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

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

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Abstract

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

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

Results: According to Chinese Pharmacopoeia, 30.51% (541) samples were detected with at least one over-limit metal. The over-limit ratio for Pb was 5.75% (102), Cd at 4.96% (88), As at 4.17% (74), Hg at 3.78% (67), and of Cu, 1.75% (31). For exposure assessment, Pb, Cd, As, and Hg have resulted in higher than acceptable risks in 25 kinds of herbs. The maximal Estimated Daily Intake (EDI) of Pb in seven herbs, of Cd in five, of Hg in four, and As in three exceeded their corresponding Provisional Tolerable Daily Intakes (PTDI). In total 25 kinds of herbs present an unacceptable risk as assessed with the HQ (Hazard Quotient) or HI (Hazard Index). Particularly, Plantaginis herba (HI=11.47) is more than 11 times over the limit.

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

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

Background

Having been utilized as traditional folk remedies for thousands of years [1], medicinal plants gained an increasingly important role in the pharmaceutical, health food, and natural cosmetic industries[2]. It was reported that a total of 60,107 COVID-19 cases (85.20% of the total cases) in China were treated by Chinese herbal medicines with positive results in all infection stages, including significant symptom management, lower rates of deterioration and mortality, faster recovery as well as disease prevention on 17 February 2020 [3]. Over years, however, concerns grew regarding the safety of herbal medicines after studies indicated that high levels of heavy metals were present in some herbal medicines. This was a problem more frequently encountered in traditional medical therapy, considered to be a cause of several health disorders [1]. Samples taken from both developed and developing countries have shown high levels of potentially toxic heavy metals in products available to the public [4]. It is known that numerous traditional medicines can give rise to severe adverse renal pathology, the mechanism of which is yet not fully certain but has been associated with heavy metal toxicity [4]. Uptake of heavy metals by plants and subsequent accumulation along the food chain is a potential threat to animal and human health. Particularly as heavy metals are known to have low renal excretion rates, potentially resulting adverse effects in humans even at very low concentrations. They are not easily metabolized by body and are found to accumulate in the soft tissues. They produce toxic effects due to their interference in many know normal biochemical and metabolic processes [5]. Several health problems were linked to excessive uptake of dietary heavy metals, including decreased; immunity, cardiac dysfunction, fetal malformation, impaired psychosocial and neurological behavior [6]. Pb and Cd are not essential elements that are required neither in the human body nor in plants, and which cause various bimolecular adverse functional effects at low level doses [1]. Though an essential component of many enzymes, excessive intake of Cu can cause dermatitis, irritation of the upper respiratory tract, abdominal pain, nausea, diarrhea, vomiting, and liver damage [6]. While As and Hg can damage pulmonary, nervous, renal and respiratory systems, as well as causing skin pathology [7]. It may also induce disorders in the central nervous system, liver, lungs, heart, kidney and brain. Leading to hypertension, abdominal pain, skin eruptions, intestinal ulcer and is associated with various types of cancers [8]. It is therefore necessary and a matter of urgency to conduct a comprehensive risk assessment of heavy metal contamination in herbal medicines.

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

Methods

Sample collection and detection

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

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

In our experiment, standard solutions of Cu (GSB04-1725, 1000 mg·L^{-1}), As (GSB04-1714, 1000 mg·L^{-1}), Cd (GSB04-1721, 1000 mg·L^{-1}), Hg (GSB04-1729, 1000 mg·L), Pb (GSB04-1742, 1000 mg·L^{-1}) were purchased from the National Nonferrous Metals and Electronic Materials Analysis and Testing Center, while Analytical Reagents (AR) nitric acid (HNO_3) and hydrofluoric acid (HF) were purchased from Merck Co., Ltd., and guaranteed reagents (GR) hydrogen peroxide and hydrochloric acid from Sinopharm Chemical Reagent Co., Ltd. Each single-element standard solution was measured precisely and then diluted with 5% HNO_3 to make a mixed solution containing 1 μg mL^{-1}. For a reference stock solution, single-element standard solutions of the five metals were separately taken and diluted with 5% nitric acid to make solutions containing 5 μg of Pb and As, 50 μg of Cu, 2.5 μg of Cd, and 0.5 μg of Hg. For preparation of a reference standard curve, precise measurement were taken of the above stock solution diluted with 5% nitric acid to make standard mixtures with: 0, 1, 5, 10, 20, and 50 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 Cd per 1 mL; with 0, 0.1, 0.5, 1, 2, and 5 ng Hg per 1 mL [6].

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

Quantification of heavy metal contamination

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

Over-limit ratio of five heavy metals

In total, 27 currently available permissible limits containing five heavy metals were obtained from 20 countries or regions and seven international organizations (Table S2). The detailed calculation of both general over-limit ratio and that of each metal in different producing areas were shown in supplementary material (Table S3). Herbal medicines were classified into five categories based on which part of the plant is employed for medicinal use: flos, folium & cortex, fructus & semen, herba & others, and radix & rhizome. Numbers of over-limit samples and metals were calculated from within across these five medicinal herbal properties.

Over-limit ratio= $(C - Limit) \times 100\%$ (1)

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

Three health risk assessments of heavy metal contamination in herbal medicines

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

Exposure assessment

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

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

Non-carcinogenic risk assessment

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

$$HQ=\frac{C \times IR \times Ef \times Ed \times t}{AT \times BW \times RfD} \tag{4}$$

IR is the daily dosage of herbal medicine, and according to a questionnaire on herbal medicine consumption of 20917 people, the 95th percentile of daily dosage of general herbal medicine consumption is 0.5 kg; Ef is exposure frequency, here the 95th percentile of annual consumption on herbal medicine was adopted which was set 90 days per year; Ed is the exposed days over a lifetime which was set as 20 years; AT is the average lifetime=365 days×70 years, while t is the transfer rate of heavy metal to herbal detection, which is 14% for Cu and Pb, 35% for As, and 24% for Hg [25]. RfD refers to Oral reference dose ($mg^{-1}\cdot kg^{-1}\cdot day$), which is 0.0035 for Pb, 0.0005 for Cd, 0.0003 for As, 0.0003 for Hg and 0.04 for Cu [26] .

$$HI=\sum HQ \tag{5}$$

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

$$\text{Contribution of } HQ_m \text{ to } HI=(HQm-HI)\times 100\% \tag{6}$$

Carcinogenic risk assessment

$$CR=\frac{C\times IR\times Ef\times Ed\times t\times CSF}{AT\times BW\times RfD}\times 10^{-6} \tag{7}$$

CSF ($mg\cdot kg^{-1}\cdot day^{-1}\cdot bw$) is the cancer severity factor, only three heavy metals were proven with certain 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 metals in the same herbal medicine was also summed up to give total CR of single herbal medicine. If CR is higher than 10^{-6}, which means one case of cancer over one million exposed people, it is considered unacceptable [28].

Results

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

In a total of 541 samples (30.51%) were detected at levels over the CP (2020 edition) standard, and 433 samples detected with one over-limit metal, 75 samples with two over-limit metals, three samples with 24 over-limit metals and nine samples with four over-limit metals (Fig.2). The order of over-limit ratio of five heavy metals based on CP (2020 edition) standard is Pb (102, 5.75%) > Cd (88, 4.96%) > As (74, 4.17%)> Hg (67, 3.78%) > Cu (31, 1.75%). The times of highest concentration detected over the standards of CP (2020 edition) were 1.70 for Cu (Schisandrae chinensis fructus), 6.27 for As (Plantaginis herba), 5.20 for Cd (Curcumae longae rhizome), 66.17 for Hg (Chrysanthemi flos), and 9.02 for Pb (Euodiae fructus). The highest concentration detected over the limit of European Union (EU) and United Kingdom (U.K.) is Hg (133.35 times). As per over-limit ratios of each metal in five medicinal properties, 40.00% samples were detected with over-limit concentrations in flos, 34.39% in folium & cortex, 7.69% in fructus & semen, 58.16% in herba & others, 37.20% in radix & rhizome. For Cu (*n*=31), there are 12.90% samples detected over-limit in flos, 9.68% in folium & cortex, 16.13% in fructus & semen, 41.94% in herba & others, and 19.35% in radix & rhizome; For As (*n*=74), 9.46% samples were detected with concentrations above the threshold in flos, 6.76% in folium & cortex, 8.11% in fructus & semen, 75.68% in herba & others, and none in radix & rhizome; For Cd (*n*=416), 27.16% samples detected over-limit in flos, 10.10% in folium & cortex, 5.29% in fructus & semen, 39.90% in herba & others, and 17.55% in radix & rhizome; For Hg (*n*=67), there are 25.37% samples detected over-limit in flos, 5.97% in folium & cortex, 34.33% in fructus & semen, 34.33% in herba & others, and none in radix & rhizome; For Pb (*n*=102), there are 11.76% samples detected over-limit in flos, 9.80% in folium & cortex, 9.80% in fructus & semen, 59.80% in herba & others, and 8.82% in radix & rhizome. Of note, we found that heavy metals prefer to accumulate in fructus & semen, while herba & others were detected with the highest over-limit ratio. Notably, heavy metal Pb was presented with the highest over-limit ratio, followed by Cd and As.

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

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

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

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

Discussion

Based on this study with a large-spatio-temporal-scale herbal medicine samples, 30.51% (541) of samples were detected with at least one over-limit heavy metal. Five heavy metals (As, Pb, Cu, Hg and Cd) were widely detected in cultivated herbal medicines according to our experiment, which is in accordance with other published results [30-31]. In Nigerian herbal remedies, 100% of the samples also contained elevated amounts of heavy metals [32], which revealed that the Nigerian herbs contained high levels of Fe, Ni, Cd, Cu, Pb, Se, and Zn sufficient to cause adverse health effect when regularly taken as recommended. In our study, 27 (31.40%) different kinds of herbal medicines, mostly with fructus & semen part with medicinal applications posed unacceptable health risk due to heavy metal accumulation though herba & others were detected with the highest over-limit ratio. Toxic element As posed the most serious health risk according to exposure, carcinogenic and non-carcinogenic risk assessments, as health risk assessment employed by Ren, indicated that As and Pb generated from industrial sites and traffic sites has a potential to pose serious health risks [33]. It was also found that As was the major metal found for water pollution [29], exceeding its permitted daily exposure dosages and suggested a potential health risk for *P. notoginseng* consumers [34]. Considering that the content of As detected is total As and As exists in different states. The toxicity of As in different states varies tremendously. Both organic and inorganic As exist in the soil, while the states of As may transform in biological activities both in plants and human metabolism. So it is hard to say if high concentration of total As would absolutely harm human health. However, As is certainly necessitating special attention and further study on its state transformation. Furthermore, Bolan employed a study and asserted that the concentrations of Cd, Hg, and Pb in Ayurvedic medicines exceeded their daily intake amounts [35]. According to the study by Lee SD, levels of Cd exceeding WHO reference values were observed in 10 samples and the weekly intakes of Pb, Cd, Cr, Cu, Hg from herbs [36]. While in Iran, maximum bioaccumulate of Pb and Hg was noted in *Artemisia dracunculus* L and *Spinacia oleracea* L, respectively [37]. The non-carcinogenic risks target hazard quotients (THQs) of Al and Cr from individual herbs were over 1, which might impart risk for human consumption. It could be concluded that heavy metals As, Pb, Cd, and Hg all impose significant risk to health due to herbal consumption. The highest HI was presented in herbal medicine Plantaginis Herba (HI=11.47) and highest CR in Andrographis herba (CR=5.27E-09), which are in need of special dosage control and monitoring. Furthermore, the highest over-limit ratios of five heavy metals based on different producing areas are Cu (7.69%) in Chongqing, As (20.21%) in Gansu province, Cd(0.77%) in Chongqing, Hg (9.89%) in Hunan province, and Pb (25.00%) in Fujian province (Tables S3). However, Principle Components Analysis (PCA) didn't showcase that there was significant statistic difference regarding the five heavy mental accumulations in five medicinal plant properties. While according to Pearson Correlation Analysis, Pb and As were correlated to flos, folium & cortex, fructus & semen, and herba & others, while Pb and Cd in radix & rhizoma (Fig.S3, 4). Additionally, based on the Analysis of Similarities (ANOSIM), it was indicated that the difference within groups of five medicinal plant properties is less significant than the one throughout the five groups (R=0.165, P=0.001) (Fig.S5).

Generally, five reasons explain the levels of heavy metal contents in herbal medicines. The first is the variable exposure to environmental pollution including industrial encroachment, contaminated soil or atmosphere. The physicochemical properties of soil including pH, temperature, redox potential, translocation exchange capacity and organic matter may influence the availability of metal to plants. Secondly, the phytological characteristics of medical plants themselves such as reduced biomass, root length and shoot length are common indicators of heavy metal toxicity. Furthermore, the interactions of soil-plant roots-microbes play vital roles in regulating heavy metal movement from the soil to edible plant parts. Certain plants are "hyper-accumulators" which grow on metalliferous soils and accumulate extraordinarily high levels of heavy metals without displaying phytotoxic effects. Thirdly, herbal plants could be contaminated during manufacturing and agronomic processes [38] including growing, harvesting, transportation, processing and storage, due to pesticide formulations, chemical fertilizers and irrigation with poor-quality water [6,35]. For example, Cd and Pb may enter the soil due to fertilizer impurities [39], non-ferrous smelters, lead and zinc mines, sewage slug application and combustion of fossil fuels [40]. Additionally, fumigants containing heavy metals may also be applied for preventing rats and mildew [41]. Fourthly, plant uptake is one of the major routes of dietary exposure to heavy metals in the soil, and the wide variations in metal concentrations in the analyzed herbs could be attributed to differences in the plant metal uptake and translocation capabilities. Studies have shown wide variations in concentration factor for different metals among different plant species and sampling sites. Certain species have higher tendency to accumulate Cd [6]. Lastly, the bioavailability of heavy metals could have an impact on their concentrations, such as; soil pH, the metal levels already resident in the soil, the oxidation-reduction potential of the soil, and other chemical and physical factors [6] (Table S5).

Here we propose a solution for heavy metal control in herbal medicines. We consider, given the results found here and those of others previously there is an urgent need to implement a regular monitoring and surveillance program, controlling extrinsic contamination of herbal medicines along the supply chain from field to consumer [35]. Secondly, research, such as identifying ways in which heavy metals reach herbal products; development and validation of kinetic models linking processing techniques with metal speciation and bioavailability; bioavailability tests of heavy metals in herbal medicines; experiments on regression relationships between speciation and bioavailability of heavy metals, clinical studies examining the toxicity of heavy metals, etc. [6]. Soil amendments, including mitigation and preservation management for the growth performance of biomass and metal accumulation in contaminated soils, is necessary [2]. Lastly, tolerant medicinal plants with high phytoremediation potential and capability for phytostabilization and phytoextraction [2] can be cultivated as an approach for the management and targeted bio-extraction of heavy metals from moderately polluted lands [28], together with a combination of different agents such as pH change-inducing chemical immobilization, alkaline materials including lime based materials, fly ash, and biochar, calcite, dolomite, oyster and egg shell [2]. Sorption agents such as phosphate materials, compost, zeolite and iron compounds, activated carbon, and bentonite, or materials that decrease dissolved organic carbon (DOC) such as gypsum treatment, Solanum nigrum, microbes, chelating agents, Extracellular polysaccharides or Exopolysaccharides (EPS) [42], and eco-friendly biocarbon technology [43]. These materials increase soil pH, favor deprotonation and the formation of oxides, metal-carbonate precipitates, complexes and secondary minerals that all decrease the phytoavailable heavy metal concentrations [1]. Phytoremediation has been perceived to be a more low-cost, low-impact, low-tech alternative, visually benign and environmentally sound comparing to more active and intrusive remedial methods [43] (Fig.5).

Conclusion

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

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

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

Japonicae Flos (19#) herbal medicines (n=3). **Table S1.8** Methodological verification of ICP-MS for determination of five heavy metals in Chrysanthemi Flos (26#) herbal medicines (n=3). **Table S1.9** Methodological verification of ICP-MS for determination of five heavy metals in Farfarae Flos (7#) herbal medicines (n=3). **Table S1.10** Methodological verification of ICP-MS for determination of five heavy metals in Forsythiae Fructus (6#) herbal medicines (n=3). **Table S1.11** Methodological verification of ICP-MS for determination of five heavy metals in Chaenomelis Fructus (3#) herbal medicines (n=3). **Table S1.12** Methodological verification of ICP-MS for determination of five heavy metals in Ligustri Lucidi Fructus (2#) herbal medicines (n=3). **Table S1.13** Methodological verification of ICP-MS for determination of five heavy metals in Taraxaci Herba (27#) herbal medicines (n=3). **Table S1.14** Methodological verification of ICP-MS for determination of five heavy metals in Lonicerae Flos (13#) herbal medicines (n=3). **Table S1.15** Methodological verification of ICP-MS for determination of five heavy metals in Corni Fructus (16#) herbal medicines (n=3). **Table S1.16** Methodological verification of ICP-MS for determination of five heavy metals in Ziziphi Spinosae Semen (1#) herbal medicines (n=3). **Table S1.17** Methodological verification of ICP-MS for determination of five heavy metals in Euodiae Fructus (8#) herbal medicines (n=3). **Table S1.18** Methodological verification of ICP-MS for determination of five heavy metals in Schisandrae Chinensis Fructus (10#) herbal medicines (n=3). **Table S1.19** Methodological verification of ICP-MS for determination of five heavy metals in Houttuyniae Herba (2#) herbal medicines (n=3). **Table S1.20** Methodological verification of ICP-MS for determination of five heavy metals in Gardeniae Fructus (14#) herbal medicines (n=3). **Table S1.21** Methodological verification of ICP-MS for determination of five heavy metals in Aurantii Fructus (4#) herbal medicines (n=3). **Table S1.22** Methodological verification of ICP-MS for determination of five heavy metals in Perillae Folium (10#) herbal medicines (n=3). **Table S1.23** Methodological verification of ICP-MS for determination of five heavy metals in Plantaginis Herba (4#) herbal medicines (n=3). **Table S2** Permissible limits of heavy metals in medicinal herbs (products) from different standards**. Table S3** Over-limit ratio (%) of five heavy metals in 32 producing areas. **Table S4** The contributions of each metal in HI>1 herbal medicines**. Table S5** Chronic and acute adverse effects of five heavy metals on health**. Fig.S1.** The over-limit Estimated Daily Intakes (EDI) of four heavy metals in herbal medicines. **Fig.S2.** The over-limit Hazard Quotients (HQ) of four heavy metals in 21 herbal medicines. **Fig.S3.1** Spearman correlation coefficient of metal relations in flos**. Fig.S3.2** Spearman correlation coefficient of metal relations in folium & cortex**. Fig.S3.3** Spearman correlation coefficient of metal relations in fructus & semen**. Fig.S3.4** Spearman correlation coefficient of metal relations in herba & others**. Fig.S3.5** Spearman correlation coefficient of metal relations in radix & rhizoma. **Fig.S4.1** Principle Components Analysis (PCA) of five heavy metal contents in five plant properties**. Fig.S4.2** PCA of five heavy metals in 32 producing areas. **Fig.S5** Analysis of Similarities (ANOSIM) of five heavy metal contents in five medicinal plant properties.

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Authors' contributions

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

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

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Availability of data and materials

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

Consent for publication

Not applicable.

Competing interests

All authors declare no competing interests.

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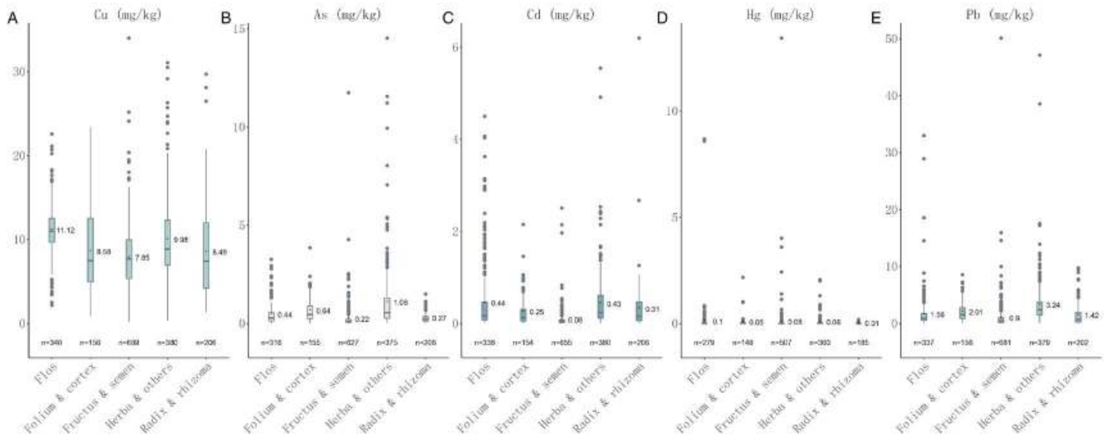

Fig.1. Plotbox showing concentrations of five heavy metals in five medicinal properties. (A) Concentrations of Cu in five medicinal properties. (B) Concentrations of As in five medicinal properties. (C) Concentrations of Cd in five medicinal properties. (D) Concentrations of Hg in five medicinal properties. (E) Concentrations of Pb in five medicinal properties.

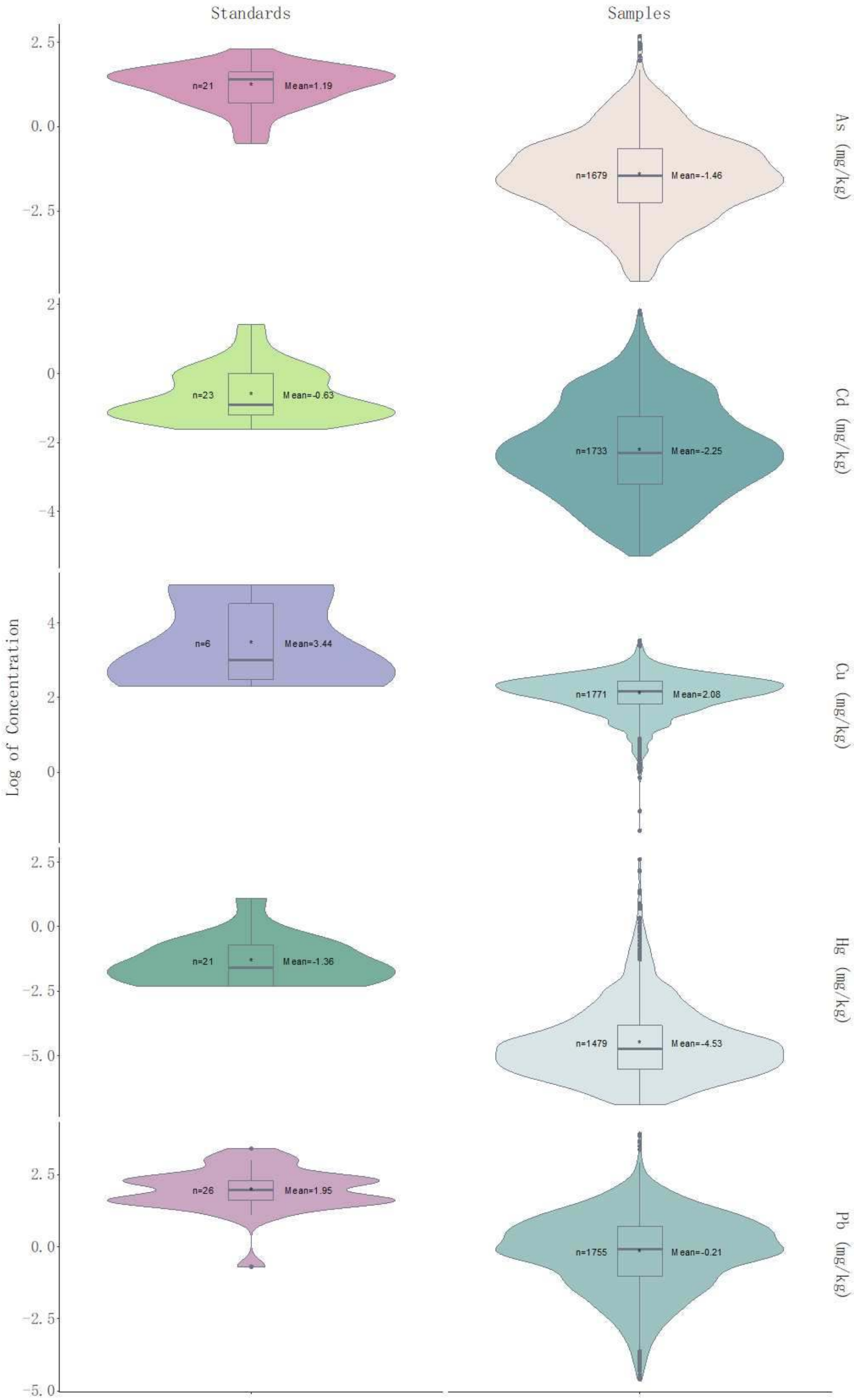

Fig.2. Violin plot showing $\log_2$ (concentration) values of five heavy metals in 1773 samples and permissible limits in different countries of each heavy metal (see Table S2 for detailed standards of each heavy metal).

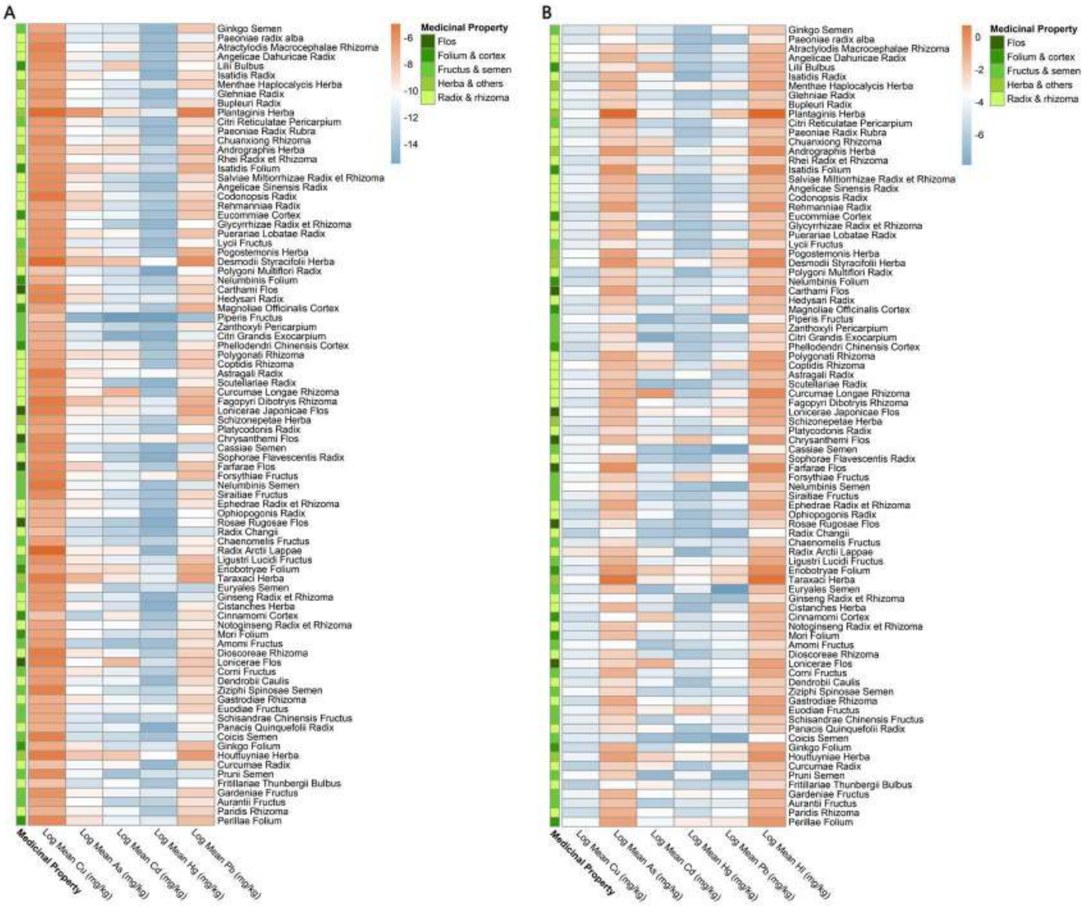

Fig.3. Heatmap of EDI and HI by mean concentrations detected for five heavy metals in 86 kinds of herbal medicines (A) Heatmap showing $\log_2$ (Estimated Daily Intake, EDI) values by mean concentrations detected of five heavy metals in 86 herbal medicines; (B) Heatmap showing $\log_2$ (Hazard Index, HI) values by mean concentrations detected of five heavy metals in 86 herbal medicines.

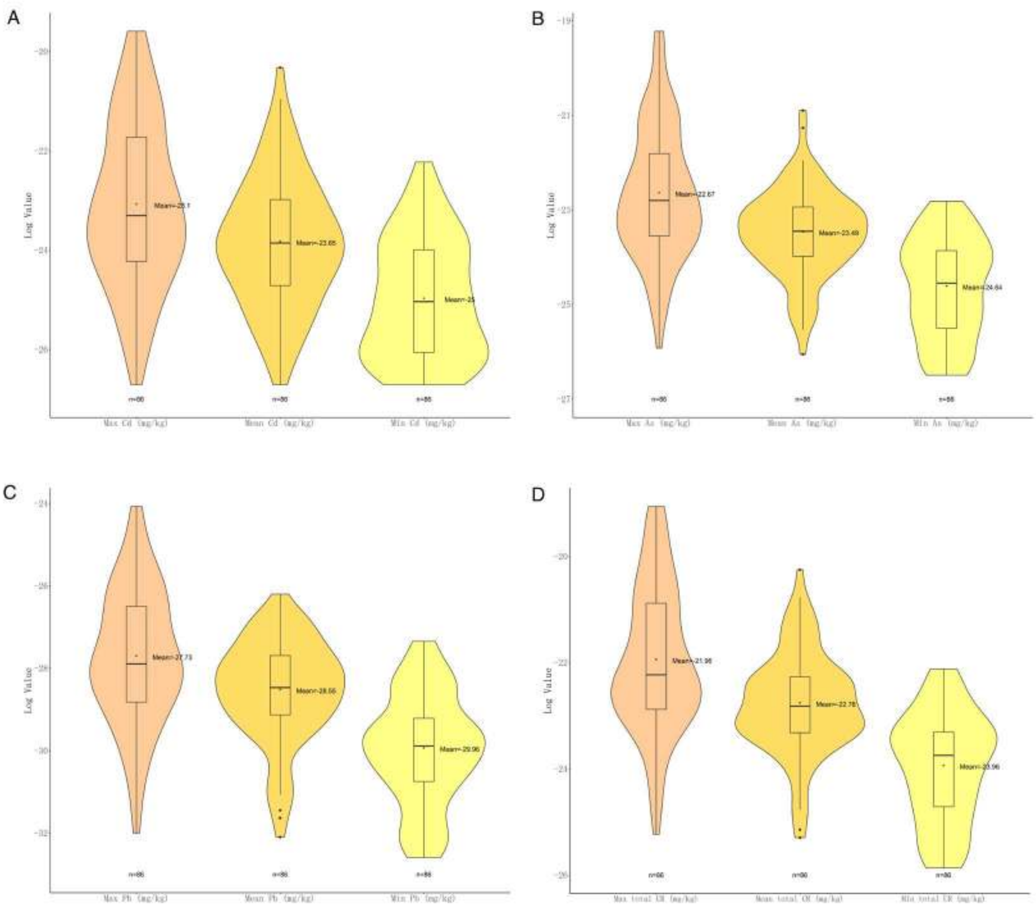

Fig.4. Violin plot of carcinogenic risk (CR) of three carcinogenic metals with maximal, minimal and mean concentrations detected

(A) Violin plot showing $\log_2$ (CR) values of Cd with maximal, minimal and mean concentrations; (B) Violin plot showing $\log_2$ (CR) values of As with maximal, minimal and mean concentrations; (C) Violin plot showing $\log_2$ (CR) values of Pb with maximal, minimal and mean concentrations; (D) Violin plot showing total $\log_2$ (CR) values with maximal, minimal and mean concentrations.

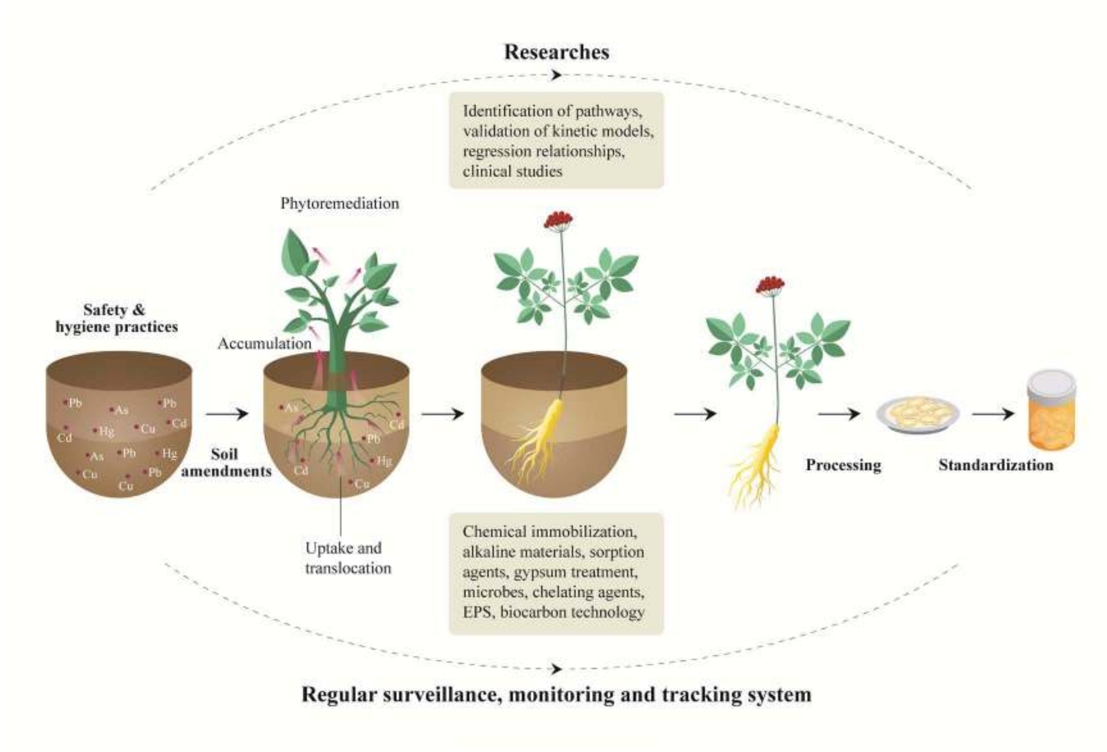

Fig.5. From-cradle-to-grave heavy metal control strategy of herbal medicine production

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

Heavy metals		Cu ($mg\cdot kg^{-1}$)	As ($mg\cdot kg^{-1}$)	Cd ($mg\cdot kg^{-1}$)	Hg ($mg\cdot kg^{-1}$)	Pb ($mg\cdot kg^{-1}$)
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
	75th percentile	11.39	0.52	0.29	0.02	2.02
	Median	8.78	0.23	0.10	0.01	0.92
	25th 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 Longae Rhizoma (6.20)	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)

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

Herbal medicine	Medicinal plant property	Max EDI ($mg\cdot kg^{-1}\cdot day^{-1}\cdot bw$)				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		0.001			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		0.001	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		0.002		0.004	1.97				3.42				
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		0.001				1.02			1.43		3.11E-09		
Coptidis rhizoma	Radix & rhizoma									Coptidis rhizoma				
Lonicerae japonicae flos	Flos				0.005					2.12				

Chaenomelis fructus	Fructus & semen									1.48				
Lonicerae flos	Flos		0.001		0.004					1.81				

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

Figures

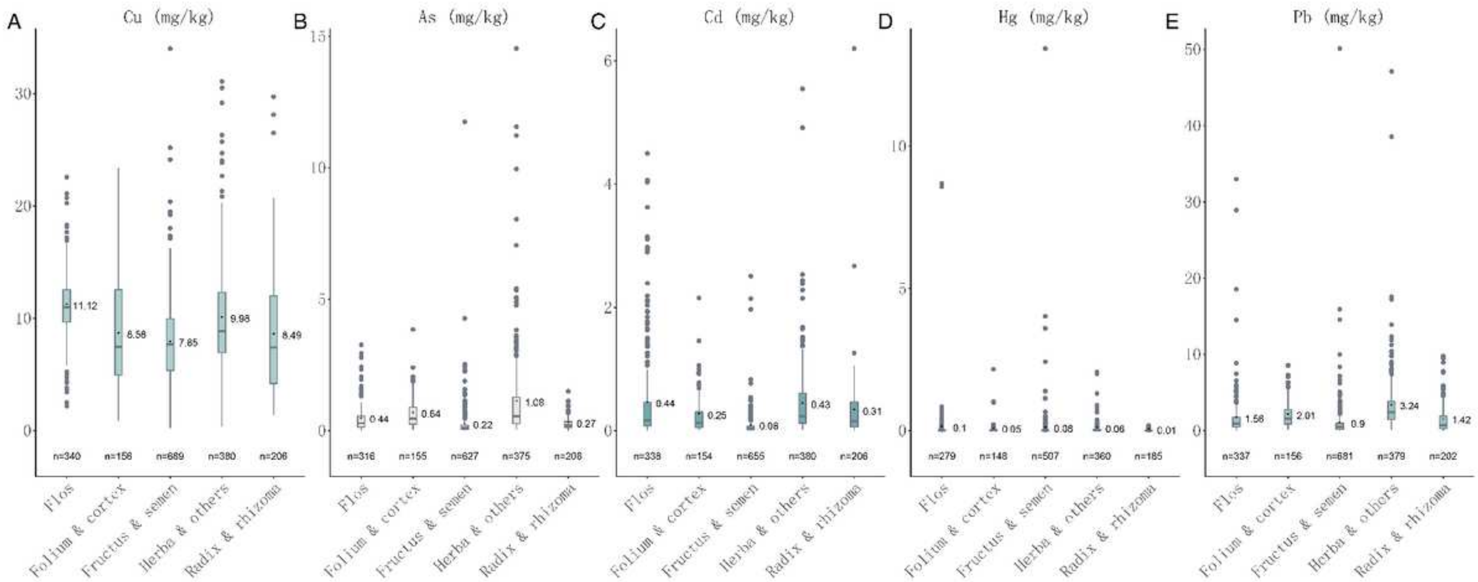

Figure 1

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.

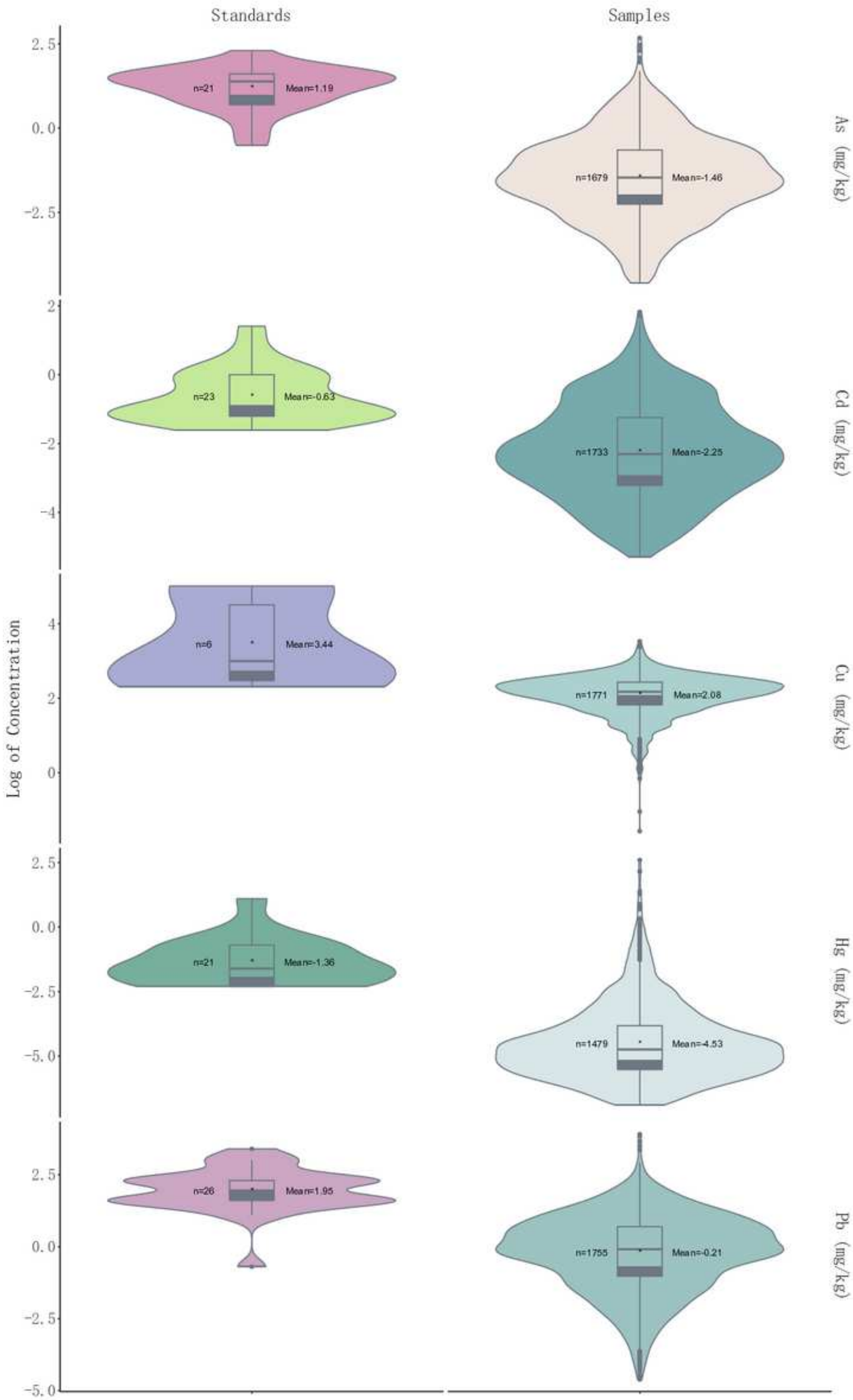

Figure 2

Violin plot showing log2 (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).

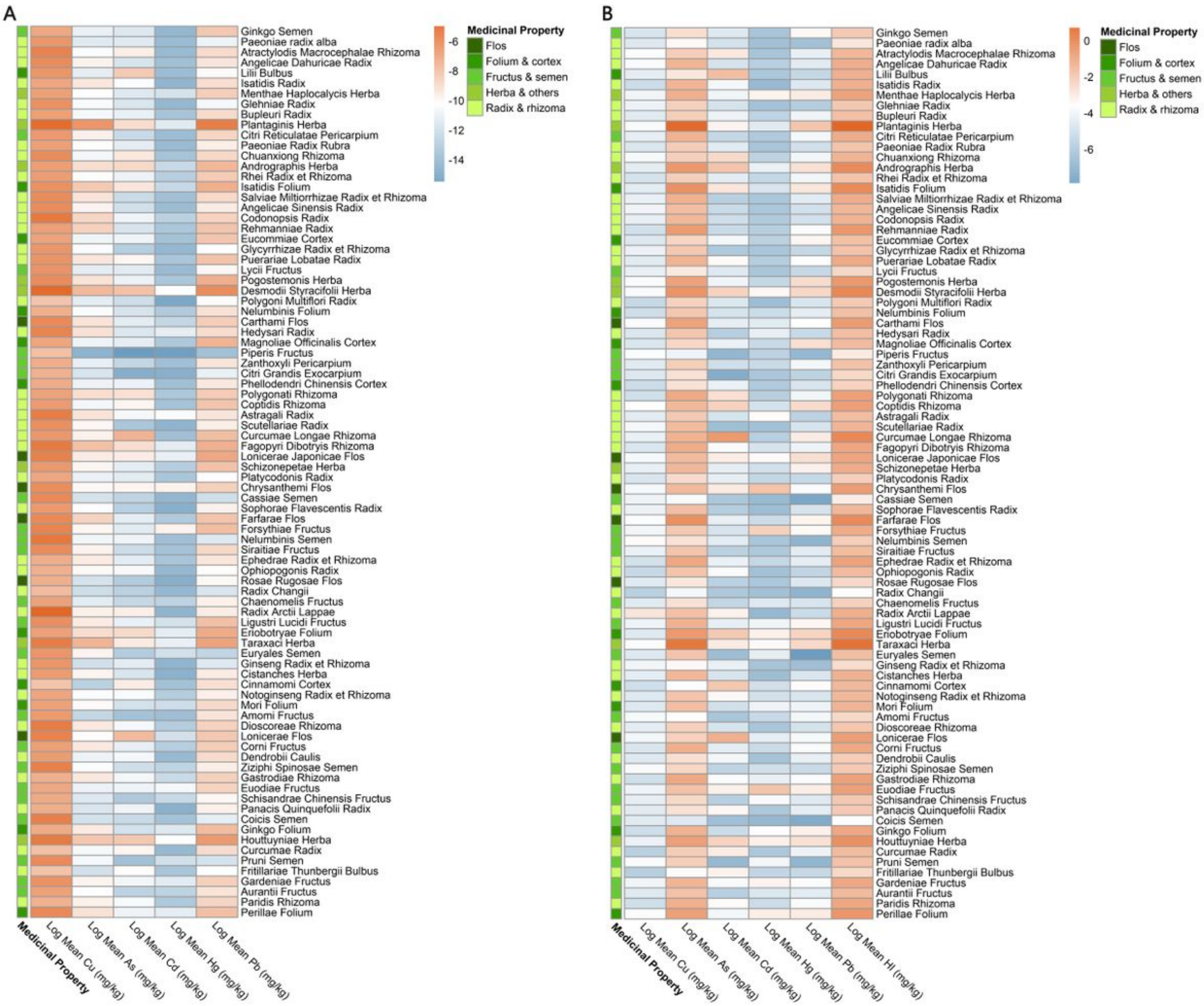

Figure 3

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.

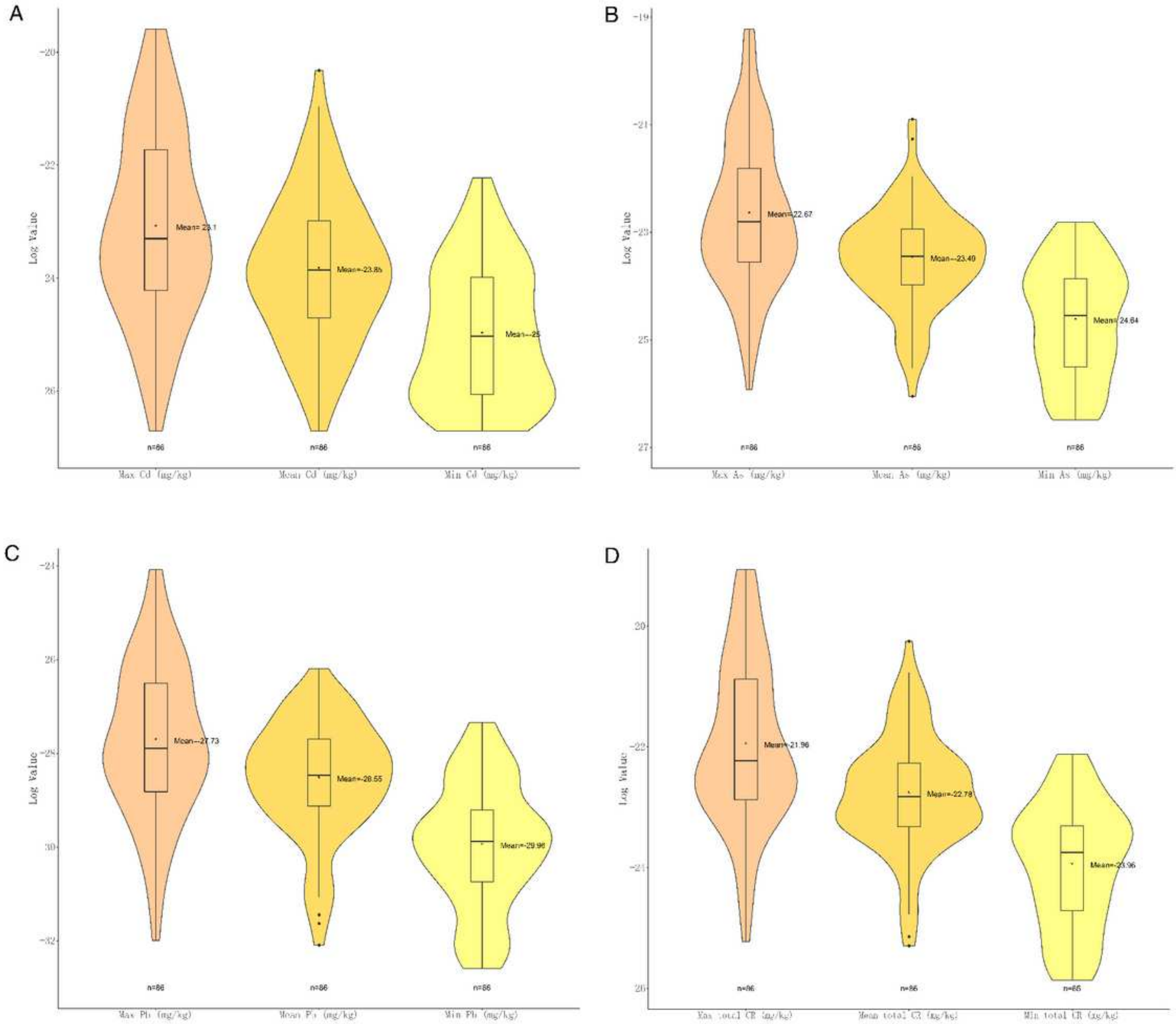

Figure 4

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.

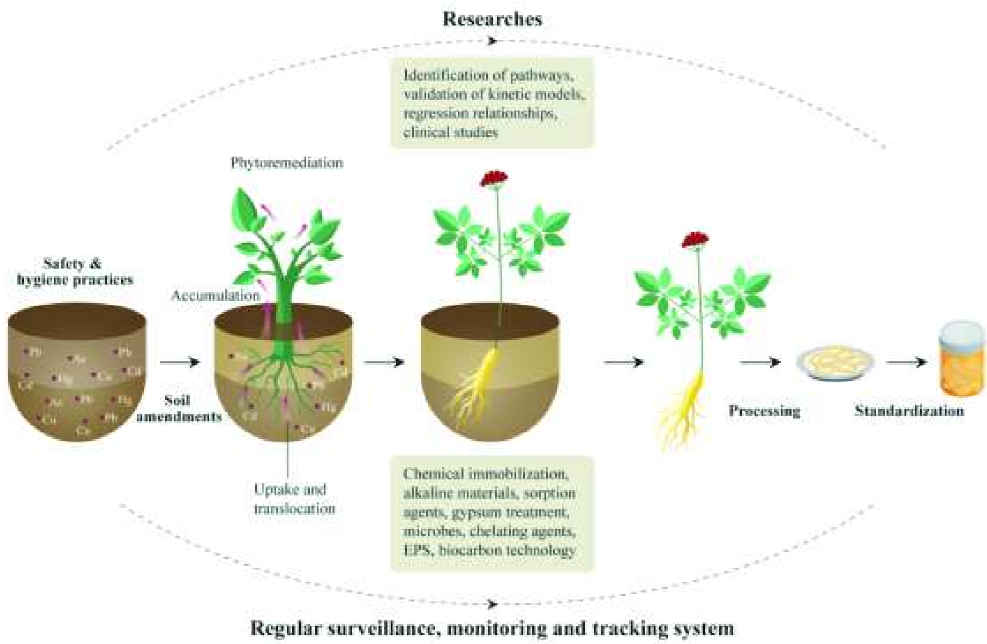

Figure 5

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

Supplementary Files

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- GA.pdf
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