Ecological differentiation in *Rumex crispus* L. natural populations in metal mining areas

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Abstract

Background: Variations in phenotypic traits of various plants living in either normal or stressed environments have been well studied, but ecological responses of plants to long-term persistent toxic metal pollution have little been reported. In this study, in order to explore the effects of continuous metal pollution in soil on variation and differentiation in the plants, *Rumex crispus* L. populations exposed to different levels of long-term persistent toxic metal pollution were studied, and corresponding *R. crispus* populations that had not been exposed to pollution were used as controls.

Results: Six phenotypic traits of *R. crispus*—root diameter, leaf area, leaf length, leaf width, leaf perimeter, and leaf length-to-width ratio—differed significantly among and within populations. Traits ranked in descending order of coefficient of variation were leaf area, leaf perimeter, root diameter, leaf length, leaf width, leaf length-to-width ratio. The average coefficient of variation was 46%. Phenotypic variation in *R. crispus* was much greater among populations (92.69%) than within populations (6.55%). The mean phenotypic differentiation coefficient (Vst) of 93.37% indicates that the interpopulation variability was the main source of phenotypic variation in *R. crispus*. Finally, root diameter was significantly positively correlated with metal factors, but leaf area, leaf length, and leaf aspect ratio were significantly negatively correlated with Pb, Zn, Mn, and Fe contents. Overall, underground growth is superior to aboveground growth in populations that have experienced long-term exposure to toxic metal pollution, and there were phenotypic differences between uncontaminated and contaminated populations.

Conclusions: These results indicate that *R. crispus* adapts to the heterogeneous environment caused by toxic metal pollution through rich phenotypic variation, and ecological differentiation has occurred among different populations.

Background

Many human activities, such as industry, metallurgy, mining, the use of fertilizer containing heavy metal, and transportation, have resulted in the transfer, redistribution, and bioaccumulation of heavy metals in the natural environment [1, 2]. Pollution of the soil with heavy metals poses a serious threat to all living systems, including humans, and has become a major global environmental problem [3]. The impact of long-term persistent heavy metal pollution on organisms, in particular on genetic diversity, is currently an important issue in both pollution ecology and environmental biology.

Genetic diversity is an important part of biodiversity. Research methods for determining genetic diversity include morphology, cytology, biochemistry, and the use of molecular markers. Phenotypic shape is an indirect estimate of genetic diversity. Variation in phenotypic traits is of great significance for both adaptability and evolution, and studying phenotypes can help researchers more intuitively predict both population genetic structure and the number of evolutionary adaptations [4]. Understanding the effects of environmental factors on plants through the study of plant morphology can also reveal the way in which plants adapt to heterogeneous environments and the relationships between plants and natural factors. Wang [5] found that long-term persistent toxic metal pollution changes plants' morphological structure and promotes ecological differentiation. In recent years, research on phenotypic variation in natural populations has mostly focused on species affected by geography, climate, and physical and chemical properties of the soil [6–9]. Other research has included basic studies on phenotypic diversity in plant resources for conservation [2, 10] and toxicity. There are few reports on ecological differentiation caused by metal pollution.

The Yunnan Province of China is known as the "The Kingdom of Non-ferrous Metals," and toxic metal contamination in the soil is increasing because of mining activity [11]. The city of Gejiu has a wide variety of mineral resources, and heavy metal pollution in mining process is more complicated in this area than in other parts of the country. The complexity of the city's toxic metal pollution makes this region an ideal area for studying the relationship between plants and soil contaminated with toxic metals. Rumex crispus is a perennial wild plant that grows well in mining areas. Xue and Liu [12] found that R. crispus is resistant to a variety of heavy metals, including certain accumulations of Pb, Zn, and Cu, but a poor accumulation of Cd, which can be used for soil remediation and restoration in mining areas. However, the adaptation of R. crispus to persistent toxic metal pollution and the ecological implications of phenotypic variation for characterizing and indicating the long-term persistent toxic metal pollution environment has not been studied.

In this study, in order to explore the ecological differentiation and the ecological response of plants to long-term persistent toxic metal pollution, we investigated differentiation in Rumex crispus populations exposed to long-term toxic metal pollution and those that had not been exposed to pollution. We measured phenotypic traits of each R. crispus population and levels of metal in the soil and used nested analyses of variance, coefficients of variation, correlation analyses, and cluster analyses to analyze phenotypic variation within and among populations. We also explored correlations between plant phenotypic variation and metal factors to better understand plant strategies for adapting to polluted environments.

Materials And Methods

Sampling

Our study area was different metal mining areas in the city of Gejiu in southwestern China. Gejiu is located in a low-latitude subtropical high-prototype humid monsoon climate zone with an average annual rainfall of 2026.5 mm and an average annual temperature of 16.4 °C. In April 2019, seven populations around these mines that had experienced continuous toxic metal pollution were investigated, and two populations in the neighboring city of Mengzi that had not been exposed to pollution were selected as controls (Table 1). We first measured latitude and longitude with GPS and measured altitude. Depending on the size of the original population, 7 to 13 individuals from each population were randomly selected for inclusion in the study. Three basal leaves were selected from each R. crispus plant, and leaf traits were measured with a portable handheld laser leaf area meter (CL-203, American CID Corporation). The root diameter where the root is connected to the stem of each plant was measured with Vernier calipers. We took mixed soil samples at a depth of 0–30 cm near the collected plants and brought these samples to the laboratory for analyses.

Table 1
Sampling locations and basic situation of sampling

Population	Region (City)	Locatity (Town)	Latitude	Longitude	Altitude (m)	Number of plants
CA	Gejiu	Laochang	23°17'49"	103°13'10"	2330	12
CB	Gejiu	Laochang	23°17'45"	103°12'60"	2360	12
CC	Gejiu	Laochang	23°118'6"	103°12'24"	2370	10
WA	Gejiu	Wugushao	23°19'14"	103°10'13"	1940	11
WB	Gejiu	Wugushao	23°19'11"	103°10'11"	1870	12
WC	Gejiu	Wugushao	23°19'5"	103°9'56"	1970	13
Y	Gejiu	Yangbadi	23°17'25"	103°16'18"	1870	9
SH	Mengzi	Shuanghe	23°56'58"	103°16'17"	1137	7
XG	Mengzi	Xinguang	23°45'31"	103°27'40"	1291	7

Contaminated population: CA, CB, CC, WA, WB, WC, Y; uncontaminated populations: SH, XG.

Table 2

Basic physical and chemical properties and metal content in the soil at sampling points of Rumex crispus populations

Population	SM (%)	pH	SOM (g/kg)	TP (g/kg)	TN(g/kg)	Metal content (mg/kg)						
						Sn	Pb	Zn	Cu	Mn	Fe	As
CA	12	7.66 ± 0.17	5.52 ± 1.38	0.35 ± 0.01	0.42 ± 0.12	1374 ± 33	7920 ± 88	6543 ± 89	1572 ± 43	17237 ± 234	130368 ± 1226	2154 ± 52
CB	7	7.29 ± 0.28	5.04 ± 0.69	0.44 ± 0.04	0.61 ± 0.31	3277 ± 47	15392 ± 151	9281 ± 114	1730 ± 47	35864 ± 389	191722 ± 1684	746 ± 64
CC	34	7.23 ± 0.2	67.85 ± 9.16	0.63 ± 0.03	1.81 ± 0.06	2243 ± 36	4220 ± 49	4500 ± 62	1355 ± 35	7583 ± 136	83403 ± 758	1871 ± 37
WA	2	7.63 ± 0.05	46.67 ± 0.73	0.87 ± 0.07	0.89 ± 0.03	9363 ± 99	2367 ± 37	2569 ± 53	5733 ± 91	2311 ± 84	170711 ± 1593	8572 ± 94
WB	15	7.69 ± 0.05	69.11 ± 1.53	0.44 ± 0.01	2.74 ± 0.09	2411 ± 38	1797 ± 28	1811 ± 38	2671 ± 52	2372 ± 79	95292 ± 873	2560 ± 37
WC	2	7.4 ± 0.13	65.55 ± 2.99	0.57 ± 0	2.57 ± 0.05	2880 ± 41	3564 ± 44	1609 ± 36	1879 ± 43	2940 ± 86	111260 ± 996	3151 ± 45
Y	4	7.7 ± 0.03	52.39 ± 33.82	0.56 ± 0.02	2.95 ± 0.21	1784 ± 32	4219 ± 48	2494 ± 44	1469 ± 38	6495 ± 119	124705 ± 1054	1753 ± 36
SH	10	7.27 ± 0.06	72.38 ± 2.38	0.79 ± 0.01	5.45 ± 0.25	124 ± 21	736 ± 14	1333 ± 27	178 ± 16	875 ± 55	44291 ± 400	242 ± 12
XG	19	7.46 ± 0.11	32.48 ± 29.19	0.31 ± 0.02	0.52 ± 0.09	0 ± 736	225 ± 8	382 ± 18	155 ± 18	1023 ± 57	93104 ± 773	108 ± 8

M = soil moisture; SOM = soil organic matter; TN = total nitrogen; TP = total phosphorus.

Determination Of Soil Environmental Factors

The collected soil samples were first weighed to determine soil moisture, then air-dried in an air-drying room. When the samples were completely dry, they were ground up. Then they were mixed thoroughly on a colorless polyethylene film, passed through a 2 mm sieve and a 0.15 mm sieve, and put into Teflon bags until their indicators could be measured.

Determination of metal content of the soil: Air-dried soil samples passed through a 0.15 mm sieve were placed in a sample preparation mold, and metal contents were measured with X-ray fluorescence spectrometry (Vanta handheld portable X-ray fluorescence analyzer, Olympus, USA). Each test was set up so that three parallel samples were measured simultaneously. Measurements were repeated three times, and the detection time was set above 120 s.

Determination of physical and chemical properties of the soil: Soil moisture was measured with the gravimetric method. Soil pH was measured with the glass electrode method (ST5000 laboratory pH meter, Ohaus Instrument Changzhou). Organic matter was measured by external heating method with potassium dichromate oxidation. The Kjeldahl nitrogen method (K1305A semi-automatic azotometer, Shanghai Shengsheng Automatic Analysis Instrument) was used to determine total nitrogen. Acid solution-molybdenum antimony colorimetry (UV1901PCS double-beam UV-spectrophotometer, Shanghai Youke Instrument) was used to determine total phosphorus.

Calculation of the coefficient of variation and phenotypic differentiation coefficient

We calculated the coefficient of variation (CV) of phenotypic traits according to the following formula: CV = SD/Mean. In this formula, SD is standard deviation.

The phenotypic differentiation coefficient was used to further measure the degree of phenotypic differentiation among populations according to the following formula: $V_{st} = \delta_{2t/s} / (\delta_{2t/s} + \delta_{2s})$. In this formula, V_{st} is the phenotypic differentiation coefficient, $\delta_{2t/s}$ is the variance among populations, and δ_{2s} is the variance within populations.

Data Analyses

Data on six phenotypic traits of 93 R. crispus in nine populations were summarized with Microsoft Excel 2016, and means and standard deviations were calculated. Analyses of variance and Duncan analyses in SPSS 16.0 were used to analyze differences in phenotypic traits among populations. To quantify the sources of variation in the phenotypic traits of R. crispus, we divided sources of variation into intra- and interpopulation variation and intraindividual variation. Nested analyses of variance were performed with SPSS 16.0, and sources of variation and distribution ratios were explained by the variance components of each group. Pearson correlation analyses were used to analyze correlations between phenotypic traits and soil environmental factors, and R Language was used to perform UPGMA cluster analyses based on Euclidean distance and to draw a tree diagram. To avoid the influence of differences in scale, we normalized the average value of each trait.

Results

Phenotypic variation in Rumex crispus

The phenotypic traits of R. crispus differed significantly among populations (Table 3). Leaf area, leaf length, leaf width, leaf perimeter, and root diameter were largest in the WC population, followed by the CC population. The CC population had the thickest roots, followed by the WC population. The SH population had the smallest and most narrow leaves, the shortest perimeter, the largest leaf aspect ratio, and the thinnest roots. The leaf shape for this population was closer to elliptic. The Y population had the shortest leaf length and smallest leaf aspect ratio, and the leaf shape was closer to round.

The coefficient of variation quantifies the degree of variability in a trait. The larger the coefficient of variation, the more discrete the trait (Table 4). The average coefficient of variation among the six phenotypic traits was 46% (range = 23–81%). The traits, listed from greatest to least variation, were on the order leaf area, leaf perimeter, root diameter, leaf length, leaf width, and leaf length-to-width ratio. The least variation was in the leaf length-to-width ratio (CV = 23%), and the largest was in leaf area (CV = 81%), which was about 3.5 times the leaf length-to-width ratio. We found great differences in the degree of variation in each population. Variation among populations, listed from greatest to least, was on the order Y, CC, SH, CA, CB, WC, WA, WB, and XG. The variation among populations ranged from 26–51%; the population with the most variation was Y (CV = 51%), and the population with the least was XG (CV = 26%). In all populations, with two exceptions, the most and least variable traits were leaf area and leaf length-to-width ratio, respectively. The exceptions were that root thickness was the most variable trait in population WA, and leaf width was the least variable trait in population Y.

Table 3

Analyses of variance in phenotypic traits among Rumex crispus populations exposed to long-term toxic metal pollution

Population	Leaf area (cm^2)	Leaf length (cm)	Leaf width (cm)	Leaf perimeter (cm)	Leaf aspect ratio	Root diameter (cm)
CA	33.39 ± 17.16ab	8.58 ± 2.55c	4.71 ± 1.38ab	26.35 ± 9.26ab	1.86 ± 0.39c	1.52 ± 0.65 cd
CB	33.95 ± 17.52ab	8.13 ± 2.77ab	5.36 ± 1.61b	26.58 ± 7.7ab	1.58 ± 0.45b	1.56 ± 0.44 cd
CC	88.62 ± 64.57c	13.61 ± 5.52f	6.96 ± 2.68c	40.3 ± 20.4c	1.95 ± 0.33 cd	1.82 ± 0.54d
WA	48.64 ± 19.1ab	11.16 ± 2.6de	5.36 ± 1.05b	36.4 ± 14.72c	2.08 ± 0.2cde	1.58 ± 0.69 cd
WB	54.25 ± 24.99b	12.18 ± 3.02ef	5.67 ± 1.2b	32.87 ± 8.25bc	2.15 ± 0.28ef	1.28 ± 0.36bc
WC	106.72 ± 56.45c	17.04 ± 4.85 g	7.82 ± 2.14c	52.7 ± 17.85d	2.22 ± 0.41ef	1.64 ± 0.49 cd
Y	28.57 ± 25.35a	6.34 ± 3.26a	4.98 ± 1.76b	23.48 ± 12.33a	1.3 ± 0.54a	0.92 ± 0.35ab
SH	27.11 ± 19.94a	8.94 ± 3.25c	3.74 ± 1.13a	23.02 ± 8.02a	2.4 ± 0.56f	0.46 ± 0.17a
XG	33.38 ± 16.05ab	9.6 ± 2.51bc	4.21 ± 1.11ab	26.91 ± 7.2ab	2.18 ± 0.51def	0.74 ± 0.23a
Mean	56.07 ± 45.57	11.31 ± 4.77	5.68 ± 2.07	34.17 ± 16.23	2 ± 0.48	1.35 ± 0.61
F	19.560**	24.108**	17.013**	16.900**	17.439**	7.662**

Identical lowercase letters indicate no significant differences; different lowercase letters indicate significant differences at $p < 0.05$; ** $p < 0.01$.

Table 4

Coefficients of variation for phenotypic traits among Rumex crispus populations exposed to long-term toxic metal pollution (%)

Population	Leaf area	Leaf length	Leaf width	Leaf perimeter	Leaf aspect ratio	Root diameter	Mean
CA	51	30	29	35	21	43	35
CB	52	34	30	29	29	28	34
CC	73	41	39	51	17	30	41
WA	39	23	20	40	10	44	29
WB	46	25	21	25	13	28	26
WC	53	28	27	34	18	30	32
Y	89	51	35	52	42	38	51
SH	74	36	30	35	23	37	39
XG	48	26	26	27	11	32	28
Mean	81	42	36	47	23	45	46

Phenotypic Differentiation And Sources Of Variation

The variance components for phenotypic traits within and among populations differed markedly (Table 5). The average variance component percentages for the six phenotypic traits among and within populations were 93.17% and 6.09%, and the remaining 0.74% were from individuals. The mean phenotypic differentiation coefficient was 93.84%, and the phenotypic differentiation coefficient of each phenotypic trait was greater than 80%.

Table 5
Variance components and phenotypic differentiation coefficients of phenotypic traits among Rumex crispus populations

Phenotypic trait	Random error	Variance component		Variance component percentage (%)			Phenotype differentiation coefficient (%)
		Among populations	Within populations	Within individuals	Among populations	Within populations	
Leaf area	2.84	25700	1310	0.01	95.13	4.86	95.14
Leaf length	0.30	319	13.23	0.09	95.93	3.98	96.02
Leaf width	0.13	48.67	2.86	0.25	94.21	5.54	94.45
Leaf perimeter	1.01	2970	176	0.03	94.38	5.58	94.41
Leaf aspect ratio	0.03	2.51	0.14	1.09	93.54	5.37	94.57
Root diameter	0.06	1.82	0.24	3.00	85.82	11.19	88.47
Mean				0.74	93.17	6.09	93.84

Correlations between phenotypic traits of Rumex crispus and soil environmental factors

Root diameter was positively correlated with all metals in the soil ($p < 0.05$; Table 6). Leaf area, leaf length, and leaf length-to-width ratio were negatively correlated with Pb, Zn, Mn, and Fe. Leaf perimeter was negatively correlated with Pb, Zn, and Mn ($p < 0.05$), but positively correlated with Sn, Cu, and As ($p < 0.05$). As and leaf length were also positively correlated ($p < 0.05$). Correlations between traits and metals, listed from greatest to least, were on the order root diameter, leaf length-to-width ratio, leaf length, leaf perimeter, leaf area, and leaf width. Leaf phenotypic traits were negatively correlated with pH ($p < 0.05$) and positively correlated with organic matter, total phosphorus, and total nitrogen ($p < 0.05$). Root diameter was negatively correlated with total nitrogen ($p < 0.05$). Correlations between traits and physical and chemical properties of the soil, listed from greatest to least, were on the order leaf length, leaf area, leaf perimeter, leaf width, root diameter, and leaf aspect ratio.

The comprehensive correlation between phenotypic traits and soil factors was the sum of the absolute values of the significant correlations of each trait, listed from greatest to least: organic matter, Zn, Mn, Pb, Fe, pH, total nitrogen, total phosphorus, As, Sn, Cu, and soil moisture. Metal content related to phenotypic traits, listed from largest to smallest, was on the order Zn, Mn, Pb, Fe, As, Sn, and Cu. Relations between physical and chemical properties of the soil and phenotypic traits, listed from largest to smallest, were on the order organic matter, pH, total nitrogen, total phosphorus, and soil moisture.

Table 6
Correlations between phenotypic characteristics of Rumex crispus and soil factors

Soil Factor	Leaf area	Leaf length	Leaf width	Leaf perimeter	Leaf aspect ratio	Root diameter	Sum |r|
Sn	0.049	0.085	0.089	0.161**	−0.019	0.311**	0.472
Pb	−0.145*	−0.250**	−0.011	−0.152*	−0.441**	0.269**	1.257
Zn	−0.179**	−0.292**	−0.057	−0.198**	−0.443**	0.313**	1.425
Mn	−0.213**	−0.315**	−0.085	−0.224**	−0.429**	0.211*	1.392
Fe	−0.174**	−0.214**	−0.05	−0.081	−0.323**	0.261*	0.972
Cu	0.025	0.083	0.064	0.138*	0.009	0.291**	0.429
As	0.101	0.162**	0.104	0.213**	0.087	0.273**	0.648
SWC	0.069	0.017	0.012	−0.073	0.037	0.038	0
pH	−0.224**	−0.163**	−0.199**	−0.147*	−0.01	−0.123	0.733
SOM	0.315**	0.372**	0.222**	0.273**	0.308**	−0.185	1.49
TP	0.154*	0.176**	0.130*	0.221**	0.089	0.113	0.681
TN	0.167**	0.208**	0.121	0.143*	0.170**	−0.225*	0.688

* $p < 0.05$, ** $p < 0.01$.

Clustering Of Populations Based On Phenotypic Traits

The nine R. crispus populations clustered based on the six phenotypic traits. After dividing by the Euclidean distance of 2.5, we divided the nine R. crispus populations into three groups (Fig. 1) not strictly clustered by geographic distance. The two populations not exposed to pollution, SH and XG, were a group.

Discussion

Ecological differentiation in Rumex crispus after exposure to persistent toxic metal pollution

The phenotypic traits of the R. crispus population contaminated by long-term toxic metal pollution in this mining area were quite different from those of populations that had not been exposed to pollution (Fig. 1). Phenotypic variation in plants is inseparable from their genes and the environment in which they grow. Rich morphological variation in plants is a manifestation of their ability to adapt to heterogeneous environments [2]. Plant leaves are more sensitive to different environments than other traits. Leaves are crucial to photosynthesis, respiration, and water exchange. They are the starting point for material circulation and energy flow. Leaves can directly affect the basic behavior and function of plants and can be a main indicator of plant genetic variation, which in turn reflects the survival strategies plants use to adapt to environmental changes [13, 14].

The six phenotypic traits we studied (leaf area, leaf length, leaf width, leaf perimeter, leaf length-to-width ratio, and root diameter) across the nine R. crispus populations differed significantly among populations, which indicates that this plant has abundant phenotypic variation. The coefficient of variation measures the degree of variation in a trait. A larger coefficient of variation indicates greater variation and more abundant diversity. The average coefficient of variation for the six phenotypic traits was 46%, and variation in all phenotypes ranged from 26% to 51%, which indicates that phenotypic variation in R. crispus populations was great, and these plants are highly adaptable to heterogeneous environments.

The six phenotypic traits of R. crispus, listed from greatest to least coefficient of variation, were on the order leaf area, leaf perimeter, root diameter, leaf length, leaf width, and leaf length-to-width ratio. The leaf length-to-width ratio had the least variation within populations (CV = 24%) and the most stability, but differences among populations were significant in analyses of variance, which indicates that the trait is variable among populations but stable within populations. Leaf area had the largest variation (CV = 81%), which shows that it has the least stability but high plasticity and thus is susceptible to the influence of individual development and environment. It differed significantly within and among populations.

Reasons for ecological differentiation in Rumex crispus populations after exposure to continuous toxic metal pollution

A total of 93.17% of the variation in the six phenotypic traits measured in these nine R. crispus populations originated from interpopulation variation, which was much higher than intrapopulation variation (6.09%) or intraindividual variation (0.74%). The mean phenotypic differentiation coefficient was 93.84%, which indicates that phenotypic variation among populations accounted for 93.84%, within-population variation accounted for 6.16%, and the contribution among populations was 15 times that within populations. Once again, interpopulation variation was the main source of phenotypic variation in R. crispus. The greater genetic differentiation among populations and the low genetic diversity within populations could be attributed to genetic segregation among different populations, which is likely due to decreased gene flow caused by severe habitat fragmentation [15-17]. This could also explain why phenotypic differentiation has occurred among R. crispus populations in the mining area despite the limited geographic distribution of these populations.

Adaptive mechanisms of Rumex crispus exposed to persistent toxic metal pollution

The phenotypic characteristics of R. crispus populations growing in the mining area are the result of long-term adaptation to soil toxic metal pollution. R. crispus growing in the mining area generally has only one main root and almost no fibrous roots, and its main roots are thicker. The root diameter is mostly greater than 1 cm. The coefficient of variation for root diameter was also greater, at 45%, which indicates that variation in root diameter is high among different populations. The correlation between root diameter and soil metal factors was the strongest ($p < 0.05$; Table 6), which means that the more severe the toxic metal pollution in the soil, the thicker the root. R. crispus is similar to Arabis alpina L., which has larger root diameters in locations with high levels of toxic metals [18]. The increase in root diameter may be due to the thickening of the root epidermis to reduce the absorption of toxic metal elements.

The roots of R. crispus growing in the mining area are generally long. The average root length of the plants in the area was as long as 20 cm, and the longest root was 30 cm. This is different from eggplant, which inhibit root elongation under high concentrations of heavy metals [19], but similar to Sedum alfredii, the concentrations of Zn of S. alfredii were positively correlated with root length [20]. This may have to do with the resistance of different plants to different heavy metals, and R. crispus is a plant that is resistant to a variety of heavy metals [12]. Therefore, the roots of the plant in the mining area were long and thick, which contributes to good growth despite continuous metal pollution.

The leaf area, leaf length, and leaf aspect ratio of R. crispus were negatively correlated with Pb, Zn, Mn, and Fe ($p < 0.05$), which is similar to the response of Arachis hypogaea L. to heavy metals—the increase in concentrations of Zn, Cu and Cd leads to a decrease in leaf area [21]. Overall, the phenotypic traits of R. crispus show that underground growth is better than aboveground growth for populations that experience long-term persistent toxic metal pollution. Under conditions of such pollution, plants tend to have a reduced leaf area, and underground growth leads to the morphological development of plants in the direction of dry biochemicals [22, 23]. R. crispus in the polluted area needs a longer root system to absorb water to adapt to physiological dehydration caused by heavy metal pollution, and the smaller leaf area may also be to cope with physiological water shortage, reduce transpiration and improve adaptability to metal pollution. This xeromorphic adaptive trait variation has also occurred in plants in other metal-contaminated areas，such as Arabidopsis arenosa from a lead zinc waste heap in southern Poland [24],and this trait variation may be heritable. It is through morphological variation in the direction of dry biochemicals that R. crispus can survive in this mining area, which has long been polluted with toxic metals.

Conclusions

In a heterogeneous environment characterized by toxic metal pollution in a mining area, Rumex crispus has rich phenotypic variation within and among populations, and a large degree of phenotypic differentiation has occurred among populations. Morphological differences between populations growing in polluted and unpolluted areas are also obvious. R. crispus adapts to long-term continuous toxic metal pollution by increasing underground growth and reducing aboveground growth. Finally, ecological differentiation has occurred among different populations.

Declarations

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Authors’ contributions

CD conceived and funded the study. YZ and SY analyzed the data and wrote the first draft. YZ, HY, YW and CT conducted the field work. CL and YZ provided editorial advice. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

The datasets supporting the conclusions of this article are included within the article. Raw data are available from the corresponding author upon reasonable request.

Consent to publish

Not applicable.

Ethics approval and consent to participate

We declare that our experiments were performed in the respect of ethical rules.

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Figures

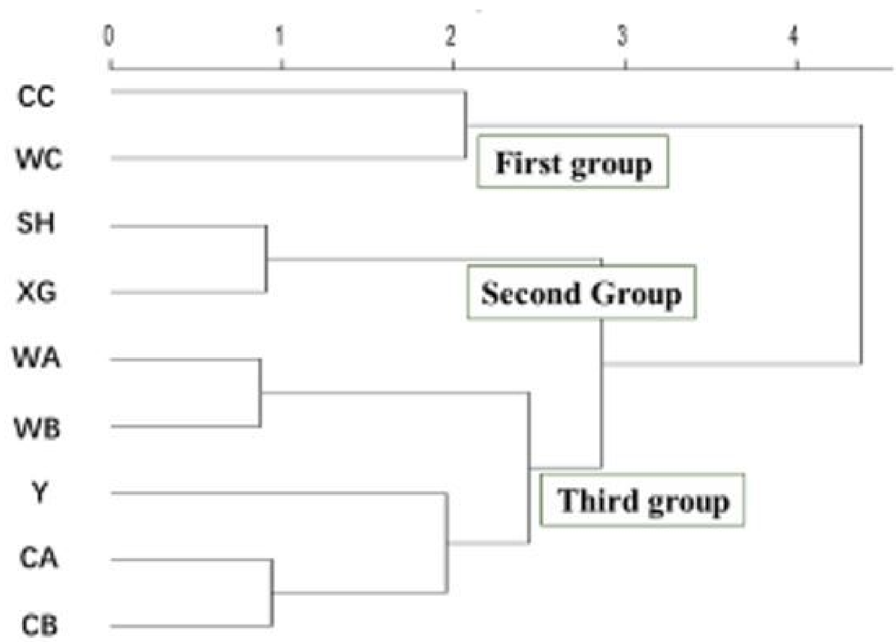

Figure 1

Cluster analysis of nine Rumex crispus populations based on variation in phenotypic traits.