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Concentrations and biomagnification of multiple metals/metalloids are higher in rice than in sugarcane agroecosystems of southern China

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ABSTRACT

Agricultural lands have been increasingly reported to be contaminated by metals and metalloids, leading to exposure to these toxins for humans and wildlife. Previous studies on metal/metalloid contamination have reported biomagnification of total elemental Hg (THg) and, less consistently, of Cd, in both aquatic and terrestrial ecosystems. Whether other metals or metalloids can biomagnify in food webs, especially those that produce food for humans, is uncertain. We aimed to compare metal/metalloid contamination and their biomagnification patterns between rice paddy and sugarcane agroecosystems. We collected samples at an inactive Pb-Zn mine site, and two reference sites in southwestern Guangxi, southern China. Samples were widely distributed across the food web, including soil, rice grain and leaves, sugarcane leaves, crickets, grasshoppers, spiders, and frogs. We found that Cr, Cd, Pb, THg, and Zn were the metals associated with mining activities, while As and Cu were higher at the reference sites. In soil, and in the majority of species for which there were significant differences between agroecosystems, concentrations of Cd, Cr, Cu, Pb, THg, and Zn were higher in rice, suggesting that the rice paddy ecosystem is particularly sensitive to metal contamination. When quantitatively rating the patterns of biomagnification using post-hoc multiple comparisons among species' bioaccumulation factors (BAFs), rice paddies had stronger patterns of biomagnification (3 strong relationships [e.g., all insectivores had higher or as high BAFs compared to all other species], 7 medium relationships [e.g., one of the insectivores had the highest BAFs, but another had an intermediary value, similar to an herbivore], 6 weak [e.g., one insectivore had the highest BAFs, but the other was as low as a primary producer], and 3 no relationships [e.g., no significant differences among species in BAFs]) than sugarcane ecosystems (1 strong, 2 medium, 8 weak, and 9 no relationships; Repeated measures *t*-test, P-value = 0.0023) across 6 metals (Cr, Cd, Cu, Hg, Pb, Zn). The strongest biomagnification was evident for THg and Zn, whereas an intermediary level of biomagnification was found for Cd, Cr, Cu, and Pb; biodilution was observed for As and Mn. Therefore, focusing on metal/metalloid apportionment to better understand the sources of the metals at our study sites would be of utility to lower the exposure of wildlife and people. Most importantly, the local authorities should check metal concentrations in any farming product from the mining area and even the close reference site to safeguard human health.

1. Introduction

Metals and metalloids have detrimental effects and may reside in the environment for a prolonged time, even if the primary sources of the pollution have not been in operation for years (Gall et al., 2015). Metals and metalloids are neither metabolized nor degraded by living organisms (Wasi et al., 2013). Their chemical compounds may cycle within ecosystems, thereby threatening or harming biodiversity (Abdu et al., 2017; Alloway, 2013a; Burger and Gochfeld, 2000, 2004; Picone et al., 2019). Further, metals and metalloid pollution in croplands can make the produced farm products unfit for use, threatening food safety, security, and human well-being either in local areas or areas to which

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they are exported (Li et al., 2020; Liu et al., 2013; Qin et al., 2021). For instance, metals and metalloids with no known biological functions, e.g., cadmium (Cd), mercury (Hg), arsenic (As), and lead (Pb) may show toxicity in organisms and the environment even at small dosages (Fairbrother et al., 2007).

Contamination of agroecosystems by metals and metalloids is a particular problem in China (Chen, 2007; Li et al., 2007). Mining activities, in particular, are reported as the primary sources for releasing various metals and metalloids in the environment, which has led to ecosystem-wide pollution in several areas of China (Hu et al., 2020; Qin et al., 2021). Another source of metal/metalloid contamination into agricultural ecosystems is through the application of various agrochemicals (i.e. pesticides and fertilizers) to control pests and diseases, as well as supplementing the necessary nutrients needed for proper growth of crops (Alloway, 2013b; Qin et al., 2021; Zhao et al., 2015). Other than pesticides and fertilizers, the use of sewage sludge (Luo et al., 2009) and irrigation of crops using sewage wastewater, both treated and untreated, are also significant contributors of metals/metalloids in agricultural ecosystems (Natasha et al., 2022; Peng et al., 2019; Singh et al., 2004; Turan et al., 2018).

Different kinds of agroecosystems may be differentially sensitive to metal/metalloid contamination. Rice (*Oryza sativa*) is grown mostly under flooded or wetland conditions (Ali et al., 2019; Elphick, 2010), which enhances the production of methylmercury (MeHg). Methylmercury is produced by bacteria from inorganic Hg in anoxic or reduced conditions, and is more bioavailable than inorganic Hg, driving bioaccumulation and biomagnification (AMAP/UN Environment, 2019; Driscoll et al., 2013; Ullrich et al., 2001). Rice is a leading staple cereal, consumed by more than 50% of the population worldwide; more than 90% of the rice in the world is being produced and consumed in Asian countries (Mu et al., 2019). In China, over 65% of the people are dependent on rice as the primary staple crop (Huang et al., 2013; Lou et al., 2013). These characteristics of rice produce a regional trend in Asia wherein Hg exposure from rice may be greater than from fish, at least for inland communities (Zhang et al., 2010). Yet the cycling of metals/metalloids through other agroecosystems has been in contrast little explored. For example, sugarcane (*Saccharum officinarum*) is a major agronomic plant in southern China, with China ranking third globally after Brazil and India in sugar production (Li and Yang, 2015). Given that sugarcane is cultivated in areas where there is no irrigation, or its use is limited (Li and Yang, 2015), sugarcane can serve as a useful comparison to rice to understand variation in metal/metalloid cycling associated with different kinds of crops.

Different metals/metalloids may vary in their cycling through the food webs of agroecosystems. For example, numerous studies have reported on biomagnification of Hg (Abeysinghe et al., 2017; Cristol et al., 2008; Jiang et al., 2022; Sun et al., 2020; Zhao et al., 2013) and, less consistently, of Cd (Cardwell et al., 2013; Reyes-Márquez et al., 2022; Tasneem et al., 2020; Zhao et al., 2013), in both aquatic and terrestrial ecosystems. In contrast, manganese (Mn) has been demonstrated to show biodilution, because animals located in the middle to the top trophic levels of the food chain are capable of regulating Mn concentrations through homeostatic mechanisms (Ikemoto et al., 2008). Whether other metals (e.g., Pb, As, Cu, Zn, Mn, and chromium [Cr]) are able to biomagnify in food chains in some situations is uncertain. In addition, aquatic ecosystems are more studied in this context than terrestrial ones. This is because aquatic ecosystems consist of longer food chains, which enhance the potential for biomagnification, in contrast to terrestrial ecosystems (Dietz et al., 2000; Palma et al., 2005). Yet some food chains in contaminated terrestrial ecosystems can be long enough to biomagnify elements like Hg strongly, particularly if carnivorous arthropods like spiders themselves are consumed by vertebrates (Cristol et al., 2008). Thus, for the study of metals/metalloids in agroecosystems, it is important to sample a range of trophic levels and include both vertebrate and invertebrate consumer species.

Here we investigated the concentrations of multiple metals/metalloids near an inactive lead/zinc mine in southern China, and in two reference sites. The main objective of this project was to compare metal/metalloid contamination in two different agricultural ecosystems that are common in tropical countries, rice paddy and sugarcane. More specifically, we aimed to study which metals/metalloids were associated with mining contamination, and compare among them in their biomagnification properties. Our research questions were: (1) For what metals was there contamination at the mine site? (2) How were metal concentrations different between rice paddy and sugarcane agricultural ecosystems? (3) In what metals and agricultural ecosystems was there evidence for biomagnification? We hypothesized that:

1) In addition to Pb and Zn at a lead–zinc mining site, Cd would also be high, because Cd is often found as a secondary product of Pb-Zn mines (Alloway, 2013b);
2) Mercury would biomagnify, and Cd would also to a lesser extent, as shown in previous studies;
3) Rice paddies would be particularly vulnerable to metal/metalloid pollution due to the centrality of water to their growth and development. We thus predicted greater levels of metals/metalloids in these ecosystems and more consistent patterns of biomagnification there.

2. Materials and methods

2.1. Study region, sites and locations

We conducted our research in Chongzuo Prefecture, southwestern Guangxi Zhuang Autonomous Region, southern China (Fig. 1). This region has a subtropical marine monsoon climate with an annual precipitation of 1350 mm (Pan et al., 2020). Significant portions of this part of Guangxi are composed of limestone karst; additionally, the region is characterized by poor surface soils, which are being degraded through surface runoff, erosion, and rocky desertification (Li et al., 2007).

Guangxi is both a major agricultural and mining province. The Guangxi economy is dominated by agriculture, and it is the largest source of sugarcane for China (Li and Yang, 2015). It is also among the major non-ferrous metal mining producers in China, and its soils have higher background levels of metals/metalloids (specifically Cd, Pb, Hg, Zn, Cu, and Cr) compared to other provinces (He et al., 2020; Hu et al., 2020; Yin et al., 2019). The effects of mining may be particularly felt by the agricultural sector in Guangxi, due to the severe scarcity of arable lands for agricultural activities (Li et al., 2007).

One sampling site for the project was in the mine tailings of the Daxin Lead-Zinc Mining District. The underground mine was under large-scale operation for about 40 years before it was shut down in 2001, owing to reduced productivity of the mining activities and environmental pollution (He et al., 2020). Due to these mine tailings' presence, many problems, including metal contamination, have been reported in nearby agricultural ecosystems (Mtemi et al., 2023; Pan et al., 2020) and in the biodiversity of the mine site (He et al., 2020; Liu et al., 2022; Liu et al., 2021), as well as in human hair and urine samples (Lv, 2014). We collected samples from three locations within the Daxin Mining District (located in Daxin County, Fig. 1), two of which (Dongling and Shangyu villages) had agriculture focused on rice, and one of which (Chang village) focused on sugarcane.

We also collected samples at close and far reference sites (see Fig. 1). The close reference site was situated 6 km away from the mine site in Daxin County without any connecting waterway, consisting of two sampling locations (Longmen village, focused on rice, and Changming village, focused on sugarcane). The far reference site was located near the Nonggang National Nature Reserve in Longzhou County, approximately 60 km away from Daxin Lead-Zinc Mining District, and was composed of two sampling locations (Shetun village, focused on rice, and Nonggang village, focused on sugarcane).

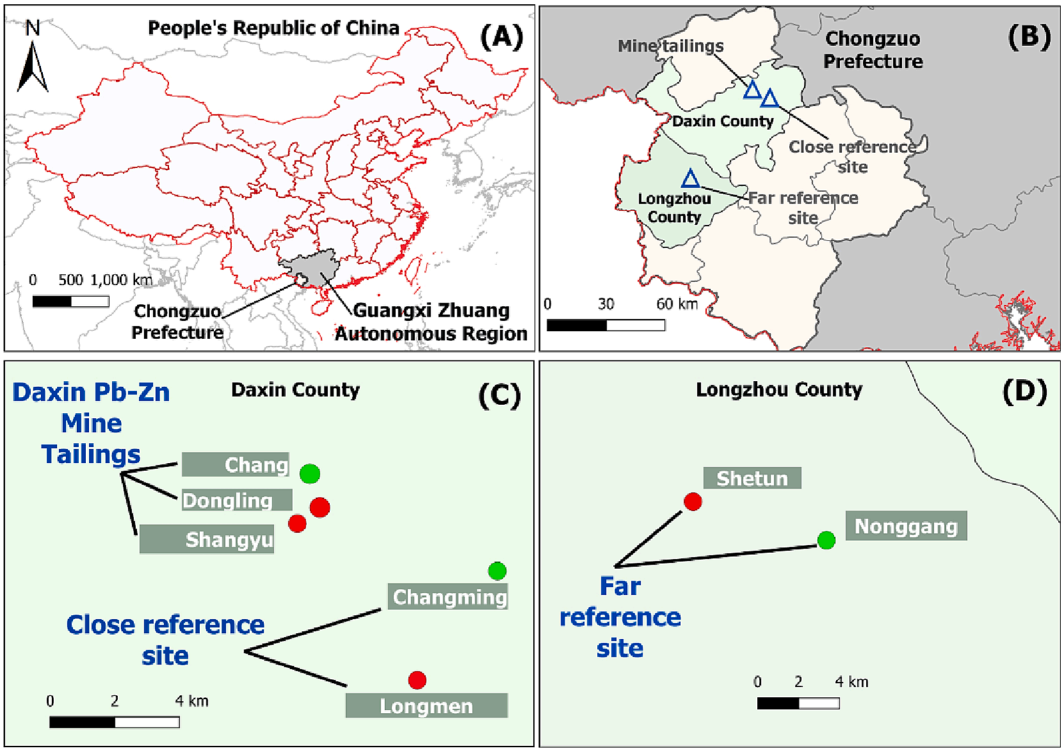

Fig. 1. A map describing the three study sites and seven sampling locations in Chongzuo Prefecture, Guangxi Zhuang Autonomous Region, China. Red locations were rice paddy ecosystems, whereas green ones were sugarcane ecosystems. Subfigure (A) indicates the location of Chongzuo Prefecture in Guangxi Zhuang Autonomous Region, China. Subfigure (B) shows the three sites in Chongzuo Prefecture such as the mine tailings and close reference site (in Daxin County) and far reference site (in Longzhou County). Subfigures (C) and (D) indicate sampling locations (rice paddy and sugarcane ecosystems) at each site. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2.2. Sample collection

Sampling was conducted during the summer (June to August) of 2020 and spring (March to May) of 2021 with soil, plant and animal samples taken at the same locations and times. At each location, sampling was conducted in an area of about 500 m × 500 m. Within this area, we established 50 1 m × 1 m quadrats, which were randomly distributed and spaced at least 10 m apart from each other.

Subsamples of soil were collected using an auger, sampling at a depth of 0–20 cm, collecting 12 samples of 50 g each, and then combining them to make a composite sample. The composite soil sample was spread over a nylon sheet and unwanted matters such as stones and roots removed. Then we collected 500 g of the composite sample into clean polyethylene sampling bags, and labelled, and stored them before further procedures in the laboratory (Davidson, 2013).

For plants, we concentrated on above ground plant tissues, as we were sampling primarily above ground herbivorous insects. For rice samples, 4–5 panicles and several rice leaves were collected at each quadrat in rice paddy and combined to make a composite sample. Likewise, 4–5 aboveground sugarcane leaves were sampled at each quadrat in sugarcane to obtain a composite sample. We collected samples when rice plants were about 1–1.2 m in height, and sugarcane plants were about 2–5 m tall. All the collected composite samples were stored in polyethylene bags before chemical analysis.

Animals were collected through an area search of the entire 500 m × 500 m sampling area in rice and sugarcane fields; although they were not necessarily collected in the same 1 × 1 m quadrats as the soil and plant samples, we ensured that sampling was spread throughout the sampling area. We used sweep nets (38 cm diameter) to trap 188 crickets (*Teleogryllus emma*) and 320 grasshoppers, including 107 *Atractomorpha sinensis* and 213 *Oxya chinensis*. A total of 522 spiders, including 136 *Pirata piraticus*, 139 *Tetragnatha nitens* and 247 *Lycosa grahami*, were directly collected by hand. A total of 161 frogs, including 13 *Duttaphrynus melanostictus*, 35 *Microhyla pulchra* and 113 *Fejervarya multistriata*, were captured by long-handled aquatic nets. The sample size of each animal species collected at each site in each agricultural ecosystem is shown in Table S1. The collected animals were euthanized using 95% ethyl alcohol and preserved in polyethylene bags at −14 °C, before cryopreservation in the laboratory at −80 °C.

2.3. Sample preparation

All the glassware, plastic tubes, and other used equipment were first soaked in 5% HNO_3 solution overnight, rinsed thrice in the ultrapure water and then oven-dried at 60 °C for about 8 h before use (Komarnicki, 2000). For soil, we air-dried samples for about two weeks in the laboratory and ground the samples with a well-cleaned agate mortar and pestle, into fine soil particles to attain the homogeneity of soil particles (Davidson, 2013). The soil samples were sieved through a 2 mm sieve made of stainless steel, then labelled and stored in polyethylene bags prior to the metals/metalloids analysis. Plant samples were washed three times using tap water to remove the impurities, and then rinsed three times using ultrapure water. They were then air-dried for about two weeks in the laboratory until they reached a constant dry weight (Yung et al., 2019). Washing for invertebrates and frogs followed similar procedures to those for plants. For frogs, we then dissected and sampled leg muscles since they have been shown to positively correlate with other internal tissues in their metal/metalloid residues (Hothem et al., 2010). Both invertebrate and vertebrate samples were air dried for 24 h (Yung et al. 2019).

2.4. Laboratory analysis

We measured Hg separately using the Direct Mercury Analyser

(DMA-80, Milestone Srl, Italy) at Guangxi University. This technique measures THg, although it is methylated Hg that is more bioavailable and ultimately toxic (Campbell et al. 2005; see discussion). Prior to analysis, plant and animal materials were cut into small pieces using sterilized stainless steel scissors. We combined several air-dried individuals of the same species for the smaller invertebrates to make a single sample weighing at least 0.0100 g. Dry weights for the tested samples were 0.0300 to 0.0550 g for soil, 0.0388 to 0.1610 g for plant material, 0.0100 to 0.4000 g for invertebrate samples, and 0.0150 to 0.5000 g for frog samples. THg concentrations in all samples were then analysed through thermal decomposition, amalgamation, as well as atomic absorption spectrophotometry at 254 nm, using the DMA-80, Milestone Srl, Italy.

All other metals/metalloids were analysed using the Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES; model ICP-5000, Focused Photonics Inc., China). Prior to analysis, we digested soil samples using 65% HNO_3 (guarantee reagent [GR]) through the graphite furnace digestion method in the graphite digestion furnace (PROD60, Zerom, China), following guidelines by Zhao et al. (2018). Plant samples were oven-dried at 60 °C for 48 h in the laboratory (Pereira et al., 2006). We then digested plant samples using 65% HNO_3 (GR) through the graphite furnace digestion method in the graphite digestion furnace (PROD60, Zerom, China), following the procedures by Huang et al. (2016). Animal samples were oven-dried at 60 °C for 24 h (Pereira et al., 2006). We then digested these samples using 65% HNO_3 (GR) through the graphite furnace digestion method in the graphite digestion furnace (PROD60, Zerom, China), following the procedures by Ma et al. (2012). Dry weights for the tested samples were 0.2200 to 0.5600 g for soil, 0.1400 to 0.5200 g for plant material, 0.0200 to 0.2500 g for invertebrate samples and 0.0400 to 0.3100 g for frog samples. ICP-OES was conducted at Guangxi University (model ICP-5000, Focused Photonics Inc., China). We present all the results in mg/kg d.w. of metal residues.

2.5. Quality control

For the THg analysis, we ran sample blanks (i.e., empty sample boats) and blind samples before starting each THg concentration reading, to ensure there were no Hg residues present in the sample boats and the machine. We considered sample boats valid for use if they had an absorbance of <0.05 μg/kg. We then used the human hair standard reference (IAEA-086) to draw a linear calibration curve, with a regression coefficient (R^2) of 0.9999. We tested blind samples and human hair standard reference material (IAEA-086) after every ten samples to ensure the accuracy, as well as precision of our method and equipment. The obtained THg concentrations of the analyzed samples were considered to be valid if when they were within the range of 0.534 to 0.612 mg/kg, which is the 95% confidence interval (CI) of the human hair standard reference (IAEA-086) (Bleise et al., 2000). The percentage Hg recovery rate of the human hair standard reference material (IAEA-086) was 100.7 ± 3.8% (all measurements of variability are standard deviations). The detection limit of the DMA-80 is 0.2 μg/kg Hg.

For other metals, we tested the certified soil (GBW07404a [GSS-4a]), plant (GBW10010a [GSB-1a]) and animal (GBW10051 [GSB-29]) reference material and spiked standard solutions after every fifteen samples to check for accuracy and precision. The mean recovery rates for the certified reference materials and the spiked standard solutions are indicated in Table S2. We prepared sample blanks in the same way as the other samples to determine the method detection limits, as well as the limit of quantification of the instrument ([ICP-OES; model ICP-5000, Focused Photonics Inc., China]; for these measurements see Table S2).

2.6. Statistical analysis

We analyzed our data using the RStudio (R version 3.6.3). We checked whether the response variables (i.e. metal/metalloid concentrations and bioaccumulation factors [BAFs]) met or violated the parametric assumptions through visual assessment of residual graphs. When assumptions were not met, we log or square-root transformed the soil concentrations and organisms' BAFs. We pooled together the two rice sampling locations at the mine site, due to the absence of significant variation between the two locations.

To understand the differences in metal contamination levels between the mine site and the two reference sites, we ran eight different ANOVA (analysis of variances) tests (i.e. one for each metal/metalloid) separately for soil and for each living species in each agricultural ecosystem, with sampling site (three levels: mine, close reference site, far reference site) as the independent variable. ANOVA was followed by multiple comparisons, using Tukey Honest Significant Difference (HSD) method.

To compare the differences in metal concentrations between rice paddy and sugarcane agricultural ecosystems at a site for soil and for the species that they had in common, we used two-sample t-tests for each metal/metalloid.

To investigate the potential biomagnification patterns of metals, we first calculated bioaccumulation factors (BAFs) for each plant and animal species, as the metal/metalloid concentrations in the organism's tissue divided by the metal/metalloid concentrations in the surrounding medium (Ali and Khan, 2019; Conder et al., 2012; Zenker et al., 2014), which was the mean soil concentration at each sampling location. BAF values were averaged across individuals for a species. We then compared BAF values among species, with ANOVA models for each metal/metalloid in each agricultural ecosystem at each site, followed by Tukey HSD multiple comparisons.

To measure the strength of biomagnification or biodilution patterns, we classified the patterns into five categories (very strong, strong, medium, weak, and no relationship) based on which pairwise comparisons were significant, and the trophic levels of the species involved. The trophic levels of species are summarized in Table S3. A very strong result for biomagnification was when all insectivorous species had (significantly) higher BAFs than any other species, herbivorous species were intermediate, and primary producers had the lowest BAFs. A strong biomagnification result was when all insectivores were higher than or as high as all other species, but herbivores could be as high as them, or as low as primary producers. A medium biomagnification result was when one of the insectivore species had the highest BAFs, the herbivores and the other insectivores were intermediate, and primary producers had the lowest BAFs. A weak biomagnification result was when one of the insectivore categories had the highest BAFs, but in this case, the herbivore or the other insectivore species were not intermediate; i.e., the herbivore could be as low as the primary producers, or as high as the highest insectivore, or another insectivore could be as low as the primary producers. Finally, we categorized no relationship when the herbivore had the most extreme BAFs, or there were no significant differences among the species. Biodilution patterns were likewise classified in the opposite direction (i.e., primary producers with highest BAFs).

To compare the strength of biomagnification patterns between the two agricultural ecosystems and between the study sites, we assigned scores to the patterns corresponding to their strength. We scored 3 for strong biomagnification (there were no very strong results), 2 for medium biomagnification, 1 for weak biomagnification, 0 for no relationship, and −1 for weak biodilution (in one instance for Cr residues). These values were assigned to Cd, Cr, Cu, Pb, THg, and Zn residues; As and Mn were excluded in this analysis because they primarily showed biodilution. Then, we used a repeated measures *t*-test (i.e., each metal was sampled at each site in both the agroecosystems), and repeated measures ANOVA, to compare the strength of biomagnification patterns between the two agricultural ecosystems and between the three study sites, respectively. The repeated measures ANOVA was followed by pairwise paired t-tests between the groups, with the significance level adjusted for multiple tests using the Bonferroni method.

To examine whether there were relationships between biomagnification patterns and metal/metalloid contamination in soil, we ran eight simple linear regression models (one for each metal/metalloid, each with six datapoints representing each sampling location) determining how the strength of biomagnification was related to soil contamination level. In this and other tests, we considered P-values < 0.05 to be statistically significant. Other than multiple comparisons, we did not correct our P-values for the many tests we made, given controversy on this topic (Armstrong, 2014; Lee and Lee, 2018), as some authors believe no correction should be done at all (Rothman, 1990), while others consider correction should be compulsory (Moyé, 1998). However, recognizing that the many tests raise the likelihood of Type I errors, we emphasize results that were found across species, sites or agricultural ecosystems.

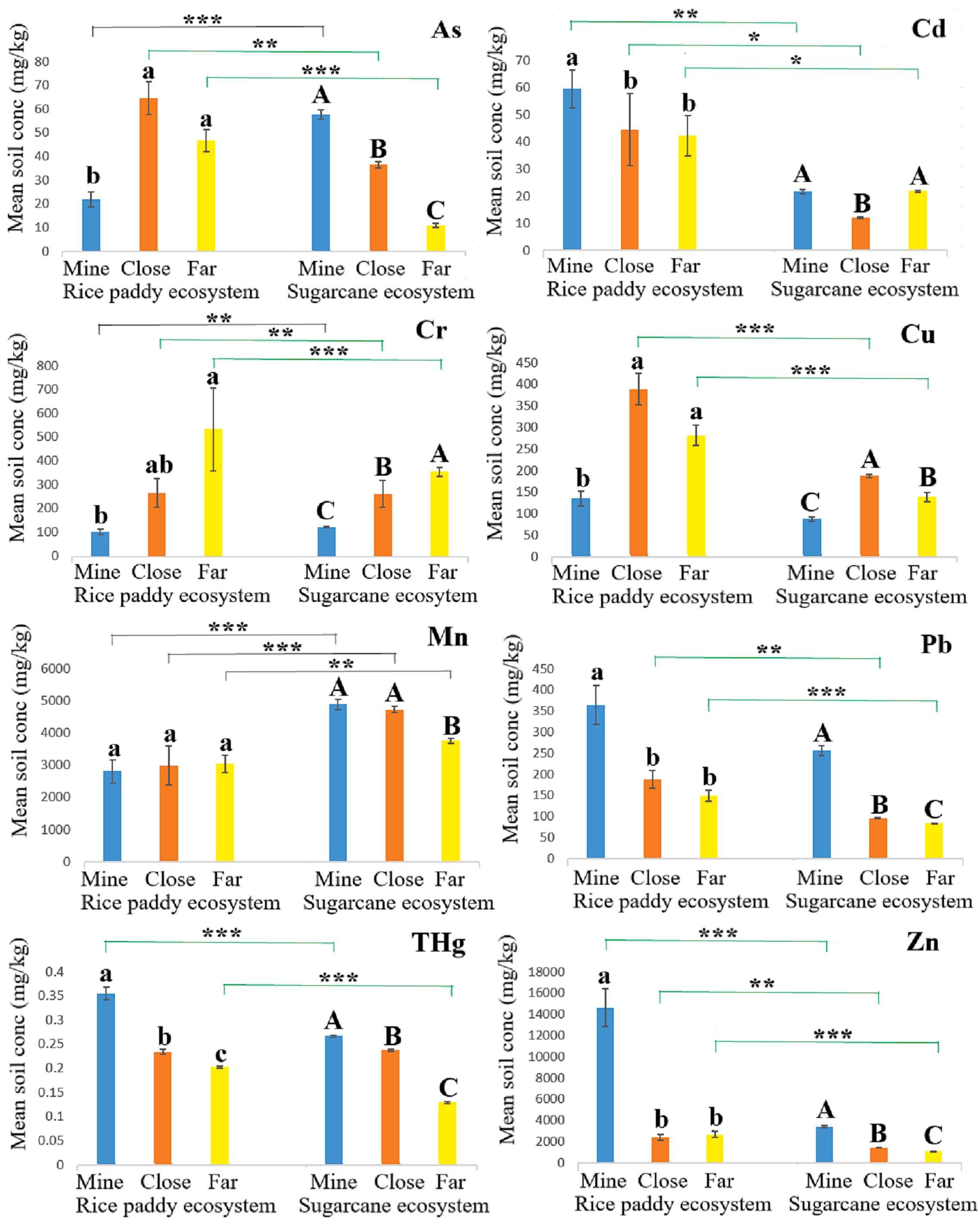

Fig. 2. Metal/metalloid concentrations (in mg/kg) in soil compared between the mine and the two reference sites, and between the rice paddy and sugarcane ecosystems. Small letters and capital letters above the bar charts represent the ANOVA results, followed by multiple comparisons, using Tukey Honest Significant Difference (HSD) method in rice paddy and sugarcane ecosystems, respectively; different letters indicate comparisons between the sites that were significantly different from each other in each ecosystem. The bar columns, colored blue, orange, and yellow, indicate the mine site, the close reference site, and the far reference site, respectively. Green lines above the bar charts indicate metals that were significantly higher in the rice paddy ecosystems compared to the sugarcane ecosystems, whereas gray lines show metals that were significantly higher in the sugarcane ecosystems. * = $p < 0.05$: ** = $p < 0.01$: *** = $p < 0.001$ based on two-sample t-tests. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3. Results

3.1. For what metals/metalloids was there contamination at the mine site?

For soil samples, the metals that had the most consistent evidence of mine-contamination were Pb, THg, and Zn, each of which was found to have significantly higher mean concentrations at the mine site than at both reference sites in both rice paddy and sugarcane ecosystems (Fig. 2). Arsenic and Cd were observed to be considerably higher at the mine site compared to both reference sites in one agroecosystem (sugarcane for As, rice paddy for Cd). Less strong results (e.g., mine site being higher than one of reference sites) were observed for Cd and Mn both in sugarcane ecosystems. In contrast, Cu was found to have significantly higher concentrations at both reference sites than at the mine site in both rice paddy and sugarcane agricultural ecosystems. Arsenic and Cr were found to have considerably elevated concentrations at both reference sites compared to the mine site in one agroecosystem – rice paddy for As, sugarcane for Cr – and Cr also had one reference site with higher concentrations than the mine site in rice paddies.

For living species, the metals that had the most strong, consistent results were Cr, Pb and Zn, each of which was found in three species in an agricultural system to have significantly higher concentrations at the mine site than at both reference sites (Table 1 lists such consistent, strong results; comparisons for all species are shown in Table S1). Cadmium and Mn were found to be significantly elevated at the mine site compared to both reference sites in one species each. In contrast, As and THg were found to have significantly higher concentrations at both reference sites than at the mine site in one species each. Less strong results (e.g., one reference site different from the mine site) generally had similar directions (Table S1).

3.2. How were metal/metalloid concentrations different between rice paddy and sugarcane agricultural ecosystems?

Zinc and Cd were observed to have elevated concentrations in rice paddy soil than in sugarcane soil at all three sites (mine, close and far reference sites; Fig. 2). Arsenic, Cr, Cu, and Pb were found to have higher residues in paddy soil compared to sugarcane soil at both the reference sites. Total Hg was also observed to be significantly higher in paddy soil than in sugarcane soil at two sites (the mine site and far reference site). In contrast, Mn was seen to be significantly higher in sugarcane soil compared to rice paddy soil at all three sites (mine, close and far reference sites). Arsenic and Cr were observed to be higher in sugarcane soil than in rice paddy soil at the mine site.

For living species, the analysis was limited to five cases (31 tests for different metals) in which the same species were seen in both rice and sugarcane agroecosystems at the same site. Copper and Zn were found to have higher concentrations in a species of frog in rice paddy compared to sugarcane agricultural ecosystems at two sites (both reference sites; Table S4). Cadmium, Cr, and Pb were observed to be higher in rice paddy compared to sugarcane ecosystems each in two species at the close reference site. In contrast, Cd, Cr, Mn and THg, were found to have significantly higher concentrations in sugarcane agricultural ecosystems than in rice paddy agricultural ecosystems, each for one species in one site.

3.3. In what metals/metalloids and agricultural ecosystems was there evidence for biomagnification?

All metals and metalloids showed significant differences across species at all sites in rice paddy ecosystems (Table S5). Similarly, all metals and metalloids showed significant differences across species at all sites in sugarcane ecosystems, with two exceptions: As and Mn did not show significant variations among species at the far reference site.

When we looked for differences between species at different trophic levels, there were no very strong patterns, in which all species of insectivores had highest BAFs, herbivores were intermediate, and primary producers were lowest. However, there were four strong patterns (all species of insectivores had highest BAFs, but herbivores were not intermediate) – three for THg, one for Zn; THg and Zn also showed two and one medium biomagnification patterns, respectively (Fig. 3 shows cases of such strong or medium patterns across trophic levels; full results are shown in Fig. S1A-S1F, and broken down by agricultural system, site and metal/metalloid in Table S6). Cadmium and Cr concentrations demonstrated two medium biomagnification patterns, and Cu and Pb had one each. In contrast, Mn showed two medium biodilution patterns, and As concentrations had one. Weak patterns were generally consistent with these results, with weak biomagnification patterns shown for Cu (five patterns), Cd (four patterns), Pb (two patterns), Zn (two patterns), and THg (one pattern), and weak biodilution patterns shown for Mn (three patterns), As (two patterns), and Cr (one pattern; Fig. S1A-S1F).

Overall, rice paddy agricultural ecosystems across all three sites had stronger patterns of biomagnification (3 strong, 7 medium, 6 weak, 3 no relationships) than sugarcane ecosystems (1 strong, 2 medium, 8 weak, 9 no relationships; $t = 3.60$, $df = 17$, p-value $= 0.0023$; Table S6). There was also a tendency for more magnification at the mine site (1 strong, 5 medium, 3 weak, 3 no relationships), and the close reference site (3 strong, 2 medium, 5 weak, 3 no relationships), than the far reference site (0 strong, 2 medium, 6 weak, 6 no relationships), although even the mine vs. far reference site comparison was not significant (P-value of the repeated measures ANOVA $= 0.06$, P-value for mine vs. far reference site, Bonferroni corrected $= 0.47$).

There was a connection between soil contamination and biomagnification, because of the 11 cases in which rice paddy had greater magnification than sugarcane for a certain metal/metalloid (not including As and Mn), 8 also had higher soil contamination in rice than sugarcane (Table S7). However, the effect was not very direct: of the eight regression analyses between soil contamination and biomagnification, only one was nearly significant (Cd; P-value $= 0.0602$, $R^2 = 0.53$), acknowledging of course that regressions with six datapoints do not have high power (Fig. S2).

4. Discussion

The objective of this project was to compare metal/metalloid contamination in two different agricultural ecosystems that are common in subtropical and tropical countries, rice paddy and sugarcane. Below we discuss our results in relation to the questions and hypotheses, after first discussing some limitations of this study.

One limitation of this study is that we measured THg and not MeHg, which is the primary biomagnifying compound for Hg trophodynamics (Campbell et al., 2005). We expect that patterns of biomagnification found for THg would be stronger, if we had the capacity to test for MeHg. Our study was also not ideal because it measured a single gradient away from a single mine, lacked sampling of higher trophic animals such as birds and snakes, and had few species that were found in both rice and sugarcane agroecosystems. We hope that future studies with multiple mines and a wider range of sampled taxa could expand on our findings.

4.1. For what metals/metalloids was there high contamination at the mine site?

As might be expected at a former Pb/Zn mine, Pb and Zn were elevated at the mine site, with each of these metals higher at the mine site than either reference sites in both agroecosystems in soil, as well as in multiple living species (three consistent results each). However, in addition, other metals also showed some signatures of mine-associated contamination. The elevated concentrations of some of these non-target metals are likely linked to the presence of mine tailings. There are no natural ores where Cd can be extracted or mined as the primary target metal, but it is produced during the extraction of other metals,

Table 1

Metal/metalloid concentrations (mean ± SD [standard deviation] in mg/kg) compared between the mine and the two reference sites. Each row of the table represents one analysis of a species in one agricultural ecosystem, and presents ANOVA results, followed by Tukey Honest Significant Difference (HSD) multiple comparisons. This table shows only those analyses that were found to be significant (P < 0.05) and to have consistent results for both reference sites. Orange shaded cells represent those metals that were significantly higher at the mine site than at both reference sites; blue shaded cells show metals that were significantly higher at the reference sites than at the mine site. Full results are shown in Supplemental Table 1. R1 and R2 indicate the close and far reference sites, respectively.

Metals	Plant or animal species	Agricultural ecosystem	Sites						Statistical summaries				
			Mine		Close reference		Far reference		ANOVA results			Tukey HSD Tests	
			N	mean±sd	N	mean±sd	N	mean±sd	F	df	p-value	Difference	p- value
Cr	Frogs *Fejervarya multistriata*	Sugarcane	7	12.1151± 4.7134	7	0.4974± 0.6154	7	0.3435± 0.3228	9.599	(2, 18)	0.0015	Mine vs. R1 Mine vs. R2	0.0029 0.0045
	Frogs *Microhyla pulchra*	Sugarcane	7	10.9509± 4.6419	7	3.1862± 5.9411	7	4.4289± 10.2824	6.433	(2, 18)	0.0078	Mine vs. R1 Mine vs. R2	0.0078 0.0456
	Spiders *Lycosa grahami*	Sugarcane	36	7.7406± 11.6216	54	1.5303± 4.4115	52	0.2057± 0.7876	10.34	(2, 139)	< 0.0001	Mine vs. R1 Mine vs. R2	0.0226 < 0.0001
Pb	Sugarcane leaves *Saccharum officinarum*	Sugarcane	40	2.5917± 1.6400	48	1.6007± 1.5586	48	1.8648± 2.0865	5.785	(2, 133)	0.0039	Mine vs. R1 Mine vs. R2	0.0119 0.0073
	Frogs *Fejervarya multistriata*	Sugarcane	7	43.7811± 49.9414	7	1.2783± 0.4907	7	2.1416± 1.7136	40.34	(2, 18)	< 0.0001	Mine vs. R1 Mine vs. R2	< 0.0001 < 0.0001
	Spiders *Lycosa grahami*	Sugarcane	36	28.1750± 34.0761	54	5.0037± 7.3715	52	2.9445± 5.1410	5.49	(2, 139)	0.0050	Mine vs. R1 Mine vs. R2	0.0257 0.0050
Zn	Rice *Oryza sativa*	Rice paddy	36	184.318± 79.7498	36	122.478± 46.0776	36	75.9485± 35.8159	22.080	(2, 105)	< 0.0001	Mine vs. R1 Mine vs. R2	0.0020 < 0.0001
	Sugarcane leaves *Saccharum officinarum*	Sugarcane	40	129.118± 68.3383	48	95.5270± 41.1107	48	86.5803± 19.1343	9.042	(2, 133)	0.0002	Mine vs. R1 Mine vs. R2	0.0036 0.0003
	Spiders *Lycosa grahami*	Sugarcane	36	1815.715 ±1746.97	54	655.538± 498.522	52	798.100± 676.410	7.752	(2, 139)	0.0006	Mine vs. R1 Mine vs. R2	0.0006 0.0070
Cd	Frogs *Fejervarya multistriata*	Sugarcane	7	9.5388± 6.0799	7	0.1394± 0.1331	7	0.3396± 0.2535	17.26	(2, 18)	< 0.0001	Mine vs. R1 Mine vs. R2	0.0001 0.0030
Mn	Sugarcane leaves *Saccharum officinarum*	Sugarcane	40	506.019± 137.545	48	402.910± 246.638	48	183.442± 51.5686	59.950	(2, 133)	< 0.0001	Mine vs. R1 Mine vs. R2	0.0014 < 0.0001
As	Frogs *Microhyla pulchra*	Sugarcane	7	0.0511± 0.1330	7	6.0042± 6.0507	7	2.3977± 1.8830	6.253	(2, 18)	0.0087	Mine vs. R1 Mine vs. R2	0.0280 0.0119
THg	Frogs *Fejervarya multistriata*	Rice paddy	13	0.0448± 0.0397	11	0.3869± 0.3532	13	0.0990± 0.0409	15.75	(2, 34)	< 0.0001	Mine vs. R1 Mine vs. R2	< 0.0001 0.0168

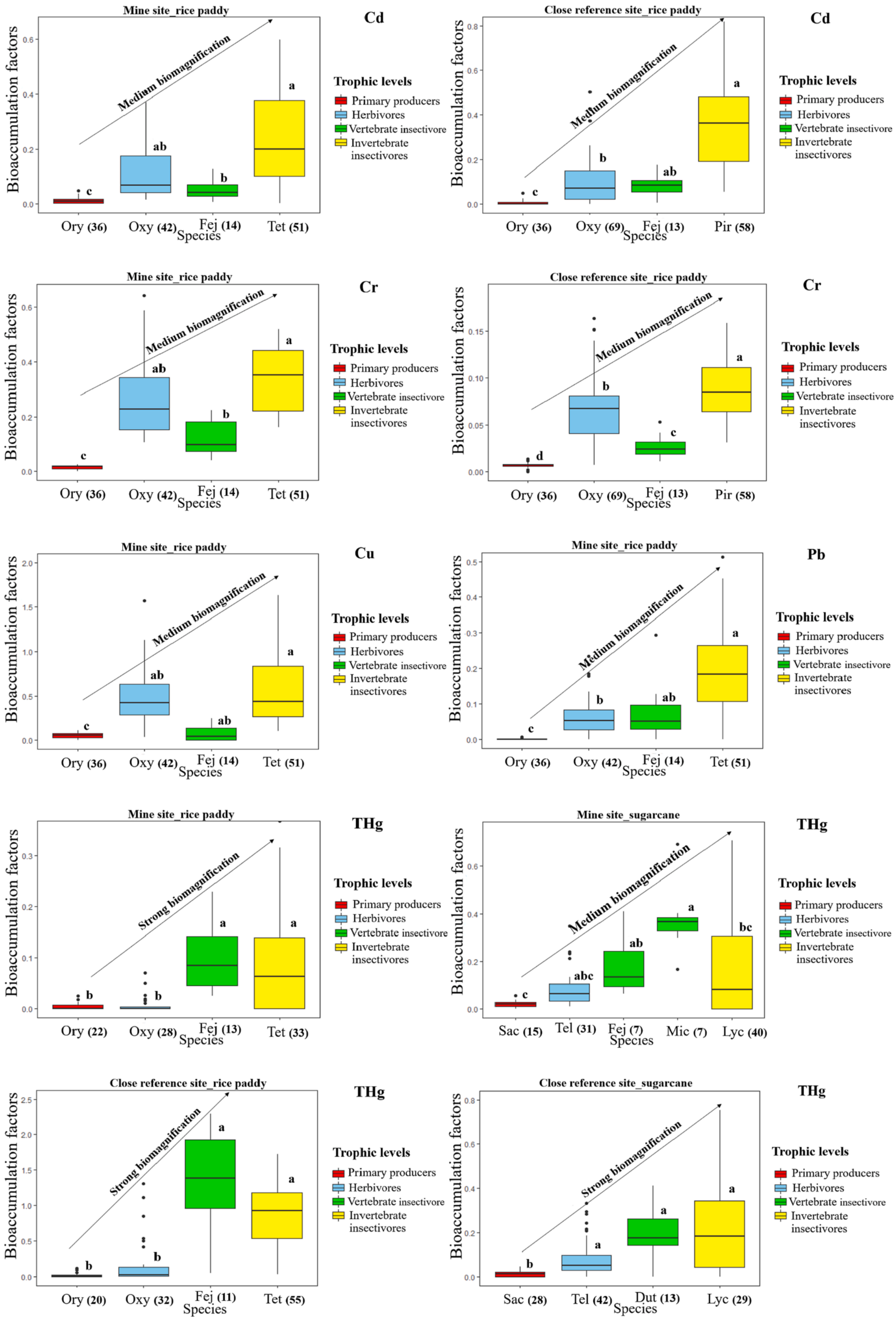

Fig. 3. Evidence for patterns of biomagnification (rising arrow) or biodilution (falling arrow) across trophic levels for different metals and metalloids. This figure only shows strong or medium patterns; see methods for definitions of all terms, and Fig. S1A-S1F for all results. Species with different letters were significantly different from each other, according to Tukey HSD multiple comparisons following ANOVA. Species abbreviations: Ory: "*Oryza sativa*", Sac: "*Saccharum officinarum*", Atr: "*Atractomorpha sinensis*", Oxy: "*Oxya chinensis*", Tel: "*Teleogryllus emma*", Dut: "*Duttaphrynus melanostictus*", Fej: "*Fejervarya multistriata*", Mic: "*Microhyla pulchra*", Lyc: "*Lycosa grahami*", Pir: "*Pirata piraticus*", and Tet: "*Tetragnatha nitens*". Number in parentheses represents sample sizes.

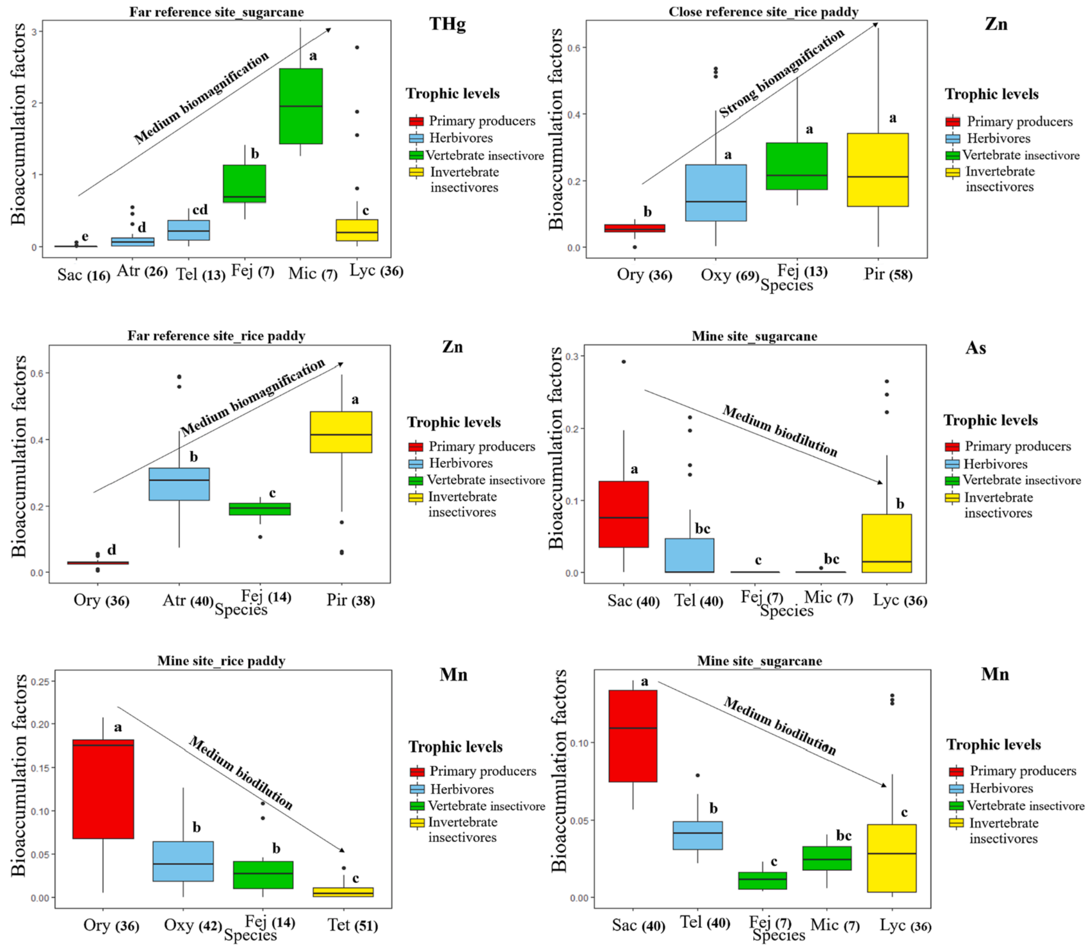

Fig. 3. (*continued*).

and notably from Pb-Zn mines as a secondary product (Alloway, 2013b; Zubair et al., 2021). Additionally, studies show that Pb-Zn mine tailings can contain elevated Cr and Mn concentrations (Du et al., 2019; Huang et al., 2020; Shu et al., 2003). Mercury residues have also been reported in Zn ores (Alloway, 2013b).

One interesting pattern is that the close reference site (6 km away from the mine) was intermediary between the concentrations of the mine and the far reference site (60 km away from the mine) for soil concentrations of As, Mn, Pb, THg, and Zn; four of these findings were in sugarcane ecosystems. This suggests that the contamination may have spread at least 6 km from the mine site, although there was no above-ground water flow between the close reference and mine sites. Contamination could perhaps be associated with an underground water source. Acid mine drainage flowing from mining areas to other neighbouring regions has been identified as a route of exposure to metals/metalloids elsewhere (Choudhury et al., 2017; Zhang et al., 2013). Or metal-bearing dust could be blown from the mine tailings (Kabala et al., 2020; Pierwoła et al., 2020), although the luxuriant vegetation in this area would seem likely to stabilize the soil and block the wind.

For situations in which soil metal/metalloid concentrations were higher in the reference sites (such as in Cu, Cr and As), explanations could involve variations between sites in agricultural treatments or background levels of the metals dictated by geology. For example, Cu in particular may be used as an additive in animal feeds to control for disease (Liu et al., 2020; Luo et al., 2009; Peng et al., 2019). Guangxi is also well known for having high background levels of many metals/metalloids, including As and Cr (Hu et al., 2020; Li et al., 2007; Yin et al., 2019).

4.2. *How were metal/metalloid concentrations different between rice paddy and sugarcane agricultural ecosystems?*

We found that there were substantially higher concentrations in soil and living organisms in rice paddy ecosystems compared to sugarcane ecosystems. Concentrations of seven out of the eight studied metals/metalloids (Mn being the exception) were higher in rice paddy soil than in sugarcane soil in at least two of the three sites. Similar patterns were observed in living species: five metals (such as Cd, Cr, Cu, Pb, and Zn) had higher concentrations in living organisms in rice paddy compared to sugarcane in at least two species, or two sites.

Water used during irrigation in rice paddy fields may be responsible for this difference. In China, over 80% of sugarcane is cultivated where irrigation is not present or is limited (Li and Yang, 2015); and our sugarcane sites were not irrigated. It is also possible that a combination of water and agricultural treatment is responsible for the difference between the two agroecosystems. Over-application of fertilizers is a major problem in China: not only escalating production costs, but also leading to detrimental impacts in the environment, including acidification of agricultural lands (Guo et al., 2010). The effects of acidification may be pronounced in rice paddy soil because of excessive application of chemical fertilizers, particularly nitrogen (N) fertilizers (Guo et al., 2010; Guo et al., 2018). Double-rice cropping patterns practiced in southern China could be another reason for vulnerability of rice paddies to acidification, due to the consumption of larger quantities of irrigation water and fertilizers (Zhao et al., 2015), especially if acid mine drainage is involved, as discussed above. Acidification can lead to increased bioavailability of some cationic metals such As, Cd, Cu, Hg, and Pb in soil, plants and consequently in the entire food chain (Gall et al., 2015;

Zhao et al., 2015).

4.3. In what metals/metalloids and agricultural ecosystems was there evidence for biomagnification?

We found that biomagnification in both vertebrate and invertebrate consumers was more widely spread than just THg, which is well-known to biomagnify (Abeysinghe et al., 2017; Cristol et al., 2008; Jiang et al., 2022; Sun et al., 2020; Zhao et al., 2013). Other metals that showed a medium or weak tendency to biomagnify included Zn, Cd, Cr, Cu and Pb. As for nonphysiological elements, including Cd, Hg, and Pb, many species are not able to regulate their levels through excretion (Rainbow, 2002). Instead, these organisms may effectively avert the metals' toxicity by storing them in less or nontoxic forms that involve the process of metals binding to metal-binding proteins (i.e. metallothionein molecules) or incorporated in non-soluble granules (Fairbrother et al., 2007; Foulkes, 1993). Therefore, animals depending on sequestration as a sole way of detoxification may accumulate metals at high exposure levels owing to the low depuration of firmly bound metals (Veltman et al., 2007), which could translate into biomagnification potential (Cheng et al., 2013). As for physiologically important elements such as Cr, Cu, and Zn, their concentrations are homeostatic and regulated by living organisms (Rainbow, 2002; Veltman et al., 2007). However, the homeostatic regulation could be overwhelmed when there is high exposure; hence biomagnification could occur in such situations (Veltman et al., 2007), which could underlie the biomagnification patterns shown by Cr, Cu and Zn in this study. There are other studies that have showed these metals to biomagnify: Cd (Cardwell et al., 2013; Tasneem et al., 2020; Zhao et al., 2013), Zn (Campbell et al., 2005; Quinn et al., 2003; Shilla et al., 2019; Sun et al., 2020; Tasneem et al., 2020), Cu (González et al., 2008; Tasneem et al., 2020), Pb (Jiang et al., 2022; Sun et al., 2020; Tasneem et al., 2020), and Cr (Gu et al., 2022; Liu et al., 2019). This study is among a few studies that have shown multiple metals to biomagnify in the same ecosystems (Campbell et al., 2005; Ikemoto et al., 2008; Jiang et al., 2022; Sun et al., 2020; Tasneem et al., 2020).

Other metals/metalloids such as As and Mn showed patterns of biodilution. For Mn, this is most likely because it is a physiologically important element that plays the part as a cofactor of some enzymes (Ikemoto et al., 2008), and most living organisms, especially animals located in the middle to the top trophic levels of the food chain, are capable of regulating Mn concentrations through homeostatic mechanisms (Ikemoto et al., 2008). Whereas, for As, the most toxic metalloid, all of its forms (organic and inorganic) are easily absorbed from the gastrointestinal tract, and then eliminated from the body, thus lowering their concentrations, as well as their transfer across trophic levels in the food web (Campbell et al., 2005; Zhao et al., 2013).

Overall, the rice paddy agricultural ecosystems showed stronger patterns of biomagnification than sugarcane ecosystems. One reason for this could be simply that there were higher concentrations there; biomagnification might not be detectable if there is very little of the metal in the environment. For instance, Quinn et al. (2003) found biomagnification of Zn both in contaminated and non-contaminated streams, but biomagnification strength was more noticeable and stronger in a contaminated stream receiving acid mine drainage. Likewise, Watanabe et al. (2008) found biodilution patterns of Pb to change with severity of contamination in all contaminated sites, so that at more contaminated sites, Pb biodilution patterns were more pronounced. In our study, 8/11 cases where biomagnification was greater in rice paddies occurred also in sites where contamination was higher in rice paddies. Nevertheless, a direct link between contamination levels and biomagnification was not very clear: none of the regressions between these values for the different metals/metalloids was significant and four (such as Cr, Cu, Mn, and Zn) were not positive, making it likely that something else about the agroecosystem of rice paddies made them biomagnify more metals.

Rice paddies are known to be an especially sensitive ecosystem for Hg biomagnification (Abeysinghe et al., 2017; Zhang et al., 2010), and it is possible that this sensitivity is also true for other metals/metalloids. Rice paddies are particularly vulnerable to metal/metalloid pollution due to the centrality of water to their growth and development (Ali et al., 2019). Water influences the dissolving, solubilizing, transporting, and detaching of metals from soil particles, sediments, and organic matters that make them bioavailable, and transferrable in tissues of living organisms in ways distinctive to their trophic levels (Gall et al., 2015; Neilson and Rajakaruna, 2012), which could lead to more consistent patterns of metal biomagnification.

5. Conclusion and recommendations

The novelty of this research was based on the comparison of rice paddy to sugarcane agricultural ecosystems in their metal/metalloid contamination and their biomagnification patterns. We found three major findings that may be interconnected: metal/metalloid concentrations in soil, and in living organisms, were higher in rice paddy than in sugarcane, and so was the degree of biomagnification. Indeed, we found several metals other than Hg (including Cd, Cr, Cu, Pb and Zn) that showed some level of biomagnification. A next step may be to study metal/metalloid apportionment to better understand the sources of the metals at these study sites and thereby lower the exposure of wildlife and people. Finally, the local authorities should check metal concentrations in farming products from the mining area, and even the close reference site, to safeguard human health.

Ethical guidelines

The use of animals in this study was approved by Animal Ethics Committee, Guangxi University (GXU2018-046). We collected common and not threatened species (i.e., no species considered threatened by China or the IUCN), and sacrificed them as immediately and painlessly as possible.

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Data accessibility.

Upon acceptance, we will upload data on the concentrations of all samples on an on-line data archive (e.g. Dryad) or as supplemental data.

CRediT authorship contribution statement

Wambura M. Mtemi: Conceptualization, Methodology, Investigation, Data curation, Visualization, Formal analysis, Writing – original draft. **Shilong Liu:** Methodology, Software, Investigation, Formal analysis. **Kangmei Liu:** Methodology, Software, Investigation, Formal analysis. **Lini Wei:** Methodology, Software, Investigation, Formal analysis. **Xueli Wang:** Methodology, Software, Investigation, Formal analysis. **Aiwu Jiang:** Resources, Supervision, Validation, Project administration, Funding acquisition, Writing – review & editing. **Eben Goodale:** Resources, Supervision, Validation, Project administration, Funding acquisition, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2023.110266.

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