Research Article

Selection of Suitable Extractants for the Determination of Available Copper in Soils of Mulberry Garden

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Abstract

Copper (Cu) is an essential micronutrient required for activation of enzymes involved in the growth and development of mulberry. Deficiency of Cu in mulberry garden results in alteration of enzymatic activity leading to the production of poor-quality leaf which in turn affect the quality of cocoon. Therefore, assessment of available Cu in soil of mulberry garden is essential in order to manage its deficiency. The available Cu content in soil of mulberry garden was estimated by employing the commonly used chemical extractants (DTPA, AB-DTPA, HCl and Mehlich-3) having different modes of extraction. Amount of Cu extracted with DTPA extractant was lesser than that of other extractants used. The Cu extracted by Mehlich-3 maintained significant positive correlation with organic carbon, but negative correlation with soil pH. Of the four extractants tested, Cu extracted by using Mehlich-3 showed highest relationship with plant Cu concentration also (R^2= 0.768) and thus, Mehlich-3 may be used as a suitable extractant for assessing the available Cu content in soils of mulberry garden.

Keywords: Available copper, chemical extractants, mulberry garden, plant copper

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Introduction

Mulberry (*Morus alba L.*) is primarily cultivated for its foliage which is utilized as the sole feed for the silkworm *Bombyx mori* L. [1]. As such mulberry leaf with optimum nutritional quality is fed to the silkworm. It is worthwhile to note that the nutritional quality of mulberry leaf determined the quality of cocoon [2, 3]. Accordingly, mulberry is being cultivated with a balanced nutrient supplement to achieve the desired quality leaf production. The role of macronutrients and micronutrients in mulberry growth and development is well known [4-6]. Although the requirement of micronutrients is less, they play a significant role in mulberry growth and nutrition. Moreover, their role in silkworm growth and nutrition is also well studied [7, 3]. Metabolic activities responsible for protein, sugar and enzyme synthesis are being influenced by micronutrients application which in turn helps in the production of quality mulberry leaf. It was reported that multiple deficiencies of micronutrients deteriorate the leaf quality besides reducing the leaf yield to the tune of 50% [8]. Bose and Majumder [9] reported that micronutrients application improved the yield and quality of mulberry leaf that influenced the quantitative traits of silkworm. Zinc and iron stimulated the metabolic activity in silkworm subsequently improving the rearing performance and silk content [8]. Alike to Zn and Fe, Cu participates in the biosynthesis of protein in mulberry and serves as a catalyst for enzyme activation in plants [6] as well as in natural spinning process of silkworms [10]. Deficiency of copper in soils results in poor growth of mulberry [6] which consequently influences the performance of silkworm [10].

Availability of Cu in soil is controlled by the various soil properties especially pH, organic carbon, clay [11, 12] and oxides of Fe and Al [13]. Adsorption, desorption and solubility relations between solid and solution phases regulate the Cu availability in soil [14]. McLaren et al. [15] reported that the adsorption and desorption reaction of Cu in soil is strongly influenced by clay, pH, cation exchange properties, organic matter and pedogenic oxides. At pH below 5.0, Cu^{2+} activity and Cu concentration were close, whereas at pH greater than 5.0 there was a divergence in Cu^{2+} activity and Cu concentration in soil solution due to increasing complexation of Cu by inorganic and organic ligands which in turn regulate the Cu availability in soil [16]. Thus, estimating the bioavailability of Cu in soils through a suitable method is essential in order to manage its deficiency or toxicity. Suitability of different extractants for estimating plant available nutrients is a function of soil and crop types [17]. For example, DTPA served as a suitable extractant for assessment of available Cu in different soils for nutrition of wheat [18] and in calcareous soil for nutrition of barley [19]. But, Mehlich-3 was found suitable for estimating plant available Cu for the nutrition of spinach in organic production system [20]. Several chemical extractants have been tested for assessing the bioavailability of Cu in different soils and for different crops [18, 21, 22]. But, suitability of extractant for estimating plant available Cu for mulberry nutrition is hardly being studied [23]. Therefore, different chemical extractants were evaluated in the present study for estimating the available Cu in mulberry soils.

Materials and Methods

The experiment was conducted at Central Sericultural Research and Training Institute, Mysuru. The experimental site was located between 12.18^{0}N latitude and76.42^{0}E longitude with 759.9 from the msl. The climate of the experimental site is characterized by hot semi-arid. Mean annual maximum and minimum temperature were33^{0}Cand 18^{0}C respectively. Soil type was red sandy loam.

The experiment comprised of five nutrient management practices *viz*., control, 100%RDF (N:P:K:: 350:140:140kg/ha/year), 80%RDF, 60%RDF with four mulberry genotypes *viz*., AGB8, MSG2, G4 and V1 and a fallow plot (no cultivation since initiation of the experiment). The experiment was laid down in split plot design with three replications.

Fifty-one soil samples (0-0.3m depth) were collected from all the treatments in three replicates. The samples were air dried, grinded and passed through 2.0mm sieve and used for analysis of soil properties viz., pH [24], electrical conductivity [24], organic carbon [25]. The initial pH of the experimental soil was 6.50.

Different chemical extractants were employed for estimation of available Cu in soil (**Table 1**). The chemical extractants used for the present study operate through different mechanisms and also multi-nutrient extractants can save time and minimize cost of analysis. Plant samples from the respective treatments were collected and air dried followed by drying in hot air oven at 60^{0}C to a constant weight. Plant samples were dry ashed in muffle furnace and dissolved in 6N HCl followed by measuring the Cu concentration in Atomic absorption spectrophotometer.

Table 1 Details of the methodologies used for extraction of plant available Cu content in soils of the experimental site

Extractants used	**Extractants composition**	**Soil: extractant ratio**	**Shaking time**
DTPA [26]	0.005M DTPA+ 0.1M TEA+ 0.01M $CaCl_2$, pH 7.3	1:2	2hrs
AB-DTPA [27]	Mixture of 1.0 M NH_4HCO_3 and 0.5 M DTPA, pH 7.6	1:2	15 min
HCl [28]	0.1 N HCl	1:5	30min
Mehlich-3 [29]	0.2 MHOAc, 0.25 M NH_4NO_3, 0.015 M NH_4F, 0.013 M $HNO_3$and 0.001 M EDTA (pH 2.5 ± 0.1)	1:10	5min

Statistical analysis

Means of three replicates and standard errors of the means were calculated for all the parameters analysed. The data were analysed using split plot design. Statistical analysis was performed by DOS-based SPSS version 20.0. The SPSS procedure was used for analysis of variance (ANOVA) to determine the statistical significance of treatments as well as of difference in varieties. Two factor factorial ANOVA was used to determine the existence of interaction effect between treatments and varieties. Simple correlation coefficients and regression equations were also developed to evaluate relationships between the response variables using the same statistical package. The 5.0% probability level is regarded as statistically significant.

Result and Discussion

Extractable Cu in soils

Nutrient management practices influenced the extractable Cu content in the experimental soil. The DTPA extractable Cu (μg g^{-1}) ranged from 1.35 in control to 1.61 in 100% RDF treatment (**Table 2**). The amount of Cu extracted by DTPA was smaller than that extracted by Mehlich-3, HCl and AB-DTPA in the experimental soils. Mehlich-3 could extract Cu that has close relationship with DTPA extractable Cu and it was 2.45times more Cu than DTPA extraction [30]. Vidal-vazquez et al. [31] reported that Mehlich-3 could extract more micronutrients than DTPA-TEA solution. Mehlich-3 due to its high acidity, could extract partly the adsorbed Cu on the oxide surfaces. Moreover, the presence of chelating agent EDTA can extract higher amount of micronutrients [32] causing the observed increase in Cu extraction compared with the other extractants used. Further, the presence of NH_4^+ in Mehlich-3 can displace more of the exchangeable cations [33, 34] making it more efficient in extracting Cu from soils. Chelating agents such as DTPA or EDTA reduce their activity in solution by complexation, causing the dissolution of the labile forms of micronutrient cations in soils while the dilute acid solution such as 0.1N HCl could only able to solubilize partially the soil Cu [35].

Table 2 Impact nutrient management practices on extractable soil Cu and plant Cu content

Treatments	DTPA_Cu	AB-DTPA_Cu	HCl_Cu	Mehlich-3_Cu	Plant Cu
		$\mu g g^{-1}$			
Varieties					
V1	1.49	1.96	1.95	2.9	22.1
AGB8	1.49	1.75	1.91	2.9	21.6
MSG2	1.46	1.71	1.88	2.8	21.1
G4	1.48	1.83	1.92	3.0	22.1
SEd	**0.063**	**0.081**	**0.085**	**0.103**	**0.997**
CD (0.05)	**0.127**	**0.162**	**0.172**	**0.211**	**2.014**
Fertilizer doses					
Control	1.44	2.19	1.60	2.5	20.0
100%RDF	1.61	1.55	2.36	3.4	22.9
80%RDF	1.52	1.81	1.87	3.1	22.4
60% RDF	1.35	1.70	1.82	2.7	21.7
SEd	**0.054**	**0.069**	**0.074**	**0.091**	**0.863**
CD (0.05)	**0.110**	**0.141**	**0.149**	**0.183**	**1.744**
F x V **SEd**	**0.108**	**0.139**	**0.148**	**0.179**	**1.73**
CD (0.05)	**0.219**	**0.281**	**0.297**	**0.362**	**3.49**
Fallow	1.51	1.72	1.94	2.86	-

Of the five nutrient management practices, 100%RDF always had higher amount of extractable Cu across the varieties and extractants used (except AB-DTPA) compared to control(Table 2). This also was true for 80% RDF treatment. This effect of 100%RDF and 80%RDF was most conspicuous when Cu was extracted with Mehlich-3 (34.2 &22.8%), HCl (47.0&16.7%) but least for DTPA (12.2&5.9%) (**Figure 1**). The fallow soil is not put under cultivation which resulted an increase accumulation of organic C (30.2%) than control that might have helped to retain higher Cu in soil. It was also found that the extractable Cu content in the experimental soils was above the critical limit established for different extractants. Mulberry requires less amount of Cu for its growth and development and this might be the reason for sustained availability of Cu in control. However, the application of FYM in treatments viz., 100% RDF, 80% RDF and 60% RDF add substantial quantity of Cu into soils. Continuous cultivation and balanced application of nutrients could increase in available status of Cu in soils by mobilizing the native micronutrient cations [34]. The extractable Cu content of the experimental soils followed the order of Mehlich-3 > HCl > AB-DTPA > DTPA.

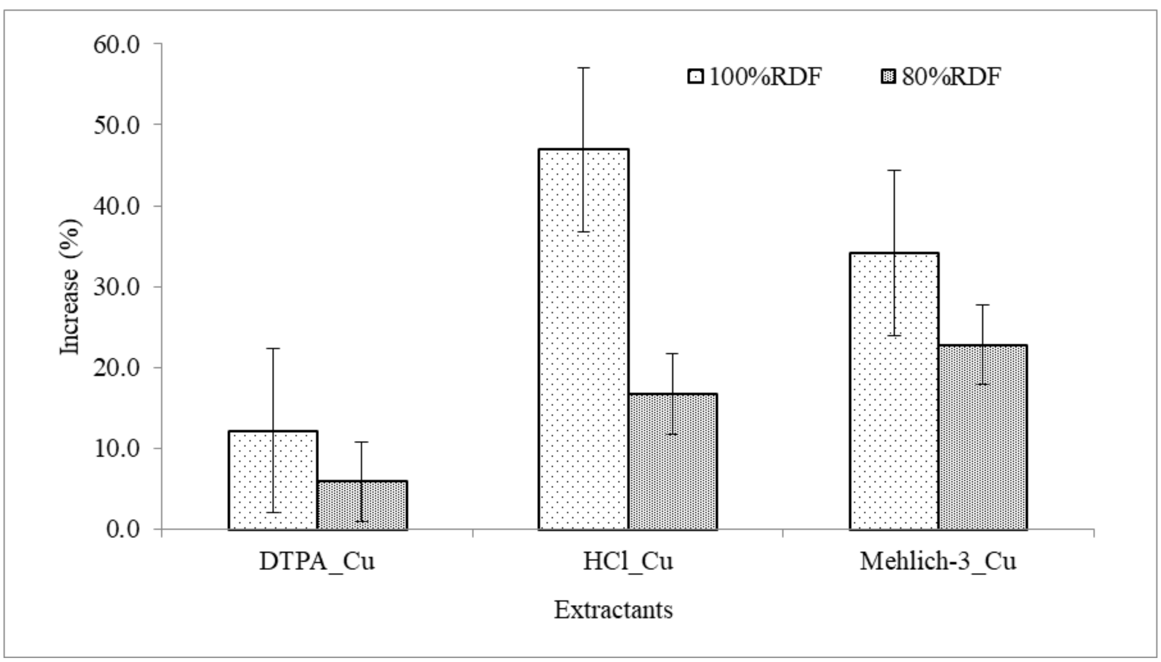

Figure 1 Relative increase in extractable Cu content in 100%RDF and 80% RDF treatment over control

Relationships among the extractable Cu

Dynamic relationships found among the extractable Cu of the experimental soils (**Table 3**). The DTPA extractable Cu maintained significant positive correlations with HCl (0.759*) and Mehlich-3 (0.879**). Significant positive correlations between the amounts of Cu extracted by the former extractants indicated that they extract Cu from similar pools in soil contributing to plant available amounts. Similar relationship among the extractable Cu in soil was earlier reported by Pradhan et al. [34]. However, AB-DTPA extractable Cu maintained negative correlations with other extractable fractions.

Relationship between extractable Cu and important soil properties

The amount of Cu extracted by DTPA, HCl and Mehlich-3 showed a significant positive correlation with organic C, but negative correlations with pH of the soils (**Table 4**). This indicated that the extractable Cu content of the soils would increase with increasing organic C and decrease with increasing soil pH. Similar relationships of soil extractable Cu with organic carbon and pH have been reported by others [23, 34]. Copper extracted with Mehlich-3 showed significant positive correlation with organic carbon which indicated organic matter could largely hold Cu [36].

Table 3 Relationship among the extractable Cu content of the experimental soils

	DTPA_Cu	AB-DTPA_Cu	HCl_Cu	Mehlich-3_Cu
DTPA_Cu	1	-0.321	0.759*	0.879**
AB-DTPA_Cu		1	-0.758*	-0.659
HCl_Cu			1	0.910**
Mehlich-3_Cu				1

Table 4 Pearson correlation coefficients (r) between extractable Cu and important soil properties

Extractable Cu/ Soil properties	DTPA_Cu	AB-DTPA_Cu	HCl_Cu	Mehlich-3_Cu
pH	-0.953**	0.362	-0.853**	-0.895**
SOC	0.710*	-0.765*	0.885**	0.950**

*&** significant at 0.05 & 0.01level respectively

Plant Cu concentration and suitability of extractants for assessing available Cu in soil

Plant Cu concentration indicated the relative plant Cu availability in soils. Application of organic and inorganic nutrients had a significant effect on plant Cu concentration. However, mulberry varieties didn't show any significant difference in their plant Cu concentration. Plant Cu concentrations among the treatments ranged from 20.0 µg g^{-1}in control to 22.8 µg g^{-1} in 100% RDF. The mean magnitude of increase in plant Cu concentrations was 14.4, 12.0 and 8.40% with 100% RDF, 80% RDF and 60% RDF over control respectively. The amount of Cu extracted by DTPA, HCl and Mehlich-3 showed significant positive correlation (r) with Cu concentration of mulberry (**Figures 2-5**). On average, the relations (r) were greater with Mehlich-3 and HCl irrespective of varieties indicating their superiority over DTPA and AB-DTPA extractants for assessing plant available Cu in soils. However, to make the assessment Cu in soil robust, inclusion of important soil properties such as pH, organic carbon, oxides of Fe and Al is essential in the regression analysis. Step-wise multiple regression analysis was computed using the different extractants used and the previously mentioned soil properties as independent variable and plant Cu concentration as dependent variable. The inclusion of soil properties particularly pH and organic C in the regression equation significantly improved the R^2 values from 0.29 to 0.55 for DTPA, 0.31 to 0.54 for AB-DTPA, 0.60 to 0.64 for Mehlich-3 and 0.54 to 0.60 for HCl respectively (**Table 5**).

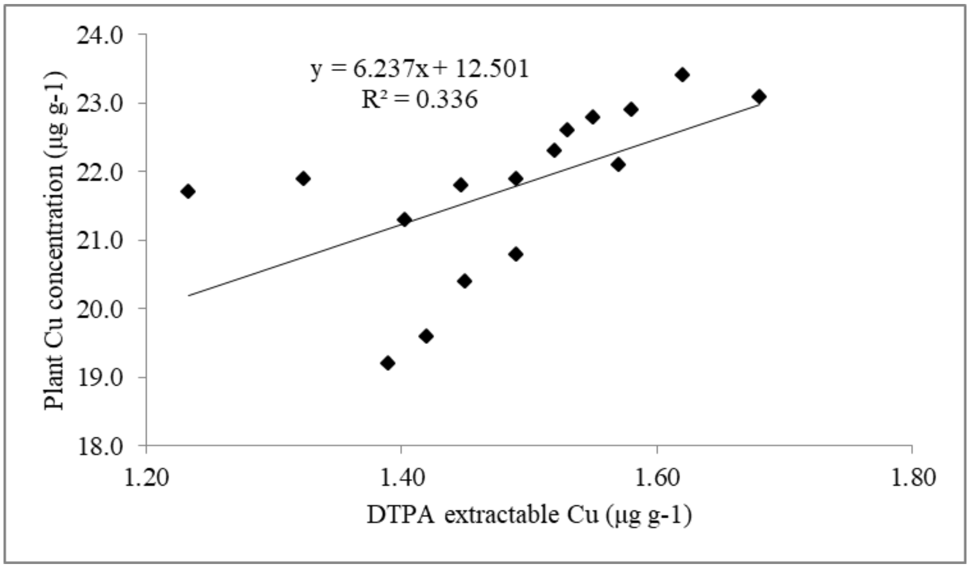

Figure 2 Relationship between DTPA extractable Cu and plant Cu concentration

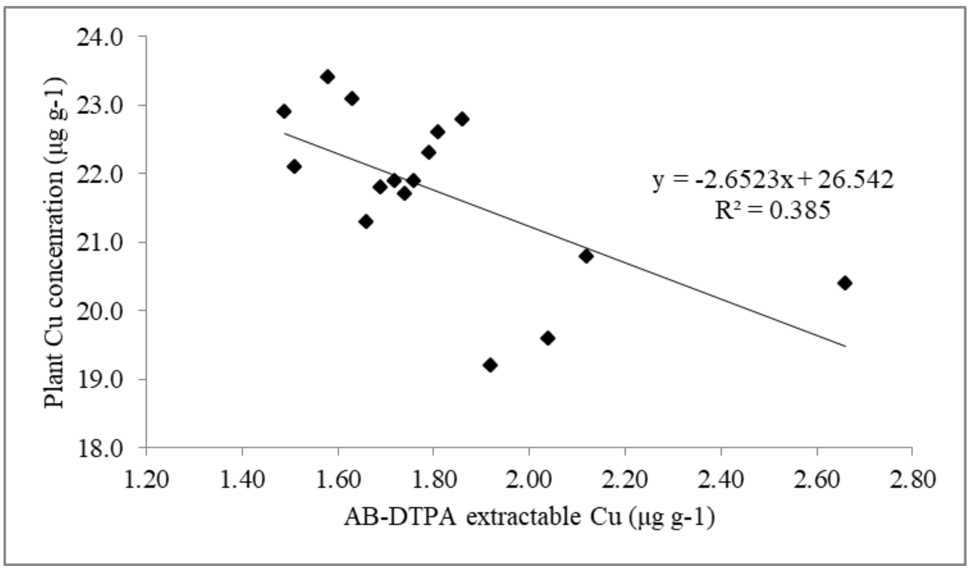

Figure 3 Relationship between AB-DTPA extractable Cu and plant Cu concentration

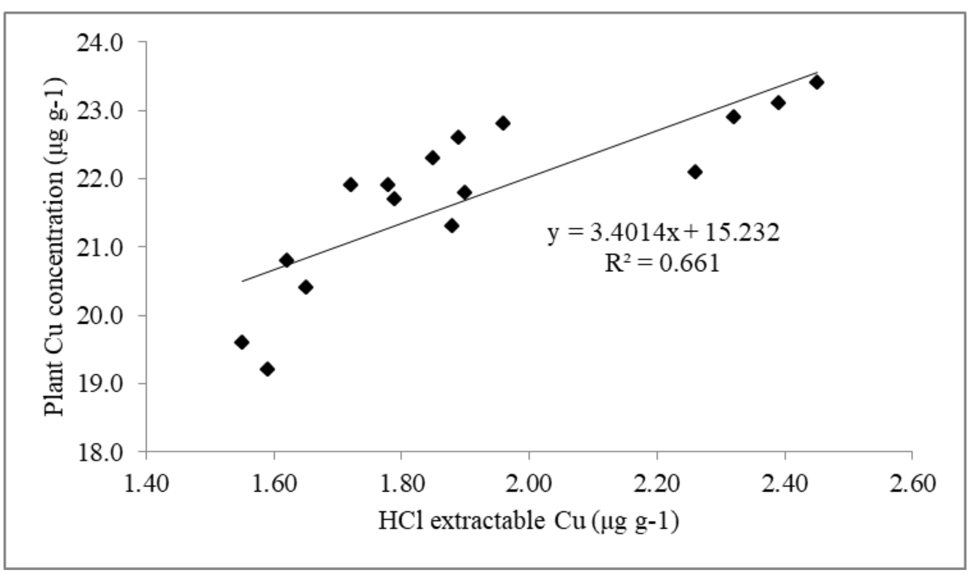

Figure 4 Relationship between HCl extractable Cu and plant Cu concentration

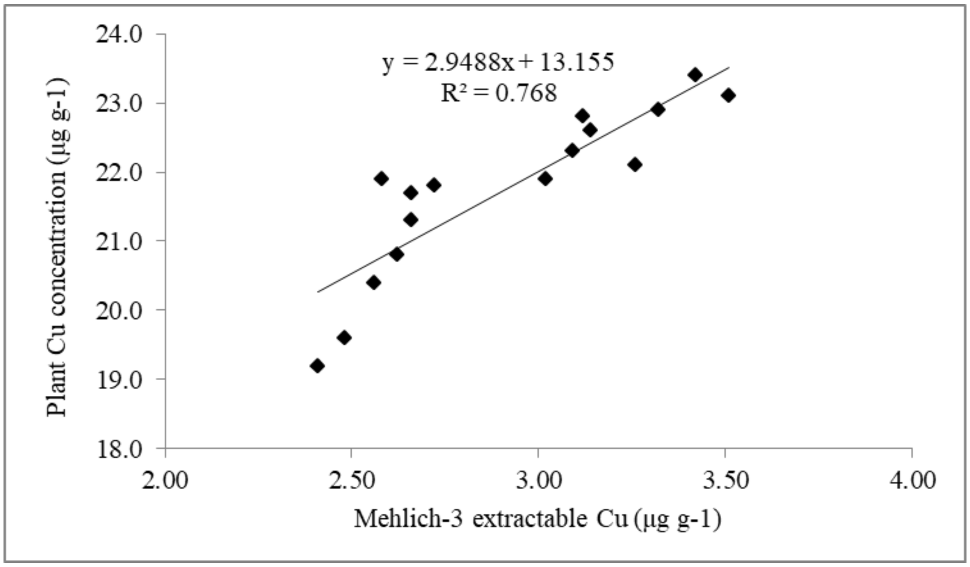

Figure 5 Relationshp between Mehlich-3 extractable Cu nd plant Cu concentration

Table 5 Multiple regression equation showing relationship between plant Cu concentration and extractable Cu

Y= Cu concentration in mulberry	**Regression Equation**	**R^2**	**Adj R^2**	**SE (Est)**
DTPA	Y= 13.162+ (0.521) DTPA-Cu**	0.27	0.25	1.10
	Y= 10.012+ (0.212) DTPA-Cu+ (0.612) pH**- (0.020) SOC	0.55	0.53	0.87
AB-DTPA	Y= 26.341- (0.561) AB-DTPA-Cu**	0.31	0.29	1.06
	Y= 18.775- (0.182) AB-DTPA-Cu- (0.091) SOC + (0.578) pH**	0.54	0.51	0.88
HCl	Y= 15.498+ (0.739) HCl-Cu**	0.54	0.53	0.86
	Y= 13.388+ (0.444) HCl-Cu**- (0.023) SOC+ (0.379) pH*	0.60	0.58	0.82
Mehlich-3	Y= 13.754+ (0.779) Mehlich-3-Cu***	0.60	0.59	0.80
	Y= 11.679+ (0.546) Mehlich-3-Cu**- (0.01) SOC+ (0.302) pH**	0.64	0.62	0.78

Conclusion

From the present study, it was concluded that the available Cu extracted by using Mehlich-3 extractant has shown a positive relationship with plant Cu concentration. Hence, Mehlich-3 may be used as a suitable extractant for the assessment of available Cu in soil of mulberry garden.

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