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Clonal variation depending on Cu, Fe and Zn element concentrations in seed orchards

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Abstract

In order to increase the existing forest assets and to maintain sustainable forestry, breeding studies are carried out in forestry. One of the most important practices in these breeding studies is seed orchards. The purpose of seed orchards is to obtain better quality seeds per unit area. In this study, plant nutritional elements were used for the first time to determine clonal variation. The antagonism of nutrients and their accumulation rate in clones were investigated. The research was carried out in the Scots pine (*Pinus sylvstris*) clonal seed orchard in Taşköprü. Needle samples were taken from 8 ramets of 30 clones in the seed orchard in 3 replications and 240 trees were randomly sampled. In addition, soil samples were taken from the seed orchard. The samples taken were first washed in the laboratory and then two different drying processes were applied to the samples. After drying, samples were prepared by the melting method and then Cu, Fe and Zn element concentration analyzes were performed with the ICP-OES device. The results obtained were evaluated by analysis of variance and Tukey test with the help of Minitab 18 package program and differences between clones were tried to be determined depending on element concentrations. In the findings obtained, it was determined that there were significant differences between the clones in terms of the studied elements at the $p<0.05$ probability level.

Keywords: Seed orchard; Clonal variation; Element; Needle; Antagonist**.**

1. Introduction

Due to the increasing world population and industrialization, the need for forest products and wood raw materials is also increasing [1-3]. In addition, extreme conditions created by global warming and climate changes threaten the existence of forests in the world day by day [4-10]. In order to protect forest assets and increase the efficiency in production, breeding studies have been initiated in forestry practices [11]. The purpose of breeding practices is to increase the quality and quantity obtained from unit area [12].

Breeding practices in Turkish forestry were started in 1994 with the "Turkish National Tree Breeding Program [13]. One of the most important facilities that form the basis of breeding studies are seed orchards. Seed orchards; These are area established to obtain abundant of seeds of known origin, high quality and efficiency. Therefore, clonal differences and variation in seed gardens have an important role in conservation genetic diversity in the future [14].

Because the genetic diversity in seed orchards must be in a position to ensure the sustainability of the forests that will be established with the seeds obtained from these orchards in the future [15]. High genetic diversity in a species means that the species is more resistant and adaptable to changing habitat conditions [16].

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Knowing the genetic structures of populations within forestry breeding studies forms the basis of breeding practices. In these practices, different methods such as isoenzyme analyses, quantitative characters and markers are used when determining the genetic diversity of forest trees [17]. In addition to genetic diversity, plant nutrition and heavy metal accumulation are also very important issues in seed orchards [18]. Because the usefulness or accumulation of nutritional elements in plants can affect the plant positively or negatively [19-22]. Some of the plant nutrients can cause toxic effects when accumulated in the plant [23-25]. Therefore, plant nutrition has an important role in the management of seed orchards. However, there are very few studies on plant nutrition and heavy metal accumulation in the management of seed orchards in Türkiye.

It has been estimated that populations that are tolerant to heavy metals and air pollution may differ genetically to tolerate [26]. Therefore the elements Cu (copper), Fe (iron) and Zn (Zinc), which are vital for the plant but create toxic effects when accumulated in high amounts, were investigated in the research [27]. Another reason for studying these elements is the antagonistic relationship between them. [28]. The intake of one element negatively affects the intake of the other.

Iron element is effective on many vital activities such as photosynthesis reactions, respiration, growth and protein synthesis in plants. The copper element plays an important role in many vital activities such as chlorophyll production and respiration, as well as in providing the plant with resistance to diseases [27]. Zinc element has important functions in protein synthesis and genetic structure formation in the plant [30].

Especially in recent years, global climate change, which has been defined as an irreversible problem worldwide and will affect forest ecosystems the most, makes the genetic diversity of forests much more important [31-35]. In the sustainability of forests, it is aimed to guarantee the seeds in the future and to ensure high productivity from these areas by conservation the genetic diversity of seed orchards which are the source of seeds. However, the narrowing of the gene pool in seed orchards established with selective breeding method reduces genetic diversity and raises concerns that productivity may decrease in the long term. In this study aimed to develop a different method such as element analysis in order to determine how the concentrations of plant nutritional elements affect each other and to reveal clonal differences.

2. Materials and Methods

2.1. Material

The Scots pine species that is the subject of the research is among the forest tree species that need to breeding within the scope of "Türkiye National Tree Breeding and Seed Production Program" [13]. Scots pine is a valuable forest tree with economic value in our country and in the world. Although it is one of the target species in the breeding program, there are not many studies on the genetic diversity of Scots pine in our country. Evaluation of clonal variation in terms of element concentrations, which is the aim of this study, is a different approach in the practices of breeding.

The subject of the research, Taşköprü Tekçam Scots pine clonal seed orchard with NRN 151 (National Registration Number), is located within the borders of Kastamonu province. The location of the seed orchard is within Taşköprü Forest Nursery Directorate and the distance to the city center is 65 km. The altitude of the seed orchard is 1160 m and the area is 7 ha. The origin of the seed orchard is 169 NRN Seed Stand and originates from Arac-Dereyayla. The seed orchard was established in 1995 with a spacing of 6x6 m in accordance with the random plot trial design. There are 1868 ramet from 30 clones in the seed orchard [36].

2.2. Method

In the research, samples were taken from the last year's needles of the trees in the seed orchard. Samples were collected from 30 clones in the seed orchard. 8 ramets from each clone were used. A total of 240 trees were randomly sampled in 3 replicates by taking 3 samples from each ramet. While collecting needle samples, the seed orchard was divided into blocks and needle samples were randomly collected from ramets belonging to 30 clones in each block. Care was taken to take a needle sample from the longest branch on the tree facing southwest. The collected needles were labeled and placed in transparent bags. In addition, soil samples were taken from each block. Three each soil samples were taken from the subsoil and topsoil at the same point in all blocks.

The needle samples taken were brought to the laboratory and ramets belonging to the same clone were grouped. Afterwards, the shells and branch pieces on the needles were cleaned and the washing process was applied. The washed and cleaned samples were re-labeled, laid on paper to dry and checked for 15 days. The dried needle samples were placed in glass petri dishes and dried again in drying ovens at 45°C. This controlled drying process took about a week. After the drying process was completed, the needle samples were pulverized with a blender in the laboratory and prepared using the dissolution method in HNO3. After the soil samples were sifted and cleaned, they were placed in petri dishes, labeled and left in the room to dry for about two weeks. Afterwards, the soil samples were dried in ovens at 45°C for a week. The dried soil samples were liquefied by the melting method and taken into aqueous solution and made ready for analysis. Copper (Cu), iron (Fe) and zinc (Zn) elemental analyzes were carried out on the prepared needle and soil samples with the help of the ICP-OES (Inductively Coupled Plasma Optical Emission Spectrometer) device. This method has been frequently used in recent years to determine the elemental content of both plant materials [37-39] and soil [40-42]. Analysis of variance and Tukey tests were applied to the elemental concentration results using Minitab 18 statistical program.

3. Results

In the study, the accumulation of copper (Cu), iron (Fe) and zinc (Zn) element concentrations in both soil and clones was investigated.

3.1. Results on Copper (Cu), Iron (Fe) and Zinc (Zn) Element Accumulation of Soil Samples

The results of variance analysis regarding the element concentrations of the data obtained from the soil samples taken in the seed orchard are given in Table 1.

Table 1 Result of variance analysis for element concentrations in soil samples

Elements	Source of Variance	Degrees of freedom	Sum of squares	Mean squares	F-value	P-value
Fe	Block	7	480873995	68696285	5.19***	0.000
	Error	40	529263999	13231600		
Cu	Block	7	353421507	50488787	9.22***	0.000
	Error	40	219068323	5476708		
Zn	Block	7	3672	524,58	15.90***	0.000
	Error	40	1319	32,98		

*Different at 0.05 and ***0.001 probability level, ns:Not statistically different

As a result of the variance analysis, it was determined that there were differences between the blocks in terms of copper (Cu), iron (Fe) and zinc (Zn) element concentrations. Therefore the Tukey test was applied to the data obtained to compare soil samples and blocks.

3.2. Investigation of Element Concentration Variation in Soil Samples between Blocks

In the research, the arithmetic means ($\bar{x}$), standard deviation (s) and coefficients of variation (Cv) of the measured values were calculated in order to detect the concentration variations of the elements investigated in the soil samples and to compare the blocks.

3.2.1. Results of related to iron (Fe) element concentration in blocks

Statistical values calculated for the Fe element concentration in the soil sample in each block are given in Table 2.

Table 2 Results of related to Fe element concentration in soil samples of the blocks.

Block	Mean (($\bar{x}$) (ppm)	Standard deviation (s)	Minimum	Maximum	Variation (Cv)
1	29180 ^{AB}	4286	26179	32181	14.7
2	26025 ^{B}	4123	23024	29026	15.8
3	23777 ^{B}	1622	20776	26778	6.8
4	28507 ^{AB}	6588	25506	31509	23.1

5	27738 AB	2268	24736	30739	8.2
6	32832 A	3754	29830	35833	11.4
7	34071 A	1843	31070	37072	5.4
8	27419 AB	1348	24417	30420	4.9

*Letters in the mean column indicate the groups formed as a result of the Tukey test. Blocks containing the same letter do not differ from each other for the corresponding character in a column (p = 0,05).

When the soil samples regarding the iron element are investigated, it is detected that the highest accumulation is in block number 7 with 34071 ppm. Then blocks 6 and 1 followed. It was determined that the lowest iron element concentration was in block number 3. While block number 4 had the highest variability among the blocks in terms of iron element (Cv=23.1), block number 8 showed the lowest variability (Cv=4.9).

3.2.2. Results of related to copper (Cu) element concentration in blocks

The analysis results obtained for the copper element in the investigated soil samples are given in Table 3.

Table 3 Results of related to Cu element concentration in the soil samples of the blocks.

Block	Mean (($\bar{x}$) (ppm)	Standard deviation (s)	Minimum	Maximum	Variation (Cv)
1	21911 B	2007	19980	23842	9.2
2	21679 B	1761	19748	23610	8.1
3	19840 BC	1048	17909	21770	5.3
4	21145 BC	3285	19214	23076	15.5
5	19742 BC	2642	17811	21673	13.4
6	27284 A	3490	25353	29215	12.8
7	22333 B	1738	20402	24263	7.8
8	17178 C	1616	15247	19108	9.4

*Letters in the mean column indicate the groups formed as a result of the Tukey test. Blocks containing the same letter do not differ from each other for the corresponding character in a column (p = 0,05).

When the table is analyzed, it is seen that block number 6 has the highest value regarding copper element accumulation among the blocks. This was followed by blocks 7 and 1, respectively. It was observed that block number 8 had the lowest copper element accumulation. Block 4 (Cv=15.5) had the highest variability in copper element concentration while block 3 (Cv=5.3) showed the lowest variability.

3.2.3. Results of related to zinc (Zn) element concentration in blocks

The results of the statistical analysis of zinc element in the soil samples investigated in the seed orchard are given in the table.

Table 4 Results of related to Zn element concentration in soil samples of the blocks.

Block	Mean (($\bar{x}$) (ppm)	Standard deviation (s)	Minimum	Maximum	Variation (Cv)
1	52.75 B	9.9	48	57.5	18.9
2	51.46 B	6.4	46.7	56.2	12.3
3	52.29 B	5.1	47.5	57.1	9.7
4	39.88 C	4.1	35.1	44.6	10.2
5	46.42 BC	4.8	41.7	51.2	10.4
6	68.46 A	6.5	63.7	73.2	9.5

7	65.33 A	3.2	60.6	70.1	4.9
8	51.00 B	2.2	46.3	55.7	4.3

*Letters in the mean column indicate the groups formed as a result of the Tukey test. Blocks containing the same letter do not differ from each other for the corresponding character in a column (p = 0,05).

When the analysis results regarding zinc element concentration were examined, it was observed that the highest element accumulation was in the 6th block, followed by the 7th and 1st blocks. It was determined that the block with the most variable and heterogeneous structure among the blocks was block number 1 (Cv = 18.9). Block number 8 (Cv=4.31) showed the least variability among the blocks.

3.3. Results of Copper (Cu), Iron (Fe) and Zinc (Zn) Element Concentrations Among Clones

The variance analysis table for the clones in terms of the element concentrations examined is given in table 5.

Table 5 Results of variance analysis for clone element concentrations.

Elements	Source of Variance	Degrees of freedom	Sum of squares	Mean squares	F-value	P-value
Fe	Clones	29	1.58339E+11	5459952383	3.49***	0.000
	Error	690	1.08091E+12	1566540652		
Cu	Clones	29	40123149	1383557	3.06***	0.000
	Error	420	189974117	452319		
Zn	Clones	29	4279191698	147558334	6.70***	0.000
	Error	690	15191506376	22016676		

* Different at 0.05 and ***0.001 probability level, ns: Not statistically different.

When the results of the analysis were evaluated, it was determined that there were significant differences between the clones in terms of the element concentrations. Therefore Tukey test was applied to the data obtained.

3.4. Investigation of Element Concentration Variation in Clones

In the research, arithmetic means ($\bar{x}$), standard deviation (s) and coefficients of variation (Cv) of the measured parameters were calculated in order to detect the element concentration variability in the seed orchard and to compare the clones.

3.4.1. Clonal values for iron (Fe) element concentration

The statistical values calculated in terms of iron element concentration of each clone are given in Table 6.

Table 6 Results of related to Fe element concentration in clones.

Clone No	Mean (($\bar{x}$) (ppm)	Standard deviation (s)	Minimum	Maximum	Variation (Cv)
11	80556 BC	26703	64693	96419	33.1
12	86026 BC	29837	70163	101889	34.7
13	79563 BC	13049	63701	95426	16.4
14	86885 BC	46356	71022	102748	53.4
15	76918 BC	24836	61056	92781	32.3
16	95725 A	46409	79863	111588	48.5
17	77439 BC	16400	61576	93302	21.2
18	98415 A	33548	82552	114278	34.1

19	111454 A	44440	95591	127316	39.9
20	115620 A	36440	99757	131482	31.5
21	88919 BC	26037	73056	104782	29.3
22	97757 A	34879	81895	113620	35.7
23	83288 BC	23182	67426	99151	27.8
24	87151 BC	28661	71288	103013	32.9
25	78321 BC	28931	62458	94184	36.9
26	85667 BC	37109	69804	101529	43.3
27	102849 A	41682	86987	118712	40.5
28	99965 A	41485	84102	115827	41.5
29	91000 BC	42450	75137	106862	46.7
30	135993 A	134209	120130	151856	98.7
31	72042 CD	24290	56179	87904	33.7
32	79641 BC	16561	63778	95503	20.8
33	61933 D	14918	46071	77796	24.1
34	79658 BC	28524	63795	95520	35.8
35	82739 BC	24248	66877	98602	29.3
36	82274 BC	29588	66411	98137	35.9
37	76648 CD	36419	60786	92511	47.5
38	64283 D	20385	48420	80145	31.7
39	86808 BC	26065	70946	102671	30
40	82136 BC	32877	66273	97999	40

*Letters in the mean column indicate the groups formed as a result of the Tukey test. Clones bearing the same letter do not differ from each other for the corresponding character in a column (p = 0,05).

When the values in Table 6 were analyzed, it was determined that the clone with the highest average iron element accumulation among the clones was clone number 30 (135993 ppm). The clone with the lowest element concentration average was clone number 33 (61933 ppm). It was observed that this clone was followed by clone number 38. It was observed that the clone with the most variability in terms of iron element concentration among the clones was clone number 30 (Cv = 98.7). The clone with the lowest variability among the clones was clone number 13 (Cv = 16.4)

3.4.2. Clonal values for zinc (Zn) element concentration

The zinc element accumulation values of the clones are shown in the table.

Table 7 Results of related to Zn element concentration in clones

Clone No	Mean (($\bar{x}$) (ppm)	Standard deviation (s)	Minimum	Maximum	Variation (Cv)
11	14570 CD	4706	12689	16450	32.3
12	13758 DE	1886	11877	15638	13.7
13	14169 DE	2142	12289	16050	15.1
14	14936 CD	2835	13055	16816	18.9
15	18405 C	4516	16524	20285	24.5
16	17617 CD	3538	15736	19497	20.1

17	14027 [DE]	1960	12146	15907	13.9
18	24020 [A]	11716	22139	25900	48.8
19	14992 [CD]	5834	13111	16873	38.9
20	16575 [CD]	4590	14695	18456	27.7
21	15092 [CD]	3062	13211	16972	20.3
22	15026 [CD]	2135	13145	16906	14.2
23	12682 [E]	3381	10801	14562	26.7
24	15055 [BC]	5037	13174	16935	33.5
25	14861 [CD]	3674	12981	16742	24.7
26	13463 [DE]	3597	11583	15344	26.7
27	14255 [DE]	2884	12374	16135	20.2
28	14911 [CD]	5405	13030	16791	36.2
29	16469 [CD]	3877	14588	18349	23.5
30	13871 [DE]	3252	11990	15751	23.4
31	18264 [BC]	6702	16384	20145	36.7
32	15965 [CD]	2467	14085	17846	15.4
33	17493 [CD]	5486	15613	19374	31.4
34	15130 [CD]	5531	13250	17011	36.6
35	12182 E	3169	10302	14063	26
36	14871 [CD]	4331	12991	16752	29.1
37	12322 [E]	2524	10441	14202	20.5
38	19377 [AB]	7606	17497	21258	39.3
39	12231 [E]	3862	10350	14111	31.6
40	12961 [DE]	5754	11080	14841	44.4

*Letters in the mean column indicate the groups formed as a result of the Tukey test. Clones bearing the same letter do not differ from each other for the corresponding character in a column (p = 0,05).

When the analysis results among the clones for the zinc element were evaluated, it was determined that the clone with the highest average element accumulation was clone number 18, followed by clone number 38. The lowest element average was clone number 35. While clone number 18 (Cv = 48.8) was observed to be the clone with the most variable and heterogeneous structure among the clones, clone number 12 showed less variability than the other clones in terms of the examined values.

3.4.3. Clonal values for copper (Cu) element concentration

Values for copper element accumulation among clones are given in Table 8.

Table 8 Results of related to Cu element concentration in clones.

Clone No	Mean (($\bar{x}$) (ppm)	Standard deviation (s)	Minimum	Maximum	Variation (Cv)
11	1705 [BC]	734	1364	2046	43.1
12	719 [D]	630	378	1061	87.6
13	1752 [BC]	786	1411	2093	44.9
14	1715 [BC]	816	1374	2056	47.6

15	1213 CD	554	901	1524	45.7
16	1246 BC	500	905	1587	40.1
17	902 D	412	560	1243	45.7
18	1144 CD	695	803	1486	60.7
19	1390 BC	810	1049	1731	58.3
20	1253 BC	649	911	1594	51.8
21	1085 CD	693	743	1426	63.9
22	1391 BC	625	1050	1733	44.9
23	1103 CD	496	762	1444	44.9
24	938 D	497	597	1280	52.9
25	1507 BC	684	1165	1848	45.4
26	1109 CD	491	768	1451	44.3
27	1149 CD	654	808	1490	56.9
28	1262 BC	804	920	1603	63.7
29	1397 BC	644	1016	1779	46.1
30	1404 BC	652	1063	1745	46.4
31	1424 BC	734	1083	1766	51.5
32	1297 BC	752	956	1638	57.9
33	1875 A	753	1534	2216	40.2
34	919 D	462	578	1260	50.3
35	1159 CD	623	818	1500	53.7
36	1382 BC	646	1041	1724	46.7
37	1518 BC	609	1176	1859	40.1
38	2124 A	972	1783	2465	45.8
39	1392 BC	783	1051	1733	56.3
40	1215 BC	695	874	1557	57.2

*Letters in the mean column indicate the groups formed as a result of the Tukey test. Clones bearing the same letter do not differ from each other for the corresponding character in a column (p = 0.05).

When the values of copper element concentration were compared between clones, it was seen that the highest concentration average belonged to clone number 38 with 2124 ppb, followed by clone number 33. Among the clones, clone number 12 (Cv = 87.6) had the highest variability in terms of copper element concentration.

4. Discussion

In this study, the variation of iron (Fe), copper (Cu) and zinc (Zn) element concentrations, which have antagonistic relationships with each other, among clones and blocks in the Scots pine seed orchard was investigated. At the end of the study, it was determined that element concentrations varied in all clones and blocks.

In the research conducted, unlike previous studies used to determine clonal variation, plant nutritional elements were used and an unusual method was followed. In this study, the variation between clones was tried to be determined by looking at the concentrations of iron, copper and zinc elements, which are both important nutritional elements and have antagonistic effects, on a clonal basis and in soil samples.

In the research, element concentration variations were investigated in soil samples taken from the blocks in the seed orchard. Among the blocks, the ones with the highest iron element concentration were blocks 7, 6 and 1, while block number 3 was found to have the lowest iron element concentration. It was determined that the blocks with the highest accumulation of copper and zinc element concentrations were blocks 6, 7 and 1.

When the element concentrations variation between clones throughout the seed orchard is examined; It was determined that clone number 30 had the highest iron element accumulation. It was determined that clones 33 and 38 had the lowest iron element concentration. It was observed that the clones with the highest copper element concentration were clones 38 and 33. It was determined that clone number 18 (Cv = 48.8) had the highest variability in terms of zinc (Zn) element concentration, and clone number 12 (Cv = 87.6) had the highest variability in terms of copper (Cu) element concentration.

It was observed that clone number 30 (Cv = 98.7) had the highest variability in terms of iron (Fe) element concentration among the clones. the clone with the highest coefficient of variation in terms of zinc (Zn) element concentration is clone number 18 (Cv=48.8), and The most variable copper (Cu) element accumulation average was found to be clone number 12 (Cv=87.6).

Antagonism could be clearly observed throughout the seed orchard in clones in which copper and zinc elements, which have an antagonistic relationship with the iron (Fe) element accumulated. It was observed that clone number 38 had the highest accumulation of copper and zinc elements which had the lowest accumulation of iron element. In addition, it was determined that clone number 33, which had the lowest accumulation for the iron element, had the highest accumulation for the copper element.

In order to better detect the diversity of this relationship between clones and elements, the element contents in the clones of blocks 1 and 7 which have the highest element concentration accumulation, were investigated. As a result, it was determined that the clone with the highest iron (Fe) element concentration in block number 1 was clone number 30. It was observed that clone number 33 had the lowest iron (Fe) element concentration. It was determined that the clone with the highest concentration of copper (Cu) element was clone number 38, and the clone with the highest concentration of zinc (Zn) element was clone number 29. In block number 7, it was determined that the highest iron (Fe) element concentration was in clone number 40, and the highest concentration of zinc (Zn) and copper (Cu) elements was found in clone number 38.

Although plant nutritional elements are important for plants to carry out their vital activities, their accumulation in plants causes toxicity, causing them to lose their usefulness and become harmful. The elements subject to research, iron (Fe), copper (Cu) and zinc (Zn), are among the nutritional elements that have an important place in plant metabolism, and their high accumulation can cause toxic effects on the plant [43].

Copper (Cu) element plays a role in many vital activities in the plant such as enzyme activation, protein synthesis, photosynthesis, and also plays an important role in physiological and reproductive activities such as the development of the plant's roots and formation of seeds. Additionally, the copper element is important for the plant to use water efficiently [30]. In addition, the copper element plays an important role in increasing the plant's resistance to fungal and other harmful diseases [27]. However, increasing copper concentration in plants may cause toxic effects [44].

In the plant, in addition to the copper element, the iron (Fe) element plays a vital role in nitrogen uptake, protein and chlorophyll synthesis and enzymatic reactions. In the deficiency of iron element, chlorophyll synthesis decreases and therefore the vital activity cycle of the plant is interrupted. Accumulation of the iron element is not very common in open field conditions [30].

Zinc element, just like copper element, is among the micronutrient elements that play an important role in protein synthesis and enzyme activities and are vital for the plant [45]. Because the element zinc is a very effective nutrient element in both the productivity and quality of the plant [46].

Heavy metals such as copper (Cu), iron (Fe), and zinc (Zn) can serve as vital nutritional elements for plants when they do not exceed a certain amount. However, when they are above a certain limit, they can negatively affect vital activities such as photosynthesis, DNA, RNA and protein synthesis in plants and cause damage to plant metabolism [43]. It is known that as a result of high accumulation of heavy metals in plants, they can interact with the nuclear proteins that make up the genetic structure of the plant and thus affect the genetic structure in plants [47-48].

In a study conducted in Greece in 2023 on the black pine (*Pinus nigra*) species, it was found that genetic diversity decreased in populations in places with high air pollution. It has been predicted that the genetic structure of populations that can tolerate air pollution may change [26]. In a study conducted on Scots pine (*Pinus sylvestris*) in Poland, it was determined that heavy metal ions had an effect on the genetic structure of Scots pine and that the Scots pine population was tolerant to heavy metal pollution [49].

In a different study on Scots pine, it was observed that Scots pine populations with high genetic diversity were less affected by air pollution and air pollution was less effective on the growth of the populations [50]. In a study conducted on peach clones in Brazil in 2015, it was observed that copper element content was at normal levels in some clones with low iron element content [51].

In a study conducted in 2019 on heavy metal accumulation in plane-leaved maple due to traffic density, it was found that the accumulation of iron elements increased in the leaves, branches and seed organs of the plant. It was observed that the highest accumulation in the leaves was at 579.67 ppm [52]. In the study, it was determined that the highest iron accumulation was higher than this level. In a different study conducted on Scots pine, the copper element concentration in the needles was examined and the highest accumulation amount in the needles was found to be 1909.5 ppm [4]. In this study, the highest value for copper element concentration was found to be 2124 ppb (2,124 ppm) and was detected at a much lower level than the value found in the above study.

In a research conducted in various regions of Scots pine in Türkiye in 1978, it was determined that the amount of copper element in Scots pine needles was 2.7 - 8.8 ppm, the amount of zinc element was 26-105 ppm and the amount of iron element was 34-280 ppm [53]. In this study, the highest copper element concentration in the needles in the seed garden was determined as 2124 ppb (2,124 ppm), zinc element concentration as 24020 ppm and iron element concentration as 135993 ppm. Among these values, copper element concentration was found to be at a low level compared to Dündar's study [53] while the values found for iron and zinc were found to be very high.

It is estimated that the amount of copper element in the soil is in the range of 2-100 ppm [54]. In the research, the highest copper element accumulation in the soil in the seed orchard was measured as 27284 ppb (27.3 ppm) and was found to be within the predicted range.

In this research, clonal variation and antagonist relationship in terms of iron (Fe), copper (Cu), zinc (Zn) element contents were tried to be revealed for the first time in a seed orchard. The elements used in the study are both vital for the plant and at the same time have antagonist properties (preventing each other's uptake). For this reason, in this research, both the accumulation of elements between clones and blocks and whether mutual element concentration affects the uptake amounts at the clonal level were investigated. Study results reveal that elements vary significantly on a clonal basis. As it is known, all phenotypic characters in plants are shaped under the mutual interaction of genetic structure [55-58] and environmental conditions [59-64]. Clonal seed orchards are established in areas with relatively homogeneous soil and climatic conditions [12].

5. Conclusion

Therefore, the differences between clones are largely due to the effect of genetic structure. As a result, it was determined that the concentrations of the studied elements Cu, Fe and Zn differed on clonal basis and mutual element concentration amounts were affected at clonal level. According to the results of the study, it was observed that element concentration accumulations revealed clonal differences.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that they no conflict of interest. The none of the authors have any competing interests in the manuscript.

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