



Metal profiles and health risk assessment of the most consumed rice varieties in Spain

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ABSTRACT

There are numerous publications describing the mineral content of row rice and its impact on human health. However, there are scarce studies about the bioaccessibility and health risk assessment of metallic and metalloid elements from cooked and digested rice. Thus, the aims of the present study were i) to determine the concentration of toxic metals (Al, As, Cd, Pb) and trace and essential elements (Ni, Se, Cr, Cu, Mn and Zn) in cooked and digested rice; ii) to evaluate the adult daily intake and iii) to estimate the non-carcinogenic and carcinogenic human risk related to cooked and digested rice consumption. The bioavailability of the elements studied and their contribution to human health risk was obtained by an in vitro digestion method simulating the human digestion. The results obtained evidenced that Pb had the lowest bioavailability, in contrast with As, which had the highest. Concerning the non-carcinogenic risk test, all values obtained in cooked and digested rice were below the toxic threshold. Furthermore, Pb was the smallest contributor to carcinogenic risk, while Cr-values are the closest to the limit (10^{-4}). Thus, it could be stated that human metallic exposure through rice consumption is overestimated when metals are measured in row rice.

1. Introduction

Nowadays, food safety has become a major concern worldwide. A wide range of diseases is directly linked to the presence of contaminants in food. Environmental contaminants such as dioxins, polychlorinated biphenyls (PCBs), heavy metals and metalloids, phthalates, and bisphenol A are under strict supervision to avoid their presence in the food chain (Kamala and Kumar, 2018). Both acute and chronic exposure to metallic elements can lead to toxic effects, depending on the time and concentration of exposure (Rajkumar and Gupta, 2021). Some metallic elements such as cadmium (Cd), lead (Pb) or chromium (Cr), and metalloids such as arsenic (As) can represent a serious public concern in animal and human health (Engwa et al., 2019; Tchounwou et al., 2012). Effects on the digestive, renal, nervous, immune, and cardiovascular dysfunction, skin disorders, teratogenic effects, or cancer are some examples of toxicity caused by the exposure to these elements (Dastgiri et al., 2010; Martinez et al., 2011; Niño et al., 2019; Rahman et al., 2009; Singh et al., 2011; Tchounwou et al., 2012). In this regard, continued

exposure to metals such as cobalt (Co) and aluminum (Al) is mainly related to neurotoxic effects such as Alzheimer's and Parkinson's diseases (Catalani et al., 2012; Exley, 2016; Igbokwe et al., 2019; Simonsen et al., 2012; Skalny et al., 2021). Moreover, essential metals such as copper (Cu), selenium (Se), or manganese (Mn) after exposure to concentrations above the functional threshold can generate neurotoxicity, cardiovascular toxicity, hepatotoxicity, or even infant mortality (Hadrup and Ravn-Haren, 2020; MacFarquhar et al., 2010; O'Neal and Zheng, 2015; Peres et al., 2016; Tapiero et al., 2003).

Metal contamination can have a natural or anthropogenic origin, and it is characterized by its persistence in soil. Ash from volcanoes or forest fires, waters contaminated by the uncontrolled use of agrochemical fertilisers, or industrial waste, among others, lead to the accumulation of metals in the environment and thus their entrance in the food chain. Although metallic and metalloid elements can be bioaccumulated after their inhalation or through dermal contact, 90% of the bioaccumulated metals came from their intake (Başaran, 2022a; Zhang et al., 2019).

Rice (*Oryza sativa* L.) is a staple food for more than half of the world's

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population, with an annual production in 2018 of 510 million tons (Olayiwola et al., 2020). Furthermore, for a high percentage of the population, it is the only source of protein and carbohydrates. Rice, as other cereals, has a high capacity to accumulate metallic elements (Zulkaffee et al., 2021), which could lead to a risk for human health. These metallic elements are bioaccumulated in the edible parts of rice plants and, thus, reach the trophic chain. Due to high rice consumption and increased concern about food safety, it is vital to perform exhaustive controls to evaluate the concentration of metallic elements.

The bioaccessible form of metallic and metalloid elements is defined as “the fraction of total metallic and metalloid elements ingested that is available to be absorbed by the digestive tract”. Determination of bio-accessible forms of metallic and metalloid elements can be performed through *in vitro* digestion models (Brodkorb et al., 2019). Bio-accessibility determination has the aim of simulating a realistic physiological scenario, since the effect of actual human health risk bioaccumulation of metals is overestimated (Versantvoort et al., 2005).

Publications describing the mineral content of rice in Spain and its impact on human health are scarce. Some similar studies with other cereals can be found (Rubio-Armendáriz et al., 2021), although there are no bioaccessibility and health risk assessment studies of metallic and metalloid elements from rice in Spain. Only some similar bio-accessibility studies in rice have been carried out in other countries (Omar et al., 2013; Praveena and Omar, 2017). As far as we know the present study is the first in Spain to analyse the bioaccessibility of fourteen of the most consumed rice varieties in this country in order to get a better understanding of the impact of the accumulation of metals from rice on human health. Taking this into account, the main goals of the present study were: i) to determine the concentration of ten metallic and metalloid elements (Al, As, Cd, Cr, Cu, Mn, Ni, Pb, Se, and Zn) in cooked and digested rice; ii) to evaluate the adult daily intake and iii) to estimate the non-carcinogenic and carcinogenic risk to human health related to cooked and digested rice consumption.

2. Materials and methods

2.1. Chemicals, reagents and standards

All reagents used were of analytical grade or higher, and were purchased from Panreac (Barcelona, Spain) and Sigma-Aldrich (Madrid, España). Type I water ($> 18 \text{ M}\Omega \text{ cm}$) was obtained from an Elix-Milli-Q water purification system (Millipore, Bedford, MA, USA). All labware used was made of plastic and cleaned by soaking at least 5 h in 20% (v/v) HNO_3 . Nitric acid was obtained in-house by double distillation in the DST-1000 sub-boiling system purchased from Savillex (Eden Prairie, MN, USA). Following the directions stated in EPA method 200.8 (EPA, 2000), labware was rinsed three times with type I water and dried in a laminar flow hood. Reagent blank consisted of 1% (v/v) HNO_3 . ICP multielement calibration standard solution ICP-MS-200.8-CAL1-1 was purchased from AccuStandard (New Haven, CT, USA) and diluted as needed to obtain a working range of 0–250 mg/g. The digestive enzymes, i.e. pepsin (porcine, 367 units per mg solid, measured as TCA-soluble products using haemoglobin as a substrate) and pancreatin (porcine, $8 \times \text{USP}$ specifications of amylase, lipase, and protease), were also purchased from Sigma-Aldrich. Salts ($\text{CaCl}_2(\text{H}_2\text{O})_2$, NaOH, HCl, KCl, KH_2PO_4 , NaHCO_3 , NaCl, $\text{MgCl}_2(\text{H}_2\text{O})_6$, $(\text{NH}_4)\text{CO}_3$, $\text{CaCl}(\text{H}_2\text{O})_2$, and Tris-HCl buffer) were obtained from Sigma-Aldrich.

2.2. Sampling and study design

A total of 42 samples (triplicate of each of the 14 varieties) of the most widely marketed and consumed rice varieties in Spain were analyzed. The samples were collected from the most popular Spanish supermarkets, taking samples of all the brands, including those commercialized by the supermarket under their own brand. When the brand had more than one type of grain, all types were collected. The

samples were collected in triplicate, taking the same brand for the triplicate and ensuring the same batch whenever possible, noting the identification data of each sample (origin, type of grain, concentration of starch, year of collection, color of the grain and aromaticity) (Table 1). To remove the metallic environmental contamination, all rice samples were washed twice with deionised water and dried at 65°C for 48 h in a furnace. In all cases, the assays were carried out in triplicate.

2.3. Cooking process

One triplicate of samples of each rice variety was cooked before being subjected to the *in vitro* digestion process to calculate the amount of metallic and metalloid elements absorbed in the digestive tract. Regarding the cooking process, 100 g of each sample was boiled, at a ratio of 1:2 (v/w) for 15 min and slowly cooled. All cooked samples were dried at 65°C for 48 h in a furnace to obtain a constant weight. Samples were kept in a desiccator until the following step.

2.4. *In vitro* digestion process

Five grams of each cooked rice sample were digested following a standard *in vitro* digestion method previously described by Brodkorb et al. (2019). Briefly, the method consists of an *in vitro* simulation of human digestion process, which is subdivided into three stages or phases. i) Oral phase, in which the mastication process was simulated in a ceramic mortar to avoid the presence of metallic elements. The simulated salivary fluid (SSF) electrolyte stock solution (pH 7) was added to rice in a final proportion of 50/50 (w/v). Human salivary-amylase (Enzyme Commission (EC) number: 3.2.1.1) and CaCl_2 were combined with SSF to obtain a final concentration of 75 U/mL and 0.75 U/mL, respectively. Samples were incubated in a Q5000 shaker (Thermo Fisher Scientific Inc. Waltham, MA, USA) at 37°C and 95 rpm for 2 min. ii) Gastric phase, where the stock electrolyte solution of simulated gastric fluid (SGF) (pH 3) is combined with five parts of the oral bolus until a 50/50 bolus / SGF ratio is obtained (w/v). Porcine pepsin (EC 3.4.23.1) and CaCl_2 were added to obtain 2000 U/mL and 0.075 nM in the final concentration. Samples were incubated in a Max Q5000 shaker at 37°C and 95 rpm for 2 h. iii) Intestinal phase, where five parts of gastric chime were mixed with four parts of the stock electrolyte solution of the Simulate Intestinal Fluid (SIF) (pH 7) in a final gastric chime/SIF relationship of 50/50 (w/v). In this case, CaCl_2 was added until a final concentration of 0.3 mM was achieved. Furthermore, a mixture of bile extract and pancreatin (trypsin activity 100 U/mL) was added to the gastric chime/SIF combination. The samples were incubated in a Max Q5000 shaker at 37°C and 100 rpm, for 2 h. After the digestive process, whole samples were centrifuged at 30,000 g (Eppendorf 5430/ 5430 R) for 2 hours to obtain an aqueous micellar phase, in which the absorbable metallic elements are found. The composition of each electrolyte solution from each simulated fluid stock is detailed in our previous work Aguilera-Velázquez et al. (2021).

2.5. Bioavailability

The estimation of the fraction of an ingested biocomponent (metallic and metalloid elements in our case) that becomes accessible for absorption through the epithelial layer of the gastrointestinal tract (GIT) or bioavailability was estimated using Eq. (1).

$$\text{BioaccessibilityMetals} = \frac{\text{mgmetalindigestiblefraction}}{\text{mgmetalincokedrice}} \times 100 \quad (1)$$

2.6. Metallic elements determination

2.6.1. Sample treatment

One gram of each sample, cooked and digested rice, was placed in a pressure vessel previously decontaminated. Then, 5 mL HNO_3 65%

Table 1
Origin and type of grain of the rice samples analyzed.

Rice varieties	Origin	Type of grain	Concentration of starch	Collecting samples	Colour	Aromatic
Barone	Greek	Short	High	2020	White	No
Guadiamar	Spain	Short	High	2020	White	No
Hispamar	Spain	Short	High	2020	White	No
Marisma	Spain	Short	High	2020	White	No
Perlado	Spain	Short	High	2020	White	No
Piñana	Spain	Short	Low	2020	White	No
Sendra	Spain	Short	High	2020	White	No
Sole	Italy	Short	High	2021	White	No
Barone	Greek	Short	High	2020	White	No
Basmati	India	Long	Low	2021	White	Yes
Jasmine	Vietnam	Long	Low	2020	White	Yes
Puntal	Spain	Long	Low	2020	White	Yes
Memby	Argentina	Long	Low	2021	White	Yes
Sona Masoori	India	Long	Low	2021	White	Yes
Thaiparla	Spain	Long	Low	2021	White	Yes

*Long grain, 6.61–7.50 mm; short grain < 6.61 mm (International Rice Research Institute, IRRI). Low starch content, 10–20%; Intermediate starch content, 20–25%; High starch content, > 25% (Chatterjee, L. and Das, P., (2018)).

+ 5 mL H₂O + 1 mL H₂O₂ 30% were added to each vessel, and the digestion program was run (Table S1). (Aguilera-Velázquez et al., 2021).

2.6.2. Analytical methods

Multielemental analysis was carried out by ICP-MS (model 8800 ICP-MS from Agilent Technologies). Some of the most relevant operating conditions of ICP-MS, such as instrumental parameters, LOD and LOQ values, or the isotopes analysed for each element are indicated in Tables S2–S7. Also, calibration curves are included as Figs. S1–S10.

2.7. Human health risk assessment

The human health risk associated with the accumulation of metallic and metalloid elements can be divided into two categories, non-carcinogenic and carcinogenic health risks (Basaran, 2022).

2.7.1. Calculation of daily intake

Estimated daily rice intake (EDI) was calculated according to Eq. (2) shown below,

$$EDI = \frac{(EF \times ED \times FIR \times MC)}{(BW \times AT)} \quad (2)$$

where EF is the exposure frequency (in this case 365 days/year), ED is the duration of the exposition for a Spanish adult (54 years), RF is the food ingestion rate (10 g of rice/day per capita) (Ministerio de Agricultura, 2018). The MC value is the metal concentration found in rice (mg/g dry weight), BW would correspond to average Spanish body weight (75 kg) (Evers and Hughes, 2002). Moreover, AT is the averaging time for both effects: non-carcinogenic risk (54 years x 365 days) and carcinogenic risk (70 years x 365 days) (Sharafi et al., 2019b).

2.7.2. Non-carcinogenic risk assessment

To estimate the non-carcinogenic risk associated with metallic element intake, the Target Hazard Quotient (THQ) was calculated according to Eq. (3) (Başaran, 2022b; Chien et al., 2002).

$$THQ = \frac{EDI}{RfD} \quad (3)$$

In this equation, RfD is the Oral Reference Dose expressed in (mg/kg day), which values used in this study are listed in Table S8 (USEPA, 2016). As EPA has not derived a reference dose (RfD) or reference concentrations (RfC) for Pb or total Cr, thus, THQ of these elements were not calculated.

Total Target Hazard Quotient (TTHQ) was used to assess the non-carcinogenic risk associated with the entire group of metallic elements. It is expressed as the sum of the individual THQs of each metal

and is calculated according to Eq. (4). If the THQ or TTHQ values were less than one, the non-carcinogenic risk associated with accumulated metallic elements would be considered below the chronic toxicity threshold. However, if one or more of these factors were higher than one, the non-carcinogenic risk would be considered as unacceptable.

$$TTHQ = \sum THQ_{metallic\ elements} \quad (4)$$

2.7.3. Carcinogenic risk assessment

The incremental lifetime cancer risk (ILCR) method was used to evaluate the carcinogenic risk associated with long-term exposure of metallic elements, following Eq. (5).

$$ILCR = EDIXOSF \quad (5)$$

From this equation, OFS is the cancer slope factor, which is understood as the risk of developing cancer throughout life by exposure to carcinogenic elements at a concentration of 1 mg/kg of body weight per day. The OFS values of the carcinogenic element are described in Table S9. As EPA has not derived an OFS for Pb, Cd or total Cr, the ILCR of these elements were not calculated. In the rest of the cases, ILCR > 10⁻⁶ represents very low or acceptable risk, ILCR: 10⁻⁶ – 10⁻⁵ represents low risk, ILCR: 10⁻⁵-10⁻⁴ means medium risk, ILCR: 10⁻⁴-10⁻³ corresponding to a high risk and ILCR: > 10⁻³ poses a very high risk.

2.8. Statistical analysis

Each data was expressed as the mean ± standard deviation (SD). The normality of continuous variables in groups was determined by the Shapiro–Wilk test. Results were statistically analysed using Analysis of Variance (ANOVA) tests; a value of P ≤ 0.05 was considered statistically significant. Data were analysed using SPSS for Windows (24.0).

3. Results and discussion

3.1. Metallic elements concentration and bioavailability

The mean concentration of metallic and metalloid elements expressed in mg/kg and the standard deviation (SD) of the cooked and digested rice samples are shown in Tables 2 and 3, respectively.

Taking into account that there are only a few studies about metallic levels in cooked and digested rice, our results will be also compared with those that detected metals in raw rice or cereals. In general, all data obtained are within the same order of magnitude as the data provided by other researchers in raw, cooked, or digested rice (Kukusamude et al., 2021; Sharafi et al., 2019a; Zulkafflee et al., 2019). Specifically, the toxic metals analyzed followed the descending order of Al > Cr ≥ As > Ni > Pb > Cd, both in cooked and digested rice, similar to those

Table 2

Concentration of metallic elements in cooked rice. Results are expressed as mean±standard deviation (mg/kg) (n = 3).

Rice varieties	Al	Cr	Mn	Ni	Cu	Zn	As	Se	Cd	Pb
Barone	16.00 ± 0.10 a	0.44 ± 0.01 a	47.00 ± 0.44 ab	0.28 ± 0.16 ab	12.00 ± 0.10 cd	130.00 ± 0.00 abc	0.73 ± 0.01 ab	0.42 ± 0.01 b	0.12 ± 0.00 cd	0.19 ± 0.00 c
Basmati	8.10 ± 0.15 a	0.27 ± 0.01 a	65.00 ± 1.50 d	0.24 ± 0.02 e	97.00 ± 0.07 de	79.00 ± 0.89 d	6.00 ± 0.12 f	0.26 ± 0.01 a	0.03 ± 0.00 cd	0.10 ± 0.00 bc
Guadamar	37.00 ± 1.00 b	0.84 ± 0.04 a	58.00 ± 2.10 a	0.30 ± 0.24 bc	19.00 ± 1.00 ab	190.00 ± 7.00 ab	0.30 ± 0.01 a	0.56 ± 0.00 a	0.05 ± 0.00 a	0.68 ± 0.02 abc
Hisparmar	8.00 ± 0.17 a	0.35 ± 0.02 a	33.00 ± 0.15 bc	0.14 ± 0.01 ab	7.50 ± 0.33 bcd	45.00 ± 1.70 ab	0.14 ± 0.01 a	0.21 ± 0.01 a	0.03 ± 0.00 ab	0.07 ± 0.00 ab
Jasmine	11.00 ± 0.19 a	0.53 ± 0.02 a	22.00 ± 0.21 ab	0.34 ± 0.01 cd	6.60 ± 0.10 abc	86.00 ± 1.00 bc	0.24 ± 0.00 a	0.30 ± 0.01 ab	0.05 ± 0.00 c	0.11 ± 0.00 ab
Marisma	8.40 ± 0.14 a	0.35 ± 0.01 a	33.00 ± 0.31 ab	0.15 ± 0.00 abc	11.00 ± 0.14 e	61.00 ± 0.47 abc	0.14 ± 0.00 a	0.22 ± 0.00 a	0.02 ± 0.00 a	0.07 ± 0.00 a
Memby	11.00 ± 0.01 a	0.57 ± 0.00 a	66.00 ± 0.63 d	0.90 ± 0.01 f	12.00 ± 0.19 de	10.00 ± 0.63 abc	1.10 ± 0.01 bc	0.33 ± 0.01 a	0.04 ± 0.00 de	0.13 ± 0.00 ab
Perlado	14.00 ± 0.23 a	0.73 ± 0.02 a	0.04 ± 0.00 ab	0.00 ± 0.00 a	7.90 ± 0.07 abc	91.00 ± 0.91 ab	0.20 ± 0.01 a	0.38 ± 0.01 a	0.02 ± 0.00 ab	0.15 ± 0.00 ab
Piñana	8.20 ± 0.14 a	0.62 ± 0.01 b	30.00 ± 0.71 ab	0.48 ± 0.01 e	5.60 ± 0.09 ab	49.00 ± 0.43 a	0.14 ± 0.00 a	0.26 ± 0.06 a	0.02 ± 0.00 a	0.08 ± 0.00 ab
Puntal	7.30 ± 0.15 a	0.31 ± 0.00 a	28.00 ± 0.30 ab	0.10 ± 0.00 abc	7.40 ± 0.08 cd	52.00 ± 0.15 ab	0.16 ± 0.00 a	0.27 ± 0.01 a	0.02 ± 0.00 b	0.08 ± 0.00 abc
Sendra	6.90 ± 0.15 a	0.29 ± 0.01 A	51.00 ± 1.50 cd	0.15 ± 0.00 ab	8.50 ± 0.10 bcd	69.00 ± 1.50 abc	0.16 ± 0.00 a	0.27 ± 0.01 a	0.02 ± 0.00 ab	0.07 ± 0.00 bc
Sole	1.00 ± 0.17 a	0.61 ± 0.00 a	38.00 ± 0.41 bc	0.74 ± 0.02 ab	4.60 ± 0.08 a	0.08 ± 0.00 abc	0.40 ± 0.00 ab	0.26 ± 0.01 a	0.07 ± 0.00 f	0.10 ± 0.00 ab
Sona Masoori	1.00 ± 0.60 a	0.54 ± 0.02 a	0.02 ± 0.00 ab	0.30 ± 0.00 d	8.80 ± 0.60 de	0.07 ± 0.00 abc	0.20 ± 0.00 a	0.46 ± 0.02 c	0.09 ± 0.00 f	0.09 ± 0.00 ab
Thaiparla	0.01 ± 0.00 a	0.29 ± 0.01 a	46.00 ± 0.27 abc	0.26 ± 0.03 a	6.90 ± 0.13 acb	0.08 ± 0.00 abc	0.13 ± 0.09 c	0.35 ± 0.00 a	0.04 ± 0.00 e	0.13 ± 0.00 abc
Average	10.00	0.47	44.10	0.36	15.00	77.60	0.26	0.35	0.04	0.14
Standard deviation	8.70	0.20	15.00	0.25	23.00	42.20	0.20	0.09	0.03	0.15

Different letters in the same columns indicate significant differences by ANOVA test ($p \leq 0.05$).

obtained by Rubio-Armendáriz et al. (2021) and Brizio et al. (2016) in cereals from Canary Islands (Spain) and Italy, respectively. In the present study, Al is the toxic metal that reached the maximum value in Guadamar variety from Spain (37 ± 1 mg/kg in cooked and 11 ± 3 mg/kg in digested samples). However, despite the toxicological considerations of this neurotoxic element, current European legislation does not include maximum levels of Al in food. Furthermore, the highest concentration of Pb (0.68 ± 0.02 mg/kg in cooked rice and 0.19 ± 0.01 mg/kg in digested one) was detected also in Guadamar variety. However, the highest As concentration was detected in Basmati rice from India (6.00 ± 0.12 mg/kg in cooked rice and 4.10 ± 0.01 mg/kg in digested one), which is in agreement with some studies that showed As as the main contaminant in Indian soils (Khandare et al., 2021). These results agree with those obtained by Zulkafflee et al., (2019) who detected a range of 0.20–0.50 mg/kg and 0.19–0.54 mg/kg of Pb and As, respectively, in digested rice. However, heavy metal concentrations detected in 22 varieties of rice cooked and digested from Malaysia were lower than those detected in the present study. In Malaysian rice, As was the metalloid detected in lower quantities (around 0.09 mg/kg in cooked rice and 0.02 mg/kg in digested ones), while Al and Cr were the toxic metal detected at the highest concentrations, although in lower levels than in the present study (0.14–17 mg/kg and 0.60–9.10 mg/kg, respectively in cooked rice, and around 0.88 mg/kg and 0.10 mg/kg,

respectively, in digested ones) (Praveena and Omar, 2017).

The statistical analysis did not show significant differences ($P > 0.05$) in the concentrations of Al, Cr, Mn, and Se between all cooked rice in our study environment. However, significant differences in levels of Cu, Zn, As, Cd, and Pb within the varieties employed were detected. These differences could be due to the ability of each rice variety to bioaccumulate these metals in combination with their concentration in the croplands. In digested rice, there are significant differences in all metals except Pb due to the different digestibility degree in each rice variety.

However, when comparing metallic concentration between short and long-grain rice, the values are very similar and show no significant differences except Al. Short-grain rice presented a hundred-fold higher Al-level ($100 \cdot 10^{-4} \pm 93 \cdot 10^{-4}$ mg/kg) than the long grain once ($1.1 \cdot 10^{-4} \pm 17 \cdot 10^{-4}$ mg/kg) (Table 4).

Each rice variety shows a different degree of digestibility, leading to a different degree of bioavailability of the metallic and metalloid elements is shown in Table 5. Bioavailability was calculated as the percentage of metals found in the absorbed phase (in vitro digestion methods) in relation to the metals measured in the cooked rice. The mean values of the bioavailability of the metallic elements of all varieties studied decrease in the following order: As>Al>Ni>Mn>Cu>Cd>Zn>Cr>Se>Pb. Inorganic arsenic was the

Table 3
Concentration of metallic elements in the digestible fraction of digested rice. Results are expressed as mean ± standard deviation (mg/kg) (n = 3).

Rice varieties	Al	Cr	Mn	Ni	Cu	Zn	As	Se	Cd	Pb
Barone	7.00 ± 0.05 e	0.20 ± 0.00 ab	21.00 ± 0.10 c	0.13 ± 0.01 bc	5.30 ± 0.01 e	59.00 ± 0.50 gh	0.33 ± 0.00 c	0.19 ± 0.00 cde	0.05 ± 0.00 f	0.09 ± 0.00 a
Basmati	5.50 ± 0.10 bc	0.19 ± 0.00 a	44.00 ± 1.00 e	0.16 ± 0.01 d	6.5 ± 0.01 f	54.00 ± 0.60 f	4.10 ± 0.01 f	0.17 ± 0.01 d	0.02 ± 0.00 c	0.07 ± 0.00 a
Guadamar	11.00 ± 0.30 f	0.24 ± 0.01 cde	17.00 ± 0.60 b	0.09 ± 0.01 a	5.30 ± 0.30 e	54.00 ± 0.20 fg	0.09 ± 0.00 a	0.16 ± 0.00 bcd	0.01 ± 0.01 ab	0.19 ± 0.01 a
Hisparmar	4.80 ± 0.10 ab	0.02 ± 0.01 abc	20.00 ± 0.90 c	0.09 ± 0.00 a	4.50 ± 0.20 cd	27.00 ± 0.10 a	0.08 ± 0.00 a	0.13 ± 0.01 a	0.02 ± 0.00 b	0.00 ± 0.00 a
Jasmine	5.10 ± 0.09 abc	0.26 ± 0.01 de	11.00 ± 0.10 a	0.16 ± 0.01 de	3.20 ± 0.05 b	41.00 ± 0.50 cd	0.10 ± 0.00 a	0.14 ± 0.00 ab	0.02 ± 0.00 c	0.05 ± 0.00 a
Marisma	5.30 ± 0.09 abc	0.22 ± 0.00 bcd	21.00 ± 0.20 c	0.09 ± 0.00 a	7.10 ± 0.01 f	39.00 ± 0.31 bc	0.09 ± 0.00 ab	0.14 ± 0.00 ab	0.01 ± 0.00 a	0.04 ± 0.00 a
Memby	5.10 ± 0.04 abc	0.27 ± 0.02 ef	31.00 ± 0.30 d	0.43 ± 0.01 h	5.60 ± 0.09 e	51.00 ± 0.31 ef	0.52 ± 0.00 d	0.16 ± 0.00 bc	0.02 ± 0.00 b	0.06 ± 0.00 a
Perlado	6.30 ± 0.10 de	0.32 ± 0.01 g	17.00 ± 0.30 b	0.31 ± 0.00 f	3.50 ± 0.00 b	40.00 ± 0.40 c	0.09 ± 0.00 a	0.17 ± 0.01 bcd	0.01 ± 0.00 a	0.07 ± 0.00 a
Piñana	5.70 ± 0.10 cd	0.43 ± 0.00 h	21.00 ± 0.50 c	0.34 ± 0.01 fg	3.90 ± 0.06 bc	34.00 ± 0.30 b	0.10 ± 0.00 ab	0.18 ± 0.00 cd	0.01 ± 0.00 a	0.06 ± 0.00 a
Puntal	4.80 ± 0.10 ab	0.20 ± 0.00 abc	18.00 ± 0.20 bc	0.08 ± 0.00 a	4.90 ± 0.05 de	34.00 ± 0.10 b	0.10 ± 0.00 abcde	0.18 ± 0.00 cd	0.01 ± 0.00 ab	0.05 ± 0.00 a
Sendra	4.60 ± 0.10 a	0.20 ± 0.01 ab	34.00 ± 1.00 d	0.10 ± 0.00 e	5.70 ± 0.07 de	46.00 ± 1.00 de	0.11 ± 0.00 ab	0.18 ± 0.01 cd	0.01 ± 0.00 a	0.05 ± 0.00 a
Sole	5.00 ± 0.08 abc	0.29 ± 0.01 fg	19.00 ± 0.20 bc	0.36 ± 0.01 g	2.20 ± 0.00 a	37.00 ± 0.50 bc	0.21 ± 0.00 bc	0.13 ± 0.01 a	0.03 ± 0.00 d	0.05 ± 0.00 a
Sona Masoori	5.10 ± 0.30 abc	0.27 ± 0.01 ef	9.40 ± 0.50 a	0.15 ± 0.01 cd	4.40 ± 0.30 cd	34.00 ± 2.00 b	0.1 ± 0.00 ab	0.23 ± 0.00 e	0.04 ± 0.00 e	0.05 ± 0.00 a
Thaiparla	5.00 ± 0.10 abc	0.22 ± 0.00 abc	0.34 ± 0.20 d	0.19 ± 0.00 e	5.10 ± 0.00 de	0.60 ± 0.10 h	0.97 ± 0.01 e	0.26 ± 0.00 f	0.32 ± 0.00 d	0.10 ± 0.00 a
Average	5.74	0.26	22.67	0.19	4.80	43.57	0.23	0.18	0.02	0.06
Standard deviation	1.60	0.06	9.60	0.11	1.30	10.40	0.03	0.04	0.01	0.04

Different letters in the same columns indicate significant differences by ANOVA test (p ≤ 0.05).

Table 4
Mean and SD of metal in relation with type of grain (mg/Kg) (n = 3).

	Al	Cr	Mn	Ni	Cu	Zn	As	Se	Cd	Pb
Average of metal in long grain	1.1·10 ⁻⁴	5.6·10 ⁻⁴	390·10 ⁻⁴	5.5·10 ⁻⁴	70·10 ⁻⁴	820·10 ⁻⁴	3.6·10 ⁻⁴	2.9·10 ⁻⁴	0.5·10 ⁻⁴	1.3·10 ⁻⁴
Standard deviation of long grain	17·10 ⁻⁴	1.4·10 ⁻⁴	210·10 ⁻⁴	2.7·10 ⁻⁴	19·10 ⁻⁴	170·10 ⁻⁴	23·10 ⁻⁴	0.7·10 ⁻⁴	0.2·10 ⁻⁴	2.1·10 ⁻⁴
Average of metal in short grain	100·10 ⁻⁴	4.9·10 ⁻⁴	420·10 ⁻⁴	4.5·10 ⁻⁴	73·10 ⁻⁴	790·10 ⁻⁴	3.1·10 ⁻⁴	2.8·10 ⁻⁴	0.5·10 ⁻⁴	1.2·10 ⁻⁴
Standard deviation of short grain	93·10 ⁻⁴	1.9·10 ⁻⁴	100·10 ⁻⁴	2.4·10 ⁻⁴	43·10 ⁻⁴	420·10 ⁻⁴	2.1·10 ⁻⁴	1.2·10 ⁻⁴	0.4·10 ⁻⁴	2.0·10 ⁻⁴

element with the highest bioavailability (79.09%) in contrast to the bioavailability of Pb (11.9%). These data are within the same order of magnitude as previously published by Praveena and Omar, (2017). In addition the concentration of metal found in digested rice was reduced by about half with respect to cooked rice. This fact has a direct influence on the estimation of the human risk. Understanding the effect of the digestion process on the bioavailability of each metal is of great relevance to avoid the overestimation of the human risk, as already indicated by some authors (Omar et al., 2013). These differences could be due to the action of the dietary fiber as a chelating agent to remove metallic elements through two pathways: partial or total inhibition of bioavailability, and inhibition of intestinal reabsorption (Borowska et al., 2020; Kachenpukdee and Oonsivilai, 2019). Similarly, the degree

of bioavailability of metallic elements decreases in the following order: Sona Masoori > Jasmine > Hisparmar > Sole > Memby > Puntal > Barone > Basmati > Guadamar > Thaiparla, with values ranging between 58.7 ± 6.1% in the case of Sona Masoori variety and 32.6 ± 4.6% in Thaiparla variety. A thorough study should be carried out to understand how metallic and metalloid elements interact with dietary fiber matrices using in vivo models. However, to our knowledge, there are no studies in which the absorbed metal values were used to calculate the non-carcinogenic and carcinogenic health risks.

3.2. Adult dietary intake assessment

Tables 6 and 7 show the estimated daily intake (EDI) values

Table 5
Metallic bioavailability values.

Rice varieties	Bioaccessibility (%)									
	Al	Cr	Mn	Ni	Cu	Zn	As	Se	Cd	Pb
Barone	73.2	24.9	41.7	70.2	41.2	25.0	63.6	20.7	20.7	12.3
Sona Masoori	78.4	21.9	93.1	70.8	58.9	42.1	87.8	52.6	69.3	12.4
Perlado	79.4	15.6	54.5	25.5	46.1	25.1	98.9	12.0	39.4	10.4
Jasmine	74.7	16.8	70.2	96.5	52.0	36.7	90.6	18.3	37.6	11.6
Sole	80.8	16.7	63.8	30.8	50.0	34.3	98.2	13.1	95.4	12.0
Memby	79.8	18.3	57.3	87.7	47.1	29.2	59.6	10.8	70.7	10.0
Marisma	71.3	17.4	41.8	78.8	43.7	36.3	93.7	12.9	25.6	12.2
Sendra	82.1	27.0	42.9	91.9	34.7	29.6	92.6	8.3	32.5	17.4
Piñana	80.7	33.9	37.7	87.2	35.8	26.6	88.0	10.0	29.2	12.2
Hispanmar	92.3	28.7	59.1	95.5	42.9	41.9	90.2	11.0	20.9	16.3
Basmati	80.0	28.5	43.2	55.2	36.7	33.5	37.0	12.1	43.3	13.8
Puntal	91.7	21.6	43.7	62.5	44.8	32.0	89.3	11.7	43.5	14.1
Guadiamar	43.8	32.1	40.1	59.2	25.7	19.6	89.5	8.1	16.8	4.3
Thaipерla	82.0	20.9	32.1	39.9	30.8	21.2	39.2	9.6	42.5	8.2

Table 6
EDI values in cooked rice (mg/kg).

Cooked rice	EDI (mg/kg)									
	Al	Cr	Mn	Ni	Cu	Zn	As	Se	Cd	Pb
Barone	0.75	0.01	1.29	0.01	0.32	2.17	0.03	0.006	0.002	0.002
Basmati	0.65	0.01	2.79	0.04	0.35	2.64	0.22	0.003	0.002	0.001
Guadiamar	0.67	0.01	0.98	0.02	0.20	1.55	0.01	0.002	0.000	0.001
Hispanmar	0.65	0.01	1.72	0.01	0.28	1.66	0.01	0.002	0.001	0.001
Jasmine	0.56	0.01	1.08	0.02	0.24	2.24	0.02	0.004	0.001	0.001
Marisma	0.56	0.01	1.28	0.02	0.45	2.05	0.01	0.003	0.001	0.001
Memby	0.60	0.01	2.64	0.06	0.39	2.13	0.05	0.003	0.002	0.001
Perlado	0.73	0.01	1.32	0.01	0.23	1.47	0.01	0.003	0.001	0.001
Piñana	0.67	0.02	1.14	0.04	0.20	1.33	0.01	0.003	0.001	0.001
Puntal	0.65	0.01	1.17	0.02	0.32	1.61	0.01	0.003	0.001	0.001
Sendra	0.82	0.01	2.14	0.01	0.29	1.99	0.01	0.002	0.001	0.001
Sole	0.59	0.01	1.73	0.02	0.16	1.86	0.03	0.002	0.005	0.001
Sona Masoori	0.59	0.01	1.28	0.03	0.38	2.10	0.01	0.018	0.005	0.001
Thaipерla	0.60	0.01	1.60	0.01	0.23	1.86	0.06	0.004	0.002	0.001

Table 7
EDI values in digested rice (mg/kg).

Digested rice	EDI (mg/kg)									
	Al	Cr	Mn	Ni	Cu	Zn	As	Se	Cd	Pb
Barone	0.46	0.01	1.39	0.01	0.35	3.91	0.02	0.01	0.004	0.006
Basmati	0.55	0.02	4.37	0.02	0.65	5.33	0.40	0.02	0.002	0.007
Guadiamar	0.44	0.01	0.70	0.00	0.22	2.26	0.00	0.01	0.001	0.008
Hispanmar	0.42	0.02	1.74	0.01	0.40	2.37	0.01	0.01	0.001	0.004
Jasmine	0.36	0.02	0.74	0.01	0.23	2.94	0.01	0.01	0.002	0.004
Marisma	0.50	0.02	1.93	0.01	0.66	3.59	0.01	0.01	0.001	0.004
Memby	0.36	0.02	2.21	0.03	0.39	3.48	0.04	0.01	0.001	0.005
Perlado	0.41	0.02	1.06	0.02	0.22	2.57	0.01	0.01	0.001	0.004
Piñana	0.58	0.04	2.11	0.03	0.40	3.50	0.01	0.02	0.001	0.006
Puntal	0.47	0.02	1.78	0.01	0.48	3.39	0.01	0.02	0.001	0.005
Sendra	0.45	0.02	3.33	0.01	0.56	4.51	0.01	0.02	0.001	0.005
Sole	0.35	0.02	1.31	0.03	0.16	2.63	0.02	0.01	0.002	0.003
Sona Masoori	0.37	0.02	0.69	0.01	0.32	2.50	0.01	0.02	0.003	0.003
Thaipерla	0.55	0.02	3.71	0.02	0.56	6.54	0.11	0.03	0.004	0.011

considering the estimated rice consumption in Spain and the mean content of the corresponding element. In order to compare the values of EDI calculated in the present study, some safe limits for the studied elements were taken into account when available. Also, the percentages of contribution to the reference values for adults were calculated taking into account the mean EDI for all the varieties (Table 8). Thus, daily average consumption of rice does not significantly expose the Spanish consumer to unsafe levels of Cr if the element detected was Cr (III), Ni, Pb, and Cd with 0.29%, 0.000018%, 0.000017%, and 0.04% of dietary contribution, respectively. However, the percentage of contribution of

Al is higher (45.40%), especially considering that Al is an element that is found in all foods in variable concentrations. Similar results were found by Rubio-Armendáriz et al. (2021) in some types of cereal (barley gofio and corn) which had around a 30% of the contribution of Al to get the TWI. Finally, rice provides a large contribution to the global dietary intake of Cu and Zn (72.63% and 62.25%, respectively). Therefore, it is necessary to regulate the content of these metals in rice by setting maximum concentration levels, mainly in those countries where rice is an important and essential part of the diet.

Table 8
Mean EDI for all the varieties in digested and cooked rice and Intake limit values of the toxic metals analyzed.

Toxic Metal	Mean EDI digested rice	Mean EDI cooked rice	Parameter	Intake Limit Values	Organization	References
Cr(III)	0.02	0.01	TDI	0.3 mg/kgbw/day	EFSA	EFSA, 2014
Ni	0.02	0.02		13 g/kgbw/day		EFSA, 2020
Pb	0.01	0.00	BMDL	0.63 g/kgbw/day 1 1.50 g/kgbw/day 2		EFSA, 2010
Al	0.45	0.65	TWI	1 mg/kgbw/week	EFSA	EFSA, 2011a
Cd	0.002	0.002		2.5 g/kgbw/week		EFSA, 2011b
Cu	0.40	0.29	SL	14–21 mg/person/week		Ellenhorn, 1997
Zn	3.54	1.90		15 mg/person/week		Hernández and Sastre, 1999

EFSA, European Food Safety Authority; WHO, World Health Organization; BMDL, benchmark dose level; TDI, Tolerable daily intake; TWI, tolerable weekly intake; bw, body weight; nephrotoxicity 1 and cardiovascular effects 2.

3.3. Relationship among the elements

Mineral elements exist together or separately in nature. The presence of these elements may have different features and their co-existence may intensify the toxicity of the metals and metalloids with their intake, increasing the potential risk. On the other hand, metals with physiological functions could exert a protective role against damage caused by toxic elements, acting as their antagonists (Brizio et al., 2016; Basaran, 2022).

Thus, a correlation analysis was carried out in order to find the potential relationships among metallic and metalloid elements in rice from different locations (Spanish and imported). The sample correlation matrix indicates the degree of correlation between the several metal-

pairs of variables at a glance. However, in order to statistically prove the possible correlation, according to the before-mentioned non-normality distribution of the elements, the non-parametric technique of Spearman (Miller and Miller, 2000) was applied. The most important correlations were those with a Spearman coefficient close to 1 value. Significant correlations ($p \leq 0.05$) between pairs with a Spearman coefficient R close to 1 of the detected elements were observed (Figs. 1a and 1b), this is a consequence of the strong interrelation of these elements. Differences were found between rice from different origins. Strong correlations $R > 0.5$ ($p \leq 0.05$) between Cr-Al, Zn-As, Zn-Se, Se-As, and Pb-Se were detected in rice from Spain (Fig. 1a) while the correlations $R > 0.5$ ($p \leq 0.05$) was observed in rice imported and sold in local markets in Pb-Al, Pb-Zn, As-Mn and Ni-Cr (Fig. 1b). These

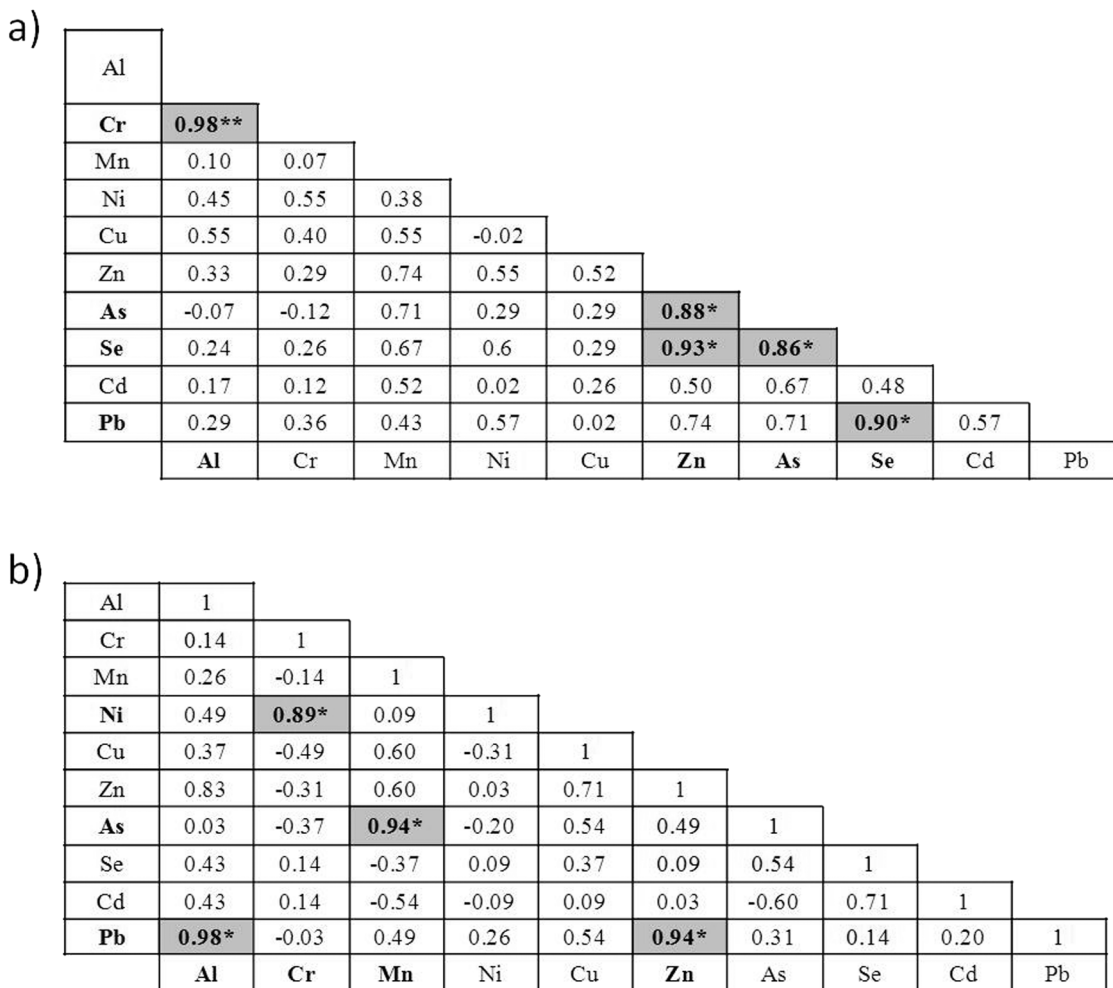


Fig. 1. Spearman coefficients (R) between several pairs of variables (metals and metalloids) in a) rice from Spain b) imported rice. * $p \leq 0.05$, ** $p \leq 0.01$. The bold meaning is that the R value are nearest of one value.

differences are related to soil composition and the agronomic bio-fortification programs of the different countries (Kumar et al., 2018; Roda et al., 2020). Regarding correlations in rice from Spain, a significant correlation between Cr and Al was observed. Both of them are present in the Earth's crust, thus, most rocks and soils can contain appreciable amounts of these two elements at the same time (Ali et al., 2011). Indeed, an increase of Cr levels in the environment has been monitored, due to the increased use with industrial applications, such as electroplating, catalysts production, refractory steel production, and metallurgical applications (Ranieri et al., 2022). Excessive levels of toxic elements in the soil will increase the risk of ingesting toxic metals into the human body through the food chain or soil dust, especially for children (Xu et al., 2022). Thus, human exposure to Cr is mainly derived from soil-crop systems, because Cr is easily integrated into the food chain (Anand et al., 2018; Ao et al., 2022). This fact was demonstrated by Adhikari et al. (2022), who detected an exceeded content not only for Cr but also for Al and other potentially toxic elements in leaves from medicinal plants and vegetables. Also, strong correlations between Zn-Se As-Se, and Pb-As can be observed in Spanish rice. Despite the decrease of Pb levels in the environment in the last decades due to its removal from fuel, there are other many anthropogenic sources of this metal, such as intensive agronomic activities, by the use of pesticides and fertilizers, or mining activities (Chamannejadian et al., 2013; Xu et al., 2022). Furthermore, Zn in soils is mainly associated with mining activities as well. Moreover, trace metal contamination in paddy soil-rice systems through mining activities following the release of untreated wastes into the environment is considered a significant issue worldwide (González-Corrochano et al., 2014). One of the main rice-growing areas of Spain would be the South of the country, in the province of Seville, near an important mining region in Huelva (minas de Río Tinto), which could explain the presence of this element in the environment. Other elements such as As, or Cr, trace elements comprising pesticides, are present in paddy soils increasing the uptake of these elements by paddy rice (Ali et al., 2011). Also, Se is present naturally in soils but its bioavailability to the crop is pH dependent, so Se levels in rice usually come from its use as fertilizer to improve crop growth (López-Bellido, 2014).

In rice of foreign origin, several correlations can be observed with Pb, which could be explained not only by agricultural or industrial activities as in Spanish rice but also by the presence of Pb in the fuels in those countries where is not suppressed (India or Vietnam). Moreover, the contamination status of paddy soil-rice systems in major rice-growing countries, including India or Vietnam has been proven (Ali et al., 2020), which could explain the rest of significant correlations observed such as As-Mn and Cr-Ni. In addition, the correlation between Pb and Zn could be explained by the application of Zn in paddy soil contaminated with Pb in order to improve biomass yield (Mardomi et al., 2019).

Table 9
Target Hazard Quotients.

Variety	Cr	Mn	Ni	Cu	Zn	As	Se	total
Barone	0.000	0.002	0.000	0.002	0.003	0.009	0.001	0.017
Basmati	0.000	0.005	0.000	0.002	0.003	0.008	0.001	0.019
Guadamar	0.001	0.002	0.000	0.002	0.003	0.008	0.000	0.015
Hispamar	0.001	0.002	0.000	0.002	0.001	0.006	0.000	0.012
Jasmine	0.001	0.001	0.000	0.001	0.002	0.007	0.000	0.012
Marisma	0.001	0.002	0.000	0.003	0.002	0.007	0.000	0.014
Memby	0.001	0.003	0.000	0.002	0.002	0.008	0.000	0.017
Perlado	0.001	0.002	0.000	0.001	0.002	0.008	0.000	0.015
Piñana	0.001	0.002	0.000	0.001	0.002	0.009	0.001	0.016
Puntal	0.001	0.002	0.000	0.002	0.002	0.009	0.001	0.015
Sendra	0.000	0.004	0.000	0.002	0.002	0.009	0.001	0.018
Sole	0.001	0.002	0.000	0.001	0.002	0.006	0.000	0.012
Sona Masoori	0.001	0.001	0.000	0.002	0.002	0.011	0.001	0.017
Thaiparla	0.001	0.004	0.000	0.002	0.003	0.013	0.001	0.022

3.4. Human health risk assessment

3.4.1. Non-carcinogenic risk assessment

The values of Individual Target Hazard Quotients (THQ) are given in Table 9. The range of THQ for each metal was: Al (0.001–0.36), Mn (0.001–0.005); Cu (0.001–0.003); Zn (0.001–0.003); As (0.006–0.013) and Se (0.000–0.001), giving the general order of Al > As > Zn > Cu > Mn > Se > Ni > Cd for target adults in all samples. Similar rank order was observed in Abtahi et al. (2017), giving the highest THQ value for As, and the lowest for Ni. It should be noted that the THQ values have not shown any value beyond 1 in any of the cases, indicating that the effects of metallic elements accumulated by rice ingestion do not involve a significant health risk. Similarly, the sum of each THQ does not pose any health problem to the rice consumer, because none of the values is beyond 1, which would be considered unacceptable in relation to non-carcinogenic health risk. The data obtained in this study are in agreement with those published previously by Gaudie et al. (2022), which showed that the values of THQ in Ethiopian rice (red and white) and in imported were within the safe level in all the samples, posing no potential health risks on consuming rice in this country. Also, in a systematic review Abtahi et al. (2017) concluded that since the THQ value was lower than 1, consumers were not exposed to carcinogenic risk by consumption of rice cultivated in Iran. However, Vatanpour et al. (2020) observed high levels of metal accumulation in cultivated rice in Iran, resulting in a THQ value higher than 1, suggesting a considerable health risk for regular rice consumers. Similarly, THQ values higher than 1 were detected for Ni, Cu, As, and Cd in rice and vegetables in Bangladesh, indicating significant health risk for consumers (Proshad et al., 2020). Bielecka et al. (2020), stated that the THQ of rice produced in Asia and consumed by the polish population was 60–120 times higher than rice not produced in Asia. However, in the present study, rice marketed in Spain, even of Asian origin, does not show values dangerous to human health.

3.4.2. Carcinogenic risk assessment

The incremental lifetime cancer risk (ILCR) was calculated for inorganic As, Cd, Cr, Ni, and Pb which are either recognized or regarded as probable human carcinogens (Baan, 2012). In the present work, no apparent carcinogenic risk could be associated with all the elements studied, since ILCR values were considered within safe limits (10^{-4}) (Table 10), being the acceptable risk limit established by USEPA (2011) values between 10^{-6} to 10^{-4} (Rapant et al., 2011). The ILCR values of As, Cd, and Cr were around 10^{-5} and 10^{-4} , respectively, while Pb and Ni led to ILCR values significantly lower with values around 10^{-8} . The ILCR value related to the exposition of As in the present work (about 10^{-5}), poses a small contribution to increase the carcinogenic risk. However, As could be underestimated, since As in rice is present not only as an inorganic component but also as organic derivative (Leone, 1984). In this regard, findings from Proshad et al. (2020) for As contaminated rice

Table 10
Estimation of Incremental lifetime cancer risks (ILCR) in all varieties studied.

Variety	Ni	Cd	Cr	As	Total
Barone	1.3•10 ⁻⁸	5.7•10 ⁻⁵	1.4•10 ⁻⁴	8.4•10 ⁻⁵	2.8•10 ⁻⁴
Basmati	7.7•10 ⁻⁹	2.5•10 ⁻⁵	1.3•10 ⁻⁴	1.0•10 ⁻⁴	1.6•10 ⁻⁴
Guadamar	1.7•10 ⁻⁸	1.4•10 ⁻⁵	1.7•10 ⁻⁴	2.2•10 ⁻⁵	2.1•10 ⁻⁴
Hispanar	9.6•10 ⁻⁹	1.8•10 ⁻⁵	1.5•10 ⁻⁴	2.1•10 ⁻⁵	1.9•10 ⁻⁴
Jasmine	1.2•10 ⁻⁸	2.5•10 ⁻⁵	1.8•10 ⁻⁴	2.9•10 ⁻⁵	2.4•10 ⁻⁴
Marisma	1.0•10 ⁻⁸	1.3•10 ⁻⁵	1.6•10 ⁻⁴	2.3•10 ⁻⁵	2.0•10 ⁻⁴
Memby	1.2•10 ⁻⁸	1.8•10 ⁻⁵	2.0•10 ⁻⁴	1.3•10 ⁻⁴	3.5•10 ⁻⁴
Perlado	9.7•10 ⁻⁸	1.1•10 ⁻⁵	2.3•10 ⁻⁴	2.3•10 ⁻⁵	2.6•10 ⁻⁴
Piñana	7.2•10 ⁻⁹	1.1•10 ⁻⁵	3.1•10 ⁻⁴	2.5•10 ⁻⁵	3.5•10 ⁻⁴
Puntal	7.9•10 ⁻⁸	1.4•10 ⁻⁵	1.5•10 ⁻⁴	2.6•10 ⁻⁵	1.9•10 ⁻⁴
Sendra	1.7•10 ⁻⁹	1.2•10 ⁻⁵	1.4•10 ⁻⁴	2.8•10 ⁻⁵	1.8•10 ⁻⁴
Sole	1.2•10 ⁻⁸	3.5•10 ⁻⁵	2.1•10 ⁻⁴	5.4•10 ⁻⁵	3.0•10 ⁻⁴
Sona Masoori	1.2•10 ⁻⁸	4.7•10 ⁻⁵	1.9•10 ⁻⁴	2.5•10 ⁻⁵	2.7•10 ⁻⁴
Thaipera	6.1•10 ⁻⁹	3.4•10 ⁻⁵	1.5•10 ⁻⁴	2.5•10 ⁻⁵	4.4•10 ⁻⁴

in Bangladesh or those by Sharafi et al. (2019b) detecting As in three different rice types in Iran showed ILCR values higher than the acceptable limit for cancer risk. Other authors have observed that the cancer risk of Pb was tolerable in China with ILCR values into the range 10⁻⁶ to 10⁻⁴ though the cancer risk levels of As, Cd and Cr was unacceptable (ILCR above 10⁻⁴) (Mao et al., 2019).

4. Conclusions

The metallic and metalloid levels detected in all the varieties studied are below the toxic threshold. The metallic element with the lowest bioavailability in all the analyzed rice varieties was Pb; while As was the metalloid with the highest bioavailability. Different correlations were observed in rice produced in Spain with those imported, indicating that the presence of metals and metalloids in rice is mainly impacted by soil composition. The non-carcinogenic risk of metals and metalloids in all rice varieties studied is below the toxicity threshold, which is in the range of 1–10% of the toxicity threshold. Regarding the carcinogenic risk, the metal with less contribution to the carcinogenic risk was Pb, in contrast to Cr, which was close to the limit value of 10⁻⁴. In addition, rice provides a large contribution to global dietary intake of Al, Cu, and Zn (30%, 72.63%, and 62.25%, respectively). Lastly, the results obtained in the present work approach a more realistic metal and metalloid exposure through rice intake, as the cooking and digestion procedures were taken into account, which makes a more realistic risk assessment, performed in most of the cases with measurements obtained in raw material. Thus, performing studies of digestion and bioavailability of these foods in raw, in order to perform a better risk assessment of these pollutants.

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CRediT authorship contribution statement

Juan Bautista: Conceptualization. **Isabel Moreno:** Conceptualization. **José Raúl Aguilera-Velázquez:** Methodology, Writing – original draft. **Esteban Alonso:** Resources. **Ana Calleja:** Writing – review & editing. **Esteban Alonso:** Writing – review & editing.

Conflicts of interest

The authors declare no conflict of interest.

Data Availability

Data will be made available on request.

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Appendix A. Supporting information

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References

- Abtahi, M., Fakhri, Y., Oliveri Conti, G., Keramati, H., Zandsalimi, Y., Bahmani, Z., Hosseini Pouya, R., Sarkhosh, M., Moradi, B., Amanidaz, N., Ghasemi, S.M., 2017. Heavy metals (As, Cr, Pb, Cd and Ni) concentrations in rice (*Oryza sativa*) from Iran and associated risk assessment: a systematic review. *Toxin Rev.* 36, 331–341. <https://doi.org/10.1080/15569543.2017.1354307>.
- Adhikari, S., Marcelo-Silva, J., Beukes, J.P., van Zyl, P.G., Coetsee, Y., Boneschans, R.B., Siebert, S.J., 2022. Total and hexavalent chromium and other potentially toxic element contamination of useful plant leaves in a polluted mining-smelting region of south africa and health risks. *SSRN Electron. J.* 9, 100260 <https://doi.org/10.2139/ssrn.4102798>.
- Aguilera-Velázquez, J.R., Carbonero-Aguilar, P., Martín-Carrasco, I., Hinojosa, M.G., Moreno, I., Bautista, J., 2021. Nutritional quality of the most consumed varieties of raw and cooked rice in Spain submitted to an in vitro digestion model. *Foods* 10, 2584. <https://doi.org/10.3390/foods10112584>.
- Ali, S., Zeng, F., Qiu, B., Cai, S., Qiu, L., Wu, F., Zhang, G., 2011. Interactive effects of aluminum and chromium stresses on the uptake of nutrients and the metals in barley. *Soil Sci. Plant Nutr.* 57, 68–79. <https://doi.org/10.1080/00380768.2010.549444>.
- Anand, N., Kundu, A., Ray, S., 2018. A validated method for the determination of neonicotinoid, pyrethroid and organochlorine residues in human milk. *Chromatographia* 81, 315–325. <https://doi.org/10.1007/s10337-017-3436-6>.
- Ao, M., Chen, X., Deng, T., Sun, S., Tang, Y., Morel, J.L., Qiu, R., Wang, S., 2022. Chromium biogeochemical behaviour in soil-plant systems and remediation strategies: A critical review. *J. Hazard. Mater.* 424, 127233 <https://doi.org/10.1016/j.jhazmat.2021.127233>.
- Baan, Robert, 2012. Chemical agents and related occupations. *IARC Monogr. Eval. Carcinog. Risks Hum.* 100, 9–562.
- Basaran, B., 2022. Comparison of heavy metal levels and health risk assessment of different bread types marketed in Turkey. *J. Food Compos. Anal.* 108, 104443 <https://doi.org/10.1016/j.jfca.2022.104443>.
- Başaran, B., 2022a. An assessment of heavy metal level in infant formula on the market in Turkey and the hazard index. *J. Food Compos. Anal.* 105, 104258 <https://doi.org/10.1016/j.jfca.2021.104258>.
- Başaran, B., 2022b. Assessment of aluminum via baby foods consumption in turkey: estimated early-life dietary exposure and target hazard quotient. *Biol. Trace Elem. Res.* 200, 3892–3901. <https://doi.org/10.1007/s12011-021-02961-2>.
- Bielecka, J., Markiewicz-zukowska, R., Nowakowski, P., Grabia, M., Puścion-Jakubik, A., Mielcarek, K., Gromkowska-Kępka, K.J., Soroczyńska, J., Socha, K., 2020. Content of toxic elements in 12 groups of rice products available on polish market: human health risk assessment. *Foods* 9, 1906. <https://doi.org/10.3390/foods9121906>.
- Borowska, S., Tomczyk, M., Strawa, J.W., Brzóska, M.M., 2020. Estimation of the chelating ability of an extract from *Aronia melanocarpa* L. Berries and its main polyphenolic ingredients towards ions of zinc and copper. *Molecules* 25, 6–8. <https://doi.org/10.3390/molecules25071507>.
- Brizio, P., Benedetto, A., Squadrone, S., Curcio, A., Pellegrino, M., Ferrero, M., Abete, M.C., 2016. Heavy metals and essential elements in Italian cereals. *Food Addit. Contam.: Part B* 9 (4), 261–267. <https://doi.org/10.1080/19393210.2016.1209572>.
- Brodkorb, A., Egger, L., Alminger, M., Alvito, P., Assunção, R., Ballance, S., Bohn, T., Bourlieu-Lacanal, C., Boutrou, R., Carrière, F., Clemente, A., Corredig, M., Dupont, D., Dufour, C., Edwards, C., Golding, M., Karakaya, S., Kirkhus, B., Le Feunteun, S., Lesmes, U., Macierzanka, A., Mackie, A.R., Martins, C., Marze, S., McClements, D.J., Ménard, O., Minekus, M., Portmann, R., Santos, C.N., Souchon, I., Singh, R.P., Vegarud, G.E., Wickham, M.S.J., Weitschies, W., Recio, I., 2019. INFOGEST static in vitro simulation of gastrointestinal food digestion. *Nat. Protoc.* 14, 991–1014. <https://doi.org/10.1038/s41596-018-0119-1>.
- Catalani, S., Rizzetti, M.C., Padovani, A., Apostoli, P., 2012. Neurotoxicity of cobalt. *Hum. Exp. Toxicol.* 31, 421–437. <https://doi.org/10.1177/0960327111414280>.
- Chamannejadian, A., Sayyad, G., Moezzi, A., Jahangiri, A., 2013. Evaluation of estimated daily intake (EDI) of cadmium and lead for rice (*Oryza sativa* L.) in calcareous soils. *Iran. J. Environ. Heal. Sci. Eng.* 10, 1. <https://doi.org/10.1186/1735-2746-10-28>.
- Chien, L.C., Hung, T.C., Choang, K.Y., Yeh, C.Y., Meng, P.J., Shieh, M.J., Han, B.C., 2002. Daily intake of TBT, Cu, Zn, Cd and As for fishermen in Taiwan. *Sci. Total Environ.* 285, 177–185. [https://doi.org/10.1016/S0048-9697\(01\)00916-0](https://doi.org/10.1016/S0048-9697(01)00916-0).
- Dastgiri, S., Mosafieri, M., Fizi, M.A.H., Olfati, N., Zolali, S., Pouladi, N., Azarfam, P., 2010. Arsenic exposure, dermatological lesions, hypertension, and chromosomal abnormalities among people in a rural community of northwest Iran. *J. Heal. Popul. Nutr.* 28, 14–22. <https://doi.org/10.3329/jhpn.v28i1.4519>.

- Engwa, G.A., Ferdinand, P.U., Nwalo, F.N., Unachukwu, M.N., 2019. Mechanism and Health Effects of Heavy Metal Toxicity in Humans. *Poisoning Mod. World - New Tricks an Old Dog?* <https://doi.org/10.5772/INTECHOPEN.82511>.
- Evers, J.M., Hughes, C.G., 2002. ANALYSIS | Chemical analysis. *Encycl. Dairy Sci.* 34–40. <https://doi.org/10.1016/B0-12-227235-8/00015-8>.
- Exley, C., 2016. The toxicity of aluminium in humans. *Morphologie* 100, 51–55. <https://doi.org/10.1016/J.MORPHO.2015.12.003>.
- González-Corrochano, B., Esbrí, J.M., Alonso-Azcárate, J., Martínez-Coronado, A., Jurado, V., Higuera, P., 2014. Environmental geochemistry of a highly polluted area: the La Unión Pb-Zn mine (Castilla-La Mancha region, Spain). *J. Geochem. Explor.* 144, 345–354. <https://doi.org/10.1016/j.gexplo.2014.02.014>.
- Guadie, A., Mohammed, I., Beshaw, T., Tefera, M., 2022. Analysis and health risk assessments of some trace metals in Ethiopian rice (white and red) and imported rice. *Heliyon* 8, e09374. <https://doi.org/10.1016/j.heliyon.2022.e09374>.
- Hadrup, N., Ravn-Haren, G., 2020. Acute human toxicity and mortality after selenium ingestion: a review. *J. Trace Elem. Med. Biol.* 58, 126435 <https://doi.org/10.1016/J.JTEMB.2019.126435>.
- Igbokwe, I.O., Igbwagu, E., Igbokwe, N.A., 2019. Aluminium toxicosis: a review of toxic actions and effects. *Interdiscip. Toxicol.* 12, 45. <https://doi.org/10.2478/INTOX-2019-0007>.
- Kachenpukdee, N., Oonsivilai, R., 2019. Modification of Dietary Fiber from Cassava Pulp to Reduce Heavy Metal by Assessing Their Heavy Metal Bioaccessibility Inhibition 12, 13–21.
- Kamala, K., Kumar, V.P., 2018. Food products and food contamination. *Microb. Contam. Food Degrad.* 1–19. <https://doi.org/10.1016/B978-0-12-811515-2.00001-9>.
- Kukusamude, C., Sricharoen, P., Limchoowong, N., Kongsri, S., 2021. Heavy metals and probabilistic risk assessment via rice consumption in Thailand. *Food Chem.* 334, 127402 <https://doi.org/10.1016/J.FOODCHEM.2020.127402>.
- Kumar, D., Dhaliwal, S.S., Naresh, R.K., Salaria, A., 2018. Agronomic biofortification of paddy through nitrogen, zinc and iron fertilization: a review. *undefined* 7, 2942–2953. <https://doi.org/10.20546/JJCMAS.2018.707.344>.
- Leone, I., 1984. Effects of Gaseous Air Pollution in Agruculture and Horticulture.137, 469–470. [https://doi.org/10.1376-0469\\$02.00/0](https://doi.org/10.1376-0469$02.00/0).
- López-Bellido, F.J., 2014. Biofortificación agronómica de los cultivos: selenio | INNOVAGRI/INNOVAGRI [WWW Document]. URL <https://www.innovagri.es/nutricion-del-cultivo/biofortificacion-agronomica.html> (accessed 8.3.22).
- MacFarquhar, J.K., Broussard, D.L., Melstrom, P., Hutchinson, R., Wolkin, A., Martin, C., Burk, R.F., Dunn, J.R., Green, A.L., Hammond, R., Schaffner, W., Jones, T.F., 2010. Acute selenium toxicity associated with a dietary supplement. *Arch. Intern. Med.* 170, 256. <https://doi.org/10.1001/ARCHINTERNMED.2009.495>.
- Mao, C., Song, Y., Chen, L., Ji, J., Li, J., Yuan, X., Yang, Z., Ayoko, G.A., Frost, R.L., Theiss, F., 2019. Human health risks of heavy metals in paddy rice based on transfer characteristics of heavy metals from soil to rice. *Catena* 175, 339–348. <https://doi.org/10.1016/j.catena.2018.12.029>.
- Mardomi, S., Najafi, N., Reyhanitabar, R., Dehgan, G., 2019. Antioxidant enzyme activities and dry matter of rice plant as affected by interactions of lead, phosphorus and zinc. *Philipp. Agric. Sci.* 102, 310–321.
- Martinez, V.D., Vucic, E.A., Becker-Santos, D.D., Gil, L., Lam, W.L., 2011. Arsenic exposure and the induction of human cancers. *J. Toxicol.* 21, 431287 <https://doi.org/10.1155/2011/431287>.
- Ministerio de Agricultura, P. y A., 2018. Informe del Consumo Alimentario en España 2020. *Gob. Esp.* 538.
- Niño, S.A., Morales-Martínez, A., Chi-Ahumada, E., Carrizales, L., Salgado-Delgado, R., Pérez-Severiano, F., Díaz-Cintra, S., Jiménez-Capdeville, M.E., Zarazúa, S., 2019. Arsenic exposure contributes to the bioenergetic damage in an Alzheimer's disease model. *ACS Chem. Neurosci.* 10, 323–336. <https://doi.org/10.1021/acchemneuro.8b00278>.
- O'Neal, S.L., Zheng, W., 2015. Manganese toxicity upon overexposure: a decade in review. *Curr. Environ. Heal. Rep.* 2, 315–328. <https://doi.org/10.1007/S40572-015-0056-X>.
- Olayiwola, K., N'Zue, F.F., 2020. The China-US Trade Dispute: Economic Implications for the ECOWAS Region. *Global Trade Cust* 15 (11-12), 609–627.
- Omar, N.A., Praveena, S.M., Aris, A.Z., Hashim, Z., 2013. Bioavailability of heavy metal in rice using in vitro digestion model. *Int. Food Res. J.* 20, 2979–2985.
- Peres, T.V., Schettinger, M.R.C., Chen, P., Carvalho, F., Avila, D.S., Bowman, A.B., Aschner, M., 2016. Manganese-induced neurotoxicity: a review of its behavioral consequences and neuroprotective strategies. *BMC Pharmacol. Toxicol.* 17, 57. <https://doi.org/10.1186/S40360-016-0099-0>.
- Praveena, S.M., Omar, N.A., 2017. Heavy metal exposure from cooked rice grain ingestion and its potential health risks to humans from total and bioavailable forms analysis. *Food Chem.* 235, 203–211. <https://doi.org/10.1016/j.foodchem.2017.05.049>.
- Proshad, R., Kormoker, T., Islam, M.S., Chandra, K., 2020. Potential health risk of heavy metals via consumption of rice and vegetables grown in the industrial areas of Bangladesh. *Hum. Ecol. Risk Assess.* 26, 921–943. <https://doi.org/10.1080/10807039.2018.1546114>.
- Rahman, M.M., Ng, J.C., Naidu, R., 2009. Chronic exposure of arsenic via drinking water and its adverse health impacts on humans. *Environ. Geochem. Health* 31, 189–200. <https://doi.org/10.1007/s10653-008-9235-0>.
- Rajkumar, V., Gupta, V., 2021. Heavy Metal Toxicity. *Pak. J. Med. Heal. Sci.* 10, 324. <https://doi.org/10.4172/2161-0495.s3-007>.
- Ranieri, E., Gikas, P., Ranieri, F., D'Onghia, G., Ranieri, A.C., 2022. Phytoextraction by Moso Bamboo under high level chromium stress in mediterranean conditions. *J. Environ. Manag.* 317, 115479 <https://doi.org/10.1016/j.jenvman.2022.115479>.
- Rapant, S., Fajčíková, K., Khun, M., Cvečková, V., 2011. Application of health risk assessment method for geological environment at national and regional scales. *Environ. Earth Sci.* 64, 513–521. <https://doi.org/10.1007/s12665-010-0875-x>.
- Roda, F.A., Marques, I., Batista-Santos, P., Esquivel, M.G., Ndayiragije, A., Lidon, F.C., Swamy, B.P.M., Ramalho, J.C., Ribeiro-Barros, A.I., 2020. Rice biofortification with zinc and selenium: a transcriptomic approach to understand mineral accumulation in flag leaves. *Front. Genet.* 11, 543. <https://doi.org/10.3389/FGENE.2020.00543/BIBTEX>.
- Rubio-Armendáriz, C., Soraya, P., Gutierrez, Á.J., Gomes Furtado, V., González-Weller, D., Revert, C., Hardisson, A., 2021. Toxic metals in cereals in cape verde: risk assessment evaluation. *Int. J. Environ. Res. Public Health* 18, 3833. <https://doi.org/10.3390/IJERPH18073833>.
- Sharafi, K., Nodehi, R.N., Yunesian, M., Hossein Mahvi, A., Pirsahab, M., Nazmara, S., 2019a. Human health risk assessment for some toxic metals in widely consumed rice brands (domestic and imported) in Tehran, Iran: Uncertainty and sensitivity analysis. *Food Chem.* 277, 145–155. <https://doi.org/10.1016/J.FOODCHEM.2018.10.090>.
- Sharafi, K., Yunesian, M., Mahvi, A.H., Pirsahab, M., Nazmara, S., Nabizadeh Nodehi, R., 2019b. Advantages and disadvantages of different pre-cooking and cooking methods in removal of essential and toxic metals from various rice types- human health risk assessment in Tehran households, Iran. *Ecotoxicol. Environ. Saf.* 175, 128–137. <https://doi.org/10.1016/j.ecoenv.2019.03.056>.
- Simonsen, L.O., Harbak, H., Bennekou, P., 2012. Cobalt metabolism and toxicology—a brief update. *Sci. Total Environ.* 432, 210–215. <https://doi.org/10.1016/J.SCITOTENV.2012.06.009>.
- Singh, R., Gautam, N., Mishra, A., Gupta, R., 2011. Heavy metals and living systems: an overview. *Indian J. Pharmacol.* 43, 246. <https://doi.org/10.4103/0253-7613.81505>.
- Skalný, A.V., Aschner, M., Jiang, Y., Gluhcheva, Y.G., Tizabi, Y., Lobinski, R., Tinkov, A. A., 2021. Molecular mechanisms of aluminum neurotoxicity: Update on adverse effects and therapeutic strategies. *Adv. Neurotoxicol.* 5, 1. <https://doi.org/10.1016/BS.ANT.2020.12.001>.
- Tapiero, H., Townsend, D.M., Tew, K.D., 2003. Trace elements in human physiology and pathology. *Copp. Biomed. Pharm.* 57, 386–398. [https://doi.org/10.1016/S0753-3322\(03\)00012-X](https://doi.org/10.1016/S0753-3322(03)00012-X).
- Tchounwou, P.B., Yedjou, C.G., Patlolla, A.K., Sutton, D.J., 2012. Heavy metals toxicity and the environment. *EXS* 101, 133. https://doi.org/10.1007/978-3-7643-8340-4_6.
- Vatanpour, N., Feizy, J., Hedayati Talouki, H., Es'haghi, Z., Scesi, L., Malvandi, A.M., 2020. The high levels of heavy metal accumulation in cultivated rice from the Tajan river basin: Health and ecological risk assessment. *Chemosphere* 245, 125639. <https://doi.org/10.1016/j.chemosphere.2019.125639>.
- Versantvoort, C.H.M., Oomen, A.G., Van De Kamp, E., Rompelberg, C.J.M., Sips, A.J.A.M., 2005. Applicability of an in vitro digestion model in assessing the bioaccessibility of mycotoxins from food. *Food Chem. Toxicol.* 43, 31–40. <https://doi.org/10.1016/j.fct.2004.08.007>.
- Xu, H., Croot, P., Zhang, C., 2022. Exploration of the spatially varying relationships between lead and aluminium concentrations in the topsoil of northern half of Ireland using Geographically Weighted Pearson Correlation Coefficient. *Geoderma* 409, 115640. <https://doi.org/10.1016/j.geoderma.2021.115640>.
- Zhang, H., Mao, Z., Huang, K., Wang, X., Cheng, L., Zeng, L., Zhou, Y., Jing, T., 2019. Multiple exposure pathways and health risk assessment of heavy metal(loid)s for children living in fourth-tier cities in Hubei Province. *Environ. Int.* 129, 517–524. <https://doi.org/10.1016/J.ENVINT.2019.04.031>.
- Zulkafflee, N.S., Adillah, N., Redzuan, M., Selamat, J., Ismail, M.R., 2021. Evaluation of heavy metal contamination in paddy. *Plants* . *Plants* 10, 3. <https://doi.org/10.3390/plants10010003>.
- Zulkafflee, N.S., Adillah, N., Redzuan, M., Hanafi, Z., Selamat, J., Ismail, M.R., Praveena, S.M., Razis, A., 2019. Heavy Metal in Paddy Soil and its Bioavailability in Rice Using In Vitro Digestion Model for Health Risk Assessment. *Int. J. Environm. Res. Public Health* 16, 4769. <https://doi.org/10.3390/ijerph16234769>.