



## DRIS formulas and PCA approach for evaluation of the nutritional status of walnut trees in the Hawraman area

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Received 10 September 2023; revised 28 November 2023;  
accepted 12 December 2023; available online 26 January 2024

DOI: 10.24271/PSR.2023.415676.1388

### ABSTRACT

It is essential to understand the proper nutrient levels for obtaining the highest yield when analyzing the foliar composition of walnut leaves using DRIS techniques that consider the interaction between nutrients. The study aims to identify the nutritional deficiencies in walnut orchards responsible for low production by analyzing the data using DRIS and PCA methods. Measured nine elements in the walnut tree leaf: N, P, K, Ca, Mg, and microelements Fe, Zn, Cu, and Mn. Used the Diagnosis and Recommendation Integrated System (DRIS) to assess nutrient status in the leaf, detect deficits or imbalances, and make specific recommendations for optimal growth. Along with Principal component analysis, diagnose deficient nutrients and deal with problems. In the present work, 42 samples of walnut leaves were analyzed and collected from the walnut orchard at the Balkha/Hawraman, located at 35°11'59.0"N 46°08'59.1"E, altitude 695 m.a.s.l. A high-yielding population of walnut trees forms up 71.4% of the total, while a low-yielding population makes up the remaining 28.6%. Results showed that the most deficient nutrients ascendingly were: Fe>Cu> Zn>Mn>N=Ca>K>P>Mg. The PCA results show that the DRIS index and the low- and high-yield subgroups' nutrient concentrations were represented by 78.8%, 87.2%, and 93.5%, respectively of the total variance. From previous results and field observation, the walnut orchards need more attention and studies in other aspects to fulfill knowledge about soil quality index, climatic changes, and diseases related to these precious trees to make decisions for best monitoring and management.

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Keywords: Walnut, DRIS, PCA, Nutrient imbalance index, Hawraman area.

### 1. Introduction

Growing walnut trees (*Juglans* spp.) has historically been a major undertaking for growers and horticulturists all over the world. To maintain the best development and output, these precious trees—respected for their valuable timber, nourishing nuts, and aesthetic appeal—need careful maintenance. However, it can be challenging to comprehend the complex relationship between nutrient management and walnut tree growth traits <sup>[1, 2]</sup>.

The Diagnosis and Recommendation Integrated System (DRIS) has become an effective tool in the field of plant nutrition in recent years. It is specialized advice for achieving optimum growth. Through this ground-breaking method, walnut tree growers can unlock their orchards' full potential, resulting in healthier trees, higher yields, and greater profitability <sup>[3]</sup>.

Positive correlations are expected when a specific nutrient is a limiting factor. The key to improving nutrient status should be a balanced and sufficient supply of macro- and micronutrients for growth and yield that are focused on nutrient requirements. Understanding the ideal nutrient concentrations or sufficient

ranges of nutrients is essential to accurately diagnose and improve the nutrient status of cultivated plants <sup>[4-6]</sup>.

Tissue analysis is thought to be a more direct means of evaluating a plant's nutritional status than soil analysis; the latter approach inevitably requires a precise study of plant components. The primary sampling material among the several tissues that should be taken into account for nutritional diagnostic purposes is leaves. The development of tissue analysis techniques made it possible to compare the outcomes of various soil-type fertilization trials <sup>[7, 8]</sup>.

It is crucial to understand their growth characteristics to optimize walnut tree development and ensure sustainable farming methods. At different stages of development, these trees have varying nutritional requirements, and any imbalance can significantly affect their overall growth<sup>[9]</sup>. Therefore, to achieve thriving orchards and maximize the economic benefits, a thorough understanding of the nutritional requirements of walnut trees is essential, along with an accurate diagnostic system like DRIS <sup>[10]</sup>.

Farmers and horticulturists must employ cutting-edge methods that maximize production efficiency while minimizing

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Peer-reviewed under the responsibility of the University of Garmian.

environmental effects as the demand for high-quality walnuts rises. A promising approach to attaining these goals is incorporating the DRIS method into walnut tree farming. Growers may increase their yields and support careful nutrient management [11].

A multivariate mathematical reduction technique called principal component analysis (PCA) condensed a large data set of variables (called a principal component, or PC), which accounted for most of the original data set's information. Several investigators have used PCA on DRIS indices [12, 13].

To interpret the results of leaf analysis for a variety of plants, including lentils [14], oak [15], wheat [16], soybeans [17], sunflowers [18], broccoli [19], walnut [20], and onion [21], several authors in Iraq have successfully used the DRIS method.

The availability of information on the nutrient balance of a walnut tree for local agricultural management and orchard producers who complain about the lack of production of these trees was studied previously [20].

In the present investigation, the DRIS approach was employed to interpret leaf nutrient analysis data collected from different walnut orchards in the Hawraman region of Iraq. The availability and deficiency ranges were derived with the DRIS technique, which were used to monitor the nutrient status of walnut trees. The study aims to diagnose the nutrient deficiency in walnut orchards that caused a low yield by interpreting the data using DRIS and PCA techniques.

## 2. Methods and Materials

### 2.1 Sample collection

Hawraman is a mountainous region located within the Halabja province in north-eastern Iraq. The region's climate is characterized by lengthy, snowy winters and short, cold summers [22]. Walnut is the most abundant tree in the area besides apple and pomegranate trees [23]. Walnut orchards in Balkha village were selected for the present investigation. Samples were taken with the proprietors of the walnut orchards' prior consent, using a Global Positioning System (UTM): Balkha village (35°11'59.0"N 46°08'59.1"E), at elevation 695 m.a.s.l. Samples of leaves from walnut trees were collected randomly and homogeneously in a circular manner from the tree branches and in all directions. The annual precipitation is nearly 720mm.

### 2.2 Laboratory analysis

The leaf samples were washed and oven-dried at 65 °C for 48 hours, then powdered. The wet digest (H<sub>2</sub>SO<sub>4</sub>:H<sub>2</sub>O<sub>2</sub>) procedure has been used for powder samples [24]. According to [25], nitrogen (N) was measured from the digestive samples using the Micro-Kjeldahl method, phosphorus (P) was measured using the Molybdenum Blue method and quantified by the Spectrophotometer, potassium (K) was determined using the Flame Photometer, calcium (Ca) and magnesium (Mg) were determined using the EDTA titrimetric method. Meanwhile, Zn, Fe, Mn and Cu was determined in dry leaves powdered by the XRF technique [26, 27].

### 2.3 DRIS calculation

For both high-yield and low-yield populations, the mean, variance, and coefficient of variation (CV) computing ratios of pairs of nutrients (e.g., N:P, K:Ca) based on their concentrations in the samples [28]. The mean and CV ratios for each nutrient pair that maximized the variance ratio between the low and high populations were chosen as the pair's norms according to [29]. The whole population was divided into two sub-groups, namely low- and high-yielding orchards, using 12.6 kg tree<sup>-1</sup> or 3.54 t.ha<sup>-1</sup> as the cut-off yield after the establishment of the data bank of leaf nutrient concentration for walnut trees from a previous study [20]. High-yielding orchards produced more than 12.6 kg of walnuts per tree while low-yielding orchards produced less than 12.6 kg per tree.

Nutrient ratios or products can be arranged using DRIS into an accurate description known as the DRIS index. The average departure of the ratios containing a certain nutrient from their corresponding optimum or standard deviation is referred to as the nutrient index. All indicators were nearly balanced. Nutrient indices, therefore, are to sum zero. The more insufficient a nutrient is compared to other nutrients used in the diagnosis, the more negative an indicator is. While a positive index indicated that the corresponding nutrients existed in relatively high concentrations. For nutrients, the following generalized equation could be used to obtain DRIS indices [30]

$$N \text{ index} = \left[ f\left(\frac{N}{P}\right) + f\left(\frac{N}{Ca}\right) + \dots - f\left(\frac{Cu}{N}\right) - f\left(\frac{Zn}{N}\right) \right]$$

$$\text{When } \frac{N}{P} > \frac{n}{p} \text{ then } f(N/P) = \left(\frac{\frac{N}{P}}{\frac{n}{p}} - 1\right) \left(\frac{1000}{CV}\right)$$

$$\text{When } \frac{N}{P} < \frac{n}{p} \text{ then } f(N/P) = \left(1 - \frac{\frac{N}{P}}{\frac{n}{p}}\right) \left(\frac{1000}{CV}\right)$$

$$\text{When } \frac{N}{P} = \frac{n}{p} \text{ then } f\left(\frac{N}{P}\right) = 0$$

Where N/P is the ratio of nutrients from analyzed leaf tissue, and n/p is the ratio of nutrients from the population's DRIS norm of paired nutrients or high-yield population references, CV is the coefficient of variance (n/p).

The nutrient imbalance index (NBI) is calculated by summing the absolute values of the nutrient indices. The lesser imbalance among nutrients leads to decreased absolute sum (NBI). However, the more imbalanced nutrients, the higher the sum index [31].

### 2.4 Principal component analysis (PCA)

PCA calculations were conducted separately using the DRIS indices and nutrient concentration data for low- and high-yield populations [32]. PCs are considered significant if their eigenvalues are greater than 1. The selection criterion (SC) was used to determine which PC loadings in the eigenvectors were significant. The following was the selection criterion:

$$SC = 0.5 / (PC \text{ eigen values})^{0.5}$$

### 2.5 Statistical analysis

The SPSS program version 25 and Excel spreadsheets were used for conducting statistical analysis, descriptive, multivariate (Principal Component Analysis), normal distribution, and DRIS calculations for parameters performed [33].

### 3. Results and Discussions

#### 3.1 Diagnosis and Recommendation Integrated System (DRIS)

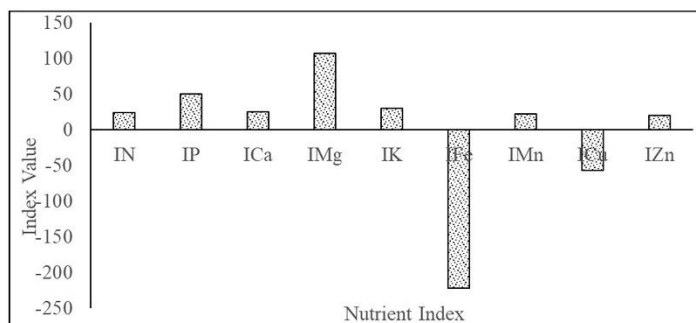
It is common knowledge that optimal plant growth depends the availability and total amount of nutrients. Table 1 shows the results of the descriptive statistics for the walnut tree leaf tissues. For both subpopulations, low-yielding and high-yielding the data of skewness for most nutrients is less than one. It indicates the normal distribution of data and suitability for using it for DRIS norm calculation. For the present study, 71.4% of walnut trees are considered a high-yielding population, and the remaining 28.6% are considered a low-yielding population, with higher concentrations for the most assessed nutrients than the previous population.

All nutrient ratios (72 ratios) were studied and selected 32 ratios of nutrients were with the highest ratio variants between low and high-yielding populations to make the DRIS norm for walnut trees. Used these norms were to calculate nutritional indices and nutrient imbalance index.

According to the findings in Figure 1, if the N/P ratio is higher than 0.048, nitrogen concentration increases while phosphorus

concentration decreases; in this situation, the plant's need for phosphorus increases. Additionally, if the N/P ratio is less than 0.042, plants require more nitrogen; the same is true for other nutrient ratios. The same finding was mentioned by [20].

Depending on (Figure 2), the most limiting nutrients in descending order are as follows: Fe>Cu>Zn>Mn>N=Ca>K>P>Mg. Fe and Cu are the most deficient nutrients, while Mg is the most available. The physicochemical characteristics of the soil control nutrient availability [34]. Low soil Fe availability, a high calcium carbonate content, and high P application cause plants' Fe deficit [35]. The same finding was

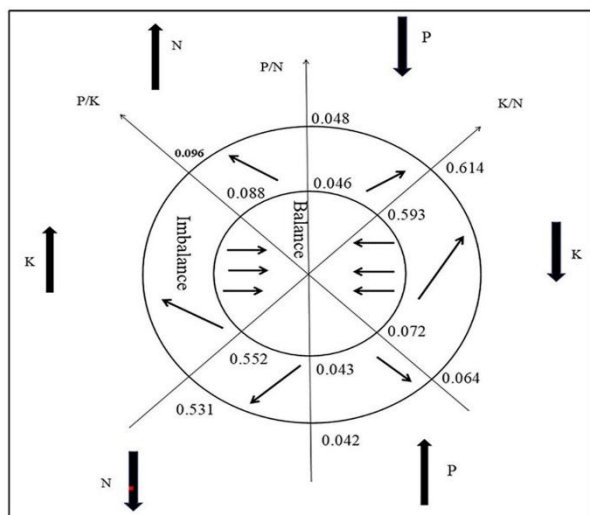


obtained by [20], who reported that Fe deficiency was abundant in

**Figure 1:** Nutrient index values in walnut leaf tissue for Hawraman orchards.

such orchard soil. Fe deficiency may occur in plants growing in alkaline soils rich in Ca and Mg. In addition, the excessive phosphate anion could inhibit plants' ability to absorb and uptake iron [36].

Plants growing on calcareous soils are more likely to have zinc deficiency. Ca is also regarded as one of the most important nutrients for plants because it is related to plant defense and may impact crop species' growth, anatomy, morphology, and chemical composition [37]. Several unfavorable environmental variables for its uptake and utilization contribute to zinc deficiencies. Poor Zn availability and the appearance of Zn deficiency in calcareous soils are primarily caused by the adsorption and occlusion of Zn by carbonates [38].



**Figure 2:** The optimal and critical values of NPK ratios are explained by the DRIS indices chart.

**Tabel 1:** Descriptive statistics of nutrients in the walnut leaves from low and high-yielding populations for Hawraman orchards.

Low yield population (n=12)										
	N	P	K	Ca	Mg	Fe	Mn	Cu	Zn	
	%			(mg/kg)						
Minimum	0.42	0.110	1.270	4.160	0.830	35.29	344.5	11.78	33.83	
Maximum	1.62	0.140	1.410	5.000	1.501	171.2	366.3	17.27	53.34	
Mean	1.018	0.120	1.345	4.376	1.208	103.5	355.4	14.52	43.58	
SD	0.045	0.012	0.071	0.347	0.023	12.97	8.996	2.113	9.630	
Skewness	0.00	1.262	-0.041	1.573	-0.613	-0.031	0.000	0.000	0.000	

High yield population (n=30)									
	N	P	K	Ca	Mg	Fe	Mn	Cu	Zn
Minimum	1.180	0.070	1.130	3.330	1.500	12.94	200.0	10.36	28.50
Maximum	3.720	0.150	1.520	5.000	3.330	124.7	432.0	28.77	50.40
Mean	2.289	0.102	1.309	4.520	2.291	71.29	294.2	16.66	37.79
SD	0.071	0.014	0.033	0.653	0.055	3.730	7.090	2.075	5.970
Skewness	0.651	0.503	0.198	-0.123	-0.012	-0.057	0.873	1.130	0.490

### 3.2 Principal component analysis (PCA)

The three PCs in the low-yielding subgroup explained 93.5% of the total variance (Table 2). Chose the nutrient with an eigenvector larger than the selection score (SC) under each PC. The first PCs had positive loading for N (0.916), K (0.929), Ca (0.624), and Zn (0.967), while negative loading to P (-0.502), Mg (-0.752), and Cu (-0.941), were expressed as (N+P-K+Ca+Mg-Cu-Zn+). Due to the high concentration of calcium carbonate (CaCO<sub>3</sub>) in Iraqi soil, phosphorus is chemically fixed, decreasing its availability [39, 40]. However, in the second PC, P showed an

inverse relation to Fe and Mn variables, designated as (P-Fe+Mn+) (Figure 3). The direct components of photosynthesis are P and Fe. Photosynthesis will decrease significantly if these components are absent in adequate amounts [41]. For PC3 the variables Ca and Mn had the same direction of change and opposite to that obtained by Mg (Ca+Mg-Mn+). The main element in chlorophyll structure is Mg. Plant productivity is reduced when Ca and Mg levels within leaf tissues are insufficient [42].

**Table 2:** Principal component analysis (data loading after log transformation) for nutrient concentration of high, low yield populations, and DRIS indices in walnuts.

Nutrients	Low yield			High yield				DRIS index	
	PC1	PC2	PC3	PC1	PC2	PC3	PC4	PC1	PC2
N	0.916*	0.288	-0.107	-0.102	0.033	0.903*	0.097	0.905*	0.239
P	-0.502*	-0.798*	-0.230	0.807*	0.059	0.124	-0.434*	0.951*	-0.153
K	0.929*	0.264	0.158	0.332	0.123	0.563*	-0.713*	0.670*	0.256
Ca	0.624*	0.049	0.714*	0.484*	-0.337	-0.519*	0.438*	0.022	0.907*
Mg	-0.752*	0.213	-0.608*	-0.918*	0.032	0.181	-0.166	0.644*	0.703*
Fe	0.040	0.990*	0.035	0.060	0.016	0.218	0.900*	-0.093	0.992*
Mn	-0.117	0.520*	0.724*	-0.402*	0.831*	0.279	-0.038	0.346*	0.648*
Cu	-0.941*	0.012	-0.247	-0.041	0.709*	0.563*	0.065	0.877*	-0.064
Zn	0.967*	0.113	0.141	0.283	0.863*	-0.208	-0.097	0.801*	0.309*
Eigenvalue	4.745	2.100	1.576	2.096	2.073	1.935	1.751	4.127	2.970
% Variance	52.72	23.33	17.51	23.29	23.03	21.50	19.45	45.86	32.90
Total Variance %	52.72	76.05	93.55	23.29	46.31	67.82	87.27	45.86	78.81

\* Significant over selection criteria

Low yield

PC1=N+P-K+Ca+Mg-Cu-Zn+

PC2=P-Fe+Mn+

PC3=Ca+Mg-Mn+

High yield

PC1=P+Ca+Mg-Mn-

PC2=Mn+Cu+Zn+

PC3=N+K+Ca-Cu+

PC4=P-K-Ca+Fe+

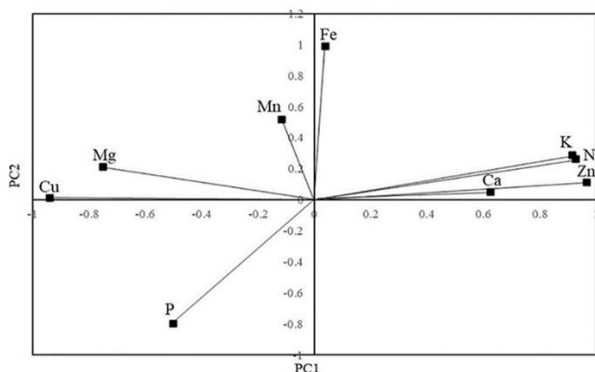
DRIS index

PC1=N+P+K+Mg+Mn+Cu+Zn+

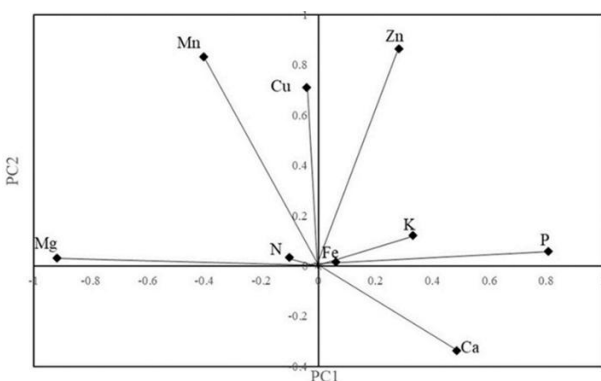
PC2=Ca+Mg+Fe+Mn+Zn+

Conducted PCA for a high-yielding population produced four PCs with total variance up to 87.2% and eigenvalue of more than 2.09. The PC1 had negative loading with Mg, Mn, and inverse positive load with P and Ca as represented (P+Ca+Mg-Mn-), (Figure 4). Ca is essential for maintaining membrane permeability, protein synthesis, and the transfer of carbohydrates. The calcium's primary function in maintaining membrane stability and cellular integrity may include an interchange of calcium ions with other cations at these binding sites, preferably on membrane surfaces, via bridging phosphate and carboxylate groups of phospholipids and proteins [43]. PC2 is designated as

(Mn+Cu+Zn+) with variables in the same change direction. While PC3 and PC4 were expressed as (N+K+Ca-Cu+) and (P-K-Ca+Fe+) respectively. For PC3 N, K, and Cu they had the same direction of changes, representing their importance for walnut growth. In contrast, a negative relation was obtained with Ca. Insufficient calcium can result in poor fruit quality, weakened cell walls, and impaired defensive mechanisms [42]. On the other hand, P, and K had negatively loaded in the PC4 and opposite positive directions of changes were obtained with Ca and Fe. Phosphorus shortage can reduce chlorophyll production and weaken photosynthetic ability [6, 43].



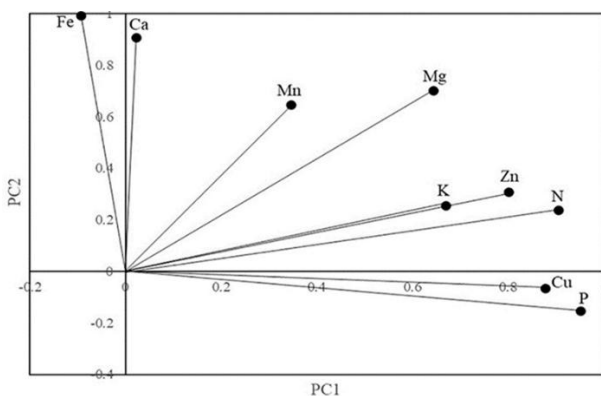
**Figure 3:** Principal component analysis (PCA) scatterplot for the low-yielding population of Hawraman walnut trees.



**Figure 4:** Principal component analysis (PCA) scatterplot for the high-yielding population of Hawraman walnut trees.

### 3.3 DRIS index

Only two PCs were obtained for the DRIS index with total variances of 45.8% and 78.8% respectively, and eigenvalue of more than 4.1 for the first PCs (Figure 5). For both PCs, all variables had changed in the same direction with a positive relationship between studied nutrients obtained with the highest load for N and P for PC1 and Ca, Fe for PC2, as designated (N+P+K+Mg+Mn+Cu+Zn+) and (Ca+Mg+Fe+Mn+Zn+) respectively. All of these nutrients are necessary for the metabolism of plants, including protein synthesis, enzyme activity, chlorophyll production, osmoregulation, phosphorylation, and enzyme activation [44].



**Figure 5:** Principal component analysis (PCA) scatterplot for DRIS index of Hawraman walnut trees.

### Conclusion

The results found that the use of DRIS norm and PCA tools facilitates the process of diagnosing the deficiency of the nutrients affecting the decreases in walnut production. The following nutrients are the most deficient, in decreasing order: Fe>Cu> Zn>Mn>N=Ca>K>P>Mg. The most lacking nutrients are Fe and Cu, while the most available nutrient is Mg. It can be recommended to use fertilizers containing Fe and Cu or add biofertilizers enrich with these elements. Additional studies prefer on soil quality, climatic factors and plant diseases that affect the walnut trees, which are directly related to productivity.

### Conflict of interests

None

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