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Impacts of chemical elements on fluorosis in children and the formation of "Safe Islands" in the fluorosis area: Case analysis in Weinan region, Shaanxi Province, China

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Abstract

Weinan region is a typical water-drinking endemic fluorosis area in China. However, during the investigation of fluorosis in Weinan, we found that there were specific areas with low or even no prevalence of fluorosis under high-fluoride drinking water conditions. These areas were defined as "Safe Islands". The present study investigated the prevalence of dental fluorosis among 8- to 12-year-old children and the drinking water fluoride content in all townships in Weinan region, and 18 Safe Island areas were selected as the research objects. In total, 5 typical townships with high dental fluorosis prevalence were selected as the control group, and the effects of 23 soil chemical elements on differences in the condition prevalence were analyzed. Statistical analysis was performed using correlation analysis, the Standard Coefficient Method and the Rank Sum Test. The results showed that phosphorus (P), copper (Cu), zinc (Zn) and lead (Pb) had significant effects on the dental fluorosis prevalence in children, and the contribution values of the four soil elements were 1.212, -4.267, 3.102 and 1.733, respectively. The results of the Rank Sum Test revealed that the contents of P and Cu in the soil were significantly different between the two groups, suggesting that these two elements probably caused abnormal fluorosis prevalence and affected the formation of a Safe Island in the Weinan fluorosis area.

Keywords: Fluorosis in children · cultivated soil · Safe Island · Soil chemical elements· Impacts · Weinan fluorosis area

Introduction

Endemic fluorosis is a kind of geochemical disease caused by excessive amount of fluoride in the environment in a certain area through drinking water, food, and air, which can results in physiologic and pathologic changes in human body [1-2]. The main manifestations of endemic fluorosis are dental fluorosis and skeletal fluorosis. According to its causes, endemic fluorosis can be divided into water-drinking, tea-drinking and coal-combustion types. Water-drinking type fluorosis is the most popular form of this condition. In China, the standard for determining endemic fluorosis of the water-drinking type is usually a fluoride concentration of more than 1.0 mg·L^{-1} in drinking water (according to the World Health Organization) and a prevalence of dental fluorosis in children that is higher than 30%.

A number of researchers have studied the relationship between the amount of fluoride intake in the human body and the fluorosis prevalence. Although the link between the fluoride geochemistry of water in an area and the incidence of dental and skeletal fluorosis is a well-established geochemical relationship [3], the relationship is not a simple correlation. The investigated of the water defluoridation in Guangdong Province found that the fluoride content in the drinking water of the Enping, Yangchun, Luoding, Xinxing and Shixing regions was 0.18, 0.37, 0.67, 0.10 and 0.73 mg·L^{-1}, respectively, which met the water safety standard. However, the corresponding dental fluorosis prevalence of the five cities was 78.80%, 58.40%, 41.82%, 36.99% and 26.10%, respectively, which was higher than 30% in most areas [4]. The water-drinking fluorosis in Tibet was monitored in another study, and it was observed that the F content in the drinking water in Xiementong county was approximately 0.18-0.34 mg·L^{-1}, while up to 50.78% of the children were affected by dental fluorosis[5]. On the other side, recent research has found that despite the tea fluoride content in Jimsar, Nilek, Fuyun, Haba River, Xinyuan and Zhansu counties being very high, with an average value of 417.0 mg·kg^{-1} (national standard value, 300 mg·kg^{-1}), the prevalence of dental fluorosis in children was as low as 4.47%, which indicated an abnormal phenomenon of high fluoride intake and low fluorosis prevalence [6]. An investigation in nine counties of the Ningxia Hui Autonomous Region also showed that the prevalence of skeletal fluorosis was 38.99% in the low environmental fluoride area, which was higher than the prevalence of 31.12% that was reported in the areas with high fluoride drinking water [7]. The abovementioned researches proved that a relationship did not necessarily exist between environmental fluoride content and fluorosis prevalence in the population of an area. A lower fluorosis prevalence may occur in a high fluoride environment, or vice versa. The above situation also occurred in Weinan region. Weinan is a typical water-drinking fluorosis area with a wide distribution of high-fluoride groundwater and high prevalence of dental fluorosis in children. However, during a previous investigation in this region, it was found that the prevalence of dental fluorosis in children in some villages and towns remained low (prevalence rate of <30%) under a long-term consumption of high-fluoride water (with a fluoride concentration of >1.0 mg·L^{-1}). We think this phenomenon indicates that besides the fluoride content of drinking water, the prevalence of fluorosis in Weinan has other influencing factors. Until now, however, few studies have noticed this problem or examined the causes of the abnormal relationship between fluoride intake and fluorosis in humans. Even today, a lot of local residents in Weinan fluorosis area think that the excessive fluoride content in their drinking water is the only predictive factor for fluorosis. Some studies in the coal-combustion fluorosis areas in Southwest China have found that chemical elements in the soil have a certain influence on the prevalence of fluorosis. Previous research in Chongqing reported that the prevalence of dental fluorosis in children is related to the contents of cadmium (Cd), copper (Cu), zinc (Zn) and selenium (Se) in surface soil, and a weak positive correlation was detected between the soil nickel(Ni)/mercury(Hg) content and the dental fluorosis [8-9]. A study on the fluorosis-affected rural area within the Three Gorges Dam region in China also revealed that the high Cd levels in rocks and soils may increase health risks to an epidemiological level, irrespective of fluoride levels [10]. These studies suggested that soil element could have significant effects on endemic fluorosis; however, related studies in Weinan fluorosis area have not been reported to date.

Humans are an integral part of the natural environment, and their growth, development and reproduction are restricted by environmental factors. The imbalance of various factors in nature can induce endemic diseases, and the effects of geochemical elements are particularly prominent [11]. Endemic fluorosis is a typical biogeochemical disease caused by locally different geochemical conditions [12]. Therefore, both hydrology and soil in the environment may have important impacts on the prevalence of endemic fluorosis, especially in the agricultural planting areas such Weinan. Even today, most local citizen in Weinan fluorosis area think that the excessive F content in their drinking water is the only influence factor for fluorosis. The present study examines the influence of trace elements in cultivated soil on the human body, and their potential effect in selected typical villages and townships drinking high fluoride water and low dental fluorosis prevalence (defined as Safe Islands) in Weinan. The Standard Coefficient Method and Rank Sum Test were used to analyze whether the content of trace elements in the cultivated soil affected the formation of Safe Islands in Weinan fluorosis areas, so as to provide a reference for the future prevention and treatment of fluorosis in this area.

Materials and methods

Study area

Regional overview

Weinan is located in the eastern part of the Weihe Plain in the Guanzhong region of Shaanxi Province (34°13′—35°52′N， 108°50′—110°38′E), belonging to the arid area of Northwest China (Figure 1). This area has a warm temperature, semi-humid and semi-arid monsoon climate, with four distinct seasons and a rain-heat corresponding period. The average temperature in Weinan is ~11.5-13.6°C, the frost-free period lasts ~199-224 days, the annual sunshine duration is ~2009-2528.1 hours, and the annual precipitation is ~508-608 mm [13]. Weinan has rich soil resources and many kinds of crops can be planted in the region; the cultivated area is 5.46×10^5 hm^2, accounting for 96% of the whole region, and the land carrying capacity is high. There are three main rivers in Weinan, namely the Luohe River, Weihe River and Yellow River, and the total amount of water resources is about $2.0\times10^9 m^3$. Due to these natural conditions, Weinan is a key agricultural production area and one of the five major commodity grain bases in China, and the diet of people in Weinan is mainly provided locally.

Fig.1 Location of the study area

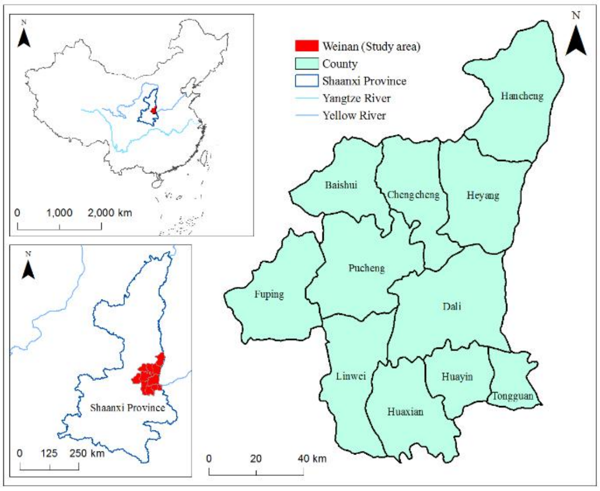


Table 1 The fluoride content in drinking water and children's dental fluorosis prevalence of samples

Sample ID	Sample sites	Fluoride content in drinking water ($mg \cdot L^{-1}$)	Prevalence (%)	Sample ID	Sample sites	Fluoride content in drinking water ($mg \cdot L^{-1}$)	Prevalence (%)
1-1	Anli	1.46	0.00	1-13	Chengguan	1.28	14.18
1-2	Luojiawa	1.20	0.00	1-14	Gaoming	1.2	14.55
1-3	Zhuangtou	1.18	0.00	1-15	Shuangquan	1.29	17.19
1-4	Shicao	1.67	2.79	1-16	Zhangjia	2.29	18.00
1-5	Duanjia	1.38	5.19	1-17	Daoxian	1.4	21.25
1-6	Buchang	1.46	5.73	1-18	Anren	1.4	21.56
1-7	Zhangqiao	1.40	7.05	2-1	Zhaoyi	2.46	34.54
1-8	Qiangbai	1.52	9.87	2-2	Boshi	1.54	36.34
1-9	Jiaoxie	1.68	10.00	2-3	Yongfeng	1.44	36.75

1-10	Fanjia	1.20	13.25	2-4	Xiazhai	3.12	40.19
1-11	Liangyi	1.25	13.50	2-5	Xiangcun	1.15	51.22
1-12	Liuji	1.40	14.08				

Prevalence of dental fluorosis in Weinan

Weinan is a typical water-drinking fluorosis area in China, and groundwater is the main drinking water source. According to survey data of Weinan government from 1981, the excess rate of fluoride content in drinking water in this area was as high as 50.6%. Since the end of the 1980s, Weinan has begun to improve its drinking water quality, and 95.5% of the villages received the water renovation project. However, the major method of improving the drinking water quality is changing to a lower-fluoride groundwater source: After groundwater exploration and water quality assessment, the government selected groundwater with better quality and lower fluoride as the drinking water source, and directly supplied this water to residents. Due to the wide distribution of high-fluoride groundwater in the area and the lack of a process for fluoride removal from the water, the current fluoride content of drinking water is still in poor condition at this region. According to survey data from 2015, the fluoride content in drinking water of the entire fluorosis area is ~0.36-6.06 $mg \cdot L^{-1}$, and there are 613 villages with fluoride content above the standard in drinking water. Taking township as the unit to carry on the statistics, a total of 64 out of the 92 townships surveyed in the whole area was found to have excessive fluoride content in their drinking water.

According to survey data of the Weinan Health and Epidemic Prevention Station collected during 2015-2016, the fluorosis area in Weinan region currently involves 8 counties, 92 townships and 804 villages, and the threatened population (population of the fluorosis area) is 1.771 million. There are 215,631 patients with dental fluorosis, of which 16,605 are children aged ~8-12 years, accounting for 31.9% of all children. Therefore, these data suggest that the situation of fluorosis in Weinan is serious.

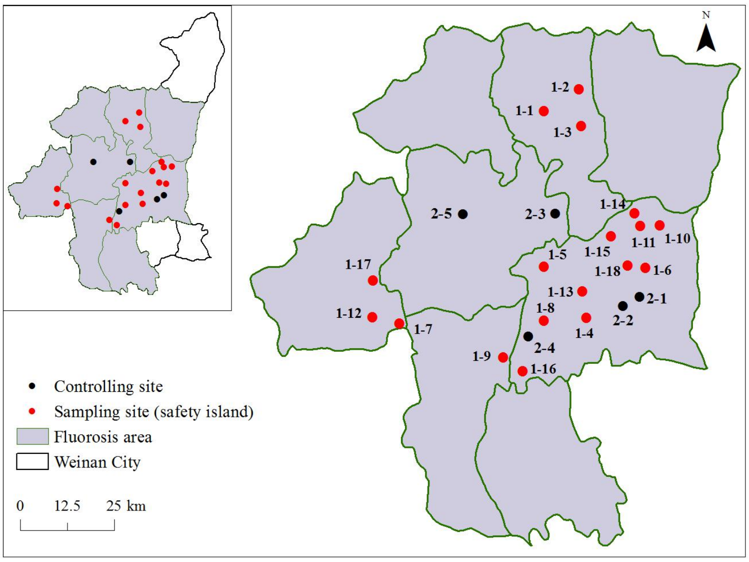
Research data acquisition

Sampling and basic data

With the support from the Leading Group for Endemic Disease Prevention, the fluoride content in drinking water and the prevalence of dental fluorosis in children were investigated in each town of Weinan. The fluoride selective electrode method was adopted for the determination of fluoride content in drinking water (GB5750-85); the dental fluorosis in children was diagnosed by technology professionals according to Dean's Index.

The investigation data of Weinan were screened on the conditions that the drinking water fluoride is more than 1.0 mg·L^{-1} and the dental fluorosis prevalence in children is less than 30%. It was found that 15.6% of the villages and townships were characterized as Safe Islands (high-fluoride drinking water and low dental fluorosis prevalence). Among these townships, 18 were selected randomly as the research objects, namely the Safe Island group (group 1). Additionally, 5 townships with similar drinking water conditions, but with high dental fluorosis prevalence, were selected as the control group (group 2). The fluoride content in drinking water and the prevalence of dental fluorosis in children in all samples in groups 1 and 2 are shown in Table 1.

Fig.2 Fluorosis area in Weinan and sampling locations of the two groups



Soil sample collection and experimental detection

Grid Method with multi-point hybrid sampling was used in all the selected towns to collect soil samples in each township according to the 5×5 km specification. One composite soil sample (approximately 1.0 kg) was created from four soil cores collected from each grid. The sampling depth of the soil was approximately 0-20 cm. All sampling sites were of cultivated soil that was away from traffic roads.

After indoor air-drying, the soil samples were sieved through a 2-mm polyethylene sieve to remove animal and plant residues, and other substances. Next, 100 g soil of each sample was taken according to the quadruple method, and was grinded to ensure that the soil can pass through the 200 mesh polyethylene sieve. To measure the concentration of the chemical elements, the prepared soil samples were processed using the microwave digestion method. A mass of 0.1 g of soil sample was placed into the polytetrafluoroethylene digestion tank, and then 4.5 mL HNO_3 (68%, *v/v*), 4.5 mL HF (38%, *v/v*) and 1.5 mL HCl (38%, *v/v*) were added. Subsequently, the substances were mixed evenly, and the polytetrafluoroethylene digestion tanks were placed into the microwave digestion instrument (Anton Paar Multiwave GO), followed by digestion at 180℃ and 80% standard atmosphere pressure for 90 mins. After digestion, 1.0 mL $HClO_4$ (~70-72%, *v/v*) was added to the soil digestion solution, and then the solution was heated for 6 hours (120℃) in the acid-driving device until it evaporated to 1.0 mL. Finally, the soil solution was diluted to 10 ml by adding pure water and filtered using a 0.22-μm filter head. All chemicals were purchased from commercial sources, and were of analytical grade purity.

Standard solutions were prepared for the elements, including aluminum (Al), silicon (Si), potassium (K), calcium (Ca), iron (Fe), phosphorus (P), titanium (Ti), vanadium (V), chromium (Cr), manganese (Mn), cobalt (Co), nickel (Ni), copper (Cu), zinc (Zn), gallium (Ga), arsenic (As), rubidium (Rb), strontium (Sr), yttrium (Y), zirconium (Zr), niobium (Nb), barium (Ba) and lead (Pb), and the concentration of each element in the soil samples was analyzed using an Inductive Coupled Plasma-Optical Emission Spectrometer (ICP-OES; SHIMADZU ICPE-9000). The analytical accuracy, controlled by duplicates and blanks, was better than 10%.

Statistical analysis

Rank Sum Test

The Rank Sum Test can be used to analyze whether the difference of a certain factor between different sample sets is statistically significant, so as to further determine whether this factor dominates the sample set difference. In this study, the application of rank sum test has two aspects: on the one hand, to judge the difference of fluoride content and prevalence in drinking water between the study group and the control group; on the other hand, to judge the difference of soil chemical elements content between the study group and the control group. The Mann-Whitney U Test, a type of Rank Sum Test, has no requirements for data normal distribution, homogeneity of variance, etc., and was thus suitable for the present study. Taking the fluorine content in drinking water as an example, first of all, combine all the data of the two sample sets and perform descending order. Next, the number of the times the first set of observations is greater than the second set is calculated and marked as U_1, and then the number of the times the second set of observations is greater than the first set is calculated and marked as U_2. Mann-Whitney U statistics are then constructed using U_1 and U_2, and the significance probability is obtained according to the distribution function table, and compared with the significance level α to determine whether to reject the null hypothesis or not. The calculation process of Mann-Whitney U Test was performed by SPSS22.0 software.

Standard Coefficient Method

There are many variables involved in this study. Standard coefficient method can eliminate the influence of dimensions among different variables and accurately measure the impact of variables on children's fluorosis. Before using the Standard Coefficient Method to analyze data, mean standardization of each variable was performed to eliminate the influence of the dimension, as follows:

$$\mathrm{Z}X_{ij} = X_{ij}/\overline{X}_i,\ \ \mathrm{ZY}_i = Y_i/\overline{Y} \qquad (1)$$

Where X_{ij} is the content of each chemical element in the soil, Y_i is the prevalence of dental fluorosis in children of each sample site, and $\mathrm{Z}X_{ij}$ and ZY_i are the standardized values of X_{ij} and Y_i, respectively.

After the standardization of the variables, the mean value of each variable was 1, thus eliminating the impact of different weights or units in the subsequent analysis, making the variables comparable.

Subsequently, the multivariate linear regression model was used to analyze the relationship between soil elements and the prevalence of dental fluorosis in children, as follows:

$$\widehat{\mathrm{ZY}} = m_1X_1 + m_2X_2 + \cdots + m_iX_i + b \qquad (2)$$

Where, $\widehat{\mathrm{ZY}}$ is the regression value of dental fluorosis prevalence in children, X_i is the content of the soil element, and m_i is the regression coefficient of X_i. According to the regression coefficient, the importance of each soil element on the fluorosis prevalence can be judged preliminarily. Since the standard error of each m_i is different, the regression coefficient must be greater than its corresponding standard error in order to be statistically significant. Based on the regression coefficient and its corresponding standard error, the contribution of each soil element to the fluorosis prevalence can be calculated as follows:

$$W_i = \mathrm{S}X_i m_i/\sigma_i \qquad (3)$$

Where W_i is the contribution value, and SX_i is the effect of the soil element on the fluorosis prevalence. If the prevalence is positively related to an element, $SX_i = 1$; otherwise, $SX_i =- 1$. When $|W_i| > 1$, the corresponding chemical element has a significant regression on the prevalence of dental fluorosis in children. A greater $|W_i|$ indicated a greater the contribution of the corresponding chemical element to the prevalence.

Results

Examination of significant differences between samples

Rank Sum Test was used to test the difference in the fluoride content in drinking water and the difference in the dental fluorosis prevalence in children between groups 1 and 2. According to the results shown in Table 2, the *P* value of fluoride content between the two groups was 0.178, which was greater than 0.05, indicating that there was no significant difference in the fluoride content in drinking water between the two groups. However, the *P* value of the prevalence between the two groups was 0.001, which was less than 0.05, showing that the dental fluorosis prevalence in children was significantly different between the two groups. The aforementioned results suggested that the difference in the dental fluorosis prevalence in children between group 1 (Safe Island) and group 2 (control) was not caused by the difference in the fluoride content in drinking water. Therefore, the undifferentiated water fluoride content and the significantly different dental fluorosis prevalence between the two groups allows for studying the effects of other factors on the dental fluorosis prevalence in children.

Table 2 Rank Sum Test results of fluoride content in drinking water and children's dental fluorosis prevalence between group 1 and group 2

	Mann-Whitney U-test				
	Sum of Ranks				
	Sample (group1) *(N=18)*	Control (group 2) *(N=5)*	Mann-Whitney U	Z	*P*
Fluoride content in drinking water	198	78	27.000	-1.347	0.178
Dental fluorosis	171	105	0.000	-3.357*	0.001

prevalence *

** Mann-Whitney U test, sig<0.01, very significantly different Detection results of soil element content

Through the ICP-OEC detection, the content of each chemical element in the soil was obtained. SPSS22.0 was used to analyze the detection data, including the variation range, mean value, standard deviation and coefficient of variation (Table 3). The coefficient of variation reflects the degree of dispersion of data, eliminating the effect of different dimensions or mean values on the comparison of two or more variables. Generally, the coefficient of variation (=15%) is used as the criterion for judging the degree of dispersion.

As seen in Table 3, the mean values of Al, Si, K, Fe, V, Cr, Mn, Co, Ni, Cu, Ga, As, Rb and Ba in group 1 are higher than those of group 2, whereas the mean values of Ca, P, Ti, Zn, Sr, Y, Zr, Nb and Pb in group 1 are lower than those of group 2. Except for P, Cr and Ni in group 1, and Ca, P and Zr in group 2, the variation coefficients of the other elements are all less than 15%, indicating that the dispersion of most elements in the same group is very small, indicating that there is no significant difference in each element content between the sample sites of one same group.

Table 3 Concentrations of elements in soil for descriptive statistics

(unit in $mg\cdot kg^{-1}$ except where indicated)

	group 1 *(N=18)*					group 2 *(N=5)*				
	min	max	mean	Std. Deviation	CV[b] (%)	min	max	mean	Std. Deviation	CV[b] (%)
Al[a]	5.89	6.74	6.49	0.192	2.96	6.06	6.50	6.38	0.181	2.84
Si[a]	24.93	28.86	26.47	0.927	3.50	25.01	28.53	26.38	1.365	5.17
K[a]	1.81	2.04	1.95	0.066	3.38	1.86	1.93	1.90	0.035	1.84
Ca[a]	3.46	6.16	4.76	0.670	14.08	3.27	5.79	4.82	1.009	20.93
Fe[a]	2.79	3.57	3.35	0.177	5.28	2.99	3.43	3.29	0.176	5.35
P	629.73	1489.	1021.8	230.968	22.6	1246.0	1703.0	1342.7	201.567	15.0

		79	2		0	9	5	8		1
Ti	3633.61	3955.91	3748.48	139.397	3.72	3691.47	3820.63	3761.75	57.305	1.52
V	68.56	87.76	80.66	4.264	5.29	73.72	81.76	79.10	3.214	4.06
Cr	63.68	142.71	72.40	17.664	24.40	66.57	67.93	67.46	0.518	0.77
Mn	471.30	674.29	602.77	44.885	7.45	556.63	601.23	589.35	18.643	3.16
Co	9.11	13.66	12.07	1.288	10.67	10.23	13.28	12.05	1.654	13.73
Ni	26.72	50.07	29.60	5.246	17.72	24.14	26.79	27.06	1.915	7.08
Cu	20.52	24.58	22.41	1.376	6.14	18.74	22.11	20.01	1.271	6.35
Zn	59.36	70.05	63.84	3.365	5.27	60.19	77.71	68.43	8.613	12.59
Ga	14.06	16.11	15.25	0.508	3.33	14.54	15.06	14.84	0.189	1.27
As	9.13	12.95	11.81	0.886	7.50	9.31	13.05	11.37	1.453	12.78
Rb	88.32	100.82	95.55	3.251	3.40	90.18	94.49	93.28	1.795	1.92
Sr	189.69	239.87	215.62	19.895	9.23	211.93	225.09	216.34	5.503	2.54
Y	22.71	26.86	25.19	0.949	3.77	24.87	25.92	25.77	0.807	3.13
Zr	219.63	312.52	259.70	25.078	9.66	245.27	362.39	282.84	51.200	18.10
Nb	12.47	14.99	14.22	0.521	3.66	13.82	14.76	14.32	0.440	3.07
Ba	440.49	500.57	463.64	13.521	2.92	430.19	455.83	445.87	10.683	2.40

Pb	20.00	26.55	23.14	0.651	2.81	22.81	24.29	23.51	0.559	2.38

a: Unit is g·kg^{-1}

b: Coefficient of variation

Effects of soil elements on dental fluorosis prevalence

Correlation analysis

In order to preliminarily reveal the relationships between soil element and the dental fluorosis prevalence in children, correlation analysis was first carried out, and the results are shown in Table 4. *P* value is the significance test index, and r value is the correlation coefficient. When the *P* value was <0.05, the correlation was considered as statistically significant (marked with an asterisk, *), and when the *P* value was <0.01, the correlation was considered as highly significant (marked with two asterisks, **) [14]. The correlation analysis results revealed that P, Cu, Zn, Y and Pb were significantly correlated with the dental fluorosis prevalence in children, and the correlation coefficients were 0.545, -0.438, 0.494, 0.432 and 0.474, respectively. The elements P, Zn, Y and Pb were positively correlated with the prevalence, suggesting that an increase in the content of these soil elements may cause an increase in the dental fluorosis prevalence. By contrast, Cu was negatively correlated with the prevalence, suggesting that an increase of Cu content in the soil would result in a downward trend in the dental fluorosis in children. The changing trend in fluorosis prevalence with respect to the soil element content can be seen in Figure 3.

Table 4 Correlation between the prevalence of children's dental fluorosis and 23 elements in soil

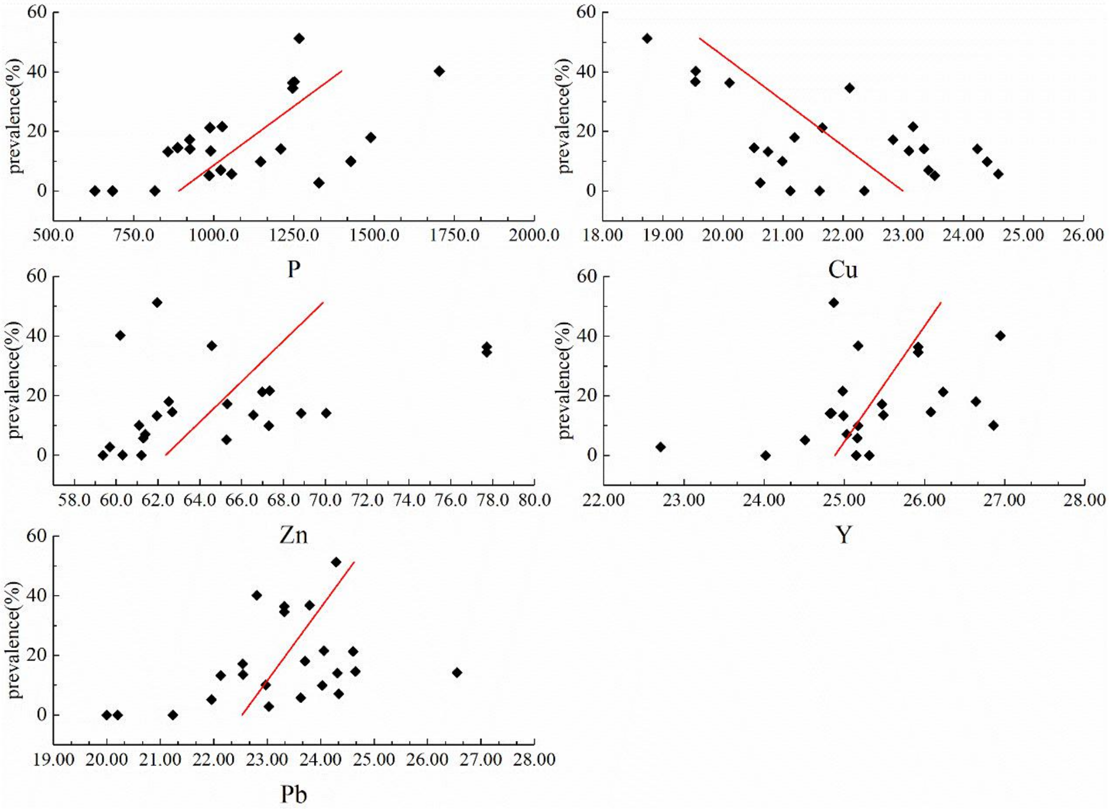
	r value	*P* value		r value	*P* value		r value	*P* value
Al	0.001	0.996	Cr	-0.122	0.579	Rb	0.077	0.728
Si	0.136	0.536	Mn	0.151	0.492	Sr	-0.170	0.439
K	0.060	0.785	Co	-0.115	0.602	Y	0.432*	0.039
Ca	-0.219	0.315	Ni	-0.210	0.336	Zr	0.137	0.533
Fe	0.083	0.706	Cu	-0.438*	0.036	Nb	0.094	0.669
P	0.545**	0.007	Zn	0.494*	0.017	Ba	-0.190	0.384

Ti	0.366	0.086	Ga	-0.074	0.738	Pb	0.474*	0.022
V	-0.050	0.821	As	-0.161	0.462			

*Correlation analysis, 0.05<P<0.01, significant correlation

** Correlation analysis, P<0.01, very significant correlation

Fig.3 Trend of dental fluorosis prevalence in children with respect to the content of P, Cu, Zn, Y and Pb in the soil



Standard coefficient method

Since the soil elements that were significantly related to the prevalence of dental fluorosis in children were determined by correlation analysis, the prevalence was taken as a dependent variable, and the five soil elements, including P, Cu, Zn, Y and Pb, were considered as the independent variables. The data were standardized, and a multiple linear regression model between dependent and independent variables was built, as follows:

$$\hat{y} =- 2.711 + 0.681x_1 - 6.717x_2 + 4.390x_3 + 1.889x_4 + 3.167x_5$$

(F=10.146, P=0.000, R^2=0.675)

Where $\hat{y}$ is the regression value of the dental fluorosis prevalence in children, while x_1, x_2, x_3, x_4 and x_5 indicate the content of P, Cu, Zn, Y and Pb in the soil of Weinan, respectively. According to the results of regression analysis, the regression coefficients of the five elements were $\sigma_1 = 0.562$, $\sigma_2 = 1.574$, $\sigma_3 = 1.415$, $\sigma_4 = 3.090$ and $\sigma_5 = 1.827$, respectively.

Since the correlation analysis results showed that the prevalence is positively correlated with P, Zn, Y and Pb, and negatively correlated with Cu, the SX_i of P, Zn, Y and Pb is 1, while that of Cu is -1. Next, the contribution of the five soil elements on dental fluorosis prevalence in children was calculated based on Eq. (6), as follows:

$$\text{P:}\quad |W_1| = 0.681/0.562 = 1.212 > 1$$

$$\text{Cu:}\quad |W_2| = |-6.717/1.574| = 4.267 > 1$$

$$\text{Zn:}\quad |W_3| = 4.390/1.415 = 3.102 > 1$$

$$\text{Y:}\quad |W_4| = 1.889/3.090 = 0.611 < 1$$

$$\text{Pb:}\quad |W_5| = 3.167/1.827 = 1.733 > 1$$

The $|W|$ value of P, Cu, Zn and Pb was greater than 1, suggesting that these four soil elements have a meaningful regression on the prevalence of dental fluorosis in children. By contrast, the $|W|$ of Y was less than 1, indicating that the regression coefficient of Y is less than its corresponding standard error and thus the effect of Y on the prevalence is not significant.

According to the abovementioned analysis, it is determined that four elements in the soil, including P, Cu, Zn and Pb, have significant effects on the prevalence of dental fluorosis in children. P, Zn and Pb have a positive effect on fluorosis prevalence, while Cu has a negative effect on the prevalence. Of all the soil elements studied, Cu contributes the most to the prevalence of dental fluorosis and its contribution is -4.267, followed by Zn (3.102), Pb (1.733) and P (1.212).

Dominant factors in the formation of the Safe Island in Weinan fluorosis area

After determining that the soil elements P, Cu, Zn and Pb are influencing factors of the dental fluorosis in children, the content of these elements in group 1 and group 2 were compared and analyzed to determine whether these elements dominated the difference in prevalence and the formation of the Safe Island. The Rank Sum Test was carried out for P, Cu, Zn and Pb content in the two groups, and the results are shown in Table 5. The *P* values of P and Cu were 0.014

and 0.006, which is less than 0.05, indicating that both P and Cu were significantly different between the two groups; however, the *P* value of Zn and Pb was 0.412 and 0.766, which is greater than 0.05, indicating that there was no significant difference between the groups.

Content distributions of these four elements in the two groups are shown in Figure 4. As shown in the figure, the content of P in the soil of the Safe Island (group 1) is lower than that of the control group (group 2), while the opposite effect is observed for Cu. Correlation analysis demonstrated that P in the soil was positively correlated with the prevalence of dental fluorosis in children (r=0.545), suggesting that higher P content in the soil could aggravate fluorosis in children; while Cu in the soil was negatively correlated with the prevalence (r=-0.438), a higher Cu content could inhibit children's fluorosis and reduce the prevalence.

Combined with the results of the Rank Sum Test, we believe that a higher Cu content and a lower P content in the soil can help reduce the prevalence of dental fluorosis in children, thus promoting the formation of a Safe Island in Weinan fluorosis area.

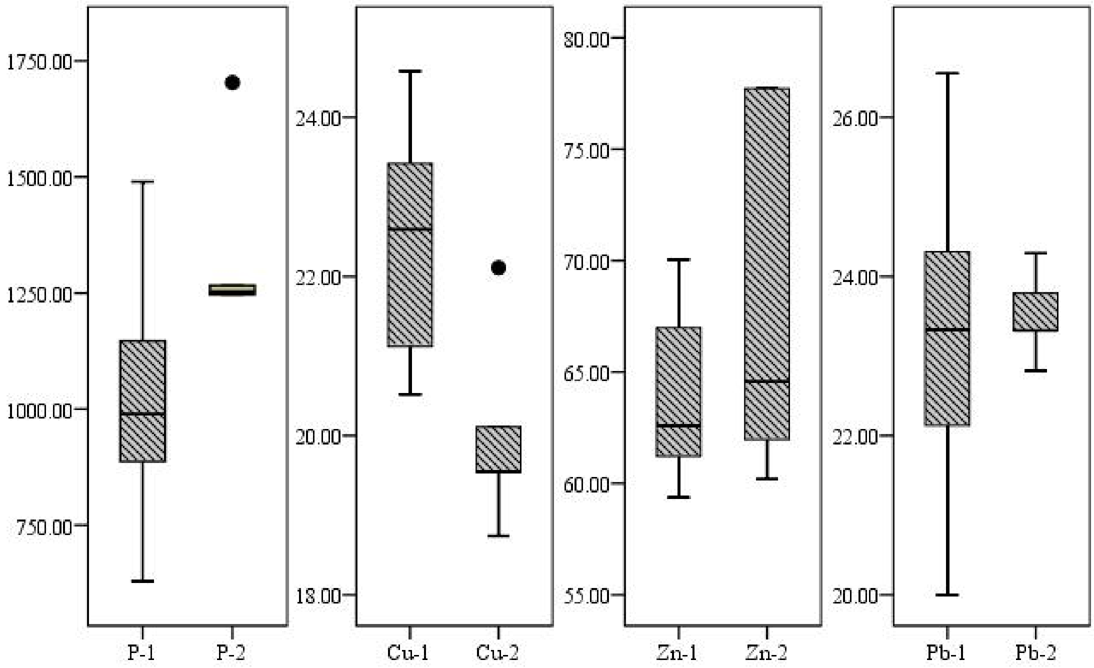
Table 5 Rank Sum Test results of P, Cu, Zn and Pb in soil between group 1 and group 2

	Mean Value (mg.kg^{-1})		Sum of Ranks		Mean Rank		Mann-Whitney U	Z	*P*
	group1	group2	group1	group2	group1	group2			
P	1021.82	1342.78	183	93	10.17	18.60	12.000	-2.460*	0.014
Cu	22.41	20.01	253	23	14.06	4.60	8.000	-2.759**	0.006
Zn	63.84	68.43	205	71	11.39	14.20	34.000	-0.820	0.412
Pb	23.14	23.51	212	64	11.78	12.80	41.000	-0.298	0.766

* Mann-Whitney U test, 0.01<sig<0.05, significantly different

** Mann-Whitney U test, sig<0.01, very significantly different

Fig.4 Content distributions of P, Cu, Zn and Pb in the soil in the two groups



Discussion

The significant difference in the content of P and Cu in the soil between the Safe Island and the control group suggests that the two elements contribute greatly to the formation of a Safe Island, while the contribution of Zn and Pb was excluded due to the negligible difference. However, it is undeniable that these four soil elements are all factors that need to be considered when studying fluorosis.

The pathological changes of fluorosis on the human body are complex and diverse, and the pathogenesis has not been fully elucidated. Some scholars have studied the formation mechanism of dental fluorosis at the molecular level, And experimental studies have shown that high concentrations of fluoride can inhibit the expression of bone morphogenetic protein (BMP), transforming growth factor-β (TGF-β) and fibroblast growth factor (FGF) in ameloblasts and osteoblasts, ultimately leading to the formation of dental fluorosis and even skeletal fluorosis [17-19]. Besides, due to the high electronegativity of fluoride, it is easily attracted by positively charged calcium ions in teeth and bones; therefore, excessive fluoride intake can lead to pathological changes in teeth and bones [20]. There are many influencing factors and pathways of chronic toxic effects of fluoride on the human body.

The present study found that the content of Cu in the soil has the greatest effect on the prevalence of dental fluorosis in children (contribution value = -4.267). Cu is an important trace element, and its content in human body is only inferior to that of Fe and Zn. Krishnamachari conducted a survey between 1973 and 1985, and found that Cu deficiency can cause osteoporosis and knee valgus, and pointed out that no fluorosis was observed in the high-fluoride drinking water area of Punjab, India, probably due to the high Cu intake in this region [21]. In China, the relationships between endemic fluorosis and chemical elements in the environment were investigated by Chen and his group, revealing that Cu content in food and vegetables in endemic fluorosis areas was significantly lower than that in normal areas [22]. Therefore, it is believed that endemic fluorosis is closely related to trace elements, such as Cu. Our results are consistent with the findings of the abovementioned studies,. [23]. Cu is ubiquitous in food, which is mainly consumed through diet in human body, and the Cu content in soil of farming areas greatly influences the Cu intake of local residents. The effect of Cu on bone development is through the influence of Cu-containing cell oxidase on the maturation of bone collagen and elastin, regulating bone mineralization. An adequate Cu content can increase the resorption rate of bones, whereas the lack of Cu can lead to cartilage and bone tissue development disorders. Fluoride can inhibit some proteinaceous substances that contribute to osteogenesis, while the physiological function of Cu makes it possible to antagonize the toxicity of fluoride [24].

The contribution of Zn in the soil to the prevalence of dental fluorosis in children in Weinan was found to be 3.102. Zn is the component of more than 200 enzymes, such as superoxide dismutase, and the activator of many enzymes that are critical in the antioxidant process. Since oxidative stress is considered to be one of the causative factors of fluorosis [25-26], it is believe that Zn deficiency may weaken the antioxidant process of the human body, leading to abnormal bone growth and development, and having ill effects on people in the fluorosis area [27]. But studies on the effects of Zn on fluorosis have been increasing; however, the conclusions of such studies are not consistent. The relationship between Zn and coal-burning endemic fluorosis in Chongqing has been studied, and Zn was found to be generally deficient in patients with fluorosis, suggesting that Zn deficiency might cause or aggravate fluorosis [28]. By contrast, an investigation on the tea-drinking fluorosis in Qinghai Province showed that the levels of Zn in the serum of individuals with different degrees of skeletal fluorosis were similar to those of normal individuals, indicating that Zn had no significant effect on fluorosis [29]. However, Xiong conducted a survey on drinking water quality and dietary nutrient intake in children with dental fluorosis in many provinces in China, and his research showed that dietary Zn was a risk factor for the dental fluorosis, suggesting that increased Zn intake will aggravate the severity of dental fluorosis in children [30]. Based on the aforementioned studies, we believe that it cannot be concluded whether Zn and fluoride have an antagonistic or synergistic relationship in the human body so far. The effect of Zn on fluorosis may vary in different areas, or there may be a certain threshold for the effect of Zn on fluorosis, which needs further research. Besides, an interesting fact was revealed in the process of literature review: Zn is medically considered as the main factor that promotes brain development, and medical statistics demonstrate that children with higher Zn content in the body usually have higher IQ tests and reaction capacity compared with children with lower Zn content. However, the former children generally have a short height and a low Cu content, while the latter are generally taller and have a higher Cu content. This phenomenon probably indicates that Zn contributes to brain development but inhibits bone growth, whereas Cu has the opposite effect.

The contribution of soil Pb content to the prevalence of the dental fluorosis in children in Weinan area was found to be 1.733. With the increase in Pb content in the soil, the fluorosis in children will be aggravated. Pb is one of the most common heavy metals that can be found in almost all environmental media and biological systems. Adults absorb 5-15% of Pb, while young children absorb significantly more Pb (about 30-40%) than adults due to differences in physiology and metabolism [31]. Pb is mainly distributed in the blood, soft tissues (kidneys, bone marrow, liver and brain) and mineral tissues after entering the body [32], which is very similar to fluoride. The distribution of Pb in bones increases with age, from about 70% in childhood to 95% in adulthood [33-34]. At present, there are many studies on the mechanism of the effect of Pb on human health, which confirmed that Pb can damage human bones and teeth, and that the action pathways of Pb are multi-faceted. Pb is considered to be an enzyme inhibitor, and the most typical example is the inhibition of δ-aminolevulinic acid dehydratase. Gerlach’s group proved that Pb can inhibit enamel proteases (including metalloproteinases), thereby damaging tooth enamel [35]. It has also been reported that Pb has a high affinity for osteoprotein and can promote the release of bone calcium, thus causing damage to bones [36] . In addition, the absorption of Pb by human body interferes with the ability of bone cells to regulate hormones, affects the function of osteoblasts and damages the cells or macromolecular active proteins in the skeletal system through oxidative stress [37]. Therefore, we believe that children may be very sensitive to Pb content found in the soil and their diet.

Furthermore, Pb may have synergistic effects with fluoride, especially in children, directly promoting fluoride toxicity. The damage caused by Pb itself to the skeletal system may also indirectly affect the degree of fluorosis. Several studies have surveyed the relationship between Pb and fluorosis in endemic fluorosis areas, and verified the effect of Pb on human fluorosis. For example, Leite et al. tested the hypothesis that co-exposure to Pb and fluoride can alter the severity of enamel fluorosis, and found that Pb can exacerbate dental fluorosis in rodents, while co-exposure to Pb may affect the degree of fluorosis [38]. Another study by Jiao et al. found a weak positive correlation between Pb in the soil and the fluorosis prevalence in Wushan County, China, indicating that Pb can promote fluorosis in the population [39]. The results of the present study are consistent with the abovementioned researches. As for its contribution to the formation of the Safe Island, Rank Sum Test results showed that there was no significant difference in the soil Pb content between the Safe Island and the control group; thus, the role of soil Pb in the formation of the Safe Island could not be determined so far. However, it is noteworthy that the background value of soil Pb content in Shaanxi Province is 21.40 $mg \cdot kg^{-1}$, while the Pb contents in the two groups tested are both higher than this value (Safe Island: ~20.00-26.55 $mg \cdot kg^{-1}$; control group: ~23.32-24.29 $mg \cdot kg^{-1}$). Therefore, whether the widespread distribution of high Pb content in the soil has an impact on the formation of the whole fluorosis area in Weinan requires further in-depth study.

The element P is the last factor affecting the prevalence of children's dental fluorosis in Weinan, and its contribution value was found to be 1.212. With the increase of soil P content, the prevalence of dental fluorosis in children was increased. Meanwhile, the significant difference in soil P content between the Safe Island and the control group proves that P is one of the influencing factors for the formation of Safe Island in Weinan fluorosis area. P is an essential element of life and the second most valuable mineral nutrient in the human body. Approximately 80-90% of the bone mineral content of the human body is composed of Ca and P. Although P is an essential nutrient, excessive amounts may be detrimental to bones. It has been demonstrated that a higher P intake could increase serum P levels, leading to a decrease in free calcium levels in the serum, thus resulting in hyperparathyroidism and increased parathyroid hormone (PTH) secretion [40]. PTH is an 84-amino-acid polypeptide hormone that functions as a mediator of bone remodeling and as an essential regulator of calcium homeostasis [41-42] . There are many similarities between the effects of excessive PTH and fluorosis on the skeleton, and PTH may play an important role in the anabolic effect of excessive fluoride on bone turnover of skeletal fluorosis [42]. Studies have shown that PTH increases bone resorption, dissolution and decalcification, i.e. bone loss, leading to osteoporosis and softening, and ultimately resulting in osteoporotic skeletal fluorosis [43-44]. In addition, high P intake can cause bone calcium decline and promote bone fluoride absorption, and the accumulation of fluoride in the skeleton will ultimately aggravate the degree of fluorosis [42]. It is believed that the P in the soil will enter the human body through plant absorption and human feeding, thus adversely affecting bone metabolism. Our research results regarding the effect of P content in the soil are consistent with the above studies. However, there is a noteworthy problem: The background value of soil P in Shaanxi Province is 483.0 mg.kg^{-1}, while the content of P in the soil of the Safe Island and the control group are ~629.73-1489.79 and ~1246.09-1703.05 mg·kg^{-1}, which is 2.11 times and 2.78 times of the background content, respectively. Although the soil P content in Weinan is much higher than the background content, the contribution of P to the prevalence of dental fluorosis in children is only 1.212, which is lower than that of Cu, Zn and Pb. The reason behind this may be that P in the soil is less effective, and the P supply capacity in the soil solution is limited. Zheng et al. evaluated the soil nutrients in the main apple producing areas of Shaanxi Province and found that the average effective P content in Weinan region was only 13.7 mg·kg^{-1}, which was at a low level in the whole province [45]. This may result in less P being absorbed directly from the soil by local residents of Weinan, and thus the impact of soil P on the human body is less obvious than that of Cu, Zn and Pb.

Conclusions

Weinan region is the most typical water-drinking fluorosis area in China. Although it has been clear that the main pathogenic factor is drinking water with a high fluoride content, the causes and influencing factors of the special areas with low fluorosis prevalence in Weinan deserve attention. These special areas with low fluorosis prevalence in high fluoride drinking water conditions were defined as “Safe Islands”. Through investigation and experiments, including correlation analysis and the Standard Coefficient Method, it was determined that Cu in the soil could inhibit children's dental fluorosis, while Zn, Pb and P could aggravate the degree of the disease. The mechanism underlying the effect of these elements on fluorosis has been relatively clear, except for that of Zn. Combined with the results of the Rank Sum Test, we concluded that the formation of Safe Island areas was probably affected by high Cu content in the soil. In addition, the significantly higher soil P content of in the control group areas may further aggravate the dental fluorosis prevalence in children, and enlarged the difference in dental fluorosis prevalence between the control group and the Safe Island.

The present study suggests that the influencing factors of fluorosis are complex, and the impacts of soil elements must receive further attention. The content of soil Cu in the high prevalence area is obviously lower, which may cause Cu deficiency in children, reducing their ability to resist the toxicity of fluoride. Furthermore, the high content of P in the soil may cause children to consume excessive P through their diet, affecting bone metabolism and aggravating the degree of fluorosis. In future studies, we will further determine the relationship between elements and fluorosis by investigating the content of elements in the serum of children, and provide effective suggestions for the prevention and treatment of fluorosis.

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Conflict of Interest

The authors declare that they have no conflict of interest to declare.

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