

## ORIGINAL RESEARCH ARTICLE

### Analysis of Inorganic Elements in *Vespa mandarinia Smith*

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#### ABSTRACT

**Objective:** To analyze the inorganic elements of *Vespula vulgaris Smith* from different regions systematically, create an inorganic element fingerprint spectrum, and establish the basis to control the quality of *Vespula vulgaris Smith*. **Methods:** Microwave digestion with inductively coupled plasma (ICP) was used to determine 20 types of inorganic elements in *Vespula vulgaris Smith* and create a fingerprint spectrum for inorganic elements, conducting analyses of metal elements. **Results:** The species of metal elements in *Vespula vulgaris Smith* are rich in P, K, Na, Mg, Ca, which account for 92.32% of the total. The content of harmful elements such as Pb, Cd, As, Hg, Cu meets the requirements of National Pharmacopoeia Standards. There are different metal elements in *Vespula vulgaris Smith* in different regions. According to the inorganic element spectrum, the content of inorganic elements exhibits a similar distribution tendency in the order of the atomic numbers. **Conclusion:** *Vespula vulgaris Smith* contains a rich variety of metal elements, including many beneficial microelements, and the content of harmful metal elements meets the requirements of the Pharmacopoeia. The inorganic element fingerprint spectrum of *Vespula vulgaris Smith* can provide a reference for the identification of *Vespula vulgaris Smith*.

**Keywords:** *Vespa mandarinia Smith*; different producing areas; inorganic elements; fingerprint

*Vespa mandarina Smith* is the largest in Vespidae *Vespa*. Predatory carnivorous insects with fierce temperament, commonly known as “tiger headed bee”<sup>[1]</sup>, also known as Chinese Tiger headed bee. Spotted wasp. Peach wasp. Head bee, etc. Its larvae. All. Bee venom and honeycomb can be used as medicine. The medicinal material is called Bumblebee (all). Wasp seed (larva). Exposed beehive (honeycomb), mainly for rheumatism and arthralgia, is a common folk Chinese medicine<sup>[2]</sup>. Bee venom made from golden ring wasp steeping wine can dispel wind dampness, treat acute and chronic rheumatic pain and rheumatoid arthritis, and has the same effect as its larvae (rhubarb wasps)<sup>[3,4]</sup>, which has a long history of use in Chinese folk.

Modern research shows that the inorganic components in traditional Chinese medicine are closely related to the curative effect. The inorganic elements in traditional Chinese medicine are essential elements that participate in and regulate metabolism in the organism, and can interact with the nitrogen contained in the organism. Oxygen. Sulfur ligands form coordination bonds, coordinate the material balance in the body, and have positive synergistic or antagonistic effects on drug efficacy<sup>[5-7]</sup>. In addition, heavy metals and harmful elements are one of the important detection indicators for the import and export of traditional Chinese medicine.

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Accurate determination of the content of heavy metals in traditional Chinese medicine can provide a scientific basis for the quality control in the production of traditional Chinese medicine and the safety of clinical medication<sup>[7,8]</sup>.

At present, the research on *Vespa aurata* mainly focuses on pharmacological effects. Research on DNA bar code identification and other aspects<sup>[9]</sup>. In this experiment, microwave digestion ICP method was used to determine the inorganic elements in *Vespa aurata* from different production areas in Yunnan, and the fingerprint of inorganic elements of *Vespa aurata* was made to analyze the correlation between the content of inorganic elements of *Vespa aurata*. It provides a reference for the research on the quality characteristics of inorganic elements of *Vespa aurata*, and provides a new basis for the research on the quality control of *Vespa aurata*.

## 1. Instruments and materials

### 1.1. Instrument

7200 inductively coupled plasma spectrometer (German thermoelectric company); MARS6 microwave digestion instrument (CEM company), AL204 Mettler electronic analytical balance; AFS-9530 Dual Channel Atomic Fluorescence Photometer (Beijing haiguang Instrument Co., Ltd.), milli-Q pure water instrument.

### 1.2. Reagents

Single element standard solution (GSB G62027-903301, general iron and Steel Research Institute of national iron and steel materials testing center); 70% nitric acid (MOS grade, Tianjin fengchuan chemical reagent Technology Co., Ltd.), hydrogen peroxide (30% aqueous solution, CMOS, Sinopharm Chemical Reagent Co., Ltd.).

### 1.3. Sample information and preparation

The experimental samples came from 14 different regions in Yunnan Province. All samples were identified by Professor guoyunjiao of Dehong normal college, *Vespa mandarinia Smith*. The information of each sample is shown in **Table 1**. After collecting the samples, freeze them at low temperature.

**Table 1.** Sample information.

Serial No.	Collection place
1	Mang City, Dehong Prefecture
2	Yingjiang County, Dehong Prefecture
3	Longchuan County, Dehong Prefecture
4	Longchuan County, Dehong Prefecture
5	Yingjiang County, Dehong Prefecture
6	Longchuan County, Dehong Prefecture
7	Longchuan County, Dehong Prefecture
8	Longchuan County, Dehong Prefecture
9	Mang City, Dehong Prefecture
10	Guangnan County, Wenshan Prefecture
11	Guangnan County, Wenshan Prefecture
12	Guangnan County, Wenshan Prefecture

## 2. Methods and results

### 2.1. Sample solution preparation

Accurately weigh 0.3 g of homogenized *Vespa aurea* in the digestion tank, add 8 mL of nitric acid, pre digest at 100 °C for 20 min, place it in the microwave digestion instrument, digest it according to the heating procedure (rise to 120 °C for 10 min, keep it for 5 min, rise to 150 °C for 10 min, keep it for 5 min, rise to 185 °C for 10 min, keep it for 35 min), take it out after cooling, drive out the acid at 100 °C for 1 h, then fix the volume of 25.00 mL with ultra pure water, and determine it by ICP method. Among them, the sample solution for the determination of arsenic is taken out after cooling, the acid is driven to about 1 mL at 150 °C, the volume is fixed to 22.5 mL with 1 mol/L hydrochloric acid, and 2.5 mL of 50 g/L thiourea ascorbic acid is added, which is determined by atomic fluorescence method after 0.5 H (as shown in table 2).

**Table 2.** 20 linear regression equations of inorganic elements.

Element	Regression equation	R	Linear range
Al	$Y = 284X + 15760$	1	0–10mg/L
B	$Y = 1648X + 4743$	0.9978	0–1mg/L
Ba	$Y = 88.3X + 828800$	0.9998	0–1mg/L
Cu	$Y = -0.5859X + 31910$	0.9997	0–0.5mg/L
Fe	$Y = 57.65X + 20330$	0.9999	0–5mg/L
Mn	$Y = 18.62X + 61370$	0.9993	0–0.5mg/L
Ni	$Y = 128.9X + 5736$	0.9995	0–5mg/L
Sr	$Y = 563.7X + 1613000$	0.9994	0–1mg/L
Ti	$Y = 193.4X + 31350$	0.9996	0–1mg/L
V	$Y = 29.78X + 10650$	0.9996	0–0.5mg/L
Zn	$Y = 119X + 15120$	0.9998	0–5mg/L
Ca	$Y = 1203X + 13600$	0.9995	0–80mg/L
K	$Y = 28.58X + 485.6$	0.9998	0–80mg/L
Mg	$Y = 38.67X + 1912$	0.9997	0–80mg/L
Na	$Y = 50.32X + 407.9$	0.9999	0–80mg/L
P	$Y = -0.1733X + 549.5$	0.9998	0–80mg/L
Hg	$Y = 496.208X + 19.914$	0.9997	0–4μg/L
As	$Y = 102.505X - 16.404$	0.9996	0–10μg/L
Pb	$Y = 0.00780X + 0.02109$	0.9989	0–10μg/L
Cd	$Y = 0.12234X + 0.02021$	0.9985	0–3.2μg/L

### 2.2. Preparation of standard curve

### 2.3. Methodological review

#### 2.3.1. Precision

Take the same mixed standard solution for continuous determination for 6 times, and the RSD of each element is 0.05%–1.26%, indicating that the precision of the instrument is good.

#### 2.3.2. Repeatability

Take No. 1 sample, do three groups of parallel experiments, and calculate the relative standard deviation between the three groups by using the average test results. Through calculation, the precision between the three groups is between 0.02% and 1.14%, indicating that the method deviation is small.

### 2.3.3. Stability

Take the No. 1 sample, prepare the sample according to 2.1, and measure 20 metal elements in the sample solution at 0, 2, 4, 6, 8, 16, and 24 h. The element is stable within 24 h.

### 2.3.4. Method recovery

The recovery rate of spiked sample 1 was tested, and the recovery rate of the method was the average value of three parallel determinations. Results the recoveries of spiked samples were between 80% and 120%. The method was accurate and met the requirements of determining the content of elements in traditional Chinese medicine.

## 2.4. Determination results and analysis of metal elements of *Vespa aurata* from different places of origin

The results of the determination of 20 metal elements in wasps from different production areas are shown in Table 3. It can be seen from Figure 1 that the content of Ca, K, Mg, Na and P in the golden-ringed *Vespa* is relatively large, the content of 20 inorganic metal elements is large, which is 2507.1629 mg/kg, and the content of Hg is the lowest, which is 0.0107 mg/kg. B, Cd, and Pb elements were not detected in wasps in many production areas. The 2020 edition of the Pharmacopoeia stipulates harmful metal elements in Chinese medicinal materials. Pb, Cd, As, Hg, and Cu cannot exceed 5, 1, 2, 0.2, 20 mg/kg, the contents of Pb, Cd, As, Hg, and Cu in the 14 different producing areas of *Vespa* were all higher than the limit values of the Chinese Pharmacopoeia, indicating that the harmful metal elements in the *Vespa* complied with the requirements of the National Pharmacopoeia. Among the 20 metal elements, the coefficient of variation of Sr element is the largest, indicating that the Sr element fluctuates greatly in different production areas, while the coefficient of variation of Ca element is the smallest, indicating that the fluctuation of Ca element in the golden-ringed wasp from different production areas is small. It can be seen that the content of P element in the golden-ringed *Vespa* is relatively high, accounting for 47.12%, the content of K element is 25.05%, the content of Na element is 9.29%, the content of Mg element is 6.23%, the content of Ca element is 4.63%, and the rest Elements accounted for 7.68%, indicating that the golden-ringed wasp is rich in nutrients.

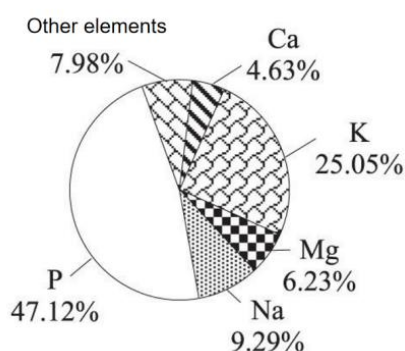


Figure 1. Analysis of the proportion of inorganic elements in *Vespa aurata*.

Table 3. Determination results of metal elements in fresh wasps (mg/kg).

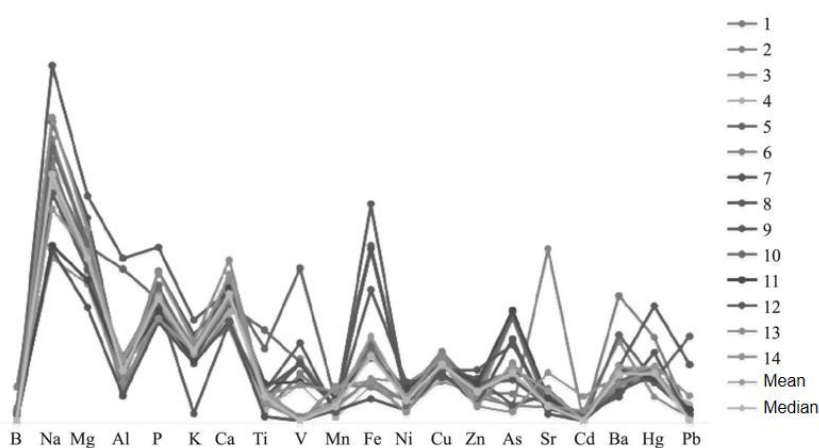
Number	Al	B	Ba	Cu	Fe	Mn	Ni	Sr	Ti	V	Zn
1	57.7600	2.2321	2.5078	7.8356	82.8056	30.2737	0.3786	0.3480	1.6825	0.0944	55.2353
2	129.8263	6.7818	1.1094	13.9344	158.1097	43.3843	0.5597	3.4530	5.5316	0.0083	61.3936
3	74.0978	1.5267	1.5914	11.6722	84.7411	24.3447	0.7643	0.2371	2.5399	0.1245	43.0028
4	102.7904	Not detected	0.7795	8.3786	69.6482	6.6667	0.2044	0.2868	2.2221	0.0721	50.4765
5	304.0420	1.9420	1.7226	12.7378	351.6171	41.3725	0.4769	0.6345	14.4550	0.3059	71.7324

6	108.0528	2.5849	0.6924	13.2370	66.8235	68.7297	0.3920	0.2723	3.4918	Not detected	46.4422
7	49.6457	Not detected	0.5942	9.7525	43.9799	20.5043	0.2092	0.1324	0.8194	Not detected	32.1938
8	108.0774	Not detected	1.0577	8.7007	262.7732	21.8213	0.7772	0.3073	4.6358	0.1136	71.9831
9	325.5352	1.0968	1.0764	10.5219	434.5564	17.1126	0.3934	0.5054	6.0796	0.1554	84.5402
10	90.0188	Not detected	0.7904	11.3663	148.3649	27.3487	0.3374	0.2693	18.1523	0.1174	63.2740
11	97.7950	Not detected	0.9713	11.2246	125.6730	50.6634	0.6494	0.4480	7.4815	0.0777	75.1964
12	81.5372	Not detected	0.4758	10.0609	344.2862	26.2840	0.4139	0.2304	6.2573	0.1144	102.0759
13	77.0850	Not detected	0.7338	12.8072	132.6728	59.0063	0.4897	0.2918	5.0547	0.0715	28.1446
14	103.0059	Not detected	0.9044	11.6134	80.9785	67.0220	0.1637	0.9645	3.5995	Not detected	29.1786
Average value	122.0907	-	1.0719	10.9888	170.5022	36.0382	0.4436	0.5986	5.8574	-	58.205
Maximum value	304.042	-	2.5078	13.9344	351.6171	68.7297	0.7772	3.453	18.1523	-	102.0759
Minimum value	57.76	-	0.4758	8.3786	43.9799	6.6667	0.1637	0.1324	0.8194	-	28.1446
Range	246.282	-	2.032	5.5558	307.6372	62.063	0.6135	3.3206	17.3329	-	73.9313
Standard deviation	84.43	-	0.54	1.88	125.67	19.33	0.19	0.85	4.87	-	21.80
Coefficient of variation/%	69.15	-	50.45	17.07	73.71	53.64	43.55	141.69	83.09	-	37.45

Number	Ca	K	Mg	Na	P	Hg	Cd	Pb	As
1	186.3197	1317.3483	310.7797	521.3059	2280.2926	0.0167	Not detected	0.0096	0.0539
2	284.6252	1417.7697	302.8855	490.3359	2520.2846	0.0084	Not detected	0.0122	0.0252
3	214.5174	1154.4972	273.9524	325.4752	2055.5373	0.0048	0.0203	0.0063	0.2060
4	216.8136	1393.9267	297.0097	480.7419	2465.3466	0.0084	Not detected	0.0326	0.0915
5	260.7579	1417.1042	349.8203	496.3336	2425.3594	0.0081	Not detected	0.1691	0.1003
6	266.0596	1461.7011	357.7300	564.4860	2617.4952	0.0098	Not detected	Not detected	0.0301
7	199.1978	1149.5334	226.9966	342.7227	1979.7806	0.0092	Not detected	0.0137	0.1640
8	196.5293	141.4959	303.6274	455.5538	2357.3464	0.0076	Not detected	0.0131	0.0817
9	261.2009	2014.6692	450.4298	712.0329	3475.2676	0.0230	Not detected	0.1124	0.0313
10	232.3710	1272.3202	353.7896	548.1893	2710.6991	0.0106	Not detected	Not detected	0.2061
11	265.7022	1212.4616	281.6073	351.2500	2204.9401	0.0086	Not detected	0.0224	0.2205
12	248.3798	1722.7839	406.8493	599.1343	2995.5345	0.0137	0.014	Not detected	0.1526
13	322.5957	1590.9947	383.2891	608.2201	2969.8384	0.0101	0.02	Not detected	0.1165
14	293.6817	1393.6077	338.9688	423.8752	2042.5588	0.0110	0.0488	0.0502	0.0170
Average value	246.3394	1332.8724	331.2668	494.2612	2507.1629	0.0107	-	-	0.1069
Maximum value	322.5957	2014.6692	450.4298	712.0329	3475.2676	0.023	-	-	0.2205
Minimum value	186.3197	141.4959	226.9966	325.4752	1979.7806	0.0048	-	-	0.017
Range	136.276	1873.1733	223.4332	386.5577	1495.487	0.0182	-	-	0.2035
Standard deviation	40.29	413.71	58.19	110.43	422.01	0.00	-	-	0.07
Coefficient of variation/%	16.36	31.04	17.57	22.34	16.83	42.27	-	-	67.71

## 2.5. Establishment of inorganic element fingerprint of *Vespa aurata*

The quantitative levels of the 20 inorganic elements in the golden-ringed *Vespa* were quite different. In order to draw fingerprints more accurately, the contents of some elements are enlarged or reduced to the same order of magnitude, among which B, Cu, Ti are enlarged by 10 times, K, P are reduced by 10 times, Ba, Ni, Sr are enlarged by 100 times, V, Pb and As are enlarged by 1000 times, and Hg is enlarged by 10000 times. According to the order of atomic numbers of 20 inorganic elements, with the elements as the abscissa and the element content as the ordinate, the fingerprints of the inorganic elements of the golden-ringed *Vespa* are drawn, as shown in **Figure 2**. It can be seen that there are differences in the content of metallic elements in different production areas, but the inorganic element content in the whole production area presents a similar distribution trend in the order of atomic number. It can be seen from **Figure 2** that the inorganic element spectrum of Golden-ringed *Vespa* has strong characteristics and consistency, and the generated inorganic element fingerprint can provide a certain reference for the identification of Golden-ringed *Vespa*.



**Figure 2.** Fingerprint of inorganic elements of *Vespa aurata*.

## 2.6. Correlation analysis of inorganic element content of *Vespa aurata*

See **Table 4** for the content correlation of inorganic metal elements in golden ring wasp. The results show that 16 pairs of metal elements in golden ring wasp are significantly positively correlated ( $p < 0.01$ ), and 10 pairs of elements are positively correlated ( $p < 0.05$ ). The positive correlation shows that the accumulation process of inorganic metal elements in golden ring wasp is synergistic. The correlation analysis of the content of inorganic elements in *Vespa aurata* can explore the synergistic effect of the accumulation of various elements in the growth process, which can provide a useful reference for further regulating the feeding mechanism in the growth and development process of *Vespa aurata*.

**Table 4.** Correlation analysis of inorganic element content of *Vespa aurata*.

	Al	B	Ba	Cu	Fe	Mn	Ni	Sr	Ti	V	Zn	Ca	K	Mg	Na	P	Hg	Cd	Pb	As
Al	1	0.181	0.172	0.234	0.767**	-0.083	0.029	0.137	0.402	0.659*	0.431	0.255	0.369	0.542*	0.458	0.49	0.417	-0.216	0.909**	-0.346
B		1	0.342	0.493	-0.012	0.197	0.213	0.835**	-0.048	-0.114	0.031	0.174	0.149	-0.063	0.074	0.008	-0.048	-0.252	0.046	-0.459
Ba			1	-0.204	0.012	-0.096	0.261	0.066	-0.028	0.428	0.01	-0.354	-0.109	-0.153	-0.111	-0.263	0.132	-0.127	0.29	-0.158
Cu				1	0.015	0.684**	0.176	0.495	0.336	-0.044	-0.176	0.771**	0.316	0.201	0.079	0.127	-0.284	0.195	0.13	-0.082
Fe					1	-0.259	0.224	0.019	0.423	0.691**	0.786**	0.137	0.243	0.688**	0.583*	0.671**	0.491	-0.191	0.610*	-0.133
Mn						1	-0.011	0.221	0.042	-0.31	-0.36	0.726**	0.147	0.142	0.005	-0.11	-0.165	0.447	-0.059	-0.267
Ni							1	0.112	0.067	0.3	0.299	-0.059	-0.47	-0.125	-0.21	-0.046	-0.349	-0.186	-0.106	0.287
Sr								1	0.022	-0.244	0.025	0.381	0.101	-0.075	-0.008	-0.018	-0.101	-0.005	0.037	-0.427
Ti									1	0.586*	0.4	0.205	0.085	0.342	0.25	0.283	-0.021	-0.209	0.361	0.315
V										1	0.516	-0.113	0.056	0.332	0.223	0.263	0.109	-0.245	0.705**	0.2
Zn											1	-0.124	0.117	0.444	0.383	0.498	0.377	-0.425	0.26	0.11
Ca												1	0.516	0.493	0.342	0.376	0.04	0.432	0.167	-0.249
K													1	0.614*	0.573*	0.586*	0.573*	0.13	0.28	-0.185
Mg														1	0.910**	0.891**	0.671**	0.122	0.319	-0.335
Na															1	0.934**	0.720**	-0.152	0.2	-0.441
P																1	0.657*	-0.202	0.182	-0.217
Hg																	1	-0.092	0.261	-0.393
Cd																		1	-0.073	-0.115
Pb																			1	-0.268
As																				1

Note: \*  $P < 0.05$ , \*\*  $P < 0.01$ .

### 3. Conclusion

ICP method was used to analyze and measure the contents of 20 inorganic elements in *Vespa aurata* from 14 different production areas, and the fingerprint of inorganic elements of *Vespa aurata* was established. The results showed that there were many kinds of metal elements in *Vespa aurata*, including many beneficial trace elements, and the inorganic element was P. K. Na. Mg. Ca is dominant, P is the highest, followed by K. Na. Mg. Ca, five elements account for 92.32% of the total elements determined. Through experiments, it is concluded that there are differences in the content of metal elements in different production areas, which may be related to the living environment (altitude) of wasps. Climate). Type of food. The accumulation law of metal elements in wasps. Pb in *Vespa aurata* from 14 different production areas. Cd. As. Hg. The content of Cu did not exceed the limit value of Chinese Pharmacopoeia, indicating that the harmful metal elements in *Vespa aurata* met the requirements of national pharmacopoeia standards. Through the content analysis of metal elements, the establishment of the fingerprint of inorganic elements of *Vespa aurata* can provide a certain reference for the identification of *Vespa aurata*.

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### Conflict of interest

The authors declare no conflict of interest.

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