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Research article

Bio-accumulation effects of heavy metals Pb, Zn and Cd on *Procecidochares utilis* parasitism to *Eupatorium adenophorum* at Suzu metal mines, Yunnan

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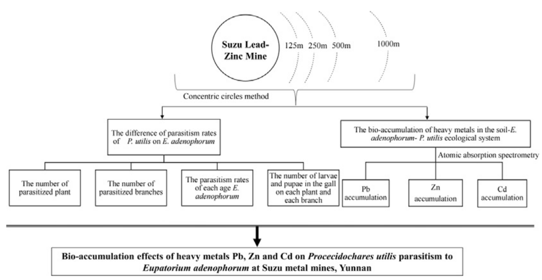
HIGHLIGHTS

- Concentric circles method was used to assess heavy metals accumulation.
- Heavy metals pollution in mining area reduced the amount of *Procecidochares utilis*.
- The content of heavy metals uptrend alongside drawing closer to mine center.
- The parasitic rate is positively correlated with the distance from mine center.
- Metals bioaccumulation lowered parasitic effect of *P. utilis* on Crofton weed.

GRAPHICAL ABSTRACT



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ABSTRACT

Procecidochares utilis is an obligatory parasitic insect to *Eupatorium adenophorum*. Both organisms have been spread to some metal mines areas. The objective of this study is to comprehend the trend of heavy metals transfer and the process of their bio-accumulation in the soil-*E. adenophorum*-*P. utilis* system and particularly their impact on the parasitic effect of *P. utilis* to *E. adenophorum* to reflect the impact of heavy metals on obligate parasitic insect and its host. Therefore, a detailed investigation was carried out at the Suzu Lead–Zinc Mine in Yunnan Province using the concentric circle's method. The results showed that the parasitic rate of *P. utilis* to a single plant and branch is positively correlated with distance. The metals content of the soil in *E. adenophorum* and *P. utilis*, decreased dramatically with an increase in distance away from the center of the mining area. From which is cleared that these metals could enter to *E. adenophorum* and *P. utilis* through the soil-*E. adenophorum*-*P. utilis* system which likely to affect its parasitic activities. In addition, the parasitic rate is impacted by per Zn content greatly, and the parasitic rate per plant is affected by Cd content enormously. This work could provide important basis of data for further understanding and clarifying the effects of bioaccumulation and heavy metals pollution on various aspects of the food chain. Simultaneously, it could clarify and simplify whether heavy metal contamination affects the parasitic behaviour of some obligatory parasitic insects.

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1. Introduction

Heavy metals are produced by a variety of industrial processes, agricultural activities, domestic trash, and automotive emissions. Due to their high level of toxicity, persistent nature, and bio-accumulation, these are considered among the most serious pollutants in the environment (Alshahri, 2017; Gonzalez-Macias et al., 2006; Li et al., 2008; Li and Zhang, 2010 a,b; Rampley et al., 2020; Zohra and Habib, 2016). The biggest environmental challenge posed by heavy metals is their infiltration into the soil around metal mining areas (Karaca et al., 2018; Pérez-Esteban et al., 2014; Sun et al., 2019). These toxic metals enter the bodies of plants, animals and humans by passing through the food chain in the soil ecological system (Gall et al., 2015; Heikens et al., 2001; Li et al., 2018; Sarwar et al., 2017; Zhang et al., 2018). The harmful effects from high concentrations of these heavy metals have resulted in the diminishing of insects, animals, and some plants species (Goretti et al., 2020; Heikens et al., 2001; Nieminen et al., 2001; Sarwar et al., 2017; Satta et al., 2012). Several studies have indicated that heavy metals hyper-accumulators and tolerant plant species could effectively take up heavy metals from the soil (Li et al., 2019; Pandey et al., 2019; Salati et al., 2010; Sarwar et al., 2017; Zu et al., 2005).

Eupatorium adenophorum (syn. *Ageratina adenophora*, common name: Crofton weed), is now a widespread noxious invasive weed, which is spreading rapidly in China and causing substantial damage to the ecological environment currently (Oelrichs et al., 1995; Sharma et al., 1998; Wan et al., 2010; Wan et al., 2010, 2010; Wang and Wang, 2006; Wu et al., 2020; Yu et al., 2005; Zheng et al., 2009). Studies have shown that, in some metal mines where *E. adenophorum* had spread, it has been proven to be an efficient accumulator of lead (Pb), Zinc (Zn), and cadmium (Cd) (Chen et al., 2014, 2016; Li et al., 2016; Li and Zhang, 2008; Song et al., 2016; Wang et al., 2013; Wang and Zhang, 2008; Zu et al., 2005). These metals deposited in *E. adenophorum* may be transmitted and bio-accumulated in natural enemies, which will frequently exert significant negative effects on natural enemies, influencing its parasitic activity, for example, *Procecidochares utilis* Stone (Wang et al., 2013; Ma et al., 2018).

P. utilis Stone (Diptera: Tephritidae), is an obligatory parasitic natural enemy of *E. adenophorum*, arrests its growth and multiplication (Wan et al., 2010; Zhang et al., 1988). Female gall flies normally lay eggs on or near the apical buds of *E. adenophorum*. Larvae then move to the base of the leaves and form tunnels in the stem, where they feed on plant tissues, resulting in the formation of galls (Bennett and van Staden, 1986; Li et al., 2006). *P. utilis* propagates fast and spread out rapidly (at an annual rate of 10000–25000 m) and has established and developed into a natural population covering a 5000 m^2 area in Yunnan province. The presence of these gall flies was recorded in some heavy metal mining areas in this province, and it was confirmed that Pb, Zn, and Cd had transferred and bio-accumulated in the soil-*E. adenophorum*-*P. utilis* system (Wang et al., 2013). The metals transfer from *E. adenophorum* to *P. utilis*, affected the development and protective enzymes (superoxide dismutase, peroxidase and catalase) in the larvae of *P. utilis* (Ma et al., 2018). This may affect the parasitic behaviour of *P. utilis* on *E. adenophorum*. However, no relevant research has been carried out in these heavy metal mining areas to assess this effect (Li and Zhang, 2008; Wang et al., 2013; Wang and Zhang, 2008; Zu et al., 2005).

In this study, we used the concentric circles approach to evaluate the trend of heavy metal transfer and bio-accumulation in the soil-*E. adenophorum*-*P. utilis* system at the Suzu Lead–Zinc Mine in Yunnan Province, and we examined their implications on the parasitic effect of *P. utilis* on *E. adenophorum*. The findings provide a critical theoretical foundation for future understanding and clarification of the consequences of heavy metals contamination on numerous components of the food chain, and to a certain extent revealed the impact of heavy metals pollution on obligate parasitic insects.

2. Materials and methods

2.1. Study area

The study area was located at Suzu Pb–Zn Deposit in Jianshui County, in Yunnan Province, China (103° 03′ E, 23° 57′ N, Figure S1). It has a subtropical humid climate with a mean annual temperature of 18 °C and 950 mm of annual precipitation.

2.2. Investigation of the difference in parasitism rates of P. utilis on E. adenophorum at different positions in the mining area

2.2.1. Determination of the age of E. adenophorum plants

Eupatorium adenophorum blooms once in a year, after which the floral stalk dries up later as the fruit ripens. During the next growth season, lateral buds growing on tips of live stems form a new layer of branches on the plants. In a few years, these plants grow into large bushes by iteration of the same process (Sun et al., 2005). In this study, the age of the plants was estimated based on the number of floral stalks on the main stem, the levels of the branches on the plants and the root system (Sun et al., 2005).

2.2.2. Data collection on the parasitic status of P. utilis on E. adenophorum

The data collection procedure followed the method of Li et al. (2006). The survey area covered the Suzu Pb–Zn deposit at the center (0 m) and at distances of 125 m, 250 m, 500 m, and 1000 m away from this central point. Patches of similar eco-environmental conditions and uniform growth of healthy *E. adenophorum* mature plants with >80% average ground coverage were selected. For each age group (1–4 years old), a simple random sampling was done to collect samples of five biological replicates, each consisting of a pool of 10 plants. The numbers of plants parasitized, parasitized branches, insect galls, larvae and pupae in the gall on each plant and each branch were recorded.

2.3. The biological accumulation of Pb, Zn, and Cd in the soil-E. adenophorum-P. utilis ecological system

2.3.1. Sample collection

The concentric circle diagram sampling method was used to collect the samples from the Suzu Pb–Zn Deposit. Within a 4000 m radius from the center of the metal mine, different sampling sites were selected by equal proportion as D1: 125 m; D2: 250 m; D3: 500 m; D4: 1000 m away from the center. The sample surveyed at 4000 m was used as the control treatment (CK). Samples were taken from sites with the good parasitic status of *P. utilis*. Branches of *E. adenophorum* were chosen randomly and soils were taken from the rhizospheric area at the top 0–20 cm soil layer. On each of the equidistant circles, three replicates of each sample, which consisted of a pool of 5 individual samples were collected.

2.3.2. Analysis of plant samples

Followed the method of Wang and Zhang (2008) and Zu et al. (2005). Plant tissues were washed in tap water to remove all the dirt and other contaminants. The roots, stems and leaves were then separated and continued to be rinsed with deionized water 2–3 times until there were no impurities, and then blotted dry with filter paper and dried 48 h in the 60 °C oven. After drying, it was chopped and well ground, and then passed through 80 mesh sieve. Finally, 1 g of plant sample was digested with 15 mL of mixed acid ($HClO_4$: HNO_3 = 1: 5 v/v) on the adjustable electric heating plate. After the digested was completed and cooled, it was diluted with 5 mL of 0.2% HNO_3. The contents of Pb, Zn and Cd in samples were determined based on absorbance using a TAS-990 atomic absorption spectrometer (Beijing General Instrument Co., Ltd.). The use of the instrument, same as below.

2.3.3. Analysis of soil samples

Followed the method of Zu et al. (2005). Samples were then dried in the room temperature 6 d, then ground and passed through 0.25 mm sieve. Finally, 0.5 g of soil sample was digested with 15 mL of mixed acid (HNO_3: HCl: $HClO_4$ = 1:2:2 v/v/v) on the adjustable electric heating plate. After the digested was completed and cooled, it was diluted with 5 mL of 0.2% HNO_3.

2.3.4. Analysis of insect samples

These samples were collected following the method of Wu et al. (2009). The larvae or pupae of the same age were collected. Each sample had 30 heads of insects which were freeze-dried by a freeze dryer, then determined its dry weight and crush it. Followed by treatment in mixed acid nitration. Finally, the digested was performed using 1 mL of mixed acid ($HClO_4$: HNO_3 = 1: 5 v/v) on the adjustable electric heating plate. After its digested was completed and cooled, it was diluted with 25 mL of distilled water.

2.4. Data analysis

Data analysis of the numbers of parasitized plants, parasitized branches, insect galls, larvae and pupae in the gall on each plant and each branch were performed by using ANOVA (setting the α level to 0.05) with Duncan's New Multiple Range Test for multiple comparisons in the DPS program (Tang and Feng, 2002). The capability of a plant (or any organism) to take up heavy metals was measured by the bio-accumulation factor. It was calculated as the ratio of heavy metals content in *E. adenophorum*/heavy metals content in soil, or heavy metals content in *P. utilis*/heavy metals content in *E. adenophorum* (Bitterli et al., 2010). The significant difference was tested using single factor randomized block analysis of variance and the multiple comparisons of the means were carried out using Duncan's new multiple range test (MRT). Data processing was done by using the DPS program (Tang and Feng, 2002). The correlation between parasitism rate and distance from the center of the mine, as well as the correlation between parasitism rate and heavy metal bioaccumulation were analyzed by linear regression.

3. Results

3.1. Difference between parasitism rates of *P. utilis* on *E. adenophorum* at different positions of the mining area

The parasitic rates of *P. utilis* on *E. adenophorum* per plant showed a linear relationship with distance from the mine center (y = 0.02272x + 77.63, R^2 = 0.7603, P = 0.0539), increased with increasing distance (Figure 1A). The parasitic rates per branch also showed a linear relationship with the distance (y = 0.02749x + 12.12, R^2 = 0.9802, P = 0.0012), increased with increasing distance (Figure 1B). This indicates that with the increase of distance, the heavy metal content decreases, which ultimately leads to the increase of parasitism rate.

The amount of galls per branch increases steadily as one moves out from the mine centre. There is a linear relationship between the two factors, which appear a positive correlation (y = 0.0001800x + 0.1185, R^2 = 0.9498, P = 0.0048) (Figure 2A). The number of gall flies per branch also showed a positive correlation of linear relationship with the distance (y = 0.0002620x + 0.2578, R^2 = 0.9680, P = 0.0025) (Figure 2B). The number of galls per branch reflect the parasitism rate, and the number of gall flies depicts the fecundity of *P. utilis*. These two factors were negatively correlated with the content of heavy metals.

The rate of parasitized branches by *P. utilis* on different aged *E. adenophorum* is positively related with the distance from the mine center (one-year-old: y = 0.007268x + 28.38, R^2 = 0.9869, P = 0.0006; two-year-old: y = 0.01114x + 25.04, R^2 = 0.7525, P = 0.0568; three-year-old: y = 0.01186x + 10.21, R^2 = 0.8863, P = 0.0169; four-year-old: y = 0.01226x + 12.95, R^2 = 0.7681, P = 0.0512) (Figure 3). Meanwhile, heavy metals accumulated in the plants, increased with the increase of time. The parasitized rate on one-year-old and two-year-old branches is higher than three-year-old and four-year-old. It is concluded that the parasitized rate on branches increases with the increase of distance and decrease of plant age.

3.2. Bio-accumulation of heavy metals in the soil-*E. adenophorum*-*P. utilis* system

3.2.1. Bio-accumulation of Pb in the soil-*E. adenophorum*-*P. utilis* system

The bio-accumulation factor of Pb in the soil-*E. adenophorum*-*P. utilis* system is shown in Table 1. The Pb content in the soil (F = 43.9, P < 0.01), *E. adenophorum* (F = 33.8, P < 0.01) and *P. utilis* (F = 114, P < 0.01), decreased significantly as the distance increased from the center of the mine. The soil Pb contents from the center of the mine to the 1000 m distance (within the range, 811–941 mg/kg) were 100 times higher than the highest standard level (80 mg/kg), as specified in the environmental quality standard for soils (China-GB15618-2008). The Pb content at 1000 m away from the center of the mine was still 10.1-fold higher than the standard level, and all the heavy metal contents from 0-1000 m were significantly higher than the data from CK. These results indicated that the soil in the mining area was highly polluted by Pb accumulation. The Pb content of *E. adenophorum* was within the range, 79.4–129 mg/kg. The Pb content in *P. utilis* decreased from 1277 mg/kg to 487 mg/kg as they fed on *E. adenophorum* from the center of the mine to 1000 m away.

The bio-accumulation factor of Pb from soil to *E. adenophorum* was within the range, 0.10–0.14 and the values recorded from the different locations of the mine were significantly different (F = 14.6, P < 0.01). This showed that *E. adenophorum* had a weak ability to accumulate the lead. The bio-accumulation factor from *E. adenophorum* to *P. utilis* was within the range, 2.65–9.87 and decreased significantly as the distance increased from the center of the mine (F = 31.0, P < 0.01). This indicated that *P. utilis* had a strong ability to accumulate lead.

a. The values are means ± standard deviation. In the same column, there are significantly different among the data followed by the different capital letters at P < 0.01 (Duncan's multiple range test). The same case is for below.

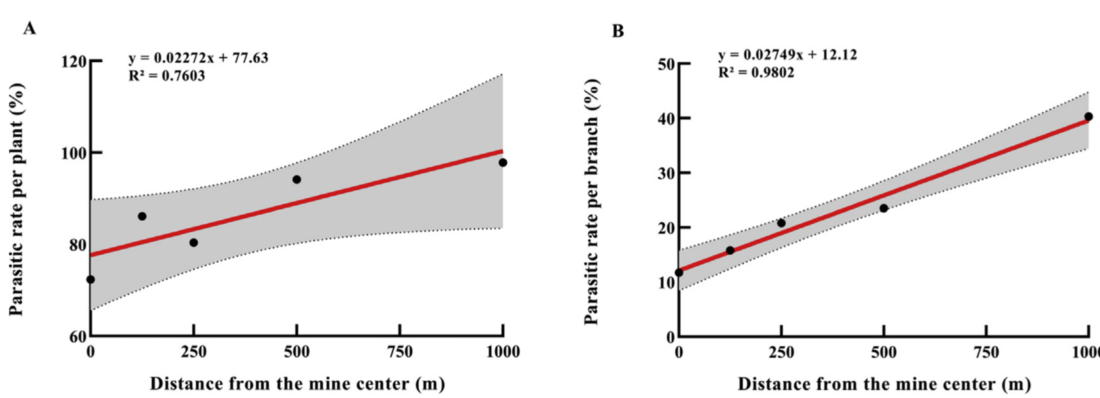

Figure 1. The parasitic rate per plant of *P. utilis* on *E. adenophorum* growing in the mining area. (A) The parasitic rate per plant; (B) The parasitic rate per branch.

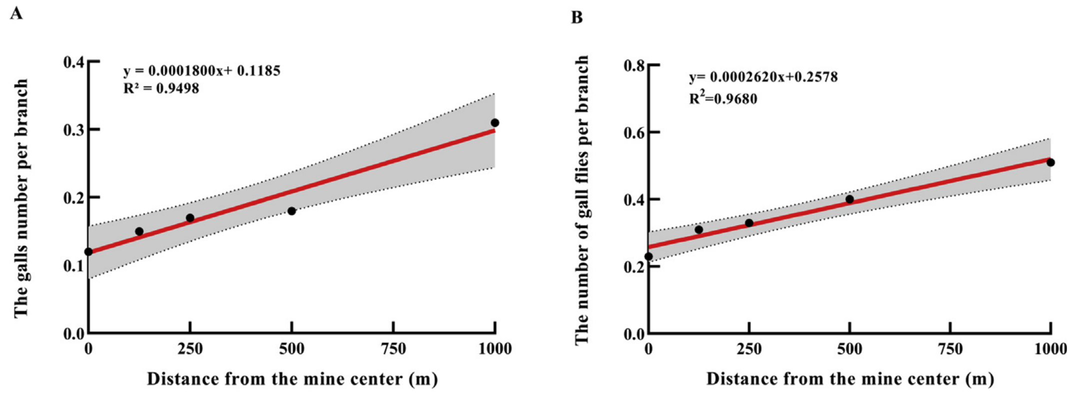

Figure 2. The number of galls and gall flies/*P. utilis* on *E. adenophorum* growing in the mining area. (A) The number of galls per branch; (B) The number of gall flies per branch.

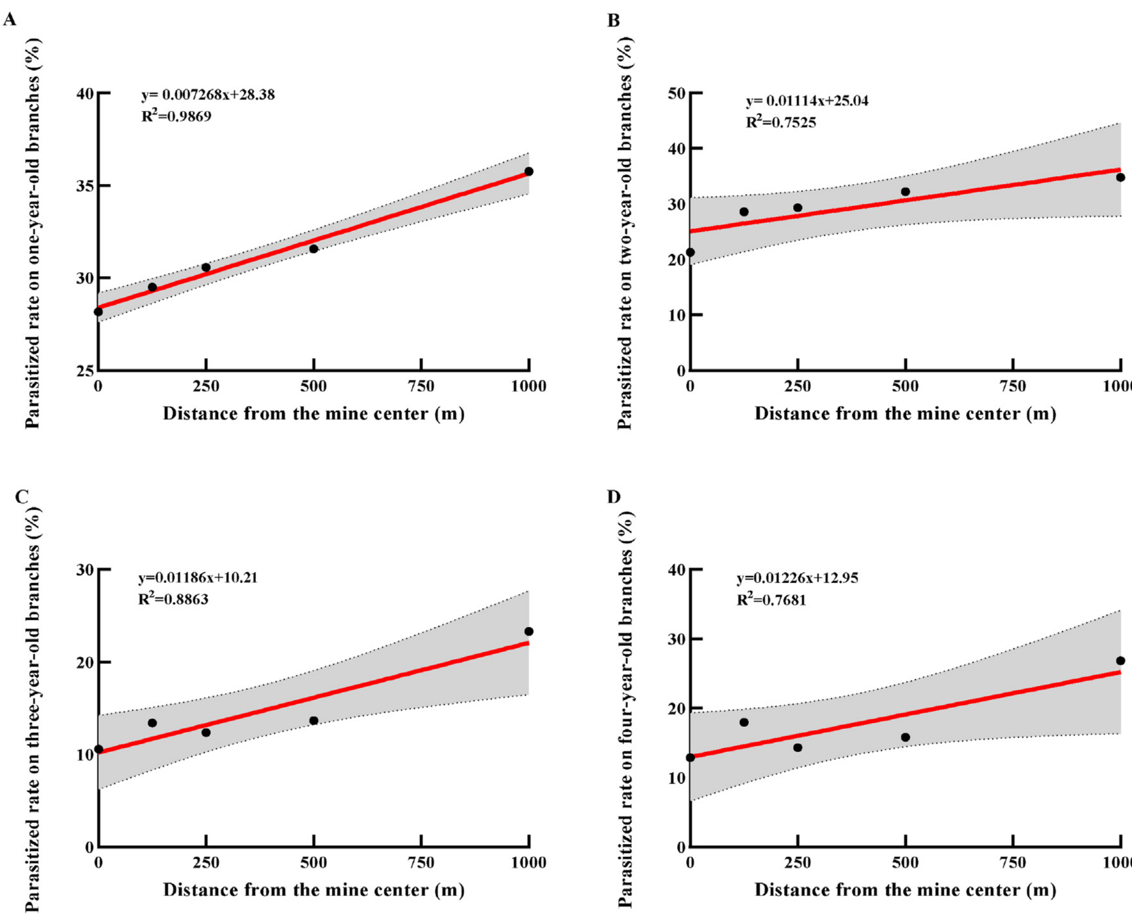

Figure 3. Percentage of parasitized branches by *P. utilis* on different aged *E. adenophorum* growing in the mining field. (A) Parasitized rate on one-year-old branches; (B) Parasitized rate on two-years-old branches; (C) Parasitized rate on three-years-old branches; (D) Parasitized rate on four-years-old branches.

b. The zero standard deviation that appears in Table 1 is caused by the small value of the standard deviation and does not mean that the value of the standard deviation is zero.
c. The CK in the table is the data of 4000 m, the same below.

3.2.2. Bio-accumulation of Zn in the soil-*E. adenophorum*-*P. utilis* system

The Zn content in the soil-*E. adenophorum*-*P. utilis* system is shown in Table 2. It decreased gradually as the distance increased from the center of the mine. The soil Zn content was within the range, 139–171 mg/kg and that recorded from most locations did not exceed the standard level (300 mg/kg) as specified in the environmental quality standard for soils (GB15618-2008) of the Ministry of Environmental Protection of the People's Republic of China. The Zn content of *E. adenophorum* was within the range, 286–367 mg/kg and the values recorded from different locations of the mine were significantly different ($F = 14.2$, $P < 0.01$). The Zn content in the body of *P. utilis* was within the range, 344–659 mg/kg and decreased significantly as the distance increased from the center of the mine ($F = 44.7$, $P < 0.01$). The bio-accumulation factor of Zn for *E. adenophorum* was within the range, 1.91–2.15, and that for *P. utilis* was within the range, 1.20–1.80. This showed that *E. adenophorum* and *P. utilis* both had a strong ability to accumulate zinc.

Table 1. Bio-accumulation of Pb in soil-*E. adenophorum-P. utilis* system.

Distance from the mine center(m)	Pb content (mg/kg)			Bio-accumulation factor	
	Soil	*E. adenophorum*	*P. utilis*	*E. adenophorum/soil*	*P. utilis/E. adenophorum*
0	941.46 ± 11.59A	129.38 ± 5.72A	1277.39 ± 63.87A	0.14 ± 0.01A	9.87 ± 0.49A
125	893.47 ± 19.89B	118.91 ± 9.37A	1131.62 ± 72.34B	0.13 ± 0.01A	9.52 ± 0.61A
250	857.96 ± 15.60C	103.12 ± 6.66B	839.87 ± 41.94C	0.12 ± 0.01AB	8.13 ± 0.41B
500	847.47 ± 5.94C	89.10 ± 3.75BC	740.63 ± 37.03C	0.11 ± 0.00 BC	8.31 ± 0.42B
1000	810.67 ± 5.54D	79.39 ± 3.04C	486.85 ± 24.34D	0.10 ± 0.00C	6.13 ± 0.31C
CK	473.72 ± 12.60E	48.69 ± 1.79D	129.15 ± 6.46E	0.10 ± 0.00BC	2.65 ± 0.13D

Table 2. Bio-accumulation of Zn in soil-*E. adenophorum-P. utilis* system.

Distance from the mine center(m)	Zn content (mg/kg)			Bio-accumulation factor	
	Soil	*E. adenophorum*	*P. utilis*	*E. adenophorum/soil*	*P. utilis/E. adenophorum*
0	170.89 ± 9.23A	366.89 ± 18.34A	659.41 ± 27.81A	2.15 ± 0.11B	1.80 ± 0.08A
125	161.20 ± 5.27AB	345.45 ± 17.27A	563.88 ± 23.31B	2.14 ± 0.11B	1.63 ± 0.07AB
250	161.75 ± 5.17AB	332.65 ± 16.63A	527.30 ± 28.42B	2.07 ± 0.10B	1.59 ± 0.09AB
500	151.03 ± 1.34BC	289.09 ± 14.45B	436.09 ± 41.25C	1.91 ± 0.10B	1.51 ± 0.14B
1000	139.28 ± 7.94C	286.39 ± 14.32B	344.29 ± 32.68D	2.06 ± 0.10B	1.20 ± 0.11C
CK	92.69 ± 4.36D	232.90 ± 11.65C	246.38 ± 15.15E	2.51 ± 0.13A	1.06 ± 0.07C

Table 3. Bio-accumulation of Cd in soil-soil-*E. adenophorum-P. utilis* system.

Distance from the mine center(m)	Cd content (mg/kg)			Bio-accumulation factor	
	Soil	*E. adenophorum*	*P. utilis*	*E. adenophorum/soil*	*P. utilis/E. adenophorum*
0	158.42 ± 7.94A	88.06 ± 4.75A	106.73 ± 6.18A	0.56 ± 0.03A	1.21 ± 0.07B
125	139.40 ± 3.85B	82.64 ± 9.39A	99.04 ± 3.18AB	0.59 ± 0.07A	1.20 ± 0.04B
250	121.55 ± 5.13C	77.58 ± 9.98A	88.53 ± 6.81BC	0.64 ± 0.08A	1.14 ± 0.09B
500	111.09 ± 8.03CD	70.12 ± 7.79AB	80.78 ± 6.87C	0.63 ± 0.07A	1.15 ± 0.10B
1000	103.69 ± 3.99D	53.67 ± 2.07B	74.61 ± 7.61C	0.52 ± 0.02A	1.39 ± 0.14B
CK	50.52 ± 3.98E	9.59 ± 2.47C	22.65 ± 3.79D	0.19 ± 0.05B	2.36 ± 0.40A

3.2.3. Bio-accumulation of Cd in the soil-E. adenophorum-P. utilis system

The distribution of Cd contents in the Soil–*E. adenophorum-P. utilis* system is described in Table 3. The Cd content in the soil ($F = 39.9$, $P < 0.01$), *E. adenophorum* ($F = 9.71$, $P < 0.01$) and *P. utilis* ($F = 12.9$, $P < 0.01$) decreased as the distance increased from the center of the mine. The soil Cd content was within the range, 104–158 mg/kg from the center to 1000 m away. According to the environmental quality standard for soils (GB15618-2008) of the Ministry of Environmental Protection of the People's Republic of China, the highest limit for Cd content in soils is 0.25 mg/kg. Therefore, the Cd content of the soil within the whole mining area was 100 times higher than this limit. Even at 1000 m away, the soil Cd content was 415-fold higher than the regulatory limit. These results indicated that in the mining area, the soil was heavily polluted by Cd from metal mining. In the *E. adenophorum*, Cd content was within the range, 53.7–88.1 mg/kg. Compared to the national standard for non-polluted vegetables (upper limit: 0.05 mg/kg), the Cd content in *E. adenophorum* at 1000 m away from the center of the mine was 215-fold higher. In the *P. utilis*, the Cd content decreased from 107 at the center of the mine to 74.6 mg/kg at 1000 m away.

The bio-accumulation factor of Cd for *E. adenophorum* was between 0.52-0.64 and for *P. utilis* it was within the range, 1.14–1.39. This showed that *E. adenophorum* had a weak ability to accumulate cadmium, and *P. utilis* had an ability to accumulate cadmium.

3.3. Correlation analysis of parasitic rates and bioaccumulation of heavy metals

The parasitic rates per plant and per branch of *P. utilis* on *E. adenophorum* both had a good linear regression relationship 0.9 and close to 1. We usually consider that the closer this R^2 value is to 1, then the linearity is better after the linear fit.–> with the Pb, Zn, and Cd contents in *E. adenophorum*, and the regression equations were $y_a = 1.8182 + 0.002x_1 - 0.0038x_2 + 0.001x_3$ and $y_b = 0.2936 - 0.0076x_1 + 0.0024x_2 - 0.009x_3$, respectively (Table 4). It can be seen from the above equations, the influence of Zn content in *E. adenophorum* on the parasitic rate per plant is the largest among the three heavy metal ions, and there is a negative correlation with the parasitic rate. And the parasitic rate per branch is extremely affected by Cd content in *E. adenophorum*, which appeared a negative correlation. The parasitic rates per plant and per branch of *P. utilis* on *E. adenophorum* also had a good linear regression

Table 4. Correlation analysis of parasitic rates of *P. utilis* on *E. adenophorum* and bioaccumulation of heavy metals in *E. adenophorum* or *P. utilis*.

Regression equation	Correlation coefficient (R^2)	F value	p-value
$y_a = 1.8182 + 0.002x_1 - 0.0038x_2 + 0.001x_3$	0.9241	5.8521	0.0904
$y_b = 0.2936 - 0.0076x_1 + 0.0024x_2 - 0.009x_3$	0.9681	14.9402	0.0262
$y_a' = 1.3739 + 0.0004x_1' - 0.0019x_2' + 0.0009x_3'$	0.9881	41.2412	0.0061
$y_b' = 0.5771 - 0.0002x_1' - 0.0005x_2' + 0.0007x_3'$	0.9828	28.3474	0.0106

y_a, y_a': the parasitic rate per plant of *P. utilis* on *E. adenophorum*; y_b, y_b': the parasitic rate per branch of *P. utilis* on *E. adenophorum*; x_1, x_2 and x_3 represent the contents of Pb, Zn and Cd in *E. adenophorum*, respectively; x_1', x_2' and x_3' represent the contents of Pb, Zn and Cd in *P. utilis*, respectively.

relationship with the Pb, Zn, and Cd contents in *P. utilis*, and the regression equations were $y_a' = 1.3739 + 0.0004x_1' - 0.0019x_2' + 0.0009x_3'$ and $y_b' = 0.5771 - 0.0002x_1' - 0.0005x_2' + 0.0007x_3'$, respectively (Table 4). It can be seen from the above equations, the influence of Zn content in *P. utilis* on the parasitic rate per plant is the strong among the three heavy metal ions, and Cd content in *P. utilis* has the greatest effect on the parasitism rate. Furthermore, the effect of Zn content is negatively correlated, while the effect of Cd content is positively correlated. In conclusion, whether in *E. adenophorum* or in *P. utilis*, Zn content has the greatest impact on the parasitic rate per plant, and Cd content in has the greatest impact on the parasitic rate per branch. The reason for this finding may be that *P. utilis* parasitize *E. adenophorum*, therefore, the same Bio-accumulation has happened in both.

4. Discussion

This investigation found that the parasitism rate of *P. utilis* and the percentage of branches of *E. adenophorum* parasitized are positively correlated with the distance, which increases with increase of distance from the center of the mining field. The parasitism status showed a dramatic change in the field at 1000 m away from the center of the mine compared to the area within the 500 m distant circle.

For the one-, two-, three-, and four-year-old branches, the parasitism rates have a positive correlation with distance from the center of mine. Therefore, regardless of the age of plant, the parasitic rate follows increased of increasing with distance. Moreover, the relationship between the parasitic rate and the ages of branches was similar to the findings of Li et al. (2006). *P. utilis* had the highest parasitic rate on one-year-old branches, it started to decline on the two-year-old branches and then further on the three-year-old branches. One possible elucidation for this is that the one-year-old plants may have contained a lower amount of heavy metals, which did not have much impact on the feeding habits of *P. utilis*. Because of its selective feeding, the high level of heavy metals accumulating in the tissues of the two-year and older branches may have inhibited the *P. utilis* from establishing on their host plants that ultimately leads to lowering the parasitism rate.

We found differences between the results of the present study and those of Zu et al. (2005) on the accumulation of Pb, Zn, and Cd by a variety of plants, and *E. adenophorum* had the highest cumulative content of Zn in the present investigation, while in the study of Zu et al. (2005) it was Pb. In both studies, it is noteworthy that neither Zn nor Pb was the most abundant heavy metal in the soil. The data in Tables (1–3) show that the content of each heavy metal in soil and *P. utilis* largely decreases with increasing distance. However, in plants, Zn and Cd showed almost no significant difference between the groups at 0–250 m and decreased significantly after >250 m. We speculate that the heavy metal content varies from one survey area to another, so that the bio-accumulation of each heavy metal by *E. adenophorum* varies. However, what exactly is the effect of each specific heavy metal on *E. adenophorum*, such as whether it raises the content of some of these components, or whether it produces resistance, etc. that affects the parasitism rate of *P. utilis*. These need to be further investigated using molecular biology techniques (Li and Zhang, 2008; Wang and Zhang, 2008).

Our results showed that Pb, Zn, and Cd were available for plant uptake. The *E. adenophorum* bio-accumulation factors of Pb, Zn, and Cd were 0.10–0.14, 1.91–2.15, and 0.52–0.64, respectively, in the Suzu Pb–Zn mine. These results showed that *E. adenophorum* had high levels of bioaccumulation of Zn, and had a weak ability to accumulate Pb and Cd from Pb–Zn mining soils. The range of bioconcentration factors of Pb, Zn, and Cd in the soil-*E. adenophorum* system was 0.058–0.079, 0.222–0.398, and 0.205–0.614, respectively (Wang et al., 2013). The enrichment coefficient and translocation factor of Pb from soil to *E. adenophorum* in Lanping Pb–Zn mine of Yunnan were 0.45 and 0.78, respectively (Zu et al., 2005). The translocation factor of Pb, Zn, and Cd from soil to *E. adenophorum* in Shuiyindong gold deposit of Guizhou were 1.88, 1.67, and 0.88, respectively (Wang and Zhang, 2008). The bioconcentration factors of Pb, Zn, and Cd from soil to *E. adenophorum* in Lannigou gold deposit of Guizhou were 1.70, 1.24, and 0.91 respectively (Li and Zhang, 2008). By comparing our findings with previous studies. The degree of soil heavy metal contamination or rather the heavy metal content varied from region to region. It leads to differences in the degree of enrichment of heavy metal ions by the same plant. However, in general, the enrichment factors of *E. adenophorum* mays for three heavy metals, Pb, Zn and Cd, were not very high. According to the method of Wang and Zhang (2008), the enrichment coefficient can only reach a maximum of relative enrichment when compared with the reference value.

In addition, referring to the method of Zhang et al. (2008), the insects were divided into five uptake sequences according to their uptake coefficients of elements: weak and very weak uptake ($n \times 10^{-2} - n \times 10^{-1}$), weak uptake ($n \times 10^{-1}$), weak accumulation and moderate uptake ($n \times 10^{-1} - n \times 10^{0}$.), strong aggregation class ($n \times 10^{0} - n \times 10^{1}$), and very strongly aggregated elements ($n \times 10^{1} - n \times 10^{2}$). As shown in Tables (1–3), the enrichment coefficients of *P. utilis* for Pb, Zn and Cd were 6.13–9.87, 1.20–1.80 and 1.14–1.39, respectively. The Zn and Cd are strong aggregating elements, and Pb is a strong aggregating element. They can affect the metabolism of carbohydrates, lipids and proteins as well as the normal physiological functions of the cells in the flies (Ma, 2013).

The data analysis indicated that the parasitic rates of *P. utilis* on *E. adenophorum* may be relevant to heavy metals bioaccumulation in *E. adenophorum* (Table 4). Lead and cadmium could induce biochemical and ultra-structural changes of *E. adenophorum* (Liu et al., 2018). Likewise, changes in plant volatile compounds (for example, camphene, 2-carene, α-oenanthene, α-limonene, α-sesquioene, α-gurrene, α-erythromyrcene) could be induced by the accumulation of Cd, Pb, or Zn in *E. adenophorum*, resulting in the inability of female *P. utilis* to effectively locate the host plant and the reduction of its parasitism rate on the host *E. adenophorum* (Ma, 2013; Ma et al., 2015). This may explain the relationship observed between the parasitism rate and heavy metals content accumulated in *E. adenophorum* in this study. In addition, some insects avoid contaminated sites, especially during oviposition. Females of *Drosophila melanogaster* (Diptera, Drosophilidae), *Plutella xylostella* (Lepidoptera, Plutellidae), *Pieris rapae* (Lepidoptera, Pieridae) and some invertebrates, for example, land snail *Cepaea nemoralis* (Helicidae) avoid oviposition on metal-rich plant materials (Freeman et al., 2006; Notten et al., 2006; Bahadorani and Hilliker, 2009).

In the present investigation, *P. utilis* accumulated 487–1277 mg/kg of Pb, 344–659 mg/kg of Zn, and 74.6–107 mg/kg of Cd in its body by feeding on the host plant (*E. adenophorum*) which also had already accumulated these metals. Furthermore, the levels of Pb, Zn, and Cd content are closely correlated with the different distances from the center of the mine. Insects collected from the center of the mine had a higher Pb, Zn, and Cd content of 9.89-, 2.68-, and 4.71-fold respectively, than those 4000 m (CK) away from the central area. The data analysis indicated that the parasitic rates of *P. utilis* on *E. adenophorum* may be linked to heavy metals bioaccumulation in *P. utilis* (Table 4). The transfer of Pb, Zn, and Cd from *E. adenophorum* to *P. utilis* likely to directly cause physiological toxicity of *P. utilis*, which in turn affect the processes such as the metabolism of carbohydrates, lipids and proteins, egg-laying, larval feeding, development, subsequent pupation, emergence, etc (Ma et al., 2018). Likewise, these heavy metals may also affect the developmental duration of the insects, percentage of pupation, body weight, fertility, total egg production and egg hatching rate, although the concentrations of metals that induced adverse effects to differ for each invertebrate and each metal (Behmer et al., 2005; Gall et al., 2015; Gao et al., 2011). Ultimately, the decline of these features would lead to the thinning of the population size of *P. utilis*, which will eventually lower its control effect on *E. adenophorum*.

5. Conclusion

The aim of this investigation was to assess the influence of heavy metals on the parasitic effect of *P. utilis* to the invasive plant

E. adenophorum, and to understand the process and trend of heavy metals transfer and bio-accumulation in the soil-*E. adenophorum*-*P. utilis* system, at the same time this work could clarify whether heavy metal pollution will affect the parasitic behavior of some obligate parasitic insects or not. The study showed that heavy metals pollution had severe impacts on the effective parasitism of *P. utilis* to the *E. adenphorum*. It was found that the parasitism rates on individual plants and branches both followed an upward trend away from the center of the mine. Therefore, we speculate that with the development of heavy metal pollution, more lead (Pb), zinc (Zn) and cadmium (Cd) are enriched in *E. adenphorum*, which negatively affects the parasitism of *P. utilis* on *E. adenphorum.* In addition, this work provides important data to further understand and clarify the effects of heavy metal contamination on various aspects of the food chain and the effects of heavy metals on the parasitism of specialized parasitic insects. Later, we will go even further to analyze the effect of which specific heavy metal or metals on the parasitic condition. In order to understand the specific causes of the effects of heavy metals such as Pb, Zn and Cd on the parasitism of *P. utilis* to *E. adenophorum.*

Declarations

Author contribution statement

Mingxian Lan and Shuquan Zeng: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Mehboob Hussain, Ping Tang, Sha Ma, Jing Yi, Lifang Li and Jixiu Wang: Contributed reagents, materials, analysis tools or data.

Guoxing Wu: Conceived and designed the experiments; Analyzed and interpreted the data.

Xi Gao: Conceived and designed the experiments; Performed the experiments; Wrote the paper.

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Data availability statement

Data included in article/supp. material/referenced in article.

Declaration of interest's statement

The authors declare no conflict of interest.

Additional information

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