# Climate Change and Soil-Plant-Environment Interactions



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#### **INTERACTIONS**

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## PREFACE

Climate change is a major global concern, with increasing temperatures and extreme weather events threatening the delicate balance of our planet's ecosystems. One of the areas most affected by these changes is the interaction between soil, plants, and the environment. Soil plays a vital role in supporting plant growth and is a crucial component of healthy ecosystems, but the effects of climate change on soil health and productivity can be significant.

The interactions between soil, plants, and the environment are complex and multi-faceted. Changes in temperature, precipitation, and other climate factors can have a wide range of effects on soil properties, nutrient availability, and the growth and survival of plants. These effects can be exacerbated by human activities such as deforestation, agricultural practices, and land-use changes, which can further degrade soil quality and exacerbate the impacts of climate change.

Understanding the complex relationships between climate change, soil, and plant health is critical for developing effective strategies to mitigate and adapt to the impacts of climate change. This involves not only understanding the direct effects of climate change on soil and plant health but also the feedback mechanisms that can amplify or dampen these effects. It also requires a deep understanding of the biological and chemical processes that govern soil-plant interactions and the role of soil soil microorganisms and other organisms in supporting plant growth and ecosystem health. This preface aims to provide an overview of the current state of knowledge in this area, highlighting the key challenges and opportunities for future research. By increasing our understanding of the relationship between climate change, soil, and plant health, we can work towards developing sustainable solutions to mitigate the impacts of climate change and safeguard our planet's natural resources for future generations. The challenges we face are significant, but with a concerted effort, we can develop the tools and knowledge needed to address this critical issue and build a more resilient and sustainable future for ourselves and the planet.

Sincerely Yours, June, 2023

Assoc. Prof. Dr. Korkmaz BELLİTÜRK Assoc. Prof. Dr. Ahmet ÇELİK Dr. Miraç KILIÇ PhD. Candidate Fatih BÜYÜKFİLİZ



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**Research Interests:** Soil Fertility, Soil Fauna, Soil Chemistry, Plant Nutrition, Soil Biology, Ecological Management for Soil Quality, Soil Pollution, Composting and Vermicomposting, Sustainable and Organic Agriculture, Sewage Sludge Compost, Fertilizers (Chemical, Organic and Organo-mineral Fertilizers).



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**Research Interests:** Digital Soil Mapping, Sustainable Agricultural Land Suitability Assessment, Soil quality assessment models, Soil and Water Pollution, Soil Ecology, Ecological Risk Assessment.



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**Research Interests:** Soil Fertility, Plant Nutrition, Organic Farming, Composting and Vermicomposting.

# PLANT AND SOIL DATA MANAGEMENT VIA INTELLIGENT AGRICULTURAL MACHINERY AND FIELD ROBOTS

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#### **1. Introduction**

A source of spatial information on crops, soil, and environmental variables that is high-density, high-speed, and reasonably priced is required in order to accomplish the goal of demand-oriented input and variable-rate fertilization for a wide range of agricultural situations and types of crops. This is necessary to achieve the goal of demand-oriented input (Gonzalez-de-Santos et al., 2020). With this data, a prescription map for agricultural productivity can be made to guide decisions regarding variable-rate irrigation, variable-rate pesticide application, and variable-rate fertilization (Saiz-Rubio and Rovira-Más, 2020).

Conventional methods such as surveys, field sampling and laboratory analysis have been the most common approaches utilized in the past when compiling data on crop nutrition, crop growth, crop yield, and soil nutrition. In the past, this task has been predominantly accomplished. In the field of precision agriculture, production managers can collect historical data, current data, point data, and any other necessary materials in order to form opinions regarding variable-rate activities (Bechar and Vigneault, 2016). The most time-consuming and expensive method of collecting geographical data about farmlands is now the most significant obstacle standing in the way of widespread implementation of precision agriculture (Mark and Griffin, 2016). This section investigates ways for gathering spatial data about farmlands by remote sensing equipment in order to satisfy the goals of precision agriculture.

The history of the development of automated agricultural devices is somewhat extensive (Lowenberg-DeBoer and Erickson, 2019). When

electronics for monitoring and control were first implemented into agricultural machinery in the 1970s, a significant amount of forward movement occurred. While progress towards fully autonomous machinery has been gradual, it was not until the advent of precision agriculture (PA) in the 1990s that significant strides were made in this direction (Lee et al., 2010). An intense management effort of the spatial and temporal diversity of fields is required for PA. As a result, the operation of machines must shift towards becoming more automated or autonomous. One of the most important PA techniques, variable-rate application of inputs, for instance, requires that the rate of application be altered while the process is in progress, and in some cases, even within every square meter of a field (Han et al., 2015). The equipment and its control cannot be operated manually, thus there is no other option. Hence, the device should have map-based, autonomous steering and speed regulation.

Soil sampling, crop reconnaissance, site-specific weed management, and selective harvesting are just a few examples of the many tasks in PA that call for the use of small, smart devices (robots) (Shamshiri et al., 2018). Robotic applications are not only desirable in some agricultural situations, but they are also more economically feasible than the alternatives, which are traditional methods (Ditzler and Driessen, 2022).

## 2. Assessment of Crop Data Using Remote Sensing Techniques

The growth of a crop can be examined using a variety of plant parameters, including its leaf area, leaf color, leaf inclination angle, plant height, and stem diameter, amongst other plant traits. Because of their direct connection to the process of plant development, additional factors, such as crop nitrogen, leaf area index, and biomass, are also used in the classification of plants. The foundation for the management and regulation of crop growth is the observation and analysis of the crop's nutritional status. When compared to healthy vegetation during the growth phase, nitrogen-deficient vegetation may experience a range of physiological, biochemical, and canopy structural changes. These changes can be attributed to several different factors. Little leaves and decreased biomass in the vegetation may result from decreased formation of proteins, nucleic acids, and lipids, which contain organic nitrogen.

The measurement of water stress is crucial for both the irrigation strategies that are used and the drought assessments that are performed on natural populations. Water stress is one of the most common constraints to photosynthesis and plant primary productivity (Penuelas et al., 1993). The spectra of the plants would exhibit a particular shift in response to changes in these factors. The use of remote sensing makes it possible to determine the amount of nitrogen present in crops as well as the amount of water present in crops based on this change, which serves as the physical basis for the determination (Serrano et al., 2002).

## 2.1. Crop Chlorophyll Content

The essential component for photosynthesis in plants is chlorophyll. Moreover, it serves as a gauge for crop growth. The chemo metrics approach is one of the common ways to measure the amount of chlorophyll and SPAD for nondestructive measuring (Haboudane et al., 2002).

When there is an insufficient supply of fertilizer, the chlorophyll level will normally increase in the upper leaves while decreasing in the lower leaves. Chlorophyll has properties that appear to absorb light in the visible spectrum, and these properties have a strong connection to nitrogen in plants. Canopy chlorophyll density (CCD) was found to be a sensitive indication of N deficits in wheat (Zhao et al., 2012). Chlorophyll content has been estimated using different spectral indices throughout the past few decades (Zhao et al., 2015). The chlorophyll content of maize leaves was estimated from their visible to near-infrared (400-1000 nm) spectra by (Li, D. et al., 2017) using a continuous wavelet transform (CWT).

## 2.2. Nitrogen

For crop growth, nitrogen (N) is a crucial nutritional element. Wheat output can be increased, environmental contamination can be reduced, and N use efficiency can be increased with timely and ideal N fertilizer supply (NUE). N fertilizer recommendations can be made effectively using laboratory-based techniques, such as soil NO<sub>3</sub>-N (nitrate N) or NH<sub>4</sub><sup>+</sup> -N (ammonium N) testing, and plant tissue (either sap or petiole) assays (Demestichas and Daskalakis, 2020).

Prior research has demonstrated a strong correlation between plant leaf N concentration (LNC) and leaf chlorophyll content (Jiang et al., 2018).

So, in order to measure the crop's N status, it is necessary to measure the spectral reflectance of the leaf or canopy. The vast majority of agricultural nitrogen status evaluations are constructed on the basis of correlations between the spectral properties of a single leaf or the entire canopy and the leaf-level nitrogen content (LNC) of the plant. The use of remote sensing makes it possible to determine the amount of nitrogen present in crops as well as the amount of water present in crops based on this change, which serves as the physical basis for the determination (Alchanatis et al., 2005).

A sensitive diagnostic for spotting N deficiency in wheat is canopy N density (CND) (Zhou et al., 2017). CND can be determined using the following formula, where CND is defined as the total leaf nitrogen per unit of land area:

LNC + SLW + LAI = CND

SLW stands for specific leaf weight, LNC for leaf nitrogen content, and LAI for leaf area index.

Old leaves' nitrogen migrates to young leaves when there is a nitrogen shortage. Therefore, under nitrogen stress, the lower leaves turn yellow, and this condition eventually spread to the top leaves. As a result, it is beneficial to consider the vertical distribution of N as well as spectral response that comes along with it. Few field investigations have focused on the difficult problem of employing remote sensing to measure the distribution of leaf N in the crop canopy (Chen et al., 2022). The utilization of hyper spectral data allows for the classification of the

existing studies into three categories. One class used spectral information from top-view observations to estimate leaf N content of various vertical levels (Huang et al., 2007). In the third unit, students investigated the spectral reflectance and fluorescence properties of several vertical leaf layers, as well as their correlations to the N or chlorophyll content of the corresponding leaf. Another group used data on the canopy's reflectance from multiple angles (Zhu et al., 2007). These investigations have significantly improved our ability to remotely detect the spread of leaf N.

## 2.3. Leaf Area Index

As a measure of a crop's development, LAI is vital for determining when and how much fertilizer to apply. It can also serve as a manual for drip or trickle irrigation systems (Kar et al., 2020) computed LAI by taking sum of the leaf areas of all the plants in a given area. Measurement of leaf area is at the heart of both direct and semi direct approaches. This can be done directly with leaf area meter or indirectly with a known link between dimension and area through shape coefficient (Zarco-Tejada et al., 2008). The LAI-2000 is another gadget that can measure LAI (LI-COR, USA).

Spectral variation of crops is utilized by the LAI inversion technique that is based on remote sensing technology. Appropriate field management tactics in agricultural production benefit from timely, accurate, and dynamic acquisition of crop LAI. Statistical algorithms (Liang et al., 2015), nonparametric algorithms (Verrelst et al., 2015), physical models (Ke et al., 2016), and data assimilation algorithms are the most used techniques (Li, H. et al., 2017).

## 2.4. Water Content

Because of its role in photosynthesis and transpiration, water is a crucial component of plants. Also, it's an essential factor in the success of irrigation systems for farms. It is usual practice to utilize procedures including weighting in order to estimate the water content of plant tissue, which serves as an indicator of the physiological state of the plant (Tian et al., 2022).

Each leaf has its own distinct spectral qualities because water, pigments, and dry components in leaves absorb and scatter light in different ways. Most significantly, water in leaves absorbs light at a frequency that is double or combined with the oscillation of water molecules, broadening the spectrum of the leaf (e.g., 1200, 1450, and 1950 nm) (Murphy et al., 2019).

The use of remote sensing to detect liquid water in vegetation is extremely beneficial to agriculture and forestry (Swain, 2012). Measuring water stress is necessary for irrigation planning and understanding how drought affects natural populations since it is one of the most frequent factors impacting photosynthesis and plant primary productivity (Liu et al., 2004). Leaf spectral reflectance is primarily affected by its water content because of its ability to absorb photons. Other biological components including protein, lignin, and cellulose rarely have an impact on the spectral reflectance of green plants in the 1300–2500 nm region (Danson and Bowyer, 2004). The NIR reflectance of a leaf's surface can be affected by a number of factors, including the leaf's internal organization, the amount of dry matter (mainly protein, lignin, and cellulose), and two minute water-related absorption bands located at 975 and 1200 nm (Ma et al., 2019). The radiative properties of water also fall short of explaining the secondary effects of water content on reflectance. The transmissive properties of water, as opposed to its absorptive ones, have an impact on some of tertiary effects of the water content on the leaf reflectance. Variations in NIR reflectance may occur as LWC decreases due to changes in interior leaf structure, such as the proportion of air holes in the spongy mesophyll (Davidson et al., 2006).

## 3. Assessment of Cropland Data

The term "acquisition of agricultural information by machine" denotes the process of gathering data about farmland through the use of machines, such as tractors and reaper that are equipped with calculators and sensors. This section provides a full description for the rapid collecting of information regarding crop output and soil nutrition by machine-borne equipment (Wang et al., 2014).

An example is the Trimble WeedSeeker, which uses sensors to detect and spray herbicides only on weeds, reducing the amount of chemicals applied and improving efficiency. Intelligent machinery can also be used for soil mapping and analysis, such as the Veris Technologies iScan, which uses electromagnetic induction technology to map soil properties and identify variations in soil texture and nutrient content (Peteinatos et al., 2014).

## 3.1. Crop Yield Data Assessment through Combine Harvester

The first steps in precision agriculture involve obtaining information about crop yields on the plot and creating a map of the geographical distribution of crops. Decisions about scientific input regulation and agriculture policy can be based on this (Chapman et al., 2014). The AFS system (CASE, USA), FieldStar system (Massey Ferguson, UK), GreenStar system (John Deere, USA) (Singh et al., 2012), and the PF system (Pioneer Farm Systems, Canada) (Amado et al., 2020) are the most common commercially available yield estimating systems installed on a combine harvester (Ag Leader, USA) (Sirikun et al., 2021).

The end product is utilized for harvest analysis and as a foundation for variable-rate farming. It also manages crop data by harvest time and place. At predetermined user-defined intervals or at regular intervals, the yield statistics are categorized. The field conditions and low-yield areas can be identified thanks to the use of different colors for each classification.

A Differential GPS device (DGPS), speed sensors for the wheels, an intelligent terminal, and the grain elevator, a grain water content sensor, a header height potentiometer, a memory card, a grain flow sensor, and graphical software make up the yield estimation system (CASE) for axial flow type combine harvesters (Figure 3.1). The grain flow sensor for the elevator is fixed to the top of the elevator. The sensor's impact

plate is where the grain, guided by the deflector, makes contact as it descends from the elevator's hopper. As an output, electric signal is generated from the impact signals. Signal strength is proportional to grain throughput. The rotational speed of a grain elevator can be determined with the help of a Hall sensor. The sensor's output signals are employed to modify the flow sensor's output signals and place constraints on the sensor's operational state. The signal processing unit receives data from grain flow sensor, the header height potentiometer, grain water content sensor, elevator rotation speed sensor, and wheel speed sensor. Monitoring is done for the machine's operating area, travel distance, transient grain water content, and transient grain flow. DGPS is used to provide this positional data. Software utilizing in-situ calibration successfully removes the measurement inaccuracies as these signals get to the smart terminal. After that, crop yield of all plots is calculated for each location based on the information that is provided on the data card. After bringing data card back to office with you, the specialist will use specialized data processing software to develop a spatial distribution graphic of the yield. The completed product is put to use in the yield analysis and serves as foundation in the implementation of the variable-rate farming. Instant Yield Map is part of software that can produce point diagrams, the grid maps, the smooth grid maps, and the other line graphs of harvest based on the original information stored in data card. In addition to managing yield data by the harvest time and location, this software can also produce yield diagrams using CASE. CASE is a program that is used to make yield diagrams. The yield data are categorized at predetermined intervals,

which can be regular intervals or intervals chosen by the user. It is possible to rapidly detect the field conditions and locate portions of the field that produce low yields thanks to the utilization of a variety of colors for each classification.

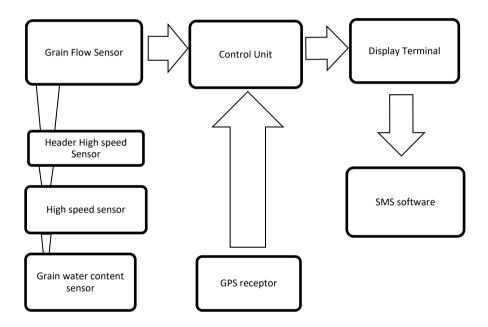


Figure 1. Yield Estimation System on an Axial Flow-type Combine Harvester

## 4. Remote Sensing Data Interpretation for Crops

Remote sensing technology is becoming increasingly important in agriculture for monitoring soil and plant health, predicting crop yields, and optimizing management practices. Recent advancements in remote sensing technology have improved the accuracy and resolution of data collected, allowing for more precise and targeted decision-making (Li et al., 2020).

One recent development in remote sensing is the use of hyperspectral imaging, which allows for the detection of subtle variations in soil and plant characteristics. Hyperspectral imaging sensors can detect a wide range of wavelengths of light, providing a detailed picture of the chemical composition and health of crops (Lu et al., 2020). This technology has been used for mapping soil properties, identifying nutrient deficiencies in plants, and monitoring water stress (Virnodkar et al., 2020).

Another recent advancement in remote sensing is the use of unmanned aerial vehicles (UAVs) or drones for collecting data. UAVs equipped with cameras or other sensors can be used to collect high-resolution images of crops, allowing for detailed analysis of plant health and growth. This technology has the potential to significantly reduce labor costs and improve the efficiency of monitoring practices (Gaffey and Bhardwaj, 2020).

The integration of remote sensing data with machine learning algorithms is also an area of active research. Machine learning can be used to analyze large datasets and predict crop yields, identify crop stress and diseases, and optimize management practices based on the collected data (Khanal et al., 2018).

The use of spectral theory in agricultural remote sensing places an emphasis on the spectral data of the ground objects, such as soil and plants, and makes use of technology. The field of spectral theory is applied to both the items on the ground and the green vegetation. The features of absorption, scattering, and reflection at a variety of determined by physiological and metabolic wavelengths are components of leaves of green plants. These characteristics serve as basis for agricultural remote sensing because they are the foundation for the technology that underpins the practice. At the moment, important biological and physicochemical properties of crops can be extracted from images captured by remote sensing technologies. These aspects include chlorophyll, nitrogen, the leaf area index (LAI), aboveground biomass, the amount of water, and the type of plant (Dyson et al., 2019). It is of the utmost importance to have a solid understanding of the relationships between the characteristics of the crop and the spectrum reflection properties of canopy, particularly in visible, near-infrared, and the middle-infrared bands. This is because these relationships have a significant impact on the crop's ability to absorb and reflect light. The geometric structure of canopy, the biochemical content of leaves, internal structure of tissue are all examples of relationships that exist between these elements. The use of remote sensing as a tool for gathering information on agricultural operations is one that has previously been demonstrated to be successful (Kimes, 1980). Yet, precision agriculture cannot make direct use of remote sensing data. In order to construct models showing how remote sensing data relates to the growth of crops, information interpretation is essential. It is necessary to have both the method for inverting agricultural parameter space and ways for generating maps to facilitate the management of farmland productivity (Waldhoff et al., 2012).

## 5. Framework for Intelligent Machine Design

There are many different ways to characterize an "intelligent machine." One school of thought holds that an intelligent machine behaves similarly to a human in the same situation. According to this definition, intelligent machines must possess traits like thinking, perception, learning, control, and supervision (Choi et al., 2021). Another line of reasoning leads to a definition that is more applicable to real-world situations: an intelligent machine is one that accomplishes a predetermined purpose despite the existence of ambiguity and variety (Steward et al., 2019). Here, the bar for what constitutes an intelligent machine is lowered. Due of the high levels of uncertainty and variable inherent in agriculture, this description likely applies to the vast majority of automated farm equipment. Agricultural machinery at the present technological stage falls between fully automated machines, which can do a set of preprogrammed duties with little to no human input, and fully intelligent machines, which can think for themselves and engage in more complex behaviors.

In order to make machines that are more intelligent and to build on the level of technology that is currently available, theoretical frameworks have been developed to categorize necessary machineries for intelligent agricultural machines. This has been done to build on the level of technology that is currently available. The reason for this is to improve upon the level of technology that is now available. This effort was done with the intention of improving upon the existing level of technological capability. Several authors have viewed these classifications as a collection of components that can be used to construct agricultural machinery (Tabile et al., 2011).

### 6. Intelligent Machine Categorization

There is a wide variety of applications for artificial intelligence and automation technology in the agricultural sector. In order to have a discussion that is more narrowly focused on intelligent machines, one must first devise a method for the classification of the various machine systems. The varieties of plants and animals that can be successfully cultivated with the assistance of various intelligent agricultural devices can be used to broadly categorize the devices (Bagheri, 2017). Irrigation networks, livestock barns, orchard equipment, greenhouses, and farm tractors are all part of the agricultural production infrastructure (Figure 2).

Intelligent agriculture machinery refers to the use of advanced technologies, such as sensors, artificial intelligence, and big data, to optimize agricultural operations and improve crop yield. These technologies can be used to monitor soil and plant health, automate irrigation and fertilization, and predict weather patterns, among other applications. One example of intelligent agriculture machinery is the John Deere ExactEmerge planter, which uses sensors and GPS technology to precisely place seeds and optimize planting density (Idoje et al., 2021).

This form of organization is geared towards conserving water through the mechanization of irrigation processes. Typically, automated technology will adjust the amount of water given to the crop based on the condition of the crop and the soil. Changing the pace at which water is released from the nozzles or the irrigation system passing over the crop is how variable-rate irrigation works (Yang et al., 2016).

Field robots are a new technology in agriculture that have shown great potential for improving crop yield and reducing labor costs. These robots are designed to operate autonomously in a field, collecting data and performing tasks related to soil and plant health. One example of such a robot is the TerraSentia robot, developed by engineers at the University of Illinois. The TerraSentia robot is equipped with sensors that can measure soil properties such as moisture, temperature, and texture, as well as plant characteristics such as height and biomass. This data can be used to optimize irrigation, fertilization, and other management practices, leading to increased yields and reduced resource use (Bao et al., 2021).

In addition to the TerraSentia robot, there are other field robots in development for agriculture, such as the BoniRob, developed by Bosch Deepfield Robotics, and the SwagBot, developed by the University of Sydney. These robots are also designed to perform tasks such as weed detection and removal, crop monitoring, and harvesting (Wisse et al., 2020).

The use of field robots in agriculture has the potential to revolutionize the industry, improving efficiency and sustainability. According to a report by Research and Markets, the global market for agricultural robots is expected to grow from \$864 million in 2020 to \$4.2 billion by 2026. However, the adoption of these technologies may be hindered by high initial costs and limited availability in certain regions (Market—Growth, 2021).

Another type of agricultural automation system is the greenhouse plant production system, which allows for precise regulation of a wide range of environmental factors. Temperature, humidity, luminosity, and carbon dioxide concentration are examples. It's possible that AI and models of plant physiology will be used in some of the many existing methods of control (Ferentinos, 2018). A similar category could be made for greenhouse-specific robots.

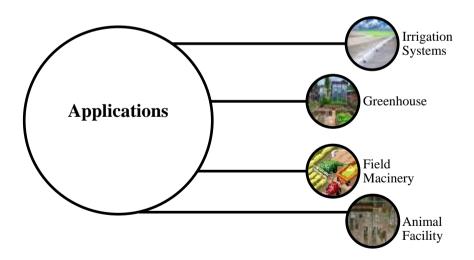


Figure 2. Yield Estimation System on an Axial Flow-type Combine Harvester

Automation and mechanization techniques are being rapidly developed and implemented for the fruit production in the orchard crops in industrialized countries due to a lack of available labor and high labor expenses. Among the specific horticultural procedures that have been advanced are automated methods of pruning and hedging, fruit thinning, the application of chemicals, and the gathering of produce. Research and development efforts have been focused on robotic fruit picking because of the unique difficulties it presents and the potentially large impact it could have on fruit output. In addition, tree crop monitoring technologies have emerged (Zhang and Pierce, 2013).

Animal sanctuaries also make use of automation technology. One use is ensuring optimal conditions for the health and well-being of animals housed indoors by controlling factors including temperature, humidity, and gas concentrations (Purswell and Gates, 2013). Automatic feed distribution systems provide for precise feeding management of single or multiple animal populations (Tantalaki et al., 2019). In the same vein as these automated processes are AMSs, or automated milking robots. Milk from dairy cows is mechanically collected by AMS, eliminating the requirement for human labor during the milking process. Rapid adoption of these systems in North America and Europe (Bhoj et al., 2022) is altering many aspects of dairy production. This includes the traditional farmer's position, current dairy management practices, and the dynamics between farmers and their cows (Smith, 2018).

## 7. Precision Management Techniques: Prescriptions

Experiments in precision agriculture have the potential to yield copious amounts of information on farming. Prescription maps for decision making can be created with the use of these data, which necessitate management, analysis, and processing. In addition to graphic processing and math, the development of prescription maps and decision-making also involve the representation and inference of practice and the knowledge. Understanding how to leverage data technology to support the variable-rate activities is essential for precision agriculture (Larson et al., 2008).

Precision agriculture relies on both historical and real-time data. The historical context is broken down into two categories: yield and soil nutritional data. Data depicting plant growth in real time includes spaceborne and airborne remote sensing information (for the inversion of chlorophyll and LAI) and other data collected in real time (by SPAD reading, LAI) (Thenkabail, 2003).

#### 8. Conclusion and Future Prospective

Agriculture must produce more food for an expanding population. Land, water, and production inputs are limited. Hence, efficiencyimproving technology must supply 70% of the extra food (Liu et al., 2021). Precision Agriculture (PA) uses machine automation. Automation improves productivity and solves labor shortages and excessive labor costs caused by the ageing farm population. Automation, variable-rate application, section control, machine coordination, and logistical assistance have improved since the early 2000s. Automation technology is rapidly transforming agriculture. Robotic farming is still far off. Several technologies needed for intelligent agricultural machinery are still in development. The most challenging aspects are mission planning, implement monitoring, and machine health awareness and protection.

Future intelligent production machinery small robots may farm. Very tiny autonomous robots have numerous benefits. Large agricultural machinery can compact soil, although tiny machines can reduce this. Small machines may cut energy use in field operations, according to research (Toledo et al., 2014).

The use of intelligent agriculture machinery has the potential to improve efficiency, reduce input costs, and increase crop yields. According to a report by MarketsandMarkets, the global market for precision agriculture, which includes intelligent machinery, is expected to reach \$12.9 billion by 2027, growing at a CAGR of 13.5% from 2020 to 2027. However, the adoption of these technologies may be hindered by high costs and limited availability in certain regions. Additionally, there may be concerns about the ethical and environmental implications of increased reliance on technology in agriculture (MarketsandMarkets, 2021).

There are very few instances of agricultural robots being successfully commercialized. Technical and economic factors account for the majority of the tardy commercialization. When human operators are removed from a machine that is carrying out a field activity, the responsibility of the machine's supervisory control mustbe placed on machine intelligence systems according to the theory. To achieve this level of intelligence up to this point in time has been a difficult task. Economically, it is now impractical to cover the huge expense of giving the machine the requisite intelligence. However, due to recent technology developments in areas including as sensors and the controls, the precision guidance, the machine communications, data management, and the power electronics, the robotic farming could become an authenticity in the near future.

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## **CHAPTER 2**

# THE EFFECT OF DIFFERENT ORGANOMINERAL AND MINERAL FERTILIZER APPLICATIONS ON SOME PHYSIOLOGICAL PROPERTIES IN RED RADISH (*Raphanus sativus var. sativus*) GROWING

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#### **1. INTRODUCTION**

New approaches in plant production gain importance due to environmental problems caused by agricultural chemicals used extensively in traditional methods. It has been stated that the application of inorganic and organic fertilizers by combining not only increases the yield of vegetables, but also can be a method used to prevent environmental problems (Yusheng et al., 2005). In this context, animal waste, compost, etc. materials have been widely used (Cıtak and Sonmez, 2010). Some physical and chemical properties of hazelnut husk, which has a large waste potential, can be evaluated as an organic material. However, due to the high C/N, it should not be used directly, but by composting (Çalışkan et al., 1996). In many studies, it has been shown that some physical and chemical properties of composted hazelnut husk have values that can be evaluated in terms of its use as an organic material (Özenç and Şenlikoğlu, 2017).

Due to the volume of today's agricultural practices, even the most fertile soils alone are not sufficient to meet all the needs of crop production. The physical, chemical and biological structure of the soil deteriorates rapidly due to rapid and large quantities of crops being grown. This situation causes low productivity and therefore economic loss. At the beginning of the solutions developed for this situation is the enrichment of the soil in terms of plant nutrients by using chemical fertilizers. However, this solution is expensive and the effects are short-lived due to the characteristics of the fertilizers used. In addition, chemical fertilizer applications without sufficient knowledge and technological opportunities pose a significant risk for human health and the natural environment, especially water resources. At the same time, fertilizer applications only improve the chemical properties of the soil and do not contribute to the physical and biological properties of the soil, which has very important functions for ideal plant growth (Özer, 2017).

the new generation fertilizers. Organomineral fertilizers are Organomineral fertilizers are new fertilizers that contain the mineral plant nutrients and organic matter contained in chemical fertilizers together. Organomineral fertilizers, which combine two different fertilizer groups, combined the advantages of organic and mineral fertilizers. Organomineral fertilizers contain N, K, P, Zn and S plant nutrients together with humic-fulvic and compost-containing organic materials. It is applied as base and top fertilizer. Organomineral fertilizers contain humic and fulvic acids from humic substances. sustainability of soil efficiency, physical, chemical and biological benefits. For this reason, organic matter increases the mineral holding capacity of soils (cation exchange), air and water retention, trace element levels, balances the pH level and regulates the microorganism balance. The soil structure-improving effects of the organic matter in the organomineral content affect the crop yield positively (Süzer and Culhacı, 2017).

Radish (*Raphanus sativus* L.) is a nutrient-rich vegetable belonging to the Brassicaceae (Cruciferae) family, with a wide variation, spread area and production especially in China, Japan, Korea and South Asia, which has an important place in meeting the fresh vegetable needs of people (Wang and He, 2005). The root part consumed in radishes has different shapes, colors and sizes. Among the radish genotypes, those with small and red roots are hazelnut; the white ones are called chestnut and the black ones are called horseradish (Vural et al., 2000).

It is known that it is a vegetable with a rich nutritional content, as well as having an important place in meeting the needs of people for fresh vegetables. 100 g radish 90-95% water, 5-10% dry matter, 17 cal energy, 0.1 g fat, 3.6 g carbohydrate, 1 g protein, 26 mg vitamin C, 10 IU vitamin A, 0.03 mg Thiamine and Riboflavin contain 0.3 mg Niacin, 30 mg Ca, 1 mg Fe, 18 mg Na, 31 mg P and 322 mg K (Günay 2005; Güllüce et al., 2012),

In the long term, it is thought that the widespread use of organomineral fertilizers in our country will contribute to the increase in the organic matter content of the soils and increase the yield and quality. However, despite the increase in the production and use of organomineral fertilizers in our country in recent years (it is stated that approximately 350 thousand tons of OMF was produced in 2019), studies on the effectiveness of chemical and organomineral fertilizers in our country are not sufficient (Atici, 2020).

In parallel with the increasing population, agricultural production should be increased in order to meet the nutritional needs. Fertilization takes the biggest share in increasing the productivity based on agricultural production. Innovations in fertilizer technology and improving the efficiency of use are of great importance.

# 2. MATERIAL and METHOD

# 2.1. Material

In this research, red radish (*Raphanus sativus* var. *sativus*) belonging to Arzuman company, which is widely grown in Turkey, was used (Figure 1). In the research, organomineral and mineral fertilizers (Figure 2) were supplied from ASOS Process Machinery Industry and Trade Inc. (Çorlu/Tekirdağ).

For this purpose, 2 organomineral fertilizers (8:8:8 NPK + 22% organic matter and 11:11:11 NPK + 10% organic matter) with different organic matter amounts obtained by using ash and urea as organomineral fertilizers and 2 mineral fertilizers (15:15:15 NPK+ trace elements and 12:12:17 NPK + trace elements) in total 4 types of fertilizers were used. Information on organomineral and mineral fertilizers used in the research is given in Table 1 and Table 2 below.



Figure 1. Seeds of the Red Radish Vegetable (Savci and Deveci, 2022)



**Figure 2.** Organomineral and Mineral Fertilizers Procured from ASOS Process Machinery Industry and Trade Inc (Savci and Deveci, 2022)

No	No Input		8:8	3:8	11:11:11		
INO			Quantit	%	Quantit	%	
1	Ash	g	624,2	31	315	32	
2	Organik	g	432,4	22	101	10	
3	Urea	g	273,4	14	171	17	
4	MKP	g	0	0	22,5	2	
5	DAP	g	0	0	63	6	
6	K2SO4	g	28,8	1	50,6	5	
7	H2SO4	g	480,4	24	180	18	
8	H3PO4	g	88,8	4	29,4	3	
9	7%	g	72,0	4	67,5	7	
TOT	AL		2000				

 Table 1. 8:8:8 and 11:11:11 Organomineral granular fertilizer content

Table 2. 15:15:15 and 12:12:17 Mineral fertilizer conten

No	No Input		15:15	:15	12:12:17		
140	mput	Unit	Quantity	%	Quantit	%	
1	Waste Plant Ash	g	730,0	14,60	1000,0	20,0	
2	H <sub>2</sub> SO <sub>4</sub> % 98	g	255,5	5,11	300,0	6,0	
3	Urea	g	1250,0	25,00	980,0	19,6	
4	DAP	g	1041,8	20,84	1050,0	21,0	
5	MAP	g	400,0	8,00	100,0	2,0	
6	K2SO4	g	1322,7	26,45	1470,0	29,4	
TO	TAL		5000,0	100,0	5000,0	100,0	

## 2.2. Method

The cultivation of plants in the research was carried out in Tekirdağ Namık Kemal University Faculty of Agriculture, Horticulture Department laboratories and climate room (Figure 3).



Figure 3. Tekirdağ Namık Kemal University Faculty of Agriculture Department of Horticulture Climate chamber (Savci and Deveci, 2022)

Chemical analyzes were carried out at the Faculty of Agriculture, Horticulture laboratory, Namık Kemal University Scientific and Technological Research Application and Research Center (NABİLTEM) and Tekirdağ Commodity Exchange laboratories.

Our experiment was set up in a climate chamber whose temperature can be adjusted between +40 °C and -20 °C under controlled conditions. All experiments were carried out in a climate chamber with a light intensity of 400  $\mu$ mol m<sup>-2</sup>s<sup>-1</sup>, at 22-23 °C during the day, 17-18 °C at night, 65-70 % humidity, 10/14 (bright/night) hour photoperiodical order.

Red radish seeds will be planted in plastic pots on the growing tables in the climate room. The plants were grown in garden soil filled in plastic pots with a volume of 800 ml (13x11cm), and 2 seeds were planted in each pot, and after the seedlings germinated, the dilutions were completed as 1 plant in each pot (Figure 4).



**Figure 4.** Plastic Pots With A Volume of 800 ml (13x11cm) on the Growing Tables in the Climate Chamber (Savci and Deveci, 2022)

Soils from two different soil layers were obtained for the establishment of the experiment and samples from these soils were sent for analysis (Table 3). These soils were moist, first the weight of the wet soil for one pot was taken and laid in the laboratory to dry to make it suitable before placing in the pots (Figure 5). The laboratory was brought to the appropriate temperature for the soils to dry.

In the experiment, garden soil sieved through a 4 mm sieve was placed in plastic pots. As a result of the soil analysis, the texture and chemical composition of the soil were determined.

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11000		37,88	400.04	97.40	29990.00	3,80	3,81	11,419	0.47	8,24	0.010	0.07	- 0.1
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000	1	32,55	813,38	444,018	2909,201	1.01	3.17		8,73		11,000	1.10,000	- 60

**Table 3.** NABİLTEM Analysis Results of Soil Samples Filled in Plastic Pots

After planting the seeds and germination, the pots were irrigated automatically every 3 days with the drip irrigation system. The cultivation of vegetables was carried out according to Şalk et al., 2008.

In the fertilizations, the garden soil to be placed in the pots was used without fertilizer, the soil in the range of macro-micronutrients that should be in vegetable cultivation, and the soils with 5 different fertilizations (Control, 2 organomineral and 2 mineral fertilization).



**Figure 5.** Drying of the Soil to be Used in the Experiment (Savc1 and Deveci, 2022) Two different doses were used for mixing organomineral and mineral fertilizers into the garden soil. Accordingly, the pots were fertilized at 10 and 20 kg/da N. Organomineral and mineral fertilizers were given

to the pots in 3 equal parts. Half of the total fertilizer (5 and 10 kg/da N) in the pots before the first fertilization, 10 days after the first true leaf emergence in the second fertilization (2.5 and 5 kg/da N), 20 days after the first true leaf emergence in the last fertilization (2, 5 and 5 Kg/da N) were added to the pots to provide the determined concentrations.

The research was established according to the 3-factor factorial experiment design in random plots in the climate chamber. Each production period will be evaluated on its own. In each replication of the experiment, 2 different soils, 5 different fertilizers (without fertilizer, 2 organomineral and 2 mineral fertilizers), 2 fertilizer doses (10 and 20 kg/da N) were applied. In each type of vegetable, there will be 2 soils, 5 fertilizers, 2 fertilizer doses and 3 replications, a total of 60 plots, 3 plants in each plot and a total of 360 plants in the whole experiment.

Statistical analyzes of the data obtained from the experiment were made using the MSTAT version 3.00 /EM package program. For the significant differences, the groups that were differentiated by the LSD control method were determined (Akdemir et al., 1994). The trial plan for the application of different factors on red radish in the experiment is given in Table 4.

The solution, for which the necessary calculations were made, was prepared and applied to the pots to be applied to the soil to be improved in the experiment. The fertilizers to be applied were prepared as 800 ml each according to the calculations made before. Some pictures of the trial period are given in Figure 6-12.

Soil	Fertilizer	Dose (kg/da	Practice
		N)	
	Control (Without Fertilizer)		GS+C
Garden Soil (GS)	Organomineral 1 (OM <sub>1</sub> )	10	$GS+OM_1+\%10$
		20	$GS+OM_1+\%20$
	Organomineral 2 (OM <sub>2</sub> )	10	$GS+OM_2+\%10$
		20	$GS+OM_{2}+\%20$
	Mineral 1 (M <sub>1</sub> )	10	$GS+M_1+\%10$
		20	$GS+M_1+\%20$
	Mineral 2 (M <sub>2</sub> )	10	$GS+M_2+\%10$
		20	$GS+M_2+\%20$
Garden Soil with	Control (Without Fertilizer)		IGS+C
Added Macro-	Organomineral 1 (OM <sub>1</sub> )	10	$IGS{+}OM_1{+}\%10$
Micronutrient		20	$IGS+OM_1+\%20$
Element in Red	Organomineral 2 (OM <sub>2</sub> )	10	IGS+OM <sub>2</sub> +%10
Radish		20	$IGS{+}OM_2{+}\%20$
Cultivation	Mineral 1 ( $M_1$ )	10	$IGS+M_1+\%10$
(Improved		20	$IGS+M_1+\%20$
Garden Soil)	Mineral 2 (M <sub>2</sub> )	10	$IGS+M_2+\%10$
Garuen Soll)		20	IGS+M2+%20

**Table 4.** Trial Plan of The Application of Different Factors on Red Radish



Figure 6. 20 g of Leonardite was Applied to Each Pot to Prepare Improved Garden Soil (Savc1 and Deveci, 2022)



Figure 7. 1.2 g Zinc Sulphate was Added to 4,000 ml of Water Prepared for Improved Garden Soil (Savc1 and Deveci, 2022)



Figure 8. 60 gr DKP was Added to 4,000 ml of Water Prepared for Improved Garden Soil (Savcı and Deveci, 2022)



Figure 9. 10 ml of Liquid Sulfur was Added to 4,000 ml of Water Prepared for Improved Garden Soil (Savc1 and Deveci, 2022)



Figure 10. 6.6 g of Iron Chelate was Added to 4,000 ml of Water Prepared for Improved Garden Soil (Savc1 and Deveci, 2022)



Figure 11. The Mixture Prepared for Improved Garden Soil (4,000 ml) was Applied to Each Pot as 20 ml (Savcı and Deveci, 2022)



Figure 12. Growing Plants Under Controlled Conditions in the Climate Chamber (Savcı and Deveci, 2022)

## 2.3. Experimental Measurements

## 2.3.1. Marketable plant weight (g)

Plants with roots cut, outer leaves removed and marketable plants were weighed on a precision balance sensitive to 0.0001g.

## 2.3.2. Determining Color Values

Color measurements were performed using the HunterLab D25LT (Hunter Associates Laboratory Inc., Virginia, USA) colorimeter, which has a very large measurement area, especially suitable for measuring the colors of non-homogeneous materials (Figure 13). Color parameters

measured with each device; color brightness (L\*) and color coordinates (a\* and b\*). The L\* value ranges from 0 to 100 and 0 indicates black and 100 indicates white. The color coordinates a\* and b\* do not have a specific measurement range and take positive and negative values. The value a\* represents the red-green axis, positive values represent red, negative values represent green, while 0 is neutral. In the 2nd color coordinate b\*, positive values show yellow and negative values show blue (Figure 14) (Eryılmaz Açıkgöz et al., 2015).



Figure 13. Determination of the Color Values of the Red Radish body in the Hunter Lab Device (Savc1 and Deveci, 2022)

The measurements were carried out using 3 different plants from each plot and selecting the most developed leaf from each plant during the harvest period. Measurements made on selected leaves were carried out on the leaf with at least 3 replications.

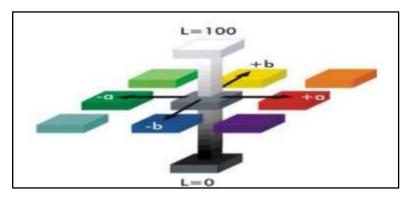


Figure 14. Hunter Lab Color Scale

#### 2.3.3. Amount of chlorophyll (SPAD Value)

For the amount of chlorophyll, in the leaves of 4 randomly selected plants from each plot during the harvest period, two regions of the leaf close to the main vein were measured with a "Konica Minolta SPAD-502" portable chlorophyllmeter (Figure 15) (Geravandi et al., 2011).



Figure 15. Measuring Plant Leaves with A Portable Chlorophyll Meter (Savcı and Deveci, 2022)

## 2.3.4. Amount of water-soluble dry matter (%)

The amount of water-soluble dry matter of the leaf juices obtained from the leaves during the harvest period was measured with a hand refractometer (Figure 16).



Figure 16. Measuring the Water Obtained from the Leaves During the Harvest Period with a Hand Refractometer (Savcı and Deveci, 2022)

## 3. RESULTS

## 3.1. Determination of Red Radish Color Values

## 3.1.1. Red Radish Leaf Color L \*Value

The results of the measurements made to determine the color L \*values of red radish are as in Table 5. We have 3 main factors in this experiment.

We have soil main factor, these are GS and IGS. We have the main dose factor, these are fertilizer doses of 10 kg/ha and 20 kg/ha. And our last main element is Fertilizer types, these are we have 1 control and 4

fertilizers. 2 of them are organomineral, 2 of them are mineral fertilizers.

#### 3.1.2. Red Radish Leaf Color a\* Value

The measurement results to determine the color  $a^*$  values of red radishes are as in Table 6. If we examine the table, when we look at the results only in terms of soil type, it is seen that the IGS type has higher results than the GS type, as in the color L\* value. And again, as in color L\*, when we examine the main effect of the fertilizer dose, it is seen that 20 kg/da of fertilizer contains higher results than 10 kg/da of fertilizer.

When the table is examined according to our fertilizer type, it is understood from our table that mineral 2 (M2) fertilizer gives the highest result statistically out of 5 fertilizer applications.

Main			Contro			Minera	Minera	Main Effect
Effect and Int.	Soil and	Soil and Doses		OM 1	OM 2	11	12	and Int.
Soil X Fertilizer	Garden So	il (GS)	41,19	42,59	51,99	41,04	42,79	43,92
Int. and Soil Main Effect	Improved Soil for Red Radish Cultivation (IGS)		41,55	42,51	51,91	42,72	43,81	44,50
Dose X Fertilizer	10 Kg/da		42,23	42,37	40,53	40,09	41,67	41,38
Int. and Dose Main Effect	20 Kg/da		41,95	41,50	51,95	43,57	44,84	44,76
Soil X	Garden	10 Kg/da	42,83	42,75	40,46	39,13	42,94	41,62
Dose X Fertilizer	Soil (GS)	20 Kg/da	41,63	41,99	50,60	41,04	44,40	43,93
Int. and	Improved Soil for	10 Kg/da	41,99	42,25	51,99	40,72	45,12	44,41
Soil X Dose Int.	Red Radish Cultivatio n (IGS)	20 Kg/da	42,91	40,75	52,91	42,41	47,35	45,27
Fertiliz	er Types M Effect	lain	42,04 b	42,09 b	49,04 a	44,34 b	44,12 b	43,72

**Table 5.** The Effect of Organomineral and Mineral Fertilizer Applications on the Leaf Color L\* Value of Red Radish in Different Garden Soil Types and Their Groups According to LSD Test\*

**Table 6.** The Effect of Organomineral and Mineral Fertilizer Applications on RedRadish Leaf Color a\* Value in Different Garden Soil Types and Their GroupsAccording to LSD Test\*

Main Effect and Int.	Fertiliz Type Soil and I	S	Control	OM 1	OM 2	Minera l 1	Minera l 2	and
Soil X Fertilizer	Garden (GS)		- 6,86	- 6,95	- 8,01	- 7,17	- 7,88	Int. - 7,37
Int. and Soil Main Effect	d Improved Soil for Red Radish iin Cultivation		- 7,18	- 7,35	- 7,58	- 7,65	- 7,80	- 7,51
Dose X Fertilizer Int. and	10 Kg/da 20 Kg/da		- 7,84	- 7,62	- 7,25	- 6,78	- 7,84	- 7,44
Dose Main Effect			- 7,26	- 6,63	- 7,67	- 8,33	- 7,20	- 7,45
0. 'I V	Garden	10 Kg/d a	- 7,49	- 6,43	- 7,46	- 8,52	- 6,81	- 7,35
Soil X Dose X Fertilizer	Soil (GS)	20 Kg/d a	- 7,8	- 7,72	- 7,38	- 6,97	- 7,80	- 7,48
Int. and Soil X Dose Int.	Improve d Soil for Red	10 Kg/d a	- 7,88	- 7,52	- 7,12	- 6,60	- 7,88	- 7,39
	Radish Cultivati on (IGS)	20 Kg/d a	- 7,03	- 6,82	- 7,88	- 8,13	- 7,59	- 7,55
	r Types Ma Effect	ain	- 7,02	- 7,15	- 7,79	- 7,41	- 7,84	-7,44

#### 3.1.3. Red Radish Leaf Color b\* Value

The measurement results to determine the color b\* values of red radishes are as in Table 7. When we examine the table in terms of soil type, it is seen that the GS type has higher results. When we examine

the main effect of the fertilizer dose, it is seen that 10 kg/da fertilizer contains higher results. When the table is examined according to our fertilizer type, it is understood from our table that the control group, which was not fertilized at all, gave the highest result statistically out of 5 fertilizer applications.

**Table 7.** The Effect of Organomineral and Mineral Fertilizer Applications on Red

 Radish Leaf Color b\* Value in Different Garden Soil Types and Their Groups

 According to LSD Test\*

Main Effect and	Fertilizer		Contro l	OM 1	OM 2	Minera l 1	Minera 12	Main Effect and
Int.	Soil and Doses		-					Int.
Soil X Fertilizer	Garden So	Garden Soil (GS)		16,99	15,06	16,47	15,89	16,37
Int. and Soil Main Effect	Improved Soil for Red Radish Cultivation (IGS)		16,90	15,63	13,64	16,55	14,71	15,49
Dose X Fertilizer Int. and	10 Kg/da 20 Kg/da		15,71	12,99	16,51	16,51	17,44	16,31
Dose Main Effect			17,89	17,63	16,73	15,18	12,72	15,55
Soil X	Garden	10 Kg/da	16,29	13,82	16,47	16,47	18,05	16,82
Dose X Fertilizer	Soil (GS)	20 Kg/da	17,96	17,10	17,81	15,93	13,82	15,92
Int. and	Improve d Soil for	10 Kg/da	15,13	12,16	16,55	16,55	16,83	15,80
Soil X Dose Int.	Red Radish 20 Cultivati on (IGS)		17,82	18,16	15,64	14,43	11,61	15,18
Fertilizer T	ypes Main	Effect	17,18	16,31	14,35	16,51	15,30	15,93

## 3.2. Amount of chlorophyll (SPAD Value)

The average of the results of the chlorophyll amount in terms of SPAD value is as seen in Table 8. When we examine Table 8, the results of the fertilizer main effect were found to be statistically significant. If we examine in more detail, when we look at the results only in terms of soil type, it is seen that the IGS type has more chlorophyll content than the GS type.

In the main effect of only the fertilizer dose, it is seen that 20 kg/da of fertilizer contains more color than 10 kg/da of fertilizer.

When we examine according to fertilizer type, it is understood from our table that organomineral 2 (OM2) fertilizer gives the highest result statistically out of 5 fertilizer applications.

## 3.3. Amount of water-soluble dry matter (%)

In Table 9, the averages of dry matter dissolved in water are shown as percentages. It is understood from the table that soil type, fertilizer doses and fertilizer type are statistically significant. Similar to our other results in this table, IGS is higher according to soil type and 20 kg/da dose creates higher dry matter amount in terms of fertilizer dose. According to the fertilizer type, the highest result is in organomineral 2 (OM2).

The research was carried out in the Climate Chamber of the Department of Horticulture, Faculty of Agriculture, Tekirdağ Namık Kemal University. Matador red radish was used as the material. Marketable yield (kg/da), color values (color L\*, a\* and b\* value), chlorophyll content (SPAD), water-soluble dry matter content (%) were determined in red radish whose stems were eaten. The findings obtained during the harvest period of the experiment are given in the tables. As a result of the collective evaluation of these charts, in red radish.

**Table 8.** The Effect of Organomineral and Mineral Fertilizer Applications on the

 Chlorophyll Value (SPAD) of Red Radish in Different Garden Soil Types and Their

 Groups According to LSD Test\*

Main	fect			0154		Minera	Minera	Main Effect
Effect and Int.			Contro	OM 1	OM 2	11	12	and Int.
Soil X Fertilizer	Garden Soi	l (GS)	15,73	29,17	31,1	29,05	30,23	27,06
Int. and Soil Main Effect	Improved Soil for Red Radish Cultivation (IGS)		15,77	29,47	30,73	31,9	30,65	27,7
Dose X Fertilizer Int. and	10 Kg/o	da	15,75	30,27	15,75	31,57	30,58	26,56
Dose Main Effect	20 Kg/da		30,37	28,15	26,75	31,88	32,73	28,2
Soil X	Garden	10 Kg/da	15,73	32,53	15,73	29,67	27,47	25,68
Dose X Fertilizer	Soil (GS)	20 Kg/da	15,77	28,00	15,77	33,47	33,7	27,44
Int. and	Improved Soil for	10 Kg/da	30,1	27,27	27	31,93	34,03	27,97
Soil X Dose Int.	Red Radish 20 Cultivation Kg/da (IGS)		30,63	29,03	26,5	31,83	31,43	28,43
Fertiliz	er Types Ma Effect	ain	15,75 b	29,32 a	30,92 a	30,48 a	30,44 a	27,38

**Table 9.** The Effect of Organomineral and Mineral Fertilizer Applications on Water-Soluble Dry Matter (%) of Red Radish in Different Garden Soil Types and TheirGroups According to LSD Test\*

Main Effect	ffect		Control	OM 1	OM 2	Minera		Main Effect
and Int.			control	0.011	01112	1	2	and Int.
Soil X Fertilizer	Garden S (GS)	oil	2,85 1	3,50 g	2,93 h	3,80 f	3,90 e	3,40 b
Int. and Soil Main Effect	Improved Soil for Red Radish Cultivation (IGS)		2,90 h	4,60 c	5,70 a	4,70 b	4,40 d	4,46 a
Dose X Fertilizer Int. and	10 Kg/d	a	4,30 c	4,90 a	4,20 d	3,90 e	3,40 f	3,78 b
Dose Main Effect	20 Kg/da		2,88 g	4,40 b	2,88 g	4,23 d	4,20 d	4,08 a
Soil X	Garden Soil (GS) Improved Soil for Red	10 Kg /da	4,20 f	3,60 h	3,40 1	4,80 d	3,00 j	3,33 d
Dose X Fertilizer		20 Kg /da	4,40 e	4,20 c	5,00 c	5,00 e	3,80 c	4,22 b
Int. and Soil X		10 Kg /da	2,85 k	2,85 k	2,85 k	3,00 j	3,40 1	3,46 c
Dose Int.	Radish Cultivatio n (IGS)	20 Kg /da	2,90 k	5,60 b	2,90 k	5,80 a	5,00 c	4,70 a
	r Types Ma Effect	in	2,88 e	4,05 d	4,31 a	4,25 b	4,15 c	3,93

In all criteria, garden soil (GS) without fertilization and additional nutritional supplement gave the lowest results as soil type. Cultivation of the other soil type, improved garden soil (IGS), gave the best results.

In the study, fertilizers were given in 2 different doses (10 and 20 kg/da). In the statistical calculations of the data obtained, when the results were examined only in terms of the main effect of the fertilizer dose, the highest results were obtained in all criteria from the 20 kg/da fertilizer dose in red radish. In the study, 2 organomineral and 2 mineral fertilizations were applied. The 2nd organomineral (OM2) fertilizer (11:11:11) gave the highest results in these criteria. These results were followed by the 1st mineral (M1) fertilization (15:15:15), which is sometimes different and sometimes in the same group. These results were followed by the 2nd mineral (M2) fertilization (12:12:17) and the 1st Organomineral (OM1) fertilization (8:8:8), while the garden type soil with no fertilization (control) gave the lowest results.

In conclusion, In the improved garden soil type (IGS) 20 Kg/da fertilizer dose application and Organomineral 2 type (11:11:11) fertilization, the best results were obtained.

## 4. CONCLUCSION

It has been determined that 11:11:11 Organomineral granular fertilizer, which gives the same and many times better results than the mineral fertilizers used in classical vegetable cultivation, can be an alternative to mineral fertilizers and has alternative fertilizer properties in vegetables that are both leaf and stem edible.

## ACKNOWLEDGEMENTS

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## **CHAPTER 3**

## INTEGRATED NITROGEN MANAGEMENT FOR IMPROVING SALT TOLERANCE IN CEREALS

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### **1. Introduction**

Climate change is a most concerning issue of the present era have been shown its noteworthy impact on environment over a long period, particularly in agricultural crops grown in these environments. Intergovernmental panel on climate change (IPCC) had reported that stresses experienced by plants are the consequences of climate change in environment which is considered as most impelling factor affecting yield and production in agricultural sector (Andy, 2016). As a result of climate change carbon dioxide in atmosphere is increasing every next day which is the major cause of food production due to rise in temperature in environment (Hirayama and Shinozaki, 2010). To cope with this hazardous issue most of the researchers are now looking for new strategies adapting this severe change in climatic conditions (Ladha et al., 2016). Stresses induced by climate change are termed as abiotic stressors influencing various plant species (Wheeler and Von Braun, 2013). Drought and salinity, waterlogging, temperature, rainfall and intensity of sunlight are included in abiotic stressors. The fight against all these stresses is now have become an imperative challenge under climate change. Among all these abiotic stresses drought and salinity is major concerning issues due to high temperature and low rainfall particularly in arid and semi-arid regions of the world.

Salt stress refers to accretion of soluble salts in high concentration in the soil which modifies normal growth of plant due to disturbance in physiological functions of plant (Cramer, 2010). Accumulation of salts in soil create a severe hazard for health and agriculture yield (Kamran et al., 2019). There are two types of salinity on the basis of source: primary and secondary salinity. Primary salinity spreads due to degradation of rocks eventually release of different salts and secondary salinity prevails due to reasons of anthropogenic actions such as over grazing, intensive cropping pattern and irrigation system (Ashraf, 1994).

## 2. Importance of nitrogen for crop growth and productivity

Nitrogen is an essential macro nutrient for plants having a dominant position for plants due to its key role in plant growth and regulation of plant metabolism. Plants require almost 3-5% N for its proper growth and development which is highest concentration among macronutrients such as carbon, hydrogen, oxygen which do not perform key role during management practice to improve fertility status of soil. Due to its involvement in chlorophyll synthesis which aided plants in sugar formation in the presence of carbon, oxygen and water, is considered a vital nutrient. It is also a major constitute of amino acids and take part in protein synthesis it is considered structural component of plant cells. Nitrogen is also an important nutrient to improve enzymatic activity in plants like nitrate reductase (NR), nitrite reductase (NiR); N assimilation enzymes, and plant growth hormones such as auxins and gibberellins.

In soil nitrogen is present in three form including organic N, ammonical form  $(NH_4^+)$  and nitrate form  $(NO_3^-)$ . Almost 95% of organic source of N is present in soil in the form of plant and animal residues. Organic form of N is not available directly to plants except urea. It has to convert

in to inorganic form with the help of soil microbes. Plants uptake N in inorganic form such as  $NH_4^+$  and  $NO_3^-$ .  $NH_4^+$  form of N attaches to soil exchange site of soil while  $NO_3^-$  which is negatively charged ion become precipitated as a soluble salts.

Plants receives N mainly from mineral source and atmospheric source. Mineral sources supply very small amount of N hence atmospheric N is considered a main source of N nutrition for plants where it is present in inert N<sub>2</sub> form which is converted to useful form by bacteria for plants. This conversion of organic to inorganic form of N and vice versa through microbial activities is performed by a cycle is called as N cycle. However nitrogen is very important nutrient for all types of crops but for the growth of cereals it is considered very important. Because almost in all countries cereal crops are the main sources of intake as food and cultivated in 90 % regions of the world (Guerrieri and Cavaletto, 2018). A number of factors are involved to influence growth and yield of cereals in which deficiency of N nutrition is very significant due to its high requirements in all types of soils degraded or fertilized soils in all regions of the world (Ladha et al., 2016).

## **3.** Need for integrated nitrogen management for salt tolerance in cereals

Under a biotic stress, most of the essential nutrients in the form of synthetic fertilizers become unavailable to plant like N and K. So, integrated use of organic source of fertilizer including farmyard manure (FYM) with chemical fertilizer can play its vital role in minimizing the effects of a biotic stress and expensive use of chemical fertilizer without disturbing fertility status of soil. The application of organic fertilizers with synthetic forms of fertilizers can sustain agricultural output without influencing uptake of other nutrients by plant. Therefore, it should be a part of our cropping scheme (Teh et al., 2016). In prevailing cropping system, use of organic manures can improve soil health and sustainability (Timsina and Conner, 2001). Generally under stress conditions, level of ethylene increased which create hindrance in plant growth resulting reduction in yield. Plant growth promoting rhizobacteria (PGPR) produces growth hormones in plant which is cause of enhanced nutrients supply to plants because organisms reside in rhizosphere have a significant effect to increase growth of plant. These PGPR restricted the roots to support the plant growth and plant yield (Shahzad et al., 2014). For example in young plants, ACCdeaminase is synthesized to reduce level of ethylene under salinity stress by improving the uptake of water and nutrients through roots (Verma et al., 2010).

Therefore, this section is intended to review the problems in growth and yield response of plants due to climate change and their management techniques.

## **3.1. Effects of salinity on nitrogen use efficiency in cereals**

There is a very intricate association between salinity and N uptake which depends upon plant species, time period of plant under salinity stress, types of salts, growth phase and form of N in root zone but sufficient supply of N nutrition is considerable for plant tolerance under salt stress conditions (Teh et al., 2016). Salt amassing to the root zone of plant causes interruption in metabolism regulation mediated by N due to less availability of N to plant which ultimately create changes in enzymatic activities (NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup> assimilating enzymes) and increase the activity of hydrolyzing enzymes involving protease, DNase, Rnase and many of others (de Souza et al., 2016).

Under salinity stress, N has its antagonistic effect with salts such as Cl<sup>-</sup> ions compete with NO<sub>3</sub><sup>-</sup> form of N and Na salt compete with uptake of NH<sub>4</sub><sup>+</sup>. High accumulation of salts diminishes the mobility and availability of N in the form of NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup> which ultimately disrupts metabolism of plant. Different plant species showed different types of competition between salts and nutrients (Dai et al., 2015).

Besides all these antagonistic effects, supply of N nutrition can also be diminished due to reduced water uptake by plants under accumulated salts in root zone. Moreover, Van Hoorn et al. (2001) also concluded that limited N supply under saline conditions is the main cause of reduced photosynthetic process which effects the internal N requirement at which plant roots uptake N. Salinity also minimizes the N mineral production through biological activity and its conversion to organic N.

## 4. Mechanisms of salt stress in plants

Under high salt concentration plants develop various types of mechanisms to survive. Some of them are discussed as under one by one. Tolerance mechanisms vary from plant-to-plant species.

#### 4.1. Ion homeostasis

The ability of an organism or cell to maintain its internal steady state under stress conditions is called ion homeostasis. Developing Ion homeostasis mechanism in response equal influx and out flux of ions and its fixation in vacuole in the form of compartments is not only significant but also imperative process for normal plant growth under salt stress (Niu et al., 1995). Regardless of their nature, both types of plant halophytes and glycophytes can't endure excessive amount of salt in their cytoplasm. Consequently, high amount of salts is either accumulated in vacuole or secluded in older plant tissues which ultimately are forfeit, thus defensive for plant against salt stress (Zhu, 2003). Mostly the salt affected soils are dominant with NaCl hence the focal point in our investigation will be the mechanism of Na<sup>+</sup> ion transportation and its fixation in plant vacuoles. The movement of Na<sup>+</sup> ions from cytoplasm to vacuole is occurred via sodium and hydrogen (Na<sup>+</sup>/H<sup>+</sup>) anti-porters. Vacuolar type H<sup>+</sup>-ATPase (V-ATPase) and the vacuolar pyrophosphatase (V-PPase) are two types of hydrogen pumps (H<sup>+</sup>) available in the vacuolar membrane of plant cells. Of these two hydrogen pumps, V-ATPase is the most dominant within the plant cell because under salt stressed condition plant growth and development relies on the movement of V-ATPase (Dietz et al., 2001). The mechanism of transportation is done by different types of proteins like diverse bearer proteins, channel proteins, antiporters, symporters, channel proteins and carrier proteins. In salinity conditions, maintenance of Na<sup>+</sup> and K<sup>+</sup> ions within plant cells is indispensable for

plant growth and endurance. Potassium performs a significant job in constructing the turgor within plant cell. The cytosol within the plant has a capacity to contain an elevated level (100 mM) of  $K^+$  for enzymatic activities of cytoplasm. Inside the vacuole  $K^+$  concentration goes somewhere in the range of 10 and 200 mM. The vacuole acts as a principal pool for  $K^+$  within the plant cell. In response to salinity stress, high Na<sup>+</sup> concentration in the soil competes with  $K^+$  ions for the transporter as they both of them share a similar transport mechanism, in that way declining the  $K^+$  uptake (Munns and Tester, 2008).

From the above views it is concluded that in ion homeostasis emission of excessive Na<sup>+</sup> or formation of compartments into cellular vacuoles is a significant adaption approach for plants under salinity stress, which facilitates cereal plants to prevent lethal effects of excessive Na<sup>+</sup> and reduces the osmotic potential consequently showing osmoregulation.

## 4.2. Compatible solute accumulation and osmotic protection

Organic solutes produced by plant under stress conditions to protect from adverse effects of salinity is called compatible solute. These are also known as osmolytes or osmoprotectants and this process of compatible solute accretion by plant is known as osmoregulation or osmotic adjustment. It is major salt tolerance mechanism proceeded at cellular level by plant in order to mitigate the salinity effects on plant especially at first rapid phase of stress induced by salinity. Osmoregulation confronted with accrual of solutes under salt affected soils to decline the water gradient without falling required concentration of water content (Serraj and Sinclair 2002). The induction of soluble salt /osmolytes as a result of salinity stress involves proline, polyols, glycine betaine, soluble sugars, alcohols and organic acids. Kaya et al. (2010) evaluated that proline content uplift in maize plant in the consequence of salinity stress. According to an estimate leaves of sweet corn accreted almost greater than 600  $\mu$ mol g<sup>-1</sup> proline contents under stress conditions induced by 400 mM NaCl (Murtaza et al., 2009).

The process of osmoregulation in root system of plant helps to alleviate the adverse effects of osmotic stress under salt stress. The most important osmoprotectants under salt stress environment responsible for the process of osmoregulation are glycinebetaine and proline in maize plant. Appearance of proline content within plant cells under stress conditions also act as an organic source of N along with stimulation of tolerance mechanisms to recuperate stress damage in plant cells. Matysik et al. (2002) studied that proline acts as an oxygen scavenger due to its antioxidant potential and improves activity of antioxidant enzymes and make strong defense system of plant under stress environment.

Glycinebetaine; another compatible solute, also contributes its significant role in alleviating adverse effects of salinity because it is non-toxic in nature and increases osmolarity in cell amid stress phase because of its distinctive structural attribute. It intermingles with both hydophilic and hydrophobic molecules such as proteins and enzymes. Glycinebetaine is produced with in plant cell by glycine or choline. It also prevent the plant cell from stress by improving process of osmotic adjustment and provide a shield around photosynthetic apparatus in plant cell to save it from stress damage (Gadallah, 1999). Similarly, polyols are also compatible solutes that act as scavenger for ROS species. Sugar alcohol is also a category of polyols. Polyols are alienated in two types known as cyclic and acyclic. Cyclic polyols includes pinitol and acyclic groups contain mannitols. Mannitol induction started during the stress period of plant by means of mannose-6-phosphate reductase that relies on NADPH. It also functions as enzyme stabilizer and acts as a defender for membrane stability in plant that is susceptible for dehydration and cell damage (Thomas et al., 1995). In the same way pinitol also shows decisive responsibility in stress mitigation.

### 4.3. Regulation of antioxidant tolerance mechanism

Plant contains a number of antioxidant enzymes including catalase (CAT), superoxide dismutase (SOD), peroxidase (POD) and ascorbate peroxidase (APX). These enzymes play its key role in protecting plant from stress. Tolerance mechanism in plant under salinity has direct relation with the activity of antioxidant enzymes (Gupta et al., 2005). Antioxidant activity increases by increasing salinity level. These enzymes also detoxify reactive oxygen species that are produced in response to salinity to lessen the damage of stress. These ROS species include molecular oxygen (O2) acts as a driver of ROS species and takes part in increasing their concentration. Likewise hydroxal radical (OH<sup>-</sup>), singlet oxygen (1O<sub>2</sub>) and hydrogen per oxide are hazardous for stability of cell because of oxidizing agents (Grob et al., 2013).

# **4.3.1.** Effects of salt stress on nitrogen uptake, assimilation and translocation

Generally, nitrogen has its dominant position among other macronutrients owing to its main role in plant growth and its metabolism. Due to stress, availability of N diminishes and further it is the essential component of all amino acids synthesized in proteins. Under saline conditions, all these amino acids including proline and glycinebetaine, amino acids, amids and polyamines are accreted in plant and play a significant part in tolerance against stress by various mechanisms (Siddiqui et al., 2010). Under salt affected soils, uptake of nutrients is minimized due to accumulation of salts and deficiency of nutrients in plant appears which triggers disordered growth pattern in plant. (Ashraf et al., 2017).

## 4.3.2. Nitrate assimilation under saline soils

In higher plants  $NO_3^-$  is considered as a chief form of N as it is assimilated by plants in two steps. In first step nitrate is transformed into NO<sub>2</sub> by nitrate reductase (NR) enzymes which is further reduced rapidly into  $NH_4^+$  by the involvement of nitrite reductase (NiR) enzymatic activity in  $NO_3^-$  assimilatory path (Crawford, 1995). As  $NO_2^$ is very reactive, therefore, it is transferred from cytosol to chloroplasts in the form of  $NH_4^+$  by NiR activity (Sadale and Karadge, 2013) which becomes the part of amino acids by GS/GOGAT course of action (Hirel and Lea, 2002). Assimilation of  $NO_3^-$  is very receptive to salinity due to which salinity creates hindrance in  $NO_3^-$  transformation and uptake by varying species of crops such as *Glycine max* L. (Queiroz et al., 2012). In case of NO<sub>3</sub><sup>-</sup>, salinity shows its inhibitory effects in its uptake than its reduction. When plant is subjected to salinity nitrate availability becomes more at root than shoots which indicates the transport of NO<sub>3</sub><sup>-</sup> in root xylem is salt receptive step (Debouba et al., 2007). This may demonstrate brutal cost during assimilation for plant for the reason that under saline soils  $NO_3^-$  has major effect on NR expressions and its functions which modifies the loading pattern and uptake of nitrate. Likewise, Yu et al. (2015) also performed an experiment using salt sensitive and salt tolerant varieties of potato at 100 mM NaCl level. He observed that the activities of GS and NADH-GOGAT enzymes in salt sensitive varieties were diminished as compared to salt tolerant cultivars of sweet potato. Sodium chloride disrupts the assimilation channel of NH<sub>4</sub><sup>+</sup> due to the efflux of NH<sub>4</sub><sup>+</sup> from NH<sub>4</sub><sup>+</sup> cellular pool. Iqbal et al. (2006) evaluate the effect of different types of salts at varying levels and concluded that salts decrease the reduction process of  $NO_3^-$  in all cultivars of wheat. He also observed that reduction process of N was decreased almost 35% in wheat cultivars due to inhibited activity of NR enzymes.

## 4.3.3. Ammonium assimilation under salinity stress

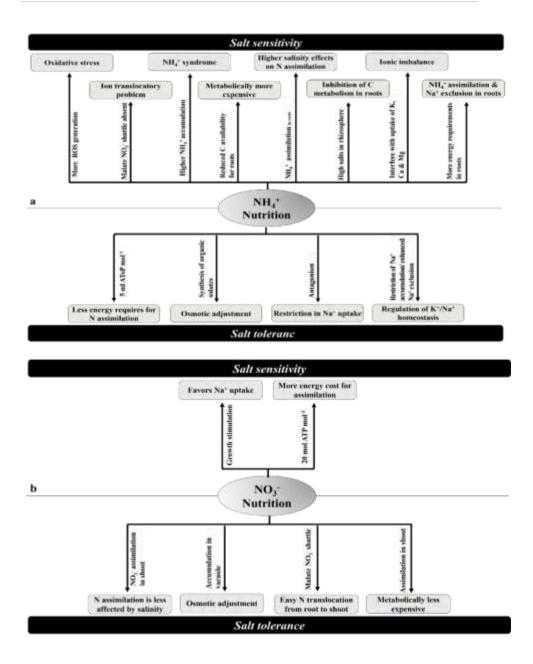
Glutamine synthetase (GS) and glutamate synthase (GOGAT) is assumed as a major channel for ammonium (NH4<sup>+</sup>) assimilation by plants which starts to diminish under salt affected salts and ultimately confines the synthesis of amino acids and glutamine. However, under salt medium accretion of NH4<sup>+</sup> occurs which triggers glutamate dehydrogenase (GDH) pathway an alternative process to assimilate NH4<sup>+</sup> under salinity. Under normal soil conditions, NH4<sup>+</sup> becomes part of soil by GS/GOGAT activity, GS catalyses the activity of enzymes to assimilate NH4<sup>+</sup> but under saline environment NH4<sup>+</sup> adopt another process of GDH to incorporate (Wickert et al., 2007). Among adverse effects of salinity, it is also believed that salinity has its direct relation with down regulation of genes (OsFd-GOGAT, OsNR1, OsGS1; and OsGS2) concerning with NH4<sup>+</sup> digestion owing to dearth of NO3<sup>-</sup> resulting in inhibited behavior of N assimilation under salinity stress. (Wang et al., 2012). Salinity throws its bad impact on NH<sub>4</sub><sup>+</sup> assimilation into plant regulation process depending upon gradient of salinity and factors related to plant and soil (Yu et al., 2015). Therefore, plants grown in salty environment could adopt GDH pathway as a substitute of GS/GOGAT for assimilation of NH4<sup>+</sup>. Kumar et al. (2000) found that improved activity of GDH in Oryza sativa L. grown in saline environment is due to enzymatic sustainability of plant metabolism by providing a transition to the tricarboxylic acid cycle. Salinity is also cause of NH<sub>3</sub> accumulation and storage of free amino acids in plant metabolism. So there are only two paths to detoxify NH<sub>3</sub> from metabolism of higher plant; GDH path and GS/GOGAT path (Wang et al., 2012). The accumulation of Na and Cl in excess amount may transform the assimilation pathway of NH<sub>4</sub><sup>+</sup> by inhibiting the enzymes (GS/GOGAT) activity and prompting the other GDH pathway which is implicated in catabolism of glutamate to oxoglutarate. Meng et al. (2016) performed his hydroponic research on *Populus simonii*. The plants were grown by using NaCl salt at the level of 75 mM that were fertilized with 1 mM NH<sub>4</sub>Cl or KNO<sub>3</sub> as a N source. The results

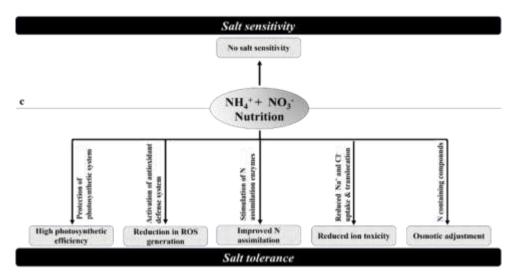
indicated that stress showed minor impact on activity of enzymes; NR and NiR in both NH4<sup>+</sup> and NO3<sup>-</sup> supplemented plants. But it showed its impact on activity of GDH enzyme which was minimized by 75 % in roots and 65% in leaves. Hence, NaCl illustrated its effect on down regulation of genes and minimize the role of GS/GOGAT pathway. Sacala et al. (2005) conducted a study on two different cultivars of maize (*Zea mays* L.), Limko and Cyrkon under saline solutions. He uses saline and minimize the role of GS/GOGAT pathway. Sacala et al. (2005) conducted a study on two different cultivars of maize (*Zea mays* L.), Limko and Cyrkon under saline solutions. Results demonstrated that saline solution inhibited the nitrate activity in cyrkon cultivar up to 30% and about 60% decrease was observed in Limko cultivar. It was also observed by researcher that Nr activity was diminished due to antagonistic effect of chloride.

## 4.3.4. Molecular and physiological responses of cereals to salinity

Soil salinity is a major restriction in coping the demand of food for world's population. Because over 20% of the agricultural land around the world is under salt stress and this type of land is increasing rapidly. On the basis of adoption mechanisms plants can be categorized in two types: the glycophytes and the halophytes. Plants that cannot tolerate salinity stress and die are called Glycophytes similarly the plants that can tolerate salinity stress are called halophytes. Major crop species belong to this first type (Glycophytes). Subsequently salinity is one of the most vicious natural abiotic stresses that reduce plant growth and crop yield (Flowers, 2004; FAO, 2009). The changes in metabolic and

physiological functions of plants under salt stress depend upon its time period and brutality which ultimately hinders crop production (James et al., 2011). Soil salinity effects plant growth in two phases significantly: osmotic stress and ion toxicity (Rahnama et al., 2010). During the initial stages of salinity stress, ability of plant roots to absorb water diminishes because of osmotic stress of high concentration of salts in soil and plants and in this way salinity stress is likewise considered as hyperosmotic stress (Munns, 2005). Osmotic stress causes different changes in physiology of plant, for example, disruption of membrane, disables the capacity to detoxify responsive oxygen species (ROS), ion homeostasis, slower the photosynthetic process, and reduction in stomatal openings are consequences of osmotic stress under salinity (Rahnama et al., 2010). Salinity stress is also called as a hyper ionic stress due to ion toxicity. One of the most injures impacts of salinity stress is the accretion of sodium and chloride ions in tissues of plant due to high concentration of NaCl in salt affected soils (Figure 1) (Ashraf et al., 2018). Uptake of both Na<sup>+</sup> and Cl<sup>-</sup> ions into plant cells causes ion imbalance and ultimately physiological disorder(s) occur during plant growth (Figure 1).





**Figure 1.** Forms of nitrogen ( $NH_4^+$  and  $NO_3^-$ ) effects on plant adaptation to saline environment (Ashraf et al., 2018).

Accretion of Na<sup>+</sup> salt restrain uptake of other essential ions like K<sup>+</sup> ions which is an essential macronutrient for growth that shows results in the form of lower productivity and even lead to the death of plant (James et al., 2011). The generation of reactive oxygen species (ROS) like superoxide, singlet oxygen and hydrogen peroxide is increased due to salt stress condition (Apel, 2004). Salinity-induced ROS formation can lead to oxidative damages in various cellular components such as proteins, lipids, and DNA interrupt vital cellular functions of plants. These induced ROS species can prompt oxidative harms in different cell segments, for example, lipids, proteins and DNA formation hindering cells of cells and molecules in plant structure.

# **5.** Strategies for integrated nitrogen management for improving salt tolerance in cereals

## 5.1. Soil management practices

The restoration process of disturbed land into cultivable land or cultural practices to dissolve salts from rhizosphere is termed as reclamation while soil management is a broader term which involves all management practices either physical chemical or biological is call management.

## 5.2. Improving soil physical properties

Soils confining with higher concentration of soluble salts is termed as saline soils and these soils are reclaimed by irrigating with good quality water in order to leach down the soluble salts from the rhizosphere. In such case amount of water to be applied is most important factor such as level of salinity and water contents in soil, soil texture and water application methods etc. Saline, sodic and saline sodic soils could be managed by adopting different ways including physical, chemical, biological, hydro technical, electro-reclamation and synergistic manners. Beside this soils could also be managed by improving physical properties of soil such as porosity, infiltration rate, structure, hydraulic conductivity and decreased bulk density (Murtaza et al., 2009) by using effective physical methods and cultural practices. These physical methods include Sub-soiling, deep ploughing, sanding and hauling are the useful physical methods to improve salt affected soils

by mechanical or physical means. All these methods are discussed below one by one.

Sub-soiling is done by a tillage implement known as sub-soiler comprised of iron or steal tines. By using this cultural implement soil permeability is increased owing to losing soil and opening soil pores. Sub-soiler breaks down the layer of lime which is favorable for soil and plant growth till many years.

Ploughing of soil from 40 cm to 150 cm depth is known as deep ploughing. It is another soil management strategy which is appropriate for stratified soils having impermeable membrane and gypsiferous subsoils. While, under those soils where subsoil is sodic this technique is not suitable. In this type of management sand is mixed with fine textured soil to increase its porosity and ultimately its permeability. This technique of sanding changes texture of soil permanently to promote easy root penetration, air and water permeability and infiltration rate and subsequently leaching of salts through root zone. Furthermore, mixing of sand to at least 10 cm soil surface shows better output. Replacement of salt affected layer of soil with good quality soil layer is termed as hauling. This technique is very useful but not suitable for every place because it is very expensive.

## 5.3. Soil amendments and bio-fertilizers

Physical properties of soils could also be improved by use of chemical amendments either inorganic or organic in nature.

## 5.3.1. Chemical amendments

Plant growth and soil properties are also improved by using any chemical amendment according to its cost, availability, handling approaches and particularly its time of application to minimize the effect of salts. Sodic and saline sodic soils are generally subjected to amendments; categorized into three groups including organic and inorganic amendments.

Cereal Crop	Treatment	Mechanism	Effect	Reference
Maize (Zea mays L.)	Treatments containing Nacl, Na <sub>2</sub> SO <sub>4</sub> and their combination at different levels (7, 10, 13 and 16 dS $m^{-1}$ ) of salinity.	The soluble salts around plant roots causes osmotic effect and reduces uptake of essential element.	NaCl was more harmful than Na <sub>2</sub> SO <sub>4</sub> and their combination at all salinity levels.	Javed et al. (2022)
Sorghum (Sorghum bicolor L.)	Plants were grown for 33 d at 5 Si levels (0, 50, 100, 150 and 200 mM) and 3 saline levels (0, 1.5 and 2 M).	Direct competition between Cl <sup>-</sup> and NO3 <sup>-</sup> ions for the same carrier and/or alterations in membrane integrity.	2 M saline solution at 0 mM of Si reduced NO <sub>3</sub> <sup>-</sup> uptake by 61% in roots and 79% in leaves compared to control.	de Souza et al. (2016)
Rice ( <i>Oryza sativa</i> L.)	Cultivars MR 220 and MR 253 were grown under non-saline and 150 mM NaCl using 5 and 10 mM proline for 30 d.	Salinity-induced reduction in plant growth and hence N demand and Cl <sup>-1</sup> antagonism.	Salinity-induced reduction in plant growth and hence N demand, and Cl <sup>-1</sup> antagonism.	Teh et al. (2016)
Wheat ( <i>Triticum</i> astivem L.)	Salinity was artificially induced in sandy clay soils by mixing different salts (MgCl <sub>2</sub> + CaCl <sub>2</sub> + Na <sub>2</sub> SO <sub>4</sub> ) in different concentrations (EC 2.16, 4.0, 6.0, 8.0 and 10.0 dS·m <sup>-1</sup> )	Salinity stress reduced essential nutrients particularly N and K having antagonistic association with salt ions (Na <sup>+</sup> , Cl <sup>-</sup> and Mg).	Maximum effect of salinity was recorded where salts (MgCl <sub>2</sub> + CaCl <sub>2</sub> + Na <sub>2</sub> SO <sub>4</sub> ) were applied @ 10 dS m <sup>-1</sup> than at 8, 6 and 4 EC dS m <sup>-1</sup> by reducing physiological as well as yield attributes and K <sup>+</sup> /Na <sup>+</sup> ratio.	Kalhoro et al.(2016)
Barley (Hordeum Vulgare L.)	15-day-old seedlings of 4 cultivars Dasht, Lisivy, Sahra and Sahand were exposed to 2 levels of NaCl (0 and 100 mM) for 10 days.	Specific ion toxicity is a main cause of adversely effecting barley seedling in which Na accumulation lowers the K uptake and subsequently increased Na <sup>+</sup> /K <sup>+</sup> ratio over control.	100 mM NaCl increased leaf Na <sup>+</sup> by 99% in Sahand, 73% in Sahra, 31% in Dasht and 15% in Lisivy while, decreased leaf K <sup>+</sup> by 16% in Dasht and Lisivy, 25% in Sahra and 36% in Sahand by reduction in K <sup>+</sup> : Na <sup>+</sup> ratio by 36% in Dasht, 26% in	Yousufinia et al. (2013)

**Table 1.** Effect of salinity stress and mechanisms in different cereal crops.

Rice (Oryza sativa L.)	7-d-old seedlings were transferred to hydroponics. After 12 d, plants were subjected to 0 (control) and 100 mmol NaCl /L.	Salinity-induced NO <sub>3</sub> - deficiency caused down regulation of genes involved in NH4 <sup>+</sup> C assimilation n (OsGS1; 2, OsGS2, OsNR1 and OsFd, GOGAT).	Lisivy, 55% in Sahra and 72% in Sahand compared to control. In shoot, OsNR1 was down regulated by 72.18%, OsGS1; 1 27.15, OsGS2 87.74 and OsFd- GOGAT by 78.67% while, OsGDH2 activity was up regulated by 54.85%.	Wang et al. (2012)
Wheat (Triticum aestivum L.)	Three cultivars were grown in pots having sandy loam soil at 5 and 10 dS m <sup>-1</sup> by adding Na <sub>2</sub> SO <sub>4</sub> , CaCl <sub>2</sub> , MgCl, and NaCl at the ratio of 70: 35: 10: 23, respectively. N reduction was measured at vegetative and reproductive growth stages.	Reduction in activity of Nitrate reductase and its synthesis under stress condition.	At the vegetative stage of plants, N reduction was decreased by 35% in wheat cultivar LU-26S, 39% in Sarsabaz and 44% in Pasban-90 at 10 dS m <sup>-1</sup> compared to control. While, at the reproductive stage, N was decreased by 42% in LU-26S, 53% in Sarsabaz and 42% in Pasban-90 at 10 dS m <sup>-1</sup> than control	Iqbal et al. (2006)
Maize (Zea mays L.)	After 12 d growth in nutrient solution, seedlings were exposed for 1 d to 150 mM NaCl by adding 50 mM NaCl daily	Reduced leaf NO <sub>3</sub> <sup>-</sup> accumulation due to restricted NO <sub>3</sub> <sup>-</sup> loading into root xylem	NaCl caused considerable reduction in leaf NR activity (about 50% of the control plants) while root NR activity was slightly increased under saline conditions	Abd-El Baki et al. (2000)

## 5.3.2. Organic amendments

In organic amendments normal and saline soils are improved by adding organic matter or organic amendment including poultry manure, farm yard manure, green manure etc by improving chemical and physical characteristics including soil structure, water holding capacity and its permeability. Similarly by products of sugar industries in the form of press mud and molasses are also effective in reclaiming sodic and saline-sodic soils.

## 5.3.2.1. Compost

Organic material produced by biological putrefaction due to microbial activity under controlled aerobic environment at thermophilic as well as mesophilic temperature is designated as compost. (AAPFCO, 2017). Compost is comprised of chemical attributes including pH, bulk density and nutrient composition in the response of material origin and its composition and methods of composting (Asses et al., 2018). Composting material is comprised of animal and human waste, industrial and municipal waste, yard waste and agricultural waste etc. (Ahmed et al., 2016). All these forms of wastes are decomposed to form compost which ultimately diminishes the dependency on limited resources to encourage the sustainable agricultural practices (Qadir and Oster, 2004). The compost is a source of basic essential nutrients for plant growth present in the form of nitrogen, phosphorus and potassium etc. Nitrogen is an essential component of plant metabolism and taken up by plants in the form of nitrate (NO<sub>3</sub><sup>-</sup>) and ammonium (NH<sub>4</sub><sup>+</sup>). Nitrogen also plays an important role to assimilate other essential nutrients found in applied compost or chemical fertilizers. Like N, P and K are also important for agricultural and other plants. Beside all these nutrients compost consists of also other micro and macro nutrients, heavy metals, and salts etc. Therefore, compost improves chemical and physical characteristics of soil to alleviate the vegetative growth of plants. Though compost is considered as a beneficial product for plant growth but due to non-uniformity of nutrients and salts in compost it is misunderstanding in for compost application in saline and sodic soils because of phytotoxic behavior for high concentration of salts such as sodium (Na<sup>+</sup>) and chloride (Cl<sup>-</sup>) (Gondek et al., 2020).

### 5.3.2.2. Biochar and organic matter

Like compost, application of biochar and organic matter to soil is also considered as beneficial amendment under salt affected soils. Biochar is a carbonated product formed under anaerobic environment at 300 °C to almost 1000 °C temperature. It is also an emerging technique to improve chemical, physical and biological attributes of salt affected soils (Wang et al., 2012). Organic matter application in the form of farm yard manure, poultry manure or other animal waste also make soil capable to increase nutrient availability under salt stress conditions. Application of biochar or organic matter at recommended rate to soil improves soil structure by increasing soil porosity in response to aggregation. Aggregated soils has ability to hold water and air in pores. Due to high porosity salt affected soils improved by minimizing effect of salts owing to leaching. Organic matter and biochar also improve microbial activity in rhizosphere soil because these are rich source of carbon utilized by microbes as food.

Application of organic matter in the form of compost, farmyard manure (FYM), poultry manure and crop residues enhance N availability in soil and subsequently improve production of crops (Ahmad et al., 2007). Application of organic matter increases fertility status of soil particularly carbon and N concentration in soil which subsequently improve plant nutrition. (Marzi et al., 2020).

## 5.3.3. Inorganic amendments

Salt affected soils are usually reclaimed by organic and inorganic amendments. Saline soils are increasing every next day which could be reclaimed by using various organic and in organic amendment. In inorganic amendments gypsum application is considered as a significant strategy to minimize the effects of salts in soil. Though other chemical amendments could also be used such as application of elemental sulfur (S), polysulfides, sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) and hydrogen sulfite etc. According to researchers application of gypsum (CaSO4·2H2O) to saline soils is most effective amendment for reclamation (Lastiri-Hernández et al., 2019). Gypsum is involved to improve physical characteristics including soil structure, bulk density and infiltration rate as well as chemical characteristics such as soil reaction (pH), sodium adsorption ratio (SAR), exchangeable sodium percentage, cation exchange capacity (CEC) and electrical conductivity, organic carbon and nutrient availability (N, P and Ca) in saline soils. It also take part in improving plant nutritional status and biomass (Wang et al., 2012).

Microbial population and its activity is also improved by gypsum fertilization (Alcívar et al., 2018).

## 5.3.3.1. Gypsum efficiency in saline soil

Gypsum application is a cheap source (Gonçalo Filho et al., 2019) of chemical amendment which is produced as a result of byproducts of phosphoric and sulfuric acid production in industries. Most of the salt affected soils are calcareous in nature having high concentration of calcium carbonate (CaCO<sub>3</sub>) is also a natural source of gypsum when elemental sulfur is applied to calcareous soils. Because sulfur and calcium carbonate reacts to form gypsum as a final product. Therefore in calcareous soils application of sulfur minimizes the uptake of toxic elements and improves fertility of saline soils (Ahmad et al., 2016). Sulfur application also alleviate gypsum concentration in calcareous soil which is known as acid former. Hence application of sulfur to calcareous soils increase acidity of soil by reducing soil pH under moist conditions and optimum temperature due to oxidation process (Gonçalo Filho et al., 2019). Application of elemental sulfur (S) is also a source of sulfuric acid formation in saline and calcareous soils which further reacts with CaCO<sub>3</sub> to form carbonate and bicarbonates in soil which helps to leach sodium sulphate (Weil and Brady, 2017).

Another way to minimize salinity effect of gypsum is to lower down the Na concentration by increased Ca contents at exchange sites of soil colloids by antagonistic effect of nutrients which ultimately improves soil structure and permeability (Ahmad et al., 2016). Because high concentration of sodium ions in soil causes dispersive behavior among soil particles, hence by application of Ca in high concentration lowers Na salts and improve soil structure by aggregation due to flocculation of soil particles which subsequently improve soil porosity and water holding capacity of soil. Calcium also helps plant to tolerate soil salinity by increasing hydraulic conductivity and surface area of plant leaves. High Ca contents in soil also improve cell membrane integrity and selectivity in plant which restricts uptake of sodium and chloride ions (Gonçalo Filho et al., 2019).

## 5.4. Fertilizer application methods

Imbalance fertilization to crops is also considered as a major cause of soil salinization. Therefore in order to diminish the deleterious effects of fertilizers application and lowering salinity effects, methods, timing and composition of fertilization and water quality for irrigation must kept in mind while excessive fertilization must be discouraged. On the other end chloride free, pure and low saline fertilizers should be applied. Nutrients in soil, fertilizers and nutrients present in irrigation water are the sources for crops; grown in irrigated regions, to meet its need. Plant could meet its requirement from irrigation water only having high concentration of nutrients such as calcium, nitrate, magnesium, boron and sulfur etc. Most of the cultivated regions of the world have high concentration of N due to nitrate (NO<sub>3</sub><sup>-</sup>) leaching from chemical fertilizers. Hence application of nitrogenous fertilizers through irrigation water can minimize salinization and improve fertilizer use efficiency owing to improved availability of nutrients and controlled timing of fertilizer application and its supply to plant. The application of fertilizers through irrigation water is termed as fertigation (Machado et al., 2008). The care should be taken during fertilizers application of high EC value through irrigation water. In arid zone sulfuric acid and nitric acids fertigation have responded well to diminish the negative effects of salinity and sodicity. Chemical amendments i.e. nitric acid through fertigation increase calcium concentration in soil and reduce soil pH which is favorable to minimize salt injury due to Ca<sup>2+</sup> and Na<sup>+</sup> antagonism. Similarly nitrate form of N also compete with chloride ions and reduce salinity in rhizosphere (Weil and Brady, 2017).

Another way to minimize salinity effects on plant growth and to increase tolerance level is foliar application of essential nutrients including silicon (Si), salicylic acid, phosphorous (P), potassium (K), nitrate ( $NO_3^-$ ) and calcium (Ca<sup>2+</sup>).

## 5.5. Nutrient management practices

Arid and semi-arid regions of the world are experiencing salt affected soils having low organic matter and meager concentration of essential nutrients. Non-essential nutrients like Na<sup>+</sup> and Cl<sup>-</sup> are dominant over essential nutrients in salt affected soil due to low moisture and minor leaching of salts. Accumulation of salts in root zone effects plant growth in two phases; first is rapid phase and termed as osmotic effect because in osmotic effect plant reduces water potential and loses membrane integrity owing to which plant wilt. In second phase plant faces nutrient imbalance or specific ion effect in which salt ions accumulated in high concentration and influence the uptake of essential nutrients due to antagonistic effect. Therefore, nutrients application

particularly N as ammonium (NH4<sup>+</sup>) and nitrate (NO3<sup>-</sup>), Ca, Mg, K, Zn and Fe in optimum concentration in these soils is proved better approach for cultivation of salt affected soils. Thus nutrient management under saline soils application methods are of great concern because non judicious application of fertilizers is also among the major sources of salinization.

## 5.6. Nitrogen as a management strategy

Decomposition of organic matter in salt affected soils is very poor due to hyperthermic nature of soil. Because salt effected soils receive high temperature causing rapid decomposition of organic matter and low concentration of organic carbon subsequently poor N availability hence these soils respond immediately while applying N fertilizers (Swarup, 1998). Salt affected soils have high concentration of salts causing reduction in nitrate uptake due to antagonistic effect with chloride ions and SO4<sup>2-</sup> ions. Salt affected soils also disturb N fixation due to its hazardous effects on rhizobia and ultimately root nodules. Under salt affected soils plant growth is very poor which ultimately effects utilization and use efficiency of N fertilizers and its transformation from soil to plants. Therefore in order to manage N use efficiency under salt affected soils, it is essential to understand the fate of N present in soil and its transformation.

In case of grain crops such as wheat and rice, the main reason of low utilization efficiency of N is its loss through volatilization according to following reaction

$$NH_4^+ + HCO_3 - 4NH_3 + H_2O + CO_2 \dots Eq. 1.$$

The degree of loss of N depends upon pH, evaporation process of soil surface and nitrogen as  $NH_4^+$  - N concentration flooding or soil, flood water depth, wind velocity and evaporation. Bhardwaj and Abrol (1978) reported that almost applied N in sodic soil is lost in the range of 33-52 % range.

Salt affected soils are usually considered deficient in essential nutrients particularly N which is universally deficient in salt affected soils. Therefore, for proper growth of plant and its N use efficiency under salt affected soils application of N fertilizers in 20-25 % higher concentrations than recommended doses (120 kg ha<sup>-1</sup> for normal soil) for grain crops had proved better approach as a nutrient management practice (Tiwari et al., 1989). Application of N fertilizer in higher concentration depends upon severity of salinity and sodicity. A study performed by Javed et al (2021) to investigate effect of different doses of N on maize under different salinity levels. He reported that N application @ 293 kg ha<sup>-1</sup> had shown best results with respect to growth and yield of maize crop. So N application is imperative for proper management of salt affected soil.

## 5.7. Integrated nitrogen management

The application of N fertilizer in higher concentrations, the recovery of degraded lands is still low and sole application of synthetic N fertilizer is very costly for farmers therefore under such situations integrated application of organic and inorganic sources of N should be applied to improve N use efficiency and minimize N losses (Javed et al., 2021). Integrated use of organic matter and synthetic nitrogen fertilizers at a

specific ratio have ability to improve salt affected soils by improving physical and chemical attributes of soil.

Under a biotic stress, most of the essential nutrients in the form of synthetic fertilizers become unavailable to plant like N and K. Therefore integrated use of organic source of fertilizer including FYM with chemical fertilizer can play its crucial role in minimizing the effects of a biotic stress and expensive use of chemical fertilizer without disturbing fertility status of soil. The application of organic fertilizers with synthetic forms of fertilizers can sustain agricultural output without influencing uptake of other nutrients by plant. Therefore, it should be a part of our cropping scheme (Teh et al., 2016). In prevailing cropping system, use of organic manures can improve soil health and sustainability (Timsina and Conner, 2001). Generally under stress conditions, level of ethylene increased which create hindrance in plant growth resulting reduction in yield. Plant growth promoting rhizobacteria (PGPR) produce growth hormones in plant which is cause of enhanced nutrients supply to plants (Shahzad et al., 2014) because organisms reside in rhizosphere have a significant position in increasing growth of plant and pathogens in soil. These PGPRs restricted the roots to support the plant growth and plant yield (Shahzad et al., 2014). For example in young plants, ACC-deaminase is synthesized to reduce level of ethylene under salinity stress by improving the uptake of water and nutrients through roots (Verma et al., 2010). Kausar et al. (2018) indicated that integrated use PGPR or sole application of PGPR have positive impact on the growth of groundnut plants and had also

exhibited its positive impact on soil health significantly compared to respective control. PGPR are an important source of N and improve crop yield therefore, it is an inexpensive source of fertilizers as well as eco-friendly than chemical fertilizers. Abd El-Ghany et al. (2015) conducted an experiment on maize to assess the influence of PGPR on growth under stress conditions. They applied PGPRs in the form of P. fluorescens, A. vinelandii and P. putida as treatments. They observed positive effects on maize growth in the form of increased biomass. protein content, K<sup>+</sup>/Na<sup>+</sup> ratio and chlorophyll content while in case of antioxidants (POD, CAT and SOD), PGPR decreased their activity under saline soils. Overall, results were positive significantly when compared to control treatment. A similar study in pots was also carried out by Ahmad et al. (2014) on maize to estimate the effect of PGPR, chemical N, biogas slurry and their combination under saline soils. They concluded that all amendments in sole application increased plant growth but when applied in combined form the increase in plant growth and yield was more efficient than sole application of PGPR, chemical nutrients and slurry. Combined application not only improved plant growth and yield, it also improves soil health.

After nutrient management and organic and inorganic amendments in salt affected soils, an emerging approach to manage salt affected soil is choice of crop. Several researches have endorsed this reclamation strategy of salt affected soils. Growing of halophyte plant under slat affected soils may assist crops to adopt saline environment along their contribution in improving physical and chemical properties of soil and food quality (Javed et al., 2021). Numerous food and cereal halophyte plants including cotton, mustard, millet, wheat, barely, oil seed rape, guar, grape, mango, guava are very important (Murphy et al., 2018). Growing of halophytic plants for few seasons improve soil fertility status and make same soil suitable for glycophytes. Application of gypsum along with growing of halophytes is also very useful practice to minimize the effects of salts. Gypsum fertilization along with planting of artiplex species had proved better to reduce salinity level, SAR and ESP in salt affected soils (Abdel-Fattah, 2015). This practice of growing halophytes in salt affected soils is also termed as saline agriculture.

#### 6. Future research directions

# 6.1. Development of salt-tolerant cereals through breeding and genetic engineering

Use of salt tolerant verities and modifying gene expressions by genetic engineering in DNA of plant have now emerged as a key approach to eliminate the issues regarding crop quality and nutrition. Production of transgenic crops by modifying the crop gene expressions related to N uptake and its translocation in different parts of plant generate capability to balance N nutrition to aggravate plant growth under stress environment. For example generating *OsAMT1; 1* expression in genetically modified rice improved N supply and transport to different parts of plant and maintain N nutrition. Owing to the improvement in plant nutrition, nitrogen use efficiency (NUE) also increased to promote photosynthesis reaction, sugar and amino acid levels and ultimately

grain yield of rice. (Ranathunge et al., 2014). In the same way stimulation of (*OsGOGAT1*) and *OsAMT1;2* in rice also proved better engineering strategy to improve NUE by improving N uptake and assimilation which are attributes of increased yield in rice (Lee et al., 2020). In case of maize under stress conditions expression of *Dof1; a stimulator of genes influence plant growth and yield by improving organic acid metabolism ad amino acids, is induced to promote NUE and its concentration in plant* (Murphy et al., 2018). Another genetically modified gene in rice is *ZmDof1* which is responsible of N uptake its assimilation and also improve level of B and C concentration in rice crop plants. (Kurai et al., 2011). Similarly in case of wheat overexpression of TabZIP15 and AtbZIP24 improve salt tolerance and root growth and maintain osmotic potential (Bi et al., 2021).

## **6.2. Identification of new plant-microbe interactions for enhancing** nitrogen use efficiency and salt tolerance in cereals

Management of salt affected soils depend upon salinity type and its available amendment. Introduction of microbes in soil to ameliorate salt affected soils is also another strategy through different organic sources. The term for this purpose is use bio-organic amendments. In this type of amendment, organic source of microbe is either used alone or in combination of other amendment such as gypsum to improve soil chemical, physical and biological properties by increasing organic matter concentration in soil. Organic matter acts as a nutrient bank in soil which have ability to augment nutrients availability particularly N and P in soil, water holding capacity of soil and to improve soil structure and microbial activity which are the main attributes to improve crop growth and yield (Bello and Yusuf, 2021). Plant growth promoting rhizobacteria and AMF are considered beneficial microorganisms for plant growth and make soils capable to promote better in the form of crop production. The use of beneficial microbes in the form of PGPR or as biofertilizers is also an important approach to improve salt tolerance in plants under saline conditions (Mbarki et al, 2017) by induction of growth hormones and osmoprotectants such as proline or glycinbetain in plants to minimize the adverse effect of salinity (Shrivastava and Kumar, 2015).

Application of double inoculation of AMF and PGPR of various species also has now been recommended to improve tolerance level of salinity in different cereal crops by aggravating nutrient availability, root colonization of AMF and microbes to improve symbiotic relations with plants to amass root development for nutrient uptake and production of osmoprotectants especially proline in leaf of maize crop (Krishnamoorthyet al., 2016).

Microbial application in soil through different sources such as bio fertilizers, PGPRs and AMF play their important contribution in nutrient cycling through mineralization and immobilization to make nutrients available for plants and improving soil structure by increasing aeration and organic matter concentration (Xu et al, 2015). *Trichoderma harzianum* and *Pseudomonas stutzeri* have proved better to enhance tolerance to salinity in glycophytes (Bacilio et al, 2016). For example tomato yield as well as soil fertility was increased by inoculation of *T. harzianum* potentially under salinity (Kitila et al., 2020) because its inoculation lowers the SAR and P availability in salt affected soil. (Daliakopoulos et al., 2019). Organic sources including green manuring, compost, straw, biochar, humic materials are also considered best for improving salt stressed soils. After decomposition of organic materials plant, concentration of CO<sub>2</sub> and organic acids in soil increased which play their significant role in improving photosynthetic apparatus in plants and augmenting microbial activity in soil (Kitila et al., 2020). Hence owing to significant effect of soil microbes and organic amendment in soil fertility, application of these organic sources with integration of gypsum also has become beneficial to improve soil physical, chemical and biological characteristics.

#### 7. Conclusion

For sustainable agricultural production and to reduce food security threats for over population it is noteworthy to cope with a biotic stresses particularly drought and salinity. Area of salt affected soils is increasing every next day which is threatening for global population with respect to food security and is considered a major constraint for optimum agricultural production. Therefore sustainable production is imperative prerequisite to meet global requirement of food which could be attained by reclaiming and securing salt affected soils using various reclamation approaches either organic or inorganic approaches or integration of both organic and inorganic strategies. Other strategies including genetic engineering technologies, introduction of plant microbes are also very beneficial for salt affected soils and crop improvement. However genetic approach is very long lasting expensive but application of organic amendment along with microbial inoculation and gypsum is considered better approach for reclamation. Because it is an urgent need to improve N concentration in salt affected soil there understanding of N dynamics is necessary for its reclamation. All approaches are considered direct or indirect sources of N improvement by improving organic matter, microbial activities, maintaining EC, pH, reducing SAR and ESP of salt affected soils. So by using current available approaches, there is a dire need to research further in the area of salt affected soils at green house or field level.

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### **CHAPTER 4**

## SOIL EROSION INCREASING AND FRUIT TREES YIELD DECREASING UNDER CLIMATE CHANGES CONDITIONS

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#### **1.1. INTRODUCTION**

There is no soil phenomenon on a global scale more destructive than erosion caused by wind and water. Since prehistoric times, mankind has carried the scourge of soil erosion on its body and suffers from its consequences, namely malnutrition and hunger. In low-income countries, the ratio of population to usable agricultural land, which was already very high, is increasing. While farming has been concentrated in flat fertile lands, and has helped in providing more food, many nations are forced to expand their cultivated lands and burn and cut forests on steep slopes. Population pressure has also led to excessive grazing of livestock in pastures and excessive extraction of wood resources. All these activities cause the destruction or removal of vegetation, and the exposure of the sensitive underlying soil of this area to erosion. Decreasing productivity of fields, forests and pastures tells only part of the sad story of erosion. The soil particles washed away or blown away from erosion areas will later be deposited in other places such as lowlands near rivers and streams or in reservoirs and anchorages downstream. The environmental and economic damage in the areas where eroded soil materials are deposited may be as much as or more than the erosion areas from which the soil is separated. Displaced soil materials cause water and air pollution problems and will result in heavy economic and social costs in the society. Fortunately, in recent decades, we have witnessed many advances in understanding the mechanism of erosion and inventing methods that can effectively and justifiably curb soil wastage in most cases.

Effective factors in the instability of an ecosystem:

1) Forestry

- 2) Population increasing
- 3) Turning pastures into pasture fields
- 4) Using pastures regardless of their capacity
- 5) Use of lands regardless of their use

6) Improving the health situation and increasing the level of culture of the society

Consequences of an unstable ecosystem:

1) Landslide

2) Loss of soil

- 3) Erosion and sedimentation
- 4) Flood and drought
- 5) Land desertification
- 6) Pollution of water, soil and air
- 7) Decrease in soil fertility

Solutions to improve unstable ecosystems:

- 1) Reducing the number of livestock from pastures
- 2) Preventing the conversion of pastures into rainfed fields
- 3) Preventing the cutting of forests and bushes
- 4) Increasing product efficiency per surface unit
- 5) Preparation of protein sources other than red meat
- 6) Converting low-yielding rainfed fields into perennial fodder

Effective factors in rapid soil erosion:

- 1) Forestry
- 2) Conversion of pasture to rainfed
- 3) Improper agricultural operations
- 4) Using pastures regardless of their capacity
- 5) Wrong road construction and improper exploitation of mines

6) Plow and furrow in sloping lands, that too in the direction of the slope

Erosion is derived from the Latin root "Eroderi", which means wear and tear, and is the wearing away of the earth's surface. In general, erosion is a process in which soil particles are separated from their original substrate and transported to another place with the help of a transfer agent.

In a general classification, we have two types of erosion:

#### 1) Natural or geological or normal or conventional erosion:

In natural erosion that takes place over a long period of time, the depth of the soil does not decrease but increases. In fact, natural erosion eventually leads to the formation of soil. And in another way, it can be said that in this type of erosion, soil formation is more than soil erosion, and as a result, the depth of the soil profile increases.



Figure 1. Natural Erosion

#### 2) Fast or destructive erosion:

This erosion started when humans set foot in nature and destroyed the plant cover. In rapid erosion, the amount of erosion is greater than soil formation and the depth of the soil profile is also reduced. And as you know, the maximum acceptable erosion is up to the level of soil construction (Saganeiti et al., 2018).



Figure 2. Destructive Erosion

#### **1.2. CLIMATIC FACTORS**:

Among the climatic factors, rain, hail, snow, frost, temperature and wind can be effective factors in the emergence of water erosion.

#### **1.2.1.** The role of rain in soil erosion

Amount of rain, intensity of rain, critical intensity, size of raindrops, size distribution of raindrops, limit speed or final speed of rain, rain erosion index are the rain factors that affect on the soil erosion.

#### 1.2.2. The role of snow and hail in soil erosion

The erosion caused by hail is much more than the erosion caused by heavy rains due to the high mass and coarseness of the grains and the high speed of the drops. But this is not the case with the erosion caused by snow and the intensity of this type of erosion is much less than the erosion caused by rain (Xu et al., 2019).

### **1.3. ERODIBILITY**:

According to the definition of soil erodibility, it is the resistance of the soil against the separation and transfer of particles. From the past to the present day, various methods have been used to determine the erodibility of soil, one of which is the Boykas method, and in the soil (% Sand + % Silt) / % Clay, he mentioned that he believed that the erodibility with Ratios where this ratio is smaller are less erosion (Lasanta et al., 2017).

### 1.3.1. The role of infiltration rate in soil erodibility

The higher the penetration rate in the soil, the lower the amount of runoff and as a result soil erosion. Therefore, in order to reduce erosion, it is better to provide conditions in the soil so that the soil becomes permeable (Cillis et al., 2021).

# **1.3.2.** The role of total soil water capacity (water storage capacity) in soil erodibility

The higher the total soil water capacity, the more rainwater it absorbs, and as a result, the amount of runoff and erosion will be less. Soils that have more total porosity, as a result, their water storage capacity is also higher, and ultimately erosion is less in them. And another point is that the amount of this capacity increases with the increase of soil depth, so if other conditions are the same, erosion in deep soils is less than erosion in shallow soils (Quaranta et al., 2020).

#### 1.3.3. The role of soil water holding capacity in soil erodibility

The force with which the soil can hold part of the water is called the water holding capacity of the soil. The higher the soil water storage capacity, the less water runoff and as a result erosion. The amount of this capacity is about 9% in sandy soils, about 18% in medium-textured soils, and about 36% in clay soils (Pacheco et al., 2018).

#### 1.3.4. The role of soil texture in soil erodibility

There is a close relationship between the amount of silt in a soil and its erodibility. The higher the amount of silt in the soil, the more its erodibility increases. And in general, very fine silt and sand particles are highly erodible.

#### 1.3.5. The role of soil structure in soil erodibility

Soils that have a granular structure wear out later than soils that have a hard structure or a hollow structure. In general, coarse and stable soil grains are resistant to erosion. Factors that are effective in the size and stability of soil grains and as a result in reducing erosion are: soil texture, type of ions in exchangeable cation complex, type of clay minerals, organic materials and type of cement materials (Sourn et al., 2022).

#### 1.3.6. The role of soil colloids in soil erodibility

The types of soil colloids are effective in soil erosion due to their effect on permeability. The more soil colloids swell due to moisture absorption, the more their pore diameter decreases. It is for this reason that the resulting water will be more in completely clayey soils composed of silty clays. Of course, soils containing hydrophilic (hydrophilic) colloids also reduce water absorption and limit erosion (Nole et al., 2015).

#### **1.4. SLOPE:**

Characteristics of the slope that are involved in soil erosion include: degree, length, shape and direction of the slope.

#### **1.5. PLANT COVER**:

The effect of vegetation in reducing erosion depends on the type, height, density and growth stage of the plant. Minimal Soil Disruption, Cover Cropping, Optimized Plant Nutrition, Optimized Plant Genetics, Optimized Pest Management, Labor Efficiencies, The Logical Use of Protected Culture, A Logical Balance of Fresh, Frozen and Canned Output are plant cover group factors that affect on the soil erosion (Tucci et al., 2021).



Figure 3.a. Bare area among tree is result of the soil erosion



Figure 3.b. Bare area among tree is result of the soil erosion

## 1.6. THE EFFECT OF EROSION ON PLANT PRODUCTION ANA TREE YIELD

Since the soil is the basis of human food supply, the production of food products is directly dependent on the quantity and fertility of the soil. Escalating erosion has reduced soil fertility and increased the risk of global food shortages. The rate and speed of the effect of soil erosion on crop production and as a result food security is very complex and variable. The effects of food insecurity on about 807 million people are especially severe in tropical and subtropical regions of sub-Saharan Africa, South Asia, Latin America, and Central Asia.

The main reason for the reduction of agricultural production due to erosion can be attributed to the loss of the surface layer. Physical obstruction in root growth, reduction of available water reserves and reduction in food reserves. In other words, the effects of soil erosion within the region reduce crop production by disrupting the vital processes of the soil (Samela et al., 2022).

#### 1.6.1. Reducing the thickness of the surface layer of the soil

If the amount of soil erosion is greater than the regeneration of the surface layer, its thickness will gradually decrease; Therefore, reducing the amount of soil erosion is necessary to maintain agricultural production and increase them. With other factors being constant, crop growth and production will decrease with a decrease in the thickness of the upper layer. The upper layer of the soil has more suitable physical characteristics than its other parts, this part has the largest amount of available water and stored nutrients, and there is a concentration of plant roots in this area.

#### 1.6.2. Physical inhibition of root growth

Accelerator erosion causes an increase in surface sealing, crusting and compaction of the soil. Soils that have a hard surface or deep layer and under normal conditions have an obstacle for plants to root in them, with the start of soil erosion, crop production in them will decrease more strongly (Coluzzi et al., 2022).



Figure 4. Yied decreasing by the reducing the thickness of the surface layer of the soil

#### 1.6.3. Reduction of water available to plants

The decrease in the amount of water available to the plant due to soil erosion is one of the effective factors in reducing the production of plant products. By reducing the amount of available water in the root area of plants, the amount of crop production is reduced. Erosion by reducing the thickness of the surface layer of the soil as well as the loss of organic matter reduces the ability to hold water in the soil. It should also be noted that the impact of raindrops destroys the soil grains into smaller particles, and with the transfer of fine grain materials to the empty spaces of the soil, blinding or reduction of porosity occurs, which itself plays an important role in reducing permeability and increasing runoff and intensifying soil erosion. In addition to these cases, the amount of water available for plants is reduced in the lower layers due to clay having more adhesion properties and high absorption of water in clay. Perhaps the fertility of the eroded soil can be restored again, but since the amount of water available to the plant depends on the effective depth of the roots, after soil erosion, it is difficult to return the amount of water available to the plant to its previous state (Buttner et al., 2004).

#### 1.6.4. Loss of organic matter and nutrients

Most of the organic matter and nutrients are located on the soil surface. Organic matter has kept most of the nutrients with it. These materials are easily affected and transported by soil erosion due to their low specific weight. Since the macro and micro elements are attached to the particles of organic matter, the amount of product production is directly related to the amount of organic matter. In addition to this, organic matter affects acidity, cation exchange and other processes in the soil and thus affects crop production. Finally, organic matter increases the activity of bacteria and micro-organisms, which has an effect on the cycle and availability of nutrients in the soil. Therefore, with the erosion and transfer of organic matter, the amount of product production decreases. Crop production is not only affected by the presence of nutrients, but the interaction of a set of soil processes leads to crop production. However, chemical and organic fertilizers minimize the negative effects of soil erosion (Muscate., 2023).

## **1.7. THE RELATIONSHIP BETWEEN SOIL EROSION AND CROP PRODUCTION**

With the increase of soil erosion, the amount of crop production decreases; but this decrease is not always linear with the increase of erosion. Rather, this reduction takes place in different ways: linear, exponential, exponential, etc.

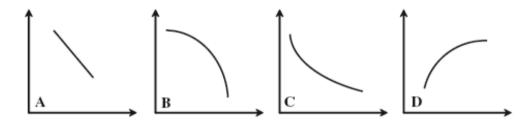


Figure 5. Types of relationship between soil erosion and crop production Understanding the relationship between soil erosion and crop production is important for better land management and water and soil protection planning.

In some soils, the reduction of the thickness of the surface soil may cause the roots of plants to reach the underground water, in this case, some of the reduction in production due to soil erosion will be compensated. Also, in some areas, fertile soils have been buried by sediments. In this case, with the increase of soil erosion and the gradual loss of surface sediments, the amount of product production increases (Terranova et al., 2009).

## **1.8. METHODS OF STUDYING THE EFFECT OF EROSION ON THE AMOUNT OF PLANT PRODUCTION**

The relationship between erosion and crop production is complex, and the use of fertilizers and other chemicals causes the effect of erosion on crop production to be covered. In this case, indirect methods are used to evaluate the effects of soil erosion on crop production in past, present and future time scales. In this case, it is possible to use the addition and removal models, SWAT and EPIC in the field of simulating the production of plant products, such as making the surface layer of the soil and checking the natural conditions of soil erosion. Surfce layer removal, increasing surface layer and natural soil erosion condition evaluation are the factors that should be studied in this case (Zhen et al., 2021).

### **1.9. EROSION AND SOIL QUALITY**

Erosion, by affecting the physical and chemical conditions, reduces the various functions of the soil. In the initial definition, soil quality is related to the issue of fertility, and only the ability of the soil to produce a specific product was considered. However, in the current definition of sustainability, environmental quality and climate change are also considered.

However, in the current definition of sustainability, environmental quality and climate change are also considered. In other words, this concept refers to the inherent ability of the soil to perform its duties correctly and also refers to the ability to resist human destruction, maintain production and fertility, reduce pollutants, watershed and improve the quality of water and air. Therefore, a good quality soil not only produces a good crop, but also protects the environment. The new term soil quality was used for the first time in the soils of temperate regions, but gradually it was used in other regions as well.

In temperate regions, non-point pollutants are an important environmental problem. While in humid areas, the reduction of nutrients causes a decrease in agricultural production and environmental problems (Sholagberu et al., 2019).

## 1.10. ECONOMIC EVALUATION OF THE EFFECTS OF SOIL EROSION

In general, the economic evaluations of soil erosion are divided into two parts: internal and external costs.

Internal effects are loss of soil, loss of nutrients, loss of soil organic matter, reduction of physical, chemical and biological properties of soil, reduction of the possibility of germination and growth of seeds and seedlings, reduction of the area of agricultural land, reduction of production, reduction of sales and profit from it. External effects are sedimentation in rivers and lakes, reducing the capacity of water resources, increasing the risk of flooding, destroying communication routes, reducing the capacity of waterways for sea transportation, nutrition, reducing aquatic biodiversity, reducing water quality, negative effects on treatment plants, reducing the efficiency of hydroelectric dams, problems for use. Resorts from water sources increase food supply costs.

Because it takes a long time to produce soil, its waste imposes an irreparable cost on natural resources that was never considered in the calculations. In general, the costs within the region are related to the reduction of soil thickness, the destruction of soil structure, the reduction of food and organic matter in the soil, and as a result, the reduction of the production of plant products, the increase of production costs in connection with the increase in the cost of fertilization and irrigation.

The most important extra-regional effect of soil erosion is related to deposition of sediments. The costs in this part are related to the cost of improving water quality, the cost of hydropower generation, the cost of dredging waterways and water supply channels, reducing water storage and sometimes reducing flooding (Kust et al., 2017).

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# **CHAPTER 5**

#### SOILS AND CLIMATE CHANGE

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### 1. Introduction

Soils are native materials of various ages that develop with soil formation events under the influence of parent rock, climatic conditions, relief and organisms. The development of soils is closely related to the development of life on Earth. In the Precambrian period, which ended about 600 million years ago, the first bacteria and algae emerged in subhydric soils. Semi-terrestrial soils and finally terrestrial soils were formed with the development of the first terrestrial plants in the Silurian period, which is the geological time period that ended about 430 million years ago and the rock systems were formed. Soils (S) are defined as a function of parent material (P), climate (C), relief (R), organisms (O), age (T) and human (H) factor:

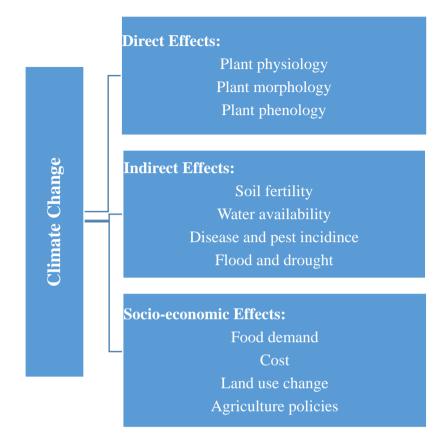
S = f (P, C, R, O, H).T (Blume et al., 2016).

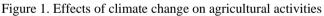
Climate is one of the two dominant factors on soil formation (the other is organisms). Solar energy, one of the climate components, is the most effective factor in soil formation with temperature changes. The other climatic factors is precipitation, air temperature, air humidity and wind. The soil temperature has a direct effect on weathering, mineral formation processes and decomposition. The effect of chemical weathering and decomposition on soil formation increases with increasing temperatures. Climate change will affect the formation rate of soils and all properties of soil (physical, chemical and biological) in different ways. This is an important environmental problem that ultimately affects soil fertility and crop production. Changes in rainfall due to global climate change may affect the surface moisture availability, which becomes important for germination and crop stand establishment in the rainfed areas. While it is estimated that a temperature increase of 2 °C in the world will cause a 5% decrease in grain yields and a temperature increase of 4 °C will cause a 10% decrease in yield, it is reported that the decrease in yield will reach 25-35% in the Mediterranean region (ÇŞB, 2012). In our country, arid and semi-arid regions such as South East and Central Anatolia, which are under the threat of desertification, and semi-humid Aegean and Mediterranean regions that do not have enough water will be more affected by global warming (Öztürk, 2002).

Due to climate change, global warming is expected to have direct effects on plant physiology, morphology and phenology. Indirect effects can be listed as the decrease in soil fertility and the amount of available water in the soil, the increase in the incidence of plant diseases and pests, and the increase in flood and drought events. Climate change will also affect the socio-economic systems of countries. Many countries will have difficulty in meeting the food needs of the increasing human population and production costs will increase. World countries need to be prepared for these possible changes and change their agricultural policies in this direction (Figure 1).

While the agricultural sector is affected by global climate changes, this sector also has a negative impact on global climate changes. Greenhouse gas emissions, the release of excess CO<sub>2</sub> into the atmosphere, the use of excessive water, fertilizers and chemicals are initiatives that can disrupt the natural balance collectively. It is

predicted that climatic changes will affect the availability and prices of food, restrict the access of low-income societies to sufficient food, and even create a food safety problem (Türkeş, 2020). When the effects of recent climatic changes on the world are examined, it has been determined that Turkey is among the risk group countries. It is stated that the decrease in precipitation together with the increase in temperature expected to occur in the Mediterranean Basin in the near future will further reduce the already insufficient water resources and cause serious problems in many countries. Drought will continue to be an important obstacle in agricultural production and in the transformation of these products into value-added products after agricultural production.





The results of climate change are conjectured primarily through alteration in soil moisture content, rise in soil temperature, and increase in CO<sub>2</sub> levels. The effects of climate change on soil properties are summarized as follows (Mondal et al., 2021):

The effects of rise in temperature:

Salinization of soils Increasing of decomposition rate of SOM SOM loss Decreasing of soil porosity Increasing of soil compactness Decreasing of CEC of soil Decreasing of soil fertility Soil degradation Increasing of soil erosion Reduction of WRT Increasing of CO<sub>2</sub> release from soil Reduction of SOC soil organic C Increasing of ammonia volatilization Rising of rhizospheric temperature Increasing of nutrient intaking Increasing of microbial activity in soil Increasing of N and P bioavailability

The effects of excess rainfall:

Destruction of soil aggregate Increasing of soil erosion Increasing leaching of alkalin cations Acidification of soils Decreasing of CEC of soil Increasing of loss of soil nutrients Development of hypoxic condition in poorly drained soil Increasing of toxicities of trace elements (Fe, Mn, Al, and B) Increasing of denitrification

The effects of low rainfall:

Increasing of salt content Decreasing in soil moisture Decreasing in diffusion of water-soluble nutrients Drought problems Loss of nutrient by erosion Reducing in nutrient intaking capacity of root system Loss in N-fixation by legumes The effects of increase in atmospheric CO<sub>2</sub>:

Increasing of C availability in soil Increasing of microbial activity in soil Increasing of fungal population in soil

SOM: Soil Organic Matter; CEC: Cation Exchange Capacity; WRT: water retention capacity; SOC: soil organic C

## 2. The effect of climate change on soil properties

Due to global warming, organic matter that is preserved in the soil will decompose rapidly and more carbon dioxide gas will be released into the environment as a result of decomposition. As soil temperature increases, the degradation and decomposition of organic matter will increase, while the uptake of plant nutrients by plants will naturally accelerate. Excessive rains will increase surface runoff and erosion in the soil. In addition to soil losses due to erosion, this situation will also negatively affect crop production. The increased runoff will cause the dams and irrigation systems to be filled with sediments and the agricultural lands to become unproductive due to the most fertile top soil carried. The decrease in precipitation, as well as the increase in precipitation, will cause different problems. In this case, wind erosion will come into play and cause the loss of soils. It is inevitable that this situation will prevent plant production (Parry et al., 1999). As a result, all erosion types (water, surface and wind erosion) will cause both soil loss and reduction of agricultural production. This situation will also reflect on the producers and social and economic difficulties will begin.

### 2.1. The effect of climate change on soil physical properties

Climate change may cause deterioration of the physical properties of soils, especially their structures, clogging of soil pores, prevention of water and air movement in the soil, salinization and alkalinization. Among the important problems of agricultural soils are the loss of organic matter in the soil, the loss of soil water, soluble salts and carbonates, as well as the loss of clay minerals, the most active and fertile part of the soil, sesqui oxides and silicon. Even the absence of water in the soil will prevent the formation of new minerals as well as soil formation events. Among these materials, the water-soluble ones will rapidly move away from the soil and lead to the impoverishment of the soil. High temperatures and low precipitation will also increase the carbon dioxide concentration in soils. This increase will lead to the deterioration of the C/N balance in the environment, the change in the physicochemical properties of the soils, the decrease in the amount of useful water in the soil, the increase in the risk of salinization, the deterioration of the biodynamic properties of the soil and the decrease in the reserves of plant nutrients in the soil (Benbi and Kaur, 2009). In addition to the fertility of a soil whose physical properties deteriorate, many other properties will also be lost. The negative effects of climate change are also effective on the structure, which is one of the physical properties of the soil. In conditions where there is not enough water, the formation of clay minerals will be prevented and the formation of sandy soils, which do not have much agricultural value, will accelerate.

Climatic events have direct and indirect effects on the formation of soil texture (Scharpenseel et al., 1990).

Events such as tillage, warming, swelling and shrinking, freezing and thawing of soils have important effects on soil structure, but the realization of these events is directly dependent on the regularity of precipitation, temperature, water, that is, climatic events. Structural stability, which refers to the bonding of soil particles as secondary particles, provides the resistance of aggregates to water and wind. Storage of soil water, microbial activity in the soil, development of plant roots, soil aeration, plant species to be grown, irrigation system to be applied, may vary depending on soil structure (Karmakar et al. 2016). Soil structure is an important indicator of soil health. The arrangement of soil aggregates, which is formed by the binding of sand, clay and clay particles in the soil by organic and inorganic colloids, reveals various soil structure types. The rapid decomposition of organic matter in the soil due to global warming will have negative effects on the soil structure.

Soil porosity is defined as the percentage voids in the soil. Porosity has a direct effect on the mobility of the roots in the soil, the availability of plant nutrients, the mobility of soil water and air in plant production (Reynolds et al. 2002). The amount of the pore volume (or the void ratio) depends on the soil organic matter content, the texture and on the soil development (Blume et al., 2016). It is expected that the pore size distribution and porosity of the soils will also be affected by global warming. Infiltration, which is the movement of water coming into the soil by entering into the soil, will be one of the parameters that will be rapidly affected by these changes in porosity. This will directly affect the amount of water available to plants in the soil. Decreases in the amount of water available in the soil may also cause decreases in field capacity, soil degradation or soil saturation with excessive water, the air in all the pores being thrown out and the filling of water instead, salinity and alkalinity. These changes, in addition to the deterioration of soil properties, will lead to changes in many things from plant species grown to soil cultivation methods or to take precautions regarding them.

Bulk density is weight (gr) of the soil with its pores in one cubic centimeter of soil. This value is an important parameter that reveals the fertility status of the soil, which gives us information about the compaction conditions of the soils, the organic matter contents, and the air and water contents. It is undesirable to have a high volume weight value in soils. Since organic materials added to the soil will cause a decrease in the soil volume weight value, it is beneficial to add all kinds of organic materials to the soil. Therefore, there is a positive relationship between the volume weight value and the organic matter content and or organic carbon content of the soil (Weil and Magdoff, 2004). Therefore, the reduction of organic matter in the soil in various ways will cause compaction in the soil and many problems with it. Activities such as tillage in extremely dry soils may cause the compaction of the lower soil layers, the formation of impermeable layers, and the accumulation of ions that will create various salinity and alkalinity in the soil. While the increase in soil volume weight will

cause crusting of the soil surface layer, the entry of water into the soil, the transfer of plant nutrients from the surface soil to the lower layers and the movement of soil air will decrease, and oxidation reactions in soils will increase. In order for the seeds to germinate, the formation of the crust layer of the soil must be prevented (Box et al., 1996). If the crust layer is not prevented, salinization and alkalization may occur in the soil, and this will cause further deterioration in the physicochemical properties of the soil.

Although soil temperature is important for seed germination, excessive heating of both the atmosphere and the soil has the same negative effects. Extreme heat will cause plants to overgrow, while on the other hand, it will cause more carbon to be stored in soils. High temperatures will increase the decomposition and mineralization of organic matter in the soil and decrease the organic carbon content. The deterioration of the temperature balance of the soil directly affects 3 main factors. The first of these is soil organic carbon content, the second is soil erosion and transport by water and wind erosion, and the third is changes in soil moisture content. One of the most important sources of CO<sub>2</sub> emissions in soils is the drainage of organic soils for agriculture. This value is equivalent to 20-40 tons of CO<sub>2</sub> per hectare per year. The most effective method for managing carbon stocks in soils is to preserve these stocks in soils and especially large stocks in peatlands by minimizing climate change. Soils and forests in prairie lands are a natural carbon sink, which is thought to store up to 80 million tons of carbon per year. However, in arable agricultural areas, this value is assumed to be 10-40

million tons of carbon per year. Irregular temperature fluctuations in soils will also affect heat conduction, gas and water vapor movements along the soil profile.

Increasing soil temperature will support the rapid degradation and decomposition of organic matter, while promoting microbiological activity and rapid nutrient release. As nitrification in will accelerate, chemical decomposition of soil minerals will increase. In other words, the effects of possible climate changes on both the carbon storage capacities of soils and soil respiration will occur differently in every geography and in every climatic condition. These effects are not expected to occur immediately. Possible changes in soil temperature and climate will also be effective on vegetative diversity and there will be a change in plant species. While species that can adapt to extreme heat will begin to dominate, plant species resistant to thirst will begin to cover the environment. Climate change will adversely affect all these parameters and adversely affect the soil health, which is the result of them. Changes in the climate will also affect the parts of the plants under the ground. Changes that may occur to find water, especially at the soil root depth, will cause negative effects on plant growth due to insufficient water content of plants (Birkas et al., 2009). Exposure of plants to long-term dehydration will also increase the concentration of undesirable salt compounds in the soil. The variety of these salt compounds can change the ionic composition of the soil surface, and as a result, the exchange of water and air and the availability of plant nutrients in the soil will decrease and may lead to the formation of anaerobic conditions in the soil. It is inevitable that this situation will reflect on yield as well as plant production.

### 2.2. The effect of climate change on soil chemical properties

Climate changes will also have negative effects on the chemical properties of the soil. The pH value of the soil, expressed as the negative logarithm of the concentration of  $H^+$  ions in the soil, is a parameter that occurs depending on factors such as vegetation, precipitation, microorganism activities, and parent material. The pH value is rapidly affected by many processes occurring in the soil. The organic matter in the soil acts as a buffer against these sudden changes. The reduction of organic matter will not prevent these sudden pH changes. This change in organic matter will lead to changes in soil C content, decrease in water in the soil, deterioration of plant nutrient balance and changes in pH value. Changing the pH value even less than one unit for some sensitive plants will lead to rapid yield reductions in crop production.

The salt content of the soil is also important in terms of both the physicochemical properties of the soils and the soil fertility. Climate changes will also cause changes in the salt values (EC) of the soils. The increase in the salt content of the soil and the elements in the composition of these salts may cause some problems in the soil. For example, sodium compounds will completely change the physical properties of the soil in a negative way and the soils will begin to become unproductive. An increase in element concentrations such as

sodium, chlorine or carbonate will adversely affect the microbial activity in the soil. Decreasing the microbial population will also reduce the availability of plant nutrients. The water and air balance of the soils will deteriorate, and thus, the crop production in these lands will decrease rapidly.

The cation exchange capacity, which is another chemical property of the soil, expresses the amount of cation adsorbed in 100 g of soil. In addition to adsorb the available nutrients to the plants, KDK is important in the immobilization of some toxic elements for plants. In general, the reasons for the high KDK is the amount of clay and organic matter in the soil. For this reason, the KDK values of sandy soils are lower than clay textured soils. The effects of climatic changes or extreme temperatures on the loss of organic matter, as well as the negative effects of soil formation events and the formation of clay minerals, will also adversely affect the KDK of the soil. In addition to leaching the clay and organic matter from the soil, excessive precipitation causes the alkali elements to be removed from the soil, and the KDK of the soil and endly the plant nutrient content of the soils are negatively affected by this situation. Nitrogen, which is the most important plant nutrient in the soil, is directly related to the soil organic matter. Excessive precipitation and extreme temperatures will affect and change the nitrogen concentration in the atmosphere and therefore the nitrogen content of the soils. These negative changes will not only be limited to nitrogen, but will also cause changes in the concentrations of other plant nutrients such as P, K, Ca, and Mg.

The organic matter content of the soils may be at different levels according to the soil of each region or the characteristics of the soils. (Weil and Magdoff, 2004). Organic matter has positive effects on all the properties of the soil. Extreme temperatures will cause a decrease in plant production and ultimately a decrease in the organic matter content of the soil. A large part of the organic matter already consists of plant residues. Organic matter is also very important in adsorbing available plant nutrients. In addition to the storage of carbon and nitrogen, which are important plant nutrients, it also carries out the cycles of elements such as phosphorus and sulfur. Organic matter positively affects the physical properties of soil, such as bulk density, porosity, structural stability, water retention and hydraulic permeability. In parallel with the decrease in the organic matter content of the soil, the sensitivity of the soils to erosion will increase, the biological productivity will decrease, bulk density will increase and the soils will begin to compact rapidly. The presence of organic matter in the soil and its good management will also prevent the release of excess carbon dioxide gas into the atmosphere. Preventing the destruction of excessive raindrops on the soil surface, the penetration of these waters into the soil instead of the surface flow, the storage of water in the soil, the easy movement of plant roots due to high porosity, the increase of the KDK of the soil, the prevention of sudden pH changes in the soil are closely related to the presence of organic matter.

### 2.3. The effect of climate change on soil biological properties

The increase in CO<sub>2</sub> concentration in the atmosphere, the slow increase in temperatures and the changing precipitation distribution have predominantly indirect effects on soil organisms. Increasing atmospheric CO<sub>2</sub> concentrations cause to an increase in plant growth, and also a change in the quality and quantity of available substrates (litter materials, rhizodeposites) for soil microorganisms. The higher water use efficiency of plants under high CO<sub>2</sub> concentration in the atmosphere cause to an increase in soil moisture. This situation affect positivly plants and soil organisms, especially during dry periods during vegetation. A lot of studies have shown that soil microorganisms rapidly transform additional available substrate and additional fixed carbon is rapidly remineralized. However, in the long term, changes in litter composition (higher C/N ratio) may lead a change in soil microbial community composition towards higher dominance of soil fungi. Since soil fungi use carbon more effectively than soil bacteria, this mechanism can lead to carbon storage in the soil, and therefore store more carbon in fungal biomass. The rising of soil temperature has a direct effect on soils by stimulating the activity and growth of soil organisms, and an indirect effect on soils through changes in physicochemical soil properties (volume, pressure, viscosity of fluids, etc.). However, the relationships between soil organisms and all climatic factors (temperature, precipitation distribution, and climatic gases) have not been sufficiently studied to date. For this reason, there is a need for studies that examine the changes in the biological properties of the soil due to climate change (Blume et al., 2006).

### 3. Conclusion

Although the organic matter of the soil is proportionally the smallest component of the soil, it has important effects on the physical, chemical and biological properties of the soil. Decreases in soil organic matter due to global warming and irregularity in precipitation may adversely affect all soil properties. Some of these effects are; cause soil salinization, reduction of soil fertility, increase risk of soil erosion, increase CO<sub>2</sub> release from soil, reduction of water retention capacity and soil organic C, stimulation of nutrient acquisition, enhance soil microbial activity and increases bioavailability of N and P from organic matter. There are some measures that can be taken to minimize the effects of global warming on soils. The first of these is to take conservation agricultural measures to increase resilience against climatic variability. These are: 1) Protecting/Increasing the organic matter content of the soil, 2) Implementing sustainable agricultural systems, 3) Conservation tillage, 4) Effective use of pesticides and chemical fertilizers, 5) Preventing soil compaction, 6) Use of cover crops, 7) Crop rotation, 8) Plant waste management, 9) Increasing water use efficiency and improving agricultural drainage, and 10) Erosion control. Another measure is the reduction greenhouse gas emissions. With proper management of N and C in agricultural areas, greenhouse gas emissions can be reduced and C sinks can be increased. Leaching and evaporation rates can be reduced by applying fertilizers at the appropriate dose and time. By increasing the efficiency of fertilizer use, the amount of washing and evaporation of fertilizers can be reduced.

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# CHAPTER 6

# USE OF ALTERNATIVE WATER RESOURCES FOR COPING WITH CLIMATE CHANGE IN AGRICULTURE

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### 1. Introduction

In the conventional applications, irrigation water including agricultural irrigation, livestock watering and cleaning, aquaculture, is provided either from groundwater through wells or from flowing (rivers) and non-flowing (lakes, reservoirs) surface waters. The key idea at this point is to keep consuming water without polluting the environment especially the water bodies at which the agricultural return flow is discharged as a diffuse source. This practice in irrigation however, has not been successfully carried out by the farmers over the years due to overuse and/or high withdrawal of water for irrigation purposes. Consequently, high return flow started to pollute the freshwater sources. Moreover, global population increase brought together the higher water demands for food production together with the food safety implications. On the contrary, global warming and climate change effects started to disturb the precipitation pattern together with the frequency and the amount of water received by the world over the years with increase in temperature (Elouissi et al., 2017; Zhao et al, 2021). That situation urged the humans to search for alternative water resources (AWR) as available freshwater resources have become scarcer (Haldar et al., 2022).

Contamination of limited freshwater resources, increase in water demand by sectors including domestic, agricultural and industrial needs brought together the term 'sustainability' to our lives. Among AWR known and practiced so far, one can address treated urban wastewater, harvested rainwater and desalinized seawater (IWA, 2015). There appear many areas where these water sources can be used. In this chapter, emphasis will be given to those AWR that can be used as irrigation water in agricultural activities. The quality of AWR is of utmost importance in agriculture in the sense of protecting human health from any exterior contamination that would affect the well-being of humans (Cabrera et al., 2018). Accordingly, food safety implications and regulations at different countries and societies will also be underlined. Last but not the least, use of alternative water resources in agriculture is a social and cultural issue that should be cared as underlined by Ricart (2019). Public acceptance on this application is a problem that necessitates public education and raise of awareness in especially developing countries. Final words will be devoted to this socio-cultural behavior.

Water use in irrigation varies around the world basically depending on the development level of the countries. Highly developed and industrialized countries like USA spent less irrigation water compared to world's average consumption ratio. On the contrary, developing countries are more dealing with agricultural activities consume more water for irrigation (Anonymous-1, 2023). Therefore, the subject of concern is an important global problem for many countries; thus, AWR seems to be a convenient option to cope with this crisis.

Not only the use of AWR; but the suitable irrigation method selection is equally important for water minimization, and so far, drip irrigation is one of the most efficient methods for delivering water to crops with minimal waste. Currently, it is used on less than 2% of irrigated land in the world but it can reduce water use by 30-70% (Anonymous-2, 2023). Besides, concentrating on the best available irrigation methods, farmers have to be also trained on the utility of AWR. Otherwise as water consumption exceeds the sustainable rates, higher amounts of pollutants will continue to join the limited freshwater sources. By 2030, the world is projected to face a 40% global water deficit under the business-as-usual scenario (UN, 2016). In addition to this, climate change will lead to more recognizable extreme events like droughts and storm coupled with higher air temperatures necessitating greater water use for crop irrigation and livestock. Application of AWR must not be regarded as an innocent exercise as it bears many disadvantages like potential scales, costs, and environmental impacts as recently noted by Qin and Horvath (2020).

### 2. Alternative Water Resources in Irrigation

In this section, AWR used in agricultural irrigation are examined under three headings as rainwater harvesting, desalinated water and recycled water followed by an evaluation of the food safety effects and public acceptance of the use of AWR in agriculture.

## 2.1. Rainwater Harvesting in Irrigation

The term "rainwater harvesting (RWH)" includes the collection, processing and use of precipitation. Today, this definition is used for human intervention to rainwater for both agricultural purposes and other uses. Archaeological findings show that rainwater harvesting is especially important for people living in arid regions. Since ancient

times, people have met their water needs by collecting and storing them in cavities in a piece of land or rock (Yannopoulos et al., 2019; Mays et al., 2013).

The main purpose of using RWH in agriculture is to ensure the availability of sufficient water for crops during the planned growing season (FAO, 2014; Velasco-Muñoz et al., 2019). It is widely practiced in arid and semi-arid regions where rainfall is insufficient to grow crops. The most common use is supplementary irrigation in plant growth stages, during water shortage or water stress conditions. In these applications, runoff from areas is collected, stored and made available for use whenever and wherever there is water shortage. Precipitation and runoff events depend on many factors and occur in intermittent periods. Therefore, in practice, it is necessary to store the maximum possible amount of rainwater during rainy periods (Qadir et al., 2007; Oweis and Hachum, 2003).

## 2.1.1 Applications of RWH in irrigation

There are numerous examples on the use of RWH for agricultural irrigation throughout history. It has been an important part of agriculture, especially in arid and semi-arid regions of India, the Middle East and Africa. It is known that the use of RWH in India dates back 4000-5000 years. Simple floodwater harvesting methods have been used for irrigation by many indigenous peoples in the arid and semi-arid regions of the Americas (Singh et al., 2022).

The interest in RWH and the number of studies on the subject have increased in recent years. Nowadays, on small farms, greenhouses and gardens that do not have access to surface water or groundwater, RWH is the optimal alternative for water access to meet crop requirements during drought periods. On the other hand, RWH is also important for "urban agriculture".

However, high concentration of pollutants that can be found in rainwater in urban environments is an issue that should be considered in the use of collected rainwater for irrigation purposes (Deng, 2021; FAO, 2014). There are many studies on the use of RWH for irrigation during dry periods to increase crop yields (Sacolo and Mkhandi, 2021; Sharma et al., 2009). It has been determined that 30-50% of rainwater can be used for irrigation purposes by using appropriate harvesting techniques in arid regions (Oweis and Hachum, 2003). Especially in sub-Saharan Africa, RWH systems are considered as a climate change adaptation strategy (Lebel et al., 2015). In a study, it was stated that the amount of arable land can be increased by 3-5% with the use of RWH systems in arid regions (Bruins et al., 1986).

A study conducted in the Caribbean by the Food and Agriculture Organization of the United Nations (FAO) confirmed that the rainfall pattern in the Caribbean is suitable for RWH systems, and found that the application of RWH may be sufficient for small-scale production of many crops grown during dry periods (FAO, 2008). Calculations of water demand and cumulative runoff were made in the study and findings showed that collected rainwater may be sufficient to extend well-planned planting seasons to at least 10 months of the year (FAO, 2014).

Eswatini, located in the south of Africa, is a region of irregular and low rainfall. Following the decline in agricultural production on the Lubombo Plateau RWH and soil conservation techniques have been proposed to improve maize production. According to the results of the study, it was determined that approximately 60% of the catchment area is suitable for RWH and 45% of the crop's water requirement deficit can be met (Sacolo and Mkhandi, 2021). A study evaluating the best alternatives for crop growing systems in the semi-arid regions in Gansu, China, shows the importance of creating rainwater catchment areas on barren land and the increased usefulness of collected rainwater combined with water conservation and leakage prevention techniques (Yuan et al., 2003). Since 2006, approximately 600 RWH systems for agricultural irrigation have been built in Beijing, China. The results of a study analyzing the economic and financial performance of these RWH systems built in rural areas determined that RWH systems are economically viable and their financial feasibility varies depending on the system size and groundwater fee (Liang et al., 2011). Low-cost rainwater collection structures with a storage capacity of 30 000 liters, built in agricultural land in Dhansiripar village of India, are used as a source of irrigation for high-value winter vegetables in dry periods (Anonymous-3, 2023).

# 2.1.2 System Design of RWH for irrigation

A typical RWH system designed for use in irrigation generally consists of the following four components (FAO, 2014; Singh et al., 2022):

<u>Catchment/collection area and surfaces</u>: It is the surface area where rainwater is collected directly for a particular irrigation area. These areas can be natural or artificially sloped. Roof tops, roads, airport strips, slopes, tree trunks, canopies, greenhouse roofs, and plasticcoated ground surfaces in the crop field can be used for this purpose.

<u>Conveyance system</u>: These are systems that carry the collected water to the storage area with gutters, channels and/or pipes. All drainage areas should be made of chemically inert material like plastic, aluminum, etc. not to adversely affect the water quality.

<u>Storage facility:</u> It is the area where the collected water is kept until use. It can be designed as a collection volume such as barrels, tanks, or a collection area such as a pond, small reservoir.

<u>Application area</u>: It is the target agricultural area where harvested rainwater is applied.

The design of components in RWH systems is made by considering the probability analysis of serial precipitation data, crop water requirement, evapotranspiration rate, and amount of rainwater (FAO, 2014). The most important aspects that determine the efficiency of the RWH systems are precipitation, terrain characteristics, elevation and slope information, soil structure, and water requirements for the crop. Planning and management of limited stored water is an essential part of

successful RWH systems, as with all irrigation activities (Velasco-Munoz et al., 2019; Fiaz et al., 2018). The most commonly used RWH systems for irrigation purposes are as follows:

<u>Rooftop RWH system:</u> This system is the most frequently used type, regardless of the end-use purpose (Figure 1). In this application, the rainwater collected from the roof surface by a discharge pipe, gutter etc. is directed to the surface or underground storage tanks and stored. Roof surface properties affect system efficiency.

<u>Farm ponds</u>: It is designed as covering a part of the planting area with a plastic surface and creating a rainwater collection surface (Figure 2). It is one of the most frequently used methods in RWH applications for irrigation purposes. Depending on the soil type, permeability and geological features, the use of sealing plastic may not be necessary (Figure 2).

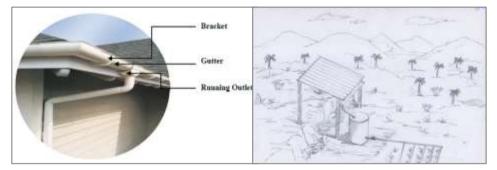


Figure 1. Rooftop RWH system (Source: FAO, 2014)



Figure 2. Farm ponds with and without plastic sealing (Source: FAO, 2014)

<u>Greenhouse (GH) roof:</u> In these systems, the plastic coating surface of the GH roof is used as the collecting area (Figure 3). The collection tank is usually at the low sloping end of the GH. The system is equipped with pipe, gutter, storage pool/tank, irrigation connection, etc.

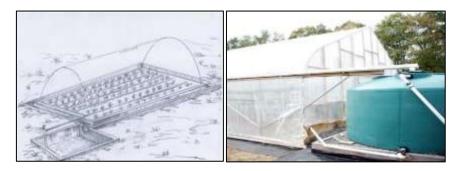


Figure 3. RWH system with GH plastic roof (Source: FAO, 2014; Anonymous-4, 2023)

<u>Natural slope collection system:</u> It is the collection of rainwater in a pool or tank by directing it with the natural slope of the land, stop ditches, collection channels. It is a common practice especially in areas with short-term heavy rainfall (Figure 4).



(b)

Figure 4. (a) RWH system with natural slope (Source: FAO, 2014), (b) Mini dams (Source: FAO, 2014)

<u>Mini dams</u>: Building mini dams to create reservoirs for irrigation is a common form of storage, especially in seasonal waterways and temporary streams. It is suitable for fields located on slopes (Figure 5). In this way, large amounts of water can be stored.

The basic condition of RWH is to provide adequate storage of the needed rainwater in the field. Requirements for successful RWH are specific regions with suitable climatic, geological, hydrological and meteorological conditions. There are a number of advantages to using RWH systems in irrigation. Captured water provides some of the water demand for irrigation, and through storage alleviates drought caused during dry seasons. The system is simple. Investment and operating costs are generally low. Besides RWH, it also has benefits such as erosion control and soil degradation prevention (Velasco-Muñoz et al., 2019, Bafdal and Dwiratna, 2018, Qadir et al., 2007).

The major disadvantage of RWH systems is their dependence on limited and uncertain precipitation. Collection and storage systems require large lands. All of the roof, collection and storage systems should be of materials that will not adversely affect the water quality. The system may be damaged during severe meteorological events. Collection and storage equipment require regular maintenance. In general, it is more suitable for products with low density and lower water needs compared to other irrigation systems (Qadir et al., 2007; Wenhua et al., 2010). All details regarding collection, storage, maintenance should be determined by regulations, and aspects of use should be standardized with limit values.

### 2.2. Desalinated Water for Irrigation

Desalination is a process in which dissolved solids (salts) are removed from seawater, brackish water, municipal/domestic and industrial wastewater. Desalination plants are used by many sectors/industries such as oil, energy, mining, gas, food/beverage, medicine, agriculture and municipalities (Barron et al., 2021). The technology of obtaining freshwater by desalination has a history of more than a century. Among the desalination methods, thermal processes have been used for the last 60 years and membrane processes and desalination have been employed for the last 40 years (Greenlee et al., 2009). While primitive forms of desalination have been applied since ancient times, industrial-scale desalination methods only began to be used in the mid 20th century. It was first practised in coastal communities. Today, approximately 300 million people in more than 150 countries obtain water from approximately 16 000 desalination plants every day. The world population based on desalinated seawater is expected to increase from 7.5% in 2015 to 18% in 2050 (Ricart et al., 2020).

Starting from the 1950s, desalination plants have been established, especially in regions suffering from chronic drought, such as the Middle East. Today, about half of Israel's drinking water is met by desalination, and desalination facilities continue to be opened in the USA state of California, which is struggling with drought (Anonymous-5). The largest desalination plants are located in the United Arab Emirates, Saudi Arabia and Israel (Anonymous-6).

Desalination is an energy-intensive technology. In Saudi Arabia, which supplies 60% of its water from seawater, 300 000 barrels of oil are used every day for desalination (Anonymous-5). Advances in technology have made desalination feasible in high yield agriculture in arid regions where water costs may be excessive due to distance or depth from the water source (Ben-Gal et al., 2009). This technology is not common in Türkiye, except for small-scale facilities in a few places. With increasing drought conditions and urbanization, desalination is becoming a technology that attracts the attention of touristic facilities, municipalities, industrial facilities such as iron, steel and textile, especially in coastal areas. For example, Avşa Island Municipality of Balıkesir has been providing desalinated water to the citizens for several years (4000 m<sup>3</sup>/day) (Anonymous-5). The cost of desalination of wastewater effluents or brackish groundwater, often found in arid regions, is typically half or less than the cost of desalination of seawater. This type of desalinated brackish water is increasingly preferred by farmers for irrigation (Ben-Gal et al., 2009).

In the following sections, information about the technology, advantages and disadvantages of the desalination process, and the use of the desalination process in the world are mentioned.

### 2.2.1. Water quality characteristics

Although desalination is expensive, it is a reliable and clean water source used for both human consumption and agricultural irrigation, especially in areas where water is scarce. Small-scale desalination plants help to ensure water security. Larger facilities play an important role in providing access to safe and reliable drinking water (Anonymous-7). Many water resources, such as groundwater, contain elements such as calcium ( $Ca^{2+}$ ), magnesium ( $Mg^{2+}$ ), and sulfur ( $S^{2-}$ ), which are essential nutrients for plants. When these substances are removed together with harmful ions such as sodium and chloride, there may be a decrease in yield due to the deficiency of these elements in plants. The water produced from desalination systems does not contain sufficient level of nutrients required for the cultivation of agricultural products. In order to provide the required nutrient level, nutrients can be added to the water used in agricultural irrigation or these elements may need to be enriched with fertilizers (Suwaileh et al., 2020). This is one of the major problems encountered in desalination. Since it also removes the carbonate  $(CO_3^{2-})$  hardness in the water, the buffer capacity of the water is also reduced. Therefore, water must be stabilized by adding sources of bicarbonate (HCO<sub>3</sub>). The reverse osmosis system also destroys viruses and bacteria (Anonymous-7).

The salinity of seawater or brackish water fed to desalination plants is about 1000 mg/L Total Dissolved Solids (TDS) to 60,000 mg/L. As known, seawaters contain salinity between 30.000-45.000 mg/L. Reverse osmosis membranes (ROS) used in the desalination of seawater is used to purify waters in the TDS range of 10.000–60.000 mg/L. Such membranes are used to purify water resources (such as underground water resources) in the TDS range of 1000–10,000 mg/L during desalination of brackish water (Greenlee et al., 2009).

Desalinated water is of high quality and has fewer adverse effects on soil and crops than using brackish water directly. Desalination of brackish water to be used for agricultural production is cost-effective compared to desalination of seawater (Beltrán et al., 2004). According to Birnhack et al. (2010), the quality parameters to consider when using desalinated water for agricultural irrigation are: electrical conductivity, Cl<sup>-</sup>, N<sup>+</sup>, B, Ca<sup>2+</sup>, Mg<sup>2+</sup> and SO4<sup>2-</sup>, alkalinity, the water stability index, calcium carbonate precipitation potential and pH (Kumar et al., 2018). In the study by Greenlee et al. (2009), Israel's experience in desalination for agricultural use was shared. Accordingly, the recommended water quality parameters values for agricultural irrigation are given in Table 1.

Parameter	Recommended values for domestic and
	irrigation water
Electrical conductivity (dS/m)	<0.3
$Cl^{-}(mg/L)$	<20
$Na^{+}(mg/L)$	<20
$Ca^{2+}(mg/L)$	32-48
$Mg^{2+}$ (mg/L)	12-18
$SO_4^2$ -S (mg/L)	>30
B (mg/L)	<0.4
Alkalinity (mg CaCO <sub>3</sub> /L)	>80
pH	<8.5

Table 1. Recommended value of water quality parameters for agricultural irrigation after desalination based on Israeli experience (Kumar et al., 2018).

#### 2.2.2. Positive and negative impacts

There are several benefits of using desalinated water in the agricultural sector. The most common of these is to obtain a new water source. Costs are a major limitation in this technology. ROS is seen as relatively inexpensive among these technologies. Most of the world's desalination capacity is provided by ROS (Burn et al., 2015). Desalination method is an expensive technology as it requires energy. At the same time, large storage areas are needed for the system to operate efficiently after the technology applied (Anonymous-8). Features such as crop irrigation schedule and seasonality, irrigation efficiency techniques and soil type in use in agricultural irrigation have important implications for the cost effectiveness of desalination plants (Barron et al., 2021).

The desalination process used in agricultural irrigation has several benefits as given below (Barron et al., 2021):

<u>Water security:</u> The most important benefit of this process to agriculture is water security. While site-specific, these technologies support agricultural resilience to drought and climate variability. Thus, the risks of agricultural enterprises can be reduced. Desalinated seawater can represent an abundant and stable water source, effectively removing climatological factors (eg. droughts) and hydrological constraints that affect traditional water sources (Martínez-Alvarez et al., 2016).

<u>Improvement of water quality:</u> The reduction of salinity in the water increases the yield of the product. For example, 25% reduction in water salinity in one study resulted in a 100% increase in grape-vine crop productivity.

<u>Conditioning of water:</u> Controlled conditioning can be done by adding fertilizer and/or minerals (fertigation) to the permeate (directly to the irrigation water) according to the needs of the crop and the soil.

<u>Water use efficiency</u>: Use of desalinated water for agricultural irrigation leads to the development of water-saving irrigation techniques and to increase the value of products.

<u>Increase in agricultural production:</u> Purification of the permeate after desalination ensures the production of suitable water.

Implementing desalination process has several challenges, including (Barron et al., 2021):

<u>Variability of seasonal water demands</u>: Wide variability of irrigation water demand (daily, monthly and yearly) can lead to a high cost of desalination if used as an 'emergency source'. Desalination plants operate at a constant rate, that is, at 100% capacity continuously, with the lowest unit water cost. To solve this problem, the water formed in the desalination plants can be stored.

<u>Current water price</u>: The unit cost of desalinated water may vary depending on feed water quality, salinity and brine disposal methods. Depending on these features, the costs can be quite high.

<u>Perception</u>: Lack of expertise and lack of confidence in practice are factors limiting the wide use of desalinated water in agriculture.

Desalination of sea or brackish water has positive effects on the environment, such as creating AWR and recycling water. However, it also has many negative effects. The most important adverse effects are the removal of desalination residues with brine, chemical additives used for antifouling, corrosion inhibitors, etc., noise and greenhouse gas emissions (Beltrán et al., 2004).

Desalination processes have several disadvantages. The most important disadvantages are; high energy footprint, dense salty wastewater released back into the ocean/sea and harmful effects on marine life. More than half of the seawater used in desalination results in more concentrated salty wastewater (brine). High-pressure jets discharge this wastewater back into the ocean. Desalination plants around the world produce 160 million(M) m<sup>3</sup> of hypersaline concentrate per day (Anonymous-9). This volume of brine amounts to a significant portion of the inflow. Desalination of seawater usually contains more salt at 50-65% of the inflow. This salt concentration is approximately twice the initial concentration (Beltrán et al., 2004). Standards for releasing wastewater back into the ocean vary considerably from country to country. In some areas, particularly in the Persian Gulf, Red Sea, Mediterranean and Gulf of Oman, desalination plants are often

clustered together and warm discharges are continuously given into shallow coastal waters. This situation increases the seawater temperature and salinity, reduces the overall water quality and may adversely affect coastal marine ecosystems. Another risk occurs when saltwater is withdrawn from the sea. Withdrawing water from the sea also causes fish, larvae and plankton to be drawn into the desalination plant and cause the death of these creatures. Millions of fish and invertebrates are trapped in desalination plants each year (Anonymous-10).

## 2.2.3. Categories of desalination systems

There are three categories of desalination commonly used today: Thermal technologies (distillation), Membrane technologies, Chemical processes.

Of these desalination systems, membrane and thermal technologies are the most widely used ones. Large-scale desalination for agricultural irrigation is usually accomplished with thermal or osmotic- based membrane technologies. There are also those based on electrochemical processes as membrane technology. However, these electrochemical methods are used less frequently (Beltrán et al., 2004).

In the thermal desalination method, the water is boiled until it evaporates, and the water vapor is condensed and collected elsewhere. Quite high thermal energy is required for this process. The thermal energy required to carry out this process on a large scale is obtained from steam generators, waste heat boilers or steam from power station turbines (Figure 5a).

Membrane processes such as electro-dialysis and reverse osmosis were developed after the thermal distillation process. The basic technology of the membrane separation method involves applying high pressure to the water through the semi-permeable membrane. The most widely used membrane system is ROS. The schematic view of the ROS is shown in Figure 5b. These membranes allow water to pass, but not dissolved salts. This is a very high-energy process. The most common membrane process, the ROS, was first developed in the 1950s and commercialized in the 1970s.

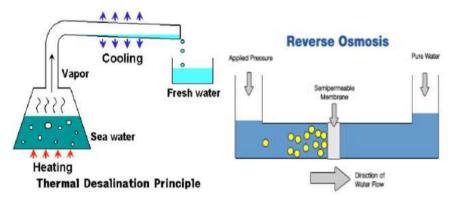


Figure 5. (a) Thermal desalination system, (b) ROS (Source: Anonymous-11)

Reverse osmosis desalination can remove most salts from water, although there is some selectivity towards specific ions. The amount of salt removed in the reverse osmosis process mostly depends on the pressure and the ratio between the amount of desalinated water produced and the amount of brine rejected and discharged as waste (Figure 6). In this process, two streams are formed, treated water (permeate) and concentrated water (brine to be disposed of) (Barron et al., 2021). As seen Figure 6, the sum of the permeate flow and the brine flow is equal to the flow of the feed water.

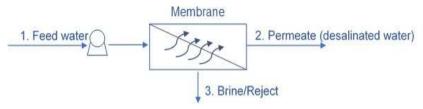


Figure 6. Permeate and brine flows in ROS process (Source: Anonymous-7)

The reverse osmosis process can be applied for two different sources: seawater and brackish water reverse osmosis (Figure 7). There are differences between these two processes in terms foulants, salinity, brine removal options, and plant location (Greenlee et al., 2009).

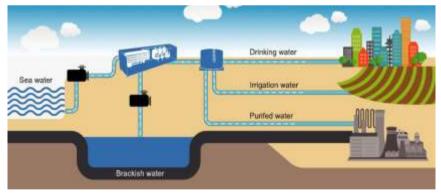


Figure 7. Usage areas of reverse osmosis system (Source: Anonymous-12)

# 2.2.4. Practices around the world

Many countries, especially arid countries, use desalination systems for their water supply. Saudi Arabia, Algeria, Bahrain, Egypt, Tunisia and countries such as China, Indonesia, Hong Kong, Cuba and Nigeria have installed desalination plants for obtaining drinking water. 50% of Malta's water supply comes from desalination of seawater (Ramadane El Zarroug et al., 2020). The volume of desalinated water used for agricultural irrigation has increased significantly in recent years in countries such as Spain, Israel, and the United Arab Emirates (Ismail and Kassem, 2015).

Among the Middle Eastern countries, Saudi Arabia is the leading country using desalination, followed by the United Arab Emirates. According to the World Resources Institute's list of the worlds' most water-stressed countries, the United Arab Emirates ranks 6th and Saudi Arabia 9<sup>th</sup>. Among the other rich Arab countries, the most waterstressed countries are Bahrain, Kuwait and Qatar, respectively (Anonymous-9). By the mid-1950s, Israel was well on its way to becoming a national-scale Water Carrier, having extended its irrigation pipes into the Negev desert. In these years, desalination was used for drinking water in Eilat. In the late 1950s, Israeli government was investing heavily in R&D on desalination. During these years, Israel has become an exporter of various desalination technologies. After periodic and prolonged droughts, the Israel Water Commission has planned mega-scale desalination solutions to bridge the growing gaps between supply and demand, and to prevent further degradation of groundwater. In 1997, the Desalination Master Plan was completed. In this plan, various water resources and demand scenarios have been extensively studied. A detailed planning has been made, including the

optimum locations and capacities of the desalination plants, and the costs and benefits of desalination. On April 4, 2002, the program for the establishment of 4 desalination plants with a total capacity of 400 Mm<sup>3</sup>/year was accepted by government decision. In July 2007, the desalination master plan was updated so that 5 coastal facilities were projected. Israel has 2 desalination plants under development. One of them is targeted to be operational by 2023 and their annual capacity is planned to be 300 Mm<sup>3</sup> (Garb, 2008).

The first desalination plants were built in the Spanish Canary Islands in the 1960s to provide water to the public. In the 1980s and 1990s, these facilities increased rapidly. In Spain, it has been necessary to seek AWR due to the excessive use and pollution of groundwater due to agricultural activities and temporal irregularities in river flows. After these obligations, the capacity of the desalination plants in the country reached 1.2 Mm<sup>3</sup>/day in 2000 and the number of plants reached 750. This value reached 1.55 Mm<sup>3</sup>/day in 2005 and 2.8 Mm<sup>3</sup>/day in 2010. This rise is expected to make Spain the 4<sup>th</sup> country in the world in terms of desalinated water production capacity (Palomar and Losada, 2010).

Between the late 1990s and 2009, the Millennium Drought afflicted southeastern Australia. Between these years, the amount of water in the water storage systems in the region has decreased to very low levels. Therefore, the use of desalination systems has increased rapidly in Perth, Melbourne and other cities of the country. The Melbourne desalination plant, which supplied its first water in 2017, now provides a third of the city's supply. In the USA, more than 400 municipalities have installed desalination microplants, mostly in California, Texas, and Florida. Many of these plants are located close to natural gas plants to take advantage of the residual heat from power plants. Most of the African continent countries are facing water problem. The drought is mostly concentrated in North Africa and the Western Cape of Southern Africa. Especially North African governments are increasing their capacity to obtain water through desalination. Algeria is focused on desalinating seawater for its drinking water supply. Tunisia has accelerated the production of multi-million-dollar power plants to meet the energy needs of desalination plants (Anonymous-9).

#### 2.3. Recycled Water in Irrigation

Water recycling or water reuse, is defined as the reuse of water from various sources for purposes such as agricultural irrigation, groundwater recharge, use in industrial processes, for use environmental restoration, and use as drinking water, etc (Anonymous-13). Recycling and reuse of wastewater is considered as one of the tools that contribute to better management of water resources as the stress of water shortage increases. Some of the increasing water demand in the world, especially in the agricultural sector, which needs very high amounts of water, can be met with treated wastewater considering appropriate criteria. This practice, which has been implemented by many countries, is of great importance in terms of both the efficient use of freshwater resources and the reduction of the environmental effects of treated wastewater.

Recycled water has many valuable uses such as (Figure 8) (Anonymous-14):

- Agricultural irrigation
- Park/garden irrigation
- Groundwater recharge
- Fire extinguishing
- Dust control on construction sites
- Flush water in toilets
- Artificial water pond
- Industrial processes
- Restoration of wetlands

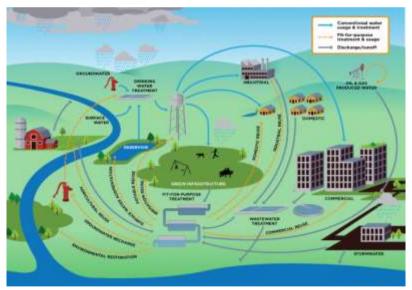


Figure 8. Examples of water sources and use applications (Anonymous-13)

Many countries in the world use wastewater for agricultural irrigation after treating it appropriately. 80% of the wastewater generated in Israel is used for agricultural irrigation (Zhang and Shan, 2019). Agriculture is the most important water consumer, using 70% of all freshwater in the world. The need for water for drinking, use and irrigation is increasing. This need makes wastewater reuse an effective solution to solve the problem of water scarcity, save drinking water and reduce the use of chemical fertilizers in agriculture (Ungureanu et al., 2018). The use of wastewater in agricultural irrigation plays an important role in the recycling of water. According to FAO, more than 20 M hectares of land worldwide are treated with untreated or partially treated wastewater (Zhang and Shan, 2019).

The use of treated wastewater in the world for agricultural, industrial and domestic purposes is 70%, 20% and 10%, respectively. These rates vary in different parts of the world depending on the characteristics of the region. Reuse of wastewater for some purposes, such as agricultural irrigation, is an indispensable part of integrated water management (Katip, 2018).

# 2.3.1. Water quality characteristics

Treated wastewater can contain a wide variety of pollutants depending on the sources (industrial, urban, domestic, agricultural etc.). According to studies, treated urban wastewater contains particulate and dissolved organic matter and inorganic substances (eg N, P, K<sup>+</sup>, Na<sup>+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup>, Cl<sup>-</sup> and B) as well as pathogens and various microorganisms. It also contains trace metals, xenobiotics and natural or semi-synthetic compounds classified as toxic, resistant and/or bioaccumulative chemicals (Metcalf and Eddy, 2003). Considering this complex nature of wastewater, detailed chemical and biological characterization must be done to evaluate the quality of treated wastewater. However, it is always difficult to predict exactly the effects that may arise from its reuse (Becerra-Castro et al., 2015).

Recycled water usually contains about 300 to 400 mg/L more dissolved substances than the drinking water from which it was produced. Therefore, the water quality of the recycled water should be analyzed periodically (Anonymous-13). The parameters that need to be analyzed to determine the irrigation water quality are given below (Pedrero et al., 2010; Polat, 2013):

Salinity: Electrical conductivity, TDS

Cations and Anions: Ca2+, Mg2+, Na+, CO32-, HCO3-, Cl-, SO42-

Miscellaneous: B, pH, Sodium Adsorption Ratio (SAR)

Users should follow this water quality data to adjust irrigation practices such as fertilization schedule, depth and wash rate. Such adjustments are critical to the successful and beneficial use of recycled water (Polat, 2013). In addition to the conventional parameters given above, wastewater from urban wastewater treatment plants often contains a wide variety of organic chemicals. Chemicals remaining in the water after treatment have the potential to contaminate soil and water resources, and drinking water supplies. Products in irrigated areas are exposed to chemicals and are transported to the edible parts of fruits and vegetables and can enter the food chain through accumulation. Helmecke et al. (2020) conducted a study summarizing the risks of using wastewater in agricultural irrigation in terms of organic micropollutants. Despite the benefits of water recycling, it has been brought with several concerns and challenges for human health and the environment, including chemical risks. It was also emphasized in the study that although many regulations and directives regarding the reuse of water have been successfully implemented at the international level, the existing information on chemical risks has not been sufficiently considered in the context of legislation (Helmecke et al., 2020).

The communiqué and its annexes on the usability of treated wastewater in Türkiye were published in the Official Gazette dated 25 October 2022 and numbered 31994 (Official Gazette, 2022). In the annex of this communique, the properties sought in wastewater to be used in irrigation are explained in detail. The chemical quality criteria of irrigation waters are given in the annex of the communiqué. Chemical criteria include ECw, TDS, sodium, chloride, boron and SAR (Official Gazette, 2022). Pollutants that can be found in wastewater such as total dissolved solids (salts), boron, heavy metals and various toxic substances can accumulate, be up-taken by plants or remain in water depending on climatic conditions and soil properties. For this reason, in case treated wastewater is used and disposed of in the field, factors such as soil characteristics, climate, plant species and irrigation method should be considered, as well as the compliance of the water with the prescribed limit values (Official Gazette, 2022).

# 2.3.2. Positive and negative impacts

Depending on the characteristics of whether treated, partially treated or untreated, wastewater can be used in agricultural irrigation. While it is necessary to treat and recycle/reuse of wastewater for irrigation purposes, there are some associated risks (Zhang and Shan, 2019). The use of untreated wastewater in agricultural irrigation within the framework of standards, without taking the necessary precautions, has various risks listed below (Bingül and Altıkat, 2017):

- There is a serious risk to the health of people who come into contact with untreated wastewater for a long time and consume vegetables irrigated with this water.
- It causes pollution in groundwater.
- It leads to accumulation of chemical pollutants in the soil.
- It can change the pH of the soil with its buffering capacity.
- It may cause heavy metal accumulation.
- It may create an medium where diseases can settle.
- It can damage irrigation systems.
- It can cause eutrophication in channels carrying wastewater.

Recycling of water has 2 important benefits: Treated wastewater is used as a water source and is kept away from streams, lakes and beaches to prevent contamination of surface and groundwaters. Secondly, with the water cycle, economic savings, valuable material and heat recovery can be achieved (Polat, 2013). The flow of freshwater resources is variable according to the seasons, climatic conditions and precipitation pattern. The volume of water obtained by recycling wastewater is not affected by these conditions, allowing farmers to grow crops throughout the year. In addition, wastewater contains nutrients that can increase plant growth and reduce the use of chemical fertilizers. In a study, it was stated that compared to irrigation with clean water, savings of up to 45% in fertilizer applied in wheat and up to 94% in alfalfa (Zhang and Shan, 2019).

Irrigation with improperly treated wastewater can cause soil hardness, enrichment with heavy metals and groundwater pollution (Gola et al., 2016). Irrigation with wastewater can cause exposure to parasitic worms in food and, accordingly, the spread of diseases.

Appropriate irrigation methods as well as appropriate treatment methods can reduce negative environmental impacts. Irrigation methods can be in different forms such as flood irrigation, spray irrigation and drip irrigation. Of these methods, the flood irrigation method has more adverse effects due to both the excessive use of water and the high probability of contact with pollutants. Wastewater used in spray irrigation must at least undergo secondary treatment and disinfection. Drip irrigation is the most environmentally and healthfriendly application due to the low risks to groundwater and less contact with water (Tripathi and Rajput, 2016).

#### 2.3.3. Suggested treatment processes for water recycling

Water sources that can be used for recycling include municipal wastewater, industrial process and cooling waters, rainwater and agricultural runoff. These waters are treated appropriately for the process or activity in which they will be used. It is expected that the water treated for reuse does not endanger public health after treatment and does not pollute environmental resources (Anonymous-13). The treatment processes required for the recycle of wastewater vary depending on the characteristics of the wastewater and the purpose for which it will be reused after treatment. The quality standards of wastewater to be used in agricultural irrigation are determined according to the legislation of the country applying this method. However, water quality parameters that cause the most important problem are pathogens. In addition to this parameter, TDS and salinity are other important parameters to be eliminated. As is known, salinity level of wastewater is high and the highest treatment cost is spent on desalination (Bingül and Altıkat, 2017).

Physical, biological and chemical treatment processes are used for the treatment of wastewater to be recycled and reused. The treatment of wastewater using mechanical and physical processes is called primary treatment, and the treatment using biological processes is called secondary treatment. Further treatment processes in addition to these treatment processes are called tertiary treatment. The most basic treatment system example that can be applied for the recycle/reuse of wastewater is given in Figure 9.

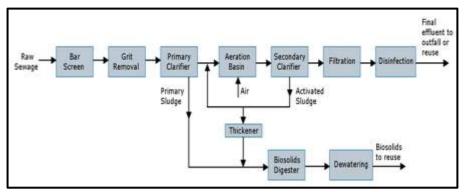


Figure 9. Basic treatment system for the reuse of wastewater (Source: Anonymous-15)

Norton-Brandão et al. (2013) conducted a comprehensive study on the reuse of wastewater by removing salinity, pathogens, nutrients and heavy metals. It has been observed that treatment with sedimentation, filtration and disinfection technologies using chlorine dioxide, UV and TiO<sub>2</sub> ensures the usability of wastewater for irrigation purposes. It has been stated that the use of membrane bioreactors can remove pathogens with high efficiency and in addition, heavy metals can be removed at high rates. It has been observed that oxidants used for artificial wetlands, artificial ponds and disinfection are efficient in the removal of microorganisms, but are not suitable for the removal of salinity. In a study, it was stated that the treatment system should be decided by considering the operation and maintenance costs and environmental effects, as well as the removal efficiency of the parameters required for use in wastewater for irrigation purposes (Becerra-Castro et al., 2015).

#### 2.3.4. Practices around the World

Although recycling is considered to be one of the main solutions for water scarcity, few countries actually practice it. The main countries, where water is recycled/reused, are; USA, Western Europe, Australia and Israel (Polat, 2013). Agricultural irrigation with wastewater is a common practice in arid and semi-arid countries. In countries experiencing drought and water scarcity, reuse of treated wastewater for irrigation is an important issue. Currently, the reuse volume of wastewater is between 10%-29% per year in Europe, the United States and China, and around 41% in Australia (Rusanescu et al., 2022).

In the study of Kellis et al. (2013), it was stated that the agricultural water needs of Paris and its surroundings have been met with purified water for nearly 100 years. In addition, it has been mentioned that urban wastewater is used for toilet flushing and industrial processes in factories in different regions (Aydoğan, 2021).

There is a growing interest in protecting water resources in Tunisia. Unconventional types of water use in the country are seawater desalination and reuse of treated wastewater. Reuse of treated wastewater for agricultural irrigation has been a practice for decades. Especially forage crops are irrigated with this method. In addition, farmers pay separately for the use of treated wastewater (Barcelo et al., 2011).

In major cities of India, Mumbai, New Delhi, Kolkata, Bangalore, Hyderabad and Ahmedabad, it is seen that untreated or treated wastewater is used for agricultural irrigation. In a study of Mekala in 2006, it was referred that along the Musi river in Hyderabad, rice was grown on an area of 2,100 hectares and grass was grown on an area of 10,000 hectares. In another study, it was shown that when 118 farmers irrigated their jasmine fields with wastewater, they gained more per ha in 8-9 months of work, and rose and marigolds were grown. Secondary treated wastewater in Hyderabad is used to irrigate parks and wooded roads. Near the Keshopur and Okhla treatment plants in New Delhi, about 12.000 farmers have grown eggplant, zucchini, coriander and okra in summer and mustard, spinach, cauliflower and cabbage in winter on 1.700 hectares of land (Aydoğan, 2021). It is estimated that wastewater generated by urban activities in India is 26.4 km<sup>3</sup> per year. 28% of this wastewater is treated. This amount has the potential to irrigate approximately 2.1M hectares of agricultural land, contribute 4 M Mg of plant nutrients, create employment for 2.8M people per day and reduce GH gas emissions of 73.7 M Mg-CO<sub>2</sub>-e (Minhas et al., 2022).

In China, the issue of urban wastewater treatment has received considerable attention over the past decade. In the city of Taiyuba, where access to water is limited, terracing of the agricultural land and damage to the natural ground caused sandstorms, and greatly reduced the water holding capacity of the soil. Most of the water used for domestic consumption is met by groundwater and agricultural land arrangements have led to a decrease in groundwater. For this reason, a water master plan has been prepared and put into practice for the sustainable use of water, and in this context, the reuse of treated water has begun to become widespread. For example, water for process in industries was reused after being purified (Aydoğan, 2021).

The Orange Country groundwater regeneration system in California is the world's largest reuse wastewater treatment system. It receives treated wastewater that is discharged into the Pacific Ocean after going through microfiltration+ROS and advanced oxidation processes. The system has been in operation since January 2008; It can produce approximately 265.000 m<sup>3</sup> of high-quality water every day. This water is supplied for 600.000 people in northern California and Orange County. Wastewater from the treatment plant, which serves 460.000 people, is used for urban irrigation in the Central Contra Costa sanitary region, which is also a part of California. Recovered water is distributed through a pipeline different from the drinking water distribution line and used in golf courses, parks, campuses and industrial irrigation (Solak, 2018).

The Orange County and the City of Orlando have developed a water reclamation program because their state prohibits discharge into surface waters. This project, named as Water Conserv II, has been implemented by the City of Orlando, Orange County, and the Agricultural Community. The project is the world's largest recycling project combining aquifer replenishment via rapid infiltration ponds for further utilization in agricultural irrigation. Rapid infiltration ponds were used in the project to recharge the Florida aquifer, Florida's primary source of drinking water. Water recovered with Water Conserv II has met public reuse standards and is permitted for use in all public areas, including residences, food crops, golf courses, plants and tree farms, nurseries, firefighting, pasture land and cement production (Anonymous-16; Chaudhary, 2019).

In 1961, the Irvine Ranch Water District was established in Orange County, California. Recycled water has been obtained from the Michelson and Los Alisos Water Treatment Facilities in the region since 2000. A recycled water distribution system was installed for a 133-square-mile service area of 316.000 residents. In Michelson and Los Alisos wastewater treatment plants, wastewater is treated and made suitable for use in non-potable waters such as irrigation water. Recycled water is distributed through a dual distribution system that includes more than 300 miles of pipelines, 12 storage reservoirs and 15 pumping stations. Recycled water is still used in landscape irrigations such as parks, schoolyards, golf courses, cemeteries, highway views, urban landscaping, front and back gardens of residences. This water is also used for watering food products, flushing toilets and urinals in 12 double plumbing office buildings and commercial office cooling towers (Chaudhary, 2019).

Israel is a rather arid country with insufficient natural water resources. Wastewater reuse and desalination systems have become the main sources of water in the country to reduce future risks of water scarcity. Almost 70% of urban and industrial wastewater in Israel is reused in agriculture after being treated in biological treatment plants across the country. The Dan District Reclamation Project (Shafdan) in the country is the largest wastewater treatment project in Israel (Icekson-Tal et al., 2003). Spain, which is the second country in the world to treat and reuse

wastewater, recycles 20% of its wastewater, while this rate is 87% for Israel (Anonymous-17).

Wastewater used for irrigation in Israel is generally subjected to tertiary treatment. But, there are cases where even lower levels of purification are allowed for food crops. For example, secondary biological treatment is used for irrigation of dates in the Dead Sea Region (EPA, 2023). The first pilot-scale MBR system in Türkiye was established on the Middle East Technical University (METU) campus, and the water treated in the system was given to the irrigation system, resulting in an annual water saving of 240.000 TL (Adalı and Yalılı Kılıç, 2020).

# **3.** Food safety implications and public acceptance on the use of alternative water resources in agriculture

Along with population growth and changing climate, an estimated 56% of irrigated land on the world is located in regions that face high water stress (Intriago et al., 2018). This condition urged the arid and semi-arid countries to practice non-traditional irrigation like is the case in Israel as the leading country of the world in applying almost 86% of domestic effluent in agricultural activities (Tal, 2016). Almost in other 60 countries, such applications are ongoing; however, reliable and systematic data covering the amount reused and the degree of treatment are unavailable and/or missing (Thebo et al., 2017). The outcomes exhibit 1600% increase in the economic value of the crop products as mentioned by Tal (2016) coupled with a parallel increase in land management policies.

As well-known these days, reuse applications lead to cope with microbiological, chemical and physical pollutants together with residual pharmaceuticals and personal care products (Sapkota, 2019). Researches on the emerging pollutants from reuse applications urged the scientists to establish A Centre of Excellence at the Nexus of Sustainable Water Reuse, Food and Health (CONSERVE) in 2016 through funding from USA, Department of Agriculture, National institute for Food and Agriculture (USDA-NIFA) to the University of Maryland School of Public Health. CONSERVE aims a systems-based approach to consider the accessibility of non-traditional irrigation water resources in the most scientific manner with a multidisciplinary team (Anonymous-17). Food safety is still a great challenge all over the world and thus, efficient and cheaper ways of testing the food safety is a necessity. In that respect, biosensors are regarded as a feasible option as referred by Griesche and Baeumner (2020).

The major concern in the reuse applications is surely water quality standards and criteria to be obeyed by the producers/farmers regarding public health. In this context, USA is the leading country that deals with the outbreaks of *E. coli* in the crop products in which treated wastewater is used in irrigation. So far, fruit and vegetable safety provoked public attention in this developed part of the world. Therefore, new Federal Regulations was released in January 2013 by the USA Food and Drug Administration (FDA) through which a science-based standard was put into force covering the Standards for Growing, Harvesting, Packing and Holding Produce for Human Consumption within the context of Food

Safety Modernization Act (FSMA) (Anonymous-18; Rock et al., 2019). The main idea behind this attempt is to reduce the water-borne and food-borne illnesses to protect the human health. As there appears no federal regulations on the reuse of effluent, states have already developed their own regulations based on the soil and climatic properties.

EU, Canada and WHO have also stated their microbiological recommendations for recreational waters besides USA (Rock et al., 2019). Crop yield, soil and human implications based on giardiasis epidemiology has been searched by Leonel and Tonetti (2021) in Brazil representing a developing country. Especially in such countries, epidemiological circumstances need to be taken into consideration with great care. The recent study of Partyka and Bond (2022) suggests that the guidelines for reuse applications depend on the geographic location, technologies used for treatment, crop cultivation types, and finally on the public health concerns after reviewing multiple epidemiological and quantitative risk assessment models. Therefore, there appears no single universally accepted recommendation for treated water quality; rather, every country has to accept their individual standards that would fit to their case.

Even though reuse activities have been extending all over the world, there is a need for further scientific studies to solve the still unknown critical issues like fertigation (recovery of water and nutrients) practices resulting in accumulation of emerging pollutants linked with public health. One of the most recent studies involve the development of a new techno-economical approach for assessing the sustainability of effluent use in different climatic conditions where different crops are cultivated (Mainardis et al., 2022). On the other hand, water reuse is regarded as a feasible option in water scarce areas as treated effluent constitutes part of ecosystem services; therefore, within the context of circular economy, promotion of water reuse is to be emphasized as mentioned by Bellver-Domingo and Hernandez-Sancho (2022).

Mediterranean Region is an important agricultural area in Europe. The opinion of key stakeholders was analysed to understand the attitudes and willingness to reuse treated effluent in agriculture in southeastern part of Italy by Saliba et al. (2018). Farmers and consumers reflected high acceptance on the reuse policy with 59 and 87%, respectively. The objections basically relied on the health problems that might arise due to use of some toxic chemical compounds that do not degrade at the ordinary wastewater treatment plants. The results of the survey put forth the reality that a comprehensive water management policy is necessary in order to accomplish high quality reuse water and to increase public awareness on the reuse option. Therefore, the policy should cover a close linkage between farmers and consumers to attain success in reuse activities as noted by Ricard and Rico (2019). As such, the key driving factors like risks, regulations and the yuck factor may be achieved. The authors conducted a thorough literature review between 2007 and 2017, and reached to a conclusion that the best way to convince public on the reuse attempts is to establish some pilot scale sites using treated effluent in irrigation, and conduct demonstrations prior to passing to full scale

activities. Such site visits will aim to increase public interest and knowledge so that the acceptance ratios will rise.

There have been several studies and surveys conducted on public awareness linked with non-traditional water use in the entire USA. One of the recent ones carried out in southwest USA indicate that the majority of the respondents to the survey were concerned with water availability, and less than the half thought the use of non-traditional water in agriculture was an important water saving method (Dery et al., 2019). As understood from this survey, people show willingness to use reclaimed/recycled water in agriculture; however, there seem to be gaps in public knowledge necessitating more training and education, and this situation is also valid for the farmers.

Study of Deh-Haghi et al. (2020) refers to the outcomes of a survey carried out among farmers in Iran representing another developing country on acceptance and willingness to pay for effluent used in irrigation. The findings prove that agricultural documentation and recording on water consumption amounts were still lacking; however, the farmers were almost fully aware of limited freshwater resources. Their acceptance on using treated effluent was quite high; but willingness to pay comparatively less amounts based on the treated water quality appeared to be much more preferred. Increasing knowledge on public health indicators seemed to be highly important. This reality has been already underlined by Fielding and Roiko (2014).

Passing more information on the utility of recycled water together with major public health concern linked with the potential pollutants

attracted the attention of public, and those who received satisfactory information and knowledge, accepted the use of treated water comparably more than those who had no information. Therefore, the importance of education and training to both farmers and end-users is an outstanding aspect that should be considered by the governmental and local authorities in charge of agricultural activities. Another waterstressed country is Saudi Arabia located in the Middle East Region of the world. Mu'azu et al. (2020) performed a survey on public acceptability of reusing treated water in non-domestic applications. Even in the highly educated group, the acceptance level was quite low. This fact necessitated changing the negative public perception through establishing a strategic management plan coupled with implications and recommendations. Another representative survey on the social attitudes of reusing treated water held in Belgium showed that feelings of disgust and contamination anxiety are still among the leading challenges on governing the social behaviour against the reuse applications (Verhoest et al., 2022).

#### 4. Conclusion

The nexus of water reuse, food production and public health is an evolving topic that raises the interest of multidisciplinary scientists in today's world that faces population growth under climate changing. Therefore, the freshwater crisis rising reuse of treated/untreated/partially treated wastewater use in especially irrigation of crops lead to food safety and public health issues. It is actually a recent global challenge that brings together the policy

implications. By year 2050, global population is expected to reach 9.7 billion, which in turn, will increase the water demand for agriculture sector as the dominating requirement of humans will be food production in the safer manner.

Utility of AWR particularly in irrigation will therefore be the key aspect in the coming decades as frequent drought conditions and continuous temperature increases are regarded as major signs of climate change. At this stage, more scientific studies are expected on the potential accumulation of emerging pollutants on the crops irrigated by AWR that are not sufficiently treated. Under such possibility, these pollutants may enter the food chain resulting in public health risks. It is of utmost importance to focus on these problems in future.

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## **CHAPTER 7**

# THE EFFECT OF DIFFERENT DRYING METHODS ON *IN VITRO* GAS PRODUCTION PARAMETERS IN EARTHWORMS

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#### 1. Introduction

Among the invertebrates (Invertebrata), insects, flies, spiders & worms constitute 95% of the animal kingdom in terms of biomass. In addition, they are widely produced & consumed in large areas (De Castro et al., 2018). It is advantageous that they have a high feed conversion ratio, contain high protein, & require little water, feed & land for their production (Van Huis et al., 2013; Barbi et al., 2020; Van Huis, 2017; Mulia, 2019). Insects are not only qualified as feed. At the same time, it has been determined that antioxidant peptides, chitin & antimicrobial peptides in their structures can stimulate the immune system. It is stated that it can regulate the intestinal microbiome in animals that consume insects (Erten et al., 2022). It has been determined that insects have an anti-inflammatory effect on fish Gasco et al., 2018; Henry et al., 2018). It has been reported to increase growth performance & improve carcass quality in broilers (Benzertiha et al., 2020; Lokman et al., 2019). In the in vitro study, it was reported that some insect species had similar fermentation effects with fish meal, while they had less gas production & methane production than rapeseed, meal soybean meal & sunflower meal (Renna et al., 2022). In a different study, it was reported that cricket, earthworm meal & black soldier fly larvae had less methane production than soybean meal (Jayanegara et al., 2017). In another study, it was not observed any negative effect on rumen fermentation & digestibility by using of 25% insect meal instead of fish (Ahmed et al., 2021). Methanogenic archaea use H<sub>2</sub> as an energy source (Beauchemin et al., 2020). Haryati et al. (2019) stated that chitin &

chitosan extract obtained from the black soldier fly reduced the acetate ratio in the rumen & this caused a decrease in  $H_2$  & CO<sub>2</sub>, in this case, it was determined that methanogenesis would decrease with the decrease of  $H_2$  in the environment.

The use of insects, flies, spiders & worms as ruminant feed is currently banned in most developed countries (Canada, China, Japan & USA). In contrast, many countries don't have a specific law against their use as ruminant feed (Lähteenmäki-Uutela et al., 2017). In *in vitro* studies, it is seen that these creatures are a good feed source for ruminant animals with their high protein & fatty acid content. In addition, insects, flies, spiders & earthworms produced less methane than forage plants with high protein value & have less impact on greenhouse gas emissions (Renna et al., 2022).

The earthworm (*Lumbricina*), which takes its place among the commonly used species in studies where animal products are used as feed, is a terrestrial invertebrate belonging to the *Annelida* phylum. It has a hydrostatic skeletal structure. They contain approximately 14.57% dry matter (DM), 59.00% crude protein (CP), 9.00% ether extract (EE), 2.6% crude fiber (CF) (Karabulut et al., 2016). It is similar to flour (Chiu et al., 2016). If the living conditions of the earthworm are made suitable, the number of earthworms becomes approximately double every 45 days & their population can multiply exponentially. Thus, they are widely used (Figure 1) (Bellitürk & Goldmann Benardete, 2020).



Figure 1. Evaluation of The Earthworm's Life Cycle & Its Bioeconomic Status.

Earthworm production with animal waste is important economically & ecologically as well. It has been stated that up to 10 kg of earthworms can be produced, equivalent to a conversion efficiency of 100% based on one ton of animal waste (Edwards, 1985). In a different study, it was reported that 40 kg of vermicompost & 6 kg of worms were produced from 450 tons of cattle manure (Hennuy & Gaspar 1986).

Drying can be defined as the reduction of moisture content in plant & animal products. It is one of the oldest known product preservation methods. As the percentage of water decreases as a result of drying, microbial activity is observed at minimum levels (Alves-Filho & Stranmen, 1996). One of the purposes of drying is that it can better preserve its solid properties such as shelf life, nutritional value, taste & smell, & slow or prevent mold & bacterial growth (Radoiu, 2020).

Because of mass transfer & heat during the drying process, many chemical, physical & biochemical changes occur that affect the quality characteristics of the dried product such as colour, structure, aroma & nutritional value (Vega-Galvez et al., 2009). Many industrial drying methods have been developed to obtain high & Standardquality dried products. Among them, osmotic dehydration, microwave, hot air, vacuum & freeze drying are the most widely used drying methods (Sagar & Kumar, 2010).

The convective drying method is called hot air drying & the heat required for evaporation is transmitted to the vegetable & animal product by convection. Compared to contact drying, its thermal efficiency is lower. The most important factor affecting drying in convection drying is the speed of the drying air (Figure 2). Air velocity is important as it determines the rate of removal of the evaporated moisture from the environment. When the drying reaches a certain value, the air velocity has no additional effect on the drying. In practice, there are various types of fluid dryers (bed, tunnel & spray dryer) (Yağcıoğlu, 1999; Cemeroğlu, 2011).

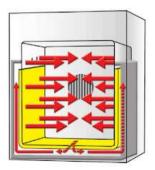
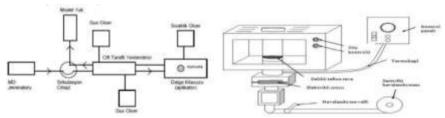


Figure 2. Convective Drying Method.

The microwave drying method is based on the volumetric heating mode with electromagnetic radiation. The response of a nutrient-containing product to dielectric heating results in rapid energy binding to moisture in the material, followed by heating & drying (Figure 3). The short drying time in microwave drying can lead to an improvement in product quality (Feng et al., 2012).



**Figure 3.** Microwave Circuit & Assisted Air Drying System (Ulcay et al. 2002; Reyes et al. 2007).

Lyophilization process is carried out in 3 states as freezing of the product, sublimation (Primary Drying) & Desorption (Secondary Drying). It is ensured that the product is completely frozen & transformed into crystal form. Vacuum is applied to the frozen product & as the temperature drops, the high energy molecules spontaneously fly out, thus drying without damaging the heat-sensitive products (Figure 4).

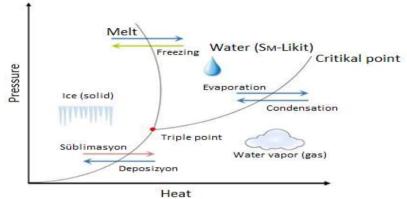


Figure 4. Lyophilization Phase Diagram, Malyer, C. (2018).

## 2. Material and Method

## 2.1. Obtaining earthworm meal

The analyses were made suitable by washing the earthworms used in the study from the soil & fertilizers on it (Figure 5).



Figure 5. Earthworms Used In The Study.

The cleaned earthworms were kept in the freezer for 48 hours to make them inactive. Afterwards, 3 types of drying methods were applied to the earthworms as lyophilization (L), oven (O) & microwave (M) & then they were subjected to grinding process. In each drying method, approximately 30 g of dry feed samples were analysed in 3 replications & a 3x3 trial design was applied (Figure 6).

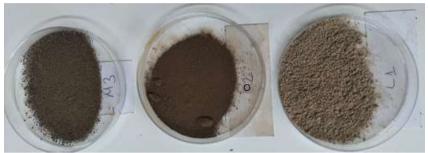


Figure 6. Earthworm Flours Dried With Different Drying Methods (Microwave, Oven, & Lyophilization).

2.2. Drying methods

## 2.2.1. Lyophilization Drying Method (LDM)

Earthworms used in this study were kept in lyophilization tubes (vials) overnight at -80°C. The samples were freeze-dried in a lyophilizer (Christ alpha 2-4 LD plus, Germany) at -76°C at 0.0010 mbar for 48 hours & then milled (Figure 7).



Figure 7. The Lyophilization Device Used In The Study.

## 2.2.2. Oven Drying Method (ODM)

Earthworms were placed in 90 millimeter (mm) petri dishes. The drying process was carried out in an oven with a dry air sterilizer (Nüve, FN055, Turkey) at 50°C for 72 hours. At the end of the application, earthworm samples were subjected to the grinding process (Figure 8).



Figure 8. The Oven Device Used In The Study.

## 2.2.3. Microwave Drying Method (MDM)

After decontamination & inactivation of earthworms, were placed on oiled paper, each of 100 gr, & placed in the erlenmeyer. The inactive earthworms in the erlenmeyer were heated for 8 minutes (min) at 2450 MHz, 540 W in the microwave (Arçelik MD 594 Microwave Oven, Turkey). At the end of the application, earthworm samples were ground & made ready for analysis (Figure 9).



Figure 9. The Microwave Device Used In The Study.

#### 2.2.4. Chemical analyzes

Dry matter (DM), ash (A), crude protein (CP), ether extract (EE) & crude fiber (CF) of feed analyzes were performed according to the method reported in AOAC (2006).

### 2.3. In vitro gas production parameters

The Gas Production Technique reported by Menke & Steingass (1988) was used to determine the in vitro gas production values. Rumen fluid is taken from Holstein cattle slaughtered in the slaughterhouse, which has completed its rumen maturity. The rumen fluid was kept at 38-40 °C in a thermos & brought to the laboratory. Solid particles were separated by filtration in a CO<sub>2</sub> environment. 200±10 mg of feed sample was put into glass syringes with a volume of 100 ml. Then, 30 ml of rumen fluid/buffer solution (10 ml rumen fluid / 20 ml buffer) was added to the glass syringes in which the feed sample was placed. Glass injectors containing feed & rumen liquid were kept in the incubation cabinet at 39 °C & the gas values formed at the 3<sup>rd</sup>, 6<sup>th</sup>, 12<sup>th</sup>, 24<sup>th</sup>, 48<sup>th</sup>, 72<sup>nd</sup> & 96<sup>th</sup> hours of the incubation. As a result of the data obtained, *in* vitro organic matter digestible (OMD), metabolic energy (ME) & net energy lactation (NE<sub>L</sub>) values were calculated. The gas formed at 24, 48, 72 & 96 hours of the incubation was taken with the help of an injector & the methane (CH<sub>4</sub>) values were measured with the MX6 IBRID Multi-Gas detector.

$$ME = 2.2 + 0.136 * GP + 0.0057 * CP + 0.00029 * EE^{2}$$
$$NE_{L} = 0.101 * GP + 0.051 * CP + 0.112 * EE$$
$$OMD = 14.88 + 0.889 * GP + 0.45 * CP + 0.0651 * A$$

GP: Gas production amount produced in 24 hours (ml); CP: Crude protein in feed samples (g/kg DM); EE: Ether extract in feed samples (g/kg DM); A: Ash content in feed samples (g/kg DM). ME; Metabolic energy (MJ/kg, DM), NE<sub>L</sub>; Net energy lactation (MJ/kg, DM), OMD; *In vitro* organic matter digestible (%).

### 2.4. Statistical analysis

Statistical analyses of the obtained data were made using the Statistica package program. In the statistical evaluation of the data, one-way analysis of variance was used to determine the difference between groups, & Duncan's multiple comparison tests were used to compare group effects. Pearson correlation analysis test was applied to examine the relationship between parameters (Soysal 2000).

### 3. Results and Discussion

### 3.1. Nutrient analysis

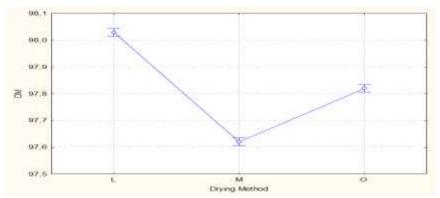
The analysis results of the nutrient values of the earthworm meal dried with different drying methods are given in Table 1, Figure 10, 11, 12 & 13.

	Nutritional Values								
Drying Method	DM	Α	СР	EE	CF				
L	98.03	6.31	65.62	15.22	0				
0	97.82	7.67	61.25	20.33	0				
Μ	97.62	5.96	56.88	18.56	0				

 Table 1. Nutrient values of earthworm meal dried with different drying methods (%DM).

L: Lyophilization, O: Oven, M: Microwave, DM: Dry matter, A: Ash, CP: Crude protein, EE: Ether extract, CF: Crude fiber.

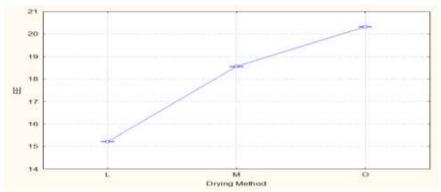
The DM values of the earthworm flours varied between 97.62-98.03 (%). While the highest DM value was observed in the LDM, the lowest was determined in the MDM. Drying in the oven & in the microwave caused a large amount of DM loss. The reason for this is the effect of freeze-drying rather than heat treatment in lyophilization (Vega-Galvez et al., 2009).



**Figure 10.** DM Values of Earthworm Meal Dried With Different Drying Methods (%).

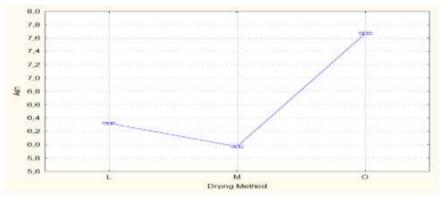
The EE values varied between 15.22-20.33 (%DM). While the highest EE value was observed in the ODM, the lowest was determined in the LDM. The reason for the higher amount of ether extract in earthworm

flours dried in an oven & microwave is due to the fact that it has been treated with more heat (Vega-Galvez et al., 2009).



**Figure 11.** EE Values of Earthworm Meal Dried With Different Drying Methods (%DM).

Ash values varied between 5.96-7.67 (%DM). While the highest ash value was observed in the ODM, the lowest was determined in the MDM.

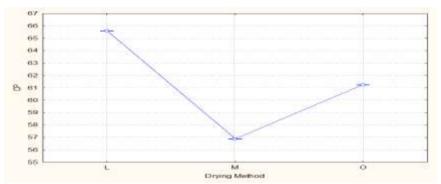


**Figure 12.** Ash Values of Earthworm Meal Dried With Different Drying methods (%DM).

The CP values varied between 56.88-65.62 (%DM). The highest CP value was observed in the LDM, while the lowest was seen in MDM. Compared to the LDM, the ODM & MDM has a lower CP value

because protein fragmentation & losses occur with heat treatment. According to the different drying methods & drying times, there are differences in the nutrient values of the feeds. While the protein values of earthworms were found to be 66.2% after drying in an oven at 90 °C for 4 hours, it was determined as 61.51% after drying at 60 °C for 4 hours (Gunya et al., 2016; Isea-León et al., 2019). While it was determined as 66.60% by LDM, it was found to be 59.56% by MDM (Dada et al., 2022; Valente et al., 2015).

In a different study on this subject, Bou-Maroun et al., (2013) showed that nutrients were affected by heat treatment. In the study, they stated that the heat denatured protein obtained from earthworms with different drying methods above 42°C. Researchers associated different volatile compounds with lipid oxidation & also the Maillard reactions that occur during the preparation of flour.



**Figure 13.** CP Values of Earthworm Meal Dried With Different Drying Methods (%DM).

No results were found regarding the amount of CF in earthworm meal.

#### 3.2. In vitro gas production values

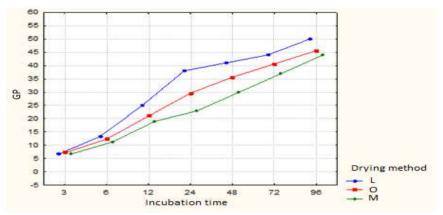
*In vitro* gas production (GP) amounts of the earthworm meal dried with different drying methods are given in Table 2 & Figure 14.

GP (ml)									
Hour									
Drying Method	3	6	12	24	<b>48</b>	72	96		
L	6.67 <sup>a</sup>	13.33	25.00 <sup>a</sup>	38.04 <sup>a</sup>	41.04 <sup>a</sup>	44.04 <sup>a</sup>	50.04		
0	7.17 <sup>a</sup>	12.33	21.00 <sup>b</sup>	29.65 <sup>b</sup>	35.65 <sup>b</sup>	40.65 <sup>ab</sup>	45.65		
$\mathbf{M}$	6.67 <sup>a</sup>	11.33	19.00 <sup>b</sup>	22.94°	29.94°	36.94 <sup>b</sup>	43.94		
SEM	0.264	0.471	0.928	2,220	1.687	1.286	1.535		
Р									
Method	0.729	0.244	0.001	0,000	0.001	0.048	0.274		

Table 2. In vitro GP of earthworm meal dried by different drying methods (ml).

<sup>a-c</sup>: The difference between groups containing different letters in the same column is statistically significant. GP: Gas production, L: Lyophilization, O: Oven, M: Microwave, SEM: Standard error of the mean.

There was no statistical difference between the groups in the GP formed at the  $3^{rd} \& 6^{th}$  hours of the incubation period (P>0.05). The GP amount of the earthworm flours dried with the LDM at the  $12^{th}$  hour was increased rapidly & it was found statistically significant compared to the other drying methods (P<0.001). The highest GP was observed in the LDM at the  $24^{th} \& 48^{th}$  hours of incubation, while the lowest was observed in the MDM (P<0.001). The reason for this is that the loss of nutrients is less in earthworm flours dried by lyophilization. Although the highest GP amount was again in the LDM at the  $72^{nd}$  hour of incubation (P<0.05), when the GP amounts at the  $96^{th}$  hour were examined, there was no statistical difference between the drying methods (P>0.05).



**Figure 14.** *In Vitro* Gas Production of Earthworm Meal Dried With Different Drying Methods (ml).

*In vitro* methane production (MP) amounts of earthworm meal dried with different drying methods are given in Table 3, Figure 15 & Figure 16.

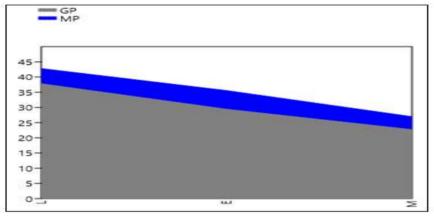
MP (ml)								
Hour								
Drying Method	24	48	72	96				
L	4.75 <sup>ab</sup>	5.02 <sup>ab</sup>	5.11 <sup>b</sup>	5,856 <sup>at</sup>				
0	5.89 <sup>a</sup>	6.05 <sup>a</sup>	6.84 <sup>a</sup>	7.27 <sup>a</sup>				
Μ	4.03 <sup>b</sup>	4.09 <sup>b</sup>	4.40 <sup>b</sup>	5.10 <sup>b</sup>				
SEM	0.313	0.323	0.401	0.374				
		Р						
Method	0.016	0.014	0.006	0.022				

Table 3. In vitro MP of earthworm meal dried by different drying methods (ml).

<sup>a-b</sup>: The difference between groups containing different letters in the same column is statistically significant. MP: Methane production, L: Lyophilization, O: Oven, M: Microwave, SEM: Standard error of the mean.

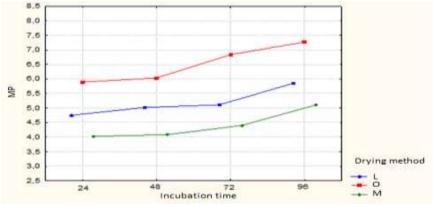
*In vitro* MP of earthworm meal dried with different drying methods varied between 7.27-4.03 ml. While the highest MP was observed in the ODM from the 24th to the 96th hour of incubation, the lowest was

determined in the MDM. This is because a small amount of methane is produced due to GP with the loss of nutrients in the microwave.



**Figure 15.** The Relationship Between *In Vitro* GP & MP of Earthworm Meal Dried by Different Drying Method (ml).

Low MP in LDM was reported by Haryati et al. (2019). It can be explained by the fact that chitin, antioxidant & antimicrobial peptides in earthworms reduce  $H_2$  gas & inhibit the proliferation of methanogenic archaea.



**Figure 16.** *In Vitro* Methane Production (ml) of Earthworm Meal Dried by Different Drying Method.

The OMD, ME & NE<sub>L</sub> values of earthworm meal dried with different drying methods are given in Table 4 & Figure 17.

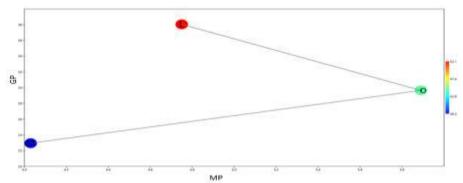
The OMD values of earthworm meal ranged between 38.21-52.06%. While the highest OMD value was found in earthworm meal dried by LDM, the lowest was observed in earthworm meal dried by MDM & the difference between them was statistically significant (P<0.001).

**Table 4.** OMD, ME & NE<sub>L</sub> values of earthworm flours dried with different drying methods.

Gas Production Parameters						
Drying Method	OMD	ME	NEL			
L	52.06a	7.81a	4.54a			
0	44.49b	6.70b	3.62b			
Μ	38.21c	5.74c	2.81c			
SEM	2.032	0.304	0.255			
		Р				
Method	0.000	0.000	0.000			

<sup>a-c</sup>: The difference between groups containing different letters in the same column is statistically significant. L: Lyophilization, O: Oven, M: Microwave, OMD: Organic matter digestibility (%), ME: Metabolic energy (MJ/kg, DM), NE<sub>L</sub>: Net energy lactation (MJ/kg, DM), SEM: Standard error of the mean.

The ME values of earthworm flours varied between 5.74-7.81 (MJ/kg, DM). While the highest ME value was found in earthworm meal dried by LDM, the lowest was observed in earthworm meal dried by MDM & the difference between them was statistically significant (P<0.001). The NE<sub>L</sub> values of earthworm meal ranged between 2.81-4.54 (MJ/kg, DM). While the highest ME value was found in earthworm meal dried by LDM, the lowest was observed in earthworm meal dried by MDM & the difference between them was statistically significant (P<0.001).



**Figure 17.** The Relationship Between *In Vitro* Gas Production<sub>24</sub>, Methane Production & OMD Values of Earthworm Meal Dried With Different Drying Methods.

### **3.3. Pearson Correlation Analysis**

Pearson correlation analysis results between nutrient & in *vitro* gas production parameters of earthworm meal dried with different drying methods are given in Table 5 & Figure 18.

Correlation M	atrix D	Μ	A	СР	EE	GP M	P ON	1D
DM	Pearson's							
	p-value	_						
Α	Pearson's	0.180	—					
	p-value	0.885	_					
СР	Pearson's	$1.000^{**}$	0.194					
	p-value	0.009	0.876					
EE	Pearson's	-0.654	0.626	-0.644	_			
	p-value	0.546	0.569	0.555	_			
GP	Pearson's	0.999*	0.130	$0.998^{*}$	-0.691	_		
	p-value	0.032	0.917	0.041	0.514			
MP	Pearson's	0.371	0.980	0.384	0.460	0.324	_	
	p-value	0.758	0.127	0.749	0.696	0.790		
OMD	Pearson's	0.999*	0.141	0.999*	-0.684	$1.000^{**}$	0.334	
* n < 05 ** n < 01 m	p-value	0.025	0.910	0.034	0.521	0.007	0.783	

 Table 5. Pearson correlation analysis results between nutrient & in vitro gas production parameters.

\* <sup>p < .05, \*\* p < .01,</sup> DM: Dry matter, A: Ash, CP: Crude protein, EE: Ether extract, GP: Gas production, MP: Methane production, OMD: Organic matter digestibility.

In parallel with the increase in DM values, an increase in CP values was observed in earthworm flours in which different drying methods were applied, & a strong positive relationship was found between them (P<0.01). Increasing DM value also increased *in vitro* GP & OMD values (P<0.05). Parallel to the increase in CP value, an increase in *in vitro* GP & MP was observed & a positive relationship was found between them (P<0.05). At the same time, a negative relationship was found between EE values & *in vitro* GP & OMD values. A strong positive correlation was found between *in vitro* GP & OMD values (P<0.01).

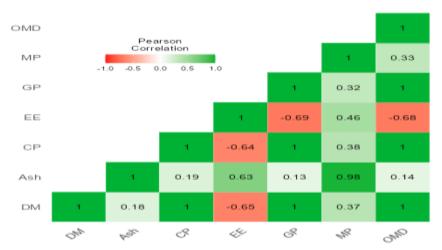


Figure 18. Pearson Correlation Analysis Results Between Nutrient & In Vitro Gas Production Parameters

#### 4. Conclusion

Insects, flies, spiders & worms contain high amounts of protein & fatty acids, making them a good feed source for ruminant animals. In addition, when these creatures replace forage plants with high protein in the ration, less methane is produced. This is due to the chitin, antioxidant & antimicrobial peptides contain in their bodies. This situation also has a positive effect on the digestive system microbiome of animals that consume these creatures. It also provides an increase in the amount of healthy animal production.

The high cost of animal proteins has led researchers into new searches in animal husb&ry. Production amounts of insects, flies, spiders & earthworms are increasing day by day. They can be an alternative feed to animal protein sources with the rapid reproduction of these creatures & the high amount of essential amino acids & fatty acids they contain. Although the use of these creatures in ruminant feeding is prohibited in some countries, they are used in many countries. Its use may become widespread in the future.

In this study, the effects of flours obtained as a result of drying of earthworms by three different methods, lyophilization, oven & microwave, on nutrient values & *in vitro* gas production parameters were investigated. In this study, lyophilization method for drying has a positive effect on digestibility by preventing nutrient loss & also reduces methane production, which is dangerous for greenhouse gas emissions.

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## **CHAPTER 8**

### **USE OF BIOCHAR AS AN ADSORBENT**

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## 1.Introduction

Changes in population density have led to an unstoppable increase in energy demand. The scarcity of resources has forced people to search for other alternative energy sources. Especially renewable energy sources are gaining more and more importance due to high energy potential and environmental compatibility.

Environmental pollution, which has become a global problem today, is caused by the discharge of various polluting factors into water and air ecosystems. Especially heavy metals and organic contaminants have negative effects on human, animal and plant health and disrupt the functioning of all ecosystems. As a result, there is an increasing need for effective and sustainable solutions to reduce the negative effects of these contaminants.

The aim of this article is to synthesize the existing information about biochar as an adsorbent and to reveal the mechanisms and factors affecting the adsorption capacity, adsorption performance of biochar by examining the literature. In this way, it is aimed to contribute to our understanding of the role of biochar in the fight against environmental

## 2. Biochar

Biomass is carbonized by pyrolysis under controlled conditions with or without oxygen to produce "biochar". This carbon-rich by-product is called "biochar", coming out when organic biomass such as agricultural product wastes, sewage sludge, straw waste, is heated by "pyrolysis" in an environment with no or limited oxygen (Lehmann et al., 2006). Theoretically, biochar can be prepared from any type of biomass. Biochar can also be produced by recycling or processing different biowaste such as bark, sawdust. Different materials such as fertilizers, domestic solid waste, sewage sludge can be the source of biochar (Zhang et al., 2017).

The properties of biochar are a result of the properties and the carbonization conditions of the biomass. Biomass generally consists of cellulose, hemicellulose, lignin and a small amount of volatiles, and their ratios vary from biomass to biomass (Akgül, 2017).

Biochar has become a very popular material today due to its physicochemical properties, large surface area, highly porous structure and ability to adsorb various contaminants. Therefore, soil, water and air purification systems are versatile candidates for soil remediation and sustainable resource use (Üstündağ, 2022).

Biochar has a wide variety of usage. It is used as soil improver, as adsorbent, as animal feed agent and silage additive, for sequestration of gases (H<sub>2</sub>S), in adsorption of contaminants such as pesticides, heavy metals and hydrocarbons, in energy storage, as a catalyst, as a building material for the absorption of electromagnetic radiation in buildings or for insulation purposes, in the production of functional clothing in the textile industry, in the production of graphene oxide from carbonized material, in the pharmaceutical industry and in the steel industry (Akgül, 2017; Cabrera et al., 2011).

#### 2.2. Physical and Chemical Properties of Biochar

The chemical property of biochar is an important indicator in determining the area to use it.

Pyrolysis temperature, heating time and the type of biomas used have a great influence on the properties of the obtained biochar. The resulting changes are mainly due to pyrolysis and are largely under the influence of the pyrolysis temperature. In the early stages of pyrolysis, water loss begins with the increase in temperature, and then pyrolytic volatiles from the biomass begin to release gradually. Due to the loss of volatile organic compounds, mineral matter is enriched in C-rich ash, resulting in higher P, K, Ca and Mg contents compared to crude biomass (Figueiredo et al., 2018). Biochar consists mainly of carbon with varying amounts of oxygen, hydrogen, nitrogen and other elements. Aromatic compounds exhibit a complex chemical structure that includes polyphenols, aliphatic hydrocarbons, and functional groups such as carboxyl, hydroxyl, and phenolic groups. These functional groups contribute to the reactivity and sorption capacity of biochar. The cation exchange capacity of biochar varies. The highest cation exchange capacity is found in biochars produced at relatively low production temperatures, where the specific surface area is high but sufficient functional groups remain in the structure and provide negative charges. During biochar production, an increase occurs in carbon content with the separation of functional groups containing oxygen and hydrogen. As the pyrolysis temperature increases, the

carbon content of the biochar increases as well (Weber & Quicker, 2018).

Biochar is mainly composed of water, stable carbon and labile carbon (McLaughlin et al., 2009). The proportions of these major elements in biochar are closely related to temperature, time of pyrolysis and biomass used. In this sense, produced biochar is not the same as other biochars in terms of content or physical composition (Spokas, 2010).

Stable carbon is a strong structure that is relatively resistant to oxidation and enzymes and very difficult to decompose, so it can remain in the soil for hundreds or even thousands of years. Unstable carbon decomposes within a few years and returns to the atmosphere as CO<sub>2</sub>. A good biochar is characterized by its high carbon content and stability. The stable carbon in biochar remains intact in the soil for up to thousands of years (McLaughlin et al., 2009).

The density of biochar depends on the nature of the biomass and how the pyrolysis process is carried out. The density of biochar is directly proportional to the increase in pyrolysis temperature and pyrolysis time. The higher the pyrolysis temperature and the pyrolysis time, the higher the density of the biochar. In addition, biochars with low volatile matter content have high solid densities (Lehman, 2007). Density is independent of the heating rate and depends simply and directly on the final pyrolysis temperature (Kercher & Nagle, 2003).

Due to its high carbon content, biochar has a much higher energy content than materials such as charcoal. It also has a large surface area due to the micropores formed during the pyrolysis process. It can be used for filtration and adsorption of contaminants due to its high micropores (Lee et al., 2013). The utilization efficiency of biochar varies greatly depending on the production conditions. The potential adsorption capacity of different biochars varies greatly depending on the specific properties of the biomass used for biochar production (Yin et al., 2018).

An increase in the pyrolysis temperature is expected to increase the hydrophobicity of the biochar. This increase is associated with removal of more polar surface functional groups and with increased aromaticity (Weber & Quicker, 2018).

Pores are divided into three classes based on their internal diameter (ID): macropores (ID > 50 nm), mesopores (2 nm) < ID < 50 nm) and micropores (ID < 2 nm). Micropores also contribute to the surface area of biochar, and are responsible for high adsorption capacity for small molecules such as gases and solvents. Mesopores are involved in adsorption processes. On the other hand, macropores affect aeration and hydrology and provide microbial habitat (Lehmann & Joseph, 2009).

### 2.3. Surface Chemistry of Biochar

Biochar is a complex carbonaceous material with many physical and chemical parameters that control its reactivity towards inorganic and organic substances (Alam & Alessi, 2019).

The surface chemistry of biochar plays an important role in its interaction with various substances such as organic and inorganic contaminants, plant nutrients and microorganisms. Functional groups of biochar such as carboxyl, hydroxyl, phenol and quinone contribute to the adsorption and desorption processes and chemical reactions that occur on biochar surfaces. The presence of these functional groups ensures that it has a high adsorption capacity for contaminants such as organic contaminants and heavy metals. Understanding the surface chemistry of biochar is important for optimizing its performance in applications such as remediation and soil fertility (Üstündağ, 2022).

The most important factors determining the surface chemistry of biochar are specific surface area, porosity, low bulk density, high cation exchange capacity, high carbon content and its pH value (Lehmann & Joseph, 2009).

## 3. Adsorption Mechanisms

Adsorption is defined as the adhesion/accumulation of particles/molecules of gas, vapor or liquids to any solid surface or the change in concentration (Demir & Yalçın). The substance adhering to the surface is called "adsorbed substance" and the solid on which adsorption takes place is called "adsorbent". (Şengül & Küçükgül, 1995). The return of molecules attached to the surface to the environment is called "Desorption" (Yurtsever, 2008).

Adsorption is grouped under four different headings: Physical adsorption, chemical adsorption, biological adsorption and ionic adsorption. Physical adsorption is a type of adsorption that does not require activation energy and includes electrostatic forces. Adsorbed materials are attached to the surface by Van der Walls forces. Chemical adsorption requires a high activation energy. Compared to physical adsorption, the forces acting on adsorption are stronger. It is an irreversible reaction (Güneş, 2018). There are chemical bonds between the adsorbed substance and the adsorbent, which are affected by the temperature increase (Karaman, 2010). Ionic adsorption is a type of adsorption in which ionic substances are attached to the adsorbent surface by electrostatic forces (Güneş, 2018). Biosorption is the uptake of ions from aqueous media by biomass (Hamutoğlu et al., 2012).

The mechanisms for separating contaminants from aqueous solutions are collected in three basic groups. The three basic reactions include ion exchange, complexation and precipitation reactions (Akça, 2020).

The main types of adsorbents with environmental importance, carbon adsorbents (active carbons, active nanomaterials), mineral adsorbents (silica gels, active alumina, metal oxides, metal hydroxides, zeolites, clay minerals, porous clay heterostructures, inorganic nanomaterials) and synthetic polymers, metalorganic microporous and mesoporous materials, composite adsorbents (Dabrowski, 2001).

## 3.1. Factors Influencing Adsorption Performance

The main factors affecting the efficiency of the adsorption process are the physical and chemical properties of the adsorbent and the adsorbed substance. Similarly, the characteristics of the environment where the adsorption takes place also affect the adsorption efficiency. The properties of the adsorbed substance include surface area, pore structure and pore size distribution, particle size and functional groups formed on the surface during interaction. Surface area, the kind and size of the pores, and the functional groups produced on the surface are some of the characteristics of the adsorbed material. The temperature of the medium, the pH and the concentrations of other dissolved substances in the medium are other factors affecting the adsorption (Dabrowski, 2001).

# 3.2. Adsorption Capacities of Biochar

Biochar is a material with a porous structure and large surface area with a number of functional groups that can immobilize heavy metal cations in soils through adsorption and complexation (Beesley & Marmiroli. 2011).

Adsorption mechanisms of biochar to remove organic and inorganic contaminants are examined under four headings: electrostatic interaction, ion exchange, pore filling and precipitation. These mechanisms are affected by physicochemical properties such as biochar amount, pyrolysis temperature, ambient/waste water pH (Rehman et al. 2017).

There are plenty of oxygen-containing functional groups on the biochar surface and these functional groups are very important for metal adsorption. During adsorption, heavy metals are usually strongly bound to active sites provided by functional groups that are suggested to play a role in complex formation (Akça, 2020). In addition, increased surface area, changes in porosity and increase in functional groups may affect heavy metal adsorption (Jin et al., 2014).

## 3.2.1. Organic Contaminants

Biochar has the feature of limiting the movement of organic contaminants in the soil and even preventing their decomposition. In this (Guo et al.,2020). It also showed that the treatment effect of the wastewater treatment system containing biochar on organic pollutants was significantly improved compared to the system without biochar (Dai et al., 2019).

The different mechanism involved in the organic pollutants' adsorption is pore filling, hydrophobic interaction, partitioning, electrostatic interaction and electron donor–acceptor interaction (Ambaye et al., 2021).

Partitioning: In general, when the biochar has a high volatile matter content and at high concentrations of organic pollutants, the partitioning process is more apparent and extremely effective. In this procedure, the adsorbate substance diffuses into the pores of the biochar's uncarbonized area. It is simple for this fraction to interact with the organic adsorbate, which results in its sorption (Keiluweit et al.2010).

Pore filling: The pore filing method is influenced by the organic contaminant's polarity, nature, and biochar type (Ambaye et al., 2021).

Electrostatic interaction: The electrostatic interaction-mediated adsorption of ionizable organic molecules to the positively charged surface of biochar is considered to be the predominant mechanism for their removal. This mechanism is influenced by the pH and ionic strength of the aqueous solution, which determine the extent to which contaminants are attracted or repelled. These factors play a crucial role in determining the effectiveness of biochar in adsorbing and removing ionizable organic molecules (Zheng et al. 2013; Ambaye et al., 2021).

Electron donor and acceptor interaction: The adsorption of aromatic chemicals on biochar, which exhibits a structure resembling graphene, often involves the mechanism of electron donor and acceptor interaction. To attain complete graphitization, a temperature exceeding 1100 °C is typically required during the production of biochar (Ambaye et al., 2021).

Hydrophobic interaction: Despite its importance in solvent extraction, the hydrophobic effect alone is of little interest when it comes to separating metal ions with the same valency because it causes similar changes in the free energy of partition of isostructural metal complexes, which do not actually distinguish the metal ions in practice (Narbutt, 2020).

# **3.2.2. Inorganic Contaminants**

Biochar can be used as an adsorbent in heavy metal contaminated environments. The factors affecting adsorption can be listed as heavy metal concentration in solution, solution pH, equilibrium temperature, specific surface area of biochar and affinity between biochar and heavy metal (Akça, 2020). Solution pH is an important factor that affects metal adsorption. Equilibrium pH affects adsorption in three different ways; affinity between molecules, the ion exchange process, and the distribution of metal species such as soluble or insoluble and cation or anion (Chen et al., 2015). Decomposition of carboxyl and phenolic groups in biochar increases soil pH and promotes metal retention (Qiao et al., 2018).

Unlike leaching and extraction, removal of pollutants in heavy metalcontaminated soils occurs by converting toxic elements into less soluble and less bioavailable forms by stabilizing (Figure 1), rather than removing heavy metals (Liu. L. et al., 2018; Guo et al., 2020).

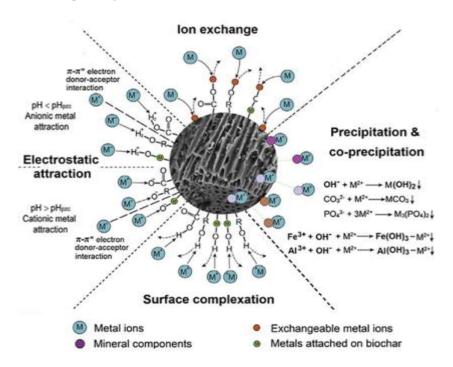


Figure 1. Mechanisms via which heavy metals in polluted soils are stabilized by biochar (Guo et al., 2020).

The amount of lignin in the biomass affects the yield of biochar. As the amount of lignin increases, the biochar yield increases. One of the adsorption mechanisms of heavy metals is electron transfer complex adsorption. Lignin itself is a material with high electron-donating capacity, and electron-donating groups increase with pyrolysis. Therefore, biochars obtained from biomass with high lignin content are very important for metal adsorption (Wu et al., 2022).

## 3.3. Applications of Biochar as an Adsorbent

Biochars are an effective adsorbent in the removal of pollutants in various environments due to their high specific surface area and functional groups. Therefore, biochar is becoming more and more important as a solution to remove contaminants to remediate the contaminated environment (Wang et al., 2017).

## **3.3.1.** Waste Water Treatment

Ammonium contamination of water is a very important problem with adverse effects on human and animal health. Shang et al. (2018) obtained biochar from spruce sawdust. Afterwards, they modified the biochars with HNO<sub>3</sub> and Na<sub>2</sub>CO<sub>3</sub> to obtain a low-cost and highefficiency adsorbent. In that study, they investigated the factors affecting the removal of ammonium from aqueous solutions and the mechanism of adsorbing ammonium on modified biochar. It was determined that pH and the existing ions affect the ammonium adsorption capacity of the modified biochar. Studies have shown that modified biochar can be used to remove ammonium from waste water due to its high adsorption capacity.

Pentachlorophenol and atrazine are the two most widely used pesticides in agriculture. Rice straw biochar and phosphoric acid-modified rice straw biochars exhibit significantly high adsorption for imidacloprid and atrazine from agricultural wastewater. Both biochars display a significantly higher adsorption capacity for imidacloprid and atrazine (Mandal and Singh, 2017).

Wang et al (2021), obtained biochar by pyrolysis method from straw, pig and chicken manure and biogas production plant wastes at different temperatures. They used biochars to remove ammonium and chemical oxygen from the liquid medium by adsorption. As a result of the study, it was determined that the adsorption ability increased with the pyrolysis temperature.

In recent years, many methods such as chemical oxidation and biodegradation have been used to remove PAHs from wastewater. However, these applied chemical remediation methods have disadvantageous aspects. These are the overuse of chemicals that can lead to cross-contamination through inappropriate reagent ratios and the formation of potentially hazardous aerobic species. Although it is a promising method for PAH removal, the survival rate of PAH degradation products in sewage treatment plants is low. Therefore, new methods must be used to remove PAHs. The use of biochar for the adsorption of PAHs from the aqueous medium has been the method preferred in recent years. The ability of biochar to adsorb contaminants is affected by its surface chemistry, pore distribution and surface area. In addition, many chemical agents such as sulfuric acid, phosphoric acid, KOH and ZnCl<sub>2</sub> are used to activate biochar (Qiao et al., 2018).

Biochar can absorb nutrients in the aqueous phase such as nitrogen and phosphorus. Ammonium, nitrate and phosphate are common reactive forms of nitrogen and phosphorus in wastewater and can lead to eutrophication. Modifying biochars increases nitrogen and phosphorus adsorption. This is because modified biochars have higher specific surface area, surface functional groups and more reaction activity (Xiang et al., 2020).

# 3.3.2. Soil Remediation

Previous studies have found that biochar applications immobilize heavy metals and hydrocarbons in contaminated soils and reduce their bioavailability through precipitation, electrostatic interaction, surface adsorption, structural sequestration, and facilitated decomposition (Yuan et al.,2019).

Park et al. (2011) obtained biochar from poultry manure and green plant waste in their study. They used the obtained biochar to examine the uptake of heavy metals and the reduction of phytotoxicity. The use of biochar reduced the amount of ammonium nitrate extractable Cd, Cu and Pb. It was determined that both biochars significantly reduced the accumulation of Cd, Cu and Pb in Indian mustard, and the decrease in heavy metal accumulation except Cu was directly proportional to the amount of biochar used.

## 3.3.3. Air Pollution Control

A growing corpus of recent research suggests that biochars can also remove chemical pollutants that are gaseous in nature, including those found in industrial flue gases. Inorganic pollutants cause health risks to the general population as well as the environment since they are toxic to living things, especially at high concentrations. These pollutants include heavy metals including Hg, Pb, Cd, Cu, Zn, and Ni besides other non-metals that can occasionally be found in aquatic systems and industrial flue gases such as nitrate (NO<sub>3</sub><sup>-</sup>), ammonium (NH<sub>4</sub><sup>+</sup>), ammonia (NH<sub>3</sub>), and hydrogen sulfide (H<sub>2</sub>S). Both organic and inorganic pollutants can be successfully removed using biochars. There are few studies on the removal methods for gaseous contaminants using biochar. However, comparisons can be made with the elimination of comparable contaminants from aqueous systems (Gwenzi et al., 2021).

### 4. Conclusion

Biochar has a large adsorption capacity due to its large surface area and complex structure. It effectively adsorbs various pollutants, especially organic compounds. In addition, the adsorption performance of biochar may vary depending on the factors and characteristics of the production process. Factors such as biomass type, pyrolysis conditions and activation method can affect the adsorption capacity of biochar. Appropriate production and optimization of biochar therefore offers potential as an effective pollution remediation strategy. Using biochar as a sorbent could be a promising solution for removing pollutants from various media such as water and air. However, further research and development will lead to a deeper understanding of the adsorption mechanism of biochar and increase its application efficiency.

# Annotation

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# **CHAPTER 9**

# LUPINES (*Lupinus spp.*) AS A SUSTAINABLE AND ALTERNATIVE FEEDSTUFF IN POULTRY DIETS

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## **1.1. Introduction**

Legume seeds are also called as "pulses" (Rawal and Navarro, 2019). Lupin (*Lupinus spp.*) belongs to *Leguminosae* (or *Fabaceae*) family. There are 400-500 lupin varieties, however, only few of them have been cultivated and used for human consumption. *Lupinus alb*us (white lupin), *Lupinus angustifolious* (blue/narrow-leafed lupin), and *Lupinus luteus* (yellow lupin) are commonly produced ones. Edible lupin varieties have been originated from mainly Africa and the Mediterranean regions, and Australia (Pilegaard and Gry, 2008). Although different varieties have been shown variations in nutrient ingredient composition, due to differences in cultivar, geographical location, agronomy, growing season, and analytical techniques (Hejdysz et al., 2019).

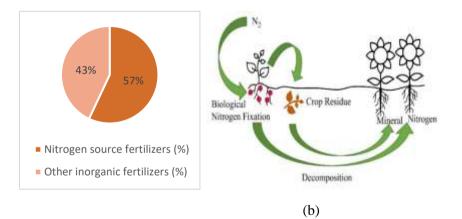


Figure 1. (a) Fertilizer usage in 2019 (FAO, 2021); (b) Nitrogen fixation in legume crops

FAO (2021) reported that total inorganic fertilizer usage in 2019 were approximately 190 million tonnes, and ratio of nitrogen source

fertilizers were %57 (Figure 1a). This data also highlights the importance of the issue from the sustainability perspective. Legumes are important to have symbiotic relationship with nitrogen-fixing bacteria in structures called root nodules.

Most of the nitrogen, which is produced by soil-borne rhizobia bacteria, is used for growth of plant. In addition when the bacteria is died, and plant decomposes in the soil, some of the nitrogen is transferred to the soil. Consequently legume crops do not need additional nitrogen fertilizer, and synthetic nitrogen fertilizer requirement might decrease for the following crop, either (Figure 1b; Rawal and Navarro, 2019). Due to that environmental and economical advantages, using lupin plant for intercropping and crop rotation is a promising choice (Pueyo et al., 2021).

Countries	Area harvested (ha)	Production (tonnes)	Yield (kg/da)
Australia	603941	865618.66	143.33
Belarus	2833	4918.60	173.61
Chile	19072	37049.45	194.26
Czechia	2090	2630	125.84
Ecuador	3497	1358.77	38.86
France	6690	15130	226.16
Germany	29000	53400	184.14
Greece	11610	15830	136.35
Lithuania	4620	4210	91.13
Morocco	85349	56856.03	66.62
Peru	11088	15790.04	142.41
Poland	139120	221390	159.14
Russian Federation	39896	69723	174.76
South Africa	9736	10144	98.76
Spain	2760	2810	101.81
Ukraine	3700	5140	138.92
World	984191	1384963.65	140.72

Table 1. Production values of *Lupinus spp.* in 2021 were derived from FAOSTAT (2023) data



Figure 2. Lupin productions of top ten countries according to 2021 data from FAOSTAT (2023)

Top ten countries, which produce lupin, are Australia, Poland, Morocco, Germany, Chile, Russia, Greece, Peru, France, and South Africa, respectively (Figure 2). In addition, total world lupin production was 1 384 963 tonnes in 2021 (FAOSTAT, 2023). Production values of *Lupinus spp.* in 2021 were derived from FAOSTAT (2023) data and shown in Table 1. Lupin yield were reported as 140.72 kg/da in the World.

## **1.2. Nutrient Properties of Lupin Varieties**

Nutrient composition of some main feed raw materials were shown in Table 2, and Table 3 (INRAE, 2022a). Lupin seeds contains 29-35% crude protein, 4-10% ether extract, 15-22% cellulose+hemicellulose, 39% total dietary fiber, and 2-6% starch (Martínez-Villaluenga et al., 2008; Straková et al., 2010; Uzun, 2023). In addition, starch in pulses is resistant and slowly digestible. Insoluble dietary fiber makes up the

most of total legume dietary fiber (approximately 85%-93%) and cellulose, hemicellulose, and lignin constitute the major parts of the insoluble dietary fiber. Additionally, soluble dietary fiber is mostly found in the cotyledon and it involves low molecular weight polysaccharides such as  $\alpha$ -galactosides (stachyose, raffinose, and verbascose), oligosaccharides, and pectins (Martínez-Villaluenga et al., 2008).

Table 2. Nutrient constituents of some mainly used feedstuffs (as fed, INRAE 2022a)

	DM	СР	CF	EE	Ash	Insol.	NDF	ADF	WICW	Starch	Sugars
Feedstuff	%	%	%	%	%	ash %	%	%	%	%	%
Blue lupin	90.1	30.0	14.3	5.4	3.2	0.07	21.4	17.1	32.5	4.2	5.2
White lupin	88.1	33.5	11.6	8.5	3.5	0.06	18.9	14.1	29.9	6.2	6.0
SBM*	88.0	46.2	6.0	1.5	6.2	0.5	12.5	7.4	18.5	5.0	8.1
Maize	86.3	7.6	2.3	3.6	1.2	0.05	10.7	2.6	8.3	63.8	1.7
Fish meal	92.1	65.2	0	9.2	16.8	0.5	5.3	0.5	4.5	0	0

DM: Dry matter; CP: Crude protein; CF: Crude fiber; EE: Crude fat; Insol. ash: Insoluble ash; NDF: Neutral detergent fiber; ADF: Acid detergent fiber ; WICW: Water insoluble cell walls; Sugars: Total sugars; \*SBM: Soybean meal (48% protein, extruded, oil<5%); Fish meal: 65% crude protein

Electrolyte balance, Ca, P, and phytase activities of lupin varieties are higher from maize seeds. However, soybean meal constitute more Ca, P levels, higher electrolyte balance, and lower phytase activity compared to the white and blue lupin seeds (Table 3, INRAE, 2022a).

Macro elements (especially sodium) contents of the lupin seeds might vary due to the soil type. Additionally, trace element contents might vary according to the precipitation period. However, macro and micro element levels in lupin seeds might not be a problem while using the seeds in feed mix. Because feed raw materials, which are grown in different regions and soil types, are being used in feed preparations, and also mineral premixes added according to birds' daily requirements (Şenköylü, 2001).

	Ca	Р	Phytate P	EB	Phytase	AMEn	AMEn
Feedstuff	g/kg	g/kg	g/kg	mEq/kg	IU/kg	cockerel	broiler
					_	kcal/kg	kcal/kg
Blue lupin	2.5	3.4	2.0	290	140	2070	2030
White lupin	2.7	3.8	2.3	251	140	2390	2330
Soybean meal*	3.4	6.2	3.7	534	20	2300	2260
Maize	0.4	2.5	1.9	67	20	3160	3090
Fish meal (65%)	41.3	26.4	0	200	-	3180	3180

Table 3. Nutrient constituents of some mainly used feedstuffs (as fed, INRAE 2022a)

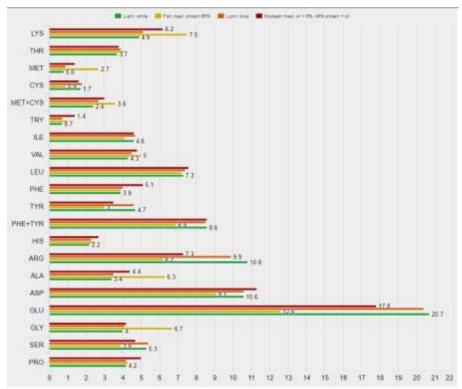
EB: Electrolyte balance.

\* 48% protein, extruded, oil<5%

According to the results of vitamin analysis (mg/kg) of sweet lupin (*L. angustifolius*) grown in Australia;  $\beta$ -carotene (provitamin A) 3,5; thiamine (B1) 5.3; ribolflavin (B2) 2.8; biotin 0.04 (B7); folic acid 0.4 (B9); choline (B4) 3.0-3.5; niacin (B3) 3.6; pantothenic acid (B5) 1.6;  $\alpha$ -tocopherol (vitamin E) 2.2. Higher amounts of  $\alpha$ -tocopherol (Merrit, 2.3-4.6 mg/kg; Gungurru, 3.0-4.2 mg/kg) were detected in lupin grown in the later period (Petterson, 2000).

Amino acid profiles of white lupin, blue lupin, fish meal (65%), and soybean meal (48%) were shown in Figure 3 (INRAE, 2022b). Eightyfive percent of total proteins of lupin seeds are conglutins (globulin group proteins which is specific to lupin). One of the main characteristics of legume is to have low amounts of sulphur-containing amino acids [methionine (~0.3% in seed), cysteine (~0.6% in seed)], and lysine (~1.7% in seed). However, arginine levels of lupin seeds are found higher compared to the other legumes (3.3-4.1% in seed) (INRAE, 2022b). In addition, Protein Efficiency Ratio (PER) of lupin seeds, was found similar to soybean. PER values for *L. albus* meal (0.8) are similar to those of soybeans (0.9) but lower than rapeseed meal (1.9). However, the addition of 0.2% DL-methionine in rat diets was improved the PER value up to that of egg albumin or casein (Petterson, 2000).

Figure 3. Amino acid levels' comparison of some protein sources used in diets (g/16g N) (INRAE, 2022b)



ALA: Alanine; ARG: Arginine; ASP: Aspartic acid; CYS: Cystine; GLU: Glutamic acid; GLY: Glycine; HIS: Histidine; ILE: Isoleucine; LEU: Leucine; LYS: Lysine; MET: Methionine; PHE: Phenylalanine; PRO: Proline; SER: Serine; THR: Threonine; TRY: Tryptophan; TYR: Tyrosine; VAL: Valine.

Cotyledon part of the lupin seeds constitute of 95-98% of oil (Smulikowska et al., 1995). Fatty acid (FA) profiles of white lupin, blue

lupin, fish meal (65%), and soybean meal (48%) were given in Figure 4 (INRAE, 2022b). However, FA profiles might be varied according to varieties. Researchers have also emphasized that oleic, linoleic, and  $\alpha$ -linolenic acid are main fatty acids in lupin seeds. Due to that, n-3/n-6 ratios (range between 0.49-0.79) were found higher than most plant source oils (such as soybean, canola, olive) (RothMaier ve Kirchgessner, 1993; Uzun et al., 2007; Boschin et al., 2007; 2008a; INRAE, 2022c). This might enhance the nutrient quality of poultry products. Mieczkowska and Smulikowska (2005) reported higher lupin levels in diets caused higher oleic, and  $\alpha$ -linolenic acids in poultry fats. Straková et al. (2010) revealed that increasing lupin meal ratio in poultry diets concluded with decrease in saturated fatty acids (mainly palmitic acid) in breast and thigh meats ( $P \le 0.05$ ). Also, they observed an increase in monounsaturated fatty acids (especially in oleic acid) and n-3 polyunsaturated fatty acids levels in the same meat parts ( $P \le 0.05$ ).

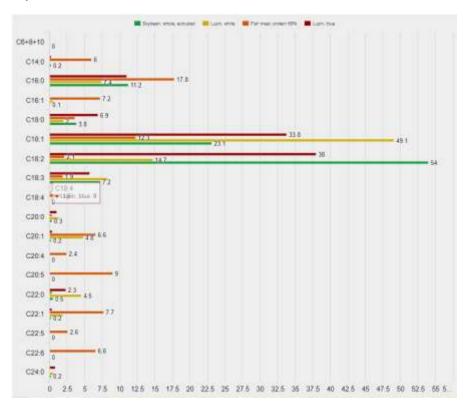


Figure 4. Fatty acid levels' comparison of some protein sources used in diets (% total fatty acids) (INRAE, 2022c)

Additionally, researchers have been stated hypocholestreomic effect of legumes on animals and humans (Kaczmarska et al., 2018; Acquah et al., 2021). For example, Viveros et al. (2007) reported that consuming lupin seeds might have a positive effect on reducing cholestreol absorption and serum glucose level in poultry. They also indicated lupin seeds might have a potential effect as a cholesterol-lowering agent.

Samtiya et al., (2020) indicated, legume grains might consist of desirable nutrient ingredients such as high protein concentration, potassium, fiber, and low glycemic index that could improve nutritional

quality for consumers. Also, they added consuming legume seed might have a strong effect on blood pressure reduction and antioxidant benefits.

# **1.3.** Anti-nutritional Factors in Lupin Seeds and Reduction Strategies

While pulses are a highly nutritious and beneficial feed sources, they also have some antinutritional factors which might affect their nutritional values, and limit their usage levels in diets (Şenköylü, 2001; Boeck et al. 2021). Plants produce antinutritional factors to protect themselves from predators, birds, bugs, humans, and animals, even from seasonal climate challenges (Ünver et al., 2004). Antinutrient factors have major effect on bioavailability of nutrients digestibility and absorption of edible crops such as legumes and cereals (Samtiya et al., 2020). Additionally, these effects might result in performance and health losses in animals (Ramteke et al., 2019). An overview of altered effects of antinutrient factors were summarized in Figure 3.

Antinutritional factors, limiting amino acids, and some nutritional contents of common lupin varieties (such as white, blue and yellow) that should be considered in the preparation of poultry diets were given in Table 4 (Hejdysz et al., 2018a).

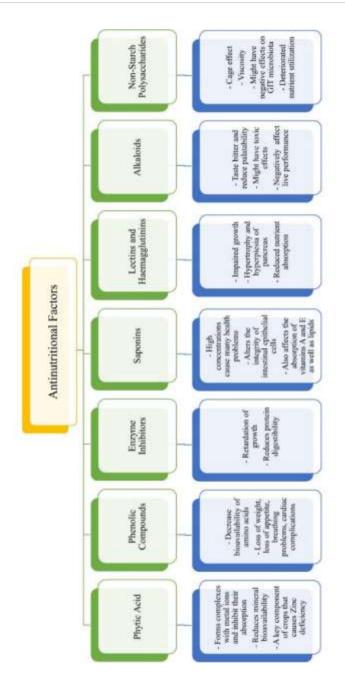


Figure 5. An overview of negative effects of some antinutrient factors (Şenköylü, 2001; Samtiya et al., 2020; Acquah et al., 2021)

#### 1.3.1. Non-Starch Polysaccharides (NSP)

Lupin's NSPs mainly consist of cellulose, arabinoxylans, and pectic polysaccharides. It has been reported that pectic polysaccharides have a complex, multi-branched structure consisting of rhamnogalacturonan main chains and  $\alpha$ -arabinan and  $\beta$ -galactan side chains (Konieczka and Smulikowska, 2018). Total NSP and soluble NSP contents of lupin varieties differ between 29.7-41.0%, 5.8-18.2%, respectively (Table 4; Hejdysz et al., 2018a). Santos (2006) indicated that high amounts of water soluble NSPs are related to the small intestinal digesta viscosity. Increase in the viscosity affects feed passage rate, microbial balance and gastrointestinal health of birds (Hetland and Svihus, 2001; Santos, 2006).

Hejdysz et al. (2018a) investigated the effects of corn and soybeanbased control diet and three lupin species (blue, yellow and white lupin) at 5 different use levels (50, 100, 150, 200, 250 g/kg) in a 35-day trial period. In the study, they used sialic acid excretion to understand the effects of anti-nutritional factors of lupin grains in broilers. Feedderived cellulose and anti-nutritional factors in feeds can affect mucin production and increase endogenous losses. Sialic acid is also part of the cellular mucin content and might be an indicator of endogenous secretion losses when detected in digestive or fecal content. The sialic acid concentration varies depending on the mucin content. Because mucin in the intestinal lumen is used as a substrate by intestinal bacteria and is broken down into free sialic acid.

	White	Narrow-	Yellow
Chemical compositions (g/kg DM)	lupin	leafed lupin	lupin
Dry matter	881	884	895
Starch	11.3	10.2	10.3
Phytate P	5.20	6.23	7.89
Alkaloids	0.13	0.43	0.41
NDF	231	228	266
Simple sugars	12.4	10.0	14.4
Total oligosaccharides (ROFs)	80.8	59.8	113.7
-Raffinose	7.24	12.5	11.1
-Stachyose	64.4	29.3	63.1
-Verbascose	9.17	19.4	38.0
Total NSP	297	410	324
Soluble NSP	65.9	181.8	58.3
Water extract viscosity (cP)	1.65	2.24	1.62
AMEn (MJ/kg)	297	410	324
Crude protein	345	257	416
-Lys	45.9	43.8	45.6
-Met+Cys	21.0	16.8	26.1
-Thr	36.8	30.8	29.6
-Val	39.5	35.6	32.0

Table 4. Chemical composition and antinutritional factors of three lupin varieties (Hejdysz et al., 2018a).

NDF: Neutral detergent fiber; RFOs: raffinose family oligosaccaharides; NSP: Nonstarch polysaccharides; Soluble NSP were calculated from difference between NSP and NDF; Simple sugars: includes glucose and fructose.

The total amount of sialic acid in the stool is related to the total mucin production. Mucin production is dependent on anti-nutritional factors. Hejdysz et al. (2018a) revealed that soluble NSP and raffinose had a significant effect on sialic acid concentration. As raffinose and soluble NSP content increased in narrow-leafed lupin contained diets, total and free sialic acid content also increased. This relationship was not found for yellow and white lupin supplemented diets. This is probably due to the much lower concentration of raffinose and soluble NSP in the white and yellow lupin seeds (Table 4). Montagne et al. (2004) revealed an increase in the amount of mucin on the mucosal surface may reduce nutrient absorption. In addition, Cowieson et al. (2003) reported that as a result of excessive sialic acid excretion, mucin secretion negatively affects the AMEn value and nutrient digestibility of the diet, due to the high nutrient requirement. As a result, the use of blue lupin containing higher soluble NSP and raffinose resulted in increased endogenous secretion losses, possibly because endogenous secretion losses could cause depression in nutrient digestibility, AMEn values and broiler performance. Losses are likely to increase.

Similarly, Kubiś et al. (2020) also emphasized that high amounts of NDF and ADF in white lupin seeds might cause an irritable impact on the intestinal mucosal surface and provoke an increase in mucin production. Increasing mucin production in the gut surface might affect absorption of nutrients negatively, and might induce endogenous energy losses. Both this circumstances might lead decrease in birds' performance (Montagne et al., 2003; Kubiś et al., 2020).

## 1.3.1.1. Viscosity

The gut microbiota has a key role in health, welfare, digestion and absorption of poultry. However, gut microbiota composition, diet ingredients, bird health, rearing conditions, and stress, that all the factors mentioned might be mutually affected by each other (Geigerová et al., 2017).

It has been reported in studies that a high content of a water-soluble NSP-rich raw material in the diet may cause gelation in the intestinal tract, resulting in an increase in viscosity (Şenköylü, 2001; Leeson and Summers, 2009). High viscosity negatively affects utilization of nutrients in intestine, deteriorates microbial balance and also leads bacterial fermentation in distal parts of the gut (Şenköylü, 2001; Konieczka and Smulikowska, 2018; Ayres et al., 2019). There are many factors that contribute to variability in broiler viscosity studies, including bird age, lupine seed variety and quality, feed preparation and processing, NSP solubility, and substrate concentrations (Ayres et al., 2019).

Konieczka and Smulikowska (2018) found a negative correlation between ileal viscosity, diet AMEn value (r = -0.577, P < 0.001), digestive tract N retention (r = -0.413, P < 0.001), fat digestibility (r = - 0.269, P = 0.029), and lupin seeds AMEn values (r = - 0.381, P < 0.002). According to bacterial enzyme activity, ileal digesta viscosity (immediately measured) was positively correlated with ileal  $\alpha$ - and  $\beta$ glucosidase activity (P < 0.003 and P = 0.016, respectively), however it was negatively correlated with ileal  $\alpha$ -galactosidase (P = 0.045) and caecal  $\alpha$ -glucosidase activity (P < 0.022).

Additionally, Ayres et al. (2019) reported that there is a negative correlation between in vitro dietary viscosity and live weight gain (1-21 days of age) (r = -0.725) and positive correlation with intestinal viscosity (r = 0.604). They also reported that intestinal viscosity negatively correlate with bird live weight (1-21d) (r = -0.615).

Konieczka and Smulikowska (2018) indicated that blue lupin kernels could be part of the broiler diets, however some varieties (>30% usage) might cause high viscosity in the small intestine. They observed that as the lupin (250-320 g/kg) content of the diet increased, in the contrary the AMEn value of the diet decreased and viscosity of the digestive content increased. High viscosity reduces the passage speed of digestive tract content, also adversely affects the nutrient digestion and absorption in the small intestine. Due to these conditions, undigested nutrients might move towards hindgut and might cause a change in microbiota profile and deteriorate the microbial balance.

## 1.3.1.2. Gut morphology, and microbiota

Hejdysz et al. (2018a) stated the ileum crypt depth (mm) decreased in all lupin consumed groups compared to the control (P < 0.0001). In the viscosity of ileal digestive content, the highest viscosity was observed in groups consuming blue lupin (especially feeds containing  $\geq 150$  g/kg lupin).

*Escherichia coli* is one of the common intestinal bacterium that most of their strains are commensal. However, some strains of *E. coli* can cause disease (Geigerová et al., 2017). Also, Zdunczyk et al. (2014) emphasized that rise in microbial  $\beta$ -glucuronidase activity might be the sign of undesirable changes in the intestinal microbiota, such as increase in *E. Coli* and *Clostridium* counts. They also reported the inclusion of 20% blue lupin in laying hens diets induced only neglectable increases in ileum viscosity and improved activity of 5 bacterial enzymes in caecum part of the intestine. Additionally, some

positive changes were observed in the numbers of the major bacterial groups of the caecum of 20% blue lupin fed-hens. For example; *Enterococcus, Bifidobacterium sp., Lactobacillus* counts were increased, in the mean time *E. coli* and bacteria of the genera *Bacteroides, Prevotella* and *Porphyromonas* counts were decreased compared to the control groups.

## 1.3.2. Raffinose family oligosaccharides (RFO)

Lupins also contains oligosaccharides, mainly alpha-galactosides.  $\alpha$ -Galactosides are also known as raffinose family oligosaccharides (RFO) (Martínez-Villaluenga et al.. 2008: Konieczka and Smulikowska, 2018). Raffinose family oligosaccharides (RFO), such as raffinose, stachyose, and verbascose, are low molecular weight sugars and cannot be hydrolyzed in chickens' small instestine by digestive enzymes. This is due to the inability of poultry to produce an endogenous enzyme, alpha-1,6 galactosidase. They might play an important role in enhancing the activity of glycolytic bacterial enzymes in the hind gut (Kaczmarek et al., 2014; 2016; Hejdysz et al., 2018a; Konieczka and Smulikowska, 2018; Kubiś et al., 2020). Kubiś et al. (2020) suggested that high levels of RFO in the gastrointestinal tract might induce water retention and due to that passage rate might be affected. This condition might lead a negative impact on the utilization and absorption of the nutrients and low AMEn levels.

Additionally, Bedford (1996a; 1996b) suggested that oligosaccharides of legumes can affect the transit of GIT digesta because of its hygroscopic properties by increasing the intestinal osmolarity. However, lupin seeds contain high levels of RFO (~twice as much RFO as soybean meal), and this might cause adverse affects (Kubiś et al., 2020).

Lupin grains have high raffinose content and it has been stated that it should be taken into account that this issue might have critical importance in their use in broiler diets. There are studies showing that raffinose negatively affects the AMEn value and nutrient utilization of lupin (Kaczmarek et al., 2014; 2016).

Martínez-Villaluenga et al. (2008) indicated that  $\alpha$ -galactosides might be effective on storability of seeds and also protect seeds against frost, and drying damages. Another beneficial impact of  $\alpha$ -galactosides is to act as a prebiotic and boost favorable bacteria (lactobacilli, and bifidobacteria) growth in the distal end of the gut.

# 1.3.3. Phytic acid

Not all of the total phosphorus in plant sources are available for monogastric animals (Antoniewicz et al., 1992; Şenköylü, 2001). Most of the P in plants is in the indigestible form of phytic-P, and digestion by monogastric animals is at very low levels due to the small amount of endogenous phytase found in their digestive tracts. Phytic acid combines with minerals (such as Fe<sup>2+</sup>, Zn<sup>2+</sup>, Ca<sup>2+</sup>, and Mg<sup>2+</sup>), protein, amino acids, and proteolytic enzymes and causes a reduction in the digestion of amino acids. The phytate form forms complexes with minerals such as Mg, Ca, Cu and Zn, reducing the availability and digestibility of minerals. The phytate-calcium complex adversely

affects the digestion of fats in the digestive tract. Combined with fatty acids, they form soap in an insoluble form in the intestinal lumen. In addition, phytic acid binds with starch, negatively affecting the digestion of carbohydrates (Kubiś et al., 2020; Boeck et al., 2021).

Some plant sources are identified in which part of the seed contains phytate. For example; 90% of corn phytate content is in the germ portion of the kernel. Phytate in sunflower seeds, cotton seeds, and peanuts is concentrated in substructures called crystalloids or globoids, which are located within the protein body membrane. In oilseeds such as soy, phytate is bound to protein bodies and evenly distributed throughout the seed. Nevertheless, there are no data on the location of phytates in legume seeds, so it might cause difficulties to predict the effect of phytase in legume seeds (Kubiś et al., 2020).

Phytates are reported as heat stable. Although small losses of phytate in cooking processes can be observed, it is attributed to leaching into cooking water. Plant sources might also contain phytase, however, soaking the material might degradate and adversely affect the enzyme activity (Boeck et al., 2021).

# 1.3.4. Alkaloids

Secondary metabolites that naturally found mainly in plant sources are named alkaloids. Alkaloids are the protection mechanisms of the plants against pathogen, pests, herbivores, drought, and frost. They are generally contain nitrogen in the 6-membered ring, and synthesized by plants from aminoacids. Lupin species contain bitter tasting and toxic alkaloids, called "quinolizidine". Bitter taste might have negative impact on palatability, and might cause a decrease in feed intake, and performance parameters such as weight gain (Boschin et al., 2008b; Villacrés et al., 2020; Estivi et al., 2022; Akinboye et al., 2023; Keuth et al., 2023). Boschin et al. (2022) indicated that when total quinolizidine alkaloids content of the diet was below 0.2 mg/g, no negative effects were observed in the animals.

With the exception of some irregular Quinolizidine alkaloids (QAs), QAs are derived from lysine amino acid and can be divided into eight classes: lupanine, angustifoline, lupinine, sparteine, structural multiflorine, aphylline, anagyrine and cytisine. However, the last two QAs are usually cannot be found in lupins. Sparteine and lupanine considered to be the two most toxic QA to humans and laboratory animals (Francis et al., 2001; Frick et al., 2017; Thakur et al., 2019; Mancinotti et al., 2022; Osorio and Till, 2022). Quinolizidine core, QAs, and the biosynthesis pathway of QAs were reviewed from different literatures and shown in Figure 4 (Frick et al., 2017; Święcicki et al., 2019; Mancinotti et al., 2022; Keuth et al., 2023). Every lupin variety has different QAs pattern. Also, toxicity of QAs is affected by lupin variety, environmental conditions, regions, soil P level, and climatic conditions such as drought, rain etc. (Boschin et al., 2008b; Boschin et al., 2022).

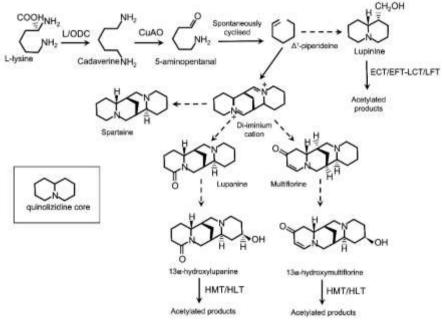
Estivi et al. (2022) used an electronic tongue to profile taste of *Lupinus albus* seeds w/wo debittering process. Higher bitterness and umami tastes were defined at untreated seeds compared to the seeds treated

with water, NaCL (0.5%, 1%), and citric acid (0.5%, 1%) solutions. Citric acid treated groups demonstrated higher debittering results with lowest bitter and umami taste. They suggested that the reduction in umami taste might be related to presence of peptides, nucloetides, aminoacids, and especially glutamic acid in lupin seeds. Glutamic acid has a carboxylic group and is highly soluble molecule in aqueous and polar solvents, and this might induced a reduction by debittering methods.

Breeding low alkaloid lupin varieties has got attention early of 1900s in Germany. After selecting and breeding low alkaloid ( $\leq$ 500 mg/kg DM) contained varieties, they were called as "sweet lupin". Also, for lupin varieties with high alkaloid ( $\geq$ 10,000 mg/kg DM) content has been used the term as "bitter lupin" (Pilegaard and Gry, 2008; Boschin et al., 2008b; Boschin et al., 2022).

Keuth et al. (2023) were collected totally 30 samples of lupin flours (13 flours), products (such as lupin grists, instant-coffe substitute, drink, whole bread, yoghurt, spreads), and seeds (2 bitter, 1 sweet lupin varieties) in Germany, and analyzed for five major quinolizidine alkaloids (angustifoline, lupanine, 13-OH-lupanine, lupinine, sparteine). The sum quinolizidine alkaloids of bitter seeds were found as 20,000-21,000 mg/kg, whereas it was 152 mg/kg for sweet lupin seed variety. They also indicated that a safety margin of sparteine for human consumption was referred as 0.2 mg, per kilogram body weight. However, Keuth et al. also added that European Food Safety Authority

(EFSA) suggested 0.16 mg/kg body weight per day for sparteine consumption.



LDC: lysine decarboxylase; CuAO: copper amine oxidase; ECT/EFT-LCT/LFT: *p*-coumaroyl-CoA/Feruloyl-CoA: (+)-epilupinine/(–)-lupinine O-coumaroyltransferase/(+)-epilupinine/(–)-lupinine O-feruloyltransferase; HMT/HLT: (–)-13 $\alpha$ -hydroxymultiflorine/(+)-13 $\alpha$ -hydroxylupanine O-tigloyltransferase; Dotted lines denote uncharacterized or multistep reactions.

Figure 6. Quinolizidine alkaloids, their biosynthesis pathway (Frick et al., 2017; Święcicki et al., 2019; Mancinotti et al., 2022; Keuth et al., 2023)

#### 1.3.4. Health-promoting effects

Antinutritional factors limit lupin seeds use in diets, especially for young animals. Although, optimum usage levels are correlated with health-promoting effects (Hejdysz et al., 2018b). There are studies showing that legume proteins such as lysine have antioxidant properties (Kaczmarek et al., 2014; Hejdysz et al., 2018b). Antioxidants (such as

selenium and vitamin E) and microelements (Zn, Cu, Mn) support the immune system and form the beneficial parts of legume grains (Boschin and Arnoldi, 2011; Hejdysz et al., 2018b).

Pulses contain phenolic compounds that have phytoestrogenic, antimicrobial, antioxidant impacts. Phenolic compounds in plants, such as polyphenols, phenolic acids, flavonoids, and lignans, play a noteworthy role against pathogens, and stress factors (Boeck et al., 2021). Phytoestrogens naturaly found in the plant sources and divided into flavonoids (isoflavones, coumestans, prenyl flavonoids), and nonflavonoids (lignans). Bartkiene et al. (2015) reported total lignans content of blue lupin is  $114.6 \,\mu\text{g}/100 \text{ g}$ . They also added that solid state fermentation increased the lignan content of blue lupin to  $175.2 \,\mu g/100$ g (Bartkiene et al., 2015). Phytoestrogens are important due to their oestrogenic, anticarcenogenic, antioxidant, cardiovascular disease (such as atherosclerosis) preventing, cholesterol, and LDL (low density lipoprotein) decreasing properties (Konar et al., 2011; Soldamli and Arslanoglu, 2019). Flavanoids (anthocyanidins, flavones, isoflavones, flavanols, and flavanones), also take part in host-symbiont signaling to nitrogen-fixing rhizobia during germination (Boeck et al., 2021).

Fontanari et al. (2012) drew attention to the hypocholesterolaemic effect of lupin cultivars. They found decrease in plasma cholesterol, and increase in plasma HDL (high density lippoprotein) by including White lupin meal and/or White lupin protein isolate to hamster diets. This might be devoted to the lupin proteins and their effects on metabolism,

such as lupin proteins are capable of stimulating the activity of LDL receptors, increasing the capture of LDL from the plasma to the cells.

In addition, they indicated that there is an increase in the faecal excretion of cholesterol and bile acids, also lower fat accumulation in the liver comparing to control group animals. These results might be related with high protein content, fibres, bio-active components, phytosterols and saponins of legumes. Because these compounds might be active in the intestinal tract to combine miselles with dietary cholesterol and bile acids (Fontanari et al., 2012).

Tocopherols have antioxidant effects, and sub-divided to four groups, such as alpha, beta, gamma, and delta. Each one has different biological duties; generally alpha-T, which is the main contributor of the vitamin E activity, and gamma-T shows the highest antioxidant activity. Frias et al. (2005) revealed that white lupin grains (*L. albus* L. var. Multolupa) contain of 20.1 mg/100 g DM of  $\gamma$ -tocopherol, 0.25 DM mg/100 g of  $\delta$ -tocopherol, and 0.19 mg/100 g DM of  $\alpha$ -tocopherol. The vitamin E activity of lupins was 2.21  $\alpha$ -TE mg/100 g.

Boschin and Arnoldi (2011) indicated Vitamin E activities of lupin varieties by using an equation, which was given below to calculate vitamin E activity as tocopherol equivalent (alpha-TE; Table 5);

Vitamin E activity = (1.0 x alpha-T) + (0.5 x beta-T) + (0.1 x gamma-T) + (0.03 x delta-T) [Equation 1]

	Vitamin E activity	
	Seeds	Oil
	(mg/100g)	(mg/100g)
Lupinus angustifolis	1.25	19.4
Lupinus albus	1.06	10.7
Lupinus mutabilis	1.03	6.01

Table 5. Vitamin E activities of major lupin varieties (Boschin and Arnoldi, 2011)

#### 1.4. Reducing or Eliminating Methods of Anti-nutritional Factors

Traditional and emerging debittering methods, such as dehulling, soaking, boiling, roasting, milling, extrusion, autoclaving, fermentation, germination, cold plasma, ultrasound etc., have been using to eliminate the antinutritional factors in legume seeds (Table 6). The main goal in these methods is to reduce limiting factors and to enhance the nutritional quality of the legumes in sustainable ways (Popova and Mihaylova, 2019; Thakur et al., 2019; Samtiya et al., 2020; Acquah et al., 2021; Ramireddy and Radhakrishnan, 2021; Šerá et al., 2021).

Table 6. Different methods to reduce and/or remove antinutritional factors (Kaczmarska et al., 2018; Thakur et al., 2019; Andersone-Trezina and Kince, 2022; Barros et al., 2022)

Methods	Procedures	
Soaking	Treatment with water and salt solutions	
Roasting	Dry heating (120-250°C)	
Blanching	Boiling (75-90°C) to inactivate endogenous enzymes	
Autoclaving	Heating	
Extrusion	Short time, high temperature application (95-200°C)	
Processing chemical	Application with sulphite, thiols, Cu-salts (± ascorbic acid)	
Germination	A basic method, easy to apply	
Cold-plasma	ionized gas state (below 60°C)	

#### 1.4.1. Dehulling

In this method, lupin seeds are separated from their coat. Due to that, cellulose, non-starch polisaccharides, and mineral contents might be reduced as well.

#### 1.4.2. Soaking

Soaking method is one of the traditional methods, widely used. In the method, lupin seeds can be stored in a natural water source, or in the tap water for 2-144 hours while renewing water at every 12h (Acquah et al., 2021; Estivi et al., 2022; Yaver and Bilgiçli, 2023). Traditionally, a combination of boiling (75 min) and soaking (5-6 days) methods were also using, respectively (Yaver and Bilgiçli, 2023). The disadvantage of this debittering method is excessive volume of water usage (Villacrés et al., 2020). Also, using water at the room temperatures with addition of supplements such as NaCl, NaHCO<sub>3</sub>, citric acid etc. and different washing times might affect final alkaloid content of the lupin seeds (Villacrés et al., 2020; Estivi et al., 2022).

#### 1.4.3. Thermal treatments

Boiling, roasting, autoclaving, and extrusion might be used for reducing and/or eliminating antinutritional factors, except lectins and phytates, cause they are thermally stable. Protein denaturation might be an important adverse effect of these methods, and heat source and duration should be carefully considered while aplying these methods (Acquah et al., 2021; Boeck et al., 2021). Villacrés et al. (2020) used two thermal debittering methods (aqueous, and saline) for three Lupinus mutabilis sweet lupin varieties (INIAP-450, INIAP-451, and Criollo). They applied three stages as hydration, cooking, washing (water changes with multiple times), respectively. They indicated that both methods were decreased 80% of alkaloid levels and reduced to the safe levels of QAs for human consumption (as 2.5-3.5g/kg). Application time and water volume in the saline thermal treatment was lower and due to that it was found more efficient. However, they underlined that the impact of debittering was related to lupin variety.

Extrusion process differs in two moisture levels (as low-moisture, and high-moisture extrusions) and process temperatures range between 95-200°C at 20MPa pressure (Andersone-Trezina and Kince, 2022). Andersone-Trezina and Kince (2022) indicated that high-moisture extrusion shows better results, if moisture content of the material is higher than 40%.

# 1.4.4. Milling

Two stages are involved in this method, First step is to separate the seed brans, and second is to ground the seeds into flour. However, the bran part of the lupins has great amount of minerals. The disadvantage of this method is mineral losses due to the separation of the seed bran (Acquah et al., 2021).

# 1.4.5. Germination

Seed germination is a basic method that is easy to apply. This process leads to significant changes in the nutritional composition, flavor, and antinutritional factors of the seeds. However, there are some parameters (such as freshness of seeds, absence or presence of light, colour of the light, humidity, temperature, and duration time) that affect germination process criticially (Kaczmarska et al., 2018; Acquah et al., 2021; Uzun, 2023).

De Cortes Sánchez et al. (2005) suggested that 3 days of germination in lupin seeds would be the optimum duration time to reduce quinilizodine alkaloids and also to prevent production of quinolizidine alkaloid esters which might form toxic compounds for animals.

Frias et al. (2005) reported that germination cause an increase in vitamin C,  $\alpha$ -tocopherol levels, and antioxidant capacity (TEAC) and a decrease in  $\gamma$ -tocopherol content.

# 1.4.6. Fermentation

Fermentation process might lead to form new compounds that could improve the flavor of the lupin seeds by reducing/masking it (Schindler et al., 2011). The process might cause changes in the nutrient composition, and alter antinutritional factors of lupin seeds by the activity of natural and/or commercial bacteria. Also, a probiotic effect might be an additional advantage of the process (Kaczmarska et al., 2018; Acquah et al., 2021; Boeck et al., 2021). Frias et al. (2005) indicated that fermentation caused a significantly important decrease in the tocopherol isomers, and however, vitamin C could not be detected.

## 1.4.7. Cold plasma

"Plasma" is an energized or ionized gas state, and referred as the fourth state of the matter. It is contained of positive/negative ions, atoms, ultraviolet radiation, reactive molecules (such as reactive oxygen species and reactive nitrogen species), free electrons, neutral molecules. Plasma is categorized according to the energy levels, as lowtemperature, and high-temperature plasma. Low-temperature plasma is being used in the agricultural and food industries. If temperature of the gas after ionisation is close to the room temperature (below 60°C) and also gas lacks of equilibrium, it is called as "cold (non-thermal =nonequilibrium) plasma". This is an emerging method for surface microbial inactivation, improving seed germination and germination rate, inactivating enzymes, modification in the structure of starches, and proteins. Cold plasma process can be applied to the heat sensitive products, such as eggshell surface, cheese, cooked meat, apple, strawberry, lupin, etc. without any negative effect on their nutrient compositions and tastes. Additional advantages of this method is to be non-destructive, chemical-free, water-free, and environmentally safe with low energy consumption and short application times. However, there are some disadvantages such as equipment requirements, and installation costs for the process (Gupta et al., 2017; Mravlje et al., 2021; Šerá et al., 2021; Ucar et al., 2021; Barros et al., 2022).

## 1.4.8. Ultrasound

Estivi et al. (2022), carried out this technique to *Lupinus albus* with combination of different soaking times (with different solvents) and cooking. They indicated solvent and application time were significantly reduced alkaloids. However, ultrasound did not show an improvement on the debittering process. They emphasized that cooking might lead to the this result, due to their similar impact on cellular membrane denaturation.

## 1.5. Usage Possibilities of Lupin Seeds in Poultry Diets

Lupin seeds are promising food and feed sources with high quality nutrient composition and their beneficial effects on sustainability, diversity, and soil quality (Pueyo et al., 2021). However, the alkaloid content limit is specified as 200 mg/kg according to the EU (Prusinski, 2017; Konieczka and Smulikowska, 2018). For laying hens, 0.77-0.90 mg of QA per kilogram body weight per day is indicated as the tolerable dose (EFSA CONTAM Panel, 2019).

Olkowski et al. (2001) stated that to feed broiler chickens (1-21 days of age) with high levels (35-40%) of sweet lupin seed (*L. angustifolius cv. Troll*) supplemented diets may cause adverse effects such as decrease in feed intake, growth rate, leg weakness. Moreover, they also reported some birds has shown muscle paralysis and skeletal deformity signs during week 2-3.

## 1.5.1. Broiler Diets

Although there are very different and contradictory study results about usage levels of lupin seeds in birds with some reporting lowered performance, and some showing similar performance results with control groups (Hejdysz et al., 2018b). For example; Kubiś et al. (2020) stated that 10% lupin addition to diet would be safe for poultry and would not affect the performance parameters. However, some researchers suggested maximum inclusion level of lupin seeds in the broiler diets that does not adversely affect the performance is 20% (Nalle et al., 2011; Jeroch et al., 2016; Rutkowski et al., 2016; Hejdysz et al., 2019). Although, Smulikowska et al. (2014) emphasized that not to use at starter diets. On the other perpective, Nalle et al. (2011) stated that it would make no harm to add 20% lupine seeds to the starter diets of young chicks. Moreover, lupin seed variety and nutrient contents are important factors for determining inclusion limitations (Hejdysz et al., 2019).

# 1.5.2. Laying Hen and Quail Diets

Jeroch et al. (2016) reviewed different literatures, and reported that usage levels of lupin varieties differ between 6% to 30% for laying hen diets.

Increasing the amount of lupin seeds' use in feed for laying hens and quails improved egg yolk colour intensity, and it is a desirable parameter which might affect consumers' choices (Krawczyk et al., 2015; Kowalska et al., 2020; Timová et al., 2020; Struţi et al., 2023).

Krawczyk et al. (2015) indicated that increasing (0, 10, 20, 30%) yellow lupin seeds levels (between 32-48 wk age) did not show any negative effects on laying performance, no effect on sensory properties of eggs, linear increase in n-6 PUFA content in diets. No effect on egg yolk lipids. However, Timová et al. (2020) observed changes on egg yolk fatty acid composition such as increase in n-6 (linoleic, and eicosadienoic acids) and n-3 ( $\alpha$ -linolenic, eicosapentaenoid, and docosapentaenoic acid) PUFA by increasing levels of yellow lupin seed inclusion. The experiment lasted starting from 17 wk of age til the end of the performance period of birds. Also, they indicated yellow lupin seed meal might be used substituted of soybean meal for partly (50%) or totally (100%) for laying hens without adverse impacts on performance and health.

Struți et al. (2023) suggested that *Lupinus albus* (cv. Amiga) from lowalkaloid varieties might be used in laying quail (at 24 wk-age) diets up to 20% (% of feed), as an alternative source to proteins of soybean meal, without any negative effect on productive performance, and health of birds. However, feed conversion ratio (FCR) values were conducted to the highest in the 25% lupin added groups. Additionally, 20% with enzyme supplementation was resulted in the highest egg production levels. White lupin might be used as part of a global strategy to improve the nutritional quality of egg yolk fats (by increasing the n-3 FA and carotenoid content and decreasing cholesterol concentration, n-6/n-3 ratio), although data have shown a negative effect on eggshell thickness. However, the FA profile and health lipid indices of the yolk fats were negatively influenced by the enzyme complex supplemented lupin diets.

In addition, Struţi et al. (2023) emphasized that use of lupin in the quail diets compared to the quails fed with control diets led to obtaining eggs with health lipid indices favorable to the consumer's health (while hypercholesterolemic effect, the atherogenic and thrombogenic index decreased, hypocholesterolemic effect increased). This positive impact attributed to the presence of quality lipids in lupin seeds.

The possible causes of these different lupin inclusion level suggestions of researchers might be as below;

- It has been stated that it may be caused by the differences between lupin varieties,

- the NOP content differences according to the species and varieties,

- the unbalanced diets such as energy and amino acids requirements of poultry.

- High levels of raffinose family oligosaccharides (RFO) found in lupin grains (approximately twice as much as SBM) may also have adverse effects (Kubiś et al., 2020).

In addition, while preparing lupin-based diets should need to be careful about methionine and cycteine amino acid levels, and diets should be supported with syntetic and/or natural amino acid sources (Rubio et al., 2003; Pietras et al., 2021).

#### **1.6.** Conclusions

Due to suggested Lupinus spp. inclusion levels to broiler diets are differing from 5%-20%. However, usage amounts of lupin seeds changes between 6%-30% for laying hens' diets. Lupin seeds might be a promising alternative feedstuff for economical and ecological sustainability. However, there are variations between the research results on the use of lupin. These might be attributed to lupin variety, seasonal differences in nutrient contents, usage of raw seeds and/or different debittering methods, bird age, differences in diets (phytase supplementation, enzyme mix supplementation, support with syntetic amino acids and/or animal protein sources, preparing balanced/unbalanced diets for birds).

Debittering methods, such as dehulling, soaking, boiling, roasting, milling, extrusion, autoclaving, fermentation, germination, cold plasma, ultrasound, might represent encouraging results on the antinutritional factors of lupin varieties.

In addition, lupin seeds might positively affect the product quality by increasing n-3 polyunsaturated fatty acid levels, decreasing plasma cholesterol and glucose levels.

#### 1.7. Implication/Recommendation/Suggestion

Besides traditional methods, germination and cold plasma show promising effects to reduce the antinutritional factors, and/or to enhance nutrient contents of lupin seeds. However, more studies should be carried out to reveal the mechanism of action, optimal application times, and doses of new processes. Additionally, the results should be supported by *in vivo* researches for more detailed perspective of subject.

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# **CHAPTER 10**

# EVALUATION OF SOME OILSEED CROPS FOR DROUGHT TOLERANCE

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#### 1. Introduction

Climate change brought about by global warming has negative effects in many areas environmentally, economically and socially. It causes the melting of glaciers and the rise of sea level in the poles with increasing temperature level. In addition, climate change can show itself as drought in some regions while appearing as increased precipitation in other regions (Temur, 2017; Anonymous, 2023a).

In the last 100 years, the global climate has warmed by about 0.5°C due to greenhouse gas emissions from anthropogenic activities. This warming process continues due to today's intense economic activities and increases in greenhouse gases released into the atmosphere (Akalın, 2014).

Increases in temperature and increased carbon dioxide as a result of climate change may seem to have a positive impact on the quantity of agricultural products in some regions in the short term, but in the long term, these components can lead to decreases in product quality and production quantity. In addition, global climate change and increasing temperatures directly or indirectly affects the drought tolerance of many cultivated crops (Mendelsohn et al., 1994; Akalın, 2014). As a result of the changes in the climate in recent years, it has been revealed by the climatic modeling studies that the temperatures will increase by a few degrees at the end of the 21<sup>st</sup> century (0.3-4.8°C) and the water level will decrease further in the middle latitude regions, and as a result the effects of the drought will be felt more severely (Stocker, 2013;

Pachauri et al., 2014). This will increase the importance of drought tolerant crops for sustainable agricultural production.

Vegetable or edible oils, which are basic foods, are obtained from oilseed crops. Oilseed crops rich in primary and secondary metabolites (fat, protein, carbohydrates, vitamins, minerals etc.) constitute an essential source of raw material for human and animal nutrition as well as for the industrial sector (Y1lmaz et al., 2021).

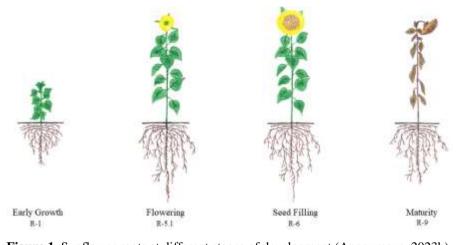
#### 2. Drought Tolerance

Drought as critical environmental stress negatively affects and limits the growth and production of crop plants (Joshan et al., 2019). There are many factors affecting the drought tolerance of crops in the environment in which they are grown. The most important limiting factor for plant production in arid and semi-arid areas is the condition of the water in the environment where the crops are grown. Water has a crucial role in plant metabolism at the cellular and plant level. The crops grown in arid environments make some physiological and morphological changes to adapt to these conditions. Stoma size, reducing leaf area, increasing leaf thickness, increasing leaf hairiness and waxiness, and increasing root/shoot ratio can be counted as some examples (Chaves et al., 2003). Genotypes that can make these changes and minimize their losses during the dry period are least affected by drought damage and these genotypes are defined as drought tolerant crops (Soheili et al., 2023). Among oilseed crops, sunflower, safflower, linseed and camelina can be counted for drought tolerant crops. The relationship of some physiological and morphological characteristics of these plants with yield under different environmental conditions is of great importance. These oilseed crops yield higher yields under arid conditions than drought sensitive genotypes. Especially in the coming years, when the global climate change is felt intensely, the importance of drought tolerant oilseed crops will increase.

## 2.1. Sunflower (Helianthus annuus L.)

Sunflower is the 4th most produced major oilseed crops after palm oil, soybean and canola oil in vegetable oil production in the world. In the marketing year of 2022/2023, it is predicted that the amount of oilseed production in the world will be 647.2 million tons. Soybean 61%, rapeseed 12.4%, peanut 7.9% and sunflower 7.8% of the total production (Faostat, 2023). Approximately 60% of the sunflower cultivation areas in the world are located in the Black Sea Region countries (Çolak et al., 2020).

Sunflower is a relatively drought resistant due to its strong and deep root system (Hussain et al., 2018) (Figure 1). As in other cultivated crops, one of the most important factors affecting seed and oil yield in sunflower is whether there is enough water in the soil at the depth that the roots can reach or not. Sunflower, which has a deep root system, is tolerant of short-term water stress, but the tolerance of the plant varies depending on the developmental stage of drought (Andrade et al., 2005; Ahmad et al., 2009). The most critical growth stages of crop development relative to moisture stress are bud initiation, flowering and seed filling (R2 to R7 growth stages). The 20 days before and after flowering are extremely important stages in terms of water stress in sunflower. The water stress in this stage significantly affects the oil content, protein content and seed yield in sunflower (Jocic et al., 2015; Hong et al., 2017).



**Figure 1.** Sunflower roots at different stages of development (Anonymous, 2023b) Sunflower is usually produced under dryland conditions but infrequently is used on irrigated land where late planting or very limited water supply favors their inclusion. It has good drought tolerance due to the well developed root systems and the ability to withstand temporary wilting. However, drought stress can limit crop productivity in some years.

The genetics of the sunflower cultivar planted also affects drought tolerance. Wild, native sunflowers have excellent drought tolerance, and this genetic reservoir has been used to enhance many commercial varieties (Graham et al., 2021).

Clarifying the scientific basis of drought tolerance of genotypes grown in order to increase seed yield and quality of sunflower will contribute to sunflower production (Balkan Nalçaiyi, 2018).

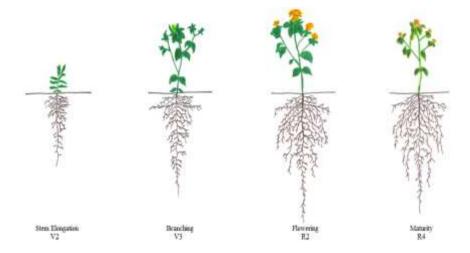
## 2.2. Safflower (Carthamus tinctorius L.)

Safflower (*Carthamus tinctorius* L.) is an alternative oilseed crop that can grow in arid and semi-arid environments because of its tolerance for drought stress (Mosupiemang et al., 2022). *Carthamus tinctorius* L. which belongs to the *Asteraceae*, is one of the oldest cultivated plants that started to be cultivated 3000 years ago. It contains 25-45% oil in its seeds, has two different types as linoleic ( $\omega$ -6) and oleic ( $\omega$ -9), has high quality edible oil, is suitable for biodiesel production, is cultivated in the form of residue and mixture and is considered as animal feed (Arslan et al., 2012; Culpan and Arslan, 2022). On the other hand, drought tolerant and cultivation without irrigation enable especially availability of fallow areas (Arslan and Culpan, 2018).

Safflower develops a strong and deeper root system talented to endure long periods of drought in arid and semi-arid regions (Emongor, 2010; Bahrami et al., 2014). Grown under drought stress, it has been reported to have a high root to shoot ratio (Hojati et al., 2011; Anjum et al., 2017).

Traits of safflower roots play an important role in plant drought stress tolerance (Figure 2). In safflower, root length incline to increase more under drought stress. Increased root length in safflower happens under drought stress (Hojati et al., 2011). Properties of safflower roots related

to maintaining plant performance under water stress include root diameters, long roots, and higher root density (Xia, 2010; Comas, 2013).



**Figure 2.** Safflower roots at different stages of development (Anonymous, 2023c) Leaf area (LA) plays an important role in photosynthetic activity and accumulation (Mosupiemang et al., 2022). It is significantly lower under drought stress (Zhou et al., 2017). Water stress reduces leaf number, leaf size, its color and vigor in many crops (Hossain et al., 2016). Water deficit and drough stress significantly decreased leaf area, leaf chlorophyll content and the membrane stability index in safflower (Amini et al., 2013). Decreases in shoot length, shoot and root dry matter, and relative growth rate were observed for safflower under water deficit conditions (Hojati et al., 2011; Amini et al., 2014).

#### 2.3. Linseed (Linum usitatissimum L.)

Linseed (*Linum usitatissimum* L.) is a traditional oilseed crop that represents a valuable alternative for fallow areas due to its adaptability to unfavorable soils and its high economic value relative to the high quality of the seed oil (Zanetti et al., 2013). It is grown either for its fiber (fiber flax) or for its oil (oilseed flax) (Hall et al., 2016). Its oil is the best source of the n-3 fatty acid,  $\alpha$ -linolenic acid, which constitutes nearly 55 % of its total fatty acids. This value is 5.5 times more than the next best sources of  $\alpha$ -linolenic acid (Bloedon and Szapary, 2004).

Alpha-Linolenic acid (ALA) is an omega-3 ( $\omega$ -3), essential fatty acid. ALA is found in many seeds and oils, including linseed, walnuts, chia, hemp, and many common vegetable oils (Chen et al., 2002; Blondeau et al., 2015).

Linseed is an alternative oilseed crop and has unique drought tolerance; in extreme conditions, it can complete its life cycle in climates in which annual rainfall is only 200 mm (Li and Wang, 2016). It is drought tolerant plant (Nematallahi and Saeidi, 2011) but, genotype  $\times$ environment interactions have been shown to be high for linseed (Diepenbrock et al., 1995), and seed yield change significantly between production years, depending on location and climate conditions.

The linseed is sensitive to drought especially at the first development stage (first true leaves), at flowering and during early seed development (Figure 3), indicating that seed yield and oil content can be maximized by maintaining soil moisture to adequate levels during the corresponding periods (Gabiana et al., 2005; Ceh et al., 2020). In addition, oilseed flax (linseed) is more drought tolerant than fiber flax. However, the coinciding of the dry periods with the flowering period and the periods after flowering causes a decrease in seed yield and quality (Arslan and Culpan, 2021).

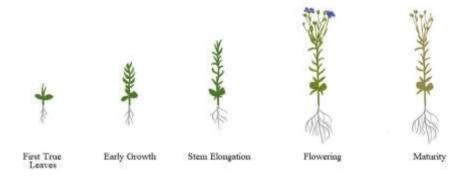


Figure 3. Linseed at different stages of development (Anonymous, 2023d)

Water stress and drought limits linseed growth and development by hastening physiological maturity, and consequently, the period of seed oil synthesis and deposition (Ceh et al., 2020). For this reason, under water deficit or arid condition, linseed plants produce less oil and fibre compared with irrigated condition (Bauer et al., 2015). Diepenbrock et al. (1995) reported that high genotype  $\times$  environment interactions in Europe, with seed yield of linseed varying significantly between production years and locations. Nematallahi and Saeidi (2011) found significant differences in the response of several linseed genotypes to drought, with some being drought tolerant and others being drought sensitive.

#### 2.4. Camelina (Camelina sativa (L.) Crantz)

Camelina (*Camelina sativa* (L.) Crantz) is ancient oilseed that blongs to *Brassicaceae* family that is grown worldwide (Righini et al., 2019; Schillinger, 2019). Several characteristics of camelina make it an alternative oilseed crop, indeed a potential oilseed crop. In recent years, camelina has started to gain importance in the international arena again and many new researches have been carried out on it (Sevilmiş et al., 2019).

Many researchers documented that camelina is drought and heat tolerant (Angelini et al., 1997; Zubr, 1997; Blackshaw et al., 2011). It (*Camelina sativa* (L.) Crantz) is more adaptive to drought conditions than other oil seeds crops such as canola (Raza et al., 2015). Recent studies showed that drought stress considerably reduced leaf area, photosynthesis rate, plant height, number of branches and pods per plant, 1000 seed weight, and seed yield in camelina (Waraich et al., 2017). Gao et al. (2018) reported that camelina is more tolerant of drought stress and potentially has greater adaptability to dryland production than canola. George et al. (2017) observed that camelina exhibited a greater drought tolerance than canola in California. The seed yield of camelina ranged from 1177 kg ha<sup>-1</sup> under drought conditions in Saskatchewan to 3012 kg ha<sup>-1</sup> in northern Alberta (Francis and Campbell, 2003). Zubr (1997) reported seed yields of 2600 kg ha<sup>-1</sup> and 3300 kg ha<sup>-1</sup> for spring and winter varieties, respectively.

## 3. Conclusion

Drought is one of the most destroying environmental stresses, limiting the productivity of crop in the worldwide. It has usually caused remarkable decreases in crop production, which are caused by continuous global warming. Drought tolerant crops continue their metabolic activities in their tissues at low water potential. Among oilseed crops, sunflower, safflower, linseed and camelina can be counted for drought tolerant crops. Optimum seed yield can be obtained from these plants even under limited water conditions. In addition, these crops grown in arid environments make some physiological and morphological changes to adapt to drought conditions. The relationship of some physiological and morphological characteristics of these plants with yield under different environmental conditions is of great importance.

Especially in the coming years, when the global climate change is felt intensely, the importance of drought tolerant oilseed crops (sunflower, safflower, linseed and camelina etc.) will increase.

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# **CHAPTER 11**

# CLIMATE CHANGE: EFFECTS ON AGRICULTURAL AND LIVESTOCK PRODUCTION, ADAPTATION AND MITIGATION

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### 1. Introduction

Current agricultural systems including livestock are most vulnerable to climate change and it is expected that vulnerability will increase in future due to weak adaptive capacity against climate change to minimize its negative effects. Climate change adversely effects the livestock production, directly causes the heat stress which leads to more than 15% decrease in feed intake, 30% losses in milk and meat production, 27% fall in reproductive performance of animal, increase in health issues and rate of mortality. Indirectly climate change leads to decrease in quality and availability of feed, reduce in water availability, manifestation in normal physiological functions of body and increase in livestock diseases. In the livestock sector losses due to climate change in USA and Pakistan are 2.36 billion and 39.54 million US dollars per annum respectively. Future climate projections of Pakistan indicate the increase in average temperature of 1.69 °C and 3.39 °C for representative concentration pathway (RCP) scenario of 4.5 and 1.81 °C and 3.93 °C in RCP 8.5 scenario of 29 general circulation models (GCMs) mean ensemble during the time of near-term (2010-2039) and (2040-2069), respectively. Likewise, mid-century changes in precipitation are projected and would range -8 to 9% and -18 to 12% under high emission scenarios of RCP 8.5 till the period of 2039 and 2069, respectively. Appropriate adaptation and mitigation strategies should be followed to diminish the impact of climate change in livestock sector.

The indirect contribution of livestock to agricultural production is the use of their excrement as organic fertilizer. Especially in the production of compost and vermicompost, the manures of livestock are widely used (Bellitürk, 2016; Bellitürk, 2018; Bellitürk and Soytürk, 2020; Koç et al., 2021; Bellitürk et al., 2022).

Progressive livestock production diversification has occurred within the agricultural sector since the beginning of the green revolution. As a part of the agricultural sector, its contribution to the nation's GDP has been steadily increasing, while the crop sector has seen a fall. Even though, over the past few decades, the national GDP contribution of agriculture and related industries has decreased, the share of livestock in agricultural GDP has steadily climbed. As a result, the role of livestock in expanding the agricultural sector in developing nations is growing by increasing employment and lowering rural poverty, and livestock help to promote socioeconomic development. In addition to providing draught force and organic manure to the crop sector, livestock is employed in industries for their hides, skins, bones, blood, and fiber. Therefore, livestock is a significant source of employment and income, assisting in reducing poverty and evening out income distribution among small landowners and the landless, who make up most livestock owners and the majority of the rural population (Benchaar *et al.*, 2001).

The development of the livestock industry could face an increasingly difficult challenge from climate change. Heat stress (HS) is projected to pose a danger to animal productivity, given that global climate change is expected to increase temperatures by 1.5-5.8 °C by the year

2100. The multifaceted effects of climate change on animals include effects on their distribution, growth, the occurrence of illnesses, availability of prey, production, and, in the worst situations, even the extinction of entire species owing to habitat loss. Information on the direct effects of climate change on animals is limited. However, both domestic and wild animals, including insects, amphibians, birds, and mammals, have been observed to be affected (Nardone *et al.*, 2010). The higher ambient temperature is primarily responsible for the effects of climate change.

Climate change has complicated effects on the domestic livestock production system (Figure 1), impacting feed availability, taxing thermoregulatory mechanisms, and leading to thermal stress, the emergence of new diseases due to changes in disease epidemiology, and many other indirect effects. The animal production system is impacted by global warming in both directions. Animals' health, nutrition, reproduction, and growth is affected by the direct effects of climate change. These affects lead to reduced productivity, poor performance, drop in production quality, disease outbreak, and decline in growth rate. While the indirect effects on livestock productivity are because of change in fertility of soil, vegetation pattern, desertification, decline in feedstuff production, and degradation of rangeland. Numerous studies show the wide range of immediate consequences of environmental conditions, including climate change, on reproductive efficiency, which in turn causes changes at the genetic, phenotypic, and behavioral levels (Benchaar et al., 2001). Due to the consequences of climate change,

everyday environmental stressors like temperature and humidity are predicted to intensify. These stressors can greatly impact growth, milk supply, estrus expression, oocyte maturation, fertilization, and embryo development. Alterations in functioning of ovaries and development of embryo devalue the fertilization competence of oocyte and reduce embryo to develop, which contributes significantly to the effect of heat stress (HS) on the establishment and maintenance of pregnancy. Heat stress-induced apoptosis, chromatin changes, and spindle microtubule disruptions may all play a role in the reduced development of oocytes and embryos under heat stress (HS). One way to reduce early embryonic wastage appears to be to increase the oocytes' and embryos' thermotolerance (Nardone *et al.*, 2010; Henry *et al.*, 2012).

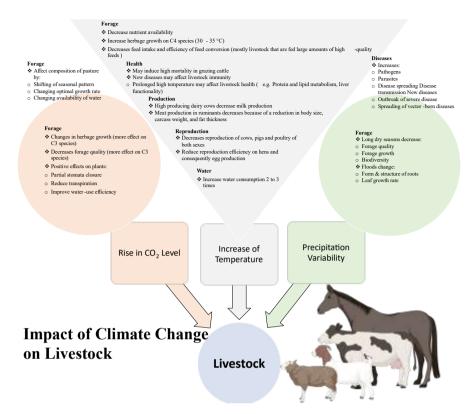


Figure 1. Impact of climate change on livestock

According to the IPCC sixth assessment report, there is variability in climatic conditions as the average temperature of the earth's surface is increased by up to 1.5% by 2022, and these results are based on the experimental studies (Thornton *et al.*, 2009; IFAD, 2010; Chapman *et al.*, 2012; Polley *et al.*, 2013). Climate change affects biodiversity (Reynolds *et al.*, 2010), availability of water (Thornton *et al.*, 2009; Nardone *et al.*, 2010; Henry *et al.*, 2012), and reproduction (Nardone *et al.*, 2010). These influences are mainly because of the rise in the level of carbon dioxide (CO<sub>2</sub>) and temperature, variation in humidity level, and blend of these aspects (Aydinalp and Cresser, 2008; Thornton *et al.*, 2009; Reynolds *et al.*, 2010; IFAD,

2010; Nardone *et al.*, 2010; Henry *et al.*, 2012; Polley *et al.*, 2013). Rising atmospheric temperature primarily affects water availability, animal production and reproduction, and general body conditions. Figures 2 and 3 explain climate change's direct and indirect effects on livestock.

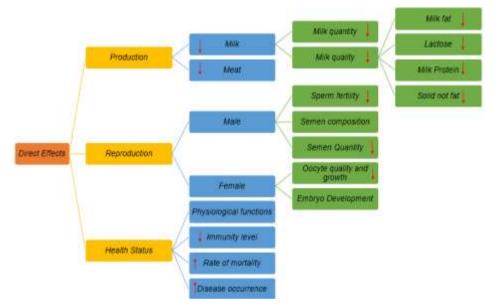


Figure 2. Direct effects of Climate Change on livestock.

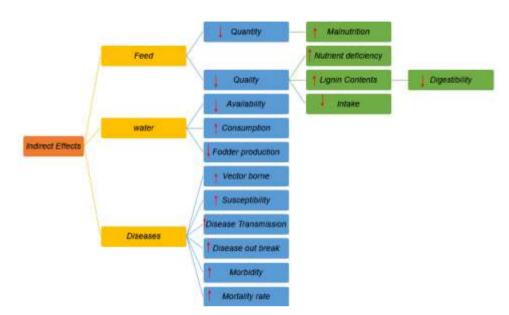


Figure 3. Indirect effects of climate change on livestock

# 2. Feed quality and quantity

Feed quality and quantity are mainly influenced by a rise in the level of environmental temperature and CO<sub>2</sub> (Chapman *et al.*, 2012). These impacts of changing climate on feed quality and quantity depend on the management system, land area under cultivation, and species (IFAD, 2010). The impacts of the raised level of atmospheric CO<sub>2</sub> concentration on crops resulted in a change in growth rate; their effects are more significant on C<sub>3</sub> plants and lesser on C<sub>4</sub> plants (Thornton *et al.*, 2009; Hatfield and Praeger, 2011; Chapman *et al.*, 2012). Increased concentration of atmospheric CO<sub>2</sub> also has beneficial effects as it leads to incomplete stomata closing, decreased water evaporation from plant leaves, and enhanced plant efficiency in using water (Rotter and van de

Geijn, 1999; Wand et al., 1999). As C<sub>4</sub> plants, less than 1% of plants in the universe are present in hot environmental conditions, which are more efficient in using water than C<sub>3</sub> plants. The increase in temperature from 30 to 35 °C can lead to an increase in plant growth rate, with more significant effects on C<sub>4</sub> plants. These effects may vary concerning geographical location, agriculture production system, and species of plants (Thornton et al., 2009; IFAD, 2010; Thornton, 2010; Hatfield and Prueger, 2011). Alteration in climatic conditions, primarily due to temperature and CO<sub>2</sub> level, affects plant growth rate and can affect pasture composition because of changes in species dynamics (Thornton et al., 2008; Thornton et al., 2009; IFAD, 2010). Vying among plant species is influenced by seasonal water availability alterations (Polley et al., 2013). The overall pasture production may increase due to changes in its composition because of an increase in temperature, nitrogen deposition, and precipitation (IPCC, 2007). Forage and feed crop quality may change due to a rise in temperatures and drought conditions because of variations in water-soluble nitrogen and carbohydrate composition.

An increase in temperature may lead to an increase in lignin and other cell wall contents (Polley *et al.*, 2013; Sanz-Saez *et al.*, 2012). A decrease in the digestion of plants by animals due to increased lignin and cell wall components leads to decreased availability of nutrients to animals (Thornton *et al.*, 2009; IFAD, 2010; Polley *et al.*, 2013; Cortignani and Dono, 2018). Increased concentration of CO<sub>2</sub> leads to improved forage quality of C<sub>3</sub> plants than C<sub>4</sub> plants. Crude protein and

digestibility of C<sub>3</sub> plants are more remarkable than C<sub>4</sub> plants (Wand *et al.*, 1999; Thornton *et al.*, 2009; Polley *et al.*, 2013). Severe climatic conditions affect plants' root structure, decreasing leaves progression rate and inclusive yield (Baruch and Mérida, 1995). Forage quality and quantity are both affected by changing climatic conditions and these effects are mainly dependent on land use and season of growth (Polley *et al.*, 2013; Hristov *et al.*, 2018). The rise in temperature up to 2 °C has positive effects on livestock production in humid temperate regions and adverse impacts in arid and semiarid areas of the world. Availability and quality of forage also depend on the length of the growing season of forage. The decline in the quality of forage leads to increased methane gas emissions with gross energy production (Benchaar *et al.*, 2001). So, to minimize methane gas emissions from livestock, we must maintain proper quality of forage or forage replacement with grains (Polley *et al.*, 2013).

### 3. Water

The agriculture sector is the largest water user in the world as it uses about 70% of freshwater resources worldwide (Thornton *et al.*, 2009). Water requirement is increasing daily due to the temperature rise, increased depletion of water resources, and water scarcity. Therefore, it is assumed that by 2050, more than 64 % of the world population will face severe water scarcity conditions (Rosegrant *et al.*, 2002). These water shortage matters will impair their adverse effects mainly on the livestock sector, which use water in crop production, feed production, and processing to meet animal demand (Thornton *et al.*, 2009; Nardone et al., 2010). Animals are already using about 8% of human water globally, which may rise to double or triple due to a temperature rise. To compete with these situations, it is need of the time to raise animals and produces their feeds which require a minimum amount of water or in areas where water is in abundance (Nardone et al., 2010). Due to changes in climatic conditions, there is a rise in sea level, which leads to the addition of salt water in freshwater sources (Karl et al., 2009). An increase in salt contents, contaminants (chemical and biological origin), and heavy metal concentration affects forage and livestock production (Nardone et al., 2010; Duran et al., 2017). Increased salt contents in water bodies affect animals' metabolic activity, digestibility, and fertility. Heavy metals and chemical contaminants affect the functioning of the cardiovascular, nervous, respiratory, and skeletal systems, and they also decline the hygienic quality of livestock products (Nardone et al., 2010). Research related to climate change and its effects on water quality and availability is minimal (Thornton et al., 2009). Therefore, to ensure maximum animal production, it is essential to focus on water quality and availability.

### 4. Livestock diseases

The occurrence of different livestock disease is mainly influenced by climate change because it depends on the ecological location, type of land used, disease characteristics, and susceptible animal species (Thornton *et al.*, 2009). The health status of the animals is affected by climatic conditions directly or indirectly, mainly due to a temperature rise (Nardone *et al.*, 2010). The rate of morbidity and mortality in

different diseases of the livestock population increased because climate change affects the pathogen communities (parasites or microbes), the spread of vector-borne diseases, the development of resistance in the host, increase in water scarcity and host susceptibility towards infections (Patz et al., 2000; Harvell et al., 2002; Tubiello et al., 2008; Thornton et al., 2009; Karl et al., 2009; Nardone et al., 2010). Climate change leads to a shift in the spread of diseases, the introduction of new pathogenic infections, and the outbreak of existing severe conditions, affecting livestock health, production, and reproduction (Thornton et al., 2009). Global warming influences the host resilience, disease dynamics, and spread of vector-borne diseases Viz, the illness due to flies, mosquitoes, and ticks (Thornton et al., 2009). Transmitted vectorborne diseases are more in warm climatic conditions (Thornton et al., 2009). The simulated model applied on the Australian livestock population shows that there are more than 18% losses in livestock due to increased tick burden (White *et al.*, 2003). In Iberia, a model was also used to simulate the response of blue tongue disease vector *Culicoides* imicola by Wittmann et al. (2001), which shows an increase of 2 °C in global mean temperature leads to an increased spread of Culicoides *imicola*. We can confirm the spread of these vector-borne diseases with the help of disease surveillance, modern techniques viz; DNA fingerprinting, genome sequencing, and tests to understand resistance and cross-breeding (Perry and Sones, 2009; Thornton, 2010). There are chances of developing new pathogens, which will be the genetic mixture of humane and animal pathogens, which will spread at a higher rate than existing strains of the pathogen. Therefore, it is difficult to

assess the actual disease risk due to continuous exposure of animals and the increase in factors responsible for disease occurrence (Randolph, 2008; Duran *et al.*, 2017).

### 5. Heat stress

Each animal has a thermo-neutral zone (TNZ), which ranges in temperature while it exhibits its normal physiological functions (FAO, 1986). Livestock keeps their body temperature within the range of  $\pm 5$ °C during the day hours (Henry et al., 2012). Animals become under heat stress when the body temperature exceeds the upper limit of temperature (FAO, 1986). During heat stress, animals develop a phenotypic response called acclimation (Fregley, 1996; Lacetera et al., 2003; Nardone et al., 2010). There are different factors viz; humidity, temperature, species, life stage, nutritional status, and genetic potential on which the severity of heat stress depends. Livestock populations located at lower altitudes are better adapted to high temperatures than livestock populations in higher latitudes. That's why livestock at higher altitudes show a more aggressively detrimental response against heat stress (Thornton et al., 2009). Climate control sheds of animal production offer a high rate of production due to no effects of climate change on them (Rotter and van de Geijn, 1999) because heat stress leads to a decrease in forage intake, production, feed conversion ratio, and overall performance of animals (Wyman et al., 1962; McDowell, 1968; Haun, 1997; Duran et al., 2017). High temperature and increased humidity lead to heat stress in animals, leading to variations in

behavioral and metabolic activities. Following are the effects of heat stress under different categories:

### 6. Feed consumption and nutrient utilization

Animals require different nutrients, including minerals, protein, fat, carbohydrates, and vitamins, the amount of these nutrients varies according to animal species and its productivity (Thornton *et al.*, 2009). As the feed intake of animal decrease during heat stress, it causes malnutrition, negative energy balance, and a reduction in productivity (Lacetera *et al.*, 1996, 2003). A decrease in water intake leads to the severity of heat stress conditions and decreased feed intake (Henry *et al.*, 2012). Dietary incompetency leads to metabolic and digestive problems as deficiency of dietary cations (Na and K) may lead to metabolic alkalosis and an increase in anima's respiration rate (Mader 2003; Chase 2012). Mainly the research about feed intake and dietary issues are focused on cattle, and animals fed a high-quality diet (Wyman *et al.*, 1962; McDowell, 1968; Haun, 1997; Mader and Davis, 2004; Thornton *et al.*, 2009; Rojas *et al.*, 2017).

# 7. Animal productivity

Heat stress occurs due to extreme climatic conditions, is among the foremost causes of decreased productivity of dairy and beef animals and leads to large economic losses (Nardone *et al.*, 2010). In the United States, economic losses due to heat stress are from 1.69 billion to 2.36 billion dollars per annum, and 50% percent of these losses are in livestock (St-Pierre *et al.*, 2003). In Pakistan, these losses are more than

5.5 billion PKR. The large amount and high energy feed intake lead to the production of a large amount of metabolic heat, which aggravates the condition of heat stress. High-producing cattle have more intake of high-energy diets than low producing, so they are more prone to heat stress than low-producing cattle. Along with heat stress increased amount of metabolic heat leads to massive losses in animal productivity (Kadzere et al., 2002; Berman, 2005). Almost all the milk producing ruminants decreased under heat stress conditions (Olsson and Dahlborn, 1989; Finocchiaro et al., 2005; Nardone et al., 2010). Generally, sheep are more prone to high temperatures and humidity (Finocchiaro et al., 2005). In goats, during the hot days of the summer season, there is an activation of the water reduction mechanism, which reduces the water loss in urine so that more water should be available for milk production. Still, heat stress inserts adverse effects on goat milk quality and quantity (Olsson and Dahlborn, 1989). Exposure of buffalo to the hot season leads to disturbance in normal physiological conditions of animals and, ultimately, milk production decreases (Seerapu et al., 2015). Beef cattle are more prone to adverse effects of climate change than low-producing dairy animals because beef animals have thick body coats, heavyweight, and mainly dark coat color (Nardone et al., 2010). Extreme climatic conditions lead to a decrease in fat deposition, body size, and weight of livestock (Mitloehner et al., 2001; Nardone et al., 2010).

When the environmental temperature exceeds 30 °C, it also affects poultry birds' production (Esminger *et al.*, 1990). In poultry, heat stress leads to a reduction in gain of body weight, feed intake, carcass yield,

protein contents, and muscle calories (Novero *et al.*, 1991; Tankson *et al.*, 2001; Nardone *et al.*, 2010). In hens, heat stress declines reproductive performance: decrease in egg production, reduction in egg and eggshell quality, and interruption in ovulation (Mashaly *et al.*, 2004; Rojas *et al.*, 2017); ultimately, there is a decline in the rate of fecundity.

### 8. Reproduction

Heat stress adversely affects the reproductive efficiency of both sexes of livestock: in the female, it leads to a decline in oocyte quality and growth, diminishes the development of an embryo, and ultimately decreases the pregnancy rate (Nardone *et al.*, 2000; Ronchi *et al.*, 2001; Barati *et al.*, 2008). In the case of a male, it leads to a decrease in sperm concentration, fertility, and quality of semen (Mathevon *et al.*, 1998; Karaca *et al.*, 2002; De Rensis and Scaramuzzi, 2003; Kunavongkrita *et al.*, 2005; King *et al.*, 2006).

### 9. Metabolic activity

One of the significant effects of climate change is a disturbance in animal health (Nardone *et al.*, 2010). Effects of climate change on animal health are reduced metabolism of glucose, lipid and protein, reduced cholesterol and albumin levels, non-esterified fatty acids, and quantity and quality of saliva (Ronchi *et al.*, 1999; Bernabucci *et al.*, 2002). A decreased feed intake and metabolic activities due to heat stress led to energy deficiency affecting the cow's fitness (King *et al.*, 2006). Lack of cations (Na and K) in diet may lead to metabolic

alkalosis and increased animal respiration rate (Mader, 2003; Chase, 2012).

# 10. Health

Heat stress occurs mainly due to warm and humid climatic conditions, leading to an increase in the rate of mortality of livestock as Howden *et al.* (2008) informed that an increase of 1-5 °C in environmental temperature leads to an increase in the rate of mortality in grazing cattle. Therefore, adaptive measures are followed during heat stress, such as a sprinkling of water, provision of shadow, and other management practices to make the animal cool. The relation between heat waves and mortality rate in livestock was explored by Sirohi and Michaelowa (2007) from 1994 to 2006 in USA and Europe.

Still, there is a need for information about the mechanism of heat stress in animals, which leads to reduced feed intake, metabolism, animal production, health, and reproduction. Whenever we have information about how heat stress affects the animal body's nutritional and metabolic processes, these issues will be solved by removing the cause or by adaptation of different managemental practices.

# **11. Biodiversity**

Biodiversity, the disparity of natural life in the world, deals with the variation of life due to hereditary, class, and habitat factors. Due to the warm climatic conditions of the equator, terrestrial biodiversity is mainly found there. About 90% of the different species of the universe are in tropical areas, which cover only 10% of the overall scope of the

earth (Swingland, 2001; MEA, 2005). Life on the planet began about 4.54 billion years ago, which is decreasing over time. Among different causes of loss of lives, climate change is one of the major causes. Being a driving force climate change leads to the loss of about 17% of the world population (Thomas et al., 2004; UNEP, 2012). An increase in temperature affects species' distribution, migration, reproduction, morbidity, and mortality (Steinfeld et al., 2006). IPCC report provides information that a rise of 2-3 °C temperature at the pre-industrial level may lead to a loss of biodiversity of up to 30 % (IPCC, 2014). About 16% of animal breeds (bovines, caprine, and equines) are lost by 2000 (Thornton et al., 2009). Furthermore, about 20% of livestock breeds are near extinction, and one breed is extinct each month (FAO, 2007). Among extinct livestock breeds, cattle breeds are at number one, but the animal whose species are at risk of extinction are shown in descending order: poultry 33% > pigs 18% >cattle 16%. Extinction of animal breeds mainly depends on the area, as in the developing regions where modern farming of a few species is practiced, about 20 to 28% of livestock breeds are at risk (FAO, 2007). Loss in biodiversity is mainly due to livestock practices, the climatic condition of the area, and the rearing of high-producing breeds (Thornton et al., 2009). Animal species confined to their habitat, with a small population and low reproduction rate, are more vulnerable to extinction (Steinfeld et al., 2006). Loss in biodiversity is mainly due to harsh climatic conditions. Therefore, the need of the time is to focus on increasing the capabilities of breeds to resist extreme climatic conditions.

#### 12. Agro-ecological zones

There are different agricultural and livestock production practices around the world. To elaborate on these variations, the International Institute for Applied System Analysis and the Food and Agricultural Organization of the United Nations developed the agroecological zones (AEZs) (FAO, 2017). These AEZs are based on climatic conditions, soil type, landform, soils, and land in use (FAO, 1996). These AEZs include the earth's tropics, subtropics, temperate, boreal, and arctic zones (FAO, 1996). Furthermore, the division in AEZs is not necessarily based on climatic conditions, but each agricultural region has different effects of climate change. Climate change has both positive and negative impacts in each AEZ, as an increase in the level of CO<sub>2</sub> leads to a rise in the rate of photosynthesis, efficiency of water use, and productivity but an increase in temperature leads to an increase in plant diseases, water deprivation and decrease in plant production (Fischer et al., 2002). Such effects of climate change on plants indirectly affect livestock production.

Most of the animals are found in tropical and subtropical areas of the world; meanwhile the extreme climatic conditions are also there, directly affecting animal health, production, and reproduction (Herrero *et al.*, 2012). About 90% of the different species of animals are found in tropical areas, including about 10% of the earth's area (Swingland, 2001; MEA, 2005). The highest animal productivity is noticed in temperate AEZ (Seo and Mendelsohn, 2008; Renaudeau *et al.*, 2012; Herrero *et al.*, 2012). An increase in temperature and CO<sub>2</sub> level has adverse effects on animal productivity, but on plants, along with

adverse effects, they also have positive impacts on plant productivity (Rowlinson, 2008; Seguin, 2008). Increased temperature leads to increased arthropod-borne diseases of livestock (Rowlinson, 2008). Climate change positively affects agriculture and livestock in the boreal AEZs (Iglesias *et al.*, 2007; Bajzelj and Richards, 2014).

### 13. What impact does climate change have on agriculture?

A study on the impacts of climate change on agriculture, specifically on cropping systems, pasture and grazing areas, and animal management, was published by the U.S. Department of Agriculture in May 2008 (Backlund *et al.*, 2008). The report's conclusions are taken in full below:

- The growth cycle of grain and oilseed crops is predicted to go forward more quickly with rising carbon dioxide and higher temperatures.
- The marketable yield of many horticultural crops, such as tomatoes, onions and fruits, is very likely to be more sensitive to climate change than grain and oilseed crops.
- Weeds are probably going to migrate farther north as a result of climate change. More weeds than most commercial crops benefit from rising carbon dioxide levels.
- With earlier springtime and warmer winters, there will likely be more disease strain on domestic animals and crops.
- Forage production will probably continue into the late autumn and the early spring as a result of anticipated temperature rises and a prolonging of the growing season.
- Rangelands are already seeing variations in plant species brought on by climate change. Early in the growing season, the availability of soil water is being reduced by the formation of perennial herbaceous plants.
- In the summer, higher temperatures will almost certainly result in decreased animal output; however, higher winter time

temperatures will somewhat make up for these losses (Backlund *et al.*, 2008).

### 14. What impact does agriculture have on climate change?

Activities related to agriculture operate as both generators and sinks of greenhouse gases. The biological process of carbon sequestration has absorbed carbon from the atmosphere and stored it in agricultural sinks for greenhouse gases. The manufacturing of nitrogen-based fertilizers, the burning of fossil fuels such coal, gasoline, diesel fuel, and natural gas, as well as waste management, are the main sources of greenhouse gases in agriculture. Methane emissions occur from livestock enteric fermentation, or the fermentation that happens in ruminant animals' digestive tracts. By the process of photosynthesis, carbon dioxide is taken from the atmosphere and changed into organic carbon. Respiration is the process through which organic carbon storage may be achieved by conservation tillage, organic farming, cover crops, and crop rotations.

According to the Intergovernmental Panel on Climate Change, between 10 and 12 percent of all greenhouse gas emissions that were created by human activity worldwide in 2005 were attributable to agriculture (IPCC, 2007b). Agriculture is responsible for 8% of all greenhouse gas emissions in the US, and these emissions have been rising since 1990 (Congressional Research Service, 2008). Since greenhouse gases have different potentials to increase global warming, climatologists use carbon dioxide equivalents to determine an overall assessment of greenhouse gas emissions.

# 15. The contribution of agriculture to reducing climate change

By improving carbon storage in soils, keeping the carbon that already exists in soil, and lowering carbon dioxide, methane, and nitrous oxide emissions, a number of agricultural techniques and technologies may lower greenhouse gas emissions and stop climate change.

# 16. Conservation tillage and cover crops

The term "conservation tillage" refers to a variety of methods and procedures for planting new crops among the remnants of older ones that have been purposefully left on the soil's surface. Lowering tillage lessens soil disturbance and helps limit the atmospheric release of soil carbon.

# 17. Crop improvement and organic systems

Several studies looked at how organic farming may lower greenhouse gas emissions (Rodale Institute, 2008). By the use of cover crops and composted animal manures, organic methods of agriculture increase the amount of organic matter in the soil. Systems for growing organic crops also remove the emissions caused by the manufacture and delivery of synthetic fertilizers.

### 18. Irrigation management

The amount of water and nitrogen applied to the cropping system can be significantly decreased by increasing water use efficiency through techniques like irrigation system mechanical improvements combined with a reduction in operating hours, drip irrigation technologies, and center-pivot irrigation systems. This lowers water demands and nitrous oxide greenhouse gas emissions.

# **19. Effective usage of nutrients**

Nitrous oxide emissions may be decreased by increasing fertilizer effectiveness via techniques like precision farming with GPS monitoring. The use of cover crops, animal and green manures, nitrogen-fixing crop rotations, composting and compost teas, and integrated pest control are further tactics.

# 20. Soil use modifications and restoration

Typically, greenhouse gas emissions are reduced through land restoration and land use adjustments that promote the preservation and enhancement of soil, water, and air quality. Greenhouse gas emissions may be reduced by changing grazing techniques, such as adopting sustainable stocking rates, rotational grazing, and seasonal usage of rangeland. Maximizing carbon storage on land that is less suited for agriculture involves converting marginal farmland to trees or grass.

# **21.** Catching methane

Substantial emissions of nitrous oxide and methane are attributed to the handling of animal manure, particularly in dairies. Covered lagoons and full mix and plug flow digesters are two examples of agricultural methane collecting and combustion technologies. By trapping methane and limiting its emission into the atmosphere, anaerobic digestion turns animal waste into energy.

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## **CHAPTER 12**

## AN INSIGHT INTO COOL CLIMATE VITICULTURE

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### 1. Introduction

Grapevine is a plant species that overly susceptible to climatic conditions and the prevailing climatic conditions of the vineyard region play a key role in the growth and development of the grapevine plant and in determining the grape quality (Kok & Çelik, 2003; Kok, 2020; Keller, 2020).

Grape growing can be performed on six of the seven continents, typically occurring approximately between latitudes of  $4^{\circ}$  and  $51^{\circ}$  in the northern hemisphere and between  $6^{\circ}$  and  $44^{\circ}$  in the southern hemisphere, across a wide range of climate conditions (Kok, 2014; Jones & Schultz, 2016).

Temperature classifications in viticulture are used to categorize grapegrowing regions based on their mean temperatures during the growing season. By using these classification systems, grape growers may choose the most convenient grape varieties and vineyard management practices for their regions. Given the mean temperatures of the growing season of different viticulture regions, viticulture consists of three main groups such as cool climate viticulture, temperate climate viticulture and tropical climate viticulture (Jackson & Schuster, 1986; Lavee, 2020; Keller 2020):

Today, the climate classification system for plant species is pervasively used to better understand which plants are best suited for growing in a particular region. The climate classification system is a way of categorizing the world's climates based on certain climatic parameters, including temperature, precipitation and vegetation. The Köppen-Geiger Climate Classification System is one of the most broadly employed climate classification systems (Table 1). In this system, the world's climate regions are divided into five main categories with subcategories based on temperature and precipitation patterns to understand and compare climate conditions in various regions of the earth (Rubel & Kottek, 2010).

There has recently been an increasing interest in cool climate viticulture in grape-growing regions of the world where the climate conditions are suitable. All grape varieties grown in cool climate viticulture regions have wine characteristics (Keller, 2020; Jones et al. 2022; van Leeuwen, 2022).

Climate main categories	Climate subcategories	General attributes of climate
	Tropical rainforest climate (Af)	The mean temperature of every month is
A zone (Equatorial climate)	Tropical monsoon climate (Am)	higher than +18°C, with significant rainfall
	Tropical savanna climate (Aw)	
B Zone (Arid climate)	Desert climate (BW)	Low annual rainfall
	Steppe climate (BS)	
	Humid subtropical climate (Cfa)	
	Temperate oceanic climate (Cfb)	
C zone (Warm-temperate climate)	Subpolar oceanic climate (Cfc)	
	Monsoon-influenced humid subtropical climate (Cwa)	The mean temperature of the coldest month
	Subtropical highland climate (Cwb	is between $-3^{\circ}$ C and $+ 18^{\circ}$ C
	Subpolar highland climate (Cwc)	
	Hot-summer Mediterranean climate (Csa)	
	Warm-summer Mediterranean climate (Csb)	
	Subarctic climate (Csc)	
	Hot-summer humid continental climate (Dfa)	
	Warm-summer humid continental climate (Dfb)	
D zone (Continental climate)	Subarctic climate (Dfc)	The mean temperature of the warmest
	Hot-summer humid continental climate (Dwa)	month is higher than +10°C and mean
	Warm-summer humid continental climate (Dwb)	temperature of the coldest month is below -
	Subarctic climate (Dwc)	3°C
E zone (Polar climate)	Tundra climate (ET)	The mean temperature of the warmest
	Ice cap climate (EF)	month is lower than +10°C

Table 1. Main and subcategories of the world's climate based on the Köppen-Geiger climate classification system

As a result of their study, Jones and Schultz (2016) declared that numerous new potential cool climate regions were coming to light owing to climate change at higher latitudes, whereas existing cool climate regions were becoming more convenient as the climate evolved.

Bader & Wahl (1996), contrary to the findings of many researchers, found out that soil has a small effect on wine quality in cool climate regions and that climate is a more prominent factor than soil in the sensory properties of wine.

Site selection for vineyard establishment is a primary means for vineyard terroir management in terms of temperature. The best vineyard sites are typically found between temperate and cool climates in viticulture. In order to grow high-quality grapes in the northern hemisphere, the best vineyard sites in warm climates are north-exposure slopes or higher elevations, which are cooler locations, whereas south-exposure slopes might be the best location in cool climates (Morlat & Bodin, 2006).

The choice of grape variety for a particular site is another outstanding means to provide ripeness in an ideal period to optimize terroir expression When establishing a new vineyard, early grape varieties should be preferred for cool climate regions and late-ripe grape varieties for warm climate regions (Happ, 2000).

Because aromatic expression and complexity are optimized, wine grapes grown in very cold regions are likely to produce wonderful white and sparkling wines. However, it is difficult to produce well-structured red table wines from wine grapes that are deficient in phenolic compounds in cool regions (Morlat & Bodin, 2006).

Excessive low temperatures are a crucial climatic attribute, limiting grape production in cold viticulture regions. Frost events may harm above-ground organs of grapevines such as winter buds, canes, cordons and trunks. Yields of grapevines are restricted and production costs increase in cold viticulture regions due to re-planting in vineyards and re-training the grapevines (Zabata, 2007).

Today, due to their higher resistance to midwinter cold, the use of cold climate interspecific hybrid grape (CCIHG) varieties in cool climate viticulture regions is becoming increasingly common. But severe weather incidents, especially polar vortexes and the high frequencies of autumn and spring freezes, end up with yield losses and deaths in grapevines in cool climate viticulture regions (Atucha, 2018).

North et al. (2021) assessed variations in winter bud cold hardiness of five cold climate interspecific hybrid grape (CCIHG) varieties grown in Wisconsin, United States, in their research. For this purpose, a forecasting model for winter bud cold hardiness was proposed to CCIHG varieties cultivated in cold climate regions in order to identify the relative risks for frost damage status throughout the dormant period by researchers. In conclusion, it was determined that the cold hardiness forecasting model for CCIHG varieties was a beneficial tool that could assist grape growers in their decision-making to decrease frost damage and grapevine yield losses.

Based on the results of their studies, Allen et al. (1991) and Lacey et al. (1991) determined that wines produced from the Sauvignon Blanc grape variety grown in colder climates have significantly higher concentrations of 3-isobutyl-2-hydroxypyrus (IBMP) than those produced in warmer climates.

Balint & Reynolds (2014) performed a study about the effects of different irrigation strategies on the grape composition and sensory profiles of the *Vitis vinifera* L. Cabernet Sauvignon grape variety grown in a cool climate region. In the results of the study, it was determined that the grape composition of the Cabernet Sauvignon variety in cool climates was generally improved by irrigation treatments, including partial root zone drying (PDR) or regulated deficit irrigation (RDI) during the dry and warm years.

Balint & Reynolds (2017) carried out research concerning the impacts of irrigation level and time of imposition on grapevine physiology, yield components, grape composition and wine quality of the Chardonnay grape variety grown in a cool climate region. Study results indicated that medium soil water deficits were extremely correlated with positive sensory features in wines. Deficit irrigation treatments mostly enhanced grapevine water status and favorably affected the sensory profiles of wines.

In cool climate viticulture regions characterized by cool and short growing seasons with prominent annual variability, grape biochemistry may be adversely affected by various environmental determinants. In viticulture, some grapevine canopy management practices may be used to improve cluster zone microclimate conditions (Howell, 2001).

Frioni et al. (2017) conducted research regarding the influences of cluster zone leaf removal and cluster thinning, applied in combination during the véraison period, on grape composition and wine quality in cool climate conditions. As a result of the study, treatments of cluster thinning and leaf removal in the cooler summer period improved grape composition at harvest time, whereas no difference was observed between treatments in the warmer summer period due to ideal temperature and light conditions.

Walker et al. (2022) carried out research relating to the effects of doubling rates of annual nitrogen (18 kg/N/ha) and irrigation (530 L/grapevine) on grapevine yields and wine compositions of Chardonnay and Pinot Noir grape varieties. In the study, the influences of increased nitrogen treatment rates on yeast assimilable nitrogen (containing ammonium ions and free alpha-amino acids) in grape must were also examined under additional irrigation conditions. As a consequence, more nitrogen in the vineyard near the vérasion period raised yeast assimilable nitrogen without causing excessive vegetative development of the grapevine, leading to increased grape quality and wine chemical composition in cool climate viticulture regions. Ascending irrigation rates could be useful in seasons with high crop loads, but existing irrigation rates were found to be sufficient.

#### 2. Climate Classifications in Viticulture

Climate is a term that refers to the long-term mean of weather conditions in a given grape-growing region over a period of time, including temperature, precipitation, solar radiation and wind (Keller, 2020).

Climate is a substantial aspect of grape cultivation since it affects the growth and development of grapevines as well as the ripening of grapes. Understanding the climate of a particular region is crucial to deciding which grape varieties will grow there and what viticulture techniques will be required to maximize grape production and quality (Jackson & Lombard, 1993; Jones, 2007).

Today, there are several climate classification systems that are commonly used to describe specific growing conditions for grape varieties. Among the climate classification systems, the Köppen-Geiger Climate Classification System is of great importance in the classification of viticulture regions and this climate classification system takes into account climatic factors such as average temperature, precipitation and evaporation rates, which are critical in determining the suitability of grape varieties for a particular viticultural climate zone. Grape growers may identify viticulture regions that are appropriate for growing a particular grape variety depending on its temperature range, water requirements and other environmental factors using this climate classification system (Table 1). The current climate classification system may also give grape growers an idea of what might be the best vineyard management practices for different viticulture regions (Dougherty, 2012).

Considering the mean temperature values seen during the growing seasons in various viticulture regions, viticulture types are divided into three basic classes, including cool climate viticulture, temperate viticulture and tropical viticulture (Table 2).

 Table 2. Typical mean temperature values of the growing season depend on viticulture type

Viticulture type	The mean temperature values of the growing season
Cool climate viticulture	10-18°C
Temperate climate	18-22°C
viticulture	
Tropical climate viticulture	22-28°C

*1-Cool climate viticulture:* Cool climate regions have a mean temperature range of 10°C to 18°C in the course of the growing season. These regions are characteristically characterized by a shorter growing season and a longer dormancy period. Cool climate regions are especially known for producing wines with high acidity, lower alcohol levels and distinct varietal flavors (Jackson & Schuster, 1986).

2-Temperate climate viticulture: Temperate climate regions have a mean temperature range of 18°C to 22°C in the course of the growing season. These regions possess a longer growing season than cool climates with a shorter dormancy period. Temperate climate regions are known for producing wines with good acidity, moderate alcohol levels and balanced flavors (Lavee, 2020).

*3-Tropical climate viticulture:* Tropical (hot) climate regions have a mean temperature range of 22°C to 28°C in the course of the growing season. These regions possess long growing seasons with little to no dormancy. Tropical climate regions are known for producing wines with low acidity, high alcohol levels and rich, ripe flavors (Kok, 2014).

The grape ripening capability of a viticulture region is related to some climatic indexes, such as growing degree days or the mean temperature of the warmest month calculated for that region (Kok, 2020). Considering this situation, the Latitude-Temperature Index (LTI) was proposed by Jackson & Cherry (1988) and grape varieties were divided into four different groups in terms of ripening characteristics based on this index (Table 3).

Climate group	Class interval	Climate	Grape varieties grown
			Gewürztraminer, Madelaine,
Group A	LTI < 190	Very cool	Angevine, Reichensteiner, Perle,
			Schönburger, Müller-Thurgau,
			Triomphe d'Alsace
			Pinot gris, Pinaot blanc, Chasselas,
		Cool	Sylvaner, Chardonnay, Faber,
			Acheurebe, Auxerrois, Baccus
Group B	LTI :190-270	Cool-	Riesling, Pinot noir
		warm	
			Cabernet Sauvignon, Cabernet Franc,
Group C	LTI: 270-380	Warm	Merlot, Malbec, Sauvignon Blanc,
			Sémillon. These grape varieties may
			occasionally be grown in cooler
			regions of Group B
			Carignane, Grenache, Shiraz, Sultana,
Group D	LTI>380	Warm-hot	Cinsaut, Zinfandel. Some of these
			grape varieties may rarely ripen in
			Group C

**Table 3.** Grape-growing regions of the world depend on the Latitude-Temperature Index (LTI)

The latitude of a vineyard region also plays a key role in the quality of the grape and the development and growth of the grapevine. The effects of the latitude degree of the vineyard site on the grapevines and grapes are realized through the factors such as temperature, sunlight, and the altitude of the vineyard The factors mentioned are the following characteristics related to the vineyard location (Gladstones, 2016):

*1-Climate:* Latitude affects the climate, which in turn impacts grapevine development, growth and quality. At lower latitudes, warmer temperatures and longer growing seasons may result in higher sugar content in grapes, whereas cooler temperatures at higher latitudes may lead to more acidic grapes.

*2-Sunlight:* The angle of the sun's rays varies with latitude, and lower latitudes receive more direct sunlight. More sunlight exposure may cause greater photosynthesis, resulting in more potent grapevines and a higher sugar content in the grapes.

*3-Altitude:* Latitude is often associated with altitude, which can also affect the development and growth of grapevines and grape quality. Higher altitudes have cooler temperatures and grapevines at higher altitudes are exposed to more UV radiation, resulting in thicker grape skin and more robust grapevines.

*4-Grape varieties:* Grape varieties possess varying requirements in terms of temperature, sunlight and water. Some grape varieties flourish in cooler climates, while others require warmer climates to grow and

develop. Therefore, the choice of grape variety for a vineyard location is frequently affected by that vineyard location's latitude.

*5-Harvest time:* Latitude may also affect the timing of the grape harvest. In cooler climate regions, the growing season can be shorter, leading to an earlier harvest in grape varieties, whereas warmer climates can allow for a longer growing season and a later harvest in grape varieties.

#### 3. Cool Climate Viticulture

Cool climate viticulture is a worldwide phenomenon that occurs in some regions of the world. These regions have developed special viticultural practices that allow them to produce premium-quality wines despite various challenges, such as lower temperatures and shorter growing seasons.

Cool climate viticulture regions are delineated by their lower mean temperatures, which may range from 10°C to 18°C in the course of the growing season (Dougherty, 2012; Jackson & Schuster, 1986; Keller, 2020).

Cool climate viticulture is best suited to viticulture regions with long, sunny days and cool nights, which allow grapes to ripen slowly and develop complex flavors. The regions where some cool climate viticulture may be performed among the world's climate regions based on the Köppen-Geiger climate classification system are shown in Table 4 (Rubel & Kottek, 2010; Dougherty, 2012). In addition, according to the Latitude-Temperature Index developed by Jackson & Cherry (1988), wine grape varieties suitable for cool climate viticulture can be grown in A, B and C group regions (Table 3).

The main factor that distinguishes cool climate viticulture from other viticulture types is its climatic attributes. Among the viticulture activities carried out in various climate regions all around the world, cool climate viticulture has a considerable role (Jackson & Schuster, 1986). Cool climate viticulture is particularly pivotal to the wine industry, producing high-quality wines with unique and distinctive flavors. Lower temperatures in this climate system may cause wine grapes to ripen more slowly, producing wines with higher acid contents, lower alcohol rates and more complex and nuanced flavors (Jackson & Schuster, 1986; Robinson, 2015).

Climate main categories	Climate subcategories	General attributes of climate	Associating cool climate viticulture regions with the Köppen-Geiger climate classification system
A zone (Equatorial climate)	Tropical climate (Af)rainforest restTropical climate (Am)monsoon climate (Am)Tropical climate (Aw)savanna climate (Aw)	Themeantemperatureofeverymonthhigherthan +18°C,withsignificantrainfall	
B Zone (Arid climate)	Desert climate (BW) Steppe climate (BS)	Low annual rainfall	
C zone (Warm- temperate climate)	Humidsubtropicalclimate (Cfa)Temperateoceanicclimate (Cfb)Subpolaroceanicclimate (Cfc)Monsoon-influencedhumidsubtropicalclimate (Cwa)Subtropicalhighlandclimate (CwbSubpolarhighlandclimate (Cwc)Hot-summerMediterraneanclimate(Csa)	The mean temperature of the coldest month is between -3°C and + 18°C	Temperate oceanic climate (Cfb) Subpolar oceanic climate (Cfc)

**Table 4.** Specifying the location of cool climate viticulture regions in the Köppen-Geiger climate classification system (to be continued)

**Table 4.** Specifying the location of cool climate viticulture regions in the Köppen-Geiger climate classification system

Climate main categories	Climate subcategories	General attributes of climate	Associating cool climate viticulture regions with the Köppen-Geiger climate classification system
	Hot-summer humid		
	continental climate		
D zone	(Dfa)	The mean	
(Continental	Warm-summer	temperature of	Warm-summer
climate)	humid continental	the warmest	humid continental
	climate (Dfb)	month is higher	climate (Dfb)
	Subarctic climate	than +10°C and	Subarctic climate
	(Dfc)	the mean	(Dfc)
	Hot-summer humid	temperature of	
	continental climate	the coldest month	
	(Dwa)	is below -3°C	
	Warm-summer		
	humid continental		
	climate (Dwb)		
	Subarctic climate		
	(Dwc)		
E zone	Tundra climate (ET)	The mean	
(Polar climate)	Ice cap climate (EF)	temperature of	
		the warmest	
		month is lower	
		than +10°C	

Some important regions in the world where cool climate viticulture may be successfully performed are shown in Table 5.

Continent	Cool Climate Regions
North America	Pacific North West: Oregon and Washington States; Finger
	Lakes region of New York; parts of Canada such as the Niagara
	Peninsula
Europe	Northern Europe: Champagne and Chablis regions of France;
	Mosel and Rheingau regions of Germany and Douro Valley in
	Portugal
Oceania	Adelaide Hills and Tasmania in Australia; Marlborough and
	Central Otago in New Zealand
South America	Casablanca and San Antonio Valleys in Chile; some parts of
	Argentina
Africa	Elgin and Walker Bay regions in South Africa

**Table 5.** Some of the world's leading cool climate viticulture regions

Grape varieties grown in cool climate regions are early-ripening varieties with wine characteristics (Happ, 2000).

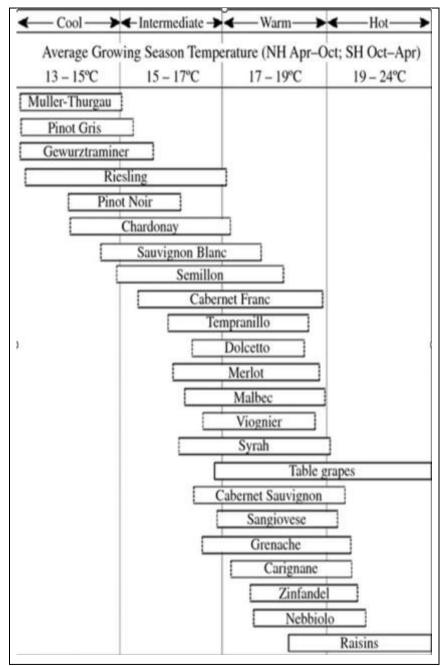
In cool climate viticulture, maturation periods of grape varieties correspond with cool periods at the end of the growing season in the northern hemisphere (September or October) and the southern hemisphere (March or April) (van Leeuwen, 2022).

Some remarkable grape varieties grown in cool climate viticulture regions and their general characteristics are given in Table 6.

Grape variety	General characteristics of grape variety
Marquette	A red grape variety that is known for its cold hardiness, disease
	resistance and ability to produce high quality wines with deep
	color and good structure
Frontenac	A red grape variety that is known for its cold hardiness, disease
	resistance and ability to produce wines with dense fruit flavors
	and high acidity
La Crescent	A white grape variety is known for its cold hardiness, disease
	resistance and ability to produce aromatic wines with floral
	and citrus notes
Vidal Blanc	A white grape variety is known for its cold hardiness, disease
	resistance and ability to produce wines with high acidity and
	fruity flavors
Traminette	A white grape variety is known for its cold hardiness, disease
	resistance and ability to produce wines with spicy and floral
	aromas
Pinot Noir	A red grape variety that is known for its light to medium body,
	high acidity and delicate flavors
Chardonnay	A white grape variety that is known for its full body, richness
	and complexity
Riesling	A white grape variety that is known for its high acidity, floral
	aromas and citrus flavors
Gamay	A red grape variety that is known for its light body, low tannins
	and fruity flavors

Table 6. Some of the well-known wine grape varieties for cool climate regions

These grape varieties are commonly grown in cool climate viticulture regions where the winters may be long. But it should be considered that the suitability of a grape variety for cool climate viticulture may also depend on the specific climate and growing conditions of the region (Figure 1).



**Figure 1.** Grouping of grape varieties according to their ability to ripen in various climate types (Jones, 2012)

Cool climate viticulture is an onerous but rewarding practice that allows wine grape growers to produce unique and high-quality wines. Some major advantages of cool climate viticulture are as follows (Robinson, 2015; Reynolds et al., 2019):

*1-Longer ripening period in grape varieties:* Cool climate viticulture regions have a longer growing season, which allows grape varieties to ripen more slowly and develop more complex flavors and aromas. But grape growing techniques for cool climates should take into consideration the challenges of low temperatures, frost, limited sunlight, uneven grape ripening and the need for careful canopy management.

2-*High acid rates in grape varieties:* Cool climate grapes constantly have higher levels of acidity, which may give wines a refreshing and lively attribute.

*3-Low sugar rates in grape varieties:* Lower temperatures may limit sugar production in grapes, leading to lower alcohol levels in the resulting wines.

4-Distinct flavor profiles in grape varieties: Cool climate wines often have distinct flavors and aromas, including green apple, citrus, and minerality in white wines and cherry, strawberry and earthiness in red wines.

*5-Matchless terroir attributes:* Cool climate regions give different characteristics to the wines to be obtained with their soil, topography and climatic conditions.

*6-Sustainability:* Cool climate viticulture may be more sustainable due to requiring less water. Additionally, fewer pests and diseases are observed in cooler climates, which decreases the need for chemical practices.

The following factors should be taken into account in order to successfully cultivate grapes in cool climate viticulture regions (Reynolds et al., 2019; Keller, 2020; Robinson, 2015):

*1-Sunlight:* The amount of sunlight required for cool climate viticulture may vary depending on a variety of factors, including grape variety, vineyard location and local weather conditions. But cool climate viticulture needs a minimum of 1200 to 1400 hours of sunlight per year to attain full ripening of grape varieties.

*2-Temperature:* Cool climate viticulture generally requires mean temperatures that range from 10°C to 18°C in the course of the growing season. The temperature range may vary depending on the grape variety. However, cooler temperatures help to maintain acidity and stimulate the development of complex aromas and flavors in grapes. Cool climate viticulture requires careful attention to temperature patterns throughout the growing season to obtain ideal grape quality.

*3-Site selection:* Choosing a proper site with good soil drainage and sufficient exposure to sunlight is crucial for successful grape growing in cool climate regions. When selecting a site for a vineyard in a cool climate region, many factors should be considered, such as temperature, sunlight, soil, water availability, topography, and elevation. A site that

is suitable for cool climate viticulture can be carefully chosen by considering these factors.

4-Grape variety selection: Preferring grape varieties that are wellsuited to cooler climates is a substantial issue to ensure high-quality wine grapes and wines. Some grape varieties are known to be well adapted to cool climates. When selecting grape varieties for cool climate regions, some factors, including cold hardiness, ripening period, disease resistance, flavor profile, and marketability, should be considered.

5-Winter pruning for grapevines: Winter pruning is a notable practice in cool climate viticulture that may affect grape quality and grapevine health. When pruning grapevines in cool climate regions, many factors should be considered, like timing, severity, training system, disease management and frost protection. In cool climate regions, winter pruning should be commonly carried out with the aim of controlling grapevine vigor and enhancing grape quality while considering disease management and frost protection. It is important for cool climate regions that winter pruning be frequently conducted later in the season to delay winter bud burst and prevent damage from spring frost.

*6-Grapevine training:* By using different grapevine training systems, grapevine growth and grape development may be promoted. The choice of training system should depend on specific grape varieties, site conditions and desired results like grape quality and disease management. Each of the grapevine training systems has advantages and disadvantages. So, it should be chosen based on what is best suited

to existing vineyard conditions. In cool climate viticulture regions, it is generally utilized with different vertical shoot positioning systems.

7-Crop management: In cool climate viticulture regions, crop management is important to grow high-quality wine grapes and ensure optimal grapevine health. Some important crop management practices considered in cool climate regions are crop load management, irrigation, fertilization, pest management and canopy management.

8-*Irrigation:* In cool climate viticulture regions, irrigation may be required to supplement rainfall and assure that grapevines receive sufficient water. But irrigation should be administered carefully to avoid overwatering, which can result in poor grape quality and disease susceptibility.

*9-Frost protection:* Frost protection is a key component of cool climate viticulture, as frost events may kill the grapevines or severely harm them in cool climate regions. Widespread methods of frost protection for cool climate viticulture are site selection, wind machines, sprinklers, heaters, cover crops, monitoring weather conditions and adjusting management practices. Each of the frost protection methods has advantages and disadvantages, and thus, the best approach should be preferred depending on the specific circumstances of the vineyard.

*10-Grape harvest timing:* In cool climate viticulture, grape harvest timing is critical to attaining high-quality wine grapes and producing high-quality wines. The ideal time for grape harvest in cool climates is typically in the late summer or early autumn, when the grapes reach full

ripeness and have the desired sugar and acid rates. Grape harvest timing depends on an array of factors, such as grape variety, climatic conditions, and the desired style of wine. Overall, cool climate grapes tend to ripen later and have a higher acidity level than grapes grown in warmer climates. Determining the optimum time for grape harvesting in cool climate viticulture regions requires careful monitoring and assessment.

#### 4. Conclusion

Although cool climate viticulture has some challenges, it also has some advantages and offers novel and interesting wines for consumers to discover and enjoy. The benefits of cool climate viticulture to the wine industry are briefly summarized as follows: The cooler climate conditions may help preserve the natural acidity of wine grapes, which may make the wines more refreshing and aged. The development of tannins and phenolic compounds that can positively affect the structure and aging capacity of wines in grapes grown in cool climate regions can be supported by keeping the grapes on the grapevine for a longer time. The cooler climates result in slower wine grape ripening, resulting in wines with lower alcohol content and higher acid levels, which balance the grape flavors in wine and provide a refreshing and crisp taste that is highly prized by wine enthusiasts. As a result, some of the world's finest wines could be produced from wine grape varieties grown in cool climate viticulture regions by means of careful viticultural management and winemaking practices.

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## **CHAPTER 13**

# THE EFFECTS OF IRON AND LIQUID SEAWEED FERTILIZER APPLICATIONS IN DIFFERENT DOSES ON SOME AGRO-MORPHOLOGICAL FEATURES OF THE CHARD (*Beta vulgaris L. var. cycla*) PLANT AND SOME MACRO-MICRO NUTRIENTS IN THE SOIL AND PLANT

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#### INTRODUCTION

Agricultural production systems in the coming years; it will be based on the adoption of agricultural production practices that reduce adverse environmental impacts, improve the sustainability of crop production, mitigate and adapt to climate change to ensure food security. In plants that are often deficient in micronutrients; there is a significant increase in susceptibility to abiotic stresses (Jin et al., 2009; Hajiboland, 2012). Climate change; negatively affects soil quality and microbial activity in the soil. This situation also negatively affects soil organic matter (Parajuli et al., 2018).

Swiss chard, also known as *Beta vulgaris* L. *var. cycla*, is a popular leafy vegetable that is valued for its health benefits. The edible parts of the plant are its leaves and stem, which are rich in phenolic acid and flavonoids compounds (Pyo et al., 2004). Swiss chard is also a good source of vitamins A, B, and C, as well as minerals such as Ca, Fe and P (Huxley et al., 1992; Tindall, 1993; Maynard and Hochmuth, 1997; Bozokalfa et al., 2011; Bozokalfa et al., 2016; Libutti and Rivelli, 2021; Rivelli and Libutti, 2022).

Organic fertilizers have several advantages over chemical fertilizers. They not only increase the nutrient content of the soil, but also improve its physical and biological properties, such as water retention, soil structure, and microbial activity. Additionally, organic fertilizers are environmentally friendly, as they do not contain harmful chemicals that can damage the soil and water resources. They are also cost-effective and sustainable, as they can be produced locally from readily available materials such as manure, compost, and crop residues. Furthermore, the use of biofertilizers, which are made from beneficial microorganisms such as bacteria, fungi, and algae, can further enhance the effectiveness of organic fertilizers. Biofertilizers can increase nutrient uptake by plants, improve soil fertility and health, and reduce the use of chemical fertilizers. Overall, the adoption of a balanced fertilization program that incorporates the use of both organic and chemical fertilizers, as well as biofertilizers, can help to improve the sustainability and productivity of agriculture while minimizing negative impacts on the environment (Duan, 2013; Sitienei et al., 2018).

Organic fertilizers, including those made from algae, can provide a wide range of these plant nutrients to the soil in a sustainable and environmentally friendly way (Eryilmaz Acikgoz et al., 2017; Gurgan and Adiloglu 2021).

It is observed that organic fertilizer production from algae has an important place. Seaweed fertilizer is popular because it is a natural and organic source of nutrients for plants. Seaweed has been used for many purposes throughout history, such as food, medicine, and industrial applications. In addition to being a source of fertilizer, it has also been used as a soil conditioner due to its ability to improve soil structure and water-holding capacity. Seaweed extracts have also been found to have beneficial effects on plant growth, including increased seed germination, root growth, and nutrient uptake. Furthermore, seaweed has been used as a natural source of bioactive compounds for various industries, such as pharmaceuticals, cosmetics, and bioplastics (Unschuld, 2010; Satana et al., 2016). Liquid seaweed fertilizers contain alginic acids, amino acids, plant growth regulators, vitamins, nucleotides, humic acids and micro and macro plant nutrients (Duan, 2013; Bender and Sen, 2017). Seaweed contains a variety of nutrients, including nitrogen, potassium, phosphorus, calcium, and magnesium, as well as trace elements such as iron, zinc, and copper. These nutrients are readily available for plants to absorb and use for growth and development. Seaweed fertilizer is also known to improve soil health by increasing the microbial activity and promoting the growth of beneficial microorganisms in the soil. This can lead to improved soil structure, increased water-holding capacity, and better nutrient availability for plants. Another advantage of seaweed fertilizer is that it can help plants to resist stress and disease. Seaweed contains natural compounds that can stimulate the plant's immune system and enhance its ability to cope with adverse growing conditions. Overall, seaweed fertilizer is a sustainable and eco-friendly choice for gardeners and farmers who want to promote healthy plant growth and improve soil health without using synthetic chemicals (Eryilmaz Acikgoz et al., 2015a; Eryilmaz Acikgoz et al., 2015b; Yagcilar et al., 2015; Moh et al., 2018).

These plant nutrients are classified into two categories: macronutrients and micronutrients. Macronutrients are required in larger amounts and include elements such as nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), and sulfur (S). Micronutrients, on the other hand, are required in smaller amounts and include elements such

as iron (Fe), zinc (Zn), copper (Cu), manganese (Mn), boron (B), molybdenum (Mo), and chlorine (Cl). Both macronutrients and micronutrients play essential roles in plant growth and development, and their availability in the soil is crucial for obtaining high-quality and high-yield crops. Iron is an essential micronutrient for plant vegetative growth. It plays a crucial role in photosynthesis, which is the process by which plants convert light energy into chemical energy to fuel their growth. Iron is also important for the formation of chlorophyll, the green pigment that gives plants their color and is essential for photosynthesis. In many cases, the amount of iron present in the soil may not be sufficient to meet the needs of plants. This can lead to iron deficiency, which can cause a range of problems such as yellowing of leaves, stunted growth, and reduced yield. Iron deficiency can be particularly common in alkaline soils or in soils with high levels of phosphorus or calcium, which can make it difficult for plants to absorb iron. Applying iron fertilizer can help to overcome this deficiency and ensure that plants have enough iron to grow and thrive. Iron fertilizers come in a variety of forms, including chelated iron, iron sulfate, and iron oxide. The specific type of iron fertilizer used will depend on the specific needs of the plant and the soil conditions (Adiloglu, 2007; Adiloglu, 2021; Gurgan and Adiloglu, 2021). In addition, iron is involved in the formation of proteins and carbohydrates, as well as the respiration process and the activity of enzymes. However, in soils with high pH or lime content, iron can become less available for plants to uptake, leading to iron deficiency. When iron deficiency occurs, plant growth and development can be stunted, and the quality and yield of crops can be negatively affected (Karaman et al., 2012; Schmidt et al., 2020).

The total amount of iron found in most soils is around 3-5%. In some lands, this figure reaches 10%. It is well known in practice that iron deficiency is caused by events such as soil compaction, irregular irrigation, and successive plant cultivation, which are frequently encountered in greenhouse soils. Elimination of iron deficiency is also more difficult than other trace elements. Because there are many factors that affect the availability of iron to plants. Leaf fertilizers are successful in eliminating iron deficiency. Excess organic matter is a factor that reduces iron deficiency. While there is iron deficiency problem in 37.22% of soils with less than 1% organic matter content, iron deficiency is seen in only 5% of soils in soils with organic matter content above 4% (Zuo and Zhang, 2011).

In line with the adoption of agricultural production practices that adapt to climate change, the aim of this study is; to investigate how different applications of iron and liquid seaweed fertilizer affect the nutrient contents of both the plant and the soil, as well as the agro-morphological characteristics of chard plants grown in iron-deficient soil.

## MATERIALS AND METHODS

The experiment was carried out autumn in Tekirdag city-Türkiye (40°98' N, 27°48' E) in high tunnel cold greenhouse covered by polyethylene (PE) with UV additive which belongs to Tekirdag Namik Kemal University, Vocational College of Technical Sciences, Plant and

Animal Production Department. The experiment was designed as three replications according to randomized block experimental design. Chard (*Beta vulgaris* L. *var. cycla*) was used for the research (Figure 1). Seeds were sown in multi-celled trays filled with peat in October (Figure 2). Some properties of the used peat are: 160-260 mg. L<sup>-1</sup> N, 180-280 mg. L<sup>-1</sup> P<sub>2</sub>O<sub>5</sub>, 200-150 mg. L<sup>-1</sup> K<sub>2</sub>O, 80-150 mg. L<sup>-1</sup> Mg, pH: 6, 70 % organic matter and 35 % C. When the seedlings became 2 to 3 true leaves (25th days for chard after seed sowing) they were planted to pre-prepared places in high tunnel cold greenhouse with 50x50 cm intervals and 6 plants in each parcel (Figure 2).



Figure 1. Chard Plants (original)



Figure 2. Research Area (original)

There is a lack of iron (Fe) element in the greenhouse soil where the research was conducted (Table 1). The amount of Fe that the plant will receive from the plant root zone through its roots depends on the presence of organic matter in the soil (Marschner, 1995). Considering this, the applications in the research were designed as follows. Control, iron (as Fe-EDDHA) to be fixed; it was added to doses with 1000 gda<sup>-</sup> <sup>1</sup> calculation in plant root zone. I. dose (1000 gda<sup>-1</sup> Fe calculation in plant root zone +  $200 \text{ ml}.100\text{L}^{-1}$  water liquid seaweed fertilizer as spray on plant leaves), II. Dose (1000 gda<sup>-1</sup> Fe calculation in plant root zone + 300 ml.100L<sup>-1</sup> water liquid seaweed fertilizer as spray on plant leaves), III. Dose (1000 gda<sup>-1</sup> Fe calculation in plant root zone + 400 ml.100L<sup>-1</sup> water liquid seaweed fertilizer as spray on plant leaves), Fe only from the soil 1000 gda<sup>-1</sup> in plant root zone, liquid seaweed fertilizer only from the soil (300 ml. 100L<sup>-1</sup> water as spray in plant root zone), liquid seaweed fertilizer only from the leave (300 ml.100L<sup>-1</sup> water liquid seaweed fertilizer as spray on plant leaves) were applied before a month from harvesting time and plants were harvested after 84 days from seed sowing (Figure 3). The length, width and leaf stalk length measurements of the plant leaves were determined as cm with the help of a ruler without wasting time after harvest. The wet weight (g) of the plant samples was determined by weighing with a balance with an accuracy of 0.01 g. The harvested leaves were dried in a drying cabinet at 72°C for 72 hours and their dry weights (g) were determined. Color indications were taken using the Hunter Lab D25LT Colorimeter. Color parameters were determined as brightness (L) (Anonymous, 1996). Measurements were made on randomly selected leaves of all plants

grown. Some macro and micro nutrient elements (N, P, K, Ca, Mg, Fe, Cu, Mn and Zn) contents of plants and soil of research were determined via ICP-OES instrument (Kacar and Inal 2010).

The results were analyzed with ANOVA followed by Duncan multiple comparison test using SPSS 21 software.



Figure 3. Plant and Soil Samples Prepared for Analysis (original)

Some chemical characteristics of the soil sample used in this study are given in Table 1.

Some properties of the liquid seaweed fertilizer used in the study are given in Table 2.

Some average climate data in cold greenhouse during the research where the plants are grown are given in Table 3.

Pesticides were not used during the growing period.

Results	
7.04	
1620	
5.65	
1.88	
Clay Loam (CL)	
36.40	
253.80	
0.70	
1.83	
0.40	
0.87	
-	7.04         1620         5.65         1.88         Clay Loam (CL)         36.40         253.80         0.70         1.83         0.40

Table 1. Some Chemical Characteristics of the Soil Sample.

+: (%), ++: (mg.kg -1)

Table 1 provided shows various chemical properties of the soil used in a study. The pH of the soil is 7.04, which indicates that it is neutral. The electrical conductivity (EC) of the soil is  $1620 \ \mu s/cm$ , which is an indication of the soils less salinity level. The CaCO<sub>3</sub> content of the soil is 5.65%, which is a measure of the soil's little calcium carbonate content. The organic matter content of the soil is 1.88%, which is a measure low organic matter content of the soil's carbon-based materials. The texture of the soil is classified as clay loam (CL), which is a mixture of clay, silt, and sand. The available phosphorus (P) in the soil is 36.40 ppm, which is a measure of the soil's medium available phosphorus fertility. The exchangeable potassium (K) in the soil is 253.80 ppm, which is an indication of the soil's ability to hold onto potassium ions and sufficient exchangeable potassium. The available manganese (Mn) in the soil is 0.70 ppm, which is a measure of the amount of this element available to plants. The available copper (Cu) in the soil is 1.83 ppm, which is a measure of the amount of this element available to plants. The available iron (Fe) in the soil is 0.40 ppm. Research soil is deficient in iron content. The total amount of iron found in most soils is around 3-5%. Finally, the available zinc (Zn) in the soil is 0.87 ppm, which is a measure of the amount of this element available to plants.

Liquid Seaweed Fertilizer	
рН	6,4
EC, dS m <sup>-1</sup>	2,01
Organic material, % w/v	15
Alginic acid, % w/v	0.5

Table 2. Some Properties of the Liquid Seaweed Fertilizer

Table 2 provides information on the properties of a liquid seaweed fertilizer. The pH of the fertilizer is 6.4, which indicates that it is slightly acidic. The electrical conductivity (EC) of the fertilizer is 2.01 dS m<sup>-1</sup>, which is an indication of its salinity level. The high organic material content of the fertilizer is 15% w/v. The alginic acid content of the fertilizer is 0.5% w/v, which is a measure of the concentration of this compound in the fertilizer.

Table 3. Some Average	Climate Data in Cold	Greenhouse During the Research.

Month	Maximum	Minimum	Mean
	temperature ( <sup>0</sup> C)	temperature ( <sup>0</sup> C)	humidity (%)
October	31	27	48,2
November	18	6,1	76
December	13,4	4,1	78
January	14,3	2,4	70,6

Table 3 provides mean climate data for a cold greenhouse during the months of October, November, December, and January. The maximum temperature in October was 31°C, while the minimum temperature was 27°C. The mean humidity during this month was 48.2%. In November, the maximum temperature was 18°C, while the minimum temperature was 6.1°C. The mean humidity during this month was 76%. In December, the maximum temperature was 13.4°C, while the minimum temperature was 4.1°C. The mean humidity during this month was 78%. Finally, in January, the maximum temperature was 14.3°C, while the minimum temperature was 2.4°C. The mean humidity during this month was 70.6%.

#### **RESULTS AND DISCUSSION**

The effects of different applications on some agro-morphological properties of chard plant are given in Table 4.

Table 4 shows effects of different applications on some agromorphological properties of chard plant. The applications evaluated include a control group, I. dose, II. dose, III. dose, Fe only from the soil, liquid seaweed fertilizer only from the soil, and liquid seaweed fertilizer only from the leaf. The table also indicates statistical significance levels, average values of three replications, and individual parameter evaluations.

Applications	Leaf	Leaf	Number of	leaf stalk	Color of the	Fresh	Dry matter
	width	height	leaves (unit)	length (cm)	leave (L)	weight (g)	weight (g)
	(cm)	(cm)					
Control	8,41b	24,08bc	20,00b	12,16b	33,43c	45,58bc	8,58b
I. dose	8,40b	24,93bc	24,65a	17,0a	39,34bc	61,14b	14,50a
II. dose	8,40b	21,46c	16,00b	11,66 b	47,00a	34,66c	6,70b
III. dose	7,33b	19,06c	18,00b	10,66b	39,21bc	27,53c	5,73b
Fe only from the soil	11,59a	33,12a	19,89Ь	17,26a	35,44c	82,25a	17,16a
liquid seaweed	8,38b	23,08bc	15,80b	11,56b	42,25ab	36,11c	8,85b
fertilizer only from the soil							
liquid seaweed	10,79a	28,58ab	17,30b	12,46b	35,60c	42,33bc	6,74b
fertilizer only from the							
leave							

**Table 4.** The Effects of Different Applications on Some Agro-Morphological Properties of Chard Plant<sup>\*</sup>, <sup>+</sup>, <sup>++</sup>, <sup>+++</sup>

\*: p<0.05, +: values are average of three replications, ++: each parameter evaluated individually, +++: mean square error

The results suggest that the different applications had varying effects on the some agro-morphological properties, with some applications resulting in significant improvements in some agro-morphological properties compared to the control group.

Table 4 presents the effects of different applications on some agromorphological properties of chard plants. The table includes measurements for leaf width (cm), leaf height (cm), number of leaves (unit), leaf stalk length (cm), color of the leaf (L) as brightness, fresh weight (g), and dry matter yield (g). The control group had a leaf width of 8.41 cm, leaf height of 24.08 cm, number of leaves 20.00, leaf stalk length of 12.16 cm, a leaf color of 33.43, fresh weight of 45.58 g, and dry matter yield of 8.58 g. Application I, with the I. dose, resulted in a leaf width of 8.40 cm, leaf height of 24.93 cm, number of leaves 24.65,

leaf stalk length of 17.0 cm, a leaf color of 39.34, fresh weight of 61.14 g, and dry matter weight of 14.50 g, which was significantly different from the control group. Application II, with the II. dose, resulted in a leaf width of 8.40 cm, leaf height of 21.46 cm, number of leaves 16.00, leaf stalk length of 11.66 cm, a leaf color of 47.00, fresh weight of 34.66 g, and dry matter weight of 6.70 g, which was significantly different from the first dose and the control group. Application III, with the III. dose, resulted in a leaf width of 7.33 cm, leaf height of 19.06 cm, number of leaves 18.00, leaf stalk length of 10.66 cm, a leaf color of 39.21, fresh weight of 27.53 g, and dry matter weight of 5.73 g, which was significantly different from the first dose and the control group. Fe only from the soil application resulted in a leaf width of 11.59 cm, leaf height of 33.12 cm, number of leaves 19.89, leaf stalk length of 17.26 cm, a leaf color of 35.44, fresh weight of 82.25 g, and dry matter weight of 17.16 g, which was significantly different from all other applications. Liquid seaweed fertilizer only from the soil application resulted in a leaf width of 8.38 cm, leaf height of 23.08 cm, number of leaves 15.80, leaf stalk length of 11.56 cm, a leaf color of 42.25, fresh weight of 36.11 g, and dry matter weight of 8.85 g, which was not significantly different from the control group. Liquid seaweed fertilizer only from the leaf application resulted in a leaf width of 10.79 cm, leaf height of 28.58 cm, number of leaves 17.30, leaf stalk length of 12.46 cm, a leaf color of 35.60, fresh weight of 42.33 g, and dry matter weight of 6.74 g, which was significantly different from the control group.

It is seen that II. dose (300 ml.100L<sup>-1</sup> water liquid seaweed fertilizer) application gives successful results in the some agro-morphological properties of chard plant relatively. It's worth noting that there are some statistically significant differences (p<0.05) between the treatments and the control group or between different treatments, but the interpretation of the results requires more information on the experimental design, the cultivar, and other factors.

The combined use of seaweed and Fe as organic matter in the research had a positive effect on the some agro-morphological properties of chard plant. Inorganic and organomineral fertilizer applications have a positive effect on the morphological characteristics of the plant (Suge et al,2011; Gore and Sreenivasa, 2011; Ayeni et al.,2012; Sayg1, 2022).

The effects of different applications on some macro-micro nutrient element contents of chard plant are given in Table 5.

Table 5 shows the effects of different applications on some macromicro nutrient element contents of chard plant. The applications evaluated include a control group, I. dose, II. dose, III. dose, Fe only from the soil, liquid seaweed fertilizer only from the soil, and liquid seaweed fertilizer only from the leaf. The table also indicates statistical significance levels, average values of three replications, and individual parameter evaluations. The results suggest that the different applications had varying effects on the nutrient contents of the chard plants, with some applications resulting in significant improvements in some nutrients compared to the control group. According to the Table 5; on the (N) content of chard plant was obtained 5,58 % the application liquid seaweed fertilizer only from the soil, on the (P) content of chard plant was obtained 0,43 % the application Fe only from the soil, on the (K) content of chard plant was obtained 3,03 % the application I. dose, on the (Ca) content of chard plant was obtained 1,33 % control group, on the (Mg) content of chard plant was obtained 0,86 % the application Fe only from the soil, on the (Fe) content of chard plant was obtained 656,33 mg.kg<sup>-1</sup> the application Fe only from the soil, on the (Cu) content of chard plant was obtained 11,73 mg.kg<sup>-1</sup> the application I. dose, on the (Mn) content of chard plant was obtained 50,19 mg.kg<sup>-1</sup> control group and on the (Zn) content of chard plant was obtained 204,70 mg.kg<sup>-1</sup> control group.

**Table 5.** The Effects of Different Applications on Some Macro-Micro Nutrient

 Element Contents of Chard Plant<sup>\*</sup>, <sup>+</sup>, <sup>++</sup>, <sup>+++</sup>

Applications	N	Р	K	Ca	Mg	Fe	Cu	Mn	Zn
	(%)	(%)	(%)	(%)	(%)	(mgkg <sup>-1</sup> )	(mgkg <sup>-1</sup> )	(mgkg <sup>-1</sup> )	(mgkg <sup>-1</sup> )
Control	3,81f	0,25f	2,05b	1,33a	0,86b	158,00e	8,71d	50,19a	204,70a
I. dose	4,45e	0,31d	3,03a	1,05c	0,76c	643,33b	11,73a	36,82c	174,20b
II. dose	5,05b	0,33b	1,95d	1,10c	0,79b	385,33d	10,67b	36,56c	130,46c
III. dose	4,92c	0,30e	2,00c	0,86e	0,68e	591,66c	9,25c	32,20e	113,50de
Fe only from the soil	5,06b	0,43a	1,85e	1,17b	0,86a	656,33a	9,37c	42,90b	126,33cd

liquid	seaweed	5,58a	0,34b	1,56f	0,93d	0,73d	595,66c	7,96e	37,30c	105,93e
fertilizer	only from									
the soil										
liquid	seaweed	4,58d	0,32c	1,78g	0,92d	0,74cd	658,66a	8,32de	34,47d	118,17cde
fertilizer	only from									
the leave										

\*: p<0.05, +: values are average of three replications, ++: each parameter evaluated individually, +++: mean square error

It is seen that II. dose (300 ml.100L<sup>-1</sup> water liquid seaweed fertilizer) application gives successful results in the macro-micronutrient content of the chard plant except for the K and Fe relatively. It's worth noting that there are some statistically significant differences (p<0.05) between the treatments and the control group or between different treatments, but the interpretation of the results requires more information on the experimental design, the cultivar, and other factors.

The combined use of seaweed and Fe as organic matter in the research had a positive effect on the macro-micronutrient content of the plant. The change in the amount of seaweed is effective in the content of macro and micro nutrients of the plant (Challen and Hemingway, 1965). Using bio-enrichment; obtainable crop and soil management applications will increase the micronutrient concentration in portions of the edible crop (Mayer et al., 2008). According to Marschner, 1995 and Makinde et al., 2011, adding organic matter to iron deficient soil; It will positively affect the amount of Fe that the plant will receive from the plant root zone. The application of Fe together with organic fertilizer, its uptake to the plant positively affected the nutrient content of the plant (Toprak, 2019).

The effects of different applications on some macro-micro nutrient element contents of research soil is given in Table 6.

Table 6 shows the effects of different applications on some macromicro nutrient element contents of research soil. The applications evaluated include a control group, I. dose, II. dose, III. dose, Fe only from the soil, liquid seaweed fertilizer only from the soil, and liquid seaweed fertilizer only from the leaf. The table also indicates statistical significance levels, average values of three replications, and individual parameter evaluations. The results suggest that the different applications had varying effects on the nutrient contents of the research soil, with some applications resulting in significant improvements in some nutrients compared to the control group

**Table 6.** The Effects of Different Applications on Some Macro-Micro Nutrient

 Element Contents of Research Soil<sup>\*</sup>, <sup>+</sup>, <sup>++</sup>, <sup>+++</sup>

Applications	Ν	Р	K	Ca	Mg	Fe	Cu	Mn	Zn
	(%)	(%)	(%)	(%)	(mgkg1)	(mgkg1)	(mgkg1)	(mgkg1)	(mgkg1)
Control	0,12ab	43,56d	592,66b	268,66b	726a	2,64ab	0,25b	68,58d	0,50a
I. dose	0,11ab	35,43f	609,33b	245,33d	728a	2,63ab	0,26b	69,05d	0,48b
II. dose	0,12a	50,12c	655,66a	330,66a	736a	2,57b	0,26b	71,04c	0,48b
III. dose	0,10c	51,40b	581,66b	258,01bc	745a	2,66a	0,27a	73,36b	0,51a
Fe only from the soil	0,11b	34,44g	531,33c	254,33cd	663b	2,37d	0,23c	65,24e	0,46c
liquid seaweed fertilizer only from the soil	0,13a	41,64e	546,33bc	251,66cd	738a	2,47c	0,23c	68,97d	0,46c
liquid seaweed fertilizer only from the leave	0,12ab	54,35a	607,33b	258,66bc	729a	2,58b	0,26ab	107,08a	0,50a

\*: p<0.05, +: values are average of three replications, ++: each parameter evaluated individually, +++: mean square error

According to the Table 6; on the (N) content of research soil was obtained 0,13 % the application liquid seaweed fertilizer only from the soil, on the (P) content of research soil was obtained 54,35 % the application liquid seaweed fertilizer only from the leave, on the (K) content of research soil was obtained 655,66 % the application II. dose, on the (Ca) content of research soil was obtained 330,66 % II. dose, on the (Mg) content of research soil was obtained 745 % the application III. dose, on the (Fe) content of research soil was obtained 2,66 mg.kg<sup>-1</sup> the application III. dose, on the (Cu) content of research soil was obtained 0,27 mg.kg<sup>-1</sup> the application III. dose, on the (Mn) content of research soil was obtained 107,08 mg.kg<sup>-1</sup> liquid seaweed fertilizer only from the leave and on the (Zn) content of research soil was obtained 0,51 mg.kg<sup>-1</sup> III. dose. The results show that the different applications had varying effects on the nutrient contents of the research soil.

It is seen that II. dose (300 ml.100L<sup>-1</sup> water liquid seaweed fertilizer) application gives successful results in the macro-micronutrient content of the research soil, except for the P and Mn relatively. It's worth noting that there are some statistically significant differences (p<0.05) between the treatments and the control group or between different treatments, but the interpretation of the results requires more information on the experimental design, the cultivar, and other factors.

The combined use of seaweed and Fe as organic matter in the research had a positive effect on the macro-micronutrient content of the research soil. According to Hong et al., 1995 contained in seaweed; trace elements, macro and micro nutrients, plant growth regulators and betaines the presence of nutrients in the soil is positively affected. According to Soyergin 2003, seaweed content affects the nutrient content of the soil. According to Blunden et al., 1992 Seaweeds which are a source of macro and micro nutrients for plants. Not taken up by the plant in the soil highest in micronutrients (like iron) rate they provide. And they stabilize them in the plant.

#### CONCLUSIONS

As a result of the research, it can be recommended that II. dose (1000 gda<sup>-1</sup> Fe calculation in plant root zone + 300 ml.100L<sup>-1</sup> water liquid seaweed fertilizer) application gives successful results the some agromorphological properties of chard plant and macro-micronutrient content of the chard plant and research soil, relatively.

Iron and seaweed fertilizers can be used together to provide a balanced and comprehensive nutrient supply to plants. Iron is an essential micronutrient that plants require for many physiological processes, including chlorophyll synthesis, respiration, and nitrogen fixation. On the other hand, seaweed fertilizer contains a variety of macronutrients, micronutrients, and plant hormones that can enhance plant growth and development, improve stress tolerance, and increase resistance to pests and diseases. When iron and seaweed fertilizers are used together, they can provide a complete and well-rounded nutrient supply to plants. The iron fertilizer can address any iron deficiencies in the soil, while the seaweed fertilizer can provide a range of other nutrients and growthpromoting substances that can boost plant health and vigor. Additionally, the use of seaweed fertilizer can help to increase the uptake and utilization of iron and other nutrients by plants, making them more efficient in their nutrient use. Overall, the use of iron and seaweed fertilizers together can help to promote healthy plant growth, improve plant performance, and increase crop yields. However, it's important to ensure that the application rates of each fertilizer are appropriate for the specific crop and growing conditions to avoid -over or under- fertilization. It's also important to note that the effectiveness of using iron and seaweed fertilizers together may vary depending on the specific types and formulations of the fertilizers, as well as the soil and environmental conditions in which they are used. Therefore, it's recommended to consult with a professional agronomist or horticulturist to determine the best fertilization strategy for specific crops and growing conditions.

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# **CHAPTER 14**

## EFFECTS OF ÇANAKKALE DOMESTIC SEWAGE SLUDGE ON SOME PLANT NUTRIENTS (N, K) AND HEAVY METAL CONTENT OF GRASS (*Lolium perenne* L.)

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#### **1. INTRODUCTION**

Sewage sludges are natural final products of microbial food chain in the process of waste water treatment. Adapting these wastes with the environment within the concept of the environmental consciousness is quite important with no doubt. Sewage sludge, also named as raw sludge, is a smelly, black liquid or half-solid material which forms by precipitation through certain processes in the waste water treatment plants, and is rich in organic matters and nutrition, containing around 0.25-12% solid. Raw sludge is named as "sewage sludge" when they made ready for usage. Sewage sludge is also named as "biosolid" in some countries (Bilgin et al., 2002).

The contents of sewage sludge change according to the treatment plant, and contain plant nutrient elements and heavy metals (Taşatar, 1997). Sewage sludge can be applied to soil, burned or used as terrestrial backfill material after some treatment processes. Inhibition of new pollution is essential in the treatment of sewage sludges. Common sewage sludge elimination methods are thermal methods, composting, sanitary landfilling, burning or dumping to sea. In recent years, sewage sludges have been used in agricultural lands, landscapes, seedling and sapling cultivation, grass and pasture lands, forests and lands to be remediated.

In the light of all this information, increasing amount of sewage sludge due to the increasing number of waste water treatment plants, and the pollution caused by the wastes in Türkiye bring up the topics of elimination of the sewage sludge to the agenda. The high amount of organic matter and plant nutrient elements in this material necessitates the research about the use of it in agriculture as fertilizer material. The quite low organic content of soil in Türkiye, bring the use of sewage sludge in agriculture into prominence.

Due to the plant nutrients and organic matter they contain, sewage sludge is successfully used in plant production in many countries and studies are carried out on these issues (BÆrug and Martinsen, 1977; Furre et al., 1984; Pakhnenko et al., 2009; Delibacak and Ongun, 2015, Delibacak and Ongun 2016a; Delibacak and Ongun 2016b; Belhaj et al., 2016; Ongun and Delibacak, 2017; Ongun and Delibacak, 2018a; Ongun and Delibacak, 2018b; Melo et al., 2018; Delibacak and Ongun, 2018, Kayıkçıoğlu and Delibacak, 2018; Kayıkçıoğlu et al., 2019, Tepecik et al., 2022).

Research should be conducted about the use of such wastes foremost in agriculture so that they would not lead to serious environmental and health problems in Türkiye as well as in the world.

For this purpose, in this study, the effect of sewage sludge of Çanakkale Municipality on the contents of N, K, Pb, Cd, Ni, Cr, Cu and Zn in English Grass (*Lolium perenne* L.) was investigated.

# 2. MATERIAL and METHODS

In this study, sewage sludge obtained from Çanakkale Municipality Advanced Wastewater Treatment Plant, soil from Çanakkale Onsekiz Mart University, Faculty of Agriculture Dardanos Campus, and perennial English Grass (*Lolium perenne* L.) were used as trial materials. The characteristics of trial material and methods of detection and analysis results of soil samples and sewage sludge are given in Table 1, 2 and 3.

Characteristics of Trial Material	Methods of Detection
Total N in soil and sludge	LECO C-N elemental analysis device (Kirsten, 1983)
Total P in soil and sludge	
Total K in soil and sludge	Wet Digestion, Perkin Elmer Optima 8000, ICP-OES device
Total and extractable heavy metals in soil and sludge	Wet Digestion-DTPA, Perkin Elmer Optima 8000, ICP-OES device
Organic matter in soil	Modified Walkley-Black method (Jackson, 1958).
Organic matter in sludge	DIN EN ISO 1172
Soil texture	(Bouyoucos, 1951).
Field capacity of soil and sludge	(Klute, 1986).
Moisture in soil and sludge	Determined due to the loss of mass of samples which became constant mass at 105 °C for soil and 70 °C for sludge (Allmaras and Gardner, 1956).
pH and EC in soil and sludge	(Richards, 1954; Grewelling and Peech, 1960).

Table 1. Characteristics of Trial Material and Methods of Detection

Attributes	Soil	Sewage Sludge
Organic Matter (%)	1.81	42.73
рН	8.01	6.39
EC (dS/m)	0.42	1.46
$CaCO_3(\%)$	11.86	
Texture	Loam (%51 Sand. %35 Loam. %14 Clay)	
Total N (%)	0.07	4.36
Total P (ppm)	932	19291
Total K (ppm)	1652	1728
Total Ca (ppm)	26798.9	33620.9
Total Mg (ppm)	8628.85	4688.77
Total Na (ppm)	660.82	2059.78
Moisture (%)	4.21	9.65
Field Capacity (%)	21.98	48.66

	Total (mg	g kg <sup>-1</sup> )	Extractable (mg kg <sup>-1</sup> )		
Metals	Sewage Sludge	Soil	Sewage Sludge	Soil	
Zn	729.34	42.44	366.0	1.202	
Cu	46.33	8.959	1.009	1.587	
Cd	0.621	0.228	0.120	0.026	
Cr	32.35	11.00	5.911	0.028	
Ni	24.41	49.55	5.251	0.996	
Pb	16.21	11.00	5.911	0.028	

Table 3. Total and Extractable Heavy Metals in Soil Samples and Sewage Sludge

The results of total heavy metal contents in soil and sewage sludge revealed that total Zn content of sewage sludge was 729.34 mg kg<sup>-1</sup>, while that of soil was 42.44 mg kg<sup>-1</sup> (Table 3). Zinc can be the most critical heavy metal in the sewage sludge of Çanakkale Municipality Domestic Wastewater Treatment Plant since its content was quite high. The maximum dose tried in the trial (Table 4) was calculated according to the Zn amount in sludge and the regulations (Anonymous, 2010).

Dosages	Sewage Sludge Amount (g)	Soil Amount (g)
<sup>1</sup> / <sub>2</sub> X Maximum	430	4000
Maximum	860	4000
2X Maximum	1720	4000
Control	0	4000

Propylene (plastic) pots of sizes 33.5 x 46 x 8.5 cm and 10.5 L volume were selected to grow grass plants taking into account of insolation,

harvesting ease and effective root depth of grass plants. Four kg of soil was added to the pots with the addition of different amounts of sewage sludge as given in Table 4. No chemical fertilizer was added to the pots. The English grass used in this experiment was hand harvested 1-2 cm above the surface 4 times on the dates 19.06.2017, 01.07.2017, 14.07.2017 and 30.07.2017 using scissors with steel razors which was cleaned in between the harvests. This way contamination of heavy metals to the plant samples was inhibited. Contents of N, K, Pb, Cd, Ni, Cr, Cu and Zn were determined in a total of 64 grass plants (4 replications x four sewage sludge doses x four harvests) according to the methods given in Table 1.

This study was designed according to randomized blocks design. The data obtained were analyzed according to randomized blocks design using ANOVA following by LSD in MINITAB 16.

## **3. RESULTS and DISCUSSION**

#### 3.1. Nitrogen

ANOVA table of changes in N content of grass according to harvesting time and dosages is given in Table 5.

The effects of harvesting times and sewage sludge together was statistically significant on the N contents of grass as seen in Table 5 (P<0,001). The N contents of grass according to harvesting times and dosages are given in Table 6.

Source of Variation	Degrees of freedom	Sum of squares	Mean squares	F value	P value
Dosage	3	33019433	11006478	109.51	0.000
Harvest	3	52102185	17368395	172.80	0.000
Dosage X Harvest	9	4009482	445498	4.43	0.000
Replicates	3	528389	176130	1.75	0.170
Error	45	4522896	100509		
Sum	63	94185385			

Table 5.	ANOVA	Table of	of N Conte	ent of Grass
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Table 6. N Content of Grass

N (mg kg <sup>-1</sup> )	Control	<sup>1</sup> / <sub>2</sub> Max. dose	Max. dose	2 x Max. dose	Average
1 <sup>st</sup> Harvest	44256	45254	45183	46055	45187
2 <sup>nd</sup> Harvest	45081	46309	46736	47022	46287
3 <sup>rd</sup> Harvest	45963	47438	48069	47477	47237
4 <sup>th</sup> Harvest	46083	47565	48461	47797	47476
Average	45346	46641	47112	47088	46547

The interaction of Harvest time X sewage sludge doses was found to be significant. These two factors were also found to be significantly effective at P $\leq$ 0.001. The content of N was the highest in the case of maximum dose application in the 4<sup>th</sup> harvest (P $\leq$ 0.001). Moreover, the least N content was obtained in the 1<sup>st</sup> harvest of control condition. The N contents of grass samples were between 44256-48461 mg kg<sup>-1</sup> (Table 6). This corresponds to 4.42-4.84% N content. Jones et al., 1991, reported that the limit values for N in grass was between 4% and 5%. Higher N content in grass can be related to the high organic content and high N content in the sewage sludge.

Nitrate (NO<sub>3</sub>) which is the extractable form of N can freely move in soil. That is why nitrate coming with sewage sludge can increase nitrate compounds in soil this may increase N in the plant. Another reason for

the increased N content may be the increased environmental temperature. Temperature is one of the main factors affecting the N mineralization in soil. Temperature can differ due to the color and amount of sewage sludge. The increase of N in grass in the process of time in the plants grown on the application of sewage sludge may lead the use of sewage sludge as N source for grass in the long term.

Topçuoğlu et al., 2003, reported an increase in N content of tomato plant with the application of sewage sludge compared to the control condition.

#### 3.2. Potassium

ANOVA table of changes in K content of grass according to harvesting time and dosages is given in Table 7.

Source of Variation	Degrees of freedom	Sum of squares	Mean squares	F value	P value
Dosage	3	125008.5	41669.5	175.15	0.000
Harvest	3	23506.7	7835.6	32.94	0.000
Dosage X Harvest	9	10617.5	1179.7	4.96	0.000
Replicates	3	1739.0	579.7	2.44	0.077
Error	45	10705.7	237.9		
Sum	63	171577.5			

**Table 7.** ANOVA Table of K Content of Grass

The effects of harvesting times and doses were statistically significant on the K contents of grass as seen in Table 7. The K contents of grass according to harvesting times and dosages are given in Table 8.

K (mg kg <sup>-1</sup> )	Control	<sup>1</sup> / <sub>2</sub> Max. dose	Max. dose	2 x Max. dose	Average
1 <sup>st</sup> Harvest	1589	1642	1662	1635	1632
2 <sup>nd</sup> Harvest	1594	1684	1714	1704	1674
3 <sup>rd</sup> Harvest	1599	1712	1717	1699	1682
4 <sup>th</sup> Harvest	1571	1701	1705	1701	1670
Average	1588	1685	1699	1685	1664

Table 8. K Content of Grass

"Harvest time X sewage sludge doses" interaction was found to be statistically significant. The two factors were also found to be significantly effective separately. The highest K content was found to be in the plant grown with the application of sewage sludge in the  $3^{rd}$  harvest time (P≤0,001). Moreover, the lowest K content was observed in the fourth harvest time in the control condition.

The content of K in grass plants was observed to be between 1571-1717 mg kg<sup>-1</sup> (Table 8). This corresponds to 0.15-0.17%. The lowest limit value for K in grass was reported to be 2.50%, while the highest to be 2.70% (Jones et al., 1991). The reason for the potassium level to be low is the high level of P in soil and sewage sludge (Ekleme and Sümer, 2018), and the low K content can be explained by high lime content in soil. Kacar and Katkat, 2010, reported that there was an antagonistic relationship between K, Mg and Ca; there was increase in the levels of Mg and Ca due to high lime and K level decreased.

Ok, 2012, was another researcher reporting the interaction of soil lime and application of sewage sludge on English grass and its effect of K level of the plant being statistically significant.

## 3.3. Lead

The effects of harvesting times and dosages on Pb contents of grass are given in the ANOVA table in Table 9.

Source of Variation	Degrees of freedom	Sum of squares	Mean squares	F value	P value
Dosage	3	0.0041406	0.0013802	4.92	0.005
Harvest	3	0.0050306	0.0016769	5.98	0.002
Dosage X Harvest	9	0.0058808	0.0006534	2.33	0.030
Replicates	3	0.0003126	0.0001042	0.37	0.774
Error	45	0.0126124	0.0002803		
Sum	63	0.0279769			

Table 9. ANOVA Table of Pb Content of Grass

The effects of harvesting times and doses were not statistically significant on the Pb contents of grass as seen in Table 9. The Pb contents of grass according to harvesting times and dosages are given in Table 10.

Table 10. Pb Content of Grass

Pb (mg kg <sup>-1</sup> )	Control	<sup>1</sup> / <sub>2</sub> Max. dose	Max. dose	2 x Max. dose	Average
1 <sup>st</sup> Harvest	0.14	0.16	0.18	0.13	0.15
2 <sup>nd</sup> Harvest	0.16	0.16	0.17	0.17	0.17
3 <sup>rd</sup> Harvest	0.16	0.16	0.18	0.17	0.17
4 <sup>th</sup> Harvest	0.16	0.18	0.18	0.19	0.18
Average	0.16	0.17	0.18	0.16	0.17

"Harvest time X sewage sludge doses" interaction was found not to be statistically significant. The two factors were found to be significantly effective separately at P $\leq$ 0.005 level. The highest Pb content was found to be in the plant grown with "maximum X 2" dose of sewage sludge

in the 4<sup>th</sup> harvest time (P $\leq$ 0,001). The lowest Pb was detected in control plant in the 4<sup>th</sup> harvest time.

The Pb content of grass samples were found to be between 0.13-0.19 ppm (Table 10). The highest Pb content level is 2 ppm (FAO-WHO, 2003). The reason for the low Pb content in grass was the low content in soil and sewage sludge.

Türkmen et al., 2001, investigated the extractability of some heavy metals from soil by barley in greenhouse conditions, and found that heavy metals except Cu and Pb were in the allowed limits.

## 3.4. Cadmium

The effects of harvesting times and dosages on Cd contents of grass are given in Table 11.

Source of Variation	Degrees of freedom	Sum of squares	Mean squares	F value	P value
Dosage	3	0.0006404	0.0002135	34.28	0.000
Harvest	3	0.0015542	0.0005181	83.20	0.000
Dosage X Harvest	9	0.0005441	0.0000605	9.71	0.000
Replicates	3	0.0000240	0.0000080	1.29	0.290
Error	45	0.0002802	0.0000062		
Sum	63	0.0030430			

Table 11. ANOVA Table of Cd Content of Grass

The effects of harvesting times and sewage sludge doses were statistically significant on the Cd contents of grass as seen in Table 11. The Cd contents of grass according to harvesting times and dosages are given in Table 12.

Cd (mg kg <sup>-1</sup> )	Control	<sup>1</sup> / <sub>2</sub> Max. dose	Max. dose	2 x Max. dose	Average
1 <sup>st</sup> Harvest	0.01	0.02	0.02	0.02	0.02
2 <sup>nd</sup> Harvest	0.02	0.03	0.04	0.03	0.03
3 <sup>rd</sup> Harvest	0.02	0.03	0.03	0.03	0.03
4 <sup>th</sup> Harvest	0.02	0.02	0.03	0.02	0.02
Average	0.02	0.03	0.03	0.03	0.02

Table 12.	Cd Content of Gras	s

The interaction of "Harvest time X sewage sludge" doses was found to be significant. These two factors were also found to be significantly effective at P $\leq$ 0.01. The content of Cd was the highest in the case of maximum dose application in the 2<sup>nd</sup> harvest (P $\leq$ 0.01). Moreover, the lowest Cd content was found in the first harvest time in control condition. The Cd content in the grass samples differed in between 0.01-0.04 ppm (Table 12). The highest limit value for Cd in grass was 0.5 ppm (FAO-WHO, 2003). The reason for low Cd content in grass was low Cd in soil and sewage sludge. Türkmen, 2004, observed increased total and extractable soil Cd by barley in the case of sewage sludge application.

## 3.5. Nickel

The effects of harvesting times and dosages on Ni contents of grass are given in Table 13.

Source of Variation	Degrees of freedom	Sum of squares	Mean squares	F value	P value
Dosage	3	0.60262	0.20087	17.30	0.000
Harvest	3	4.60181	1.53394	132.11	0.000
Dosage X Harvest	9	0.31823	0.03536	3.05	0.006
Replicates	3	0.03258	0.01086	0.94	0.431
Error	45	0.52249	0.01161		
Sum	63	6.07773			

 Table 13. ANOVA Table of Ni Content of Grass

The effects of harvesting times and sewage sludge doses were statistically significant on the Ni contents of grass as seen in Table 13. The Ni contents of grass according to harvesting times and dosages are given in Table 14.

Ni (mg kg <sup>-1</sup> )	Control	<sup>1</sup> / <sub>2</sub> Max. dose	Max. dose	2 x Max. dose	Average
1 <sup>st</sup> Harvest	0.57	0.71	0.72	0.93	0.73
2 <sup>nd</sup> Harvest	0.90	0.93	0.89	0.88	0.90
3 <sup>rd</sup> Harvest	1.15	1.22	1.26	1.51	1.29
4 <sup>th</sup> Harvest	1.18	1.30	1.49	1.56	1.38
Average	0.95	1.04	1.09	1.22	1.07

#### Table 14. Ni Content of Grass

The interaction of "Harvest time X sewage sludge" doses was found to be significant. These two factors were also found to be significantly effective at P $\leq$ 0.001. The content of Ni element was the highest in the case of maximum dose application in the 4<sup>th</sup> harvest time (P $\leq$ 0.001). Moreover, the lowest Ni content was found in the first harvest time in control condition.

The Ni content in the grass samples differed in between 0.57-1.56 ppm (Table 14). The highest limit value for Cd in grass was 5 ppm (FAO-WHO, 2003). The reason for low extractable Ni content in grass was low Cd in soil and sewage sludge.

Küçükhemek et al., 2006, reported that Ni in grass plants was increased upon application of sewage sludge. Waste sludge is typically a mixture of organic and inorganic substances obtained from domestic and industrial sources. Nickel is one of the heavy metals found in waste sludge, making the nickel content of waste sludge an important consideration. The nickel content of waste sludge can affect soil quality. High levels of nickel can disrupt soil structure, reduce biological activity, and create an unsuitable environment for plant growth. It is important to note that the specific effects of waste sludge and nickel can vary depending on factors such as waste sludge composition, application rates, soil properties, and plant species. Adequate monitoring and management practices are necessary to ensure the safe handling and disposal of waste sludge containing nickel (Sümer et al., 2013; Adiloğlu et al., 2017; Adiloğlu and Duban, 2022; Gürgan et al., 2022).

## 3.6. Chromium

The effects of harvesting times and dosages on Cr contents of grass are given in ANOVA Table in Table 15.

Source of Variation	Degrees of freedom	Sum of squares	Mean squares	F value	P value
Dosage	3	0.101017	0.033672	17.47	0.000
Harvest	3	1.054255	0.351418	182.33	0.000
Dosage X Harvest	9	0.213851	0.023761	12.33	0.000
Replicates	3	0.009503	0.003168	1.64	0.193
Error	45	0.086731	0.001927		
Sum	63	1.465357			

 Table 15. ANOVA Table of Cr Content of Grass

The effects of harvesting times and sewage sludge doses were statistically significant on the Cr contents of grass as seen in Table 15. The Cr contents of grass according to harvesting times and dosages are given in Table 16.

Cr (mg kg <sup>-1</sup> )	Control	<sup>1</sup> / <sub>2</sub> Max. dose	Max. dose	2 x Max. dose	Average
1 <sup>st</sup> Harvest	0.27	0.32	0.33	0.37	0.32
2 <sup>nd</sup> Harvest	0.34	0.64	0.62	0.58	0.55
3 <sup>rd</sup> Harvest	0.66	0.74	0.67	0.60	0.67
4 <sup>th</sup> Harvest	0.61	0.63	0.54	0.56	0.58
Average	0.47	0.58	0.54	053	0.53

Table 16. Cr Content of Grass

The interaction of "Harvest time X sewage sludge" doses was found to be significant. These two factors were also found to be significantly effective at P $\leq$ 0.001. The content of Cr was the highest in the case of "maximum/2 dose" application in the 3<sup>rd</sup> harvest (P $\leq$ 0.001). Moreover, the lowest Cr content was found in the first harvest time in control condition.

The Cr content in the grass samples differed in between 0.27-0.74 ppm (Table 16). The reason for low Cr content in grass was low extractable Cr in soil and sewage sludge.

Küçükhemek et al., 2006, reported that application of sewage sludge increased the Cr content in grass compared to control and Cr was the element with the highest increase among the other elements. High concentrations of chromium in waste sludge can have toxic effects on plants, animals, and microorganisms. Certain forms of chromium, such as hexavalent chromium (Cr(VI)), are known to be highly toxic and can pose serious health risks to living organisms. The mobility of chromium in waste sludge depends on its chemical form, soil characteristics, and environmental conditions. Chromium can undergo leaching, adsorption, or transformation processes in soil, affecting its potential for migration and environmental contamination (Adiloğlu 2016; Adiloğlu et al., 2021).

### 3.7. Copper

The effects of harvesting times and dosages on Cu contents of grass are given in Table 17.

Source of Variation	Degrees of freedom	Sum of squares	Mean squares	F value	P value
Dosage	3	51.7251	17.2417	601.92	0.000
Harvest	3	4.2879	1.4293	49.90	0.000
Dosage X Harvest	9	2.2406	0.2490	8.69	0.000
Replicates	3	0.0049	0.0016	0.06	0.982
Error	45	1.2890	0.0286		
Sum	63	59.5475			

Table 17. ANOVA Table of Cu Content of Grass

The effects of harvesting times and sewage sludge doses were statistically significant on the Cu contents of grass as seen in Table 17. The Cu contents of grass according to harvesting times and dosages are given in Table 18.

Table 18. Cu Content of Grass

Cu (mg kg <sup>-1</sup> )	Control	<sup>1</sup> / <sub>2</sub> Max. dose	Max. dose	2 x Max. dose	Average
1 <sup>st</sup> Harvest	3.65	4.31	5.26	5.25	4.62
2 <sup>nd</sup> Harvest	3.57	5.16	5.81	6.36	5.23
3 <sup>rd</sup> Harvest	3.68	5.19	6.09	6.01	5.24
4 <sup>th</sup> Harvest	3.61	5.23	5.94	5.88	5.16
Average	3.63	4.97	5.78	5.87	5.06

The interaction of "Harvest time X sewage sludge" doses was found to be significant. These two factors were also found to be significantly effective at P≤0.001. The content of Cu was the highest in the case of

"maximum X 2 dose" application in the  $2^{nd}$  harvest (P $\leq 0.001$ ). Moreover, the lowest Cu content was found in the  $2^{nd}$  harvest time in control condition.

The Cu content in the grass samples differed in between 3.57-6.36 ppm (Table 18). The lowest limit value for Cu in grass was 4-5ppm while the highest 8ppm (Jones et al., 1991). The reason for sufficient Cu content in grass was high Cu in soil and sewage sludge, and very high soil organic matter and lime (Kacar and Katkat, 2010).

Bozkurt et al. (2000), reported that sewage sludge doses increased the Cu levels in soil.

# 3.8. Zinc

The effects of harvesting times and dosages on Zn contents of grass are given in Table 19.

Source of Variation	Degrees of freedom	Sum of squares	Mean squares	F value	P value
Dosage	3	1728.31	576.10	297.27	0.000
Harvest	3	652.90	217.63	112.30	0.000
Dosage X Harvest	9	166.59	18.51	9.55	0.000
Replicates	3	14.10	4.70	2.43	0.078
Error	45	87.21	1.94		
Sum	63	2649.11			

Table 19. ANOVA Table of Zn Content of Grass

The effect of harvesting times and dosages significantly affected the Zn contents of grass plants (Table 19). Zn contents of samples according to harvesting times and dosages are given in Table 20.

Zn (mg kg <sup>-1</sup> )	Control	<sup>1</sup> / <sub>2</sub> Max. dose	Max. dose	2 x Max. dose	Average
1 <sup>st</sup> Harvest	25.35	23.98	35.96	36.74	30.51
2 <sup>nd</sup> Harvest	25.76	30.56	38.71	40.64	33.91
3 <sup>rd</sup> Harvest	31.25	35.12	44.16	40.88	37.85
4 <sup>th</sup> Harvest	31.54	37.42	44.72	39.84	38.38
Average	28.48	31.77	40.89	39.53	35.16

Table 20. Zn Content of Gras
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The interaction of "Harvest time X sewage sludge" doses was found to be significant. These two factors were also found to be significantly effective at P $\leq$ 0.001. The content of Zn was the highest in the case of "maximum dose" application in the 4<sup>th</sup> harvest (P $\leq$ 0.001). Moreover, the lowest Zn content was found in the second harvest time in control condition under "maximum/2 dose".

The Zn content in the grass samples differed in between 23.98-44.72 ppm (Table 20). The highest limit value for Zn in grass was 10-13 ppm, while the highest was 20 ppm (Jones et al., 1991). The reason for high Zn content in grass was high Zn in soil and sewage sludge and high temperature (Kacar and Katkat, 2010). There is an antagonistic relationship between phosphorus and Zn. The low uptake of phosphorus (Ekleme and Sümer, 2018) increased the Zn transfer and Zn content in plant. Moreover, the quality content of chemicals used in the flocking of sewage sludge might have increased the Zn value.

Küçükhemek et al., 2006, detected the Zn content in grass by sewage sludge applications as 18.6 mg/kg and 56.4 mg/kg as maximum and minimum. Micro-plant nutrients play a vital role in the growth and development of plants. When it comes to waste sludge, which is a mixture of organic and inorganic materials, the presence of micro-plant

nutrients can have both positive and negative implications. Waste sludge can contain micro-plant nutrients such as iron (Fe), manganese (Mn), copper (Cu), zinc (Zn), and molybdenum (Mo). When properly treated and managed, waste sludge can serve as a potential source of these nutrients, enriching the soil and supporting plant growth (Adiloğlu, 2007; Çetinkaya et al., 2010; Adiloğlu, 2012).

### 4. CONCLUSION

Sewage sludge amount is increasing in Çanakkale year by year and causes local pollution. The content of Çanakkale domestic wastewater sewage sludge was detected by detailed physically and chemically analyses. In this study, Çanakkale domestic sewage sludge was analyzed, and aimed to investigate the effects of it on plant nutrient elements and heavy metals and to provide the controlled use of it as organic fertilizer on English grass (*Lolium perenne* L.) after several processes.

In this study, Cd, Ni, Cr, Cu and Zn contents increased in the plants grown in soils with applied sewage sludge compared to the control condition, and this increase was the highest for Cd, Cr and Ni. Among the plant nutrient elements N and K increased in plants grown with the application of sewage sludge compared to the control.

The contents of Cu and Zn of grass applied with sewage sludge increased compared to control but were in sufficient level, however, the contents of Cd, Pb, Cr and Ni were quite lower than the toxic limit levels. The contents of N and P nutrient elements in grass plant with the application of sewage sludge were higher than the control and in sufficient levels and the content of K was lower.

The order of heavy metals in grass grown with the application of sewage sludge compared to the control was as follow: Zn> Cu> Ni> Cr> Pb> Cd. Among the nutrient elements, N>K was observed in the grass grown with application of sewage sludge compared to the control.

The results of our experiment which aimed to determine whether sewage sludge could be used as organic fertilizer in plant cultivation revealed that the plant nutrient elements and heavy metals (except Pb) increased in the green parts of grass compared to the control in conjunction with the amount of sewage sludge.

Sewage sludge can be suggested to be used as fertilizer for grass since the maximum dose of sewage sludge was inside the acceptable limits regulations for limits to be used in agricultural lands. Sewage sludge can be evaluated as N source for plants in the long term. The deficiency of Cu in grass was eliminated according to harvesting time and dosages.

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# **CHAPTER 15**

# PHYTOREMEDIATION TECHNOLOGIES: FROM LAB SCALE TO FOREST SCALE JOURNEY

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#### INTRODUCTION

Soil pollution caused by heavy metals is a serious problem which takes much attention recently. Studies on heavy metal pollution in soil focus on sources and fate of heavy metals, effects on public health and environment, about polluted regions and their analyses, remediation methods, techniques and risk assessment. The most significant effect of soil pollution on environmental health is the accumulation of pollutants in plants and transfer to human body via direct consumption of those plants or indirect transfer by consumption of the animals fed by them. Besides, the results of skin contact of polluted soil, swallowing of soil colloids and inhaling heavy metals such as mercury vaporizing during drying should be determined. Heavy metals reaching to toxic levels when contaminate soil affect soil productivity, biodiversity, microbial activity and environmental factors. Accumulation of micro elements in toxic levels in soil, water and atmosphere negatively affect health of organisms. This negative effect of heavy metals in soil cause serious problems. Researches have shown that organic and inorganic pollutants cause severe health problems and environmental pollution. The heavy metals that can accumulate in high levels are really harmful pollutants due to their persistency in the environment. This persistency causes toxic, genotoxic, teratogenic and mutagenic effects (Dixit et al. 2015). Moreover, they cause even in low concentrations (Yadav, 2010).

# **Remediation classifications**

Remediation can be classified as traditional and biological

Traditional class includes follows:

- Dredging
- Extracting soil vapor
- Solidification and stabilization
- Soil leaching
- > Air surfacing (air injection and distribution)
- Pumping and treatment
- Chemical oxidation
- ➢ Burning

Bioremediation methods are as follows:

- Bioremediation (Using microorganisms)
- Phytoremediation

# **Traditional remediation methods**

# Dredging

This technique involves the transportation of polluted soil or other materials from one region to another. The most frequently used method of soil remediation is dredging. It can be as easy as digging and transportation of soil away or can involve more complicated processes such as aeration depending n the type of pollutant. Dredging is physically removing the precipitated pollutants from fresh water or sea/ocean in order to decrease human and environmental health risk. Soil vapor extraction (SVE) benefits from pumps to make an air flow in air to clean pollutants distributed among all soil phases. The extracted

vapor is later processed and released to atmosphere or injected back to underground if allowed. SVE is more frequently used when the soil pollution very intense, or when dredging cannot be applied due to presence of some physical barriers (e.g., trees, buildings). Polluted soil and sediment water can be simultaneously by SVE technique.

#### Solidification or stabilization

It implicates blending of polluted medium with a certain bounding compound in order to make harmful pollutants less soluble, less mobile and less harmful forms. At present this technique is applied to treat wastes coming from wastewater effluents from tannery metal processing industries. The ingredients of such wastes are usually made up of heavy metals, organic compounds and salts.

# Soil leaching

This process expresses *ex situ* or *in situ* methods that use physical and/or chemical protocols to remove metal pollutants. Soil leaching is usually suitable when soil is granule soils with lower clay content polluted with inorganics. Soil leaching is one of the alternatives to long lasting processes to clean metals from soil. If the pollutants are organic, they must be removed, then some solvents or surface-active agents can be used as washing agent.

#### **Air Surfacing**

Air in injected to the polluted field in this technique. The injected air helps to leach (bubbling) the pollutant deep inside the unsaturated region where vapor extraction system is generally applied in relation with air surfacing to eliminate the vapor pollution. Air surfacing is an *in-situ* remediation used to reduce the petroleum products molecular components (benzene, ethyl benzene, toluene and xylene) lighter than gas. Air surfacing is a time efficient and cheap technique for the cleaning up volatile and/or biodegradable pollutants.

# **Pumping and treatment**

This technique involves pumping polluted underground water from underground and remediation of it before returning to surface. Pumping and treatment offers an important option with rapid reaction time, high compatibility and reliability. It is frequently used for the remediation of underground water and water restoration due to the excellence in the control of remediation and transfer of pollutants.

# **Chemical oxidation**

This technique aims to mineralize or at least convert CO<sub>2</sub>, water (H<sub>2</sub>O) and inorganic pollutants into unhazardous biodegradable compounds. Chemical oxidation process is used for a very long time in wastewater industry to treat petroleum hydrocarbons, carbonous compounds like chlorinated solvents and many other pollutants.

# Burning

It is a heat treatment (with high temperature) to get rid of harmful wastes. Burning of wastes converts the waste into ash, smoke and heat (Ikonomou et al., 2002). Ash is usually composed of inorganic compounds o the waste and can be in a form of particle or solid soil

carried by smoke gas. Smoke gases should be cleaned to get rid of gas and particulate pollutants before released to atmosphere.

### Bioremediation

Use of microorganisms to clean pollutants from air, water and soil. It is carried out by eliminating polluted material in situ or carrying out the polluted material away to eliminate somewhere else. Different microorganisms (bacteria, fungi, alga ad plats) are used to remediate pollutants and specific species of them are usually selected for this process.

# **Microbial diversity**

This expresses the presence of microbial organisms which are various bacterial and fungal species in a polluted area.

Macrobenthos diversity is the combination of aquatic plants and animals having high potential for lowering biochemical oxygen demand, chemical oxygen demand, ammonium, nitrate and turbidity.

#### PHYTOREMEDIATION

In recent years environmentally friendly, cost-effective biological technologies have been preferred to eliminate, eradicate or remediate toxic pollutants from contaminated areas. Using plants to clean the environment is an efficient technology for remediation of polluted soils and waters (Clemens et al., 2002; Schulze et al., 2005; Adiloğlu 2021; Yer Çelik et al., 2021).

Most of the physicochemical technologies for soil treatment completely destroy biological activity and convert the soil medium into inappropriate environment for plant growth. On the other hand, phytoremediation preserves physical and biological structure of the soil. However, in order to use phytoremediation efficiently, molecules, biochemical processes and physiological processes that characterize the accumulation of heavy metals in plants (Khan et al., 2000).

Phytoremediation is a bioremediation method that uses plants and associated microorganisms to remove, transfer, stabilize or eliminate the pollutants in air, soil, sediment waste waters and underground waters. Plants can show the presence of toxic organic or inorganic compounds, can prevent them, or can accumulate them. Therefore, they significantly contribute to the trail of chemicals and are applicable to clean the undesired compounds from the biosphere. It does not allow the use of plants in highly polluted areas, but make use of plants in lower levels of pollution (Hamutoğlu et al., 2012). The success of phytoremediation depends on the 3 criteria of plants to be used:

1) These plants should absorb and consume the heavy metals by its roots,

2) These plants should transfer and accumulate in the shoots and shoots should be processed after harvesting,

These plants should have mechanisms to protect themselves from the toxic effects of high concentrations of heavy metals (Yer Çelik et al., 2021). The first group of microorganisms negatively affected from

many environmental pollutions and especially from heavy metal pollution is the plants. Plants have the ability to accumulate metals some can accumulate 100 times more than others without any negative effect. Plants with the ability to tolerate and accumulate higher amounts of heavy metals are known as hyper accumulators. Contaminated soil has become a global problem for agriculture, forest and environmental scientists. Researches on use of hyper-accumulators to remediation of polluted areas have been increasing in recent years, due to the capacity of hyper accumulators to take up heavy metals from soil and accumulate them in their above ground biomass. Hyper-accumulators are plants that can accumulate more than 0.1% Ni, Co, Cu or 1% Zn and Mn in leaves based on dry weight independent of metal concentration in soil (Raskin et al., 1994; Adiloğlu et al., 2021). Phytoremediation method has been applied in recent years densely and successfully to remediate soils with heavy metal pollution to make them healthier. The mentioned method is both environmentally friendly and natural, and also cheaper than the 2020). other chemical and physical methods (Carşambalı, Phytoremediation technology includes many different technologies. They can be classified as phytostabilization, phytoextraction, phytodegradation, phytovolatilization, rhizodegradation, rhizofiltration, hydraulic control, vegetative cover systems and riparian buffer strips. Each of these technologies can be used for different aims.

### Phytoextraction

Phytoextraction makes use of plants or algae to remediate the pollutants from soil, water and sediment. In this method plants take up pollutants

from the soil by their own root systems and either keep them in their roots or transfer to their stems or leaves. Plants continue to take up pollutants until they are harvested. If a certain cleaning level is not achieved after the harvest, growth/harvest cycle should be carried out for a few products. The main positive aspect of this technique is that it is environmentally friendly. This does not cause lower soil quality. Other advantage of it is being cheaper than other methods. This process takes longer than any traditional soil remediation methods since it is controlled by plants. At the end of harvesting of phytoextraction process, plants can be recycled as biological metal mine via drying, composting or burning and obtaining the ash (Memon et al., 2000).

# Phytostabilization

Plants arrest the chemical contaminants in water and soil medium. Pollutants are uptake by roots and deposited in the rhizosphere. This inhibits the entrance of pollutant to the food web. The maintenance of soil contents and also enrichment of soil contents are the advantages of this method. The significant disadvantage is the leaching of pollutants by different factors to reach to the underground water since the pollutants stay in the field for a long time (Henry, 2000).

# Phytovolatilization

Water containing organic pollutants is uptake by plants in this mechanism, and pollutants are releases to the air via the leaves. Pollutants can be converted during the transfer of water to the leaves via the vascular bundles, and then evaporate to the air surrounding the

plant, or can be volatilized. The roots' going down deep for the remediation of underground water is important for the applicability of this method. The most important positive aspect of this technology is the conversion of toxic compounds to different forms and decrease or complete elimination of negative aspects. The negative aspect of it is the return of toxic compounds back to the atmosphere via transpiration (EPA, 2000).

### Phytodegradation

Organic pollutants are metabolized and destructed in plant tissues in this mechanism. The small pollutant molecules can later be used as metabolites when they grow, so they can be part of plant tissues. The main mechanism is the uptake of pollutant by plants, storage in plants and being metabolized. This process takes place in rhizosphere region of plants. Plant species, solubility, soil characteristics, and half-life of organic compounds are important in their accumulation. Phytodegradation, namely vegetative decomposition can be used for remediation of soil, sediment, mud, and underground water (Pivetz, 2001). Through this method various pollutants such as oil, solvents in underground water, or organic aromatic compounds in air (Newman and Reynolds, 2004).

#### Rhizofiltration

This method uses plants which live both in water and land to absorb metals from polluted water (surface or underground), concentrate and precipitate them. Söğüt et al. (2002) express rhizofiltration as adsorption of contaminant to the root surface. The pollutant can be absorbed and transported by the plant. The main criteria are limiting the mobility of pollutant in the plant body. Pollutants can later be taken away from the plants with different methods. This method can be applied to waste waters, ground or underground waters. The usability of both terrestrial and aquatic plants is a great advantage. Moreover, the system can be applied in both natural or artificial environments. The pollutant can be remediated *in situ* or *ex situ* (Aybar et al., 2015).

# Rhizodegradation

Plants release general nutrients to soil microorganisms through their roots in this mechanism. This way microorganisms increase biological decomposition. For example, plant roots release carbohydrates (e.g., sugars, alcohols, organic acids) for soil microbiota. Such compounds upgrade microbial reproduction and functions, and moreover plays as chemotactic signal for microorganisms. According to Söğüt et al. (2002), microorganisms are the main architects of this technology. Remediation method by microorganisms in rhizosphere region is called rhizodegradation. It is the decomposition of organic pollutants by the activities of soil microorganisms. Fatty acids, sterols, amino acids, nucleotides, sugars, organic acids, growth factors, flavanone and enzymes play role in the activation of the method since they affect the microbial activity in under soil. The significant benefit of the method is the remediation of pollutants in the natural environment. However, they can be transferred to ecological areas when present in low concentrations (Aybar, Bilgin and Sağlam, 2015).

# **Hydraulic control**

In this mechanism, trees treat the underground water. It is also known as "phytohydraulic control" or "hydraulic plume control". The aim of this system is to transfer of polluting factors or to inhibit or to control the accumulation of them in underground waters by the use of plants. This system is applicable for the cleaning up the ground or underground waters. The most significant disadvantage is the changing water need of plants depending on the climate and season. The most significant advantage is large area of effect without maintenance of any artificial system, due to the spread of roots to a larger area than pumps. Willow and *Eucalyptus* species are shown to be effective in this method. Water usage capabilities of trees such as the transpiration of a single willow tree being equal to 5000 gallons of water, or uptake of 100-200 liters of water by a five years old Populous tree make them important to be used for the aim of this method (Pivetz, 2001).

#### Vegetative covering systems

It is the retaining of pollutants from fertile layers of soil naturally by perennial plants. Vegetative cover treats or inhibits the loss of water by evaporation. Plants minimize the water loss in the soil and maximizes the water retention. The mobility of pollutants as well as their washing out is inhibited. In phytoremediation, plants minimize the filtration of water and decrease the effects of pollutants by removal from the medium down to the lower layers (EPA, 2000).

# **Riparian buffer strips**

Planting accurate plants in the sides of rivers along the strip following the flow is called riparian buffer strip. In this technique the aim is to remediate the pollutants melting into the above and underground waters. Inhibiting the mixing of pollutants to underground water and spreading to the environment are provided with this technique. This technique also controls the system erosion and sediment is reduced. Research in Canada showed the reduction of herbicide flow by 42-70%, soil erosion by around 90%. The concentrations of nitrogen, phosphorus, sediment, fecal coliform and pesticides can be reduced by 67-96%, 27-97%, 71-91%, 70-74%, and 8-100%, respectively (Gabor et al., 2001).

### Advantages

- Phytoremediation is cheaper than the old "pumping and treatment" of polluted water.
- > Phytoremediation is cheaper than dredging of polluted soil.
- Phytoremediation does not require of any maintenance after setup.
- > Phytoremediation is also aesthetic since plants are used.
- After the plantation of trees, wild life can develop in places that were once unavailable.
- Solar energy can be used for remediation technology.

#### Disadvantages

Phytoremediation is limited in the areas with low pollutant concentration.

- Phytoremediation is limited with the depth of roots in the polluted areas.
- > Toxic compounds can negatively affect the food chain.
- The burnt plat material can release toxic chemicals which can pollute air.

#### Some forest hyper-accumulators

The plants to be used in phytoremediation should can live under high heavy metal concentration, have a strong root system, can accumulate high concentration of metals in harvested parts, have a high growth rate, and produce high biomass in the field. Hyperaccumulators are plants which can grow on some elements with solubilities in soil that can harm many other plants (Reeves and Baker, 2000).

Around 400 hyperaccumulator plans have been reported and they usually belong to Asteraceae, Lamiaceae, Brassicaceae, Fabaceae, Caryophyllaceae, Cyperaceae, Cunouniaceae, Flacourtiaceae, Poaceae, Violaceae, and Euphobiaceae. Brassicaceae families (EPA, 2004).

A recent study reported *Vaccinium myrtillus Aesculus hippocastanum* L., from Ericaceae family which adapted to moderate climates. There are many species grown in cool and uplands of its native land Northern Hemisphere. Generally, there are wild species living in Northern Europe, Rocky Mountains in USA and high altitudes heathery and forests in Black Sea region of Turkiye.

Some examples of forest accumulators are as follows: *Silene vulgaris* is a perennial herbaceous plant belonging to *Caryophyllaceae* family

and lives in fields and hillsides. Thlaspi caerulescens can be used to clean polluted areas, there are related research even from 300 years ago. Thlaspi caerulescens L. and Viola calaminaria L. were shown to accumulate high levels of metals inn late 19th century. Solanum nigrum L, is a forest plant belonging to Solanaceae. Hypericum species are perennial plants (Robson and Adams, 1968). Hypericum amblysepalum live in Europe and dry regions of Northern America. Turkiye is a center for *Hypericum* species: 43 of the present 89 species live in Turkiye. Narrowleaf plantain (Plantago lanceolata) is a member of Plantaginaceae family. It is a perennial plant in rosette shape. Medicinal plants have been reported to accumulate toxic heavy metals in large amounts. Moreover, heavy metals accumulated in these plants were reported not to affect secondary metabolite contents of these plants (Yaldız and Şekeroğlu, 2012; Özbek 2015). Poplar taxon are among the plants preferred for phytoremediation. Use of poplars for bioremediation is an efficient approach due to their long and rich root system. Poplars are the most frequently used taxon in phytoextraction, phytostabilization, phytodegradation, and phytovolatilization (Vanlı, 2007; Aybar et al., 2015).

Poplar trees (Populus spp.) have been extensively studied for their phytoremediation potential in various environmental contexts, including heavy metal pollution. These fast-growing trees are known for their ability to tolerate and accumulate high levels of heavy metals in their tissues. Here are some key points regarding the relationship between poplar trees and phytoremediation: > Phytoremediation Capability: Poplar trees have shown promise in remediation efforts due to their ability to take up, accumulate, and tolerate heavy metals, such as cadmium, zinc, copper, and lead. They can absorb these contaminants from the soil through their root systems.

➤ Hyperaccumulator Properties: Certain poplar species, such as Populus nigra, Populus deltoides, and their hybrids, exhibit hyperaccumulator characteristics. Hyperaccumulator plants can accumulate higher concentrations of heavy metals compared to other plant species, making them effective for phytoremediation purposes.

➤ Rhizofiltration: Poplar trees can also aid in phytoremediation through a process called rhizofiltration. This involves the uptake and filtration of contaminants by the roots, which can help improve water quality by reducing the levels of pollutants, including heavy metals.

> Transpiration and Volatilization: Poplar trees can transpire large amounts of water through their leaves, a process that can facilitate the movement of contaminants from the soil to the atmosphere. This process, known as phytovolatilization, can be beneficial in reducing the concentration of certain volatile contaminants.

➤ Field Studies and Application: Poplar-based phytoremediation has been implemented in various field studies and pilot-scale projects to address heavy metal-contaminated soils, mine tailings, and industrial sites. These studies have provided valuable insights into the effectiveness of poplar trees in removing heavy metals from contaminated environments.

It's important to note that the effectiveness of poplar trees for phytoremediation can depend on various factors, including the specific contaminants present, soil conditions, and the species or hybrid used. Ongoing research continues to explore the potential of poplar trees and optimize their use in phytoremediation efforts (Raskin et al.,1997; Adiloğlu, 2018). Poplar species are also important due to their high growth rate and administration with short rotation. They usually accumulate cadmium in their roots. Some researchers reported o cadmium accumulated in fruits of the plant. Cadmium accumulated in the plant in the following order: roots>shoot>leaves>fruit>seeds (Hall, 2002; Benavides et al., 2005). Poplar and willow species were reported to be used in phytostabilization of boron in rhizosphere area and increase filtration capacity of the soil. While Salix alba, Popolus alba and S. babylonica have a better stabilization performance in terms of boron in wet lands, *Populus nigra* and *Salix anatolica* have the capacity have high boron phytoextraction capacity (Velioğlu et al.. 2020). Cadmium accumulation of some poplar species in their vegetative parts are given in Table 1.

**Table 1.** Cd accumulations among taxon in terms of plant parts (Yer Çelik et al.,2021).

Species	The order of Cd accumulation in			
	plant parts			
Populus nigra, Geyve	Root>leaf>branch			
Populus nigra N. 03.368A	Root>branch>leaf			
Populus euroamericana I-214	Root>branch>leaf			
Populus tremula	Leaf>root>branch			
Populus deltoides Samsun	Root>branch>leaf			
(77/51)				
Populus alba	Root>branch>leaf			

Remediation potential of metals (Cu, Cd, Mn, Cr, Co, Zn, Pb, and Ni) in *Colocasia esculenta* (L.) Schott plant was evaluated. The applicability of the plant for the treatment of municipality waste water was studied. While copper, cadmium, cobalt, lead and nickel accumulated in root tissue, manganese and zinc accumulated in the shoots. *Colocasia esculenta* (L.) Schott was shown to be used for the stabilization of Cu, Cd, Co, Pb and Ni heavy metals (BCF> 1 and TF <1). Bioconcentration and translocation factors in *Colocasia esculenta* (BCF> 1 and TF> 1) showed that this plant could be used for treatment of municipality waste water which contain Mn and Zn heavy metals (Rana and Maiti, 2018).

*Baccharis trimera* belonging to Asteraceae family was reported to have the highest copper concentration (586.2 mg kg<sup>-1</sup>) among the plants grown spontaneously in the wastewater of copper mining. The same study also reported that this plant was hyper accumulator for vanadium and barium. Both *Baccharis dracunculifolia* and *Baccharis trimera* showed a high concentration (Adiloğlu and Pamay, 2021).



**Figure 2.** *Baccharis trimera.* (Left to right: bush form, cylindirical leafless branches or stalkless leaves, grinded plant sample) (Adiloğlu and Pamay, 2021)



Figure 3. Baccharis dracunculifolia ssp. tandilensis (Adiloğlu and Pamay, 2021).

Willow trees (Salix spp.) are widely recognized for their phytoremediation potential, particularly in the context of water and soil pollution. Here are some important points about the relationship between willow trees and phytoremediation:

 $\succ$  Water Remediation: Willow trees are commonly used in phytoremediation projects focused on water bodies, such as lakes, rivers, and wetlands. They can effectively remove contaminants, including heavy metals, nutrients, organic compounds, and pesticides, from the water through a process called phytoremediation or rhizofiltration.

 $\succ$  Phytoextraction: Willow trees have the ability to extract and accumulate contaminants, particularly heavy metals, from the soil through their roots. This process, known as phytoextraction, involves the uptake of pollutants by the roots, followed by their translocation to the above-ground parts of the tree. Willow trees can accumulate significant concentrations of contaminants in their tissues.

 $\succ$  Rhizodegradation: Willow trees release root exudates into the soil, which stimulate microbial activity. This, in turn, enhances the degradation and detoxification of organic pollutants in the vicinity of

their root zone. The process is called rhizodegradation and can contribute to the overall remediation of contaminated soil.

 $\succ$  Erosion Control and Soil Stabilization: The extensive root systems of willow trees help bind the soil, reducing erosion and stabilizing slopes. This can be particularly beneficial in areas affected by mining activities or other disturbances, where soil erosion and subsequent runoff can contribute to pollution.

➤ Wastewater Treatment: Willow trees are commonly used in constructed wetlands or wastewater treatment systems for their ability to absorb and metabolize nutrients, such as nitrogen and phosphorus, from wastewater. This helps in the removal of these pollutants and improves the quality of the effluent.

It's worth noting that specific willow species and hybrids, such as Salix viminalis, Salix alba, and their cultivars, are commonly utilized in phytoremediation projects due to their robust growth and tolerance to various environmental conditions (Pandey et al., 2017; Meers, et al., 2005).

Vetiver grass is commonly used for phytoremediation due to its extensive root system, which helps stabilize soil and remove organic pollutants.

Vetiver grass (Chrysopogon zizanioides), also known as Vetiver, is a plant with significant phytoremediation potential. It is widely recognized for its ability to remediate various types of environmental pollution. Here are some important points about Vetiver grass and its role in phytoremediation:  $\succ$  Soil Erosion Control: Vetiver grass has a dense and deep root system that helps stabilize the soil and prevent erosion. Its extensive network of roots binds the soil particles together, reducing the loss of topsoil due to water runoff.

Contaminant Uptake: Vetiver grass has the ability to take up and accumulate a wide range of contaminants, including heavy metals, organic pollutants, and nutrients, from the soil. The contaminants are absorbed by the roots and transported to the plant tissues, thus removing them from the soil environment.

➤ Nutrient Uptake and Water Purification: Vetiver grass is known for its capacity to absorb excess nutrients, such as nitrogen and phosphorus, from water bodies. By taking up these nutrients, it helps improve water quality and reduces the risk of eutrophication in aquatic ecosystems.

➤ Rhizodegradation: Vetiver grass releases root exudates into the soil, which stimulate the growth of beneficial microorganisms. These microorganisms play a vital role in the degradation and detoxification of organic pollutants through a process known as rhizodegradation.

 $\triangleright$  Phytovolatilization: Some studies suggest that Vetiver grass may also have the ability to volatilize certain organic contaminants, releasing them into the atmosphere. This process, known as phytovolatilization, can contribute to the overall removal of pollutants from the environment.

Vetiver grass is commonly used in phytoremediation projects for contaminated sites, wastewater treatment, and soil improvement. Its resilience, adaptability to different soil conditions, and low maintenance requirements make it an attractive choice for remediation purposes (Kumar and Chopra, 2017; Truong et al., 2012).

#### CONCLUSION

Pollution is one of the leading problems of today's world. Phytoremediation and hyper accumulator plants are very important for the remediation of pollution as the most cost effective and applicable method. The hyper accumulator plants in forest ecosystems, whose most significant characteristics are forming high amount of biomass, should be studied and determined. Phytoremediation is the process of using plants to remove, degrade, or stabilize pollutants in the environment. Hyperaccumulator plants have the ability to absorb and accumulate high levels of pollutants from the soil or water. They can extract heavy metals, organic compounds, and other toxic substances from contaminated sites. Forest ecosystems are particularly important in the context of phytoremediation because they provide a diverse range of plant species, including hyperaccumulators. These ecosystems offer a rich source of potential plants that can play a role in remediating pollution. Studying and determining hyperaccumulator plants in forest ecosystems is crucial for several reasons. First, it allows us to identify and understand the specific plant species that have the ability to accumulate high levels of pollutants. By studying their characteristics and mechanisms, we can develop a better understanding of how phytoremediation works and how to optimize its effectiveness. Second, identifying hyperaccumulator plants in forest ecosystems helps in selecting appropriate species for specific polluted sites. Different plants accumulate specific pollutants, have varying abilities to SO understanding which plants are most effective for a particular type of pollution is essential for successful remediation efforts. Lastly, studying hyperaccumulator plants in forest ecosystems can contribute to the conservation and management of these ecosystems. Forests are invaluable in terms of biodiversity and ecosystem services. By recognizing the hyperaccumulator plants within these ecosystems, we can promote their preservation and potentially enhance their role in pollution remediation. In conclusion, phytoremediation, especially utilizing hyperaccumulator plants, is an important and cost-effective method for addressing pollution. Studying and determining hyperaccumulator plants in forest ecosystems can significantly contribute to the remediation of pollution, improve our understanding of phytoremediation mechanisms, and aid in the conservation of forest ecosystems.

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