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ICRETS 2021: International Conference on Research in Engineering, Technology and Science

Treatment of Egg Shells with Hydrogen Peroxide and Sodium Carbonate as a Disinfectant with Bactericidal Effect to Eliminate *Salmonella Entereditis*

Nora RRAHIMI HASANI
University of Pristina

Abstract: The objective of this study was to discover the effects of applying two antimicrobial drugs (sodium carbonate and hydrogen peroxide) on chicken eggshell on a poultry farm in Kosovo. Salmonella infections in egg contents may be related to external contamination of the eggshell. The first study was performed by applying sodium carbonate to the eggshell at different concentrations and pH of 10, 11 and 12 versus some Salmonella concentrations found in the analyzed eggs. A high amount of total bacteria was observed in all samples, but after the application of disinfectants we saw a decrease of these bacteria. The second study was performed to determine which concentration of H_2O_2 would give the maximum reduction of bacteria. In work 2, treatments were performed on 1) dry sample, 2) 0.5% H_2O_2, 3) 1% H_2O_2, 4) 1.5% H_2O_2, 5) 2% H_2O_2, 6) 2.5% H_2O_2 and 7) 3% H_2O_2.For shell tests, inactivation of Salmonella Enteritidis occurred at lower concentrations at pH 12 than at pH 11 and pH 10. The time of contact between the chemicals and Salmonella apparently results in accelerated bacterial inactivation.

Keywords: Egg shell, Antimicrobial, Sodium carbonate, Hydrogen peroxide.

Introduction

Eggs are one of the important sources of human food. In recent years, Salmonella enteritidis infections in humans have been traced to contaminated eggs(Coufal et al.2003, Gates, 1930). Salmonella is easily capable of contaminating bird eggs through vertical and horizontal transmission. Vertically transmitted salmonella contaminate the eggs during the laying process(Turnbull & Snoevenbos, 1974), while horizontal transmission occurs when the eggshell is contaminated by external sources such as incubators, the environment, or other infected birds (Smeltzer et al.1979). The calcareous skin surrounding the egg is porous and permeable to bacteria(Solomon, 2010) (Figure 1). The cuticle is a protein film covering the egg shell that provides a natural barrier to help prevent internal bacterial contamination (Peebles &Brake, 1986), however, defects in the shell or thinning of the cuticle can lead to invasion of the egg shell by bacteria in surface (Mayes &Takeballi, 1983). Salmonella can easily penetrate the egg cuticle and contaminate the internal contents (Williams et al, 1968, Wang & Slavik, 1998).

In egg shells, the total number of aerobic mesophilic bacteria can reach 3.75 to 7.07 $\log_{10}$ colony forming units (CFU) per egg. Therefore, reducing the microbial load of the egg shell through disinfection procedures would improve the quality of the egg to be incubated and reduce the incidence of bacterial infections in newborn embryos and hens. The salmonella enterica serotype is one of the most common serotypes associated with human salmonellosis (Rodriges et al, 1990). Studies have shown that contamination of egg contents can occur in the reproductive tract during the egg formation process. This microorganism, which is present in the feces, can also infect the contents of the egg by penetrating the shell through the shell pores or damaged areas (Humphrey, 1994, Protais et al.1989). Emphasis is placed on HACCP-based programs for identifying and preventing

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potential microbiological hazards that may arise from raw material, processing stages, product, and food plants (De Reu et al.2006, Kinner & Moats, 1981).

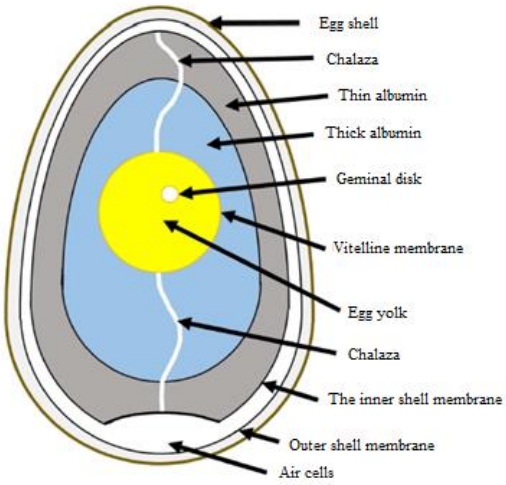

Figure 1. Chicken egg diagram

Hydrogen peroxide (H_2O_2) is a very strong oxidizer that forms free radicals exerting a destructive effect on cell membranes. As a result, it has found wide application as a biocidal (Linley et al. 2012). When O_3 is exposed to UV the net reaction results in the formation of hydrogen peroxide (H_2O_2) (Peyton & Glanze, 1988) and any hydroxyl radicals formed when O_3 reacts with UV are unable to escape this solvent cage(Peyton & Glanze, 1988). Although the advanced oxidation process (PAO) O_3 / UV is an effective disinfectant, the bactericidal properties are the result of the production of hydrogen peroxide instead of the hydroxyl radicals formed by the initial O_3 molecule.

The net photolysis of H_2O_2 yields 2 hydroxyl radicals, per quantum of absorbed radiation, which can continue to form peroxyl radicals leading to secondary oxidation reactions (Legrini et al. 1993). The H_2O_2 / UV photolytic reaction is one of the most widely used PAOs (Bustillo-Lecompte & Mehrvan, 2015), and has been demonstrated to effectively inactivate vegetative bacteria, bacterial spores, and viruses. be equal to or better than formaldehyde in hatching eggs for hatching (Sheldon & Brake, 1991). Hydrogen peroxide has previously been demonstrated to effectively reduce Salmonella contamination from experimentally contaminated eggs (Cox et al. 2000). After egg treatment, H_2O_2 evaporates easily without leaving any chemical residue and poses minimal safety issues for workers or embryo development (Sheldon & Brake, 1991, Cox et al.2000, Scott & Swetman 1993a, Padron, 1995, Keita et al. 2016). The bactericidal effects of H_2O_2 increase after UV photolysis (Ikai et al.2010).

Other benefits of using this system include the commercial availability of H_2O_2, its endless water solubility, and its lower health risk than O_3 for workers (Legrini et al.1993, Scott & Swetman 1993a). These benefits together with its effectiveness as a cleaner make the H_2O_2 / UV PAO system an attractive method of disinfection for eggs. These advanced oxidation processes are proving to be a new approach that effectively and safely reduces Salmonella contamination in egg shells. At the same time, a large variety of chemical agents have been developed, marketed as detergents, detergent-cleaners or detergents for cleaning and disinfecting eggs.

In practice, the cleaning and disinfection efficiency of agents is often determined by standard laboratory tests based on bacterial suspension tests, which are used to determine recommended concentrations (Best et el.1988). However, the applicability of these recommendations in harsh conditions is sometimes problematic because effective concentrations in suspension may be less active against bacteria sticking to a surface, especially a porous surface such as the eggshell.

Several other factors such as the number of bacteria present, the time of contact between the chemicals and the cells, and the pH of the microbial solutions also affect the effectiveness of the cleaning and cleaning agents. Commercial egg cleaners typically use alkaline ingredients to clean eggs, such as sodium carbonate and chlorine cleaners that operate at a pH ranging from 9 to 12 (Moats, 1978). However some authors have suggested that pH values> 11 be used to minimize the bacterial load on the eggshell and bath water (Cox et al. 2007, Frank & Wright, 1956).

The main objective of this study was to evaluate the effects of the application of two egg cleansing compounds contaminated with Salmonella Enteritidis and to determine the influence of pH and contact time on the activity of these antimicrobial compounds (sodium carbonate) and H_2O_2, as well as the amount the lowest chemical product needed to eliminate organisms on egg shell surfaces on a poultry farm in Kosovo.

Materials and Methods

Bacterial Registration Procedure

For the following experiments, bacterial counting was performed in the same manner. After individual eggs from the treated and dry sample groups were placed in Whirl-Pak bags, the bags were then filled with sterile 50 ml PBS (Phosphate-buffered saline). Each egg was massaged by hand into the bag for 1 min to remove bacteria located on the outer shell of the egg. Upon completion of the massage, the bags were opened and 10 ml of rinsing solution was aseptically collected in an empty tube of sterile culture. The rinsing solutions collected from all eggs receiving any treatment in the following 4 works were not serially diluted. However, 2 types of dilutions were required for all control eggs due to high bacterial loads. Once the dilutions were complete, 0.5 ml of all the rinsing samples and dilutions were spread in a non-selective medium showing the total bacterial count (agar plates). All samples were collected in duplicate. The plates were then incubated for 48 hours at 37°C. After the incubation period, the plates were removed and the colonies were counted. All results are reported as $\log_{10}$ cfu / egg.

During the experiment, 110 chicken eggs were used for microbiological evaluation. The eggs were bought from a commercial poultry farm in Kosovo, which uses brown chickens raised on the floor. For microbiological evaluation, 110 eggs were divided into 3 groups: 1) 10 eggs without disinfection; 2) 50 contaminated eggs which are then treated with sodium carbonate (30, 50 and 60ppm) in 2-12 minutes; 3) 50 eggs contaminated and then disinfected with H_2O_2 in different percentages and at different pH. A total of 110 eggs were randomly selected, cracked eggs were discarded and dispersed in disinfection treatments.

Realized works

A total of 110 apparently clean eggs were collected from chickens of a poultry farm in Kosovo and divided into 3 groups: 1) 10 eggs without disinfection; 2) 50 contaminated eggs which are then treated with sodium carbonate (40, 50 and 60ppm) in 2, 5 and 10 minutes; 3) 50 eggs contaminated and then disinfected with H_2O_2. 10 eggs tested for Salmonella positive served us as a negative control.For the egg procedure without disinfection, the egg rows were kept in the same room where the other treatments were performed, but the eggs did not undergo any disinfection procedure. Room temperature and humidity were recorded, from 26.7 to 30.5^{o}C and from 49 to 53%, respectively. The second experiment was a combination of three concentrations 40, 50 and 60ppm, three pH (10, 11 and 12), and three exposure periods (2, 5 and 10 min).

The third experiment was performed to determine which concentration of H_2O_2 would give the maximum reduction of bacteria in the eggshells. In work 3, treatments were done in 1) in the sample without treatment, 2) 0.5% H_2O_2, 3) 1% H_2O_2, 4) 1.5% H_2O_2, 5) 2% H_2O_2, 6) 2.5% H_2O_2 and 7) 3% H_2O_2 . 50 ml of disinfectant solution was sprayed on 50 eggs at the same time using a hand sprayer. The temperature of the solution was measured with a thermometer and ranged from 26 to 29ºC. To reach the entire surface of the eggs with the solution, the egg boxes were placed on a horizontal surface and the spraying was carried out in two steps: 25 ml of the solution was dispersed on one side of the eggs; and after we turned them over and 25 ml were dispensed on the other side. An average of 1 mL of hydrogen peroxide was sprayed on each egg, and 9 to 12 min were spent for this procedure. After each collection, shortly before and 1 hour after disinfection, the eggs from each treatment were selected to count the microbes in the egg shell. The eggs, collected with disposable gloves, were placed in groups in autoclaved bags, which were properly identified according to each treatment and then refrigerated at 4ºC. The samples were transported to the laboratory, where microbiological analyzes were performed 24 hours after cooling. Each bag was opened, and the eggs were transferred to another autoclaved bag, to which 50 ml PBS (Phosphate-buffered saline) solution was added. The eggs were massaged for 5 min to remove bacterial cells from their surfaces. Then, a 1.0 ml sample of PBS was taken from each bag, the plate was planted on agar, in order to obtain the Salmonella count. The plates were incubated at 37°C for 24 to 48 hours and, subsequently, bacterial colonies were counted and recorded. The microbial count was expressed as $\log_{10}$ CFU 1.0 mL^{-1} batch of eggs.

Results and Discussion

The purpose of this study was to evaluate the efficacy of chemicals used as disinfectants in chicken eggs. Salmonella contamination is considered an important hygienic issue, especially on small-scale farms that are not controlled by an authorized agency or when a Critical Risk Analysis (HACCP (Moats, 1978)), plan is not available. In this study, two commercial egg washings with sanitizing ingredients such as: sodium carbonate and hydrogen peroxide, in different concentrations, at pH values of 10, 11 and 12 were examined for their effectiveness in inactivating Salmonella Enteritidis in the shells of contaminated eggs.Compounds that do not demonstrate microbial effect at these concentrations were then examined until one found the effective amount of each cleaning agent. Figure 1 shows the bacterial count of Salmonella enterica calculated as $\log_{10}$ cfu / egg which will serve as a negative control. As seen in the picture we have a number of bacteria starting from 0.98$\log_{10}$ cfu/egg to 2.89$\log_{10}$ cfu/egg.

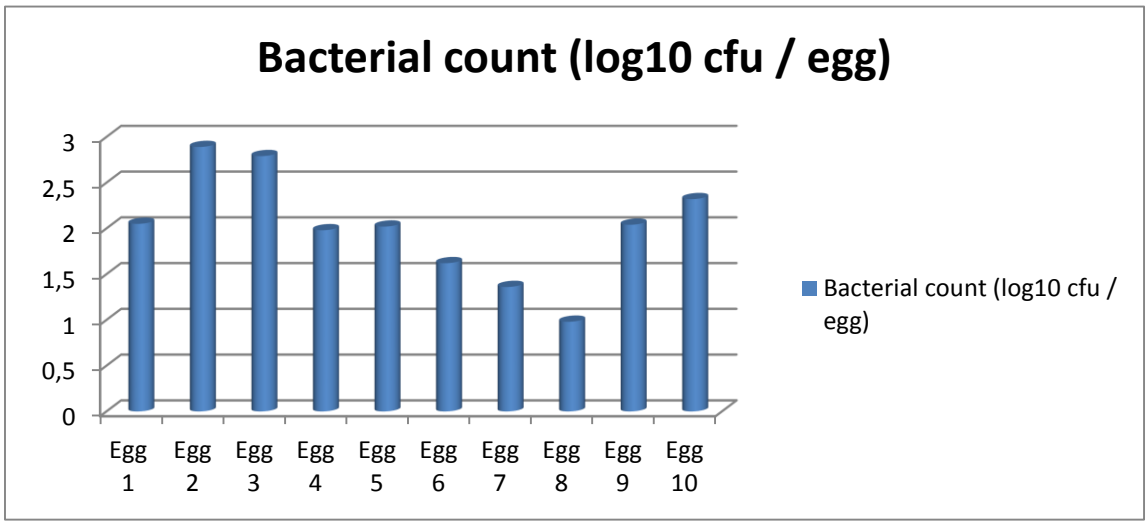

Figure 2. Bacterial counting at these sample eggs

In the second work on sodium carbonate, the effective concentrations were similar to those of other chemicals and were 5 to 6 times higher than the concentrations used in this work. The initial level of contamination of the eggshell can play an important role in the effectiveness of chemical agents. Because the numbers of bacteria present in the shell can affect the bactericidal activity of egg cleaning compounds, heavily contaminated eggs should not be placed in the egg washing machine. Moreover, before the cleaning operation they should be kept inside an environment that prevents the multiplication of bacteria in the husk.

Table 1. Bacterial count (log10 cfu/egg) in Na Carbonate treated eggs at different pH and time

		Bacterial count ($\log_{10}$ cfu/egg)								
		pH 10			pH 11			pH 12		
Product		2 min	5 min	10 min	2 min	5 min	10 min	2 min	5 min	10 min
Na	100ppm	2.02	1.89	1.56	1.85	1.65	1.41	1.52	1.36	1.12
Carbonate	150ppm	1.84	1.63	1.33	1.65	1.52	1.20	1.41	1.32	1.03
	200ppm	1.51	1.36	1.20	1.40	1.21	1.11	1.36	1.21	0.85

In work 3, all treatment groups gave significantly lower counts of bark bacteria compared to the dry sample (Figure 2). It is clearly seen from the diagram that with the increase of % of H_2O_2, the bacterial reduction calculated with $\log_{10}$ cfu/egg also increases.Bactericidal activities of any chemical treatment were determined against Salmonella Enteritidis in this paper. In this evidence, eggs with Salmonella were taken as experimental eggs, two disinfectant ingredients, such as sodium carbonate, at pH values of 10, 11, and 12 and for a contact time of 2, 5 or 10 min, and hydrogen peroxide at different concentrations were reviewed for their effectiveness in inactivating bacteria.

As shown in the results the analyzes showed differences between the values of Salmonella for both products; hydrogen peroxide showed a lower number of bacteria than Na carbonate. Bacterial values decreased significantly with an increase in pH or contact time: the number of bacteria at pH 10 was significantly higher than at pH 11, and the number of bacteria at pH 11 was significantly higher than at pH 12; the number of

bacteria for the time 2 min was not much higher than that for the time 5 min, although the values of the number of bacteria for both times were very significant and higher than for the time 10 min.

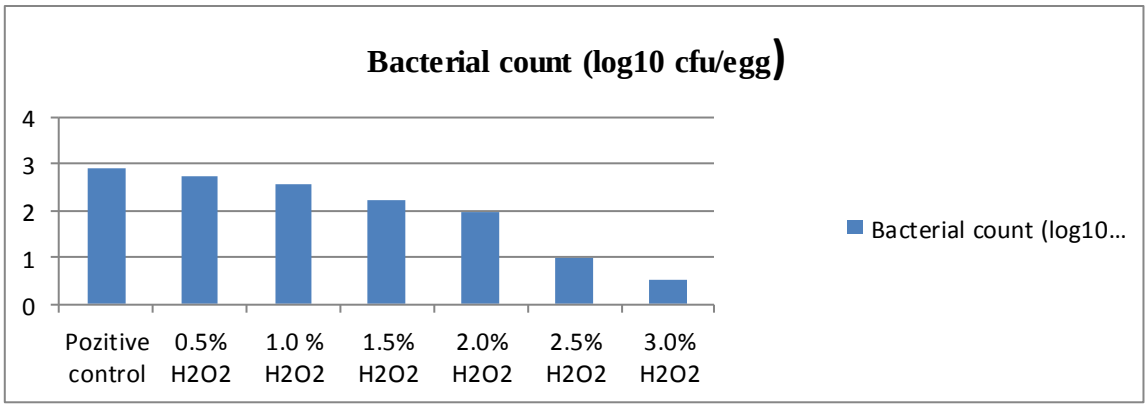

Figure 3. Comparison of different concentrations of H_2O_2 used alone in disinfection of egg shells N=50 eggs

Conclusion

This study was conducted to determine the minimum concentrations of the two chemicals needed to destroy Salmonella Enteritidis on egg surfaces. Our results indicate that very strong concentrations of these compounds were required, much higher than those commonly used in processing plants, were needed to inactivate Salmonella Enteritidis in contaminated shells, even when heat (48^0C) and alkaline environment (pH 10 to 12) were used as solutions. Therefore, preventive measures aimed at reducing or eliminating possible contamination of Salmonella peel should be a major concern when trying to control Salmonella. Further research in this area is needed to ensure egg industry with the most effective means of preventing and controlling Salmonella Enteritidis contamination. Furthermore, the use of these high concentrations of cleaning agents may be impractical for industrial application due to side effects on the eggshells and corrosive effect on equipment.As shown in the results the analyzes showed differences between the values of Salmonella for both products; hydrogen peroxide showed a lower number of bacteria than carbonate.

Scientific Ethics Declaration

The author declares that the scientific ethical and legal responsibility of this article published in EPSTEM journal belongs to the author.

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Prediction of Rheological Parameters of Asphalt Binders with Artificial Neural Networks

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Abstract: Recycling of industrial, agricultural etc. wastes is economically and environmentally important. In recent years, researchers was focused on the using wastes in structural materials. In this study, modified asphalt binders were obtained by adding 7 different ratios waste engine oil (2%, 4%, 6%, 8%, 10%, 12% and 14%), which released as a result of routine maintenance of automobiles, to the pure asphalt binder. Then, Dynamic Shear Rheometer (DSR) experiments were applied on pure and modified asphalt binders. The rheological properties of asphalt binders at different temperatures and frequencies (loading rates) were evaluated by performing the DSR Test at 4 different temperatures (40°C, 50°C, 60°C and 70°C) and 10 different frequencies (0.01-10Hz). Then, the obtained complex shear modulus and phase angle values were estimated with Artificial Neural Networks. The results showed that the addition of 2% waste mineral (engine) oil improved the elastic properties of the asphalt binder by increasing the complex shear modulus and decreasing the phase angle values. In addition, it was concluded that the rheological parameters of asphalt binders can be successfully obtained with Artificial Neural Networks, by estimating the results with low error rate and high accuracy.

Keywords: Waste Engine Oil, Recycling, Asphalt, Modification, Artificial Neural Networks

Introduction

Bitumen, which is used as a binder material in hot mix asphalts, is obtained by refining petroleum (Hunter et al., 2015). Bitumen, which has a very complex structure, ages due to effects such as traffic loads, climatic conditions, very high and very low temperatures, and loses its flexibility properties at the beginning of its service life over time (Yan et al., 2020; You et al., 2019). Pure bitumen may not provide the desired properties when used in the mixture. For this reason, additives are used to provide features such as temperature sensitivity, strength, flexibility at low temperatures. Polymer materials are most commonly preferred, and the bitumen to which polymer materials are added is called “Polymer Modified Bitumen (PMB)” (Paliukaite et al., 2016; Qian et al., 2018; Shadmani et al., 2018; Shan et al., 2019).

With the cost of the used additives and the increase in the amount of waste materials in the world day by day, the idea of using waste materials in engineering materials has emerged. For this purpose, various waste materials can be used as additives in bitumen and hot mix asphalts. The type of waste material used is very diverse, such as plastics materials, steel slags, polymers, vehicle tires, construction waste (Aldagari et al., 2021; Hake et al., 2020; Hu et al., 2018; Kamoto et al., 2020; Mashaan et al., 2021; Murugan et al., 2020; Padhan et al., 2013; Qian et al., 2018; Seyma Seyrek et al., 2020; Shadmani et al., 2018; Shruthi et al., 2020). In recent

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years, waste oils have also been used as additives or rejuvenators (Asli et al., 2012; Eriskin et al., 2017; Fernandes et al., 2018; Rahman et al., 2017; Zargar et al., 2012).

In this study, waste engine oil, which is produced as a result of periodic maintenance of vehicles, was used as an additive in bitumen binders, and its effect on the rheological parameters of bitumen was evaluated. For this purpose, modified bitumen samples containing waste motor oil in 7 different ratios (2%, 4%, 6%, 8%, 10%, 12% and 14%) were obtained, and then frequency sweep test was applied on these samples with a Dynamic Shear Rheometer device. Frequency sweep test was carried out at four different temperatures, 40°C, 50°C, 60°C and 70°C, and under ten different frequencies between 0.01-10Hz. Then, Artificial Neural Network (ANN) models were created and rheological parameters obtained from engine oil modified bitumen were estimated by ANN models.

Materials and Methods

The bitumen that used in the study (B50/70) was supplied from TÜPRAŞ Batman refinery. Waste engine oil (WEO) was obtained from a local auto repair shop. The compositions and physical properties of the waste motor oil used are given in Table 1, and the image of the oil is given in Figure 1.

Table 1. Compositions and physical properties of WEO

Items	Nitrogen (%)	Carbon (%)	Hydrogen (%)	Sulfur (%)	Flash Point (°C)	Density (g/cm^3)
WEO	-	83.18	13.91	0.408	210	0.8816

Figure 1. Image of waste engine oil

While obtaining modified bitumen, WEO, were added to the binder (2%, 4%, 6%, 8%, 10%, 12% and 14% by weight of the bitumen), then mixed in a mechanical mixer at a constant temperature of 180°C at 1000 rpm for 1 hour. The schematic representation of the modification process is given in Figure 2.

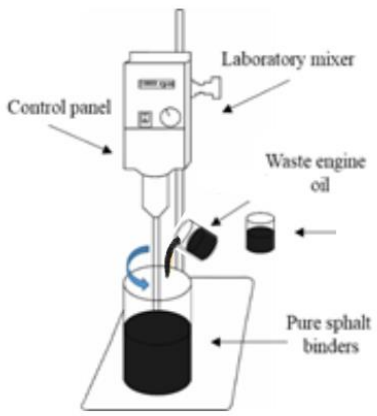

Figure 2. Schematic representation of the modification process

Traditional Binders Experiments

Penetration, softening point and flash point tests were carried out on modified bitumen samples. While the penetration test was applied according to the ASTM D5 standard, the softening point test was performed according to ASTM D36 and the flash point test was carried out according to ASTM D3143. The traditional binder test results of original and modified bitumen are presented in Table 2.

Table 2. Conventional binder test results of original and waste motor oil modified binders

Type of Binder	Penetration (mm^{-1})	Softening Point (°C)	Flash Point (°C)
Pure	62	53.3	245
2% WEO	64	53.25	242
4% WEO	69	52.2	240
6% WEO	77	51.1	238
8% WEO	88	49.6	232
10% WEO	105	48.15	228
12% WEO	121	46.95	224
14% WEO	136	44.65	217

Table 2 shows that the addition of WEO decreases the softening point values while increasing the penetration values of the original binders. With the addition of WEO, the flash point values decreased compared to the original binder. The use of WEO resulted in a softer behavior of original bitumen.

Dynamic Shear Rheometer (DSR)

The behavior of binders under load, strain and time is generally determined by DSR experiments. The DSR device is given in Figure 3. During the test, a bituminous binder sample is placed between two metal plates kept at constant temperature. One of the plates makes sinusoidal movements and the other is fixed. When torque is applied, the DSR motor goes from point A to point B, returns to point A and moves to point C. Then it reaches point A again. This process creates a single rotation and is repeated throughout the experiment (Fig. 4) (Yalçın, 2020).

Figure 3. Dynamic Shear Rheometer test device

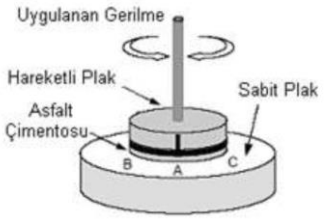

Figure 4. Movements of the binder sample in the DSR test (Yalçın, 2020).

As a result of DSR experiment, complex shear modulus and phase angle values were obtained. A high complex shear modulus means a high resistance to rutting. If high complex modulus values are obtained against low

phase angle values, it is said that the bitumen binder behaves more elastically. A phase angle of 0° is considered to be completely elastic, while if this value is 90°, the material is considered completely viscous (Figure 5) (Yalçın, 2020).

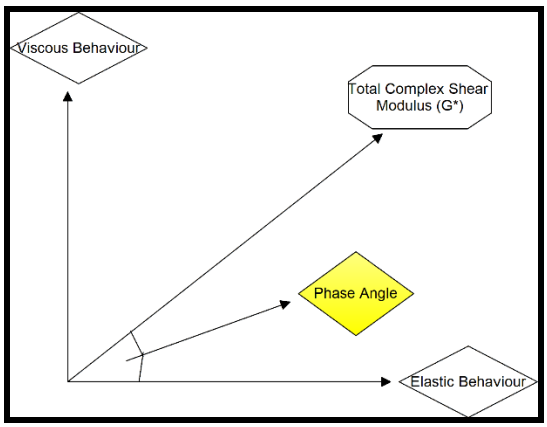

Figure 5. Representation of elastic and viscous behaviors according to DSR Experiment

In the study, sample geometry was chosen as 25mm diameter and 1mm height. Frequency sweep experiment was carried out on bitumen binders at 4 different temperatures (40°C, 50°C, 60°C and 70°C), 10 different frequencies (0.01Hz-10Hz).

Artificial Neural Network (ANN)

ANNs are an information processing process inspired by the human brain. It consists of many interconnected processing elements (neurons) that work in concert to solve specific problems. There are five parts in an ANN structure: inputs (X1,X2,X3…), weights (W1,W2,W3…), combining (addition) function, activation (transfer) function and output (Y). Inputs enter neurons with weights, pass through the coupling function and are transferred to the activation function (Graupe, 2013; Öztemel, 2008; Sönmez Çakir, 2019). Then the cell output values are calculated. The general structure of the ANN cell is presented in Figure 6.

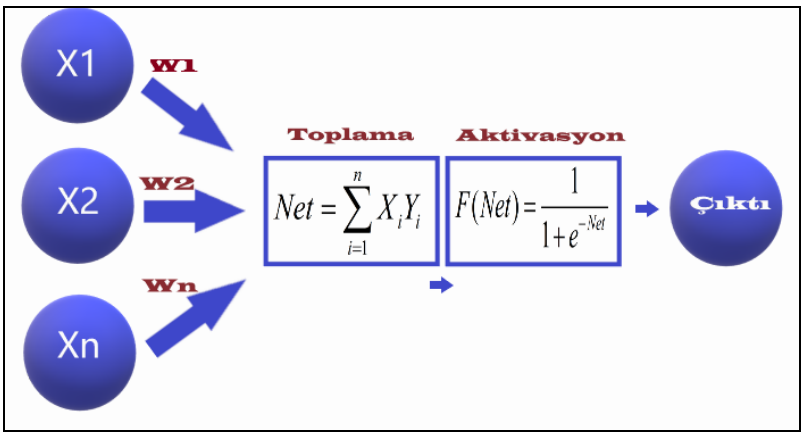

Figure 6. General structure of the ANN neuron

The artificial neural network models that created in the study were run in the MATLAB environment. Two models were created separately for the complex shear modulus and phase angle values, and a sigmoid feed-forward network structure was preferred for both models. The training algorithm is important in ANN analysis. The training algorithm to be used should be both fast and give high accuracy results with low error. For this reason, Levenberg-Marquardt back-propogation training algorithm was preferred for both models. After 70% of the data set was randomly selected for the training process, the remaining 30% was divided equally and selected for the validation (15%) and testing phases (15%). In order to determine the number of neurons in the hidden layer, different iterations and hidden layer numbers were tried to find the optimum value. The model that provides the lowest error rate with the highest accuracy should be preferred. In this study, 12 neurons were found suitable for both models.

The schematic representation of the artificial neural network model is given in Figure 7. In both models, temperature, frequency and waste engine oil content are defined as inputs. The output is the complex modulus and phase angle.

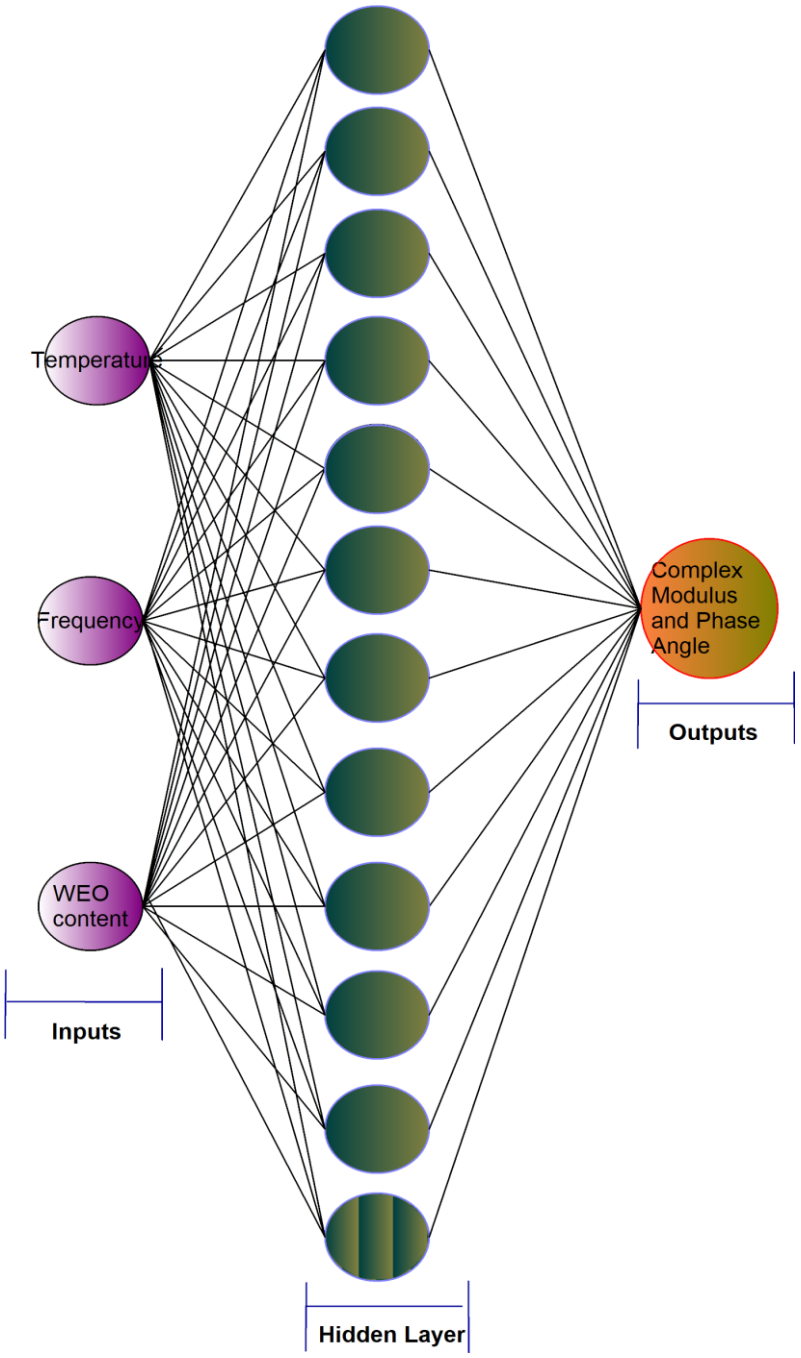

Figure 7. ANN Model

Results and Discussion

DSR Results

In the study, dynamic shear rheometer experiments were carried out on original and waste engine oil modified binders at 40°C, 50°C, 60°C and 70°C and at 10 different frequencies between 0.01 Hz and 10 Hz. The obtained complex modulus (G*) and phase angle curves are given in Figures 8 and 9.

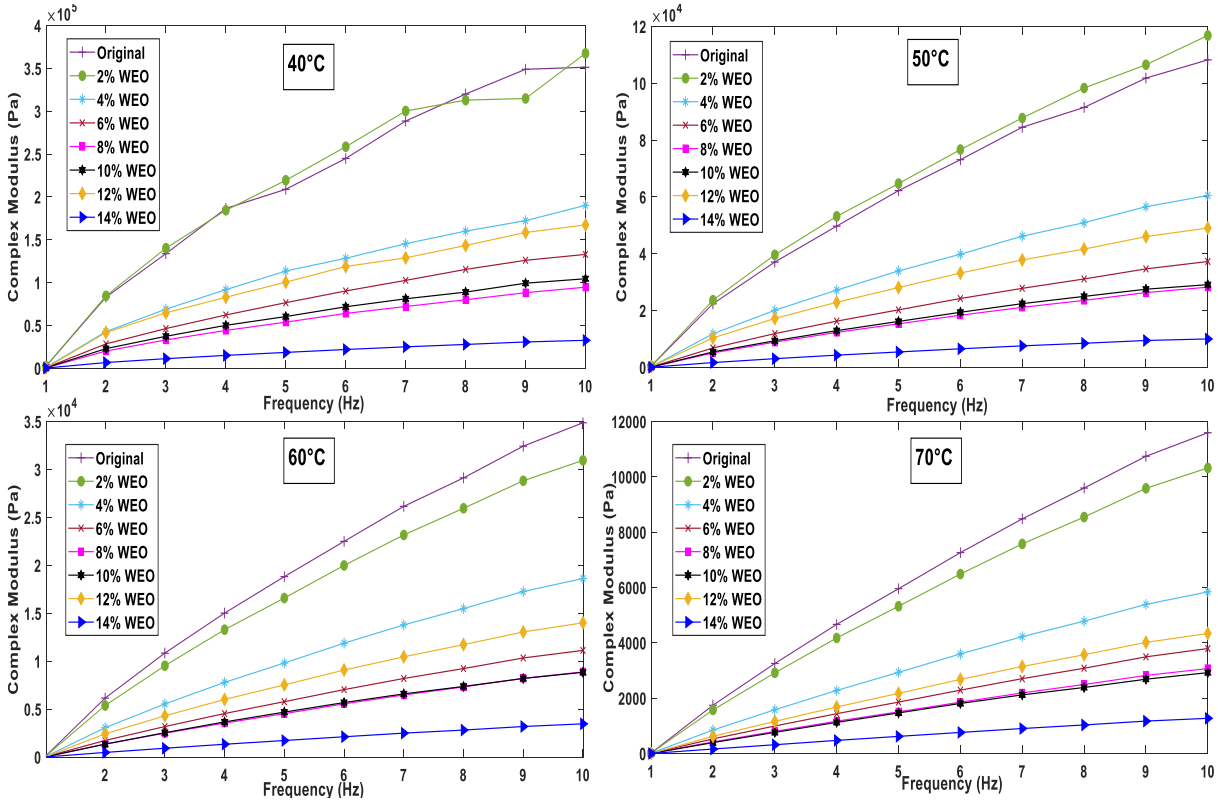

Figure 8. Complex module curves

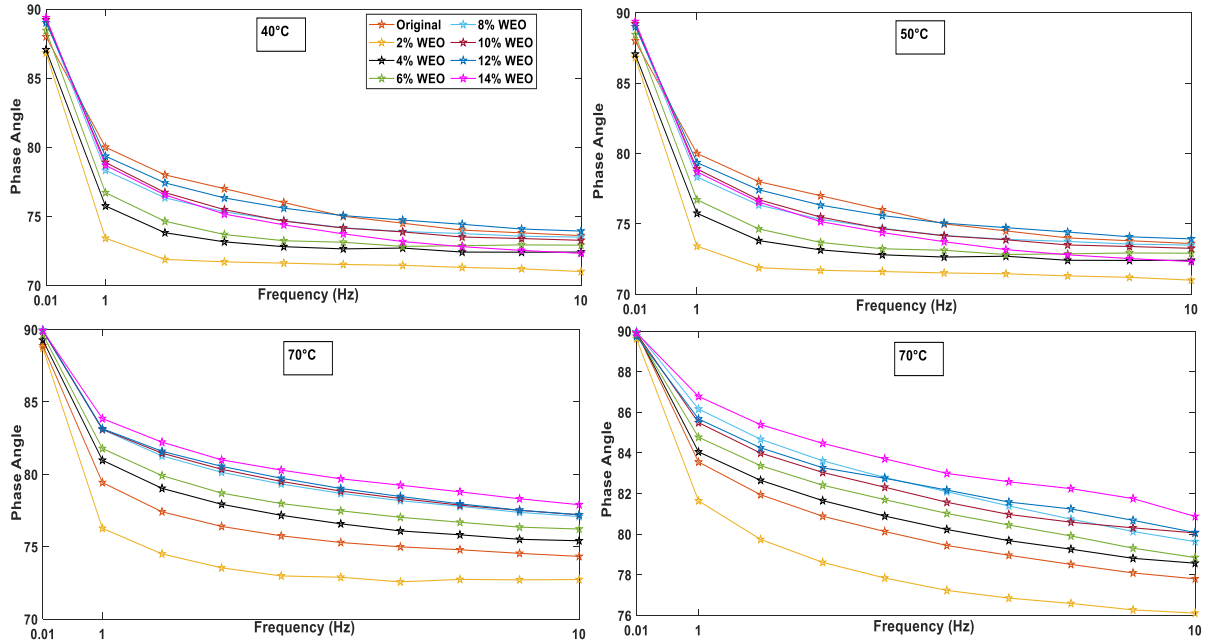

Figure 9. Phase angle curves

When Figure 8 is examined, the increase in the frequency, in other words, the loading speed, caused an increase in the complex module values. The complex modulus values of the binders generally decreased with the increase in the waste engine oil ratio. When the results corresponding to low frequency values are examined, the effect of adding engine oil is seen more clearly. However, the effect ratio decreased at higher frequencies. Original binders offered higher complex modulus values at each frequency.

Obtaining a high complex shear modulus against a low phase angle value is a measure of the elastic behavior of the bituminous binder. When Figure 9 is examined, it is seen that the phase angle values decrease as the frequency increases.

Artificial Neural Networks (ANN) Results

The regression graphs created to represent the relationship between the predicted data of both ANN models and the experimental data are given in Figure 10 for complex modulus values and Figure 10 for phase angle values.

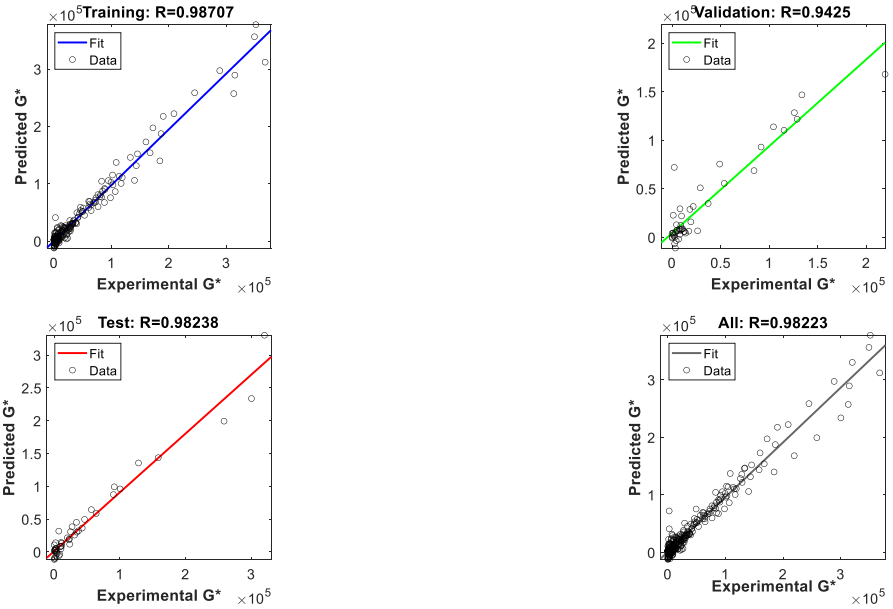

Figure 10. Relationship between experimental complex modulus data and ANN predicted data

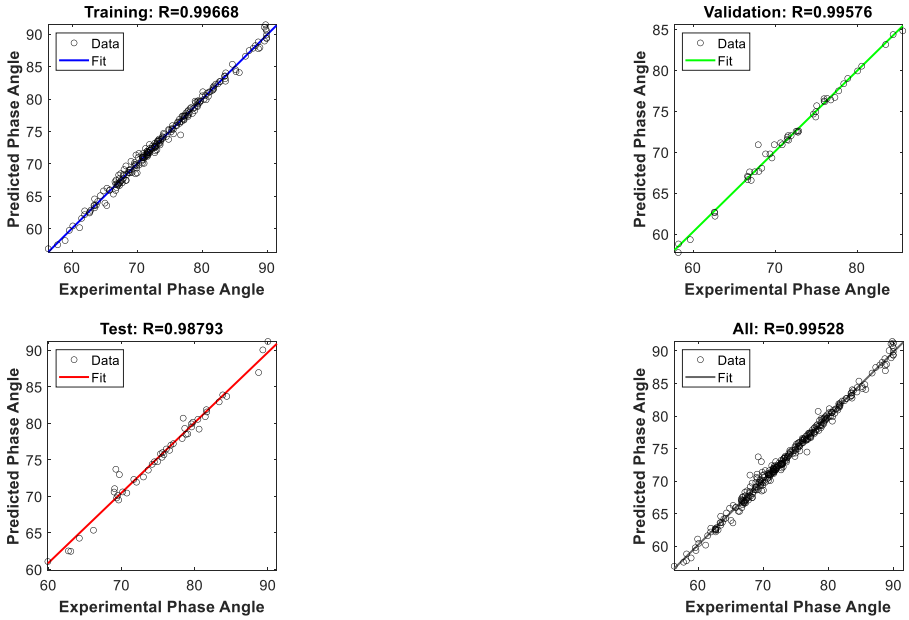

Figure 11. Relationship between experimental phase angle data and ANN predicted data

When Figures 10 and 11 are examined, it is clearly seen that both models provide high accuracy results. The correlation (R^2) between the data obtained as a result of the proposed models and the experimental data was found to be above 0.98 when evaluated overall for both models. Figure 12 was created to show how precisely the neural network model predicts output values. As the number of cycles increases, the error rate generally decreases. The training process is terminated when the error rate in the validation dataset starts to decrease so that the model is not subject to overfitting. The best performance corresponds to the number of cycles giving the lowest error rate. According to Figure 12, the error rate decreased rapidly as the network learned. When Figure 12 is examined, it is seen that the best verification performance is obtained in 25 cycles (epochs) for the

complex module and 38 cycles for the phase angle. These results show that the properties of bituminous binders can be predicted by neural networks with low error rate and in a very short time.

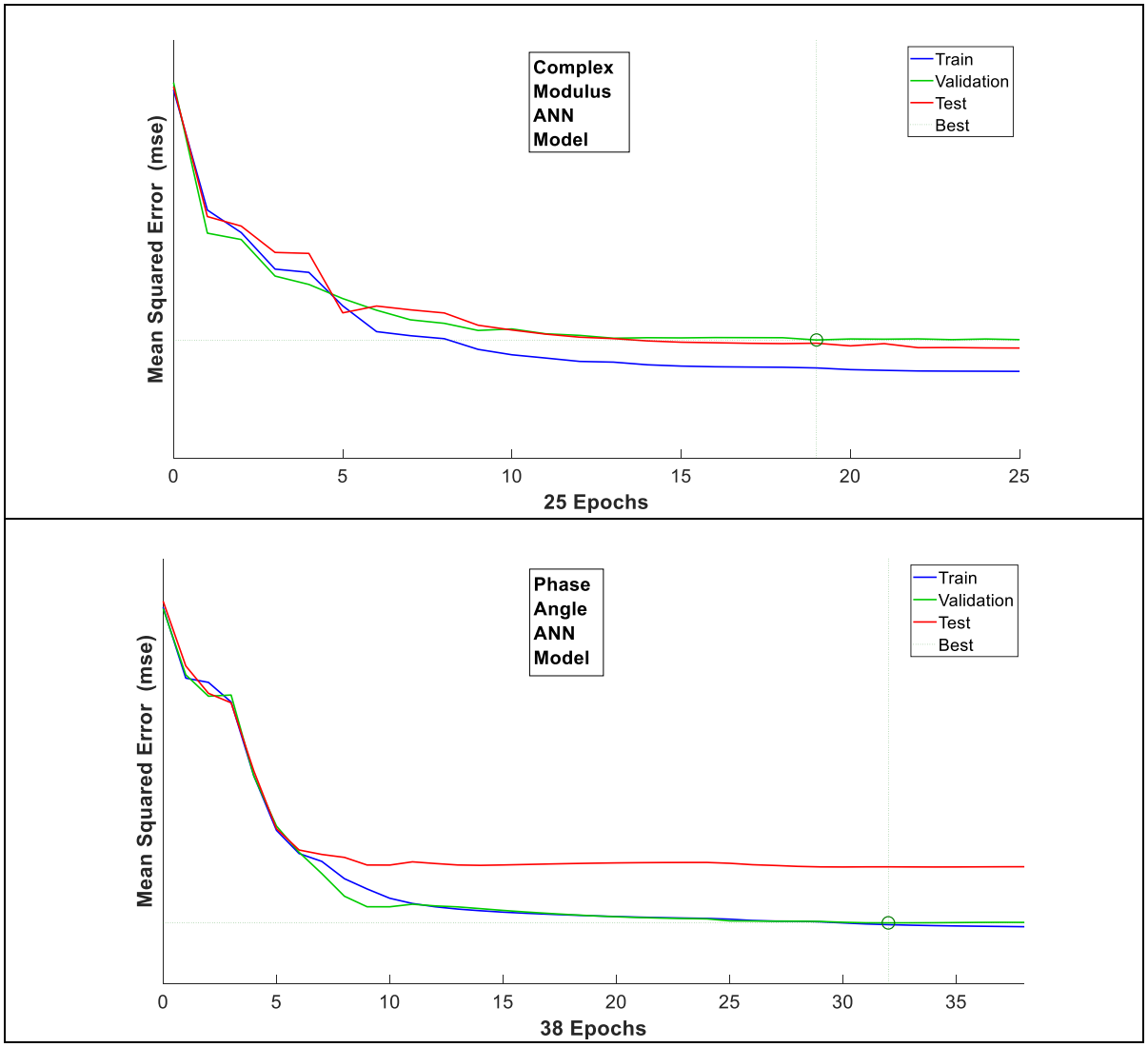

Figure 11. ANN performance plot, a) Complex modulus ANN model, b) Phase angle ANN model

Conclusions

In this study, 2%, 4%, 6%, 8%, 10%, 12% and 14% of waste engine oil that released as a result of the periodic maintenance of the vehicles were added to the original bitumen. Then, frequency sweep test was carried out on the modified bitumen samples with DSR device at four different temperatures (40, 50, 60 and 70°C) and under 10 different frequencies (0.01Hz-10Hz). The rheological parameters of the binders were estimated with the artificial neural network model and the two data sets (experimental versus predicted) were compared.

According to the results, it was determined that the complex modulus values generally decreased with the addition of waste engine oil and the increase in the additive ratio. Original binders offered higher complex modulus values at all frequencies and temperatures. This shows that the addition of waste engine oil weakens the resistance properties of bitumen against rutting and renders them weak against deformations. When the phase angle values were evaluated, the increase in the frequency values decreased the phase angle values. It is thought that the addition of waste engine oil can improve the elastic properties by increasing the phase angle values.

When the artificial neural network model results were evaluated, the ANN model created for both the complex module and the phase angle gave high accuracy results with low errors. In addition, the ANN model reached the

result quickly with a small number of iterations. As a result, it has been determined that the rheological parameters of bituminous binders can be successfully predicted by ANN. Being able to predict the experiments in the laboratory with artificial intelligence methods will provide benefits in terms of both time and material.

Scientific Ethics Declaration

The authors declare that the scientific ethical and legal responsibility of this article published in EPSTEM journal belongs to the authors.

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Effects of Nigerian Plant Gum Binder in the Optimized Multi-response Performance of Cashew Nut Shells Based Composites for Automobile Brake Pads

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Abstract: There are emerging growing needs to develop automobile brake pad materials from agro-biomass sustainable sources which are lung and environmentally friendly towards replacing asbestos which has been reported to cause other cancerous diseases. It has been established that many research works have been done towards replacing asbestos with other environmentally friendly reinforcement materials but not much has been reported on the replacement of inorganic resins like Phenolic or Formedehyde and the epoxy resins mostly used as the binder which have toxic health concerns and also known to corrode plates of brake pad assembly. Therefore, Agro-biomass such as agricultural residues, wastes and products especially from fibers and plant exudates have now emerged as the new and inexpensive materials that could be used to form parts of brake pads composite matrix that are commercially viable and environmentally acceptable. This study reveals a substitute research direction with the replacement of the inorganic resin binder with Plant Gum binder like the Nigerian Gum Arabic-NGA. Taguchi Design of Experimental method was deployed to generate twenty seven (27) trial composites of particle size 300 µm for the brake pads production. The Analysis of Variance (ANOVA) from the optimized Grey Relational Analysis indicated that the Nigerian Gum Arabic-NGA has significant effects on the multi-response performance of the developed brake pads with percentage contribution (p-value) of 18.503%.

Keywords: Brake-pad, Binder, Plant-Gum, Taguchi, Composites

Introduction

The components of brake pads in automobiles consist of composites made of reinforcement materials and some additives that a bonded together in the matrix mix. Brake pads are placed in wheel assembly to continuously clamp holding the wheels to slow down or stop a moving automobiles (Aigbodion & Agunsoye, 2010). Since over 117 years ago, the production of these automobile materials are usually made with blends of asbestos, metals and ceramics and the commonly used binders are epoxy phenol or formaldehyde resins. Many efforts in recent times of researchers is geared primarily towards replacing of the asbestos which Bala *et al.* (2016)

- Selection and peer-review under responsibility of the Organizing Committee of the Conference

reported to cause lung and other cancerous diseases. Interestingly, very few works have reported efforts towards alternative binders to replace the commonly used inorganic resins with a biomass that do not corrode any parts of the brake pads assembly. Deepika, *et al.* (2013) developed and evaluated the performance of a composite materials for wear resistance application, made use of palm kernel shells –PKS as filler material with sulphur, brass, ceramics, liquid of cashew nut shells, quartz, calcium carbonate, iron ore, and carbon black. In 1930s, Ferodo changed to thermosetting resins and produced moulded instead of knitted linings. Moulded linings were made by combining fiber with resin and polymerizing resin under elevated pressure and temperature (Deepika *et al.*, 2013). It was reported that the additive effects of different non-asbestos materials on friction lining has sensitized and increased the use of asbestos-free organic, semi-metallic and metallic friction lining materials, (Blau, 2001).

Bashar *et al.* (2012) uesd coconut shells to produce hazard free and non-asbestos brake pads experimentally. Their constituent materials included grounded coconut shells as filler, reinforced with iron chips, catalyzed with methyl ethyl ketone peroxide, Cobalt nephthanate as the accelerator, silica and iron serving as the abrasive component with brass modifying the friction. The binder used in the matrix was epoxy resin. Yawas, Aku and Amaren (2013) produced brake pads from Periwinkle shells that was characterized for its morphology and properties. The formulation of periwinkle shell powder, engine oil (SEA 20/50), water were bonded with phenolic resin (an inorganic binder).

A geological studies on kaolin clay group within the sedimentary mineral material zone of Ise-Orun-Emure local government areas of Ekiti State, Nigeria carried out by Aderiye (2014) examined, beneficiated, processed and characterized clay for automobile brake frictional materials. The study revealed kaolin clay to be of good heat resistance for friction lining material in automotive industry, and its refractoriness was not only suitable for electronic products, technical works and ceramic manufacturing industries but can also be a material used for eco-friendly and asbestos free brake pads production. Similarly, in the research work by Aderiye (2014), kaolin clay was explored, exploited and employed specifically for ceramic disk brake pads by investigating the thermal properties of kaolin samples between 1000°C to 1400^{o}C temperatures in order to ascertain their suitability for producing automobile brake pads.

Idris *et al*. (2015) investigated the production of banana peels based brake pads with phenol formaldehyde as the binder. The binder was varied from 5% to 30% weight and the physical, mechanical, wear and morphological properties of brake pad were determined. The results showed that compressive strength, hardness and specific gravity of the samples increased with increased in percentage weight of resin addition. Edokpia, *et al*. (2014) used Egg Shells (ES) based eco-friendly (biodegradable) materials to develop brake pads that was evaluated for physical, tribological and mechanical properties. Interestingly, a plant gum known as Gum Arabic (GA) was used as the binder in the mix. The study investigated the possible replacement for asbestos and formaldehyde resin which are carcinogenic in nature and non-biodegradable. The brake pad formulation was produced by varying the GA from 3 to 18 wt%. Tests carried out on samples included wear rate, thickness swelling in water and SAE oil, thermal resistance, specific gravity, compressive strength, hardness values and microstructure. Results showed that formulations containing 15 to 18 wt% of GA produced fair bonding with the sample containing 18 wt% of GA in ES particles giving the best brake lining properties.

Recent efforts by many researchers have been on to discover eco-friendly material replacement not only for asbestos constituents in brake lining pad composites but also finding an alternate agro-based biomass materials as organic binder in the composite mix that is human, ecological, economic and environmentally friendly.Brake pads are frictional components bound to the surface of brake disc in wheel assembly of automobiles to continuously clamp and hold wheels to slow down or completely stop their motion (Aigbodion *et al.*, 2010). Brake pads control the speed of moving automobile by converting the kinetic energy to thermal energy by friction and dissipating the heat produced through brake disc to the surroundings.

Therefore, this research does not only strives to address the hazardous health concerns associated with asbestos based brake pad materials by using cashew nut shells, but to present an alternative material to replace the toxic inorganic resins commonly used with plant gum binder of an acacia species exudates known as Gum Arabic. The plant binder provide a green based, natural resin that contains arabin which is a semi solidified sticky fluid oozing from incision made on bark of acacia trees. Nigeria produces different grades of exudates and is ranked as the second largest world producer after Sudan with average production of 20,000 tonnes in 2005 (Ademoh & Abdullahi, 2010).

Cashew Nut Shells

The tree called cashew botanically named *Anarcardium Occidentale* is a native of Brazil in Southern America. The tree has nuts in a kernel with a colorful cashew apple shown in figure 1, is an overdeveloped pedicel which is edible. The cashew nut (kernel) is the primary commercial product of cashew plantation as it is very appreciated as a snack and is also used in the Asiatic cuisines, especially Thai and Chinese. Cashew nuts can be processed industrially and commercially into jam, juice, syrup, chutney and beverage (Winterhaler, 1991). According to Ohler (1979) ,the world production and consumption has increased rapidly with an estimated over 1,260,000tonnes for the next twenty years from major producing countries like Nigeria, India, Mozambique, Malawi, Thailand, Tanzania, Sri Lanka, Kenya, Madagascar, Malaysia, Indonesia, Senegal and Angola. In 2010,Nigeria produced 594,000 tonnes of cashew nuts and there has been need to increase the production for the economic development in Nigeria (Olife *et al.*,2013).This pericarp of a nut which is the cashew nutshell has an alveolar mesocarp filled with a dark and caustic oily substance called cashew nutshell liquid (CNSL), obtained as a by-product during the industrial processing of the cashew nut as reported by Akinhanmi, (2008), Idah *et al.*, (2014) and Okele *et al.*, (2016).

The physical and mechanical properties of cashew nut shells and kernels have unique characteristics which set them apart from other engineering materials to be used as reinforcement material for the production of agro-based brake pad was used for this research. Okele *et al.*, (2016), Bart-Plange *et al.*, (2012), and Teye and Abano, (2012) have extensively determined these physical and mechanical properties by these researchers with favorable results to exploited.

Figure 1. Cashew pedicel, fruit, and mesocarp filled with CNSL, cashew nut and cashew kernel (Source: Lomonaco *et al*., 2017 and Bart-Plange *et al*., 2012).

Plant Gum Binder – Nigerian Gum Arabic

The plant gum known as Gum Arabic was obtained from the dried exudates obtained from stems and branches of *Acacia Senegal* wild plant belonging to *Acacia species* and the *Fabaceae* family (Tunde, 2018). It is a semi solidified sticky fluid oozing from incision made on bark or branch of acacia trees as shown in Figure 2.

Figure 2. Gum Arabic as seen on an Acacia tree

(Source: REUTERS/Mohamed Nureldin Abdallah, 2012 and www.finelib.com/, 2019)

These tree species that produces the plant gum is found in the dry, hot, and barren regions of African countries like Nigeria, Mauritania, Senegal, Mali, Niger, Sudan and Chad. Nigeria happened to be ranked the second largest producer in the World with an average production of 20,000 tonnes in 2005 after Sudan (Ademoh & Abdullahi, 2010). The major Nigerian producing states are Adamawa, Sokoto, Niger, Taraba, Borno, Yobe, Bauchi, Jigawa, Kebbi, Gombe, Plateau, Nasarawa, Katsina, and Zamfara state as shown in Figure 3. There are three major grades of Gum Arabic as shown in Figure 4 that are produced in commercial quantities.

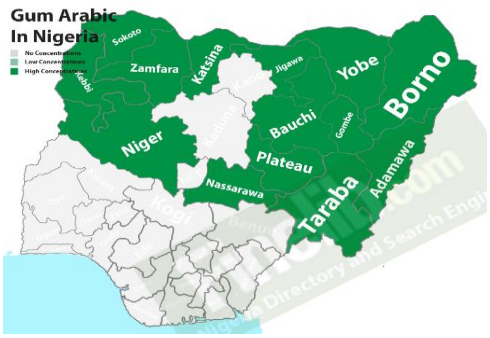

Figure 3: Map of gum Arabic producing states in Nigeria
(Source: www.finelib.com, 2019; Lawal, 2021).

Figure 4: Different grades of gum arabic (Source: Tunde, 2018).

Materials

The constituent compositions used in this study were sourced locally while the equipments in the workshop and Laboratory at the Federal Institute of Industrial Research, Oshodi, (FIIRO) in Lagos State, Nigeria were used. The base materials for the formulation and production of the composites were Cashew Nut Shell (CNS), Nigerian Gum Arabic (NGA), Steel Dust (SD), Graphite (G) and Silicon Carbide (SC). The base and other additive materials bonded with plant gum binder that were selected and their roles for their selection according to Lawal, (2021) are shown in Table 1.

Table 1. Materials selected and their roles

S/No	Materials	Role	Reason(s) for Material Choice
1	Cashew Nut Shell	Base/Filler Material	Cheaply available as Agro-allied material, rarely used and chosen to investigate its usage to replace asbestos, improve resilience in the binder system and reduces brake noise
2	Nigerian Gum Arabic	Binder	Good bindery properties, chosen for the production of brake pad as binder and to increase the chances of substantially green agro-allied (plant) based brake pad
3.	Steel Dust	Reinforcement and Abrasiveness	It is a reinforcement that influences adhesion and dispersion of polymer composite fabrication, it also has abrasive functions.
4	Silicon Carbide	Thermal Conductivity and Abrasiveness	Easy and cheap to obtain. It increases friction and also helps in controlling the build-up of friction film. That is, it effectively controls the thermal conductivity of the brake pad during usage
5.	Graphite	Friction Producer/Modifier	Cheap and widely used and it is non-hazardous for improving wet friction

The Equipment used

The equipment that were used for the study are: Hammer Crushing and Milling Machine, Ball Milling Machine, Hydraulic Press Model Piooeh-type, 100T-Capacity, Serial No 38280, Electric Oven, Europer Bench Grinder of

MD-250F, 750W, 380V-50Hz, R 29500 rpm, Ø(50-27)mm by 65mm mould, and Digital Weighing Machine. Other equipment used were BS 410 standard sieve sizes of aperture 300µm, micrometer screw gauge, Stirrer, Bowls, Optical Electron Microscope(OEM), Steel Spatula, and desiccators.

Method

The study was experimentally designed and carried out by adopting the Taguchi $L_{27}3^5$ orthogonal array Design of Experiment (DOE) for twenty seven (27) different formulations of five constituent materials (Sadiq *et al.*, 2020). The Cashew Nut Shells, Silicon Carbide and Nigerian Gum Arabic compositions were varied while the other constituents such as Graphite and Steel Dust were kept constant. The five (5) input factors (ingredients) composition is presented as percentage (%) as well as in grams in Tables 2 and 3.

Table 2. Factor levels for composition parameters in percentage

		Percentage Composition (%)		
		Level 1	Level 2	Level 3
1	Cashew Nut Shell	35	45	55
2	Steel Dust	15	15	15
3	Graphite	5	5	5
4	Silicon Carbide	20	15	10
5	Nigerian Gum Arabic	25	20	15

Table 3. Factor levels for composition parameters in grams

		Percentage Composition (Grams)		
		Level 1	Level 2	Level 3
1	Cashew Nut Shell	52.5	67.5	82.5
2	Steel Dust	22.5	22.5	22.5
3	Graphite	7.5	7.5	7.5
4	Silicon Carbide	30	22.5	15
5	Nigerian Gum Arabic	37.3	20	22.5

The experimental Layout of the $L_{27}3^5$ orthogonal array as obtained from Minitab 17 is shown in Table 4 while Table 5 is the eventual Experimental Design Matrix.

Development of the Composites

The development of the composites involved the preparation of the constituent materials into 300µm particle size and formulated into twenty seven (27) different compositions as shown in Table 5 as carried out by Lawal, (2021). The powder metallurgy method also known as the compression moulding method was successfully adopted and reported by Yawas, *et al* (2013), Fono and Koya (2013) and Bashar, *et al* (2012) were used in the development of the brake pad composites. The powdered Cashew Nut Shells was sieved into grades of 300 µm and the component materials of Cashew Nut Shells Powder, Gum Arabic Powder, the Steel Dust, Silicon Carbide and Graphite were weighed in the Digital weighing Machine correspondingly with formulations designed via Taguchi. The composition of the constituents was thoroughly mixed using Homogenizer or Mixer of Model 89.2 Rid Scale & Co Ltd, Middleborough, England.

The mixing of the composition was done for 20 to 30 minutes to achieve almost complete homogeneous mixture inside the mixer before pouring into the mould kept in a hot plate press at temperature of 150°C and 100,000N/cm^2 pressure for two minutes. They were then subjected to cold pressing and hot pressing before being allowed to cool at room temperature. After removing from hot press, the composites were removed from the mould and properly cleaned. It was then heat treated at a temperature of 120°C for 8 hours in the hot air oven. These procedures were repeated for all the twenty seven (27) formulations to produce the respective composites. Grey Relational Analysis as outlined by Yiyo *et al*, (2008) and successfully deployed by Abutu *et al*, (2018) and Lawal, (2021) was used to optimize the multi-response performance of the Wear rate, Coefficient of Friction, Hardness and Compressive Strength of the brake pad samples produced while ANOVA was used to determine the significant contribution of the Nigerian Plant Binder in the optimized performance of the brake pad samples.

Table 4. Experimental layout of the $L_{27}3^5$ orthogonal array

Trial No	CNS	SD	G	SC	NGA
1	1	1	1	1	1
2	1	1	1	1	2
3	1	1	1	1	3
4	1	2	2	2	1
5	1	2	2	2	2
6	1	2	2	2	3
7	1	3	3	3	1
8	1	3	3	3	2
9	1	3	3	3	3
10	2	1	2	3	1
11	2	1	2	3	2
12	2	1	2	3	3
13	2	2	3	1	1
14	2	2	3	1	2
15	2	2	3	1	3
16	2	3	1	2	1
17	2	3	1	2	2
18	2	3	1	2	3
19	3	1	3	2	1
20	3	1	3	2	2
21	3	1	3	2	3
22	3	2	1	3	1
23	3	2	1	3	2
24	3	2	1	3	3
25	3	3	2	1	1
26	3	3	2	1	2
27	3	3	2	1	3

Table 5. Three (3) Level $L_{27}3^5$ orthogonal array experimental matrix for the compositions

Trial No	Cashew Nut Shells CNS (Grams)	Steel Dust SD (Grams)	Graphite G (Grams)	Silicon Carbide SC (Grams)	Nigerian Gum Arabic (Grams)
1	52.5	22.5	7.5	30	37.5
2	52.5	22.5	7.5	30	30
3	52.5	22.5	7.5	30	22.5
4	52.5	22.5	7.5	22.5	37.5
5	52.5	22.5	7.5	22.5	30
6	52.5	22.5	7.5	22.5	22.5
7	52.5	22.5	7.5	15	37.5
8	52.5	22.5	7.5	15	30
9	52.5	22.5	7.5	15	22.5
10	67.5	22.5	7.5	15	37.5
11	67.5	22.5	7.5	15	30
12	67.5	22.5	7.5	15	22.5
13	67.5	22.5	7.5	30	37.5
14	67.5	22.5	7.5	30	30
15	67.5	22.5	7.5	30	22.5
16	67.5	22.5	7.5	22.5	37.5
17	67.5	22.5	7.5	22.5	30
18	67.5	22.5	7.5	22.5	22.5
19	82.5	22.5	7.5	22.5	37.5
20	82.5	22.5	7.5	22.5	30
21	82.5	22.5	7.5	22.5	22.5
22	82.5	22.5	7.5	15	37.5
23	82.5	22.5	7.5	15	30
24	82.5	22.5	7.5	15	22.5
25	82.5	22.5	7.5	30	37.5
26	82.5	22.5	7.5	30	30
27	82.5	22.5	7.5	30	22.5

All the produced brake pad samples were tested and evaluated for the physical, mechanical and tribological properties such as Microstructure analysis, Brinell hardness, compressive strength, Wear rate, (abrasion resistance), Ash content (Flame Resistance), Density Test, Specific Gravity, Swell Growth Analysis, and Oil/Water absorption.

Results and Discussion

The experimental responses for the wear rate, Compressive Strength, Coefficient of Friction and Hardness were characterized as presented in Table 6. These values were used to obtain the respective Signal-to-Noise Ratio as in Table 6, as well obtaining Grey Relational Generating, Grey Relational Coefficient and the Grade as presented in Table 7.

Table 6. Experimental responses and S/N values

Runs	Experimental Responses				Signal-to-Noise Ratios			
	Wear Rate (mg/m)	Compressive Strength (N/mm2)	Coefficient of Friction	Hardness (BHN)	Wear rate (dB)	Compressive strength (dB)	Coefficient of friction (dB)	Hardness (dB)
1	2.31	3.643	0.3868	31.83	-7.272	11.229	-8.250	30.057
2	4.24	6.714	0.4554	47.49	-12.547	16.540	-6.832	33.532
3	3.47	6.332	0.3868	31.83	-10.807	16.031	-8.250	30.057
4	0.77	8.417	0.426	76.66	2.270	18.503	-7.412	37.691
5	8.48	7.976	0.4162	47.49	-18.568	18.036	-7.614	33.532
6	4.62	5.879	0.4358	76.66	-13.293	15.386	-7.214	37.691
7	1.16	4.398	0.3966	47.49	-1.289	12.865	-8.033	33.532
8	1.54	7.138	0.426	31.83	-3.750	17.072	-7.412	30.057
9	8.48	9.863	0.4456	76.66	-18.568	19.880	-7.021	37.691
10	7.71	4.209	0.4064	76.66	-17.741	12.484	-7.821	37.691
11	6.55	6.956	0.4456	76.66	-16.325	16.847	-7.021	37.691
12	5.01	5.145	0.3868	47.49	-13.997	14.228	-8.250	33.532
13	8.48	6.365	0.475	47.49	-18.568	16.076	-6.466	33.532
14	5.78	5.509	0.4848	47.49	-15.239	14.821	-6.289	33.532
15	3.47	12.187	0.4456	47.6	-10.807	21.718	-7.021	33.552
16	8.86	4.781	0.475	31.83	-18.949	13.590	-6.466	30.057
17	6.17	7.851	0.4848	47.49	-15.806	17.898	-6.289	33.532
18	3.08	5.432	0.4064	47.49	-9.771	14.699	-7.821	33.532
19	3.85	3.568	0.4554	31.83	-11.709	11.048	-6.832	30.057
20	3.08	3.927	0.4848	47.49	-9.771	11.881	-6.289	33.532
21	8.09	3.758	0.4554	76.66	-18.159	11.499	-6.832	37.691
22	3.85	5.699	0.4554	47.49	-11.709	15.116	-6.832	33.532
23	2.7	3.099	0.4554	76.66	-8.627	9.824	-6.832	37.691
24	7.71	3.081	0.426	31.83	-17.741	9.774	-7.412	30.057
25	4.24	2.267	0.478	22.26	-12.547	7.109	-6.411	26.951
26	5.01	4.437	0.4848	47.49	-13.997	12.942	-6.289	33.532
27	2.31	6.405	0.4848	47.49	-7.272	16.130	-6.289	33.532

Table 7. Results of grey relational generating (GRG) and grey relational coefficient (GRC)

Runs	GRG				GRC				
	Wear Rate	Compressive Strength	Coefficient of Friction	Hardness	Wear rate	Compressive strength	Coeff. of friction	Hardness	Grade
X_o	1.000	1.000	1.000	1.000					
1	0.450	0.282	0.000	0.289	0.476	0.411	0.333	0.413	0.408
2	0.698	0.646	0.723	0.613	0.624	0.585	0.644	0.564	0.604
3	0.616	0.611	0.000	0.289	0.566	0.562	0.333	0.413	0.469

4	0.000	0.780	0.427	1.000	0.333	0.694	0.466	1.000	0.623
5	0.982	0.748	0.324	0.613	0.965	0.665	0.425	0.564	0.655
6	0.733	0.567	0.528	1.000	0.652	0.536	0.514	1.000	0.676
7	0.168	0.394	0.111	0.613	0.375	0.452	0.360	0.564	0.438
8	0.284	0.682	0.427	0.289	0.411	0.611	0.466	0.413	0.475
9	0.982	0.874	0.627	1.000	0.965	0.799	0.572	1.000	0.834
10	0.943	0.368	0.219	1.000	0.898	0.442	0.390	1.000	0.682
11	0.876	0.667	0.627	1.000	0.802	0.600	0.572	1.000	0.744
12	0.767	0.487	0.000	0.613	0.682	0.494	0.333	0.564	0.518
13	0.982	0.614	0.910	0.613	0.965	0.564	0.847	0.564	0.735
14	0.825	0.528	1.000	0.613	0.741	0.514	1.000	0.564	0.705
15	0.616	1.000	0.627	0.615	0.566	1.000	0.572	0.565	0.676
16	1.000	0.444	0.910	0.289	1.000	0.473	0.847	0.413	0.683
17	0.852	0.739	1.000	0.613	0.771	0.657	1.000	0.564	0.748
18	0.567	0.520	0.219	0.613	0.536	0.510	0.390	0.564	0.500
19	0.659	0.270	0.723	0.289	0.594	0.406	0.644	0.413	0.514
20	0.567	0.327	1.000	0.613	0.536	0.426	1.000	0.564	0.631
21	0.963	0.301	0.723	1.000	0.931	0.417	0.644	1.000	0.748
22	0.659	0.548	0.723	0.613	0.594	0.525	0.644	0.564	0.582
23	0.514	0.186	0.723	1.000	0.507	0.380	0.644	1.000	0.633
24	0.943	0.182	0.427	0.289	0.898	0.379	0.466	0.413	0.539
25	0.698	0.000	0.937	0.000	0.624	0.333	0.889	0.333	0.545
26	0.767	0.399	1.000	0.613	0.682	0.454	1.000	0.564	0.675
27	0.450	0.618	1.000	0.613	0.476	0.567	1.000	0.564	0.652

The summary of GRA-Grade results along with their corresponding factor levels are shown in Table 8.

Table 8. Summary of GRA-grade values and factor levels

	Experimental Factors					GRA-Grade values
Run	CNS	SD	G	SC	NGA	300 μm
1	1	1	1	1	1	0.408
2	1	1	1	1	2	0.604
3	1	1	1	1	3	0.469
4	1	2	2	2	1	0.623
5	1	2	2	2	2	0.655
6	1	2	2	2	3	0.676
7	1	3	3	3	1	0.438
8	1	3	3	3	2	0.475
9	1	3	3	3	3	0.834
10	2	1	2	3	1	0.682
11	2	1	2	3	2	0.744
12	2	1	2	3	3	0.518
13	2	2	3	1	1	0.735
14	2	2	3	1	2	0.705
15	2	2	3	1	3	0.676
16	2	3	1	2	1	0.683
17	2	3	1	2	2	0.748
18	2	3	1	2	3	0.500
19	3	1	3	2	1	0.514
20	3	1	3	2	2	0.631
21	3	1	3	2	3	0.748
22	3	2	1	3	1	0.582
23	3	2	1	3	2	0.633
24	3	2	1	3	3	0.539
25	3	3	2	1	1	0.545
26	3	3	2	1	2	0.675
27	3	3	2	1	3	0.652

Factor Levels of Main Effects

The factor effects for 300 µm shown in Table 9 were obtained using the Grade values from GRA as presented in Table 8.

Table 9. Resulting factor effects of experimental factors (300 µm)

Factor level	CNS	SD	G	SC	NGA
Level 1	0.5758	0.5909	0.5739	0.5944	0.5790
Level 2	0.6656	0.6470	0.5647	0.6421	0.6522
Level 3	0.6131	0.6166	0.6410	0.6050	0.6234

Main Effect Plot

The main effect plots for GRA shown Figure 5 was plotted for the optimal compositions for the multi- response properties and characterization.

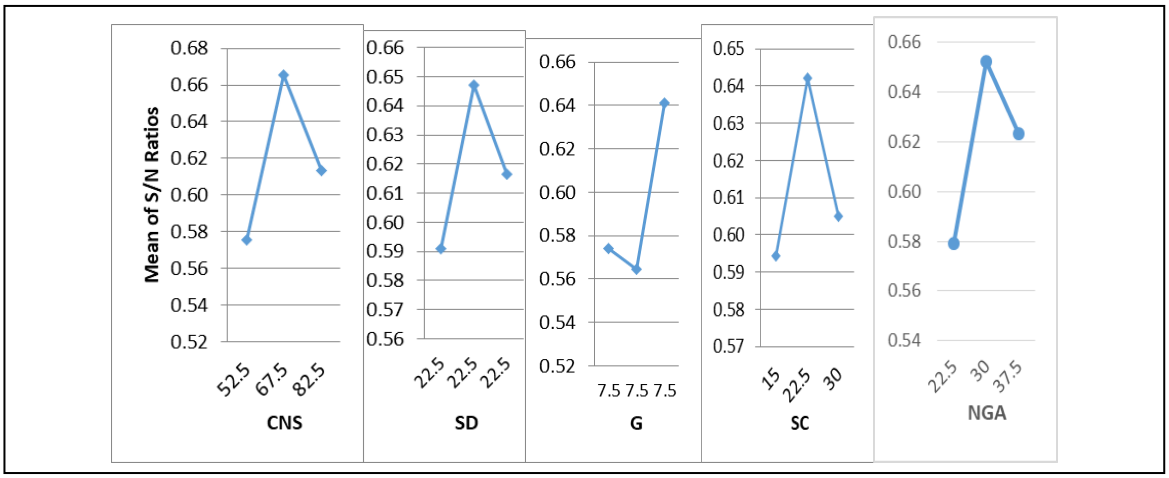

Figure 5: Main effect plot for Grey relational analysis

From the Main Effect Plot in Figure 5, the optimal composition in grams of the formulation of Cashew Nut Shells (CNS), Steel Dust (SD), Graphite (G), Silicon Carbide (SC), and Nigerian Gum Arabic (NGA) are 67.25, 22.5, 7.5, 22.5 and 30grams respectively.

Analysis of Variance (ANOVA)

ANOVA was conducted using the multi-response GRA and Grade values with the aim of identifying the significant effects of the Plant Gum Binder which affect the quality characteristics of the composite. This analysis was conducted using $\alpha = 0.05$ significance level, at 95 % confidence level. These results are shown in Tables 10.

Table 10: ANOVA for Grey relational analysis

Factor	DOF	SS	MS	F	P
CNS	2	0.069	0.034	15.197	22.467
SD	2	0.046	0.023	10.233	15.129
G	2	0.058	0.029	12.921	19.102
SC	2	0.040	0.020	8.774	12.972
NGA	2	0.057	0.028	12.516	18.503
Error	16	0.036	0.002		11.827
Total	26	0.306	0.012		100.000

The ANOVA 300 µm shown in Table 10 indicates that the CNS has the highest significant effects on the multi-response performance of the developed composites with percentage contribution (p-value) of 22.467 %

Conclusion

From the results obtained the following conclusions are drawn: Agro-biomass of Cashew Nut Shells and Plant Gum Binder of Nigerian Gum Arabic are alternative replacement materials for Asbestos and inorganic resins such epoxy, Phenolic and Formaldehyde respectively. The optimal composition of the formulation for the multi response performance of 300μm particle sizes of Cashew Nut Shells (CNS), Steel Dust (SD), Graphite (G), Silicon Carbide (SC), and Nigerian Gum Arabic (NGA) are 67.25, 22.50, 7.50, 22.50 and 30.00 respectively. The Significant Contributions of the Nigerian Gum Arabic in the Mix for the multi response performance is 18.50%.

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Scientific Ethics Declaration

The authors declare that the scientific ethical and legal responsibility of this article published in EPSTEM journal belongs to the authors.

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Extraction and Purification of the Potential Allergen Proteins from Botryotinia Fuckeliana

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Abstract: An allergy is a disease in which the immune system makes an inflammatory response to a harmless antigen. Any antigen that causes an allergy is called an allergen. Allergens may be inhaled or ingested, or they may come into contact with the skin. According to the data of the World Allergy Organization (WAO), the prevalence of allergies in different countries varies between 10-40%. Pollen, mold, animal hair, house dust mite, medicines, and foods are the most common allergen agents. Common mushrooms in nature have the potential to produce allergenic proteins. *Penicillium,Botryotinia, Aspergillus, Rhizopus,* and *Mucor* species, which are allergic fungi, are widely found in nature. In recent years, the cases of allergies caused by molds have increased significantly and studies to determine the causing allergens have accelerated. *Botryotinia fuckeliana* Pers. is a cosmopolitan necrotrophic pathogen infecting more than 200 plant species in temperate and subtropical climates, some of which are of high economic importance (e.g., grapes, strawberries, solanaceous vegetable). In Europe, maximal spore counts occur during late summer. Prevalence of skin test reactivity, i.e., immediate wheal and flare, to B. cinerea in Europe is comparable to that of Aspergillus spp. and greater than that of Cladosporium and Penicillium spp. Since the prevalence of *B. fuckeliana* spores and skin test reactivity is significant, further characterization of these allergens is warranted. *Botryotinia fuckeliana* was used in our study. *Botryotinia fuckeliana* produced in our laboratory was collected and allergen fungus protein was extracted by 2 different extraction methods. By preparing protein samples from prepared mushroom extracts, the total concentration of potential allergen proteins was determined by the BCA method. According to the data obtained, it was determined that the protein concentration of the mushroom samples dried by that were subjected to dialysis was higher than ethanol.

Keywords: Allergy, *Botryotinia fuckeliana*, Allergen protein, BCA

Introduction

Allergies develop when a person's immune system overreacts to substances that are usually harmless. The first time a person is exposed to an allergen, they do not usually experience a reaction. It often takes time for the immune system to build up a sensitivity to the substance. In time, the immune system learns to recognize and remember the allergen. As it does so, it starts making antibodies to attack it when exposure occurs. This buildup is called sensitization. Allergic diseases usually affect the skin and mucosal tissues such as sinuses, lungs, and intestines (Tao & Raz, 2015).

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According to the data of the World Allergy Organization, the prevalence of allergies in different countries varies between 10-40% (Pawankar, 2011). It is possible to encounter allergic diseases seasonally or throughout the year. Seasonal allergic reactions are caused by fungal spores, pollen, insecticides, indoor and outdoor mold fungi, house dust, and animal hairs that persist throughout the year (Şimşekli, 1994). In recent years, many researches have been carried out in the field of aeromicology, which is very popular in the world, sports calendars of many cities in our country and abroad have been published, and atmospheric sports concentrations are announced and announced to the public through meteorological bulletins (Çeter & Pınar, 2008).

Pollen, fungi, and house dust mites are the most common allergens. Fungi or fungal spores that can be found in the other environment can hang in the environment for a long time due to the effect of the airflow from their location (Simon-Nobbe et al., 2008). Mushrooms, which are among the most harmful organisms for humans, are equally useful organisms due to their use in different areas such as the decay of organic substances in the ecosystem, the production of species consumed as food, and the development of biotechnology, such as the synthesis of biofuels, enzymes and drug active substances (Kendrick, 2000; Esch, 2017). Mushrooms, which have the most species after insects, have the potential to produce allergenic proteins. These organisms, which have a wide distribution area, are estimated to constitute more than 90% of the biomass in the world (Kendrick, 2000).

Airborne fungal spores occur widely and often in far greater concentrations than pollen grains. Immunoglobulin E-specific antigens (allergens) on airborne fungal spores induce type I hypersensitivity (allergic) respiratory reactions in sensitized atopic subjects, causing rhinitis and/or asthma. The prevalence of respiratory allergy to fungi is not known with certainty, but is estimated to be between 20% and 30% of atopic (allergy-prone) individuals or up to 6% of the general population.

Diagnosis and immunotherapy of allergy to fungi require well-characterized or standardized extracts that contain the relevant allergen(s) of the appropriate fungus. Production of standardized extracts is difficult since fungal extracts are complex mixtures and a variety of fungi are allergenic. Penicillium, Aspergillus, Rhizopus, and Mucor species, which are allergic fungi, are widely found in nature. In our country, it has been reported that 614 patients with respiratory tract allergies develop allergic reactions against *Aspergillus fumigatus*, *Trichophyton rubrum*, Mucor, Penicillium notatum, *Aspergillus niger*, and *Alternaria tenius* (Güneser et al., 1994).

In connection with allergy and asthma, the typical fungal genera investigated are Cladosporium, Alternaria, Aspergillus and Penicillium, probably because they are very often the most prevalent genera in ambient air. However, the diversity of species can be rather high, both within the 2–4 most prevalent genera and in less prevalent general (Lugauskas et al., 2003). For example, 100 genera containing 359 species when investigating the fungal composition indoors. Furthermore, many fungal species from at least 80 genera, have been shown to have allergenic potential. One of these fungi present in ambient air is *Botryotinia fuckeliana*.

Botryotinia fuckeliana, an airborne necrotrophic fungus that attacks more than 200 plant species, causes gray mold disease of many economically important crops, including vegetables, ornamentals, bulbs and fruits (Plesken et al., 2015a).

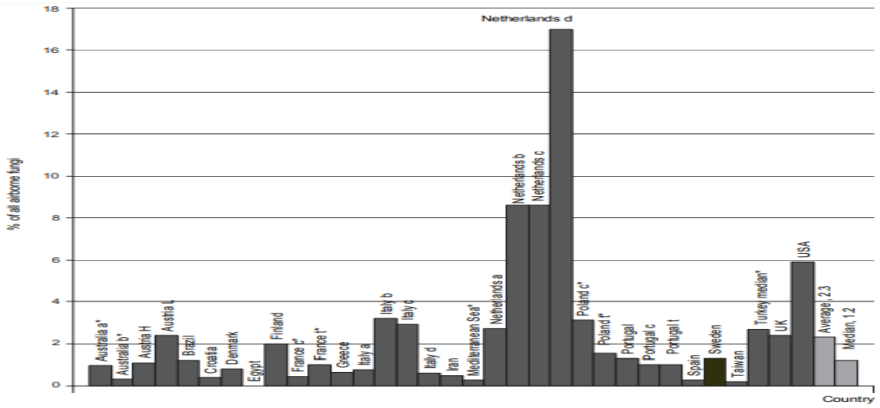

Figure1. Prevalence of outdoor *Botryotina* in percent of all airborne fungi (black bars).

Method

Preparation of *Botryotinia fuckeliana* Extracts

The mushroom samples used in our study were purchased commercially and reproduced in our laboratory. *Botryotinia fuckeliana* spores, which were cultivated on PDA medium, were left to incubate for 7-14 days at +25°C. It was collected after morphological examination by staining with cotton blue. The resulting mushrooms were treated in chloroform-methanol and ethanol on a magnetic stirrer for 24 hours. The dried mold samples were digested in PBS and dialyzed. Protein concentrations were determined by extracting the obtained extracts into %5 SDS Buffer.

Determination of Total Protein Concentration

The total protein concentration of the mushroom extracts was made using the bisinconic acid (BCA) method proposed by Smith et al. (1985). Commercially purchased BCA Macro Assay Kit (Serva Electrophoresis GmbH) was used to determine protein concentration. BCA analysis was performed following the protocol suggested by the manufacturer (Walker, 2002).

Figure 2. BCA working diagram

Results and Discussion

In our study, proteins of *Botryotinia fuckeliana*, one of the allergenic fungi, were extracted with 2 different extraction protocols. The amount of extracted proteins was measured by the BCA method. In the protocol of the mushroom extracts prepared in the study using ethanol, sufficient protein amount could not be obtained and it was observed that it was not a suitable protocol for our study. In the protocol using chloroform-methanol, the amount of protein was determined as 0.660 mg/mL. Similar results were obtained with the amount of protein stated in previous studies in chloroform-methanol extraction (Wójcicka, 2014).

Table 1. Total protein concentration values of *Botryotinia fuckeliana* extracts measured by BCA assay

Allergen Name	Protein Concentration (mg/mL)
Botryotinia fuckeliana (Ethanol)	----------
Botryotinia fuckeliana (chloroform-methanol	0,660

Recommendations

In recent years, allergy cases caused by molds have been increasing. For this reason, studies to determine the allergen proteins of fungi commonly found in nature have gained importance. In our study, protein concentrations were determined by preparing *Botryotinia fuckeliana* extracts, which is one of the allergen fungi and used in allergen kits. The data obtained from this study form the basis for the production of alternative domestic kits to imported kits used in the diagnosis and treatment of allergy patients with advanced studies.

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Assessment of Benthic Free-Living Nematode Assemblages Diversity in Kune – Vain Wetland (Adriatic Coast, Albania)

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Abstract: This work presents preliminary data on benthic free-living nematode assemblages from Kune-Vain Wetland Complex, situated in the northern part of the Adriatic coast in Albania. The study was carried out during a joint ecological approach, to collect chemical-physical and biological data of the wetland and evaluate the hydrological intervention in improving the environmental situation and increase resilience to climate change. The sampling was conducted bimonthly from July 2018 – July 2019, in three main water body component of Kune-Vain wetland, Ceka, Zaje and Merxhani. Nematode assemblages were analyzed in term of composition and structure, determining taxonomic composition up to genus level and biological traits. Nematodes were the dominant component of meiobenthos in all three water bodies, comprising 98-100%. Their taxonomic composition reflected the chemical features of transitional habitat dominated by brackish genera. Genera composition revealed different diversity patterns among water bodies. The most dominant genera were Sabatieria and Terschellingia, both deposit feeders and characteristic of bad ecological quality status.

Keywords: Adriatic coast, Wetland, Meiobenthos, Nematode assemblage diversity, Biological traits

Introduction

The study on nematode assemblages it is the first of its kind in Kune- Vain wetland and among the very few in Albania. In faunistic studies, free-living nematodes are considered permanent members of meiobenthos, fulfilling whole their lifespan in benthic environments and in meiofaunal body size-range. Until lately, the study of meiobenthic communities has been neglected in Albania, thus skipping the study of many benthic organisms with a ubiquitous distribution and high abundances, founding in all aquatic environments and often dominant of benthic communities both in numbers and species richness (Baguley et al., 2019). Study of nematode assemblages was carried out during a joint ecological approach and the aim was to collect chemical-physical and biological data of the wetland and evaluate the hydrological intervention in improving the environmental situation and increase resilience to climate changes, part of Long-Term Monitoring and Research Strategy for the *ecosystem-based adaptation (EBA)* and climate change adaptation interventions.

Ecological Assessments Based on Nematode Assemblages

Meiobenthic organisms play important ecological roles, contributing in the cycling of the minerals and nutrients (Baguley et al., 2019) which are fundamental processes in ecosystems maintaining. They occupy several trophic levels, flowing the energy from primary productions to higher trophic levels of macroscopic consumers (Coull,

1990). With the advancement of multi-disciplinary scientific studies on dynamics and functioning of aquatic ecosystems, meiobenthic researchers have addressed their studies in evidences of biological responses for environmental monitoring approaches and providing data for management purposes. Recently, meiobenthos organisms have received an increased attention in monitoring and evaluating impact of factors that disturb the natural states of sedimentary environment (Radziejewska, 2014). Meiobenthos communities exhibit biological features addressing hydrological regime shifts (Netto & Fonseca, 2017), anthropogenic impacts and climate changes (Zeppilli *et al*., 2015). Analyses of nematode assemblages provides information on succession and changes in decomposition pathways in the soil food web, nutrient status, fertility and acidity of soil, and the effects of soil contaminants (Bongers & Ferris, 1999). Nematodes are considered as one of the most appropriate groups that best fulfills the characteristics of the indicator organisms (Wilson & Kakouli-Duarte, 2009) and recently are proposed as suitable for ecological quality evaluation of marine ecosystems according to the Water Framework Directive (WFD) (Moreno et al., 2011). As biological indicators in environmental assessments, they express the ecological state with the incidence of certain nematode species with biological traits adapted to environment conditions. In disturbed environments they are still present, but with a simplified structure and low diversity, both taxonomic and functional.

Study Area and Characteristics

Kune-Vain wetland is located in the north part of Albania, in Lezha district, along Adriatic coast, extended in both sides of the estuary of River Drini of Lezha (Fig. 1). This wetland represent the first protected area in the history of nature protection in Albania as a Hunting Reserve in 1960. Kune-Vain is the most important wetland in northern Albania, with a high socio-economic importance, extending over an area of more than 30 km^2, of which 11 km^2 are covered with water. The wetland consists of two main sites: Kune and Knalla wetland in the northern part of Drini Delta, with Merxhani lagoon as the most important aquatic water body, and Vaini wetland in the south. Ceka lagoon consists of two main parts, Ceka in the south and Zaje in the north and bordering with Drini riverbanks. Ceka and Zaje are separated by a land belt. They communicate between them through two artificial underground channels. Zaje also communicates with Drini River by some artificial channels. Ceka lagoon communicate with the sea with a channel which represents the old Drini riverbed. Ceka lagoon is also fed by freshwaters by some wells and a pumping stations contributing with a freshwater input. Merxhani lagoon communicate directly with the sea through a channel in central part, which allows a good water exchange with the sea. The above watery bodies show up a mean depth of 0.7 m and a maximum depth of 1.3 m. (Miho et al., 2013).

Material and Method

The study of nematode assemblages was performed in three lagoons of the wetland: Ceke, Zaje and Merxhani, with one station in each lagoon (Fig. 1, Tab. 1). The sampling was carried out bimonthly, from July 2018 to May 2019. Sediment samples were collected using a manual corer with a diameter of 5 cm, with a depth penetration to the sediment of 5cm, collecting a sediment volume of 98 cm^3 from a surface of 19.6 cm^2. From each sampling site were collected three replicates to minimize the impact of patchy patterns distribution characteristic of meiobenthic organisms in small scale related with the availability of food resources.

Table 1. Sampling sites and characteristics

Sampling site	Depth (m)	Latitude	Longitude
Ceka	0.8	41°43'40.00"N	19°35'16.15"E
Zaje	0.7	41°44'53.95"N	19°34'43.72"E
Merxhani	0.6	41°45'34.82"N	19°35'49.25"E

Sample Processing

Sampling sediments were preserved in 4% in buffered formaldehide water solution. Later in the laboratory was proceeded with the extraction of organisms from the sediment, using a sieve of 42 μm mesh size for retaining the organisms. The organisms collected by the sieve were preserved again in solution of 4% formaldehyde and stained with Rose Bengal. All the meiobenthic organisms were counted and classified according to taxon under a stereomicroscope. The meiofaunal densities were expressed as number of individuals/10 cm^2, as a unit suitable in meiobenthic studies. After counting and sorting of meiobenthic organisms, about 50 nematodes/sampling site were randomly picked up and transferred to a series of ethanol – glycerol solution for

the dehydration (Vincx, 1996) and then mounted in permanent microscope slide for inspection at high magnification under the microscope. Nematodes in the permanent slides were identified up to genus taxonomic level according to World Database of Nematodes – NeMys (Bazerra et al., 2021) and using the pictorial keys Platt and Warwick (1983), (1988); Warwick et al., (1998); Weiser (1954); Weiser (1959); Schuurmans-Stekhoven (1950). Nematode identification was performed under a light microscope Motic BA 310 equipped with a digital camera 1/2" COMOS 3MP-2048x1536 pixels with USB 2.0 output.

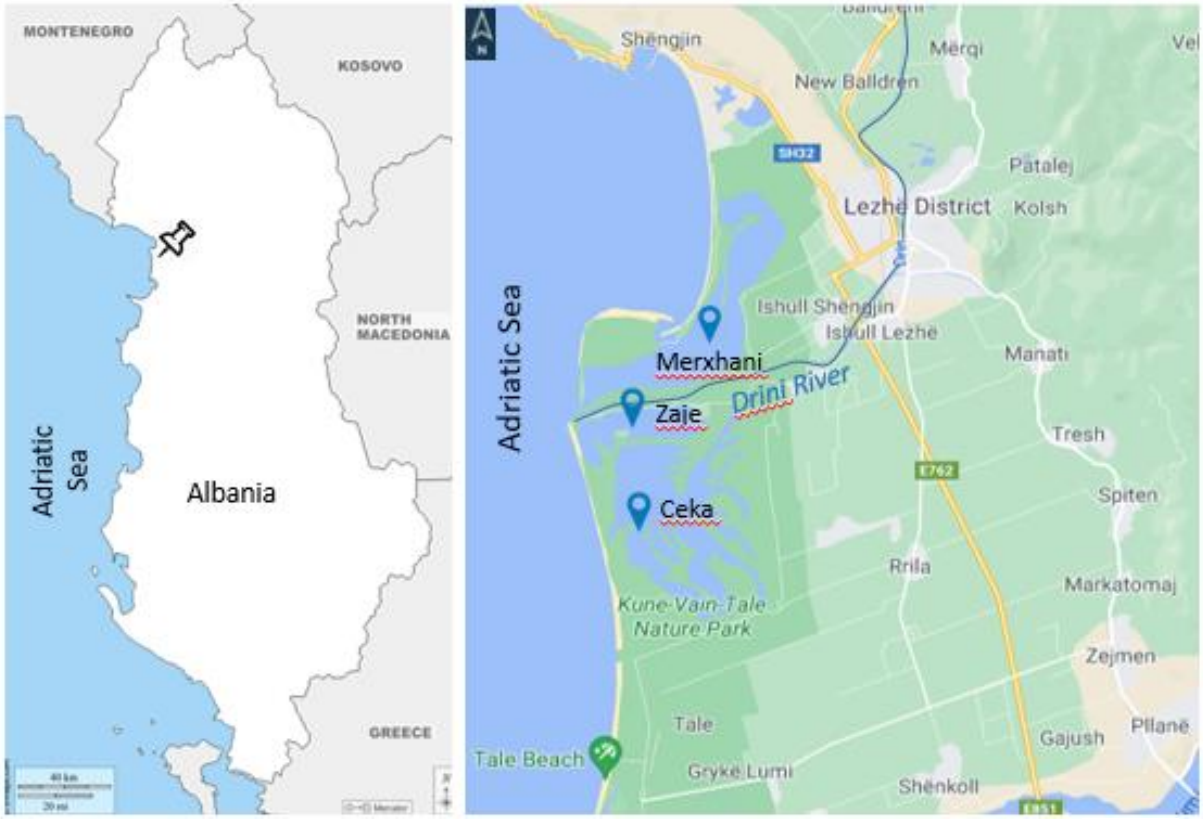

Figure 1. Geographic position of Kune - Vain wetland and location of sampling sites (Map sources: https://d-maps.com; https://maps.google.com)

Biological traits and functional diversity of nematode assemblages

After taxonomic analysis, for each identified genus was determined habitat affiliation based on data in the World Database of Nematodes; trophic group based on oral morphology according to Wieser (1953, 1959); and ecological values of c-p (c-colonizer; p-persisters) based on the colonizing and competitive abilities of the genera according to Bongers (1990) and Bongers *et al*., (1991). Nematodes were classified into five habitat affiliation from marine to terrestrial; into four trophic groups: 1A- selective deposit feeders; 1B- non-selective deposit feeders; 2A- epistrate feeders; 2B- predators/omnivores: and into five c-p (colonizer- persisters) ecological classes: c-p 1 colonizers; c-p 2 tolerant; c-p 3 moderate; c-p 4 sensitive to stress; c-p 5 persisters (Tab. 2). Dates on trophic groups and c-p values on genus level are applied respectively in the estimation of Trophic Diversity Index (ITD) (Heip *et al*., 1985) and Maturity Index (MI) (Bongers, 1990; Bongers *et al*., 1991) according to formulas: ITD = $\Sigma\theta^2$, where, θ represent the ratio between the density of each trophic group of nematodes and the total density of nematodes and $MI=\sum v(i)*f(i)/n$, where, $v(i)$ is the c-p value of the taxon i (in this case genus level) and $f(i)$ is the frequency of that taxon. The values of Trophic diversity Index vary between the values 0.25-1, where the value 0.25 represents the largest trophic diversity, when each of the four trophic groups accounts for 25% of the abundance of nematodes; value 1 represents the lowest trophic diversity, when a trophic group constitutes 100% of the abundance of nematodes. Maturity Index estimates the changes in the structure of nematode populations during ecological succession and environmental conditions based on the principle that different taxa have different sensitivities to environmental disturbances. Its low value reflects stressed and disturbed environmental conditions.

Results and Discussion

Faunistic analysis of meiobenthic communities revealed a poor diversity, showing representatives of only four major groups where nematodes appeared as the most abundant taxa. Density of nematode assemblages ranged from 20 – 195 ind./10cm^2. 644 individuals of nematodes mounted in microscopic slides were subject of taxonomic study up to the genus level. Other microscopic slides are still in process. Totally, in all studied area and period up to now, 14 nematode genera were recorded, representing two classes, 6 orders and 13 families. The most diversified class was Chromadorea, with 4 orders, 8 families and 11 genera. The most diversified order was Monhysterida, with 5 genera representing 3 families. Family Xyalida was represented by three genera, Axonolaimidae by two genera and the rest of the families were represented by one genus only. The assemblages were quantitatively dominated by class Chromadorea (95%), with the most abundant orders Monhysterida (52.%), and Araeolaimida (38.%). The species of those orders are recognized as species inhabiting both freshwaters and marine habitats. Monhysterida and Araeolaimida orders are both dominated significantly by one genus each, respectively, Terschelingia and Sabatieria, each accounting over 35% of the all identified nematodes. The aquatic water bodies shared between them only 4 genera, Sabatieria, Terschelingia, Theristrus and Chromadorina.

Table 2. Habitat affiliation, feeding type, c-p value of the indentified genera and their relative frequency according the lagoons

				Relative frequency (%)		
Genus	Habitat	FT	c-p	Ceka	Zaje	Merxhan
Ascolaimus	Marine	1B	2	0	0	0,8
Odontophora	Marine	IB	2	0	0	0,8
Sabatieria	marine, brackish	1B	2	2,4	56,9	62,4
Camacolaimus	marine, brackish	2A	3	0	1,7	0
Chromadorina	marine, brackish, fresh	2A	3	9,4	1,7	1,6
Marylynnia	Marine	2A	3	2,4	0	0.8
Terschellingia	marine, brackish, fresh	1A	3	59,8	20,7	20,0
Sphaerolaimus	marine, brackish	2B	3	0	5,2	0
Paramonhystera	Marine	1B	2	6,3	0	0
Daptonema	marine, brackish, fresh	1B	2	0	0	3,2
Theristus	marine, brackish, fresh, terrestrial	1B	2	18,9	3,4	2,4
Viscosia	marine, brackish, fresh	2B	3	0	0	4
Anoplostoma	marine, brackish, fresh	1B	2	0	6,9	0,8
Eudorylaimus	fresh, terrestrial	2B	2	0	3,4	2,4

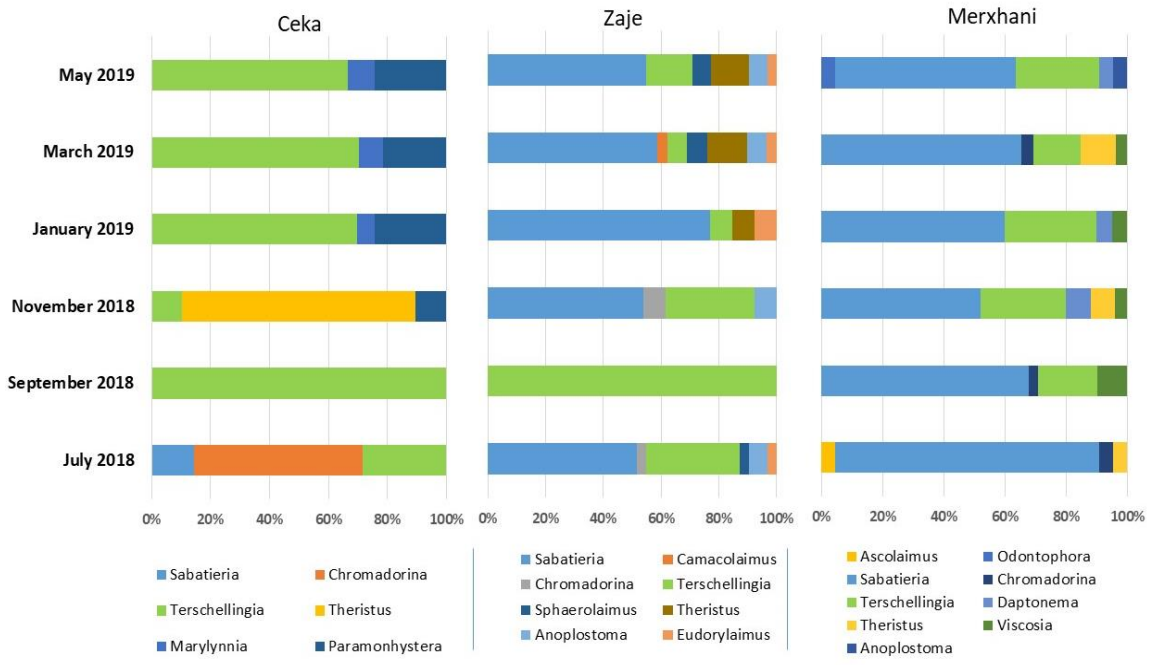

Figure 2. Genus composition according lagoons and sampling months

Merxhani represented the highest number with 9 genera, followed by Zaje with 8 genera, and Ceka with 6 genera. Merxhani and Zaje lagoons were significantly dominated by the same genus, Sabatieria, accounting about 62% and 57%, followed in both by Terschelingia, accounting about 20% and 21% respectively. Differently, in Ceka, the nematode assemblages were significantly dominated by Terschelingia, with 60% of identified nematodes in this lagoon, followed by Theristrus. Whereas the Sabatiera was the least present, less than 3% of the identified nematodes in this lagoon (Tab. 2).

Figure 2 shows the genus composition according sampling months and lagoons. In this figure the same tendency of genus dominance is observed for each lagoon. The figure indicate the same tendency of gender dominance in different sampling months according the lagoon. In Ceka lagoon, the genus Terchellingia dominates significantly in four sampling. In two other sampling months there is an increase of genus Chromadorina and a 100% dominance of Theristrus, respectively in July 2018 and November 2018. In Merxhani lagoon, the genus Sabatiera dominates significantly in all sampling, with a relative frequency over 50% throughout the sampling period. Also, genus Sabatieria dominates significantly throughout the sampling period in Zaja, with the exception of September 2018, dominated 100% by Terschellingia.

Genus habitat affiliation

Nematode assemblages in Kune-Vain lagoon complex represented free-living genera encountered in all possible habitats from marine to terrestrial (Tab.2). Relative frequency of genera according to their habitat affiliation highlights the link between taxonomic composition and ecosystem habitat far as transitional water represent areas between the land and the sea, which are partly in saline due to their proximity to coastal waters and influence of freshwater flows. The most frequent are the genera encountered into three different habitats, *marine, brackish* and *freshwater*, followed by those encountered in *marine* and *brackish* habitats only. The lower frequency represented by the genera encountered only in marine habitats, indicates the possibility of entering of marine nematodes in the lagoon via sea but failing colonization in brackish habitats.

During the whole study, the water in Merxhani, Ceka, and Zaje fluctuated respectively within limits of Euhaline, polihaline and mesohaline saline classes. Nematode assemblages according lagoons reflected the differences in their salinity. In Ceka lagoon are encountered genera of all habitat affiliation, followed by 4 habitat affiliation in Merxhani and habitat affiliation in Zaje. Ceka and Merxhani are dominated by *marine- brackish* genera, whereas Zaje is dominated by *marine-brackish-freshwater* genera (Fig. 3).

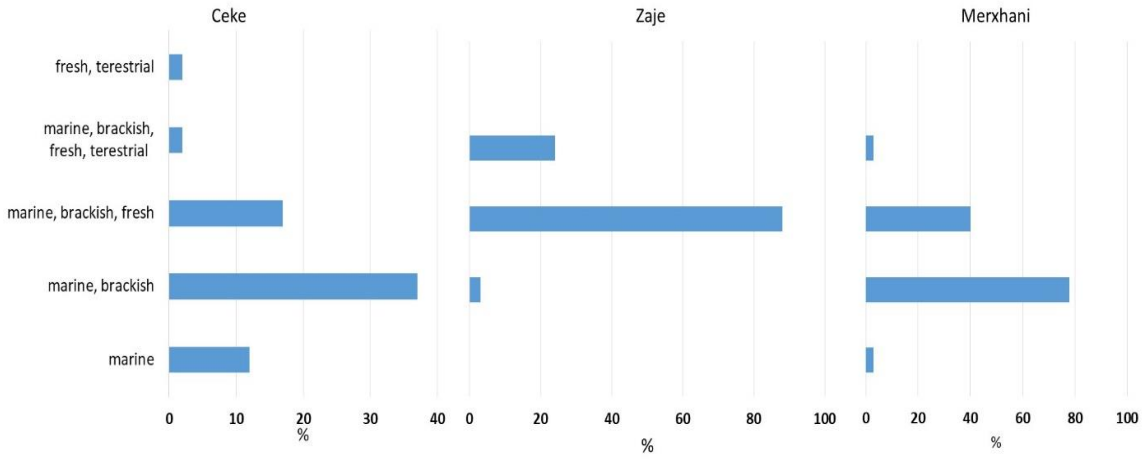

Figure 3. Relative abundance of genera according habitat affiliation

Biological traits and functional diversity

All four trophic groups have been observed (Tab. 2), but with a pronounced dominance of two trophic groups. Zaja and Merxhani lagoons are dominated by the same trophic group, IB- Non selective deposit feeders, followed by trophic group IA- Selective deposit feeders. In Ceka lagoon we have an inversion of these two groups, with significant dominance of trophic group IA. The predatory / omnivore trophic group is the least represented group in Zaje and Merxhan and is absent in the Ceka (Fig. 4).

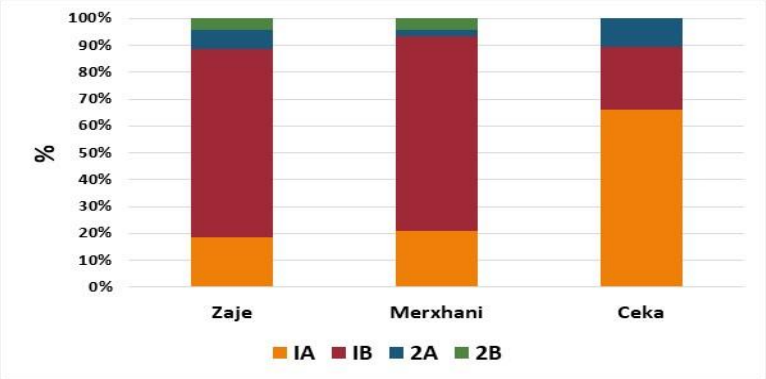

Figure 4. Relative frequency of trophic groups

All genera present in the study belonging only into two c-p classes: c-p 2 and c-p 3 (Tab.3). Zaje and Merxhani were dominated significantly by genera with c-p 2 value, whereas Ceka were dominated my genera with c-p 3 value. The dominance of the genera with c-p 2 is indicative of "r" reproductive strategies, where most of the energy is used for reproduction. Table 3 presents the values of the Maturity and Trophic Diversity Indexes and their implementation in the evaluation of ecological quality status (EQS) based on thresholds proposed by Moreno et al., (2011).

Table 3. Maturity and Trophic Diversity Indexes (Moreno et al., 2011).

Index	Zaje	Merxhani	Ceka
MI	2,26	2,22	2,77
% c─p 2	59.1	72.1	23,5
% c-p 4	0	0	0
ITD	0,53	0,57	0,5
EQS	Poor	Poor	Moderate

Conclusion

This paper brings the first data on the diversity of nematode assemblage in the Kune-Vain wetland and among the few in Albania, contributing to the knowledge of the fauna and biological diversity of aquatic ecosystems in our country and in providing biological data for use in environmental assessments and developing management plans of these ecosystems.The taxonomic composition of nematodes reflects the characteristics of trasitional ecosystems, dominated by genera with brackish habitat affiliation and biological features adapted to mud sediments.

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Scientific Ethics Declaration

The authors declare that the scientific ethical and legal responsibility of this article published in EPSTEM journal belongs to the authors.

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Evaluation of Cytotoxic Activities of Disinfectants on Human Healthy Cells

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Abstract: The Covid-19 pandemic, including our country, has affected 34 million people around the world and caused high death rates and still is. One of the most important points in this process is disinfection, and both surface and air disinfectants have been used frequently. Disinfectants, which are extremely diversified in the market, have started to create danger while protecting us from the virus. It is known that the disinfectants used prevent the contagion of the virus, but we do not have clear information about whether they have destructive effects on the healthy cells of the people who use them. Therefore, in our study, it was aimed to determine the effects of hand disinfectants produced by various brands on our healthy cells. In our study, first of all, the most widely used disinfectants were obtained from the market, and then the cytotoxic (lethal) activity of these disinfectants on healthy cells was determined by in vitro MTT ((3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) analysis. As a result of this study, it was observed that apart from the disinfectant containing hypochlorous acid, other disinfectants have cytotoxic activity against healthy human cells. Despite the increasing need during the pandemic process, we recommend that these disinfectant substances, which are put on the market in large numbers and in a wide variety, should be inspected and necessary measures should be taken so that they can be sold after passing through the quality control stages.

Keywords: Disinfectant, Cytotoxic Activity, Toxicity, Covid-19

Introduction

For the last 1.5-2 years, we, as humanity, have been struggling vitally with Covid-19, which is a pandemic epidemic. This new type of coronavirus, which emerged in Wuhan, the capital of the Hubei region of China, on December 1, 2019, was named SARS-COV-2 because it causes an atypical pneumonia and does not respond to drugs and various vaccines. It has been determined that the virus, which can be transmitted from person to person, has increased in the transmission rate in mid-January 2020. Then, virus cases started to be reported in Europe, North America and Asia-Pacific, and a global epidemic was declared by the World Health Organization (WHO) on March 11, 2020. Since then, there have been 195,886,929 confirmed cases of COVID-19, including 4,189,148 deaths, reported to WHO. Coronavirus, which is the causative agent of upper respiratory tract infection, is usually transmitted by close contact with infected people. The route of transmission is mostly through the respiratory route. Today, although vaccination and vaccine production studies continue, social

isolation and personal protection measures against these viruses still maintain their importance. Studies have shown that Covid-19 is sensitive to disinfectants (Beşirbellioğlu, 2007). For this reason, we have given disinfectants an important place in our lives in order to protect against the virus. Recently, these disinfectants are available everywhere we can reach, including our homes, market entrances, pharmacies, hospitals, public transportation vehicles, and their use is quite common. The frequency and misuse of disinfectants has reached a level that threatens human health. So much so that acute and then chronic upper respiratory tract diseases and skin disorders, especially in children, have reached significant levels due to excessive use of disinfectants. While disinfectants used as aerosols increase asthma and allergic reactions; skin disinfectants cause irritation, allergies and eczema. When the studies in the literature are examined, it is seen that there are many clinical cases (Chen, 2020).

Disinfection; It is the whole of the processes carried out to remove microorganisms and prevent contamination in case of suspected presence of disease-causing microorganisms in the environment (Abbasoglu, 2007). Disinfectants; These are chemical substances used for places where pathogenic microorganisms are or suspected to be found, and for devices or materials that may be a source of contamination (Öztürk, 2002). A good disinfectant should be large enough to act against bacteria, viruses, protozoa and fungi. It is also important that it is not corrosive. It should have an antimicrobial effect at low concentrations, be effective at normal temperatures, and should not cause structural defects in the applied area (Metin and Öztürk, 1995; Mısırlı, 2009). Disinfectants are available in different types as high, medium and low level disinfectants according to the active substances in their composition (Favero and Bond, 1991). Disinfectants that eliminate most bacterial spores and kill microorganisms are in the group of high-level disinfectants. In long-term contact with bacterial spores, the spores die, but it should not be used unless necessary due to its toxic effect on human health at the cell and DNA level. This group includes glutaraldehydes (2-3%), hydrogen peroxide (6%), formaldehyde (3-8%), sodium hypochlorite, peracetic acid (0.23%), orthophthalaldehyde (0.55%) (Sagripanti and Bonifacino, 1999; Alev, 2014). Intermediate disinfectants are chemicals that do not affect bacterial spores, but are effective against mycobacteria, non-enveloped viruses and other microorganisms. Those in this group are effective on tuberculosis bacillus and other microorganisms within 10 minutes. Generally, the composition of medium-level disinfectants; ethyl alcohol (70%), isopropyl alcohol (60-90%), phenol compounds (0.4-5%) and iodophor (30-50 ppm free iodine) form (Sagripant and Bonifacino, 1999). Low-level disinfectants, which are considered safer for humans; Although it inactivates vegetative bacteria, some fungi and viruses in 10 minutes, it has no effect against endospores of microorganisms and tuberculosis bacillus. Ethyl alcohol, phenol, iodophor, quaternary ammonium compounds can be given as examples of the compounds in these disinfectants (Sagripant and Bonifacino, 1999). From this point of view, we have planned to reveal the cytotoxic effect, which is the underlying cause of the clinical cases seen. The disinfection process and the content of disinfectants are of fundamental importance in these applications. Depending on the ingredients, the extent of the damage to human health can also vary.

Disinfectants, which have become an indispensable part of our lives due to the Covid-19 pandemic, are chemical products used to purify surfaces with pathogenic microorganisms, and these products are expected to have high efficacy but also not have any side effects. In the fight against Covid-19, disinfectants, which have come to the fore to protect against the virus, have been used in a highly diversified and uncontrolled way to meet the need. Therefore, our concerns about the use of disinfectants have also increased. While these products have a negative effect on the viability of the virus, how do they affect our healthy cells? In this study, which we designed to answer this question, which is important for our health, it was aimed to investigate the cytotoxic effects of disinfectants, which are widely sold in the market, on healthy cells.

Method

Supply of disinfectants

4 different brands of disinfectants sold in the market and frequently preferred were purchased. First disinfectant igredient Hypochlorous acid, second has ethyl alcohol and ammonium derivates, the third one contains isopropyl alcohol and chlorhexidine digluconate (Table1).

Cell culture

In this study, human umbilical vein endothelial cells (HUVEC) cell lines were used (obtained from Gaziantep University, Department of Biology). Cells were grown in Dulbecco's Modified Eagle Medium (DMEM)

medium containing 10% Fetal Bovine Serum (FBS), 1% L-glutamine and 1% penicillin/streptomycin in an incubator at 5% CO_2 and 37°C. The morphological structures of the produced cells were observed daily under an inverted microscope.

Table 1. The main ingredients of the disinfectants used in the study

Dis 1 (HOCl)	Dis 2 (EtDiAl)	Dis 3 (IsoChl)	Dis 4 (EtIso)
Hypochlorous acid	Ethyl Alcohol Didecyldimethylammonium Chloride Alkyldimethylbenzylammonium Chloride	Isoprophyl Alcohol Chlorhexidine Digluconate	Ethyl Alcohol Isoprophyl Alcohol

Cytotoxic Activity Assay

MTT ((3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) viability test, which is one of the enzymatic test methods used in the study, is a simple, colorimetric and economical method used to measure cell viability, proliferation or cytotoxicity. Viable cells in a cell population can be detected colorimetrically and quantitatively by the MTT method.This method is based on the principle of fragmentation of the tetrazolium ring of MTT.Formazan formation is only seen in living cells with active mitochondria.This is considered a marker of cell viability and crystals The absorbance values determined spectrophotometrically by dissolving it are associated with the number of viable cells.For the MTT test, HUVEC cells were seeded with 5×103 cells in each well of the 96-well cell dishes and incubated for 24 hours at 37oC in an environment containing 5% CO2.At the end of the incubation, disinfectants were applied to the cells in 5 different in concentration (1, 1/2, 1/4, 1/8, 1/16) have been added. After 15 hours of incubation, 40 µl of MTT dye was added to each well and the cells were incubated for another 4 hours. To solubilize the formazan crystals formed as a result of incubation, 80 µl of DMSO was added to all wells. Then, the color intensity formed in the cells was measured in a spectrophotometer (Thermo Scientific, USA) at a wavelength of 570 nm, and their absorbance was taken. In the experiment, each concentration was repeated in three independent wells (Mossman, 1983). The values read in the spectrophotometer were calculated with the % vitality equation and graphed.

% Viability = [100× (Mean of compound-treated cell absorbance / non-medicated control cell (MO) viability)

In this equation, the viability of the untreated control cell (MO) was assumed to be 100%, and the viability rates of the sample-treated cells were calculated.

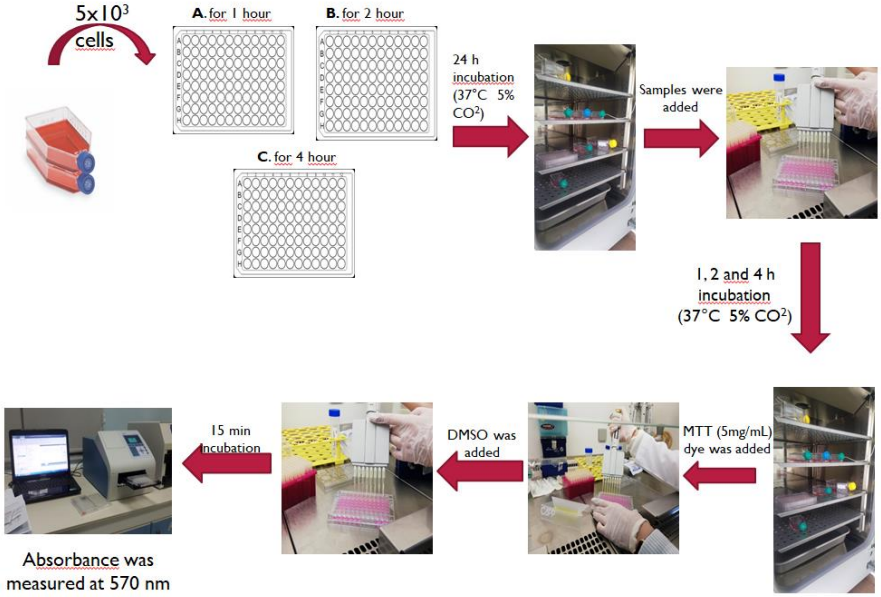

Figure 1. The overall scheme depicting the perform of the MTT assay

Results and Discussion

According to our findings, it was observed that all of the disinfectants with the material in the study had a cytotoxic effect on healthy cells (Figure 2). When the cytotoxic activity results were examined, the group containing ethyl alcohol and ammonium derivatives, which is the second group, showed a serious toxic effect for healthy cells at 3 different hours. In fact, at the end of the 4th hour, all of the cells were dead. Although chlorhexidine and isopropyl alcohol in the 3rd group showed less lethal effect in the first hour at low doses, the viability still decreased to 60%. The situation in the 4th group containing isopropyl and ethyl alcohol is similar to the 3rd group. In the first group, it was observed that the cells remained viable at all concentrations and at all hours. It has been determined that hypochlorous acid in this group does not have a toxic effect on our healthy cells.

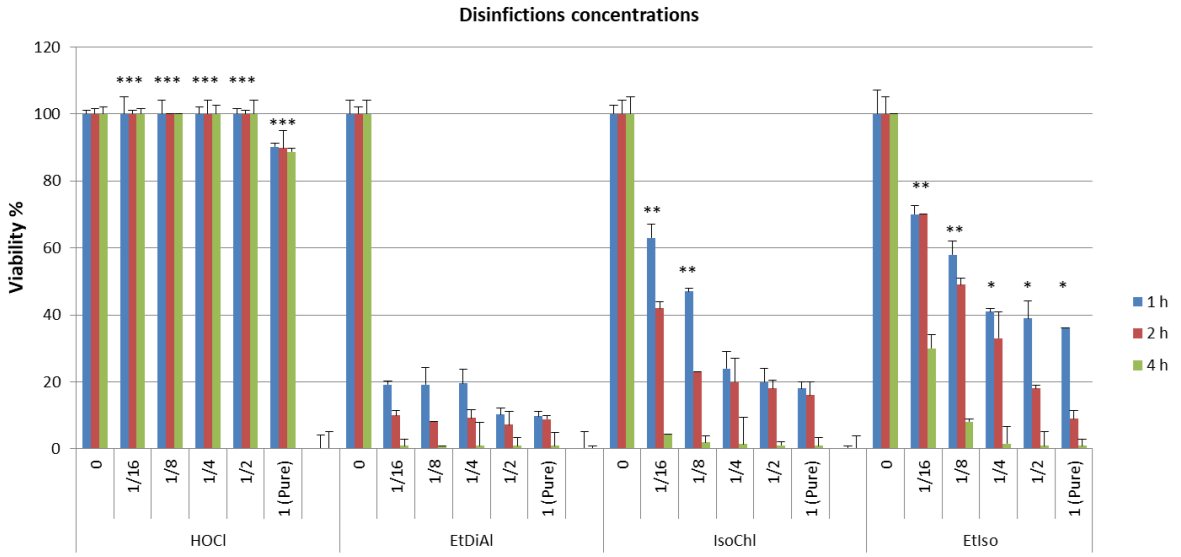

Figure 2. Disinfectants concentration-cell viability on HUVEC cell line

In addition to social isolation in the prevention of COVID-19 disease, the widespread use of masks and disinfectants in the society raises some questions. The fact that disinfectants, which we consider as friendly, are chemical products and contain very harmful components, brings to mind that these products can be harmful to health (Dindarloo et al., 2020). These products, which help us to be protected from viruses, also have the potential to threaten our cells. In this respect, it is very important to observe the effects of disinfectants at the cell level. In this study, it was aimed to observe whether disinfectants obtained from different brands have cytotoxic effects against human umbilical cord vessel endothelial cells, which are healthy cells. According to the data obtained in this study, it is seen that 4 different disinfectants can show lethal results on healthy cells. Commercial forms of these substances, which we frequently use to provide protection against disease-causing agents, have strong cytotoxic activity and therefore may have harmful effects on the human body. No significant reduction in lethality was observed even when diluted at the concentrations sold. This makes us doubt the reliability of disinfectants. When the literature data is examined, it is seen that skin irritation is observed in people who are exposed to disinfectants a lot, and disinfectants trigger asthma disease (Su et al., 2019).

In addition, 50% inhibition on human, monkey and mouse cells in a previous cytotoxicity study with liquid disinfectants (Sagripanti and Bonifacino, 2000) showed that our study also supports the literature. There is information in the literature that alcohol-based disinfectants cause irritant contact dermatitis and allergic contact dermatitis (Wilhelm, 1996; Ale and Maibach, 2014; Tan and Oh., 2020), ammonium salt derivatives cause contact dermatitis and cause lung damage (Ruiz et al., 2011). Also its known that Chlorhexidine Digluconate triggers oxidative stress and organelles damages (Ohnuma et al., 2011). Despite this, it is known that HOCL inactivates viruses, bacteria, endospores, and fungi and is safe for human tissues (including eye, lung, and skin) (Rasmussen et al., 2017). It should be noted that hocl applications have already been approved by FDA for eyelid infection management (Stroman et al., 2017), skin (Abu-Soud et al., 2014), and cosmetics (Lai et al., 2020) with repeated daily exposure for a set period of time.

Conclusion

The non-toxic property of the hocl substance on healthy cells is quite remarkable. While this chemical, which has lethal activity against viruses, can be described as quite safe, other disinfectant chemicals have been found to be harmful to human health.

Recommendations

We think that this study contributes to the development of measures to be taken against COVID-19. Also we believe that the study of HOCl in a dose-dependent manner and with different healthy cell lines should be elaborated, and its effectiveness on corona virus should be evaluated.

Scientific Ethics Declaration

The authors declare that the scientific ethical and legal responsibility of this article published in EPSTEM journal belongs to the authors

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Selecting Image Processing Supplier for Quality Control AI Software in Metal Cataphoretic Painting Firm by Using Fuzzy AHP and Fuzzy Topsis

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Abstract: Nowadays many companies try to adopt Industry 4.0 technology in their production lines, supply chains, etc. Industry 4.0 makes a company more profitable and automatized. There are many beneficial impacts of Industry 4.0 in companies unless there is a wrong application. Companies usually buy Artificial Intelligence software and they implement applications for them. The selection of the supplier is the most important part of applying this technology in a company. The application of Industry 4.0 in companies starts with defining the issue or eligible ways of developing. After AI solution is selected, the next phase is defining needs, budget and materials. All phases depend on the supplier. There are many qualitative and quantitative criteria in selecting the supplier of quality control AI software and this causes a lot of complicated processes. As Industry 4.0 is more popular globally, there is a significant increase in supplier numbers. When companies need to select a supplier, they want the selection process simpler and more accurate. Uncertainty in the future makes supplier selection more difficult. Therefore, Fuzzy AHP and Fuzzy TOPSIS methodologies are proper and when companies deal with uncertain situations. As a case study, Karakaya86 firm wants to implement Image Processing technology with Artificial Intelligence in their Quality Control processes for cataphoretic painting metal to the surface, and the study shows which supplier will fit with their needs for quality control defect software by using Fuzzy AHP and Fuzzy TOPSIS. We have not seen any paper similar to our study.

Keywords: quality control defect detection, chemistry, artificial intelligence, image processing, supplier selection, fuzzy topsis, fuzzy ahp

Introduction

Cataphoretic painting is an important technological improvement for painting and protecting parts from outer impacts. For controlling the quality of the painted part, it must be inspected visually after coating. There are different defects may be done in the painting process such as roughness, crates, redissolutions, dirt, streaking, etc. Every type of defect needs a specific variety of actions (Bračun & Lekše, 2019), so that, quality control is a difficult process to automate for having complicated processes to identify all types of defects. So, it generally makes this process manual and human required with a huge amount of time and less reliable results. To automate the quality control phase needs visual inspection systems and computerized identification. Although improvements are applied easily in production processes, they can not be applied as easily in quality control processes for being complicated and mutable. Hence, companies want to work with the best supplier. However,

in competitive markets there are a lot of similar alternatives, and sometimes seeing differences between them is not easy as thought. Instead of selecting a supplier with instinctive approach, selecting with well-known methods provide the highest potential. In this circumstance, Karakaya86 wants to implement image processing technology in their quality control processes for eliminating manual processes and human errors. In this case, Fuzzy AHP and Fuzzy TOPSIS will be applied for selecting the best image processing supplier.

Purpose of Thesis

In the cataphoretic coating sector, there is no important research about selecting a supplier for implementing image processing to the quality control process. This thesis aims to define the most important criteria and uses them with the fuzzy logic to obtain accurate decision.

Cataphoretic Coating

Cataphoretic painting is called different in different countries such as e-coating, CED (Cathodic Electro Deposited) coating, cathodic dip painting (CDP), electro coating, and cataphoretic coating, and German based publication described as KTL coating (Kathodische Tauchlackierung, which means cathodic dip painting). (Almeida et al., 2003; Fedel et al., 2010). This method can be applied to any electrically conductive surface. This technological painting method makes huge differences in the metal coating industry. Hylák states that, "Cataphoretic painting is considered as a tremendously economical method of corrosion protection for metals in these times. The concept of a special organic coating method is a technology that produces an electrophoretic coating on parts, and it is known by effective at small dimensions and complex geometric shapes. Coating by electrophoresis is based on the electrodeposition of the particles in the solution of the paint by the electric current. The difference in potential between the anode and the cathode creates the conditions where the surface is covered with a thin coating of the excluded paint." (Hylák, 2019). This well-known method is significantly important for protecting parts from environmental impacts and prevent defects made by them. Cataphoretic coating is used by numerous industries to coat products in different kinds of categories such as, agricultural equipment, automobiles, automotive parts, marine components, metal office furniture, lawn & garden equipment and furniture, and much more.

Cataphoretic Coating Process

Cataphoretic coating has several steps that should be done properly. Sometimes different processes can be added to customize the final product. These steps are: pretreatment which contains acid and phosphate baths, rinsing the excess chemicals, cataphoretic coating, oven, and quality control. The whole process needs nearly fifteen baths depending on the product and required conditions.

Cataphoretic Coating Quality Control

Cataphoretic coating has different quality control processes, first cataphoretic process quality control must be done before pretreatment for controlling products' condition such as dirt and shape. Another quality control process can be done after the rinsing processes. The other quality control process is finished products' controlling. The traditional quality control process is carried out manually for detecting finished products' defects. There are a significant variety of defects that may be found in this process, such as craters which have 1mm length bowl shaped depressions, randomly distributed small pinholes, dirt and rinsing based defects, air entrapment, gloss variations, thin coating, and orange peel. This method may lead to human mistakes and errors, and this evaluation is subjective, depends on employees.

Image Processing

Image processing has different varieties of use cases, such as checking for presence, object detection, localization, measurement, differentiation, identification, and verification. The information which is coming from the camera is not directly processed in applications. Firstly, the quality of an image should be preprocessed for enhancing image quality. For instance, noise reduction, brightness, and contrast enhancement are

preprocesses for increasing image quality. After an image has been restored, the processing starts. Arithmetic and logical operations are used for detecting and differentiating the image.

Figure 1. Dirt based defects after cataphoretic coating.

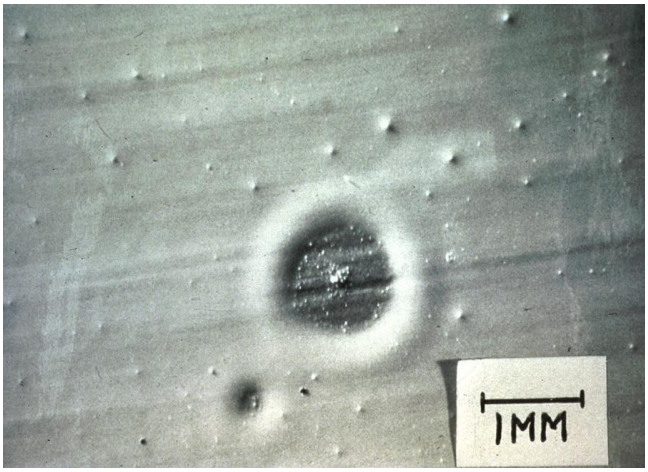

Figure 2. Crater defects after cataphoretic coating.

Figure 3. Pinhole defects after cataphoretic coating.

There are some specific solutions for image processing in cataphoretic coating. Coating defects are generally differentiated with the different lighting conditions. There are no important differences in pigmentation. Thus, the system should take images in different lighting conditions and be considered as one image. The information obtained from the last image gives specific features about the finished part. Artificial intelligence algorithms should be in this part for learning new defects (Bračun & Lekše, 2019).

Supplier Selection

Selecting of the supplier is trending in the competitive markets for choosing the best alternative for the company's needs. Selection is based on creating criteria and measures to evaluate differences between alternatives. Multi criterion decision making is important for evaluating criteria and alternatives. In this case, decreasing alternatives makes the selection process simpler (Kahraman et al, 2003).

Generally creating criteria is based on the performance of supplier and product, cost criterion, reputation, and expertise. This process needs an important amount of knowledge, insights, and analysis. After creating criteria, there should be an evaluation of criteria by experienced professionals. Once the criteria importance's is determined alternatives should be evaluated. Important objectives in the supplier selection process are minimization of purchase risk and cost, maximization of the efficiency of supplier, and performance.

Method

Fuzzy Sets Theory

Fuzzy sets were introduced to find out how to deal with human thought's uncertainty, vagueness, and subjectivity in 1965 by Zadeh (Zadeh, 1996). After that theory, uncertainties became essential to science and this theory shows researchers how to deal with uncertain situations (vague, inconsistent, imprecision). Zadeh introduces a theory whose values are sets with the upper and the lower limit and the outcome is in that boundaries instead of binary values. This theory also allows attribute quantitative values to linguistic, qualitative, variables. For instance, there is no single quantitative value that defines exactly the term young or old. Young and old words depend on the humans thought, some of them prefer young as a 20 and some others 30. When we evaluate the young word with the fuzzy set, young may be considered between 20 and 30 (Klir & Yuan, 1995). Fuzzy Set Theory uses fuzzy logic and creates a new concept which is works between 0 and 1. Elements in a fuzzy set have varying degrees of membership.

Fuzzy AHP

Analytic Hierarchy Process (AHP) is created by Thomas L. Saaty in 1977 for including non-quantitative data into decision making process. It is widely used to make decisions in multi criteria in a hierarchy system. AHP is known for being simple and accurate to make decisions in a certain environment (Roy & Dutta, 2018). AHP calculates criteria weights based on pairwise comparisons. The simplicity of this method makes it one of the most widely used multi criteria decision making (MCDM) methods. In the other hand AHP has drawbacks such as depends on experts' preferences and uncertain judgment.

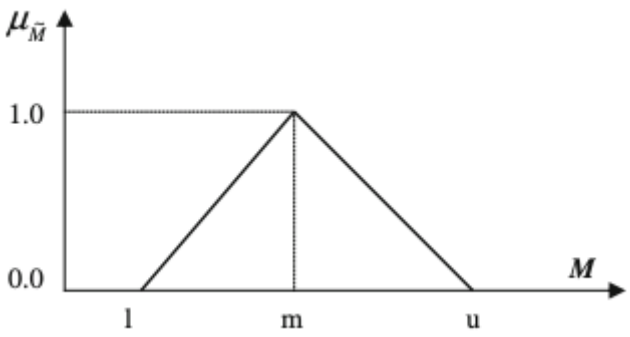

Figure 4. Lower and upper boundaries of TFN.

For example, decision makers may not be giving their opinion exactly or giving value to words that can not express their thoughts (Somsuk & Laosirihongthong, 2013). When the traditional AHP Method and fuzzy logic come together, AHP gains more accurate judgments. It allows decision makers to express their thoughts as a fuzzy set instead of single numbers. (Somsuk & Laosirihongthong, 2013) Decision makers give their pairwise comparison opinions as triangular fuzzy numbers (TFNs). A TFN consist of triplets, such as (a,b,c) a is lower bound which limits the value on the lower side, c is upper bound which is also limits the possible evaluation in the upper side, and b is the most favorable value. (Somsuk & Laosirihongthong, 2013)

Fuzzy TOPSIS

Technique for Order of Preference by Similarity to Ideal Solution (TOPSIS) method is developed by Hwang and Yoon in 1981. TOPSIS method is one of the most known MCDM methods. This method is based on the concept that chooses to the shortest distance from Positive Ideal Solution (PIS) and Negative Ideal Solution (NIS). In the traditional method, the weight of criteria and performance rating defined as crisp values. (Nădăban et al., 2016). Cheng introduced the extension of the triangular fuzzy TOPSIS method to express decision makers' thoughts correctly.

He also states that crisp data are inadequate for many situations and using linguistic variables may be a more realistic approach to decision makers opinion. We can transform the decision matrix into a fuzzy decision matrix and create a weighted normalized fuzzy decision matrix after adding the fuzzy ratings of the decision makers (Kekilli et al., 2021). We used positive ideal solution (PIS) and negative ideal solution (NIS) according to TOPSIS concept. Later, in this article, a vertex method is proposed to calculate the distance between two triangular fuzzy gradations. Using the Euclidian distance method, we can calculate the distance of each alternative to PIS and NIS, respectively. Finally, a closeness coefficient of each alternative is defined to determine the ranking order of all alternatives. A higher value of the closeness coefficient indicates that an alternative is simultaneously closer to the PIS and farther from the NIS (Chen, 2000).

Proposed Methodology

Table 1. Criteria of MCDM

CRI	Criteria	Unit
C1	Defect Detection Rate	Fuzzy
C2	Solution Speed	Fuzzy
C3	Customization	Fuzzy
C4	AI Software Implementation Time	Fuzzy
C5	Total Cost	Fuzzy
C6	Reputation of Supplier	Fuzzy
C7	Expertise in Coating Sector	Fuzzy

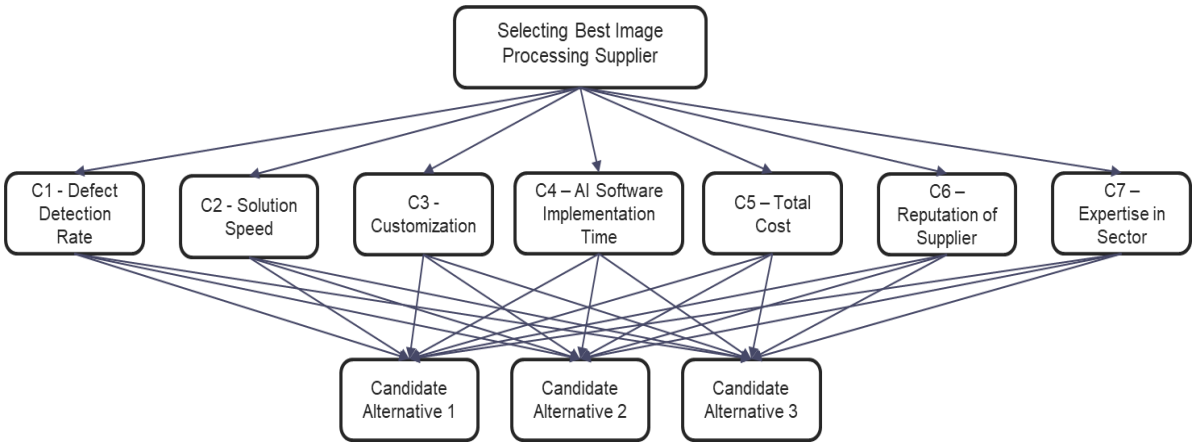

Figure 5. Hierarchical model of MCDM

The first criterion of the supplier selection MCDM is defect detection rate. This criterion is calculated with detected defects / total defects (percentage). The second criterion is solution speed. How much time is spent for processing images for detecting defects. The third one is customization. How many types of product can be automated with the algorithm. Others are total cost which contains annual maintenance and initial cost this criterion is also fuzzy because there is no exact cost value in the current phase and maintenance cost of the next year or breakdowns can't be predicted exactly, reputation in supplier, expertise in coating sector. The first level of the hierarchical model defines the objective of MCDM which is selecting the best supplier for implementing image processing software in the quality control process. The second level explains the criteria and the last one shows the alternatives.
Step 2 is selecting alternatives. Alternatives are selected from the real market but called as A1, A2, and A3 instead of sharing their names. A1 is a local image processing supplier which is experienced in the sector with giving consultancies and had made different varieties of quality control process automated. A2 is not an experienced firm in the market but they have capabilities of transforming traditional quality control processes to automated with AI image processing quality control. A3 is a global firm that has a lot of experience in the sector for a long time and they used high quality equipment for transforming processes.
Step 3 is asking several experts' opinions for evaluating criteria importance.
Step 4 is calculating criteria weights according to experts' opinions and Fuzzy AHP calculation methodology.
Step 5 is asking several experts to their thoughts about alternatives considering criteria.
Step 6 is converting this information to the Fuzzy TOPSIS methodology and calculating average values of decision makers.
Step 7 is making a normalized fuzzy decision matrix according to the average matrix.
Step 8 is multiplying normalized fuzzy decision matrix with criteria weights obtained from Fuzzy AHP.
Step 9 is calculating distances from Positive Ideal Solution (PIS) and Negative Ideal Solution (NIS).
Step 10 Select the best possible alternative which has the biggest Closeness Coefficient (CC).

Application

Karakaya86

Karakaya86 company, which adopts customer satisfaction as a principle, started its working life in Kartal in 1986 with zinc electrolysis activity. In 1997, it continued by establishing the first commercial cataphoretic coating line in Turkey. To provide high quality service to its customers with economical solutions, it started its investment studies in 2003. The company, which performs the coating processes of metal parts of the automotive and white goods sector, extends the service life of metal parts with many coating methods such as cataphoretic coating. The company has more than 350 employees, 1 central factory, 3 branches, and 1 R&D Center. The whole production area is 56.000 m2 with +25.000 m2 closed indoor area. There are more than 500 different product types coated in Karakaya86 in different lines. Applying image processing to all products and all lines may not be feasible thus, the products and their lines which has more production than others selected.

Fuzzy AHP Application

Before obtaining experts' opinion, the importance of criterion is determined as linguistic variables and their respective TFN's like in Table1.

Table 2. Linguistic variables with TFN's

Triangular Fuzzy Numbers	Linguistic Variable
(1,1,1)	Equal Importance
(2,3,4)	Moderate Importance
(4,5,6)	Strong Importance
(6,7,8)	Very Strong Importance
(9,9,9)	Extremely Strong Importance
Others	Intermediate Values

Importance of criteria asked the 3 experienced professionals. Their responses are expected to be like in Table 5.1. One of them is the director of R&D center at cataphoretic coating, the other one is production planner and C level manager at the coating firm, and the last one is experienced in consultancy about manufacturing.

Table 3. Pairwise comparison matrix according to DM1's response

DM1	C1			C2			C3		
C1	1,00	1,00	1,00	2,00	3,00	4,00	6,00	7,00	8,00
C2	0,50	0,33	0,25	1,00	1,00	1,00	4,00	5,00	6,00
C3	0,17	0,14	0,13	0,25	0,20	0,17	1,00	1,00	1,00
C4	0,14	0,13	0,11	0,20	0,17	0,14	0,50	0,33	0,25
C5	0,14	0,13	0,11	0,20	0,17	0,14	0,20	0,17	0,14
C6	0,11	0,11	0,11	0,14	0,13	0,11	0,14	0,13	0,11
C7	0,14	0,13	0,11	0,20	0,17	0,14	0,20	0,17	0,14

After all calculations are done with the rules of Fuzzy AHP and geometric mean method criteria weights are shown in Table 4.

Table 4. Average and normalized criteria weights according to all DM's

DM	AW	NW	RANK
C1	0,406298	0,40269	1
C2	0,287789	0,285234	2
C3	0,097849	0,09698	3
C4	0,052273	0,051809	5
C5	0,091806	0,09099	4
C6	0,023643	0,023433	7
C7	0,049302	0,048864	6
Total	1,00896		

After the calculations have been done, Criterion1 which is defect detection rate is selected as the most important criterion for all decision makers. Criterion2 which is solution speed is the second important criterion. Importance of criterion:

C1>C2>C3>C5>C4>C7>C6

Fuzzy TOPSIS Application

Firstly, linguistic variables and their respective TFN's are determined.

Table **Hata! Belgede belirtilen stilde metne rastlanmadı.**. Linguistic variables and TFN's

	TFN's		
Very Poor (VP)	0	0	0,2
Poor (P)	0,1	0,2	0,3
Medium Poor (MP)	0,2	0,35	0,5
Fair (F)	0,4	0,5	0,6
Medium Good (MG)	0,5	0,65	0,8
Good (G)	0,7	0,8	0,9
Very Good (VG)	0,8	1	1

Table 6. DM1's evaluation of alternatives w.r.t. each criterion

DM1	C1			C2			C3		
A1	0,7	0,8	0,9	0,5	0,65	0,8	0,7	0,8	0,9
A2	0,5	0,65	0,8	0,2	0,35	0,5	0	0	0,2
A3	0,8	1	1	0,7	0,8	0,9	0,5	0,65	0,8

Table 6 (Continued). DM1's evaluation of alternatives w.r.t. each criterion

C4			C5			C6			C7		
0,7	0,8	0,9	0,7	0,8	0,9	0,2	0,35	0,5	0,5	0,65	0,8
0,4	0,5	0,6	0,4	0,5	0,6	0,5	0,65	0,8	0,5	0,65	0,8
0,5	0,65	0,8	0,5	0,65	0,8	0,7	0,8	0,9	0,7	0,8	0,9

Table 7. DM2's evaluation of alternatives w.r.t. each criterion

DM2	C1			C2			C3		
A1	0,7	0,8	0,9	0,5	0,65	0,8	0,5	0,65	0,8
A2	0,8	1	1	0,2	0,35	0,5	0,2	0,35	0,5
A3	0,2	0,35	0,5	0,7	0,8	0,9	0,5	0,65	0,8

Table 7 (Continued) DM2's evaluation of alternatives w.r.t. each criterion

C4			C5			C6			C7		
0,4	0,5	0,6	0,7	0,8	0,9	0,4	0,5	0,6	0,4	0,5	0,6
0,1	0,2	0,3	0,2	0,35	0,5	0,7	0,8	0,9	0,2	0,35	0,5
0,5	0,65	0,8	0,1	0,2	0,3	0,8	1	1	0,2	0,35	0,5

After obtaining experts opinions about alternatives each criterion's normalized fuzzy matrixes, weighted fuzzy matrixes and their distance from PIS and NIS calculated separately. L M U represents the average values of lower middle and upper values. After that normalized fuzzy decision matrix shown in the tables. Weighted represents the weighted normalized fuzzy decision matrix with the criteria weights calculated at the Fuzzy AHP application (Table 14). Normalized fuzzy decision matrix, weighted normalized fuzzy decision matrix, and distances from PIS and NIS for criterion 5

Table 8. Normalized fuzzy decision matrix, weighted normalized fuzzy decision matrix, and distances from PIS and NIS for criterion 1.

Alt/CR	Criteria	DM1			DM2			L	M	U	Max
A1	C1	0,7	0,8	0,9	0,7	0,8	0,9	0,7	0,8	0,9	1
A2		0,5	0,65	0,8	0,8	1	1	0,5	0,825	1	
A3		0,8	1	1	0,2	0,35	0,5	0,2	0,675	1	

Table 8 (Continued). Normalized fuzzy decision matrix, weighted normalized fuzzy decision matrix, and distances from PIS and NIS for criterion 1.

Normalized			Weighted			Distance	
L	M	U	L	M	U	d-	d*
0,7	0,8	0,9	0,2819	0,3222	0,3624	0,12	0,02
0,5	0,825	1	0,2013	0,3322	0,4027	0,08	0,05
0,2	0,675	1	0,0805	0,2718	0,4027	0,02	0,12
		A+	0,2819	0,3322	0,4027		
		A-	0,0805	0,2718	0,3624		

Table 9. Normalized fuzzy decision matrix, weighted normalized fuzzy decision matrix, and distances from PIS and NIS for criterion 2.

Alt/CR	Criteria	DM1			DM2			L	M	U	Max
A1	C2	0,5	0,65	0,8	0,5	0,65	0,8	0,5	0,65	0,8	0,9
A2		0,2	0,35	0,5	0,2	0,35	0,5	0,2	0,35	0,5	
A3		0,7	0,8	0,9	0,7	0,8	0,9	0,7	0,8	0,9	

Table 9 (Continued). Normalized fuzzy decision matrix, weighted normalized fuzzy decision matrix, and distances from PIS and NIS for criterion 2.

Normalized			Weighted			Distance	
L	M	U	L	M	U	d-	d*
0,5	0,65	0,8	0,2013	0,2617	0,3222	0,07	0,08
0,2	0,35	0,5	0,0805	0,1409	0,2013	0,12	0,20
0,7	0,8	0,9	0,2819	0,3222	0,3624	0,12	0,02
		A+	0,2819	0,3222	0,3624		

Table 10. Normalized fuzzy decision matrix, weighted normalized fuzzy decision matrix, and distances from PIS and NIS for criterion 3.

Alt/CR	Criteria	DM1			DM2			L	M	U	Max
A1	C3	0,7	0,8	0,9	0,5	0,65	0,8	0,5	0,725	0,9	0,9
A2		0	0	0,2	0,2	0,35	0,5	0	0,175	0,5	
A3		0,5	0,65	0,8	0,5	0,65	0,8	0,5	0,65	0,8	

Table 10 (Continued)**.** Normalized fuzzy decision matrix, weighted normalized fuzzy decision matrix, and distances from PIS and NIS for criterion 3.

Normalized			Weighted			Distance	
L	M	U	L	M	U	d-	d*
0,5	0,725	0,9	0,2013	0,292	0,3624	0,07	0,06
0	0,175	0,5	0	0,0705	0,2013	0,16	0,25
0,5	0,65	0,8	0,2013	0,2617	0,3222	0,07	0,08
		A+	0,2013	0,292	0,3624		
		A-	0	0,0705	0,2013		

Table 11. Normalized fuzzy decision matrix, weighted normalized fuzzy decision matrix, and distances from PIS and NIS for criterion 4.

Alt/CR	Criteria	DM1			DM2			L	M	U	Max
A1	C4	0,7	0,8	0,9	0,4	0,5	0,6	0,4	0,65	0,9	0,9
A2		0,4	0,5	0,6	0,1	0,2	0,3	0,1	0,35	0,6	
A3		0,5	0,65	0,8	0,5	0,65	0,8	0,5	0,65	0,8	

Table 11 (Continued). Normalized fuzzy decision matrix, weighted normalized fuzzy decision matrix, and distances from PIS and NIS for criterion 4.

Normalized			Weighted			Distance	
L	M	U	L	M	U	d-	d*
0,4	0,65	0,9	0,1611	0,2617	0,3624	0,05	0,08
0,1	0,35	0,6	0,0403	0,1409	0,2416	0,11	0,20
0,5	0,65	0,8	0,2013	0,2617	0,3222	0,07	0,08
		A+	0,2013	0,2617	0,3624		
		A-	0,0403	0,1409	0,2416		

Table 12. Normalized fuzzy decision matrix, weighted normalized fuzzy decision matrix, and distances from PIS and NIS for criterion 5.

Alt/CR	Criteria	DM1			DM2			L	M	U	Max
A1	C5	0,7	0,8	0,9	0,7	0,8	0,9	0,7	0,8	0,9	0,9
A2		0,4	0,5	0,6	0,2	0,35	0,5	0,2	0,425	0,6	
A3		0,5	0,65	0,8	0,1	0,2	0,3	0,1	0,425	0,8	

Table 12 (Continued). Normalized fuzzy decision matrix, weighted normalized fuzzy decision matrix, and distances from PIS and NIS for criterion 5.

Normalized			Weighted			Distance	
L	M	U	L	M	U	d-	d*
0,7	0,8	0,9	0,2819	0,3222	0,3624	0,12	0,02
0,2	0,425	0,6	0,0805	0,1711	0,2416	0,09	0,18
0,1	0,425	0,8	0,0403	0,1711	0,3222	0,07	0,17
		A+	0,2819	0,3222	0,3624		
		A-	0,0403	0,1711	0,2416		

Table 13. Normalized fuzzy decision matrix, weighted normalized fuzzy decision matrix, and distances from PIS and NIS for criterion 6.

Alt/CR	Criteria	DM1			DM2			L	M	U	Max
A1	C6	0,2	0,35	0,5	0,4	0,5	0,6	0,2	0,425	0,6	1
A2		0,5	0,65	0,8	0,7	0,8	0,9	0,5	0,725	0,9	
A3		0,7	0,8	0,9	0,8	1	1	0,7	0,9	1	

Table 13 (Continued). Normalized fuzzy decision matrix, weighted normalized fuzzy decision matrix, and distances from PIS and NIS for criterion 6.

Normalized			Weighted			Distance	
L	M	U	L	M	U	d-	d*
0,2	0,425	0,6	0,0805	0,1711	0,2416	0,09	0,18
0,5	0,725	0,9	0,2013	0,292	0,3624	0,07	0,06
0,7	0,9	1	0,2819	0,3624	0,4027	0,13	0,02
		A+	0,2819	0,3624	0,4027		
		A-	0,0805	0,1711	0,2416		

Table 14. Normalized fuzzy decision matrix, weighted normalized fuzzy decision matrix, and distances from PIS and NIS for criterion 7.

Alt/CR	Criteria	DM1			DM2			L	M	U	Max
A1	C7	0,5	0,65	0,8	0,4	0,5	0,6	0,4	0,575	0,8	0,9
A2		0,5	0,65	0,8	0,2	0,35	0,5	0,2	0,5	0,8	
A3		0,7	0,8	0,9	0,2	0,35	0,5	0,2	0,575	0,9	

Table 15. Normalized fuzzy decision matrix, weighted normalized fuzzy decision matrix, and distances from PIS and NIS for criterion 7.

Normalized			Weighted			Distance	
L	M	U	L	M	U	d-	d*
0,4	0,575	0,8	0,1611	0,2315	0,3222	0,06	0,10
0,2	0,5	0,8	0,0805	0,2013	0,3222	0,05	0,15
0,2	0,575	0,9	0,0805	0,2315	0,3624	0,02	0,13
		A+	0,1611	0,2315	0,3624		

A-	0,0805	0,2013	0,3222

After all calculations completed, all distances between alternatives and PIS and NIS are used to determine which alternative has nearest solution to PIS and farthest from NIS. It is also called Closeness Coefficient (CC) and the bigger ratios are much closer than others.

Table 16**.** Alternative evaluation with closeness coefficient.

ALT\CRI	Di-	Di+	CC	Rank
A1	0,5787	0,5438	0,5156	1
A2	0,6709	1,0747	0,3844	3
A3	0,5102	0,6234	0,45	2

A1>A3>A2

Conclusion

As technology continues to advance at this rate, the rate of competition in the market will increase. So, customers will start asking companies for more. Especially B2B businesses will have difficulty in meeting the needs of their customers unless they follow the technology. on the other hand, Industry 4.0 accelerated the development of technology even more. Here, the market adapts to this and implements industry 4.0 applications very quickly. In fact, it has adapted so quickly that leads to many alternatives in the market have been able to do this job, so, the alternatives in the market have increased. Therefore, the supplier selection process has become a more challenging and complex process for companies. In this study, the company Karakaya86, which made cataphoretic coatings for the metal parts, decided to transform the quality control process of the most used production lines from the traditional manual inspection method, with automated AI image processing technology. Fuzzy AHP and FUZZY TOPSIS, two of the most well-known MCDM methods, were used to select the supplier according to their needs. For the Fuzzy AHP process, 3 expert opinions were received and a pairwise comparison was requested among 7 criteria. At the end of this process, Defect Detection Rate and Solution Speed emerged as the two most important criteria. Then, the Fuzzy TOPSIS stage was started to evaluate the alternatives with calculated criteria weights in Fuzzy AHP. At this stage, the opinions of 2 experts in the market and sector were taken. Fuzzy TOPSIS is based on the working principle of taking the distances from the best and worst solutions, and after the necessary calculations have been made, it has been revealed that the 1st alternative has a higher potential than the others and is more compatible with the needs of the company.

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Scientific Ethics Declaration

The authors declare that the scientific ethical and legal responsibility of this article published in EPSTEM journal belongs to the authors.

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Importance of the Factors Affecting the Delays in the Projects of Construction of Collective Housing in Algeria - Program Rent-Purchase

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Abstract: Faced with rapid urbanization and increasing demographic pressure on Algerian cities, housing policy in Algeria has evolved considerably over the past twenty years. However, the construction process is subject to many variables and unpredictable factors, which result in delays in project completion. It is therefore essential to identify and evaluate the relative importance of the main factors affecting delays in collective housing construction projects according to the vision of professionals and experts established in Algeria. Indeed, we focus our attention on the point of view of clients, consultants, designers and contractors to assess the relative importance of 67 delay factors. In total, 37 valid responses were analyzed and ranked. The results indicate that the 5 most important causes are Delay in payment of performed work, Shortage in skilled workers, Bureaucracy in public administrations, Delay in the provision of on-site public services and difficulties in financing the project by contractor. This study is hoped to help the practitioners to implement the mitigation measure at planning stage in order to achieve successful construction projects.

Keywords: Construction, Time overrun, Project, Management, Collective housing, Algeria

Introduction

Although projects are routinely conceived to operationalise strategic goals or to meet operational needs. Consequently, projects contribute to the operational and financial success of the organization (Anantatmula & Rad, 2018). Nevertheless, delays are inevitable in construction and are considered by the construction industry to be expensive, complicated and risky. Thus, construction delays have to be resolved promptly by apportioning the liabilities among the parties fairly and appropriately (Perera, 2019). For this reason, a plethora of studies have addressed the issue of project delay and identified its main causes according to country, region, project type and procurement methods, as well as from the perspectives of various stakeholders (Durdyev, 2018). In most developing countries, the biggest customer of the construction industry is the government and the procurement strategy adopted is the traditional contract in which the contract is awarded to the lowest bidder. This has led to significant delays resulting in overruns in initial time and cost estimates in the majority of projects (Ramanathan, 2012).

In Algeria, the owner-related causes are the most severe and important sources of delay (Zemra, 2019). Algeria's housing policy consists in generalizing the right of ownership of family housing. The Programme Rent-Purchase projects are awarded to design and build companies that are selected from a shortlist of companies approved by the Ministry of Housing that are generally good performers. Substantial investment of 3,500-4,500 billion DZD (32-41 billion USD) was committed to the various government housing programs during the 1999-2019 budget periods, but only 3.1 million of the planned 5.5 million units were actually built and delivered.

Our research aims to provide insights that can enrich previous work on the risk events related to delays in public housing construction projects in Algeria.

Literature Review

Several researchers have carried out literature reviews to provide an updated compilation of previous studies on the ranking of causes of delay. In order to identify a universal set of causes of delay that affect the performance of construction projects, (Durdyev &. Hosseini, 2018) presented a systematic review of studies published between 1985 and 2018. A total of 149 causes of delay were identified in an in-depth review of 97 selected studies. However, the results reveal that there are common causes that have been reported by the majority of researchers in this field. Thus, due to the concentration of resources on the most damaging causes, only the ten most frequently cited causes were considered and discussed in depth: Weather/climate conditions; poor communication, lack of coordination and conflicts between stakeholders; ineffective or improper planning; material shortages; financial problems; payment delays; equipment/plant shortage; lack of experience/qualification/competence among project stakeholders; labour shortages, and poor site management.

Ramanathan, et al. (2012) concluded that none of the studies are comparable to another and each study has a different ranking for the groups/categories/sources/factors/causes of delays and cost overruns. The groups most influential in earlier studies (in 1995) are now (2010) not considered high risk factors. The possible variations in the ranking results are most unlikely to be because of the different respondents. It would therefore appear that the groups and factors causing delays are country, location and project-specific and that there are no root causes that can be generalised.

Durdyev, et al (2017), investigated the key causes of delays, which are specific to the operational and socio-cultural context of the Cambodia's residential building sector. Results showed that the most 10 influential factors agreed by the contractors and consultants as the main causes of project delays are : shortage of materials on site ; unrealistic project scheduling ; late delivery of material ; shortage of skilled labour ; complexity of project ; labour absenteeism ; rain effect on construction activities ; design changes ; delay by subcontractor, and accidents due to poor site safety. It was recommended that construction frontline players should focus their efforts on the identified key factors in relation to their magnitudes of influence, which will ultimately lead to the on time project completion.

Ariffin et al. (2018), showed that weakness in management by developer and financial crisis are the main factors leading to the abandonment of housing project in Malaysia. Based on real data from projects in Egypt, Saudi Arabia, the United Arab Emirates and Qatar, Badawy et al. (2020), concluded that the cost of late payments in residential buildings can be estimated considering the total value of the contract, the duration of late payments, and the total project duration.

Aiyetan and Dillip (2018), indicated that the shortage of skilled labourers affects significantly the quality of work, causing rework and low productivity, followed by construction delays of projects in the Eastern Cape Province of South Africa. The strategies to improve the availability of skilled labourers were suggested namely: enhancing investment in labour wages ; investing in talent management and staff development programmes, and ensuring a better work environment with improved health and safety.

Research Methodology

This section is devoted to the precise definition of the initial causes that could defeat the project objective. These causes were identified through an extensive review of previous literature and a real exchange with experts in the construction industry. The specific research method aims to collect data from a large sample, it includes an online questionnaire in Google Forms, it has a practicality and relative simplicity, as all returned answers can be easily analyzed. The questionnaire included an ordinal measurement scale (Likert scale) ranking the level of importance of each identified risk in an "ascending" order from 1 to 5.

Finally, the evaluation consists of quantifying the probability of occurrence of each identified risk and estimating the severity of the consequences on the project objectives. The combination of these two parameters is used to measure and judge the criticality of an identified risk in relation to the others. The analysis of survey is done by Relative Importance Index (RII) of each cause. The following formulas were used to calculate the different indexes (Zemra et al. 2018).

Frequency Index

$$F = \frac{1}{4} \times \sum_{i=1}^{5} Wf_i \times \left(\frac{n_i}{N}\right) \times 100(\%) \quad \text{.....(1)}$$

where Wfi is the constant weighting given to each response (0 for Not relevant up to 4 for Always), ni is the frequency of the ith response and N is the total number of responses.

Severity Index

$$S = \frac{1}{4} \times \sum_{i=1}^{5} Ws_i \times \left(\frac{n_i}{N}\right) \times 100(\%) \quad \text{..... (2)}$$

where Wsi is the constant weighting given to each response (0 for Not relevant up to 4 for Extreme), ni is the frequency of the ith response and N is the total number of responses.

Relative Importance Index

$$R = [F\ (\%) \times S(\%)] \div 100(\%) \text{..... (3)}$$

Results and Discussion

The ranking indexes of frequency, severity and relative importance were used to rank delay causes from the viewpoint of respondents. In order to analyse the delay causes, Table 1 shows the importance index of each cause and the rank of the top 10 causes in the overall ranking obtained from the combined data of the all respondents.

Table 1. Ten most important causes

ID	Description of delay causes	Index	Rank
C43	Delay in payment of performed work	59,49	1
C9	Shortage in skilled workers	56,25	2
C6	Bureaucracy in public administrations	52,43	3
C4	Delay in the provision of on-site public services	50,09	4
C52	Difficulties in financing the project by contractor	49,94	5
C50	Slow variation orders in extra quantities	49,72	6
C11	Lack of qualified professionals in construction project management	49,65	7
C55	Ineffective planning and scheduling of project by contractor	49,27	8
C34	Work start before completion of the execution study	49,19	9
C54	Poor site management and supervision by contractor	48,75	10

The sources of each cause of delay are summarised below :

1- Delay in Payment of Performed Work :

The funding logic of the public authorities, which is focused on the social sector but in an excessive manner, makes the economic logic absent in their behaviour. This has put the administration on projects of intention or declaration rather than on real projects which must be technically prepared, programmed and budgeted both financially and in time.

2- Shortage in Skilled Workers :

The status of skilled labour in the public housing sector has not been considered at all levels, especially in terms of remuneration, working conditions, retraining and especially further training. Moreover, this category is involved in both private and self-employed work.

3- Bureaucracy in Public Administrations :

The public authorities have remained in a logic of a state that distributes rent, although the context has changed and the administration is no longer that authority of public sense, synonymous with spending. Now, value is created by people who work, setting clear rules and ensuring the equality of actors that is something concrete and not a speech. Social pressure is now present, but leaders must make decisions on a technical and political level.

4- Delay in the Provision of on-Site Public Services :

Lack of knowledge of the legislation in terms of economy or technical regulations by the implementing body. We find ourselves, therefore, with an administration that responds as many times differently as there are situations.

5- Difficulties in Financing the Project by Contractor :

The lack of rationalisation of the budgetary choice accounts for the importance of the deviation of the results from the target. There is an efficiency problem. The strongest signal is that the exchange rate is not stable and that a bureaucratic administrative staff is given responsibility for designing and controlling economic projects.

6- Slow Variation orders in Extra Quantities :

Prices are capped and short deadlines are imposed on companies in favour of free market rules. However, the housing programmes are not precisely defined and evaluated, and in fact, during the course of the project, the expenditure is re-evaluated and revised, and as a result, the budget is systematically increased, thus increasing the budget deficit.

7- Lack of Qualified Professionals in Construction Project Management :

Sometimes political influence plays a big role, especially when a situation requires an emergency call to save the situation. In addition to the rationalisation of technical choices, the competences of certain institutions such as independent experts and assistants to the project owner should be associated in terms of control and strength of proposal, and also in making recommendations to be taken up by the executive.

8- Ineffective Planning and Scheduling of Project by Contractor :

The resources actually implemented are different from the resources foreseen by the planning tools, so constraints due to problems of availability of building land, sufficient financial resources, qualified personnel and the choice of competent companies as well as the suitability of the project environment are not taken into account in the planning phase.

9- Work Start before Completion of the Execution Study :

Social pressure forces the administration to announce the start of a project before the execution files have matured, the project lacks technical preparation, as well as programming and budgeting, both financially and in time.

10- Poor Site Management and Supervision by Contractor :

Use of obsolete means and methods, Lack of internal control on site and Execution of all lots by the general contractor rather than engaging subcontractors in the different phases of the project in an official manner.

Conclusion

This study was conducted to identify and evaluate the relative importance of the main factors affecting delays in collective housing construction projects. Sixty-seven delay causes were identified through a comprehensive literature survey and considerable contributions of construction experts and professionals established in Algeria.

The specific research method aims to collect data from a large sample, it includes an online questionnaire in Google Forms, we focus our attention on the point of view of clients, consultants, designers and contractors to assess the relative importance of 67 delay factors. In total, 37 valid responses were analyzed and ranked.

The results indicate that the 10 most important causes are Delay in payment of performed work, Shortage in skilled workers, Bureaucracy in public administrations, Delay in the provision of on-site public services, Difficulties in financing the project by contractor, Slow variation orders in extra quantities, Lack of qualified professionals in construction project management, Ineffective planning and scheduling of project by contractor, Work start before completion of the execution study and Poor site management and supervision by contractor.

A number of measures to mitigate the causes of delay were recommended following feedback from respondents in order to achieve successful construction projects.

Recommendations

In order to rationalise both the budgetary and the technical choice, one must work on a housing programme which should be identified and evaluated in a precise manner, and for which one should monitor the execution and estimate the rate of achievement of the objectives in terms of improving the social situation of society and trying to improve the efficiency of public expenditure.

Scientific Ethics Declaration

The author declares that the scientific ethical and legal responsibility of this article published in EPSTEM journal belongs to the author.

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Allergenic Proteins of Tilia Cordata

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Abstract: Pollen forms large amount of aeroallergens in the atmosphere that spread through winds and insects. Allergenic pollen grains are water-soluble glycoprotein or protein bioparticles and its weight is approximately 5-80 kDa. There are many factors that cause allergies and considering the recently changing climatic conditions and their ability to spread over a wide geographical areas, pollens are among the major factors causing allergies. In this study, it was aimed to detect potential allergen pollen proteins of *Tilia cordata* which is a potent allergen. The pollen samples belonging to *T. cordata* were dried and then they were separated with sieve. Acetone washing and dialysis processes were applied to purificate pollen. Afterwards, the amount of protein in the pollens of *T. cordata* was determined by the bicinchoninic acid (BCA) method. In order to detect allergen proteins, gel runing was performed by SDS-PAGE. Silver staining method was applied to make visible the bands of *T. cordata* pollen proteins obtained from the gel. Five different allergenic pollen proteins, weights 10, 23, 40, 50 and 80 kDa, were detected as a result of SDS-PAGE. This is the second study in the literature related with the identification of *T. cordata* allergenic proteins and we showed two different protein bands compared with the former study result. Besides, our study is original in this perspective. We think that this study contributes to the literature on allergenic proteins of *T. cordata* and should be supported by future studies.

Key words: Allergy, allergen, allergenic protein, pollen allergy, T. cordata

Introduction

Allergy is a kind of disorder that occurs as a result of an overreaction of the immune system against a very small amount of a certain substance made up of proteins called allergens, where the balance is disturbed (Jae-Won, 2018). Allergens stimulate the production of allergic antibodies or sensitive cells. This response is mediated by the allergen-specific immunoglobulin IgE antibody. Mast cells and basophils are activated after IgE binding and initiate a series of cellular and molecular events that result in clinical sypmtoms of allergic disease (Jae-Won, 2018). Allergens are commonly found in pollen, molds, animal skin cells and hair, house dust mites, insects, drugs, and even foods such as milk, egg, soy, wheat or nut, fish and shellfish. These allergens can enter the body by injection through the respiratory tract (Chinen et al., 2009). Aeroallergens are particles in the atmosphere that can cause respiratory or conjunctival allergy (King et al., 1995). Pollen is one of the important allergen sources among the aeroallergens. Since the size of pollens that are aeroallergens is in the range of 20-60 µm and their allergenic components are proteins with molecular weights between 10-40kDa., they have a role in the pathogenesis of allergic rhinitis, bronchial asthma and hypersensitivity pneumonia (Stewart, 2000).

Pollen is produced in the microsporangia in the male cone of a conifer or other gymnosperm or in the anthers of an angiosperm flower. Pollen grains have a wide variety of species-specific shapes, sizes, and surface marks (Pleasants et al., 2001). In angiosperms, during flower development the anther consists of a mass of cells that appear undifferentiated apart from a partially differentiated dermis. As the flower develops, four groups of sporogenous cells form within the anther. After the formation of the four microspores contained in the callose walls, the development of the pollen walls begins. The callus wall is broken down by an enzyme called callase, and the released pollen grains grow in size and develop their characteristic shape, forming a resistant outer wall called exine and an inner wall called intin (Furness and Rudall, 2001). It is known that the allergen properties of pollen are caused by lipoprotein, polysaccharide and proteins found in the intin and exin layers (Pehlivan, 1984; 1995; Esch et al., 2001). Recent studies on pollen allergy show that most of the major allergens are found in the pollen in amyloplasts, sometimes in the cytoplasm, depending on cell organelles, and rarely in the cytoplasm alone or attached to the pollen wall (Grote, 2001). In addition to genetic factors in pollen allergy (sensitivity), environmental factors such as temperature, air pressure, winds and pollution are also effective. Sensitivity to pollen is determined either by a skin prick test or by detection of the level of allergen-specific IgE in peripheral blood (Radauer et al., 2008; Traidl-Hoffmann et al., 2009).

Linden (*Tilia sp.*) is a large tree of variable form. In woodland up to 30 m (rarely to 37 m) high, with a cylindrical trunk up to about 1 m in diameter at breast height, tapering gradually and unbranched to two-thirds of its height. Lower branches of the first-order horizontal and arching; branches of the second-order horizontal, ascending or vertical: upper branches ascending or vertica. This description applies to the species in the strict sense but *Tilia cordata* may also be regarded as a collective species, which extends from western Europe to Eastern Asia and includes at least seven species or subspecies (Pigott, 1991). The small-leaved linden- *T. cordata* - is found throughout Europe and most parts of North America. The dried leaves are used as herbal tea. The height of *T. cordata* can reach up to 30m. Its leaves are half-heart-shaped, dark green, 4-8cm long. Pollen dissemination continues from June to July. Sometimes it can continue until August. During these period, *T. cordata* pollen cause to severe allergy symptoms. However, its allergenic proteins has not known in detail for diagnosis *T. cordata* allergy. In this purpose, we aimed to detect total allergen proteins of *T. cordata* and identificate the potential allergenic proteins.

Method

Pollen Collection

First, we collected *T. cordata* pollens in campus area of Gaziantep University, Turkey. Pollen extraction was prepared as fresh according to the method by Aytug and Peremeci (1987). The most suitable period for this is the phase immediately after the anthers are opened. Considering these, pollens were collected by appropriate methods during the dissemination period of the plant to be used. We dried its flowers and poured onto a clean blotter by hitting the dried flowers. The spilled pollen was placed in dark glass bottles and kept in a desiccator for 24 hours to dry. After drying, the flowers were separated from their pollen by sieving with 3 different pore diameters (180, 90, 63 µm). Then, washing with acetone was performed to separate the pollen from foreign materials such as plant parts. The pollens were then dried in a climate cabinet at 20-37 ℃.

Pollen Exctract Preperation

T. cordata pollen was mixed in 1:12 (w/v) 125mM NH_4HCO_3 solution at + 4 °C for 12 h on a low speed magnetic stirrer. Then the pollen residues will be removed by settling in a centrifuge (13000xg, + 4 °C, 1 h) The upper liquid phase was first passed through the 125mm thick Whatman paper and then through the filtration system. The filtrate obtained was transferred to the dialysis tube. Dialysis was performed at + 4 °C for 48 h in a shaker against pure water.

Total Protein Concentration in Pollen Extracts

The protein concentration in the pollen extracts was determined according to the bicinchoninic acid (BCA) method, and this method is based on the processing of proteins in alkaline solution with biurea reagent, reduction of Cu (II) ions to Cu (I) ions and spectrophotometric measurement of the complex formed by Cu (I) ions with BCA. First of all, the bovine serum albumin (BSA) protein included in the BCA kit was dissolved in water and diluted in appropriate proportions, and standards were prepared in certain concentrations. Standards

were prepared with distilled water dissolved in powdered proteins. Distilled water was used as a blank. The BCA indicator was obtained by mixing the reagent solutions in the kit in a certain ratio. 200µL reagent was added onto the protein samples, standards and blank. Then it was incubated for 30 min in a 37°C incubator. After the incubation process, absorbance values at 562 nm were measured in the spectrophotometer. The protein concentrations were determined by placing the absorbance values of the protein values into the line equation.

Pollen Morphology

Pollen samples of T. cordata species used in this study were prepared by Woodhouse (1935) method and microscope images were taken from the prepared preparations. T. cordata pollen imaged with X 40 lens.

SDS-PAGE Electrophoresis

For identification the potential pollen allergen proteins of *T. cordata*, extracted total proteins were run in Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) method proposed by Walker (2002). First, the solutions, buffers for SDS were prepared. After, the separating gel was prepared and emptied with a pipette to fill 3/4 of the gel cassette. The contact of the gel with oxygen was cut off, by adding 96% isopropanol on the gel. After the separating gel polymerized, a 5% stacking gel was prepared and added. Following to polymerization stacking gel, the samples were loaded into wells. The electrophoresis tank was filled with 1X tank buffer. The protein extract sample dissolved in PBS, BSA and 5µL protein marker were mixed with sample application buffer at a ratio of 1:1 (v:v) and loaded onto the gel at 20 µg protein per well. After the charging process was completed, electrophoresis was carried out at constant volt (235V) for approximately 90 min. At the end of the running, silver ($AgNO_3$) staining was performed to analyze the bands of the samples.

Result and Discussion

As a result of morphological examinations, it was determined that the diameter of pollen was approximately 83 micrometers (Figure 1). Compared to different allergen pollens, it was observed that the pollen of *T. cordata* was larger. These images and literature comparisons suggest that *T. cordata* has a higher allergenicity. Pollen grains penetrate the upper respiratory tract, they rarely reach the bronchi as their size is always greater than 10µm. Therefore, pollen usually causes allergies in the upper respiratory tract.

After we calculated the protein concentration as 5.193,32 µg/mL in the measurements we made at 562nm. *T. cordata* has a very high concentration when compared with the protein concentrations of other allergens in the literature.

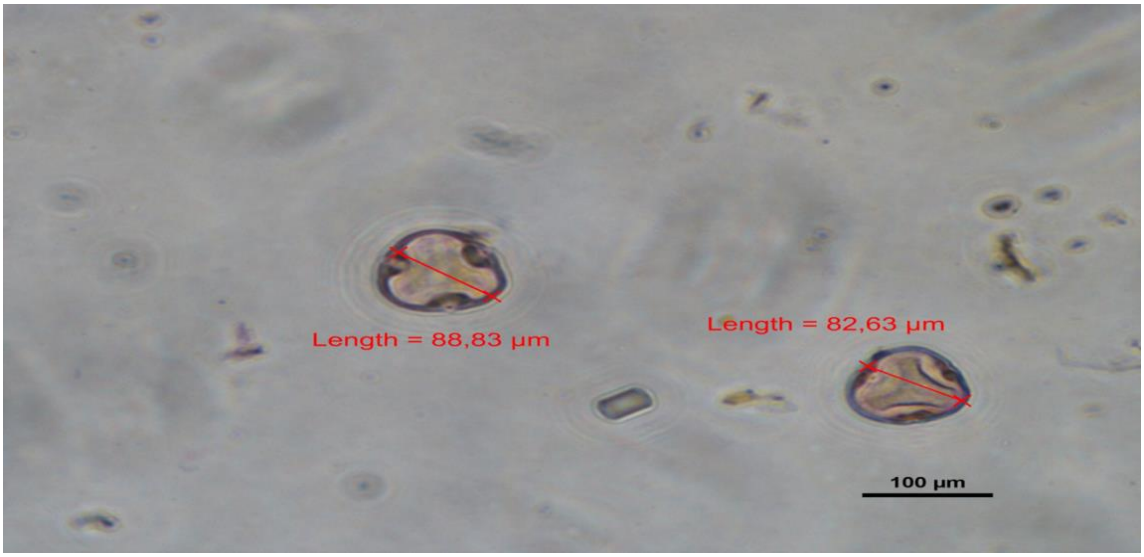

Figure 1. Pollen images of *T. cordata*

In a previous study, it was seen that *T. cordata* had 3 different allergen proteins which weights 10, 21, 50kDa, and these proteins had not been characterized (Mur et al., 2001). In our study, in addition to bands of 10, 21, 50kDa, we observed two different allergen protein bands around 40 and 80kDa. Figure 2 shows the band images of these allergen proteins.

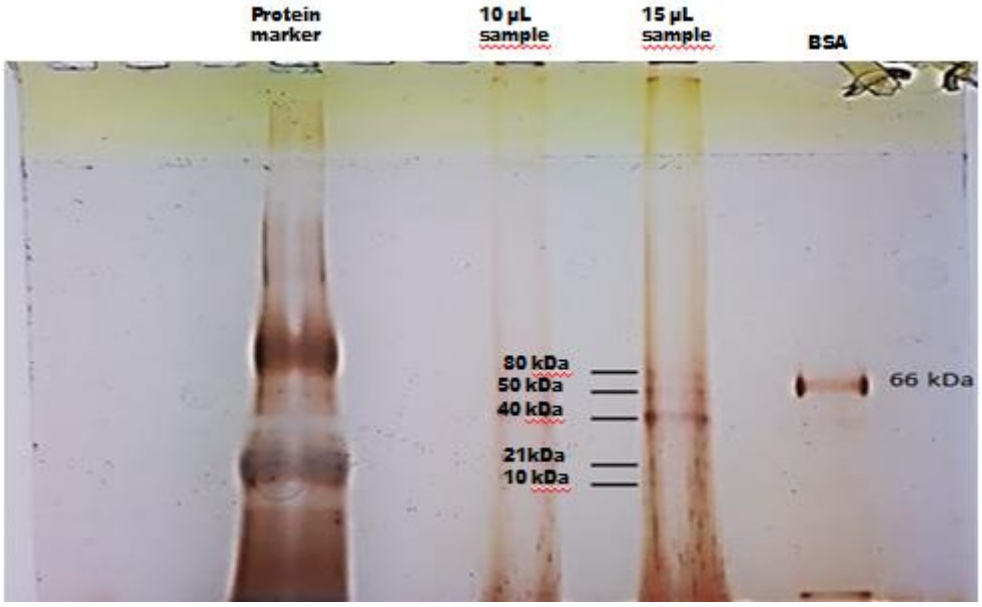

Figure 2. Allergenic proteins of *T. cordata*

According to the data we have obtained, *T. cordata* has 5 different allergen proteins, which support its allergenicity is clearly high. Between 10 and 35% of European young adults have IgE antibodies to pollen allergens (D'amato et al., 2007). In the USA, the prevalence of allergic diseases is around 30% (Pincus et al., 2011). The World Health Organization estimates that more than 400 million people worldwide suffer from allergic rhinitis and 300 million suffer from asthma (Gamble et al., 2008). When the data on allergic diseases are evaluated, the disease significantly affects public health and quality of life today. Along with these problems, the detection of allergens increases the importance of research on treatment methods against allergen diseases. Our study shows that *T. cordata* has allergen proteins that will cause allergic reactions. The fact that there are only a few studies on this subject and the absence of advanced studies and patient data clearly shows that there is a need for comprehensive studies. Our study supports the need for more both *in vivo* and *in vitro* studies on t cordata allergenicity. We hope that our study will shed light on future studies.

Scientific Ethics Declaration

The authors declare that the scientific ethical and legal responsibility of this article published in EPSTEM journal belongs to the authors

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Determination of Complex Modulus Values of Low-Density Polyethylene Modified Bitumen Obtained by Using Two Different Waste Types with Artificial Neural Networks

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Abstract: The present study aims to solve an important environmental problem and improve the performance properties of bitumen by using two types of waste low density polyethylene (LDPE). For this purpose, two types of additives, LDPE (A) and LDPE (B), were added to the pure binder at the rates of 1%, 2%, 3% and 4% to obtain modified binders. Then, Dynamic Shear Rheometer experiments were applied on the binders under different temperatures and frequencies, and their behavior under these conditions was investigated. The complex shear modulus values obtained as a result of the experiment were estimated with Artificial Neural Network models created by training with different training algorithms. Experimental results showed that both additives increased the complex modulus values of the binder, with the LDPE (A) additive having higher complex modulus values compared to the LDPE (B) additive. In addition, it was determined that the model obtained with the Levenberg-Marquardt training algorithm gave the best results and it was concluded that the complex module values of asphalt binders can be successfully estimated using Artificial Neural Networks.

Keywords: Waste Materials, Recycling, Asphalt, Modification, Artificial Neural Networks

Introduction

Waste plastic has been one of the most remarkable materials for the last few years (Y. Huang et al., 2007; Ingrassia et al., 2019). However, the waste plastic recycling rate in the US is quite low compared to other countries that report recycling rates between 30% and 60%. Japan has the highest recycling rate with a value of 78% (Khoo, 2019). Plastic waste mixtures are difficult materials to recycle due to their complex structure and inefficient mechanical recycling processes. Instead of sending waste to developing countries, Australia is taking proactive steps to develop an alternative to using recycled plastic, which contributes significantly to the country's waste generation (Chin & Damen, 2019). In addition, new alternatives are sought for using waste plastics in the USA. In China and India, the import of waste plastics is prohibited (Cockburn, 2019).

Reducing the use of plastic may be the best way to directly reduce waste plastic. For example, a perspective has been proposed to move towards zero waste by banning single-use plastics (Walker & Xanthos, 2018). This prohibition can be difficult to enforce. Therefore, other options should be sought to reduce the plastic waste problem. Researchers and engineers are working to produce wood-plastic composites (Keskisaari & Kärki, 2018), concrete blocks (Meng et al., 2018) and mortars (Makri et al., 2019; Ramli & Akhavan Tabassi, 2012) that can be used in construction infrastructures by evaluating waste plastic materials. Ramli and Tabassi (Ramli

& Akhavan Tabassi, 2012) found that polymer modified mortars exhibited better engineering properties than conventional mortar mixtures. Arulrajah et al., investigated the possibility of using plastic granules in combination with crushed brick and recycled asphalt pavement (RAP) wastes used as base fill material (Arulrajah et al., 2017) .

Utilizing waste materials in bituminous coatings is an environmentally friendly approach that has gained great importance in recent years. Bitumen is the most commonly used binder in pavement and is derived from a non-renewable resource, petroleum (Ingrassia et al., 2019). On the one hand, researchers make use of various wastes such as waste motor oil, cooking oil, pig manure and coffee grounds within the scope of environmental studies. This can undoubtedly reduce harmful environmental impacts and raw material consumption. Engineers, on the other hand, are hesitant to advocate the use of large amounts of recycled materials unless such a pavement infrastructure with recycled materials is proven to perform as well as those without recycled materials. There are many studies on the use of various waste polymers on roads (Poulikakos et al., 2017; Sangita et al., 2011) . However, there is a gap in the literature to fully understand the performance of asphalt pavements containing various dosages and types of recycled plastics.

Low density polyethylene (LDPE) is often used in bitumen modification to improve rutting resistance at high temperature and significantly reduce temperature sensitivity. In this study, the effect of using low density polyethylene (LDPE) with two different chemical contents on the rheological properties of bitumen binders was investigated. Two different LDPE binders were added to the pure bitumen in 4 different ratios (1%, 2%, 3% and 4%). Dynamic Shear Rheometer experiment was carried out. In addition, Artificial Neural Network models were obtained with two different training algorithms and various neuron numbers, then complex modulus values were obtained through these models.

Material

In this study, 50/70 penetration grade pure asphalt binder procured from Batman Refinery by TÜPRAŞ (Turkish Petroleum Refineries Corporation) was preferred. The procured binder was modified with two different low-density polyethylene additives. The physical properties of LDPE (A) and LDPE (B), which are two different additives used, are given in Table 1. The additive rate used for the modification process was determined as 1%, 2%, 3% and 4%.

Table 1. Physical properties of LDPE (A) and LDPE (B)

Property	LDPE (A)	LDPE (B)
Specific gravity (g/cm^3)	0.913	0.916
Tensile strength (MPa)	20	15
Melting temperature (°C)	120	130
Impact strength (kJ/m^2)	5	5

Method

Dynamic Shear Rheometer (DSR)

The dynamic shear rheometer test is carried out to determine the time-dependent deformation and elastic behavior of asphalt binders at medium and high temperatures. As a result of the experiment, the complex shear modulus (G*), which represents the total deformation resistance of the bitumen binders, and the phase angle (δ), which is defined as the delay between the applied shear stress and the resulting deformation, are obtained.

The frequency scanning test performed with the DSR device can simulate the speed of a vehicle traveling on an asphalt pavement. A loading frequency of 10 Hz corresponds to a speed of 60 km/h, while a loading frequency of 15 Hz corresponds to a speed of 90 km/h. The complex modulus and phase angle values vary significantly with temperature and frequency (W. Huang et al., 2019).

In this study, different temperature and frequency effects were investigated on two different LDPE modified asphalt binders by applying frequency scanning test at 40°C, 50°C, 60°C and 70°C temperatures and in the range of 0.01-10Hz.

Artificial Neural Network (ANN)

ANN consists of cells inspired by the working principles of the human brain and are structures that can be programmed for learning with the information given to them. The ANN has structures (neurons) resembling human neurons (Öztemel, 2008). ANN takes information from the available data for the problem to be solved, learns, and uses this information to produce solutions and make predictions (Graupe, 2013).

An ANN structure has a total of five parts: inputs (X1,X2,X3…), weights (W1,W2,W3…), aggregation (addition) function, activation (transfer) function, and output (Y). The inputs enter the neurons together with the weights and pass through the joining function to the activation function. In this study, the Sigmoid (Logsig) Function was used as the activation function.

ANN must be trained to work optimally and produce results. It is important to select suitable algorithms for the training process (Sönmez Çakir, 2019). In this study, ANNs were run in MATLAB environment and two different training algorithms, Levenberg-Marquardt (LM) and Scaled Conjugate Gradient (SCG), were preferred. In the established model, three inputs (temperature, frequency, and additive ratio) were used for both additive types and the complex modulus (G*) values of the asphalt binder were obtained as the output.

70% of the dataset was allocated for training, 15% for validation and 15% for testing. While the number of neurons required in the ANN layers when starting the training can be found by various calculations, this number can be found by trial and error until the desired performance values are obtained. Therefore, for each training algorithm, the model was retested with three different neuron numbers (8,10,12) to obtain the highest accuracy with the lowest error rate. The exemplary architecture of one of the models (with eight neurons) used in the study is shown in Figure 1. Explanation of ANN models given in Table 2.

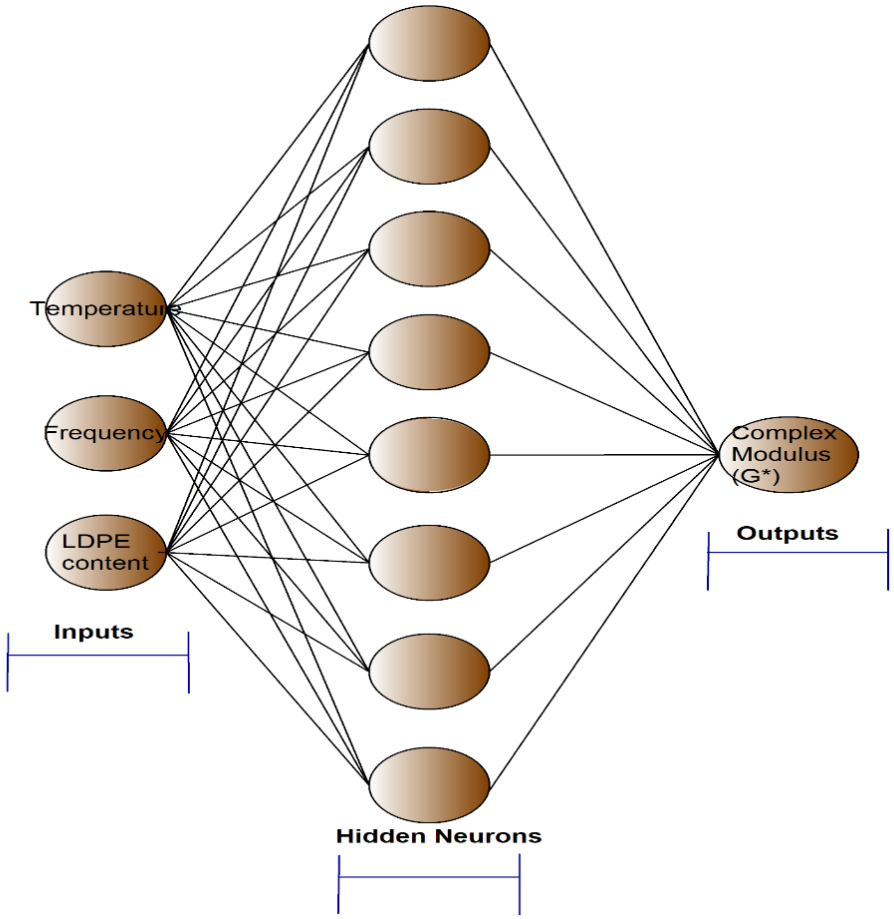

Figure 1. ANN architecture

Table 2. Explanation of ANN models

Model Name	Explanation
Lev-8_A	Levenberg-Marquardt training algorithm with 8 neurons / LDPE (A)
Lev-10_A	Levenberg-Marquardt training algorithm with 10 neurons / LDPE (A)
Lev-12_A	Levenberg-Marquardt training algorithm with 12 neurons/ LDPE (A)
Sca-8_A	Scaled Conjugate Gradient training algorithm with 8 neurons/ LDPE (A)
Sca-10_A	Scaled Conjugate Gradient training algorithm with 10 neurons/ LDPE (A)
Sca-12_A	Scaled Conjugate Gradient training algorithm with 12 neurons/ LDPE (A)
Lev-8_B	Levenberg-Marquardt training algorithm with 8 neurons/ LDPE (B)
Lev-10_B	Levenberg-Marquardt training algorithm with 10 neurons/ LDPE (B)
Lev-12_B	Levenberg-Marquardt training algorithm with 12 neurons/ LDPE (B)
Sca-8_B	Scaled Conjugate Gradient training algorithm with 8 neurons/ LDPE (B)
Sca-10_B	Scaled Conjugate Gradient training algorithm with 10 neurons/ LDPE (B)
Sca-12_B	Scaled Conjugate Gradient training algorithm with 12 neurons/ LDPE (B)

A total of three statistical methods, namely Coefficient of Determination (R2), Mean Square Error (MSE) and Root Mean Square Error (RMSE), were used to evaluate model performance and errors.

$Y^{experimental}$ = Experimental data,

$Y^{predicted}$ = Estimated data and n is the number of experimental data:

$$R^2 = 1 - \frac{\sum_{i=1}^{n} (Y_i^{predicted} - Y_i^{experimental})^2}{\sum_{i=1}^{n} (Y_i^{experimental})^2} \quad (1)$$

$$MSE = \frac{\sum_{i=1}^{N} (Y_i^{predicted} - Y_i^{experimental})^2}{n} \quad (2)$$

$$RMSE = \sqrt{\frac{\sum_{i=1}^{N} (Y_i^{predicted} - Y_i^{experimental})^2}{n}} \quad (3)$$

Results and Discussion

Dynamic Shear Rheometer (DSR) Results

In the study, dynamic shear rheometer test was applied on pure and modified binders at four different temperatures, 40°C, 50°C, 60°C and 70°C, at ten different frequencies in the range of 0.01-10Hz. The complex modulus values obtained at different frequencies as a result of the experiment are given in Figure 3 for LDPE (A) and Figure 4 for LDPE (B).

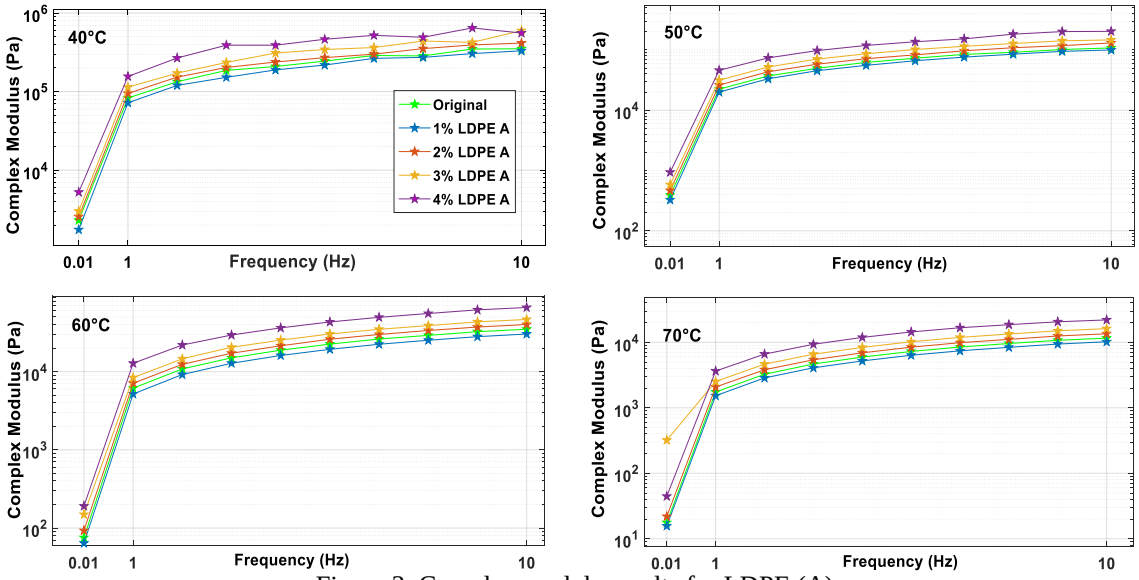

Figure 3. Complex module results for LDPE (A)

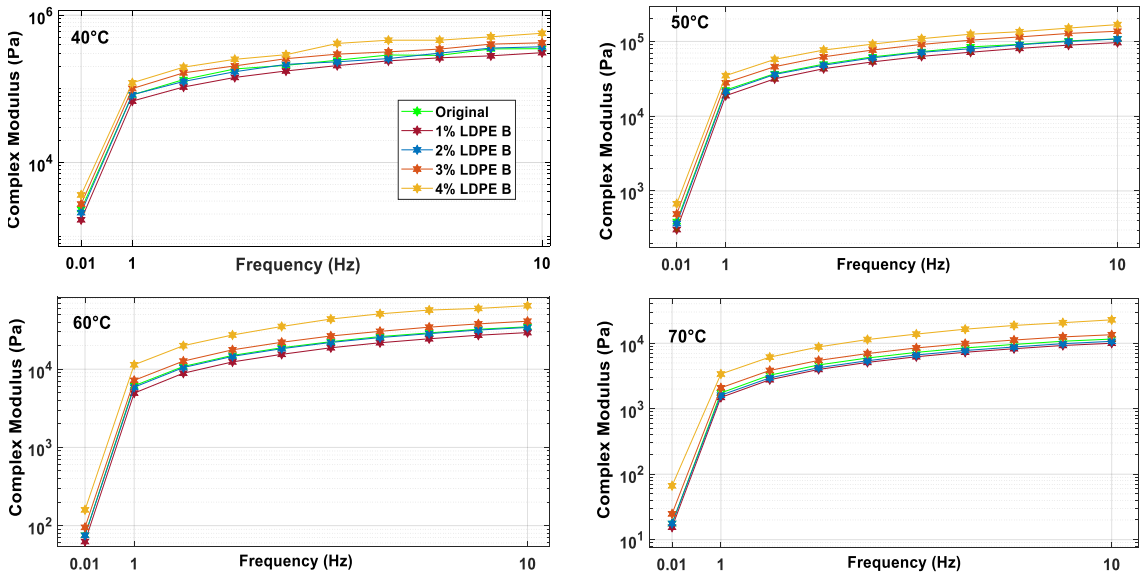

Figure 4. Complex module results for LDPE (B)

The figures clearly show that the complex modulus values increase with the increase in frequency (loading rate). It was determined that the complex modulus values of the binders increased as the amount of waste additive used as a modifier in asphalt binders increased. Also, the difference in complex modulus values became more pronounced with increasing LDPE content. The LDPE (A) additive produced greater changes in complex modulus values compared to the LDPE (B) additive. It was observed that LDPE (A) modified binders had lower complex modulus values compared to LDPE (B) modified binders.

Artificial Neural Network (ANN) Results

The R2, MSE and RMSE results obtained to evaluate the performances of the created ANN models are given in Tables 3 and 4, respectively, for each contribution type. Considering the performances of the models created for LDPE (A), the highest R2 value was obtained with the Lev-8_A model (0.9878), while the lowest R2 value was obtained with the Sca-12 model (0.6424). Table 3 shows that the Lev-10_A model gives the results with high accuracy (98%) and the lowest error (30.122). Considering that the G* values are between 102 and 104, the RMSE, that is, the distance between the actual and predicted values as 30.12, indicates that the model performance is quite high. In Table 4, it is clear that the Lev-10_B model performs better than other models.

Table 3. Error values for LDPE (A)

LDPE A	R2	MSE	RMSE
Lev-8_A	0.98788978	498806.5327	706.2623681
Lev-10_A	0.98662916	907.3571236	30.12236916
Lev-12_A	0.968757957	300146.9421	547.8566802
Sca-8_A	0.957851173	14815431.44	3849.081895
Sca-10_A	0.904859996	4283121.59	2069.570388
Sca-12_A	0.642444886	16581287.09	4072.012658

Table 4. Error values for LDPE (B)

LDPE B	R2	MSE	RMSE
Lev-8_B	0.996919861	6998.412564	83.65651537
Lev-10_B	0.996004047	5153.020205	71.78454015
Lev-12_B	0.987051443	91855.23165	303.0762802
Sca-8_B	0.955447955	11135010,8	3336,916361
Sca-10_B	0.979690252	509762.3127	713.976409
Sca-12_B	0.972448819	6982088.272	2642.364145

The performances of the model, which has ten neurons and obtained by using the Levenberg-Marquardt training algorithm, in the training, validation and testing stages are given in Figure 5 and Figure 6 for LDPE (A) and (B), respectively.

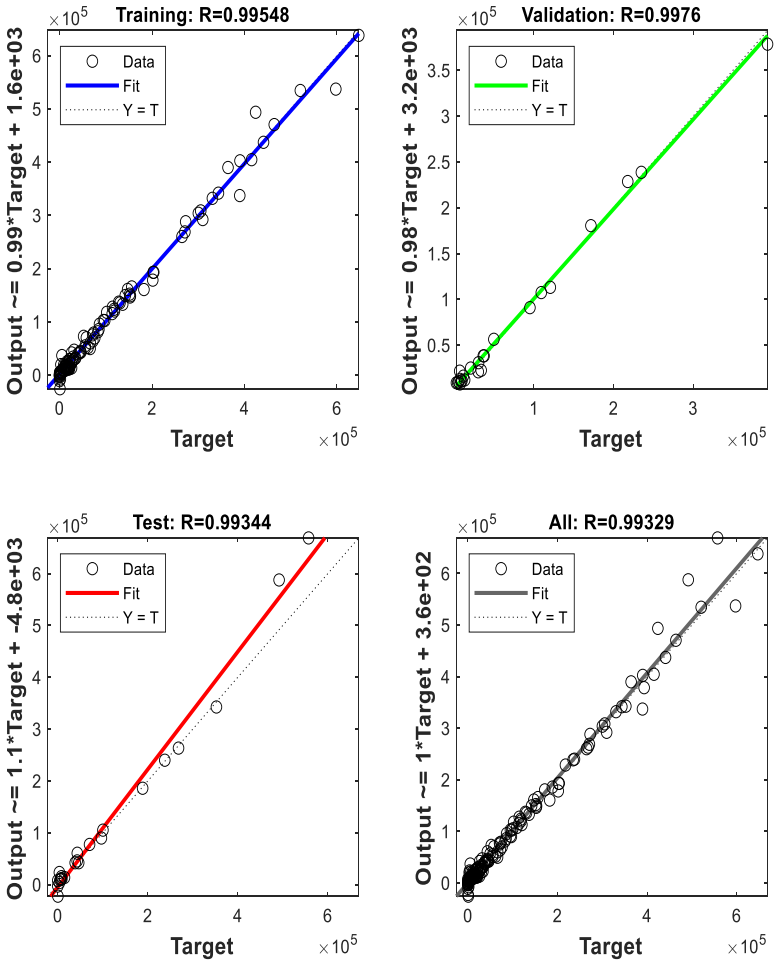

Figure 5. Training, validation and testing performances of the Lev-10_A model

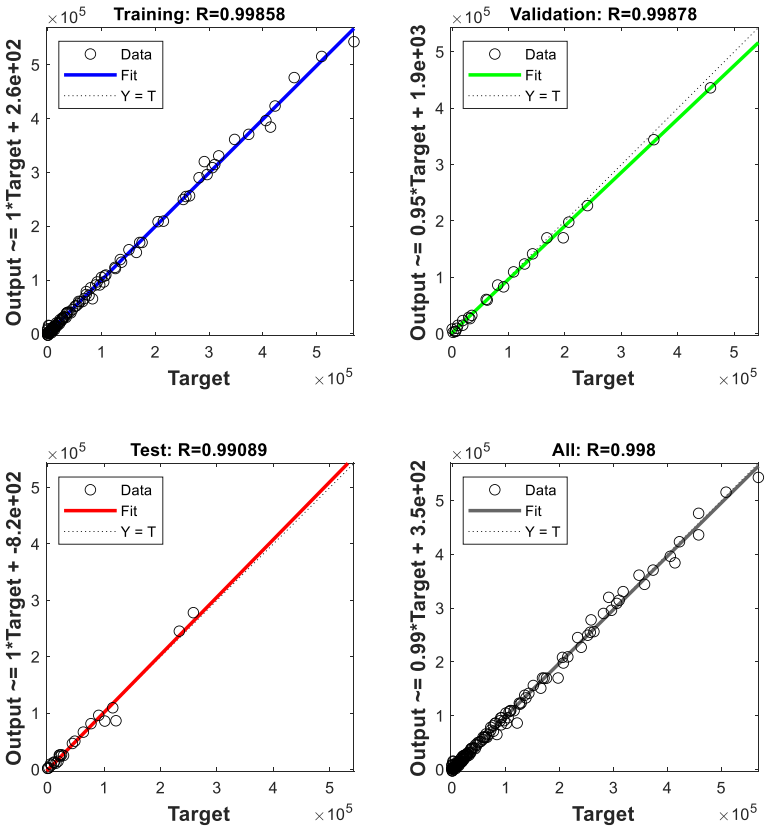

Figure 6. Training, validation and testing performances of the Lev-10_B model

Figures 5 and 6 show that Lev-10_A and Lev-10_B models are quite successful in both training, validation, and testing phases. The correlation ratio between predicted and experimental values is quite high. Comparison of the best and worst neural network models for LDPE (A) is presented in Figure 7. When Figure 7 is examined, it is seen that the Lev-10_A model successfully predicts the complex modulus values of the modified bitumen binder. It is clearly seen in the graph that the most unsuccessful model, Sca-12_A, cannot provide high accuracy rates and its prediction performance is low.

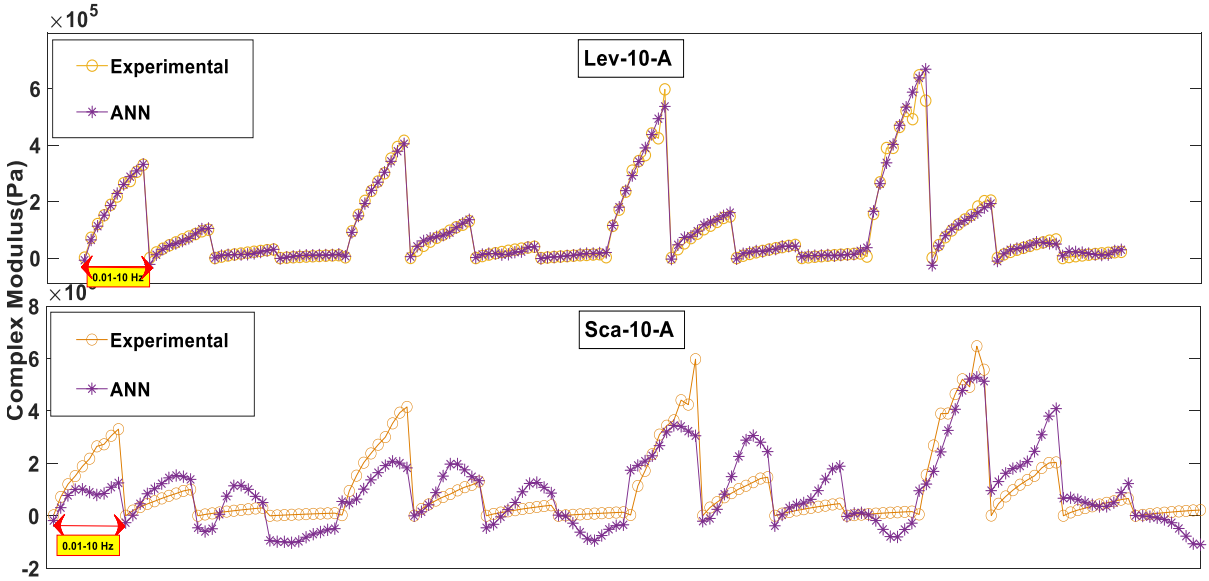

Figure 7. Comparison of the most successful and most unsuccessful neural network models

Conclusion

In the present study, a Dynamic Shear Rheometer experiment was performed on pure and modified binders by modifying the pure asphalt binder with two different types of waste low density polyethylene (LDPE A, LDPE B). Within the scope of the experiment, the binders were tested at four different temperatures including 40°C, 50°C, 60°C and 70°C and ten different frequencies in the range of 0.01-10Hz and complex modulus values were examined. In addition, Artificial Neural Network models were created with two different training algorithms and various neuron numbers and complex module values were obtained through these models. The results are given below:

- When the complex modulus values were examined, it was determined that the complex modulus values of the asphalt binders increased as the additive content increased, thus contributing to the resistance shown against deformations.

-When LDPE A and LDPE B were compared, it was seen that LDPE A contribution gave more positive results compared to the other contribution.

-It was seen that the artificial Neural Network models created successfully predicted the complex modulus values for both additive types. It was determined that the preferred number of neurons significantly affected the model performance.

-It was seen that the models trained with the Levenberg-Marquart training algorithm obtained more accurate results compared to the Scaled Conjugate Gradient training algorithm and had the lowest error rate when compared to the experimental data. The artificial neural network model trained with the Levenberg-Marquart training algorithm, which has ten neurons with the lowest error rate, gave the most accurate result.

-When the experimental data and the estimation data were compared in general, it was concluded that the complex modulus values of artificial neural networks and asphalt binders could be successfully estimated.

In this study, it was aimed to provide environmental and economic benefits by using two types of waste materials that are harmful to the environment as modifiers in bitumen binders. In addition, a lot of time and materials are spent for experiments performed in laboratories. Predicting possible results using artificial intelligence techniques is quite useful in terms of both time and materials used.

Scientific Ethics Declaration

The authors declare that the scientific ethical and legal responsibility of this article published in EPSTEM journal belongs to the authors

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Assessing Fire Safety in Sports Halls: An Investigation from Samsun

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Abstract: Sport is essential in terms of physical health. For this reason, sports halls are one of the most important places for a life intertwined with sports. Together with its own sports halls, Turkey is one of the countries that understand the importance of sports. Beyond that, sports, along with their benefits, contains many risks. These risks may be caused by personal injuries and disabilities and equipment, materials, and physical space. There are measures to be taken due to the importance of sports halls, which are insufficient numbers to meet the needs of the sport in Turkey. One of the leading measures is against fire. Precautions to be taken against fire in sports halls are extremely important for athletes and spectators due to many people's physical structure and building. Fire is an event that occurs suddenly and is a danger that can seriously harm life and property. Fire safety is all of the measures taken to prevent and reduce fire to avoid the loss of life and property and to minimize the loss when it reveals. In addition, these precautions taken against fire from the design stage to the usage stage enable the users in the fire environment to move away from there quickly. Despite the importance of sports halls, unfortunately, Turkey has no clear statistical information regarding fires in sports halls and their causes. However, some historical data is vital in terms of measures to be taken against fire. It is considered essential to investigate and analyze fire safety and possible fire hazards in sports halls in this context. In this study, a sample sports hall is examined in the context of fire safety. Due to the importance of fire hazards, precautions to be taken against fire in the building are emphasized, and various suggestions are made.

Keywords: Sports, Sports Hall, Fire Safety

Introduction

The time to be devoted to rest and entertainment, which arises due to the fast living habit in today's conditions, is necessary for physically and mentally healthy individuals (Timur et al., 2011). Sports halls are among the most important places for a healthy life and a life intertwined with sports. Sports fields are open and closed spaces with athletics, swimming pools, tennis, volleyball, basketball, and football that meet the sport of people of all ages. Besides, urban and natural areas are also used for sports activities. In addition, sports halls include the purpose of creating an athlete infrastructure as well as meeting individual sports needs (Gençlik ve Spor Bakanlığı, 2019a).

The Ministry of Youth and Sports carries out stadiums, swimming pools, football fields, sports halls, athletics tracks, and similar sports facilities throughout the country and the maintenance, repair, and modernization of existing facilities. In addition, The Ministry builds sports facilities to be used in various international sports organizations it undertakes (Gençlik ve Spor Bakanlığı, 2020). In the 2019-2023 Strategic Plan of the Sports Services General Directorate affiliated to the Ministry, it is stated that there are 3567 sports halls in the current situation. In addition, it is mentioned that it is aimed to increase to 3863 in total, adding 48 of them in 2019, 80 in 2020, 65 in 2021, 50 in 2022, and 53 in 2023 (Gençlik ve Spor Bakanlığı, 2019b). Based on these data, it is seen that the presence of sports halls is sufficient for Turkey. The fact that sports are an element that entertains society and is the basis of a healthy life enables sports to reach the masses. As a result, participation in sports for entertainment and health increases day by day (Denizci, 2019). In addition, regardless of the establishment purpose and characteristics of sports facilities, certain general principles should be followed in the architectural

design. Ergonomic approaches, the comfort of use, health and safety principles should be considered for the human factor that will use the spaces (Karel, 2011).

There are some measures to be taken due to the importance of the sports halls, which are insufficient to meet Turkey's sports needs. One of the leading measures is against fire. Precautions to be taken against fire in sports halls are extremely important for athletes and spectators. It is a building with collective use. Unfortunately, Turkey has no ready-made statistical data regarding fires in sports halls. However, some historical data is vital in terms of precautions to be taken against fire.

Fire is an event that occurs suddenly and is a danger that can seriously harm life and property. At the same time, although there is a risk of fire in buildings, it is a precautionary phenomenon to prevent its onset and to minimize the damage (Şimşek & Çatıkkaş, 2020). Fire safety is all of the measures taken against fire to prevent and reduce fire to prevent loss of life and property and minimize the loss. In addition, measures are taken against fire to ensure that the users in the environment move away from the environment quickly. These measures against fire can be taken from the design stage to the usage stage. In this respect, the study aims at examining Mustafa Dağıstanlı Sports Hall, adjacent to Ondokuz Mayıs University Fine Arts Campus, in the context of fire safety, and drawing attention to the issue due to the importance of fire hazard.

Research Area

Within the scope of this study, Mustafa Dağıstanlı Sports Hall, located next to Ondokuz Mayıs University Fine Arts Campus, is analyzed in the context of fire safety. The Sports Hall is located within the borders of Samsun province, İlkadım district and takes its name from the Olympic and world champion freestyle wrestler Mustafa Dağıstanlı. Figure 1 shows its location and immediate surroundings.

Figure 1. Location of the building (Source: Google maps)

The sports hall is located near Atatürk Boulevard, one of the leading transportation roads, and Adnan Menderes Boulevard in Samsun. The building entrance and its surroundings, which are not affected by heavy traffic, generally do not encounter parking problems during the day except for sports competitions. Since it is in a central area, it is possible to reach the hall by different means of transportation such as tram, bus or minibus. Athletes who come to the hall with their parents provide transportation by private vehicle. There are no problems in terms of ambulance and fire brigade transportation.

The main entrance of the sports hall was built from the east, considering Adnan Menderes Boulevard. In addition, there is an entrance to the building from the west and north directions, and internal and external stairs can provide transportation between floors. The ground floor level is kept lower than Adnan Menderes

Boulevard, and the stair steps and ramps at the entrance are used to reach the ground level. Figure 2 shows the facades and entrances of the hall.

Figure 2. Entrances of the building (Source: Google maps)

Mustafa Dağıstanlı Sports Hall was opened in June 2013. The hall has three floors and has a spectator capacity of 2000 people. Except for pandemic conditions, it is possible to do sports in the hall every day of the week. There are basketball, volleyball, shooting, judo, taekwondo, karate, kickboxing, kung fu, muaythai, boxing, wrestling, fencing, table tennis, weightlifting, badminton, chess, goalball, wheelchair, basketball branches in the hall (Mustafa Dağıstanlı Spor Salonu, 2021b). There are free sports opportunities in these sports branches, but people who want to do sports must obtain a medical report and a license.

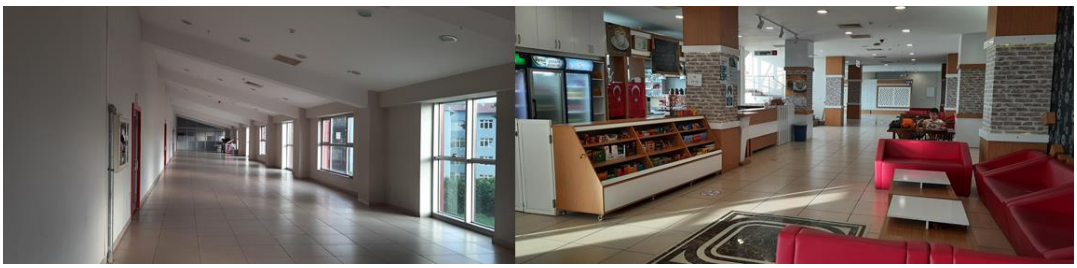

Figure 3. Some views from the interior volume (Source: personal archives)

Figure 3 shows some images from the interior volume of the hall. There is a canteen for athletes and spectators. The canteen service is located on the top floor, taking into account the number of spectators coming to the basketball court. Other athletes who want to benefit from the canteen service have to reach the service from other floors. The basketball court area, which constitutes the largest area of the sports hall and hosts other organizations such as national celebrations and various concerts, is covered with a steel roof that crosses a long distance. The structural system was built as a reinforced concrete carcass. Figure 4 contains images from the basketball court.

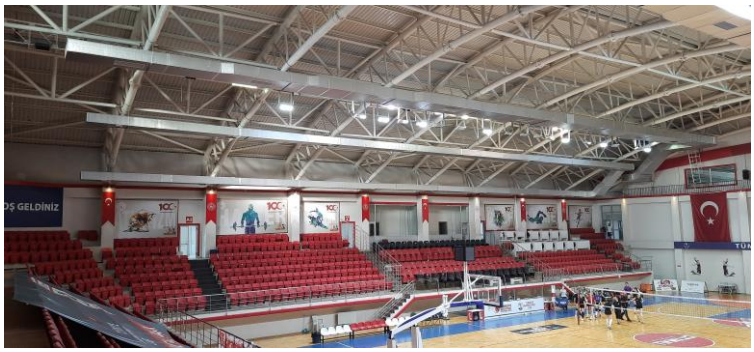

Figure 4. The basketball court (Source: Personal archives)

Within the scope of this study, various investigations have been made in the context of the measures taken against a fire that will occur in terms of athletes and spectators using the building, and the subject is tried to be analyzed in-depth within the scope of the findings.

Findings

In this study, Mustafa Dağıstanlı Sports Hall Building is examined within the framework of fire safety. Much progress has been made on fire safety in developed countries, but the history of fire protection measures in Turkey is very recent. In Turkey, The Regulation on the Protection of Buildings from Fire (BYKHY) entered into force in 2002 (Bodur, 2020). The examinations and analyzes carried out in this study within the scope of the regulation are detailed below.

The usage classes of the buildings are explained in the third part of the regulation. Accordingly, the sports hall included in the research is in buildings for collective use in the regulation. In addition, the hazard classes of the buildings are also specified in the regulation. However, there is no precise regulation about which type of danger class open and closed sports areas fall into. On the other hand, considering that other meeting areas such as museums, cinemas, theaters, concert halls are in the middle danger class, it can be stated that open and closed sports halls are also in the middle danger class.

Figure 5. Site plan (Source: Google maps)

When the location and site plan of the hall is examined, it is seen that there are no problems in terms of transportation and access. The layout plan of the sports hall is shown in Figure 5. Access to the hall located adjacent to the Fine Arts Campus is accessible from the coastal road (Adnan Menderes Boulevard) and Atatürk Boulevard. It will be straightforward for the fire brigade to reach the building during a fire, and this situation is considered positive regarding fire safety.

In the Regulation (Article 22), it is stated that "The horizontal distance from the last point where fire trucks can approach to any point on the exterior of the building can be 45 m at most". This provision could be provided for the building. As shown in Figure 5, fire trucks can access it from every facade of the building in case of a fire. In addition, there are hydrants on both sides of the building entrance for the fire brigade to use. Figure 6 shows the location of the hydrants.

Figure 6. Fire hydrants (Source: Personal archives)

Figure 7 shows the 1st-floor plan of the sports hall. As shown in Figure 7, the building's structural system is reinforced concrete, and the roof is covered with steel material. The fire resistance of the carrier system is evaluated by its load-carrying capacity, integrity, and insulation (Regulation, Article 23).

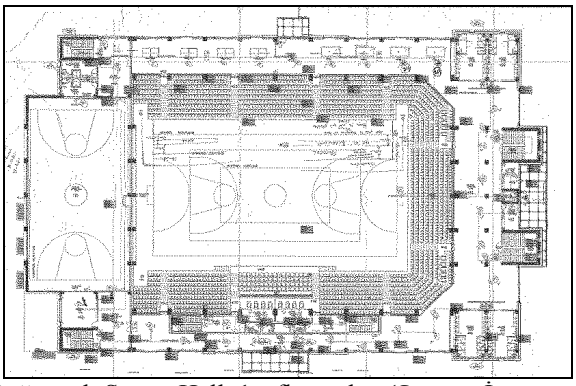

Figure 7. Mustafa Dağıstanlı Sports Hall, 1st floor plan (Source: İremnur Öztürk et al., 2020)

The primary purpose here is to evacuate people from the area in a sufficient period during the fire. For this to happen, the reinforced concrete and steel bearing system must be designed to remain stable. Provided that other standards and regulations are complied with, according to the BYKHY regulation, it is considered sufficient to insulate the steel elements in a temperature not exceeding 540ºC. It can be stated that the selected building was designed in terms of the structural system to allow people to evacuate from the area in case of a fire.

There are no fire compartments in the building. The criteria for fire compartments are given in article 24 of the regulation. According to this article, it is stated that "For non-residential buildings with a building height of more than 21.50 m and in residential buildings with a building height of more than 30.50 m, a maximum of three floors are arranged as a fire compartment". For this reason, it is not necessary to build a fire compartment in the sports hall, which does not pose a problem in terms of fire safety for the building. In addition, the regulation states that firewalls are built to separate adjacent regular structures from each other. Mustafa Dağıstanlı Sports Hall does not have an adjacent regular structure; therefore, firewalls were not required. In the fire regulation, it is stated that

1. all floors must be firewall nature,
2. The material interposed in gear floors should be the least hardly flammable,
3. The floor coverings should be covered with a screed layer of at least 2 cm thickness if normal flammable and if easily flammable heat insulation is applied.

When the building is evaluated, it seems complicated to say that an adequate and effective measure was taken against fire. In Figure 8, the floor coverings of the wrestling, weightlifting, taekwondo, and table tennis halls are shown, respectively. As seen in the figure, some halls have rubber-based flooring. Accordingly, it does not seem very easy to say that the floor coverings meet the regulation requirements.

Figure 8. Floor coverings of some training halls (Source: Personal archives)

The materials used in exterior facades are in A1 class and do not contribute to burning during a fire, as shown in Figure 9. It can be accepted that there will not be a problem in terms of fire safety, as the regulation accepts that these materials provide all the specified features sufficiently.

Article 28 of the regulation is about the measures to be taken against fire in the construction of roofs. The roof of the sports hall has a steel structure. In case of fire, it may be possible for the roof covering to form flame

drops, move to the neighboring roof in case of fire, and spread the fire under and inside the roof. This rule is not the case in terms of the selected building.

Figure 9.View from the building facade (Source: Personal archives)

What should be for construction materials is dealt with within the scope of Article 29. In the context of fire safety, it is not allowed to use easily flammable building materials in construction, except when converting into a regular flammable material in a composite. It is reported that structures with more than 100 people are made of the least hardly flammable material. In some training halls in the building, the flammability of the materials on the walls stands out as a factor that reduces the resistance to fire. Figure 10 shows images from some training halls.

Figure 10. Images from some training halls (Source: Personal archives)

Escape security principles are specified in Article 30. Escape safety is designed to enable people to move away quickly from the environment in emergencies such as fires. According to the regulation, escape measures should not be considered individually. They should protect people from the dangers arising from heat, smoke, and panic. They should have a suitable environment for all users to escape, and each exit should be visible. There are necessary directions for escape routes in the sports hall, but the corridors and stairs used for escape are the same as those used daily. In addition, Article 30 states that "… any gate or road leading to an exit that does not have an exit characteristic shall be arranged or marked so as not to be confused with the actual exit…". If this provision is not followed, the user will likely turn towards the building and other floors rather than the exit. Not all stairs in this building are fireproof, and this situation is not considered appropriate in terms of fire safety.

Article 31 explains the scope of escape routes. In the regulation, *escape routes* are defined as the whole of the continuous and unobstructed road from any point of a building to the street at ground level. Escape routes include exits from rooms and other independent spaces, corridors and similar passageways on each floor, floor exits, stairs reaching the ground floor, roads leading from the stairwells to the last exit of the building on the same floor, and the final exit. However, elevators are not included in escape routes. However, the exit capacity and escape distance are covered under article 32. According to the article, the building exit and capacities are designed sufficient in the Mustafa Dağıstanlı Sports Hall. Figure 11 shows an escape route in the building.

According to the regulation (article 33), the number and width of the escape routes cannot be less than the value found by multiplying the total number of users in the usage areas on a floor by the number of people passing through the unit width by 0.5 m. Accordingly, the user load coefficient in the hall should be three m² / person, and the escape route width should not be less than 150 cm. It can be stated that the number and width of escape routes are sufficient in Mustafa Dağıstanlı Sports Hall. In the 34th article of the regulation, the standards of fire

safety halls should be explained. Unfortunately, there are no fire safety halls in the building. With the emergency elevator, it is mandatory to build a fire safety hall in front of the escape ladder in buildings with a building height of more than 51.50 m. Since the building height of the sports hall is less than 51.50 m, there was no need for fire safety halls in the building.

Figure 11. View from an escape route (Source: Personal archives)

According to Article 35, as long as the building is in use, the obligatory exits should be easily accessible, the doors can be opened, and there should be no obstacles in front of them. In this respect, there is no problem in the building. The doors can be opened, and there are no obstructions in front of them. There are no protected internal escape corridors and passages (although specified in article 36) in the building. This situation is not favorable in terms of fire. External escape passages specified in Article 37 are not available in the building. However, this situation does not create a negative situation for the building in terms of fire safety.

Information on escape ladders is described in Article 38. According to the regulation, the standard stairs of the building that can be used in case of fire and other emergencies are accepted as escape stairs. Escape ladders are part of the whole of escape routes used in fire and other emergency evacuation and cannot be designed independently from other escape routes. When evaluated from this point of view, there are no stairs in the building that can be described as escape stairs. According to Article 39, in all structures, unless otherwise specified, at least two exits must be established, and the exits must be protected and as far from each other as possible. In this respect, the building appears to meet the requirements.

According to Article 40 of the Regulation, no matter where the fire occurs, escape routes and escape stairs must be positioned as alternatives to each other in order to ensure the exit of all people at that level. Escape routes and escape ladders must not be built side by side, and the location of the staircase nests should be chosen to facilitate the safe escape of the people in the building. In basements and high buildings, escape stairs must be entered through a fire safety hall or a protected hall. When evaluated from this point of view, it can be stated that the stairs in the examined building were designed to be alternatives to each other. However, they were not made conservatively, and no special precautions were taken, and this situation is not suitable for fire safety.

The features of the escape ladder are specified in Article 41. It is impossible to talk about a protected staircase in the form of an escape ladder in the building. Fire safety in the building is found to be negative in this respect. External escape stairs are dealt with in article 42, circular stairs in article 43, and escape ramps in article 44. There are no external escape stairs, circular stairs, and escape ramps in the building.

In Article 45, the essential features of escape ladder ventilation are specified. In Article 46, the conditions of the basement floor escape stairs are explained. According to this article, in case of an emergency, the ground-level platform of the staircase should be separated from the basement staircase by a door or similar physical obstacle, or an appropriate direction should be made. In the sports hall, regular stairs go down to the basement floor, but there is no proper orientation in the building. According to article 47 of the regulation, escape route doors must be at least 80 cm and have no thresholds. Besides, revolving doors and turnstiles cannot be used as exit doors. The building does not have a revolving door, and the door widths are sufficient.

Special regulations for buildings intended for collective use are explained in Article 51 of the Regulation. However, the article contains explanations for theaters, cinemas, auditoriums, concert halls, and no specific explanation for sports halls. In the "Building Sections and Facilities" section of the regulation, the measures to be taken for the parts of the buildings such as boiler rooms, fuel tanks, stoves and chimneys, shelters, parking lots, kitchens, roofs, elevators, lightning protection installations, transformers, and generators are shown. Adequate measures for these areas were taken in the examined building.

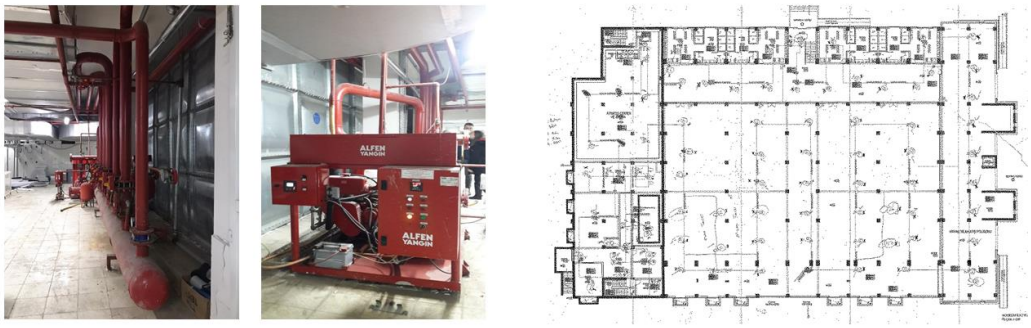

Figure 12. Fire extinguishing system and detection plan (Source: İremnur Öztürk et al., 2020)

Figure 12 shows the fire alarm and extinguishing systems in the building. As seen in Figure 12, there is also a fire detection system in the building. The detection system is directly related to the alarm and extinguishing system (the building has a sprinkler system) and is automatically activated in the event of a fire.

Evaluation and Conclusion

This study was planned to examine the Mustafa Dağıstanlı Sports Hall within the fire legislation framework and suggest the subject. The sports hall has a collective use. In the 15th article of BYKHY Regulation, a building for collective usage refers to all buildings or parts used for this purpose, where 50 or more people can come together for reasons such as ceremony, worship, entertainment, eating, drinking, transportation, and vehicle waiting. Hence, The fact that the number of users and their characteristics are variable and high in sports halls increases the importance of the measures to be taken against fire. For this reason, passive measures such as escape ladders, compartments, and active systems such as detection, warning, and guidance systems must be considered together against fire.

In buildings such as sports halls, due to the high number of users and why the users do not know the building sufficiently, the exit places and access routes to the escape stairs should be clearly described with direction signs and made visible with illuminated signs. These measures are of vital importance in terms of life and property safety. It is observed that in the sports hall examined within the scope of the study, the necessary conditions are met in terms of the absence of an adjacent regular structure, the access of fire trucks to the building, the fire evacuation plan, the sprinkler system, and the presence of directional signs. The most critical deficiencies in the measures taken within the fire safety framework in the building can be stated as the unsuitability of escape stairs and the possibility of the materials used in the flooring of some halls to release toxic gas during a fire. For this reason, measures for escape routes and stairs of sports halls should be put forward at the design stage. Considering the spread of smoke in the building, materials that will produce toxic smoke pose a significant risk. Minimizing the use of such materials would be appropriate in terms of fire safety. In addition, to prevent the fire from spreading to other areas, it is necessary to take measures such as a suitable compartment to prevent the spread of smoke between the sections or smoke screening.

It is essential for passive fire safety precautions that the sport hall managers know the fire hazard. Sports hall management and employees should be given training at regular intervals; drills should be made. It should be ensured that they are cautious against the situations that may arise during the fire. In the event of a fire, daily sports activities will be affected, and athletes and spectators. For this reason, it is crucial to train the personnel in terms of fire and control active and passive fire precautions.

People using sports halls must protect themselves in the event of a fire and leave the building safely. The measures not taken against fire will have severe consequences for the users. For this reason, every precaution must be taken against fire in buildings such as sports halls. One of the most important of those is the measures to prevent the outbreak of fire. In conclusion, one of the most critical ways of not putting users at risk in sports halls is through fire-fighting measures.

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Scientific Ethics Declaration

The author declares that the scientific ethical and legal responsibility of this article published in EPSTEM journal belongs to the author.

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IMSD: Interactive Methods for Finding Similar or Diverse Answer Sets

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Abstract: Answer set programming (ASP) is a modeling language in knowledge representation, rooted in Logic Programming and Nonmonotonic Reasoning, which has been gaining increasing attention during the last years. In recent years, many of the researchers developed integrated development environments (IDE) for ASP programs including editors and debuggers. Other researchers focused on analyzing the answer sets, they introduced offline and online methods to find specific solutions of a given problem in answer set programming in different approaches such as phylogeny reconstruction. However, with an enormous number of answer sets could be available, the user is not interested in all of them. Thus, a navigation of the search space could be a solution to help the user to access the specific answer sets. To this end, we aim at finding similar/diverse solutions of the answer sets with a new method. The intuition behind this navigation is to make the search faster than other methods and explore information that is related to the user's query. Afterward, we implement a tool performing the above approach in order to simplify the search task and show the applicability and effectiveness of our method. We conclude by testing the performance of the proposed tool into a real-world example of ASP programs.

Keywords: Answer set programming, Navigation approach, Diversity, Similarity

Introduction

It is worth finding solutions of the answer sets which are similar/diverse to each other. For instance, in planning, it might be useful to compute a set of similar plans. Therefore, when the execution of the plan fails, one can switch to a very similar one. Towards this goal, we represent a problem at hand by a logic program, such that its answer sets correspond to solutions. These solutions characterize the solutions of the original problem, and then, use an answer set solver to find such solutions. In the last few years, many solvers are developed, such as, Clasp (in conjunction with Gringo), DLV, Clingo (Gebser et al., 2014), and SMODELS (*Computing the Stable Model Semantics*, n.d.).

On one hand, the researchers have turned their attention to develop different integrated development environment (IDE) for ASP programs including editors and debuggers (e.g., APE (Fandinno et al., 2019), iGROM, SeaLion (Busoniu et al., 2013)), and like the online development environment for answer set programming (Marcopoulos et al., 2017). On the other hand, some of them have developed tools to visualize the answer sets and their relations by means of a directed graph, such as, ARVi tool (Ambroz et al., 2013).

Despite these improvements, there is a lack of attention to analyze the answer sets themselves. In some particular problems, a massive amount of answer sets could be available. However, the user is not interested in all of them. In (Afeefi, 2019) we implemented different navigation approaches, such as, one case of finding diverse/similar solutions to help the user to access the specific answer sets. To this end, we are looking into another two cases for finding diverse/similar solutions. The intuition behind this navigation method is to make the search faster and explore information that is related to the user's query.

Related Work

Analysis of answer-set programming (ASP) is one wide field that is increasingly growing in the last few years. At first, several tools have been designed to support the user in developing ASP applications, and the visualization aspects of these tools focus on the representation of single answer set. Eiter et al introduce offline and online methods to find similar or diverse solutions of a given problem in answer set programming in phylogeny reconstruction (Eiter et al., 2009). They study two kinds of computational problems related to finding similar/diverse solutions of a given problem, in the context of ASP: one problem asks for a set of n solutions that are k- similar (resp. k-diverse), the other one asks for a solution that is k-close (k-distant) to a given set of solutions.

On the other hand, different tools for developing ASP programs have been proposed including editors and debuggers. Koziarkiewics implemented iGROM (*IGROM Download | SourceForge.Net*, n.d.) which is an IDE for ASP programs specifically those written in DLV (and its frontends) and Smodels. It provides some features, such as syntax highlighting for DLV and its dialects, error detection for DLV and dialects.

In (Sureshkumar et al., 2007) Sureshkumar et al. implement an Integrated Development Environment (IDE) for ASP, the AnsProlog* Programming Environment (APE). It offers many features, like syntax highlighting, automatic syntax checking, integration of editor; LPARSE and SMODELs, and display dependency graph of program.

Recently, Oetsch et al. in (Busoniu et al., 2013) design an IDE for ASP (SeaLion) as a plug-in for Eclipse platform. This tool provides source-code editors for the languages of Gringo and DLV. It offers functionalities, like syntax highlighting, syntax checking, code completion, visual program outline, and refactoring functionality.

Ambroz et al. (Ambroz et al., 2013) present a new tool, ARVis. The main purpose of ARVis is to visualize answer sets and their relations by means of a directed graph. The general idea for this tool is passing the answer sets of a first user-specified ASP encoding to a second user-specified encoding which specified the relations between them. Obviously, ARVis is not designed to obtain a high performance since a potential exponential number of answer sets of the first program has to be processed by the second one.

As discussed above, some approaches are much more focused on editing and debugging ASP programs. Others are developed for a certain problem. To the best of our knowledge there does not exist a tool yet that is capable of navigating the space of answer sets for general problems.

Computing Similar/Diverse Solutions

Towards this goal, we study finding similar/diverse solutions in answer set programming. The computation of similar and diverse solutions is symmetric. Thus, we focus on finding the diverse solutions. This section introduces Preliminaries, a preprocessing and so-called (modified) interactive method to compute the diversity of solutions.

Preliminaries

We introduce the graph structures used to internally represent the answer sets. Then, we continue with Hamming and Jaccard distances which are the measures for similarity/diversity of the solutions.

1) Graph: Graphs are common fundamental data structures in knowledge representation. We use graphs to represent a set of objects and the relationship between pairs of objects. A graph is defined as the structure $G = \langle V, E \rangle$ representing a set of vertices V (also called nodes) and a set of edges $E \subseteq V \times V$. There are two types of graphs, directed and undirected. In our work, we consider an undirected graph. All the edges in the undirected graph are bidirectional. A complete graph is a simple undirected graph in which every pair of distinct nodes is connected by a unique edge. The complete graph on n nodes has $n(n-1)/2$ edges.

Given a graph G, each edge in E of G might be associated with a real number, then called its weight. G together with these weights on its edges, is called a weighted graph. We therefore exploit this property to express the weight of the edges by a distance, e.g., Hamming distance or Jaccard distance. In this work, the answer sets are internally represented as the nodes of the graph. The edges are labeled by Hamming or Jaccard distances between pairs of nodes.

2) Clique: A clique in a graph G is a complete subgraph in G, that means, it is a subset S of the vertices V such that each two vertices in S are joined by an edge in G. A maximal clique is a clique with the maximum number of vertices; no more vertices can be added. In this work, we are interested to find a maximal clique to obtain a largest complete subgraph. To demonstrate, if the user needs n answer sets that differ in k atoms, we need to find a maximal clique with size n or greater than n that differ in k atoms. Reporting the maximal cliques of a graph is a major problem arising in graph structures. The output of maximal clique enumeration algorithm may be exponentially sized, so that an algorithm with provably good running time w.r.t. the input size is not possible. However, any algorithm reporting all maximal cliques should be output sensitive.

There is a comprehensive bibliography of clique enumeration algorithms. For instance, Bron-Kerbosch (BK) algorithm (Bron & Kerbosch, 1973) and new algorithms with an alternative strategy based on matrix multiplication (Makino & Uno, 2004). Recently, all papers acknowledged BK algorithm as the best one in practice (Baum, 2004). We choose herein BK algorithm to find diverse/similar answer sets. In (Bron & Kerbosch, 1973), Bron and Kerbosch report two algorithms, version 1 and version 2. Version 2 is an optimization of version 1 based on pivots or fixed points. In this work, we implement Bron-Kerbosch (version 2) algorithm to find maximal cliques of the graph whose nodes are the answer sets.

3) Hamming Distance: Hamming distance is used to measure similarity and diversity between two sequences. It is limited to cases when two sequences have the same length. The Hamming distance is defined to be the number of positions at which the corresponding symbols are different. The sequences may be strings or binary vectors (*Fakecineaste : How to Calculate the Hamming Code*, n.d.). Similarly, for answer sets, the length of two answer sets (number of atoms) should be the same. Each answer set is represented as a vector of boolean values. We compare the first contents of the two indexes in each vector. If they are the same, record a "0", otherwise, record a "1" for that index.

4) Jaccard Distance: A very simple and often effective approach to measure the similarity and dissimilarity between non-empty finite sample sets is the Jaccard index. The Jaccard index (Deng et al., 2012), also known as Jaccard coefficient is used to compare the similarity and diversity of non-empty finite sample sets. The Jaccard coefficient is defined as the size of the intersection divided by the size of the union of the sample sets. The Jaccard distance is complementary to Jaccard coefficient and is obtained by subtracting the Jaccard coefficient from 1. Similarly, we can define the Jaccard distance by dividing the difference of the sizes of the union and the intersection of two sets by the size of the union.

The Jaccard coefficient measures the similarity between the non-empty finite sample sets, but the Jaccard distance measures the dissimilarity between the non-empty finite sample sets. For the answer sets, we use this measure when we have different lengths of the answer sets.

Preprocessing

In the preprocessing method, we compute all the answer sets of an ASP program by running an ASP solver. The answer sets are stored with their cardinality in a database. At the same time, we get and store the set of all ground atoms of the answer sets in a text file. After that, we create a hash mapping data structure which maps each ground atom in the file to an integer number. From the set of all ground atoms, we can check whether is in an answer set or not in order to build the boolean vector for computing distance purpose. We build a complete undirected graph $K = \langle S, E \rangle$ whose nodes S correspond to the answer sets AS and edges $E = \left\{ \{s_i, s_j\} \mid \forall s_i, s_j \in S, s_i \neq s_j \right\}$ are labeled by a function $L : e \rightarrow \mathbb{N}$ that maps each $e \in E$ to a natural number (the distance), such that, $L(\{s_i, s_j\}) = d(s_i, s_j)$. The distances between the corresponding answer sets are calculated by Jaccard or Hamming distance. Additionally, we store the value of maximum (resp., minimum) distance, denoted by d_{max} (resp., d_{min}) between the answer sets for computing diversity/similarity.

Interactive Method (IM)

We study various problems to find similar/diverse answer sets of the given ASP program. As in illustration, the user can specify the number of the answer sets that differ in a certain number of k atoms. More precisely, if the user needs n different answers and specifies a relational operator (e.g., $\leq$) and k atoms then, the result n should be different in $\leq k$ atoms. There are two distances we use for edges in this work. Hamming distance for the answer sets with the same length, and the Jaccard distance for the answer sets with different lengths. After we build a complete undirected graph K in the preprocessing method, we check whether there exists a complete subgraph (or a clique) of size n in K whose distance is specified by the user. In this work, we find a maximal clique to obtain the largest complete subgraph (Makino & Uno, 2004). In detail, if the user needs n answer sets that differ in k atoms, a maximal clique will be found with size greater than or equal to n. Each node in the maximal clique corresponds to an answer set, so it represents exactly one answer set.

Definition. Let A and B be sets. The set A corresponds to the set B, denoted by $\approx c$, where $A = \{a_1, \ldots, a_n\}$ and $B = \{b_1, \ldots, b_n\}$, such that

$$A \approx c\, B \;\; iff \;\; \forall b_i \in B \;\; a_i = b_i$$

where $1 \leq i \leq n$.

We are mainly interested in two cases of problems related to the computation of a diverse/similar answer sets:

Case 1 (n-Most Diverse Answer Sets (resp., n-Most Similar Answer Sets))

Instance. Given a complete graph $K = \langle S, E \rangle$ whose nodes $S \approx c\, AS$ (answer sets) of an ASP program P where $E \subseteq S \times S$, a non-negative integer n, and the value of the maximum distance d_{max}.

Question. Does there exist a set S_1 with the cardinality $|S_1| \leq n$ where a complete subgraph (clique) $K{\downarrow_{S_1}} := (S_1, E_1 \subseteq S_1 \times S_1)$, $E_1 \subseteq E$ and $S1 \subseteq S$, and the distance of the set S_1, denoted by $d_s(S_1)$, is maximum (resp., minimum) distance between each pair of its elements such that

$$d_s(S_1) = d_{max} \;\; iff \;\; \forall s_i, s_j \in S_1$$

$$d_{max} = max\{d(s_i, s_j) \mid \{s_i, s_j\} \in E\}$$

where $1 \leq i, j \leq n$.

To demonstrate, given a complete undirected graph K whose nodes are the answer sets and the edges are labeled by the distance between pairs of the nodes. The user specifies an integer number n and the value of maximum distance d_{max} (and for minimum distance d_{min}) are stored during the preprocessing method; n is the number of the answer sets that differ in d_{max} atoms. A clique of the size at most n with distance equal to dmax is picked from K. The motivation for finding the clique with distance d is to find corresponding answer sets which are different in d_{max} atoms. Algorithm 1 shows Case 1 of the interactive method.

As an illustration, we find n-most diverse answer sets (resp., n-most similar answer sets) by calculating the maximum (minimum) value of the distance and check whether there exists a maximal clique with this distance. If there is no maximal clique, the distance will be decreased by 1 (resp., increased by 1) until the maximal clique is found (Figure 1).

Notations. We shall denote the graph $K' = \langle S, E' \rangle$. Given a node $u \in S$, $nbrs(u)$ denotes the neighbors of u, i.e. $nbrs(u) = \{v \mid (u, v) \in E'\}$.

Algorithm 1: `IMCase1`

Input: A complete graph $K = \langle S, E \rangle$.
A negative integer n.
The maximum distance d_{max} and the minimum distance d_{min}.
Output: A set S_1 of at most n answer sets whose distance is $d_s(S_1) = d_{max}$.

S_1 = **IMAlgo**($\langle S, E \rangle$, n, d_{max}, $=$)
if $|S_1| = \emptyset$ **then**
  while $|S_1| = \emptyset$ *and* $d_{max} \geq d_{min}$ **do**
    $d_{max} \leftarrow d_{max} - 1$
    S_1 = **IMAlgo**($\langle S, E \rangle$, n, d_{max}, $=$)
return S_1

Algorithm 2: `IMAlgo`

Input: A complete graph $K = \langle S, E \rangle$.
Two non-negative integers n and k.
A relational opertor op is one of $\{=, <, >, \leq, \geq\}$.
Output: A set S_1 of at most n answer sets whose distance is $d_s(S_1)$ op k.

$E' \leftarrow \{\{s_j, s_i\} \mid s_j \neq s_i$ denote $s_j, s_i \in S, d(s_j, s_i)$ op $k\}$
$P = \{S\}$
$R = \{\}$
$X = \{\}$
S_1 = **BronKerbosch**(P, R, X, E')

Algorithm 3: `BronKerbosch(`P, R, X, E'`)`

if $P \cup X = \emptyset$ **then**
  if $|R| \geq n$ **then**
    pick R as a maximal clique of size at least n
    $S_1 \leftarrow$ report the first n elements of R
    Output S_1
    terminate
choose a pivot $u \in P \cup X$
for *each vertex* $v \in P \backslash nbrs(u)$ **do**
  BronKerbosch($P \cap nbrs(v)$, $R \cup v$, $X \cap nbrs(v)$)
  $P \leftarrow P \setminus v$
  $X \leftarrow X \cup v$

Algorithm 4: `MIMCase2`

Input: The answer sets (AS)
Two non-negative integers n and k.
A relational opertaor op is one of $\{=, <, >, \leq, \geq\}$.
Output: A set S_1 of at most n answer sets whose distance is $d_s(S_1)$ op k.

$S \leftarrow$ Define a set of $|AS|$ vertices
$E \leftarrow \{\{s_j, s_i\} \mid s_j \neq s_i$ denote $s_j, s_i \in S, d(s_j, s_i)$ op $k\}$
S_1 = **IMAlgo**($\langle S, E \rangle$, n, k, op)

Figure 1.Algorithms

Case 2 Modified Interactive Method (MIM)

Instead of building a graph $K = \langle S, E \rangle$ of all the answer sets (nodes S) in the preprocessing method and then a clique is picked with specific distance value in the interactive method, we can build a complete subgraph (clique) with only the edges with the specific distance value that the user specifies. Our intuition behind building a clique during the interactive method which is so-called modified interactive method is to present only to the user the answer sets (AS) he is interested in. Thus, we save the memory since the complete graph K is not built in the preprocessing method. Additionally, the execution time is reduced of the preprocessing method. Algorithm 4 shows the modified interactive method case.

The inputs of the above algorithm are the answer sets (AS) that the user specifies, the two non-negative integer numbers n and k; n is the number of the answer sets that differ in k atoms, and a relational operator op with $op \in \{<, >, \leq, \geq, or =\}$. At first, a complete undirected graph K_m is built from AS whose nodes are S and the edges E are labeled by the distance between pairs of nodes. Then, the IMAlgo algorithm is invoked with specific arguments, such as, the complete graph K_m, n, k, and the relational operator op, to find the maximal clique and return a set S_1 of at most n answer sets whose distance is $d_s(S_1)$ op k. The interactive method and the modified interactive method are different only in the inputs.

NAVAS Tool

In this section, we describe the graphical user interface of Navigation Approaches for Answer Sets (NavAS) and explain step by step how to use the tool.

Description

The start window of NavAS tool is as depicted in Figure 2.

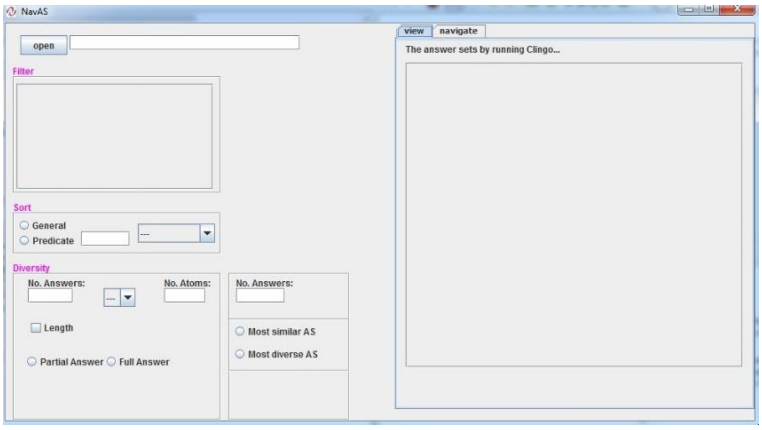

Figure 2. NavAS user interface

The starting point is to select a file with an *.lp* extension by the open button which is at the left top corner. Clicking on this button brings up a dialog box allowing to browse for the data file on the local file system. Once the user clicks on the open button, the answer sets as a result of running the Clingo solver, are displayed in a scrollable text area in the tab view in the right top corner.

Diversity Box

The box contains many components to find the diversity between the solutions. (see Figure 3). To illustrate, there are three components available on this box:

- ***No. Answers***. NavAS allows the user to type the number of solutions that he wants to show.
- ***No. Atoms***. The number of atoms that the solutions are different in.

The working scenario of the box is as following: "Show me the answer sets (No. Answers) that are different in one of $\{=, <, >, \leq, \geq\}$ of (No. Atoms)."

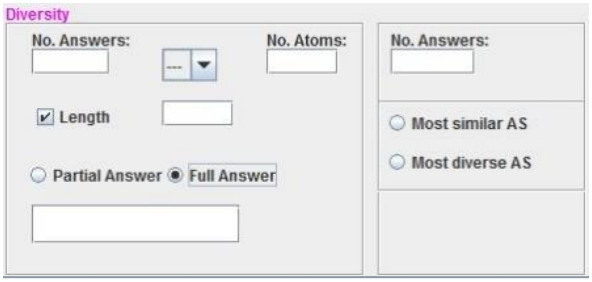

Figure 3. Diversity box

There is a length option which is applied when the solutions have different number of ground instances. The user can decide which answer set he wants as a pivot to find the diversity by either typing the full answer set or a part of it. To the right of the diversity box is the box for finding the most similar and diverse answer sets. The number of answer sets specifies by the user (No. Answers).

Evaluation

In this section, we make an evaluation of the performance of the NavAS tool. We consider a pizza configuration. We discuss the experiment of result on this example. We ran the experiment on an Intel machine with processor

2.30 GHz Intel Core i5 and memory 4 GB 665.1 MHz DDR3.

Pizza Example

This example is implemented in ASP to generate different pizza configurations. The input of the ASP program are the toppings with their categories to specify the type of pizza and the price of each of them. To accomplish this task, several rules and constraints on toppings have to be satisfied. Thus, a different number of toppings provide many different configurations of pizza.We consider herein a sample of the code to explain the facts of the program as input. In the following, we give a listing of some facts of the program (Figure 4).

```
price(bacon,110).
has_category(bacon,meat).
price(chicken,120).
has_category(chicken,meat).
price(peas,140).
has_category(peas,veg).
```

Figure 4. Facts of the program

We have a topping bacon which is indicated under meat category, and its price is `price(bacon,110)`. The same thing for other facts. We add many facts to the pizza program to increase the search space (Figure 5).

```
Answer: 1

on(dough) on(tomato_sauce)
on(mozzarella) on(oregano) on(bacon)
on(broccoli) on(caper) total(870)
normal

Answer: 2

on(dough) on(tomato_sauce)
on(mozzarella) on(oregano) vegetarian
on(caper) on(basil) total(750)
```

Figure 5. Code for increase the search space

Usually there are too many answer sets of pizza example computed by an ASP solver. The user needs to compare these answer sets, by analyzing the similar/diverse ones with respect to some distance measure. To this end, we use the tool on this example to evaluate the performance of it. For similarity and diversity, we do experiment for testing the computation time of finding diverse/similar solutions. We measure the execution time of the preprocessing and the interactive method. We take five samples for each value and compute the average of them.

We consider several parameters to assess the performance of the tool with respect to finding similar/diverse answer sets:

(1) number of answer sets, (2) preprocessing method, (3) (modified) interactive method. Table 1, 2, 3, and 4 report the time of the case of interactive and modified interactive method with different values of k (number of different atoms), $k = 1, 2$, respectively, and the number of solutions n (the maximum size of the maximal cliques corresponding to the value of k).

From Table 1 and 2, we note that the execution time for the preprocessing method is quite high because of the storing and building a complete undirected graph K. We can see that the execution time for the number of answer sets 2000 is higher than 1000. For the number of atoms that the answer sets are different, we note that the execution time for $k = 2$ is higher than the execution time for $k = 1$. In fact, this depends on the configurations of the answer sets of a problem, the distance between them, and the ordering of the answer sets in the complete graph K.

From Table 3 and 4, we note that the execution time for the preprocessing method is less than the execution time for the preprocessing method in the previous tables. The preprocessing method herein is only used to store the answer sets. The graph K is built during the modified interactive method with a specific answer sets that the user specifies.

Table 1. Time execution for $(k = 1 \text{ and } n = 3)$ for pizza example

ANSWER SETS (AS)	*PREPROCESSING (MINUTES)*	*INTERACTIVE METHOD CASE1 (MINUTES)*
1000 (137KB)	0.5441	0.0046
2000 (278KB)	1.3237	0.0150

Table 2: Time execution for $(k = 2 \text{ and } n = 8)$ for pizza example

Answer Sets (AS)	*Preprocessing (minutes)*	*Interactive Method Case 1 (minutes)*
1000 (137KB)	0.5441	0.0107
2000 (278KB)	1.3237	0.0505

Table 3: Time execution for $(k = 1 \text{ and } n = 3)$ for pizza example

Answer Sets (AS)	*Preprocessing (minutes)*	*Modified Interactive Method Case 2 (minutes)*
1000 (137KB)	0.4669	0.0525
2000 (278KB)	0.7868	0.4039

Table 4: Time execution for $(k = 2 \text{ and } n = 8)$ for pizza example

Answer Sets (AS)	*Preprocessing (minutes)*	*Modified Interactive Method Case 2 (minutes)*
1000 (137KB)	0.4669	0.0454
2000 (278KB)	0.7868	0.4073

In general, we note that the execution time for the modified interactive method case is higher than the execution time for the interactive method in the previous tables, because the time needed to build the graph K is included in the execution time for the modified interactive method.

Conclusion

There is a large number of the answer sets available of an ASP program, all of them are not of the user's interest. Thus, a navigation of the search space could be a solution to help the user to access a specific answer sets. We studied finding similar/diverse solutions of the answer sets. We offered scenarios to find similar/diverse solutions. To this purpose, we introduced preprocessing and interactive methods and applied some distance measures. Regarding practical use, we presented NavAS, a tool for navigating the answer sets for general ASP programs. Finally, we made an evaluation of the performance of NavAS tool with Pizza example. We recorded the execution time for finding diverse/similar answer sets.

Scientific Ethics Declaration

The author declares that the scientific ethical and legal responsibility of this article published in EPSTEM journal belongs to the author.

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Researching on Baby Diaper Performance with 3D Absorbent Layer

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Abstract: Disposable diaper manufacturers have focused on absorbent zone layers as a way to ensure consumers achieve optimum comfort and minimize the environmental impact of products. The producers who want to achieve the desired performance values in the absorbent core layers have been investigated the amounts / mixing ratios of raw materials such as superabsorbent polymer (SAP) and wood pulp (pulp) or their distribution on the absorbent layer. When the absorbent core layers consisting of homogeneous SAP / pulp mixtures are examined in the current diapers, it is seen that the urine is concentrated in the middle section and this amount decreases towards the front and back parts of the diaper. It can be said that the SAP / pulp mixtures, which are sectionally evenly distributed in the absorbent zone layer, are used less in the front and rear sections, that is, the absorbent layer has a 3D structure. It can be said that the SAP/pulp mixtures, which are distributed sectionally on the absorbent layer, are absorbed more intensely in the middle region, so the amount in the front and back parts has less effect on the performance of the diaper. When the diaper machines currently in our company were examined, it was seen that it has not be possible to produce diapers with a 3D absorbent zone layer. In this study the effect of transferring some of the SAP/pulp mixtures homogeneously distributed in the absorbent layer from the back sections to the center of the core section on the diaper performance was investigated with the 3D mold system designed in our company. As result of the tested diapers, it was observed that the performance of the product increased or remained constant by moving the SAP / pulp mixes moved behind the absorbent layer to the center of the core layer. At the same time, it can be said that it is a cost-reducing work since raw materials that are not used in the front and back sections are saved.

Keywords: Baby Diaper, Core Distribution, Sectional Retention, 3D Absorbent Layer, SAP, Fluff Pulp

Introduction

Disposable absorbent hygiene products are designed to absorb and retain body fluids and faecal matter (Malarvizhi, 2015; Shanmugasundaram, 2010). Among these products that appeal to a wide range of users; diapers, sanitary pads, tampons, incontinence products, panties and mostly disposable wet wipes (Krafchik, 2016). The global market for hygiene products is growing significantly, especially with disposable products. Although absorbent hygiene products face the challenges of rising raw material prices due to the global economy and the limitation of bio-based resources, many consumers prefer these products (Kumar, 2014). While the world diaper market size was 50.5 billion dollars in 2019, it is expected to increase to 5.7% growth between 2020 and 2030. The growing adult population around the world, the high rate of urbanization and the number of working women are some of the main growth factors driving the diaper industry. The COVID-19 pandemic, which started around the world in late 2019, has also seriously affected the diapers market. The

epidemic weakened the production capacities of diaper manufacturers due to the full or partial restrictions of most countries, causing the global diaper market to decline. Diapers with high absorbency have attracted great attention recently and took place in the diaper category with the largest market share in 2019. In addition, it is expected that high absorbent diapers will continue to dominate the market throughout the years 2020-2030. These diapers, which prevent leakage more than other diapers, are widely preferred because of the developments in Super absorbent polymer (SAP) technology. The growing demand for biodegradable, environmentally friendly diapers is another major driver for the worldwide diaper market. Disposable diapers only 2% of US landfill waste, meaning that most of them will not biodegrade for many years. Since conventional disposable diapers are non-biodegradable by nature, environmentally friendly biodegradable diapers are considered to be a will contribute contributor to the diaper market by attracting great interest recently. Biodegradable diapers are safe for babies' sensitive skin as they are not made from environmentally friendly petroleum-derived chemicals or skin irritants (Prescient & Strategic Intelligence, 2020).

When the diaper structure is analyzed simply; Urine is first rapidly absorbed from the soft porous nonwoven surface called the topsheet, and the liquid is transmitted downwards. In order to prevent wetness on the baby's skin, the urine absorbed by the upper surface should not exist from the upper surface to skin. Urine is then rapidly absorbed at the ADL (acquisition and distribution layer) and transmitted to the absorbent (core) layer. The polyester fibers in the ADL provide spread of the urine and transfer the liquid to the absorbent section evenly along the fiber lengths. The backsheet under the core layer of the diaper is typically produced from non-bioactive hydrophobic films that act as a microporous barrier. This layer is designed to have small pores, preventing water droplets from leaking the clothes (Counts et al., 2017).

Figure 1. Baby diaper

Absorbent core; It is the layer on which two different raw materials such as pulp (wood pulp) and SAP (super absorbent polymers) homogeneously distributed between two nonwoven surfaces are wrapped. Superabsorbent polymers in the core section hold about 30 times their own weight, and wood pulps hold about 1.5 times their weight. The purpose of pulp usage inside a diaper is to have very well urine distribution and a bit absorption.

Pulp consists of high density, homogeneous and uniform short fibers. Standard wood pulp liquid absorption capacity is about 10 cc/gr. However, when it is subjected to 5 KPa pressure, its liquid absorption capacity will be less than 2 cc. Pulp layers are usually derived from special trees grown in the North American continent. Liquid flows through the cavities of the fiber. The overall fiber length for wood pulp fibers used in diapers is approximately 2.6mm.

Super Absorbent Polymer or SAP, also known as Super Absorbent Material: It is used in fine-grained form (like table salt). It Increases the retention capacity in the diaper. This ensures thinner product with less wood pulp but higher performance. Sodium polyacrylate is the chemical material widely used as SAP. When the SAP contacts with water, sodium leaves itself by leaving carboxyl ions. These carboxyl ions are negatively charged, so they are sprayed together. It dissolves and absorbs water absorbed by sodium ions thanks to SAP polymer structure. With water, the polymer becomes gel. Gel is connected to each other with a three-dimensional structure by cross-linking, which causes high molecular density. Hydrogen inside the water (H-O-H) is retained by acrylate thanks to forces between polarity forces. The superabsorbent properties of SAP are provided by electrolytes in the fluid (urine contains 0.9% mineral electrolyte). Electrolytes reduce polarity. This ensures the superabsorbent

capacity required for fluid retention. Therefore, diapers with SAP should never be tested with pure water, but the actual capacity can be seen with 0.9% saline solution. Mixture and distribution of these two important materials in the diaper is also very important

The mixture can be concentrated in a particular section of diaper instead of being evenly distributed, it is predicted that these two important raw materials can be used more efficiently. In other words, the absorbent layer of the diaper needs to be made in 3 dimensions. It is seen that the diapers of the well-known brands in the sector also confirm this prediction. With that improvement it also reduces usage of pulp (cellulose) and chemicals (SAP) which supports reducing disposable wastage and sustainable green world.

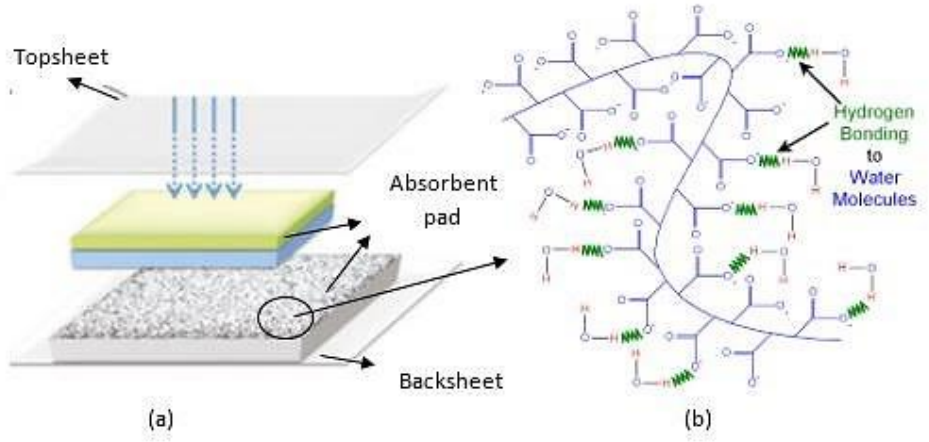

Figure 2. (a) Liquid absorption mechanism in disposable diapers; (b) attraction between water molecules and sodium polyacrylate monomer in the absorbent (Kotz, et al., 2014)

In this study, it is intended to achieve maximum benefit from SAP material by moving it to the middle zone. Towards that target, a new system developed to move collect most of SAP material at the middle zone of diaper. Performance values of diapers produced with new system were analyzed. Mixture and distribution of these two important raw materials in the diaper is also very important. The mixture can be concentrated in a particular section of diaper instead of being evenly distributed, it is predicted that these two important raw materials can be used more efficiently. In other words, the absorbent layer of the diaper needs to be made in 3D. It is seen that the diapers of the well-known brands in the market also confirm this prediction. With that improvement it also reduces usage of pulp (cellulose) and chemicals (SAP) which supports reducing disposable wastage and sustainable green world

Material and Method

Material

In the absorbent core generally utilise fiberized wood pulp, often termed “fluff pulp”. With the use of pulp in the baby diaper, cellulose fibers help to easily transmit the liquid in the absorbent sectional and provide the integrity of the absorbent layer together with the SAPs, and also give softness and volume. Fluff pulp raw material properties used in the absorbent core section are given in Table 1.

Table 1. Technical properties of fluff pulp fibres used in disposable diapers

Properties	Standart	Unit
Brightness (%)	ISO 2470	87
Basis Weight (g/m2)	T-410 0M-02	675 g/m2
Fiber Length (mm)	T-271 0M-02	3,1
Mullen (kPa)	T-807 0M-03	900
Density (g/cc)	T-411 0M-05	0,64
Moisture (%)	T-412 0M-02	8
Ash Content (%)	T-211 0M-02	0.1
SCAN Absorbency Capacity (g/g)	SCAN-C 33:80	11,4
SCAN Absorbency Time(s)	SCAN-C 33:80	3,2

While the diaper is free, 1 g of cellulose fiber has a water absorption capacity of 10 cc, while its absorption capacity decreases to 2 cc under pressure. That's why SAPs that hold liquid even under pressure were needed. SAP raw material properties used in the absorbent core section are given in Table 2.

Table 2. Properties of SAPs required for use in disposable diapers

Properties	Standard	Unit
Moisture content (w--t%)	WSP 230.3	~2%
Centrifuge retention capacity (g/g)	WSP 241.3	~ 33,5 g/g
Absorbency under pressure at 0.7 psi (g/g)	WSP 242.3	~ 22 g/g
Permeability dependent absorption under pressure (g/g)	WSP 243.3	~ 11g/g
Particle size distribution on >850µm (%)	WSP 220.3	max 1%
Particle size distribution on >150µm (%)	WSP 220.3	max ~15%
Flow rate (g/s)	WSP 250.3	~ 12 g/s
Apparent Bulk density (g/l)	WSP 250.3	650 - 750

3D mold modifications are designed in the machine to carry the amounts of SAP and Pulp used in the back sections of the absorbent layer to the middle section. The high vacuum amount under the mold enables the SAP and Pulp to be transported by sticking to the mold. It is possible to prevent this amount of vacuum on the mold by blocking the pores of the mold. In order to prevent homogeneous distribution of SAP and Pulp on existing molds, product trials have been made with non-permanent modifications.

These designs prevented an even (non-sectional) distribution of the homogeneous SAP/pulp mixture in the absorbent section. For this reason, the absorbent core layers of the diapers produced with 3 different molds were divided into 5 sections and examined.

Method

Sample productions were carried out at the Fameccanica diaper line at a production speed of 450 m/min and an output speed of 1000 pads/min. In the study, the core mix ratios were determined as 11/9 grams. During the production of diapers, 1% tension was applied. Core section is bonded with hot melt adhesive applied in the temperature between 150-170 °C.

Table 3. Sample Productions

Code	Content
C	Current Production
S1	1st mold production
S2	2 nd mold production
S3	3 rd mold production

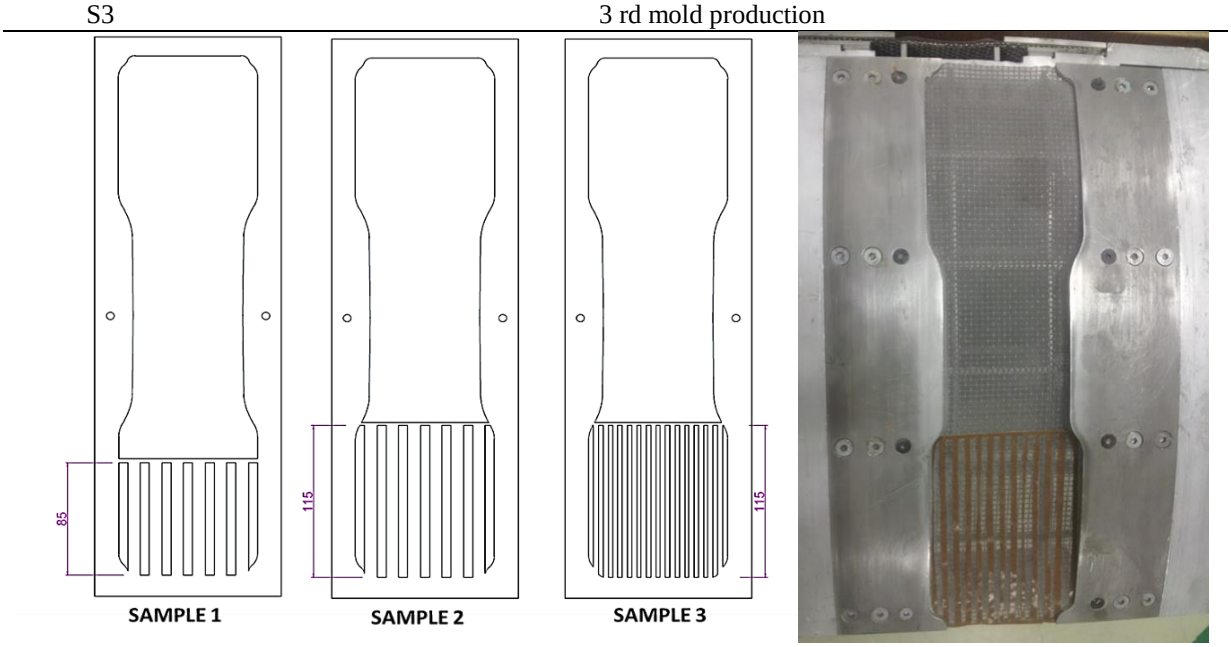

Figure 3. Technical drawing of baby diaper absorbent layer mold modified with sticking shim (metal plate) in 3 different ways

For the absorbent core section mold with a length of 425 mm, shim metal sheets with lengths of 85 and 115 mm were designed. In the first sample, the shim metal designed for the posterior section formed 20% of the mold. In the second sample, the shim metal designed for the rear area formed 27% of the mold. In the third sample, the shim metal designed for the rear area formed 27% of the mold. At the same time, in the 3rd sample, by using 50% thinner shim sheets than the 1st and 2nd molds, the closed areas were increased 42% more.

Table 4. Applied tests

Test Type	Tests and Standards
Physical	Determination of Diaper Dry Weight [g/m²] NWSP 130.1. R0 (15)
Liquid Absorption Properties	Rewet (Repeated Fluid Release) [g] DEGUSA S. SUSE.205-4.1
	Absorption Time (Repeated Fluid Release Time) [s]
	Maximum Absorption [g] DEGUSA S. SUSE.202-4.1
	Maximum Retention [g] DEGUSA S. SUSE.202-4.1
	Leakage (PAKTEN PR-46)
	Sectional Retention Method [g] DEGUSA S.SUSE- AWT.-203e

For dry diaper weight determination; Due to the differences that may arise from that may arise in the absorbent section from the SAP-pulp raw material, 5 samples for each product were tested on a Precisa brand NWSP 130.1. R0 (15) precision balance under normal atmospheric conditions. For the determination of leakage; the specific weight placed on the diaper and the solution was poured on the filter paper, and it was visually tested on 5 diapers for each sample, according to PAKTEN PR-46 standards. In the maximum absorption capacity test; after the diapers were kept in pure water solution with 0.9% NaCl for 30 minutes without applying any pressure, the amount of liquid absorbed by the diaper was calculated by weighing and subtracting from the weight of the first diaper. Determination of maximum holding capacity; After the maximum absorbent capacity test, the first diaper weight was subtracted from the weight after the diapers were kept in a centrifuge at 2800 rpm for 45 seconds. For maximum absorption and maximum holding capacity tests, 3 diapers were tested according to DEGUSA S. SUSE.202-4.1 standards. In the Rewet (Repeated Fluid Release Amount) test; After pouring 0.9% NaCl solution into the diaper placed in a hook designed according to the baby's anatomy, the amount of liquid left by the hygienic product under 11 kg weight was calculated by testing 3 diapers for each sample according to DEGUSA S. SUSE.205-4.1 standards. In the absorption time (Repeated Liquid Release Time) test; The duration of the amount of liquid left by the diaper used for the rewet test under 11 kg weight was calculated. 3 diapers tested according to DEGUSA S. SUSE.205-4.1 standards

Retention of Diapers by Section

For SAP determination; The diaper is weighed and five sections of equal size are drawn on to the diaper. The sections are then cut out along the marked dividing lines using scissors. The sections are then weighed, sealed in teabags, and immersed in the test solution. After a defined immersion time the teabags are hung up in order to remove the non-absorbed liquid. In order to determine the retention, the teabags are centrifuged. Then the individual diaper sections (without teabag) are weighed. 0.9% NaCl weight solution was used in the tests

A triple determination is carried out in each case. The test method is given below

a. The dry diaper is weighed (WT).
b. The diaper is spread out tautly on a table. Five sections are drawn on (see Figure 4). The length of the sections is determined by the minimum width of the absorbent core. The section width is determined by the length of the absorbent core divided by five (section 1 is located in the area of the tape).
c. The individual sections are then cut out along the marked dividing lines using scissors.
d. The sections are weighed G, (weighed accuracy: 0.1g) and heat-sealed in teabags.
e. The teabags are placed in the photographic dish, which is filled with test liquid, and gently submerged to ensure complete wetting. The test solution has to be changed after each set of diapers (3) tested
f. After 30 minutes the teabags are hung up with pegs for 5 minutes in order to allow the excess fluid to drip off the teabag.
g. The teabags are weighed (Gn1-5) in order to determine the absorption per section.
h. In order to determine the retention, the teabags are centrifuged for 45 seconds in the 2,800-rpm centrifuge
ı. For final weighing of the individual diaper sections Gg1-5, the teabags are removed (weighing accuracy: 0.1 g)

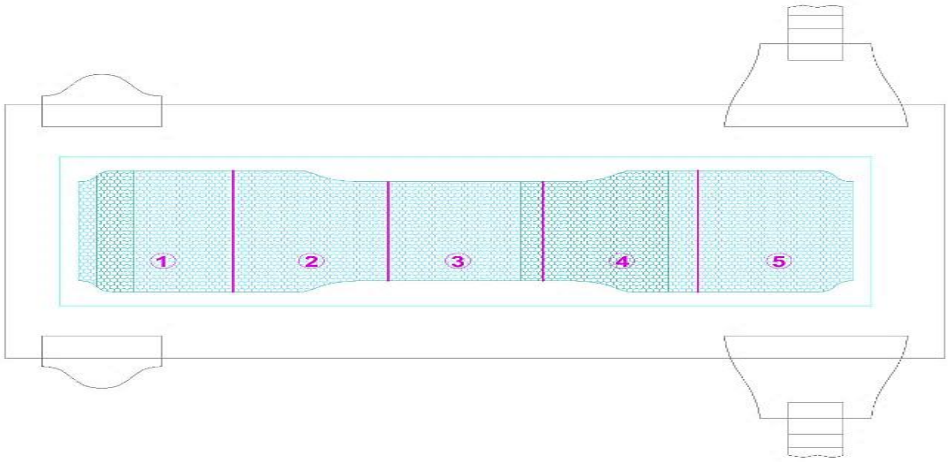

Figure 4. Technical drawing of baby diaper absorbent layer mold modified with sticking shim (metal plate) in 3 different ways

Evaluation

A1-5 = Gn1-5 – Gtr1-5 [g]
R1-5 = Gg1-5 – Gtr1-5 [g]

mSAP = ((mAbs. Mat x (RetAbs. Mat – RetFI)) / (RetSAP – RetFI) [g]

If the retention of the fluff pulp cannot be determined, an average retention of 1.5g/g is included in the formula.

A1-5: Absorption (diaper sections 1 - 5) [g]
R1-5: Retention (dipaer section 1-5) [g]
Gn1-5: Weight of the wet sections (1-5) [g]
Gtr1-5: Weight of the dry sections (1-5) [g]
m SAP: quantity of superabsorber [g]
m FI: quantity of fluff pulp [g]
m Abs.Mat.: quantity of absorptive material [g]

Ret SAP: superabsorber retention [g/g]
Ret FI: fluff pulp retention [g/g]
Ret Abs.Mat.: absorptive material retention [g/g]
(= retention of the hygiene article [g] / quantity of absorptive material [g]) (Evonik, 2007).

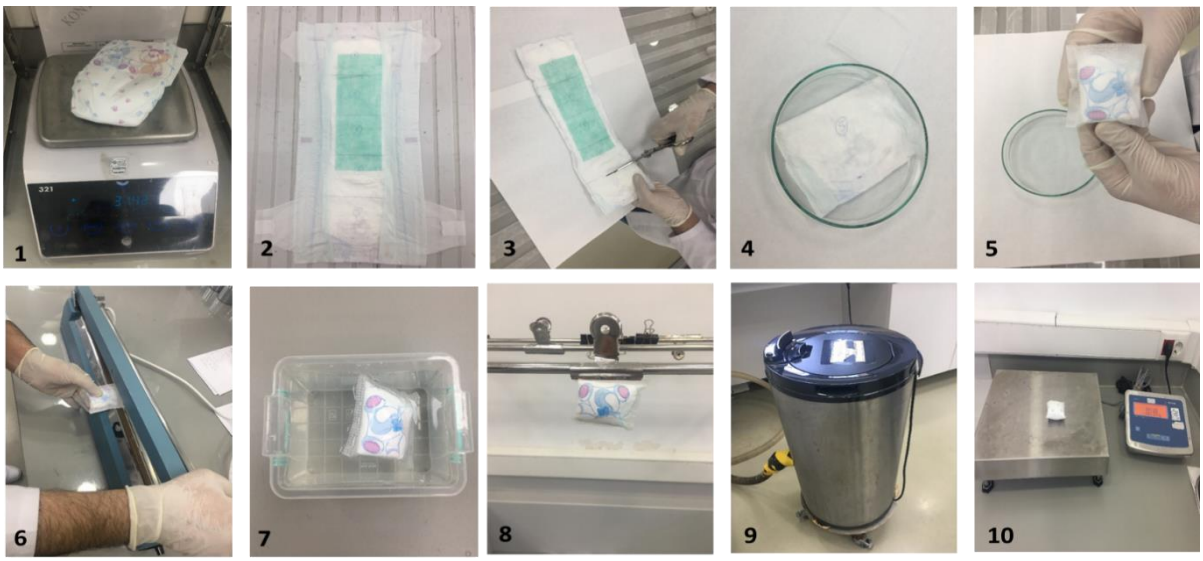

Figure 5. Retention of diapers by section test steps

Results and Discussion

In this study, the distributions of SAP & pulp amounts of diapers produced with 3 different molding techniques were investigated.

Determination of Sectional Absorbent Core Fluid Retention

Before transferring the studies to concentrate the amount of SAP & pulp used on the front and back of the absorbent layer to the middle parts by mold modification on the machine, the sectional distributions of SAP & pulp in 3 different competitor diapers in the market was examined.

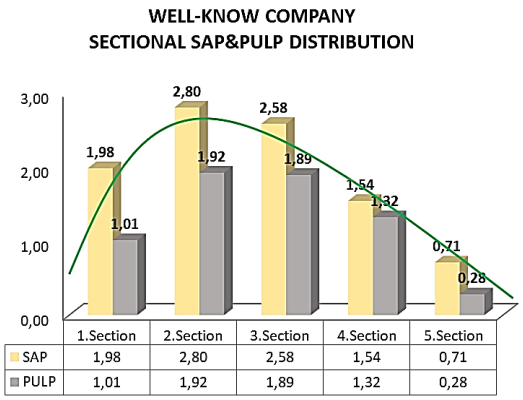

Figure 6. Comparison of well-known company's sectional SAP&Pulp Distribution. (Yellow columns are SAP & grey columns are PULP amount)

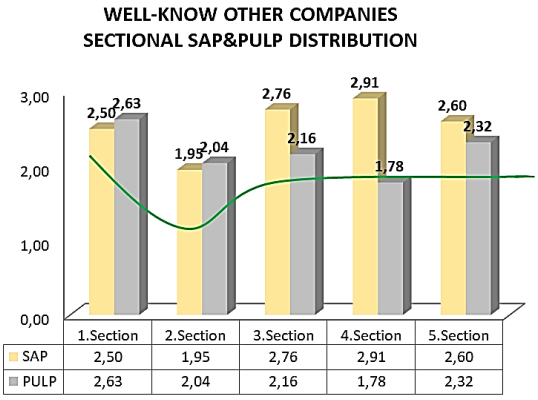

Figure 7. Comparison of well-known other companies sectional SAP&Pulp Distribution. (Yellow columns are SAP & grey columns are PULP amount)

When the product of the company (Figure 6), which as proven itself in the international arena, is examined; Parabolic increases in SAP & Pulp amounts are seen towards the 2nd and 3rd middle sections. Less SAP and Pulp content were detected in the 4th and 5th sections of the back part of the diaper. This 3D core structure, made with large investments in the machine, shows the desired curve of the work. However, considering the superior cost policy of the company manufacturer, the same performance is aimed with the molds produced by our company at lower costs. When examined in Figure 7, similar curves of competitor diapers without 3D core structure can be seen.

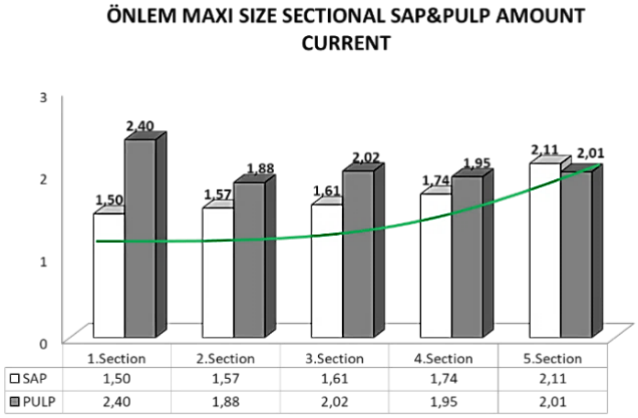

Figure 8. Current baby diaper

The sectional SAP & pulp distributions of the existing Önlem brand diapers were analysing before starting the trial production with the mold. While the amount of SAP & Pulp was higher in the posterior parts, it was observed that this amount decreased in the middle parts.

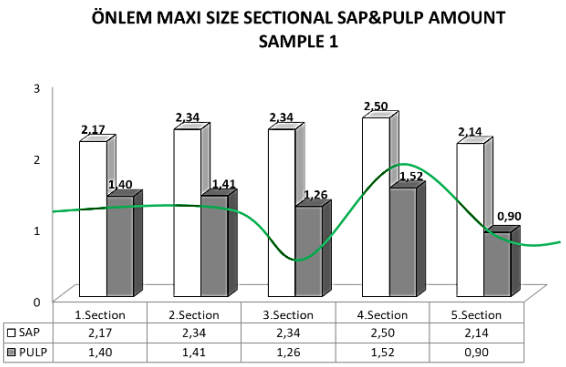

Figure 9. Sample 1

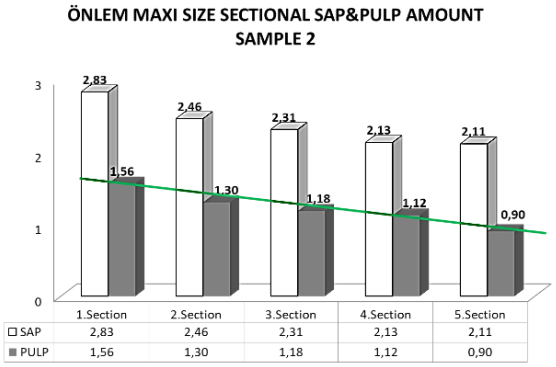

Figure 10. Sample 2

In the first sample (Fig. 9) we closed back side of diaper with shim. When 1, sample analyzed shows that the diapers have an uneven SAP & Pulp distribution from the front to the back. This situation was far from expectations. For the 2nd sample (Figure 10), the die length was increased by 35% compared to the 1st trial, and the back part was closed a little more. The amount of SAP and Pulp decreased towards the back of the diaper, but they were not high enough in those regions, although they should have been concentrated in regions 2 and 3. This formwork system was close to expectations, but not exactly as desired.

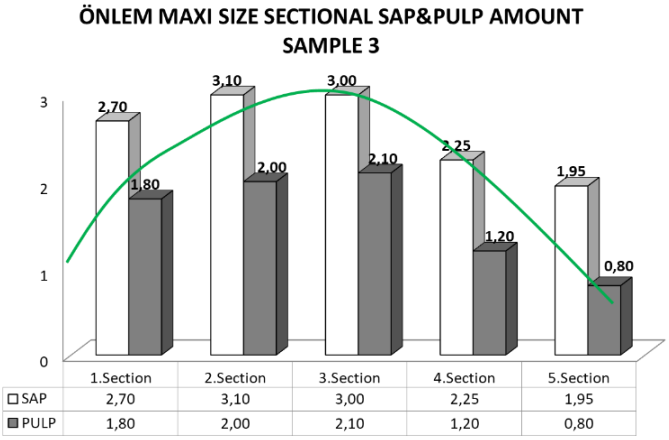

Figure 11. Sample 3

In the last trial, sheets with thinner and less spacing compared to the 1st and 2nd trials (50% thinner) were used. As a result of this study, it was ensured that the product amount was collected in the middle section. It is seen in the graph that the desired absorbent section structure is obtained.

Absorbent Core Liquid Absorption Time Comparison

The performance comparison of the liquid absorption times with the ideal core mold with the competitor companies and the current product was made.

Table 5. Liquid absorption time comparison

		3D CORE TRIAL	CURRENT PRODUCT	WELL-KNOWN COMPANY 1	WELL-KNOWN COMPANY 2	WELL-KNOWN COMPANY 3
	Unit	AVG	AVG	AVG	AVG	AVG
Diaper Weight	g	30,6	30,6	25,7	32,5	29,7
1st Liquid Absorption Time	sec	27	42	40	48	52
2nd Liquid Absorption Time	sec	59	68	47	115	129
3rd Liquid Absorption Time	sec	71	81	108	119	215

Lower data for liquid absorption time means that it absorbs liquid in a shorter time, so it can be understandable that its performance is better and the liquid trapped in a shorter time will not leave wetness on baby skin. As a further output of this study, we found that the 3rd liquid absorption time in the diaper improved up to 12% compared to our current product. As seen in Table 5, the 3rd suction time was shortened by allowing the product amount to accumulate more in the middle section

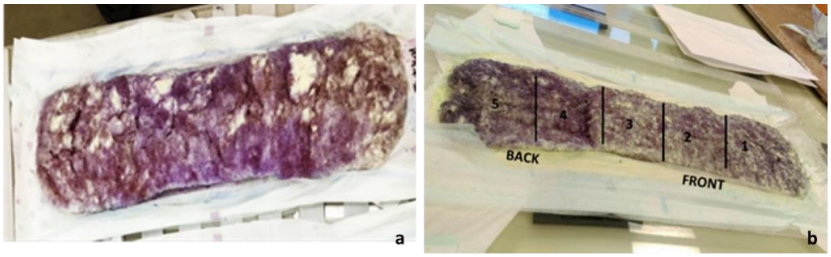

Figure 12. Image of absorbent core that acquisition liquid a) current b) sample 3

Rewet Amount (gr) and Time (sec)

It is desirable that the diapers absorb the liquid quickly and not give it back even under pressure, so that the baby's skin does not feel wet and there are no leakage problems. In this context, it is expected that the cellulose/SAP mixture in the absorbent layer will absorb the liquid quickly and not transfer the liquid back even under pressure. In do to determine these values, absorption time and rewet tests are performed.

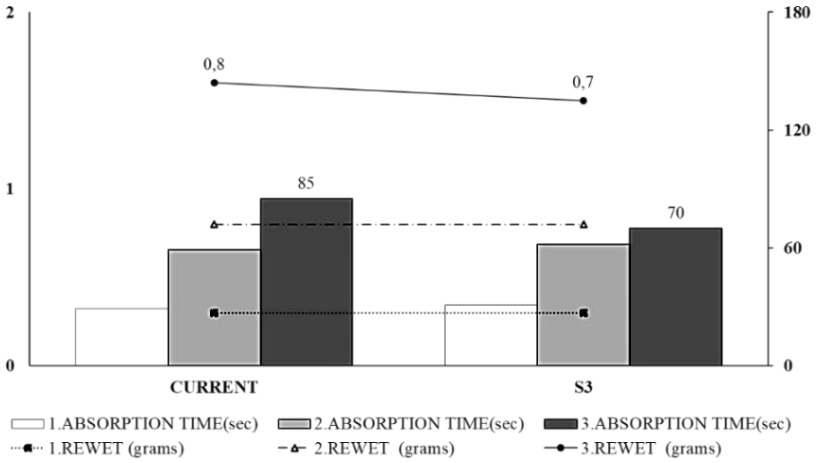

Figure 13. Rewet and absorbtion time

When the 3rd rewet, values are examined, it is seen that the S3 sample developed with the new mold has lower rewetting values than the existing sample. The main reason for this can be explained as the trapping of the liquid in the desired area with the newly designed mold, unlike the existing mold system. When the 3rd absorption times are examined, it is seen that the fastest absorption time is in the S3 sample. While it is desired to complete the liquid absorption time for diapers as soon as possible, it has been determined that the current sample has a higher absorption time. It can be said that the main reason for this is the SAP & pulp mixture, which is intensely present in the 2nd and 3rd sections of the developed product, as opposed to the mixture that is evenly distributed to the regions in the existing product.

Maximum Absorption and Maximum Retention (grams)

In the maximum absorption and maximum retention tests, 0.9% saline solution is used to simulate urine. With this test, it is observed how much the diapers absorb and how much urine they hold. Accordingly, although there is no significant difference for the two different samples tested, it is seen that it absorbs and retains similar amounts of saline solution.

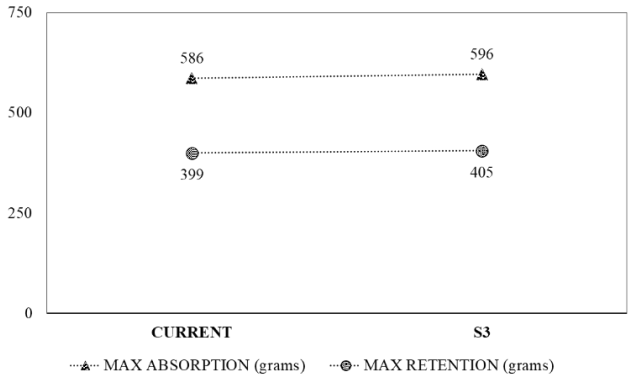

Figure 14. Maximum absorption and maximum retention (grams)

Conclusion

In this study, it was aimed to increase the SAP &pulp density in different parts of the diaper instead of distributing the SAP &pulp amounts in the absorbent section of the diapers equally. Before this study, it was found that the amount of SAP & Pulp increased towards the back of the diaper. With raising the concentration of SAP and pulp in the middle section, as the saturation ratio and liquid absorption capacity will increase, the skin of the baby will remain drier. For this, firstly, the absorbent parts of the cloth were divided into 5 different regions and marked. Afterwards, it was aimed to increase the amount of SAP & Pulp on the 2nd and 3rd regions where urine is intense. At this point, three different molds were designed and regional density was realized for the distribution of SAP &pulp amounts. In order to develop the product, the products of competing companies were examined. As a result of the tests carried out by determining the absorbent regions of a famous brand with 3D absorbent regional density, the presence of more SAP &pulp in the 2nd and 3rd regions showed that we are progressing in the right direction in our work. The effect of this distribution difference on product performance is analyzed. We found that the 3rd liquid absorption time in the diaper improved up to 12% compared to our current product. In the rewet and absorbent capacity test, it was seen that the baby diapers produced with 3rd Sample mold were superior to the existing product. Finally, more environmentally friendly diapers are produced than the current product, since less raw material (SAP & Pulp) can be used. A consumer survey was conducted with 25 parents in comparison with the current diaper. Considering the preferences in terms of satisfaction, the 3D core structured diaper (61%) was preferred more than the existing diaper (39%) in terms of equal distribution of the liquid, no leakage and sagging.

Scientific Ethics Declaration

The authors declare that the scientific ethical and legal responsibility of this article published in EPSTEM journal belongs to the authors.

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Determination of Potential Anti-Alzheimer Activity of Gentiopicroside and Isoorientin Using Molecular Docking Studies

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Abstract: Alzheimer's disease (AD) is the most common type of dementia worldwide, involving a multifactorial combination of environmental, genetic and epigenetic factors. It is characterized by the accumulation of abnormal amyloid beta (Aß) and tau fibrillar tangles, oxidative stress, neuroinflammation, and disruption of autophagy mechanisms. The agents used for the treatment of the disease only prevent the symptoms of the disease. Epigenetic modifications such as DNA methylations and histone modifications that occur in learning and memory processes have come to the fore in the search for new and reliable potential therapeutic agents for AD. Against these multiple mechanisms of AD, natural products are currently considered an alternative strategy for the discovery of new multipotent drugs. Phytocompounds of Gentiana olivieri, Gentiopicroside and Isoorientin, which have been known to have many benefits for health, act as a neuroprotective effect by acting as an anti-inflammatory and antioxidant. Based on this, in order to determine the possible effects of Gentiopicroside and Isoorientin phytocompounds on Sirtüin-1 (SIRT1), Sirtüin-2 (SIRT2), Sestrin 2 (SESN2), Histone deacetyl transferase-6 (HDAC6) and divalent metal transporter 1 (DMT1) enzymes, which are seen as targets in AD, molecular docking analysis was carried out. AutoDock 4.0 software was used to predict the interaction of ligands with possible active binding sites on the target molecule crystal structure. As a result of the analyzes, the best coupling occurred between the Gentiopicroside and DMT1 and HDAC6 enzymes. In the light of this information, it can be suggested that the molecular clamping analysis is carried out by the neuroprotective effect of Gentiopicroside by DMT1 enzyme inhibition, while Izoorientine performs through the HDAC6 enzyme. As a result, it is thought that our results will contribute to the search for new therapeutic agent studies using epigenetic approaches against AD.

Keywords: Alzheimer, molecular docking, epigenetic modifications, Isoorientin, Gentiopicroside

Introduction

Alzheimer Disease (AD) is the most common progressive neurodegenerative and dementia disease characterized by the accumulation of amyloid-beta (Aβ) peptides and the formation of neurofibrillary tangles in the brain (Alzheimer Fact and Figures, 2020). ADs, the etiology and pathogenesis of which cannot be determined exactly; β amyloid storage, tau hyperphosphorylation, inflammation, oxidative stress, energy metabolism, and errors in cell cycle and apoptosis entry of cells, which occur with the effect of many genes and environmental factors, in

which the expression of thousands of genes can change, multiple pathogenic pathways occur (Mastroeni et al., 2011). Besides these pathogenites epigenetic and autophagy mechanisms allows to have information at the intersection of Alzheimer's pathophysiological processes and different risk factors (Mastroeni et al., 2011). Histone deacetylation, which is one of the histone modifications that includes processes such as suppression or activation of gene expression by affecting nucleosome, histone-histone interaction stability (Suganuma & Workman, 2008), suppresses transcription by histonedeacetylases (HDAC) (Wang et al., 2013).

In human, four classes HDAC enzymes: class I HDACs 1, 2, and 8; class IIa HDACs 4, 5, and 7; class IIb HDACs 6 and 10; HDACs III also known as Sirtuins (SIRT) 1-7; and finally class IV HDACs 11 (Gregorotti et all., 2004). Dysregulation of acetylation mechanism has been associated with several impairments such as signaling, proliferation, inflammation, apoptosis, and neuronal plasticity (Lu et al., 2015). HDAC6 levels increased both in the hippocampus and cortex AD humans and in animal models of AD (Ding et al., 2008). Morever, HDAC6 influences tubulin acetylation, tau phosphorylation and degredation (Konsoula & Barile, 2012). Reducing HDAC6 level can increase neural survival while increasing clearance of tau aggregates as well as reducing the formation of tau aggregates (Konsoula & Barile, 2012). In addition to them, Sirtuins have important role in synaptic plasticity and memory so that associated with patogenesis of AD. SIRT1 activation in AD can inhibit tau aggregation by autophagy-mediated mechanism (Heinisch & Brandt, 2016). SIRT2 is higly a higly conserved lysine deacetylase that is necessary for protection againist oxidative stress ,highly associated with AD, and that higher SIRT2 activation in neurodegeneration may be compansatory against neuronal stress (Cacabelos et al., 2019). In primary rat cortical nerve cell cultures, it has been determined that Aβ causes an increase in sestrin2 (SESN2), one of the three sestrin enzyme families with free radical scavenging and autophagy inducing effects, and activates antioxidant and autophagy pathways (Chen et al., 2014). Other AD associated protein is Divalent Metal Transporter 1 (DMT1) that transporting divalent cation metals such as iron and cooper, an increase in DMT1 level, which directs APP proteolysis to the amyloidogenic pathway, was also detected in Swedish mutant SH-SY5Y cells overexpressing APP (Zheg et al., 2009).

Gentiopicroside (Figure 1) has been used in Chinese medicine for pain relief and treatment of rheumatism for more than 200 years, and is an active component of the plant called *Gentiana olivieri* (Deng, 2018). The protective effect of gentiopicroside was found in chronic pain, depression and pain/depression models in the Central Nervouss Systems (CNS) of mice. (Chen et al. 2008; Liu et al. 2014). In neurotoxicity caused by inflammation, it was determined that gentiopicroside has a protective effect on neurons, through antioxidant pathway mechanisms (Deng et al., 2018).

Another pyhtochemical in our invastigation is Isoorientin (Figure 2) that C-glycosyl flavonoid isolated from *Gentiana olivieri*. In studies conducted with 6-hydroxydopamine and Aß-induced neurotoxicity, isoorientin has been found to have a neuroprotective effect (Lin et al., 2009; Liang et al., 2016). In a study designed by in vitro and molecular docking study, it inhibited Glycogen synthase kinase 3ß (GSK3ß), which plays a key role in tau hyperphosphorylation; In an in vitro study, it has been shown that isoorientin reduces tau phosphorylation via GSK3ß and has a neuroprotective effect against ß-amyloid-induced tau hyperphosphorylation and neurotoxicity (Liang et al., 2016).

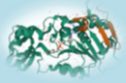
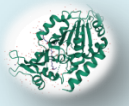
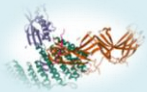
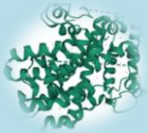
It has become increasingly important to use computer techniques in the drug design and development process. Molecular docking, which is one of these techniques and is generally used to predict the binding orientations of drug candidates against target proteins in order to predict drug affinity and activity, is more preferred due to its ease of use and is being developed day by day (Nagoor et al., 2017). In this invastigation, based on literatüre survey, the binding kinetics of five important AD-associated proteins (HDAC6, SIRT1, SIRT2, SESN2 and DMT1) with isoorientin and gentiopicroside were analyzed using molecular docking.

METHOD

Protein and Ligand Preparation

A total of five proteins which related to ADs were selected on the basis of literature survey (Table 1). Crystal structures of protein structures were obtained from Protein Data Bank (www.rcsb. Org). All polar hydrogens have been added with the Discovery Studio 2020 (Biovia, 2016) modeling package to reduce the tension of the crystal structure and make the proteins available for use in the Autodock simulation program. Gentiopicroside and ısoorientin were taken from PubChem (https://pubchem.ncbi.nlm.nih.gov) database in sdf format. And then these format converted to pdb format.using Discovery Studio 2020 (Biovia, 2016) program.

Table 1. Targeted receptor proteins associated with Alzheimer disease along with structures

Target Protein	PDB Code	3D Structure
SIRT1	4IF6	
SIRT2	4Y6L	
HDAC6	6CEE	
DMT1	5F0P	
SESN2	5DJ4	

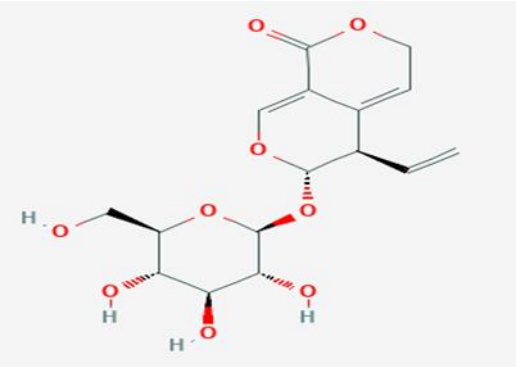


Figure 1. Gentiopicroside 2D structure (taken from PubChem)

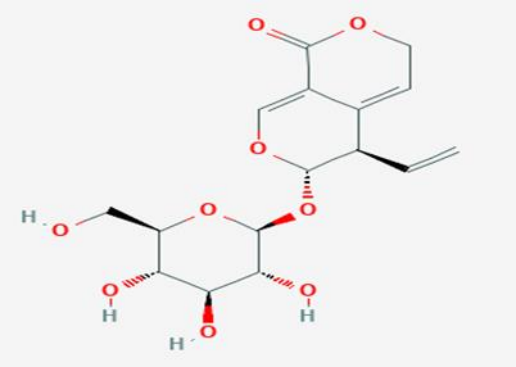


Figure 2. Isoorientin 2D Structure (taken from PubChem)

Molecular Docking

In the modeling, in which the herbal active substances are mainly selected as the test substance, the target molecule (Sirtuin 1 (SIRT1), Sirtuin 2 (SIRT2), Sestrin 2, HDAC6, DMT1, amyloid ß (Aß), tau protein) with possible active binding sites on the crystal structure. Molecular docking was simulated using AutoDock 4.2 software to predict the interaction of ligands with (gentiopicroside, isoorientin, and Aß1-42). The current version of AutoDockTools was used to prepare the target and ligand molecules before starting the coupling analysis using AutoDock 4.2. During modeling, polar hydrogen atoms in target and ligand molecules were eliminated and nonpolar hydrogens were included in the model. Following this, Gasteiger loads will be calculated with AutoDockTools (ADT) (Nasab et al., 2017; Ricci & Netz, 2009). During the docking experiment, all possible rotations of the ligand were allowed and then the prepared target and ligand constructs were saved in PDBQT format. As the program configuration; The grid box size was set on a scale of 60 × 60 × 60 Å and a grid spacing of 0.375 Å. After 25 independent docking studies for ligand molecules, all possible binding modes were clustered by the program and on the basis of the lowest (negative) binding free energy (Gibbs free binding energy) (ΔGbinding) i.e. the conformation with the best docking position (kcal/mol) are listed. Among these findings, those with Gibbs free binding energies lower than −6.0 kcal / mol (<−6.0 kcal / mol) were considered important in terms of chelation (Shityakov and Förster 2014). In addition, the best docking position obtained between possible binding sites on the active site of ligand and target molecules using AutoDock 4.2 was analyzed using BIOVIA Discovery Studio Visualizer 2021.

Results and Discussion

The data obtained as a result of the docking processes were evaluated based on the standard threshold binding free energy (-6 kcal/mol) level, which is considered important, and it was determined that the lowest binding energies for gentiopicroside and isooorientin were between DMT1 and HDAC6 enzymes, respectively. The results of the docking score of selected ligand and target proteins are presented in table 2.

Table 2. Docking score of gentiopicroside and isoorientin aganist ADs associated target protein receptors. Docking score was expressed in terms of kcal/mol.

Result Analysis Software	Visualization Sofware	Protein	Ligand	Docking score (kcal/mol)
Autodock 4.2	3 D BIOVIA Discovery Stduio Visualizer	5F0P (DMT1)	Gentiopicroside	-7.44
Autodock 4.2	3 D BIOVIA Discovery Stduio Visualizer	6CEE (HDAC6)	İsoorientin	-6.97

Gentiopicroside and isoorientin formed generally hydrogen, van der Walls and alkyl bonds with residues of enzymes(HDAC6 and DMT1) and especially detailed shown in Figure 3 and 4.

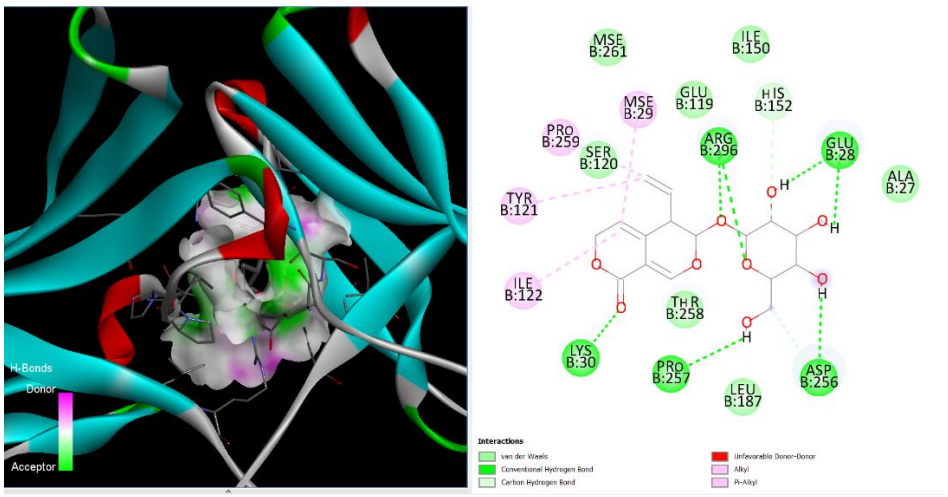


Figure 3. 3D H bond receptor surface ınteraction and 2D receptor ligand ınteraction between gentiopicroside and DMT1

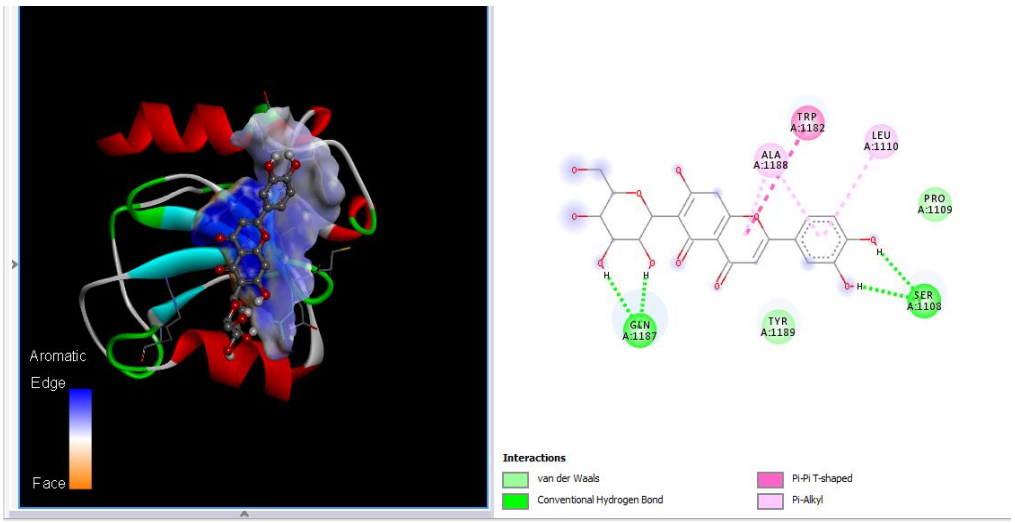


Figure 4. 3D H bond receptor surface ınteraction and 2D receptor ligand ınteraction between ısoorientin and HDAC6

The concentration of metal ions, such as active redox transition metals, which are retained in low concentrations in the brain under physiological conditions, is particularly under the control of the blood-brain barrier (Duce & Bush, 2010). However, with aging, the functions of the BBB begin to decrease and the transition of these metal ions from the blood to the brain and from the brain to the blood becomes easier (Tiiman et al., 2013). Although copper, iron and zinc concentrations are higher in the brain compared to other tissues under physiological conditions, these values increase significantly in Alzheimer's patients (Tiiman et al., 2013). DMT1 is a proton-coupled metal ion transport protein expressed in neurons and known to actively transport Fe^{2+}, $Zn^{2+,}$ Mn^{2+}, Co^{2+}, Cd^{2+}, Cu^{2+}, Ni^{2+} and Pb^{2+} (Ingrassia et al., 2019). DMT1 has a critical and important role in ion-mediated pathogenesis of AD and that pharmacological occluding DMT1, may provide novel therapeutic agents against AD (Zheng et al., 2009). Our molecular docking result between DMT1 and gentiopicroside, with -7.44 kcal/mol the lowest binding energy, is supporting these evaluation and that we are thinking gentiopicroside may be a potantial therapeutic agents againist AD through blockage of DMT1 protein.

Reversbl protein acetylation mechanisms in living organisms that cellular functions of nuclear and cytoplasmic proteins can be regulated by lysine acetylation-deacetylation cycles in epigenetics, cell signaling,and metabolism (Hai & Christianson, 2016). Three classes protein are involved in the chemical biogly of the acetylome which including HDAC (Verdin & Ott, 2015). The HDACs are especially considareble in that upregulated activity is associated with tumorogenesis (Ma et all., 2016) and may also function as therapeutic agents for other disease, such as neurodegenerative diseases (Falkenberg & Johnstone, 2014). HDAC6 is the one of the four HDAC enzymes that contain two catalytic domain and a carboxyl-terminal Zn^{+2} binding site (Hai & Christianson, 2016). Hsp90 (Kowacks et all., 2005) and microtubule associated protein Tau (Hubbert et all., 2002) are HDAC6 substrats that implicating HDAC6 in the pathology of AD (Cohen et all., 2011). HDAC6

enzyme catalyzes deacetylation of K40 in the α tubulin subunit of the microtobulues, attachment in the cytoplasm by serin/glutamate-rich repeat motif (Bertos et all., 2004), consequently regulation microtubule dynamics: overactivation of HDAC6 reduce tubulin acetylation levels and increase cell motitiy. while these enzymes inhibition cause hyperacetylation of α-tubulin and suppression of microtubule Dynamics (Szyk et all., 2014). Based on the relationship between AD and HDAC6 in the literature, it was concluded that HDAC6 inhibitors could be therapeutic agents for AD. Accordingly our Autodock analysis, the lowest binding energy between isoorientin and HDAC6 enzyme was determined as -6.97 and suggest that isoorientin may be a potantial therapeutic agents againist AD through inhibition of HDAC6 enzyme.

Scientific Ethics Declaration

The authors declare that the scientific ethical and legal responsibility of this article published in EPSTEM journal belongs to the authors.

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Lignocellulosic Ezymes of Basidial Fungi-Isolated from Different Ecological Niches of Georgia

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Abstract: Great interest in basidiomycetes for targeted technological treatment of agro-industrial plant substrates are conditioned by their ability to produce lignocellulosic enzymes. Consisting of 29 soil-climatic zones, Georgia (South Caucasus area) represents a unique place for the isolation of microorganisms. In order to study the biochemistry and physiology of wood-degrading basidiomycetes, samples of wood-degrading basidiomycetes were collected from different taxonomic niches of Georgia. 45 strains were obtained as pure cultures and 32 ones – identified. Producers of lignocellulosic enzymes were revealed among test fungi under solid-state and submerged cultivation conditions. Pleurous ostreatus GV10, Pleurotus drynus IN 11 and Fomes fomentarius GK33 were found to be the best producers of cellulosic enzymes using orange and mandarin peels, wheat and Potato straw, wheat bran as substrates, and Ganoderma lucidum GB03 – the best producer of laccase during cultivation on orange and mandarin peels waste. The influence of lignocellulose on the accumulation of the enzymes laccase, xylanase and Filter paper assay was studied.

Keywords: lignocellulosic materials, Basidiomycetes, laccase, cellulase, xylanase.

Introduction

The increasing expansion of agro-industrial activity over the last few years has led to the accumulation of a large quantity of lignocellulosic residues all over the world (Mahesh et al., 2013). Mentioned the process of waste biodegradation should be noted the role of white rot basidial fungi capable of synthesizing hydrolyzing and oxidizing enzymes, which play an important role in the degradation of polysaccharides and lignin of plant biomass, should be especially noted (Huang et al., 2008). Cellulolytic enzymes play the most indispensable and centralized role in the hydrolysis of cellulosic material. Effective enzymatic hydrolysis is one of the major prerequisites in the development of a successful lignocellulosic bio refinery (Dey et al. 2021).

The major obstruction in the biological conversion of lignocelluloses is the physical protection of cellulose by lignin against cellulolytic enzymes (Keller et al., 2003). The different degrees of lignin degradation with respect to other wood components depend on the environmental conditions and the fungal species involved. White-rot fungi have been studied extensively for application in biological pulping and bleaching because they are the only organisms that are able to degrade lignin efficiently (Heinzkill et al., 1998). Fungal laccases have higher redox potential than bacterial or plant laccases and their action seems to be relevant in nature finding also important applications in biotechonology. Thus, fungal laccases are involved in the degradation of lignin or in the removal of potentially toxic phenols arising during lignin degradation (Thurston, 1994). Therefore, it is necessary to identify new laccases with lower cost but higher activity (Fang et al., 2015). Environmental factors influence the ability of fungi to produce enzymes by high activity and different strains react differently to these conditions. One should thus select the strains capable of producing high concentrations of suitable enzymes and then optimize conditions for enzymes production by the selected organism. It is therefore not surprising that these enzymes yet remains a topic of intense research today.

Method

Microorganisms

Were test of the 45 Basidial fungi strains, collected from different ecological niches of Georgia.

Lignocellulosic substrates

Orange, banana, tea and mandarin peelings, wheat bran, wheat and Potato straw, apples residual, sawdust, bagasse. Lignocellulosic wastes available at the local market and bagasse existent at the international market were applied in studies. All residues were dried at 50oC and milled to dust extent (<1 mm).

Fungal Inoculant

Were prepared by growing the fungi on a rotary shaker at 180 rpm, at 27oC in 500-ml flasks containing 100 ml of synthetic medium with the following composition (g/l): glucose – 15.0; ammonium nitrate – 3.0; yeast extract – 3.0; NaH2PO4 – 0.9; K2HPO4 – 0.3; MgSO4 – 0.5; initial pH was adjusted to 5.7 prior to sterilization. The nutrient media was sterilized at 121ºC for 20 min. After 7-9 days of fungi cultivation, mycelium was inoculated to conduct the SSF and SF of selected lignocellulosic materials. (Tsiklauri et al., 2014)

Cultivation Conditions

Solid-state fermentation (SSF) of selected plants residues was carried out at 27ºC in 100-ml flasks containing 5 g of lignocelluloses substrates moistened with 18 ml of the nutrient medium (g /l): NaNO3 – 2.0; yeast extract – 3.0; NaH2PO4 – 0.9; K2HPO4 – 0.3; MgSO4×7H2O – 0.5; 0.2mM CuSO4×5H2O; final pH 5.8. The flasks were inoculated with 5 ml of mycelial homogenate. After the 7th, 15th and 22nd days of cultivation, the extracellular enzymes were extracted from the whole biomass twice with 25 ml of distilled water (total volume 50 ml). The extract was centrifuged at 10000g for 15 min at 4ºC.The filtrate was used for the determination of biologically active compounds.

Submerged fermentation (SF) of fungi was performed at 27oC on a rotary shaker at 180 rpm in 500-ml flasks containing 100 ml of above-mentioned medium, (g/l): Lignocellulosic substrate – 5%; NaNO3 –3.0; yeast extract – 2.0; NaH2PO4 – 0.9; K2HPO4 – 0.3; MgSO4×7H2O – 0.5; 0.2mM CuSO4×5H2O; Initial pH was adjusted to 5.8. The flask was sterilized at 121ºC for 35 min. The flasks were inoculated with 10 ml of mycelial homogenate. On the 6th and 10th days of cultivation, the culture liquid was centrifuged at 10000g for 15 min at 4oC, and the filtrate was used for the determination of enzyme activities (Tsiklauri et al., 2014).

Enzyme Assays

Xylanase activity was determined by mixing 70 µl appropriately diluted samples with 630 µl of birchwood xylan (Roth 7500) (1% w/v) in 50 mM citrate buffer (pH 5.0) at 50oC for 10 min. Carboxymethyl cellulase

(CMCase) activity was assayed according to IUPAC recommendations by mixing 100 µl appropriately diluted samples with 100 µl of low-viscosity carboxymethyl cellulose (1% w/v) in 50 mM citrate buffer (pH 5.0) at 50oC for 10 min (Ghose, 1987).

Glucose and xylose standard curves were used to calculate cellulase and xylanase activities. In all assays, the release of reducing sugars was measured using the dinitrosalicylic acid reagent method (Bailey et al., 1992)
Filter paper assay for saccharifying cellulase (FPA) activity was assayed using Whatman filter paper №1 according to IUPAC recommendations (Yennamalli et al., 2013). One unit of enzyme activity was defined as the amount of enzyme, releasing 1 µmol of reducing sugars per minute. Laccase activity was determined by monitoring the A420 change related to the rate of oxidation of 1 mM 2,2-azino-bis-[3-ethylthiazoline-6-sulfonate] (ABTS) in 100 mmol sodium tartrate buffer (pH 4.5). Assays were performed in 1 ml spectrophotometric cuvette at 30 ± 1oC with adequately diluted culture liquid. One unit of laccase activity was defined as the amount of enzyme, which leads to the oxidation of 1 mmol of ABTS per minute (Bourbonnais, 1990).

Results and Discussion

Basidiomycetes Enzyme Activity In Solid-State Fermentation (SFF).

SSF is suitable for the production of enzymes by using natural substrates such as agricultural residues because they mimic the conditions under which the fungi grow naturally. The lignin, cellulose ,and hemicelluloses are rich in sugar and promote fungal growth in the fermentor and make the process more economical (Brijwani et al., 2010).

At the initial stage to estimate enzyme production, basidial fungi were screened during their cultivation on the wheat straw. The mentioned substrate is a complex plant waste containing all those polymers (cellulose, hemicellulose, lignin), degradation ability of which possess higher filamentous fungi.45 strains of different genera and species were chosen for testing, At SSF testing of basidiomycetes on the wheat straw substrate, their various potentials were revealed in terms of accumulation both hydrolyzing (CMCase, xylanase, FPA) and oxidizing (laccase) enzymes (Table 1. Abbreviated). Activities of synthesized enzymes were tested on the 7th, 15th, and 22nd days of cultivation, as it is known that enzyme synthesis by microorganisms significantly depends on the period and conditions of cultivation. Pleurotus ostreatus GK10 was found to be the best producer of hydrolyzing enzymes (CMCase, xylanase, FPA). Enzyme activities were 10.4 U/ml, 18.3 U/ml and 0.42 U/ml, respectively. However, Ganoderma sp. GB 03 displayed rather high laccase activity – 45.5 U/ml.

Table 1. Basidiomycetes enzyme activities (U/ml), during in solid-state fermentation of Wheat straw

Fungus	*Laccase*	*CMCase*	*Xylanase*	*FPA*
Fomes fomentarius GK33	0.9±0.2	5.0±0.1	6.9±0.5	0.42±0.05
Fomes sp. KA 35	2.9±0.9	1.0±0.5	4.9±0.7	0.23±0.07
Fomitopsis sp. IK46	2.2±0.4	3.9±0.3	3.3±0.2	0.12±0.05
Ganoderma applanatum IN10	13.4±1.2	2.2±0.9	3.4±1.2	0.15±0.10
Ganoderma sp. GB 03	45.5±2.3	3.0±0.3	2.1±0.7	0.11±0.03
Ganoderma lucidum IG74	10.3±1.3	3.5±0.1	5.3±0.4	0.09±0.02
Ganoderma sp. IN59	5.3±0.7	2.2±0.3	1.5±0.1	0.07±0.02
Lentinus edodes GK97	4.1±0.8	1.3±0.2	0.44±0.1	0.05±0.01
Pleurotus ostreatus IN20	3.5±0.7	5.2±0.5	2.5.±0.6	0.11±0.03
Pleurotus ostreatus GK10	5.1±0.5	10.4±1.2	18.3±1.1	0.42±0.05
Pleurotus drynus IN 11	22.5±3.3	5.4±0.6	8.6±1.0	0.34±0.03
*Trametes sp.*GK 60	22.5±0.8	1.6±0.4	4.0±0.1	0.05±0.01
Sabaduri 16	9.1±1.2	3.1±0.5	3.8±0.1	0.10±0.06
Bordjomi 23	7.2±1.7	3.7±0.4	6.2±0.3	0.28±0.05

Basidiomycetes Enzyme Activity in Submerged Fermentation (SF) on Orange Peels.

As seen in Table 2, all fungi somewhat displayed the ability to synthesize enzymes on the orange peels substrate. Pleurotus ostreatus GK10 was found to be the best producer of hydrolyzing enzymes – CMCase, xylanase and

the FPA with activities 14.4 U/ml, 26.3 U/ml, and 0.82 U/mI, respectively. Enzyme production has been found to be highly dependent on the conditions for fungus cultivation and media. As for laccase, the highest activity (87.5U/mL) showed the strain Ganoderma sp. GB 03. High ability to synthesize laccase also displayed the strains –Ganoderma applanatum IN10, Pleurotus drynus IN 11 and Trametes sp.GK 60. It is noteworthy that fungi in the range of the same genus revealed different activities of enzymes: e.g. Pleurotus drynus IN11 displayed a high ability to accumulate all four test enzymes; Pleurotus ostreatus GK10, representative of the same genus was distinguished by synthesis of hydrolases only and Pleurotus ostreatus IN20 showed low all four test enzymes activity.

Table 2. Basidiomycetes enzyme activities (U/ml), during submerged fermentation on orange peels

Fungus	*Laccase*	*CMCase*	*Xylanase*	*FPA*
Fomes fomentarius GK33	0.9±0.2	7.0±0.2	8.9± 1.1	0.42±0.05
Fomes sp. KA 35	2.9±0.9	2.0±0.5	7.9±0.7	0.23±0.07
Fomitopsis sp. IK46	2.2±0.4	3.9±0.3	4.3±0.2	0.19±0.05
Ganoderma applanatum IN10	30.4±1.2	3.2±0.9	5.4±1.2	0.25±0.10
Ganoderma sp. GB 03	87.5±3.3	4.0±0.6	3.1±0.9	0.17±0.05
Ganoderma lucidum IG74	10.3±1.3	3.5±0.1	5.3±0.4	0.09±0.02
Ganoderma sp. IN59	9.3±0.7	2.2±0.3	1.5±0.1	0.09±0.02
Lentinus edodes GK97	6.1±0.8	1.3±0.2	0.44±0.1	0.05±0.01
Pleurotus ostreatus IN20	3.5±0.7	5.2±0.5	2.5.±0.6	0.11±0.03
Pleurotus ostreatus GK10	5.1±0.5	14.4±2.2	26.3±1.3	0.82±0.08
Pleurotus drynus IN 11	39.5±3.3	10.4±0.4	11.6±1.3	0.34±0.03
*Trametes sp.*GK 60	32.5±0.8	1.6±0.4	4.0±0.1	0.05±0.01
Sabaduri 16	10.9±1.2	3.1±0.5	3.8±0.1	0.20±0.06
Bordjomi 23	8.2±1.7	4.7±0.4	6.2±0.3	0.28±0.05

Effect of the Lignocellulosic Substrates on Fungi Enzymes Activity

For efficient development of microbial technologies, selection of proper plant raw materials, on which synthesis of targeted enzymes occurs more effectively is of main importance for cultivation of fungi (Rosales et al., 2007; Levin et al., 2008). The effect of various lignocellulosic substrates on the accumulation of enzymes by the selected strains basidiomycetes was studied. Basidiomycetes strains of the genus Pleurotus were the best producers of the hydrolyzing enzymes while growing on substrates: mandarin peels, orange peels, wheat bran and tea peels) Ganoderma sp. GB 03 proved to be the best laccase producer while cultivation on almost all tested lignocelluloses with, the highest activity 93.5 U/ ml when grown on mandarin peels (Fig. 1, 2).

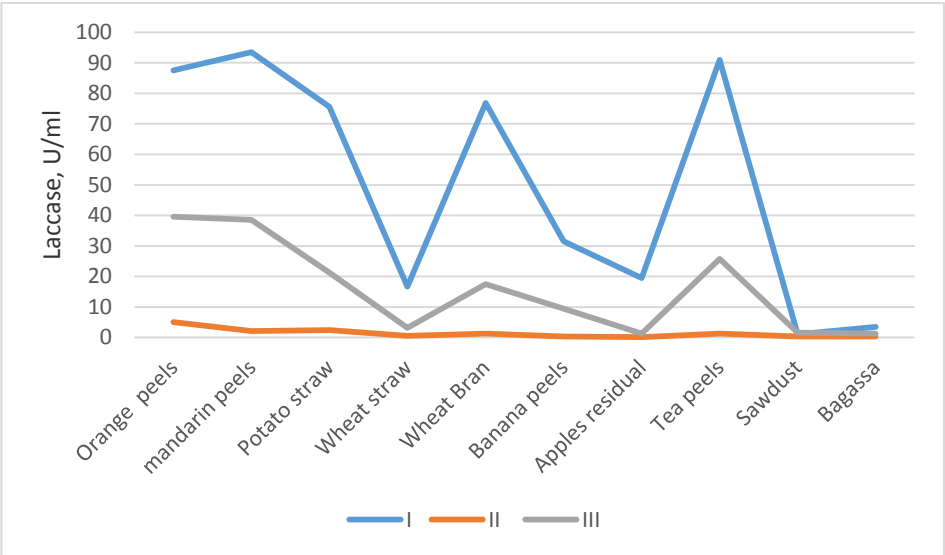

Figure 1. Effect of lignocellulosic substrates on the laccase activity while SF of: I - Ganoderma sp. GB 03; II - P. ostreatus GK10; III- P. drynus IN 11.

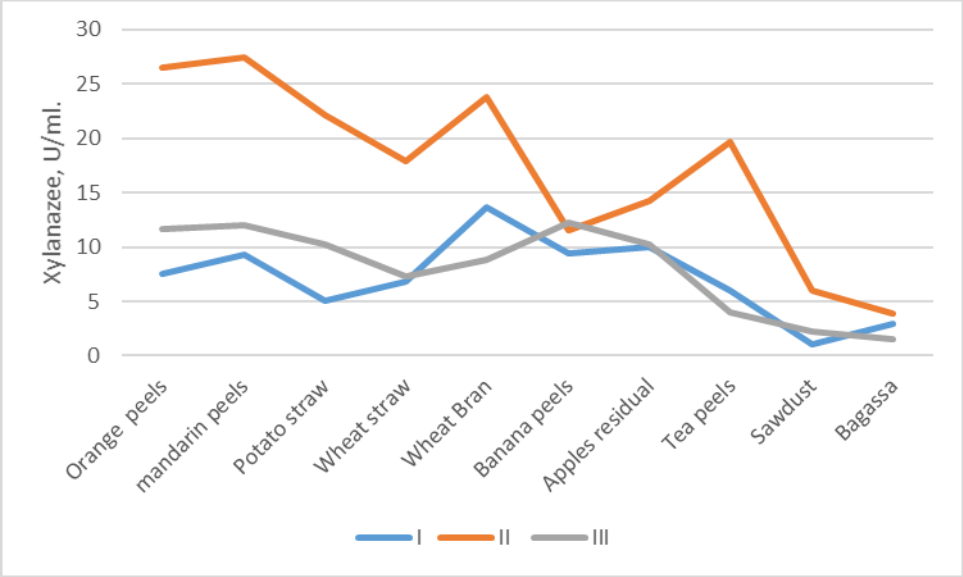

Figure 2. Effect of lignocellulosic substrates on the Xylanase activity while SF of: I- Ganoderma sp. GB 03; II- P. ostreatus GK10; III - P. drynus IN 11.

It should be mentioned that for the accumulation of enzymes, in both types of cultivation, the best was orange and mandarin peel and next came wheat bran and tea peels. Hence, how the process of delignification proceeded in mentioned plant substrates is the issue of further investigations.

Conclusion

The production of ligninolytic enzymes by 45 basidiomycetes species isolated from various geographical and ecological regions of Georgia was investigated under submerged (SF) and solid-state fermentation (SSF) of lignocellulosic materials. Notable intergeneric and intragenic differences were revealed with regard to the extent of oxidases activity. The regulatory role of lignocellulosic materials in laccase and hydrolyzing enzymes secretion by the selected fungi was shown. The studies revealed promising strains of the genera Pleurotus, Ganoderma and Fomes producers of lignocellulose deconstruction enzymes. The best producer of oxidative enzymes is Ganoderma sp. GB03; the best producer of hydrolizing enzymes is Pleurotus ostreatus GK10, Pleurotus drynus N11 and Fomes fomentarius GK33. The best condition for accumulation of laccase for all test strains was SF, when the index of laccase activity was significantly high in comparison of with that of SSF.

Recommendations

Conducted studies showed that the selection of plant biomass for the production of target enzymes is of special importance playing a decisive role in the effective development of technologies. The results obtained indicate that the production of biologically active compounds of basidiomycetes is a regulatory process. subject to extensive regulation and that understanding involved will be important for the development of modern technologies of their production.

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Scientific Ethics Declaration

The authors declare that the scientific ethical and legal responsibility of this article published in EPSTEM journal belongs to the authors.

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Extraction and Purification of the Potential Allergen Proteins from Aspergillus Fumigatus

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Abstract: Allergic diseases are an important public health problem today and are increasing rapidly around the world. According to the research conducted by the World Allergy Organization (WAO), %22 of the world population is struggling with allergic diseases. In our country, one out of every five citizens complains of at least one form of allergy, and it is the third most common chronic disease in children. Some of the allergic diseases develop suddenly and are life threatening. The diagnosis and treatment of allergic diseases is one of the most expensive diseases in our country and in the world. It is triggered by the production of IgE(Immunoglobulin E) in the body against antigens in individuals sensitive to allergen substances. Allergens that play a role in the development of allergic diseases are protein molecules that stimulate IgE synthesis. Pollen, fungus and house dust mites are the most common allergens due to their widespread presence in nature. The presence of fungal spores in the atmosphere for a long time with the effect of air flow increases the possibility of people coming into contact with these allergens. It is very important for health to determine which factors individuals have allergies to. Among the current diagnostic methods; respiratory function tests, skin tests with allergens (prick tests, interdermal tests, patch tests), serum total IgE determination and procovation tests. All of the existing tests used in the identification of allergic diseases in our country are of foreign origin and are imported. One of the fungi that cause allergies is Aspergillus fumigatus from the genus Aspergillus. In our study, we aimed to purify Aspergillus fumigatus allergen proteins and produce allergens that can be used in allergy diagnosis methods. For this purpose, protein isolation and purification of Aspergillus fumigatus, which was cultured and multiplied in SDA (Sabouraud Dextrose Agar) medium in the laboratory, was performed with 2 different methods. The protein amount of the product obtained was determined by the BCA(Bicinchoninic Acid) method. In our studies, it was determined that the protein content of the extract prepared with chloroform/methanol solution was higher than the protein amount of the extract prepared with ethanol solution.

Keywords: Allergy, Aspergillus fumigatus, Allergen protein, Fungal allergy, BCA (Bicinchoninic Acid)

Introduction

Allergic diseases are a very important health problem that affects many people from all age groups, significantly affecting the quality of life of patients and their families, and the number of patients increases over time. With the acceleration of industrialization, there has been a parallel increase in atmospheric pollution, which has led to an increase in allergic diseases around the world. Allergy can be transmitted genetically as well as can occur

with environmental factors. These environmental factors can be biological or chemical. Among the most important biological factors causing allergies are mites, pollen and fungal spores (ASAN 2002).

The habitats of fungi have very large areas. They can be found in the atmosphere, soil, aquatic ecosystems, dead plants and animals, although they are usually seen in damp places such as kitchens and bathrooms and in corners that cannot be ventilated, in foods such as vegetables, fruits and bread. Fungal spores carried by air in the atmosphere can cause very serious diseases when inhaled by humans and animals. Fungi that cause the most allergic diseases are Aspergillus, Alternaria, Penicillium and Cladiosporium genera (CORSİCO 1998).

Fungi take part in the ecosystem as saprophytes, but they can also survive as parasites on some living things. There are spores in the atmosphere, which are the reproductive structures of many fungi. Fungi love moist environments and the number of spores in the atmosphere increases when it rains. These fungal spores cause allergic reactions in humans and animals (DURUGBO 2013).

Allergen substances are antigens that form their specific IgE (Immunoglobulin E) antibody and bind to this antibody. Allergic Bronchopulmonary Aspergillosis (ABPA) is an important lung disease that affects more than 4 million people worldwide, caused by the allergens of Aspergillus fumigatus. An increase in Aspergillus-specific serum IgE (Immunoglobulin E) is observed in the serum of patients with ABPA (KURUP 2000). The average size of Aspergillus fumigatus spores can be 2.0 μm to 3.0 μm. Due to such a small size, the spores can easily become airborne. In addition, as Aspergillus fumigatus has the capacity to reproduce very easily and quickly, the human respiratory tract is always at risk of contracting ABPA infection (LATGE 2001).

It is very important for health to determine which factor individuals are allergic to. Among the available diagnostic methods; respiratory function tests, skin tests with allergens (Prick tests, interdermal tests, patch tests), serum total IgE determination and procovation tests are included. All of the current tests used in the identification of allergic diseases in our country are of foreign origin and are imported. In our study, we aimed to purify Aspergillus fumigatus allergen proteins and produce allergens that can be used in allergy diagnosis methods. For this purpose, the protein amount of Aspergillus fumigatus cultured and grown in SDA (Sabouraud Dextrose Agar) medium in the laboratory was determined by BCA (Bikinchoninic Acid) method.

Method

Preparation of *A. Fumigatus* Extracts

Aspergillus fumigatus strain 9197 was purchased commercially and propagated in our laboratory. Aspergillus fumigatus spores inoculated on SDA medium were incubated for 15 days at 25°C. After morphological examination, samples were collected with the help of sterile wooden cotton swab. The collected mycelium was transferred into chloroform/methanol (2:1) solution and mixed in a falcon tube at 4°C for 24 hours on a magnetic stirrer. The degreased mycelium was left to dry with blotting paper at room temperature (25°C) for 24 hours. The powdered materials were mixed in the prepared phosphate buffered saline (PBS) (1:50 g/ml) at 4°C for 72 hours on a magnetic stirrer. The solution was centrifuged at 5000 rpm for 30 min at 4°C. The supernatant was taken and centrifuged again at 5000 rpm for 30 min at 4°C. The supernatant was dialyzed in 25% PBS at 4°C for 6 hours and then dialyzed again in distilled water at 4°C for 6 hours. The solution was frozen at -20°C for 72 hours and then lyophilized. Protein extraction of dialysis and lyophyse samples was performed according to the extraction method of Ziwei Li et al. (2018). Protein concentrations were determined by extracting the obtained extracts into SDS buffer solution.

Determination of Total Protein Concentration

The total protein concentration of the mushroom extracts was determined using the bicinchoninic acid (BCA) method proposed by Smith et al. A commercially purchased BCA Macro Assay was used to determine protein concentration. BCA analysis was performed following the protocol recommended by the manufacturer (Walker, 2002). The protein amounts of the mushroom extracts prepared in the study were determined with the help of the standard graph drawn with bovine serum albumin (BSA) standards.

Results and Discussion

In our study, Aspergillus fumigatus proteins, one of the allergenic fungi, were extracted with 2 different extraction protocols. Extracted protein amounts were measured by the BCA method. The protein amounts of the mushroom extracts prepared in the study were determined with the help of a standard graphic drawn with bovine serum albumin (BSA) standards.

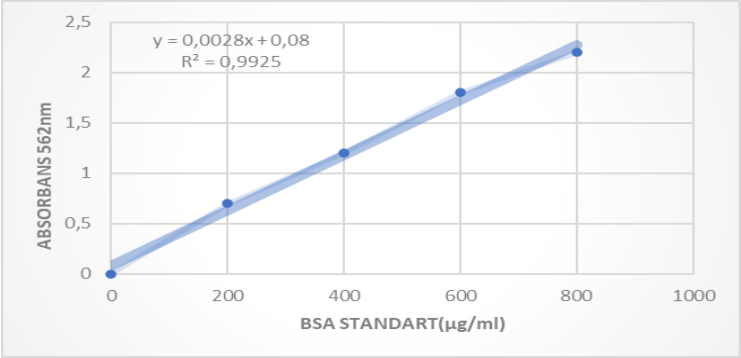

Figure 1. Standard graph used to determine protein concentration

Table 1. Total protein concentrations of Aspergillus fumigatus extracts measured by BCA method

Alergen name	Absorbance Measurements	Absorbance Average	Protein concentration (µg/mL)
Aspergillus fumigatus (Chloroform/Methanol)	0,802/0,791/0,801	0,798	228
Aspergillus fumigatus (Ethanol)	0,774/0,767/0,775	0,772	218

In our study, Aspergillus fumigatus proteins, one of the allergenic fungi, were extracted. Extracted protein amounts were measured by the BCA method. As a result of the data obtained, the total protein amount of Aspergillus fumigatus was 228 µg/mL according to the Chloroform/Methanol protocol and 218 µg/mL to the ethanol protocol. The amount of protein obtained from the Chloroform/Methanol protocol was higher than the amount of protein obtained from the ethanol protocol. The protein amounts obtained in previous studies of Aspergillus fumigatus (Kahlert 1996) and the protein amounts obtained in our study support our study.

Conclusion

In recent years, cases of allergies caused by fungi have been increasing. For this reason, studies on the determination of allergen proteins of fungi, which are common in nature, have gained importance. In our study, Aspergillus fumigatus extracts, which is one of the allergenic fungi and used in allergen kits, were prepared and their protein concentrations were determined. The data obtained from this study form the basis for the production of alternative domestic kits to the imported kits used in the diagnosis and treatment of allergy patients with subsequent studies.

Scientific Ethics Declaration

The authors declare that the scientific ethical and legal responsibility of this article published in EPSTEM journal belongs to the authors.

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Investigation of Antimicrobial, DNA Protective and Cytotoxic Activity of Red Cabbage (*Brassica Oleracea* L. *Var. Capitata F. Rubra*) Plant

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Abstract: The need to search for natural products that offer a safe alternative to drugs and industrial products that often cause side effects and harm human health is growing over the years. In this study, the water and methanol extracts of the anthocyanin-rich red cabbage (Brassica oleracea L. var. capitata f. rubra) which is available on markets throughout the year, were examined for their antibacterial activity on Escherichia coli, Staphylococcus aereus and Stenotrophomonas maltophilia, and for their antifungal activity on Yarrowia lipolytica, Candida albicans and Candida maltophilia. The DNA protective activity of extracts on the pBR322 plasmid was evaluated by exposing pBR322 to UV and adding hydrogen peroxide. Furthermore, the MTT assay was performed on the H1299 lung cancer cell line and the HUVEC cell line as a negative control to evaluate the cytotoxicity of the extracts. The red cabbage extracts were found to have no antibacterial or antifungal activity on the tested microorganisms. The results of DNA protective activity have shown that at a concentration of 50 mg/mL there is a DNA protective activity of the extracts. No effect of the red cabbage extracts on the H1299 cell line as shown by MTT assay results. On the other hand, the decrease in the viability of HUVEC cells started at a concentration of 25 µg/mL and above, reaching 70% at 100 µg/ mL for the water extract and 74 % at 100 µg/ mL for the methanol extract. Further studies to investigate the active components of the DNA protective activity in the red cabbage extracts are required. Further studies are also needed to explore the possibility of incorporating these components in cosmetic products such as sunscreens without causing skin damage.

Keywords: Red Cabbage, Antibacterial Activity, DNA Protective Activity, MTT test.

Introduction

Red cabbage (Brassica oleracea L. var capitate f. rubra) is one of the economically important vegetables consumed all over the world as a fresh, cooked and fermented form known for its enriched bioactive constituents (Fernandes et al., 2019). Red cabbage acts as an antioxidant that helps prevent chronic diseases. It is an abundant source of potassium, manganese, iron, and magnesium minerals. It also contains A, B, C, K vitamins and a litany of phyto-chemicals (Draghici et al., 2013). The wonderful purple/red color of red cabbage is due to the presence of anthocyanin. Cabbage leaves vary in color depending on the pH of the soil, red at low pH and violet to green at high pH (Fernandes et al., 2019).

While the search is increasing daily for natural products that provide a healthy alternative to industrial drugs, this study aimed to study the effect of water and methanol extracts of red cabbage as anti-bacterial and anti-fungal, as well as the possibility of protecting DNA and the possibility of working as an anti-cancer drug.

Antimicrobial, DNA protective and anticancer activity of red cabbage

Antibiotic resistance is increasing as a result of its excessive and unconscious use, and this problem is the focus of researchers' attention. Therefore, studies and research are continuing to develop new drugs, especially those of natural origin that are used as antibiotics in order to avoid the side effects that may be caused by industrial drugs.

Reactive oxygen species may result either from internal processes such as metabolism, inflammation and exercise, or from external sources such as environmental pollutants, radiation, smoking and industrial solvents. An imbalance between free radical production and antioxidant defenses leads to what is called oxidative stress. Oxidative stress leads to cell damage, causing a number of cancers, inflammation, aging and neurological disorders. Reactive oxygen species primarily target DNA, proteins, and lipids (Kada et al., 2017; Lobo et al., 2010).

The rapid increase in the number of people with different cancers poses a great challenge for researchers to discover effective natural medicines that can be used safely, whereas many studies have shown that some natural products can effectively affect the prevention of a number of malignant cancers. Some studies have focused on the anti-cancer activity of red cabbage, which is rich in phenolic compounds and anthocyanin (Hafidh et al., 2013). Cytotoxicity tests for extracts are the first commonly used preclinical tests and thus cell culture and potential therapeutic agent screening have become commonplace (Van Tonder et al., 2015).

Tetrazolium Bromide (MTT) Assay

The MTT test is a common cytotoxicity test that is a colorimetric test based on the reduction of the yellow tetrazolium pigment through the metabolic activity of the enzyme and its conversion to formazan violet crystals in living cells.

Method

Red cabbage collection and extraction preparation

Red cabbage was collected from a local market in Gaziantep, Turkey. The red cabbage leaves were washed well, shade dried for 10 days then were ground into a fine powder. A Gerhardt EV 14 soxhlet apparatus was used to obtain water and methanol extracts of red cabbage leaves. The extracts were placed in Petri dishes and the solvents were left at room temperature 2-3 days until they evaporated (Topçu et al., 2007).

Antimicrobial assay – agar diffusion method

Escherichia coli ATCC 25322, *Staphylococcus aereus* ATCC 29213, *Stenotrophomonas maltophilia, Yarrowia lipolytica* NCAIM Y00591, *Candida albicans* ATCC 10231, *Candida parapsilosis* ATCC 22019 are bacterial and fungal strains examined in our research that were obtained from the Molecular Biology laboratory at biology department at Gaziantep University. Standard disk diffusion method recommended by EUCAST (European Committee on Antimicrobial Susceptibility Testing) was used to determine the antimicrobial activity of red cabbage leaf extracts. The red cabbage powder was dissolved in the same solvent, water, methanol to final concentration of 50 mg/mL and loaded in 6 mm sterile blank discs. Screening was carried out by disc diffusion using 100 ml of microbial suspension 0.5 McFarland density and was spread on the surface of Mueller Hinton agar plates. The impregnated discs were placed on the agar surface and incubation for 24 hours was implemented. Inhibition zone diameters were measured in mm. For the control, the results were checked in the EUCAST QC Tables at http://www.eucast.org (EUCAST, 2021).

DNA protective activity

The DNA protective activity of red cabbage extracts was tested on pBR322 plasmid (Vivantis, Czech Republic). pBR322 plasmid (172 ng/µL) was treated with 30% H_2O_2 and UV in the presence and absence of 50 mg/mL of red cabbage extracts. UV irradiation was continued for 5 min by UV transilluminator with intensity 8000 µW/cm^2 at 302 nm under room temperature. The mixture was loaded on 1.5% agarose gel for electrophoresis. Electrophoresis was performed at 100 V for 45 min. samples without extracts were used as controls (Tepe et al., 2011).

Cytotoxic activity

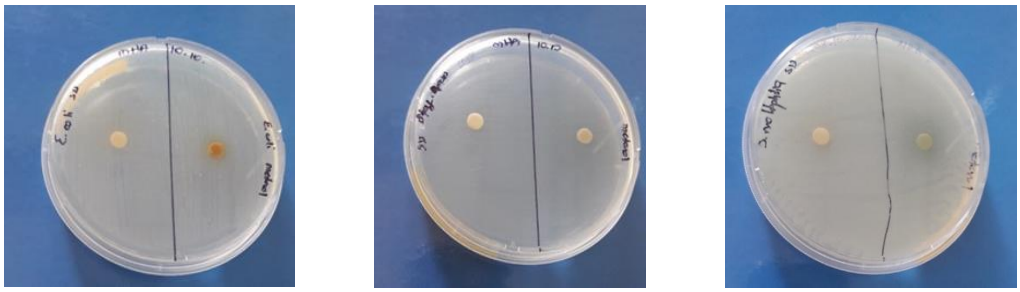
The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used to study the cytotoxic activity of red cabbage extracts. H1299 (human non-small cell lung carcinoma cell line) carcinoma cells was tested for cell viability after treating with extracts and HUVEC (Human Umbilical Vein Endothelial Cells) normal cells as a control. The cells were seeded in 96-well plates at a density of 5000 cells/well and incubated for 24 hours at 37 °C in a CO_2 incubator. After incubation, cells were treated with 100 µL of different concentrations of extracts (100, 50, 25, 12.5, 6.25 µg/mL) and incubated in 5% CO_2 for 48 hours at 37 °C. 40 µl of MTT solution was added to wells and incubated again for 4 hours at 37 °C. The medium was removed and 80 µL DMSO was added to dissolved formazan crystals. Finally, the absorbance was measured in a spectrophotometer (Thermo Scientific, USA) at 540 nm (Bahuguna et al., 2017). Cell viability was calculated using the following formula after reading the absorbance values (Kilic, 2020):

Viability % = 100 × (mean absorbance of cells treated with extract / control cell viability without extract).

Results and Discussion

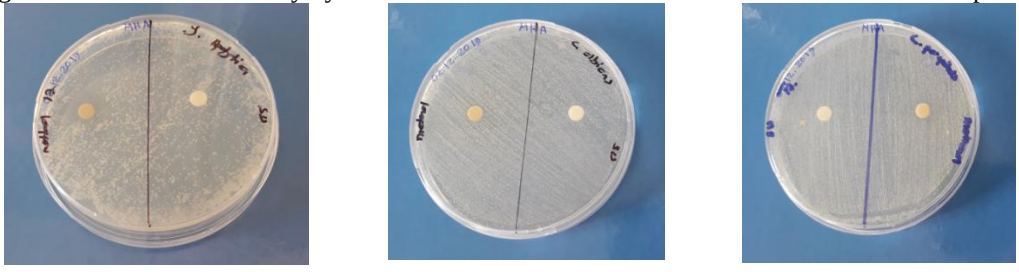
Antimicrobial Effect of Extracts

Red cabbage extracts did not show any antibacterial or antifungal activity whreras pathogens continued growth in petri dishes with zero growth inhibition zone. The results of the disk diffusion test are shown in Figure 1 and Figure 2. Ayshwarya and Rameshwari (2015) have reported that red cabbage extracts (100 µg -200 µg) have antibacterial effects on *E. coli* and *S. aereus*.



A B C

Figure 1. Antibacterial activity by disc diffusion method. A. E. coli. B. S. aereus. C. S. maltophilia.



A B C

Figure 2. Antimicotic activity by disc diffusion method. A. Y. lipolytica. B. C. albicans. C. C. parapsilosis.

The extraction method used in the study depends on homogenizing the ground sample with solvent and keeping the preparation for 72 hours at room temperature then evaporating the solvent may be more effective. In addition, the extract of *B. oleracea* obtained from 2.4 mol/L HCl methanol proved to have antibacterial activity for *E. coli* and *S. aereus* bacteria (Hafidh et al., 2011). It is thought that the solvent used in Hafidh et al. (2011) study made a difference from our study results. According to Santhosh and Vaithyanathan (2018) natural red cabbage dye has a clear antimicrobial activity on *S. aereus* bacteria. It is believed to be related to the red cabbage extract extraction method.

DNA Protective Activity

DNA damage protective activity of red cabbage water and methanol extracts after UV irradiation of DNA plasmid in H_2O_2 presence with 50 mg/mL concentration of the extracts is shown in figure 3. Untreated pBR322 plasmid DNA (K1) showed a bright faster band on agarose gel electrophoresis and conformed to the original shape of supercoiled (scDNA). The slower moving band was the open circular form (ocDNA) and linear form (linDNA) was in the middle. The detrimental effect on treated plasmid DNA (K2) without extracts was clearly visible where only one faint band (scDNA) appeared. (LS) and (LM) were samples in which water and methanol extracts were added to the reaction mixture (DNA plasmid + UV + H_2O_2), respectively. It was noted that adding red cabbage extracts (50 mg/mL) to the DNA with (UV + H_2O_2) induced a good recovery level of DNA especially with methanol extract. These results are consistent with Kada et al. (2017) study which showed the DNA protective effect of *Hertia cheirifolia* extracts. This is the first time that DNA protective activity of red cabbage extracts was demonstrated and there is not any such previous research in the literature till now. Further investigations to search for the active components of the DNA protective activity in the red cabbage extracts are required.

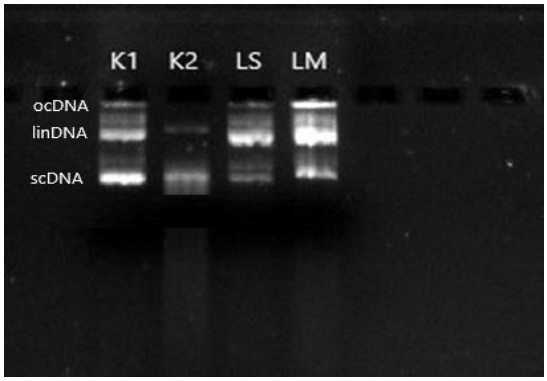


Figure 3. DNA protective activity

K1: Control: DNA plasmid, **K2:** Control: DNA plasmid + UV + H_2O_2,

LS: DNA plasmid + red cabbage water extract + UV + H_2O_2 , **LM:** DNA plasmid + red cabbage methanol extract + UV + H_2O_2

Cytotoxic Activity

The cytotoxic effect of red cabbage water and methanol extracts was investigated on H1299 cancerous cell line and HUVEC normal cell line as a control. The viability (%) graphs are given in Figure 4 and Figure 5. Cancer cells (H1299) maintained a vitality rate of over 90 % even at the highest applied concentration (100 µg/mL) of the extracts. While the vitality rate of HUVEC normal cells, which used as a control, decreased gradually with increasing concentration of extracts until it reached about 74.16% and 69.54% at the concentration of 100 µg/mL of water and methanol extracts, respectively. These results are in agreement with Farag and Abdel Motaal study (2010) which showed weak anticancer activity of red cabbage aqueous extracts against A-549 cell line (human lung carcinoma) as cell vitality was 73% at 500 µg/mL concentration. In Devi and Thangam study (2012), anticancer activity of isolated sulforaphane (SFN) from red cabbage was approved against HEp-2 (human epithelial carcinoma) and Vero cells. Ye et al. (2020) purified antifungal protein from red cabbage seeds and demonstrated its antiproliferative activity on nasopharyngeal cancer NE-1 cells and hepatoma HepG2 cells. Three cancerous cell lines Caco-2 (human epithelial colorectal adenocarcinoma cell), KYSE-30 (human esophageal squamous carcinoma cell) and MCF-7 (human adherent and epithelial breast cancer cell line) were

searched for their anticancer effect and compared with HFF-3 (human adherent and fibroblast normal cell from foreskin tissue). The prepared concentrations in the study ranged from 625-20000 µg/mL, and the study showed the presence of anti-cancer activity for the studied cancerous cell lines, especially at higher doses. Red cabbage extract also decreased the cell viability of HFF-3 normal cells (IC_{50} = 6.4 mg/mL) thus red cabbage extract should not be used at higher concentration than 6.4 mg/mL to avoid normal cell damage (Tajalli et al., 2020).

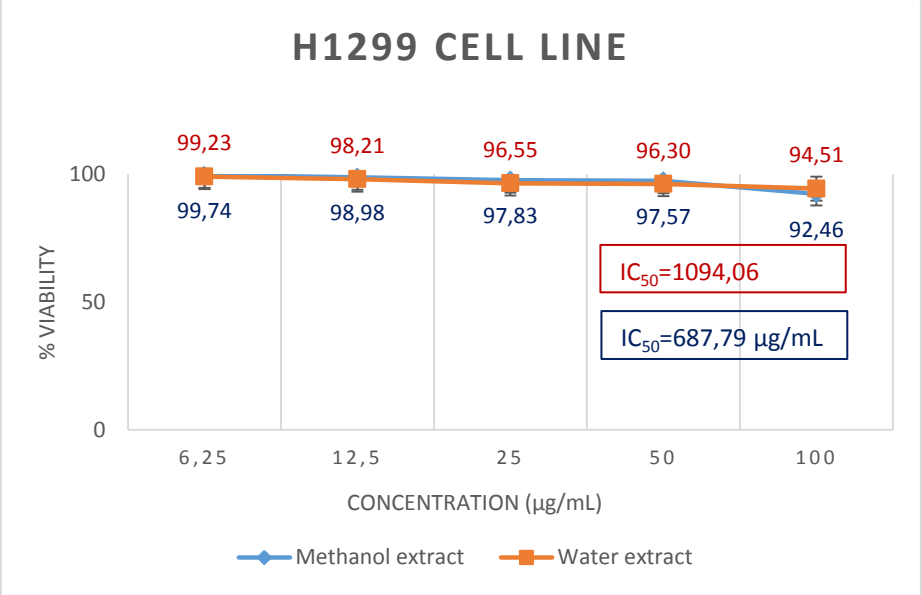


Figure 4. Viability (%) graph of red cabbage extracts (H1299).

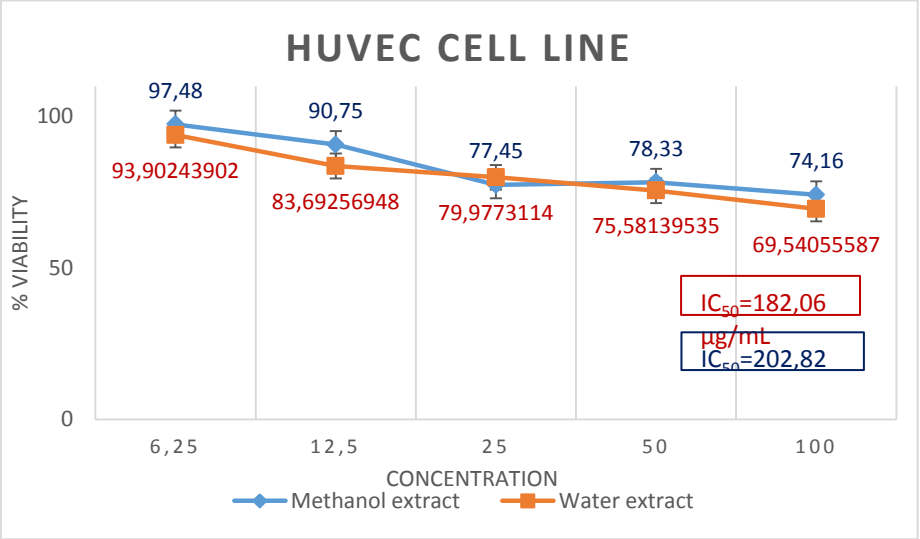


Figure 5. Viability (%) graph of red cabbage extracts (HUVEC)

Conclusion

According to our study and reviewing of previous literature, red cabbage extracts do not have any anti-fungal activity, but they may have anti-bacterial activity against some types of bacteria and that by using 2.4 mol/L HCl methanol as a solvent and extracting by soaking the plant powder in solvent, then filtering the extract and evaporating the solvent at room temperature. Red cabbage water and methanol extracts have a DNA protective activity at a concentration of 50 mg/mL of the extracts. Previous literature did not report any study on the effect of red cabbage extracts on DNA protection activity and this study opens new horizons for research in this field. There is no anticancer activity of red cabbage extracts. However, some isolated components from red cabbage such as sulforaphane and antifungal protein possess anti-cancer activity against some types of cancer.

Recommendations

Further studies are needed to investigate the optimal concentration of red cabbage extracts that gives the best DNA protection effect as well as further studies to explore the effective ingredients in this process and the possibility of incorporating these ingredients into cosmetics such as sunscreens without causing skin damage.

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Scientific Ethics Declaration

The authors declare that the scientific ethical and legal responsibility of this article published in EPSTEM journal belongs to the authors.

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