Influence of soil chemistry on metal and bioessential element concentrations in nymphal and adult periodical cicadas (Magicicada spp.)

G.R. Robinson Jr. a,⁎, P.L. Sibrell b, C.J. Boughton b, L.H. Yang c

a U.S. Geological Survey, 954 National Center, Reston, Virginia, 20192, USA
b U.S. Geological Survey, 11649 Leetown Road, Kearneysville, West Virginia, 25430, USA
c Section of Evolution and Ecology, University of California, One Shields Avenue, Davis, California, 95616, USA

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Abstract

Metal and bioessential element concentrations were measured in three species of 17-year periodical cicadas (Magicicada spp.) to determine how cicada tissue chemistry is affected by soil chemistry, measure the bioavailability of metals from both uncontaminated and lead-arsenate-pesticide contaminated soils, and assess the potential risks of observed metal contamination for wildlife.

Periodical cicada nymphs feed on root xylem fluids for 13 or 17 years of underground development. The nymphs then emerge synchronously at high densities, before leaving their nymphal keratin exoskeleton and molting into their adult form. Cicadas are an important food source for birds and animals during emergence events, and influence nutrient cycles in woodland ecosystems.

Nymphal exoskeletons and whole adult cicadas were sampled in Clarke and Frederick Counties, Virginia and Berkeley and Jefferson Counties, West Virginia during the Brood X emergence in May and June, 2004. Elements, such as Al, Fe, and Pb, are strongly enriched in the nymphal exoskeleton relative to the adult body; Cu and Zn are enriched in bodies. Concentrations of Fe and Pb, when normalized to relatively inert soil constituents such as Al and Ce, are similar in both the molt exoskeleton and their host soil, implying that passive assimilation through prolonged soil contact (adhesion or adsorption) might control these metal concentrations. Normalized concentrations of bioessential elements, such as S, P, K, M, Cu, Zn, and Mo, and chalcophile (sulfur-loving) elements, such as As, Se, and Au, indicate strong enrichment in cicada tissues relative to soil, implying selective absorption and retention by xylem fluids, the cicada nymphs themselves, or both. Element enrichment patterns in cicada tissues are similar to enrichment patterns observed in xylem fluids from tree roots. Chalcophile elements and heavy metals accumulate in keratin-rich tissues and may bind to sulfhydryl groups. Metal concentrations in the nymphal exoskeleton show a positive correlation with soil metal concentrations, with Au exhibiting particularly strong enrichment in the exoskeleton relative to soil concentrations. Metal concentrations in adult bodies do not correlate with soil chemistry. Bioessential elements S, Ca, Mn, Fe, and Zn differed by sex in adults, whereas Na, Mg, K, Ca, Mn, Fe, Zn, and As differed by species. Body concentrations of Ca differed by site conditions (orchard or reference setting). The high Pb contents of orchard soils contaminated by arsenical pesticide residues might inhibit Ca uptake by cicada nymphs. The adult cicadas contain concentrations of metals similar to, or less than, other invertebrates, such as earthworms. There does not appear to be a dietary

⁎ Corresponding author. Tel.: +1 703 648 6113; fax: +1 703 648 6383.
E-mail address: grobinson@usgs.gov (G.R. Robinson).

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threat to birds or other consumers of adult cicadas based on Maximum Tolerable Dietary Level (MTDL) Guidelines developed for agricultural animals.

Keywords: Periodical cicadas (*Magicicada* spp.); Biogeochemistry; Metals; Orchard; Soil; Keratin; Lead arsenate; Gold

1. Introduction

Periodical cicadas are widespread insect herbivores throughout the deciduous forests of the eastern United States. Geographically separate broods of periodical cicadas emerge synchronously at high densities over large areas from underground burrows after 13 to 17 years of nymphal development. During their long underground development, periodical cicada nymphs feed on xylem fluids from tree roots (Williams and Simon, 1995; Day et al., 2002).

Periodical cicada emerge at such high densities that they commonly satiate the foraging abilities of their predators. They have been recognized as important events in the geochemical cycles of forest ecosystems (Wheeler et al., 1992; Yang, 2004).

This study examined two focus points of the biogeochemical cycle of bioessential elements and metals: 1) differences in source factors between natural soils and soils contaminated with arsenical pesticide residues, and 2) the bioavailability of bioessential elements, metals, and metalloids from these soils through xylem fluids and direct exposure to cicadas.

This study addresses four questions:

1. Do concentration levels of metals, metalloids, and bioessential elements in cicada tissues vary systematically with changes in soil chemistry?
2. How does cicada tissue chemistry vary by sex or species?
3. To what degree does cicada tissue chemistry reflect homeostatic processes to regulate the minerals in the

Fig. 1. Area of emergence in May–June 2004 of brood X periodic cicada, showing study area in northern Virginia and northeastern West Virginia. Figure modified from online map at College of Mount St. Joseph (2004).
body by adjusting physiological processes in response to environmental conditions versus passive uptake of constituents from soil by adhesion and/or adsorption?

4. Do concentrations of arsenic and heavy metals in periodical cicadas emerging from contaminated orchard sites represent a risk to birds and other cicada consumers?

We test the hypothesis that the chemistry of body tissues reflects the concentrations of constituents in the soil zone due to the long residence time and feeding behavior of cicada nymphs in soil. We characterize cicada tissues by species, type and sex, and compare the bioavailability of metals and metalloids with the geochemical behavior of bioessential elements.

2. Background

2.1. Periodical cicadas

Brood X 17-year periodical cicadas (*Magicicada* spp.) emerged during May-June, 2004, in the eastern United States (Fig. 1). Cicada densities of ≤30,000 per hectare to >3.5 million per hectare are known to occur during emergence in orchard and forest habitats (Williams and Simon, 1995). Other cicada broods emerge in other areas in other years.

Cicadas feed on root xylem fluids underground for 13 or 17 years before emerging to molt into their adult form. Cicada nymphs pass through five nymphal instars during their belowground development. *Instars* are insect developmental periods between successive exoskeleton moltings. The duration of these instars is variable in periodical cicadas, but the fifth nymphal stage typically lasts 5 years or more (Marlatt, 1907; White and Lloyd, 1975), during which time the exoskeleton is not shed until emergence. The feeding depth of cicada nymphs seems to depend largely on the depth of available plant roots. Maier (1980) observed that 5th instar nymphs fed at shallower depths in orchard soils compared to forest soils, and that most 5th instar nymphs were found between 7.6 and 37.5 cm depth. Cicada nymphs have also been observed to feed at depths of more than 60 cm (Williams and Simon, 1995). However, the soils developed over the carbonate bedrock of the study area are typically thin, clay-rich and rocky; tree roots, and likely cicada nymph feeding sites, tend to be localized at shallow depths.

Nymphs emerge at dusk over a period of a few days during their 13th or 17th year, depending on the species. Adult cicadas typically shed their nymphal exoskeleton within a few meters of their emergence site the next morning. Adult cicadas have limited dispersal, rarely traveling more than 50 m in a single flight (Karban, 1981). They congregate at chorus centers, often close to their emergence sites.

3. Materials and methods

3.1. Study area

A sampling program for soils, cicada molt exoskeletons and live mature adults was conducted from May to June 2004 at orchards, former orchards, and non-orchard reference sites in Clarke and Frederick Counties, Virginia and Berkeley and Jefferson Counties, West Virginia. Cicada and soil sampling sites in relation to site conditions are shown in Fig. 2. Sites were mostly located on carbonate bedrock overlain with thin clay-rich soils. The orchard locations are based on aerial photographs and topographic maps (showing orchard areas) prepared using information from the time period of extensive use of arsenical pesticides between the 1920s and 1960s. A digital dataset identifying orchard areas where arsenical pesticides were likely used in the study area is available from Reed et al. (2006).

Cicada molt exoskeleton samples were collected at emergence sites and adults were collected at emergence sites and chorus centers. Soils and cicadas were sampled at approximately 5 to 6 individual collection points within a 1 km² area at each site. The sex and species of adults was determined. Cicada samples were frozen until analysis, then dried at 60 °C. Dried adult insects were macerated and analyzed individually; molt exoskeletons were composited into one sample for each individual collection point.

Representative soil samples (B horizon) were collected to depths of 15 to 25 cm (depending on depth of regolith) by soil auger and as grab samples. The soil sample intervals overlap much of the observed depth range of 5th instar cicada nymphs, particularly in orchard soils (Maier, 1980). Soil samples were air dried, homogenized, sieved to <200 micrometers (80 mesh), and pulverized before analysis.

Element concentrations in cicada tissue and soil samples were determined by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) following sample digestion in aqua regia using the Ultratrace-2 method performed by Activation Laboratories, Ancaster, Ontario, Canada. Mercury was measured using cold-vapor analysis techniques using the Code 1G method performed by Activation Laboratories, Ancaster, Ontario, Canada. Duplicate samples were submitted at a rate of 5% of total samples. Data were accepted if the relative standard deviation was <15% at five times the
limit of detection for the duplicate samples. Analytical methods, quality assurance and control methods, and laboratory accreditation information are described in more detail in Actlabs (2006).

3.2. Statistical methods

Normality tests were done on the geochemical data for soils, cicada bodies and nymphal exoskeletons. Most constituents analyzed for this report were neither normally nor lognormally distributed and a few sample measurements were below detection limits for some elements. Concentrations less than detection limits were reported as one-half the detection limit to calculate sample means. We employed nonparametric methods, which do not require assumptions about the distributions of the data, to compare sample group means.

The data for soil site chemistry were grouped by land-use status into orchard and former orchard sites or reference sites. The data for cicada body chemistry were grouped by land-use status, species type, and sex. The cicada exoskeleton samples were not classified according to species or sex and were grouped by land-use status.

Tests for differences in chemical distributions in soils by site status and in cicada tissues by site status, sex, and species classifications were performed using Mann–Whitney (binary group) and Kruskal–Wallis (multiple group) tests. The null hypothesis of these tests is that there is no significant difference between the means of the ranks of the concentrations of a chemical constituent among the sample groups (Sokal and Rohlf, 1969; Conover and Iman, 1981; Helsel and Hirsch, 2002). Rejection of the null hypothesis at the 95-percent confidence level (alpha=0.05) was considered to be evidence supporting the alternative hypothesis, that is, the existence of a significant difference in chemistry between groups. The general linear model (GLM) procedure (SAS Institute Inc., 1999) was used to do the non-parametric tests because the number of samples was not the same for all of the category groups (Helsel and Hirsch, 2002). Multiple comparison tests, based on Kruskal–Wallis rank sums and comparisons of means of ranks (Tukey) tests, were used to identify which groups differed from others (Hollander and Wolfe, 1973). Tukey’s significant-difference test (Sokal and Rohlf, 1969; Stoline, 1981; Helsel and Hirsch, 2002) was used.
to discriminate which category group or groups of data differed when the analysis of variance rejected the null hypothesis. Rank–order correlation (Spearman’s rho) was used to determine if the concentration levels of elements in the paired cicada tissue and soil samples were associated.

4. Results and discussion

4.1. Soil chemistry

Soil concentrations at the collection sites varied over one order of magnitude for bioessential elements and many metals and over two orders of magnitude for As, Pb, and Au (Table 1). Approximately one third of the soil sites were from orchard sites where arsenical pesticides were used. Relative to non-orchard sites, orchard site soils contain elevated Pb, As and other heavy metal concentrations (Tables 1 and 2), consistent with the presence of Pb-arsenate pesticides residues at these sites (Peryea, 1998). With the exception of As and Pb values, the chemistry of B-horizon soils was similar to other untilled soils in the eastern United States (Shacklette and Boerngen, 1984).

4.2. Cicada tissue chemistry

Average adult cicada body concentrations for As, Cu, Hg, Pb, and Zn were 3, 62, 0.015, 0.4, and 166 mg/kg (dry weight), respectively (Table 1). The adult cicadas contained concentrations of metals similar to other invertebrates, such as earthworms (Saxe et al., 2001), consistent with the results of Clark (1992). The average chemistry of adult cicada bodies from orchard sites was compared to the Maximum Tolerable Dietary Level (MTDL) Guidelines (Table 3) developed for small...
barnyard animals and poultry (NRC, 1980, Table 1) to evaluate if the heavy metal content of cicadas pose a dietary threat for birds and other cicada consumers. In all cases, the average cicada body concentrations were less than half of the MTDL guidelines, implying no significant dietary risk to consumers, even if adult cicadas were consumed at twice normal dietary requirements during the emergence event (Clark, 1992).

Periodical cicada emergence events coincide with, and provide abundant food for, the nestling–rearing activities of many birds. These nestlings might represent a special population sensitive to heavy metal and toxic element loading. Based on our data and on feeding rates calculated from previous field and lab studies, Red-Winged Blackbird (Agelaius phoeniceus) nestlings feeding on cicadas may consume between 0.08–0.19 mg As, 0.01–0.03 mg Pb, 1.8–4.6 mg Cu, and 0.42–1.10 ng Hg from adult cicadas in the first 10–12 days before fledging (Strehl and White, 1986; Yasukawa and Searcy, 1995; Pope et al., 2001; Cartwright et al., 1998; Fiala and Congdon, 1983, A. Clark and L. Yang, personal communication). The xenobiotic metals, such as Pb and Hg, may accumulate and concentrate in specific target organs of the nestlings, such as the liver and kidney (Hunter and Johnson, 1982). However, the concentration levels of As, Pb, Cu, and Hg in adult cicadas from orchard sites contaminated with arsenical pesticide residues are comparable to the concentrations of these elements in

### Table 2
Summary of attained significance levels (p-values) for Mann–Whitney and Kruskal–Wallis tests on element concentrations on sample groups defined by sample criteria

<table>
<thead>
<tr>
<th>Sample criteria</th>
<th>Element</th>
<th>Soil site</th>
<th>Exoskeleton site</th>
<th>Cicada body</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site</td>
<td>Ce</td>
<td>0.558</td>
<td>0.908</td>
<td>0.544</td>
<td>0.224</td>
</tr>
<tr>
<td></td>
<td>Na</td>
<td>0.557</td>
<td>0.497</td>
<td>0.159</td>
<td>0.647</td>
</tr>
<tr>
<td></td>
<td>Mg</td>
<td>0.693</td>
<td>0.032</td>
<td>0.528</td>
<td>0.794</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>0.912</td>
<td>0.633</td>
<td>0.458</td>
<td>0.057</td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>0.912</td>
<td>0.090</td>
<td>0.676</td>
<td>0.0001</td>
</tr>
<tr>
<td></td>
<td>K</td>
<td>0.613</td>
<td>0.524</td>
<td>0.577</td>
<td>0.406</td>
</tr>
<tr>
<td></td>
<td>Ca</td>
<td>0.370</td>
<td>0.391</td>
<td>0.014</td>
<td>0.0001</td>
</tr>
<tr>
<td></td>
<td>Sr</td>
<td>0.884</td>
<td>0.313</td>
<td>0.030</td>
<td>0.0001</td>
</tr>
<tr>
<td></td>
<td>Ba</td>
<td>0.446</td>
<td>0.972</td>
<td>0.648</td>
<td>0.319</td>
</tr>
<tr>
<td></td>
<td>Mn</td>
<td>0.013</td>
<td>0.025</td>
<td>0.086</td>
<td>0.0001</td>
</tr>
<tr>
<td></td>
<td>Fe</td>
<td>0.083</td>
<td>0.507</td>
<td>0.795</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Pb</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.092</td>
<td>0.016</td>
</tr>
<tr>
<td></td>
<td>Cu</td>
<td>0.018</td>
<td>0.0001</td>
<td>0.161</td>
<td>0.398</td>
</tr>
<tr>
<td></td>
<td>Zn</td>
<td>0.0007</td>
<td>0.0005</td>
<td>0.969</td>
<td>0.0001</td>
</tr>
<tr>
<td></td>
<td>As</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.307</td>
<td>0.548</td>
</tr>
<tr>
<td></td>
<td>Hg</td>
<td>0.128</td>
<td>0.026</td>
<td>0.969</td>
<td>0.843</td>
</tr>
</tbody>
</table>

| n               | 72      | 55        | 127              | 127         | 127      |

<table>
<thead>
<tr>
<th>(Sample numbers by criteria)</th>
</tr>
</thead>
</table>

The p-values show the probability that the observed differences are due to chance rather than the factor tested; p-values significant at alpha=0.05 (95% confidence) are shown in bold. The number of samples evaluated in each sample group is denoted by “n”. The comments column identifies the direction of the differences within sample groups by sample criteria and identifies the number of samples tested by sample criteria.

### Table 3
Chemistry of adult cicada bodies and pre-molt nymph relative to maximum tolerable dietary level limits defined for small animals

<table>
<thead>
<tr>
<th>Site</th>
<th>Mn (mg/kg dry weight)</th>
<th>Fe (mg/kg dry weight)</th>
<th>Cu (mg/kg dry weight)</th>
<th>Zn (mg/kg dry weight)</th>
<th>Al (mg/kg dry weight)</th>
<th>As (mg/kg dry weight)</th>
<th>Pb (mg/kg dry weight)</th>
<th>Hg (mg/kg dry weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult cicada</td>
<td>193</td>
<td>210</td>
<td>59</td>
<td>167</td>
<td>&lt;100</td>
<td>2</td>
<td>0.4</td>
<td>0.01</td>
</tr>
<tr>
<td>Nymph</td>
<td>218</td>
<td>642</td>
<td>56</td>
<td>168</td>
<td>260</td>
<td>3</td>
<td>4.2</td>
<td>0.02</td>
</tr>
<tr>
<td>MTDL† (NRC, 1980)</td>
<td>400</td>
<td>500</td>
<td>200</td>
<td>500</td>
<td>200</td>
<td>50</td>
<td>30</td>
<td>2.00</td>
</tr>
</tbody>
</table>

† Maximum Tolerable Dietary Level, minimum of poultry–rabbit levels.

The estimate of mean cicada nymph chemistry is based on a mass-weighted average of nympha exoskeleton and adult body means.
other food sources, from uncontaminated sites, that are likely to be consumed by nestlings, such as earthworms (Saxe et al., 2001). It is unlikely that these metal loadings exceed the detoxification capacity or homeostatic regulatory capability of the nestlings, although the evaluation of biomagnification of heavy metals in natural ecosystem foodchains is a controversial issue (Laskowski, 1991; van Straalen and Ernst, 1991).

Average elemental concentrations in cicada tissues differ by tissue type (Table 1; adult body vs exoskeleton). Bioessential elements, such as Na, K, P, S, and Mg, were concentrated in adult body tissues. Heavy metal concentrations, with the exception of Cu, were low in body tissues. Al, Fe, Ce, Pb, and Au were concentrated in the nymphal exoskeleton. Ca, Mn, As, and Hg show approximately similar distributions.

Many nocturnal predators, such as mice and shrews, consume the vulnerable cicada nymphs before they shed their nymphal exoskeleton. Using a mass-weighted average of the mean exoskeleton and adult body chemistry as an estimate of nymph chemistry, we estimate that the mean Al and Fe values for cicada nymph slightly exceed MTDL guidelines, whereas other element concentrations were below the MTDL guidelines (Table 3).

The cicada populations sampled in this study appear to constitute a safe diet for birds and small mammals and probably have lower average contamination levels than do other invertebrates that may be consumed in this geographic area. Consistent with the results of Clark (1992), adult cicada bodies have higher concentrations of Cu than host soil and other invertebrates, such as earthworms. Periodical cicadas from areas with histories of Cu contamination may need to be analyzed before the safety of these cicadas can be evaluated. However, Cu is an essential trace element in animal nutrition and efficient homeostatic regulation of Cu is observed in field populations of small mammals in areas of metal contamination (Hunter and Johnson, 1982; Laskowski, 1991).

4.3 Soil and cicada chemistry in relation to site and sample characteristics

Mann–Whitney (binary group) and Kruskal–Wallis (multiple group) tests were used to identify differences in chemical distributions in soils by site status and in cicada tissues by site status, sex, and species classifications (Table 2). Cicada exuviae and soils collected from orchard site soils contain elevated Pb, As and other heavy metal concentrations (Cu, Zn) relative to non-orchard (reference) sites (Table 2), consistent with the presence of lead-arsenate and other metal-based pesticide residues at these sites (Peryea, 1998). The elevated Mg and Hg contents of cicada exuviae from orchard sites relative to reference sites may reflect interaction effects with other metals in the contaminated soils. The group differences in Mn concentrations between soil (and exoskeleton) by site classification likely reflect natural variation, not contamination, because Mn compounds are not known to have been used as agricultural chemicals.

Except for Ca and Sr concentrations, the chemistry of adult cicada bodies collected from orchard sites did not differ from non-orchard sites (Table 2). The lower concentrations of Ca and Sr in cicada bodies from orchard sites cannot be explained by differences in Ca and Sr in the orchard site soils. Calcium and Sr have similar chemical properties. The high Pb contents of orchard soils contaminated by arsenical-pesticide residues might limit Ca uptake and retention by cicada nymph. Lead has been shown to inhibit nutrient assimilation and water uptake in plant roots (Malecka et al., 2001; Burzynski, 1988) and to disrupt Ca-ion channel functions regulated by Ca-channel proteins in animals (Anetor et al, 2005). The inhibition of Ca uptake and retention may occur either at the soil–root tip–xylem fluid interface (where Pb concentrations are highest) or at the cicada nymph–xylem fluid interface.

The bioessential elements S, Ca, Mn, Fe, and Zn differed in cicada bodies by sex; all elemental concentrations were greater in female bodies. These sex-based differences may influence the dietary resources available to cicada predators, as female cicadas are preferentially consumed by some predators (Steward et al., 1988; Karban, 1983). Both Red-Winged Blackbirds (Agelaius phoeniceus) and House Sparrows (Passer domesticus) preferentially forage on female cicadas, perhaps due to their higher nutrient content or lack of a disturbance squawk behavior (Steward et al., 1988; Karban, 1983).

Concentrations of Na, Mg, K, Ca, Mn, Fe, Zn, and As differed in cicada bodies by species, and these differences persist when the data are split by sex. For all elements, concentrations in M. cassini are greater than in M. septendecim or M. septendecula species (Table 2). The observation of higher elemental concentrations in M. cassini cicadas might reflect different habitat preferences between the different cicada species. M. cassini prefers wetter floodplain sites compared to the drier upland habitats of M. septendecim and M. septendecula (Dybas and Davis, 1962). These wetter habitats might explain the observed species differences in elemental composition either through increased concentration of some elements in these soils, or due to increased absorption and assimilation rates from
wetter soils. An alternate hypothesis is that since _M. cassini_ is substantially smaller than _M. septendecim_ (Karban, 1983), this species has a higher proportional ratio of body mass to exoskeleton that might lead to a concentration of elements in body tissues. However, _M. septendecula_ is almost the same size as _M. cassini_, but does not have the same elevation of chemical constituents, favoring the habitat hypothesis.

### 4.4. Soil–cicada tissue correlation

Metal concentrations in nymphal exoskeletons tended to vary in relation to soil chemistry as indicated by the consistency in elemental group differences for exoskeleton and soil chemistry as classified by site (Table 2) and by the rank-correlation (Spearman's rho) coefficients between paired soil and tissue samples (Table 4). Spearman correlation coefficients, relating concentrations of elements such as Ce, Ca, Mg, Fe, Cu, Pb, and As in exoskeleton to soil, were consistently positive and low to moderate (0.385 < r < 0.754) in value (Table 4). For soil and exoskeleton chemical data normalized relative to Ce, a relatively inert soil constituent, the Spearman correlations increased in value and are significant for more elements (Ce-normalized data in Table 4). These results imply that assimilation through prolonged soil contact may influence the concentrations of these elements in the nymphal exoskeleton.

In contrast, the cicada body-soil correlation coefficients for these elements were low (−0.371 < r < 0.487), inconsistent by sex category, and generally not significant at the 95% confidence level. Ce-normalized Spearman correlation coefficients for cicada bodies relative to soil are not shown in Table 4, but were similar to the raw data correlations in being inconsistent by sex category and generally not significant at the 95% confidence level. These data indicate that biological regulation processes influenced cicada body chemistry, consistent with the geochemical features of other soil invertebrates, such as earthworms (Saxe et al., 2001).

### 4.5. Soil–exoskeleton comparison

The soils contained approximately 1 to 12% of Al-bearing clay minerals, by weight. These clay minerals are relatively insoluble in soil fluids and soil concentrations of Al were high relative to the concentration of Al in soil pore waters and xylem fluids (Smith and Shortle, 2001). The high concentration of Al in cicada exoskeleton, in contrast to the low concentration of Al in the adult cicada body, was likely the result of 1) direct exposure to insoluble clay minerals in soil involving adhesion and adsorption processes, or 2) efficient sequestration of Al derived from xylem fluids by the nymph into exoskeleton.

The role of passive assimilation (adhesion–adsorption) resulting from cicada nymph exposure to soil relative to other biological regulation processes was assessed by normalizing the cicada tissue chemistry to relatively insoluble, but abundant, soil constituents such as Al and Ce (the precision for Ce is better than Al for our dataset). For cicada exoskeletons, Al:Ce:Pb ratios were similar to soil ratios and the data follow a 1:1 trend line over two orders of magnitude (Fig. 3A). Concentrations of Pb, Al, and Ce in cicada exoskeleton appear to be largely controlled by soil adsorption or adhesion.

Normalized plots for bioessential elements (Ca, P, and Na) indicate correlation with soil chemistry and demonstrate consistent fractionation into exoskeleton tissue (Fig. 3B). These bioessential elements are expected to fractionate into biological tissues. Sulfur and As also preferentially fractionate into exoskeleton (data not shown in graph), but their fractionation appears to be biologically regulated as well as varying as a function of soil chemistry (resulting in weak Ce-normalized Spearman correlations, Table 4).

Similar plots for chalcophile elements (Cu and Au are shown examples, Fig. 3C and D) exhibit consistent and strong fractionation into exoskeleton tissue from soil. The strong enrichment in Au (fractionation factor of approximately 50) is surprising for a reported bio-

### Table 4

Summary of Spearman's rho rank-correlation coefficients for element concentrations between paired soil and tissue samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>Ce</th>
<th>Na</th>
<th>K</th>
<th>P</th>
<th>S</th>
<th>Ca</th>
<th>Mg</th>
<th>Fe</th>
<th>Cu</th>
<th>Pb</th>
<th>As</th>
<th>Au</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exoskeleton</td>
<td>0.658</td>
<td>(0.196)</td>
<td>(−0.280)</td>
<td>(0.265)</td>
<td>(−0.028)</td>
<td>0.385</td>
<td>0.581</td>
<td>0.647</td>
<td>0.473</td>
<td>0.754</td>
<td>0.669</td>
<td>0.747</td>
</tr>
<tr>
<td>Exoskeleton; Ce-normalized data</td>
<td>0.606</td>
<td>0.351</td>
<td>0.396</td>
<td>0.499</td>
<td>0.535</td>
<td>0.786</td>
<td>0.848</td>
<td>0.662</td>
<td>0.907</td>
<td>0.636</td>
<td>0.638</td>
<td></td>
</tr>
<tr>
<td>Male body</td>
<td>(0.038)</td>
<td>(0.124)</td>
<td>(−0.099)</td>
<td>(−0.070)</td>
<td>(0.022)</td>
<td>(0.108)</td>
<td>(−0.060)</td>
<td>0.140</td>
<td>(0.132)</td>
<td>(0.094)</td>
<td>(−0.005)</td>
<td>−</td>
</tr>
<tr>
<td>Female body</td>
<td>(0.298)</td>
<td>(0.110)</td>
<td>(0.271)</td>
<td>(0.216)</td>
<td>(0.078)</td>
<td>0.444</td>
<td>(0.142)</td>
<td>0.487</td>
<td>(−0.039)</td>
<td>(0.290)</td>
<td>(−0.371)</td>
<td>−</td>
</tr>
</tbody>
</table>

Significant correlations, the probability that the observed correlations are due to the relation tested rather than to chance, at α = 0.05 (95% confidence level), are shown without brackets. Ce-normalized data correlation coefficients for cicada bodies are similar to the raw data correlations; most are not significant at 95% confidence.
nonessential element, but is consistent with some other data reported for keratin samples, such as Desert Tortoise scute (keratin fraction of shell) (Haxel et al., 2000) and other biota (Reith, 2003).

4.6. Cicada tissue–soil comparison

The general pattern of biogeochemical fractionation into cicada tissues is shown in the plot of normalized cicada tissue chemistry relative to soil chemistry (Fig. 4). The fractionation factors, shown on the log-transformed Y axis, are cicada tissue chemistry, normalized by Ce concentrations, divided by Ce-normalized soil concentrations. Symbols occur at the median fractionation factor, bounded by the 10–90 percentile envelopes, for the sample population groups. A fractionation factor of 1 indicates a pattern consistent with passive assimilation resulting from soil contact. Fraction factors >1 indicate preferential incorporation of the element from soil conditions into biological tissues.

Normalized concentrations of Al, Pb and the transition metals Fe, Co, and Ni in both exoskeleton (Fig. 4a) and cicada body tissues (Fig. 4b) indicate fractionation ratios of approximately 1, consistent with their concentrations being controlled by passive assimilation through dermal exposure. Bioessential (Ca, P, Na) and chalcophile (Cu, Zn, Mo, Au) elements show strong enrichment in cicada tissues relative to soil and the fractionation patterns are similar for both nymphal exoskeleton and adult body tissues (although the
Fractionation values are generally higher for the body tissues. The strongest elemental enrichments for both tissue types are for S and for the chalcophile elements, consistent with the hypothesis that chalcophile elements may be bound to sulfhydryl groups in organic tissues.

Divergence of the mean fractionation values for male versus female bodies in Fig. 4b indicate preferential enrichment in female bodies of the elements: S, Ca, Cr, Mn, and Mo, consistent with the differences shown in Table 2.

Cicadas feed on xylem fluids from plant roots, and xylem sap is one component of the linkage between the soil environment and cicada physiology. We could not identify the host tree for the individual adult cicadas collected in our study and we did not sample tree root sap for comparison with soil chemistry. Although the chemistry of xylem sap in tree roots varies in relation to soil element availability and geochemical discrimination in root uptake by plant species, the ionic chemistry of xylem sap appears to be largely regulated by the plant (physiologic homeostatic control) in response to conditions in their soil environment (Smith and Shortle, 2001). Xylem fluid from various host plants for periodical cicada show similarities in ionic composition (Cheung and Marshall, 1973). We use some limited, but available, data on tree root sap chemistry, in relation to soil data, to illustrate what we believe is likely to be a general pattern of homeostatic control on xylem chemistry relative to soil chemistry (Fig. 4b). The tree root sap (xylem) enrichment values shown in Fig. 4, derived from data for red spruce reported by Smith and Shortle (2001), are not from a host tree for periodical cicada in the study area. However, this data is probably representative of the likely variation in element composition of typical root xylem fluids relative to host soil chemistry. The pattern of geochemical enrichment in cicada tissues relative to soil conditions is similar to those observed in these reference xylem fluids from tree roots. The cicada body composition appears to reflect xylem composition (Fig. 4b) much more than soil composition (Table 2), whereas the nymphal exoskeleton reflects soil (Table 2) better than xylem fluid (Fig. 4).

4.7. Gold in cicada tissues

The observed correlation between Au concentrations in paired soil and nymphal exoskeleton samples (Table 4, Fig. 3D) and the strong enrichment in Au concentrations in the nymphal exoskeleton relative to host soil concentrations (Table 1) implies the presence of mechanisms to dissolve and transport Au in the soil.
 Concentrations of potentially toxic elements in adult cicada bodies are low and probably do not pose a significant risk to birds and other consumers that preferentially feed on cicadas during emergence years. These findings support the conclusions of previous studies investigating metal accumulation in cicadas (Clark, 1992). Periodical cicadas can represent a substantial flux of nutrients (Yang, 2004, 2006) and other chemicals from soil environments into above-ground food webs during a substantial resource pulse. These data suggest that Fe, Al, Pb, and As are concentrated in the nymphal exoskeleton, which is shed within hours of emergence. Metal concentrations, except for Cu, are low in adult cicada bodies. The shedding of the nymphal skin may reduce the risk of metal exposure to predators of adult cicadas, but nocturnal consumers of the cicada nymphs, such as mice and shrews, may experience greater exposure to accumulated metals.

Normalized concentrations of bioessential and chalcophile elements are enriched in cicada tissues relative to soil. These metals may bind to sulfhydryl groups in organic tissue. The patterns of element enrichment relative to soil concentrations are similar between nymphal exoskeleton and adult body tissues. The enrichment patterns in the tissues are similar to enrichment patterns reported in the literature for tree root sap, implying that cicada nymphs might be able to minimize their internal energy requirements for homeostatic control by utilizing the homeostatic processes exerted by their host tree biology. Although soil chemistry does not appear to strongly influence cicada body chemistry, our data support the hypothesis that high Pb soils might inhibit Ca (and Sr) uptake and retention by cicada nymphs.

The strongest fractionation observed is for Au, and might reflect the solubilization and mobility of Au in the soil environment by organic acids, thiosulfate, and cyanogenic metabolites exuded by tree roots and excreted by cicada nymph. Soluble Au has a strong affinity for keratin (c.f. Ishikawa and Suyama, 1998) and Au concentrations measured in nymphal exoskeleton are very high relative to soil conditions.

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References
