

The distribution characteristics and bioaccumulation of arsenic in food webs around a realgar mine, China

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Abstract Arsenic (As) is a ubiquitous but carcinogenic metalloid element. Shimen realgar mine has the largest As mine in Asia with a mining history of 1500 years, therefore it could induce substantial increase of arsenic concentration in the environment and living organisms, and ultimately into human bodies. The total As in surface soil, sediments and water were in the range of 35-5000 mg/kg, 43-4543 mg/kg and 5-3293 µg/l, respectively. The As concentrations were decreasing with the distance from the central mining sites, indicating the As mining activities had produced eminent impact on As contamination in Shimen. Throughout the study sites, the rank order of As in biota was tadpole>earthworms>crab>frog>loach>other fishes. The total As concentrations in soil, litter and earthworms showed a significant positive relationship. The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ value in the same species of organisms varied greatly with sites, which suggest the impact of diet difference on the trophic levels of the same species in the food chain. The trophic levels for various species of organisms were determined as from 1.25 to 3.76 based on $\delta^{15}\text{N}$ values. In streams, As levels were much higher in lower trophic organisms, but were quite stable at higher trophic levels, indicating no bio-magnification of As in the food chain, however, the reservoir showed slight arsenic concentrations and obvious biomagnification through the food webs. Moreover, the As speciation, including iAs(III), iAs(V), MMA, DMA, AsB and other species were analyzed to explore the biotransformation of As and the sources and bio-transfer of organic As in food chain. It found that the organic As species increased with trophic level in the contaminated streams, while the reservoir showed contrast pattern.

Keywords: arsenic speciation, HPLC-ICP-MS, bioaccumulation, transformation, trophic level

1. Introduction

Arsenic (As) is a pervasive environmental toxicant and carcinogen that can pose significant health risks to humans and animals (Mandal and Suzuki, 2002; Smedley and Kinniburgh, 2002). As toxicity depends on not only its total concentration but also its chemical speciation (Ng, 2005). The inorganic forms of As (arsenite, As(III), and arsenate, As(V)) are predominant in the environment and

are considered to be more toxic than the simple methylated As species such as monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) (Mandal and Suzuki, 2002). However, trivalent forms of the methylated metabolites, MMA(III) and DMA(III), are generally regarded as extremely toxic (Hughes, 2002). Arsenobetaine (AsB), arsenocholine (AsC) and arsenosugars (AsS) are relatively non-toxic (Kaise *et al.*, 1985). Therefore, much attention has been given to the As speciation in environmental and biological samples.

Mining and smelting activities have been reported to be the major sources that significantly contribute to the elevation of As levels in the environment (Smedley and Kinniburgh, 2002; Garelick *et al.*, 2008). As loading from mines has long been a public concern, with serious health consequences for people living in As-contaminated areas. Shimen Realgar Mine, a historic mine that contains significant amounts of harmful metals and metalloids as a result of single As-rich ore processing, was targeted for a case study. The mining activities could induce substantial increase of arsenic concentration in the environment and living organisms, and ultimately into human bodies. To explore the As accumulation and transformation throughout the food webs, surface water, sediment, soil, aquatic organisms and terrestrial invertebrates were collected from Zaoshi Reservoir used as a reference site and five study sites along the streams where metal contaminants had been discharged from the abandoned mine. The findings of this research could provide important information for understanding the mechanisms of As bioaccumulation in As-contaminated environments and for assessing the potential environmental impacts of elevated As concentrations on mining activities at the realgar mine area.

2 Materials and methods

a. Total arsenic analysis

The total As content in samples was determined by ICP-MS (DRC-e, PerkinElmer, Waltham, USA.) following acid digestion. Initially, about 200-300 mg freeze-dried powders were directly weighed into a 25 ml

graduated vessels. After which 5ml of concentrated HNO₃ was slowly added to the samples, and then left overnight in order to digest gently covered with a perforated glass stopper. In the following morning, the vessel was carried out on a hot plate at 120°C-140°C for complete digestion until the solution became transparent and about 1 ml solution remained. After cooling, about 2ml H₂O₂ was added and heated for an hour, and then transferred to a 20-ml tube. The tube was brought to volume with ultra-pure water for analysis. To determine the As concentration in soil samples, a portion (0.3±0.02 g) was weighed and digested with 10 ml HNO₃-HClO₄ (v/v 9:1) at 180°C on an electric hot plate.

b. Extracted and determination of As species

The dried samples (100-300 mg) was weighed into a 50 ml polyethylene tube and extracted using a methanol-water mixture (15 ml, 1+1 v/v). The tubes were then placed on a shaker at 150 rpm under room temperature overnight. Then the extracted solution was sonicated in an ultrasonic bath for 30 min before centrifugation at 3000 rpm for 15 min. The supernatant was transferred into a 25 ml tubes while the residue was re-extracted with 5 ml of 1:1 methanol: water mixture following the former procedure. The mixture was maintained shaking for 1 hour, sonicated for 30min and centrifuged for 20min. The two combined extracts were mixed and evaporated using a pressured N₂ gas-blowing concentrator until less than 5 ml solution remained. Before analysis, the samples were filtered with a 0.45µm disposable syringe filters into HPLC vials and stored at 4°C.

Six As chemical species (As(III), As(V), DMA, MMA, AsB and AsC) were detected using the high-performance liquid chromatography inductively coupled plasma mass spectrometry (HPLC-ICP-MS) method. After optimization, the experimental condition were determined. Anion-exchange HPLC separation were performed on a PRP-X100 column (4.1mm i.d×250 mm length, 10µm diam. particles) from Hamilton (Reno, USA). The mobile phase consisted of 30 mM (NH₄)₂CO₃ at pH=9.3 (adjusted with aqueous ammonia), and the mobile phase flow rate was 1.2 ml/min and injection volume was 30 µl. The dynamic reaction cell (DRC) technology with O₂ as reaction gas was used for the element quantification of ⁹¹AsO⁺, as it can eliminate the extent of ArCl⁺ (*m/z* 75) formation on As⁺ (*m/z* 75). All calibration graphs and subsequent sample concentrations were carried out by using peak areas.

2. Results and discussion

Analysis of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) stables isotopes provides the numerical characterization of trophic level, and as such, we combined it with As to evaluate the biomagnification/biodilution within a freshwater ecosystem. The total As in surface soil, sediments and water were in the range of 35-5000 mg/kg, 43-4543 mg/kg and 5-3293 µg/l, respectively(Fig.1). The As concentrations were decreasing with the distance from the central mining sites, indicating the As mining activities

had produced eminent impact on As contamination in Shimen.

Throughout the study sites, the rank order of As in biota was tadpole>earthworms>crab>frog>loach>other fishes. The total As concentrations in soil, litter and earthworms showed a significant positive relationship. It is noted that the total As levels ranged from 1.13 mg/kg to 4.95 mg/kg in muscle and 5.19 mg/kg to 51.65 mg/kg in feathers (data not given). The As bioaccumulation followed the tissue-specific order of stomach contents>liver>feather>stomach>claw> muscle≈heart in the magpie sample. The arsenic concentration in aquatic organism from contaminated streams were in the range of 0-2450 mg/kg, while 0-7.5 mg/kg in reservoir(Fig 2, Fig 3).

The main As compounds identified by HPLC-ICP-MS were inorganic As (arsenite, As(III), and arsenate, As(V)), dimethylarsinic acid (DMA) and monomethylarsonic acid (MMA), while arsenobetaine (AsB) and arsenocholine (AsC) were mostly present as minor species. In our study, the proportions of DMA and MMA and the As(III) accounts for higher proportion in the muscle tissue compared to those in the water. To explain these variations in the As forms between the feathers and muscle, it can be hypothesized that methylation occurs in birds consisting of alternating steps of reduction (e.g., As(V)+2e⁻→As(III)) and oxidative addition of a methyl group (e.g., As(III)+CH₃⁺→MMA(V)) (Challenger, 1945).

The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ value in the same species of organisms varied greatly with sites, which suggest the impact of diet difference on the trophic levels of the same species in the food chain. The trophic levels for various species of organisms were determined as from 1.25 to 3.76 based on $\delta^{15}\text{N}$ values. In streams, As levels were much higher in lower trophic organisms, but were quite stable at higher trophic levels, indicating no bio-magnification of As in the food chain, however, the reservoir showed slight arsenic concentrations and obvious biomagnification through the food webs. It is noteworthy that MMA and DMA were the dominant form of methylated As in the higher trophic level in contaminated streams. The same trends results were reported in the team group previously, which indicating that the lower trophic levels of organisms have a greater ability to accumulate arsenic and higher trophic levels of organisms have a greater ability to methylate arsenic. While the opposite pattern was found in the organisms in reservoir. The reason and mechanisms need further researches.

3. Conclusion

Despite the arsenic concentration in various media, its mode of action in freshwater is poorly understood. Arsenic contamination from mines is obvious in freshwaters. There are a number of reports documented biodilution of As with increasing trophic levels in freshwater food webs which can be related to many factors. This study give a full picture of As characterization in both aquatic and terrestrial ecosystem in the As-contaminated area in China. However, a contrast pattern can be found in both total arsenic and its species. No conclusive evidence from the field study has been presented yet, thus further studies are needed.

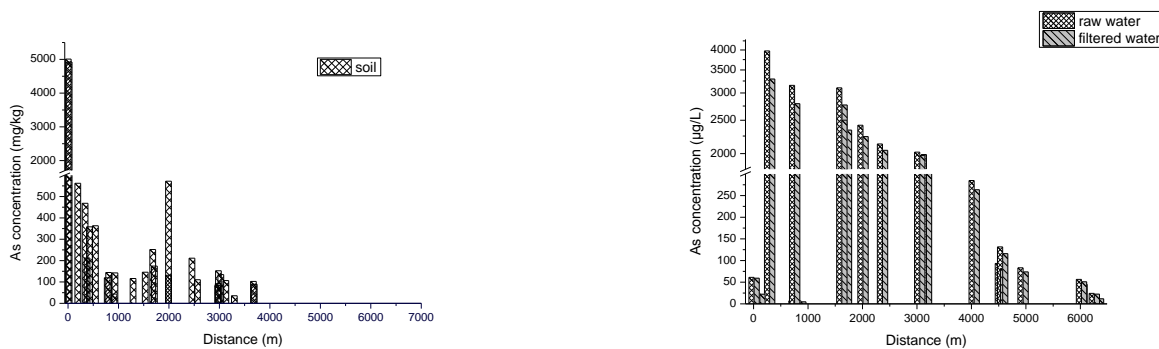


Figure 1. Total arsenic in soil (left) and water (right) collected from the realgar mine.

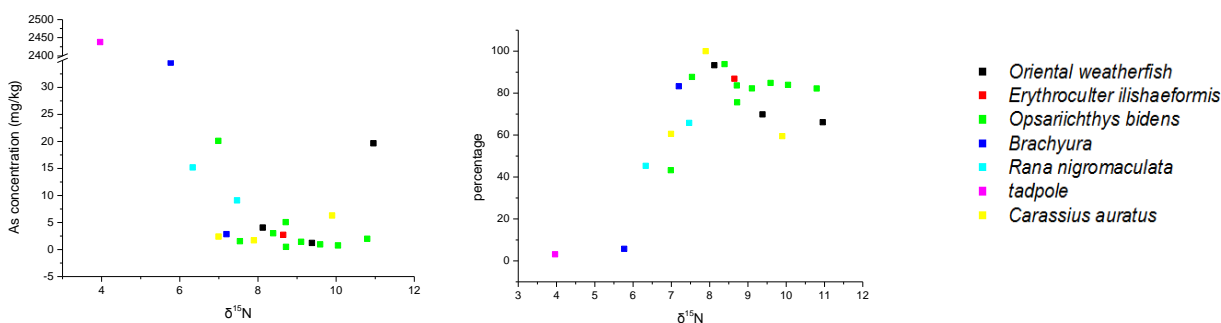


Figure 2. Total arsenic (left) and arsenic speciation (right) of aquatic organisms from the contaminated streams.

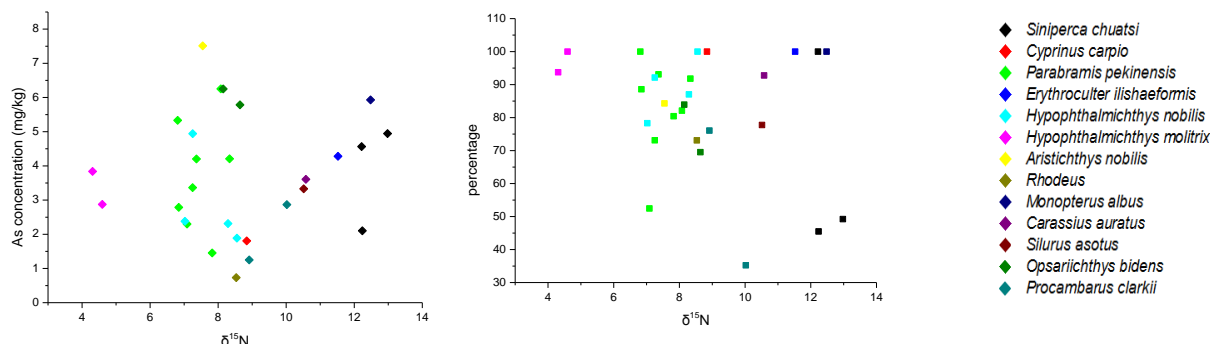


Figure 3. Total arsenic (left) and arsenic speciation (right) of aquatic organisms from the reservoir

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