**Simultaneous determination and comprehensive evaluation of mineral elements in Yellow Croaker Ear-stone on sale in China**

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Abstract

Yellow Croaker Ear-stone or Yunaoshi, is actually two kinds of fish otolith in China and has received increased attention in recent years as important folk medicine. For better understanding of this crude drug, a chaotic market circulation status investigation was carried out and seventeen samples with different varieties or producing areas were collected. In this study, pharmacodynamic components of nineteen varieties mineral elements of the seventeen samples were simultaneous determined by Inductively coupled plasma mass spectrometry (ICP-MS) method. The detected elements were categorized into the beneficial (Na, Mg, Ca, K, Fe, Mn, Zn, Sr, B) and unbeneficial elements (Cu, As, Cd, Hg, Al, Pb, Co, Ba, Cr and Ni) kinds and their concentrations were quantified. Then the principal component analysis (PCA) and hierarchical clustering analysis (HCA) were further applied to launch an exploratory analysis for Yunaoshi samples. The results showed that samples 1-3, 15-8, 15-3 ranked the top three from the perspective of beneficial elements and samples 1-3, 1-4, 15-2 ranked the top three based on the unbeneficial elements sides. Combined with HCA results, all samples can be used as the substitutes for Yunaoshi except for samples 1-3, 1-4 and 15-2 only judging from the perspective of mineral elements concentrations. In conclusion, simultaneous determination of mineral elements accompanied with PCA and HCA can not only provide pharmacogenetic reference for the medicinal material of Yunaoshi, but also establish a feasibility for exploring new crude resources or substitutes to this medicine.

Keywords:
Yellow Croaker Ear-stone; Mineral elements; Inductively coupled plasma mass spectrometry (ICP-MS); Principal component analysis (PCA); Hierarchical clustering analysis (HCA)
1 Introduction

Yellow Croaker Ear-stone (Chinese name: Yunaoshi), a rarely used medicinal herb in Chinese folk medicine, is actually the fish otolith or ear bones located underneath the braincase of *Pseudosciaena crocea* or *Pseudosciaena polyactis* Bleeker (Clinical Chinese Medicine Dictionary, 1994; Traditional Chinese Medicine Records, 1998). Its medicinal value was first recorded in the ancient works of Materia Medica of the Kaibao Reign (A.D. 973) in the Song Dynasty. According to the theory of traditional Chinese Medicine (TCM), Yunaoshi is sweet and salty in flavor, cold in nature and attributive to the bladder meridian (Traditional Chinese Medicine Records, 1998). It has the efficacy of fossil drenching and anti-inflammation and can be used for the treatment of stranguries, dysuria, otitis media, rhinitis, cerebral leakage and other diseases in clinical practice. Recently, with the rise of Folk Medicine application, Yunaoshi is now drawing considerable attention due to its convenient use, low price and great curative effect in TCM formulas.

Modern pharmacological studies of Yunaoshi have been reported to display a variety of biological activities including antipyretic, diuresis, anti-inflammatory and so on (Traditional Chinese Medicine Records, 1998). These curative effects are popularly believed to be an integrative result of a number of bioactive compounds, which are mainly composed of calcium carbonate (aragonite) precipitated around an organic core of extra-cellular matrix protein, mineral elements and trace fibrin\(^1\). Among them, although mineral elements are in trace amounts, they play a vital role in pharmacodynamic effect just because they are important components of many coenzymes and involve in many physiological and biochemical reactions as activators of enzyme reactions.\(^2,3\) Besides, mineral elements are usually combined with organic compounds in TCM to form a variety of coordination compounds, forming bioactive
drugs in vivo, thus affecting the efficacy of TCM. 4) Till now, over 30 mineral elements have been identified in otoliths, but they are mainly studied for the protection of fish species and fishery management. The past decades witnessed the fact that the diversification and concentrations of mineral elements in fish otoliths are favored to be analyzed with fingerprints to record environmental information. 4-7) It was also used for stock or sub-populations identification of marine species and life history interpretation other than pharmacognosy study. 8, 9, 10, 11)

Although fish otolith exists in the skulls of many fishes, only those from *Pseudosciaena crocea* (large yellow croaker) and its close ally *Pseudosciaena polyactis* Bleeker (small yellow croaker) were listed the genuine source in authoritative Chinese herbal medicine literatures (Traditional Chinese Medicine Records, 1998). The large yellow croaker and small yellow croaker not only are the representative species of the Sciaenidae but also used to be two of the four most important marine fisheries in China, which distributed in the four major coastal regions of our country. However, due to a prolonged period of intensive fishing in the 1960s and 1970s, the resources of wild *Pseudosciaena crocea* were seriously destroyed. 12) In 2004, this species is even listed on the easy endangered species catalog just because the population has decreased by 30% in the past decade and is expected to reduce further. Thus, this resource from *Pseudosciaena crocea* almost collapse domestically and instead various species of fish otolith were found to be sold as Yunaoshi in the crude drug market. Previous investigation in the market of Anhui Bozhou and Guangxi Yulin, which are the two largest circulation markets of crude drug in China, confirmed that the market circulation species of Yunaoshi are relatively chaotic and that from *Pseudosciaena crocea* even not yet been found. Another question is that lacking efficient, scientific and reliable identification methods for
Yunaoshi clinically used in form of powder would present a challenge to accurately distinguishing genuine products from their close relatives, inferior substitutes, adulterants and counterfeits.

For better understanding of Yunaoshi’s current circulation status in market, we collected seventeen samples with different varieties or producing areas, then performed simultaneous determination of its nineteen mineral elements which are very important in the participation of active efficacy. Principal component analysis (PCA) method was then performed to evaluate the correlation matrix of elements and hierarchical clustering analysis (HCA) was applied to investigate samples clustering features. PCA method, usually been regarded as a sophisticated tactic for reducing the dimensions of multivariate variations, was applied to select several main factors of the elements and combine linear equation of the original variables. The HCA, an algorithm that groups similar objects into clusters, further categories the seventeen samples into different groups. In this study, the samples similar to the genuine Yunaoshi were screened through the two multivariate analysis methods of PCA and HCA, devoting a contribution to the development and research of Yunaoshi and their substitutes. We hope that this study will provide helpful references and beneficial directions for future studies of this traditional medicine, and will help in the exploitation of new natural resources of Yunaoshi.

2 Materials and methods

2.1 Sample collection and pretreatment

A total number of seventeen samples were collected in December 2019 from Bozhou (Anhui Province) and Yulin (Guangxi Province) herbal market, which are the two largest crude drug market in China. The seventeen samples are shown in Table 1 and Fig.1. The samples were initially compared with the reference of Otolith Atlas of
Chinese Marine Fish, and then identified by Professor Shao Lin, and the voucher samples were stored in the Shandong University of TCM.

To avoid contamination, all sample manipulations were performed with extreme care under clean room conditions (class 100). After being soaked overnight in 20% HNO$_3$ (v/v), all samples were rinsed with ultrapure water, air-dried and then finely ground to powder. A blank reagent was prepared for each digestion to assess possible contamination for the sample preparation.

2.2 Reagents and Instrumentation

All solutions were prepared using high purity deionized 18 MV water. Nitric acid (Merck, Germany) with analytical purity were used in the digestion process. Analytical curves were prepared by successive dilutions of certified standard solutions (National Metal Testing Center, China): 10$\sim$1000 $\mu$g/L for K, Al, As, B, Ba, Cd, Co, Cr, Cu, Fe, Mn, Ni, Sr, Zn, Pb and 0.2$\sim$20mg/L for Na, Mg, Ca, Hg a in 0.2% (v/v) nitric acid, respectively.

2.3 Mineral concentration determination using ICP-MS

Analytical portions of approximately 0.5 g each sample was accurately weighted and mineralized by adding 6 mL of 65 % HNO$_3$ in Speedwave$^{\text{TM}}$MWS3+ oven (MARS 6 CLASSIC, CEM company, USA). The digestion residue was then subjected to simultaneous determination of mineral elements. The microwave digestion system was operated to increase 120$^{\circ}$C from room temperature within 10 min, then maintain it for 5 min, rinse from 120$^{\circ}$C to 150$^{\circ}$C within 5 min, then maintain it for 5 min, from 150$^{\circ}$C to 190$^{\circ}$C within 5 min, and then maintain the digestion for 20 minutes. After completing the process, the cooled and digested samples were transferred from polytetrafluorethylene tubes to volumetric flasks and diluted to 50 mL with ultrapure deionized water, filtered and then subjected to elemental analysis with Inductively
coupled plasma-mass spectrometry (ICP-MS, Agilent 7900, Agilent Com, USA). ICP-MS was performed with carrier gas flow rate of 0.8 L/min, plasma gas flow rate of 15 L/min, the spray chamber temperature is 2°C and rise speed is 0.3 r/s, the power output for the RF generator is 1500 W. Before testing, the sensitivity and resolution of the instrument has been adjusted to the standard provided by the instrument. All analyses were carried out in triplicate and analytical blanks were also prepared following the same procedure used for the samples.

2.4 Statistical Analysis

Data analysis was performed using SPSS version 25.0 (SPSS Inc. USA) and the data was presented as mean ± standard deviation.

Principal component score F was calculated using SPSS by the formula as follow:

\[
F = XNa \times ZNa + XMg \times ZMg + XCa \times ZCa + XK \times ZK + XFe \times ZFe + XMn \times ZMn + XZn \times ZZn + XSr \times ZSr + XB \times ZB.
\]

Where, F represents the PCA scores of different samples, through which different samples can be analyzed and compared more intuitively. Z is the standardized value of the primary data obtained by SPSS software. X represents the correlation coefficient of the corresponding original variable of the principal component. The greater the absolute value of X is, the greater the representation of the principal component to this variable is. In addition, a comprehensive elements evaluation model was obtained by SPSS software based on F:

\[
Q = \left( \lambda_1 F_1 + \lambda_2 F_2 + \lambda_3 F_3 \right) \times \frac{1}{\lambda_1 + \lambda_2 + \lambda_3}
\]

whether Q is the comprehensive score, and \( \lambda_1, \lambda_2, \lambda_3 \) stand for the characteristic root of the major eigenvalues.
3 Results

3.1 Method validation and linear equation establishment

The analytical parameters including linear range, regression correlation coefficient (R), and limit of detection (LOD) of the nineteen elements obtained for the method validation were shown in Table 2. The regression curves were drawn with the standard mass concentration as the abscissa (\(x\)) and the ratio of the analytical peak signal value to the reference peak response value in the internal standard is the ordinate (\(y\)). All the correlation coefficient is higher than 0.999, so the linearity is considered acceptable.

3.2 Element Concentrations in Yunaoshi samples

According to whether it is beneficial to human body or not, the nineteen elements were categorized into beneficial elements (Group I, \(n=9\)) and unbeneficial elements groups respectively (group II, \(n=10\)). Table 3 and Table 4 displayed the concentrations of two groups of mineral elements in Yellow Croaker Ear-stone.

3.3 PCA of beneficial elements

Principal components analysis was applied to select several main factors which have no correlation to represent all the variables and create variables that are linear combinations of the original nine variables. The result was shown in Table 5 and Fig 2. Table 5 demonstrated that the first three principal components (F1, F2, F3) contained 85.43% of the information of the original nine elements and had a great contribution to explain the cumulative variance. Table 5 and Figure 2 showed the contribution level of nine elements to three principal components. The first principal component has a high positive correlation with Mn, Fe, Na, Zn, Sr and B, the second principal component is closely associated with Zn, Sr, Ca and B. The third principal component has a clear relationship with Ca and K.
From the eigenvalue (Table 5) and the normalized beneficial elements concentration (Z), the principal component score F and the comprehensive scores Q were calculated according to the following formulas:

\[
F_1 = 0.668 \times Z_{Na} + 0.374 \times Z_{Mg} - 0.243 \times Z_{Ca} - 0.389 \times Z_{K} + 0.793 \times Z_{Fe} + 0.857 \times Z_{Mn} + 0.853 \times Z_{Zn} + 0.771 \times Z_{Sr} + 0.850 \times Z_{B}
\]

\[
F_2 = -0.544 \times Z_{Na} - 0.797 \times Z_{Mg} + 0.355 \times Z_{Ca} + 0.185 \times Z_{K} - 0.215 \times Z_{Fe} - 0.215 \times Z_{Mn} + 0.483 \times Z_{Zn} + 0.458 \times Z_{Sr} + 0.482 \times Z_{B}
\]

\[
F_3 = -0.267 \times Z_{Na} + 0.329 \times Z_{Mg} + 0.739 \times Z_{Ca} + 0.628 \times Z_{K} + 0.392 \times Z_{Fe} + 0.385 \times Z_{Mn} - 0.02 \times Z_{Zn} - 0.141 \times Z_{Sr} - 0.037 \times Z_{B}
\]

\[
Q = 0.4674 \times F_1 + 0.2066 \times F_2 + 0.1606 \times F_3
\]

The comprehensive scores Q and ranking of seventeen Yunaoshi samples were obtained (Table 6). Generally, the varieties and concentrations of effective components of Chinese herbal medicine can reflect their pharmacological action. As for Yunaoshi, mineral elements are the most important effective constituents, so their concentrations can reflect the efficacy to some extent. However, there are two kinds of mineral elements in Yunaoshi, one is beneficial for the body within a certain dosage range and the other kind can cause harm to the body in a very small dose. It is vital to evaluate the content of the essential and toxic elements in various samples, which might cause opposite action to the body. In addition, the contents of a single element are more incomplete in terms of the quality of Yunaoshi. So, the comprehensive scores Q of two kinds of elements were adopted to evaluate some characteristic of Yunaoshi, such as the pharmacognostical similarity or identification. For example, the higher sample ranking or the higher Q value is, the higher concentrations of beneficial elements and the better curative effect is. The samples 1-3, 15-8 and 15-3 ranked the top three in the comprehensive scores, indicating that their mineral elements...
concentrations and pharmacodynamic value were comparatively higher than other samples.

3.4 PCA of unbeneficial elements

Cr was removed from the variables in the PCA model because its concentration was 0 μg/g. The PCA model for nine unbeneficial mineral elements results (except Cr) are displayed in Table 7 and Fig 3. The result of Table 7 demonstrated that the first two principal components (F1, F2) contained 84.21% of the information provided by the original variable and had a great contribution to explaining the cumulative variance. Table 7 and Figure 3 shows the contribution of ten elements to two principal components. The first principal component has a positive correlation with Cu, As, Cd, Hg, Al, Pb, Co, and Ni, the second principal component is closely associated with Hg.

From the eigenvalue (Table 7) and the normalized unbeneficial elements concentrations (Z), the principal component score F and the comprehensive scores Q were calculated according to the following two formulas:

\[
F1 = 0.395 \times Z_{\text{Cu}} + 0.367 \times Z_{\text{As}} + 0.396 \times Z_{\text{Cd}} + 0.006 \times Z_{\text{Hg}} + 0.289 \times Z_{\text{Al}} + 0.394 \times Z_{\text{Pb}} + 0.395 \times Z_{\text{Co}} + 0.395 \times Z_{\text{Ni}} - 0.036 \times Z_{\text{Ba}}
\]

\[
F2 = -0.061 \times Z_{\text{Cu}} + 0.072 \times Z_{\text{As}} - 0.055 \times Z_{\text{Cd}} + 0.695 \times Z_{\text{Hg}} + 0.199 \times Z_{\text{Al}} - 0.071 \times Z_{\text{Pb}} - 0.039 \times Z_{\text{Co}} - 0.058 \times Z_{\text{Ni}} - 0.675 \times Z_{\text{Ba}}
\]

\[
Q = 0.7031 \times F1 + 0.1390 \times F2
\]

The comprehensive scores Q and ranks of seventeen samples of 9 unbeneficial elements were shown in Table 8. Samples 1-3, 1-4 and 15-2 ranked the top three in the comprehensive scores, indicating their unbeneficial mineral elements concentrations were comparatively higher than other samples and should be cautious used.

3.5 Results of hierarchical clustering analysis
A hierarchical agglomerative cluster analysis is a well-known method for distinguishing samples. The results were shown in Figure 4 and Figure 5. Figure 4 clearly indicated the seventeen samples were divided into three categories based on the concentrations of nine beneficial elements: sample 1-3 as a cluster, sample 15-8 as a cluster, and the rest samples gather into the last category. Based on the ten unbeneiful elements concentrations, all samples turned out to be two clusters:1-3 as one cluster and the rest as one clusters.

4 Discussion

From the Song Dynasty, Yunaoshi has been medicinally used for more than a century. During this long period, especially in the Song and Ming Dynasty, benefiting from the rapid development of maritime and fishing industry, numerous otolith from a large number of fishes of various family were recorded in ancient works as Yunaoshi to treat diseases. In modern times, with the variety arrangement of Chinese herbal medicine resources, numerous modern medical books in China (Compilation of Chinese Herbal Medicine, 1975; Chinese Medicine Sea, 1993; Chinese Materia Medica, 1999; New Chinese Medicinal Herbal, 2002) established *Pseudosciaena crocea* and *Pseudosciaena polyactis* Bleeker as the only genuine sources mainly on the basis of their yellow brown fish body, earbones size and distribution sea-area of fish shoal. However, some other medical books, such as Medicinal marine life in China (1977) insisted that otoliths from some other fishes of the family Sciaenidae possess equal medicinal efficacy and can be used as Yunaoshi in clinic. Thus, it is necessary to re-undertstand the boundary between the genuine and fake resource. From the perspective of chemical composition, whether the so called genuine or the fake Yunaoshi both contain the same kind of bioactive constituents, which are calcium carbonate, mineral elements and trace fibrin. From the aspect of resource, they all
come from the stones with different shapes and sizes formed in the brains of aquatic fishes, possess the same kind of nature and taste properties and attributive to the same meridian tropism, leading them obey the same illness-curing criteria.

In view of the above points, we carried out investigation on the market-circulation species, collected circulation samples, and then launched simultaneous determination on their main active ingredients of mineral elements by ICP-MS for the purpose of better development and utilization of Yunaoshi resource. Among the seventeen samples, sample 15-1, 15-9, 15-10, 15-11, 15-12 were all derived from *Pseudosciaena polyactis* Bleeker and clustered together for the information of nine helpful elements. Hierarchical agglomerative cluster analysis indicated that ten samples of 1-1, 15-4, 15-5, 15-7, 15-6, 1-2, 1-4, 15-2, 1-5 and 15-3 can be clustered with the genuine samples of 15-1, 15-9, 15-10, 15-11, 15-12 together. Only judging from the perspective of these beneficial mineral elements concentrations and varieties, the ten sample can all be used as the substitutes for Yunaoshi.

Although sample 1-3 ranked the top in the comprehensive scores of beneficial mineral elements concentrations, it also contains the highest concentrations of the measured nine kind of unbeneficial metals (Cu, As, Cd, Hg, Al, Pb, Co, Ni, Ba), especially its Cu concentration is more than forty-five times that of genuine sample 15-12. However, it is a pity that no officially standards for these metal concentrations of Yunaoshi were issued till now, so there is no ready-made limit for our reference. For the sake of safety, we had to abandon sample 1-3. The same applies to sample 1-4 whose Hg concentration obviously higher than other samples and sample 15-5 who has a considerable higher one of Ba.

Considering that the Yunaoshi commodity in market is traditionally identified by character and microscopic method, which does not work when facing the powder
form, we hope this paper can provide a new research method supplementing its morphology and microscopic identification.

5 Conclusions

In conclusion, simultaneous determination of mineral elements accompanied with PCA and HCA can not only provide pharmacognostical reference for the medicinal material of Yunaoshi, but also establish a feasibility for exploring new crude resources or substitutes to Yunaoshi.

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Conflict of interest

The authors declare no conflict of interest.
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| No | Collection /origin site | Origin | No | Collection /origin site | Origin |
|----|--------------------------|--------|----|--------------------------|--------|
| 1-1 | Bozhou/UN | Argyrosomus pawak Lin | 15-5 | Bozhou/Brazil | UD |
| 1-2 | Bozhou/UN | Leiognathus rivulatus (Temminck et Schlegel) | 15-6 | Bozhou/Australia | UD |
| 1-3 | Bozhou/UN | Umbrina russels Cuvier et Valenciennes | 15-7 | Bozhou/Australia | UD |
| 1-4 | Bozhou/UN | Lutjanus kasmira (Forsskål) | 15-8 | Bozhou/Shandong | Scorpaenopsis neglecta Heckel |
| 1-5 | Bozhou/UN | Saurida tumbil (Bloch) | 15-9 | Bozhou/Weihai | Pseudosciaena polyactis (Bleeker) |
| 15-1 | Yulin/UN | Pseudosciaena polyactis (Bleeker) | 15-10 | Bozhou/Qingdao | Pseudosciaena polyactis (Bleeker) |
| 15-2 | Yulin/UN | Apogon quadrifraosciatus Cuvier et Valenciennes | 15-11 | Bozhou/Yantai | Pseudosciaena polyactis (Bleeker) |
| 15-3 | Bozhou/Shandong | Johnius belengeri (Cuvier et Valenciennes) | 15-12 | Bozhou/UN | Pseudosciaena polyactis (Bleeker) |
| 15-4 | Bozhou/Zheji | Synodus macrops Tanaka |  |  |  |

UN: unknown; UD: undefined
**Table 2** Results of regression analysis on calibration curves

| Element | Linear range[μg/L] | Regression | R     | LOD [μg/L] |
|---------|--------------------|------------|-------|------------|
| Na      | 0.2-20000          | $y=11.366x+1.973$ | 0.999 | 6.790      |
| Mg      | 0.2-20000          | $y=0.006x+0.052$ | 0.999 | 0.143      |
| Ca      | 0.2-20000          | $y=0.018x+0.001$ | 1.000 | 25.640     |
| K       | 10-20000           | $y=3.075x+0.222$ | 0.999 | 4.040      |
| Fe      | 10-1000            | $y=0.065x+0.578$ | 0.999 | 1.507      |
| Mn      | 10-1000            | $y=0.035x+0.009$ | 0.999 | 0.045      |
| Co      | 10-1000            | $y=0.230x+0.002$ | 0.999 | 0.002      |
| Ni      | 10-1000            | $y=0.068x+0.014$ | 1.000 | 0.021      |
| Zn      | 10-1000            | $y=0.021x+0.098$ | 0.999 | 0.136      |
| Sr      | 10-1000            | $y=0.059x+0.028$ | 1.000 | 0.067      |
| B       | 10-1000            | $y=2.932E-004x+0.001$ | 0.999 | 1.627      |
| Ba      | 10-1000            | $y=0.003x+0.005$ | 1.000 | 0.303      |
| Al      | 10-1000            | $y=0.002x+0.031$ | 1.000 | 1.227      |
| Cr      | 10-1000            | $y=0.085x+0.006$ | 0.999 | 0.026      |
| Cu      | 10-1000            | $y=0.187x+0.030$ | 1.000 | 0.033      |
| As      | 10-1000            | $y=0.014x+3.647E-004$ | 1.000 | 0.033      |
| Cd      | 10-1000            | $y=0.004x+1.033E-005$ | 1.000 | 0.003      |
| Hg      | 0.2-20000          | $y=6.357E-004x+2.530E-005$ | 0.999 | 0.037      |
| Pb      | 10-1000            | $y=0.007480x+0.000000E$ | 0.999 | 0.021      |

R: correlation coefficient; LOD: limit of detection
Table 3 Results of nine beneficial elements in Yunaoshi by ICP-MS

| No   | Na (g kg\(^{-1}\)) | Sr (g kg\(^{-1}\)) | K (g kg\(^{-1}\)) | Ca (g kg\(^{-1}\)) | Fe (μg g\(^{-1}\)) | Mn (μg g\(^{-1}\)) | Mg (μg g\(^{-1}\)) | Zn (μg g\(^{-1}\)) | B (μg g\(^{-1}\)) |
|------|-------------------|--------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-----------------|
| 1-1  | 1.93±0.29         | 1.96±4.21          | 0.91±0.41         | 243±3.24          | 6.60±1.18         | 2.90±0.83         | 33.80±3.23        | 182±0.23         | 0               |
| 1-2  | 2.08±0.05         | 2.42±2.12          | 0.25±0.71         | 232±5.21          | 7.10±2.34         | 4.60±0.75         | 30.00±3.12        | 178±0.12         | 0               |
| 1-3  | 2.67±0.13         | 3.73±1.25          | 0.53±1.23         | 243±3.24          | 71.60±2.43        | 25.00±0.87        | 30.00±0.98        | 6729±0.12        | 400±0.09        |
| 1-4  | 2.08±0.26         | 1.98±0.23          | 0.21±1.23         | 241±4.32          | 9.90±2.35         | 6.70±1.23         | 34.90±0.21        | 187±0.03         | 0               |
| 1-5  | 3.09±0.19         | 2.21±1.43          | 0.74±2.12         | 235±5.76          | 14.30±3.12        | 2.90±2.12         | 121.00±0.09       | 198±0.04         | 0               |
| 15-1 | 1.97±0.09         | 1.85±0.21          | 1.07±2.15         | 233±6.32          | 8.00±1.23         | 3.20±0.67         | 25.90±0.08        | 129±0.12         | 0               |
| 15-2 | 2.52±0.12         | 2.00±0.75          | 0.24±1.26         | 253±3.23          | 14.80±1.23        | 7.80±1.23         | 41.70±0.07        | 233±0.13         | 0               |
| 15-3 | 2.37±0.04         | 2.00±0.53          | 0.93±0.89         | 260±1.34          | 76.20±1.34        | 9.60±0.89         | 33.90±0.21        | 320±0.09         | 0               |
| 15-4 | 1.12±0.06         | 1.65±0.75          | 0.87±0.34         | 255±2.45          | 4.30±2.34         | 4.60±0.75         | 73.40±1.31        | 143±0.07         | 0               |
| 15-5 | 1.49±0.08         | 1.91±0.36          | 0.88±0.43         | 260±2.43          | 6.80±3.56         | 10.20±2.14        | 29.80±2.12        | 228±0.05         | 0               |
| 15-6 | 1.49±0.06         | 2.98±0.18          | 0.64±1.35         | 260±3.54          | 7.10±2.34         | 6.30±2.12         | 31.90±2.13        | 186±0.09         | 0               |
| 15-7 | 1.44±0.11         | 2.13±1.23          | 1.27±2.46         | 258±1.46          | 9.00±2.45         | 8.00±2.15         | 35.00±3.12        | 308±0.12         | 0               |
| 15-8 | 3.06±0.13         | 2.06±0.75          | 0.66±3.56         | 242±5.21          | 66.70±3.56        | 26.70±1.25        | 2555±0.05         | 183±0.23         | 0               |
| 15-9 | 1.70±0.15         | 1.93±1.24          | 0.96±1.35         | 250±3.56          | 13.10±3.45        | 5.50±0.21         | 30.40±0.12        | 169±0.32         | 0               |
| 15-10| 1.66±0.21         | 1.97±10.98         | 0.95±1.34         | 252±7.23          | 13.30±5.32        | 5.40±0.89         | 41.90±2.12        | 147±0.17         | 0               |
| 15-11| 1.63±0.14         | 2.01±0.87          | 0.89±2.34         | 247±2.45          | 15.20±2.45        | 6.50±0.31         | 29.90±2.14        | 239±0.23         | 0               |
| 15-12| 1.64±0.22         | 1.98±0.31          | 1.02±3.12         | 250±3.56          | 9.80±2.21         | 5.50±0.21         | 29.70±1.23        | 129±0.21         | 0               |
| No | Cu (μg g⁻¹) | As (μg g⁻¹) | Cd (μg g⁻¹) | Hg (μg g⁻¹) | Al (μg g⁻¹) | Pb (μg g⁻¹) | Cr (μg g⁻¹) | Co (μg g⁻¹) | Ni (μg g⁻¹) | Ba (μg g⁻¹) |
|----|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
| 1-1| 446±2.53    | 0.39±1.36   | 14±2.66     | 48±2.56     | 16.30±0.53  | 18.10±2.73  | 0           | 30±1.42     | 0.36±2.67   | 5.30±1.06   |
| 1-2| 432±1.23    | 0.16±0.04   | 15±1.34     | 20±3.12     | 17.30±1.12  | 16.10±1.23  | 0           | 31±1.34     | 0.32±1.58   | 3.70±2.12   |
| 1-3| 12817±0.91  | 2.56±1.23   | 590±0.24    | 310±1.23    | 52.30±0.45  | 694.3±0.05  | 0           | 210±0.12    | 11.20±1.23  | 6.10±2.01   |
| 1-4| 463±0.04    | 0.57±0.34   | 23±2.14     | 5208±0.23   | 27.10±1.23  | 21.90±0.14  | 0           | 23±2.31     | 0.39±0.32   | 1.70±1.21   |
| 1-5| 447±0.32    | 1.02±1.23   | 13±2.34     | 1545±0.56   | 16.60±1.21  | 22.1±0.32   | 0           | 23±2.12     | 0.30±0.21   | 7.50±0.98   |
| 15-1| 350±0.76    | 0.07±0.01   | 11±0.23     | 530±2.12    | 14.50±0.87  | 9.90±1.32   | 0           | 18±1.23     | 0.25±1.23   | 5.30±1.06   |
| 15-2| 627±0.98    | 0.07±0.08   | 39±1.23     | 189±1.34    | 50±0.65     | 16.70±2.32  | 0           | 22±2.87     | 0.49±2.12   | 1.80±2.12   |
| 15-3| 701±0.78    | 0.23±0.03   | 30±2.43     | 56±2.23     | 22.80±1.23  | 32.50±2.12  | 0           | 29±0.06     | 0.58±1.34   | 9.30±0.91   |
| 15-4| 335±1.23    | 0.05±0.23   | 11±0.23     | 128±1.23    | 12.80±2.32  | 11.70±1.22  | 0           | 17±0.98     | 0.18±1.35   | 3.70±2.12   |
| 15-5| 515±1.23    | 0.11±1.23   | 23±3.21     | 85±0.89     | 17.10±2.12  | 31.90±0.98  | 0           | 18±0.65     | 0.38±0.09   | 38.80±0.98  |
| 15-6| 446±0.91    | 0.16±1.23   | 14±2.16     | 17±1.56     | 16.80±1.23  | 19.40±2.12  | 0           | 17±0.23     | 0.30±1.23   | 3.50±1.23   |
| 15-7| 684±1.23    | 0.16±0.98   | 25±2.13     | 17±1.76     | 19.90±0.21  | 31.90±1.86  | 0           | 21±0.43     | 0.50±1.43   | 6.90±1.34   |
| 15-8| 372±1.98    | 0.31±1.23   | 19±2.34     | 4±0.54      | 30.00±2.12  | 16.70±2.32  | 0           | 26±0.54     | 0.40±2.12   | 6.60±2.41   |
| 15-9| 379±1.23    | 0.36±2.13   | 13±2.13     | 4±0.23      | 15.20±1.23  | 16.10±1.23  | 0           | 15±0.23     | 0.32±1.23   | 7.70±1.34   |
| 15-10| 344±0.07    | 0.34±2.13   | 12±0.25     | 8±0.21      | 17.20±3.21  | 14.20±2.54  | 0           | 14±0.32     | 0.20±2.34   | 8.10±2.32   |
| 15-11| 532±0.05    | 0.71±0.38   | 24±0.56     | 0           | 20.10±1.11  | 22.80±2.31  | 0           | 22±1.23     | 0.42±1.23   | 9.61±1.45   |
| 15-12| 284±1.23    | 0.24±2.15   | 12±0.21     | 0           | 12.9±0.34   | 21.60±2.45  | 0           | 16±2.12     | 0.19±2.15   | 8.20±1.34   |
Table 5 Principal component analysis of nine beneficial elements

| Elements | First principal component (F1) | Second principal component (F2) | Third principal component (F3) |
|----------|-------------------------------|--------------------------------|-------------------------------|
| XNa      | 0.668                         | -0.544                        | -0.267                        |
| XMg      | 0.374                         | -0.797                        | 0.329                         |
| XCa      | -0.243                        | 0.355                         | 0.739                         |
| XK       | -0.389                        | 0.185                         | 0.628                         |
| XFe      | 0.793                         | -0.215                        | 0.392                         |
| XMn      | 0.857                         | -0.215                        | 0.385                         |
| XZn      | 0.853                         | 0.483                         | -0.024                        |
| XSr      | 0.771                         | 0.458                         | -0.141                        |
| XB       | 0.85                          | 0.482                         | -0.037                        |

Eigenvalue | 4.206 | 1.86 | 1.445 |
Proportion (%) | 46.735 | 20.662 | 16.056 |
Accumulates (%) | 46.735 | 67.397 | 83.453 |

Note: XNa, XMg, XCa, XK, XFe, XMn, XZn, XSr, XB represent the principal component load coefficient of each element, or the correlation coefficient of the corresponding original variable of the principal component. The greater the absolute value of X is, the greater the representation of the principal component to this variable is. Eigenvalue is the variances of the principal components. Proportion (%) represents the percentage of variance of each principal component. Accumulates (%) represents the cumulative percentage of variance accounted for by the current and all preceding principal components.
Table 6 Principal component score and rank of samples based on beneficial elements

| No | F1  | Rank | F2  | Rank | F3  | Rank | Q   | Rank |
|----|-----|------|-----|------|-----|------|-----|------|
| 1-1| -1.01 | 15 | 0.02 | 10 | 0.97 | 4 | -0.31 | 11 | 15-4 | -1.35 | 17 | 0.62 | 4 | -1.75 | 16 | -0.78 | 17 |
| 1-2| 0 | 4 | -0.40 | 13 | 1.53 | 2 | 0.16 | 4 | 15-5 | -0.82 | 10 | 0.61 | 5 | -1.65 | 15 | -0.52 | 15 |
| 1-3| 6.41 | 1 | 2.55 | 1 | -0.60 | 12 | 3.42 | 1 | 15-6 | -0.10 | 5 | 1.30 | 2 | -2.30 | 17 | -0.15 | 6 |
| 1-4| -0.25 | 7 | -0.54 | 15 | 0.38 | 8 | -0.17 | 8 | 15-7 | -0.94 | 13 | 0.95 | 3 | 0.57 | 6 | -0.15 | 7 |
| 1-5| -0.39 | 8 | -1.04 | 16 | 0.04 | 11 | -0.39 | 12 | 15-8 | 2.81 | 2 | -4.18 | 17 | 0.33 | 9 | 0.51 | 2 |
| 15-1| -1.05 | 16 | -0.30 | 11 | 0.63 | 5 | -0.45 | 13 | 15-9 | -0.91 | 12 | 0.28 | 8 | 1.63 | 1 | -0.11 | 5 |
| 15-2| -0.22 | 6 | -0.54 | 14 | 0.23 | 10 | -0.18 | 9 | 15-10 | -0.91 | 11 | 0.37 | 7 | -0.96 | 13 | -0.50 | 14 |
| 15-3| 0.36 | 3 | -0.35 | 12 | 1.50 | 3 | 0.34 | 3 | 15-11 | -0.66 | 9 | 0.25 | 9 | 0.44 | 7 | -0.19 | 10 |
| 15-12| -0.98 | 14 | 0.40 | 6 | -0.98 | 14 | 0.53 | 16 |

F represents the PCA scores of different samples, through which different samples can be analyzed and compared more intuitively. F1 is the first principal component, F2 is the second principal component, and F3 is the third principal component. Q is the comprehensive score.
Table 7 Principal component analysis of nine unbeneficial elements

| Elements | First principal component (F1) | Second principal component (F2) |
|----------|--------------------------------|---------------------------------|
| XCu      | 0.395                          | -0.061                          |
| XAs      | 0.367                          | 0.072                           |
| XCd      | 0.396                          | -0.055                          |
| XHg      | 0.006                          | 0.695                           |
| XAl      | 0.289                          | 0.199                           |
| XPb      | 0.394                          | -0.071                          |
| XCo      | 0.395                          | -0.039                          |
| XNi      | 0.395                          | -0.058                          |
| XBa      | -0.036                         | -0.675                          |

Eigenvalue  
6.328  
1.251

Proportion (%)  
70.311  
13.902

Accumulates (%)  
70.311  
84.213

Note: XCu, XAs, XCd, XHg, XAl, XPb, XCo, XNi, XBa represent the principal component load coefficient of each element, or the correlation coefficient of the corresponding original variable of the principal component. The greater the absolute value of X is, the greater the representation of the principal component to this variable is. Eigenvalue is the variances of the principal components. Proportion (%) represents the percentage of variance of each principal component. Accumulates (%) represents the cumulative percentage of variance accounted for by the current and all preceding principal components.
Table 8 Principal component score F and ranks of samples based on unbeneificial elements

| No  | F1  | sort | F2  | sort | Q   | sort | No  | F1  | sort | F2  | sort | Q   | sort |
|-----|-----|------|-----|------|-----|------|-----|-----|------|-----|------|-----|------|
| 1-1 | -0.59 | 8    | -0.07 | 9    | -0.43 | 8    | 15-4 | -1.05 | 17   | 0.02 | 8    | -0.74 | 16   |
| 1-2 | -0.70 | 10   | 0.03  | 6    | -0.49 | 10   | 15-5 | -0.92 | 14   | -2.79 | 17   | -1.03 | 17   |
| 1-3 | 9.68  | 1    | -0.29 | 12   | 6.77  | 1    | 15-6 | -0.83 | 13   | 0.05  | 5    | -0.58 | 11   |
| 1-4 | -0.20 | 3    | 3.24  | 1    | 0.31  | 2    | 15-7 | -0.61 | 9    | -0.19 | 10   | -0.46 | 9    |
| 1-5 | -0.27 | 4    | 0.65  | 3    | -0.10 | 4    | 15-8 | -0.34 | 6    | 0.02  | 7    | -0.23 | 5    |
| 15-1| -0.99 | 16   | 0.14  | 4    | -0.67 | 14   | 15-9 | -0.80 | 11   | -0.29 | 13   | -0.60 | 12   |
| 15-2| 0.10  | 2    | 0.81  | 2    | 0.18  | 3    | 15-10| -0.80 | 12   | -0.29 | 11   | -0.60 | 13   |
| 15-3| -0.41 | 7    | -0.32 | 14   | -0.33 | 7    | 15-11| -0.33 | 5    | -0.34 | 15   | -0.28 | 6    |
| 15-12| -0.94 | 15   | -0.39 | 16   | -0.72 | 15   |      |      |      |      |      |      |      |

F represents the PCA scores of different samples, through which different samples can be analyzed and compared more intuitively. F1 is the first principal component, F2 is the second principal component, and F3 is the third principal component. Q is the comprehensive score.
Fig. 1 The seventeen samples of Yunaoshi

(Color figure can be accessed in the online version.)
Fig. 2 The Score Scatter 3D plot of nine beneficial elements

$R^2_X$ can be thought of as the square of the percentage of original data information retained in the different directions of the X-axis. The ellipse represents the 95% confidence interval of the sample with no outliers. (Color figure can be accessed in the online version.)
**Fig. 3** The Score Scatter 3D plot of nine unbeneficial elements

R²X can be thought of as the square of the percentage of original data information retained in the X-axis direction. (Color figure can be accessed in the online version.)
Fig. 4 Hierarchical clustering analysis (HCA) of seventeen samples based on beneficial elements concentrations.
Fig. 5 Hierarchical clustering analysis (HCA) of seventeen samples based on 
unbeneficial elements concentrations