

DOKUZ EYLÜL UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

**GREEN SYNTHESIS OF SILVER
NANOPARTICLES BY CAULERPA SPP**



by

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July, 2019

İZMİR

GREEN SYNTHESIS OF SILVER NANOPARTICLES BY CAULERPA SPP

**A Thesis Submitted to the
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Science in Biotechnology**

**by
Yeşim YILMAZ ABEŞKA**

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İZMİR**

M.Sc THESIS EXAMINATION RESULT FORM

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GREEN SYNTHESIS OF SILVER NANOPARTICLES BY CAULERPA SPP

ABSTRACT

Nanomaterials have been the subject of many researches in the literature as they have very different and useful properties compared to bulk materials. These distinct chemical, physical and biological properties are utilized in many areas of industry such as water treatment, catalysis and medicine. Green synthesis of silver nanoparticles has many superiorities compared to physical and chemical methods such as low cost, nontoxicity, and eco-sensitive. Silver nanoparticles (AgNPs) were synthesized by using honey, native seaweed *Caulerpa prolifera* and invasive seaweed *Caulerpa cylindracea* that its invasion has not been prevented since 1991. Absence of any scientific paper related to the synthesis of silver nanoparticles by using invasive *C. cylindracea* and native *C. prolifera* is the originality of this thesis. Silver nanoparticles can be widely used in different industrial areas. In this thesis, experimental conditions related to the green synthesis of silver nanoparticles by honey and *Caulerpa* spp extracts were optimized. Optimization parameters were selected as agitation time, agitation rate, pH, temperature, the concentration of *Caulerpa* spp extracts, honey concentration, silver nitrate concentration. Synthesized silver nanoparticles were characterized thorough Scanning Electron Microscopy, UV-Visible Spectroscopy, Fourier Transform Infrared Spectroscopy and X-Ray Diffraction. Synthesized silver nanoparticles were used for antifouling paint formulation and decolourization of Congo red. In addition, experimental conditions related to the green synthesis of silver nanoparticles by *Caulerpa* spp. were modelled using artifical neural network. In conclusion, natural and invasive seaweeds can be used to synthesize AgNPs to be evaluated in the industry.

Keywords: Green synthesis, silver nanoparticles, nanobiotechnology, *Caulerpa* spp., antifouling paint, decolourization, artifical neural network

CAULERPA TÜRLERİ KULLANILARAK GÜMÜŞ NANOPARTİKÜLLERİN YEŞİL SENTEZİ

ÖZ

Nanomateriyaller ham materyallere kıyasla oldukça farklı ve kullanışlı özelliklere sahip oldukları için literatürde pek çok araştırmanın konusu olmuştur. Bu farklı kimyasal, fiziksel ve biyolojik özelliklerinden endüstride su arıtma, kataliz ve tıp gibi pek çok alanda yararlanılır. Gümüş nanoparçacıkların yeşil sentezi düşük maliyetli, toksik olmayan ve çevreye duyarlı olması gibi fiziksel ve kimyasal yöntemlere kıyasla birçok üstünlüğe sahiptir. Gümüş nanoparçacıkları bal, istilacı olmayan deniz yosunu *Caulerpa prolifera* ve istilası 1991'den beri önlenememiş olan istilacı deniz yosunu *Caulerpa cylindracea* kullanılarak sentezlendi. İstilacı *C. cylindracea* ve yerli *C. prolifera* kullanılarak gümüş nanoparçacıkların sentezi ile ilgili bilimsel bir makalenin bulunmaması bu tezin özgünlüğüdür. Gümüş nanopartiküller farklı endüstriyel alanlarda yaygın olarak kullanılabilir. Bu tezde, gümüş nanopartiküllerin bal ve *Caulerpa* türleri ekstraktları ile yeşil sentezi ile ilgili deneysel koşullar optimize edilmiştir. Optimizasyon parametreleri karıştırma süresi, karıştırma hızı, pH, sıcaklık, *Caulerpa* türleri ekstraktlarının konsantrasyonu, bal konsantrasyonu, gümüş nitrat konsantrasyonu olarak seçildi. Sentezlenmiş gümüş nanoparçacıklar, Taramalı Elektron Mikroskopu, UV-Görünür Bölge Spektroskopisi, Fourier Dönüşüm İnfrared Spektroskopisi ve X-Işını Kırınımı kullanılarak karakterize edildi. Sentezlenmiş gümüş nanoparçacıklar, antifouling boya formülasyonu ve Kongo kırmızısının dekolorizasyonu için kullanılmıştır. Ek olarak, *Caulerpa* türleri tarafından gümüş nanoparçacıkların yeşil sentezi ile ilgili deneysel koşullar yapay sinir ağı kullanılarak modellenmiştir. Sonuç olarak, sanayide değerlendirilmek üzere doğal ve yayılımcı yosunlar kullanılarak AgNP sentezlenebilir.

Anahtar Kelimeler: Yeşil sentez, gümüş nanoparçacıklar, nanobiyoteknoloji, *Caulerpa* türleri, antifouling boya, dekolorizasyon, yapay sinir ağları

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CHAPTER ONE

INTRODUCTION

1.1 Nanotechnology Definition, History and Classification

Nanotechnology is a scientific field of characterization, production and application of nanoscale particles sized 1-100 nm (Ramsden, 2018; Singh, 2015). The word nano is a Greek word that is synonymous with the dwarf. Because of their large surface area and small sizes, nanoparticles have different properties such as mechanical, electrical, magnetic, and chemical properties compared to bulk materials (Ramsden, 2018; Lloyd, Byrne & Coker 2011). As the optical, chemical and mechanical properties of nanomaterials are defined, their applications have increased. Nanoparticles can be synthesized using many materials having different chemical properties. However, metals, metal oxides, biomolecules, silicates, polymers, carbon and organic molecules are materials commonly used to synthesize nanoparticles. The diversity of nanoparticles is the origin of its chemical nature, morphology, dispersion, media and numerous possibilities for surface modification.

Although nanotechnological applications gained momentum in the late 20th century, the use of nanotechnology began much earlier. It was used around 2600 BC in textile applications and were used to colour the church glass in the Middle Ages (Chan, 2008; Dolez, 2015). Another example is that there was the carbon nanotubes in the Damascus steel structure, produced in the Middle East in the 12th to the 18th century (Reibol et al., 2009). In addition, the use of nanomaterials is seen in traditional Chinese medicine, medieval Italian paintings and Mayan (José-Yacamán, Rendón, Arenas & Puche, 1996; Padeletti & Fermo 2003; Peiyan, Zhengyi, Yanli & Jingjing 2006). In 1857, Michael Faraday described the important properties of nanoparticles in his article “The experimental relation of gold and other metals” (Faraday, 1857). More recently, in 1910, carbon black was used to reinforce the tires (Ngô & Van de Voorde, 2014). The first scientist to draw attention to nanotechnology was Richard Feynman. In 1959, he challenged scientists to write the 24 volume of the Encyclopedia Britannica at the needle point in his speech titled "There's plenty of room at the bottom"

(Feynman, 1960). The word nanotechnology was first used by Professor Norio Taniguchi in 1974 (Taniguchi, Arakawa & Kobayashi, 1974). However, nanotechnology showed its original development in 1981 with the discovery of scanning tunnelling microscopy by IBM researchers Gerd Binnig and Heinrich Rohrer (Binnig, Rohrer, Gerber & Weibel, 1982).

Nanomaterials can be classified according to their composition, morphology, origin and dimension and in different ways. Here, the origin-based and dimension-based classification of nanomaterials will be explained.

Nanomaterials can be divided into two classes as natural and synthetic (engineered) nanomaterials. Natural nanomaterials are found in nature spontaneously (Jeevanandam, Barhoum, Chan, Dufresne & Danquah, 2018). Synthetic nanomaterials can be synthesized by physical, biological, chemical or hybrid methods. Magnetic nanoparticles, metal and metal oxide nanoparticles, quantum dots, semiconductor nanoparticles and other nanomaterials can be produced using these synthesis routes (Jeevanandam et al., 2018).

The classification of nanomaterials can be based on their dimensionality. Nanomaterials with all external dimensions on the nanoscale are categorized as zero-dimensional (Dolez, 2015). In zero-dimensional nanomaterials, the electron remains in a dimensionless field (Gleiter, 2000; Jeevanandam et al., 2018). Nanoparticles, nanodots, quantum dots are an example of zero dimensional nanomaterials. One-dimensional (1D) nanomaterials have two dimensions on a nanoscale and one dimension is outside the nanoscale (Dolez, 2015). Electrons of one-dimensional nanoparticles move along the x-axis (Gleiter, 2000; Jeevanandam et al., 2018). Examples of one-dimensional nanomaterials are nanotubes, nanowires and nanorods. Two-dimensional (2D) nanomaterials have two dimensions outside the nanometric size range (Dolez, 2015). Their electrons move along the x-y axis (Gleiter, 2000; Jeevanandam et al., 2018). Nanomaterials in this class have plate-like shapes. Graphene, nanofilms and nanolayers belong to this class. Three-dimensional nanomaterials do not have an external dimension on the nanoscale and their electrons move along x, y and z-axis (Dolez, 2015; Gleiter, 2000; Jeevanandam et al., 2018). Bulk powders, multi-nanolayers and nanotubes are examples.

1.2 Applications of Nanoparticles

Nanoparticles have many applications in the different areas because of their unique physical, chemical, optical and mechanic properties.

- Heterogeneous catalysis (Jia & Schüth, 2011; Yu, Tam & Tsang, 2008)
- Antimicrobial additives (Fondevila, Herrero, Casallas, Abecia & Ducha, 2009; Martinez-Gutierrez et al., 2010)
- UV protection (Schlossman & Shao, 2005; Shi, Shan, Ju, Aikens & Prud'homme, 2012)
- LCD-technology (Jamil, Ahmad, Rhee & Jeon, 2011; Shiraishi et al., 2002;)
- Fluorescent biological labels (Wang, Mamedova, Kotov, Chen, & Studer, 2002; Bruchez, Moronne, Gin, Weiss, & Alivisatos, 1998)
- Drug and gene delivery (Cho, Wang, Nie, & Shin, 2008; Sun, Lee, & Zhang, 2008)
- Bio-detection of pathogens (Sanvicens, Pastells, Pascual, & Marco, 2009; Zhang, Huarng, & Alocilja, 2010)
- Detection of proteins (Nam, Thaxton & Mirkin, 2003; You, 2007)
- Probing of DNA structure (Taylor, Fang, & Nie, 2000; Wang, Liu, Hu, Song, & Fan, 2006)
- Tissue engineering (Goldberg, Langer & Jia, 2007; Peter et al., 2010)
- Separation and purification of cell parts, cells and biological molecules (Chiang, Sung, Wu, Chen & Hsu, 2005; Lee et al., 2006)
- MRI contrast agents (Mulder, Strijkers, van Tilborg, Griffioen & Nicolay, 2006; Na, Song & Hyeon, 2009)
- Aerospace (Haynes & Asmatulu, 2013; Voevodin, Kurdziel & Mantz, 2006)
- Automotive (Asmatulu, Nguyen & Asmatulu, 2013; Im, Cho, Kim & Kim, 2010)
- Cosmetic (Müller, Radtke & Wissing, 2002; Pardeike, Hommoss & Müller, 2009)
- Electronics (Ko et al., 2007; Li, Wu & Ong, 2005)
- Energy (Frey, Peng, Cheng & Sun, 2009; Wu, Jiang, Cao, Guo & Wan, 2009)

- Food (Espitia et al., 2012; Weir, Westerhoff, Fabricius, Hristovski & Von Goetz, 2012)
- Textile (Kathirvelu, D'souza & Dhurai, 2009; Xue, Jia, Zhang & Tian, 2009)
- Coating, paint (Anyagou, Fedorov & Neckers, 2008; Kumar, Vemula, Ajayan & John, 2008)

1.3 Green Chemistry

Green chemistry is the procedure that interested in designing of chemical goods and processes for reducing or eliminate the use and production of hazardous substances (Anastas & Eghbali, 2010). The Green Chemistry concept was created about 29 years ago (Collins, 1997). Since then many books and papers were written about Green Chemistry and its applications. Design is the most important thing for Green Chemistry and with designing chemical processes, accidents are not happened (Anastas & Eghbali, 2010). The Twelve Principles of Green Chemistry were formed for helping a chemist to teach goal of sustainability and these principles are instructions for designing (Anastas & Eghbali, 2010). Succeeding sustainability at the molecular level is the main goal of the Green Chemistry and because of that, it is usable for many industry sectors. The concept of Green Chemistry is not only important for academia but also industry, environment and education.

The Twelve Principles of Green Chemistry were formulated by Paul Anastas and John Warner in 1998 as followed (Anastas&Warner, 1998);

1. Prevention
2. Atom Economy
3. Less Hazardous Chemical Synthesis
4. Creating Safer Compound
5. Degredable Designs
6. Reduce Derivatives
7. Catalysis
8. Energy Efficient Designs

9. Use of Renewable Stocks
10. Safer Solvents and Auxiliaries
11. Real-Time Analysis for Pollution Prevention
12. Inherently Safer Chemistry for Accident Prevention

1.4 Green Synthesis

Because of global problems such as environmental pollution, environment-friendly green processes are gaining popularity. Many books and papers about green chemistry and synthesis have been published in the last 30 years. Metal nanoparticle, metal oxide nanoparticle, magnetic nanoparticle and quantum dot synthesis can be performed by using green synthesis methods (Luo, Shanmugam & Yeh, 2015). Many metals such as silver, gold, palladium, iron, copper, lead, titanium, lithium, zinc, cerium and metal oxide nanoparticles are synthesized by green synthesis method (Sharma, Yngard & Lin, 2009; Philip, 2010; Nadagouda & Varma, 2008; Zain, Stapley & Shama, 2014; Elango & Roopan, 2015; Sundrarajan & Gowri, 2011; Álvarez et al., 2015; Sangeetha, Rajeshwari & Venckatesh, 2011; Arumugam et al., 2015).

According to Sharma et al. (2009), there are 5 different methods for green synthesis and these are Polyoxometalates method, Tollens method, Polysaccharide method, Irradiation method, and Biological method. We explain the Biological method here.

In recent years, many types of research have been carried out to develop synthesis methods using natural reduction, coating and stabilizing agents to prepare the desired morphology and size nanoparticles. Plants, fungi, microorganisms and biodegradable polymers are used in the green synthesis of nanoparticles as a source of reduction, capping and stabilizing agents (Kharissova, Dias, Kharisov, Pérez & Pérez, 2013). Biological synthesis is a bottom-up process like chemical synthesis (Iravani, 2011; Velusamy, Kumar, Jeyanthi, Das & Pachaiappan, 2016).

According to Iravani et al. (2011), for the synthesis of well characterized and highly stable nanoparticles, the following aspects should be considered.

1. Selection of the best organisms

2. Enzyme activation and optimal conditions
3. Optimal reaction conditions

1.4.1 Green Synthesis of Nanoparticles Using Microorganisms

Microorganisms are important synthesis tools as they are environmentally friendly and cost-effective and do not require toxic chemicals and high energy in the synthesis of metal nanoparticles (Xie, Lee, Wang & Ting, 2007). Metal nanoparticles, metal oxides and salts, and sulfides were synthesized using microorganisms (Kharissova et al., 2013). Microorganisms can induce metal salts into metal nanoparticles owing to various reductase enzymes in their structure (Mandlik, Bejugam, Singh, 2016). These properties are very important for detoxification of heavy metals. The synthesized nanoparticles may have different sizes and morphologies depending on the kind of microorganism and synthesis conditions used. Understanding of interactions between metal and microorganisms is very important in the synthesis of metal nanoparticles.

Since bacteria are easy to obtain and genetic modifications, they are preferred in the synthesis of nanoparticles (Velusamy et al., 2016). Extracellular or intracellular synthesis of inorganic structures has been carried out with many microorganisms. Extracellular synthesis is more preferred inasmuch as it does not require downstream processes, cell membrane degradation and different purification steps (Singh, Kim, Zhang & Yang, 2016). Metal-resistant genes, proteins, peptides, enzymes, reducing cofactors and some organic structures serve as reducing and coating agents, stabilizers. Klaus et al. synthesized silver nanoparticles using *Pseudomonas stutzeri* AG259 which is a metal-accumulating bacteria and they obtained it from a silver mine. They synthesized silver nanoparticles with different compositions and sizes in a wide range of sizes (Klaus, Joerger, Olsson & Granqvist, 1999). Examples of bacteria used in nanoparticle synthesis are *Escherichia coli* (Du, Jiang, Liu & Wang, 2007), *Pseudomonas aeruginosa* (Kumar & Mamidyala, 2011), *Weissella oryzae* (Singh, Kim, Wang, Mathiyalagan & Yang, 2016), *Bacillus subtilis* (Kannan & Subbalaxmi, 2011), *Brevibacterium casei* (Kalishwaralal et al., 2010).

Nanoparticle synthesis using fungi is termed as mycosynthesis (Singh, Kim, Zhang et al., 2016). Fungi are used in nanoparticle synthesis for reasons such as greater bioaccumulation capabilities, easy downstream processes and ease of culture (Singh, Kim, Zhang et al., 2016). Nanoparticle synthesis by fungi attracts attention due to their higher protein content and efficient synthesis among all microorganisms (Banerjee & Rai, 2018). Their tolerance for metals is higher than bacteria (Singh, Kim, Zhang et al., 2016). In most nanoparticle synthesis with fungi, it was found that NADPH-dependent reductase enzymes were responsible for the reduction of the metal atom (Shankar, Ahmad & Sastry, 2003; Sadowski, Maliszewska, Grochowalska, Polowczyk & Kozlecki 2008; Balaji et al., 2009; Sanghi & Verma, 2009). *Fusarium solani* (Ingle, Rai, Gade & Bawaskar, 2009), *Penicillium fellutanum* (Kathiresan, Manivannan, Nabeel & Dhivya, 2009), *Aspergillus flavus* (Vigneshwaran et al., 2007), *Phomosis* sp. (Bharathidasan, & Panneerselvam, 2012) are examples of fungi used in nanoparticles synthesis.

Also, yeasts have been examined for the synthesis of nanoparticles among other microorganisms. Yeasts are mainly used in the synthesis of semiconductors compared to other microorganisms (Velusamy et al., 2016). Yeasts are very similar to fungi with their cellular organisation however, they are separated from them by unicellular structure (Banerjee & Rai, 2018). Examples of yeasts used in nanoparticle synthesis are strain MKY3 (Kowshik et al., 2002), *Yarrowia lipolytica* NCIM 3589 (Agnihotri, Joshi, Kumar, Zinjarde & Kulkarni, 2009), *Rhodospiridium diobovatum* (Seshadri, Saranya & Kowshik, 2011), *Pichia capsulate* (Subramanian, Alikunhi & Kandasamy, 2010).

Viruses are unicelled organisms that damage the replication mechanism and cellular activity of the host cell (Lodish et al., 2000). There are DNA or RNA in their structure and this surrounded by a shell which its structure may contain protein or lipid (Lodish et al., 2000). There are chemical groups in the structure of viruses that allow modification because of that they can be used for nanosize devices as a scaffold (Velusamy et al., 2016). Viruses are used in the production of nanowires that can be used in battery electrodes and supercapacitors (Nam et al., 2006). There is a need for a template that can be biological materials like amino acids and fatty acids for semiconductors nanocrystal synthesis (Douglas & Young, 1999). Also, viruses are

useful as a template (Narayanan & Sakthivel, 2010). Tobacco mosaic virus (TMV) and cowpea mosaic virus (CPMV) are examples of viruses used as a template (Dujardin, Peet, Stubbs, Culver & Mann, 2003; Blum et al., 2004).

1.4.2 Green Synthesis of Nanoparticles Using Plants

Phytonanotechnology offers environmentally friendly, cost-effective, fast and simple methods for nanoparticle synthesis. Many plant parts such as fruits, leaves, roots, stems, pericarp, latex and extracts can be used in nanoparticle synthesis (Singh, Kim, Zhang et al., 2016; Saratale et al., 2018). The mechanisms and compounds responsible for the synthesis of nanoparticles using plants are not fully known.

Plants can reduce metal ions in various tissues and organs. Research on plants has revealed that they store metals in the form of nanoparticles (Harris & Bali, 2008). Particularly plants with strong metal ion hyperaccumulation and reduction capacity are used to remove precious metals and it's called phytomining (Makarov et al., 2014). The entire plant can be used in the generation of metal nanoparticles. However, some restrictions arise when synthesizing at an industrial level. Particularly the shape and size of the nanoparticle depends on which part of the plant it is produced furthermore different metal contents and amounts can also be found in different plant parts. These factors may affect the initiation of synthesis of nanoparticles (Gardea-Torresdey et al., 2002). Phytomining may not be suitable for large amounts of synthesis because applications require nanoparticles with homogeneous size distribution and shape. Additionally, the isolation and refining of nanoparticles from the plant is a compelling process. Therefore, in vitro approaches including reduction of metal ions to nanoparticle form by using plant extracts have gained importance. This approach allows targeted nanoparticle synthesis with changes in ambient conditions and purification steps are easier than phytomining. In recent years, metal nanoparticles have been synthesized in vitro using many metal salts and plants (Chandran, Chaudhary, Pasricha, Ahmad & Sastry, 2006; Jain, Daima, Kachhwaha & Kothari, 2009; Ghaedi, Yousefinejad, Safarpour, Khafri & Purkait, 2015). The properties of synthesized nanostructures depend on interactions between metal ions and

biomolecules in plant extracts (Shankar, Rai, Ahmad & Sastry 2004). There are different biomolecules in different plants and this condition may clarify morphological differences in synthesized nanoparticles (Raveendran, Fu & Wallen, 2003; Haverkamp & Marshall, 2009).

It is suggested that in many literature proteins, vitamins, flavonoids, polyphenols, alkaloids, saponins, terpenoids, phenols, polyols, amino acids, sugars in plant structure may involve in reduction and stabilization (Kharissova et al., 2013; Sharma et al., 2009; Velusamy et al., 2016; Arulkumar & Sabesan, 2010; Philip, 2011; Antony et al., 2011; Vijayaraghavan, Nalini, Prakash & Madhankumar, 2012; Geethalakshmi & Sarada, 2012; Lukman, Gong, Marjo, Roessner & Harris, 2011; Ghosh et al., 2012). Terpenoids are a modified form of terpenes with different functional groups and are composed of 5-carbon isoprene units in plants and they have high antioxidant activity. Shankar et al. (2003) in the study with geranium leaves stated that terpenoids are involved in the synthesis. Flavonoids are a group of polyphenolic compounds, additionally, they have the ability to chelate and reduce metals. It is assumed that the tautomeric conversion of the flavonoids may result in reactive hydrogen release from the structure which may reduce metal ions (Ahmad et al., 2003). In addition, some flavonoids like quercetin can chelate with metal ions by means of carbonyl groups and π electrons in their structures (Ravichandran, Rajendran & Devapiriam, 2014). These mechanisms may explain the role of flavonoids in reducing metals. Monosaccharides such as glucose in plant extracts can also reduce metals. Monosaccharides that have a keto-group such as fructose can only exhibit antioxidant activity after conversion from ketone to aldehyde (Makarov et al., 2014). Glucose is a more powerful reducing agent than fructose because the ability of fructose to reduce depends on tautomeric shifts (Panigrahi, Kundu, Ghosh, Nath & Pal, 2004). The feature of disaccharides and polysaccharides to reduce metals hinge on the ability of monosaccharides in their structures to convert to open chain form. Only in this way, the metal ions reach the aldehyde group and are reduced. Maltose and lactose are disaccharides which capable of reducing. The aldehyde group of glucose is oxidized to the carboxyl group after the nucleophilic attack of the hydroxyl ion. As a result, metal ions are reduced and metal nanoparticles are formed (Shankar et al., 2003). When FTIR analyses of the synthesized nanoparticles are examined, the data on the proteins stand out (Zayed, Eisa

& Shabaka, 2012). Amino acids can bind to metals and reduce them and this ability is not the same for every amino acid (Gruen, 1975). Tan et al. (2010) investigated the ability of 20 natural α -amino acids for defining the ability of binding and reducing metals (Tan, Lee & Wang, 2010). Aminoacids can attach to metal ions via carboxyl and amino groups in their main or side chains (Glusker, Katz, Bock, 1999). When amino acids form peptides, their ability to bind and reduce metals could change. For example, since the carboxyl and amino groups of the amino acid will participate in peptide formation, they cannot interact with metals. Amino acids in the structure of proteins can affect the properties of nanoparticles to be synthesized (Glusker et al., 1999).

Synthesis of metal nanoparticles using plants and plant extracts takes place in 3 steps (Makarov et al., 2014; Glusker et al., 1999; Si & Mandal, 2007);

1. The activation step
2. Growth step
3. Termination step

Advantages of green synthesis using plant extracts (Kharissova et al., 2013; Benelli & Lukehart, 2017);

- ✓ Plants easy availability,
- ✓ There are many biomolecules in plant extracts for reducing, capping and stabilizing of metal nanoparticles.
- ✓ Usually, extracts are prepared with water. In the synthesis of nanoparticles using biological methods, although not very often, different solvents than water are also used. These solvents are ethanol, methanol, acetone, ethyl acetate and petroleum ether (Raghunandan et al., 2011; Zargar et al., 2011; Bankar, Joshi, Kumar & Zinjarde, 2010; Kasthuri, 2009a; Kasthuri, 2009b).
- ✓ No need for special conditions.
- ✓ Waste that originates from green synthesis methods is biocompatible.
- ✓ Convenient for large-scale production.
- ✓ Low cost.
- ✓ Eco-friendly.

1.4.3 Green Synthesis of Nanoparticles Using Marine Algae

Many compounds from marine organisms are used in industrial and biotechnological products. In recent years, marine organisms have been used in also nanoparticle synthesis. In recent years, marine organisms have been researched to synthesize nanoparticles. Since the conditions of the marine ecosystem are quite different compared to land, marine plants are quite different from terrestrial plants. Because of this reason, bioactive compounds of marine plants may have dissimilarities (Ravikumar, Gnanadesigan, Suganthi & Ramalakshmi, 2010). Algae contain polysaccharides such as alginate, fucoidan; polyphenols, proteins, vitamins and minerals in their structure (Kushnerova et al., 2010; Mizuno et al., 2009).

In general, the synthesis of nanoparticles using algae is carried out in three steps (Thakkar, Mhatre & Parikh, 2010);

1. Extract preparation using water or other organic solvents under certain conditions
2. Preparation of ionic solutions of metallic compounds
3. Mixing of extract and ionic metal solution under certain conditions

Silver, gold, palladium, platinum, cadmium sulfide, copper oxide, iron oxide nanoparticles which have different size and morphology were synthesized using algae extracts (Kathiraven, Sundaramanickam, Shanmugam & Balasubramanian, 2015; Singh, Kalaivani, Manikandan, Sangeetha & Kumaraguru, 2013; Momeni & Nabipour, 2015; Ramkumar et al., 2017; Abboud et al., 2014; Mahdavi, Namvar, Ahmad & Mohamad, 2013).

Algae are highly suitable for green synthesis owing to high metal deposition capabilities (Davis, Volesky & Mucci, 2003). The synthesis of metal nanoparticles is affected by changes in pH, mixing, temperature, extract and metal ion concentration. According to Chakraborty et al. (2009), enzymes found in algal cells performed as reducing agent and after it plays role nucleation and growth of metal nanoparticles by the way medium conditions are very important in this step.

Since different biomolecules and redox systems work in the reduction of metal ions, the mechanism has not been fully explained. There are also differences in the mechanisms of intracellular and extracellular synthesis of nanoparticles.

The formation of algal metallic nanoparticles involves independent biosorption of metabolites on the cell surface and subsequent aggregation of metal nanoparticles. (Shankar et al., 2016; Chakraborty, Pal, Ramaswami, Nayak, La, 2006; Barwal, Ranjan, Kateriya & Yadav, 2011; Wang & Chen, 2009).

1.4.4 Factors of Affecting Green Synthesis of Silver Nanoparticles

The physical properties of the synthesized metal nanoparticles are highly influenced by the reaction medium. The change in pH is very important in nanoparticle synthesis since it will cause changes in the charge of phytochemicals in the extract (Gan & Li 2012; Armendariz et al., 2004). It affects the ability of phytochemicals to bind and reduce to metal ions, in consequence, it may affect the size, morphology and yield of the synthesized nanoparticles (Sathishkumar, Sneha, Yun, 2010).

Temperature variable affects the synthesis of particles in the nanoscale. The rise in temperature increases the reaction rate so the yield of the reaction rate increase (Cruz et al., 2010). It is assumed that the increase in temperature also affects the morphology of the nanostructures (Lin et al., 2010).

Plants have limited ability to reduce metal ions because of that electrochemical potential of metal ions is very significant. Because if the metal ion has a high positive charge, the possibility of reduction by biomolecules increases (Haverkamp & Marshall, 2009).

Studies have shown that rise at the concentration of reducing agent may cause polydispersity (Von White et al., 2012). Peaks for silver nanoparticles appear in the UV-Vis spectrum in the range of 400-480 nm. The increase in silver ion concentration results in an increase in the absorbance of the peaks. Several publications have indicated that this may be indicative of an increase in particle size (Dubey, Lahtinen & Sillanpää, 2010; Park, 2014). Zeta potential gives information about the distribution

of particles and surface charge. According to Dubey et al. (2010), the zeta potential of silver nanoparticles at high acidic pH is low and at alkali pH is high.

1.5 Silver Nanoparticles Synthesis, Toxicities and Applications

Silver is an element with atomic number 47 and it has two stable isotopes ^{107}Ag and ^{109}Ag . The abundance of isotopes in nature is very close to each other, 51.839% and 48.61%, respectively. It is a soft, bright, white transition metal with an atomic weight of 107.8682 g / mol. It has high electrical and thermal conductivity. It is found in nature as alloys with gold or other metals. Silver shows high antimicrobial activity even at very low concentrations. Silver can be found in three oxidation states; Ag^0 , Ag^{2+} , Ag^{3+} and the first two are abundant, while the latter is unstable in aqueous media (Ramya & Subapriya, 2012).

The antimicrobial characteristics of silver have been used since the Babylonian and Greek times (Khan T., Khan M. & Nadhman 2015). Silver nanoparticles are the most targeted nanoparticle synthesis by green synthesis methods (Hu, Wang S., Wang K., Zhang & Yu, 2008; Sunkar & Nachiyar, 2012; Rao, Kotakadi, Prasad, Reddy & Gopal, 2013).

Examples of applications of silver nanoparticles include catalysis, textile manufacturing, microelectronics, biomolecular detection, waste treatment and agriculture, antibacterial agent (Singh, Jain, Upadhyay, Khandelwal, Verma, 2010; Jagtap & Bapat, 2013; Niraimathi, Sudha, Lavanya & Brindha, 2013).

1.5.1 Synthesis of Silver Nanoparticles

The synthesis of nanoparticles is generally divided into two approaches; top-down and bottom up. These approaches are based on Feynman's 1959 speech at the American Physical Society (Feynman, 1960). Top-down approaches involve breaking up bulk materials to obtain nanostructures. The bottom-up approach is to construct larger structures in the nanometric size range using chemical or physical forces with atomic

level units. Silver nanoparticles can be synthesized using biological, chemical and physical methods. Biological methods are explained in “Green Synthesis” section.

1.5.1.1 Silver Nanoparticles Synthesis by Physical Methods

The physical synthesis of silver nanoparticles is carried out using the laser ablation technique and the evaporation-condensation method (Iravani, 2011). Using these two approaches, large amounts of high purity AgNP can be synthesized without the use of toxic chemicals. However, agglomeration may be a problem because the stabilizer is not used. These two methods consume large amounts of energy, have a long synthesis time and require the use of different equipment, which in turn increases the cost.

The evaporation-condensation technique uses a gas phase route using a tube furnace to synthesize nanoparticles. The synthesis takes place at atmospheric pressure and nanospheres are obtained (Kruis, Fissan & Rellinghaus, 2000). There is a vessel with metal welding inside the tube furnace and the metal is evaporated in carrier gas which allows the synthesis of nanoparticles. The shape, size and yield of the nanoparticles can be controlled by making changes in the reaction medium. Ag, Au, Pb and fullerene nanoparticles are successfully synthesized by this method (Kruis et al., 2000; El-Nour, Eftaiha, Al-Warthan & Ammar, 2010). Disadvantages of this method; the tubular furnace covers a large area, requires a long time to maintain thermal stability, requires high energy to vaporize the metal source (Jung, Oh, Noh, Ji & Kim, 2006).

The laser ablation method, which provides nanoparticle synthesis in a narrow size range, is used in the synthesis of silver nanoparticles. Silver nanoparticles can be synthesized by laser ablation of the bulk metal source placed in a liquid medium (Chen & Yeh, 2002). After irradiation with laser, there are no other compounds, ions and reducing agents other than silver nanoparticles (Chen & Yeh, 2002). The type of metal source, the properties of the liquid medium, the power of the laser and the irradiation time affect the properties of the nanoparticles formed (Mafuné, Kohno, Takeda, Kondow & Sawabe, 2000).

1.5.1.2 Silver Nanoparticles Synthesis by Chemical Methods

Methods of chemical synthesis include the reduction of metal salts as a colloidal dispersion in an aqueous solution or organic solvent. Salts of these metals are used to synthesize related metal nanoparticles such as gold, silver, iron, zinc oxide, copper, palladium, platinum (Kinnear, Moore, Rodriguez-Lorenzo, Rothen-Rutishauser & Petri-Fink, 2017). Different reducing and capping agents can be used to produce silver nanoparticles with the desired properties (Pillai & Kamat, 2004). For the efficiency and safety of the method, the choice of solvent media, the use of less toxic and environmentally friendly chemicals are important. Nucleation and growth of nanoparticles depend on various reaction parameters including pH, reaction temperature, the molar ratio of surfactant/stabilizer and precursor, precursor type, concentration, reducing and stabilizing agents, and the like (Dakal, Kumar, Majumdar, & Yadav, 2016). In the synthesis of nanoparticles sodium citrate, hydrazine, ascorbate, dextrose, sodium borohydride, elemental hydrogen, hydrazine hydrate, poly (ethylene glycol), Tollens reagents, N, N-dimethylformamide and glucose are used as reducing agent. (Evanoff & Chumanov, 2005; Goulet & Lennox, 2010; Dong et al., 2009). Capping agent/surfactants such as chitosan, oleylamine, polymethylmethacrylate (PMMA), gluconic acid, polymethacrylic acid (PMAA), cellulose, poly N-vinyl-2-pyrrolidone (PVP) and polyethylene glycol (PEG), are used to stabilise nanoparticles (Chen & Yeh, 2002).

1.5.2 Toxicity of Silver Nanoparticles

Although silver nanoparticles are used more and more frequently in many applications due to their unique physical and chemical properties, there are also studies proving their toxicity. Silver nanoparticles may step in the cell by way of phagocytosis, macropinocytosis and endocytosis and later be distributed to cytoplasmic compartments such as mitochondria and nuclei. The release of Ag^+ ions into the cell causes oxidative damage (Wilkinson, White & Chipman, 2011). The mechanism of

toxicity of silver ions depends on concentration, surface modifications, shape, size and target cell type (Otsuki, Yoshioka & Holian, 2016).

1.5.3 Applications of Silver Nanoparticles

Antibacterial: Bacteria had gained resistance towards the antibiotic within time and alternative treatments had gained necessity (Sharma et al., 2009). It is reported that Gram(-) and Gram(+) bacteria are significantly affected by silver nanoparticles (Stoimenov, 2002). Antimicrobial activities of silver nanoparticles involve case like reactive oxygen species (ROS); intracellular ion flow; denaturation of sulfur, phosphorus and DNA, proteins and enzymes; destruction of cell walls and membranes (Kanmani & Lim, 2013).

Silver nanoparticles are used for wound dressing, bone cement, catheters because of their antibacterial effect (Becker, 1999; Rupp et al., 2004; Alt et al., 2004). In addition, its use in water purification has been approved by the World Health Organization (Pedahzur, Lev, Fattal & Shuval, 1995).

Antifungal: Since the past, extreme fungal infections have been problematic for living things (Martin, 2003). Efficacy of silver nanoparticles against common fungi such as *Aspergillus spp*, *Candida spp* and *Saccharomyces spp* have been studied (Khatoon, Mishra, Alam, Manzoor & Sardar, 2015; Kim et al., 2007).

Antiviral: Silver nanoparticles have been used to inhibit HIV interaction into host cells and also can be used for preventing infection after surgeries (Sun et al., 2005; Elechiguerra et al., 2005).

Antitumor: Silver nanoparticles cause death and oxidative stress in human fibrosarcoma and skin carcinoma cells, and there are many articles on this subject in the literature (Premkumar, Lee & Geckeler, 2010).

Biosensors and Bioimaging: The plasmonic properties of silver nanoparticles depend largely on the size, shape and medium and these properties enable them to be used in biosensing (Kreibig & Vollmer, 2013). Because of their plasmonic properties,

AgNPs can be used numerous optical microscopy techniques and can also be used on fluorescent organic dyes during imaging (Schrand, Braydich-Stolle, Schlager, Dai, & Hussain, 2008).

1.6 Artificial Neural Network

Artificial Intelligence (AI) has been developed in recent years and it has been an important part of our daily life nowadays. AI applications are commonly used in information technologies including mobile phones and also their apps. One of the AI applications is an artificial neural network (ANN). ANN has many superiorities on traditional modelling methods. On the other hand, ANN provides an important contribution to the understanding of complex situation including non-linear systems. ANN has so far been used in the estimation of retention time in HPLC, development and validation of anti-cancer drugs, drug discovery, diagnosis tool for tuberculosis, diagnosis of osteoporosis (Mert, Topcam, Cavas, 2014; Pivetta et al., 2013; Mandlik, Bejugam, Singh, 2016; Dande & Samant, 2018; Yu, Ye, Xiang, 2016). ANN has been attracted by a wide of scientists from different fields ANN can solve the problems based on unknown relationships among the data. Based on training, validation and testing steps, ANN develops a learning process and then it generalizes the knowledge for prediction of the response (Pivetta et al., 2013).

1.7 The Aims of the Thesis

The aims of the thesis are to synthesize AgNPs by honey, native seaweed *Caulerpa prolifera* and invasive seaweed *Caulerpa cylindracea*. Th experimental data is also aimed to be modelled by using an artificial intelligence method: artificial neural network. The output of the thesis, AgNPs was used in the preparation of antifouling paint composition and also decolouring process.

CHAPTER TWO

MATERIALS AND METHODS

2.1 Materials and Instruments

AgNO_3 (Macron Fine Chemicals) and Congo Red (Merck) were used for synthesizing of silver nanoparticles. Deionized water used in experiments was generated with ELGA model OS007XXM1. NaBH_4 (Merck) was used for catalytic activity assessment. Honey was purchased from a local market. pH meter and IKA RCT basic IKAMAG® shaker were used for synthesizing procedure. BioTek Microplate Reader and Shimadzu UV-1801 spectrophotometer were used for measurement of silver nanoparticle synthesis.

2.2 Methods

2.2.1 Traditional Synthesis of Silver Nanoparticles (AgNPs)

Chemical reduction of AgNPs is a traditional synthesis method for AgNPs. Traditional synthesis of AgNPs was done according to Solomon et al. (2007). Sodium borohydride (NaBH_4) was used for the reduction of ionic silver and stabilization of AgNPs. A 1 M, 10 ml of silver nitrate (AgNO_3) was added dropwise into 30 ml of 2 ml NaBH_4 solution that had been cooled. The experiment was performed above agitator and the reaction mixture was stirred vigorously. The colour of the reaction was turned light yellow after the addition of AgNO_3 .

2.2.2 Research Permissions

Required research permission was taken from Republic of Turkey Ministry of Agriculture and Forestry, General Directorate of Nature Conservation and National Parks and Republic Of Turkey Ministry Of Agriculture And Forestry since the

collection of invasive *Caulerpa* species have been forbidden in along Turkish coastlines (Figure 2.1).


T.C.
İZMİR VALİLİĞİ
İl Gıda Tarım ve Hayvancılık Müdürlüğü

Sayı : 65578134-140.03.03-E.661423
Konu : Araştırma İzni

02.03.2018

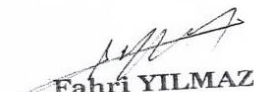
DOKUZ EYLÜL ÜNİVERSİTESİ REKTÖRLÜĞÜNE
(Fen Fakültesi Dekanlığı)

Üniversiteniz Fen Fakültesi Kimya Bölümü öğretim üyesi Prof. Dr. Levent ÇAVAŞ danışmanlığında yürütülecek olan "Green Synthesis of Silver Nanoparticles by Caulerpa spp" yüksek lisans tezinin arazi çalışmaları için Bakanlığımız tarafından verilen izin Bakanlığımız Balıkçılık ve Su Ürünleri Genel Müdürlüğünün 22.02.2018 tarih ve 67852565-140.03.03-E.570270 sayılı yazısı ile bildirilmiştir.

İlgi yazı gereğince Prof. Dr. Hasan Levent ÇAVAŞ ve Yüksek Lisans öğrencisi Yeşim YILMAZ'ın; Şubat 2018 - 1 Şubat 2020 tarihleri arasında, Dikili, Çeşme ve Seferihisar kıyılarında, mevsimlik periyotlarla, 0-5 metre derinlikte, el ile toplanarak, 10 kg *Caulerpa* spp örneğinin alınmasına, çalışma yapılacak güne ait çalışma takviminin, İl Müdürlüğümüze önceden bildirilmesi, çalışmaların İl Müdürlüğümüzde görevli en az bir teknik elemanın katılımıyla gerçekleşmesi, 4/1 Numaralı Ticari ve 4/2 Numaralı Amatör Su Ürünleri Avcılığını Düzenleyen Tebliğlerde belirtilen diğer hükümlere riayet edilmesi, yakalanan su ürünlerinin ticarete konu edilmemesi, çalışmalara yabancı araştırmacının katılmaması, proje çalışma sonuçlarının Bakanlığımıza gönderilmesi, örneklerin izin almadan yurt dışına çıkartılmayacağı ve arazi çalışmaları araştırmacılardan en az bir kişinin bulunması şartlarına uyulması hususunda;

Bilgilerinizi ve gereğini arz ederim.

e-imzalıdır
Musa BAKAN
İl Müdür V.


Fahri YILMAZ
Memur
Güvenli Elektronik İmza
Aşlı İle Aynıdır
05 Mart 2018

Arz ederim
14.03
Sulcu

T.C. DOKUZ EYLÜL ÜNİVERSİTESİ FEN FAKÜLTESİ	
Kayıt Tarihi	13.3.18
Kayıt No	596

Not: 5070 sayılı Elektronik İmza Kanunu gereği bu belge elektronik imza ile imzalanmıştır.

Evrak Doğrulama Kodu: PDYBCZNBZDWSMEUNUMEI Evrak Takip Adresi: <https://www.turkiye.gov.tr/gida-tarim-hayvancilik-bakanligi-ebys>
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Bilgi için: Erkan METE
Mühendis

Figure 2.1 Research permission was taken from Republic Of Turkey Ministry Of Agriculture And Forestry

2.2.3 Collection of *Caulerpa* Species

C. cylindracea and *C. prolifera* were collected from Dikili and Çeşme via free diving from 0-5 meter depths. After collection, *Caulerpa* samples were washed and then dried under the sunlight and ground. The average particle diameter of the ground sample was found as 500 μm .

2.2.4 Extraction Protocols

Ground *Caulerpa* samples were extracted according to the method of Edison et al. (2016) and Kathiraven et al. (2015). The extraction densities were chosen as 0.001 g/ml, 0.01 g/ml and 0.075 g/ml.

Previously, Edison et al. (2016) and Kathiravenn et al. (2015) proposed the methods to extract *Caulerpa* spp for the synthesis of AgNPs. The joint parts of their methods are to add 1 g *Caulerpa* samples into 20 mL deionised water after washing and drying protocols. Edison et al. (2016) applied 80° C for 30 min. On the other hand, Kathiraven et al. (2015) stayed their samples 24 hours at room temperature. In this thesis, in addition to the above mentioned protocols, *Caulerpa* samples were washed and dried and then powdered. 1 g of *Caulerpa* powder was added into 20 ml deionized water and heated for 1 hour at 80°C. After that extract was cooled and filtered using Whatman 40 filter paper.

2.2.5 Silver Nanoparticles (AgNPs) Synthesis

AgNPs were synthesized using honey, *C. cylindracea* and *C. prolifera* as reducing agents.

2.2.5.1 Silver Nanoparticles (AgNPs) Synthesis using Honey

The concentration of the honey solution and AgNO₃ solution, pH, temperature, agitation time and agitation rate were worked for the optimization of AgNPs synthesis. 5 g of honey was dissolved in 20 ml deionized water. 15 ml of honey solution were added into 20 ml, 0.001 M AgNO₃ solution and pH was adjusted using NaOH. This solution colour was light yellow almost colourless. After 10-minute agitation time colour was turned into brown-yellow. This colour change indicates the formation of AgNPs. The synthesized AgNPs were separated via centrifugation at 10,000 rpm for 30 minutes. Table 2.1 shows the statistics of the data for the optimization process for honey-based AgNPs.

Table 2.1 Statistics of data for the optimization process for honey based AgNPs synthesis

[AgNO ₃], M	[Honey], g/ml	Temperature, °C	pH	Agitation rate	Agitation time
0.0010	0.10	25	3.0	0	0-390 min
0.0025	0.25	45	6.0	100	
0.0050	0.50	65	6.5	200	
0.0075	0.75	85	7.0	400	
0.0100			7.5		
0.0250			8.0		
0.0500			8.5		
0.0750			9.0		
0.1000					

2.2.5.2 Silver Nanoparticles (AgNP) Synthesis by using *C. cylindracea* and *C. prolifera*

The concentration of *Caulerpa spp* extract and AgNO₃ solution, pH, temperature, agitation time and agitation rate were worked for optimization of AgNPs synthesis. 1 g of *Caulerpa spp* was added into 100 ml deionized water and heated for 30 minutes at 80°C and then filtered using Whatman 40. 10 ml of *Caulerpa spp* extract, 10 ml 0.001 M AgNO₃ solution and 30 ml deionized water mixed and pH was adjusted using NaOH and HCl. This solution colour was light yellow after 20-minute agitation colour

turned into reddish brown. This colour change indicates the formation of AgNPs. The synthesized AgNPs were separated via centrifugation at 10,000 rpm for 30 minutes. Table 2.1 shows the statistics of the data for the optimization process for *Caulerpa* based AgNPs.

Table 2.2 Statistics of the data for the optimization process for *Caulerpa* spp. based AgNPs synthesis

[AgNO ₃], M	[<i>Caulerpa</i>], g/ml	Temperature, °C	pH	Agitation rate	Agitation time
0.0010	0.001	25	3.0	0	0-360 min
0.0025	0.010	40	4.5	200	
0.0050	0.075	55	6.0	300	
0.0075		70	7.5	400	
0.0100		85	9.0		
0.0250			10.5		
0.0500					
0.0750					
0.1000					
0.2000					

2.2.6 Characterization of Silver Nanoparticles (AgNPs)

AgNPs exhibit light yellow to brown colour. The synthesis of AgNPs was monitored using Shimadzu UV-1801 UV-VIS spectrophotometer. The spectrum of the solution was recorded between 200-800 nm. BioTek Microplate Reader was also used for optimization experiments.

The Fourier transform infrared (FT-IR) spectrometry was used to define the surface characteristics of the extract and also synthesized nanoparticles. FT-IR was carried out for the biosynthesized AgNPs and *Caulerpa* samples. 100 mg of KBr powder and 1 mg of synthesized AgNPs were mixed and pellets were prepared.

The X-ray diffraction (XRD) analysis was performed to identify the crystallographic nature of the synthesized AgNPs. Thermo Scientific ARL X'TRA was used for analysis. The analysis was performed between 5-90 degree angles with 2 degrees/minute scan rate and using Ni-filtered CuK α radiation.

The scanning electron microscopy (SEM) analysis was performed to identify the morphology of the synthesized AgNPs.

2.2.7 Catalytic Activities of Synthesized Silver Nanoparticles (AgNPs)

Catalytic activity experiments were carried out according to Edison et al. (2016). In optimization studies, concentration of Congo red and AgNPs solution, temperature and agitation rate were studied (Table 2.3). 0.0005 M, 10 ml of Congo red dye and 0.005 M, 5 ml of NaBH₄ solution were mixed and 0.05 ml AgNPs added. Approximately after 10 minutes, the colour of the mixture changed from red to white with regard to the concentration of Congo red dye.

Table 2.3 Statistics of the data for the optimization process for catalytic activity of AgNPs

[Congo red], g/ml	[AgNP], g/ml			Temperature, °C	Agitation rate, rpm
	Honey	<i>C.cylindracea</i>	<i>C.prolifera</i>		
2x10 ⁻⁵	7.00x10 ⁻⁴	5.00x10 ⁻⁴	6.20x10 ⁻⁴	25	200
3x10 ⁻⁵	3.50x10 ⁻⁵	2.50x10 ⁻⁴	3.10x10 ⁻⁴	40	300
4x10 ⁻⁵	1.75x10 ⁻⁵	1.25 x10 ⁻⁵	1.60x10 ⁻⁴	55	400
5x10 ⁻⁵	8.75x10 ⁻⁶	6.25 x10 ⁻⁶	7.75x10 ⁻⁵		
6x10 ⁻⁵	4.38x10 ⁻⁶	3.13 x10 ⁻⁶	3.39x10 ⁻⁵		
7x10 ⁻⁵					
8x10 ⁻⁵					
9x10 ⁻⁵					
10x10 ⁻⁵					

2.2.8 Antifouling Tests in the Field

The antifouling performance of green synthesized AgNPs was assessed in a seawater immersion test for 33 days (from September 24 to October 26, 2018) at Levent Marina, İzmir, Turkey. The plates were painted with different antifouling

paints which had different contents for testing antifouling effects of green synthesized AgNPs. Antifouling paints were prepared in our laboratory. Antifouling paints consisted of rosin, xylene, lutanol, Cu₂O and AgNPs. Firstly, the plates were sandpapered and primer was painted. After drying of primer plates were painted with antifouling paints. Painted plates were immersed in sea water. Figure 2.2 shows antifouling paint coated plates.



Figure 2.2 Antifouling paint coated plates

2.2.9 Artificial Neural Network (ANN) Study

The data related to the synthesis of AgNPs was modelled by using neural networks toolbox of MATLAB (R2016b). The input parameters in these networks were agitation time, agitation rate, temperature, pH, AgNO₃ concentration and honey concentration, extract of *C. cylindracea* and *C. proliferata* concentration. The output data was absorbance value. Before creating network hidden neuron number was optimized under default settings of MATLAB. The MSE and R² values in the ANN calculated by the following equations;

$$MSE = \frac{1}{n \sum_{i=1}^n (a_i - b_i)^2} \quad (2.1)$$

$$R^2 = 1 - \frac{\sum_{i=1}^n (a_i - b_i)^2}{\sum_{i=1}^n (a_i - a_m)^2} \quad (2.2)$$

Where a_i is the target value, b_i is the output value, a_m is the average of the target values and n is the total number of training patterns. Backpropagation algorithms used in this study were given in Table 2.4.

Table 2.4 The backpropagation algorithms used in the study

Backpropagation Algorithms	Function
BFGS quasi-Newton backpropagation	<i>trainbfg</i>
Powell–Beale conjugate gradient backpropagation	<i>traincgb</i>
Fletcher–Reeves conjugate gradient backpropagation	<i>traincgf</i>
Polak–Ribière conjugate gradient backpropagation	<i>traincgp</i>
Batch gradient descent	<i>traingd</i>
Batch gradient descent with momentum	<i>traingdm</i>
Variable learning rate backpropagation	<i>traingdx</i>
Levenberg–Marquardt backpropagation	<i>trainlm</i>
One step secant backpropagation	<i>trainoss</i>
Resilient backpropagation (Rprop)	<i>trainrp</i>
Scaled conjugate gradient backpropagation	<i>Trainscgp</i>

ANN structure consists of two-layer feed-forward networks with sigmoid and linear functions, in our study (Figure 2.3).

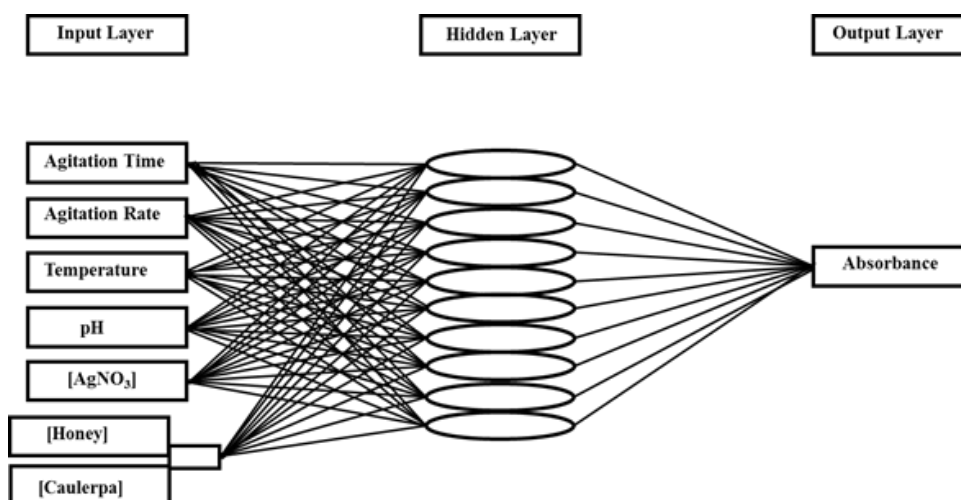


Figure 2.3 The architecture of ANN created in the study

2.2.9.1 Artificial Neural Network (ANN) Study for Honey Based AgNP

In our network creation training, validation and testing data were selected randomly by MATLAB. The total numbers of data were 129. 114 of the data were used for training, testing and validation in ANN modelling. Furthermore, training, validation and testing percentages were studied and the percentage which had the highest R^2 and the lowest mean square error (MSE) values were selected for ANN design. The 70%, 20% and 5% of datasets were used for training, validation and testing. 15 of the total data were randomly chosen to compare the experimental and predicted values for testing the efficiency of the developed ANN model. In order to find the best performance parameters, 11 different backpropagation algorithms were tested.

2.2.9.2 Artificial Neural Network (ANN) Study for C. cylindracea and C. prolifera Based AgNP

Training, validation and testing data were selected randomly by MATLAB for network creation. Total numbers of data were 126 and 111 data were chosen for training, testing and validation in ANN modelling. Moreover, training, validation and test percentages were examined. Percentages, which had the highest R^2 and the lowest mean square error (MSE) values, were selected for ANN design. Hidden neuron numbers were studied using default settings which 70%, 20% and 5% of datasets for training, validation and testing. 15 of the 126 data were selected as sample parameters for using after ANN optimization as estimating data. 11 different backpropagation algorithms were tested in order to find the best performance parameters.

CHAPTER THREE

RESULTS

3.1 Traditional Synthesis

In this thesis, the traditional synthesis of AgNPs was performed. The traditional synthesis of AgNPs was explained in the materials and methods section. The synthesized AgNPs was initially characterized by UV-VIS spectrophotometry and the maximum absorbance peak was found in 400 nm as 0.678 (Figure 3.1). The results obtained are compatible with the results published in the scientific literature (Guzmán, Dille & Godet, 2009; Song, Lee, Park & Lee, 2009).

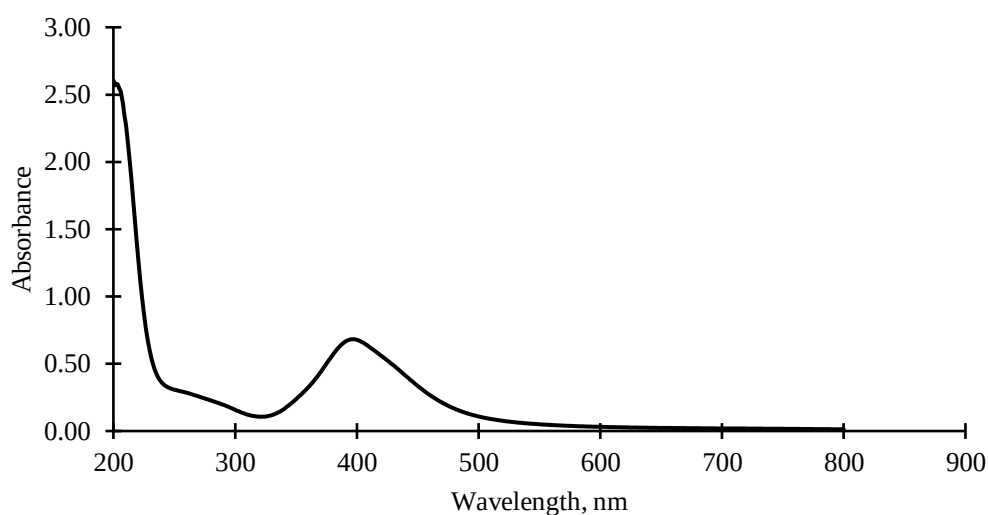


Figure 3.1 UV-Vis spectrum of synthesized AgNPs

3.2 Green Synthesis of AgNPs Through Honey

AgNPs were synthesized by using honey to optimize the conditions. In the first experiments, we wanted to optimize the wavelength for the kinetic experiments. 405, 450, 490, 590 and 630 nm were monitored during the AgNP synthesis by honey. It was found that max absorbance values were obtained at 405 nm (Figure 3.2). The

experiment was carried out during 390 minute and absorbance values were recorded. There were fluctuations in the diagram but absorbance value of AgNPs in different minutes was not divergent. The optimum agitation time was determined as 240 min.

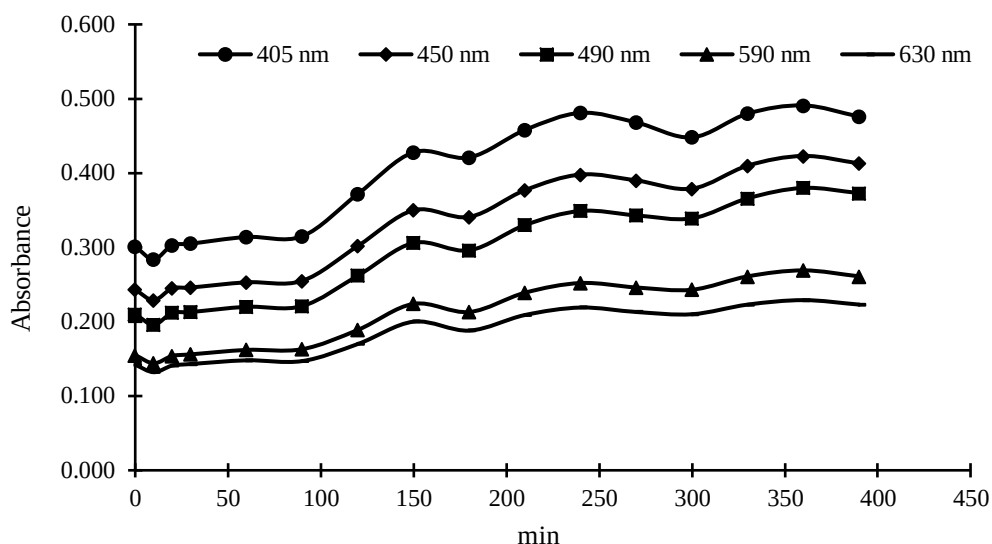


Figure 3.2 Absorbance values of honey solution at different wavelengths during 360 minute ([Honey] =0.25 g/ml, [AgNO₃] =0.001 M, Temperature=25°C, Agitation rate=200 rpm, Agitation time=240 min, pH=6.00)

AgNPs' shape and size are affected from the pH value of the experiment solution. Colour of the solution changed yellow to black according to the pH value of solution and aggregations were observed at different pH values. Optimization of pH values for AgNPs synthesis was done to avoid aggregations. The experiments were carried out in the pH range of 3-7. Figure 3.3 reveals that the optimum pH value was 8.5. Absorbance value at pH 3 was found as 0.302 and then was observed a decline after that pH value showed fluctuations and absorbance value at pH 9 was found as 0.249.

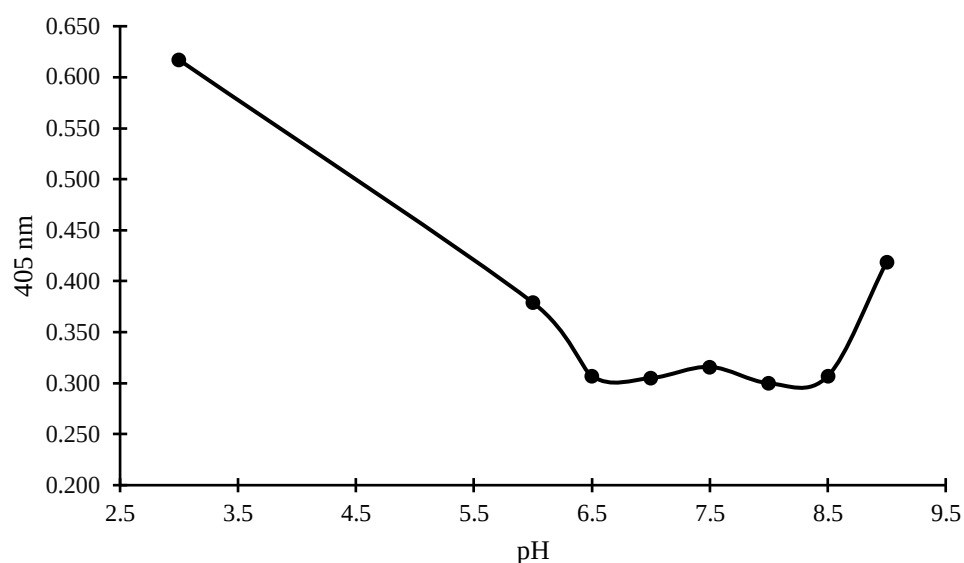


Figure 3.3 Effect of different pH values on AgNPs synthesis ([Honey] =0.25 g/ml, [AgNO₃] =0.001 M, Temperature= 25°C, Agitation rate=200 rpm, Agitation time=240 min)

Agitation rate may affect the connection of capping agents so this may affect the stability of AgNPs. We found out that agitation rate has not been studied before in the literature to the best of our knowledge by other papers according to our knowledge. Agitation rate was studied in the range of 0-400 rpm. We observed that there was a linear increase and absorbance value at 400 rpm was found as 0.437 at 25° C (Figure 3.4).

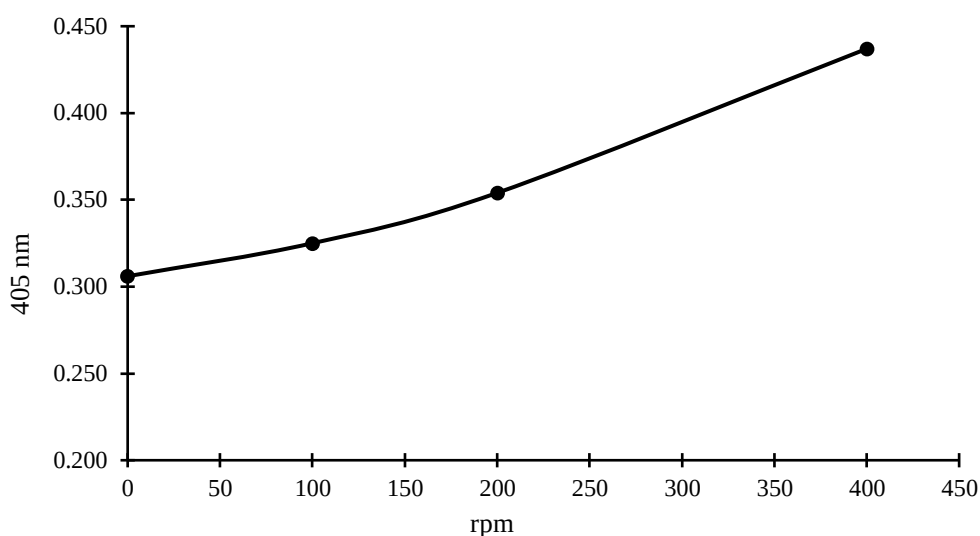


Figure 3.4 Effect of different agitation rate values on AgNPs synthesis ([Honey] =0.25 g/ml, [AgNO₃]=0.001 M, Temperature=25°C, Agitation time=240 min, pH=6.00)

Temperature values effect kinetics of a synthesis reaction. The experiments were performed in the temperature range 25-85° C. Absorbance value at 25° C was found as 0.332 after that was observed increment at 65° C as 0.562 and then absorbance value was decreased to 0.400 at 85° C (Figure 3.5)

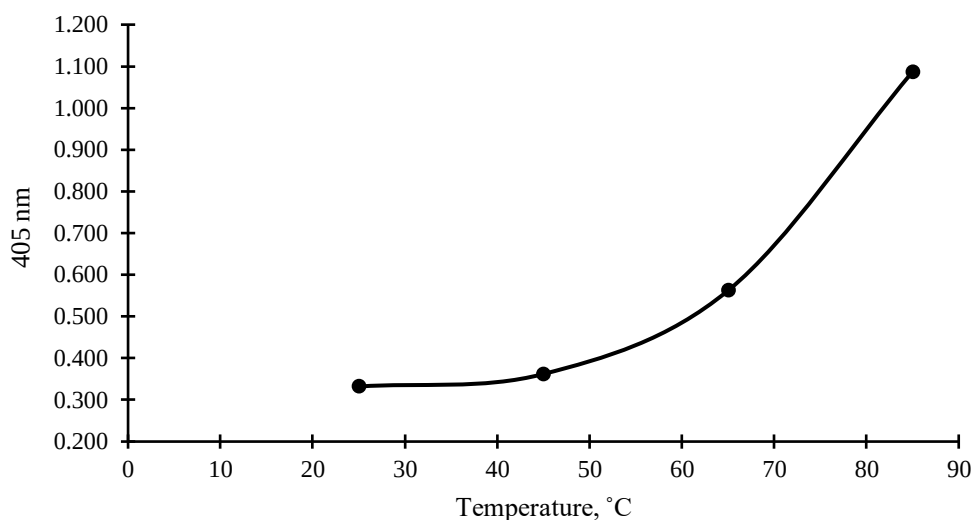


Figure 3.5 Effect of different temperature values on AgNPs synthesis ([Honey] =0.25 g/ml, [AgNO₃] =0.001 M, Agitation rate=200 rpm, Agitation time=240 min, pH=6.00)

The effects of AgNO_3 concentration was studied in this work. According to Figure 3.6, there was a sharp increase from the concentration of 0.001 M to 0.0075 M. After this point, there was a smooth decline and then showed fluctuations. The decrease in the synthesized AgNPs concentration can be explained with a decrease of reducing agent concentration in honey.

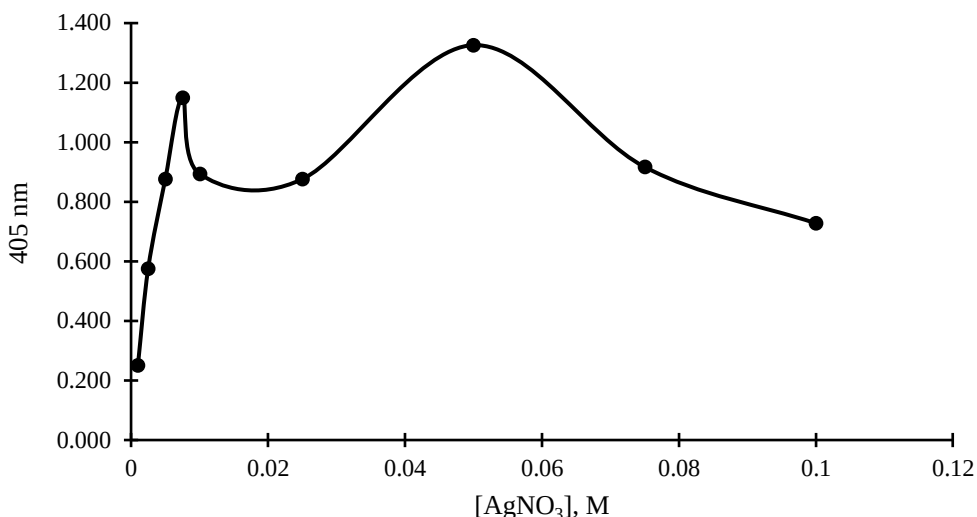


Figure 3.6 Effect of different AgNO_3 concentrations on AgNPs synthesis ([Honey] =0.25 g/ml, Temperature=25°C, Agitation rate=200 rpm, Agitation time=240 min, pH=6.00)

Honey concentration was worked at a range of 0.1 g/ml – 0.75 g/ml. According to Figure 3.7, there was a linear increase and absorbance value was 0.321 at 0.75 g/ml honey concentration.

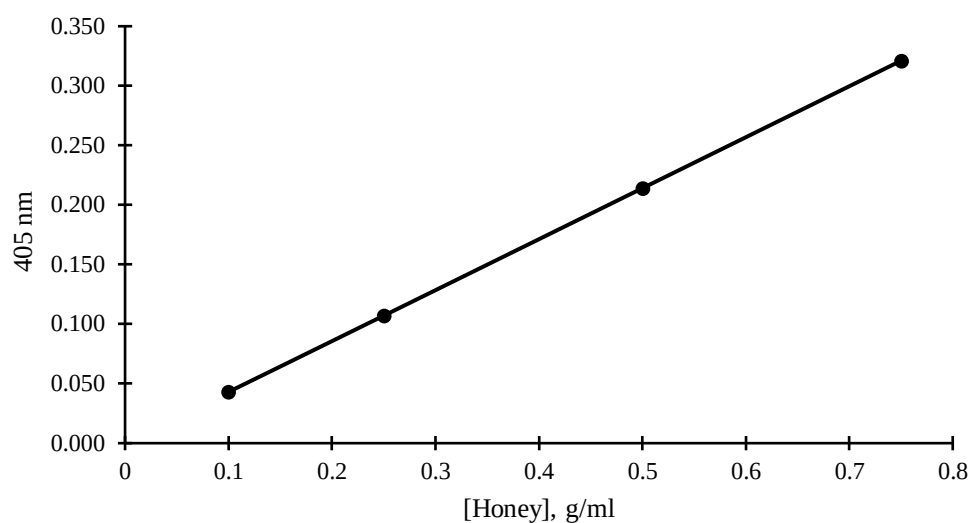


Figure 3.7 Effect of different honey concentrations on AgNPs synthesis ($[AgNO_3] = 0.001$ M, Temperature= $25^\circ C$, Agitation rate=200 rpm, Agitation time=240 min, pH=6.00)

3.3 Green Synthesis of AgNPs through *C. cylindracea*

As described in the material and method section, three different methods were performed for the extraction of *C. cylindracea* sample. According to Figure 3.8, the highest absorbance value was found for the procedure of Edison et al. (2016), absorbance value was found as a 0.178 after 240 minutes. There were fluctuations in the diagram after 60-minute absorbance values for 30min/ $80^\circ C$ increased drastically. Absorbance values of other extraction protocols were not changed many in comparison with 30min/ $80^\circ C$. For 1h / $80^\circ C$, highest absorbance value was found as 0.126 at 240 min and for 24h/ $25^\circ C$ highest absorbance value was 0.09 at the beginning.

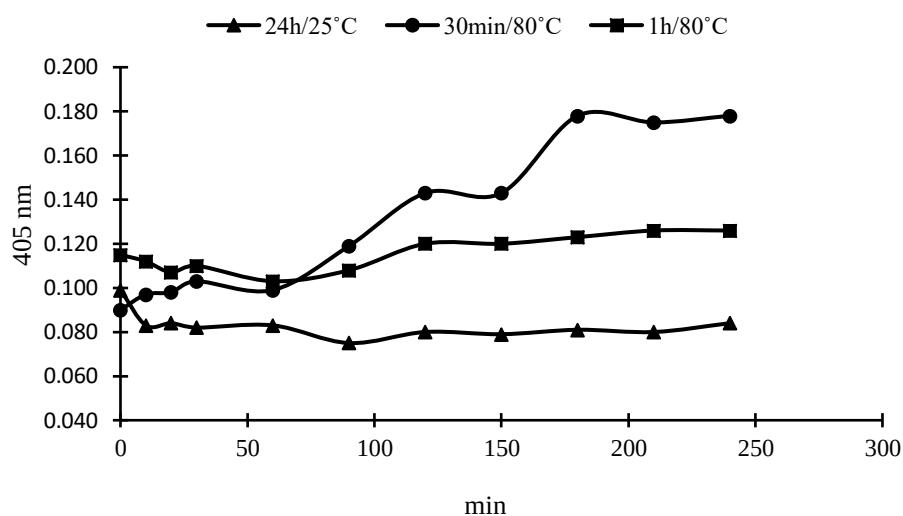


Figure 3.8 Effect of different extraction protocols for *C. cylindracea* on AgNPs synthesis (pH=6.50, Temperature=60° C, *C. cylindracea* concentration=0.02 g/mL, [AgNO₃] =0.001 M, Agitation rate=0 rpm)

0.001, 0.01 and 0.075 g/ml values were chosen for optimization of extract concentration. In our work, we observed that absorbance values of synthesized AgNPs increased when extract concentration increased. This can be seen in Figure 3.9.

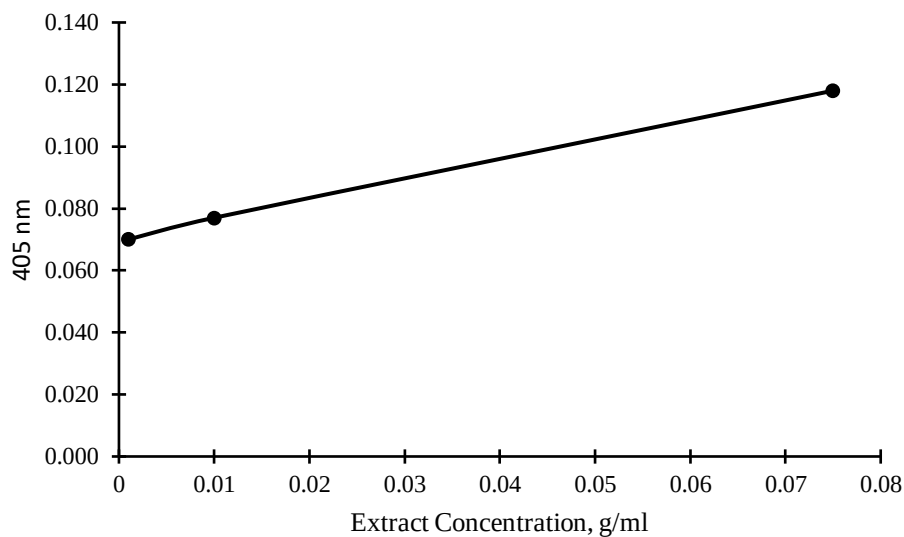


Figure 3.9 Effect of *C. cylindracea* extract concentration on the AgNPs synthesis (pH=6.5, Temperature=25° C, [AgNO₃] =0.001 M, Agitation rate=0 rpm)

Effect of time on the synthesis of AgNPs was studied in the range of 0-390 minute. According to Figure 3.10, there were fluctuations and the experiment time was chosen as 240 minutes because the highest absorbance value was 0.082 at that point.

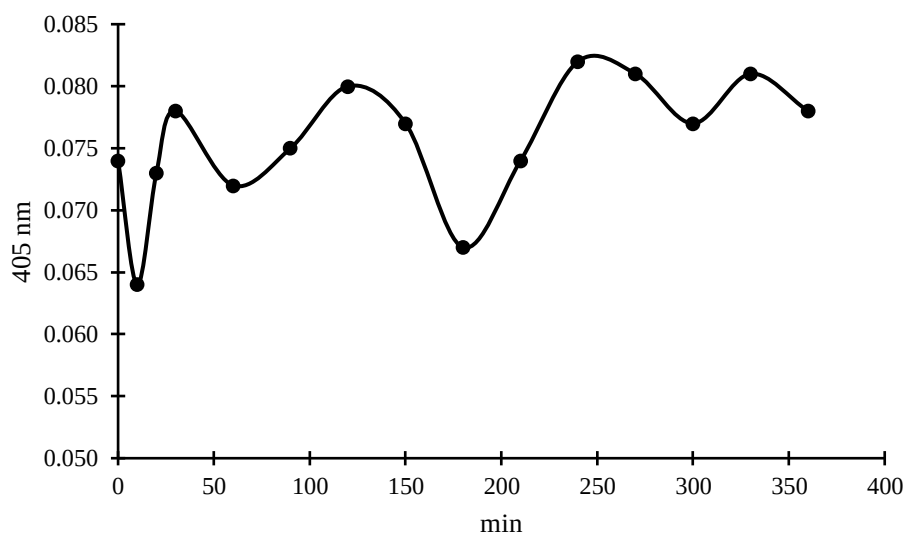


Figure 3.10 Effect of time on AgNPs synthesis ($[\text{AgNO}_3] = 0.001 \text{ M}$, *C. cylindracea* concentration=0.01 g/mL, pH=6.00, Temperature=25°C, Agitation rate=0 rpm)

The experiment was performed at different values of AgNO_3 concentrations. According to Figure 3.11, the highest absorbance value was found as a 0.206 at 0.1 M AgNO_3 . Under the light of Figure 3.12, it is seen that when the concentration of AgNO_3 rise, absorbance values of synthesized AgNPs also rise.

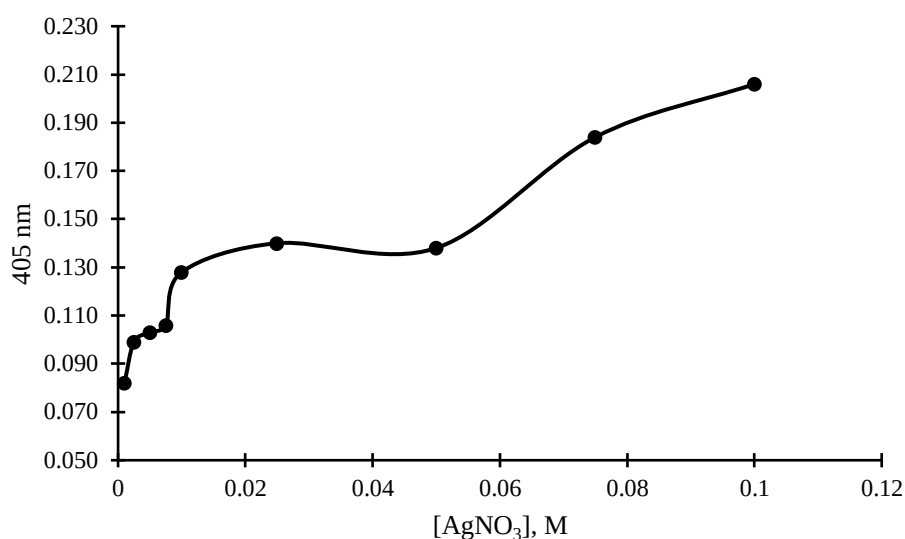


Figure 3.11 Effect of different AgNO₃ concentrations on AgNPs synthesis (*C. cylindracea* concentration=0.01 g/mL, pH=6.00, Temperature=25°C, Agitation rate=0 rpm, Agitation time=240 min)

For the optimization of green synthesis of AgNPs, the effect of temperature values to the synthesis was worked. Temperature values were chosen in the range of 25-85°C and the highest absorbance value was found as a 0.623 at 85°C. According to Figure 3.12, along with the increasing of temperature firstly absorbance values slightly decreased after that absorbance values started to increase.

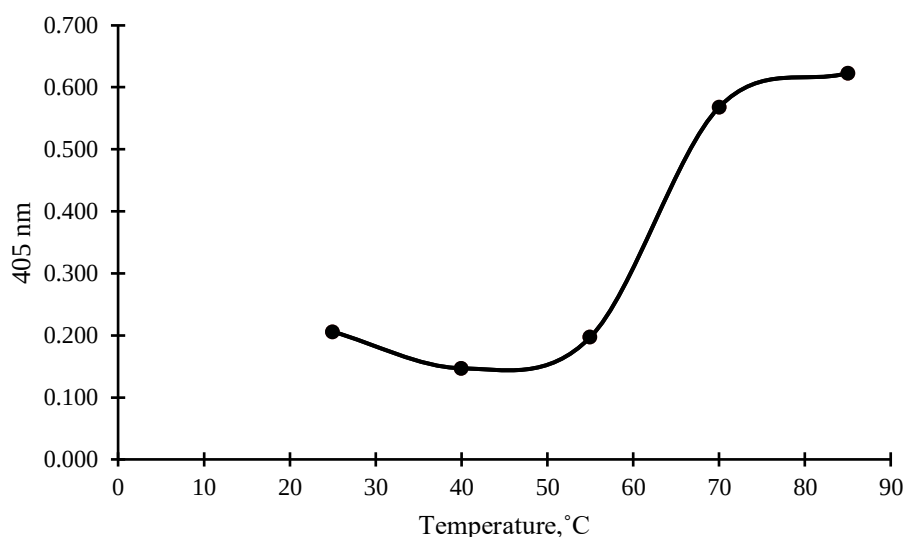


Figure 3.12 Effect of different temperature values on AgNPs synthesis ([AgNO₃] =0.1 M, *C. cylindracea* concentration=0.01 g/mL, pH=6.00, Agitation rate=0 rpm, Agitation time=240 min)

Effect of pH values was examined in the pH range of 3-10. As regards Figure 3.13, the highest absorbance value was found as 1.727 at pH 10.5 and the colour of the experiment solution was changed yellow to black as pH values increased. According to our observations, the size of AgNPs was increased and aggregation started.

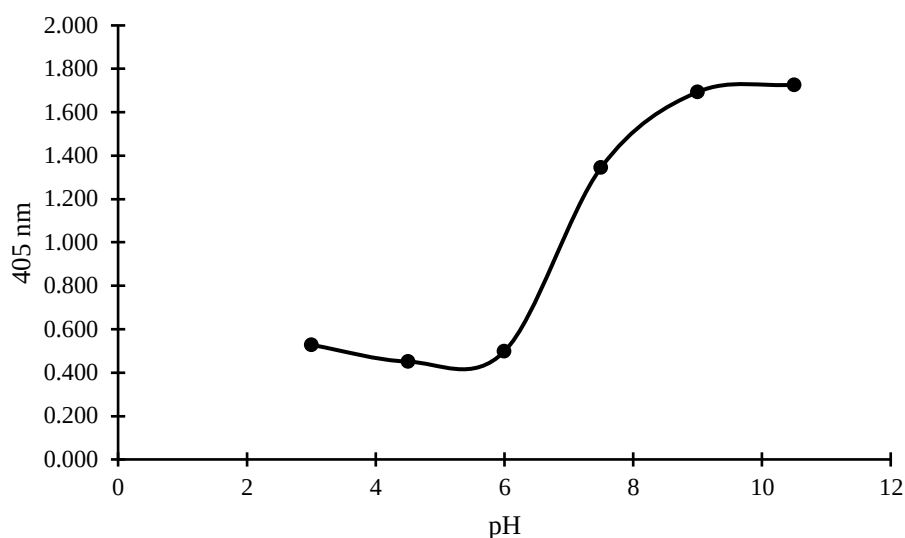


Figure 3.13 Effect of different pH values on AgNPs synthesis ($[AgNO_3] = 0.1$ M, *C. cylindracea* concentration=0.01g/mL, Temperature=70°C, Agitation rate=0 rpm, Agitation time=240 min)

Agitation rate is one of the affected parameters on the synthesis of AgNPs nanoparticles. There is an increasing with the rising agitation rates in the absorbance values and the highest absorbance value was 2.928 at 400 rpm. Because of the high absorbance values at over 1 and this may cause to error we did not display diagram of this experiment. Colour of the reaction solution turned reddish brown to black and particles aggregated and size' increased. Agitation rate was choosen as a 200 rpm for further experiments.

3.4 Green Synthesis of AgNPs Through *C. prolifera*

The extraction optimization is important because reduction agents which are found in *C. prolifera* should be transferred to the water at high levels. 3 optimization method for extraction were done and the procedure of Edison et al. (2016) was chosen for

extraction because of high absorbance value. According to Figure 3.14, there are fluctuations in the diagram and 30min/80°C had high absorbance value as 0.687 at the 240 minutes.

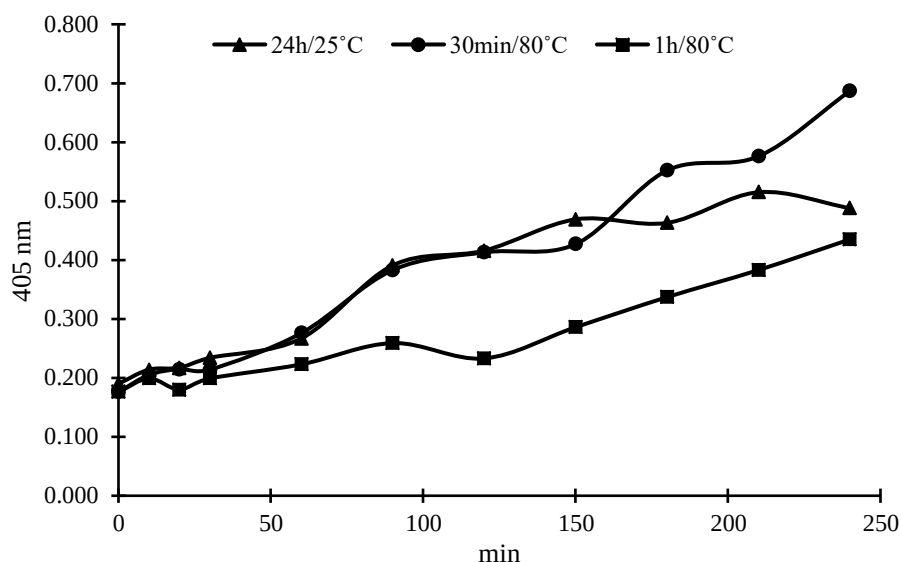


Figure 3.14 The effect of different extraction protocols for *C. proliferans* on AgNPs synthesis (pH=6.50, Temperature=60° C, *C. proliferans* concentration=0.02 g/mL, [AgNO₃] =0.001 M, Agitation rate=0 rpm)

3 value of extraction concentrations were chosen for study of extraction optimization. A linear increase was observed and high absorbance value was 0.158 at 0.075 g/ml that was seen in Figure 3.15. This concentration value was chosen for further experiments.

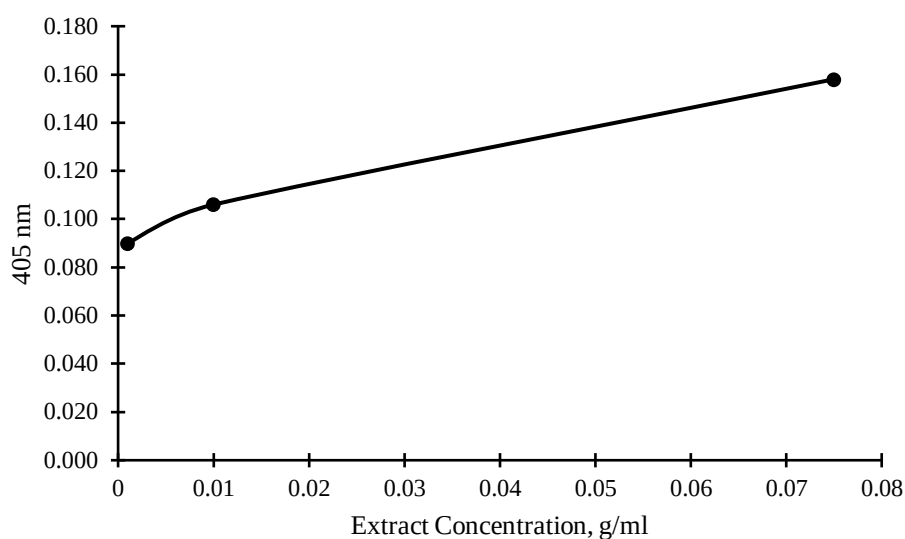


Figure 3.15 Effect of *C. proliferata* extract concentration on the AgNPs synthesis (pH=6.5, Temperature=25° C, [AgNO₃]=0.001 M, Agitation rate=0 rpm)

In order to determine the effect of time on the AgNPs synthesis, experiments performed for 390 min. According to Figure 3.16, the highest absorbance value is 0.147 at 210 min, because of this, experiment time were selected as a 210 min.

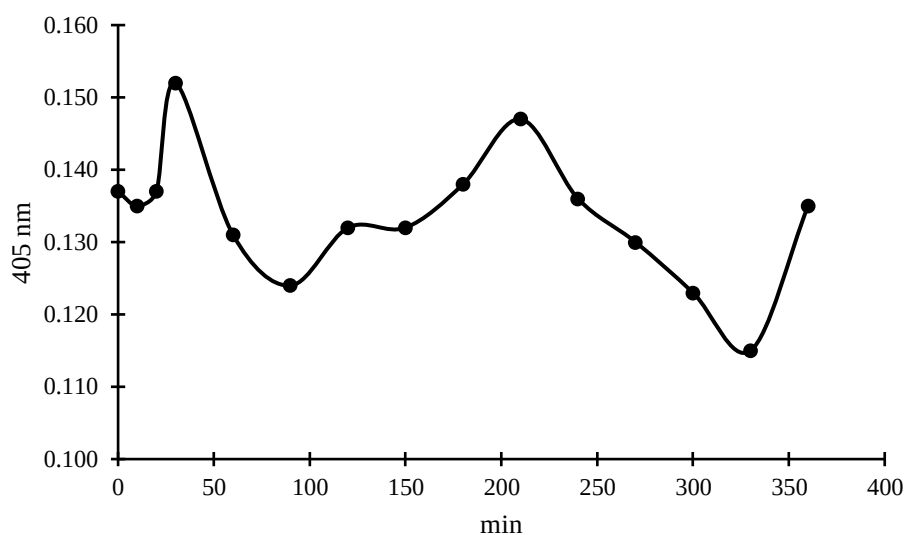


Figure 3.16 Effect of time on AgNPs synthesis ([AgNO₃]=0.001 M, *C. proliferata* concentration=0.01g/mL, pH=6.00, Temperature=25°C, Agitation rate=0 rpm)

The concentration of AgNO_3 is one of the important parameters for the green synthesis of AgNPs because it affects the yield of the reaction. According to Figure 3.17, there is peak as 0.414 at 0.075 M and this highest absorbance value. Figure 3.17 is a different diagram in comparison with other diagrams in terms of the concentration of AgNO_3 since there are an approximately linear increase in other diagrams but in this diagram, the highest absorbance value is not 0.1 M.

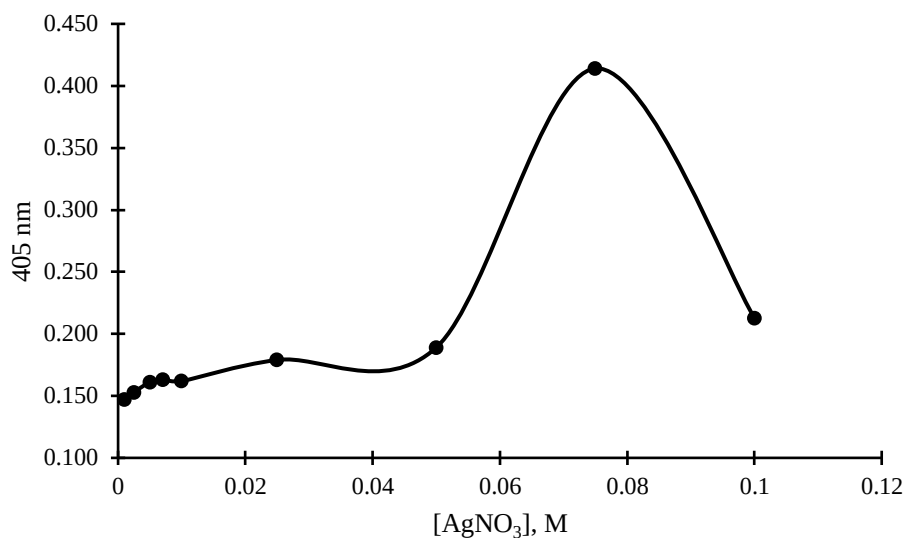


Figure 3.17 Effect of different AgNO_3 concentrations on AgNPs synthesis (*C. prolifera* concentration=0.01 g/mL, pH=6.00, Temperature=25°C, Agitation rate=0 rpm, Agitation time=210 min)

The temperature was varied between 25 °C and 85 °C to evaluate the effect of temperature on the synthesis of AgNPs. The highest absorbance value was found as 2.88 at 85°C. Since absorbance values at over 1 could lead to deviation from Bragg equation the diagram for effect of temperature values on AgNP synthesis is not displayed. Temperature value of 70°C was chosen as an optimum value for the synthesis of AgNPs.

Effect of different pH values was studied in the range of 3-10.5 for optimization of synthesis of AgNPs. Colour changing were observed in different pH values as yellow to black in our experiments as similar to experiments which were done with *C. cylindracea*. The highest absorbance value was found as 2.854 at pH 6. For further experiments as a pH of reaction solution pH 7 was chosen.

According to Figure 3.18, there is a decreasing trend in the diagram at increasing value of agitation rates in contrast with the study of optimization of agitation rates using *C. cylindracea*. The highest absorbance value was found as 1.1 when agitation was not used. But our experiments were performed at 200 rpm.

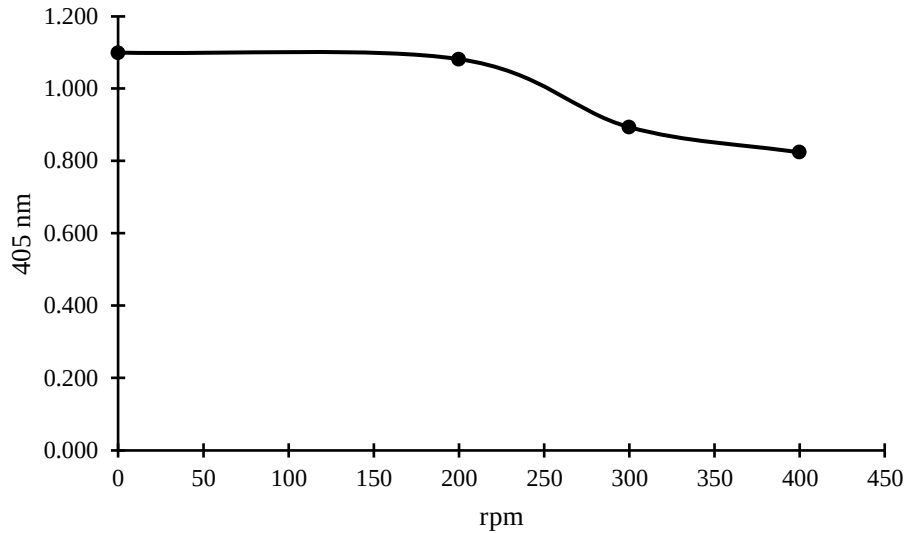


Figure 3.18 Effect of different agitation rate values on AgNPs synthesis ([AgNO₃] =0.075 M, *C. prolifer*a concentration=0.01g/mL, pH=7.5, Temperature=70°C, Agitation time=210 min)

3.5 Catalytic Activity Assessment

Industrial effluent leads to pollution in the stream and affects the micro and macro organisms lived in there. Congo red as a model dye for investigating decolourization kinetics were chosen and worked. As explained in the Materials and Methods section, the decolourization of Congo red study was performed.

The pseudo-first order kinetic model is rationalized with the Langmuir-Hinshelwood model, which is arranged for reactions ensuing in a solid-liquid interface (Turchi & Ollis 1990; Poulios, Micropoulou, Panou & Kostopoulou, 2003).

$$r_0 = -\frac{dC}{dt} = \frac{k_r K C_{eq}}{1 + K C_{eq}} \quad (3.1)$$

Where r_0 is the initial fading rate of the organic substrate, C_{eq} is the equilibrium bulk-solute concentration and K symbolize the equilibrium constant for adsorption. k_r

is the limiting constant of the reaction in the case of maximum coating. The equation can be applied when data provides linearity as follows:

$$\frac{C_{eq}}{r_0} = \frac{1}{k_r K} + \frac{C_{eq}}{k_r} \quad (3.2)$$

The effect of the change in equilibrium concentration of Congo red at constant AgNPs concentration on the initial rate of degradation is seen in Figure 3.19, Figure 3.21 and Figure 3.23. Using the initial concentration of Congo red dye in the range of 2×10^{-5} - 10×10^{-5} g/ml, the r_0 values were obtained from C_{eq} -t diagram. At constant AgNPs concentration, the relationship between C_{eq}/r_0 and C_e is shown in Figure 3.20, Figure 3.22 and Figure 3.24. The k_r ve K values were calculated according to equation 2 as using honey based AgNPs $k_r=0.1677 \text{ mg.l}^{-1}\text{min}^{-1}$, $K=0.9769 \text{ mg}^{-1}\text{l}$; using *C. cylindracea* based AgNPs $k_r=0.1069 \text{ mg.l}^{-1}\text{min}^{-1}$, $K=3.4441 \text{ mg}^{-1}\text{l}$; using *C. proliferata* based AgNPs $k_r=0.4192 \text{ mg.l}^{-1}\text{min}^{-1}$, $K=1.9368 \text{ mg}^{-1}\text{l}$.

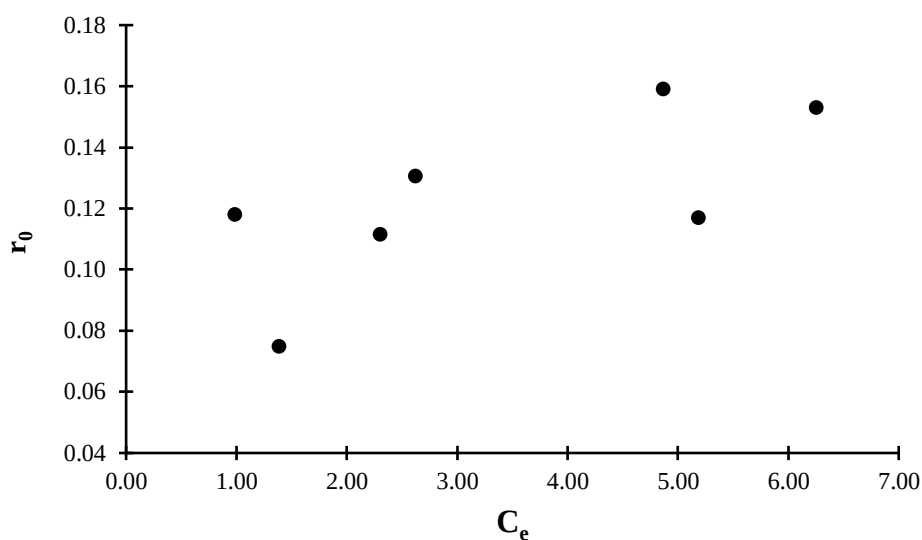


Figure 3.19 Plot of r_0 vs. C_{eq} for Congo red at different initial concentrations for the constant concentration of honey based AgNPs

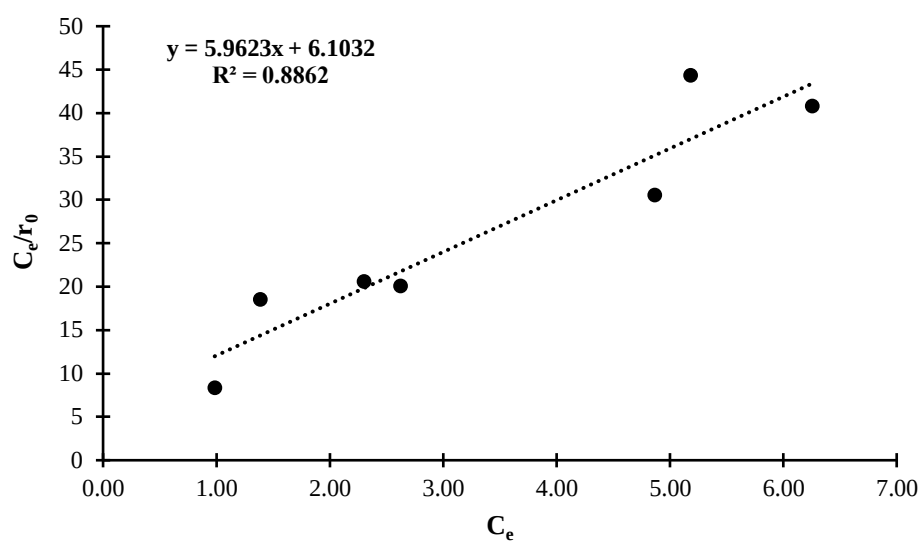


Figure 3.20 Linear transformation of C_e/r_0 vs. C_e according to Equation 3.2 data from Figure 3.19

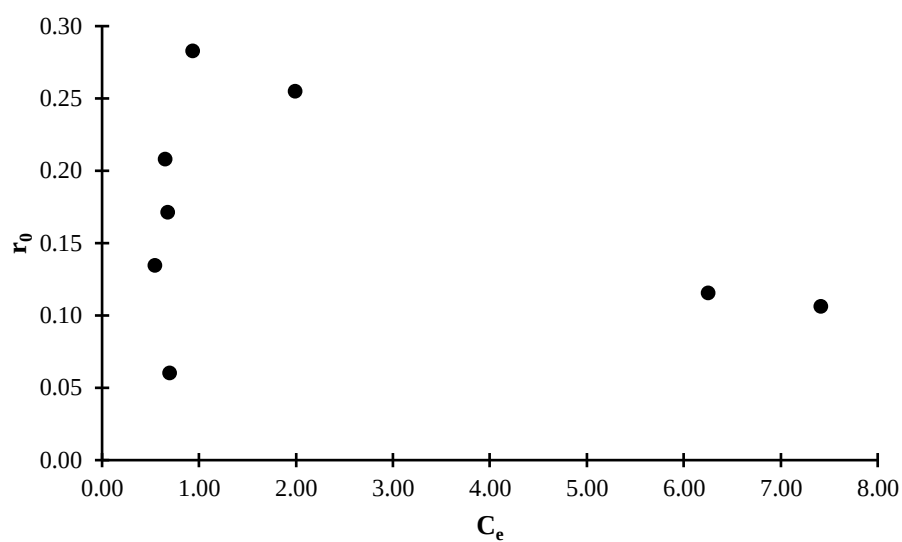


Figure 3.21 Plot of r_0 vs. C_e for Congo red at different initial concentrations for the constant concentration of *C. cylindracea* based AgNPs

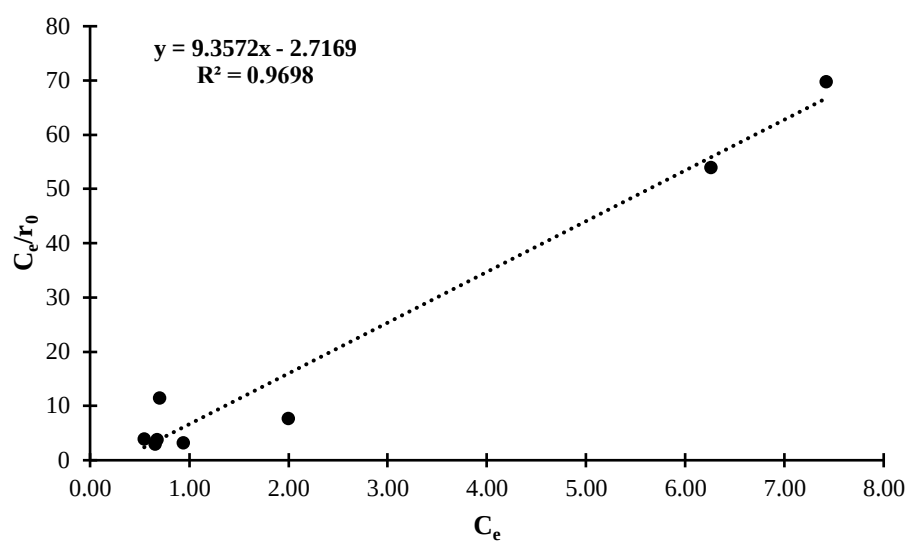


Figure 3.22 Linear transformation of C_e/r_0 vs. C_e according to Equation 3.2 Data from Figure 3.21

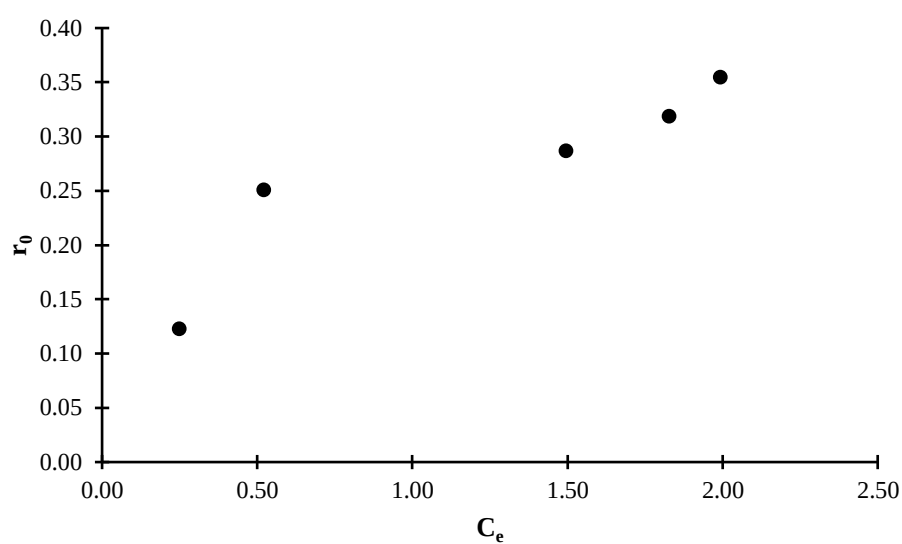


Figure 3.23 Plot of r_0 vs. C_e for Congo red at different initial concentrations for the constant concentration of *C. prolifer* based AgNPs

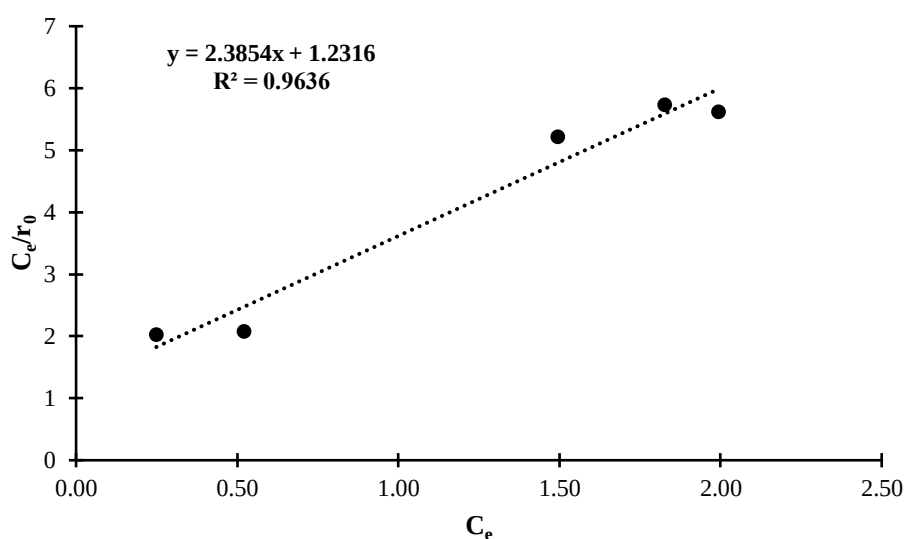


Figure 3.24 Linear transformation of C_{eq}/r_0 vs. C_{eq} according to Equation 3.2 Data from Figure 3.23

Decolourization of Congo red occurs by electron exchange between NaBH_4 and Congo red. In this reaction, NaBH_4 electron donor Congo red is the receiver. AgNPs act as catalysts by accelerating electron transfer and reaction occurs on the surface of AgNP. It is seen from the linear correlation of t versus $\ln A_t / A_0$ graph that the reaction is compatible with pseudo first order kinetic law. Pseudo first order kinetic formulated with the following equation (Lagergren, 1898; Ho & McKay, 1999):

$$\ln \left(\frac{C_t}{C_0} \right) = -k \cdot t \quad (3.3)$$

C_t = The concentration of Congo red in t reaction time.

C_0 = The initial concentration of Congo red.

k = Rate constant.

According to Figure 3.25 - 27, the rate constants were calculated from the slope of the line and found as 0.0455 min^{-1} , 0.0234 min^{-1} and 0.0791 min^{-1} for honey, *C. cylindracea* and *C. prolifera* based AgNPs, respectively.

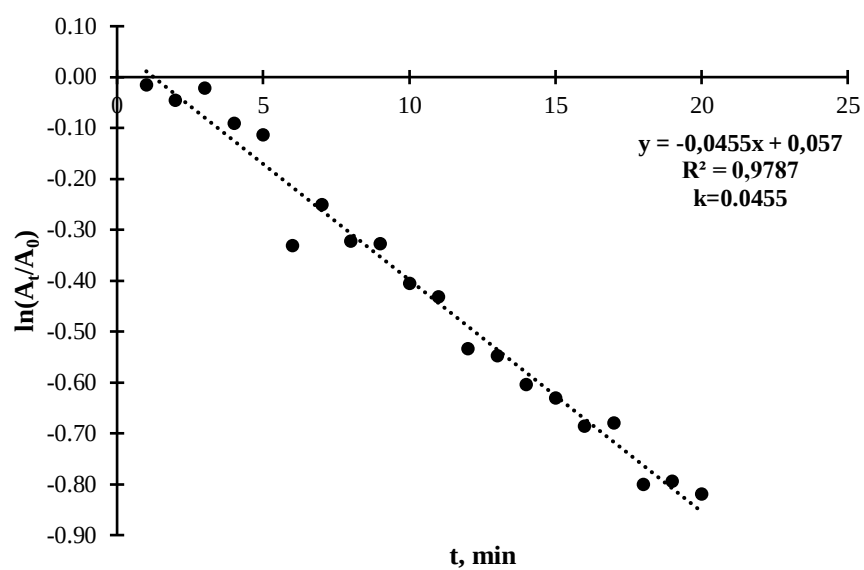


Figure 3.25 First order kinetics plot of $\ln(A_t/A_0)$ versus time for decolourization of Congo red using honey based AgNPs

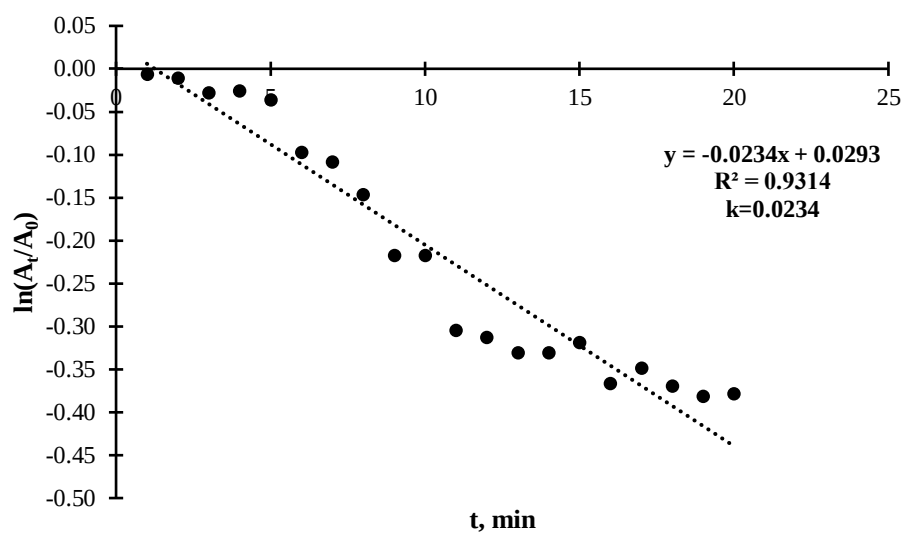


Figure 3.26 First order kinetics plot of $\ln(A_t/A_0)$ versus time for decolourization of Congo red using *C. cylindracea* based AgNPs

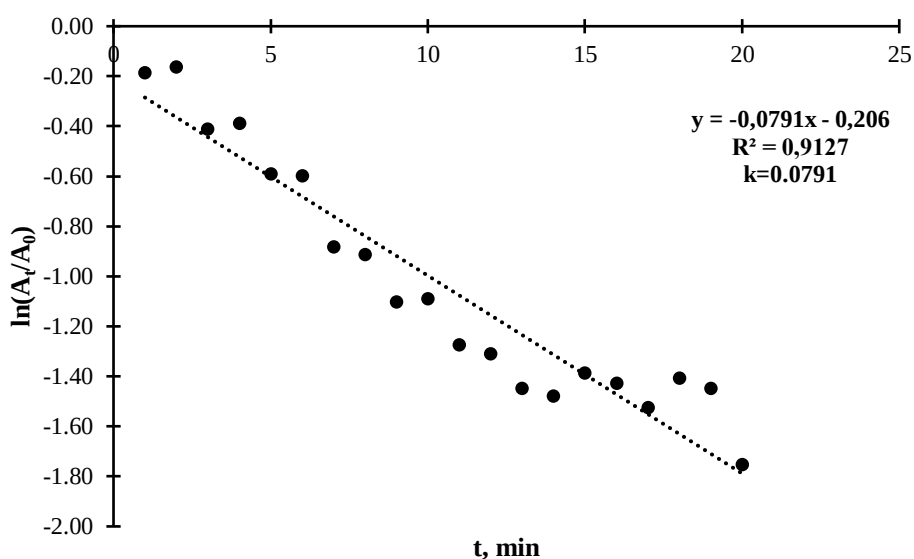


Figure 3.27 First order kinetics plot of $\ln(A_t/A_0)$ versus time for decolourization of Congo red using *C. proliferans* based AgNPs

3.6 UV-Visible Spectrophotometry Analysis

The optical properties, which described by UV-Vis spectroscopy and surface plasmon resonance, of metal and semiconductor nanoparticles are quite different compared to bulk materials. The UV-Vis spectrum shows the optical properties of the synthesized AgNPs. Surface plasmon resonance (SPR), which is distinctive for each nanomaterial, can be defined as the release of free electrons on the surface of nanoparticles.

The reaction between biologic reduction agent and AgNO_3 solution was physically indicated by a change colour from light yellow to reddish brown, which shows a reduction of Ag^+ to Ag^0 . The observed colour change results from the characteristic vibrations as a result of the changes in the electronic energy levels of the metal nanoparticles (Daisy, 2009).

In the event of honey-based AgNPs, the highest absorbance peak was in the range of 450-475 nm and it changes according to reaction time. Absorption band for 24 hours was highest and centred on 465 nm (Figure 3.28).

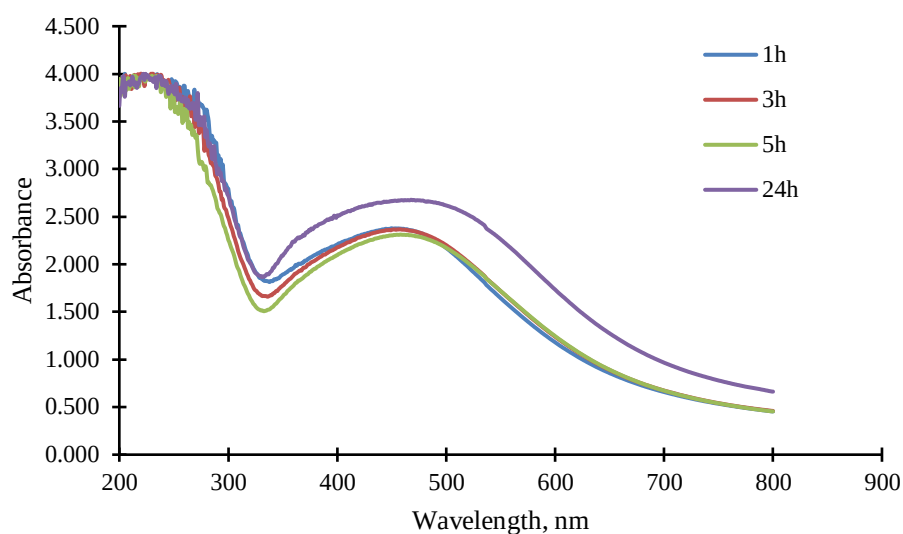


Figure 3.28 UV-Vis spectrum of honey-based AgNPs

According to Figure 3.29, there is not a regular increase in absorbance values. UV-Vis spectrum of *C. cylindracea* based AgNPs shows that there is increasing in absorbance values over time and the highest absorbance value can be seen at 445 nm.

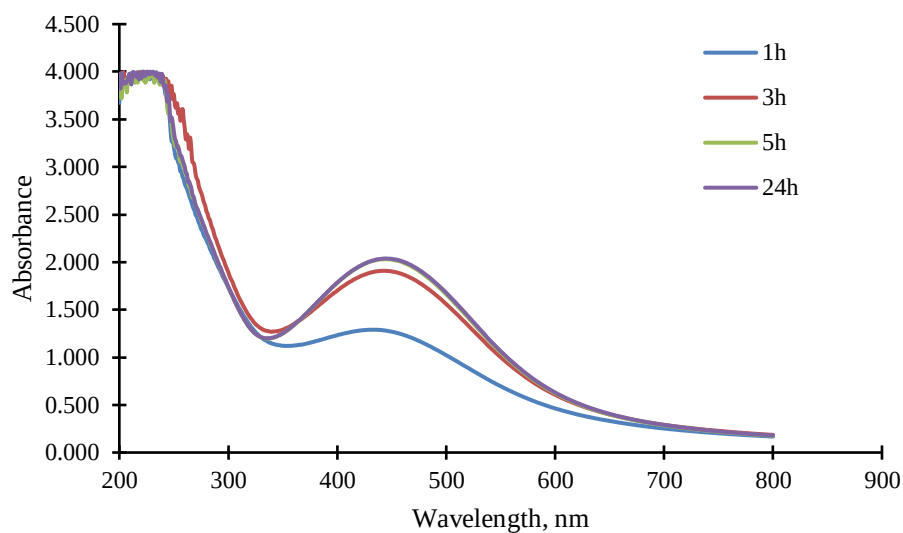


Figure 3.29 UV-Vis spectrum of *C. cylindracea* based AgNPs

There is regular rise for absorbance value in terms of time (Figure 3.30). Highest absorbance peaks are found in the range of 448-450 nm wavelengths for different times and 24 hours have the highest absorbance value at 451 nm.

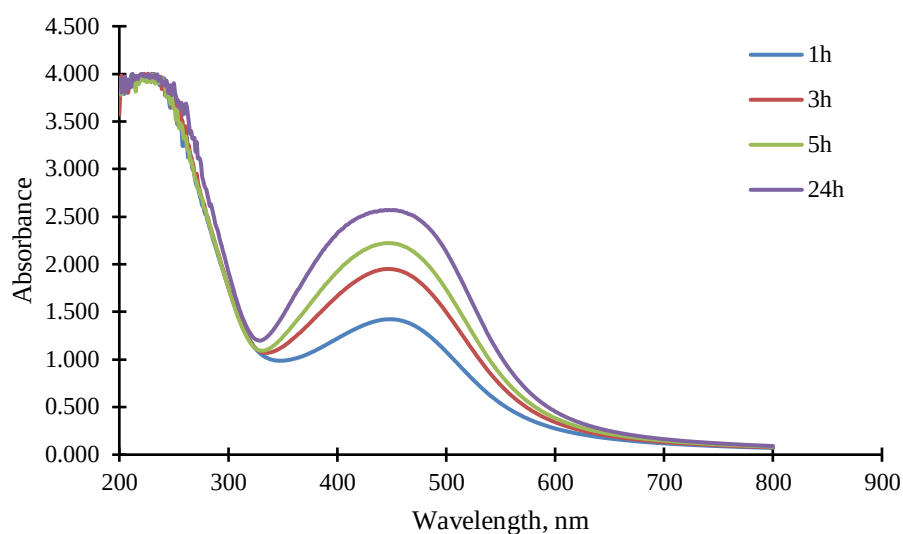


Figure 3.30 UV-Vis spectrum of *C. proliferata* based AgNPs

3.7 Fourier Transform Infrared (FT-IR) Analysis

FT-IR analysis is used to determine the potential biomolecules involved in the reduction. For detecting, the active functional groups responsible for reduction FTIR analysis performed in the range of 400–4000 cm^{-1} .

According to Figure 3.31, FTIR revealed that there are absorptions peaks at 3356 cm^{-1} , 2914 cm^{-1} , 1636 cm^{-1} , 1534 cm^{-1} , 1334 cm^{-1} and 1053 cm^{-1} . The 3356 cm^{-1} peak attributed to OH stretching, 2914 cm^{-1} peak correspond to CH stretching vibrations. 1636 and 1534 cm^{-1} peaks illustrate the presence of the amide I and II bonds (Haiza, 2013). The 1053 cm^{-1} peak can be attributed to C-O-C symmetric stretching and C-O-H bending vibrations. Honey contains fructose, glucose, sucrose, minerals and vitamins. The amide I and II bands originate from carboxyl stretch and N-H

deformation vibrations in the proteins. The COO⁻ (carboxylate ions) groups of amino acids may work for stabilization of the system. (Philip, 2010).

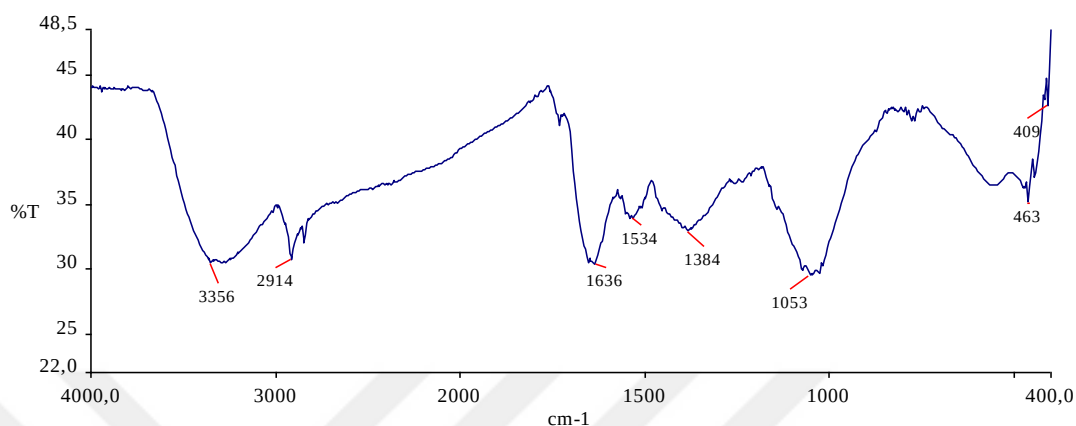


Figure 3.31 FT-IR spectrum of honey based AgNPs

There are FT-IR diagrams as to *C. cylindracea* and *C. cylindracea* based AgNPs for specifying which seconder metabolites responsible for reduction and stabilization. According to Figure 3.32, there are peaks at 3416 cm⁻¹, 1650 cm⁻¹, 1042 cm⁻¹ and 613 cm⁻¹. In the FT-IR spectrum of *C. cylindracea* based AgNPs, the considerable peaks at 3416 cm⁻¹, 3436 cm⁻¹, 2380 cm⁻¹, 1759 cm⁻¹, 1383 cm⁻¹, 1009 cm⁻¹ and 823 cm⁻¹ (Figure 3.33). The peak at the 3416 cm⁻¹ and 3436 cm⁻¹ may be attributed to OH stretching in proteins, polysaccharides or polyphenols (Song, Jang, & Kim 2009). The peaks positioned at 2939 cm⁻¹ and 2967 cm⁻¹ are because of CH stretching of alkanes. A small band at 1629 cm⁻¹ and 1637 cm⁻¹ could be owing to the stretching vibration of the group. The presence of group (NH)=O indicates that the cyclic peptides may have acted as stabilizing agents (Kathiraven et al., 2015). The bands presented at 1042 cm⁻¹ and 1009 cm⁻¹ a credit to C-N vibration of aliphatic amines. Furthermore, *C. cylindracea* based AgNPs indicate sharp peak at 1383 cm⁻¹ and this originates from NO₃⁻ ion in the AgNO₃ solution (Edison, 2016).

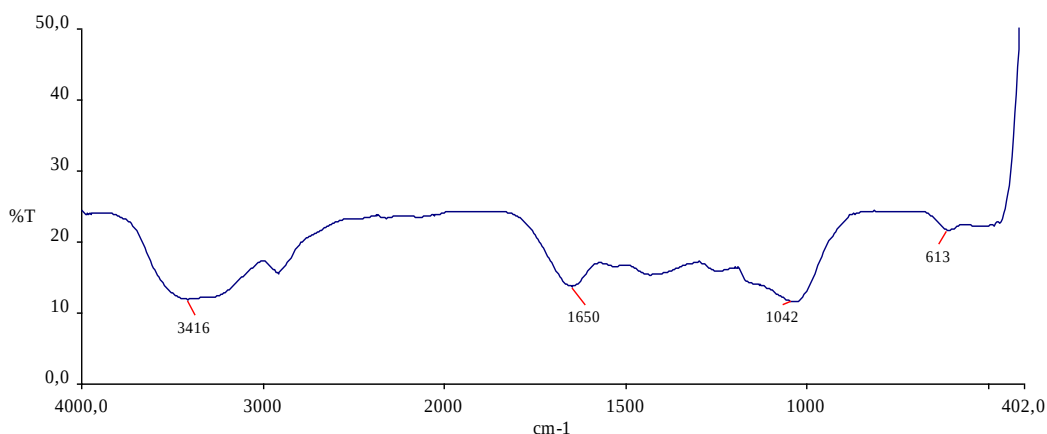


Figure 3.32 FT-IR spectrum of *C. cylindracea*

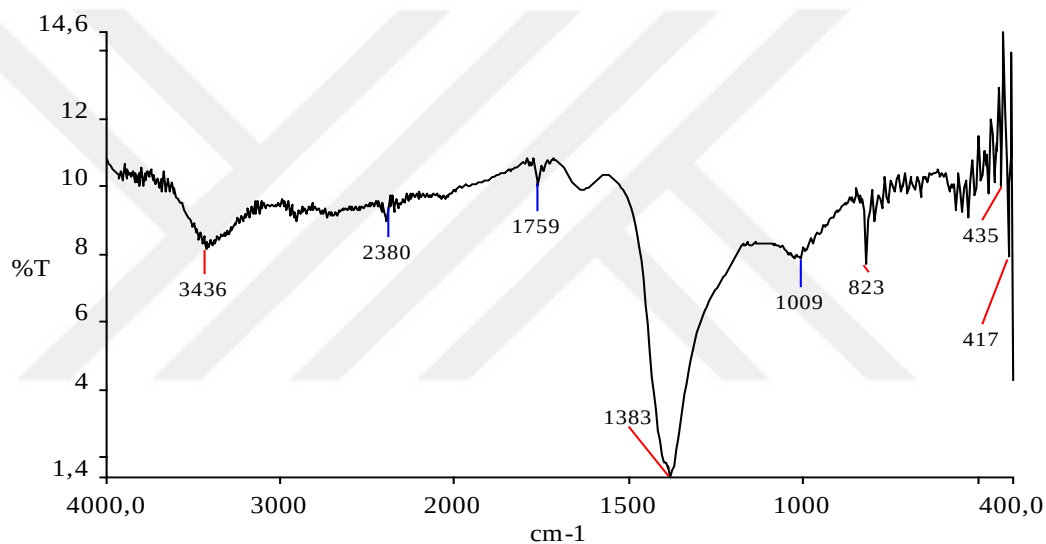


Figure 3.33 FT-IR spectrum of *C. cylindracea* based AgNPs

FT-IR spectrums of *Caulerpa* extract (Figure 3.34) have peaks at 3517 cm⁻¹, 3302 cm⁻¹ and 2940 cm⁻¹ which attributed to stretching vibrations of OH and CH. Moreover, peaks at 1647 cm⁻¹, 1264 cm⁻¹ and 1062 cm⁻¹ originate from the (NH=O), C-O and C-N vibration. According to Figure 3.35, there are peaks at 3421 cm⁻¹ and 2361 cm⁻¹ which arise from stretching vibration of OH and NH. The sharp peak at 1381 cm⁻¹ is characteristic for NO₃⁻ ion found in AgNO₃ solution.

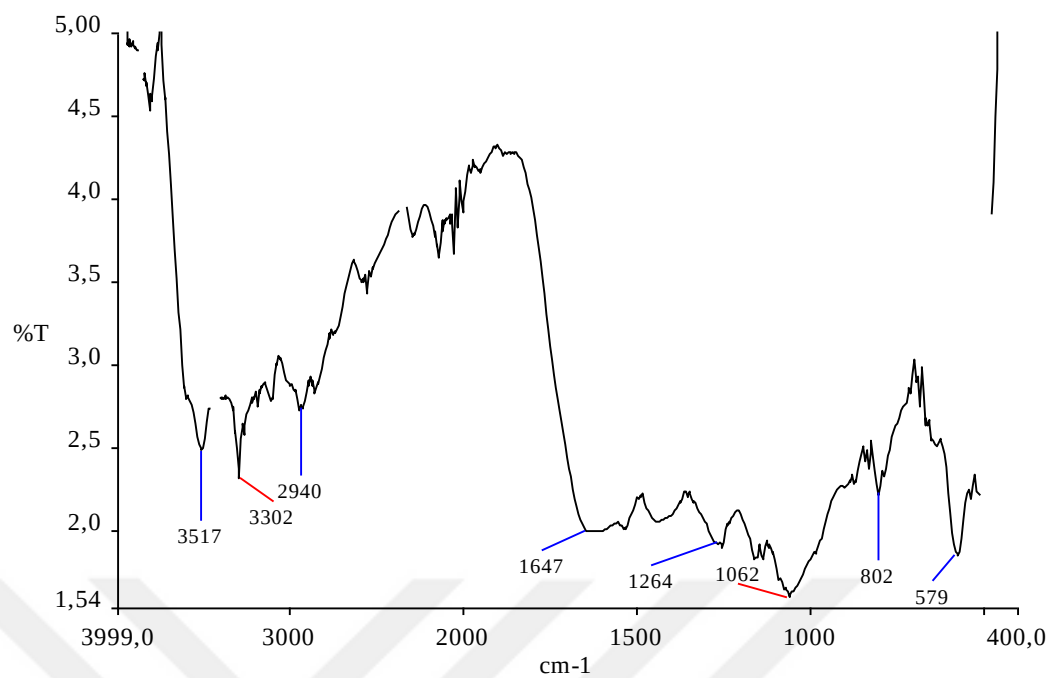


Figure 3.34 FT-IR spectrum of *C. prolifera*

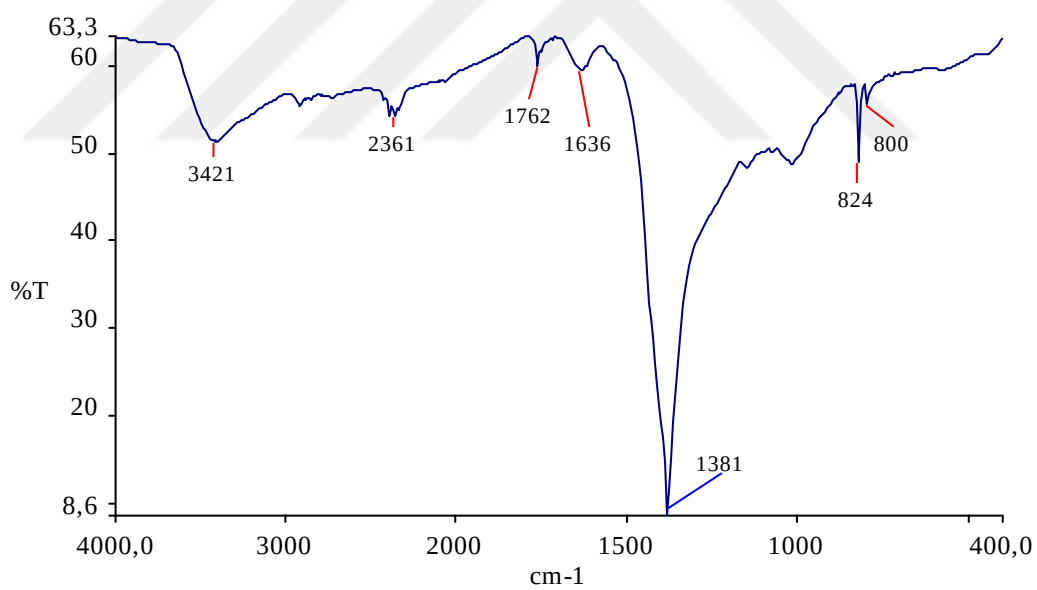


Figure 3.35 FT-IR spectrum of *C. prolifera* based AgNPs

3.8 X-ray Diffraction (XRD) Analysis

Figure 3.36 indicates the XRD pattern of honey based AgNPs without calcination. Since the peak intensity is high, the calcination process was not done to honey based

AgNPs. Diffraction peaks show up at 28.04° , 32.46° and 46.46° . The average size calculated according to the Scherrer equation using peak at 32.46° and found as 16.63 nm.

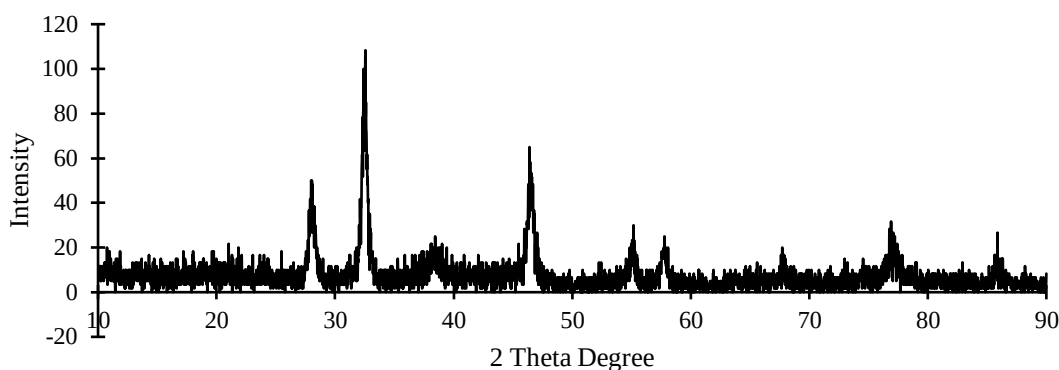


Figure 3.36 XRD spectrum of honey based AgNPs without calcination

Calcination process was done for removing the high noise and augmenting intense of peaks at 400° 2 hours. Figure 3.37 shows XRD spectrum of *C.cylindracea* based AgNPs without calcination process. Diffraction peaks can be seen at 29.72° , 32.08° and 46.06° as consistent with Figure 3.38. Particle size was found as 34.7 nm using full width at half maximum belong to 46.06° with the Scherrer equation.

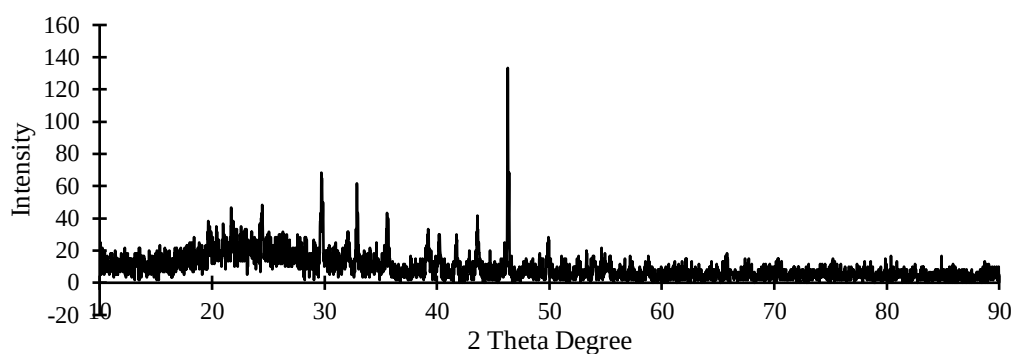


Figure 3.37 XRD spectrum of *C. cylindracea* based AgNPs without calcination

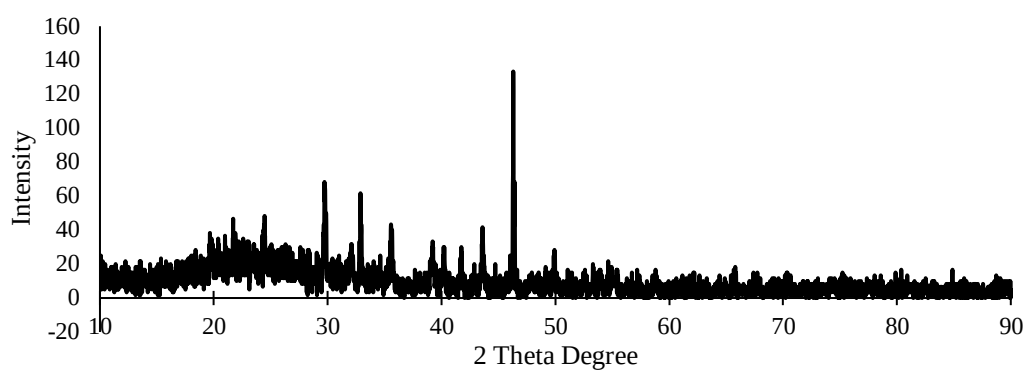


Figure 3.38 XRD spectrum of *C. cylindracea* based AgNPs with calcination

Difference between peaks found in Figure 3.39 and Figure 3.40 originates from the calcination step. The noise in the spectrum pretty decreased after this step. According to Figure 3.43, peaks are found at 38.04° , 44.2° , 64.36° and 77.3° . The size of AgNPs was calculated as 27.45 nm using full width at half maximum belong to 46.06° with the Scherrer equation.

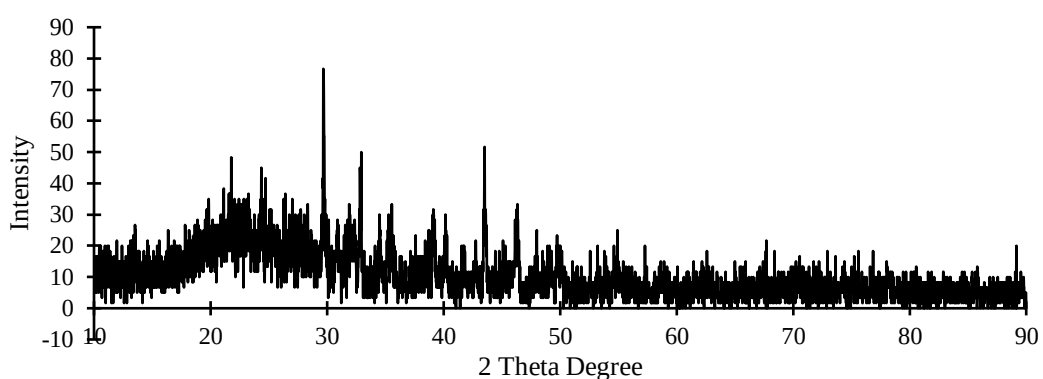


Figure 3.39 XRD spectrum of *C. a proliferata* based AgNPs without calcination

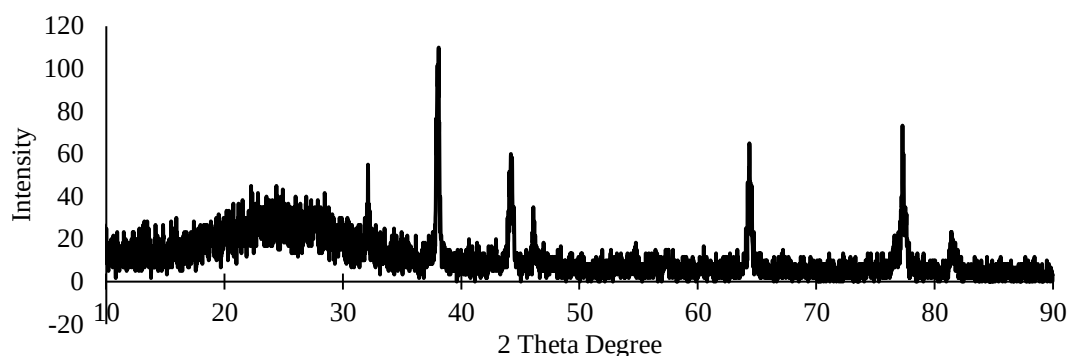


Figure 3.40 XRD spectrum of *C. proliferata* based AgNPs with calcination

XRD pattern of AgNPs that synthesized using the chemical reduction method can be seen in Figure 3.41. The size of AgNPs was calculated as 27.46 nm using full width at half maximum belong to 38.2° with the Scherrer equation. There are peaks at 38.2° , 44.42° , 64.6° and 77.5° . Peaks position accordant with patterns, which belong to AgNPs, synthesized using the biological method. In the light of this report, we can assume that our XRD spectrum belongs to AgNPs.

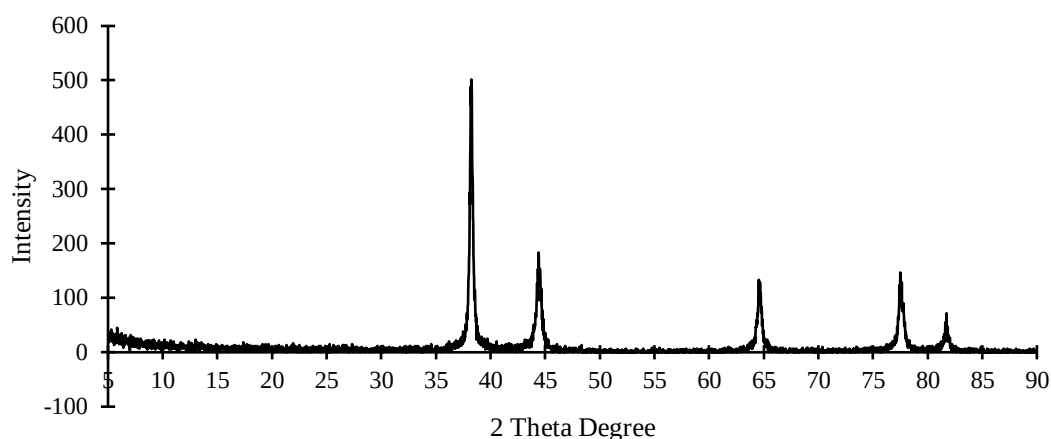


Figure 3.41 XRD spectrum of AgNPs that were synthesized using a chemical reduction method.

If the crystal size of the powder samples is small enough, the max diffraction peak expands inversely proportional to the crystal size and using this additional expansion, the crystal size can be measured (Langford & Wilson, 1978). The particle sizes of the

powder samples can be determined by the Scherrer equation using XRD peaks. Scherrer equation;

$$L = \frac{K\lambda}{\beta \cdot \cos\theta} \quad (3.4)$$

Where K and λ are Scherrer shape factor, θ is Bragg angle and β is full width at half maximum. K varies from 0.62 to 2.08. In our calculation, K and λ were taken as 0.94, 1.45178 Å respectively.

3.9 Scanning Electron Microscopy (SEM) Analysis

SEM analysis was performed due to determine shape, size and morphology of synthesized AgNPs. AgNPs size were found about micrometre level (Figure 3.42). Reason of this situation can be explained with aggregation of AgNPs owing to lack of capping agents found in *Caulerpa* species. Seasonal fluctuations in the level of seconder metabolites and carbohydrates for *Caulerpa* species have been reported (Cavas, Cengiz, Karabay, 2012). Since we worked in the solid state, nanoparticles must have been aggregated after drying process.

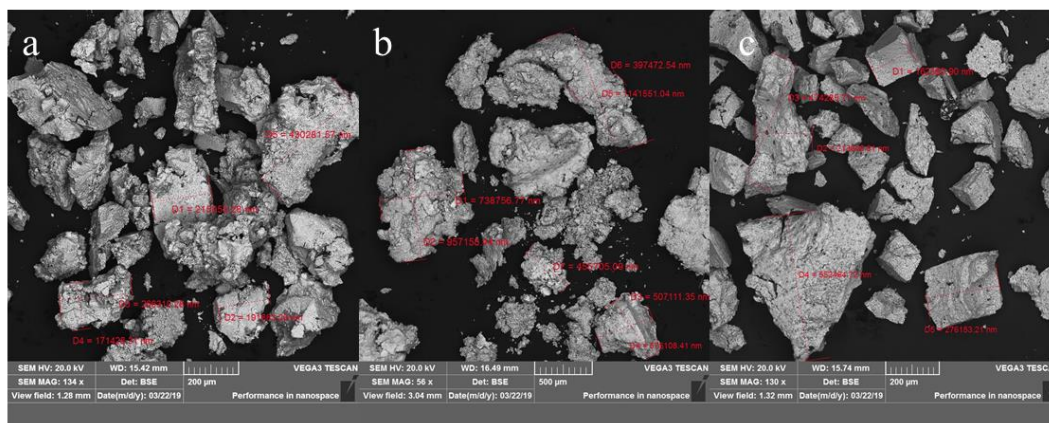


Figure 3.42 SEM images of AgNPs a) *C.cylindracea* based b) *C. prolifera* based c) honey based

As seen in Table 3.1, there are different elements as C, N, O, Mg, Al, Si, P, S, Cl which originate from extracts and of Ag shows synthesized AgNPs. Elemental

composition analysis of AgNPs according to the reduction agent source can be seen in Figure 3.43-45.

Table 3.1 Results of composition analysis for *C. cylindracea*, *C. prolifera* and honey based AgNPs

	Percentages of Elements										
	C	N	O	Na	Mg	Al	Si	P	S	Cl	Ag
<i>C.cylindracea</i>	2.98	5.11	11.39	0.28	-	0.14	0.33	0.24	0.14	5.06	74.33
<i>C.prolifera</i>	5.55	5.88	13.48	0.28	0.13	0.20	0.19	0.15	0.26	2.52	71.35
Honey	26.31	2.85	5.52	-	-	0.09	1.15	0.20	0.50	12.22	51.17

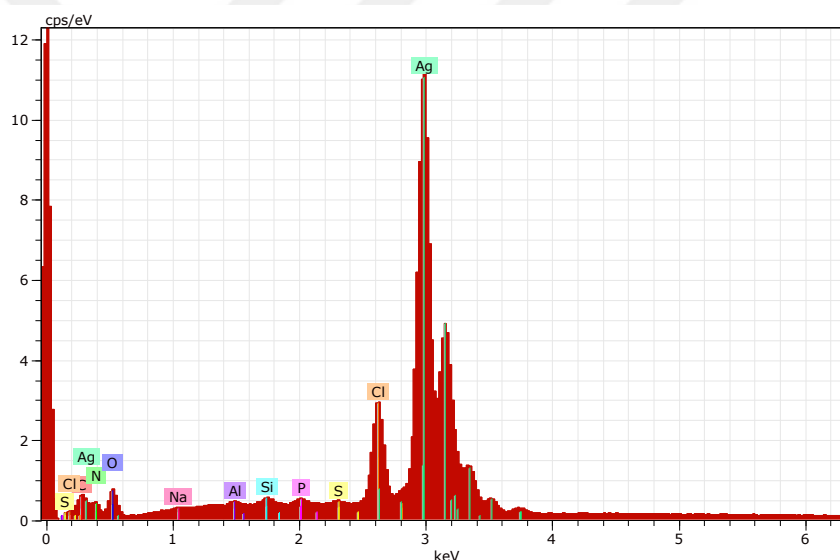


Figure 3.43 Elemental composition analysis of *C. cylindracea* based AgNPs

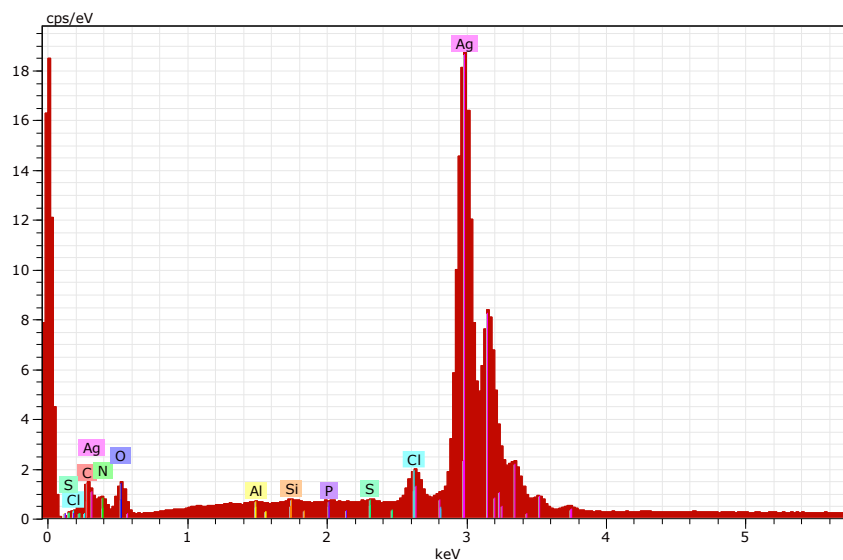


Figure 3.44 Elemental composition analysis of *C. proliferata* based AgNPs

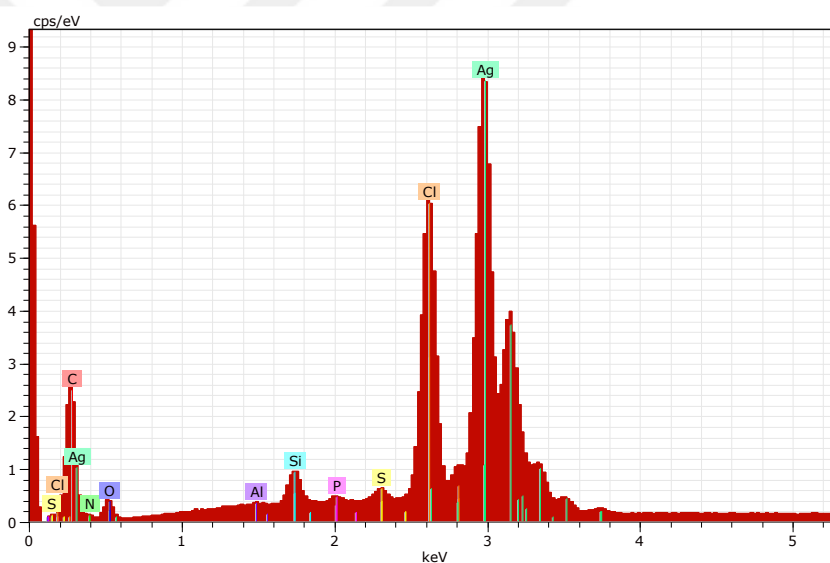


Figure 3.45 Elemental composition analysis of honey based AgNPs

3.10 Antifouling Performance

The field tests were performed to evaluate the antifouling effects of AgNPs from September 24 to October 26, 2018, at Levent Marina, İzmir, Turkey. Antifouling paint ingredient can be seen in Table 3.2.

Table 3.2 Paint composition of prepared rosin based antifouling paint

Paint Composition	Percent by weight (w/w%)
Rosin	23
Xylene	40
Lutanol M40	2
Cu ₂ O	34
AgNP	1

Many criteria have been specified in the literature to determine the performance of antifouling paints (Arimura, Hiyoshi, Nakamura & Tsuboi, 2004; Okimoto, Mukunoki, Ashida, Ono, 2005; Karabay, 2011). Under the light of these papers fouling criteria consist of 5 levels.

- ✓ Level I. There are no fouling organisms (micro- or macro-) on the surface.
- ✓ Level II. There is microfilm layer on the surface.
- ✓ Level III. There are the microfilm layer and the beginning of macrofilm layer on the surface.
- ✓ Level IV. There are microfilm layer, macrofilm layer and crustaceans at the beginning state on the surface.
- ✓ Level V. More than %50 of the surface is covered by macrofouling organisms.

Table 3.3 The evaluation of fouling levels on the surface of plates covered by antifouling paint containing AgNP

Plate No	Fouling Level	Plate No	Fouling Level
1	IV	1'	IV
2	III	2'	III
3	III	3'	III
4	III	4'	III
5	III	5'	IV
6	III	6'	III
7	IV	7'	IV
8	III	8'	III
9	IV	9'	IV
10	III	10'	III
11	III	11'	III
12	III	12'	III
13	III	13'	III
14	III	14'	III
15	IV	15'	IV
16	II	16'	II
17	II	17'	II
18	II	18'	II
19	II	19'	II
20	II	20'	II
21	II	21'	II
22	II	22'	II
23	II	23'	II

Table 3.3 shows the evaluation of fouling levels on the surface of plates covered by antifouling paint containing AgNP. As seen in Figure 3.46, all plates that painted with antifouling paint were non-homogenously covered with micro slime layer. Organisms attached to test panels were barnacles (*Balanus amphitrite*), bryozoans (*Bugula neritina*) and serpulidae (*Hydroides elegans*). The uncoated plate was highly covered by macro-fouling organisms (Figure 3.46, plate 1). The best antifouling performance is seen in AgNPs and copper oxide contain antifouling paint.

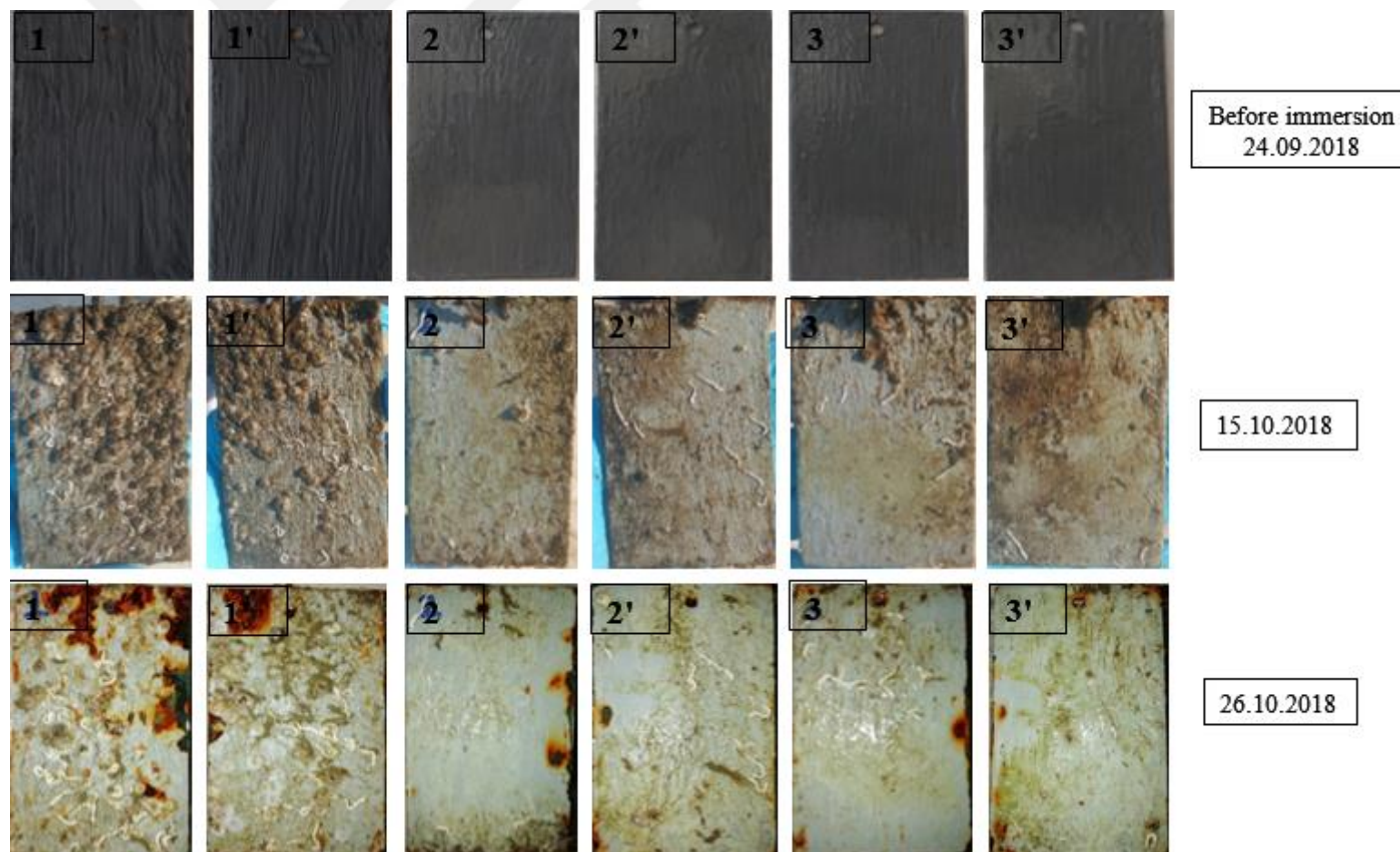


Figure 3.46 AgNPs used antifouling paint coated plates at different time intervals (Plate 1 coated with just primer)

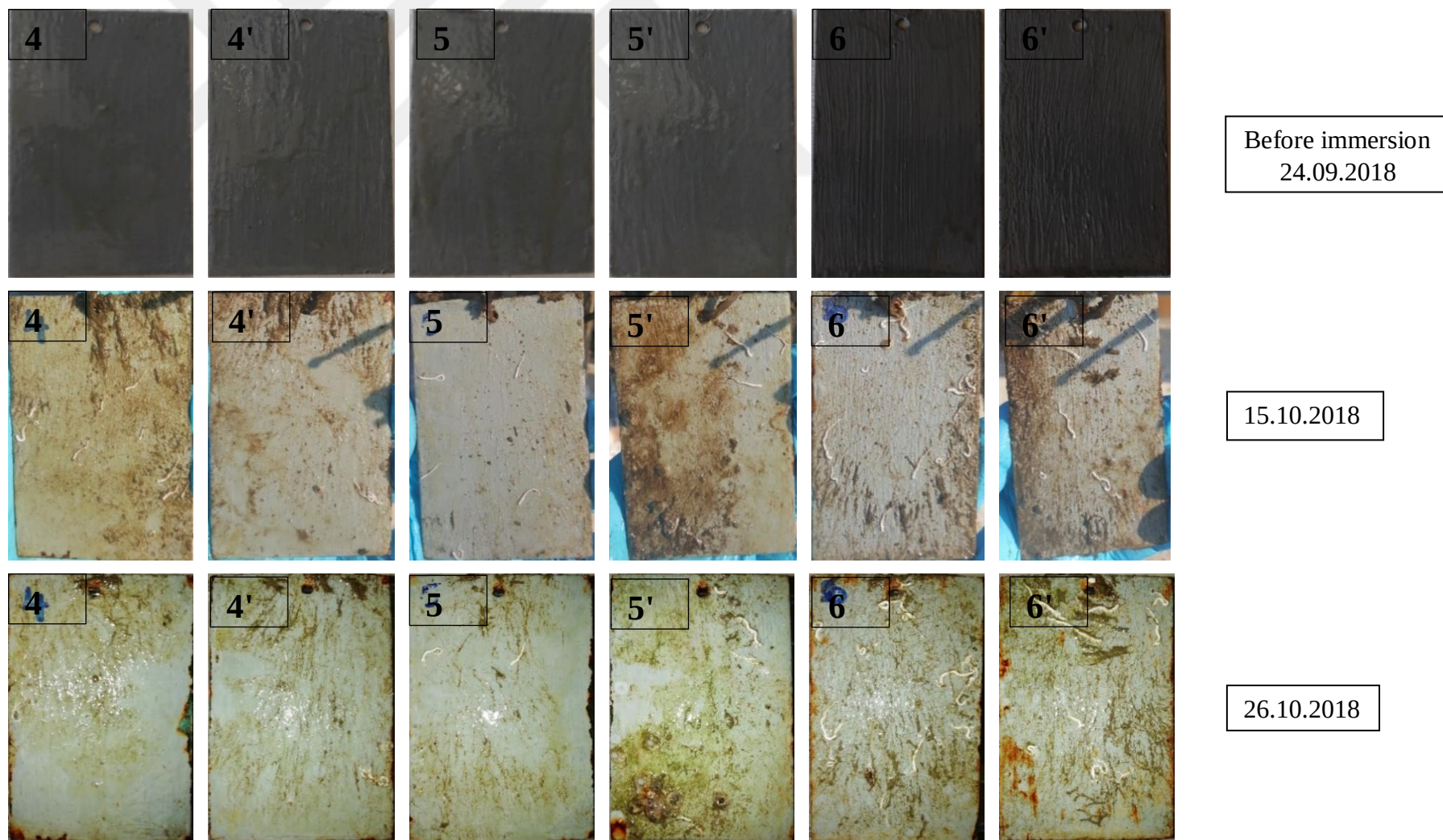


Figure 3.46 continues

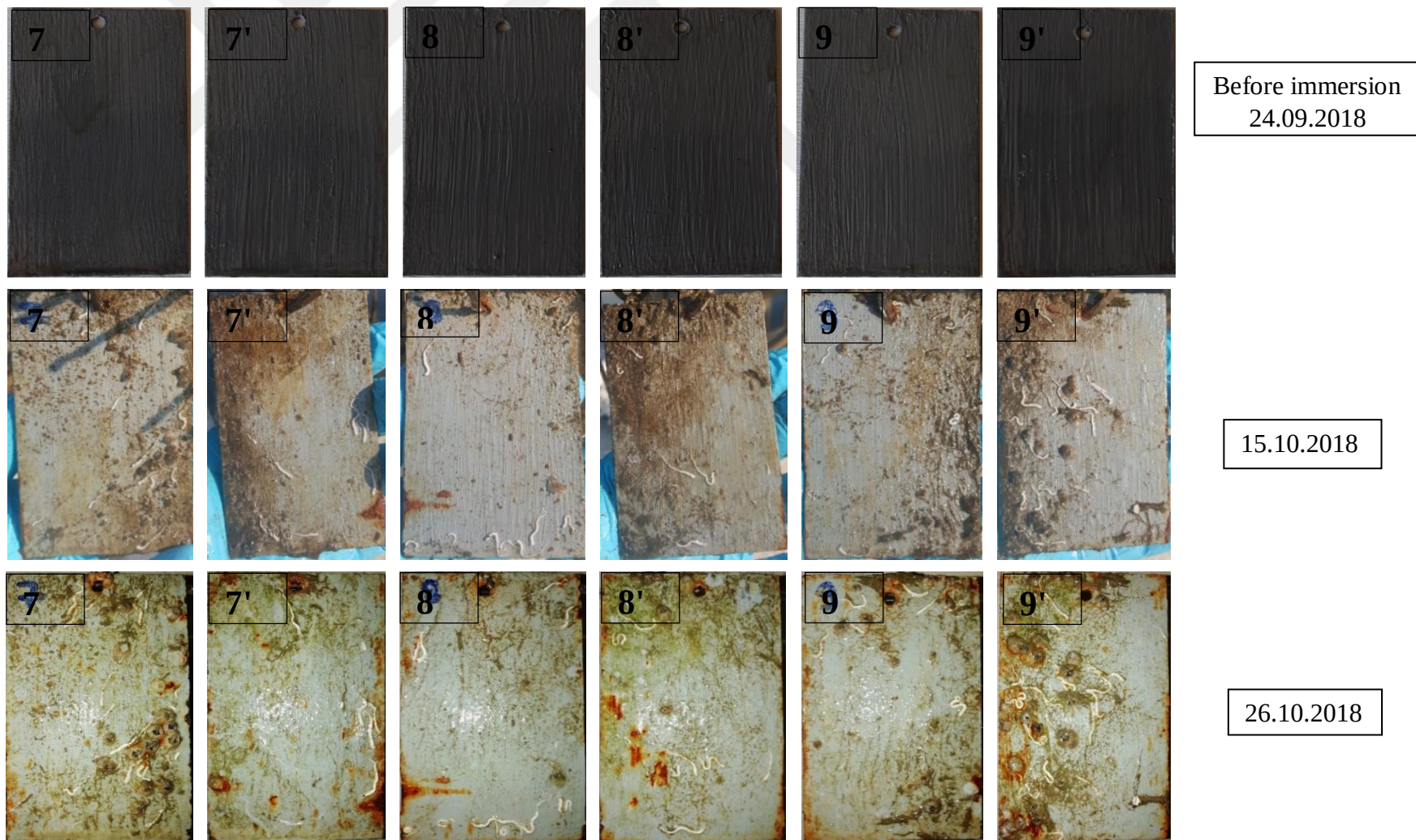


Figure 3.46 continues

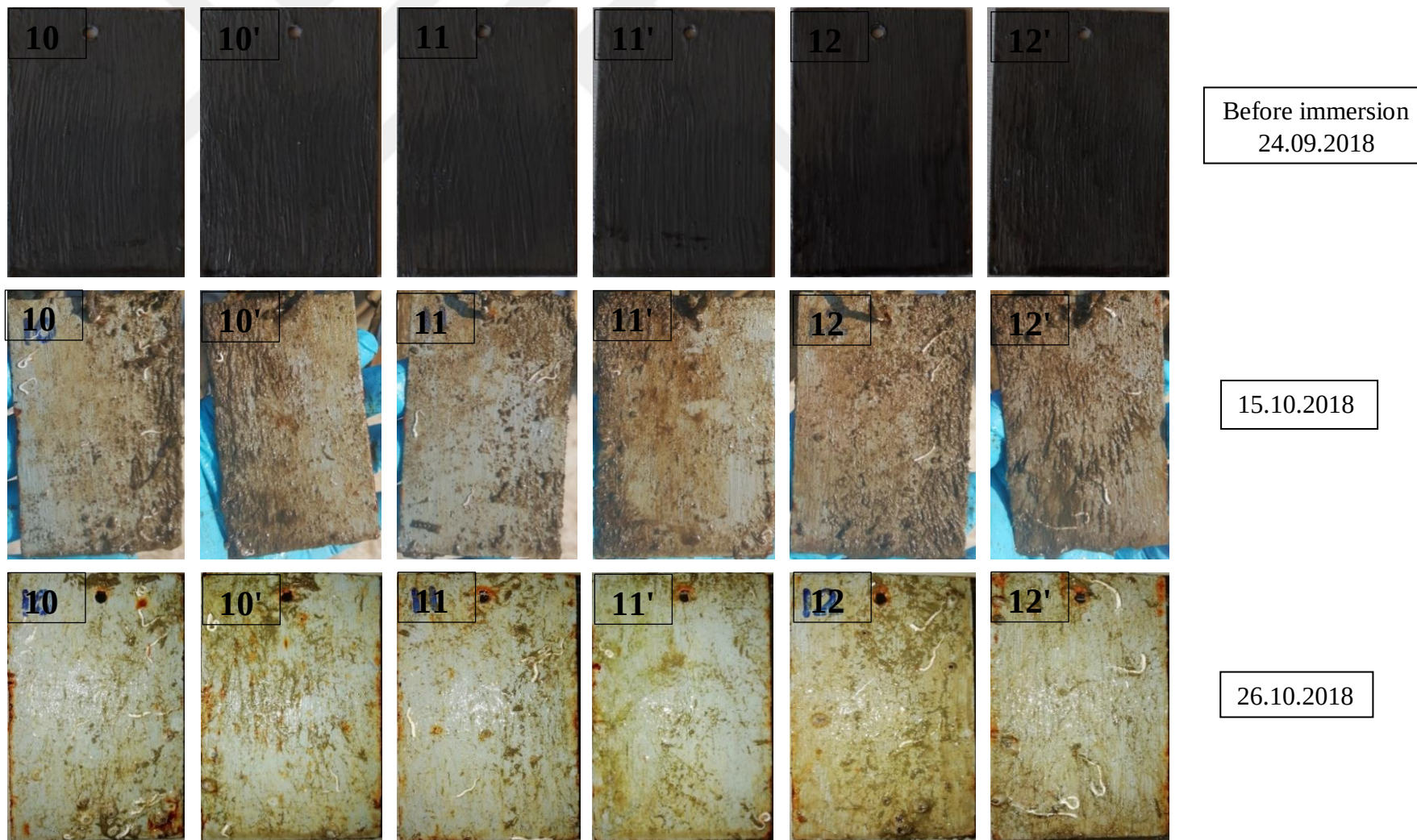


Figure 3.46 continues

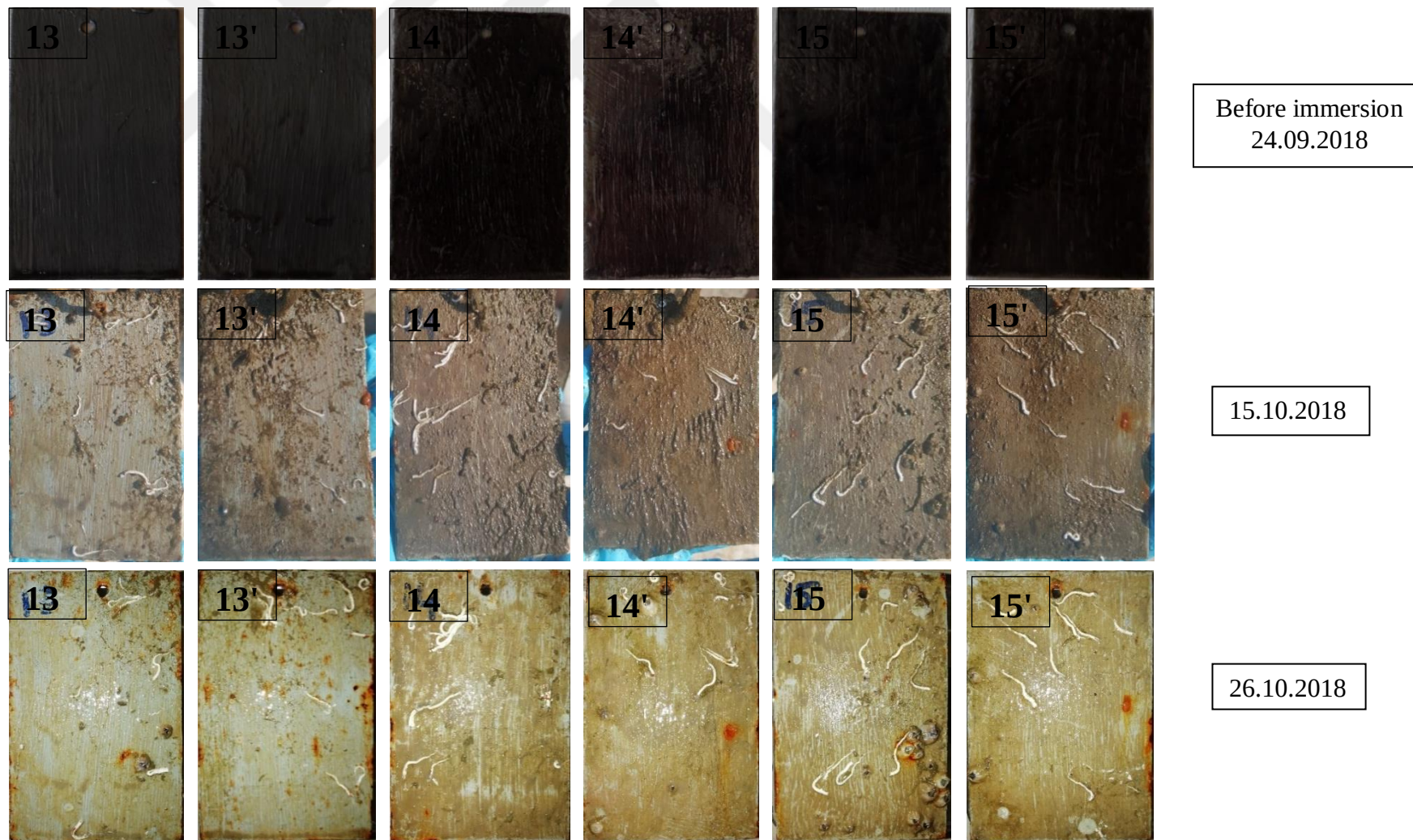


Figure 3.46 continues

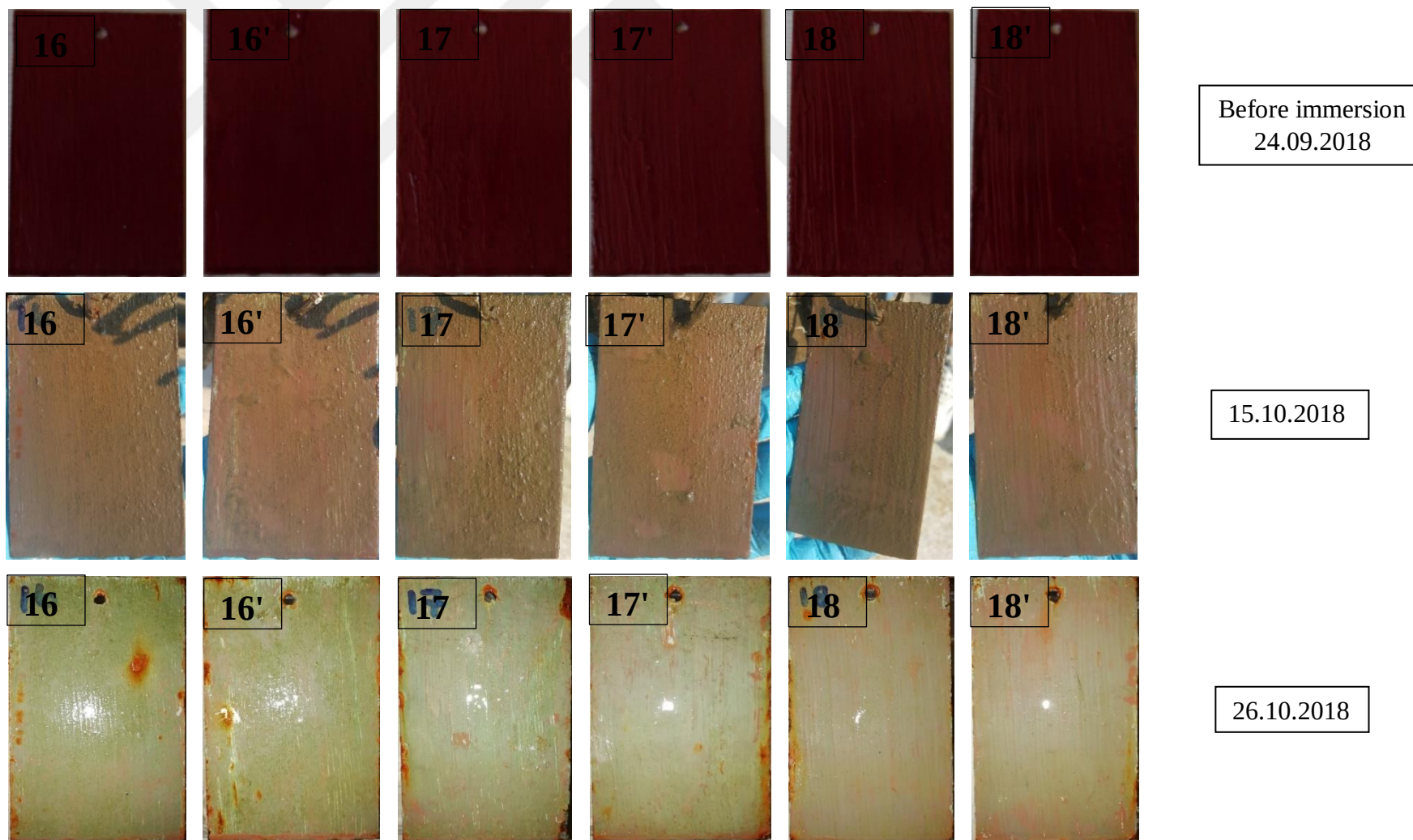


Figure 3.46 continues

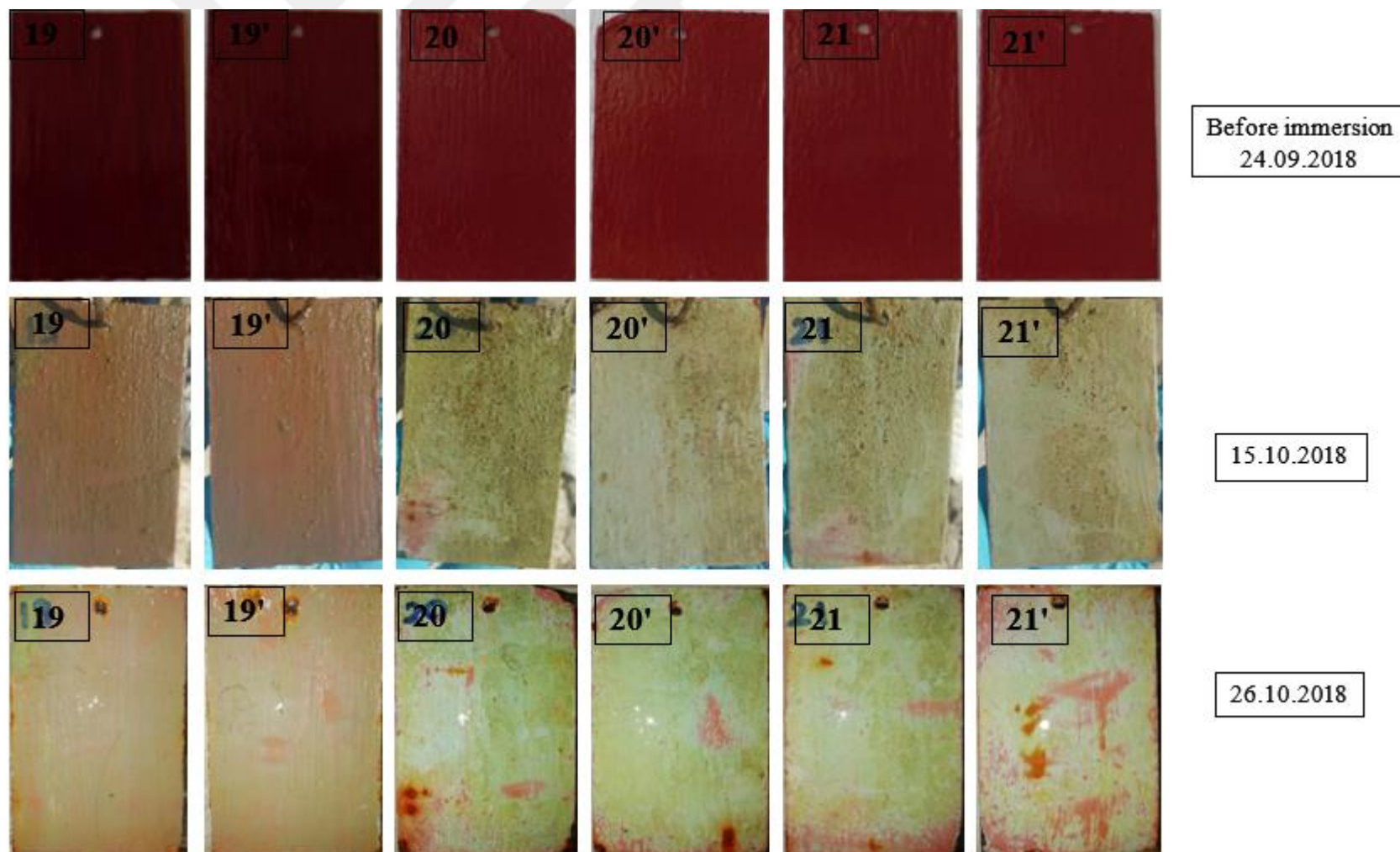


Figure 3.46 continues

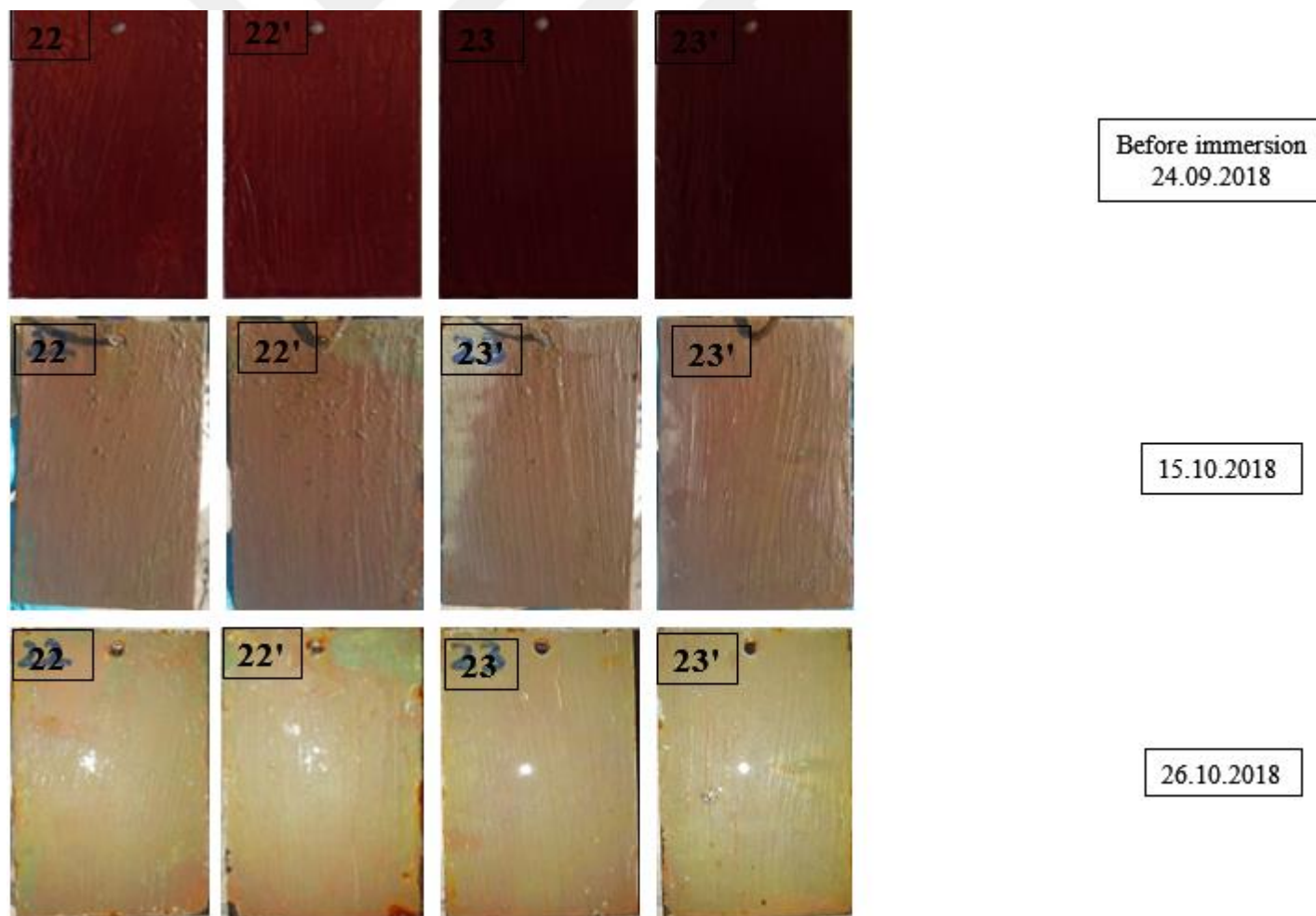


Figure 3.46 continues

3.11 Artificial Neural Network

3.11.1 Artificial Neural Network Study for Honey Based AgNPs

Green synthesis of AgNPs was carried by using honey solution. During the optimization process, the experimental data related to agitation time, agitation rate, temperature, pH, honey and AgNO₃ concentration were collected. Table 3.4 shows the data statistics of model variables. This data was used to create an artificial neural network by MATLAB. According to the results, the optimum hidden neuron number was found as 40 when default settings (the percentages of training, validation and testing are 70, 15 and 15%, respectively, Levenberg-Marquardt backpropagation algorithm) were applied. The R² values of training, validation and testing are 0.9967, 0.9887 and 0.9792, respectively, when the hidden neuron number was 40 (Table 3.5).

Table 3.4 The data statistics of model variables

Variables	Data Statistics Range	Mean ± S.D.
Agitation Time (min)	0-360	158 ± 118.61
Agitation Rate (rpm)	0-400	175 ± 147.90
Temperature (°C)	25-85	55 ± 22.36
pH	3-9	6.94 ± 1.76
AgNO ₃ Concentration (mol.L ⁻¹)	0.001-0.1	0.03 ± 0.03
Honey Concentration (g.mL ⁻¹)	0.1-0.75	0.4 ± 0.25
Absorbance	0.240-1.057	0.448±0.247

Table 3.5 Effects of hidden neuron number on the ANN performance

70-15-15 (%)	R ²				MSE			
Hidden Neuron Number	Training	Validation	Testing	All	Training	Validation	Testing	Iteration
1	0.7001	0.9572	0.6382	0.6863	0.0628	0.0107	0.1403	10
2	0.5142	0.2584	0.4152	0.4644	0.0886	0.0392	0.1668	8
3	0.9906	0.9950	0.9845	0.9103	0.0020	0.0019	0.0018	79
4	0.9954	0.9949	0.9982	0.9948	0.0008	0.0046	0.0016	196
5	0.9746	0.9948	0.9843	0.9980	0.0054	0.0031	0.0019	23
6	0.9943	0.9853	0.9897	0.9927	0.0016	0.0028	0.0010	53
7	0.9950	0.9927	0.9971	0.9953	0.0012	0.0006	0.0010	61
8	0.9351	0.9372	0.9680	0.9431	0.0142	0.0042	0.0183	15
9	0.9905	0.9986	0.9755	0.9914	0.0022	0.0006	0.0025	33
10	0.9947	0.9792	0.9788	0.9894	0.0014	0.0083	0.0018	42
11	0.9968	0.9965	0.7557	0.8567	0.0007	0.0017	0.3432	160
12	0.9202	0.7487	0.3525	0.7756	0.0149	0.1731	0.1835	9
13	0.9945	0.9950	0.4180	0.7227	0.0010	0.0042	0.7191	42
14	0.9873	0.9813	0.9885	0.9859	0.0020	0.0082	0.0045	21
15	0.9678	0.9837	0.9694	0.9681	0.0071	0.0022	0.0133	17
16	0.9937	0.9851	0.9953	0.9931	0.0013	0.0019	0.0038	31
17	0.9125	0.9646	0.9232	0.9279	0.0153	0.0012	0.0305	11
18	0.9810	0.9763	0.9690	0.9795	0.0057	0.0021	0.0035	14
19	0.8109	0.0249	0.4159	0.6260	0.0680	0.4164	0.0479	8
20	0.9978	0.9896	0.9142	0.9748	0.0005	0.0047	0.0325	52

Table 3.5 continues

70-15-15 (%)	R ²				MSE			
Hidden Neuron Number	Training	Validation	Testing	All	Training	Validation	Testing	Iteration
21	0.9917	0.9916	0.9979	0.9931	0.0013	0.0007	0.0059	37
22	0.9070	0.2592	0.8016	0.7839	0.0272	0.2396	0.0143	8
23	0.9962	0.9972	0.9934	0.9953	0.0006	0.0053	0.0009	70
24	0.9964	0.9971	0.8188	0.9080	0.0008	0.0029	0.1659	95
25	0.7828	0.8169	0.7679	0.7432	0.0349	0.0230	0.1998	8
26	0.9622	0.9956	0.9553	0.9747	0.0060	0.0028	0.0078	13
27	0.9695	0.8872	0.9862	0.9649	0.0087	0.0109	0.0018	11
28	0.9977	0.9872	0.9958	0.9956	0.0004	0.0025	0.0027	78
29	0.9492	0.9713	0.9893	0.9596	0.0115	0.0033	0.0040	9
30	0.9919	0.7925	0.8275	0.9632	0.0026	0.0211	0.0262	16
31	0.9795	0.9796	0.9901	0.9810	0.0050	0.0026	0.0035	11
32	0.9687	0.9894	0.9810	0.9724	0.0072	0.0055	0.0033	10
33	0.9534	0.9732	0.9651	0.9537	0.0114	0.0137	0.0060	10
34	0.9941	0.9936	0.2965	0.9477	0.0009	0.0024	0.0751	41
35	0.9930	0.9758	0.8909	0.9835	0.0016	0.0090	0.0092	36
36	0.9966	0.9811	0.9816	0.9944	0.0009	0.0006	0.0037	84
37	0.9952	0.9980	0.9950	0.9947	0.0008	0.0015	0.0043	34
38	0.9914	0.9033	0.7595	0.9731	0.0024	0.0152	0.0154	14
39	0.9514	0.9341	0.3596	0.6179	0.0120	0.0016	101.86	9
40	0.9967	0.9887	0.9792	0.9945	0.0009	0.0007	0.0037	41

Table 3.6 Effects of data percentage on the performance of ANN Modelling

Hidden Neuron Number	Training, Validation, Testing (%)	R ²				MSE			Iteration
		Training	Validation	Testing	All	Training	Validation	Testing	
40	90-5-5	0.9929	0.9799	0.9237	0.9928	0.0018	0.0004	0.0009	34
40	85-5-10	0.9886	0.9991	0.9968	0.9907	0.0022	0.0054	0.0003	21
40	80-5-15	0.9865	0.9806	0.9859	0.9862	0.0027	0.0002	0.0076	13
40	75-5-20	0.9332	0.9973	0.9862	0.9566	0.0126	0.0026	0.0040	9
40	70-5-25	0.9915	0.9796	0.9825	0.9875	0.0018	0.0021	0.0063	15
40	65-5-30	0.4885	0.6464	0.5712	0.5256	0.0038	0.4053	0.1419	8
40	60-5-35	0.9897	0.9982	0.4769	0.8220	0.0025	0.0014	0.1449	19
40	85-10-5	0.9711	0.9812	0.9130	0.9697	0.0073	0.0012	0.0116	14
40	80-10-10	0.9927	0.9963	0.9525	0.9923	0.0020	0.0005	0.0019	45
40	75-10-15	0.9964	0.9881	0.9991	0.9949	0.0005	0.0065	0.0107	57
40	70-10-20	0.5703	0.4884	0.4819	0.5281	0.1312	0.4300	0.2730	7
40	65-10-25	0.9737	0.9858	0.9879	0.9808	0.0043	0.0101	0.0034	15
40	60-10-30	0.9913	0.9925	0.9949	0.9932	0.0015	0.0006	0.0020	29
40	55-10-35	0.9818	0.9997	0.4230	0.6662	0.0035	0.0027	0.3594	15
40	80-15-5	0.9808	0.8709	0.9327	0.9611	0.0048	0.0314	0.0155	10
40	75-15-10	0.9881	0.9662	0.9918	0.9854	0.0023	0.0067	0.0064	19
40	70-15-15	0.9911	0.9374	0.9688	0.9876	0.0025	0.0032	0.0042	18
40	65-15-20	0.9812	0.9959	0.7678	0.9131	0.0045	0.0042	0.0909	15
40	60-15-25	0.9930	0.8301	0.7088	0.9034	0.0016	0.0717	0.0458	13
40	55-15-30	0.7167	0.3326	0.5943	0.6129	0.1195	0.1641	0.1589	7

Table 3.6 continues

Hidden Neuron Number	Training, Validation, Testing (%)	R ²				MSE			Iteration
		Training	Validation	Testing	Training, Validation, Testing (%)	Training	Validation	Testing	
40	50-15-35	0.9758	0.7711	0.8001	0.9203	0.0084	0.0380	0.0240	10
40	75-20-5	0.9960	0.9955	0.9118	0.9957	0.0011	0.0007	0.0009	85
40	70-20-10	0.9857	0.9967	0.7974	0.9883	0.0034	0.0009	0.0010	21
40	65-20-15	0.9977	0.9880	0.9848	0.9956	0.0007	0.0020	0.0012	68
40	60-20-20	0.9568	0.6350	0.2588	0.5979	0.0014	0.1077	0.8010	8
40	55-20-25	0.9973	0.9979	0.4563	0.7454	0.0004	0.0047	0.3313	32
40	50-20-30	0.9631	0.7595	0.9248	0.9390	0.0162	0.0230	0.0087	10
40	45-20-35	0.9962	0.9906	0.9764	0.9909	0.0011	0.0033	0.0029	7
40	70-25-5	0.7357	0.5595	0.8783	0.6597	0.0500	0.1979	0.0344	7
40	65-25-10	0.9880	0.9434	0.9136	0.9786	0.0036	0.0060	0.0109	18
40	60-25-15	0.9974	0.9822	0.9960	0.9928	0.0003	0.0037	0.0072	78
40	55-25-20	0.9662	0.3428	0.3066	0.6242	0.0111	0.2891	0.3534	8
40	50-25-25	0.9895	0.9918	0.9871	0.9887	0.0020	0.0036	0.0037	27
40	45-25-30	0.9685	0.9008	0.3190	0.4897	0.0036	0.0279	0.5218	20
40	40-25-35	0.9955	0.7600	0.6169	0.8325	0.0014	0.0749	0.0749	11
40	65-30-5	0.8346	0.2532	0.9506	0.6266	0.1055	0.5586	0.1048	7
40	60-30-10	0.9662	0.7982	0.9830	0.9562	0.0069	0.0142	0.0160	10
40	55-30-15	0.9804	0.2578	0.2350	0.4995	0.0067	0.4798	0.9202	9
40	50-30-20	0.8891	0.6451	0.7520	0.7402	0.0020	0.2131	0.6681	7
40	45-30-25	0.9833	0.9910	0.9587	0.9814	0.0041	0.0054	0.0042	11

Table 3.6 continues

		R ²				MSE			
Hidden Neuron Number	Training, Validation, Testing (%)	Training	Validation	Testing	All	Training	Validation	Testing	Iteration
40	40-30-30	0.9504	0.8585	0.3213	0.6506	0.0229	0.0339	0.2501	8
40	35-30-35	0.9799	0.2391	0.1247	0.2576	0.0022	0.3115	0.4572	19
40	60-35-5	0.9158	0.6529	0.4389	0.7563	0.0043	0.1807	0.5919	8
40	55-35-10	0.9921	0.7094	0.9324	0.8122	0.0014	0.1371	0.0440	23
40	50-35-15	0.9844	0.5447	0.6716	0.6879	0.0016	0.1254	0.1201	12
40	45-35-20	0.9414	0.4985	0.5728	0.6213	0.0132	0.1450	0.2221	8
40	40-35-25	0.9858	0.3318	0.5545	0.6680	0.0076	0.1451	0.1795	10
40	35-35-30	0.9346	0.7885	0.8220	0.8349	0.0216	0.0600	0.0617	8
40	30-35-35	0.99834	0.2397	0.4753	0.4189	0.0001	0.2601	0.1506	24

Table 3.7 Performances of different backpropagation algorithms on the prediction of experimental values

		ANN Algorithms						
#	Experimental	Trainbfg	Trainbr	Traincgb	Traincgf	Traincgp	Traingd	Traingdm
1	0.273	0.195	0.353	0.445	0.473	0.227	0.309	0.209
2	0.395	0.195	0.381	0.346	0.461	0.220	0.358	0.208
3	0.400	0.195	0.566	0.210	0.470	0.231	0.544	0.312
4	0.321	0.195	0.533	0.250	0.421	0.216	0.350	0.276
5	0.385	0.195	0.418	0.208	0.566	0.212	0.408	0.212
6	0.238	0.195	0.335	0.196	0.362	0.210	0.290	0.220
7	0.262	0.195	0.405	0.202	0.334	0.217	0.300	0.211
8	0.255	0.195	0.427	0.207	0.327	0.224	0.314	0.209
9	1.171	0.195	0.412	0.200	0.425	0.201	0.650	0.372
10	0.859	0.195	0.602	0.195	0.288	0.219	0.475	1.269
11	0.744	0.195	0.956	0.195	0.241	0.550	0.714	1.907
12	0.278	0.195	0.391	0.205	0.442	0.201	0.638	0.329
13	0.432	0.195	0.622	0.709	0.375	0.327	0.513	1.444
14	0.356	0.195	0.732	0.914	0.395	0.208	0.743	0.267
15	0.402	0.195	1.006	1.891	0.318	0.594	0.598	0.212

Table 3.8 Performances of different backpropagation algorithms on the prediction of experimental values

		ANN Algorithms						
#	Experimental	Traingda	Traingdx	Trainlm	Trainoss	Trainr	Trainrp	Trainscg
16	0.273	0.655	0.657	0.308	0.195	0.356	0.220	0.313
17	0.395	0.459	0.619	0.334	0.195	0.397	0.233	0.319
18	0.400	0.451	0.699	0.364	0.195	0.419	0.488	0.510
19	0.321	0.524	0.775	0.365	0.195	0.464	0.342	0.392
20	0.385	0.427	0.423	0.382	0.195	0.428	0.298	0.384
21	0.238	0.294	0.254	0.251	0.195	0.288	0.286	0.339
22	0.262	0.247	0.248	0.240	0.195	0.220	0.236	0.340
23	0.255	0.246	0.243	0.253	0.195	0.214	0.243	0.352
24	1.171	0.410	0.405	1.057	0.195	0.784	0.636	0.718
25	0.859	0.694	0.606	0.906	0.195	0.998	0.900	0.916
26	0.744	1.008	0.988	0.721	0.195	0.749	1.214	1.053
27	0.278	0.385	0.389	0.282	0.195	0.715	0.512	0.674
28	0.432	0.865	0.720	0.436	0.195	0.435	0.444	0.435
29	0.356	0.519	0.513	0.364	0.195	0.752	0.531	0.632
30	0.402	0.772	1.578	0.399	2.145	1.940	0.683	2.113

In the present study, the effects of data percentages were also studied. The highest R^2 values were found as 70, 20 and 5% for training, validation and testing, respectively (Table 3.6).

After creating the network, we also studied the performances of the 11 different backpropagation algorithms. Levenberg-Marquardt backpropagation algorithm gave the best results (Table 3.7-8).

In order to compare the experimental and ANN predicted values, Figure 3.47 was drawn. This figure showed that the created ANN model can estimate the experimental values precisely. On the other hand, other backpropagation algorithms did not give promising results.

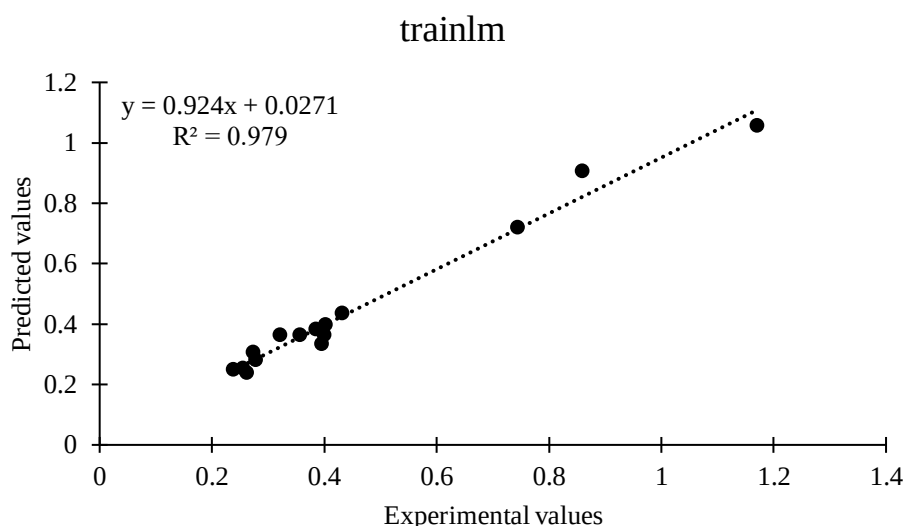


Figure 3.47 Comparison of experimental values with ANN predicted values (Trainlm: Levenberg–Marquardt backpropagation)

3.11.2 Artificial Neural Network Study for *C. cylindracea* Based AgNPs

C. cylindracea extract was used for green synthesis of AgNPs and during the synthesis parameters like agitation time, agitation rate, temperature, pH and concentration of AgNO_3 and extract were studied and experiments data were collected.

Table 3.9 shows the data statistics of model variables. The data was used for creating an artificial neural network by MATLAB.

After a study of MATLAB, the optimum hidden number was found as 17 when default settings (the percentages of training, validation and testing are 70, 15 and 15%, respectively, Levenberg-Marquardt backpropagation algorithm) were applied (Table 3.10). When the hidden number was 17, the R^2 values as to training, validation and testing was found as a 0.994327, 0.997549 and 0.991847 respectively. Also, the effects of data percentages were studied and the highest R^2 values were found as a 0.994195, 0.999989 and 0.999664 when percentages of training, validation and testing were 85, 5 and 10, respectively (Table 3.11). The performance of the 11 backpropagation algorithms was also studied (Table 3.12-13). The R^2 values of 3 of backpropagation algorithms were close to each other and these algorithms were Bayesian regularization backpropagation, scaled conjugate gradient backpropagation and Levenberg-Marquardt backpropagation algorithms. The R^2 values of this backpropagations were found as 0.9921, 0.9919 and 0.9918, respectively. In an attempt to compare the experimental and ANN predicted values Figure 3.48-50 were drawn. These tables show that the created ANN model can estimate the experimental values precisely but the other backpropagation algorithms were not give promising results for this work.

Table 3.9 The data statistics of model variables

Variables	Data Statistics Range	Mean $\pm$ S.D.
Agitation Time (min)	0-360	158 $\pm$ 118.61
Agitation Rate (rpm)	0-400	175 $\pm$ 147.90
Temperature ($^{\circ}$ C)	25-85	55 $\pm$ 22.36
pH	3-10.5	6.94 $\pm$ 1.76
AgNO ₃ Concentration (mol.L ⁻¹)	0.001-0.2	0.03 $\pm$ 0.03
Extract Concentration (g.mL ⁻¹)	0.001-0.075	0.4 $\pm$ 0.25
Absorbance	0.06-2.993	0.448 $\pm$ 0.247

Table 3.10 Effects of hidden neuron number on the ANN performance

%70-15-15	R ²				Mean Square Error (MSE)			
Hidden Neuron Number	Training	Validation	Testing	All	Training	Validation	Testing	Iteration
1	0.7717	0.6950	0.8361	0.7479	0.5105	0.1634	0.3274	16
2	0.9860	0.9879	0.9841	0.9855	0.0168	0.0086	0.0185	18
3	0.9935	0.9975	0.9950	0.9937	0.0083	0.0006	0.0060	40
4	0.9941	0.9929	0.9962	0.9941	0.0054	0.0083	0.0097	22
5	0.9955	0.9954	0.9977	0.9937	0.0057	0.0134	0.0051	14
6	0.9931	0.9959	0.9950	0.9930	0.0074	0.0041	0.0123	18
7	0.9727	0.9761	0.8497	0.9566	0.0374	0.0229	0.1242	8
8	0.9946	0.9956	0.9819	0.9932	0.0067	0.0031	0.0143	71
9	0.9970	0.9863	0.9888	0.9939	0.0032	0.0091	0.0196	10
10	0.9967	0.9904	0.9863	0.9921	0.0027	0.0114	0.0314	28
11	0.9978	0.9926	0.9838	0.9938	0.0023	0.0072	0.0269	20
12	0.9675	0.9919	0.9668	0.9690	0.0360	0.0068	0.0563	10
13	0.9968	0.9763	0.9820	0.9939	0.0038	0.0010	0.0246	16
14	0.9922	0.9928	0.8236	0.9820	0.0097	0.0263	0.0614	8
15	0.9935	0.9986	0.9687	0.9915	0.0073	0.0224	0.0205	10
16	0.9929	0.9889	0.9579	0.9905	0.0137	0.0218	0.0211	8
17	0.9943	0.9975	0.9918	0.9941	0.0063	9.8E-05	0.0118	42
18	0.9932	0.9943	0.9894	0.9927	0.0081	0.0110	0.0061	11
19	0.9979	0.9988	0.9506	0.9882	0.0025	0.0056	0.0649	11
20	0.9952	0.9964	0.9908	0.9944	0.0062	0.0025	0.0089	17

Table 3.10 continues

%70-15-15	R ²				Mean Square Error (MSE)			
Hidden Neuron Number	Training	Validation	Testing	All	Training	Validation	Testing	Iteration
21	0.9938	0.9616	0.9961	0.9907	0.0061	0.0307	0.0074	26
22	0.9795	0.9719	0.9904	0.9796	0.0286	0.0246	0.0206	8
23	0.9976	0.9855	0.9969	0.9944	0.0019	0.0262	0.0046	55
24	0.9957	0.9959	0.9779	0.9942	0.0048	0.0087	0.0138	11
25	0.9927	0.9980	0.9954	0.9932	0.0064	0.0050	0.0135	66
26	0.9936	0.9969	0.9924	0.9938	0.0068	0.0073	0.0078	10
27	0.9945	0.6866	0.9914	0.9923	0.0062	0.0111	0.0142	571
28	0.9928	0.9422	0.9825	0.9886	0.0100	0.0071	0.0358	10
29	0.9981	0.9970	0.9732	0.9934	0.0022	0.0002	0.0346	12
30	0.9794	0.9888	0.9575	0.9762	0.0401	0.0335	0.0714	8
31	0.9977	0.9542	0.9686	0.9892	0.0028	0.0351	0.0292	9
32	0.9953	0.9720	0.9877	0.9948	0.0064	0.0016	0.0057	28
33	0.9953	0.9932	0.9970	0.9942	0.0052	0.0088	0.0084	13
34	0.9915	0.9754	0.9866	0.9876	0.0128	0.0189	0.0310	8
35	0.9924	0.9830	0.9937	0.9856	0.0047	0.0942	0.0118	11
36	0.9938	0.9952	0.9980	0.9948	0.0058	0.0067	0.0037	19
37	0.9941	0.9966	0.9982	0.9948	0.0066	0.0032	0.0026	20
38	0.9854	0.9358	0.9570	0.9769	0.0330	0.0241	0.0400	8
39	0.9766	0.9977	0.9759	0.9762	0.0312	0.0071	0.0400	10
40	0.9955	0.8829	0.6155	0.9569	0.0058	0.1523	0.1462	9

Table 3.11 Effects of data percentage on the performance of ANN Modelling

Hidden Neuron Number	Training, Validation, Testing (%)	R ²				MSE			Iteration
		Training	Validation	Testing	All	Training	Validation	Testing	
17	90-5-5	0.9951	0.9991	0.9900	0.9950	0.0057	0.0057	0.0028	17
17	85-5-10	0.9942	0.9999	0.9997	0.9950	0.0061	0.00002	0.0015	69
17	80-5-15	0.9972	0.9436	0.9754	0.9917	0.0031	0.00008	0.0441	26
17	75-5-20	0.9960	0.9999	0.9826	0.9932	0.0064	0.0217	0.0125	8
17	70-5-25	0.9949	0.8577	0.9871	0.9935	0.0066	0.0087	0.0073	9
17	65-5-30	0.9954	0.9999	0.9808	0.9916	0.0054	0.0025	0.0177	13
17	60-5-35	0.9893	0.9974	0.9749	0.9814	0.0089	0.0073	0.0430	12
17	85-10-5	0.9952	0.9971	0.9929	0.9950	0.0056	0.0006	0.0095	22
17	80-10-10	0.9926	0.9993	0.9967	0.9947	0.0052	0.0025	0.0125	31
17	75-10-15	0.9938	0.9999	0.9999	0.9951	0.0069	0.00008	0.0007	78
17	70-10-20	0.9926	0.8452	0.9913	0.9918	0.0099	0.0107	0.0095	9
17	65-10-25	0.9894	0.9763	0.9853	0.9874	0.0126	0.0047	0.0257	8
17	60-10-30	0.9665	0.9979	0.9437	0.9642	0.0420	0.0030	0.0463	8
17	55-10-35	0.9981	0.9969	0.9867	0.9923	0.0016	0.0036	0.0205	69
17	80-15-5	0.9946	0.9988	0.9995	0.9950	0.0058	0.0017	0.0085	15
17	75-15-10	0.9864	0.9451	0.9992	0.9875	0.0163	0.0099	0.0014	10
17	70-15-15	0.9954	0.9980	0.9925	0.9947	0.0060	0.0078	0.0031	37
17	65-15-20	0.9882	0.9371	0.9625	0.9800	0.0154	0.0231	0.0482	9
17	60-15-25	0.9940	0.9997	0.9933	0.9936	0.0062	0.0005	0.0133	11
17	55-15-30	0.9927	0.9973	0.7815	0.9180	0.0080	0.0032	0.2819	10

Table 3.11 continues

Hidden Neuron Number	Training, Validation, Testing (%)	R ²				MSE			Iteration
		Training	Validation	Testing	All	Training	Validation	Testing	
17	50-15-35	0.9839	0.9222	0.9506	0.9560	0.0142	0.1361	0.0647	9
17	75-20-5	0.9978	0.9915	0.9990	0.9946	0.0026	0.0098	0.0384	12
17	70-20-10	0.9948	0.9927	0.9901	0.9930	0.0060	0.0162	0.0033	10
17	65-20-15	0.9965	0.9660	0.9934	0.9916	0.0047	0.0170	0.0203	12
17	60-20-20	0.9958	0.9903	0.9863	0.9919	0.0061	0.0152	0.0153	9
17	55-20-25	0.9967	0.9918	0.8290	0.9275	0.0026	0.0121	0.3084	135
17	50-20-30	0.9769	0.9892	0.9919	0.9822	0.0203	0.0270	0.0219	9
17	45-20-35	0.9972	0.9848	0.97759	0.9853	0.0133	0.0282	0.0263	8
17	70-25-5	0.9970	0.9976	0.9923	0.9942	0.0033	0.0030	0.0662	53
17	65-25-10	0.9992	0.9801	0.9980	0.9940	0.0010	0.0199	0.0169	11
17	60-25-15	0.9986	0.9771	0.9926	0.9896	0.0012	0.0328	0.0161	45
17	55-25-20	0.9943	0.9931	0.9921	0.9937	0.0066	0.0100	0.0030	55
17	50-25-25	0.9940	0.9862	0.9932	0.9908	0.0052	0.0198	0.0090	9
17	45-25-30	0.9983	0.9739	0.9840	0.9913	0.0025	0.0197	0.0106	388
17	40-25-35	0.9993	0.9695	0.9912	0.9885	0.0007	0.0328	0.0117	19
17	65-30-5	0.9949	0.9952	0.9965	0.9946	0.0067	0.0046	0.0017	34
17	60-30-10	0.9853	0.9925	0.9975	0.9871	0.0156	0.0141	0.0017	17
17	55-30-15	0.9931	0.9862	0.9697	0.9862	0.0103	0.0113	0.0362	9
17	50-30-20	0.9961	0.9843	0.9969	0.9899	0.0049	0.0182	0.0270	9
17	45-30-25	0.9974	0.9869	0.9905	0.9895	0.0018	0.0360	0.0182	35

Table 3.11 continues

		R ²				MSE			
Hidden Neuron Number	Training, Validation, Testing (%)	Training	Validation	Testing	All	Training	Validation	Testing	Iteration
17	40-30-30	0.9996	0.9851	0.9815	0.9854	0.0002	0.0259	0.3375	14
17	35-30-35	0.9933	0.7574	0.9423	0.9405	0.0075	0.1054	0.0971	10
17	60-35-5	0.9967	0.9395	0.9999	0.9904	0.0048	0.0206	0.0022	10
17	55-35-10	0.9984	0.9710	0.9699	0.9889	0.0021	0.0185	0.0484	25
17	50-35-15	0.9646	0.9736	0.9755	0.9648	0.0431	0.0410	0.0629	11
17	45-35-20	0.9841	0.9667	0.9839	0.9773	0.0198	0.0324	0.0258	8
17	40-35-25	0.9921	0.9729	0.9556	0.9749	0.0079	0.0510	0.0424	120
17	35-35-30	0.9984	0.9926	0.9596	0.9819	0.0021	0.0069	0.0655	32
17	30-35-35	0.9970	0.9258	0.9870	0.9697	0.0035	0.0782	0.0139	10

Table 3.12 Performances of different backpropagation algorithms on the prediction of experimental values

		ANN Algorithms						
#	Experimental	Trainbfg	Trainbr	Traincgb	Traincgf	Traincgp	Traingd	Traingdm
1	0.074	0.107	0.077	0.071	0.060	0.060	0.073	0.151
2	0.084	0.100	0.082	0.080	0.060	0.060	0.081	0.128
3	0.082	0.110	0.091	0.105	0.060	0.060	0.131	0.244
4	0.098	0.105	0.091	0.100	0.060	0.060	0.117	0.187
5	0.179	0.097	0.168	0.138	0.060	0.060	0.120	0.190
6	0.193	0.216	0.223	0.288	0.060	0.060	0.106	0.302
7	0.212	0.100	0.201	0.168	0.060	0.060	0.113	0.199
8	0.430	0.669	0.615	0.445	0.122	0.060	0.698	0.278
9	0.699	1.044	0.550	0.766	0.782	0.060	1.257	0.474
10	0.474	0.388	0.415	0.162	0.062	0.060	0.445	0.129
11	1.587	1.930	1.710	1.948	2.996	2.996	1.824	2.377
12	1.964	2.530	2.217	2.296	2.994	2.927	2.461	1.604
13	2.794	2.802	2.929	2.954	2.996	2.996	2.739	2.075
14	0.081	0.103	0.087	0.103	0.060	0.060	0.143	0.160
15	0.114	0.216	0.119	0.082	0.060	0.060	0.066	0.869

Table 3.13 Performances of different backpropagation algorithms on the prediction of experimental values

		ANN Algorithms						
#	Experimental	Traingda	Traingdx	Trainlm	Trainoss	Trainr	Trainrp	Trainscg
16	0.074	0.131	0.182	0.069	0.060	0.109	0.078	0.070
17	0.084	0.127	0.227	0.080	0.060	0.094	0.081	0.066
18	0.082	0.141	0.264	0.085	0.060	0.122	0.089	0.068
19	0.098	0.133	0.266	0.092	0.060	0.109	0.087	0.067
20	0.179	0.125	0.408	0.170	0.062	0.130	0.099	0.077
21	0.193	0.137	0.721	0.199	0.143	0.443	0.193	0.176
22	0.212	0.126	0.456	0.196	0.069	0.157	0.113	0.087
23	0.430	0.711	0.408	0.657	0.590	0.540	0.706	0.547
24	0.699	1.037	0.417	0.580	0.660	0.867	0.704	0.657
25	0.474	0.487	0.385	0.432	0.185	0.250	0.585	0.374
26	1.587	2.360	0.513	1.728	1.911	2.022	1.992	1.783
27	1.964	2.244	0.324	2.174	2.265	2.488	2.272	2.327
28	2.794	2.842	0.612	2.979	2.967	2.869	2.957	2.948
29	0.081	0.149	0.260	0.072	0.060	0.114	0.090	0.074
30	0.114	0.101	0.144	0.231	0.060	0.284	0.078	0.061

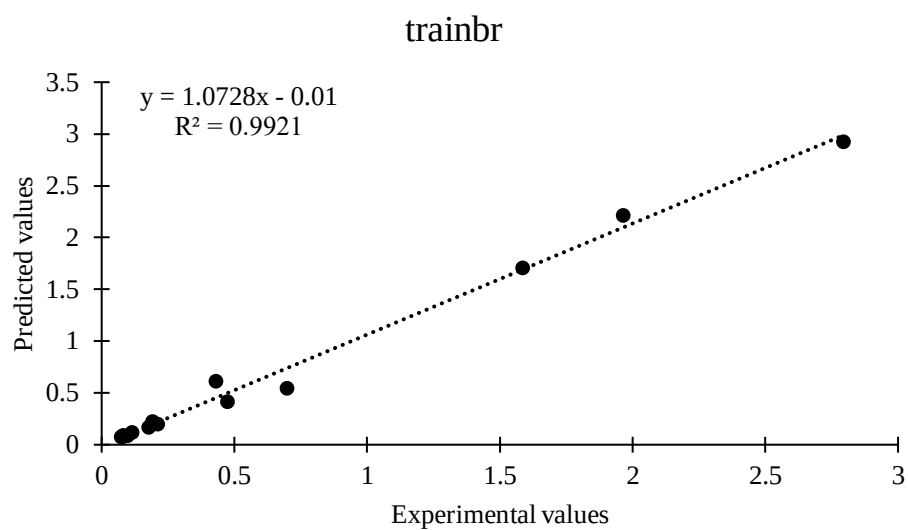


Figure 3.48 Comparison of experimental values with ANN predicted values (Trainbr: Bayesian regularization backpropagation)

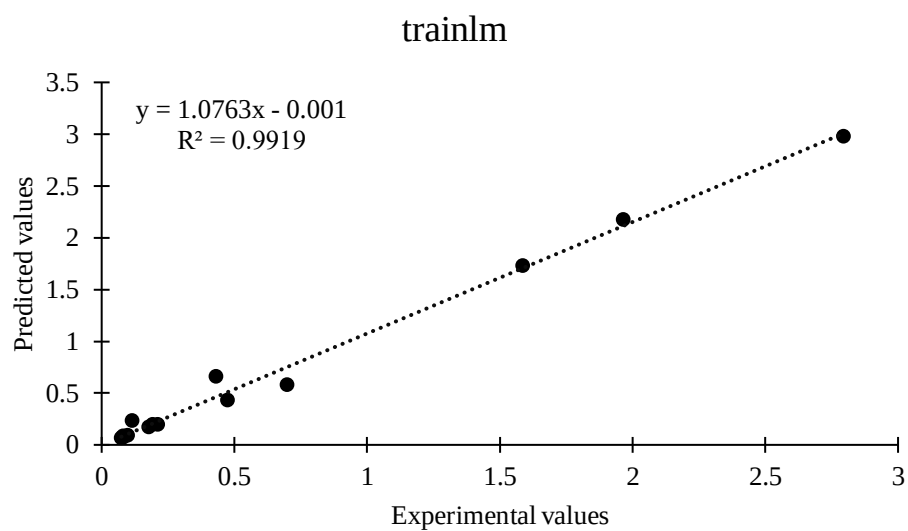


Figure 3.49 Comparison of experimental values with ANN predicted values (Trainlm: Levenberg–Marquardt backpropagation)

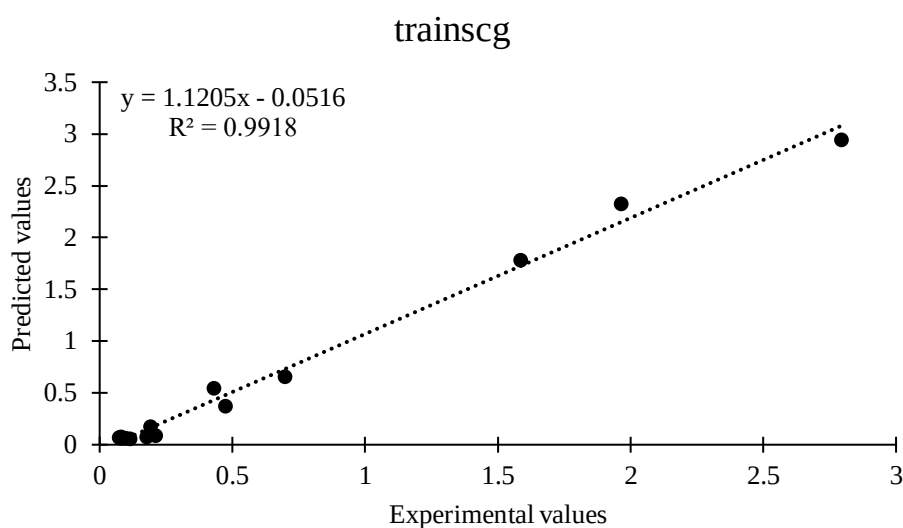


Figure 3.50 Comparison of experimental values with ANN predicted values (Trainscg: scaled conjugate gradient method)

3.11.3 Artificial Neural Network Study for *C. prolifer* Based AgNPs

Green synthesis of AgNPs was performed using *C. prolifer* extract as a reduction agent. The experimental data were collected working parameters like agitation time, agitation rate, temperature, pH and concentration of AgNO_3 and extract. Table 3.18 shows the data statistics of model variables. This data was used for creating an artificial neural network by MATLAB. The optimum hidden neuron number was found as 29 with the study of MATLAB when default settings (the percentages of training, validation and testing are 70, 15 and 15%, respectively, Levenberg-Marquardt backpropagation algorithm) were applied. The R^2 values for training, validation and testing were found as 0.998103, 0.992759 and 0.986334, respectively when the hidden number was 29 (Table 3.15). Besides, the effects of data percentages were studied and the highest R^2 values were found for 75, 5 and 20% (training, validation and testing) and R^2 values were 0.994298, 0.998833 and 0.997350, respectively (Table 3.16). Also, the performance of the 11 backpropagation algorithms was studied and the highest R^2 values were found for Bayesian regularization backpropagation algorithm as a 0.99. Usually, Levenberg-Marquardt backpropagation algorithm are known as a best

backpropagation algorithm and best estimation results are found with this backpropagation algorithm. In addition, Levenberg-Marquardt backpropagation algorithm were given the highest R^2 values for the other experiments performed by us (Table 3.17-18). For comparing experimental and ANN estimated data Figure 3.51 were drawn. These tables show that the created ANN model can estimate the experimental values precisely but the other backpropagation algorithms are not give promising results for this work.

Table 3.14 The data statistics of model variables

Variables	Data Statistics Range	Mean $\pm$ S.D.
Agitation Time (min)	0-360	158 ± 118.61
Agitation Rate (rpm)	0-400	175 ± 147.90
Temperature ($^{\circ}\text{C}$)	25-85	55 ± 22.36
pH	3-10.5	6.94 ± 1.76
AgNO_3 Concentration (mol.L^{-1})	0.001-0.2	0.03 ± 0.03
Extract Concentration (g.mL^{-1})	0.001-0.075	0.4 ± 0.25
Absorbance	0.082-2.913	0.448 ± 0.247

Table 3.15 Effects of hidden neuron number on the ANN performance

%70-15-15	R ²				Mean Square Error (MSE)			
Hidden Neuron Number	Training	Validation	Testing	All	Training	Validation	Testing	Iteration
1	0.8880	0.7610	0.9530	0.8674	0.1516	0.3267	0.2191	9
2	0.7849	0.8745	0.8797	0.8069	0.3168	0.2699	0.2169	9
3	0.9575	0.9946	0.9414	0.9600	0.0604	0.0077	0.0787	23
4	0.9422	0.8855	0.93050	0.9275	0.0801	0.2239	0.0771	12
5	0.9936	0.9978	0.9920	0.9937	0.0081	0.0043	0.0164	44
6	0.9913	0.9871	0.9854	0.9883	0.0101	0.0192	0.0455	18
7	0.9936	0.9581	0.9807	0.9329	0.0076	0.0994	0.8002	16
8	0.9819	0.9749	0.8603	0.9575	0.0265	0.0388	0.2473	15
9	0.9947	0.9939	0.9961	0.9940	0.0082	0.0123	0.0061	42
10	0.9934	0.9962	0.9519	0.9900	0.0083	0.0109	0.0443	27
11	0.9937	0.9032	0.9540	0.9672	0.0077	0.1772	0.1047	18
12	0.9923	0.9299	0.9993	0.9299	0.0114	0.1587	0.0010	17
13	0.9960	0.9978	0.9937	0.9944	0.0059	0.0036	0.0230	51
14	0.9146	0.8687	0.9556	0.9195	0.1497	0.0508	0.1607	10
15	0.9920	0.9620	0.9995	0.9903	0.0112	0.0324	0.0071	17
16	0.9966	0.9851	0.9900	0.9936	0.0056	0.0191	0.0202	14
17	0.9956	0.9990	0.9404	0.9658	0.0057	0.0002	0.4238	38
18	0.9925	0.9101	0.9926	0.9753	0.0148	0.1514	0.0119	13
19	0.9583	0.9960	0.6487	0.9372	0.0655	0.0196	0.2430	12
20	0.9727	0.9961	0.9602	0.9731	0.0322	0.0083	0.0931	11

Table 3.15 continues

%70-15-15	R ²				Mean Square Error (MSE)			
Hidden Neuron Number	Training	Validation	Testing	All	Training	Validation	Testing	Iteration
21	0.9760	0.9881	0.9552	0.9746	0.0328	0.0480	0.0552	10
22	0.9958	0.9931	0.9933	0.9922	0.0063	0.0276	0.0214	13
23	0.9916	0.9853	0.9956	0.9901	0.0126	0.0305	0.0045	16
24	0.8956	0.8490	0.7830	0.8765	0.2391	0.3003	0.3334	8
25	0.9958	0.9919	0.9454	0.9826	0.0057	0.0194	0.1185	87
26	0.9942	0.9057	0.9871	0.9882	0.0080	0.0411	0.0343	66
27	0.9759	0.9829	0.9683	0.9755	0.0339	0.0033	0.0665	13
28	0.9952	0.9928	0.9958	0.9941	0.0080	0.0120	0.0076	16
29	0.9981	0.9928	0.9863	0.9940	0.0033	0.0024	0.0422	24
30	0.9973	0.9846	0.9918	0.9927	0.0050	0.0363	0.0150	11
31	0.9773	0.9472	0.9604	0.9521	0.0295	0.2635	0.1715	12
32	0.9961	0.9847	0.9920	0.9941	0.0060	0.0148	0.0133	28
33	0.8832	0.7972	0.7475	0.8341	0.1558	0.4119	0.4431	8
34	0.9950	0.9912	0.9857	0.9921	0.0077	0.0123	0.0352	13
35	0.9967	0.9693	0.9801	0.9877	0.0055	0.0675	0.0276	13
36	0.9956	0.9956	0.9972	0.9949	0.0057	0.0094	0.0115	19
37	0.9951	0.9957	0.9968	0.9946	0.0067	0.0128	0.0061	22
38	0.6652	0.6674	0.7204	0.6755	0.5134	0.3725	0.5282	8
39	0.9930	0.9212	0.9965	0.9899	0.0096	0.0393	0.0099	12
40	0.9856	0.9962	0.9635	0.9835	0.0217	0.0060	0.0501	11

Table 3.16 Effects of data percentage on the performance of ANN Modelling

Hidden Neuron Number	Training, Validation, Testing (%)	R ²				MSE			Iteration
		Training	Validation	Testing	All	Training	Validation	Testing	
29	90-5-5	0.9946	0.8468	0.9320	0.9947	0.0082	0.0005	0.0008	15
29	85-5-10	0.9942	0.9999	0.9771	0.9932	0.0092	0.0005	0.0234	14
29	80-5-15	0.9950	0.9630	0.9092	0.9864	0.0084	0.1149	0.0447	39
29	75-5-20	0.9943	0.9988	0.9974	0.9949	0.0076	0.0018	0.0078	29
29	70-5-25	0.9942	0.9996	0.9613	0.9872	0.0086	0.0032	0.0480	27
29	65-5-30	0.9981	0.9987	0.9902	0.9943	0.0022	0.0180	0.0191	17
29	60-5-35	0.9956	0.9995	0.9825	0.9904	0.0068	0.0031	0.0283	29
29	85-10-5	0.9961	0.9987	0.9964	0.9987	0.0061	0.0005	0.0357	82
29	80-10-10	0.9958	0.9969	0.9908	0.9949	0.0062	0.0011	0.0209	51
29	75-10-15	0.9949	0.9927	0.9971	0.9943	0.0077	0.0128	0.0071	34
29	70-10-20	0.9960	0.99884	0.9376	0.9820	0.0062	0.0028	0.1125	46
29	65-10-25	0.9724	0.9723	0.9866	0.9770	0.0402	0.0545	0.0288	12
29	60-10-30	0.9938	0.9984	0.9889	0.9929	0.0089	0.0076	0.0141	15
29	55-10-35	0.9899	0.9974	0.9241	0.9691	0.0132	0.0169	0.1038	16
29	80-15-5	0.9905	0.9857	0.9984	0.9907	0.0140	0.0167	0.0133	15
29	75-15-10	0.9669	0.8890	0.6955	0.9107	0.0497	0.1575	0.6337	9
29	70-15-15	0.8580	0.7544	0.9104	0.8444	0.1877	0.3808	0.2299	8
29	65-15-20	0.9990	0.9889	0.9885	0.9934	0.0013	0.0363	0.0196	20
29	60-15-25	0.9982	0.9648	0.9876	0.9856	0.0020	0.0743	0.0300	27
29	55-15-30	0.9940	0.9858	0.9797	0.9863	0.0048	0.0382	0.0371	36

Table 3.16 continues

		R ²				MSE			
Hidden Neuron Number	Training, Validation, Testing (%)	Training	Validation	Testing	All	Training	Validation	Testing	Iteration
29	50-15-35	0.9888	0.9867	0.9838	0.9843	0.0233	0.0174	0.0609	11
29	75-20-5	0.9779	0.9855	0.9968	0.9779	0.0260	0.0528	0.0689	14
29	70-20-10	0.9955	0.9945	0.9891	0.9943	0.0062	0.0065	0.0240	18
29	65-20-15	0.9781	0.9871	0.9794	0.9809	0.0290	0.0244	0.0295	11
29	60-20-20	0.9972	0.9566	0.9516	0.9774	0.0036	0.0688	0.0818	14
29	55-20-25	0.9934	0.7629	0.8738	0.9437	0.0149	0.2150	0.1579	12
29	50-20-30	0.9624	0.8865	0.9093	0.9316	0.0661	0.1440	0.1254	12
29	45-20-35	0.9946	0.9858	0.9887	0.9907	0.0104	0.0187	0.0220	13
29	70-25-5	0.9944	0.9917	0.9974	0.9917	0.0083	0.0099	0.0093	14
29	65-25-10	0.9958	0.9825	0.9724	0.9889	0.0057	0.0293	0.0457	14
29	60-25-15	0.9889	0.9797	0.9624	0.9744	0.0134	0.0604	0.0842	12
29	55-25-20	0.9981	0.9813	0.9491	0.9869	0.0059	0.0583	0.0178	13
29	50-25-25	0.9952	0.9960	0.9882	0.9924	0.0061	0.0112	0.0199	25
29	45-25-30	0.9319	0.9031	0.8621	0.8731	0.1216	0.3138	0.3354	12
29	40-25-35	0.9574	0.4577	0.2262	0.5064	0.1539	1.5927	1.9071	8
29	65-30-5	0.9966	0.9418	0.9950	0.9743	0.0042	0.1113	0.0002	18
29	60-30-10	0.9961	0.9927	0.9916	0.9946	0.0066	0.0049	0.0222	65
29	55-30-15	0.9884	0.9092	0.8676	0.9437	0.0274	0.1112	0.2007	9
29	50-30-20	0.9693	0.9456	0.9884	0.9604	0.0500	0.0884	0.0254	10
29	45-30-25	0.9963	0.9472	0.8788	0.9576	0.0060	0.0860	0.1334	39

Table 3.16 continues

		R ²				MSE			
Hidden Neuron Number	Training, Validation, Testing (%)	Training	Validation	Testing	All	Training	Validation	Testing	Iteration
29	40-30-30	0.9995	0.9690	0.9777	0.9845	0.0007	0.0449	0.0313	22
29	35-30-35	0.9520	0.8073	0.7460	0.7669	0.1485	0.3750	0.4695	7
29	60-35-5	0.9948	0.9898	0.9998	0.9922	0.0078	0.0177	0.0005	13
29	55-35-10	0.9543	0.8736	0.9943	0.9024	0.0566	0.3029	0.0505	14
29	50-35-15	0.9972	0.8079	0.7733	0.8516	0.0030	0.4916	0.5253	15
29	45-35-20	0.9932	0.8860	0.7718	0.9401	0.0162	0.1480	0.2050	16
29	40-35-25	0.9171	0.5894	0.7746	0.7621	0.5693	0.9899	0.5497	9
29	35-35-30	0.9807	0.7697	0.9469	0.8920	0.0394	0.3162	0.0836	9
29	30-35-35	0.9917	0.9006	0.9023	0.9276	0.0268	0.1287	0.1391	12

Table 3.17 Performances of different backpropagation algorithms on the prediction of experimental values

		ANN Algorithms						
#	Experimental	Trainbfg	Trainbr	Traincgb	Traincgf	Traincgp	Traingd	Traingdm
1	0.141	0.091	0.135	0.213	0.095	0.093	0.099	0.347
2	0.126	0.091	0.137	0.252	0.095	0.092	0.098	0.304
3	0.129	0.091	0.134	0.256	0.098	0.094	0.104	0.248
4	0.161	0.091	0.137	0.275	0.096	0.092	0.100	0.288
5	0.406	0.091	0.393	0.363	0.093	0.092	0.120	0.534
6	0.200	0.091	0.196	0.741	0.099	0.109	0.520	1.484
7	0.417	0.091	0.393	0.363	0.093	0.092	0.120	0.534
8	2.674	2.665	2.923	1.764	2.766	2.610	2.099	0.471
9	2.907	2.979	2.878	2.062	2.963	2.974	2.675	0.490
10	0.999	2.355	0.780	1.694	1.848	1.145	1.679	0.754
11	1.297	0.177	1.170	1.785	0.441	0.376	1.084	0.309
12	1.077	0.718	1.086	1.340	1.285	1.702	1.465	1.607
13	0.854	0.112	0.810	0.921	0.836	0.788	0.476	2.659
14	0.082	0.091	0.126	0.240	0.096	0.092	0.100	0.239
15	0.156	0.091	0.162	1.346	0.094	0.134	0.132	0.971

Table 3.18 Performances of different backpropagation algorithms on the prediction of experimental values

		ANN Algorithms						
#	Experimental	Traingda	Traingdx	Trainlm	Trainoss	Trainr	Trainrp	Trainscg
16	0.141	0.118	0.186	0.180	0.093	0.254	0.113	0.345
17	0.126	0.104	0.185	0.135	0.093	0.174	0.119	0.315
18	0.129	0.097	0.262	0.145	0.093	0.183	0.147	0.302
19	0.161	0.098	0.205	0.134	0.093	0.177	0.141	0.295
20	0.406	0.113	0.184	0.219	0.093	0.191	0.197	0.195
21	0.200	0.217	0.298	0.567	0.295	0.296	0.238	0.330
22	0.417	0.113	0.184	0.219	0.093	0.191	0.197	0.195
23	2.674	2.243	2.044	0.170	2.628	1.991	2.691	2.442
24	2.907	2.778	2.598	0.151	2.942	2.234	2.963	2.815
25	0.999	1.716	2.214	0.188	2.165	1.748	1.832	2.595
26	1.297	0.600	1.453	0.092	0.292	1.270	0.757	0.551
27	1.077	1.107	1.296	0.166	1.536	1.596	1.250	1.013
28	0.854	0.759	0.675	0.482	1.118	1.093	0.635	0.705
29	0.082	0.099	0.203	0.153	0.093	0.171	0.153	0.343
30	0.156	0.175	0.602	0.461	0.099	0.161	0.124	0.196

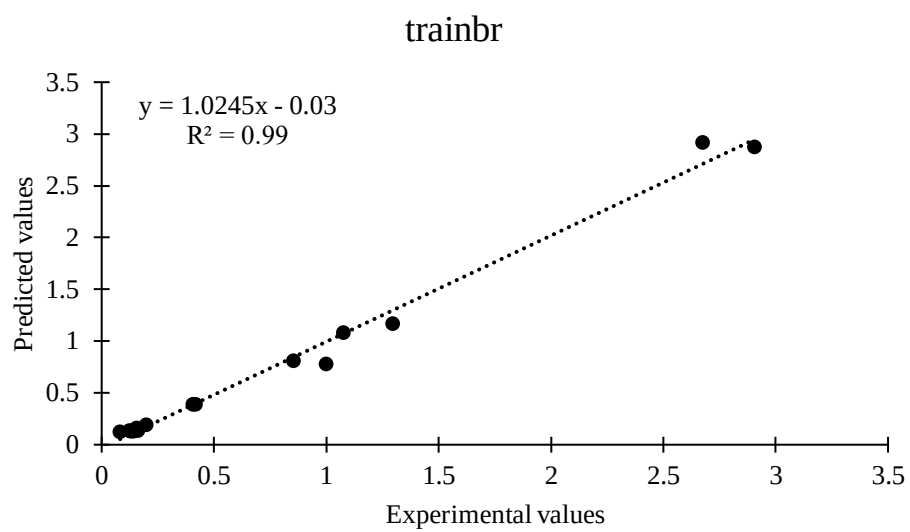


Figure 3.51 Comparison of experimental values with ANN predicted values (Trainbr: Bayesian regularization backpropagation)

CHAPTER FOUR

CONCLUSION

In this study, *C. cylindracea* and *C. prolifera* collected from İzmir coastline and were used for the green synthesis of AgNPs. After that catalytic activity of synthesized AgNPs were examined. Additionally, AgNPs were synthesized with traditional methods for comparing with synthesized AgNPs with green synthesis. The antifouling performance of synthesized AgNPs via green synthesis were evaluated. In order to model experimental conditions related to the green synthesis of AgNPs, Artificial Neural Network were used. Additionally, for evaluating catalytic activity of AgNPs, experiments relevant to decolourization of Congo red were done. Owing to lack of capping agents found in *Caulerpa* spp, the stabilization of nanoparticles was not accomplished and AgNPs aggregated. Because of that reason size of nanoparticles were found using XRD results and Scherrer equation. SEM, XRD, FT-IR and UV-Vis spectrometry were used for characterization of synthesized AgNPs.

Kathiveran et al. (2015) studied the antibacterial activity of the synthesized AgNPs by *Caulerpa racemosa*. They characterized AgNPs through FT-IR, XRD, TEM and UV-Vis spectrometry. They found out that from UV-Vis spectrum surface plasmon resonance band of AgNPs was smooth, narrow and centred at 413 nm which is characteristic of virtually spherical nanoparticles. From the FT-IR results, they expressed that peptides may participate in the synthesis of AgNPs. Kathiveran et al. TEM results indicated that morphology of synthesized AgNPs was spherical and size was between 5-25 nm. They did antibacterial tests against *P. mirabilis* and *S. aureus* with different concentrations. They concluded that AgNPs were synthesized extracellularly by *C. racemosa* and synthesized AgNPs stable in solution because of capping agents like proteins present in the extract and this AgNPs could be used as an antimicrobial agent after many trials.

Edison et al. (2016) studied the catalytic activity of synthesized AgNPs on the degradation of methylene blue. AgNPs were synthesized using *Caulerpa racemosa*. They used FT-IR, XRD, HR-TEM, EDS and UV-Vis spectroscopy for the characterization of synthesized AgNPs. Synthesized AgNPs size was 25 nm with a

distorted spherical shape. Synthesized AgNPs were highly crystalline nature to selected area electron diffraction (SAED) pattern. They tested the catalytic activity of synthesized AgNPs using UV-Vis spectroscopy. They found out that in the presence of just NaBH_4 the degradation of methylene blue did not take place but with the addition of AgNPs to the solution degradation of methylene blue occurred. They expressed that the reasonable mechanism for the AgNPs supported catalytic degradation of methylene blue was elucidated with the Langmuir-Hinshelwood model. In this model NaBH_4 is electron donor and hydrogen source in addition to that AgNPs play a role as an electron transfer mediator between BH_4^- ion and methylene blue. Their results are consistent with other previous reports (Edison, Atchudan, Kamal & Lee, 2016)

Aboelfetoh et al. (2017) synthesized AgNPs using *Caulerpa serrulata* and studied reaction optimization, catalytic and antibacterial activity of synthesized AgNPs. They used FT-IR, XRD, HR-TEM and UV-Vis Spectroscopy for the characterization of synthesized AgNPs. Synthesized AgNPs size was 10 ± 2 nm. They examined pH values, contact time, extract concentration and temperature for reaction optimization. According to Aboelfetoh et al., the antibacterial activity of AgNPs depended on the concentration of AgNPs. They did an antibacterial test against *E. coli* and *S. typhi* eventually maximum inhibition zone was found in *E. coli* (21 mm for 75 μL). Additionally, their studies confirmed that silver ions which released from the surface of nanoparticles contribute to their toxicity. For optimization of degradation percentage of Congo red, they studied the effect of NaBH_4 concentration, extract concentration and catalytic dosage.

Previous studies and Aboelfetoh et al. (2017) study reported that the formation of AgNPs in the acid medium was with large size whereas in alkaline medium was with small size. They expressed that as the temperature was increased reactants were consumed rapidly and nanoparticles formed. Their study showed that extract not only reducing agent but also stabilizer (Aboelfetoh, El-Shenody & Ghobara, 2017).

Abdel-Raouf et al. (2018) synthesized AgNPs using marine red alga *Laurencia catarinensis* and they characterized synthesized AgNPs using FT-IR, TEM, DLS (Dynamic Light Scattering) and UV–Visible spectroscopy. Synthesized AgNPs size

was in the range of 39.41–77.7-1 nm and shapes were spherical, triangular, rectangle, polyhedral and hexagonal. They synthesized AgNPs with algal powder and with algae extracts which prepared with ethanol and chloroform (Abdel-Raouf, Alharbi, Al-Enazi, Alkhulaifi & Ibraheem, 2018).

Azizi et al. (2013) synthesized AgNPs using the aqueous extract of brown macroalgae *Sargassum Muticum*. They used FTIR, XRD, TEM and UV–Vis spectroscopy for characterization. According to the TEM image, AgNPs size were in the range of 5-15 nm. They concluded that sulphated polysaccharides found in *S.muticum* were responsible for the reduction of silver ions. (Azizi, Namvar, Mahdavi, Ahmad, M. & Mohamad, 2013).

de Aragao et al. (2016) studied the antibacterial activity of AgNPs which synthesized using polysaccharides extracted from red algae *Gracilaria birdie*. They performed different polysaccharides concentrations, pH values for optimization. In order to characterize AgNPs, they used FTIR, DLS, UV-Vis spectroscopy and TEM. They found that synthesized AgNPs size were between 20.2 nm and 94.9 nm. They tested antibacterial activity using *Escherichia coli* and *Staphylococcus aureus* and then concluded that all synthesized silver nanoparticle samples showed antibacterial activity against *Escherichia coli* (de Aragao et al., 2016).

Shabanzadeh et al. (2013) studied neural network modelling for the prediction of the size of silver nanoparticle prepared by green method. They synthesized AgNPs using soluble starch. Their input parameters were the volume of NaOH, temperature, starch and AgNO₃ concentration and output parameter was the size of nanoparticles. For training, validation and testing steps they used 20, 5, 5 sample respectively. They found a number of a hidden neuron as 10. R² values of their best predictive model for training, validation and testing were 0.9839, 0.9778 and 0.9787, respectively (Shabanzadeh, Senu, Shameli, & Ismail, 2013)

Shabanzadeh et al. (2014) also worked neural network modelling for in their paper named Neural Network Modelling for AgNPs in montmorillonite/starch synthesis by chemical reduction method. Their input parameters were AgNO₃ and NaBH₄ concentration, temperature, the weight percentage of starch and output parameter was the size of silver nanoparticle in their ANN model. Their percentage of datasets for

training, validation and testing were 70%, 15% and 15%, respectively. They found a number of hidden neurons as 10. R^2 values that they found for training, validation and testing were 0.9979, 0.9952 and 0.9984 (Shabanzadeh, Yusof, & Shameli, 2014).

Venkatesan et al. (2016) synthesized AgNPs using the extract of *Ecklonia cava* and investigated anticancer, antioxidant and antimicrobial effects of AgNPs. In order to characterize synthesized AgNPs they used TEM, DLS, UV-Vis spectroscopy, FTIR and XRD analysis. They stated that bioactive molecules like phenolic compounds found in *Ecklonia cava* acted as capping and reducing agent. Synthesized AgNPs size and shape were 43 nm and spherical. They tested antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli*. Additionally, they used 1,1-diphenyl-2-picrylhydrazyl (DPPH) for antioxidant activity assessment. Also, they found that synthesized AgNPs displayed anticancer activity toward human cervical cancer cells (Venkatesan, Kim, Shim, 2016).

Aravindan et al. (2014) synthesized AgNPs using *Caulerpa racemosa* and *Ulva lactuca* also investigated phytotoxicity of AgNPs via seed germination test. In order to characterize AgNPs, they used UV-Vis spectroscopy, SEM, XRD and TEM, FTIR. They synthesized AgNPs in the range of 35-75 nm. For evaluating antimicrobial activity, they worked with *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, and *Vibrio cholera* (Aravindan, Azeez, Nallamuthu, 2014).

Ibraheem et al. (2016) studied the synthesis of AgNPs using an alcoholic extract of *Acanthophora specifera* which is a red alga. They used XRD and FTIR for characterizing AgNPs. They sought antimicrobial activity of AgNPs using *Candida albicans*, *Salmonella spp.*, *Staphylococcus aureus*, *Escherichia coli* and *Bacillus subtilis*. Size of AgNPs which they synthesized was between 33-81 nm (Ibraheem, Abd-Elaziz, Saad, Fathy, 2016).

Salari et al. (2016) examined antibacterial activity of AgNPs which they synthesized by extract of macroalgae *Spirogyra varians*. They investigated the size and morphology of AgNPs using UV-Vis spectroscopy, SEM, XRD and FTIR. They synthesized uniform AgNPs with a size of 17.6 nm. They evaluated antibacterial activity against many microorganisms like *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli* and *Klebsiella* (Salari, Danafar, Dabaghi, Ataei, 2016).

Rajeshkumar et al. (2014) analyze usage of *Sargassum longifolium* as a reducing and capping agent for the synthesis of AgNPs and antifungal activity of AgNPs. They worked about experiment parameters like pH and incubation time for optimization of synthesis. They used UV-Vis spectroscopy, SEM, TEM, XRD and FTIR for characterization of AgNP. In order to evaluate the antifungal activity, they used *Fusarium* sp., *Candida albicans*, and *Aspergillus fumigatus* (Rajeshkumar et al., 2014).

Green chemistry is an important and innovative issue in terms of sustainability and pollution prevention. Green chemistry methods are very easy to apply, the use of toxic chemicals is limited and does not require the use of expensive tools. Because of these reasons they can easily be a subject of research. Since the mechanism behind the green synthesis of nanoparticles is not fully known and differs from source, it is always an area where new information can be discovered. In the light of the results obtained within the scope of the thesis, *Caulerpa* species found on the coasts of our country can only be used in the synthesis and stabilization of nanoparticles when collected in certain seasons. Further studies should be conducted to prevent aggregation. The resulting silver nanoparticles were used in decolourization of Congo red and in developing antifouling dye formulation. Especially promising results were obtained from study of decolourization of Congo red and comprehensive investigations should be conducted. As far as we know, the use of silver nanoparticles produced by green chemistry methods in antifouling paint formulation has not been studied before. We conclude that silver nanoparticles can be used to inhibit fouling organisms, but further research is needed in terms of toxicity and proportion in dye. Artificial intelligence applications are used successfully in many fields. Its use in the field of chemistry is also important in terms of providing ideas for us before entering the laboratory. Considering that green chemistry is actually the design of experimental processes and products, the use of artificial intelligence applications is quite suitable for this field.

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