

Heavy Metal Contaminations in Herbal Medicines: Determination, Comprehensive Risk Assessments, and Solutions

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Research

Keywords: heavy metal, herbal medicine, extrinsic contamination, risk assessment

Posted Date: August 7th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-53256/v1>

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Version of Record: A version of this preprint was published at Frontiers in Pharmacology on January 14th, 2021. See the published version at <https://doi.org/10.3389/fphar.2020.595335>.

1 Heavy Metal Contaminations in Herbal Medicines: Determination,
2 Comprehensive Risk Assessments, and Solutions

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8

9 **Abstract**

10 **Background:** Heavy metal contamination in herbal medicines is a global threat to human beings
11 especially at levels above known threshold concentrations.

12 **Methods:** The concentrations of five heavy metals cadmium (Cd), lead (Pb), arsenic (As), mercury (Hg)
13 and copper (Cu) were investigated using Inductively Coupled Plasma Optical Mass Spectrometry
14 (ICP-MS) with 1773 samples. Exposure assessment, Non-carcinogenic risk assessment, and
15 carcinogenic risk assessment were applied to measure their risks in human body.

16 **Results:** According to Chinese Pharmacopoeia, 30.51% (541) samples were detected with at least one
17 over-limit metal. The over-limit ratio for Pb was 5.75% (102), Cd at 4.96% (88), As at 4.17% (74), Hg
18 at 3.78% (67), and of Cu, 1.75% (31). For exposure assessment, Pb, Cd, As, and Hg have resulted in
19 higher than acceptable risks in 25 kinds of herbs. The maximal Estimated Daily Intake (EDI) of Pb in
20 seven herbs, of Cd in five, of Hg in four, and As in three exceeded their corresponding Provisional
21 Tolerable Daily Intakes (PTDI). In total 25 kinds of herbs present an unacceptable risk as assessed with
22 the HQ (Hazard Quotient) or HI (Hazard Index). Particularly, *Plantaginis herba* (HI=11.47) is more

23 than 11 times over the limit.

24 **Conclusions:** Heavy metal contamination in herbal medicines was borderline or higher than the safety
25 level with the majority of the herbal plants were within acceptable risks. Notably, As posed the highest
26 risk in all indicators including EDI, HI, and CR, inducing the most serious risks in all five metals.
27 Herbal medicines *Euodiae fructus*, *Plantaginis herba*, and *Desmodii styracifolii* were considered
28 the most risk-inducing herbal medicines. Therefore, it is of great advantage to establish universal
29 standards and quality requirements for hazardous elements in herbal medicines so that this natural
30 resource can continue and expand further, to benefit health globally.

31

32 **Key words:** heavy metal; herbal medicine; extrinsic contamination; risk assessment

33

34 **Background**

35

36 Having been utilized as traditional folk remedies for thousands of years [1], medicinal plants
37 gained an increasingly important role in the pharmaceutical, health food, and natural cosmetic
38 industries[2]. It was reported that a total of 60,107 COVID-19 cases (85.20% of the total cases) in
39 China were treated by Chinese herbal medicines with positive results in all infection stages, including
40 significant symptom management, lower rates of deterioration and mortality, faster recovery as well as
41 disease prevention on 17 February 2020 [3]. Over years, however, concerns grew regarding the
42 safety of herbal medicines after studies indicated that high levels of heavy metals were present in
43 some herbal medicines. This was a problem more frequently encountered in traditional medical
44 therapy, considered to be a cause of several health disorders [1]. Samples taken from both

45 developed and developing countries have shown high levels of potentially toxic heavy metals in
46 products available to the public [4]. It is known that numerous traditional medicines can give rise
47 to severe adverse renal pathology, the mechanism of which is yet not fully certain but has been
48 associated with heavy metal toxicity [4]. Uptake of heavy metals by plants and subsequent
49 accumulation along the food chain is a potential threat to animal and human health. Particularly as
50 heavy metals are known to have low renal excretion rates, potentially resulting adverse effects in
51 humans even at very low concentrations. They are not easily metabolized by body and are found
52 to accumulate in the soft tissues. They produce toxic effects due to their interference in many
53 know normal biochemical and metabolic processes [5]. Several health problems were linked to
54 excessive uptake of dietary heavy metals, including decreased; immunity, cardiac dysfunction,
55 fetal malformation, impaired psychosocial and neurological behavior [6]. Pb and Cd are not
56 essential elements that are required neither in the human body nor in plants, and which cause various
57 bimolecular adverse functional effects at low level doses [1]. Though an essential component of many
58 enzymes, excessive intake of Cu can cause dermatitis, irritation of the upper respiratory tract,
59 abdominal pain, nausea, diarrhea, vomiting, and liver damage [6]. While As and Hg can damage
60 pulmonary, nervous, renal and respiratory systems, as well as causing skin pathology [7]. It may also
61 induce disorders in the central nervous system, liver, lungs, heart, kidney and brain. Leading to
62 hypertension, abdominal pain, skin eruptions, intestinal ulcer and is associated with various types
63 of cancers [8]. It is therefore necessary and a matter of urgency to conduct a comprehensive risk
64 assessment of heavy metal contamination in herbal medicines.

65

66 To explore and guarantee the safety of herbal medicines, multiple studies regarding heavy metal

67 contamination in herbal medicines have been carried out in China [9], India [10], Iran [11],
68 Egypt [12], South Africa [13], United States [6], Brazil [14], and Australia [2], etc. Though a
69 number of studies have been conducted regarding heavy metal contamination in herbal plants,
70 most were with limited sample numbers and categories. Given the severe consequence it may
71 bring to health and environment, exposure risk assessment [15], hazard quotient [9], and
72 ecological risk assessment [4], have been conducted by researchers, showing that heavy metal
73 contamination in herbal medicines is an area requiring immediate attention, with potential risk to
74 human health having now being demonstrated. Though studies now show that exposure of heavy
75 metals through general dietary consumption contribute negatively to human health [16], very few
76 have conducted comprehensive health risk assessments, with large sample numbers focusing on
77 herbal medicines. Furthermore, specific identification of metals is required for accurate diagnosis
78 due to considerable overlap between various clinical syndromes associated with heavy metal
79 poisoning [5]. Therefore a study of accurately quantified heavy metal contents in herbal medicines
80 appears necessary to further assess and justify of the dosage of herbal formulas. This study
81 assesses contamination levels and the health risk to humans posed by heavy metals more
82 specifically in herbal medicines, providing an evidence base on which to further build prevention
83 measures, establish relative standards, and control external contamination. Through investigation
84 and suggested recommendations in able to significantly reduce or eliminate the levels of heavy
85 metals in herbal medicines.

86

87 **Methods**

88 **Sample collection and detection**

89 A total of 1773 samples from 2014 to 2019, representing 86 different kinds of commonly used herbal
90 medicines were collected for examination of heavy metals. As a part of a heavy metal detection project
91 for Chinese Pharmacopoeia (2020 edition) [17] and based on the principle that only herbal medicines
92 from large-scale production areas would be considered, at least three samples for each herbal medicine
93 were collected from one to 13 different sampling locations. Sampling locations were chosen according
94 to areas of traditional production of the herbal medicines, without regard to possible pollution sources.
95 Duplicate specimens were taken from each same herbal medicine sample and every specimen was
96 tested three times and the final mean concentration was adopted for further data analysis. Each bulk
97 sample was harvested, cleaned, and processed according to the method required by Chinese
98 Pharmacopoeia [17]. Precisely 0.5 g sample Electronic balance (Mettler Toledo) was first ground into
99 powder, then soaked in polytetra fluoroethylene (PTFE) tank with 6 ml nitric acid (HNO_3) added
100 overnight. It was then predigested for one hour on a temperature controlled electronic hotplate with 1
101 mL hydrogen peroxide solution (H_2O_2) added. After cooling down, more HNO_3 was added to up to
102 volume of 7 mL. The PTFE tank was then placed in Multiwave PRO Microwave Digestion Apparatus
103 (Anton Paar) for further digestion before being placed on a 130°C electronic hotplate, until reduced to 1
104 mL. The tank was then removed for cooling and 50 mL of the digested liquid transferred to a graduated
105 flask. The tank was flushed with low volume amounts of water multiple times and the complete
106 washings added to the graduated flask, 200 μL of single-element standard solution ($1 \text{ mg}\cdot\text{L}^{-1}$) was
107 added. Then diluted up to the mark of the graduated flask with water, shaken and set aside. The blank
108 solution was prepared in the same way except that no standard solution of single element and sample
109 powder were added [18].

110

111 The resulting digestate was analyzed using a Scientific X Series Inductively-Coupled Plasma Mass
112 Spectrometer (ICP-MS) (Thermo Fisher Scientific, Waltham, MA). Heavy metal results from the
113 ICP-MS were quantified against standard curves generated from 1 blank and at least 4 standard
114 reference solutions (High-Purity Standards, Charleston, SC) run separately. Quality control was
115 assessed by running a laboratory reagent blank after every 10 samples. The limit of detection (LOD)
116 achieved for each metal was 0.1 mg·kg⁻¹ for Cu, 0.01 mg·kg⁻¹ for As, 0.005 mg·kg⁻¹ for Cd, 0.001
117 mg·kg⁻¹ for Hg, and 0.01 mg·kg⁻¹ for Pb. The detection limit was based on consideration of the blank
118 runs, concentration of the low standard in the calibration curve and the sample preparation procedure.
119 Based on this method, the limit of detection was considered equivalent to the limit of quantification
120 [19-20].

121 In our experiment, standard solutions of Cu (GSB04-1725, 1000 mg·L⁻¹), As (GSB04-1714, 1000
122 mg·L⁻¹), Cd (GSB04-1721, 1000 mg·L⁻¹), Hg (GSB04-1729, 1000 mg·L), Pb (GSB04-1742, 1000
123 mg·L⁻¹) were purchased from the National Nonferrous Metals and Electronic Materials Analysis and
124 Testing Center, while Analytical Reagents (AR) nitric acid (HNO₃) and hydrofluoric acid (HF) were
125 purchased from Merck Co., Ltd., and guaranteed reagents (GR) hydrogen peroxide and hydrochloric
126 acid from Sinopharm Chemical Reagent Co., Ltd. Each single-element standard solution was measured
127 precisely and then diluted with 5% HNO₃ to make a mixed solution containing 1 µg mL⁻¹. For a
128 reference stock solution, single-element standard solutions of the five metals were separately taken and
129 diluted with 5% nitric acid to make solutions containing 5 µg of Pb and As, 50 µg of Cu, 2.5 µg of Cd,
130 and 0.5 µg of Hg. For preparation of a reference standard curve, precise measurement were taken of the
131 above stock solution diluted with 5% nitric acid to make standard mixtures with: 0, 1, 5, 10, 20, and 50

132 ng As or Pb per 1 mL; 0, 10, 50, 100, 200, and 500 ng Cu per 1 mL; with 0, 0.5, 2.5, 5, 10, and 25 ng
133 Cd per 1 mL; with 0, 0.1, 0.5, 1, 2, and 5 ng Hg per 1 mL [6].

134

135 The conditions for Inductively coupled plasma mass spectrometry (ICP-MS) conditions were: Radio
136 Frequency (RF) power: 1400 W; sampling depth: 15mm; auxiliary gas (argon) flow rate: 0.8 L min⁻¹;
137 cooling gas flow rate: 13.0 L min⁻¹; peristaltic pump speed: 30.0 L min⁻¹; channel three; repeat for three
138 times; scan for 100 times, automatic detection. Before samples were measured, the instrument was
139 optimized to perform under the optimal conditions. Microwave digestion was set on a digestion
140 program. For the first stage, 1100 W, maintained for 10 minutes then further, for the second stage, 1400
141 W for 15 minutes and held for 20 minutes[21]. The detailed methodology is listed in the appendix
142 (Table S1).

143

144 **Quantification of heavy metal contamination**

145

146 Mean concentrations, general detection rate, and detection rates of each metal were calculated. Figures
147 were plotted using *R* language [no IDE (integrated development environment), *R* from the linux
148 terminal *R* version 3.5.1. (2018-007-02)--"Feather Spray" Copyright (C) 2018, the *R* Function for
149 Statistic Computing Platform: x86_64-pc-linux-gnu (64-bit)] [22].

150

151 **Over-limit ratio of five heavy metals**

152 In total, 27 currently available permissible limits containing five heavy metals were obtained from 20
153 countries or regions and seven international organizations (Table S2). The detailed calculation of both

154 general over-limit ratio and that of each metal in different producing areas were shown in
155 supplementary material (Table S3). Herbal medicines were classified into five categories based on
156 which part of the plant is employed for medicinal use: flos, folium & cortex, fructus & semen, herba &
157 others, and radix & rhizome. Numbers of over-limit samples and metals were calculated from within
158 across these five medicinal herbal properties.

$$159 \text{ Over-limit ratio} = (C - \textit{Limit}) \times 100\% \quad (1)$$

$$160 \text{ Times over permissible limit} = \frac{C - \textit{Limit}}{\textit{Limit}} \times 100\% \quad (2)$$

161

162 **Three health risk assessments of heavy metal contamination in herbal medicines**

163 Exposure assessment, non-carcinogenic risk assessment, and carcinogenic risk assessment were
164 employed to explore the potential health impacts from heavy metal contamination in herbal medicines.
165 The minimal, mean and maximal concentrations of each metal in each herbal medicine were applied
166 for calculations with the equations below:

167

168 **Exposure assessment**

169

$$170 \text{ EDI} = \frac{C \times \text{IRD}}{\text{BW}} \quad (3)$$

171

172 The estimated daily intake (EDI, $\text{mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1} \cdot \text{bw}$) of each metal in each sample was calculated,

173 before comparison with its corresponding provisional tolerable daily intake (PTDI). The PTDIs
174 ($\text{mg}\cdot\text{kg}^{-1}\cdot\text{d}^{-1}$) of As, Cu, Hg, Pb, and Cd are 0.00214, 0.5, 0.00057, 0.00357, and 0.00083, respectively
175 [23]. *C* in the equation here refers to the concentrations detected of each metal in herbal medicines
176 (maximal, mean and minimal concentrations were all considered). IRD refers to daily ingestion rate,
177 which signifies the daily dosage of herbal medicines. Here the maximal dosage specified in CP (2020
178 edition) was applied. BW is body weight, and average human weight of 60 kg was applied in the
179 equation[24].

180

181 **Non-carcinogenic risk assessment**

182

183 The non-cancer risk was evaluated by comparing an exposure level over a specified time period (e.g.,
184 lifetime) with a reference dose derived for a similar exposure period. The non-cancer risk can be
185 characterized as a hazard quotient (HQ) [23].

186

$$187 \text{HQ} = \frac{C \times \text{IR} \times \text{Ef} \times \text{Ed} \times t}{\text{AT} \times \text{BW} \times \text{RfD}} \quad (4)$$

188

189 IR is the daily dosage of herbal medicine, and according to a questionnaire on herbal medicine
190 consumption of 20917 people, the 95th percentile of daily dosage of general herbal medicine
191 consumption is 0.5 kg; Ef is exposure frequency, here the 95th percentile of annual consumption on
192 herbal medicine was adopted which was set 90 days per year; Ed is the exposed days over a lifetime
193 which was set as 20 years; AT is the average lifetime=365 days×70 years, while *t* is the transfer rate of
194 heavy metal to herbal detection, which is 14% for Cu and Pb, 35% for As, and 24% for Hg [25]. RfD

195 refers to Oral reference dose ($\text{mg}^{-1}\cdot\text{kg}^{-1}\cdot\text{day}$), which is 0.0035 for Pb, 0.0005 for Cd, 0.0003 for As,
196 0.0003 for Hg and 0.04 for Cu [26].

197

$$198 \quad \text{HI} = \sum \text{HQ} \quad (5)$$

199

200 HQs of five heavy metals in each herbal medicine were summed up to obtain non-carcinogenic Hazard
201 Index (HI). If HQ or HI is less than 1, there will not be obvious risk for exposed population from metal
202 exposure in herbal medicine. If HQ or HI is equal to or above 1, the risk will be considered
203 unacceptable. As the HQ or HI increases, the risk also does. The contributions of HQs of each metal
204 (HQ_m) to the total HI were calculated to explore which metal contributed the most serious risks [27].

$$205 \quad \text{Contribution of } \text{HQ}_m \text{ to HI} = (\text{HQ}_m - \text{HI}) \times 100\% \quad (6)$$

206 **Carcinogenic risk assessment**

207

$$208 \quad \text{CR} = \frac{\text{C} \times \text{IR} \times \text{Ef} \times \text{Ed} \times \text{t} \times \text{CSF}}{\text{AT} \times \text{BW} \times \text{RfD}} \times 10^{-6} \quad (7)$$

209

210 CSF ($\text{mg}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}\cdot\text{bw}$) is the cancer severity factor, only three heavy metals were proven with certain
211 CSF: 6.1 for Cd, 1.5 for As and 0.0085 for Pb, while 10^{-6} is the conversion factor. CR of these three
212 metals in the same herbal medicine was also summed up to give total CR of single herbal medicine. If
213 CR is higher than 10^{-6} , which means one case of cancer over one million exposed people, it is
214 considered unacceptable [28].

215

216 Results

217

218 Heavy metals were detected in all 1773 samples. The order of detection rates of heavy metal
219 concentrations in herbal medicines is Cu (1771, 99.89%) > Pb (1755, 98.98%) > Cd (173, 97.74%) > As
220 (1679, 94.70%) > Hg (1497, 84.43%). The contents ($\text{mg}\cdot\text{kg}^{-1}$) of each medicinal property was also
221 calculated, the highest content of Cu, Cd and Hg was 11.12 ± 2.73 , 0.439 ± 0.686 and 0.081 ± 0.667 ,
222 respectively, in Flos ($n=340$); the highest content of As and Pb was 1.06 ± 1.56 and 3.23 ± 3.83 , in Herba
223 & others ($n=380$) (Table 1). The orders of mean concentrations detected of five heavy metals in five
224 plant properties are: for Cu, flos > herba & others > folium & cortex > radix & rhizoma > fructus &
225 semen; for As, herba & others > folium & cortex > flos > radix & rhizoma > fructus & semen; for Cd,
226 flos > herba & others > radix & rhizoma > folium & cortex > fructus & semen; for Hg, flos > fructus &
227 semen > herba & others > folium & cortex > radix & rhizoma; while for Pb, herba & others > folium &
228 cortex > flos > radix & rhizoma > fructus & semen (Fig.1). The highest concentration detected for Cu
229 was in herbal medicine *Schisandrae chinensis fructus* ($34.01 \text{ mg}\cdot\text{kg}^{-1}$), the highest concentration of As
230 was in *Plantaginis herba* ($14.53 \text{ mg}\cdot\text{kg}^{-1}$), of Cd was in *Curcumae longae rhizome* ($6.20 \text{ mg}\cdot\text{kg}^{-1}$), of
231 Hg was in *Chrysanthemi flos* ($8.69 \text{ mg}\cdot\text{kg}^{-1}$), of Pb was in *Euodiae fructus* ($50.11 \text{ mg}\cdot\text{kg}^{-1}$) (Table 1). In
232 conclusion, all five heavy metals were widely detected in herbal medicines, particularly, Cu and Pb,
233 most notably in flos and herba parts of medicinal plants.

234

235 In a total of 541 samples (30.51%) were detected at levels over the CP (2020 edition) standard, and 433
236 samples detected with one over-limit metal, 75 samples with two over-limit metals, three samples with
237 24 over-limit metals and nine samples with four over-limit metals (Fig.2). The order of over-limit ratio

238 of five heavy metals based on CP (2020 edition) standard is Pb (102, 5.75%) > Cd (88, 4.96%) > As
239 (74, 4.17%)> Hg (67, 3.78%) > Cu (31, 1.75%). The times of highest concentration detected over the
240 standards of CP (2020 edition) were 1.70 for Cu (*Schisandrae chinensis fructus*), 6.27 for As
241 (*Plantaginis herba*), 5.20 for Cd (*Curcumae longae rhizome*), 66.17 for Hg (*Chrysanthemi flos*), and
242 9.02 for Pb (*Euodiae fructus*). The highest concentration detected over the limit of European Union
243 (EU) and United Kingdom (U.K.) is Hg (133.35 times). As per over-limit ratios of each metal in five
244 medicinal properties, 40.00% samples were detected with over-limit concentrations in flos, 34.39% in
245 folium & cortex, 7.69% in fructus & semen, 58.16% in herba & others, 37.20% in radix & rhizome.
246 For Cu ($n=31$), there are 12.90% samples detected over-limit in flos, 9.68% in folium & cortex, 16.13%
247 in fructus & semen, 41.94% in herba & others, and 19.35% in radix & rhizome; For As ($n=74$), 9.46%
248 samples were detected with concentrations above the threshold in flos, 6.76% in folium & cortex, 8.11%
249 in fructus & semen, 75.68% in herba & others, and none in radix & rhizome; For Cd ($n=416$), 27.16%
250 samples detected over-limit in flos, 10.10% in folium & cortex, 5.29% in fructus & semen, 39.90% in
251 herba & others, and 17.55% in radix & rhizome; For Hg ($n=67$), there are 25.37% samples detected
252 over-limit in flos, 5.97% in folium & cortex, 34.33% in fructus & semen, 34.33% in herba & others,
253 and none in radix & rhizome; For Pb ($n=102$), there are 11.76% samples detected over-limit in flos,
254 9.80% in folium & cortex, 9.80% in fructus & semen, 59.80% in herba & others, and 8.82% in radix &
255 rhizome. Of note, we found that heavy metals prefer to accumulate in fructus & semen, while herba &
256 others were detected with the highest over-limit ratio. Notably, heavy metal Pb was presented with the
257 highest over-limit ratio, followed by Cd and As.

258

259 As per exposure assessment, though the majority of herbal medicines were considered within the

260 acceptable limit, the concentrations detected in all five heavy metals in few herbal medicines have
261 surpassed their corresponding Provisional Tolerable Daily Intakes (PTDI), demonstrating unacceptable
262 risk to health. The EDIs of three heavy metals (Cd, Hg, and Pb) in *Desmodii styracifolii herba* have
263 surpassed their corresponding PTDIs (Fig.3, Fig.S1, Table S4). Notably, a total of 12 herbal medicines
264 out of 86 have presented with EDIs above their corresponding PTDIs. The maximal EDI of As in three
265 herbal plants *Plantaginis herba* (0.007), *Taraxaci herba* (0.002), and *Corni fructus* (0.002); of Cd in five
266 herbal plants *Desmodii styracifolii herba* (0.001) *Andrographis herba* (0.0008), *Curcumae longae*
267 *rhizoma* (0.001), *Lonicerae flos* (0.001), and *Houttuyniae herba* (0.002), of Hg in four herbal plants
268 *Desmodii styracifolii herba* (0.001), *Chrysanthemi flos* (0.001), *Forsythiae fructus* (0.001), and *Euodiae*
269 *fructus* (0.001); and of Pb in seven herbal plants *Plantaginis herba* (0.024), *Taraxaci herba* (0.004),
270 *Desmodii styracifolii herba* (0.008) *Lonicerae flos* (0.004), *Houttuyniae herba* (0.004), *Euodiae fructus*
271 (0.004), *Lonicerae japonicae flos* (0.005) exceeded their corresponding PTDIs (Fig.3).

272

273 For non-carcinogenic risk, the majority of the herbal medicines were calculated with risks within the
274 acceptable limit (<1). The HIs of a total of 86 herbal medicines ranged from 11.47 (*Plantaginis Herba*)
275 and 0.02 (*Chaenomelis Fructus*) and HIs in a total of 25 out of 86 kinds of herbal medicines (29.07%)
276 showed values over 1, thus considered unacceptable risk. HQs of As in 20 herbal medicines, of Hg in
277 five herbal medicines, of Pb in two herbal medicines, and of Cd in one herbal medicine exceeded 1,
278 considering as unacceptable risks. It was also shown that heavy metal As contributed the most in HQ>1
279 herbal medicines (The highest was 92.94% in *Corni Fructus*). Heavy metal As has shown the highest
280 non-carcinogenic (HQ=9.95), presenting more severe risks than other four heavy metals (Fig.3, Table
281 2, Fig. S2, Table S4).

282

283 For carcinogenic risks, all CRs were found to be within the acceptable limit (10^{-4}) [29]. The highest
284 risk of As was found in Plantaginis herba ($4.48E-09$), the lowest was in Ziziphi spinosae semen
285 ($3.12E-12$); the highest of Cd was in Curcumae longae rhizome ($3.11E-09$), the lowest in Citri grandis
286 exocarpium ($2.51E-12$); and the highest of Pb was in Euodiae fructus ($3.50E-11$), the lowest were in
287 Puerariae lobatae radix and Ziziphi spinosae semen ($6.99E-15$). Andrographis herba presented with the
288 highest total carcinogenic risk ($5.27E-09$), while Ziziphi spinosae semen the lowest ($5.83E-12$). Among
289 these top risk-inducing herbs, nine belong to fructus & semen (Fig.4). Heavy metal As has shown
290 carcinogenic risks ($CR=4.48E-09$), which, again, presented with the highest carcinogenic risk.

291

292 In total here, 25 (29.07%) different kinds of herbal medicines ($n=86$), presented with unacceptable risks
293 based on exposure assessment, among which, nine belonged to fructus & semen, six belonged to herba
294 & others, five belonged to the flos category, three belonged to radix & rhizoma, and two belonged to
295 the folium & cortex. Plantaginis herba presented with the highest non-carcinogenic risk ($HI=11.47$),
296 while Andrographis herba with the highest carcinogenic risk ($CR=5.27E-09$). Heavy metal As has
297 shown both the highest non-carcinogenic ($HQ=9.95$) and carcinogenic risks ($CR=4.48E-09$) in herbal
298 medicines. As these particular herbal medicines and heavy metals have the potential to cause health
299 problems, they are in need of special monitoring to reduce potential risk (Table 2).

300

301 **Discussion**

302 Based on this study with a large-spatio-temporal-scale herbal medicine samples, 30.51% (541) of

303 samples were detected with at least one over-limit heavy metal. Five heavy metals (As, Pb, Cu, Hg
304 and Cd) were widely detected in cultivated herbal medicines according to our experiment, which
305 is in accordance with other published results [30-31]. In Nigerian herbal remedies, 100% of the
306 samples also contained elevated amounts of heavy metals [32], which revealed that the Nigerian
307 herbs contained high levels of Fe, Ni, Cd, Cu, Pb, Se, and Zn sufficient to cause adverse health
308 effect when regularly taken as recommended. In our study, 27 (31.40%) different kinds of herbal
309 medicines, mostly with fructus & semen part with medicinal applications posed unacceptable
310 health risk due to heavy metal accumulation though herba & others were detected with the highest
311 over-limit ratio. Toxic element As posed the most serious health risk according to exposure,
312 carcinogenic and non-carcinogenic risk assessments, as health risk assessment employed by Ren,
313 indicated that As and Pb generated from industrial sites and traffic sites has a potential to pose
314 serious health risks [33]. It was also found that As was the major metal found for water pollution
315 [29], exceeding its permitted daily exposure dosages and suggested a potential health risk for *P.*
316 *notoginseng* consumers [34]. Considering that the content of As detected is total As and As exists
317 in different states. The toxicity of As in different states varies tremendously. Both organic and
318 inorganic As exist in the soil, while the states of As may transform in biological activities both in
319 plants and human metabolism. So it is hard to say if high concentration of total As would
320 absolutely harm human health. However, As is certainly necessitating special attention and further
321 study on its state transformation. Furthermore, Bolan employed a study and asserted that the
322 concentrations of Cd, Hg, and Pb in Ayurvedic medicines exceeded their daily intake amounts [35].
323 According to the study by Lee SD, levels of Cd exceeding WHO reference values were observed
324 in 10 samples and the weekly intakes of Pb, Cd, Cr, Cu, Hg from herbs [36]. While in Iran,

325 maximum bioaccumulate of Pb and Hg was noted in *Artemisia dracuncululus* L and *Spinacia*
326 *oleracea* L, respectively [37]. The non-carcinogenic risks target hazard quotients (THQs) of Al
327 and Cr from individual herbs were over 1, which might impart risk for human consumption. It
328 could be concluded that heavy metals As, Pb, Cd, and Hg all impose significant risk to health due
329 to herbal consumption. The highest HI was presented in herbal medicine Plantaginis Herba
330 (HI=11.47) and highest CR in Andrographis herba (CR=5.27E-09), which are in need of special
331 dosage control and monitoring. Furthermore, the highest over-limit ratios of five heavy metals
332 based on different producing areas are Cu (7.69%) in Chongqing, As (20.21%) in Gansu province,
333 Cd(0.77%) in Chongqing, Hg (9.89%) in Hunan province, and Pb (25.00%) in Fujian province
334 (Tables S3). However, Principle Components Analysis (PCA) didn't showcase that there was
335 significant statistic difference regarding the five heavy metal accumulations in five medicinal plant
336 properties. While according to Pearson Correlation Analysis, Pb and As were correlated to flos, folium
337 & cortex, fructus & semen, and herba & others, while Pb and Cd in radix & rhizoma (Fig.S3, 4).
338 Additionally, based on the Analysis of Similarities (ANOSIM), it was indicated that the difference
339 within groups of five medicinal plant properties is less significant than the one throughout the five
340 groups (R=0.165, P=0.001) (Fig.S5).

341

342 Generally, five reasons explain the levels of heavy metal contents in herbal medicines. The first is the
343 variable exposure to environmental pollution including industrial encroachment, contaminated soil or
344 atmosphere. The physicochemical properties of soil including pH, temperature, redox potential,
345 translocation exchange capacity and organic matter may influence the availability of metal to plants.
346 Secondly, the phytological characteristics of medical plants themselves such as reduced biomass, root

347 length and shoot length are common indicators of heavy metal toxicity. Furthermore, the interactions of
348 soil-plant roots-microbes play vital roles in regulating heavy metal movement from the soil to edible
349 plant parts. Certain plants are “hyper-accumulators” which grow on metalliferous soils and accumulate
350 extraordinarily high levels of heavy metals without displaying phytotoxic effects. Thirdly, herbal plants
351 could be contaminated during manufacturing and agronomic processes [38] including growing,
352 harvesting, transportation, processing and storage, due to pesticide formulations, chemical fertilizers
353 and irrigation with poor-quality water [6,35]. For example, Cd and Pb may enter the soil due to
354 fertilizer impurities [39], non-ferrous smelters, lead and zinc mines, sewage slug application and
355 combustion of fossil fuels [40]. Additionally, fumigants containing heavy metals may also be applied
356 for preventing rats and mildew [41]. Fourthly, plant uptake is one of the major routes of dietary
357 exposure to heavy metals in the soil, and the wide variations in metal concentrations in the analyzed
358 herbs could be attributed to differences in the plant metal uptake and translocation capabilities. Studies
359 have shown wide variations in concentration factor for different metals among different plant species
360 and sampling sites. Certain species have higher tendency to accumulate Cd [6]. Lastly, the
361 bioavailability of heavy metals could have an impact on their concentrations, such as; soil pH, the
362 metal levels already resident in the soil, the oxidation-reduction potential of the soil, and other
363 chemical and physical factors [6] (Table S5).

364 Here we propose a solution for heavy metal control in herbal medicines. We consider, given the results
365 found here and those of others previously there is an urgent need to implement a regular monitoring
366 and surveillance program, controlling extrinsic contamination of herbal medicines along the supply
367 chain from field to consumer [35]. Secondly, research, such as identifying ways in which heavy metals
368 reach herbal products; development and validation of kinetic models linking processing techniques

369 with metal speciation and bioavailability; bioavailability tests of heavy metals in herbal medicines;
370 experiments on regression relationships between speciation and bioavailability of heavy metals, clinical
371 studies examining the toxicity of heavy metals, etc. [6]. Soil amendments, including mitigation and
372 preservation management for the growth performance of biomass and metal accumulation in
373 contaminated soils, is necessary [2]. Lastly, tolerant medicinal plants with high phytoremediation
374 potential and capability for phytostabilization and phytoextraction [2] can be cultivated as an approach
375 for the management and targeted bio-extraction of heavy metals from moderately polluted lands [28],
376 together with a combination of different agents such as pH change-inducing chemical immobilization,
377 alkaline materials including lime based materials, fly ash, and biochar, calcite, dolomite, oyster and egg
378 shell [2]. Sorption agents such as phosphate materials, compost, zeolite and iron compounds, activated
379 carbon, and bentonite, or materials that decrease dissolved organic carbon (DOC) such as gypsum
380 treatment, *Solanum nigrum*, microbes, chelating agents, Extracellular polysaccharides or
381 Exopolysaccharides (EPS) [42], and eco-friendly biocarbon technology [43]. These materials increase
382 soil pH, favor deprotonation and the formation of oxides, metal-carbonate precipitates, complexes and
383 secondary minerals that all decrease the phytoavailable heavy metal concentrations [1].
384 Phytoremediation has been perceived to be a more low-cost, low-impact, low-tech alternative, visually
385 benign and environmentally sound comparing to more active and intrusive remedial methods [43]
386 (Fig.5).

387

388 **Conclusion**

389 In conclusion, heavy metal contamination in herbal medicines was borderline or higher than the safety
390 level. There are 30.51% samples detected with at least one over-limit heavy metal according to Chinese

391 Pharmacopoeia (CP, 2020 edition) standards [17]. The risk assessments have demonstrated that the
392 majority (70.93%) of the herbal plants were within acceptable risks. Notably, As posed the highest risk
393 in all indicators including EDI, HI, and CR, inducing the most serious risks in all five metals. Herbal
394 medicines *Euodiae fructus*, *Plantaginis herba*, and *Desmodii styracifolii* were considered the most
395 risk-inducing herbal medicines. Extrinsic contamination in herbal medicines is well demonstrated and
396 clearly poses a serious potential risk to health. Furthermore, trace metals play a significant role in
397 reactions which lead to formation of the active chemical plant constituents and are, therefore,
398 responsible in-part for their curative as well as toxic properties. The analysis of toxic metals can be
399 useful to evaluate the dosage of the herbal drugs prepared from these plants. Therefore, it is of great
400 advantage to establish universal standards and quality requirements for hazardous elements in herbal
401 medicines so that this natural resource can continue and expand further, to benefit health globally.

402

403 **Additional file 1: Table S1.1** Methodological verification of ICP-MS for determination of five heavy
404 metals in *Menthae Haplocalycis Herba* (18#) herbal medicines (n=3). **Table S1.2** Methodological
405 verification of ICP-MS for determination of five heavy metals in *Menthae Andrographis Herba* (12#)
406 herbal medicines (n=3). **Table S1.3** Methodological verification of ICP-MS for determination of five
407 heavy metals in *Menthae Isatidis Folium* (14#) herbal medicines (n=3). **Table S1.4** Methodological
408 verification of ICP-MS for determination of five heavy metals in *Lycii Fructus* (10#) herbal medicines
409 (n=3). **Table S1.5** Methodological verification of ICP-MS for determination of five heavy metals in
410 *Desmodii Styracifolii Herba* (9#) herbal medicines (n=3). **Table S1.6** Methodological verification of
411 ICP-MS for determination of five heavy metals in *Carthami Flos* (4#) herbal medicines (n=3). **Table**
412 **S1.7** Methodological verification of ICP-MS for determination of five heavy metals in *Lonicerae*

413 Japonicae Flos (19#) herbal medicines (n=3). **Table S1.8** Methodological verification of ICP-MS for
414 determination of five heavy metals in Chrysanthemi Flos (26#) herbal medicines (n=3). **Table S1.9**
415 Methodological verification of ICP-MS for determination of five heavy metals in Farfarae Flos (7#)
416 herbal medicines (n=3). **Table S1.10** Methodological verification of ICP-MS for determination of five
417 heavy metals in Forsythiae Fructus (6#) herbal medicines (n=3). **Table S1.11** Methodological
418 verification of ICP-MS for determination of five heavy metals in Chaenomelis Fructus (3#) herbal
419 medicines (n=3). **Table S1.12** Methodological verification of ICP-MS for determination of five heavy
420 metals in Ligustri Lucidi Fructus (2#) herbal medicines (n=3). **Table S1.13** Methodological verification
421 of ICP-MS for determination of five heavy metals in Taraxaci Herba (27#) herbal medicines (n=3).
422 **Table S1.14** Methodological verification of ICP-MS for determination of five heavy metals in
423 Lonicerae Flos (13#) herbal medicines (n=3). **Table S1.15** Methodological verification of ICP-MS for
424 determination of five heavy metals in Corni Fructus (16#) herbal medicines (n=3). **Table S1.16**
425 Methodological verification of ICP-MS for determination of five heavy metals in Ziziphi Spinosae
426 Semen (1#) herbal medicines (n=3). **Table S1.17** Methodological verification of ICP-MS for
427 determination of five heavy metals in Euodiae Fructus (8#) herbal medicines (n=3). **Table S1.18**
428 Methodological verification of ICP-MS for determination of five heavy metals in Schisandrae
429 Chinensis Fructus (10#) herbal medicines (n=3). **Table S1.19** Methodological verification of ICP-MS
430 for determination of five heavy metals in Houttuyniae Herba (2#) herbal medicines (n=3). **Table S1.20**
431 Methodological verification of ICP-MS for determination of five heavy metals in Gardeniae Fructus
432 (14#) herbal medicines (n=3). **Table S1.21** Methodological verification of ICP-MS for determination of
433 five heavy metals in Aurantii Fructus (4#) herbal medicines (n=3). **Table S1.22** Methodological
434 verification of ICP-MS for determination of five heavy metals in Perillae Folium (10#) herbal

435 medicines (n=3). **Table S1.23** Methodological verification of ICP-MS for determination of five heavy
436 metals in Plantaginis Herba (4#) herbal medicines (n=3). **Table S2** Permissible limits of heavy metals
437 in medicinal herbs (products) from different standards. **Table S3** Over-limit ratio (%) of five heavy
438 metals in 32 producing areas. **Table S4** The contributions of each metal in HI>1 herbal medicines.
439 **Table S5** Chronic and acute adverse effects of five heavy metals on health. **Fig.S1.** The over-limit
440 Estimated Daily Intakes (EDI) of four heavy metals in herbal medicines. **Fig.S2.** The over-limit Hazard
441 Quotients (HQ) of four heavy metals in 21 herbal medicines. **Fig.S3.1** Spearman correlation coefficient
442 of metal relations in flos. **Fig.S3.2** Spearman correlation coefficient of metal relations in folium &
443 cortex. **Fig.S3.3** Spearman correlation coefficient of metal relations in fructus & semen. **Fig.S3.4**
444 Spearman correlation coefficient of metal relations in herba & others. **Fig.S3.5** Spearman correlation
445 coefficient of metal relations in radix & rhizoma. **Fig.S4.1** Principle Components Analysis (PCA) of
446 five heavy metal contents in five plant properties. **Fig.S4.2** PCA of five heavy metals in 32
447 producing areas. **Fig.S5** Analysis of Similarities (ANOSIM) of five heavy metal contents in five
448 medicinal plant properties.

449

450 **Acknowledgements**

451 The authors thank Hubei Institute for Drug Control, China for conducting the heavy metal detection
452 experiments.

453

454 **Authors' contributions**

455 Shilin Chen: Conceptualization and Supervision; Linlin Dong: Conceptualization, Methodology and
456 Sample Collection; Lu Luo: Data analysis, Original Draft, Visualization, Review & Editing. Bo Wang:

457 Heavy Metal Analysis; Jingwen Jiang, Qin Huang, Jiqing Zhang and Chenyuyan Yang: Data analysis
458 and Visualization; Zheng Yu and Hui Li: Validation; Hui Zhang: Review & Editing. All authors proved
459 the final text.

460

461 **Funding**

462 This project was supported by Beijing Nova Program (Z181100006218020), Fundamental Research
463 Funds for the central public welfare research institutes (ZZ13-AQ-049), Major science and technology
464 projects of Yunnan province (2018ZF011), National Key R&D Plan (2018YFC1706302), National
465 Science and Technology Major Project for “Significant New Drugs Development”
466 (2019ZX09201005-006-001), and National Natural Science Foundation (No. 81973429).

467

468 **Availability of data and materials**

469 All data related to this research has been included in the supplementary materials.

470

471 **Consent for publication**

472 Not applicable.

473

474 **Competing interests**

475 All authors declare no competing interests.

476

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503

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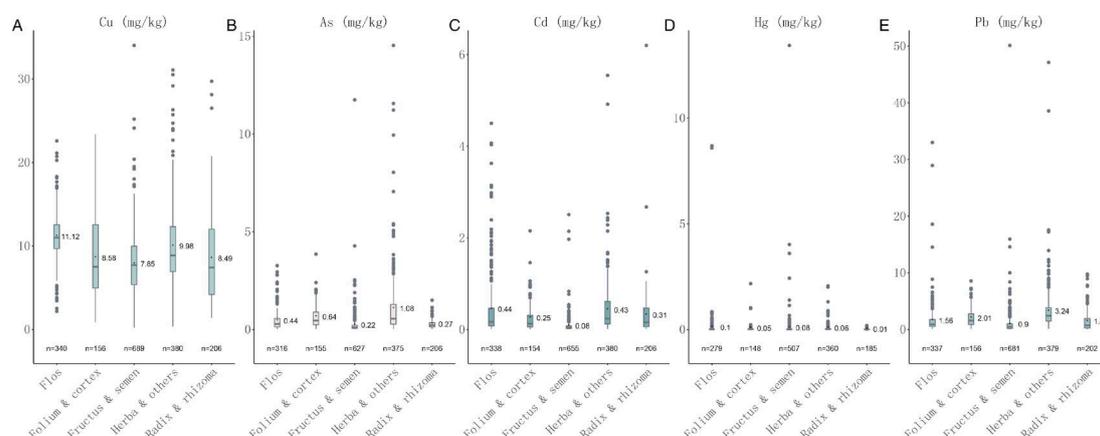
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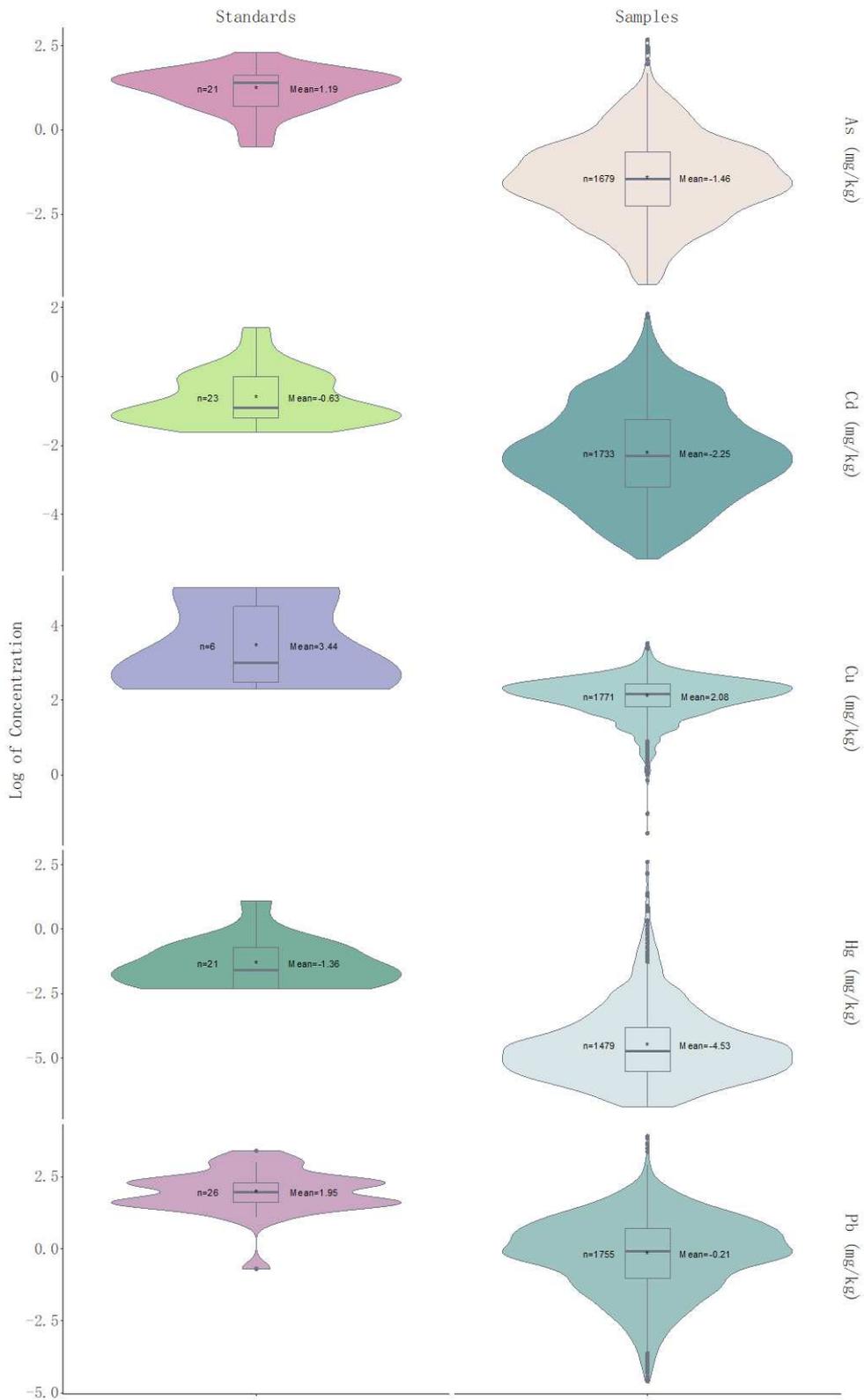
619

620 **Fig.1.** Plotbox showing concentrations of five heavy metals in five medicinal properties. (A)

621 Concentrations of Cu in five medicinal properties. (B) Concentrations of As in five medicinal

622 properties. (C) Concentrations of Cd in five medicinal properties. (D) Concentrations of Hg in five

623 medicinal properties. (E) Concentrations of Pb in five medicinal properties.

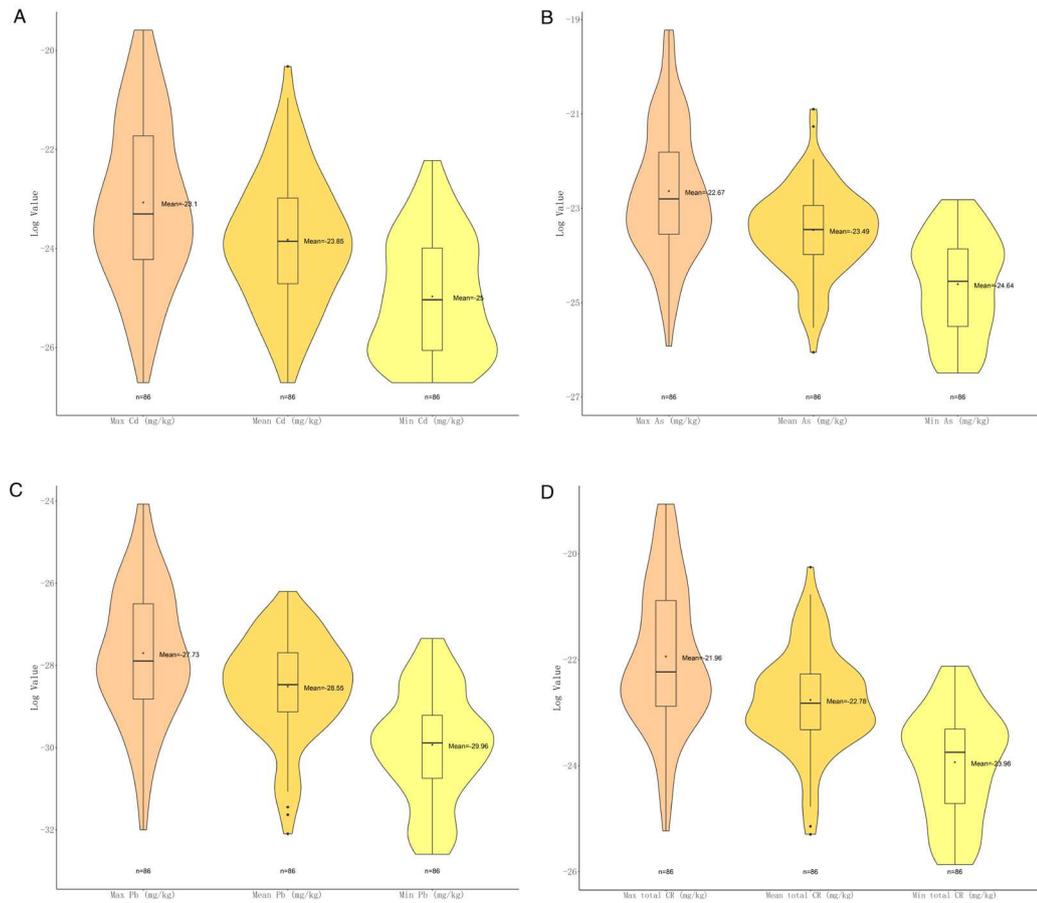


624

625 **Fig.2.** Violin plot showing \log_2 (concentration) values of five heavy metals in 1773 samples and

626 permissible limits in different countries of each heavy metal (see Table S2 for detailed standards of

627 each heavy metal).



636

637 **Fig.4.** Violin plot of carcinogenic risk (CR) of three carcinogenic metals with maximal, minimal and
 638 mean concentrations detected

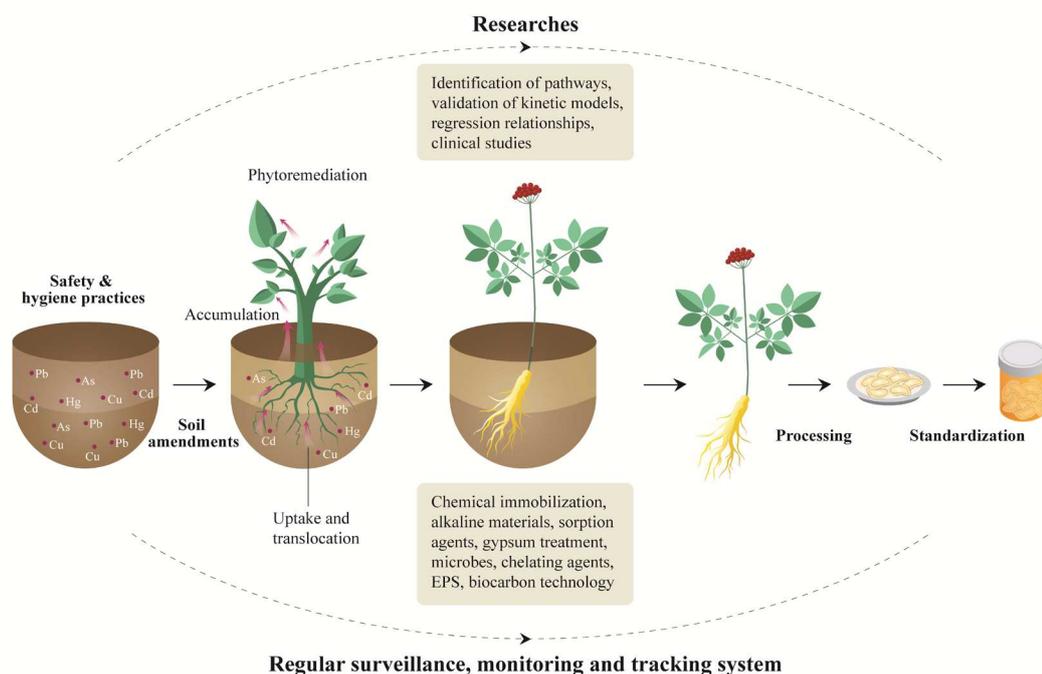
639 (A) Violin plot showing $\log_2(\text{CR})$ values of Cd with maximal, minimal and mean concentrations; (B)

640 Violin plot showing $\log_2(\text{CR})$ values of As with maximal, minimal and mean concentrations; (C)

641 Violin plot showing $\log_2(\text{CR})$ values of Pb with maximal, minimal and mean concentrations; (D)

642 Violin plot showing total $\log_2(\text{CR})$ values with maximal, minimal and mean concentrations.

643



644

645 **Fig.5.** From-cradle-to-grave heavy metal control strategy of herbal medicine production

646

647 **Table 1** Contents and detection rates of five heavy metals in herbal medicines

648

Heavy metals		Cu (mg·kg ⁻¹)	As (mg·kg ⁻¹)	Cd (mg·kg ⁻¹)	Hg (mg·kg ⁻¹)	Pb (mg·kg ⁻¹)
Concentrations of each heavy metal	Mean±SD	9.07±4.21	0.50±0.93	0.27±0.48	0.07±0.51	1.69±2.85
	Max	34.01	14.53	6.20	13.43	50.11
	75 th percentile	11.39	0.52	0.29	0.02	2.02
	Median	8.78	0.23	0.10	0.01	0.92
	25 th percentile	6.17	0.11	0.04	0.004	0.36
	Min	0.21	0.01	0.01	0.001	0.01
Mean	Flos (n=340)	11.12±2.73	0.4±0.49	0.439±0.686	0.081±0.667	1.55±2.8327

concentrations of five medicinal plant properties	Folium & cortex (n=157)	8.52±4.73	0.64±0.57	0.244±0.307	0.049±0.206	1.99±1.6128
	Fructus & semen (n=689)	7.85±3.64	0.2±0.55	0.07±0.18	0.08±0.57	0.89±2.28
	Herba & others (n=380)	9.98±4.53	1.06±1.56	0.43±0.54	0.06±0.19	3.23±3.83
	Radix & rhizoma (n=207)	8.45±5.26	0.27±0.22	0.31±0.52	0.01±0.02	1.38±1.74
Detection rates		99.89%	94.70%	97.74%	84.43%	98.98%
Top five herbal medicines with highest concentrations (mg·kg⁻¹)	Schisandrae Chinensis Fructus (34.01)	Plantaginis Herba (14.53)	Curcumae Rhizoma (6.20)	Longae Chrysanthemi Flos (8.69)	Euodiae Fructus (50.11)	
	Plantaginis Herba (31.08)	Corni Fructus (11.75)	Andrographis Herba (5.55)	Chrysanthemi Flos (8.58)	Plantaginis Herba (47.12)	
	Taraxaci Herba (30.52)	Plantaginis Herba (11.57)	Houttuyniae Herba (4.92)	Forsythiae Fructus (4.02)	Plantaginis Herba	

					(38.56)
Radix Arctii Lappae (29.73)	Plantaginis Herba (11.23)	Lonicerae Flos (4.50)	Forsythiae Fructus (3.60)	Lonicerae Japonicae Flos (32.98)	
Taraxaci Herba (29.18)	Taraxaci Herba (9.95)	Lonicerae Flos (4.07)	Gardeniae Fructus (2.42)	Lonicerae Japonicae Flos (28.93)	

649

650 **Table 2 Health risk assessment scores of top risk-inducing herbal medicines**

Herbal medicine	Medicinal plant property	Max EDI (mg·kg ⁻¹ ·day ⁻¹ ·bw)				Max HQ				HI	Max CR			
		As	Cd	Hg	Pb	As	Cd	Hg	Pb		As	Cd	Pb	Total CR
		Menthae haplocalycis herba	Herba & others					1.54					2.28	
Plantaginis herba	Herba & others	0.007			0.024	9.95			1.11	11.47	4.48E-09			
Andrographis herba	Herba & others		0.00 1			5.51				7.02				5.27E-09
Isatidis folium	Folium & cortex					1.64				2.21				
Puerariae lobatae radix	Radix & rhizoma					1.03				1.35				
Desmodii styracifolii herba	Herba & others		0.00 1	0.001	0.008	1.79				3.47				
Carthami flos	Flos					2.02				2.41				

Chrysanthemi flos	Flos			0.001		1.90		4.08		6.31			
Farfarae flos	Flos					2.24		1.89		2.65			
Forsythiae fructus	Fructus & semen			0.001		1.29				3.51			
Ligustri lucidi fructus	Fructus & semen					1.74				2.91			
Taraxaci herba	Herba & others	0.002			0.004	6.82				8.62			
Corni fructus	Fructus & semen	0.002				8.05				8.66			
Ziziphi spinosae semen	Fructus & semen					1.58				1.83			
Euodiae fructus	Fructus & semen			0.001	0.004	1.69		6.31	1.18	9.53			3.50E-11
Schisandrae chinensis fructus	Fructus & semen					1.07				1.49			
Houttuyniae herba	Herba & others		0.00		0.004					3.42			
			2			1.97							
Gardeniae fructus	Fructus & semen					2.93		1.14		4.57			
Aurantii fructus	Fructus & semen					1.08				1.28			
Perillae folium	Folium & cortex					2.64		1.02		4.02			
Curcumae longae rhizoma	Radix & rhizoma		0.00					1.02		1.43			3.11E-09
			1										
Coptidis rhizoma	Radix & rhizoma									Coptidis rhizoma			
Lonicerae japonicae flos	Flos				0.005					2.12			

Chaenomeles fructus	Fructus & semen									1.48				
Lonicerae flos	Flos		0.00		0.004					1.81				

651 The estimated daily intakes (EDI, $\text{mg}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}\cdot\text{bw}$) above their corresponding provisional tolerable daily intakes (PTDI) were
652 shown (The PTDIs ($\text{mg}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$) of As, Hg, Pb, and Cd are 0.00214, 0.00057, 0.00357, and 0.00083, respectively). The
653 non-carcinogenic hazard quotient (HQ) and non-carcinogenic Hazard Index (HI) above one were shown. The carcinogenic risks
654 (CR) higher than 10^{-6} , which means one case of cancer over one million exposed people, is considered unacceptable, thus shown
655 here. All scores in this table were calculated with maximal concentrations of each herbal medicine.

656

Figures

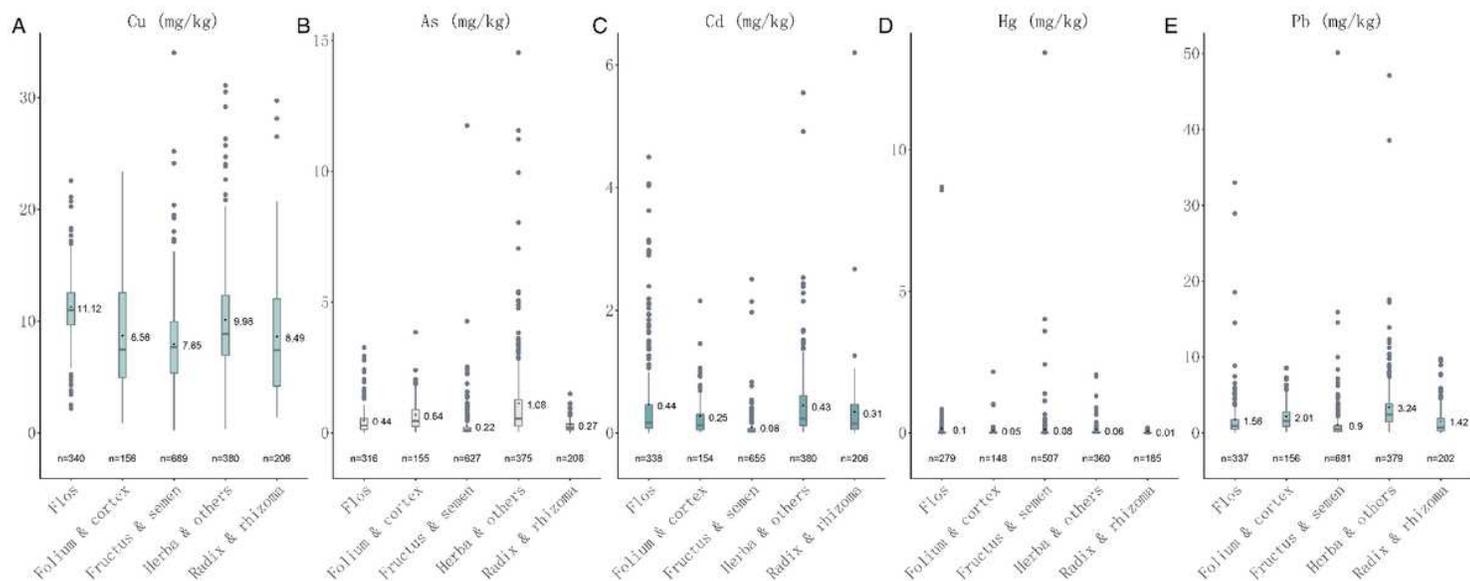


Figure 1

Plotbox showing concentrations of five heavy metals in five medicinal properties. (A) Concentrations of Cu in five medicinal properties. (B) Concentrations of As in five medicinal properties. (C) Concentrations of Cd in five medicinal properties. (D) Concentrations of Hg in five medicinal properties. (E) Concentrations of Pb in five medicinal properties.

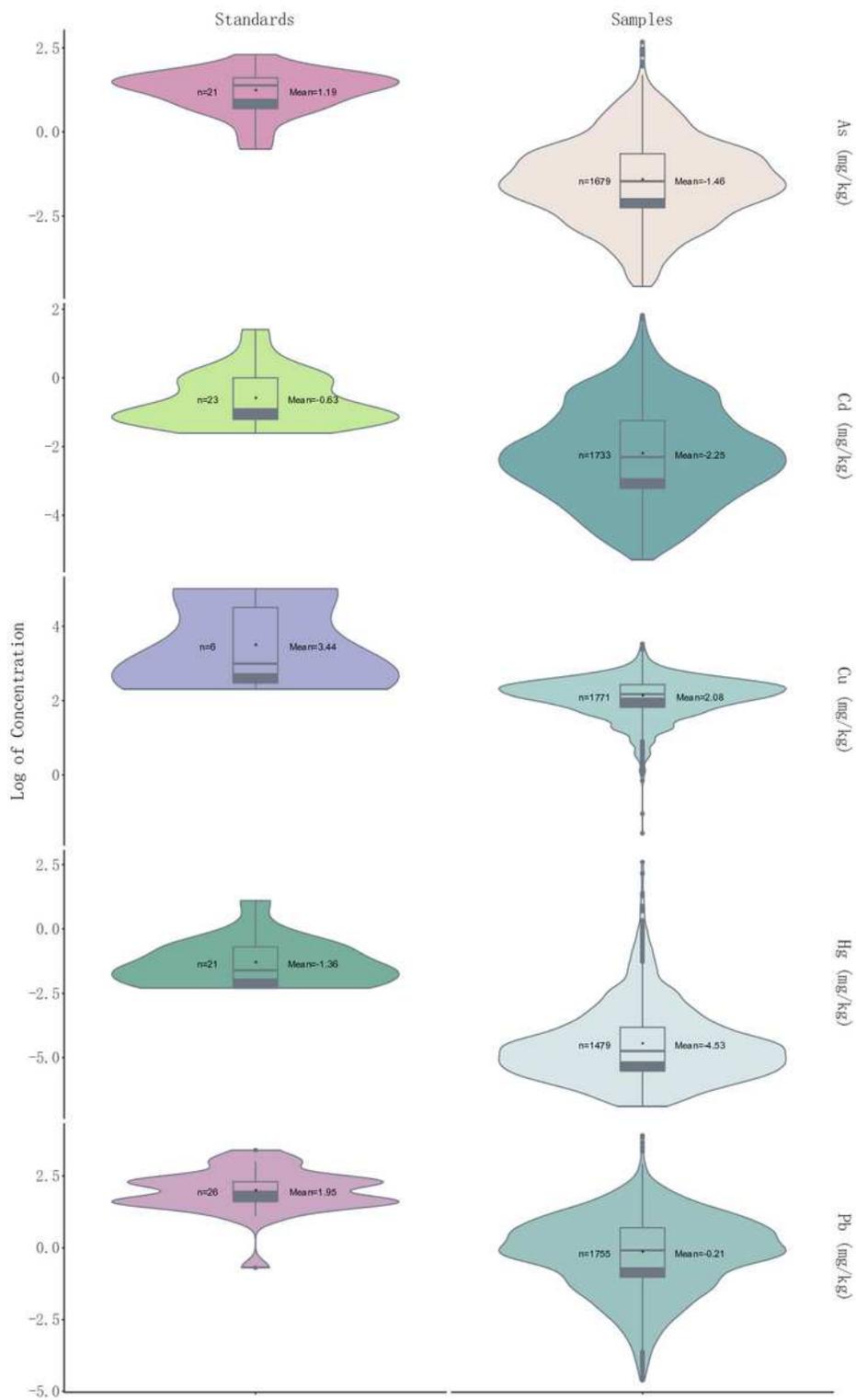


Figure 2

Violin plot showing \log_2 (concentration) values of five heavy metals in 1773 samples and permissible limits in different countries of each heavy metal (see Table S2 for detailed standards of each heavy metal).

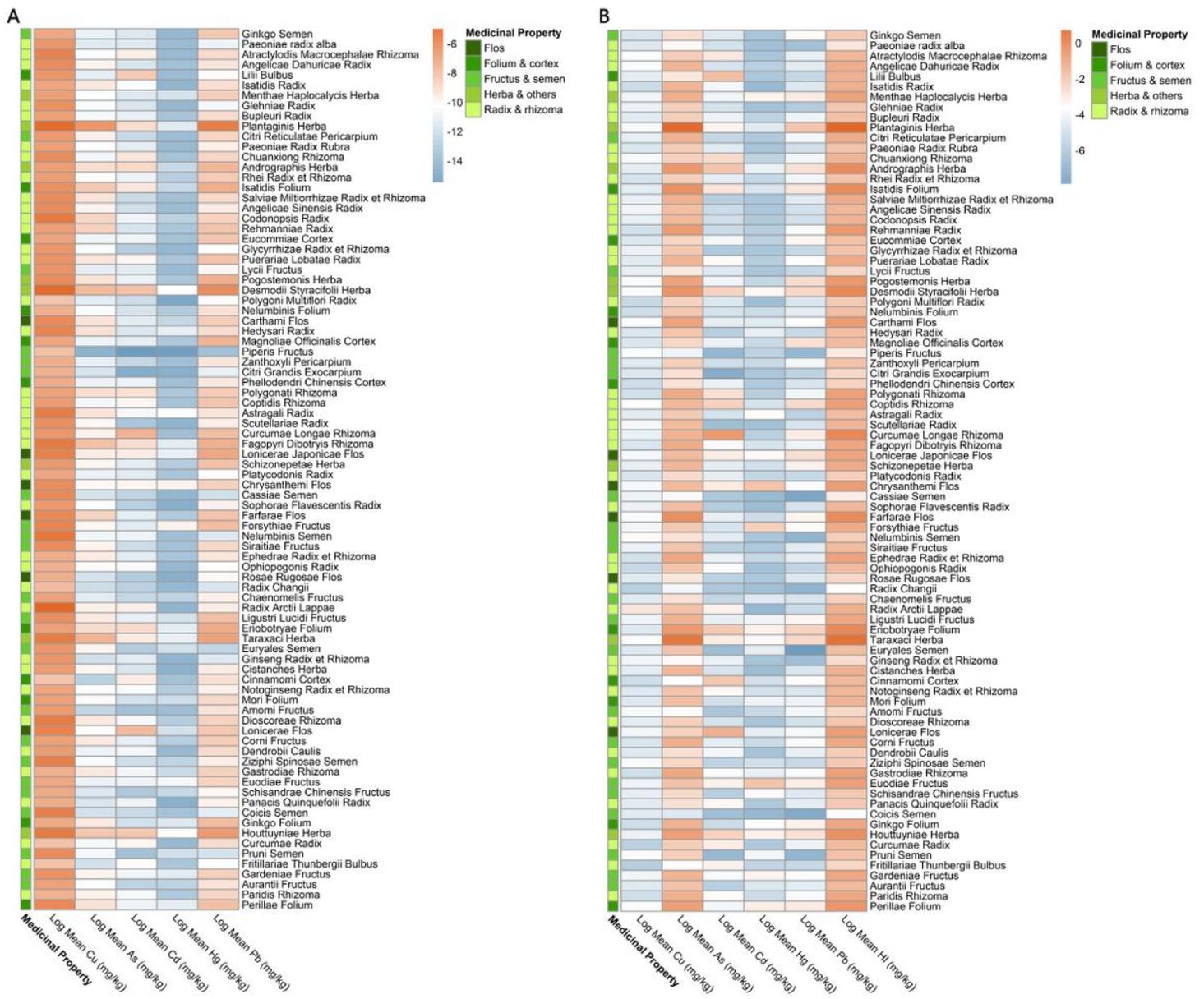


Figure 3

Heatmap of EDI and HI by mean concentrations detected for five heavy metals in 86 kinds of herbal medicines (A) Heatmap showing log₂ (Estimated Daily Intake, EDI) values by mean concentrations detected of five heavy metals in 86 herbal medicines; (B) Heatmap showing log₂ (Hazard Index, HI) values by mean concentrations detected of five heavy metals in 86 herbal medicines.

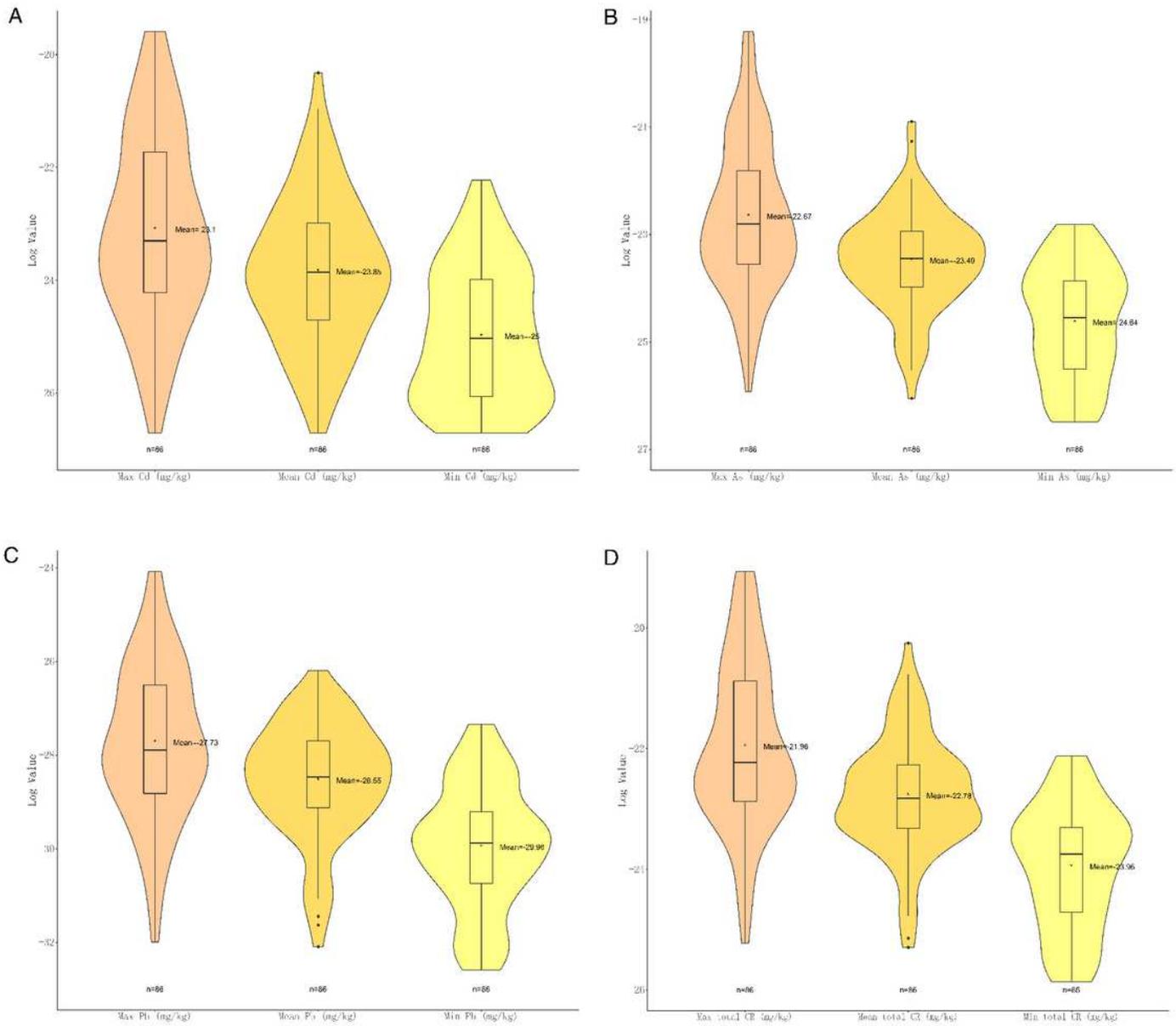


Figure 4

Violin plot of carcinogenic risk (CR) of three carcinogenic metals with maximal, minimal and mean concentrations detected (A) Violin plot showing log₂ (CR) values of Cd with maximal, minimal and mean concentrations; (B) Violin plot showing log₂ (CR) values of As with maximal, minimal and mean concentrations; (C) Violin plot showing log₂ (CR) values of Pb with maximal, minimal and mean concentrations; (D) Violin plot showing total log₂ (CR) values with maximal, minimal and mean concentrations.

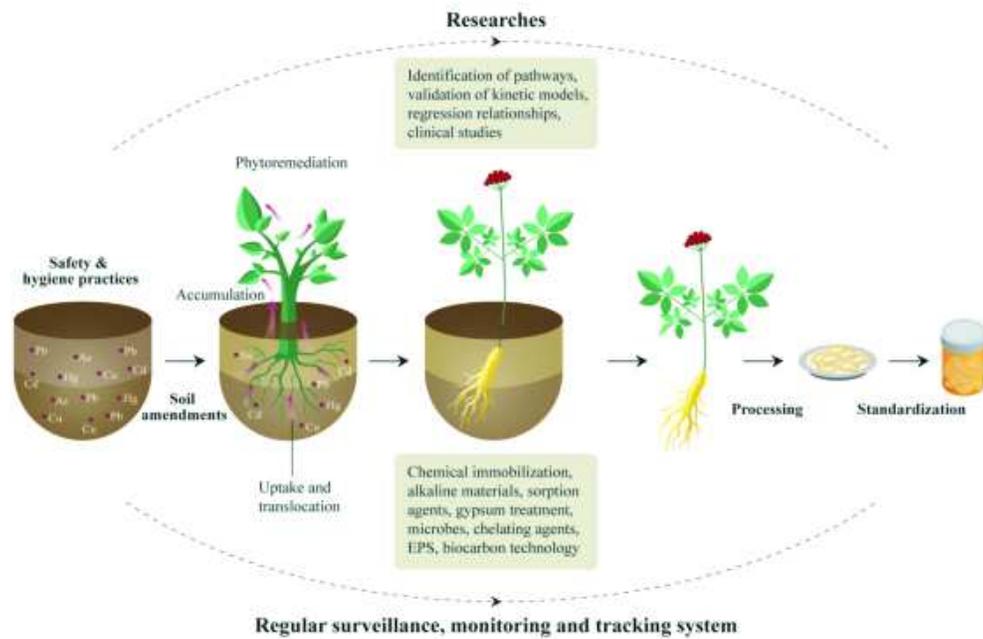


Figure 5

From-cradle-to-grave heavy metal control strategy of herbal medicine production

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