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Review

Arsenic transfer and biotransformation in a fully characterized freshwater food web

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ABSTRACT

X-ray absorption near edge spectroscopy (XANES) was combined with ICP-MS to understand arsenic transfer and transformation within the freshwater Montezuma Well (central Arizona, USA) food web. Montezuma Well water contains 110 $\mu g\,L^{-1}$ arsenic (100% H_3AsO_4), which was shown previously to originate from ore deposits approximately 30 km to the west, and transported underground to enter Montezuma Well through vents in the bottom of this collapsed travertine spring. The Montezuma Well food web contains three trophic levels with only five organisms in the top two levels, making it possible to account for the arsenic in >90% of the biomass of the food web. Arsenic diminution generally occurs between trophic levels, with 702 $mg\,kg^{-1}$ As in the primary producers, 3.4 $mg\,kg^{-1}$ As in the second trophic level, and <1.3 $mg\,kg^{-1}$ As for the top-tier of the littoral zone food web. A notable exception to the biodiminution trend is the very high total arsenic content (2810 $mg\,kg^{-1}$) of *Motobdella montezuma*, an endemic leech and top predator. The biotransformed (sulfur-coordinated) arsenic in *M. montezuma* appears to be present almost entirely on the surface of the organism, possibly suggesting a detoxification mechanism. XANES sample spectra were fit by a linear combination of model arsenic compound spectra and indicated that arsenic enters the food web from the Well water entirely as inorganic As(V) and is transformed within the food web into more reduced and organic arsenic species, including sulfur-coordinated and methylated compounds.

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1. Introduction

Arsenic deserves a special place among the chemical elements because of its chemistry and its interesting history. Arsenic has been known as a substance since antiquity and was named "arsenikon" by the Greeks, their name for the yellow mineral orpiment, As_2S_3. Arsenic was identified as a chemical element by Albertus Magnus in the 1200s [1]. Arsenic is the 20th most abundant element of the Earth's crust and over 245 arsenic bearing minerals are known [2]. Arsenic trioxide is an important industrial chemical. The United States imported more than 20,000 tons of arsenic trioxide annually during 2001–03. This quantity dropped to 6100 tons when the use of chromated copper arsenate as a wood preservative was banned. High-purity arsenic (99.9999%) is used in the electronics industry to manufacture semi-conductors, solar cells, and specially coated optical materials [3].

Hippocrates (460–357 BC) used orpiment (As_2S_3) and realgar (As_2S_2) as a corrosive salve and to treat ulcers [4]. Pliny the Elder (23–79 AD) described the preparation of an arsenic liniment made with vinegar that was suggested as a remedy for asthma, cough, and several other ailments [1]. Modern medicinal uses of arsenic include using sodium cacodylate and sodium arsanilate to treat pellagra, malaria, and sleeping sickness [1,4,5]. In 1909, arsphenamine was proposed by Paul Ehrlich as a treatment for syphilis and trypanosomiais. This practice continued for nearly 40 years until penicillin became available [4]. Arsphenamine is considered the first chemotherapeutic agent [6].

The toxic properties of arsenic were reported by Aristotle in 340 BC when he wrote that realgar (As_2S_2) kills horses and cattle when put into water [1,7]. Arsenic's poisonous properties have been used to change the course of history. Nero is reported to have poisoned Britannicus in 55 AD to secure his Roman throne, and Pope Alexander IV, and his son, Cesare Borgia, are legendary for their use of arsenic solutions to kill enemies [4]. The Chinese are credited with using arsenic sulfides as an insecticide as early as 900 AD, and arsenic oxide was reportedly used in ant bait in Europe in 1699 [8].

The widespread global use of arsenical insecticides over the past 150 years represents one of society's major environmental catastrophes. Copper acetoarsenite, commonly known as Paris Green, was first used in 1867 on the Colorado potato beetle in the USA. Paris Green sprays were soon used by fruit growers to control the codling moth (*Cydia pomonella*), an insect pest capable of destroying up to 98% of a farmer's apple production [8,9]. Paris Green was also used internationally in mosquito abatement programs for which it was applied directly to water bodies as a powder. Lead arsenate was first used as an insecticide against the gypsy moth (*Lymantria dispar*) in 1892 in Massachusetts, USA. Lead arsenate proved to be more effective than Paris Green sprays for the gypsy moth because of its lower solubility in water and its tendency to adhere to the surfaces of plants. Lead arsenate became the insecticide of choice for many agricultural crops including apples, cherries, cotton and many vegetables [8–10]. Lead arsenate use increased steadily in the United States, peaking in 1944 at 86.4 million pounds annually. Sophisticated sprayers were designed to apply lead arsenate over large areas of farmland and orchards. Adding lead arsenate to irrigation water became a common practice in some locations [9].

The discovery of DDT's insecticidal properties in 1947 provided a convenient alternative to lead arsenate. Lead arsenate insecticides were quickly replaced by DDT, largely because the insect pests had built up resistance to lead arsenate and DDT was more effective at lower doses. All insecticidal uses of lead arsenate in the United States were officially banned in 1988 [8].

Unfortunately, hundreds of thousands of hectares of contaminated soil are the legacy that remains from a century of relying on arsenic-based insecticides. Elevated levels of arsenic and lead were discovered in apples as early as 1919 when experiments were begun to identify washing procedures to eliminate the toxic residues. Although lead remains in the top 5–20 cm of soil, the arsenic may be transported through runoff or through groundwater because of its higher solubility. In soluble forms, arsenic can be transported through the root system into plants or to wells where they can be ingested through drinking water. Transport via groundwater has resulted in millions of people living in the Bengal Delta Plain in Bangladesh and the West Bengal area of India to acquire arsenic poisoning from their drinking water, although in this case the underlying source of arsenic in the water is geologic, rather than human activity [11–13].

Rice accumulates arsenic at higher levels than other terrestrial plants. Indeed, dietary arsenic from rice grown in contaminated fields is known to be a contributing factor for arsenic poisoning in the Bengal Delta [14,15]. The American Chemical Society addressed this concern in a recent symposium on arsenic contamination in food and water [16], and the U.S. Department of Agriculture has a program to measure the arsenic content of rice grown in the United States [17].

It is essential to understand the environmental chemistry of arsenic in order to develop methods for remediating contaminated arsenic soil and water. The solubility of arsenic, and therefore its ability to be transported in an aqueous environment, is governed by the oxidation state of arsenic, the presence of other metals in the soil or aquifer material and their oxidation states [2,18–20]. The toxicity of arsenic also depends on the oxidation state and on the ligands that surround the arsenic atom, making the chemical form of the arsenic as important as the amount of arsenic when assessing environmental hazards [21]. Until recently, the literature on environmental arsenic has emphasized arsenic quantity present in environmental samples and has paid less attention to its chemical form.

1.1. Environmental transformations of arsenic

More than 50 organoarsenic compounds are found in nature [22,23]. Table 1 lists the most common arsenic substances found in aquatic environments. Inorganic arsenic species predominate in aqueous environments, and depending upon the redox properties of the system, the arsenic may be present in the +5 oxidation state as arsenic acid (H_3AsO_4) or in the +3 oxidation state as arsenous acid (H_3AsO_3) [20,21]. Most arsenic compounds isolated from biological organisms are organic species that are produced through metabolic processes in phytoplankton, bacteria or in the organs of vertebrates [21,24,25]. Two recent review articles document arsenic concentrations in an extensive list of organisms and the species of arsenic that have been identified in these organisms

Table 1
Chemical forms of arsenic substances found in aquatic environments, their formulas and abbreviations [22,23].

	Name	Formula	Abbreviation
1	Arsenous acid or arsenite	H_3AsO_3	As(III)
2	Arsenic acid or arsenate	H_3AsO_4	As(V)
3	Monomethylarsonous acid	$CH_3As(OH)_2$	MMA(III)
4	Monomethylarsonic acid	$CH_3AsO(OH)_2$	MMA(V)
5	Dimethylarsinous acid	$(CH_3)_2As(OH)$	DMA(III)
6	Dimethylarsinic acid	$(CH_3)_2AsO(OH)$	DMA(V)
7	Trimethylarsine	$(CH_3)_3As$	TMA
8	Trimethylarsine oxide	$(CH_3)_3AsO$	TMAO
9	Arsenocholine	$(CH_3)_3As^+CH_2CH_2OH$	AsC
10	Arsenobetaine	$(CH_3)_3As^+CH_2CO_2^-$	AsB
11	Arsenosugars	$(CH_3)_2AsOCH_2C_4H_4O–R$[a]	AsS
12	Arsenic glutathione complex	$(GS)_3As(III)$[b]	$(GS)_3As(III)$

[a] R can be an alcohol, phosphate, sulfate, sulfite, carboxylic acid or a heterocycle.
[b] GS represents the glutathione anion.

[21,26]. The transformation of inorganic arsenic to organoarsenic substances proceeds via a series of biomethylation steps involving S-adenosylmethionine, following a pathway first proposed by Frederick Challenger [21,27–29].

Mammals metabolize inorganic arsenic via methylation in the liver to methylarsonic acid, MMA(V), and dimethylarsinic acid, DMA(V) [30]. DMA(V) and MMA(V), the final arsenic metabolic products in mammals, are excreted from the body through urine. Methylation of arsenic was long considered a detoxification process for this reason [22]. However, reduced forms of the methylated metabolites, MMA(III) and DMA(III), are highly toxic and have been observed in urine [22,30].

Freshwater phytoplankton convert inorganic arsenic into the less toxic DMA(V), which is then excreted from the organism [31]. Biotransformation of inorganic arsenic to organoarsenicals is therefore, considered the main detoxification/defense mechanism of phytoplankton [26]. substances, but they are not typically found in the aqueous environment. Arsine and methylated arsines are produced by fungi in reducing environments [32].

1.2. Chemistry of arsenic in food webs

Giddings and Eddlemon were the first to demonstrate the movement of arsenic between trophic levels [33]. Using arsenic-74, a radioactive isotope, to follow arsenic concentrations, bioaccumulation factors of 965, 192 and 11 were reported for the first (phytoplankton), second (zooplankton), and third (snail) trophic levels, respectively. Lindsay and Sanders [34] similarly observed significant arsenic uptake by phytoplankton in an estuarine system, but only a slight increase in arsenic concentrations in the grass shrimp that feed on the phytoplankton. Further examining the seasonality of arsenic uptake, Chen and Folt [35] found that arsenic concentrations in the freshwater lake phytoplankton peaked during the summer at 25 mg kg^{-1}. Arsenic concentrations diminished with each successive level in the four-step food chain, with arsenic concentrations in fish of <1 mg kg^{-1}.

Maeda et al. improved our understanding of arsenic metabolism in freshwater food chains by adding arsenic speciation information. In a study of a three-trophic level system (an autotroph (alga, *Chlorella* sp.), a grazer (zooplankton, *Moina* sp.) and a carnivore (goldfish, *Carassius* sp.) [36], water arsenic levels of 30 and 100 mg L^{-1} resulted in alga arsenic concentrations of 745 and 2850 mg kg^{-1}, respectively. More than 98% of the algae arsenic was found to be non-methylated inorganic. While the relative concentration of methylated arsenic *increased* successively moving up the three-step freshwater food chain, the total arsenic accumulation *decreased* by an order of magnitude with each trophic level. The authors also demonstrated that the bioaccumulated arsenic came from both water and food in the two higher trophic levels by raising the zooplankton and carnivores in water that contained different concentrations of arsenic. The trends of biodiminution and increased relative abundance of methylated arsenic species with trophic level was observed in another three-step food chain consisting of green alga (*Chlorella vulgaris*), shrimp (*Neocaridina denticulate*) and killifish (*Oryzias latipes*) [37]. In fact, methylated arsenic compounds became the dominant form of arsenic in the second and third trophic levels.

Arsenic transfer between phytoplankton and zooplankton and changes in speciation in three Canadian lakes was studied by Caumette et al. [38]. This study is noteworthy because of the highly variable arsenic concentrations in each lake (7–250 μg L^{-1}), due to historic mining activities in the area. Phytoplankton arsenic levels ranged from a low of 154 mg kg^{-1} in Grace Lake to a high of 894 mg kg^{-1} in Kam Lake, proportional to arsenic concentrations in the water. Similarly, zooplankton arsenic levels varied in proportion to water and phytoplankton concentrations, from 7 mg kg^{-1} in Grace Lake to 340 mg kg^{-1} in Kam Lake. Only inorganic and predominantly oxidized forms of arsenic were observed within the phytoplankton. Zooplankton contained smaller percentages of inorganic arsenic, ranging from 38% in Grace Lake to 98% in Kam Lake. DMA(V) and MMA(V) were observed in zooplankton at levels ranging from 0.1 to 2%. Arsenosugars were observed in zooplankton samples from all three lakes, but Grace Lake, with the lowest ambient arsenic concentration, contained a greater variety and higher relative abundance of arsenosugars. Arsenobetaine was observed in zooplankton from Grace Lake, but was absent in samples from Kam Lake and Long Lake.

Table 2
Chemical characteristics of Montezuma Well water (concentrations in mg L^{-1}, except water As concentration, which is expressed in μg L^{-1} and sediment As concentration, which is expressed as mg kg^{-1} dry wt) [39,41].

Ca^{2+}	117.0 ± 4.5
Mg^{2+}	37.4 ± 2.2
Na^{+}	50.6 ± 0.2
K^{+}	5.10 ± 0.02
SO_4^{2-}	10.8 ± 0.8
Cl^{-}	44.6 ± 3.4
HCO_3^{-}	595.0 ± 5.0
SiO_2	21.9 ± 3.0
As (water)	110
As (sediment)	427
CO_2	500
O_2 (daytime)	>12
O_2 (nighttime)	<4
pH	6.5 ± 0.02

1.3. Montezuma Well ecosystem

Montezuma Well, a collapsed travertine spring, is located in central Arizona, USA, in an upper Sonoran Desert grassland (34°39′ N, 111°45′ W). Water enters the Well through four large vents in the floor of the spring (4150–7300 m^3 day^{-1}) and exits at the southeast corner, where it passes through a limestone cave system to eventually merge with Wet Beaver Creek [39]. The feed water is supersaturated with carbon dioxide and outgassing occurs at the bottom of the Well, resulting in dissolved CO_2 levels exceeding 500 mg L^{-1}. The feed water contains dissolved arsenic transported underground over a distance of 30 km, resulting in an arsenic concentration of 110 μg L^{-1} [40]. The source of arsenic in Montezuma well is ore deposits located near Jerome, AZ on the west side of the Verde Valley [41]. The water surface of the Well is circular with a diameter of 112 m, and the maximum depth, at the center of the pool, is 17 m. Water chemistry of Montezuma Well is summarized in Table 2.

Exceedingly high levels of CO_2, abundant sunshine, and unusually constant water temperature year round within Montezuma Well result in some of the highest primary productivity numbers reported [42]. On an annual basis, 1698 g-carbon (dry weight) is produced by photosynthetic activity for each m^2 of Montezuma Well [43]. The unusual water chemistry and the isolated location have resulted in an ecosystem with a high proportion of endemic species. The dominant organisms in the Montezuma Well food web are listed in Table 3.

A striking feature of the food web is the small number of organisms that are involved. Sixty-five percent of the phytoplankton community consists of only two species (*Coccomyxa minor* and *Nannochloris bacillaris*) and only five species (*Ankistrodesmus* sp., *Chlorella* sp., *Monoraphidium* sp., *Nephrocytium* sp. and *Scenedesmus* sp.) make up the next 12% of the phytoplankton assemblage [41]. The zooplankton community is dominated by *Hyalella montezuma*, an endemic freshwater amphipod. *H. montezuma* is so critical to the viability of the Well's food web that it is labeled a keystone organism. The highest trophic level consists of only four insects

Table 3
Dominant organisms of the Montezuma Well ecosystem [41].

Primary producers			
Phytoplankton	*Coccomyxa minor*	Cyanobacteria	*Chroococcus*
Phytoplankton	*Nannochloris bacillaris*	Cyanobacteria	*Gloeocapsa*
Phytoplankton	*Ankistrodesmus* sp.	Cyanobacteria	*Merismopedia*
Phytoplankton	*Chlorella* sp.	Cyanobacteria	*Oscillatoria*
Phytoplankton	*Monoraphidium* sp.	Diatoms	*Achnanthidium*
Phytoplankton	*Nephrocytium* sp.	Diatoms	*Cocconeis*
Phytoplankton	*Scenedesmus* sp.	Diatoms	*Gomphonema*
Zooplankton			
Amphipod	*Hyalella montezuma*	Amphipod	*Hyalella azteca*
Copepod	*Tropocyclops prasinus mexicanus*		
Insects			
Water scorpion	*Ranatra montezuma*	Damsel fly	*Telebasis salva*
Giant water bug	*Belostoma bakeri*	Dytiscid beetle	*Cybister ellipticus*
Other invertebrates			
Leech	*Motobdella montezuma*		

in the littoral zone (*Ranatra montezuma*, *Belostoma bakeri*, *Telebasis salva* and *Cybister ellipticus*) and an endemic leech (*Motobdella montezuma*) in the limnetic zone. The four insects listed constitute over 80% of the insect biomass at Montezuma Well. Half of the organisms in the Well's food web are endemic [43].

Freshwater amphipods commonly feed on dead organic matter and associated microbes. *H. montezuma* is unique among North American amphipods, being the sole planktonic filter feeder. *H. montezuma* swims freely in the water column, feeding on phytoplankton by filtering an average of 15.6 mL of water a day. The four insects and the endemic leech, *M. montezuma*, feed exclusively on *H. montezuma* [43]. These feeding behaviors make it possible to study the movement of arsenic through the entire food web.

The leech, *M. montezuma*, feeds in the open water column of Montezuma Well at night where it senses its prey, *H. montezuma*, from the waves created by swimming. During the daytime, *M. montezuma* retreats to the sediments at the bottom of the well to rest and wait for darkness. The insects that feed on *H. montezuma* live in the water plants surrounding the edge of the Well and require daylight to see their prey. To avoid predation, *H. montezuma* migrates between the open water column during the day and the water plants at the edge of the Well at night [43]. This behavior is illustrated in Fig. 1, which shows the dynamics of the Montezuma Well food web.

2. Sample collection and total arsenic analysis

Samples were collected from Montezuma Well in May and September during the peak productivity season. The following samples were collected for analysis: *H. montezuma, Hyalella azteca*, *R. montezuma*, *B. bakeri*, *T. salva*, *M. montezuma*, neuston (a phytoplankton assemblage) and sediment. *B. bakeri*, *T. salva*, *R. montezuma* and *M. montezuma* were collected by hand, frozen in liquid nitrogen at the site and transported to the laboratory in individual centrifuge tubes. *H. montezuma* and *H. azteca* were collected in the water column with a 254-μm pore-size plankton tow net. *H. montezuma* was sampled in the open water column, and *H. azteca* was collected along the shoreline. *M. montezuma* was collected at dusk in the open water column using a hand-operated dip net. Neuston was collected by skimming the water surface with a plankton hand net and manually removing the material collected with a stainless steel spatula and placing it into centrifuge tubes. Sediment samples were collected with a plastic scoop, placed in Ziplock bags, sealed and placed on ice for transport to the laboratory. All samples were stored at −80 °C until analyzed.

Total arsenic in samples was determined by ICP-MS after digestion in a Milestone Ethos closed-vessel, temperature-controlled microwave digestion system. Approximately 0.5 g of sample was weighed into Teflon digestion vessels, into which 9.0 mL of HNO_3 and 3.0 mL of H_2O_2 were added. Each digestion set of 12 samples contained 2–4 SRMs, 6–8 samples and two digestion blanks (reagents only). Sample vessels were heated up to 180 °C over 5.5 min and held at 180 °C for 11 min at 600 W, allowed to cool in a water bath, and diluted to 50.0 mL with distilled deionized water. Trace-metal grade nitric acid was used for all digests and dilutions.

A Perkin-Elmer Elan 500 ICP-MS with a glass nebulizer and water-cooled spray chamber was used to determine total arsenic in sediment, soil, and organisms. Sample was delivered to the nebulizer by a peristaltic pump at a flow rate of 1.5 mL min^{-1}. Gallium was used as an internal standard and mass-to-charge (m/z) ratios were measured by peak hopping at 75 and 69. Calibration standards, ranging from 0 to 1000 μg L^{-1}, were prepared in 2% nitric acid by serial dilution of Spex plasma standards. A calibration blank and a calibration standard were run with every 10 samples. A lab duplicate and a spike of the samples were also run with each 10 samples. The calculated detection limit was 0.5 μg L^{-1}, based on three times the standard deviation of the blanks. Table 4 summarizes the reference materials used and the percent recoveries obtained from these procedures.

2.1. XANES data collection and analysis

X-ray absorption near-edge spectroscopy (XANES) is a sensitive tool for probing the local atomic and electronic environment of an element, such as arsenic in a complex environment. It probes all forms of arsenic in all phases (solid, aqueous, etc.) and can, with careful analysis, be used to quantify the different forms of arsenic present in a particular sample [44,45]. Arsenic K-edge X-ray absorption spectroscopy was carried out on beamline 7–3 of the Stanford Synchrotron Radiation Laboratory using a Si(2 2 0)

Table 4
Standard reference materials used for arsenic analysis.

Standard reference material	Sample matrix represented	Certified arsenic value (mg kg^{-1})	Percent recovery
Estuarine sediment, NIST 1646	Sediment	11.6 ± 1.3	94 ± 6 (n = 6)
Montana soil, NIST 2711	Soil	105 ± 8	113 ± 4 (n = 4)
Dogfish tissue, DORM-2 NCR	Invertebrate	18.0 ± 1.1	99 ± 2 (n = 6)

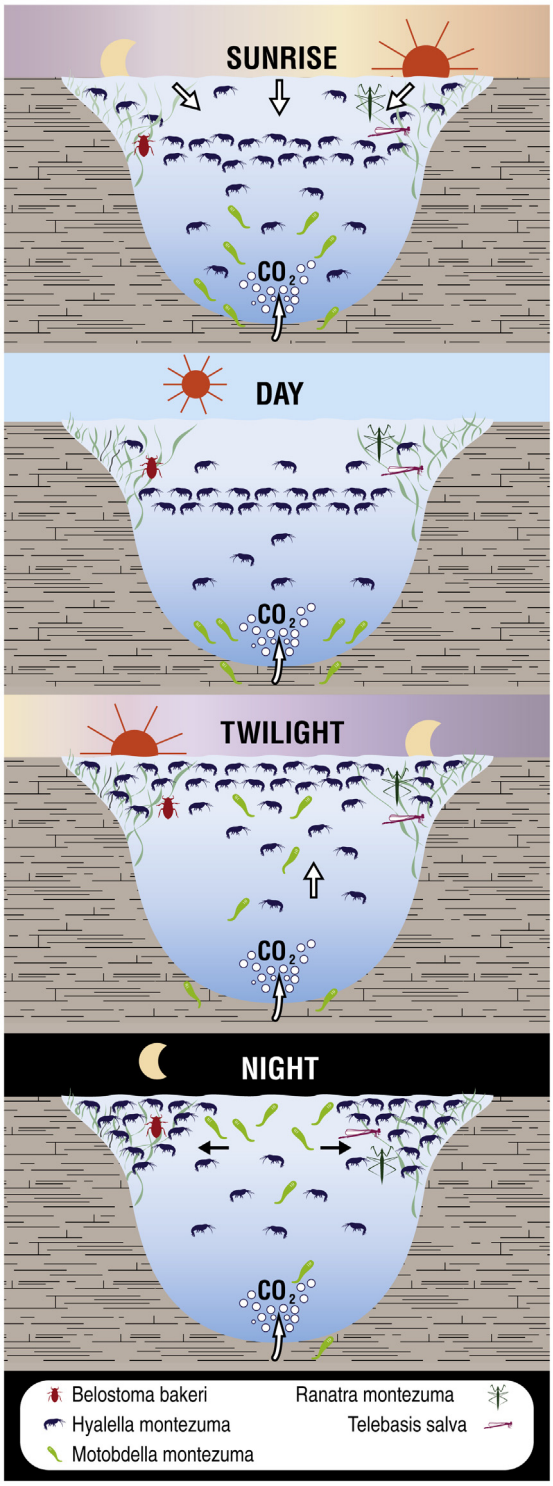

Fig. 1. Dynamics of the Montezuma Well food web. The endemic fresh water amphipod *Hyalella montezuma* feeds on phytoplankton floating freely in the main water column of Montezuma Well. *H. montezuma* is the exclusive food source for the endemic leech, *Motobdella montezuma* and four insects, *Cybister ellipticus* (not shown), *Belostoma bakeri*, *Ranatra montezuma* and *Telebasis salva*. The leech feeds in the open water column at night, and the insects feed during the day in the weed beds along the shore. This feeding behavior leads to a daily migration for the amphipod, which spends nights in the weeds along the shore and days in the open water column in the center of the well [43].

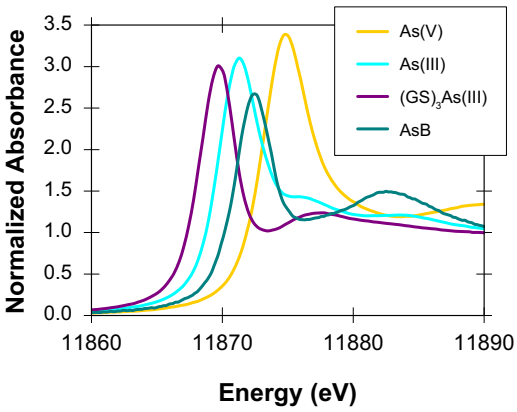

Fig. 2. As K-edge X-ray absorption spectra of model arsenic species.

double crystal monochromator, an upstream vertical aperture of 1 mm, and no focusing optics. Incident and transmitted intensities were measured using N_2-filled ion chambers, and the X-ray absorption spectrum of the sample was recorded by monitoring As Kα fluorescence using a Canberra 30-element germanium detector. During data collection, samples were maintained at approximately 10 K in a flowing liquid helium cryostat. A spectrum of elemental arsenic was collected simultaneously with that of each sample in order to calibrate the energy scale; the first energy inflection of the As(0) was taken as 11,867.0 eV. Data collection was carried out using the program XAS_COLLECT [46], and data reduction was carried out using the EXAFSPAK suite of programs (http://ssrl.slac.stanford.edu/exafspak.html) according to standard methods.

Near-edge spectra were fit to a linear combination of spectra from arsenic model compounds in a least squares procedure using the program DATFIT in EXAFSPAK. All spectra are normalized, and hence, the fractional contribution of the arsenic model spectrum to the fit is quantitatively equivalent to the fraction of arsenic present as that type of species in the sample under scrutiny. Analytical standards were purchased from Sigma–Aldrich and were of the highest quality available. Model compounds for fitting were selected to represent the types of arsenic species expected in a freshwater environment and included the following: aqueous solutions of arsenate (pH 9) and arsenite (pH 5.5), representing inorganic As(V) and As(III); and As(III)-tris-glutathione [$(GS)_3$As(III)] and arsenobetaine, representing organic compounds with arsenic coordinated to sulfur and methyl groups, respectively. Solutions of arsenic model compounds had a concentration of between 5.0 and 7.5 mM after the addition of 30% glycerol as a glassing agent. Arsenic (III)-tris-glutathione complex was made by adding a 10-fold excess of glutathione to a solution of sodium arsenite to give a pH 5.5 solution [44,47]. Dimethyl arsenate, DMA(V), commonly known as cacodylate) was also tested, but gave inferior fits to those using only arsenobetaine. Aqueous solutions were pipetted into 2-mm path-length Lucite sample holders and frozen in liquid nitrogen. The model arsenic spectra should be considered to be representative of a type of environment, rather than a specific molecule. For example, the determination of a fraction of $(GS)_3$As(III) indicates As(III) coordinated by sulfur, which may or may not be from glutathione. Component contributions were set to zero in the final fit if they contributed <1% of the fit.

3. Results and discussion

Fig. 2 shows the near-edge spectra of select arsenic models, demonstrating the sensitivity of the As K-edge not only to oxidation

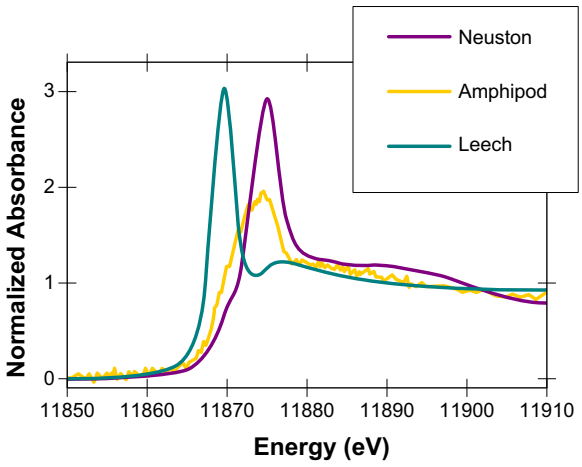

Fig. 3. As K-edge X-ray absorption spectra of food web organisms.

state but also to chemical environment. The pronounced peak on top of each of the edges is due to dipole-allowed 1s → 4p transitions.

XANES spectra for three food web components, neuston (a phytoplankton assemblage), *H. montezuma* and *M. montezuma* have strikingly diverse spectra (Fig. 3). Sample spectra were fit by a linear combination of model spectra to determine the % abundance of each type of arsenic species in the sample. The precision of the model compound contributions, expressed as three times the estimated standard deviation, was <5% for all models and samples. Overall model fit residuals, expressed as $100 \times \Sigma(I_o - I_c)^2/N$, where I_o and I_c are the observed and calculated intensities, respectively, and the summation is over the number of data points, N, were <0.136, except for the insect samples, which ranged from 0.298 to 1.70.

The phytoplankton assemblage (neuston), at the bottom of the food web, shows the most oxidized spectrum (80%). Although the phytoplankton spectrum is fit well by the aqueous arsenate model in the near-edge region, the spectra do not correspond well in the post-edge region (11880–11900 eV) [45,48,49]. Variations in this part of the spectrum are indicative of changes in longer-range order [50], and the arsenate in these samples may be adsorbed to a mineral surface or coordinated in some other way.

The amphipod spectra differed from the phytoplankton in showing a significant (~1/3) contribution of methylated arsenic, with the remainder being predominantly inorganic As(V) and sulfur-coordinated As(III). As might be expected, the two amphipod species analyzed (*H. montezuma* and *H. azteca*) showed arsenic distributions that were substantially similar (data not shown). The insect samples had very low total arsenic levels and consequently, XANES spectra for these samples suffered from noisier spectra and higher fit residuals. Although the lower quality data limit the reliability of the exact numerical abundances of a given arsenic model within these samples, it still allows a general assessment of the relative presence reduced and oxidized forms. The lower trophic *R. montezuma* contains a greater proportion of oxidized arsenic (~65%) than the higher trophic *B. bakeri* (~26%) and *T. salva* (~41%). Finally, the top predator, the *M. montezuma* leech, contains arsenic solely in the reduced and sulfur-coordinated form.

Total arsenic concentration data for food web organisms [51] are shown with the speciation data in Fig. 4, which illustrates the transfer and transformation of arsenic through the Montezuma Well food web. Based on the trophic structure and productivity model developed by Blinn and co-workers [42,52–54], we have identified the quantity and the speciation type of arsenic for >90% of the biomass in the Montezuma Well food web. This is possible because Montezuma Well is a simple closed system *and* the feeding characteristics of the endemic species are well known.

Total arsenic content in Montezuma Well organisms generally decreases between successive trophic levels, a pattern that is consistent with the results from previous studies [35,38]. The phytoplankton assemblage had a total arsenic concentration of 702 mg kg^{-1}, and the total arsenic content for the second trophic level, the freshwater amphipod *H. montezuma*, is 3.4 mg kg^{-1}, a 200-fold decrease. The littoral zone insects, *R. montezuma*, *B. bakeri* and *T. salva* have arsenic levels ranging from 1.3 to <0.5 mg kg^{-1}, representing approximately an order of magnitude decrease between the second and third trophic levels.

The extremely high total arsenic content of *M. montezuma*, the endemic leech provides a notable exception to the overall diminution trend. The measured 2810 mg kg^{-1} is, to our knowledge, the highest arsenic level reported in any organism. Earthworms collected from contaminated mining sites [55–57] are reported to have the highest arsenic levels for living organisms, with one specimen, *Lumbricus castaneous*, from an abandoned gold mine in Nova Scotia, Canada reported to have an arsenic body burden of 2200 mg kg^{-1} [56].

The leech feed exclusively on the amphipod, *H. montezuma*. Most leech specimens we collected contained noticeable bulges, which upon further examination yielded carcasses of recently consumed amphipods. The only other arsenic sources for the leech would be the water (110 μg L^{-1}, 100% H_3AsO_4) or the sediments where the leech resides at night (427 mg L^{-1}). XANES spectra show 100% of the leech arsenic is sulfur-coordinated. There is no evidence for arsenate or non-sulfur containing organoarsenic complexes in the XANES spectrum. *M. montezuma* uses enzymes to digest the internal fluids of *H. montezuma* and excrete the empty carcasses [43] and then appears to store dietary arsenic as a glutathione-like (i.e., sulfur-coordinated) complex. In a separate experiment, two leech specimens were washed with a 0.1 M sodium phosphate solution and re-analyzed for total arsenic. Total arsenic in the washed samples dropped to 45.5 mg kg^{-1}, suggesting that most of the arsenic–sulfur complex was on the surface of the organism and not located within the tissue. This observation suggests that the leech may be transforming arsenic from its diet to a sulfur-coordinated complex and excreting it to the membrane on its surface (which is visible by microscope). This may indicate that the biotransformed As is stored in this manner as a detoxification mechanism, a possibility that will be examined in future work.

The only arsenic source for Montezuma Well is the feed water that enters from vents at the bottom of the water column, and it was shown previously that the only form of arsenic in the water is arsenate, H_3AsO_4 [40]. Many studies have shown that phytoplankton ingests arsenic from water, consistent with our data [33–36,38,58]. Other studies report the form of arsenic in phytoplankton is predominantly a mixture of arsenate with a small percentage as arsenite. MMA(V), DMA(V) and arsenobetaine have been reported in phytoplankton, but always at much smaller percentages than inorganic arsenic [59]. The Montezuma Well phytoplankton assemblage shows a similar pattern.

The endemic amphipod, *H. montezuma* contains less arsenic than the phytoplankton upon which it feeds, 702 mg kg^{-1} As for the phytoplankton assemblage compared to 3.4 mg kg^{-1} As for the amphipod. The percentage of inorganic arsenic is significantly reduced between trophic levels as well from 86 to 43%. Three insects living in the aquatic plants around the edge of the well, the littoral zone, constitute the third trophic level, in addition to the leech mentioned previously, and they feed exclusively on this endemic amphipod. Total arsenic levels show approximately an order of magnitude decrease from the second trophic level, and the arsenic speciation varied considerably within these three organisms. The fact that dietary arsenic from the same food source leads to different speciation between these three insect species suggests that ingested arsenic is metabolized differently, i.e.

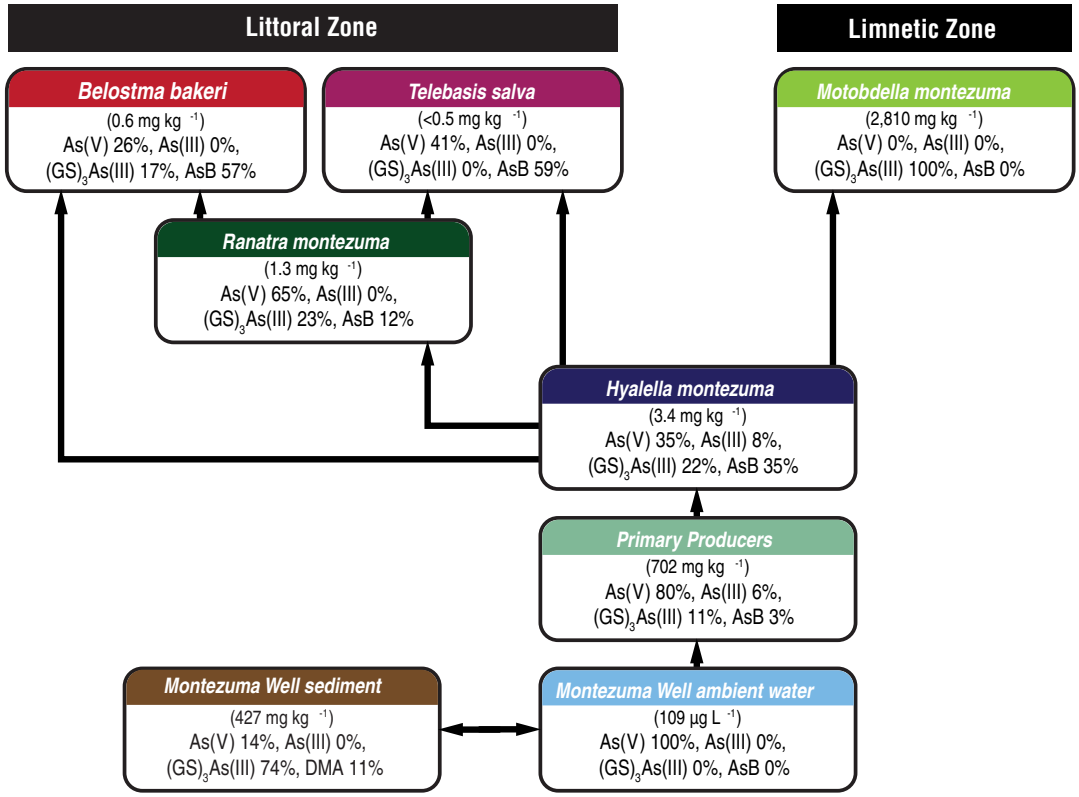

Fig. 4. Total arsenic concentrations determined by ICP-MS and relative abundance of arsenic species types (expressed as model compounds) determined by XANES for the organisms in the Montezuma Well food web. This figure is adapted from a model for the trophic structure, production, and energy flow in the littoral and limnetic zones in Montezuma Well [42,53,54]. Because of the high XANES fit residuals for the insects, speciation abundances are for informational purposes only. Total arsenic concentrations are reported as dry weight.

arsenic speciation is species-specific [60]. It is likely that the As(V) observed for the three insects was arsenate adsorbed to the surface of their exoskeleton which could have been picked up from the water in which they live.

4. Conclusions

We have shown for the first time how arsenic is distributed in >90% of the biomass of a freshwater food web. Our data show a general biodiminution of arsenic in a freshwater food web, even as the top predator, *M. montezuma*, has a higher arsenic content than any previously reported organism. The biotransformed (sulfur-coordinated) arsenic in *M. montezuma* appears to be present almost entirely on the surface of the organism, however, suggesting a detoxification process. Our insect data illustrate that the transformations of arsenic in the Montezuma Well ecosystem appear to be species-specific.

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References

[1] J.M. Azcue, J.O. Nriagu, in: J.O. Nriagu (Ed.), Arsenic in the Environment Part I: Cycling and Characterization, John Wiley & Sons, Inc., New York, USA, 1994, pp. 1–16.
[2] B.K. Mandal, K.T. Suzuki, Talanta 58 (2002) 201–235.
[3] U.S. Geological Survey, Mineral Commodity Summaries 2013, U.S. Geological Survey, Reston, Virginia, 2013.
[4] M.S. Gorby, in: J.O. Nriagu (Ed.), Arsenic in the Environment, Part II: Human Health and Ecosystem Affects, John Wiley & Sons, Inc., New York, 1994, pp. 1–16.
[5] M.F. Hughes, B.D. Beck, Y. Chen, A.S. Lewis, D.J. Thomas, Toxicol. Sci. 123 (2011) 305–352.
[6] K.J. Williams, J. R. Soc. Med. 102 (2009) 343–349.
[7] J.O. Nriagu, in: W.T. Frankenberger Jr. (Ed.), Environmental Chemistry of Arsenic, Marcel Dekker, New York, 2002.
[8] F.J. Peryea, 16th World Congress of Soil Science, Montpellier, France, 1998.
[9] T. Schooley, M.J. Weaver, D. Mullins, M. Eick, J. Pestic. Saf. Educ. 10 (2008) 22–32.
[10] J.O. Nriagu, J.M. Azcue, in: J.O Nriagu, M.S. Simmons (Eds.), Food Contamination from Environmental Sources, John Wiley & Sons, Inc., New York, USA, 1990, pp. 121–144.
[11] A. Chatterjee, D. Das, B.K. Mandal, T.R. Chowdhury, G. Samanta, D. Chakraborti, Analyst 120 (1995) 643–650.
[12] T.R. Chowdhury, G.K. Basu, B.K. Mandal, B.K. Biswas, G. Samanta, U.K. Chowdhury, C.R. Chanda, D. Lodh, S.L. Roy, K.C. Saha, S. Kabir, Q. Quamruzzaman, D. Chakraborti, Nature 401 (1999) 545–547.

[13] A.B. Mukherjee, P. Bhattacharya, Environ. Rev. 9 (2001) 189–220.
[14] E. Lombi, K.G. Scheckel, J. Pallon, A.M. Carey, Y.G. Zhu, A.A. Meharg, New Phytol. 184 (2009) 193–201.
[15] P.N. Williams, A.H. Price, A. Raab, S.A. Hossain, J. Feldmann, A.A. Meharg, Environ. Sci. Technol. 39 (2005) 5531–5540.
[16] B. Burton-Freeman, L. Jackson, J. Johnston, J. Maclachlan, W.R. Mindak, 245th ACS National Meeting, American Chemical Society, New Orleans, LA, 2013.
[17] D.T. Heitkemper, K.M. Kubachka, P.R. Halpin, M.N. Allen, N.V. Shockey, Food Addit. Contam. B: Surveill. 2 (2009) 112–120.
[18] J.G. Hering, P.E. Kneebone, in: W.T. Frankenberger Jr. (Ed.), Environmental Chemistry of Arsenic, Marcel Dekker, New York, 2002, pp. 155–161.
[19] J. Matschullat, Sci. Total Environ. 249 (2000) 297–312.
[20] P.L. Smedley, D.G. Kinniburgh, Appl. Geochem. 17 (2002) 517–568.
[21] K.J. Reimer, I. Koch, W.R. Cullen, in: A. Sigel, H. Sigel, R.K.O. Sigel (Eds.), Organometallics in Environment and Toxicology: Metal Ions in Life Sciences, Royal Society of Chemistry, 2010, pp. 165–229.
[22] E. Dopp, A.D. Kligerman, R.A. Diaz-Bone, in: A. Sigel, H. Sigel, R.K.O. Sigel (Eds.), Organometallics in Environment and Toxicology: Metal Ions in Life Sciences, Royal Society of Chemistry, 2010, pp. 231–265.
[23] K.A. Francesconi, D. Kuehnelt, in: W.T. Frankenberger Jr. (Ed.), Environmental Chemistry of Arsenic, Marcel Dekker, New York, 2002, pp. 51–94.
[24] B.M. Adair, S.B. Waters, V. Devesa, Z. Drobna, M. Styblo, D.J. Thomas, Environ. Chem. 2 (2005) 161–166.
[25] R.S. Oremland, J.F. Stolz, Science 300 (2003) 939–944.
[26] M.A. Rahman, H. Hasegawa, R.P. Lim, Environ. Res. 116 (2012) 118–135.
[27] R. Bentley, T.G. Chasteen, Microbiol. Mol. Biol. Rev. 66 (2002) 250–271.
[28] F. Challenger, Chem. Rev. 36 (1945) 315–361.
[29] F. Challenger, Adv. Enzymol. Relat. Areas Mol. Biol. 12 (1951) 429–491.
[30] M. Vahter, Toxicology 181–182 (2002) 211–217.
[31] H. Hasegawa, Y. Sohrin, K. Seki, M. Sato, K. Norisuye, K. Naito, K. Naito, M. Matsui, Chemosphere 43 (2001) 265–272.
[32] W.T. Frankenberger Jr., M. Arshad, in: W.T. Frankenberger Jr. (Ed.), Environmental Chemistry of Arsenic, Marcel Dekker, New York, 2002, pp. 363–381.
[33] J.M. Giddings, G.K. Eddlemon, Arch. Environ. Contam. Toxicol. 6 (1977) 491–505.
[34] D.M. Lindsay, J.G. Sanders, Environ. Toxicol. Chem. 9 (1990) 391–395.
[35] C.Y. Chen, C.L. Folt, Environ. Sci. Technol. 34 (2000) 3878–3884.
[36] S. Maeda, R. Inoue, T. Kozono, T. Tokuda, A. Ohki, T. Takeshita, Chemosphere 20 (1990) 101–108.
[37] T. Kuroiwa, A. Ohki, K. Naka, S. Maeda, Appl. Organomet. Chem. 8 (1994) 325–333.
[38] G. Caumette, I. Koch, E. Estrada, K.J. Reimer, Environ. Sci. Technol. 45 (2011) 9917–9923.
[39] A.D. Konieczki, S.A. Keake, Hydrogeology and Water Chemistry of Montezuma Well and Montezuma Castle National Monument and Surrounding Area, Arizona, U.S. Department of the Interior, Tucson, AZ, 1997.
[40] R.D. Foust Jr., P. Mohapatra, A.M. Compton-O'Brien, J. Reifel, Appl. Geochem. 19 (2004) 251–255.
[41] R.D. Foust Jr., M. Brandstrom, G. Evans, P. Nez, D. Waupoose, E. Hillman, Arsenic in Soil and Groundwater Environment, Elsevier Ltd., 2007, pp. 85–100.
[42] C. Runck, D.W. Blinn, Limnol. Oceanogr. 39 (1994) 1800–1812.
[43] D.W. Blinn, in: L.E Stevens, V.J. Meretsky (Eds.), Aridlands Springs in North America: Ecology and Conservation, University of Arizona Press, Tucson, 2008, pp. 98–126.
[44] G.N. George, R.C. Prince, S.P. Singh, I.J. Pickering, Mol. Nutr. Food Res. 53 (2009) 552–557.
[45] I.J. Pickering, G.N. George, AIP Conf. Proc. 882 (2007) 311–315.
[46] M.J. George, J. Synchrotron Radiat. 7 (2000) 283–286.
[47] I.J. Pickering, R.C. Prince, M.J. George, R.D. Smith, G.N. George, D.E. Salt, Plant Physiol. 122 (2000) 1171–1177.
[48] J.J.H. Cotelesage, M.J. Pushie, P. Grochulski, I.J. Pickering, G.N. George, J. Inorg. Biochem. 115 (2012) 127–137.
[49] G.N. George, I.J. Pickering, M.J. Pushie, K. Nienaber, M.J. Hackett, I. Ascone, B. Hedman, K.O. Hodgson, J.B. Aitken, A. Levina, C. Glover, P.A. Lay, J. Synchrotron Radiat. 19 (2012) 875–886.
[50] S.M. Webb, J.F. Gaillard, L.Q. Ma, C. Tu, Environ. Sci. Technol. 37 (2003) 754–760.
[51] A.-M. Compton-O'Brien, R.D. Foust Jr., M.E. Ketterer, D.W. Blinn, in: Y Cai, O.C. Braids (Eds.), Biogeochemistry of Environmentally Important Trace Elements, American Chemical Society, Washington, DC, 2003, pp. 200–209.
[52] B. Dehdashti, D.W. Blinn, Freshw. Biol. 25 (1991) 131–141.
[53] C. Runck, D.W. Blinn, J. N. Am. Benthol. Soc. 9 (1990) 262–270.
[54] C. Runck, D.W. Blinn, J. N. Am. Benthol. Soc. 12 (1993) 136–147.
[55] M. Button, G.R.T. Jenkin, C.F. Harrington, M.J. Watts, J. Environ. Monit. 11 (2009) 1484–1491.
[56] M. Button, I. Koch, K.J. Reimer, Environ. Pollut. 169 (2012) 74–80.
[57] M. Button, M.M. Moriarty, M.J. Watts, J. Zhang, I. Koch, K.J. Reimer, Chemosphere 85 (2011) 1277–1283.
[58] S. Maeda, K. Kusadome, H. Arima, A. Ohki, K. Naka, Appl. Organomet. Chem. 6 (1992) 399–405.
[59] S. Maeda, in: J.O. Nriagu (Ed.), Arsenic in the Environment, Part I: Cycling and Characterization, John Wiley & Sons, Inc., New York, USA, 1994, pp. 155–188.
[60] M.A. Rahman, C. Hassler, Aquat. Toxicol. 146 (2014) 212–219.