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Effects of Cement Dust on Chlorophyll and Metabolism Products

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Abstract

Aim of study: Due to recent developments in the world, various industrial establishments began to operate without the pressures they create on the environment being taken into account. Some of these businesses are established on agricultural lands or on natural meadows and pasture areas. This study investigated the effects of dust particles, one of airborne contaminants, on some plant species.

Area of study: The changes in protein, total chlorophyll, carbohydrate and dry matter amounts of plant samples collected from the near and far periphery of a cement plant were investigated.

Material and Methods: Elm (*Ulmus glabra*), maple (*Acer negundo*) and yellow pine (*Pinus sylvestris*) plants were included in the study.

Main results: Cement dust emissions spreading in the environment had adverse effects on plant development. Significant reductions were observed for chlorophyll ($p<0.01$), carbohydrates ($p<0.01$), dry matter in leaves ($p<0.01$) and dry matter in trunks ($p<0.005$), as well as protein levels in pine ($p<0.01$) and elm ($p<0.005$).

Highlights: It was found that dust emissions clogged the pores in plant leaf tissue, negatively affecting photosynthesis and consequently causing a decrease in the levels of metabolic products.

Keywords: Cement dust, total chlorophyll, protein, dry matter, total carbohydrate

Çimento Tozlarının Klorofil ve Metabolizma Ürünlerine Etkileri

Öz

Çalışmanın amacı: Dünyada gelişmeye bağlı olarak çeşitli sanayi kuruluşları işletmeye açılmıştır. İşletmelerin bir kısmı tarım arazileri ya da doğal çayır ve meralar üzerine kurulmuştur. Bu çalışmada, hava kökenli kirletici parametrelerden olan toz partiküllerin bazı bitkiler üzerine etkisi incelenmiştir.

Çalışma alanı: Çimento Fabrikası'nın yakın ve uzak çevresinden toplanan bitki örneklerinin protein, toplam klorofil, karbonhidrat ve kuru madde miktarlarının nasıl ve hangi yönde değiştiği araştırılmıştır.

Materyal ve Yöntem: Karaağaç (*Ulmus glabra*), akçaağaç (*Acer negundo*) ve sarıçam (*Pinus sylvestris*) bitkileri incelenmeye alınmıştır.

Sonuçlar: İstatistiki olarak, sırasıyla klorofil ve karbonhidrat miktarı açısından önemli anlamı taşıyan $p<0.01$, kuru madde miktarı için ise yapraklarda önem seviyesi açısından $p<0.01$ ve gövdelerde bu sonuç oldukça önemli olan $p<0.005$ olarak azalma bulunmuştur. Protein açısından çamda $p<0.01$ ve karaağaçta ise $p<0.005$ olarak azalma tespit edilmiştir

Önemli Vurgular: Toz emisyonlarının özellikle bitki yaprak dokusundaki gözenekleri tıkayarak fotosentezi olumsuz etkilediği ve buna bağlı olarak metabolik ürünlerin düzeylerinde azalmalara neden olduğu saptanmıştır.

Anahtar kelimeler: Çimento tozu, toplam klorofil, protein, kuru madde, toplam karbonhidrat

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Introduction

Plants, which form the basis of the relationship between energy and matter in ecosystems, are faced with dangers of air, soil and water pollution, as well as drought. For this reason, distorted soil structure, mineral insufficiency, extreme temperatures, high light intensity and irresponsible soil management cause severe destruction in the nature (Kadıoğlu, 2004; Turfan, Savacı & Sarıyıldız, 2016). Physical-chemical properties and water holding capacity of soil, amount of oxygen, pH, mineral content, and fading point are some of the parameters which can tell us what type of plants can grow in a certain environment. Environmental factors, however, are also important for development of plants (Berges L, Chevalier, Dumas, Franc & Gilbert, 2005; Sarıyıldız and Küçük, 2009; Sağlam and Elvan, 2017). In Turkey, especially since 1970s, the terms "environmental issues" and "environmental pollution" have become frequently encountered and highly spoken terms in everyday life. It is recommended that necessary precautions to solve environmental problems or prevent environmental pollution should start primarily in the sources of pollution. Therefore, discharge of any waste into the environment should have a composition that is the least harmful for the nature (Öner, Ayan, Sıvacıoğlu & İmal, 2007). Solid cement dusts affect the quality of air negatively. The source of these particles, which are air pollutants and harmful to vegetation, are mainly cement factories, but these also include power plants, stone crushing quarries, agricultural operations, iron and steel production and forest products facilities (Vandergrift et al., 1971). Dust and other gaseous pollutants in various sizes that are formed during cement production are significant air pollutants. Cement dust, creating air pollution, is non-toxic, non-flammable and non-explosive, and does not seriously harm human health. However, pollutants accumulate in the "alveoli" region of the lungs. This is because there are no hairs called "flickering hairs" in this region that are used to filter and remove particles. Particles smaller than 0.1 microns in size travel to the air bags of the lungs due to the Brownian motion and settle in the "alveoli". In general, particles larger than 1 micron are retained before reaching this region (Karpuzcu, 1988). Since cement factories are often located far from large settlements, they have a negative impact on rural areas in particular (Anonymous, 1983). Additionally, these dusts spread over a wide area under the influence of atmospheric conditions, accumulating on surrounding plants and preventing their normal development (Brandt and Rhoades 1973; Cireli, 1975; Katırcıoğlu and Iren 1988; Bayhan, 2016) affecting floristic composition (Brandt and Rhoades 1972; Sheikh, Öztürk & Vardar, 1976), as well as altering the chemical structure of soil (Brands and Rhoades, 1973; Sheikh et al., 1976; Voran, 1984; Bayhan et al., 2002) and negatively affecting microbial species and activity (Lux, 1974; Bilen, 2010). In order to determine these adverse effects, chlorosis, leaf and branch drying or even complete drying were observed in *Pinus sylvestris* trees in Germany (Lux, 1974) and *Ouercus* trees in the United States (Rhoads, 1976). It was found that the lateral growth of the four selected species of forest vegetation in southwestern Virginia was adversely affected (Brandt and Rhoades, 1973). Lafraguta, Garcia-Criado, Arranz & Vazquez-de-Aldana (2014), on the other hand, found that cement dust solution had a negative effect on the germination of alfalfa (*Medicago sativa),* which is a feed crop plant. The effects of cement dust on olive trees near Bornova in İzmir, Turkey were investigated by Sheikh et al. (1976), indicating the harmful effects on growth and development. Additionally, changes in wheat beans, apples and landscape plants were investigated by cement dust applications in different time periods and quantities in controlled environments (Singh and Rao 1981; Katırcıoğlu and Iren, 1988; Çetin, 2016).

This study investigated the effects of dust particles emitted from cement factories on chlorophyll a, chlorophyll b, total chlorophyll, carbohydrate, dry matter and dissolved protein quantities of plants.

Material and Methods

Material

The pre-revised periphery of Aşkale Cement Factory in the northeastern part of the district of Aşkale in the province of Erzurum, Turkey was selected as the study area. In this study, five separate samples were collected for each tree species from near the cement plant (at about 100 m.) and from further points where pollution was less visible (at about 500 m.). The collection and preservation processes of the elm (*Ulmus glabra*), maple (*Acer negundo*) and yellow pine (*Pinus sylvestris*) specimens obtained for the purpose of investigation were made according to the method by Kaçar (1972). The chemicals that were used in the study were of analytical purity.

Method

For the analysis of chlorophyll a and chlorophyll b, the plant samples were initially washed under tap water, and prepared for processing. Analyses were performed on five different specimens for each plant species. These procedures were carried out for both leaf and trunk samples. The plant specimens were weighed after they were dried, then left in an 80 °C incubator until they reached constant weight, and their dry matter quantities were determined (Singh and Rao, 1981). Chlorophyll a and chlorophyll b analyses were performed to determine the amount of chlorophyll in fresh leaf tissues. For this purpose, fresh leaf tissues were weighed and crushed in 20 ml of 80% acetone (Acetone:Water, 4:1, V/V). The same method was applied for each sample. The resulting mixture was filtered and centrifuged at 3000 rpm for 15 minutes. The optical density of the chlorophyll extract was determined by a computerized colorimeter (Shimadzu, UV-160 A, UV-Spectrophotometer) which could be adjusted to 645 and 663 nanometer wave lengths. Chlorophyll a and b amounts were determined as mg/g in fresh leaves. By adding the amounts of chlorophyll a and b in our experiment, the total amount of chlorophyll in 1 g of fresh leaf tissue was determined by the following formulas (Sing & Rao, 1981).

$$\text{Chlorophyll a} = \frac{1{,}23 \times D_{663} - 0{,}86 \times D_{645}}{\text{d} \times 1000 \times \text{W}} \times V \quad (1)$$

$$\text{Chlorophyll b} = \frac{1{,}93 \times D_{645} - 3{,}6 \times D_{663}}{\text{d} \times 1000 \times \text{W}} \times V \quad (2)$$

W: Weight of fresh leaf;
V: Volume of acetone used;
D: Absorbance value

The values were found as mg chlorophyll/g fresh leaves.

The Norris and Ribbons (1971) phenolic-sulfur method was used to determine the amount of carbohydrate in leaf tissue. The spectrophotometer was calibrated with standard solutions to 488 nanometers for hexoses and 480 nanometers for pentoses, and the standard curves in Figures 1 and 2 were obtained, respectively. With this method, hexose and pentose levels in the tissue were determined spectrophotometrically. Based on the findings, plant samples that were affected and not affected by cement dust were compared among themselves. The results were statistically analyzed by t-test. Evaluations were made according to the method by Düzgüneş (1963) and significant changes were identified.

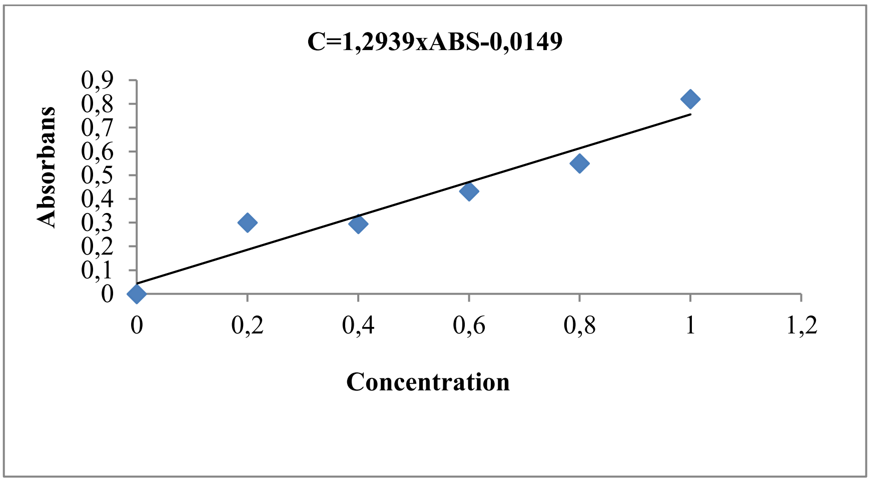

Figure 1.UV standard curve for hexose sugar, (488 nm)

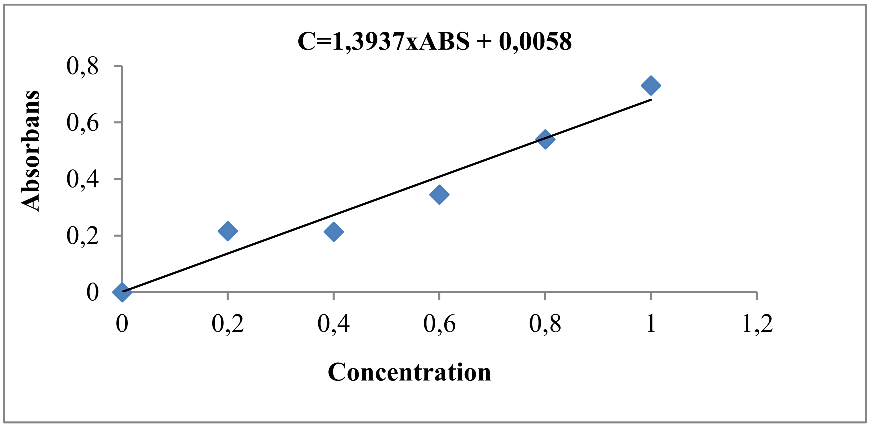

Figure 2.UV standard curve for pentose sugar, (480 nm)

To determine the protein amounts, chlorophyll-removed leaves were crushed in a 0.05 M phosphate buffer solution and the resulting liquid was filtered, transferred to tubes, and centrifuged. Protein amount was measured spectrophotometrically. The device was calibrated using standard protein solutions (Bradford, 1976). The standard curve is shown in Figure 3. After taking 0.5 g of the prepared samples, 0.5 ml of purified water, 3 ml of "Coomassie Brilliant Blue G-250" dye solution and 4 ml of phosphate buffer solution were added, and the absorbance values were measured at 595 nm wavelength. The amount of protein in 1 gram of fresh leaf was calculated as mg.

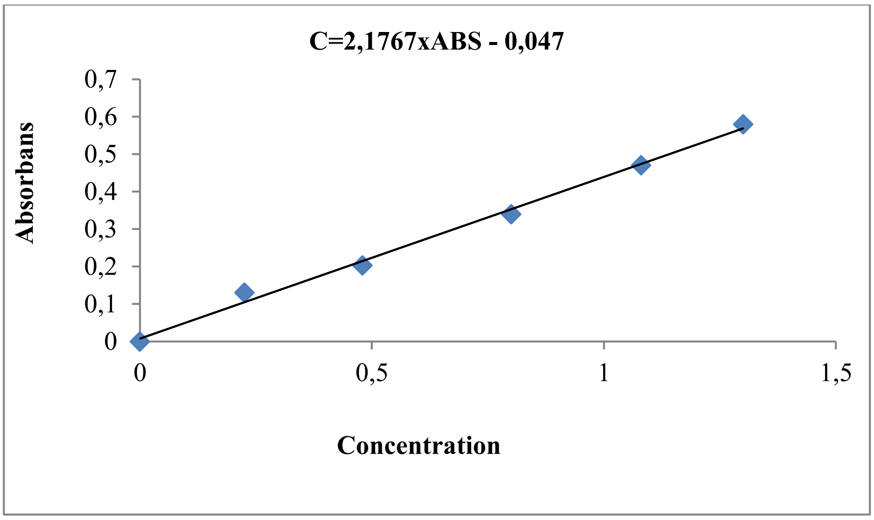

Figure 3.UV standard curve for protein, (595 nm)

The dry matter amount was determined on specimens taken from leaves and trunks. These samples were weighed immediately after being collected and their wet weights were found. They were then placed in a petri dish and dried at 80 °C in an incubator until they reached constant weight, and their dry weights were determined. Based on the difference between the values obtained, the quantities of dry matter in 1 g of fresh-tissue were determined.

Results and Discussion

The effects of dusts emitted by a cement factory on various plant species were examined in this study. For this purpose, chlorophyll a, chlorophyll b and total chlorophyll, carbohydrate, dry matter and soluble protein parameters were evaluated in plant samples.

Plants are greatly affected by the ambient conditions they are in. As a result, changes occur in plant organelles and metabolic products (Gond, DePury, Veroustraete & Ceulemans, 2012; Kurtar, 2012). As it is known, chloroplasts, one of the most important organelles that enable plants to grow and develop, play an active role in the synthesis of organic materials. For this reason, chlorophyll a, chlorophyll b and total chlorophyll levels of plant samples taken from polluted areas were compared to plant samples taken from unpolluted areas. The results are given in Table 1.

Table 1. The amount of chlorophyll

Plant Species	Sampling	Measured Parameters	The amount of chlorophyll (mg/g) I	II	III	IV	V	Averg.	% Variation
Elm	Dustless	Chlorophyll a	0,836	0,726	0,792	0,939	1,006	0,860	29,9
		Chlorophyll b	0,450	0,445	0,465	0,463	0,643	0,493	
		Total Chlorophyll	1,286	1,172	1,256	1,402	1,649	1,353	
	Dusty	Chlorophyll a	0,466	0,418	0,681	0,625	0,703	0,579	
		Chlorophyll b	0,279	0,290	0,425	0,364	0,489	0,369	
		Total Chlorophyll	0,745	0,708	1,105	0,989	1,192	0,948	
Maple	Dustless	Chlorophyll a	0,787	0,752	0,733	0,875	0,946	0,818	19,3
		Chlorophyll b	0,424	0,354	0,449	0,412	0,509	0,430	
		Total Chlorophyll	1,210	1,106	1,182	1,286	1,455	1,248	
	Dusty	Chlorophyll a	0,588	0,681	0,592	0,545	0,602	0,602	
		Chlorophyll b	0,392	0,454	0,411	0,368	0,401	0,405	
		Total Chlorophyll	0,980	1,135	1,002	0,914	1,003	1,007	
Yellow pine	Dustless	Chlorophyll a	0,683	0,629	0,673	0,666	0,722	0,675	8,8
		Chlorophyll b	0,455	0,420	0,468	0,463	0,481	0,457	
		Total Chlorophyll	1,138	1,049	1,142	1,129	1,203	1,132	
	Dusty	Chlorophyll a	0,563	0,613	0,619	0,630	0,628	0,617	
		Chlorophyll b	0,391	0,426	0,396	0,420	0,442	0,415	
		Total Chlorophyll	0,954	1,039	1,015	1,051	1,100	1,032	

As seen in Table 1, the chlorophyll a and chlorophyll b analysis of fresh leaf tissues revealed significant reductions ($p<0.01$) in total chlorophyll levels of plants growing in areas affected by dust in comparison to plant samples taken from dust-free areas. In particular, it was seen that the decrease in total chlorophyll levels was very significant in maple ($p<0.005$). The mean chlorophyll values determined for elm, maple and yellow pine samples obtained from unpolluted areas were determined to be 1.353, 1.248 and 1.032, respectively, whereas these values for the same plants taken from polluted areas were 0.948, 1.007 and 1.032, respectively. The decreases in chlorophyll contents were also found to be 29.9%, 19.3% and 8.8%, respectively. Similar findings were obtained in studies on sunflower (Borka, 1980), wheat (Singh & Rao 1981), maize and poplar (Cireli, 1975). Additionally, Katırcıoğlu & İren (1988) found a 29% reduction in chlorophyll in bean and apple plants affected by dust emissions. Czaja (1966) and Lerman (1972) showed that a decrease in chlorophyll levels directly affected photosynthesis, and hence plant growth and development, negatively. They proved in various experiments that, as accumulated dusts turned into an alkaline solution, they progressed from the epidermis to palisade and sponge parenchyma, damaging the chloroplasts and the tissues (Katırcıoğlu & İren, 1984). Various studies have been carried out on the effects of cement dusts, which cause a decrease in chlorophyll levels and thus photosynthesis rate, and reduce dry matter and carbohydrate amounts in tissues to elicit adverse effects on plant growth and development.

Carbohydrates, which are among the metabolic products in plants, are found in various forms. The carbohydrates subject to this study were hexoses and pentoses, which are the most common monosaccharides in plants. In the experiments examining the changes in carbohydrate levels in plants, samples were obtained from polluted areas affected by cement dusts, and unpolluted areas. Analyses of hexose, pentose and total carbohydrate levels were performed on these samples and the results are shown in Table 2.

Table 2. The amount of carbohydrate

Plant Species	Sampling	Measured Parameters	The amount of carbohydrate (mg/g)					Averg	% Variation
			I	II	III	IV	V		
Elm	Dustless	Hexose	0,777	0,667	0,746	0,612	0,706	0,702	25
		Pentose	0,763	0,530	0,469	0,721	0,735	0,644	
		Total carbohydrate	1,540	1,197	1,216	1,333	1,441	1,345	
	Dusty	Hexose	0,738	0,531	0,645	0,548	0,468	0,586	
		Pentose	0,437	0,200	0,240	0,711	0,535	0,425	
		Total carbohydrate	1,175	0,731	0,885	1,259	1,003	1,010	
Maple	Dustless	Hexose	0,602	0,510	0,835	0,558	0,561	0,613	23
		Pentose	0,455	0,470	0,669	0,430	0,467	0,498	
		Total carbohydrate	1,057	0,980	1,504	0,988	1,028	1,111	
	Dusty	Hexose	0,488	0,455	0,537	0,355	0,460	0,459	
		Pentose	0,372	0,371	0,485	0,342	0,406	0,395	
		Total carbohydrate	0,860	0,826	1,022	0,697	0,866	0,854	
Yellow pine	Dustless	Hexose	0,684	0,555	0,579	0,584	0,631	0,607	12
		Pentose	0,288	0,306	0,363	0,371	0,431	0,352	
		Total carbohydrate	0,972	0,861	0,942	0,955	1,062	0,958	
	Dusty	Hexose	0,454	0,372	0,421	0,469	0,463	0,436	
		Pentose	0,384	0,356	0,418	0,431	0,440	0,406	
		Total carbohydrate	0,838	0,728	0,838	0,900	0,903	0,841	

Table 2 shows that the mean carbohydrate amounts in plants collected from cleaner areas where dust emissions were not effective were 1.345, 1.111 and 0.958 mg/g for elm, maple and yellow pine, respectively. For samples collected from areas affected by cement dust, the mean carbohydrate levels were 1.010, 0.854 and 0.841, for elm, maple and yellow pine, with decreases of 25%, 23% and 12%, respectively. At the same time, significant reductions in carbohydrate amounts for elm and yellow pine were determined at locations where dust emissions were effective ($p<0.01$), but no significant change was observed for maple. Some researchers obtained similar findings in their studies. A decrease was reported in starch and sugar levels due to increased dust levels by Lerman (1972) in peas, by Anda (1986) in corn, and by Katırcıoğlu & İrem (1988) in beans and apples. When Table 2 is examined, it is seen that in the plants growing in polluted areas, there were significant reductions in comparison to plants growing in unpolluted areas. The significance level for dry matter in elm and maple leaves was $p<0.01$, while the significance level in the trunks was $p<0.005$.

Amount of dry matter was determined on the samples taken from leaves and trunks in the plants selected for use in this study. The results are shown in Table 3.

Table 3.The amount of dry matter

Plant Species	Sampling		The amount of dry matter (mg/g) I	II	III	IV	V	Averg.	% Variation
Elm	Leaf	Dustless	0,52	0,42	0,41	0,37	0,48	0,44	20,5
		Dusty	0,37	0,36	0,31	0,32	0,39	0,35	
	Bole	Dustless	0,62	0,75	0,67	0,67	0,68	0,68	19,18
		Dusty	0,56	0,60	0,53	0,54	0,54	0,55	
Maple	Leaf	Dustless	0,36	0,40	0,41	0,39	0,33	0,38	21
		Dusty	0,34	0,36	0,32	0,21	0,26	0,30	
	Bole	Dustless	0,55	0,60	0,54	0,57	0,62	0,58	13,80
		Dusty	0,52	0,50	0,45	0,44	0,58	0,50	
Yellow pine	Leaf	Dustless	0,51	0,49	0,48	0,45	0,50	0,48	10,42
		Dusty	0,44	0,47	0,44	0,40	0,42	0,43	
	Bole	Dustless	0,55	0,59	0,61	0,64	0,57	0,59	10,17
		Dusty	0,55	0,53	0,48	0,57	0,51	0,53	

Table 3 shows that the amount of dry matter was lower in areas affected by dust in comparison to dust-free areas. The decrease in the amount of dry matter was on different levels for leaf and trunk tissues. Reductions in the amount of dry matter in the leaf tissue were found to be insignificant in maple, while a significant decrease was observed for elm and yellow pine ($p<0.01$). In trunk tissues, a highly significant decrease was observed in elm ($p<0.005$), while no significant change was observed in maple and yellow pine. Similar results were obtained in other studies on the effects of pollutants on the amount of dry matter in plants. Various studies have shown that the amount of dry matter in olive trees decreased considerably (Sheik et al., 1976), and decreases were observed in the amount of dry matter in corn plants (Anda, 1986). Cireli (1975) reported that dry matter production decreased in various plants under the effect of pollutants, and this effect occurred due to inhibition of light available for photosynthesis in plants by dust.

Another important metabolic product in living organisms is protein (Yadeghari, Heidari & Carapetian, 2008). Proteins constitute approximately 50-80% of the dry weight of cells. Most of the substances that create seeds and other plant parts are in protein form. Additionally, all enzymes are in protein form (Akman, 1980). Separate assessments were performed for dusty and dust-free regions to analyze soluble protein levels, and the results are given in Table 4.

Table 4.Amounts of soluble protein

Plant Species	Sampling	Amounts of soluble protein (mg/g) I	II	III	IV	V	Averg.
Elm	Dustless	2,925	3,139	3,070	2,967	2,366	2,893
	Dusty	2,143	2,186	2,514	2,235	2,322	2,280
Maple	Dustless	2,152	1,972	1,714	2,000	2,033	1,974
	Dusty	2,033	1,539	1,368	1,520	1,421	1,576
Yellow pine	Dustless	2,014	2,343	2,175	2,273	2,754	2,312
	Dusty	2,004	1,847	1,672	1,795	1,977	1,853

When Table 4 is examined, a decrease may be seen in the amount of protein in the plants growing in highly polluted areas. This decrease was significant in yellow pine ($p<0.01$), and elm ($p<0.005$). Although no significant decrease was found in maple, a 20% decrease was observed.

Conclusions

In conclusion, chlorophyll a and chlorophyll b analyses of fresh leaf tissues revealed that total chlorophyll content of the plants growing in areas affected by dust showed a significant decrease in comparison to the plant samples obtained from dust-free areas. In particular, it was seen that chlorophyll levels decreased considerably in maple. This shows that maple is more sensitive to dust than the other plants.

According to the results of the carbohydrate analysis, it was found that carbohydrate levels significantly decreased in elm and yellow pine in the samples obtained from the polluted area in comparison to the samples collected from the unpolluted area. The amount of dry matter, one of the benchmarks for plant growth and development, was also found to decrease in the samples collected from the polluted area in comparison to the samples collected from the unpolluted area. Decreases in the amount of dry matter in the leaves were significant in elm and yellow pine, whereas in the trunk region, a significant decrease was observed only in elm.

Soluble protein amounts were also compared among the plant samples collected from clean and polluted areas. Except for elm, no significant reductions were found in protein levels. It was found that dust accumulating on the plants formed a covering layer on the leaves, preventing photosynthesis, which is highly important for plants, as well as reacting with moisture and transforming into an alkaline solution. Thus, dust emissions damage chloroplasts in the leaves, thereby reducing chlorophyll levels and indirectly slowing the photosynthetic metabolism.

Our findings indicate that the dust emissions of the studied cement factory had an adverse effect on meadows and pasture areas, as well as other vegetation. For this reason, in order to protect the nature, it has become necessary to consider environmental impact assessments seriously before establishing facilities such as cement factories.

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