

1 **A study on toxic and essential elements in rice from the Republic of**
2 **Kazakhstan. Comparing the level of contamination in rice from the**
3 **European Community**

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19

20 **Abstract**

21

22 Selected toxic elements (total As, Cd, Cr, Hg, Pb, Sr, U and V) and essential elements (Co,
23 Cu, Fe, Mn and Zn) were analyzed using an inductively coupled plasma mass spectrometry
24 (ICP-MS) in unpolished and milled rice collected from Kazakhstan and milled rice from
25 Spain and Portugal to evaluate the potential health risk to the population. Arsenic species
26 (arsenite, arsenate, arsenobetaine, dimethylarsinate and monomethylarsonate) were
27 analyzed using HPLC-IC-MS. From 146 samples analyzed none of them exceeded the
28 maximum limit set by the European Legislation for Cd or Pb or values recommended by
29 the Codex Alimentarius. Concentrations of Sr, U and V were below LOD and those of Hg,
30 Pb, Co, Cr between < LOD and 0.54 mg/kg (highest concentration of Cr) in milled rice.
31 Portuguese rice samples contained the highest mean concentration of As, Hg, Pb, Co, Cr,
32 Cu, Mn and Zn. The highest mean of arsenobetaine (0.001 mg/kg), dimethylarsinate (0.27
33 mg/kg), monomethylarsonate (0.02 mg/kg) were found in Spanish rice and those of arsenite
34 (0.30 mg/kg) in Kazakh rice. Inorganic As in samples from Kazakhstan was above the ML
35 (0.2 mg/kg) proposed by FAO/WHO but in seven samples from Spain and in four from
36 Portugal were above the limit. The estimated weekly intake of total or inorganic As (III,
37 V), Cd, Hg and Pb for rice consumption by Kazakh, Spanish and Portuguese adults and
38 children was lower than the provisional tolerable weekly intake established by Joint
39 FAO/WHO Expert Committee on Food Additives and the European Food Safety
40 Authority.

41

42 **Keywords:** Rice, toxic elements, essential elements, weekly intake, Kyzylorda province,
43 Kazakhstan.

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46 **Introduction**

47

48 Rice is the dominant staple food for over half of the world's population, especially
49 in developing Asian countries, where it contributes to over 70% of the energy provided by
50 their daily food intake (Qian et al. 2010). According to market research on cereals in the
51 Republic of Kazakhstan, 53% of the total consumption of cereals consists of rice and
52 accounted for several cereals, except for wheat consumption, during this study. Residents
53 of the Republic of Kazakhstan consume approximately 7 kg of rice per person per year
54 (Good Creative Marketing PR 2010). Kyzylorda Province, the main rice-growing region in
55 the country, produces 90% of the total rice produced in the country (Temen 2011).

56 Overall, Kyzylorda Province has significant mineral resources, such as zinc, lead,
57 uranium, vanadium, gas and petroleum. One hundred twenty-five gold-mining and oil
58 enterprises and suppliers of shungite (it is a black, lustrous, non-crystalline mineraloid
59 consisting of more than 98% of carbon), gold, titanium and vanadium are currently
60 operated in the area (Temen 2011). These mining activities have certainly increased the
61 dispersal of toxic elements in Kyzylorda Province. The presence of elements such as
62 cadmium (Cd), copper (Cu), lead (Pb) and zinc (Zn) in soil and water samples obtained
63 from Kyzylorda Province (Republic of Kazakhstan) has already been reported
64 (Berdenkulova 2008). Rice is grown under inundation cultivation systems and could
65 accumulate As from the soil and water due to the important mobility of this compound in
66 these conditions (Liang et al. 2010). Williams and collaborators indicated that transference
67 of As from soil to grain was greater in rice than in wheat or barley, leading to baseline
68 levels of As in rice that are approximately 10-fold higher than those in wheat (Williams et
69 al. 2007). Erdinger and collaborators reported that some organic arsenicals may have been

70 used as pesticides in the former Soviet Union to irrigate cotton fields and orchards in the
71 territory of Southern Kazakhstan and Uzbekistan (Erdinger et al. 2004). Other researchers
72 studying the town of Aralsk in the Kyzylorda Province have recently reported the detection
73 of As in soil with a mean As concentration of 6.7 (n=45, range 7-9 mg/kg) (Musabekov et
74 al. 2009).

75 Paddy fields used for rice cultivation in Kyzylorda Province are located on the bank
76 of the Syrdarya River. An investigation of the contamination of the Syrdarya River water
77 by sulfates, copper, nitrite, magnesium, oil products, phenols and pesticides was carried
78 out from 2006 to 2008 by Askarova (2010) and classified this river as "moderately
79 polluted". It has been reported that the maximum concentrations of the soluble forms of
80 toxic elements in water from the Syrdarya River were related to the disposal of industrial
81 and domestic sewage from cities and human settlements (Askarov 2010).

82 It should be noted that high levels of toxic elements have also been measured in
83 biological samples collected from Kazakhstan. As and Hg have been detected in urine
84 samples of children from Aralsk and Akchi, cities of Kazakhstan (Erdinger et al. 2004) and
85 Pb, Cd and Zn have been measured in meat products (liver, kidneys, lungs and muscles) of
86 cattle, horse and sheep from Oskemen at the centre of the east Kazakhstan región (Farmer
87 and Farmer 2000).

88 The aim of the present research study was to investigate toxic elements (As, Cd, Cr,
89 Hg, Pb and U) and essential elements (Co, Cu, Fe, Mn, Sr, V and Zn) in rice samples
90 obtained from the Kyzylorda Province of the Republic of Kazakhstan and to compare them
91 with rice samples obtained from Spain and Portugal. Spain and Portugal have been selected
92 for comparison as Spain is with Italy one of the main producers of rice in the European
93 Community and share 30% and 50% of the European rice production. Portugal, Greece and
94 France also have a noticeable impact on the European rice market (Cerdeño 2012).

95 Additionally, the presence of arsenic species (arsenobetaine (AsB), dimethylarsinate
96 (DMA), monomethylarsonate (MMA), arsenite As(III) and arsenate As(V)) were analyzed
97 in those rice samples where the concentration of total As was higher than 0.3 µg/kg which
98 is the maximum level (ML) proposed by FAO/WHO. To the best of our knowledge, there
99 are no published data regarding levels of both toxic and essential elements in rice from the
100 Republic of Kazakhstan. Additionally, data about rice produced in the Kyzylorda Province
101 are necessary because there is evidence of contamination related to mines and/or other
102 environmental sources, which might be reflected in rice samples.

103

104 **Materials and methods**

105 *Sampling*

106 For the Republic of Kazakhstan samples, two types of rice samples were collected:
107 unpolished and polished (milled rice). A total of 51 unpolished rice samples were collected
108 from local farms from 6 districts of Kyzylorda Province (Republic of Kazakhstan) from
109 September to November 2011 . Additionally, a total of 95 milled rice samples were bought
110 from local markets and supermarkets in the Republic of Kazakhstan, Portugal and Spain.
111 Samples obtained from the Republic of Kazakhstan (45 samples) were of the following
112 varieties: “Kuban”, “Kuban-3”, “Leader”, “Marzhan”, “Red Marzhan”, “Novator” and
113 “Yantar” (Figure 1) . Those obtained from Spain (30 samples) were of the following
114 varieties: “Largo”, “Bomba” and according to the treatment processes, “Integral”,
115 “Vaporizado” and “Ecologico” (organically farmed). Those bought in Portugal (20
116 samples) were of the following types: “Agulha”, “Biologico” and “Carolino”.

117

118 *Sample treatment*

119 For the unpolished rice, the grains were separated from the hull mechanically. Rice
120 grains were ground to a fine powder using a Grandomix mill (Haan Rheinische, Germany),
121 and 1.0 g was placed into a vessel in which 5 mL of HNO₃ (Panreac, Barcelona, España)
122 (67-69%), 5 mL of MilliQ water and 1 mL of H₂O₂ (Panreac) 33% (w/v) were added. The
123 vessel was closed, and the sample was digested using a microwave oven decomposition
124 system from Milestone Ethos 1 (Sorisole, Italy). Decomposition was performed according
125 to the following program: 85°C (3 min), 145°C (9 min), 180°C (4 min) and 180°C (15
126 min), with the power maintained during the 15 min period. After cooling to room
127 temperature, the solution was transferred into a 25-mL PET bottle and diluted with Milli-Q
128 water. Reagent blanks were processed simultaneously to deduce the error induced by the
129 analytical procedure. As was obtained from SCP Science (Clark Graham, Canada); Cd, Co,
130 Cr, Cu, Fe, Hg, Mn, Pb, Sr and Zn were obtained from Merck (Darmstadt, Germany); and
131 V was obtained from Panreac (Barcelona, España). The solvent was prepared with 20%
132 HNO₃.

133 All measurements were conducted using an ICP MS-820 (Varian; Mulgrave,
134 Australia) and high-purity argon (99.999%), which used a low (400 µL/min) glass
135 concentric nebulizer connected to a double pass spray chamber. Sample data were acquired
136 with 3 replicates and a minimum dwell time of 200 µs. The following isotopes were
137 analyzed in all the rice samples: ⁷⁵As, ¹¹²Cd, ⁵⁹Co, ⁵³Cr, ⁶³Cu, ⁵⁶Fe, ²⁰⁰Hg, ⁵⁵Mn, ²⁰⁷Pb ⁸⁸Sr,
138 ²³⁸U, ⁵¹V and ⁶⁶Zn.

139 The method limit of detection (LOD) and limit of quantification (LOQ) were
140 calculated as the concentration associated with 3 and 10 times the standard deviation of the
141 background noise recorded for 30 reagent blanks digested as real samples. Calibration
142 standard solutions were analyzed at the beginning and end of each sample sequence which
143 consisted of 30 samples. Table 1 shows LOD, LOQ and calibration range of each element.

144 Ge was employed as internal standard for V, Cr, Mn, Fe, Co, Cu, Zn and As and Tb for Sr,
145 Cd, Hg, Pb and U.

146

147 *Arsenic speciation analysis*

148 For the arsenic speciation 0.5 g ground rice was placed into a vessel in which 10
149 mL of HNO₃ at 1% (Panreac, Barcelona, España) were added. The vessel was closed, and
150 the sample was extracted using a microwave oven decomposition system from Milestone
151 Ethos 1 (Soriso, Italy). The oven temperature was kept for 90 minute at 90 °C. After
152 cooling to room temperature, the solution was transferred into a polystyrene centrifuge
153 tubes, subsequently centrifuged (3000 rpm) and filtered through a disposable 0.45 µm
154 membrane filter fromALBET® LabScience (Daseel Germany).

155 Standard solutions were prepared daily and stored at 4°C in the dark. As(V),
156 As(III), AsB, DMA and MMA were obtained from Sigma-Aldrich (St Louis, MO), AB
157 was obtained from the Institute of Reference materials Measurements (Geel, Belgium).
158 Eluents were filtered through a disposable 0.45 µm membrane filter fromALBET®
159 LabScience (Daseel Germany) for As speciation analysis.

160

161 For analysis of arsenic species (As(III), As(V), AsB, DMA and MMA) a HPLC-
162 230 (Varian; Prostar, Agilent Technologies, Stockport, Cheshire. UK) connected to an
163 ICP-MS-820 (Bruker; Mulgrave, Australia) was used. The ICP-MS detector was equipped
164 with Collision Reaction Interface for interference management and a glass concentric
165 nebulizer connected to a double pass spray chamber. The chromatographic column was
166 obtained from Hamilton and consisted of a PRP-X100 10 µm anion-exchange column (250
167 x 4.1mm). The injection volume was 50 µL and the mobile phase flow rate was maintained
168 at 1 mL/min. employing of 12.5 mM ammonium carbonate (NH₄)₂CO₃ (mobile phase A)

169 and 30 mM ammonium carbonate (NH₄)₂CO₃ (mobile phase B), both mobile phases were
170 adjusted to pH 9 with ammonia. The gradient elution program employed was: t=0 min
171 100% A; t=2 min 80% A; t=2.9 min 60% A; t=3 min, 30% A; t=12.5min 0% A.

172 High purity deionized water (resistivity 18.2 M \square cm) used in sample analysis and
173 solution preparation was obtained using a Milli-Q water purification system (Millipore,
174 Bedford, MA, USA). Plastic bottles, vessels and conical tubes (Rubi, Barcelona, España)
175 were cleaned by soaking them into HNO₃ (10%) for 24 h and then rinsed with Milli-Q
176 water.

177

178 *Quality assurance and quality control*

179 Standard reference materials (SRM) of rice flour (SRM 1568b) and apple leaves
180 (SRM 1515) were obtained from the National Institute of Standards and Technology
181 (Gaithersburg, Maryland, USA) and were used to calculate method trueness and
182 precision.

183

184 *Estimated weekly intake*

185 Estimated weekly intakes (EWI) through rice for the Kazakh, Spanish and
186 Portuguese populations were calculated for each one of the elements investigated based on
187 the average content obtained during this research and weekly rice consumption. Body
188 weight was set at 70 kg for a standard male adult and at 24 kg for children, as described by
189 Ferré-Huguet et al. (2008). The rice consumption data for the Kazakh population were
190 calculated as the median of data from studies from the Ministry of Labor and Social
191 Protection and the Statistical Agency of the Republic of Kazakhstan (2005), data from the
192 leading company in consumer studies in Kazakhstan (Good Creative Marketing PR 2010)
193 and data from the Food and Agriculture Organization of the United Nations (FAO 2003).

194 The data to calculate the EWI for the Spanish population were obtained from
195 Cerdeño (2012). Finally, for the Portuguese population, the data regarding rice
196 consumption was obtained from the Centre for the Promotion of Imports from developing
197 countries (CBI 2010).

198 To calculate the EWI, equation 1 was used:

199

$$200 \quad EWI=CxM/Bw \quad \text{Equation (A.1)}$$

201

202 where C ($\mu\text{g/g}$) is the average chemical element concentration in rice obtained
203 during this study (Table 2, Table 5 and Table 6) and M (kg) is the amount of rice
204 consumed weekly in the Republic of Kazakhstan, Portugal or Spain.

205

206 *Statistical analyses*

207 To evaluate significant differences in mineral concentrations between the rice
208 types or rice origins, a one-way ANOVA was performed. Similarly, linear discriminant
209 analysis (LDA) was used to verify the classification of the rice samples according to
210 the data obtained. All statistical analyses were performed using Statgraphics
211 Centurion[®] software for Windows (SAS Institute, North Carolina, USA).

212

213 **Results and discussion**

214

215 *Total arsenic and arsenic speciation in rice*

216 Taken into consideration that As is essential for human but it is also toxic at high
217 concentration or with long term exposure, the FAO/WHO set up maximum levels for
218 inorganic As of 0.3 mg/kg for raw rice and 0.2 mg/kg for polished rice. As FAO/WHO

219 indicate that total As can be used as an screening method of Inorganic As (FAO/WHO
220 2012). Therefore, for this research study total As was measured first and results are shown
221 in Table 2. Levels of total As in Kazakh unpolished rice were similar to those observed in
222 rice collected from a typical E-waste recycling area in Zhejiang Province, Southeast China
223 (Fu et al. 2008) but were higher compared to those collected from an As-affected district in
224 India (Bhattacharya et al. 2010), from a high-risk industrialized tungsten-mining area in
225 China (Liu et al. 2010) and from milled rice samples collected from Brazil (Poletti et al.,
226 2014). The total As concentrations in the unpolished rice collected from different areas of
227 Kazakh stan showed significant differences ($p<0.05$) being higher the concentration in rice
228 collected from the Central area of Kyzylorda Province. Significant difference ($p<0.05$) was
229 also observed between unpolished and milled rice samples obtained from the Republic of
230 Kazakhstan. The average concentration of total As in the unpolished rice (0.24 mg/kg) was
231 double than in the milled rice (0.10 mg/kg). This is an expected result after milling brown
232 rice to white rice as was indicated in the Joint FAO/WHO Food Standard Programme,
233 Codex Committee on Contaminants in Foods (WHO 2011). The highest average
234 concentration of total As in milled rice was measured in Portuguese rice (0,36 mg/kg)
235 which was higher than those measured in Italian rice by others researchers (Sommella et
236 al., 2013).

237 Total As concentration in Kazakh milled rice ranged from 0.03 to 0.15 mg/kg and
238 did not exceed the above mentioned MLs (FAO/WHO 2012) nor 0.2 mg/kg the one
239 established by the Kazakh legal regulations for total As in cereals (Health Ministry of the
240 Republic of Kazakhstan 2010)

241 When total As measured in the milled rice obtained from the Republic of
242 Kazakhstan were compared with those obtained from the Spanish and Portuguese rice
243 samples (Table 2) significant difference ($p<0.05$) was observed. While none of the

244 Kazakhstan milled rice samples contained levels of total As above the ML, 13 from
245 Portugal and 11 from Spain showed total As concentrations above 0.3 mg/kg and also 11
246 unpolished rice samples from Kazakhstan. All these samples, those with concentrations of
247 total As above 0.3 mg/kg, were analyzed to separate organic (AsB, DMA and MMA)
248 and inorganic As (As(III), As(V)). Several studies have shown that the main species of As
249 detected in rice was inorganic (Williams et al. 2006; Liang et al. 2010).

250 In milled rice, Spanish and Portuguese, As speciation ranked in the order
251 As(III)>MMA>As(V)>DMA>AsB (Table 3), while in natural rice the order was
252 As(III)> As (V)>DMA>MMA>AsB, the fact that As III was the most abundant in both
253 type of rice is important due to its toxicity. Our results were similar to those obtained by
254 other researchers from Bangladesh, China and Europe countries, where inorganic As
255 predominated the organic As (Liang et al. 2010; Williams et al. 2006). Only mean
256 inorganic As in Spanish rice samples exceed 0.20 mg/kg, the ML set up for milled rice.
257 Out of 35 samples, 7 samples from Spain, four from Portugal and 9 from Kazakhstan
258 exceeded the ML set up by the FAO/WHO.

259 DMA was the predominant species in Spanish and Portuguese rice samples with
260 53% and 56% of the As being organic, respectively. However, in Kazakh rice inorganic As
261 was predominant with 90% of the As detected classified as inorganic, with a mean of 0.36
262 mg/kg which is slightly higher than the ML of 0.3 mg/kg for inorganic As in raw rice
263 proposed by FAO/WHO (WHO 2012). As(III) represented the most abundant As species
264 extracted in analyzed Kazakh samples. There are evidences regarding the use of pesticides
265 with As in Kyzylorda region, likewise some researchers have reported As concentrations
266 higher in urine measured in children in Kyzylorda province than the concentrations of total
267 As in the urine of children from Europe (Erdinger et al. 2004). The present research
268 supports those previous results published by Erdinger and collaborators. To the best of our

269 knowledge, no data was available on the As concentrations in rice from Kazakhstan.
270 Unpolished rice is not commonly consumed in Kazakhstan as in Spain or Portugal, where
271 population frequently consumes unpolished or brown rice. It can be stated that inorganic
272 species does not cause danger to the Kazakh population, because of bran of unpolished rice
273 has 10-20-fold higher inorganic As concentration than polished rice (Sun et al. 2008).
274 Because of the relatively high content of As in rice it would be interesting to investigate
275 the total As, and other toxic metal contents in water used in boiling rice. It would be likely
276 to expect an increase in total As concentrations in rice after boiling (Domingo 2011).

277

278 *Cd, Hg and Pb concentrations*

279 The total Cd and Hg concentrations in the unpolished rice collected from different
280 areas of the Republic of Kazakhstan showed significant differences ($p < 0.05$) within the
281 concentrations of these metals, which were higher in rice collected from the central area of
282 Kyzylorda Province. These results are consistent with data from Berdenkulova (2008),
283 who demonstrated differences between the South and the North of Kyzylorda Province for
284 concentrations of toxic metal ions from soil and water samples. However, no significant
285 differences ($p > 0.05$) in the Pb concentration were observed for these two areas of
286 Kazakhstan. The minimum, maximum and average concentrations of, Cd, Hg and Pb in the
287 milled rice obtained from markets in the Republic of Kazakhstan are summarized in Table
288 2. Even though concentration of As in Kazak rice was similar to data reported previously
289 for others Asian countries, concentrations of Cd, Hg and Pb were lower than to those
290 reported from Asian countries and the Unites States (Al-Rmalli et al. 2012; Fu et al. 2008;
291 Liu et al. 2007; Zhuang et al. 2009; Qian et al. 2010; Park et al. 2011) and similar to those
292 reported by Cao et al. (2010) for China. Overall, it could be said that concentrations of Cd,
293 Hg and Pb in rice from Kazakhstan were between the LOD and the LOQ (Table 1).

294 When the concentrations of total Cd, Hg and Pb measured in the milled rice
295 obtained from the Republic of Kazakhstan were compared with those obtained from the
296 Spanish and Portuguese rice samples (Table 2), Hg concentrations were found to be
297 significantly different ($p < 0.05$). The highest average value for Hg concentrations were
298 found in the Spanish and Portuguese milled rice, with an average value of 0.01 mg/kg.
299 While Portuguese rice showed the highest mean for Cd and Pb, those from Spain and
300 Kazakhstan were below LOD.

301 In cereals, legal regulations from the Republic of Kazakhstan established MLs for
302 Cd, Hg and Pb of 0.1, 0.03 and 0.5 mg/kg, respectively (Health Ministry of the Republic of
303 Kazakhstan 2010), whereas Regulation 1881/2006 of the European Communities
304 established MLs of 0.2 mg/kg for Cd and Pb and the Codex Alimentarius indicated an ML
305 of 0.4 mg/kg for Cd. Based on these legislations, it can be stated that the concentrations for
306 Cd, Hg and Pb in the commercial rice from the Republic of Kazakhstan, Spain and
307 Portugal analyzed during this study were below the established ML limits.

308 The aim of this research work was to evaluate the presence of toxic elements in rice
309 sample and evaluate the possible risk for Kazakh population through the consumption of
310 rice. From our results, with regards to Cd, Hg and Pb, it can be stated that there is no risk
311 to the population due to the consumption of local Kazakh rice, especially considering that
312 the Kazakh population does not consume integral or unpolished rice. Following this
313 suggestion, it must be stated that high concentrations of Cd, Pb and Zn were found
314 previously in animal meat (cattle, horse and sheep) products of the (Farmer and Farmer
315 2000) and could be a higher risk for the population than the rice.

316

317

318

320 Co, Cu, Mn Fe and Zn are elements that need provided by dietary source however
321 at high concentration they can also produce toxic effects. The average, minimum and
322 maximum concentrations of Co, Cu, Fe, Mn and Zn in the rice samples are summarized in
323 Table 4. The concentrations of Co, Cu and Mn in unpolished rice collected from central
324 and southern areas of Kyzylorda Province (Republic of Kazakhstan) were significantly
325 different ($p < 0.05$). Rice collected in the central area showed higher average concentrations
326 of Co (0.02 mg/kg), Cu (2.51 mg/kg) and Mn (16.48 mg/kg). The Zn and Fe concentrations
327 were not significantly different ($p > 0.05$) between the central and southern Kyzylorda
328 province areas. On the other hand, the Co, Cu, Fe and Mn concentrations were
329 significantly different in the unpolished and milled rice from the Republic of Kazakhstan,
330 Spain and Portugal ($p < 0.05$). The average Cu concentration was higher in the unpolished
331 rice (2.35 mg/kg) compared to the level obtained for the Kazakh milled rice (1.84 mg/kg).
332 The levels of Co and Mn were double in the unpolished rice, and the level of Fe was 3
333 times higher in the unpolished rice compared with data obtained from milled samples from
334 Kazakhstan. The latter result is likely due to the declining gradient for the Fe concentration
335 from the outer to the inner space of the grain (Prom-u-thai et al. 2007). The Fe
336 concentration in unpolished rice ranged from 9.99 to 65.15 mg/kg with a mean of 18.55
337 mg/kg. These results were lower than those reported for China (mean 32 mg/kg, range
338 0.40-147 mg/kg (Zeng et al. 2010)) and higher than those reported in India (mean 14.06
339 mg/kg; range 1.25-39.19 mg/kg (Anandan et al. 2011)). Dialysability studies demonstrate
340 that the availability of iron in rice is low, for example, in cereal diets; Fe is not available to
341 the human body due to the presence of inhibitory phytate compounds. Although the
342 cooking process degrades phytates, processing rice could increase iron absorption (Prom-
343 u-thai et al. 2007). Mean Fe concentrations in Milled rice were similar in rice from

344 Kazakhstan, Spain and Portugal and similar to those reported in a study conducted in
345 Kingdom of Saudi Arabia ([Shraim](#) 2014).

346 With regards to Co, Cu, Mn and Zn, several recent studies on rice collected from mining
347 and industrialized areas in Kazakhstan have found similar or higher levels of Co, Cu, Mn
348 and Zn (Heikens et al. 2005; Liu et al. 2005; Fu et al. 2008; Zhuang et al. 2009; Anandan
349 et al. 2011) in other Asian and European countries and in the United States.

350

351 *Concentrations of Cr, Sr, U and V in rice samples*

352 The concentrations of Cr, Sr, V and U in the rice grains collected in Kazakhstan, Spain and
353 Portugal are shown in Table 4. A significant difference ($p>0.05$) was observed in the Cr
354 and V concentrations between samples from the central and southern parts of Kyzylorda
355 Province (Republic of Kazakhstan), with the samples obtained from the central part of the
356 province containing higher concentrations of Cr and V. However, no significant
357 differences were observed for the Sr and U concentrations. The Cr, Sr and V
358 concentrations were significantly different ($p<0.05$) in the unpolished and milled rice from
359 the Republic of Kazakhstan. Higher Cr, Sr and V average values were measured in the
360 unpolished rice. In particular, the Sr mean concentration was 1.33 mg/kg in unpolished rice
361 and below LOD (0.86 mg/kg) in milled rice. The Sr concentrations in the Kazakh, Spanish
362 and Portuguese milled rice were significantly different ($p<0.05$), Sr was mainly detected in
363 Kazakh rice with a maximum concentration of 2.47 mg/kg. Mean V concentration was
364 above the LOD only for unpolished samples of Kazakhstan and Cr mean concentration
365 were similar in samples from Kazakhstan, Spain and Portugal. It could be stated that U was
366 not detected in the rice samples analyzed, it is important to highlight this results because
367 Kazakhstan was a leader in the global uranium mining industry in 2012, providing

368 approximately 37% of the world's uranium production. Some uranium mines are located in
369 the rice-growing Kyzylorda Province, and, likewise, this province disposes of 65% (the
370 largest percentage for a province in Kazakhstan) of the V stocks in Kazakhstan. Thus, the
371 little information available on these elements in Kazakh rice has led to studies on the
372 content of these metals in rice to obtain more information for evaluating the health risk to
373 the local population from rice consumption.

374 Referring to Cr, some studies performed with rice have demonstrated its uptake by
375 the rice plants from soils (Bhattacharya et al. 2005; Zeng et al. 2008). The measured Cr
376 concentration in the Kazakh rice (0.03-0.054 mg/kg) was similar to that obtained from
377 other Asian countries, such as China (Fu et al. 2008), Iran (Zazouli et al. 2006) and Korea
378 (Jung et al. 2005). Related to U concentration in rice, authors working in high uranium
379 content areas (i.e., near areas surrounding uranium mines) have demonstrated the
380 bioaccumulation of this element in plants (0.134 to 1.293 mg/kg) due to dust transport near
381 uranium mines when data was compared with unpolluted areas (Zoriy et al. 2010). Thus, it
382 appears that the main rice production area in Kazakhstan (Kyzylorda Province), which was
383 sampled in the present work, is not contaminated with this element. The average
384 concentration of V (0.04 mg/kg) in the unpolished rice analyzed in this study was similar
385 or even higher compared to those obtained from milled rice (0.02 mg/kg) and the V
386 concentrations reported by Myron et al. (1977) and Antoine et al. (2012).

387

388 *Sample classification using discriminant analysis*

389 The classification of the 146 rice samples was performed using the data from the 13
390 elements studied using the discriminant analysis. The toxic and essential element contents
391 were used as chemical descriptors in the analysis to classify the different rice samples. All
392 samples belonging to the Kazakhstan unpolished or milled rice were correctly classified

393 using the 13 variables studied (Figure 2); however, some samples, belonging to the
394 Portuguese or Spanish milled rice groups, were not well classified due to similarities in
395 mineral contents. From Figure 2, it can be established that all the milled rice samples are
396 relatively comparable according to their mineral content and can therefore be easily
397 discriminated from the "Unpolished rice" group. Among the 146 rice samples used to fit
398 the model, 130 (89.04%) were classified correctly. Other researchers have previously used
399 this statistical tool to classify and establish a geographical traceability for rice according to
400 mineral element content (González et al. 2011). With regards to the unpolished rice,
401 sample groups from the central and southern areas of Kyzylorda Province were well
402 classified (98%) separately from one another. With regards to these classifications, the
403 standardized coefficient of the discriminant function shows that the independent variables
404 that most affect the discriminant function were the Fe and V concentrations. The
405 standardized coefficient of the discriminant function describes the relative burden of each
406 independent variable in determining the valor of the function. This coefficient is consistent
407 with the results obtained during this study, showing that the V concentration in the
408 unpolished rice was significantly different between the central and southern areas of
409 Kyzylorda Province. In fact, one of the major V-producing companies in Kazakhstan is
410 located in the southern part of the Kyzylorda Province. However, as stated previously, no
411 significant differences were observed for Fe concentrations between the central and
412 southern areas.

413

414 *Estimated Weekly Intake of metals from Kazakh, Spanish and Portuguese rice*

415 WHO and EFSA have established the following provisional tolerable weekly intake
416 (PTWI): 0.5-3.0 µg/kg for As (WHO, 2010); 2.5 µg/kg for Cd (EFSA, 2011); 23.3 µg/kg
417 for Cr (Lin et al., 2004; Zazouli et al., 2006); 3500 µg/kg for Cu (WHO, 1982); 4 µg/kg for

418 Hg (WHO, 2010); 25 µg/kg for Pb (WHO, 1993) and 7000 µg/kg for Zn (WHO, 1982).
419 The weekly intake was calculated for Kazakh, Spanish and Portuguese population taken in
420 to consideration concentration of total As, inorganic As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Pb,
421 Sr, U, V in Kazakh, Spanish and Portuguese rice (Table 5). EWI for total As (0.22-1.69
422 µg/kg to adults; 0.33-4.10 µg/kg to children), inorganic As (0.29-0.84 µg/kg to adults;
423 0.70-2.04 µg/kg to children), Cd (0.02-0.14 µg/kg to adults; 0.07-0.33µg/kg to children),
424 Cr (0.15-0.61 µg/kg to adults; 0.35-0.70 µg/kg to children), Cu (2.51-12.94 µg/kg to
425 adults; 5.95-31.46 µg/kg to children), Hg (0.01-0.04 µg/kg to adults; 0.02-0.09. µg/kg to
426 children), Pb (0.01-0.04 µg/kg to adults; 0.02-0.10 µg/kg to children) and Zn (20.46-
427 110.29 µg/kg to adults; 48.57-268.08 µg/kg to children) from rice consumption by Kazakh,
428 Spanish and Portuguese adults and children were lower than the PTWI values established
429 by WHO and EFSA. EFSA has withdrawn its PTWI for Pb (EFSA 2010). According to
430 inorganic As, the Joint Food and Agriculture Organization of the United Nations
431 (FAO)/WHO Expert Committee on Food Additives (JECFA) determined the lower limit
432 on the benchmark dose for a 0.5% increased incidence of lung cancer (BMDL_{0.5}) from
433 epidemiological studies to be 3.0 µg/kg body weight per day (2-7 µg/kg body weight per
434 day based on the range of estimated total dietary exposure) FAO/WHO (2010). Based on
435 these data, it can be assumed that the amounts of total or inorganic As, Cd, Cr, Cu, Hg, Pb
436 and Zn consumed through rice do not support a risk for the Kazakh, Spanish and
437 Portuguese population. Due to the abundant supply of accessible minerals throughout
438 Kazakhstan, the metal intake of the population could be from other food sources.
439 References concerning the risk assessment for metals from rice consumption in
440 Kazakhstan were not found. Thus, it is very important to have relevant information to
441 estimate the health risk for rice consumption by the Kazakh population. During this study,
442 the EWI for total As, Cd, Pb, Cu, Cr and Zn calculated from the milled rice, which is

443 consumed by Kazakh adults and children, were lower than intakes previously estimated in
444 other Asian countries, such as Taiwan (Lin et al. 2004) and Iran (Zazoli et al. 2006), and
445 the EWI values for the Spanish and Portuguese population were lower than those available
446 data reported by Ferré-Huguet et al. (2008) and Martorell et al. (2011) in Spain.

447

448 **Conclusions**

449

450 The rice results gathered during this study provide a better understanding of the
451 current state of the rice obtained from the Republic of Kazakhstan, Spain and Portugal and
452 its potential risk to human health. Based on the data obtained, it can be stated that the rice
453 samples analyzed did not contain concentrations of toxic elements such as Cd, Hg and Pb
454 above the ML established by different European and Kazakh legislations. However, 90%
455 of As detected in Kazakh unpolished rice was inorganic (n=11) which values were slightly
456 higher than the ML of 0.3 mg/kg. As (III) was the predominant species in the unpolished
457 rice samples which should be of concern to health population who consume rice. Further
458 studies need to be conducted with more samples in order to state As level in rice grain
459 from Kazakhstan. Likewise in Spanish and Portuguese milled rice samples which exceeded
460 the level of 0.3 mg/kg (11 and 13 samples respectively) most of the As was organic DMA.

461 The environmental distribution of Kyzylorda Province contamination may reflect
462 the results for some mineral concentrations in samples from this province (i.e., V
463 concentration differences between central and southern zones). However, although
464 Kazakhstan environmental contamination appears to be higher than that of the European
465 countries included in this study, the results for the Kazakh unpolished samples indicate a
466 lesser impact for the concentrations of elements studied in the composition of rice than
467 might be expected. In spite of the importance of the present work, some data presented in

468 this study must be carefully considered because rice sampling in Kazakhstan is currently
469 very complex due to the significant amount of territory encompassed by Kyzylorda
470 Province (226.019 km²) and the relatively low population concentration in rural rice-
471 producing areas.

472

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474

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478

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480

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659 TABLE 1: Quality control parameter

Element	LOD (mg/kg)	LOQ (mg/kg)	Truness ^a (%)	Truness ^b (%)	Recoveries ^c (%)	Repetibility ^c	Reproducibility ^c
As ⁷⁵	0.03	0.09	104,70		105,3	6,27	38,50
Cd ¹¹¹	0.01	0.04	84,10	101,04	100,6	6,11	8,11
Co ⁵⁹	0.01	0.02	90,27		100,9	17,82	25,49
Cr ⁵³	0.05	0.18		100,79	88,9	29,03	32,49
Cu ⁶³	0.03	0.10	94,49	103,90	104,2	3,86	4,47
Fe ⁵⁶	1.60	5.32	118,89	95,81	104,6	7,53	12,39
Hg ²⁰²	0.01	0.02	75,16	96,42	89,8	19,70	14,81
Mn ⁵⁵	0.03	0.09	114,20	99,65	110,0	5,35	11,18
Pb ²⁰⁸	0.05	0.18	168,11	55,89	85,8	15,08	5,54
Sr ⁸⁸	0.86	2.86		90,54	104,8	6,38	9,71
U ²³⁸	0.03	0.10		74,20	61,0	13,27	12,85
V ⁵¹	0.03	10,00		73,99	95,5	12,99	12,22
Zn ⁶⁶	1.48	4.93	95,97	121,98	106,6	8,79	16,18
As III	0,05	0,15					
As V	0,04	0,15					
As Inorganic	0,09	0,29	98,27			27,17	
AsB	0,01	0,02					
DMA	0,00	0,01	73,34			23,19	

MMA 0,02 0,07 106,04 10,05

As
Speciation 0,11 0,35 83,18 24,62

660 ^aTruness of reference material of rice; ^bTruness of reference material of apple leave; ^cData obtained with rice samples fortified at 1 and 5 µg/kg

661

662 **Table 2:** Concentrations of As, Cd, Hg and Pb in rice samples from the Republic of
 663 Kazakhstan, Spain and Portugal (mg/kg)

Element	Area	N	A ^a	A.S.D ^b	Minimum	Maximum
As	Unpolished (Kazakhstan)	51	0.24	0.09	0.04	0.36
	Milled (Kazakhstan)	45	0.10	0.03	0.03	0.15
	Milled (Spain)	30	0.28	0.24	< LOD	1.16
	Milled (Portugal)	20	0.36	0.14	0.22	0.78
Cd	Unpolished (Kazakhstan)	51	< LOD	< LOD	< LOD	< LOD
	Milled (Kazakhstan)	45	< LOD	< LOD	< LOD	< LOD
	Milled (Spain)	30	< LOD	< LOD	< LOD	0.03
	Milled (Portugal)	20	0.03	0.02	< LOD	0.08
Hg	Unpolished (Kazakhstan)	51	< LOD	< LOD	< LOD	0.01
	Milled (Kazakhstan)	45	< LOD	< LOD	< LOD	0.01
	Milled (Spain)	30	0.01	< LOD	< LOD	< LOD
	Milled (Portugal)	20	0.01	< LOD	0.01	0.01
Pb	Unpolished (Kazakhstan)	51	< LOD	< LOD	< LOD	0.25
	Milled (Kazakhstan)	45	< LOD	< LOD	< LOD	0.08
	Milled (Spain)	30	< LOD	< LOD	< LOD	0.04
	Milled (Portugal)	20	0.01	< LOD	< LOD	0.02

664 ^aaverage values, ^barithmetic standard deviation, < LOD: below limit of detection

665

666 **Table 3:** Arsenic speciation of Kazakh, Spanish and Portuguese rice samples showing an amount
667 of As with values above 3.0 mg/kg
668

Element	Area	N	A ^a	A.S.D ^b	Minimum	Maximum
AsB	Unpolished (Kazakhstan)	11	0.0001	0.0005	0	0.002
	Milled (Spain)	11	0.001	0.002	0	0.004
	Milled (Portugal)	13	0.001	0.002	0	0.01
DMA	Unpolished (Kazakhstan)	11	0.03	0.02	0	0.06
	Milled (Spain)	11	0.27	0.15	0.12	0.63
	Milled (Portugal)	13	0.22	0.14	0.12	0.65
MMA	Unpolished (Kazakhstan)	11	0.004	0.004	0	0.01
	Milled (Spain)	11	0.02	0.01	0	0.04
	Milled (Portugal)	13	0.01	0.01	0	0.05
AsB+DMA+MMA	Unpolished (Kazakhstan)	11	0.04	0.02	0	0.06
	Milled (Spain)	11	0.29	0.16	0.12	0.66
	Milled (Portugal)	13	0.23	0.15	0.13	0.71
As (III)	Unpolished (Kazakhstan)	11	0.30	0.05	0.21	0.36
	Milled (Spain)	11	0.19	0.10	0.09	0.46
	Milled (Portugal)	13	0.13	0.05	0.08	0.26
As (V)	Unpolished (Kazakhstan)	11	0.06	0.01	0.04	0.09
	Milled (Spain)	11	0.07	0.05	0.02	0.16
	Milled (Portugal)	13	0.05	0.04	0.02	0.13
As (III)+ As (V)	Unpolished (Kazakhstan)	11	0.36	0.02	0.25	0.45
	Milled (Spain)	11	0.25	0.16	0.15	0.55
	Milled (Portugal)	13	0.18	0.15	0.10	0.30
Total As determined as a sum of five arsenic species	Unpolished (Kazakhstan)	11	0.40	0.06	0.29	0.49
	Milled (Spain)	11	0.55	0.26	0.30	1.21
	Milled (Portugal)	13	0.41	0.17	0.26	0.91

669 ^aaverage values, ^barithmetic standard deviation

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671
672
673

Table 4: Concentrations of other heavy metals and essential elements in rice samples from the Republic of Kazakhstan, Spain and Portugal (mg/kg).

Element	Area	N	A ^a	A.S.D ^b	Minimum	Maximum
Co	Unpolished (Kazakhstan)	51	0.02	0.01	0.01	0.04
	Milled (Kazakhstan)	45	0.01	0.004	< LOD	0.02
	Milled (Spain)	30	0.01	0.01	< LOD	0.06
	Milled (Portugal)	20	0.02	0.02	0.01	0.08
Cr	Unpolished (Kazakhstan)	51	0.21	0.11	0.06	0.54
	Milled (Kazakhstan)	45	0.13	0.07	< LOD	0.37
	Milled (Spain)	30	0.13	0.06	< LOD	0.28
	Milled (Portugal)	20	0.13	0.05	0.08	0.31
Cu	Unpolished (Kazakhstan)	51	2.35	0.53	1.40	3.94
	Milled (Kazakhstan)	45	1.84	0.54	0.69	2.95
	Milled (Spain)	30	2.13	0.81	1.05	5.22
	Milled (Portugal)	20	2.78	0.64	1.99	4.07
Fe	Unpolished (Kazakhstan)	51	18.55	8.07	9.99	65.15
	Milled (Kazakhstan)	45	5.27	3.22	2.68	18.49
	Milled (Spain)	30	5.01	3.14	2.53	19.23
	Milled (Portugal)	20	4.86	0.97	3.48	7.06
Mn	Unpolished (Kazakhstan)	51	15.47	2.96	8.21	24.17
	Milled (Kazakhstan)	45	6.14	1.46	3.56	10.49
	Milled (Spain)	30	9.98	6.49	3.70	40.08
	Milled (Portugal)	20	13.97	4.42	9.17	24.72
Sr	Unpolished (Kazakhstan)	51	1.33	< LOD	< LOD	2.47
	Milled (Kazakhstan)	45	< LOD	< LOD	< LOD	< LOD
	Milled (Spain)	30	< LOD	< LOD	< LOD	< LOD
	Milled (Portugal)	20	< LOD	< LOD	< LOD	< LOD
U	Unpolished (Kazakhstan)	51	< LOD	< LOD	< LOD	< LOD
	Milled (Kazakhstan)	45	< LOD	< LOD	< LOD	< LOD
	Milled (Spain)	30	< LOD	< LOD	< LOD	< LOD
	Milled (Portugal)	20	< LOD	< LOD	< LOD	< LOD
V	Unpolished (Kazakhstan)	51	0.04	0.07	< LOD	0.46
	Milled (Kazakhstan)	45	< LOD	< LOD	< LOD	< LOD
	Milled (Spain)	30	< LOD	< LOD	< LOD	< LOD
	Milled (Portugal)	20	< LOD	< LOD	< LOD	< LOD
Zn	Unpolished (Kazakhstan)	51	20.95	4.59	9.00	29.43
	Milled (Kazakhstan)	45	19.98	21.89	9.35	152.52
	Milled (Spain)	30	17.36	7.65	6.55	40.66
	Milled (Portugal)	20	23.68	6.16	13.23	36.28

^aaverage values, ^barithmetic standard deviation, < LOD: below limit of detection

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676 **Table 5:** Average estimated weekly intake of heavy metals and essential elements by
 677 eating rice in Kazakh, Spanish and Portuguese population (70 kg body weight for adults
 678 and 24 kg body weight for children)
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Elements	Kazakh adults	Kazakh children	Spanish adults	Spanish children	Portuguese adults	Portuguese children
	150 ^a	125	82.47	67.12	326.03	271.7
Total As	0.22 ^b	0.53	0.33	0.78	1.69	4.10
Inorganic As	0.77 ^b	1.88	0.29	0.70	0.84	2.04
Cd	0.03	0.07	0.02	0.05	0.14	0.33
Co	0.03	0.06	0.01	0.02	0.06	0.13
Cr	0.29	0.70	0.15	0.35	0.61	0.68
Cu	3.94	9.57	2.51	5.95	12.94	31.46
Fe	11.29	27.45	5.91	14.02	22.65	55.05
Hg	0.01	0.03	0.01	0.02	0.04	0.09
Mn	12.83	31.19	10.36	24.60	62.39	151.64
Sr	0.77	1.88	0.25	0.60	0.55	1.34
Pb	0.03	0.08	0.01	0.02	0.04	0.10
U	0.0004	0.001	0.0003	0.001	0.001	0.002
V	0.03	0.07	0.02	0.04	0.09	0.22
Zn	42.80	104.04	20.46	48.57	110.29	268.08

680 ^aRice consumption media (grams per week) for each population group, ^bAverage estimated
 681 weekly intake are given in µg/kg.

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