Probabilistic Analysis of Human Health Risks Linked to Procambarus Clarkii Consumption in Almaden Hg Mining District

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Abstract

The American crayfish (*Procambarus clarkii*) is considered a bioindicator of Potentially Toxic Elements (As, Hg, Cd, Pb, Cu, Zn, and Sb); on this basis, this study aims to assess the human health risk associated with its consumption by the local population in the Almadén mercury mining district, in terms of Hazard Quotient and Cancer Risk. A probabilistic Bayesian approach was applied based on the concentrations of each PTE in the sampled crayfish and survey data collected from the local population. The results show that consumption of crayfish represents a health risk, mainly due to its As, Hg and Sb content.

Keywords: Bayesian probabilistic approach, mercury pollution, bioaccumulation, food safety, human health risk, potentially toxic elements

Introduction

The Almadén Mercury Mining District (AMMD), located in Spain, has been historically impacted by intense mercury mining activities. These impacts have been exacerbated by inadequate mine closure practices, leading to widespread contamination of soil, water, sediments, and biota with potentially toxic elements (PTEs) such as mercury (Hg), arsenic (As), and cadmium (Cd) (Higueras *et al.*, 2016; Jiménez-Oyola *et al.*, 2020; Barquero *et al.*, 2023). This contamination poses significant ecological and human health risks, particularly for populations living in proximity to the mining district.

Among the various exposure pathways, dietary intake of contaminated aquatic species has emerged as a critical concern. In the AMMD, the freshwater crayfish Procambarus clarkii is frequently consumed by the local population. This species is known for its high capacity to bioaccumulate PTEs, especially mercury, in its tissues (Anandkumar *et al.*, 2020). While several studies have assessed environmental contamination in the region, there is a notable lack of research evaluating the health risks associated with the consumption of bioindicator species such as *Procambarus clarkii*, particularly in historically contaminated mining environments.

This study aims to assess both carcinogenic and non-carcinogenic human health risks in adults associated with the consumption of abdominal muscle (AbM) from Procambarus clarkii in the AMMD. To address this objective, a Bayesian probabilistic framework is employed. Bayesian statistics provide a robust method for incorporating uncertainty and prior knowledge into the analysis of environmental health risks. Based on Bayes' theorem, this approach allows the estimation of the probability of health outcomes while correcting for potential errors or limitations in empirical data (Wu *et al.*, 2014). Importantly, it is especially suitable for cases where sample sizes are limited, as it enables the integration of prior information to enhance the reliability of results (Aven and Eidesen, 2007).

In this study, probabilistic distributions were assigned to both the concentrations of PTEs measured in crayfish tissues and the exposure parameters obtained from surveys conducted among the local population. These distributions were constructed by combining in situ data with well-established probability density functions (PDFs) from the scientific literature (Iribarren *et al.*, 2009). This framework was used to estimate key health risk metrics, including the Hazard Quotient (HQ), Cancer Risk (CR), Hazard Index (HI), and Total Cancer Risk (TCR) for each sampling point.

The results contribute to a deeper understanding of the long-term environmental and public health implications of historical mining activities in the AMMD. Furthermore, the findings highlight the value of *Procambarus clarkii* as a reliable bioindicator species, providing a basis for future monitoring programs and environmental health assessments in similarly impacted regions.

Methods

The study area was located along a stretch of the Valdeazogues River and its tributaries within the AMMD area in Ciudad Real, Spain. A total of 10 sites (S1, S2, S3, S4, S5, S6, S7, S8, S9, and S10) were sampled.

A total of 300 *Procambarus clarkii* specimens were collected. The crayfish were rinsed with Milli-Q water and stored in airtight polyethylene bags. Upon arrival at the laboratory, they were euthanized by hypothermia.

After a minimum of one week, the crayfish were dissected using a stainless-steel dissection kit to extract the AbM. The extracted tissues were weighed to calculate parameters for the Hazard Quotient (HQ). They were then freeze-dried using a Telstar Cryodos apparatus (at 0.1 mbar and approximately -50 °C). The dried samples were powdered and homogenized with a KINEMATICA Microtron MB 800 B blade mill for further analysis.

The AbM samples were analyzed using Energy Dispersive X-ray Fluorescence (ED-XRF) to determine the concentrations of Potentially Toxic Elements (PTEs), including

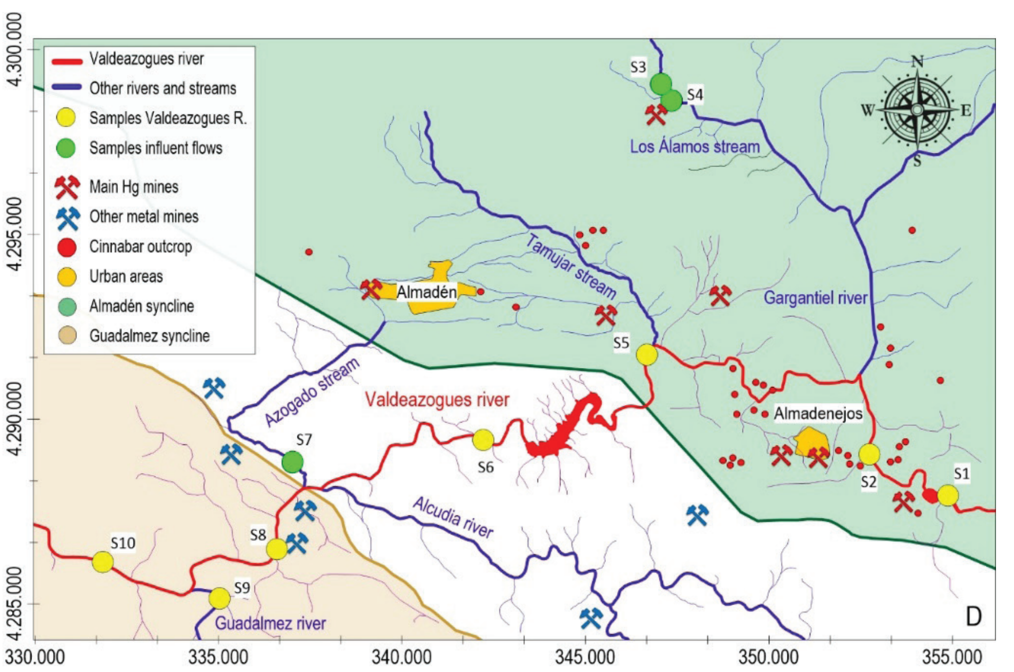

Figure 1 *Location of the sampling sites, main mines and Hg outcrops.*

As, Cd, Pb, Cu, Zn, and Sb. Additionally, total mercury (HgT) concentration was quantified using Zeeman-corrected atomic absorption spectrometry (Sholupov and Ganeyev, 1995).

A structured survey was administered to the adult local population, following the privacy policy of Spain, to gather key exposure parameters, including body weight (BW), exposure frequency (EF), ingestion rate (IR), and exposure duration (ED). These variables are essential for characterizing the population in the context of human health risk assessment.

In order to perform the risk assessment, it has been done the estimation of the absorbed dose (ADD: mg kg^{-1} day^{-1}) which was estimated using the equation:

$$ADD = \frac{C \times EF \times IR \times ED}{AT \times BW} \quad (1)$$

Where C is the concentration (mg kg^{-1}), EF is the exposure frequency (days year^{-1}), IR is the ingestion rate (kg day^{-1}), ED is the exposure duration (years), AT is the average exposure time (365 days × 30 years for non-carcinogenic or 365 days × 70 years for carcinogenic risk assessment) and BW is the body weight (kg).

The non-carcinogenic (HQ) and carcinogenic risk (CR) were calculated according to equation 2 and 3, respectively.

$$HQ = \frac{ADD}{RfD} \quad (2)$$

$$CR = ADD \times SF \quad (3)$$

The oral reference dose (RfD) and the slope factor (SF) were obtained from the Risk Assessment Information System Website (RAIS, 2020). RfD: 0.0003 mg kg^{-1} for As, 0.001 mg kg^{-1} for Cd, 0.04 mg kg^{-1} for Cu, 0.0001 mg kg^{-1} for Hg, 0.004 mg kg^{-1} for Pb, 0.0004 mg kg^{-1} for Sb and 0.3 mg kg^{-1} for Zn. And the SF: 1.5 mg kg^{-1} day^{-1} for As and 0.0085 mg kg^{-1} day^{-1} for Pb.

Finally, the cumulative non carcinogenic risk, expressed as the Hazard Index (HI), has been calculated ($HI=\Sigma HQ_i$). Similarly, the Total Carcinogenic Risk (TCR) has been estimated ($TCR=\Sigma CR_i$). The acceptable threshold values are 1 for HI and 1E-05 for the TCR. (USEPA 2004a, 2001)

The probabilistic distributions used in the risk assessment were selected based on previous documentation and expert opinion. Additionally, EasyFit 5.5 software was used

***Table 1** Probabilistic distributions and their priors used for each parameter.*

Parameters	Distributions
C_{As}, C_{Cd}, C_{Hg}, C_{Pb}, C_{Sb}	LogNormal (muC, tauC)
	C = LogNormal (0, 0.1)
	tauC = Gamma (1.0E-04, 1.0E-04)
C_{Cu}, C_{Zn}	Gamma (alpha, beta)
	alpha = Gamma (1, 1)
	beta = Gamma (1, 1)
IR	Normal (muIr, tauIr)
	Ir = Normal (0, 0.1)
	tauIr = Gamma (0.07, 1.0E-04)
BW	Normal (muBw, tauBw)
	Bw = Normal (1, 1)
	tauIR = Gamma (2, 1)
EF	Beta (a, b)
	a = Normal (5, 1.0E-04)
	b = Normal (5, 1.0E-04)

to determine the best-fit distributions for collected data. The distributions and priors used for each parameter are found in Table 1. The probabilistic modelling was conducted in RStudio using the RJags package, obtaining 1000 values for each parameter (Jiménez-Oyola *et al.*, 2021).

Results and discussion

The metal concentrations detected at the sampling points are summarized in Table 2. Since measurements were taken on a dry weight basis, and assuming a water content of 70% for all analyzed samples, appropriate adjustments were made. Zn and Cu exhibited the highest concentrations, as they are essential elements required in trace amounts by organisms (Anadkumar *et al.*, 2020). In contrast, more than 50% of the samples exceeded the legal limit for Hg, Pb, and Cd (0.5 mg kg^{-1}, fresh weight), as established by the European Union (UE 2023/915, 2023).

As shown in the Fig. 2a, the HQ_{As} and HQ_{Sb} exceeds the acceptable limit of 1 at 97.5 percentile and HQ_{Hg} at 75 percentile. HQ_{Cu} also exceeds the limit in the 99.8 percentile. As regards the carcinogenic risk, the CR_{As} exceeds the permissible value of 1.E-05 (USEPA 2004a) in the 75 percentile, while Pb doesn't represents a carcingenic risk as it doesn't exceed this value (Fig. b). These results indicates that the consumption of crayfish in the Almadén mercury mining district may represent a risk for consumers.

As shown in Table 3, the HI exceeds the permissible value of 1 at each sampling site. In the case of the carcinogenic risk, all the sampling points also surpasses the limit for TCR of 1E-05.

Although all values exceed the maximum permissible limit, each site can be categorized into three levels based on HI values: low (1 to 2), medium (2 to 3), and high (≥3). Specimens from sampling site S3 exhibits the lowest HI value, placing it within the low-level category, probably because it's a point of non-influence, upstream Las Cuevas mine. Sampling sites S1, S2, S5, S6, S8, and S9 fall within the medium level, while sites S4, S7, and S10 are classified as high-level sites, all of them downstream a mining affected zone.

Among the high-HI sites, S7 (Arroyo Azogado) receives wastewater from nearby towns and runoff from the Almadén Hg mine, which likely contributes to its elevated HI value. Similarly, site S10 (Presa Mendoza) is located downstream the main mining zones; acting like a barrier as it receives water from the DMMA and the Alcudia Valley.

Among the medium-HI sites, S2 collects water draining from El Entredicho mine, potentially leading to a significant influx of As and Hg into the Valdeazogues River at this location. Unlike S7, which is affected by the Almadén Mercury mine—a site with historical metallurgical activity—S2 is influenced solely by El Entredicho mine, where no metallurgy was present. The S5 site, on the other hand, is influenced by two distinct sources: treated wastewater from the town of Almadén and runoff from the industrial area of the same town; this combination of inputs may explain its classification within the medium-HI range.

A significant difference in HI and TCR values can also be observed between sites S3 and S4, with S3 located upstream of Las Cuevas mine and S4 downstream of it. This contrast highlights the strong influence of

Table 2 *Metal concentrations of the sampled points (mg* kg^{-1}*, f.w.)*

	Minimum	p50	Mean	p97.5	Maximum
As	0.46	2.11	2.45	6.44	7.46
Cd	0.21	0.70		1.84	1.89
Cu	50.85	112.37	125.50	219.59	227.71
Hg	0.48	1.02	1.22	2.47	2.50
Pb	0.28	0.70		2.95	3.15
Sb	1.61	5.53	5.76	9.34	10.33
Zn	91.91	253.75	261.37	332.57	355.63

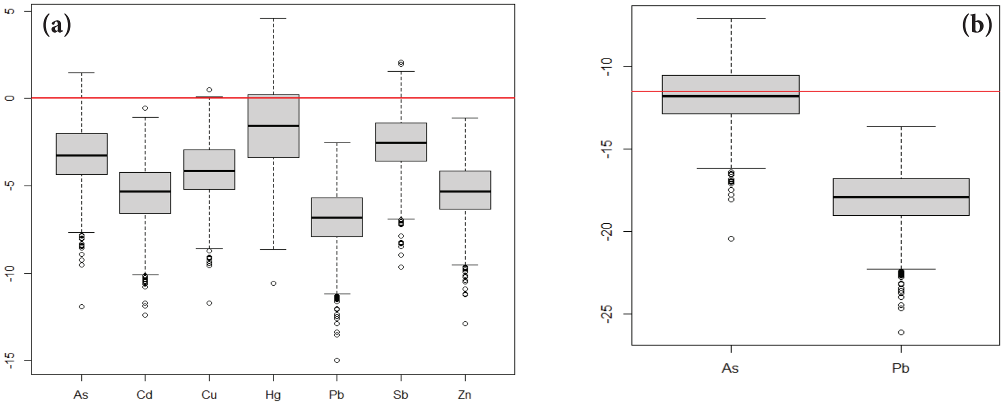

Figure 2 Boxplot of the (a) Hazard Quotient and (b) Cancer Risk (values in logarithm scale). The red line represents the safe exposure threshold.

Table 3 Values of the HQ, CR, HI, and TCR for each PTE and sampling site.

Sampling Site	HQ							HI	CR		TCR
	As	Cd	Cu	Hg	Pb	Sb	Zn		As	Pb	
S1	0.47	0.06	0.13	0.46	0.03	1.06	0.06	2.27	9.10E-05	3.66E-07	9.13E-05
S2	0.22	0.09	0.14	1.23	0.01	1.06	0.06	2.81	4.18E-05	1.69E-07	4.19E-05
S3	0.22	0.02	0.14	0.53	0.02	0.48	0.03	1.45	4.18E-05	3.16E-07	4.21E-05
S4	0.61	0.06	0.34	1.59	0.01	0.91	0.05	3.56	1.17E-04	1.69E-07	1.17E-04
S5	0.38	0.09	0.17	0.80	0.01	1.23	0.07	2.75	7.31E-05	1.41E-07	7.32E-05
S6	0.43	0.06	0.21	0.58	0.02	0.73	0.05	2.07	8.20E-05	2.20E-07	8.22E-05
S7	0.46	0.03	0.31	0.97	0.01	1.23	0.07	3.08	8.95E-05	1.30E-07	8.96E-05
S8	0.68	0.05	0.18	0.43	0.02	1.30	0.06	2.73	1.32E-04	3.55E-07	1.32E-04
S9	0.61	0.06	0.14	0.35	0.02	0.89	0.06	2.13	1.18E-04	2.65E-07	1.19E-04
S10	1.34	0.06	0.31	1.15	0.02	0.65	0.07	3.59	2.59E-04	2.76E-07	2.59E-04

abandoned mines on the aquatic ecosystem in the region and, consequently, their potential implications on human health through the trophic chain.

Conclusions

The findings suggest that consumption of crayfish sampled in the context of the AMMD pose a health risk, primarily due to their As, Hg and Sb content. Arsenic contributes to a cancer and non-cancer risk at all sampling sites. On the other hand, Hg and Sb are the main contributors to non-cancer risk. This finding suggests that sediments in the crayfish habitat may contain elevated levels of these PTEs.

The primary driver of human health risks associated with crayfish consumption through the food chain appears to be the large number of abandoned mines in the region. Due to inadequate closure, these sites continue to influence the aquatic ecosystem, particularly downstream of the mines, where the highest HI and TCR levels are recorded.

Upstream sampling points exceed the maximum permissible limit for HI, indicating that although pollution levels are lower in these areas, they remain significant. This contamination is likely influenced by both anthropogenic and geogenic sources within the exploitation zone.

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