (< 5 nm). Similar profiles were observed in the other samples, as seen in Figure S3.

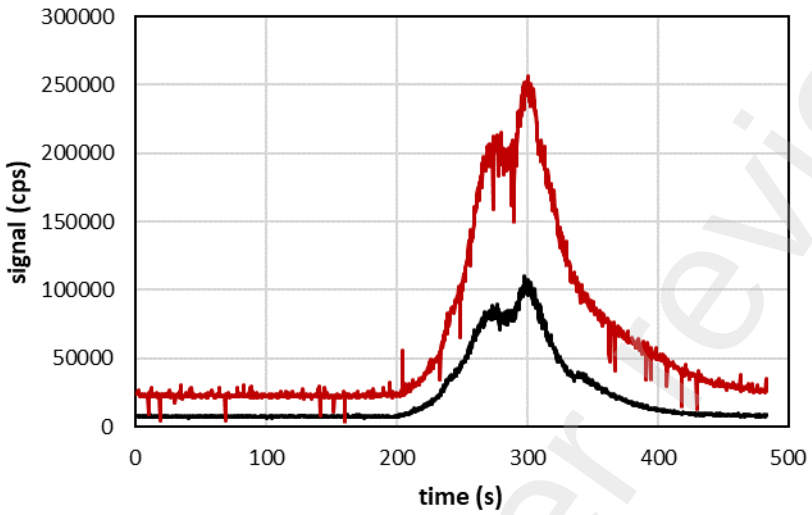


Figure 3. Chromatographic profiles for ^{57}Fe (black trace) and ^{27}Al (red trace) for (A) *A. domesticus* 1.

The peak broadening does not provide a specific size value for the detected NPs but instead indicates the presence of a wide dispersion of small NP sizes in the samples, as expected. Moreover, peaks with retention times closer to the ionic elution region (above 360 s) can represent the presence of low molecular mass species containing Al and Fe, which is expected due to the complex biological origin of the samples analyzed.

The same extracts were taken to TEM and HR-TEM to permit elemental analysis by EDX. Some of the obtained results are shown in Figure 4 for the samples corresponding to *A. domesticus* and *L. migratoria* extracts. As can be seen, nanostructures of different sizes containing either Fe, Al, or both metals can be observed and are responsible for the undefined chromatographic profile observed in Figure 4. However, these results confirm the formation of Fe and Al biogenic nanoparticles in the analyzed samples.

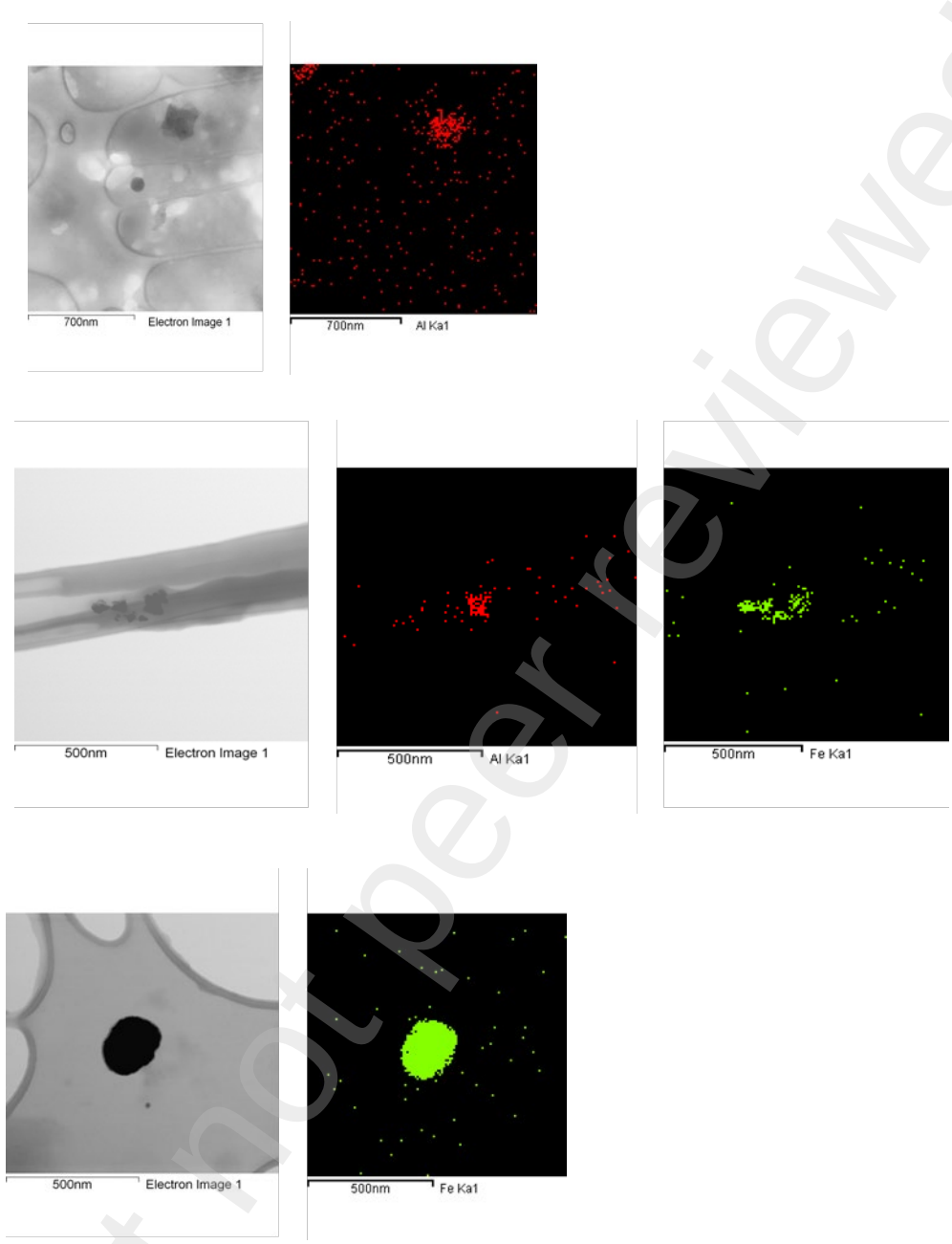


Figure 4. TEM images and EDX elemental analysis for structures found in extracts of A) *A. domesticus* with Al elemental analysis; B) *L. migratoria,* with Al and Fe elemental analysis respectively and C) *L. migratoria* only Fe in a larger structure

Conclusions

The characterization of the metal content and the *in vitro* bioaccessibility assay based on the INFOGEST protocol was successfully applied to three different types of edible insects. Zinc was the element that presented the highest %BF of all, ranging from 86.0 to

97.6 %, which can be related to the formation of more soluble species for this element. Also, in the case of Fe, high %BF ranging from 92.6 to 93.2 % were obtained for *T. molitor* and *L. migratoria*. The contribution of the studied elements to the RDA, considering a 50 g portion, showed that the studied insects constitute excellent sources of Cu and Zn to the human diet, contributing significantly to the RDA of women over 19 years, covering between 44.4 and 66.7 % of Cu RDA and above 85% of Zn RDA. Moreover, consuming 50 g of *A. domesticus* would fully cover the Zn RDA, demonstrating that this species could be a major source of this element in the diet. The analyzed insects incorporate increasing amounts of Se upon increasing exposure concentrations, which affects the incorporation of iron; however, they could be an additional source for Se supplementation.

This study found that only Fe and Al nanoparticles could be reliably measured among the accumulated elements by SP-ICP-MS. However, in combination with HPLC-ICP-MS, this technique revealed a heterogeneous distribution of these elements in a nanoparticulated form. In the case of *L. migratoria*, the mass of Fe per nanoparticle was significantly higher than that of the other samples. The bioaccessibility of the NPs would need to be further addressed in future studies.

Disclosure statement

The authors declare that there is no conflict of interest regarding the publication of this article.

Acknowledgements

Ignacio Machado would like to thank the Comisión Sectorial de Investigación Científica (CSIC, Uruguay), Agencia Nacional de Investigación e Innovación (ANII, Uruguay) and the Programa de Desarrollo de las Ciencias Básicas (PEDECIBA-Química, Uruguay).

This study was also supported by the Czech Science Foundation (GAČR, project number 21–47159L) and the Slovene Research Agency (Program Group P1-0143 and project J7-3155). The authors gratefully acknowledge the financial support from the Spanish MICINN (Spanish Ministry for Science and Innovation, Project Numbers PID2019-104334RB-I00 and PID2021-123854OB-I00) and FICYT (Grant number: SV-PA-21-AYUD/2021/51399). The instrumental support from Thermo Instrument is also acknowledged.

References

Alghuthaymi, M.A. (2022). Antibacterial action of insect chitosan/gum Arabic nanocomposites encapsulating eugenol and selenium nanoparticles. *Journal of King Saud University – Science*, 34, 102219. https://doi.org/10.1016/j.jksus.2022.102219

Álvarez-Fernández García, R., Corte-Rodríguez, M., Macke, M., LeBlanc, K.L., Mester, Z., Montes-Bayón, M., & Bettmer, J. (2020), Addressing the presence of biogenic selenium nanoparticles in yeast cells: analytical strategies based on ICP-TQ-MS. *Analyst*, 145, 1457. https://doi.org/10.1039/c9an01565e

Anastopoulos, I., Bhatnagar, A., Bikiaris, D.N., & Kyzas, G.Z. (2017). Chitin adsorbents for toxic metals: A review. *International Journal of Molecular Sciences*, 18, 114 https://doi.org/10.3390/ijms18010114

Belluco, S., Mantovani, A., & Ricci, A. (2018). Edible insects in a food safety perspective. In A. Halloran, R. Flore, P. Vantomme, & N. Roos (Eds.), *Edible insects in sustainable food systems* (pp. 109–126). Springer International Publishing.

Bhattacharyya, A., Bhaumik, A., Usha Rani, P., Mandal, S., & Epidi, T. T. (2010). Nano-particles - A recent approach to insect pest control. *African Journal of Biotechnology*, 9(24), 3489–3493.

Brodkorb, A., Egger, L., Alminger, M., Alvito, P., Assunção, R., Ballance, S., Bohn, *et al.* (2019). INFOGEST static in vitro simulation of gastrointestinal food digestion. *Nature Protocols*, 14(4), 991–1014. https://doi.org/10.1038/s41596-018-0119-1

Chen, Z., Lu, Y., Dun, X., Wang, X., & Wang, H. (2023). Research progress of selenium-enriched foods. *Nutrients*, 15(19), 4189. https://doi.org/10.3390/nu15194189

Corte-Rodríguez, M., Álvarez-Fernández, R., García-Cancela, P., Montes-Bayón, M., & Bettmer, J. (2020). Single cell ICP-MS using online sample introduction systems: Current developments and remaining challenges. *Trends in Analytical Chemistry*, 132, 116042. https://doi.org/10.1016/j.trac.2020.116042

Delgado Calvo-Flores, L., Garino, C., Moreno, F.J., & Broll, H. (2022). Insects in food and their relevance regarding allergenicity assessment. *EFSA Journal*, 20(S2), e200909. https://doi.org/10.2903/j.efsa.2022.e200909

Devkota, B., & Schmidt, G.H. (2000) Accumulation of heavy metals in food plants and grasshoppers from the Taigetos Mountains, Greece. *Agriculture, Ecosystems and Environment*, 78(1), 85–91. https://doi.org/10.1016/S0167-8809(99)00110-3

FAO. (2021). Looking at edible insects from a food safety perspective. Challenges and opportunities for the sector. Rome. https://doi.org/10.4060/cb4094en

Fernandez-Cassi, X., Supeanu, A., Vaga, M., Jansson, A., Boqvist, S., & Vagsholm, I. (2019). The house cricket (*Acheta domesticus*) as a novel food: a risk profile. *Journal of Insects as Food and Feed*, 5(2), 137–157. https://doi.org/10.3920/JIFF2018.0021

Gamborg, C., Röcklinsberg, H., & Gjerris, M. (2018). Sustainable proteins? Values related to insects in food systems. In A. Halloran, R. Flore, P. Vantomme, & N. Roos (Eds.), *Edible insects in sustainable food systems* (pp. 199–211). Springer International Publishing.

García Fernández, J., Sánchez-González, C., Bettmer, J., Llopis, J., Jakubowski, N., Panne, U., & Montes-Bayón, M. (2018). Quantitative assessment of the metabolic products of iron oxide nanoparticles to be used as iron supplements in cell cultures. *Analytica Chimica Acta*, 18, 24. https://doi.org/10.1016/j.aca.2018.08.003

Genchi, G., Lauria, G., Catalano, A., Sinicropi, M.S., & Carocci, A. (2023). Biological activity of selenium and its impact on human health. *International Journal of Molecular Sciences*, 24(3), 2633. https://doi.org/10.3390/ijms24032633

Heckmann, L.H., Andersen, J.L., Gianotten, N., Calis, M., Fischer, C.H., & Calis, H. (2018). Sustainable mealworm production for feed and food. In A. Halloran, R. Flore, P. Vantomme, & N. Roos (Eds.), *Edible insects in sustainable food systems* (pp. 321–328). Springer International Publishing.

Helfrich, A., Brüchert, W., & Bettmer, J. (2006).Size characterisation of Au nanoparticles by ICP-MS coupling techniques. *Journal of Analytical Atomic Spectrometry*, 21, 431-434. https://doi.org/10.1039/B511705D.

566 Iaquinta, F., Pistón, M., & Machado, I. (2021). *In vitro* bioaccessibility of Cu and Zn in cooked beef cuts.
567 *LWT - Food Science and Technology*, 150, 112027. https://doi.org/10.1016/j.lwt.2021.112027
568 Iaquinta, F., Rodríguez, N., & Machado, I. (2023). *In vitro* bioaccessibility of copper, iron, and zinc from
569 common meat substitutes, influence of exogenously added garlic/onion and contribution to the diet. *Journal*
570 *of Food Composition and Analysis*, 115, 104910. https://doi.org/10.1016/j.jfca.2022.104910
571 Jaiswal, A., Pathania, V., & Lakshmi, J. (2021). An exploratory trial of food formulations
572 with enhanced bioaccessibility of iron and zinc aided by spices. *LWT – Food Science and Technology*, 143,
573 111122. https://doi.org/10.1016/J.LWT.2021.111122
574 Jensen, K., Kristensen, T.N., Heckmann, L.H.L., & Sørensen, J.G. (2017). Breeding and maintaining high-
575 quality insects. In A. Van Huis, & J.K. Tomberlin (Eds.), *Insects as food and feed: From production to*
576 *consumption* (pp. 174–198). Wageningen Academic Publishers.
577 Klotz, K., Weistenhöfer, W., Neff, F., Hartwig, A., van Thriel, C., & Drexler, H. (2017). The health effects
578 of aluminum exposure. *Deutsches Ärzteblatt International*, 114(39) 653–659.
579 https://doi.org/10.3238%2Farztebl.2017.0653
580 Latunde-Dada, G.O., Yang, W., & Aviles, M.V. (2016). *In vitro* iron availability from insects and sirloin
581 beef. *Journal of Agricultural and Food Chemistry*, 64(44), 8420–8424.
582 https://doi.org/10.1021/acs.jafc.6b03286
583 Marone, P.A. (2016). Food safety and regulatory concerns. In A.T. Dossey, J.A. Morales-Ramos, & M.G.
584 Roja (Eds.), *Insects as sustainable food ingredients* (pp. 203–221). Elsevier.
585 Michel, P., & Begho, T. (2023). Paying for sustainable food choices: The role of environmental
586 considerations in consumer valuation of insect-based foods. *Food Quality and Preference*, 106, 104816.
587 https://doi.org/10.1016/j.foodqual.2023.104816
588 Muzzarelli, R.A.A., Boudrant, J., Meyer, D., Manno, N., DeMarchis, M., & Paoletti, M.G. (2012). Current
589 views on fungal chitin/chitosan, human chitinases, food preservation, glucans, pectins and inulin: A tribute
590 to Henri Braconnot, precursor of the carbohydrate polymers science, on the chitin bicentennial.
591 *Carbohydrate Polymers*, 87, 995–1012. https://doi.org/10.1016/j.carbpol.2011.09.063
592 Mwangi, M.N., Oonincx, D.G.A.B., Stouten, T., Veenenbos, M., Melse-Boonstra, A., Dicke, M., & Van
593 Loon, J.J.A. (2018). Insects as sources of iron and zinc in human nutrition. *Nutrition Research Reviews*,
594 31(2), 248–255. https://doi.org/10.1017/S0954422418000094

NIH (2023). Recommended Dietary Allowances and Adequate Intakes, Elements. Retrieved from https://www.ncbi.nlm.nih.gov/. Accessed December 6, 2023.

Oonincx, D.G.A.B. (2017). Environmental impact of insect production. In A. Van Huis, & J.K. Tomberlin (Eds.), *Insects as food and feed: From production to consumption* (pp. 79–93). Wageningen Academic Publishers.

Pace, H.E., Rogers, N.J., Jarolimek, C., Coleman, V.A., Higgins, C.P., & Ranville, J.F. (2011). Determining Transport Efficiency for the Purpose of Counting and Sizing Nanoparticles via Single Particle Inductively Coupled Plasma Mass Spectrometry. *Analytical Chemistry*, 83, 9361. https://doi.org/10.1021/ac201952t

Paoletti, M. G., Norberto, L., Cozzarini, E. & Musumeci, S. (2009). Role of chitinases in human stomach for chitin digestion: AMCase in the gastric digestion of chitin and chitotriosidase in gastric pathologies. In S. Musumeci, & M.G. Paoletti (Eds.), *Binomium chitin–chitinase: Recent issues* (pp. 339–358). Nova Biomedical Books.

Regulation (EU) 2015/2283 of the European Parliament and of the Council of 25 November 2015 on novel foods. Retrieved from https://eur-lex.europa.eu/. Accessed December 6, 2023.

Rodríguez Pescador, A., Gutiérrez Romero, L., Blanco-González, E., Montes-Bayón, M., & Sierra, L.M. (2022). Intracellular Biotransformation of Ultrasmall Iron Oxide Nanoparticles and Their Effect in Cultured Human Cells in Drosophila Larvae In Vivo. *International Journal of Molecular Sciences*, 23, 8788. https://doi.org/10.3390/ijms23158788

Roos, N. (2018). Insects and human nutrition. In A. Halloran, R. Flore, P. Vantomme, & N. Roos (Eds.), *Edible insects in sustainable food systems* (pp. 83–93). Springer International Publishing.

Sezer Tunçsoy, B. (2018). Toxicity of nanoparticles on insects: A Review. *Artıbilim: Adana Bilim ve Teknoloji Üniversitesi Fen Bilimleri Dergisi*, 1(2), 49-61.

Sikora, D., Proch, J., Niedzielski, P., & Rzymski, P. (2023). Elemental content of the commercial insect-based products available in the European Union. *Journal of Food Composition and Analysis*, 121, 105367. https://doi.org/10.1016/j.jfca.2023.105367

So, J., Choe, D.H., Rust, M.K., Trumble, J.T., & Lee, C.Y. (2023). The impact of selenium on insects. *Journal of Economic Entomology*, 116(4), 1041–1062. https://doi.org/10.1093/jee/toad084

Suliburska, J., & Krejpcio, Z. (2014). Evaluation of the content and bioaccessibility of iron, zinc, calcium and magnesium from groats, rice, leguminous grains, and nuts. *Journal of Food Science & Technology*, 51(3), 589–594. https://doi.org/10.1007/s13197-011-0535-5

Tissot, F., & Machado, I. (2020). Evaluation of essential and potentially toxic elements in popcorn: Study of cooking effect and *in vitro* bioaccessibility. *Journal of Food Measurement and Characterization*, 14, 1842–1849. https://doi.org/10.1007/s11694-020-00431-2

Turck, D., Castenmiller, J., De Henauw, S., Hirsch-Ernst, K.I., Kearney, J., Maciuk, A., Mangelsdorf, I., McArdle, H.J., Naska, A., Pelaez, C., Pentieva, K., Siani, A., Thies, F., Tsabouri, S., Vinceti, M., Cubadda, F., Frenzel, T., Heinonen, M., Marchelli, R., Neuhäuser-Berthold, M., Poulsen, M. Prieto Maradona, M., Schlatter, J.R., van Loveren, H., Azzollini, D., & Knuts, H.K. (2021). Safety of frozen and dried formulations from migratory locust (*Locusta migratoria*) as a novel food pursuant to Regulation (EU) 2015/2283. *EFSA Journal*, 19(7), 6667. https://doi.org/10.2903/j.efsa.2021.6667

Usman, M., Ayoub, A., Mustafa, G., Rasheed, K., Nadeem, M.K., Ashfaq, H., Usman Shahid, M., & Parveen, M. (2022). Nanotechnology: A new technology in insect and disease control. *Annals of R.S.C.B.*, 26(1), 1605–1615.

Van Huis, A. (2020). Insects as food and feed, a new emerging agricultural sector: a review. *Journal of Insects as Food and Feed*, 6(1), 27-44.

Van Huis, A. (2021). Welfare of farmed insects. *Journal of Insects as Food and Feed*, 7(5), 573-584.

Ververis, E., Boué, G., Poulsen, M., Monteiro Pires, S., Niforou, A., Thomsen, S.T., Tesson, V., Federighi, M., & Naska, A. (2022). A systematic review of the nutrient composition, microbiological and toxicological profile of *Acheta domesticus* (house cricket). *Journal of Food Composition and Analysis*, 114, 104859. https://doi.org/10.1016/j.jfca.2022.104859

WHO. (2020). WHO guidance helps detect iron deficiency and protect brain development. Retrieved from https://www.who.int/news/. Accessed December 6, 2023.

Zhang, G., Zhang, J., Xie, G., Liu, Z., & Shao, H. (2006). Cicada wings: A stamp from nature for nanoimprint lithography. *Small*, 2(12), 1440–1443. https://doi.org/10.1002/smll.200600255

Zhang, Z., Song, X., Wang, Q., & Lu, X. (2012). Cd and Pb contents in soil, plants, and grasshoppers along a pollution gradient in Huludao City, Northeast China. *Biological Trace Element Research*, 145, 403–410. https://doi.org/10.1007/s12011-011-9199-2