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Dear Colleagues,

TOJSAT welcomes you. TOJSAT would like to thank you for your online journal interest. The online journal system has been diffused very fast for last ten years. We are delighted that a lot of academicians from around the world have visited TOJSAT. It means that TOJSAT has continued to diffuse new trends in science and technology to all over the world since January, 2011. We hope that the volume 9, issue 3 will also successfully accomplish our global science and technology goal.

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TOJSAT thanks and appreciate the editorial board who have acted as reviewers for one or more submissions of this issue for their valuable contributions.

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**July 01, 2019**

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The journal favours papers addressed to inter-disciplinary and multi-diciplinary articles shown in the section of scopes. In this issue of the online journal, selected peer reviewed papers are published.

I will thank to the readers for their supports by sending their valuable scientific works to publish in this journal.

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## Table of Contents

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|  |     |
|--|-----|
| INVESTIGATION OF FACTORS AFFECTING SURFACE ROUGHNESS OF HEAT TREATED 5040 STEEL BY EXPERIMENTAL DESIGN METHOD  | 215 |
| <i>Ayhan AYTAÇ, Muhammed İLİVAN</i>  |     |
| NITROREDUCTASES FROM THERMOPHILIC BACILLUS SPECIES   | 223 |
| <i>Kemal GÜVEN, Fatma MATPAN BEKLER, Reyhan GÜL GÜVEN</i>  |     |
| PROTECTING MASONRY SURFACES FROM MOLD GROWTH WITH MINIMUM RISK ANTIMICROBIAL AGENTS  | 227 |
| <i>Benton Allen, Emily M. Hunt</i>   |     |
| THE USE OF NEW TECHNOLOGIES IN THE ELDERLY IN THE NORTH OF PORTUGAL  | 233 |
| <i>Vitor RODRIGUES, Marta GONÇALVES, Arsénio REIS, Carlos TORRES, Maria João MONTEIRO, João BARROSO, Tiago RODRIGUES, Hugo PAREDES, Vitor FILIPE, Rui XAVIER</i> |     |



## INVESTIGATION OF FACTORS AFFECTING SURFACE ROUGHNESS OF HEAT TREATED 5040 STEEL BY EXPERIMENTAL DESIGN METHOD

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**Abstract:** One of the main problems to be tried to solve in machining is machinability. Manufacturer seeks answer of how he can produce his product cheaper and more quality. One of the main purposes in machining is to bring surface roughness to the top level. The stage where the most decisive work can be done in improving the product quality is the parameter design phase for both product and process design. In order to determine the most effective parameters and to evaluate the results more efficiently, the Taguchi experiment design technique is preferred to realize the experiments in a shorter time. This performed study was done by the aim of investigating the effect of change in terms of hardness, feed rate, and cutting tool of “Dual Phase” steel, which is a class of HSLA (High Strength Low Alloy) steels that increases usage and importance day by day, on surface quality in turning process in the pieces subjected to reasonable forces in machine, device, car manufacturing. Experimental design In the Minitab statistical analysis program, Taguchi experiment design technique and 9 trials according to L9 orthogonal design. Experiments were performed in dry cutting conditions in CNC Turning Table that has 1.5 kW power and rotates with maximum 2000 rpm. Variance analysis and signal / noise ratio were used in the evaluation of the test results. . It was possible to achieve the intended results with only one third of the number of experiments required in full factorial design (9 experiments instead of 27). In the experiments, the cutting tool type (CBN, Ceramic and Carbide cutter), the feed rate (0,02, 0,04 and 0,06 mm/cycle) and the material hardness (Material with three different hardness values obtained by annealing at 745, 760, and 775 degrees obtained after heat treatment) were used as the independent variable (factor). The mean surface roughness value (Ra) as a dependent variable was determined from measurements taken at 6 different points in three trials. As a result, the most effective parameters on the surface quality are the feed rate, the material hardness (microstructure) and the cutting tool. The results obtained are interpreted together with the evaluations which have been entered into the literature before.

**Keywords:** Taguchi, Dual Phase, Surface Quality, Machinability.

### Introduction

Importance of heat treatment applied in the process of improving mechanical properties has gradually increased with growing technology. Heat treatment applied to steel plays an important role on defining machinability properties of steel (Uzkut and Ark., 2001., Çeviker, 1991., Demir and Ark., 2011). Material technology and cutting tool technology growing in recent years allows machinability of heat treated steels easily and quality (Elbestawi and Ark., 1996, Yaka and Ark., 2016). After heat treatment applied of steels, internal strains occur in material. This causes problems such as warping in workpiece, burning on surface, and micro crack. Arising these problems can be eliminated with various processes applied after heat treatment Çolak, 2006., Daghini ve Nicolescu., 2007., Binali and Ark., 2018).

Dewes et al., processed AISI H13 material with 52 HRC hardness by using WC solid milling machine coated with TiCN. They observed that cutting speed increase temperature, temperature increases as directly proportional with cutting speed, and temperature in cutting area decreases with increasing tool radius. Asilturk and Akkus (2011), investigated effect of cutting speed, cutting depth, and feed rate on surface roughness in turning process of hardened AISI 4140 (51 HRC) with coated carbide. As a result of experimental studies, they showed that feed rate has the most significant effect on Ra and Rz.

Ren et al. (2014) investigated the study about optimization of cutting geometry in last milling process on Ti-5Al-5Mo-5V-1Cr-1Fe alloy with Taguchi method. They aimed at reaching combination to minimize cutting forces and surface roughness, and to optimize cutting speed by changing milling cutter geometry in the study. As a result, they found that multi performance characteristics can be improved with grey-Taguchi method. Zhao (2017), performed an experimental study to understand the effect of cutting edge radius on workpiece machining performance with regards to surface roughness and tool abrasion in AISI52100 steel. Three groups of cutter

(CBN) with 20, 30, 40  $\mu\text{m}$  nominal edge radius were used in the study. Change in cutting edge radius was evaluated with an optical microscope. The effect of surface radius and tool abrasion on cutting edge radius was investigated in different machining conditions with different machining tests by designing three-leveled, two-factored experiments with Taguchi. Variations tends to lower with increasing nominal values of cutting radius, and also, it was resulted that cutting radius has an important effect on surface roughness and tool abrasion.

The situation defined above is an example of typical problem showing up machining tool that is appropriate to tolerance in engineering and research and development studies. It is required to improve surface quality which is a measurement of machinability, to make experiment to investigate effect of tool type, cutting speed and feed rate on performance, and make optimization by evaluating these experiments.

The purpose of this study is to make optimization and investigate effect of material and cutting parameters (feed rate and cutting tool type) on workpiece surface roughness that is an important machinability criteria by doing machinability experiments with turning method on steels used in machine production industry. In this study, dual-phased steel specimen obtained in three different hardness after heat treatment was performed to turning process with three different feed rate by using three different cutter type by evaluating factors affecting turning surface quality after literature review. Results obtained from Taguchi optimization were evaluated with regards to adaptation to literature.

### Experimental Study

#### Used Material and Properties

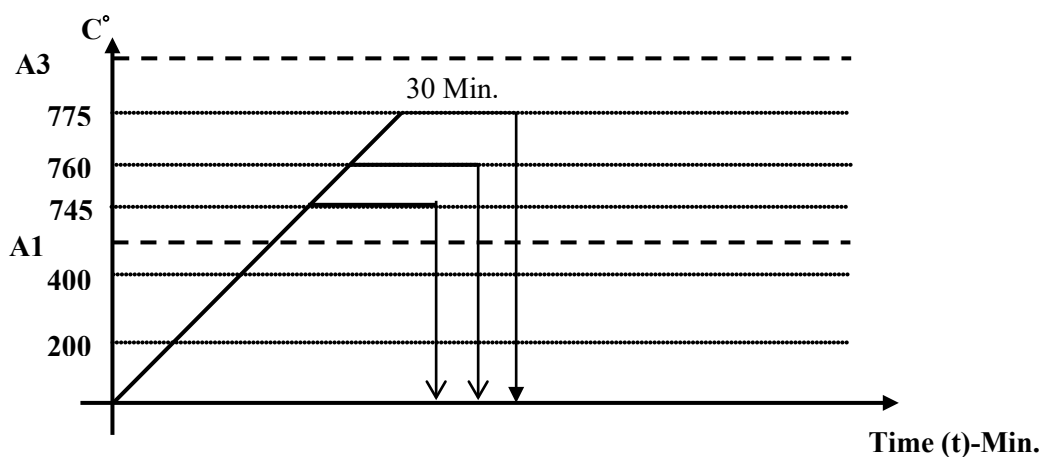
5040 ERDEMIR quality numbered SAE 1040 Standard Tool produced as hot mill product in Ereğli Iron and Steel Factories (ERDEMIR) T.A.S and given chemical composition in Table 1 was used by preparing 12 mm diameter, and hardness measurement was performed by doing heat treatment.

**Table 1:** Chemical Composition of 3936 quality steel

| Quality | Standard | Chemical Composition (% Weight) |      |       |       |       |       |
|---------|----------|---------------------------------|------|-------|-------|-------|-------|
|         |          | C                               | Mn   | P     | S     | Si    | Al    |
| 5040    | SAE 1040 | 0.38                            | 0.75 | 0.010 | 0.016 | 0.210 | 0.058 |

It was utilized from previous studies to define relevant annealing temperatures. Temperatures values in the study performed related to mechanical properties of materials having same chemical composition (Tayanç and Toktas, 2001).

It was given water in water to turning specimen annealed 30 minutes in 745, 760, and 775  $^{\circ}\text{C}$  temperatures on the purpose of obtaining three different hardness on same material in total. During preparation of specimen, it was waited to chill oven for two different temperatures to prevent different heat treatment conditions. Specimen were subjected to cooling in water after annealing process. Temperature-time diagram (T-t) belong to aforesaid heat treatment was shown in Figure 1.



**Figure 1.** Temperature (T)-Time(t) diagram.

**Microhardness Measurement**

Hardness measurement of specimens were performed with micro Vickers method in Qness Q10 microhardness test equipment. HV 0,5 load and 10 seconds main loading values were defined as test parameters, and trace image was taken by the help of 40X lens. Hardness measurements of materials were obtained as Vickers (HV) in Qness Q10 microhardness equipment. Results were given in Table 2.

**Table 2:** Hardness Values

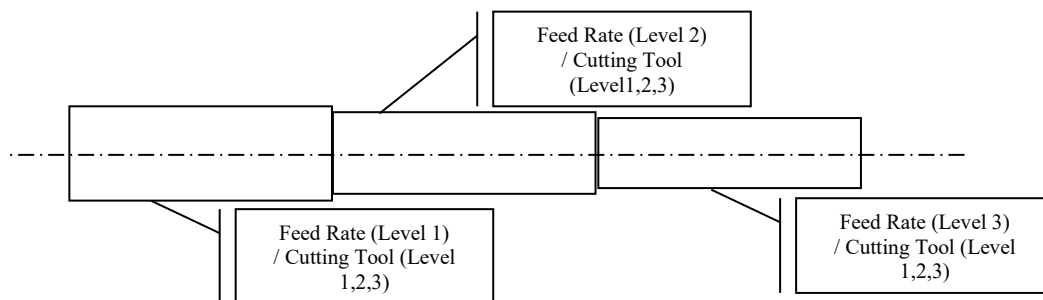
| Materil               | 745°  | 760°  | 775°  |
|-----------------------|-------|-------|-------|
| Meaurement 1 (HV)     | 174   | 360   | 429   |
| Measurement 2 (HV)    | 175   | 389   | 434   |
| Average Hardness (HV) | 174,5 | 374,5 | 431,5 |

**Surface Roughness Measurement**

Surface roughness was measured with TIME TR200 surface roughness equipment. Three measurement trace to parallel and vertical to cutting direction were measured. The mean of three arithmetical average surface roughness measurement (Ra) in the direction and through cutting were used to show surface roughness of specimen.

**Choosing Cutting Parameters and its Levels**

Experimental studies within study were performed in CNC Turning Table that has 1.5 kW power and rotates with maximum 2000 rpm. Dual phase steels is a new class of high strenght-low alloy steels (HSLA). A cylindrical workpiece made from 5040 number steel having 0,38 % C ratio that is produced by ERDEMIR as special wheel steel was processed with Al<sub>2</sub>O<sub>3</sub> coated Cementite Carbide, Ceramic, and CBN cutting tools by applying three different feed rate in dry cutting conditions in the study. Cutting area order is shown in Figure 2. Factors used in machining and its levels were defined with user experience and were specified in Table 3.



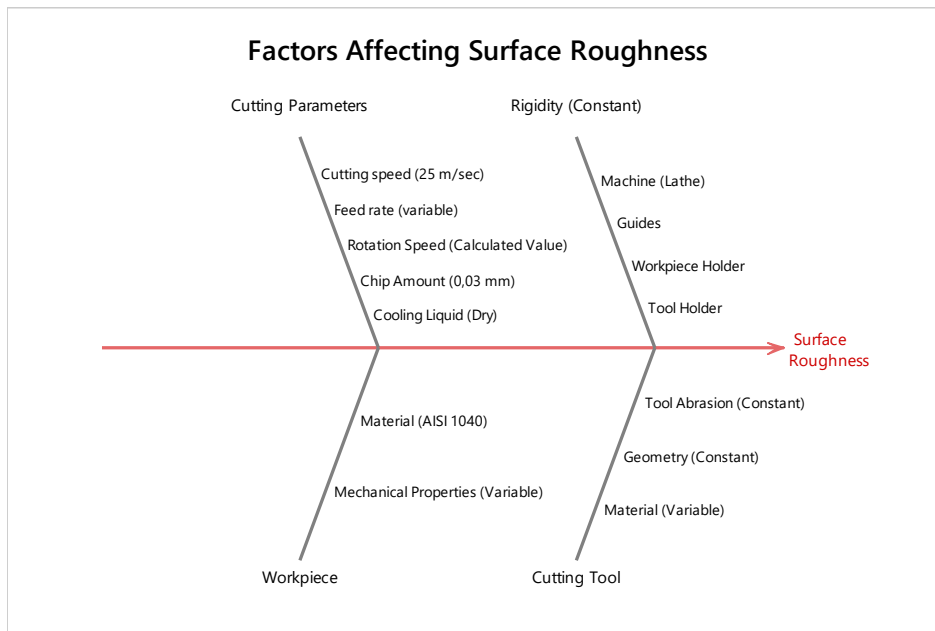
**Figure 2.** Cutting Area Order

Fishbone diagram is one of output in designing experiments. A fishbone diagram can be created to see relations defined factors to each other's exactly (Şirvancı, 1997). This diagram specifies all factors representing product or process quality and affecting measured values (Savaskan at al., 2004). It was decided variable and constant factors with the help of fishbone diagram. Factors affecting machinability are collected under four main categories (cutting parameters, rigidity, workpiece, cutting tool) as shown in Figure 3.

Values of variable parameters except factors that has to be constant and that cannot be controlled were taken as compatible with real working environment values as much as possible. Because cooling liquid usage will have positive effect to surface quality, experiments were planned in dry condition to keep experiment numbers in certain amount.

**Table 3:** Cutting Parameters

| Factors             | Unit   | Symbol | Level 1 | Level 2 | Level 3 |
|---------------------|--------|--------|---------|---------|---------|
| Cutting Tool        | -      | A      | Carbide | Ceramic | CBN     |
| Feed Rate           | Mm/dev | B      | 0,02    | 0,04    | 0,06    |
| Heat                | /Hv0.5 | C      | 745°C   | 760°C   | 775°C   |
| Treatment /Hardness |        |        |         |         |         |



**Figure 3.** Evaluation of Factors Affecting Surface Roughness with Fishbone Diagram

**Taguchi Experiment Design**

Choosing optimum process conditions is an extremely important subject since it defines surface quality of produced pieces and dimensional sensitivity. Contact surfaces of machine elements working together are desired to finish with particular rough, especially in machine design. Sometimes, sensitive surfaces are required, and sometimes rough surfaces are suitable to work machine properly, as well. Therefore, it is important to define surface roughness in design step, and to control in production step. After, surfaces can be operated in desired roughness values (Karayel, 2009). It is needed to optimize surface quality and to define optimum cutting parameters on the purpose of machining machine pieces as suitable to environment they will work. For this purpose, feed rate, cutting tool, and material hardness was defined as parameters to use in this study. Machining experiments were performed by considering Taguchi one patterned (each factor was taken three levels) L9 orthogonal design. Experiment index was given in Table 4.

**Table 4:** Taguchi L9 experiment design

| Experiment Nu. | Control Factors  |               |              |
|----------------|------------------|---------------|--------------|
|                | Cutting Tool (A) | Feed Rate (B) | Hardness (C) |
| 1              | 1                | 1             | 1            |
| 2              | 1                | 2             | 2            |
| 3              | 1                | 3             | 3            |
| 4              | 2                | 1             | 2            |
| 5              | 2                | 2             | 3            |
| 6              | 2                | 3             | 1            |
| 7              | 3                | 1             | 3            |
| 8              | 3                | 2             | 1            |
| 9              | 3                | 3             | 2            |

**Analysis of S/N Ratios**

Taguchi experiment design and analysis were performed in Minitab 16.2 package program, and basic leveled (three level) L<sub>9</sub> orthogonal index was used. “Smallest-the best” formula specified equation 1 was used to evaluate obtained Signal-Noise Ratios (S/N).

$$\frac{S}{N} = -10 * \log \left[ \frac{\sum_{i=1}^n Y_i^2}{n} \right] \quad \text{Eq. (1)}$$

S/N ratios were calculated by using “smallest-the best” equation after obtained surface roughness ratios in machining experiments of ERDEMİR 5040 quality steel according to performed Taguchi L9 experiment design. Surface roughness values and S/N ratios obtained after machining were shown in Table 5.

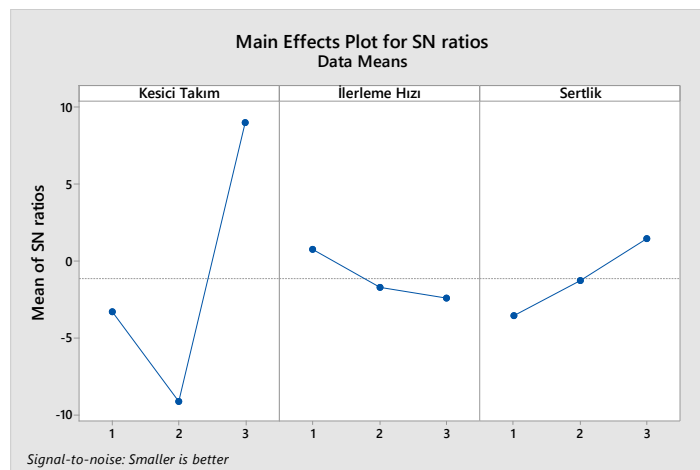
**Table 5:** Surface roughness values and S/N ratios obtained after machining

| Experiment Nu. | Control Factors |           |          | Average Surface Roughness Value (Ra) μm | S/N Ratios (dB) |
|----------------|-----------------|-----------|----------|---|-----------------|
|                | Cutting Tool    | Feed Rate | Hardness |   |                 |
| 1              | 1               | 1         | 1        | 2,148                                   | -6,6407         |
| 2              | 1               | 2         | 2        | 1,359                                   | -2,6644         |
| 3              | 1               | 3         | 3        | 1,083                                   | -0,6926         |
| 4              | 2               | 1         | 2        | 2,012                                   | -6,0726         |
| 5              | 2               | 2         | 3        | 3,118                                   | -9,8775         |
| 6              | 2               | 3         | 1        | 3,727                                   | -11,4272        |
| 7              | 3               | 1         | 3        | 0,180                                   | 14,8945         |
| 8              | 3               | 2         | 1        | 0,432                                   | 7,2903          |
| 9              | 3               | 3         | 2        | 0,570                                   | 4,8825          |

Effect of control factors on surface roughness values was analyzed by using S/N response table. S/N response table was given in Table 6 for surface roughness. This table, which is created with Taguchi method to get optimum surface roughness value, shows optimum levels besides factor effect range. S/N values of control factors for surface roughness were shown in Figure 4.

**Table 6:** S/N response table for surface roughness

| Level               | Cutting Tool | Feed Rate | Hardness |
|---------------------|--------------|-----------|----------|
| 1                   | -3,3325      | 0,7271    | -3,5925  |
| 2                   | -9,1258      | -1,7505   | -1,2848  |
| 3                   | 9,0225       | -2,4124   | 1,4415   |
| <b>Delta</b>        | 18,1482      | 3,1395    | 5,0340   |
| <b>Effect Range</b> | 1            | 3         | 2        |



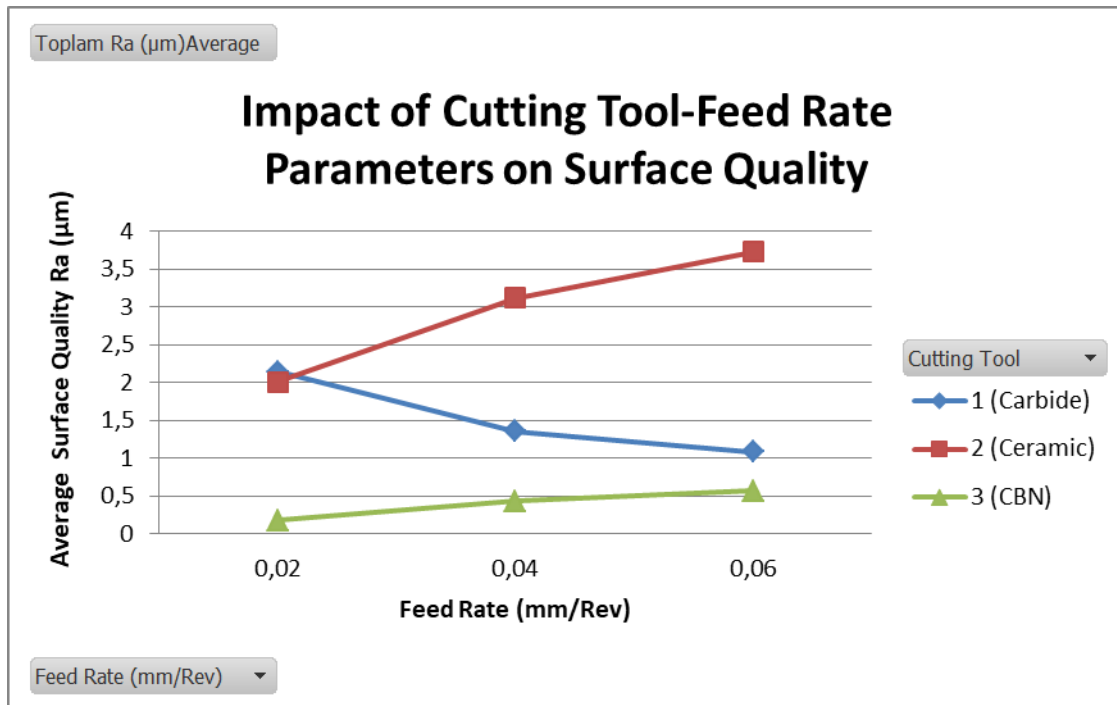
**Figure 4.** S/N values of control factors for surface roughness

Optimum levels of control factors for surface roughness for A cutting tool (CBN-Level 3), B feed rate (0,02 mm/cyc-Level 1), C Hardness (775 C° Heat Treatment, Level 3) was measured 0,180 µm surface roughness value. However, anticipated S/N ratio is 13,47 and average value is 0,0501778 µm in Minitab program when optimum levels are given.

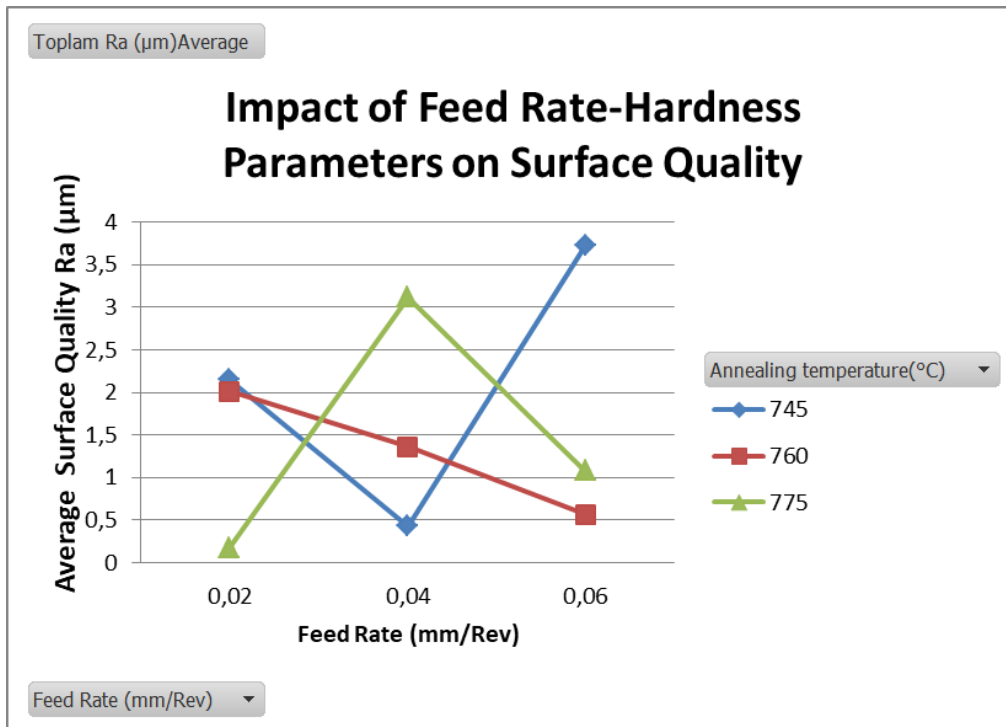
| Prediction |           |
|------------|-----------|
| S/N Ratio  | Mean      |
| 13,4744    | 0,0501778 |

### Evaluation of Experiment Results

Change in surface roughness depending on cutting tool and feed rate were explained in Figure 5 in turning ERDEMIR 5040 quality steel. While surface roughness values are almost equivalent in 0,02 mm/cyc for carbide ceramic cutter, surface roughness values showed an increase in 0,04 and 0,06 mm/cyc for ceramic cutters. Using CBN cutters in 0,02-0,04-0,06 feed rate increased surface roughness. Results correcting performance of CBN cutters, which supports optimization obtained from S/N ratios are clearly seen in graph. The best surface roughness values were obtained with CBN cutters in each three feed rates.

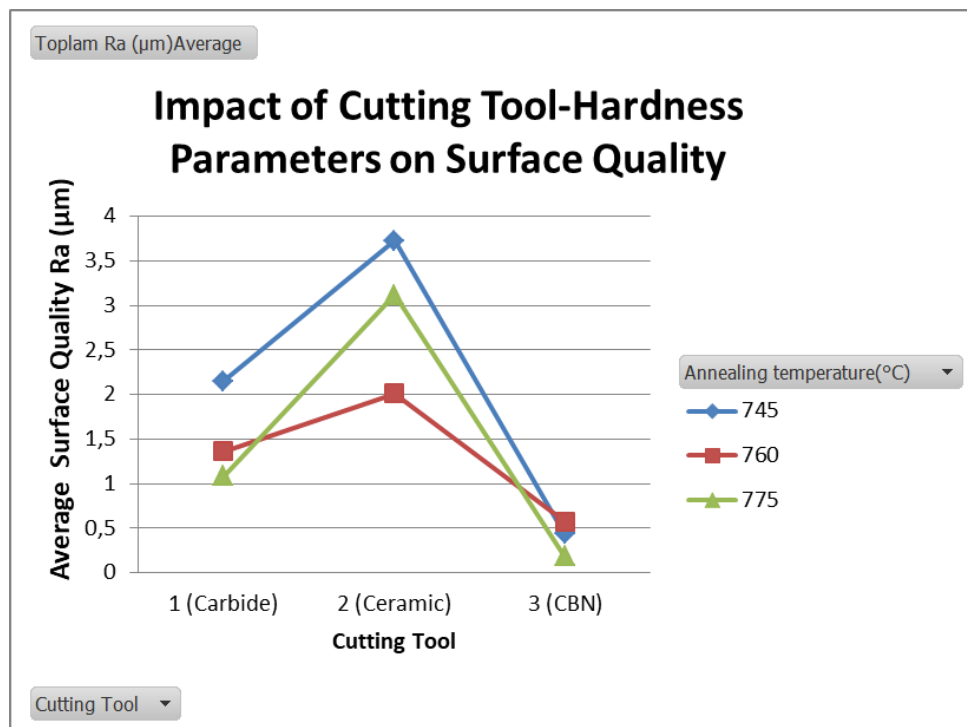


**Figure 5.** Change of surface roughness depending on cutting tool and feed rate in turning ERDEMIR 5040 Quality Steel



**Figure 6.** Change of surface roughness depending on hardness and feed rate in turning ERDEMİR 5040 Quality Steel

Change of surface roughness depending on hardness of workpiece and feed rate of cutting tool was explained in Figure 6. Lowest surface roughness in 0,02 mm/cyc feed rate was obtained from steel heat treated at 775 C°. Almost equivalent surface roughness values in same feed rate were obtained in steels heat treated at 745 ve 760 C°. Surface roughness value decreased when feed rate increased in steel heat treated at 760 C°. Ideal cutting conditions for every three cutters were occurred in medium steel heat treated at 760 C°, and a decrease in surface roughness was observed depending on feed rate (3,5,16).



**Figure 7.** Change of surface roughness depending on cutting tool and heat treatment (hardness) in turning ERDEMİR 5040 Quality Steel

Change in surface roughness depending on cutting tool and hardness level is explained in Figure 7. While CBN cutters exhibits the best performance for every three hardness, surface roughness value in ceramic cutters is higher than carbide cutters. Better surface quality was obtained with CBN cutters when high hardenability steel is used. This can be explained in two ways. Ceramic cutters were more unstable depending on heating in the effect of increasing friction forces when martensite distribution in material micro structure condensates, and CBN cutters kept stability. Tool abrasion in non-homogenous micro structure resulted in increase in surface roughness by increasing cutting surface (3,16).

## Results

In this study, three different paramaters were evaluated and optimized with regards to machinability because Taguchi experiment design has wide usage area, and it enables to obtain results with both less experiments and lower costs compared with traditional experiment design.

Optimisation of cutting parameters affecting surface roughness values obtained from turning of ERDEMİR 5040 Quality steel was performed in this study. A cutter tool (CBN-Level 3), B feed rate (0,02 mm/cyc, Level 1), C hardness (775 C° heat treated-Level 3) surfce roughness value 0,180 µm was measured for lowest surface roughness value in the study made. According to analyse result, it was seen that the most efficient parameter on surface roughness was feed rate with 68,93 % content. Micro hardness values increased with martensite increase taking place in micro structure depending on heat treatment of ERDEMİR 5040 Quality steel. CBN exhibited the best performance depending on hardness.

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## NITROREDUCTASES FROM THERMOPHILIC BACILLUS SPECIES

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**Abstract:** Nitroreductases (NTR) are members of the NAD(P)H/FMN oxidoreductase that exists widely in bacteria. NTRs have raised a great interest due to their potential in biomedicine, especially in prodrug activation for chemotherapeutic cancer treatments, in bioremediation and enzyme-based biosensors for nitro-sensitive compounds. Thermophilic *Bacillus* species isolated from hot water springs in Turkey were identified by 16S rRNA gene sequence analysis. The previously identified species, *Bacillus paralicheniformis* (5NK) and *Bacillus paralicheniformis* (FMB2) with sequence similarity of 97% and 99.65, respectively, were used. They were all found to possess the putative NADPH nitroreductase genes. Genomic DNAs for isolation of nitroreductase genes were isolated by conventional phenol-chloroform extraction method. The nitroreductase genes from thermophilic *Bacillus* species were amplified by PCR, using the designed three primer pairs. The corresponding DNA fragments were fractionated by agarose gel electrophoresis and PCR products were purified and concentrated for cloning and DNA sequencing.

**Keywords:** Nitroreductases, Thermophilic *Bacillus*, PCR

### Introduction

Most nitroaromatic compounds are toxic for living organisms, which are released to the environment during manufacturing and handling, through filtration and losses of the storage tanks and during transport and intensive military activities, especially TNT and other explosives. There have been human health risk with regard to nitrosubstituted compounds due to the metabolites causing genotoxic and mutagenic effects and the generation of reactive nitrogen oxide species, which readily react with biological macromolecules (Cortial et al. 2010).

It has been found that various microorganisms have developed reductive pathways to degrade or transform these compounds. Bacterial nitroreductases including *Bacillus* species are flavoenzymes that catalyze the NAD(P)H-dependent reduction of the nitro groups on nitroaromatic and nitroheterocyclic compounds. Therefore, the nitroreductases are used for several biotechnological applications for bioremediation, as biosensors and for clinical uses (Gwenin et al. 2015, Roldán et al. 2008, Chaignon et al. 2006).

Particularly, prodrug activation gene therapy is a promising approach to cancer treatment, whereby prodrug-activating enzymes are expressed in tumour cells. Following administration of a non-toxic prodrug, the nitroreductase enzyme expressed in tumour cells converts it to cytotoxic metabolites which directly kill the cancer cells. The enzyme nitroreductase, isolated from *Escherichia coli* B, converts CB1954 ((5-aziridin-1-yl)-2,4-dinitro-benzamide) into a potent DNA-crosslinking cytotoxic agent that kills tumor cells (Drabek et al. 1997).

The use of any prodrug activating enzyme is, however, limited by factors such as its stability of which is needed when for example conjugated to an antibody. From this point of view, enzymes from thermophilic organisms have considerable potential because they are resistant and remain fully active under denatured conditions compared to most enzymes from mesophilics. On the other hand, thermophilic stable enzymes still need to have sufficient catalytic activity at 37 °C if they are utilised in prodrug therapy (Emptage 2009).

Turkey has lots of different ecological areas, which possesses a broad microbial diversity. Turkey is well known for its geothermal activity and there are so many thermal springs all over the country. Therefore, there should be a great deal of opportunities for newly isolated microorganisms from extreme environments, including thermophilic ones with numerous biotechnological applications. For many decades, the *Bacillaceae* family members have been good sources in biotechnological processes concerning whole cells or enzymes. In Turkey, the isolated and identified thermophilic members of the *Bacillaceae* family include *Anoxybacillus*, *Geobacillus*, *Bacillus*, *Brevibacillus*, *Aeribacillus*. Members of *Bacillus* genus are well known to be widespread all over the world in various extreme and geographical areas including hot springs of Turkey (Güven et al 2018).

In the present study, we report on the identification and characterization of nitroreductase genes from the Gram-positive thermophilic *Bacillus* species isolated from hot springs in Turkey.

## Material and Methods

### Bacteria, Plasmids and Media

*Bacillus paralicheniformis* 5NK, *Bacillus paralicheniformis* FMB2 and *E. coli* DH5 $\alpha$  strains used for transferring the nitroreductase gene in to pET28a + vector.

Bacterial strains and *E. coli* DH5 $\alpha$  maintained at -20 ° C in medium containing 30% v / v glycerol. Bacterial strains were produced in Luria Bertani Broth (LB) medium (Bacto Trypton 10 g / L, Bacto-Maya Extract 5 g / L, NaCl 10 g / L, pH 7.0), 40-55 ° C and aerobic conditions. *E. coli* DH5 $\alpha$  strains were produced at 37 ° and aerobic conditions in LB broth. 1.5% w / v agar was added to breeding media for solid medium.

### Isolation of chromosomal DNA from *Bacillus* strains

Bacteria were produced in aerobic conditions in LB medium and chromosomal DNA was isolated from the produced bacteria using the Thermo Fischer Scientific DNA isolation kit. The amount and purity of DNA in the spectrophotometer was measured and prepared for the PCR reaction.

### Agarose Gel Electrophoresis

For the volume required, SeaKem® GTG® pure agarose was weighed and added to the volume of 1X TAE buffer in a mulled beer and kept on the flame until dissolved. After cooling to the tangible temperature (45-50 ° C), the EtBr solution was added to give a final concentration of 0.5  $\mu$ g / mL. The gel was poured carefully into the container and gel tray was placed and waited for about 40 minutes to freeze. 1X TAE buffer was added to the gel electrophoresis tank until it was covered. DNA samples (3  $\mu$ L BFB and 10  $\mu$ L DNA) were inserted into the wells formed by removing the comb. One DNA standard (4  $\mu$ L) was loaded from the wells to calculate the DNA size. After the progress of the molecules in the electric field, the gel was kept in the pure water for 10 minutes and the gel was observed by translucinator.

### PCR

A nucleotide BLAST search of the *Bacillus* genome was performed using the *nfnB* gene sequence of *E. coli*. *nfnB* gene was identified as BC\_1619a: putative oxygen-insensitive NADPH nitroreductase, Primers were obtained from Sentegen (Ankara/Turkey). Primers for amplification of the *nfnB* gene were ATAGGATCCATGACTAACTCAGTAAAGAC (5' primer) and ATCAAGCTTTTATTTCCATTCAGCAAC (3' primer), *Bam*HI and *Hind*III sites are underlined. PCR products were purified using the QIAquick PCR Purification Kit (QIAGEN Ltd., UK) according to the manufacturer's instructions. The purity and approximate size of the PCR products were confirmed using agarose gel electrophoresis.

### Cloning

The purified PCR products obtained from the bacteria and the vector DNA to be used for cloning were partially cleaved by *Bam*HI and *Hind*III restriction enzymes. 500 ng of chromosomal DNA and 50 ng of plasmid DNA digested with *Bam*HI and *Hind*III were ligated. Prior to transformation, the ligation mixture was subjected to ethanol precipitation and ligase samples were inactivated by incubation at 65°C for 15 min prior to transformation. The inactivated self ligation and ligation samples were transformed with the prepared competent bacteria. All of the liquids in the transformation tube were first separately produced at 37 ° C for 1 hour, poured into S.O.C liquid media. With this application, the bacteria that have become sensitized by the competing processes are returned to their normal state, and the adaptation of the bacteria to the antibiotic fattening, which will be inoculated after one hour, is provided. At the end of one hour, the bacterium was removed and plated on a LB/Kanamycin petri dishes, and the plates were dried for 5-10 min, then inverted and left at 37 ° C for 1 night. The next day, transformants were transformed with recombinant (expanded) and non-recombinant (widely closed) plasmid DNAs. Recombinant colonies were selected by means of a sterile toothpick and transferred to tubes containing 10 mL of LB/Amp100 nutrient and produced at 37 ° C with shaking for 1 night and plasmid isolation was performed as described below with the aid of QIAGEN mini-prep kit. The amount of plasmid DNA was measured and then the purity was tested by agarose gel. Sequence analysis was carried out by Sentegen (Ankara/Turkey). Nitroreductase gene sequences were examined in the Blast program at the National Center for Biotechnology Information (NCBI) website (<http://www.ncbi.nlm.nih.gov/BLAST/>), CLC Workbench v 4.0 Software (CLC bio, Aarhus, and other *Bacillus* species, and their homology affinities were determined.

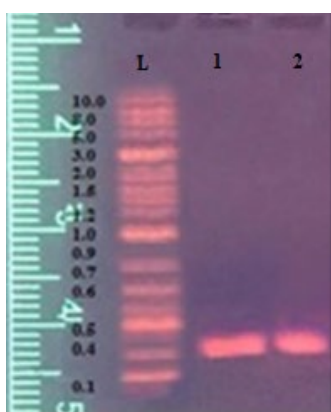
### Results and Discussion

Thermophilic *Bacillus* species isolated from hot water springs in Turkey were identified by 16S rRNA gene sequence analysis. The previously identified species, *Bacillus paralicheniformis* (5NK), *Bacillus paralicheniformis* (FMB2), with sequence similarity of 97% and 99.65% respectively, were used. Genomic DNAs of *Bacillus* species were isolated and shown in Figure 1.

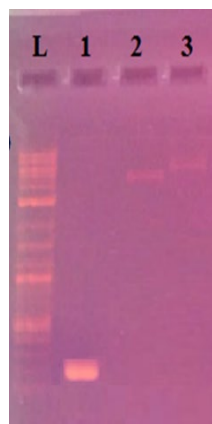


**Figure 1:** Genomic DNAs of *Bacillus* species, **B2:** Genomic DNA of *Bacillus paralicheniformis* (FMB2), **5NK:** Genomic DNA of *Bacillus paralicheniformis* (5NK), **L:** DNA marker (New England Biolabs)

One gene from *Bacillus* species were successfully amplified during PCR using the primer BC\_1619a (putative oxygen-insensitive NADPH nitroreductase) among three primers designed (Figure 2). The primers were designed on the basis of *Bacillus cereus* nitroreductase genes which have been found to metabolise prodrug CB1954 and its metabolites as superior cell killing ability, which were a promising candidate for enzyme prodrug therapy (Gwenin et al. 2015).



**Figure 2:** PCR products, **L:** DNA marker (New England Biolabs), **1:** PCR products of *Bacillus paralicheniformis* (FMB2), **2:** PCR products of *Bacillus paralicheniformis* (5NK)



**Figure 3:** **L:** DNA marker (New England Biolabs), **1:** PCR products of *Bacillus paralicheniformis* (5NK) after ligation, **2:** pET28a+, **3:** Ligation (double digested DNA of strain 5NK and double digested pET28a+)

These PCR products were successfully inserted into pET28a+ expression vector, which consists N-terminal His-tag for ease of protein purification (Figure 3).

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## PROTECTING MASONRY SURFACES FROM MOLD GROWTH WITH MINIMUM RISK ANTIMICROBIAL AGENTS

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**Abstract:** Sanitation efforts in public institutions quietly operate behind the scenes to ensure comfort and safety of the building occupants. Without extremely consistent, detail oriented service, the aesthetic appeal and sanitation of surfaces can quickly deteriorate to a level that puts the occupants of a building at risk. Public restrooms are one of the most mission-critical areas in a janitorial regimen due to the number of revolving occupants and the activities conducted therein. Though a significant amount of research has been dedicated to developing sanitary architectural design practices, many of these practices were not utilized in prior construction and are not being adopted in new construction. This creates a critical and immediate need for coatings and sealants that can aid janitorial efforts in keeping surfaces clean and hygienic. Researchers from West Texas A&M University (Canyon, Texas, USA), in collaboration with Buffalo Technology Group LLP (Canyon, Texas, USA) explored several mixtures of antimicrobial additives that could be incorporated into commercially available masonry sealants to provide tile grout and other masonry surfaces with protection from fungi and bacteria. These additive mixtures were subjected to standardized microbiologically testing procedures against mold (*Aspergillus niger*) by agar diffusion disk method. Results from microbiological testing indicate that the sealants enhanced with the antimicrobial additives could provide surfaces that are inhospitable to fungi growth and propagation. These results are significant because the additive material eliminates the primary sources of aesthetic and hygienic contamination on masonry surfaces in public areas and can be easily and safely applied to existing and new surfaces without the additional environmental concerns of common sterilizing cleaners.

**Keywords:** Mold Resistant Coatings, Public Sanitation, Building Products, Minimum Risk Pesticides

### Introduction

Fungi grow well in warm, moist environments, and are perfectly suited to the ecosystem within a large number of households throughout the world. Mudarri and Fisk report that *47 percent of all homes in the United States have substantial mold and dampness* (2007), and these unwanted house guests are wreaking havoc on our health and economy. In the US alone there are an estimated *4.6 million cases of asthma attributed to household mold growth* (2007). The travesty of this preventable sickness is also causing significant economic damage. Americans are estimated to spend \$22.4 billion on various illnesses attributed to household mold (Mudarri, 2016). Researchers have recognized the importance of creating surfaces that resist microbiological growth and propagation, including that of mold and mildew, through surface chemistry (Morones (1998), Zhao (1998), Faille (2002)) and surface roughness (Vasilev, 2009; Zhao, 1998; Faille, 2002) in a host of industries. One method of creating surfaces that are inhospitable to microbiological growth is by doping a coating matrix with antimicrobial compounds. The authors recently demonstrated the feasibility and benefits of enhancing coatings to resist microbiological growth by enhancing a coating matrix with antimicrobial additives in a variety of industries and coating systems (Hunt et. al, 2017, Hunt, et. al, 2017, Hunt, et. al., 2016, Chiu, et. al, 2016). This method of enhancing existing coatings to provide more robust protection against microbiological organisms is a growing trend to fulfill the call of industry for a solution to the problems associated with microbial growth.

At the same time that there is a growing need for surfaces that resist microbiological growth, there is also an increasing demand from consumers for antimicrobial solutions that are more environmentally friendly and less toxic (Laroche, 2007). This demand, along with the growing concern over pharmacologically resistant strains of harmful microorganisms “Super Bugs” has produced a resurgence in the utilization of naturally derived

antimicrobial agents to protect surfaces from microorganisms. These materials, some of which are classified as Minimum Risk Pesticides by the United States Environmental Protection Agency (EPA), inherently resist the growth of mold, mildew, and bacteria on their surfaces. These materials can be optimized for use in various coating matrices to resist microbial growth without the use of environmentally ambiguous compounds and procedures. One type of coating matrix that that could reap substantial benefits by possessing an antimicrobial surface from environmentally friendly materials is masonry sealant. Figure 1 shows mold growth in a bathroom with exposed grout and standard masonry sealant.

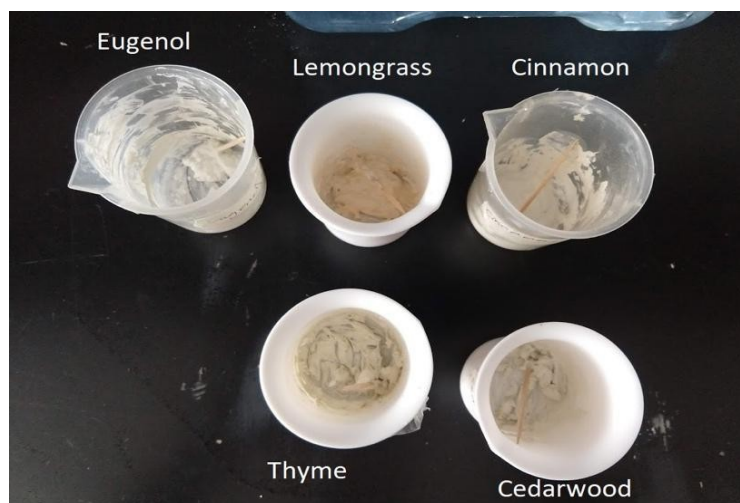


**Figure 1.** Mold growth on grouted surface in tiled tub surround

Tiled surfaces are a popular finish style for commercial and residential buildings throughout the world. Within tiled surfaces, the surface area of grout can be up to 50% of the total covered area depending upon the size of tiles and the thickness of the grout lines. The morphology of grout surfaces inherently collects and holds moisture while simultaneously resisting scrubbing and disinfecting efforts. These characteristics provide an excellent platform for microbiological growth and propagation on grouted surfaces. Sealing the grout with a variety of commercially available grout sealants can decrease the hospitality of the surfaces to harboring microbiological organisms, but even when coated with a sealant, grout lines are routinely the most microbial dense areas of a tiled surface and are often the first to become impacted by staining due to mold and mildew. By doping commercially available grout sealants with combinations of antimicrobial agents it may be possible to decrease microbial growth on grouted surfaces without the use of harsh chemicals or excessive scrubbing.

## Materials and Methods

Experiments were performed to evaluate the effectiveness of several additive mixtures against *Aspergillus Niger* by utilizing agar diffusion disk method. A list of 6 potential antimicrobial agents were selected from the United States Environmental Protection Agency list of Minimum Risk Pesticides. Figure 2 shows the experimental coating additive solutions as mixed prior to experimentation.



**Figure 2.** Mixtures of experimental antimicrobial agents

The independent agents shown in Table 1 were selected based on an extensive literature review and preliminary testing against various mold strains. The list features several essential oils as well as the active ingredient (Eugenol) in several more essential oils. Zinc Oxide, a known fungicidal compound, is also included in the list and has been used historically in cosmetics and sunscreens.

**Table 1:** Potential antimicrobial agents classified as minimum risk by the US Environmental Protection Agency.

| Antimicrobial Agent | CAS Number |
|---------------------|------------|
| Thyme oil           | 8007-46-3  |
| Cedarwood oil       | 8000-27-9  |
| Cinnamon leaf oil   | 8007-80-5  |
| Lemongrass oil      | 8007-02-01 |
| Eugenol             | 8000-34-8  |
| Zinc Oxide          | 1314-13-2  |
| Thyme oil           | 8007-46-3  |
| Cedarwood oil       | 8000-27-9  |

Using these chemicals, experimental sealant solutions, displayed in Table 2, were prepared using an industry leading, water-based commercial grout sealer. Lecithin was added to the experimental solutions to help suspend the active ingredient within the solution. Circular disks (6±0.5 mm diameter) of filter paper (Whatman Grade 1: 11 µm) were immersed in the various solutions and allowed to dry on waxed paper. Once dry, the samples were then placed on petri dishes filled with agar and inoculated with spores of *Aspergillus Niger*. The petri dishes were incubated at 25 C for 24 hours before examination.

**Table 2:** Experimental grout sealant solutions.

| Sample | Components   |
|--------|--|
| A      | Thyme India 20% in Coating w/ Lecithin & Water       |
| B      | Cedarwood Virginia 20% in Coating w/Lecithin & Water |
| C      | Lemongrass 20% in Coating w/Lecithin & Water         |
| D      | Cinnamon Leaf 20% in Coating w/Lecithin & Water      |
| E      | Eugenol 20% in Coating w/Lecithin & Water            |
| F      | Control Coating                                      |
| G      | Composite Oil Mixture with Zinc Oxide                |
| H      | Control Coating                                      |

**Results and Discussion**

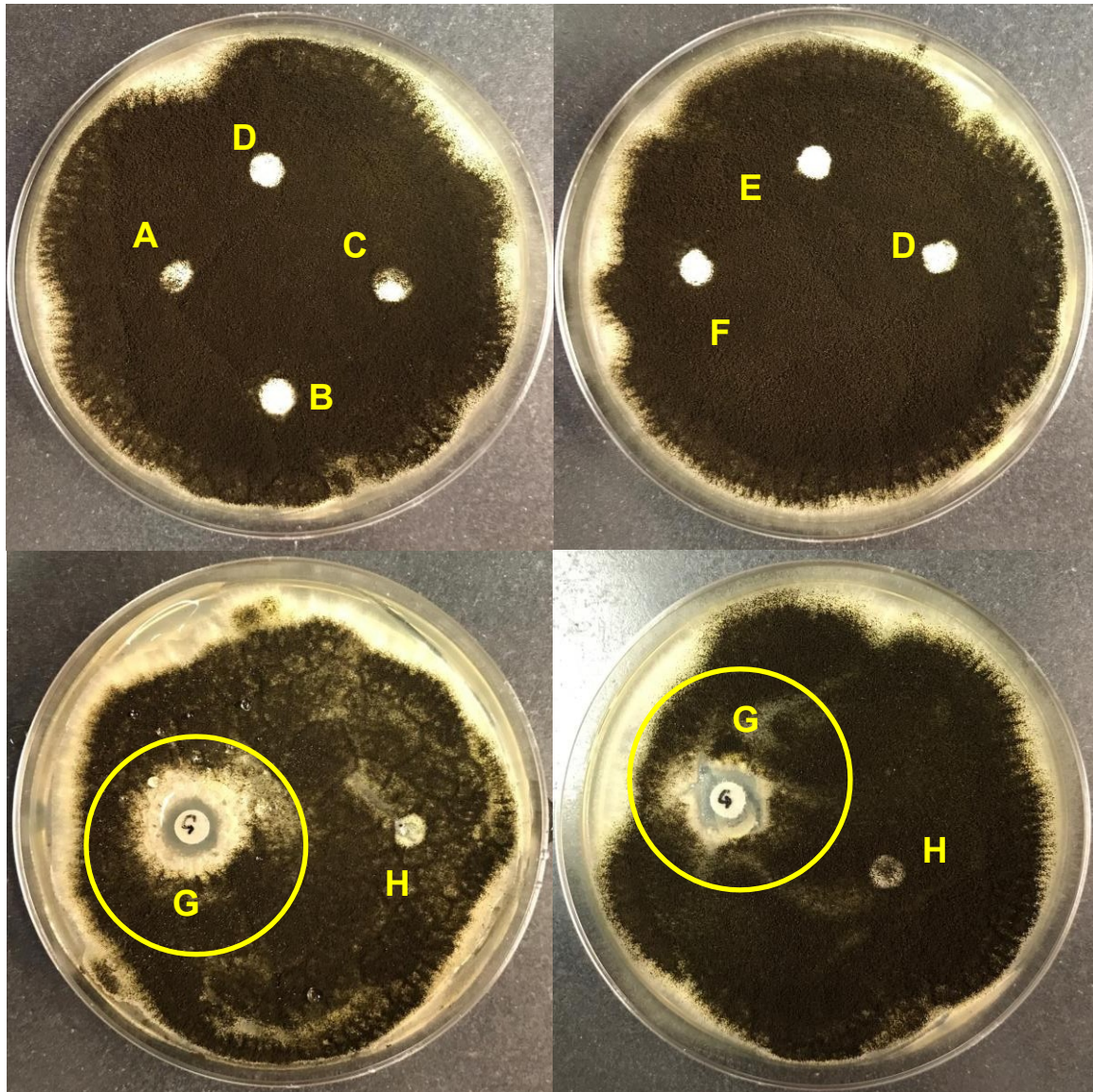
Qualitative analysis was performed on images of the petri dishes captured 24 hours after inoculation of the mold spores. The antimicrobial efficacy of each disk was categorized by the zone of inhibition created around the disk. Each disk was categorized as having no impact on the mold growth (0) whereby the boundaries of the disk were overcome by mold, having limited impact on mold growth (1) whereby the disks maintained their boundaries, or having significant impact on mold growth (2) whereby a zone of growth inhibition was created around the disks. These qualitative results are displayed in Table 3.

**Table 3:** Qualitative zone of inhibition results of experimental antimicrobial grout sealant mixtures against *Aspergillus Niger*.

| Sample | Observation        | Score |
|--------|--------------------|-------|
| A      | No impact          | 0     |
| B      | Limited impact     | 1     |
| C      | No impact          | 0     |
| D      | Limited impact     | 1     |
| E      | Limited impact     | 1     |
| F      | Limited impact     | 1     |
| G      | Significant impact | 2     |
| H      | No impact          | 0     |

As described in Table 3 and shown in the photographs of Figure 3, samples B, D, E, and F showed slight inhibition of the mold. These samples maintained the boundary of the disks and were not overcome by mold growth. Furthermore, Sample G displayed significant impact to mold growth by creating a sizeable zone of inhibition around each of the sample disks. Samples A, C, and H had no impact on retarding the spread of mold onto the disks.





**Figure 3.** Zone of inhibition of experimental solutions 24 hours after mold inoculation.

Based on data from the images in Figure 3, G (composite oil mixture with zinc oxide) created the largest zone of inhibition and is the most effective solution for inhibiting mold growth. Sample H, which consisted of the leading commercial grout sealer on the market with no additional additive, did not inhibit mold growth in any way. In fact, it was overrun with mold growth. By using H as a baseline, it is apparent that every experimental solution from Table 2 improved the mold resistance of the coating.

**Conclusion**

The results of this study show that a composite solution of zinc oxide and various essential oils can be mixed within a commercial grout sealant to create an effective barrier against mold growth in laboratory settings. The solution created a significant zone of mold growth inhibition and could be a suitable solution for mold growth on masonry surfaces. This technology leverages the antimicrobial efficacy of materials classified as minimum risk by the US Environmental Protection Agency to create a solution to mold growth on masonry surfaces that is safe, effective, and exhibits limited environmental impact. Further investigation will be performed to determine the minimum concentration of antimicrobial agents necessary to inhibit mold growth on coated surfaces as well as explore any potential impact the additives have on clarity, surface morphology, and life expectancy of the coating.

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## THE USE OF NEW TECHNOLOGIES IN THE ELDERLY IN THE NORTH OF PORTUGAL

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**Abstract:** The elderly, especially those living in institutions, tend to have many health limitations, such as reduced vision, hearing and locomotion, which necessarily implies the risk of loneliness. An exploratory and cross-sectional study was carried out. Data collection took place between February and May 2018, in 5 residential structures for the elderly in the north of Portugal. The study included 130 elderly people, 68,5% female and 31,5% male, with an average age of 82,43 years and an average stay in the institution of 4,5 years and 57,7% are widowers. 43,1% of the elderly would see a robot that interacted with them as interesting and very interesting. The data found in this study shows that it is essential to provide the elderly with new forms of social relationships with the help of new technologies in order to avoid loneliness and to contribute to a better quality of life.

**Keywords:** New Technologies, Elderly, Robots

### Introduction

The elderly, many of them without family support, with several chronic diseases, characteristic of aging, as well as various health limitations, such as decreased visual, auditory and locomotion acuity, also due to the normal aging process, live in residential structures for the elderly (Conselho Económico e Social, 2013).

In Portugal, between 2015 and 2080, the number of elderly people will increase from 2.1 to 2.8 million and the aging rate goes from 147 to 317 elderly people per 100 young people in 2080 (INE, 2017).

Aging is a natural and biological process that necessarily introduces some limitations, which implies effects on the person's lifestyle and well-being, introduces changes in their social relations, and increases the risk of isolation and loneliness (Reis, et al, 2017).

It is necessary that the elderly in these institutions do not feel changes in their social role, their routines, and their involvement in stimulating activities (Dillin, Gottschling, Nyström, 2014). Given the quality of life of the elderly, social and family interactions, the decreasing risk of loneliness in the institutionalized elderly, the challenge is placed on the development of technological and communication resources, such as solutions based on social robotics that ensure this challenge (Reis, et al, 2017<sup>b</sup>).

Recently, several technologies have emerged with the aim to provide innovative and efficient ways to help the elderly in their daily lives and reduce the cost of health care (Khosravia, Ghapanchia, 2016).

The increase of the life expectancy of the elderly comes to settle in the health area, through the use of social robotics, namely, with the so-called telepresence robots. These are already used in telemedicine, whether in hospitals or in nursing homes, and mobile telepresence robots add an added value to the activities of daily living that people have to perform (Laniel, et al, 2017).

Telepresence robots can be a significant help in promoting a life with as much independence as possible in the elderly, helping the most fragile and reducing loneliness (Pripfl et al, 2016), and because more and more attention is given to the aging of the population, as well as to new forms of social relationship (Bedaf, Marti, Amirabdollahian, Luc de Witte, 2017).

In 2016, through a systematic review of the literature made between 2000 and 2014 on assistive technologies used in care of the elderly, it was recorded that support technologies are a reality and can be applied to improve the quality of life, especially, of the older ones (Khosravia, Ghapanchia, 2016).

In this perspective, a study was developed with the objective of identifying the appetite of the elderly for the use of new technologies.

**Materials and Methods**

An exploratory and cross-sectional study was carried out, using a form consisting of socio-demographic characteristics, clinical antecedents and issues related to the use of new technologies. Data collection took place between February and May 2018, in 5 residential structures for the elderly in the north of Portugal.

The data was collected in the Institutions by 2 properly trained research scholarship students and oriented to the project objectives. The collection of data was always done in a way that did not interfere with the activities of the Institution.

The elderly who participated in the study were aged 65 or over, oriented in time and space and gave their informed consent.

Also note that all institutions authorized the data collection and the UTAD - Ethics Committee, gave a favorable opinion to carry out the study.

**Results and Discussion**

The study included 130 elderly people, 68,5% are female and 31,5% are male, with an average age of 82,43 years and an average stay in the institution of 4,5 years. 57,7% of elderly are widowers.

The institutionalization happened on 23,8% because the family did not have time to take care of them, on 20% there was worsening of health status, and on 19,2% of the elderly lived alone, see (Table 1).

**Table 1:** Reasons for Institutionalization

|  | N          | %            |
|--|------------|--------------|
| The family lives in a distant geographical area  | 10         | 7,7          |
| The family did not have time to take care of him | 31         | 23,8         |
| Worsening of health status                       | 26         | 20,0         |
| Weak housing conditions in the face of needs     | 2          | 1,5          |
| Physical dependence                              | 7          | 5,4          |
| Difficulty performing tasks of daily living      | 8          | 6,2          |
| Death of spouse                                  | 7          | 5,4          |
| Loss of autonomy                                 | 8          | 6,2          |
| Lived alone                                      | 25         | 19,2         |
| Other  | 1          | ,8           |
| Total  | 125        | 96,2         |
| Missing  | 5          | 3,8          |
| <b>Total</b>                                     | <b>130</b> | <b>100,0</b> |

95,4% of the elderly reported having health problems, 98,5% take medication and 86,9% suffer from some disability, see (Table 2), namely 22,3% in hearing, 59,2% in mobility and 50% in vision, see (Table 3).

**Table 2:** Health problems, disabilities and medication

| Variables           |     | N   | %    |
|---------------------|-----|-----|------|
| Health problems     | Yes | 124 | 95,4 |
|                     | No  | 6   | 4,6  |
| Does medication     | Yes | 128 | 98,5 |
|                     | No  | 2   | 1,5  |
| Have any disability | Yes | 113 | 86,9 |
|                     | No  | 17  | 13,1 |

**Table 3:** Presence of disabilities

| Variables |         | N  | %    |
|-----------|---------|----|------|
| Hearing   | Yes     | 29 | 22,3 |
|           | No      | 84 | 64,6 |
|           | Missing | 17 | 13,1 |
| Mobility  | Yes     | 77 | 59,2 |
|           | No      | 36 | 27,7 |
|           | Missing | 17 | 13,1 |
| Vision    | Yes     | 65 | 50,0 |
|           | No      | 48 | 36,9 |
|           | Missing | 17 | 13,1 |

Probably one of the reasons for the existence of 46,9% of the 130 elderly people interviewed, reported already having had falls in the institution, is due to the fact that the majority of the elderly presents limitations in mobility and vision.

Only 37,7% of the elderly use the mobile phone and only 2,3% use a computer, see (Table 4). In this context it should be noted that for 40,8% of the elderly, contact with family, friends and former co-workers has decreased.

**Table 4:** Phone and computer use

| Variables    |     | N   | %    |
|--------------|-----|-----|------|
| Mobile phone | Yes | 49  | 37,7 |
|              | No  | 81  | 62,3 |
| Computer     | Yes | 3   | 2,3  |
|              | No  | 127 | 97,7 |

81,5% of the elderly, would like to have more frequent contact with the family, and 70,8% with friends, see (Table 5). 50,8% of the elderly do not contact family, friends and former co-workers more frequently because of lack of resources.

**Table 5:** Most frequent contacts with

| Variables         |     | N   | %    |
|-------------------|-----|-----|------|
| Family            | Yes | 106 | 81,5 |
|                   | No  | 24  | 18,5 |
| Friends           | Yes | 92  | 70,8 |
|                   | No  | 38  | 29,2 |
| Former co-workers | Yes | 82  | 63,1 |
|                   | No  | 48  | 36,9 |

43,1% of the elderly would see a robot, that interacted with them as interesting and very interesting, 53% of the elderly would see a robot, that would make it easier to do some activities and daily tasks as interesting and very interesting and 39,2% of the elderly would see a robot, who proposed playful activities as interesting and very interesting. However, it should be noted that the majority of the elderly have no interest or did not respond (70%) to the robot's help in reading the emails, as well as 65,4% of the elderly, the robot has no interest to divulge activities of your family members on social networks, see (Table 6).

**Table 6:** How do you see a robot

| Variables   |                     | N  | %    |
|---|---------------------|----|------|
| To interact with you by proposing you some social activities according to your state of mind through the voice  | No interest         | 30 | 23,1 |
|   | Little Interesting  | 23 | 17,7 |
|   | I would like to try | 21 | 16,2 |
|   | Interesting         | 35 | 26,9 |
|   | Very interesting    | 21 | 16,2 |
| That would make it easier for you to do some daily activities and tasks, such as taking medication correctly  | No interest         | 34 | 26,2 |
|   | Little Interesting  | 17 | 13,1 |
|   | I would like to try | 10 | 7,7  |
|   | Interesting         | 51 | 39,2 |
|   | Very interesting    | 18 | 13,8 |
| That could manage your e-mail, for example read your messages   | No interest         | 52 | 40,0 |
|   | Little Interesting  | 8  | 6,2  |
|   | I would like to try | 5  | 3,8  |
|   | Interesting         | 16 | 12,3 |
|   | Very interesting    | 10 | 7,7  |
| That gives you information about the activities of your family members on social networks, such as presenting the new publications and photos of your family on facebook on a daily basis | Missing             | 39 | 30,0 |
|   | No interest         | 48 | 36,9 |
|   | Little Interesting  | 12 | 9,2  |
|   | I would like to try | 6  | 4,6  |
|   | Interesting         | 19 | 14,6 |
| To propose playful activities, such as a card game  | Very interesting    | 8  | 6,2  |
|   | Missing             | 37 | 28,5 |
|   | No interest         | 38 | 29,2 |
|   | Little Interesting  | 23 | 17,7 |
|   | I would like to try | 18 | 13,8 |
|   | Interesting         | 29 | 22,3 |
|   | Very interesting    | 22 | 16,9 |

With regard to the use of robots in the elderly, in Austria a study was carried out with 7 elderly people over 75 years old living alone (Pripfl et al, 2016). The results showed that the elderly value the robot enough to have lifted objects off the ground and transported them. However, these seniors felt that the robot had not been able to increase their own independence and their sense of security at home.

In the Netherlands, a study was carried out with 10 elderly people, with an average age of 79.3 years with the objective to report the experience of living at home with a robot. The elderly considered the robot very limited and reported that it should perform more complex tasks (Bedaf, Marti, Amirabdollahian, Luc de Witte, 2017)

### Conclusion

The data found in this study indicate that it is essential to provide the elderly with new forms of social relationships with the help of new technologies in order to avoid loneliness and contribute to a better quality of life (Ministério da Saúde, 2018; Pripfl, Körtner, Batko-Klein, Hebesberger, Weninger, Gisinger, 2016).

Technologies to aid the elderly have a positive impact not only on the elderly, but also on those who work with them, as they promote a more independent life, increased security, increased social connectivity and advances in mobility (Khosravia, Ghapanchia, 2016).

Robots should be able to perform more complex tasks and related to the limitations of the elderly, as well as perform preferential tasks for each one of the elderly (Bedaf, Marti, Amirabdollahian, Luc de Witte, 2017).

On the other hand, it is fundamental that the new information and communication technologies, real scenarios of the 21st century, do not exclude the elderly and can create appropriate interfaces for their use (Tavares, Souza, 2012).

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