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Evaluation of Soil Texture, EC, pH and Primary Macro Elements in Five Mango Orchard Soils of Kotri, Sindh, Pakistan

Abdul Saleem Babar[1*], Gaji Bux Bugti[2], Mehruinsa Memon[1]

[1]Department of Soil Science, Sindh Agriculture University Tandojam, Sindh, Pakistan
[2]Directorate of Agriculture Research, Sibi, Balochistan

*Email: msaleemb80@gmail.com

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Abstract: Analysis of soil is a very important practice for evaluating the nutrient status and ultimately establishing the fertilizer application program for soil health, fruit quality and production. The aim of present study was to assess the soil texture, electrical conductivity (EC), pH, and primary macronutrients such as N, P, K at 0-15 and 15-30cm depths from five orchards such as Aijaz farm (Orchard 1), Zardari farm (Orchard 2), Shahnawaz farm (Orchard 3), Ismail farm (Orchard 4), and Malik Azad farm (Orchard 5) of Kotri, Sindh Pakistan. The results indicated that most of the soils were sandy clay loam in textural class. The maximum electrical conductivity was found 0.65 dS m^{-1} in orchard 5, whereas the lowest electrical conductivity was found as 0.42 dS m^{-1} in orchard 1 at 15-30cm. All the orchards were found alkaline in nature at both 0-15 and 15-30cm depths. The total N content was found 80% low and 20% adequate at 0-15cm depth and 100% orchards were found low in total N content in soil at 15-30cm depth as compared with standard values. The AB-DTPA-P content in orchards were found 60% marginal and 40% adequate at 0-15cm depth, whereas 20% low, 40% marginal and 40% adequate were found at 15-30cm depth. AB-DTPA-K (mg kg^{-1}) was found 20% low and 80% marginal at 0-15 and 15-30cm depths. Future studies should focus on assessing the soil biological properties, plant analysis, soil interaction mechanism, amended with press mud compost, farmyard manure, biochar, modified biochar, nano-material, minerals and low-cost additives.

Keywords: Primary macro elements, soil texture, chemical properties, mango orchard, Kotri, Sindh.

Introduction

The king of fruits in Asia "Mango" is a tropical fruit which belongs to the genus *Magnifera indica* L. and dicotyledonous family, Anacardiaceae (Yadav & Singh, 2017; Aga & Gagabo, 2024). It is produced in various agro-climatic environments of Pakistan and is the most popular fruit liked all over the world. Pakistani mangoes fetch good price in Europe, Canada, Gulf and Far Eastern countries (Das et al., 2019). Mango trees have different growth stages and the successful production lies in the proper and timely application of essential nutrients (Ganeshamurthy et al., 2018). Bud induction, flowering and fruit setting in mango are most critical phonological events which demand continuous supply of nutrients for better crop production (Swamy, 2012; Makhmale et al., 2016). Pakistan is the 4th largest producer and third largest exporter of mango with an average annual production of 1.85 million tons (Evans et al., 2017). Total area of 1.03 million hectares constituting around 52.4% in Punjab and 45.6% in Sindh produced 66% and 32.5% mango, respectively. Remaining less than 2% production was shared both by Baluchistan and KPK (Maqbool et al., 2007). Sindh from 59.2 thousand hectares produced 0.38 million tons in 2010-11 (GOP, 2011). District Jamshoro is not a main mango growing district in Sindh province nevertheless during 2008-09, 810 to 520 tons of mangoes were produced from the area of 117 to 75 hectares (GOP, 2009), with Kotri being the main mango growing tract. There is big potential to increase mango production principally in southern Sindh where soil and climatic conditions are favorable (Zahid et al., 2022). Nutrients play an important role in the growth, development, production and quality of fruit and mango is no exception. Nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and sulphur (S) are the major nutrients in addition to some micronutrients required for growth and quality of mango production (Lebaka et al., 2021). These nutrients are required for different tree functions at different times (Durán-Zuazo et al., 2005). The optimum supply of these nutrients at right time forms the basis for mango production on sustainable basis and ensures the fruit quality (Ganeshamurthy et al., 2018). Among primary macronutrients, N is the most important one, and plays a vital role in qualitative and quantitative production. It is required for mango tree vigour, vegetative and floral growth (Silber et al., 2022). Application of N in excess or at wrong time may develop undesirable vegetative growth, while application at proper times may reduce irregular bearing in mango (Zhang et al., 2019). Deficiencies of N can be observed, particularly at the vegetative growth stage (El-Motaium et al., 2019). Considering the relationship between vegetative and reproductive parts i.e. the floral buds and fruit formation, N deficiency may adversely affect yield of mango (Durán-Zuazo et al., 2005). Regular supplies of N develop shoots resulting into viable panicle formation (Babu et al., 2022). Phosphorus enhances root growth, a sturdy trunk and fruit maturity of mango, and it plays a major role in the cell division, growth and formation of sugar-phosphate molecules (Asis & Niscioli, 2024). Low supply of P may result in a poor root growth resulting in reduced uptake of nutrients and delayed fruit maturation (Fujita et al., 2003). In addition, the quality of fruit is improved, especially the color of fruit, aroma, size and shelf life (Bibi et al., 2019). Potassium plays a role for resistance against pathogens and insect pests, regulating water uptake and fruit quality (Sardans & Peñuelas, 2021). Adequate supply of K protects mango trees from dry, cold, salinity, disease and pest attacks (Thind & Mahal, 2021). During last few years, some physiological stresses and quality related issues have been raised in mango orchards in Pakistan (Ullah et al., 2024). It was observed that unbalanced fertilization, macro and micronutrients deficiencies, poor tree management and inadequate cultural practices are mainly responsible for orchard related quality issues (Chatzistathis et al., 2021). Jilani et al. (2006) evaluated physico-chemical characteristics of various mango cultivars of thirty years old grown in D. I. Khan. Farm yard manure was applied at 50 kg per plant, whereas N, P and K were applied at 3-3-2 kg per plant in December.

Moyin-Jesu & Adeofun (2008) carried out research work on comparative evaluation of different organic fertilizers on the soil fertility, leaf mineral composition and growth performance of mango seedlings. Rahayu et al. (2013) reported low quality of mangoes from West Nusa Tenggara province, Indonesia which was due to lack of good agricultural practices. Reviewing factors affecting the yield of high-density mango orchards, it was found there are no any ideal planting densities well-defined or standardized, but the canopy management is very important for nutrients solubility and production (Menzel & Le Lagadec, 2017). Anderson et al. (2021) studied the potential of deep learning for predicting the fruit load, quantification and yield of Australian mango orchards. Hassan et al. (2024) examined the farmer's perceived socio-ecological influences of transforming mango orchards into housing areas in Multan, Pakistan. To the best of knowledge, no any previous study was done in this area to assess the fertility status of mango orchards soil of Kotri, Sindh. Therefore, this study was carried out to evaluate the nutrient contents in mango orchards of Kotri with the specific objectives of evaluating the N, P, K, soil texture, EC and pH at 0-15 and 15-30cm depths from five mango orchards soil of Kotri, Sindh, and to compare the determined values with the established critical levels.

Materials and Methods

Study Area

In this study, soil samples were collected from five mango orchards such as Aijaz farm (Orchard 1), Zardari farm (Orchard 2), Shahnawaz farm (Orchard 3), Ismail farm (Orchar 4), and Malik Azad farm (Orchards 5) of Kotri, aiming to analyze the selected parameters such as oil texture, EC, pH, and N, P, K in the five mango orchards to understand the fertility status and proper fertilization, and gaining higher orchard fruit production.

Sample Collection and Processing

Present study comprises of five mango orchards from taluka Kotri, district Jamshoro. Three soil samples each were taken from mango, underneath the tree canopy one meter away from the trunk and 5 trees at 0-15 and 15-30 cm depths from each orchard. In addition, 15 soil samples were taken from 0-15 cm, and 15 soil samples were collected from 15-30 cm depth from each orchard. The mango tree plants belonging to each orchard were randomly selected and tagged depth wise. Soil samples were packed in polythene bags, and labeled with permanent marker. In the laboratory soil samples were air-dried, ground using wooden pestle mortar, passed through 2 mm sieve and stored in plastic containers for further analysis.

Soil Analysis

Particle size distribution of the soil collected from various mango orchards was carried out by hydrometer method as outlined by Bouyoucos (1962). Electrical conductivity and pH analysis were carried out by first preparing a soil water extract followed by their determination. Soil water extracts were prepared in 1:2 soil water ratio and EC (McLean, 1982). All the samples were analyzed for Kjeldahl's N and ABDTPA extractable P and K. Kjeldahl's N was estimated by digesting the contents in sulfuric acid followed by distillation and finally titrating the distillate with acid (Jones, 1991), Available P and K were extracted with AB-DTPA (Soltanpour & Schwab,

1977) and the P in the extracts was determined by developing a blue color method (Cottenie, 1980), while K was analyzed directly by emission spectroscopy using flame photometer (Knudsen et al., 1982).

Statistical Analysis

The mean value and standard error of obtained data were calculated by using Excel 2016. All the graphs were designed by using Origin Pro 8.5.

Results and Discussion

Particle Size Distribution (%)

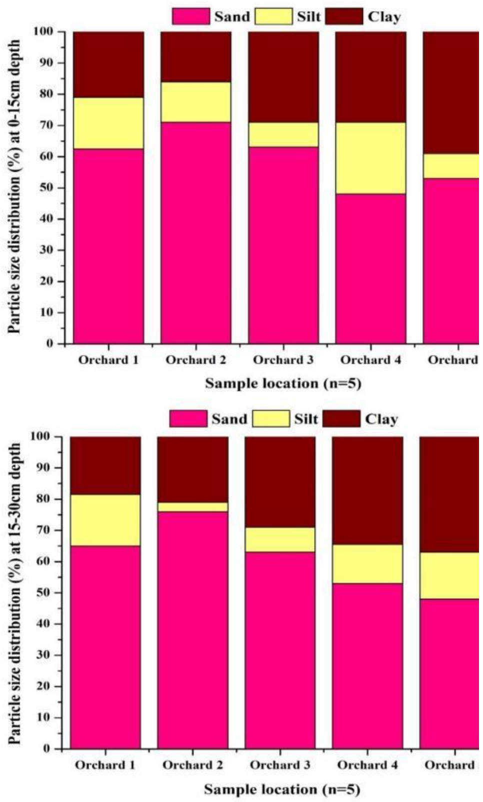

Fig. 1 Particle size distribution (%) at 0-15 and 15-30cm depths of five mango orchards soil.

The particle size analysis of various mango orchard soils at 0-15 and 15-30cm soil depth is shown in Figure 1. The data regarding particle size distribution showed a 62.5% sand, 16.5% silt, and 21% clay (Sandy clay loam textural class) at 0-15 cm, 65% sand, 16.5% silt, and 18.5% clay (Sandy loam) at 15-30cm depth for Aijaz farm (Orchard 1). The particle size distribution showed 71% sand, 13% silt, and 16% clay (Loamy sand) at 0-15 cm, 76% sand, 3% silt, and 21% clay (Sandy clay loam) at 15-30cm depth for Zardari farm (Orchard 2). The particle size distribution was 63% sand, 8% silt, and 29% clay (Sandy clay loam) at 0-15 cm, 63% sand, 8% silt, and 29% clay (Sandy clay loam) at 15-30cm depth for Shahnawaz farm (Orchard 3). The particle size distribution showed 48% sand, 23% silt, and 29% clay (Sandy clay loam) at 0-15 cm, 53% sand, 12.5% silt, and 34.5% clay (Sandy clay loam) at 15-30cm for Ismail farm (Orchard 4). The particle size distribution was found as 53% sand, 8% silt, and 39% clay (Sandy clay) at 0-15 cm, 48% sand, 15% silt, and 37% clay (Sandy clay loam) at 15-30cm depth for Malik Azad farm (Orchard 5). The results clearly indicated that the majority of studied orchards were found sandy clay loam in texture class. The mango orchards of Kotri were tested to quantify the soil texture, EC, pH and primary macronutrient contents in soil. The soils of mango orchards were mostly sandy clay loam in texture. The sandy clays are mostly recommended for best production and yield (Tambe et al., 2024). Faloye et al. (2024) applied different parts of mango plant made biochar to silt loam and sandy loam, and as a result found an increase in soil porosity, water retention, and plant available water.

Electrical Conductivity and pH

The data regarding electrical conductivity of various mango orchard soils at 0-15 and 15-30 cm depths presented in Figure 2 depict higher electrical conductivity at surface (0-15cm) compared to the lower depths. Electrical conductivity values of Kotri soils ranged between 0.46 and 0.65 with an average value of 0.55 dS m^{-1} at surface and 0.42 and 0.49 with average value of 0.46 dS m^{-1}. The electrical conductivity values of individual surface orchard soils were 0.46, 0.48, 0.49, 0.54 and 0.65 dS m^{-1} in Aijaz farm (Orchard 1), Zardari farm (Orchard 2), Shahnawaz farm (Orchard 3), Ismail farm (Orchard 4), and Malik Azad farm (Orchard 5), respectively. The sub-surface soils at 15-30 cm had electrical conductivity values of 0.42 dS m^{-1} in Aijaz farm (Orchard 1), 0.44 dS m^{-1} in Zardari farm (Orchard 2), 0.43 dS m^{-1} in Shahnawaz farm (Orchard 3), 0.46 dS m^{-1} in Ismail farm (Orchard 4), and 0.59 dS m^{-1} in Malik Azad farm (Orchard 5), respectively. The data regarding pH of five mango orchards soil of taluka Kotri, district Jamshoro at 0-15 and 15-30cm soil depth is presented in Figure 2. The pH values of Kotri surface soils ranged between 7.96 and 8.27 with an average value of 8.12, and the subsurface soils at 15-30cm ranged between 7.95 and 8.28 with an average value of 8.13.

The average pH values of individual orchards were 8.04, 8.27, 8.20, 8.11 and 7.96 in Aijaz farm (Orchard 1), Zardari farm (Orchard 2), Shahnawaz farm (Orchard 3), Ismail farm (Orchard 4), and Malik Azad farm (Orchard 5), respectively. For sub-surface soils at 15-30cm, the pH values of 8.28, 8.21, 8.14, 8.07 and 7.95 were recorded in Aijaz farm (Orchard 1), Zardari farm (Orchard 2), Shahnawaz farm (Orchard 3), Ismail farm (Orchard 4), and Malik Azad farm (Orchard 5), respectively. Soil electrical conductivity was measured in 1:2 soil and water extract of mango orchards. The results of this study showed that all the mango orchards were found to be non-saline having average values of 0.55 dS m^{-1} and 0.46 dS m^{-1}. It was elaborated by Grisso et al. (2009) that the electrical conductivity of soils varies depending on the amount of moisture held by soil particles and may strongly correlate with soil particle size and texture. The soil pH of different mango orchards showed alkaline nature ranging between 7.96 and 8.27 at surface, and 7.95 and 8.28 at subsurface. This alkaline pH range is due to presence of high calcium level and low organic matter. Our results are in line with the pH of the soil which varied from 6.3 to 7.9 in the 20 mango orchards (Salehin et al., 2020).

Kjeldhals N Content, AB-DTPA Extractable P and K (mg kg-1) in Five Orchards

Total N contents were examined in Aijaz farm (Orchard 1), Zardari farm (Orchard 2), Shahnawaz farm (Orchard 3), Ismail farm (Orchard 4), and Malik Azad farm (Orchard 5) of Kotri, Sindh. The total proportion of N in top of five mango orchards at 0-15cm was compared to 15-30cm depth. It could be due to decomposition of plant leaves and application of organic manures in soil, which may be responsible to increase the organic matter content in the top layer of soil medium. The mean contents ranged between 0.020 and 0.050 % with an average value of 0.034% at surface, and 0.020 and 0.040 % with an average value of 0.026% at subsurface. The Zardari farm (Orchard 2) at 0-15 and 15-30cm and subsurface (15-30cm) of Shahnawaz farm (Orchard 3) and Malik Azad farm (Orchard 5) had the N content of 0.02%, whereas the Shahnawaz farm (Orchard 3) at (0-15 cm) and Ismail farm (Orchard 4) at (0-15 and 15-30 cm) had N content of 0.03%. Remaining Orchards: Aijaz farm (Orchard 1) at (0-15 and 15-30 cm) and at Malik Azad farm (Orchard 5) at (0-15 cm), had N content of 0.05 and 0.04 %, respectively (Fig. 3). It has been noted that 80% soils were low in total N content and 20% soils were adequate at 0-15cm depth. However, 100% soils were found to be low in total N content in the studied orchards as compared to critical standards (Table 2).

The results indicated that Aijaz farm (Orchard 1) was found to be higher in total N content in soil as compared to other orchards. Figure 3 shows the AB-DTPA extractable P and K contents in mango orchards viz., Aijaz farm (Orchard 1), Zardari farm (Orchard 2), Shahnawaz farm (Orchard 3), Ismail farm (Orchard 4), and Malik Azad farm (Orchard 5) of Kotri at two soil depths (0-15 and 15-30 cm). The AB-DTPA extractable P contents ranged between 4.48 and 17.44 mg kg^{-1} with average contents of 10.96 mg kg^{-1} at surface and 2.34 and 11.52 mg kg^{-1} with average contents of 6.93 mg kg^{-1} at subsurface soils of various mango orchards. The data of each orchard depict a wide variation among orchards. The depth wise trend shows higher contents at surface compared to the lower ones. The surface soils were observed for 17.44, 17.42, 8.86, 7.44 and 4.48 mg P kg^{-1} and subsurface ones for 11.52, 11.11, 5.54, 5.99, and 2.34 mg P kg^{-1} in Aijaz farm (Orchard 1), Zardari farm (Orchard 2), Shahnawaz farm (Orchard 3), Ismail farm (Orchard 4), and Malik Azad farm (Orchard 5), respectively. It has been observed that 60% soils were found to be marginal and 40% were adequate in AB-DTPA extractable P at 0-15cm, whereas 20% low, 40% marginal and 40% soils were found adequate in AB-DTPA extractable P at 15-30cm as compared with critical values given in Table 2.

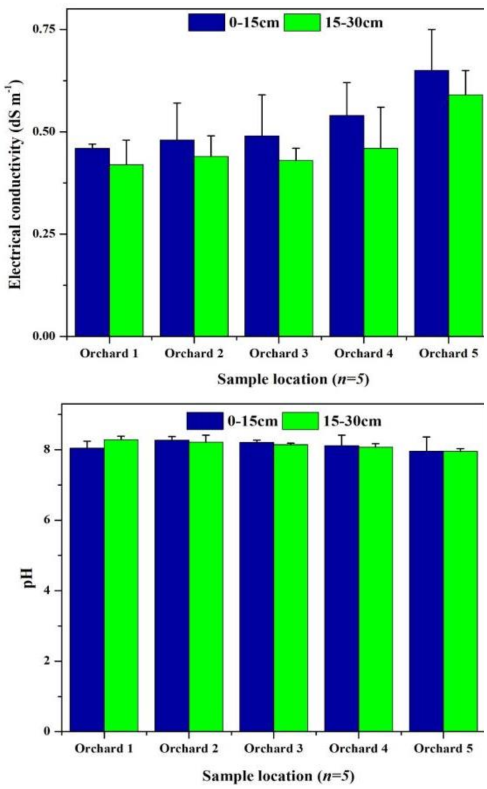

Fig. 2 Electrical conductivity and pH of five mango orchards soil in Kotri, Sindh.

The results highlighted that the maximum AB-DTPA extractable P was observed in Aijaz farm (Orchard 1), Zardari farm (Orchard 2) at 0-15cm depth as compared to other orchards. The AB-DTPA extractable K content of surface soils (0-15cm) ranged between 51 and 117 mg kg^{-1} with average value of 110.70 mg kg^{-1} (Fig. 3).

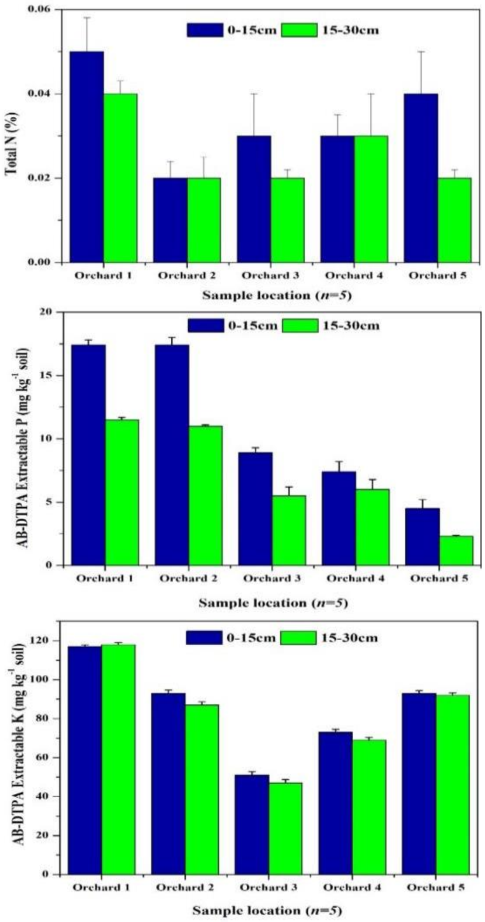

Fig. 3 Total nitrogen (%) contents, ammonium bicarbonate extractable phosphorus (mg kg-1), and ammonium bicarbonate extractable

Whereas, the subsurface soils (0-15cm) had K content between 47 and 118 mg kg^{-1} with average value of 82.30 mg kg^{-1}. Looking at K content of surface soils, the highest value was observed in Aijaz farm (Orchard 1) having 117 mg kg^{-1} K, followed by Zardari farm (Orchard 2) and Malik Azad farm (Orchard 5), each containing 93 mg kg^{-1} K. While, Ismail farm (Orchard 4) had 73 mg kg^{-1} K, and Shahnawaz farm (Orchard 3) contained 51 mg kg^{-1}. The sub-surface soils followed the same pattern. The values were highest in Aijaz farm (Orchard 1) 118 mg kg^{-1}, followed by Malik Azad farm (Orchard 5) 92 mg kg^{-1}, Zardari farm (Orchard 2) 87 mg kg^{-1}, Ismail farm (Orchard 4) 69 mg kg^{-1}, and Shahnawaz farm (Orchard 3) 47 mg kg^{-1}, respectively. The results indicated that the AB-DTPA extractable K was found to be 20% low and 80% marginal at 0-15 and 15-30cm, respectively as compared with critical values (Table 2). The above results indicated that the highest AB-DTPA extractable K content was observed in Aijaz farm (Orchard 1) at 15-30cm depth as compared to other orchards. Continuous and proper supply of nutrients at each phonological mango growth stage forms the basis for good quality fruit and yield.

Chemical analysis of mango soil samples is a useful tool for establishing the current nutrient levels in an orchard. The analysis of primary macronutrient was compared with established critical limits to find out the current status in these orchards (Table 2). Most agricultural soils of Pakistan are poor in N in particular and P in general (Ahmad & Rashid, 2003). It could be due to low content (<1%) of organic matter which hinders enhanced yield of different crops. High temperature, low rainfall and removal of almost all the crop residues are some of the major reasons for low organic matter content. Nitrogen is the main constituent for vegetative growth and quality of fruit, therefore, it becomes obligatory to assess N levels and evaluate its effective utilization at different stages of growth. Soils tested for primary macronutrient status suggested that out of five macronutrients (N, P and K), only N was found low according to critical categorization. However, most of the times, the critical limits suggested by many workers are crucial. Total N contents in soil were 0.020-0.050 % at surface and 0.020-0.039 % at sub-surface, respectively.

Eighty percent of the soils contained below <0.05% N with only 20% being between 0.05-0.1% (moderate) in Kjeldhal's N. Even these N contents were at the starting edge of moderate range. Bozkurt et al. (2010) reported that one of the major reasons for lower fertility is the insufficient organic matter of the agriculture soils. In case of AB-DTPA extractable soil P, the present study revealed that 60% and 40% surface soil samples were between 4-7 mg kg^{-1} and >7 mg kg^{-1}, respectively and 20%, 40% and 40% subsurface samples were respectively <4 mg kg^{-1} in available P content. For this reason, high soil P contents in two orchards may be due to fertilizer application in these areas as (Table 1). Because P moves very little in soil, and the current tested values present in soil give picture about the currently applied inorganic stocks. The P reaches the plant at least after one year, especially in case of fruit trees. This was further supported by Prado (2010) that P fertilization can enhance the contents in soil but the plant performance is influenced only after the second year of cultivation. Their results showed that the effects of P application on mango growth are very slow and the fruit set was not influenced by P fertilization. In case of K, 80% soils of the area had K contents between 60-120 mg kg^{-1} with only

20% being below <60 mg kg^{-1} K. 100% soils of Kotri were categorized as high in Ca (>2000) and Mg (>480 mg kg^{-1}) contents. The leaf tissue analysis was in line with the soil analysis, where 100% soils were sufficient in K and Ca ranging between 0.40-1.0% and 2.0-5.0 % and high in Mg (>0.50%) content, respectively. Excessive amounts of K in soil can cause worse absorption of Ca and Mg (Pietranek & Jadczuk, 2005). Likewise, the high Ca levels in soil can be compensated by potassium fertilization (Szucs, 2005). Salehin et al. (2020) found the available nitrogen, phosphorus, potassium 190 - 510, 39 - 196, 36 – 206 mg/kg contents, respectively in 20 mango orchards. Shahidin et al., (2024) revealed that the total nitrogen, available P, and exchangeable potassium contents in the soil cultivated with Harumanis mangoes varied with phenological periods (Fig. 3). Total nitrogen (%) contents, ammonium bicarbonate extractable phosphorus (mg kg^{-1}), and ammonium bicarbonate extractable potassium (mg kg^{-1}) of five mango orchards soil in Kotri, Sindh are shown in Table 2.

Table 1. Application of fertilizers and organic manure practices in five orchards.

Orchards	Farm area	Chemical fertilizer application rate	Organic manure application rate
Aijaz farm (Orchards 1)	32 acre	Urea 2 and DAP 2 bags/acre per year	1 truck per year
Zardari farm (Orchards 2)	10 acre	Urea 2 and DAP 2 bags/acre per year	I truck/acre after 6 months
Shahnawaz farm (Orchards 3)	120 acre	Urea 1 and DAP 1 bag/acre after 6 months	5 trucks/acre
Ismail farm (Orchards 4)	235 acre	Urea 1, DAP 2 potassium sulphate 1 kg /tree after 6 months	120 kg/tree per year
Malik Azad farm (Orchards 5)	176 acre	DAP 3 and NPK 3 kg/tree	I truck/acre per year

Table 2. Soil primary macronutrients in mango orchards of Kotri- categorization based on established critical limits.

Parameters	Soil depth (cm)	Range	Mean ±standard deviation	Categorization (% samples)		
Kjeldahl's N (%)				**Low <0.05**	**Moderate 0.05-0.1**	**Adequate >0.1**
	0-15	0.020-0.050	0.034±0.01	80%	20%	-
	15-30	0.020-0.039	0.026±0.01	100%	-	-
				Low	**Marginal**	**Adequate**
AB-DTPA-P (mg kg^{-1})				**<4**	**4-7**	**>7**
	0-15	4.48-17.44	11.12±5.96	-	60%	40%
	15-30	2.30-11.50	7.26±4.00	20%	40%	40%
AB-DTPA-K (mg kg^{-1})				**<60**	**60-120**	**>120**
	0-15	51-117	85±25	20%	80%	-
	15-30	47-118	83±27	20%	80%	-

Source: Melherb, 1953; Soltanpour & Schwab 1977.

Conclusion

The present study concluded that the majority of orchards were found sandy clay loam in texture. All the mango orchards were found to be non-saline in nature. The soil pH of different mango orchards showed alkaline nature. It has been noted that soils tested for primary macronutrient status suggested that out of five macronutrients (N, P and K). Total N content was found higher in the Aijaz farm (Orchard 1), whereas the lowest total N content was found in Zardari farm (Orchard 2) at 0-15 and 15-30cm depths. The highest AB-DTPA-P content was observed in Aijaz farm (Orchard 1) at 0-15cm depth, but the lowest AB-DTPA-P was detected in Malik Azad farm (Orchard 5) at 15-30cm depth. The AB-DTPA-K in Aijaz farm (Orchard 1) was observed higher, whereas lowest AB-DTPA-K content was found in Shahnawaz farm (Orchard 3) as compared to other orchards. It is suggested that soil analysis should be carried at each phonological stage, and on yearly basis to have a clear view of the nutrient utilization. Future studies should be focused on assessment of soil biological properties, nutrients dynamics, soil-plant interaction mechanism amended with press mud compost, biochar, modified biochar, hydrochar, nano-material and organic fertilizers mixed with chemical fertilizers for increasing nutrient uptake by plant, plant growth, and development of fruit quality and enriched fruits for living beings. It is suggested that future research work should focus on application of remote sensing, geographic information system (GIS), and machine learning to assess and predict the soil fertility and mango production.

Conflicts of Interest: The authors declare no conflicts of interest in this research.

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