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Original Research

The Distribution of Phosphorus Forms in Wuyi Rock Region and Its Effect on Tea Quality-Related Constituents in Tea Garden Soil

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Abstract

Phosphorus is an essential element for life, an innate constituent of soil organic matter, and a major anthropogenic input to terrestrial ecosystems. This paper employed the sequential extraction method for phosphorus fraction to study the distribution of phosphorus in different region in Wuyishan. Hierarchical cluster and correlation were used for investigating the relationship between the phosphorus form and tea quality-related constituents in Zhengyan Rock Tea (ZYRT), Banyan Rock Tea (BYRT) and Zhou Rock Tea (ZRT). The result showed that the concentration of phosphorus in different regions followed this order: ZYRT>BYRT>ZRT. The relative contribution of phosphorus fraction in different regions show the similar trend: Metal Oxide Bound Phosphorus (NaOH-P)>Calcium Bound Phosphorus (HCl-P)> Reduced Phosphorus (BD-P)>Adsorbed Phosphorus (NH_4Cl-P). The maximum content of water extract (WE), caffeine (CF), tea polyphenols (TPP) and total amount of amino acids (TAA) in tea leaves is in ZYRT, followed BYRT, and least in the ZRT. The phosphorus forms seem to have no obvious effects on the tea quality in the ZYRT and BYRT area, while TPP and NaOH-P, TPP and HCl-P are significantly positively correlated ($r = 0.912$, $r = 0.956$, $r = 0.938$; $p<0.01$) in ZRT.

Keywords: phosphorus forms, Wuyi Rock tea, Tea quality-related constituents, chemical extraction

Introduction

Tea is one of the most consumed nonalcoholic beverages, which possesses numerous benefits for human health, including anticancer, antioxidant properties, reduction of free radicals, and other unique health advantages [1-3]. As the birthplace of Chinese oolong and black teas, Wuyishan has a long history of tea production [4]. According to the different geographical environment, Wuyi Rock Tea has been classified as Zhengyan Rock Tea (ZYRT), Banyan Rock Tea (BYRT) and Zhou Rock Tea (ZRT). It is noted that the tea quality in Wuyishan is followed this order: ZYRT>BYRT>ZRT [5].

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There are many factors contribute to tea quality, such as the characteristic of soil [6], the trace elements in the soil and phosphorus [7]. The functions of phosphorus cannot be neglected in the processes of photosynthesis and respiration of tea plant. Phosphorus in the soil plays an important role in the growth and yield of tea bush [8]. Phosphorus deficiency reduces the content of WE, TPP, TAA and theanine in green tea leaves, but increases the content of water-soluble sugar and phenolic ammonia ratio [9].

Studies have shown that TPP, TAA and WE of tea leaves have no significant relationship with the content of total phosphorus (TP) and organic phosphorus (OP) in soil [10, 11]. However, it is not sufficient to draw conclusions about effect of the phosphorus on the tea-related constituents. Phosphorus exists in a variety of complex chemical forms, and not all the forms of phosphorus are likely to be released from particulates. Its morphologies and contents are affected by many factors, and may be subjected to variations in response to the evaluation of physical, chemical, and biological conditions. The form of phosphorus determines its migration, transformation, and bioavailability in the environment [12, 13], and different forms of phosphorus might make different contributions to tea quality.

Phosphorus fraction's assessment can be made by determining the share of phosphorus in the mobile or immobile fraction [14]. Zhang et al. employed the chemical extraction to investigate the fate of fertilizer phosphorus in soil and phosphorus fractions with long-term phosphorus addition and depletion [15]. Previous studies [16] adopted the sequential extraction method for phosphorus fraction to study the distribution of phosphorus forms in riparian soils and peripheral river sediments. And result indicated that phosphorus, especially NaOH-P and HCl-P, in riparian soil were mainly affected by agricultural activities. Therefore, it is a good choice for using the chemical extraction to analysis the correlation between phosphorus fraction in the tea garden soil and tea quality. However, few literatures reported the distribution of phosphorus fraction on the tea quality, especially in the Wuyi Rock tea.

Therefore, the objectives of this study are focusing on the distribution of phosphorus fraction in the tea garden soil of Wuyishan and the relationship between the phosphorus fraction and tea-quality constituents. Sequential extraction was conducted to determine the fractions of phosphorus. The samples (soil and tea leaves) are from 29 different sites (9 samples in ZYRT area, 11 samples in BYRT area and 9 samples in ZRT area) in Wuyishan. Clarifying the correlation between phosphorus forms and tea quality would help understand the characterization of phosphorus in Rock tea garden soil and provide a reference for the protection of ecological environment of the tea gardens, which ensures the utilization of phosphorus fertilizer.

Material and Methods

Sample Collection

The sample sites were conducted at Wuyishan, located in the Fujian province, China, at May 2017. The tea garden (spring tea) from tea gardens are harvested only once a year in April or May, and are further cultivated and weeded in the fall. Tea seed cakes and oil-tea cakes are often used to fertilize and control pests in most of the rock tea. The basic information of the tested samples from various rock tea gardens are present in Table 1. Rack tea garden could be separated into ZYRT, BYRT and ZRT. Shoots comprising four leaves and an apical bud was harvested. The soil in the

Table 1. Tea plantations characteristics.

Sample No.	Rock area	Sample ID	Tea type	Age (years)	Soil type	Longitude (E)	Latitude (N)	Altitude (m)	The amount of fertilizer
1	ZYRT	Scenic Area Center	Shuixian	30	Moist sandy soil	117°55'58.96"	27°38'15.14"	222	0.1 kg/m^2
2	ZYRT	Scenic Area Center	Shuixian	30	Moist sandy soil	117°57'45.64"	27°39'40.2"	346	0.1 kg/m^2 (OF)
3	ZYRT	Scenic Area Center	Shuixian	30	Moist sandy soil	117°57'50.96"	27°39'54.64"	349	0.15 kg/m^2
4	ZYRT	Scenic Area Center	Shuixian	30	Moist sandy soil	117°57'37.9"	27°39'56.42"	338	0.15 kg/m^2 (OF)
5	ZYRT	Scenic Area Center	Shuixian	30	Moist sandy soil	117°57'20.89"	27°39'58.04"	363	0.2 kg/m^2
6	ZYRT	Scenic Area Center	Shuixian	30	Moist sandy soil	117°57'50.83"	27°38'23.56"	228	0.2 kg/m^2 (OF)
7	ZYRT	Scenic Area Center	Shuixian	30	Moist sandy soil	117°57'24.79"	27°38'32.44"	248	0.2 kg/m^2 (OF)
8	ZYRT	Scenic Area Center	Shuixian	30	Moist sandy soil	117°58'2.14"	27°38'30.41"	218	0.2 kg/m^2 (OF)

Table 1. Continued.

9	ZYRT	Scenic Area Center	Shuixian	30	Moist sandy soil	117°57'44.75"	27°38'24.63"	226	0.2 kg/m^2 (OF)
10	BYRT	Xing Village	Shuixian	30	Purple soil	117°59'56"	27°44'18"	219	0.1 kg/m^2
11	BYRT	Xing Village	Shuixian	30	Purple soil	117°59'57"	27°44'20"	227	0.1 kg/m^2
12	BYRT	Xing Village	Shuixian	30	Purple soil	117°5**9′**41″	27°**36′**34**″**	258	0.1 kg/m^2
13	BYRT	Xing Village	Shuixian	30	Purple soil	117°5**9′**42″	27°**36′**33**″**	258	0.1 kg/m^2
14	BYRT	Xing Village	Shuixian	30	Purple soil	117°56′52.30″	27°48′34.68″	258	0.15 kg/m^2
15	BYRT	Xing Village	Shuixian	30	Purple soil	117°54'21.57"	27°37'58.65"	246	0.1 kg/m^2 (OF)
16	BYRT	Xing Village	Shuixian	30	Purple soil	117°54'24.14"	27°37'58.95"	257	0.15 kg/m^2 (OF)
17	BYRT	Xing Village	Shuixian	30	Purple soil	117°55'57.87"	27°36'59.61"	298	0.2 kg/m^2 (OF)
18	BYRT	Xing Village	Shuixian	30	Purple soil	117°55'57.87"	27°36'59.61"	298	0.15 kg/m^2
19	BYRT	Xing Village	Shuixian	30	Purple soil	117°55'56.89"	27°36'55.43"	322	0.2 kg/m^2
20	BYRT	Xing Village	Shuixian	30	Purple soil	117°19'57"	27°36'04"	194	0.2 kg/m^2 (OF)
21	ZRT	Wufu	Shuixian	30	Yellowish red soil	118°20'87"	36°02'79"	193	0.2 kg/m^2 (OF)
22	ZRT	Wufu	Shuixian	30	Yellowish red soil	118°18'54"	27°36'7.08"	202	0.2 kg/m^2
23	ZRT	Wufu	Shuixian	30	Yellowish red soil	118°17'61"	36°06'56"	235	0.2 kg/m^2 (OF)
24	ZRT	Wufu	Shuixian	30	Yellowish red soil	118°20'43"	36°04'03"	188	0.15 kg/m^2 (OF)
25	ZRT	Wufu	Shuixian	30	Yellowish red soil	118°9'50"	27°36'28"	450	0.2 kg/m^2 (OF)
26	ZRT	Wufu	Shuixian	30	Yellowish red soil	118°9'41"	27°36'34"	400	0.1 kg/m^2
27	ZRT	Wufu	Shuixian	30	Yellowish red soil	118°9'42"	27°36'33"	420	0.1 kg/m^2 (OF)
28	ZRT	Wufu	Shuixian	30	Yellowish red soil	117°56'52.30"	27°48'34.68"	325	0.2 kg/m^2
29	ZRT	Wufu	Shuixian	30	Yellowish red soil	117°56'46.86"	27°48'29.62"	328	0.15 kg/m^2

Note: Organic Fertilizer (OF)

study area are characterized as Nitisols with a friable clay texture and derived from 0 to 100 mm top layer.

Sample Processing

The soil samples were first cleaned of gravel, plant roots, dead branches, and other impurities to perform the element fraction analysis. Subsequently, the soil samples were milled, dried in natural atmosphere, and further purified by a 20-mesh sieve (0.841mm). Finally, the samples were mixed uniformly and quartered. For each measurement, 200 g of the soil samples were taken out, ground in an agate grinder, passed through a 100-mesh sieve (0.147 mm), and properly stored.

The tea leaves samples in fresh condition were rinsed repeatedly with tap water to remove adhesive dust and debris, and further rinsed two or three times with deionized water. The clean leaves were then dried in air at room temperature, followed by another drying at 60ºC until constant weight. Subsequently, the dried leaves were crushed and filtered by an 80-mesh (0.177 mm).

Methods

Tea Quality Measurement

The collected tea leaves samples were subjected to the following biochemical analyses according ISO test methods to check their compliance to the ISO requirements (Table 2).

The ratio of tea polyphenols and amino acids (RAS) of the sample was calculated with the following equation:

$$RAS=\frac{m_0}{m_1} \quad (1)$$

Note: m_0 and m_1 is the amount of TPP and TAA, respectively.

Coefficient of variation (CV) of the sample was calculated with the following equation:

$$CV=\frac{SD}{MN}*100\% \quad (2)$$

...were the SD and MN is stand for the standard deviation and average of sample.

Determination of Soil Chemical Properties

Soil samples are analyzed with total nitrogen (TN) measured using the concentrated H_2SO_4 digestion method and total phosphorus (TP) determined by the molybdate colorimetric method with perchloric acid digestion. pH was measured with a glass electrode in a slurry of 10 g of soil and 25 cm^3 of deionized water. The organic matter (OM) in the sediments is measured after treatment with $K_2Cr_2O_7/H_2SO_4$ according to the Walkley-Black method.

TP in sediments can be divided as inorganic phosphorus (IP) and organic phosphorus (OP). IP can be divided as weakly adsorbed phosphorus (NH_4Cl-P), reduced phosphorus (BD-P), metal oxide bound phosphorus (NaOH-P) and calcium bound phosphorus (HCl-P). Among them, NH_4Cl-P, BD-P and NaOH-P are the bioavailable forms of phosphorus (BAP = NH_4Cl-P + BD^-P + NaOH-P = IP - HCl-P), while HCl-P mainly comes from clastic rocks and autogenous sources [21]. Indeed, HCl-P is the most stable form and can be regarded as a permanent sink of phosphorus. In other words, IP is difficult to be utilized by organisms. The extraction processes of phosphorus are shown in Fig. 1. Each phosphorus fraction was quantitatively assessed by the molybdenum blue/ascorbic acid method, and more details of extraction can be found in the references. The experiments were repeated for three times and the relative deviations of the results were kept below 5%.

Table 2. ISO recommended chemical parameters for tea.

Parameter	Test method	Reference
Water extract (%) (WE)	ISO 9768	[17]
Tea polyphenols (%) (TPP)	ISO 14502-1	[18]
Caffeine (%) (CF)	ISO 4052	[19]
The total amount of amino acids (%) (TAA)	Ninhydrin method	[20]

Data Analysis

In this study, hierarchical cluster and correlation were used for factors selecting. Hierarchical cluster analysis (HCA), an unsupervised learning process, divides similar objects into group or more subsets through static classification, and members in the same subset have similar properties, which use Euclidean distances [22] to calculate the distance (similarity) between different categories of data points. A more general alternative is the weighted Euclidean distance between two vectors x_i and x_j

$$d_{i,j}=\left[\sum_{k=1}^{K} w_k \left(x_{i,k}-x_{j,k}\right)^2\right]^{1/2} \quad (3)$$

For w_k = 1 for k-1, …, K, Equation (2-3) reduces to the ordinary Euclidean distance.

Correlation analysis refers to the analysis of two or more related variable elements to measure the closeness of the two variables factors [23]. The Person correlation coefficient, the covariance of the two variables divided by the product of their standard deviations, was used to measure the degree of correlation in this study. Correlation analysis and one-way ANOVA were performed using SPSS (version 22.0 for windows) and the figure in this paper was compiled by Origin 9.0 and Seaborn in Python 3.7.

Results and Discussion

The Distribution of Elements in the Rock Tea Garden Soil

The elements in tea garden soil are shown in Fig. 2, Si, Al, and Fe are the main elements in the soil [24]. The element of Si in the ZYRT area was highest with 51.56%, and in the ZRT area soil was lowest with 39.84%. Compared with BYRT and ZRT, the amount of Al and Fe in ZYRT area was lowest, with 9.91% and 2.60%, respectively. The present of Al and Fe would have the effect on the phosphorus in the NaOH-P [25]. In these three different areas, the element of Ca and Mn also have some discrepancy. The proportion of Ca in

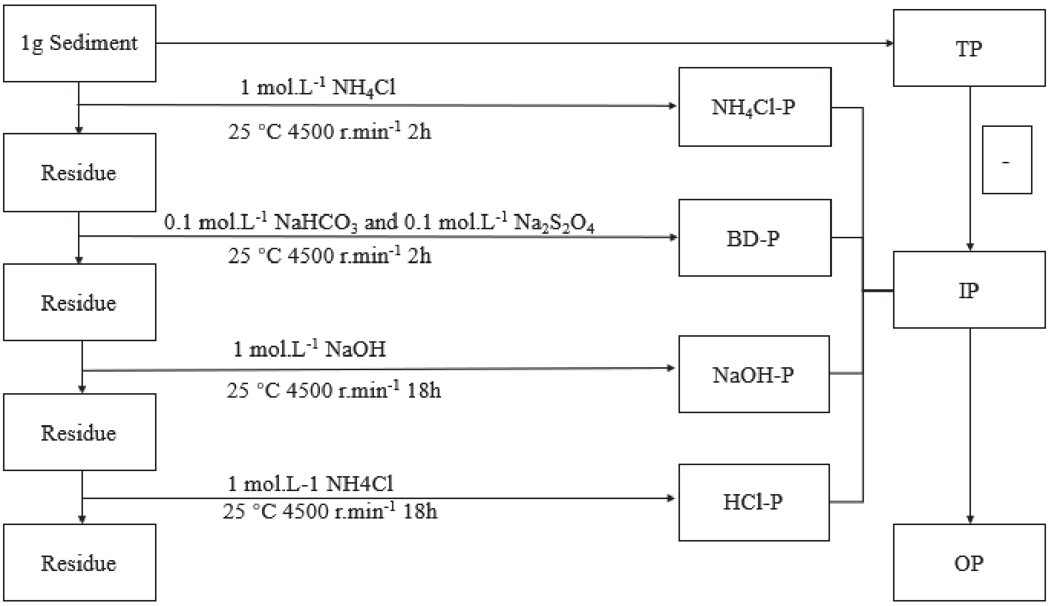

Fig. 1. The extraction processes of phosphorus in sample.

ZRT area is lower than other place, and the percentage of Mn in this place is 0.12%. Some researchers [26] suggested that the form of phosphorus like HCl-P may be subjected to the element of Ca and Mn.

Chemical Characteristics of the Rock Tea Garden Soil

Fig. 3 shows the chemical characteristics of the rock tea garden soil in different regions. The range of pH value in ZYRT, BYRT, and ZRT soil area were 4.32, 4.51, and 4.59, respectively, as the Fig. 3 shown. Literatures [27] have shown that pH optima of tea growth condition were between 4.2 and 5.5, which indicate that the soil of Wuyi Rock area is suitable for tea growth.

The value of OM, TN, TP, AN and AP followed the order: ZYRT>BYRT>ZRT. The OM content in the ZYRT, BYRT and ZRT were 31.52, 29.55, and 28.85 mg/kg, respectively. It indicated that Wuyishan has a fertile soil and its fertility meets the standard of first-class soil fertility in the regulation of Technical Condition of a Tea-Producing Area (NY/T 853-2004) [28].

Compared with BYRT and ZRT, the content of TN in ZYRT is 1626 and 1407 mg/kg, respectively, in Fig. 3. Meanwhile, the scale of AN in ZYRT and BYRT are approximately equal (110 and 101 mg/kg). By contrast, the main AN distribution in ZRT was 80 mg/kg. as the available nitrogen is also vital factor for the tea bush growth, the discrepancy of AN in ZRT, BYRT and ZRT maybe result in the tea quality [29]. Fig. 3 plots that both the amount of TP and AP in ZYRT are higher than in other area, which may contribute to the reason that the tea quality in ZRT better than other places [30].

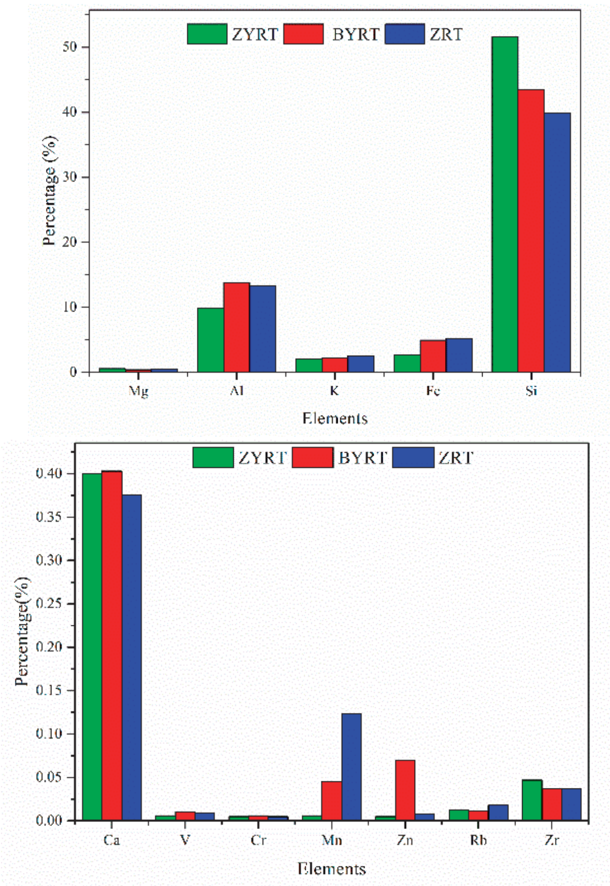

Fig. 2. Comparison of the elements in the tea soil garden tea in different regions.

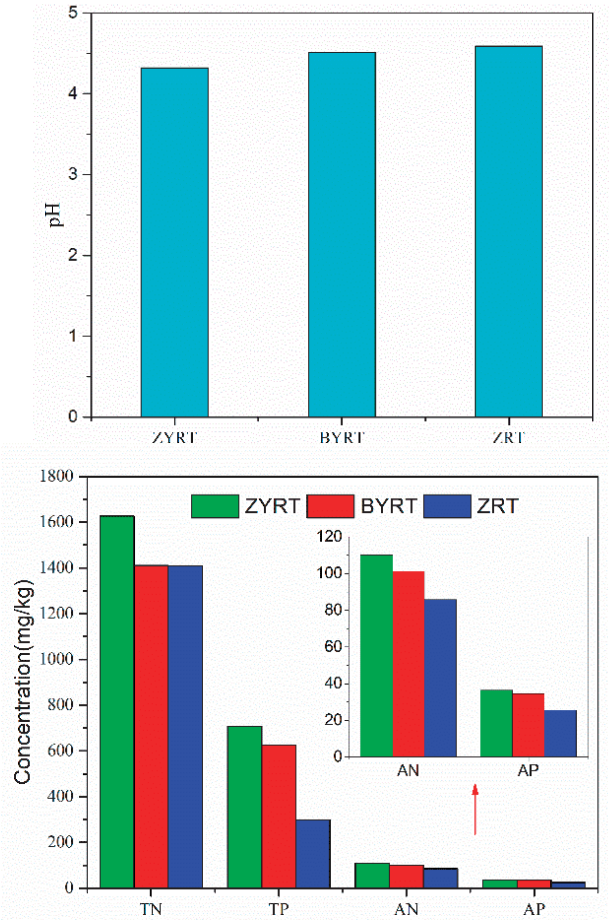

Fig. 3. Distribution of nutrient elements in tea garden soil.

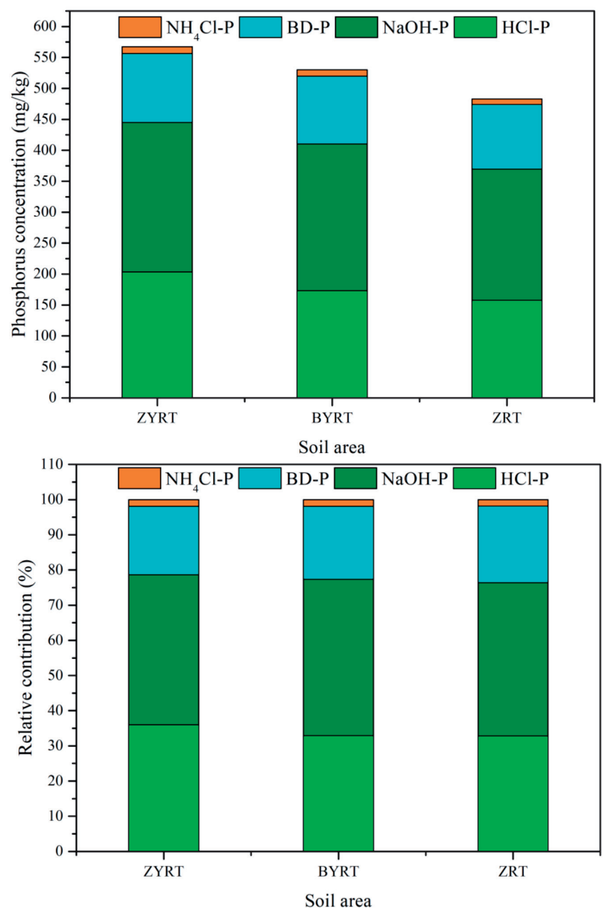

Fig. 5. Average of concentrations and proportions of phosphorus fraction in different regions.

Effect of the Different Type of Soils on the Form of Phosphorus

In order to further study the characteristics of phosphorus fractions, the proportion of phosphorus fraction contents and relative contribution values associated with different phosphorus forms in different regions was plotted in Fig. 4. In ZYRT, the concentration of phosphorus increased with the amount of applying fertilizers. Inputting 0.1 kg/m², 0.15 kg/m², and 0.2 kg/m² compound fertilizer in the 1st, 3rd, and 5th

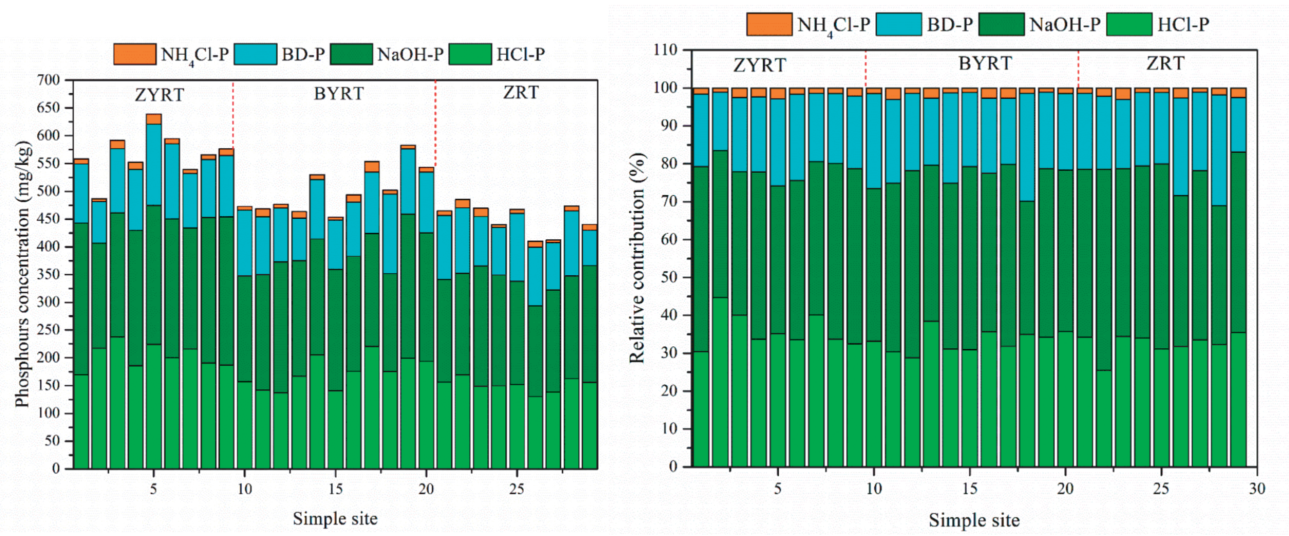

Fig. 4. Concentrations and proportions of phosphorus fraction in different regions.

sample site, respectively, and phosphorus concentration follow this order: 5th (639.248 mg/kg) >3rd (591.66 mg/kg) >1st (558.44 mg/kg). Meanwhile, the 2nd (0.1 kg/m^2 organic fertilizer), 4th (0.15kg/m^2 organic fertilizer) and 6th (0.2 kg/m^2 organic fertilizer) sample sites also show the similar trends.

As for BYRT tea garden soil, the first and second largest amount of phosphorus in the soil at the 19th and 17th with 582.84 mg/kg and 553.55 mg/kg. The data of phosphorus at the 10th, 11th, 12th,13th and 16th sample sites were not obvious different. Related to ZRT garden soil, phosphorus concentration is almost at the same level except the 26th and 27th. These two simple sites were applied compound fertilizer (0.1 kg/m^2) and organic fertilizer (0.1 kg/m^2), respectively.

The distribution of phosphorus forms at all sample sites except 2nd and 7th in ZYRT tea garden mainly follow this pattern: NaOH-P>HCl-P>BD-P>NH_4Cl-P. While the distribution of phosphorus fraction at 2nd and 7th sample sites follows this order: HCl-P>NaOH-P>BD-P>NH_4Cl-P. By contrast, the ranking of four phosphorus forms in BYRT and ZRT follow an unchanged pattern, NaOH-P>HCl-P>BD-P>NH_4Cl-P at all sampling point. And phosphorus fraction in the BYRT and ZRT main in the NaOH-P and HCl-P form, with total percentage of its in NaOH-P and HCl-P to IP more than 70%.

Fig. 5 plotted the average of concentrations and proportion of phosphorus fraction in different regions. The concentration of phosphorus in different regions followed this order: ZYRT>BYRT>ZRT, which is in agreement with others researchers' conclusions [31]. While the relative contribution of phosphorus fraction in different regions show the similar trend: NaOH-P>HCl-P>BD-P>NH_4Cl-P, which indicated that the phosphorus

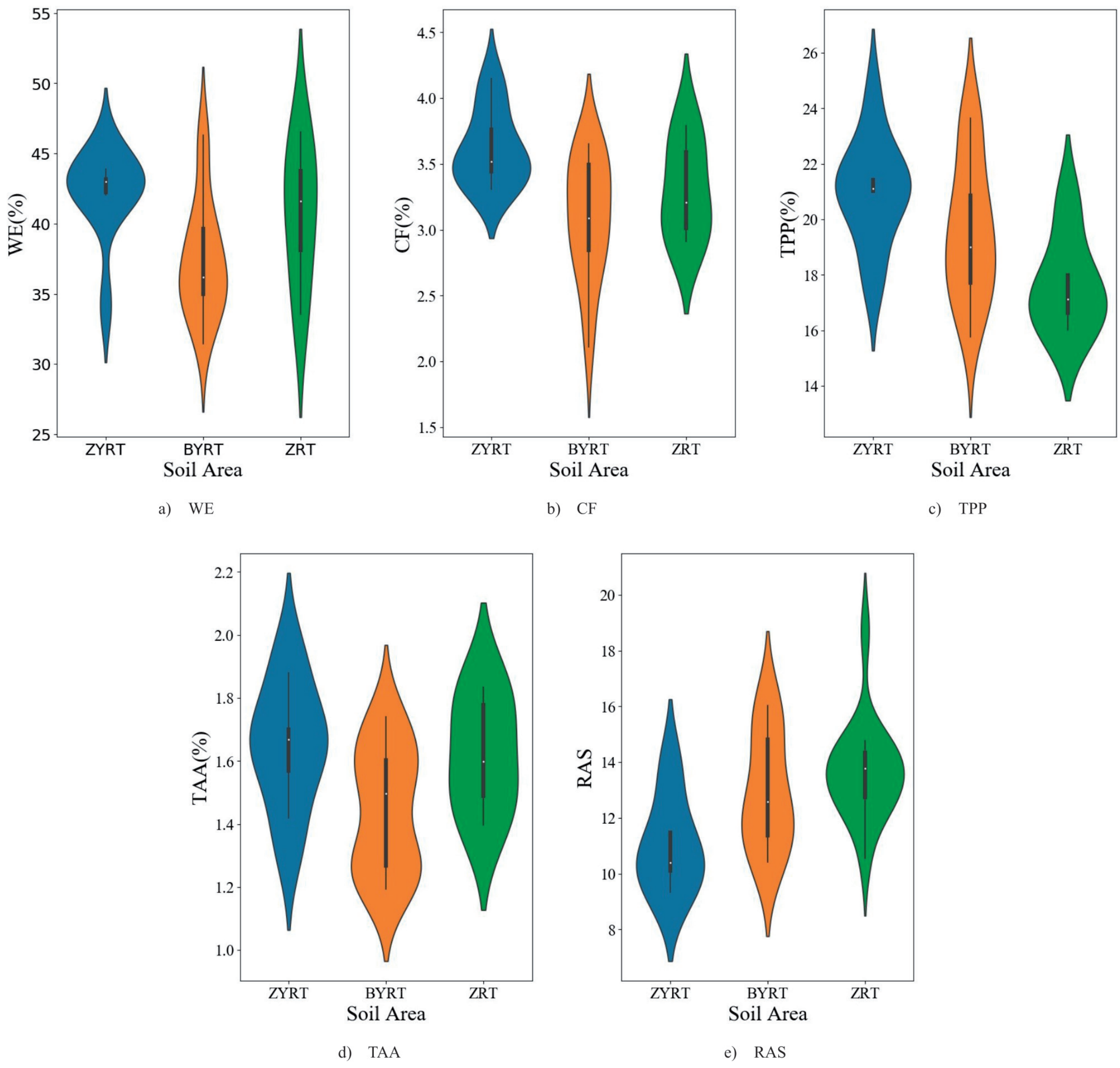

Fig. 6. the distribution of tea quality-related constituents.

forms in the different regions show the same trends in Wuyi rock tea.

The Characteristics of Tea Quality

The violin plot features a kernel density estimation of the underlying distribution. Fig. 6 plots the distribution of tea quality-related constituents (WE, CF, TPP, TAA and RAS) in ZYRT, BYRT and ZRT garden area. WE, CF, TPP, TAA and RAS are crucial indexes to evaluate the tea quality [32].

As plotted in the Fig. 6a), the possibility of WE from the ZYRT leaves was 40~45%, while the content of WE in BYRT area was lower than that in ZYRT, with 30~40%. Compared with the leaves from ZYRT and BYRT area, the amount of WE in tea bush from the ZRT ranged from 27% to 53%. This phenomenon indicated that the content of WE in tea leaves was fluctuated, which may lead to the tea quality from this area is unstable.

The distribution of CF from tea leaves seems more concentrated than that in another place, the CV of CF in ZYRT, BYRT and ZRT was in 6.58%, 14.33% and 10.21%, respectively (Fig. 6b). TPP in tea leaves have the ability to reduce the risk of a variety of diseases and it also cannot be neglected parameter for tea quality [33]. The probability of TPP content in ZYRT and ZRT show the "spindle" shape, but the value of TPP in ZYRT area is higher than that in ZRT area. As for BYRT soil area, the scale of TPP values cover the range from 13% to 27%.

Amino acid mainly represents the N-Compounds in tea quality-related constituents and the main material makes the tea soup fresh and fragrant [34]. Fig. 6d) indicated that the percentage of TAA in tea leaves from ZYRT, BYRT and ZRT area was 1.1~2.2%, 0.8~1.8%, and 1.1~2.1%, respectively. Many researchers [35] suggested that it is not reasonable to use the amino acid to evaluate the tea quality, because the type of amino acid is various and different.

Therefore, it seems that using the RAS is more available than only Amino index. The average of RAS in ZYRT, BYRT and ZRT was 11.02, 12.94 and 13.69, respectively. The fresh and brisk taste increases as the ratio of RAS decreases. In other word, the higher the ratio of RAS, the poorer the fresh and brisk taste.

HCA and Correlation Analysis

HCA

A heat map (Fig.7) was created using seaborn (version 1.0) to visualize the content composition in different regions: red, purple and cyan areas indicate high, low, and moderate levels of chemical composition, respectively. Hierarchical cluster analysis, an unsupervised learning process, divides similar objects

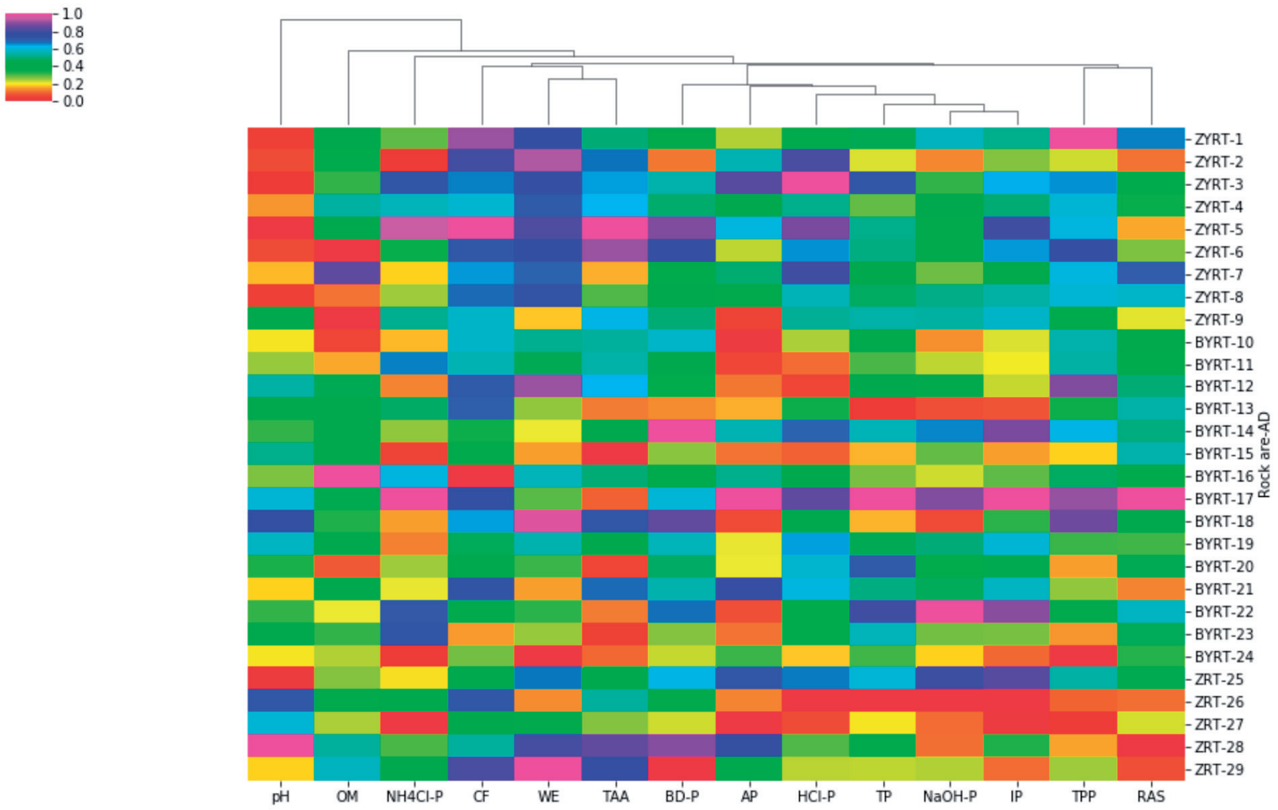

Fig. 7. Heat map of 14 variables and the results of hierarchical clustering for origins.

into groups or more subsets through static classification, and members in the same subset have similar properties, which use Euclidean distance to calculate the distance (similarity) between different categories of data points. 14 variables could be divided into four categories: I (IP, NaOH-P, TP, HCl-P, AP, BD-P), II (TPP and RAS), III (TAA, WE and CF), and other (NH_4Cl-P, OM, and pH). The distance from I to II and III is approximately equal, which indicate that the phosphorus fraction except NH_4Cl-P have significant correlation with tea indexes.

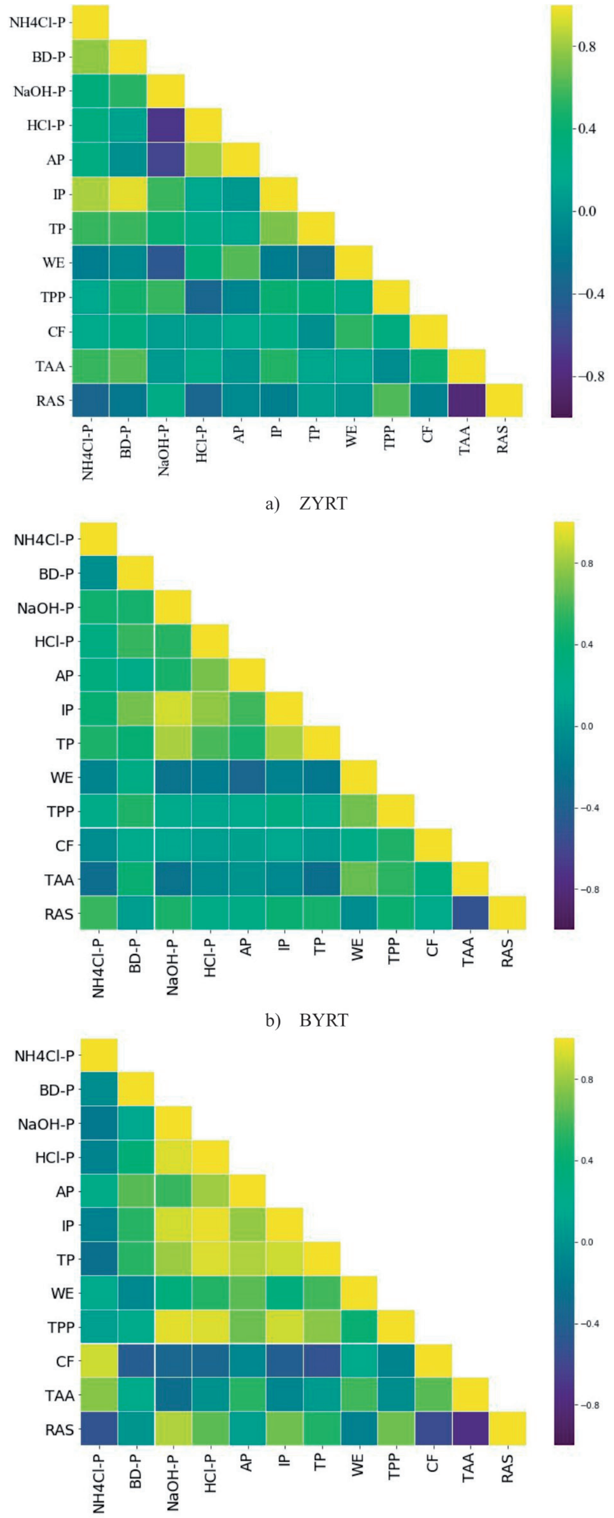

Fig. 8. Person's correlation coefficients of p distribution and tea quality in different regions: a) ZYRT; b) BYRT; c) ZRT.

Correlation Analysis

The Pearson correlation coefficient matrix of phosphorus forms in different regions is shown in Fig. 7a), which shows that the NH_4Cl-P with BD-P and IP contents in ZYRT areas are significantly positively correlated (r = 0.774, p<0.01; r = 0.84, p<0.05). And the correlation coefficient between BD-P contents and IP contents value reached 0.959 (p<0.05). while the relationship between NaOH-P contents and HCl-P is negative (r = -0.691, p<0.01). It seems that the phosphorus form may have some relationship with tea quality-related indexes. there are some indexes indicated the negatively correlated in ZYRT garden soil: WE content and NH_4Cl-P (r = -0.163), WS contents and BD-P (r=-0.065), WS contents and NaOH-P (r = -0.483), and TAA contents and HCl-P (r = -0.345). On the contrary, the rest of tea quality constituents and phosphorus fraction seem positive relationship, especially between TPP content and NaOH-P content (r = 0.454), TAA and BD-P content (r = 0.558).

As for BYRT area, phosphorus fraction shows the similar correlation in WE and TAA: BD-P have positive correlation with WE (r = 0.259) and TAA (r = 0.408), while the relationship with the rest of phosphorus fraction (NH_4Cl-P, NaOH-P, and HCl-P), WE and TAA show a negative trend.

In the ZRT garden area, TPP and NaOH-P, TPP and HCl-P are significantly positively correlated (r = 0.912, r = 0.956, r = 0.938; p<0.01), suggesting that the tea quality like CF and TPP are subjected by HCl-P and NaOH-P. While other phosphorus fraction has negative correlation with CF, the correlation value of CF and BD-P, NaOH-P, and HCl-P is -0.43, -0.348, and -0.344, respectively.

In conclusion, it seems that the tea quality indexes and phosphorus forms only in ZRT area have high correction, and it is not obvious in ZYRT and BYRT rock area.

Conclusions

We have investigated the distribution of phosphorus fraction in different tea garden soil and the effect of phosphorus form on the tea quality in Wuyishan. The result indicated that the content of OM, TN, TP, AN and AP in different regions in Wuyishan followed this order: ZYRT> BYRT>ZRT. The concentration of phosphorus in different regions also followed same order. In addition, the relative contribution of phosphorus fraction in different regions show the similar trend:

NaOH-P> HCl-P> BD-P> NH_4Cl-P. The content of WE, CF, TPP and TAA in tea leaves is largest in ZYRT, followed BYRT, and last in the ZRT. The phosphorus forms seem have not obviously effects on the tea quality in the ZYRT and BYRT area, while TPP and NaOH-P, TPP and HCl-P are significantly positively correlated ($r = 0.912$, $r = 0.956$, $r = 0.938$; $p<0.01$) in ZRT.

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